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Regulatory Role of Endogenous Interleukin-10 in Cutaneous Inflammatory Response of Murine Wound Healing

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This study investigated the role of endogenous interleukin (IL)-10 in cutaneous wound healing. Both IL-10 mRNA and protein were detectable in murine incised wounds for 10 days after injury. The IL-10 protein level peaked 3 h after incision, returned to the normal level by 24 h, but increased again to another peak at 72 h. *In situ* hybridization studies and immunostaining revealed that epidermal cells and infiltrating mononuclear cells were the major source of IL-10. Neutralizing antibody studies demonstrated that IL-10 inhibited the infiltration of neutrophils and macrophages toward the site of injury. IL-10 also inhibited overexpression of C-C chemokines (monocyte chemoattractant protein-1, macrophage inflammatory protein-1α) and proinflammatory cytokines (IL-1β, IL-6, tumor necrosis factor-α) *in vivo.* These results suggest that IL-10 may play an important regulatory role in the phase-specific infiltration of neutrophils and macrophages as well as the cytokine production in the inflammatory response of cutaneous wound healing.

The inflammatory response is an important component of wound healing and involves the ordered migration of neutrophils and then macrophages. Within wounds, these neutrophils and macrophages secrete various inflammatory mediators that modulate the healing process (1). To accomplish successful wound repair and tissue regeneration, the inflammatory response must be tightly regulated *in vivo.* Interleukin (IL)-10 is known to be a major regulator in suppressing the inflammatory response (2). IL-10 inhibits the synthesis of proinflammatory cytokines such as IL-1β, IL-6 and tumor necrosis factor-α (TNF-α) in activated macrophages (3, 4). Similar effects have been observed on neutrophils (5). IL-10 also inhibits leukocyte migration toward the site of inflammation by, in part, inhibiting the synthesis of several chemokines, including monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α) (6, 7). Both of the C-C chemokines promote monocyte accumulation and MIP-1α is also a potent neutrophil chemoattractant in mice (8). Moreover, recent studies have shown that MCP-1 and MIP-1α play prominent roles in macrophage recruitment into wounds during cutaneous wound repair (9–11). Because IL-10 can be produced by resident skin cells such as keratinocytes as well as inflammatory cells involved in the healing process (2), it is predicted that IL-10 is involved in the cutaneous wound healing as a regulator of the inflammatory response. The role of endogenous IL-10 in cutaneous wound healing was examined in this study.

MATERIALS AND METHODS

Expression of IL-10 during Cutaneous Wound Healing

Animal protocol. Male 8-week-old Crj-CD1 (ICR) mice, weighing 30–37 g (Charles River Breeding Laboratories, Japan), were anesthetized with intraperitoneal administration of sodium pentobarbital (5 μg/g). After shaving the dorsal region, a 2 cm full-thickness incision was made on the dorsal skin using a scalpel. At 1, 3, 6, 12, 24, 72, 144 and 240 h following the incision, a 2 $\times$ 1 cm area surrounding the wound was excised. As a control, skin specimens from mice without incision were examined. Animals were cared for in accordance with the Guidelines for the Care and Use of Laboratory Animals at Takara-machi Campus of Kanazawa University.

Reverse transcriptase-polymerase chain reaction (RT-PCR). Total RNA was isolated from each skin specimen using the RNA extraction kit ISOGEN (Nippon Gene, Japan). The total RNA (2 μg) was used to generate cDNA using the First-Strand cDNA Synthesis Kit (Life Science, FL). The cDNA was used for PCR with 40 cycles of amplification for IL-10 and 30 cycles for β-actin with an annealing temperature of 65°C. The primers used were previously described (12). Aliquots of the PCR products were subjected to agarose gel electrophoresis then stained with ethidium bromide. The PCR products were visualized under UV light.

