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Anton V. Gorbachev, Nancy A. DiIulio and Robert L. Fairchild

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IL-12 Augments CD8+ T Cell Development for Contact Hypersensitivity Responses and Circumvents Anti-CD154 Antibody-Mediated Inhibition

Anton V. Gorbachev, Nancy A. Dilullo, and Robert L. Fairchild

During sensitization with dinitrofluorobenzene for contact hypersensitivity (CHS) responses, hapten-specific CD8+ T cells develop into IFN-γ-producing cells, and CD4+ T cells develop into IL-4/IL-5-producing cells. Administration of IL-12 during sensitization skews CD4+ T cell development to IFN-γ-producing cells, resulting in exaggerated CHS responses. In the current report we tested the role of IL-12 on CD8+ T cell development during sensitization and elicitation of CHS to dinitrofluorobenzene. Administration of IL-12 during hapten sensitization induced the expression of IL-12Rβ2 on both CD4+ and CD8+ T cells, augmented IFN-γ production by these T cell populations, and increased the magnitude and duration of the CHS response to hapten challenge. CHS responses were virtually identical in wild-type and IL-12 p40−/− mice. Since engagement of CD40 on APC may stimulate IL-12 production, we also tested the role of CD40-CD154 interactions on the development of IFN-γ-producing CD4+ and CD8+ T cells following hapten sensitization. Development of IFN-γ-producing CD4+ T cells during hapten sensitization was absent in wild-type mice treated with anti-CD154 mAb or in CD154−/− mice. In contrast, the absence of CD40-CD154 signaling had little or no impact on the development of IFN-γ-producing CD8+ T cells. These results demonstrate that the development of hapten-specific Th1 effector CD4+ T cells in CHS requires both CD40-CD154 interactions and IL-12, whereas the development of IFN-γ-producing effector CD8+ T cells can occur independently of these pathways. The Journal of Immunology, 2001, 167: 156–162.

Contact hypersensitivity (CHS) is a T cell-mediated inflammation of the skin of hapten-sensitized individuals in response to contact with the sensitizing hapten (1, 2). During sensitization Langerhans cells process the hapten and migrate from the sensitized epidermis to the skin-draining lymph nodes where hapten-specific T cells are primed (3, 4). Subsequent hapten contact results in cutaneous infiltration of the primed T cells and their activation to mediate local inflammation within the skin challenge site. Both clinical and experimental studies have suggested roles for CD4+ and CD8+ T cells as the effector T cells mediating CHS (5–8). Studies from this and other laboratories have indicated that CD8+ T cells are the primary effector cells in CHS responses to the model hapten 2,4-dinitrofluorobenzene (DNFB) and oxazolone (Ox) and that CD4+ CHS responses of increased magnitude and duration. However, the role of IL-12 in CHS and its effect on hapten-specific CD8+ T cell development remain undefined. In the current report we have extended our previous studies and have demonstrated that endogenous IL-12 is not required for CHS, but that exogenous IL-12 amplifies IFN-γ-producing CD8+ T cell development independently of the CD4+ T cell compartment. Anti-CD154 mAb and CD154−/− mice were used to show that the IL-12-driven development of hapten-specific CD4+ T cells to IFN-γ-producing cells is dependent on CD40-CD154 interactions, whereas the development of CD8+ T cells in CHS occurs independently of this costimulatory signal. The results presented indicate for the first time the role of endogenous IL-12 and the effect of exogenous IL-12 on the development of hapten-specific CD8+ T cell development.

