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Dendritic Cells Produce IL-12 and Direct the Development of Th1 Cells from Naive CD4$^+$ T Cells

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Dendritic cells are APCs that are unique in their potency to stimulate proliferation of primary Ag-specific responses in vitro and in vivo. In this study, we demonstrate that dendritic cells can produce IL-12, a dominant cytokine involved in the development of IFN-γ-producing T cells. This finding resulted from our observations that dendritic cell-induced Th1 development from total CD4$^+$ T cells upon neutralization of endogenous levels of IL-4 was IL-12-dependent. Furthermore, we demonstrate that dendritic cells can induce the development of Th1 cells from Ag-specific naive LECAM-1^{bright} CD4$^+$ T cells obtained from αβ-TCR transgenic mice, provided that CD4$^+$ LECAM-1^{dull} T cells, which produce significant levels of IL-4, are not present in the primary cultures. Production of IL-12 by dendritic cells was confirmed by positive immunofluoresence staining with Abs specific for the inducible IL-12 p40 subunit. This suggests that in addition to inducing proliferation and clonal expansion of naive T cells, dendritic cells, by their production of IL-12, play a direct role in the development of IFN-γ-producing cells that are important for cell-mediated immune responses. *The Journal of Immunology,* 1995, 154: 5071–5079.

Dendritic cells have a highly developed function in the immune system as specialized APCs for the initiation of T cell-dependent immune responses (1–3). Their strategic positioning in nonlymphoid tissues (e.g., Langerhans cells of the skin and interstitial dendritic cells) and their ability to circulate via blood and lymph (4–9) to lymphoid organs as the interdigitating dendritic cells of T cell areas demonstrate their important role in the initiation of immune responses against invading pathogens. The ability of dendritic cells to act as potent APCs for induction of T cell responses to a variety of Ags both in vitro (1, 2, 9, 10) and in vivo (11–13) may be greatly attributable to their ability to express high levels of accessory and/or costimulatory molecules (2, 14, 15). Dendritic cells have been shown to induce several cytokines during the MLR (16–20), although it is now likely that apart from IL-2 most of these cytokines may be produced by "memory" T cells (21). However, the ability of dendritic cells to direct the development of discrete Th cell subsets from naive CD4$^+$ T cells in the absence of exogenously added cytokines is still unclear (22–24).

Diseases resulting from infection with certain organisms (e.g., *Leishmania major*, *Myobacterium leprae*, and *Schistosoma mansoni*) may be resolved according to the type of Th cell response mounted (25). Many studies have indicated that the selective induction of CD4$^+$ T cells with distinct lymphokine profiles (26) may be dictated by the dose and type of Ag as well as by the route of immunization (27–30). These factors may favor the involvement of a particular APC as well as the production of particular cytokines by either accessory cells/APCs or T cells in the microenvironment. The role of soluble factors in inducing the development of particular Th cell subsets is well documented (31, 32), with dominant cytokines such as IL-4 (23, 33–35) and IL-12 (36–38) directing Th2 and Th1 responses, respectively.

Antigen-presenting cells, such as macrophages, have been shown to direct Th1 development by secretion of IL-12 (24, 36). This cytokine is heterodimeric and composed of two distinct but unrelated gene products. The

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40-kDa (p40) and 35-kDa (p35) glycosylated protein subunits must be assembled for biologically active IL-12 to be secreted (39–41). IL-12 augments NK cell- and T cell-mediated cytotoxicity and stimulates IFN-γ production and proliferation of NK cells and T cells (39, 42–45) alone or in synergy with IL-2 (42). Although IL-12 is clearly produced by monocytes and macrophages upon activation (46–48), and the cDNA encoding human IL-12 was originally isolated from a B lymphoma (41, 49), it is not clear at this stage what other normal cell types produce this cytokine. Attempts to identify cytokines produced by dendritic cells have been negative to date (22), with the exception of reports of IL-1 and macrophage inflammatory protein 1α in freshly isolated epidermal Langerhans cells (50–53). The capacity of dendritic cells to produce cytokines such as IL-1α and TNF-α is controversial, and this may reflect the maturational state and activation signals used in different systems (50–53).

We have recently shown that dendritic cells induce strong Ag-specific proliferation of $CD4^+$ T cells from mice expressing an OVA-specific transgenic TCR, but addition of *Listeria*-activated macrophages producing significant levels of IL-12 was required for Th1 development (24, 36). However, we now show that dendritic cells can themselves drive Th1 development by their production of IL-12 upon removal of endogenous IL-4.

Materials and Methods

Animals

Mice transgenic for the D011.10 TCR-$\alpha\beta$ on a BALB/c genetic background (54) were identified at age 4 to 6 wk by staining PBL with the anti-TCR clonotype-specific mAb KJ1–26 (55). All transgenic mice used were heterozygous for the TCR α and β transgenes. Female nontransgenic BALB/c mice between 6 and 10 wk old were purchased from Simonsen Laboratories (Gilroy, CA).

