CD28 is a major costimulatory signal receptor for T cells. We have used human naive CD4+ cells from cord blood to analyze the effect of the CD28/B7 costimulatory pathway on development of T helper (Th) subsets. We show that CD28 costimulation is critical for development of the Th2 cytokine-producing cells and that in the absence of CD28 costimulation, cells are not primed to produce Th2 cytokines and consequently "default" to the Th1 subset, independent of the presence of exogenous cytokines. After CD28 costimulation, cells differentiate into a subset that produces Th2 cytokines. However, further CD28 costimulation is not required to maintain Th2 cytokine production. We conclude that CD28 costimulation is critical for the development of Th0 and Th2 subsets, but not for the maintenance of cytokine production.

**Critical Role of CD28/B7 Costimulation in the Development of Human Th2 Cytokine-Producing Cells**

By Louise M.C. Webb and Marc Feldmann

CD28 is a major costimulatory signal receptor for T cells. We have used human naive CD4+ cells from cord blood to analyze the effect of the CD28/B7 costimulatory pathway on development of T helper (Th) subsets. We show that CD28 costimulation is critical for development of the Th2 cytokine-producing cells and that in the absence of CD28 costimulation, cells are not primed to produce Th2 cytokines and consequently "default" to the Th1 subset, independent of the presence of exogenous cytokines. After CD28 costimulation, cells differentiate into a subset that produces Th2 cytokines. However, further CD28 costimulation is not required to maintain Th2 cytokine production. We conclude that CD28 costimulation is critical for the development of Th0 and Th2 subsets, but not for the maintenance of cytokine production.

**Critical Role of CD28/B7 Costimulation in the Development of Human Th2 Cytokine-Producing Cells**

By Louise M.C. Webb and Marc Feldmann

CD28 is a major costimulatory signal receptor for T cells. We have used human naive CD4+ cells from cord blood to analyze the effect of the CD28/B7 costimulatory pathway on development of T helper (Th) subsets. We show that CD28 costimulation is critical for development of the Th2 cytokine-producing cells and that in the absence of CD28 costimulation, cells are not primed to produce Th2 cytokines and consequently "default" to the Th1 subset, independent of the presence of exogenous cytokines. After CD28 costimulation, cells differentiate into a subset that produces Th2 cytokines. However, further CD28 costimulation is not required to maintain Th2 cytokine production. We conclude that CD28 costimulation is critical for the development of Th0 and Th2 subsets, but not for the maintenance of cytokine production.

**Critical Role of CD28/B7 Costimulation in the Development of Human Th2 Cytokine-Producing Cells**

By Louise M.C. Webb and Marc Feldmann

CD28 is a major costimulatory signal receptor for T cells. We have used human naive CD4+ cells from cord blood to analyze the effect of the CD28/B7 costimulatory pathway on development of T helper (Th) subsets. We show that CD28 costimulation is critical for development of the Th2 cytokine-producing cells and that in the absence of CD28 costimulation, cells are not primed to produce Th2 cytokines and consequently "default" to the Th1 subset, independent of the presence of exogenous cytokines. After CD28 costimulation, cells differentiate into a subset that produces Th2 cytokines. However, further CD28 costimulation is not required to maintain Th2 cytokine production. We conclude that CD28 costimulation is critical for the development of Th0 and Th2 subsets, but not for the maintenance of cytokine production.

**Critical Role of CD28/B7 Costimulation in the Development of Human Th2 Cytokine-Producing Cells**

By Louise M.C. Webb and Marc Feldmann

CD28 is a major costimulatory signal receptor for T cells. We have used human naive CD4+ cells from cord blood to analyze the effect of the CD28/B7 costimulatory pathway on development of T helper (Th) subsets. We show that CD28 costimulation is critical for development of the Th2 cytokine-producing cells and that in the absence of CD28 costimulation, cells are not primed to produce Th2 cytokines and consequently "default" to the Th1 subset, independent of the presence of exogenous cytokines. After CD28 costimulation, cells differentiate into a subset that produces Th2 cytokines. However, further CD28 costimulation is not required to maintain Th2 cytokine production. We conclude that CD28 costimulation is critical for the development of Th0 and Th2 subsets, but not for the maintenance of cytokine production.

