Delayed Wound Healing in the Absence of Intercellular Adhesion Molecule-1 or L-Selectin Expression

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Inflammatory cells play a crucial role in wound healing, but the role of adhesion molecules including L-selectin and intercellular adhesion molecule-1 (ICAM-1) is not known in this process. We examined skin wound repair of excisional wounds in mice lacking L-selectin, ICAM-1, or both. The loss of ICAM-1 inhibited wound healing, keratinocyte migration from the edges of the wound toward the center, and granulation tissue formation. By contrast, L-selectin deficiency alone did not affect any of these parameters. However, the loss of both L-selectin and ICAM-1 resulted in inhibition of keratinocyte migration and granulation tissue formation beyond those caused by loss of ICAM-1 alone. Treatment of platelet-derived growth factor to the wounds normalized delayed wound healing in ICAM-1^{-/-} mice, but not in L-selectin/ICAM-1^{-/-} mice. Therefore, although ICAM-1 contributes to wound repair to a greater extent than L-selectin, a role for L-selectin was revealed in the absence of ICAM-1. The impaired wound repair was associated with reduced infiltration of neutrophils and macrophages in ICAM-1^{-/-} and L-selectin/ICAM-1^{-/-} mice. These results demonstrate a distinct role of ICAM-1 and L-selectin in wound healing and that the delayed wound healing in the absence of these molecules is likely because of decreased leukocyte accumulation into the wound site. (*Am J Pathol* 2000, 157:237-247)

Leukocyte recruitment into inflammatory sites is achieved using distinct constitutive or inducible adhesion molecules.¹⁻³ L-selectin (CD62L) is constitutively expressed by most leukocytes whereas P-selectin (CD62P) and E-selectin (CD62E) are expressed by activated endothelial cells.⁴ These selectins primary mediate leukocyte capture and rolling on the endothelium.⁴ Intercellular adhe-

sion molecule-1 (ICAM-1, CD54) is constitutively expressed by endothelial cells and is rapidly up-regulated during inflammation, resulting in increased leukocyte-endothelial cell adhesion.⁵ Leukocytes express β_2 integrins, including lymphocyte function-associated antigen-1 (CD11a/CD18), which interact with ICAM-1. The ICAM-1/ β_2 integrin interactions predominantly mediate firm adhesion and transmigration of leukocytes at sites of inflammation.³

The generation of adhesion molecule-deficient mice has provided considerable insight into the molecular interactions that occur during inflammation *in vivo*. L-selectin-deficient (L-selectin^{-/-}) mice have decreased trauma-induced rolling of leukocytes in the exteriorized mesentery and decreased rolling in cremaster muscle venules after tumor necrosis factor- α treatment.⁶⁻⁸ L-selectin^{-/-} mice also demonstrate decreased leukocyte recruitment into an inflamed peritoneum at early and late time points, decreased delayed-type hypersensitivity responses, delayed rejection of allogeneic skin transplants, and resistance to lipopolysaccharide-induced septic shock.^{6,9-12} ICAM-1^{-/-} mice have significantly reduced numbers of infiltrating neutrophils during the early stages of peritonitis, reduced susceptibility to lipopolysaccharide-induced septic shock, and impaired delayed-type hypersensitivity reactions, although allogeneic skin graft rejection is normal.^{9,13,14} Recent studies in L-selectin/ICAM-1^{-/-} mice demonstrate a direct role for ICAM-1 in leukocyte rolling as the frequency of rolling leukocytes in L-selectin^{-/-} mice treated with tumor necrosis factor- α is decreased significantly by the additional loss of ICAM-1 expression.¹⁵ Furthermore, the loss of both L-selectin and ICAM-1 expression reduces leukocyte recruitment into sites of inflammation beyond what is observed with loss of either receptor alone in various experimental models of inflammation.¹⁶ Therefore, L-selectin and ICAM-1 mediate optimal leukocyte accumulation during inflammation through overlapping as well as synergistic functions.

