A Role for CD4 in Peripheral T Cell Differentiation

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Summary

Naive $CD4^+$ T helper cells (Th) differentiate into one of two well-defined cell types during immune responses. Mature Th1 and Th2 cells regulate the type of response as a consequence of the unique cytokines that they secrete. CD4 serves a prominent role in potentiating antigen recognition by helper T cells. We have examined the role of CD4 in peripheral T cell differentiation by studying helper T cells from mice with a congenital defect in CD4 expression. After protein immunization or infection with *Leishmania major*, CD4-deficient mice were incapable of mounting antigen-specific Th2 responses, but retained their Th1 potency. CD4-deficient, T cell receptor transgenic T cells were also incapable of Th2 differentiation after in vitro activation. Expression of a wild-type CD4 transgene corrected the Th2 defect of CD4-deficient mice in all immune responses tested. To investigate the role of the cytoplasmic domain, mice reconstituted with a truncated CD4 molecule were also studied. Expression of the tailless CD4 transgene could not rescue the Th2 defect of CD4-deficient mice immunized with protein or CD4-deficient transgenic T cells activated in vitro, raising the possibility that the cytoplasmic domain of CD4 may influence Th2 generation. Expression of the tailless transgene was, however, capable of restoring Th2 development in CD4-deficient mice infected with *L. major* or CD4-deficient transgenic T cells activated in the presence of recombinant IL-4, demonstrating that the cytoplasmic domain is not absolutely required for Th2 development. Together, these results demonstrate a previously undescribed role of the CD4 molecule. The requirement for CD4 in Th2 maturation reflects the importance of molecules other than cytokines in the control of helper T cell differentiation.

A key functional characteristic of $CD4^+$ helper T cells is their capacity to secrete cytokines when stimulated with peptides presented by MHC class II molecules. During an immune response, a differentiation process gives rise to one of two types of mature helper T cells (see reviews in references 1 and 2). Th1 cells produce IFN-γ and provide help for cell-mediated immunity, whereas Th2 cells produce IL-4 and support the generation of humoral immunity. These two types of helper cells derive from a common naive precursor that has a fate determined in large part by exposure to cytokines. In particular, IL-12 is a potent inducer of the Th1 fate while IL-4 drives cells toward the Th2 fate (1, 2).

Although cytokines have a dominant role in helper T cell differentiation, there is mounting evidence that TCR signaling and costimulation can also influence helper T cell fate (2–4). Of the many cell surface molecules involved in T cell activation, CD4 has a unique capacity to enhance TCR signaling through direct interactions with MHC class II molecules on antigen presenting cells (5) and through its intracellular association with the src-related tyrosine kinase $p56^{lck}$ (6–10). The possible involvement of CD4 in regulating the differentiation of helper T cells has been suggested by several studies (11–13), but remains unclear.

We have studied the functional properties of a unique population of helper T cells that develops in CD4-deficient ($CD4^\circ$)[1] mice. Earlier studies have demonstrated the capacity of these cells to become Th1 cells (14). To examine the role of CD4 in the full spectrum of peripheral differentiation, we have generated $CD4^\circ$ mice on the Th2-prone BALB/c background and examined their response in several well-characterized models of helper T cell maturation. Under all circumstances tested, we found that helper T cells lacking CD4 are defective in their capacity to adopt the Th2 fate. $CD4^\circ$ mice were bred with mice expressing a

[1]*Abbreviations used in this paper:* β2m, β2-microglobulin; $CD4^\circ$, CD4-deficient; $CD4^{\Delta cyt}$, CD4 mutant lacking the cytoplasmic tail; $CD4^{WT}$, wild-type CD4; mRNA, messenger RNA.

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transgene encoding either wild-type CD4 or a mutant form of the molecule lacking its cytoplasmic portion (15). While the full-length molecule was capable of reconstituting Th2 development, the tailless molecule was only partially capable of rescuing Th2 maturation. Thus, our results define a novel function for CD4 in the determination of peripheral T cell fate through its selective potentiation of Th2 maturation.

Materials and Methods

CD4-deficient Mice. CD4° mice used in these experiments were from the fifth backcross to C57BL (C57BL/6 or B10.D2) or fifth backcross to BALB/c. Heterozygote and wild-type littermates were used as controls. Mice expressing transgenes encoding a wild-type or truncated CD4 molecule have been previously described (15). CD4° mice and CD4 transgenic mice were also bred to mice expressing a transgenic TCR specific for a peptide from chicken OVA presented by I-A^d (16; provided by Dennis Loh, Washington University, St. Louis, MO). All TCR transgenic experiments were performed using cells from mice of the BALB/c background. Mice were housed in a specific pathogen-free environment. Genotypes were determined by flow cytometry using stains for commercially conjugated fluorescent mAbs against CD4 (CALTAG Labs., South San Francisco, CA). All work was performed in accordance with University of Chicago (Chicago, IL) guidelines for animal use and care.

