American Journal of Pathology, Vol. 150, No. 5, May 1997

Role of Endothelial Selectins in Wound Repair

Meera Subramaniam,* Simin Saffaripour,* Livingston Van De Water,† Paul S. Frenette,* Tanya N. Mayadas,‡ Richard O. Hynes,§ and Denisa D. Wagner*‡

From the Center for Blood Research, the Shriners Burns Institute,† and Departments of Pathology‡ and Surgery,† Harvard Medical School, Boston, and the Howard Hughes Medical Institute, Center for Cancer Research, Department of Biology,§ Massachusetts Institute of Technology, Cambridge, Massachusetts*

P- and E-selectins are adhesion molecules expressed on activated endothelium and platelets at sites of vascular injury and inflammation. The selectins are important for leukocyte recruitment. Because little is known about the role of selectins in wound healing, we studied cutaneous wound repair of full-thickness excisional skin wounds in mice lacking P-selectin, E-selectin, or both of these selectins. The absence of either selectin alone had no notable effect on healing, and the only deficit observed was a delay in early neutrophil extravasation in the P-selectin-deficient mice. Mice deficient in both P- and E-selectins had markedly reduced recruitment of inflammatory cells and impaired closure of the wounds. Wound sections, studied up to 3 days after wounding, showed significant impairment of neutrophil influx. Macrophage numbers were also reduced in the double mutants at 3 and 7 days after wounding as compared with wild-type mice. Additionally, a wider epithelial gap in the wounds of the P- and E-selectin-double-deficient mice 3 days after wounding indicated delayed keratinocyte migration. These results demonstrate an important combined role for P- and E-selectins in processes leading to wound healing. (Am J Pathol 1997, 150:1701–1709)

P- and E-selectins are adhesion molecules of the selectin family expressed by stimulated endothelium and activated platelets. P-selectin is stored in α-granules of platelets and in Weibel-Palade bodies of endothelial cells.[1–4] P-selectin is rapidly expressed on the endothelial cell surface upon degranulation, and its synthesis can be further increased by cytokines.[5] E-selectin is expressed only after a delay, as it is up-regulated by cytokines and requires *de novo* synthesis.[6] Leukocytes roll on these endothelial selectins, subsequently forming firm adhesions through integrins and migrating across the endothelium to reach the injured or inflamed tissues. By promoting the first step in emigration of inflammatory cells, the selectins play important roles in inflammatory and immune responses.[7–10] P-selectin-deficient mice have shown significant impairment of neutrophil, macrophage, and lymphocyte influx in several acute and chronic inflammatory models[7,11–13]; this impairment is further augmented in mice that are deficient in both P-selectin and E-selectin.[14,15] Although adhesion of leukocytes to E-selectin has been demonstrated *in vitro*,[16] E-selectin-deficient mice have not shown any major phenotype thus far.[17]

Inflammatory cells play a crucial role in wound repair, yet it is not known whether selectins contribute to the wound-healing process by mediating the recruitment of these cells. In the initial inflammatory phase of wound healing, neutrophils and macrophages are recruited. Neutrophils provide defense against invading microorganisms, release oxygen radicals, produce degradative enzymes, and secrete cytokines. Many neutrophils are eventually present in the clot covering the wound and slough off with the eschar as healing progresses.[18,19] Macrophages are critical for proteolysis and removal of debris as well as reconstructive processes.[19–21] They phagocytose organisms, express specific fibronectin variants,[22,23] release cytokines and growth factors including angiogenic factors, and promote the formation of granulation tissue that replaces the provisional matrix.[19–21] Re-epithelialization of the wound takes place by the migration of the keratinocytes from the edges of the wound toward the center

Supported by National Institutes of Health grants HL53756 (D. D. Wagner) and HL41484 (R. O. Hynes). R. O. Hynes is an investigator of the Howard Hughes Medical Institute. P. S. Frenette is a fellow of the Medical Research Council of Canada.

Accepted for publication February 6, 1997.

Address reprint requests to Dr. Denisa D. Wagner, Center for Blood Research, Harvard Medical School, 800 Huntington Avenue, Boston, MA 02115.

and overlaps with the inflammatory phase.[21,24,25] Subsequently, granulation tissue rich in new blood vessels develops and transforms into a scar.[19,21]

Two lines of evidence suggest that leukocytes exert important functions in wound healing. First, depletion of macrophages by corticosteroids and anti-macrophage serum delays wound healing,[20] although no obvious defects were seen in animals made neutropenic with antisera.[26] Second, delayed wound healing was observed in leukocyte adhesion deficiency type I patients who lack all $\beta 2$ integrins and who have defects in several neutrophil functions including neutrophil-endothelial interactions. These defects result in a markedly impaired recruitment of leukocytes, especially neutrophils, into sites of infections in these patients.[27]

As it is well established that selectins expressed on blood vessels are important for recruitment of leukocytes, in the present study we examined the function of the endothelial selectins in wound healing. The approach taken was to analyze cutaneous wound repair in mice lacking P-selectin or E-selectin and in mice deficient in both of these selectins.

Materials and Methods

Mice

Two- to four-month-old 129Sv/C57B1/6 age-matched, wild-type, P-, E-, or P/E- (double-deficient) selectin-deficient female mice generated by gene targeting[11,14] were used in this study. All experimental procedures were approved by the Animal Care and Use Committees of the New England Medical Center and the Center for Blood Research, Boston, MA.