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Healing of cutaneous wounds is a complex process that progresses through three general stages: 1) an inflammatory stage which consists of platelet aggregation and recruitment of inflammatory cells to the wound site; 2) a proliferative phase which involves the migration and proliferation of keratinocytes, fibroblasts, and endothelial cells, leading to re-epithelialization and granulation tissue formation; and 3) a long remodeling phase.¹⁷⁻¹⁹ Migration of inflammatory cells to the wound site is important in wound repair. Initially, neutrophils begin accumulating at the wound sites within minutes of injury.¹⁸ Infiltrating neutrophils form a first line of defense against local infections by clearing foreign particles and bacteria. Recent studies have shown that neutrophils are also a source of pro-inflammatory cytokines that probably serve as some of the earliest signals to activate fibroblasts and keratinocytes.¹⁹ Neutrophils are then extruded with the eschar or phagocytosed by macrophages. Macrophages enhance the debridement by phagocytosis of microorganisms and fragments of extracellular matrix.²⁰ In addition, macrophages are important producers for a battery of growth factors such as platelet-derived growth factor (PDGF), transforming growth factor- β , basic fibroblast growth factor (bFGF), heparin binding epidermal growth factor, and transforming growth factor- α .^{17,18} These factors stimulate the synthesis of extracellular matrix by local fibroblasts, generate new blood vessels, promote the granulation tissue formation, and enhance re-epithelialization that takes place by the migration of the keratinocytes from the edges of the wound toward the center.^{17,18}

The later stages of wound repair have been suggested to be strongly dependent on the initial inflammatory phase of the healing process. Depletion of macrophages by corticosteroids and anti-macrophage serum delays wound healing.²¹ Although depletion of neutrophils by anti-neutrophil antibody does not result in delayed wound repair,²² recent studies have shown that pro-inflammatory cytokines, which are predominantly expressed in neutrophils during the early phase of wound repair, are significantly reduced in healing-impaired corticosteroid-treated mice.¹⁹ In addition, there is increasing evidence that several adhesion molecules play a critical role in wound repair. Poor wound healing is observed in leukocyte adhesion deficiency type 1 patients who lack expression or function of β_2 integrins and thereby have impaired neutrophil migration into the sites of inflammation.^{23,24} Recent studies in P-selectin/E-selectin^{-/-} mice demonstrate a direct role of P-selectin and E-selectin in wound healing *in vivo*.²⁵ However, it is not known whether L-selectin or ICAM-1 contribute to the wound healing process by mediating the recruitment of leukocytes.

As a critical role for L-selectin and ICAM-1 in various inflammatory models has been demonstrated,^{15,16} we examined the *in vivo* function of L-selectin and ICAM-1 in wound healing. For this purpose, we analyzed cutaneous wound repair in mice lacking either L-selectin or ICAM-1, or both. The results demonstrate that ICAM-1 and L-selectin contribute to wound healing by mediating accumulation of leukocytes through synergistic functions.

Materials and Methods

Animals

L-selectin^{-/-} mice were produced as described.⁶ ICAM-1^{-/-} mice¹³ were obtained from The Jackson Laboratory (Bar Harbor, ME). These ICAM-1^{-/-} mice express residual amounts of ICAM-1 splice variants in the thymus and spleen but not in other organs including skin.²⁶ Mice lacking both L-selectin and ICAM-1 were generated by crossing F1 offspring from crosses of homozygous L-selectin^{-/-} mice with homozygous ICAM-1^{-/-} mice as described.¹⁵ All mice were healthy, fertile, and did not display any evidence of infection or disease. All mice were backcrossed between five to 10 generations onto the C57BL/6 background. Mice were 7 to 12 weeks old for all experiments and age-matched wild-type littermates or C57BL/6 mice (Jackson Laboratory) were used as controls. All mice were housed in a specific pathogen-free barrier facility and screened regularly for pathogens. All studies and procedures were approved by the Committee on Animal Experimentation of Kanazawa University School of Medicine.

Wounding and Macroscopic Examination

Mice were anesthetized with diethyl ether and their backs were shaved and wiped with 70% alcohol. Four full-thickness excisional wounds per mouse were made using a disposable sterile 6-mm punch biopsy (Maruho, Osaka, Japan), as described elsewhere.²⁵ After surgery, mice were caged individually. At 3 days and 7 days after wounding, mice were anesthetized, and areas of open wounds were measured by tracing the wound openings onto a transparency. Any signs suggestive for local infection were not detected in the wounded skin. For macroscopic analysis of wound closure, 14 mice were used in each group. After distorted wounds were excluded, one wound was randomly selected in each mouse for the analysis.

