RESEARCH ARTICLE | FEBRUARY 01 2006

Potentiation of Antigen-Stimulated Vγ9Vδ2 T Cell Cytokine Production by Immature Dendritic Cells (DC) and Reciprocal Effect on DC Maturation[1]

Marie-Claire Devilder; ... et. al

J Immunol (2006) 176 (3): 1386–1393.
https://doi.org/10.4049/jimmunol.176.3.1386

Related Content

In Vivo Immunomanipulation of Vγ9Vδ2 T Cells with a Synthetic Phosphoantigen in a Preclinical Nonhuman Primate Model
J Immunol (October,2005)

Early Triggering of Exclusive IFN-γ Responses of Human Vγ9Vδ2 T Cells by TLR-Activated Myeloid and Plasmacytoid Dendritic Cells
J Immunol (September,2009)

Partial and Ineffective Activation of Vγ9Vδ2 T Cells by Mycobacterium tuberculosis-Infected Dendritic Cells
J Immunol (August,2010)

Downloaded from http://journals.aai.org/jimmunol/article-pdf/176/3/1386/1217988/1386.pdf by guest on 27 January 2023

Potentiation of Antigen-Stimulated Vγ9Vδ2 T Cell Cytokine Production by Immature Dendritic Cells (DC) and Reciprocal Effect on DC Maturation[1]

Marie-Claire Devilder,[2]* Sophie Maillet,[2]* Isabelle Bouyge-Moreau,* Emmanuel Donnadieu,† Marc Bonneville,[3]* and Emmanuel Scotet[3]*

Vγ9Vδ2 T cells, a major γδ PBL subset in human adults, have been previously implicated in dendritic cell (DC) licensing, owing to their high frequency in peripheral tissues and their ability to produce inflammatory cytokines upon recognition of a broad array of conserved Ags. Although these observations implied efficient recognition of Ag-expressing immature DC (iDC) by Vγ9Vδ2 T cells, the role played by DC subsets in activation of these lymphocytes has not been carefully studied so far. We show that iDC, and to a lesser extent mature DC, potentiated Th1 and Th2 cytokine, but not cytolytic or proliferative responses, of established Vγ9Vδ2 T cell clones and ex vivo memory Vγ9Vδ2 PBL stimulated by synthetic agonists. The ability of iDC to potentiate Vγ9Vδ2 production of inflammatory cytokines required for their own maturation suggested that Vγ9Vδ2 T cells, despite their strong lytic activity, could promote efficient iDC licensing without killing at suboptimal Ag doses. Accordingly Vγ9Vδ2 cells induced accelerated maturation of Ag-expressing iDC but not "bystander" DC, even within mixed cell populations comprising both Ag-expressing and nonexpressing iDC. Furthermore Vγ9Vδ2 cells induced full differentiation into IL-12-producing cells of iDC infected by Vγ9Vδ2-stimulating mycobacteria that were otherwise unable to induce complete DC maturation. In conclusion the ability of iDC to selectively potentiate cytokine response of memory Vγ9Vδ2 T cells could underlie the adjuvant effect of these lymphocytes, and possibly other natural memory T cells, on conventional T cell responses. *The Journal of Immunology,* **2006, 176: 1386–1393.**

Mammalian CD3$^+$ lymphocytes can be separated into two lymphocyte subsets bearing T cell receptors composed of either αβ or γδ heterodimers, for which expression is mutually exclusive. Specificity and physiology of conventional MHC-restricted αβ T cells is by now well understood, whereas the primary physiological functions of γδ T cells remain debated. Most of our understanding of the biological functions of human γδ T cells has been drawn from analysis of Vγ9Vδ2 cells, a major peripheral blood γδ subset representing up to 5% of the PBL pool in adults (1, 2). Like CD1d-restricted invariant NKT cells, Vγ9Vδ2 cells have been referred to as innate-like T cells owing to: 1) their ability to recognize conserved Ag expressed by a broad range of infected, stressed and/or transformed cells, 2) their preactivated status resulting from an early (postnatal) acquisition of memory markers, and 3) their high frequency in particular tissue locations (1, 3, 4). Two kinds of Ag selectively stimulating Vγ9Vδ2 T cells have been identified: 1) small phosphorylated compounds referred to as phosphoantigens (5, 6), which are produced through the isoprenoid biosynthetic pathway of mammalian (such as isopentenyl pyrophosphate (IPP)[4]) (7) and nonmammalian cells (such as 4-hydroxy-3-dimethylallyl pyrophosphate) (8) and 2) complexes comprising ATP synthase subunits, which are found on the surface of some tumor cells (9). Besides pharmacological agents acting upstream (like statins) or downstream (like aminobisphosphonate (ABP)), IPP biosynthesis has been shown to inhibit or enhance target cell lysis by Vγ9Vδ2 T cells (10, 11).

The cellular requirements for activation of innate-like T cells, including Vγ9Vδ2 cells, remain ill defined. Vγ9Vδ2 cells are generally poor producers of IL-2 and, therefore, can proliferate upon Ag stimulation in vitro and in vivo only in the presence of Th cells or exogenous IL-2 (12). Whether optimal activation of Vγ9Vδ2 cells requires additional factors provided, e.g., by professional APC, remains unclear. Owing to their memory status, these cells are classically considered as dendritic cell (DC)-independent, and accordingly can efficiently expand in vitro in response to phosphoantigen and IL-2 in the absence of any adherent cells. However optimal activation of Vγ9Vδ2 T cells by ABP clearly requires the presence of myelomonocytic or transformed cells (13–15). The explanation classically put forward is that ABP can only act on cells showing high pinocytic activity such as DC, macrophages, or tumor cells. However the need for additional factors specifically provided by APC could not be ruled out in the absence of any detailed comparison of Vγ9Vδ2 T cell responses against soluble phosphoantigen in various cellular contexts. In this regard, several

*Institut National de la Santé et de la Recherche Médicale, Unité 601, Nantes, France; and †Institut National de la Santé et de la Recherche Médicale, Unité 567, Centre National de la Recherche Scientifique, Unité Mixte de Recherche 8104, Paris, France

Received for publication August 25, 2005. Accepted for publication November 8, 2005.

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.

[1] This work was supported by grants from the Institut National de la Santé et de la Recherche Médicale, from the Commission of the European Union Programme 6FP, Contract No. LSHP-CT-2003-503367, "TB-VAC: An integrated project for design and testing of vaccine candidates against tuberculosis: identification, development and clinical studies," and from the Association pour la Recherche sur le Cancer Contract No. 3662.

[2] M.-C.D. and S.M. contributed equally to this work.

[3] Address correspondence and reprint requests to Dr. Marc Bonneville, INSERM U601, Institut de Biologie, 9 Quai Moncousu, 44093 Nantes Cedex, France; E-mail address: bonnevil@nantes.inserm.fr or Dr. Emmanuel Scotet, INSERM U601, Institut de Biologie, 9 Quai Moncousu, 44093 Nantes Cedex, France; E-mail address: Emmanuel.Scotet@nantes.inserm.fr

[4] Abbreviations used in this paper: IPP, isopentenyl pyrophosphate; DC, dendritic cell; iDC, immature DC; mDC, mature DC; B-LCL, B lymphoblastoid cell line; ABP, aminobisphosphonate; BrHPP, bromohydrin pyrophosphate; BCG, bacillus Calmette-Guérin; MOI, multiplicity of infection.

Copyright © 2006 by The American Association of Immunologists, Inc.

0022-1767/06/$02.00

Downloaded from http://journals.aai.org/jimmunol/article-pdf/176/3/1386/1217988/1386.pdf by guest on 27 January 2023

indirect observations suggest that DC can enhance in vivo activation and proliferation of other innate-like (16–18) or conventional memory T cell subsets (19–21).

The functions played by Vγ9Vδ2 T cells are still unclear. Owing to their potent lytic and bactericidal activities, these cells can directly contribute to elimination of infected or tumor cells (22–25). Like invariant NKT cells, they may also enhance NK and conventional T cell responses through release of proinflammatory cytokines (such as IFN-γ and TNF-α) and DC priming (26). In this regard, several studies have reported in vitro maturation of DC upon coculture with phosphoantigen- or ABP-stimulated Vγ9Vδ2 cells (15, 27). However in neither case have the outcome of Ag-expressing DC been precisely studied in these in vitro systems. In particular the possibility that DC maturation could exclusively result from a "bystander" process (28), which would involve DC not interacting with Vγ9Vδ2 T cells but exposed to inflammatory factors released by the latter cells upon interaction and subsequent killing of Ag-expressing DC, could not be ruled out. Besides such experiments have been performed in quite "artificial" Ag-stimulating conditions but not yet in the context of infection by Vγ9Vδ2-stimulating pathogens.

