Roles of alternatively activated M2 macrophages in allergic contact dermatitis

Kotaro Suzuki a, *, Kazuyuki Meguro a, Daiki Nakagomi b, Hiroshi Nakajima a

a Department of Allergy and Clinical Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan
b Department of Third Internal Medicine, University of Yamanashi, Yamanashi, Japan

Abstract

Alternatively activated macrophages (M2 macrophages) play key roles in the suppression of Th1 cell responses and the orchestration of tissue repair. However, recent studies have shown that M2 macrophages have potentials to produce high levels of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α, suggesting that M2 macrophages may exacerbate inflammation in some settings. In this regard, we have recently shown that large numbers of M2 macrophages accumulate in the sites of hapten-induced contact hypersensitivity (CHS), an animal model of allergic contact dermatitis, and that M2 macrophages exacerbate hapten-induced CHS by producing matrix metalloproteinase 12 (MMP12). We have also shown that suppressor of cytokine signaling-3 (SOCS3), a member of SOCS family proteins that are cytokine-inducible negative regulators of the JAK/STAT signaling pathways, is highly and preferentially expressed in M2 macrophages in hapten-induced CHS and that SOCS3 expressed in M2 macrophages is involved in the attenuation of CHS by suppressing MMP12 production. These findings underscore the importance of M2 macrophage-derived MMP12 in the development of CHS, and suggest that inhibition of M2 macrophages or MMP12 could be a potential therapeutic strategy for the treatment of allergic contact dermatitis.

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Introduction

Allergic contact dermatitis, one of the most prevalent skin diseases, is caused by delayed-type hypersensitivity reactions to foreign substances or hapten-modified proteins. The agents are frequently included in latex materials, protective equipment, soap and cleansers, and resins. A large number of studies using contact hypersensitivity (CHS), which is induced by epicutaneous exposure of hapten in sensitized mice, revealed detailed immunological mechanisms underlying allergic contact dermatitis. In addition, recent studies on CHS have revealed that new subsets of CD4+ T cells, such as Foxp3+ regulatory T cells and Th17 cells, are also involved in the regulation of allergic contact dermatitis. Moreover, the identification of langerin-positive dermal dendritic cells has questioned the relevance of epidermal Langerhans cells as key antigen-presenting cells in allergic contact dermatitis. Furthermore, we have recently shown that M2 macrophages, which are believed to be involved in tissue repair, are involved in the induction of CHS. In this review, we will summarize recent advance which changes some key dogmas of allergic contact dermatitis and also introduce our findings regarding a novel role of M2 macrophages in CHS.

Immunological mechanisms underlying contact hypersensitivity

CHS is composed of two phases; sensitization phase and elicitation phase. During the sensitization phase of CHS, keratinocytes are activated upon hapten application and produce various...
Because the information on Mb and Md is limited, they do not appear in this Table.

During the elicitation phase of CHS, hapten skin painting activates skin-resident cells such as keratinocytes, Langerhans cells, and skin dendritic cells. Keratinocytes are thought to be the important source of chemokines as the activated keratinocytes produce multiple chemokines, such as CXCL1, CXCL2, CXCL8, CXCL10, CCL8, CCL17, and CCL27. Among these chemokines, CXCL10, a ligand for CXCR3 that is highly expressed on Th1 cells, has been shown to play an important role in the infiltration of antigen-specific T cells into the skin.11-18 Infiltrated T cells are activated by skin antigen-presenting cells and produce cytokines including IFN-γ and IL-17 at the site. The cytokines produced by activated T cells stimulate skin-resident cells and lead to further infiltration of T cells, resulting in the amplification of the inflammatory responses.

