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Original Article

CD22 and CD72 cooperatively contribute to the development of the reverse Arthus reaction model

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ABSTRACT

Background: Local type III hypersensitivity reactions are acute inflammatory events induced by immune complex (IC) deposition. CD22 and CD72 are B cell-specific cell surface molecules that negatively regulate B cell function.
Objective: To elucidate the roles of CD22 and CD72 in the development of IgG-mediated type III hypersensitivity reactions.
Method: The reverse Arthus reaction model in the skin was induced in mice lacking CD22 ($CD22^{-/-}$), CD72 ($CD72^{-/-}$), and both of them ($CD22^{-/-}/CD72^{-/-}$). Edema at 4 h and hemorrhage at 8 h after IC challenge were evaluated. Inflammatory cell infiltration and cytokine and chemokine expression were also examined.
Results: Edema and hemorrhage were significantly reduced in $CD22^{-/-}/CD72^{-/-}$ mice compared with wild-type mice. The loss of both membrane proteins resulted in a greater decrease in edema at 4 h, but not hemorrhage at 8 h, than the loss of each protein alone. Infiltration of neutrophils, macrophages, and T cells, and the expression of TNF-α, IL-6, MIP-1α, and CCR5 mRNA were also diminished in the knockout mice compared to wild-type mice, and most significantly reduced in $CD22^{-/-}/CD72^{-/-}$ mice. Regulatory T (Treg) cells in the spleen were significantly increased in all knockout mice at 4 h. Significant differences in the severity of edema and hemorrhage between wild-type and knockout mice were lost when Treg cells were depleted in the knockout mice.
Conclusion: These results demonstrate that CD22 and CD72 expression contribute to the development of the reverse Arthus reaction model and CD22 and CD72 might be therapeutic targets for human IC-mediated diseases.

1. Introduction

The formation and deposition of immune complexes (IC) in particular tissues induces local type III hypersensitivity reaction, termed an Arthus reaction [1,2]. The IgG-containing IC injury is connected with the pathogenesis of systemic lupus erythematosus, rheumatoid arthritis, and vasculitis syndrome. In the classical model for the Arthus reaction, horse serum was repeatedly injected intradermally into rabbits; the response included edema, hemorrhage, and neutrophil infiltration. In contrast, the "reverse" Arthus reaction model has been used in most experimental models due to its simplicity and reproducibility. The term "reverse" is used for the following reasons. In the original model, the antigen was intradermally administered, whereas in the reverse model, antibody is injected at the site where the investigator wants the inflammatory response to develop and antigen is injected intravenously immediately before or after antibody injection [1,2].

Neutrophil and mast cell recruitment is a necessary step for IC-mediated vascular tissue damage, leading to edema and hemorrhage [3,4]. How these leukocytes accumulate at the injection sites is a complex process regulated by various chemokines and the cooperation of adhesion molecules on endothelial cells and the leukocytes [4–7].

CD22 is a B cell-specific transmembrane molecule which is a member of the sialic acid-binding immunoglobulin-like lectin family of adhesion molecules [8]. The cytoplasmic domain of CD22

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contains six tyrosine residues, including immune receptor tyrosine-based inhibition motifs (ITIMs) [9] and the overall function of CD22 on BCR signaling is inhibitory [10].

CD72 is a type II membrane protein and contains an ITIM and an ITIM-like sequence in its cytoplasmic tail. Upon recruitment of SHP-1 to phosphorylated ITIM, CD72 negatively regulates BCR signaling [11]. However, a potential collaboration between CD22 and CD72 in the reverse Arthus reaction model has not been examined in detail.

Deficiency of inhibitory molecules on the B cell surface enhances the immune response in several murine disease models. For instance, mice deficient in FcγRIIB, the inhibitory Fc receptors for IgG-containing IC, exhibited augmented IgG-mediated passive cutaneous anaphylaxis [12] as well as reverse passive alveolitis [13]. Therefore, CD22 and CD72 may suppress inflammation in the reverse Arthus reaction model. However, contrary to this hypothesis, the present study showed that the combined loss of CD22 and CD72 resulted in reduced edema and hemorrhage. Given their proinflammatory role, CD22 and CD72 might be potential therapeutic targets for the treatment of human IC-mediated diseases.

2. Materials and methods

2.1. Mice

C57BL/6 wild-type mice were purchased from CLEA Japan, Inc. (Shizuoka, Japan). CD22-deficient ($CD22^{-/-}$) (C57BL/6 × 129) mice were generated as described [10] and backcrossed onto a C57BL/6 strain 12 times. CD72-deficient ($CD72^{-/-}$) mice were purchased from The Jackson Laboratory. Mating these $CD22^{-/-}$ mice with $CD72^{-/-}$ mice generated $CD22^{+/-}/CD72^{+/-}$ mice. Then, we generated $CD22^{-/-}/CD72^{-/-}$ mice by crossing the $CD22^{+/-}/CD72^{+/-}$ parents. To verify the CD22 or CD72 genotype, we conducted polymerase chain reaction amplification of each gene using genomic DNA from each mouse. All mice used in experiments were 8 to 12 weeks of age and were housed in a specific pathogen-free barrier facility and screened regularly for pathogens. The Committee on Animal Experimentation of Kanazawa University Graduate School of Medical Science approved all studies and procedures.

