**In Vivo** Effects of Interleukin-10 on Contact Hypersensitivity and Delayed-Type Hypersensitivity Reactions

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Interleukin (IL) 10 is a recently discovered cytokine, originally isolated from T-helper 2 (Th2) cells, which inhibits cytokine production of T-helper 1 (Th1) cells. Because Th1 cells appear to be of importance during the contact hypersensitivity reaction (CHS) we hypothesized that IL-10 might modulate the outcome of CHS *in vivo*. Intrapерitoneal injection of murine recombinant IL-10 (1000 ng) into naïve mice 24, 72, or 120 h before sensitization by epicutaneous application of 2,4-dinitrofluorobenzene (DNFB) did not affect ear swelling when ears were challenged 5 d later. However, intraperitoneal injection of IL-10 into already sensitized mice 24 h before challenge resulted in a significant suppression of the ear swelling response, suggesting that under the conditions employed IL-10 is able to block the effector phase, but not the induction phase of CHS *in vivo*. The suppression could be reversed by the concurrent injection of an IL-10 antibody. Moreover, heat inactivation of native IL-10 resulted in loss of the inhibitory capacity. When mice were sensitized by subcutaneous injection of trinitrophenyl-coupled spleen cells (DTH) instead of epicutaneous application of the hapten (CHS), intraperitoneally-injected IL-10 suppressed the effector phase, but also the induction phase of DTH. IL-10 did not inhibit the toxic ear-swelling response induced by topical application of two irritants tested (croton oil or benzalkonium chloride). The capacity of IL-10 to suppress the effector phase of CHS and DTH supports an important role for this cytokine in the downregulation of type IV immune reactions *in vivo*. The finding that IL-10 suppresses the induction of DTH, but not of CHS, further suggests that CHS and DTH are related but distinct immune reactions. **Key words:** interleukin-10/contact hypersensitivity/delayed-type hypersensitivity/suppression. *J Invest Dermatol* 103:211–216, 1994

The molecular mechanisms modulating the cellular interactions in contact allergic reactions are still poorly understood. However, there is strong evidence that the cytokine network and the interplay of accessory signals are part of this complex reaction. In particular, the involvement of cytokines offers promising aspects with regard to pharmacologic intervention and therapeutic options including the application of downregulating cytokines or cytokine antagonists. This is of major relevance because contact dermatitis is a quite frequently observed disease and can, particularly in the chronic stage, be a disabling disorder.

There is good evidence that T-helper 1 (Th1) cells are critically involved in delayed-type hypersensitivity (DTH) and contact hypersensitivity (CHS) reactions [1]. T-helper 2 (Th2) clones were shown to secrete interleukin(IL)-10, a cytokine that inhibits the production of interferon-γ and IL-2 by Th1 cells [2]. It was subsequently shown that IL-10 mediated its effects on T-cell function indirectly by inhibiting the antigen-presenting function of purified macrophages and monocytes, but not B cells [3,4]. In addition, IL-10 acts as a mast cell [5] and T-cell growth factor [6], and enhances B-cell viability and major histocompatibility complex class II expression on B cells [7]. IL-10 also suppresses the release by monocytes/macrophages of a variety of inflammatory mediators including IL-1, IL-6, IL-8, granulocyte/macrophage colony-stimulating factor, granulocyte colony-stimulating factor, and tumor necrosis factor-α (TNF-α) [8,9]. These biologic abilities strongly suggest an important role for IL-10 in the downregulation of inflammatory and immune reactions, and support its possible clinical use as an immunosuppressor and antiinflammatory agent [9].

In view of the critical role that Th1 cells play during CHS we investigated the role of IL-10 during this reaction [1]. Our approach was to determine whether contact allergic reactions can be modulated *in vivo* by injection of IL-10 at the time of hapten sensitization or hapten challenge. Our results indicate that IL-10 can inhibit CHS *in vivo*.

**Materials and Methods**

**Mice** Balb/c and C3H/HeN mice between 8 and 12 weeks old were purchased from the Versuchstierzuchtanstalt, Hannover, Germany.

