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T Cell-Dependent Activation of Dendritic Cells Requires IL-12 and IFN-γ Signaling in T Cells

Francesca Miro,* Cinzia Nobile,* Nicolas Blanchard,* Marianne Lind,* Orchidée Filipe-Santos,† Claire Fieschi,‡ Ariane Chapgier,‡ Guillaume Vogt,† Ludovic de Beaucoudrey,† Dinakantha S. Kumararatne,‡ Françoise Le Deist,‡ Jean-Laurent Casanova,† Sebastian Amigorena,* and Claire Hivroz‡*

Patients presenting with genetic deficiencies in IFNGR1, IFNGR2, IL-12B, and IL-12RB1 display increased susceptibility to mycobacterial infections. We analyzed in this group of patients the cross-talk between human CD4+ T lymphocytes and dendritic cells (DCs) that leads to maturation of DC into producers of bioactive IL-12 and to activation of T cells into IFN-γ producers. We found that this cross-talk is defective in all patients from this group. Unraveling the mechanisms underlying this deficiency, we showed that IL-12 signaling in T cells is required to induce expression of costimulatory molecules and secretion of IL-12 by DCs and that IFNGR expression is required on both DCs and CD4+ T cells to induce IL-12 secretion by DCs. These data suggest that CD4+ T cell-mediated activation of DCs plays a critical role in the defense against mycobacterial infections in humans. The Journal of Immunology, 2006, 177: 3625–3634.

Humans with defective response to IFN-γ or IL-12 share a common vulnerability to infections due to nontuberculous mycobacteria or vaccine-associated bacille Calmette-Guérin (BCG) and to a lesser degree to *Salmonella* and some intracellular bacteria (1). They also display modest vulnerability to ~20% of common viruses (2, 3). This susceptibility to mycobacteria, BCG, and other intracellular opportunistic pathogens is shared by another group of patients presenting with mutations in CD40L, who were first described for their hyper-IgM syndrome (4–6) and have been shown to develop localized disease due to BCG and severe tuberculosis (6, 7).

CD40L/CD40 interactions, IFN-γ, and IL-12 are all major players of the cross-talk between dendritic cells (DCs) and Th cells, cross-talk that regulates the Ag-presenting functions of DCs and influences the polarization of Th1 responses and priming of CTL (8, 9) (reviewed in Ref. 10). Indeed, although the process of DC maturation, which is required for naive T cell priming (11), is initially triggered by microbial products through TLR (12), interactions of maturing DCs with various lymphocyte populations orientate the priming capacity of mature DCs. CD4+ Th lymphocytes have been shown to license or educate DCs to prime CTLs or to orientate CD4+ T cell priming toward Th1 or Th2 responses (reviewed in Refs. 13, 14).

Mouse studies have shown that CD4+ T cells can be replaced by agonistic anti-CD40 Abs for the induction of CD8+ T cell priming (15–17), suggesting a major role for CD40-CD40L interactions in the induction of full DC maturation. In vitro, CD40-deficient DCs are partially defective for CD8+ T cell priming, suggesting a major, but not exclusive, role for CD40-CD40L interactions in DC licensing (18). In human models, anti-CD40 Abs, soluble trimeric CD40L, or CD40L-transfected cell lines have been shown to induce expression of costimulatory molecules (19, 20) and secretion of bioactive IL-12 by DCs (21, 22). Moreover, it has been shown that effective human CTL priming in vitro requires the presence of Ag-specific CD4+ T cells and TNF-α-activated DCs (23). Thus, although the Ag-specific encounter between CD4+ T lymphocytes and immature or maturing DCs is generally recognized as a major step in the development of an adaptive immune response, little is known about the molecular players involved at this level.

The present study was designed to determine: 1) whether the cross-talk between CD4+ T cells and DCs from patients presenting with mutations in CD40L, IL-12B, IL-12RB1, IFNGR1, and IFNGR2 was efficient; and 2) the relative contribution of T cell and DC responses to IL-12 and IFN-γ in this cross-talk.

**Materials and Methods**

**Medium and reagents**

Medium used was as follows: RPMI 1640 Glutamax, 1% pyruvate, 5 × 10−3 M 2–2-ME, 100 U/ml penicillin, 100 μg/ml streptomycin (Invitrogen Life Technologies), and 10% FCS (Biowest). Human rIL-4 and GM-CSF were purchased from BRUCELLS; IFN-γ from Roussel; IL-12p70, IL-23, TNF-α, and anti-IL-12 from R&D Systems; and anti-IFN-γ from BD Pharmingen. The agonist anti-human CD40 mAb (clone G28-5) was a gift from Y. Richard (Institut Paris-Sud sur les Cytokines, Clamart, France), Recombinant bacterial superantigen, toxic shock syndrome toxin 1 (TSST1), was purchased from Toxin Technology, and LPS and brefeldin A were obtained from Sigma-Aldrich.

