Interleukin 10 but Not Interleukin 4 Is a Natural Suppressant of Cutaneous Inflammatory Responses

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Summary

We have examined the role of endogenously produced interleukin (IL) 4 and IL-10 in the regulation of inflammatory and immune reactions in the skin. In these experiments, irritant and contact hypersensitivity (CH) responses were elicited in mice with targeted disruptions of the IL-4 (IL-4T) or IL-10 (IL-10T) gene. Our study showed that IL-4T and wild-type (wt) mice exhibited equivalent responses to the irritant croton oil. In contrast, the response of IL-10T mice challenged with croton oil was abnormally increased. When IL-10T mice were exposed to a higher dose of irritant, irreversible tissue damage occurred. By comparison, any treatment of wt mice with croton oil resulted in far less tissue damage and resolution of inflammation. Neutralizing antibody studies demonstrated that the necrosis that occurred in IL-10T mice was due to the overproduction of tumor necrosis factor. The anti-tumor necrosis factor antibody treatment of IL-10T mice did not significantly reduce the edema or the influx of inflammatory cells, suggesting that these changes were due to the uncontrolled production of other proinflammatory cytokines. T cell–dependent immune responses were also evaluated using the contact sensitizer oxazolone. The response of IL-4T mice did not differ from wt mice. In contrast, IL-10T mice mounted an exaggerated CH response, increased in both magnitude and duration as compared with wt mice. Based on these studies, we have concluded that IL-10, but not IL-4, is a natural suppressant of irritant responses and of CH, and it limits immunopathologic damage in the skin.

IL-4 and IL-10 were first identified as products of Th2 clones (1, 2) and are part of the unique cytokine pattern that distinguishes Th2 cells from other types of T cells. Th2 cells are believed to provide major support for the development of humoral immunity. This is due, in part, to the strong B cell–stimulating activities of IL-4 and IL-10 (3–6). Numerous studies have shown that IL-4 and IL-10 also possess potent immunosuppressive activity. IL-4 and IL-10 can inhibit the synthesis of proinflammatory cytokines by activated macrophages (7–12). Furthermore, IL-4 and IL-10 can inhibit, directly or indirectly, the synthesis of cytokines by Th1 clones (12–17). Th1 cells, in contrast to Th2 cells, produce IFN-γ and TNF-β and thus have been associated with the generation of cell-mediated immunity and delayed-type hypersensitivity reactions (18). Based on the suppressive actions ascribed to IL-4 and IL-10 in various in vitro assays, it has been predicted that IL-4 and IL-10 may negatively regulate inflammatory and cell-mediated responses in vivo.

Several investigations have focused on the potential of IL-4 and IL-10 to influence immunological reactions in the skin. The possibility that IL-4 and IL-10 are normally involved in modifying skin reactions seemed likely, since it was found that these proteins can be made by certain cell types that reside in the skin. Recent studies have indicated that, in addition to Th2 cells, IL-4 can also be produced by connective tissue mast cells (19), which are found in large numbers in the skin (20). Production of IL-4 has been documented during the sensitization phase of contact hypersensitivity (CH)[1] (21, 22). Moreover, IL-4 messenger RNA (mRNA) expression has been associated with the initiation of acute skin inflammation in patients with atopic dermatitis (23). IL-10 is produced in skin by keratinocytes (24–26) and macrophages (10). IL-10 mRNA and protein have been detected in intact skin,

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[1] *Abbreviations used in this paper:* CH, contact hypersensitivity; H & E, hematoxylin and eosin; IL-4T, IL-4 gene with targeted disruption; IL-10T, IL-10 gene with targeted disruption; IL-10T, IL-10 gene with targeted disruption; mRNA, messenger RNA; TNCB, trinitrochlorobenzene; wt, wild type.

and their expression is increased after exposure to trinitrochlorobenzene (TNCB) (24, 26) and UV irradiation (27).

In a model of CH, it was found that IL-10 can regulate both the sensitization and elicitation stages. Injection of mice with IL-10 shortly before sensitization with TNCB induced anergy (28), and administration of IL-10 before rechallenge diminished the recall response (26, 29). In contrast, the infusion of mice with IL-4 failed to inhibit the sensitization step (30). Nevertheless, IL-4 was able to reduce the magnitude of the recall response. These results provided evidence that exogenous IL-4 and IL-10 may be administered to suppress CH reactions. To determine whether IL-4 and IL-10 normally function as regulators of CH, neutralizing antibodies were given to ablate the activities of endogenously produced proteins. In mice treated with anti–IL-10 antibodies, the duration of the CH response was prolonged (26). In mice treated with anti–IL-4 antibodies, there was no effect on the induction of CH (21, 30), whereas the effect on the magnitude of the response upon rechallenge was variable (21, 30).

We have evaluated the importance of IL-4 and IL-10 in the regulation of inflammatory and immune reactions in skin using mice deficient in the production of IL-4 or IL-10. These mutant mice were generated by targeted disruptions of the IL-4 (IL-4T; 31) or IL-10 (IL-10T; 32) gene. Gene-targeted animals have proven to be valuable models for delineating the activities of various cytokines in complex immunologic responses. Our study has focused on the role of endogenously produced IL-4 and IL-10 to modify two types of skin responses, the irritant response and CH.