Enzyme-linked immunosorbent assay (ELISA). The skin specimens were minced and homogenized with 0.5 ml phosphate-buffered saline (PBS) on ice. The homogenized samples were centrifuged at 12000 rpm for 20 min at 4°C and the supernatant was collected. The total protein levels in the samples were measured by Lowry's method. The quantitation of IL-10 was performed using an IL-10

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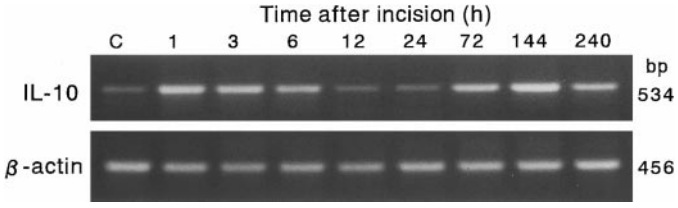

FIG. 1. RT-PCR analysis of IL-10 mRNA expression during cutaneous wound healing. Incised wounds were made on mice and the expression of IL-10 mRNA in skin specimens was examined using RT-PCR as described under Materials and Methods. The size in base pairs of the amplified cDNA fragments is indicated. Lane C indicates the results of normal mice (control). The results represent 5 independent experiments.

ELISA kit (Endogen, MA) according to the manufacturer's instructions. The IL-10 level in each skin specimen was determined using the formula described in our previous manuscript (13).

In situ hybridization (ISH). To obtain sense and antisense digoxigenin (DIG)-labeled RNA probes for IL-10 mRNA, RT-PCR product was obtained using the following primer pair with the addition of T7- and Sp6-RNA polymerase promoter to the 5′ end of each sense and antisense primer, respectively; sense, 5′-CAGCCGGGAAGACAATAACTGCAC-3′, antisense, 5′-GAGGGTCTTCAGCTTCTCACCC-3′. The sense and antisense probes corresponding to gene transcripts were obtained by in vitro transcription (DIG RNA Labeling Kit, Boehringer Mannheim Biochemica, Germany) according to the manufacturer's instructions. The sense probe was used as a negative control.

ISH was performed as previously described (14). Briefly, serial 10 μm-frozen sections were fixed with 4% paraformaldehyde (PFA) in PBS for 15 min and incubated at 37°C for 10 min with 2.0 μg/ml proteinase K in 10 mM Tris-HCl and 1 mM EDTA. After postfixation for 10 min in 4% PFA in PBS, the slides were dehydrated in graded concentrations of ethanol and air-dried. The sections were incubated for 16 h at 50°C with a hybridization mixture containing RNA probes, 50% deionized formamide, 10 mM Tris-HCl, pH 7.6, 10% dextran sulfate, 1× Denhardt's solution, yeast transfer RNA and salmon sperm DNA. The slides were washed in 2× sodium chloride-sodium citrate solution (SSC) containing 50% formamide for 20 min at 50°C, twice in 2× SSC for 30 min at 50°C and in 0.1× SSC for 30 min at 50°C. Then, the slides were incubated with a 1:500 dilution of alkaline phosphatase-conjugated anti-DIG polyclonal antibody (Boehringer Mannheim Biochemicals) for 1 h at room temperature (RT) and were incubated with a color-substrate solution containing nitroblue tetrazolium salt and 5-bromo-4-chloro-3-indolyl phosphate toluidinium salt.

Immunohistochemistry. The skin specimens were fixed with 10% formaldehyde in PBS and 4-μm paraffin-embedded tissue sections were prepared. The sections were pretreated with 3% H_2O_2 in methanol, and then blocked with 2% normal rabbit serum. The slides were incubated with a 1:100 dilution of goat IgG anti-mouse IL-10 polyclonal antibody (Santa Cruz Biotechnology, Inc., CA) for 16 h at 4°C. The secondary biotinylated rabbit anti-goat IgG antibody diluted 1:200 in PBS was reacted for 1 h at RT, followed by a 10-min incubation with avidin-biotin-peroxidase complexes (LSAB2 kit, DAKO, Denmark). Color development was performed with 3,3′-diaminobenzidine (DAB) and the slides were counterstained with hematoxylin. Control sections were incubated with primary antibody absorbed with the respective peptide antigen.

Effects of Anti-IL-10 Treatment

Neutralization of IL-10. A rat IgG1 anti-mouse IL-10 monoclonal antibody (Genzyme, MA) was used for the neutralization of IL-10. Mice were treated with the anti-IL-10 antibody by hypodermic injection into the proximal part of the wound margin at the time of incision (50 μg/injection). As a control, equivalent amounts of rat IgG1 were also given to another group of mice. The skin specimens were excised 6 h after incision. Similarly, mice were treated with either anti-IL-10 antibody or control IgG1 daily on days 0 to 2 (50 μg/day) and the skin specimens were excised 72 h after incision. As an additional control, mice without incision were similarly treated with either anti-IL-10 antibody or control IgG1 (50 μg/day) and the skin specimens were excised at 6 and 72 h.