Culture medium, cytokines, Abs, and antigens

RPMI 1640 (JR Scientific Inc., Woodland, CA) supplemented with 10% FCS (JR Scientific), 0.05 mM 2-ME (Sigma Chemical Co., St. Louis, MO), 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES buffer, and 1 mM sodium pyruvate was used as culture medium (cRPMI). For dendritic cell isolations, RPMI 1640 Dutch modification medium (Life Technologies Ltd., Paisley, Scotland) was used with glutamine, FCS, 2-ME, penicillin, streptomycin, and sodium pyruvate as described above.

Recombinant mouse cytokines were as follows: IFN-γ (Schering Research, Bloomfield, NJ), IL-4 (Dr. S. Menon, DNAX), TNF-α (Genzyme Corp., Cambridge, MA), and IL-1α (a kind gift from Dr. P. Lomedico, Roche, Nutley, NJ). Recombinant mouse IL-12 was obtained by transfecting COS7 cells with the cDNA encoding p35 and p40, as described previously (56), which was obtained by PCR cloning using published sequences (56). Supernantants from mock-transfected cells were used as a control and showed no effects in the concentration range of IL-12 used. The IL-12 content of the supernatants ranged from 2000 to 3000 U/ml (48).

Purified 11B11 rat anti-mouse IL-4 Ab, previously described (57), and an isotype-matched control were supplied by Dr. J. Abrams (DNAX) (58). IL-12-specific mAbs C15.6.7 and C15.1.2 were as described (59). MAbs used for flow cytometric sorting or analysis included: biotinylated anti-mouse Mac-1 (M1/70; Caltag, San Francisco, CA), biotinylated hamster anti-mouse N418 (hybridoma from American Type Culture Collection, Rockville, MD) (60), biotinylated anti-mouse CD8α, B220 (RA3-6B2), and IA^d (all from PharMingen, San Diego, CA), and anti-mouse CD4-phycoerythrin and lymphocyte endothelial cell adhesion molecule-1 (LECAM-1)[3]-FITC (both from PharMingen). Biotinylated NLDC-145 was kindly provided by Dr. G. Kraal, Free University, Amsterdam, The Netherlands (61). Additional mAbs for ELISA, including anti-mouse IL-4 and IFN-γ reagents were purified from serum-free hybridoma supernatants as previously decribed (24, 58).

The antigenic OVA peptide from chicken OVA ($OVA_{323-339}$) was synthesized on an Applied Biosystems Model 430 peptide synthesizer (Biosynthesis, Inc., Lewisville, TX).

Preparation of T cells and APC

$CD4^+$ T cells were enriched by negative selection using magnetic activated cell sorting with a mixture of biotinylated anti-mouse CD8α, IA^d, B220, and Mac-1 Abs, as previously described (24) (Miltenyi, Sunnyvale, CA). T cells staining positive for CD4 were further purified by positive selection using a $FACStar^{Plus}$ flow cytometer (Becton Dickinson, Mountain View, CA) to achieve 99.8% $CD4^+$ T cells. Staining did not alter the function of the T cells (not shown). In some experiments, $CD4^+$ T cells were further subdivided on the basis of LECAM-1 (Mel-14) expression (21).

Dendritic cells were enriched from normal BALB/c spleen cell preparations, as described previously (24). by first removing adherent cells by overnight culture in plastic flasks, followed by overlayering onto 2-ml metrizamide gradients (analytical grade, 13.7%; Nycomed Pharma AS, Oslo, Norway) and centrifugation for 10 min at 600 $\times$ g to give a population enriched for dendritic cells in the low density fraction. Enriched dendritic cells ($N418^{high}Mac\text{-}1^{low}$) were then further purified to homogeneity by FACS, as previously described (62).

Stimulation of transgenic $CD4^+$ T cells for cytokine production

Primary stimulations of $CD4^+$ T cells (2.5×10^5/well) were conducted using OVA peptide (0.3 μM) and dendritic cells (10^5 or 10^4/well, 1500 rad) or RBC-lysed spleen cells (5×10^6/well, 3000 rad) as APCs in a total volume of 2 ml in 24-well plates. In addition, some cultures received cytokines (IL-4 (200U/ml) or IL-12 (1:80 dilution of COS supernatant)) or mAbs to block endogenous cytokines, (anti-IL-4 (10 μg/ml), anti-IFN-γ (10 μg/ml), anti-IL-12 (100 μg/ml)). Supernatants were collected at 48 h. T cells were expanded threefold into fresh medium, containing the same additives at 72 h. Cells were harvested on day 6, washed three times, counted, and restimulated with fresh BALB/c APC (splenocytes) and 0.3 μM OVA peptide at the cell concentrations described for primary cultures. Supernatants were collected at 48 h, and in some cases 72 h, for measurement of IL-4 and IFN-γ.

Cytokine assays

IFN-γ was detected using a two-site sandwich ELISA (63), which has a lower limit of sensitivity of 125 pg/ml (1 U/ml = 0.1 ng/ml). The ELISA for IL-4 has been described previously (58) with a lower limit of sensitivity of 150 pg/ml.