Materials and Methods

Animals. IL-10T mice generated on a C57Bl/6/129 (B6/129) background (32) and wild-type (wt) littermate controls were derived by cesarean section at Simonsen Laboratory (Gilroy, CA) and maintained in our animal facility at DNAX Research Institute (Palo Alto, CA). Healthy 5–6-wk-old IL-10T mice were used for this study. IL-4T mice (31) bred onto the 129J or C57Bl/6J background were kindly provided by Dr. R. Coffman (DNAX Research Institute). wt 129J and C57Bl/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

Reagents and Antibodies. Oxazolone (ethoxymethylene-2 phenyl oxazolone) and croton oil were obtained from Sigma Chemical Co. (St. Louis, MO). Oxazolone was resuspended in 100% EtOH, and croton oil was diluted in acetone. Purified rat anti–mouse TNF (XT22) and an isotype-matched control mAb, anti-β-galactosidase (GL-117), were kindly provided by Dr. J. Abrams (DNAX Research Institute). The anti-TNF antibody XT22 was elicited against TNF-α, but it neutralizes both TNF-α and TNF-β (Abrams, J., personal communication).

Experimental Protocols. The irritant response was induced by applying 10 μl of 1% (vol/vol) croton oil (low dose) or undiluted croton oil (high dose) to the dorsal and ventral aspects of the right ear. The reaction was evaluated by measuring the increase in ear thickness with time. To induce a chronic skin lesion, undiluted croton oil (50 μl) was applied to a 1-cm^2 shaved area on the back of wt or IL-10T mice. The resulting inflammatory response was monitored by measurement of ulcer formation as well as by microscopic examination.

CH was elicited by painting the shaved abdomens of mice with 150 μl of a 3% solution of oxazolone. After 6 d, 10 μl of 1% oxazolone was applied to the dorsal and ventral sides of the right ear. The reaction was evaluated by measuring ear thickness with an engineer's caliper (Mitutoyo/MTI, Paramus, NJ). The amount of swelling at 24, 48, 72, and 96 h after challenge was determined by measuring the difference in ear swelling in challenged (right) and unchallenged (left) ears.

Histologic Analysis. Tissues from mutant and normal mice were fixed in 10% neutral buffered formalin, routinely processed, sectioned at 6 μm, and stained with hematoxylin and eosin (H & E). Tissue sections were graded semiquantitatively by the same pathologist (M. W. Leach) as 0 (no change) to 4 (most severe).

Treatment with Anti-TNF Antibodies. Mice were injected intraperitoneally with 2 mg of anti-TNF mAb or anti-β-galactosidase mAb 4 h before the application of croton oil. The same lot and amount of the anti-TNF mAb used here has been shown to ablate the effects of endogenously produced TNF in several in vivo models (17, 33, 34).

Statistical Analysis. Significant differences between experimental groups were evaluated by the nonparametric Mann Whitney U test.

Results

Irritant Responses of IL-4T and IL-10T Mice. Data presented in Fig. 1 show the irritant responses of IL-4T and wt mice after challenge with the irritant, croton oil. After exposure to 1% croton oil, maximal swelling was detected at 24 h, with a significant decrease by 48 h (Fig. 1 *A*). The IL-4T mice did not differ from wt in their response to the irritant. When the mice were challenged with a higher dose of irritant, increased swelling was observed at 24 h and maintained at 48 h (Fig. 1 *B*). As with the low-dose treatment, there was no difference between IL-4T and wt mice.

The data presented in Fig. 2 show that the irritant responses of IL-10T and wt mice were markedly different. After exposure to 1% croton oil, the swelling in IL-10T mice was twice that of wt mice (Fig. 2 *A*). Furthermore, compared with the 24-h measurement, the swelling observed in the IL-10T ears was only slightly decreased after 48 h, whereas the swelling at 48 h in wt mice was diminished by nearly 50%. To further evaluate the altered irritant responses in IL-10T mice, we used croton oil at a higher concentration. We found that the swelling in IL-10T mice did not differ significantly from wt mice at 24 h (Fig. 2 *B*). At 48 h the reaction in the wt ears remained high, whereas, unexpectedly, the ears of IL-10T mice showed no evidence of swelling (Fig. 2 *B*).

WT and IL-10T ears were examined microscopically (Fig. 3). After challenge with 1% croton oil, wt ears (Fig. 3 *C*) had a predominantly dermal infiltrate of granulocytes and mononuclear cells, mild edema, and mild hemorrhage. Occasional intraepidermal pustules were also present. The IL-10T ears elicited with 1% croton oil (Fig. 3 *D*) showed a more extensive inflammatory cell infiltrate in the dermis, with more edema, moderate hemorrhage, and focal areas of epithelial necrosis. There was also an increase in the frequency and size of intraepidermal pustules.

Exposure to high-dose croton oil–induced lesions in wt ears was consistent with a severe inflammatory response. The

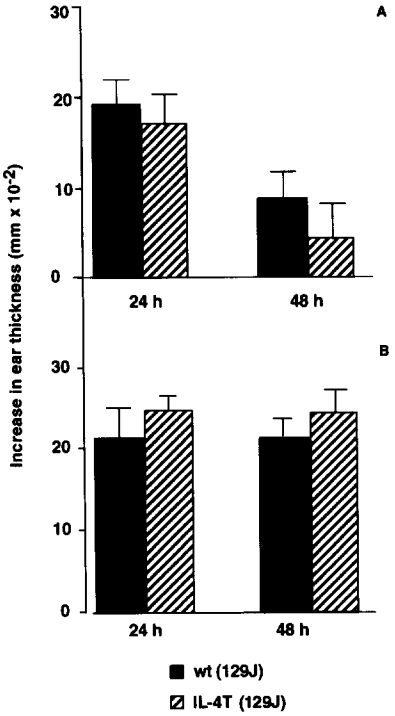


Figure 1. Irritant responses of IL-4T mice. The increases in ear thickness of IL-4T and wt mice on a 129J background (129J) were measured 24 and 48 h after application of 1% croton oil (low dose; *A*) and undiluted croton oil (high dose; *B*). Data presented are the mean values ± SD of six mice per group and are representative of two independent experiments. Differences between the two groups were not significant, $P > 0.05$.

