Activation of Human Dendritic Cells through CD40 Cross-linking

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Summary

Dendritic cells, the professional antigen-presenting cells (APC) involved in T cell priming, express CD40, a molecule which triggering plays a key role in B cell growth and differentiation as well as monocyte activation. Herein we demonstrate that dendritic Langerhans cells (D-Lc) generated by culturing cord blood $CD34^+$ progenitor cells with granulocyte/macrophage colony-stimulating and tumor necrosis factor α (TNF-α) express functional CD40 at a density higher than that found on B cells. Culturing D-Lc on CD40-ligand (CD40L) transfected L cells allowed D-Lc survival as 50 ± 15% of seeded cells were recovered after 4 d while only 5% survived over control L cells. CD40 activation induced important morphological changes with a reduction of cytoplasmic content and a remarkable increase of dendrite development as well as an altered phenotype. In particular, CD40 triggering induced maintenance of high levels of major histocompatibility complex class II antigens and upregulation of accessory molecules such as CD58, CD80 (B7-1) and CD86 (B7-2). CD40 engagement also seems to turn on D-Lc maturation as illustrated by upregulation of CD25, a molecule usually expressed on interdigitating dendritic cells of secondary lymphoid organs. Finally, CD40 activated D-Lc secreted a limited set of cytokines (TNF-α, IL-8, and macrophage inflammatory protein 1α [MIP-1α]) whereas a similar activation induced elutriated monocytes to secrete IL-1α, IL-1β, IL-6, IL-8, IL-10, TNF-α, and MIP-1α. As D-Lc activated T cells upregulated CD40L, it is likely that CD40 activation of D-Lc observed herein with a fibroblast cell line stably expressing CD40L, mimics physiological interactions between dendritic cells and T cells.

The CD40 antigen (1, for review) was identified by monoclonal antibodies reacting with carcinomas and B cells (2) and showing costimulatory effects for B lymphocytes (3). It is a 50-kD glycoprotein which belongs to the TNF receptor superfamily (4). Cross-linking of CD40, in conjunction with IL-4, was found to induce B cells to undergo long-term growth, as well as isotype switching, whereas addition of IL-10 results in B cell differentiation as well as isotype switch (5–8). The use of a CD40-Fc fusion protein allowed the isolation of a cDNA encoding for a CD40 ligand (CD40L)[1], a new member of the TNF superfamily mainly expressed on activated T cells (9). Interaction between CD40 and CD40L has now been shown in vitro to be essential during T cell–dependent B cell activation (10, 11). In vivo studies in mice have demonstrated that an antibody to CD40L can inhibit primary and secondary antibody production and establishment of B cell memory (12). The crucial role of CD40/CD40L in humoral responses in human is further demonstrated in patients suffering from hyper-IgM syndrome which is characterized by genetic alterations of the CD40L gene localized on chromosome X, leading to the critical lack of circulating IgG and IgA and the absence of germinal centers (13–17). Functional CD40 molecules were found to be expressed on cells other than mature B cells. In particular, upon CD40 cross-linking, human progenitor B lymphocytes express CD23 and proliferate in response to IL-3 (18). Thymic epithelial cells secrete GM-CSF in response to CD40 engagement (19). Finally, monocytes express high levels of CD40 after exposure to IFN-γ, IL-3, and GM-CSF and CD40 cross-linking induces cytokine secretion as well as cytotoxicity (20). Since cultured Langerhans cells, as well as circulating and interdigitating dendritic cells, the professional antigen presenting cells involved in T cell priming (21), express CD40 (22, 23), we wondered whether dendritic cell CD40 would be functional. To this end, we studied the biological effects of CD40-L-transfected L cells on human dendritic Langerhans cells (D-Lc) generated in vitro by culturing human $CD34^+$ he-

[1] *Abbreviations used in this paper:* CD40-L, CD40 ligand; D-Lc, dendritic Langerhans cells; HPC, hematopoietic cells; ICAM-1, intercellular adhesion molecule 1; MFI, mean fluorescences intensities; MIP-1α, macrophage inflammatory protein 1α.

matopoietic progenitors in presence of GM-CSF and TNF-α (24). CD40 triggering was found to induce survival and morphological changes of D-Lc. In addition, CD40 engagement signals D-Lc to upregulate accessory molecules such as CD58, B7-1 (CD80), and B7-2 (CD86) and to secrete a limited set of cytokines (TNF-α, IL-8, and macrophage inflammatory protein 1α [MIP-1α]).

Materials and Methods

Hematopoietic Factors. Recombinant human (rh)GM-CSF (sp act, 2×10^6 U/mg; Schering-Plough Research Institute, Kenilworth, NJ) was used at a saturating concentration of 100 ng/ml (200 U/ml). rhTNF-α (sp act, 2×10^7 U/mg; Genzyme Corp., Cambridge, MA) was used at an optimal concentration of 2.5 ng/ml (50 U/ml) (25).

Collection and Purification of Cord Blood CD34$^+$ Cells. Umbilical cord blood samples were obtained according to institutional guidelines. Cells bearing CD34 antigen were isolated from nonadherent mononuclear fractions through positive selection by indirect immune "panning" using anti-CD34 mAb (Imu-133.3; Immunotech, Marseille, France). A second purification step was performed using a cocktail of mAbs and immunomagnetic beads (Dynabeads M450; Dynal, Oslo, Norway), as described (26). Thus, in all experiments the isolated cells were 95–99% CD34$^+$ as judged by staining with anti-CD34 mAb.

Purification of Adult Peripheral Blood T cells and Monocytes

T cells. Mononuclear cells were isolated from adult peripheral blood and depleted of adherent cells. Total T lymphocytes were then purified by immunomagnetic depletion using a cocktail of mAbs IOM2 (CD14), ION16 (CD16), ION2 (HLA-DR) (Immunotech), NKH1 (CD56) (Ortho Diagnostic Systems, Raritan, NJ), 4G7 (CD19), and mAb 89 (CD40) (27). After two rounds of bead depletion, the purity of total T cells was routinely higher than 95%.

Monocytes. Total PBMC were isolated from healthy donors by Ficoll-Hypaque centrifugation; monocytes were isolated by elutrial centrifugation. The preparation was >90% pure as controlled by flow cytometry with FITC-conjugated anti-CD14 (Leu-M3; Becton Dickinson & Co., Mountain View, CA) and by May Grünwald Giemsa staining.

Generation of D-Lc from CD34$^+$ HPC. Cultures were established in the presence of GM-CSF and TNF-α in medium consisting of RPMI 1640 (GIBCO BRL, Gaithersburg, MD) supplemented with 10% (vol/vol) FCS, 10 mM Hepes, 2 mM L-glutamine, 5×10^{-5} M 2-mercaptoethanol, 100 U/ml penicillin, and 100 μg/ml streptomycin (referred to as complete medium). CD34$^+$ cells were seeded for expansion in 24-well culture-plates (Linbro; Flow Laboratories Inc., McLean, VA) at 2×10^4 cells/ml. Optimal conditions were maintained by splitting these cultures every 4–5 d. Cells were routinely collected at day 12 when the cultures contained between 40 and 80% of CD1a$^+$ dendritic cells including Langerhans cells that express Birbeck granules (24). These cultured cells will be referred to as D-Lc throughout the text. For certain experiments, a second purification step was performed by FACS® sorting (Becton Dickinson & Co.) according to CD1a expression.

CD1a$^+$ Cells FACS® Sorting. For certain experiments, after 12 d of culture in the presence of GM-CSF+TNF-α cells generated from CD34$^+$ hematopoietic progenitor cells (HPC) were collected and labeled with FITC-conjugated OKT6 (CD1a) (Ortho Diagnostic Systems) and PE-conjugated Leu-M3 (CD14). Cells were separated according to CD1a and CD14 expression into CD1a$^-$ CD14$^+$ and CD1a$^+$CD14$^-$ fractions using a FACStar® (Becton Dickinson & Co.). The procedure of staining and sorting was performed in presence of 5 mM EDTA in order to avoid cell aggregation. Reanalysis of the sorted populations showed a purity higher than 98%.

