MIP-1α as a Critical Macrophage Chemoattractant in Murine Wound Repair

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Abstract

At sites of injury, macrophages secrete growth factors and proteins that promote tissue repair. While this central role of the macrophage has been well studied, the specific stimuli that recruit macrophages into sites of injury are not well understood. This study examines the role of macrophage inflammatory protein 1α (MIP-1α), a C–C chemokine with monocyte chemoattractant capability, in excisional wound repair. Both MIP-1α mRNA and protein were detectable in murine wounds from 12 h through 5 d after injury. MIP-1α protein levels peaked 3 d after injury, coinciding with maximum macrophage infiltration. The contribution of MIP-1α to monocyte recruitment into wounds was assessed by treating mice with neutralizing anti–MIP-1α antiserum before injury. Wounds of mice treated with anti–MIP-1α antiserum had significantly fewer macrophages than control (41% decrease, $P < 0.01$). This decrease in wound macrophages was paralleled by decreased angiogenic activity and collagen synthesis. When tested in the corneal micropocket assay, wound homogenates from mice treated with anti–MIP-1α contained significantly less angiogenic activity than control wound homogenates (27% positive for angiogenic activity versus 91% positive in the control group, $P < 0.01$). Collagen production was also significantly reduced in the wounds from anti–MIP-1α treated animals (29% decrease, $P < 0.05$). The results demonstrate that MIP-1α plays a critical role in macrophage recruitment into wounds, and suggest that appropriate tissue repair is dependent upon this recruitment. (*J. Clin. Invest.* 1998. 101:1693–1698.) Key words: monocyte • macrophage • chemoattractant • wound healing • neovascularization

Introduction

The tissue macrophage plays a pivotal role in modulating the repair process. Within wounds, macrophages phagocytose debris, become activated, and secrete a large number of bioactive substances (1). The functional importance of the wound macrophage was first demonstrated in 1975, when Leibovich and Ross showed that monocyte and macrophage depleted guinea pigs exhibited significantly delayed wound repair (2). Subsequently, many studies have provided additional information about the multifunctional role of macrophages within wounds. For example, macrophages have been shown to affect the extracellular matrix composition after injury via the production of extracellular matrix molecules, proteases, and protease inhibitors (3–6). Macrophages also affect tissue repair through the production of soluble growth factors that influence the proliferation and differentiation of endothelial cells, fibroblasts, keratinocytes, and other cell types (7–9). The capability of the macrophage to accelerate wound healing has been substantiated by in vivo studies showing that the application of macrophage-activating substances accelerates the wound repair response (10, 11). The injection of additional macrophages into healing wounds also augments the repair response in both normal and impaired wound healing models (12).

Despite intense research interest in the functional capacity of the macrophage within the healing wound, little is known about the mechanisms of sustained recruitment of monocytes into the wound bed. Among the many candidates for a wound monocyte chemoattractant is macrophage inflammatory protein 1α (MIP-1α)[1] (13). MIP-1α is an LPS-inducible chemokine of the C–C family that is a strong chemoattractant for monocytes (14). Recent studies have documented the importance of this cytokine in mediating leukocyte infiltration in certain viral infections, autoimmune diseases, and other inflammatory states (15–20). This study examines the role of MIP-1α in wounds, and provides evidence for a critical role of this chemokine in the tissue repair response.

Methods

Wound repair model. Animal use followed institutional guidelines and was performed with Loyola University Institutional Animal Care and Use Committee approval. Female Balb/C mice (3 mo old) were anesthetized by inhalation of methoxyfluorane. The hair was shaved from the dorsum and six full-thickness dermal punch wounds (3 mm in diameter) extending through the panniculus carnosus were produced on the dorsal skin of each mouse. At specific times after injury, mice were again anesthetized, killed by cervical dislocation, and the wounds harvested for analysis. Each 3-mm wound was removed from the skin using a 5-mm diameter punch. Individual wounds from each animal were processed separately for examination in the assays described below.

