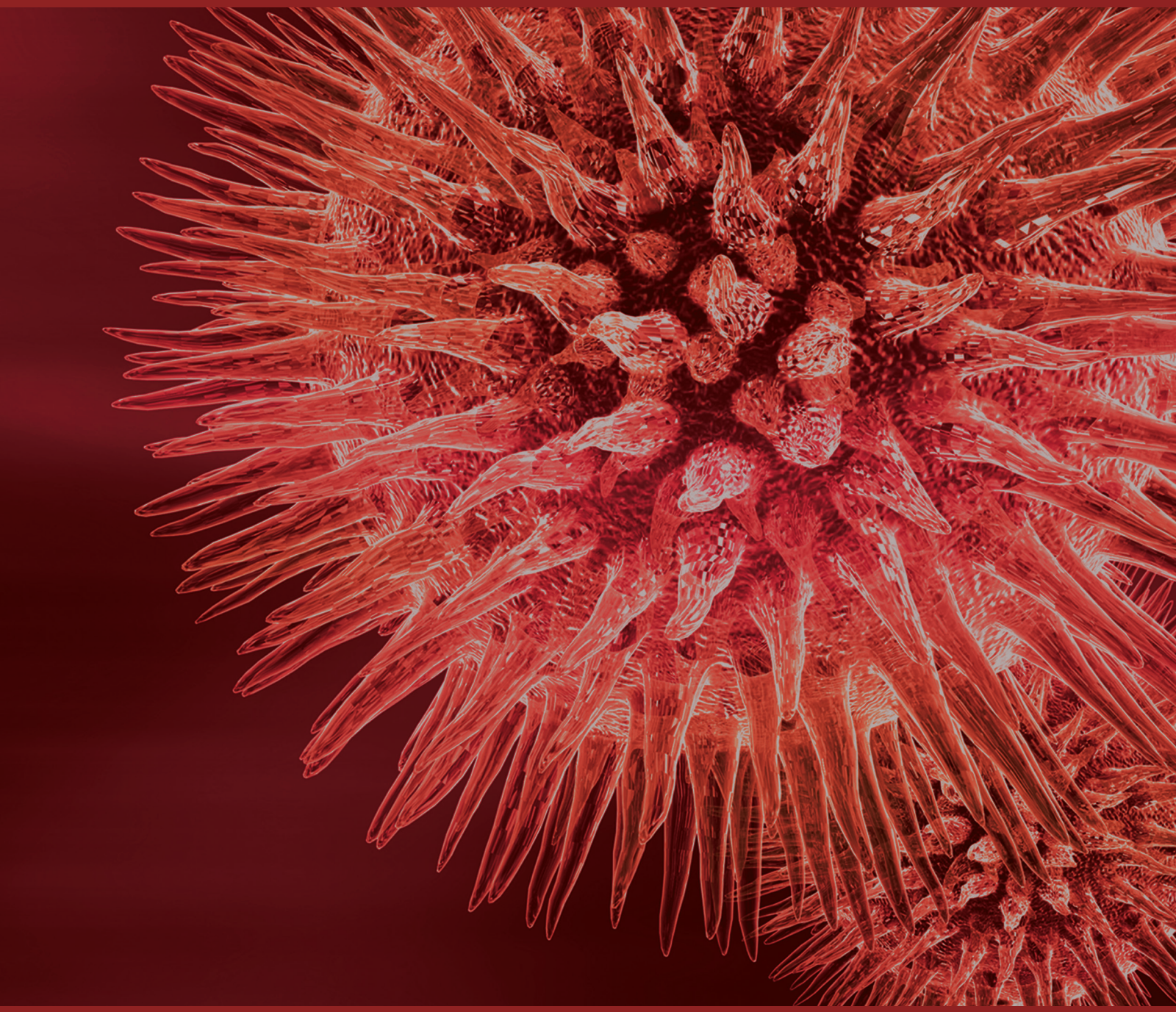
BioMed Research International

Immune Mechanisms in Vascular Disease and Stroke

Guest Editors: Ban-Hock Toh, Alex Bobik, Tin Soe Kyaw, Grant Drummond, Chris Sobey, and Tomasz Guzik



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Editorial

Immune Mechanisms in Vascular Disease and Stroke

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This special issue is a direct offshoot of a meeting of The Australian-European Consortium on Immune Mechanisms in Vascular Disease and Stroke held on October 10–12, 2013, in Monash University Prato Centre, Prato, Italy, cohosted by Monash University, Australia, and the Faculty of Medicine, Jagiellonian University Medical College, Poland, and organised by Grant Drummond, Chris Sobey, and Tomasz Guzik.

In this special issue, the authors addressed issues related to the immunopathogenesis, diagnosis and therapeutic reversal of atherosclerosis, and the role of the immune system in the development of hypertension and stroke.

In studies of immune activation in atherosclerosis, M. Maddaluno et al. reported that that while murine primary aortic smooth muscle cells express MHC class II and can acquire exogenous antigens, they fail to activate T cells through a failure in antigen presentation and a lack of costimulatory molecule expression. The findings do not support a role of antigen presentation by vascular smooth cells in the activation of T cells in atherosclerosis development. T. Kurita-Ochiai and M. Yamamoto examined the role of *P. gingivalis* or *A. actinomycetemcomitans* in augmenting inflammatory mechanisms and oxidative modification in the formation and activation of atherosclerotic plaques in ApoE-deficient mouse model fed a high-fat diet. They also examined whether mucosal vaccination with a periodontal pathogen or the anti-inflammatory activity of catechins can reduce periodontal pathogen-accelerated atherosclerosis. J. Maciag et al. examined endothelial function and major vascular disease risk factors in patients with dentures with and without clinical and microbiological features of patients' denture-related stomatitis. Patients with denture-related stomatitis had significantly lower flow mediated dilatation suggesting that it is associated with endothelial dysfunction that may predispose to atherosclerosis development. S. Visentin et al. examined relationships between levels of adipocytokine (adiponectin and leptin), markers of inflammation (tumor necrosis factor α, interleukin-6, and C reactive protein), and vascular remodelling in pregnancies complicated by intrauterine growth restriction. They found that these foetuses had a higher intima medial thickness that was associated with decreased blood adiponectin levels and increased adipocytokines concentrations that might be related to a greater risk of vascular remodeling.

I. A. Sobenin et al. examined low density lipoprotein-containing circulating immune complexes (LDL-CIC) and found that the titer of LDL-CIC in blood serum significantly correlated with atherosclerosis progression in humans and has the highest diagnostic value among other measured serum lipid parameters. They suggested that elevated CIC-cholesterol might well be a possible risk factor of coronary atherosclerosis.

M. A. Ulleryd et al. reported that metoprolol significantly reduced atherosclerotic plaque area and macrophage content in the thoracic aorta, accompanied by reduced serum levels

of TNFα and CXCL1 chemokine while total cholesterol levels were not affected. The findings suggest a potential role for metoprolol in the therapeutic management of patients with atherosclerosis.

Q. Dinh et al. reviewed evidence from human and animal studies that inflammation, oxidative stress, and endothelial dysfunction lead to the development of hypertension. Other potential proinflammatory conditions such as aging and elevated aldosterone that contribute to hypertension were also discussed. The evidence suggested that inflammation can lead to the development of hypertension and that oxidative stress and endothelial dysfunction are involved in the inflammatory cascade. The findings point to the potential therapeutic benefit of anti-inflammatory drugs and statins as antihypertensive therapy. C. T. Chan et al. described the protein targets of antibodies that are elevated in individuals with essential and pregnancy-related hypertension and the likely pathophysiological consequences of antibody binding to these targets. Potential mechanisms that underlie elevated antibody levels in hypertensive individuals were discussed and therapeutic opportunities that could arise with a better understanding of how and why antibodies are produced in hypertension were outlined. X. Xu and Y. Jiang reviewed the role of the innate immune system in stroke.

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Research Article

Murine Aortic Smooth Muscle Cells Acquire, Though Fail to Present Exogenous Protein Antigens on Major Histocompatibility Complex Class II Molecules

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In the present study aortic murine smooth muscle cell (SMC) antigen presentation capacity was evaluated using the Eα-GFP/Y-Ae system to visualize antigen uptake through a GFP tag and tracking of Eα peptide/MHCII presentation using the Y-Ae Ab. Stimulation with IFN-γ (100 ng/mL) for 72 h caused a significant ($P < 0.01$) increase in the percentage of MHC class II positive SMCs, compared with unstimulated cells. Treatment with Eα-GFP (100 μg/mL) for 48 h induced a significant ($P < 0.05$) increase in the percentage of GFP positive SMCs while it did not affect the percentage of Y-Ae positive cells, being indicative of antigen uptake without its presentation in the context of MHC class II. After IFN-γ-stimulation, ovalbumin- (OVA, 1 mg/mL) or $OVA_{323–339}$ peptide-(0.5 μg/mL) treated SMCs failed to induce OT-II $CD4^+$ T cell activation/proliferation; this was also accompanied by a lack of expression of key costimulatory molecules (OX40L, CD40, CD70, and CD86) on SMCs. Finally, OVA-treated SMCs failed to induce DO11.10-GFP hybridoma activation, a process independent of costimulation. Our results demonstrate that while murine primary aortic SMCs express MHC class II and can acquire exogenous antigens, they fail to activate T cells through a failure in antigen presentation and a lack of costimulatory molecule expression.

1. Introduction

Atherosclerosis is an immunoinflammatory process [1, 2] in which smooth muscle cells (SMCs) play a critical role [3–5]. SMCs produce a broad range of immunoinflammatory mediators contributing to vascular inflammation [6] and participate in the formation of arterial tertiary lymphoid tissue in experimental atherosclerosis [7]. Human SMCs express class II major histocompatibility complex molecules (MHC class II) in atherosclerotic plaques [8] and following IFN-γ stimulation [9–11]. In addition, SMC MHC class II expression increases following vascular injury in rodent models [12]. However, the possibility that SMCs can act as antigen presenting cells (APCs) and consequently activate vascular T cell response remains, to date, controversial. In mice it has been demonstrated that brain microvessel SMCs/pericytes can induce a proliferation of syngenic $CD4^+$ T cells *in vitro* in a MHC class II dependent manner [13]. SMCs/pericytes were able to process and present exogenous antigens to T cell hybridoma [14] and preferentially activated Th1 T cell clones as compared with Th2 T cells of the same antigen specificity [15]. In contrast to syngeneic cocultures using wild type $CD4^+$ T cells, microvascular SMCs did not support proliferation of antigen specific T cell receptor (TCR) transgenic $CD4^+$ T cells [16]. Others demonstrated that murine SMCs pulsed with antigen increased the expression of the IL-2 receptor on T cells but were not able to induce T cell proliferation [17].

Human saphenous vein SMCs expressing MHC class II molecules were unable to activate allogeneic memory T cells [18] and failed to effectively support T cell proliferation to the polyclonal activator, phytohemagglutinin [19]. This inability resulted from a defect in costimulatory function, particularly the lack of OX40 ligand (OX40L) [19]. SMCs from different tissues may behave differently; for example, cultured human airway smooth muscle cells were capable of presenting the superantigen, staphylococcal enterotoxin A, via MHC class II molecules to $CD4^+$ T cells [20]. More selective approaches are required to investigate SMC antigen presentation capacity.

Here we utilized the Eα-GFP/Y-Ae model that allows visualization of antigen uptake through a GFP tagged Eα peptide and tracking of antigen presentation using the Y-Ae Ab. The Eα-GFP protein is internalized and processed by APCs to generate Eα peptide for presentation on MHC class II. The monoclonal Ab Y-Ae detects Eα only when bound to MHC class II molecules (I-A^b) [21–24]. We demonstrate that while murine primary aortic SMCs express MHC class II and can acquire exogenous antigens, they fail to activate T cells through a failure in antigen presentation and a lack of costimulatory molecule expression.

2. Materials and Methods

2.1. Animals. C57BL/6 mice (Harlan, Shardlow, UK) were used to prepare SMCs and dendritic cells (DCs). OT-II (CD45.1) mice bred in house were used as donors of Tg T cells. These transgenic mice express the mouse alpha-chain and beta-chain T cell receptor that pairs with the CD4 coreceptor and is specific for chicken ovalbumin 323–339 in the context of I-A^b. Animals were maintained on a 12/12-hour light/dark cycle with free access to food and water and all the procedures were performed in accordance with local ethical and UK Home Office regulations.

2.2. Cell Cultures and Cocultures. Murine primary SMCs were derived from the thoracic aorta of C57BL/6 mice as previously described [25, 26] and grown in DMEM supplemented with L-glutamine, 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin (all from Gibco, Paisley, UK). Before initiation of the assays, the SMCs were starved into DMEM supplemented with 0.1% fetal bovine serum for 48 hours [25, 27]. Cells were characterized by immunofluorescence microscopy using FITC labeled anti-smooth muscle α-actin (α-SMA) monoclonal antibody (Ab) (clone 1A4; Sigma-Aldrich, Dorset, UK). Studies were performed with cells at passages 3–6. OVA specific TCR transgenic OT-II $CD4^+$ T cells were isolated from OT-II/CD45.1 mice using the MicroBead-based $CD4^+$ T Cell Isolation Kit II (Miltenyi Biotec, Bisley, UK) according to manufacturer's instructions and grown in complete RPMI (containing L-glutamine, 10% fetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin). The DO11.10-GFP hybridoma cells [28] were grown in complete RPMI containing geneticin (0.5 mg/mL, Sigma-Aldrich) as previously described [29]. DCs were obtained by flushing the bone marrow of C57BL/6 mice and grown in complete RPMI containing 10% granulocyte-macrophage colony stimulating factor (GM-CSF) for 7 days [30]. All cells used were kept in a humidified incubator at 37°C in 5% CO_2.

Murine SMCs were cultured in 48 multiwell plates until 80% confluence. Subsequently cells were stimulated with IFN-γ (100 ng/mL; R&D Systems, Abingdon, UK) for 72 h to enhance their MHC class II expression and then treated with OVA (1 mg/mL; InvivoGen, Toulouse, France) or $OVA_{323-339}$ peptide (0.5 μg/mL; InvivoGen) overnight. Isolated OT-II $CD4^+$ T cell or DO11.10-GFP hybridoma cell preparations were then introduced into the murine SMC cultures at a 1 : 5 ratio, for 24, 48, and 72 h or 24 h, respectively. OVA-treated DCs, cocultured with both OT-II $CD4^+$ T cells and DO11.10-GFP hybridoma cells at the same ratio of SMCs, were used as positive control. Subsequently, OT-II $CD4^+$T cells or DO11.10-GFP hybridoma cells were collected by rinsing the cocultures three times followed by staining and preparation for flow cytometric analysis. For the analysis of costimulatory molecule expression murine SMCs were cultured in 6 multiwell plates and stimulated with IFN-γ (100 ng/mL) for 72 h before flow cytometry. In a separate set of experiments, SMCs were stimulated with IFN-γ (100 ng/mL) for 72 h and then treated with fluorescein labeled-chicken OVA (FITC-OVA, 1 mg/mL, Molecular Probes) overnight. Subsequently, the supernatant was removed and the cells washed with PBS. The FITC-OVA uptake was visualized using the EVOS FL Cell Imaging System (Life Technologies Ltd., Paisley, UK).

2.3. Ealpha-GFP Preparation and Treatment. To assess the ability of murine SMCs to act as APCs, we employed the Ealpha- (Eα-) GFP/Y-Ae system as previously described [22–24]. A recombinant *Escherichia coli* strain expressing the Eα-GFP fusion protein was grown to midlog phase before induction of protein expression. Protein expression was induced by addition of isopropyl β-D-1-thiogalactopyranoside (IPTG; Sigma-Aldrich) to a final concentration of 1 mM and cultures were incubated overnight at 30°C with agitation (200 rpm). The Eα-GFP fusion protein was purified from the bacterial lysates using HisPur Cobalt Spin Columns (Thermo Scientific, Loughborough, UK) and endotoxin was removed using Detoxi-Gel Endotoxin Removing Columns (Thermo Scientific). Murine SMCs were cultured in 6 multiwell plates, as described above, stimulated with IFN-γ (100 ng/mL) for 72 h, and then treated with Eα-GFP (100 μg/mL). After 1, 24, and 48 h of treatment, cells were collected for flow cytometric analysis. DCs cultured under the same conditions and treated with Eα-GFP (100 μg/mL) for 24 h were used as a positive control.

2.4. Flow Cytometry. Aliquots of cells were washed and resuspended in Fc block (2.4G2 hybridoma supernatant) for 25 mins at 4°C to block Fc receptors. Subsequently, cells were incubated with Abs (in PBS containing 2% FBS) for 30 mins at 4°C, washed twice and then, where necessary, incubated with streptavidin for additional 20 mins at 4°C. Following washing, cells were analyzed on a FACScalibur using CellQuest-Pro (BD Biosciences, Oxford, UK), or on

a MACSQuant Analyzer (Miltenyi Biotec). Data analysis was performed using 6 FlowJo (Tree Star Inc., Olten, Switzerland).

Murine SMCs were stained with the following primary Abs: Y-Ae-Bio (specific for I-Eα 52–68 presented on I-A^b; clone: eBioY-Ae), anti-MHC II (I-A/I-E)-APC (clone: M5/114.15.2), anti-CD11c-APC (clone: N418), anti-CD54-PE (clone: 3E2), anti-CD44-FITC (clone: IM7), anti-OX40L-Bio (clone: RM134L) followed by streptavidin-PerCP, anti-CD80-FITC (clone: 16-10A1), anti-CD40-PE (clone: 3/23), anti-CD86-APC (clone: GL1), and anti-CD70-Bio (clone: FR70) followed by streptavidin-PerCP. OT-II $CD4^+$ T cells were stained with primary mAbs anti-CD4-PerCP (clone: RM4-5), anti-CD25-APC (clone: PC61), anti-CD44-PE (clone: IM7), and anti-CD69-Bio (clone: H1.2F3) followed by streptavidin-Pacific Blue. DO11.10-GFP hybridoma cells were stained with the primary Ab anti-DO11.10 TCR-APC (clone: KJ 1-26). Isotype-matched Abs were used as negative control. Y-Ae Ab, anti-CD11c, and anti-MHC II Ab were from eBioscience (Hatfield, UK); streptavidin-Pacific Blue was from Life Technologies Ltd.; all other Abs were from BD Biosciences.

2.5. CFSE Staining. OT-II $CD4^+$ T cells were labeled with the fluorescent dye carboxyl fluorescein succinimidyl ester (CFSE, Molecular Probes) as previously described [31]. The cells were washed and then cocultured with SMCs or DCs (used as a positive control) for 72 h. The level of fluorescence intensity from the CFSE labeling was measured by flow cytometry. Incremental loss of CFSE intensity showed proliferation.

2.6. Statistical Analysis. Results are expressed as mean ± SEM of 3 experiments run in triplicate. The results were statistically analyzed by the t-test or ANOVA (Two-Tail P value) and the Bonferroni post hoc test. The level of statistical significance was $P < 0.05$ per test.

3. Results

3.1. Assessment of Antigen Uptake/Presentation by SMCs Using the Eα-GFP/Y-Ae System. Stimulation with IFN-γ (100 ng/mL) for 72 h resulted in a significant ($P < 0.01$) 5- to 6-fold increase in the percentage of MHC class II positive SMCs compared with unstimulated cells (Figure 1(a)). Similar results were observed in IFN-γ-stimulated SMCs subsequently treated with Eα peptide (100 μg/mL) for 1 and 24 h ($P < 0.05$), while no significant changes were observed after 48 h of treatment (Figure 1(a)). As shown in Figure 1(b), SMC treatment with Eα peptide induced an increase in the percentage of GFP positive cells, both in presence or absence of IFN-γ-stimulation, being indicative of antigen uptake. The increase in GFP positive cells observed was significant only at 48 h ($P < 0.05$). No significant changes were observed in the percentage of Y-Ae positive SMCs after IFN-γ-stimulation and/or treatment with Eα peptide (Figure 1(c)) suggesting that, although SMCs internalize the antigen, they are not able to present the Eα peptide in the context of MHC class II. Treatment of DCs with Eα peptide (100 μg/mL), used as positive control, caused an increase in the percentage of Y-Ae positive cells (Figure 1(d)).

3.2. SMCs Fail to Induce OT-II $CD4^+$ T Cell Activation and Proliferation. We next assessed the ability of SMCs to activate OVA-specific transgenic $CD4^+$ T cells. In preliminary experiments by using FITC-OVA we confirmed the uptake of the model antigen by SMCs (data not shown). Using CFSE to track proliferation, we evaluated the number of Tg T cells undergoing proliferation after 72 h of coculture with SMCs or bone marrow derived DCs, used as positive control. The proportion of dividing T cells (expressed as percentage of $CFSE^-$ $CD4^+$ cells) was approximately 0.5–1% in both presence and absence of cocultured unstimulated SMCs (Figure 2). Neither stimulation with IFN-γ nor treatment with OVA or $OVA_{323-339}$ peptide of SMCs affected the proliferation of OT-II $CD4^+$ T cells. In contrast, coculture with OVA-treated DCs significantly ($P < 0.01$) increased the proportion of dividing OT-II $CD4^+$T cells by around 20% (Figure 2).

We also examined cell surface expression of activation markers such as CD25, CD44, and CD69 on OT-II $CD4^+$ T cells after coculture with SMCs or bone marrow derived DCs. CD25 and CD69 were detected in approximately 2% of OT-II $CD4^+$ T cells, alone or cocultured for 24, 48, and 72 h with unstimulated SMCs, IFN-γ-stimulated SMCs, or IFN-γ-stimulated SMCs treated with OVA or $OVA_{323-339}$ peptide. Moreover, the percentage of CD25 and CD69 positive T cells did not change after SMC treatment with OVA or $OVA_{323-339}$ peptide alone, while a significant ($P < 0.001$) increase was observed only after coculture with OVA-treated DCs at all of the time points considered (Figure 3). The percentage of CD44 positive OT-II $CD4^+$ T cells was about 7% at all of the time points considered, in both presence and absence of unstimulated SMCs. Stimulation with IFN-γ and/or treatment of SMCs with OVA or $OVA_{323-339}$ did not affect CD44 expression. A significant ($P < 0.01$) increase in CD44 positive OT-II $CD4^+$ T cells was observed after 48 and 72 h of coculture with OVA-treated DCs (Figure 3). These data demonstrate that antigen-pulsed aortic murine SMCs are not able to induce antigen-specific T cell activation/proliferation.

3.3. Effect of IFN-γ Stimulation on Costimulatory/Adhesion Molecules Expression by Murine SMCs. Previous studies have correlated the inability of human SMCs to activate memory T cells with the lack of costimulation [19]. Thus we examined whether murine SMCs express costimulatory/adhesion molecules at baseline and after IFN-γ (100 ng/mL) stimulation for 72 h. As shown in Figure 4, unstimulated SMCs expressed CD54 (ICAM-1), CD80, and CD44 (30%, 11%, and 87% positive cells, resp.). The stimulation with IFN-γ caused a 2-fold increase in the percentage of both ICAM-1 ($P < 0.01$) and CD80 ($P < 0.001$) positive cells while it did not affect the percentage of CD44 positive cells. In contrast, only low levels of OX40L, CD40, CD70, and CD86 expression were detectable in unstimulated SMCs. IFN-γ stimulation did not increase the percentage of SMCs positive to these molecules. The failure of SMCs to respond to IFN-γ, in this case, was selective for the costimulatory molecules because

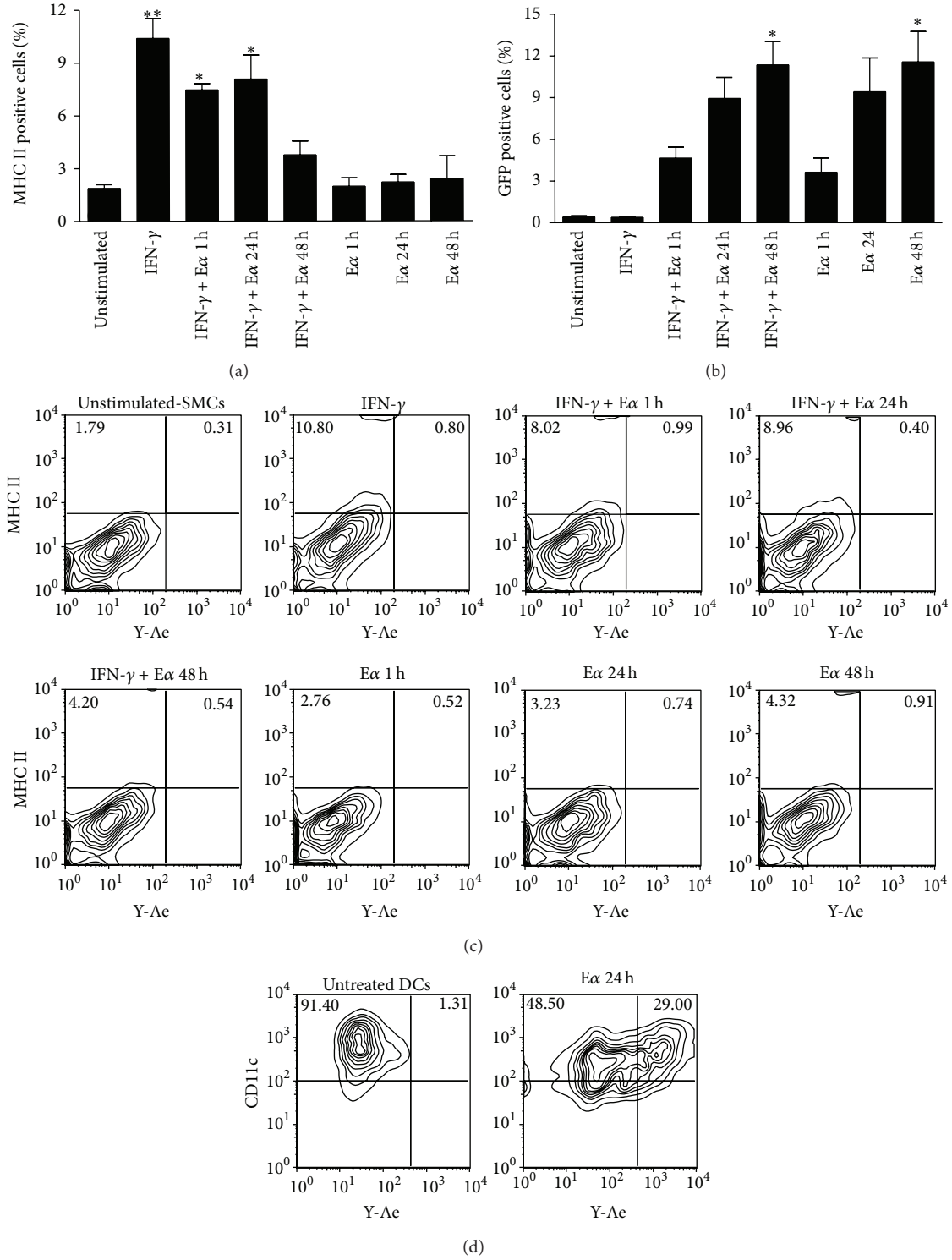

Figure 1: SMCs acquire exogenous antigens but fail to present them in the context of MHC class II. Evaluation of antigen uptake/presentation by murine SMCs. SMCs were stimulated with IFN-γ (100 ng/mL) for 72 h and subsequently treated with Eα-GFP peptide (100 μg/mL) for the indicated time points. (a) MHC class II expression. (b) GFP expression. (c) Representative flow cytometry plots showing no positivity of SMCs to the Y-Ae Ab or (d) positivity of DCs, used as a positive control. Results are expressed as mean ± SEM from three separate experiments. $^{*}P < 0.05$, $^{**}P < 0.01$, versus unstimulated cells.

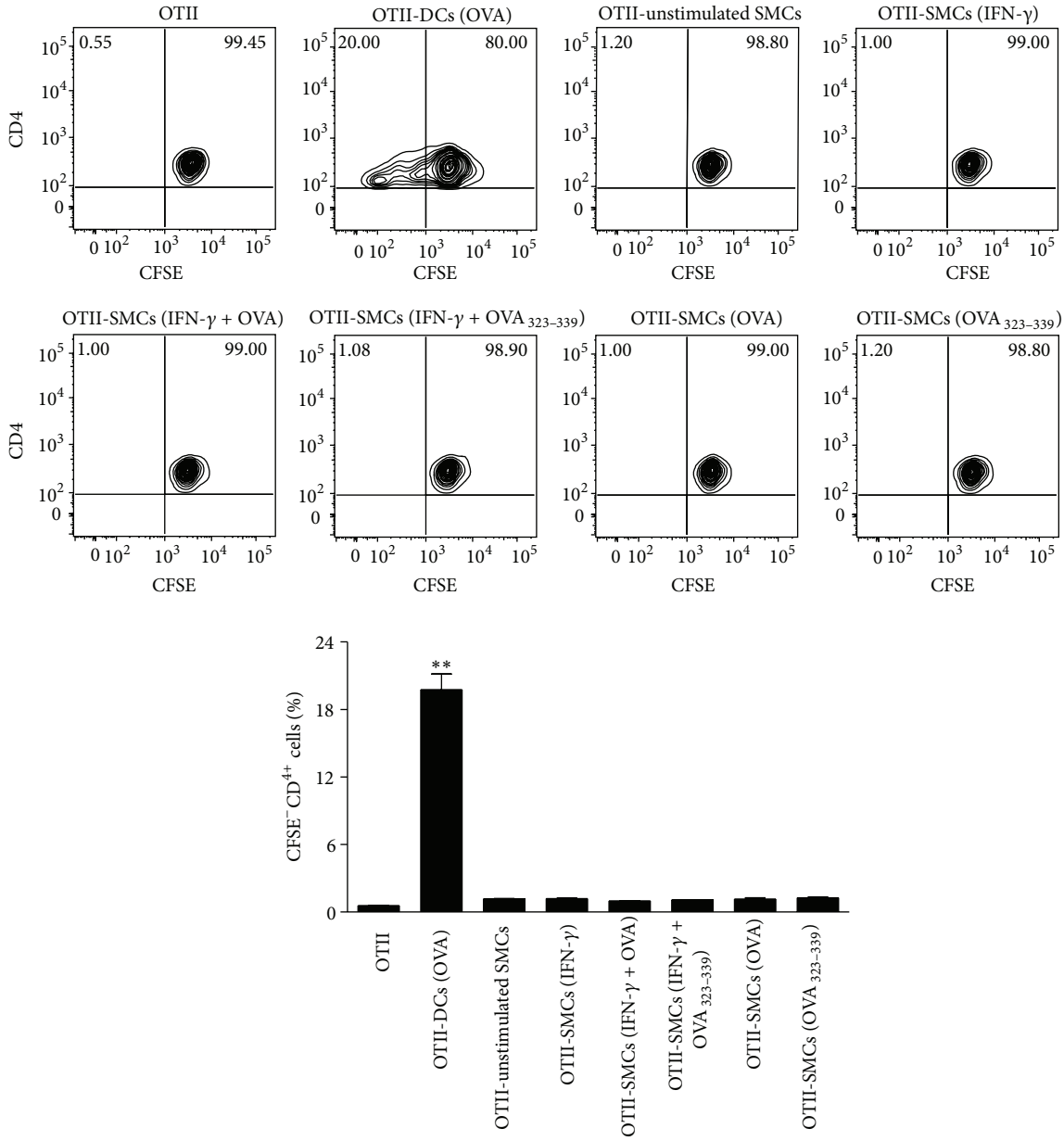

Figure 2: SMCs fail to induce OT-II $CD4^+$ T cell proliferation. Representative plots and relative statistical analysis showing the effect of SMCs on OT-II $CD4^+$ T cell proliferation. IFN-γ-stimulated SMCs were treated with OVA (1 mg/mL) or $OVA_{323-339}$ peptide (0.5 μg/mL) overnight and then cocultured with CFSE-labeled OT-II $CD4^+$ T cells for 72 h. OVA-treated DCs were used as a positive control. Results are expressed as mean ± SEM from three separate experiments run in triplicate. $^{**}P < 0.01$ versus OT-II $CD4^+$ T cells alone.

the percentage of MHC class II molecules was increased after IFN-γ stimulation under the same conditions (Figure 4).

3.4. SMCs Do Not Activate DO11.10-GFP Hybridoma Cells. The murine DO11.10-GFP hybridoma was originally obtained by stably transfecting a DO11.10 T cell hybridoma with a construct in which GFP expression is under the control of a nuclear factor of activated T cells (NFAT) regulated promoter [28]. Thus, once activated, hybridoma cells, detectable using the KJ1-26 clonotypic antibody, become GFP-positive. DO11.10 hybridoma cells express the TCR recognizing $OVA_{323-339}$ peptide in the context of either $I\text{-}A^d$ or $I\text{-}A^b$ MHC class II [32] without any requirement for costimulation [29]. Coculture with unstimulated SMCs had no effect on GFP expression by DO11.10-GFP hybridoma cells and similar results were obtained after stimulation with IFN-γ and/or treatment of SMCs with OVA or $OVA_{323-339}$ peptide. On the contrary, DCs treated with OVA, used as positive control, caused a significant ($P < 0.001$) increase in GFP expression by hybridoma cells (Figure 5). These data confirm that SMCs are unable to present exogenous protein antigens in the context of MHC class II.

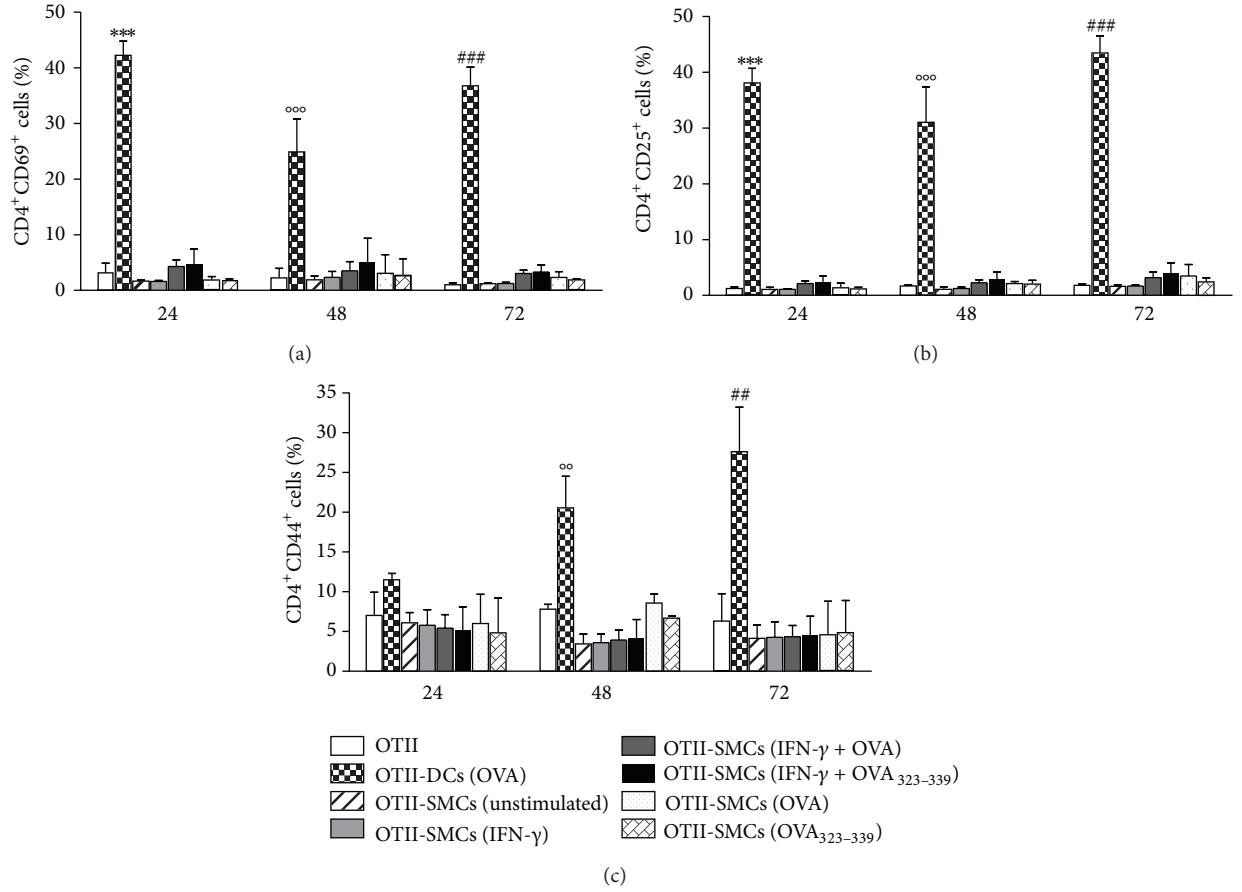

FIGURE 3: SMCs fail to induce OT-II $CD4^+$ T cell activation. Expression of CD69, CD25, and CD44 on OT-II $CD4^+$ T cells cocultured with OVA- or $OVA_{323–339}$ peptide-treated SMCs or OVA-treated DCs (used as positive control). Results are expressed as mean ± SEM from three separate experiments run in triplicate. $^{***}P < 0.001$ versus OT-II $CD4^+$ T cells alone at 24 h; $^{\circ\circ}P < 0.01$, $^{\circ\circ\circ}P < 0.001$ versus OT-II $CD4^+$ T cells alone at 48 h; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ versus OT-II $CD4^+$ T cells alone at 72 h.

4. Discussion

In the present study, we demonstrated that (1) cultured primary murine SMCs express MHC class II molecules after stimulation with IFN-γ and are able to acquire/uptake antigens; however, they fail to present the peptide antigen in the context of MHC class II, as demonstrated by using the specific Ealpha- (Eα-) GFP/Y-Ae system; (2) OVA-treated SMCs fail to induce activation/proliferation of OT-II $CD4^+$ T cells, data consistent with a defect in MHC class II-restricted Ag presentation and in the expression of costimulatory molecules, such as OX40L, CD40, CD70, and CD86; (3) SMCs also fail to promote activation of OVA responding DO11.10-GFP hybridoma T cells that do not require any costimulatory signal for activation.

The first finding that murine aortic SMCs express MHC class II molecules is in line with previous data showing MHC class II expression in atheroma SMCs [8] and in rodent arteries in response to injury [12], as well as in human SMCs in culture following IFN-γ-stimulation [18]. Murray and colleagues [18] demonstrated that class II molecules on human saphenous vein SMCs were functional, since they induced CD25 expression on resting $CD4^+$ T cells. Additional studies demonstrated that survival and activation of T cells occurred as a result of the specific interaction between TCR on T cells and MHC molecules on SMCs, since treatment with antibodies directed toward MHC class II blocked the proliferation of $CD4^+$ T cells cocultured with syngeneic SMCs [13, 16]. On the contrary, in the context of nonspecific generalized T cell stimulation or in the presence of polyclonal activators such as phytohemagglutinin SMCs did not activate $CD4^+$ T cells [18, 19].

In order to understand whether an antigen specific stimulation leads to immunological competence of SMCs, engaging MHC molecules, we employed a novel and selective approach such as the Eα-GFP/Y-Ae model that allows visualization of antigen uptake through a GFP tagged Eα peptide and tracking of antigen presentation using the Y-Ae Ab. The Eα-GFP protein is internalized and processed by APCs to generate Eα peptide for presentation on MHC class II. The monoclonal Ab Y-Ae detects Eα only when bound to MHC class II molecules ($I\text{-}A^b$) [21–24]. Eα-GFP treatment of SMCs increased the percentage of GFP positive cells, without affecting the percentage of SMCs positive to

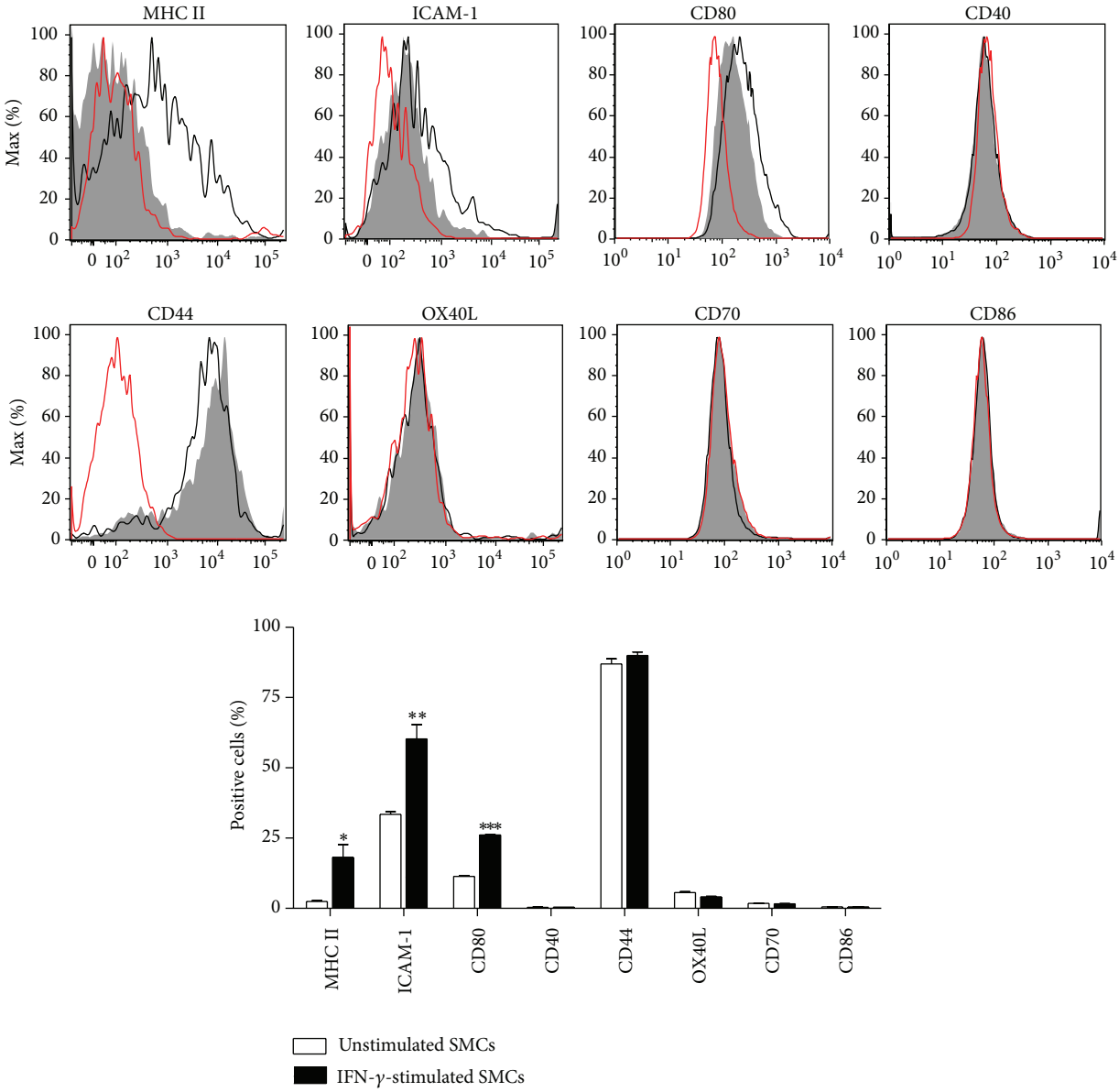

FIGURE 4: SMCs lack key costimulatory molecules. Representative flow cytometry histograms and relative graph showing the effect of IFN-γ (100 ng/mL) on costimulatory/adhesion molecules expression in murine SMCs. Red empty histograms: isotype control; gray filled histograms or white columns: unstimulated SMCs; black empty histograms or black columns: IFN-γ-stimulated SMCs. Results are expressed as mean ± SEM from three separate experiments run in triplicate. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ versus unstimulated cells.

the monoclonal Ab Y-Ae. These results clearly demonstrate that primary aortic murine SMCs fail to present exogenous protein antigens in the context of MHC class II.

Our results also prove the inability of SMCs in inducing OVA specific OT-II $CD4^+$ T cell activation and proliferation. A possible explanation for these observations is that SMCs fail to activate T cells through a failure in antigen presentation and a lack of costimulatory molecule expression. Indeed, although human SMCs express the costimulatory molecules CD44, CD54, CD58, and CD59 [18], they lack OX40L, which is considered essential for T cell activation [19]. We also observed lack of costimulatory molecule expression (OX40L, CD40, CD70, and CD86) on SMC surface following IFN-γ stimulation, supporting the hypothesis that impaired costimulation function contributes to the inability of SMCs to induce T cells activation/proliferation.

In order to analyze this point further, we cocultured SMCs with the DO11.10-GFP hybridoma cells that, in presence of the model Ag OVA, undergo activation without requiring any costimulatory signal [28, 29]. Importantly, SMCs failed to activate DO11.10-GFP hybridoma cells, demonstrating that other mechanisms, apart from a defect in costimulation function, are liable for the limited capacity of SMCs to activate T cells.

One possibility could be that SMCs cannot process protein antigens, rather than not being able to present

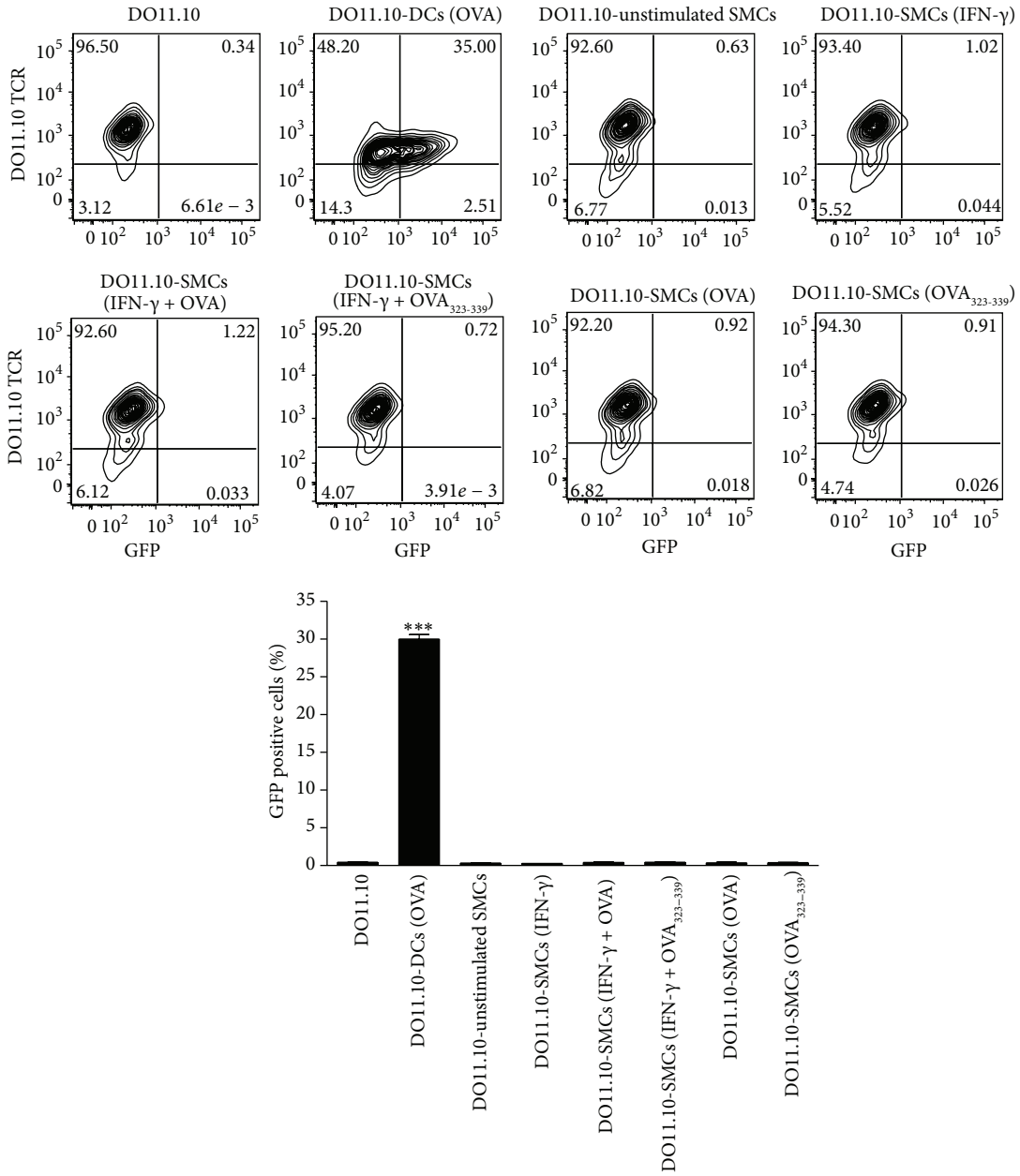

Figure 5: Effect of SMCs on DO11.10-GFP hybridoma cell activation. IFN-γ-stimulated SMCs were treated with (OVA, 1 mg/mL) or $OVA_{323-339}$ peptide (0.5 μg/mL) overnight and then cocultured with DO11.10-GFP hybridoma cells for 24 h. OVA-treated DCs were used as positive control. Results are expressed as mean ± SEM from three separate experiments run in triplicate. $^{***}P < 0.001$ versus DO11.10-GFP hybridoma cells alone.

them. Nevertheless, in our experiments, treatment of SMCs with $OVA_{323-339}$ peptide, which does not require any processing to be presented in the context of MHC molecules, did not affect activation/proliferation of neither OT-II $CD4^+$ T cell nor DO11.10-GFP hybridoma cells. This observation demonstrates that the SMC inability in presentation cannot lie in a defect in the antigen processing; thus further investigations will be necessary to understand the mechanisms underlining this deficiency.

5. Conclusions

In summary, our work demonstrates that while murine primary aortic SMCs express MHC class II and can acquire exogenous antigens, they fail to activate T cells through a failure in antigen presentation and a lack of costimulatory molecule expression. Our results do not preclude the possibility that SMCs could act as APCs, depending on the environment (e.g., in atherosclerotic arteries) and the vascular bed; however, they suggest that antigen presentation

may not be the key immunological feature of SMCs in the initiation of vascular inflammation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

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References

[1] E. Galkina and K. Ley, "Immune and inflammatory mechanisms of atherosclerosis," *Annual Review of Immunology*, vol. 27, pp. 165–197, 2009.

[2] P. Libby, A. Lichtman, and G. Hansson, "Immune effector mechanisms implicated in atherosclerosis: from mice to humans," *Immunity*, vol. 38, no. 6, pp. 1092–1104, 2013.

[3] G. K. Owens, M. S. Kumar, and B. R. Wamhoff, "Molecular regulation of vascular smooth muscle cell differentiation in development and disease," *Physiological Reviews*, vol. 84, no. 3, pp. 767–801, 2004.

[4] A. C. Doran, N. Meller, and C. A. McNamara, "Role of smooth muscle cells in the initiation and early progression of atherosclerosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 5, pp. 812–819, 2008.

[5] S. Allahverdian, P. S. Pannu, and G. A. Francis, "Contribution of monocyte-derived macrophages and smooth muscle cells to arterial foam cell formation," *Cardiovascular Research*, vol. 95, no. 2, pp. 165–172, 2012.

[6] H. Loppnow, M. Buerke, K. Werdan, and S. Rose-John, "Contribution of vascular cell-derived cytokines to innate and inflammatory pathways in atherogenesis," *Journal of Cellular and Molecular Medicine*, vol. 15, no. 3, pp. 484–500, 2011.

[7] K. Lötzer, S. Döpping, S. Connert et al., "Mouse aorta smooth muscle cells differentiate into lymphoid tissue organizer-like cells on combined tumor necrosis factor receptor-1/lymphotoxin β-receptor NF-κB Signaling," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 30, no. 3, pp. 395–402, 2010.

[8] G. K. Hansson, L. Jonasson, J. Holm, and L. Claesson-Welsh, "Class II MHC antigen expression in the atheroscerotic plaque: smooth muscle cells express HLA-DR, HLA-DQ and the invariant gamma chain," *Clinical and Experimental Immunology*, vol. 64, no. 2, pp. 261–268, 1986.

[9] S. J. C. Warner, G. B. Friedman, and P. Libby, "Regulation of major histocompatibility gene expression in human vascular smooth muscle cells," *Arteriosclerosis*, vol. 9, no. 3, pp. 279–288, 1989.

[10] S. Stemme, G. Fager, and G. K. Hansson, "MHC class II antigen expression in human vascular smooth muscle cells is induced by interferon-gamma and modulated by tumour necrosis factor and lymphotoxin," *Immunology*, vol. 69, no. 2, pp. 243–249, 1990.

[11] G. Butticè, J. Miller, L. Wang, and B. D. Smith, "Interferon-γ induces major histocompatibility class II transactivator (CIITA), which mediates collagen repression and major histocompatibility class II activation by human aortic smooth muscle cells," *Circulation Research*, vol. 98, no. 4, pp. 472–479, 2006.

[12] L. Jonasson, J. Holm, and G. K. Hansson, "Smooth muscle cells express Ia antigens during arterial response to injury," *Laboratory Investigation*, vol. 58, no. 3, pp. 310–315, 1988.

