CD4⁺ T Cells Regulate CD8⁺ T Cell-Mediated Cutaneous Immune Responses by Restricting Effector T Cell Development through a Fas Ligand-Dependent Mechanism

Anton V. Gorbachev and Robert L. Fairchild

J Immunol 2004; 172:2286-2295; doi: 10.4049/jimmunol.172.4.2286
http://www.jimmunol.org/content/172/4/2286

References This article cites 36 articles, 21 of which you can access for free at: http://www.jimmunol.org/content/172/4/2286.full#ref-list-1

Subscription Information about subscribing to The Journal of Immunology is online at: http://jimmunol.org/subscription

Permissions Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
CD4⁺ T Cells Regulate CD8⁺ T Cell-Mediated Cutaneous Immune Responses by Restricting Effector T Cell Development through a Fas Ligand-Dependent Mechanism

Anton V. Gorbachev and Robert L. Fairchild

The magnitude and duration of CD8⁺ T cell-mediated responses in the skin to hapten sensitization and challenge, contact hypersensitivity (CHS), is negatively regulated by CD4⁺ T cells through an unknown mechanism. In this study we show that CD4⁺ T cells restrict the development and expansion of hapten-specific CD8⁺ T cells mediating CHS responses to 2,4-dinitrofluorobenzene. In the absence of CD4⁺ T cells, high numbers of hapten-specific CD8⁺ T cells producing IFN-γ were detected in the skin-draining lymph nodes on day 5 postsensitization, and these numbers decreased slightly, but were maintained through day 9, correlating with the increased magnitude and duration of CHS responses observed in these mice. In the presence of CD4⁺ T cells, the number of hapten-specific CD8⁺ T cells producing IFN-γ detected on day 5 postsensitization was lower and quickly fell to background levels by day 7. The limited development of effector CD8⁺ T cells was associated with decreased numbers of hapten-presenting dendritic cells in the lymphoid priming site. This form of immunoregulation was absent after sensitization of Fas ligand-defective gld mice. Transfer of wild-type CD4⁺ T cells to gld mice restored the negative regulation of CD8⁺ T cell priming and the immune response to hapten challenge in gld-recipient mice. These results indicate that CD4⁺ T cells restrict hapten-specific CD8⁺ T cell priming for CHS responses through a Fas ligand-dependent mechanism. The Journal of Immunology, 2004, 172: 2286–2295.

Contact hypersensitivity (CHS) is a T cell-mediated immune response to cutaneous sensitization and subsequent challenge with hapten contactants. Examples of haptens inducing these responses are urushiol, the reactive agent in poison ivy, and the model hapten 2,4-dinitrofluorobenzene (DNFB), oxa-azolone (Ox), and FITC. After application to the epidermis, haptens are acquired and processed by the epidermal dendritic cells (DC), Langerhans cells (LC), which then migrate to skin-draining lymph nodes and present hapten-MHC complexes to specific T cell populations (1, 2). Subsequent hapten challenge results in cutaneous infiltration of the hapten-primed T cells and their activation to mediate the characteristic edema or spongiosis that peaks 24–48 h after challenge and then quickly resolves (3, 4).

Early studies in mouse models and clinical studies using T cell clones isolated from allergic contact dermatitis patients have supported a role for both CD4⁺ and CD8⁺ T cells as cellular mediators of the CHS response (5–8). Subsequent studies in mice by Gosinski and Tigelaar (9) and repeated by this laboratory (10) demonstrated that depletion of CD4⁺ T cells before hapten sensitization resulted in CHS responses of high magnitude and long duration, whereas depletion of CD8⁺ T cells resulted in either absent or reduced CHS responses. Similar results were observed using MHC class I and class II knockout mice as models of CD8⁺ and CD4⁺ T cell deficiencies, respectively (11). In vitro analyses of hapten-primed T cells indicated that sensitization for CHS induces two distinct polarized populations of hapten-reactive T cells: CD8⁺ T cells that produce IFN-γ in response to hapten stimulation and hapten-specific CD4⁺ T cells that produce IL-4, IL-5, and IL-10 and little or no IFN-γ (10). Collectively, these studies have indicated that the primary effector cells of CHS responses to most haptens are IFN-γ-producing CD8⁺ T cells and that CD4⁺ T cells restrict the magnitude and duration of the response.