CD40 Activation. L cells transfected with the ligand for human CD40 (van Kooten, C., and I. Banchereau, manuscript in preparation, and reference 28) (CD40L L cells), were used to induce CD40 triggering on D-Lc. CD32 transfected L cells (CD32 L cells) were used for control cultures. 2.5×10^4 L cells were seeded together with 5×10^5 cells (either total GM-CSF+TNF-α cells, CD1a$^+$CD14$^-$ cells, or elutriated monocytes) per well (24-well culture plate; Linbro; Flow Laboratories Inc.) in presence or absence of GM-CSF (100 ng/ml) in 1 ml culture medium and cultured for 2–4 d. Cell survival was monitored by enumeration of cells excluding Trypan blue. For determination of cytokine production, supernatants were recovered after 48 h. For phenotypic and morphological analysis, cells were recovered after 4 d of culture.

Cytofluorimetric Cell Surface Phenotyping. Indirect immunofluorescence was performed according to standard techniques, using a panel of murine mAbs revealed by PE-conjugated sheep F(ab')$_2$ anti-mouse immunoglobulin (Ortho Diagnostic Systems). Double-color fluorescence was carried out by sequential incubation of cells with unconjugated mAbs, PE-conjugated anti-mouse immunoglobulin, normal mouse serum, and OKT6 (CD1a) or Leu-M3 (CD14) mAbs directly labeled with FITC. Negative controls were performed with unrelated murine mAbs. Indirect mAbs used were as follows: anti-CD11b, anti-CD14, anti-CD15, anti-CD16 (Becton Dickinson & Co.); anti-CD11a, anti-CD23, anti-CD25, anti-CD32, anti-CD54, anti-CD58, anti-HLA-DR, anti-HLA-DP, anti-HLA-DQ (Immunotech); anti-CD11c, anti-CD18 (Dakopats, Glostrup, Denmark), anti-CD40 (27), anti-CD80 (29), and anti-CD86 (30). Fluorescence analysis was determined with a FACScan® (Becton Dickinson & Co.).

Cytokine Determination. Cytokine concentrations in dendritic cell supernatants were measured by two-site sandwich ELISAs, as previously described for IL-4 (31), IL-6, GM-CSF, and IL-10 (32) (sensitivities, 50 pg/ml). Kits of dosage were purchased from Medgenix Diagnostics (Brussels, Belgium) for TNF-α and IL-8; Immunotech for IL-1α and IL-1β; and R&D Systems (Minneapolis, MN) for MIP-1α.

Results

D-Lc Express CD40 which Cross-linking Induces Their Survival and Triggers Morphological Changes. CD40 is expressed spontaneously or upon activation on most APC including B cells (3), monocytes (20), and dendritic cells (22). Accordingly, we wondered whether D-Lc generated by culturing cord blood CD34$^+$ cells for 12 d in presence of GM-CSF+TNF-α (later on referred to as 12-d D-Lc), expressed CD40 as measured by double staining using anti-CD40 and anti-CD1a mAbs. As shown in Fig. 1, all CD1a$^+$ D-Lc expressed high levels of CD40. The intensity of CD40 expression on D-Lc was three- to fourfold higher than that of freshly isolated tonsil B cells (not shown). Note that a significant proportion of cultured cells expressed CD40 without CD1a.

We next wondered whether CD40 cross-linking would affect D-Lc. Thus, cultured D-Lc were incubated with CD40L

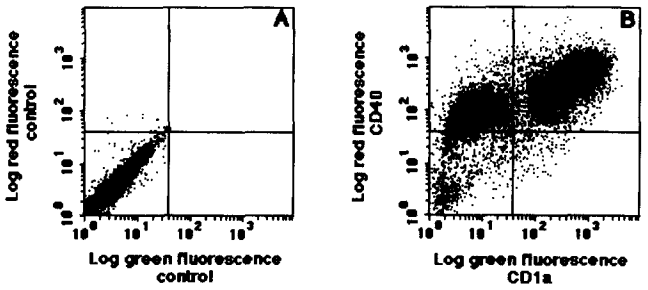

Figure 1. In vitro-generated D-Lc express CD40. D-Lc generated, after 12 d of culture of $CD34^+$ cord blood HPC in the presence of GM-CSF+TNF-α, were processed for double staining. Cells were labeled with anti-CD40 (mAb 89) revealed by PE-conjugated anti-mouse Ig followed by FITC anti-CD1a (OKT6) after saturation in 2% mouse serum. *A* shows isotype controls and *B* shows CD1a/CD40 staining.

or CD32 (as a negative control) transfected L cells with or without 100 ng/ml GM-CSF and cell viability was determined 2 and 4 d later by trypan blue exclusion. As shown in Fig. 2, in the absence of GM-CSF, D-Lc cultured on CD32 L cells died rapidly with 50% recovery (range 40–75, n = 6) after 2 d of culture and 3% recovery (range 1.3–3.7, n = 6) after 4 d. Upon CD40 triggering (without GM-CSF), the recovery was 75% (range 65–100, n = 6) after 2 d and 40% (range 32–65, n = 6) after 4 d. In presence of GM-CSF and CD32 L cells, no major decrease in D-Lc viability was observed after 2 d (recovery range 75–80, n = 6), but a cell viability reduced to 50% was observed after 4 d (range 50–56, n = 6). A combination of CD40 triggering and GM-CSF resulted in sustained cell viability detected both after 2 d (recovery range 95–100, n = 6) and after 4 d (recovery range 75–80, n = 6). In presence of CD40L L cells and GM-CSF, D-Lc can be maintained without major cell loss for at least one week (not shown). TNF-α was not included in those experiments as pilot studies indicated its lack of effect on the survival of 12 day D-Lc (not shown).

During the culture, morphological changes could be identified by simple phase contrast microscope observation. In the absence of GM-CSF, cells died when cultured on CD32 L cells (Fig. 3 *A*) whereas CD40L activated D-Lc survived and developed extensive dendrites (Fig. 3 *B*). The morphology of D-Lc cultured for 4 d in presence of GM-CSF on CD32 or CD40L L cells is shown after May Grünwald Giemsa staining. Most D-Lc cultured on CD32 L cells with GM-CSF appeared as large cells with very small dendrites at the membrane surface (Fig. 4 *A*). In contrast, D-Lc cultured on CD40L L cells with GM-CSF appeared as a homogenous population of smaller cells with a lower cytoplasm/nucleus ratio but with a very dense and uniform system of dendrites at the membrane surface (Fig. 4 *B*). This system of dendrites represented a very strong extension of the plasma membrane surface when compared with unactivated D-Lc. These morphological changes, similarly observed in absence of GM-CSF (not shown) were thus due to CD40 triggering.

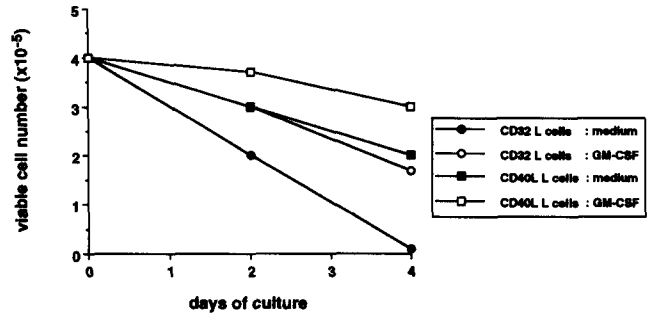

Figure 2. CD40 triggering allows survival of D-Lc. 12-d D-Lc were cultured for 4 d either on control L cells or CD40L L cells, in the presence or absence of GM-CSF. At the indicated time points, cells were harvested and counted. The results are representative of six experiments.

