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Midkine in Inflammation

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The 13 kDa heparin-binding growth factor midkine (MK) was originally identified as a molecule involved in the orchestration of embryonic development. Recent studies provided evidence for a new role of MK in acute and chronic inflammatory processes. Accordingly, several inflammatory diseases including nephritis, arthritis, atherosclerosis, colitis, and autoimmune encephalitis have been shown to be alleviated in the absence of MK in animal models. Reduced leukocyte recruitment to the sites of inflammation was found to be one important mechanism attenuating chronic inflammation when MK was absent. Furthermore, MK was found to modulate expression of proinflammatory cytokines and the expansion of regulatory T-cells. Here, we review the current understanding of the role of MK in different inflammatory disorders and summarize the knowledge of MK biology.

KEYWORDS: Leukocytes, cytokines, immunity.

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1. INTRODUCTION

The cytokine midkine (MK) was first discovered in the eighties of the last century as a molecule involved in embryonic development [1]. MK was found to be rarely expressed in the adult organism, but different types of cancer cells showed high MK expression associated with a poor prognosis of the patients [2–6]. Several studies revealed that overexpression of MK promoted tumor growth, survival, invasion, and tumor angiogenesis [7–9]. However, there is growing evidence that MK may also play an important role in chronic inflammatory disorders including, for example, kidney diseases, rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis as summarized in Table 1 [10–13]. The fact that these diseases exhibit high clinical and epidemiological relevance and affect the patients' quality of life dramatically makes it worthwhile to take a deeper look at a new factor possibly contributing to the induction and/or maintenance of these pathological conditions. In this paper, we summarize the findings on the role of MK in chronic inflammatory diseases and give an overview of MK biology including its gene and protein structure as well as its receptors and signaling.

2. GENE AND PROTEIN

MK was first identified in mouse embryonic carcinoma cells in studies on early stages of embryogenesis. In this model, differentiation of embryonic carcinoma cells by application of retinoic acid, one of the key players coordinating embryogenesis, led to increased MK mRNA expression in these cells [1]. In mouse embryos, MK was found to be induced at day 7 and showed a complex expression pattern at day 11 when organogenesis had started. After the midgestation stage, MK expression rapidly decreased and was thereafter only detectable at restricted sites such as the kidney. In situ hybridization technique in mouse embryos between days 7 and 13 revealed strong MK expression in epithelial tissues interacting with mesenchymal tissues during organ formation, in neuronal tissues, in the mesoderm where remodeling occurred, in the anterior lobe of the pituitary gland, in the retina, and in the kidney. This mode of expression considering gestation in mice lasting about 21 days was one of the reasons to give this molecule the name midkine (midgestation, kidney) [14].

While the human MK gene (MDK) is located on chromosome 11, the mouse MK gene was identified on chromosome 2 [15, 16]. The coding sequence of the human and mouse MK gene consists of 4 exons. In the promoter region of the MK gene, a retinoic acid response element (RARE), a hypoxia-responsive element (HRE), and a binding site for the product of the Wilms tumor suppressor gene WT-1 leading to decreased MK expression upon WT-1 binding were found [17–19].

As shown by NMR technique, the human 13 kDa protein MK consists of two similar domains each containing three antiparallel β-strands which are connected via disulfide bonds (Figures 1(a) and 1(b)) [20, 21]. MK is rich in basic amino acids forming two clusters responsible for heparin binding which are located in the C-terminal domain, namely, cluster 1 (K79, R81, and K102 in human MK) and cluster 2 (K86, K87, R89 in human MK). Although the N-terminal domain also contains several basic residues, the heparin-binding activity is very weak which is probably due to the existence of several acidic amino acids in this domain [20]. The C-terminal domain has been found to be functionally more important than the N-terminal domain [22]. Accordingly, different MK functions, for example, the promotion of neurite outgrowth or plasminogen activator activity are mediated by the C-terminal domain [23–25]. However, only the full length molecule, not the C-terminal domain by itself, was able to promote survival of embryonic brain neurons [23]. For some of its functions as, for example, enhancement of plasminogen activator activity, MK needs to dimerize which is mediated by a head-to-head conformation involving heparin being sandwiched by the cluster 2 heparin binding sites of two MK molecules [20, 26]. Transglutaminase activity and heparin have been found to be capable of promoting MK dimerization [26, 27].