**Contact Hypersensitivity** Mice were sensitized by painting 25 μl of 2,4-dinitrofluorobenzene (DNFB; Sigma Corp., St. Louis, MO) solution (acetone:olive oil, 4:1) on the shaved abdomen on day 1 and day 2, as reported previously [10]. On day 6 the left ear was challenged by applying 20 μl 0.5% DNFB, and the right ear was treated with acetone/olive oil alone. The degree of ear swelling was measured with a spring-loaded micrometer (Mitutoyo, Japan) 24 h after challenge. Contact hypersensitivity
was determined as the amount of swelling of the hapten-challenged left ear compared to the thickness of the vehicle (acetone: olive oil)-treated right ear in sensitized animals and expressed in cm × 10⁻³ (mean ± SD). Mice that were ear challenged without prior sensitization served as negative controls. Each treatment group consisted of at least seven animals, and each experiment was performed at least three times.

**Delayed Type Hypersensitivity** Spleens were removed aseptically from naive donor mice (C3H/HeN) and teased apart in Hank's balanced saline solution (HBSS). The suspensions were filtered through nylon gauze to remove clumps and washed, red blood cells were lysed with Tris-NH₄Cl solution for 2 min, refiltered, and washed twice with HBSS. Cells were conjugated with trinitrophenyl by incubating them for 15 min at 37°C in 10 mM recrystallized trinitrobenzene sulfonate in HBSS at pH 7.0. After incubation, the cells were washed three times to remove unconjugated hapten. For sensitization, 5 × 10⁴ trinitrophenyl-coupled spleen cells (TNP-SC) were injected subcutaneously at two sites on the dorsal side of the mice. Challenge was performed after 7 d by injecting 2 × 10⁴ TNP-SC (suspended in 40 μl) subcutaneously into the left footpad. The respective control groups consisted of mice that were either sensitized or challenged with uncoupled spleen cells. Footpad swelling was measured with a spring-loaded micrometer 24 h after challenge. Delayed type hypersensitivity was determined as the amount of swelling of the hapten-challenged left footpad compared to the thickness of the untreated right footpad in sensitized mice and expressed in cm × 10⁻³ (mean ± SD).

**Irritant Dermatitis** For induction of irritant dermatitis 20 μl of 1% croton oil (Serva, Heidelberg, Germany) dissolved in acetone were applied on the left ear [11]. Ear swelling was measured 8 h after irritant application with a spring-loaded micrometer. Irritant reaction was determined as the amount of swelling of the irritant-treated left ear compared to the vehicle (acetone)-treated right ear and expressed in cm × 10⁻³ (mean ± SD). In some experiments, a 5% solution of benzalkonium chloride (Sigma Corp.) in acetone was used to elicit irritant dermatitis. Application and measurement was performed identically as with croton oil.

**IL-10 Treatment In Vivo** Recombinant mouse IL-10 was expressed in Escherichia coli [12] and renatured and purified using ion exchange and hydrophobic interaction chromatography [13]. Mice were injected intraperitoneally with IL-10 diluted in sterile endotoxin-free saline in a total volume of 100 μl at the times indicated in each experiment. Control mice were treated intraperitoneally with equal volumes of saline, which had no effect on the outcome of both the sensitization and challenge procedure. Likewise, intraperitoneal injection of IL-10 into naive mice did not affect ear thickness by itself. Heat inactivation of IL-10 was performed by heating at 90°C for 30 min. Biologic activity of IL-10 was monitored using the thymocyte proliferation assay as described previously [14]. Neutralizing antibody experiments utilized a rat monoclonal immunoglobulin (IgG1) antibody directed against murine IL-10 (UBI Company, Lake Placid, NY) or an isotype control (rat IgG1, The Binding Site Inc., Birmingham, U.K.).

