Targeting Myeloid-Derived Suppressor Cells Is a Novel Strategy for Anti-Psoriasis Therapy

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Received 25 January 2020; Revised 24 April 2020; Accepted 15 May 2020; Published 28 June 2020

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Psoriasis is a common immune-mediated, chronic inflammatory genetic-related disease that affects patients’ quality of life. Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of progenitor and immature myeloid cells which are expanded in psoriatic skin lesions and peripheral blood. However, the role of MDSCs in the pathogenesis of psoriasis remains unclear. Here, we confirmed that the accumulation of human MDSCs is remarkably increased in skin lesions of psoriasis patients by flow cytometry. Depleting MDSCs by Gemcitabine significantly suppresses IMQ-induced psoriatic inflammation and epidermal thickening as well as Th17 and Treg cell accumulation. Moreover, through the RNA-Seq technique, we validated some differentially expressed genes on CD4+ T-cells of IMQ-induced-MDSC-depleted mice such as IL-21 and Timd2, which are involved in Th17-cell differentiation or T-cell activation. Interestingly, neutralizing IL-21R by antibody reduces IMQ-induced epidermal thickening through downregulating the infiltration of MDSCs and Th17 cells. Our data suggest that targeting myeloid-derived suppressor cells is a novel strategy for antipsoriasis therapy. IL-21 may be a potential therapeutic target in psoriasis.

1. Introduction

Psoriasis is a common immune-mediated, chronic inflammatory skin disease, which has been characterized by epidermal acanthosis, hyperkeratosis, parakeratosis, and extensive inflammatory cell infiltration including T-lymphocytes, macrophages, mast cells, and neutrophils [1]. Accumulating evidence showed that the psoriatic keratinocytes (KCs) not only have been shown uncontrollable proliferation but also respond to cytokines such as IL-22 or IL-17A/IL-17F released from Th17 or Th22 cells, which facilitate the secretion of proinflammatory factors such as AMP activating dendritic cells to initiate specific T-cell-related immune responses [1, 2]. More importantly, psoriatic KCs recruit immune cells into psoriatic skin lesions through the production of chemokines or cytokines including myeloid-derived suppressor cells (MDSCs) [3–6].

MDSCs (myeloid-derived suppressor cells) are a heterogeneous population of progenitor and immature myeloid cells, which have been generated during a variety of pathologic conditions such as cancer, infectious diseases, and autoimmune disorders [7–9]. Murine MDSCs are characterized by coexpression of CD11b and Gr-1, whereas human MDSCs are most commonly identified by CD11b+ and CD33+ with low levels of HLA-DR, the major histocompatibility complex (MHC) class II molecule [7, 10]. MDSCs consist of two large groups of cells: granulocytic or polymorphonuclear MDSCs (PMN-MDSCs, CD11b+CD14−CD15+CD33+HLA-DR−/lo)
and monocytic MDSCs (M-MDSCs, CD11b⁺CD14⁺CD15⁻CD33⁺HLA-DR⁻/lo) [9]. Moreover, it was reported that CD14⁺HLA-DR⁻/hi monocytic MDSCs are more suppressive than PMN-MDSCs and have emerged as important mediators of tumor-induced immunosuppression [9, 11].

In normal conditions, MDSCs have differentiated into mature granulocytes, macrophages, or dendritic cells (DCs) in bone marrow [9]. However, under pathological conditions such as cancer, chronic inflammatory diseases, and immune diseases, those undifferentiated immature myeloid cells have been recruited and infiltrated into the specific organ from bone marrow [7]. Although MDSCs have been shown a remarkable ability to suppress T-cell responses in cancer, it becomes more heterogeneous and complicated in autoimmune diseases. Recent studies revealed that expanded MDSCs induce immune responses in systemic lupus erythematosus (SLE), autoimmune arthritis (RA), and autoimmune encephalomyelitis [12–15]. Interestingly, studies showed that the population of MDSCs has been expanded in psoriasis patients, which produce cytokines including IL-23, IL-1β, and CCL4 [16–18]. Moreover, MDSCs isolated from psoriasis patients fail to suppress T-cell activation and express reduced programmed cell death protein-1 (PD-1), as a consequence of losing the ability to induce regulatory T-cell conversion compared with those cells from healthy controls or melanoma patients [16, 19], indicating MDSCs showed great heterogeneity under different pathogenesis.

