

CD1-mediated γ/δ T Cell Maturation of Dendritic Cells

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Abstract

Immature myeloid dendritic cells (DCs) express only low levels of major histocompatibility complex (MHC) class II but express high levels of CD1 a, b, and c antigen-presenting molecules at the cell surface. As $V\delta 1^+$ γ/δ T cells are the main tissue subset of γ/δ T cells and they are known to recognize CD1c in the absence of specific foreign antigen recognition, we examined the possible interaction of these T cells with immature DCs. We show that CD1-restricted γ/δ T cells can mediate the maturation of DCs. DC maturation required cell-cell contact and could be blocked by antibodies against CD1c. The maturation process was partially mediated by tumor necrosis factor α . Importantly, immature DCs matured in the presence of lipopolysaccharide and CD1-restricted γ/δ T cells produced bioactive interleukin-12p70. In addition, these DCs were able to efficiently present peptide antigens to naive $CD4^+$ T cells. CD1-restricted γ/δ T cell recognition of immature DCs provides the human immune system with the capacity to rapidly generate a pool of mature DCs early during microbial invasion. This may be an important source of critical host signals for T helper type 1 polarization of antigen-specific naive T cells and the subsequent adaptive immune response.

Key words: T cell antigen receptors γ/δ • CD1 • dendritic cells • antigen-presenting cell • tumor necrosis factor

Introduction

Dendritic cells (DCs)* represent a pool of professional APCs that plays an essential role in the initiation of host T cell immunity (1). The maturational state of these cells is critical in determining their function. DCs residing in peripheral tissues exist in an immature state where they are uniquely adapted for antigen internalization and processing (2). After interaction with microbial antigens or host cellular signals, maturation and migration to regional lymph nodes occurs. Mature DCs in lymphoid sites down-regulate their rate of antigen uptake and express increased cell surface levels of MHC class II and costimulatory molecules such as B7.2 (CD86) that enable them to present antigens to naive $CD4^+$ and $CD8^+$ T cells promoting the adaptive immune response (3).

During host infection, microbial antigens such as LPS provide critical innate signals for DC maturation via cell surface Toll-like receptors (TLRs). However, maturation of DCs by microbial stimuli alone may promote a short-lived burst of IL-12 leading to an "exhausted" DC population unable to produce this cytokine upon subsequent encounter with naive T cells in the lymph node (4). The ability of the host to successfully defend against microbial invasion is, therefore, dependent upon both innate signals provided by microbial products as well as T cell signals. Studies in mice have demonstrated the important role of host T cells in both the development of normal DCs as well as the ability of these DCs to efficiently prime naive T cells to respond to microbial invasion. For example, $Rag2^{-/-}$ mice do not develop normal numbers of DCs and the ability to efficiently present antigens to naive T cells by DCs is impaired (5). In addition, members of the TNF family of cell surface receptors, expressed by host T cells, including CD40L (6), RANKL (7), and FasL (8) have been demonstrated to be important mediators of human DC maturation in vitro and in mice, in vivo. Finally, it has be-

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*Abbreviations used in this paper: DC, dendritic cell; MFI, mean fluorescence intensity; OPG, osteoprotegerin.

come increasingly clear that optimal production of inflammatory cytokines such as heterodimeric IL-12 by the DCs, promoting efficient Th1 polarization of naive T cells, is also influenced by signals imparted by host T cells. Yet, the specific T cell populations capable of providing this variety of T cell signals for DC maturation remain poorly defined.

As immature DCs express high levels of CD1a, b, and c molecules (9), we considered the possibility that T cells recognizing CD1 might be capable of interacting with immature DCs. Here, we provide evidence that a subpopulation of V δ 1⁺ γ/δ T cells can selectively stimulate monocyte-derived dendritic cells to undergo maturation. Importantly, these γ/δ T cells recognize CD1 on the surface of immature DCs in the absence of a specific foreign antigen. Upon recognition of CD1c, they secrete TNF- α and other factors that, together with microbial products such as LPS, induce immature DCs to mature and to produce heterodimeric IL-12p70.

Materials and Methods

mAbs and Blocking Reagents. The following mAbs were used for flow cytometry and blocking experiments: P3 (IgG control), F10/21A3 (anti-CD1c; reference 10), 10D12 (anti-CD1a; reference 11), OKT6 (anti-CD1a; reference 12), IgG1-FITC, IgG1-PE, IgG2a-PE, CD83-PE, CD86-PE (anti-B7.2), anti-CD40 ligand-PE, anti-TNF- α , anti-IFN- γ , anti-Fas ligand (all from BD Biosciences), osteoprotegerin (OPG; PeproTech), and δ TCS1(anti-V δ 1-J δ 1; Pierce Chemical Co./Endogen).

T Cell Lines and Clones. CD1c-reactive V δ 1⁺ γ/δ clones JR.2 and XV.1 were derived by limited dilution cloning from normal donor peripheral blood as described previously (13). V δ 1⁺ γ/δ clones 10G4 and 20H8 were isolated from peripheral blood lymphocytes from normal donors by Ficoll-Hypaque centrifugation. Cells were stained with saturating concentrations of anti-V δ 1 mAb δ TCS1-FITC and positive cells were directly sorted using a Beckman Coulter EPICS Elite fluorescence-activated cell sorter (Beckman Coulter) at one cell per well into 96-well round-bottomed tissue culture plates in a volume of 200 μ l containing 5×10^4 irradiated (5,000 rads) heterologous PBMCs and 5×10^4 irradiated (7,500 rads) EBV-transformed B lymphoblastoid cells as feeders in RPMI medium supplemented with 10% fetal bovine serum (Hyclone), PHA-P (1:4,000 final dilution; Difco), and 2 nM IL-2 (Chiron Corp.). The bisphosphonate reactive V γ 2V δ 2 line was generated by culture of normal donor PBMCs in 10 μ M Risedronate and 2 nM IL-2 for 28 d.

