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$CD4^+$ T Cells Regulate $CD8^+$ T Cell-Mediated Cutaneous Immune Responses by Restricting Effector T Cell Development through a Fas Ligand-Dependent Mechanism[1]

Anton V. Gorbachev[2]* 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)[3] 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 haptens 2,4-dinitrofluorobenzene (DNFB), oxazolone (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-Tnfsf6^{gld}) 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-A^b), CD4, CD8, CD11c, CD11a, CD44, Fas, and FasL and capture and detection mAbs for IFN-γ and IL-4-specific

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[3] Abbreviations used in this paper: CHS, contact hypersensitivity; DC, dendritic cells; DNFB, 2,4-dinitrofluorobenzene; FasL, Fas ligand; LC, Langerhans cells; LNC, lymph node cells; Ox, oxazolone.

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ELISPOT assays were purchased from BD PharMingen (San Diego, CA). Polyclonal rat IgG was purchased from Southern Biotechnology Associates (Birmingham, AL).

Hapten sensitization and elicitation of CHS

For sensitization to DNFB, mice were painted on 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 each footpad. On day 5, sensitized and control unsensitized mice were challenged with 10 μl of 0.2% DNFB on both sides of each ear. Ear thickness was measured in a blinded manner at 24-h intervals after challenge using an engineer's micrometer (Mitutoyo, Elk Grove Village, IL) and was expressed in units of 10^{-4} in. as previously described (10). The magnitude of the ear-swelling response is presented as the mean increase in each group of four sensitized or nonsensitized mice (i.e., eight ears) $\pm$ SEM over the thickness measured just before hapten challenge on day 5.

$CD4^+$ T cell depletion and T cell enrichment

For in vivo depletion of $CD4^+$ T cells, mice were injected with 200 μg of each anti-CD4 mAb, YTS 191, and GK1.5 i.p. on 3 consecutive days before hapten sensitization on days 0 and 1 as previously described (13). This treatment resulted in $\geq$99% depletion of the target population as assessed by Ab staining and flow cytometric analysis of spleen and lymph node cells (LNC) compared with cells from control, rat IgG-treated mice. Treated mice were rested 1–3 days before sensitization with hapten. For in vitro enrichment of $CD4^+$ or $CD8^+$ T cells, LNC from hapten-sensitized mice were incubated with anti-CD8 or anti-CD4 mAb-coated magnetic beads, respectively, and $CD4^+$ or $CD8^+$ 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; Polifiltronics, Rockland, MA) were coated with 100 μl of 4 μg/ml anti-IFN-γ mAb R26A2 or anti-IL-4 mAb 11B11 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 DNBS (100 μg/ml), and used as stimulator cells. Responder LNC were resuspended in serum-free HL-1 medium (BioWhittaker, Walkersville, MD) and cultured at 2×10^5 cells/well with 5×10^5 stimulator cells/well 24 h at 37°C in 5% CO_2. In all experiments responder cells cultured with unlabeled splenocytes and LNC from naive mice cultured with DNBS-labeled stimulator cells were used as negative controls. After 24 h, cells were removed from the culture wells by extensive washing with PBS and then PBS/0.2% Tween 20. Biotinylated anti-IFN-γ mAb XMG1.2 or anti-IL-4 mAb BVD6-24G2 (2 μg/ml) was added, and the plate was incubated overnight at 4°C. The following day the wells were washed three times with PBS/0.2% Tween 20 and incubated with anti-biotin alkaline phosphatase conjugate for IFN-γ or with streptavidin-HRP for IL-4. After 2 h at room temperature, the wells were washed with PBS, nitro blue tetrazolium-5-bromo-4-chloro-3-indolyl substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD) was added for the detection of IFN-γ, and 3-amino-9-ethylcarbazole (Pierce, Rockford, IL) was added for the detection of IL-4. The resulting spots were counted on an ImmunoSpot Series 1 Analyzer (Cellular Technology, Cleveland, OH) that was designed to detect spots with predetermined criteria on size, shape, and colorimetric density.

Flow cytometry

Two-color flow cytometric analysis to assess the phenotype of DC and $CD4^+$ and $CD8^+$ T cells was performed as previously described (14, 15). To prevent nonspecific Ab binding, cells were incubated with rat serum (Rockland, Gilbertsville, PA) diluted 1/1000 in staining buffer (Dulbecco's PBS with 2% FCS/0.2% NaN_3) for 20 min on ice. Stained cells were washed five times, resuspended in staining buffer, and analyzed by two-color flow cytometry using a FACScan (BD Immunocytometry Systems, San Jose, CA) To detect FITC-presenting DC in the lymph nodes, mice were painted with 100 μl of 1% FITC on the shaved abdomen and 5 μl on each footpad. One to 5 days after FITC sensitization LNC were isolated and stained with PE-labeled anti-CD11c mAb HL3. To detect DC in DNFB-sensitized mice, LNC were stained with PE-labeled anti-I-A^b mAb and FITC-labeled anti-CD11c mAb HL3.

