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# IL-1 Plays a Critical Role in Oral, But Not Dermal, Wound Healing

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**Wound healing is a well-orchestrated complex process leading to the repair of injured tissues. After injury, proinflammatory cytokines act as important modulators of the inflammatory process. IL-1 expression has been regarded as necessary for healing; however, its effects have also been implicated in delayed wound repair. Currently, there is no consensus or direct evidence that IL-1 activity plays a central role in the healing process. The present investigation was undertaken to define the role of IL-1R signaling in the healing outcome of an excisional wound in the palate or scalp of mice that had targeted deletions of the IL-1R type 1 (IL-1R1$^{-/-}$) compared with matched wild-type mice. Histomorphometric analysis was undertaken to assess the degree of healing and the recruitment of polymorphonuclear and mononuclear phagocytes. After 14 days, wild-type mice exhibited complete closure of intraoral wounds, while IL-1R1$^{-/-}$ animals had only partial closure (50%). In the IL-1R1$^{-/-}$ mice, healing tissues exhibited a persistent inflammatory cell infiltrate, which did not occur in wild-type animals. Treatment with antibiotics significantly diminished the persistent inflammatory infiltrate and improved healing in the experimental animals. In contrast to oral wounds, the rate of healing and recruitment of polymorphonuclear cells in scalp wounds was similar in IL-1R1$^{-/-}$ and wild-type mice. The present data underscore the importance of IL-1 in wound healing in a challenging environment and identify its principal role in facilitating the healing process by protecting an open wound from bacterial insult. In a less challenging environment, the production of new connective tissue and its coverage by migrating epithelium are minimally affected by the absence of IL-1 activity.** *The Journal of Immunology,* **2001, 167: 5316–5320.**

Wound healing has three principal phases (1). Healing is initiated by an inflammatory phase in which cytokines and other inflammatory mediators are generated. This is followed by a proliferation phase, particularly of fibroblasts and endothelial cells. The last phase involves the production and reorganization of extracellular matrix leading to repair or regeneration. The inflammatory phase is thought to be important in the repair process because it leads to the recruitment of leukocytes that produce growth factors and remove the debris of the wound. A number of inflammatory mediators are up-regulated during the healing process, including IL-1. Surprisingly, there is little direct evidence establishing whether this up-regulation is functionally important. However, mice with targeted deletion of both P- and E-selectins exhibit reduced recruitment of inflammatory cells (neutrophils and macrophages) and impaired closure of the wounds, demonstrating the functional importance of inflammatory events (2). This is consistent with earlier studies demonstrating impaired healing in animals depleted of monocytes (3). Thus, expression of inflammatory cytokines, including IL-1, may play a central role in the early events of wound healing, in part because they stimulate the recruitment of leukocytes.

IL-1 is a potent proinflammatory cytokine that regulates many aspects of the immune response (4). There are two IL-1 ligands with agonist activity, IL-1$\alpha$ and IL-1$\beta$. Both bind to IL-1Rs termed type I and type II. The type I IL-1R (IL-1R1) is responsible for specific signaling, while the type II receptor functions as a nonsignaling decoy receptor. The generation of mice with targeted functional deletions of IL-1R1 (IL-1R1$^{-/-}$) has helped elucidate the role of IL-1R signaling in several processes (5, 6). These mice develop normally, do not exhibit gross abnormalities, and are capable of developing Abs to exogenous Ag stimulation. In most, but not all studies, mice with deficient IL-1 activity exhibit an attenuated inflammatory response as measured in the turpentine abscess formation model, and are often more susceptible to infectious agents (6–8).

In healing tissue, IL-1 is produced primarily by cells of the epithelium, and exogenous IL-1 has been shown to accelerate epidermal healing (9, 10). However, findings that IL-1 is overexpressed in wounds that heal poorly have cast doubt on the positive role of IL-1 in the healing process (11, 12). Further evidence indicates that application of exogenous IL-1R antagonist can partially reverse the negative impact of TNF on healing (13). That wound healing is enhanced in mice that have a lower level of cytokine expression compared with normals also suggests that the expression of cytokines may inhibit repair even under normal conditions (14). Thus, there are reports indicating that proinflammatory cytokines such as IL-1 are expressed during wound healing, but it is not known whether IL-1 ultimately is required for normal repair.