Monocyte-derived DC Maturation Assays. Monocyte-derived DCs were generated from human blood monocytes that were isolated from the by-products of platelet pheresis and induced to differentiate and express CD1a, CD1b, and CD1c by incubation with GM-CSF and IL-4 as described (14). Immature DC were then cultured in 24-well tissue culture plates at a concentration of 1×10^6 cells/ml for 1–3 d in RPMI complete medium alone or in the presence of 10 ng/ml *Escherichia coli* LPS (Sigma-Aldrich), 50 ng/ml TNF- α , or γ/δ T cell clones (T:DC ratio ranging from 1:3 to 1:27) as maturation stimuli. mAbs used in blocking studies were added at a final concentration of 20 μ g/ml. After coculture of DCs with T cells or the maturation stimuli above, cells were washed and stained with PE-conjugated mAbs against CD83 and CD86 and analyzed by flow cytometry (FACSortTM; Becton Dickinson). CD83 results are expressed as percentage of positive

cells compared with negative cells stained with an isotype-matched control antibody. CD86 results are expressed as mean fluorescence intensity (MFI).

FITC-Dextran Endocytosis. Measurement of soluble antigen uptake by immature DCs was performed by culturing immature DCs in the presence of medium, TNF- α (50 ng/ml) of the γ/δ T cell clone JR.2 (T:DC ratio = 1:10) for 48 h to induce maturation. DCs were then washed and resuspended at 10^6 cells/ml. FITC-Dextran (MW = 40,000; Sigma-Aldrich) was added at a concentration of 1 mg/ml and cells were incubated from 0–60 min at 37°C to allow endocytosis to occur. This concentration is saturating for uptake via the mannose receptor so that differences in uptake primarily reflect changes in macropinocytosis (15). After the incubation period, DCs were washed in medium and FITC-Dextran MFI was determined by flow cytometry as described.

Confocal Microscopy. After maturation for 48 h in the presence of medium alone, 50 ng/ml TNF- α or γ/δ T cell clone JR.2, DCs were adhered on glass slides by cytospin, fixed, and permeabilized as described previously (16). The cells were then double-labeled with FITC-conjugated LB3.1 mAb to HLA-DR and rabbit polyclonal antiserum against LAMP-1 (17), followed by incubation with Texas Red-conjugated donkey F(ab')₂ antibody to rabbit IgG (Jackson ImmunoResearch Laboratories). Labeled cells were then examined using a Leica TCS-NT confocal laser scanning microscope fitted with krypton and argon lasers.

Measurement of Soluble Cytokines by ELISA. Immature monocyte-derived DCs were harvested following 48 h of culture in IL-4 and GM-CSF. Cell surface expression of CD1a, CD1b, and CD1c was confirmed by mAb staining and flow cytometry and the DCs were plated in 96-well round bottom plates at a concentration of 10^6 /ml in RPMI complete medium. γ/δ clone JR.2, at a T cell:DC ratio of 1:9, and/or LPS (10 ng/ml) with and without blocking mAbs against CD1a, CD1c, IFN- γ , or isotype-matched control antibody. After coculture for 24 h, supernatants were harvested and analyzed for TNF- α , IFN- γ , IL-12p70, IL-4, and IL-13 by sandwich ELISA assay using antibody pairs purchased from Pierce Chemical Co./Endogen. Results were expressed as ng/ml \pm SD.

Presentation of KLH to CD45RO⁻CD4⁺ Naive T Cells by DCs. Immature monocyte-derived DCs were isolated as described by culture in GM-CSF and IL-4 for 3 d. DCs were cultured in 24-well tissue culture plates at 10^6 cells/ml for 72 h in the presence of TNF- α (50 ng/ml), γ/δ clone JR.2 (T cell:DC ratio = 1:10), or medium alone with and without 25 μ g/ml Keyhole Limpet Hemacyanin (Calbiochem). DC expression of CD83 and CD86 was then measured by mAb staining and flow cytometry to assess phenotypic maturation. These DCs were washed, irradiated (5,000 rads), and then cultured with 5×10^4 autologous CD4⁺CD45RA⁺ naive T cells (isolated using a negative selection column (R&D Systems) per well at a DC:T cell ratio of 1:10 for 5 d. The T cells derived were >98% positive for CD4 and CD45RA. During the final 12 h of culture, cells were pulsed with 1 μ Ci [³H]thymidine (2 Ci/mmol; New England Nuclear) and then harvested using a Tomtec harvester. Filter mats were counted on a Betaplate scintillation counter (Wallac). Results were expressed as relative stimulation index.

Allogeneic Mixed Lymphocyte Reaction. Immature monocyte-derived DCs were isolated as described previously. DCs were cultured in 24-well tissue culture plates at 10^6 cells/ml for 72 h in the presence of TNF- α (50 ng/ml), γ/δ clone JR.2 (T cell:DC ratio = 1:3), or medium alone and DC expression of CD83 and CD86 was then measured to assess phenotypic maturation. These DCs were washed, irradiated (5,000 rads), and then cul-

tured with 10^5 allogeneic CD4⁺ T cells (isolated by immunomagnetic selection (Miltenyi Biotec) per well for 7 d. During the final 12 h of culture, cells were pulsed with 1 μ Ci [³H]thymidine (2 Ci/mmol; New England Nuclear) and counted as described previously.

Results

γ/δ T Cells Promote Maturation of Immature DCs. Outside the thymus, only myeloid dendritic cells are known to express all of the group I (CD1a, CD1b, and CD1c) and group2 (CD1d) CD1 isoforms (9). As we had previously isolated human γ/δ T cells expressing V δ 1 TCRs that recognize CD1c in the absence of exogenous microbial antigens (13), we hypothesized that this T cell population might mediate the maturation of immature monocyte-derived dendritic cells. Fresh blood monocytes were cultured with GM-CSF and IL-4 for 2 to 3 d, in vitro, to obtain a pool of immature monocyte-derived DCs. Cocultures of such DCs with CD1-reactive γ/δ T cell clones resulted in increased cell surface expression of the costimulatory molecule CD86 (B7.2) and an increased percentage of cells expressing the maturation marker CD83, consistent with DC maturation. For example, culture of the CD1c-restricted γ/δ T clones JR.2 and XV.1 with immature DCs at ratios ranging from 1:3 to 1:27 T cells per DC for 48 h led to a 9- and 13-fold increase in DC cell surface expression of CD86 for JR.2 and XV.1, respectively (from MFI = 196.7 to MFI = 2,565.1 for JR.2 and MFI = 1,742.6 for XV.1), as well as an increase in the percentage of CD83⁺ DCs (from 3.5 to 75.5% for JR.2 and 61.7% for XV.1) when compared with DCs cultured in medium alone. (Fig. 1 A).