RT-PCR. The expression of mRNA for MCP-1, MIP-1α, IL-1β, IL-6 and TNF-α in each skin specimen was examined using the RT-PCR procedure described above. The primers used were previously described (7, 14, 15). All amplification reactions were performed for 28 cycles with an annealing temperature of 65°C except those for MCP-1, which was cycled 30 times, and IL-1β, which was cycled 26 times for skin specimens obtained 72 h after incision. Preliminary experiments on cycle titration revealed that the amplification reaction did not plateau within the cycles used for any primer pair. The PCR products were visualized under UV light on agarose gels.

ELISA. The protein levels for the cytokines were measured using commercial ELISA kits for MCP-1 (R&D Systems, MN), MIP-1α (R&D Systems), IL-1β (Genzyme), IL-6 (Endogen) and TNF-α (Endogen). Sample preparation and evaluation of cytokine levels were performed using the procedure described above.

Histological evaluation. To investigate the effect of anti-IL-10 treatment on the cellular infiltrate, paraffin-embedded tissue sections of skin specimens taken 6 and 72 h after incision were prepared. After staining with naphthol AS-D chloroacetate (16), the total number of infiltrating neutrophils was counted in 5 random ×200 high power fields within the wound area and the mean was calculated for 6 serial sections for each mouse. To quantitate macrophages, immunostaining was performed using a rabbit anti-mouse macrophage antibody (Inter-Cell Technologies, Inc., NJ) as a primary antibody. Briefly, the sections were pretreated with 3% H_2O_2, and then blocked with 2% normal goat serum. The slides were incubated with a 1:500 dilution of the primary antibody for 1 h at RT. Then, the EnVision+ (DAKO) was reacted for 30 min at RT. Color development was performed with DAB and the slides were counter-

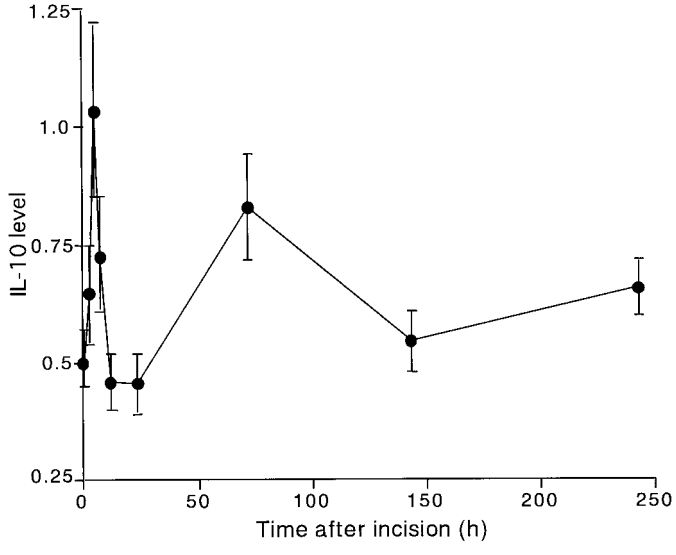

FIG. 2. Temporal expression of IL-10 protein during cutaneous wound healing. The protein levels in skin specimens were determined using ELISA as described under Materials and Methods. The results represent the mean ± SEM of 5 mice per group. The data of 0 h after incision represent the results obtained from normal mice.