These experiments demonstrate that CD40 cross-linking signals D-Lc.

CD40-activated D-Lc Upregulate Accessory Molecules and CD25/Tac. As CD40 triggering on D-Lc is likely to occur during an interaction with T cells, we wondered whether such signaling would induce modulation of accessory molecules on the D-Lc surface. 12-d D-Lc were incubated on either CD40-L or CD32 transfected L cells, in presence of GM-CSF in order to maintain cell viability. After 4 d of culture, cells were double stained with a panel of mAbs revealed by PE-conjugated goat anti-mouse Ig then anti-CD1a FITC mAb. Fig. 5 shows the phenotype of $CD1a^+$ D-Lc before and after culture. Mean fluorescence intensities (MFI) of a given antigen were measured for 12-d D-Lc and for D-Lc cultured for an additional 4 d on either CD32 L cells (D-Lc 4 d CD32) or CD40L L cells (D-Lc 4 days CD40L). This permitted us to calculate for a given antigen two ratios: R_{CD32} = (MFI D-Lc 4 d CD32)/(MFI 12-d D-Lc) and R_{CD40L} = (MFI D-Lc 4 d CD40L)/(MFI 12-d D-Lc). Table 1 represents mean ratio values calculated from six independent experiments. A value ≥ 1.8 represents a significant increase in antigen expression while a value ≤ 0.5 represents a significant decrease in antigen expression. In all conditions, cells were $CD14^-$ and $CD15^-$ (not shown) but, upon CD40 engagement the intensity of CD1a decreased significantly (R_{CD40L} = 0.5 ± 0.1). The Fcγ receptor FcγRIII (CD16), expressed on 12-d D-Lc was not specifically affected by culturing upon CD40L L cells. A slight increase of FcγRII (CD32) expression was detected on 12-d D-Lc recultured both on CD32 and CD40L L cells (Table 1). The FcϵRII (CD23) absent on 12-d D-Lc was not upregulated by culturing upon CD40L L cells (Table 1). Likewise, neither the α (CD11a) nor the β (CD18) chain of the LFA-1 complex were affected significantly. In contrast, the counter-receptor intercellular adhesion molecule 1 (ICAM-1) (CD54), was lost on CD32 L cells (R_{CD32} = 0.2 ± 0.1), but was maintained on CD40L L cells (R_{CD40L} = 0.8 ± 0.2). Concerning the other integrins, CD40 ligation upregulated CD11c (R_{CD40L} = 2.7 ± 1.0), but not the complement receptor CR3 (CD11b) (R_{CD40L} = 0.8 ± 0.2). In response to CD40 triggering, the accessory molecules LFA-3 (CD58) and B7-1 (CD80) were increased (R_{CD40L} = 3.9 ± 3.1 and 3.1 ± 1.8 respectively). In addition the alternative

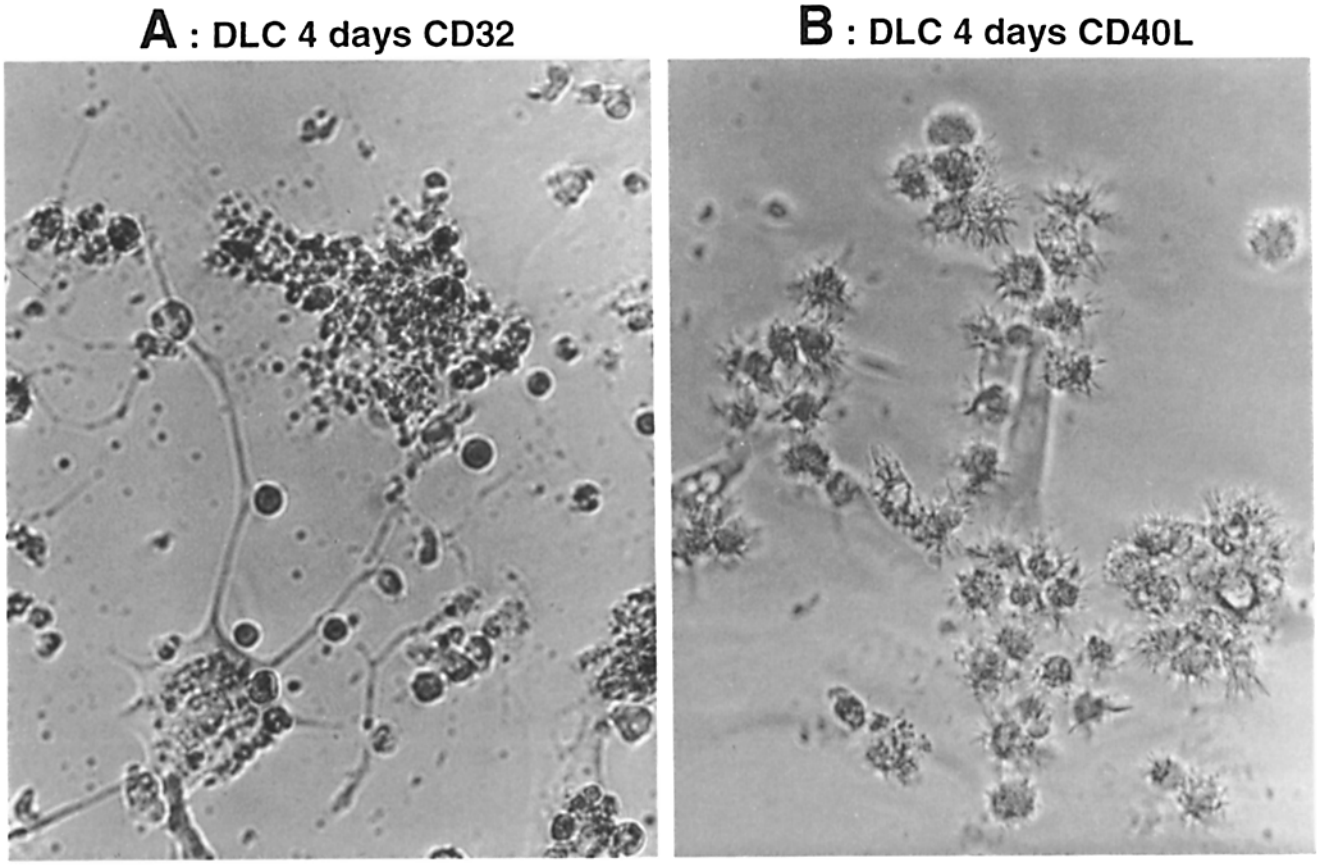

Figure 3. L cells transfected with CD40L induce morphological change of CD1a$^+$ D-Lc: phase contrast observation. 12-d CD1a$^+$ D-Lc were FACS® sorted, then cultured for 4 d either on CD32 L cells (*A*) or CD40L L cells (*B*), in absence of GM-CSF. Original magnification, ×200.

ligand for CD28/CTLA4, B7-2 (CD86), was strongly upregulated by CD40 ligation (R_{CD40L} = 22.2 ± 13.5). The expression of a third ligand for CD28/CTLA4 as determined by CTLA4-Ig binding in presence of competing anti-CD80 and anti-CD86 mAbs was never detected (not shown). Additionally, CD40 ligation enhanced the expression of all the MHC class II molecules (R_{CD40L} = 3.0 ± 2.4 for HLA-DR, 8.8 ± 5.7 for HLA-DP, and 7.0 ± 5.3 for HLA-DQ). Note that CD40 engagement upregulated CD40 expression when compared to the initial population (R_{CD40L} = 2.0 ± 0.2). Importantly CD40 triggering induced D-Lc to express the low affinity IL-2 receptor α chain Tac (CD25) (R_{CD40L} = 5.0 ± 2.6), which, in vivo, is detected on interdigitating dendritic cells of secondary lymphoid organs. These alterations of phenotype, similarly observed in absence of GM-CSF (not shown) were due to CD40 triggering, and could be detected at day 2 (not shown) but were more marked at day 4. 12-d D-Lc cultured with GM-CSF and CD32 L cells or with GM-CSF alone display similar phenotypes (not shown) indicating that CD32 L cells represent an inactive control.

