The Journal *of* Immunology

This information is current as of May 7, 2021.

Inhibition of Functional T Cell Priming and Contact Hypersensitivity Responses by Treatment with Anti-Secondary Lymphoid Chemokine Antibody During Hapten Sensitization

Tara M. Engeman, Anton V. Gorbachev, Ronald P. Gladue, Peter S. Heeger and Robert L. Fairchild

---

References   This article **cites 41 articles**, 25 of which you can access for free at:
http://www.jimmunol.org/content/164/10/5207.full#ref-list-1

Why *The JI*? Submit online.

- **Rapid Reviews! 30 days*** from submission to initial decision

- **No Triage!** Every submission reviewed by practicing scientists

- **Fast Publication!** 4 weeks from acceptance to publication

*average

Inhibition of Functional T Cell Priming and Contact Hypersensitivity Responses by Treatment with Anti-Secondary Lymphoid Chemokine Antibody During Hapten Sensitization[1]

Tara M. Engeman,* Anton V. Gorbachev,* Ronald P. Gladue,§ Peter S. Heeger,‡ and Robert L. Fairchild[2]*†‡

Recent studies have suggested a pivotal role for secondary lymphoid chemokine (SLC) in directing dendritic cell trafficking from peripheral to lymphoid tissues. As an extension of these studies, we examined the consequences of anti-SLC Ab treatment during Ag priming on T cell function in an inflammatory response. We used a model of T cell-mediated inflammation, contact hypersensitivity (CHS), where priming of the effector T cells is dependent upon epidermal dendritic cell, Langerhans cells, and migration from the hapten sensitization site in the skin to draining lymph nodes. A single injection of anti-SLC Ab given at the time of sensitization with FITC inhibited Langerhans cell migration into draining lymph nodes for at least 3 days. The CHS response to hapten challenge was inhibited by anti-SLC Ab treatment in a dose-dependent manner. Despite the inhibition of CHS, T cells producing IFN-γ following in vitro stimulation with anti-CD3 mAb or with hapten-labeled cells were present in the skin-draining lymph nodes of mice treated with anti-SLC Ab during hapten sensitization. These T cells were unable, however, to passively transfer CHS to naive recipients. Animals treated with anti-SLC Ab during hapten sensitization were not tolerant to subsequent sensitization and challenge with the hapten. In addition, anti-SLC Ab did not inhibit CHS responses when given at the time of hapten challenge. These results indicate an important role for SLC during sensitization for CHS and suggest a strategy to circumvent functional T cell priming for inflammatory responses through administration of an Ab inhibiting dendritic cell trafficking. *The Journal of Immunology,* 2000, 164: 5207–5214.

C ontact hypersensitivity (CHS)[3] is a T cell-mediated inflammatory response of the epidermis occurring in sensitized individuals who contact the sensitizing hapten. In industrialized countries, CHS is among the most common dermatoses, affecting as much as 3% of the population (1, 2). Results from both clinical studies and studies using experimental models of CHS have supported a role for CD4+ and CD8+ T cells as the effector cells of the response (3–5). Experimental strategies using either Ab-mediated depletion of CD4+ vs CD8+ T cells or mice with targeted deletions of class II MHC genes have implicated CD8+ T cells as the primary effector cells in CHS responses to the model haptens dinitrofluorobenzene (DNFB) and oxazolone (Ox) (5–8). These results are supported by studies from this laboratory demonstrating that most of the IFN-γ-producing cells induced by epicutaneous sensitization with DNFB or Ox are hapten-specific CD8+ T cells (9). In contrast, most of the hapten-specific CD4+ T cells develop to IL-4/IL-10-producing cells. After these T cell populations are primed during sensitization, hapten challenge induces recruitment of the primed T cells to the challenge site and production of the proinflammatory cytokines, resulting in the characteristic edema of the response (10, 11).

During sensitization of the epidermis, the reactive hapten is covalently coupled to cell surface proteins. Langerhans cells (LC), the dendritic cells (DC) of the epidermis, capture the hapten, enter lymphatic vessels, and migrate to skin-draining lymph nodes where hapten-MHC complexes are presented to the T cell (12–14). The important role of LC has been demonstrated by the absence of CHS responses observed when epidermal areas lacking LC are sensitized with hapten (15, 16). In addition, exposure of skin to UV B irradiation before hapten sensitization inhibits LC migration to the lymph nodes and T cell priming for CHS (17, 18). These results suggest that inhibition of DC migration might be an effective strategy to inhibit T cell-mediated responses such as CHS.

The chemotactic factors directing LC trafficking from hapten sensitization sites to draining lymph nodes are unclear. Chemokines are a superfamily of cytokines with chemoattractant properties for leukocytes (19, 20). Neutralization of specific chemokines has been shown to inhibit leukocyte infiltration and pathology in several experimental models of inflammation, demonstrating their critical role in directing cell infiltration into inflammatory sites (21–24). Recent studies have also indicated the role of chemokines in directing cellular traffic into and within lymphoid tissues. Secondary lymphoid chemokine (SLC), a member of the C-C chemokine family, is constitutively expressed at high levels in the endothelium of the afferent lymphatics and high endothelial venules of lymph nodes (25, 26). The in vivo importance of this chemokine has recently been suggested in *plt* mice where an absence of SLC production was associated with the inability of DC to migrate from peripheral to lymphoid tissues (27). In further support of the role

of SLC in DC trafficking, treatment of wild-type mice with anti-SLC mAb has been shown to inhibit DC migration to lymphoid organs (28). However, the functional consequences of this inhibition on an immune response have not been tested. Since effector T cell priming in CHS is dependent on LC migration from the skin to draining lymph nodes, we hypothesized that treatment with anti-SLC mAb may be an effective method of inhibiting the T cell-mediated response.

In this study, we examined the effect of anti-SLC Ab given at the time of hapten sensitization on the elicitation of the subsequent CHS response. The results demonstrate that administration of anti-SLC Ab during hapten sensitization inhibits the development of effector T cells and CHS responses, suggesting a novel strategy directed at SLC for inhibition of T cell-mediated skin allergies.

Materials and Methods
Animals

BALB/c mice were obtained through Dr. Clarence Reeder (National Cancer Institute, Frederick, MD). Adult females of 6–10 wk of age were used throughout this study.

Abs and cytokines

Ab to mouse SLC and control Ig were obtained from R&D Systems (Minneapolis, MN). mAb from the culture supernatant of the IgG-producing hybridomas N418 (anti-mouse CD11c), 145.2C11 (anti-CD3ϵ), and KJ23a (anti-Vβ17a) were purified by protein G chromatography. Capture and detecting Abs for cytokine-specific ELISA and recombinant IFN-γ and IL-4 for standardization of assays were purchased from PharMingen (San Diego, CA).

Sensitization and elicitation of contact hypersensitivity

Mice were sensitized and challenged to elicit CHS responses to DNFB and to Ox as described previously (8, 9). For the induction of CHS to DNFB, groups of three to four mice were either sensitized by two daily paintings (days 0 and +1) with 25 μl of 0.25% DNFB (Sigma-Aldrich, St. Louis, MO) on the shaved abdomen and 5 μl on the footpads or were sensitized by a single painting with 50 μl of 1% DNFB on the shaved abdomen and 5 μl on each footpad. For the induction of CHS to Ox, mice were sensitized by a single painting (day 0) of 25 μl of 3% Ox on the shaved abdomen and 5 μl on the footpads. For sensitization to FITC, mice were painted once (day 0) with 100 μl of 1% FITC (isomer I; Sigma-Aldrich) on the shaved abdomen and 5 μl on each footpad. On day +5, the ear thickness of sensitized and unsensitized control animals was measured with an engineer's micrometer (Mitutoyo, Elk Grove Village, IL) and each animal was challenged by applying 10 μl of 0.2% DNFB, 0.6% Ox, or 0.5% FITC to each side of both ears. Increase in ear swelling was measured in a blinded manner 24 h after challenge and expressed in units of 10^{-4} inches. The magnitude of ear swelling is given as the mean increase of each group of three to four individual animals (i.e., six to eight ears) $\pm$ SEM. The change in ear thickness is calculated as ear thickness at the indicated time after challenge minus ear thickness before challenge. The statistical significance of ear-swelling responses between experimental groups of mice was determined using Student's t test.

