

HUMAN & MOUSE CELL LINES

Engineered to study multiple immune signaling pathways.

Transcription Factor, PRR, Cytokine, Autophagy and COVID-19 Reporter Cells
ADCC, ADCC and Immune Checkpoint Cellular Assays



The Journal of Immunology

RESEARCH ARTICLE | JANUARY 15 2002

IL-1 β Enhances CD40 Ligand-Mediated Cytokine Secretion by Human Dendritic Cells (DC): A Mechanism for T Cell-Independent DC Activation¹ **FREE**

Thomas Luft; ... et. al

J Immunol (2002) 168 (2): 713–722.

<https://doi.org/10.4049/jimmunol.168.2.713>

Related Content

Prostaglandin E₂ Is Generally Required for Human Dendritic Cell Migration and Exerts Its Effect via EP2 and EP4 Receptors

J Immunol (January,2006)

Lactoferrin Acts as an Alarmin to Promote the Recruitment and Activation of APCs and Antigen-Specific Immune Responses

J Immunol (May,2008)

IL-27 in Human Secondary Lymphoid Organs Attracts Myeloid Dendritic Cells and Impairs HLA Class I–Restricted Antigen Presentation

J Immunol (March,2014)

IL-1 β Enhances CD40 Ligand-Mediated Cytokine Secretion by Human Dendritic Cells (DC): A Mechanism for T Cell-Independent DC Activation¹

Thomas Luft,[†] Michael Jefford,^{*} Petra Luetjens,^{*} Hubertus Hochrein,[‡] Kelly-Anne Masterman,^{*} Charlie Maliszewski,[§] Ken Shortman,[‡] Jonathan Cebon,^{*} and Eugene Maraskovsky^{2*}

CD40 ligand (CD40L) is a membrane-bound molecule expressed by activated T cells. CD40L potently induces dendritic cell (DC) maturation and IL-12p70 secretion and plays a critical role during T cell priming in the lymph nodes. IFN- γ and IL-4 are required for CD40L-mediated cytokine secretion, suggesting that T cells are required for optimal CD40L activity. Because CD40L is rapidly up-regulated by non-T cells during inflammation, CD40 stimulation may also be important at the primary infection site. However, a role for T cells at the earliest stages of infection is unclear. The present study demonstrates that the innate immune cell-derived cytokine, IL-1 β , can increase CD40L-induced cytokine secretion by monocyte-derived DC, CD34⁺-derived DC, and peripheral blood DC independently of T cell-derived cytokines. Furthermore, IL-1 β is constitutively produced by monocyte-derived DC and monocytes, and is increased in response to intact *Escherichia coli* or CD40L, whereas neither CD34⁺-derived DC nor peripheral blood DC produce IL-1 β . Finally, DC activated with CD40L and IL-1 β induce higher levels of IFN- γ secretion by T cells compared with DC activated with CD40L alone. Therefore, IL-1 β is the first non-T cell-derived cytokine identified that enhances CD40L-mediated activation of DC. The synergy between CD40L and IL-1 β highlights a potent, T cell-independent mechanism for DC activation during the earliest stages of inflammatory responses. *The Journal of Immunology*, 2002, 168: 713–722.

Maturation of dendritic cells (DC)³ is a critical event for the induction of an immune response. Upon pathogen entry or tissue damage, inflammatory processes in the affected microenvironment induce DC maturation, which in turn can induce DC to migrate out of the inflamed peripheral tissues and into the lymphatic organs. DC maturation is characterized by a significant change in the cell's Ag expression pattern and functional characteristics. These mature APC can induce quiescent T cells to become activated (1).

IL-12p70 is a cytokine secreted by activated macrophages and DC that stimulates IFN- γ secretion by T lymphocytes and NK cells (2–4). Although cytokines such as TNF- α , IL-1 β , and IFN- α are potent inducers of DC maturation, they are not sufficient to induce secretion of IL-12p70 (5–7). Indeed, TNF- α and IL-1 β in-

hibit the capacity of monocyte-derived DC (MoDC) to secrete IL-12 in response to IL-12-inducing stimuli (7). IL-12 is induced by CD40 ligand (CD40L) (5, 8), a TNF family member up-regulated on activated CD4⁺ Th cells, or by pathogens and their derivatives (e.g., LPS, CpG-DNA, dsRNA) (9–13).

A critical role for CD40L during the priming phase of adaptive immune responses in the lymph nodes has now been established. Here naive CD4⁺ T cells up-regulate CD40L after interaction with CD70, the ligand for CD27. CD70 is expressed on CD8⁺ T cells following interaction with DC (14). This suggests an important stimulatory loop, which involves DC, CTL, and Th cells. The crucial function of CD4⁺ Th cells in priming naive CD8⁺ CTL can be completely replaced by preactivating DC with anti-CD40 or soluble CD40L (15–17). In humans, CD40L expression is not restricted to T cells but has also been reported on activated B lymphocytes (18), eosinophils (19), platelets (20), smooth muscle cells (21), vascular endothelial cells (21), macrophages (21), and DC (22, 23). Because CD40L can be expressed by non-T cells, it is possible that non-T cells may contribute to CD40L-mediated effects at sites of infection, providing an alternative, T cell-independent CD40L pathway. Although maximal CD40L-mediated IL-12p70 secretion by DC requires T cell-derived cytokines (IFN- γ (9) or IL-4 (24–26)), thereby suggesting a dependence on T cell assistance, a role for T cells at the earliest stages of infection is unclear. Presumably, the presence of T cells (and T cell-derived cofactors such as IFN- γ and IL-4) at such sites would be temporally delayed and restricted to the memory effectors that eventually arrive after successful priming in the lymph nodes. A role for CD40L at the primary infection site may still be possible if other cofactors produced by non-T cells were present. A recent report indicates that IL-12p70 secretion by DC in vivo can only be amplified by CD40L in the presence of innate signals initiated by

*Melbourne Tumor Biology Branch, Ludwig Institute for Cancer Research, Austin and Repatriation Medical Center, Heidelberg, Victoria, Australia; [†]University of Heidelberg, Heidelberg, Germany; [‡]Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia; and [§]Immunex Corporation, Seattle, WA 98101

Received for publication June 20, 2001. Accepted for publication November 16, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

¹This work was supported by the Sylvia and Charles Viertel Foundation. T.L. is supported by the Anti-Cancer Council of Victoria, Australia. P.L. and H.H. are supported by a Deutsche Krebshilfe fellowship. M.J. was supported by a grant from the Stewardson Family Trust.

²Address correspondence and reprint requests to Dr. Eugene Maraskovsky, Melbourne Tumor Biology Branch, Ludwig Institute for Cancer Research, Austin and Repatriation Medical Center, Studley Road, Heidelberg, Victoria 3084, Australia. E-mail address: eugene.maraskovsky@ludwig.edu.au

³Abbreviations used in this paper: DC, dendritic cell; MoDC, monocyte-derived DC; PBDC, peripheral blood DC; FL, Flt3 ligand; CD40L, CD40 ligand; rh, recombinant human; SN, supernatant; MIP, macrophage-inflammatory protein; CM, conditioned medium.

microbial stimuli. They exclude IFN- γ as the innate signal but are unable to directly define it (27).

The present study demonstrates that IL-1 β is a potent modulator of CD40L-induced cytokine secretion by three different human DC subsets: MoDC, CD34⁺-derived DC, and peripheral blood DC (PBDC). This can occur independently of IL-4 and IFN- γ and result in the induction of IFN- γ secretion by T cells. Because monocytes and MoDC rapidly secrete high levels of IL-1 β following encounter with intact microbes (such as bacteria), our results identify a novel mechanism for the rapid induction of inflammatory responses, which can occur at the infection site independently of T cells and T cell-derived cytokines.

Materials and Methods

Cell sources

For CD34⁺ progenitor cells, leukapheresis harvest samples were obtained from either normal donors or patients of the Department of Medical Oncology, Austin and Repatriation Medical Center (Heidelberg, Australia). Patients with non-Hodgkin's lymphoma or solid tumors received stem cell-mobilizing chemotherapy and G-CSF as part of their treatment. PBDC were isolated from the blood of patients with stage III and IV melanoma enrolled in a Phase I clinical study (LUD97-012), receiving 14 consecutive days of Flt3 ligand (FL) (25 μ g/kg/day) followed by peptide vaccines. Blood for PBDC was taken at day 15. The protocol was approved by the Ludwig Institute's Investigators Review Board and the Ethics Committee at the Austin and Repatriation Medical Center, and informed consent was obtained from all patients. Monocytes and PBDC were also isolated from the PBMC fraction of healthy volunteer donors provided by the Australian Red Cross Blood Bank (Southbank, Melbourne, Australia) and used to produce MoDC.

Media

MoDC and PBDC were grown in RPMI 1640 (Trace Biosciences, Melbourne, Australia) supplemented with 20 mM HEPES, 60 mg/l penicillin G, 12.6 mg/l streptomycin, 2 mM L-glutamine, 1% nonessential amino acids, and 10% heat-inactivated FCS (CSL, Melbourne, Australia) in a 5% CO₂ incubator. The serum-free medium X-Vivo 20, used for the generation of CD34⁺-derived DC, was purchased from BioWhittaker (Walkersville, MD). MLR were performed in IMDM (Life Technologies, Grand Island, NY) and 5% pooled normal human serum (gift of the Victorian Tissue Typing Service, Royal Melbourne Hospital, Melbourne, Australia) in a 10% CO₂ incubator.

mAbs, ELISA kits, and cytokines

Flow cytometric analysis of DC was performed using the following mAbs: FITC-conjugated IgG1 isotype control, PE-conjugated IgG1 isotype control, anti-CD34 (HPCA-2), anti-CD11c-FITC, anti-CD14-APC, anti-CD16-PE, anti-CD33-PE, anti-CD123w-PE, anti-HLA-DR-biotin (BD PharMingen, San Diego, CA), and anti-CD1c-FITC; (BioSource International, Camarillo, CA). Neutralizing mAbs against the IL-1R and IL-4 were purchased from BD PharMingen. Cytokine ELISA kits (Opteia) for IL-1 β , IL-6, IL-10, and IL-12p70 were purchased from BD PharMingen. Capture and HRP-conjugated detection Abs for IFN- γ ELISAs were a kind gift from CSL. The following cytokines were added to DC cultures: recombinant human (rh)TNF- α (10 ng/ml; R&D Systems, Minneapolis, MN), rhGM-CSF (40 ng/ml; Schering-Plough, Sydney, Australia), rhIL-4 (500 U/ml; Schering-Plough, Kenilworth, NJ), rhIL-1 β (1–2 ng/ml; R&D Systems), PGE₂ (1 μ M final concentration; ICN Biomedicals, Aurora, OH), IFN- γ (1000 IU/ml; PeproTech, Rocky Hill, NJ), rhIL-6 (50–100 ng/ml) and soluble rhIL-6R (100 ng/ml) were a kind gift of Dr. R. Simpson (Ludwig Institute for Cancer Research, Melbourne, Australia). CD40L-trimer (1 μ g/ml final concentration) was a gift from Immunex (Seattle, WA).