We next compared the CD1c-reactive γ/δ clone JR.2 to other γ/δ T cells that are not restricted by CD1 in terms of their ability to mediate maturation of immature DCs. Again, coculture of JR.2 with immature DCs led to increased cell surface expression of CD86 (from MFI =

177.9 to MFI = 2,517.4) and an increase in the percentage of CD83⁺ DCs (from 20.49 to 92.32%). This level of maturation was greater than that seen when DCs were cultured in the presence of 50 ng/ml TNF- α as a maturation stimulus both in terms of CD86 expression (MFI = 2,517 for JR.2 vs. 901 for TNF- α) and the percentage of CD83⁺ cells (92% for JR.2 vs. 78.31% for TNF- α). In contrast, coculture of immature DCs with T cell clones 10G4 and 20H8, which express V δ 1 TCRs but are not CD1-restricted, as well as a V γ 2V δ 2 γ/δ line reactive against a bisphosphonate antigen (V δ 2), had no effect upon CD86 cell surface expression or the percentage of DCs expressing CD83 further supporting a role for CD1c in the maturation process (Fig. 1 B).

γ/δ T Cell Maturation of Immature DCs Is Mediated by CD1c. As only certain γ/δ T cells were able to induce the maturation of DCs, we wished to determine if recognition of CD1c by these T cells was critical for inducing DC maturation. During a 48-h coculture of γ/δ clone JR.2 with immature DCs at a T cell:DC ratio of 1:9, treatment with anti-CD1c mAb F10/21A3 inhibited up-regulation of CD83 and CD86 by 64 and 78%, respectively, when compared with DCs cultured in medium alone. Treatment with a mAb directed against CD1a (OKT6) had no such effect (Fig. 2). These data indicate that CD1c recognition by certain γ/δ T cells is required for the ability to induce in vitro monocyte-derived DC maturation.

DCs Cultured with CD1c-restricted γ/δ T Cells Display the Morphologic and Functional Characteristics of Mature DCs. To further evaluate the characteristics of DC maturation that occurred, we assessed morphologic and functional markers of DC maturation. In immature DCs, antigen uptake via macropinocytosis and expression of pattern recognition receptors (PRRs) for microbial products equip these cells to internalize a wide array of soluble antigens in peripheral tissues (18).

We first investigated the ability of DCs matured in the presence of CD1c-reactive γ/δ T cells to internalize solu-

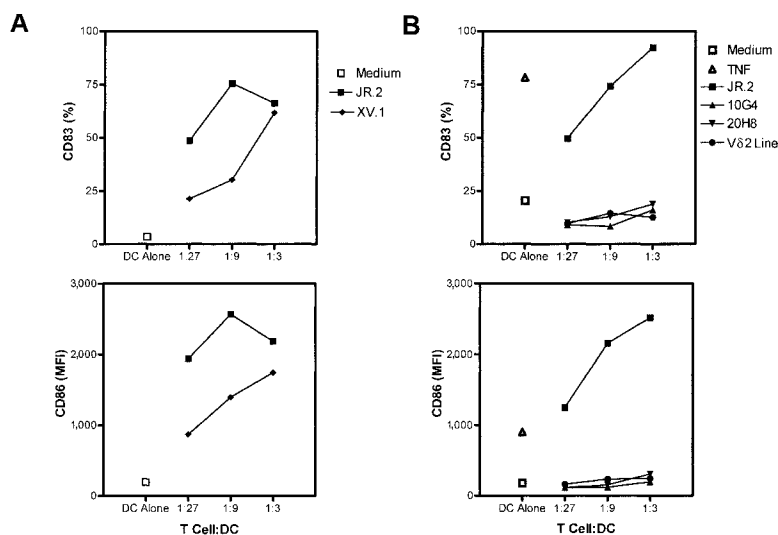


Figure 1. CD1-restricted γ/δ T cells induce phenotypic DC maturation. (A) Immature monocyte-derived DCs cultured with V δ 1⁺ CD1c-restricted T cell clones JR.2 and XV.1 for 48 h expressed increased CD83 and CD86 cell surface molecules when compared with DCs cultured in medium alone as assessed by mAb staining and flow cytometry. (B) Immature DCs cultured with CD1c-restricted γ/δ clone JR.2 or TNF- α (50 ng/ml) for 48 h expressed increased levels of CD83 and CD86 cell surface molecules. Culture of DCs with V δ 1⁺ clones 10G4, 20H8, and a V δ 2⁺ γ/δ line (which are not CD1-restricted) all failed to promote maturation of DCs as assessed by CD83 and CD86 cell surface expression. Open squares, medium alone; open triangles, TNF- α ; filled squares, JR.2; diamonds, XV.1; triangles, 10G4; inverted triangles, 20H8; circles, V δ 2 line. These results are representative of four independent experiments using different DC donors.

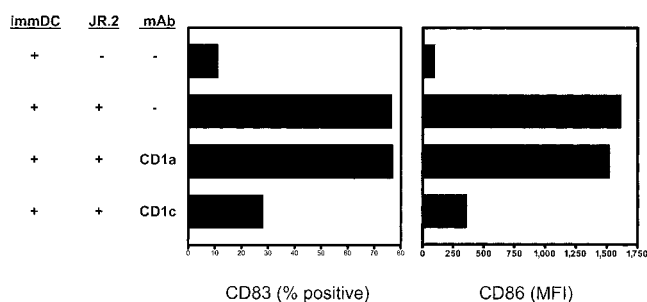


Figure 2. γ/δ T cell-induced DC maturation is mediated by CD1c. Immature monocyte-derived DCs were cultured with the CD1c-restricted γ/δ clone JR.2 at a T cell:DC ratio of 1:9 for 48 h in culture medium or in the presence of anti-CD1 mAb blocking. Treatment with anti-CD1c mAb F10/21A3 inhibited up-regulation of CD83 and CD86 by 64 and 78%, respectively. Blocking with mAb directed against CD1a (OKT6) did not inhibit maturation. These results are representative of three independent experiments using different DC donors.

ble antigens. After a 48-h culture with γ/δ clone JR.2 (T cell:DC ratio = 1:9), TNF- α (50 ng/ml), or medium alone, DCs were assessed for their ability to internalize

the soluble fluorescent antigen FITC-Dextran (MW = 40,000; 1 mg/ml concentration) during a 60-min time course. DCs cultured in the presence of JR.2 (MFI = 132) or TNF- α (MFI = 100) showed decreased uptake of FITC-Dextran at 60 min when compared with immature DC cultured in medium alone (MFI = 369), consistent with their functional maturation (Fig. 3 A).