Flow cytometry

Two-color flow cytometry analyses to detect LC in the skin-draining lymph nodes of FITC-sensitized mice was performed as described previously (29). One to 3 days after FITC sensitization, lymph node cells (LNC) from two animals per group were pooled, and 5 $\times$ 10^5 cell aliquots were washed twice with staining buffer (Dulbecco's PBS with 2% FCS/0.2% NaN$_3$) and then incubated on ice in 100 μl rat serum (Rockland, Gilbertsville, PA) diluted 1/1000 in the staining buffer. After 20 min, the cells were washed twice, resuspended in 200 μl of staining buffer, and stained with biotinylated N418 Ab for 30 min on ice. The cells were washed five times and then incubated for 20 min with streptavidin-PE. After 30 min, the cells were washed five times, resuspended in staining buffer, and analyzed by two-color flow cytometry using a FACScan (Becton Dickinson, San Jose, CA). Sample data were collected on 20,000 cells. Each experiment was performed three times, and the results from a single representative experiment are shown.

Cell culture to stimulate cytokine production and ELISA

Cells were stimulated to produce cytokines by culture on anti-CD3 Ab-coated tissue culture wells. The 96-well U-bottom tissue culture plates were precoated with 30 μl/well of anti-CD3ϵ Ab or, as a negative control, anti-Vβ17a Ab at 25 μg/ml for 90 min at 37°C and then washed extensively. LNC cells were obtained from nonsensitized and hapten-sensitized mice on day +4, suspended in complete medium, and 2 $\times$ 10^5 LNC from each group were delivered to each well in 200 μl complete medium. After 48 h, culture supernatants were harvested and assayed for cytokine production by ELISA. Each experiment was performed three times, and a single representative experiment is shown in *Results*. Previous results have demonstrated that lymph node T cells from hapten-sensitized, but not from naive, mice produce cytokines during anti-CD3 Ab-mediated stimulation similar to cytokine production during culture with isolated hapten-presenting LC (9).

Cytokine ELISAs for determining quantity of IFN-γ and IL-4 were performed as described previously (9). Polyvinylchloride ELISA plates were coated with capture Ab in 0.1 M bicarbonate buffer (pH 8.6) overnight at 4°C and then blocked with 5% FCS/0.5% gelatin in PBS. Duplicate aliquots of all supernatant dilutions were tested undiluted and in at least two dilutions. Each plate also included titrated recombinant cytokine as a positive control and to obtain a standard curve for quantitation. Following incubation overnight at 4°C, each plate was washed and the biotin-labeled anti-cytokine- detecting mAb added. The plate was incubated for 2 h at 37°C, washed, and alkaline phosphatase-conjugated streptavidin (Fisher Scientific, Pittsburgh, PA) added. Following a final incubation for 1 h at 37°C, the plate was washed and the assay developed by addition of the substrate p-dinitrophenylphosphate (Sigma-Aldrich). Results were read at 405 nm and mean values were calculated. The amount of cytokine in each test supernatant was then calculated using the standard curve on each plate.

ELISA spot assays for enumeration of IFN-γ-producing cells

ELISA spot assays for IFN-γ were performed as described previously (29, 30). Briefly, ELISA spot plates (Unifilter 350; Polyfiltronics, Rockland, MA) were coated with 4 μg/ml IFN-γ-specific mAb R46A2 and incubated overnight at 4°C. The plates were blocked with 1% BSA in PBS for 1 h at room temperature and then washed four times with PBS. LNC from unsensitized or DNFB-sensitized mice treated with control or anti-SLC Ab were prepared on day +4 after sensitization and used as responder cells. Syngeneic spleen cells from naive BALB/c mice were labeled with 100 μg/ml DNBS as described previously (31), treated with 50 μg/ml mitomycin C, and used as stimulator cells. After washing in RPMI 1640 medium, responder and stimulator cells were resuspended in serum-free HL-1 medium (BioWhittaker, Walkersville, MD) supplemented with 1 mM L-glutamine. Stimulator cells were plated at 5 $\times$ 10^5 cells/well with 2.5 $\times$ 10^5 responder cells/well. Responder cells plated with unlabeled splenocytes were used as a negative (hapten-specificity) control. After 24 h of cell culture at 37°C in 5% CO$_2$, cells were removed from the plate by extensive washing with PBS/0.05% Tween 20 (PBS-T). Biotinylated anti-IFN-γ mAb XMG1.2 (4 μg/ml) was added and the plate was incubated overnight at 4°C. The following day the plate was washed three times with PBS-T and conjugated streptavidin-alkaline phosphatase was added to each well. After 2 h at room temperature, the plates were washed with PBS-T and nitroblue tetrazolium/5-bromo-4-chloro-3-indoyl substrate was added (Kirkegaard & Perry Laboratories, Gaithersburg, MD). The resulting spots were counted with an ELISA spot image analyzer (developed at Case Western Reserve University) using Optimas (Optimas, Botheled, WA) that was designed to detect ELISA spots with predetermined criteria on size, shape, and colorimetric density.

Adoptive transfer of CHS

Hapten immune T cells were obtained from mice treated with 25-μg aliquots of anti-SLC mAb or control Ig the day of Ox sensitization (i.e., day 0). On day +4, LNC suspensions were prepared, washed, and resuspended at 125 $\times$ 10^6/ml in PBS and 400-μl aliquots (50 $\times$ 10^6 cells) were injected i.v. Immediately following cell transfer, ears were challenged with hapten and the increase in ear swelling was measured 24 h later.

Results
Anti-SLC Ab inhibits LC migration following hapten sensitization

Following hapten sensitization of the skin, LC migrate from the epidermis to the skin-draining lymph nodes where they present
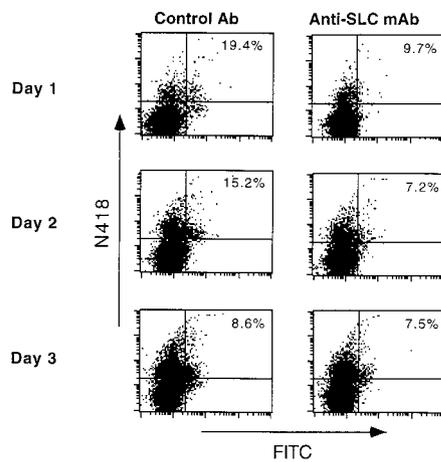
FIGURE 1. Anti-SLC Ab-mediated inhibition of LC migration from the epidermis to draining lymph nodes. BALB/c mice were injected i.v. with 100 μg of control Ig or anti-SLC Ab and were sensitized with 0.5% FITC on day 0. LNC from the skin-draining lymph nodes were prepared on days +1 through +3 and were stained with biotinylated N418 followed by PE-streptavidin. The number in the *upper right-hand corner* indicates the percentage of FITC+N418+ cells in the LNC population.