The mechanism expressed by the CD4⁺ T cells to mediate regulation of CHS has remained undefined. Recent studies from this laboratory indicated that CD4⁺ T cells negatively influence the stimulatory capacity of LC during priming of hapten-specific CD8⁺ T cells (12). These results led us to postulate that CD4⁺ T cells may regulate CHS by restricting the development of effector CD8⁺ T cells during hapten priming in the skin-draining lymph nodes. In the current study the regulatory role of CD4⁺ T cells in CD8⁺ T cells priming for CHS was tested. The results demonstrate for the first time CD4⁺ T cell restriction of hapten-specific CD8⁺ T cell development during hapten sensitization through a Fas ligand (FasL)-mediated pathway.

Materials and Methods

Animals

C57BL/6 mice were purchased from Dr. C. Reeder (National Cancer Institute, Frederick, MD). FasL-defective gld mice on the C57BL/6 genetic background (B6Smn.C3H-Tufsf/+), were purchased from The Jackson Laboratory (Bar Harbor, ME). Adult female mice, 8–10 wk old, were used throughout these studies.

Antibodies

The mAbs from the culture supernatant of the IgG-producing hybridomas YTS191.1.2 and GK1.5 (anti-mouse CD4 mAb) and N418 (anti-CD11c) were purified by protein G chromatography, PE- and FITC-labeled mAbs specific for mouse MHC class II (I-A¹), CD4, CD8, CD11c, CD11a, CD44, Fas, and FasL, and capture and detection mAbs for IFN-γ and IL-4-specific.
ELISPOT assays were purchased from BD PharMingen (San Diego, CA). Polyclonal rat IgG was purchased from Southern Biotechnology Associates (Birmingham, AL).


dnfb-sensitized mice, Lnc were stained with PE-labeled anti-I-Ab mAb each footpad. One to 5 days after FITC sensitization Lnc were isolated vitro enrichment of CD4– T cells, respectively, and CD4+ and CD8+ T cells were enriched by negative selection following the manufacturer’s instructions (Dynabeads, Dynal Biotech, Oslo, Norway). The efficiency of this negative selection was >95% as assessed by flow cytometry.


cytokine-specific ELISPOT assays

ELISPOT assays to enumerate IFN-γ and IL-4-producing cells were performed as previously described (12). Briefly, ELISPOT plates (Unifilter 350; Polyscreen, Rockland, MA) were coated with 100 µl of 4 µg/ml anti-IFN-γ mAb R26A2 or anti-IL-4 mAb 1B11 and incubated overnight at 4°C. The plates were blocked with 1% BSA in PBS for 90 min at 37°C and washed four times with PBS. Lnc from DNFB-sensitized mice were prepared on day 5 after sensitization and used as responder cells. Syngeneic spleen cells from naive mice were treated with 50 µg/ml mitomycin C, labeled by incubation with DNFB (100 µg/ml), and used as stimulator cells. Responder Lnc were resuspended in serum-free HL-1 medium (Bio-Whittaker, Walkersville, MD) and cultured at 2 × 105 cells/well 24 h at 37°C and washed three times with PBS. Lnc from DNFB-sensitized mice were prepared on day 5 after sensitization and used as responder cells. Syngeneic spleen cells from naive mice were treated with 50 µg/ml mitomycin C, labeled by incubation with DNFB (100 µg/ml), and used as stimulator cells. Responder Lnc were resuspended in serum-free HL-1 medium (Bio-Whittaker, Walkersville, MD) and cultured at 2 × 105 cells/well 24 h at 37°C and washed three times with PBS. Lnc from DNFB-sensitized mice were prepared on day 5 after sensitization and used as responder cells.
The hapten-primed effector CD8+ T cells are contained within the CD44high/CD11ach high LNC population in sensitized mice (14). To further assess the level of CD8+ T cell activation in mice with and without CD4+ T cells during hapten sensitization, the temporal expression of activation determinants by CD8+ T cells in the lymph nodes of DNFB-sensitized mice was examined by flow cytometry. The percentages of CD8+ T cells expressing high levels of the activation markers CD11a and CD44 within the CD8+ T cell compartment were compared in sensitized mice with CD4+ T cells and CD4– T cell-depleted mice. Consistent with the results showing increased expansion of hapten-specific CD8+ T cells in the absence of CD4+ T cells, the numbers of CD8+ T cells expressing CD44 (Fig. 2) and CD11a (not shown) were increased and sustained in the lymph nodes of CD4– T cell-depleted mice.

Similar to IFN-γ-producing CD8+ T cells, hapten-specific CD4+ T cells producing IL-4 in the lymph nodes of DNFB-sensitized mice were also detected in high numbers on day 5 and decreased to background levels thereafter (Fig. 1C). This suggested that the development of both hapten-specific CD4+ and CD8+ T cells during sensitization for CHS is regulated in a similar manner. Collectively, the results presented in Figs. 1 and 2 demonstrate that CD4+ T cells restrict the magnitude and duration of effector CD8+ T cell expansion during hapten sensitization.