Many factors, including the delivery of various costimulatory signals and the cytokine environment during Ag priming, influence CD4+ T cell development to a particular cytokine-producing phenotype (12). The roles of such factors during priming of hapten-specific CD4+ and CD8+ T cells for CHS remain unclear. Previous studies from this laboratory indicated that administration of IL-12 during hapten sensitization skewed the development of CD4+ T cells from an IL-4-producing to an IFN-γ-producing phenotype (13). The consequences of this treatment were elimination of the regulatory component of the response, resulting in CHS responses of increased magnitude and duration. However, the role of IL-12 in CHS and its effect on hapten-specific CD8+ T cell development remain undefined. In the current report we have extended our previous studies and have demonstrated that endogenous IL-12 is not required for CHS, but that exogenous IL-12 amplifies IFN-γ-producing CD8+ T cell development independently of the CD4+ T cell compartment. Anti-CD154 mAb and CD154−/− mice were used to show that the IL-12-driven development of hapten-specific CD4+ T cells to IFN-γ-producing cells is dependent on CD40-CD154 interactions, whereas the development of CD8+ T cells in CHS occurs independently of this costimulatory signal. The results presented indicate for the first time the role of endogenous IL-12 and the effect of exogenous IL-12 on the development of hapten-specific CD8+ T cell development.

Materials and Methods

Animals

BALB/c and C57BL/6 mice were purchased through Dr. C. Reeder (National Cancer Institute, Frederick, MD). CD4−/−, CD8−/−, and CD154−/− mice on the C57BL6 genetic background and IL-12 p40−/− mice on the BALB/c background were purchased from The Jackson Laboratory (Bar Harbor, ME). Adult female mice, 8–10 wk old, were used throughout these studies.
Abs and cytokines

mAb from the culture supernatant of the IgG-producing hybridomas YT8191.1.2 and GK1.5 (anti-mouse CD4 mAb), 2.43 (anti-mouse CD8 mAb), 145.2C11 (anti-mouse CD3 mAb), J23a (anti-β2m aAb), and MR1 (anti-mouse CD154 mAb) and 15.12 and 15.6.7 (anti-mouse IL-12 mAbs) were purified by protein G chromatography. Capture and detection mAbs for IL-4- and IFN-γ-specific ELISA and IFN-γ and IL-4 were purchased from PharMingen (San Diego, CA). Polyclonal hamster IgG was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). Recombinant mouse IL-12 was a gift from Genetics Institute (Cambridge, MA).

Hapten sensitization and elicitation of CHS

For sensitization to DNFB, mice were painted on days 0 and 1 with 25 μl 0.25% DNFB (Sigma, St. Louis, MO) on the shaved abdomen and 5 μl on each at 4°C. Each site was washed with 10 μl of 0.2% DNFB on both sides of each ear. The increase in ear swelling was measured 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 (8). The magnitude of the ear swelling responses is presented as the mean increase in each group of four sensitized or nonsensitized mice (i.e., eight ears) ± SEM.

Cell culture

Lymph node cells (LNC) were obtained from nonsensitized mice and from hapten-sensitized mice on day 4. For in vitro depletion of CD4^+ or CD8^+ T cells, LNC were incubated with specific Ab-coated magnetic beads (Dynabeads, Dynal, Oslo, Norway). Ab staining and flow cytometric analyses performed in each experiment indicated that the efficiency of this depletion was 95% for the target T cell population (data not shown). Enriched CD4^+ and CD8^+ T cell populations were stimulated to produce cytokines by culture on anti-CD3 mAb-coated wells. The 96-well U-bottom tissue culture plates were coated with 10 μl per well anti-CD3 mAb (145.2C11 (25 μg/ml) for 90 min at 37°C. As a negative control, wells were coated with an Ab to a Vβ region not expressed by BALB/c or C57BL/6 mice, anti-Vβ17a mAb KJ 23a (4). The wells were washed, and 2 × 10^5 cells were added to each well. After 24 h of culture on anti-CD3 mAb, these cells were removed from the plate by extensive washing with PBS.