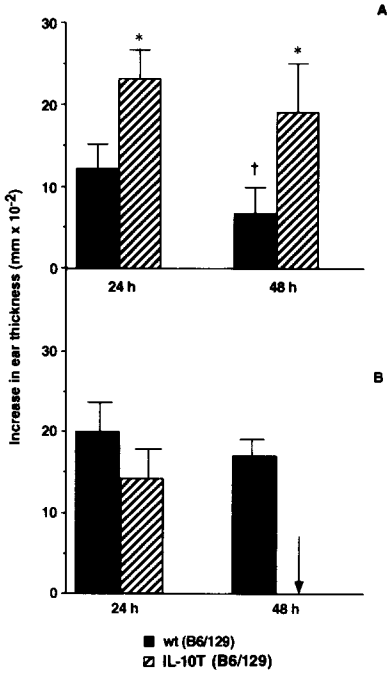


Figure 2. Irritant responses of IL-10T mice. The increases in ear thickness of IL-10T and wt mice on a B6/129 background were measured 24 and 48 h after application of 1% croton oil (low dose; *A*) and undiluted croton oil (high dose; *B*). Data presented are the mean values ± SD of six mice per group and are representative of three independent experiments. An arrow indicates that no increase in ear thickness was detected. $^*P < 0.01$ compared with values obtained with wt mice at the same time point; † $P < 0.01$ compared with wt at 24 h.

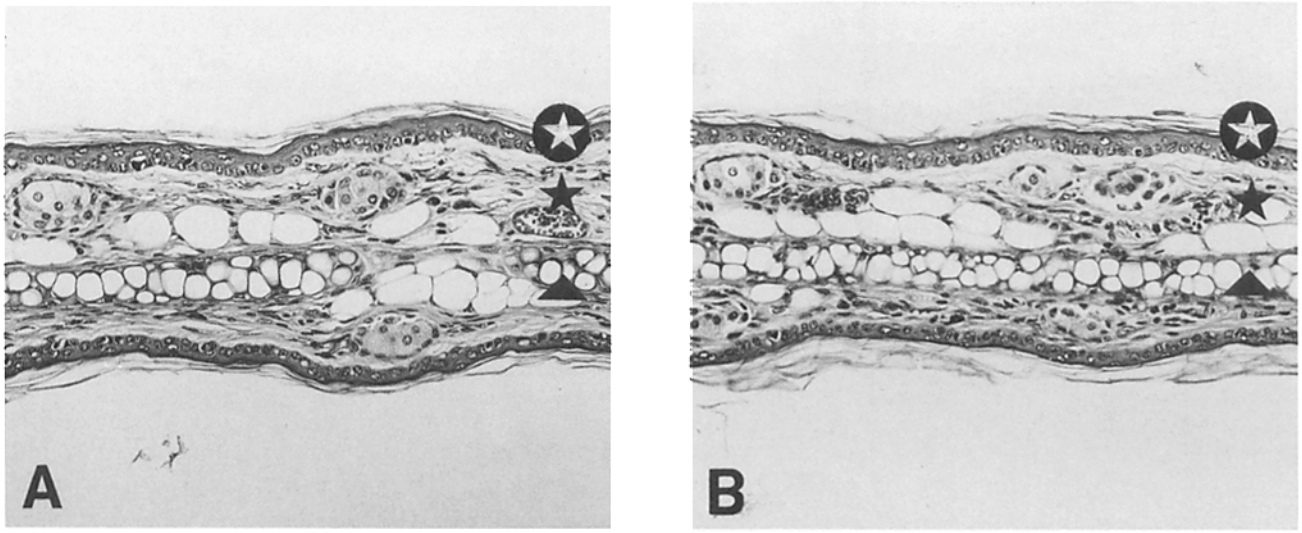
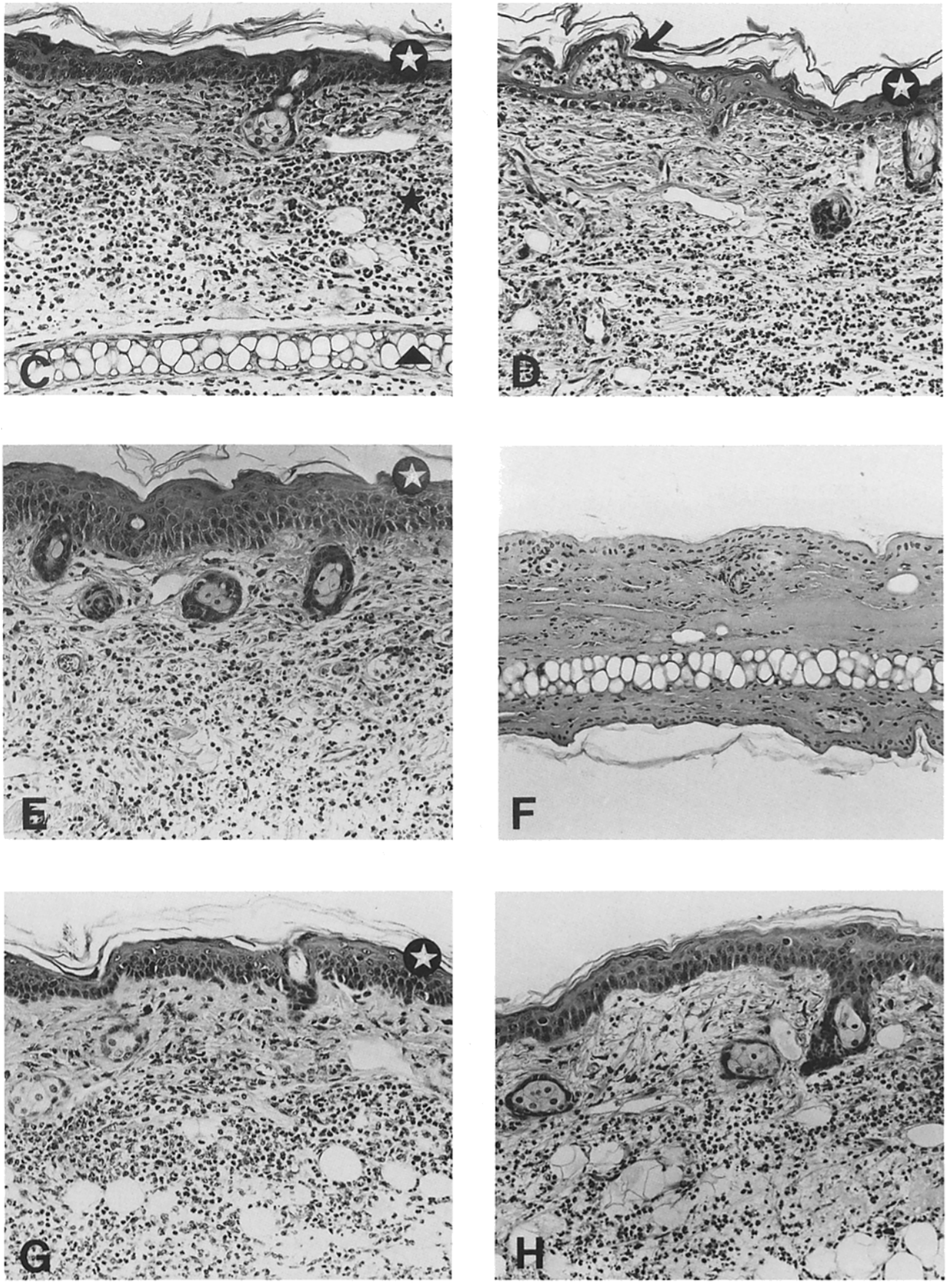