CD34⁺-derived DC

Serum-free cultures of CD34⁺-derived DC were performed as described (28). Briefly, PBMC were obtained from leukapheresis harvests, red cells were lysed using NH₄Cl, and CD34⁺ progenitor cells were separated with the MACS CD34 isolation kit (Miltenyi Biotec, Sunnyvale, CA). CD34⁺ cells (1 \times 10⁶/ml) were cultured in 500 μ l of X-Vivo 20 in 24-well plates (Nunc, Roskilde, Denmark) in GM-CSF (40 ng/ml) and TNF- α (20 ng/ml). Fresh medium containing cytokines was added twice weekly. IL-4 (1000 U/ml) was added to the CD34⁺-derived DC cultures on day 7. On day 14,

cells were pooled, readjusted to 1 \times 10⁵ DC/well, and stimulated with maturation-inducing factors.

MoDC

For MoDC generation, CD14⁺ monocytes (5 \times 10⁵) were affinity-purified using the MACS CD14 isolation kit (Miltenyi Biotec) and cultured in 1 ml of RPMI 1640, 10% FCS, GM-CSF (40 ng/ml), and IL-4 (500 IU/ml) in 24-well plates. At day 7, MoDC represented >90% of cultured cells. All wells were pooled and readjusted to a concentration of 1 \times 10⁵ DC/ml. Maturation-inducing factors were added on day 7 and cells and supernatants were harvested on day 10 for functional assessment. MoDC-conditioned medium refers to the medium from MoDC cultures after 7 days of culture in GM-CSF and IL-4.

Enrichment of PBDC from FL-treated patients

PBDC were enriched from frozen PBMC samples obtained from a Phase I randomized study performed in HLA-A2⁺ patients with evaluable stage III and IV malignant melanoma receiving FL (Immunex) with or without peptide vaccines (LUD97-012). Informed consent was obtained from all individuals and the protocol and consent forms were approved by an Investigators Review Board of the Ludwig Institute for Cancer Research. After thawing, CD14⁺ monocytes were depleted using immunomagnetic beads (MACS; Miltenyi Biotec) according to the manufacturer's instructions. These CD14-depleted PBMC underwent a second round of depletion using MACS beads coupled to anti-CD3, anti-CD14, anti-CD19 (Miltenyi Biotec), and purified anti-CD16, anti-CD56, and anti-glycophorin A (BD PharMingen) in combination with rat anti-mouse IgG MACS beads (Miltenyi Biotec). This depletion procedure yielded >80% CD1b/c⁺CD14⁺HLA-DR⁺ PBDC as assessed by FACS. These immature PBDC were then cultured in 96-well plates (1 \times 10⁵/well) in RPMI 1640–10% FCS for 3 days with various combinations of stimuli before examination of function. In later experiments, CD1b/c⁺HLA-DR⁺ PBDC were sorted by FACS on a MoFlo cell sorter (Cytomation, Fort Collins, CO).

Blocking of CD40L-mediated IL-12p70 secretion with anti-IL-4 or anti-IL-1R mAb

MoDC (1 \times 10⁵/ml) were stimulated in their own conditioned medium (CM) with CD40L (1 μ g/ml) for 24 h. Neutralizing anti-IL-4 or IL-1R mAb or both (BD PharMingen) were added at the initiation of culture in a range of concentrations (0.05–2 μ g/ml). An isotype-matched mouse mAb was also used as control (BD PharMingen). After 24 h, supernatants (SN) were harvested and examined for IL-12p70 secretion by ELISA.

Measurement of Ag uptake

MoDC were harvested after culture in maturation-inducing conditions. Following incubation with 1 mg/ml FITC-dextran (44 and 260 kDa; Sigma-Aldrich, St. Louis, MO) for 30–60 min at 0 or 37°C. Cells were washed three times in PBS 5% FCS and then incubated with PE-anti-CD11c. FITC-dextran uptake was quantified as mean fluorescence intensity on gated CD11c⁺ cells. Nonspecific FITC signal was assessed by incubating MoDC with FITC-dextran at 0°C. Phagocytosis was assessed by incubating cells with 1 mg/ml PE-latex beads (Sigma-Aldrich) for 90 min at 37°C. In some conditions, cells were pretreated with 10 μ M cytochalasin D (Sigma-Aldrich) for 30 min at 37°C to depolymerize actin. To verify that the flow cytometry-based FITC signal represented internalized dextran or beads, cells were analyzed by epifluorescence and phase-contrast microscopy.

Measurement of cell migration

Lower chambers of Transwell plates (8- μ m pore size; Costar, Corning, NY) were filled with 500 μ l of IMDM/5% human serum with or without chemokines: CCL21 (macrophage-inflammatory protein (MIP)-3 β) (3–300 ng/ml) or CCL19 (6CKine) (5–250 ng/ml). A total of 1–2 \times 10⁴ MoDC were added in 50 μ l of IMDM/5% human serum into the upper chamber. After 2 h, cells in the lower chambers were harvested, concentrated to 50- μ l volumes in Eppendorf tubes, and counted microscopically with a hemocytometer. Each stimulation condition was performed in replicate wells.

Cytokine ELISAs

ELISAs (IL-1 β , IL-6, IL-10, IL-12p70, and IFN- γ) were performed according to the manufacturer's instructions using Maxisorp plates (Nunc). The HRP substrate was tetramethylbenzidine peroxidase substrate (Kirkegaard & Perry Laboratories, Gaithersburg, MD); the color reaction was terminated by adding 100 μ l of ortho-phosphoric acid (1 M). Plates were read in a Thermomax microplate reader (BioMediq, Melbourne, Australia).

Mixed leukocyte reaction

Graded numbers of DC subsets were cultured in replicates in 96-well round-bottom plates (Falcon, Franklin Lakes, NJ) with 10^5 allogeneic T cells for 5 days in RPMI 1640 with 10% human serum. T cells were prepared from healthy volunteers by first rosetting with 2-aminoethylisothiuronium bromide-treated SRBC and then further fractionating by negative enrichment using anti-CD4 ($CD8^+$ T cells) or anti-CD8 ($CD4^+$ T cells) MACS beads (Miltenyi Biotech). After 5 days, $200 \mu\text{l}$ of supernatants were harvested and fresh medium containing $1 \mu\text{Ci}/\text{well}$ [^3H]thymidine (DuPont, Sydney, MA) was added for 8 h. Cells were transferred onto a glass fiber filter (Wallac, Turku, Finland), and [^3H]thymidine incorporation was measured using an LKB 1205 Betaplate scintillation counter (Wallac).

Results

Cytokine secretion by CD40L activated MoDC in the presence or absence of their own CM

It has previously been shown that the induction of high levels of IL-12p70 secretion by MoDC requires a combination of either T cell signals (CD40L) (5, 8) with IFN- γ (9), or pathogen signals (LPS) (10–14). We evaluated cytokine secretion by MoDC following stimulation with CD40L. When added directly into MoDC cultures, CD40L alone induced IL-12p70 secretion (200–1000 pg/ml; $n > 10$). These culture SN did not contain measurable levels of IFN- γ as assessed by ELISA (sensitivity, 10 pg/ml; $n > 40$). This suggested that the CM of MoDC cultures after 7 days of culture in GM-CSF and IL-4 may contain factor(s) other than IFN- γ that can enhance CD40L-induced IL-12p70 secretion.

Fig. 1 shows the effect of MoDC-CM on the secretion of IL-6, IL-10, and IL-12p70 by MoDC following 3 days of stimulation with either CD40L or IFN- γ or the combination of CD40L and IFN- γ . MoDC-CM contained high levels of IL-6 (>5000 pg/ml), in the absence of stimulation, and this was not enhanced by either IFN- γ or CD40L stimulation (Fig. 1A). In contrast, washed MoDC cultured in fresh RPMI 1640/FCS (in the absence of exogenous GM-CSF and IL-4) produced low levels of IL-6 (<500 pg/ml) over the 3-day culture period which could somewhat be enhanced by CD40L (max 1000 pg/ml) but not by IFN- γ (Fig. 1A). The combination of IFN- γ and CD40L enhanced IL-6 secretion by MoDC in either the presence or absence of MoDC-CM. Similarly, IL-10 was detected at significant levels in unstimulated (day 7) MoDC-CM and was even detected in the CM of washed MoDC recultured for 3 days in fresh RPMI 1640/FCS (Fig. 1B). CD40L, but not IFN- γ , induced IL-10 secretion by MoDC in both the presence and absence of MoDC-CM. Unlike with CD40L-mediated IL-6 secretion, IFN- γ did not augment CD40L-mediated IL-10 secretion.

