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Intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 cooperatively contribute to the cutaneous Arthus reaction

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Abstract

Immune complex-induced inflammation is mediated by inflammatory cell infiltration, a process that is highly regulated by expression of multiple adhesion molecules. The roles and interactions of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), the major regulators of leukocyte firm adhesion, were examined in the cutaneous reverse passive Arthus reaction using ICAM-1-deficient (ICAM-1^{-/-}) mice and blocking monoclonal antibodies (mAbs) against VCAM-1. Within 8 hours, immune complex challenge of wild-type mice induced edema, hemorrhage, interstitial accumulation of neutrophils and mast cells, as well as production of tumor necrosis factor- α and IL-6. All of these inflammatory parameters were reduced significantly in ICAM-1^{-/-} mice. The blockade of VCAM-1 in wild-type mice did not affect any inflammatory parameters. In contrast, ICAM-1^{-/-} mice treated with anti-VCAM-1 mAbs had significantly reduced edema, hemorrhage, and neutrophil infiltration. Furthermore, VCAM-1 blockade in ICAM-1^{-/-} mice suppressed cutaneous tumor necrosis factor-alpha and IL-6 production. Thus, VCAM-1 plays a complementary role to ICAM-1 in the cutaneous Arthus reaction by regulating leukocyte accumulation and proinflammatory cytokine production.

Introduction

The pathogenesis of autoimmune diseases frequently involves the formation of IgG-containing immune complexes (IC) inducing inflammatory responses with significant tissue injury, commonly referred to as type III hypersensitivity reaction. This IC injury has been implicated in the pathogenesis of vasculitis syndrome, systemic lupus erythematosus, rheumatoid arthritis, and cryoglobulinemia [1]. The mechanisms by which the immune system controls effector responses to IC are of central importance for developing therapeutic strategies. The standard animal model for the inflammatory response in these IC-mediated diseases is the Arthus reaction [2]. Analyses using gene knockout mice have revealed that activation of the complement system, especially C5a and its interaction with C5a receptor, and of Fc receptors for IgG on inflammatory cells, particularly mast cells, are both required to initiate the Arthus reaction [3-8]. In addition, accumulation of neutrophils and mast cells is necessary for the progression of the IC-mediated vascular tissue damage, which results in edema and hemorrhage [3-8].

Leukocyte recruitment from the circulation into an extravascular site is pivotal to the inflammatory response. Leukocytes first tether and roll on vascular endothelial cells, before they are activated to adhere firmly and then crawl and transmigrate to the extravascular space. This multistep process is regulated by the cooperative action of adhesion molecules on both the endothelial cell and the leukocyte [9, 10]. The selectins primarily mediate tethering and rolling of leukocytes, while immunoglobulin (Ig) superfamily members, including intercellular adhesion molecule-1 (ICAM-1, CD54) and vascular cell adhesion molecule-1 (VCAM-1, CD106) are critical for the firm adhesion through the interaction with their integrin ligands, lymphocyte function-associated antigen-1 (LFA-1; CD11a/CD18) and very late antigen-4 (VLA-4; CD49d/CD29), respectively [11]. ICAM-1 is constitutively expressed at low levels on the

surface of endothelial cells, but its expression is significantly increased upon endothelial cell activation with cytokines or endotoxin [12]. These stimuli also induce the expression of VCAM-1, which interacts with most leukocytes, except neutrophils, which express VLA-4 [13]. VCAM-1 plays essential roles in various aspects of biological responses including the development of inflammation. Deletion of the VCAM-1 gene produces a phenotype similar to deletion of the α_4 integrin gene, with embryonic lethality due to cardiac and placental developmental abnormalities, although a few VCAM-1^{-/-} mice survive [14, 15].

We previously reported roles and interaction of the selectins, major regulators of the initial step of leukocyte migration, in the cutaneous Arthus reaction. Arthus reaction was reduced by deficiency of either P-selectin (CD62P) or L-selectin (CD62L), while it was not inhibited by loss of E-selectin (CD62E) expression [6, 16]. Furthermore, both E- and P-selectin blockade resulted in more reduction relative to P-selectin deficiency alone, suggesting the cooperative roles of these selectins in the cutaneous Arthus reaction [16]. We also reported that ICAM-1 plays a critical role in the cutaneous Arthus reaction, since ICAM-1-deficient (ICAM-1^{-/-}) mice exhibit reduced Arthus reaction that is associated with decreased infiltration of neutrophils and mast cells [17]. However, the relative contribution and interaction of ICAM-1 and VCAM-1, both of which predominantly regulate firm adhesion process of leukocyte migration, to the Arthus reaction remains unknown. In this study, to clarify the role of VCAM-1 and interaction of ICAM-1 and VCAM-1 in the Arthus reaction, we examined inflammation induced by IC in mice lacking ICAM-1 and wild-type mice with anti-VCAM-1 monoclonal antibody (mAb).

Materials and Methods

Mice.

ICAM-1^{-/-} mice [18] expressing residual amounts of ICAM-1 splice variants in the thymus and spleen but not in other organs, including skin [19], were obtained from The Jackson Laboratory (Bar Harbor, ME). All mice were healthy, fertile, and did not display evidence of infection or disease. All mice were backcrossed between 5 and 10 generations onto the C57BL/6 genetic background. Mice used for experiments were 12 to 16 weeks old. Age-matched wild-type littermates and C57BL/6 mice (The Jackson Laboratory) were used as controls with equivalent results so all control results were pooled. All mice were housed in a pathogen-free barrier facility and screened regularly for pathogens. All studies and procedures were approved by the Committee on Animal Experimentation of Kanazawa University Graduate School of Medical Science.

