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Chemokine changes during oral wound healing

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Abstract

The oral mucosa is susceptible to tissue injury from many causes, including infection, autoimmune disorders, surgical and accidental trauma, and gingival and periodontal inflammation; however, little is known about the events that influence wound healing in the mouth. Recent studies in non-oral tissues have implicated immune system-derived factors, in particular chemokines, in the wound healing process. Tissues from mice with experimental gingival wounds were studied for expression of genes for four chemokine ligands or receptors (CCL19, CCL20, CCL25, and CCR5) that are important in leukocyte trafficking or inflammation. Notably, during the peak phase of wound healing, chemokine gene expression was up-regulated for CCL19, CCL20, and CCL25, and down-regulation of CCR5, suggesting an orchestrated process of chemokine-mediated recruitment or retention of lymphocytes and macrophages into wound areas, while simultaneously suppressing a potentially adverse inflammatory response. These findings have implications for developing therapeutic strategies aimed at promoting more effective tissue healing at oral surfaces.

Keywords: Chemokines; Gingiva; Wound repair; CCL19; CCL20; CCL25; CCR5

Wound healing is a dynamic, multi-faceted physiological process that involves a wide range of biological mediators [1–3]. Additionally, a role for the immune system in the wound healing process has been suggested from a number of studies. For example, macrophage inflammatory protein (MIP)-1α has been linked to enhanced macrophage influx, angiogenic activity, and collagen production in dermal punch wounds in mice [4]. Curiously, wound re-epithelialization was not appreciably different in MIP-1$\alpha^{-/-}$ mice or in mice treated with anti-MIP-1α antibody, although it was significantly delayed in monocyte chemotactic protein (MCP)-$1^{-/-}$ mice [5,6]. Thus, MCP-1 may function as an effector molecule involved in activating other cellular or molecular responses in the healing process. Other studies have linked MCP-1 and monocytes to TNFα in wound repair [7], further pointing to a role for immune system-derived factors in that response. T cells, in particular γδ T cells that are frequently found in epidermal and epithelial tissues, have been shown to be involved in wound healing, and also to secrete cytokines and chemokines that act beneficially in the healing process [8–10].

As part of our previous studies into the immune response within the oral mucosa, we conducted a series of experiments to evaluate the changes that occur in chemokine gene expression following local antigen deposition. An unexpected finding from that work was the observation that CCL25, thymus expressed chemokine (TECK), and its receptor (CCR9) were expressed in normal mouse buccal epithelia, and that expression of CCL25 increased in animals that had been challenged locally with antigen [11]. Those findings were of particular interest since until then CCL25 was known to have a highly selective distribution, limited to the thymus and the intestine where it is believed to be involved in regulating lymphocyte trafficking [12–14].

In the present study, we have examined changes that occur in the expression of four chemotactic and inflammatory mediators in gingival wound tissues of mice.

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These studies provide new evidence for a potential role for chemokines involved in leukocyte recruitment or retention within mucosal wound tissues while simultaneously curtailing the development of a strong inflammatory response in aseptic conditions.

Materials and methods

Mice. Eight- to 10-week-old C57BL/6 mice were purchased from Harlan Sprague–Dawley (Houston, TX). Mice were used in accord with The University of Texas Health Science Center at Houston Animal Welfare Guidelines.

Gingival wounding. Wounds were made in the mandibular gingiva of anesthetized mice by a lateral incision using a #11 scalpel below the lower right incisor. The cut was made to the width of the incisor and down to the periosteum through the epithelium and mucosa. On the appropriate day, mice were euthanized, tissues were removed by loosening from the tooth and bone with a cleoid-discoid #3 instrument. Tissues were cut adjacent to the wounded area using fine iris scissors, placed in formalin, and prepared for hematoxylin and eosin sections, or were used as fresh tissues for RT-PCR analysis.

Evaluation of histological sections. Histological sections were evaluated using a CCD camera. The area of the epithelial layer was quantified from four mice at four time points (2, 24, 48, and 72 h) after wounding, and compared to that of tissues from non-wounded mice. Tissues were examined at 20× magnification, thus permitting the wound region to be measured in a standardized manner for all specimens using an Image-Pro Plus imaging system with Image-Pro Plus V4.5 data analysis software. Wounds were characterized based on the area of the epithelium, lymphocytic infiltration, and degree of hyperkeratosis.