KLH Immunization. BALB/c or C57BL mice plus normal littermate controls were immunized in the hind footpads with 150 μg KLH (Calbiochem Corp., San Diego, CA) emulsified in complete Freund's adjuvant (Sigma Chemical Co., St. Louis, MO). 7–10 d after immunization, draining popliteal lymph nodes were harvested and single cell suspensions were prepared in complete Iscove's media (supplemented with 10% fetal calf serum, 2 mM 1-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, and 50 μM 2-mercaptoethanol). For the restimulation assay, 10^6 cells were plated in each well of a flat-bottom 96-well microtiter plate (Costar Corp., Cambridge, MA) with either media or KLH at 30 μg/ml. Supernatants were harvested at 48 h and analyzed for IL-4 and IFN-γ by ELISA using commercial mAb pairs (PharMingen, San Diego, CA) according to the manufacturer's instructions.

Infection with Leishmania major. Infections were performed by injection of 5×10^5 metacyclic promastigotes (WHOM/IR/-/173) into each hind footpad. Lesion size was measured weekly with a metric caliper. *Leishmania*-infected mice were killed at 6–8 wk after infection for cytokine analysis. Single cell suspensions of popliteal lymph node cells from individual animals were examined for antigen-specific cytokine production by restimulation in vitro. In brief, $0.5–1 \times 10^6$ lymph node cells/well were cultured in a 96-well round-bottom microtiter plate in complete media with and without soluble *Leishmania* antigen (100 μg/ml). MHC class II was blocked using mAb M5/114 (rat IgG2b anti-$A^{b,d}$ and -$E^{d,k}$), whereas mAb 10-2-16 served as an isotype control (mouse IgG2b anti-A^k). Supernatants from the restimulation cultures were removed at 48 h and analyzed for IL-4 and IFN-γ production by ELISA.

In Vitro Priming of Transgenic T Cells. $CD8^-$ T cells were isolated from spleens and lymph nodes of fifth BALB/c $CD4^+$ or CD4° T cell receptor transgenic mice (16) by depletion of $B220^+$ and $CD8^+$ cells using mAbs and magnetic beads (PerSeptive Biosystems, Cambridge, MA). Where indicated, CD4° mice also expressed a transgene encoding either a full-length or truncated CD4 molecule. An aliquot was stained with mAb KJ1-26 to calculate the percentage of cells expressing clonotypic antigen receptors. 2.5×10^5 clonotypic cells were cultured in 1 ml with 2.5×10^6 irradiated (2,500 rads) T-deficient splenocytes and 0.3 μM OVA peptide in the presence or absence of *r*IL-4 (20,000 U/ml). After 5 d, cells were washed extensively and 2×10^5 cells were restimulated with 8×10^5 irradiated splenocytes and 7.5 μM OVA peptide in 200 μl. Supernatants were harvested at 48 h for quantitation of IL-4 and IFN-γ by ELISA.

Anti-CD3 Stimulation of T Cells. $CD8^-$ T cells (>95% pure) were isolated from lymph nodes of B10.D2 $CD4^+$ or CD4° mice by depletion of $B220^+$, $CD8^+$, $NK1.1^+$, and MHC class II^+ cells with mAbs and magnetic beads. 5×10^5 cells were cultured in wells precoated with anti-CD3 (2C11; 10 μg/ml; a gift of Dr. Jeff Bluestone, University of Chicago, Chicago, IL) plus *r*IL-4 (10,000 U/ml) in 1 ml. After 5 d, cells were washed extensively and 10^5 cells were restimulated in anti-CD3–coated 96-well flat-bottom plates in triplicate for an additional 48 h. Cell number and anti-CD3 concentrations vary where indicated. IL-4 and IFN-γ were quantitated from supernatants by ELISA. For determination of thymidine incorporation, 1 μCi of methyl-[^{3}H]thymidine (Amersham Life Science, Arlington Heights, IL) was added to wells in 25 μl after 48 h. Cultures were harvested 12–14 h later onto filters, and radioactivity was measured (Betaplate 1205 counter; Wallac, Turku, Finland).