**Critical Role of CD28/B7 Costimulation in the Development of Human Th2 Cytokine-Producing Cells**

By Louise M.C. Webb and Marc Feldmann

CD28 is a major costimulatory signal receptor for T cells. We have used human naive CD4+ cells from cord blood to analyze the effect of the CD28/B7 costimulatory pathway on development of T helper (Th) subsets. We show that CD28 costimulation is critical for development of the Th2 cytokine-producing cells and that in the absence of CD28 costimulation, cells are not primed to produce Th2 cytokines and consequently "default" to the Th1 subset, independent of the presence of exogenous cytokines. After CD28 costimulation, cells differentiate into a subset that produces Th2 cytokines. However, further CD28 costimulation is not required to maintain Th2 cytokine production. We conclude that CD28 costimulation is critical for the development of Th0 and Th2 subsets, but not for the maintenance of cytokine production.

**Critical Role of CD28/B7 Costimulation in the Development of Human Th2 Cytokine-Producing Cells**

By Louise M.C. Webb and Marc Feldmann

CD28 is a major costimulatory signal receptor for T cells. We have used human naive CD4+ cells from cord blood to analyze the effect of the CD28/B7 costimulatory pathway on development of T helper (Th) subsets. We show that CD28 costimulation is critical for development of the Th2 cytokine-producing cells and that in the absence of CD28 costimulation, cells are not primed to produce Th2 cytokines and consequently "default" to the Th1 subset, independent of the presence of exogenous cytokines. After CD28 costimulation, cells differentiate into a subset that produces Th2 cytokines. However, further CD28 costimulation is not required to maintain Th2 cytokine production. We conclude that CD28 costimulation is critical for the development of Th0 and Th2 subsets, but not for the maintenance of cytokine production.

**Critical Role of CD28/B7 Costimulation in the Development of Human Th2 Cytokine-Producing Cells**

By Louise M.C. Webb and Marc Feldmann

CD28 is a major costimulatory signal receptor for T cells. We have used human naive CD4+ cells from cord blood to analyze the effect of the CD28/B7 costimulatory pathway on development of T helper (Th) subsets. We show that CD28 costimulation is critical for development of the Th2 cytokine-producing cells and that in the absence of CD28 costimulation, cells are not primed to produce Th2 cytokines and consequently "default" to the Th1 subset, independent of the presence of exogenous cytokines. After CD28 costimulation, cells differentiate into a subset that produces Th2 cytokines. However, further CD28 costimulation is not required to maintain Th2 cytokine production. We conclude that CD28 costimulation is critical for the development of Th0 and Th2 subsets, but not for the maintenance of cytokine production.

**Critical Role of CD28/B7 Costimulation in the Development of Human Th2 Cytokine-Producing Cells**

By Louise M.C. Webb and Marc Feldmann

CD28 is a major costimulatory signal receptor for T cells. We have used human naive CD4+ cells from cord blood to analyze the effect of the CD28/B7 costimulatory pathway on development of T helper (Th) subsets. We show that CD28 costimulation is critical for development of the Th2 cytokine-producing cells and that in the absence of CD28 costimulation, cells are not primed to produce Th2 cytokines and consequently "default" to the Th1 subset, independent of the presence of exogenous cytokines. After CD28 costimulation, cells differentiate into a subset that produces Th2 cytokines. However, further CD28 costimulation is not required to maintain Th2 cytokine production. We conclude that CD28 costimulation is critical for the development of Th0 and Th2 subsets, but not for the maintenance of cytokine production.

**Critical Role of CD28/B7 Costimulation in the Development of Human Th2 Cytokine-Producing Cells**

By Louise M.C. Webb and Marc Feldmann

CD28 is a major costimulatory signal receptor for T cells. We have used human naive CD4+ cells from cord blood to analyze the effect of the CD28/B7 costimulatory pathway on development of T helper (Th) subsets. We show that CD28 costimulation is critical for development of the Th2 cytokine-producing cells and that in the absence of CD28 costimulation, cells are not primed to produce Th2 cytokines and consequently "default" to the Th1 subset, independent of the presence of exogenous cytokines. After CD28 costimulation, cells differentiate into a subset that produces Th2 cytokines. However, further CD28 costimulation is not required to maintain Th2 cytokine production. We conclude that CD28 costimulation is critical for the development of Th0 and Th2 subsets, but not for the maintenance of cytokine production.