**Thymocyte Proliferation Assay** Thymocytes obtained from 7-week-old Balb/c mice (1 × 10⁶ per well) were cultured in flat-bottom 96-well plates with IL-10 (native or heat inactivated) at various concentrations in the presence of IL-2 (500 U/ml) and IL-4 (250 U/ml) in 300 μl of RPMI medium (Gibco, Gaithersburg, MD) supplemented by L-glutamine (200 mM), modified Eagle’s medium (MEM) amino acids, MEM vitamins, sodium bicarbonate, penicillin/streptomycin (Sigma), 5 × 10⁻⁴ M 2-mercaptoethanol, and 10% fetal bovine serum (Gibco) for 4 d [14]. The wells were then pulsed with 1 μCi/well [³H]thymidine and harvested 18 h later. Activity is expressed as cpm (mean of triplicates).

**Histology** The ears were fixed in buffered formaldehyde, embedded in paraffin, sectioned, and stained with hematoxylin/eosin.

**Statistical Analysis** The statistical significance of differences in the means for each experimental group was calculated with the Student t test. Mean differences were considered significant when p < 0.05.

**RESULTS**

**Effect of IL-10 on the Induction of CHS** To evaluate the effect of IL-10 on the induction phase of CHS, groups of Balb/c mice were injected intraperitoneally with varying amounts of murine IL-10 in 100 μl saline 24 h before epicutaneous sensitization with DNFB. In three independent experiments of this type, IL-10 did not alter the outcome of CHS response upon ear challenge 6 d later (Fig 1A). Because suppression of the induction of CHS has been observed upon injection of supernatants obtained from UV-irradiated murine keratinocytes 120 h before sensitization [10], a kinetic analysis was performed by injecting 1000 ng IL-10 24, 72, or 120 h before hapten application. However, induction of CHS was not affected under any of these conditions (Fig 1B).

**Administration of IL-10 Results in Suppression of the Effect Phase of CHS** DNFB-sensitized Balb/c mice received 1-2000 ng of IL-10 i.p. 24 h before hapten challenge on the ears. In each of five experiments, a statistically significant reduction in ear swelling compared with control animals was observed upon injection of 1000 ng or more (Fig 2A). Interestingly, there was no real dose-dependent effect because it was not possible to show an increase of suppression by increasing the amounts of IL-10 injected. Optimal reduction of CHS was seen when 1000 ng was administered 24 h before challenge (Fig 2B); injection of IL-10 less than 12 h before challenge did not affect ear swelling. Application of DNFB to the ears of sensitized mice caused both epidermal and dermal alterations, in particular, spongiosis, edema, hemorrhage, and leukocyte infiltration (Fig 3A). Administration of IL-10 24 h before elicitation inhibited both the dermal and epidermal component of the CHS reaction (Fig 3B). The inhibitory effect of IL-10 on the elicitation of CHS was not strain specific, because similar data were obtained with C3H/HeN mice (Table 1).
IL-10 produces proliferation of thymocytes in a dose-dependent manner, with some variation in the ear thickness of challenged mice compared to control animals the differences were not statistically significant (Table I).

Figure 2. Effect of IL-10 on the effector phase of CHS. IL-10 was injected intraperitoneally at the concentrations indicated into DNFB-sensitized Balb/c mice. Twenty-four hours later challenge was performed on the left ear (A). IL-10 (1000 ng) was injected intraperitoneally into DNFB-sensitized Balb/c mice at the timepoints indicated before performance of ear challenge (B). Ear swelling was evaluated 24 h after ear challenge. CHS response is expressed as the difference (cm X 10^-3, mean ± SD) of the thickness of the challenged left ear minus the thickness of the vehicle-treated right ear. Ear thickness (mean ± SD) of the control was 25.7 ± 0.9 cm X 10^-3 (A) and 21.8 ± 0.9 cm X 10^-3 (B). Negative controls consisted of unsensitized mice that were ear challenged only. Experiments were repeated at least three times; results show one representative experiment. * p < 0.05, ** p < 0.01, *** p < 0.001 significantly different from positive control.

To prove that the inhibition of the elicitation was indeed mediated by IL-10, a neutralizing anti-IL-10 antibody was used. Concurrent injection of the antibody (50 μg) blocked the IL-10-mediated suppression of the effector phase of CHS. In contrast, the isotype did not affect the inhibitory activity of IL-10 (data not shown). As a further control, the anti-IL-10 antibody was administered in the absence of IL-10. Although there was some variation in the ear thickness of challenged mice compared to positive control animals the differences were not statistically significant (Table I).