Detection of cytokines by immunofluorescence

Cells were fixed and stained by the paraformaldehyde-saponin procedure as described previously (64) and modified (65). Briefly, the fixation and staining was performed on Bio-Rad adhesion slides (Bio-Rad Laboratories, GMBH, Munich, Germany). Before staining, the slides were washed once using HBSS–0.1% saponin (Sigma). For staining, 10 μl of rat anti-mouse IL-12 p40 Ab (C15.67.6) (59) at 2.5 μg/ml diluted in HBSS–saponin was added, and the slides were incubated for 30 min. After one wash in HBSS–saponin, 10 μl of FITC-labeled rabbit anti-rat IgG (Vector, Burlingame, CA) diluted 1:100 in HBSS–saponin was added and the slides were incubated for 30 min. After cells were washed 3 times in HBSS (no saponin), a biotinylated anti-mouse dendritic cell Ab (NLDC-145) (61) was added at 5 μg/ml and the slides were incubated for 30 min in HBSS. The slides were then washed 3 times in HBSS, and rhodamine-labeled avidin (Vector) at 1:200 was added for 30 min. Slides

[3] Abbreviations used in this paper: LECAM-1, lymphocyte endothelial cell adhesion molecule-1; OVA, OVA-peptide Ag.

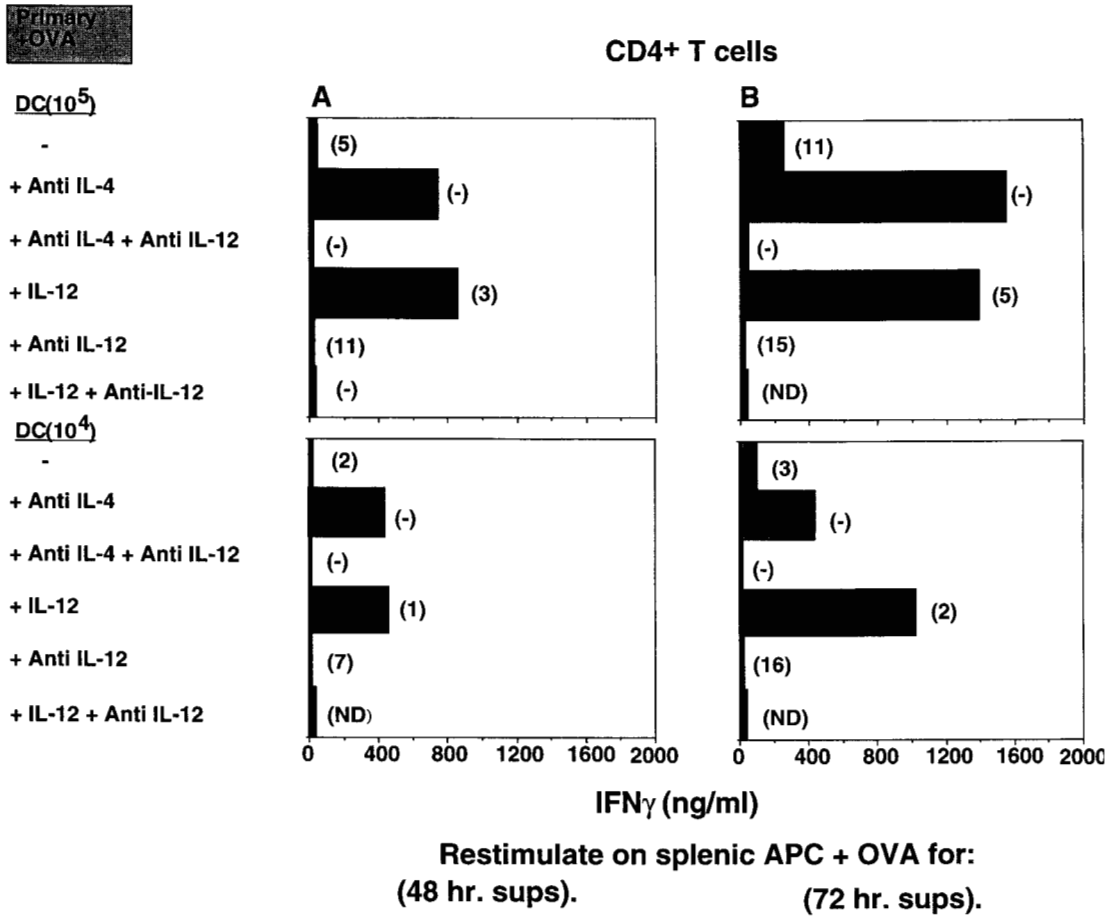

FIGURE 1. Development of Th1 cells resulting from neutralization of endogenous IL-4 is IL-12-dependent. FACS-purified TCR-transgenic CD4+ T cells (2.5×10^5/well) were cultured with FACS-purified dendritic cells (10^5 or 10^4/well) prepared from normal mice and OVA peptide (0.3 μM) in 2-ml volumes in 24-well plates. During primary stimulation, the cultures contained IL-12 at 100 U/ml, anti-IL-4 at 10 mg/ml, or medium alone either separately or together with anti-IL-12 mAbs (100 μg/ml; C15.6.7 and C15.1.2). After 2 days, supernatants were removed to analyze cytokine production in primary cultures. After 3 days, the cultures were split into three in complete medium containing the additives indicated. After 7 days, the cells from primary cultures were harvested, washed, and restimulated (2.5×10^5 T cells) with fresh splenocytes and OVA peptide, without additives, and supernatants were harvested and tested for cytokines at 48 h (*A*) and 72 h (*B*). IFN-γ levels are shown in the histogram; IL-4 levels (in ng/ml) are shown in parentheses. This figure shows a representative experiment from five experiments conducted.

were washed again and mounted with buffered glycerol as previously described (64). All experiments included isotype-matched controls for both intracellular and extracellular proteins.

Results

Dendritic cell-driven Th1 development resulting from neutralization of endogenous IL-4 is IL-12-dependent

We have previously demonstrated that dendritic cells, potent APCs for induction of proliferation and clonal expansion of OVA-specific $CD4^+$ T cells from unimmunized mice, did not induce development of either a polarized Th1 or Th2 phenotype (24). Addition of recombinant IL-12, or appropriately activated macrophages producing high levels of IL-12, was required for the development of Th1 cells (24). These data suggested that dendritic cells, although capable of expressing high levels of membrane-bound accessory or costimulatory molecules, did not produce cytokines necessary for induction of discrete Th