In immature DCs, a majority of MHC class II molecules are stored in an acidic lysosomal compartment, where peptide loading is proposed to occur. As DCs mature, a dramatic shift in the cellular location of MHC class II occurs as peptide antigen-loaded MHC class II molecules are efficiently transported to the plasma membrane. Concurrently, internalization of MHC class II-peptide complexes is down-regulated, promoting an increased ability of the mature DCs to present peptide antigens to MHC class II-restricted T cells (2). As a shift in the cellular location of the predominant pool of MHC class II molecules from intracellular lysosomes to the plasma membrane occurs as DCs mature, we next performed two-color confocal laser microscopy to assess for these morphologic changes during

A

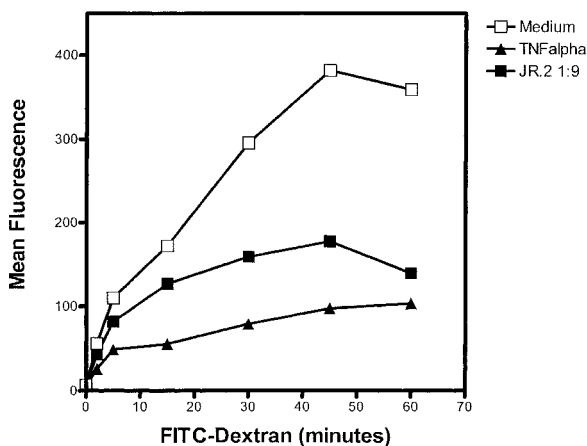
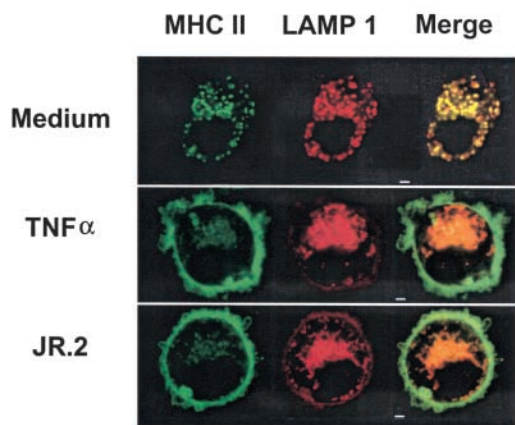
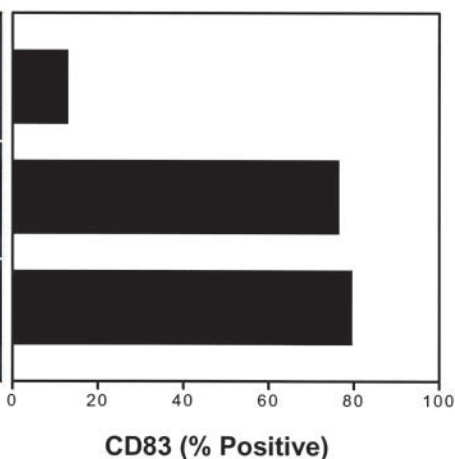


Figure 3. CD1c-reactive T cells induce functional DC maturation. (A) Decreased uptake of soluble FITC dextran by mature DCs. Immature DCs were cultured in the presence of TNF- α (50 ng/ml), γ/δ clone JR.2 (T cell:DC ratio = 1:9), or medium alone for 48 h. These DCs were then washed and cultured in the presence of FITC-dextran during a 60 min time course and antigen uptake was assessed by flow cytometry. Note that DCs matured in the presence of clone JR.2 or TNF α had decreased uptake of FITC-dextran consistent with functional maturation. Open squares, medium; filled squares, JR.2; triangles, TNF- α . (B) Redistribution of MHC class II molecules in maturing DCs. Confocal microscopy was performed on DCs matured in the presence of TNF- α , JR.2 (T cell:DC ratio = 1:9) or medium alone. Cells were labeled with mAb against MHC class II (green staining) and the lysosomal marker LAMP 1 (red staining). Colocalization of these molecules is represented by the merged image (yellow). Note that DCs matured in the presence of JR.2 or TNF- α have increased staining of cell surface MHC class II and minimal colocalization with LAMP 1. Bar = 2 μ m. (C) DC expression of cell surface CD83. DCs cultured in the presence of TNF- α or JR.2 expressed increased levels of the maturation marker CD83 as assessed by mAb staining and flow cytometry. These results are representative of three independent experiments using different DC donors.

B



C



culture of immature DCs with CD1c-restricted γ/δ T cells. After 48 h culture of immature DCs in the presence of γ/δ clone JR.2 (T cell:DC ratio = 1:9), TNF- α (50 ng/ml), or medium alone, DCs were permeabilized with Saponin and stained with mAbs against MHC class II (HLA-DR-FITC; green staining) and the late endosome/lysosomal marker LAMP-1 (LAMP-1-Texas Red; red staining). Immature DCs cultured in medium alone showed a high degree of colocalization of MHC class II and LAMP-1 (yellow staining) in intracellular vesicles with low intensity MHC class II cell surface staining (green). In contrast, DCs cultured in the presence of either TNF- α or the γ/δ clone JR.2 demonstrated minimal colocalization of MHC class II and LAMP-1 molecules but intense staining of MHC class II on the cell surface (Fig. 3 B). These results are consistent with a shift in MHC class II molecules from lysosomes to the plasma membrane, as well as decreased recycling of MHC class II molecules indicating increased DC maturation. Mature DCs up-regulate the expression of costimulatory molecules such as CD80, CD86, and CD40, increasing their ability to stimulate these antigen-specific T cells. To confirm that phenotypic maturation paralleled the changes in antigen uptake and MHC class II redistribution, we assessed the percentage of CD83⁺ DC during the same assay. Confirming our previous observations, the percentage of cells expressing CD83 increased in the presence of TNF- α (76%) or the CD1c-restricted γ/δ

clone JR.2 (79%) but not in culture with medium alone (16%; Fig. 3 C).