Unlike with IL-6 and IL-10, IL-12p70 was not detected in either the MoDC-CM of unstimulated MoDC or the CM of washed MoDC (Fig. 1C). Furthermore, CD40L only induced IL-12p70 secretion by MoDC in the presence of MoDC-CM. The addition of IFN- γ did not induce IL-12p70 secretion in either the presence or absence of MoDC-CM. As reported previously (9), the addition of IFN- γ to CD40L enhanced IL-12p70 secretion even in the absence of MoDC-CM (Fig. 1C); however, the highest IL-12p70 secretion was induced in the presence of MoDC-CM (1–15 ng/ml; $n > 10$). Finally, the finding that CD40L-mediated IL-10 secretion was not enhanced by IFN- γ suggests that IL-12p70 and IL-10 secretion are differentially regulated. These results indicate that a factor(s) present in MoDC-CM synergize(s) with CD40L or CD40L and IFN- γ to induce IL-12p70 secretion and augment IL-10 secretion.

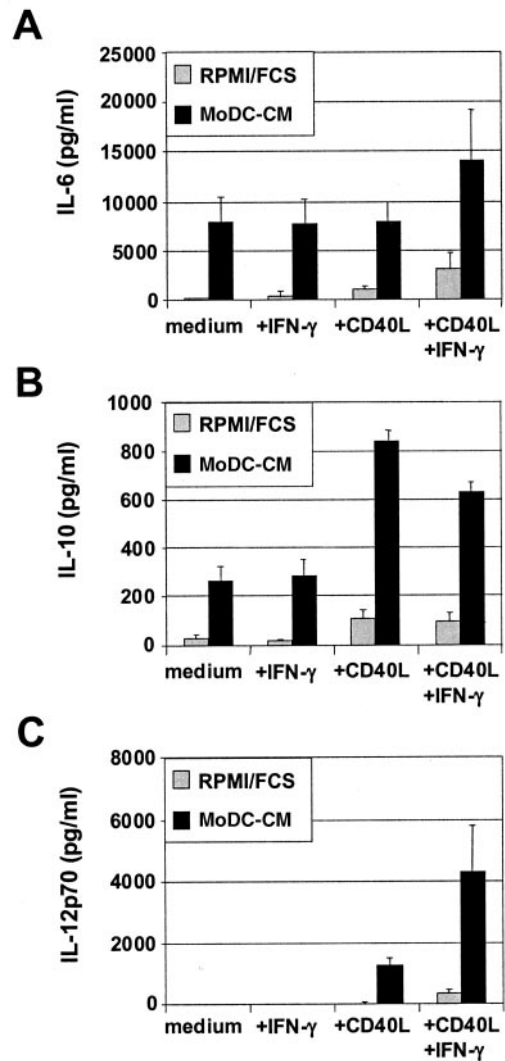


FIGURE 1. Cytokine secretion by MoDC stimulated with IFN- γ , CD40L, or CD40L plus IFN- γ in the presence or absence of their conditioned culture medium. MoDC were prepared by culturing purified $CD14^+$ monocytes for 7 days in GM-CSF and IL-4. On day 7, the cultures were washed twice and recultured ($1 \times 10^5/\text{ml}$) for a further 3 days in either fresh medium (RPMI 1640/FCS) or their own conditioned culture medium (MoDC-CM), with or without IFN- γ (1000 IU/ml), or CD40L (1 $\mu\text{g}/\text{ml}$), or CD40L and IFN- γ . Culture SN were examined for cytokine production by ELISA. A, Secretion of IL-6. B, Secretion of IL-10. C, Secretion of IL-12p70. Data represent the means \pm SEM of experiments from four separate donors.

Effect of IL-1 β , IL-4, IL-6/IL-6R, or TNF- α upon CD40L-induced cytokine secretion

MoDC-CM contains several known soluble factors. Some are exogenous cytokines which are added at the initiation of culture (e.g., GM-CSF, IL-4), while others are secreted by monocytes and MoDC during the 7-day culture period (e.g., IL-6/IL-6R, IL-1 β , and TNF- α). To investigate which cytokines present in MoDC-CM contribute to CD40L-mediated cytokine secretion, we systematically added GM-CSF, IL-4, IL-6/IL-6R, IL-1 β , or TNF- α to MoDC that were washed and recultured in fresh RPMI 1640–10% FCS. First, none of the cytokines tested directly induced cytokine secretion by MoDC, this function requiring the presence of CD40L (Fig. 2 and data not shown). Second, neither IL-6 (50–100 ng/ml) in combination with soluble IL-6R (100 ng/ml) nor TNF- α (20

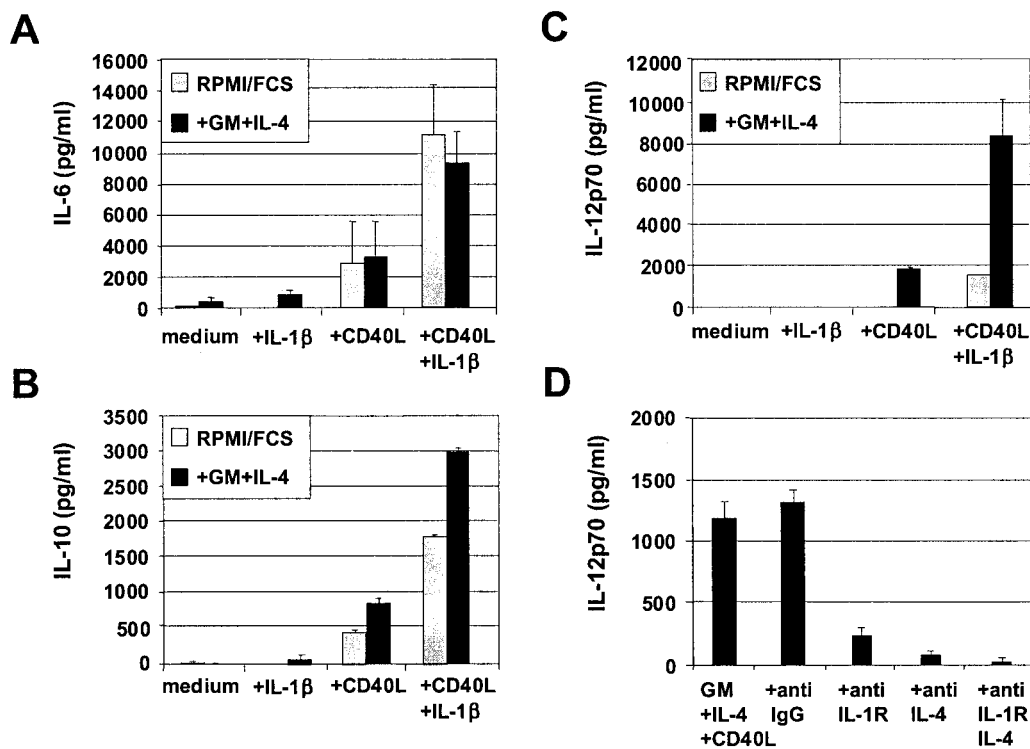


FIGURE 2. IL-1 β and IL-4 independently enhance CD40L-induced cytokine secretion. MoDC were washed on day 7 and recultured (1×10^5 /ml) for a further 3 days in either fresh culture medium alone (RPMI 1640/FCS) or medium containing GM-CSF and IL-4, with or without the indicated cytokine stimuli. Culture SN were examined for cytokine production by ELISA. **A**, Secretion of IL-6. **B**, Secretion of IL-10. **C**, Secretion of IL-12p70. Addition of IL-1 β alone or in combination with GM-CSF and IL-4 was tested separately and did not induce substantial cytokine secretion in these cultures. **D**, Blocking of CD40L-mediated IL-12p70 secretion by anti-IL-1R and anti-IL-4 mAb. MoDC were stimulated with CD40L for 24 h in the presence of either anti-IL-1R (0.5 μ g/ml) or anti-IL-4 (1 μ g/ml), or both, or with isotype-matched IgG1 control mAb. Culture SN were then harvested and examined for IL-12p70 production by ELISA. Data represent the mean \pm SD of three replicate wells. The figure is a representative experiment from three to four separate donors.

ng/ml) could replace the activity of MoDC-CM responsible for the augmentation of CD40L-mediated cytokine secretion ($n = 2$; data not shown). In contrast, IL-1 β potently enhanced CD40L-mediated IL-6, IL-10, and IL-12p70 secretion either in the presence of fresh RPMI 1640–10% FCS alone (medium) or medium containing GM-CSF and IL-4 (Fig. 2, A–C).

[GRAPHIC]In contrast, GM-CSF and IL-4 enhanced CD40L-mediated IL-10 (Fig. 2B) and IL-12p70 secretion (Fig. 2C) in the absence of MoDC-CM. This confirms the recent reports showing that IL-4 is an important regulator of CD40L-mediated IL-12p70 secretion (23–25). Surprisingly, IL-1 β alone (Fig. 2C, \square) was as potent as GM-CSF and IL-4 (Fig. 2C, \blacksquare) at enhancing CD40L-mediated IL-12p70 secretion in the absence of MoDC-CM (Fig. 2C) and was more potent than GM-CSF and IL-4 at enhancing CD40L-mediated IL-10 production (Fig. 2B). Furthermore, the combination of IL-1 β with GM-CSF and IL-4 induced the highest levels of IL-12p70 and IL-10 secretion by MoDC (Fig. 2, B and C). Finally, the addition of either anti-IL-1R or anti-IL-4 mAb blocked CD40L-mediated IL-12p70 secretion by MoDC, indicating that both of these cytokines are important cofactors in CD40L-mediated cytokine secretion (Fig. 2D).

Effect of IL-1 β on DC maturation

Given that IL-1 β was identified as a potent cofactor for CD40L-mediated cytokine secretion, we next examined whether IL-1 β could also act as a maturation factor when added to immature MoDC. Fig. 3A shows that immature MoDC express negligible levels of the maturation markers CD25, CD80, CD83, and CD86 and low to intermediate levels of surface HLA-I and HLA-II. Up-

regulation of all of these molecules was achieved when immature MoDC were stimulated with CD40L alone but not by IL-1 β alone (Fig. 3A). Furthermore, the combination of IL-1 β with CD40L was no more potent than CD40L alone at inducing phenotypic maturation of immature MoDC (Fig. 3A). This suggests that IL-1 β is not a potent factor for the induction of DC phenotypic maturation.