Reverse passive Arthus reaction.

For cutaneous reverse passive Arthus reactions, mice anesthetized by inhalation of diethyl ether were shaved on their dorsal skin and wiped with 70% alcohol. Rabbit IgG anti-chicken egg albumin Abs (60 µg/30 µl; Cappel, Durham, NC) were injected intradermally with a 29-gauge needle, followed immediately by an intravenous injection of chicken egg albumin (20 mg/kg; Sigma-Aldrich, St. Louis, MO) [5]. The intradermal injection of purified polyclonal rabbit IgG (60 µg/30 µl, Sigma-Aldrich) followed by intravenous installation of chicken egg albumin served as a control. The solution of chicken egg albumin contained 1% Evans blue dye (Sigma-Aldrich). For a blocking study using mAbs to VCAM-1 (clone 429 MVCAM.A, rat IgG2a, 30

µg per mouse; BD PharMingen, San Diego, CA), mAbs were injected intravenously 30 min before IC challenge. Irrelevant isotype-matched, purified rat IgG1 mAb (R3-34) and rat IgG2a mAb (R35-95) served as controls (30 µg per mouse, BD PharMingen).

Quantitation of Edema and Hemorrhage.

Edema was evaluated by measuring the vascular leak 4 hr after IC challenge [5]. Mice were sacrificed and the skin containing the injection site was removed at the level of fascia above skeletal muscle and was reversed. The diameter of extravascular Evans blue dye on the fascia side of the injection site was measured directly. Evans blue dye binds to serum proteins and thereby can be used to quantify alterations in vascular permeability. The diameter of the major and minor axis of the blue spot was averaged for analysis. The amount of hemorrhage was assessed 8 hr after IC challenge by direct macroscopic measurement of the purpuric spot. The diameter of the major and minor axis of the purpuric spot was averaged for analysis.

Histological Examination and immunohistochemical staining.

Tissues were harvested 4 or 8 hr after IC challenge using a disposable sterile 6-mm punch biopsy (Maruho, Osaka, Japan) and assessed for tissue damage and number of infiltrating neutrophils and mast cells. Tissues were cut into halves, fixed in 3.5% paraformaldehyde, and then embedded in paraffin. Sections (6 µm) were stained using hematoxylin and eosin for neutrophil evaluation and toluidine blue for mast cell staining. Neutrophil and mast cell infiltration was evaluated by counting extravascular neutrophils and mast cells in the entire section and averaging the numbers present in 10 serial skin sections from the injection site. Each section was

examined independently by three investigators in a blinded manner, and the mean was used for analysis.

For immunohistochemistry, tissue sections of skin biopsies were fixed with acetone and then incubated with 10% normal rabbit serum in PBS (10 min, 37°C) to block nonspecific staining. Sections were then stained with rat mAbs specific for mouse VCAM-1 (429 MVCAM. A, BD PharMingen) as described previously [13]. Rat IgG2a (BD PharMingen) was used as a control for nonspecific staining. Sections were then incubated sequentially (20 min, 37°C) with biotinylated rabbit anti-rat IgG secondary Abs (Vectastain avidin-biotin complex method; Vector Laboratories, Burlingame, CA), then HRP-conjugated avidin-biotin complexes (Vectastain ABC method; Vector Laboratories). Sections were finally developed with 3,3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide and counterstained with methyl green.

RNA isolation and real-time PCR.

Tissues were harvested 4 or 8 hr after IC challenge using a disposable sterile 6-mm punch biopsy (Maruho, Osaka, Japan) and cut into halves. All skin samples were snap-frozen in liquid nitrogen and stored at -80°C before use. Total RNA was isolated from frozen tissue with QIAGEN RNeasy spin columns (QIAGEN Ltd., Crawley, UK) and then was reversely transcribed into cDNA according to the protocol for the Reverse Transcription System (Promega, Madison, WI). The expressions of VCAM-1, IL-6, and tumor necrosis factor (TNF)- α mRNA were analyzed using a real-time PCR quantification method according to the manufacture's instructions (Applied Biosystems, Foster City, CA). Sequence-specific primers and probes were designed by Pre-Developed TaqMan® Assay Reagents (Applied Biosystems). Real-time PCR (one cycle of at 50°C for 2 min, one at 95°C for 10 min; 40 cycles at 92°C for 15 sec and at 60°C for 60 sec)

was performed on an ABI Prism 7000 Sequence Detector (Applied Biosystems). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used to normalize the mRNA. To compare target gene and housekeeping GAPDH gene mRNA expression, the relative expression of real-time PCR products was determined using the $\Delta\Delta C_t$ method [20]. The fold induction = $2^{-[\Delta\Delta C_t]}$, where C_t = the threshold cycle, and $\Delta\Delta C_t = [C_t \text{ gene interest (unknown sample)} - C_t \text{ GAPDH (unknown sample)}] - [C_t \text{ gene interest (calibrator sample)} - C_t \text{ GAPDH (calibrator sample)}]$. 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.

Statistical Analysis.

The Mann-Whitney U test was used to determine the level of significance of differences between the sample means, and Bonferroni's test was used for multiple comparisons. A p value of less than 0.05 was considered statistically significant. All values are shown as the mean \pm SEM.

Results

VCAM-1 expression in ICAM-1^{-/-} mice in the cutaneous reverse passive Arthus reaction.

