Regulation of Contact Hypersensitivity by Interleukin 10

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Summary

Contact hypersensitivity (CHS) responses require the participation of T cells, along with a variety of cytokines and adhesion molecules. In the classical CHS, antigen-specific T cells are recruited to a site of antigenic challenge, where they react with antigen, release cytokines, and attract other inflammatory cells. In the mouse model of CHS, this reaction is elicited in sensitized mice by application of the immunogen 4–7 d after immunization. The reaction peaks at 24 h, is slightly reduced by 48 h, and can return to normal by 72 h. This is in spite of the fact that some antigen is still present at the site of challenge. Here we examined the hypothesis that locally produced interleukin 10 (IL-10) regulates the duration of the response. Our data show that IL-10 protein peaked 10–14 h after antigenic challenge and returned to background by 24 h. The production of IL-10 protein corresponded with, and followed IL-10 mRNA transcription as detected by reverse transcriptase–polymerase chain reaction. During peak IL-10 production after antigenic challenge, it was not possible to transfer CHS with immune lymphoid cells, unless neutralizing antibody to IL-10 was given first. Additionally, when sensitized mice were given neutralizing anti-IL-10 antibody at the time of antigenic challenge, the duration of CHS was prolonged well beyond the natural course of the response. Finally, we demonstrate that rIL-10, when injected into the skin before antigenic challenge, prevented the elicitation of CHS in previously sensitized mice. Taken together, our data show an important role for IL-10 in the natural regulation of CHS responses in vivo.

The control of the magnitude and duration of immune responses has been attributed to several factors. These include the retention/release of antigen (1), induction of tolerance (2, 3), activation of suppressor cells (4, 5), and the release of inhibitory cytokines (6, 7). The classic experimental system for inflammatory cell–mediated immunity in skin is contact hypersensitivity (CHS)[1] (1–18). This response requires the participation of $CD4^+$ and $CD8^+$ T cells (13, 14), as well as other inflammatory cells (8–10). Numerous cytokines (6, 7) have been shown to be involved, as well as adhesion molecules (9–11) that are committed to helping cells migrate to the skin. In the typical reaction, application of the immunizing agent to skin results in the activation of antigen-specific T cells in the draining lymph nodes. Subsequent to the initial sensitizing dose (typically 4–7 d), the reapplication of the same antigen to skin results in the influx of antigen-specific T cells, which react to the antigen locally, release cytokines, and attract other inflammatory cells (macrophages and PMNs) to the site. The resultant edema can then be measured as a record of the immune response. The reaction proceeds in an antigen-specific manner (8) and can be passively transferred to naive mice with T cells (9, 10). The response typically peaks at 24 h, with some reduction at 48 h, and by 72 h the area can return to normal size in spite of the continued presence of antigen. Thus, it has been suggested that the magnitude and duration of the response is controlled locally by the production of "factors" that control the duration of the response (6, 19).

Recent studies have shown that a number of cytokines are produced in the skin after the application of antigen for CHS (6, 7). These include TNF-α, IFN-γ, GM-CSF, IL-1α, IL-1β, and IL-10. Data indicate that an interesting temporal relationship exists between the upregulation of the mRNA for these molecules and the elicitation of the CHS reaction (6). We were interested in examining those cytokines that might be involved in downregulating the response. IL-10 is a multifunctional molecule produced by a number of cell types (19–37). Besides being a growth factor for several cell types (20, 24, 25), a major function of this cytokine is the inhibition of Th1 cell function. These are the cells shown to be

[1] *Abbreviations used in this paper:* CHS, contact hypersensitivity; DTH, delayed type hypersensitivity; G3PDH, glyceraldehyde 3-phosphate dehydrogenase; TNBS, 2,4,6 trinitrobenzene sulfonic acid; TNCB, 2,4,6-trinitro-1-chlorobenzene.

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the mediators of delayed type hypersensitivity (DTH/CHS) reactions (32–34). Therefore, we tested the hypothesis that production of IL-10 at sites of antigenic challenge regulates the T cell responses in CHS. Our results demonstrate that IL-10 is involved in the natural control of CHS responses in skin.

Materials and Methods

Mice. BALB/cByJ mice were purchased from The Jackson Laboratory (Bar Harbor, ME). In all in vivo experiments, groups consisted of five or more animals. Experiments were repeated at least three times with similar results before they were reported.