To address some of these issues, we have studied the APC requirements for optimal in vitro activation of Vγ9Vδ2 T cells and the consequences of Vγ9Vδ2 T cell activation on the phenotypic and functional status of Vγ9Vδ2 Ag-expressing APC. Our results show an unexpected ability of immature DC (iDC) to potentiate memory Vγ9Vδ2 cytokine responses with far better efficacy than any other APC tested, including mature DC (mDC). This observation could underlie the capacity of Vγ9Vδ2 cells to promote full maturation of Ag-expressing iDC, and possibly a general adjuvant effect of innate-like memory T cell on conventional T cell responses.

Materials and Methods

Abs and reagents

The following mAbs from Immunotech Beckman Coulter were used for flow cytometry: FITC-conjugated Vδ2, CD83 and MHC class I, PE-conjugated CD86 and MHC class II, and PC5-pan γδ. For intracellular staining of cytokines, PE-conjugated mAbs specific of TNF-α, IFN-α, IL-4, and IL-12p40/70 were obtained from BD Pharmingen. Flow cytometry acquisitions and analysis were performed using LSR and FACSCalibur systems and CellQuest Pro software (BD Biosciences). IL-12p70 pair of mAbs designed for ELISA was purchased from R&D Systems. SNARF-1 (seminaphthorhodafluor-1) cell tracking dye and fura 2 were purchased from Molecular Probes. Phosphostim (bromohydrin pyrophosphate (BrHPP)) was provided by Innate Pharma (29). Pamidronate supplied as disodium salts was obtained from Calbiochem. Brefeldin A, PMA, ionomycin, and LPS from *Escherichia coli* were purchased from Sigma-Aldrich. Recombinant human GM-CSF, IL-4, and TNF-α were obtained from AbCys. *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) and GFP-BCG strains were supplied, respectively, by G. Marchal and B. Gicquel (Institut Pasteur, Paris, France).

DC isolation

Human monocytes were isolated from peripheral blood samples of healthy donors. Briefly, PBMC were collected after Ficoll-Hypaque (Eurobio) density centrifugation, resuspended at 7×10^6 cells/ml and allowed to adhere in culture flasks (RPMI 1640 supplemented with 2 mM L-glutamine, streptomycin, penicillin, 5% FCS) for 1 h 30 at 37°C. Nonadherent cells were removed by gentle pipetting and adherent cells were rinsed five times with PBS. The resulting adherent monocyte populations ($CD14^+$ >90%) were routinely checked for the presence of contaminant cells as well as for the expression of monocyte-specific markers by flow cytometry. Monocytes were further differentiated for 5–6 days in the same medium supplemented with GM-CSF (350 U/ml) and IL-4 (500 U/ml). The resulting cell preparations routinely contained >90% of iDC as assessed by flow cytometric analysis. For induction of maturation, cultures of monocyte-derived iDC were supplemented with 1 μg/ml LPS and 2.5 ng/ml TNF-α for 24 or 48 h. Phenotype of mDC was routinely checked by analysis of up-regulation of surface markers such as CD80, CD83, CD86, and MHC class I and class II. iDC and mDC functional status was confirmed by MLR assays using naive T cell populations (data not shown). In some experiments iDC were stained with SNARF-1 (2 μM) tracking dye for 10 min at 37°C and washed extensively before being used in T cell/DC coculture assays. ABP treatment of DC was performed by incubating cells for 16 h with various doses of pamidronate, and DC were extensively washed before use. No significant ABP-induced maturation of iDC could be detected following pamidronate sensitization of DC. For mycobacterial infections, iDC (1×10^6/ml) were washed in complete medium without antibiotics and infected for 5 or 16 h with BCG at various multiplicity of infection (MOI) values. BCG-infected iDC were extensively washed in medium before use in T cell/DC cocultures. Internalization of GFP-mycobacteria in DC was assessed by using standard ethidium bromide staining and fluorescence microscopy.

γδ T cells

Vγ9Vδ2 T cell clones (G42 and G115) were obtained and expanded as already described (30). T cell clones were cultured in RPMI 1640 supplemented with 2 mM L-glutamine, 10 μg/ml streptomycin, 100 U/ml penicillin, 10% FCS, and human IL-2 (300 U/ml). Fresh ex vivo nonadherent cells from healthy donors blood samples were collected following plastic adherence process.

Determination of cytokine levels

γδ T cells (1.5×10^5) were activated by soluble, endogenous, or mycobacterial phosphoantigens in the absence or in the presence of different APC (T cell, B lymphoblastoid cell line (B-LCL), iDC, or mDC) generally at a T cell to DC ratio of 1:1, unless indicated. After 2 h, accumulation of cytokines was induced by adding brefeldin A (10 μg/ml). After 5 h, cells were collected, subsequently stained for surface γδ TCR expression, and fixed with 2% paraformaldehyde. T cells were further permeated at 4°C with 0.4% saponin and incubated with fluorochrome-conjugated mAbs specific for cytokines. Double-stained cells were analyzed by flow cytometry. For Transwell assay, T cells and DC were cocultured with BrHPP (3 μM) as indicated in 24-well plate or separated by a permeable membrane (12 mm diameter; 0.4-μm pore size) in Transwell chambers (Corning). After 5 h, cytokine accumulation was determined by intracellular staining in T cells seeded in a lower chamber. In some experiments, DC (3×10^5) were pretreated with LPS for 3 h at 37°C, washed, and cocultured together with γδ T cell clones (1×10^5) in the presence of BrHPP (3 μM). After 48 h, supernatants from T cell/DC cocultures in triplicates were harvested and subsequently analyzed for IL-12p70 secretion by ELISA, following standard procedures.

^{51}Cr release assay

Cytolytic activity of phosphoantigen-activated Vγ9Vδ2 T cell clones was measured by a standard 4-h ^{51}Cr release assay (31). Percentage of specific target cell lysis was calculated according to the following formula: ((experimental release − spontaneous release)/(maximum release − spontaneous release)) × 100. Maximum and spontaneous release were determined by respectively adding 0.1% Triton X-100 or medium to target cells in the absence of effector cells. Data are presented as the mean of triplicate samples.

Single cell Ca^{2+} video imaging

Measurement of the intracellular Ca^{2+} concentration was performed as previously described (32). In brief, DC (1×10^5) were left to adhere to glass coverslips for 45 min and washed with mammalian saline buffer (33) supplemented with 2% of FCS. γδ T cells were incubated for 20 min at 37°C with 1 μM fura 2-AM. Cells were then washed and added to the DC layer simultaneously with BrHPP (3 μM). Ca^{2+} video imaging was performed at 37°C in mammalian saline buffer with an Eclipse TE300 inverted microscope (Nikon), equipped with a ×20 UV permissive objective. Images were acquired and analyzed with Metafluor imaging software (Universal Imaging). Fura 2-AM loaded T cells were excited at 350 and 380 nm. Emissions at 510 nm were used for analysis of Ca^{2+} responses. Transmission light images acquired to visualize the DC interacting with γδ T cells were taken every 30 s in turn with fluorescence images. T cells were considered as responsive when the amplitude of their response reaches at least twice that of the background.

Results

TNF-α response of phosphoantigen-stimulated Vγ9Vδ2 T cells is enhanced by iDC, and to a lesser extent by mDC

To study the cellular requirements for optimal Vγ9Vδ2 T cell activation by phosphoantigens, we compared the ability of

Downloaded from http://journals.aai.org/jimmunol/article-pdf/176/3/1386/1217988/1386.pdf by guest on 27 January 2023