Competitive PCR Analysis. 10^6 purified $CD8^-$ lymph node cells were cultured in the presence of *r*IL-4 (10,000 U/ml) on plates precoated with anti-CD3 (10 μg/ml). After 48 h, the cells were pelleted and RNA was extracted with TRIzol Reagent (GIBCO BRL, Gaithersburg, MD) according to the manufacturer's instructions. RNA was reverse transcribed using random hexamer primers (Pharmacia, Piscataway, NJ). Semiquantitative PCR was performed as described (14). In brief, cDNA was amplified in the presence of a polycompetitor construct that contains addition mutations of the authentic cDNA. Resolved on agarose gels, the larger molecular weight bands provide an internal standard for the relative amounts of the lower molecular weights experimental cDNAs. Concentrations of cDNAs are adjusted using the housekeeping gene hypoxanthine phosphoribosyl transferase before assay of lymphokine gene transcription. Results were verified by repetition of both individual samples and whole experiments.

Results

Impaired In Vivo Th2 Responses in the Absence of CD4. To induce T helper subset differentiation in vivo, CD4° mice were immunized with the protein antigen, KLH. Draining lymph node cells were restimulated 7 d later with antigen in vitro, and the cytokine profile was assessed. Cultures from $CD4^+$ animals produced abundant IL-4, as well as moderate amounts of IFN-γ, whereas the cells from CD4° mice made the opposite response, producing very little IL-4 and accentuated levels of IFN-γ (Fig. 1 *A*). The addition of an anti–MHC class II mAb to the cultures blocked all antigen-driven cytokine production, confirming that the responses were indeed mediated by MHC class II–restricted helper T cells (data not shown). Moreover, the CD4-deficient helper T cells manifested the same Th2 defect in the absence of MHC class I expression in doubly deficient CD4°β2-microglobulin (β2m)° mice (Fig. 1 *B*). Thus,

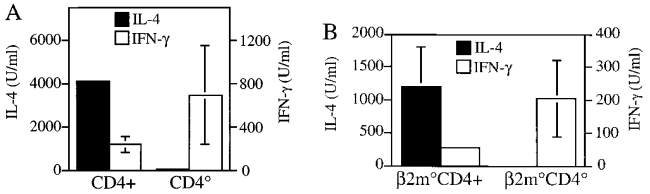

Figure 1. The absence of CD4 impairs Th2 differentiation during immunization. (*A*) BALB/c CD4$^+$ or CD4° mice were immunized with KLH, and draining lymph node cells were harvested 7–10 d later for a restimulation assay as described in Materials and Methods. Supernatants were harvested at 48 h for quantitation of IL-4 (*filled bars*) and IFN-γ (*open bars*) by ELISA. The results shown are the mean of triplicate determinations from two CD4$^+$ and 3 CD4° mice, and are representative of 16 separate experiments involving 6 BALB/c CD4$^+$, 9 BALB/c CD4°, 25 C57BL CD4$^+$, and 24 C57BL CD4° mice. Cells cultured without the addition of KLH produced <25 U/ml of IL-4 and 0.5 U/ml of IFN-γ. Standard deviations are expressed as y-axis error bars throughout the figures. (*B*) β2m-deficient mice (*β2m°CD4+*) and double mutant (*β2m°CD4°*) were immunized with KLH and restimulated as described above. Results are the mean cytokine measurements of triplicate determinations using three animals in each group.

in vivo Th2 differentiation in response to KLH immunization is dependent on CD4 expression.

To examine T helper cell differentiation in response to chronic infection with a parasite, we challenged mice with *L. major*. Infected BALB/c CD4$^+$ mice developed progressively, enlarging footpad lesions. In striking contrast, BALB/c CD4° mice developed minimal footpad swelling, which resolved over time and was indistinguishable from genetically resistant controls (Fig. 2 *A*). Parasite cultures from the feet and spleens of infected mice confirmed that BALB/c CD4° mice had resolved infection (data not shown). When lymph node cells from infected animals were restimulated in vitro with parasite antigens, the cells from CD4$^+$ mice produced high levels of IL-4 and low levels of IFN-γ, whereas the cells from CD4° mice produced high levels of IFN-γ and minimal IL-4 (Fig. 2 *B*). The addition of an anti–MHC class II antibody to the cultures blocked all cytokine production, again confirming that class II–restricted helper T cells mediated these effects (Fig. 2 *B*). Thus, like the response to KLH, *L. major* infection in BALB/c CD4° mice is characterized by defective Th2 differentiation.