**Decreased numbers of hapten-presenting DC are associated with CD4+ T cell regulation of effector CD8+ T cell during sensitization for CHS**

Recent studies suggested that CD4+ T cells may attenuate DC activity during hapten-specific CD8+ T cell priming (12). As hapten-presenting DC are the cells priming the effector CD8+ T cells during sensitization for CHS, the numbers of hapten-bearing DC in the lymph nodes of sensitized control vs CD4+ T cell-depleted mice were compared. Application of FITC allows detection of hapten-presenting DC, including epidermal LC, as FITC+/CD11c+ cells in the skin-draining lymph nodes (1, 15, 16). Mice were painted with FITC on day 0, and LNC suspensions were prepared 3 days later and were stained for the DC marker CD11c. Low numbers of FITC+/CD11c+ resident DC were present in the lymph nodes of naive mice. LNC from FITC-sensitized animals contained a readily detectable population of FITC+/CD11c+ cells, indicating the presence of hapten-presenting DC that had emigrated from the epidermis to the lymph nodes. The number of CD11c+/Hapten-expressing cells

### Table I. Sustained development of hapten-specific IFN-γ producing CD8+ T cells in the absence of CD4+ cell-mediated regulation*

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>2.50</td>
<td>4.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Immune control</td>
<td>24.0</td>
<td>10.0</td>
<td>5.00</td>
</tr>
<tr>
<td>Immune CD4-depleted</td>
<td>21.0</td>
<td>16.0</td>
<td>15.0</td>
</tr>
</tbody>
</table>

*Equal numbers of axillary, brachial, and inguinal lymph nodes (six per mouse) were harvested from naive and DNFB-sensitized control or CD4+ T cell-depleted mice (two mice per group) at different times post-sensitization. Lymph nodes in each group were pooled, and the total number of LNC was counted using trypan blue. Aliquots of CD8+ T cells prepared from each group using negative selection by Dynabeads were stimulated in vitro with DNFB-labeled spleen cells and tested for the number of IFN-γ-producing cells by ELISPOT. The total number of IFN-γ-producing hapten-specific CD8+ T cells in a pool of six skin-draining lymph nodes was calculated as a proportion of the LNC number for each group.
in the lymph nodes of CD4⁺ T cell-depleted mice was sustained on day 3 (72 h) postsensitization, whereas the number of these cells decreased 2-fold in control-treated mice with an intact CD4⁺ T cell compartment (Fig. 3). Similar results were obtained when LNC from DNFB-sensitized mice with and without CD4⁺ T cells were stained with anti-CD11c and anti-I-A^b mAb for the detection of DC (not shown). To ensure that the increase in the numbers of hapten-bearing DC in the lymph nodes of CD4⁺ T cell-depleted mice was not due to an increase in their frequency after CD4 depletion, total numbers of FITC-labeled DC were calculated for each individual mouse based on the total LNC number and the number of DC per LNC aliquot. Total numbers of FITC-bearing DC in the lymph nodes of control and CD4⁺ T cell-depleted mice were compared at different post-sensitization time points. The number of hapten-bearing DC in the lymph nodes of control mice with an intact CD4⁺ T cell compartment peaked at 24–48 h after sensitization with FITC and then significantly declined at 96 h post-sensitization. The number of FITC⁺ DC in the lymph nodes of CD4⁺ T cell-depleted mice was similar to that in control mice at 24 h post-sensitization (Fig. 4). In contrast to control mice, the FITC⁺ DC numbers increased at 48–72 h post-sensitization.

**FIGURE 2.** Expansion of activated CD8⁺ T cells is regulated by CD4⁺ T cells. LNC from control, rat IgG-treated and CD4⁺ T cell-depleted mice prepared on days 5, 7, and 9 after sensitization with 0.25% DNFB were stained with FITC-labeled anti-CD8 mAb and PE-labeled anti-CD44 mAb. The CD8⁺ T cell population was gated and analyzed for CD44 expression vs cell size (forward scatter). Numbers in the upper right corner represent the percentage of CD44^high^ cells within the gated CD8⁺ T cell population. Results are representative of two individual experiments.