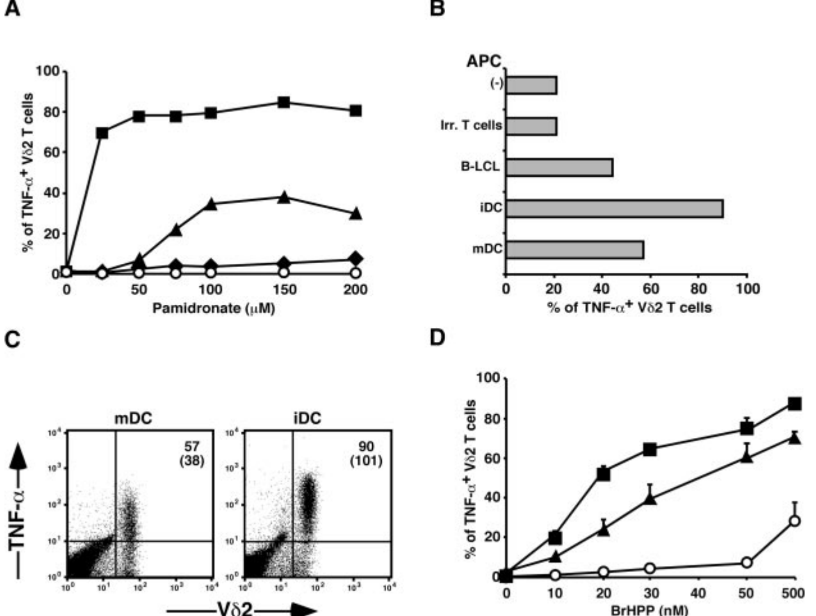
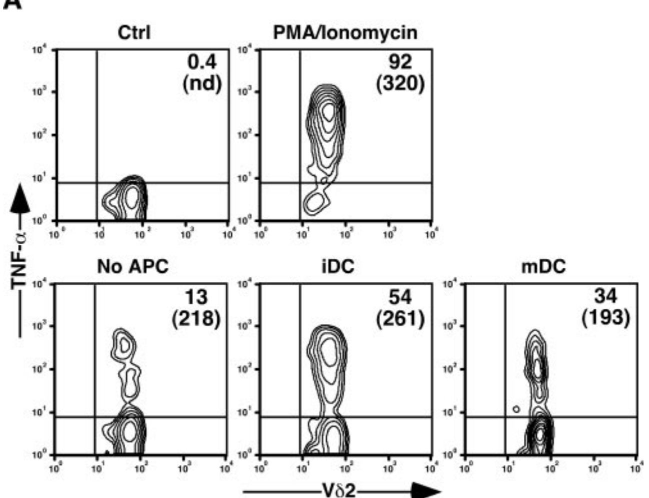


FIGURE 1. iDC potentiate TNF-α production by phosphoantigen-activated human Vγ9Vδ2 T cells. *A*, iDC (■), mDC (▲), B-LCL (◆) and irrelevant T cells (○) were sensitized with grading doses of pamidronate and cocultured together with Vγ9Vδ2 T cells (G42) at a 1:1 ratio. Intracellular TNF-α was detected in V$\delta 2^+$ cells by flow cytometry. *B*, Vγ9Vδ2 T cells (G42) were activated by BrHPP (3 μM) in the presence of different APCs: no APC (−), irrelevant T cells (Irr.T), B-LCL, iDC, and mDC. Intracellular TNF-α was detected in V$\delta 2^+$ cells by flow cytometry. *C*, Dot plot representation of experiment described in *B* showing TNF-α production by V$\delta 2^+$ T cells in the presence of iDC and mDC. Values for the percentage of cytokine-positive cells (top) and mean fluorescence intensity (bottom parentheses) are indicated in *upper right-hand* corner of quadrant. *D*, Vγ9Vδ2 T cells (G42) were activated with grading doses of BrHPP in the presence of iDC (■), mDC (▲), or irrelevant T cells (○). Intracellular TNF-α was detected in V$\delta 2^+$ cells by flow cytometry. One representative result of at least three experiments is shown.

different APCs to enhance the cytolytic and cytokine responses of Vγ9Vδ2 T cells activated by either an ABP (pamidronate) or a synthetic phosphoantigen (BrHPP). We first measured TNF-α response of Vγ9Vδ2 T cell clones cocultured with iDC, mDC, or $\alpha\beta$ T cells that have been preincubated with increasing doses of pamidronate. As indicated in Fig. 1*A*, pamidronate-treated monocyte-derived DC promoted stronger Vγ9Vδ2 TNF-α responses than irrelevant $\alpha\beta$ T cells and among the former cells, iDC were much more potent stimulators than mDC. This observation could reflect either differential responses of iDC vs mDC to pamidronate and/or a general ability of iDC to potentiate cytokine response of Ag-stimulated Vγ9Vδ2 cells. To test this idea, we performed similar coculture experiments using Vγ9Vδ2 cells stimulated by a soluble phosphoantigen (BrHPP) at saturating concentrations. Like pamidronate-stimulated cultures, the frequency of TNF-α producing cells was higher within BrHPP-stimulated Vγ9Vδ2 lymphocytes cocultured with DC than within Ag-stimulated Vγ9Vδ2 cells alone or cocultured with $\alpha\beta$ T cells or B-LCL. Furthermore among DC, iDC were better stimulatory APC than mDC (Fig. 1*B*). Individual Vγ9Vδ2 T cells accumulated more TNF-α when cocultured with iDC than mDC, as indicated by increased mean fluorescence intensity of intracellular TNF-α staining measured by flow cytometry (Fig. 1*C*). Similar results were obtained with other T cell clones (data not shown), ex vivo memory Vδ2 T cells (Fig. 2), or using suboptimal doses of BrHPP (Fig. 1*D*). Taken together these results indicate that iDC are more potent stimulators than mDC of TNF-α responses of Ag-stimulated Vγ9Vδ2 T cells.

iDC potentiate both Th1 and Th2 cytokine responses of phosphoantigen-activated Vγ9Vδ2 T cells but not their cytolytic activity

To test the overall effect of DC on the activation of Ag-stimulated Vγ9Vδ2 cells, we studied their impact on the proliferative activity as well as on several Vγ9Vδ2 effector responses such as Th1/Th2 cytokine production and cytolytic activity. Like TNF-α response, IFN-γ response of BrHPP-stimulated Vγ9Vδ2 cells was significantly enhanced by iDC, and to a lesser extent by mDC, when compared with Vγ9Vδ2/T cell cocultures (Fig. 3*A*). This potentiation effect was also observed on IL-4 responses of Th0/Th2 Vγ9Vδ2 T cell clones (Fig. 3*B*). By contrast, cytolytic activities of



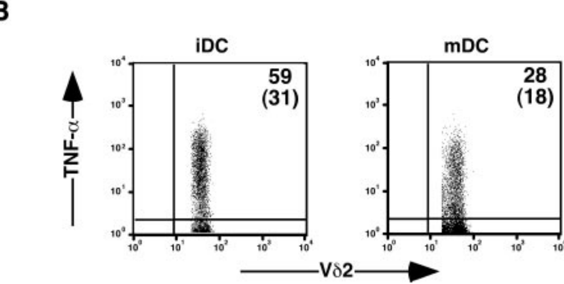


FIGURE 2. iDC potentiate TNF-α production of ex vivo phosphoantigen-activated human Vγ9Vδ2 T cells. *A*, Fresh nonadherent cells isolated from PBMC of a healthy donor (donor no. 569) were activated for 5 h using BrHPP (3 μM) in the presence of different APCs: no APC, autologous iDC, or mDC (Ctrl: nonstimulated). *B*, iDC and mDC were preincubated with pamidronate (250 μM) washed and cocultured with fresh nonadherent cells isolated from PBMC of a healthy donor (donor no. 961) for 5 h at a 5:1 ratio. Intracellular TNF-α was detected in V$\delta 2^+$ cells by flow cytometry. Values for the percentage of cytokine-positive Vδ2 T cells (top) and mean fluorescence intensity (bottom parentheses) are indicated in the *upper right-hand* corner of quadrant.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/176/3/1386/1217988/1386.pdf by guest on 27 January 2023

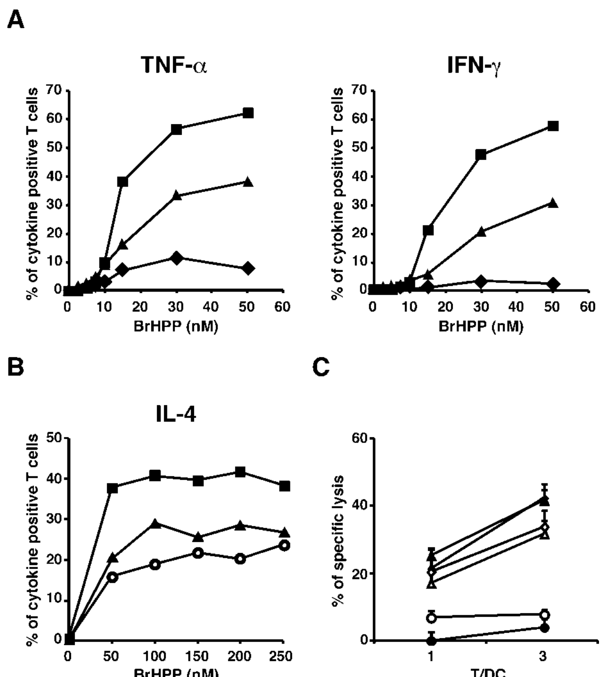
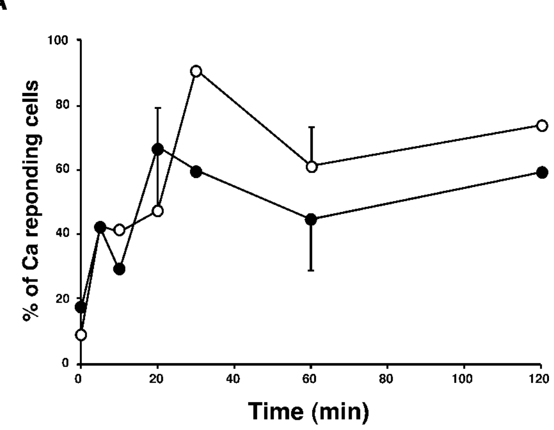