[13] Z. Fabry, M. M. Waldschmidt, L. Van Dyk, S. A. Moore, and M. N. Hart, "Activation of CD4+ lymphocytes by syngeneic brain microvascular smooth muscle cells," *Journal of Immunology*, vol. 145, no. 4, pp. 1099–1104, 1990.

[14] Z. Fabry, M. M. Waldschmidt, S. A. Moore, and M. N. Hart, "Antigen presentation by brain microvessel smooth muscle and endothelium," *Journal of Neuroimmunology*, vol. 28, no. 1, pp. 63–71, 1990.

[15] Z. Fabry, M. Sandor, T. F. Gajewski et al., "Differential activation of Th1 and Th2 CD4+ cells by murine brain microvessel endothelial cells and smooth muscle/pericytes," *Journal of Immunology*, vol. 151, no. 1, pp. 38–47, 1993.

[16] B. J. Swanson, D. C. Baiu, M. Sandor, Z. Fabry, and M. N. Hart, "A small population of vasculitogenic T cells expands and has skewed T cell receptor usage after culture with syngeneic smooth muscle cells," *Journal of Autoimmunity*, vol. 20, no. 2, pp. 125–133, 2003.

[17] J. Suttles, R. W. Miller, and C. F. Moyer, "T cell-vascular smooth muscle cell interactions: antigen-specific activation and cell cycle blockade of T helper clones by cloned vascular smooth muscle cells," *Experimental Cell Research*, vol. 218, no. 1, pp. 331–338, 1995.

[18] A. G. Murray, P. Libby, and J. S. Pober, "Human vascular smooth muscle cells poorly co-stimulate and actively inhibit allogeneic CD4+ T cell proliferation in vitro," *Journal of Immunology*, vol. 154, no. 1, pp. 151–161, 1995.

[19] P. Zhang, T. D. Manes, J. S. Pober, and G. Tellides, "Human vascular smooth muscle cells lack essential costimulatory molecules to activate allogeneic memory T cells," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 30, no. 9, pp. 1795–1801, 2010.

[20] H. Veler, A. Hu, S. Fatma et al., "Superantigen presentation by airway smooth muscle to $CD4^+$ T lymphocytes elicits reciprocal proasthmatic changes in airway function," *The Journal of Immunology*, vol. 178, no. 6, pp. 3627–3636, 2007.

[21] A. A. Itano, S. J. McSorley, R. L. Reinhardt et al., "Distinct dendritic cell populations sequentially present antigen to CD4 T cells and stimulate different aspects of cell-mediated immunity," *Immunity*, vol. 19, no. 1, pp. 47–57, 2003.

[22] K. A. Pape, D. M. Catron, A. A. Itano, and M. K. Jenkins, "The humoral immune response is initiated in lymph nodes by b cells that acquire soluble antigen directly in the follicles," *Immunity*, vol. 26, no. 4, pp. 491–502, 2007.

[23] C. M. Rush, T. J. Mitchell, and P. Garside, "A detailed characterisation of the distribution and presentation of DNA vaccine encoded antigen," *Vaccine*, vol. 28, no. 6, pp. 1620–1634, 2010.

[24] N. MacRitchie, G. Grassia, S. R. Sabir et al., "Plasmacytoid dendritic cells play a key role in promoting atherosclerosis in apolipoprotein e-deficient mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 32, no. 11, pp. 2569–2579, 2012.

[25] G. Grassia, M. Maddaluno, A. Guglielmotti et al., "The anti-inflammatory agent bindarit inhibits neointima formation in both rats and hyperlipidaemic mice," *Cardiovascular Research*, vol. 84, no. 3, pp. 485–493, 2009.

[26] G. Grassia, M. Maddaluno, C. Musilli et al., "The IκB kinase inhibitor nuclear factor-κB essential modulator-binding

domain peptide for inhibition of injury-induced neointimal formation," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 30, no. 12, pp. 2458–2466, 2010.

[27] M. Maddaluno, G. Grassia, M. V. di Lauro et al., "Bindarit inhibits human coronary artery smooth muscle cell proliferation, migration and phenotypic switching," *PLoS ONE*, vol. 7, no. 10, Article ID e47464, 2012.

[28] D. M. Underhill, M. Bassetti, A. Rudensky, and A. Aderem, "Dynamic interactions of macrophages with T cells during antigen presentation," *The Journal of Experimental Medicine*, vol. 190, no. 12, pp. 1909–1914, 1999.

[29] S. Culshaw, O. R. Millington, J. M. Brewer, and I. B. McInnes, "Murine neutrophils present Class II restricted antigen," *Immunology Letters*, vol. 118, no. 1, pp. 49–54, 2008.

[30] T. R. Ghimire, R. A. Benson, P. Garside, and J. M. Brewer, "Alum increases antigen uptake, reduces antigen degradation and sustains antigen presentation by DCs *in vitro*," *Immunology Letters*, vol. 147, no. 1-2, pp. 55–62, 2012.

[31] V. B. Gibson, R. A. Benson, K. J. Bryson et al., "A novel method to allow noninvasive, longitudinal imaging of the murine immune system in vivo," *Blood*, vol. 119, no. 11, pp. 2545–2551, 2012.

[32] P. Marrack, C. Hannum, and M. Harris, "Antigen-specific, major histocompatibility complex-restricted T cell receptors," *Immunological Reviews*, vol. 76, pp. 131–145, 1983.

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Review Article

Roles of Inflammation, Oxidative Stress, and Vascular Dysfunction in Hypertension

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Hypertension is a complex condition and is the most common cardiovascular risk factor, contributing to widespread morbidity and mortality. Approximately 90% of hypertension cases are classified as essential hypertension, where the precise cause is unknown. Hypertension is associated with inflammation; however, whether inflammation is a cause or effect of hypertension is not well understood. The purpose of this review is to describe evidence from human and animal studies that inflammation leads to the development of hypertension, as well as the evidence for involvement of oxidative stress and endothelial dysfunction—both thought to be key steps in the development of hypertension. Other potential proinflammatory conditions that contribute to hypertension—such as activation of the sympathetic nervous system, aging, and elevated aldosterone—are also discussed. Finally, we consider the potential benefit of anti-inflammatory drugs and statins for antihypertensive therapy. The evidence reviewed suggests that inflammation can lead to the development of hypertension and that oxidative stress and endothelial dysfunction are involved in the inflammatory cascade. Aging and aldosterone may also both be involved in inflammation and hypertension. Hence, in the absence of serious side effects, anti-inflammatory drugs could potentially be used to treat hypertension in the future.

1. Introduction

Hypertension is the most common cardiovascular risk factor [1] and contributes to widespread morbidity and mortality worldwide [2]. Hypertension is a complex condition, and about 90% of cases are classified as essential hypertension, where the precise cause is unknown [3]. A small minority of hypertensive patients have secondary hypertension, in which a known factor is specifically responsible for raising blood pressure. Many secondary causes of hypertension include primary aldosteronism, obstructive sleep apnea, and renovascular disease [4]. An association between hypertension and inflammation has now been clearly demonstrated; however, it is presently unclear whether inflammation is predominately a cause or effect of hypertension. This brief review will describe evidence that inflammation might lead to the development of hypertension (Figure 1).

2. Inflammation in Human Hypertension

Current therapies for human hypertension include angiotensin II (Ang II) type 1 receptor (AT1R) inhibitors, angiotensin converting enzyme (ACE) inhibitors, diuretics, calcium channel antagonists, and β-blockers. Treatment with commonly used antihypertensives reduces the risk of total major cardiovascular events, and importantly, it appears that the larger the reduction in blood pressure, the larger the reduction in cardiovascular risk [5]. Insulin resistance contributes causally toward the pathogenesis of hypertension [6, 7]. Indeed, hypertension has been found to be associated with hyperinsulinemia and insulin resistance in humans [8]. Yet, while the above-mentioned therapies successfully lower blood pressure in most individuals, there are a group of patients who are resistant to such treatments. Furthermore, even when blood pressure targets are achieved, many hypertensive patients remain at risk for

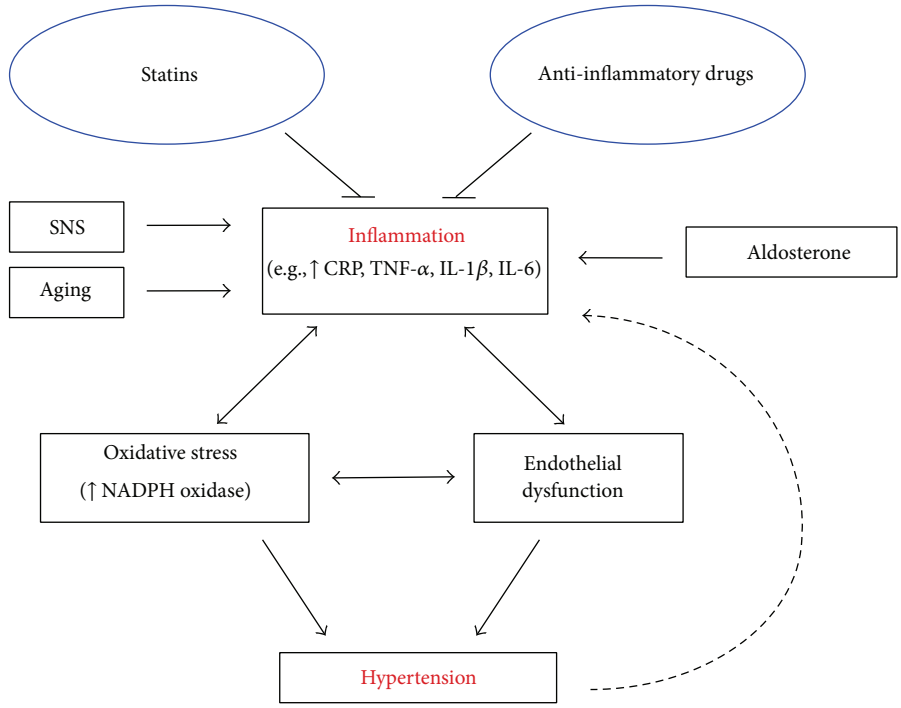

Figure 1: Schematic diagram illustrating the relationship between inflammation and hypertension and the contributing factors involved. Anti-inflammatory drugs and statins may be effective antihypertensive due to their anti-inflammatory properties.

a cardiovascular event, which may be due to underlying inflammation.

Inflammation is a protective response to injury or infection. It is a complex process that involves inflammatory cells first identifying the affected tissue, leukocyte recruitment into tissue, elimination of the offending agent, and repair of the site of injury. Inflammation requires interactions between cell surfaces, extracellular matrix, and proinflammatory mediators [9]. Excessive inflammation can have detrimental effects and contribute to the progression of chronic and/or prolonged diseases such as atherosclerosis [10], rheumatoid arthritis [11], and systemic lupus erythematosus [12].

The acute phase protein, C-reactive protein (CRP), is involved in innate immune responses and has roles that include activating the complement system and enhancing phagocytosis [13]. CRP can stimulate monocytes to release proinflammatory cytokines such as interleukin-6 (IL-6), interleukin-1 beta (IL-1β), and tumour necrosis factor alpha (TNF-α) [14] and also endothelial cells to express intracellular adhesion molecule (ICAM)-1 and vascular cell adhesion molecule (VCAM)-1 [15], effects which will further promote inflammation.

CRP is considered the inflammatory marker with the strongest association with hypertension. It has been demonstrated in numerous clinical trials that hypertensive patients commonly have increased plasma CRP levels [16–21]. Both males and females participated in these studies and the ages of these patients varied from young [16] to middle aged [17]. Prehypertensive patients generally have higher plasma CRP levels than normotensive patients [22], and higher baseline CRP levels are reportedly associated with a higher risk of developing overt hypertension [23–25], consistent with the concept that systemic low-grade inflammation may precede hypertension. Systemic low-grade inflammation can be defined as a 2- to 3-fold increase in plasma levels of cytokines and acute phase proteins [26]. As discussed below, hypertensive patients have been reported to have higher plasma concentrations of proinflammatory cytokines. Nonhypertensive offspring of hypertensive parents tend to have higher serum CRP levels than offspring of nonhypertensive parents [27]. Studies have also demonstrated higher plasma IL-6 [28–30], IL-1β [31, 32], and TNF-α [28, 33, 34] levels in hypertensive patients compared to normotensive patients.

There is also evidence for involvement of immune cells in human hypertension. Patients with hypertensive nephrosclerosis have higher renal infiltration of $CD4^+$ and $CD8^+$ T cells than normotensive control patients [35]. Furthermore, C-X-C chemokine receptor type 3 (CXCR3) chemokines are well-known tissue-homing chemokines for T cells, and circulating levels of CXCR3 chemokines have been reported to be elevated in hypertensive patients [35]. Acquired immunodeficiency syndrome (AIDS) patients have reduced $CD4^+$ T cells and the incidence of hypertension has been reported to be lower in AIDS patients than in HIV-negative participants. The highly active antiretroviral therapy (HAART) which can raise T cell levels increases the incidence of hypertension in AIDS patients similar to HIV-negative participants after treatment for less than 2 years, and the incidence of hypertension in AIDS patients is higher than in HIV-negative participants after treatment for 2 to 5 years [36]. In addition, it is becoming increasingly recognized that both neonatal and childhood health and disease are linked to the prenatal

environment. Indeed, infants born following intrauterine inflammation are at increased risk of perinatal morbidity and mortality than infants born to healthy mothers [37–39].

3. Inflammation in Experimental Hypertension

Findings from animal studies have also suggested a role for inflammation in the pathophysiology of hypertension. Spontaneously hypertensive rats (SHR) are a genetic model of essential hypertension that develop hypertension as they age. SHR at 3 weeks of age are not yet hypertensive, yet their kidneys have higher levels of infiltrating lymphocytes and macrophages and activation of nuclear factor-kappaB (NF-κB) than in Wistar Kyoto (WKY) normotensive control rats. These inflammatory changes in the kidneys continue to increase, together with blood pressure, with age in the SHR [40]. Junctional adhesion molecule (JAM)-1 is involved in leukocyte binding to the endothelium and has been found to be upregulated in the brainstem of SHR compared to WKY rats. Overexpression of JAM-1 in WKY rats resulted in elevated systolic blood pressure [41].

Macrophage colony-stimulating factor (m-CSF) acts as a chemotactic factor for monocytes and regulates both effector functions of mature monocytes and macrophages, and production of cytokines. Mice deficient in m-CSF [42], IL-6 [43], TNF-α [44], or interleukin-17 (IL-17) [45] develop a lower blood pressure in response to a hypertensive dose of Ang II compared with control mice. RNA interference knockdown of IL-6 in rats has also been shown to inhibit hypertension [46]. Suppression of NF-κB reportedly inhibits the increase in blood pressure that normally occurs in SHR [47]. Lipopolysaccharide (LPS)- endotoxin from gram-negative bacteria elicits a strong immune response and intraperitoneal injection of LPS is a well-characterised model of systemic inflammation in rodents. In rats, LPS-induced increases in plasma levels of CRP, TNF-α, and IL-1β are associated with an increase in blood pressure [48]. Inhibition of Cox-2 in LPS-treated rats inhibited the increase in blood pressure, suggesting that inflammation in response to LPS treatment contributed to the hypertensive effect [48]. Offspring with prenatal exposure to LPS [49, 50] or IL-6 [51] have higher blood pressure than control offspring.

Immune cells have been implicated to play a role in the development of hypertension. RAG-1 deficient mice, which lack T and B cells, do not develop hypertension in response to Ang II [52], DOCA-salt [52], or chronic stress [53]. Adoptive transfer of T cells but not B cells restored the hypertensive effect in RAG-deficient mice [52]. An analogous effect has been reported to occur in macrophage-depleted mice in response to Ang II infusion [54].

T cells have been demonstrated to express AT1R (the main target receptor of Ang II), ACE, angiotensinogen, renin, and the renin receptor, all important components of the renin angiotensin aldosterone system (RAAS) [55]. The RAAS is a key regulator of blood pressure, and excessive stimulation of this system can cause hypertension. While T cells are capable of producing Ang II [56], which can cause vasoconstriction, stimulate inflammation, and aldosterone production [57], the role of T cell-derived Ang II in such effects is uncertain. There is some evidence that inflammation can overstimulate the RAAS. Inflammatory markers such as CRP [58], TNF-α, and IL-1β [59] have been found to upregulate AT1R. Furthermore, central administration of LPS to rats increases AT1R mRNA expression in the hypothalamus [60].

Given the importance of this recent concept that immune cells are involved in hypertension, we will briefly discuss a hypothesis presented in the literature by Harrison's group regarding how immune cells are activated in hypertension. Hypertensive stimuli, including salt, overactivity of the RAAS, oxidative stress, and inflammation lead to an initial elevation in blood pressure (mainly because of central actions but also due to endogenous hormones such as Ang II and aldosterone), which results in protein modifications. These altered proteins are no longer recognized as self (i.e., they serve as neoantigens), and T cells are activated. T cell-derived signals promote entry of macrophages (and other inflammatory cells) into the vasculature and kidney which results in cytokine release. In the vasculature, activated T cells promote vasoconstriction and remodeling. Together with the promotion of sodium and water retention in the kidney, more severe hypertension can result [61, 62].

4. Inflammation and Endothelial Dysfunction in Hypertension

One potential mechanism by which inflammation may promote hypertension is by causing endothelial dysfunction. The endothelium is a single cell layer that lines the luminal surface of blood vessels and is involved in regulation of vascular tone and structure. Nitric oxide (NO) derived from endothelial nitric oxide synthase (eNOS) is a signalling molecule important in regulating vascular tone. When NO is released from endothelial cells it causes smooth muscle relaxation and subsequent vasodilation (Figure 2) [63]. Endothelial dysfunction may contribute to increased systemic vascular resistance and thus lead to the development of hypertension and is commonly manifested as impaired endothelium-dependent vasodilation due to an imbalance between vasoconstrictors and vasodilators [64]. Inflammation can alter the rates of synthesis and degradation of vasoconstrictors and vasodilators including NO, and impaired NO bioactivity is associated with hypertension. Rats chronically treated with the NO synthase inhibitor, N-nitro-L-arginine methyl ester, have higher blood pressure than controls [65]. Inflammation has been shown to downregulate NO synthase activity. For example, CRP [66] and TNF [67] have both been demonstrated to attenuate NO production by destabilising eNOS mRNA, which reduces NOS protein expression, and inhibition of TNF restores endothelial-dependent vasodilation in humans [68] (Figure 2). IL-17 has been reported to cause endothelial dysfunction by activating Rho-kinase, which leads to phosphorylation of the inhibitory eNOS residue, threonine 495 [69]. Inhibition of eNOS increases vascular tone [70] and consistent with this, Rho-kinase has been shown to contribute to cerebral vascular tone *in vivo* and this

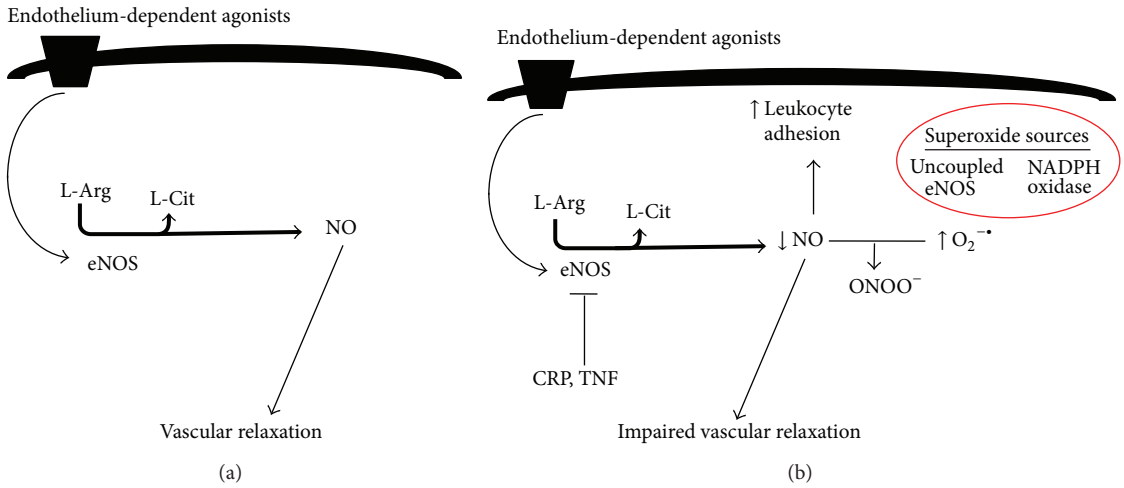

Figure 2: (a) Diagram illustrating endothelium-dependent relaxation during health. (b) Diagram illustrating mechanism by which inflammation and oxidative stress cause endothelial dysfunction. Inflammatory mediators such as CRP and TNF destabilise eNOS mRNA (thus inhibiting eNOS). NO protects endothelium by inhibiting leukocyte adhesion; thus, impaired NO function results in increased leukocyte adhesion. Increased superoxide (derived from NADPH oxidase or uncoupled eNOS) impairs NO bioavailability and leads to impaired vascular relaxation. The figure is based on text.

is enhanced during chronic hypertension. In contrast, protein kinase C was not found to contribute to cerebral vascular tone in either normotensive or hypertensive animals [71]. Increased serum CRP levels also correlate with reduced NO bioavailability in coronary artery disease patients [72]. Mice deficient in m-CSF were found to develop less endothelial dysfunction and vascular oxidative stress in response to Ang II compared to wild type mice [42].

Importantly, normal healthy endothelium exerts anti-inflammatory effects such as NO-dependent inhibition of leukocyte adhesion [73]. Inhibition of eNOS activity augments expression of leukocyte adhesion molecules and chemokines such as monocyte chemotactic protein 1 (MCP-1) [74, 75]. Furthermore, gene therapy to augment vascular NO synthase activity attenuates hypercholesterolemia-induced leukocyte adhesion molecule expression and monocyte infiltration [76]. Therefore, endothelial dysfunction can further exacerbate vascular inflammation, which may in turn contribute to the development of hypertension.

5. Inflammation and Oxidative Stress in Hypertension

Chronic inflammation can also trigger oxidative stress, which has been associated with hypertension [77]. As mentioned, inflammation is the primary immune response to eliminate pathogens or to repair tissue damage. Innate immune cells, such as neutrophils and macrophages, produce reactive oxygen species (ROS) such as superoxide and hydrogen peroxide in order to kill pathogens [77]. Nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase is a major source of ROS in immune cells and also in the vasculature [78]. Inflammatory processes continue until the pathogens are destroyed or the tissue repair process has been completed. However, sustained inflammation can lead to an overproduction of ROS. Oxidative stress (defined as an imbalance between the production and breakdown of ROS) is a major cause of endothelial dysfunction, primarily through reducing NO bioavailability via the direct chemical reaction of superoxide with NO, resulting in the formation of peroxynitrite [79]. The reaction between superoxide and NO is faster [80] than the breakdown of superoxide via superoxide dismutase [81]. Furthermore, peroxynitrite formation may result in further impairment of NO levels and enhanced oxidative stress by inhibiting eNOS activity through oxidation of 4-tetrahydrobiopterin (BH_4), a cofactor of eNOS. This leads to eNOS uncoupling, where eNOS produces superoxide instead of NO [82]. Excessive ROS levels can also induce cellular damage by interacting with DNA, lipids, and proteins [83], which may further impair vascular structure and function. Immune cells such as T cells, macrophages, and neutrophils express NADPH oxidase subunits and produce ROS. In the setting of Ang II-induced hypertension, T cells express higher levels of $p47^{phox}$, $p22^{phox}$, and NOX2, components of NOX2 oxidase. Furthermore, adoptive transfer of T cells deficient in NADPH oxidase results in lower superoxide production and hypertension in response to Ang II [52]. Oxidative stress can promote inflammatory processes by activating transcription factors such as NF-κB [84]. CRP levels have been shown to correlate with the level of oxidative stress in inflammatory cells from hypertensive patients [85].

The kidney is an important organ involved in regulating blood pressure, and chronic kidney disease is one of the most common causes of secondary hypertension [86]. Elevated renal oxidative stress can be seen in the early stages of chronic kidney disease [87], and inflammation [88] and oxidative stress [87] increase as renal dysfunction progresses.

Prehypertensive SHR from 2-3 weeks of age have elevated renal inflammation and oxidative stress compared to age-matched WKY rats [89]. Renal artery stenosis results in reduced renal perfusion and pressure at the afferent arteriole, thus stimulating the release of renin and, hence, activation of the RAAS [90]. Renal artery stenosis can cause renovascular hypertension, a secondary form of hypertension due to kidney dysfunction. Indeed, reduced blood flow to the kidney decreases renal function and can lead to chronic kidney disease [91]. Renal artery stenosis is the most common primary disease of renal arteries, and up to 90% of cases are caused by atherosclerosis [92], a chronic inflammatory disease that predominately occurs in large arteries, and oxidative stress plays a major role in its development [93].

Overall, with oxidative stress being able to act as a key trigger of both inflammation and hypertension, it remains unclear whether inflammation is predominately a cause or effect of hypertension, with evidence to support either scenario in a likely vicious cycle.

6. The Sympathetic Nervous System and Inflammation in Hypertension

Sympathetic nervous system (SNS) activation is a common feature of hypertension and can contribute to the development of hypertension [94]. Essential hypertension patients are reported to have increased renal sympathetic outflow [95]. Autonomic dysfunction is characterised by increased sympathetic and decreased parasympathetic activity, and the SHR has been shown to be a good rodent model of human autonomic dysfunction [96]. The SNS innervates primary and secondary lymphoid organs and most immune cells express receptors for catecholamines such as noradrenaline [97]. The SNS can enhance inflammatory responses. For example, deletion of extracellular superoxide dismutase in the circumventricular organs of mice increased sympathetic outflow, modestly elevated blood pressure and increased T cell activation [98]. Renal sympathetic nerves have been suggested to play a role in kidney inflammation. Rats that have undergone renal sympathetic denervation have reduced renal macrophage levels and cortical TNF expression [99]. Neuroinflammation has been associated with increased sympathetic drive during cardiovascular disease. Inflammation may also promote SNS activation. Central administration of LPS to rats is reported to increase renal sympathetic drive and blood pressure [60]. Blood-borne pro-inflammatory cytokines may also act on receptors in the microvasculature of the brain to induce Cox-2 activity and the production of prostaglandins which penetrate the blood brain barrier to activate the SNS [100]. Overall, these studies suggest a potentially important link between the SNS and inflammation in the development of hypertension.

Increased sympathetic drive to the kidneys causes the release of renin and subsequently raises blood pressure [101]. Catheter-based renal denervation is a promising therapeutic approach to treat resistant hypertension [102]. Catheter-based renal denervation involves the application of radiofrequency energy in short bursts along the main renal arteries to disrupt the renal nerves. However, the recent SYMPLICITY HTN-3 clinical trial has reported that renal denervation does not result in a significant reduction in systolic blood pressure in resistant hypertensive patients with systolic blood pressure readings above 160 mmHg when compared to sham control [103]. The previous SYMPLICITY HTN clinical trials [102, 104] had reported that renal denervation can cause large reductions in blood pressure; however, these clinical trials were unblinded, had small sample sizes, and lacked sham controls as opposed to SYMPLICITY HTN-3. This does not necessarily mean that renal denervation is ineffective in lowering blood pressure as SYMPLICITY HTN-3 used the Medtronic catheter and there are other catheters available; hence, future clinical trials could study the use of other catheters to lower blood pressure. Currently there is a clinical trial examining Covidien's OneShot device [105].

7. Aging and Chronic Inflammation

More than half of the elderly (above 65 years of age) have hypertension [106], and the prevalence of hypertension increases with age [107]. Secondary causes of hypertension such as obstructive sleep apnea, chronic kidney disease, and renal artery stenosis, which are all associated with inflammation, are highly prevalent in the elderly [108]. Chronic low-grade inflammation commonly occurs with aging and this has been termed "inflammaging" [109]. Inflammaging is characterised by an imbalance of proinflammatory markers and anti-inflammatory markers. Levels of proinflammatory markers such as IL-6, TNF-α, and CRP are elevated, while anti-inflammatory cytokines such as interleukin-10 are reduced [110]. Inflammaging is believed to be caused by continuous lifelong exposure to antigens, due either to infection and/or nonpathogenic antigens.

8. Aldosterone and Inflammation

Patients with primary aldosteronism have elevated aldosterone levels, and as mentioned earlier, primary aldosteronism is a common secondary cause of hypertension. More than 10% of hypertensive patients have raised aldosterone levels [111], and drugs that block the mineralocorticoid receptor (MR), the main target receptor of aldosterone, are used to treat hypertension that is resistant to ACE inhibition and AT1R antagonism [112]. Aldosterone is involved in the RAAS whereby a fall in blood pressure under physiological conditions leads to Ang II generation which, through its action on the AT1R in the adrenal zona glomerulosa, stimulates the release of the mineralocorticoid, aldosterone. Aldosterone activates the MR in the distal renal tubule of the kidney to increase sodium and water retention, and potassium excretion, leading to an increase in blood volume and thus blood pressure [113]. Actions of aldosterone were, until recently, believed to be restricted to the kidney, but it is now understood that aldosterone can target other tissues relevant to blood pressure control, including the brain [114], vasculature [115], and heart [116].

Aldosterone has been reported to exert proinflammatory effects that appear to be MR-mediated. Administration of exogenous aldosterone to experimental animals results in elevated levels of ICAM-1, MCP-1, and TNF-α in coronary arteries [117], and increased vascular infiltration of macrophages and lymphocytes [118]. In the heart, aldosterone can increase vascular expression of ICAM-1, MCP-1, osteopontin, and COX-2, which can be blocked by the MR antagonist, eplerenone [116], indicating involvement of the MR. Proinflammatory effects of aldosterone have also been reported to occur in the kidney, where aldosterone and salt treatment caused MR-dependent leukocyte infiltration and elevation of osteopontin, IL-6, IL-1β, and MCP-1 levels [119]. Association between aldosterone and inflammation has been reported in essential hypertensive patients, where high plasma aldosterone levels were correlated with high levels of circulating CRP and leukocytes [120].

9. Anti-Inflammatory Drugs and Hypertension

Currently, anti-inflammatory drugs are not used to treat hypertension. Of the different classes of anti-inflammatory agents, immunosuppressant drugs could potentially be used to treat hypertension. Mycophenolate mofetil, which blocks T cell and B cell proliferation by inhibiting inosine monophosphate dehydrogenase, has been demonstrated to reduce hypertension in SHR [121], in Dahl salt-sensitive rats (another rodent model of hypertension) [122, 123], and in psoriasis and rheumatoid arthritis patients [124]. Another immunosuppressant, tacrolimus, a calcineurin inhibitor which blocks T cell activation, is reported to reduce hypertension in Dahl salt-sensitive rats [125]. Furthermore, chronic kidney disease patients with hypertension and who are on immunosuppressant drugs were found to require less antihypertensive medication than patients who were not taking immunosuppressant drugs [126]. Based on these studies, T cells may be a potential target in treating hypertension. T cells are an important component of the immune system and are involved in various aspects such as regulating immune responses by secreting anti- and proinflammatory cytokines. Ang II and aldosterone have proinflammatory effects; hence, targeting the RAAS could also target inflammation in hypertension. Ang II has been demonstrated to stimulate T cell proliferation and T cells deficient in the AT1R proliferate much less than T cells from wild type mice [127]. Vaccines that target the RAAS are currently in development to treat hypertension. The CYT006-AngGb vaccine which targets Ang II has been reported to reduce blood pressure in patients with mild to moderate hypertension with no serious safety issues in a phase IIa clinical trial [128]. Recently, the ATRQβ-001 vaccine which targets the AT1R has been found to be successful in lowering blood pressure in Ang II-induced hypertensive mice and SHR [129]. However, further studies are required to demonstrate the long-term safety of vaccines which target the RAAS, as one of the earlier vaccines developed which targeted renin was found to cause fatal autoimmunity [130].

Immunosuppressants can have serious side effects [131]; hence, their potential use to treat hypertension should be carefully considered and is not currently justified. Moreover, it is noteworthy that nonsteroidal anti-inflammatory drugs can increase blood pressure by causing sodium retention [132]. The use of immunosuppressants to treat treatment-resistant hypertension could possibly be justified. Treatment-resistant hypertension is defined as blood pressure that is above the patient's goal, despite treatment with at least three different classes of antihypertensive drugs, including a diuretic, at optimal doses. Patients with controlled blood pressure, and taking at least four antihypertensive medications, are also considered to be treatment-resistant [133]. It is difficult to determine the exact prevalence of treatment-resistant hypertension, but clinical trials have suggested that this may include up to 30% of hypertensive patients [134]. Currently, there are few therapeutic options available to treat resistant hypertension.

Statins were developed to inhibit cholesterol synthesis by blocking HMG-CoA reductase, but these drugs may also have anti-inflammatory effects and can cause a small reduction in systolic blood pressure in hypercholesterolemic patients, an effect that is greater in patients with a higher baseline blood pressure [135]. Statins can reduce levels of proinflammatory cytokines such as IL-1, IL-6, TNF-α, ICAM-1, and CRP [136]. Even in participants without hyperlipidemia, rosuvastatin has been shown to reduce CRP levels in association with a lower incidence of cardiovascular events [137]. The anti-inflammatory effects of statins may occur through the mevalonate pathway (i.e., HMG-CoA reductase pathway), which is responsible for cholesterol synthesis [138]. Pitavastatin has been shown to reduce IL-6 and IL-8 secretions and mRNA expression of IL-6, IL-8, and granulocyte macrophage colony-stimulating factor (GM-CSF) in LPS-stimulated human bronchial epithelium cells. These effects were inhibited by treatment with mevalonate, the initial product of HMG-CoA reductase [139]. The anti-inflammatory effects of statins may also occur through NO. Statins have been shown to enhance mRNA and protein expression of eNOS in human endothelial cells by inhibiting Rho-kinase geranylgeranyl-phosphorylation [140], and as discussed earlier NO has protective anti-inflammatory effects. Statins may also improve eNOS coupling by reducing plasma asymmetrical dimethylarginine (ADMA) [141], as ADMA has been associated with eNOS uncoupling [142].

10. Conclusion

It is unclear whether inflammation is a cause or effect of hypertension, but as discussed in this review there is evidence from human and animal studies suggesting that inflammation can lead to the development of hypertension. Oxidative stress and endothelial dysfunction are known to be associated with inflammation and can contribute to hypertension, at least in part, by exacerbating the inflammatory response. Other factors that contribute to hypertension such as SNS activation, aging, or aldosterone are also associated with inflammation (summarized in Figure 1). Future studies should focus on whether anti-inflammatory drugs are beneficial in reversing hypertension, oxidative

stress, and endothelial dysfunction in experimental models of hypertension. Hence, in the absence of serious side effects, anti-inflammatory drugs could potentially be used not only to treat hypertension in the future but also to treat other cardiovascular diseases by minimizing the impact of oxidative stress and endothelial dysfunction.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] H. O. Ventura, S. J. Taler, and J. E. Strobeck, "Hypertension as a hemodynamic disease: the role of impedance cardiography in diagnostic, prognostic, and therapeutic decision making," *The American Journal of Hypertension*, vol. 18, no. 2, pp. 26S–43S, 2005.

[2] P. M. Kearney, M. Whelton, K. Reynolds, P. Muntner, P. K. Whelton, and J. He, "Global burden of hypertension: Analysis of worldwide data," *The Lancet*, vol. 365, no. 9455, pp. 217–223, 2005.

[3] F. H. Messerli, B. Williams, and E. Ritz, "Essential hypertension," *The Lancet*, vol. 370, no. 9587, pp. 591–603, 2007.

[4] E. Onusko, "Diagnosing secondary hypertension," *The American Family Physician*, vol. 67, no. 1, pp. 67–74, 2003.

[5] F. Turnbull, "Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials," *The Lancet*, vol. 362, no. 9395, pp. 1527–1535, 2003.

[6] S. Bhanot and J. H. McNeill, "Insulin and hypertension: a causal relationship?" *Cardiovascular Research*, vol. 31, no. 2, pp. 212–221, 1996.

[7] L. T. Tran, V. G. Yuen, and J. H. McNeill, "The fructose-fed rat: a review on the mechanisms of fructose-induced insulin resistance and hypertension," *Molecular and Cellular Biochemistry*, vol. 332, no. 1-2, pp. 145–159, 2009.

[8] R. A. DeFronzo and E. Ferrannini, "Insulin resistance: a multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease," *Diabetes Care*, vol. 14, no. 3, pp. 173–194, 1991.

[9] M. P. Keane and R. M. Strieter, "Chemokine signaling in inflammation," *Critical Care Medicine*, vol. 28, no. 4, pp. N13–N26, 2000.

[10] A. J. Lusis, "Atherosclerosis," *Nature*, vol. 407, no. 6801, pp. 233–241, 2000.

[11] S. E. Sweeney and G. S. Firestein, "Rheumatoid arthritis: regulation of synovial inflammation," *International Journal of Biochemistry and Cell Biology*, vol. 36, no. 3, pp. 372–378, 2004.

[12] Y. F. Asanuma, "Accelerated atherosclerosis and inflammation in systemic lupus erythematosus," *Japanese Journal of Clinical Immunology*, vol. 35, no. 6, pp. 470–480, 2012.

[13] R. F. Mortensen and W. Zhong, "Regulation of phagocytic leukocyte activities by C-reactive protein," *Journal of Leukocyte Biology*, vol. 67, no. 4, pp. 495–500, 2000.

[14] S. P. Ballou and G. Lozanski, "Induction of inflammatory cytokine release from cultured human monocytes by C-reactive protein," *Cytokine*, vol. 4, no. 5, pp. 361–368, 1992.

[15] V. Pasceri, J. T. Willerson, and E. T. H. Yeh, "Direct proinflammatory effect of C-reactive protein on human endothelial cells," *Circulation*, vol. 102, no. 18, pp. 2165–2168, 2000.

[16] C. Stumpf, J. Jukic, A. Yilmaz et al., "Elevated VEGF-plasma levels in young patients with mild essential hypertension," *European Journal of Clinical Investigation*, vol. 39, no. 1, pp. 31–36, 2009.

[17] L. E. Bautista, J. E. Atwood, P. G. O'Malley, and A. J. Taylor, "Association between C-reactive protein and hypertension in healthy middle-aged men and women," *Coronary Artery Disease*, vol. 15, no. 6, pp. 331–336, 2004.

[18] G. J. Blake, N. Rifai, J. E. Buring, and P. M. Ridker, "Blood pressure, C-reactive protein, and risk of future cardiovascular events," *Circulation*, vol. 108, no. 24, pp. 2993–2999, 2003.

[19] E. M. Stuveling, S. J. L. Bakker, H. L. Hillege et al., "C-reactive protein modifies the relationship between blood pressure and microalbuminuria," *Hypertension*, vol. 43, no. 4, pp. 791–796, 2004.

[20] K. C. Sung, J. Y. Suh, B. S. Kim et al., "High sensitivity C-reactive protein as an independent risk factor for essential hypertension," *American Journal of Hypertension*, vol. 16, no. 6, pp. 429–433, 2003.

[21] T. Xu, Z. Ju, W. Tong et al., "Relationship of C-reactive protein with hypertension and interactions between increased C-reactive protein and other risk factors on hypertension in Mongolian people, China," *Circulation Journal*, vol. 72, no. 8, pp. 1324–1328, 2008.

[22] C. Chrysohoou, C. Pitsavos, D. B. Panagiotakos, J. Skoumas, and C. Stefanadis, "Association between prehypertension status and inflammatory markers related to atherosclerotic disease: the ATTICA Study," *The American Journal of Hypertension*, vol. 17, no. 7, pp. 568–573, 2004.

[23] H. D. Sesso, J. E. Buring, N. Rifai, G. J. Blake, J. M. Gaziano, and P. M. Ridker, "C-reactive protein and the risk of developing hypertension," *Journal of the American Medical Association*, vol. 290, no. 22, pp. 2945–2951, 2003.

[24] V. Dauphinot, F. Roche, M. P. Kossovsky et al., "C-reactive protein implications in new-onset hypertension in a healthy population initially aged 65 years: the Proof study," *Journal of Hypertension*, vol. 27, no. 4, pp. 736–743, 2009.

[25] L. Niskanen, D. E. Laaksonen, K. Nyyssönen et al., "Inflammation, abdominal obesity, and smoking as predictors of hypertension," *Hypertension*, vol. 44, no. 6, pp. 859–865, 2004.

[26] R. Tkacova, "Systemic inflammation in chronic obstructive pulmonary disease: may adipose tissue play a role? Review of the literature and future perspectives," *Mediators of Inflammation*, vol. 2010, Article ID 585989, 2010.

[27] W. Lieb, M. J. Pencina, T. J. Wang et al., "Association of parental hypertension with concentrations of select biomarkers in nonhypertensive offspring," *Hypertension*, vol. 52, no. 2, pp. 381–386, 2008.

[28] L. E. Bautista, L. M. Vera, I. A. Arenas, and G. Gamarra, "Independent association between inflammatory markers (C-reactive protein, interleukin-6, and TNF-alpha) and essential hypertension," *Journal of Human Hypertension*, vol. 19, no. 2, pp. 149–154, 2005.

[29] C. U. Chae, R. T. Lee, N. Rifai, and P. M. Ridker, "Blood pressure and inflammation in apparently healthy men," *Hypertension*, vol. 38, no. 3, pp. 399–403, 2001.

[30] J.-M. Fernandez-Real, M. Vayreda, C. Richart et al., "Circulating interleukin 6 levels, blood pressure, and insulin sensitivity in

apparently healthy men and women," *The Journal of Clinical Endocrinology & Metabolism*, vol. 86, no. 3, pp. 1154–1159, 2001.

[31] S. Zhao, Q. Li, L. Liu, Z. Xu, and J. Xiao, "Simvastatin reduces interleukin-1beta secretion by peripheral blood mononuclear cells in patients with essential hypertension," *Clinica Chimica Acta*, vol. 344, no. 1-2, pp. 195–200, 2004.

[32] G. N. Dalekos, M. Elisaf, E. Bairaktari, O. Tsolas, and K. C. Siamopoulos, "Increased serum levels of interleukin-1β in the systemic circulation of patients with essential hypertension: additional risk factor for atherogenesis in hypertensive patients?" *Journal of Laboratory and Clinical Medicine*, vol. 129, no. 3, pp. 300–308, 1997.

[33] X. Yu, Z. Yang, and M. Yu, "Correlation of tumor necrosis factor alpha and interleukin 6 with hypertensive renal damage," *Renal Failure*, vol. 32, no. 4, pp. 475–479, 2010.

[34] T. Furumoto, N. Saito, J. Dong, T. Mikami, S. Fujii, and A. Kitabatake, "Association of cardiovascular risk factors and endothelial dysfunction in Japanese hypertensive patients: implications for early atherosclerosis," *Hypertension Research*, vol. 25, no. 3, pp. 475–480, 2002.

[35] J. Youn, H. T. Yu, B. J. Lim et al., "Immunosenescent $CD8^+$ T clls and C-X-C cemokine receptor type 3 chemokines are increased in human hypertension," *Hypertension*, vol. 62, no. 1, pp. 126–133, 2013.

[36] E. C. Seaberg, A. Muñoz, M. Lu et al., "Association between highly active antiretroviral therapy and hypertension in a large cohort of men followed from 1984 to 2003," *AIDS*, vol. 19, no. 9, pp. 953–960, 2005.

[37] N. A. Hodyl, M. J. Stark, A. Osei-Kumah, and V. L. Clifton, "Prenatal programming of the innate immune response following in utero exposure to inflammation: a sexually dimorphic process?" *Expert Review of Clinical Immunology*, vol. 7, no. 5, pp. 579–592, 2011.

[38] M. E. Msall, G. M. Buck, B. T. Rogers et al., "Multivariate risks among extremely premature infants," *Journal of Perinatology*, vol. 14, no. 1, pp. 41–47, 1994.

[39] F. Mwanyumba, I. Inion, P. Gaillard, K. Mandaliya, M. Praet, and M. Temmerman, "Placental inflammation and perinatal outcome," *European Journal of Obstetrics Gynecology and Reproductive Biology*, vol. 108, no. 2, pp. 164–170, 2003.

[40] B. Rodríguez-Iturbe, Y. Quiroz, A. Ferrebuz, G. Parra, and N. D. Vaziri, "Evolution of renal interstitial inflammation and NF-κB activation in spontaneously hypertensive rats," *American Journal of Nephrology*, vol. 24, no. 6, pp. 587–594, 2004.

[41] H. Waki, B. Liu, M. Miyake et al., "Junctional adhesion molecule-1 is upregulated in spontaneously hypertensive rats: evidence for a prohypertensive role within the brain stem," *Hypertension*, vol. 49, no. 6, pp. 1321–1327, 2007.

[42] C. De Ciuceis, F. Amiri, P. Brassard, D. H. Endemann, R. M. Touyz, and E. L. Schiffrin, "Reduced vascular remodeling, endothelial dysfunction, and oxidative stress in resistance arteries of angiotensin II-infused macrophage colony-stimulating factor-deficient mice: evidence for a role in inflammation in angiotensin-induced vascular injury," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 10, pp. 2106–2113, 2005.

[43] W. Zhang, W. Wang, H. Yu et al., "Interleukin 6 underlies angiotensin II-induced hypertension and chronic renal damage," *Hypertension*, vol. 59, no. 1, pp. 136–144, 2012.

[44] S. Sriramula, M. Haque, D. S. A. Majid, and J. Francis, "Involvement of tumor necrosis factor-α in angiotensin II-mediated effects on salt appetite, hypertension, and cardiac hypertrophy," *Hypertension*, vol. 51, no. 5, pp. 1345–1351, 2008.

[45] M. S. Madhur, H. E. Lob, L. A. McCann et al., "Interleukin 17 promotes angiotensin II-induced hypertension and vascular dysfunction," *Hypertension*, vol. 55, no. 2, pp. 500–507, 2010.

[46] P. Crosswhite and Z. Sun, "Ribonucleic acid interference knockdown of interleukin 6 attenuates cold-induced hypertension," *Hypertension*, vol. 55, no. 6, pp. 1484–1491, 2010.

[47] B. Rodríguez-Iturbe, A. Ferrebuz, V. Vanegas, Y. Quiroz, S. Mezzano, and N. D. Vaziri, "Early and sustained inhibition of nuclear factor-κB prevents hypertension in spontaneously hypertensive rats," *Journal of Pharmacology and Experimental Therapeutics*, vol. 315, no. 1, pp. 51–57, 2005.

[48] K. L. H. Wu, S. H. H. Chan, and J. Y. H. Chan, "Neuroinflammation and oxidative stress in rostral ventrolateral medulla contribute to neurogenic hypertension induced by systemic inflammation," *Journal of Neuroinflammation*, vol. 9, no. 1, article 212, 2012.

[49] Y. L. Wei, X. H. Li, and J. Z. Zhou, "Prenatal exposure to lipopolysaccharide results in increases in blood pressure and body weight in rats," *Acta Pharmacologica Sinica*, vol. 28, no. 5, pp. 651–656, 2007.

[50] X.-Q. Hao, H.-G. Zhang, Z.-B. Yuan, D. Yang, L. Hao, and X. Li, "Prenatal exposure to lipopolysaccharide alters the intrarenal renin-angiotensin system and renal damage in offspring rats," *Hypertension Research*, vol. 33, no. 1, pp. 76–82, 2010.

[51] A. Samuelsson, I. Öhrn, J. Dahlgren et al., "Prenatal exposure to interleukin-6 results in hypertension and increased hypothalamic-pituitary-adrenal axis activity in adult rats," *Endocrinology*, vol. 145, no. 11, pp. 4897–4911, 2004.

[52] T. J. Guzik, N. E. Hoch, K. A. Brown et al., "Role of the T cell in the genesis of angiotensin II-induced hypertension and vascular dysfunction," *Journal of Experimental Medicine*, vol. 204, no. 10, pp. 2449–2460, 2007.

[53] P. J. Marvar, A. Vinh, S. Thabet et al., "T lymphocytes and vascular inflammation contribute to stress-dependent hypertension," *Biological Psychiatry*, vol. 71, no. 9, pp. 774–782, 2012.

[54] P. Wenzel, M. Knorr, S. Kossmann et al., "Lysozyme M-positive monocytes mediate angiotensin ii-induced arterial hypertension and vascular dysfunction," *Circulation*, vol. 124, no. 12, pp. 1370–1381, 2011.

[55] M. Jurewicz, D. H. McDermott, J. M. Sechler et al., "Human T and natural killer cells possess a functional renin-angiotensin system: further mechanisms of angiotensin II-induced inflammation," *Journal of the American Society of Nephrology*, vol. 18, no. 4, pp. 1093–1102, 2007.

[56] N. E. Hoch, T. J. Guzik, W. Chen et al., "Regulation of T-cell function by endogenously produced angiotensin II," *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, vol. 296, no. 2, pp. R208–R216, 2009.

[57] S. Kim and H. Iwao, "Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases," *Pharmacological Reviews*, vol. 52, no. 1, pp. 11–34, 2000.

[58] C. Wang, S. Li, R. D. Weisel et al., "C-reactive protein upregulates angiotensin type 1 receptors in vascular smooth muscle," *Circulation*, vol. 107, no. 13, pp. 1783–1790, 2003.

[59] D. Gurantz, R. T. Cowling, N. Varki, E. Frikovsky, C. D. Moore, and B. H. Greenberg, "IL-1β and TNF-α upregulate angiotensin II type 1 (AT 1) receptors on cardiac fibroblasts and are associated with increased AT1 density in the post-MI heart," *Journal of Molecular and Cellular Cardiology*, vol. 38, no. 3, pp. 505–515, 2005.

[60] Z. H. Zhang, Y. Yu, S. Wei, and R. B. Felder, "Centrally administered lipopolysaccharide elicits sympathetic excitation

via NAD(P)H oxidase-dependent mitogen-activated protein kinase signaling," *Journal of Hypertension*, vol. 28, no. 4, pp. 806–816, 2010.

[61] D. W. Trott and D. G. Harrison, "The immune system in hypertension," *Advances in Physiology Education*, vol. 38, no. 1, pp. 20–24, 2014.

[62] D. G. Harrison, T. J. Guzik, H. E. Lob et al., "Inflammation, immunity, and hypertension," *Hypertension*, vol. 57, no. 2, pp. 132–140, 2011.

[63] S. Chrissobolis, A. A. Miller, G. R. Drummond, B. K. Kemp-Harper, and C. G. Sobey, "Oxidative stress and endothelial dysfunction in cerebrovascular disease," *Frontiers in Bioscience*, vol. 16, no. 5, pp. 1733–1745, 2011.

[64] S. Chrissobolis and F. M. Faraci, "The role of oxidative stress and NADPH oxidase in cerebrovascular disease," *Trends in Molecular Medicine*, vol. 14, no. 11, pp. 495–502, 2008.

[65] C. Baylis, B. Mitruka, and A. Deng, "Chronic blockade of nitric oxide synthesis in the rat produces systemic hypertension and glomerular damage," *Journal of Clinical Investigation*, vol. 90, no. 1, pp. 278–281, 1992.

[66] S. Verma, C. Wang, S. Li et al., "A self-fulfilling prophecy: C-reactive protein attenuates nitric oxide production and inhibits angiogenesis," *Circulation*, vol. 106, no. 8, pp. 913–919, 2002.

[67] G. Yan, B. You, S. Chen, J. K. Liao, and J. Sun, "Tumor necrosis factor-α downregulates endothelial nitric oxide synthase mRNA stability via translation elongation factor 1-α 1," *Circulation Research*, vol. 103, no. 6, pp. 591–597, 2008.

[68] K. M. Mäki-Petäjä, F. C. Hall, A. D. Booth et al., "Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by anti-tumor necrosis factor-α therapy," *Circulation*, vol. 114, no. 11, pp. 1185–1192, 2006.

[69] H. Nguyen, V. L. Chiasson, P. Chatterjee, S. E. Kopriva, K. J. Young, and B. M. Mitchell, "Interleukin-17 causes Rho-kinase-mediated endothelial dysfunction and hypertension," *Cardiovascular Research*, vol. 97, no. 4, pp. 696–704, 2013.

[70] I. Fleming, J. Bauersachs, A. Schafer, D. Scholz, J. Aldershvile, and R. Busse, "Isometric contraction induces the Ca2+-independent activation of the endothelial nitric oxidesynthase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 3, pp. 1123–1128, 1999.

[71] S. Chrissobolis and C. G. Sobey, "Evidence that Rho-kinase activity contributes to cerebral vascular tone in vivo and is enhanced during chronic hypertension: comparison with protein kinase C," *Circulation Research*, vol. 88, no. 8, pp. 774–779, 2001.

[72] S. Fichtlscherer, S. Breuer, V. Schächinger, S. Dimmeler, and A. M. Zeiher, "C-reactive protein levels determine systemic nitric oxide bioavailability in patients with coronary artery disease," *European Heart Journal*, vol. 25, no. 16, pp. 1412–1418, 2004.