FIGURE 2. Anti-SLC Ab-mediated inhibition of the CHS response to FITC. BALB/c mice were injected with 100 μg of anti-SLC Ab or control Ig i.v. and were sensitized with 1% FITC on day 0. On day +5, the ears of sensitized and unsensitized control mice were challenged with 0.5% FITC and the change in ear thickness was measured 24 h later. The mean increase in ear thickness for each group following FITC challenge is shown in units of 10^{-4} inches ± SEM.

hapten-MHC complexes and prime the T cells mediating and regulating CHS following hapten challenge. Application of the hapten FITC allows detection of migrating epidermal LC as FITC+/CD11c+ cells in the skin-draining lymph nodes (12, 14, 18, 29). Previous studies have demonstrated the decreased LC migration in response to FITC application in *plt* mice that are defective in SLC production (27). To test the ability of anti-SLC Ab to inhibit LC migration from the skin to draining lymph nodes in wild-type mice, groups of mice were treated with 100 μg of anti-SLC Ab or control Ig i.v. and then sensitized with 0.5% FITC on the shaved abdomen and each footpad. LNC were prepared from the mice at days +1, +2, and +3 postsensitization and stained with anti-CD11c mAb, N418. LNC from animals treated with the control Ig contained readily detectable FITC-labeled CD11c+ cells, indicating migration of the hapten-presenting LC from the epidermis. The presence of FITC-labeled DC were considerably reduced in animals treated with anti-SLC Ab, indicating that hapten-presenting LC migration to the lymph nodes was inhibited by the Ab (Fig. 1). Furthermore, the inhibitory effect of the anti-SLC Ab treatment was maintained through at least day 3 after sensitization.

Anti-SLC Ab treatment inhibits primary CHS responses

Since treatment with anti-SLC Ab in FITC-sensitized mice inhibited LC migration to the lymph nodes, we next evaluated the functional significance of this inhibition by testing whether the anti-SLC Ab would inhibit CHS to FITC. Groups of mice were injected with 100 μg of anti-SLC Ab or control Ig i.v. and then sensitized with 1% FITC on day 0. On day +5, the sensitized animals and a group of unsensitized animals were challenged by applying 0.5% FITC to each side of both ears and the increase in ear thickness was measured 24 h later. Readily detectable ear-swelling responses to FITC were observed in mice treated with control Ig during hapten sensitization when compared with unsensitized mice (Fig. 2, group 1 vs group 3). Treating mice with the anti-SLC Ab at the time of sensitization inhibited the magnitude of the CHS response to near background levels (group 2).

Next, we tested the effect of anti-SLC Ab treatment on the CHS response to sensitization with the hapten Ox. Mice were injected i.v. with dose levels 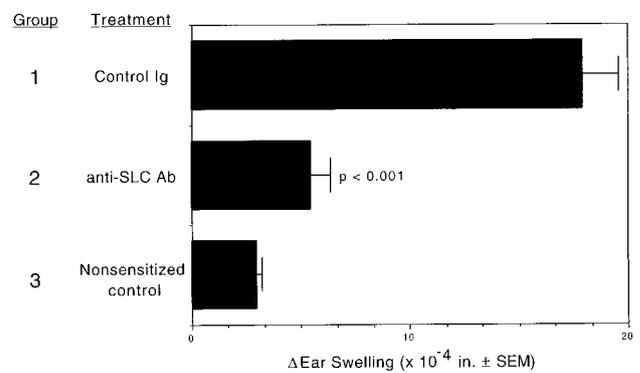of anti-SLC Ab ranging from 10 to 100 μg at the time of sensitization with 3% Ox. Five days later, the mice were challenged on the ears with 0.6% Ox and the increase in ear thickness was measured every 24 h thereafter. Sensitized mice treated with 100 μg of control Ig (Fig. 3A, group 1) but not the unsensitized control animals (group 5) had readily apparent ear-swelling responses to challenge with Ox. Treatment of Ox-sensitized animals with anti-SLC Ab inhibited the CHS response in a dose-dependent manner (groups 2–4). Treatment with 25 μg of anti-SLC Ab resulted in 74% inhibition of the CHS response. This inhibitory effect was long-lasting as an ear-swelling response was not observed in treated animals through 72 h after challenge (Fig. 3B). Inhibition of CHS to Ox was also observed when 100 μg of anti-SLC Ab was administered i.p. at the time of sensitization (data not shown).

We also tested the effect of anti-SLC Ab treatment on the CHS response to a second hapten, DNFB. Sensitization to DNFB is normally elicited by painting the skin on 2 consecutive days (days 0 and +1). We evaluated the effect of single vs dual injections of anti-SLC Ab on CHS responses in mice sensitized with single vs dual applications of DNFB. In contrast to the anti-SLC Ab-mediated inhibition of CHS to Ox, giving 25 μg of anti-SLC Ab i.v. at the time of hapten sensitization was not sufficient to inhibit CHS to DNFB (data not shown). The ear-swelling response in mice sensitized a single time with 1% DNFB was inhibited by a higher dose, 75 μg, of anti-SLC Ab (Fig. 4, group 1 vs group 2). In contrast, CHS responses of mice sensitized with 0.25% DNFB on 2 consecutive days and challenged with the hapten on day +5 were not inhibited by either single or dual administration of 75 μg of anti-SLC Ab (group 3 vs groups 4 and 5).

To further examine the contrasting effects of anti-SLC Ab treatment to sensitization with one vs two hapten paintings, groups of mice were given 100 μg of anti-SLC Ab or control Ig i.p. at the time of sensitization with 3% Ox. Mice were sensitized with a single application of Ox (day 0), a dual application (days 0 and +1), or a painting with an irritant (acetone-olive oil) followed by Ox (days 0 and +1). As previously observed, treatment with the anti-SLC Ab, and not with the control Ig, inhibited CHS in response to a single sensitization with Ox (Fig. 5, groups 1 and 2). CHS responses to painting with the irritant followed by Ox or a dual sensitization with Ox were not inhibited by administration of the anti-SLC Ab (groups 3 and 4).
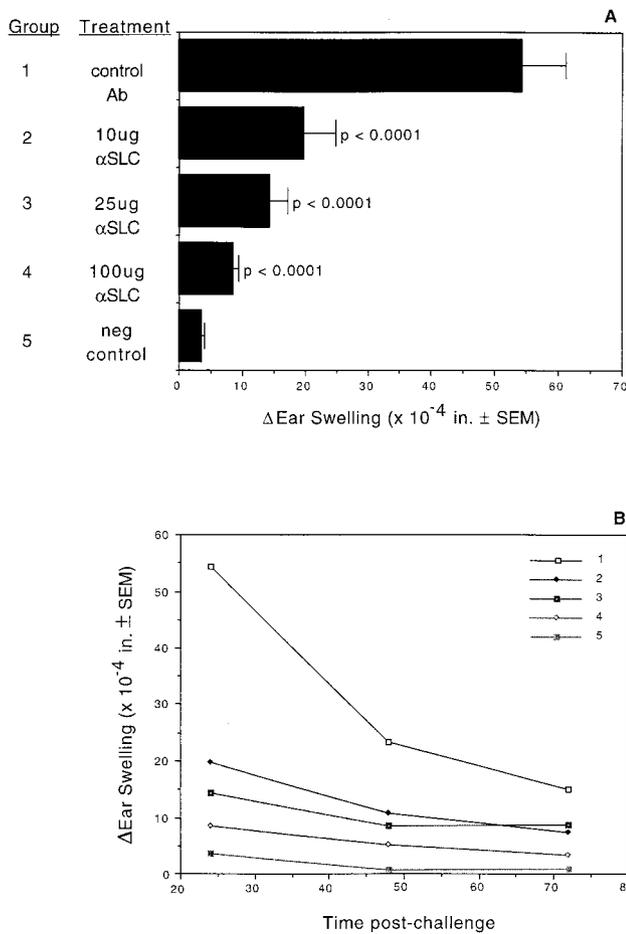
FIGURE 3. Anti-SLC Ab-mediated inhibition of the CHS response to Ox. BALB/c mice were injected with the indicated doses of anti-SLC Ab or 100 μg of control Ig i.v. and were sensitized with 3% Ox on day 0. On day +5, the ears of sensitized and unsensitized control mice were challenged with 1% Ox and the change in ear thickness was measured 24 h later (A). Ear thickness was measured in 24-h intervals until the thickness decreased to background levels (B). The mean increase in ear thickness following Ox challenge for each group is shown in units of 10^{-4} inches $\pm$ SEM.