**Patients**

PBMC were obtained from two unrelated patients presenting with CD40L mutations, resulting in a complete defect in CD40L expression (6), one...
patient with recessive complete IFNGRI deficiency described previously (1) and one patient with a homozygous mutation of the IFNGR2-encoding gene (24), resulting in both cases in complete deficiencies in IFN-γ response, one patient with a homozygous deletion in the IL-12p40-encoding gene (25) and three unrelated patients presenting with mutations in the gene encoding IL-12β1 (26). This study has been approved by the Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale of Necker Hospital.

**DC preparation**

Anti-CD14-conjugated magnetic microbeads (Miltenyi Biotec) were used to purify monocytes from controls’ or patients’ PBMCs. DCs were generated, as described (27), by culturing monocytes in medium supplemented with 100 ng/ml GM-CSF and 40 ng/ml IL-4 for 5 days. Populations of immature DCs obtained were 100% CD1a⁺/CD14⁺.

**Sorting of CD4⁺ T cells and purification of CD45RA⁺ and CD45RO⁺ CD4⁺ T cells**

After depletion of CD14⁺ cells (see above), the CD4⁺ T cell isolation kit II from Miltenyi Biotec was used to negatively select CD4⁺ T cells. Sorted CD4⁺ T cells were 97–99% CD4⁺/CD3⁺. Isolation of CD45RO⁺ memory or CD45RA⁺ naive CD4⁺ T cells was performed by incubation of CD4⁺ T cells with anti-CD45RA (Alb11; Beckman Coulter) or anti-CD45RO mAbs (UCHL1; a gift from P. Beverley, Edward Jenner Institute for Vaccine Research, Compton, U.K.), respectively, and depletion with anti-mouse IgG magnetic beads (Dynal Biotec; Invitrogen Life Technologies). The memory CD45RA⁺ CD4⁺ and naive CD45RO⁺ CD4⁺ T cell populations obtained were 95–98% CD45RO⁺ and CD45RA⁺, respectively.

**In vitro DC activation assay**

Cocultures of immature DCs and CD4⁺ T cells (5 × 10⁴ DCs and equal number of T cells, unless otherwise stated) were performed in flat-bottom 96-well plates. In experiments addressing trans activation, 2 × 10⁶ monocyte-derived DCs were cocultured with 2 × 10⁵ T cells in 24-well plates containing cell culture inserts with a permeable membrane (0.4-μm pore size, Transwell from BD Biosciences); several combinations of cells were used in the upper and lower well. Twenty-four hours later, cytokine production in supernatants and expression of maturation markers by DCs and CD4⁺ T cells were analyzed.

**Cytokine detection**

Cytokine production was measured in the supernatants by ELISA using matched paired Abs specific for IL-12p70 (DuoSet; R&D Systems), IL-2, or IFN-γ (OptiEA; BD Biosciences). In some experiments, the cytometric bead array human inflammation kit (BD Biosciences) was used to measure inflammatory production.

**FACS analysis**

The following murine mAbs, anti-CD1a FITC, anti-CD14 PE, anti-CD86 FITC, anti-HLA-DR FITC, anti-CD80 PE, anti-CD83 PE, anti-CD40 PE, anti-CD4 PE, and anti-CD69 allophycocyanin, and IgG1 PE, IgG1 allophycocyanin, and IgG2a FITC isotypic controls were purchased from BD Pharmingen. Anti-TCRVβ2 FITC was from Beckman Coulter. Samples were analyzed on a FACSCalibur using the CellQuest software (BD Biosciences). Intracellular production of IFN-γ was measured by FACS. Brefeldin A (5 μg/ml) was added during the last 3 h of cocultures. Cells were then labeled with anti-CD4 mAbs coupled to FITC, fixed with 3% paraformaldehyde, and permeabilized with the Cytoperm/Wash kit from BD Biosciences before labeling with anti-IFN-γ mAb coupled to PE (Beckman Coulter).

**Immunolabeling and fluorescence microscopy**

After cocultures, cells were settled in RPMI 1640 onto polylysine-coated coverslips for 15 min. After one PBS wash, cells were fixed with 3% paraformaldehyde (Carlo Erba) for 20 min and incubated for 10 min in 10 mM PBS glycine to quench free aldehyde groups. Cells were then permeabilized and labeled for 1 h by incubation with anti-IL-12p70 and anti-IFN-γ Abs diluted in PBS, 0.2% BSA (Sigma-Aldrich), 0.05% saponin (ICN Biomedicals), and secondary Abs labeled with Alexa 647-conjugated F(ab’), anti-species-specific Abs from Molecular Probes diluted in the same buffer. Cells were then labeled with either anti-CD1a FITC (BD Biosciences) or anti-TCRVβ2 FITC (Beckman Coulter). Coverslips were finally mounted onto glass slides using Fluoromount-G (Southern Biotechnology Associates). Fluorescence images were acquired using a Leica TCS SP2 confocal scanning microscope equipped with a 100 Å–1.32 NA HCX objective.