[73] P. Kubes, M. Suzuki, and D. N. Granger, "Nitric oxide: an endogenous modulator of leukocyte adhesion," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 88, no. 11, pp. 4651–4655, 1991.

[74] A. M. Zeiher, B. Fisslthaler, B. Schray-Utz, and R. Busse, "Nitric oxide modulates the expression of monocyte chemoattractant protein 1 in cultured human endothelial cells," *Circulation Research*, vol. 76, no. 6, pp. 980–986, 1995.

[75] H. Tomita, K. Egashira, M. Kubo-Inoue et al., "Inhibition of NO synthesis induces inflammatory changes and monocyte chemoattractant protein-1 expression in rat hearts and vessels," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 18, no. 9, pp. 1456–1464, 1998.

[76] H. Qian, V. Neplioueva, G. A. Shetty, K. M. Channon, and S. E. George, "Nitric oxide synthase gene therapy rapidly reduces adhesion molecule expression and inflammatory cell infiltration in carotid arteries of cholesterol-fed rabbits," *Circulation*, vol. 99, no. 23, pp. 2979–2982, 1999.

[77] S. D. Crowley, "The cooperative roles of inflammation and oxidative stress in the pathogenesis of hypertension," *Antioxidants & Redox Signaling*, vol. 20, no. 1, pp. 102–120, 2014.

[78] G. R. Drummond, S. Selemidis, K. K. Griendling, and C. G. Sobey, "Combating oxidative stress in vascular disease: NADPH oxidases as therapeutic targets," *Nature Reviews Drug Discovery*, vol. 10, no. 6, pp. 453–471, 2011.

[79] R. M. Touyz, "Reactive oxygen species and angiotensin II signaling in vascular cells—implications in cardiovascular disease," *Brazilian Journal of Medical and Biological Research*, vol. 37, no. 8, pp. 1263–1273, 2004.

[80] R. E. Huie and S. Padmaja, "The reaction of no with superoxide," *Free Radical Research Communications*, vol. 18, no. 4, pp. 195–199, 1993.

[81] A. Cudd and I. Fridovich, "Electrostatic interactions in the reaction mechanism of bovine erythrocyte superoxide dismutase," *Journal of Biological Chemistry*, vol. 257, no. 19, pp. 11443–11447, 1982.

[82] J. Vásquez-Vivar, B. Kalyanaraman, P. Martásek et al., "Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 16, pp. 9220–9225, 1998.

[83] E. A. Hassoun, F. Li, A. Abushaban, and S. J. Stohs, "Production of superoxide anion, lipid peroxidation and DNA damage in the hepatic and brain tissues of rats after subchronic exposure to mixtures of TCDD and its congeners," *Journal of Applied Toxicology*, vol. 21, no. 3, pp. 211–219, 2001.

[84] T. G. Canty Jr., E. M. Boyle Jr., A. Farr, E. N. Morgan, E. D. Verrier, and T. H. Pohlman, "Oxidative stress induces NF-κB nuclear translocation without degradation of IκBα. Circulation," *Circulation*, vol. 100, no. 19, pp. II-361–II-364, 1999.

[85] K. Yasunari, K. Maeda, M. Nakamura, and J. Yoshikawa, "Oxidative stress in leukocytes is a possible link between blood pressure, blood glucose, and C-reacting protein," *Hypertension*, vol. 39, no. 3, pp. 777–780, 2002.

[86] R. N. Foley and A. J. Collins, "End-stage renal disease in the United States: an update from the United States Renal Data System," *Journal of the American Society of Nephrology*, vol. 18, no. 10, pp. 2644–2648, 2007.

[87] E. Dounousi, E. Papavasiliou, A. Makedou et al., "Oxidative stress is progressively enhanced with advancing stages of CKD," *American Journal of Kidney Diseases*, vol. 48, no. 5, pp. 752–760, 2006.

[88] E. M. Stuveling, H. L. Hillege, S. J. L. Bakker, R. O. B. Gans, P. E. De Jong, and D. De Zeeuw, "C-reactive protein is associated with renal function abnormalities in a non-diabetic population," *Kidney International*, vol. 63, no. 2, pp. 654–661, 2003.

[89] S. K. Biswas and J. B. L. de Faria, "Which comes first: renal inflammation or oxidative stress in spontaneously hypertensive rats?" *Free Radical Research*, vol. 41, no. 2, pp. 216–224, 2007.

[90] D. Lao, P. S. Parasher, K. C. Cho, and Y. Yeghiazarians, "Atherosclerotic renal artery stenosis—diagnosis and treatment," *Mayo Clinic Proceedings*, vol. 86, no. 7, pp. 649–657, 2011.

[91] M. R. Khatami, "Ischemic nephropathy: more than a simple renal artery narrowing," *Iranian Journal of Kidney Diseases*, vol. 7, no. 2, pp. 82–100, 2013.

[92] K. J. Hansen, M. S. Edwards, T. E. Craven et al., "Prevalence of renovascular disease in the elderly: a population-based study," *Journal of Vascular Surgery*, vol. 36, no. 3, pp. 443–451, 2002.

[93] C. Weber and H. Noels, "Atherosclerosis: current pathogenesis and therapeutic options," *Nature Medicine*, vol. 17, no. 11, pp. 1410–1422, 2011.

[94] P. G. Guyenet, "The sympathetic control of blood pressure," *Nature Reviews Neuroscience*, vol. 7, no. 5, pp. 335–346, 2006.

[95] M. P. Schlaich, E. Lambert, D. M. Kaye et al., "Sympathetic augmentation in hypertension: role of nerve firing, norepinephrine reuptake, and angiotensin neuromodulation," *Hypertension*, vol. 43, no. 2 I, pp. 169–175, 2004.

[96] Z.-Y. Tan, Y. Lu, C. A. Whiteis et al., "Chemoreceptor hypersensitivity, sympathetic excitation, and overexpression of ASIC and TASK channels before the onset of hypertension in SHR," *Circulation Research*, vol. 106, no. 3, pp. 536–545, 2010.

[97] D. M. Nance and V. M. Sanders, "Autonomic innervation and regulation of the immune system (1987–2007)," *Brain, Behavior, and Immunity*, vol. 21, no. 6, pp. 736–745, 2007.

[98] H. E. Lob, P. J. Marvar, T. J. Guzik et al., "Induction of hypertension and peripheral inflammation by reduction of extracellular superoxide dismutase in the central nervous system," *Hypertension*, vol. 55, no. 2, pp. 277–283, 2010.

[99] R. Veelken, E. Vogel, K. Hilgers et al., "Autonomic renal denervation ameliorates experimental glomerulonephritis," *Journal of the American Society of Nephrology*, vol. 19, no. 7, pp. 1371–1378, 2008.

[100] Y. Yu, Z. Zhang, S. Wei, J. Serrats, R. M. Weiss, and R. B. Felder, "Brain perivascular macrophages and the sympathetic response to inflammation in rats after myocardial infarction," *Hypertension*, vol. 55, no. 3, pp. 652–659, 2010.

[101] R. D. Bunag, I. H. Page, and J. W. McCubbin, "Neural stimulation of release of renin," *Circulation Research*, vol. 19, no. 4, pp. 851–858, 1966.

[102] M. D. Esler, H. Krum, M. Schlaich, R. E. Schmieder, M. Böhm, and P. A. Sobotka, "Renal sympathetic denervation for treatment of drug-resistant hypertension: one-year results from the symplicity htn-2 randomized, controlled trial," *Circulation*, vol. 126, no. 25, pp. 2976–2982, 2012.

[103] D. L. Bhatt, D. E. Kandzari, W. W. O'Neill et al., "A controlled trial of renal denervation for resistant hypertension," *The New England Journal of Medicine*, vol. 370, no. 15, pp. 1393–1401, 2014.

[104] H. Krum, "Catheter-based renal sympathetic denervation for resistant hypertension: durability of blood pressure reduction out to 24 months," *Hypertension*, vol. 57, no. 5, pp. 911–917, 2011.

[105] S. Verheye, "TCT-62 preliminary result of the rapid renal sympathetic denervation for resistant hypertension using the maya medical OneShot(tm) Ablation System (RAPID) study," *Journal of the American College of Cardiology*, vol. 62, no. 18, supplement 1, p. B20, 2013.

[106] P. Lloyd-Sherlock, J. Beard, N. Minicuci, S. Ebrahim, and S. Chatterji, "Hypertension among older adults in low- and middle-income countries: prevalence, awareness and control," *International Journal of Epidemiology*, vol. 43, no. 1, pp. 116–128, 2014.

[107] C. T. M. van Rossum, H. van de Mheen, J. C. M. Witteman, A. Hofman, J. P. Mackenbach, and D. E. Grobbee, "Prevalence, treatment, and control of hypertension by sociodemographic factors among the Dutch elderly," *Hypertension*, vol. 35, no. 3, pp. 814–821, 2000.

[108] M. A. Rafey, "Resistant hypertension in the elderly," *Clinics in Geriatric Medicine*, vol. 25, no. 2, pp. 289–301, 2009.

[109] C. Franceschi, M. Capri, D. Monti et al., "Inflammaging and anti-inflammaging: a systemic perspective on aging and longevity emerged from studies in humans," *Mechanisms of Ageing and Development*, vol. 128, no. 1, pp. 92–105, 2007.

[110] D. B. Bartlett, C. M. Firth, A. C. Phillips et al., "The age-related increase in low-grade systemic inflammation (Inflammaging) is not driven by cytomegalovirus infection," *Aging Cell*, vol. 11, no. 5, pp. 912–915, 2012.

[111] J. W. Funder, R. M. Carey, C. Fardella et al., "Case detection, diagnosis, and treatment of patients with primary aldosteronism: an endocrine society clinical practice guideline," *Journal of Clinical Endocrinology and Metabolism*, vol. 93, no. 9, pp. 3266–3281, 2008.

[112] A. Struthers, H. Krum, and G. H. Williams, "A comparison of the aldosterone-blocking agents eplerenone and spironolactone," *Clinical Cardiology*, vol. 31, no. 4, pp. 153–158, 2008.

[113] F. C. Luft, "Mendelian forms of human hypertension and mechanisms of disease.," *Clinical Medicine & Research*, vol. 1, no. 4, pp. 291–300, 2003.

[114] Z. Zhang, Y. Yu, Y. Kang, S. Wei, and R. B. Felder, "Aldosterone acts centrally to increase brain renin-angiotensin system activity and oxidative stress in normal rats," *The American Journal of Physiology: Heart and Circulatory Physiology*, vol. 294, no. 2, pp. H1067–H1074, 2008.

[115] E. Leibovitz, T. Ebrahimian, P. Paradis, and E. L. Schiffrin, "Aldosterone induces arterial stiffness in absence of oxidative stress and endothelial dysfunction," *Journal of Hypertension*, vol. 27, no. 11, pp. 2192–2200, 2009.

[116] R. Rocha, A. E. Rudolph, G. E. Frierdich et al., "Aldosterone induces a vascular inflammatory phenotype in the rat heart," *American Journal of Physiology: Heart and Circulatory Physiology*, vol. 283, no. 5, pp. H1802–H1810, 2002.

[117] Y. Sun, J. Zhang, L. Lu, S. S. Chen, M. T. Quinn, and K. T. Weber, "Aldosterone-induced inflammation in the rat heart: role of oxidative stress," *The American Journal of Pathology*, vol. 161, no. 5, pp. 1773–1781, 2002.

[118] D. A. Kasal, T. Barhoumi, M. W. Li et al., "T regulatory lymphocytes prevent aldosterone-induced vascular injury," *Hypertension*, vol. 59, no. 2, pp. 324–330, 2012.

[119] E. R. Blasi, R. Rocha, A. E. Rudolph, E. A. G. Blomme, M. L. Polly, and E. G. McMahon, "Aldosterone/salt induces renal inflammation and fibrosis in hypertensive rats," *Kidney International*, vol. 63, no. 5, pp. 1791–1800, 2003.

[120] V. Tzamou, G. Vyssoulis, E. Karpanou, S.-. Kyvelou, T. Gialernios, and C. Stefanadis, "Aldosterone levels and inflammatory stimulation in essential hypertensive patients," *Journal of Human Hypertension*, vol. 27, no. 9, pp. 535–538, 2013.

[121] B. Rodríguez-Iturbe, Y. Quiroz, M. Nava et al., "Reduction of renal immune cell infiltration results in blood pressure control in genetically hypertensive rats," *The American Journal of Physiology: Renal Physiology*, vol. 282, no. 2, pp. F191–F201, 2002.

[122] N. Tian, J.-W. Gu, S. Jordan, R. A. Rose, M. D. Hughson, and R. D. Manning Jr., "Immune suppression prevents renal damage and dysfunction and reduces arterial pressure in salt-sensitive hypertension," *The American Journal of Physiology—Heart and Circulatory Physiology*, vol. 292, no. 2, pp. H1018–H1025, 2007.

[123] D. L. Mattson, L. James, E. A. Berdan, and C. J. Meister, "Immune suppression attenuates hypertension and renal disease in the Dahl salt-sensitive rat," *Hypertension*, vol. 48, no. 1, pp. 149–156, 2006.

[124] J. Herrera, A. Ferrebuz, E. G. MacGregor, and B. Rodriguez-Iturbe, "Mycophenolate mofetil treatment improves hypertension in patients with psoriasis and rheumatoid arthritis," *Journal of the American Society of Nephrology*, vol. 17, supplement 3, no. 12, pp. S218–S225, 2006.

[125] C. de Miguel, C. Guo, H. Lund, D. Feng, and D. L. Mattson, "Infiltrating T lymphocytes in the kidney increase oxidative stress and participate in the development of hypertension and renal disease," *The American Journal of Physiology—Renal Physiology*, vol. 300, no. 3, pp. F734–F742, 2011.

[126] C. J. Ferro, N. C. Edwards, C. Hutchison et al., "Does immunosuppressant medication lower blood pressure and arterial stiffness in patients with chronic kidney disease? An observational study," *Hypertension Research*, vol. 34, no. 1, pp. 113–119, 2011.

[127] C. Nataraj, M. I. Oliverio, R. B. Mannon et al., "Angiotensin II regulates cellular immune responses through a calcineurin-dependent pathway," *Journal of Clinical Investigation*, vol. 104, no. 12, pp. 1693–1701, 1999.

[128] A. C. Tissot, P. Maurer, J. Nussberger et al., "Effect of immunisation against angiotensin II with CYT006-AngQb on ambulatory blood pressure: a double-blind, randomised, placebo-controlled phase IIa study," *The Lancet*, vol. 371, no. 9615, pp. 821–827, 2008.

[129] X. Chen, Z. Qiu, S. Yang et al., "Effectiveness and safety of a therapeutic vaccine against angiotensin II receptor type 1 in hypertensive animals," *Hypertension*, vol. 61, no. 2, pp. 408–416, 2013.

[130] J.-B. Michel, C. Guettier, M. Philippe, F. X. Galen, P. Corvol, and J. Ménard, "Active immunization against renin in normotensive marmoset," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 84, no. 12, pp. 4346–4350, 1987.

[131] E. Tepperman, D. Ramzy, J. Prodaer et al., "Surgical biology forthe clinician: vascular effects of immunosuppression," *Canadian Journal of Surgery*, vol. 53, no. 1, pp. 57–63, 2010.

[132] A. G. Johnson, T. V. Nguyen, and R. O. Day, "Do nonsteroidal anti-inflammatory drugs affect blood pressure? A meta-analysis," *Annals of Internal Medicine*, vol. 121, no. 4, pp. 289–300, 1994.

[133] A. Myat, S. R. Redwood, A. C. Qureshi, J. A. Spertus Director, and B. Williams, "Resistant hypertension," *BMJ*, vol. 345, no. 7884, Article ID e7473, 2012.

[134] E. Pimenta and D. A. Calhoun, "Resistant hypertension: incidence, prevalence, and prognosis," *Circulation*, vol. 125, no. 13, pp. 1594–1596, 2012.

[135] P. Strazzullo, S. M. Kerry, A. Barbato, M. Versiero, L. D'Elia, and F. P. Cappuccio, "Do statins reduce blood pressure? A meta-analysis of randomized, controlled trials," *Hypertension*, vol. 49, no. 4, pp. 792–798, 2007.

[136] S. Kinlay, G. G. Schwartz, A. G. Olsson et al., "High-dose atorvastatin enhances the decline in inflammatory markers in patients with acute coronary syndromes in the MIRACL study," *Circulation*, vol. 108, no. 13, pp. 1560–1566, 2003.

[137] P. M. Ridker, E. Danielson, F. A. H. Fonseca et al., "Rosuvastatin to prevent vascular events in men and women with elevated C-reactive protein," *The New England Journal of Medicine*, vol. 359, no. 21, pp. 2195–2207, 2008.

[138] J. A. Tobert, "Lovastatin and beyond: the history of the HMG-CoA reductase inhibitors," *Nature Reviews Drug Discovery*, vol. 2, no. 7, pp. 517–526, 2003.

[139] A. Iwata, R. Shirai, H. Ishii et al., "Inhibitory effect of statins on inflammatory cytokine production from human bronchial epithelial cells," *Clinical and Experimental Immunology*, vol. 168, no. 2, pp. 234–240, 2012.

[140] U. Laufs and J. K. Liao, "Post-transcriptional regulation of endothelial nitric oxide synthase mRNA stability by Rho GTPase," *Journal of Biological Chemistry*, vol. 273, no. 37, pp. 24266–24271, 1998.

[141] T.-M. Lu, Y.-A. Ding, H.-B. Leu, W.-H. Yin, W. H.-H. Sheu, and K.-M. Chu, "Effect of *rosuvastatin* on plasma levels of asymmetric dimethylarginine in patients with hypercholesterolemia," *The American Journal of Cardiology*, vol. 94, no. 2, pp. 157–161, 2004.

[142] C. Antoniades, C. Shirodaria, P. Leeson et al., "Association of plasma asymmetrical dimethylarginine (ADMA) with elevated vascular superoxide production and endothelial nitric oxide synthase uncoupling: implications for endothelial function in human atherosclerosis," *European Heart Journal*, vol. 30, no. 9, pp. 1142–1150, 2009.

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Research Article

Metoprolol Reduces Proinflammatory Cytokines and Atherosclerosis in ApoE$^{-/-}$ Mice

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A few studies in animals and humans suggest that metoprolol (β1-selective adrenoceptor antagonist) may have a direct antiatherosclerotic effect. However, the mechanism behind this protective effect has not been established. The aim of the present study was to evaluate the effect of metoprolol on development of atherosclerosis in ApoE$^{-/-}$ mice and investigate its effect on the release of proinflammatory cytokines. Male ApoE$^{-/-}$ mice were treated with metoprolol (2.5 mg/kg/h) or saline for 11 weeks via osmotic minipumps. Atherosclerosis was assessed in thoracic aorta and aortic root. Total cholesterol levels and Th1/Th2 cytokines were analyzed in serum and macrophage content in lesions by immunohistochemistry. Metoprolol significantly reduced atherosclerotic plaque area in thoracic aorta ($P < 0.05$ versus Control). Further, metoprolol reduced serum TNFα and the chemokine CXCL1 ($P < 0.01$ versus Control for both) as well as decreasing the macrophage content in the plaques ($P < 0.01$ versus Control). Total cholesterol levels were not affected. In this study we found that a moderate dose of metoprolol significantly reduced atherosclerotic plaque area in thoracic aorta of ApoE$^{-/-}$ mice. Metoprolol also decreased serum levels of proinflammatory cytokines TNFα and CXCL1 and macrophage content in the plaques, showing that metoprolol has an anti-inflammatory effect.

1. Introduction

Cardiovascular events are most often caused by complications to atherosclerotic disease. β-Blockers have been shown to reduce the risk of cardiovascular events after myocardial infarction [1, 2]. The mechanisms behind this cardioprotective effect have been attributed to the many positive effects that β-blockers have on cardiac function: antiarrhythmic effects, improvement of myocardial function, lowering of cardiac oxygen consumption, and lowering of blood pressure. In addition, a few studies have also suggested that β-blockers may have direct antiatherosclerotic effects in rabbits and monkeys, as well as in humans [3, 4].

In landmark studies, Kaplan and coworkers showed that atherosclerosis development was accelerated in dominant male cynomolgus monkeys living in unstable hierarchies [5, 6]. The effect on atherosclerosis was inhibited by nonselective β-blockade using propranolol [7]. In later experiments, blockade with metoprolol protected against acute endothelial injury induced by unstable social conditions in the same animal model, suggesting that stress-induced atherogenesis is mediated via the β_1-receptor [8]. The protective effect of metoprolol was also seen under nonstressed conditions in rabbit models of atherosclerosis [9]. It has also been shown in two separate studies that low-dose metoprolol administered during three years slows the progression of intima media thickness (IMT) in humans [10, 11] and alters the grey scale of carotid plaques [12]. However, despite these convincing data, the mechanisms behind this protective effect have not yet been established.

We hypothesized that metoprolol would reduce progression of atherosclerosis in ApoE$^{-/-}$ mice and therefore

studied whether long-term treatment with metoprolol would reduce plaque area. We also studied the potential effects of metoprolol on proinflammatory cytokines known to play a role in development of atherosclerosis.

2. Methods

2.1. Animals. Mice were housed at 21 to 24°C in a room with a 12 h light/12 h dark cycle. Water and food were available *ad libitum*. All procedures involving mice were approved by the Regional Animal Ethics Committee at the University of Gothenburg, in accordance with the European Communities Council Directives of 24 November 1986 (86/609/ECC).

2.2. Study I: Dose-Finding

2.2.1. Experimental Design. To find an appropriate dose of metoprolol we randomly divided male C57BL/6 mice (Taconic, Denmark), 9 weeks of age, into four groups: (i) Control (*C*, $n = 4$), (ii) Metoprolol Dose 1 (1.4 mg/kg per hour, Sigma Aldrich St. Louis, Missouri, USA, $n = 4$), (iii) Metoprolol Dose 2 (2.5 mg/kg per hour, $n = 4$), and (iv) Metoprolol Dose 3 (4.1 mg/kg per hour, $n = 4$).

At 10 weeks of age, mice were implanted with ECG telemetry probes (PhysioTel Transmitters TA-F20, weight 3.9 g, Data Science International, Inc., St. Paul, MN, USA) as previously described [13]. At 12 weeks of age osmotic minipumps (Alzet model 1002, DURECT Corporation, ALZET Osmotic Pumps, Cupertino, CA, USA) with the three different doses of metoprolol were implanted as previously described [14]. Mice were anesthetized with isoflurane for 5–10 minutes and minipumps were implanted subcutaneously on the back of the mouse.

2.2.2. Effects of Metoprolol on Heart Rate. At 13 weeks of age heart rate was measured with radiotelemetry in an undisturbed room during 24 hours at baseline conditions. Further, heart rate was recorded one hour prior to air-jet stress (baseline) and two hours during air-jet stress (stress) for baseline and stress measurements, respectively, as previously described [13].

2.2.3. Plasma Concentration of Metoprolol. Plasma concentrations of metoprolol were measured with liquid chromatography coupled with tandem mass spectrometry (LC/MS/MS) from blood samples collected at termination.

2.3. Study II: Effects on Atherosclerosis

2.3.1. Experimental Design. Male ApoE$^{-/-}$ mice (Taconic Transgenic Models Strain B6.129P2-Apoe^{tm1Unc}/N11 Taconic, Denmark), 6 weeks of age, were randomly divided into two groups: (i) Control (*C*, $n = 12$) and (ii) Metoprolol infusion (2.5 mg/kg per hour, Met, $n = 12$). From 9 weeks of age and throughout the experiment, mice in both groups were fed a high fat, cholesterol enriched diet, "western diet" (21% fat, 0.15% cholesterol; R638, Lantmännen, Sweden).

At 9 weeks of age, mice were implanted with osmotic minipumps (Alzet model 2006) delivering saline or metoprolol, as described above. Since the duration of the minipumps was six weeks, minipumps were replaced once during the experiment.

2.3.2. Termination and Fixation. The mice in the atherosclerosis study were sacrificed at 19 weeks of age with an overdose of pentobarbital (Apoteksbolaget, Sweden, 0.9 mg/g BW i.p.), as previously described [15]. Briefly, blood was collected from the right ventricle into standard coated tubes for Li-heparin and serum. The heart and vascular tree were perfused by intracardiac saline infusion to clear the lumen from blood. The heart and aorta were then fixed in 4% paraformaldehyde. After fixation, the thoracic aorta (from the left common carotid artery to the right renal artery) was cleared from surrounding fat and tissue and kept in 4% paraformaldehyde.

2.4. Quantification of Atherosclerosis and Immunohistochemistry

2.4.1. En Face Quantification of Thoracic Aorta. The thoracic aorta was analyzed *en face*, as previously described [15]. Briefly, the aortas were cut open longitudinally, pinned onto silicone dishes, and stained with Sudan IV for lipids. Images were captured with Canon Utilities Remote Capture 2.7 (Canon Inc., Tokyo, Japan) using a digital camera connected to a dissection microscope. The outline of the intima was manually traced using Adobe Photoshop, by a blinded observer, to calculate the total area of the vessel. Lesions were outlined in the same manner and plaque area was calculated as the percentage of the total vessel area covered with lesions.

2.4.2. Cross-Sectional Quantification of Aortic Root. The aortic root was serially sectioned and stained with Oil Red O as previously described [16]. Briefly, the aortic root was serially sectioned at six different levels, 100–600 μm from the aortic valves. The cross-sections of the vessel were then stained with Oil Red O and lesions were measured by a blinded observer. Lesion size was normalized by IEL length.

2.4.3. Immunohistochemistry. Immunostaining was performed on formalin-fixed cross-sections of the aortic root as previously described [14, 17]. The following antibodies were used: Mac-2 (CL8942AP: Cedarlane Laboratories Ltd., Burlington, Ontario, CA) and biotinylated rabbit anti-rat IgG (BA-4001: Vector Laboratories, Burlingame, CA, USA). Binding was visualized by DAB kit (Vector Laboratories) and counterstained with Hematoxylin (HARRIS HTX Histolab: Histolab Procuts AB, Gothenburg, Sweden). Images were produced with an Olympus BX60F5 microscope with a 10X objective connected to an Olympus DP72 camera. Positive staining for Mac-2 was automatically traced using cellSens Dimension analysis software (Version 1.5, Olympus Optical Company, Hamburg, Germany) and normalized to lesion

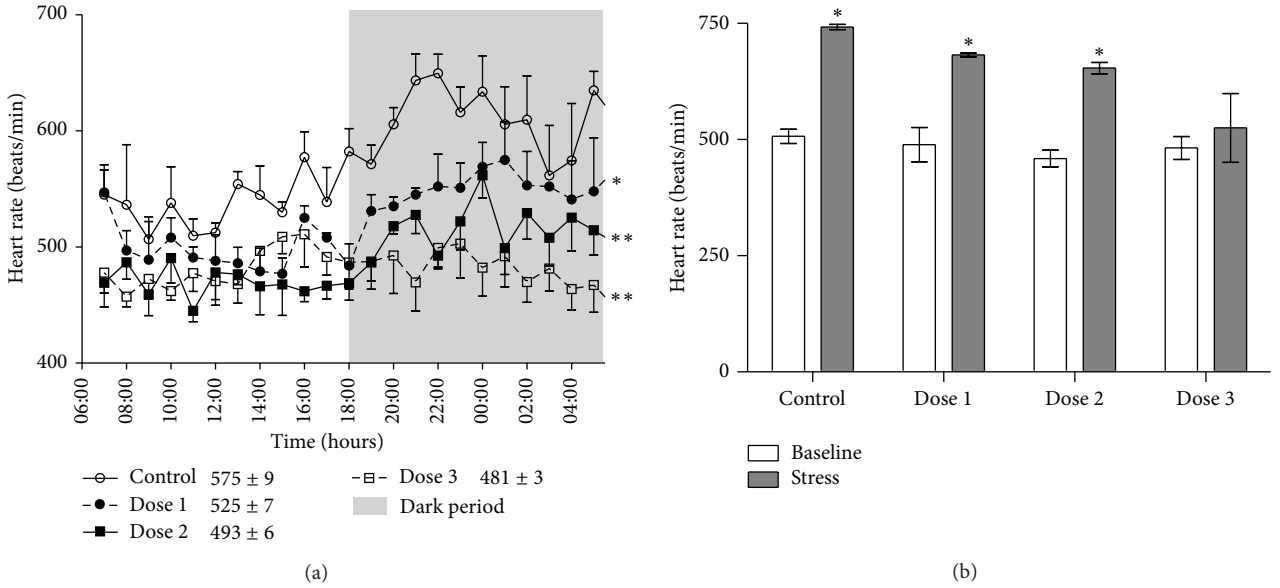

Figure 1: Metoprolol dose-finding (Study I). (a) 24-hour heart rate during baseline conditions after three different doses of metoprolol compared with Control mice. Metoprolol was administrated to C57BL76 mice via osmotic minipumps delivering 1.4 mg/kg per hour (dose 1, $n = 4$), 2.5 mg/kg per hour (dose 2, $n = 4$), or 4.1 mg/kg per hour (dose 3, $n = 4$). Average 24-hour values for each group are given in the figure; $^{*}P < 0.05$, $^{**}P < 0.01$ versus Control. (b) Heart rate increased for Control mice and metoprolol treated mice receiving dose 1 and dose 2 (1.4 mg/kg per hour and 2.5 mg/kg per hour, resp.) during air-jet stress compared to baseline. For mice receiving metoprolol dose 3 (4.1 mg/kg per hour) heart rate did not increase during air-jet stress, compared to baseline. $^{*}P < 0.05$ versus baseline. Mice received metoprolol treatment for one week prior to heart rate measurements. Data are expressed as mean ± SEM.

area. Data are presented as the average staining from two consecutive sections.

2.5. Blood Sample Analysis

2.5.1. Analysis of Th1 and Th2 Cytokines. Serum samples were used for analysis of Th1 cytokines: IL-1β, IL-2, IL-12 total, IFN-γ, TNFα, and CXCL1 and Th2 cytokines: IL-4, IL-5, and IL-10, using a Mouse Th1/Th2 Multiplex ELISA (Meso Scale Discovery, Gaithersburg, Maryland, USA) according to the manufacturer's protocol.

2.5.2. Total Cholesterol. Total serum cholesterol was determined colorimetrically after enzymatic hydrolysis and oxidation using a cholesterol kit (cholesterol enzymatic endpoint method, RANDOX Laboratories Ltd., United Kingdom), according to the manufacturers protocol.

2.6. Statistics. 24-hour heart rate was analyzed using repeated measurement ANOVA followed by Dunnett's post hoc test (SPSS Statistics version 17.0, Chicago, IL, USA). All other data was analyzed with the nonparametric Mann-Whitney U test (SPSS). One mouse in the metoprolol treated group in Study II was considered an outlier in SPSS regarding plaque area. This mouse was removed from all analysis in the study. All data are expressed as mean ± SEM. $P < 0.05$ was considered as statistically significant for all data except for Th1/Th2 cytokines. To reduce the risk for mass significance in the analysis of Th1/Th2 cytokines we used a 99% significance level ($P < 0.01$) for this data.

3. Results

3.1. Study I: Dose-Finding

3.1.1. Metoprolol Lowered 24-Hour Heart Rate. To find an appropriate dose of metoprolol for the atherosclerosis study we tested three different doses and their effects on heart rate. All three doses of metoprolol lowered resting heart rate during a 24-hour period. Although both doses 1 and 2 significantly reduced heart rate (Dunnett's, $P < 0.05$ and $P < 0.01$, resp.), the circadian rhythm was similar to the control situation. Dose 3, on the other hand, disrupted the circadian rhythm as well as markedly reducing heart rate ($P < 0.01$, Figure 1(a)). To further validate the metoprolol doses we performed air-jet stress. Air-jet stress markedly increased heart rate in control animals. Metoprolol doses 1 and 2 decreased the air-jet induced rise in heart rate; however, there was still a significant increase in heart rate. Only dose 3 abolished the heart rate response to air-jet stress ($P < 0.05$, Figure 1(b)). Hence, dose 2 was chosen for the following atherosclerosis study.

3.1.2. Plasma Metoprolol Concentrations. Plasma concentrations of metoprolol were 79 ± 11, 119 ± 15, and 225 ± 18 nM, respectively, for the three different doses of metoprolol.

3.2. Study II: Effects on Atherosclerosis

3.2.1. Metoprolol Reduced Atherosclerotic Plaque Area. Eleven weeks of metoprolol treatment significantly decreased

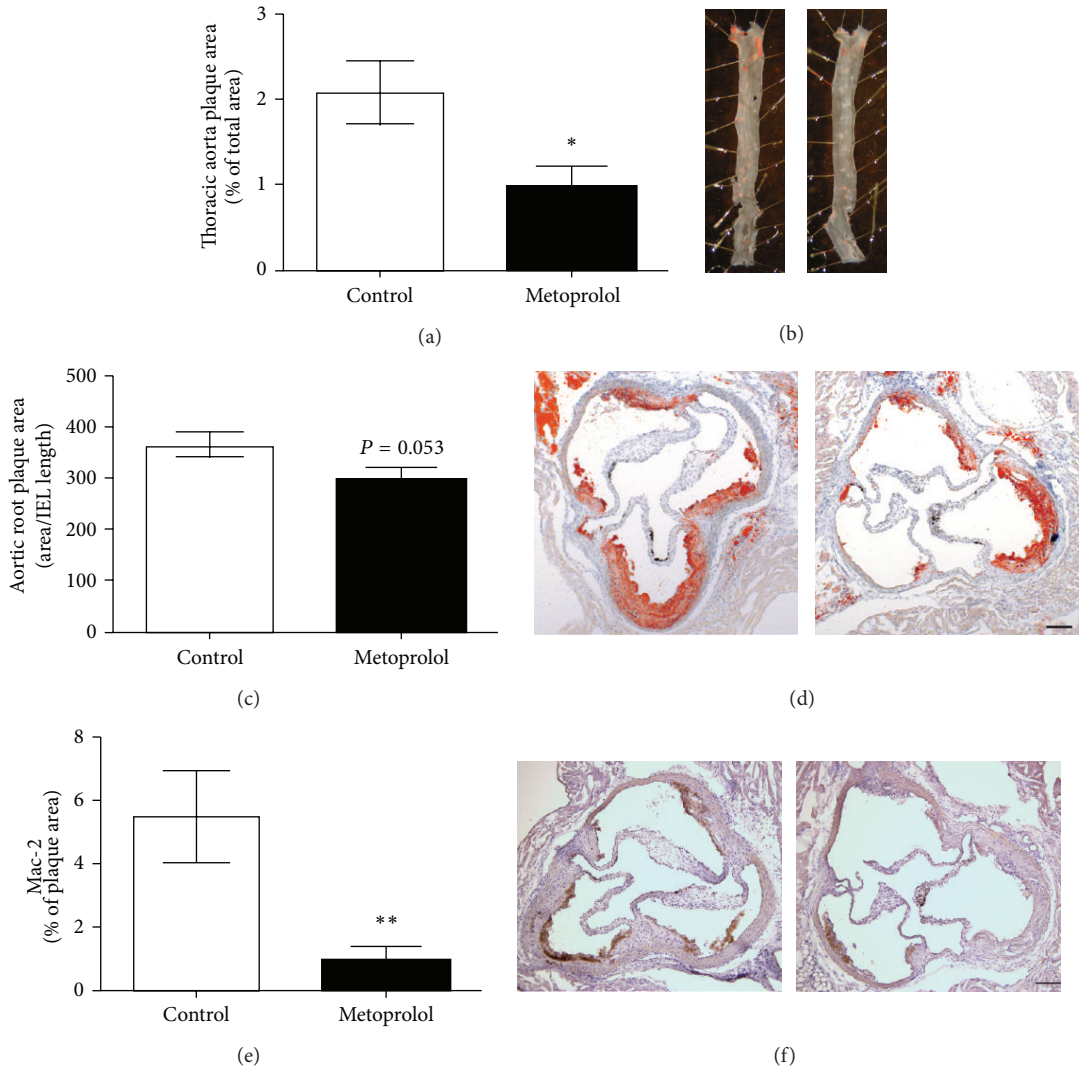

Figure 2: Metoprolol decreases atherosclerosis. (a)-(b) Metoprolol treatment (2.5 mg/kg per hour) decreased atherosclerotic plaque area in thoracic aorta. (c)-(d) A similar trend was seen in the aortic root, although this did not reach significance ($P = 0.053$). (e)-(f) Metoprolol treatment decreased the macrophage content in aortic lesions. Representative micrographs of thoracic aorta stained with Sudan IV (b), aortic root stained with Oil Red O (d), and macrophage marker Mac-2 (f). Left panel: Controls; right panel: metoprolol treated. Scale bar represents 200 μm. $^{*}P < 0.05$, $^{**}P < 0.01$ versus Control.

atherosclerotic plaque area in thoracic aorta ($P < 0.05$, Figures 2(a) and 2(b)). A similar pattern was seen in the aortic root; however, this did not reach significance ($P = 0.053$, Figures 2(c) and 2(d)).

3.2.2. Metoprolol Reduced Lesion Macrophages and Serum Levels of TNFα and CXCL1. Macrophage marker Mac-2 was significantly decreased in metoprolol treated compared to Control mice ($P < 0.01$, Figures 2(e) and 2(f)). Further, metoprolol treatment reduced serum levels of the Th1 cytokines TNFα and CXCL1, by approximately 30%, compared to untreated controls ($P < 0.01$, Table 1).

3.2.3. Total Cholesterol. Metoprolol treatment was not associated with changes in total plasma cholesterol levels (Control 12.3 ± 0.5 versus Metoprolol 11.6 ± 0.4 mmol/L).

4. Discussion

In the present study we demonstrated that treatment with a moderate dose of the selective β_1-adrenoceptor antagonist metoprolol reduced atherosclerotic plaque area in ApoE$^{-/-}$ mice. Further, metoprolol reduced macrophage content in atherosclerotic lesions and decreased serum levels of the proinflammatory Th1 cytokines TNFα and CXCL1. Our study confirms the atheroprotective effect of metoprolol as previously reported [18] and complements the previous study by showing an effect on proinflammatory cytokines.

Different mechanisms may contribute to the decreased atherogenesis in the current study. Metoprolol is known to lower heart rate and reduce blood pressure. Hypertension is a well-known risk factor for atherosclerosis in humans and we cannot exclude that the antihypertensive effects of

Table 1: Effects of metoprolol treatment on Th1/Th2 cytokines (Study II).

	Th1						Th2		
	IL-1β (pg/mL)	IL-2 (pg/mL)	IL-12 total (pg/mL)	IFN-γ (pg/mL)	TNFα (pg/mL)	CXCL1 (pg/mL)	IL-4 (pg/mL)	IL-5 (pg/mL)	IL-10 (pg/mL)
Control	4.8 ± 0.2	7.8 ± 0.5	3016 ± 106	4.0 ± 1.0	1.6 ± 0.1	241 ± 18	7.8 ± 1.7	7.2 ± 0.8	81.8 ± 14
Metoprolol	4.9 ± 0.5	21.3 ± 14	2904 ± 95	5.9 ± 3.3	1.1 ± 0.1	161 ± 18	13.0 ± 7.4	8.0 ± 1.7	121 ± 52
P value (Mann-Whitney)	0.712	0.538	0.389	0.735	**0.008**	**0.005**	0.601	0.951	1.000

Serum cytokine concentrations (pg/mL) were analyzed with the Mann-Whitney U test. Data are expressed as mean ± SEM. $P < 0.01$ was considered statistically significant.

metoprolol explain the reduced atherogenesis in the present study. However, in normotensive patients metoprolol has little impact on blood pressure [11] and in normotensive mice blood pressure lowering seems to have little influence on atherogenesis [19]. The only valid way to study possible metoprolol effects on arterial blood pressure in the present experiments would be to perform long-term blood pressure telemetry experiments in ApoE$^{-/-}$ mice with simultaneous chronic metoprolol infusions. We do not have such data. However, in a recent study investigating the effects of different β-antagonists on atherosclerosis none of the β-antagonists used, including metoprolol given at a similar dose as in the current study, influenced blood pressure [18]. Our suggestions are that blood pressure reduction may come into play but it is unlikely to be the sole effect on the decreased atherosclerosis.

Another, perhaps neglected, mechanism is the reduction in central sympathetic drive [20, 21]. The lipophilic property of metoprolol allows this β-blocker to enter the central nervous system and acts by decreasing sympathetic activity and increases vagal activation [22, 23]. This is of particular interest since recent studies show an anti-inflammatory signaling mediated via the vagus nerve [24]. Stimulation of the vagus nerve decreases release of the proinflammatory cytokine TNFα in a model of endotoxemia and vagotomy increases serum levels of TNFα [25]. It is interesting to speculate whether the anti-inflammatory effect of metoprolol could be attributed to decreased sympathetic activity and increased vagal tone. Indeed, in the current study we found decreased serum levels of the proinflammatory cytokines TNFα and CXCL1.

Previous studies show that metoprolol reduces the expression of adhesion molecules VCAM-1, ICAM-1, and MMP-1 in atherosclerotic lesions in rabbit aorta [26]. In line with this, we show that metoprolol reduces serum levels of TNFα, which is known to induce VCAM, ICAM, and MMPs [27]. Inhibition of TNFα or depletion of the TNFα gene reduces the progression of atherosclerosis in ApoE$^{-/-}$ mice [28–30]. Transcription of TNFα is regulated by transcription factors such as NF-κB [31], which has been shown to be activated by the sympathetic nervous system and to be inhibited by adrenergic blockade [32]. Hence, it is possible that metoprolol inhibits TNFα production through the inhibition of NF-κB, with a subsequent reduction in expression of adhesion molecules.

The chemokine CXCL1, also known as KC (keratinocyte-derived chemokine), is the murine homologue of human GROα (growth-regulated-oncogene-α). We have previously shown that sympathetic activation by social disruption stress in ApoE$^{-/-}$ mice not only accelerates atherosclerosis but also increases plasma levels of CXCL1 [16]. On this note, we here demonstrate reduced serum levels by metoprolol treatment. CXCL1 and its receptor CXCR2 are present in atherosclerotic lesions and play an important role in adhesion of monocytes and accumulation of macrophages in lesions in mice [33–35]. Consequently, we found a decreased amount of macrophages in the atherosclerotic lesions after metoprolol treatment. Interestingly, beta receptor antagonism can also decrease the LDL affinity for arterial proteoglycans [36], thereby decreasing the accumulation of LDL in the arterial wall. In contrast, beta receptor antagonists may also decrease HDL levels and increase triglyceride levels, two proatherogenic features [36]. In the current study only total cholesterol levels were measured and we cannot exclude a change in lipoproteins. Nevertheless, it is plausible that not only decreased infiltration of macrophages but also reduced LDL accumulation could explain the decreased atherogenesis.

In this study we used a moderate dose of metoprolol, 2.5 mg/kg per hour. We chose this dose since it did not blunt heart rate reactivity during air-jet stress, allowing the mouse to react normally to a stressful situation. Further, heart rate was significantly decreased at baseline conditions during a 24-hour period, but more importantly the circadian rhythm was still virtually intact.

5. Conclusions

To conclude, metoprolol treatment reduces the progression of atherosclerosis in ApoE$^{-/-}$ mice. A possible mechanism for the decreased atherogenesis is the anti-inflammatory effect of metoprolol, manifested as decreased serum levels of proinflammatory cytokines TNFα and CXCL1 and, consequently, decreased macrophage content in the atherosclerotic plaques.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Marcus A. Ulleryd and Evelina Bernberg contributed equally to this paper.

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References

[1] J. Wikstrand and M. Kendall, "The role of beta receptor blockade in preventing sudden death," *European Heart Journal*, vol. 13, supplement D, pp. 111–120, 1992.

[2] "Effect of metoprolol CR/XL in chronic heart failure: metoprolol CR/XL Randomised Intervention Trial in Congestive Heart Failure (MERIT-HF)," *The Lancet*, vol. 353, pp. 2001–2007, 1999.

[3] B. Ablad, J.-A. Bjorkman, D. Gustafsson, G. Hansson, A.-M. Ostlund-Lindqvist, and K. Pettersson, "The role of sympathetic activity in atherogenesis: effects of β-blockade," *American Heart Journal*, vol. 116, no. 1, pp. 322–327, 1988.

[4] J. Wikstrand, G. Berglund, B. Hedblad, and J. Hulthe, "Antiatherosclerotic effects of β-blockers," *The American Journal of Cardiology*, vol. 91, no. 12, supplement 1, pp. 25–29, 2003.

[5] J. R. Kaplan, S. B. Manuck, T. B. Clarkson, F. M. Lusso, and D. M. Taub, "Social status, environment, and atherosclerosis in cynomolgus monkeys," *Arteriosclerosis*, vol. 2, no. 5, pp. 359–368, 1982.

[6] J. R. Kaplan, S. B. Manuck, T. B. Clarkson, F. M. Lusso, D. M. Taub, and E. W. Miller, "Social stress and atherosclerosis in normocholesterolemic monkeys," *Science*, vol. 220, no. 4598, pp. 733–735, 1983.

[7] J. R. Kaplan, S. B. Manuck, M. R. Adams, K. W. Weingand, and T. B. Clarkson, "Inhibition of coronary atherosclerosis by propranolol in behaviorally predisposed monkeys fed an atherogenic diet," *Circulation*, vol. 76, no. 6, pp. 1364–1372, 1987.

[8] H. B. Skantze, J. Kaplan, K. Pettersson et al., "Psychosocial stress causes endothelial injury in cynomolgus monkeys via β1-adrenoceptor activation," *Atherosclerosis*, vol. 136, no. 1, pp. 153–161, 1998.

[9] A. M. Ostlund-Lindqvist, P. Lindqvist, J. Brautigam, G. Olsson, G. Bondjers, and C. Nordborg, "Effect of metoprolol on diet-induced atherosclerosis in rabbits," *Arteriosclerosis*, vol. 8, no. 1, pp. 40–45, 1988.

[10] B. Hedblad, J. Wikstrand, L. Janzon, H. Wedel, and G. Berglund, "Low-dose metoprolol CR/XL and fluvastatin slow progression of carotid intima-media thickness: main results from the β-blocker cholesterol-lowering asymptomatic plaque study (BCAPS)," *Circulation*, vol. 103, no. 13, pp. 1721–1726, 2001.

[11] O. Wiklund, J. Hulthe, J. Wikstrand, C. Schmidt, S. Olofsson, and G. Bondjers, "Effect of controlled release/extended release metoprolol on carotid intima-media thickness in patients with hypercholesterolemia: a 3-year randomized study," *Stroke*, vol. 33, no. 2, pp. 572–577, 2002.

[12] G. Östling, I. Gonçalves, J. Wikstrand, G. Berglund, J. Nilsson, and B. Hedblad, "Long-term treatment with low-dose metoprolol CR/XL is associated with increased plaque echogenicity: the Beta-blocker Cholesterol-lowering Asymptomatic Plaque Study (BCAPS)," *Atherosclerosis*, vol. 215, no. 2, pp. 440–445, 2011.

[13] E. Bernberg, I. J. Andersson, S. Tidstrand, M. E. Johansson, and G. Bergström, "Repeated exposure to stressors do not accelerate atherosclerosis in ApoE$^{-/-}$ mice," *Atherosclerosis*, vol. 204, no. 1, pp. 90–95, 2009.

[14] M. E. Johansson, E. Bernberg, I. J. Andersson et al., "High-salt diet combined with elevated angiotensin II accelerates atherosclerosis in apolipoprotein E-deficient mice," *Journal of Hypertension*, vol. 27, no. 1, pp. 41–47, 2009.

[15] M. E. Johansson, A. Wickman, S. M. Fitzgerald, L. Gan, and G. Bergström, "Angiotensin II, type 2 receptor is not involved in the angiotensin II-mediated pro-atherogenic process in ApoE$^{-/-}$ mice," *Journal of Hypertension*, vol. 23, no. 8, pp. 1541–1549, 2005.

[16] E. Bernberg, M. A. Ulleryd, M. E. Johansson, and G. M. L. Bergström, "Social disruption stress increases IL-6 levels and accelerates atherosclerosis in ApoE$^{-/-}$ mice," *Atherosclerosis*, vol. 221, no. 2, pp. 359–365, 2012.

[17] M. E. Johansson, A. Wickman, O. Skøtt, L. Gan, and G. Bergström, "Blood pressure is the major driving force for plaque formation in aortic-constricted ApoE$^{-/-}$ mice," *Journal of Hypertension*, vol. 24, no. 10, pp. 2001–2008, 2006.

[18] K. Shimada, E. Hirano, T. Kimura, M. Fujita, and C. Kishimoto, "Carvedilol reduces the severity of atherosclerosis in apolipoprotein E-deficient mice via reducing superoxide production," *Experimental Biology and Medicine*, vol. 237, no. 9, pp. 1039–1044, 2012.

[19] H. Lu, L. A. Cassis, and A. Daugherty, "Atherosclerosis and arterial blood pressure in mice," *Current Drug Targets*, vol. 8, no. 11, pp. 1181–1189, 2007.

[20] A. Gourine, S. I. Bondar, K. M. Spyer, and A. V. Gourine, "Beneficial effect of the central nervous system β-adrenoceptor blockade on the failing heart," *Circulation Research*, vol. 102, no. 6, pp. 633–636, 2008.

[21] G. W. Parker, L. H. Michael, C. J. Hartley, J. E. Skinner, and M. L. Entman, "Central β-adrenergic mechanisms may modulate ischemic ventricular fibrillation in pigs," *Circulation Research*, vol. 66, no. 2, pp. 259–270, 1990.

[22] B. Åblad, T. Bjurö, J.-A. Björkman et al., "Metoprolol, but not atenolol, reduces stress induced neuropeptide Y release in pigs," *Scandinavian Cardiovascular Journal*, vol. 44, no. 5, pp. 273–278, 2010.

[23] L. Gullestad, J. Pernow, T. Bjurö et al., "Differential effects of metoprolol and atenolol to neuropeptide y blockade in coronary artery disease," *Scandinavian Cardiovascular Journal*, vol. 46, no. 1, pp. 23–31, 2012.

[24] U. Andersson and K. J. Tracey, "Neural reflexes in inflammation and immunity," *Journal of Experimental Medicine*, vol. 209, no. 6, pp. 1057–1068, 2012.

[25] L. V. Borovikova, S. Ivanova, M. Zhang et al., "Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin," *Nature*, vol. 405, no. 6785, pp. 458–462, 2000.

[26] C. Liang, L. Xiaonan, C. Xiaojun et al., "Effect of metoprolol on vulnerable plaque in rabbits by changing shear stress around plaque and reducing inflammation," *European Journal of Pharmacology*, vol. 613, no. 1–3, pp. 79–85, 2009.

[27] H. Zhang, Y. Park, J. Wu et al., "Role of TNF-α in vascular dysfunction," *Clinical Science*, vol. 116, no. 3, pp. 219–230, 2009.

[28] L. Brånén, L. Hovgaard, M. Nitulescu, E. Bengtsson, J. Nilsson, and S. Jovinge, "Inhibition of tumor necrosis factor-α reduces atherosclerosis in apolipoprotein E knockout mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 11, pp. 2137–2142, 2004.

[29] H. Ohta, H. Wada, T. Niwa et al., "Disruption of tumor necrosis factor-α gene diminishes the development of atherosclerosis in ApoE-deficient mice," *Atherosclerosis*, vol. 180, no. 1, pp. 11–17, 2005.

[30] M. Canault, F. Peiretti, M. Poggi et al., "Progression of atherosclerosis in ApoE-deficient mice that express distinct molecular forms of TNF-alpha," *Journal of Pathology*, vol. 214, no. 5, pp. 574–583, 2008.

[31] R. De Martin, M. Hoeth, R. Hofer-Warbinek, and J. A. Schmid, "The transcription factor NF-kappa B and the regulation of vascular cell function," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 20, no. 11, pp. E83–E88, 2000.

[32] A. Bierhaus, J. Wolf, M. Andrassy et al., "A mechanism converting psychosocial stress into mononuclear cell activation," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 4, pp. 1920–1925, 2003.

[33] W. A. Boisvert, R. Santiago, L. K. Curtiss, and R. A. Terkeltaub, "A leukocyte homologue of the IL-8 receptor CXCR-2 mediates the accumulation of macrophages in atherosclerotic lesions of LDL receptor-deficient mice," *Journal of Clinical Investigation*, vol. 101, no. 2, pp. 353–363, 1998.

[34] W. A. Boisvert, D. M. Rose, K. A. Johnson et al., "Up-regulated expression of the CXCR2 ligand KC/GRO-α in atherosclerotic lesions plays a central role in macrophage accumulation and lesion progression," *The American Journal of Pathology*, vol. 168, no. 4, pp. 1385–1395, 2006.

[35] Y. Huo, C. Weber, S. B. Forlow et al., "The chemokine KC, but not monocyte chemoattractant protein-1, triggers monocyte arrest on early atherosclerotic endothelium," *The Journal of Clinical Investigation*, vol. 108, no. 9, pp. 1307–1314, 2001.

[36] T. Linden, G. Camejo, O. Wiklund, I. Warnold, S. O. Olofsson, and G. Bondjers, "Effect of short-term beta blockade on serum lipid levels and on the interaction of LDL with human arterial proteoglycans," *Journal of Clinical Pharmacology*, vol. 30, pp. S124–S131, 1990.