Wounding and Tissue Preparation

Mice were anesthetized with tribromoethanol (0.15 ml/10 g body weight) or methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL), and hair was removed from the back with electric clippers. The skin was cleaned with 70% alcohol, and full-thickness wounds were made by picking up a fold of skin, placing it over dental wax (Polysciences, Warrington, PA), and using a disposable sterile 4-mm punch biopsy (Baker Cummins Dermatological, Lakewood, NJ) to punch through the two layers of skin on one flank. In this manner, two wounds were made at the same time on the left side. Later, two wounds were made simultaneously on the right side. For some experiments, wounds were made in mice treated orally with trimethoprim (24 mg/dl) and sulfamethoxazole (120 mg/dl)[14] starting 3 days before wounding and continuing for 7 days. The mice were housed in individual cages. At various intervals after wounding, mice were anesthetized, and a transparency was placed over the wound so the wound could be traced. The tracings of the wound openings were photocopied on paper and were cut out and weighed. The weight of the cut out copy was converted to area by weighing a piece of paper of a known area. The mice were sacrificed, and wounds were harvested with 1 to 2 mm of normal skin tissue around them. The wounds were cut in half, fixed in 4% paraformaldehyde (Baxter Diagnostics, Deerfield, IL), and embedded in paraffin, and 7-μm sections were stained with hematoxylin and eosin (H&E). Extravascular neutrophils were counted in the entire section outside the blood vessels using a light microscope (Olympus BX40F at $\times$50 magnification). The neutrophil infiltrate in the clot/eschar above the wound was very dense; hence, neutrophils could not be counted. Instead the neutrophils were scored based on their density and the area of the clot/eschar they occupied. In the scoring scheme, 0 indicated absence of a neutrophil band in the clot/eschar and 4 indicated that almost the entire clot/eschar was occupied by neutrophils. The epithelial gap (distance between the leading edge of migrating keratinocytes) was measured in H&E-stained paraffin sections of wounds using a linear grid at $\times$10 magnification. The area of the granulation tissue in H&E-stained tissues was visualized by a light microscope through a Hitachi CCD camera and was quantitated by an image analysis system (Leica Q-500MC). The paraffin sections were stained for macrophages with F4/80 antibody (American Type Culture Collection, Rockville, MD) modified from the method described by Hume et al[28] using the biotin-streptavidin system (Zymed Laboratories, San Francisco, CA) and Vecta stain ABC kit (Vector Laboratories, Burlingame, CA).

All sections were examined independently by two investigators without knowledge of the genotype.

Myeloperoxidase Assay

Excised wound tissue was washed in phosphate-buffered saline and homogenized in 1 ml of 50 mmol/L potassium phosphate buffer, 0.5% hexadecyl trimethyl ammonium (Sigma Chemical Co., St. Louis, MO), 5 mmol/L EDTA, pH 5.5, at 4°C using a Polytron homogenizer (four bursts of 15 seconds at 10,000 speed). The samples were sonicated for 20 seconds, freeze-thawed three times, and centrifuged at 47,807 $\times$ *g* at 4°C. Supernatants were collected and assayed for myeloperoxidase activity by

adding 0.30 ml of supernatant to 0.970 ml of 50 mmol/L potassium phosphate buffer, pH 5.5, containing 0.2% *O*-dianosine dihydrochloride (Sigma) and 0.0001% hydrogen peroxide and measuring the change in absorbance at 460 nm over 4 minutes. The assay used was a modification of the method described by Trush et al.[29]

Statistical Analysis

The *P* values were obtained using Student's *t*-test for all statistical analysis except for scoring of neutrophils in clot/eschar, which was analyzed using the Mann-Whitney *U* test.

Results

Neutrophil Recruitment

Neutrophil numbers in the tissue were analyzed 1 hour, 4 hours, 1 day, and 3 days after wounding in the selectin-deficient and corresponding wild-type mice. Neutrophils that had migrated outside the blood vessels into tissues 1 and 4 hours after wounding were counted microscopically in H&E-stained sections. In the P-selectin-deficient mice, significantly less neutrophil infiltration was present in the tissues in 1-hour wounds compared with wild-type mice (Figure 1A and Figure 2, A and B); however, by 4 hours, many neutrophils emigrated out of the blood vessels into the wound sections in both the mutant and wild-type mice, and the difference between genotypes was no longer significant (Figure 1A and Figure 2, D and E). E-selectin-deficient mice showed no decrease in neutrophil infiltration at either time point (Figure 1B). In contrast, mice deficient for both P-selectin and E-selectin had markedly impaired neutrophil influx at 1 hour as well as 4 hours (Figure 1C and Figure 2, C and F). By day 1, the large number of neutrophils in the tissues precluded microscopic quantitation. Therefore, myeloperoxidase activity was assayed from the wound sections 1 or 3 days after injury. There was a small increase in myeloperoxidase activity in P-selectin-deficient mice compared with wild-type mice at day 1 (Figure 1D). Again, no difference in the neutrophil influx in the E-selectin-deficient mice was observed (Figure 1E). In contrast, a significant reduction in neutrophil recruitment persisted in mice deficient for both P-selectin and E-selectin in the 1- and 3-day wounds, although the difference between the mutant mice and the wild-type mice narrowed by day 3 (Figure 1F).