RT-PCR analysis. RNA from gingival tissues was extracted using a 4PCR Kit #1914 (Ambion; Austin, TX). cDNAs were prepared with an RT-PCR Kit #1402-2 (Clontech; Palo Alto, CA) as previously described [11]. Primer sequences were:

CCR5	Forward	5′-CACTGCTGCCTAAACCCTGT-3′
	Reverse	5′-TTCCTACTCCCAAGCTGCAT-3′
CCL19	Forward	5′-TCTCCTCCCTCCCCTTAGAA-3′
	Reverse	5′-CGGCTTTATTGGAAGCTCTG-3′
CCL20	Forward	5′-CGTCTGCTCTTCCTTGCTTT-3′
	Reverse	5′-AGGAGGTTCACAGCCCTTTT-3′
CCL25	Forward	5′-GTGATGATGCCCAGAAAGACC-3′
	Reverse	5′-TCAGCAATCATCAATAGCCAATAG-3′
β-Actin	Forward	5′-ATGGATGACGATATGGCTG-3′
	Reverse	5′-ATGAGGTAGYCTCTAAGGT-3′

Amplification consisted of 40 cycles at 95 °C for 1 min, 59 °C for 1 min, and 72 °C for 1 min using a Biometra T-gradient thermocyler (Whatman Biometra; Göttingen, Germany). PCR products were run on a 2% agarose gel.

Results

Gingival epithelial changes during wound repair

Morphometric and histopathological changes in gingival tissues were determined using Image-Pro Plus imaging software within circumscribed regions of epithelia from non-wounded ("none") mice and in mice from 2 to 72 h post-wounding. By 24 h after wounding, gingival tissues wound regions were characterized by epithelial thickening, hyperkeratosis, and mononuclear cell infiltration as shown in Fig. 1. Kinetic changes in wound areas at 2, 24, 48, and 72 h compared to non-wounded tissue ("none") four mice per time point are shown in Fig. 2, which indicates a statistically significant ($p < 0.01$) increase in epithelial area of gingival wounds at 24 and 48 h compared to non-wounded tissues.

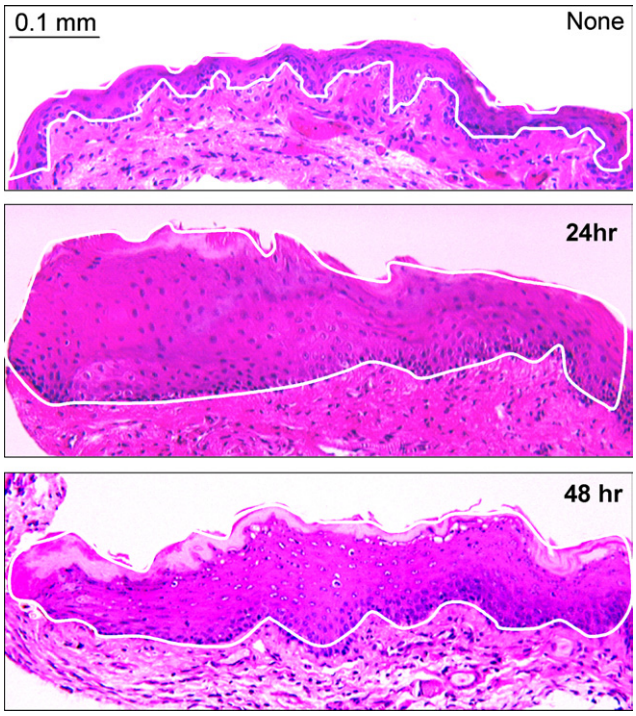

Fig. 1. Hematoxylin and eosin sections of gingival tissues from a non-wounded mouse and from mice at 24 and 48 h post-wounding. Epithelial regions were circumscribed in bitmaps (white lines) and epithelial area was calculated using an Image-Pro Plus imaging system with Image-Pro Plus V4.5 data analysis software. Note the increased epithelial area at 24 and 48 h, the hyperkeratosis at 48 h, and the accumulation of mononuclear cells in wound tissues, particularly at 48 h post-wounding.

Chemokine alterations during gingival wound repair

To determine the impact of wound healing on the expression of chemokines that are known to be involved in leukocyte migration or inflammation, gingival tissues were recovered from euthanized mice at 0 (non-wounded) and 2, 24, 48, and 72 h after wounding. RT-PCR analysis was done for CCL19 (MIP-3β), CCL20 (MIP-3α), CCL25 (TECK), and CCR5 (the receptor for MIP-1α, MIP-1β, and RANTES [regulated upon activation normal T cell expressed]). As seen in Fig. 3, there was a dynamic pattern of chemokine expression during the wound repair period in that the expression

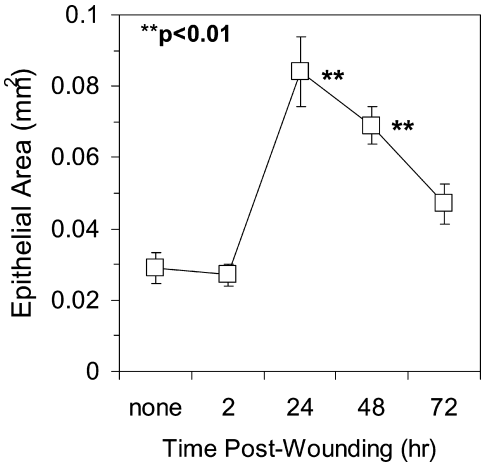

Fig. 2. Gingival epidermal tissue area from four mice each for non-wounded mice, and from mice at 2, 24, 48, and 72 h post-wounding. Note the marked increase in epithelial area at 24 and 48 h post-wounding (**$p < 0.01$ compared to non-wounded tissue area as determined by Student's t test for unpaired observations).