2.2. Reverse Arthus reaction model

The reverse Arthus reaction model was induced as previously described [4]. We shaved the abdominal skin and rabbit IgG anti-chicken egg albumin Abs (60 μg/30 μl) (Sigma-Aldrich, St. Louis, MO) were injected intradermally with a 29-gauge needle, followed immediately by i.v. injection of chicken egg albumin (20 mg/kg) (Sigma-Aldrich). The intradermal injection of purified polyclonal rabbit IgG (60 μg/30 μl) (Sigma-Aldrich) followed by i.v. administration of chicken egg albumin served as a control. The solution of chicken egg albumin contained 1% Evans blue dye (Sigma-Aldrich). Tissues were harvested 4 or 8 h later and edema, hemorrhage, and numbers of infiltrating leukocytes were assessed.

Edema was evaluated 4 h after IC challenge by directly measuring extravascular Evans blue dye diameter on the reverse side of the injection site. The diameters of the major and minor axes of the blue spots were averaged for analysis. The amount of hemorrhage was assessed 8 h after IC challenge by direct macroscopic measurement of the purpuric area. The major and minor diameters of the purpuric spots were averaged for analysis.

2.3. Histological examination and immunohistochemical staining

We removed the injection area by using a disposable sterile 6 mm punch biopsy (Kai, Gifu, Japan). These tissues were fixed in 3.5% paraformaldehyde and then paraffin embedded. Six-micrometer sections were stained using H&E for neutrophils and toluidine blue for mast cells. Extravascular neutrophils and mast cells were counted in the entire sections. The numbers of infiltrating macrophages, T cells, and B cells were assessed by immunohistochemical staining as described previously [14]. Sections were incubated with rat monoclonal Abs specific for F4/80 (A3-1) (Abcam, Cambridge, UK) or CD3 (CD3-12) (Serotec, Oxford, UK). To identify B cells, rat anti-mouse CD45R/B220 Ab (RA3-6B2) (BD Biosciences) was used. Rat anti-mouse FoxP3 Ab (FJK-16 s) (Invitrogen, Carlsbad, CA) was used to identify regulatory T (Treg) cells. Each section was examined independently by two investigators, in a blinded manner, and the mean value was used for analysis.

2.4. RT-PCR

Skin tissues were harvested 4 or 8 h after IC challenge using a disposable, sterile, 6 mm punch biopsy specimen. Total RNA was isolated from skin tissue using an RNeasy Fibrous Tissue Mini Kit (Qiagen, Hilden, Germany) and then reverse transcribed into cDNA and amplified. The expression levels of TNF-α, IL-6, MIP-1α, CCL5, CXCL1, CXCL2, CXCR2, CX3CL1, and CX3CR1 mRNAs were analyzed using a real-time polymerase chain reaction (RT-PCR) quantification method. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the mRNA. The relative expression of RT-PCR products was determined using the $\Delta\Delta C_T$ method to compare target genes with housekeeping GAPDH gene mRNA expression. One of the control samples was chosen as a calibrator sample. Each sample was examined in duplicate, and the mean C_T was used in the equation [15].

2.5. Regulatory T and B cell analyses

Single-cell suspensions of spleen were generated for Treg and regulatory B (Breg) cell analyses. In Treg cell analysis, after cell-surface staining with FITC-conjugated anti-CD4 Ab (RM 4–5) and PerCP-Cy 5.5-conjugated anti-CD25 Ab (PC61) (BioLegend, San Diego, CA), the cells were washed, fixed and permeabilized using the Cytofix/Cytoperm Kit (eBioscience, ThermoFisher Scientific, Waltham, MA), followed by staining with PE-conjugated mouse anti-FoxP3 Ab (FJK-16 s) (eBioscience). For Breg cell analysis, intracellular IL-10 expression was visualized by immunofluorescence staining, as described previously [16]. Stained cells were analyzed on a FACSCanto II flow cytometer (BD Biosciences). Data were analyzed using FlowJo software version 10.6 (Tree Star, Ashland, OR).

2.6. Treg cell depletion in the reverse Arthus reaction model

For Treg cell depletion, mice were given a single intraperitoneal (i.p.) injection of 500 μl of anti-CD25 Ab (PC61) (BioLegend) on day 0 [17]. Isotype-matched control Ab were given into wild-type mice. The reverse Arthus reaction model was induced 7 days after i.p. injection of anti-CD25 Ab or control Ab.

2.7. Statistical analysis

The Student *t*-test was used for determining the level of significance of differences in sample means, and Bonferroni's test was used for multiple comparisons. A P value < 0.05 was considered significant. All data are shown as means ± SEM. All statistical analyses were performed using RStudio software version 1.0.136.

3. Results

3.1. Edema and hemorrhage in the reverse Arthus reaction model

In the reverse Arthus reaction model, edema and hemorrhage are two distinct responses (Fig. 1A). Edema was significantly reduced in