Histological Examination and Immunohistochemistry

After the mice were sacrificed, wounds were harvested with a 2-mm rim of unwounded skin tissue. The wounds were cut into halves laterally, fixed in 3.5% paraformaldehyde, and were then paraffin embedded. Six- μ m sections were stained with hematoxylin and eosin (H&E). All sections were derived from the center of the wounds. Neutrophils were counted in the entire section outside the blood vessels at 1 hour and 4 hours after wounding. Numbers of macrophages per field (0.07 mm²) were determined by counting in paraffin sections stained with antibody directed against macrophages (clone F4/80, American Type Culture Collection, Rockville, MD) as described below. The epithelial gap, which represents distance between the leading edge of migrating keratino-

cytes, was measured in H&E-stained sections of wounds. We identified the area that consists of newly formed capillaries and the collection of fibroblasts and macrophages as granulation tissue. Wound sections were visualized in the color monitor (PVM-14M4J, OLYNPUS, Tokyo, Japan) using the CCD camera (CS-900, OLYNPUS, Tokyo, Japan). Then, the area of granulation tissue was gated and measured by the video micrometer (VM-60, OLYNPUS, Tokyo, Japan). The number of mice used for each examination was as follows: 10 in each group for count of neutrophils and macrophages and 14 in each group for measurement of the epithelial gap and the granulation tissue area. After distorted wounds were excluded, one wound was randomly selected in each mouse for every microscopic analysis.

Tissue sections of skin biopsies were acetone-fixed and then incubated with 10% normal rabbit serum in phosphate-buffered saline (PBS) (10 minutes, 37°C) to block nonspecific staining. Sections were then incubated with rat monoclonal antibodies specific for macrophages (F4/80) and mouse ICAM-1 (Coulter, Inc., Miami, FL). Rat immunoglobulin G (Southern Biotechnology Associates Inc., Birmingham, AL) was used as a control for nonspecific staining. Sections were then incubated sequentially (20 minutes, 37°C) with a biotinylated rabbit anti-rat immunoglobulin G secondary antibody (Vectastain ABC method, Vector Laboratories, Burlingame, CA), then horseradish peroxidase-conjugated avidin-biotin complexes (Vectastain ABC method, Vector Laboratories). Sections were washed 3 times with PBS between incubations. Sections were developed with 3,3'-diaminobenzidine tetrahydrochloride and hydrogen peroxide, and then counterstained with methyl green. We identified the area surrounded by both sides of unwounded skin, fascia, regenerated epidermis, and eschar as the wound bed. The measurement of macrophages was performed by averaging the number of cells positive for the F4/80 staining in nine high power fields (magnification, $\times 400$) in the wound bed per section. Among the nine fields, six fields were selected from both edges of the wound bed, and the remaining three fields were chosen from the middle of the wound bed.

Application of Growth Factors

An optimal concentration of growth factors was applied to each wound in 20 μ l of aqueous buffer, and wounds were covered with an occlusive dressing (Tegaderm, 3M Canada, London, ON). Growth factors were applied to wounds immediately after wounding and 12 hours after wounding. Growth factors and their amounts used in this study were as follows: PDGF B-B isoform (AUSTRAL Biologicals, San Ramon, CA) 800 ng/20 μ l; transforming growth factor- β 3 (Novartis Pharmaceutical, Basel, Switzerland) 800 ng/20 μ l; and bFGF (Kaken Pharmaceutical, Tokyo, Japan) 1000 ng/20 μ l. These optimal amounts of growth factors were determined elsewhere.²⁷ Macroscopic area of the open wound was measured at 3 days

and 7 days after wounding. For the analysis, 14 mice were used in each group, and one wound was randomly selected after distorted wounds were excluded in each mouse.

Statistical Analysis

Analysis of variance was used to analyze the data and Mann-Whitney *U* test was used to determine the level of significance of differences in sample means.