FIGURE 3. iDC potentiate cytokine production but not cytolytic activity of phosphoantigen-activated human Vγ9Vδ2 T cells. *A*, Vγ9Vδ2 T cells (G42) were activated using grading doses BrHPP in the presence of iDC (■), mDC (▲), or irrelevant T cells (♦). Intracellular TNF-α (*left*) and IFN-γ (*right*) were detected in Vδ2$^+$ cells by flow cytometry. *B*, IL-4-producing Vγ9Vδ2 T cells (G115) were activated using grading doses BrHPP without APC (○) or in the presence of either iDC (■) or mDC (▲). Intracellular IL-4 was detected in Vδ2$^+$ cells by flow cytometry. *C*, ^{51}Cr-labeled iDC (filled symbols) or mDC (open symbols) were incubated together with Vγ9Vδ2 T cells (clone G42) at the indicated T cell to DC ratios and BrHPP concentrations of 10 nM (circles), 100 nM (triangles), and 500 nM (diamonds). Results are expressed as the percentage of specific lysis calculated as described in *Materials and Methods*. Data represent the average value of triplicate samples ± SD. Results are representative of at least three independent experiments.



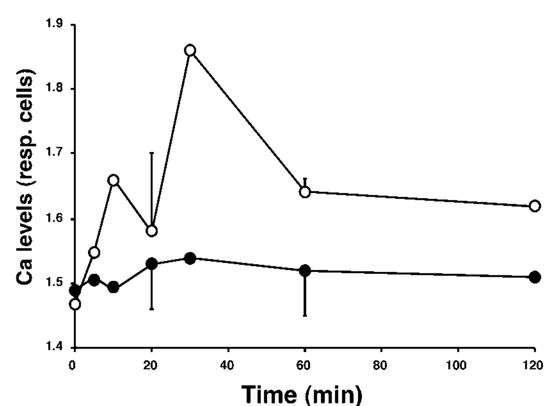


FIGURE 4. Characteristics of Vγ9Vδ2 T cell Ca^{2+} responses activated by soluble phosphoantigen in the presence of DC. *A*, Percentage of Ca^{2+} (G42) responding T cells cocultured together with iDC (○) or mDC (●) in the presence of BrHPP (3 μM). *B*, Ca^{2+} levels measured in responding Vγ9Vδ2 T cells. Results represent the mean and SD of experiments in which >30 cells were analyzed by experiment. Results are representative of three independent experiments.

Vγ9Vδ2 T cell clones incubated with either iDC or mDC in the presence of high (500 nM) or low (10 nM) doses of BrHPP were similar (Fig. 3*C*). Moreover, when activated by BrHPP in the presence of low doses of IL-2, polyclonal Vγ9Vδ2 T cells did not expand better in the presence of autologous iDC than mDC (data not shown). Altogether these results indicate that iDC potentiate both Th1 and Th2 cytokine responses of Vγ9Vδ2 T cells, but have no or limited effects on their cytotoxicity and proliferation.

Ca^{2+} responses and kinetics of cytokine production of phosphoantigen-activated Vγ9Vδ2 T cells cocultured with DC

To better characterize the early events occurring when Vγ9Vδ2 T cells are activated by phosphoantigens in the presence of DC, we compared Ca^{2+} responses evoked in γδ cells by iDC and mDC during the first 2 h following addition of soluble BrHPP. As shown in Fig. 4*A*, the percentage of Ca^{2+}-responding cells peaked at 20–30 min, decreased at 60 min, and reached a plateau for the remaining time. The overall kinetics of Ca^{2+} response were similar for Vγ9Vδ2 T cells incubated with either iDC or mDC. However the average Ca^{2+} levels in Ag-stimulated T cells at 30 min and later time points were higher in the presence of iDC than mDC (Figs. 4*B* and 5), thus suggesting strong and sustained BrHPP-induced signaling in the former but not the latter case. Accordingly although the kinetics of TNF-α and IFN-γ production of BrHPP-stimulated Vγ9Vδ2 T cells incubated with iDC or mDC were very similar, the amount of cytokine produced per T cell was dramatically increased in iDC cocultures after 90 min and rapidly reached a plateau after 180 min (Fig. 6).

iDC-mediated potentiation of cytokine production by phosphoantigen-activated Vγ9Vδ2 T cells requires close cell-to-cell contacts

To assess the respective implication of soluble vs membrane-bound stimuli to the iDC potentiation effect on Vγ9Vδ2 T cells activation, BrHPP activation of Vγ9Vδ2 cells was performed in Transwell conditions. The highest TNF-α production levels were observed when T cells and iDC were left together (>50% of TNF-α$^+$ T cells) (Fig. 7). In contrast, phosphoantigen activation was decreased to basal levels (20%) when DC and T cells were separated, indicating that T cells and DC needed to be in close contact for optimal activation. Moreover, the fact that TNF-α responses of T cells in the lower chamber were not enhanced by T cell/DC cocultures put in the upper chamber argues against the implication of soluble factors in the potentiation process.

Vγ9Vδ2 T cells induce full maturation of pamidronate- or BrHPP-treated iDC

The results described indicated that Vγ9Vδ2 T cells produced high levels of Th1 and Th2 cytokines when activated by phosphoantigens in the presence of iDC, even at Ag concentrations in which

Downloaded from http://journals.aai.org/jimmunol/article-pdf/176/3/1386/1217988/1386.pdf by guest on 27 January 2023

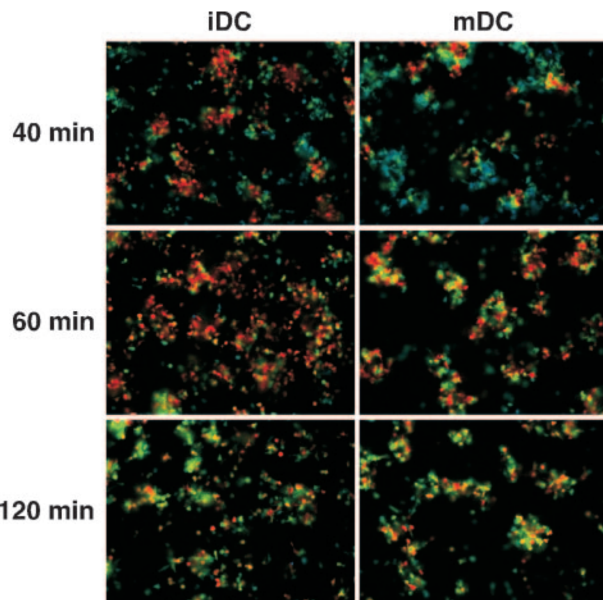


FIGURE 5. DC-induced T cell Ca^{2+} responses. Fura 2-labeled Vγ9Vδ2 T cells were cocultured with either iDC (left) or mDC (right) at 1:1 ratio in the presence of BrHPP (3 μM). Images were captured at the indicated times following addition of BrHPP. Intensity of Ca^{2+} responses is represented in pseudocolors (from blue to red). Images are representative of three independent experiments.

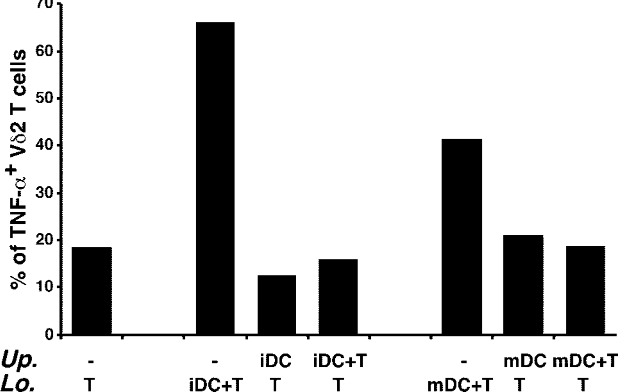


FIGURE 7. Close cell-to-cell contacts between iDC and Vγ9Vδ2 T cells are required for potentiation of cytokine production following phosphoantigen activation. iDC and Vγ9Vδ2 T cells (clone G42) were cocultured together (ratio 1:1) with BrHPP (3 μM) in transwells and distributed as upper chamber (Up.) and lower chamber (Lo.). $V\delta2^+$ cells seeded in lower chamber were harvested, and intracellular TNF-α was detected by flow cytometry. Data are representative of at least three independent experiments.