MK represents the founding member of a family of heparin-binding growth factors and is structurally unrelated to any other growth factor family known so far consisting of only one other member named pleiotrophin (PTN). MK and PTN are highly conserved within the different species. While human MK

TABLE 1: Overview of the role of MK in chronic inflammatory diseases.

Organ	Disease	Model/specimen	Publication
Kidney	Diabetic nephropathy	Streptozotocin-induced diabetic nephropathy	Kosugi et al. 2006 [28], Kosugi et al. 2007 [10]
	Tubulointerstitial injury	Ischemia/reperfusion	Sato et al. 2001 [29], Sato et al. 2005 [30]
	Drug side effects	Cisplatin-induced renal damage	Kawai et al. 2004 [31]
Joints	Rheumatoid arthritis	Human synovial fluid, human synovial tissue	Takada et al. 1997 [32]
		Antitype II collagen antibody-induced arthritis	Maruyama et al. 2004 [11]
Vascular System	Atherosclerosis	Intimal hyperplasia in vein grafts	Banno et al. 2006 [33]
		In-stent restenosis	Narita et al. 2008 [34]
		Neointima formation in restenosis	Horiba et al. 2000 [35]
Colon	Crohn's disease	Human blood	Krzystek-Korpacka et al. 2010 [12]
	Ulcerative colitis	Dextran-sulfate-sodium- (DSS-) induced colitis	Yuki et al. 2006 [36]
Central nervous system	Multiple sclerosis	Experimental autoimmune encephalomyelitis (EAE)	Liu et al. 1998 [37], Wang et al. 2008 [13]

shares 87% sequence identity with mouse MK, human MK and PTN have about 50% sequence identity (Figure 2(a)) [38–40]. All cysteine residues in both molecules are conserved in the human and murine system [21]. The basic amino acids that form the clusters for heparin-binding are also highly conserved in MK and PTN (Figure 2(b)). As an exception, the basic amino acid K84 in human MK is changed to R84 in human PTN [22]. Although MK and PTN share distinctive sequence similarities, both proteins show different expression patterns. While MK is highly expressed during midgestation as mentioned above, the expression peak of PTN in mice occurs around birth [41]. In Drosophila melanogaster, two MK/PTN homologues have been identified, named miple1 and miple2 (midkine, pleiotrophin) [42]. The amino acid sequence of miple1 and miple2 is about 60% identical to human MK and human PTN with particularly high homology to the C-terminal domain. The basic amino acids in the heparin-binding clusters are partially conserved in the miple proteins [42].

3. RECEPTORS AND SIGNALING

MK binds a variety of different receptors and an overview of these receptors is shown in Table 2. MK has been found to promote, for example, growth, survival, migration, and gene expression of different cell types probably via a multiprotein receptor complex consisting of several molecules [43]. Within this complex, the best characterized receptor represents the receptor-like protein tyrosine phosphatase β/protein tyrosine phosphatase ζ (PTPζ), which is abundantly expressed in the central nervous system mediating cell adhesion and signaling during embryonic development [44]. PTPζ is a transmembrane protein with intracellular tyrosine phosphatase activity linked to an extracellular chondroitin sulfate chain [45]. The fact that the binding affinity of MK to PTPζ decreased from a Kd of 0.56 nM to 8.8 nM after removal of the chondroitin sulfate chain indicated its importance for ligand-receptor recognition [45]. Binding of MK to PTPζ induced migration of embryonic neurons as well as UMR106 osteoblast-like cells and enhanced survival of neurons during embryonic development [45–47]. Inhibition of different kinases such as PI3 kinase, MAP kinases, Src family kinases, and protein kinase C impaired MK-dependent migration of UMR-106 cells implying the involvement of multiple kinases in downstream signaling upon MK binding to PTPζ [46].