Cytokine-specific ELISA

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

ELISPOT assay

Hapten-specific IFN-γ-producing T cells were enumerated using ELISPOT assays as previously described (14). Briefly, ELISA spot plates (Unifilter 350, Polyfiltertronics, Rockland, MA) were coated with 4 μg/ml IFN-γ-specific mAb and incubated overnight at 4°C. The plates were blocked with 1% BSA in PBS and then washed four times with PBS. LNC from unsensitized or DNFB-sensitized mice were prepared on day 5 after hapten sensitization and used as responder cells. Syngeneic spleen cells from naive mice were treated with mitomycin C, then labeled with 100 μg/ml DNBS and used as stimulator cells as previously described (15). Responder and stimulator cells were cultured in serum-free HL-1 medium (BioWhittaker, Walkersville, MD) supplemented with 1 nM T-glutamine. DNBS-labeled stimulator cells were added to each well at 5 × 10^5 cells/well with 2 × 10^5 responder cells/well. After 24 h of cell culture at 37°C in 5% CO₂, cells were removed from the plate by extensive washing with PBS. Biotinylated anti-IFN-γ mAb (2 μg/ml) was added, and the plate was incubated overnight at 4°C. The following day the plate was washed three times with PBS/0.05% Tween 20, and conjugated streptavidin-alkaline phosphatase for IFN-γ was added to each well. After 2 h at room temperature the plates were washed with PBS, and nitro blue tetrazolium-5-bromo-4-cloro-3-indolyl substrate (Kirkegaard & Perry, Gaithersburg, MD) was added for the detection of IFN-γ-producing cells. The resulting spots were counted with an ELISA spot image analyzer (developed at Case Western Reserve University, Cleveland, OH) using Optimas software (Optimas, Botheled, WA) that was designed to detect ELISA spots with predetermined criteria of spot size, shape, and colorimetric density.

RNase protection assay

LNC from naive or sensitized mice were obtained on day 5 following hapten sensitization. CD4^+ and CD8^+ T cells cell populations were positively selected using Dynabeads and lysed in TRIZol reagent (Life Technologies, Grand Island, NY). Total RNA was isolated by phenol-chloroform extraction, followed by precipitation with isopropl alcohol. IL-12 mRNA was detected by RNase protection assay, using the MCR-3 template from PharMingen.

Results

Exogenous IL-12 augments the development of IFN-γ-producing CD4^+ and CD8^+ T cells in CHS

The effect of rIL-12 administration during hapten sensitization on the development of hapten-specific CD4^+ and CD8^+ T cells was tested. To initiate these studies, CHS responses in wild-type, CD4^+/-, and CD8^+/- cells given IL-12 during DNFB sensitization were compared. Consistent with our previous observations (13), IL-12 treatment during sensitization of wild-type mice increased the magnitude of ear swelling responses following challenge (Fig. 1A). IL-12 also increased CHS responses in CD4^+/- mice (Fig. 1B). Whereas CHS was absent in sensitized and challenged CD8^+/- mice, IL-12 treatment restored the responses in these mice (Fig. 1C).

The development of IFN-γ-producing CD4^+ and CD8^+ T cells in response to DNFB sensitization with or without IL-12 treatment was examined in each group by ELISPOT assay. Although sensitization of wild-type mice did not induce many CD4^+ T cells producing IFN-γ to DNBP-labeled stimulator cells, this was reversed by IL-12 treatment (Fig. 2A). IL-12 treatment also increased the number of IFN-γ-producing CD8^+ T cells given IL-12 during sensitization of wild-type mice. Similarly, IL-12 increased the number of hapten-specific IFN-γ-producing CD8^+ T cells in DNFB-sensitized CD8^+/- mice, although not to the same degree as observed in wild-type mice (Fig. 2B). Few IFN-γ-producing CD4^+ T cells were observed in DNFB-sensitized CD8^+/- mice correlating with the absent CHS responses to hapten challenge (Fig. 2C). Again, this was reversed by IL-12 treatment during DNFB sensitization of CD8^+/- mice. These data indicated that exogenous IL-12 enhanced the development of IFN-γ-producing CD4^+ and CD8^+ T cells in response to DNFB sensitization.