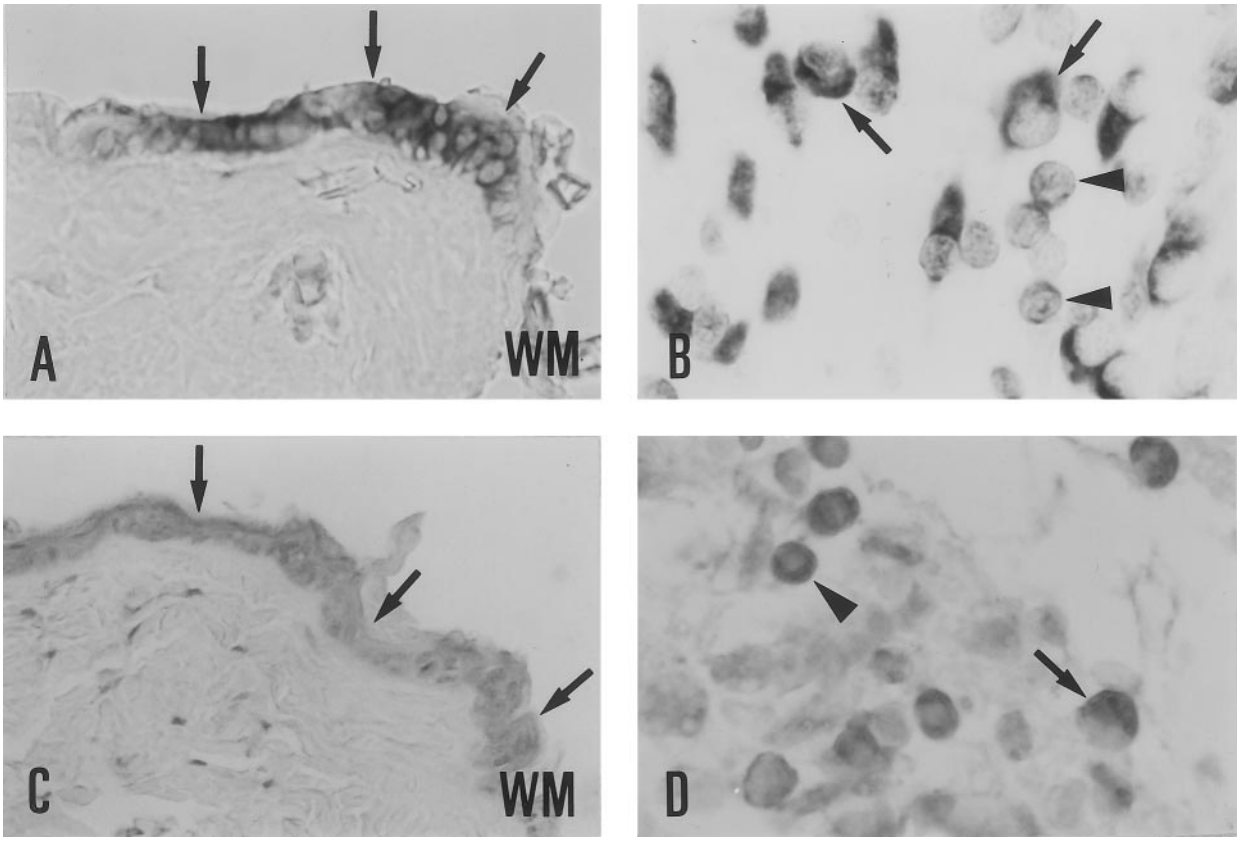

FIG. 3. Localization of IL-10 mRNA (A, B) and protein (C, D) during cutaneous wound healing. (A) 1 h after incision. Positive hybridization signals for IL-10 mRNA were detected in epidermal cells (arrows) of the wound margin (WM). (B) 3 days after incision. Two different types of infiltrating mononuclear cells (MNCs) (arrows and arrowheads) showed positive hybridization signals, probably macrophages and lymphocytes, respectively. (C) 3 h after incision. Epidermal cells (arrows) were immunoreactive for anti-IL-10 antibody. (D) 3 days after incision. Two different types of MNCs (arrow and arrowhead), which corresponded with those of IL-10 mRNA-positive cells, were immunostained. Original magnification; (A) ×200, (B) ×400, (C) ×200, (D) ×400.

stained with hematoxylin. The number of macrophages was counted using the procedure described above.

Statistics. The mean and standard deviations were calculated for the survival times after incision. The difference in the cytokine levels and the number of infiltrating cells between the anti-IL-10 treated mice and control mice was determined at the survival time. The significance was determined by Student's *t*-test with $p < 0.05$ being considered significant.

RESULTS

Expression of IL-10 mRNA and protein during cutaneous wound healing. Basal levels of both IL-10 mRNA and protein were detectable in normal skin specimens by RT-PCR and ELISA (Figs. 1 and 2). After incision, there was an increase in the IL-10 levels. The IL-10 protein level peaked at 3 h, returned to normal level by 24 h, then increased again to a peak at 72 h (Fig. 2). The protein level appeared to correlate with the induction and upregulation of IL-10 mRNA (Fig. 1).