Impaired In Vitro Th2 Differentiation in the Absence of CD4. The absence of CD4 decreases positive selection in CD4° mice resulting in a reduced number of helper T cells (14, 17). To correct for this deficiency in cell number, the capacity of CD4-deficient helper T cells to adopt the Th2 fate was examined using in vitro differentiation assays. CD4° mice were bred to mice expressing a transgenic TCR specific for a peptide from chicken OVA presented by I-A^d (16). The clonotypic TCR formed by pairing of the transgenic α and β chains is recognized by the mAb KJ1-26 (16). Equal numbers of CD4 lineage (CD8-negative) KJ1-26$^+$ helper T cells were stimulated in vitro with OVA peptide and APCs. After 5 d, cells were washed extensively and restimulated to assess their differentiation state. CD4$^+$ T cells produced high levels of IL-4 and low levels of IFN-γ, whereas CD4° T cells produced greatly reduced levels of IL-4 and accentuated levels of IFN-γ (Fig. 3). The addition of *r*IL-4 to primary cultures strongly biases toward Th2 differentiation (1). CD4° T cells activated in the presence of *r*IL-4 still yielded only minimal IL-4 production upon restimulation (Fig. 3). Thus, even when responding helper T cell numbers were equalized, the CD4° cells were still incapable of antigen-driven Th2 differentiation.

As a further test of the intrinsic differentiation capacity of CD4-deficient helper T cells, we established an antigen–MHC-independent system of T cell activation thereby eliminating differences in T cell–APC adhesion of CD4$^+$ and CD4° T cells. Highly purified CD8$^-$ helper T cells were stimulated with plate-bound anti-CD3 mAb plus *r*IL-4 for 5 d. Cells were then washed and restimulated with plate-bound anti-CD3 to assess their maturational state. CD4$^+$ T cells were capable of significant IL-4 and modest IFN-γ production, whereas the T cells from CD4° mice produced high amounts of IFN-γ with negligible IL-4 (Fig. 4 *A*). We also attempted to augment the efficiency of Th2 maturation or IL-4 secretion by increasing the density of T cells in the primary or secondary cultures, respectively. For CD4$^+$ T cells, increasing cell number in the primary cul-

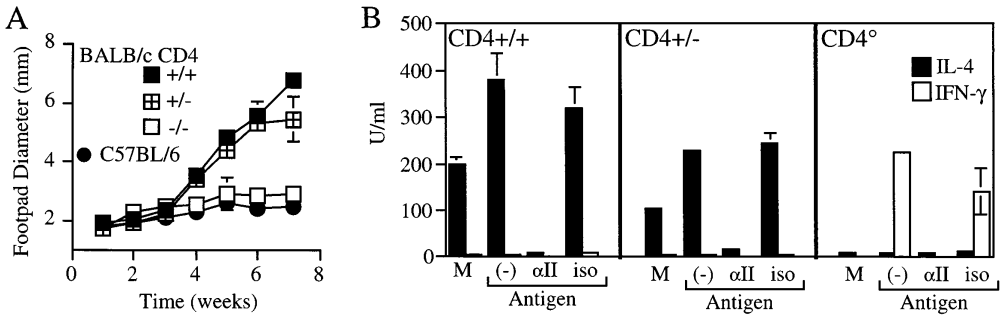

Figure 2. BALB/c CD4° mice are resistant to *L. major*. (*A*) BALB/c CD4$^+$ (+/+, +/−), CD4° (−/−) littermates, and genetically resistant C57BL/6 mice were injected with 5×10^5 metacyclic *L. major* promastigotes in each hind footpad. Disease progression was assessed by measuring footpad thickness with a metric caliper. The results shown are the mean footpad measurements of individual BALB/c mice and are representative of 10 separate experiments using 24 BALB/c CD4$^+$, and 20 BALB/c CD4° mice. (*B*). Infected BALB/c CD4+/+, CD4+/−, or CD4° mice were killed after 7 wk, and popliteal lymph node cells were cultured with either no addition (*M*) or with the addition of 100 μg/ml of soluble parasite antigens in triplicate (*Antigen*). Wells receiving antigen were cultured without antibody [(−)] or with either anti-MHC class II (*αII*) or a control antibody (*iso*). After 48 h, supernatants were harvested for quantitation of IL-4 (*filled bars*) and IFN-γ (*open bars*) by ELISA. The results shown are the mean of triplicate wells from individual mice and are representative of eight experiments using 19 CD4$^+$ and 14 CD4° mice.