Phenotypic Maturation of DCs by CD1-restricted γ/δ T Cells Is Partially Mediated by the Cytokine TNF- α . The Th1 cytokines IFN- γ (19) and TNF- α (20) have been extensively characterized as soluble factors that are important in DC maturation. For example, production of IFN- γ by host lymphocytes including CD8⁺ T cells (21) and NK cells (22) was found to promote the ability of maturing DCs to produce IL-12p70 leading to Th1 polarization of antigen specific naive T cells. Therefore, we assessed the ability of γ/δ clone JR.2 to produce these cytokines during coculture with CD1 expressing immature DCs. Both IFN- γ (1.4 ± 0.05 ng/ml) and TNF- α (14.3 ± 3.3 ng/ml) were produced by CD1-specific γ/δ T cells as assessed by ELISA, and cytokine production was inhibited by 89% using mAb against CD1c but not by mAb against CD1a (Fig. 4 A). Production of the Th2 cytokines IL-4 and IL-13 was below the limit of detection (unpublished data). We next examined the role of these cytokines in DC maturation. The addition of anti-TNF- α mAb at 20 μ g/ml partially inhibited phenotypic maturation of immature DCs cultured with JR.2 (70% decrease in CD83-positive cells compared with no antibody blocking) while addition of anti-IFN- γ at 20 μ g/ml did not inhibit DC maturation as assessed by cell surface expression of CD83 and CD86 using flow cytometry (Fig. 4 B). These results are consistent with production of a soluble factor such as TNF- α being partially responsible for the CD1-mediated

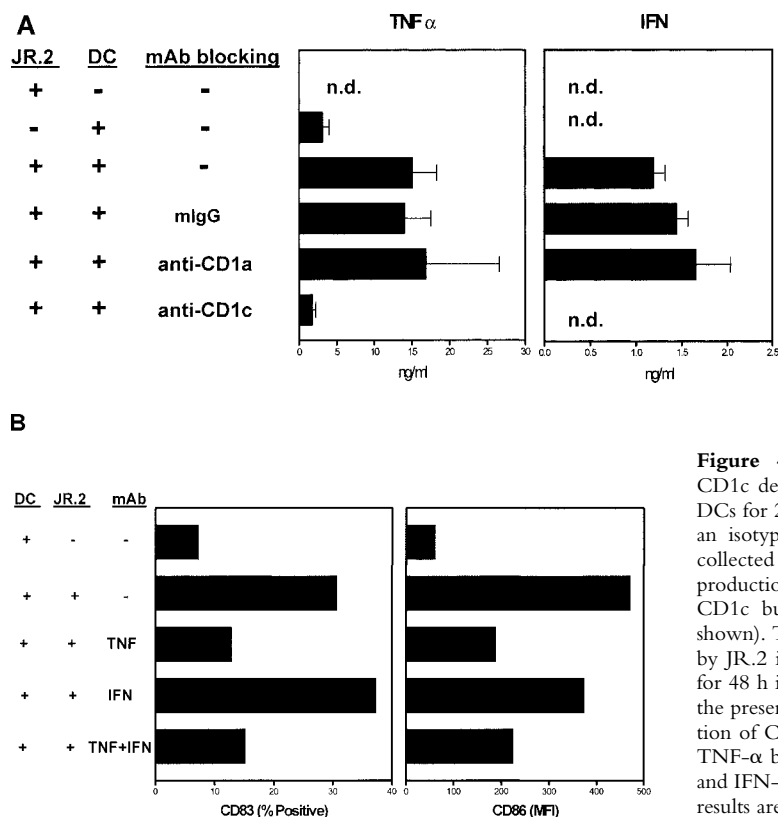


Figure 4. Th1 cytokine production during JR.2:DC coculture is CD1c dependent. (A) JR.2 γ/δ T cells were incubated with immature DCs for 24 h in the presence of blocking mAbs against CD1a, CD1c, or an isotype-matched control antibody. The culture supernatants were collected and assayed for TNF- α , IFN- γ , IL-4, and IL-13. Note that production of both TNF- α and IFN- γ was inhibited by mAb against CD1c but not CD1a. IL-4 and IL-13 were not detected (data not shown). The sensitivity of the assay was 30 pg/ml. (B) Maturation of DCs by JR.2 is partially mediated by TNF- α . Immature DCs were cultured for 48 h in medium or with γ/δ clone JR.2 (T cell:DC ratio = 1:10) in the presence of blocking mAbs against TNF- α or IFN- γ . DC up-regulation of CD83 and CD86 was inhibited by the presence of mAbs against TNF- α but not IFN- γ . The combination of mAbs against both TNF- α and IFN- γ was similar to the effect seen with TNF- α mAbs alone. These results are representative of four independent experiments using different DC donors.