We evaluated histological sections 1 day after wounding from both wild-type and P/E-selectin-double-deficient mice. Sections obtained from wild-type mice exhibited a dense infiltrate of inflammatory cells, mainly neutrophils, subjacent to the clot/eschar. In the double-deficient mice, such infiltrates of neutrophils could not be seen in most of the sections (Figure 3, A and B). In a few instances, a small band of neutrophils was observed at the wound edge. By day 3, however, dense neutrophilic infiltrates could also be seen in the P/E-mutant mice, although they were still significantly less prominent than those of wild-type mice (Figure 3, C and D). The density and area occupied by the neutrophils in the clot/eschar visually scored on a scale of 0 to 4 was significantly higher in wild-type mice ($P < 0.0003$; median P/E +/+ 4, P/E −/− 1.75), and the pattern of distribution in the clot/eschar was also different from the P/E-

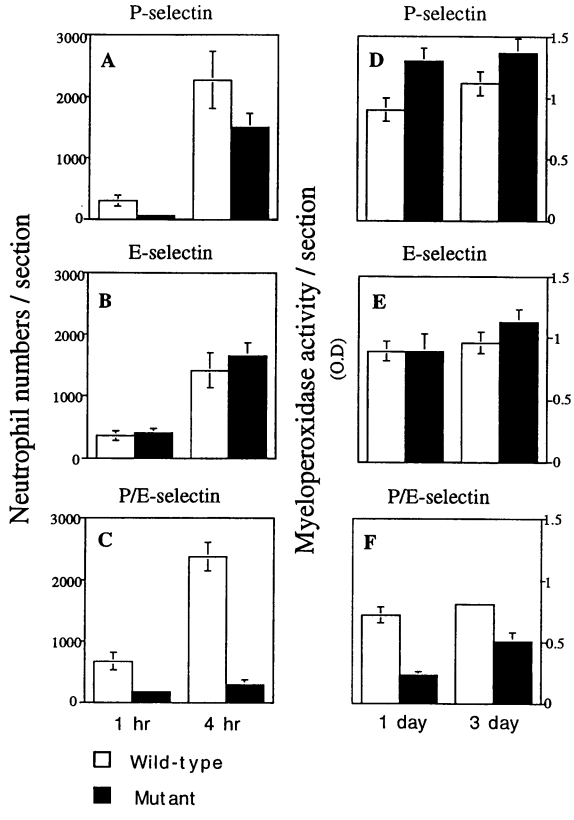

Figure 1. *Neutrophil recruitment in skin excisional wounds.* A *to* C: *Numbers of neutrophils per section were determined by counting in H&E-stained sections under the microscope.* D *and* F: *Neutrophil infiltration was established by measuring myeloperoxidase activity in the sections.* A: *Neutrophil numbers were significantly reduced 1 hour after wounding in the P-selectin-deficient mice compared with wild-type mice ($n = 4$ to 5; $P < 0.03$); however, by 4 hours, the neutrophil numbers in the P-selectin-deficient mice increased ($n = 4$; $P = 0.19$).* D: *By day 1, slightly more myeloperoxidase activity was observed in the mutant mice ($n = 11$ to 14; $P < 0.01$).* B *and* E: *No differences were observed in the E-selectin-deficient mice as compared with wild-type mice.* C *and* F: *Significantly reduced neutrophil recruitment was observed in the P/E-selectin-double-deficient mice compared with wild-type mice at 1 and 4 hours ($n = 8$ to 10; $P < 0.001$), as well as 1 and 3 days ($n = 6$; $P < 0.004$) after wounding. Values are reported as mean ± SEM and n represents the number of wound sections.*