**FIGURE 3.** CD4⁺ T cells decrease the numbers of FITC-bearing DC in the lymph nodes of FITC-sensitized mice by 72 h post-sensitization. Control or CD4⁺ T cell-depleted mice were sensitized with 1% FITC on day 0. On days 1 (24 h) and 3 (72 h) postsensitization LNC suspensions from the sensitized and naive mice were prepared and stained with PE-labeled anti-CD11c mAb. Hapten-bearing DC were detected as FITC-positive cells with high granularity (FITC vs side scatter; A) expressing CD11c (B). Numbers in the upper right corner represent the number of FITC⁺/CD11c⁺ DC per 2⁻¹⁰⁴ LNC aliquot. Results are representative of three individual experiments.
CD4+ T cells inhibit hapten-specific CD8+ T cell development

Collectively, the results of these initial experiments indicated that decreased number of hapten-presenting DC were observed in the presence of CD4+ T cells during priming of hapten-specific T cells.

Lack of regulation of CHS in FasL-defective gld mice

To examine the potential role of FasL in CD4+ T cell-mediated negative regulation of CHS responses, ear-swelling responses to DNFB sensitization and challenge were compared in wild-type vs gld mice. As previously observed, CHS responses in wild-type animals peaked at 24–48 h postchallenge and then declined. In contrast, responses in DNFB-sensitized and challenged gld mice with an intact CD4+ T cell compartment were higher at peak times and longer in duration (Fig. 5, A vs B). To ensure that CHS responses to DNFB in gld mice are mediated by hapten-specific CD8+ T cells, LNC were obtained from DNFB-sensitized gld mice and CD4+, and CD8+ T cell-enriched or CD4+/CD8+ T cell-depleted (CD4+ CD8+) cell suspensions were assayed for the number of hapten-specific, IFN-γ-producing cells by ELISPOT. As previously observed in wild-type mice, CD8+ T cells were the major population producing IFN-γ in response to in vitro stimulation with hapten (Fig. 5C). Additionally, transfer of CD8+ T

FIGURE 4. CD4+ T cells restrict presence of hapten-bearing DC in the lymph nodes of mice during T cell priming. Mice were either untreated (□) or depleted of CD4+ T cells (■) before sensitization with FITC. At a different post sensitization time, LNC were obtained from skin-draining lymph nodes of each individual mouse and stained with PE-labeled anti-CD11c mAb. Hapten-bearing DC were defined as the side scatterhigh/FITChigh/CD11chigh cell population, and numbers of these cells per 2 × 10⁴ LNC aliquot were assessed. The total number of hapten-presenting DC was calculated for each individual mouse as a proportion of total LNC obtained from the skin-draining lymph nodes. Results are presented as the mean number of hapten-presenting LC per mouse ± SEM (three mice for each group). Results are representative of three individual experiments. *, p < 0.05.

FIGURE 5. Increased and sustained CHS responses to DNFB in gld mice. C57BL/6 wild-type (A) and gld (B) mice were sensitized with 0.25% DNFB on days 0 and 1. On day 5 naive (○) or sensitized (■) mice were ear-challenged with 0.2% DNFB, and the change in ear thickness was measured at 24-h intervals. The mean increase in ear thickness after challenge with DNFB is shown as 10-1 in. ± SEM for groups of four mice. Results are representative of three individual experiments. *, p < 0.05 as compared with control wild-type mice. C, LNC were harvested from either sensitized or naive gld mice on day 5 post sensitization. CD4+, CD8+ T cell-enriched or CD4+CD8+ populations were obtained from LNC of sensitized mice using negative selection by Dynabeads. Aliquots (2 × 10⁵ cells) were cultured with 5 × 10⁴ DNBS-labeled syngeneic splenocytes on IFN-γ-coated ELISPOT plates. T cells cultured with unlabeled splenocytes were used as a negative control. After 24 h, cells were removed, and the ELISPOT assay was developed to detect IFN-γ-producing cells. The results indicate the mean number ± SEM of hapten-specific IFN-γ-producing cells in each 2 × 10⁵ cell aliquots. Results are representative of two individual experiments. ND, not detected.

FIGURE 6. Increased and sustained numbers of IFN-γ-producing CD8+ T cells in the lymph nodes of gld mice after sensitization with DNFB. C57BL/6 wild-type and gld mice were sensitized with 0.25% DNFB on days 0 and 1. On days 5 and 7 after sensitization, LNC suspensions were prepared from skin-draining lymph nodes of naive (day 0) and sensitized wild-type (□) and gld (■) mice. CD8+ T cell-enriched populations were prepared using anti-CD4 mAb and magnetic bead negative selection, and 2 × 10⁵ cell aliquots were cultured with 5 × 10⁴ DNBS-labeled syngeneic splenocytes on IFN-γ-coated ELISPOT plates. After 24 h, cells were removed, and the ELISPOT assay was developed to detect IFN-γ-producing CD8+ T cells. The total number of IFN-γ-producing, hapten-specific CD8+ T cells in a pool of skin-draining lymph nodes was calculated as a proportion of LNC number. The results represent the mean number ± SEM of IFN-γ-producing CD8+ T cells. Results are representative of two individual experiments. *, p < 0.05.
cells enriched from sensitized gld mice into naive wild-type mice induced ear swelling in these recipients in response to subsequent hapten challenge (not shown).