VCAM-1 expression on activated endothelial cells of the lung, heart, liver, kidney, and skin is induced by stimulation with proinflammatory cytokines [21]. First, VCAM-1 expression during the cutaneous Arthus reaction was examined immunohistochemically in ICAM-1^{-/-} mice. In normal skin from ICAM-1^{-/-} mice, VCAM-1 was scarcely detected on endothelial cells (Figure 1A). In contrast, 4 hr after IC induction there was substantial increase in VCAM-1 expression on the endothelium of blood vessels (Figure 1B). This up-regulation remained observed 8 hr after IC induction (Figure 1C). This expression pattern was also similarly observed in wild-type mice (data not shown). Regional VCAM-1 mRNA levels were comparable between wild-type mice and ICAM-1^{-/-} mice at 4 and 8 hrs (Figure 1D). Therefore, VCAM-1 expression may play a role in the cutaneous reverse passive Arthus reaction.

Edema and hemorrhage in the cutaneous reverse passive Arthus reaction.

Cutaneous inflammation induced by an Arthus reaction can be separated into two distinct responses: edema, which reaches a maximum at 3-4 hr after IC challenge, and hemorrhage, which peaks in intensity at 8 hr [7]. Therefore, edema and hemorrhage were evaluated 4 and 8 h after IC challenge, respectively, in wild-type or ICAM-1^{-/-} mice treated with anti-VCAM-1 mAb or control Ab. When edema was assessed by measuring the diameter of Evans blue dye in the extravascular space, edema was significantly reduced in ICAM-1^{-/-} (21%, $p < 0.0005$) compared with wild-type mice, as reported previously (Figure 2A and ref[6].). Treatment of ICAM-1^{-/-} mice with anti-VCAM-1 mAb further reduced edema compared with control Ab treatment (20%, $p < 0.05$). By contrast, wild-type mice treated with anti-VCAM-1 mAb developed edema that

was almost identical to that found in wild-type mice. Hemorrhage was macroscopically quantitated after 8 hr by measuring the size of the purpuric spot. Hemorrhage was significantly inhibited in ICAM-1^{-/-} mice compared with wild-type mice (50%, $p < 0.005$; Figure 2B), which was further reduced by anti-VCAM-1 mAb injection (63%, $p < 0.05$). Treatment with wild-type mice with anti-VCAM-1 mAb slightly suppressed hemorrhage, although there was no significant difference in comparison with wild-type mice treated with control Ab. Hemorrhage was not detected in mutant mice or their wild-type littermate controls following intradermal injection of rabbit polyclonal IgG with systemic chicken egg albumin (data not shown). Thus, VCAM-1 blockade alone does not affect cutaneous Arthus reaction, but has an additive effect to ICAM-1 deficiency.

Leukocyte infiltration in the cutaneous Arthus reaction.

Extravascular neutrophils were assessed in skin tissue sections after 4 and 8 hr of IC formation (Figures 3A and 4). Before IC challenge, there were no significant differences in cutaneous neutrophil numbers between mutant and wild-type mice. After 4 hr of IC challenge, infiltrating neutrophil numbers were significantly lower in ICAM-1^{-/-} mice than in wild-type mice, as previously reported (32%, $p < 0.05$; ref[6]). While VCAM-1 blockade did not affect neutrophil numbers in wild-type mice, it significantly reduced neutrophil infiltration in ICAM-1^{-/-} mice when compared with those treated with control Ab (41%, $p < 0.05$). Similar results were obtained after 8 hr of IC challenge. Thus, the combined blockade or loss of ICAM-1 and VCAM-1 led to greater reductions in neutrophil accumulation than the loss of ICAM-1 alone.

Mast cell numbers were also assessed in skin tissue sections stained with toluidine blue (Figures 3B and 5). Before IC challenge, skin mast cell numbers did not significantly differ

between mutant and wild-type mice. By contrast, 4 hr after IC challenge, mast cell numbers were significantly reduced in ICAM-1^{-/-} mice (28%, $p < 0.001$; ref[6]), and ICAM-1^{-/-} mice with VCAM-1 blockade (32%, $p < 0.001$) compared with wild-type mice. VCAM-1 blockade did not significantly reduce mast cell numbers in wild-type or ICAM-1^{-/-} mice. Similar results were obtained after 8 hr of IC challenge. Therefore, VCAM-1 blockade had no effect on mast cell accumulation either in wild-type mice or in ICAM-1^{-/-} mice.

Cytokine mRNA levels in the cutaneous Arthus reaction.

Cytokine mRNA expression in the skin was quantified by real-time PCR after 4 hr of IC formation. IL-6 mRNA levels were decreased in ICAM-1^{-/-} mice (44%, $p < 0.005$) relative to wild-type mice and in ICAM-1^{-/-} mice with VCAM-1 blockade (19%, $p < 0.05$) compared with ICAM-1^{-/-} mice (Figure 6A). TNF- α mRNA levels were reduced in ICAM-1^{-/-} mice (41%, $p < 0.005$) relative to wild-type mice and in ICAM-1^{-/-} mice with VCAM-1 blockade (15%, $p < 0.05$) compared with ICAM-1^{-/-} mice (Figure 6B). But both IL-6 and TNF- α mRNA were not decreased in wild-type mice treated with ant-VCAM-1 mAb compared to wild-type mice treated with control Ab. Thus, the reduced cutaneous inflammatory responses by blockade of adhesion molecules were generally associated with the reduced release of IL-6 and TNF- α .