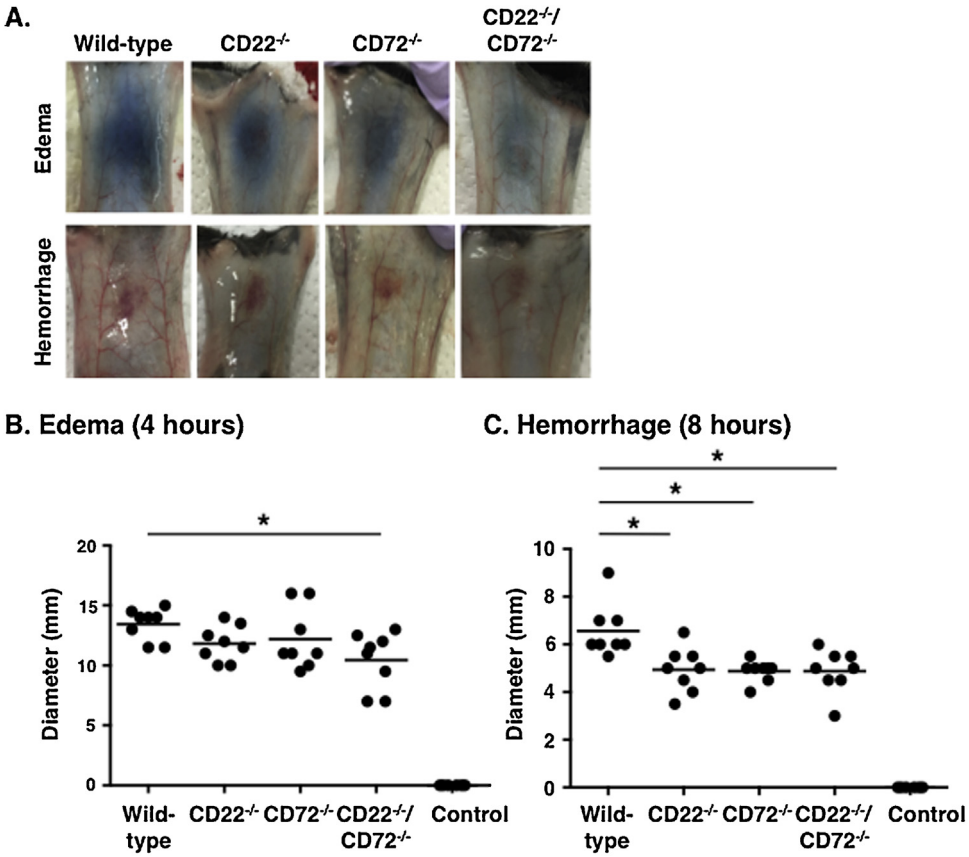

Fig. 1. Macroscopic findings, edema, and hemorrhage in the reverse Arthus reaction model **(A–C)**. Mice were injected intradermally with rabbit anti-chicken egg albumin IgG Abs followed by intravenous administration of chicken egg albumin and 1% Evans blue dye. Abdominal skin was harvested from wild-type and knockout mice after 4 or 8 h. **A.** Representative macroscopic findings of edema (4 h) and hemorrhage (8 h) in wild-type mice and knockout mice. **B.** Edema was evaluated by the diameter of the Evans blue spot after 4 h. **C.** Hemorrhage was assessed as the diameter of the purpuric spot after 8 h. Wild-type mice with intradermal injection of purified polyclonal rabbit IgG, followed by intravenous administration of chicken egg albumin served as controls. Horizontal bars indicate mean values for each group of mice. *, $p < 0.05$.

$CD22^{-/-}/CD72^{-/-}$ mice compared to wild-type mice (23%, $p < 0.05$) (Fig. 1B). $CD22^{-/-}$ mice and $CD72^{-/-}$ mice each exhibited a decrease in edema compared to wild-type mice (12% and 9%, respectively), although the differences did not reach significance. No edema was detected in wild-type mice or control knockout mice following intradermal injection of rabbit polyclonal IgG with intravenous administration chicken egg albumin (Fig. 1B and data not shown).

Hemorrhage was macroscopically assessed 8 h after the IC challenge by measuring the size of the purpuric spot (Fig. 1C). Hemorrhage was significantly inhibited in $CD22^{-/-}$ mice (17%, $p < 0.05$), $CD72^{-/-}$ mice (20%, $p < 0.05$) and $CD22^{-/-}/CD72^{-/-}$ mice (22%, $p < 0.05$) compared with wild-type mice. Hemorrhage was not seen in wild-type mice or knockout mice following intradermal injection of rabbit polyclonal IgG with intravenous injection of chicken egg albumin (Fig. 1C and data not shown). Thus, the combined deficiency of CD22 and CD72 resulted in a greater inhibition in the reverse Arthus reaction model, especially in edema at 4 h, than the loss of each surface molecule alone.

3.2. Leukocyte infiltration in the reverse Arthus reaction model

Extravascular leukocyte infiltration was assessed in skin tissue sections after 4 and 8h after IC challenge (Figs. 2 and 3). Infiltrating neutrophil numbers 4 h after IC challenge were significantly reduced in $CD72^{-/-}$ mice (64%, $p < 0.05$) and $CD22^{-/-}/CD72^{-/-}$ mice (70%, $p < 0.05$) compared with wild-type mice (Figs. 2 and 3A). $CD22^{-/-}$ mice showed a 48% decrease in the number of infiltrating neutrophils 4 h after IC challenge, but the difference did not reach significance. The number of infiltrating neutrophils was significantly reduced in $CD22^{-/-}/CD72^{-/-}$ mice (50%, $p < 0.05$), but not in $CD22^{-/-}$ mice or $CD72^{-/-}$ mice compared with wild-type mice 8 h after IC challenge (Figs. 2 and 3A).