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Review Article

Antibodies in the Pathogenesis of Hypertension

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It has long been known that circulating levels of IgG and IgM antibodies are elevated in patients with essential and pregnancy-related hypertension. Recent studies indicate these antibodies target, and in many cases activate, G-protein coupled receptors and ion channels. Prominent among these protein targets are AT_1 receptors, α_1-adrenoceptors, β_1-adrenoceptors, and L-type voltage operated Ca^{2+} channels, all of which are known to play key roles in the regulation of blood pressure through modulation of vascular tone, cardiac output, and/or Na^+/water reabsorption in the kidneys. This suggests that elevated antibody production may be a causal mechanism in at least some cases of hypertension. In this brief review, we will further describe the protein targets of the antibodies that are elevated in individuals with essential and pregnancy-related hypertension and the likely pathophysiological consequences of antibody binding to these targets. We will speculate on the potential mechanisms that underlie elevated antibody levels in hypertensive individuals and, finally, we will outline the therapeutic opportunities that could arise with a better understanding of how and why antibodies are produced in hypertension.

1. Introduction

Hypertension is defined as chronically elevated blood pressures of >140/90 mmHg. Two of the most common forms of the condition are essential hypertension, where the underlying cause is unknown, and preeclampsia or pregnancy-related hypertension. For several decades it has been known that both essential and pregnancy-related hypertension are associated with elevated serum levels of antibodies [1–4]. More recently, studies in humans and animal models of each condition have begun to identify the protein targets of these antibodies as receptors and ion channels with key roles in the regulation of blood pressure. Such studies not only offer insights into the mechanisms by which antibodies might contribute to hypertension but they also highlight potential new avenues for the clinical management of hypertension. In this brief review we will summarise the evidence in support of a role for antibodies in the pathophysiology of essential hypertension and preeclampsia. We will discuss the protein targets of the antibodies that have been identified in hypertensive individuals and provide some potential explanations for why the production of these antibodies may be elevated. Finally, we will speculate on how such findings may translate into improved clinical management of hypertension.

2. Antibodies as Causes of Disease

Antibodies, or immunoglobulins (Ig), are produced exclusively by B cells as part of the mammalian adaptive immune response [5]. Antibodies play a crucial role in adaptive immunity through their ability to bind antigens, which are normally toxic substances or fragments of pathogen-derived proteins. Such binding results in either neutralisation of the antigen itself or, when the antigen is bound to a cell (e.g., bacteria), destruction of that cell via activation of the complement system, neutrophil degranulation, or phagocytosis by macrophages (Figure 1).

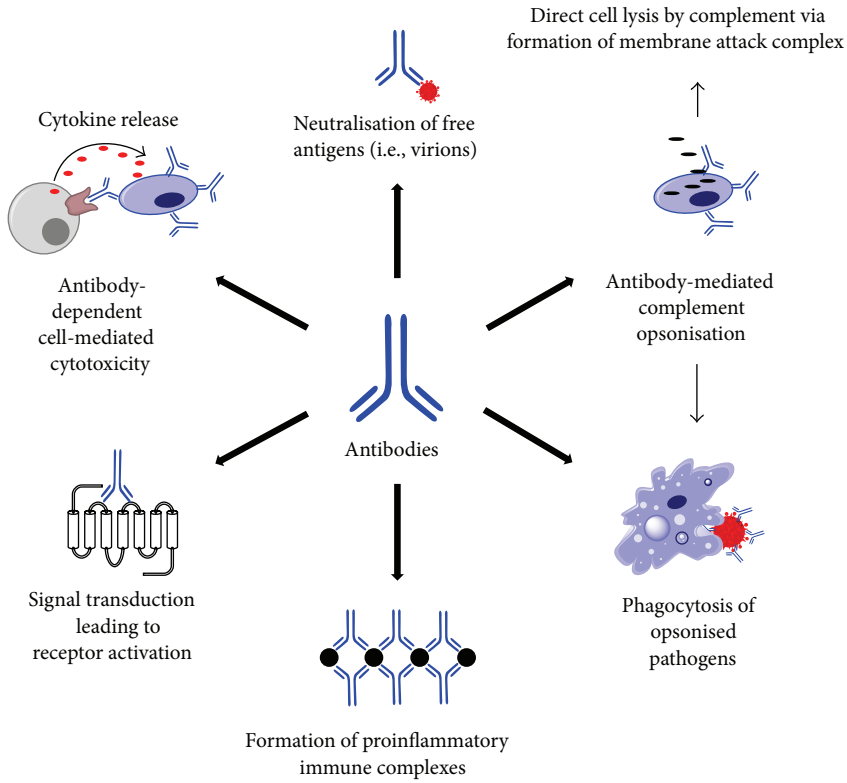

Figure 1: Schematic diagram showing the various types of antibody-mediated autoimmune responses.

For B cells to generate and secrete antibodies, they must first undergo differentiation into plasma cells. Naïve B cells detect antigens via their B cell receptors, which are membrane-bound immunoglobulins (IgM) with unique and randomly-generated antigen-binding sites [6]. Following binding, the antigen is internalised, processed, and displayed by major histocompatibility class II (MHC II) proteins on the extracellular surface of the B cell [6]. The next step in B cell differentiation involves the detection of the MHC II-presented antigen by the T cell receptor (TCR) of an activated T helper (T_H) cell that has previously encountered the same antigen. The T_H cell also provides essential costimulatory signals in the form of interactions between its CD40 ligand and the CD40 receptor on the B cell [7], as well as cytokines such as interferon-gamma and interleukin-4 [8]. Together, these signals ultimately promote the differentiation of the B cell into an antibody-secreting plasma cell. Such signals also play a crucial role in isotype switching (e.g. IgM $\rightarrow$ IgG) [9], which allows the generated antibodies to interact with different effector molecules and thereby direct the type of immune response that is mounted.

Although antibodies normally target foreign molecules, under some circumstances they may be raised against host-derived molecules. Such a loss of recognition of "self" is the basis for autoimmune diseases. There are several mechanisms by which aberrant antibody production can lead to autoimmune pathologies (Figure 1). For example, the binding of antibodies to antigens expressed on the surface of endogenous cells may lead to the destruction of these cells via complement- or leukocyte-dependent interactions. This type of response is termed a "Type II Hypersensitivity reaction" and is the cause of the loss of erythrocytes in autoimmune haemolytic anaemia [10]. Alternatively, "Type III Hypersensitivity reactions" involve the recognition of soluble antigens in the host by antibodies and the subsequent formation of "immune complexes." Immune complexes are cross-linked aggregations of antibodies and antigens that can be deposited in various tissues to cause local inflammatory responses [11]. Immune complexes are a hallmark of several autoimmune disorders including vasculitis and systemic lupus erythematosus where deposition of such complexes in the kidneys gives rise to glomerulonephritis [12, 13]. Finally, some autoimmune diseases are associated with the formation of nonimmunogenic, agonistic antibodies to receptors [14]. These types of diseases are often classified as "Type V Hypersensitivity Reactions." Agonistic antibodies stimulate receptors in a similar fashion to their cognate ligands and thus lead to overstimulation of the specific system involved. Myasthenia gravis is an example of an autoimmune disease caused by the generation of agonistic antibodies against nicotinic receptors [14, 15].

3. Protein Targets of Hypertension-Related Antibodies

Studies dating back to the 1970s demonstrated that essential hypertension in humans is associated with elevated IgG and IgM titres [1–4]. However, these early studies did not

identify the targets of these antibodies and thus provided no indication of whether they were important to the pathophysiology of hypertension. Studies from around the same time on animal models confirmed that hypertension was associated with an increase in antibody production and even went some way towards implicating a possible causative role for these antibodies. For example, Ba et al. identified autoantibodies in the serum of spontaneously hypertensive rats (SHRs) that were cytotoxic to T cells. Although the authors did not establish a precise mechanism by which these antibodies contribute to hypertension, they implied that the antibodies might induce apoptosis of "suppressor" T cells that normally prevent damage to the vascular wall and thus protect against cardiovascular disease [16]. In a separate study, it was shown that rats with hypertension induced by renal infarction had high serum levels of antibodies that bound to arteries, glomeruli, and basement membranes of the kidneys [17]. Given the key roles of the kidney and vasculature in the regulation of hemodynamic parameters, this latter study provided an indication that elevated antibody production may actually contribute to the chronic elevation in blood pressure that defines hypertension. And indeed, more recent work identifying the specific molecular targets of the antibodies present in individuals with essential hypertension and preeclampsia not only supports this idea, but also begins to shed light on how elevated antibody production might contribute to elevated blood pressure.

3.1. Angiotensin II Type-1 Receptors. The angiotensin II type-1 receptor (AT_1R) plays a crucial role in the regulation of blood pressure [18]. Stimulation of the AT_1R by its cognate ligand, angiotensin II, results in vascular smooth muscle cell (VSMC) contraction and proliferation, release of aldosterone from the adrenal glands, and activation of the sympathetic nervous system [18, 19]. Furthermore, it has recently been discovered that AT_1 receptor activation on T lymphocytes promotes a proinflammatory phenotype that contributes to hypertension [20].

AT_1R-activating IgG autoantibodies (AT_1-AAs) directed against the second extracellular loop of the AT_1R are prevalent in over 95% of patients with pregnancy-associated hypertension, and antibody titres correlate positively with disease severity [21, 22]. AT_1-AAs appear to activate a cascade of proinflammatory cytokines that contribute directly to hypertension in preeclampsia [23]. *In vivo* administration of AT_1-AAs isolated from preeclamptic humans to pregnant mice was shown to induce hypertension in those animals [24]. Furthermore, AT_1-AA treatment causes an increase in the circulating levels of tumour necrosis factor-α and interleukin-6 in pregnant mice and inhibition of these cytokines with neutralising antibodies blunts hypertension [24, 25].

AT_1-AAs have also been identified in a subset of individuals with essential hypertension [26–28]. These AT_1-AAs appear to be similar in function and specificity as those identified in preeclamptic patients as they also bind to the second extracellular loop of the AT_1R [26, 28]. The fact that essential hypertensive patients with AT_1-AAs respond with greater blood pressure reductions to AT_1R blockade by candesartan than hypertensive individuals without AT_1-AA [29, 30] suggests a causal role for AT_1-AAs in at least some cases of hypertension.

3.2. Alpha-1 Adrenergic Receptors. The alpha-1 adrenergic receptor (α_1AR) is a G-protein coupled receptor that is primarily expressed on VSMCs and proximal renal tubules [31]. Activation of the α_1AR by its endogenous ligands, noradrenaline and adrenaline, or synthetic compounds such as phenylephrine, results in VSMC contraction and increased total peripheral resistance, as well as increased Na^+ reabsorption in the kidney causing elevated blood pressure [32]. IgG receptor-activating autoantibodies against the α_1AR (α_1AR-AA) have been described in patients with essential hypertension [28, 33–35]. Unlike AT_1-AAs, which appear to bind to a similar domain of the AT_1R (i.e., the second extracellular loop) irrespective of the patient from which they were derived, α_1AR-AA from different patients display selectivity towards separate regions of the receptor, with antibodies from some individuals targeting the first extracellular loop, and antibodies from other individuals targeting the second extracellular loop [33, 34]. It is unclear whether these varying binding properties have implications for the ability of a specific antibody to modulate receptor function. It is also unclear whether α_1AR-AAs are present or elevated in individuals with preeclampsia.

3.3. Beta-1 Adrenergic Receptors. The β-1 adrenergic receptor (β_1AR) shares the same endogenous agonists with α_1ARs but differs in ligand affinity, tissue distribution, and functional outcomes following stimulation. β_1ARs are localised predominately in cardiac tissue where activation results in increased heart rate and contractility and an overall increase in cardiac output [36]. Cardiac output is a major determinant of blood pressure and thus β_1AR blockers have long been used as antihypertensive agents [37]. β_1AR agonistic IgG autoantibodies (β_1AR-AA) against the second extracellular loop of the receptors were detected in the serum of spontaneously hypertensive rats [38]. Furthermore, injection of β_1AR-AAs into healthy Lewis rats promoted cardiomyopathy and increases in systolic blood pressure [39]. Although evidence for the presence of β_1AR-AAs in human essential hypertension and preeclampsia is lacking, these antibodies have been identified in patients with idiopathic dilated cardiomyopathy [40].

3.4. L-Type Voltage Gated Calcium Channels. L-type voltage gated calcium channels (L-type VOCCs) are expressed on VSMCs in resistance vessels and, in their open state, directly contribute to vascular tone and blood pressure by facilitating the influx of extracellular Ca^{2+} [41]. It is well established that L-type VOCC expression in the vasculature is upregulated in experimental hypertension and that this contributes to increased Ca^{2+} levels in VSMCs and thus elevated vascular resistance [42–47]. The importance of L-type VOCCs in human hypertension is highlighted by the fact that inhibitors of these channels (e.g., nifedipine) continue to

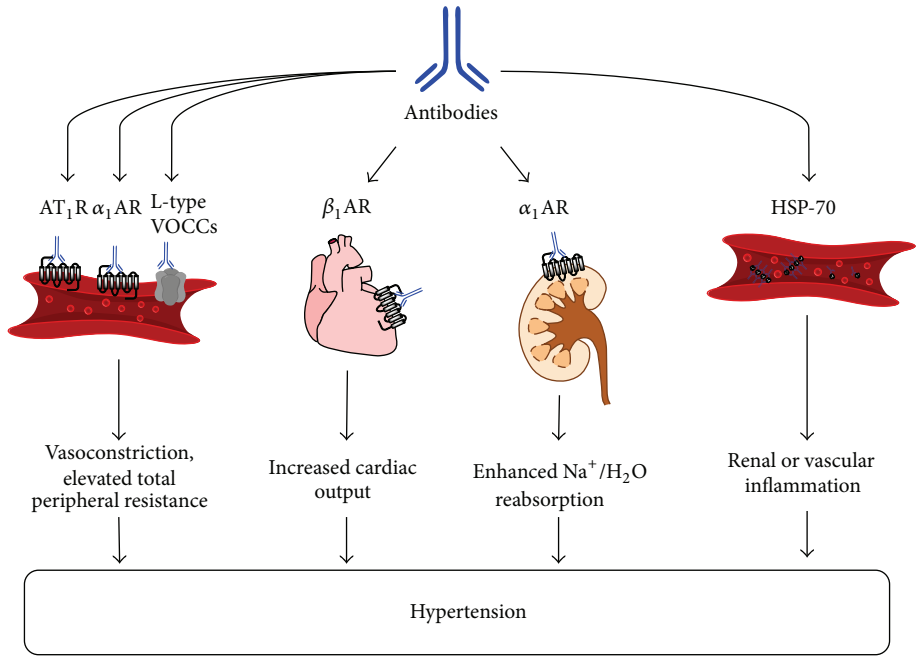

Figure 2: Schematic diagram showing the mechanism by which autoantibodies may promote increases in vascular tone, cardiac output, Na^+/water reabsorption, and renal and vascular inflammation, and thereby contribute to hypertension.

represent one of the most effective and widely-used classes of antihypertensive medications [48]. Thus, it is noteworthy that autoantibodies against L-type VOCCs were identified in some patients with essential hypertension [49]. Although the authors did not examine the effect of these antibodies on L-type VOCC function, a separate study demonstrated increased intracellular Ca^{2+} influx in pancreatic islet cells following the binding of analogous IgG and IgA autoantibodies to L-type VOCCs in the setting of Type-1 diabetes [50]. This implies that antibodies against L-type VOCCs are likely to be agonistic in nature and could thus contribute to increased VSMC Ca^{2+} influx in hypertension.

3.5. Heat Shock Proteins. Heat shock proteins (HSPs) are a family of highly-conserved proteins that provide protection against danger-related signals by acting as molecular chaperones to assist in the folding and trafficking of proteins during cellular stress [51]. Among the multitude of known mammalian HSPs, HSP-70 has received most attention in the field of hypertension research. First, *in vitro* exposure of cultured VSMCs, endothelial cells or isolated aortic rings to hypertension-relevant stimuli such as oxidative stress, cyclic strain, and angiotensin II, induces the expression of HSP-70 [52–54]. Second, levels of HSP-70 and HSP-70-reactive CD4 T cells are elevated in the kidneys in several rat models of hypertension [55–57]. Finally, HSP-70 serum concentrations are elevated in pregnancy-associated hypertension and are positively correlated with blood pressure in affected women [58].

Elevated levels of IgG and IgA antibody titres against HSP-70 have been identified in essential hypertensive individuals [59, 60]. Surprisingly, elevated anti-HSP-70 antibody levels in essential hypertension were not associated with changes in serum HSP-70 in these patients [60]. Thus, the function of anti-HSP-70 antibodies in essential hypertension remains unclear. It is possible that anti-HSP-70 antibodies could either promote inflammation via formation of circulating immune complexes or, alternatively, alleviate the proinflammatory actions of these proteins via neutralisation. A more recent study by Molvarec et al. was unable to demonstrate any changes in circulating levels of anti-HSP-70 antibodies in women with preeclampsia [61].

3.6. Miscellaneous. A study in borderline hypertensive patients described a reduction in circulating levels of anti-oxidised LDL IgG antibodies [62]. However, a follow-up investigation failed to detect any difference in levels of these antibodies between patients with clinical hypertension and normotensive controls [63], and thus the significance of anti-oxidised LDL antibodies in the pathophysiology of hypertension is unclear. Other studies in borderline hypertensive individuals detected elevations in circulating anti-endothelial cell IgG and IgM antibodies [64–66]. While data in the setting of human essential hypertension is still missing, these antibodies have been identified in women with severe preeclampsia and have been proposed to contribute to endothelial dysfunction [67].

Figure 2 provides a summary of the targets of antibodies that have been shown to be elevated in hypertension and

the potential mechanisms by which these antibodies may contribute to disease pathophysiology.

4. Mechanisms Contributing to Antibody Production

The previous discussion highlighting the association of hypertension with increased antibody levels raises the question: what are the mechanisms involved in antibody production during hypertension? There are at least three possible explanations including (1) neoantigen formation; (2) molecular mimicry; and/or (3) aberrant B cell function.

4.1. Neoantigen Formation. Harrison and colleagues recently put forward a hypothesis whereby "neoantigens" were highlighted as the central mediators of the immune cell activation that underlies hypertension [68, 69]. These authors suggested that hypertensive stimuli such as Ang II, catecholamines, and aldosterone initially induce a moderate increase in blood pressure via their "classical" actions in promoting Na^+/water retention, vasoconstriction, and/or increased sympathetic drive [68, 69]. This moderate increase in blood pressure is postulated to cause both mechanical and oxidative stress in the walls of blood vessels and also in the kidneys, leading to structural and chemical modifications to proteins such that they are no longer recognised as "self," but rather as neoantigens. These neoantigens are predicted to then invoke an adaptive immune response, leading to vascular and renal inflammation and exacerbation of hypertension [68, 69]. However, it is presently unclear whether any of the proteins that have been identified as targets of antibodies in hypertensive animals and humans (e.g., AT_1R, α_1AR, β_1AR, L-type VOCCs, or HSP-70) undergo structural or chemical alterations that may render them as potential neoantigens.

4.2. Molecular Mimicry. Another possible explanation for autoantibody production in hypertension involves molecular mimicry, where foreign or pathogen-derived antigens trigger an immune response against "self" peptides of similar homology [70]. A prominent example of this is myasthenia gravis, an autoimmune disease where agonistic antibodies are raised against nicotinic receptors [14, 15]. These antibodies show strong cross-reactivity to herpes simplex virus glycoprotein D [15].

Relating to hypertension, AT_1-AAs from women with preeclampsia were shown to recognise the VP2 caspid protein from parvovirus B19 [71]. The seroprevalence of this virus has been reported to be more than 70% of the adult population [72, 73], and its involvement in predisposing infected individuals to various autoimmune disorders has been recognised [73]. Thus, it is plausible that molecular mimicry underlies the elevations in AT_1-AAs observed in preeclampsia [74] and essential hypertension.

Although not examined in the setting of hypertension, there is evidence in other disease states that antibodies against L-type VOCCs and β_1ARs may also arise as a result of molecular mimicry. For example, autoantibodies against L-type VOCCs that are present in a subset of individuals with Type-1 diabetes also recognise the B4 VP1 protein of the coxsackievirus [50]. Interestingly, the seroprevalence of coxsackievirus infection was reported to positively associate with the incidence of hypertension in a Chinese Mongolian population [75]. Furthermore, β_1AR-AAs were demonstrated to recognise the carboxy-terminus of the ribosomal P0 and P2 proteins from *Trypanosoma cruzi,* the parasite that is responsible for chronic Chagas heart disease [76–79]. Conversely, autoantibodies against human ribosomal P proteins that are present in patients with systemic lupus erythematosus cross-react with (but do not activate) the β_1AR [78]. These findings may suggest that a high degree of sequence and/or structural homology exists between β_1AR and ribosomal P proteins.

4.3. Aberrant B Cell Function. Hypertensive stimuli such as Ang II might act to directly modify the function of B cells, such that their capacity to produce antibodies is enhanced. Na^+/H^+ ion exchangers (NHEs) are critical regulators of intracellular pH and are crucial to a variety of fundamental cellular processes such as proliferation, growth, and migration [80]. Studies from the 1990s demonstrated that B cells isolated from a subset of hypertensive patients display heightened activity of NHEs [81, 82]. Moreover, these B cells were further characterised as having enhanced G-protein activation, a higher proliferative capacity, and augmented IgG and IgM antibody secretion compared to B cells from nonhypertensive individuals [83, 84]. While the mechanism underlying this increase in NHE activity in B cells was not explored, in other cell types (e.g., VSMCs and cardiomyocytes) it is known that AT_1R stimulation can enhance NHE activity [85–87]. Indeed, B cells express AT_1R [88] and we have preliminary data showing that stimulation of B cells isolated from mice with Ang II potentiates IgM formation in response to a known B cell stimulator, the oligodeoxynucleotide CpG (Figure 3). Thus, amplified antibody production may arise as a result of elevated NHE function due to the direct activation of AT_1R expressed on B cells.

5. The Role of B Cells in Hypertension

Implicit in the previous discussion on antibodies in hypertension is a potentially important role for the cell type that produces antibodies, namely, B cells. In their seminal study, Guzik et al. showed that recombinase-activating gene-1 knockout ($RAG1^{-/-}$) mice—which lack T and B cells—displayed a blunted hypertensive response to both Ang II and deoxycorticosterone acetate/salt [89]. Whereas adoptive transfer of T cells into $RAG1^{-/-}$ mice recapitulated the full hypertensive effects of Ang II, transfer of B cells had no effect [89]. There are at least two potential explanations for the lack of effect of B cell adoptive transfer in Ang II-treated $RAG1^{-/-}$ mice. First, it is possible that the adoptively transferred B cells did not engraft in sufficient numbers to influence immune function. Indeed, in a previous study it was shown that retroviral-mediated reintroduction of the RAG1 gene into

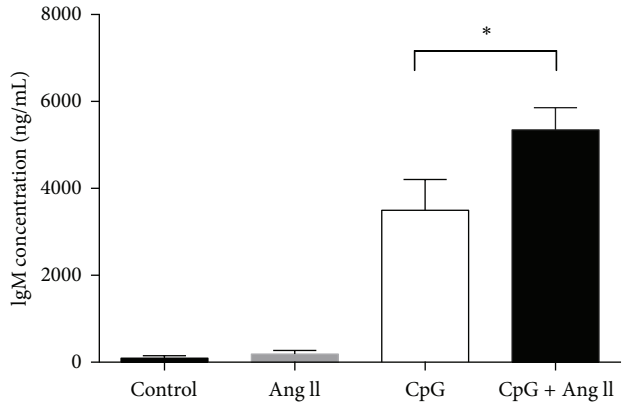

Figure 3: Effect of angiotensin II (Ang II; 0.1 μM, 48 h) or CpG oligodeoxynucleotides (CpG; 5 μg/mL, 48 h) alone or in combination on IgM antibody secretion from primary cultures of mouse B cells. Values represent mean ± S.E.M. of $n = 14$ experiments. $^{*}P < 0.05$ for Bonferroni's post-hoc test after one-way ANOVA.

RAG1$^{-/-}$ mice restored T cells numbers back to levels in wild-type mice, while B cell numbers only increased marginally [90]. This suggests that the immunological environment in RAG1$^{-/-}$ mice, while being favourable to the survival and function of T cells, may be incompatible with the growth and function of B cells. An alternative explanation may lie in the different mechanisms that activate T cells and B cells during an immune response. Whilst activation of T cells relies primarily on antigen presentation from innate immune cells such as dendritic cells (which are relatively unaffected in RAG1$^{-/-}$ mice), as discussed previously, B cell activation and differentiation into an effector phenotype normally requires interactions with T_H cells [6]. Hence, the lack of T cells in RAG1$^{-/-}$ mice may have precluded the possibility of any adoptively transferred B cells becoming activated. Indeed, a critical role of T_H cells in B cell activation during hypertension was suggested in a recent study showing that adoptive transfer of T_H cells from preeclamptic mice into normal pregnant mice induced AT_1-AA production and elevated blood pressure [91]. Importantly, a B cell depleting agent ameliorated both of these effects [91].

6. Therapeutic Implications

An understanding of the role of B cells and antibody production during hypertension could aid in the refinement of current treatment approaches and also in development of novel antihypertensive therapies. For example, by identifying the autoantibodies that are specifically elevated in a given hypertensive patient, it might be possible to "tailor" the way their disease is subsequently managed for better clinical outcomes; that is, individuals with AT_1-AAs would favourably respond to AT_1R blockers over patients with L-type VOCC autoantibodies, where calcium channel blockers such as nifedipine would be preferred.

In terms of new therapeutic approaches, identification of the specific pathogen-derived or neoantigens that lead to elevated antibody generation in hypertension could lead to immunisation strategies aimed at neutralising such antigens or steering the immune response away from one that promotes hypertension. Indeed, two vaccines against Ang II have been developed and showed some early promise in reducing blood pressure in hypertensive patients [92]. However, due to their lower efficacy compared to conventional inhibitors of the renin-angiotensin system, the vaccines did not proceed into Phase III clinical trials [93, 94], and thus further work is needed to determine if alternative immunisation strategies (i.e., involving different adjuvants and/or immunogens) will be more effective.

B cell-depleting agents, which include antibodies against the B cell specific surface receptor CD20 and the B cell activating factor BAFF, are already in clinical use for the treatment of autoimmune diseases such as lupus erythematosus [95] and could potentially be used to treat hypertension. Of course, these drugs have the potential for causing immunosuppression and hence their use might be best reserved for those hypertensive patients that do not respond to conventional therapies. Until recently, one of the main therapeutic options for individuals with resistant hypertension was surgical denervation of the renal artery [96]; however, the effectiveness of this procedure has recently been called into question [97]. Thus, B cell-modulating drugs might yet be a safer and more efficacious therapeutic option for such patients.

7. Conclusion

There is evidence that circulating antibody levels are elevated in both essential and pregnancy-related hypertension. Many of these antibodies appear to target receptors and ion channels known to be involved in the regulation of blood pressure. Further studies are required to characterise the precise impact that antibody binding has on the function of these proteins and to uncover the mechanisms responsible for aberrant antibody production in hypertension. Such studies should not only allow us to evaluate the significance of elevated antibody production in the pathophysiology of hypertension, but they may also lead to the development of new therapeutic approaches and/or the refinement of current approaches, to improve the management of clinical hypertension in the future.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] A. Ebringer and A. E. Doyle, "Raised serum IgG levels in hypertension," *British Medical Journal*, vol. 2, no. 5702, pp. 146–148, 1970.

[2] P. Suryaprahba, T. Padma, and U. Brahmaji Rao, "Increased serum IgG levels in essential hypertension," *Immunology Letters*, vol. 8, no. 3, pp. 143–145, 1984.

[3] E. Hilme, H. Herlitz, T. Soderstrom, and L. Hansson, "Increased secretion of immunoglobulins in malignant hypertension," *Journal of Hypertension*, vol. 7, no. 2, pp. 91–95, 1989.

[4] T. Gudbrandsson, L. Hansson, H. Herlitz, L. Lindholm, and L. A. Nilsson, "Immunological changes in patients with previous malignant essential hypertension," *Lancet*, vol. 1, no. 8217, pp. 406–408, 1981.

[5] T. W. Lebien and T. F. Tedder, "B lymphocytes: How they develop and function," *Blood*, vol. 112, no. 5, pp. 1570–1580, 2008.

[6] D. C. Parker, "T cell-dependent B cell activation," *Annual Review of Immunology*, vol. 11, pp. 331–360, 1993.

[7] I. S. Grewal and R. A. Flavell, "CD40 and CD154 in cell-mediated immunity," *Annual Review of Immunology*, vol. 16, pp. 111–135, 1998.

[8] J. Purkerson and P. Isakson, "A two-signal model for regulation of immunoglobulin isotype switching," *The FASEB Journal*, vol. 6, no. 14, pp. 3245–3252, 1992.

[9] S. G. Tangye, A. Ferguson, D. T. Avery, C. S. Ma, and P. D. Hodgkin, "Isotype switching by human B cells is division-associated and regulated by cytokines," *Journal of Immunology*, vol. 169, no. 8, pp. 4298–4306, 2002.

[10] B. C. Gehrs and R. C. Friedberg, "Autoimmune hemolytic anemia," *The American Journal of Hematology*, vol. 69, no. 4, pp. 258–271, 2002.

[11] A. N. Theofilopoulos and F. J. Dixon, "Immune complexes in human diseases," *The American Journal of Pathology*, vol. 100, no. 2, pp. 529–594, 1980.

[12] D. Koffler, V. Agnello, R. Thoburn, and H. G. Kunkel, "Systemic lupus erythematosus: prototype of immune complex nephritis in man," *Journal of Experimental Medicine*, vol. 134, supplement 3, pp. 169S–179S, 1971.

[13] W. L. Gross, A. Trabandt, and E. Reinhold-Keller, "Diagnosis and evaluation of vasculitis," *Rheumatology*, vol. 39, no. 3, pp. 245–252, 2000.

[14] J. M. Lindstrom, M. E. Seybold, V. A. Lennon, S. Whittingham, and D. D. Duane, "Antibody to acetylcholine receptor in myasthenia gravis: prevalence, clinical correlates, and diagnostic value," *Neurology*, vol. 51, no. 4, p. 933, 1998.

[15] P. L. Schwimmbeck, T. Dyrberg, D. B. Drachman, and M. B. A. Oldstone, "Molecular mimicry and myasthenia gravis. An autoantigenic site of the acetylcholine receptor α-subunit that has biologic activity and reacts immunochemically with herpes simplex virus," *Journal of Clinical Investigation*, vol. 84, no. 4, pp. 1174–1180, 1989.

[16] D. Ba, N. Takeichi, T. Kodama, and H. Kobayashi, "Restoration of T cell depression and suppression of blood pressure in spontaneously hypertensive rats (SHR) by thymus grafts or thymus extracts," *Journal of Immunology*, vol. 128, no. 3, pp. 1211–1216, 1982.

[17] F. N. White and A. Grollman, "Autoimmune factors associated with infarction of the kidney," *Nephron*, vol. 204, pp. 93–102, 1964.

[18] M. J. Peach, "Renin angiotensin system: biochemistry and mechanisms of action," *Physiological Reviews*, vol. 57, no. 2, pp. 313–370, 1977.

[19] L. Hunyady and K. J. Catt, "Pleiotropic AT1 receptor signaling pathways mediating physiological and pathogenic actions of angiotensin II," *Molecular Endocrinology*, vol. 20, no. 5, pp. 953–970, 2006.

[20] J. L. Silva-Filho, M. C. Souza, M. D. G. Henriques et al., "AT1 receptor-mediated angiotensin II activation and chemotaxis of T lymphocytes," *Molecular Immunology*, vol. 48, no. 15-16, pp. 1835–1843, 2011.

[21] G. Wallukat, V. Homuth, T. Fischer et al., "Patients with preeclampsia develop agonistic autoantibodies against the angiotensin AT1 receptor," *Journal of Clinical Investigation*, vol. 103, no. 7, pp. 945–952, 1999.

[22] A. H. Siddiqui, R. A. Irani, S. C. Blackwell, S. M. Ramin, R. E. Kellems, and Y. Xia, "Angiotensin receptor agonistic autoantibody is highly prevalent in preeclampsia: correlation with disease severity," *Hypertension*, vol. 55, no. 2, pp. 386–393, 2010.

[23] Y. Xia and R. E. Kellems, "Angiotensin receptor agonistic autoantibodies and hypertension : preeclampsia and beyond," *Circulation Research*, vol. 113, no. 1, pp. 78–87, 2013.

[24] C. C. Zhou, R. A. Irani, Y. Dai et al., "Autoantibody-mediated IL-6-dependent endothelin-1 elevation underlies pathogenesis in a mouse model of preeclampsia," *Journal of Immunology*, vol. 186, no. 10, pp. 6024–6034, 2011.

[25] R. A. Irani, Y. Zhang, C. C. Zhou et al., "Autoantibody-mediated angiotensin receptor activation contributes to preeclampsia through tumor necrosis factor-α signaling," *Hypertension*, vol. 55, no. 5, pp. 1246–1253, 2010.

[26] M. L. Fu, H. Herlitz, W. Schulze et al., "Autoantibodies against the angiotensin receptor (AT1) in patients with hypertension," *Journal of Hypertension*, vol. 18, no. 7, pp. 945–953, 2000.

[27] F. Zhu, Y. Sun, M. Wang et al., "Correlation between HLA-DRB1, HLA-DQB1 polymorphism and autoantibodies against angiotensin AT(1) receptors in Chinese patients with essential hypertension," *Clinical Cardiology*, vol. 34, no. 5, pp. 302–308, 2011.

[28] Y. H. Liao, Y. M. Wei, M. Wang, Z. H. Wang, H. T. Yuan, and L. X. Cheng, "Autoantibodies against AT1-receptor and α1-adrenergic receptor in patients with hypertension," *Hypertension Research*, vol. 25, no. 4, pp. 641–646, 2002.

[29] Y. Sun, Y. Liao, Y. Yuan et al., "Influence of autoantibodies against AT1 receptor and AGTR1 polymorphisms on candesartan-based antihypertensive regimen: results from the Study of Optimal Treatment in Hypertensive Patients with Anti-AT1-Receptor Autoantibodies trial," *Journal of the American Society of Hypertension*, vol. 8, no. 1, pp. 21–27, 2014.

[30] F. Wei, X. J. Jia, S. Q. Yu et al., "Candesartan versus imidapril in hypertension: a randomised study to assess effects of anti-AT1 receptor autoantibodies," *Heart*, vol. 97, no. 6, pp. 479–484, 2011.

[31] J. L. Reid, "Alpha-adrenergic receptors and blood pressure control," *The American Journal of Cardiology*, vol. 57, no. 9, pp. 6E–12E, 1986.

[32] M. T. Piascik and D. M. Perez, "α1-adrenergic receptors: new insights and directions," *Journal of Pharmacology and Experimental Therapeutics*, vol. 298, no. 2, pp. 403–410, 2001.

[33] H. P. Luther, V. Homuth, and G. Wallukat, "α1-Adrenergic receptor antibodies in patients with primary hypertension," *Hypertension*, vol. 29, no. 2, pp. 678–682, 1997.

[34] M. L. Fu, H. Herlitz, G. Wallukat et al., "Functional autoimmune epitope on α1-adrenergic receptors in patients with malignant hypertension," *The Lancet*, vol. 344, no. 8938, pp. 1660–1663, 1994.

[35] K. Wenzel, H. Haase, G. Wallukat et al., "Potential relevance of α1-Adrenergic receptor autoantibodies in refractory hypertension," *PLoS ONE*, vol. 3, no. 11, Article ID e3742, 2008.

[36] K. P. Minneman, R. N. Pittman, and P. B. Molinoff, "Beta-adrenergic receptor subtypes: properties, distribution, and regulation," *Annual Review of Neuroscience*, vol. 4, pp. 419–461, 1981.

[37] M. A. van Baak, H. A. J. Struyker Boudier, and J. F. M. Smits, "Antihypertensive mechanisms of beta-adrenoceptor blockade: a review," *Clinical and Experimental Hypertension A Theory and Practice*, vol. 7, no. 1, pp. 1–72, 1985.

[38] G. Wallukat, I. E. Blasig, R. Morwinski, H. J. Herrmann, and E. Rohde, "The sera of spontaneously hypertensive rats contain agonistic auto-antibodies against the β1-adrenoceptor," *Journal of Hypertension*, vol. 13, no. 9, pp. 1031–1036, 1995.

[39] R. Jahns, V. Boivin, L. Hein et al., "Direct evidence for a β1-adrenergic receptor-directed autoimmune attack as a cause of idiopathic dilated cardiomyopathy," *Journal of Clinical Investigation*, vol. 113, no. 10, pp. 1419–1429, 2004.

[40] Y. Magnusson, G. Wallukat, F. Waagstein, Å. Hjalmarson, and J. Hoebeke, "Autoimmunity in idiopathic dilated cardiomyopathy: characterization of antibodies against the β1-adrenoceptor with positive chronotropic effect," *Circulation*, vol. 89, no. 6, pp. 2760–2767, 1994.

[41] W. A. Catterall, "Voltage-gated calcium channels," *Cold Spring Harbor perspectives in biology*, vol. 3, no. 8, Article ID a003947, 2011.

[42] D. W. Wilde, P. B. Furspan, and J. F. Szocik, "Calcium current in smooth muscle cells from normotensive and genetically hypertensive rats," *Hypertension*, vol. 24, no. 6, pp. 739–746, 1994.

[43] R. H. Cox and I. M. Lozinskaya, "Augmented calcium currents in mesenteric artery branches of the spontaneously hypertensive rats," *Hypertension*, vol. 26, no. 6, pp. 1060–1064, 1995.

[44] Y. Ohya, T. Tsuchihashi, S. Kagiyama, I. Abe, and M. Fujishima, "Single L-type calcium channels in smooth muscle cells from resistance arteries of spontaneously hypertensive rats," *Hypertension*, vol. 31, no. 5, pp. 1125–1129, 1998.

[45] J. M. Simard, X. Li, and K. Tewari, "Increase in functional Ca2+ channels in cerebral smooth muscle with renal hypertension," *Circulation Research*, vol. 82, no. 12, pp. 1330–1337, 1998.

[46] A. Pesic, J. A. Madden, M. Pesic, and N. J. Rusch, "High blood pressure upregulates arterial L-type Ca2+ channels: is membrane depolarization the signal?" *Circulation research*, vol. 94, no. 10, pp. e97–e104, 2004.

[47] P. F. Pratt, S. Bonnet, L. M. Ludwig, P. Bonnet, and N. J. Rusch, "Upregulation of L-type Ca2+ channels in mesenteric and skeletal arteries of SHR," *Hypertension*, vol. 40, no. 2, pp. 214–219, 2002.

[48] D. J. Triggle, "L-type calcium channels," *Current Pharmaceutical Design*, vol. 12, no. 4, pp. 443–457, 2006.

[49] Z. H. Zhou, J. Wang, H. Xiao et al., "A novel autoantibody in patients with primary hypertension: antibody against L-type Ca2+ channel," *Chinese Medical Journal*, vol. 121, no. 16, pp. 1513–1517, 2008.

[50] C. Bason, R. Lorini, C. Lunardi et al., "In type 1 diabetes a subset of anti-coxsackievirus B4 antibodies recognize autoantigens and induce apoptosis of pancreatic beta cells," *PLoS ONE*, vol. 8, no. 2, Article ID e57729, 2013.

[51] M. J. Schlesinger, "Heat shock proteins," *Journal of Biological Chemistry*, vol. 265, no. 21, pp. 12111–12114, 1990.

[52] Q. Xu, G. Schett, C. Li, Y. Hu, and G. Wick, "Mechanical stress-induced heat shock protein 70 expression in vascular smooth muscle cells is regulated by rac and ras small G proteins but not mitogen-activated protein kinases," *Circulation Research*, vol. 86, no. 11, pp. 1122–1128, 2000.

[53] Q. Xu, D. G. Li, N. J. Holbrook, and R. Udelsman, "Acute hypertension induces heat-shock protein 70 gene expression in rat aorta," *Circulation*, vol. 92, no. 5, pp. 1223–1229, 1995.

[54] D. F. Liao, Z. G. Jin, A. S. Baas et al., "Purification and identification of secreted oxidative stress-induced factors from vascular smooth muscle cells," *Journal of Biological Chemistry*, vol. 275, no. 1, pp. 189–196, 2000.

[55] G. Parra, Y. Quiroz, J. Salazar et al., "Experimental induction of salt-sensitive hypertension is associated with lymphocyte proliferative response to HSP70," *Kidney International*, vol. 74, no. 111, pp. S55–S59, 2008.

[56] N. Ishizaka, T. Aizawa, M. Ohno et al., "Regulation and localization of HSP70 and HSP25 in the kidney of rats undergoing long-term administration of Angiotensin II," *Hypertension*, vol. 39, no. 1, pp. 122–128, 2002.

[57] H. Pons, A. Ferrebuz, Y. Quiroz et al., "Immune reactivity to heat shock protein 70 expressed in the kidney is cause of salt-sensitive hypertension," *The American Journal of Physiology—Renal Physiology*, vol. 304, no. 3, pp. F289–F299, 2013.

[58] A. Molvarec, Z. Prohászka, B. Nagy et al., "Association of elevated serum heat-shock protein 70 concentration with transient hypertension of pregnancy, preeclampsia and superimposed preeclampsia: a case-control study," *Journal of Human Hypertension*, vol. 20, no. 10, pp. 780–786, 2006.

[59] T. Wu, J. Ma, S. Chen et al., "Association of plasma antibodies against the inducible Hsp70 with hypertension and harsh working conditions," *Cell Stress & Chaperones*, vol. 6, no. 4, pp. 394–401, 2001.

[60] A. G. Pockley, U. de Faire, R. Kiessling, C. Lemne, T. Thulin, and J. Frostegård, "Circulating heat shock protein and heat shock protein antibody levels in established hypertension," *Journal of Hypertension*, vol. 20, no. 9, pp. 1815–1820, 2002.

[61] A. Molvarec, Z. Derzsy, J. Kocsis et al., "Circulating anti-heat-shock-protein antibodies in normal pregnancy and preeclampsia," *Cell Stress and Chaperones*, vol. 14, no. 5, pp. 491–498, 2009.

[62] R. Wu, U. de Faire, C. Lemne, J. L. Witztum, and J. Frostegård, "Autoantibodies to OxLDL are decreased in individuals with borderline hypertension," *Hypertension*, vol. 33, no. 1 I, pp. 53–59, 1999.

[63] J. Frostegard, R. Wu, C. Lemne et al., "Circulating oxidized low-density lipoprotein is increased in hypertension," *Clinical Science*, vol. 105, no. 5, pp. 615–620, 2003.

[64] D. P. Papadopoulos, T. K. Makris, P. Krespi et al., "Antiendothelial cell antibody levels in healthy normotensives with high normal blood pressure," *Clinical and Experimental Hypertension*, vol. 28, no. 8, pp. 663–667, 2006.

[65] D. P. Papadopoulos, T. K. Makris, U. Papazachou, M. Daskalaki, E. Sanidas, and V. E. Votteas, "Antiendothelial cell antibody levels in patients with masked hypertension," *International Journal of Cardiology*, vol. 130, no. 3, pp. 405–408, 2008.

[66] J. Frostegård, R. Wu, C. Gillis-Haegerstrand, C. Lemne, and U. de Faire, "Antibodies to endothelial cells in borderline hypertension," *Circulation*, vol. 98, no. 11, pp. 1092–1098, 1998.

[67] V. J. Rappaport, G. Hirata, H. K. Yap, and S. C. Jordan, "Anti-vascular endothelial cell antibodies in severe preeclampsia," *The American Journal of Obstetrics and Gynecology*, vol. 162, no. 1, pp. 138–146, 1990.

[68] D. G. Harrison, T. J. Guzik, H. E. Lob et al., "Inflammation, immunity, and hypertension," *Hypertension*, vol. 57, no. 2, pp. 132–140, 2011.

[69] D. G. Harrison, A. Vinh, H. Lob, and M. S. Madhur, "Role of the adaptive immune system in hypertension," *Current Opinion in Pharmacology*, vol. 10, no. 2, pp. 203–207, 2010.

[70] M. B. Oldstone, "Molecular mimicry and immune-mediated diseases," *The FASEB Journal*, vol. 12, no. 13, pp. 1255–1265, 1998.

[71] F. Herse, S. Verlohren, K. Wenzel et al., "Prevalence of agonistic autoantibodies against the angiotensin II type 1 receptor and soluble fms-like tyrosine kinase 1 in a gestational age-matched case study," *Hypertension*, vol. 53, no. 2, pp. 393–398, 2009.

[72] J. R. Kerr, "Parvovirus B19 infection," *European Journal of Clinical Microbiology and Infectious Diseases*, vol. 15, no. 1, pp. 10–29, 1996.

[73] C. Lunardi, E. Tinazzi, C. Bason, M. Dolcino, R. Corrocher, and A. Puccetti, "Human parvovirus B19 infection and autoimmunity," *Autoimmunity Reviews*, vol. 8, no. 2, pp. 116–120, 2008.

[74] B. Lamarca, K. Wallace, and J. Granger, "Role of angiotensin II type i receptor agonistic autoantibodies (AT1-AA) in preeclampsia," *Current Opinion in Pharmacology*, vol. 11, no. 2, pp. 175–179, 2011.

[75] L. Liu, Y. Liu, W. Tong et al., "Pathogen burden in essential hypertension," *Circulation Journal*, vol. 71, no. 11, pp. 1761–1764, 2007.

[76] I. Ferrari, M. J. Levin, G. Wallukat et al., "Molecular mimicry between the immunodominant ribosomal protein P0 of Trypanosoma cruzi and a functional epitope on the human β1-adrenergic receptor," *Journal of Experimental Medicine*, vol. 182, no. 1, pp. 59–65, 1995.

[77] P. Lopez Bergami, J. Scaglione, and M. J. Levin, "Antibodies against the carboxyl-terminal end of the Trypanosoma cruzi ribosomal P proteins are pathogenic," *The FASEB Journal*, vol. 15, no. 14, pp. 2602–2612, 2001.

[78] D. Kaplan, I. Ferrari, P. L. Bergami et al., "Antibodies to ribosomal P proteins of Trypanosoma cruzi in Chagas disease possess functional autoreactivity with heart tissue and differ from anti-P autoantibodies in lupus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 19, pp. 10301–10306, 1997.

[79] A. Mijares, L. Verdot, N. Peineau, B. Vray, J. Hoebeke, and J. Argibay, "Antibodies from Trypanosoma cruzi infected mice recognize the second extracellular loop of the β1-adrenergic and M2-muscarinic receptors and regulate calcium channels in isolated cardiomyocytes," *Molecular and Cellular Biochemistry*, vol. 163-164, pp. 107–112, 1996.

[80] M. E. Malo and L. Fliegel, "Physiological role and regulation of the Na+/H+ exchanger," *Canadian Journal of Physiology and Pharmacology*, vol. 84, no. 11, pp. 1081–1095, 2006.

[81] D. Rosskopf, R. Dusing, and W. Siffert, "Membrane sodium-proton exchange and primary hypertension," *Hypertension*, vol. 21, no. 5, pp. 607–617, 1993.

[82] D. Rosskopf, E. Fromter, and W. Siffert, "Hypertensive sodium-proton exchanger phenotype persists in immortalized lymphoblasts from essential hypertensive patients. A cell culture model for human hypertension," *Journal of Clinical Investigation*, vol. 92, no. 5, pp. 2553–2559, 1993.

[83] D. Rosskopf, K. Hartung, J. Hense, and W. Siffert, "Enhanced immunoglobulin formation of immortalized B cells from hypertensive patients," *Hypertension*, vol. 26, no. 3, pp. 432–435, 1995.

[84] W. Siffert, D. Rosskopf, A. Moritz et al., "Enhanced G protein activation in immortalized lymphoblasts from patients with essential hypertension," *Journal of Clinical Investigation*, vol. 96, no. 2, pp. 759–766, 1995.

[85] V. N. Phan, M. Kusuhara, P. A. Lucchesi, and B. C. Berk, "A 90-kD NA+-H+ exchanger kinase has increased activity in spontaneously hypertensive rat vascular smooth muscle cells," *Hypertension*, vol. 29, no. 6, pp. 1265–1272, 1997.

[86] E. Takahashi, J. I. Abe, and B. C. Berk, "Angiotensin II stimulates p90(rsk) in vascular smooth muscle cells: a potential Na+-H+ exchanger kinase," *Circulation Research*, vol. 81, no. 2, pp. 268–273, 1997.

[87] S. Gunasegaram, R. S. Haworth, D. J. Hearse, and M. Avkiran, "Regulation of sarcolemmal Na+/H+ exchanger activity by angiotensin II in adult rat ventricular myocytes opposing actions via AT1 versus AT2 receptors," *Circulation Research*, vol. 85, no. 10, pp. 919–930, 1999.

[88] J. D. Zhang, M. B. Patel, Y. S. Song et al., "A Novel role for type 1 angiotensin receptors on T lymphocytes to limit target organ damage in hypertension," *Circulation Research*, vol. 110, no. 12, pp. 1604–1617, 2012.

[89] T. J. Guzik, N. E. Hoch, K. A. Brown et al., "Role of the T cell in the genesis of angiotensin II-induced hypertension and vascular dysfunction," *Journal of Experimental Medicine*, vol. 204, no. 10, pp. 2449–2460, 2007.

[90] C. Lagresle-Peyrou, F. Yates, M. Malassis-Séris et al., "Long-term immune reconstitution in RAG-1-deficient mice treated by retroviral gene therapy: a balance between efficiency and toxicity," *Blood*, vol. 107, no. 1, pp. 63–72, 2006.

[91] S. R. Novotny, K. Wallace, J. Heath et al., "Activating autoantibodies to the angiotensin II type I receptor play an important role in mediating hypertension in response to adoptive transfer of CD4+ T lymphocytes from placental ischemic rats," *The American Journal of Physiology—Regulatory Integrative and Comparative Physiology*, vol. 302, no. 10, pp. R1197–R1201, 2012.

[92] P. Maurer and M. F. Bachmann, "Immunization against angiotensins for the treatment of hypertension," *Clinical Immunology*, vol. 134, no. 1, pp. 89–95, 2010.

[93] T. H. Do, Y. Chen, V. T. Nguyen, and S. Phisitkul, "Vaccines in the management of hypertension," *Expert Opinion on Biological Therapy*, vol. 10, no. 7, pp. 1077–1087, 2010.

[94] M. J. Brown, "Success and failure of vaccines against renin-angiotensin system components," *Nature Reviews Cardiology*, vol. 6, no. 10, pp. 639–647, 2009.

[95] M. Ramos-Casals, I. Sanz, X. Bosch, J. H. Stone, and M. A. Khamashta, "B-cell-depleting therapy in systemic lupus erythematosus," *The American Journal of Medicine*, vol. 125, no. 4, pp. 327–336, 2012.

[96] M. D. Esler, H. Krum, M. Schlaich, R. E. Schmieder, M. Böhm, and P. A. Sobotka, "Renal sympathetic denervation for treatment of drug-resistant hypertension: one-year results from the symplicity htn-2 randomized, controlled trial," *Circulation*, vol. 126, no. 25, pp. 2976–2982, 2012.

[97] D. L. Bhatt, D. E. Kandzari, W. W. O'Neill et al., "A controlled trial of renal denervation for resistant hypertension," *The New England Journal of Medicine*, vol. 370, no. 15, pp. 1393–1401, 2014.

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Research Article

Adiponectin Levels Are Reduced While Markers of Systemic Inflammation and Aortic Remodelling Are Increased in Intrauterine Growth Restricted Mother-Child Couple

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Aim of the Study. To investigate the relationships between the adipocytokine levels, markers of inflammation, and vascular remodelling in pregnancies complicated by intrauterine growth restriction (IUGR). *Materials and Methods*. This was a retrospective study. One hundred and forty pregnant patients were enrolled. Adiponectin, leptin, tumor necrosis factor α (TNFα), interleukin-6 (IL-6), and C reactive protein (CRP) were assessed in IUGR, small for gestational age (SGA), and appropriate for gestational age (AGA) mother-child couples at delivery. IUGR and SGA fetuses were defined as fetuses whose estimated fetal weight (EFW) was below 10th percentile for gestational age with and without umbilical artery (UA) Doppler abnormalities, respectively. Fetal aorta intima media thickness (aIMT) was evaluated by ultrasound in the same fetal groups. Data were analyzed by R (version 2.15.2). *Results*. There were 37 IUGR mother-child couples, 33 SGA, and 70 AGA. Leptin, TNFα, IL-6, and CRP serum levels were higher in IUGR pregnant patients ($P < 0.05$). Adiponectin levels were significantly reduced in IUGR fetuses compared to SGA and AGA, while leptin, TNFα, and IL-6 levels were higher in IUGR group ($P \leq 0.05$). Fetal aIMT was significantly higher in IUGR ($P < 0.05$) and in this group there was a negative correlation between aIMT and adiponectin/leptin ratio (A/L ratio) ($P < 0.05$) and between adiponectin and IL-6 levels ($P < 0.05$). *Conclusions*. In conclusion, compared to SGA and AGA, IUGR fetuses had reduced circulating levels of adiponectin and elevated measures of aIMT and several inflammatory markers. Moreover, adiponectin levels were negatively correlated with aIMT in IUGR fetuses suggesting a possible causal link between reduced adiponectin and vessel remodelling.