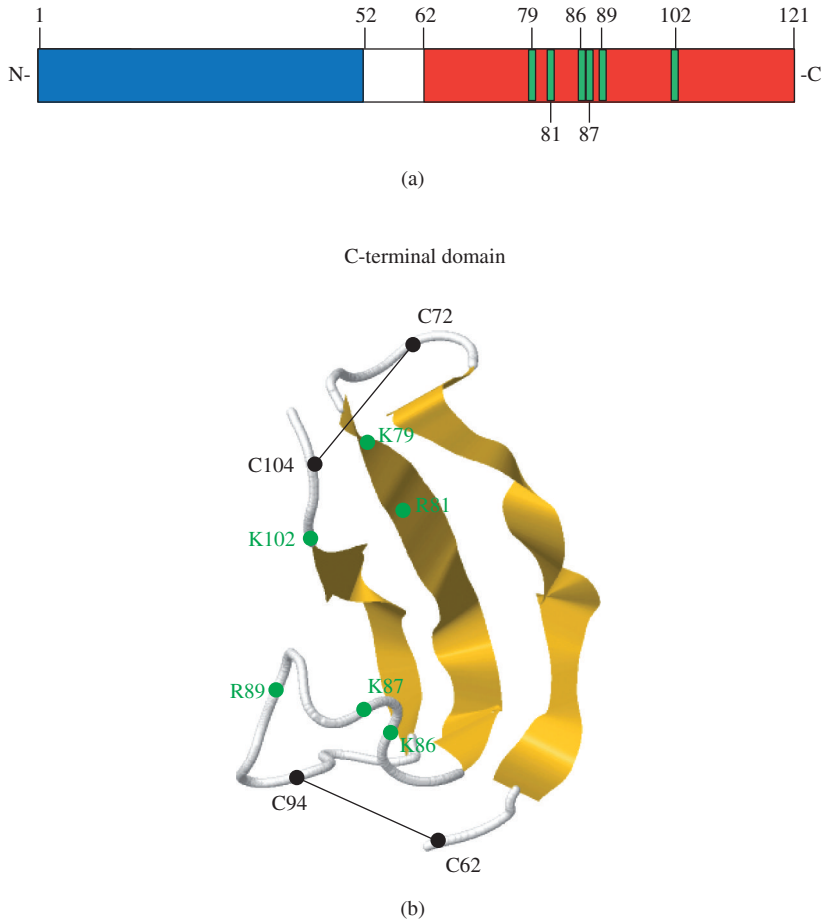

FIGURE 1: Protein structure of MK. (a) MK protein structure is shown. MK consists of two domains with similar size connected by an interdomain (white box). Numbers indicate amino acid position within the protein. The heparin-binding sites consisting of basic amino acids are located in the C-terminal domain (green boxes) [20]. (b) Tertiary structure of the C-terminal domain of MK protein "taken from the protein data bank PDB via http://www.rcsb.org/pdb/explore.do?structureId=1MKC." The C-terminal domain contains three β-strands (yellow structure). Heparin binding clusters consisting of basic amino acids (cluster 1: K79, R81, K102; cluster 2: K86, K87, R89 in human MK) are displayed as green dots. C62, C72, C94, and C104 represent highly conserved cysteine residues forming disulfide bonds (black lines) [20].

In addition to PTPζ, members of the low-density-lipoprotein receptor-related protein (LRP) family have been identified as components of the MK receptor complex including LRP-1, megalin/brushin, LRP-6, and apoE receptor-2 [47, 48]. LRP-1 induced survival of embryonic neurons and prevented hypoxic injury in mouse embryonic stem cells upon MK binding with a Kd of 3.5 nM, whereas binding affinity of MK to the other LRP family members mentioned above was significantly lower [47–49].

In 13-day-old mouse embryos, β_1 integrins were identified as functional MK receptors. MK binding to $\alpha_4\beta_1$-integrin mediated migration of UMR-106 osteoblast-like cells [51]. In these cells, MK treatment led to increased tyrosine phosphorylation of paxillin which interacts with the cytoplasmatic tail of β integrins [52, 61]. Via $\alpha_6\beta_1$-integrin binding, MK induced neurite outgrowth of embryonic neurons [51]. Furthermore, MK promoted the association of the $\alpha_6\beta_1$-integrin and the tetraspanin CD9, which belongs to a family of highly conserved receptor proteins forming multimeric complexes with other membrane proteins and thereby modulating cell adhesion, proliferation, and metastasis [52, 62]. The fact that $\alpha_4\beta_1$and $\alpha_6\beta_1$ integrins coimmunoprecipitated with the LRP-6 ectodomain and PTPζ upon MK treatment suggested a functional interaction of these molecules in an MK receptor complex [51].