maturation of DC by γ/δ T cells. We next employed a transwell system to determine if cell–cell contact was required for maturation to occur. Immature DCs were cultured in direct contact with the JR.2 γ/δ clone or were separated by a 0.2 μ transwell membrane. DCs separated from JR.2 T cells by the transwell failed to up-regulate CD83. To assess the specific role of soluble factors, DCs and JR.2 were cocultured together (T cell:DC ratio = 1:10) and additional DCs separated in the transwell were assayed for CD83 up-regulation. The percentage of CD83⁺ cells among the DCs exposed to soluble factors in this transwell increased twofold (20 to 40% positive), whereas DCs cultured in direct contact with γ/δ clone JR.2 in the absence of a transwell demonstrated a fourfold increase (20 to 80% positive; data not depicted). These results are consistent with production of a soluble factor such as TNF α being only partially responsible for the CD1-mediated maturation of DCs by γ/δ T cells. Other investigators have emphasized the role of cell surface TNF family members such as CD40 ligand (CD154; reference 6), RANK ligand (7), and Fas ligand (8) expressed by T cells in the maturation of DCs. As rapid, tightly regulated expression of these cell surface T cell molecules may provide critical signals for DC maturation, we examined the expression of CD40 ligand by γ/δ clone JR.2 during culture with immature DCs. CD40 ligand cell surface levels on JR.2 T cells increased approximately fourfold (from MFI = 21.3 to 84.23) when cultured with CD1c expressing immature DCs over 24 h when compared with JR.2 T cells cultured in tissue culture medium alone, implying a potential role for CD40 ligand in the maturation process (Fig. 5 A). Although CD40 ligand expression on the surface of the JR.2 T cells increased during coculture with immature DCs, mAb blocking studies using antibodies against CD40L led to only 20% inhibition of CD83 up-regulation. In addition, mAb blocking of FasL, as well as the RANKL antagonist OPG, also failed to significantly inhibit maturation of DC as assessed by CD83 cell surface expression (Fig. 5 B). Taken together, our results emphasize the importance of soluble TNF- α production for the maturation of DCs by the CD1-reactive γ/δ clone JR.2. In addition, cell–cell contact between the DCs and CD1-restricted γ/δ T cell clone appears to be required for optimal maturation to occur, although we could not inhibit maturation with blockage of FasL or RANKL and we observed only partial inhibition via CD40L.

Both LPS and CD1-restricted γ/δ T Cells Are Required for the Production of Heterodimeric IL-12 by Maturing DCs. Maturation of DCs by microbial products such as LPS has been proposed to lead to an “exhausted” DCs unable to produce bioactive IL-12p70 upon subsequent ligation of CD40 by antigen-specific naive CD4⁺ T cells. Increasing evidence suggests that signals derived from host T cells, including soluble factors such as IFN- γ as well as cell surface molecules such as CD40L, may provide crucial second signals to the maturing DCs that are required for the production of IL-12p70 (4). Therefore, we cultured immature DCs in the presence of low levels of LPS alone (10 ng/ml) or together with the CD1c-restricted γ/δ clone JR.2 and assayed supernatants by ELISA for production of IL-12p70.

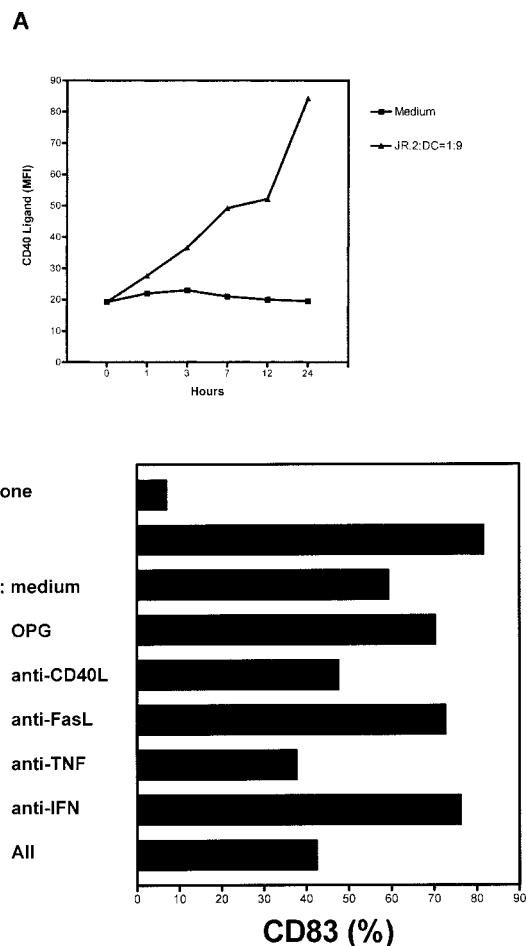


Figure 5. Role of γ/δ T cell surface molecules and soluble mediators in DC maturation. (A) CD40 ligand cell surface expression by JR.2. γ/δ T cells. CD40 ligand expression on the surface of γ/δ T cell clone JR.2 increased approximately fourfold during a 24 h coculture with immature DCs as assessed by mAb staining and flow cytometry. Triangles, culture with DC; squares, medium control. (B) Inhibition of DC maturation by blocking of γ/δ T cell surface molecules and the cytokines TNF- α and IFN- γ . Immature DCs were cocultured with LPS (10 ng/ml) or JR.2 for 48 h. In the JR.2:DC cocultures the presence of mAb against TNF- α inhibited up-regulation of DCs expressing CD83 by \sim 50%, while culture in the presence of mAbs against IFN- γ , CD40L, FasL, or the RANKL antagonist OPG had no effect. These results are representative of four independent experiments using different DC donors.

DCs cultured for 12 h in the presence of LPS or JR.2 alone failed to produce IL-12 (<30 pg/ml), whereas DCs cultured in the presence of both LPS and JR.2 (T:DC ratio = 1:10) produced significant amounts of IL-12 (18.4 \pm 7.8 ng/ml). This effect could be partially inhibited by the addition of anti-IFN- γ mAb (\sim 63% inhibition) suggesting a role for this cytokine in the ability of these DCs to make bioactive IL-12. The addition of mAb against CD1c also efficiently blocked the production of IL-12 (\sim 87% inhibition) further supporting that recognition of CD1c expressed by the immature DCs by JR.2 γ/δ T cells was critical for the production of IL-12 (Fig. 6).