1. Introduction

Intrauterine growth restriction (IUGR) is considered the second leading cause of perinatal morbidity and mortality [1]. Adverse perinatal environments influence fetal growth and may result in developmental adaptations that permanently change the physiology and metabolism of the offspring thereby predisposing individuals to metabolic, endocrine, and cardiovascular events [2]. Insulin resistance has been proposed to be the underlying pathogenic link between metabolic syndrome and cardiovascular disease [3]; both are associated with a state of low-grade aseptic markers of

systemic inflammation, whose pathogenic significance was mostly eclipsed by the vigorous advances in lipid research [4]. A growing body of evidence has recently suggested that the adipose tissue may play a major role in linking poor fetal growth to subsequent development of adult diseases [5]. IUGR is known to alter the development of fetal adipose tissue. An increase in sympathetic tone and a dyslipidemic condition (high concentration of apolipoprotein B and apolipoprotein A1 and reduction in the concentration of insulin-like growth factor 1) in IUGR fetuses could help to increase the existing endothelial damage [6]. Several human studies showed that an estimated fetal weight (EFW) below the 10th centile and fetal Doppler vessel abnormalities were associated in utero and in neonates with endothelial dysfunction, represented by a higher aorta intima media thickness (aIMT) [7, 8]. In recent years adipocyte-derived signaling molecules ("adipokines") have been implicated in intrauterine growth restriction disorders. Adipose tissue is a complex organ including adipocytes, immune cells, fibroblast, tissue resident macrophages, collagen fibers, and vascular constituents. Over the past decade, it has been recognized that it is not only a fat store tissue, but also an endocrine organ, secreting a variety of bioactive molecules which influence body metabolism and energy homeostasis [9]. High serum concentrations of proinflammatory cytokines, such as leptin, CRP, IL-6 and TNF-α, as well as a reduction in serum adiponectin levels, should be related to low intrauterine weight and might worsen this condition [10].

Circulating levels of TNFα and IL-6 are directly correlated with adiposity and insulin resistance [11]. Macrophages, crucial contributors to inflammation, are the major source of TNFα produced by white adipose tissue (WAT) and contribute approximately 50% to WAT-derived IL-6 [12]. CRP is released by adipose tissue and is an important first line host defense molecule; it recognizes damaged cells and promotes their elimination by activating the complement system [13]. Plasma leptin concentrations directly reflect the amount of adipose tissue and the control of appetite is its primary role [14]. Leptin action in regulating immunity has been fueled by early observations in animal models, protecting T lymphocytes from apoptosis and regulating T-cell activation [15]. Leptin also influences monocytes activation, phagocytosis, and cytokine production; in endothelial cells it finally induces oxidative stress and upregulation of adhesion molecules [15]. Adiponectin is the most abundant adipokine produced by the adipose tissue and belongs to a collagen superfamily, sharing significant homology with collagen X, VIII, complement factor C1q, and TNF-α, suggesting a connection with the immune system. It modulates insulin action and exerts anti-inflammatory effects, playing an important role in the pathogenesis of metabolic syndrome [16]. Several reports suggest that adiponectin exerts an antiatherogenic role protecting vessels from endothelial dysfunction by its quiescent effect on macrophages, suppressing their production of proinflammatory cytokines, such as TNFα and IL-6, and inducing the production of anti-inflammatory cytokines [16, 17]. The mechanism underlying the relationship between birth weight, inflammation, and insulin sensitivity during adulthood remains still unclear.

To investigate the hypothesis that fetuses with a low EFW and umbilical artery (UA) Doppler abnormalities would exhibit lower concentrations of serum adiponectin and higher levels of leptin, CRP, and proinflammatory cytokines (TNFα and IL-6), we studied these adipocytokines in IUGR mother-child couples in comparison with small for gestational age (SGA) and appropriate for gestational age (AGA).

2. Subjects and Methods

2.1. Population. A retrospective study was performed from January, 2011, to March, 2013, in the Department of Woman and Child Health, University of Padua, Italy. The protocol was designed to study fetuses that were IUGR and SGA and those that were AGA. They were selected during the ultrasound evaluation of the third trimester. One hundred and forty pregnant patients were included in the study. IUGR fetuses were classified as fetuses whose EFW was below the 10th percentile for gestation age with UA Pulsatility Index (PI) > 2 SD; SGA fetuses were those whose EFW was below the 10th percentile without fetal velocimetry abnormalities. All pregnancies were dated correctly by first trimester ultrasound scan until the twentieth week of gestation. Customized centile were used with respect to the Italian standards of referral [18]. The Ethical Committee of the University Hospital approved the study protocol and all included mothers provided signed informed consent before enrollment. The diagnosis of IUGR and SGA was made within the 32nd week of gestation. Excluding factors were twin pregnancy, major congenital anomalies, pregnancies complicated by maternal history of cardiovascular disease or endocrine disorders (diabetes, hypercholesterolemia, preeclampsia, thyroid, and adrenal problems), and clinical chorioamnionitis. Women who consumed alcohol, smoked, nicotine, or any medication such as ritodrine and corticosteroids (except for fetal lung maturation) were excluded, such as amniotic fluid disorders and placental abnormalities. Antenatal surveillance was performed by fetal biometry every two weeks and maternal-fetal Doppler and amniotic fluid evaluation from one up to three times a week, depending on severity. Mean PI values were found to be upper 95th centile in all IUGR fetuses with a progressive worsening in 11 ones (UA absent end diastolic flow, PI middle cerebral artery (MCA) < 2 SD, a reduction of a wave in ductus venosus), indicating the initiation of fetal blood flow redistribution.

Amniotic fluid, as assessed by the largest fluid column on the vertical plane, was decreased (<2 cm) in the same 11 IUGR fetuses. PI uterine arteries were altered in 25 IUGR fetuses. In the AGA group, mother were healthy and no smokers.

aIMT and diameter measurements were determined for each fetus at a mean gestational age of 32 weeks (range 30 to 34 weeks). All parameters were measured by high-resolution ultrasound scan using an ultrasound machine equipped with a 3.5- to 5-MHz linear array transducer (Antares, Siemens Medical Solutions, Mountain View, CA). aIMT and diameter were measured in a coronal or sagittal view of the fetus at the dorsal arterial wall of the most distal 15 mm of the abdominal aorta sampled below the renal arteries and

above the iliac arteries; gain settings were used to optimize image quality. Abdominal aIMT was defined as the distance between the leading edge of the blood-intima interface and the leading edge of the media-adventitia interface on the far wall of the vessel, as previously described [8, 19]. Three measurements were taken, and the arithmetic mean aIMT was considered for the study. All images were taken at end-diastole of the cardiac cycle to minimize the variability. All the ultrasound studies in fetuses and children were performed by two, independently, blinded, skilled practitioners (E.C, S.V.). Before starting the main research, the intraobserver and interobserver agreement were evaluated in the measurement of aorta intima media thickness (0.876 and 0.856, resp.). Data concerning women, pregnancies, and deliveries were recorded according to the routine practice of the Department of Obstetrics and Gynecology of the University of Padua. During gestation mother's age, BMI before and after gestation, parity, and obstetrical history were collected. At delivery, sex, gestational age, birth weight, length, mode of delivery, Apgar score, acid base equilibrium, and perinatal data were registered. The main clinical features are reported in Table 1.

2.2. Collection of Blood Sample. Maternal blood was collected during the first stage of labor or before receiving anesthesia in case of elective cesarean section. Umbilical vein samples were collected from doubly clamped umbilical cords, after fetal expulsion, from all IUGR, SGA, and controls. Serum leptin, adiponectin, TNFα, IL-6, and CRP were measured.

Blood was put in sterile, pyrogen-free tubes and it was centrifuged (3000 g/min for 10 min at 5°C) after clotting; the supernatant serum was kept frozen at −80°C until assay.

2.3. Maternal and Cord Serum Assays. Serum leptin levels were measured using the Kit Leptin (Mediagnost, CAT. R44, Germany), a radioimmunoassay with streptavidin coated tubes. Analytical specifications are analytical sensitivity = 0.1 μg/L; intra-assay and interassay variation (CV%), respectively, 4.4 and 5.1; measuring range = 0.1–64 μg/L.

Serum adiponectin levels were measured using the radioimmunoassay RIA KIT Human Adiponectin (Millipore, cat. number HADP-61HK). Analytical specifications are limit of sensitivity = 1 μg/L; measuring range = 1–240 μg/L; intra-assay and interassay imprecision (CV%), respectively, 3.59 and 7.85.

Human TNFα was measured using the analyzer IMMULITE One (Medical System S.p.A., Genova, Italia). Assay characteristics are measuring range = 1,7–1000,0 ng/L; analytical sensitivity = 1,7 ng/L; between assays imprecision = 17,0–788,0 ng/L (CV = 4,0–6,5%). Each sample was measured in triplicate and each experiment was repeated three times.

Serum IL-6 was measured using the analyzer IMMULITE One (Medical System S.p.A., Genova, Italia). The test is an immunoassay based on chemiluminescence. Assays characteristics are measuring range = 2,0–1000,0 ng/L; analytical sensitivity = 2,0 ng/L; between assays imprecision = 88,0–1001,0 ng/L (CV = 5,1–7,5%).

Serum CRP level determination was performed by fully mechanized latex-particle-enhanced immunonephelometric assays on the Dimension Vista (Siemens Healthcare Diagnostic Products GmbH). Intra- and interassay coefficients of variation were 11.91 mg/L, 4.8%, and 6.0%.

2.4. Statistical Analysis. Statistical analysis was performed using R system. The normal distribution of the data was determined using the Kolmogorov-Smirnov test. These data were analyzed using, when appropriate, the following tests: t-test, Wilcoxon test, chi-square test, or Fisher's exact test. Kendall's Tau was used for correlation analysis. All possible correlations were performed and only significant correlations are reported in the text. A P value < 0.05 was accepted as statistically significant.

3. Results

3.1. Description of the Groups. There were 37 IUGR, 33 SGA, and 70 AGA mother-child couples. Table 1 shows the characteristics of the samples. We found no significant differences in maternal age or parity among studied groups (mean age 32.30 ± 4.88). Gestational age at delivery and neonatal weight were significantly lower in IUGR pregnancies than in other groups ($P < 0.05$). aIMT was higher in IUGR fetuses than in SGA and AGA ($P < 0.05$), and SGA fetuses had a higher aIMT than controls ($P < 0.05$).

3.2. Maternal and Fetal Hormones Levels

3.2.1. Adiponectin. Within each group adiponectin levels in the mother-fetus couples were higher in the fetuses ($P < 0.05$). There was a statistically significant lower level of maternal adiponectin concentration in IUGR than in control group ($P < 0.05$). Also in IUGR fetuses' adiponectin levels were lower than in AGA and SGA groups ($P < 0.05$). No differences were observed between SGA and AGA fetuses (Table 2).

3.2.2. Leptin. IUGR and SGA women presented higher leptin serum concentrations than AGA ($P < 0.05$). IUGR fetuses presented higher leptin levels than SGA and AGA ($P < 0.05$). There were no differences in leptin fetus levels between SGA and AGA (Table 2).

3.2.3. IL-6, TNFα and CRP. IL-6 concentration was higher in IUGR fetuses than SGA and AGA ($P < 0.05$); there was also a significant difference between SGA and control group ($P < 0.05$) (Table 2).

TNFα levels in mother-fetus couples were significantly higher in IUGR than SGA and controls. ($P < 0.05$).

IUGR patients presented maternal serum CRP concentrations higher than SGA and AGA ($P < 0.05$). Fetal IUGR, SGA, and AGA serum CRP did not show differences (Table 2).

3.3. Correlation among Maternal-Fetal Hormone Levels, Anthropometric, and Ultrasound Measures. In all groups, maternal adiponectin positively correlated with birth weight

Table 1: Description of the samples. Data are expressed as mean ± standard deviation or percentage. The P value refers to t-test, chi-square test, or Fisher's exact test.

	IUGR (37)	SGA (33)	Controls (70)	P
Woman age (years)	32.70 (±4.4)	31.90 (±5.1)	32.00 (±4.9)	NS
Prepregnancy BMI (kg/m^2)	23.80 (±6.3)	20.80 (±2.9)	22.10 (±2.9)	(1, 3)
Nulliparous women	69%	55%	53%	NS
Mode of delivery				
Spontaneous delivery	39%	33%	54%	NS
Caesarean section	61%	67%	46%	NS
Fetal gender				
Male	42%	27%	54%	(3)
Gestational age at delivery (weeks)	36.75 (±2.82)	38.45 (±1.59)	38.57 (±2.14)	(1, 2)
Neonatal weight (grams)	2131.67 (±519.4)	2648.23 (±282.8)	3178.17 (±510.7)	(1, 2, 3)
aIMT (mm)	1.10 (±0.20)	0.80 (±0.20)	0.60 (±0.20)	(1, 2, 3)

Significant differences ($P < 0.05$) between (1) IUGR and SGA; (2) IUGR and AGA; and (3) SGA and AGA.
NS: nonsignificant differences.
BMI: body mass index.
aIMT: aorta intima media thickness.

Table 2: Differences in examinations of blood values among the evaluated groups in maternal and fetal circulation. Data are presented as median and interquartile range (IQR). The P value refers to Wilcoxon test.

	IUGR (37)	SGA (33)	Controls (70)	P
Maternal blood				
Adiponectin (μg/L)	45 (32–61)	58 (37–64)	57 (45–61)	(2)
Leptin (μg/L)	8.2 (5–11.9)	8.5 (5.2–10.7)	5.5 (3.1–7.6)	(2, 3)
Adiponectin/leptin	5.5 (2.7–8.7)	5.9 (4.6–12.5)	11.4 (7.1–18.1)	(2, 3)
IL-6 (ng/L)	4.9 (2.3–8.4)	3.9 (2.5–5.4)	4.6 (2.4–6.2)	NS
TNFα (ng/L)	5.4 (4.3–10)	5.0 (4–5.6)	4.9 (4.0–6.1)	(1, 2)
CRP (mg/L)	5.1 (3.9–12.8)	4.1 (2.8–5.7)	4.9 (2.9–5.9)	(1, 2)
Fetal blood				
Adiponectin (μg/L)	134 (105–159)	197 (122–239)	196 (134–239)	(1, 2)
Leptin (μg/L)	7.1 (5.5–8.3)	4.3 (2.7–7.8)	4.9 (2.9–6.5)	(1, 2)
Adiponectin/leptin	17.4 (14.3–24.2)	43.5 (24.4–75)	41.3 (31.1–79.7)	(1, 2)
IL-6 (ng/L)	8.6 (4.6–12.1)	5.3 (2.4–8.9)	4.7 (2.3–6)	(1, 2, 3)
TNFα (ng/L)	11.1 (8.9–14.9)	8.3 (7.5–9.1)	8.7 (7.3–9.9)	(1, 2)
CRP (mg/L)	2.8 (2.7–2.9)	2.8 (2.7–2.9)	2.8 (2.8-2.9)	NS

Significant differences ($P < 0.05$) between (1) IUGR and SGA; (2) IUGR and AGA; and (3) SGA and AGA.
NS: nonsignificant differences.

($P < 0.05$) (Figure 1(a)). In control group maternal adiponectin negatively correlated with fetal aIMT ($P < 0.05$) (Figure 1(b)). Moreover, only IUGR and SGA maternal adiponectin negatively correlated with maternal TNFα ($P < 0.05$) (Figure 1(c)). In the three groups, fetal adiponectin positively correlated with maternal adiponectin ($P < 0.05$) (Figure 1(d)).

Furthermore, fetal adiponectin positively correlated with gestational age at delivery ($P < 0.05$) (Figure 2(a)). In IUGR fetuses, adiponectin serum concentrations were negatively correlated with fetal IL-6 ($P < 0.05$) (Figure 2(b)) and fetal adiponectin/leptin ratio (A/L) presented a negative correlation with aIMT ($P < 0.05$) (Figure 2(c)). In all groups, there was a negative correlation between aIMT and birth weight ($P < 0.05$) (Figure 2(d)).

IUGR and SGA maternal serum leptin concentrations were positively correlated with gestational age at delivery ($P < 0.05$) and maternal CRP levels ($P < 0.05$) (Figures 3(a) and 3(b)).

IUGR fetal leptin levels positively correlated with fetal aIMT ($P < 0.05$) (Figure 3(c)). Only in control fetal group there was a positive correlation between adiponectin and leptin levels ($P < 0.05$) (Figure 3(d)).

4. Discussion

This study showed that fetuses with IUGR and Doppler abnormalities presented thicker aIM, higher concentrations of leptin, TNFα, IL-6, and CRP, and lower adiponectin levels

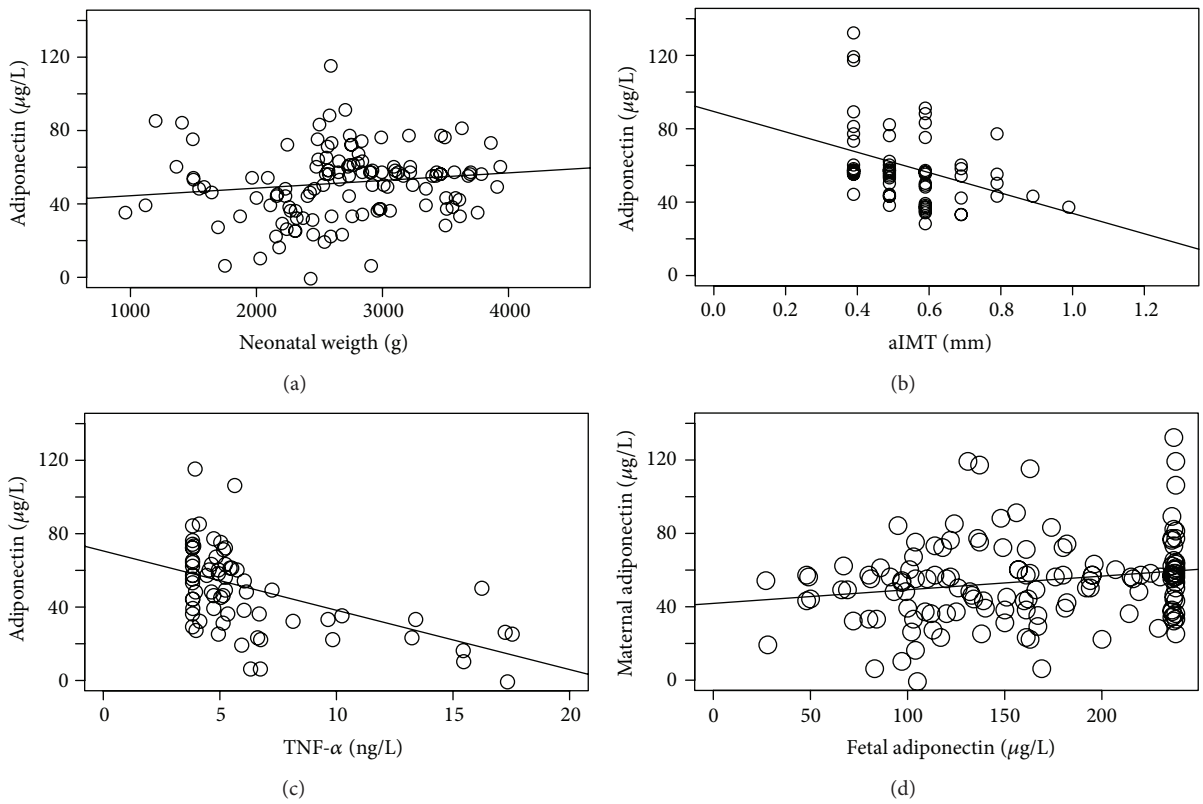

Figure 1: Correlations of maternal blood adiponectin level with other hormone levels, anthropometric, or ultrasound parameters. (a) Correlation between neonatal weight and maternal blood adiponectin levels in all the population (tau test $P < 0.05$). (b) Correlation between fetal aIMT and maternal blood adiponectin levels in AGA population ($P < 0.05$). (c) Correlation between maternal blood TNFα and adiponectin levels in IUGR and SGA the population ($P < 0.05$). (d) Correlation between neonatal and maternal blood adiponectin levels in all the population ($P < 0.05$).

than SGA or AGA. To the best of our knowledge, this is the first report demonstrating a correlation between A/L ratio and aIMT in IUGR fetuses, supposing a link between immune system and endothelial damage. This association was not found for SGA fetuses. The subdivision of IUGR disorder considering Doppler velocimetry allowed stratification into different classes of vascular risk. In SGA fetuses aIMT was lower than IUGR but higher than AGA, while adipokines and inflammatory cytokines presented only minimal differences in comparison to control group. These results confirmed previous studies in which aIMT was inversely related to EFW, showing that low birth weight and Doppler abnormalities may be correlated with an altered vascular structure causing possible endothelial damage, both in single and twin pregnancies [8, 20]. Moreover, in children who had IUGR, aIMT was greater in those with the lowest birth weight, suggesting that atherogenesis and an increased arterial stiffness may be a potential mechanism mediating the mentioned epidemiological link between impaired fetal growth and cardiovascular disease in adulthood, similar to major environmental risk factors such as cigarette smoking and hypertension [7, 21]. Postmortem studies in young adults showed an inverse correlation between birth weight and severity of aortic lesions [22]. Histochemical analysis also confirmed that the fetal aIMT observed during pregnancy by ultrasound corresponded to intima thickening. The CD68, a widely used marker for macrophages, is usually absent in normal vessels; E-selectin, a marker of activated endothelial cell (EC) and CD31, a marker for quiescent EC, were found present in the aortic wall of IUGR stillbirth. These might represent peculiar elements of preatherosclerotic lesions [23]. Experimental evidence have demonstrated that cardiovascular remodeling, triggered in response to the stress conditions in utero, persists as a permanent feature in postnatal life, including vascular dysfunction, increased blood pressure, and aorta intima media thickness [24].

In many instances, metabolic disorders as well as other disorders associated with IUGR have an endocrine origin and are accomplished by the changes in hormone bioavailability in adulthood [25]. Several independent observations have shown a relationship between low birth weight and insulin resistance; reduced insulin sensitivity might be secondary to altered programming of metabolic pathways in presence of adverse intrauterine environment [26]. IUGR fetuses showed a marked reduction in body fat mass, which mainly reflects a decreased accumulation of lipids in the adipocytes. However, although total body fat percentage is reduced, visceral adipose tissue is relatively increased and it results hyperresponsiveness to catecholamine and early insulin resistance [5]. Adipokines, bioactive molecules produced by adipose tissue,

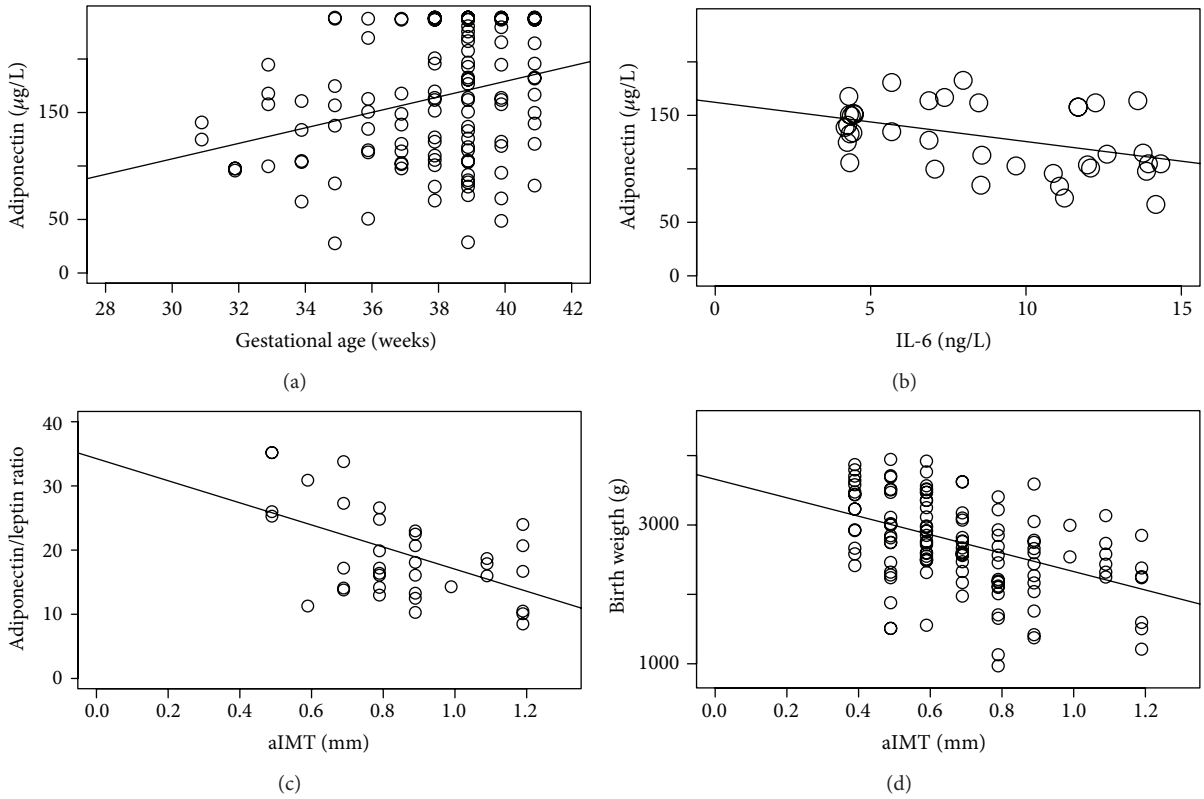

Figure 2: Correlations of fetal blood adiponectin, other hormone levels, anthropometric, or ultrasound parameters. (a) Correlation between gestational age at delivery and fetal blood adiponectin levels in all the population (tau test $P < 0.05$). (b) Correlation between fetal blood IL-6 levels and fetal blood adiponectin levels in IUGR population ($P < 0.05$). (c) Correlation between fetal aIMT and fetal adiponectin/leptin ratio in IUGR population ($P < 0.05$). (d) Correlation between fetal aIMT and neonatal weight in all the population ($P < 0.05$).

should regulate body metabolism and are implicated in fetal growth. Adiponectin influences carbohydrate metabolism, improving insulin sensitivity, and low adiponectin levels have been suggested to play a causal role in the development of insulin resistance and cardiovascular disease in adulthood [27]. In agreement with literature the present study shows that during third trimester umbilical cord blood adiponectin concentration is approximately three times higher than in maternal blood, in all groups analyzed [28]. In accordance with Lindsay, our study found that maternal adiponectin is positive with birth weight [29]. Pregnancy is a unique situation in which there is a physiological, temporary insulin resistance, gradually settled down in the third trimester, with an increase of fetal blood glucose and free fatty acid concentrations and a reduction in maternal insulinemia [30]. This could explain the reduction of maternal adiponectin at the end of pregnancy. In IUGR and SGA pregnancies, maternal adiponectin concentration negatively correlates with maternal TNFα, suggesting a prevalent inflammatory condition in a mother whose pregnancy is complicated by fetal growth restriction.

Adiponectin represents antiatherogenic and anti-inflammatory properties suppressing the macrophages proinflammatory cytokines production, such as TNFα and IL-6 [31], and inhibiting macrophage to foam cell transformation [32]. A reduction in IUGR fetuses of adiponectin level and its negative correlation with fetal IL-6 might represent the immune system's modification, which could explain the endothelial damage expressed by a thickening of aIM. Moreover, the A/L ratio, actually the most indicative sign of metabolic risk, is even negatively correlated in IUGR fetuses with aIMT [33]. Animal models reveal that the majority of macrophages in established atherosclerotic lesions are derived from local proliferation rather than from the influx of blood-borne monocytes [34]. Recent studies demonstrate the role of perivascular adipose tissue dysfunction in cardiovascular inflammation and oxidative stress [35]. Significant infiltration of macrophages and T cells in perivascular adipose tissue was accompanied by endothelial dysfunction. Decreased secretion of adiponectin and increased production of cytokines from dysfunctional adipose tissue may significantly contribute to vascular inflammation, insulin resistance, vascular stiffness, and impaired relaxation [36].

In many studies investigating the effect of low birth weight on metabolic diseases in later life, IUGR has been used equivalently to the term SGA. In contrast to SGA, IUGR implies an underlying pathological process that prevents the fetus from achieving its growth potential and can be assessed by prenatal ultrasound and Doppler examinations. In our study we found a decrease in adiponectin levels only in

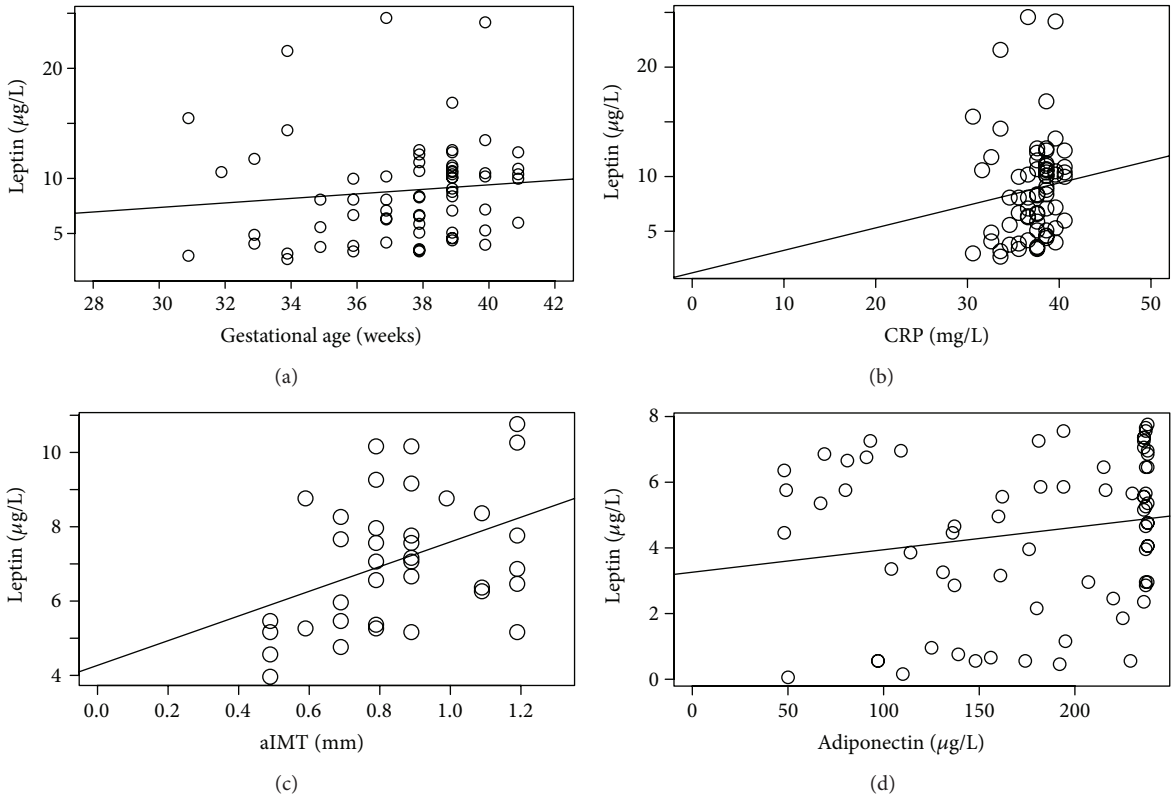

Figure 3: Correlations of blood leptin, other hormone levels, anthropometric, or ultrasound parameters. (a) Correlation between gestational age at delivery and maternal blood leptin levels in IUGR and SGA population (tau test $P < 0.05$). (b) Correlation between maternal blood CRP and leptin levels in IUGR and SGA population ($P < 0.05$). (c) Correlation between fetal aIMT and fetal blood leptin levels in IUGR population ($P < 0.05$). (d) Correlation between fetal blood adiponectin and leptin levels in AGA population ($P < 0.05$).

IUGR fetuses, and although not significant SGA presented a higher value than controls. A possible explanation for these contradictory results may be related to the different definition of IUGR, often without considering Doppler velocimetry. and in methodological aspects. Several authors described lower levels of adiponectin in SGA fetuses and children, proposing that this downregulation might be a predisposing factor for later development of insulin resistance and metabolic syndrome. Interestingly very low adiponectin levels in IUGR children should predict the subsequent development of visceral fat and insulin resistance in adulthood [37–39]. When Briana and Lindsay found similar levels of adiponectin in cord blood of SGA and control fetuses [29, 31], conversely, López-Bermejo et al. studying prepuberal children found increased adiponectin concentrations related to increased insulin sensitivity [33, 40]. Kyriakakou et al. used Doppler velocimetry in IUGR definition, finding that leptin and adiponectin serum levels were higher and lower, respectively, in IUGR fetuses, in accordance with our results [17].

Leptin seems to be a critical factor for overall fetal development. In this respect, numerous animal studies indicated that prenatal exposure to maternal under nutrition leads to the development of diet-induced obesity, hyperleptinemia, hyperinsulinism, and hypertension in the rat offspring [41]. Thus, leptin may play a role in the control of substrate utilization and in the maintenance and functional characteristics of fat mass before birth, producing permanent changes concerning adiposity and body composition in adult life [42]. In accordance with other studies, IUGR presented a positive correlation between maternal leptin and gestational age at delivery, indicating in these patients a possible preexisting metabolic alteration [40]. Moreover, in IUGR fetuses there was a positive correlation between leptin and IL-6 levels, underlying a similar proinflammatory role. The inversely correlation between fetal A/L ratio and aIMT might represents a link between endocrine function of adipose tissue and endothelial damage. In literature, there is no accordance among investigators about cord leptin concentration in this category of fetuses. Several studies demonstrated lower circulating leptin concentrations in IUGR fetuses, due to reduced fat mass and/or decreased placental production, increasing and becoming higher in IUGR infants, children, and adults [42–45], while other investigators determined similar and higher leptin concentrations [31, 46].

IUGR ovine models showed that leptin levels are inversely related to uterine blood flow and fetal/placental weight, suggesting that fetal leptin may be involved in an adaptive response [47]. Tzschoppe et al., differentiating the two groups by EFW and pathological uterine and umbilical artery Doppler velocimetry, found that leptin mRNA

and protein expression are increased in the placentas of IUGR newborns compared to AGA. Hypoxic and inflammatory processes inducing placental dysfunction might explain increased placental leptin mRNA expression. Leptin gene in fact is highly sensitive to oxygen abundance and IUGR fetuses, exhibiting severe distress and having significantly higher leptin concentrations per kilogram of weight [46, 48, 49].

TNFα and IL-6 are produced by adipose tissue monocytes and macrophages and also by the placenta. Few and contradictory data exist in the literature regarding the IUGR state [50]. Some investigators documented a reduced fetal IL-6 and TNFα levels in growth restricted fetuses [51, 52], possibly due to impaired placental insufficiency. On the other hand, an upregulation of IL-6 and TNFα in IUGR fetuses could be secondary to hypoxia and to survival mechanism, by inducing muscle insulin resistance and enabling glucose to be spared for brain metabolism [10, 53]. In this study, we hypothesized that higher levels in IUGR fetuses could be secondary to the reduction of adiponectin concentrations, which do not inhibit macrophage-cytokines release; this condition should worsen the endothelial damage of intrauterine growth restriction. In IUGR mothers this finding might reflect the state of inflammation and chronic stress, expressed also by high levels of CRP, not found among IUGR, SGA, and AGA fetuses. High sensitivity CRP was not measured, and this might explain our result.

In conclusion, a specific profile of increased leptin, IL-6, CRP, and TNFα in IUGR mothers might indicate a proinflammatory condition for the development of poor intrauterine environment. The increased umbilical leptin, TNFα, and IL-6 concentrations and the decreased adiponectin levels in IUGR fetuses might represent the inflammatory substrate that contributes to the vessel remodelling, represented by thickening of the aorta. These conditions could predispose to vascular and metabolic disorders in adult life. Differential regulation of adipocytokines and higher aIMT in utero in the IUGR state may be predictive of adult disease. Further understanding of the changes in adipocyte maturation during prenatal nutrition and their influence on molecular pathways could help explain the complex association between IUGR and adult disease risk and support the development of effective preventive strategies.

Conflict of Interests

The authors declare that they have no potential conflict of interests relevant to this paper.

References

[1] S. Sankaran and P. M. Kyle, "Aetiology and pathogenesis of IUGR," *Best Practice and Research: Clinical Obstetrics and Gynaecology*, vol. 23, no. 6, pp. 765–777, 2009.

[2] D. J. P. Barker, C. Osmond, J. Golding, D. Kuh, and M. E. J. Wadsworth, "Growth in utero, blood pressure in childhood and adult life, and mortality from cardiovascular disease," *British Medical Journal*, vol. 298, no. 6673, pp. 564–567, 1989.

[3] G. Reaven, "Why a cluster is truly a cluster: insulin resistance and cardiovascular disease," *Clinical Chemistry*, vol. 54, no. 5, pp. 785–787, 2008.

[4] R. Deepa, K. Velmurugan, K. Arvind et al., "Serum levels of interleukin 6, C-reactive protein, vascular cell adhesion molecule 1, and monocyte chemotactic protein 1 in relation to insulin resistance and glucose intolerance—the Chennai Urban Rural Epidemiology Study (CURES)," *Metabolism: Clinical and Experimental*, vol. 55, no. 9, pp. 1232–1238, 2006.

[5] D. Jaquet, S. Deghmoun, D. Chevenne, D. Collin, P. Czernichow, and C. Lévy-Marchal, "Dynamic change in adiposity from fetal to postnatal life is involved in the metabolic syndrome associated with reduced fetal growth," *Diabetologia*, vol. 48, no. 5, pp. 849–855, 2005.

[6] E. Koklu, S. Kurtoglu, M. Akcakus et al., "Increased aortic intima-media thickness is related to lipid profile in newborns with intrauterine growth restriction," *Hormone Research*, vol. 65, no. 6, pp. 269–275, 2006.

[7] M. R. Skilton, N. Evans, K. A. Griffiths, J. A. Harmer, and D. S. Celermajer, "Aortic wall thickness in newborns with intrauterine growth restriction," *The Lancet*, vol. 365, no. 9469, pp. 1484–1486, 2005.

[8] E. Cosmi, S. Visentin, T. Fanelli, A. J. Mautone, and V. Zanardo, "Aortic intima media thickness in fetuses and children with intrauterine growth restriction," *Obstetrics and Gynecology*, vol. 114, no. 5, pp. 1109–1114, 2009.

[9] N. Cinar and A. Gurlek, "Association between novel adipocytokines adiponectin, vaspin, visfatin, and thyroid: an experimental and clinical update," *Endocrine Connections*, vol. 2, pp. R30–R38, 2013.

[10] M. E. Street, P. Seghini, S. Feini et al., "Changes in interleukin-6 and IGF system and their relationships in placenta and cord blood in newborns with fetal growth restriction compared with controls," *European Journal of Endocrinology*, vol. 155, no. 4, pp. 567–574, 2006.

[11] D. R. Cottam, S. G. Mattar, E. Barinas-Mitchell et al., "The chronic inflammatory hypothesis for the morbidity associated with morbid obesity: implications and effect of weight loss," *Obesity Surgery*, vol. 14, no. 5, pp. 589–600, 2004.

[12] S. P. Weisberg, D. McCann, M. Desai, M. Rosenbaum, R. L. Leibel, and A. W. Ferrante Jr., "Obesity is associated with macrophage accumulation in adipose tissue," *Journal of Clinical Investigation*, vol. 112, no. 12, pp. 1796–1808, 2003.

[13] R. F. Mortensen, "C-reactive protein, inflammation, and innate immunity," *Immunologic Research*, vol. 24, no. 2, pp. 163–176, 2001.

[14] R. S. Ahlma, D. Prabakaran, C. Mantzoros et al., "Role of leptin in the neuroendocrine response to fasting," *Nature*, vol. 382, no. 6588, pp. 250–252, 1996.

[15] I. S. Farooqi, G. Matarese, G. M. Lord et al., "Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency," *Journal of Clinical Investigation*, vol. 110, no. 8, pp. 1093–1103, 2002.

[16] A. Shehzad, W. Iqbal, O. Shehzad, and Y. S. Lee, "Adiponectin: regulation of its production and its role in human diseases," *Hormones*, vol. 11, no. 1, pp. 8–20, 2012.

[17] M. Kyriakakou, A. Malamitsi-Puchner, H. Militsi et al., "Leptin and adiponectin concentrations in intrauterine growth restricted and appropriate for gestational age fetuses, neonates, and their mothers," *European Journal of Endocrinology*, vol. 158, no. 3, pp. 343–348, 2008.

[18] D. Paladini, M. Rustico, E. Viora et al., "Fetal size charts for the Italian population. Normative curves of head, abdomen and long bones," *Prenatal Diagnosis*, vol. 25, no. 6, pp. 456–464, 2005.

[19] E. Koklu, S. Kurtoglu, M. Akcakus, A. Yikilmaz, A. Coskun, and T. Gunes, "Intima-media thickness of the abdominal aorta of neonate with different gestational ages," *Journal of Clinical Ultrasound*, vol. 35, no. 9, pp. 491–497, 2007.

[20] S. Visentin, E. Grisan, V. Zanardo et al., "Developmental programming of cardiovascular risk in intrauterine growth-restricted twin fetuses according to aortic intima thickness," *Journal of Ultrasound in Medicine*, vol. 32, no. 2, pp. 279–284, 2013, Erratum in "Developmental programming of cardiovascular risk in intrauterine growth-restricted twin fetuses according to aortic intima thickness", *Journal of Ultrasound in Medicine*, vol. 32, no. 3, p. 550, 2013.

[21] E. Koklu, M. A. Ozturk, T. Gunes, M. Akcakus, and S. Kurtoglu, "Is increased intima-media thickness associated with preatherosclerotic changes in intrauterine growth restricted newborns?" *Acta Paediatrica*, vol. 96, no. 12, article 1858, 2007.

[22] H. C. McGill Jr., C. A. McMahan, E. E. Herderick, G. T. Malcom, R. E. Tracy, and J. P. Strong, "Origin of atherosclerosis in childhood and adolescence," *American Journal of Clinical Nutrition*, vol. 72, supplement 5, pp. 1307S–1315S, 2000.

[23] V. R. Lo Vasco, R. Salmaso, V. Zanardo et al., "Fetal aorta wall inflammation in ultrasound-detected aortic intima/media thickness and growth retardation," *Journal of Reproductive Immunology*, vol. 91, no. 1-2, pp. 103–107, 2011.

[24] A. Tintu, E. Rouwet, S. Verlohren et al., "Hypoxia induces dilated cardiomyopathy in the chick embryo: mchanism, intervention, and long-term consequences," *PLoS ONE*, vol. 4, no. 4, Article ID e5155, 2009.

[25] A. L. Fowden, D. A. Giussani, and A. J. Forhead, "Endocrine and metabolic programming during intrauterine development," *Early Human Development*, vol. 81, no. 9, pp. 723–734, 2005.

[26] C. N. Hales and D. J. P. Barker, "Type 2 (no insulin dependent) diabetes mellitus: the thrifty phenotype hypothesis," *Diabetologia*, vol. 35, pp. 595–601, 1992.

[27] J. Kewano and R. Arora, "The role of adiponectin in obesity, diabetes, and cardiovascular disease," *Journal of the CardioMetabolic Syndrome*, vol. 4, no. 1, pp. 44–49, 2009.

[28] F. O. Abessolo, J. P. Ngou-Mve-Ngou, J. Mouba, A. M. Bengone, and E. Ngou-Milama, "Adiponectin in mother-and-child couple: Is there a relation with materno-foetal transfer of nutrients, obesityand risk of type 2 diabetes?" *Biochimica Clinica*, vol. 36, no. 4, pp. 235–239, 2012.

[29] R. S. Lindsay, J. D. Walker, P. J. Havel, B. A. Hamilton, A. A. Calder, and F. D. Johnstone, "Adinopnectin is present in cord blood but is unrelated to birth weight," *Diabetes Care*, vol. 26, no. 8, pp. 2244–2249, 2003.

[30] T. A. Bucharan and A. H. Xiang, "Gestational diabetes mellitus," *The Journal of Clinical Investigation*, vol. 115, no. 3, pp. 485–491, 2005.

[31] D. D. Briana and A. Malamitsi-Puchner, "Intrauterine growth restriction and adult disease: the role of adipocytokines," *European Journal of Endocrinology*, vol. 160, no. 3, pp. 337–347, 2009.

[32] C. Tsatsanis, V. Zacharioudaki, A. Androulidaki et al., "Adiponectin induces TNF-α and IL-6 in macrophages and promotes tolerance to itself and other pro-inflammatory stimuli," *Biochemical and Biophysical Research Communications*, vol. 335, no. 4, pp. 1254–1263, 2005.

[33] A. Skvarca, M. Tomazic, R. Blagus, B. Krhin, and A. Janez, "Adiponectin/leptin ratio and insulin resistance in pregnancy," *Journal of International Medical Research*, vol. 41, no. 1, pp. 123–128, 2013.

[34] C. S. Robbins, I. Hilgendorf, G. F. Weber et al., "Local proliferation dominates lesional macrophage accumulation in atherosclerosis," *Nature Medicine*, vol. 19, pp. 1166–1172, 2013.

[35] T. Szasz, G. F. Bomfim, and R. C. Webb, "The influence of perivascular adipose tissue on vascular homeostasis," *Vascular Health and Risk Management*, vol. 9, pp. 105–116, 2013.

[36] C. Boydens, N. Maenhaut, B. Pauwels, K. Decaluwé, and J. van de Voorde, "Adipose tissue as regulator of vascular tone," *Current Hypertension Reports*, vol. 14, no. 3, pp. 270–278, 2012.

[37] S. Cianfarani, C. Martinez, A. Maiorana, G. Scirè, G. L. Spadoni, and S. Boemi, "Adiponectin levels are reduced in children born small for gestational age and are inversely related to postnatal catch-up growth," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 3, pp. 1346–1351, 2004.

[38] M. E. Street, C. Volta, M. A. Ziveri, I. Viani, and S. Bernasconi, "Markers of insulin sensitivity in placentas and cord serum of intrauterine growth-restricted newborns," *Clinical Endocrinology*, vol. 71, no. 3, pp. 394–399, 2009.

[39] T. Kamoda, H. Saitoh, M. Saito, M. Sugiura, and A. Matsui, "Serum adiponectin concentrations in newborn infants in early postnatal life," *Pediatric Research*, vol. 56, no. 5, pp. 690–693, 2004.

[40] A. López-Bermejo, P. Casano-Sancho, J. M. Fernández-Real et al., "Both intrauterine growth restriction and postnatal growth influence childhood serum concentrations of adiponectin," *Clinical Endocrinology*, vol. 61, no. 3, pp. 339–346, 2004.

[41] S. O. Krechowec, M. Vickers, A. Gertler, and B. H. Breier, "Prenatal influences on leptin sensitivity and susceptibility to diet-induced obesity," *Journal of Endocrinology*, vol. 189, no. 2, pp. 355–363, 2006.

[42] I. C. McMillen, B. S. Muhlhausler, J. A. Duffield, and B. S. J. Yuen, "Prenatal programming of postnatal obesity: fetal nutrition and the regulation of leptin synthesis and secretion before birth," *Proceedings of the Nutrition Society*, vol. 63, no. 3, pp. 405–412, 2004.

[43] M. Pighetti, G. A. Tommaselli, A. D'Elia et al., "Maternal serum and umbilical cord blood leptin concentrations with fetal growth restriction," *Obstetrics and Gynecology*, vol. 102, no. 3, pp. 535–543, 2003.

[44] L. Yildiz, B. Avci, and M. Ingeç, "Umbilical cord and maternal blood leptin concentrations in intrauterine growth retardation," *Clinical Chemistry and Laboratory Medicine*, vol. 40, no. 11, pp. 1114–1117, 2002.

[45] A. Varvarigou, C. S. Mantzoros, and N. G. Beratis, "Cord blood leptin concentrations in relation to intrauterine growth," *Clinical Endocrinology*, vol. 50, no. 2, pp. 177–183, 1999.

[46] I. Cetin, P. S. Morpurgo, T. Radaelli et al., "Fetal plasma leptin concentrations: relationship with different intrauterine growth patterns from 19 weeks to term," *Pediatric Research*, vol. 48, no. 5, pp. 646–651, 2000.

[47] P. S. Shekhawat, J. S. Garland, C. Shivpuri et al., "Neonatal cord blood leptin: its relationship to birth weight, body mass index, maternal diabetes, and steroids," *Pediatric Research*, vol. 43, no. 3, pp. 338–343, 1998.

[48] A. Buchbinder, U. Lang, R. S. Baker, J. C. Khoury, J. Mershon, and K. E. Clark, "Leptin in the ovine fetus correlates with fetal and placental size," *American Journal of Obstetrics and Gynecology*, vol. 185, no. 4, pp. 786–791, 2001.

[49] A. Tzschoppe, E. Struwe, W. Rascher et al., "Intrauterine growth restriction (IUGR) is associated with increased leptin synthesis and binding capability in neonates," *Clinical Endocrinology*, vol. 74, no. 4, pp. 459–466, 2011.

[50] J. C. Pickup, G. D. Chusney, S. M. Thomas, and D. Burt, "Plasma interleukin-6, tumour necrosis factor α and blood cytokine production in type 2 diabetes," *Life Sciences*, vol. 67, no. 3, pp. 291–300, 2000.

[51] S.-L. Opsjon, R. Austgulen, and A. Waage, "Interleukin-1, interleukin-6 and tumor necrosis factor at delivery in preeclamptic disorders," *Acta Obstetricia et Gynecologica Scandinavica*, vol. 74, no. 1, pp. 19–26, 1995.

[52] R. A. Ødegård, L. J. Vatten, S. T. Nilsen, K. Å. Salvesen, H. Vefring, and R. Austgulen, "Umbilical cord plasma interleukin-6 and fetal growth restriction in preeclampsia: a prospective study in Norway," *Obstetrics and Gynecology*, vol. 98, no. 2, pp. 289–294, 2001.

[53] J.-M. Fernández-Real and W. Ricart, "Insulin resistance and inflammation in an evolutionary perspective: the contribution of cytokine genotype/phenotype to thriftiness," *Diabetologia*, vol. 42, no. 11, pp. 1367–1374, 1999.

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Research Article

Denture-Related Stomatitis Is Associated with Endothelial Dysfunction

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Oral inflammation, such as periodontitis, can lead to endothelial dysfunction, accelerated atherosclerosis, and vascular dysfunction. The relationship between vascular dysfunction and other common forms of oral infections such as denture-related stomatitis (DRS) is unknown. Similar risk factors predispose to both conditions including smoking, diabetes, age, and obesity. Accordingly, we aimed to investigate endothelial function and major vascular disease risk factors in 44 consecutive patients with dentures with clinical and microbiological features of DRS ($n = 20$) and without DRS ($n = 24$). While there was a tendency for higher occurrence of diabetes and smoking, groups did not differ significantly in respect to major vascular disease risk factors. Groups did not differ in main ambulatory blood pressure, total cholesterol, or even CRP. Importantly, flow mediated dilatation (FMD) was significantly lower in DRS than in non-DRS subjects, while nitroglycerin induced vasorelaxation (NMD) or intima-media thickness (IMT) was similar. Interestingly, while triglyceride levels were normal in both groups, they were higher in DRS subjects, although they did not correlate with either FMD or NMD. *Conclusions*. Denture related stomatitis is associated with endothelial dysfunction in elderly patients with dentures. This is in part related to the fact that diabetes and smoking increase risk of both DRS and cardiovascular disease.

1. Introduction

Oral inflammation is an important element in the pathogenesis of vascular disease. In particular, large body of evidence has accumulated recently that chronic periodontitis is a potential novel risk factor for atherosclerosis and endothelial dysfunction [1–5]. Indeed, intensive treatment of chronic periodontitis alleviates endothelial dysfunction in a long-term follow-up, with clinical benefit lasting up to 6 months after intensive treatment [6]. The mechanisms of this association are not clearly defined but are most likely dependent on systemic inflammatory response, involving increased levels of IL-6, CRP, TNF-alpha, and other cytokines, which accompany periodontitis [6, 7]. Moreover, cellular immunity is also activated in periodontitis, including monocyte subpopulation shift as well as T cell activation with overproduction of interferon gamma and IL-17 [8].