Results

Area of Open Wound

The areas of open wounds were measured at 3 days and 7 days after wounding to assess macroscopic healing defects (Figure 1A). At 3 days after injury, open wound area was significantly larger in ICAM-1^{-/-} mice (24% increase, $P < 0.0005$) and L-selectin/ICAM-1^{-/-} mice (32%, $P < 0.0001$) than that in wild-type mice. At day 7, the difference was larger than that at day 3 as ICAM-1^{-/-} mice and L-selectin/ICAM-1^{-/-} mice exhibited 47% ($P < 0.001$) and 66% ($P < 0.0001$) larger open wound areas, respectively. By contrast, wound healing was not delayed in L-selectin^{-/-} mice relative to wild-type littermates at either day 3 or day 7. The additional loss of L-selectin in ICAM-1^{-/-} mice tended to delay wound healing relative to ICAM-1^{-/-} mice. Therefore, macroscopic wound healing was delayed in the absence of ICAM-1 whereas L-selectin loss did not affect macroscopic wound healing.

Epithelial Gap

Migration of keratinocytes under the eschar was assessed by microscopically measuring the epithelial gap that is the distance between the migrating edges of keratinocytes (Figure 1B). Keratinocyte migration was significantly inhibited in ICAM-1^{-/-} mice relative to wild-type mice at day 3 after wounding (by 27%, $P < 0.05$) and day 7 after wounding (44%, $P < 0.05$). Similarly, L-selectin/ICAM-1^{-/-} mice exhibited significant inhibition of keratinocyte migration at both day 3 (by 52%, $P < 0.0001$) and day 7 after wounding (63%, $P < 0.01$) when compared with wild-type mice. The loss of both L-selectin and ICAM-1 resulted in a significant inhibition of keratinocyte migration relative to ICAM-1 loss alone ($P < 0.05$) at 3 days after wounding, but this difference was no longer significant at 7 days after injury. The L-selectin deficiency alone did not significantly impair migration of keratinocytes at either day 3 or day 7. Thus, although keratinocyte migration was normal in L-selectin^{-/-} mice, keratinocyte migration in L-selectin/ICAM-1^{-/-} mice was significantly inhibited beyond the inhibition associated with ICAM-1 deficiency alone.

Granulation Tissue Formation

The area of granulation tissue was microscopically measured because granulation tissue formation is one of the

A. Area of open wound

B. Epithelial gap

C. Area of granulation tissue

Figure 1. Wound closure and granulation tissue formation in mutant and wild-type mice at 3 days and 7 days after wounding. Full-thickness cutaneous wounds were made using a 6-mm punch biopsy. The area of open wound was determined by tracing of the wound openings onto a transparency (A). The distance between the migrating edges of keratinocytes under the eschar (epithelial gap; B) and the area of granulation tissue (C) were measured in the tissue sections. All values represent the mean $\pm$ SEM. These results were obtained from 14 mice in each group.

Figure 2. Neutrophil recruitment in wounded skin from mutant and wild-type mice at 1 hour and 4 hours after injury. Numbers of neutrophils per section were determined by counting in H&E-stained sections under the microscope. All values represent the mean $\pm$ SEM. These results were obtained from 10 mice in each group.

most important components in wound repair (Figure 1C). At 3 days after wounding, granulation tissue formation was significantly reduced in ICAM-1^{-/-} (41%, $P < 0.0005$) and L-selectin/ICAM-1^{-/-} mice (52%, $P < 0.0001$) relative to wild-type controls. At 7 days after injury, granulation tissue formation was also inhibited in ICAM-1^{-/-} mice (23%, $P < 0.005$) and in L-selectin/ICAM-1^{-/-} mice (30%, $P < 0.005$) when compared with wild-type controls. Granulation tissue formation was significantly reduced in L-selectin/ICAM-1^{-/-} mice relative to ICAM-1^{-/-} mice at day 3 ($P < 0.05$), but not at day 7. L-selectin deficiency alone did not affect granulation tissue formation at either day 3 or day 7. Thus, although ICAM-1 loss alone impaired granulation tissue formation, the combined L-selectin/ICAM-1 loss resulted in a greater reduction in granulation tissue formation than the loss of ICAM-1 alone.