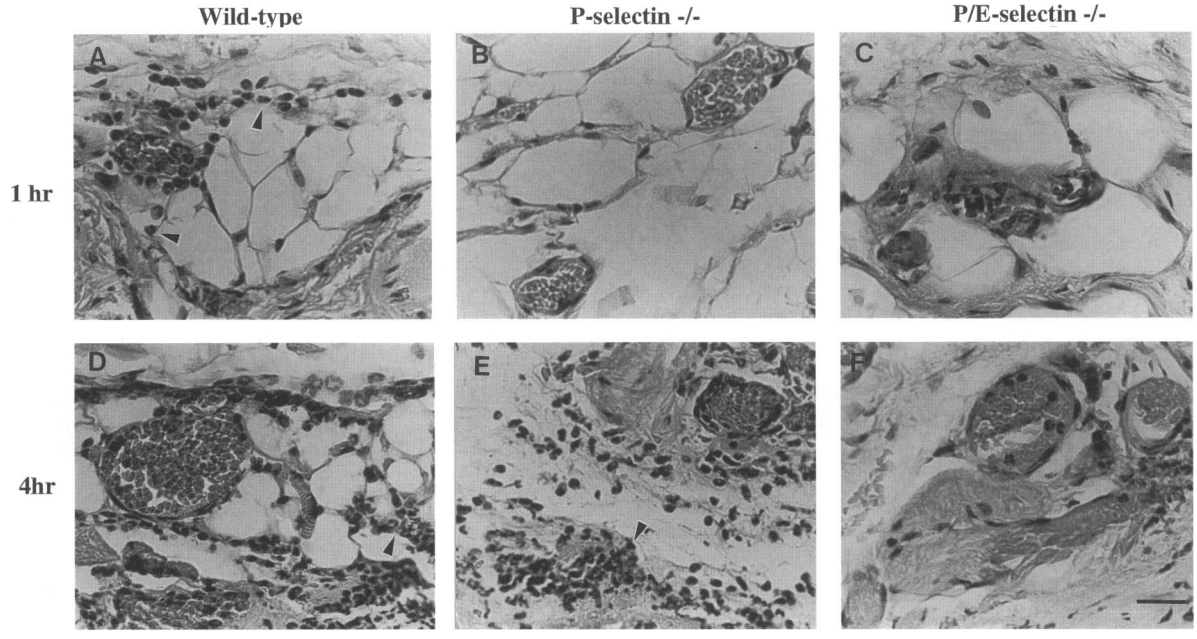

Figure 2. *Histology sections of the wound area after injury. In H&E-stained sections of the wounds, neutrophils* (arrow) *were observed extravasating from the blood vessels in the wild-type mice 1 hour after wounding* (A), *whereas very few were observed in the P-selectin-deficient mice* (B) *and the P/E-selectin-double-deficient mice* (C). *By 4 hours, neutrophil influx was observed in the P-selectin-deficient mice* (E); *however, in the P/E-selectin-double-deficient mice* (F), *reduced neutrophil recruitment compared with wild-type mice persisted* (D). *Bar, 20 μm.*

selectin-double-deficient mice. The neutrophils in the wild-type mice were observed spreading into the clot/eschar (Figure 3C), whereas in the P/E-mutant mice they were present in a thin band below the eschar (Figure 3D). There was no difference in neutrophil infiltration in the mice deficient for either molecule alone compared with wild-type mice.

Macrophage Recruitment

As macrophages are known to play an important role in wound healing, they were quantitated microscopically after macrophage-specific immunohistochemical staining in the day 3 and day 7 wound sections. Macrophage numbers in the wound tissues of either the P-selectin or E-selectin-deficient mice were similar to those in wild-type animals. However, in mice deficient for both P- and E-selectin, there was a threefold reduction of macrophage infiltration in the wounds at 3 days as well as 7 days after injury compared with wild-type mice (Table 1).

Granulation Tissue

We measured the area occupied by the granulation tissue in sections using an image analyzer. There was no significant difference in the granulation area in day 3 and day 7 wounds in any of the mutant mice compared with their wild-type counterparts (Table 2).

Macroscopic Examination of the Wounds

The wounds were also examined grossly up to 7 days after wounding to assess healing defects. There was no defect in healing on macroscopic examination or in wound area determined from tracings of the wounds in the P-selectin- or E-selectin-deficient mice. However, wound healing appeared to be impaired in the P/E-selectin-double-deficient mice at 3 days as the wound size was larger (Figure 4A).

Keratinocyte Migration

On examination of the tissue sections, we observed that keratinocytes were migrating under the eschar below the dense infiltrate of neutrophils. To evaluate the narrowing of the epithelial gap, the distance between the migrating edges of keratinocytes was measured microscopically at 3 and 7 days after wounding. There was no significant difference in the epithelial gap in wounds of either the P-selectin-deficient or the E-selectin-deficient mice compared with wild-type mice (Figure 4B). In mice that were deficient for both P- and E-selectins, the gap was significantly wider compared with the wild-type mice at 3 days, indicating a defect in migration of the epithelial cells in the mutant mice (Figure 4B). However, by 7 days, the epithelial cells in P/E-selectin-double-deficient mice had advanced similarly to