iDC lysis was minimal in short term ^{51}Cr release assay. This suggested that despite their strong lytic potential, Vγ9Vδ2 cells could mediate maturation of Ag-expressing iDC. To test this suggestion more directly, iDC were preincubated with low (10 μM) or high (150 μM) doses of pamidronate and their survival was assessed by flow cytometry after a 2-day coculture with Vγ9Vδ2 T cells at different T cell to DC ratios (Fig. 8*A*). TNF-α production by Vγ9Vδ2 T cells and CD86 up-regulation on iDC were studied in parallel (Fig. 8*B*). Significant DC death was detectable only at high T cell to DC ratio (5:1) and high doses of pamidronate (150 μM). At lower T cell to DC ratios (1:1 and 1:5), Ag-stimulated Vγ9Vδ2 T cells produced TNF-α and cocultured iDC strongly up-regulated (>4-fold) CD86 at their surface, thus indicating a T cell-driven maturation process. To more specifically study the outcome of iDC interacting with Vγ9Vδ2 cells, expression of maturation markers (CD83, CD86, and MHC class I and class II) was assessed within a mix (1/1) of pamidronate-treated and nontreated iDC after a 24- to 48-h incubation with Vγ9Vδ2 cells (Fig. 8*C* and data not shown). Although at 48 h, both pamidronate-treated and untreated DC subsets showed similar maturation phenotypes, only pamidronate-treated DC were phenotypically mature at 24 h, which indicated accelerated Vγ9Vδ2-induced maturation of Ag-expressing DC, when compared with bystander ones. Consistent with induction of full DC maturation by Vγ9Vδ2 cells, and in agreement with previous studies (15, 27), a dramatic up-regulation of IL-12p70 production was observed within iDC pretreated with as low as 1 ng/ml LPS and then incubated for 2 days with Vγ9Vδ2 cells in the presence of BrHPP (Fig. 8*D*).

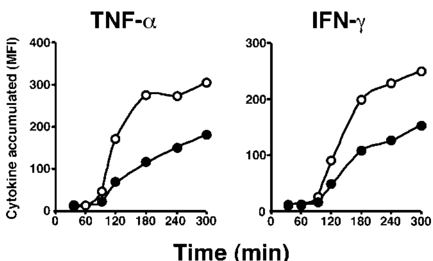


FIGURE 6. Kinetics of cytokines accumulation in phosphoantigen-activated Vγ9Vδ2 T cells. Vγ9Vδ2 cells (G42) were cocultured at 1:1 ratio with either iDC (○) or mDC (●) for 5 h in the presence of BrHPP (30 nM) and brefeldin A. At the indicated times, cells were harvested and fixed, and intracellular TNF-α (*left*) and IFN-γ (*right*) in $V\delta2^+$ cells were detected by flow cytometry.

Vγ9Vδ2 T cells mediate full maturation of BCG-infected DC

To assess the significance of our findings in a more physiological context, we studied the ability of Vγ9Vδ2 cells to induce maturation of iDC infected by a Vγ9Vδ2-stimulating pathogen. We chose the BCG infection model because this mycobacteria is known to express phosphoantigen (5, 34–36) and previous studies have described incomplete maturation of iDC infected with BCG-related mycobacterial strains (37–39). Vγ9Vδ2 T cells produced high amounts of IFN-γ and TNF-α when incubated with iDC infected with as low as 1 BCG/cell (MOI of 0.1–1) (Fig. 9, *A* and *B*), i.e., in conditions in which infected iDC lysis remained limited (Fig. 10). Importantly although BCG infection induced up-regulation of CD83, CD86, and MHC on iDC, coincubation with Vγ9Vδ2 cells led to accelerated maturation of infected iDC, as indicated by the 2-fold increase of CD86 expression levels at 24 h of coculture (Fig. 9*C*). Furthermore strong up-regulation of IL-12p70 production was observed in short-term infected iDC incubated with Vγ9Vδ2 cells but not in BCG-infected iDC alone (Fig. 9*D*). Finally, Vγ9Vδ2 T cells induced full maturation of BCG-infected but not noninfected iDC within mixed iDC populations (Fig. 9*E*). This indicated that signals delivered by activated Vγ9Vδ2 T cells upon recognition of BCG-infected cells were not sufficient to induce full maturation of noninfected iDC.

Discussion

This study demonstrates an unexpected role played by iDC in activation of a major γδ T cell subset with memory features. The fact that iDC selectively potentiated cytokine but not cytolytic or proliferative responses of Ag-stimulated γδ T cells could be related to the previously demonstrated ability of Vγ9Vδ2 T cells to induce

Downloaded from http://journals.aai.org/jimmunol/article-pdf/176/3/1386/1217988/1386.pdf by guest on 27 January 2023

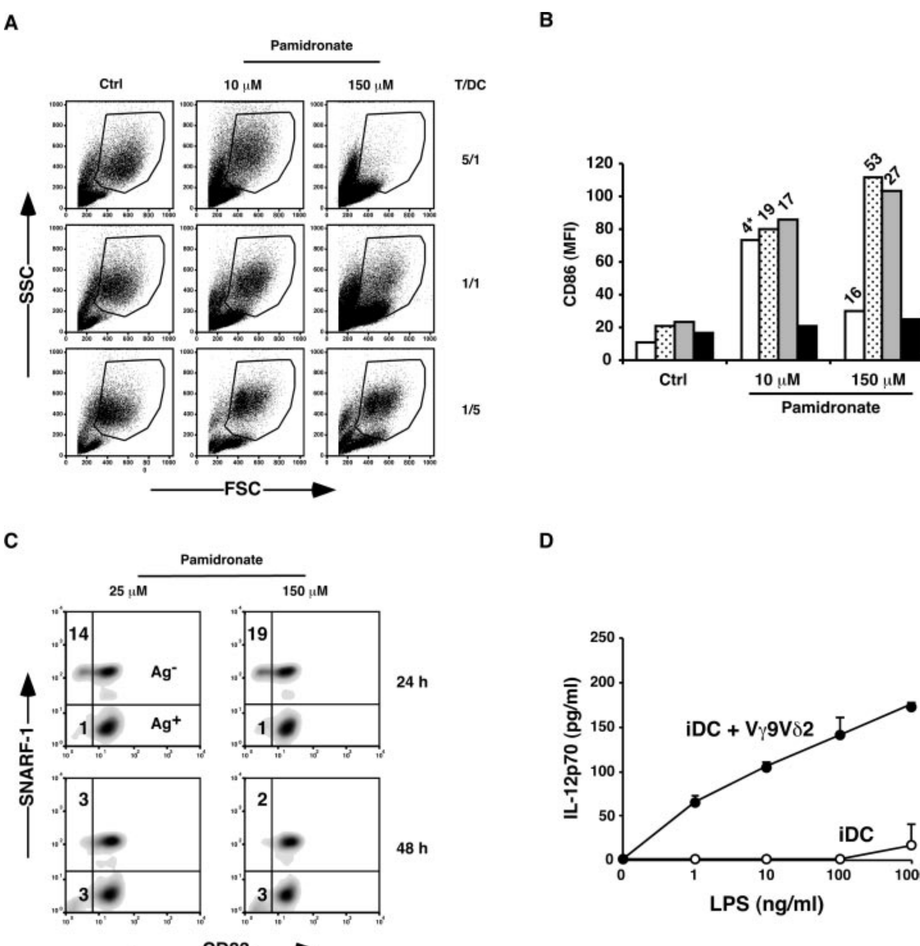


FIGURE 8. Phosphoantigen-activated Vγ9Vδ2 cells induce iDC maturation. *A*, Forward scatter (FSC) vs side scatter (SSC) plots showing the gating on pamidronate-treated (10 and 150 μM) DC cocultured for 48 h with Vγ9Vδ2 T cells (G42) at the indicated T cell to DC ratios. *B*, CD86 staining of the gated DC subset treated with pamidronate or not treated. Results are expressed as mean fluorescence intensity (MFI). T cell to DC ratio: 5:1 (□), 1:1 (dotted), 1:5 (▨), and DC alone (■). The percentage of TNF-α^+ (*) in $V\delta2^+$ T cells is indicated. *C*, Direct interactions between activated Vγ9Vδ2 and iDC are required for efficient DC maturation. SNARF-1-labeled (Ag^-) and pamidronate-treated (Ag^+) iDC were equally mixed (1/1) and cocultured with Vγ9Vδ2 T cells (G42) at T cell to DC ratio of 1:1. After 24 and 48 h, DC were stained for CD83 expression and analyzed by flow cytometry. The value for the percentage of $CD83^-$ DC in each subset of DC is *inset* in the quadrant. *D*, Phosphoantigen-activated Vγ9Vδ2 T cells promote IL-12p70 production by DC. iDC were preincubated for 3 h with grading doses of LPS, washed, and cocultured with Vγ9Vδ2 T cells (G42) in the presence of BrHPP (3 μM). After 48 h, supernatants were collected and assayed for IL-12p70 by ELISA. Data represent the average value of triplicate samples ± SD and are representative of three independent experiments.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/176/3/1386/1217988/1386.pdf by guest on 27 January 2023

iDC maturation and accordingly, such functional property could be presently documented in the context of a natural infection.