Cell transfer

LNC were collected from naive wild-type C57BL/6 mice, and $CD4^+$ T cells were purified by high affinity negative selection using a mouse T cell CD4 subset column kit following the manufacturer's instructions (R&D Systems, Minneapolis, MN). The purity of recovered $CD4^+$ T cells was 95%, as assessed by Ab staining and flow cytometry. The *gld* mice received 30×10^6 $CD4^+$ T cells in sterile PBS i.v. on day -2 before sensitization. On days 0 and 1, T cell recipient and control, nonrecipient *gld* mice were sensitized and challenged with DNFB, and the ear thickness was measured as detailed above.

Statistical analysis

Statistical analysis to determine differences between groups for immune responses was performed using Student's *t* test. The differences were considered significant at $p < 0.05$.

Results

Increased and sustained expansion of hapten-primed $CD8^+$ T cells in the absence of $CD4^+$ T cells during sensitization

Consistent with previous reports from this laboratory (10, 12), the magnitude and duration of CHS responses to DNFB were increased in mice depleted of $CD4^+$ T cells before hapten sensitization compared with responses in mice with the $CD4^+$ T cell compartment intact (Fig. 1*A*). As IFN-γ-producing $CD8^+$ T cells are the primary effector cells mediating CHS, the number of hapten-specific $CD8^+$ T cells producing IFN-γ in the skin-draining lymph nodes of DNFB-sensitized mice with and without $CD4^+$ T cells was determined in ELISPOT assays (Fig. 1*B*). On day 5 after sensitization, $CD8^+$ T cells producing IFN-γ during culture with hapten-labeled stimulator cells were easily detectable in control, rat IgG-treated mice. Within 2 days (i.e., day 7), the number of these $CD8^+$ T cells dropped to the background number observed in lymph nodes of unsensitized mice. In contrast to control treated mice, considerably higher numbers of hapten-specific $CD8^+$ T cells producing IFN-γ were observed in the lymph nodes of $CD4^+$ T cell-depleted mice on day 5 postsensitization. Furthermore, the numbers of IFN-γ-producing $CD8^+$ T cells remained high on days 7 and 9 postsensitization in the $CD4^+$ T cell-depleted mice. $CD4^+$ T cells constitute a significant component of LNC, raising the possibility that the increase in IFN-γ-producing $CD8^+$ T cell numbers in $CD4^+$ T cell-depleted mice simply reflected an alteration in the frequency of $CD8^+$ T cells due to this depletion. To examine this possibility, the total numbers of LNC and hapten-specific, IFN-γ-producing $CD8^+$ T cells obtained from skin-draining lymph nodes of control and $CD4^+$ T cell-depleted mice were compared at different postsensitization time points. On day 5 postsensitization the numbers of LNC in control and depleted animals were similar, indicating that the absence of $CD4^+$ T cells in depleted mice was already compensated at this time by the cells expanding during immune response. The total number of hapten-specific, IFN-γ-producing $CD8^+$ T cells in $CD4^+$ T cell-depleted mice was increased and sustained, whereas the number of these cells in control mice with an intact $CD4^+$ T cell compartment decreased to background levels after day 5 (Table I). The results indicate that the increase in hapten-specific $CD8^+$ T cell numbers observed in $CD4^+$ T cell-depleted mice reflected increased expansion of these cells rather than an increase in frequency due to the absence of $CD4^+$ T cells. Similar results were obtained when ear-swelling responses and numbers of hapten-specific $CD8^+$ T cells producing IFN-γ were tested in DNFB-sensitized MHC class $II^{-/-}$ mice (not shown). Thus, the augmented and prolonged ear swelling (i.e., CHS) responses observed in the absence of $CD4^+$ T cell-mediated regulation correlated with the increased and sustained numbers of hapten-specific $CD8^+$ T cells producing IFN-γ in the lymph nodes draining the sensitization site.