Discussion

In the present study, IC-induced edema and hemorrhage were significantly inhibited in ICAM-1^{-/-} mice treated with mAb to VCAM-1 compared with ICAM-1-deficiency alone, whereas they were not inhibited by blockade of VCAM-1 alone in wild-type mice (Figure 2). The results indicate that ICAM-1 and VCAM-1 cooperatively contribute to the development of the cutaneous passive Arthus reaction. ICAM-1 plays a dominant role since VCAM-1 blockade did not alter the reaction in wild-type mice but reduced it in the absence of ICAM-1 expression. Furthermore, while the reduced Arthus reaction by loss of ICAM-1 expression correlated with neutrophil and mast cell accumulation in the skin, VCAM-1 blockade in combination with ICAM-1 deficiency decreased neutrophil accumulation but did not affect mast cell infiltration. Therefore, VCAM-1 expression mainly regulates the infiltration of neutrophils but not mast cells in the cutaneous passive Arthus reaction. In contrast to the current study, a significant effect of VCAM-1 blockade has been observed in the reverse passive Arthus reaction in cremaster muscle [22]. Antibodies to VCAM-1 alone reduced adhesion and emigration to control levels. Therefore, relative contribution of VCAM-1 may be different between tissues.

Mast cells play an important role in the passive Arthus reaction [8]. Although the exact routes of mast cells into inflammatory sites have not been determined yet, immature mast cells derived from bone marrow progenitors are thought to migrate through the circulation into tissues and subsequently differentiate into mature mast cells [23]. In this study, IC challenge induced rapid mast cell accumulation, which was significantly decreased in ICAM-1^{-/-} mice, while no further inhibition was observed with a blockade of VCAM-1 in ICAM-1^{-/-} mice. There are two distinct populations of mast cells based on phenotype, histochemistry, and proteoglycan content: mucosal mast cells, found in the mucosa of intestines and the lungs, and connective tissue mast

cells located in the skin, peritoneal cavity, and other non-mucosal sites [24]. Connective tissue mast cells express substantially less VLA-4 [25]. Therefore, it is possible that mast cell accumulation in the cutaneous Arthus reaction is independent of the interaction between VCAM-1 and VLA-4.

Neutrophils are another critical effector cell in the cutaneous Arthus reaction. In contrast to mast cell accumulation, the current study has demonstrated that VCAM-1 had a complementary role to ICAM-1 in neutrophil recruitment. Although neutrophils have been generally considered to lack VLA-4 expression [26], several reports have provided evidence for the presence of VLA-4 expression on human and rodent neutrophils [27-29]. We previously reported positive expression of VLA-4 in peritoneal neutrophils from wild-type mice and ICAM-1^{-/-} mice [17]. Furthermore, studies have demonstrated that VCAM-1 and ICAM-1 play important roles in neutrophil emigration in vivo [21]. In a CD18-null mouse model of myocardial ischemia and reperfusion injury, neutrophil emigration on cardiac endothelial monolayers efficiency was further reduced (90%) after VCAM-1 Ab blockade [30]. Moreover, in a murine model of endotoxic shock, increased expression of VCAM-1 was observed in the liver. Pretreatment with anti-VCAM-1 Ab decreased neutrophil transmigration into the liver parenchyma and attenuated liver tissue damage [13]. Constitutive VLA-4 expression on resting rat neutrophils is also shown to mediate neutrophil accumulation in the inflamed joints of rats with adjuvant-induced arthritis as well as in dermal inflammation sites. Blocking VLA-4 together with LFA-1 inhibited neutrophil migration to dermal inflammatory reactions by 30-70%. Thus while VLA-4 by itself had no effect on neutrophil accumulation, it strongly inhibits neutrophil accumulation, when combined with anti-LFA-1, in cutaneous inflammation [29]. In the current study, after 4 hr of IC challenge, neutrophil numbers were significantly reduced in

ICAM-1^{-/-} mice compared with wild-type mice, but moreover, the combined loss or blockade of ICAM-1 and VCAM-1 led to greater reductions than the loss of ICAM-1 alone by approximately 40% (Figure 3A). Taken together, the results suggest that VCAM-1 plays a pivotal role in neutrophil accumulation in the passive Arthus reaction.

The current study is consistent with our previous observations that the loss or blockade of L-, E-, and P-selectins and ICAM-1 completely abrogated mast cell infiltration but not neutrophil infiltration, indicating that there exists an adhesion pathway independent of all selectins and ICAM-1 for neutrophil recruitment [17]. Additionally, a recent study has shown that VLA-4 is the predominant E- and P-selectin-independent mechanism for leukocyte migration to skin in response to various stimuli [31]. Collectively, during the process of the cutaneous Arthus reaction which selectins, ICAM-1, and VCAM-1 regulate cooperatively, VCAM-1 expression is important for neutrophil infiltration.

Proinflammatory cytokines, including TNF- α and IL-6, are produced and released from mast cells, neutrophils, and monocytes [32, 33]. TNF- α and IL-6 levels after 4 hr within skin at site of IC deposition were significantly decreased by the loss of ICAM-1 in comparison with wild-type mice. VCAM-1 blockade had an additive effect in ICAM-1^{-/-} mice but not in wild-type mice, which is likely to result from the decreased neutrophil infiltration (Figure 3A). Thus, deficiency or blocking of adhesion molecules regulates leukocyte trafficking, which in turn influences the survival and activation by their role in out-side-in signaling [34]. Therefore, these results suggest that ICAM-1 and VCAM-1 cooperatively control neutrophil accumulation or possibly their function after IC challenge and thereby influence the release of proinflammatory cytokines from these leukocytes, leading to the regulation of the Arthus reaction.