Determination of MIP-1α protein levels in wounds. Individual wounds were homogenized in 1× PBS containing 2 mM PMSF and 1 μg/ml each of antipain, aprotinin, leupeptin, and pepstatin A, followed by sonication for 2 min on ice. Homogenates were briefly centrifuged to remove debris, and filtered through a 1.2-μm pore filter. Murine MIP-1α ELISA analysis was performed as described previously (21, 22). Briefly, flat-bottomed 96-well microtiter plates (Immuno-Plate I 96-F; Nunc, Inc., Naperville, IL) were coated with 50 μl/well of the rabbit anti–murine MIP-1α polyclonal antibodies (1 μg/ul in 0.6 M NaCl, 0.26 M H_3BO_4, and 0.08 N NaOH, pH 9.6) for 24 h at

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1. *Abbreviation used in this paper:* MIP-1α, macrophage inflammatory protein 1α.

4°C and then washed with PBS (pH 7.5) 0.05% Tween 20 (wash buffer). Microtiter plate nonspecific binding sites were blocked with 2% BSA in PBS and incubated for 60 min at 37°C. Plates were rinsed three times with wash buffer and diluted (neat and 1:10), samples (50 μl/well) were added, and then incubated for 1 h at 37°C. Plates were then washed three times and 50 μl/well of biotinylated polyclonal rabbit anti–murine MIP-1α antibodies (3.5 ng/μl in PBS, pH 7.5, 0.05% Tween 20, and 2% FCS) were added, and the plates were incubated for 45 min at 37°C. Plates were then washed three times, streptavidin-peroxidase conjugate (Bio-Rad Laboratories, Richmond, CA) was added, and the plates were incubated for 30 min at 37°C. Plates were then washed three times and chromogen substrate (Bio-Rad Laboratories) was added. The plates were incubated at room temperature to the desired extinction, and the reaction was terminated with 50 μl/well of 3 M H_2SO_4 solution. Plates were read at 490 nm in an automated microplate reader (Bio-Tek Instruments, Inc., Winooski, VT). Standards were 1:2 log dilutions of recombinant murine MIP-1α (R&D Systems, Minneapolis, MN) from 100 ng to 1 pg/ml (50 μl/well). This ELISA method consistently detected specific cytokine concentrations in a linear fashion $>$ 10 pg/ml. Data were analyzed for significance using a one-way ANOVA followed by a Tukey-Kramer multiple comparisons test.

Northern analysis. Total RNA was prepared by the method of Davis et al. (23). Northern analysis was performed by electrophoresis of RNA samples through 0.8% agarose 2 M formaldehyde gels in 20 mM MOPS buffer (pH 7) containing 5 mM of sodium acetate and 1 mM EDTA. 10 μg of total cellular RNA was loaded per lane. Gels were blotted onto GeneScreen Plus (DuPont-NEN, Wilmington, DE) and hybridized according to the manufacturer's directions. The plasmid encoding GAPDH was a generous gift of Dr. H.M. Jäck (Loyola University Medical Center) (24). The MIP-1α probe was cloned by reverse transcriptase PCR of mRNA derived from LPS-stimulated murine macrophages. The primers (5′ primer, 5′ CAGCGAGTACCAGTCCCTTTT; and 3′ primer, 5′CCTCGCTGCCTCCAAGA) were derived from the published sequence of murine MIP-1α (14). First strand cDNA synthesis was performed in a 20 μl reaction mixture containing 1 μg of RNA, 1 mM dNTPs, 1× PCR buffer II (Perkin-Elmer, Branchburg, NJ), 5 mM $MgCl_2$, 20 U of RNasin (Promega Corp., Madison, WI), 2.5 μM of random hexamers, and 10 U of MuLV reverse transcriptase (Promega Corp.) which was incubated at room temperature for 10 min, 42°C for 15 min, and 90°C for 5 min. PCR was performed using 20 μl of the reverse transcriptase reaction in a 100-μl reaction containing 200 μM dNTPs, 1× PCR buffer II (Perkin-Elmer), 1 mM $MgCl_2$, 20 U of RNasin (Promega Corp.), 50 pM of each primer, and 2.5 U of *Amplitaq* (Perkin-Elmer). The reaction mixture was subjected to 1 cycle at 95°C for 2 min, 30 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, and 1 cycle at 72°C for 7 min. The expected 415-bp PCR fragment was obtained and cloned into the pT7Blue vector (Novagen, Madison, WI). The insert was sequenced and its identity confirmed with murine MIP-1α. For each probe, isolated insert was labeled with ^{32}P using the Random Primers DNA Labeling System (GIBCO BRL, Gaithersburg, MD) to a specific activity of at least 10^8 cpm/μg.