Figure 3. Histologic analysis of the irritant response. Photomicrographs of mouse ears after croton oil treatment (H & E; ×180). (*A*) wt ear, unchallenged. (*B*) IL-10T ear, unchallenged. (*C*) wt ear challenged with 1% croton oil, 24 h. Ear thickening is due to edema and moderate numbers of inflammatory cells. There is slight epidermal thickening (*open star*) secondary to cellular proliferation (acanthosis). (*D*) IL-10T ear challenged with 1% croton oil, 24 h. As in the wt, ear thickening is due to edema and infiltration of inflammatory cells. The cartilage is not seen in this field because of extensive edema. Epidermal thickening (*open star*) is secondary to edema rather than cellular proliferation. A large intraepidermal pustule is present (*upper left, arrow*). (*E*) wt ear challenged with 100% croton oil, 48 h. This is similar to *C*, but there is more edema, and marked acanthosis (*open star*) is present. (*F*) IL-10T ear challenged with undiluted croton oil, 48 h. The ear is completely necrotic. (*G*) Ear of wt mouse treated with anti-TNF antibody and challenged with 100% croton oil, 48 h. (*H*) Ear of IL-10T mouse treated with anti-TNF antibody and challenged with 100% croton oil, 48 h. Edema and inflammatory cell infiltrate are present, but there is no necrosis. An open star indicates epidermis; a closed star, dermis; and a triangle, cartilage.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lesions had a similar appearance at 24 and 48 h (a representative field at 48 h is shown in Fig. 3 *E*). Similar lesions were also observed in IL-10T ears 24 h after exposure to high-dose croton oil. In addition, blood vessels containing fibrin thrombi and small areas of hemorrhage were observed in IL-10T ears. By 48 h the ears of IL-10T mice had extensive necrosis of most of the tissue (Fig. 3 *F*). The tissue death accounted for the absence of measurable swelling at the 48-h time point shown in Fig. 2 *B*.

Effects of Anti-TNF Antibodies on the Irritant Response to Croton Oil. Anti-TNF antibodies were administered to IL-10T and wt mice 4 h before challenge with croton oil. Using the low-dose protocol, it was found that treating IL-10T mice with antibodies did not significantly reduce their ear swelling at 24 h, although a small reduction was seen at 48 h (Fig. 4 *A*). Antibody treatment of wt mice reduced their modest ear swelling by >50% at 24 h (Fig. 4 *A*).

Using the high-dose protocol, anti-TNF antibodies had no effect on the large response elicited in wt and IL-10T mice at 24 h, and significant ear swelling was observed (Fig. 4 *B*). Similarly, the anti-TNF treatment had no effect on the sustained response of wt mice at 48 h. However, anti-TNF was found to preserve tissue viability in IL-10T mice, resulting in the significant ear swelling detected at 48 h (Fig. 4 *B*).

Microscopic examination confirmed the beneficial effects of the anti-TNF treatment. The necrosis observed in the IL-10T mice was dramatically reduced (compare Fig. 3, *F* and *H*), demonstrating the contribution of TNF to the excessive tissue damage. The focal necrosis observed in wt mice at the high dose was also eliminated. However, the anti-TNF antibody treatment did not prevent edema or the accumulation of large numbers of inflammatory cells (Fig. 3, *G* and *H*).