Macrophage numbers were significantly decreased in $CD22^{-/-}/CD72^{-/-}$ mice compared with wild-type mice both 4 h (22%, $p < 0.05$) and 8 h (69%, $p < 0.01$) after IC challenge. Macrophage numbers were also significantly reduced in $CD22^{-/-}$ mice (56%, $p < 0.01$) and $CD72^{-/-}$ mice (54%, $p < 0.01$) compared with wild-type mice at 8 h, but not 4 h, after IC challenge (Figs. 2 and 3B).

$CD3^+$ T cell infiltration was also significantly decreased in $CD22^{-/-}/CD72^{-/-}$ mice compared with wild-type mice both 4 h (65%, $p < 0.01$) and 8 h (66%, $p < 0.01$) after IC challenge (Figs. 2 and 3C). The numbers of $CD3^+$ T cells in $CD22^{-/-}$ mice and $CD72^{-/-}$ mice were also significantly attenuated at both 4 h (46%, $p < 0.01$ and 40%, $p < 0.01$, respectively) and 8 h (36%, $p < 0.01$ and 52%, $p < 0.01$, respectively). At 4 and 8 h after IC challenge, the frequencies of Treg cells were at or below detection in wild-type and knockout mice (data not shown).

The knockout mice exhibited similar levels of mast cell accumulation as seen in wild-type mice, both at 4 and 8 h after IC challenge (data not shown). We detected a few $B220^+$ B cells in the inflamed skin tissue sections of wild-type and knockout mice after IC challenge (data not shown).

These results suggest that CD22 and CD72 cooperatively play important roles in the migration of leukocytes into inflamed skin tissue in the reverse Arthus reaction model. The loss of either CD22 or CD72 expression significantly reduced neutrophil, macrophage, and T cell accumulation, and the absence of both molecules further reduced neutrophil accumulation.

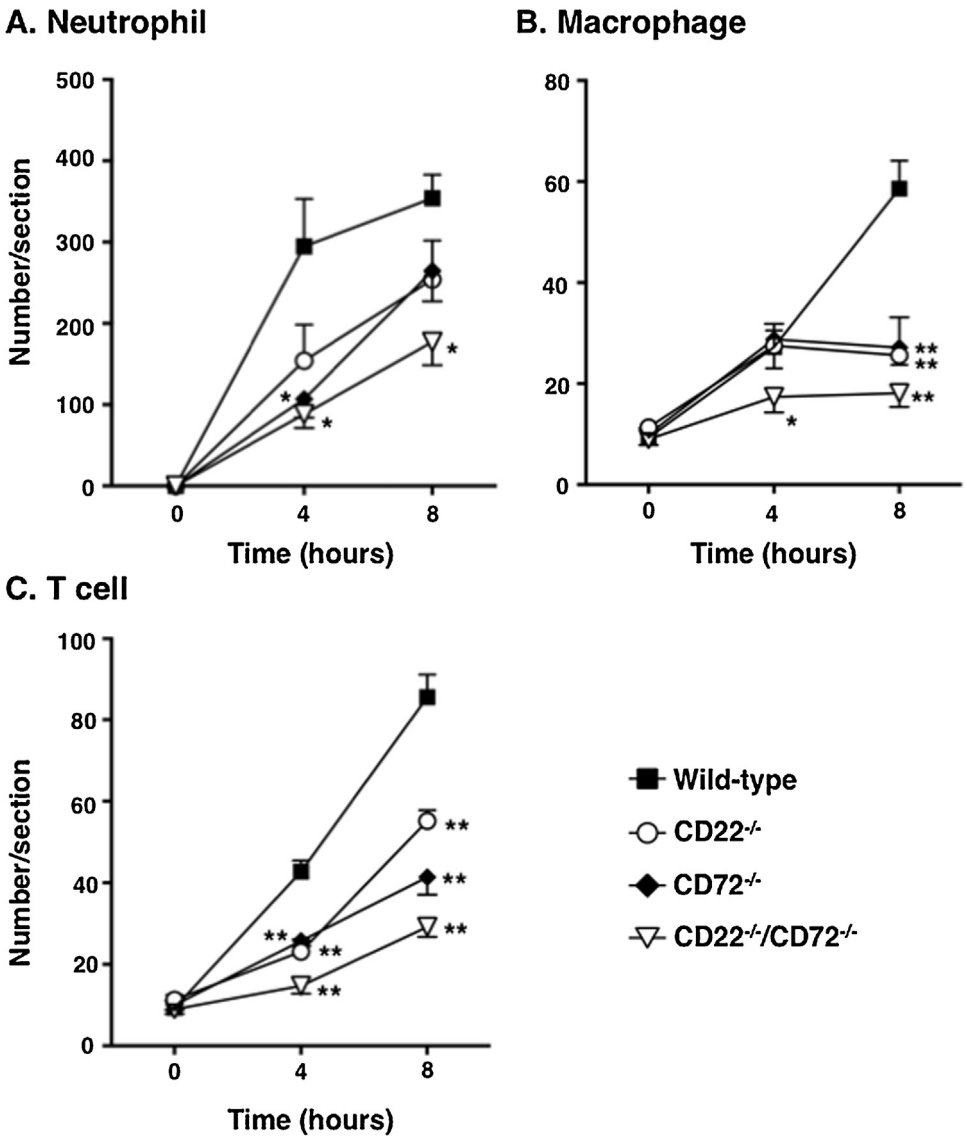

Fig. 2. Reverse Arthus reaction-induced recruitment of neutrophils **(A)**, macrophages **(B)**, and T cells **(C)** in skin from wild-type and knockout mice 4 and 8 h after IC challenge. Numbers of neutrophils, macrophages, and T cells per skin section were determined by counting H&E staining, anti-F4/80 Ab- and anti-CD3 Ab-immunohistochemical staining, respectively. All values represent the mean ± SEM of results obtained from 5 to 10 mice in each group. *, $p < 0.05$; **, $p < 0.01$.