In this study, we aim to investigate the proinflammatory roles of MDSCs in the pathogenesis of psoriasis. We found it is a novel strategy to target myeloid-derived suppressor cells for antipsoriasis therapy.

2. Materials and Methods

2.1. Human Skin Samples. This study was reviewed and approved by the local ethics Institutional Review Board (IRB) (Xiangya Hospital, Central South University, IRB-201512526). All experiments were conducted in accordance with the Declaration of Helsinki Principles. We performed a cross-sectional study of 27 patients with psoriasis and 17 healthy control subjects without inflammatory skin disease. Inclusion criteria included psoriasis patients or healthy control subjects older than 18 years of age, able to give written informed consent, and able to give skin samples. Exclusion criteria included patients on subcutaneous and intravenous systemic immunosuppressant medications. Patients were clinically evaluated for psoriasis subtype and PASI score.

2.2. IMQ-Induced Psoriasis-Like Skin Inflammation. Six- to eight-week-old mice were treated with daily topical doses of 62.5 mg of IMQ cream (5%, 3.125 mg of the active compound; Aldara, 3M Pharmaceuticals), which was applied to their shaved backs for 6 consecutive days. A scoring system based on the clinical Psoriasis Area and Severity Index (PASI) was used to evaluate the skin inflammation on the skin lesions of mice. Briefly, erythema, scale, and infiltration were graded on a scale from 0 to 4 as follows: 0, none; 1, slight; 2, moderate; 3, marked; and 4, very marked. The level of erythema was scored using a table with red tints. The cumulative score served as a measure of inflammation severity (scale: 0–12) [20]. The animal study protocol was approved by the Ethics Committee of Xiangya Hospital (Central South University, China, #2015110134).

2.3. In Vivo Treatments. Gemcitabine treatment: BALB/c mice were injected intraperitoneally with Gemcitabine (Selleckchem, Houston, TX, USA) on days -1, 1, and 3 at the dose of 40 mg/kg; IMQ was applied from day 1 to their shaved backs for 5 consecutive days topically. The mice were photographed and sacrificed for skin lesion analysis on day 8 (mice divided into 3 groups: vehicle (IMQ+vehicle), Gemcitabine (IMQ+GEM), and untreated (normal)). Anti-IL-21R antibody treatment: BALB/c mice were injected intraperitoneally with anti-mouse IL-21R antibody (4A9) (BioXCell, West Lebanon, NH, USA) on days -2, 0, 1, 3, and 5 by i.p. injection of 140 μg anti-IL-21R antibody; IMQ was applied from day 1 to their shaved backs for 6 consecutive days topically. The mice were photographed and sacrificed for skin lesion analysis on day 8 (mice divided into 3 groups: vehicle (IMQ+vehicle), anti-IL-21R antibody (IMQ+Anti-IL-21R), and untreated (normal)).

2.4. Tissue Processing. Skin lesions of psoriasis patient or mice were cut into small pieces and digested in 5 ml PBS containing 2 mg/ml collagenase type IV and 1 mg/ml dispase II (both Sigma-Aldrich, USA) while shaking at 37°C for 150 minutes. Enzyme activity was stopped using 10% FBS medium. The tissue was further homogenized with a syringe and filtered through a 40 μm cell strainer. The cell strainer was washed with 20 ml PBS followed by centrifugation (500 x g for 10 min). Single-cell suspensions from the spleens were obtained by mashing the spleens through 40 μm cell strainers. The cell strainer was washed with 20 ml PBS followed by centrifugation (500 x g at 4°C for 5 min) and then split red blood cells by means of lysing solution (BD Pharm Lyse™, USA). Single cells were then stained with fluorescence antibodies for flow cytometry.