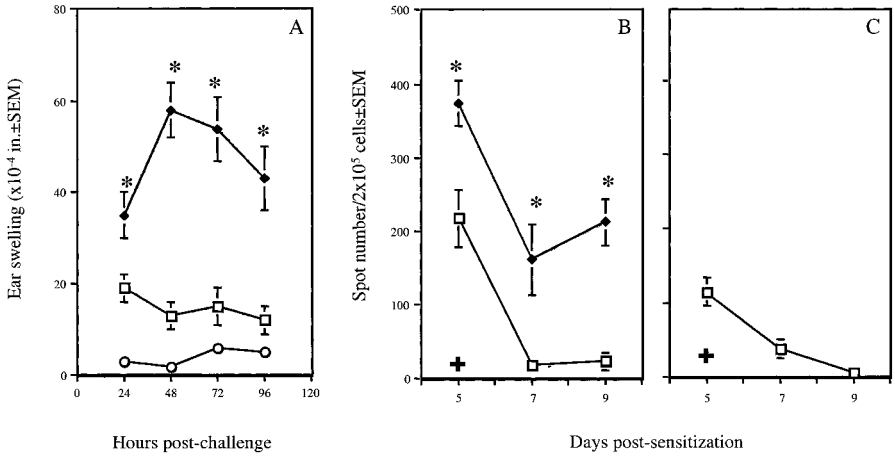

FIGURE 1. CD4+ T cell-mediated regulation of CHS responses to DNFB. *A*, C57BL/6 mice were treated with 200 μg of rat IgG (■) or anti-CD4 mAb (♦) on 3 consecutive days before sensitization with 0.25% DNFB on days 0 and 1. On day 5, sensitized mice and a group of naive mice (○) were ear-challenged with 0.2% DNFB, and the change in ear thickness was measured in 24-h intervals. The mean increase in ear thickness after challenge with DNFB was 10^{-4} in. ± SEM for groups of four mice. *B*, On days 5, 7, and 9 after DNFB sensitization, CD8+ T cell-enriched cell suspensions were prepared using anti-CD4 mAb-negative selection from the lymph nodes of sensitized control (■), sensitized CD4+ T cell-depleted (♦), or naive (✚) mice, and 2×10^5 cell aliquots were cultured with 5×10^5 DNBS-labeled syngeneic splenocytes on IFN-γ-coated ELISPOT plates. After 24 h, the cells were removed, and the ELISPOT assay was developed to detect IFN-γ production. The mean number ± SEM of IFN-γ-producing CD8+ T cells per 2×10^5 cells in triplicate cultures for two individual mice are shown after subtraction of spots from control wells containing T cells with unlabeled cells. *C*, CD4+ T cell-enriched cell suspensions were obtained from naive (✚) or DNFB-sensitized (■) mice on the indicated days after hapten sensitization, cultured with hapten-labeled spleen cells, and analyzed by ELISPOT to determine the number of IL-4-producing cells. The results are representative of three individual experiments. *, $p < 0.05$.

The hapten-primed effector CD8+ T cells are contained within the CD44^{high}/CD11a^{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. 1*C*). 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*[a]

Group	No. of LNC (×10⁶)			No. of IFN-γ-Producing CD8+ T Cells (×10³)		
	Day 5	Day 7	Day 9	Day 5	Day 7	Day 9
Naive	2.50			0.40		
Immune control	24.0	10.0	5.0	26.16	0.95	0.60
Immune CD4-depleted	21.0	16.0	15.0	43.01	12.96	15.90

[a] 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 postsensitization. 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.

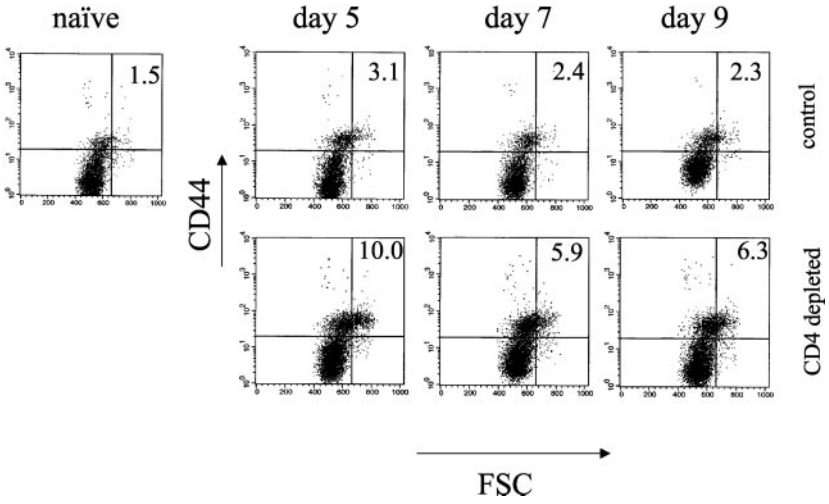

FIGURE 2. Expansion of activated $CD8^+$ T cells is regulated by $CD4^+$ T cells. LNC from control, rat IgG-treated and $CD4^+$ T cell-depleted mice prepared on days 5, 7, and 9 after sensitization with 0.25% DNFB were stained with FITC-labeled anti-CD8 mAb and PE-labeled anti-CD44 mAb. The $CD8^+$ T 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.

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 postsensitization 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 postsensitization. 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 postsensitization (Fig. 4). In contrast to control mice, the $FITC^+$ DC numbers increased at 48–72 h postsensitization.