The Arthus reaction is a useful, simplified model to evaluate direct roles of adhesion

molecules in effector phase of inflammation. The current study together with the results from our previous studies demonstrates relative contribution of these molecules: edema and hemorrhage are inhibited by 30 to 40% with L-selectin deficiency, by ~45% with ICAM-1 deficiency, by 40 to 60% with P-selectin deficiency or blockade, by ~50% with both L-selectin and ICAM-1 deficiency, by ~75% with both E- and P-selectin blockade, by 100% with both E- and P-selectin blockade in L-selectin and ICAM-1 deficiency, and by ~80% with VCAM-1 blockade in ICAM-1 deficiency [6, 17]. In this study, although VCAM-1 alone plays a minor role, orchestrated regulation with ICAM-1 takes a fundamental part in the cutaneous passive Arthus reaction.

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Figure legends

Figure 1. VCAM-1 expression in the skin from ICAM-1^{-/-} mice during a cutaneous passive Arthus reaction. VCAM-1 expression in the skin before IC challenge (A) and in inflamed skin after 4 hr (B) and 8 hr (C) of IC challenge was assessed by immunohistochemistry using anti-VCAM-1 Ab. Sections were counterstained with methyl green. Original magnification, x100. (D) VCAM-1 mRNA levels in the skin from wild-type mice or ICAM-1^{-/-} mice during a cutaneous passive Arthus reaction, before IC challenge (0 hr), and after 4 hr and 8 hr. Transcript levels were quantified by real-time RT-PCR analysis and were normalized relative to endogenous GAPDH levels. Values represent mean (\pm SEM) relative to transcript levels of wild type mice at 4 hr, from 4-5 mice in each group.

Figure 2. The effect of loss or blockade of ICAM-1 and VCAM-1 on edema and hemorrhage in the cutaneous reverse passive Arthus reaction. Mice were injected intradermally with rabbit IgG anti-chicken egg albumin Ab, followed by systemic chicken egg albumin and 1% Evans blue dye. After 4 or 8 hr, dorsal skins were assessed from ICAM-1^{-/-} mice treated with either anti-VCAM-1 mAb or control Ab and from wild-type mice treated with either anti-VCAM-1 mAb or control Ab. A: Edema was evaluated as the diameter of extravasated Evans blue spot. Wild-type mice that received an intradermal injection of polyclonal rabbit IgG followed by intravenous installation of chicken egg albumin served as control. B: Hemorrhage after 8 hr was assessed as the diameter of the purpuric spot. Edema and hemorrhage were significantly inhibited in ICAM-1^{-/-} mice treated with either anti-VCAM-1 mAb or control Ab compared with wild-type mice treated with either anti-VCAM-1 mAb or control Ab for both panels ($p < 0.05$). Horizontal bars indicate mean values for each group of mice.

Figure 3. Arthus reaction-induced recruitment of neutrophils and mast cells in the skin from ICAM-1^{-/-} mice treated with either anti-VCAM-1 mAb or control Ab and from wild-type mice treated with either anti-VCAM-1 mAb or control Ab at 4 and 8 hr after IC challenge. Numbers of neutrophils and mast cells per skin section were determined by counting in H&E- and toluidine blue-stained skin sections, respectively. All values represent the mean ± SEM of results obtained from 5 to 10 mice in each group. *, p<0.05; **, p<0.01 vs. wild-type mice with control Ig. †, p<0.05; ††, p<0.01 vs. ICAM-1^{-/-} mice with control Ig.

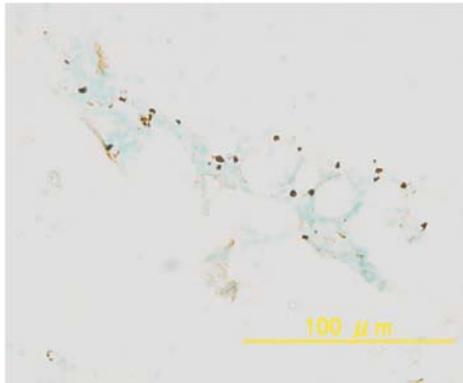
Figure 4. Histological tissue sections showing neutrophil infiltration in the skin of ICAM-1^{-/-} mice treated with either anti-VCAM-1 mAb or control Ab and of wild-type mice treated with either anti-VCAM-1 mAb or control Ab at 4 hr (A) and 8 hr (B) after IC challenge. Neutrophils were revealed by H&E staining. Original magnification, x100.

Figure 5. Histological tissue sections showing mast cell accumulation in the skin of ICAM-1^{-/-} mice treated with either anti-VCAM-1 mAb or control Ab and of wild-type mice treated with either anti-VCAM-1 mAb or control Ab at 4 hr (A) and 8 hr (B) after IC challenge. Mast cells (arrows) were detected as cells with metachromatic staining of granules in toluidine blue-stained sections. Original magnification, x100.

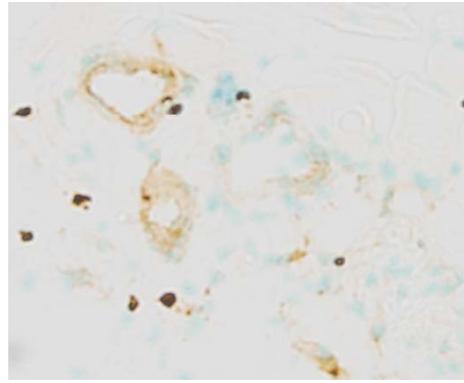
Figure 6. TNF- α and IL-6 mRNA expression in the skin of ICAM-1^{-/-} mice treated with either anti-VCAM-1 mAb or control Ab and of wild-type mice treated with either anti-VCAM-1 mAb

or control Ab at 4 hr after IC challenge. Relative mRNA expression was quantified by real-time RT-PCR. All values represent the mean \pm SEM of results obtained from 5 mice in each group.