2.5. Flow Cytometry. All utilized antibodies are summarized in Supplementary Table S2. First, Zombie Aqua™ Fixable Viability Dye was used for selecting living cells. Then, TruStain fcX anti-mouse CD16/32 was used to block Fc receptor on the immune cells of mice. For surface staining, single cells isolated from the skin or the spleens were incubated with antibodies at 4°C for 30 min, followed by washing and centrifugation (500 x g at 4°C for 5 min) and then split red blood cells by means of lysis solution (BD Pharm Lyse™, USA). Single cells were then stained with fluorescence antibodies for flow cytometry.
from ThermoFisher followed by incubation with anti-mouse/rat Foxp3 antibodies at room temperature for 40 min according to the manufacturer’s instructions. To better distinguish the border between positive and negative subsets, we set FMO-controls for markers including IL-17A, IFN-γ, CD25, and Foxp3. The acquisition was performed with FACS Canto II (BD Biosciences). Flow cytometric analysis on live, single cells was performed using FlowJo (Tree Star) software.

2.6. Quantitative RT-PCR (qRT-PCR). Total RNA was extracted with Trizol (Invitrogen), and cDNA was synthesized via reverse transcription using a HiScript Q RT Kit (Vazyme) (R123-01). qRT-PCR was performed using an UltraSYBR Mixture with ROX (CWBio, Beijing, China) according to the manufacturer’s instructions on a QuantStudio 3 RT-PCR instrument (ThermoFisher, USA). The reaction mixture contained 0.5 ml of forward and reverse mouse primers, as described in Supplementary Table S1. Values were normalized to Gapdh. All reactions were conducted in triplicate across. Relative quantification was performed using the ΔΔCT method, and the results were expressed in a linear form using the formula $2^{-\Delta\Delta CT}$.

2.7. Cell Sorting for RNA Sequencing. Splenic cells were isolated from the freshly obtained spleen of mice. CD4$^+$ T-cells were positively selected from splenic cells using magnetic CD4 microbeads (Miltenyi Biotec, San Diego, CA) with a magnet according to the manufacturer’s instructions. The purity of the CD4$^+$ T-cells after sorting was >95%. The cDNA library construction, library purification, and transcriptome sequencing were implemented according to the Shanghai Genergy Biotechnology Sequencing Company’s instructions.

2.8. Statistical Analysis. All statistical analyses were performed using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). The statistical significance between values was determined by 2-tailed unpaired Student’s t-test or one-way ANOVA with Dunnett’s post hoc test when samples were not distributed normally. All data represent the mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001, ns: not significant.

3. Results

3.1. The Accumulation of Human MDSCs Is Remarkably Increased in Skin Lesions of Psoriasis Patients. Recently, the accumulation of MDSCs has been observed in the peripheral blood or spleen of murine models in autoimmune disorders such as SLE and RA, which are positively related to disease severity [12, 13, 15] and the number of MDSCs has been found expanded in psoriasis patients [16, 19, 21]. To study the relationship between psoriasis and MDSCs, we analyzed the population of MDSCs in skin lesions of psoriasis patients.
Figure 2: Continued.
3.2. MDSC Inhibitor (Gemcitabine) Significantly Attenuates IMQ-Induced Psoriasis-Like Skin Inflammation through Downregulating Th17 and Treg Cells. Although the number of MDSCs has been found elevated in both skin lesions and peripheral blood, the effect of MDSCs on the pathogenesis of psoriasis remains to be elucidated. Gemcitabine (GEM) is well known to be an inhibitor of MDSCs, which reduces the accumulation of MDSCs with no significant influence on other immune cells such as T, B cells, NK cells, and macrophages [22]. Therefore, we treated mice with GEM to study the relationship between MDSCs and psoriasis. The specific drug use scheme is shown in Figure 2(a). The murine MDSCs have been characterized by CD11b+ and Gr-1+ [7, 10], and we found that IMQ treatment significantly induces psoriasis-like skin inflammation as well as the accumulation of MDSCs in spleen and skin lesions (Figures 2(b) and 3(a)). As expected, GEM treatment significantly reduces IMQ-induced accumulation of MDSCs in skin lesions and spleen (Figure 3(a)), therefore alleviating the phenotype of IMQ-induced psoriasis-like skin inflammation (Figure 2(b)) based on the Psoriasis Area and Severity Index (PASI) score (Figure 2(c)). In addition, GEM treatment markedly decreases IMQ-induced epidermal thickening and inhibited splenomegaly compared with the vehicle on day 6 after IMQ application for 5 consecutive days topically (Figures 2(b) and 2(d)). Moreover, GEM treatment remarkably decreases IMQ-mediated infiltration of Th17 and Treg cells in the spleen (Figures 3(b) and 3(c)), indicating depletion of MDSCs by GEM abrogates IMQ-induced psoriasis-like skin inflammation such as erythema, skin thickening, scaling, and the infiltration of Th17 and Treg cells.