Consistent with these results, IFN-γ-producing CD8+ T cells in the lymph nodes of DNFB-sensitized, wild-type mice were clearly present on day 5 and decreased to background levels by day 7 post-sensitization, whereas the numbers of these cells in sensitized gld mice were increased and sustained (Fig. 6). Increased numbers of FITC+/CD11c+ cells were observed in the lymph nodes of gld mice compared with wild-type mice with the CD4+ T cell population intact. The number of FITC-bearing DC in these mice remained at least 5-fold increased compared with DC numbers in wild-type mice by 72 h post-sensitization, indicating that the clearance of the DC was delayed in the lymph nodes of gld mice (Fig. 7). Collectively, the results observed in gld mice were similar to the effect of CD4+ T cell depletion on the development of hapten-specific CD8+ T cells and CHS responses in wild-type mice. These results indicated the lack of negative regulation during CHS responses in the presence of CD4+ T cells, but in the absence of functional FasL, and suggested that CD4+ T cells require functional FasL to restrict hapten priming of effector CD8+ T cells for CHS and the associated decreases in hapten-presenting DC.

**CD4+ T cells express FasL in the lymph nodes of sensitized mice**

To further investigate the requirement for functional FasL for CD4+ T cell-mediated regulation of the development of CHS responses, FasL expression on LNC populations of naive or sensitized mice was tested. Total LNC suspensions were obtained from naive or DNFB-sensitized mice on day 2 after sensitization and were stained with anti-CD4 or anti-CD8 mAb and anti-FasL mAb. FasL expression was clearly detected on CD4+ T cells from naive mice, and the percentage of FasL-expressing CD4+ T cells was slightly elevated in hapten-sensitized mice (Fig. 8, A and B). In contrast, FasL expression was largely absent on CD8+ T cells, and the percentage of CD8+ T cells expressing FasL was very low in the lymph nodes of either naive (not shown) or hapten-sensitized mice (Fig. 8C).

**Wild-type CD4+ T cells restore immunoregulation of CHS responses in gld mice**

To further test the requirement for CD4+ T cells expressing functional FasL in the regulation of CHS, CD4+ T cells were purified from LNC of naive, wild-type mice and transferred to gld mice before sensitization with DNFB. Preliminary experiments transferring CFSE-labeled wild-type CD4+ T cells to gld mice demonstrated the accumulation of CD4+/CFSE+ cells in the skin-draining lymph nodes 2 days after transfer (not shown). Similar to CHS responses in wild-type animals, transfer of wild-type CD4+ T cells to gld mice before sensitization resulted in CHS responses to DNFB sensitization and challenge that peaked at 24 h postchallenge and declined thereafter (Fig. 9A). These responses were considerably lower and shorter in duration than responses in gld mice that did not receive wild-type CD4+ T cells. In contrast to the transfer of wild-type CD4+ T cells, transfer of wild-type cells

![FIGURE 7](https://www.jimmunol.org/content/229/7/2291/F1.large.jpg)

**FIGURE 7.** Increased numbers of DC in the lymph nodes of DNFB-sensitized gld mice during T cell hapten priming. Wild-type C57BL/6 and gld mice were sensitized with 0.25% DNFB on days 0 and 1. On days 2 (48 h) or 3 (72 h) postsensitization, LNC suspensions were prepared from skin-draining lymph nodes and stained with PE-labeled anti-CD11c mAb. A, Hapten-bearing DC were detected as side scatter/FITC/CD11c+ cells. Numbers in the upper right corner represent the number of hapten-bearing DC per 2 × 10^6 LNC aliquot. B, The total number of hapten-bearing DC was calculated for each individual mouse as a proportion of total LNC obtained from the skin-draining lymph nodes. Results are presented as the mean number of hapten-presenting LC ± SEM for control wild-type (○) or gld mice (■, three mice for each group). Results are representative of two individual experiments. *, p < 0.05.