Passive immunization. 2 h before wounding, and then every 2 d, each mouse was injected intraperitoneally with 0.5 ml of either rabbit anti–murine MIP-1α antisera or control preimmune rabbit sera. The specificity of the antibody and administration schedule have been described previously (18–20). For quantitation of macrophages and assessment of angiogenic activity, tissue was harvested at 3 d after injury. For assessment of collagen levels, tissue was harvested 7 d after injury.

Macrophage quantitation. Wounds and surrounding tissue were snap frozen in OCT compound and stored at −80°C until sectioned. Sections were fixed in acetone at room temperature for 15 min, pretreated with 3% H_2O_2 in methanol to block endogenous peroxidase, and then blocked for nonspecific binding with normal mouse serum (1:1,000). Incubations in primary (F4/80, a rat mAb directed against mature murine macrophages [1:10]) (25), and secondary antibody (biotinylated mouse anti–rat Ig [1:100]) (Jackson Laboratories, West Grove, PA) were performed for 30 min each, followed by a 30-min incubation with avidin-biotin horseradish peroxidase complexes (ABC, Vector Labs, Burlingame, CA). Color development was performed with 3,3′-diaminobenzidine and slides were counterstained with Gill's hematoxylin. For each section, a blinded observer counted the number of macrophages within 10 random high power fields with the aid of an optical grid. Quantitation was performed on four to six sections for each mouse. Data were analyzed for significance by a one-way ANOVA followed by a Tukey-Kramer multiple comparisons test.

In vivo analysis of angiogenic activity. In vivo angiogenic activity of wounds was assayed in the avascular cornea of Long Evans rat eyes as described previously (26, 27). Briefly, equal volumes of lyophilized wound specimens normalized to total protein were combined with sterile Hydron (Interferon Sciences Inc., New Brunswick, NJ) casting solution. 5-μl aliquots were pipetted onto the flat surface of an inverted sterile polypropylene specimen container and polymerized overnight in a laminar flow hood under UV light. Before implantation, pellets were rehydrated with normal saline. Rats were given ketamine intraperitoneally (150 mg/kg) and atropine (250 μg/kg) for anesthesia. Rat corneas were anesthetized with 0.5% proparacaine hydrochloride ophthalmic solution followed by implantation of the Hydron pellet into an intracorneal pocket (1–2 mm from the limbus). 6 d after implantation, animals received heparin (1,000 U) and ketamine (150 mg/kg) intraperitoneally, followed by 10-ml perfusion of colloidal carbon via the left ventricle. Corneas were harvested and photographed. Scoring was performed by a blinded observer. Positive neovascularization responses were defined as sustained directional ingrowth of capillary sprouts and hair pin loops towards the implant. Negative responses were defined as either no growth or only an occasional sprout or hairpin loop displaying no evidence of sustained growth. To compare the groups for statistically significant differences, the data were subjected to a Fisher's exact test.