Antigens, Antibodies, and Reagents. 2,4,6-trinitro-1-chlorobenzene (TNCB) was purchased from Eastern Chemical Co. (Smithtown, NY). 2,4,6 trinitrobenzene sulfonic acid (TNBS) was purchased from Sigma Chemical Co. (St. Louis, MO). The neutralizing rat antibodies to mouse IL-10, JES5.2A5 (IgG1) and SXC-1 (IgM) (24, 36) were kindly provided by DNAX Inc. (Palo Alto, CA). For ELISA, antibodies were prepared from culture supernatants by passage over a protein G–Sepharose column. Antibodies for in vivo experiments were prepared as ascites fluid in SCID mice. Antibodies were quantitated using a sandwich ELISA for rat IgG1 and IgM. Recombinant murine IL-10 (rIL-10) was purchased from Genzyme Corp. (Cambridge, MA).

Contact Hypersensitivity. BALB/c mice were painted on shaved abdominal skin with 100 μl of 2% TNCB dissolved in acetone/olive oil 3:1 (vehicle). After 4–5 d, mice were killed and single cell suspensions were made from draining lymph nodes and spleens. For adoptive transfer, 5×10^7 cells were injected intravenously via the retro-orbital plexus into syngeneic recipients (total volume, 200 μl). Mice were ear challenged with 1% TNCB (acetone/olive oil, 3:1) and CHS was measured at 24 h as the difference between the right ear (challenged) and the left ear (unchallenged). In some cases, measurements were made at 24, 48, 72, and 96 h. All measurements were done with an engineer's micrometer by an investigator who did not know the identity of the groups. Background values (Bkg) were determined by the difference in ear swelling between the antigen challenged and unchallenged ears of naive mice. To determine the effect of rIL-10 injection, mice sensitized 4 d earlier with 2% TNCB were challenged in the right footpad with 0.033 ml of 10 mM TNBS and in the left footpad with 0.033 ml of PBS 2 h after injection of rIL-10.

Tissue Extraction and IL-10 ELISA. At various times after application of TNCB, ears were excised, weighed, and each ear was added to a 1.5-ml microcentrifuge tube. To each sample was added 0.5 ml of 0.1% Tween 20 in PBS and samples were ground for 3–5 min with a pellet pestle (Kontes Scientific Glassware/Instruments, Vineland, NJ) attached to a three-eighths-inch cordless electric drill (Sears Roebuck and Co., Chicago, IL). Samples were then quick frozen in liquid N_2, thawed in a 37°C water bath, and ground again for 3–5 min. Samples were sonicated for 15 s and then centrifuged for 5 min at 13,000 *g*. Supernatants were removed for IL-10 determination.

IL-10 in ear tissue was quantitated using a sandwich ELISA developed with the two anti-IL-10 mAbs. 96-well ELISA immunoplates (Polysorb; Nunc, Roskilde, Denmark) were coated with 2 μg/ml purified JES5.2A5 mAb diluted in coating buffer (0.1 M $NaHCO_3$/0.1 M Na_2CO_3). After overnight incubation at 4°C, wells were blocked with 1% BSA in PBS for 2 h at room temperature. Plates were then washed three times with PBS containing 0.1% Tween-20 (PBS/Tween) and the rIL-10 standard or test samples were added (100 μl/well). Standard and unknowns were diluted in PBS/Tween containing 10% BSA. Plates were incubated overnight at 4°C, wells were washed four times with PBS/Tween, and 100 μl/well of biotinylated mAb SXC-1 (1 μg/ml) was added for 45 min at room temperature. Wells were washed at least six times, followed by the addition of 100 μl of 1:500 avidin–alkaline phosphatase (Sigma Chemical Co.) for 30 min at room temperature. Wells were washed at least eight times with PBS/Tween and 100 μl of *p*-nitrophenyl phosphate (Zymed Laboratories, Inc., South San Francisco, CA) was added. Upon color development, the plate was analyzed on an ELISA plate reader at OD 405 nm. The ELISA did not react with IL-2, IL-4, TNF-α, IL-1α, or IFN-γ.