Effect of IL-1 β on DC Ag uptake capacity and migration to chemokines

Immature MoDC capture a variety of Ags using several different mechanisms (1). As immature MoDC mature, they undergo a step-wise coordinated process of reducing their Ag uptake capacity, up-regulating the expression of chemokine receptors (such as CCR7), and acquiring migratory capacity toward chemokines such as CCL21 (MIP-3 β), which can direct them to draining lymphoid tissues (1). Therefore, we examined endocytic and phagocytic capacity of immature MoDC as compared with those matured with IL-1 β or CD40L or combinations of CD40L and IL-1 β . Analysis of MoDC by FACS revealed that, as expected, immature MoDC were maximally capable of internalizing soluble dextran (260 kDa) and phagocytosing 1- μ m latex particles (Fig. 3, B and C). Maturation with IL-1 β did not affect the capacity of MoDC to ingest FITC-dextran (260 kDa) or PE-latex (1 μ m). Only CD40L or CD40L plus IL-1 β maximally reduced the ability of MoDC to ingest these particulates. This suggests that IL-1 β is not a major regulator of MoDC Ag uptake capacity. Finally, immature MoDC matured with IL-1 β did not migrate toward the CCR7 ligands, CCL21 (MIP-3 β) (Fig. 3D) or CCL19 (6Ckine) (data not shown), whereas those matured with IFN- α 2a, TNF- α and PGE $_2$ did (Fig.

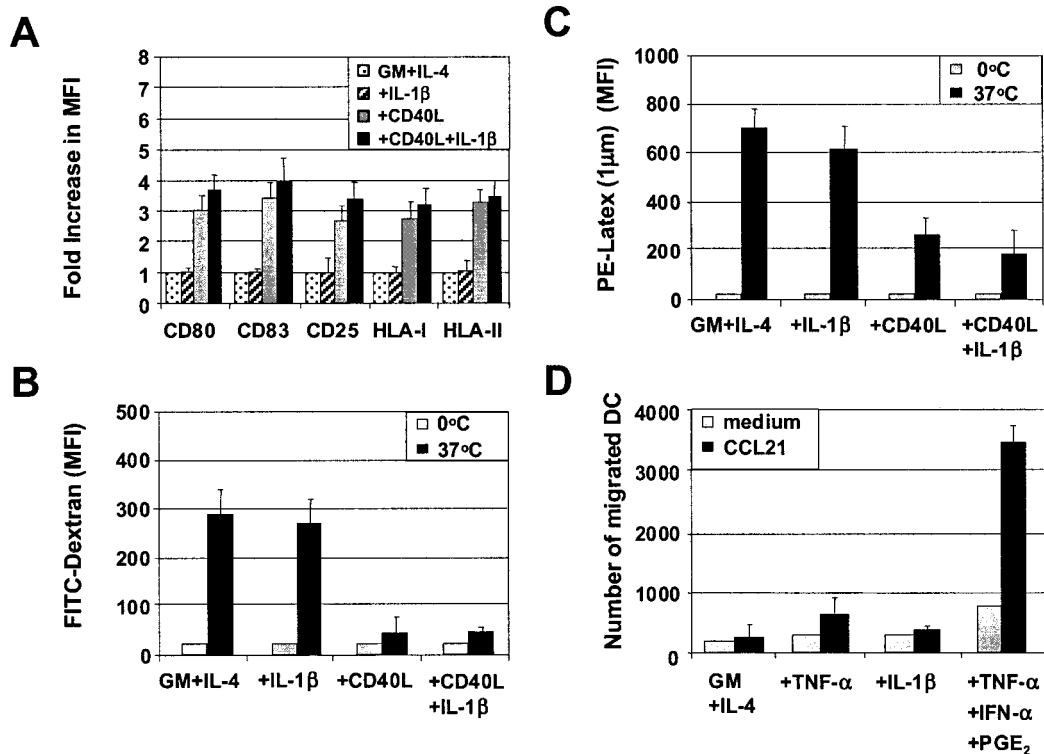


FIGURE 3. Effect of IL-1 β on immature MoDC phenotype and function. MoDC were prepared by culturing purified CD14⁺ monocytes for 7 days in GM-CSF and IL-4. On day 7, CD40L (1 μ g/ml) and/or IL-1 β were added for 3 days. *A*, Surface expression of maturation markers was examined by flow cytometry on day 10. Results are shown as increase of mean fluorescence levels relative to nonstimulated control MoDC (control, set as 1). Figures represent the means \pm SEM of three to five experiments. *B* and *C*, Effect of IL-1 β upon MoDC Ag uptake capacity. Immature and mature MoDC were incubated with FITC-dextran (*B*) or PE-Latex beads (*C*) (1 μ m) at either 4 or 37°C for 30 min. Cells were examined by flow cytometry to assess internalized FITC or PE. The data are presented as the mean fluorescence intensity (MFI) of internalized FITC or PE and represent means \pm SEM of four experiments. *D*, Analysis of migration toward CCL21 (MIP-3 β). Immature MoDC were either nonstimulated or stimulated with either TNF- α alone or IL-1 β alone or combinations of IFN- α 2a, TNF- α , and PGE₂ for 48 h, washed, and examined for migratory capacity toward CCL21 (MIP-3 β) chemokine using transwell chambers. Data are representative of four separate experiments.

3D). This indicates that although IL-1 β can synergize with CD40L to enhance IL-10 and IL-12p70 production, IL-1 β as a single agent is inefficient at altering MoDC phenotypic maturation, migration, or Ag uptake capacity.

Secretion of IFN- γ by allogeneic T lymphocytes stimulated with MoDC

IL-12p70 is an important regulator of IFN- γ secretion by T cells (2–5). We investigated whether the conditions which induced the highest levels of IL-12p70 production by MoDC translated into increased IFN- γ secretion by allogeneic T cells following DC stimulation. MoDC were washed and activated with either CD40L alone or in combination with GM-CSF and IL-4 and/or IL-1 β for 24 h, washed again, and then cultured with allogeneic T cells. Fig. 4A shows that IL-1 β could potentially enhance the ability of CD40L-activated MoDC to stimulate IFN- γ secretion by T cells. Furthermore, the cytokine combination that induced MoDC to secrete the highest levels of IL-12p70 (i.e., GM-CSF, IL-4, IL-1 β , and CD40L) also induced the highest IFN- γ production by T cells (Fig. 4A). Interestingly, DC activated with the combination of CD40L and the T cell-independent factor (IL-1 β) were as potent as DC exposed to CD40L plus IFN- γ at inducing IFN- γ secretion in alloreactive T cells (Fig. 4B).

Examination of IL-1 β secretion by MoDC, monocytes, CD34⁺-derived DC, and PBDC

IL-1 β is predominantly produced by monocytes and macrophages in response to bacterial-derived signals (28). To investigate

whether different DC subsets were comparable in their capacity to produce this cytokine, we measured IL-1 β secretion in the culture SN of monocytes, MoDC, CD34⁺-derived DC, and PBDC following stimulation. All cultures were stimulated in the presence of GM-CSF and IL-4 with either CD40L plus IFN- γ or intact *Escherichia coli*. Table I clearly shows differences with respect to basal and inducible IL-1 β production by the four types of APC examined. Only monocytes and MoDC secreted significant basal levels of IL-1 β (170–210 pg/ml), and this was increased 17- and 30-fold, respectively, following stimulation with intact *E. coli*. CD40L plus IFN- γ enhanced the level of IL-1 β produced by monocytes and to a lesser degree by MoDC (Table I). In contrast, neither CD34⁺-derived DC nor PBDC secreted IL-1 β in response to these stimuli. These results suggest that MoDC are closely related to their monocyte precursors with respect to IL-1 β production, whereas CD34⁺-derived DC and PBDC, which are poor producers of IL-1 β following stimulation, may represent functionally distinct APC populations.

Cytokine secretion by CD34⁺-derived DC and PBDC in response to CD40L and IL-1 β stimulation

Because CD34⁺-derived DC and PBDC differed from MoDC in their capacity to secrete IL-1 β following stimulation, we investigated whether CD40L-mediated cytokine secretion by CD34⁺-derived DC and PBDC could be enhanced by the addition of IL-1 β . CD34⁺-derived DC were generated under serum-free conditions as previously described using GM-CSF, TNF- α , and IL-4, and yielded CD1a⁺ DC in the range of 35–65% of cultured cells (Fig.

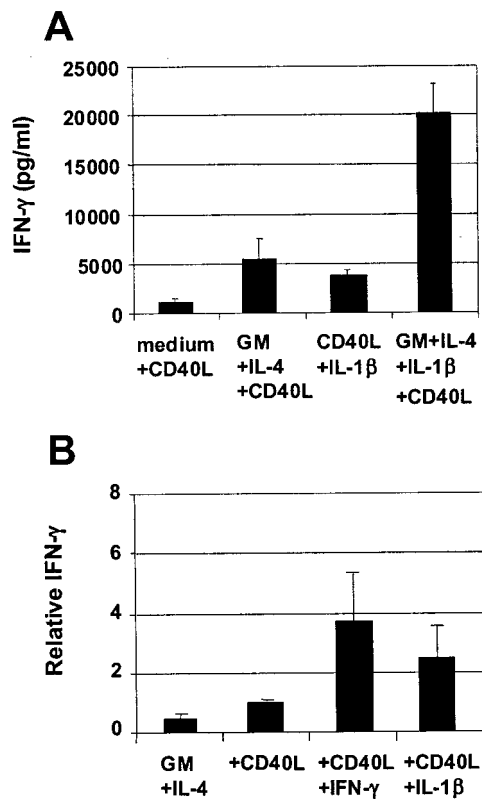


FIGURE 4. Secretion of IFN- γ by allogeneic T cells stimulated by differentially activated MoDC. MoDC were washed and activated with the indicated cytokine stimuli for 24 h. MoDC were then rewashed and DC (1×10^4 /well) were cultured with CD2 $^+$ allogeneic T cells (1×10^5 /well). At day 5, supernatants were harvested and IFN- γ secretion was detected by ELISA. **A**, Data represent the means \pm SD of replicate cultures from a representative experiment ($n = 3$). **B**, Results are shown relative to IFN- γ levels induced by CD40L-activated DC (set as 1). Data are presented as the means \pm SEM of three individual experiments.