![FIGURE 8](https://www.jimmunol.org/content/229/7/2291/F2.large.jpg)

**FIGURE 8.** FasL expression on T cell populations. LNC from naive or DNFB-sensitized mice (three mice per group) were stained with FITC-labeled anti-CD4 or anti-CD8 mAb and biotinylated anti-FasL mAb plus streptavidin-PE. The expression of FasL by CD4+ T cells from naive mice (A) or sensitized mice (B) and the expression of FasL by CD8+ T cells from sensitized mice (C) are shown on dot plots as gate R7. Numbers in the upper right corner represent the percentage of cells expressing FasL within the CD4+ or CD8+ T cell population. Results are representative of three individual experiments.
depleted of CD4+ T cells to gld mice resulted in further increased CHS responses compared with control gld mice (not shown).

The negative regulatory function of wild-type CD4+ T cells in gld mice was also examined by testing the numbers of IFN-γ-producing CD8+ T cells in the lymph nodes of CD4+ T cell-recipient and control (i.e., nonrecipient) gld mice. Consistent with the CHS responses, on day 5 after DNBFB sensitization the number of IFN-γ-producing CD8+ T cells in the lymph nodes of gld mice that had received wild-type CD4+ T cells before sensitization was ~30% of the number observed in control gld mice (Fig. 9B). Furthermore, staining LNC suspensions from the control and CD4+ T cell-recipient gld mice with anti-I-A<sup>b</sup> and anti-CD11c mAb indicated that the wild-type CD4+ T cell transfer decreased the number of DC in the lymph nodes of sensitized gld mice on day 5 after sensitization (Fig. 9C). Thus, the presence of wild-type CD4+ T cells in FasL-defective animals during hapten sensitization decreased hapten-specific CD8+ T cell development and DC numbers as well as the magnitude of the CHS response to challenge. Furthermore, CD4+ T cells transferred from wild-type mice into wild-type mice previously depleted of CD4+ T cells (e.g., CD4+ T cell-deficient mice) inhibited the development of hapten-specific, IFN-γ-producing CD8+ T cells in these recipients, as assessed by ELISPOT assay. In striking contrast, CD4+ T cells transferred from gld mice into CD4+ T cell-depleted, wild-type animals were unable to restore immunoregulation of effector CD8+ T cell development in these CD4+4-depleted recipient mice (Fig. 10). These results indicate the critical role of functional FasL in CD4+ T cell-mediated regulation of CHS responses.

**Discussion**

Studies employing either treatment with CD4+ and CD8+ T cell-depleting mAb or MHC class I<sup>-/-</sup> and class II<sup>-/-</sup> mice have demonstrated that CD8+ T cells are the primary effector cells in CHS responses and that CD4+ T cells down-regulate the magnitude and duration of CHS (9–11). Whether CD4+ T cell regulation of CHS is mediated at the sensitization or elicitation stage has remained unclear. Higher ear-swelling responses observed after adoptive transfer of hapten-specific CD8+ T cells without CD4+ T cells suggested that CD4+ T cells might regulate CHS during response elicitation (9). Studies from this laboratory indicating that the majority of hapten-prime CD4+ T cells produce type 2 cytokines such as IL-4, IL-5, and IL-10 (10) raised the possibility that these cytokines suppress CD8+ T cell-mediated elicitation of CHS. In support of this possibility, injection of IL-4 or IL-10

![Figure 9](https://www.jimmunol.org/content/173/6/2287/F1.large.jpg)

**FIGURE 9.** Adoptive transfer of CD4+ T cells from wild-type mice restores immunoregulation of CHS responses in gld mice. A, CD4+ T cells were purified from the lymph nodes of naive wild-type C57BL/6, and 30 × 10<sup>5</sup> cells were transferred by i.v. injection to gld mice. Two days later, the recipient (●) and control, nonrecipient (●) gld and wild-type C57BL/6 (●) mice were sensitized with 0.25% DNBFB on days 0 and 1. On day 5, the sensitized mice and naive wild-type C57BL/6 (△) and naive gld (■) mice were challenged with 0.2% DNBFB, and the change in ear thickness was measured at 24<h intervals. The mean increase in ear thickness after challenge with DNBFB is shown to be 10<sup>-4</sup> in. ± SEM for groups of four mice. B, On day 5 after DNBFB sensitization, CD8+ T cell-enriched populations were prepared from the skin-draining lymph nodes of the control, nonrecipient gld and gld recipients of wild-type CD4+ T cells, and 2 × 10<sup>5</sup> cell aliquots were cultured with 5 × 10<sup>5</sup> DNBFB-sensitized syngeneic splenocytes on IFN-γ-coated ELISPOT plates. After 24 h, the cells were removed, and the ELISPOT assay was developed to detect the number of IFN-γ-producing CD8+ T cells per cell aliquot. The total number ± SEM of IFN-γ-producing hapten-specific CD8+ T cells was calculated as a proportion of the total LNC number. C, LNC suspensions were prepared from the control, nonrecipient gld and gld recipients of wild-type CD4+ T cells on day 5 after DNBFB sensitization. Cell aliquots were stained with FITC-labeled anti-CD11c mAb and PE-labeled anti-I-A<sup>b</sup> mAb, and the numbers of CD11c/I-A<sup>b</sup>-expressing DC were assessed by flow cytometry. The mean number ± SEM of DC in skin-draining lymph nodes are shown. *, p < 0.05. ND, not detected.
into hapten-sensitized mice at the time of hapten challenge, but not sensitization, reduced the magnitude of the CHS response elicited (17, 18). In addition, adoptive transfer of hapten-specific CD4+ Th2 cells inhibited CHS responses when cells were transferred into mice before hapten challenge, but not before sensitization (19).