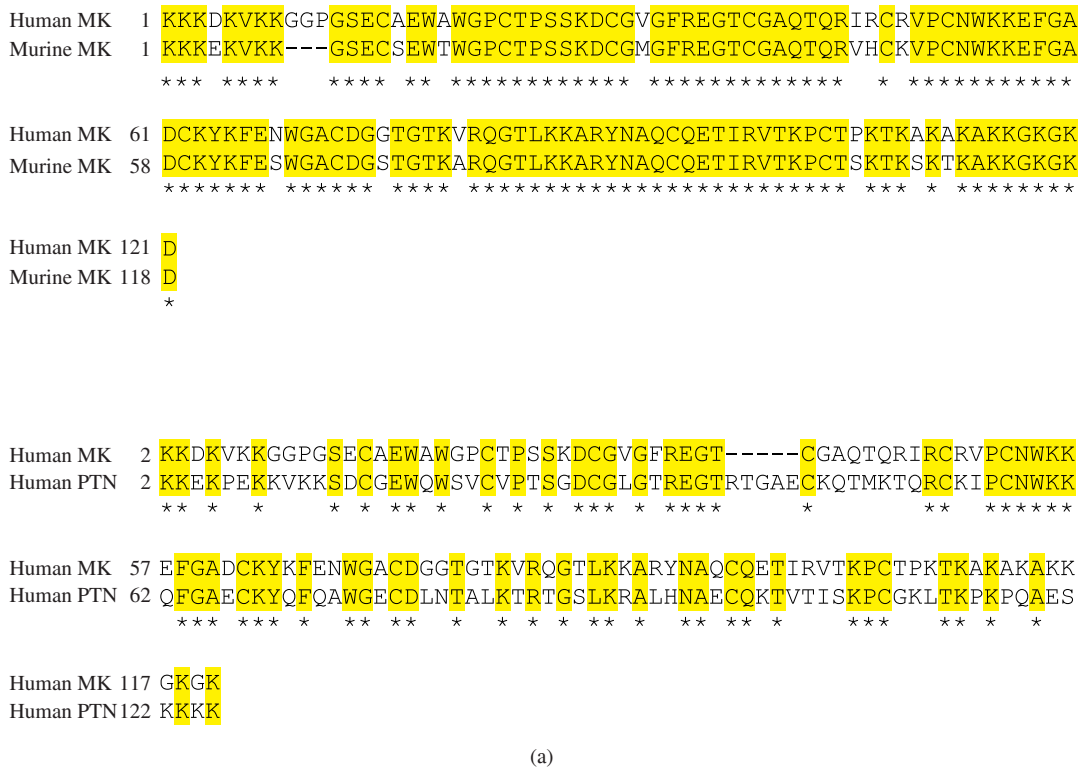

FIGURE 2: Sequence homologies of MK and PTN. (a) Amino acid sequence of human MK and murine MK as well as human MK and human PTN. Human and murine MK show 87% amino acid sequence identity; human MK and PTN share 50% sequence homology. ⋆ = residues identical in both sequences [38–40]. (b) Web logos displaying sequence homology of human MK, murine MK, chicken MK, miple1, and human PTN in information bits [50]. A large overall height indicates strong conservation of the corresponding amino acid in that position; the height within the stack shows the relative frequency of the amino acid at that position. Cysteine residues (black circle) and basic amino acids (green circle) are highly conserved within different species of the MK family.

TABLE 2: Overview of MK receptors.