5A). CD34 $^+$ -derived DC used in these experiments were phenotypically immature, as they expressed negligible levels of CD80, CD83, and CD86 and represented Langerhans-like cells expressing Lag and E-cadherin rather than interstitial DC, which express CD14 (29). Fig. 5B shows that compared with MoDC, CD34 $^+$ -derived DC did not produce detectable IL-12p70 in response to CD40L in the presence of their own CM containing IL-4. In contrast, the addition of either IL-1 β or IFN- γ to CD40L induced IL-12p70 secretion by CD34 $^+$ -derived DC, and this was further augmented when IL-1 β and IFN- γ were combined (Fig. 5B). Although IL-1 β and/or IFN- γ significantly enhanced the levels of IL-12p70 production by CD34 $^+$ -derived DC, these were still 10-fold lower than the levels produced by MoDC under the same stimulation conditions (Figs. 1C and 2C), highlighting a striking

difference in the cytokine-secreting potential of these two in vitro-generated DC populations. Furthermore, unlike MoDC, which secrete high levels of IL-10 in response to these stimuli, IL-10 was not substantially produced by CD34 $^+$ -derived DC under these conditions. Finally, as with MoDC, IL-1 β could potentially enhance the ability of CD40L-activated CD34 $^+$ -derived DC to stimulate IFN- γ secretion by T cells. However, consistent with their lower IL-12p70-secreting potential, CD34 $^+$ -derived DC induced lower levels of IFN- γ in T cells than did MoDC (Fig. 5C).

Cytokine secretion by PBDC in response to CD40L and IL-1 β stimulation

We next examined the effect of IL-1 β upon CD40L-mediated cytokine secretion by PBDC isolated from healthy donors or expanded in vivo by the administration of FL to cancer patients. FL-generated PBDC were isolated from patients with malignant melanoma. FL-generated PBDC preparations were enriched by depletion of cells expressing CD3, CD14, CD16, CD19, CD56, and glycoprotein A using Abs and magnetic beads. PBDC were also FACS sorted on the basis of CD1b/c and HLA-DR expression (Fig. 6, C and D). As previously reported, FL-generated PBDC were phenotypically immature when examined immediately ex vivo, expressing negligible levels of CD80 and CD83 and relatively low levels of CD86 and HLA-DR (Fig. 6A and Refs. 30 and 31). However, maturation was rapidly induced upon in vitro culture, resulting in up-regulation in the expression of CD80, CD83, CD86, and HLA-DR molecules (Fig. 6A). Although isolated from cancer patients, FL-generated PBDC were not functionally defective as assessed by in vitro functional studies, but rather were potent stimulators of allogeneic T cell proliferation and cytokine secretion comparable to PBDC isolated from the blood of untreated, healthy individuals (Refs. 30 and 31 and M. Jefford, T. Luft, K.-A. Masterman, T. Toy, T. Beecroft, M. Shackelton, P. Parente, I. Davis, J. Cebon, and E. Marakovskiy, manuscript in preparation). In contrast to either MoDC or CD34 $^+$ -derived DC, freshly isolated PBDC from FL-treated cancer patients (Fig. 6B) or from healthy donors (Fig. 6C) did not produce detectable levels of IL-12p70 in response to a 72-h culture with the indicated stimuli. However, CD40L plus IL-1 β did increase IL-6 and IL-10 secretion by PBDC, indicating that PBDC could be stimulated to secrete cytokines in vitro and that this was modulated by IL-1 β (Fig. 6, B and C). Finally, induction of IL-12p70 by PBDC in response to stimuli previously shown to induce IL-12p70 in MoDC (such as the combination of GM-CSF plus IL-4 plus CD40L plus IFN- γ and intact *E. coli* bacteria as a source of pathogen signals) (24–26) induced less than 100 pg/ml of IL-12p70 (Fig. 6D), this being the highest IL-12p70 level detected from three separate healthy donors. These results demonstrate that IL-1 β enhances CD40L-mediated cytokine secretion in all three DC populations examined, but that the type (i.e., IL-6, IL-10, and IL-12p70) and levels of cytokines induced varies among the DC subsets.

Table I. IL-1 β secretion by DC subsets and monocytes in response to different soluble stimuli^a

Stimuli	Monocytes ^b	McDC ^b	CD34 $^+$ -derived DC ^b	PBDC ^b
GM+IL-4 +CD40L	212 \pm 31 (8)	174 \pm 44 (7)	5 \pm 4 (15)	10 \pm 4 (5)
+CD40L	ND	166 \pm 25 (5)	4 \pm 8 (15)	10 \pm 5 (5)
+CD40L +IFN- γ	1344 \pm 417 (8)	224 \pm 41 (7)	45 \pm 27 (7)	12 \pm 5 (5)
<i>E. coli</i>	6161 \pm 2421 (8)	3024 \pm 1060 (6)	94 \pm 60 (4)	48 \pm 16 (5)

^a DC subsets and monocytes were pooled and cultured (1×10^5 cells/ml) for 3 days with the indicated stimuli in their own condition medium (McDC-CM). Culture SN were examined for IL-1 β production by ELISA. Data represent the means \pm SEM of experiments from separate donors. Number of donors is listed in parentheses.

^b Concentration of IL-1 β in picograms per milliliter.

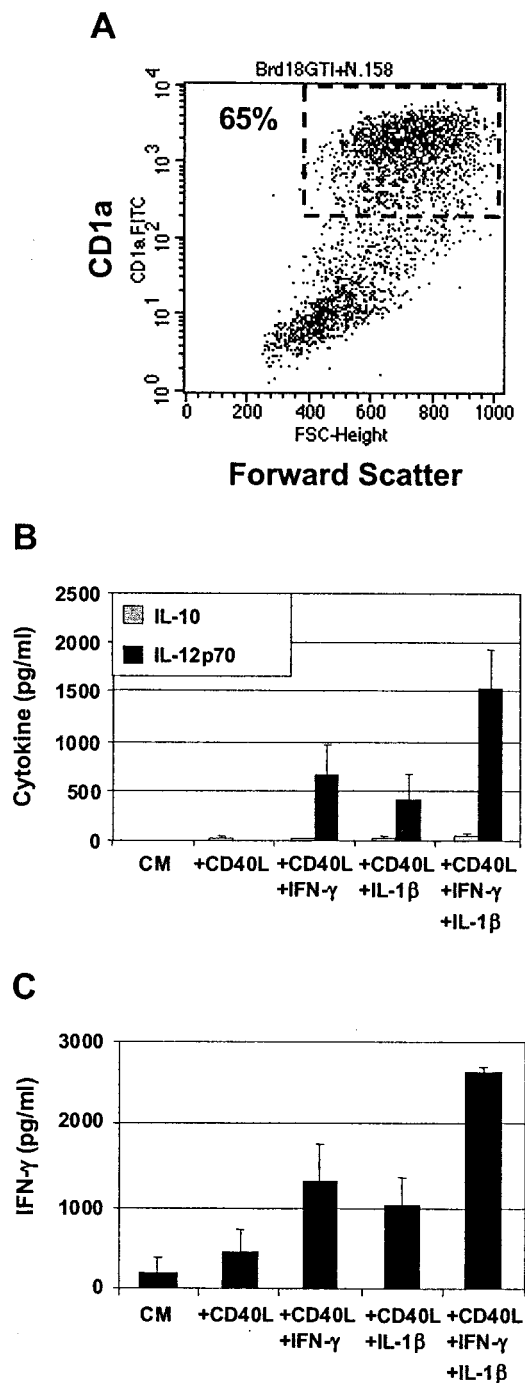


FIGURE 5. Cytokine secretion by CD34⁺-derived DC. CD34⁺-derived DC were generated from purified CD34⁺ progenitor cells in a serum-free culture system containing GM-CSF, TNF- α , and IL-4 for 14 days. CD34⁺-derived DC (1×10^5 /ml) were cultured either in their own CM or in CM with the indicated cytokine stimuli for 3 days. Culture SN were examined for cytokine production by ELISA. *A*, At day 14, the cultures contained between 35 and 65% CD1a⁺ DC. *B*, Secretion of IL-10 and IL-12p70. *C*, Secretion of IFN- γ by allogeneic T cells stimulated by differentially activated CD34⁺-derived DC. CD34⁺-derived DC were activated with the indicated cytokine stimuli for 48 h, washed, and then cultured (1×10^4 /well) with CD2⁺ allogeneic T cells (1×10^5 /well). At day 5, supernatants were harvested and IFN- γ secretion was detected by ELISA. Data represent the means \pm SEM of experiments from three separate donors.