DCs Matured by γ/δ T Cells Can Present Antigen to Naive Human T Cells. One hallmark of mature DCs is their

ability to present antigen to naive T cells encountered in specialized locations such as the draining lymph node. This interaction is critical for the development of an adaptive immune response and the generation of immunologic memory. We employed a system to determine if DCs, matured in the presence of the γ/δ T cell clone JR.2, were able to efficiently present antigens to CD4⁺ T cells. As mature DCs display an increased ability to stimulate allogeneic CD4⁺ T cells in a mixed lymphocyte reaction (MLR), we first assessed the capacity of DCs matured in the presence of γ/δ T cell clone JR.2 (T cell:DC ratio = 1:3) or TNF- α (50 ng/ml) to stimulate allogeneic CD4⁺ T cells. CD4⁺ T cells were cultured for 7 d with irradiated allogeneic immature monocyte-derived DCs that had been previously cultured for 72 h in the presence of TNF- α , JR.2, or medium alone. During the final 12 h of culture, cells were pulsed with [³H]thymidine and assayed for proliferation. DCs matured in the presence of JR.2 or TNF- α led to increased proliferation by allogeneic CD4⁺ T cells when compared with immature DCs that had been cultured in medium alone (Fig. 7 A). We next employed a system to determine if DCs, matured in the presence of the γ/δ T cell clone JR.2, were able to efficiently present KLH, an antigen encountered for the first time, to naive T cells. Human CD4⁺CD45RA⁺ naive T cells were isolated using a negative selection mAb column. Autologous immature monocyte-derived DCs were cultured for 72 h in the presence of TNF- α (50 ng/ml), γ/δ clone JR.2 (T cell:DC ratio = 1:10), or medium alone with or without 25 μ g/ml KLH. DCs cultured in either TNF- α (54% CD83 positive, CD86

MFI = 608) or the presence of JR.2 (42% CD83 positive, CD86 MFI = 664) expressed increased levels of CD83 and CD86 consistent with a mature DC phenotype. In contrast, the presence of KLH had no effect upon phenotypic maturation (2.5% CD83 positive, CD86 MFI = 16; data not depicted). On day 3 of culture, these DCs were washed, irradiated (5,000 rads), and then cultured with autologous naive T cells at a DC:T cell ratio of 1 to 10 for 7 d. During the final 12 h of culture, cells were pulsed with [³H]thymidine and assayed for proliferation. Only DCs pulsed with KLH and matured in the presence of TNF- α (SI = 3.6) or the CD1c-restricted γ/δ T cell clone JR.2

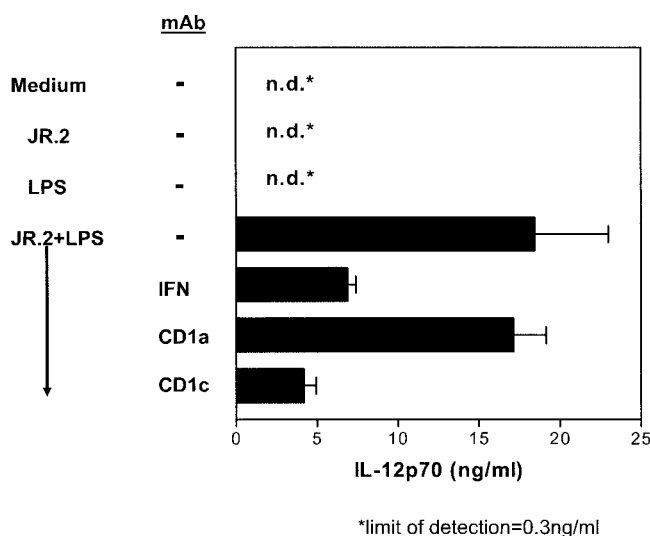


Figure 6. IL-12p70 production by DCs requires both CD1-restricted γ/δ T cells and LPS. Immature DCs were incubated for 24 h with the JR.2 γ/δ T cell clone, LPS (10 ng/ml), or both JR.2 and LPS in the presence of blocking mAbs against IFN- γ , CD1c, or CD1a. The culture supernatants were collected and assayed for IL-12p70. Note that production of IL-12p70 occurred only in the presence of both JR.2 and LPS and was inhibited by mAb against IFN- γ and CD1c but not CD1a. The sensitivity of the assay was 0.3 ng/ml. These results are representative of three independent experiments using different DC donors.

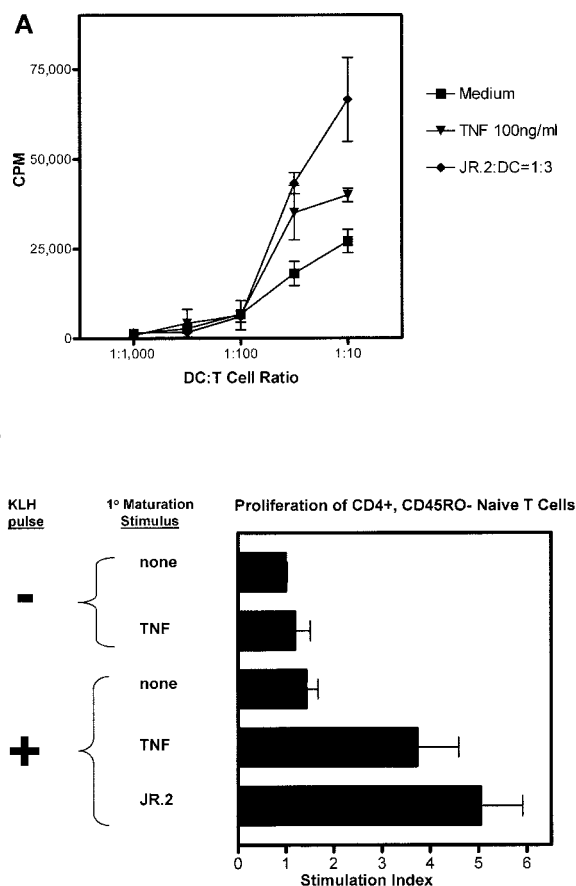


Figure 7. γ/δ clone JR.2 promotes DC antigen presentation to CD4⁺ T cells. (A) Allogeneic MLR. Immature monocyte-derived DCs were cultured for 72 h in the presence of TNF- α , γ/δ clone JR.2, or medium alone. Irradiated DC were then cultured at DC:T cell ratios from 1:1,000 to 1:10 with allogeneic CD4⁺ T cells for 7 d and proliferation was measured. DCs that had been matured in the presence of TNF- α or γ/δ clone JR.2 resulted in increased proliferation of allogeneic CD4⁺ T cells when compared with immature DCs. Squares, medium alone; inverted triangles, TNF- α ; diamonds, JR.2. (B) KLH antigen presentation. Immature monocyte-derived DCs were cultured for 72 h in the presence of TNF- α , γ/δ clone JR.2, or medium alone in the presence or absence of 25 μ g/ml Keyhole Limpet Hemacyanin (KLH). Irradiated DCs were then cultured with 5×10^4 autologous CD4⁺CD45RA⁺ naive T cells/well at a DC:T cell ratio of 1:10 for 5 d and proliferation was measured. Only DCs pulsed with KLH and matured in the presence of TNF α or γ/δ clone JR.2 resulted in proliferation by naive responder T cells. Results are expressed as relative stimulation index. Results are representative of two independent experiments using different DC donors.