Receptor family	MK receptor	Function	Publication
PTP	PTPζ	Migration of embryonic neurons	Maeda et al. 1999 [45]
		Migration of UMR106 osteoblast-like cells	Qi et al. 2001 [46]
		Survival of embryonic neurons	Sakaguchi et al. 2003 [47]
LRP	LRP-1	Survival of embryonic neurons	Muramatsu et al. 2000 [48]
		Prevention of hypoxic injury in mouse embryonic stem cells	Lee et al. 2011 [49]
	megalin/brushin		Muramatsu et al. 2000 [48]
	LRP-6		Muramatsu et al. 2000 [48], Muramatsu et al. 2004 [51]
	apoE receptor-2		Muramatsu et al. 2000 [48]
Integrins	$\alpha_4\beta_1$	Migration of UMR106 osteoblast-like cells	Muramatsu et al. 2004 [51]
	$\alpha_6\beta_1$	Neurite outgrowth of embryonic neurons	Muramatsu et al. 2004 [51], Huang et al. 2008 [52]
Notch	notch2	Epithelial mesenchymal transition of immortalized HaCaT keratinocytes	Huang et al. 2008 [52]
		Epithelial mesenchymal transition and chemoresistance in pancreatic ductal adenocarcinoma cells	Güngör et al. 2011 [53]
Receptor tyrosine kinase	ALK	Growth of SW-13 cells in soft agar	Stoica et al. 2007 [7]
Carbohydrates	Neuroglycan C	Process elongation of oligodendrocyte precursor-like cells	Ichihara-Tanaka et al. 2006 [54]
	Heparan sulfate trisulfated units		Kaneda et al. 1996 [55], Ueoka et al. 2000 [56], Zou et al. 2003 [57]
	Chondroitin sulfate E units		Ueoka et al. 2000 [56], Zou et al. 2003 [57]
	Syndecan-1		Mitsiadis et al. 1995 [58]
	Syndecan-3		Mitsiadis et al. 1995 [58], Nakanishi et al. 1997 [59]
	Glypican-2		Kurosawa et al. 2001 [60]

Recently, neuroglycan C was found to serve as an MK receptor in an oligodendrocyte precursor-like cell line [54]. Neuroglycan C is a chondroitin sulfate proteoglycan exclusively expressed in the central nervous system contributing to brain development [63]. Similar to PTPζ, the binding affinity of neuroglycan C to MK was impaired when chondroitin sulfate chains were absent [54]. In addition, MK binds to Notch2, a transmembrane protein belonging to the Notch family. Here, MK was found to be involved in induction of epithelial mesenchymal transition (EMT) of immortalized HaCaT keratinocytes indicated by a decrease of epithelial and an increase of fibroblast markers [64]. EMT is a process that occurs during embryonic development as well as in tumor metastasis and is characterized by loss of cell contacts in order to allow cell migration [64]. An interaction between MK and Notch2 promoting EMT was also shown in pancreatic ductal adenocarcinoma cells (PDACs) [53]. Furthermore, Notch2 signaling upon MK binding upregulated NFκB implying an involvement of MK in inflammatory pathways [53]. Moreover, anaplastic lymphoma kinase (ALK), a transmembrane tyrosine kinase mediating survival, proliferation, and differentiation of normal and tumor cells was identified as MK receptor [7, 65]. MK promoted colony formation of the adrenal gland SW-13 tumor cell line in soft agar and induced proliferation of human endothelial cells via ALK signaling [7]. In WI-38 human fibroblasts, MK stimulation led to phosphorylation of ALK and subsequent activation of PI3 kinase and MAP kinase [7].

The fact that most MK effects were diminished upon administration of heparin or treatment with heparitinase or chondroitinase indicated the importance of carbohydrate recognition in MK signaling. Two specific carbohydrate structures, namely, heparan sulfate trisulfated units or chondroitin sulfate E units—when present as oligomers—have been shown to bind to MK with high affinity [55–57]. As mentioned above, chondroitin sulfate chains linked to PTPζ enhanced MK binding affinity. Syndecans and glypican-2, carbohydrates involved in the neuronal development, also showed MK binding activity contributing to development of the central nervous system, neuronal cell migration, and neurite outgrowth [58–60].

In conclusion, MK represents a promiscuous ligand that binds different receptors thereby activating several intracellular signaling events. However, the intracellular signal transduction pathways that mediate the effects of MK in inflammation are widely unknown.