Thus, upon CD40 triggering, D-Lc maintain high levels or upregulate accessory molecules involved in T cell costimulation (CD54, CD58, CD80, CD86, HLA class II) and acquire surface CD25/Tac.

CD40-activated D-Lc Produce a Restricted Set of Cytokines. As CD40-activated monocytes and B cells were described to secrete cytokines (20, 33), we investigated whether CD40 cross-linking also induced D-Lc to produce cytokines. Thus, cultured D-Lc seeded at 5 × 10^5/ml were either unstimulated or stimulated with a combination of PMA and ionomycine (PMA+Iono) or with CD40L-transfected L cells. Four populations of cells were actually analyzed that included (*a*) unseparated cultured cells which contained CD1a$^+$ D-Lc as well as CD1a$^-$ cells including monocytes/macrophages; (*b*) sorted CD1a$^+$ D-Lc (>98% pure); (*c*) sorted CD1a$^-$ cells (>98% pure); and (*d*) elutriated blood monocytes. Levels of TNF-α, IL-1α, IL-1β, IL-4, IL-6, IL-8, IL-10, GM-CSF, and MIP-1α were measured in cell supernatants harvested after 2 d. Table 2 shows the ranges from 10 independent experiments. Unseparated cultured cells and sorted CD1a$^-$ cells were found to produce high levels of TNF-α (1–30 ng/ml, n = 6), IL-6 (0–8 ng/ml), IL-8 (1–30 ng/ml); and MIP-1α (1–30 ng/ml) after activation with both PMA+Iono and CD40L L cells. In contrast, IL-1α (0–0.3 ng/ml), IL-1β (0.2–0.4 ng/ml), and GM-CSF (0.6–4.1 ng/ml) were produced only upon PMA+Iono activation. IL-10 and IL-4 were never produced by any D-Lc population, regardless of the mode of activation. FACS®-sorted CD1a$^+$CD14$^-$ cells were

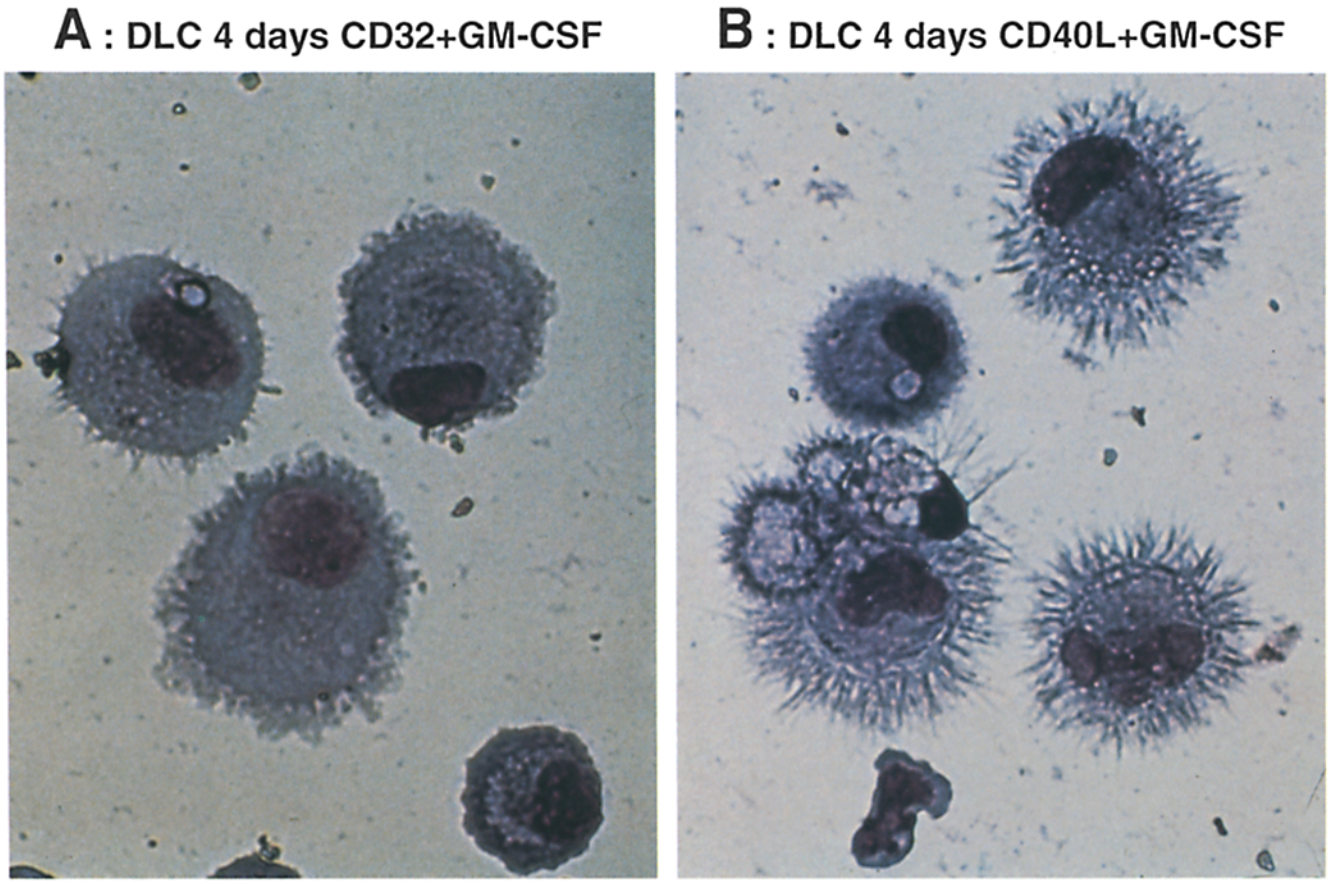

Figure 4. L cells transfected with CD40L induce morphological change of CD1a$^+$ D-Lc: May Grünwald Giemsa staining. 12-d CD1a$^+$ D-Lc were FACS® sorted, then cultured for 4 d either on CD32 L cells (*A*) or CD40L L cells (*B*), in presence of GM-CSF. May Grünwald Giemsa staining of the cultured cells is illustrated. Original magnification, ×600.