3.3. Cytokine and chemokine mRNA expression in the reverse Arthus reaction model

The mRNA expression levels of TNF-α in $CD22^{-/-}$ and $CD22^{-/-}$/$CD72^{-/-}$ mice were significantly decreased 8 h after IC challenge ($p < 0.01$ for each) (Fig. 4A and B). TNF-α mRNA expression levels in $CD72^{-/-}$ mice were also significantly reduced compared to those in wild-type mice both 4 and 8 h after IC challenge ($p < 0.05$ and $p < 0.01$, respectively). IL-6 mRNA expression levels in $CD22^{-/-}$ mice and $CD22^{-/-}$/$CD72^{-/-}$ mice were significantly decreased compared to those in wild-type mice 8 h after IC challenge ($p < 0.05$ for each). Thus, the reduced skin inflammatory responses by the loss of CD22 and/or CD72 molecules were associated with the reduced release of TNF-α and IL-6.

The expression levels of MIP-1α and CCR5 were not significantly different 4 h after IC challenge (Fig. 4A). However, MIP-1α expression levels were significantly lower in $CD22^{-/-}$ and $CD22^{-/-}$/$CD72^{-/-}$ mice compared with wild-type mice ($p < 0.05$ for each). The expression levels of CCR5 were significantly lower in $CD22^{-/-}$/$CD72^{-/-}$ mice compared with wild-type mice after 8 h ($p < 0.05$) (Fig. 4B). On the other hand, a significant association of the severity of vasculitis with the expression levels of CXCL1, CXCL2, CXCR2, CX3CL1, and CX3CR1 was not observed in this model (data not shown). Therefore, CD22 and CD72 can affect MIP-1 and CCR5 expression, leading to the regulation of inflammatory cell infiltration in the reverse Arthus reaction model.

3.4. Regulatory T and B cells in the reverse Arthus reaction model

We examined Treg and Breg cells from the spleens of wild-type mice and knockout mice before and 4 and 8 h after IC challenge. The frequency of Treg cells was significantly increased in $CD22^{-/-}$/$CD72^{-/-}$ mice compared with wild-type mice, $CD22^{-/-}$ mice, and $CD72^{-/-}$ mice before IC challenge ($p < 0.01$, $p < 0.05$, and $p < 0.01$, respectively) (Fig. 5A and Supplemental Fig. 1). The absolute Treg cell number was also significantly increased in $CD22^{-/-}$/$CD72^{-/-}$ mice compared with wild-type mice ($p < 0.01$). Although the frequency of Treg cells was significantly increased only in $CD22^{-/-}$ mice compared with wild-type mice at 4 h after IC challenge ($p < 0.01$), absolute Treg cell numbers were significantly increased

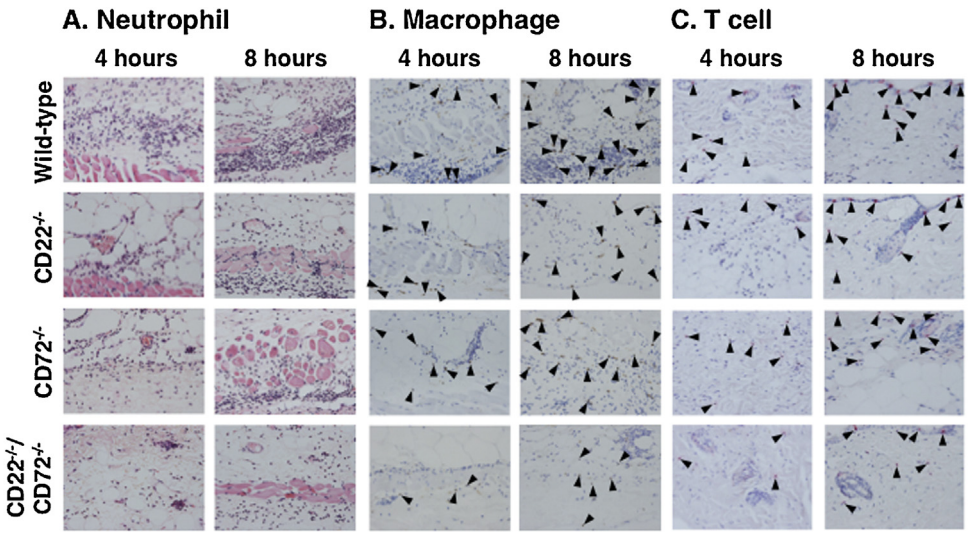

Fig. 3. Representative inflammatory cell recruitment in the skin from wild-type mice and knockout mice. **A.** Neutrophils were detected by H&E staining at 4 and 8 h after IC challenge (left columns). **B.** Macrophages (arrows) were detected as $F4/80^+$ cells by immunohistochemistry Ab. **C.** T cells (arrows) were detected as $CD3^+$ cells by immunohistochemistry. Original magnification, x200.