Many studies have shown that both CD4+ T cells and CD8+ T cells play multiple roles in inflammatory responses in the elicitation phase of CHS. It is well recognized that CD4+ T helper cells are subdivided into at least three subsets; Th1 cells characterized by the secretion of IFN-γ, Th2 cells characterized by the secretion of IL-4, IL-5, and IL-13, and Th17 cells characterized by the secretion of IL-17A, IL-17F, and IL-22. CD8+ T cells are also subdivided into at least three subsets; Tc1 cells characterized by the secretion of IFN-γ, Tc2 cells characterized by the secretion of IL-4, and Tc17 cells characterized by the secretion of IL-17A. In the elicitation phase of CHS, CD8+ T cells mainly play proinflammatory roles, whereas CD4+ T cells play both proinflammatory and anti-inflammatory roles, depending on their producing cytokines.11 When Th1 cells and Tc1 cells infiltrate into the skin lesion during the elicitation phase of CHS, they release proinflammatory cytokines including IFN-γ. Consistent with the accumulation of Th1 cells and Tc1 cells and the high levels of IFN-γ expression at the inflammatory sites of CHS,14,15 studies using IFN-γ-deficient mice or IFN-γ receptor-deficient mice have revealed that IFN-γ plays an important role in the induction of CHS. In this regard, IFN-γ has been shown to trigger resident myeloid and non-myeloid cells to secrete chemokines such as CXCL2 and CCL2 that further induce the recruitment of various mononuclear cells including monocytes.16 While the importance of Th1 cells and Tc1 cells for the induction of CHS is apparent, contributions of other Th/Tc subset in the elicitation phase of CHS may differ depending on experimental models, because each hapten has its own properties regarding the activation of Th/Tc cell subsets.17

### Macrophage polarization and plasticity

During the last decade, phenotypic heterogeneity and plasticity of macrophages has been intensively investigated. Macrophages are essential components of innate immunity and play a critical role in primary responses to pathogens, inflammation, and tissue repair.19,20 Circulating monocytes differentiate into macrophages after their migration to tissues and then macrophages acquire distinct characteristics in response to environmental factors including cytokines in the tissues. The phenotypic diversity of macrophages can be assessed by the expression of several surface markers and by their secretome. The concept of classically activated macrophages (M1 macrophages) was established in the 1980s,14 and some time later, alternatively activated macrophages (M2 macrophages) were discovered.21 To date, roles of M1 macrophages and M2 macrophages have been extensively investigated in both physiological and pathological conditions in several experimental systems.14

Th1 cytokines such as IFN-γ, proinflammatory cytokines such as TNF-α, and microbial products such as lipopolysaccharide (LPS), alone or in combination, promote M1 macrophage differentiation (Table 1). M1 macrophages play important roles in the exacerbation of the inflammation caused by proinflammatory cytokines such as IL-1β, IL-6, IL-12, IL-17A, IL-23, and TNF-α. M1 macrophages also produce toxic agents such as reactive nitrogen and oxygen intermediates which eradicate bacterial, fungal, and viral infections.12

In addition, M1 macrophages secrete chemokines such as CXCL5, CXCL9, and CXCL10 which promote the recruitment of Th1 cells and NK cells, facilitating the killing of intracellular pathogens. While these activities of M1 macrophages are beneficial for preventing infection, a chronic activation of M1 macrophages can cause tissue damage and impair wound healing.

As the opposite side of the macrophage spectrum, M2 macrophages counteract the inflammatory responses sustained by M1 macrophages. The differentiation of M2 macrophages is promoted by Th2 cytokines such as IL-4 and IL-13 (Table 1). It is generally believed that M2 macrophages play key roles in the suppression of Th1 cell responses and tissue repair, and exhibit phagocytic, proangiogenic, and pro-fibrotic capacities.15,16,17 Several in vitro studies have led to an extended classification with subdivision into four sub-groups: M2a, M2b, M2c, and M2d. The differentiation of M2a macrophages is promoted by IL-4 and IL-13. M2a macrophages express high levels of the mannose receptor (MR) and produce profibrotic factors such as fibronectin, insulin growth factor, and TGF-β, which lead to the tissue repair.19 The differentiation of M2b macrophages is induced by combined exposure to immune complexes and Toll-like receptor ligands or IL-1 receptor agonists. M2b

### Table 1

Characteristics of activated macrophages.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Markers</th>
<th>Inducer</th>
<th>Product</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>CCR7, CD86, CD117, MHCII, ITOS, TLR4</td>
<td>IFN-γ, LPS, bacteria, GM-CSF, HMGB1</td>
<td>TNF-α, IL-1β, NO, IL-6, IL-12, IL-23, CCL1, CCL5, CCL10, CCL2, CCL17, CCL18, IGF-1</td>
<td>Proinflammatory function, tissue damage, pathogen clearance</td>
</tr>
<tr>
<td>M2a</td>
<td>CD206 (MR), CD209, Fizz1, Ym1,2, RELMα, arginase-1, ST2, dectin-1</td>
<td>IL-4, IL-13, M-CSF, helmithin</td>
<td>CCL2, CCL17, CCL18, IGF-1</td>
<td>Allergic inflammation, tissue repair</td>
</tr>
<tr>
<td>M2c</td>
<td>CD163, CD206 (MR), Fizz1, Ym1,2, arginase-1, SRA-1, MerTK</td>
<td>IL-10, TGF-β, glucocorticoid</td>
<td>IL-10, TGF-β, CCL8, CCL17, MMP9, VEGF</td>
<td>Anti-inflammatory function, tissue remodeling, fibrosis</td>
</tr>
</tbody>
</table>