In this work, we present studies examining the impact of IL-1 activity on wound repair by studying the healing response in mice with targeted mutation of the IL-1R1 and matched wild-type counterparts. These studies were conducted by placing small excisional biopsies at two different sites, the scalp and the hard palate. In the latter, a relatively significant commensal flora is present. The results indicate that the capacity to recruit polymorphonuclear cells

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(PMNs)[2] and monocytes under normal conditions was not dependent upon IL-1. However, for oral wounds in the IL-1R-deficient mice, there was a failure to reduce the inflammatory infiltrate at later time points, diminished formation of new connective tissue, and delayed covering of the wound by epithelium. Treatment with antibiotics reversed these deficits to a significant extent. In contrast, healing in the scalp was minimally affected by the absence of IL-1R signaling. Thus, IL-1R signaling is critical for normal wound healing in a challenging environment, which is largely due to its essential function in up-regulating antibacterial defenses. In contrast, the absence of IL-1R signaling in a less challenging environment has only a small effect on the wound healing response. To our knowledge, the present study is the first to address the mechanistic role of IL-1 signaling in wound healing.

## Materials and Methods

### *Mice*

Experimental IL-1R1$^{-/-}$ mice on a C57BL/6 × 129 background and control C57BL/6 × 129 $F_2$ wild-type mice were purchased from The Jackson Laboratory (Bar Harbor, ME). For all data points, six animals were examined. For all procedures, mice were anesthetized with injection of ketamine (80 mg/kg) and xylazine (10 mg/kg) in sterile PBS. A 1.5-mm palatal excisional biopsy was placed anterior to the soft palate, or a 1.5-mm excisional scalp biopsy was placed at a midline between the ears after the area was carefully shaved. Animals received either a palatal or scalp biopsy, but not both. In some experiments, mice were given the antibiotics sulfamethoxazole (8 mg/ml) and trimethoprim (1.6 mg/ml) in their drinking water starting 10 days before excisional wounding and continuing thereafter. Water with fresh antibiotics was changed daily. Mice were sacrificed at the indicated time points by $CO_2$ overdose.

### *Preparation of specimens*

Following sacrifice, the head of each animal was dissected free and then placed for 48 h in cold 4% paraformaldehyde, followed by dissection of the calvaria with intact soft tissue. This tissue was decalcified by incubation in cold Immunocal (Decal, Congers, NY) for approximately 4 days, with solution changed daily. Cryostat sections (5 $\mu$m) were then prepared as previously described (15).

### *Quantitative histologic analysis*

The distance between the edges of the epithelium and connective tissue of the wound was measured with the use of Image ProPlus software (Media Cybernetics, Silver Spring, MD) from sections at the central portion of each wound. The number of PMNs was identified by their characteristic morphology in H&E-stained sections and counted at ×1000 magnification. The presence of mononuclear phagocytes was determined by immunohistochemistry using the F4/80 Ab, as we have previously described (16). Blood vessels were identified with the MECA-32 Ab, which recognizes newly formed blood vessels (17), purchased from BD Biosciences (Franklin Lakes, NJ). Measurements of mononuclear phagocytes, PMNs, and blood vessels were made in the healing connective tissue and expressed as the number per connective tissue area. All quantitative measurements were confirmed by random reanalysis of approximately one-fourth of the specimens by the same examiner and by another independent examiner to ensure consistency. The intra- and interexaminer variation was generally <15%; each data point presented represents the mean of six specimens ± SEM. In experiments with multiple time points, differences between the experimental and the wild-type groups were determined by ANOVA with significance set at the 0.05 level. Student's $t$ test was used to establish differences between experimental and wild-type groups in other experiments.

## Results

Following placement of a 1.5-mm excisional wound in the hard palate, healing was significantly delayed in the IL-1R-deficient mice, as measured by the gap between the edges of the healing connective tissue as well as the gap between the healing epithelium (Fig. 1). Significantly less closure of the wound by connective tissue or epithelium was observed in the IL-1R-ablated mice compared with the wild-type animals at days 4, 7, and 14 (Fig. 2). By day 4 in the wild-type mice, new connective tissue had covered approximately 40% of the original wound surface, and new epithelium approximately 50%. A similar degree of healing was not seen until day 14 in the IL-1R1$^{-/-}$ animals. In contrast, there was complete bridging of the wound by both connective tissue and epithelium in the wild-type animals by day 14.