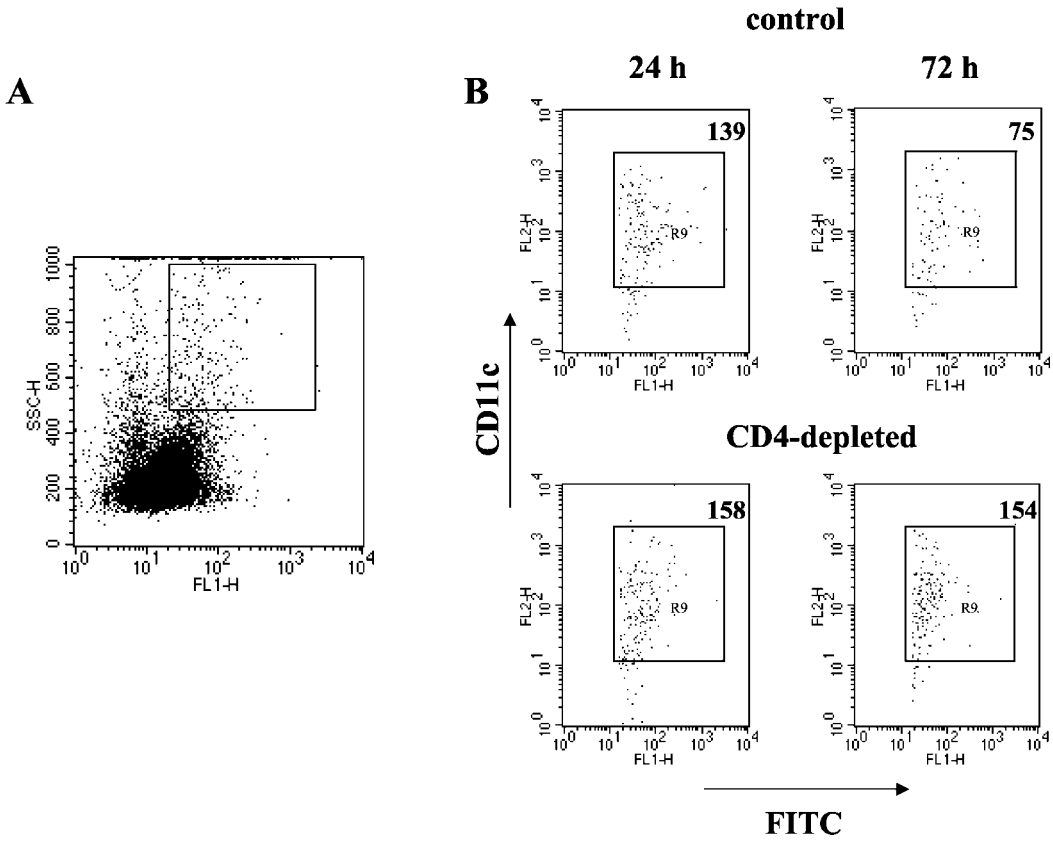

FIGURE 3. $CD4^+$ T cells decrease the numbers of FITC-bearing DC in the lymph nodes of FITC-sensitized mice by 72 h postsensitization. Control or $CD4^+$ T cell-depleted mice were sensitized with 1% FITC on day 0. On days 1 (24 h) and 3 (72 h) postsensitization LNC suspensions from the sensitized and 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 2×10^4 LNC aliquot. Results are representative of three individual experiments.

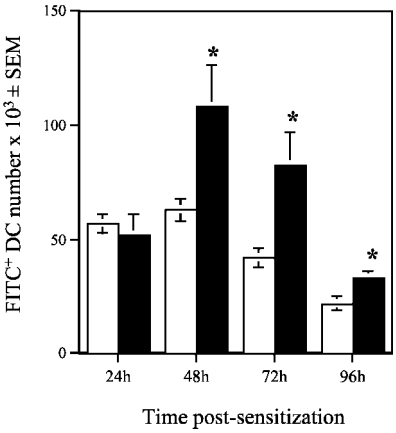

FIGURE 4. CD4+ T cells restrict presence of hapten-bearing DC in the lymph nodes of mice during T cell priming. Mice were either untreated (□) or depleted of CD4+ T cells (■) before sensitization with FITC. At a different postsensitization time, LNC were obtained from skin-draining lymph nodes of each individual mouse and stained with PE-labeled anti-CD11c mAb. Hapten-bearing DC were defined as the side scatter^{high}/FITC^{high}/CD11c^{high} cell population, and numbers of these cells per 2×10^4 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. *, $p < 0.05$.

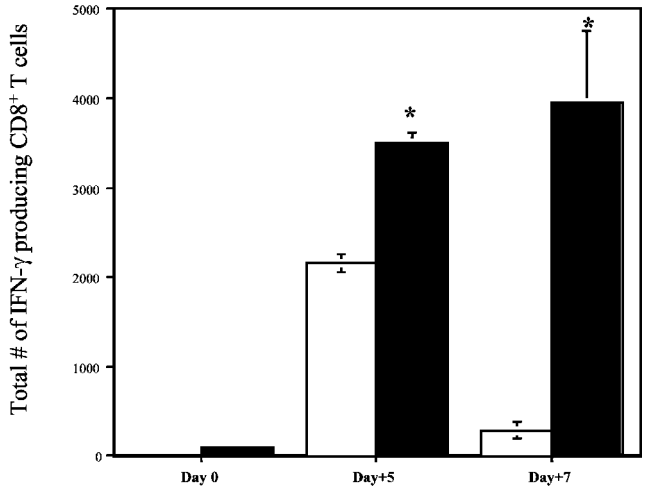

FIGURE 6. Increased and sustained numbers of IFN-γ producing CD8+ T cells in the lymph nodes of *gld* mice after sensitization with DNFB. C57BL/6 wild-type and *gld* mice were sensitized with 0.25% DNFB on days 0 and 1. On days 5 and 7 after sensitization, LNC suspensions were prepared from skin-draining 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 2×10^5 cell aliquots were cultured with 5×10^5 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. *, $p < 0.05$.