RT-PCR for IL-10. At various times after application of TNCB or vehicle, ears were excised. They were then washed once with PBS and stored at −80°C. Total RNA was extracted using guanidium thiocyanate as described by Chomczynski (38). Samples were treated with RNase-free DNase I at 37°C for 30 min, the RNA reprecipitated, and RNA concentrations determined by OD_{260}. All samples were brought to 5 ng/μl and samples tested for DNA contamination by 40 cycles of PCR using the glyceraldehyde 3-phosphate dehydrogenase (G3PDH) primers. cDNA synthesis was then performed by the instructions on a RNA PCR kit (model no. N808-0017; Perkin-Elmer Cetus, Norwalk, CT) using total RNA and the oligo $d(T)_{16}$ primer provided with the kit. The RT reaction used M-MuLV reverse transcriptase and the thermal program was run at 42°C for 1 h followed by 95°C for 5 min. cDNA products were stored at −80°C until used. Primers were synthesized by Keystone Laboratories (Menlo Park, CA) and were: G3PDH, 5′-ATCTTCTTGTGCAGTGCCAGC-3′ and 5′-TATTTCTCGTGGTTCACACCC-3′, giving a 450-bp product; IL-10, 5′-CCCAGAAATCAAGGAGCATTTG-3′ and 5′-CATGTATGCTTCTATGCAGTTG-3′, giving a 209-bp product as described (19).

PCR conditions were optimized such that only the desired product was produced. Cycle conditions were 95°C for 1 min, 55°C for 1.5 min, and 72°C for 2 min. This was repeated for 40 cycles with the G3PDH primers and for 45 cycles with the IL-10 primers and reaction mixtures contained $\alpha[^{32}P]$dCTP (1 μCi/sample). Samples were then run on 2% agarose gels and bands visualized using ethidium bromide. The observed bands were cut out and counted using a beta counter (model LS-5000 CE; Beckman Instruments, Fullerton, CA). Counts per minute values for G3PDH were used for comparison to IL-10. Values are expressed as the ratio of IL-10 cpm to G3PDH cpm, and each point represents the average of two to five samples. Experiments were repeated at least three times.

Treatment of Mice with Anti-IL-10. Normal mice were treated with 0.5 mg of anti-IL-10 24 h before application of antigen to skin. They were then treated with 0.5 mg of antibody intravenously at the time of antigen application. In some experiments where the effect of neutralizing IL-10 was determined by measuring on consecutive days after challenge, 0.5 mg of anti-IL-10 was given daily. Control antibody was 0.5 mg/mouse of rat IgG.

Statistics. Significant differences between groups was evaluated using a two-tailed Student's *t* test ($p < 0.05$).

Results

Detection of IL-10 in Skin. We wanted to examine the production of IL-10 protein in the skin of mice treated with TNCB. Thus, we developed an ELISA to detect IL-10 using two mAb specific for the protein. Data in Fig. 1 show that there was a baseline level of IL-10 detectable in skin (normal)