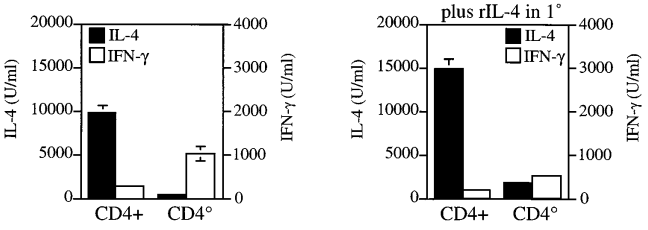

Figure 3. The absence of CD4 impairs antigen-specific Th2 responses in vitro. CD8$^-$ T cells were isolated from spleens and lymph nodes of CD4$^+$ or CD4° BALB/c D011.10 TCR transgenic mice. An equivalent number of clonotypic cells were cultured in 1 ml with irradiated T-deficient splenocytes and OVA peptide without (*left*) or with (*right*) the addition of *r*IL-4. After 5 d, cells were washed and restimulated with irradiated splenocytes and OVA peptide. Supernatants were harvested at 48 h for quantitation of IL-4 (*filled bars*) and IFN-γ (*open bars*) by ELISA. The results shown are the mean of triplicate determinations and are representative of two separate experiments.

ture was sufficient to promote Th2 maturation, even in the absence of *r*IL-4 (Fig. 4 *B*). For CD4° T cells, even the highest cell number plus the addition of *r*IL-4 failed to elicit IL-4 production (Fig. 4 *B*). Moreover, neither the addition of *r*IL-2 nor co-culture of CD4$^+$ with CD4° T cells could rescue Th2 differentiation in the CD4° population (data not shown).

Despite the inability to generate a Th2 response, there was no deficiency in the proliferative response of the CD4° T cells (Fig. 4 *C*). Thus, an inability to proliferate normally does not appear to account for the Th2 defect. Helper T cells initially transcribe multiple lymphokine genes in response to an activation stimulus (18). The addition of *r*IL-4 to cultures enhances IL-4 messenger RNA (mRNA) and suppresses IFN-γ mRNA within 48 h (18, 19). Competitive reverse transcriptase PCR was performed on highly purified helper T cells that had been stimulated for 48 h with anti-CD3 plus *r*IL-4. Although the levels of IFN-γ mRNA were higher in the CD4° T cells, both populations made equivalent levels of IL-4 mRNA at this early time point (Fig. 5). Furthermore, the viability of unstimulated CD4° helper T cells was substantially augmented by the addition of *r*IL-4 as a survival factor (data not shown). Taken together, these results suggest that the absence of CD4 impairs the intrinsic ability of T cells to commit to the Th2 fate, but does not impede their ability to proliferate, receive signals through the IL-4 receptor, or transcribe the IL-4 gene.