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. 5*C*). Additionally, transfer of CD8+ T

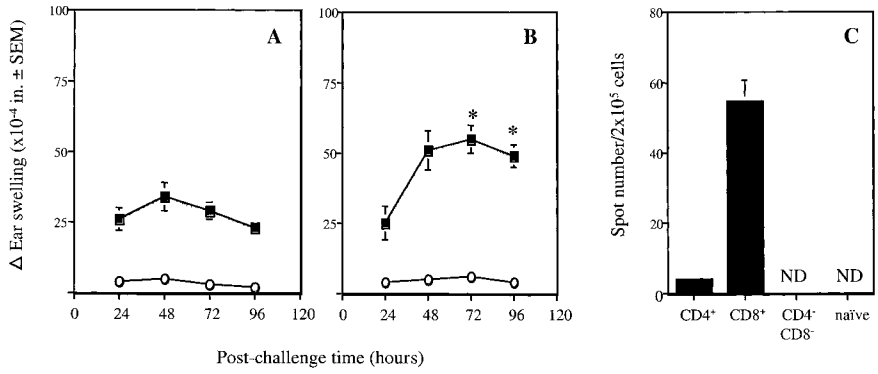

FIGURE 5. Increased and sustained CHS responses to DNFB in *gld* mice. C57BL/6 wild type (*A*) and *gld* (*B*) mice were sensitized with 0.25% DNFB on days 0 and 1. On day 5 naive (○) or sensitized (■) mice were ear-challenged with 0.2% DNFB, and the change in ear thickness was measured at 24-h intervals. The mean increase in ear thickness after challenge with DNFB is shown as 10^{-4} 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 postsensitization. CD4+, CD8+ T cell-enriched or CD4−CD8− populations were obtained from LNC of sensitized mice using negative selection by Dynabeads. Aliquots (2×10^5 cells) were cultured with 5×10^5 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 2×10^5 cell aliquots. Results are representative of two individual experiments. ND, not detected.

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 postsensitization, 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 postsensitization, 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. 8*C*).

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. 9*A*). 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

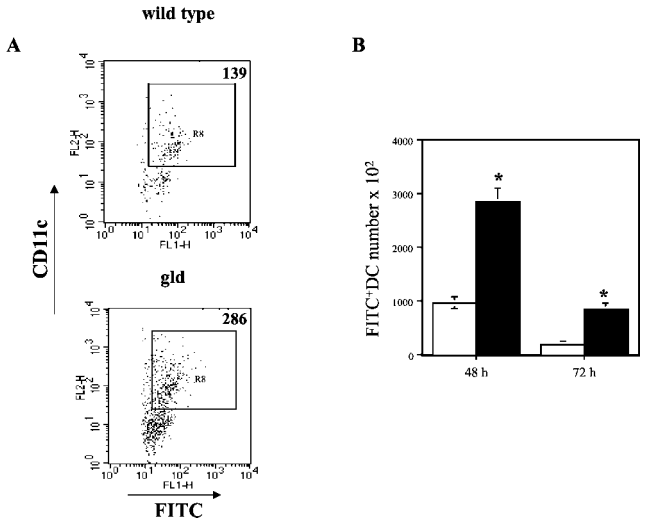

FIGURE 7. Increased numbers of DC in the lymph nodes of DNFB-sensitized *gld* mice during T cell hapten priming. Wild-type C57BL/6 and *gld* mice were sensitized with 0.25% DNFB on days 0 and 1. On days 2 (48 h) or 3 (72 h) postsensitization, LNC suspensions were prepared from skin-draining lymph nodes and stained with PE-labeled anti-CD11c mAb. *A*, Hapten-bearing DC were detected as side $scatter^{high}/FITC^{high}/CD11c^{high}$ cells. Numbers in the *upper right* corner represent the number of hapten-bearing DC per 2×10^4 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. *, $p < 0.05$.