To further confirm the specificity of the inhibitory effect of IL-10 on the effector phase of CHS, heat-inactivated IL-10 was used. Immediately prior to injection, IL-10 was heat inactivated at 90°C for 30 min. The efficacy of this treatment was monitored with the thymocyte proliferation assay demonstrating that native IL-10 induces proliferation of thymocytes in a dose-dependent manner, whereas heat-treated IL-10 did not exhibit any biologic activity (Fig 4). Heat-inactivated IL-10 injected intraperitoneally into sensitized mice 24 h before ear challenge failed to suppress the effector phase of CHS in Balb/c mice, in contrast to native IL-10, which blocked it significantly (Table II).

Effect of IL-10 on Irritant Reaction To investigate whether IL-10 also suppresses irritant reactions, IL-10 was injected intraperitoneally into Balb/c mice 3 or 24 h before application of 1% croton oil on the left ear. Toxic ear swelling, however, was not affected significantly in mice pretreated with IL-10 when compared with control animals (treated with irritant only) (Table III). Similar results were obtained when benzalkonium chloride was used as an irritant (Table III).

Administration of IL-10 Suppresses both Induction and Elicitation Phase of DTH Although CHS and DTH are both T-cell-mediated immune reactions with delayed onset it recently has been shown that both reactions induced and elicited by epicutaneous application of hapten and injection of hapten-conjugated cells, respectively, are not equivalent and interchangeable [15]. Therefore, the effect of IL-10 on DTH induced by TNP-SC was studied. We first investigated whether IL-10 has an effect on the induction of DTH. For this purpose, C3H/HeN mice were injected intraperitoneally with 1000 ng IL-10 24 h before sensitization by subcutaneous application of TNP-SC. Seven days later the mice were challenged by injecting TNP-SC into the footpads of sensitized or control mice. In contrast to the findings with CHS, injection of IL-10 24 h before sensitization significantly suppressed the induction of DTH when compared with the positive controls (normal immunized animals) (Table IV showing one representative ex-
The observation that IL-10 injected intraperitoneally before sensitization blocks the induction of DTH, but not of CHS, further supports the hypothesis that CHS and DTH are regulated by different pathways [15].

To address the effect of IL-10 on the elicitation phase of DTH, IL-10 was injected intraperitoneally 24 h before subcutaneous challenge into mice that were already sensitized by subcutaneous injection of TNP-SC. In each of three experiments a statistically significant reduction in footpad swelling was observed (Table IV showing one representative experiment). These data indicate that IL-10 is able to suppress the elicitation phase of CHS and DTH. However, IL-10 downregulated only the induction phase of DTH, but not of CHS, suggesting that different mechanisms control the modulation of these responses.

**DISCUSSION**

A number of studies are available addressing the immunomodulatory effects of IL-10 in vitro. They provide good evidence that IL-10 inhibits antigen presentation [3,4] and secretion of cytokines by macrophages [8,9]; recent data also show that IL-10 can affect T cells directly [6,16]. However, only a few reports exist addressing the in vivo effects of IL-10 in T-cell-mediated reactions. Most of these studies addressed the function of IL-10 in mouse models of parasitic infections [17]. They have shown that IL-10 is released in the course of certain parasitic infections and that it suppresses macrophage effector function and the release of interferon-γ [18,19].