CHS responses are not dependent on endogenous IL-12

The ability to alter hapten-specific T cell development and the CHS response by administration of IL-12 during sensitization suggested that the induction of CHS was not dependent on IL-12. To test this directly, groups of BALB/c mice were first depleted of CD4^+ T cells by treatment with anti-CD4 mAb on days −4, −3, and −2, and these mice and control mice were treated with control rat IgG or with two different anti-IL-12 mAb on each day of hapten sensitization (i.e., days 0 and 1). Treatment with anti-IL-12 Ab did not alter the magnitude of ear swelling observed in sensitized mice with or without CD4^+ T cells following hapten challenge (Fig. 3A). In addition, wild-type BALB/c and IL-12-deficient p40^-/- mice were sensitized with DNFB, and 5 days later the ear swelling responses to hapten challenge were compared. As shown in Fig. 3B, responses were virtually identical in the two groups. These
The number of IFN-\(\gamma\)-sensitized mice contained detectable amounts of IL-12R\(\beta\) \(g\) labeled syngeneic spleen cells on ELISPOT plates coated with anti-IFN-\(\gamma\) (Fig. 4). On day 5 DNFB-sensitized and control, unsensitized \(\bullet\) mice were ear challenged with 0.2% DNFB, and ear thickness was measured at 24-h intervals. The mean increase in ear thickness following hapten challenge is shown in units of \(10^{-3}\) in. \(\pm\) SEM. The results shown are representative of three individual experiments.

results indicated that endogenous IL-12 was not required for CHS responses.

**Up-regulation of IL-12R\(\beta\) on both CD4\(^+\) and CD8\(^+\) T cell subsets following exogenous IL-12 treatment**

To further examine the role of IL-12 during the development of T cells for CHS, the expression of the inducible component of the IL-12R (i.e., IL-12R\(\beta\)\(2\)) was tested on CD4\(^+\) and CD8\(^+\) T cells from DNFB-sensitized and naive mice. Lysates of enriched CD4\(^+\) and CD8\(^+\) T cell populations obtained from LNC of naive or hapten-sensitized mice contained detectable amounts of IL-12R\(\beta\)\(1\) mRNA (Fig. 4). However, the expression of the IL-12R\(\beta\)\(2\) component that is up-regulated during Th1 cell development (16) was not detected on either cell population from the sensitized mice. In contrast, administration of IL-12 during hapten sensitization resulted in detectable amounts of IL-12 R\(\beta\)\(2\) mRNA in lysates of both CD4\(^+\) and CD8\(^+\) T cells (Fig. 4).

**IL-12 circumvents the anti-CD154 mAb-mediated inhibition of CD4\(^+\) and CD8\(^+\) T cell development in CHS**

Recent studies from this laboratory demonstrated the development of hapten-specific CD4\(^+\) and CD8\(^+\) T cells as well as CHS responses following sensitization of CD154\(^+/−\) mice (17). Since IL-12 production is stimulated by CD40-CD154 engagement (18, 19), the ability of exogenous IL-12 to enhance CHS responses in the absence of CD40-/CD154-mediated costimulation was tested. Groups of wild-type C57BL/6 and CD154\(^+/−\) animals were sensitized to DNFB with or without IL-12 treatment, and ear swelling responses to DNFB challenge were compared (Fig. 5). The magnitude of CHS in sensitized CD154\(^+/−\) mice was slightly lower than the magnitude in sensitized wild-type animals. Treatment with IL-12 during sensitization amplified CHS in each group.

The development of hapten-specific, IFN-\(\gamma\)-producing CD4\(^+\) and CD8\(^+\) T cells in wild-type and CD154\(^+/−\) mice treated with or without IL-12 during DNFB sensitization was compared by ELISPOT assay. The number of IFN-\(\gamma\)-producing CD8\(^+\) T cells in response to hapten-labeled stimulator cells was slightly reduced in sensitized CD154\(^+/−\) mice with or without IL-12 treatment, and ear swelling responses to DNFB challenge were compared (Fig. 5). The magnitude of CHS in sensitized CD154\(^+/−\) mice was slightly lower than the magnitude in sensitized wild-type animals. Treatment with IL-12 during sensitization amplified CHS in each group.