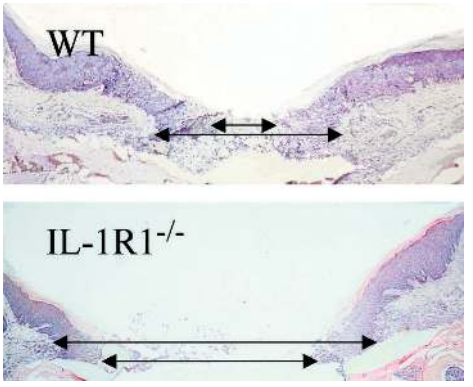


**FIGURE 1.** Healing of excisional oral wounds is delayed in IL-1R$^{-/-}$ mice. A 1.5-mm palatal excisional wound was placed in the hard palate of experimental and matched wild-type mice. Animals were sacrificed 7 days later. Specimens were prepared for cryostat sectioning and stained with H&E. The gap between the healing connective tissue is denoted by long arrows; the interepithelial gap is marked by short arrows. Original magnification, ×40.

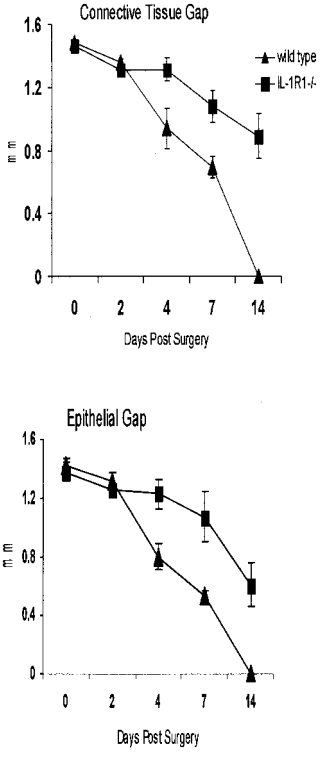


**FIGURE 2.** Healing of excisional oral wounds is delayed in IL-1R$^{-/-}$ mice. A 1.5-mm palatal excisional wound was placed in the hard palate of experimental and matched wild-type mice. Animals were sacrificed 0, 2, 4, 7, and 14 days following biopsy. The gap between the healing connective tissue and epithelium was calculated by image analysis of H&E-stained sections at the wildest part of each lesion. Differences between experimental and control groups were significant at days 4, 7, and 14 ($p < 0.05$).

[2] Abbreviation used in this paper: PMN, polymorphonuclear cell.

In Fig. 3, the data were stratified so that the animals were placed into one of three groups: 1) those that showed a high degree of connective tissue healing (covering >70% of the original wound surface), 2) moderate (covering 30–70%), or 3) little to no healing (covering <30%). For the wild-type group, the majority of animals exhibited moderate healing by day 4, whereas none of the IL-1R1$^{-/-}$ mice exhibited this degree of healing at the same time point. On day 14, all of the wild-type mice had a high degree of healing, while two-thirds of the IL-1R1$^{-/-}$ mice had only a moderate degree of healing. Taken together, these data indicate that from day 4 onward the majority of experimental mice exhibited delayed healing compared with wild-type controls.

Since angiogenesis is an important component of healing, we determined whether the formation of new blood vessels was hindered by the absence of IL-1R signaling by quantitative immunohistochemistry using an Ab that recognizes newly formed blood vessels, MECA-32. The results indicate that there were 90 ± 18 blood vessels per mm$^2$ in the wild-type and 76 ± 12 in the IL-1R$^{-/-}$ group (mean ± SD). The difference was not statistically significant ($p > 0.05$), suggesting that other mechanisms are responsible for the differences in the rates of wound repair.

Since IL-1 stimulates many of the cellular events that lead to formation of an inflammatory infiltrate, experiments were undertaken to determine whether IL-1R1$^{-/-}$ mice had a diminished capacity to recruit monocytes and neutrophils (Fig. 4). In these experiments, monocytes and PMNs were counted in the healing connective tissue of the wound. Monocytes were detected by quantitative immunohistochemistry, as CD68-positive cells and neutrophils were identified by their characteristic appearance on H&E-stained sections. On day 2, enhanced recruitment of both monocytes and PMNs was observed in all of the animals tested compared with baseline. Unexpectedly, the number of monocytes present following wounding was higher in the experimental animals compared with controls. In this group, large numbers of monocytes were recruited by day 2 and did not decrease thereafter. In contrast, the number of monocytes in wild-type mice peaked at day 4 and decreased to almost normal levels by day 14. PMNs in the wild-type group also peaked on day 4 and returned to baseline levels by day 14. In contrast, the recruitment of PMNs in IL-1R-ablated mice reached high levels by day 2 that persisted for the entire experimental period.