Effect of anti-SLC Ab treatment on cytokine production by immune T cells

We next tested the effect of anti-SLC Ab treatment on T cell priming in the lymph nodes draining the skin sensitization site by assessing cytokine production. Groups of mice were administered
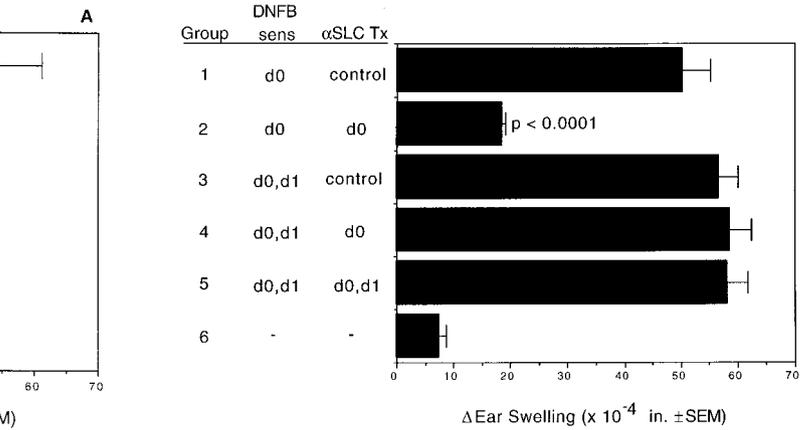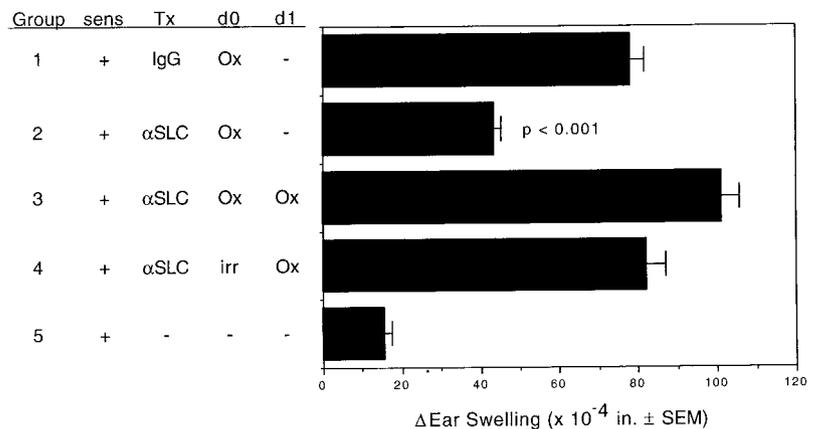

FIGURE 4. Anti-SLC Ab-mediated inhibition of CHS to DNFB. BALB/c mice were injected with 75 μg of control Ig or anti-SLC Ab i.v. and were sensitized with 1% DNFB on day 0 (groups 1 and 2) or 0.25% DNFB on days 0 and +1 (groups 3 and 4). Mice in group 5 were injected with 75 μg of anti-SLC Ab on days 0 and +1 and were sensitized with 0.25% DNFB on days 0 and +1. On day +5, all animals and a group of naive mice were challenged on the ears with 0.2% DNFB and the change in ear thickness was measured 24 h later. The mean increase in ear thickness following DNFB challenge for each group is shown in units of 10^{-4} inches $\pm$ SEM.

10–100 μg of anti-SLC Ab or control Ig i.v. and sensitized with 3% Ox. Four days later, LNC suspensions were prepared and cultured on anti-CD3 Ab-coated wells to stimulate the hapten-primed T cells to produce cytokines. The supernatants were collected 48 h later and tested by ELISA for IFN-γ and IL-4 production. Supernatants of LNC from naive mice cultured on anti-CD3 mAb-coated wells yielded low to nondetectable levels of IFN-γ and IL-4, whereas supernatants of LNC from hapten-immune mice treated with the control Ab produced readily detectable amounts of IFN-γ and IL-4 (Fig. 6). LNC from mice treated with increasing doses of anti-SLC Ab at Ox sensitization produced equivalent amounts of IFN-γ and IL-4. These results suggested that although LC migration to the lymph nodes was inhibited, anti-SLC Ab did not inhibit priming for cytokine production in the lymph nodes of hapten-sensitized mice.

ELISA spot assays were performed to test whether the LNC from the anti-SLC Ab-treated mice were producing IFN-γ in a hapten-specific manner. Mice were treated i.p. with 100 μg of anti-SLC Ab or control Ig at the time of a single painting with DNFB. Four days later, the LNC from each group of sensitized

FIGURE 5. Inability of anti-SLC Ab to inhibit CHS to dual applications of Ox. Groups of BALB/c mice were injected with 100 μg of control Ig or anti-SLC Ab i.p. and were sensitized with 3% Ox on day 0 (groups 1 and 2), 3% Ox on days 0 and +1 (group 3), or with acetone-olive oil (4:1) on day 0 and with 3% Ox on day +1 (group 4). On day +5, all animals and a group of naive mice were ear challenged with 0.6% Ox and the change in ear thickness was measured 24 h later. The mean increase in ear thickness following Ox challenge for each group is shown in units of 10^{-4} inches $\pm$ SEM.
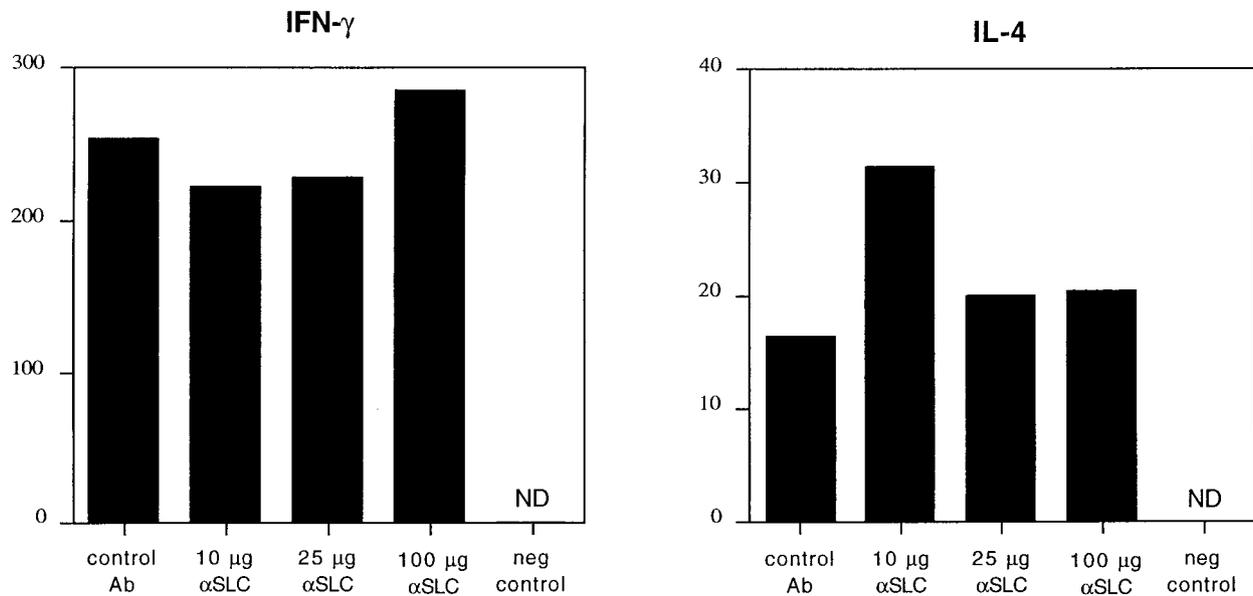
FIGURE 6. Cytokine production by LNC from mice treated with anti-SLC Ab during sensitization with Ox. BALB/c mice were injected with 100 μg of control Ig or the indicated doses of anti-SLC Ab and were sensitized with 3% Ox on day 0. On day +4, pooled LNC suspensions were prepared from each group of sensitized mice and a group of naive mice, and 2×10^5 cells were cultured on anti-CD3 Ab-coated wells. Supernatants were collected 48 h later and analyzed by ELISA for IFN-γ and IL-4 production. Culturing cells on control anti-Vβ17a Ab-coated wells did not stimulate detectable levels of cytokine production. ND, not detectable.