Cellular source of IL-10 mRNA and protein. Positive hybridization signals of the antisense probe for IL-10 mRNA were detected in epidermal cells of normal skin (data not shown). When the sense probe was used, there were no signals other than background staining observed, suggesting the specificity of positive signals for the target sequence. Intense positive signals were detected in epidermal cells proximal to the wound margin 1 h after incision (Fig. 3A). Infiltration of neutrophils was evident at 6 h, but these cells lacked positive hybridization signals. Within 24 to 72 h, the neutrophils had been largely replaced by macrophages and granulation tissue had invaded the incision space. In the cytoplasm of two different types of mononuclear cells (MNCs), localization of IL-10 mRNA signals were observed (Fig. 3B). Reepithelialization was evident at 144 h and the regenerating epidermal cells were also labeled with antisense probe for IL-10 (data not shown). The wounds were almost healed 240 h after incision.

Cell types showing IL-10 immunoreactivity corresponded with those of the IL-10 mRNA-positive cells. Figure 3C shows positive signals for IL-10 in epidermal cells 3 h after incision. Between 24 and 144 h, two different types of MNCs also showed immunoreactivity for IL-10 (Fig. 3D). The signals were abolished when

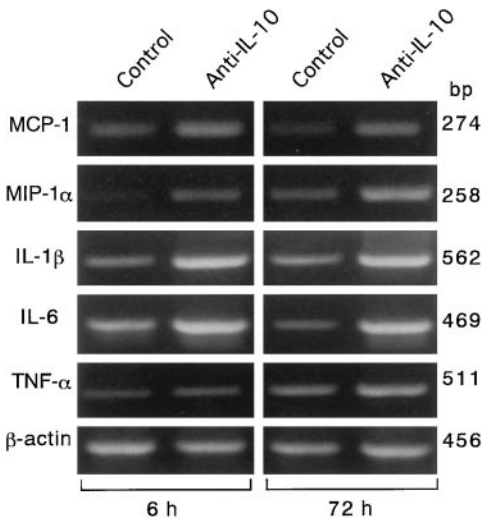

FIG. 4. Effects of anti-IL-10 treatment on mRNA expression of chemokines and proinflammatory cytokines. Mice were treated with either anti-IL-10 antibody or control IgG1 (50 μg/day) by hypodermic injection into the proximal part of the wound margin. The mRNA expression of the cytokines in skin specimens was examined 6 and 72 h after incision using RT-PCR as described under Materials and Methods. The size in base pairs of the amplified cDNA fragments is indicated. The results represent 5 independent experiments.

the primary antibody was absorbed with the respective peptide antigen.

Effects of anti-IL-10 treatment. Because there were two peaks of IL-10 (Fig. 2), the effects of the administration of anti-IL-10 antibody were examined 6 and 72 h after incision. Increased levels of mRNA expression especially for IL-1β and IL-6 were observed by RT-PCR after the anti-IL-10 treatment (Fig. 4). An increase in the protein levels for all cytokines examined was detected by ELISA following treatment (Fig. 5), and these results seemed to correlate with the findings of RT-PCR analysis. In mice without incision, there was no significant difference in the cytokine levels observed between the anti-IL-10 treated mice and control mice, suggesting that the induction of cytokine production after the treatment was caused by the incisional wounds. Histologically, the treatment caused a significant ($p < 0.05$) increase in the number of infiltrating neutrophils (Table 1) and remarkable tissue swelling 6 h after incision (Fig. 6). The treatment also caused a significant increase in the number of infiltrating macrophages and more prolonged accumulation of neutrophils 72 h after incision (Table 1).

DISCUSSION

In the present experiments, IL-10 expression was rapidly upregulated after incision, returned to normal level, but increased again at both the mRNA and protein levels. The early peak of IL-10 might reflect the production of IL-10 by epidermal cells, as was demonstrated by the ISH technique and immunostaining, and preceded the infiltration of neutrophils toward the injury site. While the later peak of IL-10 might be due to IL-10 production by infiltrating MNCs as well as regenerating epidermal cells, and seemed to be temporally linked to maximal macrophage infiltration into the wound, which usually occurs between 2 and 3 days (9–11). To clarify the role of endogenous IL-10, the effects of anti-IL-10 treatment were examined 6 and 72 h after incision.