3.3. The Effect of Depletion of MDSCs on Gene Expression Profiles of CD4+ T-Cells. To further investigate the detailed effect of MDSCs on CD4+ T-cells, we performed the RNA-seq technique to analyze transcriptional alteration of CD4+ T-cells after depletion of MDSCs by GEM. We found that 40 genes were upregulated, and 198 genes were downregulated after GEM treatment (Figure 4(a)). KEGG pathway analysis exhibited that the top significant differential expression of enriched pathways include the MAPK signaling pathway, PI3K-Akt signaling pathway, ECM-receptor interaction, and HIF-1 signaling pathway (Figure 4(b)). Next, we also performed gene-set-enrichment analysis (GSEA), which showed those differentially expressed genes are enriched in LY6C_HIGH_VS_LOW_MONOCYTE_DN and RIG_1LIKE_RECEPTOR_SIGNALING_PATHWAY (Figure 4(c)). Thus, the results of GSEA based on transcriptional profiling of those splenic CD4+ T-cells revealed enriched genes downregulated in Ly6C monocytes and the RIG-I-like receptor signaling pathway was more activated in CD4+ T-cells after depletion of MDSCs by GEM. Furthermore, we validated the expression of IL-21, Dsp, Cd109, Ackr2, Timd2, and Adamts9 in GEM-treated mice through qRT-PCR (Figure 4(d)), which have been documented to regulate Th17-cell differentiation (IL-21) [23, 24], Th1/Th17 immune skewing (Dsp) [25], T-cell activation (CD109, Timd2) [26–28], inflammatory T-cell chemotaxis (Ackr2) [29], and immune suppression (Adams9) [30].

3.4. Neutralizing IL-21R In Vivo Inhibits IMQ-Induced Epidermal Thickening, Cutaneous MDSC Infiltration, and Splenic Th17 Infiltration. Evidence revealed that IL-21 is...
Figure 3: MDSC inhibitor (Gemcitabine) attenuates IMQ-induced psoriasis-like skin inflammation through downregulating Th17 and Treg cells. (a) Representative flow cytometry panels for quantification of MDSCs in spleen and skin lesions of BALB/c mice (n = 3 – 6 mice per group). CD11b<sup>+</sup> Gr-1<sup>+</sup> cells were selected from CD45<sup>+</sup> cells. Statistical analysis data is shown in (B). (b) Representative flow cytometry panels for quantification of splenic Th17 cells of BALB/c mice (n = 4 – 6 mice per group). Statistical analysis data is shown in (B). (c) Representative flow cytometry panels for quantification and FMO-control of splenic Treg cells of BALB/c mice (n = 5 – 6 mice per group). Statistical analysis data is shown in (B). *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001; ns: not significant. One-way ANOVA with Dunnett’s post hoc test was used.
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(a)