FIGURE 2. Effect of exogenous IL-12 on the development of IFN-\(\gamma\)-producing CD4\(^+\) and CD8\(^+\) T cells. On day 5 following DNFB sensitization with or without IL-12 treatment (Tx), T cell suspensions were prepared from lymph nodes of sensitized C57BL/6 wild-type (A), CD4\(^+/−\) (B), or CD8\(^+/−\) (C) mice and from naive mice. CD4\(^+\) and CD8\(^+\) T cell-enriched suspensions were prepared, and 2 \(\times\) 10\(^5\)-cell aliquots were cultured with 5 \(\times\) 10\(^5\) DNBS-labeled syngeneic spleen cells on ELISPOT plates coated with anti-IFN-\(\gamma\) mAb. After 24 h cells were removed, and the ELISPOT assay was developed. The number of IFN-\(\gamma\)-producing CD4\(^+\) T cells (\(\square\)) and CD8\(^+\) T cells (\(\blacksquare\)) for each group represents the mean number detected in triplicate wells using an immunospot image analyzer. The results are representative of two experiments. *, \(p < 0.05\) (compared with values in the control untreated group).
cells). These results indicated that IFN-$\gamma$-producing CD8$^+$ T cell development occurred independently of CD40-CD154 interactions during hapten sensitization, but the development of type 1 CD4$^+$ T cells required CD40-CD154 costimulation, and the deficiency in this costimulatory pathway was not reversed by exogenous IL-12.

Although IFN-$\gamma$-producing CD8$^+$ T cell development and CHS are observed in CD154$^{-/-}$ mice following sensitization, the administration of anti-CD154 mAb during sensitization of wild-type or p40$^{-/-}$ mice was sensitized with 0.25% DNFB on days 0 and 1. On day 5 DNFB-sensitized and naive mice were ear challenged with 0.2% DNFB, and ear thickness was measured 24 h later. The mean increase in ear thickness following hapten challenge is shown in units of $10^{-4}$ in $\pm$ SEM. The results shown are representative of two individual experiments.

To investigate the development of T cell populations in these responses cytokine production by separated CD4$^+$ and CD8$^+$ T cells from hapten-primed LNC was tested following culture on anti-CD3 mAb-coated wells. T cells cultured on control, anti-V$\beta$17a mAb-coated wells produced nondetectable amounts of the cytokines (data not shown), and T cells from nonsensitized mice produced low to nondetectable levels of IFN-$\gamma$ and IL-4 following culture on anti-CD3 mAb-coated wells (Fig. 8). CD8$^+$ T cells from the control group of hapten-sensitized mice produced IFN-$\gamma$, and the CD4$^+$ T cells produced IL-4 and low amounts of IFN-$\gamma$ (Fig. 8). As recently reported, administration of MR1 during hapten sensitization inhibited IFN-$\gamma$ production by CD4$^+$ and CD8$^+$ T cells and enhanced IL-4 production by CD4$^+$ T cells (17). Both CD4$^+$ and CD8$^+$ T cell populations isolated from mice treated with IL-12 during DNFB sensitization produced high levels of IFN-$\gamma$, and CD4$^+$ T cell production of IL-4 was absent. IL-12 treatment during DNFB sensitization completely restored the production of IFN-$\gamma$ by CD4$^+$ and CD8$^+$ T cells from MR1-treated mice to the levels observed in the control group. However, IL-12 treatment completely inhibited the exaggerated CD4$^+$ T cell production of IL-4 observed in mice treated only with anti-CD154 mAb during sensitization. Furthermore, IFN-$\gamma$ production by CD4$^+$ T cells

![FIGURE 3.](http://www.jimmunol.org/)

![FIGURE 4.](http://www.jimmunol.org/)
Hapten-primed CD4+ T cells, such as IL-4 and IL-5 (11). However, in our experience mice treated with only IL-12 during sensitization.