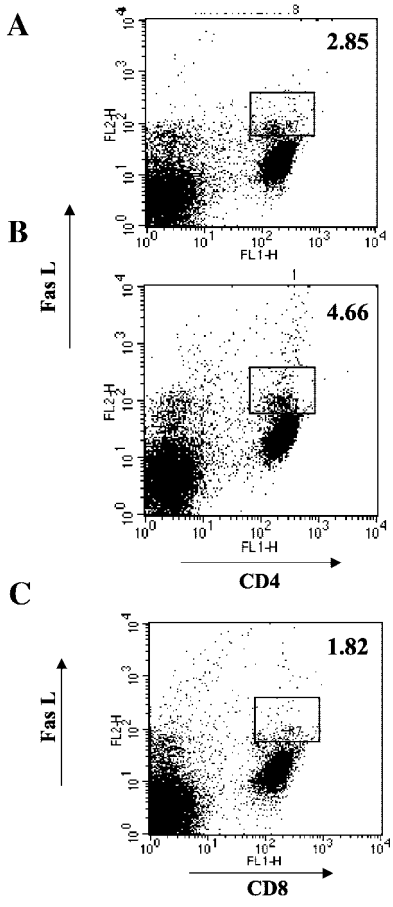

FIGURE 8. FasL expression on T cell populations. LNC from naive or DNFB-sensitized mice (three mice per group) were stained with FITC-labeled anti-CD4 or anti-CD8 mAb and biotinylated anti-FasL mAb plus streptavidin-PE. The expression of FasL by $CD4^+$ T cells from naive mice (*A*) or sensitized mice (*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.

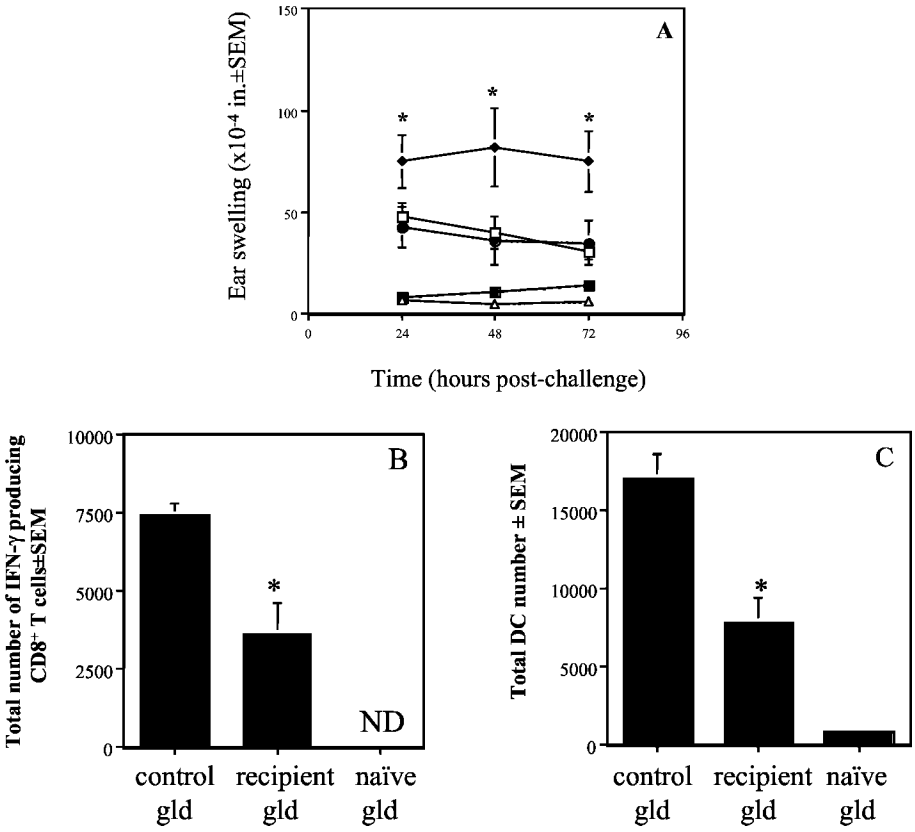

FIGURE 9. Adoptive transfer of $CD4^+$ T cells from wild-type mice restores immunoregulation of CHS responses in *gld* mice. *A*, $CD4^+$ T cells were purified from the lymph nodes of naive wild-type C57BL/6 mice, and 30×10^6 cells were transferred by i.v. injection to *gld* mice. Two days later, the recipient (●) and control, nonrecipient (♦) *gld* and wild-type C57BL/6 (□) mice were sensitized with 0.25% DNFB 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% 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 to be 10^{-4} in. ± SEM for groups of four mice. *B*, On day 5 after DNFB 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 2×10^5 cell aliquots were cultured with 5×10^5 DNBS-labeled 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 DNFB sensitization. Cell aliquots were stained with FITC-labeled anti-CD11c mAb and PE-labeled anti-$I\text{-}A^b$ mAb, and the numbers of CD11c/$I\text{-}A^b$-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.

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 DNFB 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. 9*B*). Furthermore, staining LNC suspensions from the control and $CD4^+$ T cell-recipient *gld* mice with anti-$I\text{-}A^b$ 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. 9*C*). 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-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 $I^{-/-}$ and class $II^{-/-}$ 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-primed $CD4^+$ 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