Analysis of collagen content. As an indication of total collagen content, hydroxyproline concentration was determined as described previously (28). Briefly, tissues were hydrolyzed in 2 ml of 6 N HCl for 3 h at 130°C. The solution was neutralized to pH 7 with 2.5 N NaOH and diluted 40-fold with H_2O. 2 ml of diluted solution was mixed with 1 ml of 0.05 M chloramine T solution and incubated for 20 min at room temperature. 1 ml of 3.15 M perchloric acid was added and the solution was incubated an additional 5 min at room temperature. 1 ml of 20% *p*-dimethylamino-benzaldehyde was then added and the solution incubated for 20 min at 60°C. The absorbance of each sample at 557 nm was determined, and the amount of hydroxyproline was determined by comparison to a standard curve.

Immunolocalization. Specimens were fixed in 4% paraformaldehyde for 24 h. Paraffin-embedded tissue sections were dewaxed with xylene and rehydrated through graded concentrations of ethanol. Samples were then stained for murine MIP-1α using a modification of our previously described technique (29). Briefly, nonspecific binding sites were blocked with normal goat serum (BioGenex, San Ramon, CA), washed, and overlaid with a 1:1,000 dilution of either control (preimmune rabbit) or rabbit anti–murine MIP-1α serum. Slides were then rinsed and overlaid with secondary biotinylated goat anti–rabbit IgG (1:35) and incubated for 60 min. After washing twice with Tris-buffered saline, slides were overlaid with a 1:35 dilution of alkaline phosphatase conjugated to streptavidin (BioGenex), and incubated for 60 min. Fast Red reagent (BioGenex) was used for chromogenic localization of MIP-1α antigen. After optimal color development, sections were immersed in sterile water, counterstained with Mayer's hematoxylin, and covered with cover slips using an aqueous mounting solution.

Results

MIP-1α mRNA levels and protein production correlate temporally with macrophage infiltration into wounds. To assess the

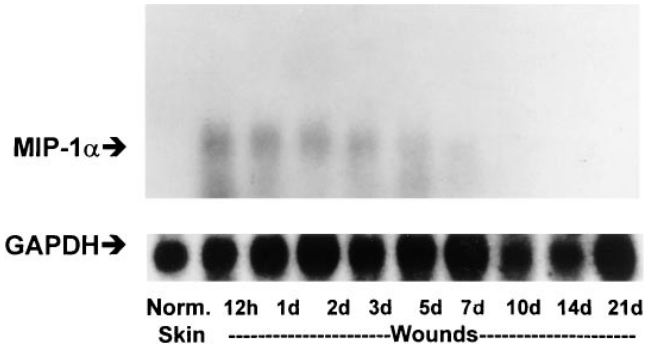

Figure 1. MIP-1α mRNA levels during wound healing. mRNA, prepared from normal murine skin and individual full-thickness excisional wounds at times from 12 h to 21 d after injury, was subjected to Northern blot analysis for MIP-1α mRNA. Blots were sequentially hybridized with (*top*) a probe specific for MIP-1α followed by (*bottom*) a probe specific for GAPDH, which served as a control for equal loading of RNA. Each lane contains 10 μg of total RNA. The analysis reveals that MIP-1α mRNA was detectable in wounds as early as 12 h after injury, and continued to be observable through day 5 after injury. This figure is representative of three separate experiments.

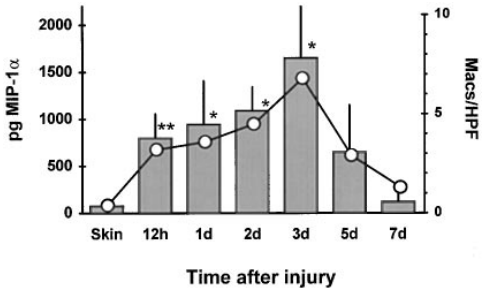

Figure 2. MIP-1α protein levels and macrophage infiltration in wounds. Tissue homogenates were prepared from normal skin and individual wounds from times 12 h to 7 d after injury. The amount of MIP-1α per wound was determined by ELISA analysis. Data are expressed as the mean±SD ($n = 6$) (*left axis*). The level of MIP-1α increased in wounds through day 3, then diminished to baseline by day 7. *$P < 0.01$ compared to normal skin. **$P < 0.05$ compared to normal skin. In a companion set of individual wounds, the number of macrophages per High-power field was determined by histologic analysis (*circles*). Data are expressed as the mean ($n = 6$) at each time point after injury (*right axis*).