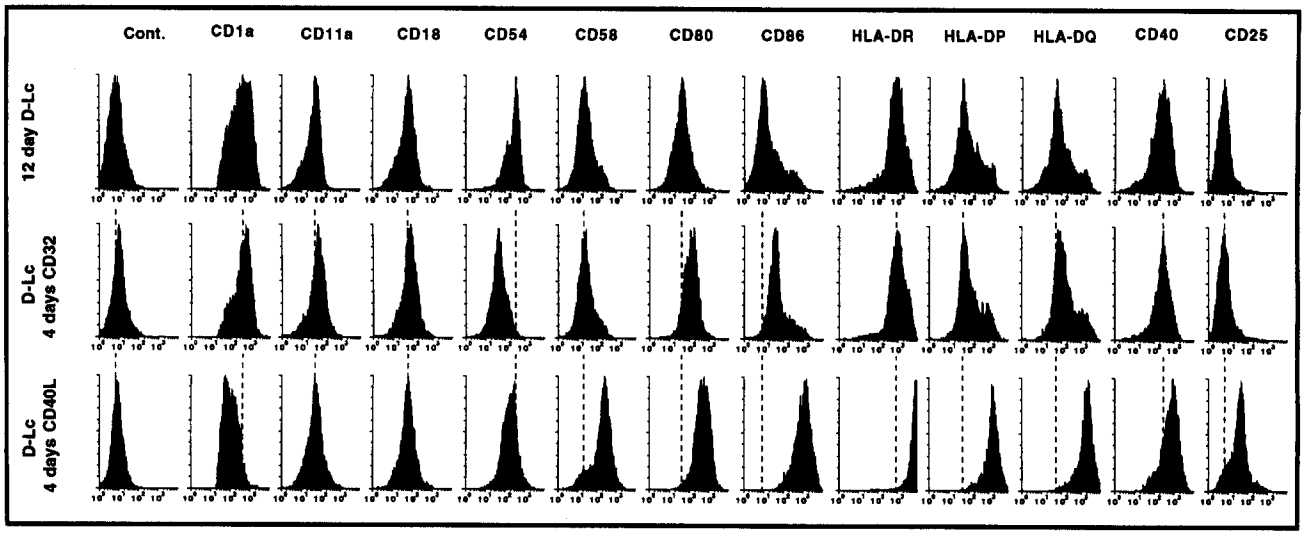

Figure 5. CD40 triggering induces maintenance or upregulation of high levels of accessory molecules. 12-d D-Lc were cultured for 4 days either on CD32 L cells (D-Lc 4 d CD32) or CD40L L cells (D-Lc 4 d CD40L), in the presence of GM-CSF. Then, cells were processed for double staining using a panel of mAb revealed by PE-conjugated anti-mouse Ig followed by FITC anti-CD1a (OKT6) after saturation in 2% mouse sera. The figure represents the staining of the mAbs on the CD1a$^+$ cells at day 12, and after 4 d on CD32 L cells, or CD40L L cells. Vertical lines corresponding to the peak channel of fluorescence intensity of a given antigen on 12-d D-Lc have been drawn to allow the comparative analysis of antigen expression levels on day 12 D-Lc that undergo different secondary culture conditions.

Table 1. *Modulation of the Expression of Antigens by Culturing D-Lc over CD32 or CD40L L Cells*

	Ratios: MFI of cultured cells/MFI of 12-d D-Lc	
	R_{CD32}/D-Lc 4 d CD32	R_{CD40L}/D-Lc 4 d CD40L
Lineage-specific antigens		
IgG1	0.9 ± 0.2	1.4 ± 0.2
CD1a	1.0 ± 0.1	0.5 ± 0.2
CD14	1.1 ± 0.4	0.9 ± 0.2
CD15	1.2 ± 0.3	1.4 ± 0.3
Fc receptors		
CD16 (Fcγ RIII)	0.9 ± 0.1	1.2 ± 0.2
CD32 (Fcγ RII)	2.2 ± 0.2	1.8 ± 0.4
CD23 (Fcε RII)	1.1 ± 0.2	1.2 ± 0.3
Costimulatory molecules		
CD11a (LFA-1α)	1.3 ± 0.3	1.4 ± 0.2
CD11b (CRIII)	0.8 ± 0.3	0.8 ± 0.2
CD11c	1.2 ± 0.3	2.7 ± 1.0
CD18 (LFA-1β)	1.1 ± 0.3	1.2 ± 0.1
CD54 (ICAM-1)	0.2 ± 0.1	0.8 ± 0.2
CD58 (LFA-3)	0.7 ± 0.3	3.9 ± 3.1
CD80 (B7-1)	1.1 ± 0.2	3.1 ± 1.8
CD86 (B7-2)	1.8 ± 0.4	22.2 ± 13.5
MHC molecules		
HLA-DR	0.8 ± 0.1	3.0 ± 2.4
HLA-DP	1.1 ± 0.6	8.8 ± 5.7
HLA-DQ	1.0 ± 0.4	7.0 ± 5.3
Activation antigens		
CD25 (TAC)	1.2 ± 0.1	5.0 ± 2.6
CD40	0.8 ± 0.2	2.0 ± 0.2

12-d D-Lc were cultured for 4 days either on CD32 L cells (D-Lc 4 d CD32) or CD40L L cells (D-Lc 4 d CD40L), in presence of GM-CSF. Cells were then processed for double staining using a panel of mAbs revealed by PE-conjugated anti-mouse Ig followed by FITC anti-CD1a (OKT6) after saturation in 2% mouse sera. MFIs of a given antigen were measured before and after culture. The following ratios were calculated: R_{CD32} = (MFI D-Lc 4 d CD32)/(MFI 12-d D-Lc) and R_{CD40L} = (MFI D-Lc 4 d CD40L)/(MFI 12-d D-Lc). Values represent mean ± SD of ratio from six independent experiments. A value ≥1.8 represents a significant increase in antigen expression whereas a value ≤0.5 represents a significant decrease in antigen expression.

found to produce high levels of TNF-α (2–10 ng/ml), IL-8 (3–17 ng/ml), and MIP-1α (≥30 ng/ml) and significant levels of IL-1β (0.1–0.5 ng/ml) and GM-CSF (0–1.1 ng/ml) but no IL-6 (0 ng/ml) upon PMA+Iono activation. Upon CD40 triggering, $CD1a^+$ cells were found to produce only TNF-α (0.6–4.5 ng/ml), IL-8 (0.6–2.5 ng/ml), and MIP-1α (0.5–1.1 ng/ml). When cells were cultured on CD32 L cells, no significant induction of cytokine production was detected when compared with medium alone (not shown). As a positive control, elutriated monocytes were found to produce high levels of TNF-α, IL-1α, IL-1β, IL-6, IL-8, MIP-1α, and IL-10 upon CD40 activation. Upon CD40 triggering, elutriated monocytes were never found (from day 2 to 6) to display the typical morphology of D-Lc, even in presence of GM-CSF (not shown).

Thus, $CD1a^+$ D-Lc produce a restricted set of cytokines (TNF-α, IL-1β, IL-8, and MIP-1α) under nonspecific activation and only TNF-α, IL-8, and MIP-1α under CD40 cross-linking.

CD40L Is Upregulated on T Cells during a Cognate Interaction with D-Lc. As D-Lc are powerful activators of T cells, we investigated whether CD40L was upregulated on T cells during an MHC-restricted interaction with D-Lc. To address this question, allospecific $CD4^+$ T cell lines were established

Table 2. *CD40 Triggering of CD1a⁺ D-Lc Induces TNF-α, IL-8, and MIP-1α Production*

Cells	Activation	TNF-α	IL-6	IL-1α	IL-1β	IL-4	IL-10	GM-CSF	IL-8	MIP-1α
						ng/ml				
	Medium	0–0.2	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.1–0.4	0–0.1
12-d D-Lc	PMA + Iono	3.1–30	1.3–8.4	0.1–0.3	0.3–0.4	<0.05	<0.05	0.9–4.1	3.7–30	⩾30
	CD40L	1.0–2.3	0–3.3	<0.05	<0.05	<0.05	<0.05	<0.05	0.7–5.7	2.1–30
	Medium	0–0.1	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0–0.2	0.1–0.2
CD1a⁺ D-Lc	PMA + Iono	1.9–10	<0.05	<0.05	0.1–0.5	<0.05	<0.05	0–1.2	3.5–17	⩾30
	CD40L	0.6–4.5	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	0.6–2.5	0.5–1.1
	Medium	0–0.1	<0.05	<0.05	<0.05	ND	<0.05	<0.05	0.4–1.0	0.2–0.4
CD1a⁻ cells	PMA + Iono	1.4–5.5	1.2–2.0	<0.05	0.2–0.4	ND	<0.05	0.6–1.8	3.9–11.9	8.7-⩾30
	CD40L	0.2–2.1	0–0.8	<0.05	<0.05	ND	<0.05	<0.05	0.8–7.6	0.8–6.4
	Medium	0.1–0.2	<0.05	<0.05	<0.05	ND	<0.05	<0.05	4.0–4.4	0.1–0.3
Monocytes	PMA + Iono	1.1–2.3	1.7–3.2	ND	ND	ND	ND	<0.05	6.2–23.2	2.5–3.5
	CD40L	1.3–3.5	3.7–15.9	0.5–1.1	0.1–6.9	ND	1.2–2.6	<0.05	8.0–9.5	6.1–6.5