In the current report CD4+ T cell-mediated regulation of hapten-specific CD8+ T cell development was tested. The numbers of IFN-γ-producing CD8+ T cells in the lymph nodes of hapten-sensitized mice with an intact CD4+ T cell compartment were high 4–5 days after sensitization, but quickly decreased to the levels observed in naive mice within 2–3 days. In contrast, depletion of CD4+ T cells before hapten sensitization or sensitization of class II MHC−/− mice resulted in increased and sustained numbers of hapten-specific CD8+ T cells producing IFN-γ. These increases in the hapten-primed CD8+ T cell compartment directly correlated with the higher magnitude and longer duration of ear-swelling responses in mice lacking CD4+ T cells, indicating that CD4+ T cells restrict the development and/or expansion of the effector CD8+ T cells during hapten sensitization.

We first considered the possibility that Th2 or other immunoregulatory cytokines inhibit the development of CD8+ effector T cells. Studies in humans have suggested that hapten-specific CD4+ T cells may regulate CHS responses through IL-10-mediated repression of APC function (20). Ab-mediated neutralization of IL-10 or TGF-β during DNFB sensitization increased the development of IFN-γ-producing CD8+ T cells and ear-swelling responses to hapten challenge. However, these effects were marginal compared with the magnitude of these responses in CD4+ T cell-depleted animals (21). Furthermore, the presence of TGF-β mRNA in the lymph nodes of hapten-sensitized mice did not correlate with the presence vs the absence of CD4+ T cells (A. Gorbachev, unpublished observations). Thus, IL-10 and TGF-β may be endogenous regulators of CHS, but did not appear to be the major regulatory mechanism by which CD4+ T cells restrict hapten-specific CD8+ effector T cell development during sensitization.

The appearance and maintenance of the hapten-primed CD4+ and CD8+ T cell compartments during sensitization for CHS were similar, peaking on day 5 postsensitization and sharply declining thereafter. This suggested a common mechanism of immunoregulation for the development of these T cells during hapten sensitization. As hapten-presenting DC prime both CD4+ and CD8+ T cells, these cells were logical targets of the CD4+ T cell-mediated immunoregulation. Recent studies from this laboratory indicated that CD4+ T cells may affect hapten-specific CD8+ T cell development indirectly, via hapten-presenting DC (12). With these studies in mind, CD4+ T cell-mediated regulation of DC presence in the lymph nodes during T cell priming for CHS was tested. On days 2 through 4 post sensitization, higher numbers of FITC-bearing DC were detected in the lymph nodes of CD4+ T cell-depleted mice than in control mice after sensitization with FITC. Similar increases in DC numbers were observed in the lymph nodes of CD4+ T cell-depleted mice sensitized with DNFB (data not shown). These increases correlated with the increased and sustained numbers of hapten-specific CD8+ T cells producing IFN-γ and the augmented ear-swelling responses in the CD4+ T cell-depleted mice sensitized and challenged with DNFB.