4. MK AS A MODULATOR OF THE INFLAMMATORY RESPONSE

4.1. Kidney

There is a growing body of evidence that MK plays a critical role during inflammation. In the adult organism, MK shows constitutive expression in proximal renal tubular epithelial cells, and different pathological kidney conditions including diabetic nephropathy have been linked to enhanced MK expression [10, 29–31]. Diabetic nephropathy caused by diabetes mellitus represents the main cause of end-stage renal failure and dialysis requirement accompanied by high mortality and exceeding cost of care [66]. During diabetic nephropathy which is associated with tubulointerstitial inflammation, glomeruli, interstitium, and tubules of patients showed highly increased MK expression in contrast to healthy patients [10]. In a diabetes mouse model where hyperglycemia was induced by application of streptozotocin which impairs insulin production by selective destruction of β-cells of the pancreas and results in elevated glucose levels, tubulointerstitial damage was less severe in mice lacking MK expression compared to WT control animals within 2 to 6 months after the onset of the experiment. This suggests that MK may aggravate the inflammatory response in diabetic nephropathy [10, 28].

Inflammation induced by ischemia/reperfusion (I/R) in the kidney, which is one of the main causes of acute renal failure in humans, led to increased MK expression in the renal tubules two days after ligation of renal arteries for 90 minutes in a mouse model. Seven days after I/R, MK expression returned to baseline values [29, 30]. In MK-deficient mice or mice treated with MK antisense oligodeoxyribonucleotides, tubulointerstitial damage was reduced compared to control conditions [29, 30]. Furthermore, renal damage caused by cisplatin—a chemotherapeutic drug used for the treatment of different kinds of cancer—was found to be reduced in MK-deficient mice implying that MK may exacerbate side effects of drugs in the

kidney [31]. However, in all pathological conditions mentioned above, reduced renal damage in the absence of MK was associated with impaired infiltration of inflammatory cells into the renal tissue [10, 29–31]. This is in line with the finding that monocyte chemotactic protein 1 (MCP-1, CCL2) and macrophage inflammatory protein (MIP-2, CXCL2) were upregulated in WT mice upon I/R of the kidney. Similarly, MCP-1 expression was found to be increased in streptozotocin-induced diabetic nephropathy [10, 29, 30]. Thus, MK may be able to contribute to the recruitment of leukocytes by induction of chemokine expression in the kidney. Both chemokines are known to mediate leukocyte infiltration and upregulation of these proteins was diminished in mice lacking MK expression [10, 29]. Studies with cultured tubular epithelial cells (TEC) revealed that high glucose levels mimicking the diabetes model and H_2O_2 mimicking conditions during ischemia induced MK expression in TEC and increased MCP-1 and MIP-1 expression in these cells. This effect was not observed in cells of MK-deficient mice or in cells treated with an MK blocking antibody [10, 29]. In addition, recombinant MK enhanced MCP-1 and MIP-1 expression in TEC [29]. Thus, induction of chemokines by MK may at least partially explain the reduced recruitment of inflammatory cells into the kidney under inflammatory conditions in the absence of MK. In addition, MK by itself has been reported to act as a haptotactic and chemotactic agent for neutrophils in a Boyden chamber [32]. However, leukocyte infiltration was diminished during Cisplatin-induced renal inflammation in the genetic absence of MK or upon downregulation of MK by antisense technique, whereas no difference in expression pattern of MCP-1 was observed [31]. Thus, the induction of chemokines may represent not the only mechanism mediating leukocyte recruitment in the kidney. Taken together, MK may aggravate different pathologies of the kidney by enhanced recruitment of leukocytes. The question whether MK is able to induce additional inflammatory chemokines besides MCP-1 and MIP-2 and how MK exactly contributes to leukocyte trafficking still needs to be investigated.