Histopathology of Chronic Lesions Elicited with an Irritant. Because the mouse ear is very thin, severe damage elicited with high-dose croton oil resulted in complete necrosis of the IL-10T ears. This event precluded additional studies of the pathology associated with chronic inflammation and tissue repair. Therefore, a high dose of croton oil was applied to the backs of IL-10T and wt mice, as the same (or greater) depth of necrosis in this location would cause ulceration yet leave an underlying bed of tissue from which repair could take place. Several days after application of croton oil, ulcerations developed in both the wt and IL-10T mice, but they were larger in the IL-10T animals. Microscopic examination revealed moderate inflammation, ulceration, and fibrosis in the skin of the wt animals (Fig. 5 *A*). In contrast, the backs of the IL-10T mice treated with croton oil showed severe inflammation, necrosis, fibrosis, and serocellular crusting (Fig. 5 *B*). While the inflammation in the wt mice involved only the skin, IL-10T mice showed extensive inflammation extending through the body wall involving muscle and the lining of the body cavity (parietal peritoneum). As a result, adhesions developed between the peritoneum and the liver.

Contact Hypersensitivity Responses of IL-4T and IL-10T Mice. To further evaluate the role of IL-4 and IL-10 in skin responses, we examined the T cell–dependent CH response in wt, IL-4T, and IL-10T mice. Data presented in Fig. 6 *A*

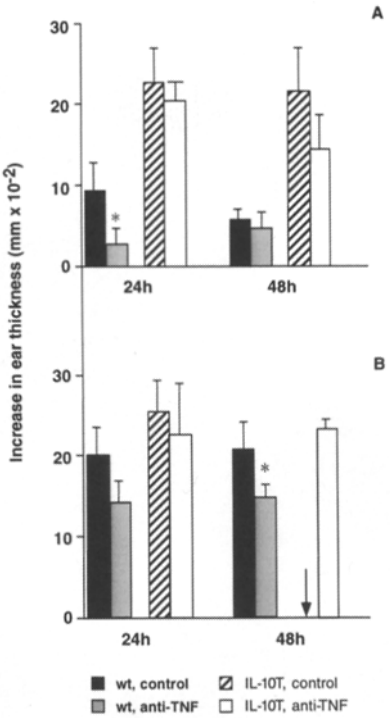

Figure 4. Effects of anti-TNF antibody treatment on the irritant response. Anti-TNF or isotype control mAbs were given to IL-10T and wt mice 4 h before application of 1% croton oil (*A*), or undiluted croton oil (*B*). Data presented are mean values ± SD of six mice per group measured at 24 and 48 h after application of the irritant and are representative of two independent experiments. An arrow indicates that no increase in ear thickness was detected. $^{*}P < 0.01$ compared with values obtained with wt mice treated with isotype control antibody.

show the CH responses of IL-4T mice and wt mice after sensitization and rechallenge with oxazolone. wt and IL-4T mice on a C57Bl/6 background gave identical responses to the sensitizing agent. Similarly, no difference was seen between wt and IL-4T mice on a 129/J background, although the magnitude of the CH response was greater than that observed with C57Bl/6J mice, demonstrating the contribution of strain differences to the CH response.

In the same type of experiment, the ear swelling of IL-10T (B6/129) mice was found to be greater than that of wt (B6/129) mice at 24 h, when the response peaked (Fig. 6 *B*). In addition, the response was greatly prolonged in IL-10T mice, as swelling was maintained 4 d after challenge. Microscopic examination revealed an exaggerated inflammatory response in the IL-10T mice. As expected, the application of oxazolone to the ears of sensitized wt mice resulted in significant edema and in the accumulation of mononuclear cells and neutrophils (Fig. 7 *A*). In the wt mice, intraepidermal pustules containing neutrophils and fewer eosinophils were noted on the ear surface. The underlying edematous dermis and skeletal muscle tissue contained scattered mixed inflammatory cells, including granulocytes and mononuclear cells. Changes in the ear of IL-10T elicited mice were qualitatively similar but were significantly more severe than those seen in the wt elicited mice (Fig. 7 *B*). Epidermal pustules were