**Critical Role of CD28/B7 Costimulation in the Development of Human Th2 Cytokine-Producing Cells**

By Louise M.C. Webb and Marc Feldmann

CD28 is a major costimulatory signal receptor for T cells. We have used human naive CD4+ cells from cord blood to analyze the effect of the CD28/B7 costimulatory pathway on development of T helper (Th) subsets. We show that CD28 costimulation is critical for development of the Th2 cytokine-producing cells and that in the absence of CD28 costimulation, cells are not primed to produce Th2 cytokines and consequently "default" to the Th1 subset, independent of the presence of exogenous cytokines. After CD28 costimulation, cells differentiate into a subset that produces Th2 cytokines. However, further CD28 costimulation is not required to maintain Th2 cytokine production. We conclude that CD28 costimulation is critical for the development of Th0 and Th2 subsets, but not for the maintenance of cytokine production.

**Critical Role of CD28/B7 Costimulation in the Development of Human Th2 Cytokine-Producing Cells**

By Louise M.C. Webb and Marc Feldmann

CD28 is a major costimulatory signal receptor for T cells. We have used human naive CD4+ cells from cord blood to analyze the effect of the CD28/B7 costimulatory pathway on development of T helper (Th) subsets. We show that CD28 costimulation is critical for development of the Th2 cytokine-producing cells and that in the absence of CD28 costimulation, cells are not primed to produce Th2 cytokines and consequently "default" to the Th1 subset, independent of the presence of exogenous cytokines. After CD28 costimulation, cells differentiate into a subset that produces Th2 cytokines. However, further CD28 costimulation is not required to maintain Th2 cytokine production. We conclude that CD28 costimulation is critical for the development of Th0 and Th2 subsets, but not for the maintenance of cytokine production.
Th1, cytokine-producing cells. In addition, once cells have differentiated into the Th2 cytokine-producing cells, further CD28 costimulation is not required for the maintenance of Th2 cytokine production.

MATERIALS AND METHODS

Reagents. T-cell activation was performed using OKT3, a monoclonal antibody (MoAb) directed against CD3 (American Type Culture Collection, Maryland) and 9.3, an IgG2a MoAb directed against CD28 donated by Dr J. Ledbetter (Bristol Myers Squibb, Seattle, WA). For direct fluorescent staining, PE-conjugated anti-CD4 MAb (anti-Leu-3a; Becton Dickinson, Palo Alto, CA), FITC-conjugated anti-CD8 (anti-Leu-2a; Becton Dickinson), FITC-conjugated anti-CD45RA (Leu-18; Becton Dickinson), FITC-conjugated anti-CD45RO (UCHL-1; donated by Prof. P. Beverley, ICRF, London, UK), FITC-conjugated anti-CD14 (HLel; Becton Dickinson), FITC-conjugated anti-CD19 (Leu-12; Becton Dickinson), recombinant IL-2, IL-4, or IL-12 were added into cultures at a final concentration of 10 ng/mL in 10% human serum RPMI. After 4 days, supernatants were collected and placed on enzyme linked immunosorbent assays (ELISA) or bioassays. For secondary culture, cells were placed onto 96-well plates coated with OKT3 and cultured as described above. For proliferation assays, 10^6 cells/mL were cultured in OKT3-coated 96-well plates as above. Costimulation was expressed as counts per minute (cpm) during the final 8 hours of culture. As shown in Fig 1, proliferation did not occur in the absence of CD3 stimulation and both IL-2 and anti-CD28 enhanced anti-CD3-induced proliferation. A marked increase in cell proliferation was consistently observed when cells were costimulated via CD3 and CD28 in the presence of IL-2 (Fig 1). An IgG2a isotype control had no effect on proliferation (counts per minute; cpms). The effects of different activation conditions on cytokine production were analyzed by using this in vitro differentiation model to analyze the effects of different activation conditions on cytokine pro-