Rather than Th2 cytokine-mediated mechanisms, the results suggest that CD4+ T cells may restrict effector CD8+ T cell development for CHS responses by limiting the presence of hapten-presenting DC in the priming site. The regulation of CD8+ T cell development and hapten-presenting DC in lymph nodes during sensitization was dependent on functional FasL. First, the increased numbers of DC and hapten-specific IFN-γ-producing CD8+ T cells in the lymph nodes of hapten-sensitized gld mice directly correlated with the increased and sustained CHS responses compared with wild-type mice. As previous studies of CHS in gld mice have shown (22, 23), the magnitude of the CHS response was similar in both wild-type and gld mice 24 h after hapten challenge. Then CHS in wild-type mice begin to decrease, whereas the response in gld mice is increased and sustained by at least 96 h postchallenge, indicating lack of CHS regulation in these mice. Second, treatment of gld mice with anti-CD4 mAb did not result in any further increase in CHS responses or in DC or hapten-specific CD8+ T cell numbers in the lymph nodes of these mice. However, CD4+ T cells remained in gld mice even after treatment with anti-CD4 mAb, which resulted in the depletion of 99% of the target cell population in wild-type mice (A. Gorbachev, unpublished observations). The reason for this resistance of FasL-defective CD4+ T cells to anti-CD4 mAb-mediated depletion remains unknown. Nevertheless, these results indicate the lack of immunoregulation of CHS responses in the presence of CD4+ T cells, but in the absence of functional FasL. Finally, presensitization transfer of wild-type CD4+, but not CD8+ T cells to gld mice resulted in decreased CHS responses and hapten-specific CD8+ T cell development in the gld-recipient mice. Just as important, transfer of CD4+ T cells from wild-type mice into wild-type recipients depleted of their CD4+ T cell compartment restored immunoregulation of hapten-specific CD8+ T cell development, whereas CD4+ T cells transferred from gld mice failed to do so. Collectively, these results indicate the crucial role of CD4+ T cells expressing functional...
CD4⁺ T CELLS INHIBIT HAPTEN-SPECIFIC CD8⁺ T CELL DEVELOPMENT

FasL in limiting the development and expansion of hapten-specific CD8⁺ T cells to regulate CHS responses.

The current results are the first to indicate that CD4⁺ T cells use FasL-dependent mechanism to restrict the development of hapten-specific CD8⁺ T cells during priming. The mechanism underlying this regulation is not entirely clear at this time. Hapten-presenting DC express Fas in the lymph nodes of sensitized mice (A. Gorbachev, unpublished observations), whereas a subpopulation of CD4⁺ T cells express FasL. Therefore, Fas-Fasl apoptosis of DC mediated by the CD4⁺ T cells would be the most direct mechanism. Both in vivo and in vitro studies have indicated the elimination of APCs as a form of immunoregulation (24–27). CD8⁺ T cells mediate the elimination of allogeneic or Ag-pulsed DC after injection into mice (28, 29). Fasl-mediated apoptosis of OVA-presenting Langerhans cell lines by Ag-specific CD4⁺ T cells has been observed in vitro (27). However, recent studies indicated the resistance of at least some DC subsets to Fasl-mediated apoptosis through CD40 signaling or expression of the apoptosis inhibitory protein FLIP (30–32).

Collectively, the results of this study demonstrate that negative regulation of CHS responses by CD4⁺ T cells is mediated by the ability of CD4⁺ T cells to restrict effector hapten-specific CD8⁺ T cell development in the skin-draining lymph nodes during sensitization. The CD4⁺ T cells require functional Fasl to mediate this immunoregulation. The development and expansion of hapten-specific CD8⁺ T cells for CHS responses are restricted presumably by reducing hapten-presenting DC numbers in the lymph nodes through a Fasl-dependent mechanism. The consequence of this would be a limited access of CD8⁺ T cells to MHC class I/hapten and costimulatory signals provided by DC during priming for the CHS response. The increased and prolonged presence of DC in the absence of FasL-expressing CD4⁺ T cells sustains the development and expansion of effector CD8⁺ T cells during CHS responses. These results suggest a novel mechanism of CD4⁺ T cell-mediated immunoregulation of CD8⁺ T cell responses to cutaneous Ags. Ag-specific CD8⁺ T cells are an important effector component of many cutaneous immune responses, including melanoma, graft-vs-host disease, and psoriasis (33–36). Many aspects concerning the development and regulation of effector CD8⁺ T cells in these responses remain poorly understood. The current studies should help in designing strategies to manipulate CD8⁺ T cell development and function during cutaneous immune responses. Ongoing autoimmune disease may be attenuated by promoting CD4⁺ T cell Fasl-mediated restriction of self-Ag-presenting DC, whereas inhibition of this regulatory activity may be useful in augmenting responses in infectious or malignant diseases.

Acknowledgments
We thank Mayumi Naramura for suggestions during the course of this work, and the staff of the Cleveland Clinic Foundation Biological Resources Unit for excellent animal care.

References
10. Xu, H., N. A. Difilio, 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 (Th1) effector CD8⁺ T cells and IL-4/IL-10 producing (Th2) negative regulatory CD4⁺ T cells. J. Exp. Med. 188:1061.