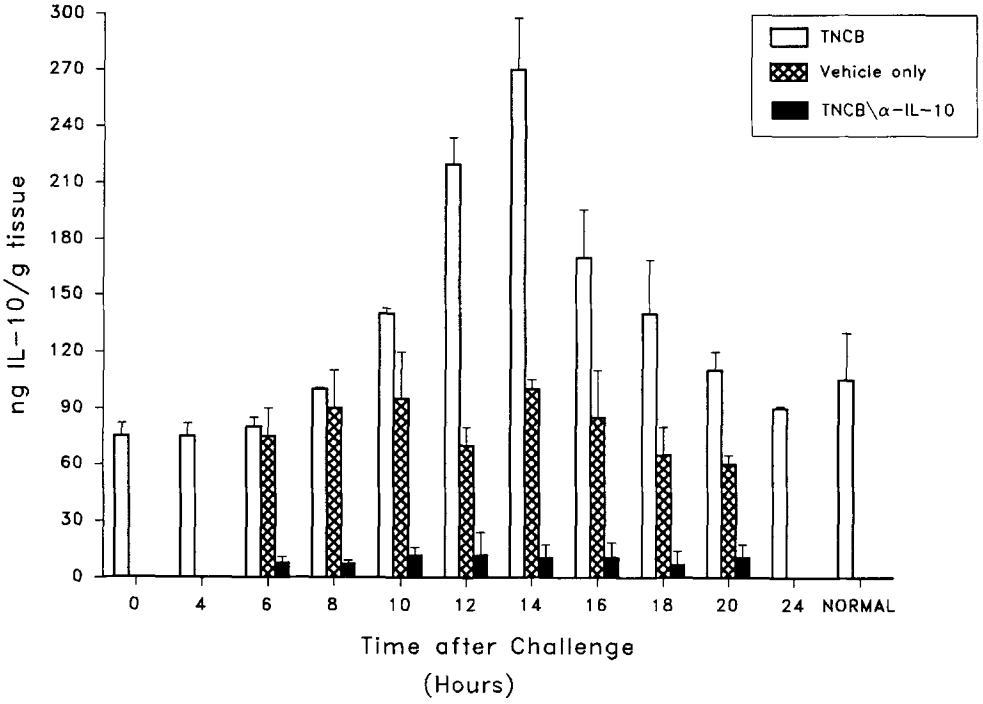

Figure 1. The amount of IL-10 protein was detected in skin using an ELISA. Normal BALB/cByJ mice were painted with 1% TNCB and the ears excised at various times. Experimental groups include TNCB only, TNCB/anti-IL-10 (JES5.2A5), and vehicle only (3:1 acetone/olive oil). After excision, the ears were weighed, protein was extracted, and the amount of IL-10 was determined by comparison to a known standard of rIL-10. Values are expressed as nanograms of IL-10 per gram of tissue. Each value represents the mean of three to five individual samples.

that remained unchanged for the first 8 h after application of TNCB. The amount of the protein increased at 10 h, reached maximum level at 14 h, and fell back to normal by 24 h. Skin treated with vehicle alone did not upregulate the amount of IL-10. We also examined the level of IL-10 protein in painted skin after treatment with neutralizing antibody to IL-10 (SXC-1). In this case, there was a barely detectable level of protein suggesting that we had neutralized (or at least bound) virtually all IL-10. Note that treatment with JES5-2A5.11 or SXC-1 resulted in nearly identical data, as noted by others (35).

In Fig. 2 we examined the production of mRNA after TNCB application. These data show that by 8–10 h after

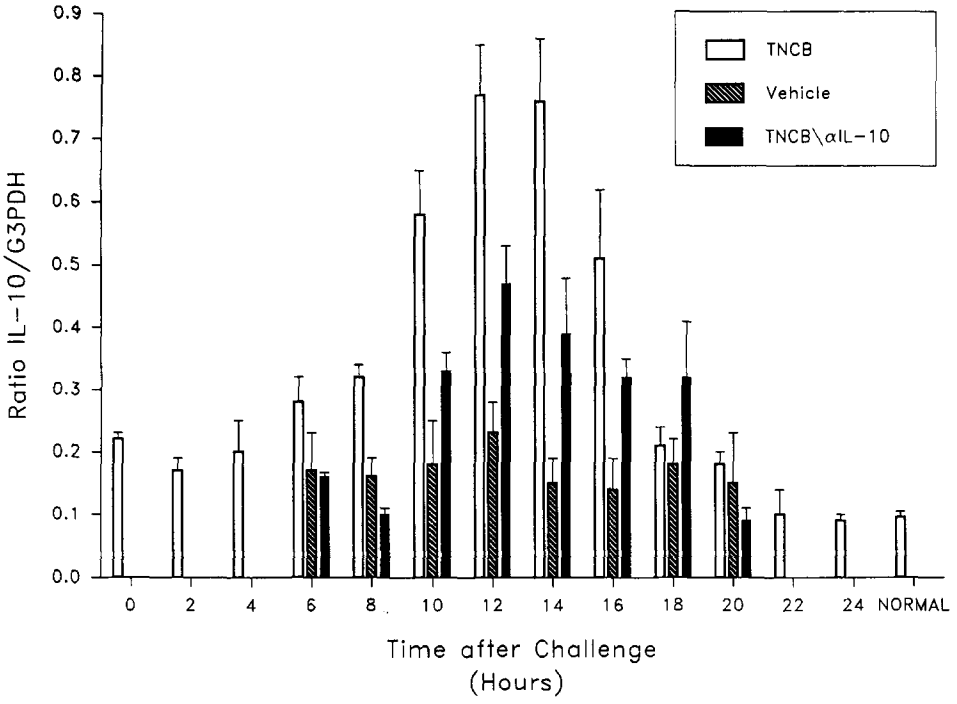

Figure 2. The amount of IL-10 mRNA was detected in skin by using RT-PCR. Normal BALB/cByJ mice were painted with 2% TNCB and the ears excised at various times. Experimental groups include TNCB only, TNCB/α-IL-10 (SXC-1), and vehicle only (3:1 acetone/olive oil). After excision, ears were weighed, mRNA was extracted, and RT-PCR was performed in the presence of $\alpha[^{32}P]$dCTP. Samples were run on 2% agarose gels, bands were cut out, and counts per minute determined. Values are expressed as the ratio of counts per minute for IL-10 product (209 bp) to G3PDH product (450 bp). Each point represents the average of two to five individual samples.

antigen application, the amount of mRNA for IL-10 was rapidly upregulated, peaking at 12–14 h and falling to baseline levels by 18 h. Treatment with anti-IL-10 resulted in less IL-10 mRNA, however, caution must be exercised since this was not a quantitative PCR. The pattern of IL-10 mRNA production in anti-IL-10–treated mice was nearly identical to that for untreated mice. Vehicle alone does not upregulate IL-10 mRNA. Thus, the mRNA correlates with the production and upregulation of IL-10 protein by application of TNCB. These data are in agreement with previously published results showing IL-10 mRNA in treated skin by RT-PCR (19), however, our data show that a constitutive level of mRNA and protein were present. Note that the levels of IL-10 protein and IL-10 mRNA are the same whether the mice are naive or presensitized to TNCB (data not shown).