RESULTS

Costimulation via CD28 enhances proliferation of human naive CD4+ cells to anti-CD3 and IL-2. To analyze the effect of CD28 costimulation on human naive CD4+ cells, we used an APC-free system to eliminate costimulation by accessory molecules present on APCs. Naive CD4+ cells were purified from cord blood mononuclear cells (CBMCs) yielding a pure (>99%) population of unactivated cells as described in experimental procedures. Purified CD4+ cells were stimulated by crosslinking the T-cell receptor (TCR)/CD3 complex using plastic-immobilized anti-CD3 MoAb. To stimulate cells via the CD28 molecule, soluble anti-CD28 MoAb was added to cultures. A predetermined optimal dose of IL-2 and/or anti-CD28 was added to cells and proliferation was assessed on day 5 by measuring [3H]thymidine incorporation (counts per minute; cpm) during the final 8 hours of culture. As shown in Fig 1, proliferation did not occur in the absence of CD3 stimulation and both IL-2 and anti-CD28 enhanced anti-CD3-induced proliferation. A marked increase in cell proliferation was consistently observed when cells were costimulated via CD3 and CD28 in the presence of IL-2 (Fig 1). An IgG2a isotype control had no effect on anti-CD3 and IL-2–induced proliferation of naive CD4+ cells (data not shown).

CD28 costimulation enhances cytokine production by human CD4+ cells. It has been shown that human naive CD4+ cells are unable to express certain lymphokine genes on primary in vitro stimulation but can be induced to undergo functional in vitro differentiation by stimulation via CD3 and growth in exogenous IL-2 for a period of 7 to 10 days. We have used this in vitro differentiation model to analyze the effects of different activation conditions on cytokine pro-
IL-12 had no significant effect on development of Th2 cells because it altered neither IL-4 nor IL-5 production (Fig 3, C and D), irrespective of the presence of anti-CD28. In contrast, while inclusion of IL-4 alone had no effect on Th2 cytokine production (Fig 3, C and D) the combination of IL-4 and anti-CD28 lead to high levels of both IL-4 and IL-5 production. IL-4 was able to enhance the production of Th2 cytokines only when cells were primed using anti-CD28 MoAb as a costimulus. In the absence of anti-CD28 MoAb, naive cells failed to develop into Th2 cytokine-producing cells, even in the presence of exogenous IL-4. Exogenous IL-12 had no effect on development of Th2 cytokine-producing cells. CD28 costimulation was essential for the development of Th2 cytokine-producing cells and was enhanced by exogenous IL-4.

Development of Th2 cytokine-producing cells is dependent on CD28 costimulation. To confirm that CD28 costimulation is a requirement for development of Th2 cytokine-producing cells, naive CD4+ cells were stimulated via CD3 in the presence of IL-2 and IL-4 along with different concentrations of anti-CD28. As shown in Fig 4, the development of Th2 cytokine-producing cells was dependent on the concentra-

IL-12 and IL-4 alter human Th subset development. Studies using murine naive CD4+ cells have shown that IL-12 and IL-4 prime cells to produce Th1 and Th2 cytokines, respectively. We analyzed the effect of CD28 costimulation on IL-12 or IL-4-induced development of human Th1 and Th2 cells by culturing naive cells as described above in the presence or absence of a predetermined optimal dose of exogenous IL-12 or IL-4. As shown in Fig 3A, the presence of IL-12 enhanced production of the Th1 cytokine, IFN-γ, in the absence of CD28 costimulation. However, inclusion of anti-CD28 MoAb enhanced this IL-12–induced IFN-γ production by threefold to fourfold. In contrast, co-culture with IL-4 had only a slight inhibitory effect on IFN-γ production even in the presence of anti-CD28.
Fig 3. IL-12 and IL-4 alter human Th subset development. Naive CD4+ cells were stimulated via CD3 or costimulated via CD3 plus CD28 and grown in IL-2, IL-2 and IL-12, or IL-2 and IL-4. After 7 days, cells were washed three times, rested for 2 hours to remove exogenous cytokines, washed twice more, and then stimulated as described in experimental procedures. Results are representative of five separate experiments using blood from different donors. (□) Cytokine production after stimulation via CD3; (■) cytokine production after costimulation via CD3 and CD28. Panels A, B, C, and D represent production of IFN-γ, IL-2, IL-4, and IL-5, respectively. Sensitivities of ELISA and bioassay were 40 pg/mL, 50 pg/mL, 120 pg/mL, and 20 pg/mL for IL-4, IL-5, IFN-γ, and IL-2, respectively.

Fig 4. Development of Th2 cytokine-producing cells is dependent on CD28 costimulation. Naive CD4+ cells were stimulated with immobilized anti-CD3 and 0, 1, 10, or 100 ng/mL of soluble anti-CD28. Cells were grown in IL-2 and IL-4 for 7 days before a 24-hour restimulation with PHA and ionomycin to determine the effect of CD28 costimulation on development of Th2 cytokine-producing cells. Sensitivity of IL-4 ELISA was 40 pg/mL.