Discussion

This study identifies IL-1 β as a T cell-independent modulator of CD40L-induced DC activation. IL-1 β alone was a poor stimulator

of DC phenotypic maturation and function (e.g., Ag uptake capacity, migration toward chemokines, and cytokine secretion). However, when combined with CD40L, IL-1 β enhanced the secretion of cytokines from three different DC populations: MoDC, CD34⁺-derived DC, and PBDC. The cytokine profile and quantities of cytokines secreted differed for these three DC types. For MoDC, IL-1 β enhanced CD40L-mediated IL-6, IL-10, and IL-12p70 secretion. For CD34⁺-derived DC, IL-1 β enhanced CD40L-mediated IL-6 (data not shown) and IL-12p70 but not IL-10, while for CD1b/c⁺HLA-DR⁺ PBDC, IL-1 β enhanced CD40L-mediated IL-6 and IL-10 but not IL-12p70 secretion. The lack of detectable IL-12p70 production by PBDC was not due to blunted function as a result of 1) cryopreservation and thaw before use in experiments, 2) expansion *in vivo* with FL, or 3) isolation from cancer patients, because sorted PBDC isolated from fresh blood from healthy donors also demonstrated low/negligible production of IL-12p70 following stimulation with CD40L (Fig. 6C). Finally, even when stimuli known to potently induce IL-12p70 secretion in MoDC were combined (e.g., GM-CSF plus IL-4 plus CD40L plus IFN- γ plus intact *E. coli*) (24, 26), they induced low levels of IL-12p70 (<100 pg/ml) by sorted PBDC in three of three healthy donors (Fig. 6D).

IL-1 β potently enhanced the ability of CD40L-activated MoDC (Fig. 4) and CD34⁺-derived DC (Fig. 5C) to stimulate IFN- γ secretion by T cells. Interestingly, PBDC were also potent stimulators of IFN- γ secretion by T cells, suggesting PBDC secrete cytokines other than IL-12p70 for the induction of IFN- γ secretion by T cells (M. Jefford, T. Luft, K.-A. Masterman, T. Toy, T. Beecroft, M. Shackleton, P. Parente, I. Davis, J. Cebon, and E. Marakovsky, manuscript in preparation). This finding suggests that PBDC are functionally different from MoDC and CD34⁺-derived DC and/or may represent a different stage of DC maturation. Finally, PBDC were similar to CD34⁺ DC in that they both secreted 10-fold lower levels of cytokines following stimulation as compared with MoDC, suggesting that MoDC were functionally distinct from either CD34⁺-derived DC and PBDC even when IL-1 β was present in the stimulation conditions.

IL-1 β not only enhanced CD40L-mediated cytokine secretion but could also synergize with IL-4 (and to a lesser extent with IL-13; $n = 3$; data not shown) to further enhance CD40L-mediated IL-12p70 secretion by MoDC. The increased IL-12p70 production resulted in increased IFN- γ secretion by alloreactive T cells following DC stimulation. Furthermore, neutralizing Abs to either IL-1R or IL-4 blocked the ability of CD40L to induce IL-12p70 secretion by MoDC, suggesting that both IL-1 and IL-4 are important cofactors for CD40L-mediated cytokine secretion. The neutralizing effects of anti-IL-4 mAb confirm the findings of Kalinski and colleagues (25).

The effect of IL-1 β as a cofactor for CD40L-mediated bioactivity for MoDC was recently demonstrated by Wesa and Galy (32). In that study, they demonstrate the role of IL-1 β on enhancing CD40L-mediated IL-12p70 secretion (32). The present study also demonstrates that monocytes and MoDC were potent producers of IL-1 β in response to intact *E. coli*, whereas CD34⁺-derived DC and PBDC were not. This suggests that MoDC are functionally related to their monocyte precursors with respect to IL-1 β secretion, whereas CD34⁺-derived DC and PBDC, which are poor producers of IL-1 β , represent functionally distinct APC populations. Furthermore, this finding also identifies a novel mechanism by which monocytes and their progeny (i.e., macrophage or MoDC) can potentiate CD40L-mediated DC activation *in vivo* by secreting IL-1 β at sites of infection and/or inflammation. Finally, because certain DC subsets and their precursors (e.g., monocytes) produce IL-1 β in response to specific stimuli while others do not (CD34⁺

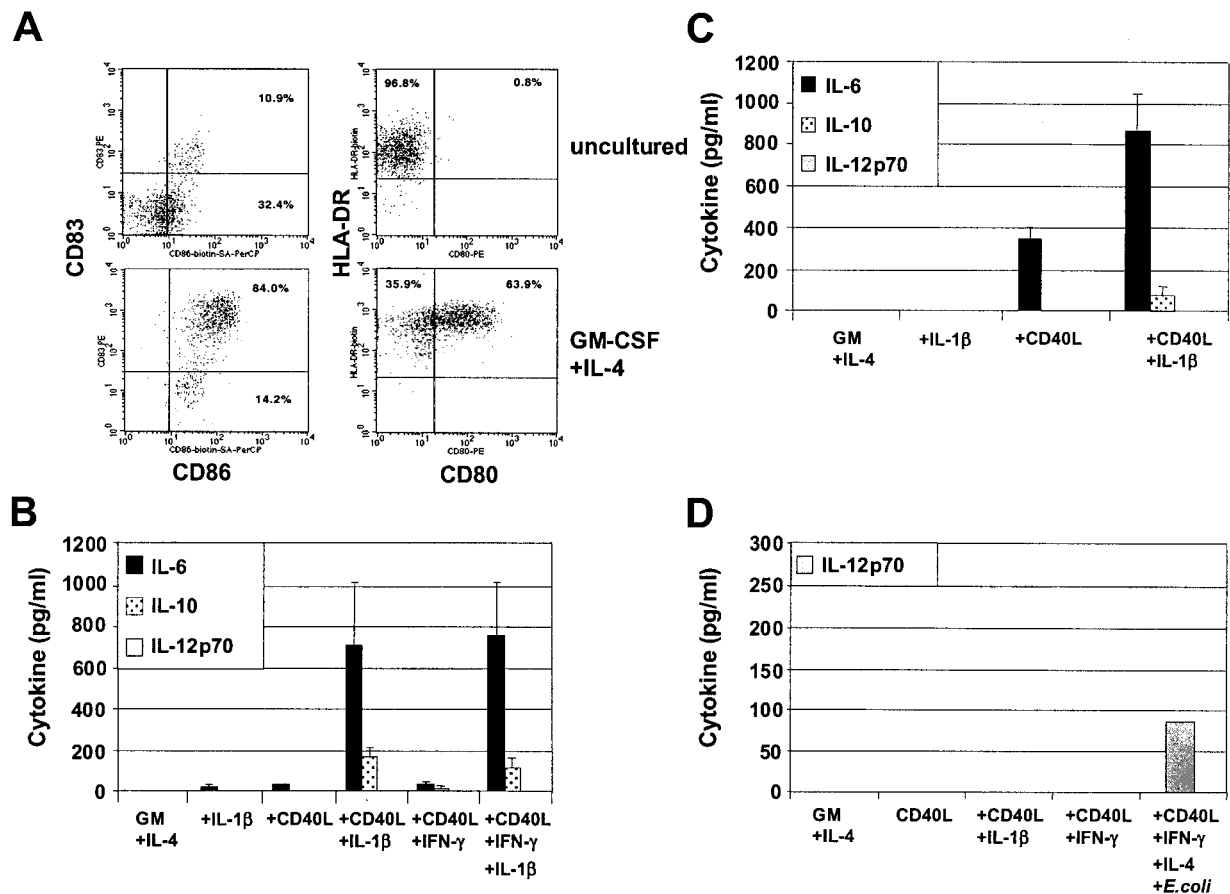


FIGURE 6. Phenotypic analysis of and cytokine secretion by PBDC. PBDC were purified by negative depletion from the peripheral blood of healthy donors or melanoma patients treated with FL for 14 consecutive days. *A*, Expression of CD80, CD83, CD86, and HLA-DR on FL-generated CD1c⁺HLA-DR⁺ PBDC immediately ex vivo or following a 15-h in vitro culture with GM-CSF and IL-4. *B–D*, FL-generated PBDC from patients with stage III malignant melanoma (*B*) or FACS sorted CD1b/c⁺HLA-DR⁺ PBDC (*C* and *D*) from healthy donors were cultured (1×10^5 /well) in the presence of GM-CSF and IL-4 with the indicated cytokine stimuli for 3 days. Culture SN were examined for cytokine production by ELISA. Data represent the means \pm SEM of experiments from seven separate FL-treated donors (*B*), three healthy donors (*C*), and a representative experiment from three separate healthy donors (*D*).

DC and CD1c⁺ PBDC, our findings suggest the potential for immunologic cross talk among different DC subsets located in or recruited to inflammatory sites.

DC secrete high levels of cytokines, such as IL-12p70, in response to two types of stimuli. These can be classified as T cell-dependent (e.g., CD40L) and T cell-independent stimuli derived from pathogens (intact bacteria, dsRNA, etc.) (10–13, 27). However, cross-linking CD40 is suboptimal at inducing IL-12p70 secretion by DC (9). Additional T cell-derived signals, such as IFN- γ (7, 9, 33, 34, 35) or IL-4 (24–26), are essential for enhancing CD40L-mediated IL-12p70 secretion. However, normal IL-12-mediated functions in IFN- γ ^{-/-} mice suggest the existence of IFN- γ -independent regulation of IL-12 (36, 37). Although IL-4 has been reported to inhibit IL-12 secretion (38), our study confirms recently published data demonstrating that IL-4 enhances IL-12p70 production by human MoDC (24–26). This is in line with reports showing that human IL-4-producing T cell clones enhance IL-12 production by DC (39).

In vivo, the potent combination of CD40L and T cell-derived cytokines (9, 24–26) required for maximal IL-12p70 production likely requires T cell-Ag interactions either during priming of rare, naive T cells in the lymph nodes or during the restimulation of memory T cells at effector sites. Therefore, an alternative mechanism of rapid CD40L access is required at the ear-

liest stages of primary infection where cognate, effector T cells will either be rare or temporally delayed by the requirement for priming in the lymph nodes. Non-T cells such as inflamed somatic cells (smooth muscle cells (21) and vascular endothelial cells (21)) and activated innate immune response effectors (eosinophils (19), activated platelets (20), macrophages (21), and DC (22, 23)) can fulfill this role by rapidly providing both CD40L and IL-1 β at the site of infection/inflammation. Therefore, in this context, the interaction of CD40L with IL-1 β in the periphery would provide a mechanism for linking innate and adaptive immunity at the earliest stages of the inflammatory response.