Anti-IL-10 Prolongs the CHS Response. If IL-10 is involved in controlling the duration of the CHS response, we should be able to alter the response by neutralizing this cytokine. Naive mice were given anti-IL-10 (JES5.2A5) or control IgG along with TNCB immune cells before ear challenge. Ears were challenged with TNCB and responses were measured on three consecutive days. Results in Fig. 3 *A* show that the normal response was maximal at 24 h, 50% less at 48 h, and near background by 72 h. When anti-IL-10 was given, however, the CHS response continued through the 72-h time point at a level comparable with the maximal 24-h response. In Fig. 3 *B* a similar experiment was performed with the SXC-1 anti-IL-10 but instead of cell transfer, treatments were done in previously sensitized mice, and measurements were taken on four consecutive days. In this case, anti-IL-10 prolonged the response for 94 h. Therefore, we concluded that the presence of this cytokine was important for the down-regulation of the CHS response.

CHS Was Not Transferable during Peak IL-10 Production. In Fig. 1 we demonstrated that IL-10 was increased between 8 and 14 h after antigenic challenge. To further test our hy-

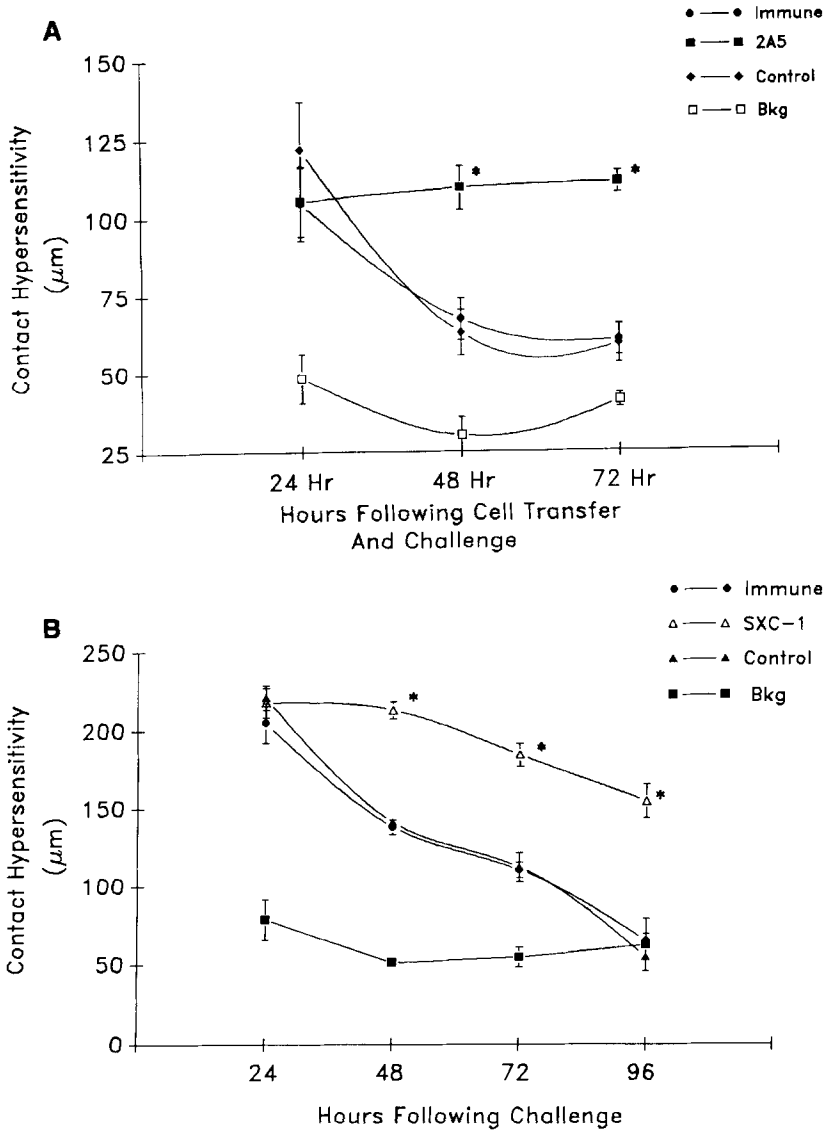

Figure 3. Anti-IL-10 prolongs the CHS response. (*A*) Mice were treated with anti-IL-10 or a control rat IgG to determine the effect on the CHS response. Naive BALB/cByJ mice received no treatment (*Immune*), 0.5 mg JES5.2A (*2A5*), or 0.5 mg control rat IgG (*Control*) and received 5 × 10^7 TNCB immune spleen and lymph node cells, and were ear challenged (right ear only) with 1% TNCB. Measurements were taken at 24, 48, and 72 h. (*B*) Naive BALB/c mice were sensitized by topical application of 2% TNCB. They were given 0.5 mg SXC-1 or 0.5 mg of rat Ig (*Control*) and were ear challenged with 1% TNCB. Measurements were taken at 24, 48, 72, and 96 h. Background values are included to demonstrate how values normally return to background levels on successive days. (*) Statistically significant difference from immune control (*Immune*).