Once the mice were treated with the anti-IL-10 antibody, a significant increase in the number of neutrophils was observed 6 h after incision. IL-10 itself seems to have little inhibitory effect on neutrophil chemotaxis (17), but it inhibits neutrophil production of MIP-1α, IL-1β and TNF-α (5, 18). MIP-1α is a potent neutrophil chemoattractant in mice (8), and IL-1β and TNF-α also indirectly elicit neutrophil extravasation by changing the adhesive properties of endothelial cells and inducing the production of several chemokines (19). Therefore, the induction of neutrophil infiltration after the treatment might be functionally associated with high levels of MIP-1α, IL-1β and TNF-α expression, and it appears that the epidermal cell-derived IL-10 may regulate the neutrophil infiltration in the early phase of cutaneous wound healing by, in part, suppressing the production of these chemotactic factors.

It is unclear whether MCP-1 and IL-6 expression can be modulated in neutrophils (10, 11, 20), but endothelial cells and dermal fibroblasts can produce both of the

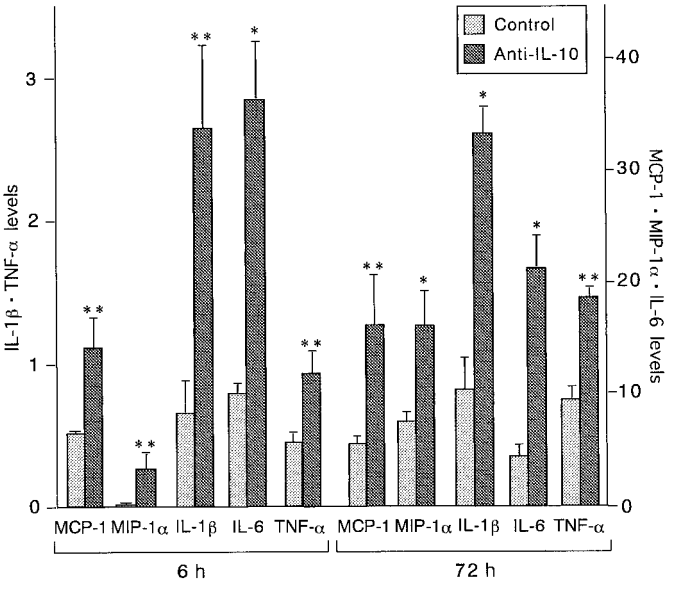

FIG. 5. Effects of anti-IL-10 treatment on the expression of chemokines and proinflammatory cytokines. The protein levels in skin specimens were determined 6 and 72 h after incision using ELISA as described under Materials and Methods. The results represent the mean ± SEM of 5 mice per group. *, $p < 0.01$; **, $p < 0.05$ (vs. control).

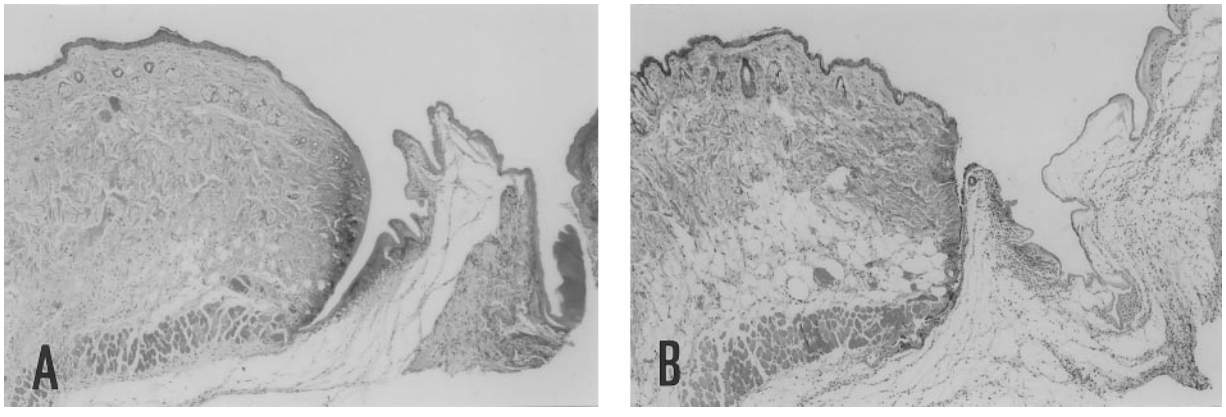

FIG. 6. Histological alterations following the anti-IL-10 treatment observed 6 h after incision. (A) untreated. (B) anti-IL-10 treatment. Five mice in each group were examined and representative results from individual mice are shown. Note the heavy infiltration of inflammatory cells (neutrophils) and remarkable tissue swelling after the treatment. HE. Original magnification, ×40.