Th1 Cytokine Production (pg/ml)  Th2 Cytokine Production (pg/ml)

Concentration of anti-CD28 (ng/ml)

Production of IL-4 (pg/ml)

CD28-induced Th2 cytokine production is not due to enhanced production of IL-2. CD28 costimulation can significantly enhance IL-2 production. Because IL-2 is also critical for IL-4 production by Th cells, we sought to determine if...
DEVELOPMENT OF TH2 CYTOKINE-PRODUCING CELLS

Fig 5. Memory Th cells produce Th2 cytokines independent of CD28 costimulation. Th1 and Th2 cytokine-producing cells were generated and restimulated as described in the legend to Fig 3 from naive CD4⁺ cells using a costimulatory protocol of anti-CD3 plus anti-CD28. After 7 to 10 days of priming, memory Th cells were restimulated via CD3 or costimulated via CD3 and CD28 and grown in IL-2, IL-2 and IL-4, or IL-2 and IL-12 for 7 days before restimulation with PMA and ionomycin to determine the resultant cytokine profiles. Results are representative of five separate experiments using blood from different donors. (a) Cytokine production after secondary culture of memory cells that were stimulated via CD3; (b) cytokine production after secondary culture of memory cells that were costimulated via CD3 and CD28. Panels A and B represent production of IL-4 and IL-5, respectively. Sensitivities of IL-4 and IL-5 ELISA were 40 pg/mL and 50 pg/mL, respectively.

CD28 costimulation induced development of Th2 cells via its ability to enhance IL-2 production. We analyzed the effect of a supra-optimal concentration of exogenous IL-2 on development of Th2 cells. Cells were cultured for 7 days in the presence or absence of anti-CD28 and/or IL-4 with two different concentrations of IL-2: an optimal dose (10 ng/mL) or a supra-optimal dose (100 ng/mL). IL-4 production by primed cells was assessed following restimulation for 24 hours with PMA and ionomycin. The increased dose of exogenous IL-2 did not induce development of IL-4-producing cells, independently of exogenous IL-4. Development of Th2 cytokine-producing cells was only seen when cells were costimulated via CD28. IL-4-producing cells developed in the absence of exogenous IL-4. The presence of IL-4 during priming enhanced subsequent production of Th2 cytokines. The increased concentration of exogenous IL-2 slightly enhanced production of IL-4 but only when cells had been costimulated via CD28 (Fig 6), in the absence of CD28 costimulation Th2 cytokine-producing cells did not develop and this was independent of the concentration of exogenous IL-2 added.

DISCUSSION

The role of CD28 costimulation in T-cell activation has been of considerable interest. In vitro studies have shown that CD28 costimulation can augment mitogen and anti-CD3-induced T-cell proliferation, amplify the production of multiple T-cell cytokines, and prevent induction ofergy in T-cell clones. CD28 costimulation can also induce responsiveness to IL-4 in both Th1 and Th2 cell clones. However, the role of CD28 costimulation during the early stages of human Th subset development has, until now, remained enigmatic.

To analyze the role of CD28 costimulation in the development of Th subsets it is essential that the starting population is antigenically naive. Approximately, 40% of CD4⁺ cells in adult blood were thought to be naive due to their expression of the CD45RA isoform and lack of the CD45RO isoform expression. However, studies have shown that a memory cell, expressing CD45RO, can revert back to CD45RA expression, implying that the once thought unidirectional progression from CD45RA to CD45RO expression is cyclical. Thus, expression of the CD45RA isoform in adult blood does not represent a naive population of cells and such cells would not be appropriate for this study. Hence, by using neonatal blood taken from umbilical cords our source of naive CD4⁺ cells are taken early in the development of the immune system. Neonatal T cells are naive with respect to antigenic stimulation, cytokine production (producing only IL-2), and also cell surface marker expression (CD45RA⁺CD45RO CD3⁺). In order to elimi