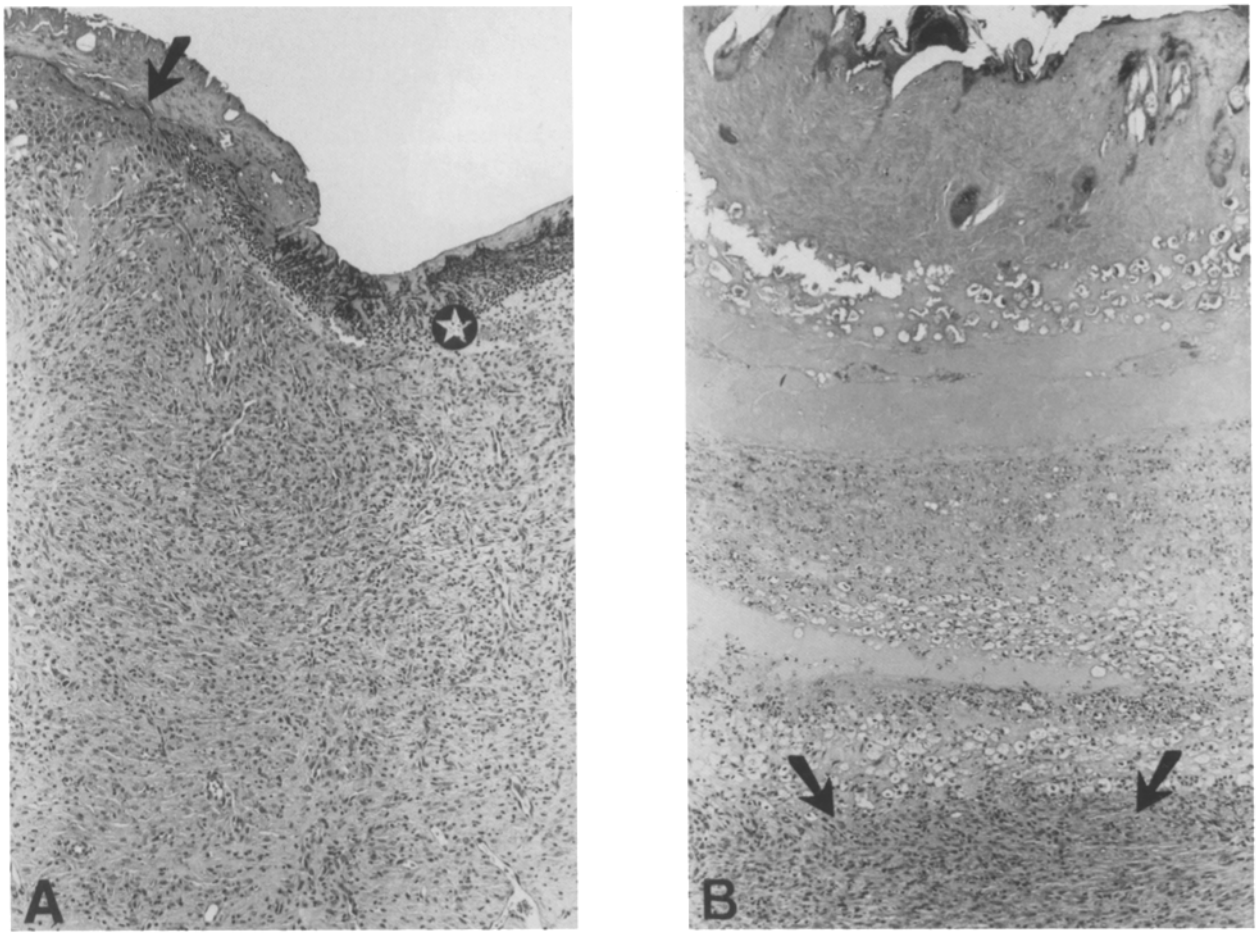

Figure 5. Histologic analysis of chronic lesions elicited with croton oil. Photomicrographs of lesions 14 d after treatment of skin with undiluted croton oil (H & E; ×68). (*A*) wt lesion showing extensive granulation tissue in the dermis. The overlying epidermis still has ulceration (*open star*), but epidermal cells are present at the edge of the ulcer (*arrow*). (*B*) IL-10T lesion, field comparable to *A*. Granulation tissue is present only in the very deep dermis (*arrows*); all the material above the arrows is necrotic. No reepithelialization is present.

larger and more frequent, and in many areas they became confluent, sometimes forming serocellular crusts on the surface.

Discussion

The skin is a site of significant immunologic activity because of its constant exposure to physical trauma, chemical irritants, and infectious organisms. A complex set of immune reactions has evolved to provide an appropriate defense under a variety of circumstances. An integral part of the immune response is a mechanism for controlling inflammatory and immune reactions to prevent immunopathologic damage. We have investigated the roles of IL-4 and IL-10 as regulators of immune responses in skin by studying the outcome of irritant responses and CH in IL-4T and IL-10T mice.

The first type of skin reaction examined was the irritant response induced by croton oil. This response is T cell independent and does not require prior sensitization. Instead, the mechanism of development depends upon the ability of a chemical compound to induce the production of inflammatory mediators by cells in the skin (35), particularly keratinocytes (36) and Langerhans cells (37). The role of IL-10 in the regulation of the irritant response has not been extensively studied. In a previous study, mRNA for IL-10 was not induced after application of the irritant, sodium lauryl sulfate (24). In another study, IL-10 administration did not alter the irritant response (29). These reports suggested that the irritant response was not regulated by IL-10, in contrast to CH. However, we have found that the application of croton oil to the skin of IL-10T mice produced intense inflammation characterized by edema and leukocyte infiltration. This response was much greater in magnitude and more persistent than that observed in similarly treated wt mice, demonstrating a clear role for IL-10 in the regulation of an irritant response. The role of IL-4 in the irritant response has not been investigated. Our studies showed no alteration in the irritant response in IL-4T animals. These data suggest that IL-10, but not IL-4, functions as a negative regulator of the irritant response in skin.

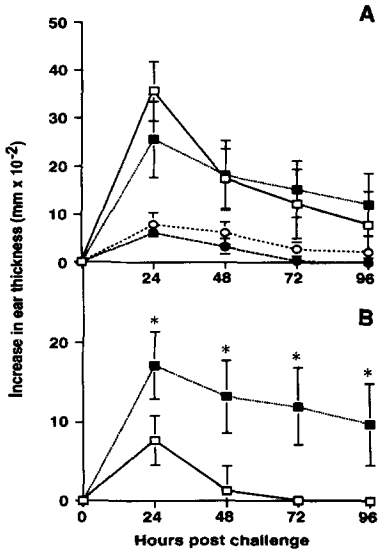

Figure 6. CH responses of IL-4T and IL-10T mice. The increases in ear thickness of sensitized interleukin-deficient and wt mice were measured daily after challenge with oxazolone. Data presented are the mean values ±SD of six mice per data point. (*A*) Differences between IL-4T and wt mice (129J) were not significant, $P > 0.05$. Data are representative of two independent experiments. Differences between IL-4T and wt mice (C57Bl/6J) were not significant, $P > 0.05$. —□—, wt (129J); —■—, IL-4T (129J); —○— wt (C57Bl/6); —●— IL-4T (C57Bl/6). (*B*) Differences between IL-10T and wt mice (B6/129) were significant, $^*P < 0.01$. Data are representative of three independent experiments. —□—, wt (B6/129); —■—, IL-10T (B6/129).

