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Microbial Compounds Selectively Induce Th1 Cell-Promoting or Th2 Cell-Promoting Dendritic Cells In Vitro with Diverse Th Cell-Polarizing Signals[1]

Esther C. de Jong,[2]*† Pedro L. Vieira,* Pawel Kalinski,* Joost H. N. Schuitemaker,* Yuetsu Tanaka,‡ Eddy A. Wierenga,* Maria Yazdanbakhsh,§ and Martien L. Kapsenberg*†

Upon microbial infection, specific Th1 or Th2 responses develop depending on the type of microbe. Here, we demonstrate that different microbial compounds polarize the maturation of human myeloid dendritic cells (DCs) into stably committed Th1 cell-promoting (DC1) or Th2 cell-promoting (DC2) effector DCs that polarize Th cells via different mechanisms. Protein extract derived from the helminth *Schistosoma mansoni* induced the development of DC2s that promote the development of Th2 cells via the enhanced expression of OX40 ligand. Likewise, toxin from the extracellular bacterium *Vibrio cholerae* induced development of DC2s as well, however, via an OX40 ligand-independent, still unknown mechanism. In contrast, toxin from the intracellular bacterium *Bordetella pertussis* induced the development of DC1s with enhanced IL-12 production, which promotes a Th1 cell development. Poly(I:C) (dsRNA, mimic for virus) induced the development of extremely potent Th1-inducing DC1, surprisingly, without an enhanced IL-12 production. The obtained DC1s and DC2s are genuine effector cells that stably express Th cell-polarizing factors and are unresponsive to further modulation. The data suggest that the molecular basis of Th1/Th2 polarization via DCs is unexpectedly diverse and is adapted to the nature of the microbial compounds. *The Journal of Immunology,* **2002, 168: 1704–1709.**

Appropriate responses against microorganisms require selective forms of specific immunity mediated by functionally polarized subsets of effector Th cells, e.g., IFN-γ-producing Th1 cells and IL-4-producing Th2 cells, which develop from a common pool of naive precursor T cells. There is accumulating evidence that microbes drive the development of protective Th1 or Th2 cells through their effects on APCs (1–4). Dendritic cells (DCs)[3] are professional APCs that are present as immature sentinel cells that efficiently sample their environment for foreign Ag at potential sites of pathogen entry. Upon activation by signals released from the microorganisms or from infected tissues, sentinel DCs undergo maturation into potent T cell stimulatory effector DCs and migrate toward the T cell areas of draining lymphoid organs. There, effector DCs will activate naive Th cells with pathogen-specific (MHC peptide complexes, signal 1) and costimulatory (B7 family molecules, signal 2) (5) molecules. In addition to signals 1 and 2, DCs carry a third signal, which determines the polarization of naive Th cells into Th1 or Th2 cells (6). Like signal 2, signal 3 is heterogeneous and can be mediated by various soluble or membrane-bound molecules, including IL-12 (7), IL-18 (8), IFN-α (9), and OX40 ligand (OX40L) (10). Importantly, in vitro studies suggested that the expression levels of these Th cell-polarizing molecules by mature effector DCs strongly depend on the conditions during their initial activation as sentinel DCs. Tissue-derived factors such as IFN-γ and PGE_2 present during the activation of human monocyte-derived sentinel DCs promote the generation of type 1 effector DCs (DC1s), which produce high amounts of IL-12 upon subsequent engagement of naive T cells, or the generation of IL-12-deficient DC2s, which drive the development of Th2 cells (11, 12). These findings imply that pathogens may promote the development of distinct DC phenotypes by provoking tissues to release mediators involved in polarization.

In addition to these indirect effects, microorganisms also directly affect sentinel DCs at the time of pathogen encounter, as has been shown previously (1–4).

In the present study, we investigate the DC-derived molecules involved in Th-cell polarization of different DC1 and DC2 subsets. Soluble egg Ags (SEA) of the helminth *Schistosoma mansoni* and the toxin of the intestinal bacterium *Vibrio cholerae* (CT), both associated with Th2 cell responses (13, 14), as well as dsRNA (poly(I:C), a mimic of viral RNA) and the toxin of the intracellular bacterium *Bordetella pertussis* (PT), both associated with Th1 cell responses (15), all promoted sentinel DCs to develop into functional effector cells with stably polarized DC2 or DC1 phenotypes, respectively. It became clear that, although the effector DC1 or DC2 populations induced similar Th1 and Th2 cell subsets, there is a heterogeneity within DC1 and DC2 subsets with respect to the expression and use of Th-polarizing molecules.