Fig 6. CD28-induced Th2 cytokine production does not occur by enhancing exogenous IL-2. Naive CD4⁺ cells were cultured as described in the legend to Fig 3 with anti-CD3 or anti-CD3 plus anti-CD28 and grown in IL-2 or IL-2 plus IL-4. The effect of optimal or supraoptimal concentration of IL-2 on development of IL-4-producing cells was assessed by using exogenous IL-2 at either 10 ng/mL or 100 ng/mL. IL-4 production was measured after a 24-hour restimulation with PMA plus ionomycin. (a) Cytokine production after costimulation via CD3 and CD28 and growth in an optimal concentration of IL-2 (10 ng/mL). (b) Cytokine production after costimulation via CD3 and CD28 and growth in a supra-optimal concentration of IL-2 (100 ng/mL). No IL-4 production was detected after priming in the absence of anti-CD28. Results are representative of three separate experiments using blood from different donors. The sensitivity of the IL-4 ELISA was 40 pg/mL.
nate costimulation by other accessory molecules, we used an APC-free culture (as assessed by flow cytometry and the lack of proliferation of cells to soluble anti-CD3). CD4+ cells were purified from CBMCs and then stimulated with plate-immobilized anti-CD3, and grown in IL-2. An optimal concentration of IL-2 was used throughout culture to ensure that any effect of co-stimulation via CD28 was not merely due to CD28-enhanced production of IL-2. To mimic the stimulatory interaction between B7 molecules and CD28, an agonistic MoAb to CD28 was added into the initial culture. For detection of cytokines, we chose to measure secreted cytokines as opposed to cytokine message, since the detection of cytokine mRNA does not confirm cytokine secretion and may be of little physiological significance.

This study of human Th subset development draws a number of parallels with development of Th subsets in the mouse. Previous murine studies have shown that IL-12 and IL-4 are the main factors that induce development of the Th1 and Th2 subsets, respectively. Our data confirm this observation in human cells.

However, by using an APC-free system, we have been able to further define the activation requirements for subset development. Murine studies have invariably used culture systems where APCs are present. APCs will express ligands for CD28 and other T-cell surface molecules (eg, ICAM-1, CD40L, CD44, etc). In one murine study, blocking the interaction of CD28 with its ligands by adding CTLA-4Ig showed that CD28 costimulation was critical for the development of both Th1 and Th2 subsets from naive cells. However, this requirement for CD28 costimulation could be overcome by addition of exogenous IL-2. In contrast, we find that human naive cells require IL-2 for development of both Th1 and Th2 cytokine-producing cells, independent of CD28 costimulation (data not shown), but human Th2 subset development shows an absolute requirement for CD28 costimulation, which cannot be overcome by excess exogenous IL-2. An increase in the concentration of exogenous IL-2 did not allow development of Th2 cells in the absence of CD28 costimulation. Thus, although CD28 induction of IL-2 production is critical for Th2 cytokine production, clearly other CD28-stimulated events are also important. CD28 costimulation not only allowed development of a Th2-like subset but also enhanced anti-CD3-induced proliferation of naive CD4+ cells and secretion of the Th1 cytokines IL-2 and IFN-γ. Thus, it could be argued that the production of Th2 cytokines seen after CD28 costimulation was merely due to an enhancement in production of all T-cell cytokines. However, even if Th2 cytokines were being secreted at levels just below the detection limits of the ELISA then CD28 costimulation enhanced Th2 cytokine production by at least 40- to 100-fold but only enhanced Th1 cytokine production by 3- to 5-fold.

CD28 has been reported to enhance transcription and stabilization of cytokine mRNAs. Hence, it is possible that CD28 costimulation may enhance transcription and stabilization of IL-4 mRNA, inducing autocrine production of IL-4 during initial stimulation, which could allow development of the Th2 subset. However, in our system, addition of IL-4 during priming could not induce Th2 subset development in the absence of CD28 costimulation (even when the concentrations of exogenous IL-4 exceeded 100 ng/mL; data not shown). In addition, we also find that CD28 costimulation does not induce production of IL-4 during priming (data not shown). Thus, mechanisms involving enhanced transcription and stabilization of IL-4 message by CD28 costimulation during priming, though probably important, are not critical for Th2 subset development. Thus, the mechanism by which CD28 costimulation induces Th2 subset development remains elusive. Our finding that Th2 cytokine production by memory cells occurs in the absence of costimulation agrees with a previous study in mice in which IL-4 secretion occurred independently of CD28 costimulation after naive cells had been first primed with APCs and Ag. In our study, IL-4 induction of Th2 subset development showed an absolute requirement for the presence of anti-CD28, but once naive cells have acquired the ability to produce Th2 cytokines, we found that subsequent Th2 cytokine production occurred independently of CD28 costimulation, suggesting that memory and naive cells differ in their costimulatory requirements. We have been able to maintain the phenotype of Th2 cytokine production for several weeks by immobilized anti-CD3 stimulation and growth in IL-2 (data not shown).