Infiltration of Neutrophils and Macrophages

Numbers of neutrophils that migrated outside the blood vessels were assessed in the wound tissues (Figures 2 and 3). At 1 hour and 4 hours after wounding, neutrophil numbers were significantly reduced in ICAM-1^{-/-} mice (53% and 45%, $P < 0.001$ and $P < 0.0005$, respectively) and L-selectin/ICAM-1^{-/-} mice (72% and 56%, $P < 0.0005$). At 1 hour after wounding, the additional loss of L-selectin in ICAM-1^{-/-} mice resulted in significantly reduced numbers of neutrophils relative to ICAM-1^{-/-} mice ($P < 0.01$), but the difference was no longer significant at 4 hours after injury. Neutrophil numbers in L-selectin^{-/-}

mice were 20% lower than those in wild-type mice at 4 hours after injury; however, the difference did not reach statistical significance ($P = 0.07$). Macrophage infiltration was assessed by immunohistochemistry using the F4/80 mAb (Figures 4 and 5). Macrophage numbers were significantly reduced in ICAM-1^{-/-} mice (~25%) and L-selectin/ICAM-1^{-/-} mice (~35%) when compared with wild-type mice at both 3 days and 7 days after injury ($P < 0.0005$). However, there was no difference in macrophage infiltration between ICAM-1^{-/-} mice and L-selectin/ICAM-1^{-/-} mice. Thus, the loss of ICAM-1 resulted in reduced infiltration of both neutrophils and macrophages, and additional loss of L-selectin in ICAM-1^{-/-} mice inhibited early neutrophil infiltration more than ICAM-1^{-/-} mice.

ICAM-1 Expression in Wound Healing

ICAM-1 has been found on the surface of keratinocytes and endothelial cells of inflamed skin and several pro-inflammatory cytokines up-regulate ICAM-1 expression on various types of cells.^{5,28} Therefore, ICAM-1 expression was assessed in the wounded skin immunohistochemically. In normal skins, ICAM-1 was detected exclusively on endothelial cells (Figure 6A). ICAM-1 expression was up-regulated on endothelial cells in granulation tissues at both 3 days and 7 days after wounding (Figure 6, B and C). At day 3, keratinocytes in the migrating edges did not express ICAM-1 (Figure 6B). By day 7, ICAM-1 expression was detected in keratinocytes, particularly basal keratinocytes, above granulation

A. 1 hour

B. 4 hours

Figure 3. Histological sections of wounded skin from ICAM-1^{-/-} mice, L-selectin/ICAM-1^{-/-} mice, and wild-type mice at 1 hour (A) and 4 hours (B) after injury. Neutrophils were detected in H&E-stained sections. Original magnification, ×600.

tissue, although its staining intensity was much weaker than that of endothelial cells (Figure 6C). By contrast, fibroblasts in granulation tissues did not express ICAM-1 at either 3 days or 7 days after wounding (Figure 6, B and

C). ICAM-1 expression was not detected in the intact or wounded skin from ICAM-1^{-/-} mice (data not shown). In addition, the loss of L-selectin expression did not affect ICAM-1 expression in the intact or wounded skin (data

Figure 4. Macrophage recruitment in wounded skin from mutant and wild-type mice at 3 days and 7 days after injury. Numbers of macrophages per field (0.07 mm²) were determined by counting in paraffin sections stained with antibody against macrophages (F4/80). All values represent the mean ± SEM. These results were obtained from 10 mice in each group.

A. Day 3

Wild-type

ICAM-1^{-/-}

L-selectin/ICAM-1^{-/-}

B. Day 7

Wild-type

ICAM-1^{-/-}

L-selectin/ICAM-1^{-/-}

Figure 5. Immunohistochemical sections of wounded skin from ICAM-1^{-/-} mice, L-selectin/ICAM-1^{-/-} mice, and wild-type mice at 3 days (A) and 7 days (B) after injury. Sections were stained with monoclonal antibodies specific for macrophages (F4/80). Original magnification, $\times 300$.

not shown). Thus, ICAM-1 was predominantly expressed on wound endothelial cells.

served in ICAM-1^{-/-} mice and L-selectin/ICAM-1^{-/-} mice.