The iDC-mediated potentiation of Vγ9Vδ2 T cell responses could be explained at least in part by a differential ability of iDC vs mDC to up-regulate and/or present Vγ9Vδ2 T cell Ag. In this regard, although it has not been possible to precisely assess iDC or mDC ability to respond to pharmacological agents promoting accumulation of Vγ9Vδ2 agonists (such as ABP), a correlation between pamidronate cell responsiveness and pinocytic activity (which is presumably higher in iDC than in mDC) has been previously reported (13). However iDC-mediated potentiation of Vγ9Vδ2 cytokine responses cannot be solely explained by an increased responsiveness of iDC to ABP because Vγ9Vδ2 cytokine responses to phosphoantigen, whose activity is not affected by cell pinocytic activity, were still higher in iDC than mDC cocultures. Furthermore the fact 1) that the lytic curves of iDC and mDC were fully overlapping and 2) that the minimal stimulatory Ag concentration was identical for both iDC and mDC, also supports the assumption that potentiation activity of iDC when compared with mDC is not merely accounted for by the differential ability of iDC vs mDC to present phosphoantigen. The higher intensity of cytokine responses induced in the presence of iDC when compared with mDC strongly suggests the existence of specific costimulatory signals provided by iDC and/or inhibitory signals provided by mDC. Also consistent with qualitative rather than quantitative processes underlying enhanced cytokine responses in iDC cocultures, the strength of Ca^{2+} signals were higher in Vγ9Vδ2 T cells incubated with iDC but the kinetics of T cell activation and cytokine responses in iDC and mDC cocultures were similar. What could be the underlying mechanisms of such a phenomenon? It cannot be merely explained by a higher overall avidity of Vγ9Vδ2 T cells for iDC because these T cells showed much higher avidity for mDC than for iDC or B-LCL as suggested by conjugate formation experiments (data not shown). Furthermore motility of Vγ9Vδ2 T cells incubated with iDC was higher than with mDC at early time points, as suggested by video microscopic analyses (data not shown). Both Transwell experiments and blocking studies using paraformaldehyde-fixed cells suggest primary involvement of either membrane-bound stimuli or local soluble factors. The possible implication of several candidate costimulatory molecules differentially expressed by iDC and mDC (such as LIGHT, ICOS, and other TNFR family members) has been studied by means of blocking reagents, but have not led to any conclusive results to date. Analysis of the effect of inhibitors of various signaling cascades as well as transcriptome analysis of maturing DC at various time points after Vγ9Vδ2 incubation should certainly help identify the mechanisms underlying such a potentiation effect.

The ability of iDC to efficiently trigger Vγ9Vδ2 production of cytokines required for their own maturation would suggest direct implication of this T cell subset in DC priming. Present and previous reports demonstrated efficient in vitro iDC maturation mediated by Ag-stimulated Vγ9Vδ2 CTL. Both membrane-bound (i.e., CD40L) and soluble (i.e., TNF-α and IFN-γ) T cell-derived signals have been implicated in iDC maturation (15, 27, 40). Accordingly, Vγ9Vδ2-mediated iDC licensing was strongly inhibited by anti-TNF-α and anti-IFN-γ mAbs (data not shown). These observations raised the possibility that iDC maturation observed in vitro mainly resulted from a bystander process (i.e., maturation of

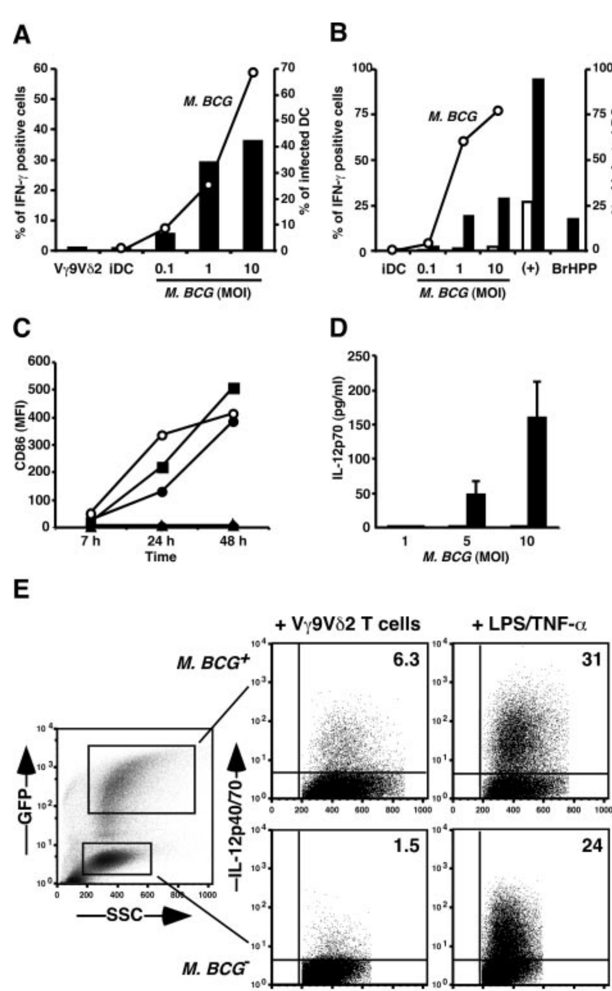


FIGURE 9. Vγ9Vδ2 T cells promote efficient maturation of *Mycobacterium bovis* BCG-infected iDC. *A* and *B*, *M. bovis* BCG-infected iDC induce IFN-γ production by Vγ9Vδ2 T cells. iDC were infected with enhanced GFP-labeled *M. bovis* BCG at indicated MOI and cocultured for 5 h with either clonal (G115) Vγ9Vδ2 T cells (*A*) or ex vivo nonadherent cells isolated from PBMC of an healthy donor. *B*, Intracellular accumulation of IFN-γ in $\gamma\delta^+$ cells (histogram) on left scale and percentage of infected iDC (○) on right scale were determined by flow cytometry. *B*, $\gamma\delta^+$ T cells (■), $\gamma\delta^-$ T cells (□), PMA/ionomycin (+) are shown. *C*, *M. bovis* BCG-infected iDC (MOI = 3) were cocultured with Vγ9Vδ2 T cells (G115) at a T cell to DC ratio of 1:1. At the indicated times, cells were harvested, stained for CD86 expression, and analyzed by flow cytometry: iDC alone (▲), iDC with Vγ9Vδ2 T cells (♦), *M. bovis* BCG-infected iDC (●), *M. bovis* BCG-infected iDC with Vγ9Vδ2 T cells (○), and iDC matured with TNF-α and LPS (■). *D*, Vγ9Vδ2 T cells promote IL-12p70 production by *M. bovis* BCG-infected DC. iDC were infected for 5 h with *M. bovis* BCG at indicated MOI and cultured alone (□) or together with Vγ9Vδ2 T cells (G42) at a T cell to DC ratio of 1:1 (■). After 24 h, supernatants were collected and assayed for IL-12p70 by ELISA. Data represent the average value of triplicate samples ± SD and are representative of three independent experiments. *E*, Vγ9Vδ2 T cells selectively induce full maturation of BCG-infected DC subset in mixed DC populations. iDC were infected for 5 h with enhanced GFP-BCG (MOI = 5), mixed with noninfected iDC (1:1) and cocultured together with Vγ9Vδ2 T cells (G42) at a T cell to DC ratio of 1:1. After 8 h of T cell/DC coculture, brefeldin A was added in the medium. After 16 h of treatment, cells were harvested, stained for intracellular IL-12p40/70 accumulation, and analyzed by flow cytometry. GFP staining of DC subsets (*left*) and IL-12p40/70 production (*right*) of *M. bovis*-infected (M.BCG$^+$) and noninfected (M.BCG$^-$) DC incubated either with Vγ9Vδ2 T cells or LPS/TNF-α (positive control). Values for percentage of cytokine-positive cells are indicated in *upper righthand* corner of quadrant. Data are representative of three independent experiments.