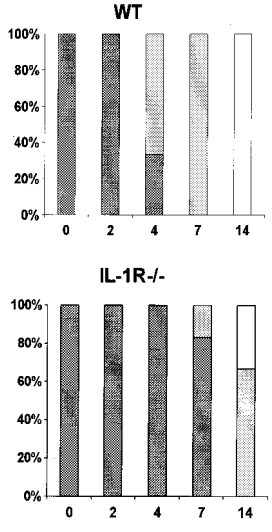


**FIGURE 3.** A high percentage of excisional oral wounds in IL-1R$^{-/-}$ mice exhibits delayed healing. Mice described in Fig. 2 were stratified into three groups: 1) those that showed a high degree of connective tissue healing (covering >70% of the original wound surface), dark bars; 2) moderate (covering 30–70%), gray bars; or 3) little to no healing (covering <30%), light bars. Clear differences between the experimental and control groups are noted on days 4, 7, and 14.

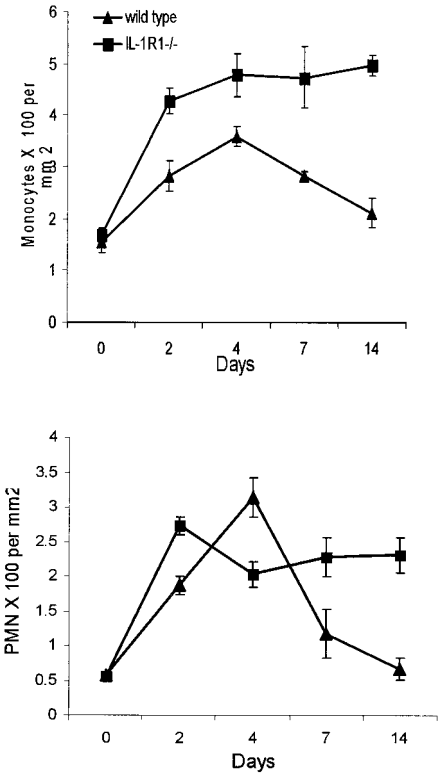


**FIGURE 4.** Healing oral wounds in IL-1R1$^{-/-}$ mice have a persistent monocyte and PMN infiltrate. Histologic sections were analyzed for the presence of mononuclear phagocytes or PMNs in the connective tissue of the healing wound. Mononuclear phagocytes were identified by immunohistochemistry using an Ab to CD68 and PMNs by their characteristic appearance in H&E sections.

We previously reported that IL-1R1$^{-/-}$ mice exhibited a limited capacity to resist infection by oral pathogens (18). We therefore tested whether the delayed oral healing observed in the IL-1R1$^{-/-}$ animals was due to a limited capacity to protect the wound site from infection. In these experiments, mice were treated with an antibiotic regimen that has been reported to substantially reduce the commensal flora in the oral cavity of mice (19). Before antibiotic treatment, high bacterial counts were obtained from a swab of the hard palate of both the experimental and control mice (Fig. 5). Just before creation of an excisional wound, the bacterial flora was reduced by >1000-fold, and 1 wk after biopsy, during the period of healing, only a few bacterial colonies were detected.

The effect of antibiotic treatment was assessed by histomorphometric analysis of H&E-stained sections from mice sacrificed on day 14, which was selected because the greatest difference in healing was noted between the experimental and control groups at this time point (see Fig. 1). Experimental mice treated with antibiotics had a significantly improved healing response (Fig. 6). Epithelial and connective tissue wound closure was improved by 50% and 65%, respectively, in the antibiotic-treated group of IL-R$^{-/-}$ animals. In the wild-type animals, healing was completed at this time point with or without antibiotics (data not shown).