**FIGURE 1.** Activation of DCs by CD4⁺ T cells. Immature monocyte-derived DCs (5 × 10⁴) and the superantigen TSST1 (10 ng/ml) or with a combination of LPS (500 ng/ml) and IFN-γ (20 U/ml). A and B. Flow cytometric analysis of DC maturation markers. A. Histograms of a representative experiment. B. For each marker, expression was plotted as a ratio between the mean fluorescence intensity (MFI) obtained in different conditions and the MFI measured in immature DC (fold increase MFI). Data are presented as mean ± SD of triplicates from 15 independent experiments performed with 11 unrelated donors. IFN-γ secretion (C) or IL-12p70 secretion (D) was measured by ELISA in supernatants from 11 and 9 individual donors, respectively. The mean of cytokine production is indicated in each column. Significant differences between the groups were assessed by Mann-Whitney’s unpaired t test (**, p < 0.0002; ***p < 0.0001).
Results
T cells induce DC activation in the presence of superantigen

We set up a human model to study T cell-induced activation of immature DCs. Human monocyte-derived immature DCs and autologous or allogenic CD4+ T cells were purified from control donors and cocultured with or without the bacterial superantigen TSST1. Maturation of DCs was studied 24 h later, by measuring the surface expression of DC maturation markers (CD40, CD80, CD83, CD86, and HLA-DR) and the production of IL-12p70.

DC activation was not significantly induced when cultured with either TSST1 or CD4+ T cells alone (Fig. 1B). In contrast, immature DCs cocultured for 24 h in the presence of CD4+ T cells and TSST1 showed increased expression of CD86, CD80, CD83, and CD40. Expression of HLA-DR was not always enhanced (Fig. 1, A and B). Statistical analysis of the data demonstrated that the expression of CD80, CD86, CD83, and CD40, and HLA-DR was significantly increased by coculture with T cells and TSST1. DC maturation induced by CD4+ T cells and TSST1 was comparable to the maturation induced by LPS + IFN-γ (Fig. 1B). It was reproducibly observed in 15 independent experiments with monocyte-derived DCs from 11 different donors.

Induction of CD69 expression on the T cells (data not shown) as well as production of IFN-γ (Fig. 1C) were observed when the CD4+ T cells were cultured with immature DCs and TSST1 (2536 ± 222.2 pg/ml; n = 46; 9 different donors). This production was 30-fold what was produced when T cells were cultured with TSST1 alone (75.90 ± 26.43 pg/ml; n = 17).

Finally, immature DCs produced IL-12p70 only when cocultured with TSST1 and CD4+ T cells (Fig. 1D). The mean concentration of IL-12p70 in supernatants of immature DCs cultured with TSST1 and CD4+ T cells was 167.5 ± 14.4 pg/ml (50 independent experiments; 11 different donors) as compared with 7927 ± 1655 pg/ml in supernatants of immature DCs activated by LPS + IFN-γ. Up-regulation of maturation markers and IL-12 production depended on the T cell number and were observed, respectively, for a DC:CD4+ T cell ratio of up to 25 DCs for 1 T cell and 5 DCs for 1 T cell (data not shown). IL-6, IL-8, IL-10, and TNF-α were also produced in the cocultures in the presence of TSST1 (Fig. 7); no detectable IL-4 was found in these conditions (data not shown).

No significant difference in DC maturation and IL-12p70 production was observed when DCs and CD4+ T cells were autologous or allogenic (data not shown).

This model may be used to study the T cell-driven activation of human immature DCs.

CD4+ T cell-driven DC activation requires direct contact between the two cell populations

We next asked whether direct contact between human CD4+ T cells and immature DCs was required to induce expression of co-stimulatory molecules and IL-12p70 secretion by DCs. Using Transwell plates, we did not observe any phenotypic maturation of the DCs when TSST1-bearing immature DCs were seeded in the lower chamber and CD4+ T cells in the upper chamber (Fig. 2A).

Therefore, direct contacts between the two cell types are required to induce DC maturation. However, DCs of the lower chamber showed moderate increased expression of CD86 and CD83, when exposed to supernatants of DCs + TSST1 + CD4 produced in the upper chamber (Fig. 2A).