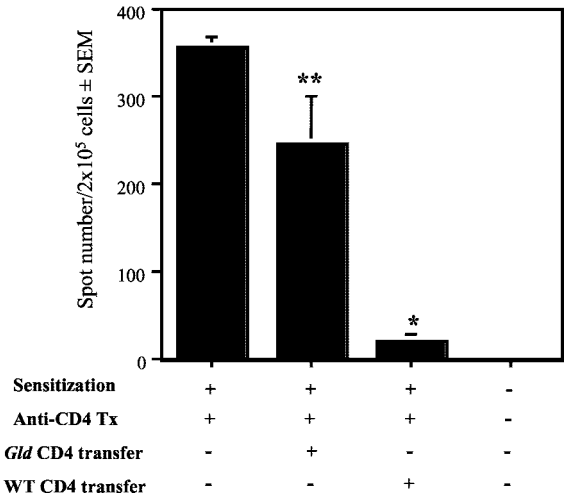

FIGURE 10. Adoptive transfer of wild-type $CD4^+$ T cells, but not FasL-defective $CD4^+$ T cells, inhibits the development of hapten-specific $CD8^+$ T cells in $CD4^+$ T cell-depleted mice. $CD4^+$ T cells were purified from either naive wild-type C57BL/6 mice or naive *gld* mice, and 30×10^6 cells were transferred by i.v. injection to wild-type mice depleted of $CD4^+$ T cells 7 days before the transfer. Two days later, the recipient and control, nonrecipient CD4-depleted mice were sensitized with 0.25% DNFB on days 0 and 1. On day 5 after DNFB sensitization, $CD8^+$ T cell-enriched populations were prepared from the lymph nodes of the control and recipient mice, and 2×10^5 cell aliquots were cultured with 5×10^5 DNBS-labeled 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 numbers ± SEM of IFN-γ-producing hapten-specific $CD8^+$ T cells per cell aliquot are indicated. *, $p < 0.05$; **, $p > 0.05$.

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 postsensitization, 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

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.

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References

1. 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.*
2. Kripke, M., S. 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.*
3. Eisen, H. N., L. Orris, and S. Belman. 1952. Elicitation of delayed allergic skin reactions with haptens: the dependence of elicitation on hapten combination with protein. *J. Exp. Med. 95:473.*
4. Gosta, R., and B. Ridell. 1979. The cellular infiltrate in contact hypersensitivity to picryl chloride in the mouse. *Acta Dermatovener. 59:191.*
5. Miller, S. D., and M. K. Jenkins. 1985. In vivo effects of GK1.5 monoclonal antibody on induction and expression of delayed-type hypersensitivity. *Cell. Immunol. 92:414.*
6. Gautam, S., J. Matriano, M. Chikkala, M. Edinger, and R. Tubbs. 1991. L3T4 (CD4$^+$) T cells that mediated contact sensitivity to trinitroclorobenzene express I-A determinants. *Cell. Immunol. 135:27.*
7. Sunday, M. E., J. Z. Weinberger, B. Benacerraf, and M. E. Dorf. 1980. Hapten-specific T cell responses to 4-hydrxy-3-nitrophenyl acetyl. IV. Specificity of cutaneous sensitivity responses. *J. Immunol. 125:1601.*
8. Sunday, M. E., and M. E. Dorf. 1981. Hapten-specific T cell response to 4-hydroxy-3-nitrophenyl acetyl. X. Characterization of distinct T cell subsets mediating cutaneous sensitivity responses. *J. Immunol. 127:766.*
9. 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.*
10. 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.*
11. Bour, H., E. Peyron, M. Gaucherand, J. L. Garrigue, C. Desvignes, D. Kaiserlian, J. 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.*
12. Gorbachev, A. V., P. S. Heeger, and R. L. Fairchild. 2001. CD4$^+$ and CD8$^+$ T cell priming for contact hypersensitivity occurs independently of CD40-CD154 interactions. *J. Immunol. 166:2323.*
13. Cobbold, S. P., A. Jayasuriya, A. Nash, T. D. Prospero, and H. Waldmann. 1984. Therapy with monoclonal antibodies by elimination of T cell subsets in vivo. *Nature 312:548.*
14. 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.*
15. Engeman, T. M., A. V. Gorbachev, R. P. Gladue, P. S. Heeger, and R. L. Fairchild. 2000. Inhibition of functional T cell priming and CHS response by treatment with anti-secondary lymphoid chemokine antibody during hapten sensitization, *J. Immunol. 164:5207.*
16. Macatonia, S. E., A. J. Edwards, and S. C. Knight. 1986. Dendritic cells and initiation of contact hypersensitivity to fluorescein isothiocyanate. *Immunology 59:509.*
17. Asada, H., J. Linton, and S. I. Katz. 1997. Cytokine gene expression during elicitation phase of contact sensitivity: regulation by endogenous IL-4. *J. Invest. Dermatol. 108:406.*
18. Schwarz, A., S. Grabbe, H. Riemann, Y. Aragane, M. Simon, S. Manon, S. Andrade, T. A. Luger, A. Zlotnik, and T. Schwarz. 1994. In vivo effects of IL-10 on contact hypersensitivity and delayed-type hypersensitivity reactions. *J. Invest. Dermatol. 103:211.*
19. Biedermann, T., R. Mailhammer, A. Mai, C. Sander, A. Ogilvie, F. Brombacher, K. Maier, A. D. Levine, and M. Rocken. 2001. Reversal of established delayed type hypersensitivity reactions following therapy with IL-4 or antigen-specific Th2 cells. *Eur. J. Immunol. 31:1582.*
20. Cavani, A., F. Nassori, C. Prezzi, S. Sebastiani, C. Albanesi, and G. Girolomoni. 2000. Human CD4$^+$ T lymphocytes with remarkable regulatory functions on dendritic cells and nickel-specific Th1 immune responses. *J. Invest. Dermatol. 14:295.*
21. Gorbachev, A. V., and R. L. Fairchild. 2001. Regulatory role of CD4$^+$ T cells during the development of contact hypersensitivity responses. *Immunol. Res. 24:69.*
22. Schwarz, A., S. Grabbe, K. Grosse-Heitmeyer, B. Roters, H. Riemann, T. A. Luger, G. Trinchiery, and T. Schwarz. 1998. Ultraviolet light-induced immune tolerance is mediated via Fas-Fas ligand system. *J. Immunol. 160:4262.*
23. Hill, L. L., V. K. Shreedhar, M. Kripke, and L. B. Owen-Schaub. 1999. A critical role for Fas ligand in the active suppression of systemic immune responses by ultraviolet radiation. *J. Exp. Med. 189:1285.*
24. Rathmell, J. C., M. P. Cooke, W. Y. Ho, J. Grein, S. E., Townsend, M. M. Davis, and C. C. Goodnow. 1995. CD95 (Fas)-dependent elimination of self-reactive B cells upon interactions with CD4$^+$ T cells. *Nature 376:181.*
25. Ashany, D., X. Song, E. Lacy, J. Nikolic-Zugic, S. M. Friedman, and K. B. Elkon. 1995. Th1 CD4$^+$ lymphocytes delete activated macrophages through the Fas/APO-1 antigen pathway. *Proc. Natl. Acad. Sci. USA 92:11225.*
26. DeSmedt, T., B. Pajak, E. Muraille, L. Lespagnard, E. Heinen, P. De Baetselier, J. Urbain, O. Leo, and M. Moser. 1996. Regulation of dendritic cell numbers and maturation by lipopolysaccharide in vivo. *J. Exp. Med. 184:1413.*
27. Matsue, H., D. Edelbaum, A. C. Hartmann, A. Morita, P. R. Bergstresser, H. Yagita, K. Okumura, and A. Takashima. 1999. Dendritic cells undergo rapid