likelihood that MIP-1α plays a significant role in tissue repair, healing wounds were analyzed for the presence of MIP-1α mRNA and protein at several time points after injury. Northern analysis of wound mRNA demonstrated that substantial MIP-1α mRNA was present in wounds as early as 12 h after injury (Fig. 1). MIP-1α mRNA continued to be detectable in wounds through day 5 after injury (Fig. 1). MIP-1α protein was also detectable at 12 h after injury, peaked at day 3 after wounding, and returned to baseline around day 7 (Fig. 2). Thus, the MIP-1α protein levels in wound correlated with the appearance of MIP-1α mRNA. In addition, the peak concentration of MIP-1α protein (at day 3 after injury) corresponded directly to the time point at which the maximum number of macrophages was observed histologically in companion wounds (Figs. 2 and 3) (30). This finding strongly suggested that MIP-1α is involved in monocyte recruitment into wounds. No constitutive MIP-1α mRNA expression and little MIP-1α protein was detected in normal skin. Immunolocalization of MIP-1α suggested that macrophages within the wound are a source of this

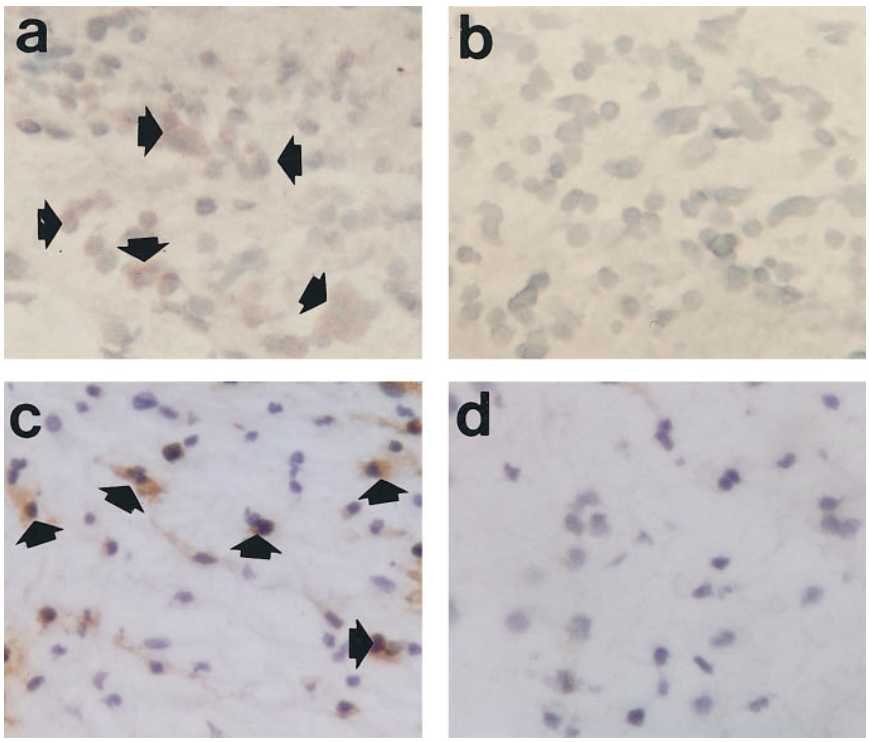

Figure 3. Immunolocalization of MIP-1α and identification of macrophages in wounds. (*a*) Immunolocalization of MIP-1α within wounds was performed by immunostaining wound sections with anti–MIP-1α antiserum. A representative section of 2-d wounds shows numerous MIP-1α immunopositive (*red*) inflammatory cells within the wound bed (*arrows*). (*b*) A 2-d wound stained with control preimmune rabbit serum contains no immunopositive cells. (*c*) Identification of macrophages within wounds was performed by immunostaining wound sections with F4/80, a rat mAb specific for murine macrophages. A representative section of a 2-d wound exhibits numerous immunopositive (*brown*) macrophages (*arrows*). In this wound model, the macrophage infiltration into the wound peaks on the third day after injury. (*d*) A wound stained with control rat IgG displays no immunopositive cells.

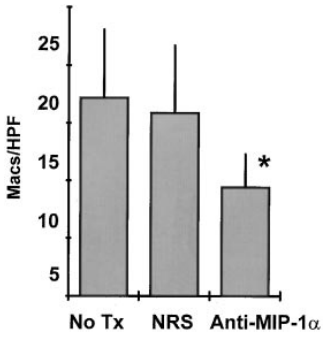

Figure 4. Macrophage infiltration into the wounds of mice treated with anti-MIP-1α neutralizing antiserum. the number of macrophages per high power field was determined histologically in wounds from untreated mice and mice receiving either preimmune normal rabbit serum of anti–MIP-1α neutralizing antiserum. The data are expressed as mean±SD ($n = 6$). *$P < 0.01$ compared to mice receiving no treatment and to mice receiving normal rabbit serum.