The present study indicates that a protective immune response is mounted via the development of polarized DC1 and DC2 subsets

Departments of *Cell Biology and Histology and †Dermatology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; ‡Department of Infectious Disease and Immunology, Okinawa-Asia Research Center of Medical Sciences, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan; and §Department of Parasitology, Medical Center Leiden, Leiden, The Netherlands

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[2] Address correspondence and reprint requests to Dr. Esther C. de Jong, Department of Cell Biology and Histology, Academic Medical Center, University of Amsterdam, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands. E-mail address: E.C.deJong@AMC.UvA.NL

[3] Abbreviations used in this paper: DC, dendritic cell; OX40L, OX40 ligand; SEA, soluble egg Ags; CT, cholera toxin; PT, pertussis toxin; SEB, *Staphylococcus enterotoxin B*; CD40L, CD40 ligand; MF, maturation-inducing factors.

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with diverse expression of signal 3 induced by factors derived from the invading pathogen.

Materials and Methods

Generation of immature DCs and their induction of maturation by different microbial compounds

Monocytes were isolated from PBMCs using density centrifugation. Immature DCs were generated by culturing monocytes for 6 days in IMDM (Life Technologies, Paisley, U.K.) containing gentamicin (86 μg/L; Duchefa, Haarlem, The Netherlands) and 1% FCS (HyClone, Logan, UT), supplemented with GM-CSF (500 U/ml; Schering-Plough, Uden, The Netherlands) and IL-4 (250 U/ml; PBH, Hanover, Germany). At day 6, maturation was induced by culturing the cells for 2 days with the following factors alone or with a combination as indicated in the text: IL-1β (10 ng/ml; PBH), TNF-α (50 ng/ml; PBH), poly(I:C) (20 μg/ml, Sigma-Aldrich, St. Louis, MO), *Schistosoma mansoni* egg Ags (30 μg/ml; prepared as described previously (16)), CT (1 μg/ml; Sigma-Aldrich), PT (1 μg/ml; Sigma-Aldrich), IFN-γ (1000 U/ml; gift from Dr. P. H. van der Meide, Utrecht University, Utrecht, The Netherlands), or PGE_2 (10^{-6} M; Sigma-Aldrich). All subsequent tests were performed after harvesting and extensive washing of the cells to remove all factors.

Expression of cell surface molecules

At day 8, the obtained effector DCs were analyzed for the expression of cell surface molecules by FACS. Mouse anti-human mAbs were used against the following molecules: CD1a (OKT6; Ortho Diagnostic Systems, Beerse, Belgium), CD83 (HB15a, IgG2b; Immunotech, Marseille, France), CD86 (1G10, IgG2a; Innogenetics, Ghent, Belgium), HLA-DR (L234, IgG2a; BD Biosciences, San Jose, CA), and OX40L (5A8) (17). All mAbs were followed by FITC-conjugated goat $F(ab')_2$ anti-mouse IgG and IgM (Jackson ImmunoResearch Laboratories, West Grove, PA). Samples were analyzed on a FACScan (BD Biosciences).

Determination of naive $CD4^+CD45RA^+CD45RO^-$ Th cell polarization by effector DCs

Highly purified $CD4^+CD45RA^+CD45RO^-$ naive Th cells (>98% as assessed by flow cytometry) were purified from PBMCs or PBLs using a human $CD4^+/CD45RO^-$ column kit (R&D Systems, Minneapolis, MN). Naive $CD4^+$ Th cells (2×10^4 cells/200 μl of IMDM with 10% FCS) were cocultured with 5×10^3 effector DCs coated with *Staphylococcus enterotoxin B* (SEB; Sigma-Aldrich; final concentration, 100 pg/ml) in 96-well flat-bottom culture plates (Costar, Cambridge, MA). As indicated in certain figures, the following neutralizing or blocking Abs were used: anti-IL-12 (10 μg/ml; kind gift from Dr. P. H. van der Meide), anti-IL-18 (10 μg/ml; MBL, Nagoya, Japan), anti-IFN-α (10 μg/ml; PBL, New Brunswick, NJ), and anti-type I IFNR (10 μg/ml; Research Diagnostics, Flanders, NJ). At day 5, human rIL-2 (10 U/ml, Cetus, Emeryville, CA) was added, and the cultures were expanded for the next 9 days. On day 14, the quiescent Th cells were restimulated with PMA (10 ng/ml; Sigma-Aldrich) and ionomycin (1 μg/ml; Sigma-Aldrich) for 6 h, and during the last 5 h, Brefeldin A (10 μg/ml; Sigma-Aldrich) was present to detect the intracellular production of IL-4 and IFN-γ (BD Biosciences). For the direct stimulation of naive Th cells in the presence of supernatants of activated DCs, plate-bound anti-CD3 (16A9; Central Laboratory for Blood Transfusions, Amsterdam, The Netherlands) and anti-CD28 (5E8, CLB) were used at a concentration of 1 μg/ml.