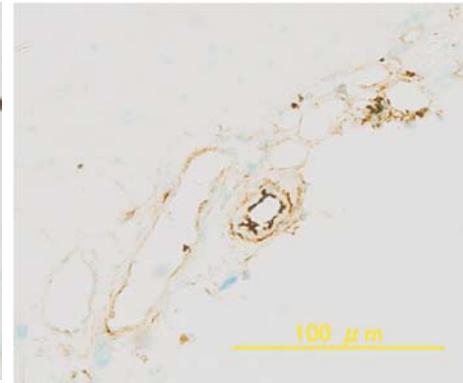
A. 0 hr



B. 4 hr



C. 8 hr



D.

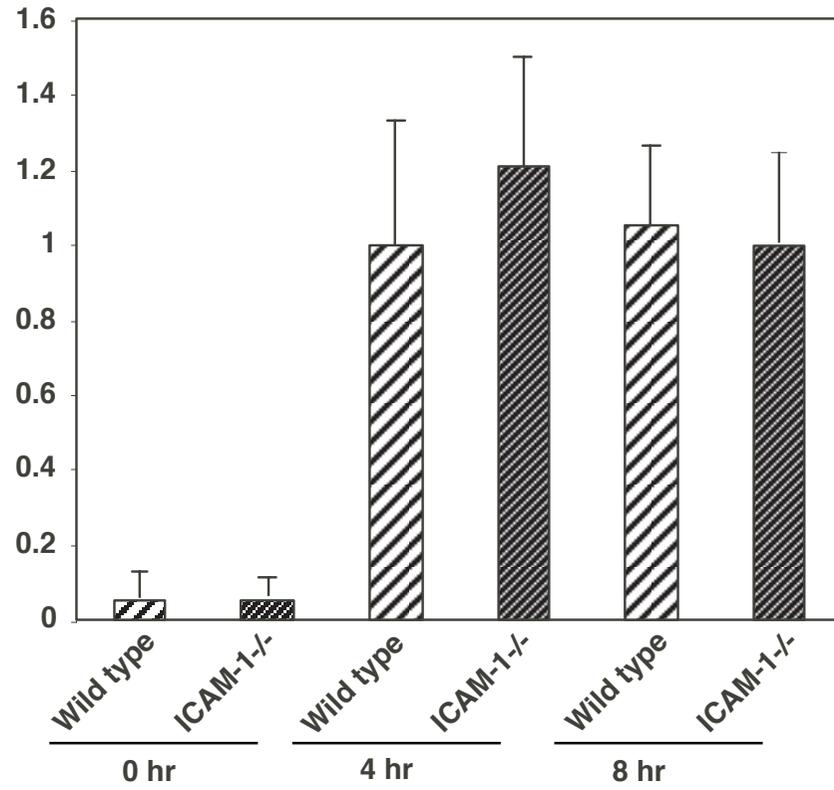


Figure 1

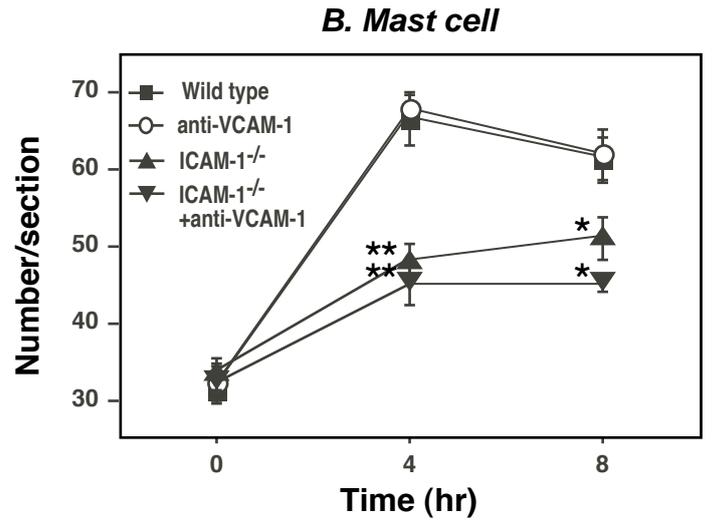
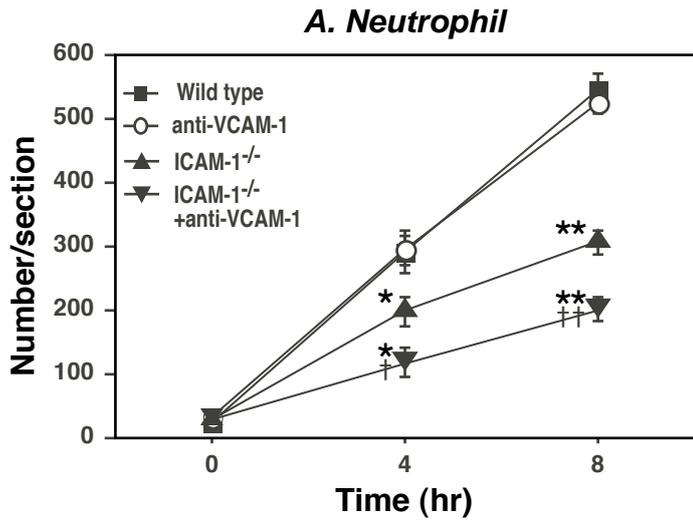