Effect of Growth Factors on Delayed Wound Repair

As neutrophils and macrophages in the initial inflammatory phase are a source of cytokines and growth factors that promote wound repair, the effect of growth factors on the delayed wound healing observed in ICAM-1^{-/-} mice and L-selectin/ICAM-1^{-/-} mice was examined (Figure 7). Wound healing was assessed by macroscopic area of the open wound at 3 days and 7 days after wounding. Topical treatment of either bFGF or PDGF that were applied at the time of injury and 12 hours after injury normalized the delayed wound healing in ICAM-1^{-/-} mice by 3 days after injury. Wound repair was also recovered by application of bFGF in L-selectin/ICAM-1^{-/-} mice to the levels observed in wild-type littermates by 3 days after wounding. By contrast, PDGF application did not normalize the delayed wound repair in L-selectin/ICAM-1^{-/-} mice at both 3 days and 7 days after wounding. Impaired wound healing in ICAM-1^{-/-} mice and L-selectin/ICAM-1^{-/-} mice was not affected by application of transforming growth factor- $\beta 3$ at either 3 days or 7 days after injury. Thus, topical application of some growth factors could normalize the delayed wound healing ob-

Discussion

In the present study, macroscopic wound healing, keratinocyte migration, and granulation tissue formation were significantly inhibited in the absence of ICAM-1 expression (Figure 1). By contrast, the L-selectin deficiency alone did not affect any of these parameters in the wound healing process. However, the loss of both L-selectin and ICAM-1 led to inhibited keratinocyte migration and retarded granulation tissue formation beyond those caused by loss of ICAM-1 alone at an early time point after wounding. Therefore, the role of ICAM-1 in cutaneous wound repair is predominant relative to L-selectin. However, L-selectin may also contribute to wound repair through overlapping and synergistic functions with ICAM-1. This is consistent with the finding that L-selectin and ICAM-1 function synergistically to mediate optimal rolling as well as to recruit leukocytes into sites of inflammation.^{15,16} The impaired wound repair was accompanied by significantly reduced infiltration of neutrophils and macrophages in ICAM-1^{-/-} mice and L-selectin/ICAM-1^{-/-} mice (Figures 2, 3, 4, and 5). As the significant difference between ICAM-1^{-/-} mice and L-selectin/

A. Normal skin

B. Day 3

C. Day 7

Figure 6. ICAM-1 expression in wild-type mice during wound healing. ICAM-1 expression in normal skin (A) and in the wounded skin at 3 days (B) and 7 days (C) after injury was assessed by immunohistochemistry using anti-ICAM-1 antibodies. **Arrows** indicate ICAM-1 expression on endothelial cells, and **arrowheads** indicate ICAM-1 expression in keratinocytes. Original magnification, $\times 300$.

A. ICAM-1^{-/-}

B. L-selectin/ICAM-1^{-/-}

Figure 7. Effect of growth factors on delayed wound repair in ICAM-1^{-/-} mice (A) and L-selectin/ICAM-1^{-/-} mice (B). Growth factors were applied to each wound in 20 μ l of aqueous buffer immediately after wounding and 12 hours after wounding. The area of open wound at 3 days and 7 days after wounding was determined by tracing of the wound openings onto a transparency. All values represent the mean $\pm$ SEM. These results were obtained from 14 mice in each group.

ICAM-1^{-/-} mice was observed only for infiltration of neutrophils at 1 hour after wounding, the reduced early neutrophil infiltration might result in the delayed keratinocyte migration and retarded granulation tissue formation in L-selectin/ICAM-1^{-/-} mice relative to ICAM-1^{-/-} mice. Therefore, the results of this study demonstrate a distinct role of ICAM-1 and L-selectin in a process of wound healing and that the delayed wound healing in the absence of these molecules is likely because of decreased leukocyte accumulation into the wound site.