subsets from naive T cells. We now present data showing that dendritic cells can drive the development of IFN-γ-producing Th1 cells from $CD4^+$ T cells in the absence of IL-4, strongly suggesting that they can indeed produce IL-12. Primarily, dendritic cells induced the development of Th1 cells that produce high levels of IFN-γ and undetectable IL-4 from total FACS-purified, OVA-specific $CD4^+$ T cells upon neutralization of endogenous levels of IL-4 (Fig. 1). As shown in Figure 1, we demonstrate that the development of a Th1 phenotype resulting from the addition of anti-IL-4 mAb is significantly inhibited by anti-IL-12 mAbs added during primary T cell activation with

Ag and dendritic cells as APCs. Addition of 100 U/ml of exogenous IL-12 during primary T cell activation led to the development of T cells that produce high levels of IFN-γ upon restimulation, although in this case IL-4 was also detectable, probably resulting from endogenous IL-4 in primary cultures. To control for the specificity of the anti-IL-12 mAb, we show that recombinant IL-12-mediated development of T cells producing high levels of IFN-γ was specifically inhibited by anti-IL-12 mAb (Fig. 1), whereas an isotype-matched control mAb showed no effect (not shown). Addition of anti-IL-12 alone to primary cultures of $CD4^+$ T cells stimulated with Ag presented by dendritic cells caused a modest increase in IL-4 production by T cells upon restimulation. Thus, whereas IL-12 probably plays an important role in stimulating the production of high levels of IFN-γ, it probably plays a minor role in suppression of the IL-4 response to achieve homeostasis. These data, demonstrate that endogenous and biologically active levels of IL-12 are present in supernatants of cultures containing FACS-purified dendritic cells, $CD4^+$ T cells, and Ag, and strongly suggest that the dendritic cells are producing IL-12.

Dendritic cells drive Th1 development from $CD4^+$, LECAM-1^{bright} T cells via an IL-12-dependent mechanism in the absence of T cells producing significant levels of IL-4 in primary cultures

The ability of dendritic cells to induce IL-12-dependent Th1 development upon addition of neutralizing anti-IL-4 Abs demonstrated the presence of endogenous IL-4 in primary $CD4^+$ T cell cultures. A possible source of this IL-4 is the LECAM-1^{dull} subset of $CD4^+$ T cells which contains the majority of the "memory/activated" $CD4^+$ T cells and has been shown to secrete significant levels of IL-4 upon primary stimulation in vitro, in contrast to the "naive" LECAM-1^{bright} subset (21, 66). Surprisingly, although the $CD4^+$ T cells were obtained from unimmunized mice bearing a TCR transgene specific for a peptide of OVA, they contained between 3 and 10% $CD4^+$ T cells that expressed low to undetectable levels of LECAM-1 (Fig. 2). Furthermore, although a proportion of these $CD4^+$ LECAM-1^{dull} T cells only expressed an endogenous TCR, a significant percentage also stained positive for the clonotype TCR transgene, either expressing only the transgenic TCR or the transgenic TCR and an endogenous TCR, as described by Padovan et al. in normal human T cells and by others in TCR-transgenic mice (67–69).