mice and a group of unsensitized mice were prepared and cocultured with either unlabeled or DNP-labeled spleen cells on anti-IFN-γ-coated filters. After 24 h, the cells were removed, the filters were developed, and the number of IFN-γ-producing cells in each group was quantified using an enzyme-linked immunospot image analyzer. In contrast to LNC from naive mice, LNC from DNFB-sensitized mice treated with control Ig contained a readily detectable population of cells that produced IFN-γ when cultured with the DNP-labeled, but not with the unlabeled, spleen cells (Fig. 7). Treatment with anti-SLC Ab did not affect the number of IFN-γ-producing cells in DNFB-sensitized mice. The results indicated that sensitization of control and anti-SLC Ab-treated mice induced equivalent hapten-specific IFN-γ-producing cell responses.
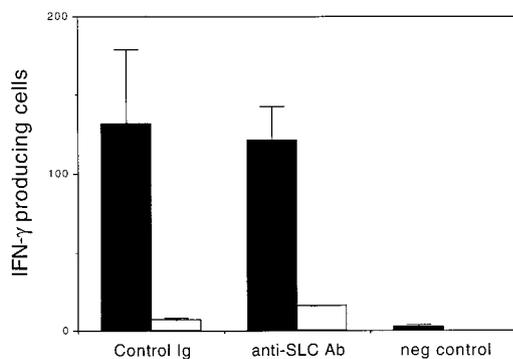
Ability of T cells from anti-SLC Ab-treated mice to passively transfer CHS

Since LNC from Ox-sensitized mice treated with anti-SLC mAb and control Ig produced comparable levels of IFN-γ, we wanted to determine whether the anti-SLC Ab treatment affected the function of the T cells in CHS. Therefore, we tested the ability of the T cells to mediate CHS responses following passive transfer to naive recipients. Donor mice were injected with 25 μg of anti-SLC Ab or control Ig i.v. and were sensitized with Ox. Four days later, pooled LNC from each group of sensitized mice and from unsensitized mice were transferred to naive recipients. Immediately after transfer, the recipients were challenged on the ears with 1% Ox.

FIGURE 7. Number of DNP-specific IFN-γ-producing cells in skin-draining lymph nodes from mice treated with control Ig or anti-SLC Ab during sensitization with DNFB. BALB/c mice were treated with 100 μg of control Ig or anti-SLC Ab i.p. at the time of sensitization with 1% DNFB (day 0). On day +4, LNC suspensions from each group of sensitized mice and a group of naive mice were prepared and cultured with DNP-labeled (black bars) or unlabeled (white bars) on anti-IFN-γ mAb-coated filters. After 24 h, the cells were removed, the assay was developed, and the number of IFN-γ-producing cells per 2.5×10^5 cells for each group was quantified using an enzyme-linked immunospot image analyzer.

FIGURE 8. Passive transfer of CHS with LNC from anti-SLC Ab-treated mice. BALB/c mice were injected with 25 μg of control Ig or anti-SLC Ab i.v. and were sensitized with 3% Ox on day 0. On day +4, LNC were harvested from the sensitized mice and from naive mice, washed, and resuspended at 125×10^6/ml in PBS, and 50×10^6 cells were injected i.v. to naive recipient BALB/c mice. Immediately following cell transfer, the recipient mice were challenged on the ears with 1% Ox and the change in ear thickness was measured 24 h later and is shown in units of 10^{-4} inches ± SEM.

FIGURE 9. Treatment with anti-SLC Ab during sensitization does not induce hapten-specific tolerance. Groups of BALB/c mice (groups 1–3) were injected with 25 μg of control Ig or anti-SLC Ab i.v. and were sensitized with 3% Ox. On day +5, mice were challenged with 1% Ox and the ear thickness was measured 24 h later. Five weeks after the initial sensitization and challenge with Ox, the mice were painted on the abdomen with either 3% Ox (groups 1 and 3) or 0.25% DNFB (group 2). At this time, control mice were also injected with 25 μg of control Ig or anti-SLC Ab i.v. and sensitized with 3% Ox (groups 4 and 5). Sensitized and unsensitized mice were challenged 5 days later with 1% Ox (groups 1 and 3–6) or 0.2% DNFB (group 2) and the change in ear thickness was measured 24 h later and is shown in units of 10^{-4} inches $\pm$ SEM.

Group	Ox immune	Ab Tx	sens	chlg
1	αSLC	-	Ox	Ox
2	αSLC	-	DNFB	DNFB
3	control	-	Ox	Ox
4	-	control	Ox	Ox
5	-	αSLC	Ox	Ox
6	-	-	-	Ox

Δ Ear Swelling (x 10^{-4} in. $\pm$ SEM)
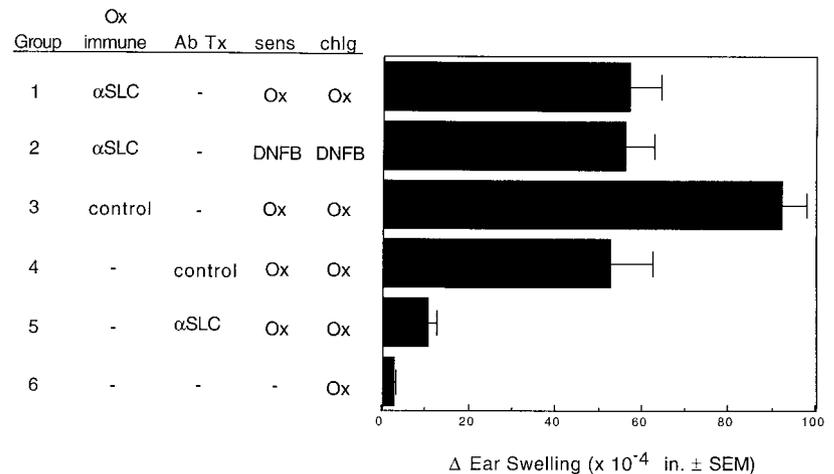
Delivery of LNC from control Ig-treated mice sensitized with Ox, but not from unsensitized donors, elicited an ear-swelling response (Fig. 8, group 1 vs group 3). Delivery of LNC from anti-SLC Ab-treated mice sensitized with Ox, however, did not induce an ear-swelling response (group 2). Therefore, T cells were induced in the lymph nodes of anti-SLC Ab-treated animals that produced IFN-γ in response to anti-CD3 mAb or to DNP-labeled spleen cells in vitro, but these cells could not elicit a CHS response to hapten challenge in vivo.