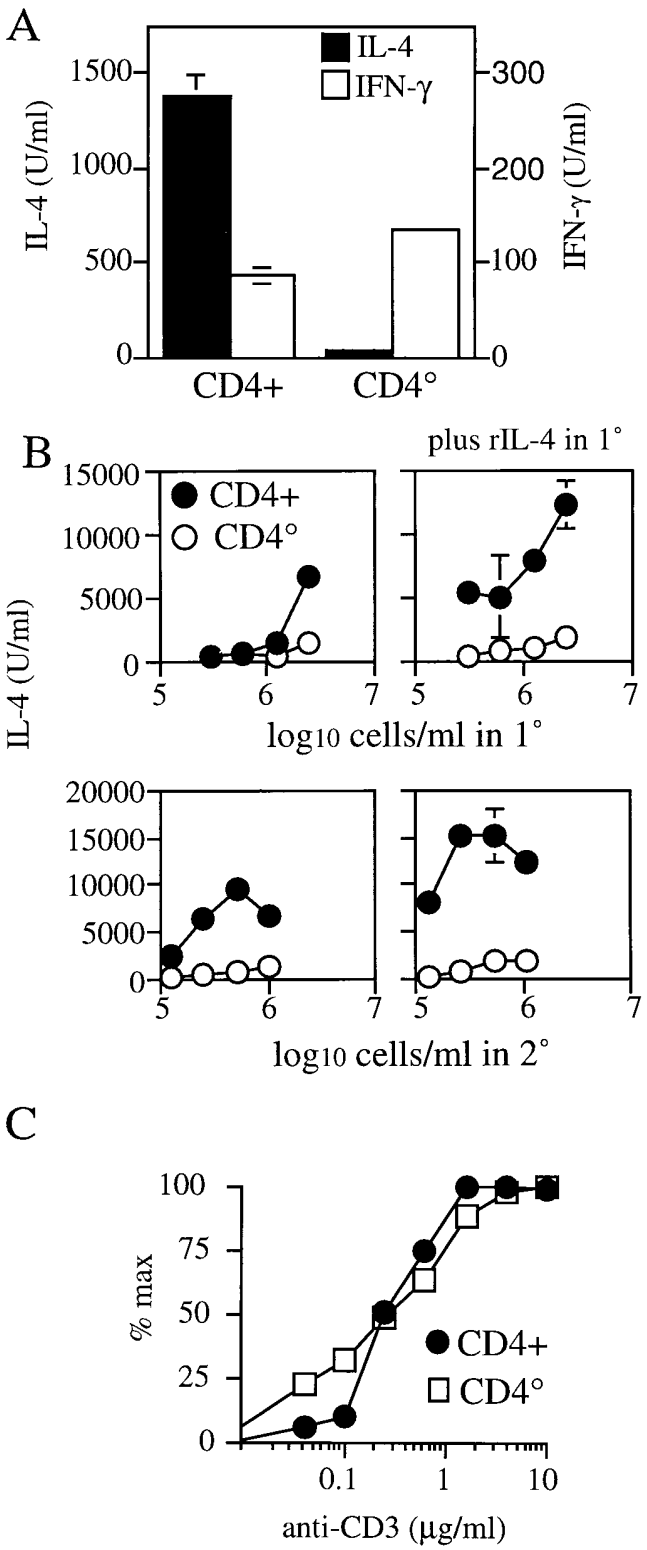

Figure 4. The absence of CD4 impairs Th2 differentiation when T cells are primed without APCs. (*A*) Purified CD8$^-$ T cells were isolated from lymph nodes of CD4$^+$ or CD4° mice, and 5×10^5 cells were plated in wells precoated with anti-CD3 (10 μg/ml) plus *r*IL-4. After 5 d, cells were washed extensively and 10^5 cells were restimulated on anti-CD3–coated plates in triplicate for an additional 48 h. IL-4 (*filled bars*) and IFN-γ (*open bars*) were quantitated from supernatants by ELISA. The results represent the mean of triplicate determinations and are representative of six separate experiments. (*B*) Purified helper lineage T cells were primed as described in *A*, using varying numbers of cells in the primary stimulation (x-axis) and 2×10^5 cells/ml in the secondary stimulation (*B*, *top*), or 2.5×10^6 cells/ml in the primary stimulation and varying numbers of cells in the secondary stimulation (x-axis; *B*, *bottom*). *r*IL-4 was withheld (*B*, *left*) or added (*B*, *right*) to the primary cultures where indicated. (*C*) CD8$^-$ T cells isolated from lymph nodes of CD4$^+$ or CD4° mice were cultured on plates precoated with the indicated concentration of anti-CD3. After 48 h, [^{3}H]thymidine was added, and plates were harvested 12–14 h later. The mean of triplicate wells was used to determine the percent maximum thymidine incorporation for each group.

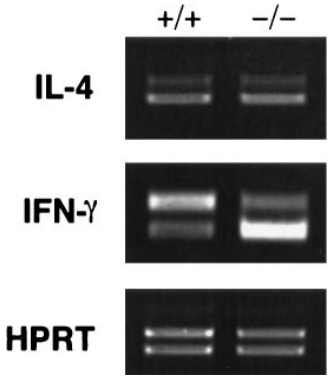

Figure 5. Early cytokine mRNA from CD4° T cells. $CD8^-$ T cells from $CD4^+$ (+/+) and CD4° (−/−) mice were stimulated with plate-bound anti-CD3 plus *r*IL-4. After 48 h, RNA was isolated for competitive reverse transcriptase PCR analysis. PCR reactions were resolved on ethidium-stained 2% agarose gels. Within each panel, the upper band corresponds to amplification of the internal standard competitor molecule, whereas the lower band corresponds to the amplification of experimental cDNAs. The results are representative of three separate experiments.

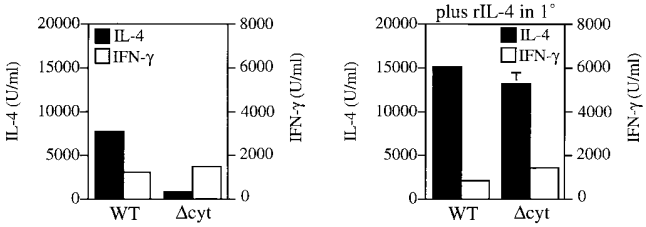

Figure 6. Rescue of Th2 differentiation by CD4 transgenes. $CD8^-$ T cells were isolated from spleens and lymph nodes of CD4° BALB/c D011.10 TCR transgenic mice that had been additionally reconstituted with transgenes encoding either full-length CD4 (*WT*) or a mutant CD4 lacking the cytoplasmic tail (*Δcyt*). An equivalent number of clonotype cells were cultured in 1 ml with irradiated T-deficient splenocytes and OVA peptide without (*left*) or with (*right*) the addition of *r*IL-4. After 5 d, cells were washed and restimulated with irradiated splenocytes and OVA peptide. Supernatants were harvested at 48 h for quantitation of IL-4 (*filled bars*) and IFN-γ (*open bars*) by ELISA. The results shown are the mean of triplicate determinations and are representative of two separate experiments.