In keeping with reported findings in normal mice (21, 66), upon primary stimulation of OVA-specific TCR-transgenic $CD4^+$ T cells with their specific Ag presented by dendritic cells, the purified $CD4^+$ LECAM-1^{dull} population produced 2 ng/ml of IL-4, whereas no IL-4 was detectable (i.e., <200 pg/ml) in supernatants from the LECAM-1^{bright} population. This supported the suggestion that the source of IL-4 in primary cultures of total $CD4^+$ populations, which inhibited the development of Th1 cells, was this population of LECAM-1^{dull} $CD4^+$ T cells. Indeed, upon removal of this IL-4-producing LECAM-1^{dull} population of $CD4^+$ T cells using flow cytometry, purified $CD4^+$ LECAM-1^{bright} T cells, in contrast to total $CD4^+$ T cells, developed into Th1 cells following priming with dendritic cells and OVA alone, without the addition of rIL-12 or neutralizing Abs directed against IL-4 (Fig. 2). Activated B cells showed only the ability to drive the development of a modest Th1 phenotype (<50 ng/ml IFN-γ per culture) and this development is not inhibited by anti-IL-12 Abs (N. Hosken and A. O'Garra, unpublished observations). The levels of IFN-γ produced by the resulting T cell population varied and could often be enhanced by the addition of rIL-12 or anti-IL-4 Abs to the priming cultures (Fig. 2), which may reflect variation in the levels of these endogenous cytokines. Indeed, the most polarized Th1 population, producing high levels of IFN-γ and undetectable IL-4, developed upon neutralization of low levels of endogenous IL-4. Development of T cell populations producing the greatest levels of IFN-γ was observed at higher doses of dendritic cells and was most evident when supernatants were obtained 3 days, rather than 2 days, after restimulation. This could reflect either lower frequencies of cells within the population that have committed to the Th1 phenotype or committed Th1 cells that are at different stages of differentiation or activation. Addition of Abs directed against IL-12 to primary cultures of $CD4^+$ LECAM-1^{bright} T cells stimulated by dendritic cells and OVA specifically inhibited their development into Th1 cells that produce high levels of IFN-γ.

These data corroborated the preceding findings, suggesting that dendritic cells can produce IL-12 and drive Th1 development from "naive" $CD4^+$ LECAM^{bright} T cells when $CD4^+$ LECAM-1^{dull} T cells, which produce significant levels of IL-4 during primary stimulations in vitro, are absent from priming cultures. The reproducible inhibition of IL-12-dependent dendritic cell-driven Th1 development in total $CD4^+$ populations is thus most undoubtedly a result of significant levels of IL-4 produced by the LECAM-1^{dull} $CD4^+$ T cells. Because adding exogenous IL-12 (100 U/ml), or *Listeria*-activated macrophages producing IL-12 (not shown), to cultures of total $CD4^+$ T cells containing the $CD4^+$ LECAM-1^{dull} population can drive the development of T cells producing high levels of IFN-γ (Fig. 1, *A* and *B*, *left panel*), it is possible that dendritic cells may produce levels of IL-12 insufficient to counteract the inhibition of Th1 development by endogenous IL-4 produced by the $CD4^+$ LECAM-1^{dull} cells. OVA-specific T cells producing high levels of IFN-γ developed from $CD4^+$ LECAM-1^{dull} T cells themselves only in the presence of a neutralizing IL-4-specific mAb or added rIL-12 (Fig. 2*B*), and IL-4 was often also present in these supernatants. Thus, the relative levels of T cell-derived IL-4 vs APC (macrophage or dendritic cell)-derived