Further support that innate immune response-derived factors are important for IL-12p70 secretion in vivo was provided by Schulz and colleagues (27), who demonstrated that CD40L is insufficient at inducing IL-12p70 secretion by DC in vivo but requires an innate signal induced by microbial stimuli. This signal was not IFN- γ , because the effect was not diminished in IFN- γ ^{-/-} mice (27). They concluded that although CD40 signaling is important for amplifying IL-12p70 secretion by DC, it is initiated by a microbe-mediated innate signal (27). One possibility is that the microbe-mediated signal inducing IL-12 is direct interaction with the pathogen (40–43). However, certain

pathogens can inhibit IL-12 production (44–46), and thus alternative IL-12-inducing signals will likely exist to ensure optimal anti-pathogen immune responses. IL-1 β , which is secreted by monocytes, macrophages, and MoDC following microbe stimulation, may represent such an innate signal which can synergize with non-T cell-derived CD40L during the earliest stages of infection and inflammation.

Synergy between CD40L and IL-1 β has been previously reported in vivo in other settings. For instance, CD40L and IL-1 synergistically enhance the production of IL-6, IL-8, and RANTES, and additively stimulated monocyte chemoattractant protein-1 production during the activation of human tubular renal epithelial cells (47). This suggests a novel role for CD40L and IL-1 in the recruitment of cells into inflammatory sites. Furthermore, IL-1 β potently enhances CD40 expression on tubular renal epithelial cells (47) and human blood DC (48) and CD40L expression on smooth muscle cells, vascular endothelial cells, and macrophages (21). Conversely, CD40 signaling up-regulates the secretion of bioactive IL-1 β by smooth muscle cells and endothelial cells (21, 49), as well as by monocytes (50) and DC (51, 52). These reports all emphasize that CD40 signaling and IL-1 β are physiologically coupled during various stages of inflammatory responses.

Finally, T cell-independent activation of DC via CD40L and IL-1 β may lack the Ag-specific dimension of memory T cell responses, thus provoking considerable pathology, including autoimmune damage. This may underlie the inflammatory and immunological responses seen in pathogen-independent diseases such as atherosclerosis. In this regard, CD40L is expressed in atheromas (21, 53), while elevated IL-1 β levels correlate with the severity of atherosclerosis (54–56). Indeed, IL-12 and IFN- γ have been found abundantly in atherosclerotic plaques (57, 58). In this way, the pathogenesis of atherosclerosis may represent the result of cytokine loops involving IL-1 β , CD40L, and IL-12 without requiring Ag-specific (autoreactive) T cells as primary etiological factors.

The findings reported in this work have several clinical implications. The capacity of nonimmune cells to express CD40L following stress or inflammation, and potentially to induce DC activation in the presence of IL-1 β , suggests the involvement of complex cellular networks at inflammatory sites that cannot easily be studied using in vitro systems. However, fully understanding these networks is critical to identifying how specific immune responses can be optimally generated or blunted, as in the case of immune evasion by pathogens or developing tumors. This study contributes to the understanding of how DC activation may be initially modulated at localized sites of infection in the absence of T cells and presence of innate signals. Our findings suggest a potential role for immunological cross-talk among a variety of different cell types located in or recruited to these inflammatory sites. This study may also highlight dual roles for CD40L in the lymph nodes during priming and in the periphery during inflammatory processes. Finally, the conspicuous absence of inflammation within the tumor microenvironment may point to specific mechanisms used by tumors to evade effective immune targeting. The complete understanding of how innate and adaptive immune responses are linked will be a crucial step in understanding mechanisms underlying autoimmunity, chronic inflammatory diseases, and immune escape by tumors.

Acknowledgments

We thank F. Battye, D. Kaminaris, and V. Lapatis for assistance with cell sorting. We thank J. Nielissen for assistance with manuscript preparation.

References

- Banchereau, J., and R.M., Steinman. 1998. Dendritic cells and the control of immunity. *Nature* 392:245.
- Hsieh, C. S., S. E. Macatonia, C. S. Tripp, S. F. Wolf, A. O'Garra, and K. M. Murphy. 1993. Development of TH1 CD4⁺ T cells through IL-12 produced by *Listeria*-induced macrophages. *Science* 260:547.
- Tripp, C. S., S. F. Wolf, and E. R. Unanue. 1993. Interleukin 12 and tumor necrosis factor α are costimulators of interferon γ production by natural killer cells in severe combined immunodeficiency mice with listeriosis, and interleukin 10 is a physiologic antagonist. *Proc. Natl. Acad. Sci. USA* 90:3725.
- Manetti, R., P. Parronchi, M. G. Giudizi, M. P. Piccini, E. Maggi, G. Trinchieri, and S. Romagnani. 1993. Natural killer cell stimulatory factor (interleukin 12 (IL-12)) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. *J. Exp. Med.* 177:1199.
- Cella, M., D. Scheidegger, K. Palmer-Lehmann, P. Lane, A. Lanzavecchia, and G. Alber. 1996. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J. Exp. Med.* 184:747.
- de Saint-Vis, B., I. Fugier-Vivier, C. Massacrier, C. Gaillard, B. Vanbervliet, S. Ait-Yahia, J. Banchereau, Y. J. Liu, S. Lebecque, and C. Caux. 1998. The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. *J. Immunol.* 160:1666.
- Kalinski, P., J. H. Schuitemaker, C. M. Hilkens, E. A. Wierenga, and M. L. Kapsenberg. 1999. Final maturation of dendritic cells is associated with impaired responsiveness to IFN- γ and to bacterial IL-12 inducers: decreased ability of mature dendritic cells to produce IL-12 during the interaction with Th cells. *J. Immunol.* 162:3231.
- Koch, F., U. Stanzl, P. Jennewein, K. Janke, C. Heufler, E. Kämpgen, N. Romani, and G. Schuler. 1996. High level IL-12 production by murine dendritic cells: upregulation via MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. *J. Exp. Med.* 184:741.
- Snijders, A., P. Kalinski, C. M. Hilkens, and M. L. Kapsenberg. 1998. High-level IL-12 production by human dendritic cells requires two signals. *Int. Immunol.* 10:1593.
- von Stebut, E., Y. Belkaid, T. Jakob, D. L. Sacks, and M. C. Udey. 1998. Uptake of *Leishmania major* amastigotes results in activation and interleukin 12 release from murine skin-derived dendritic cells: implications for the initiation of anti-*Leishmania* immunity. *J. Exp. Med.* 188:1547.
- Henderson, R. A., S. C. Watkins, and J. L. Flynn. 1997. Activation of human dendritic cells following infection with *Mycobacterium tuberculosis*. *J. Immunol.* 159:635.
- Jakob, T., P. S. Walker, A. M. Krieg, M. C. Udey, and J. C. Vogel. 1998. Activation of cutaneous dendritic cells by CpG-containing oligodeoxynucleotides: a role for dendritic cells in the augmentation of Th1 responses by immunostimulatory DNA. *J. Immunol.* 161:3042.
- Cella, M., S. Mariolino, S. Yoichi, H. Langen, I. Julkunen, and A. Lanzavecchia. 1999. Maturation, activation, and protection of dendritic cells induced by double-stranded RNA. *J. Exp. Med.* 189:821.
- Stuhler, G., A. Zobywalski, F. Grunebach, P. Brossart, V. L. Reichardt, H. Barth, S. Stevanovic, W. Brugger, L. Kanz, and S. F. Schlossman. 1999. Immune regulatory loops determine productive interactions within human T lymphocyte-dendritic cell clusters. *Proc. Natl. Acad. Sci. USA* 96:1532.
- Ridge, J. P., F. Di Rosa, and P. Matzinger. 1998. A conditioned dendritic cell can be a temporal bridge between a CD4⁺ T-helper and a T-killer cell. *Nature* 393:474.
- Bennett, S. R. M., F. R. Carbone, F. Karamalis, R. A. Flavell, J. F. Miller, and W. R. Heath. 1998. Help for cytotoxic-T-cell responses is mediated by CD40 signalling. *Nature* 393:478.
- Schoenberger, S. P., R. E. Toes, E. I. van der Voort, R. Offringa, and C. J. Melief. 1998. T-cell help for cytotoxic T lymphocytes is mediated by CD40-CD40 interactions. *Nature* 393:480.
- Gramer, A. C., M. C. Bergman, Y. Miura, K. Fujita, L. S. Davis, and P. E. Lipsky. 1995. The CD40 ligand expressed by human B cells costimulates B cell responses. *J. Immunol.* 154:4996.
- Gauchat, J. F., S. Henchoz, D. Fattah, G. Mazzei, J. P. Aubry, T. Jomotte, L. Dash, K. Page, R. Solari, D. Aldebert, et al. 1995. CD40 ligand is functionally expressed on human eosinophils. *Eur. J. Immunol.* 25:863.
- Henn, V., J. R. Slupsky, M. Gräfe, I. Anagnostopoulos, R. Förster, G. Müller-Berghaus, and R. A. Kroczeck. 1998. CD40 ligand on activated platelets triggers an inflammatory reaction of endothelial cells. *Nature* 391:591.
- Mach, F., U. Schönbeck, G. K. Sukhova, T. Bourcier, J. Y. Bonnefoy, J. S. Pober, and P. Libby. 1997. Functional CD40 ligand is expressed on human vascular endothelial cells, smooth muscle cells, and macrophages: implications for CD40-CD40 ligand signaling in atherosclerosis. *Proc. Natl. Acad. Sci. USA* 94:1931.
- Pinchuk, L. M., S. J. Klaus, D. M. Magaletti, G. V. Pinchuk, J. P. Norsen, and E. A. Clark. 1996. Functional CD40 ligand expressed by human blood dendritic cells is up-regulated by CD40 ligation. *J. Immunol.* 157:4363.
- Salgado, C. G., K. Nakamura, M. Sugaya, Y. Tada, A. Asahina, Y. Koyama, S. Irie, and K. Tamaki. 1999. Functional CD40 ligand is expressed on epidermal Langerhans cells. *J. Leukocyte Biol.* 66:281.
- Hochrein, H., M. O'Keefe, T. Luft, S. Vandenabeele, E. Maraskovsky, and K. Shortman. 2000. IL-4 is a major regulatory cytokine governing bioactive IL-12 production by mouse and human dendritic cells. *J. Exp. Med.* 192:823.
- Kalinski, P., H. H. Smits, J. H. N. Schuitemaker, P. L. Vieira, M. van Eijk, E. C. de Jong, E. A. Wierenga and M. L. Kapsenberg. 2000. IL-4 is a mediator of IL-12p70 induction by human Th2 cells: reversal of polarized Th2 phenotype by dendritic cells. *J. Immunol.* 165:1877.