Effect of CD4 Transgenes on Th2 Development. The association of CD4 with $p56^{lck}$ has been implicated in signaling and in the recruitment of CD4 to the TCR complex (20, 21). To study the role of this association in the potentiation of the Th2 fate, we used transgenic mice expressing wild-type CD4 ($CD4^{WT}$) or a mutant lacking the cytoplasmic tail ($CD4^{\Delta cyt}$). Both of these transgenes are capable of rescuing helper T cell development in CD4° mice, although overexpression of the tailless version is required for this effect (15). $CD4^{WT}$ and $CD4^{\Delta cyt}$ transgenic mice were backcrossed onto the BALB/c background and then intercrossed with CD4° mice expressing the OVA-specific transgenic TCR. The $CD4^{WT}$ transgene corrected the Th2 defect when expressed in CD4° OVA-specific T cells, but the $CD4^{\Delta cyt}$ transgene was ineffective in promoting the Th2 fate (Fig. 6). When exogenous *r*IL-4 was added to the primary culture, however, either transgene was capable of reconstituting Th2 induction (Fig. 6). Similar results were observed in vivo. The $CD4^{WT}$ transgene rescued the Th2 response to KLH immunization, whereas the $CD4^{\Delta cyt}$ transgene did not (Fig. 7). Both types of transgenes, however, restored susceptibility (Fig. 8 *A*) and Th2 responses (Fig. 8 *B*) to leishmaniasis in BALB/c CD4° mice. Thus, the Th2 defect caused by a null mutation in the CD4 gene can be fully corrected by ectopic expression of wild-type CD4, and partially corrected by a mutant form that lacks the cytoplasmic domain.

Discussion

The results presented in this paper suggest that the function of CD4 influences the outcome of helper T cell differentiation. CD4° helper T cells exhibited a consistent bias toward the Th1 fate in several types of immune responses, even under optimal in vitro conditions of cell number and in the presence of exogenous IL-4. Furthermore, the defect in Th2 differentiation was observed for CD4° mice on both the Th2-prone BALB/c background and for mice on the C57BL/6 background. We cannot fully exclude the possibility that CD4° T cells exit the thymus with an incomplete helper potential. However, CD4° T cells were capable of proliferating and generating IL-4 mRNA early in the response to TCR ligation, showing that the absence of CD4 does not impair these common aspects of T helper cell activation. Rather, the results of in vitro assays suggest that the lack of CD4 results in an intrinsic defect in the capacity of helper T cells to generate signals for proper Th2 differentiation. Thus, the results support a novel role for CD4 in the potentiation of the Th2 fate during immune responses.

In an effort to explore the mechanism by which CD4 influences the fate of T helper cells, we used two previously described lines of transgenic mice that express either $CD4^{WT}$, or $CD4^{\Delta cyt}$ (15). Th2 differentiation was fully restored by expression of the former molecule, but only partially by the latter. For example, Th2 responses to KLH required the presence of the cytoplasmic domain of CD4, but Th2-dependent susceptibility to *Leishmania* did not. The partial rescue of Th2 differentiation mediated by the tailless molecule indicates that the cytoplasmic domain of CD4 can be dispensable for Th2 differentiation under certain circumstances. This result implies that Th2 cells can be formed in the absence of any unique signaling pathway that initiates exclusively at the CD4 cytoplasmic domain. Thus, it is possible that the primary Th2-promoting effect of the tailless molecule is to stabilize the TCR engagement of pep-

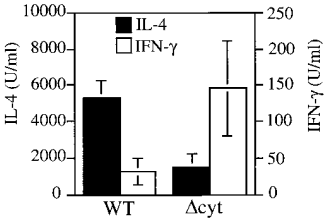

Figure 7. The cytoplasmic domain of CD4 is required for Th2 responses after KLH immunization. CD4 transgene-reconstituted BALB/c CD4° mice were immunized with KLH, and draining lymph node cells were restimulated as described in Materials and Methods. The results shown are the mean of triplicate determinations from two $CD4°CD4^{WT}$ (*WT*) and three $CD4°CD4^{\Delta cyt}$ (*Δcyt*) mice, and are representative of nine separate experiments involving 11 BALB/c $CD4°CD4^{WT}$, 12 BALB/c $CD4°CD4^{\Delta cyt}$, 9 C57BL $CD4°CD4^{WT}$, and 11 C57BL $CD4°CD4^{\Delta cyt}$ mice.