After 12 d of culture in the presence of GM-CSF + TNF-α, total D-Lc, FACS® sorted CD1a⁺ D-Lc or FACS® sorted CD1a⁻ cells were cultured for 48 h either in medium alone, PMA (1 ng/ml) plus ionomycine (1μg/ml), or CD40L L cells, in the absence of GM-CSF. Elutriated monocytes were used as positive control. Cytokine levels were determined by two-site sandwich ELISAs. Results are expressed in ng/ml and represent ranges of cytokine production obtained in 10 unrelated experiments.

by repeated cultures on the same D-Lc samples (as described in Caux, C., C. Massacrier, C. Dezutter-Dambuyant, B. Vanbervliet, C. Jacquet, D. Schmitt, and J. Banchereau, manuscript in preparation). After two rounds of expansion on D-Lc originating from a single cord blood, the CD4⁺ T cells were activated for 24 h either with immobilized anti-CD3 or with the allospecific D-Lc (10^5 D-Lc for 10^6 T cells/ml) and then analyzed for their expression of CD71 (as a control of cellular activation) and CD40L (using biotinylated CD40-Ig fusion molecule) (Fig. 6). In the absence of any activation, T cells lacked both CD71 and CD40L, whereas upon anti-CD3 activation, T cells upregulated CD71 as well as CD40L. In contrast, when D-Lc were used as stimulators, CD71 was upregulated reaching levels observed during anti-CD3 activation, while CD40L remained undetectable. However, the lack of CD40-Ig binding may be due to saturation of CD40L with a soluble form of CD40 as observed earlier with B cells (28). This is particularly relevant because D-Lc supernatants were found to contain significant levels of soluble CD40 (sCD40) (not shown). Thus, to determine whether D-Lc–derived sCD40 might prevent the CD40L detection on activated T cells, an anti-CD40 (mab-89) mAb was added to the medium during the 24 h of D-Lc/T cell coculture as it prevents binding of sCD40 to CD40L (28). In this case, high levels of CD40L could be detected on D-Lc–activated T cells (Fig. 6, *right*). When mAb-89 was added to either nonactivated or anti-CD3–activated T cells, no changes in CD71 nor CD40L expression were detected (not shown). The D-Lc–induced upregulation of CD40L on T cells was confirmed in a primary reaction using a cocktail of superan-

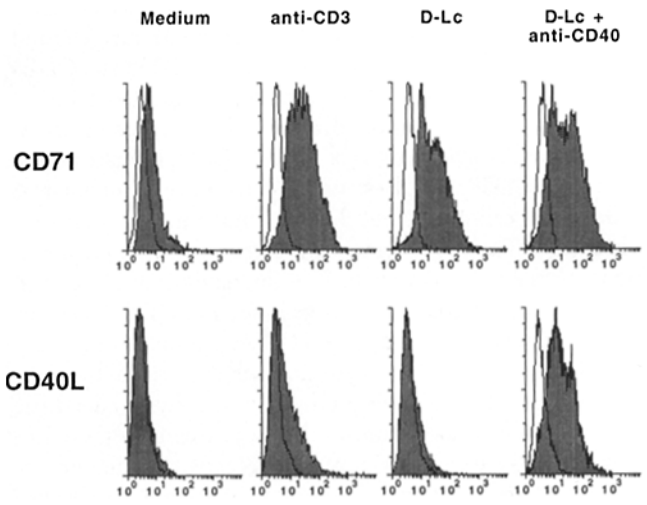

Figure 6. D-Lc–dependent activation of allospecific CD4⁺ T cells induces CD40L upregulation. Allospecific CD4⁺ T cell lines were established by repeated cultures on the same D-Lc sample (Caux, C., C. Massacrier, C. Dezutter-Dambuyant, B. Vanbervliet, C. Jacquet, D. Schmitt, and J. Banchereau, manuscript in preparation). After two rounds of expansion on the same D-Lc, the CD4⁺ T cells were activated for 24 h either with immobilized anti-CD3 or with the allospecific D-Lc (10^5 D-Lc for 10^6 T cells/ml) in presence or absence of anti-CD40 (mAb 89) and then analyzed for CD71 (control of activation) and CD40L (using biotinylated CD40-Ig fusion molecule) expression. D-Lc were excluded by gating on smaller size cells. The presence of anti-CD40 mAb during anti-CD3 activation of T cells did not affect CD71 nor CD40L expression (not shown). An isotype match mAb did not affect CD71 nor CD40L expression in any condition (not shown).

tigens (Staphylococcal enterotoxins A, B, C-1, D, and E) which allows activation of more than 70% of peripheral blood resting T cells (not shown). Furthermore, the CD40L expression was also detected by a rabbit polyclonal anti-CD40L (15) binding to epitopes not involved in CD40/CD40L interactions (not shown).

Thus, D-Lc can turn on CD40-L expression on interacting with T cells that may, in return, signal D-Lc.

Discussion

The present study demonstrates that dendritic cells derived from $CD34^+$ HPC express high levels of functional CD40. CD40 triggering of D-Lc enhances cell survival, induces striking morphological changes, maintains or increases high levels of accessory molecules and turns on the production of a restricted set of cytokines. Therefore, the expression of a functional CD40 on dendritic cells is in line with the functional role of CD40 on all positive cells studied to date (18–20).

As cognate interaction between D-Lc and T cells results in CD40L upregulation on T cells, CD40 triggering of dendritic cells is likely to occur during an antigen-dependent interaction with T cells in the paracortical T cell–rich areas of secondary lymphoid organs. Accordingly, after in vivo immunization, CD40L is mainly expressed on T cells in the periarteriolar lymphocyte sheats (34). CD40/CD40L interactions result in enhanced D-Lc survival that will permit prolonged dendritic cell/T cell interactions. In this context, the maintenance of high levels of HLA class II antigens and upregulation of accessory molecules such as CD58 and CD28 ligands (B7-1/CD80; B7-2/CD86) will concur to a strong T cell activation. The most dramatically upregulated accessory molecule turned out to be B7-2 (CD86), the alternative ligand for CD28/CTLA-4 which plays a predominant role in dendritic cell dependent T cell activation as shown elsewhere (35). The CD40 dependent extension of plasma membrane surface may facilitate further interactions of D-Lc with other antigen-specific immune cells such as CD8 T cells or B cells. In this context, upon CD40 engagement D-Lc produce MIP-1α and IL-8, cytokines characterized by their chemotactic activity. Their production by activated dendritic cells may indeed contribute to the recruitment of these immune cells, in secondary lymphoid organs. It will thus be important to establish the full repertoire of chemokines secreted by activated D-Lc and to determine the cell types chemoattracted by activated DC. In addition to MIP-1α and IL-8, D-Lc were found to secrete significant amounts of TNF-α upon CD40 engagement and IL-1β and GM-CSF in response to phorbol esters + ionomycine. TNF-α may be secreted either to enhance the activation of antigen selected B cells (36–38) or to induce apoptosis (39, 40) of bystander T or B cells. These cytokines may also play an autocrine role in D-Lc activation. This set of secreted cytokines is consistent with the production of IL-1β and MIP-1α by fresh Langerhans cells (41). However, until now, Langerhans cells have never been reported to produce TNF-α, but this lack of information may merely be due to the difficulty to obtain pure populations of dendritic cells. In view of the present study, the ability of freshly isolated dendritic cells to secrete TNF-α and IL-8 will require further analysis. It should be stressed that under similar experimental conditions, monocytes produced more of these cytokines as well as large amounts of IL-1α, IL-1β, IL-6, and IL-10. This striking difference between D-Lc and monocytes regarding cytokine production may be relevant to either monocyte/macrophage functions in nonantigen-specific cellular immunity or to a role of monocytes in skewing of T cell differentiation. Indeed the lack of influence of dendritic cells on commitment towards either TH1 or TH2 during primary T cell activation (42, 43) may be due to the limited repertoires of cytokines secreted by dendritic cells.