Wound healing is normal in both P-selectin^{-/-} mice and E-selectin^{-/-} mice, although neutrophil infiltration is reduced in P-selectin^{-/-} mice at 1 hour and 4 hours after injury.²⁵ Similarly, this study showed that wound repair was not impaired in L-selectin^{-/-} mice, although neutrophil recruitment at 4 hours after injury was slightly inhibited in these mice (Figures 1, 2, and 3). Therefore, the loss of a single selectin member is not sufficient to cause impaired wound healing. Although L-, P-, and E-selectin have distinct roles, the selectins support optimal leukocyte rolling through overlapping functions.² In the absence of L-selectin or E-selectin, P-selectin primarily mediates rolling whereas L-selectin primarily mediates rolling in P-selectin^{-/-} mice.^{8,29-31} The loss of both P-selectin and E-selectin leads to delayed wound repair and inhibited keratinocyte migration at 3 days, but not at 7 days after wounding.²⁵ Significantly decreased numbers of infiltrating neutrophils and macrophages in P-selectin/E-selectin^{-/-} mice may contribute to the delayed wound repair. Therefore, reduced leukocyte rolling by combined loss of the selectins may cause the defect in the wound healing process.

The loss of ICAM-1 expression resulted in dramatic inhibition of wound healing at both 3 days and 7 days after wounding (Figure 1). Because wound repair is delayed in P-selectin/E-selectin^{-/-} mice at 3 days, but not 7 days after injury,²⁵ the loss of ICAM-1 resulted in greater effect on wound repair than the combined loss of P-selectin and E-selectin. This suggests that ICAM-1-mediated firm adhesion and transmigration of leukocytes contribute to wound repair more than selectin-mediated rolling. In normal skin, ICAM-1 was expressed exclusively on endothelial cells (Figure 6). However, ICAM-1 expression on various types of cells, including keratinocytes and fibroblasts, is induced by stimulation with several pro-inflammatory cytokines *in vitro*.^{5,28} Furthermore, the ICAM-1 expression on fibroblasts may mediate migration of neutrophils and macrophages through fibroblast layers.^{32,33} Therefore, the loss of ICAM-1 expression on fibroblasts and keratinocytes might contribute to the delayed wound healing observed in ICAM-1^{-/-} mice. ICAM-1 expression on endothelial cells was up-regulated in granulation tissues of the wounded skin (Figure 6). However, fibroblasts in granulation tissues did not express ICAM-1 and basal keratinocytes of the epidermis above granulation tissues were weakly positive for ICAM-1. This suggests that the delayed wound healing in the absence of ICAM-1 is attributed mainly to impaired interaction between leukocytes and endothelial cells by loss of ICAM-1 expression on endothelial cells. Taken

together, these findings demonstrate a critical role of ICAM-1 in wound healing.

Treatment of bFGF normalized the delayed wound repair in both ICAM-1^{-/-} mice and L-selectin/ICAM-1^{-/-} mice (Figure 7). bFGF acts mainly as a potent angiogenic factor during wound healing because wound angiogenesis is almost completely blocked when this growth factor is experimentally depleted with monospecific antibodies raised against bFGF.³⁴ Furthermore, bFGF promotes angiogenesis during the early stage of wound healing.³⁵ Therefore, angiogenesis enhanced by exogenous bFGF application may overcome the reduced infiltration of neutrophils and macrophages by the loss of ICAM alone or both ICAM-1 and L-selectin, leading to the normalization of wound repair. bFGF is released at the wound site by macrophages and damaged endothelial cells.^{17,18} The decreased release of bFGF by reduced numbers of wound macrophages might account for the impaired wound repair in both ICAM-1^{-/-} mice and L-selectin/ICAM-1^{-/-} mice. PDGF accelerates deposition of provisional wound matrix and collagen synthesis by fibroblasts.³⁶ Early in repair, PDGF augments the acute inflammatory response, specifically recruiting and activating wound macrophages.³⁷ PDGF is also a chemoattractant for wound fibroblasts.³⁷ These various functions of PDGF may cooperatively normalize the delayed wound healing in ICAM-1^{-/-} mice. By contrast, PDGF activities were not sufficient for normalization of impaired wound repair in L-selectin/ICAM-1^{-/-} mice. This reinforces an important role of L-selectin in wound healing.

Understanding regulation of cutaneous wound repair at the molecular level is important because there are many disorders based on abnormal wound repair, including stasis ulcer, diabetic ulcer, keloids, and hypertrophic scars.¹⁷ Understanding the contributions of L-selectin and ICAM-1 to the wound repair process could provide new clues to regulating wound healing.

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