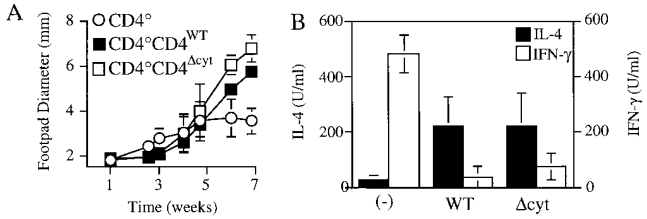

Figure 8. The cytoplasmic domain of CD4 is not essential for susceptibility or Th2 responses after *L. major* infection. (*A*) BALB/c CD4° and CD4° transgene-reconstituted mice (*CD4°CD4^{WT}*, *CD4°CD4$^{\Delta cyt}$*) were infected with *L. major* as described in Materials and Methods. The results shown are the mean footpad measurements of groups of two animals each and are representative of five separate experiments using 9 CD4°CD4^{WT} and 11 CD4°CD4$^{\Delta cyt}$ mice. (*B*) Popliteal lymph node cells from infected BALB/c CD4° (−) and CD4° transgene-reconstituted mice (*WT*, *Δcyt*) were analyzed for parasite-specific cytokine secretion after restimulation in vitro. The results shown are the mean of triplicate determinations from individual mice, and are representative of four separate experiments using eight CD4^{WT} and nine CD4$^{\Delta cyt}$ mice.

tide–MHC complexes. In this way, the tailless molecule may facilitate sustained or enhanced TCR signaling, and this may be the determining factor for Th2 differentiation. However, since some Th2 responses were not restored in the CD4$^{\Delta cyt}$ mice, it is also possible that the cytoplasmic domain of CD4 may have an intrinsic (but not exclusive) capacity to promote the Th2 fate. This Th2-promoting activity could be mediated by direct signal transduction involving p56^{lck}, or other proteins that may associate with CD4, such as CD81 or CD82 (22). Although the results obtained with the CD4$^{\Delta cyt}$ transgenic mice preclude a precise determination of how CD4 influences the differentiation process, they still provide a striking example of how the Th2 differentiative capacity of T helper cells can be profoundly affected by a strategy that impairs the function of CD4.

The requirement for CD4 in T helper cell differentiation may be consistent with models proposing that stronger interactions and activation signals favor Th2 differentiation (2–4). In this regard, it is noteworthy that the Th2 defect was evident even when CD4° T cells were activated in an MHC class II–independent fashion using anti-CD3 mAbs. This observation is perhaps paradoxical given the expectation that anti-CD3 stimulation should bypass the need for CD4 in T cell activation. However, previous work has shown that the activation of T cells with anti-CD3 antibodies can promote the recruitment of CD4 to the TCR complex (23, 24). Thus, CD4$^+$ T cells treated with anti-CD3 may receive a qualitatively or quantitatively distinct activation signal from that received by CD4° T cells, due to differential recruitment of CD4 and its associated p56^{lck}. Nonetheless, it is also possible that the absence of CD4 impairs or changes TCR/CD3 signaling in a distinct and crucial fashion, as has been suggested by studies on T cell hybridomas (25). Regardless of how CD4 exerts its effect on the differentiation process, the data presented here would still be consistent with a model that involves commitment to the Th2 fate only when the character of antigen-specific signaling is sufficiently complex or efficient.

As has been previously reported, the lack of CD4 expression on T helper cells did not impair the differentiation of Th1 cells. Interestingly, CD4° T cells showed normal levels of IL-4 mRNA, but enhanced levels of IFN-γ mRNA at 48 h after stimulation. In other published experiments, the blockade of the CD4–MHC class II interaction with an MHC class II peptide also impaired Th2 differentiation and favored the Th1 fate (11). Although these and our experiments could be consistent with passive commitment to Th1 differentiation in the absence of CD4, it is also possible that the involvement of CD4 in T cell activation normally serves an active Th1-suppressive function. This supposition may be consistent with the finding that the ligation of CD4 with mAbs can suppress the production of IFN-γ and the induction Th1 cells (12). Similarly, it is possible that chronic ligation of CD4 by the HIV envelope in HIV-infected humans delivers signals that potentiate Th2 differentiation. This may be relevant to the controversial finding that the progression to AIDS is accompanied by increased Th2-mediated allergic complications and impaired Th1 immunity (26–29). If CD4 does have an active role in suppressing Th1 differentiation, then it may be possible to induce Th2 differentiation in naive T helper cells by strategies that would bypass CD4 and directly engage the putative Th1 suppressive pathway. A better understanding of the mechanism by which CD4 regulates T helper cell fate may ultimately suggest alternative therapies for many diseases. In summary, the data support a novel role for CD4 in the determination of helper T cell lineage after thymic development. Despite the critical role of cytokines in controlling helper T cell fate, the results of this work help to refocus attention on TCR/CD4 signaling as being essential for proper Th2 development.

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