Concerning IL-12p70 production, addition of a 24-h supernatant produced by CD4+ T cells + immature DCs + TSST1 did not induce IL-12p70 production by TSST1-pulsed DCs and did not synergize with CD4+ T cells to induce more IL-12p70 secretion by TSST1-pulsed immature DCs (Fig. 2B).

We conclude that some phenotypic maturation of DCs is induced in the absence of direct contact with T cells, but that a direct contact between the two cell types is required for IL-12 production.

Memory T cells mediate T cell-driven DC activation

It has been shown previously that memory T cells induce IL-12 production by DCs (22, 28); we checked whether this was true in our model. Memory CD45RO+CD4+ T cells and naive CD45RA−CD4+ T cells were purified from control donors, and their ability to induce expression of maturation markers and secretion of IL-12p70 by DCs was compared. The same percentage (8−12%) of TSST1-specific, Vβ2+ CD4+ T cells was measured in the naive and memory CD4+ T cell populations (data not shown).

However, for all the donors tested, the induction of CD69 by TSST1-pulsed immature DCs was less pronounced in naive than in memory CD4+ T cells (see representative experiment in Fig. 3A). Naive CD45RA−CD4+ T cells were less efficient at inducing CD86, CD83, and CD40 expression by DCs than memory
CD45RO⁺CD4⁺ T cells (Fig. 3B); they also produced lower amount of IFN-γ (25 pg/ml) than memory T cells (Fig. 3C).

Finally, as shown in Fig. 3D, naive T cells induced low levels of IL-12p70 secretion by immature DCs, which was not only due to the absence of IFN-γ in the coculture because addition of IFN-γ did not restore the IL-12p70 production to level obtained with total or memory CD4⁺ T cells (Fig. 3D).

These results show, in our model, that only memory CD4⁺ T cells induce DC activation and IL-12 secretion.

**CD4⁺ T cell-driven DC activation requires CD40L (CD154) expression by T cells**

The CD40 pathway has been shown to play an important role in eliciting costimulatory molecule expression and bioactive IL-12 secretion by DCs. To directly test the role of this pathway in our model, we used CD4⁺ T cells purified from two unrelated immunodeficient patients with complete defects in CD40L expression (6).

As shown in Fig. 4A, CD40L-deficient CD4⁺ T cells (CD4⁺/CD40L⁻) were efficiently activated by the TSST1-pulsed immature DCs, as witnessed by the increased expression of CD69. Moreover, CD40L-deficient CD4⁺ T cells induced increased expression of CD80, CD86, CD83, and CD40 by DCs, which was comparable to the expression induced by CD4⁺ T cells from a control donor (Fig. 4B). In contrast, CD40L-deficient CD4⁺ T cells from the two patients, although producing significantly higher amount of IFN-γ than in the absence of TSST1, produced 7–15 times less IFN-γ than CD4⁺ T cells from control donors (Fig. 4C). Moreover, CD40L-deficient T cells did not induce any IL-12p70 production by DCs (Fig. 4D).

We next tested whether the absence of IL-12 production (Fig. 4D) was due to the low production of IFN-γ by T cells. Addition of 1000 U/ml IFN-γ to CD40L-deficient CD4⁺ T cells did not restore IL-12 production by TSST1-bearing DCs (Fig. 4D). In the same experiment, 40 U/ml IFN-γ increased 4.5-fold IL-12p70 secretion induced by LPS in immature DCs (Fig. 4D), demonstrating the biological activity of the IFN-γ we used. These results suggested that CD40 triggering by CD40L was required for IL-12p70 production by DCs and could not be replaced by IFN-γ. To test this hypothesis, we added an activating anti-CD40 mAb to the cocultures of CD40L-deficient T cells and TSST1-pulsed immature DCs. As shown in Fig. 4E, addition of the anti-CD40 mAb to the cocultures containing CD40L-deficient T cells induced the secretion of IL-12p70, whereas no IL-12p70 production was induced when a control IgG was added at the same concentration (Fig. 4E).

The IL-12p70 production observed with anti-CD40 mAb was due to the low production of IFN-γ in the cocultures containing CD40L-deficient T cells and TSST1-pulsed immature DCs. As shown in Fig. 4F, addition of the anti-CD40 mAb to the cocultures containing CD40L-deficient T cells induced the secretion of IL-12p70, whereas no IL-12p70 production was induced when a control IgG was added at the same concentration (Fig. 4F). This reciprocal activation of immature DCs and CD40L-deficient T cells leading to IL-12p70 and IFN-γ production was observed only in the presence of TSST1. Indeed, no IL-12p70 production was observed when immature DCs were cocultured with CD4⁺ T cells, the activating anti-CD40 mAb, and IFN-γ (Fig. 4E).
T cell-driven secretion of bioactive IL-12 by human DCs thus requires at least three signals, CD40 stimulation, IFN-γ, and antigenic stimulation of T cells.