The importance of IL-10's ability to dampen the irritant response was most clearly illustrated by using a higher dose of the irritant. When the ears of IL-10T mice were challenged with a high dose of croton oil, we found extensive tissue necrosis but little evidence of inflammatory cells, suggesting that tissue death occurred rapidly and was secondary to excessive inflammatory cytokine production by the resident skin cells rather than by infiltrating leukocytes. In the case of wt mice challenged with the higher dose, the inflammatory response was amplified overall with only scattered areas of hemorrhage and epithelial necrosis. These differing outcomes demonstrated that IL-10 is essential for modifying inflammatory reactions to prevent unnecessary tissue damage.

TNF appears to be the major mediator of cutaneous pathology associated with reactions to contact sensitizers and irritants (38). Previous studies have shown that intradermal injections of TNF-α into normal mice produced edema, leukocyte sequestration along the venules, and focal areas of necrosis and hemorrhage (38, 39). Conversely, anti-TNF antibody treatment of unsensitized and TNCB-sensitized mice reduced all of these components upon TNCB challenge (38). We found that anti-TNF antibody treatment of IL-10T mice challenged with a high dose of croton oil prevented hemorrhage and tissue necrosis and allowed for the development of an inflammatory response. These results clearly implicate excessive TNF production in the IL-10T mice as the cause of necrosis and suggest that the overproduction of other inflammatory mediators (e.g., IL-1, IL-6, chemokines, prostaglandins, leukotrienes, etc.) was the major cause of edema and cell recruitment.

The consequences of an uncontrolled inflammatory response became more apparent when we studied the lesions on the backs of wt and IL-10T mice painted with croton oil. The inflammation elicited in wt mice was limited to the skin, and reepithelialization was evident by 2 wk after exposure. The inflammatory response in IL-10T mice was much greater, involving not only the skin but the entire body wall. Extensive tissue necrosis occurred in IL-10T mice, and there was minimal evidence of healing. The delay in resolution may have been secondary to differences in the size of the lesions elicited; however, inhibition of healing cannot be ruled out. Previous studies have demonstrated that TNF has an inhibitory effect on wound healing (40, 41), and hence it is possible that excessive production of TNF in IL-10T mice may have impaired their ability to heal the wound.

The second type of skin reaction studied was CH, which is a T cell–dependent immune response that requires prior sensitization. CH is a complex immunologic phenomenon, and there are multiple ways in which it may be regulated by IL-10. Results of mRNA studies suggest that proinflammatory cytokines and chemokines are produced early during the initiation phase of CH (36). Keratinocytes and macrophages are major sources of these factors, as well as of IL-10, which is known to down-regulate the production of proinflammatory cytokines (10, 11, 24, 25). Based on evidence provided by in vitro (10, 11) as well as in vivo studies (28), it seems likely that the absence of IL-10 would permit the uncontrolled, local production of proinflammatory cytokines, leading to an increased response. Other potential sources of proinflammatory cytokines, as well as IL-10, are activated $CD4^+$ and $CD8^+$ T cells (15, 42), which are key participants in CH (43–45). Several studies have documented that IL-10 inhibits the APC function of macrophages (12–14) and Langerhans cells (46) that is required for the activation of cytokine production by $CD4^+$ T cells. Furthermore, IL-10 has the potential to ablate CH in vivo by inducing anergy in allergen-reactive T cells (27, 28). Therefore, IL-10 may modulate the CH response by inhibiting T cell–APC interactions.

Our data show that IL-10T mice develop an exaggerated CH response after sensitization and subsequent challenge with oxazolone. As a result, both the magnitude and duration of the response were increased beyond those observed in wt mice. Histologic analysis revealed that the responses of IL-10T and wt mice differed quantitatively but not qualitatively. Thus, the increased reaction in the ears of IL-10T mice could be accounted for by the presence of a larger cellular infiltrate and increased edema. In mice depleted of IL-10 by neutralizing antibodies (26), the duration, but not the magnitude, of the CH response was increased. The discrepancy between the results obtained with IL-10T mice and with the antibody-treated mice may reflect that, in our study, IL-10 was absent during both the sensitization and elicitation phases, whereas IL-10 was absent during only the elicitation phase in the an-