We performed ELISPOT assays to determine the number of CD4+ T cells following hapten challenge. The results represent the mean number of CD4+ T cells in triplicate wells, and data are representative of two experiments.

**FIGURE 6.** IL-12 augments the development of IFN-γ-producing CD8+ T cells in CD8−/− mice. C57BL/6 wild-type and CD154−/− mice were sensitized with 0.25% DNFB and treated with 0.2% DNFB, and ear thickness was measured 24 h later. The mean increase in ear thickness following hapten challenge is shown in units of 10−4 in. ± SEM. Results shown are representative of two experiments. *p < 0.005 (compared with control untreated groups).

**Discussion**

Studies from many laboratories have indicated that CD8+ T cells are the primary effector T cells in CHS responses to haptens such as DNFB, dimethylbenzanthracene, and Oxa and that CD4+ T cells regulate the magnitude of these responses (8–11). The direct examination of hapten-primed T cell populations from sensitized mice indicated the induction of two polarized and functionally opposed T cell populations during hapten sensitization: IFN-γ-producing CD8+ T cells and CD4+ T cells producing type 2 cytokines, such as IL-4 and IL-5 (11). However, in our experience hapten-primed CD4+ T cells often produce very low, but detectable, amounts of IFN-γ following in vitro stimulation (17). These results suggest that hapten sensitization generally skews CD4+ T cell development to the Th2 phenotype, but that a small number of hapten-specific Th1 CD4+ T cells may also develop. The cytokine and cellular interactions required for the development of these individual T cell populations in the CHS response remain under investigation in several laboratories.

The development of CD4+ T cells in response to IFN-γ-producing cells is largely, although not exclusively, dependent upon the presence of IL-12 during Ag priming (12). The development of CD4+ T cells to type 2 cytokine-producing cells during sensitization for CHS responses suggests that IL-12 is absent or at suboptimal levels during priming of hapten-specific CD4+ and CD8+ T cells by hapten-presenting Langerhans cells (hpLC). Previous results from this laboratory demonstrated that administration of rIL-12 during sensitization with DNFB or Oxa skewed hapten-specific CD4+ T cell development to IFN-γ-producing cells (13). This altered development resulted in the ability of hapten-primed CD4+ T cells to mediate ear swelling responses (e.g., in the absence of CD8+ T cells) to hapten challenge. Another consequence of this altered development was the elimination of a major regulatory component of the response resulting in ear swelling responses of increased magnitude and extended duration compared with responses in animals not given IL-12 during sensitization. As shown in the current report, IFN-γ-producing CD4+ T cell development and CHS responses were absent in CD8α−/− mice unless exogenous IL-12 was given during sensitization. Despite similar magnitudes of CHS in CD8−/− and CD4−/− mice treated with IL-12 during sensitization, the number of hapten-specific CD4+ T cells producing IFN-γ was almost 3-fold lower than the number of IFN-γ-producing CD8+ T cells from these respective mice. This result may be indicative that CD4+ T cells produce more IFN-γ on a per cell basis than the CD8+ T cells during elicitation of the response. An alternative explanation is that IFN-γ-producing CD4+ and CD8+ T cells may have differences in the ability to traffic to the hapten challenge site.