cytokines (21–23). The high levels of MCP-1 and IL-6 expression, observed 6 h after incision following the anti-IL-10 treatment, indicate that endothelial cells are not the major source of the cytokines in the early phase of cutaneous wound healing, because IL-10 is known to induce MCP-1 and IL-6 production by endothelial cells (21). However, fibroblasts can induce MCP-1 and IL-6 production in response to IL-1β and TNF-α (22, 23), thus providing a possible explanation for the high levels of MCP-1 and IL-6 expression after the treatment. Naturally, the contribution of keratinocytes to the cytokine production cannot be excluded (10, 23).

The inflammatory response is often accompanied by an increase in vascular permeability that is mediated by various factors, including histamine, prostaglandin E_2 (PGE_2), prostacyclin (PGI_2) and platelet-activating factor (PAF). IL-1β and TNF-α cause vasodilation and increase vascular permeability by producing PGE_2, PGI_2 and PAF (19). MCP-1 and MIP-1α also induce tissue swelling by releasing histamine (8). Berg *et al.* (24) demonstrated that the application of a chemical irritant to the ear of IL-10 knock-out mice produced more extensive edema than those occurred in wild type mice. Our data suggest that IL-10 may regulate the vascular permeability by suppressing the release of these vasoactive mediators during the early phase of cutaneous wound healing.

TABLE 1

Number of Infiltrating Neutrophils and Macrophages in the Wound Area of Mice with and without Anti-IL-10 Treatment

Anti-IL-10 treatment	Time after incision (h)	Number of neutrophils	Number of macrophages
−	6	252 ± 36	NE
+	6	713 ± 219*	NE
−	72	76 ± 29	33 ± 10
+	72	331 ± 147**	75 ± 21*

Note. Mice were treated with either anti-IL-10 antibody or control IgG1 (50 μg/day). The number of infiltrating neutrophils and macrophages in the incised wound area was determined under the light microscopy. The data represent the mean ± SD of 5 mice per group. NE, not examined. *, $p < 0.01$; **, $p < 0.05$ (vs. control).

The anti-IL-10 treatment also caused a significant increase in the number of infiltrating macrophages 72 h after incision. Tissue macrophage plays a critical role in modulating the healing process (1) and all the cytokines examined in this study are macrophage products. Indeed, the macrophage has been shown to be the major source of the cytokines during cutaneous wound healing (9–11, 13, 14). Moreover, recent studies on wound healing have indicated that macrophage-derived MCP-1 and MIP-1α may lead to additional macrophage recruitment (9–11). It is well established that IL-10 inhibits the macrophage production of proinflammatory cytokines (3, 4) and IL-10 also inhibits the production of MCP-1 and MIP-1α by lipopolysaccharide- or IL-1β-stimulated macrophages (22, 25, 26). Because the later peak of IL-10 appeared to correlate with maximal macrophage infiltration into the wound, it is suggested that the later peak of IL-10 is closely associated with regulating macrophage recruitment and its cytokine production in the inflammatory response of cutaneous wound healing.

The more prolonged accumulation of neutrophils after treatment suggests that IL-10 may be responsible for the decline in the number of neutrophils in the wound area that often occurs within 1 to 2 days. Based on our data as well as previously reported findings (24), it seems likely that IL-10 is required for controlling the duration and intensity of cutaneous inflammatory response, and that the anti-IL-10 treatment may lead to the delay of normal wound healing as the result of dysregulated inflammatory response. In addition, it is

possible that IL-10 may also regulate T lymphocyte chemotaxis by suppressing the production of MCP-1 and MIP-1α (27, 28), and therefore, further study is necessary.

In summary, the present study provided evidence for the involvement of IL-10 in wound healing process. IL-10 may play an important regulatory role in the phase-specific infiltration of neutrophils and macrophages as well as cytokine production during cutaneous inflammatory response of wound healing.