(b) Figure 4: Continued.
highly expressed in the psoriatic skin lesions, which stimulates the proliferation of keratinocytes [31]. Moreover, IL-21 is well known to be related to immune diseases and regulates the differentiation of CD4+ T-cells [32]. IL-21R, a receptor for IL-21, is a class I cytokine heterodimeric receptor, which mainly expressed on lymphoid cells such as circulating T-cells, B cells, NK cells, and nonlymphocytic cells and tissues including keratinocytes [31]. To verify the role of IL-21 in the progression of psoriasis, we administrated the anti-IL-21R antibody to neutralize the IL-21 signaling pathway through IMQ-induced psoriasis-like BALB/c mouse models. The experimental design scheme is shown in Figure 5(a). The result of H&E staining and quantification showed that neutralizing IL-21R in vivo inhibits IMQ-
Figure 5: Neutralizing IL-21R in vivo inhibits IMQ-induced epidermal thickening, cutaneous MDSCs infiltration, and splenic Th17 infiltration. (a) Schematic illustration of the experimental setup. (b) The H&E staining of the back skin derived from mice injected intraperitoneally with vehicle (IMQ+vehicle) or anti-mouse IL-21R antibody (IMQ+anti-IL-21R) or untreated (Normal) (one representative mouse is presented, n = 4 – 7 mice per group). Scale bars: 100 μm. Statistical analysis data is shown in (B). (c) Representative flow cytometry panels for quantification and FMO-control of cutaneous MDSCs and splenic Th17 cells of BALB/c mice (n = 4 – 7 mice per group). CD11b+ Gr-1+ cells were selected from CD45+ cells. Statistical analysis data is shown in (B). (d) Schematic illustration of targeting MDSCs attenuates IMQ-induced psoriasis-like skin inflammation. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001; ns, not significant. One-way ANOVA with Dunnett’s post hoc test was used.
induced epidermal thickening (Figure 5(b)). Moreover, neutralizing IL-21R with anti-mouse IL-21R antibody significantly reduces IMQ-mediated accumulation of MDCs in skin lesions and splenic Th17 cells (Figure 5(c)), indicating targeting IL-21 is a therapeutic approach for psoriasis.

4. Discussion

Psoriasis has been documented to be a T-cell-mediated chronic inflammatory disease [2, 33]. The IL-23/IL-17A–Th17 axis has a crucial role in the development of psoriasis [2, 34, 35]. IL-23, secreted by DCs or KCs, facilitates Th17 differentiation which produces proinflammatory cytokines including IL-17A, IL-17F, IL-6, IL-21, and IL-22, resulting in the infiltration of Th17 and high levels of Th17-mediated proinflammatory cytokines in skin lesions and peripheral blood of psoriasis patients [2, 36, 37]. The Treg cells, constitutively expressing Foxp3 (the master transcriptional factor of Treg cells), are believed to maintain immune homeostasis through suppressing the function of other lymphocytes such as Th1, Th2, and Th17, resulting in inhibition of immune and inflammatory responses [38–40].

Although the role of Treg cells in psoriasis has not been fully elucidated, studies showed that numbers of Treg cells are upregulated in psoriatic skin lesions [41–44] or peripheral blood [39, 43, 44] of psoriasis patients or murine models [45]. In addition, evidence has indicated that Foxp3+ Treg cells can converse into inflammation-associated Th17 cells under proinflammatory conditions both in psoriasis [18, 46, 47] and in rheumatoid arthritis (RA) [48]. And there is a positive correlation between Treg cells and Th17 cells in psoriasis [43]. Moreover, accumulating studies demonstrated that the polarization of Th17 cells has been related to the induction of Foxp3+ Treg cells [18, 46, 49].