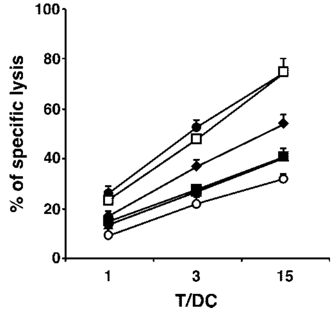


FIGURE 10. Cytolytic activity of Vγ9Vδ2 T cells activated by *Mycobacterium bovis* BCG-infected iDC. ^{51}Cr-labeled iDC, previously infected for 16 h with *M. bovis* BCG, were cocultured for 4 h together with Vγ9Vδ2 T cells (clone G115) at the indicated T cell to DC ratios. Results are expressed as the percentage of specific lysis calculated as described in *Materials and Methods*. iDC (○), *M. bovis* BCG MOI = 1 (▲), *M. bovis* BCG MOI = 5 (■), *M. bovis* BCG MOI = 10 (♦), *M. bovis* BCG MOI = 10 + BrHPP (3 μM) (□), and BrHPP (3 μM) (●) are shown.

Ag-nonexpressing iDC in response to signals delivered by activated T cells). Importantly, our present observations drawn from analysis of mixed iDC populations expressing or not Vγ9Vδ2 Ag demonstrated selective full maturation of the former subset, and thus survival of iDC having interacted in a TCR-dependent fashion with Vγ9Vδ2 CTL. These observations were further extended to a more physiological situation mimicking infection by a Vγ9Vδ2-stimulating pathogen unable to promote complete iDC maturation. In particular Vγ9Vδ2 T cells mediated full maturation into IL-12-producing cells of iDC exposed for a few hours with BCG at low MOI but not of noninfected iDC in mixed cell experiments. These in vitro results, which strongly suggest that Vγ9Vδ2 cells act as adjuvants of antimycobacterial response in vivo, are clearly in line with recent studies demonstrating IFN-γ-dependent priming of conventional tumor-specific Th1 responses by particular murine γδ subsets (41). The availability of GMP-grade synthetic Vγ9Vδ2 agonists able to promote in vivo activation and expansion of Vγ9Vδ2 counterparts in monkeys will certainly help address this issue in vivo. In any case, expression of lymph node chemokine receptors such as CCR7 by activated Vγ9Vδ2 T cells should favor their encounter with infected iDC in these sites (42). Besides iDC licensing, Vγ9Vδ2 T cells may also directly behave as professional APC for conventional T cells, as suggested by a recent study (43). However because Vγ9Vδ2 T cells are unable to produce cytokines that are key to induction of Th1 responses (such as IL-12), and because the ability of these T cells to efficiently process peptidic Ag has not be formally demonstrated yet, the significance of the professional APC properties of Vγ9Vδ2 T cells in the context of a natural infection remains to be assessed.

Owing to the memory phenotype of Vγ9Vδ2 T cells and their close functional relationships with other innate-like subsets, such as CD1-restricted T cells (4, 44), one may wonder whether the potentiating effect of iDC on T cell cytokine response is specific to Vγ9Vδ2 T cells or more generally applies to other natural or conventional memory subsets as well. Our preliminary results suggest that like Vγ9Vδ2 T cells, cytokine responses of MHC-restricted memory CD4 and CD8 T cells are potentiated in a similar way by Ag-expressing iDC when compared with mDC, although the latter are killed at least as efficiently than the former by conventional CTL (data not shown). If iDC-mediated potentiation of cytokine responses does underlie the DC priming capabilities of responding T cells, these results would suggest that not only innate-like T cells, such as γδ or CD1-restricted T cells (40, 45–47), but more

Downloaded from http://journals.aai.org/jimmunol/article-pdf/176/3/1386/1217988/1386.pdf by guest on 27 January 2023

generally memory T cells could act as adjuvants of newly generated cellular immune responses.

Acknowledgments

We thank Innate Pharma (Marseille, France) for kindly providing Phosphostim, M. J. Loirat (EFS, Nantes, France) for providing blood samples, Dr. G. Marchal and Dr. B. Gicquel (Institut Pasteur, France) for providing BCG strains. We also thank Alain Trautmann for providing critical reading and comments to the manuscript and Salvatore Valitutti for stimulating and helpful discussions.

Disclosures

The authors have no financial conflict of interest.