pothesis that IL-10 was important for downregulation of the response, we attempted to transfer CHS just before, during, and after peak IL-10 production in TNCB-sensitized skin. To do this, naive mice were challenged at various times before adoptive transfer of TNCB-immune cells, and the response measured 24 h after challenge. Data in Fig. 4 show that when cells were transferred during peak IL-10 production (12 h) it was not possible to transfer the CHS response. It is interesting to note that when cells are transferred just before peak IL-10 (8 h) or after IL-10 levels return to normal (20 h), it is still not possible to transfer CHS. At all time points, however, the barrier to transfer was overcome by prior injection of the neutralizing antibody to IL-10 (SXC-1), suggesting that IL-10 was critical to preventing CHS transfer at these time points.

IL-10 Prevented the Elicitation of Immunity. To further test the hypothesis that IL-10 is important in the regulation of CHS, we attempted to modulate the response by injecting the cytokine in vivo. Mice were sensitized to TNCB and 5 d later challenged with TNBS by injection in the footpad. One group received an injection of 150 ng rIL-10 2 h before antigenic challenge. This amount was chosen because it is an intermediate value for IL-10 as detected in sensitized skin. Data in Fig. 5 show that pretreatment of the mice with rIL-10 prevented the elicitation of immunity in previously sensitized mice.

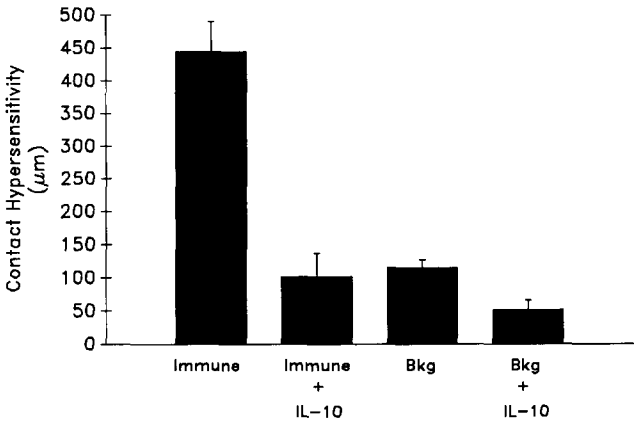

Figure 5. Pretreatment with rIL-10 blocks immunity. Mice were immunized with 2% TNCB (*Immune*) or nothing (*Bkg*) on shaved abdominal skin. 5 d later some mice received a footpad injection of 150 ng of rIL-10 in the footpad 2 h before injection of 0.033 ml of 10 mM TNBS. Footpad thickness was measured 24 h later. Values represent the difference between the right footpad (challenged) and the left footpad (unchallenged) and are expressed in micrometers.