**IL-12 signaling in CD4+ T cells is required for T cell-induced DC activation**

IL-12 is a key regulator of CD4+ T cell differentiation to the Th1 phenotype (8, 9), thus regulating IFN-γ production by CD4+ T cells. We analyzed IL-12 production by DCs in our model. As shown in Fig. 5A (lower panels), after coculture with CD4+ T cells in the presence of superantigens, anti-IL-12 Ab strongly labeled CD1a+ DCs’ dendrites, some of which enwrapped CD4+ T cells. Eighty to ninety percent of the DCs, in conjugates or not, were labeled with anti-IL-12 Abs. This IL-12 labeling of DCs was never observed in the absence of superantigen (Fig. 5A, upper panels). A kinetic analysis of the production of IL-12p70 and IFN-γ revealed a rapid production of both IL-12p70 and IFN-γ, which are detected in the supernatants after 12 h of coculture (data not shown).

We then studied the role of IL-12 secretion by DCs in the cross-talk between CD4+ T cells and immature DCs by using CD4- T cells from three unrelated patients presenting with mutations in ***IL12RB1***, resulting in a totally defective expression of this receptor (3). TSST1-pulsed immature DCs from normal donors induced CD69 expression in 15% of the CD4+IL12RB1- T cells (data not shown) witnessing their activation. However, these activated CD4+IL12RB1- T cells produced low level or no IFN-γ in coculture with TSST1-pulsed DCs (Fig. 5B), showing that IL-12Rβ1-mediated signaling is required for optimal IFN-γ production. IL-12Rβ1 is a common subunit for both IL-12R and IL-23R, which binds the IL-12p40 subunit shared by these two cytokines (10). To distinguish the requirement for these two cytokines in IFN-γ secretion by T cells, we analyzed the ability of DCs derived from a patient presenting with a total defect in IL-12p40 expression (25) to induce IFN-γ production by CD4+ T cells from control donors. As expected, IL-12p40-deficient DCs did not secrete IL-12p70 when cocultured with TSST1+CD4+ T cells from a control donor (data not shown). IL-12p40-deficient DCs induced 7 times less IFN-γ production by T cells than DCs from a control donor (Fig. 5C), yet they induced CD69 expression by 20–25% CD4+ T cells, showing T cell activation (data not shown). This result confirmed the key role of IL-12p40 in the induction of IFN-γ production by T cells. To find out the relative role of IL-12 and IL-23 in IFN-γ production by T cells, we added either IL-12p70 or IL-23 to cocultures of TSST1-pulsed IL-12p40-deficient DCs and CD4+ T cells and measured IFN-γ production in the supernatants. Whereas no effect of IL-12 or IL-23 was observed on CD69 production by T cells (data not shown), IL-12p70 was able to increase production in the supernatants (mean ± SD of triplicates).