To measure the impact of antibiotic treatment on formation of an inflammatory infiltrate, the number of PMNs was counted. When the IL-1R$^{-/-}$ group was treated with antibiotics, there was a 63% reduction in the number of PMNs so that the level was

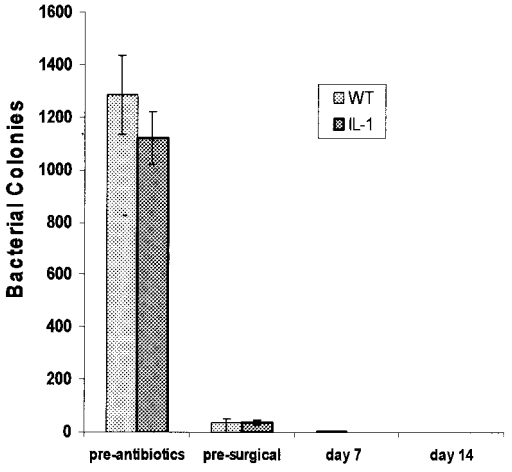


**FIGURE 5.** Antibiotic treatment reduces bacterial levels in the palates of IL-1R$^{-/-}$ and wild-type mice. One group of mice was treated with antibiotics, sulfamethoxazole (8 mg/ml), and trimethoprim (1.6 mg/ml) in their drinking water starting 10 days before inducing an oral excisional wound and continuing thereafter. Swabs of the palate were cultured under aerobic conditions to assess bacterial colony formation just before antibiotic treatment, at the time of surgery, and 7 and 14 days after wounding.

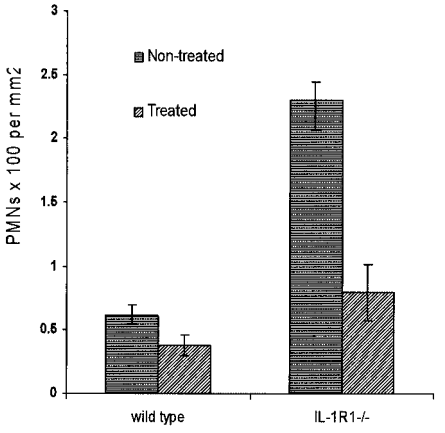


**FIGURE 7.** Antibiotic treatment reduces the size of the inflammatory infiltrate following wounding in IL-1R1$^{-/-}$ mice. Mice were treated with antibiotics, as described in Fig. 6. An excisional biopsy was placed in the palate, and animals were sacrificed 14 days later. PMNs were counted at $\times$1000 magnification in the healing connective tissue in H&E-stained sections. The difference between treated and nontreated groups was significant for IL-1R1$^{-/-}$ animals ($p < 0.05$).

similar to that in the untreated wild-type mice (Fig. 7). This indicates that much of the prolonged recruitment of PMNs in the experimental group is due to constant bacterial stimulation. Moreover, it is consistent with a subclinical infection since no overt signs of infection were present at the wound site.

Experiments were then undertaken to examine healing in experimental and wild-type animals under conditions in which bacterial challenge was less significant. To accomplish this, a 1.5-mm excisional wound was created in the scalp and the rate of healing determined (Fig. 8). At this site, the rates of healing in the wild-type and control mice were similar, except for a single time point, day 7. This contrasts sharply with different rates of healing observed in the oral cavity. When the data were stratified into groups consisting of a high degree, moderate, or little healing, the distribution was similar for wild-type and IL-1R1$^{-/-}$ mice, also indicating that the rates of healing between the two groups were similar (data not shown).

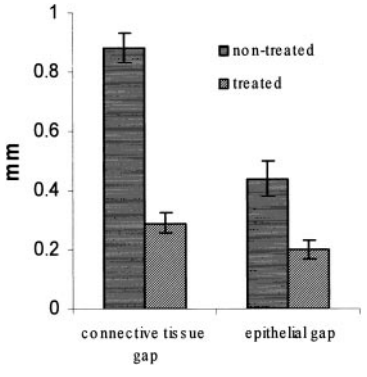


**FIGURE 6.** Antibiotic treatment enhances the rate of healing in IL-1R$^{-/-}$ mice. An excisional wound was placed in the palate of IL-1R1$^{-/-}$ mice, which were sacrificed 14 days later. In one group, mice were treated with antibiotics, as described in Fig. 6. Histologic measurements of the connective tissue and epithelial gaps were made from H&E-stained sections. The differences between the treated and untreated groups for the IL-1R1$^{-/-}$ mice were significant ($p < 0.05$).

The impact of IL-1R signaling on formation of an inflammatory infiltrate following wounding in the scalp was determined by measuring the number of PMNs (Fig. 9). In the wild-type and IL-1R1$^{-/-}$ groups, there was a significant increase in the number of PMNs on day 2. For both groups, the size of the inflammatory infiltrate decreased considerably by day 7, although they were still above baseline levels. No significant differences were noted between IL-1R-deficient and wild-type mice at any of the time points.