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REFERENCES

1. Martin, P. (1997) *Science* **276,** 75–81.
2. Moore, K. W., O'Garra, A., de Waal Malefyt, R., Vieira, P., and Mosmann, T. R. (1993) *Annu. Rev. Immunol.* **11,** 165–190.
3. Fiorentino, D. F., Zlotnik, A., Mosmann, T. R., Howard, M., and O'Garra, A. (1991) *J. Immunol.* **147,** 3815–3822.
4. de Waal Malefyt, R., Abrams, J., Bennett, B., Figdor, C. G., and de Vries, J. E. (1991) *J. Exp. Med.* **174,** 1209–1220.
5. Cassatella, M. A., Meda, L., Bonora, S., Ceska, M., and Constantin, G. (1993) *J. Exp. Med.* **178,** 2207–2211.
6. Ajuebor, M. N., Das, A. M., Virag, L., Szabo, C., and Perretti, M. (1999) *Biochem. Biophys. Res. Commun.* **255,** 279–282.
7. Ajuebor, M. N., Das, A. M., Virag, L., Flower, R. J., and Perretti, M. (1999) *J. Immunol.* **162,** 1685–1691.
8. Alam, R., Kumar, D., Anderson-Walters, D., and Forsythe, P. A. (1994) *J. Immunol.* **152,** 1298–1303.
9. DiPietro, L. A., Burdick, M., Low, Q. E., Kunkel, S. L., and Strieter, R. M. (1998) *J. Clin. Invest.* **101,** 1693–1698.
10. Engelhardt, E., Toksoy, A., Goegeler, M., Debus, S., Bröcker, E-B., and Gillitzer, R. (1998) *Am. J. Pathol.* **153,** 1849–1860.
11. DiPietro, L. A., Polverini, P. J., Rahbe, S. M., and Kovacs, E. J. (1995) *Am. J. Pathol.* **146,** 868–875.
12. Ohshima, T., and Sato, Y. (1998) *Int. J. Legal Med.* **111,** 251–255.
13. Kondo, T., and Ohshima, T. (1996) *Int. J. Legal Med.* **108,** 231–236.
14. Sato, Y., and Ohshima, T. (1999) *Int. J. Legal Med.*, in press.
15. Lukacs, N. W., Chensue, S. W., Smith, R. E., Strieter, R. M., Warmington, K., Wilke, C., and Kunkel, S. L. (1994) *Am. J. Pathol.* **144,** 711–718.
16. Yam, L. T., Li, C. Y., and Crosby, W. H. (1971) *Am. J. Clin. Pathol.* **55,** 283–290.
17. Vicioso, M. A., Garaud, J. J., Reglier-Poupet, H., Lebeaut, A., Gougerot-Pocidalo, M. A., and Chollet-Martin, S. (1998) *Eur. Cytokine Netw.* **9,** 247–254.
18. Kasama, T., Strieter, R. M., Lukacs, N. W., Burdick, M. D., and Kunkel, S. L. (1994) *J. Immunol.* **152,** 3559–3569.
19. Mantovani, A., and Dejana, E. (1989) *Immunol. Today* **10,** 370–375.
20. Cassatella, M. A. (1995) *Immunol. Today* **16,** 21–26.
21. Sironi, M., Munoz, C., Pollicino, T., Siboni, A., Sciacca, F. L., Bernasconi, S., Vecchi, A., Colotta, F., and Mantovani, A. (1993) *Eur. J. Immunol.* **23,** 2692–2695.
22. Seitz, M., Loetscher, P., Dewald, B., Towbin, H., Gallati, H., and Baggiolini, M. (1995) *Eur. J. Immunol.* **25,** 1129–1132.
23. Paquet, P., and Pierard, G. E. (1996) *Int. Arch. Allergy Immunol.* **109,** 308–317.
24. Berg, D. J., Leach, M. W., Kühn, R., Rajewsky, K., Müller, W., Davidson, N. J., and Rennick, D. (1995) *J. Exp. Med.* **182,** 99–108.
25. Berkman, N., John, M., Roesems, G., Jose, P. J., Barnes, P. J., and Chung, K. F. (1995) *J. Immunol.* **155,** 4412–4418.
26. Yano, S., Yanagawa, H., Nishioka, Y., Mukaida, N., Matsushima, K., and Sone, S. (1996) *J. Immunol.* **157,** 2660–2665.
27. Roth, S. J., Carr, M. W., and Springer, T. A. (1995) *Eur. J. Immunol.* **25,** 3482–3488.
28. Jinquan, T., Deleuran, B., Gesser, B., Maare, H., Deleuran, M., Larsen, C. G., and Thestrup-Pedersen, K. (1995) *J. Immunol.* **154,** 3742–3752.