Table I. Angiogenic Activity of Wound Homogenates from Mice Treated with Anti–MIP-1α Antiserum

Treatment	*Number of positive/total*	*%*
Preimmune serum	10/11	(91)
Anti–MIP-1α antiserum	3/11	(27)*

Tissue homogenates were prepared from 3-d wounds of mice ($n = 3$) treated with either preimmune or anti–MIP-1α antiserum. 11 tissue homogenate samples per treatment group were assessed for angiogenic activity in the rat corneal pocket assay. *$P < 0.01$, compared to the preimmune serum treatment group.

chemokine (Fig. 3). In addition, the fibrin clot was noted to stain positively, perhaps reflecting platelet-derived MIP-1α (not shown) (31).

Depletion of MIP-1α results in reduced macrophage infiltration into wounds. To assess the contribution of MIP-1α to monocyte recruitment into wounds, mice were treated with neutralizing anti–MIP-1α antiserum before injury. At day 3 after injury, the number of macrophages at the wound site was quantitated histologically. When compared to control, mice treated with anti–MIP-1α antiserum exhibited a significant decrease in the number of macrophages present in wounds (Fig. 4). 3 d after injury, the wounds of mice treated with anti–MIP-1α contained significantly less (41% decrease, $P < 0.01$) macrophages than controls. Immunohistochemical staining of sections from mice treated with rabbit antiserum confirmed that rabbit Ig was present at the site of injury (data not shown).

Depletion of wound MIP-1α results in reduced angiogenic activity. Because macrophages are believed to be key regulators of angiogenesis in wounds, the effect of the decreased macrophage population on wound angiogenesis was assessed. The angiogenic environment of the wounds of mice treated with anti–MIP-1α antiserum was examined using the in vivo corneal micropocket assay for neovascularization (Table I) (Fig. 5). Wound homogenates (day 3) from mice ($n = 3$) treated with anti–MIP-1α antiserum had a significantly diminished angiogenic response in the corneal assay ($P < 0.01$). Whereas wound homogenates from control animals produced a positive corneal angiogenic response in 10 of 11 corneas (73%), wound homogenates from mice treated with anti–MIP-1α produced a positive angiogenic response in only 3 of 11 corneas (27%).