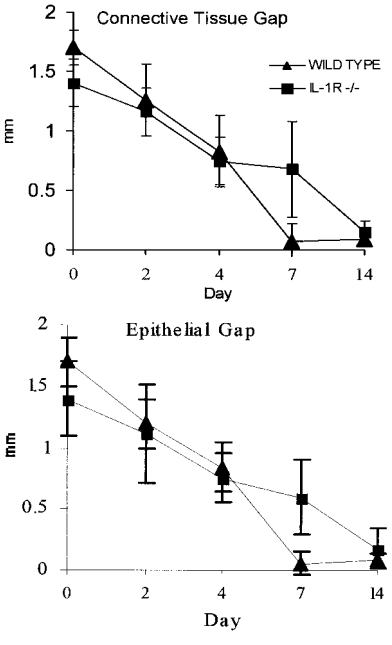


**FIGURE 8.** Healing of excisional wounds in the scalp is similar in IL-1R$^{-/-}$ and wild-type mice. A 1.5-mm biopsy was placed in the scalp of experimental and control mice at a point midway between the ears. Animals were sacrificed 0, 2, 4, 7, and 14 days later. Histologic measurements of the connective tissue and epithelial gaps were made from H&E-stained sections at the widest point of each lesion. A significant difference between experimental and control groups was observed only on day 7 ($p < 0.05$).

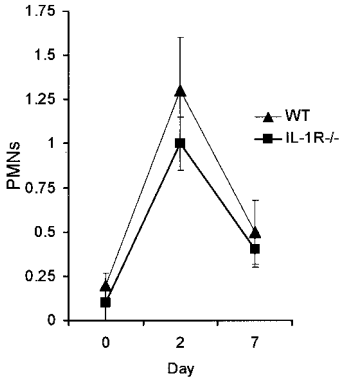


**FIGURE 9.** Recruitment of PMNs is similar in IL-1R$^{-/-}$ and wild-type mice in excisional wounds of the scalp. A 1.5-mm biopsy was placed in the scalp, as described in Fig. 7. PMNs were counted in H&E-stained sections at ×1000 magnification in the healing connective tissue. Differences between the IL-1R1$^{-/-}$ mice and wild-type mice were not significant ($p > 0.05$).

## Discussion

It has been documented in several reports that IL-1 is up-regulated during wound healing. However, there is no consensus or direct evidence that IL-1 activity plays a central role in the healing process. Thus, the significance of its expression is poorly understood. The present study addresses this issue and demonstrates that in a challenging environment the loss of IL-1R signaling causes a significant delay in excisional wound healing. In contrast, healing in the scalp is minimally affected by ablation of IL-1Rs. The difference between these two outcomes can be reconciled by the fact that a significant reduction in the oral flora greatly enhances the rate of healing in IL-1R1$^{-/-}$ mice. These data underscore the role of IL-1 in wound healing and identify its principal role in facilitating the healing process by protecting an open wound from bacterial insult.

In oral healing, monocyte and PMN infiltration was significantly reduced by day 7 in the wild-type animals, while it was maintained without a reduction in the experimental mice. Experiments in which mice were treated with antibiotics suggest that persistent bacterial challenge in the oral cavity of IL-1R1$^{-/-}$ mice may account for the long period of inflammation and duration of the inflammatory infiltrate. This is likely to result from an impairment of the host response in the experimental group, since IL-1 has been shown to significantly contribute to the antibacterial activity of PMNs and monocytes (20–22). This interpretation is consistent with our previous finding that in a model of chronic infection, IL-1R1$^{-/-}$ mice had an impaired antibacterial defense, as demonstrated by higher levels of oral pathogens at infected sites compared with wild-type animals (16), which was associated with prolonged recruitment of leukocytes. Thus, a diminished host response present in the IL-1R1$^{-/-}$ mice could lead to a mild subclinical infection at the wound site, inducing persistent inflammation, and that in turn causes impaired healing. These results may give insight into mechanisms of delayed wound healing in which there is a compromised host response in individuals such as diabetics (23).

Results from excisional wounds in the scalp demonstrate that ablation of IL-1Rs has only a small effect on formation of new connective tissue and wound coverage by epithelium. This further emphasizes that the deficit in oral healing is due to the impact of that particular environment rather than an absolute requirement for IL-1 stimulation of fibroblasts or epithelial cells. It is also consistent with studies demonstrating that reformation of an epithelial barrier following repeated treatment of the skin with acetone or tape stripping is not significantly affected by the absence of IL-1Rs (24).

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