Discussion

Control of the duration and magnitude of immune responses can be attributed to a number of biological responses, including loss of antigen from the site (1), induction of tolerance/suppression (2–5), and/or production of inhibitory cytokines (6, 7). The probable existence of controlling mechanisms is well represented by the murine CHS model. In this response, application of sensitizing antigen to previously immune mice results in a maximal response 24 h later. By 48 h the response is somewhat reduced, and by 72 h the site returns to normal. In this paper we examined the hypothesis that IL-10 plays a role in the natural control of the duration of this response, and our data make a strong argument for this. We have shown that IL-10 protein is normally present in skin, and that it is upregulated after application of antigen. Treatment of mice with anti-IL-10 was sufficient to neutralize much of the detectable IL-10 in the skin. When IL-10 was neutralized, the duration of the CHS was prolonged well beyond the natural course of the response. We further demonstrated that during peak IL-10 production at the site of antigenic challenge, it was not possible to adoptively transfer CHS with immune cells, unless the mice were previously treated with neutralizing anti-IL-10. Additional experimental evidence demonstrated that injection of IL-10 before antigen prevented the elicitation of CHS in previously sensitized mice. Thus, we conclude that IL-10 is a very important regulator of this T cell–mediated immune response.

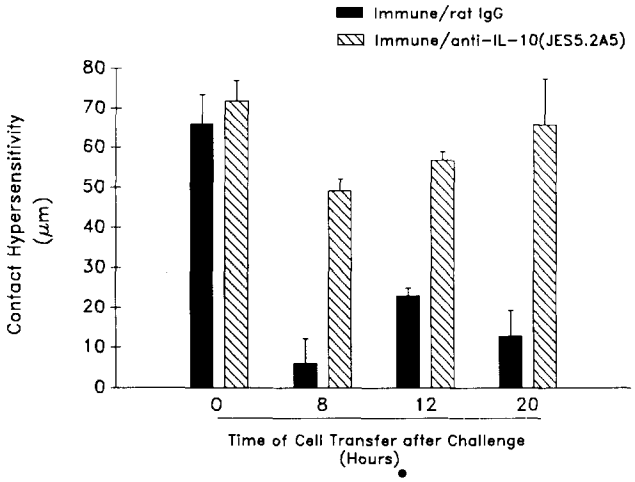

Figure 4. CHS was not transferred during peak IL-10 production. Naive BALB/c mice were given control rat IgG (*Immune/rat IgG*) or anti-IL-10 (*Immune/anti-IL-10*) (JES5.2A5) at the time of ear challenge with 1% TNCB. At 0, 8, 12, and 20 h, mice received 5×10^7 immune spleen and lymph nodes. Ear thickness was determined 24 h later. Values represent the difference between the right ear (challenged) and the left ear (unchallenged) and are expressed in micrometers. A background value (25.4 μm) represented by naive, challenged mice that did not receive immune cells was subtracted from each group.

Recent studies (20–37) have demonstrated an important regulatory role for IL-10 in other systems. IL-10 was originally described as a product of $CD4^+$ Th2 cells that inhibited IL-2 and IFN-γ production by $CD4^+$ Th1 cells (28, 32, 36). However, it has now been shown that IL-10 is made by a variety of cells including B lymphocytes (20, 24), macrophages (23), keratinocytes (19), and mast cells (21). IL-10 is a multifunctional cytokine that has been shown to promote the growth of B cells ($Ly\text{-}1^+$) (24), inhibit class II MHC on monocytes (37), and function as a CTL differentiation factor (25). IL-10 exerts many of its functions through activity on macrophages (and other APCs) such as inhibi-

tion of IL-1, IL-6, TNF-α, and GM-CSF production (22). It has also been shown to inhibit the expression of the costimulator B7 (24), which is an important molecule for T cell activation (39).

In skin, it has been shown that keratinocytes are the predominant source of IL-10. Recent studies have shown that after application of an allergen, mRNA for IL-10 is rapidly upregulated (19). In fact, the inhibitory effects of UV irradiation on skin may be mediated by the release of IL-10 from keratinocytes (40). We confirm and extend these studies by measuring the level of protein in skin. Our data show a constitutive production of the cytokine both at the mRNA and protein levels. The IL-10 is probably within the keratinocyte and not available to other cells in the area. Application of the contact sensitizer likely causes release of the cytokine for the purpose of regulation, similar to the effect of UV irradiation. The constitutive levels might be present for rapid deployment to control local, potentially harmful (if uncontrolled), immune reactions such as CHS. Similar results have been shown for IL-1, where this cytokine is present in cells constitutively (41) and cell death or injury (apoptosis) leads to its release (42). Additionally, the constitutive IL-10 may function to regulate APC function of the producing cell (keratinocyte), which has been shown to play a role in CHS induction (15, 43). Indeed, recent evidence showing that keratinocytes induce hapten-specific unresponsiveness support this.