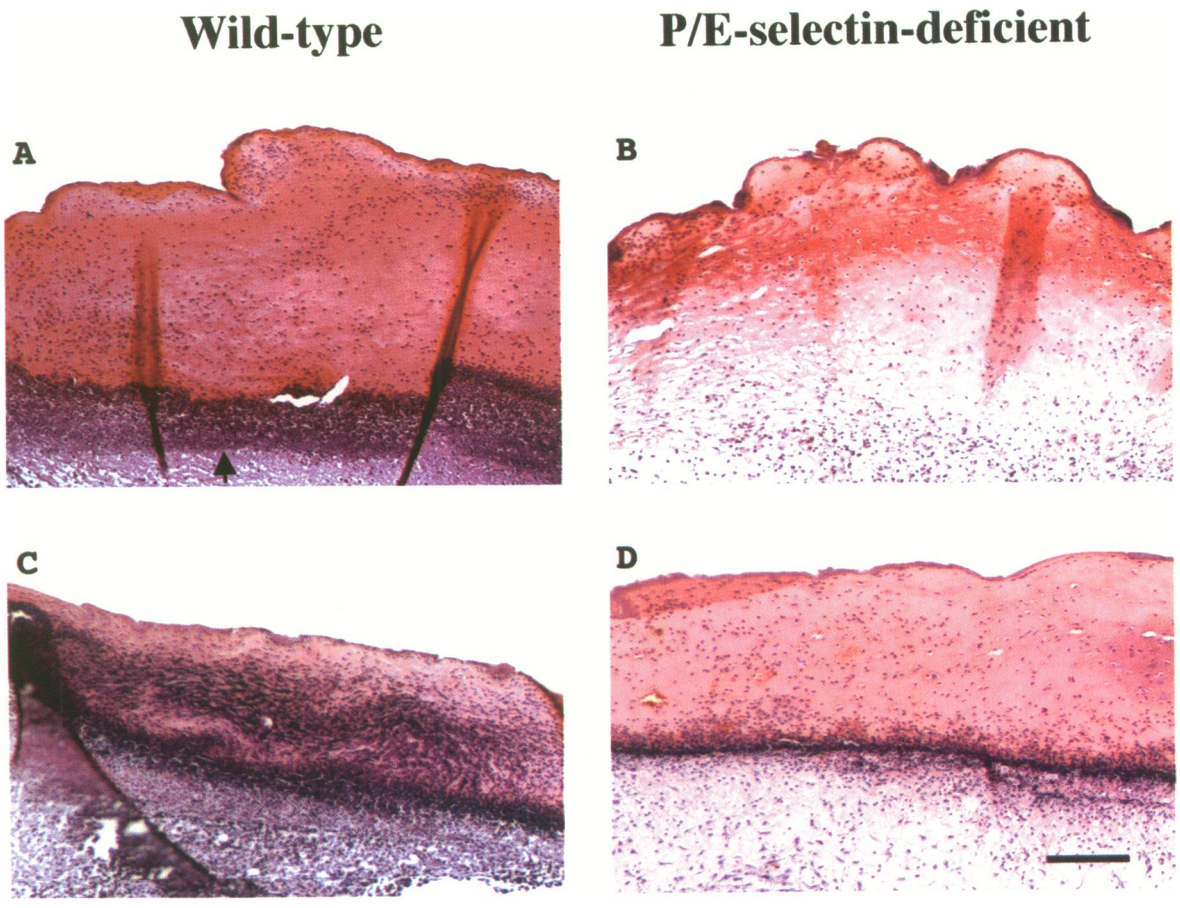

Figure 3. *Histology sections of 1- and 3-day wounds.* A: *In H&E-stained sections, dense neutrophil infiltrate* (arrow) *was seen in the clot/eschar on top of the wound in wild-type mice 1 day after wounds were made.* B: *The P/E-selectin-double-deficient mice were missing the neutrophil infiltrates in 1-day wounds.* C: *At 3 days after wounding in the wild-type mice, the neutrophils were seen spreading in the clot/eschar.* D: *A less prominent band of neutrophils was now observed in the P/E-double-deficient mice. Bar, 100 μm.*

those in the wild-type mice, and the epithelial gaps were the same size. The P/E-selectin-double-deficient mice have previously been shown to be susceptible to skin infections.[14,15] To confirm that the delay in epithelial migration was not due to infections, wounds were made in mice prophylactically treated with antibiotic regimens known to prevent spontaneous skin infections in these mice. The delay in keratinocyte migration at 3 days persisted in the antibiotic-treated double-deficient mice (P/E +/+ 1.65 mm, n = 5; P/E −/− 2.45 mm, n = 11; $P < 0.01$).

Discussion

Previously, many laboratories including our own have demonstrated an important role for endothelial

Table 1. *Macrophage Recruitment in Skin Wounds*

	P-Selectin			E-selectin			P/E-selectin		
	+/+	−/−	*P*	+/+	−/−	*P*	+/+	−/−	*P*
3 day	30 ± 12 (n = 4)	28 ± 6 (n = 6)	0.8	33 ± 4 (n = 7)	25 ± 5 (n = 6)	0.2	32 ± 4* (n = 14)	12 ± 2* (n = 11)	0.0004
7 day	27 ± 9 (n = 6)	34 ± 6 (n = 6)	0.4	27 ± 5 (n = 8)	30 ± 5 (n = 6)	0.7	18 ± 3† (n = 13)	5 ± 1† (n = 12)	0.0002

Macrophages were counted in paraffin sections stained with F4/80, an antibody directed to macrophages. Numbers are reported per field (0.15 mm^2) as mean ± SEM. n, number of wound sections. Significant reduction in recruitment of macrophages to wound sections 3 and 7 days after wounding was observed in the P/E-selectin-double-deficient mice.

*$P < 0.0004$.

†$P < 0.0002$.

Table 2. *Granulation Tissue*

	P-Selectin			E-selectin			P/E-selectin		
	+/+	−/−	*P*	+/+	−/−	*P*	+/+	−/−	*P*
3 day (mm^2)	0.63 ± 0.08 (n = 17)	0.77 ± 0.07 (n = 16)	0.2	0.91 ± 0.14 (n = 8)	0.85 ± 0.1 (n = 15)	0.7	1.27 ± 0.34 (n = 7)	1.24 ± 0.15 (n = 13)	0.9
7 day (mm^2)	1.44 ± 0.15 (n = 17)	1.36 ± 0.19 (n = 12)	0.7	1.13 ± 0.07 (n = 12)	1.26 ± 0.09 (n = 16)	0.3	2.28 ± 0.21 (n = 13)	1.92 ± 0.15 (n = 15)	0.1

No significant difference was observed in the area of granulation tissue measured by an image analysis system in any of the genotypes compared with wild-type mice 3 and 7 days after wounding. Values are expressed as mean ± SEM.

selectins in leukocyte homeostasis and emigration of leukocytes to tissues under inflammatory conditions.[7,9,11,14,15] It is apparent from several studies that adhesion molecules affect wound repair. Leukocyte recruitment as well as wound healing is defective in patients who lack β2 integrins.[27] Integrins are also involved in the migration and adhesion of keratinocytes, where the members of the integrin family are increased and redistributed to the advancing epidermal edge.[30] Up-regulation of E-selectin has been observed in the capillaries in chronic ulcers of venous insufficiency although its significance is not clear.[31] However, to our knowledge, no studies have evaluated the role of selectins in wound healing.