Because the information on Mb and Md is limited, they do not appear in this Table.

MR, mannose receptor; RELMα, resistin-like molecule α; HMGB1, high-mobility group box 1; SRA-1, scavenger receptor 1; IGF-1, insulin-like growth factor-1; MerTK, Mer receptor tyrosine kinase.
macrophages express high levels of MR and Mer receptor tyrosine kinase (MerTK) and produce high levels of IL-10, IL-1β, IL-6, and TNF-α. The differentiation of M2c macrophages is induced by IL-10 and glucocorticoids, and M2c macrophages express high levels of MR and MerTK and produce high levels of IL-10. Both M2b macrophages and M2c macrophages are generally thought to act as anti-inflammatory macrophages. The differentiation of M2d macrophages is induced by co-stimulation with Toll-like receptor ligands and adenosine A2A receptor agonists. M2d macrophages express low levels of MR and produce high levels of IL-10 and vascular endothelial growth factor (VEGF) and low levels of TNF-α and IL-12. The significant progress has been made in characterizing the phenotype and function of M2 macrophage subpopulations; however, use of different markers to identify and characterize M2 macrophage subpopulations in vitro and in vivo has created a confusing picture. In addition, recent studies have shown that in some situations, M2 macrophages have potentials to produce high levels of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α, similar to M1 macrophages, suggesting that M2 macrophages possess remarkable plasticity and may exacerbate inflammation in some pathological conditions.

Characteristics of macrophages in the inflammatory sites of CHS

Recent studies have shown that monocyes migrate to inflammatory sites and differentiate into macrophages in the elicitation phase of CHS. However, detailed characteristics of macrophages in the skin lesion of CHS remain unclear. Therefore, we examined the phenotype of F4/80+ cells in the skin lesion of 2,4-dinitrofluorobenzene (DNFB)-induced CHS. We found that upon the induction of DNFB-induced CHS, large numbers of MR-positive (MR+) F4/80+ cells and MR-negative (MR-) F4/80+ cells were accumulated in the skin lesion. We also found that MR+ F4/80+ cells expressed high levels of CCR2 and moderate levels of MerTK, whereas MR- F4/80+ cells expressed moderate levels of CCR2 and high levels of MerTK, suggesting that both MR+ F4/80+ cells and MR- F4/80+ cells in the sites of CHS are monocye-derived macrophages but not skin-resident macrophages. Moreover, we found that MR+ F4/80+ cells but not MR- F4/80+ cells expressed high levels of M2 macrophage markers such as dectin-1, Arg1, and Fizz1, suggesting that MR+ F4/80+ cells in the sites of CHS represent M2 macrophages.

Roles of M2 macrophages in DNFB-induced CHS

Although proinflammatory roles of M1 macrophages in skin inflammation including CHS have been well documented, the roles of M2 macrophages in CHS remain largely unknown. We therefore performed cell transfer experiments of bone marrow (BM)-derived M1 and M2 macrophages into the sites of CHS to address the roles of M1 and M2 macrophages in CHS. Intradermal injection of BM-derived M2 macrophages significantly exacerbated ear thickness and increased the numbers of CD4+ T cells, CD8+ T cells, neutrophils, and eosinophils in the skin lesion in DNFB-induced CHS. In contrast, intradermal injection of BM-derived M1 macrophages did not significantly exacerbate ear thickness and only modestly increased the numbers of CD4+ T cells, CD8+ T cells, neutrophils, and eosinophils in the skin lesion in DNFB-induced CHS. These findings suggest that M2 macrophages but not M1 macrophages can exacerbate DNFB-induced CHS.