While numerous studies have focused on the links between periodontitis and endothelial dysfunction, little is known about the links between other forms of oral infection and inflammation in the context of cardiovascular risk. In particular, denture-related stomatitis (DRS) is an inflammatory process of the oral mucosa in contact with a denture and is one of the most common diseases in elderly patients, affecting up to 70% of patients in the course of life [9, 10]. It is most common in complete prosthesis wearers,

edentulous subjects [9–11]. The potential links are particularly worth addressing, as major risk factors for DRS include smoking, diabetes, age, and obesity, which coincide with risk of atherosclerosis and vascular disease [9, 12, 13]. Thus it is even more surprising that this problem of concomitant incidence of both conditions has not been studied up to date. Interestingly, the relationship of DRS to dyslipidemia is not known and female sex appears to predispose to higher occurrence [9]. Clinical symptoms of DRS include erythema and swelling of palatal mucosa, sometimes combined with subjective symptoms, such as dysgeusia or burning sensation. The aetiology of the DRS is multifactorial [9]. Long-term and continuous use of dentures and poor denture and oral hygiene habits promote the development of a biofilm, called denture plaque, on the surface of the prosthesis [9, 12]. *Candida albicans* is fungal component of the physiological microflora of the human oral cavity [14, 15]; however, factors mentioned above may promote its excessive growth and, consequently, the development of infection and DRS.

While in periodontitis systemic activation of the immune system is very important in mediating increased cardiovascular risk, the extent of systemic response to DRS is poorly characterized. Systemic inflammation may affect vascular dysfunction in number of ways, which include activation of monocytes and T cells with overproduction of cytokines such as interferon γ, TNF-alpha, interleukin 6, or 17 [16], subsequently leading to atherosclerosis and hypertension [16–18] and increased cardiovascular risk. Interestingly, increased cardiovascular risk has been shown also for caries [19–21], as well as endodontic infection [22–26]. These diseases are all caused by bacterial infections, but, other microorganisms are also able to infect oral tissues. Relationship between fungal infection in oral cavity and systemic inflammatory response in context of vascular risk has not yet been studied. Therefore, the aim of this study was to determine whether the presence of DRS coincides with the clinical measures of vascular dysfunction, such as impaired endothelial function or elevated blood pressure.

2. Methods

2.1. Patients. Using 44 consecutive patients with dental prostheses for at least 6 months were included in this study. Their oral mucosa was examined by the dentist to clinically identify inflammation and DRS. The clinical signs of oral mucosa inflammation in DRS include erythema and swelling of palatal mucosa, sometimes combined with subjective symptoms, such as dysgeusia or burning sensation. These observations were confirmed by routine microbiological laboratory diagnostic tests for *Candida* species presence. Based on clinical and microbiological investigations, patients were divided into DRS ($n = 20$) group and non-DRS ($n = 24$) group. Diagnosis was confirmed by an independent observer. Control, non-DRS patients had clinically healthy oral mucosa and negative oral *Candida* swabs. Clinically healthy oral mucosa was a pale pink, smooth mucosal membrane without redness or swelling and with no pain or discomfort reported by patient. Exclusion criteria included acute inflammatory disorders other than DRS, neoplastic disease relapses or chemotherapy courses less than 5 years before the enrolment, and using antibiotics in less than 4 weeks or anti-inflammatory drugs (steroids and nonsteroidal, excluding aspirin in doses less than 80 mg) in less than 2 months before the enrolment. Patients with history of myocardial infarction, acute coronary incident or vascular inflammation in 5 weeks or less before the enrolment, chronic haematological disorders, and immunodeficiency or major medication changes during less than 5 weeks before or during study were also excluded. The study was approved by local ethics committee of Jagiellonian University. Informed consent was obtained from all patients and all work was conducted in accordance with the Declaration of Helsinki (1964).

2.2. Microbiological Investigations. Swabs were taken from the hard palate (between the second and third palatal fold). Samples were collected after an overnight fast and after at least 6 hours of continous denture usage, without the use of adhesives or rinsing the mouth with disinfectants. The material was collected in accordance with the general principles of microbial material collection.

2.3. Clinical Data. Patients' blood pressure (systolic, diastolic) was monitored for 24 hours using ambulatory blood pressure monitoring system (ABPM; SpaceLabs 90217, Ultralite device). Systolic diastolic and mean arterial pressures were recorded every 20 minutes for 24 hours. Day and night averages were calculated. One patient in control group did not agree to wear the ABPM monitor. Major risk factors for both atherosclerosis and DRS were recorded based on patient medical records and detailed patient history. Clinical risk factors were defined as follows: hyperlipidemia (total plasma cholesterol level > 5 mmol/L and/or triglycerides level > 1,7 mmol/L), diabetes (fasting glucose level $\geq$ 7 mmol/L or HbA1c > 6.5% or current treatment with insulin or oral hypoglycemic agents), hypertension (blood pressure $\geq$ 140/90 mmHg or current treatment with antihypertensive agents), and smoking (current or within last 6 months) based on [27]. Blood samples were obtained from antecubital vein and lipoprotein profile was assessed by routine diagnostic measurements of triglycerides, total cholesterol, low-(LDL), and high-(HDL) density lipoprotein cholesterol fractions. C-reactive protein (CRP) concentration was also assessed as in routine diagnostics.

2.4. Endothelial Function Measurement. Flow-mediated dilatation (FMD) method was used to determine the vascular endothelial function and NMD (nitroglycerine-mediated dilatation) for measuring endothelial-independent vasodilatation. Measurements were conducted using Toshiba Xario Diagnostic Ultrasound System after 1, 2, and 4-5 minutes after manometer cuff deflation or sublingual administration of nitroglycerine and presented as percentage of the diameter of the artery before intervention. Method validation in our laboratory has been described elsewhere [28]. Observers were blinded regarding oral status of the patients.

Table 1: Patient clinical characteristics.

	DRS group $n = 20$		Control group $n = 24$
Gender (M : F)	2 : 18	$P > 0{,}05$	6 : 18
Age [mean (SD)]	63,9 (6,6)	$P > 0{,}05$	65,9 (10,3)
BMI [median ($Q1$; $Q2$)]	28,5 (24,9; 33,6)	$P > 0{,}05$	27,8 (24,3; 29,3)
Smoking (%)	6 (30%)	$P > 0{,}05$	3 (12,5%)
Diabetes mellitus (%)	6 (30%)	$P > 0{,}05$	2 (8,3%)
Hypertension (%)	17 (85%)	$P > 0{,}05$	19 (79,2%)
Hyperlipidemia (%)	13 (65%)	$P > 0{,}05$	12 (50%)
Medications (%)			
ACE inhibitor	7 (35%)	$P > 0{,}05$	12 (50%)
Acetylsalicylic acid	3 (15%)	$P > 0{,}05$	4 (17%)
β-blocker	7 (35%)	$P > 0{,}05$	10 (42%)
Ca antagonist	3 (15%)	$P > 0{,}05$	8 (34%)
Diuretic	7 (35%)	$P > 0{,}05$	6 (25%)
Statin	6 (30%)	$P > 0{,}05$	5 (21%)
Insulin	2 (10%)	$P > 0{,}05$	2 (8,3%)
Oral antidiabetic agents	2 (10%)	$P > 0{,}05$	2 (8,3%)

ACE: angiotensin converting enzyme, BMI: body mass index, DM: diabetes mellitus, SD: standard deviation.

2.5. Subclinical Atherosclerosis Assessment. The measurements of intima-media thickness were performed in 12 different points (2 cm below common carotid arteries bulbs, ca. every 1 cm, omitting visible coronary plaques), on right and left common carotid artery, measuring the distance between the border between artery lumen and carotid artery intima and second bright line-m (border between media and adventitia) as described previously [28].

2.6. Statistical Analysis. Analysis was performed using Statsoft Statistica software. Compliance of the distribution of variables with normal distribution was tested by Shapiro-Wilk test. Most of the variables did not have normal distributions, and therefore the results are presented as medians and 25th ($Q1$), 75th ($Q3$) percentiles. For those variables nonparametric statistical tests were used, Mann-Whitney U test for continuous variables or, for dichotomous variables, χ^2 for the expected frequencies > 5 or χ^2 with Yates' correction for the expected frequencies < 5 with confirmation of Fisher's exact test and Spearman correlation. For variables with normal distribution Student's t-test was applied and data are presented as mean with standard deviation (SD). Method of presentation of results is given for each variable in the text. Values of $P < 0,05$ were considered statistically significant.

3. Results

3.1. Clinical Risk Factors in Studied Groups. Both groups were balanced in terms of age, sex, body mass index (BMI) value, and antihypertensive treatment. There were more smokers and patients with diabetes mellitus (DM) in DRS group than in control group, although these differences were not statistically significant. Higher prevalence of DM and smoking in DRS group is consistent with epidemiologic data and is understandable, as both are recognized as a risk factor for developing DRS [13]. The proportion of males in both study groups was lower than expected for general population, which is consistent with the epidemiology of DRS, which is more common in females [9]. Patients characteristics are summarized in Table 1.

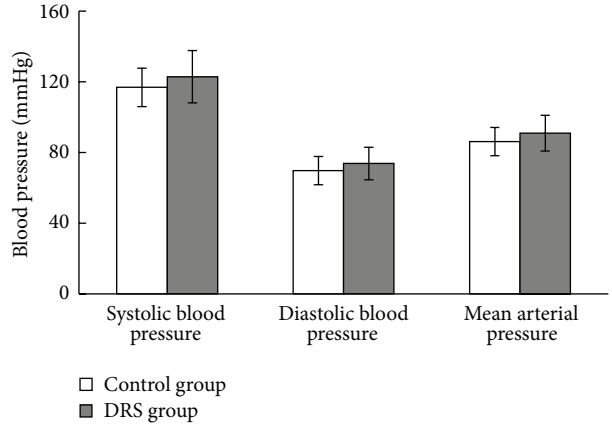

Figure 1: Ambulatory blood pressure parameters in control and DRS patients. Blood pressure parameters were assessed by 24 h measurement with ambulatory blood pressure monitoring system. Results are presented as mean (SD); n control group = 23, n DRS group = 20.

3.2. Blood Pressure in Denture Related Stomatitis. Ambulatory blood pressure monitoring has shown no significant differences in both mean systolic and mean diastolic blood pressure in DRS and control non-DRS group (Figure 1).

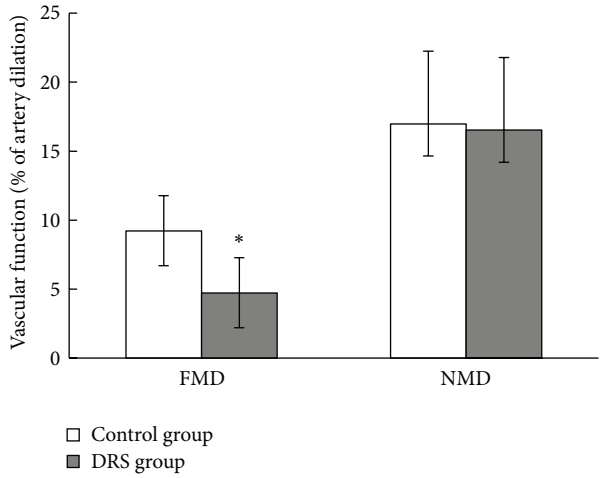

Figure 2: Vascular dysfunction in control and DRS. Vascular endothelium-dependent flow-mediated dilatation (FMD) and endothelium-independent nitroglycerin-mediated dilatation (NMD) parameters were assessed by ultrasonography. Results presented as median ($Q1$; $Q2$); $^*P < 0,005$; n control group = 24, n DRS group = 20.

Moreover, subsequent analysis of blood pressures during activity and rest periods did not show significant differences either (data not shown).

3.3. Vascular Function. Flow-mediated dilatation measurements showed a significantly reduced median percentage of arterial dilation in response to flow in the DRS group in comparison with control patients (Figure 2). At the same time there was no difference between groups in endothelium-independent vasodilatation, NMD (Figure 2). There was no difference in baseline vessel diameter between control and DRS group ($3,7 \pm 0,8$ mm versus $3,8 \pm 0,7$ mm; $P = 0,4$).

3.4. Subclinical and Clinical Atherosclerosis. Intima-media thickness evaluation showed no significant differences in either maximal or mean IMT in studied groups. It is important to point out that neither of the groups showed very high values of mean IMT (Figure 3). Moreover, presence of the atherosclerotic lesions of common carotid artery was equally distributed between groups; it was detected in 37.5% of patients from control group and in 35% of DRS group, $P = 0,86$.

3.5. Plasma Lipid Profile and CRP. As the elevated blood triglycerides, LDL and total cholesterol levels and low HDL cholesterol levels are recognized as cardiovascular risk factors; we compared their concentrations in blood samples collected from patients with oral fungal infection and with healthy oral mucosa. We found that plasma levels of total, LDL, and HDL cholesterol were similar in both groups; however, triglycerides levels were significantly elevated in DRS group (Figure 4(a)). Surprisingly, CRP levels were similar between studied groups, indicating lack of significant component of systemic inflammation in DRS (Figure 4(b)).

As the level of triglycerides was different between groups and this parameter may impact vascular function, we checked if there is a correlation between FMD or NMD and triglycerides levels. We found that these parameters were not correlated in case of FMD (R Spearman = −0,13, $P = 0,42$) and NMD (R Spearman = −0,025, $P = 0,87$) (Figure 5).

3.6. Subgroup Analysis in Female Subpopulation Only. As there was a much lower proportion of males in our study population, we performed an additional subgroup analysis in female population. It revealed that all studied vascular phenomena were observed to the same extent as in total studied population, including the difference in endothelium derived vasorelaxations (FMD (mean ± SD): $5,95 \pm 3,80\%$ in female DRS patients and $9,72 \pm 3,31\%$ in control subjects; $P = 0,0032$) and TG levels (median [Q1; Q2]: 1,61 [1,46; 1,99] in DRS versus 1,09 [0,86; 1,34] in non-DRS; $P = 0,01$).

4. Discussion

The oral health impact on the general health is evident. Oral infections and inflammation have been implicated in many disease entities, such as rheumatoid arthritis [29], obesity [30], negative pregnancy outcomes [31], DM [32], and even in epilepsy [33]. In particular, the role of oral inflammation and infection in the modulation of the risk of cardiovascular disorders has been well defined [1–5]. These studies have, however, focused mainly on periodontal inflammation and gingival bleeding. In the present study we investigated the relationships between denture-related stomatitis, a common oral inflammatory condition in elderly patients with endothelial dysfunction, blood pressure, and lipid profile. We observed that denture-related stomatitis which occurs in ca. 60% of patients wearing dentures, is associated with significant reduction of endothelial function, measured as nitric oxide bioavailability in a clinical flow-mediated dilatation study. Importantly, control, nitroglycerin induced endothelium-independent vasodilatation was not changed. As it is known that the severity of endothelial dysfunction correlates with the development of coronary artery disease and predicts future cardiovascular events [34], our results implicate that the presence of DRS may be associated with negative cardiovascular outcomes. Thus, such patients should be particularly carefully monitored in relation to their cardiovascular risk. Considering that DRS is one of the most common oral disorders in the elderly, occurring in 40–70% of subjects wearing dentures [9, 10], our finding may have important implications for clinical care of denture wearing patients. It is important to note, however, that majority of patients studied here were females which is consistent with the epidemiology of DRS, that is, more common in females [9]. We have also performed an additional analysis in female subpopulation only, which confirmed all major observations of this study.

While numerous previous studies have shown increased cardiovascular risk in subjects with oral inflammatory conditions such as periodontitis [1–5], endodontic infections [22–26], and even caries [19–21], this is the first study focusing

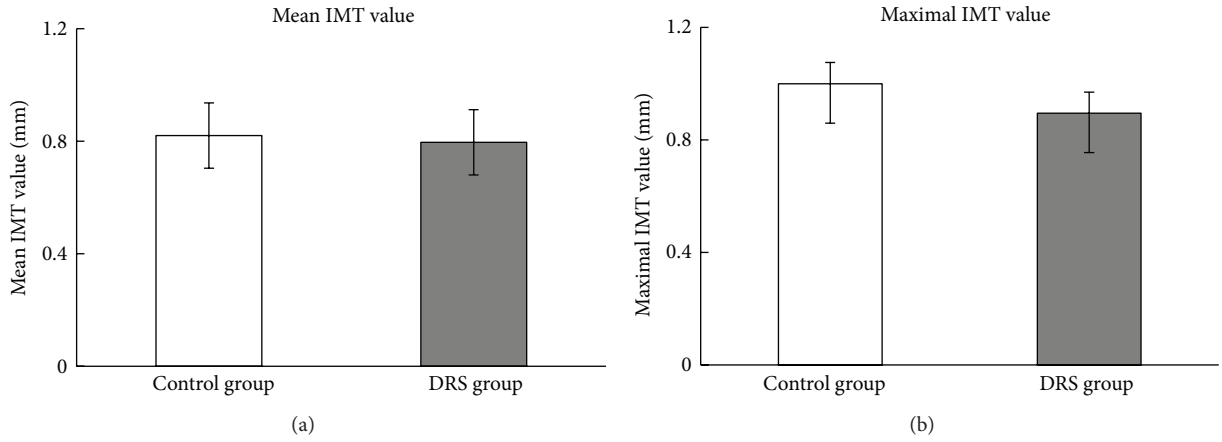

Figure 3: IMT measurements in DRS and control group. (a) Mean common carotid artery intima-media thickness. Results are presented as mean (SD); (b) maximal common carotid artery intima-media thickness. Results presented as median ($Q1$; $Q2$). (a) and (b): n control group = 24, n DRS group = 20.

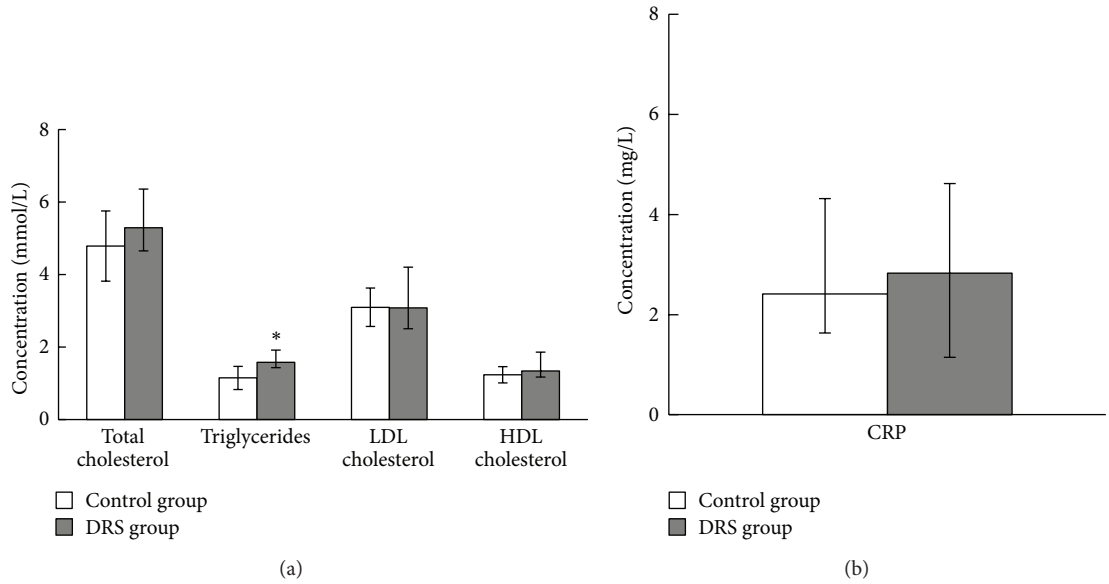

Figure 4: Plasma lipid profile and C-reactive protein levels in control and DRS patients. (a) Comparison of lipid profiles. Results are presented as median ($Q1$; $Q2$); $^{*}P < 0,05$; (b) comparison of plasma CRP concentrations. Results are presented as median ($Q1$; $Q2$). (a) and (b): n control group = 24, n DRS group = 18.

on vascular dysfunction in elderly population of patients wearing dentures. This is important, while previous studies focused on bacteria-mediated, resulting from disturbances of physiological oral microflora diseases, we have primarily studied fungal infection, as DRS is most commonly associated with *Candida* infection.

Previous studies focused on a positive association between periodontitis and vascular endothelial dysfunction. Amar et al. and Blum et al. observed that subjects with advanced periodontal disease exhibit worse endothelial function when compared to the healthy controls [35, 36]. Blum et al. [36], along with others [6, 37], reported also an improvement of endothelial function as a long-term outcome of periodontal treatment. Tonetti et al. [6] in a landmark study has shown in a proper randomized controlled trial that such improvement provides clinical benefit for up to 6 months after intensive treatment.

The mechanisms of increased cardiovascular risk in oral inflammatory conditions are multifactorial and range from chronic systemic inflammation (periodontitis) to the effects of risk factors such as diabetes, hyperlipidemia, smoking, and age which predispose to both cardiovascular diseases and oral disorders, such as periodontitis [1–5], caries [19–21], endodontic infections [22–26], or DRS [9, 12, 13]. This coincidence of risk factors is visible in the population of patients we have studied. Although the difference in occurrence of smoking or diabetes did not reach statistical significance, we can clearly see increased occurrence of these factors

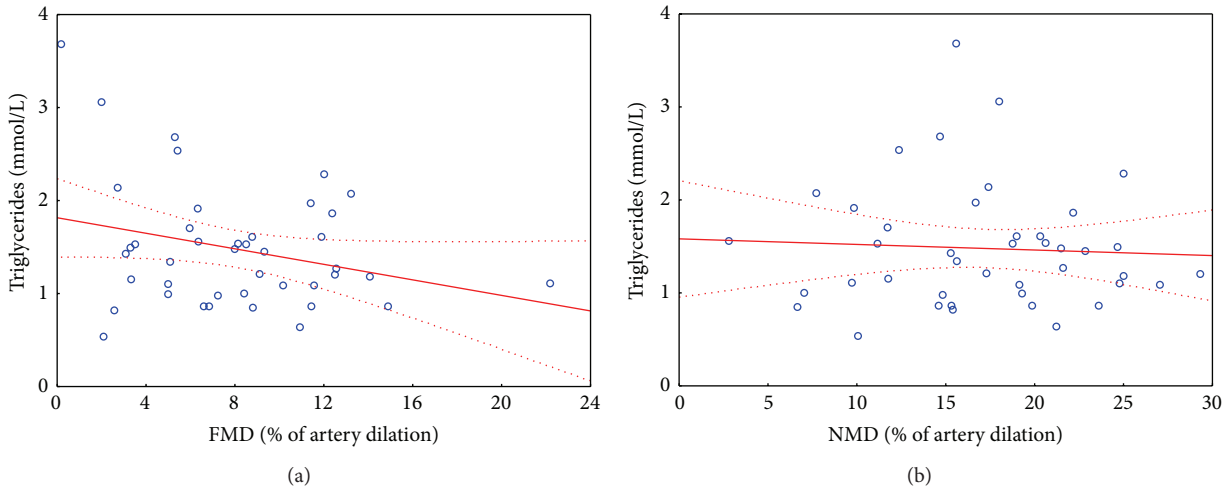

Figure 5: Spearman correlation between parameters of vascular function parameters and triglycerides levels. (a) Spearman correlation between FMD and triglycerides levels: R Spearman = −0,13, P = 0,42; (b) Spearman correlation between NMD and triglycerides levels: R Spearman = −0, 025, P = 0, 87; (a) and (b): n = 42.

in DRS. This can in part explain the increased degree of endothelial dysfunction in DRS subjects. Measurement of baseline FMD prior to developing DRS in a long-term follow-up study would unquestionably strengthen the conclusions of this study. Alternatively, a future interventional study in which the effect of treatment of DRS on endothelial function could also help to address this issue in a more cause-effect manner. Importantly, as the population we studied was relatively young for denture carriers, no significant increase in intima-media thickness was detected yet. This is in agreement with numerous cardiovascular studies which show that endothelial dysfunction precedes the development of severe atherosclerosis [38].

The role of systemic inflammation, very well defined in periodontitis, is not known in DRS. The mechanisms through which DRS could affect endothelial dysfunction are unclear. In periodontitis, bacteria lead to the activation of the local immune response, leading to systemic inflammation. Similarly, immune stimulation of T cells and monocytes has been reported in response to fungal *C. albicans* antigens [39, 40]. However, in our study we did not find significantly increased levels of total CRP, which could suggest that local *Candida*-evoked oral mucosal inflammation is not causing significant activation of systemic inflammatory response. The CRP levels among edentulous were assessed by Ajwani et al. at Helsinki Aging Study involving over 600 patients older than 75 years old [41]. They identified mucosal lesions in the edentulous as an important factor associated with elevated CRP level among elderly individuals and observed that it was significantly more common among the edentulous with complete dentures. Importantly, patients having clinical signs of oral candidosis or denture stomatitis also showed elevated levels of CRP, and authors suggested that it may be the explanation of the elevated CRP levels seen in the edentulous. In our study, we have also seen a trend toward higher CRP values in DRS patients, but it did not reach statistical significance, probably because of small numbers of patients involved and the fact that we have not measured high sensitivity hsCRP which would better characterize cardiovascular risk [42]. In the light of our results, assessing other markers of the systemic inflammation becomes a very interesting aspect for further studies. Taking into account our results, at present our data do not support the hypothesis that systemic inflammation is involved. Rather, the effect of concomitant risk factors on DRS and vascular function is most likely.

De Oliveira et al. and Rodriguez-Archilla et al. have found that oral *Candida* infection may impact peripheral blood mononuclear cells state, measured by amount of cytokines produced in vitro in response to *Candida* antigens [39, 40]. Direct impact of *Candida* cells on the vascular system is unlikely, since systemic fungal infections are characterized, in contrast to the DRS, by extremely serious symptoms; moreover, fungal DNA has not been detected in atherosclerotic lesions [43].

However our results may point us to the potential role of vascular risk factors such as diabetes, smoking, and hypertriglyceridemia in mediating vascular dysfunction. Although no relationship was found between triglyceride levels and endothelial dysfunction in a simple correlation analysis, when multivariate linear regression was introduced including diabetes, smoking, and/or triglyceride levels, the difference in endothelial function was no longer significant (data not shown). One has to however bear in mind that statistical power of such analysis in the studied group was relatively modest.

While the finding that classical risk factors may be main mediators of endothelial dysfunction may not sound exceptionally interesting, it is very important to show that this is the case in DRS subjects, and therefore this group of patients should be carefully studied in larger epidemiological trials. This is particularly important in the light of ageing population.

Despite the lack of differences in the levels of total, HDL, and LDL cholesterol in the blood of healthy subjects and

DRS, we observed significantly higher levels of triglycerides in patients with DRS. Our surprising finding that DRS is associated with selective increase in triglyceride levels is quite intriguing and could be related to the fact that denture wearing can change dietary habits. It could also suggest that increased triglyceride levels could be a risk factor of DRS, although our study was not powered to answer this question. There are no studies looking at lipid parameters in DRS, while conflicting data are available regarding periodontitis. Sandi et al. and Penumarthy et al. observed higher concentrations of total cholesterol and LDL cholesterol in the blood of patients with periodontal disease than in healthy group, but the differences in the levels of triglycerides and HDL cholesterol were shown only by Penumarthy et al., despite the smaller sample sizes [44, 45]. Simultaneously, Elter et al. did not demonstrate changes in the total cholesterol and HDL levels after treatment of periodontitis [37]. Altogether, these results can point to a potential relationship between oral infection and blood lipid profile, but conclusive evidence is still needed.

We did not observe the tendency towards elevated blood pressure in patients with DRS as compared to healthy subjects. This shows potential important difference in oral inflammatory conditions as periodontitis is potentially associated with elevated blood pressure, which was frequently observed [46]. The relationship between the use of dental prostheses and the prevalence of cardiovascular diseases, including elevated blood pressure, was discussed by Buhlin et al. [2]. They found positive association between dentures and all cardiovascular diseases but not for elevated blood pressure, myocardial infarction, or stroke. However, the group defined as "dentures" was very heterogeneous. The authors gathered together edentulous and partially edentulous denture wearers and edentulous without dentures. This creates potential bias of the presence of periodontitis in partially edentulous patients. Importantly, in our study virtually all subjects were completely edentulous (41 out of 44).

5. Conclusions

In conclusion, our study shows that patients with denture-related stomatitis are characterized by more pronounced systemic endothelial dysfunction than denture subjects without stomatitis. This difference in vascular function is likely linked with increased cardiovascular risk in DRS and indicates that such patients should be carefully monitored for cardiovascular disease. While our study identifies certain very interesting and potentially very important cardiovascular aspects of denture-related stomatitis, a larger study is warranted to finally confirm these observations. This may be very important for clinical practice considering ageing population.

Abbreviations

CRP: C-reactive protein
DM: Diabetes mellitus
DRS: Denture-related stomatitis
FMD: Flow-mediated dilatation
HDL: High density lipoprotein cholesterol
LDL: Low density lipoprotein cholesterol
NMD: Nitroglycerine-mediated dilatation
$Q1$ and $Q3$: 25th ($Q1$) and 75th ($Q3$) percentiles
SD: S tandard deviation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Joanna Maciąg and Grzegorz Osmenda contributed equally to this study.

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References

[1] S. J. Arbes, G. D. Slade, and J. D. Beck, "Association between extent of periodontal attachment loss and self-reported history of heart attack: an analysis of NHANES III data," *Journal of Dental Research*, vol. 78, no. 12, pp. 1777–1782, 1999.

[2] K. Buhlin, A. Gustafsson, J. Håkansson, and B. Klinge, "Oral health and cardiovascular disease in Sweden: results of a national questionnaire survey," *Journal of Clinical Periodontology*, vol. 29, no. 3, pp. 254–259, 2002.

[3] K. Buhlin, A. Gustafsson, S. Ahnve, I. Janszky, F. Tabrizi, and B. Klinge, "Oral health in women with coronary heart disease," *Journal of Periodontology*, vol. 76, no. 4, pp. 544–550, 2005.

[4] M. Desvarieux, R. T. Demmer, T. Rundek et al., "Relationship between periodontal disease, tooth loss, and carotid artery plaque: the oral infections and vascular disease epidemiology study (INVEST)," *Stroke*, vol. 34, no. 9, pp. 2120–2125, 2003.

[5] H.-C. Hung, W. Willett, A. Merchant, B. A. Rosner, A. Ascherio, and K. J. Joshipura, "Oral health and peripheral arterial disease," *Circulation*, vol. 107, no. 8, pp. 1152–1157, 2003.

[6] M. S. Tonetti, F. D'Aiuto, L. Nibali et al., "Treatment of periodontitis and endothelial function," *The New England Journal of Medicine*, vol. 356, no. 9, pp. 911–920, 2007.

[7] M. K. Noh, M. Jung, S. H. Kim et al., "Assessment of IL-6, IL-8 and TNF-α levels in the gingival tissue of patients with periodontitis," *Experimental and Therapeutic Medicine*, vol. 6, no. 3, pp. 847–851, 2013.

[8] A. di Benedetto, I. Gigante, S. Colucci, and M. Grano, "Periodontal disease: linking the primary inflammation to bone loss," *Clinical and Developmental Immunology*, vol. 2013, Article ID 503754, 7 pages, 2013.

[9] L. Gendreau and Z. G. Loewy, "Epidemiology and etiology of denture stomatitis," *Journal of Prosthodontics*, vol. 20, no. 4, pp. 251–260, 2011.

[10] A. E. Kossioni, "The prevalence of denture stomatitis and its predisposing conditions in an older Greek population," *Gerodontology*, vol. 28, no. 2, pp. 85–90, 2011.

[11] O. Abaci, A. Haliki-Uztan, B. Ozturk, S. Toksavul, M. Ulusoy, and H. Boyacioglu, "*Determining candida* spp. incidence in denture wearers," *Mycopathologia*, vol. 169, no. 5, pp. 365–372, 2010.

[12] C. Salerno, M. Pascale, M. Contaldo et al., "Candida-associated denture stomatitis," *Medicina Oral, Patologia Oral y Cirugia Bucal*, vol. 16, no. 2, pp. e139–e143, 2011.

[13] B. Dorocka-Bobkowska, D. Zozulinska-Ziolkiewicz, B. Wierusz-Wysocka, W. Hedzelek, A. Szumala-Kakol, and E. Budtz-Jörgensen, "Candida-associated denture stomatitis in type 2 diabetes mellitus," *Diabetes Research and Clinical Practice*, vol. 90, no. 1, pp. 81–86, 2010.

[14] M. A. Ghannoum, R. J. Jurevic, P. K. Mukherjee et al., "Characterization of the oral fungal microbiome (mycobiome) in healthy individuals," *PLoS Pathogens*, vol. 6, no. 1, Article ID e1000713, 2010.

[15] M. S. Campos, L. Marchini, L. A. S. Bernardes, L. C. Paulino, and F. G. Nobrega, "Biofilm microbial communities of denture stomatitis," *Oral Microbiology and Immunology*, vol. 23, no. 5, pp. 419–424, 2008.

[16] E. L. Schiffrin, "The immune system: role in hypertension," *Canadian Journal of Cardiology*, vol. 29, no. 5, pp. 543–548, 2013.

[17] A. P. Davel, C. F. Wenceslau, E. H. Akamine et al., "Endothelial dysfunction in cardiovascular and endocrine-metabolic diseases: an update," *Brazilian Journal of Medical and Biological Research*, vol. 44, no. 9, pp. 920–932, 2011.

[18] T. J. Guzik, N. E. Hoch, K. A. Brown et al., "Role of the T cell in the genesis of angiotensin II-induced hypertension and vascular dysfunction," *The Journal of Experimental Medicine*, vol. 204, no. 10, pp. 2449–2460, 2007.

[19] B. Larsson, I. Johansson, L. Weinehall, G. Hallmans, and T. Ericson, "Cardiovascular disease risk factors and dental caries in adolescents: effect of a preventive program in Northern Sweden (the Norsjo project)," *Acta Paediatrica*, vol. 86, no. 1, pp. 63–71, 1997.

[20] R. Kelishadi, S. Mortazavi, T. R. Hossein, and P. Poursafa, "Association of cardiometabolic risk factors and dental caries in a population-based sample of youths," *Diabetology and Metabolic Syndrome*, vol. 2, no. 1, article 22, 2010.

[21] P. V. Ylöstalo, M. R. Järvelin, J. Laitinen, and M. L. Knuuttila, "Gingivitis, dental caries and tooth loss: risk factors for cardiovascular diseases or indicators of elevated health risks," *Journal of Clinical Periodontology*, vol. 33, no. 2, pp. 92–101, 2006.

[22] E. Cotti, C. Dess, A. Piras et al., "Association of endodontic infection with detection of an initial lesion to the cardiovascular system," *Journal of Endodontics*, vol. 37, no. 12, pp. 1624–1629, 2011.

[23] K. J. Joshipura, W. Pitiphat, H.-C. Hung, W. C. Willett, G. A. Colditz, and C. W. Douglass, "Pulpal inflammation and incidence of coronary heart disease," *Journal of Endodontics*, vol. 32, no. 2, pp. 99–103, 2006.

[24] D. J. Caplan, J. B. Chasen, E. A. Krall et al., "Lesions of endodontic origin and risk of coronary heart disease," *Journal of Dental Research*, vol. 85, no. 11, pp. 996–1000, 2006.

[25] D. J. Caplan, J. S. Pankow, J. Cai, S. Offenbacher, and J. D. Beck, "The relationship between self-reported history of endodontic therapy and coronary heart disease in the Atherosclerosis Risk in Communities study," *Journal of the American Dental Association*, vol. 140, no. 8, pp. 1004–1012, 2009.

[26] J. Petersen, E. M. Glassl, P. Nasseri et al., "The association of chronic apical periodontitis and endodontic therapy with atherosclerosis," *Clinical Oral Investigations*, 2013.

[27] J. Perk, G. de Backer, H. Gohlke et al., "European guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts)," *Atherosclerosis*, vol. 223, no. 1, pp. 1–68, 2012.

[28] G. Wilk, G. Osmenda, P. Matusik et al., "Endothelial function assessment in atherosclerosis: comparison of brachial artery flowmediated vasodilation and peripheral arterial tonometry," *Polskie Archiwum Medycyny Wewnetrznej*, vol. 123, no. 9, pp. 443–452, 2013.

[29] C. O. Bingham and M. Moni, "Periodontal disease and rheumatoid arthritis: the evidence accumulates for complex pathobiologic interactions," *Current Opinion in Rheumatology*, vol. 25, no. 3, pp. 345–353, 2013.

[30] C. C. Zeigler, G. R. Persson, B. Wondimu, C. Marcus, T. Sobko, and T. Modéer, "Microbiota in the oral subgingival biofilm is associated with obesity in adolescence," *Obesity*, vol. 20, no. 1, pp. 157–164, 2012.

[31] A. J. Kim, A. J. Lo, D. A. Pullin, D. S. Thornton-Johnson, and N. Y. Karimbux, "Scaling and root planing treatment for periodontitis to reduce preterm birth and low birth weight: a systematic review and meta-analysis of randomized controlled trials," *Journal of Periodontology*, vol. 83, no. 12, pp. 1508–1519, 2012.

[32] B. Chee, B. Park, and P. M. Bartold, "Periodontitis and type II diabetes: a two-way relationship," *International Journal of Evidence-Based Healthcare*, vol. 11, no. 4, pp. 317–329, 2013.

[33] A. L. Costa, C. L. Yasuda, W. Shibasaki et al., "The association between periodontal disease and seizure severity in refractory epilepsy patients," *Seizure*, vol. 23, no. 3, pp. 227–230, 2014.

[34] E. H. C. Tang and P. M. Vanhoutte, "Endothelial dysfunction: a strategic target in the treatment of hypertension?" *Pflügers Archiv*, vol. 459, no. 6, pp. 995–1004, 2010.

[35] S. Amar, N. Gokce, S. Morgan, M. Loukideli, T. E. van Dyke, and J. A. Vita, "Periodontal disease is associated with brachial artery endothelial dysfunction and systemic inflammation," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 7, pp. 1245–1249, 2003.

[36] A. Blum, K. Kryuger, M. Mashiach Eizenberg et al., "Periodontal care may improve endothelial function," *European Journal of Internal Medicine*, vol. 18, no. 4, pp. 295–298, 2007.

[37] J. R. Elter, A. L. Hinderliter, S. Offenbacher et al., "The effects of periodontal therapy on vascular endothelial function: a pilot trial," *American Heart Journal*, vol. 151, no. 1, article 47, 2006.

[38] T. J. Guzik, N. E. West, E. Black et al., "Vascular superoxide production by NAD(P)H oxidase: association with endothelial dysfunction and clinical risk factors," *Circulation Research*, vol. 86, no. 9, pp. E85–E90, 2000.

[39] M. A. M. de Oliveira, L. P. Carvalho, M. D. S. Gomes, O. Bacellar, T. F. Barros, and E. M. Carvalho, "Microbiological and immunological features of oral candidiasis," *Microbiology and Immunology*, vol. 51, no. 8, pp. 713–719, 2007.

[40] A. Rodriguez-Archilla, M. Urquia, A. Cutando, and R. Asencio, "Denture stomatitis: quantification of interleukin-2 production by mononuclear blood cells cultured with *Candida albicans*," *Journal of Prosthetic Dentistry*, vol. 75, no. 4, pp. 426–431, 1996.

[41] S. Ajwani, K. J. Mattila, T. O. Närhi, R. S. Tilvis, and A. Ainamo, "Oral health status, C-reactive protein and mortality—a 10 year follow-up study," *Gerodontology*, vol. 20, no. 1, pp. 32–40, 2003.

[42] P. Calabrò, E. Golia, and E. T. H. Yeh, "CRP and the risk of atherosclerotic events," *Seminars in Immunopathology*, vol. 31, no. 1, pp. 79–94, 2009.

[43] A. R. L. Aquino, K. C. Lima, M. S. Paiva, I. N. Rôças, and J. F. Siqueira Jr., "Molecular survey of atheromatous plaques for the presence of DNA from periodontal bacterial pathogens, archaea and fungi," *Journal of Periodontal Research*, vol. 46, no. 3, pp. 303–309, 2011.

[44] R. M. Sandi, K. G. Pol, P. Basavaraj, N. Khuller, and S. Singh, "Association of Serum Cholesterol, Triglyceride, High and Low Density Lipoprotein (HDL and LDL) levels in chronic periodontitis subjects with risk for Cardiovascular Disease (CVD): a cross sectional study," *Journal of Clinical and Diagnostic Research*, vol. 8, no. 1, pp. 214–216, 2014.

[45] S. Penumarthy, G. P. Penmetsa, and S. Mannem, "Assessment of serum levels of triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol in periodontitis patients," *Journal of Indian Society of Periodontology*, vol. 17, no. 1, pp. 30–35, 2013.

[46] X. F. Leong, C. Y. Ng, B. Badiah, and S. Das, "Association between hypertension and periodontitis: possible mechanisms," *The Scientific World Journal*, vol. 2014, Article ID 768237, 11 pages, 2014.

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Review Article

Low Density Lipoprotein-Containing Circulating Immune Complexes: Role in Atherosclerosis and Diagnostic Value

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It has been suggested that low density lipoprotein-containing circulating immune complexes (LDL-CIC) play a role in atherogenesis and are involved in the formation of early atherosclerotic lesion. These complexes, as well as anti-LDL autoantibodies, have been found in the blood and in the atherosclerotic lesions of patients with different cardiovascular diseases, as well as in the blood of animals with experimental atherosclerosis. It can be suggested that the presence of anti-LDL antibodies in the blood is a result of immune response induced by lipoprotein modification. LDL-CIC differs from native LDL in many aspects. It has much lower sialic acid content, smaller diameter, and higher density and is more electronegative than native LDL. Fraction of LDL-CICs is fundamental to the serum atherogenicity manifested at the cellular level. LDL-CIC, unlike native LDL, is able to induce intracellular accumulation of neutral lipids, especially esterified cholesterol, in cells cultured from uninvolved human aortic intima and in macrophage cultures. After removal of LDL-CIC, the CHD patient's sera lose their atherogenic properties. Titer of LDL-CIC in blood serum significantly correlates with progression of atherosclerosis in human *in vivo* and has the highest diagnostic value among other measured serum lipid parameters. Elevated CIC-cholesterol might well be a possible risk factor of coronary atherosclerosis.

1. Introduction

Widely spread clinical manifestations of atherosclerosis such as coronary heart disease (CHD), cerebrovascular stroke, renovascular hypertension, and violation of the lower limbs vascular permeability, are the result of formation of advanced atherosclerotic lesions in a vascular wall. A trigger mechanism for the development of atherosclerotic lesions is an intracellular lipid deposition and subsequent foam cell formation with excessive production of connective tissue matrix components and, possibly, cellular proliferation and inflammatory reactions [1, 2]. Atherosclerosis can be generally described as an excessive fibrofatty, proliferative, inflammatory response to damage of the artery wall, involving several cell types, such as smooth muscle cells, monocyte-derived macrophages, lymphocytes, and platelets [3]. During the last three decades, the autoimmune hypothesis of atherosclerosis was developed and the evidence for an important role for autoantibodies against modified low density lipoprotein (LDL) and LDL-containing circulating immune complexes (LDL-CIC) in atherogenesis has been accumulated. Immunological factors appear to contribute to the development of atherosclerosis as many other factors including alterations in plasma lipid and lipoprotein levels, platelet function,

clotting factors, arterial smooth muscle cell metabolism, and blood pressure regulation. In a number of recent studies it has been suggested that the presence of LDL-CIC in the blood promotes the onset and development of atherosclerotic lesions in the vessel wall. It has been demonstrated that modified LDL and especially LDL-CIC act as the primary agents responsible for excessive cholesterol accumulation in vascular cells [4–9]. The atherogenic properties of LDL-containing immune complexes suggest them as a candidate marker for atherosclerosis.

2. LDL-CIC and Its Physicochemical Characteristics

Anti-LDL autoantibodies were first detected in the blood of patients with hyperlipidemia accompanied by myeloma or/and ischemic heart disease [10]. In 1965, Beaumont [11] described a situation in which hyperlipidemia, xanthomatosis, and atherosclerosis were apparently associated with anti-β-lipoprotein antibodies. The antibodies against lipoproteins or LDL-binding factors were found in the blood of patients suffering from various vascular diseases as well as in healthy subjects [12]. Bauer et al. established that immunoglobulins are the major LDL-binding proteins in human plasma [13]. The emergence of anti-LDL autoantibodies in the blood implies that lipoproteins can be regarded as autoantigens. The high immunogenicity and ability of homologous chemically modified LDL to generate antibodies have been demonstrated [14]. The discovery of autoantibodies in modified LDL in the blood of patients therefore seems natural. Autoantibodies against glycosylated LDL were detected in the blood of patients with diabetes mellitus [15]. Autoantibodies specific for malondialdehyde-modified LDL have been found in the blood of healthy subjects and patients with coronary artery disease, as well as in the blood of experimental animals [16]. Deposits of the immune complex components were found in vascular atherosclerotic lesions [17–19]. Autoantibodies of immunoglobulin G class against modified LDL were detected in the blood of patients with angiographically assessed coronary atherosclerosis [2, 12, 20–23]. In healthy subjects, the level of anti-LDL autoantibodies was considerably lower than that in atherosclerotic patients [2]. These autoantibodies exhibit a high affinity for desialylated LDL (neuraminidase-treated LDL) and for malondialdehyde-modified LDL. They have a lower affinity for native, oxidized, glycosylated, and acetylated LDL, as well as for LDL, which has undergone other chemical modifications. The higher affinity of autoantibodies for modified LDL compared with native LDL suggests that the antibodies are produced *in vivo* in response to the appearance of modified LDL in the blood [24, 25].

Antibodies against LDL modified with malondialdehyde (MDA) have been detected in the blood of animals with experimental atherosclerosis and in atherosclerotic lesions in humans [25–28]. Even though elevated levels of oxidized lipids, such as MDA and F_2-isoprostanes, have been found in the blood of subjects with CHD [29, 30], there is some evidence that oxidized lipids do not accumulate in noticeable amounts in human LDL since high density lipoproteins seem to detoxify and/or transfer them from the circulation to the liver [31]. On the other hand, electronegative LDL [32], small/dense LDL [33], and desialylated LDL differing from native LDL by lowered sialic acid content [2, 34, 35] were found in the blood of patients with coronary atherosclerosis.

Table 1: The properties of LDL from circulating immune complexes.

Characteristic	LDL from CIC compared to native circulating LDL
Neutral lipid content (free cholesterol, esterified cholesterol, and triglycerides)	Lowered
Phospholipid content	Lowered
Sialic acid content	Lowered
Neutral sugars content	Lowered
Electrophoretic mobility	Increased
Hydrated density	Increased
Particle size	Decreased

It can be suggested that the presence of anti-LDL antibodies in the blood is a result of immune response induced by lipoprotein modification. Tertov et al. [36] isolated circulating immune complexes from blood serum using polyethylene glycol 6000 and have found that LDL-CIC differs from native LDL in many aspects (Table 1). Specifically, it has low sialic acid content; that is, it is desialylated LDL. The neutral lipid and phospholipid contents of LDL-CIC are considerably lower than those in native LDL. Particles of LDL-CIC have a smaller diameter and higher density. The higher electrophoretic mobility shows that LDL-CIC is more electronegative than native LDL. Finally, LDL-CIC, unlike native LDL, is able to induce intracellular accumulation of neutral lipids, especially esterified cholesterol, in cells cultured from uninvolved human aortic intima. Thus, it was shown that LDL-CIC is quite similar to the multiple-modified (desialylated) LDL described earlier [37–39]. There was a strong correlation between the LDL content in circulating immune complexes and blood concentration of desialylated LDL but not of total LDL. This suggests that predominantly desialylated LDL forms complex with autoantibodies and proves that the affinity of circulating anti-LDL autoantibodiesis higher for desialylated LDL than for native LDL [36]. Moreover, anti-LDL autoantibodies bind much more effectively with LDL of patients having a high percentage of desialylated LDL than with LDL of healthy subjects having a low content of desialylated LDL [40]. Desialylated LDL has certain modifications that could stimulate the immune response: alterations in carbohydrate composition and in the tertiary structure of apo B, modification of lysine amino groups, and aggregation of lipoprotein particles [38, 39].

3. Atherogenicity of LDL-CIC

The investigation of proatherogenic role of LDL-CIC has started long ago, and it seems relevant to look into historical perspective to revive the interest in this topic. The first experimental data on the effect of LDL and anti-LDL autoantibodies

on cell metabolism were reported by Beaumont's group as far as in 1979 [41]. It was found that incubating cultured fibroblasts with LDL, forming immune complexes with antibodies, facilitated intracellular cholesterol accumulation.

Klimov et al. have demonstrated that mouse macrophages cultured in the presence of immune complexes containing LDL and rabbit anti-human LDL antibodies demonstrated an increased uptake of LDL [42]. They have also shown that incubation of human peritoneal macrophages with autologous LDL-containing immune complexes causes transformation of macrophages into foam cells [38].

Griffith et al. [43] have found that human macrophages incubated *in vitro* with insoluble LDL-containing immune complexes accumulate cholesterol and are transformed into foam cells.

It should be mentioned that the LDL of immune complexes amounts to not more than 2% of the total circulating LDL pool [4, 5]. However, this LDL fraction is fundamental to the serum atherogenicity manifested at the cellular level. In a cell culture, immune complexes isolated from the serum cause atherosclerosis-related changes similar to those caused by the whole serum [4, 5]. There is a direct correlation between the LDL content of circulating immune complexes and serum atherogenic potential [4].

Orekhov et al. [44] have shown that insoluble immune complexes containing LDL and heterologous anti-LDL antibodies induce lipid accumulation in cultured cells. The ability of antibodies to stimulate lipid accumulation was found to be dependent on the LDL content of the immune complex [44]. It was also shown that the atherogenic potential of desialylated LDL isolated from the blood of atherosclerotic patients is markedly increased if the LDL forms an immune complex with autoantibodies [2]. The addition of desialylated LDL and anti-LDL autoantibodies to cultured human aortic smooth muscle cells enhanced the intracellular cholesterol accumulation considerably [2, 45, 46]. Native LDL that did not induce intracellular cholesterol accumulation became atherogenic after interaction with autoantibodies (i.e., the LDL acquires the ability to increase the cholesterol content of cultured cells) [2, 45, 46]. Interaction of LDL with anti-LDL autoantibodies considerably increases the uptake (binding and internalization) of the lipoprotein by arterial cells, which may account for the stimulating effect of antibodies on the LDL-induced accumulation of intracellular cholesterol [2]. After being added to a cell culture together with antibodies, fibronectin and Clq complement component (which are the constituents of an immune complex) increase the LDL uptake to a greater extent than the antibodies added alone. This leads to a massive cholesterol accumulation [2].

It was demonstrated that removal of IgG and IgM as well as circulating immune complexes from atherogenic sera of CHD patients leads to a partial or complete elimination of their atherogenic properties (i.e., its ability to induce intracellular lipid accumulation). Removal of immunoglobulin G caused the greatest fall in the serum atherogenicity; the fall was lower after removal of immunoglobulin M, and atherogenicity remained virtually unchanged after immunoglobulin A removal [3]. These facts lead to the suggestion that the majority of the atherogenic LDL of an immune complex is bound with antibodies of the immunoglobulin G class; there is no evidence that IgG-containing LDL-CIC may be more atherogenic and those results only allow assuming that just IgG and, in lesser extent, IgM but not IgA participate in LDL-CIC formation. At the same time, circulating immune complexes (CIC) isolated from these sera brought about accumulation of cholesterol in cultured SMC of unaffected human aortic intima. The ability of atherogenic sera to stimulate the accumulation of intracellular cholesterol correlated with the cholesterol level in the CIC isolated from these sera. The cholesterol content in CIC isolated from sera of CHD patients, which displayed atherogenic properties in culture, was characterized by an elevated cholesterol level in CIC (33.2 ± 1.2 pg/mL) significantly ($P < 0.01$) different from the values seen in the group of healthy donors. Neither of the nonatherogenic sera had an elevated CIC-cholesterol level. These findings suggest that in most cases the cholesterol-containing immune complexes are responsible for the atherogenic properties of the serum [3]. Basing on the knowledge of the absence of atherogenicity of native LDL, it can be suggested that nonmodified LDL does not produce an immune response, and for the formation of LDL-CIC lipoprotein particles should be modified in some way; apo-B desialylation may act as one of the mechanisms of LDL immunogenic modification. At the same time, it is unknown whether LDL-CIC contain only modified LDL, or also native LDL; the last is possible due to common immunoglobulin-binding sites, which may be present in both native and modified LDL.