Anti-SLC Ab treatment during primary sensitization for CHS does not induce tolerance to the hapten

Since T cells from mice given anti-SLC Ab at the time of hapten sensitization could not mediate a CHS response, we next tested whether these mice had developed immunological tolerance to the hapten. Groups of mice were injected with 25 μg of anti-SLC or control Ig i.v. and were sensitized with 3% Ox. Five days later, the mice were challenged with 1% Ox and the ear thickness was measured 24 h later. As previously observed, the primary CHS response to Ox was inhibited in mice treated with anti-SLC Ab during hapten sensitization (data not shown). Five weeks later, these anti-SLC Ab-treated/Ox-sensitized and challenged mice were repainted with either 3% Ox or 0.25% DNFB and were challenged 5 days later (Fig. 9, groups 1–3). Mice treated with the control Ig before primary Ox sensitization had an increased response follow-ing resensitization and challenge with Ox (group 3) when compared with the primary response in mice sensitized and challenged a single time (group 4). Mice treated with anti-SLC Ab during primary sensitization and then resensitized with Ox had less of an ear-swelling response to Ox challenge than mice that were treated with control Ig during primary sensitization and resensitized 5 wk later (group 1 vs group 3). This response was equivalent, however, to the primary response in control mice sensitized and challenged a single time (group 1 vs group 4). Ear-swelling responses were also observed in response to DNFB sensitization and challenge of mice previously treated with anti-SLC Ab and sensitized with Ox (group 2). In addition, control groups of naive mice were treated with anti-SLC Ab or control Ig and were sensitized and challenged with Ox (groups 4 and 5). Again, treatment with anti-SLC Ab inhibited the primary CHS response when compared with control Ig-treated mice. These results indicated that the inhibition observed with anti-SLC Ab treatment during primary CHS responses was not sustained during subsequent sensitization and challenge. Thus, treatment with anti-SLC mAb during sensitization did not induce tolerance to the hapten.

Administration of anti-SLC Ab during hapten challenge

Finally, we tested whether hapten-immune T cell recruitment and function was inhibited by treatment with anti-SLC Ab given to sensitized animals at the time of challenge. Groups of mice were

Group	Ox sens	Tx at sens	Tx at chall
1	+	control Ab	-
2	+	αSLC	-
3	+	-	αSLC
4	-	-	-

p < 0.0001

Δ Ear Swelling (x 10^{-4} in. $\pm$ SEM)
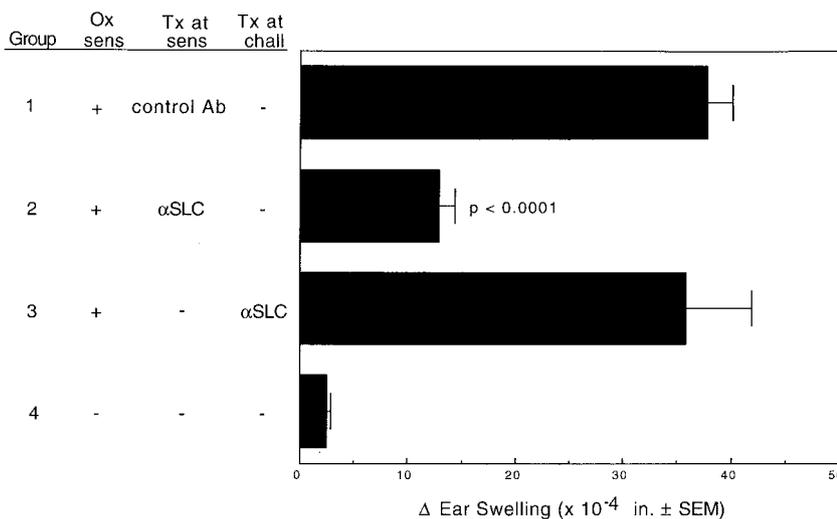
FIGURE 10. CHS responses to Ox-immune mice given anti-SLC Ab at the time of hapten sensitization. BALB/c mice were sensitized with 3% Ox on day 0 and injected with 25 μg of control Ig or anti-SLC Ab i.v. just before sensitization on day 0 (groups 1 and 2) or injected with 25 μg of anti-SLC Ab i.v. just before challenge on day +5 (group 3). Sensitized and unsensitized mice were challenged with 1% Ox and the change in ear thickness was measured 24 h later and is shown in units of 10^{-4} inches $\pm$ SEM.

5213

injected with 25 μg of anti-SLC Ab or control Ig i.v. at the time of sensitization with 3% Ox or were sensitized with Ox and given the anti-SLC Ab or control Ig at the time of hapten challenge with 0.6% Ox. As previously observed, treatment with anti-SLC Ab at the time of sensitization inhibited the ear-swelling response to Ox challenge (Fig. 10, group 1 vs group 2). Giving the anti-SLC Ab at the time of challenge, however, had no effect on the elicitation of CHS when compared with control Ig-treated immune animals (group 3 vs group 1).

Discussion

LC are the primary APC in the epidermis. Their migration from sites of epicutaneous hapten application to skin-draining lymph nodes is critical for the priming of hapten-specific T cells and the induction of CHS (14, 17, 18). The mechanisms directing LC migration from epidermal sensitization sites to draining lymph nodes is unclear and continues to be investigated by many laboratories. Recent evidence has implicated the chemokine SLC in DC migration. This is supported by studies in mice homozygous for the paucity of lymph node T cell (*plt*) mutation which display an abnormality in naive T cell homing and impaired DC migration to secondary lymph node organs (27, 32, 33). LC migration to lymph nodes of *plt* mice following epicutaneous sensitization with FITC is reduced 4-fold when compared with wild-type mice. A mechanism for the severe immunodeficiency was recently suggested in studies by Gunn et al. (27) who reported that *plt* mice are deficient in expression of SLC. A role for SLC in DC trafficking has been supported by studies demonstrating that migration of labeled DC from footpad injection sites to draining lymph nodes is inhibited by administration of anti-SLC Ab (28). These studies have suggested that strategies aimed at SLC neutralization may inhibit DC migration to lymphoid tissues and potentially prevent priming of T cells to applied or injected Ags.

In this study, we have extended these observations and demonstrated that the CHS response to Ox is inhibited in a dose-dependent manner by treating mice with anti-SLC Ab at the time of hapten sensitization. The effect of this single dose was not transient because inhibition of the ear-swelling response continued for at least 3–4 days after Ox challenge, which is the time that the response in control Ig-treated mice nears resolution. Consistent with these results, we have also shown that migration of FITC-presenting LC from skin sensitization sites to the draining lymph nodes is inhibited in mice treated with anti-SLC Ab for at least 3 days after epicutaneous application of the FITC. On the basis of these results, we predicted that T cells in the lymph nodes would not be primed to react to the hapten and therefore not be able to elicit an immune response to hapten challenge. Surprisingly, although anti-SLC Ab inhibited the CHS response, T cells from hapten-sensitized mice treated with the anti-SLC Ab were still capable of producing IFN-γ when stimulated by culture on anti-CD3 Ab-coated wells. T cells from naive mice did not produce IFN-γ during these cultures, indicating that the cytokine production observed is the result of hapten application. Similarly, equivalent numbers of hapten-specific IFN-γ-producing cells were observed in sensitized mice treated with control and anti-SLC Ab. An obvious interpretation of this result is that CD8$^+$ T cell production of IFN-γ is not critical for the elicitation of CHS and the response is mediated by other proinflammatory cytokines produced by the hapten-primed T cells. However, recent observations in our laboratory have indicated a direct correlation between hapten-primed CD8$^+$ T cell IFN-γ production and the magnitude of the CHS response observed following challenge, including the absence of CHS responses in IFN-γ-deficient mice (our unpublished observations).