MDCs are known to be a heterogeneous population of progenitor and immature myeloid cells derived from different stages and have essential roles for regulating the function of Th17 and Treg cells. The expanded MDCs enhance the differentiation of naive CD4+ T-cell precursors into Th17 cells and are positively correlated with disease severity of SLE and RA patients as well as their murine models [12–15]. Our results showed that GEM, an MDSC inhibitor, inhibits IMQ-induced epidermal thickening and the accumulation of Th17, Treg cells, and MDCs (Figure 3). Furthermore, we investigated the effect of depleting MDCs by GEM treatment on gene expression profiles of CD4+ T-cells and the results exhibited that IMQ-induced IL-21 expression has been dramatically suppressed by GEM treatment (Figures 4(a) and 4(d)). IL-21 is highly expressed in skin lesions and peripheral blood of psoriasis patients, which is required for epidermal hyperplasia and Th17-cell polarization [23, 24, 31, 50]. And it was reported that IL-21 promotes psoriatic inflammation by inducing an imbalance of Th17 and Treg cells [47]. Consistent with those results, neutralizing IL-21R by its antibody abrogates IMQ-induced epidermal thickening, MDC migration, and Th17 infiltration (Figure 5), indicating IL-21 may be a potential therapeutic target for psoriasis.

Still, there are limitations in the present study which merit consideration. For example, our intervention to deplete MDCs by GEM was at the animal level; thus, our hypothesis needs further investigations to verify. In addition, we have noticed the numerous side effects of GEM during application in humans, such as the dose-limiting toxicity (myelosuppression, thrombocytopenia, and anemia) and the minimal nonhematologic toxicity (nausea, shortness of breath, mouth sores, diarrhea, neuropathy, hair loss, etc.) [51], which may limit the chance of GEM being a useful therapy in psoriasis patients. However, we verified the significant anti-inflammatory effects by depleting MDCs. Despite the severe side effects of Gemcitabine, we still can conclude that targeting MDCs is a potential strategy for antipsoriasis therapy.

In summary, our study provided evidence that MDCs play a proinflammatory role in IMQ-induced psoriasis-like skin inflammation and regulating the infiltration of CD4+ T-cells (Figure 5(d)). Depleting MDC by its inhibitor (Gemcitabine) significantly suppresses the IMQ-mediated psoriatic phenotype as well as the accumulation of Th17 and Treg cells. Furthermore, we identified and validated the transcriptional expression changes of genes including IL-21 and Timd2 on CD4+ T-cells of GEM-treated mouse models, which are involved in Th17-cell differentiation or T-cell activation. Neutralizing IL-21R by antibody reduces IMQ-induced epidermal thickening through downregulating the infiltration of MDCs and Th17 cells (Figure 5(d)), suggesting the accumulation of MDCs exerts important function for the pathogenesis of psoriasis and IL-21 may be a potential therapeutic target in psoriasis.

5. Conclusions

Targeting myeloid-derived suppressor cells is a novel strategy for antipsoriasis therapy. IL-21 may be a potential therapeutic target in psoriasis.

Abbreviations

KC: Keratinocytes
MDCs: Myeloid-derived suppressor cells
IMQ: Imiquimod
GEM: Gemcitabine
Th17: IL-17A-producing cells
Treg cells: Regulatory T-cells

Data Availability

The RNA-seq data that support the findings of this study have been deposited in the CNSA (https://db.cngb.org/cnsa/) of CNGBdb with accession number CNP0001133.

Conflicts of Interest

The authors have no conflict of interest to declare.
Authors’ Contributions
The authors are responsible for the content and writing of the manuscript. CC conducted the research, analyzed the data, and wrote the original draft. LT conducted part of the research. WZ, YK, and JL supervised the clinical part of the research. LL and PL contributed essential reagents and materials. CP supervised the research, performed the formal analysis, and edited the paper, and XC supervised the research and formulated the research goals and aims. All authors contributed to manuscript revision and read and approved the submitted version.

Acknowledgments
The authors would like to thank the patients who were involved in this study. This work was supported by Grant No. 81830096 from the key project of the National Science Foundation and supported by Grant Nos. 81773341, No. 81830096 from the key project of the National Science Foundation and supported by Grant Nos. 81773329, 81673065, and 81974476 by the National Natural Foundation and supported by Grant Nos. 81773341, 81773329, 81673065, and 81974476 by the National Natural Science Foundation of China.

Supplementary Materials
Table S1: a list of the primer for qPCR. Table S2: a list of the antibodies for flow cytometry. (Supplementary Materials)

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