Figure 3

A. 4 hours

B. 8 hours

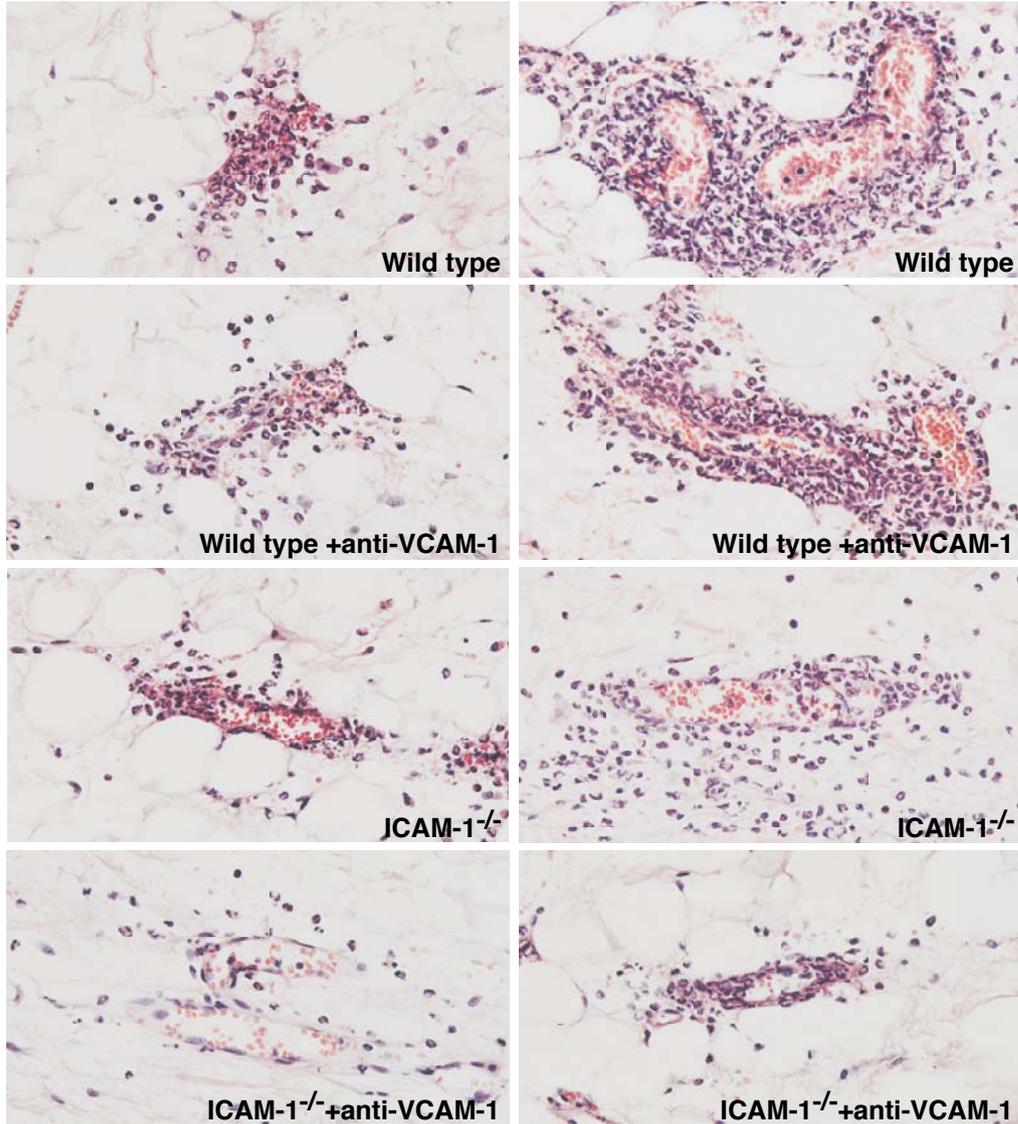


Figure 4

A. 4 hours

B. 8 hours