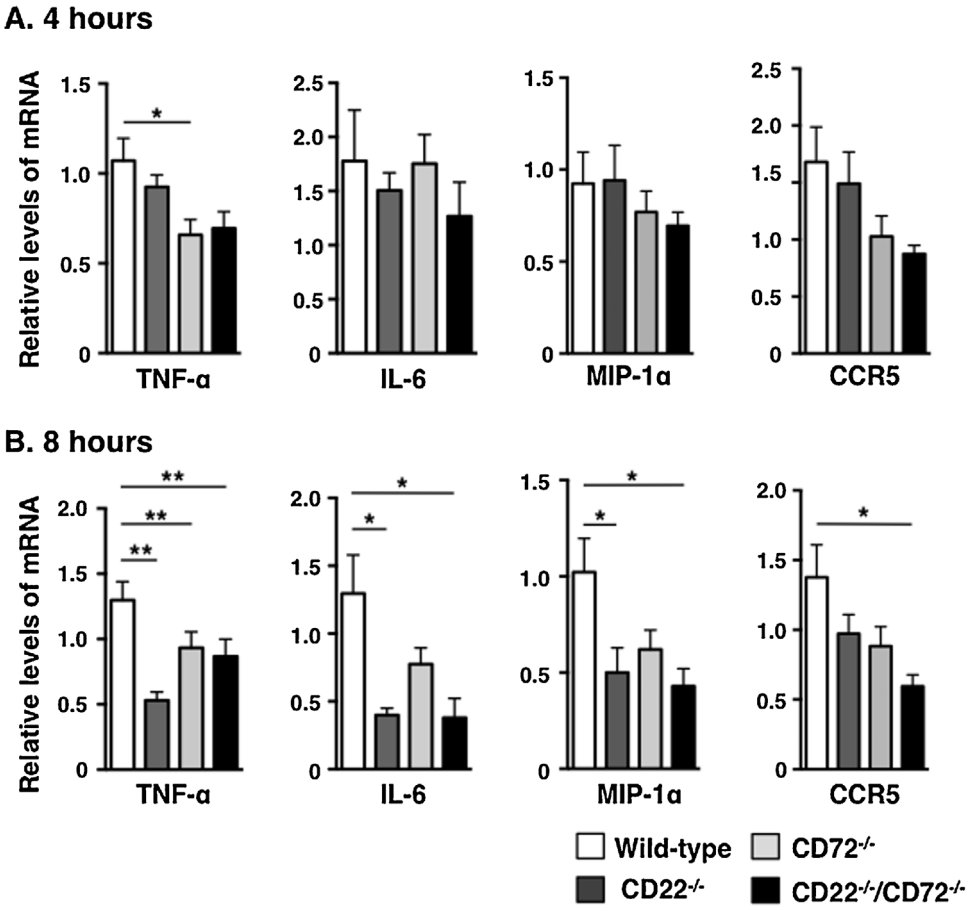

Fig. 4. TNF-α, IL-6, MIP-1α, and CCR5 mRNA expression in the skin of wild-type and knockout mice 4 h **(A)** and 8 h **(B)** after IC challenge. The mRNA amount was measured by real-time PCR and normalized to GAPDH mRNA. TNF-α, IL-6, MIP-1α, and CCL5 mRNA levels in wild-type mice were used as calibrators. All values represent the mean ± SEM of results obtained with 5 mice in each group. *, $p < 0.05$.

in $CD22^{-/-}$ mice, $CD72^{-/-}$ mice, and $CD22^{-/-}/CD72^{-/-}$ mice compared with wild-type mice ($p < 0.01$, $p < 0.05$, $p < 0.01$, respectively). At 8 h, Treg cell frequencies were comparable between all groups; however, absolute Treg cell numbers were significantly increased in $CD22^{-/-}$ and $CD22^{-/-}/CD72^{-/-}$ mice compared with wild-type mice (p 0.01 and $p < 0.01$, respectively). The absolute Treg cell numbers in $CD22^{-/-}$ mice were also significantly increased at 8 h, compared with $CD72^{-/-}$ mice ($p < 0.05$). Next, we conducted Treg cell depletion therapy in knockout mice to confirm a role of Treg cells in the reverse Arthus