**FIGURE 4.** CD40L (CD154) expression by T cells is required to induce DC activation. Immature DCs were cultured as in Fig. 1 with CD4/wild-type (WT) or T cells from two CD40L deficiencies (CD4/CD40L-1 and 2). A, FACS analysis of CD69 expression by CD4+ T cells. B, Expression of phenotypic maturation markers in DCs is plotted as in Fig. 1B. IFN-γ (C) and IL-12p70 (D and E) secretion in supernatants (mean ± SD of triplicates). D, Human rIFN-γ (rhIFN-γ) at 1000 U/ml was added to cocultures of DC + CD4 and DC + CD4 + TSST1. IL-12p70 in supernatants of DCs cultured with LPS (200 ng/ml) or LPS + IFN-γ (40 U/ml). E, A total of 3 μg/ml anti-hemagglutinin Ab or anti-CD40 Ab was added alone or in combination with IFN-γ (1000 U/ml) to the cultures. One representative experiment of two is shown in A–E.
FIGURE 5. IL-12 signaling is required for T cell-dependent DC activation. A, Fixed cells were permeabilized and labeled with anti-IL-12p70 and CD1a Abs and visualized by confocal microscopy. B and C, IFN-γ production in 24-h supernatants of: B, CD4+ T cells from control (CD4/WT) or three IL-12Rβ1-deficient patients (CD4/IL12Rβ1−/−, #1–3) with or without control DCs and/or TSST1; C, CD4+ T cells from a control donor cocultured with DCs from an allogeneic control donor (DC/WT) or from an IL-12p40-deficient donor (DC/IL12B−/−). IL-12p70 (5 ng/ml), IL-23 (5 ng/ml), or medium was added at the beginning of the coculture. D, Expression of maturation markers in DCs from control donors cultured for 24 h in the presence of CD4+ T cells from allogenic control donor (CD4/WT) or from IL-12Rβ1-deficient patients presenting with a complete defect of IL-12Rβ1 (CD4/IL12Rβ1−/−). E and F, IL-12p70 secretion in 24-h supernatants of T cells from control donor or from two IL-12Rβ1-deficient patients activated in the presence of TSST1, DC, or DC + TSST1. Various concentrations of IFN-γ (E) and 10 ng/ml TNF-α (F) were added to cocultures, and IL-12p70 secretion was measured. IL-12p70 was also measured in supernatants of DCs cultured with LPS (500 ng/ml) or LPS + IFN-γ (40 U/ml) (E). G, Expression of CD80 and CD83 by DCs cultured without (gray line) or with 10 ng/ml TNF-α (black line). One representative experiment of two is shown in all panels.
We then analyzed whether IL-12 signaling in T cells plays a role in the T cell-driven DC activation. Surprisingly, CD4⁺/IL-12Rβ1⁻ T cells induced neither expression of maturation markers nor IL-12p70 production by DCs in the presence of TSST1 (maturation markers, Fig. 5D; IL-12, Figs. 5E and 7). This absence of IL-12p70 production was accompanied by an absence of production of TNF-α, IL-10, and IL-6 (Fig. 7). Because CD4⁺/IL-12Rβ1⁻ T cells produced low amount of IFN-γ and TNF-α, we added IFN-γ or TNF-α in the cocultures and measured IL-12p70 production. Addition of 4–400 U/ml (corresponding to 20–20,000 pg/ml) IFN-γ produced by CD4⁺ T cells from control donors activated by TSST1 and immature DCs, restored neither phenotypic maturation of DCs (data not shown) nor IL-12 production induced by CD4⁺/IL-12Rβ1⁻ T cells (Fig. 5E). Nonetheless, these concentrations of IFN-γ added to LPS-induced IL-12p70 secretion by immature DCs (Fig. 5E). Addition of TNF-α did not restore IL-12 production induced by CD4⁺/IL-12Rβ1⁻ T cells either (Fig. 5F). In the same conditions, TNF-α induced some phenotypic maturation of DCs (Fig. 5G) as witnessed by the increase expression of CD80 and CD83; however, it did not increase the phenotypic maturation of DCs induced by CD4⁺/IL-12Rβ1⁻ T cells (data not shown).

These results show that IL-12 signaling in T cells is required to induce expression of costimulatory molecules and bioactive IL-12 secretion by DCs, and that this requirement is at least partially IFN-γ and TNF-α independent.

T cell-driven DC activation requires stimulation of both CD4⁺ T cells and DCs by IFN-γ

Results presented in Fig. 4E showed that IFN-γ controls IL-12p70 secretion by DCs. We thus better characterized the production of IFN-γ in the conjugates formed between CD4⁺ T cells and DCs.
Confocal analysis of immature DCs cocultured for 10 h with CD4\(^+\) T cells in the absence or presence of TSST1 (10 ng/ml) was performed. In the absence of TSST1, no IFN-\(\gamma\) labeling of T cells or DCs was observed (Fig. 6A, upper panels), confirming the ELISA results. In the presence of TSST1, 7–12% of the conjugates, depending on the donors, showed labeling for IFN-\(\gamma\) on V\(\beta\)2 T cells. Only rare DCs were labeled. The IFN-\(\gamma\)-labeling was, in most cases, polarized toward the DCs (Fig. 6A, lower panels). These figures corresponded to the percentage of T cells, which responded to TSST1, i.e., V\(\beta\)2 T cells (data not shown), and to the percentage of CD4\(^+\) T cells with intracellular IFN-\(\gamma\)-labeling by FACS (Fig. 6B). These FACS analyses also confirmed that only T cells presented intracellular IFN-\(\gamma\)-labeling. Therefore, CD4\(^+\) T cells secreted IFN-\(\gamma\)-in an Ag-specific manner when interacting with immature DCs. Moreover, IFN-\(\gamma\)-labeling is polarized toward the zone of interaction.