4.2. Joints

Rheumatoid arthritis (RA), an autoimmune disease leading to chronic joint inflammation, subsequent joint destruction, and deformities, is of high clinical and epidemiological relevance affecting approximately from 0.5% to 1% of the adults worldwide with a higher prevalence in women [67]. High levels of RF—autoantibodies against the Fc fragment of IgG—can be detected in 80% of RA patients and correlate with the susceptibility to developing RA and severe joint damage. However, RF is also elevated in other autoimmune diseases and does not correlate with disease activity [68]. Of note, synovial fluid and sera of 90% of patients suffering from RA showed enhanced MK levels. MK levels correlated positively with RF [11]. Inflamed tissues of patients with active inflammatory synovitis of RA expressed high concentrations of MK, whereas noninflamed tissue showed no MK expression at all [32]. Elevated MK expression in a large number of RA patients indicated that MK may represent a new additional biomarker for the diagnosis of RA. Furthermore, the functional relevance of MK during arthritis was investigated in a murine arthritis model where joint inflammation was induced by injection of an antitype II collagen Ab followed by intra-articular LPS administration [69]. While control mice developed severe arthritis until day 7 measured by joint cavity size, synovial membrane thickening, and accumulation of synovial fluid, the inflammatory response was almost completely absent in MK-deficient mice. However, intraperitoneal administration of recombinant MK restored this effect and led to severe arthritis in this model. As seen in the kidney, the number of extravasated neutrophils and macrophages into the synovial tissue in MK-deficient mice was decreased after 2 to 7 days when compared to WT control animals at the same time point indicating that MK may represent a critical player for pathogenesis of RA [11].

4.3. Vascular System

Although risk factors for development and maintenance of atherosclerosis including hypercholesterolemia, free radicals from cigarette smoking, diabetes mellitus, and hypertension have been clearly identified, cardiovascular diseases still represent the main cause of death in Europe and in the United States.

Atherosclerosis is a chronic inflammatory disease of large- and medium-sized arteries characterized by endothelial dysfunction and subsequent plaque formation of the vascular wall. Under healthy conditions, arteries and veins express MK at a very low level [34]. However, injury of the endothelium caused by experimental interventions led to increased MK expression in different rabbit and mouse models [33, 35]. Macrophages infiltrating the injured vascular wall after stenting have been found to be a major source of MK, whereas freshly isolated monocytes did not express MK [34]. Clinically, mechanic vessel injury is followed by an inflammatory response causing neointima formation which may lead to restenosis after therapeutic intervention. Leukocytes play an important role in this context by secreting factors which promote migration and proliferation of smooth muscle cells (SMCs) and maintain an inflammatory environment [70]. Compared to control mice, neointima formation was almost completely absent in MK-deficient mice [35]. This was in line with the finding that leukocyte recruitment to the injured vessel wall was reduced in MK-deficient mice compared to control animals. However, intra-arterial administration of recombinant MK restored neointima formation [35]. Endothelial cells of implanted vein grafts interpositioned into an artery in rabbits also strongly expressed MK. When grafts were treated with MK siRNA, intima thickness and leukocyte infiltration into the vessel wall was significantly reduced suggesting that MK promoted leukocyte infiltration into the vascular wall after intervention-associated vessel injury [33].

4.4. Colon

Approximately 1.4 million people in Europe are diagnosed—generally in late adolescence or early adulthood—with inflammatory bowel disease (IBD) which consists of two major forms, namely, ulcerative colitis (UC) and Crohn's disease (CD) [71]. Both pathologic entities are characterized by autoimmune-mediated inflammation of the gastrointestinal tract. In CD, MK serum levels showed positive correlation with Crohn's Disease Activity Index (CDAI) which includes vital parameters, clinical findings, and medical history [12, 72]. Accordingly, MK has been found to be a sensitive biomarker of diagnostic value comparable with the gold standard C-reactive protein (CRP) in this disease [12]. In an experimental colitis model, where bowel inflammation was induced by dextran sulfate sodium (DSS) and which represents a well-established model for UC, MK was abundantly expressed in fibroblasts of the mucosal and submucosal layers of the rat distal colon [36]. Here, TNF-α and IL-1β, which show high local expression during active IBD in humans leading to infiltration of innate immune cells, were expressed in the distal rat colon [36, 73]. In addition, both cytokines induced MK expression in the rat fibroblast cell line 3Y1. Moreover, MK was found to accelerate intestinal wound repair in vitro using cultured epithelial monolayer sheets suggesting that MK may contribute to mucosal repair [36]. Thus, the inflammatory cytokines TNF-α and IL-1β may facilitate wound repair by upregulating MK. However, the question whether MK is beneficial for mucosa repair or rather promotes inflammation requires further investigation.