Depletion of wound MIP-1α reduces collagen synthesis. Previous investigations suggest that wound macrophages can promote collagen synthesis (7). Therefore anti–MIP-1α treatment with its attendant decrease in macrophage population might be expected to result in decreased wound collagen synthesis. To examine this possibility, collagen synthesis was assessed by determining the amount of hydroxyproline in tissue samples derived 7 d after injury from treated and control mice (Table II). Hydroxyproline levels were 29% less in mice treated with MIP-1α than in control (normal rabbit serum) treated mice (significant, $P < 0.05$). These results suggest that the reduced macrophage infiltration can negatively affect collagen deposition.

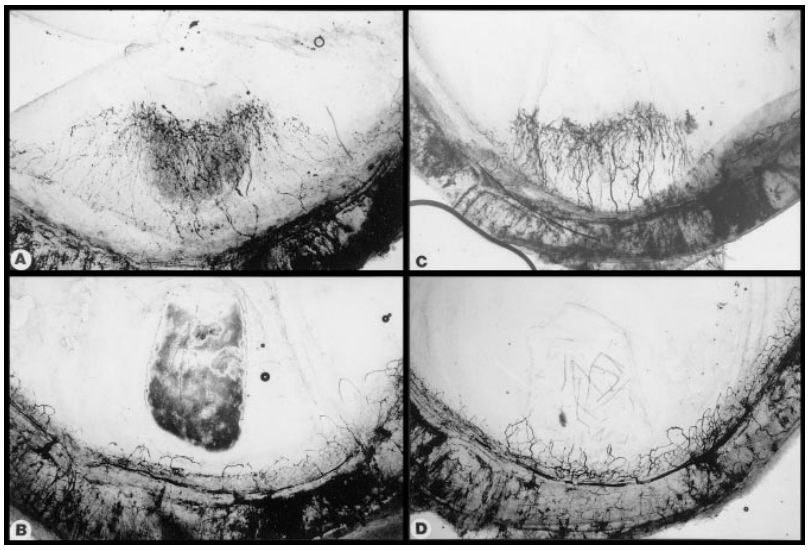

Figure 5. The effect of anti–MIP-1α treatment on the angiogenic activity in murine wounds. Wound homogenates were prepared from mice treated with anti–MIP-1α or preimmune rabbit serum. The angiogenic activity of the homogenates was then assessed in the corneal micropocket assay as described in Methods ($n = 11$). Representative photographs of the corneal assay are shown. (*a* and *c*) Wound homogenates from mice treated with control (preimmune) serum. (*b* and *d*) Wound homogenates from mice treated with anti–MIP-1α antiserum.

Table II. Collagen Levels In Wounds of Mice Treated with Anti–MIP-1α Antiserum

Treatment	
	μg/wound±SD
None	33.43±4.36
Preimmune serum	33.98±5.06
Anti–MIP-1α antiserum	24.53±9.62*

The hydroxyproline content of wounds was determined at day 7 after injury for mice receiving either no treatment, preimmune serum, or anti–MIP-1α antiserum. Data are expressed as mean hydroxyproline content per wound±SD ($n = 4$). *$P < 0.05$, compared to the group receiving no treatment. The results are representative of two experiments.

Discussion

Tissue repair involves an acute inflammatory reaction and is typified by the ordered infiltration of neutrophils and then macrophages. Depending upon the wound model, maximum macrophage infiltration generally occurs between days 3 and 5 after injury (30). The mechanism of the specific recruitment of monocytes into wounds, where they differentiate into macrophages, is poorly understood. Previous investigations in other models of inflammation have shown that monocyte recruitment depends upon both the activation of the endothelium, and the generation of monocyte chemoattractants (for review see reference 32). In healing wounds, a large array of monocyte chemoattractants is probably present, as platelets, macrophages, and other cell types produce chemokines that facilitate macrophage infiltration. At least two sources of MIP-1α occur in wounds. First, MIP-1α is found in platelets, and some MIP-1α may be quickly released from platelets as wound hemostasis occurs (31). In addition, immunolocalization suggests that macrophages within the wound are also a source of MIP-1α. Theoretically, platelet-derived MIP-1α would reach maximum levels during or soon after hemostasis. Yet the current study demonstrates that MIP-1α levels within wounds continue to rise until day 3 after injury, and that this rise directly parallels macrophage infiltration. This sequence of events suggests that maximal monocyte recruitment into wounds depends upon the sustained local production of chemoattractants such as MIP-1α during the early inflammatory phase.