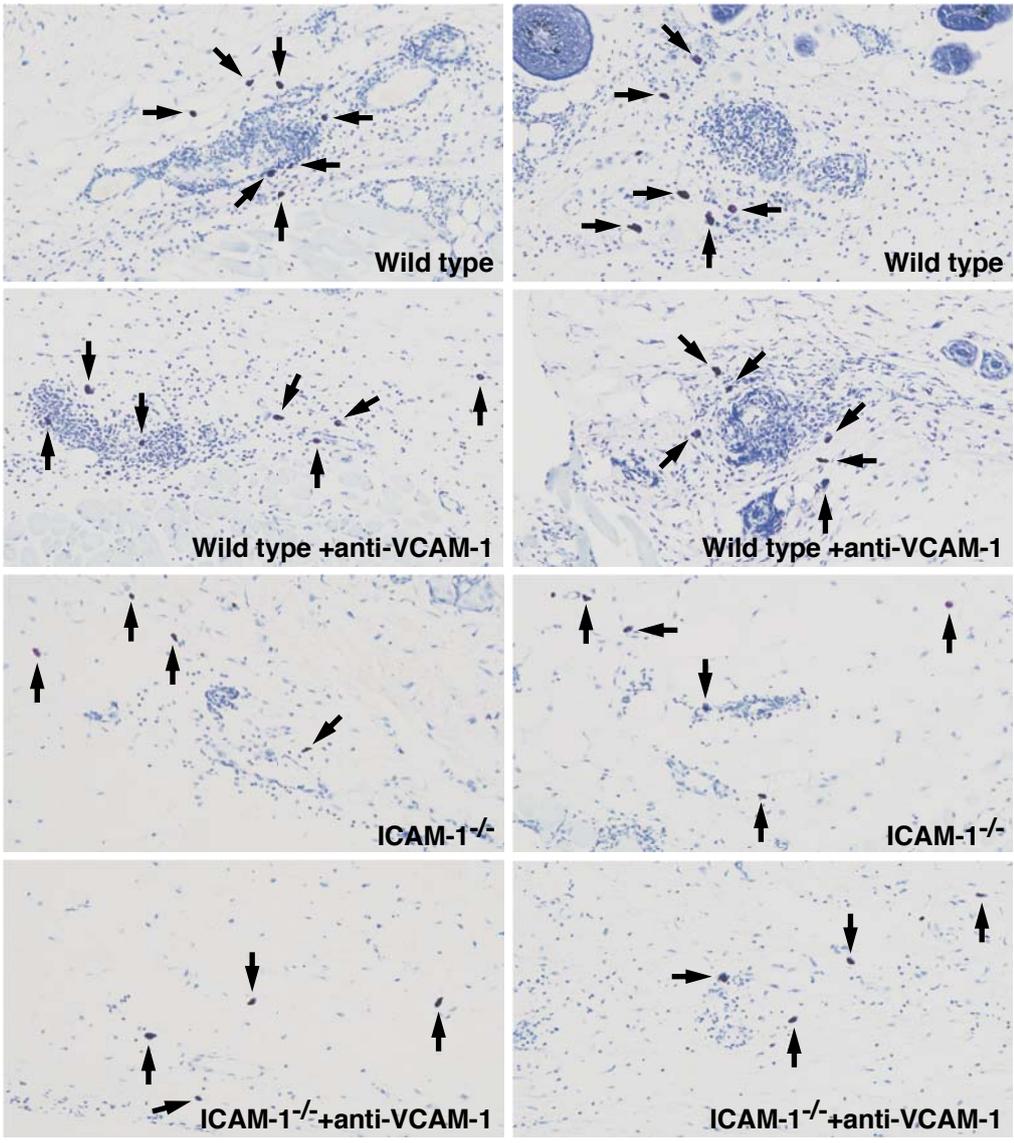


Figure 5

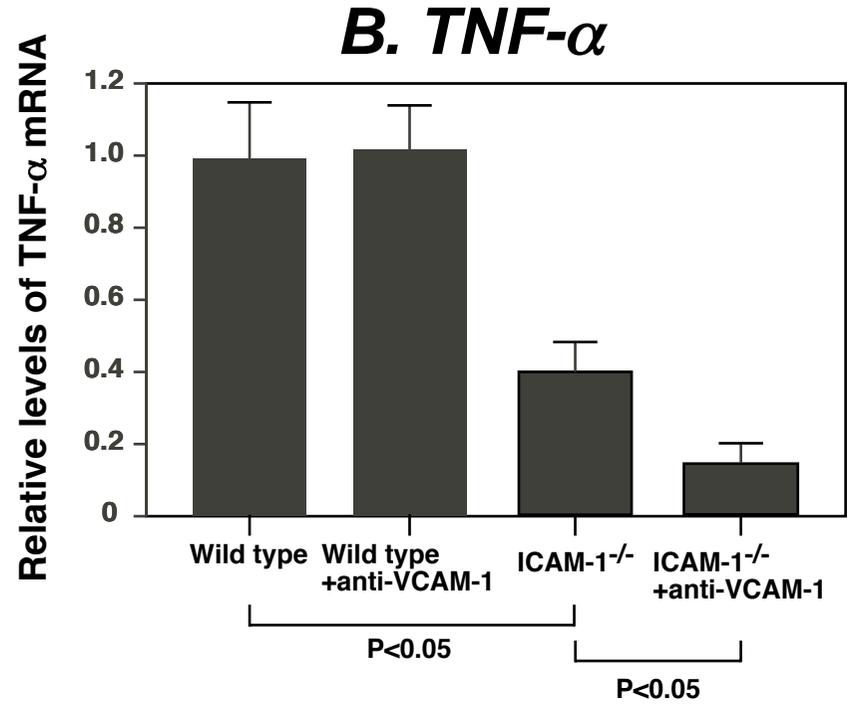
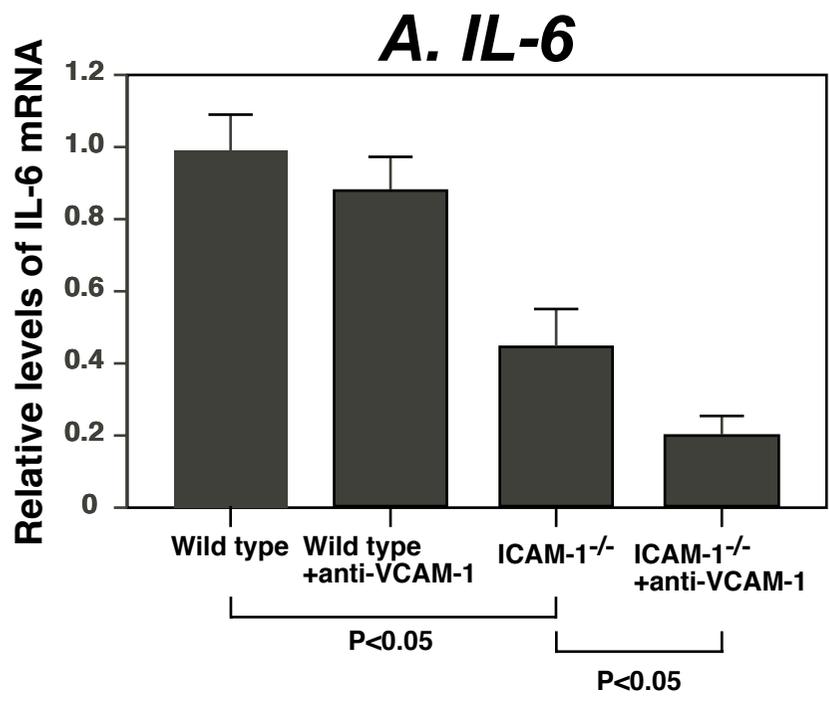


Figure 6