We studied the role of P-selectin and E-selectin in wound healing by using mice deficient in each of these selectins and in mice deficient in both selectins. In our study, the P/E-selectin-double-deficient mice showed reduced recruitment of neutrophils and macrophages. An overall reduction of neutrophils in wound sections studied up to 3 days after wounding was observed. In addition, delayed recruitment and reduced numbers were also observed in the clot/eschar 1 and 3 days after wounding. Large numbers of neutrophils were present throughout the clot/eschar in wild-type mice, whereas the P/E-double-deficient mutants displayed a thin band of neutrophils. It is not clear to us whether the difference in neutrophil distribution in the clot/eschar of the mutants is due to an overall reduction and a delay of their appearance or whether the migration of neutrophils in the clot/provisional matrix was also affected in the P/E-double-mutant mice. Perhaps P-selectin and integrins expressed on platelets and platelet particles deposited in the clot/provisional matrix may provide a surface for migration and recruitment.[32–34] In addition, macrophage recruitment was also significantly impaired in the P/E-double-deficient mice up to 7 days after wounding. No major defects in leukocyte recruitment were observed in the mice deficient for P-selectin or E-selectin alone, except for an early decrease in neutrophil recruitment in the P-selectin-deficient mice. The P-selectin-deficient mice, in fact, had increased recruitment 1 day after wounding. This has also been observed in another inflammatory model of thioglycollate-induced peritonitis where the P-selectin-mutant mice had increased neutrophil accumulation in the peritoneal cavity at 24 hours (P = 0.057).[12] One explanation for this could be a defect in the clearance mechanism of neutrophils once they are in the tissues.

It appears that the absence of either of P-selectin or E-selectin alone does not have a notable effect on neutrophil recruitment beyond the first hour of cutaneous wound repair. The severe prolonged defect in recruitment observed in the double-deficient mice supports the notion that P-selectin and E-selectin work cooperatively to recruit leukocytes. Mice with this combined deficiency have previously been shown to present a much more severe phenotype compared with singly deficient mice demonstrating

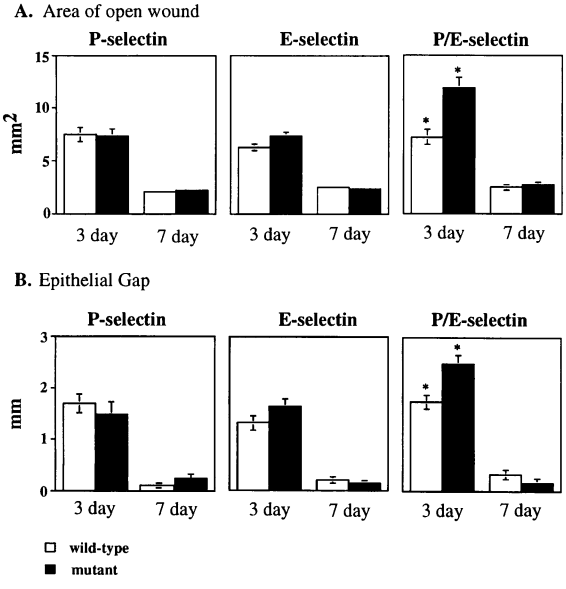

Figure 4. *Wound closure.* A: *Significantly larger open wound area was measured in P/E-double-deficient mice compared with wild-type mice 3 days after wounding (n = 11 to 45; *P < 0.001). This difference was no longer observed 7 days after wounding. No delay in wound closure was observed in the P-selectin- or E-selectin-deficient mice.* B: *Epithelial gap measured microscopically in H&E-stained sections was also wider in the P/E-double-deficient mice 3 days after wounding (n = 15 to 40; *P < 0.001). Values are expressed as mean ± SEM.*

leukocytosis,[14,15] hematopoietic alterations,[14] and opportunistic bacterial infections.[14,15]

Although the main defect in the double-deficient mice was the reduced recruitment of neutrophils and macrophages to the wounds, a modest but significant delay in early migration of keratinocytes was also observed. A provisional matrix, composed of fibrin, fibronectin, and other plasma proteins, is a prominent component of the wound bed at this time. Thus, it is possible that reduced proteolytic degradation and phagocytosis of this provisional matrix may retard the movement of keratinocytes through the provisional matrix.[25,35] Marked decreases have been observed in epithelial migration in incisional skin wounds of plasminogen-deficient mice. In these animals, it appears that, due to defects in matrix degradation, epithelial cells were not able to migrate under the eschar.[35] Keratinocytes in the wound have been thought to express regulators of plasminogen activation; however, many cell types, such as neutrophils, macrophages, and endothelial cells, possibly share this function. These cells may also secrete plasminogen activators and other proteolytic enzymes providing a pericellular environment for degradation of fibrin and other extracellular matrix proteins.[25,35–40] The effect of fibrin or its breakdown products could also be indirect, regulating keratinocyte migration on other matrix proteins. Additionally, macrophages may provide a plasmin-independent method of clot clearance through phagocytosis and degradation of fibrin.[41] Perhaps in our study, reduced recruitment of inflammatory cells may account for the delayed migration of the keratinocytes by retarding matrix degradation. In fact, as keratinocyte migration seems to be just below this neutrophil band, these neutrophils appear to be in a good position to enhance matrix degradation, thereby allowing keratinocytes to dissect their way through the wound matrix. It is also possible that growth factors and/or cytokines secreted by the neutrophils and macrophages may affect epidermal migration.[18,21] The appearance of neutrophils by day 3 could account for the fact that there is no defect in wound closure by day 7. Moreover, a possible overlap in function with other cells, such as keratinocytes, which regulate plasminogen activation[40] and are a source of growth factors,[42] may compensate for the defect. We have not observed any difference in the size of the granulation tissue; however, we do not know whether more subtle alteration of the individual components occurred.