The role of IFN-\(\gamma\)-in IL-12p70 secretion by DCs has been reported previously; however, the exact contribution of T cell and DC responses to IFN-\(\gamma\)-requirement is not clearly characterized. We thus evaluated these contributions. To do so, we prepared monocyte-derived DCs and CD4\(^+\) T cells from two patients presenting with a total defect in IFN\(\gamma\)R1 (1) (the IFN-\(\gamma\)-binding chain of the receptor) or IFN\(\gamma\)R2 expression (24) (the accessory chain that contributes to signal transduction (29)). As shown in Fig. 6C, IFN-\(\gamma\)-signaling was required in neither T cells nor DCs for the T cell-driven induction of CD86 and CD83 expression by DCs (left panel for IFN\(\gamma\)R2-deficient DCs; right panel for IFN\(\gamma\)R2-deficient CD4\(^+\) T cells). TSST1-pulsed DCs derived from healthy donors or from IFN\(\gamma\)R1- and R2-deficient patients induced similar levels of IFN-\(\gamma\)-production by control CD4\(^+\) T cells (Fig. 6D). IFN\(\gamma\)R2-deficient CD4\(^+\) T cells were also able to secrete IFN-\(\gamma\) when activated with control DCs and TSST1, whereas IFN\(\gamma\)R2-deficient T cells did not secrete any IFN-\(\gamma\) (Fig. 6D). These results highly suggest that IFN-\(\gamma\)-binding to CD4\(^+\) T cells is required to induce IFN-\(\gamma\)-secretion by T cells, but that IFN-\(\gamma\)-signaling in T cells is not required. IFN\(\gamma\)R expression by DCs or CD4\(^+\) T cells is not required either for IL-8, IL-10, or TNF-\(\alpha\)-production in cocultures (Fig. 7). In contrast, as shown in Fig. 6E, IFN\(\gamma\)R1 and IFN\(\gamma\)R2 expression were required on both DCs and CD4\(^+\) T cells to induce IL-12p70 secretion by DCs.

**Discussion**

Patients affected by the clinical syndrome known as Mendelian susceptibility to mycobacterial disease present with specific susceptibility to live BCG vaccine, poorly virulent environmental mycobacteria, *Salmonella*, and few other intracellular pathogens (reviewed in Ref. 3). These patients have been shown to present with genetically distinct germline mutations in at least five genes, *IL12B, IL12RB1, IFNRI, IFNRII, and STAT1*, but have in common a defective IL-12/IFN-\(\gamma\)-axis. Although known for 10 years now, the underlying mechanisms for this susceptibility to very specific intracellular pathogens are still unclear.

In this study, we specifically analyzed the T cell-driven maturation of DCs between highly purified human monocyte-derived DCs and CD4\(^+\) T lymphocytes from patients presenting with Mendelian susceptibility to mycobacterial disease and compared it with interactions between DCs and CD4\(^+\) T cells from control donors. We found out that the T cell-driven maturation of DCs is abolished in all the patients.

Although based on a human in vitro model using monocyte-derived DCs, purified CD4\(^+\) T cells, and recombinant superantigen, this experimental model reconstitutes a number of the known characteristics of the interactions between DCs and CD4\(^+\) T cells during DC licensing in vivo (in mice). These similarities include the absolute need for an Ag- and CD40-CD40L-dependent direct cell-cell contact for the induction of IL-12 secretion by DCs (30), because CD40L-deficient CD4\(^+\) T cells are unable to induce IL-12p70 secretion by DCs (Fig. 4). Interestingly, CD40L expression in T cells was required for the induction of IL-12p70 by human DCs, but not of other immunomodulatory cytokines (such as IL-8, IL-10, and TNF) (Fig. 7). Although CD40 plays a crucial role in the education of human DCs, anti-CD40 agonist mAbs were unable to induce IL-12p70 secretion by immature human DCs even in the presence of IFN-\(\gamma\) (Fig. 4). These results show that DCs require at least three signals coming from T cells, namely CD40L.
expression and IFN-γ production by T cells and yet another signal, which is TCR dependent and remains to be found.

Also supporting the in vivo relevance of our in vitro model, the efficiency of CD4+ T cells for inducing IL-12 secretion by immature DCs is very high: one Ag-specific T cell for 125 immature DCs is sufficient to induce significant levels of IL-12 (at a ratio of total T cells:DCs of 1:5 (data not shown), and 4–5% of total purified T cells are Vβ2* (TSSST1 responsive) with a memory phenotype). However, some of our results differ from results obtained in mice models that showed that bioactive IL-12 by DCs can be initiated by T cell-derived signals only in the presence of microbial signal (31, 32). In our study, we did not add any microbial product, and, although not completely excluded, the presence of trace amounts of endotoxins was not detected in our culture medium or in the rTSSST1 (data not shown). These discrepancies in the requirement for microbial products may be due, apart from the species diversity, to several differences in the experimental models. First, we used monocyte-derived human DCs, whereas mouse splenic CD11chigh DCs were used. Because different DC populations can have very different functions (33), this difference may be critical. Second, we show in this study that only memory human T cells are able to induce IL-12p70 secretion by immature human DCs, whereas in the murine model only newly activated naive T cells have been tested for their ability to promote IL-12p70 production by DCs (32). Third, in this study, we used a superantigen, a polyclonal activator of T cells, whereas in the mice models, monoclonal populations of transgenic T cells were activated with their cognate MHC/peptide complex.