We next investigated the transcriptome of MR+ F4/80+ cells and MR- F4/80+ cells isolated from the skin in DNFB-induced CHS to address the mechanisms underlying M2 macrophage-mediated exacerbation of CHS. RNA-sequencing analysis identified 189 genes that are more than 4-fold higher in MR+ F4/80+ cells than MR- F4/80+ cells. Among them, we picked 11 genes, Ccl8, Pf4, Mmp12, Cxcr5, Cx3cl1, Ctsk, Cpe, Gdf15, Adamt2, Bmp5, and Bmp2, according to the two categories: "extracellular region" Gene Ontology (GO) cellular component and "cytokine/chemokine" or "proteolytic enzyme" of GO molecular function (Table 2). Owing to the expression profiles available, we focused on matrix metalloproteinase 12 (MMP12), also known as macrophage elastase, among 11 genes as a candidate gene that possibly involves the M2 macrophage-mediated exacerbation of CHS. Consistent with the data of RNA-sequencing analyses, qPCR analyses revealed that MMP12 mRNA was highly expressed in MR+ F4/80+ cells as compared to that in MR- F4/80+ cells in the skin lesion of DNFB-induced CHS. In addition, MMP12 mRNA was highly expressed in BM-derived M2 macrophages as compared to that in BM-derived M1 macrophages. These findings suggest that MMP12 is highly and preferentially expressed in M2 macrophages in the skin lesion in DNFB-induced CHS. Our findings are consistent with a previous report showing that MMP12 is one of the most strongly induced genes in the skin of hapten-induced CHS in rat models.

It has been shown that IL-4-STAT6 signaling induces not only the polarization toward M2 phenotype but also the transcriptional activation of a part of M2 macrophage-specific genes in macrophages. We therefore examined the roles of IL-4-STAT6 signaling in MMP12 expression in BM-derived macrophages and found that IL-4 induced the expression of MMP12 mRNA in BM-derived macrophages of wild-type mice but not of STAT6-deficient mice. In addition, in agreement with the presence of a putative STAT6-binding site in MMP12 promoter, ChIP-qPCR analysis revealed that IL-4 induced STAT6 binding to the promoter region of MMP12 gene. These findings suggest that IL-4 induces MMP12 expression through STAT6-mediated activation of MMP12 promoter in M2 macrophages.

### Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Product</th>
<th>MR+ (FPKM)</th>
<th>MR- (FPKM)</th>
<th>MR+/MR- (Fold)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ccl8</td>
<td>Chemokine (C-C motif) ligand 8</td>
<td>969.7</td>
<td>20.0</td>
<td>48.5</td>
</tr>
<tr>
<td>Pf4</td>
<td>Platelet factor 4</td>
<td>674.0</td>
<td>114.4</td>
<td>5.89</td>
</tr>
<tr>
<td>Mmp12</td>
<td>Matrix metallopeptidase 12</td>
<td>329.2</td>
<td>67.7</td>
<td>4.86</td>
</tr>
<tr>
<td>Cxcr5</td>
<td>Chemokine (C-C motif) ligand 5</td>
<td>156.5</td>
<td>32.7</td>
<td>4.79</td>
</tr>
<tr>
<td>Cx3cl1</td>
<td>Chemokine (C-C motif) ligand 5 (M14 family)</td>
<td>53.8</td>
<td>11.8</td>
<td>4.56</td>
</tr>
<tr>
<td>Ctsk</td>
<td>Cathepsin K</td>
<td>50.6</td>
<td>10.1</td>
<td>5.01</td>
</tr>
<tr>
<td>Cpe</td>
<td>Carboxypeptidase E</td>
<td>26.4</td>
<td>1.55</td>
<td>17.0</td>
</tr>
<tr>
<td>Gdf15</td>
<td>Growth differentiation factor 15</td>
<td>16.4</td>
<td>3.36</td>
<td>4.88</td>
</tr>
<tr>
<td>Adamt2</td>
<td>ADAM metallopeptidase with</td>
<td>14.5</td>
<td>2.87</td>
<td>5.05</td>
</tr>
<tr>
<td>Bmp5</td>
<td>Bone morphogenetic protein 5</td>
<td>6.79</td>
<td>1.01</td>
<td>6.72</td>
</tr>
<tr>
<td>Bmp2</td>
<td>Bone morphogenetic protein 2</td>
<td>5.28</td>
<td>0.86</td>
<td>6.14</td>
</tr>
</tbody>
</table>