It is interesting to note that upon CD40 activation, D-Lc express CD25/Tac, an event that is observed after cells have migrated from the epidermis (22, 44–46). Likewise, the CD40-dependent downregulation of CD1a observed herein also occurs in vivo during migration of DC from the periphery into the secondary lymphoid organs (22, 47). Thus, the expression of CD25 and lack of CD1a on interdigitating dendritic cells may be a consequence of CD40 triggering. Actually, it is difficult to estimate whether this phenotypic alteration occurs in the paracortical areas or in the periphery after encounter with CD40L-expressing cells. In the latter case, CD40L-expressing cells may be either activated T cells or basophils (48). In any case, it will be important to determine whether CD40-activated DC express high affinity IL-2 receptors and if so what signals does IL-2 provide them with.

Hyper IgM patients display clinical symptoms suggestive of immune alterations not limited to B cells (49). In particular, the frequent *Pneumocystis carenii* infections indicate impaired T cell functions that might stem from altered dendritic cell/T cell interactions as would predict the present study. Altered CD40L might result in incomplete dendritic cell activation and consequently impaired T cell activation. Alternatively, since cross-linking of T cell CD40L contributes to development of cytotoxicity in naive T cells (50), cells with altered CD40L might not receive optimal signals for the full development of their cytotoxic potential.

Thus, as well as B cells, monocytes and thymic stroma, dendritic cells display functional CD40 molecules.

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References

1. Banchereau, J., F. Bazan, D. Blanchard, F. Brière, J.-P. Galizzi, C. van Kooten. Y.-J. Liu, F. Rousset, and S. Saeland. 1994. The CD40 and its Ligand. *Annu. Rev. Immunol.* 52:881.
2. Paulie, S., B. Ehlin-Henriksson, H. Mellstedt, H. Koho, H. Ben-Aissa, and P. Perlmann. 1985. A p50 surface antigen restricted to human urinary bladder carcinomas and B lymphocytes. *Cancer Immunol. Immunother.* 20:23.
3. Clark, E.A., and J.A. Ledbetter. 1986. Activation of human B cells mediated through two distinct cell surface differentiation antigens, Bp35 and Bp50. *Proc. Natl. Acad. Sci. USA.* 83:4494.
4. Stamenkovic, I., E.A. Clark, and B. Seed. 1989. A B-lymphocyte activation molecule related to the nerve growth factor receptor and induced by cytokines in carcinomas. *EMBO (Eur. Mol. Biol. Organ.) J.* 8:1403.
5. Banchereau, J., P. de Paoli, A. Vallé, E. Garcia, and F. Rousset. 1991. Long term human B cell lines dependent on interleukin 4 and anti-CD40. *Science (Wash. DC).* 251:70.
6. Rousset, F., E. Garcia, and J. Banchereau. 1991. Cytokine-induced proliferation and immunoglobulin production of human B lymphocytes triggered through their CD40 antigen. *J. Exp. Med.* 173:705.
7. Jabara, H.H., S.M. Fu, R.S. Geha, and D. Vercelli. 1990. CD40 and IgE: synergism between anti-CD40 monoclonal antibody and interleukin 4 in the induction of IgE synthesis by highly purified human B cells. *J. Exp. Med.* 172:1861.
8. Brière, F., C. Servet-Delprat, J.-M. Bridon, M.-M. Saint-Remy, and J. Banchereau. 1994. Human interleukin 10 induces naive surface immunoglobulin D^+ ($sIgD^+$) B cells to secrete IgG1 and IgG3. *J. Exp. Med.* 179:757.
9. Armitage, R.J., W.C. Fanslow, L. Strockbine, T.A. Sato, K.N. Clifford, B.M. Macduff, D.M. Anderson, S.D. Gimpel, T. Davis-Smith, C.R. Maliszewski, et al. 1992. Molecular and biological characterization of a murine ligand for CD40. *Nature (Lond.).* 357:80.
10. Noelle, R.J., J.A. Ledbetter, and A. Aruffo. 1992. CD40 and its ligand, an essential ligand-receptor pair for thymus-dependent B-cell activation. *Immunol. Today.* 13:431.
11. Spriggs, M.K., W.C. Fanslow, R.J. Armitage, and J. Belmont. 1993. The biology of the human ligand for CD40. *J. Clin. Immunol.* 13:373.
12. Foy, T.M., D.M. Shepherd, F.H. Durie, A. Aruffo, J.A. Ledbetter, and R.J. Noelle. 1993. In vivo CD40–gp39 interactions are essential for thymus-dependent humoral immunity. II. Prolonged suppression of the humoral immune response by an antibody to the ligand for CD40, gp39. *J. Exp. Med.* 178:1567.
13. Aruffo, A., M. Farrington, D. Hollenbaugh, X. Li, A. Milatovich, S. Nonoyama, J. Bajorath, L.S. Grosmaire, R. Stenkamp, M. Neubauer, et al. 1993. The CD40 ligand, gp39, is defective in activated T cells from patients with X-linked hyper-IgM syndrome. *Cell.* 72:291.
14. Allen, R.C., R.J. Armitage, M.E. Conley, H. Rosenblatt, N.A. Jenkins, N.G. Copeland, M.A. Bedell, S. Edelhoff, C.M. Disteche, D.K. Simoneaux, et al. 1993. CD40 ligand gene defects responsible for X-linked hyper-IgM syndrome. *Science (Wash. DC).* 259:990.
15. Korthäuer, U., D. Graf, H.W. Mages, F. Brière, M. Padayachee, S. Malcolm, A.G. Ugazio, L.D. Notarangelo, R.J. Levinsky, and R.A. Kroczek. 1993. Defective expression of T-cell CD40 ligand causes X-linked immunodeficiency with hyper-IgM. *Nature (Lond.).* 361:539.
16. DiSanto, J.P., J.Y. Bonnefoy, J.F. Gauchat, A. Fischer, and G. de Saint Basile. 1993. CD40 ligand mutations in X-linked immunodeficiency with hyper-IgM. *Nature (Lond.).* 361:541.
17. Fuleihan, R., N. Ramesh, R. Loh, H. Jabara, F.S. Rosen, T. Chatila, S.-M. Fu, I. Stamenkovic, and R.S. Geha. 1993. Defective expression of the CD40 ligand in X chromosome-linked immunoglubulin deficiency with normal or elevated IgM. *Proc. Natl. Acad. Sci. USA.* 90:2170.
18. Saeland, S., V. Duvert, I. Moreau, and J. Banchereau. 1993. Human B cell precursors proliferate and express CD23 after CD40 ligation. *J. Exp. Med.* 178:113.
19. Galy, A.H.M., and H. Spits. 1992. CD40 is functionally expressed on human thymic epithelial cells. *J. Immunol.* 149:775.
20. Alderson, M.R., R.J. Armitage, T.W. Tough, L. Strockbine, W.C. Fanslow, and M.K. Spriggs. 1993. CD40 expression by human monocytes: regulation of cytokines and activation of monocytes by the ligand for CD40. *J. Exp. Med.* 178:669.
21. Steinman, R.M. 1991. The dendritic cell system and its role in immunogenicity. *Annu. Rev. Immunol.* 9:271.
22. Romani, N., A. Lenz, H. Glassl, H. Stossel, U. Stanzl, O. Majdic, P. Fritsch, and G. Schuler. 1989. Cultured human Langerhans cells resemble lymphoid dendritic cells in phenotype and function. *J. Invest. Dermatol.* 93:600.
23. Freudenthal, P.S., and R.M. Steinman. 1990. The distinct surface of human blood dendritic cells, as observed after an improved isolation method. *Proc. Natl. Acad. Sci. USA.* 87:7698.
24. Caux, C., C. Dezutter-Dambuyant, D. Schmitt, and J. Banchereau. 1992. GM-CSF and TNF-α cooperate in the generation of dendritic Langerhans cells. *Nature (Lond.).* 360:258.
25. Caux, C., S. Saeland, C. Favre, V. Duvert, P. Mannoni, and J. Banchereau. 1990. Tumor necrosis factor-alpha strongly potentiates interleukin-3 and granulocyte-macrophage colony-stimulating factor-induced proliferation of human $CD34^+$ hematopoietic progenitor cells. *Blood.* 75:2292.
26. Caux, C., C. Favre, S. Saeland, V. Duvert, I. Durand, P. Mannoni, and J. Banchereau. 1991. Potentiation of early hematopoiesis by tumor necrosis factor-α is followed by inhibition of granulopoietic differentiation and proliferation. *Blood.* 78:635.
27. Vallé, A., C.E. Zuber, T. Defrance, O. Djossou, M. De Rie, and J. Banchereau. 1989. Activation of human B lymphocytes through CD40 and interleukin 4. *Eur. J. Immunol.* 19:1463.
28. van Kooten, C., C. Gaillard, J.-P. Galizzi, P. Hermann, F. Fossiez, J. Banchereau, and D. Blanchard. 1994. B cells regulate expression of CD40-ligand on activated T cells by lowering the mRNA level and through the release of soluble CD40. *Eur. J. Immunol.* 24:787.
29. Vallé, A., P. Garrone, H. Yssel, J.Y. Bonnefoy, A.S. Freedman, G. Freeman, L.M. Nadler, and J. Banchereau. 1990. Mab 104,