Equivalent CHS responses were also observed in IL-12 p40−/− mice and wild-type animals following sensitization and challenge with DNFB. These results are consistent with the proposal that IL-12 is either absent or at low levels and has little influence on CD4+ and CD8+ T cell development during priming for CHS. In contrast, results from two other laboratories have indicated the ability to inhibit the induction of CHS responses by treating mice with anti-IL-12 mAb during hapten sensitization (20, 21). In our
FIGURE 8. Differential effect of rIL-12 and anti-CD154 mAb treatment (Tx) on the development of CD4+ vs CD8+ T cell populations in response to DNFB sensitization. On day 5 after sensitization, CD4+ and CD8+ -enriched T cell populations were prepared from naive mice or mice sensitized with DNFB and treated with rIL-12 and anti-CD154 mAb as described in Fig. 7, and 2 × 10^5-cell aliquots were cultured on anti-TCR mAb-coated wells. After 48 h supernatants were collected and tested by ELISA for IFN-γ and IL-4 production by CD4+ T cells (□) and CD8+ T cells (■). The results shown are representative of three experiments.

hands, treatment with these Abs did not alter T cell development or the magnitude of CHS responses following sensitization with DNFB. However, we have recently observed that sensitization with supraoptimal doses of hapten promotes the development of IFN-γ-producing CD4+ T cells (manuscript in preparation). The possible role of endogenous IL-12 in the development of these CD4+ T cells is under investigation.

In contrast to the role of IL-12 in CD4+ T cell development, the effect of IL-12 on the development of hapten-specific CD8+ T cells during sensitization for CHS has not been previously tested. Results from the current report indicate that the expression of the IL-12Rβ2 component of the IL-12R is absent on CD8+ and CD4+ T cells from sensitized mice, but is up-regulated by administration of rIL-12. Recent in vitro studies by Wu and co-workers showed that IL-12Rβ2 expression on human CD4+ and CD8+ T cells is up-regulated by IL-12 (22). Administration of rIL-12 during sensitization also increased the number of hapten-specific, IFN-γ-producing CD8+ T cells in the skin draining lymph nodes 2- to 3-fold. This increased development was much greater than that observed for hapten-specific, IFN-γ-producing CD4+ T cells from mice treated with IL-12 during sensitization. The ability of exogenous IL-12 to enhance CD8+ T cell responses has been reported by other laboratories (23–25). In one study the ability of IL-12 to enhance Ag-driven CD8+ T cell proliferation in vitro was dependent on exogenous IL-2 (26). During sensitization for CHS IL-12-mediated enhancement of CD8+ T cell development occurred in CD4+/-/- mice, indicating that this enhancement was not dependent on CD4+ T cells. Our previous studies have indicated that hapten-primed CD4+, but not CD8+, T cells produce IL-2 (27). In this light, the current results may be indicative that IL-2 is not the growth factor promoting the IL-12-mediated enhanced growth of CD8+ T cells. Several points are worth considering. First, IL-12 stimulation may alter the phenotype of hapten-specific CD8+ T cells to produce IL-2. Alternatively, IL-12 administration may enhance the production of a growth factor used for clonal expansion of the CD8+ T cells or positively affect growth factor receptor expression on the CD8+ T cells. For example, IL-15 is required for CD8+ T cell expansion in many immune responses (28–30), and the effects of IL-12 on IL-15 production or on CD8+ T cell expression of the IL-15R α-chain have not been thoroughly investigated.