To explore the paradoxical induction of IFN-γ-producing T cells with the lack of CHS responses in mice treated with anti-SLC Ab during hapten sensitization, we passively transferred LNC from sensitized mice treated with control or anti-SLC Ab. These experiments demonstrated that even though there were hapten-specific IFN-γ-producing T cells in the lymph nodes of anti-SLC Ab-treated mice, these cells were unable to mediate CHS in naive mice following transfer and hapten challenge. This argues that treatment with anti-SLC Ab inhibited the development of a critical immune function during hapten priming of these T cells. This result raised two important questions regarding the priming of T cells in anti-SLC Ab-treated mice during hapten sensitization: 1) If the LC do not migrate to the lymph nodes, how are the T cells stimulated to produce cytokines? and 2) If the T cells can produce IFN-γ, why are they unable to mediate CHS in response to hapten challenge? It may be possible that hapten-protein complexes drain from the epidermal sensitization site into the lymph nodes through the blood and lymphatics and are picked up by resident DC in the lymphoid tissue which subsequently prime hapten-specific T cells. Such presentation may be sufficient to allow T cell priming for production of proinflammatory cytokines such as IFN-γ but not in a manner which renders the cells capable of functioning in response to epicutaneous challenge with the hapten.

One result of priming by alternative hapten-presenting cells may be the lack of T cell programming for circulation to challenge sites in the skin. Several studies have indicated the requirement for expression of specific adhesion markers on T cells that enable the cells to enter cutaneous inflammatory sites during CHS (34, 35). Recent studies in other laboratories have indicated that a specific homing receptor/endothelial ligand pair, the cutaneous lymphocyte-associated Ag and E-selectin on endothelial cells, is associated with the infiltration of human T cells into inflammatory sites in the skin (36, 37). Similar studies have been performed in mice and have suggested that only CD4$^+$ T cells expressing P- and E-selectin-binding proteins are recruited to inflammatory sites in the skin (38, 39). As such, treatment with anti-SLC mAb might inhibit critical priming factors that induce the expression of skin-homing receptors on the T cells mediating the CHS response. LC may provide such a critical component, inducing the expression of these skin-homing receptors during hapten priming. This possibility is currently being explored.

The results of this study have demonstrated the ability of a single dose of Ab to SLC to inhibit functional T cell priming for elicitation of CHS to Ox. In contrast, treatment with the Ab did not inhibit the response when more than one application of hapten or when an additional irritant were used to sensitize mice. Two applications of hapten may cause more irritation of the skin, which may increase inflammation at the site and stimulate LC or dermal DC migration to draining lymph nodes despite the anti-SLC Ab treatment. This is demonstrated by the lack of anti-SLC Ab-mediated inhibition of CHS when an irritant is applied to the skin before hapten sensitization. Consistent with this proposal, an increased dose of anti-SLC Ab was required to inhibit the CHS response to a single sensitizing application of DNFB when compared with the amount required to inhibit the response to Ox. This indicates an important difference between these two haptens during skin sensitization. A possible explanation is that the increased inflammation induced by DNFB application may either stimulate the production of higher levels of SLC or other chemokines that direct LC migration to the lymph nodes. Alternatively, this increased inflammation may stimulate a higher density of CCR7 on the LC surface, which renders this migration less sensitive to the Ab treatment.

Although a good deal is known about the role of LC in the induction of CHS, very little is known about the role these DC play in the elicitation of the response. Results from some laboratories have suggested that the presence of LC at the challenge site during elicitation of CHS enhances the magnitude of the response (40). In contrast, a recent report indicated the presence of LC at the hapten challenge site resulted in decreased CHS responses (41). Results from the current report indicate that treatment with anti-SLC Ab inhibits LC migration and CHS but does not have an effect on the elicitation of the response when administered at the time of challenge. These results suggest that inhibition of LC migration from the challenge site does not affect the magnitude of the elicited response in a positive or negative manner.

In summary, the results of this report have indicated an important role for SLC in the CHS response. The inhibition of LC migration was associated with a selective inhibition of functional T cell priming to mediate contact allergy responses to challenge with the hapten. By examining the functional consequences of anti-SLC Ab treatment, we gain insight into DC trafficking and hapten priming of specific T cells which could potentially provide new therapies involving immune regulation in autoimmunity and transplantation.