a new monoclonal antibody, recognizes the B7 antigen which is expressed on activated B cells and HTLV-1 transformed T cells. *Immunology.* 69:531.
30. Nozawa, Y., E. Wachi, K. Tominaga, M. Abe, and H. Wakasa. 1993. A novel monoclonal antibody (FUN-1) identifies an activation antigen in cells of the B-cell lineage and Reed-Sternberg cells. *J. Pathol.* 169:309.
31. Chrétien, I., A. Van Kimmenade, M.K. Pearce, J. Banchereau, and J.S. Abrams. 1989. Development of polyclonal and monoclonal antibodies for immunoassay and neutralization of human interleukin-4. *J. Immunol. Methods.* 117:67.
32. Abrams, J.S., M.-G. Roncarolo, H. Yssel, U. Andersson, G.J. Gleich, and J.E. Silver. 1992. Strategies of anti-cytokine monoclonal antibody development: Immunoassay of IL-10 and IL-5 in clinical samples. *Immunol. Rev.* 127:5.
33. Burdin, B., C. Péronne, J. Banchereau, and F. Rousset. 1993. Epstein-Barr virus transformation induces B lymphocytes to produce human interleukin 10. *J. Exp. Med.* 177:295.
34. Van den Eertwegh, A.J.M., R.J. Noelle, M. Roy, D.M. Shepherd, A. Aruffo, J.A. Ledbetter, W.J.A. Boersma, and E. Claassen. 1993. In vivo CD40-gp39 interactions are essential for thymus-dependent humoral immunity. I. In vivo expression of CD40 ligand, cytokines, and antibody production delineates sites of cognate T-B cell interactions. *J. Exp. Med.* 178:1555.
35. Caux, C., B. Vanbervliet, C. Massacrier, M. Azuma, K. Okumura, L. Lanier, and J. Banchereau. 1994. B70/B7-2 is identical to CD86 and is the major functional ligand for CD28 expressed on human dendritic cells. *J. Exp. Med.* In press.
36. Aversa, G., J. Punnonen, and J.E. de Vries. 1993. The 26-kD transmembrane form of tumor necrosis factor α on activated $CD4^+$ T cell clones provides a costimulatory signal for human B cell activation. *J. Exp. Med.* 177:1575.
37. Macchia, D., F. Almerigogna, P. Parronchi. A. Ravina, E. Maggi, and S. Romagnani. 1993. Membrane tumour necrosis factor-α is involved in the polyclonal B-cell activation induced by HIV-infected human T cells. *Nature (Lond.).* 363:464.
38. Rodriguez, C., E. Roldan, G. Navas, and J.A. Brieva. 1993. Essential role of tumor necrosis factor-α in the differentiation of human tonsil in vivo induced B cells capable of spontaneous and high-rate immunoglobulin secretion. *Eur. J. Immunol.* 23:1160.
39. Larrick, J.W., and S.C. Wright. 1990. Cytotoxic mechanism of tumor necrosis factor-α. *FASEB (Fed. Am. Soc. Exp. Bio.) J.* 4:3215.
40. Hernandez-Caselles, T., and O. Stutman. 1993. Immune functions of tumor necrosis factor. I. Tumor necrosis factor induces apoptosis of mouse thymocytes and can also stimulate or inhibit IL-6-induced proliferation depending on the concentration of mitogenic costimulation. *J. Immunol.* 151:3999.
41. Matsue, H., P.D. Cruz, P.R. Bergstresser, and A. Takashima. 1992. Cytokine expression by epidermal cell subpopulations. *J. Invest. Dermatol.* 42:42.
42. 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 (Wash. DC).* 260:547.
43. Macatonia, S.E., C.-S. Hsieh, Murphy K.M., and A. O'Garra. 1993. Dendritic cells and macrophages are required for Th1 development of $CD4^+$ T cells from $\alpha\beta$ TCR transgenic mice: IL-12 substitution for macrophages to stimulate IFNγ production is IFNγ-dependent. *Int. Immunol.* 5:9.
44. Crowley, M.T., K. Inaba, M.D. Witmer-Pack, S. Gezelter, and R.M. Steinman. 1990. Use of the fluorescence activated cell sorter to enrich dendritic cells from mouse spleen. *J. Immunol. Methods.* 133:55.
45. Larsen, C.P., R.M. Steinman, W. Witmer-Pack, D.F. Hankins, P.J. Morris, and J.M. Austyn. 1990. Migration and maturation of Langerhans cells in skin transplants and explants. *J. Exp. Med.* 172:1483.
46. O'Doherty, U., R.M. Steinman, M. Peng, P.U. Cameron, S. Gezelter, I. Kopeloff, W.J. Swiggard, M. Pope, and N. Bhardwaj. 1993. Dendritic cells freshly isolated from human blood express CD4 and mature into typical immunostimulatory dendritic cells after culture in monocyte-conditioned medium. *J. Exp. Med.* 178:1067.
47. Teunissen, M.B.M., J. Wormeester, S.R. Krieg, P.J. Peters, I.M.C. Vogels, M.L. Kapsenberg, and J.D. Bos. 1990. Human epidermal Langerhans cells undergo profound morphological and phenotypical changes during in vitro culture. *J. Invest. Dermatol.* 94:166.
48. Gauchat, J.F., S. Henchoz, G. Mazzei, J.P. Aubry, T. Brunner, H. Blasey, P. Life, D. Talabot, L. Flores-Romo, J. Thompson, K. Kishi, J. Butterfield, C. Dahinden, and J.Y. Bonnefoy. 1993. Induction of human IgE synthesis in B cells by mast cells and basophils. *Nature (Lond.).* 365:340.
49. Notarangelo, L.D., M. Duse, and A.G. Ugazio. 1992. Immunodeficiency with hyper-IgM (HIM). *Immunodef. Rev.* 3:101.
50. Cayabyab, M., J.H. Phillips, and L.L. Lanier. 1994. CD40 preferentially costimulates activation of $CD4^+$ T lymphocytes. *J. Immunol.* 152:1523.