The development of IFN-γ-producing CD4+ T cells also is dependent upon interactions between CD40 expressed by APC and CD154 expressed by the T cell (31, 32). The skewing of hapten-specific CD4+ T cell development to type 2 cytokine production might be indicative of the absence of these interactions during T cell priming for CHS. In support of this, recent studies from this laboratory revealed that the development of hapten-specific IL-4-producing CD4+ T cell and IFN-γ-producing CD8+ T cell populations as well as CHS responses were similar in DNFB-sensitized wild-type and CD154−/− mice (17). One consequence of CD40 ligation during T cell priming is the stimulation of APC to produce IL-12 (18, 19). In studies by Stuber and colleagues (33), administration of IL-12 circumvented the anti-CD154 mAb-mediated inhibition of effector, IFN-γ-producing CD4+ T cell development and restored the induction of hapten-induced colitis. Similarly, administration of rIL-12 to CD154−/− mice restored IFN-γ-producing CD4+ T cell development and protection from leishmaniasis (34). In contrast to wild-type animals, however, administration of rIL-12 during DNFB sensitization of CD154−/− animals did not rescue the development of hapten-specific CD4+ T cells producing IFN-γ. This indicates that exogenous IL-12 cannot substitute for CD40-mediated signaling and that both IL-12 and CD40-CD154 interactions are required for the development of Th1 CD4+ T cells able to mediate CHS responses. CD40-CD154 interactions during T cell priming also up-regulate many costimulatory molecules on the dendritic cell surface (35). We previously reported that T cell engagement of B7-2, but not B7-1, on hplC was necessary for priming of the IL-4–producing CD4+ and IFN-γ-producing CD8+ T cell populations during sensitization for CHS (36). It is conceivable that the induction of hapten-specific CD4+ T cell development to an IFN-γ-producing phenotype through administration of IL-12 during DNFB sensitization requires additional costimulatory signals, such as B7-1, that are initiated through CD40-CD154 interactions during T cell priming by hplC. In contrast to CD4+ T cells, CD8+ T cell development was enhanced by giving IL-12 during sensitization of CD154−/− mice, again indicating that IFN-γ-producing CD8+ T cell development is not dependent upon CD40-mediated stimulation.

Although development of IFN-γ-producing CD8+ T cells is similar in wild-type and CD154−/− animals, administration of anti-CD154 mAb during hapten sensitization of wild-type mice inhibits the development of these T cells and the CHS response (17). By contrast, the development of hapten-specific CD4+ T cells producing IL-4 is enhanced by the treatment of wild-type mice with anti-CD154 mAb during sensitization. We have recently shown that the inhibitory effects of the anti-CD154 mAb treatment are abolished by depleting mice of CD4+ T cells before hapten sensitization and anti-CD154 mAb administration (17). Together
these findings indicate that the inhibition of CD8$^+$ T cell development for CHS by anti-CD154 mAb is mediated through direct effects on CD4$^+$ T cells, rather than through blockade of CD40-CD154 interactions. Since administration of rIL-12 during hapten sensitization inhibits regulatory CD4$^+$ T cell development, we tested whether exogenous IL-12 would reverse the inhibitory effects of anti-CD154 mAb treatment. The development of ear swelling responses in mice treated with both anti-CD154 mAb and IL-12 was restored to the level in the control group, although not to the level in mice treated with IL-12 alone. Administration of IL-12 with the anti-CD154 mAb had two notable effects on the development of hapten-specific CD4$^+$ and CD8$^+$ T cells. First, IL-12 rescued the development of hapten-specific CD8$^+$ T cells producing IFN-γ. Thus, IL-12 circumvented the inhibitory effect of anti-CD154 mAb mediated through CD4$^+$ T cells. Second, in contrast to CD8$^+$ T cells, CD4$^+$ T cell development to IFN-γ-producing cells was inhibited in mice given both IL-12 and anti-CD154 mAb sensitization. We interpret these results as indicating that exogenous IL-12 primes the CD4$^+$ T cell responses in interaction with hpLC to become IFN-γ-producing cells, but that CD40-CD154 interactions are required to complete this process. In the absence of these interactions, CD4$^+$ Th1 cells receiving only IL-12 signal do not develop at optimal levels. The synergistic effects of IL-12 and CD40-CD154 interactions on the development of human CD4$^+$ T cells to IFN-γ-producing cells during stimulation with anti-TCR mAb have been recently reported (37). In contrast to CD4$^+$ T cells, the effect of IL-12 on the development of CD4$^+$ T cells for CHS is independent of CD40-CD154 engagement.

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References
13. Dilullo, N. A., H. Xu, and R. L. Fairchild. 1996. IL-12 diverts development of CD4$^+$ T cells from regulatory Th2 to effector Th1 cells in contact hypersensitiv-