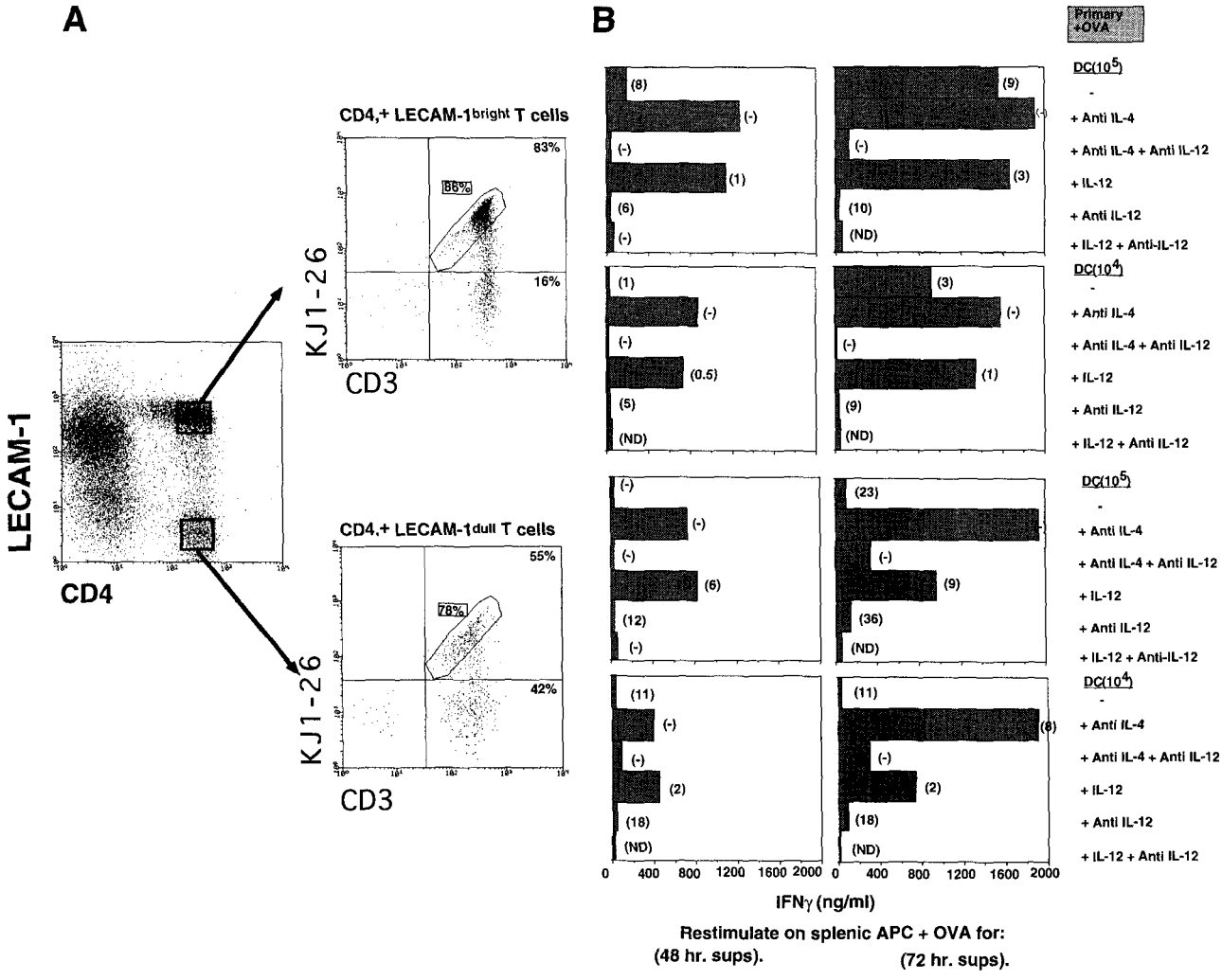

FIGURE 2. Dendritic cells drive IL-12-dependent Th1 development from "naive" $CD4^+$ LECAM-1^{bright} T cells in the absence of IL-4-producing $CD4^+$ LECAM-1^{dull} T cells. (A) Flow cytometric analysis of $CD4^+$ LECAM-1^{bright} and $CD4^+$ LECAM-1^{dull} T cell populations in OVA-specific $\alpha\beta$-TCR transgenic mice. Spleen cell suspensions from OVA-specific TCR-transgenic mice were stained with CD4–Tricolor, LECAM-1-phycoerythrin, KJ1–26–biotin/avidin–Texas red, and CD3–FITC. Cells were gated on the basis of staining LECAM-1^{bright} $CD4^+$ or LECAM-1^{dull} $CD4^+$ (as indicated in *B*), and the gated populations were then assessed for staining with KJ1–26 (transgenic TCR clonotype) vs CD3 (*A*). (*B*) Flow cytometry-sorted TCR-transgenic subpopulations of $CD4^+$ T cells (*top*, $CD4^+$, LECAM-1^{bright}; *bottom*, $CD4^+$, LECAM-1^{dull}; 2.5×10^5/well) were cultured with flow cytometry-sorted dendritic cells (10^5 or 10^4/well) prepared from normal mice and OVA peptide (0.3 μM) in 2-ml volumes in 24-well plates. Experiments were conducted as described in Figure 1. This figure shows a representative experiment from five experiments conducted.

IL-12 strictly control whether Th1 cells responsible for cell-mediated immunity develop in response to Ag.

Dendritic cells produce IL-12 p40 subunit

Our findings that dendritic cells induce IL-12-dependent Th1 development from LECAM-1^{bright} $CD4^+$ T cells strongly suggested that dendritic cells may produce IL-12. To examine this and to exclude the possibility that IL-12 production was stimulated by a small number of contaminating cells, enriched populations of dendritic cells were examined for IL-12 p40 subunit (56) expression by immunostaining with FITC-labeled Abs directed against IL-12 p40 (Fig. 3*A*, green). Cells were simultaneously counter-stained with the dendritic cell-specific Ab NLDC-145 (61) conjugated to biotin and developed with streptavidin–rhodamine (Fig. 3*C*, red). Double-stained dendritic cells stained with anti-IL-12–FITC and NLDC-145–rhodamine were yellow (Fig. 3*E*) and this represented most of the dendritic cells. The enriched dendritic cell preparation also contained infrequent IL-12-expressing cells (Fig. 3*E*, green) which did not stain positive for the dendritic cell marker. The fields *B*, *D*, and *F*, in Figure 3 show isotype-matched controls for either intracellular (IL-12) or extracellular (NLDC-145) proteins, or both proteins together,