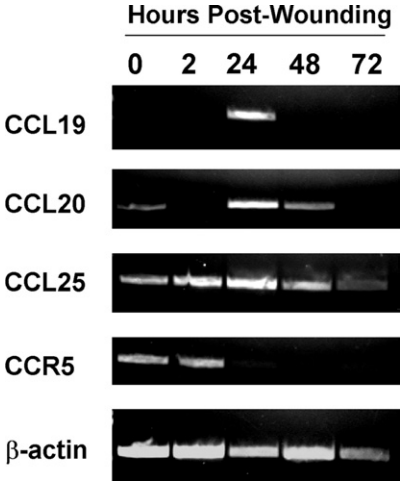

Fig. 3. CCL19, CCL20, CCL25, and CCR5 gene expression in gingival tissues of non-wounded mice and mice at 2, 24, 48, and 72 h post-wounding. Note the increase in CCL19, CCL20, and CCL25 gene expression at 24 h post-wounding, and the decrease in gene expression of CCR5 at the same time following gingival wounding. The loss in CCR5 expression may serve to restrict the inflammatory response in aseptic conditions while the increase in CCL19, CCL20, and CCL25 would promote the entry of mononuclear cells that would be beneficial to the tissue repair process.

of the proinflammatory chemokine receptor gene, CCR5, was markedly down-regulated from 24 to 72 h post-wounding. The loss of CCR5 may serve to naturally curtail the inflammatory response, which would be unwanted in an aseptic wound, although CCR5 expression could be up-regulated in the case of bacterial-driven inflammation. Notably, however, there was a precipitous increase in expression of both CCL19 and CCL20 24–48 h after wounding. Additionally, expression of CCL25 was elevated 24 h after wounding and then down-regulated by 72 h after wounding. Together, these changes point to a specific and well-regulated set of chemokine changes in oral wounds during a critical wound healing period.

Discussion

A particularly novel finding to emerge from these studies was the pattern of change that occurred in the expression of chemokine genes for CCL19, CCL20, and CCL25, and for the chemokine receptor expression, CCR5. Up-regulation of CCL19, CCL20 (MIP-3β and MIP-3α, respectively) would have beneficial effects due to their chemotactic activity in mucosal sites [15,16]. However, the temporal relationship in the expression of those chemokine ligands as seen by their peak expression at 24–48 h post-wounding, followed by down-regulation at 72 h post-wounding, strongly suggests that they are associated with the homeostatic process of tissue repair. Similarly, the recruitment of lymphocytes, dendritic cells, and other mononuclear leukocytes by CCL25 [12,15] appears to be a self-limiting event as seen by the suppressed levels of CCL25 at 72 h post-wounding—a time when wound tissue damage had mostly resolved. Additionally, recruitment of lymphocytes by CCL25, in particular γδ T cells that are known to aid the wound healing process through the elaboration of keratinocyte growth factors [9], would further facilitate efficient tissue healing.

The dramatic drop in CCR5 synthesis by 24 h post-wounding is particularly insightful for several reasons. A drop in CCR5 expression would greatly restrict the binding of MIP-1α, MIP-1β, and RANTES, all of which are involved in an inflammatory response [17–20]. However, the model used here in which wounds were made with sterile scalpels would not lend itself to an extensive inflammatory response unless septic conditions prevailed. Our experimental model, therefore, would be reflective of what generally occurs under most normal circumstances of oral tissue damage due to abrasion or dental treatment in which wounds have been created but rapidly healed. The situation involving tissue wound due to periodontal disease in which bacterial colonization has occurred would represent an exception to this. However, in that situation as well, efficient tissue healing would be desired once bacterial treatment had been initiated. A concomitant and fortuitous benefit from suppressed CCR5 expression during normal tissue healing would be the limiting effect it would have on the local entry of macrophage (M)-tropic strains of human immunodeficiency virus-I (HIV-I), since CCR5 is a receptor for M-tropic HIV-I [21,22]. That effect on CCR5 expression during wound healing would parallel the process of tissue regeneration at a time when the mucosa would be particularly susceptible to HIV infection.

Clearly, factors other than the chemokines identified here would contribute to wound healing in the mouth. It is known, for example, that salivary secretions are rich in basic fibroblast growth factor and lysophosphatidic acid [23–27], both of which would enhance tissue regeneration. The findings described here coupled with those observations indicate that multiple factors undoubtedly are involved in the wound healing process overall. As more information is acquired about the factors that affect wound healing, it may be possible to accelerate that process therapeutically. Additionally, studies now can be done to systematically evaluate the contribution of specific lymphocyte and mononuclear cell populations, in particular resident mucosal γδ T cells, in oral tissue healing.

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