Previous investigations in our laboratory, as well as others, have documented the production of several specific monocyte chemoattractants in wounds, including MCP-1 and TGF-β (30, 33). While these cytokines may play a role in monocyte recruitment into wounds, our attention was turned to MIP-1α because of its recently described importance in mediating leukocyte recruitment in several disease processes. MIP-1α has been shown to influence inflammatory infiltration in rheumatoid arthritis, the acute lung injury after endotoxemia, bleomycin-induced lung injury, and Schistosoma mansoni egg–induced granulomatous inflammation (16–20). Investigations in the MIP-1α knockout mouse suggest MIP-1α also plays a vital role in mediating leukocyte infiltration of Coxsackievirus-induced myocarditis (15). Our data suggest that MIP-1α plays a prominent role in mediating the inflammation that follows traumatic tissue damage. MIP-1α was produced within wounds at physiologic concentrations, and the time of peak protein levels corresponded with the maximum macrophage infiltration into the wound. Further, neutralization of MIP-1α resulted in a 41% decrease in the number of infiltrating macrophages. This report is the first to establish the role of a particular chemokine as a mediator of monocyte migration into wounds. Given the abundance of monocyte chemoattractants that have been reported to occur in wounds, the consequences of neutralizing MIP-1α, a single chemokine, are somewhat remarkable. One explanation is that the neutralization of MIP-1α results in an overall reverse amplification of the inflammatory response to injury. In the most simplistic model, the neutralization of MIP-1α would initially lead to a decrease in macrophage infiltration into wounds. Compared to control wounds, these wounds would subsequently produce less of all macrophage-derived monocyte chemoattractants, including MCP-1, MIP-1β, RANTES, and TGF-β. This decreased chemoattractant activity would lead to an overall decline in monocyte recruitment. This model provides a mechanistic explanation for the fact that, despite chemokine redundancy, the neutralization of a single chemokine can sometimes affect a remarkable decrease in the inflammatory response. A reverse amplification model might apply to certain investigations of the inflammatory response in knockout mice, where the elimination of a single chemokine sometimes has profound effects.

The decrease in macrophage infiltration due to anti–MIP-1α neutralizing antibody is striking, yet perhaps a more interesting observation is that this treatment leads to decreased wound angiogenic activity and collagen synthesis. While these findings underscore the critical role of MIP-1α in wound repair, the data do not establish whether the observed effect is direct or indirect. Previous reports that wound macrophages are themselves both angiogenic and fibrogenic lend support to the hypothesis that the neutralization of MIP-1α downregulates wound healing primarily by reducing macrophage recruitment and activation within the wound bed (7, 34). It is unclear whether MIP-1α directly influences angiogenesis or fibrogenesis, as a role for this chemokine in those processes has yet to be identified. For example, in in vivo assays for angiogenic activity, recombinant MIP-1α appears to induce a mononuclear infiltration that precedes the onset of maximal angiogenesis, suggesting that MIP-1α may not be a direct angiogenic factor (Strieter, R.M., unpublished observations).

The normal wound healing response, which includes an acute inflammatory phase followed by spontaneous resolution, has been well documented. In healing wounds, MIP-1α levels follow a specific temporal sequence, increasing during the inflammatory phase, and decreasing as inflammation resolves and repair proceeds. As a model system, then, the normal wound healing response may provide further insight into the regulation of production of inflammatory chemokines such as MIP-1α. In particular, the resolution phase of wound healing might provide significant information regarding the manner by which chemokine production concludes and inflammation ultimately resolves.

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