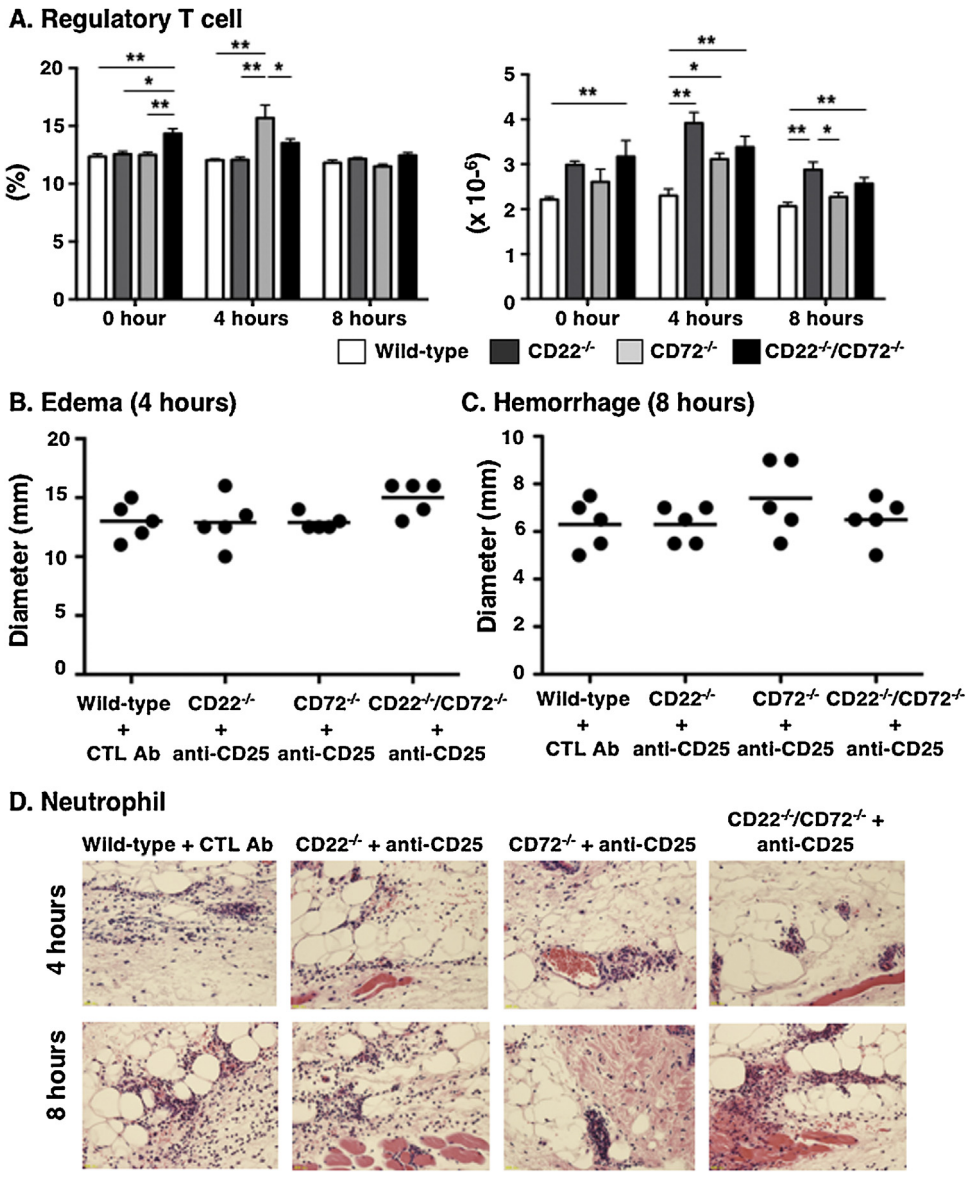

Fig. 5. Regulatory T cells (Tregs) present in the spleen of wild-type and knockout mice before and after IC challenge. **A.** Frequencies and absolute cell numbers of Tregs in wild-type and knockout mice. Bar graphs indicate the mean ± SEM of results obtained with at least 4 mice in each group. **B–C.** Edema after 4 h **(B)** and hemorrhage after 8 h **(C)** in wild-type and knockout mice treated with control Ab or anti-CD25 Ab. Horizontal bars indicate mean values for each group of mice. **D.** Reverse Arthus reaction-induced recruitment of neutrophils in the skin from wild-type and knockout mice treated with control Ab or anti-CD25 Ab 4 and 8 h after IC challenge. *, $p < 0.05$; **, $p < 0.01$.

reaction model. Significant differences of the degree of both edema and hemorrhage between wild-type mice and knockout mice were lost when knockout mice were treated with anti-CD25 Ab (Fig. 5B and C). Infiltrating neutrophil and macrophage cell numbers in the affected skin were comparable among all groups after both 4 and 8 h after IC challenge (Fig. 5D and data not shown). Therefore, Treg cells play, in part, a role in knockout mice in the reverse Arthus reaction model.

In contrast to Treg cells, significant differences in both the frequencies and absolute Breg cell numbers were not observed among wild-type mice and knockout mice before and 4 and 8 h after IC challenge (data not shown). Therefore, Breg cells do not appear to contribute to the reverse Arthus reaction model.

4. Discussion

This study is the first to elucidate the contribution of CD22 and CD72 in combination on the development of the reverse Arthus reaction model. The present study demonstrates that CD22 and CD72 cooperatively contribute to the development of the reverse Arthus reaction model by regulating the migration of leukocytes via control of Treg cells and the production of cytokines and chemokines.

Mice lacking CD22 or CD72 exhibit augmented B cell activation, since CD22 and CD72 are inhibitory co-receptors that control BCR signaling strength and determine the fate of B cells, leading to the induction of various autoimmune conditions [9–11,18]. In contrast, in the reverse Arthus reaction model, the degree of IC-mediated tissue injury was diminished in $CD22^{-/-}$, $CD72^{-/-}$, and $CD22^{-/-}/CD72^{-/-}$ mice. Sato et al. reported that CD22 negatively regulates antigen receptor signaling in the absence of antigen. However, activation of CD22-deficient B cells by prolonged IgM cross-linking resulted in modest B cell proliferation, demonstrating that CD22 positively regulates antigen receptor signaling in the presence of antigen [10]. Further experiments using other murine disease models are needed in order to confirm the contribution of CD22

and CD72 in the development of various immune-mediated diseases.