There are currently two CD28 ligands, namely B7-1 (CD80) and B7-2 (CD86). The existence of a further CD28 ligand, B7-3, has been postulated but not yet proven by molecular cloning. Numerous studies have analyzed the expression of CD80 and CD86 and have shown that these two ligands are regulated differently; CD86 expression tends to precede that of CD80 during the development of the immune response. The existence of CD28 ligands and their different patterns of expression imply that these ligands serve distinct functions. Hence, studies are currently underway to analyze the effect of CD28 engagement by CD80 and CD86 on human Th subset development.

In vitro models of human Th subset development have shown that IL-12 induces development of a Th1-like subset. We also found that addition of IL-12 enhanced Th1 cytokine production, while addition of IL-4 inhibited Th1 cytokine production, irrespective of costimulation. CD28 costimulation synergized with IL-12 to enhance Th1 cytokine production. In agreement with our study, there have been two reports that stimulation via CD3 and growth in IL-2 and IL-4 is insufficient to prime human naive CD4+ cells to a subset able to produce Th2 cytokines. In the first study, inclusion of a CD32 transfected mouse fibroblast cell line allowed development of Th2 cells and since the use of this fibroblast line was of critical importance for production of Th2 cytokines, it was postulated that they were providing an accessory signal. It is possible that ligands for CD28, present on the transfected cell line, were allowing costimulation via CD28. In agreement with our study, Kalinski et al have also found that CD28 costimulation allows development of human naive CD4+ cells to a phenotype capable of secreting IL-4. In addition, they observed that there was no IL-4 production during the priming period. The model sys-
tem used by Kalinski et al differed from ours in their source of naive cells; they used naive CD4+ cells purified from adult peripheral blood on the basis of CD45 isoform expression. Although this study clearly showed a role for CD28 costimulation in the development of adult naive CD4+ cells into Th2 cytokine-producing cells, given the controversy regarding the use of CD45 isoforms as markers of naivety, the naivety of the cells used in their study is debatable. Recently, in vivo blockade of the CD28-B7 pathway has shown differential effects on Th1 and Th2 effector subset development in experimental Leishmaniasis. Susceptible mice generate Th2 effector cells in response to Leishmania major; however, administration of CTLA-4-Ig during the first week of infection abrogates this progressive disease in susceptible mice, but has no effect on the protective immune response developed by the resistant mice, which generate Th1 effector cells. This implied that CTLA4-Ig prevented development of Th2 cells without effecting development of Th1 cells. More curiously, continuous administration of CTLA4-Ig abolished the capacity of the resistant mice to contain infection, implying that maintenance of Th1 cytokine production is dependent on CD28 costimulation. This has been elegantly demonstrated in vitro activation of murine Th subsets and also studies using human Th1 clones or primed human CD4+ T cells taken from adult blood. Taken together these data point to a reciprocal role for CD28 co-stimulation in the generation and maintenance of Th subsets. Th1 subsets develop independently of CD28 costimulation but subsequently require costimulation for survival. In contrast, Th2 cells require CD28 costimulation for their initial development but then remain CD28 independent. Thus, the ligands for CD28 can differentially regulate Th1 and Th2 cytokine production depending on the differentiation state of the T cell.

ACKNOWLEDGMENT

We are indebted to the staff of the Chelsea and Westminster hospital for the collection of cord blood samples. We also thank Drs J. Ledbetter, P. Beverley, M. Gately, P. Lomedico, P. Ramage, F. de Padova, D. Novick, and J. Abrams for generously providing reagents used in this study and Drs M. Turner, S. Cohen, and M. Londei for critically reviewing this manuscript.

REFERENCES


3486

ental T cell costimulatory requirements in CD28-deficient mice. Science 261:609, 1993
39. Linsley PS, Greene JA, Brady W, Bajorath J, Ledbetter JA, Peach R: Human B7-1 (CD80) and B7-2 (CD86) bind with similar avidities but distinct kinetics to CD28 and CTLA-4 receptors. Immunity 1:793, 1994
Critical role of CD28/B7 costimulation in the development of human Th2 cytokine-producing cells

LM Webb and M Feldmann