FPKM: fragments per kilobase of exon model per million mapped fragments; MR, mannose receptor (modified from reference 20).
Roles of M2 macrophage-derived MMP12 in CHS

MMP12 belongs to a family of matrix metalloproteinase (MMPs) that are structurally related extracellular matrix-degrading enzymes. As other MMPs, MMP12 is capable of degrading extracellular matrix components such as elastin and thought to be involved in tissue remodeling process. However, recent studies have raised the possibility that MMP12 is involved in the development of inflammatory responses. For example, it has been shown that cigarette smoke-induced accumulation of neutrophils and macrophages into the lung depends on MMP12.

To determine the roles of MMP12 in CHS, we first examined DNFB-induced CHS in MMP12-deficient mice and littermate wild-type mice. Importantly, MMP12-deficient mice showed reduced ear thickness in DNFB-induced CHS as compared with wild-type mice. Histological analyses revealed that MMP12-deficient mice exhibited less inflammatory change in the skin in DNFB-induced CHS. Flow cytometric analyses of leukocytes isolated from the skin lesion showed that the numbers of CD4^+ T cells, CD8^+ T cells, neutrophils, M1 macrophages, and M2 macrophages were significantly decreased in MMP12-deficient mice as compared to those in wild-type mice in DNFB-induced CHS. These findings suggest that MMP12 plays critical roles in the induction of DNFB-induced CHS (Fig. 1).

To elucidate the involvement of M2 macrophage-derived MMP12 in the induction of CHS, we performed cell transfer of BM-derived M1 and M2 macrophages of wild-type mice and MMP12-deficient mice into the skin lesion of DNFB-challenged MMP12-deficient mice. The intradermal injection of BM-derived M2 macrophages of wild-type mice but not of MMP12-deficient mice reconstituted ear thickness and the numbers of neutrophils in the sites of CHS in MMP12-deficient mice to the levels similar to DNFB-challenged wild-type mice. In contrast, the intradermal injection of BM-derived M1 macrophages of wild-type mice and MMP12-deficient mice only modestly increased ear thickness and the numbers of neutrophils in the sites of CHS. Taken together, these findings suggest that M2 macrophage-derived MMP12 is involved in the exacerbation of CHS.

Regarding the mechanisms underlying MMP12-mediated skin inflammation, Senior et al. have shown that elastin-derived peptides generated by proteolytic cleavage have chemotactic activity for monocytes. In addition, Dean et al. have reported that MMP12 cleaves several chemokines and some cleaved products promote neutrophil influx. Moreover, Nenan et al. have shown that instillation of recombinant MMP12 into murine airways induces the expression of chemokines such as CCL3 and CXCL1, leading to the accumulation of neutrophils and macrophages in the airways. To clarify how MMP12 is involved in the elicitation phase of DNFB-induced CHS, we examined the effect of intradermal injection of recombinant MMP12. The intradermal injection of recombinant MMP12 but not of heat-inactivated MMP12 caused ear thickness and skin inflammation and induced the expression of chemokines such as CCL1, CCL2, CCL3, and CCL2 which are capable of inducing the recruitment of lymphocytes, neutrophils, and macrophages. These results indicate that the enzymatic activity of MMP12 is required for the induction of skin inflammation and suggest that MMP12 induces skin inflammation presumably through the induction of chemokine expression (Fig. 1).

With respect to the therapeutic potential of MMP12 inhibition for allergic contact dermatitis, we examined the effect of an MMP12 inhibitor MMP408 on DNFB-induced CHS. Consistent with the findings of MMP12-deficient mice, topical application of MMP408 to the ear strongly suppressed ear thickness, inflammatory responses, and the accumulation of CD4^+ T cells, CD8^+ T cells, neutrophils, M1 macrophages, and M2 macrophages in the ear. These findings indicate that MMP12 is involved in the induction of inflammation in the elicitation phase of DNFB-induced CHS, suggesting that MMP12 inhibitors can be applicable for the treatment of allergic contact dermatitis. Since Klose et al. have shown that MMP14 expressed in macrophages/monocytes is also required for trans-endothelial migration of monocytes and T cells into the skin in CHS, combined inhibition of MMP12 and MMP14 would be a logical therapeutic approach for allergic contact dermatitis.
SOCS3 is highly and preferentially expressed in M2 macrophages