### Table I. Effect of IL-10 on the Elicitation Phase of CHS

<table>
<thead>
<tr>
<th>Mice</th>
<th>Sensitization⁴</th>
<th>Challenge⁵</th>
<th>Treatment⁶</th>
<th>Increase Ear Thickness⁷</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balb/c</td>
<td>DNBC (0.3%)</td>
<td>DNBC (0.3%)</td>
<td>IL-10</td>
<td>0.4 ± 0.5</td>
<td>73.3</td>
</tr>
<tr>
<td>Balb/c</td>
<td>DNBC (0.3%)</td>
<td>DNBC (0.3%)</td>
<td>IL-10 + Ab</td>
<td>15.0 ± 1.8</td>
<td>4.0 ± 1.5</td>
</tr>
<tr>
<td>Balb/c</td>
<td>DNBC (0.3%)</td>
<td>DNBC (0.3%)</td>
<td>Ab</td>
<td>12.8 ± 4.8</td>
<td>8.5 ± 4.5</td>
</tr>
<tr>
<td>C3H/HeN</td>
<td>DNBC (0.3%)</td>
<td>DNBC (0.3%)</td>
<td>Ab</td>
<td>1.8 ± 4.3</td>
<td>16.0 ± 3.3</td>
</tr>
<tr>
<td>C3H/HeN</td>
<td>DNBC (0.3%)</td>
<td>DNBC (0.3%)</td>
<td>Ab</td>
<td>1.8 ± 4.3</td>
<td>18.5 ± 4.3</td>
</tr>
</tbody>
</table>

⁴ Hapten was applied on the shaved abdomen on days 1 and 2.
⁵ Hapten was applied on IIL-2 car on day 6.
⁶ IL-10 (1000 ng) or anti-IL-10 Ab was injected intraperitoneally 24 h before performance of challenge.
⁷ cm x 10⁻².
⁸ p < 0.00001 significantly different from positive control.
⁹ p < 0.01 significantly different from positive control.

In contrast, induction of DTH could be effectively blocked by intraperitoneal administration of 1000 ng IL-10 24 h before sensitization by subcutaneous injection of TNP-SC. Both CHS and DTH are T-cell-mediated immune reactions and because of many similarities between CHS and DTH, they previously have been used interchangeably [15,21–23]. Accordingly, it has been assumed that they are suppressed by the same mechanism following irradiation with ultraviolet (UVB) light. Recently, however, major differences between these reactions became evident: although UVB-irradiated mice could be immunized effectively for DTH by a subcutaneous injection of TNP-SC derived from normal mice, this procedure did not restore the CHS reaction measured by painting TNCB of the ears [15]. Moreover, treatment of UVB-irradiated mice with methylprednisolone before immunization prevented the suppression of CHS, but had no effect on the suppression of CHS [15]. Thus, our findings, i.e., that IL-10 prevents induction of DTH but not of CHS, further support the hypothesis that DTH and CHS are based upon distinct mechanisms in which different pathways seem to be involved. However, we cannot exclude that by changing the experimental conditions, e.g., application of even higher doses, multiple injections, or selection of other timepoints of injection, IL-10 will be able to suppress the induction of CHS in vivo. The present data, however, are in agreement with a recent observation by Rivas and Ulrich. Using the UVB model they could show that injection of an anti–IL-10 antibody prevents UVB-induced suppression of the induction of DTH [24] but not of CHS.† These data yield indirect evidence that IL-10 is involved in the suppression of the induction of DTH, but not of CHS. Conversely, Rivas and Ulrich could demonstrate that application of TNF-α antibodies


![Figure 4. Dose-dependent growth-promoting effect of IL-10 on thymocytes in the presence of IL-2 plus IL-4. Adult thymocytes were cultured with serial dilutions of native murine recombinant IL-10 (Δ) or heat-inactivated IL-10 (▼). Mean background activity was 1302 cpm. SD were within 10%.](image-url)
Table II. Effect of IL-10 on the Elicitation Phase of CHS

<table>
<thead>
<tr>
<th>Mice</th>
<th>Sensitization</th>
<th>Challenge</th>
<th>Treatment</th>
<th>Increase ear Thickness</th>
<th>% Suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Balb/c</td>
<td>DNFB (0.3%)</td>
<td>DNFB (0.3%)</td>
<td>IL-10</td>
<td>23.6 ± 4.1</td>
<td>60.0</td>
</tr>
<tr>
<td>Balb/c</td>
<td>DNFB (0.3%)</td>
<td>DNFB (0.3%)</td>
<td>inactivated IL-10</td>
<td>23.6 ± 4.1</td>
<td></td>
</tr>
</tbody>
</table>

* Hapten was applied on the shaved abdomen on days 1 and 2.
† Hapten was applied on the left ear on day 6.
‡ Native or inactivated IL-10 (1000 ng) was injected intraperitoneally 24 h before performance of challenge.
§ cm × 10⁻².
∥ Significantly different from positive control (p < 0.001).
\ Not significantly different from positive control.