The mechanisms of intracellular lipid accumulation caused by LDL-CIC obviously are an area that needs further investigation. There is evidence that immune complexes formed between modified LDL as antigens and IgG autoantibodies may modulate the inflammation in atherosclerosis via Fc receptor signaling and complement activation; the role of antibody isotypes in atherogenesis is unclear, since IgG is regarded as potentially proatherogenic, and IgM may even play a protective role [47]. The last finding contradicts with earlier data on atherogenicity of IgM-containing LDL-CIC; however, till now there is no evidence that activation of inflammation and foam cells formation should go in parallel. It is not definitely known in which way foam cells formed as a result of intracellular lipid accumulation further drive the progression of atherosclerosis; there exists the possibility of macrophages to acquire proinflammatory phenotype after ingesting LDL-IgG through Fc gamma receptor. However, it is known that LDL-CIC may induce other atherosclerosis-related processes at the cellular level, namely, excessive production of connective tissue matrix and cellular proliferation [5, 36]. It has been shown recently that *in vitro* produced immune complexes containing oxidized LDL stimulate type IV collagen production by mesangial cells, the effect being realized via Fc gamma receptors I and III [48]. Such immune complexes also increased proliferative activity of cultured human monocytes, and this effect was mediated by cross-linking of Fc gamma receptor I; a concentration-dependent production of monocyte colony-stimulating factor was observed. These results offered a novel mechanism by which an immune reaction toward

modified LDL can play a role in local accumulation of macrophages in atherosclerotic lesions [49]. It is generally approved that LDL-CIC effects are mediated via interaction of immunoglobulin moiety with Fc receptors; since IgG and IgM antibodies do not share the same receptors, this may be the explanation for the abovementioned difference in uptake of LDL bound either to IgG or to IgM.

It has been demonstrated that the lipoprotein-antibody complexes prepared *in vitro* affect lipoprotein metabolism in human fibroblasts and monocytes [50, 51] and facilitate the accumulation of lipids in mouse macrophages [52]. Complexes of human LDL with polyclonal goat antibodies against LDL induce the deposition of lipids in cultured SMC of human aortic intima and peritoneal mouse macrophages [3].

Taken together, these findings suggest that multiple-modified desialylated LDL has immunogenic properties and circulating immune complexes containing modified LDL and anti-LDL autoantibodies are the blood components responsible for primary cholesterol accumulation in vascular cells. Since cholesterol accumulation is accompanied by stimulation of other atherosclerotic manifestations at the cellular level, it can be suggested that the presence of LDL-containing complexes in the blood promotes the emergence and development of atherosclerotic lesions in the vessel wall.

4. Diagnostic and Prognostic Value of LDL-CIC in Atherosclerosis

Using a simple method of measurement of LDL-CIC level, Orekhov et al. demonstrated that only LDL-CIC level and the apo B/apo A-1 ratio contributed strongly to the discrimination between patients with coronary and/or extracoronary atherosclerosis and those without stenosis [53]. In the same study, total cholesterol, triglycerides, HDL cholesterol, apo B, Lp[a], and apo A1 did not correlate with the presence and severity of coronary and/or extracoronary atherosclerosis. The authors concluded that LDL-CIC level might be the most reliable marker of atherosclerosis as compared to other parameters of lipid profile. LDL-CIC level was significantly correlated also with the severity of coronary atherosclerosis, and this biochemical parameter was proposed to be used as a sensitive and specific marker for atherosclerosis, possessing a high diagnostic value [5, 53, 54]. Salonen et al. have reported that the titer of antibodies against MDA-modified LDL in blood serum is associated with the progression of a carotid atherosclerosis [55]. In the recent Epidemiology of Diabetes Interventions and Complications (EDIC) Trial it has been demonstrated that cholesterol and apolipoprotein B content of immune complexes were significantly higher in patients who showed progression of the internal carotid IMT than in those showing no progression, regression, or mild progression, and cholesterol content of immune complexes was a significant positive predictor of internal carotid IMT progression [56]. High cholesterol levels in CIC are considered to be surrogate markers of modified LDL associated with increased carotid intima-media thickness and cardiovascular events. Lopes-Virella et al. have measured oxidized LDL, advanced glycation end products-modified LDL, and malondialdehyde-modified LDL in CIC, determined their relationship with increased carotid IMT in type I diabetes, and compared the strength of the association with that observed with conventional risk factors [57].

The most recent evaluation of diagnostic and predictive role of LDL-CIC (immune cholesterol) as well as other lipid parameters in early carotid atherosclerosis was carried out by Sobenin at al. in two-year prospective study [58]. The rate of atherosclerosis progression was estimated by high-resolution B-mode ultrasonography as the increase in intima-media thickness (IMT) of common carotid arteries. The patients with elevated levels of LDL-CIC were characterized by significantly higher levels of serum total and LDL cholesterol as well as significantly increased mean and maximum intima-media thickness of common carotid arteries. Cholesterol level of LDL-CIC and serum LDL cholesterol were contingent with the extent of early carotid atherosclerosis ($P = 0.042$ and $P = 0.049$, resp.). Additionally, LDL-CIC was characterized by the highest values of sensitivity and specificity as compared to commonly used lipid parameters. Only LDL-CIC, but not any other lipid parameter, was contingent with the progression of early carotid atherosclerosis ($P = 0.042$) and also had the highest levels of relative risk and odds ratio [59]. Normal level of LDL-CIC (below 16.0 μg/mL) was the only parameter that predicted the absence of carotid atherosclerosis progression for two following years at prognostic value of 78.3% (95% CI, 67.1–87.3) [59]. Normal levels of serum total cholesterol, LDL and HDL cholesterol, and triglycerides did not possess statistically significant predictive values. Thus, in spite of the absence of clinical manifestations of atherosclerosis, the elevated level of LDL-CIC is associated with increased intima-media thickness and can be regarded as a predictor for higher risk of atherosclerosis development [58, 59].

In large meta-analyses of prospective population studies in 165,544 participants without baseline CVD in 37 prospective cohorts (calendar years of recruitment: 1968–2007) with up to 15,126 incident fatal or nonfatal CVD outcomes (10,132 CHD and 4994 stroke outcomes) during a median follow-up of 10.4 years (interquartile range: 7.6–14 years), both baseline LDL and HDL cholesterol levels were strong predictors of both CHD and stroke [60]. The studies included in this cited meta-analysis described conventional lipid profile parameters, but not LDL-CIC, as predicting variables. However, the results of analysis demonstrate that the predictive biomarkers in healthy population are not the same as those for the diagnosis and prognosis of atherosclerosis; therefore, diagnostic and prognostic role of LDL-CIC should be in focus for further investigations in both nonatherosclerotic subjects and preclinical and overt atherosclerosis.

5. Conclusions

On the basis of current data, it is possible to define the role of lipoprotein-containing immune complexes in atherogenesis (Figure 1). It can be supposed that LDL-CIC may play a significant role in atherogenesis and are involved in the formation of early atherosclerotic lesion. LDL-CIC can induce massive cholesterol accumulation in cultured vascular cells

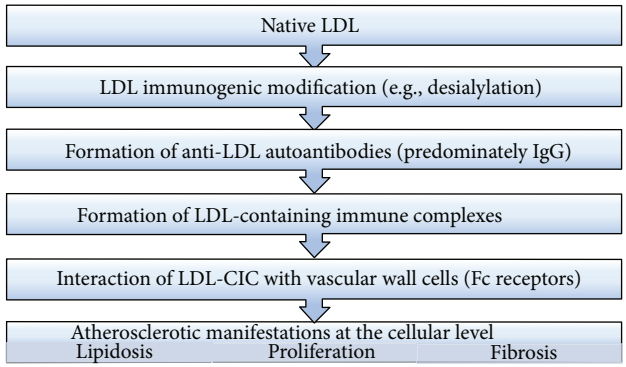

Figure 1: Schematic presentation of the role of lipoprotein-containing immune complexes in atherogenesis.

that leads to foam cell formation, cellular proliferation, and extracellular matrix production [5, 36]. Modified LDL (e.g., desialylated, glycosylated, and oxidized LDL), which has an atherogenic potential in contrast to native LDL, appears in the blood. It stimulates atherosclerotic manifestations at the arterial cell level, for example, by inducing the intracellular lipid accumulation. Atherogenic modified LDL triggers the production of anti-LDL autoantibodies which react with LDL, leading to the formation of an LDL-containing immune complex. Interaction of anti-LDL antibodies with modified LDL increases their atherogenic potential. After forming an immune complex with anti-LDL antibodies, the originally nonatherogenic, native LDL becomes atherogenic (i.e., they are capable of inducing intracellular lipid accumulation and other atherosclerosis-related alterations). On entering the subendothelial space of the arterial intima and interacting with subendothelial cells, lipoprotein-containing immune complexes may induce the whole spectrum of atherosclerotic cellular perturbations. Titer of LDL-CIC in blood serum significantly correlates with progression of IMT and has the highest diagnostic value among other measured serum lipid parameters. After removal of CIC, the sera lose their atherogenic properties. Elevation of CIC-cholesterol seems to be a characteristic feature of coronary atherosclerosis while CIC-cholesterol might well be a possible risk factor.

Abbreviations

CHD:	Coronary heart disease
CIC:	Circulating immune complexes
HDL:	High density lipoprotein
LDL:	Low density lipoprotein
LDL-CIC:	LDL-containing circulating immune complexes
MDA:	Malondialdehyde
SMC:	Smooth muscle cells
IMT:	Intima-media thickness.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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References

[1] V. V. Tertov, A. N. Orekhov, L. H. Ryong, and V. N. Smirnov, "Intracellular cholesterol accumulation is accompanied by enhanced proliferative activity of human aortic intimal cells," *Tissue and Cell*, vol. 20, no. 6, pp. 849–854, 1988.

[2] A. N. Orekhov, V. V. Tertov, S. A. Kudryashov, and V. N. Smirnov, "Triggerlike stimulation of cholesterol accumulation and DNA and extracellular matrix synthesis induced by atherogenic serum or low density lipoprotein in cultured cells," *Circulation Research*, vol. 66, no. 2, pp. 311–320, 1990.

[3] P. Libby, P. M. Ridker, and G. K. Hansson, "Progress and challenges in translating the biology of atherosclerosis," *Nature*, vol. 473, no. 7347, pp. 317–325, 2011.

[4] V. V. Tertov, A. N. Orekhov, K. S. Sayadyan et al., "Correlation between cholesterol content in circulating immune complexes and atherogenic properties of CHD patients'serum manifested in cell culture," *Atherosclerosis*, vol. 81, no. 3, pp. 183–189, 1990.

[5] V. V. Tertov, A. N. Orekhov, A. G. Kacharava, I. A. Sobenin, N. V. Perova, and V. N. Smirnov, "Low density lipoprotein-containing circulating immune complexes and coronary atherosclerosis," *Experimental and Molecular Pathology*, vol. 52, no. 3, pp. 300–308, 1990.

[6] C. Gisinger, G. T. Virella, and M. F. Lopes-Virella, "Erythrocyte-bound low-density lipoprotein immune complexes lead to cholesteryl ester accumulation in human monocyte-derived macrophages," *Clinical Immunology and Immunopathology*, vol. 59, no. 1, pp. 37–52, 1991.

[7] A. G. Kacharava, V. V. Tertov, and A. N. Orekhov, "Autoantibodies against low density lipoprotein and atherogenic potential of blood," *Annals of Medicine*, vol. 25, no. 6, pp. 551–555, 1993.

[8] G. Virella, M. F. Lopes-Virella, C. J. Binder, and J. L. Witztum, "Humoral immunity and atherosclerosis," *Nature Medicine*, vol. 9, no. 3, pp. 243–245, 2003.

[9] M. F. Lopes-Virella, N. Binzafar, S. Rackley, A. Takei, M. La Via, and G. Virella, "The uptake of LDL-IC by human macrophages: predominant involvement of the FcγRI receptor," *Atherosclerosis*, vol. 135, no. 2, pp. 161–170, 1997.

[10] J. L. Beaumont, "Hyperlipidemia with circulating anti-beta-lipoprotein auto-antibody in man. Auto-Immune hyperlipidemia, its possible role in atherosclerosis," *Progress in Biochemical Pharmacology*, vol. 4, pp. 110–121, 1968.

[11] J. L. Beaumont, "L'hyperlipidemie par auto-anticorps anti-beta-lipoproteine. Une nouvelle entile pathologique," *Comptes Rendus Hebdomadaires des Seances de l Academie des Sciences D*, vol. 261, pp. 4563–4566, 1965.

[12] E. Szondy, M. Horváth, Z. Mezey et al., "Free and complexed anti-lipoprotein antibodies in vascular diseases," *Atherosclerosis*, vol. 49, no. 1, pp. 69–77, 1983.

[13] B. J. Bauer, K. Blashfield, R. Norris, D. A. Buthala, and L. C. Ginsberg, "Immunoglobulin as the major low density lipoprotein binding protein in plasma," *Atherosclerosis*, vol. 44, no. 2, pp. 153–160, 1982.

[14] U. P. Steinbrecher, M. Fisher, J. L. Witztum, and L. K. Curtiss, "Immunogenicity of homologous low density lipoprotein after methylation, ethylation, acetylation, or carbamylation: generation of antibodies specific for derivatized lysine," *Journal of Lipid Research*, vol. 25, no. 10, pp. 1109–1116, 1984.

[15] J. L. Witztum, U. P. Steinbrecher, Y. A. Kesaniemi, and M. Fisher, "Autoantibodies to glucosylated proteins in the plasma of patients with diabetes mellitus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 81, no. 10, pp. 3204–3208, 1984.

[16] W. Palinski, M. E. Rosenfeld, S. Yla-Herttuala et al., "Low density lipoprotein undergoes oxidative modification *in vivo*," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 86, no. 4, pp. 1372–1376, 1989.

[17] W. Hollander, M. A. Colombo, B. Kirkpatrick, and J. Paddock, "Soluble proteins in the human atheroschlerotic plaque. With Spectral Reference to Immunoglobulins, C3-Complement Component, α1-Antitrypsin and α2-Macroglobulin," *Atherosclerosis*, vol. 34, no. 4, pp. 391–405, 1979.

[18] G. K. Hansson, G. Bondjers, A. Bylock, and L. Hjalmarsson, "Ultrastructural studies on the localization of IgG in the aortic endothelium and subendothelial intima of atherosclerotic and nonatherosclerotic rabbits," *Experimental and Molecular Pathology*, vol. 33, no. 3, pp. 302–315, 1980.

[19] D. Parums and M. J. Mitchinson, "Demonstration of immunoglobulin in the neighbourhood of advanced atherosclerotic plaques," *Atherosclerosis*, vol. 38, no. 1-2, pp. 211–216, 1981.

[20] L. A. Lewis and I. H. Page, "An unusual serum lipoprotein-globulin complex in a patient with hyperlipemia," *The American Journal of Medicine*, vol. 38, no. 2, pp. 286–297, 1965.

[21] J. L. Beaumont, "Autoimmune hyperlipidemia," in *Atherosclerosis (Proceedings of the 2nd International Symposium)*, R. G. Jones, Ed., Springer, Berlin, Germany, 1970.

[22] G. Noseda, W. Riesen, E. Schlumpf, and A. Morell, "Hypo-a-lipoproteinaemia associated with auto-antibodies against-lipoproteins," *European Journal of Clinical Investigation*, vol. 2, no. 5, pp. 342–347, 1972.

[23] A. N. Klimov, A. D. Denisenko, N. Zubzhitsky Yu., and E. A. Gerchikova, "Detection of autoimmune complex lipoprotein-antibody in human blood plasma and aorta wall," *Voprosy Meditsinskoj Khimii*, vol. 24, no. 4, pp. 539–543, 1978 (Russian).

[24] A. N. Orekhov, "Lipoprotein immune complexes and their role in atherogenesis," *Current Opinion in Lipidology*, vol. 2, no. 5, pp. 329–333, 1991.

[25] J. T. Salonen, "Markers of oxidative damage and antioxidant protection: assessment of LDL oxidation," *Free Radical Research*, vol. 33, pp. S41–S46, 2000.

[26] P. Avogaro, G. B. Bon, and G. Gazzolato, "Presence of a modified low density lipoprotein in humans," *Arteriosclerosis*, vol. 8, no. 1, pp. 79–87, 1988.

[27] L. K. Curtiss and J. L. Witztum, "Plasma apolipoproteins AI, AII, B, CI, and E are glucosylated in hyperglycemic diabetic subjects," *Diabetes*, vol. 34, no. 5, pp. 452–461, 1985.

[28] A. N. Orekhov, V. V. Tertov, D. N. Mukhin, and I. A. Mikhailenko, "Modification of low density lipoprotein by desialylation causes lipid accumulation in cultured cells: discovery of desialylated lipoprotein with altered cellular metabolism in the blood of atherosclerotic patients," *Biochemical and Biophysical Research Communications*, vol. 162, no. 1, pp. 206–211, 1989.

[29] U. Mutlu-Türkoğlu, Z. Akalin, E. Ilhan et al., "Increased plasma malondialdehyde and protein carbonyl levels and lymphocyte DNA damage in patients with angiographically defined coronary artery disease," *Clinical Biochemistry*, vol. 38, no. 12, pp. 1059–1065, 2005.

[30] Z. J. Zhang, "Systematic review on the association between F2-isoprostanes and cardiovascular disease," *Annals of Clinical Biochemistry*, vol. 50, part 2, pp. 108–114, 2013.

[31] B. Shao and J. W. Heinecke, "HDL, lipid peroxidation, and atherosclerosis," *Journal of Lipid Research*, vol. 50, no. 4, pp. 599–601, 2009.

[32] T. J. Lyons, R. L. Klein, J. W. Baynes, H. C. Stevenson, and M. F. Lopes-Virella, "Stimulation of cholesteryl ester synthesis in human monocyte-derived macrophages by low-density lipoproteins from Type 1 (insulin-dependent) diabetic patients: the influence of non-enzymatic glycosylation of low-density lipoproteins," *Diabetologia*, vol. 30, no. 12, pp. 916–923, 1987.

[33] M. F. Lopes-Virella, R. L. Klein, T. J. Lyons, H. C. Stevenson, and J. L. Witztum, "Glycosylation of low-density lipoprotein enhances cholesteryl ester synthesis in human monocyte-derived macrophages," *Diabetes*, vol. 37, no. 5, pp. 550–557, 1988.

[34] V. V. Tertov, I. A. Sobenin, A. G. Tonevitsky, A. N. Orekhov, and V. N. Smirnov, "Isolation of atherogenic modified (desialylated) low density lipoprotein from blood of atherosclerotic patients: separation from native lipoprotein by affinity chromatography," *Biochemical and Biophysical Research Communications*, vol. 167, no. 3, pp. 1122–1127, 1990.

[35] A. N. Klimov, A. D. Denisenko, A. G. Vinogradov et al., "Accumulation of cholesteryl esters in macrophages incubated with human lipoprotein-antibody autoimmune complex," *Atherosclerosis*, vol. 74, no. 1-2, pp. 41–46, 1988.

[36] V. V. Tertov, I. A. Sobenin, A. N. Orekhov, O. Jaakkola, T. Solakivi, and T. Nikkari, "Characteristics of low density lipoprotein isolated from circulating immune complexes," *Atherosclerosis*, vol. 122, no. 2, pp. 191–199, 1996.

[37] A. N. Orekhov, V. V. Tertov, and D. N. Mukhin, "Desialylated low density lipoprotein-naturally occurring modified lipoprotein with atherogenic potency," *Atherosclerosis*, vol. 86, no. 2-3, pp. 153–161, 1991.

[38] V. V. Tertov, A. N. Orekhov, I. A. Sobenin, J. D. Morrisett, A. M. Gotto Jr., and J. G. Guevara Jr., "Carbohydrate composition of protein and lipid components in sialic acid- rich and -poor low density lipoproteins from subjects with and without coronary artery disease," *Journal of Lipid Research*, vol. 34, no. 3, pp. 365–375, 1993.

[39] V. V. Tertov, I. A. Sobenin, Z. A. Gabbasov et al., "Multiple-modified desialylated low density lipoproteins that cause intracellular lipid accumulation: isolation, fractionation and characterization," *Laboratory Investigation*, vol. 67, no. 5, pp. 665–675, 1992.

[40] A. N. Orekhov, V. V. Tertov, A. E. Kabakov, I. Y. Adamova, S. N. Pokrovsky, and V. N. Smirnov, "Autoantibodies against modified low density lipoprotein. Nonlipid factor of blood plasma that stimulates foam cell formation," *Arteriosclerosis and Thrombosis*, vol. 11, no. 2, pp. 316–326, 1991.

[41] C. Dachet, M. F. Baudet, and J. L. Beaumont, "Cholesterol synthesis by human fibroblasts in the presence of LDL and anti-LDL IgA," *Biomedicine*, vol. 31, no. 3, pp. 80–82, 1979.

[42] A. N. Klimov, A. D. Denisenko, A. V. Popov et al., "Lipoprotein-antibody immune complexes their catabolism and role in foam cell formation," *Atherosclerosis*, vol. 58, no. 1–3, pp. 1–15, 1985.

[43] R. L. Griffith, G. T. Virella, H. C. Stevenson, and M. F. Lopes-Virella, "Low density lipoprotein metabolism by human

macrophages activated with low density lipoprotein immune complexes. A possible mechanism of foam cell formation," *Journal of Experimental Medicine*, vol. 168, no. 3, pp. 1041–1059, 1988.

[44] A. N. Orekhov, V. V. Tertov, D. N. Mukhin et al., "Insolubilization of low density lipoprotein induces cholesterol accumulation in cultured subendothelial cells of human aorta," *Atherosclerosis*, vol. 79, no. 1, pp. 59–70, 1989.

[45] A. N. Orekhov and V. V. Tertov, "Antibody-like immunoglobulins G against low density lipoprotein that stimulate lipid accumulation in cultured cells," *Advances in Experimental Medicine and Biology*, vol. 285, pp. 399–405, 1991.

[46] A. N. Orekhov and V. V. Tertov, "Atherogenicity of autoantibodies against low density lipoprotein," *Agents and Actions*, vol. 32, no. 1-2, pp. 128–129, 1991.

[47] D. F. Burut, Y. Karim, and G. A. A. Ferns, "The role of immune complexes in atherogenesis," *Angiology*, vol. 61, no. 7, pp. 679–689, 2010.

[48] S. A. Abdelsamie, Y. Li, Y. Huang et al., "Oxidized LDL immune complexes stimulate collagen IV production in mesangial cells via Fc gamma receptors I and III," *Clinical Immunology*, vol. 139, no. 3, pp. 258–266, 2011.

[49] R. Oksjoki, P. T. Kovanen, K. A. Lindstedt, B. Jansson, and M. O. Pentikäinen, "OxLDL-IgG immune complexes induce survival of human monocytes," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 3, pp. 576–583, 2006.

[50] M.-F. Baudet, C. Dachet, and J. L. Beaumont, "Interaction between fibroblasts, lipoproteins and three antilipoproteins IgA kappa," *Clinical and Experimental Immunology*, vol. 39, no. 2, pp. 455–460, 1980.

[51] M. J. Tikkanen, R. Dargar, B. Pfleger, B. Gonen, J. M. Davie, and G. Schonfeld, "Antigenic mapping of human low density lipoprotein with monoclonal antibodies," *Journal of Lipid Research*, vol. 23, no. 7, pp. 1032–1038, 1982.

[52] A. N. Klimov, A. D. Denisenko, A. V. Popov et al., "Lipoprotein-antibody immune complexes their catabolism and role in foam cell formation," *Atherosclerosis*, vol. 58, no. 1–3, pp. 1–15, 1985.

[53] A. N. Orekhov, O. S. Kalenich, V. V. Tertov, and I. D. Novikov, "Lipoprotein immune complexes as markers of atherosclerosis," *International Journal of Tissue Reactions*, vol. 13, no. 5, pp. 233–236, 1991.

[54] A. N. Orekhov, O. S. Kalenich, V. V. Tertov et al., "Diagnostic value of immune cholesterol as a marker for atherosclerosis," *Journal of Cardiovascular Risk*, vol. 2, no. 5, pp. 459–466, 1995.

[55] J. T. Salonen, S. Ylä-Herttuala, R. Yamamoto et al., "Autoantibody against oxidised LDL and progression of carotid immunoglobulin," *The Lancet*, vol. 339, no. 8798, pp. 883–887, 1992.

[56] M. F. Lopes-Virella, M. B. McHenry, S. Lipsitz et al., "Immune complexes containing modified lipoproteins are related to the progression of internal carotid intima-media thickness in patients with type 1 diabetes," *Atherosclerosis*, vol. 190, no. 2, pp. 359–369, 2007.

[57] M. F. Lopes-Virella, K. J. Hunt, N. L. Baker, J. Lachin, D. M. Nathan, and G. Virella, "Levels of oxidized LDL and advanced glycation end products-modified LDL in circulating immune complexes are strongly associated with increased levels of carotid intima-media thickness and its progression in type 1 diabetes," *Diabetes*, vol. 60, no. 2, pp. 582–589, 2011.

[58] I. A. Sobenin, V. P. Karagodin, A. A. Melnichenko, Y. V. Bobryshev, and A. N. Orekhov, "Diagnostic and prognostic value of low density lipoprotein-containing circulating immune complexes in atherosclerosis," *Journal of Clinical Immunology*, vol. 33, no. 2, pp. 489–495, 2013.

[59] I. A. Sobenin, V. A. Orekhova, A. A. Melnichenko, Y. V. Bobryshev, and A. N. Orekhov, "Low density lipoprotein-containing circulating immune complexes have better prognostic value in carotid intima-media thickness progression than other lipid parameters," *International Journal of Cardiology*, vol. 166, no. 3, pp. 747–748, 2013.

[60] E. Di Angelantonio, P. Gao, L. Pennells et al., "Lipid-related markers and cardiovascular disease prediction," *Journal of the American Medical Association*, vol. 307, no. 23, pp. 2499–2506, 2012.

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Review Article

Periodontal Pathogens and Atherosclerosis: Implications of Inflammation and Oxidative Modification of LDL

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Inflammation is well accepted to play a crucial role in the development of atherosclerotic lesions, and recent studies have demonstrated an association between periodontal disease and cardiovascular disease. *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, causative agents of destructive chronic inflammation in the periodontium, can accelerate atheroma deposition in animal models. Emerging evidence suggests that vaccination against virulence factors of these pathogens and anti-inflammatory therapy may confer disease resistance. In this review, we focus on the role of inflammatory mechanisms and oxidative modification in the formation and activation of atherosclerotic plaques accelerated by *P. gingivalis* or *A. actinomycetemcomitans* in an ApoE-deficient mouse model and high-fat-diet-fed mice. Furthermore, we examine whether mucosal vaccination with a periodontal pathogen or the anti-inflammatory activity of catechins can reduce periodontal pathogen-accelerated atherosclerosis.

1. Introduction

Periodontitis, a chronic, destructive condition affecting a large portion of the adult population, is one of the major causes of tooth loss and characterized by a chronic infection associated with gram-negative anaerobic bacteria in the dental biofilm. It leads to irreversible destruction of tissues supporting the teeth and is clinically detectable as periodontal pockets and alveolar bone loss [1, 2]. Since periodontitis often causes bacteremia, a relationship between periodontitis and systemic diseases via periodontal pathogens has been explored [3]. Two or more periodontopathic bacteria have been detected in the cardiac valve [4, 5] and aortic aneurysm [6]; therefore, periodontal infection could also affect the progression of cardiovascular disease (CVD). However, many questions regarding this causal relationship and pathological mechanism need to be answered.

This review discusses the involvement of periodontopathic bacteria in the development of atherosclerosis. Furthermore, we mention the possibility of preventing atherosclerosis by developing a vaccine for specific bacteria or common antigens, as well as by the intake of catechin, which has both antioxidative and anti-inflammatory effects.

2. The Periodontal-Systemic Relationship

Epidemiological studies have suggested that periodontal infections are associated with an increased risk of CVD [7–10]. For example, it was shown that patients with periodontitis have a 19% greater risk of CVD compared to subjects without periodontitis [11, 12]. Furthermore, severe periodontitis is associated with increased intima-media thickening [13], while a systemic antibody response to a periodontal organism is associated with coronary heart disease [14]. Although observational studies suggest that such an association is independent of known confounders such as hypertension, smoking, diabetes, and obesity, this contention has not been confirmed [2]. In contrast, a recent cross-sectional study indicated that periodontitis is associated with endothelial dysfunction in a general population [15].

Considerable evidence indicates that periodontopathic bacteria may directly or indirectly contribute to cardiovascular disease, such as blood platelet aggregation, enhanced low-density cholesterol and lipoprotein deposition in the arterial walls, invasion of cardiac and carotid endothelium, and the high level of circulating- or tissue-derived inflammatory mediators [16–18]. Periodontal bacteria may directly infect

atherosclerotic lesions, thus contributing to the inflammatory process. The fact that periodontopathic bacteria were detected from atheromatous plaques of coronary arteries suggested that periodontal bacteria such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* invade endothelial cells and induce chronic vascular inflammation [19]. Furthermore, *P. gingivalis*, *A. actinomycetemcomitans*, *Fusobacterium nucleatum*, and other oral anaerobes were shown to degrade immunoglobulin, inhibit the complement system, and produce toxic components such as lipopolysaccharide (endotoxins) and secreted molecules (exotoxins) [20, 21]. These factors sustain bacterial viability during bacteremia, which commonly occurs as a result of surgical treatments, tooth brushing, and other dental procedures [16, 22]. Chronic periodontal infections, initiated by gram-negative tooth-associated microbial biofilms, may also indirectly induce endothelial activation or dysfunction through a state of systemic inflammation as evidenced by elevated plasma acute phase proteins; proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α; and the matrix metalloproteinase (MMP) family [23–26]. Moreover, the release of bacterial products such as outer membrane vesicles [27] or gingipains [28] from *P. gingivalis* or the release of free soluble components from *A. actinomycetemcomitans* [29] into the circulation can induce proatherogenic responses in endothelial cells. Immune activation by the pathogen-derived heat-shock protein (HSP) GroEL may also result in an autoimmune response followed by atherosclerosis via the structural similarity or "molecular mimicry" between host HSP60 and GroEL. Previous studies have shown that HSP60 is selectively located in atherosclerotic lesions rather than nonatherosclerotic areas of the arterial wall [30]. In addition, a significant correlation has been observed between anti-HSP antibody (Ab) levels and the severity of atherosclerosis. High titers of anti-HSP60 Abs have been identified in patients with carotid atherosclerosis, coronary disease, and stroke [31]. HSP of a major periodontal pathogen, such as *P. gingivalis* (GroEL), was also suggested to be a key molecule linking periodontitis (an infectious disease) with atherosclerosis (an autoimmune disease) [32, 33]. Clonal analysis of the T-cells clearly demonstrated that both human HSP60- and *P. gingivalis* GroEL-reactive T-cell populations were present in the peripheral circulation of patients with atherosclerosis [34]. In addition, *P. gingivalis* can induce platelet aggregation [35], and lipopolysaccharide (LPS) from periodontal pathogens can induce the formation of foam cells [36, 37]. Therefore, we assessed the potential of periodontal infection in atherosclerosis progression.

3. Role of Periodontal Infection in Atherosclerosis Progression

Animal models have been used to examine atherosclerotic pathology caused by periodontopathic bacteria. *P. gingivalis* has been reported by our and other groups to accelerate atherosclerosis in ApoE-KO or apolipoprotein E-deficient spontaneously hyperlipidemic ($ApoE^{shl}$) mice [38–41]. *A. actinomycetemcomitans* bacteremia also aggravated atherosclerosis in $ApoE^{shl}$ mice [42, 43]. In addition to murine models, rabbits with induced periodontal disease accumulated fatty streaks in the aorta earlier than periodontally healthy animals [44]. *P. gingivalis* bacteremia also enhanced atherosclerosis in normocholesterolemic and hypercholesteromic pigs [45]. Thus, in addition to the detection of live periodontal pathogens in human atherosclerotic plaques, *in vivo* experiments in a variety of animal models have corroborated that *P. gingivalis* can enhance atherogenesis.

4. Endothelial Cell Activation

Periodontal bacteria and their products can initiate an inflammatory response in the periodontal tissues with systemic consequences. Moreover, bacteria and their products can gain access to the circulation during dental procedures such as scaling and root planning, or even after vigorous home care, especially in patients with periodontitis. Once in the blood stream, bacterial antigens can induce a systemic immune response. Periodontal bacteria in the blood can be carried to distant sites such as the heart tissues freely in the circulation or within circulating cells such as monocytes, neutrophils, or platelets and can initiate pathogenic processes. Tissue or cellular invasion is a key virulence property of many bacterial species. Periodontal pathogens including *P. gingivalis* also adhere to and invade various human vascular cells in culture and animal model [40, 46, 47]. Furthermore, *P. gingivalis* can simulate monocyte adhesion to human umbilical vein endothelial cells [40]. Endothelial activation and increased expression of adhesion molecules and chemokines comprise an initial step in the development of atherosclerotic lesions. *A. actinomycetemcomitans* infection of $ApoE^{shl}$ mice resulted in increased expression of intercellular adhesion molecule 1 (ICAM-1), monocyte chemoattractant protein 1 (MCP-1), E-selectin, P-selection, chemokine (C-C motif) ligand 19 (CCL19), CCL21, and C-C chemokine receptor 7 in the aorta [42]. Coculture of human aortic endothelial cells with *P. gingivalis* also increased the expression of ICAM-1, vascular cell adhesion molecule 1 (VCAM-1), MCP-1, and E-selectin by a fimbriae-dependent activation mechanism [48, 49]. Chemical inhibition of endocytosis blocked the upregulation of MCP-1, indicating the need of bacterial invasion for MCP-1 stimulation [50]. In contrast, stimulation of endothelial cells with LPS, an outer membrane protein, and HSP60 derived from *P. gingivalis* had only a slight stimulatory effect on ICAM-1 and VCAM-1 expression [51].

5. Toll-Like Receptor- and Nod-Like Receptor-Mediated Responses

Exposure of periodontal pathogens to endothelial cells may result in increased expression and interaction with Toll-like receptors (TLRs). *A. actinomycetemcomitans* infection of $ApoE^{shl}$ mice enhanced mRNA expression of TLR2, TLR4, TLR-9, and nucleotide binding oligomerization domain 1 (NOD-1) in the aorta [42, 43]. Treatment with the heat-killed

(H.K.) *A. actinomycetemcomitans* or *A. actinomycetemcomitans* LPS also increased TLR4, NOD1, and NOD2 expression. However, the order of atherosclerosis extent was as follows: live *A. actinomycetemcomitans* > H.K. *A. actinomycetemcomitans* > *A. actinomycetemcomitans* LPS. Furthermore, a significant difference in atherosclerotic lesion size was observed between *A. actinomycetemcomitans*-challenged mice and *A. actinomycetemcomitans* LPS-challenged mice. ApoE knockout mice challenged with *P. gingivalis* also expressed TLR2 and TLR4 in aortic tissue [39], while challenge with an invasion-impaired *P. gingivalis* fimbriae-deficient mutant did not upregulate TLRs, suggesting that innate immune recognition of invasive bacteria is a prerequisite for the acceleration of atherosclerosis. In contrast, ApoE(–/–) and TLR4(–/–) double-knockout mice were markedly more susceptible to atherosclerosis after oral infection with *P. gingivalis*, demonstrating an atheroprotective role for TLR4 in response to *P. gingivalis* infection [52].

6. Oxidative Stress-Mediated Mechanisms

Lipid peroxidation plays an important role in many diseases [53]. In particular, low-density lipoprotein (LDL) oxidation may be a key step in the development of atherosclerosis [54]. Oxidation of LDL is essential for its accumulation within the macrophages and for the formation of foam cells, which can upregulate proatherogenic chemokines and adhesion molecules [55] and induce interleukin 6 (IL-6), tumor necrosis factor alpha, and C-reactive protein secretion [56]. *A. actinomycetemcomitans* infection of ApoE^{shl} mice promoted LDL oxidation, as indicated by the marked upregulation of 4-hydroxynoneal, oxidated LDL, and phospholipase A2 in the aorta, ox-LDL, 8-oxo-2′-deoxyguanosine, and myeloperoxidase serum levels, and aortic expression of nicotinamide adenine dinucleotide phosphate oxidase, caveolin-1, and RAGE [43]. *P. gingivalis* also increased oxidative modification of LDL [57, 58] and rupture of atherosclerotic plaque through induction of MMP [59]. Indeed, patients with periodontitis have increased levels of lipid peroxidation in plasma, saliva, and gingival crevicular fluid [60], and these levels have been correlated with the severity of periodontal disease [60, 61]. Coincubation of a murine macrophage cell line with *P. gingivalis* in the presence of LDL resulted in the formation of foam cells in a dose-dependent manner [62].

7. Hyperlipidemia-Induced Atherosclerosis

CVD remains the leading cause of morbidity and mortality in the Western world. A sedentary lifestyle and Western dietary habits may contribute to this increased risk of CVD development [63, 64]. For example, consumption of a diet rich in saturated fat is positively associated with elevated plasma lipid levels and a state of subacute chronic inflammation, which are important risk factors promoting both the onset and development of CVD [65, 66]. Research on a correlation between periodontopathic bacteria and atherosclerosis has been performed using hyperlipidemic animals [38, 39, 45, 67]. Furthermore, a previous study demonstrated that an increase in atherosclerosis is associated with *P. gingivalis* infection in B6.ApoE^{shl} mice, but not in wild-type mice fed a regular chow diet (RD) [68]. In contrast, *P. gingivalis* injection significantly increased the size and lipid content of atherosclerotic lesions in C57BL/6 mice fed a high fat diet (HFD) [69]. Inoculation of *C. pneumoniae* into C57BL/6 mice fed a HFD also accelerated hypercholesterolemia-induced atherosclerosis [70]. In contrast, chlamydial inoculation into C57BL/6 mice fed the RD did not affect aortic lipid accumulation, although inflammatory changes were induced [71]. Upon infection with *P. gingivalis*, gene expression profiles of the aorta and liver in wild-type mice showed proatherogenic profiles, even in animals fed the RD [68]. Challenge with *P. gingivalis* also induced inflammation associated with elevated serum IL-6, IL-8, and MCP-1 levels, even in C57BL/6 mice fed the RD; however, plaque formation was only slightly elevated. Therefore, the inflammatory response caused by periodontal infection may act in concert with hyperlipidemia to exacerbate atherosclerotic lesion formation.

8. Prevention of Atherosclerosis by Mucosal Vaccination

Since several possible mechanisms may be involved in the acceleration of atherosclerosis by periodontal pathogens, the prevention of periodontal infection may be an effective way to reduce the induction of atherosclerosis, as well as periodontitis. Thus, the prevention of periodontitis might be relevant not only for oral, but also for systemic health. Atherosclerosis and inflammation in ApoE^{shl} and HFD-fed C57/BL6 mice challenged with *P. gingivalis* were effectively prevented by nasal immunization with the 40 kDa outer membrane protein of *P. gingivalis* [41, 69]. Furthermore, since HSP60 (GroEL) from *P. gingivalis* can trigger molecules linking infectious periodontitis and autoimmune atherosclerosis [32, 33], mucosal administration of a relevant autoantigen is an effective method of attenuating autoimmune disease by inducing an unresponsive state of tolerance [72, 73]. Sublingual immunization with *P. gingivalis* GroEL controlled the acceleration of disease by *P. gingivalis* infection with an increase in the GroEL antibody [74]. Therefore, mucosal vaccination with GroEL may control inflammation and the progression of atherosclerosis due to periodontopathic bacterial infection.

9. Prevention of Atherosclerosis Using an Anti-Inflammatory Agent

Previous studies have indicated that early activation of inflammatory mediators in response to a challenge with periodontal pathogens may be associated with periodontitis-associated atherosclerosis [41, 42, 69]. Therefore, oral inflammatory diseases and systemic inflammation caused by periodontal infection may contribute to atherosclerosis. Green tea is a popular drink worldwide, and consumption of green tea has been suggested to prevent the development of a variety of diseases, including diabetes, hypertension, cancer, and cardiovascular diseases [75]. The effects of green tea are

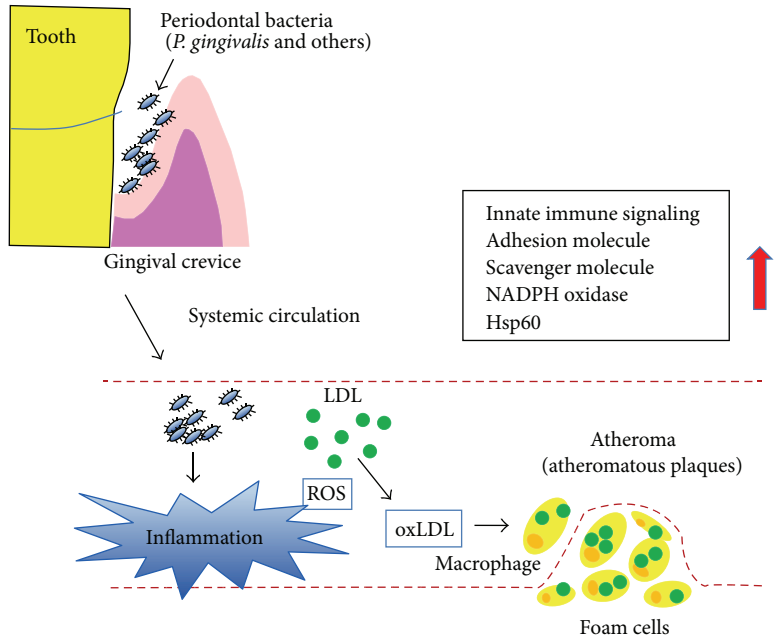

Figure 1

attributed to its abundant and biologically active ingredient catechin, or epigallocatechin-3-gallate (EGCG), which has antioxidative [76], anti-inflammatory [77], and antiangiogenic [78] effects. Consumption of EGCG in ApoE knockout mice decreased atherosclerotic lesions, proinflammatory cytokines, and inflammatory- and oxidative stress-related mediators in the serum and aorta induced by *P. gingivalis* [79]. Therefore, previous catechin consumption may be useful for the prevention of pathogen-accelerated atherosclerosis.

10. Conclusions

The association between periodontal disease and atherosclerotic cardiovascular disease is supported by a large body of evidence. Periodontal bacteria were shown to accelerate the progression of atherosclerosis in ApoE knockout mice, rabbits, and pigs. Furthermore, observations have suggested that inflammation caused by periodontopathic bacteria may play a synergistic role with other preexisting factors, such as hyperlipidemia, resulting in the development of atherosclerosis. These findings support the hypothesis that a periodontal pathogen is not an independent risk factor but instead acts in concert with hyperlipidemia to exacerbate atherosclerosis lesion formation. However, whether live bacteria are necessary for this process, or if its component alone is sufficient to cause an increase in atherosclerotic damage, remains unclear. In a complex tissue, such as an atherosclerotic lesion, innate signals can originate from several sources and promote atherogenesis through an association with pattern-recognition receptors (PRRs). These signals include various extracellular activation cascades and intracellular signaling pathways and lead to effective clearance of infectious agents and induction of inflammatory responses. Since live bacteria can activate multiple PRRs, they may induce a more significant inflammatory response than their components. Finally, the best method to prevent atherosclerosis is by blocking bacterial invasion and bacteria-induced inflammation and lipid peroxidation. Previous results have demonstrated that atherosclerosis and inflammation with lipid peroxidation are accelerated in ApoE-deficient mice after an infection of periodontal bacteria (Figure 1) and that these can be prevented by mucosal immunization with bacterial products or an anti-inflammatory agent.

Disclosure

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see http://www.textcheck.com/certificate/iW3mmS.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] W. J. Loesche and N. S. Grossman, "Periodontal disease as a specific, albeit chronic, infection: diagnosis and treatment," *Clinical Microbiology Reviews*, vol. 14, no. 4, pp. 727–752, 2001.

[2] B. Burt, "Epidemiology of periodontal diseases," *Journal of Periodontology*, vol. 76, no. 8, pp. 1406–1419, 2005.

[3] I. Olsen, "Update on bacteraemia related to dental procedures," *Transfusion and Apheresis Science*, vol. 39, no. 2, pp. 173–178, 2008.

[4] K. Nakano, H. Nemoto, R. Nomura et al., "Detection of oral bacteria in cardiovascular specimens," *Oral Microbiology and Immunology*, vol. 24, no. 1, pp. 64–68, 2009.

[5] K. Nakano, H. Inaba, R. Nomura et al., "Distribution of *Porphyromonas gingivalis* fimA genotypes in cardiovascular specimens from Japanese patients," *Oral Microbiology and Immunology*, vol. 23, no. 2, pp. 170–172, 2008.

[6] N. Kurihara, Y. Inoue, T. Iwai, M. Umeda, Y. Huang, and I. Ishikawa, "Detection and localization of periodontopathic bacteria in abdominal aortic aneurysms," *European Journal of Vascular and Endovascular Surgery*, vol. 28, no. 5, pp. 553–558, 2004.

[7] M. Sanz, F. D'aiuto, J. Deanfield, and F. Fernandez-Avilés, "European workshop in periodontal health and cardiovascular disease—scientific evidence on the association between periodontal and cardiovascular diseases: a review of the literature," *European Heart Journal Supplement*, vol. 12, pp. B3–B12, 2010.

[8] P. B. Lockhart, A. F. Bolger, P. N. Papapanou et al., "Periodontal disease and atherosclerotic vascular disease: does the evidence support an independent association? A scientific statement form the American Heart Association," *Circulation*, vol. 125, no. 20, pp. 2520–2544, 2012.

[9] T. Dietrich, P. Sharma, C. Walter, P. Weston, and J. Beck, "The epidemiological evidence behind the association between periodontitis and incident atherosclerotic cardiovascular disease," *Journal of Clinical Periodontology*, vol. 40, no. 4, supplement, pp. S70–S84, 2013.

[10] P. Bouchard, P. Boutouyrie, F. D'aiuto et al., "European workshop in periodontal health and cardiovascular disease consensus document," *European Heart Journal Supplement*, vol. 12, pp. B13–B22, 2010.

[11] Y. S. Khader, Z. S. M. Albashaireh, and M. A. Alomari, "Periodontal diseases and the risk of coronary heart and cerebrovascular diseases: a meta-analysis," *Journal of Periodontology*, vol. 75, no. 8, pp. 1046–1053, 2004.

[12] S. J. Janket, A. E. Baird, S. K. Chuang, and J. A. Jones, "Meta-analysis of periodontal disease and risk of coronary heart disease and stroke," *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*, vol. 95, no. 5, pp. 559–569, 2003.

[13] J. D. Beck, J. R. Elter, G. Heiss, D. Couper, S. M. Mauriello, and S. Offenbacher, "Relationship of periodontal disease to carotid artery intima-media wall thickness: the atherosclerosis risk in communities (ARIC) study," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 21, no. 11, pp. 1816–1822, 2001.

[14] J. D. Beck, P. Eke, G. Heiss et al., "Periodontal disease and coronary heart disease: a reappraisal of the exposure," *Circulation*, vol. 112, no. 1, pp. 19–24, 2005.

[15] B. Holtfreter, K. Empen, S. Glaser et al., "Periodontisis is associated with endothelial dysfunction in a general population: a cross-sectional study," *PLoS ONE*, vol. 8, no. 12, Article ID e84603, 2013.

[16] B. R. Dorn, W. A. Dunn Jr., and A. Progulske-Fox, "Invasion of human coronary artery cells by periodontal pathogens," *Infection and Immunity*, vol. 67, no. 11, pp. 5792–5798, 1999.

[17] A. Sharma, E. K. Novak, H. T. Sojar, R. T. Swank, H. K. Kuramitsu, and R. J. Genco, "*Porphyromonas gingivalis* platelet aggregation activity: outer membrane vesicles are potent activators of murine platelets," *Oral Microbiology and Immunology*, vol. 15, no. 6, pp. 393–396, 2000.

[18] P. Libby, P. M. Ridker, and A. Maseri, "Inflammation and atherosclerosis," *Circulation*, vol. 105, no. 9, pp. 1135–1143, 2002.

[19] L. Yuan, P. H. Rodrigues, M. Bélanger, W. A. Dunn Jr., and A. Progulske-Fox, "*Porphyromonas gingivalis htrA* is involved in cellular invasion and in vivo survival," *Microbiology*, vol. 154, no. 4, pp. 1161–1169, 2008.

[20] P. M. Fives-Taylor, D. H. Meyer, K. P. Mintz, and C. Brissette, "Virulence factors of *Actinobacillus actinomycetemcomitans*," *Periodontology 2000*, vol. 20, no. 1, pp. 136–167, 1999.

[21] I. Brook, "The role of anaerobic bacteria in upper respiratory tract and other head and neck infections," *Current Infectious Disease Reports*, vol. 9, no. 3, pp. 208–217, 2007.

[22] C. G. Daly, D. H. Mitchell, J. E. Highfield, D. E. Grossberg, and D. Stewart, "Bacteremia due to periodontal probing: a clinical and microbiological investigation," *Journal of Periodontology*, vol. 72, no. 2, pp. 210–214, 2001.

[23] E. Leivadaros, U. van der Velden, S. Bizzarro et al., "A pilot study into measurements of markers of atherosclerosis in periodontitis," *Journal of Periodontology*, vol. 76, no. 1, pp. 121–128, 2005.

[24] K. Buhlin, M. Hultin, O. Norderyd et al., "Risk factors for atherosclerosis in cases with severe periodontitis," *Journal of Clinical Periodontology*, vol. 36, no. 7, pp. 541–549, 2009.

[25] Y. Higashi, C. Goto, T. Hidaka et al., "Oral infection-inflammatory pathway, periodontitis, is a risk factor for endothelial dysfunction in patients with coronary artery disease," *Atherosclerosis*, vol. 206, no. 2, pp. 604–610, 2009.

[26] S. Fedele, W. Sabbah, N. Donos, S. Porter, and F. D'Aiuto, "Common oral mucosal diseases, systemic inflammation, and cardiovascular diseases in a large cross-sectional US survey," *The American Heart Journal*, vol. 161, no. 2, pp. 344–350, 2011.

[27] J. B. Bartruff, R. A. Yukna, and D. L. Layman, "Outer membrane vesicles from *Porphyromonas gingivalis* affect the growth and funtion of cultured human gingival fibroblasts and umbilical vein endothelial cells," *Journal of Periodontology*, vol. 76, no. 6, pp. 972–979, 2005.

[28] R. E. Fitzpatrick, L. C. Wijeyewickrema, and R. N. Pike, "The gingipains: scissors and glue of the periodontal pathogen, *Porphyromonas gingivalis*," *Future Microbiology*, vol. 4, no. 4, pp. 471–487, 2009.

[29] J. Oscarsson, M. Karched, B. Thay, C. Chen, and S. Asikainen, "Proinflammatory effect in whole blood by free soluble bacterial components released from planktonic and biofilm cells," *BMC Microbiology*, vol. 8, article 206, 2008.

[30] A. Kol, G. K. Sukhova, A. H. Lichtman, and P. Libby, "Chlamydial heat shock protein 60 localizes in human atheroma and regulates macrophage tumor necrosis factor-α and matrix metalloproteinase expression," *Circulation*, vol. 98, no. 4, pp. 300–307, 1998.

[31] K. Mandal, M. Jahangiri, and Q. Xu, "Autoimmunity to heat shock proteins in atherosclerosis," *Autoimmunity Reviews*, vol. 3, no. 2, pp. 31–37, 2004.

[32] J. I. Choi, S. W. Chung, H. S. Kang et al., "Epitope mapping of *Porphyromonas gingivalis* heat-shock protein and human heat-shock protein in human atherosclerosis," *Journal of Dental Research*, vol. 83, no. 12, pp. 936–940, 2004.

[33] J. Choi, S. Y. Lee, K. Kim, and B. K. Choi, "Identification of immunoreactive epitopes of the *Porphyromonas gingivalis* heat shock protein in periodontitis and atherosclerosis," *Journal of Periodontal Research*, vol. 46, no. 2, pp. 240–245, 2011.

[34] K. Yamazaki, Y. Ohsawa, H. Itoh et al., "T-cell clonality to *Porphyromonas gingivalis* and human heat shock protein 60s in patients with atherosclerosis and periodontitis," *Oral Microbiology and Immunology*, vol. 19, no. 3, pp. 160–167, 2004.

[35] M. A. Curtis, M. Macey, J. M. Slaney, and G. L. Howells, "Platelet activation by protease I of *Porphyromonas gingivalis* W83," *FEMS Microbiology Letters*, vol. 110, no. 2, pp. 167–173, 1993.

[36] H. K. Kuramitsu, M. Qi, I. C. Kang, and W. Chen, "Role for periodontal bacteria in cardiovascular diseases," *Annals of periodontology*, vol. 6, no. 1, pp. 41–47, 2001.

[37] M. Morishita, W. Ariyoshi, T. Okinaga, M. Usui, K. Nakashima, and T. Nishihara, "*A. actinomycetemcomitans* LPS enhances foam cell formation induced by LDL," *Journal of Dental Research*, vol. 92, no. 3, pp. 241–246, 2013.

[38] E. Lalla, I. B. Lamster, M. A. Hofmann et al., "Oral infection with a periodontal pathogen accelerates early atherosclerosis in apolipoprotein E-null mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 8, pp. 1405–1411, 2003.

[39] F. C. Gibson III, C. Hong, H.-H. Chou et al., "Innate immune recognition of invasive bacteria accelerates atherosclerosis in apolipoprotein E-deficient mice," *Circulation*, vol. 109, no. 22, pp. 2801–2806, 2004.

[40] S. Amar, S.-C. Wu, and M. Madan, "Is *Porphyromonas gingivalis* cell invasion required for atherogenesis? Pharmacotherapeutic implications," *Journal of Immunology*, vol. 182, no. 3, pp. 1584–1592, 2009.