Identification of the leukocyte subsets that play the most important roles in the reverse Arthus reaction model may depend on the experimental system. Nonetheless, neutrophils are a prominent leukocyte cell subset regardless of the model. Mast cells are also essential in most studies of the reverse Arthus reaction model [19]. In an IgE-mediated reverse Arthus reaction model, eosinophils played a substantial role in combination with neutrophils and mast cells [20]. Consistent with previous studies, neutrophils were significantly decreased in CD72$^{-/-}$ and CD22$^{-/-}$/CD72$^{-/-}$ mice at 4 h and in CD22$^{-/-}$/CD72$^{-/-}$ mice at 8 h. In contrast, a role for mast cells was not identified in the present study. Instead, the number of macrophages was significantly decreased in CD22$^{-/-}$, CD72$^{-/-}$, and CD22$^{-/-}$/CD72$^{-/-}$ mice at 8 h compared to wild-type mice, and the degree of IC-mediated tissue injury was similarly decreased. The importance of T cells in the development of the reverse Arthus reaction model was reported previously [21]. The present study showed that CD3^{+} T cells were significantly decreased in knockout mice compared with wild-type mice. Therefore, it is likely that CD22 and CD72 are required for the recruitment of neutrophils, macrophages, and T cells to the inflammatory sites in IC-mediated tissue injury.

Chemokines are thought to provide the directional cues for the movement of leukocytes in development, homeostasis, and inflammation. CCR5 is expressed on many hematopoietic cells, including lymphocytes, macrophages and granulocytes [22,23]. MIP-1α is one of the main chemokines supporting lymphocyte trafficking. MIP-1α functions as an autocrine mediator for macrophages to produce TNF-α [24], and also plays a crucial role in neutrophil recruitment in the reverse Arthus reaction model [25]. MIP-1α induced chemotaxis of CCR1- and/or CCR4-positive cells. In addition, CXCL1 and CXCL2 are also reported to be involved. In the present study, MIP-1α expression in the skin lesions of CD22$^{-/-}$/CD72$^{-/-}$ mice was significantly reduced compared to wild-type mice at 8 h (Fig. 4). Moreover, intradermal CCR5 expression was also dramatically inhibited in CD22$^{-/-}$/CD72$^{-/-}$ mice compared to wild-type mice 8 h after IC challenge. On the other hand, roles for CXCL1, CXCL2, CXCR2, CX3CL1, and CX3CR1 were not identified in this model. These data suggest that, in the reverse Arthus reaction model, CD22 and CD72 might regulate lymphocyte recruitment through MIP-1α and CCR5 chemokine-related pathways, resulting in the reduced production of pro-inflammatory cytokines, including TNF-α and IL-6.

It is now understood that a multitude of regulatory cell subsets regulate immune responses. Our results suggest that CD22 and CD72 may be involved in the development of Treg cells, since the frequency of Treg cells was significantly increased in CD22$^{-/-}$/CD72$^{-/-}$ mice in the steady state. Further experiments are required to clarify how CD22 and CD72 regulate the development of Treg cells in vivo. Our results indicated that small alterations of the frequency and absolute number of Treg cells in the spleen, but not in the skin, influence the severity of vasculitis, resulting from control of the infiltration of neutrophils, macrophages, and T cells in the affected skin lesion. This concept is supported by the results that Treg cell depletion in knockout mice exacerbated the severity of vasculitis to wild-type mice levels. The spleen is functionally linked to the systemic blood circulation [26] and, therefore, the cell population in the spleen may partially reflect the cell frequency in the circulation. Although the frequencies of Treg cells in the affected skin lesion were at or below detection in the present study, Treg cells in the blood vessels in the affected skin lesion may regulate the IC-induced vasculitis via controlling inflammatory cell infiltration and cytokine and chemokine production. Alternatively, previous studies reveal that the spleen plays a central role to control the peripheral immune response by expanding regulatory cell subsets. The spleen accumulates immunosuppressive myeloid cells after tissue remodeling [27,28]. In a murine sclerodermatous chronic graft-versus-host disease model, early administration of FTY720, an oral S1P receptor modulator, inhibited the severity of fibrosis resulting from the expansion of splenic myeloid-derived suppressor cells, Treg cells, and Breg cells [29]. In another study, combined vaccination with heat-killed BCG and Mycobacterium kansasii antigen 85B in mice decreases atopic dermatitis-like skin lesions by inducing regulatory T cells in the spleen [30]. Therefore, similar mechanism by which Treg cells in the spleen regulate the peripheral immune response may function in the reverse Arthus reaction model. However, we cannot exclude the possibility that CD72 and CD22 might affect vascular leakage in knockout mice, since we did not assess the vascular structure among wild-type and knockout mice. Regarding an association of Breg cells and CD22, Nakashima et al. reported that peritoneal B-1a cells have a regulatory role (Breg cells) in contact hypersensitivity (CHS), and CD22 deficiency results in disturbed CHS remission by impaired retention or survival of peritoneal B-1a cells that migrate into lymphoid organs [31]. However, Breg cells were not involved in the present study.

Targeting specific B cell surface molecules represents a promising approach for the treatment of autoimmune diseases. Recently, a humanized anti-CD22 Ab (Epratuzumab) was developed, the efficacy of which has been reported in the treatment of several autoimmune diseases, including systemic lupus erythematosus and Sjogren syndrome [32–34]. Our findings indicate that CD22, together with CD72, plays an important role in the development of a murine reverse Arthus reaction model, and that they could be potential targets for the treatment of IC-mediated diseases. However, the effect of simultaneous inhibition of both CD22 and CD72 may be limited, because the additive effect of inhibiting CD72 in addition to the blockade of CD22 was only observed in edema at 4 h, and single deficiency of CD22 or CD72 was enough to reduce hemorrhage 8 h after IC challenge.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jdermsci.2019.06.005.

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