Although the immunological mechanisms underlying the sensitization and elicitation phases of CHS have been intensively investigated, the mechanisms that antagonize the elicitation phase of CHS are still largely unknown. The suppressor of cytokine signaling (SOCS) proteins are a family of eight intracellular cytokine-inducible proteins, which are rapidly induced not only by cytokines but also by a variety of stimuli including Toll-like receptor (TLR) ligands and hormones.\(^29,30\) All eight SOCS family members negatively regulate JAK/STAT pathways through the association with key phosphorylated tyrosine residues of JAKs and/or cytokine receptors.\(^29,30\) Accumulating evidence suggests that SOCS family members play crucial roles in down-regulation of inflammatory responses in a number of experimental systems.\(^29,30\) In macrophages, it has been shown that SOCS expression is very low at steady-state conditions; however, SOCS1 and SOCS3 are rapidly induced upon activation with IFN-\(\gamma\) or LPS.\(^31,32\)

To determine the role of SOCS family members expressed in M2 macrophages in the regulation of CHS, we analyzed mRNA levels of SOCS family members in M2 macrophages isolated from the sites of DNFB-induced CHS by RNA-seq.\(^33\) Among eight SOCS family members, SOCS3 was highly expressed not only in M1 macrophages but also in M2 macrophages at mRNA levels. In addition, flow cytometric analyses of various cell types harvested from the sites of DNFB-induced CHS revealed that M2 macrophages expressed higher levels of SOCS3 protein than M1 macrophages, CD4\(^+\) T cells, CD8\(^+\) T cells, and neutrophils.\(^33\) These results suggest that SOCS3 is highly and preferentially expressed in M2 macrophages at the sites of DNFB-induced CHS.

It has been shown that IFN-\(\gamma\), one of the important effector cytokines eliciting hapten-induced CHS, induces the expression of SOCS3 in macrophages.\(^4\) Therefore, we examined the effect of neutralizing anti-IFN-\(\gamma\) antibody on SOCS3 expression in M2 macrophages in the elicitation phase of DNFB-induced CHS.\(^33\) As expected, the injection of anti-IFN-\(\gamma\) antibody diminished SOCS3 expression in M2 macrophages at the sites of DNFB-induced CHS. Consistently, recombinant IFN-\(\gamma\) induced SOCS3 expression in BM-derived M2 macrophages. Taken together, these findings suggest that endogenously produced IFN-\(\gamma\) at the site of CHS induces SOCS3 expression in M2 macrophages during the elicitation phase of CHS, although the molecular mechanism underlying IFN-\(\gamma\)-induced up-regulation of SOCS3 expression in M2 macrophages is uncertain. Further studies are required to elucidate the mechanism of SOCS3 induction by IFN-\(\gamma\).

It has recently been demonstrated that when SOCS3 is deleted by LysM-Cre-driven excision of the Socs3 allele in macrophages (SOCS3-cKO mice), LPS stimulation results in enhanced M1 polarization.\(^33\) Similarly, enhanced M1 gene expression in response to LPS has been reported in alveolar macrophages of SOCS3-cKO mice.\(^33\) These in vitro studies suggest that SOCS3 functions as a negative regulator of M1 polarization program in murine macrophages.

Roles of SOCS3 expressed in M2 macrophages in CHS

To clarify the role of SOCS3 expressed in macrophages in CHS, we examined DNFB-induced CHS in SOCS3-cKO mice.\(^33\) SOCS3-cKO mice exhibited enhanced ear thickness as compared with littermate control mice. Ear thickness persisted over 30 days after DNFB challenge in SOCS3-cKO mice, whereas ear thickness returned to baseline levels at 10 days after DNFB challenge in control mice. Histological analyses showed that SOCS3-cKO mice exhibited severe inflammation in the skin. Flow cytometric analyses of leukocytes harvested from the ear demonstrated that the numbers of CD4\(^+\) T cells, CD8\(^+\) T cells, neutrophils, M1 macrophages, and M2 macrophages were significantly increased in SOCS3-cKO mice as compared with those in control mice. These results suggest that SOCS3 expressed in macrophages is involved in down-regulation of hapten-induced CHS.