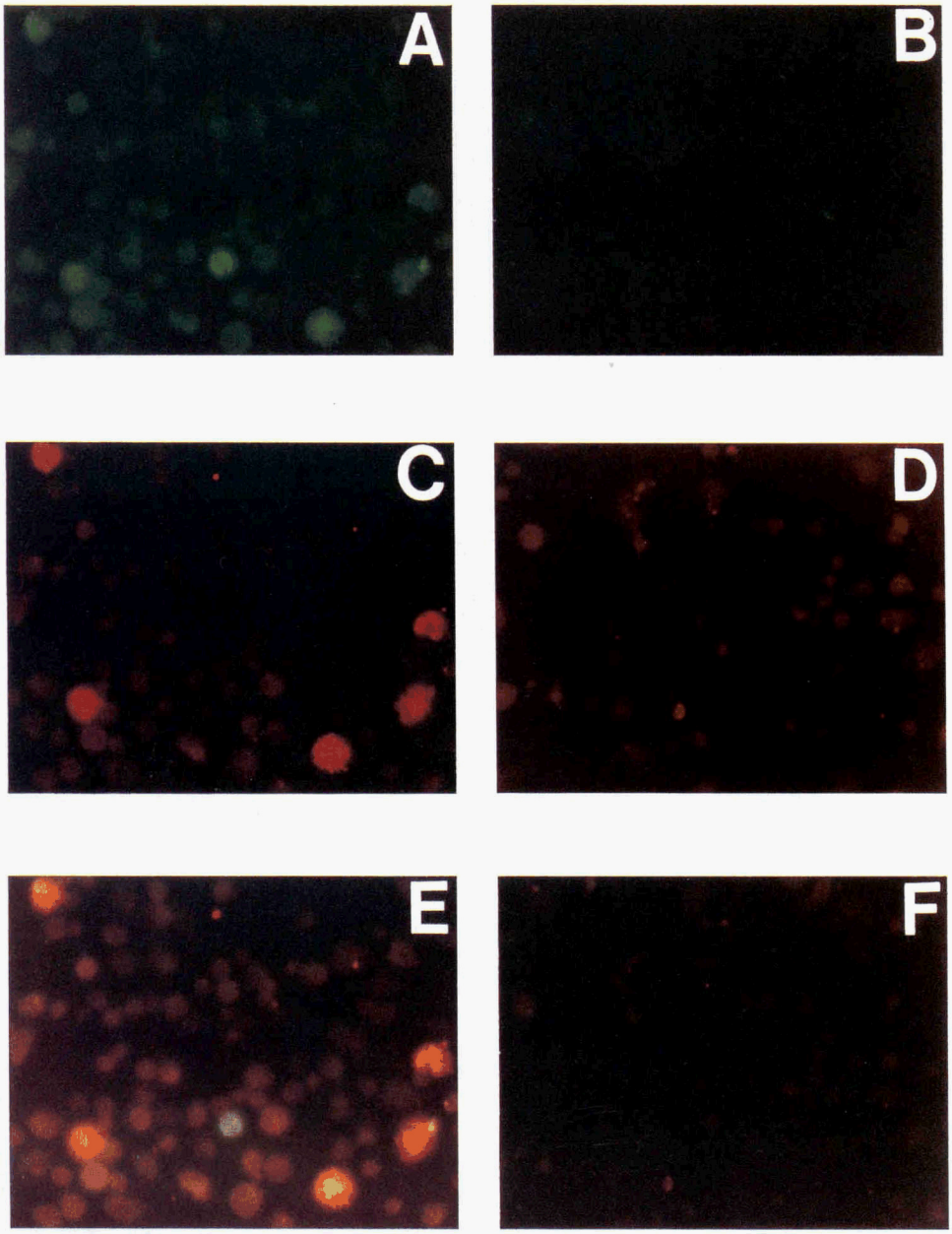

FIGURE 3. Dendritic cells produce IL-12 p40 protein. Dendritic cells were stained, as described in *Materials and Methods*, with FITC-labeled anti-IL-12 p40 Abs (green, *top left*) and then surface-stained with a dendritic cell-specific biotin/streptavidin–rhodamine-conjugated mAb, NLDC-145 (red, *middle left*). When FITC and rhodamine are combined and double exposed they reflect a yellow color (*bottom left*). *A*, *C*, and *E* show the appropriate isotype-matched controls.

respectively. To enhance IL-12 production, dendritic cells were first cultured with TCR-transgenic $CD4^+$ T cells plus (Fig. 3) or minus (not shown) OVA for 6 to 20 h, as this incubation with Ag and T cells appeared to increase the expression of IL-12 by dendritic cells. This would support findings by Germann et al. (70), who show that IL-12 expression by granulocyte-macrophage CSF-derived bone marrow macrophages is induced by culturing with syngeneic Th1 cells plus Ag. The greatest level of staining for IL-12 p40 was observed at approximately 20 h after initiation of the cultures (Fig. 3), and was localized to the Golgi apparatus in dendritic cells staining positive for NLDC-145.