apoptosis in vitro during antigen-specific interaction with $CD4^+$ T cells. *J. Immunol. 162:5287.*

28. Loyer, V., P. Fontaine, S. Pion, F. Hetu, D. C. Roy, and C. Perreault. 1999. The in vivo fate of APCs displaying minor H antigen or MHC differences is regulated by CTLs specific for immunodominant class I-associated epitopes. *J. Immunol. 163:6462.*
29. Hermans, I. F., D. S. Ritchie, J. Yang, J. M. Roberts, and F. Ronchese. 2000. $CD8^+$ T cell-dependent elimination of dendritic cells in vivo limits the induction of antitumor immunity. *J. Immunol. 164:3095.*
30. Ashani, D., A. Savir, N. Bhardwaj, and K. B. Elkon. 1999. Dendritic cells are resistant to apoptosis through the Fas (CD95/APO-1) pathway. *J. Immunol. 163:5303.*
31. Koppi, T. A., T. Tough-Bement, D. M. Lewinsohn, D. H. Lynch, and M. R. Alderson. 1997. CD40 ligand inhibits Fas/CD95-mediated apoptosis of human blood-derived dendritic cells. *Eur. J. Immunol. 27:3161.*
32. Rescigno, M., V. Piguet, B. Valzasina, S. Lens, R. Zubler, L. French, V. Kindler, J. Tschopp, and P. Riccardi-Castagnoli. 2000. Fas engagement induces the maturation of dendritic cells (DCs), the release of IL-1b and the production of IFN-γ in the absence of IL-12 during DC-T cell cognate interaction: a new role for Fas ligand in inflammatory responses. *J. Exp. Med. 192:1661.*
33. Van der Bruggen, P., C. Traversari, P. Chomez, C. Lurouin, E. De Plaen, B. Van den Eynde, A. Knuth, and T. Boon. 1991. A gene encoding an antigen recognized by cytotoxic T lymphocytes on a human melanoma. *Science 254:1643.*
34. Sprent, J., M. Schaefer, E. K. Gao, and R. Korngold. 1988. Role of T cell subsets in lethal graft-vs-host disease directed to class I vs class II H2 differences. *J. Exp. Med. 167:556.*
35. Onuma, S. 1994. Immunohistochemical studies of infiltrating cells in early and chronic lesions of psoriasis. *J. Dermatol. 21:223.*
36. Gottlieb, S. L., P. Gilleaudeau, R. Johnson, L. Estes, T. G. Woodworth, A. B. Gottlieb, and J. G. Krueger. 1995. Response of psoriasis to a lymphocyte-selective toxin (DAB389 IL-2) suggests a primary immune, but not keratinocyte pathogenic basis. *Nat. Med. 1:442.*