Table III. Effect of IL-10 on Irritant Reaction

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Ear Swelling</th>
<th>IL-10 3 h</th>
<th>IL-10 24 h</th>
<th>IL-10 48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Croton oil</td>
<td>23.5 ± 2.6</td>
<td>23.4 ± 1.9</td>
<td>20.8 ± 3.2</td>
<td>23.6 ± 4.1</td>
</tr>
<tr>
<td>Benzalkonium chloride</td>
<td>57.8 ± 20.7</td>
<td>55.4 ± 16.3</td>
<td>54.5 ± 23.9</td>
<td>23.6 ± 4.1</td>
</tr>
</tbody>
</table>

* Difference of the thickness of the irritated left ear minus the thickness of the vehicle-treated right ear (cm × 10⁻³).
† IL-10 (1000 ng) was injected intraperitoneally 3 h before irritant application.
‡ IL-10 (1000 ng) was injected intraperitoneally 24 h before irritant application.
§ 1% croton oil was applied on the left ear.
∥ Not significant compared to positive control (irritation only).
\ 5% benzalkonium chloride was applied on the left ear.

reverses UVB-induced suppression of the induction of CHS, but not of DTH.†

Interestingly, we found that IL-10 injected intraperitoneally was able to suppress the elicitation of both CHS and DTH. The inhibitory effect of IL-10 on the effector phase is specific because it could be blocked by concurrent injection of an anti–IL-10 antibody. In several control experiments injection of the IL-10 antibody alone affected ear swelling somewhat, but these changes were never significant. These variations may reflect neutralization of endogenous IL-10. On the other hand, mice treated continuously from birth until 8 weeks of age with anti–IL-10 antibody appeared to be healthy and, by numerous parameters, unaffected by the treatment [25,26]. To further prove the specificity, IL-10 was inactivated by heat treatment. Incubation at 90°C for 30 min resulted in a complete loss of bioactivity, as checked in the thymocyte proliferation assay [14]. As expected, injection of heat-inactivated IL-10 had no effect on the elicitation of CHS.

CHS-induced ear swelling was blocked significantly by injection of at least 1000 ng of IL-10. Although lower doses had some inhibitory activity, the effects were not significant. Moreover, there was no real dose-dependent effect because it was not possible to increase the inhibitory activity by increasing the amounts of IL-10. This may suggest that in this complex in situ system probably other mediators are indirectly involved. To be inhibitory, IL-10 had to be administered at least 12 h before hapten challenge; maximum suppression was observed upon injection 24 h before challenge. The need of this relatively long period suggests that the observed effect of IL-10 is not a direct one, but could be mediated indirectly, e.g., via other cytokines. In this respect, TNF-α appears to be of primary interest because it has recently been identified as a critical mediator in hapten-induced irritant and contact hypersensitivity reactions [27]. In particular, injection of anti–TNF-α antibodies into sensitized mice before application of the challenging dose of the respective hapten abrogated the ear-swelling response of the respective hapten. Accordingly, we recently found that pentoxifylline, a phosphodiesterase inhibitor known to inhibit the release of TNF-α, is able to inhibit ear challenge in sensitized mice [11]. Moreover, it was observed that pentoxifylline also reduces irritant dermatitis, as was the case by injection of anti–TNF-α antibody, suggesting that TNF-α is also of importance in the mediation of irritant reactions [27]. IL-10 efficiently blocks the in vitro production of TNF-α, as well as of other cytokines, by lipopolysaccharide-activated monocytes/macrophages [8]. Through this mechanism IL-10 may be able to prevent lethality in experimental endotoxemia [13,28]. To further elucidate whether the inhibitory effect of IL-10 on the effector phase of CHS could be due to the inhibition of the release of TNF-α, the effect of IL-10 on irritant dermatitis, another TNF-α-mediated reaction, was studied. However, in contrast to pentoxifylline and anti–TNF-α antibody, IL-10 was not able to reduce toxic ear swelling response elicited by topical application of croton oil and benzalkonium chloride, respectively. Although these data do not definitely prove it, they favor the view that inhibition of TNF-α may not be the primary mechanism by which IL-10 prevents the effector phase of CHS.