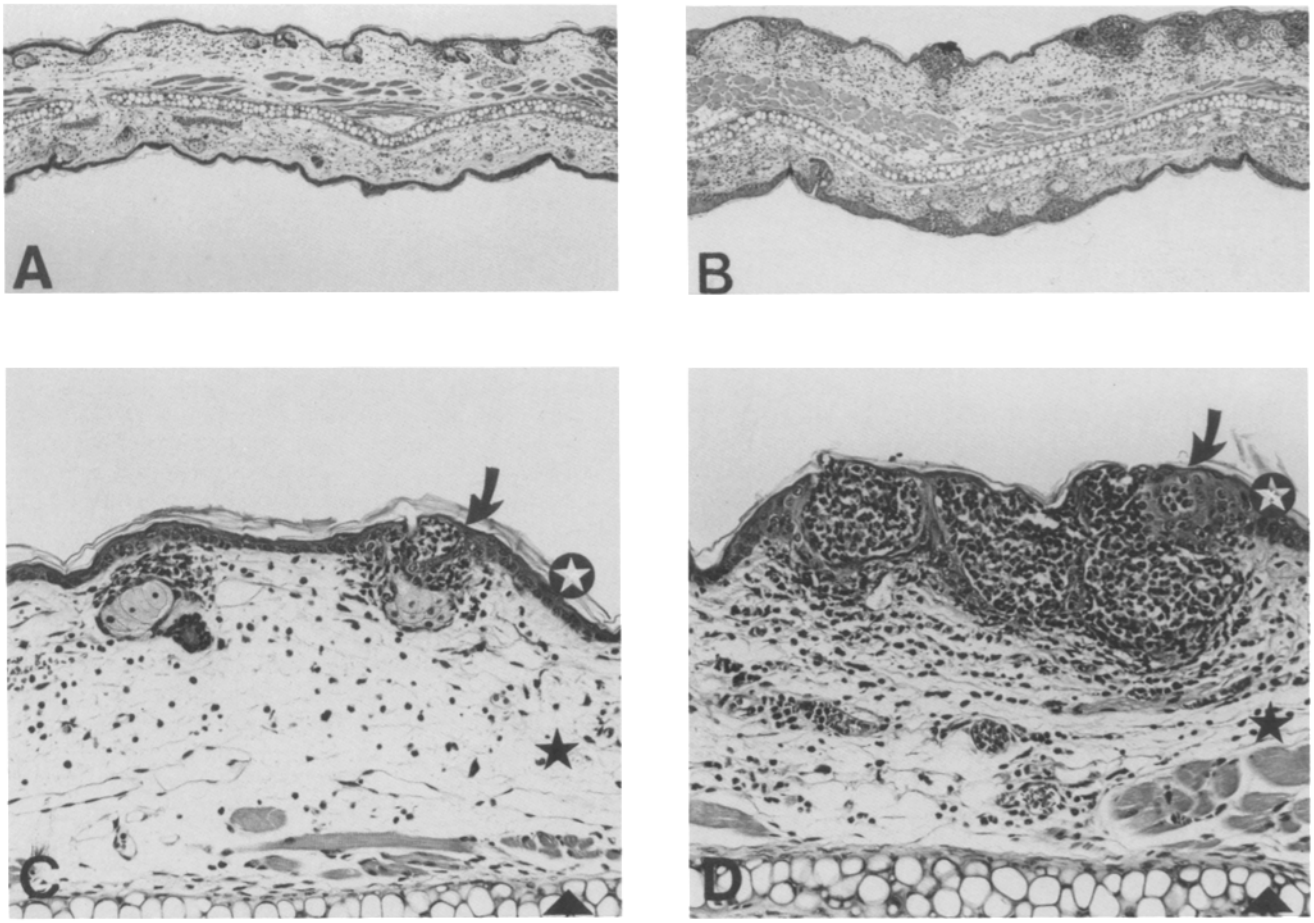

Figure 7. Histologic analysis of the CH response. (*A*) wt ear 24 h after challenge with oxazolone. Ear thickening is due to edema and mild infiltration of inflammatory cells (H & E; ×34). (*B*) IL-10T ear 24 h after challenge with oxazolone. The IL-10T ear is thicker than the wt ear because of increased edema and inflammatory cell infiltration. Of note are the numerous intraepidermal pustules (H & E; ×34). (*C*) Higher magnification of wt ear challenged with oxazolone (H & E; ×180). Intraepidermal pustules are small (*arrow*). (*D*) Higher magnification of IL-10T ear challenged with oxazolone (H & E; ×180). Intraepidermal pustules (*arrow*) are larger and more numerous, and they obscure the dermal–epidermal junction. An open star indicates epidermis; a closed star, dermis; and a triangle, cartilage.

tibody study. The lack of regulation by IL-10 of T cell–APC interactions during the sensitization phase may have contributed to the increased magnitude of the CH response seen in the IL-10T mice.

Surprisingly, our studies with IL-4T mice failed to demonstrate any alteration in their CH response due to the absence of IL-4. This was unexpected, considering the previous report of IL-4 production in vivo in response to contact sensitizers, including oxazolone (21, 22). Studies using neutralizing antibodies to IL-4 have found variable effects on the elicitation phase of CH (21, 30), and in our hands anti–IL-4 treatment of wt mice at elicitation did not result in an increased CH response (data not shown). When IL-4T mice on either the 129/J or C57Bl/6J background were evaluated, no differences from wt were detected in the CH response. Our study, using two strains of mice deficient in the production of IL-4, has led us to conclude that IL-4 does not regulate the magnitude or duration of the CH response.

In summary, our data demonstrate that endogenously produced IL-10, but not IL-4, is an essential regulator of both irritant and CH responses in the skin. There is similarity between these results and our previous finding that the absence of IL-10 resulted in the development of an exaggerated inflammatory response in the gastrointestinal tract (32). Both the skin and the gastrointestinal tract are exposed to large numbers of potential antigens and irritants. The immune response must be carefully balanced so that a proper defense is mounted without causing excessive immunopathologic damage. Our studies indicate that the role of IL-10, i.e., to prevent prolonged and exaggerated immune/inflammatory responses to antigens and irritants, is similar in both the skin and the gastrointestinal tract.

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