[41] Y. Koizumi, T. Kurita-Ochiai, S. Oguchi, and M. Yamamoto, "Nasal immunization with *Porphyromonas gingivalis* outer membrane protein decreases *P. gingivalis*-induced atherosclerosis and inflammation in spontaneously hyperlipidemic mice," *Infection and Immunity*, vol. 76, no. 7, pp. 2958–2965, 2008.

[42] T. Zhang, T. Kurita-Ochiai, T. Hashizume, Y. Du, S. Oguchi, and M. Yamamoto, "*Aggregatibacter actinomycetemcomitans* accelerates atherosclerosis with an increase in atherogenic factors in spontaneously hyperlipidemic mice," *FEMS Immunology and Medical Microbiology*, vol. 59, no. 2, pp. 143–151, 2010.

[43] R. Jia, T. Kurita-Ochiai, S. Oguchi, and M. Yamamoto, "Periodontal pathogen accelerates lipid peroxidation and atherosclerosis," *Journal of Dental Research*, vol. 92, pp. 247–252, 2013.

[44] A. Jain, E. L. Batista Jr., C. Serhan, G. L. Stahl, and T. E. van Dyke, "Role for periodontitis in the progression of lipid deposition in an animal model," *Infection and Immunity*, vol. 71, no. 10, pp. 6012–6018, 2003.

[45] N. Brodala, E. P. Merricks, D. A. Bellinger et al., "*Porphyromonas gingivalis* bacteremia induces coronary and aortic atherosclerosis in normocholesterolemic and hypercholesterolemic pigs," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 7, pp. 1446–1451, 2005.

[46] A. D. Haffajee and S. S. Socransky, "Microbial etiological agents of destructive periodontal diseases," *Periodontology 2000*, vol. 5, pp. 78–111, 1994.

[47] R. G. Deshpande, M. Khan, and C. A. Genco, "Invasion strategies of the oral pathogen *Porphyromonas gingivalis*: implications for cardiovascular disease," *Invasion and Metastasis*, vol. 18, no. 2, pp. 57–69, 1999.

[48] H.-H. Chou, H. Yumoto, M. Davey et al., "*Porphyromonas gingivalis* fimbria-dependent activation of inflammatory genes in human aortic endothelial cells," *Infection and Immunity*, vol. 73, no. 9, pp. 5367–5378, 2005.

[49] Y. Takahashi, M. Davey, H. Yumoto, F. C. Gibson III, and C. A. Genco, "Fimbria-dependent activation of pro-inflammatory molecules in *Porphyromonas gingivalis* infected human aortic endothelial cells," *Cellular Microbiology*, vol. 8, no. 5, pp. 738–757, 2006.

[50] I.-C. Kang and H. K. Kuramitsu, "Induction of monocyte chemoattractant protein-1 by *Porphyromonas gingivalis* in human endothelial cells," *FEMS Immunology and Medical Microbiology*, vol. 34, no. 4, pp. 311–317, 2002.

[51] T. Honda, T. Oda, H. Yoshie, and K. Yamazaki, "Effects of *Porphyromonas gingivalis* antigens and proinflammatory cytokines on human coronary artery endothelial cells," *Oral Microbiology and Immunology*, vol. 20, no. 2, pp. 82–88, 2005.

[52] C. Hayashi, G. Papadopulos, C. V. Gudino et al., "Protective role for TLR4 signaling in atherosclerosis progression as revealed by infection with a common oral pathogen," *The Journal of Immunology*, vol. 189, no. 7, pp. 3681–3688, 2012.

[53] M. K. Logani and R. E. Davies, "Lipid oxidation: biologic effects and antioxidants—a review," *Lipids*, vol. 15, no. 6, pp. 485–495, 1980.

[54] R. Stocker and J. F. Keaney Jr., "Role of oxidative modifications in atherosclerosis," *Physiological Reviews*, vol. 84, no. 4, pp. 1381–1478, 2004.

[55] D. Li, L. Liu, H. Chen, T. Sawamura, and J. L. Mehta, "LOX-1, an oxidized LDL endothelial receptor, induces CD40/CD40L signaling in human coronary artery endothelial cells," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 23, no. 5, pp. 816–821, 2003.

[56] J. Hulthe and B. Fagerberg, "Circulating oxidized LDL is associated with increased levels of cell-adhesion molecules in clinically healthy 58-year old men (AIR study)," *Medical Science Monitor*, vol. 8, no. 3, pp. CR148–CR152, 2002.

[57] M. Hashimoto, T. Kadowaki, T. Tsukuba, and K. Yamamoto, "Selective proteolysis of apolipoprotein B-100 by Arg-gingipain mediates atherosclerosis progression accelerated by bacterial exposure," *Journal of Biochemistry*, vol. 140, no. 5, pp. 713–723, 2006.

[58] T. Bengtsson, H. Karlsson, P. Gunnarsson et al., "The periodontal pathogen *Porphyromonas gingivalis* cleaves apoB-100 and increases the expression of apoM in LDL in whole blood leading to cell proliferation," *Journal of Internal Medicine*, vol. 263, no. 5, pp. 558–571, 2008.

[59] Y. Ding, V. J. Uitto, J. Firth et al., "Modulation of host matrix metalloproteinases by bacterial virulence factors relevant in human periodontal diseases," *Oral Diseases*, vol. 1, no. 4, pp. 279–286, 1995.

[60] F. A. Akalin, E. Baltacioğlu, A. Alver, and E. Karabulut, "Lipid peroxidation levels and total oxidant status in serum, saliva and gingival crevicular fluid in patients with chronic periodontitis," *Journal of Clinical Periodontology*, vol. 34, no. 7, pp. 558–565, 2007.

[61] A. S. M. Bastos, D. T. Graves, A. P. Loureiro et al., "Lipid peroxidation is associated with the severity of periodontal disease and local inflammatory markers in patients with type 2 diabetes," *The* , vol. 97, no. 8, pp. E1353–E1362, 2012.

[62] H. Miyakawa, K. Honma, M. Qi, and H. K. Kuramitsu, "Interaction of *Porphyromonas gingivalis* with low-density lipoproteins: implications for a role for periodontitis in atherosclerosis," *Journal of Periodontal Research*, vol. 39, no. 1, pp. 1–9, 2004.

[63] B. W. Zagol and R. A. Krasuski, "Effect of motorized scooters on quality of life and cardiovascular risk," *The American Journal of Cardiology*, vol. 105, no. 5, pp. 672–676, 2010.

[64] L. Qi, M. C. Cornelis, C. Zhang, R. M. van Dam, and F. B. Hu, "Genetic predisposition, western dietary pattern, and the risk of type 2 diabetes in men," *The American Journal of Clinical Nutrition*, vol. 89, no. 5, pp. 1453–1458, 2009.

[65] P. M. Ridker, "Clinical application of C-reactive protein for cardiovascular disease detection and prevention," *Circulation*, vol. 107, no. 3, pp. 363–369, 2003.

[66] M. A. Denke and J. L. Breslow, "Effects of a low fat diet with and without intermittent saturated fat and cholesterol ingestion on plasma lipid, lipoprotein, and apolipoprotein levels in normal volunteers," *Journal of Lipid Research*, vol. 29, no. 7, pp. 963–969, 1988.

[67] L. Li, E. Messas, E. L. Batista Jr., R. A. Levine, and S. Amar, "*Porphyromonas gingivalis* infection accelerates the progression of atherosclerosis in a heterozygous apolipoprotein E-deficient murine model," *Circulation*, vol. 105, no. 7, pp. 861–867, 2002.

[68] T. Maekawa, N. Takahashi, K. Tabeta et al., "Chronic oral infection with *Porphyromonas gingivalis* accelerates atheroma formation by shifting the lipid profile," *PLoS ONE*, vol. 6, no. 5, Article ID e20240, 2011.

[69] A. Fukasawa, T. Kurita-Ochiai, T. Hashizume, R. Kobayashi, and M. Yamamoto, "*Porphyromonas gingivalis* accelerates atherosclerosis in C57BL/6 mice fed a high-fat diet," *Immunopharmacology and Immunotoxicology*, vol. 34, no. 3, pp. 470–476, 2012.

[70] E. Blessing, L. A. Campbell, M. E. Rosenfeld, N. Chough, and C.-C. Kuo, "*Chlamydia pneumoniae* infection accelerates hyperlipidemia induced atherosclerotic lesion development in C57BL/6J mice," *Atherosclerosis*, vol. 158, no. 1, pp. 13–17, 2001.

[71] E. Blessing, T. M. Lin, L. A. Campbell, M. E. Rosenfeld, D. Lioyd, and C.-C. Kuo, "*Chlamydia pneumoniae* induces inflammatory changes in the heart and aorta of normocholesterolemic C57BL/6J mice," *Infection and Immunity*, vol. 68, no. 8, pp. 4765–4768, 2000.

[72] H. L. Weiner, "Induction of oral tolerance to the acetylcholine receptor for treatment of myasthenia gravis," *The Journal of Clinical Investigation*, vol. 104, no. 12, pp. 1667–1668, 1999.

[73] C. J. Calder, L. B. Nicholson, and A. D. Dick, "Mechanisms for inducing nasal mucosal tolerance in experimental autoimmune uveoretinitis," *Methods*, vol. 38, no. 2, pp. 69–76, 2006.

[74] M. Hagiwara, T. Kurita-Ochiai, R. Kobayashi, T. Hashizume-Takizawa, K. Yamazaki, and M. Yamamoto, "Sublingual vaccine using GroEL attenuates atherosclerosis," *Journal of Dental Research*, vol. 93, no. 4, pp. 382–387, 2014.

[75] S. Wolfram, "Effects of green tea and EGCG on cardiovascular and metabolic health," *Journal of the American College of Nutrition*, vol. 26, no. 4, pp. 373S–388S, 2007.

[76] Q. Feng, T. Kumagai, Y. Torii, Y. Nakamura, T. Osawa, and K. Uchida, "Anticarcinogenic antioxidants as inhibitors against intracellular oxidative stress," *Free Radical Research*, vol. 35, no. 6, pp. 779–788, 2001.

[77] G. L. Tipoe, T. M. Leung, M. W. Hung, and M. L. Fung, "Green tea polyphenols as an anti-oxidant and anti-inflammatory agent for cardiovascular protection," *Cardiovascular and Hematological Disorders Drug Targets*, vol. 7, no. 2, pp. 135–144, 2007.

[78] Y. Cao and R. Cao, "Angiogenesis inhibited by drinking tea," *Nature*, vol. 398, no. 6726, p. 381, 1999.

[79] Y. Cai, T. Kurita-Ochiai, T. Hashizume, and M. Yamamoto, "Green tea epigallocatechin-3-gallate attenuates *Porphyromonas gingivalis*-induced atherosclerosis," *Pathogens and Disease*, vol. 67, pp. 76–83, 2013.

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Review Article

The Yin and Yang of Innate Immunity in Stroke

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Immune system plays an elementary role in the pathophysiological progress of ischemic stroke. It consists of innate and adaptive immune system. Activated within minutes after ischemic onset, innate immunity is responsible for the elimination of necrotic cells and tissue repair, while it is critically involved in the initiation and amplification of poststroke inflammation that amplifies ischemic damage to the brain tissue. Innate immune response requires days to be fully developed, providing a considerable time window for therapeutic intervention, suggesting prospect of novel immunomodulatory therapies against poststroke inflammation-induced brain injury. However, obstacles still exist and a comprehensive understanding of ischemic stroke and innate immune reaction is essential. In this review, we highlighted the current experimental and clinical data depicting the innate immune response following ischemic stroke, mainly focusing on the recognition of damage-associated molecular patterns, activation and recruitment of innate immune cells, and involvement of various cytokines. In addition, clinical trials targeting innate immunity were also documented regardless of the outcome, stressing the requirements for further investigation.

1. Introduction

Stroke, among which ischemic stroke accounts for over 87% [1], is the leading cause of morbidity and permanent disability in adults worldwide [2]. It causes severe burdens to the individuals as well as the society, especially in the developing countries like China [3]. However, despite all the intensive researches in the recent decade, the therapeutic strategies of acute ischemic stroke remain limited. Intravenous delivery of recombinant tissue plasminogen activator (rt-PA) is by far the only therapy proved effective in clinical application [4]. Yet this therapy is faced with major drawbacks, the narrow therapeutic time window of 4.5 hours, and the increased risk of intracranial hemorrhage. In the cases delayed beyond the currently approved time window, rt-PA is not warranted since the potential risk would outweigh benefits [5]. Actually, only less than 5% of patients would benefit from the therapy [6]. Therefore, developing alternative therapies is imperative but impossible without a comprehensive understanding of the pathophysiological changes after the ischemic onset.

Ischemic onset is an insult for brain and immune system is firewall for the whole body. Immune system is divided into innate and adaptive systems. The innate immune system is the first line of defense, while immune system and central nervous system (CNS) were traditionally regarded as two distinct entities [7]. The existence of blood-brain barrier and absence of cerebral lymphatic vessels are largely impeding the communication between brain tissue and circulating immune cells and the antigen presenting T cells [8]. However, mounting evidence is challenging this viewpoint, indicating that ischemic stroke is complicated by mutual interplay between CNS and immune system.

Innate immune response plays a dual role in stroke, exerting beneficial as well as deleterious effects on the outcome [9]. Considering the Yin and Yang effects of innate immune system, an overall suppression or activation of innate immunity might not be beneficial, while the true challenge is to selectively inhibit the deleterious effects without compromising the beneficial roles of innate immune response in tissue repair, remodeling, and recovery. It means that we

should use immune system after stroke in the right time and right place.

This review mainly focused on latest research data concerning the activation of innate immune response after cerebral ischemia and function of these components.

2. Innate Immune System

Immune system monitors and preserves the homeostasis of CNS under normal and pathological conditions. Immune system consists of two mechanisms: the innate and adaptive immunity. The former reacts rapidly after ischemic insult and represents first step of inflammatory cascade [10], while the latter depends on antigen presenting and takes days to be activated. Therefore, innate immunity lays the foundation of the adaptive response and plays the key role in the integrated immune response secondary to cerebral ischemia.

The innate immune system in the brain relies on various immune cells including resident cells such as microglia and endothelia, as well as circulating immune cells from blood such as neutrophils, monocytes/macrophages, and dendritic cells, among which microglia, neutrophils, and monocytes/macrophages are most investigated. Besides the cellular component, cytokines are also involved, mainly including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α). Produced by immune cells, these cytokines function to promote as well as quell inflammation, exerting both deleterious and protective roles.

Immediately after cerebral ischemic onset, dying and dead neurons begin to release the so-called damage-associated molecular patterns (DAMPs). Innate immune system senses the DAMPs via broad-specificity receptors and responds to the cerebral ischemic injuries within minutes, and the response remains predominant throughout the first few hours to induce postischemic inflammatory cascade (Table 1) [11].

Under normal condition, the blood-brain barrier forms a nature obstacle to prevent the entrance of circulating immune cells into the brain. However, under ischemic condition, necrotic tissues would cause local inflammation, leading to the release of inflammatory mediators like cytokines, chemokine, nitric oxide, and reactive oxygen species, which eventually leads to dysfunction of the blood-brain barrier, allowing the translocation of circulating immune cells [1].

3. The Role of Innate Immune System in Stroke

3.1. Initiation of Innate Immune Response: DAMPs and Pattern Recognition Receptors. Neurons are particularly vulnerable to ischemic insult. Shortly after cerebral vascular accident, local ischemia would lead to the destruction of neurons in the ischemic core and peri-infarct zone, resulting in the release of various DAMPs including high mobility group protein B1 (HMGB1), uric acid, heat shock proteins, S100 proteins, DNA, and RNA, which attract and activate neighboring microglia [8] and thereby trigger the postischemic inflammatory cascade.

In spite of the mounting studies on DAMPs [12–15], it is still controversial which molecule represents the most important mediator that triggers the activation of innate immunity. Among all these DAMPs, high mobility group box 1 (HMGB1) stands out as the most investigated molecule [16]. HMGB1 is a nuclear protein normally localized in cell nuclei under the normal condition. Upon ischemia onset, however, neuronal necrosis causes the protein to translocate into the cytosol and then to passively enter the extracellular compartment. In clinical studies, Schulze and colleagues [12] detected an elevated plasma HMGB1 level in patients with acute ischemic stroke and verified a correlation between HMGB1 level and circulating leukocytes. Release of HMGB1 by necrotic neurons in early stage of cerebral ischemia exhibits proinflammatory activity and amplifies inflammatory damage to brain tissue. Whereas intravenous injection of anti-HMGB1 monoclonal antibody would remarkably ameliorated brain infarction in middle cerebral artery occlusion models [13]. Moreover, via electron microscopic observation, Zhang and colleagues [14] directly demonstrated that HMGB1 release induced rapid and drastic disruption of the BBB, followed by significant cerebral edema, which appeared to be in consistence with their findings by MRI. Interestingly, Hayakawa and colleagues [15, 17] found that, during the stage of stroke recovery, HMGB1 mediated beneficial plasticity and enhanced stem and progenitor cell recruitment, proliferation, and differentiation within damaged brain tissue. This effect, however, occurs in a delayed phase and is beyond the scope of this review.

HMGB1, as well as other DAMPs, is reported to induce downstream biological effects via interactions with pattern recognition receptors, including Toll-like receptors (TLRs), widely expressed on surrounding microglia, perivascular macrophages, and cerebrovascular endothelium [18]. The Toll-like receptor (TLR) pathway plays a pivotal role in the activation and amplification of innate immune response to endogenous tissue damage resulting from cerebral ischemia [19–21]. So far, 10 functional TLRs have been identified in humans as well as 12 in mice. TLR1-TLR9 are conserved in both species, while TLR 10 is not functional in mice because of a retrovirus insertion, and TLR 11, TLR 12, and TLR 13 have been lost from the human genome [22]. Among all the TLRs, TLR 2 and TLR 4 are expressed on the cell surface and detect endogenous ligands [23]. After middle cerebral artery (MCA) occlusion, TLR 2 and TLR 4 are documented to be upregulated and contribute to tissue damage by triggering the expression of inflammatory and apoptotic genes [24]. In fact, TLR 2 and TLR 4 play differential roles in acute cerebral ischemia/reperfusion injury. Hua and colleagues [25] found in genetic modified mice that TLR 4 knockout resulted in reduced infarct size, while TLR 2 knockout led to enlarged infarct size, higher mortality, and decreased neurological function, suggesting that TLR 4 contributed to cerebral ischemia/reperfusion damage, whereas TLR 2 appeared to be neuroprotective in response to cerebral ischemia.

In addition to TLRs, the intracellular NOD-like receptors (NLRs) have also recently been identified as key mediators of inflammatory and immune responses [26]. NLRP 3 contributes to neurovascular damage by regulating the release

Table 1: Activation and function of innate immune cells.

	Activation	Function
Microglia	Within minutes	M1 phenotype promotes inflammation by secreting cytokines like IL-1β, TNF-α and presenting antigens to T cell; M2 phenotype quells inflammation by secreting cytokines like IL-10, TGF-β, and tissue repair [38, 46].
Macrophages	2 days	View point 1: M1 phenotype promotes inflammation and M2 phenotype quells inflammation (similar with microglia) [29, 49]. View point 2: macrophages originating from peripheral monocytes are cytotoxic, while those from microglia are protective [50].
Neutrophils	30 minutes	N1 phenotype exacerbates the damage, whereas N2 is protective [51].
Dendritic cells	1 hour	Generally deleterious, suggested by researches to date [42–45].

of NLRP3-mediated proinflammatory mediators, and NLRP3 deficiency ameliorates cerebral ischemic injury in mice after by reducing infarcts and blood-brain barrier damage [27].

3.2. Activation of Innate Immune System

3.2.1. Activation of Local Resident Microglia in Central Nervous System. Microglia are the resident macrophages in brain that survey the CNS and eliminate debris via phagocytosis under normal and pathological conditions. In the resting state, microglia exhibit ramified appearance and once activated, these cells alter into an amoeboid morphology. Microglial activation is the initial step in CNS inflammation of numerous causes [9, 28–31]. In ischemic stroke, microglia are activated within minutes of ischemic onset and microglial products are detected as early as 1 hour after stroke [32]. Microglia express pattern recognition receptors including TLRs and NLRs to sense exogenous pathogens and endogenous danger signals [33].

3.2.2. Infiltration of Immune Cells from Peripheral Blood

Monocytes/Macrophages Accumulation. Monocytes are resting innate immune cells derived from the blood. Upon activation, these cells would undergo morphological and functional alteration and then be referred to as macrophages. Of note, it has been controversial for years regarding the precise origin of local infiltrating macrophages [34–36], due to the morphological and functional similarity between activated microglia and recruited monocytes/macrophages. Once activated, microglia alter their morphology and gene expression to develop an inflammatory phenotype, making themselves indistinguishable to circulating macrophages [33]. Yet one mostly recent research has settled the debate, proving that local reactive macrophages consist of 2 distinct populations of cells, that is, a majority originates from resident microglia and a small group recruited from circulation [37]. In contrast to the immediate response of microglia, the latter group of cells is recruited no sooner than 2 days after ischemia and remains abundant through day 3 to day 7 [38].

Neutrophil Infiltration. Within the acute phase of ischemic stroke (minutes to hours), the injured tissue would release free-radicals and proinflammatory cytokines and chemokine, which would thereby upregulate adhesion molecules on endothelial cells as well as the surface of circulating immune cells, and facilitate the recruitment and migration of leukocytes [38]. Among all the components in the circulating immune system, neutrophils are the first responders [39] that are reported to react to the acute ischemia within 30 minutes and peak in the first 3 days [32]. Via neutrophil CD11b/CD18 and endothelial ICAM-1 interactions, neutrophils adhere to activated vascular endothelium and infiltrate into the injured area, and blocking of the interactions would result in reduced leukocyte accumulation [39, 40].

Dendritic Cell Increment. As a link between the innate and adaptive arms of the immune system, dendritic cells (DCs) are key regulators in many forms of immune response [41, 42], but the regulatory role of DCs in inflammation provoked specifically by stroke has not yet been sufficiently investigated [43]. Kostulas and colleagues [44] stood among the first to provide data on DCs in cerebral ischemia and demonstrated ascending numbers of DCs in the ischemic hemispheres in rat models as early as 1 hour after permanent MCA occlusion. Later on, Gelderblom et al. [45] confirmed this finding by analyzing different subclasses of inflammatory cells using flow cytometric analysis and found in surprise that DCs showed one of the largest increases in cell numbers and accounted for a substantial portion among all the infiltrating immune cells with 20-fold increase on day 3 and still 12-fold on day 7. Consistently, a more recent study carried out by Yilmaz and colleagues [43] demonstrated in patients that the numbers of DCs decreased transiently after stroke; furthermore, by analyzing human cerebral specimens with acute ischemic or hemorrhagic stroke, the authors found numerous DCs locating in the infarct area, supporting the hypothesis that the DCs in circulation were most likely to

be their recruitment into the infarcted brain. On the other hand, it is also possible that the part of the DCs found in the lesion originates from local cerebral cells such as microglia [44].

3.3. Dual Roles of Innate Immune System

3.3.1. Cells

Microglia. Activated microglia function analogously to circulating macrophages, with the ability to eliminate necrotic tissue and secrete proinflammatory cytokines including IL-1β and TNF-α under ischemic condition, which exacerbate brain damage and promote leukocyte infiltration [38]; on the other hand, these cells also exert a neuroprotective potential by releasing anti-inflammatory cytokines like IL-10 and TGF-β to quell inflammation and benefit the outcome [38, 46]. In the emerging concept, microglia are assorted into M1 and M2 phenotypes, like macrophages. The M1 phenotype is referred to as the classically activated phenotype and processes deleterious features by secreting proinflammatory cytokines and presenting antigen to T cells, whereas M2 microglia, the alternatively activated phenotype, are involved in the neuroprotection and tissue repair after ischemic injury [33]. Existing data suggests that overall suppression of microglia fails to benefit experimental outcome but, on the contrary, results in larger infarctions and doubling apoptotic neurons after ischemia [47], indicating the significance of microglia in alleviation and recovery of injury.

Macrophages. Traditionally, macrophages are viewed as a noxious component that amplifies ischemic injury and exacerbates secondary progression of ischemic lesions. Monocytes/macrophages are recruited via CCL2/CCR2 axis, and deletion of CCR2 or CCL2 results in smaller cerebral infarcts, reduced monocytes/macrophages infiltration, and less proinflammatory mediator production, indicating a deleterious effect of these cells [48]. But the majority of studies demonstrate that macrophages in the injured region, regardless of the exact origin, are polarized into M1 and M2 phenotypes, and the M2 phenotype would show beneficial effects against ischemic damage [29, 49]. Girard and colleagues [50] reported that macrophages that originate from peripheral monocytes might be cytotoxic, independently of their phenotype, while microglia may be protective. On the other hand, Hu and colleagues [49] demonstrated that the majority of microglia/macrophages within the infarct areas experience an M2-to-M1 shift during the stroke progress. Soon after the ischemia, macrophages of the M2 phenotype were present and exerted neuroprotective effects; while being at the later stage of injury, the M2 phenotype gradually transforms into the M1 phenotype and is involved in neuronal damage.

Neutrophils. Although elevated neutrophil accumulation is often observed during cerebral ischemia/reperfusion, the exact pathogenesis role of neutrophil infiltration is uncertain, and blocking the postischemia neutrophil recruitment is not necessarily leading to improved outcome [39]. In current concept, neutrophils confer to a functional heterogeneity and polarize into 2 distinct subsets, in which N1 phenotype mediates deleterious effect, while N2 phenotype exhibits neuroprotective effects [51].

Dendritic Cells. To date the exact role of DCs was not defined comprehensively, but most studies suggested that DCs increment was associated with worsened outcome [42–45]. In murine models, the numbers of DC in the brain correlated with the size of the brain lesion after pMCAO [44], whereas in patients with transient ischemic attack, acute ischemic stroke, and acute hemorrhagic stroke, the extent of the decrease of DCs significantly correlated with the clinical stage and the radiological size of stroke [43]. Moreover, suppression of DC migration and maturation by granulocyte-colony stimulating factor contributed to attenuation of cerebral inflammation and reduction of infarct size, exhibiting neuroprotective effects in murine models of tMCAO [42].

The mechanism of how DCs lead to poorer outcome remained elusive. Theoretically, DCs presented in the infarcted areas may activate T cells, induce a long-lasting immune response, and therefore lead to further neurological damage [44]. Additionally, the transient decrease of circulating DCs might lead to immunodepression, resulting in poststroke infections to worsen the clinical outcome in stroke patients [43].

3.3.2. Cytokines.

Infiltration and activation of innate immune cells result in the production of various cytokines and inflammatory mediators, which either exacerbate or alleviate inflammatory damage to the ischemic brain tissue. Within the first 24 hours of cerebral ischemia in animal models, inflammatory cytokines interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α are upregulated dramatically by up to 40- to 60-fold and are believed to affect the infarct volume and tissue damage. Therefore, these cytokines stand among the most investigated inflammatory mediators [52].

IL-1β is one of the neurotoxic cytokines released within 30 minutes after ischemic onset [16]. Activated microglia appear to be the major source, whereas other immune cells may also express IL-1β [53]. Noxious effects of IL-1β are well documented in numerous studies [1, 53, 54]. It is considered as a neurotoxic mediator that directly induces neuronal death and enhances the expression of cytokines. Furthermore, chronic release of IL-1β is associated with increased expression of adhesion molecules and blood-brain barrier permeability, promoting further leukocyte infiltration [55]. In animal experiments, IL-1α and IL-1β double knockout significantly reduced infarct volume in cerebral ischemic mice models [56]. Additionally, meta-analysis of animal model studies also revealed that IL-1 receptor antagonist markedly reduced infarct volume by 38.2% [57].

Expressed within the first hour after ischemic onset [16], TNF-α is also an essential component involved in the early stage of cerebral ischemia [16, 38, 58, 59]. Increased TNF-α level in serum was observed after stroke in patients, and the increase correlated infarct volume and severity of neurological impairment [38]. TNF-α plays a dual role in brain injury. The neurotoxic effect of TNF-α might be attributed

to direct induction of neuronal death and indirect promotion of leukocyte infiltration by elevating the expression of adhesion molecules and chemokine. However, in addition to the deleterious roles, TNF-α also exerts beneficial effects and mitigates inflammatory injury. TNF receptor knockout was reported to be associated with enlargement of infarct volume [16]. Besides, TNF-α pretreatment would result in decreased infarct volume and reduced leukocyte infiltration after permanent middle cerebral artery occlusion in mice [60].

Compared with the former ones, reports on the role of IL-6 in experimental ischemic stroke are relatively fewer [61–65]. And current available evidence argues against a pathogenic role of IL-6 in ischemic stroke. On the contrary, initial studies indicated that IL-6 deficient has no impact on infarct volume in mice models [63]. Whereas studies later on argued that the failure of IL-6 deficient to affect infarct size might be due to hypothermia in the mice models, and with well-controlled hypothermia, IL-6 deficiency would lead to increased infarct volume and neuronal death, suggesting a neuroprotective role of IL-6 [11, 61]. Furthermore, underlying mechanisms of the neuroprotective potential of IL-6 are partially revealed in recent studies, in which IL-6 was demonstrated to participate in angiogenesis [64], and Jung and colleagues [62] verified that IL-6 exerted this ability and protected ischemic tissue probably via STAT3 pathway. Moreover, in order to depict the interactions between various cytokines participating the postcerebral ischemic inflammation, Zeng and colleagues [65] adopted Bayesian network (BN) learning procedure to explore the underlying links among circulating cytokines and discovered that IL-6 modulated TNF-α and IL-1β mRNA expression directly or indirectly, indicating that IL-6 is a key mediator of the inflammatory cytokine network during the postcerebral ischemia inflammation.

Attempts and Difficulties in Bench-to-Bedside Translation. A better knowledge of poststroke inflammatory response may give birth to novel therapeutic strategies against ischemic stroke. Thrombolysis with rt-PA is the only effective treatment to date. However, due to the time window of 4.5 hours and safety concerns, the portion of stroke patients that would benefit from this treatment is less than 5% [6]. On the other hand, immunomodulatory therapies hold a great potential. Based on current knowledge, inflammatory response reacts immediately after ischemic onset while requiring hours to days to fulfill. Therefore, immunomodulatory treatment would have extended therapeutic window. In addition, immunomodulatory therapy would not increase the risk of hemorrhage. Finally, since the inflammation would be particularly exacerbated upon reperfusion, immunomodulation would ameliorate the potential reperfusion-induced exacerbation secondary to medical intervention and recanalization [18].

As is mentioned above, poststroke inflammation is featured by significant leukocyte infiltration, which is facilitated by the upregulated adhesion molecules. Based on this theory, clinical trials are conducted to explore whether the suppression of leukocyte infiltration by blocking ICAM-1 with monoclonal antibody enlimomab benefits clinical outcome in acute ischemic stroke patients. Disappointingly, the clinical trial ended up with negative results, suggesting an even worsened outcome upon enlimomab treatment [66]. In this study, 625 patients with ischemic stroke were enrolled, of whom 317 were randomized to receive enlimomab within 6 hours after stroke onset. Patients were not enrolled if they had received rt-PA. The treatment lasted over 5 days. However, when evaluated at day 90, patients that received enlimomab exhibited significantly worse Modified Rankin Scale score and higher mortality. Additionally, patients in enlimomab group experienced more adverse events, primarily infections and fever, than the placebo group. The negative effect may be interpreted by the murine source of enlimomab and the murine antibody might activate neutrophils through complement-dependent mechanisms and therefore amplify the inflammation and damage [1].

Likewise, UK-279, 276, a recombinant glycoprotein that selectively binds to the CD11b integrin to reduce neutrophil infiltration and infarct size in murine models, failed to exhibit any benefit in patients. The study was a multicenter, double-blind, randomized, placebocontrolled clinical trial to evaluate the efficacy of UK-279,276 in acute ischemic stroke. 966 patients were enrolled, among whom 887 had ischemic stroke and 204 were cotreated with rt-PA. Unfortunately, the trial was stopped early for futility in both subgroups receiving UK-279,276 no matter with concomitant rt-PA prescription or not [67].

In addition to the above-mentioned immune cells and cytokines that complicate the immune response to stroke, free-radicals also complicate the pathophysiological progress [68, 69]. Therefore, free-radical trapping agents like NXY-059 are theoretically neuroprotective, and this hypothesis was confirmed in animal models [70]. Furthermore, SAINT I study [70] found that NXY-059 significantly reduced disability rate in patients receiving this agent and markedly lowered hemorrhagic risk in those receiving rt-PA concomitantly. Nonetheless, the subsequent SAINT II study [71] overturned both of these optimistic findings, stating that NXY-059 was ineffective for the treatment of acute ischemic stroke and had no effect on the hemorrhagic risk of rt-PA. Since SAINT II study presented larger sample size (3306 versus 1699), it is reasonable to consider the results from SAINT II study to be more reliable and the data from SAINT I may be false positive. However, even though no evidence was found of an interaction between rt-PA use and the effect of NXY-059 in either trial, we cannot completely rule out the possibility that the disparity between the two trials may derive from the higher frequency of rt-PA prescription use in SAINT II study (44% versus 29%) in which maximal improvement may be achieved already by rt-PA, in spite of NXY-059 [71].

There are several promising findings as well. In another trial, investigators adopted IL-1 receptor antagonist (IL-1Ra) in attempt to block cytokine cascade. This randomized phase II study [72] recruited 34 patients, among whom half were randomized to receive IL-1Ra and the others received placebos. None of the patients received rt-PA. Upon 3-month evaluation, patients that received IL-1Ra exhibited lower levels of inflammatory markers and better clinical outcomes. No adverse events were observed in both groups. This study

indicated that IL-1Ra was safe and well tolerated among acute stroke patients and that IL-1Ra held a great potential to be a novel therapy, whereas the efficacy required further investigation.

The trial and errors in the attempt to find novel therapeutic strategies targeting poststroke inflammation have revealed several obstacles before successful clinical translation were put forward by Macrez and colleagues [1]. Firstly, it is still uncertain whether animal models of stroke can recapitulate human pathology and predict success in clinical trials needs. Secondly, safety, tolerance, and potential adverse events associated with therapeutic immunomodulation are of relevant concern. Finally, our knowledge of immune system and stroke is still limited. The interactions between stroke and immunity are elusive, and the role of inflammation in ischemic injury is complicated and sometimes conflicting. Therefore, safe and successful bench-to-bedside translation calls for a more comprehensive understanding of immune response after ischemic stroke before it could benefit stroke patients substantially.

4. Conclusions

Innate immune system, triggered immediately and kept for a while after ischemic stroke onset, protects and hurts brain by activation of endogenous and exogenous immune cells and production of cytokines. Immunomodulatory therapies targeting the poststroke inflammation are promising with great obstacles, and a comprehensive understanding of innate immune response to cerebral ischemic attack calls for further investigation.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

[1] R. Macrez, C. Ali, O. Toutirais et al., "Stroke and the immune system: from pathophysiology to new therapeutic strategies," *The Lancet Neurology*, vol. 10, no. 5, pp. 471–480, 2011.

[2] Y. Jiang, N. Wei, T. Lu, J. Zhu, G. Xu, and X. Liu, "Intranasal brain-derived neurotrophic factor protects brain from ischemic insult via modulating local inflammation in rats," *Neuroscience*, vol. 172, pp. 398–405, 2011.

[3] G. Xu, M. Ma, X. Liu, and G. J. Hankey, "Is there a stroke belt in China and why?" *Stroke—A Journal of Cerebral Circulation*, vol. 44, no. 7, pp. 1775–1783, 2013.

[4] Y. Jiang, W. Zhu, J. Zhu, L. Wu, G. Xu, and X. Liu, "Feasibility of delivering mesenchymal stem cells via catheter to the proximal end of the lesion artery in patients with stroke in the territory of the middle cerebral artery," *Cell Transplantation*, vol. 22, no. 12, pp. 2291–2298, 2013.

[5] K. R. Lees, E. Bluhmki, R. von Kummer et al., "Time to treatment with intravenous alteplase and outcome in stroke: an updated pooled analysis of ECASS, ATLANTIS, NINDS, and EPITHET trials," *The Lancet*, vol. 375, no. 9727, pp. 1695–1703, 2010.

[6] G. C. Fonarow, E. E. Smith, J. L. Saver et al., "Timeliness of tissue-type plasminogen activator therapy in acute ischemic stroke: patient characteristics, hospital factors, and outcomes associated with door-to-needle times within 60 minutes," *Circulation*, vol. 123, no. 7, pp. 750–758, 2011.

[7] C. E. Downes and P. J. Crack, "Neural injury following stroke: are Toll-like receptors the link between the immune system and the CNS" *British Journal of Pharmacology*, vol. 160, no. 8, pp. 1872–1888, 2010.

[8] A. Chamorro, A. Meisel, A. M. Planas, X. Urra, D. van de Beek, and R. Veltkamp, "The immunology of acute stroke," *Nature Reviews. Neurology*, vol. 8, no. 7, pp. 401–410, 2012.

[9] M. A. Yenari, T. M. Kauppinen, and R. A. Swanson, "Microglial activation in stroke: therapeutic targets," *Neurotherapeutics*, vol. 7, no. 4, pp. 378–391, 2010.

[10] G. Courties, M. A. Moskowitz, and M. Nahrendorf, "The innate immune system after ischemic injury: lessons to be learned from the heart and brain," *JAMA Neurology*, vol. 71, no. 2, pp. 233–236, 2013.

[11] K. L. Lambertsen, K. Biber, and B. Finsen, "Inflammatory cytokines in experimental and human stroke," *Journal of Cerebral Blood Flow and Metabolism*, vol. 32, no. 9, pp. 1677–1698, 2012.

[12] J. Schulze, D. Zierath, P. Tanzi et al., "Severe stroke induces long-lasting alterations of high-mobility group box 1," *Stroke—A Journal of Cerebral Circulation*, vol. 44, no. 1, pp. 246–248, 2013.

[13] K. Liu, S. Mori, H. K. Takahashi et al., "Anti-high mobility group box 1 monoclonal antibody ameliorates brain infarction induced by transient ischemia in rats," *The FASEB Journal*, vol. 21, no. 14, pp. 3904–3916, 2007.

[14] J. Zhang, H. K. Takahashi, K. Liu et al., "Anti-high mobility group box-1 monoclonal antibody protects the blood-brain barrier from ischemia-induced disruption in rats," *Stroke*, vol. 42, no. 5, pp. 1420–1428, 2011.

[15] K. Hayakawa, N. Miyamoto, J. H. Seo et al., "High-mobility group box 1 from reactive astrocytes enhances the accumulation of endothelial progenitor cells in damaged white matter," *Journal of Neurochemistry*, vol. 125, no. 2, pp. 273–280, 2013.

[16] T. Shichita, T. Ago, M. Kamouchi, T. Kitazono, A. Yoshimura, and H. Ooboshi, "Novel therapeutic strategies targeting innate immune responses and early inflammation after stroke," *Journal of Neurochemistry*, vol. 123, supplement 2, pp. 29–38, 2012.

[17] K. Hayakawa, L. D. Pham, K. Arai, and E. H. Lo, "High-mobility group box 1: an amplifier of stem and progenitor cell activity after stroke," *Acta Neurochirurgica*, vol. 118, pp. 31–38, 2013.

[18] C. Iadecola and J. Anrather, "The immunology of stroke: from mechanisms to translation," *Nature Medicine*, vol. 17, no. 7, pp. 796–808, 2011.

[19] B. M. Famakin, Y. Mou, K. Johnson, M. Spatz, and J. Hallenbeck, "A new role for downstream Toll-like receptor signaling in mediating immediate early gene expression during focal cerebral ischemia," *Journal of Cerebral Blood Flow and Metabolism*, vol. 34, no. 2, pp. 258–267, 2014.

[20] M. J. Quattromani, P. Cordeau, K. Ruscher, J. Kriz, and T. Wieloch, "Enriched housing down-regulates the Toll-like receptor 2 response in the mouse brain after experimental stroke," *Neurobiology of Disease C*, vol. 66, pp. 66–73, 2014.

[21] S. C. Tang, S. J. Yeh, Y. I. Li et al., "Evidence for a detrimental role of TLR8 in ischemic stroke," *Experimental Neurology*, vol. 250, pp. 341–347, 2013.

[22] S. Akira, S. Uematsu, and O. Takeuchi, "Pathogen recognition and innate immunity," *Cell*, vol. 124, no. 4, pp. 783–801, 2006.

[23] Y.-C. Wang, S. Lin, and Q.-W. Yang, "Toll-like receptors in cerebral ischemic inflammatory injury," *Journal of Neuroinflammation*, vol. 8, article 134, 2011.

[24] T. Abe, M. Shimamura, K. Jackman et al., "Key role of CD36 in toll-like receptor 2 signaling in cerebral ischemia," *Stroke*, vol. 41, no. 5, pp. 898–904, 2010.

[25] F. Hua, J. Ma, T. Ha et al., "Differential roles of TLR2 and TLR4 in acute focal cerebral ischemia/reperfusion injury in mice," *Brain Research*, vol. 1262, pp. 100–108, 2009.

[26] M. Saleh, "The machinery of Nod-like receptors: refining the paths to immunity and cell death," *Immunological Reviews*, vol. 243, no. 1, pp. 235–246, 2011.

[27] F. Yang, Z. Wang, X. Wei et al., "NLRP3 deficiency ameliorates neurovascular damage in experimental ischemic stroke," *Journal of Cerebral Blood Flow and Metabolism*, vol. 34, no. 4, pp. 660–667, 2014.

[28] Z. Yang, T. Zhao, J. H. Zhang, and H. Feng, "Curcumin inhibits microglia inflammation and confers neuroprotection in intracerebral hemorrhage," *Immunology Letters*, vol. 14, pp. S0165–S2478, 2014.

[29] V. H. Perry, J. A. R. Nicoll, and C. Holmes, "Microglia in neurodegenerative disease," *Nature Reviews Neurology*, vol. 6, no. 4, pp. 193–201, 2010.

[30] E. Yoritsune, M. Furuse, H. Kuwabara et al., "Inflammation as well as angiogenesis may participate in the pathophysiology of brain radiation necrosis," *Journal of Radiation Research*, 2014.

[31] E. Rissanen, J. Tuisku, J. Rokka et al., "In vivo detection of diffuse inflammation in secondary progressive multiple sclerosis using PET imaging and the radioligand 11C-PK11195," *Journal of Nuclear Medicine*, 2014.

[32] R. Jin, G. Yang, and G. Li, "Inflammatory mechanisms in ischemic stroke: role of inflammatory cells," *Journal of Leukocyte Biology*, vol. 87, no. 5, pp. 779–789, 2010.

[33] R. A. Taylor and L. H. Sansing, "Microglial responses after ischemic stroke and intracerebral hemorrhage," *Clinical & Developmental Immunology*, vol. 2013, Article ID 746068, 10 pages, 2013.

[34] J. V. Faustino, X. Wang, C. E. Johnson et al., "Microglial cells contribute to endogenous brain defenses after acute neonatal focal stroke," *Journal of Neuroscience*, vol. 31, no. 36, pp. 12992–13001, 2011.

[35] A. Denes, R. Vidyasagar, J. Feng et al., "Proliferating resident microglia after focal cerebral ischaemia in mice," *Journal of Cerebral Blood Flow and Metabolism*, vol. 27, no. 12, pp. 1941–1953, 2007.

[36] M. Schroeter, S. Jander, I. Huitinga, and G. Stoll, "CD8+ phagocytes in focal ischemia of the rat brain: predominant origin from hematogenous macrophages and targeting to areas of pannecrosis," *Acta Neuropathologica*, vol. 101, no. 5, pp. 440–448, 2001.

[37] T. Li, S. Pang, Y. Yu, X. Wu, J. Guo, and S. Zhang, "Proliferation of parenchymal microglia is the main source of microgliosis after ischaemic stroke," *Brain: A Journal of Neurology*, vol. 136, no. 12, pp. 3578–3588, 2013.

[38] T. Chiba and K. Umegaki, "Pivotal roles of monocytes/macrophages in stroke," *Mediators of Inflammation*, vol. 2013, Article ID 759103, 10 pages, 2013.

[39] A. S. Easton, "Neutrophils and stroke—can neutrophils mitigate disease in the central nervous system?" *International Immunopharmacology*, vol. 17, no. 4, pp. 1218–1225, 2013.

[40] H. Morrison, D. McKee, and L. Ritter, "Systemic neutrophil activation in a mouse model of ischemic stroke and reperfusion," *Biological Research for Nursing*, vol. 13, no. 2, pp. 154–163, 2011.

[41] J. C. Felger, T. Abe, U. W. Kaunzner et al., "Brain dendritic cells in ischemic stroke: time course, activation state, and origin," *Brain, Behavior, and Immunity*, vol. 24, no. 5, pp. 724–737, 2010.

[42] B. Dietel, I. Cicha, B. Kallmünzer et al., "Suppression of dendritic cell functions contributes to the anti-inflammatory action of granulocyte-colony stimulating factor in experimental stroke," *Experimental Neurology*, vol. 237, no. 2, pp. 379–387, 2012.

[43] A. Yilmaz, T. Fuchs, B. Dietel et al., "Transient decrease in circulating dendritic cell precursors after acute stroke: potential recruitment into the brain," *Clinical Science*, vol. 118, no. 2, pp. 147–157, 2010.

[44] N. Kostulas, H.-L. Li, B.-G. Xiao, Y.-M. Huang, V. Kostulas, and H. Link, "Dendritic cells are present in ischemic brain after permanent middle cerebral artery occlusion in the rat," *Stroke*, vol. 33, no. 4, pp. 1129–1134, 2002.

[45] M. Gelderblom, F. Leypoldt, K. Steinbach et al., "Temporal and spatial dynamics of cerebral immune cell accumulation in stroke," *Stroke*, vol. 40, no. 5, pp. 1849–1857, 2009.

[46] E. Parada, J. Egea, I. Buendia et al., "The microglial alpha7-acetylcholine nicotinic receptor is a key element in promoting neuroprotection by inducing heme oxygenase-1 via nuclear factor erythroid-2-related factor 2," *Antioxidants & Redox Signaling*, vol. 19, no. 11, pp. 1135–1148, 2013.

[47] M. Lalancette-Hebert, V. Swarup, J. M. Beaulieu et al., "Galectin-3 is required for resident microglia activation and proliferation in response to ischemic injury," *The Journal of Neuroscience*, vol. 32, no. 30, pp. 10383–10395, 2012.

[48] O. B. Dimitrijevic, S. M. Stamatovic, R. F. Keep, and A. V. Andjelkovic, "Absence of the chemokine receptor CCR2 protects against cerebral ischemia/reperfusion injury in mice," *Stroke*, vol. 38, no. 4, pp. 1345–1353, 2007.

[49] X. Hu, P. Li, Y. Guo et al., "Microglia/macrophage polarization dynamics reveal novel mechanism of injury expansion after focal cerebral ischemia," *Stroke—A Journal of Cerebral Circulation*, vol. 43, no. 11, pp. 3063–3070, 2012.

[50] S. Girard, D. Brough, G. Lopez-Castejon, J. Giles, N. J. Rothwell, and S. M. Allan, "Microglia and macrophages differentially modulate cell death after brain injury caused by oxygen-glucose deprivation in organotypic brain slices," *Glia*, vol. 61, no. 5, pp. 813–824, 2013.

[51] M. I. Cuartero, I. Ballesteros, A. Moraga et al., "N2 neutrophils, novel players in brain inflammation after stroke modulation by the PPARγ agonist rosiglitazone," *Stroke—A Journal of Cerebral Circulation*, vol. 44, no. 12, pp. 3498–3508, 2013.

[52] Y. B. Ouyang, "Inflammation and stroke," *Neuroscience Letters*, vol. 548, pp. 1–3, 2013.

[53] J. Galea and D. Brough, "The role of inflammation and interleukin-1 in acute cerebrovascular disease," *Journal of Inflammation Research*, vol. 6, pp. 121–128, 2013.

[54] M. E. Edye, G. Lopez-Castejon, S. M. Allan, and D. Brough, "Acidosis drives damage-associated molecular pattern (DAMP)-induced interleukin-1 secretion via a caspase-1-independent pathway," *The Journal of Biological Chemistry*, vol. 288, no. 42, pp. 30485–30494, 2013.

[55] S. S. Shaftel, T. J. Carlson, J. A. Olschowka, S. Kyrkanides, S. B. Matousek, and M. K. O'Banion, "Chronic interleukin-1β expression in mouse brain leads to leukocyte infiltration

and neutrophil-independent blood-brain barrier permeability without overt neurodegeneration," *Journal of Neuroscience*, vol. 27, no. 35, pp. 9301–9309, 2007.

[56] H. Boutin, R. A. LeFeuvre, R. Horai, M. Asano, Y. Iwakura, and N. J. Rothwell, "Role of IL-1α and IL-1β in ischemic brain damage," *Journal of Neuroscience*, vol. 21, no. 15, pp. 5528–5534, 2001.

[57] V. Banwell, E. S. Sena, and M. R. Macleod, "Systematic review and stratified meta-analysis of the efficacy of interleukin-1 receptor antagonist in animal models of stroke," *Journal of Stroke and Cerebrovascular Diseases*, vol. 18, no. 4, pp. 269–276, 2009.

[58] M. C. Cure, A. Tufekci, E. Cure et al., "Low-density lipoprotein subfraction, carotid artery intima-media thickness, nitric oxide, and tumor necrosis factor alpha are associated with newly diagnosed ischemic stroke," *Annals of Indian Academy of Neurology*, vol. 16, no. 4, pp. 498–503, 2013.

[59] A. Maddahi, L. S. Kruse, Q. W. Chen, and L. Edvinsson, "The role of tumor necrosis factor-α and TNF-α receptors in cerebral arteries following cerebral ischemia in rat," *Journal of Neuroinflammation*, vol. 8, article 107, 2011.

[60] H. Nawashiro, K. Tasaki, C. A. Ruetzler, and J. M. Hallenbeck, "TNF-α pretreatment induces protective effects against focal cerebral ischemia in mice," *Journal of Cerebral Blood Flow and Metabolism*, vol. 17, no. 5, pp. 483–490, 1997.

[61] K. Biber, A. Pinto-Duarte, M. C. Wittendorp et al., "Interleukin-6 upregulates neuronal adenosine A1 receptors: implications for neuromodulation and neuroprotection," *Neuropsychopharmacology*, vol. 33, no. 9, pp. 2237–2250, 2008.

[62] J. E. Jung, G. S. Kim, and P. H. Chan, "Neuroprotection by interleukin-6 is mediated by signal transducer and activator of transcription 3 and antioxidative signaling in ischemic stroke," *Stroke*, vol. 42, no. 12, pp. 3574–3579, 2011.

[63] W. M. Clark, L. G. Rinker, N. S. Lessov et al., "Lack of interleukin-6 expression is not protective against focal central nervous system ischemia," *Stroke*, vol. 31, no. 7, pp. 1715–1720, 2000.

[64] K. Gertz, G. Kronenberg, R. E. Kälin et al., "Essential role of interleukin-6 in post-stroke angiogenesis," *Brain: A Journal of Neurology*, vol. 135, no. 6, pp. 1964–1980, 2012.

[65] L. Zeng, Y. Wang, J. Liu et al., "Pro-inflammatory cytokine network in peripheral inflammation response to cerebral ischemia," *Neuroscience Letters*, vol. 548, pp. 4–9, 2013.

[66] D. Sherman, A. Bes, J. D. Easton et al., "Use of anti-ICAM-1 therapy in ischemic stroke: results of the enlimomab acute stroke trial," *Neurology*, vol. 57, no. 8, pp. 1428–1434, 2001.

[67] M. Krams, K. R. Lees, W. Hacke, A. P. Grieve, J.-M. Orgogozo, and G. A. Ford, "Acute stroke therapy by inhibition of neutrophils (ASTIN): an adaptive dose-response study of UK-279,276 in acute ischemic stroke," *Stroke*, vol. 34, no. 11, pp. 2543–2548, 2003.

[68] P. A. Fraser, "The role of free radical generation in increasing cerebrovascular permeability," *Free Radical Biology and Medicine*, vol. 51, no. 5, pp. 967–977, 2011.

[69] T. Nakase, S. Yoshioka, and A. Suzuki, "Free radical scavenger, edaravone, reduces the lesion size of lacunar infarction in human brain ischemic stroke," *BMC Neurology*, vol. 11, article 39, 2011.

[70] K. R. Lees, J. A. Zivin, T. Ashwood et al., "NXY-059 for acute ischemic stroke," *The New England Journal of Medicine*, vol. 354, no. 6, pp. 588–600, 2006.

[71] A. Shuaib, K. R. Lees, P. Lyden et al., "NXY-059 for the treatment of acute ischemic stroke," *The New England Journal of Medicine*, vol. 357, no. 6, pp. 562–571, 2007.

[72] H. C. A. Emsley, C. J. Smith, R. F. Georgiou et al., "A randomised phase II study of interleukin-1 receptor antagonist in acute stroke patients," *Journal of Neurology, Neurosurgery and Psychiatry*, vol. 76, no. 10, pp. 1366–1372, 2005.