26. Ebner, S., G. Ratzinger, B. Krosbacher, M. Schmuth, A. Weiss, R. Daniela, R. A. Kroccek, M. Herold, C. Heufler, P. Fritsch, and N. Romani. 2001. Production of IL-12 by human monocyte-derived dendritic cells is optimal when the stimulus is given at the onset of maturation, and is further enhanced by IL-4. *J. Immunol.* 166:633.
27. Schulz, O., D. E. Edwards, M. Schito, J. Alberti, S. Manickasingham, A. Sher, and C. Reis e Sousa. 2000. CD40 triggering of heterodimeric IL-12p70 production by dendritic cells in vivo requires a microbial priming signal. *Immunity* 13:453.
28. March, C. J., B. Mosley, A. Larsen, D. P. Cerretti, G. Braedt, V. Price, S. Gillis, C. S. Henney, S. R. Kronheim, K. Grabstein, et al. 1985. Cloning, sequence and expression of two distinct human interleukin-1 complementary DNAs. *Nature* 315:641.
29. Luft, T., K. C. Pang, E. Thomas, C. J. Bradley, H. Savoia, J. Trapani, and J. Cebon. 1998. A serum-free culture model for studying the differentiation of human dendritic cells from adult CD34⁺ progenitor cells. *Exp. Hematol.* 26:489.
30. Pulendran, B., J. Banchemereau, S. Burkeholder, E. Kraus, E. Guinet, C. Chaloujini, D. Caron, C. R. Maliszewski, J. Davoust, J. Fay, and K. Paluka. 2000. Flt3-ligand and granulocyte colony-stimulating factor mobilize distinct human dendritic cell subsets in vivo. *J. Immunol.* 165:566.
31. Maraskovsky, E., E. Daro, E. Roux, M. Teepe, C. R. Maliszewski, J. Hoek, D. Caron, M. E. Lebsack, and H. J. McKenna. 2000. In vivo generation of human dendritic cell subsets by Flt3 ligand. *Blood* 96:878.
32. Wesa, A. K., and A. Galy. 2001. IL-1 β induces dendritic cells to produce IL-12. *Int. Immunol.* 13:1053.
33. Kato, T., R. Hakamada, H. Yamane, and H. Nariuchi. 1996. Induction of IL-12 p40 messenger RNA expression and IL-12 production of macrophages via CD40-CD40 ligand interaction. *J. Immunol.* 156:3932.
34. Kennedy, M. K., K. S. Picha, W. C. Fanslow, K. H. Grabstein, M. R. Alderson, K. N. Clifford, W. A. Chin, and K. M. Mohler. 1996. CD40/CD40 ligand interactions are required for T cell-dependent production of interleukin-12 by mouse macrophages. *Eur. J. Immunol.* 26:370.
35. Ma, X., J. M. Chow, G. Gri, G. Carra, F. Gerosa, S. F. Wolf, R. Dzialo, and G. Trinchieri. 1996. The interleukin 12 p40 gene promoter is primed by interferon γ in monocytic cells. *J. Exp. Med.* 183:147.
36. Scharon-Kersten, T. M., T. A. Wynn, E. Y. Denkers, S. Bala, E. Grunvald, S. Hieny, R. T. Gazzinelli, and A. Sher. 1996. In the absence of endogenous IFN- γ , mice develop unimpaired IL-12 responses to *Toxoplasma gondii* while failing to control acute infection. *J. Immunol.* 157:4045.
37. Heinzl, F. P., R. M. Rerko, F. Ahmed, and A. M. Hujer. 1996. IFN- γ -independent production of IL-12 during murine endotoxemia. *J. Immunol.* 157:4521.
38. Levings, M. K., and J. W. Schrader. 1999. IL-4 inhibits the production of TNF- α and IL-12 by STAT6-dependent and -independent mechanisms. *J. Immunol.* 162:5224.
39. Vezzio, N., M. Sarfati, L. P. Yang, C. E. Demeure, and G. Delespesse. 1996. Human Th2-like cell clones induce IL-12 production by dendritic cells and may express several cytokine profiles. *Int. Immunol.* 8:1963.
40. Castro, A. G., R. A. Silva, and R. Appelberg. 1995. Endogenously produced IL-12 is required for the induction of protective T cells during *Mycobacterium avium* infections in mice. *J. Immunol.* 155:2013.
41. Liu, W., and R. J. Kurlander. 1995. Analysis of the interrelationship between IL-12, TNF- α , and IFN- γ production during murine listeriosis. *Cell. Immunol.* 163:260.
42. Thompson-Snipes, L., E. Skamene, and D. Radzioch. 1998. Acquired resistance but not innate resistance to *Mycobacterium bovis* bacillus Calmette-Guérin is compromised by interleukin-12 ablation. *Infect. Immun.* 66:5268.
43. Derrico, C. A., and K. J. Goodrum. 1996. Interleukin-12 and tumor necrosis factor α mediate innate production of γ interferon by group B *Streptococcus*-treated splenocytes of severe combined immunodeficiency mice. *Infect. Immun.* 64:1314.
44. Braun, M. C., J. He, C. Y. Wu, and B. L. Kelsall. 1999. Cholera toxin suppresses interleukin (IL)-12 production and IL-12 receptor β 1 and β 2 chain expression. *J. Exp. Med.* 189:541.
45. Fugier-Vivier, I., C. Servet-Delprat, P. Rivailler, M. C. Rissoan, Y. J. Liu, and C. Rabourdin-Combe. 1997. Measles virus suppresses cell-mediated immunity by interfering with the survival and functions of dendritic and T cells. *J. Exp. Med.* 186:813.
46. Marshall, J. D., J. Chehimi, G. Gri, J. R. Kostman, L. J. Montaner, and G. Trinchieri. 1999. The interleukin-12-mediated pathway of immune events is dysfunctional in human immunodeficiency virus-infected individuals. *Blood* 94:1003.
47. van Kooten, C., X. van der Linde, A. M. Woltman, L. A. van Es, and M. R. Daha. 1999. Synergistic effect of interleukin-1 and CD40L on the activation of human renal tubular epithelial cells. *Kidney Int.* 56:41.
48. McLellan, A. D., R. V. Sorg, L. A. Williams, and D. N. Hart. 1996. Human dendritic cells activate T lymphocytes via a CD40:CD40 ligand-dependent pathway. *Eur. J. Immunol.* 26:1204.
49. Schönbeck, U., F. Mach, J. Y. Bonnefoy, H. Loppnow, H. D. Flad, and P. Libby. 1997. Ligation of CD40 activates interleukin 1 β -converting enzyme (caspase-1) activity in vascular smooth muscle and endothelial cells and promotes elaboration of active interleukin 1 β . *J. Biol. Chem.* 272:19569.
50. Kiener, P. A., P. Moran-Davis, B. M. Rankin, A. F. Wahl, A. Aruffo, and D. Hollenbaugh. 1995. Stimulation of CD40 with purified soluble gp39 induces proinflammatory responses in human monocytes. *J. Immunol.* 155:4917.
51. Caux, C., C. Massacrier, B. Vanbervliet, B. Dubois, C. Van Kooten, I. Durand, and J. Banchemereau. 1994. Activation of human dendritic cells through CD40 cross-linking. *J. Exp. Med.* 180:1263.
52. Gardella, S., C. Andrei, S. Costigliolo, L. Olcese, M. R. Zocchi, and A. Rubartelli. 2000. Secretion of bioactive interleukin-1 β by dendritic cells is modulated by interaction with antigen specific T cells. *Blood* 95:3809.
53. Mach, F., U. Schönbeck, G. K. Sukhova, E. Atkinson, and P. Libby. 1998. Reduction of atherosclerosis in mice by inhibition of CD40 signalling. *Nature* 394:200.
54. Moyer, C. F., D. Sajuthi, H. Tulli, and J. K. Williams. 1991. Synthesis of IL-1 α and IL-1 β by arterial cells in atherosclerosis. *Am. J. Pathol.* 138:951.
55. Ku, G., N. S. Doherty, J. A. Wolos, and R. L. Jackson. 1988. Inhibition by probucol of interleukin 1 secretion and its implication in atherosclerosis. *Am. J. Cardiol.* 62:77B.
56. Shimokawa, H., A. Ito, Y. Fukumoto, T. Kadokami, R. Nakaike, M. Sakata, T. Takayanagi, K. Egashira, and A. Takeshita. 1996. Chronic treatment with interleukin-1 β induces coronary intimal lesions and vasospastic responses in pigs in vivo: the role of platelet-derived growth factor. *J. Clin. Invest.* 97:769.
57. Uyemura, K., L. L. Demer, S. C. Castle, D. Jullien, J. A. Berliner, M. K. Gately, R. R. Warrier, N. Pham, A. M. Fogelman, and R. L. Modlin. 1996. Cross-regulatory roles of interleukin (IL)-12 and IL-10 in atherosclerosis. *J. Clin. Invest.* 97:2130.
58. Lee, T. S., H. C. Yen, C. C. Pan, and L. Y. Chau. 1999. The role of interleukin 12 in the development of atherosclerosis in ApoE-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* 19:734.