Induction of cytokine production by DCs

$CD1a^+$ DCs (4×10^4 cells/well) were stimulated with human CD40 ligand (CD40L)-expressing mouse fibroblasts (J558 cells; 4×10^4 cells/well; kind gift from Dr. P. Lane, University of Birmingham, Birmingham, U.K.) in the presence or absence of human rIFN-γ (1000 U/ml) in 96-well flat-bottom culture plates (Costar) in IMDM containing 10% FCS in a final volume of 200 μl. Supernatants were harvested after 24 h and stored at −20°C until the levels of cytokines were measured by ELISA.

Cytokine measurements

Measurements of IL-12p70, TNF-α, and IL-6 levels in the culture supernatants were performed by specific solid-phase sandwich ELISA as described previously (11). The limits of detection of these ELISAs are as follows: IL-12p70, 3 pg/ml; TNF-α, 10 pg/ml; IL-6, 20 pg/ml.

Statistical analysis

Data were analyzed for statistical significance (GraphPad, InStat, version 2.02) using ANOVA followed by Dunnett's multiple comparisons test. A p value <0.05 was taken as the level of significance.

Results

Pathogens induce either effector DC1 or DC2

To study the direct effects of different microbial compounds on the maturation of sentinel DCs into effector DCs, uncommitted monocyte-derived DCs were cultured in the presence of SEA, CT, PT, or poly(I:C). As a control, DCs were cultured by the combination of IL-1β and TNF-α (maturation-inducing factors (MF)). In agreement with previous reports, MF (18), CT (4), and poly(I:C) (1) induced final DC maturation within 48 h, as was evident from the loss of expression of the mannose receptor, the inability to phagocytose, the induction of CD83 expression, and the up-regulation of the CD80, CD86, and HLA-DR expression, of which the expression levels were comparable within the different DC subsets (Fig. 1*A*; data not shown for HLA-DR and CD80). SEA and PT induced DC maturation as well (Fig. 1*A*), although the degree of maturation in the case of PT varied among different donors. Because the state of maturation of DCs may influence the capacity to drive Th1 or Th2 responses, due to differences in the expression of certain cytokines or membrane-bound molecules, MF were added to the stimulations (CT, PT, and the controls PGE_2 and IFN-γ) that do not induce full maturation by themselves.

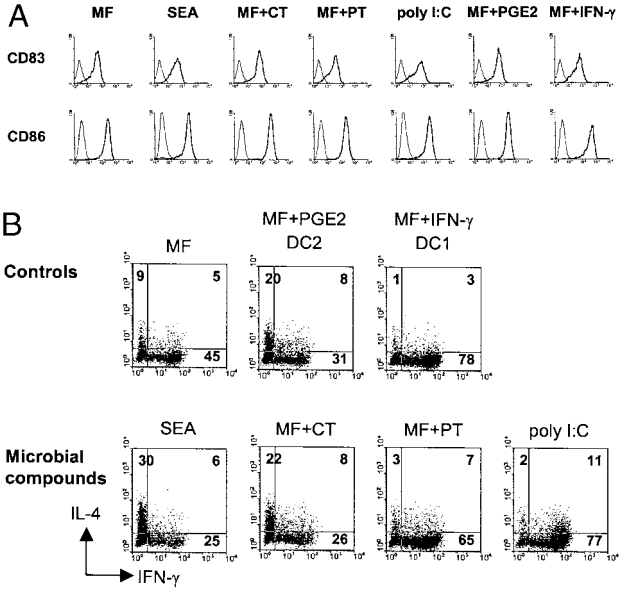

FIGURE 1. DCs matured by microbial compounds from different origins drive the development of Th1 or Th2 response and differ in their cytokine production upon CD40 ligation. Sentinel immature DCs were matured for 48 h by MF or by the microbial compounds SEA, MF + CT, MF + PT, or poly(I:C). As controls, DCs were matured with MF + IFN-γ (for DC1) and MF + PGE_2 (for DC2). *A*, Effector DCs were analyzed for the expression of CD83 and CD86 by flow cytometry. The thick line represents the specific expression of CD83 (*upper panel*) or CD86 (*lower panel*), whereas the thin line represents the isotype control. *B*, Mature, effector DCs (5×10^3 cells/well) were loaded with SEB and cocultured with naive $CD4^+CD45RA^+$ Th cells (2×10^4 cells/well). After 14 days, the responder Th cells had become quiescent and were restimulated with PMA/ionomycin for 6 h. The IL-4- and/or IFN-γ-producing cells were measured by intracellular FACS analysis and the percentages of cells are given in the respective quadrants. Results from one representative experiment of six.