References

1. Enk, A. H. 1997. Allergic contact dermatitis: understanding the immune response and potential for targeted therapy using cytokines. *Mol. Med. Today 3:423.*
2. Grabbe, S., and T. Schwarz. 1998. Immunoregulatory mechanisms involved in elicitation of allergic contact hypersensitivity. *Immunol. Today 19:37.*
3. Kalish, R. S., and K. L. Johnson. 1990. Enrichment and function of urushiol (poison ivy)-specific T lymphocytes in lesions of allergic contact dermatitis to urushiol. *J. Immunol. 145:3706.*
4. Kalish, R. S., J. A. Wood, and A. LaPorte. 1994. Processing of urushiol (poison ivy) hapten by both endogenous and exogenous pathways for presentation to T cells in vitro. *J. Clin. Invest. 93:2039.*
5. Gocinski, B. L., and R. E. Tigelaar. 1990. Roles of CD4$^+$ and CD8$^+$ T cells in murine contact sensitivity revealed by in vivo monoclonal antibody depletion. *J. Immunol. 144:4121.*
6. Bour, H., E. Peyron, M. Gaucherand, J.-L. Garrigue, C. Desvignes, D. Kaiserlian, J.-P. Revillard, and J.-F. Nicolas. 1995. Major histocompatibility complex class I-restricted CD8$^+$ T cells and class II-restricted CD4$^+$ T cells, respectively, mediate and regulate contact sensitivity to dinitrofluorobenzene. *Eur. J. Immunol. 25:3006.*
7. Abe, M., T. Kondo, H. Xu, and R. L. Fairchild. 1996. Interferon-γ inducible protein (IP-10) expression is mediated by CD8$^+$ T cells and is regulated by CD4$^+$ T cells during the elicitation of contact of hypersensitivity. *J. Invest. Dermatol. 107:360.*
8. Xu, H., A. Banerjee, N. A. DiIulio, and R. L. Fairchild. 1997. Development of effector CD8$^+$ T cells in contact hypersensitivity occurs independently of CD4$^+$ T cells. *J. Immunol. 158:4721.*
9. Xu, H., N. A. DiIulio, and R. L. Fairchild. 1996. T cell populations primed by hapten sensitization in contact sensitivity are distinguished by polarized patterns of cytokine production: IFN-γ producing (Tc1) effector CD8$^+$ T cells and Il-4/Il-10 producing (Th2) negative regulatory CD4$^+$ T cells. *J. Exp. Med. 183:1001.*
10. Roupe, G., and B. Ridell. 1979. The cellular infiltrate in contact sensitivity to picryl chloride in the mouse. *Acta Dermato-Venereol. 59:191.*
11. Piguet, P. F., G. E. Grau, C. Hauser, and P. Vassali. 1991. Tumor necrosis factor is a critical mediator in hapten-induced irritant and contact hypersensitivity reactions. *J. Exp. Med. 173:673.*
12. Macatonia, S. E., A. J. Edwards, and S. C. Knight. 1986. Dendritic cells and the initiation of contact sensitivity to fluorescein isothiocyanate. *Immunology 59:509.*
13. Macatonia, S. E., S. C. Knight, A. J. Edwards, S. Griffiths, and P. Fryer. 1987. Localization of antigen on lymph node dendritic cells after exposure to the contact sensitizer fluorescein isothiocyanate. *J. Exp. Med. 166:1654.*
14. Kripke, M., C. Munn, A. Jeevan, J. Tang, and S. Bucana. 1990. Evidence that cutaneous antigen-presenting cells migrate to regional lymph nodes during contact sensitization. *J. Immunol. 145:2833.*
15. Streilein, J. W., G. B. Toews, and P. R. Bergstresser. 1980. Langerhans cells: functional aspects revealed by in vivo grafting studies. *J. Invest. Dermatol. 75:17.*
16. Streilein, J. W., and P. R. Bergstresser. 1981. Langerhans cell function dictates induction of contact hypersensitivity or unresponsiveness to DNFB in Syrian hamsters. *J. Invest. Dermatol. 77:272.*
17. Streilein, J. W., and P. R. Bergstresser. 1988. Genetic basis of ultraviolet-B effects on contact hypersensitivity. *Immunogenetics 27:252.*
18. Bigby, M., R. Vargas, and M.-S. Sy. 1989. Production of hapten-specific T cell hybridomas and their use to study the effect of ultraviolet B irradiation on the development of contact hypersensitivity. *J. Immunol. 143:3867.*
19. Oppenheim, J. J., C. O. C. Zachariae, N. Mukaida, and K. Matsushima. 1991. Properties of the novel proinflammatory supergene "intercrine" cytokine family. *Annu. Rev. Immunol. 9:617.*
20. Rollins, B. J. 1997. Chemokines. *Blood 90:909.*
21. Smith, R. E., R. M. Strieter, S. H. Phan, N. W. Lukacs, G. B. Huffnagle, C. A. Wilke, M. D. Burdick, P. Lincoln, H. Evanhoff, and S. L. Kunkel. 1994. Production and function of murine macrophage inflammatory protein-1α in bleomycin-induced lung injury. *J. Immunol. 153:4704.*
22. Karpus, W. J., N. W. Lukas, B. L. McRae, R. M. Strieter, S. L. Kunkel, and S. D. Miller. 1995. An important role for the chemokine macrophage inflammatory protein-1α in the pathogenesis of the T cell-mediated autoimmune disease, experimental autoimmune encephalomyelitis. *J. Immunol. 155:5003.*
23. Shanley, T. P., H. Schmal, H. P. Friedl, M. L. Jones, and P. A. Ward. 1995. Role of macrophage inflammatory protein-1α (MIP-1α) in acute lung injury in rats. *J. Immunol. 154:4793.*
24. Lloyd, C. M., A. W. Minto, M. E. Dorf, A. Proudfoot, T. N. C. Wells, D. J. Salant, and J.-C. Gutierrez-Ramos. 1997. RANTES and monocyte chemoattractant protein-1 (MCP-1) play an important role in the inflammatory phase of crescentic nephritis, but only MCP-1 is involved in crescent formation and interstitial fibrosis. *J. Exp. Med. 185:1371.*
25. Tanabe, S., Z. Lu, Y. Luo, E. J. Quaackenbush, M. A. Berman, L. A. Collins-Racie, S. Mi, C. Reilly, D. Lo, K. A. Jacobs, and M. E. Dorf. 1997. Identification of a new mouse β-chemokine, thymus-derived chemotactic agent 4, with activity on T lymphocytes and mesangial cells. *J. Immunol. 159:5671.*
26. Gunn, M. D., K. Tangemann, C. Tam, J. G. Cytster, S. D. Rosen, and L. T. Williams. 1998. A chemokine expressed in lymphoid high endothelial venules promotes the adhesion and chemotaxis of naive T lymphocytes. *Proc. Natl. Acad. Sci. USA 95:258.*
27. Gunn, M. D., S. Kyuwa, C. Tam, T. Kakiuchi, A. Matsuzawa, L. T. Williams, and H. Nakano. 1999. Mice lacking expression of secondary lymphoid organ chemokine have defects in lymphocyte homing and dendritic cell localization. *J. Exp. Med. 189:451.*
28. Saeki, H., A. M. Moore, M. J. Brown, and S. T. Hwang. 1999. Secondary lymphoid-tissue chemokine (SLC) and CC chemokine receptor 7 (CCR7) participate in the emigration pathway of mature dendritic cells from the skin to regional lymph nodes. *J. Immunol. 162:2472.*
29. Xu, H., P. S. Heeger, and R. L. Fairchild. 1997. Distinct roles for B7-1 and B7-2 determinants during priming of effector CD8$^+$ Tc1 and regulatory CD4$^+$ Th2 cells for contact hypersensitivity. *J. Immunol. 159:4217.*
30. Lehmann, P. V., D. Matesic, G. Benichou, and P. S. Heeger. 1997. Induction of T helper 2 immunity to an immunodominant allopeptide. *Transplantation 64:292.*
31. Krasteva, M., J. Kehren, F. Horand, H. Akiba, G. Choquet, M.-T. Ducluzea, R. Tedone, J.-L. Garrigue, D. Kaiserlian, and J.-F. Nicolas. 1998. Dual role of dendritic cells in the induction and down-regulation of antigen-specific cutaneous inflammation. *J. Immunol. 160:1181.*
32. Nakano, H., T. Tamura, T. Yoshimoto, H. Yagita, M. Miysaka, E. C. Butcher, H. Nariuchi, T. Kakiuchi, and A. Matsuzawa. 1997. Genetic defect in T lymphocyte-specific homing into peripheral lymph nodes. *Eur. J. Immunol. 27:215.*
33. Nakano, H., S. Mori, H. Yonekawa, H. Nariuchi, A. Marsuzawa, and T. Kakiuchi. 1998. A novel mutant gene involved in T-lymphocyte specific homing into peripheral lymphoid organs on mouse chromosome 4. *Blood 91:2886.*
34. Camp, R. L., A. Scheynius, C. Johansson, and E. Pure. 1993. CD44 is necessary for optimal contact allergic responses but is not required for normal leukocyte extravasation. *J. Exp. Med. 178:497.*
35. Scheynius, A., R. L. Camp, and E. Pure. 1993. Reduced contact sensitivity reactions in mice treated with monoclonal antibodies to leukocyte function-associated molecule-1 and intercellular adhesion molecule-1. *J. Immunol. 150:655.*
36. Picker, L. J., S. A. Michie, L. S. Rott, and E. C. Butcher. 1990. A unique phenotype of skin-associated lymphocytes in humans: preferential expression of the HECA-452 epitope by benign and malignant T cells at cutaneous sites. *Am. J. Pathol. 136:1053.*
37. Berg, E. L., T. Yoshino, L. S. Rott, M. K. Robinson, R. A. Warnock, T. K. Kishimoto, L. J. Picker, and E. C. Butcher. 1991. The cutaneous lymphocyte antigen is a skin lymphocyte homing receptor for the vascular lectin endothelial cell-leukocyte adhesion molecule 1. *J. Exp. Med. 174:1461.*
38. Austrup, F., D. Vestweber, E. Borges, M. Hohning, R. Brauer, U. Herz, H. Renz, R. Hallmann, A. Scheffold, A. Radbruch, and A. Hamannn. 1997. P- and E-selectin mediate recruitment of T-helper-1 but not T-helper-2 cells into inflamed tissues. *Nature 385:81.*
39. Tietz, W., Y. Allemand, E. Borges, D. von Laer, R. Hallmann, D. Vestweber, and A. Hamann. 1998. CD4$^+$ T cells migrate into inflamed skin only if they express ligands for E- and P-selectin. *J. Immunol. 161:963.*
40. Streilein, J. W., S. F. Grammer, T. Yoshikawa, A. Demidem, and M. Vermeer. 1990. Functional dichotomy between Langerhans cells that present antigen to naive and to memory/effector T lymphocytes. *Immunol. Rev. 117:159.*
41. Grabbe, S., K. Steinbrink, M. Steinert, T. A. Luger, and T. Schwartz. 1995. Removal of the majority of epidermal Langerhans cells by topical or systemic steroid application enhances the effector phase of murine of contact hypersensitivity. *J. Immunol. 155:4207.*