CHS is a $CD4^+$- and $CD8^+$-mediated T cell reaction (13, 14). We do not know which cell types are affected by IL-10 in the skin. Since IL-10 is a growth signal for $CD8^+$ cells (25), it may function in a dual capacity by simultaneously inhibiting $CD4^+$ Th1 cell function (32), and activating $CD8^+$ cells that can regulate CHS in vivo (5). Recent evidence showing that IL-10 is chemotactic for $CD8^+$ cells (31) and that IL-10 may be a potent recruitment signal for leukocyte migration is supportive of this idea (33). Since IL-10 does not act directly on T cells (32), a possible scenario in the present system is that application of antigen results in increased production and release of IL-10 by keratinocytes. The protein is then taken up by local APCs (e.g., Langerhans cells) that, in turn, inhibit further Th1 cell activation (i.e., CHS). One recent study has shown that IL-10 inhibits the APC function of Langerhans cells for Th1, but not Th2 cells (27). Since Langerhans cells are believed to be the major APCs in skin (12, 16, 17), these cells are an obvious site of action for IL-10. It is also possible that IL-10 converts skin Langerhans cells from immunogenic to tolerogenic by downmodulating B7 (24, 27). It has been suggested that one function of IL-10 is to convert APCs from those that induce immunity to those that induce tolerance by downregulating B7 on the APCs (27). These "tolerogenic" APCs could then serve to regulate immunity by migrating to the lymph node and inducing anergy. An additional (but not mutually exclusive) mechanism might involve the recently observed effect of IL-10 on mouse macrophages. It was observed that IL-10 causes these cells to round up and become less adherent in culture (32). In TNCB skin, the release of IL-10 might induce local APCs to undergo this change as a means of preventing further APC function, or even as a prelude to the migration to the lymph nodes (16–18). By treating with anti-IL-10 mAb, we prevent these effects because the APCs do not become tolerogenic, but remain at the site for continued T cell activation. Thus, skin 12–14 h after application of TNCB may function similar to UV-irradiated skin (44) in which IL-10 is released to inhibit CHS (40). Whatever the precise mechanism, however, we have made a strong case for an important role for IL-10 in the natural regulation of the CHS response in vivo.

We do not know if infiltrating cells (T cells, B cells, etc.) would play a role in production of IL-10 in sensitized skin. These cells are known to make the cytokine (20, 36), and could contribute to the enhanced production of the protein. However, we have observed that ear tissue from naive and immune mice have nearly identical levels of IL-10 protein and mRNA. Additionally, treatment of mice with anti-IL-10 does not alter the number of ^{111}In radiolabeled cells that traffic to the ear after application of TNCB (data not shown). Thus, it appears that resident skin cells are the major source of IL-10 (19).

An interesting component of these studies was the kinetics of IL-10 production. The cytokine was upregulated after TNCB application, but returned to normal levels by 20–24 h. The first effects of IL-10 were observed at 8 h, which was the time when the barrier to transfer of CHS was evident. Even though the level of IL-10 protein and mRNA returned to normal by 18–20 h, it appeared that the function of IL-10 remained for several more hours. Even at 20 h it is not possible to produce a CHS reaction unless the IL-10 was neutralized. Perhaps the effect of IL-10 remains until skin cells (keratinocytes) are replenished or the effect is inhibited by other factors. Also suggested by our data is a role for IL-10 in regulation of its own production. Treatment with anti-IL-10 seemed to downmodulate the amount of IL-10 mRNA, suggesting a feedback loop of IL-10 regulation. However, this was difficult to determine, since we were not performing quantitative RT-PCR. We also detected less IL-10 in skin when mice were treated with anti-IL-10, however, this may be due to the large quantity of anti-IL-10 in tissue as a result of the antibody treatment. Recently it was observed that prolonged treatment with anti-IL-10 resulted in increased production of TNF-α (35). TNF-α is a cytokine critical to the induction and elicitation of CHS (7) and may be involved in regulating IL-10 production. It is also possible that the prolongation of CHS by anti-IL-10 treatment is partially due to elevated levels of TNF-α. A recent study (45) demonstrating that IL-10 inhibits production of TNF-α is also supportive of this. Whatever the effect of IL-10 on its own production, IL-10 appears to be an important regulatory molecule in cell-mediated immune reactions in vivo. Besides a role in CHS reported here, IL-10 has been shown to be a pivotal cytokine in certain parasitic infections (34), where DTH plays an important role in disease pathogenesis. Additionally, it has been shown to be a growth factor for B cells (20) and CTLs (25), and is an inhibitor of Th1 cell function (32). This polyfunctional cytokine, therefore, has potential application toward modulation of T cell function in vivo.

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