References

1. Hayday, A. C. 2000. γδ cells: a right time and a right place for a conserved third way of protection. *Annu. Rev. Immunol.* 18: 975–1026.
2. Haas, W., P. Pereira, and S. Tonegawa. 1993. γ/δ Cells. *Annu. Rev. Immunol.* 11: 637–685.
3. Bonneville, M., and J. J. Fournié. 2005. Sensing cell stress and transformation through Vγ9Vδ2 T cell-mediated recognition of the isoprenoid pathway metabolites. *Microbes Infect.* 7: 503–509.
4. Bendelac, A., M. Bonneville, and J. F. Kearney. 2001. Autoreactivity by design: innate B and T lymphocytes. *Nat. Rev. Immunol.* 1: 177–186.
5. Constant, P., F. Davodeau, M. A. Peyrat, Y. Poquet, G. Puzo, M. Bonneville, and J. J. Fournié. 1994. Stimulation of human γδ T cells by nonpeptidic mycobacterial ligands. *Science* 264: 267–270.
6. Espinosa, E., C. Belmant, H. Sicard, R. Poupot, M. Bonneville, and J.-J. Fournié. 2001. Y2K+1 state-of-the-art on non-peptide phosphoantigens, a novel category of immunostimulatory molecules. *Microbes Infect.* 3: 645–654.
7. Tanaka, Y., C. T. Morita, E. Nieves, M. B. Brenner, and B. R. Bloom. 1995. Natural and synthetic non-peptide antigens recognized by human γδ T cells. *Nature* 375: 155–158.
8. Hintz, M., A. Reichenberg, B. Altincicek, U. Bahr, R. M. Gschwind, A.-K. Kollas, E. Beck, J. Wiesner, M. Eberl, and H. Jomaa. 2001. Identification of (*E*)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate as a major activator for human γδ T cells in *Escherichia coli*. *FEBS Lett.* 509: 317–322.
9. Scotet, E., L. O. Martinez, E. Grant, R. Barbaras, P. Jenö, M. Guiraud, B. Monsarrat, X. Saulquin, S. Maillet, J. P. Estève, et al. 2005. Tumor recognition following Vγ9Vδ2 T cell receptor interactions with a surface F1-ATPase-related structure and apolipoprotein A-I. *Immunity* 22: 71–80.
10. Gober, H. J., M. Kistowska, L. Angman, P. Jeno, L. Mori, and G. De Libero. 2003. Human T cell receptor γδ cells recognize endogenous mevalonate metabolites in tumor cells. *J. Exp. Med.* 197: 163–168.
11. Kunzmann, V., E. Bauer, and M. Wilhelm. 1999. γ/δ T-cell stimulation by pamidronate. *N. Engl. J. Med.* 340: 737–738.
12. Casetti, R., G. Perretta, A. Taglioni, M. Mattei, V. Colizzi, F. Dieli, G. D'Offizi, M. Malkovsky, and F. Poccia. 2005. Drug-induced expansion and differentiation of Vγ9Vδ2 T cells in vivo: the role of exogenous IL-2. *J. Immunol.* 175: 1593–1598.
13. Miyagawa, F., Y. Tanaka, S. Yamashita, and N. Minato. 2001. Essential requirement of antigen presentation by monocyte lineage cells for the activation of primary human γδ T cells by aminobisphosphonate antigen. *J. Immunol.* 166: 5508–5514.
14. Kato, Y., Y. Tanaka, F. Miyagawa, S. Yamashita, and N. Minato. 2001. Targeting of tumor cells for human γδ T cells by nonpeptide antigens. *J. Immunol.* 167: 5092–5098.
15. Conti, L., R. Casetti, M. Cardone, B. Varano, A. Martino, F. Belardelli, F. Poccia, and S. Gessani. 2005. Reciprocal activating interaction between dendritic cells and pamidronate-stimulated γδ T cells: role of CD86 and inflammatory cytokines. *J. Immunol.* 174: 252–260.
16. Kitamura, H., K. Iwakabe, T. Yahata, S. Nishimura, A. Ohta, Y. Ohmi, M. Sato, K. Takeda, K. Okumura, L. Van Kaer, et al. 1999. The natural killer T (NKT) cell ligand α-galactosylceramide demonstrates its immunopotentiating effect by inducing interleukin (IL)-12 production by dendritic cells and IL-12 receptor expression on NKT cells. *J. Exp. Med.* 189: 1121–1128.
17. van der Vliet, H. J., N. Nishi, Y. Koezuka, B. M. von Blomberg, A. J. van den Eertwegh, S. A. Porcelli, H. M. Pinedo, R. J. Scheper, and G. Giaccone. 2001. Potent expansion of human natural killer T cells using α-galactosylceramide (KRN7000)-loaded monocyte-derived dendritic cells, cultured in the presence of IL-7 and IL-15. *J. Immunol. Methods* 247: 61–72.
18. Fujii, S., K. Shimizu, M. Kronenberg, and R. M. Steinman. 2002. Prolonged IFN-γ-producing NKT response induced with α-galactosylceramide-loaded DCs. *Nat. Immunol.* 3: 867–874.
19. Geginat, J., F. Sallusto, and A. Lanzavecchia. 2001. Cytokine-driven proliferation and differentiation of human naive, central memory, and effector memory CD4$^+$ T cells. *J. Exp. Med.* 194: 1711–1719.
20. Morel, Y., A. Truneh, R. T. Costello, and D. Olive. 2003. LIGHT, a new TNF superfamily member, is essential for memory T helper cell-mediated activation of dendritic cells. *Eur. J. Immunol.* 33: 3213–3219.
21. Zammit, D. J., L. S. Cauley, Q.-M. Pham, and L. Lefrançois. 2005. Dendritic cells maximize the memory CD8 T cell response to infection. *Immunity* 22: 561–570.
22. Viey, E., G. Fromont, B. Escudier, Y. Morel, S. Da Rocha, S. Chouaib, and A. Caignard. 2005. Phosphostim-activated γδ T cells kill autologous metastatic renal cell carcinoma. *J. Immunol.* 174: 1338–1347.
23. Poccia, F., B. Cipriani, S. Vendetti, V. Colizzi, Y. Poquet, L. Battistini, M. Lopez-Botet, J.-J. Fournié, and M. L. Gougeon. 1997. CD94/NKG2 inhibitory receptor complex modulates both anti-viral and anti-tumoral responses of polyclonal phosphoantigen-reactive Vγ9Vδ2 T lymphocytes. *J. Immunol.* 159: 6009–6017.
24. Bukowski, J. F., C. T. Morita, and M. B. Brenner. 1994. Recognition and destruction of virus-infected cells by human γδ CTL. *J. Immunol.* 153: 5133–5140.
25. Fisch, P., E. Meuer, D. Pende, S. Rothenfusser, O. Viale, S. Kock, S. Ferrone, D. Fradelizi, G. Klein, L. Moretta, et al. 1997. Control of B cell lymphoma recognition via natural killer inhibitory receptors implies a role for human Vγ9/Vδ2 T cells in tumor immunity. *Eur. J. Immunol.* 27: 3368–3379.
26. Münz, C., R. M. Steinman, and S. Fujii. 2005. Dendritic cell maturation by innate lymphocytes: coordinated stimulation of innate and adaptive immunity. *J. Exp. Med.* 202: 203–207.
27. Ismaili, J., V. Olislagers, R. Poupot, J.-J. Fournié, and M. Goldman. 2002. Human γδ T cells induce dendritic cell maturation. *Clin. Immunol.* 103: 296–302.
28. Sporri, R., and C. Reis e Sousa. 2003. Newly activated T cells promote maturation of bystander dendritic cells but not IL-12 production. *J. Immunol.* 171: 6406–6413.
29. Espinosa, E., C. Belmant, F. Pont, B. Luciani, R. Poupot, F. Romagné, H. Brailly, M. Bonneville, and J.-J. Fournié. 2001. Chemical synthesis and biological activity of bromohydrin pyrophosphate, a potent stimulator of human γδ T cells. *J. Biol. Chem.* 276: 18337–18344.
30. Allison, T. J., C. C. Winter, J.-J. Fournié, M. Bonneville, and D. N. Garboczi. 2001. Structure of a human γδ T-cell antigen receptor. *Nature* 411: 820–824.
31. Davodeau, F., M. A. Peyrat, M. M. Hallet, J. Gaschet, I. Houde, R. Vivien, H. Vie, and M. Bonneville. 1993. Close correlation between Daudi and mycobacterial antigen recognition by human γδ T cells and expression of V9JPC1γ/V2DJCδ-encoded T cell receptors. *J. Immunol.* 151: 1214–1223.
32. Real, E., A. Kaiser, G. Raposo, A. Amara, A. Nardin, A. Trautmann, and E. Donnadieu. 2004. Immature dendritic cells (DCs) use chemokines and intercellular adhesion molecule (ICAM)-1, but not DC-specific ICAM-3-grabbing nonintegrin, to stimulate CD4$^+$ T cells in the absence of exogenous antigen. *J. Immunol.* 173: 50–60.
33. Donnadieu, E., G. Bismuth, and A. Trautmann. 1994. Antigen recognition by helper T cells elicits a sequence of distinct changes of their shape and intracellular calcium. *Curr. Biol.* 4: 584–595.
34. Lang, F., M. A. Peyrat, P. Constant, F. Davodeau, J. David-Ameline, Y. Poquet, H. Vie, J.-J. Fournié, and M. Bonneville. 1995. Early activation of human Vγ9Vδ2 T cell broad cytotoxicity and TNF production by nonpeptidic mycobacterial ligands. *J. Immunol.* 154: 5986–5994.
35. Rojas, R. E., M. Torres, J.-J. Fournié, C. V. Harding, and W. H. Boom. 2002. Phosphoantigen presentation by macrophages to *Mycobacterium tuberculosis*–reactive Vγ9Vδ2$^+$ T cells: modulation by chloroquine. *Infect. Immun.* 70: 4019–4027.
36. Constant, P., Y. Poquet, M. A. Peyrat, F. Davodeau, M. Bonneville, and J.-J. Fournié. 1995. The antituberculous *Mycobacterium bovis* BCG vaccine is an attenuated mycobacterial producer of phosphorylated nonpeptidic antigens for human γδ T cells. *Infect. Immun.* 63: 4628–4633.
37. Hanekom, W. A., M. Mendillo, C. Manca, P. A. Haslett, M. R. Siddiqui, C. Barry, III, and G. Kaplan. 2003. *Mycobacterium tuberculosis* inhibits maturation of human monocyte-derived dendritic cells in vitro. *J. Infect. Dis.* 188: 257–266.
38. Gagliardi, M. C., R. Teloni, F. Giannoni, M. Pardini, V. Sargentini, L. Brunori, L. Fattorini, and R. Nisini. 2005. *Mycobacterium bovis* bacillus Calmette-Guerin infects DC-SIGN-dendritic cell and causes the inhibition of IL-12 and the enhancement of IL-10 production. *J. Leukocyte Biol.* 78: 106–113.
39. Geijtenbeek, T. B., S. J. Van Vliet, E. A. Koppel, M. Sanchez-Hernandez, C. M. Vandenbroucke-Grauls, B. Appelmelk, and Y. Van Kooyk. 2003. Mycobacteria target DC-SIGN to suppress dendritic cell function. *J. Exp. Med.* 197: 7–17.
40. Leslie, D. S., M. S. Vincent, F. M. Spada, H. Das, M. Sugita, C. T. Morita, and M. B. Brenner. 2002. CD1-mediated γ/δ T cell maturation of dendritic cells. *J. Exp. Med.* 196: 1575–1584.
41. Gao, Y., W. Yang, M. Pan, E. Scully, M. Girardi, L. H. Augenlicht, J. Craft, and Z. Yin. 2003. γδ T cells provide an early source of interferon γ in tumor immunity. *J. Exp. Med.* 198: 433–442.
42. Brandes, M., K. Willimann, A. B. Lang, K. H. Nam, C. Jin, M. B. Brenner, C. T. Morita, and B. Moser. 2003. Flexible migration program regulates γδ T-cell involvement in humoral immunity. *Blood* 102: 3693–3701.
43. Brandes, M., K. Willimann, and B. Moser. 2005. Professional antigen-presentation function by human γδ T cells. *Science* 309: 264–268.
44. Dieli, F., F. Poccia, M. Lipp, G. Sireci, N. Caccamo, C. Di Sano, and A. Salerno. 2003. Differentiation of effector/memory Vδ2 T cells and migratory routes in lymph nodes or inflammatory sites. *J. Exp. Med.* 198: 391–397.
45. Hermans, I. F., J. D. Silk, U. Gileadi, M. Salio, B. Mathew, G. Ritter, R. Schmidt, A. L. Harris, L. Old, and V. Cerundolo. 2003. NKT cells enhance CD4$^+$ and CD8$^+$ T cell responses to soluble antigen in vivo through direct interaction with dendritic cells. *J. Immunol.* 171: 5140–5147.
46. Silk, J. D., I. F. Hermans, U. Gileadi, T. W. Chong, D. Shepherd, M. Salio, B. Mathew, R. R. Schmidt, S. J. Lunt, K. J. Williams, et al. 2004. Utilizing the adjuvant properties of CD1d-dependent NK T cells in T cell-mediated immunotherapy. *J. Clin. Invest.* 114: 1800–1811.
47. Vincent, M. S., D. S. Leslie, J. E. Gumperz, X. Xiong, E. P. Grant, and M. B. Brenner. 2002. CD1-dependent dendritic cell instruction. *Nat. Immunol.* 3: 1163–1168.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/176/3/1386/1217988/1386.pdf by guest on 27 January 2023