4.5. Central Nervous System

MK has also been investigated in a chronic inflammatory disease affecting the central nervous system, namely, multiple sclerosis (MS). In Europe around 500,000 people suffer from MS [74]. MS is a chronic autoimmune disease that is characterized by demyelination of axons of the central nervous system caused by autoreactive T-cells and demyelinating antibodies. To study MS, experimental autoimmune encephalomyelitis (EAE) has been used as a widely accepted animal model where demyelination is induced by injection of myelin-oligodendrocyte-glycoprotein leading to T_H1 and T_H17 cell-mediated inflammation [75, 76]. After induction of EAE in rats, increased MK mRNA expression in the spinal cord correlated with the severity of clinical symptoms such as limb and tail weakness. Recovery of clinical symptoms led to decreased MK expression in the spinal cord again [37]. EAE induction of WT mice caused severe clinical symptoms 18 days after onset of the experiment. MK-deficient mice also developed clinical signs of encephalomyelitis. However, clinical scores were significantly lower than in WT

control animals. Administration of recombinant MK with a subcutaneously implanted microosmotic pump deteriorated clinical scores [13]. Histological analysis of the spinal cord revealed decreased infiltration of inflammatory cells in the spinal cord in MK-deficient mice compared to WT control animals, whereas MK application restored this effect showing positive correlation of clinical findings and histological analysis [13]. This indicates that MK was important for induction and development of EAE. CD4+ and CD8+ T cell populations were similar in peripheral lymph nodes of WT and MK-deficient mice and, therefore, did not explain reduced clinical and histopathological severity in MK-deficient mice. After onset of EAE, expansion of CD4+/CD25+ regulatory T-cells (T_{reg}), which have the ability to suppress autoreactive T_H1 and T_H17 cells, was significantly increased in peripheral lymph nodes in the absence of MK [13, 77]. Accordingly, administration of MK critically suppressed T_{reg} expansion in a dose-dependent manner in vitro and in vivo. When T_{reg} were targeted with a CD25 blocking antibody in MK-deficient mice, suppression of EAE was abolished [13]. These findings indicate that an increased number of T_{reg} were recruited to peripheral lymph nodes when MK was absent after induction of EAE leading to attenuated clinical and histopathological symptoms in this model [13]. Furthermore, it was shown that CD4+ T-cells from MK-deficient mice after induction of EAE expressed lower amounts of IFN-γ and IL-17, cytokines which are critically involved in the pathogenesis of several autoimmune diseases [78]. It could also be shown that targeting MK by RNA aptamers significantly increased T_{reg} expansion and improved clinical signs after induction of EAE in WT mice [13]. Taken together, MK expression has been shown to be enhanced during induction and progression of EAE which correlated positively with clinical scores and histopathological signs of inflammation of the myelin sheath. MK suppressed expansion of T_{reg} and enhanced autoreactive injury of the myelin sheath. Targeting MK led to increased T_{reg} expansion and subsequently suppressed autoreactive T_H1 and T_H17 cell populations in this model which are critically involved in the pathogenesis of autoimmune diseases.

5. OUTLOOK AND PERSPECTIVES

Being diagnosed with autoimmune diseases such as multiple sclerosis, ulcerative colitis, Crohn's disease, or rheumatoid arthritis critically affects the patients' quality of life [79, 80]. Especially the early onset and gradual progression of disease over a long period of time makes therapy and treatment for clinicians and patients very challenging [81]. Immunosuppressive drugs, corticosteroids, cytostatics, and biological drugs specifically targeting proteins involved in the inflammatory response can effectively suppress autoimmune responses. However, severe side effects, for example, increased risk of infection, cancer, and other autoimmune diseases, make treatment options unsatisfying [81]. Similar to autoimmune disorders, a number of additional widespread diseases including atherosclerosis are characterized by chronic inflammatory processes [70]. Different studies have shown that MK plays a role in inflammation by induction of leukocyte infiltration and chemokine expression as well as by suppression of T_{reg} expansion. As MK shows highly restrictive expression patterns in healthy tissues of the adult organism, targeting MK may represent a promising new approach for treatment of chronic inflammation including autoimmune diseases.

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