Discussion

We demonstrate in this study that dendritic cells can produce IL-12, a dominant cytokine involved in the development of IFN-γ-producing T cells. This finding resulted from our observations that dendritic cell-induced Th1 development from total $CD4^+$ T cells, upon neutralization of

endogenous levels of IL-4 was IL-12-dependent. Furthermore, dendritic cells induced the development of Th1 cells from Ag-specific naive LECAM-1^{bright} CD4$^+$ T cells obtained from $\alpha\beta$-TCR transgenic mice, provided that CD4$^+$ LECAM-1^{dull} T cells which produce significant levels of IL-4 were not present in the primary cultures. Production of IL-12 by dendritic cells was confirmed by positive immunofluorescence staining with Abs specific for the inducible IL-12 p40 subunit.

These data suggest that in addition to inducing strong proliferation and clonal expansion of naive T cells, dendritic cells, by their production of IL-12, may play a role in the development of IFN-γ-producing cells, which are important for cell-mediated immune responses. This underscores the importance of soluble as well as membrane-bound signals delivered between T cells and APC/accessory cells in the initiation of an immune response. The strict regulation of dendritic cell-induced Th1 development by IL-4-producing LECAM-1^{dull} CD4$^+$ T cells is probably critical for achieving the appropriate immune response from rapidly expanding as well as differentiating T cells with minimum immunopathology (71).

Many previous attempts to identify cytokines produced by dendritic cells have been negative (22), and the capacity of dendritic cells to produce cytokines such as IL-1α and TNF-α is controversial. Our data regarding the production of IL-12 by dendritic cells suggest that the interdigitating dendritic cells in the spleen may produce only low levels of IL-12, as they cannot drive the development of OVA-specific Th1 cells from total populations of CD4$^+$ T cells unless significant amounts of exogenous IL-12 are added to the system to overcome the inhibitory effects of IL-4 or IL-4-producing T cells are removed from primary cultures. This differential production of cytokines by dendritic cells may reflect the maturational state and activation signals used in different systems (50–53), which would be in keeping with a recent report that high levels of IL-12 are produced by human dendritic cells after certain conditions of activation (G. Schuler, personal communication). However, low levels of IL-12 produced by dendritic cells during cognate MHC-restricted presentation of Ag to naive CD4$^+$ T cells may be an efficient mechanism for influencing an immune response toward cell-mediated immunity by production of high levels of IFN-γ by T cells.

The potent capacity of CD4$^+$ LECAM-1^{dull} T cells containing OVA-specific TCR-transgene bearing cells, to inhibit IL-12-dependent dendritic cell-driven Th1 development, prompts speculation on their origin and as to whether they are indeed "memory/activated" cells (21). The ratio of clonotype positive cells (KJ1–26$^+$) having only one transgenic TCR, vs those expressing a transgenic TCR plus a second endogenous TCR, is very similar in the CD4$^+$ LECAM-1^{bright} and LECAM-1^{dull} populations, suggesting that neither population was enriched in the CD4$^+$ LECAM-1^{dull} subset. This suggested that if the loss of expression of LECAM-1 resulted from antigenic stimulation it was via the transgenic TCR. In contrast, there was a reproducible 2.5-fold increase in the clonotype negative CD4$^+$ LECAM-1^{dull} population, which is often higher in older mice, in keeping with a memory/activated phenotype that results from stimulation by environmental Ags. Loss of expression of LECAM-1 by the CD4$^+$, KJ1–26$^+$ T cells may have resulted from stimulation of the transgenic TCR in vivo by a cross-reactive Ag or a superantigen provided by a foreign organism. Alternatively, these CD4$^+$ LECAM-1^{dull} cells might represent a separate lineage, programmed to differentiate along this pathway in the absence of Ag exposure, as previously documented (72–75). That the CD4$^+$ LECAM-1^{dull} T cells may represent a distinct postively selected lineage may be suggested by their requirement for OVA-specific stimulation, although-IL-4 producing cells within this population may be activated via a bystander mechanism. Regardless of the evolution of the LECAM-1^{dull} CD4$^+$ T cells producing significant levels of IL-4 during primary in vitro stimulation, it is clear that they mediate an important role in regulating Th1 cell development and cell-mediated immunity.

In summary, we show that dendritic cells produce the cytokine IL-12 and can drive Th1 development in the absence of IL-4. These data underscore the importance of soluble as well as membrane-bound signals delivered between T cells and dendritic cells in the initiation of an immune response. It is likely that in most infectious diseases, a balanced immune response is required in which cell-mediated immune mechanisms and humoral responses cooperate to eradicate the pathogen. Thus, it is logical that the ability of interdigitating dendritic cells to drive Th1 phenotype development by their production of IL-12, as well as massive clonal expansion, should be under strict regulatory control. This tight regulation of dendritic cell IL-12-induced Th1 development by IL-4-producing T cells is probably critical for achieving the appropriate immune response from rapidly expanding as well as differentiating T cells with minimum immunopathology.

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