To determine the roles of SOCS3 expressed in M2 macrophages in CHS, we performed cell transfer experiments.\(^33\) Intradermal injection of BM-derived M2 macrophages of SOCS3-cKO mice tended to exacerbate ear thickness and accumulation of neutrophils, M1 macrophages, and M2 macrophages in the skin more strongly than that of BM-derived M2 macrophages of control mice. In addition, pretreatment of BM-derived M2 macrophages with IFN-\(\gamma\) attenuated the exacerbation of CHS when BM cells were derived from control mice but not from SOCS3-cKO mice. These results indicate that IFN-\(\gamma\) exhibits an anti-inflammatory property on M2 macrophage-mediated exacerbation of CHS in a SOCS3-dependent manner.

To address the involvement of MMP12 in M2 macrophage-mediated exacerbation of CHS in SOCS3-cKO mice, we examined the expression of MMP12 mRNA in M2 macrophages isolated from the skin of SOCS3-cKO mice and control mice in DNFB-induced CHS.\(^33\) qPCR analysis revealed that the expression of MMP12 in M2 macrophages was higher in SOCS3-cKO mice than that in control mice. Consistent with these in vivo data, the expression of MMP12 mRNA in BM-derived M2 macrophages was higher in SOCS3-cKO mice than that in control mice. These findings suggest that SOCS3 is involved in the suppression of MMP12 expression in M2 macrophages.

To further determine the role of MMP12 in the exacerbated CHS in SOCS3-cKO mice, we examined DNFB-induced CHS in mice lacking both SOCS3 and MMP12 in macrophages (MMP12-deficient SOCS3-cKO mice).\(^33\) Importantly, MMP12-deficient SOCS3-cKO mice exhibited reduced ear thickness and decreased numbers of CD4\(^+\) T cells, CD8\(^+\) T cells, neutrophils, M1 macrophages, and M2 macrophages as compared with SOCS3-cKO mice. Furthermore, topical application of MMP12 inhibitor MMP408 significantly suppressed DNFB-induced CHS in SOCS3-cKO mice. Taken together, these results suggest that MMP12 expressed in M2 macrophages participates in the enhanced CHS by the absence of SOCS3 expression in macrophages. Intriguingly, when compared to MMP12-deficient mice, MMP12-deficient SOCS3-cKO mice exhibited increased ear thickness and increased numbers of inflammatory cells in DNFB-induced CHS. These results suggest that SOCS3 attenuates CHS not only in MMP12-dependent mechanisms but also in MMP12-independent mechanisms.

To clarify the molecular mechanisms underlying the enhanced MMP12 expression in M2 macrophages in SOCS3-cKO mice, we examined the effect of IFN-\(\gamma\)-on IL-4-induced MMP12 expression in BM-derived macrophages in SOCS3-cKO mice and control mice.\(^33\) IFN-\(\gamma\) pretreatment inhibited IL-4-induced MMP12 expression in BM-derived macrophages in control mice but not in SOCS3-cKO mice. Consistently, IFN-\(\gamma\) pretreatment inhibited IL-4-induced STAT6 phosphorylation in BM-derived macrophages in control mice but not in SOCS3-cKO mice. Moreover, IL-4-induced STAT6 binding to the promoter region of the MMP12 gene in BM-derived macrophages was much stronger in SOCS3-cKO mice than that in control mice. Furthermore, IFN-\(\gamma\) pretreatment partly inhibited IL-4-induced STAT6 binding to the MMP12 promoter in BM-derived macrophages in control mice but not in SOCS3-cKO mice. Taken together, these results suggest that SOCS3 induced by IFN-\(\gamma\) signaling suppresses MMP12 expression by inhibiting IL-4 STAT6 pathways as a counterregulatory mechanism and thus prevents hyperactivation of M2 macrophages, resulting in the down-regulation of CHS (Fig. 1).
Concluding remarks

Recent studies have shown the importance of M2 macrophages in clearing the apoptotic cells, alleviation of inflammatory responses, and promotion of wound healing. In addition, our findings reviewed here suggest a novel role of M2 macrophages and their product MMP12 in the induction of CHS. Our findings also suggest a role of SOCS3, which is induced by endogenously produced IFN-γ along with IL-4, in the counter-regulation of CHS possibly by suppressing MMP12 expression. Although further studies are required, our findings suggest that M2 macrophages and/or MMP12 can be new therapeutic targets for the treatment of allergic contact dermatitis.

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Conflict of interest

The authors have no conflict of interest to declare.

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