As the double-deficient mice are susceptible to infections,[14,15] we were interested to know whether the abnormal epithelial closure was related to wound infections. The P/E-selectin-double-deficient mice given antibiotics showed the same delay in wound closure as was seen in P/E-double-deficient control mice, indicating that the defect was independent of any infection. The markedly impaired recruitment of inflammatory cells in the skin of these mice in response to injury may be responsible for the development of spontaneous chronic skin infections leading to chronic nonhealing ulcerative dermatitis that is reminiscent of the leukocyte adhesion deficiency type I patients.[27] The P/E-selectin-double-deficient mice may prove useful to study the role of selectins in the defense against invasion by various pathogens.

Acknowledgments

We thank Molly Ullman-Culleré for mouse husbandry, Chris Simpson for histology sections, and Caitlin Moyna for editorial comments.

References

1. Stenberg PE, McEver RP, Shuman MA, Jacques YV, Bainton DF: A platelet α-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. J Cell Biol 1985, 101:880–886
2. Berman CL, Yeo EL, Wencel-Drake JD, Furie BC, Ginsberg MH, Furie B: A platelet α-granule membrane protein that is associated with the plasma membrane after activation: characterization and subcellular localization of platelet activation-dependent granule-external membrane protein. J Clin Invest 1986, 78:130–137
3. Bonfanti R, Furie BC, Furie B, Wagner DD: PADGEM (GMP-140) is a component of Weibel-Palade bodies of human endothelial cells. Blood 1989, 73:1109–1112
4. McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF: GMP-140, a platelet α-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. J Clin Invest 1989, 84:92–99
5. Weller A, Isenmann S, Vestweber D: cloning of the mouse endothelial selectins: expression of both E- and P-selectin is inducible by tumor necrosis factor-α. J Biol Chem 1992, 267:15176–15182
6. Bevilacqua MP, Pober JS, Mendrick DL, Cotran RS, Gimbrone MA: Identification of an inducible endothelial-leukocyte adhesion molecule. Proc Natl Acad Sci USA 1987, 84:9238–9242
7. Carlos TM, Harlan JM: Leukocyte-endothelial adhesion molecules. Blood 1994, 84:2068–2101
8. McEver RP, Moore KL, Cummings RD: Leukocyte trafficking mediated by selectin-carbohydrate interactions. J Biol Chem 1995, 270:11025–11028
9. Springer TA: Traffic signals on endothelium for lympho-