Using combinations of purified CD4+ T cells from patients and DCs from control and vice versa, we showed that T cell responses to IL-12 and IFN-γ are required to induce maturation of DCs and/or IL-12 secretion by DCs. Indeed, most studies addressing the question of the role of IL-12 and/or IFN-γ have used blocking Abs (22) or, when using patients or animals presenting with individual genetic defects in the IFN-γ/IL-12 axis, have measured the responses of mixed population of cells (PBMCs or splenocytes) (34–37). These experiments did not allow discriminating the role played by each cytokine on each cell population.

We observed that CD4+/IL-12β1R− T cells were even more defective than CD4+/CD40L− T cells in inducing DC activation. This was witnessed by the low increase in the expression by DCs of CD86, CD80, CD83, and CD40 induced by CD4+/IL-12β1R− T cells and TSSST1 and the absence of IL-12, but also IL-10, and TNF-α in the supernatants of DCs cultured in the same conditions (Fig. 7). The absence of DC activation by CD4+/IL-12β1R− T cells cannot be attributed to deficient T cell triggering because TSSST1-pulsed immature DCs induced TCR down-regulation and expression of CD69 by CD4+/IL-12β1R− T cells (data not shown). Thus, an IL-12β1R-dependent T cell signaling controls T cell-driven DC activation. This signal is IL-12 and not IL-23 dependent, as shown by the experiments performed with IL-12β2-deficient DCs (Fig. 5C). Moreover, this signal does not depend on IFN-γ or TNF-α only, because addition of IFN-γ or TNF-α to IL-12β1R+/CD4+ T cell TSSST1 and immature DCs does not restore IL-12p70 secretion by DCs (Fig. 5, E and F) or expression of maturation markers to levels obtained with control CD4+ T cells (data not shown). The absence of IL-12 secretion may be due to the low level of CD40 expression by immature DCs cocultured with IL-12β1R+/CD4+ T cells, a level that may be insufficient to induce triggering of DCs.

In our study, we confirm that IFN-γ signaling in DCs is required to induce production of bioactive IL-12 by DCs because IFNGR1- and IFNGR2-deficient DCs are unable to secrete IL-12 (Fig. 6). Indeed, DCs that cannot bind IFN-γ (IFNGR1 deficient) or cannot signal through IFNGR (IFNGR2 deficient) showed an increased expression of CD83 and CD86 (Fig. 6C), but did not produce detectable amount of IL-12 (Fig. 6E). This absence of IL-12 production did not preclude IFN-γ production, which in our model was only produced by T cells. This IFN-γ may be induced by type I IFNs in an IL-12-independent manner, as already reported (38). In vivo the source of IFN-γ may also come from NK cells (39) or some populations of DCs (40).

We show that IFN-γ signaling is also required on the CD4+ T cell side for a cross-talk between CD4+ T cells and DCs that leads to IL-12 production. These results demonstrate that IFN-γ induces a signal in T cells that makes them competent to induce DC activation. What could be this signal? The first possibility is that the absence of functional cross-talk observed with IFNGR-deficient T cells is not due to an absence of IFN-γ secretion by these T cells because: 1) IFNGR2-deficient T cells are still able to produce IFN-γ when cocultured with DCs and superantigen (Fig. 6); 2) addition of IFN-γ in the cocultures did not restore IL-12 production by DCs (data not shown). IFNGR signaling in T cells may be required, as shown in CD4+ mice T cells (41), to induce IL-12β2 expression by T cells. IFNGRs would thus control IL-12 response of T cells, which as shown in this study is implicated in the T cell-driven DC activation. The second possibility is that IFNγ expression by T cells is important to correctly present IFN-γ to DCs. Indeed, a polarized delivery of several receptors at the immunological synapse has been shown, i.e., TCR (42), CD40L, and, more recently, IFNγRs (43, 44). The directional delivery of both cytokines and their receptors at the synapse probably allows the formation of a high local concentration of cytokines, which is required for functional responses. Such mechanisms may also ensure, in the case of the T cell-driven activation of DCs, that reciprocal activation of the two cells will only happen in an Ag-dependent manner.

Immunity against intracellular pathogens such as mycobacteria strongly depends upon the induction of a Th1 CD4+ T cell response. Interactions of DCs with CD4+ Th lymphocytes have been shown to license or educate DCs to prime CTLs or to orientate CD4+ T cell priming toward Th1 or Th2 responses (reviewed in Refs. 13, 14). It is thus tempting to speculate that the defective cross-talk between CD4+ T cells and DCs in patients presenting defective response to IFN-γ or IL-12 or CD40 triggering may account for their shared vulnerability to mycobacteria, Salmonella, and other discrete intracellular pathogens.

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Disclosures
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