(SI = 5) led to specific proliferation by the naive T cells re-active against KLH (Fig. 7 B).

Discussion

We have described and functionally characterized the ability of γ/δ T cells to mediate CD1-dependent maturation of monocyte-derived DCs. These CD1-reactive T cells express V δ 1 TCR specificities characteristic of the predominant γ/δ subset present in tissues and represent an attractive candidate for a host T cell population that could provide critical signals for the development of a fully functional DC population at an early point during microbial infection.

Studies in both mice and humans have implicated γ/δ T cells in host defense. For example, γ/δ T cells modulate the severity of disease or mediate a component of protective immunity in murine models of listeriosis, tuberculosis, malaria, and HSV-1 encephalitis. In humans, γ/δ T cells expressing V γ 2V δ 2 TCRs are dramatically expanded during infection with *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Brucella melitensis*, and *Ehrlichia chaffeensis* (23). Additionally, γ/δ T cells expressing V δ 1 TCR specificities are expanded during HIV (24, 25) and malarial (26) infections. Recent evidence has demonstrated that the antigens recognized by the V γ 2V δ 2 γ/δ T cell subset, the predominant γ/δ population in the blood, are small nonpeptide molecules containing alkylphosphate (27, 28), alkylamine (29), and bisphosphonate moieties (30). Further, V γ 2V δ 2 T cells display a memory-type response to these antigens in BCG-vaccinated macaque monkeys and provide immunity to subsequent challenge with *Mycobacterium tuberculosis* (31). In contrast, human γ/δ T cells bearing V δ 1-encoded TCRs account for the vast majority of γ/δ T cells in tissues such as the spleen and intestine (32) yet the antigens that they recognize remain enigmatic. Recent studies have demonstrated that a subset of V δ 1⁺ γ/δ T cells can recognize stress inducible MHC class I-like MIC molecules expressed by epithelial cells via specific TCR interactions (33). We have previously demonstrated that this major tissue population of γ/δ T cells expressing V δ 1-encoded TCRs recognizes CD1c, one member of a family of nonpolymorphic CD1 molecules expressed on the surface of DCs and B cells, that present lipid and glycolipid foreign antigens to T cells (13). Importantly, this recognition of CD1c occurred in the absence of exogenous foreign antigens, implying reactivity against as yet to be identified self-lipids.

Here, we demonstrated the capacity of V δ 1 T cells to recognize CD1 on immature DCs and induce their maturation. Hence, based upon their location in the tissues and their recognition of CD1c, these T cells may be uniquely poised to interact with immature DCs during the earliest time points during microbial infection. Although the γ/δ T cell population that we have described here expresses V δ 1 TCR specificities characteristic of the γ/δ subpopulation predominant in peripheral tissues, recent evidence suggests that these cells are also present in the lymph nodes (M. Brandes and B. Moser, personal communication).

Thus, they may encounter recently activated DCs either in tissues or in draining lymph nodes.

Signals derived from host T cells play a critical role in the generation of mature DCs capable of effectively polarizing naive antigen-specific T cells to produce Th1 cytokines. Although microbial products such as LPS can effectively promote maturation and the initial production of IL-12 by the DCs, in the absence of additional host-derived signals such as IFN- γ , these DCs rapidly lose the capacity to produce IL-12 upon subsequent CD40 ligation by naive T cells (4). Several host cell types have been proposed as candidates to provide such signals to the maturing DCs. CD45RA⁺ CD8 T cells can rapidly produce IFN- γ and TNF- α and augment the ability of the maturing DCs to polarize naive CD4⁺ T cells toward a Th1 response (21). However, in the naive host the pool of antigen-specific CD8 T cells available to provide such signals at the site of a microbial infection would likely be small, limiting the amount of these cytokines available to DCs during their initial exposure to a microbe. Similarly, NK cells can also provide IFN- γ and TNF- α to maturing DCs but this interaction would also seem to be limited by the availability of these cells at the site of infection and require prior activation of this pool of NK cells (22). In addition, it has recently been reported that phosphoantigen-reactive γ/δ T cells, which are not CD1-restricted, can promote DC maturation under specific activating conditions (34). The CD1c self-reactive γ/δ T cells characterized here, express V δ 1 TCR specificities characteristic of the subpopulation of γ/δ T cells that predominates in tissues. Additionally, these cells are able to promote DC maturation via CD1 recognition without the requirement for specific recognition of microbial antigens. Therefore, we suggest based upon their location at the site infection and their ability to mediate DC maturation via recognition of CD1 that this important population of T cells can provide the host with the ability to rapidly generate a pool of mature DCs able to polarize naive T cells during subsequent encounter in the secondary lymphoid tissues. DCs matured in the presence of CD1-restricted γ/δ T cells expressed increased levels of costimulatory molecules, decreased antigen uptake, and redistribution of MHC class II molecules consistent with functional maturation. This CD1c-dependent maturation was partially mediated by TNF- α and required cell-cell contact. In addition, these mature DCs were able to efficiently present a protein antigen not previously encountered, such as KLH, to naive CD4⁺ T cells. Finally, our data demonstrate that DCs require both a microbial signal, LPS, as well as IFN- γ produced by CD1-reactive γ/δ T cells for production of heterodimeric IL-12. Thus, CD1-reactive γ/δ T cells may provide critical signals for Th1 polarization of naive antigen-specific CD4 T cells.

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