cyte recirculation and leukocyte emigration. Annu Rev Physiol 1995, 57:827–872
10. Frenette PS, Wagner DD: Adhesion molecules. II. Blood vessels and blood cells. N Engl J Med 1996, 335:43–45
11. Mayadas TN, Johnson RC, Rayburn H, Hynes RO, Wagner DD: Leukocyte rolling and extravasation are severely compromised in P-selectin-deficient mice. Cell 1993, 74:541–554
12. Johnson RC, Mayadas TN, Frenette PS, Mebius RE, Subramaniam M, Lacasce A, Hynes RO, Wagner DD: Blood cell dynamics in P-selectin-deficient mice. Blood 1995, 86:1106–1114
13. Subramaniam M, Saffaripour S, Watson SR, Mayadas TN, Hynes RO, Wagner DD: Reduced recruitment of inflammatory cells in a contact hypersensitivity response in P-selectin-deficient mice. J Exp Med 1995, 181:2277–2282
14. Frenette PS, Mayadas TN, Rayburn H, Hynes RO, Wagner DD: Susceptibility to infection and altered hematopoiesis in mice deficient in both P- and E-selectins. Cell 1996, 84:563–574
15. Bullard DC, Kunkel EJ, Kubo H, Hicks MJ, Lorenzo I, Doyle NA, Doerschuk CM, Ley K, Beaudet AL: Infectious susceptibility and severe deficiency of leukocyte rolling and recruitment in E-selectin and P-selectin double mutant mice. J Exp Med 1996, 183:2329–2336
16. Lawrence MB, Springer TA: Neutrophils roll on E-selectin. J Immunol 1993, 151:6338–6346
17. Labow MA, Norton CR, Rumberger JM, Lombard-Gillooly KM, Shuster DJ, Hubbard J, Bertko R, Knaack PA, Terry RW, Harbison ML, Kontgen F, Stewart CL, McIntyre KW, Will PC, Burns DK, Wolitsky B: Characterization of E-selectin-deficient mice: demonstration of overlapping function of endothelial selectins. Immunity 1994, 1:709–720
18. Clark RAF: Basics of cutaneous wound repair. J Dermatol Surg Oncol 1993, 19:693–706
19. Clark RAF: Wound repair: overview and general considerations. The Molecular and Cellular Biology of Wound Repair. Edited by RAF Clark. New York, Plenum Press, 1996, pp 3–50
20. Leibovich SJ, Ross R: The role of the macrophage in wound repair: a study with hydrocortisone and antimacrophage serum. Am J Pathol 1975, 78:71–91
21. Davidson JM: Wound Repair. Inflammation: Basic Principles and Clinical Correlates. Edited by JI Gallin, IM Goldstein, R Snyderman. New York, Raven Press, 1992, pp 809–819
22. Ffrench-Constant C, Van De Water L, Dvorak HF, Hynes RO: Reappearance of an embryonic pattern of fibronectin splicing during wound healing in the adult rat. J Cell Biol 1989, 109:903–914
23. Brown LF, Dubin D, Lavigne L, Logan B, Dvorak HF, Van De Water L: Macrophages and fibroblasts express embryonic fibronectins during cutaneous wound healing. Am J Pathol 1993, 142:793–801
24. Croft CB, Tarin D: Ultrastructural studies of wound healing in mouse skin. I. Epithelial behaviour. J Anat 1970, 106:63–77
25. Donaldson DJ, Mahan JT: Keratinocyte migration and the extracellular matrix. J Invest Dermatol 1988, 90: 623–628
26. Simpson DM, Ross R: The neutrophilic leukocyte in wound repair: a study with antineutrophil serum. J Clin Invest 1972, 51:2009–2023
27. Anderson DC, Schmalsteig FC, Finegold MJ, Hughes BJ, Rothlein R, Miller LJ, Kohl S, Tosi MF, Jacobs RL, Waldrop TC, Goldman AS, Shearer WT, Springer TA: The severe and moderate phenotypes of heritable Mac-1, LFA-1 deficiency: their quantitative definition and relation to leukocyte dysfunction and clinical features. J Infect Dis 1985, 152:668–689
28. Hume DA, Gordon S: Mononuclear phagocyte system of the mouse defined by immunohistochemical localization of antigen F4/80: identification of resident macrophages in renal medullary and cortical interstitium and juxtaglomerular complex. J Exp Med 1983, 157: 1704–1709
29. Trush MA, Egner PA, Kensler TW: Myeloperoxidase as a biomarker of skin irritation and inflammation. Fd Chem Toxic 1994, 32:143–147
30. Yamada KM, Gailit J: Integrins in wound repair. The Molecular and Cellular Biology of Wound Repair. Edited by RAF Clark. New York, Plenum Press, 1996, pp 311–338
31. Veraart JCJM, Verhaegh MEJM, Neumann HAM, Hulsmans RFHJ, Arends JW: Adhesion molecule expression in venous leg ulcers. Vasa 1993, 22:213–218
32. Palabrica T, Lobb R, Furie BC, Aronovitz M, Benjamin C, Hsu Y, Sajer SA, Furie B: Leukocyte accumulation promoting fibrin deposition is mediated *in vivo* by P-selectin on adherent platelets. Nature 1992, 359:848–851
33. Diacovo TG, Roth SJ, Buccola JM, Bainton DF, Springer TA: Neutrophil rolling, arrest, and transmigration across activated, surface-adherent platelets via sequential action of P-selectin and β_2-integrin CD11b/CD18. Blood 1996, 88:146–157
34. Sheikh S, Nash GB: Continuous activation and deactivation of integrin CD11b/CD18 during *de novo* expression enables rolling neutrophils to immobilize on platelets. Blood 1996, 87:5040–5050
35. Rømer J, Bugge TH, Pyke C, Lund LR, Flick MJ, Degen JL, Danø K: Impaired wound healing in mice with a disrupted plasminogen gene. Nature Med 1996, 2:287–292
36. Unkeless JC, Gordon S, Reich E: Secretion of plasminogen activator by stimulated macrophages. J Exp Med 1974, 139:834–850
37. Vassalli JD, Wohlwend A, Belin D: Urokinase-catalyzed plasminogen activation at the monocyte/macrophage cell surface: a localized and regulated proteolytic system. Curr Top Microbiol Immunol 1992, 181:65–86
38. Granelli-Piperno A, Vassalli JD, Reich E: Secretion of plasminogen activator by human polymorphonuclear leukocytes. J Exp Med 1977, 146:1693–1706

39. Heiple JM, Ossowski L: Human neutrophil plasminogen activator is localized in specific granules and is translocated to the cell surface by exocytosis. J Exp Med 1986, 164:826–840
40. Kramer MD, Schaefer B, Reinartz J: Plasminogen activation by human keratinocytes: molecular pathways and cell-biological consequences. Biol Chem 1995, 376:131–141
41. Simon DI, Ezratty AM, Francis SA, Rennke H, Loscalzo J: Fibrin(ogen) is internalized and degraded by activated human monocytoid cells via Mac-1 (CD11b/CD18): a nonplasmin fibrinolytic pathway. Blood 1993, 82:2414–2422
42. McKay IA, Leigh IM: Epidermal cytokines and their roles in cutaneous wound healing. Br J Dermatol 1991, 124:513–518