More likely, IL-10 may exert its inhibitory effects on CHS and DTH via modulation of antigen-presenting cells. In a variety of antigen-driven secondary reactions IL-10 has been found to be inhibitory, acting primarily on antigen-presenting cells. It was shown that IL-10 inhibits the activity of macrophages, but not B cells, to induce interferon-γ secretion from Th1 cells [3,4]. However, IL-10 has no effect on the capacity of dendritic cells to stimulate proliferation of CD4+ or CD8+ T cells from unprimed mice. Although macrophage-driven proliferation is inhibited [29], a finding in accordance with data using a mitogen-driven system [30]. The effect phase of CHS and DTH reflects a primed (secondary) in vivo system. Therefore, the inhibitory activity of IL-10 on the elicitation of CHS and DTH could be explained by the effects of IL-10 on antigen-presenting cells, as demonstrated in the in vitro system [31]. On the other hand, there is recent evidence, at least in the human system, that IL-10 exerts direct downregulatory effects also on T cells [16]. In particular, it was reported that IL-10 directly inhibits IL-2 production and proliferation of activated human subsets of CD4+ T-cell clones and T cells isolated from peripheral blood [16]. In addition, IL-10 could indirectly affect either antigen-presenting cells or T cells by modulating the release of other cytokines, which could explain the relatively long period IL-10 has to be injected to block CHS and DTH. We are currently investigating whether IL-10 in our in vivo models of CHS and DTH primarily affects antigen-presenting cells, T-effector cells, or both.

In summary, the present study demonstrates a new in vivo function of IL-10, the ability to suppress induction of DTH and to block the elicitation of both CHS and DTH. In particular, the latter findings emphasize the role of IL-10 as an immunosuppressive and antinflammatory drug, giving rise to speculation about pharmacologic intervention in these types of immune reactions by application of IL-10. Moreover, the observation that IL-10 suppresses the induction of DTH, but not of CHS, further supports the concept that DTH and CHS are related but distinct immunologic reactions involving different pathways.
Table IV. Effect of IL-10 on the Elicitation Phase of DTH

<table>
<thead>
<tr>
<th>Treatment Before Sensitization</th>
<th>Sensitization</th>
<th>Treatment Before Challenge</th>
<th>Challenge</th>
<th>Increase Footpad Thickness</th>
<th>% Suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>TNP-SC</td>
<td>TNP-SC</td>
<td>TNP-SC</td>
<td>6.5 ± 1.0</td>
<td>48.3</td>
</tr>
<tr>
<td>SC</td>
<td>TNP-SC</td>
<td>TNP-SC</td>
<td>TNP-SC</td>
<td>3.8 ± 1.2</td>
<td>42.4</td>
</tr>
<tr>
<td>TNP-SC</td>
<td>TNP-SC</td>
<td>IL-10</td>
<td>TNP-SC</td>
<td>17.1 ± 1.6</td>
<td></td>
</tr>
<tr>
<td>TNP-SC</td>
<td>TNP-SC</td>
<td>IL-10</td>
<td>TNP-SC</td>
<td>8.8 ± 1.3</td>
<td></td>
</tr>
<tr>
<td>TNP-SC</td>
<td>TNP-SC</td>
<td>IL-10</td>
<td>TNP-SC</td>
<td>9.8 ± 0.8</td>
<td></td>
</tr>
</tbody>
</table>

* IL-10 (1000 ng) was injected intrapertioneally 24 h before sensitization and challenge, respectively.
* Mice were sensitized by subcutaneous injection of TNP-SC or SC (5 × 10^7) cells at two sites on the dorsal skin.
* Mice were challenged by subcutaneous injection of TNP-SC or SC (2 × 10^7) cells into the left footpad.
* Footpad swelling was measured 24 h after challenge and expressed as the difference (cm ± SD) of the thickness of the challenged left footpad minus the thickness of the untreated right footpad.
* p < 0.005 significantly different from positive control.

REFERENCES