Subsequently, the effector DCs obtained after 48 h were used to stimulate naive Th cells with the superantigen SEB to generate effector Th cells. The Th1/Th2 cytokine profiles of the resulting effector Th cells were assessed by analysis of intracellular IL-4 and IFN-γ expression (Fig. 1*B*). As expected, DCs matured by MF induced the development of a mixture of IFN-γ-producing Th1 and IL-4-producing Th2 effector cells. In contrast, DCs matured by SEA or CT became type 2 DCs (DC2s) with the intrinsic ability to bias for the development of Th2 cells, comparable to the known Th2-polarizing effect of PGE_2. In contrast, priming of DCs with PT or poly(I:C) resulted in type 1 DCs (DC1s) that biased for the development of Th1 cells. The latter effect was comparable to the induction of Th1 cells by DCs matured with the combination of MF and IFN-γ.

Cytokine production by DC subsets

Because the level of IL-12 production by myeloid DCs during activation of naive Th cells is a major factor driving the development of Th1 cells, we first studied whether DC1 and DC2 types are associated with high or low IL-12 production. Bioactive IL-12p70 production by the effector DC was measured upon ligation of CD40 by CD40L-transfected cells, thereby mimicking the engagement by T cells (Fig. 2*A*). Compared with the MF-primed control DCs, the SEA- or CT-primed DC2s showed a strongly reduced IL-12p70 production, in accordance with their Th2-driving capacity. As expected from their Th1-polarizing effect, PT primed for DC1 with enhanced IL-12p70 production, similar to MF plus IFN-γ-primed DC1s. Surprisingly, unlike the MF + PT- and MF + IFN-γ-primed DC1s, poly(I:C)-primed DC1s did not show enhanced IL-12p70 production, despite their potent Th1-promoting capacity, suggesting the existence of alternative Th1-driving mechanisms. Priming of DCs with SEA or poly(I:C) in the additional presence of MF did not alter the capacity to produce IL-12p70 upon CD40 ligation of the effector cells (data not shown).

The production of the proinflammatory cytokine TNF-α followed the pattern of IL-12 production, with the exception that the TNF-α production was consistently not enhanced in PT-primed DCs (Fig. 2*B*). IL-6 production is differentially regulated (Fig. 2*C*), being consistently low in SEA-primed DCs and high in poly(I:C)-primed DCs but unaffected in the other effector DCs. The production of IFN-α by all effector DC subsets was below or near the detection limit of the ELISA (data not shown). These findings indicate that the various subsets of DC1 and DC2 have unique cytokine profiles. Among others, SEA-primed DCs are unable to produce high levels of any of the cytokines tested (e.g., IL-12, TNF-α, and IL-6). The variable profiles may imply that the microbial compounds activate different signaling pathways in the sentinel DCs.

Role of IL-12 in the induction of Th1 cells

To analyze the contribution of DC-derived IL-12 to the development of Th1 cells, we tested the effect of neutralizing anti-IL-12 Ab in cocultures of naive Th cells and the distinct effector DC subsets (Fig. 3, *A* and *B*). In the case of most DC subsets, neutralization of IL-12 increased the development of IL-4-producing Th cells and dramatically decreased the development of IFN-γ-producing Th cells. However, in the case of poly(I:C)-primed DCs, the percentage of IFN-γ-producing cells could not be reduced below 20–40%. This is in line with the finding that poly(I:C) primes for efficient effector DC1s despite moderate IL-12 production and suggests that poly(I:C)-primed DCs express Th1-driving factors other than IL-12p70. Neutralizing Abs to IL-18, IFN-α, or type I IFNR did not substantially block Th1 development induced by the poly(I:C)-primed DCs (Fig. 3*B*), suggesting the involvement of yet another factor.

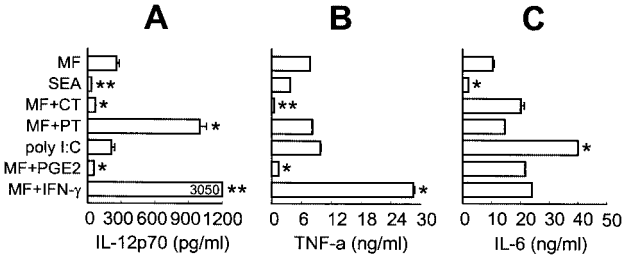

FIGURE 2. DCs matured by different microbial compounds differ in their cytokine production upon CD40 ligation. Sentinel, immature DCs were matured by MF or by the microbial compounds SEA, MF + CT, MF + PT, or poly(I:C). As controls, DCs were matured with MF + IFN-γ (for DC1) and MF + PGE_2 (for DC2). After 48 h, the cells were thoroughly washed and stimulated (4×10^4 cells/well) with the CD40L-expressing mouse fibroblast cell line J558 (4×10^4 cells/well). Supernatants were harvested after 24 h and secreted cytokines were measured by ELISA. *A*, IL-12p70; *B*, TNF-α; *C*, IL-6. Concentrations that were out of range of the axes are given in the respective bars. Results from one representative experiment of six. *, $p < 0.05$; **, $p < 0.001$.

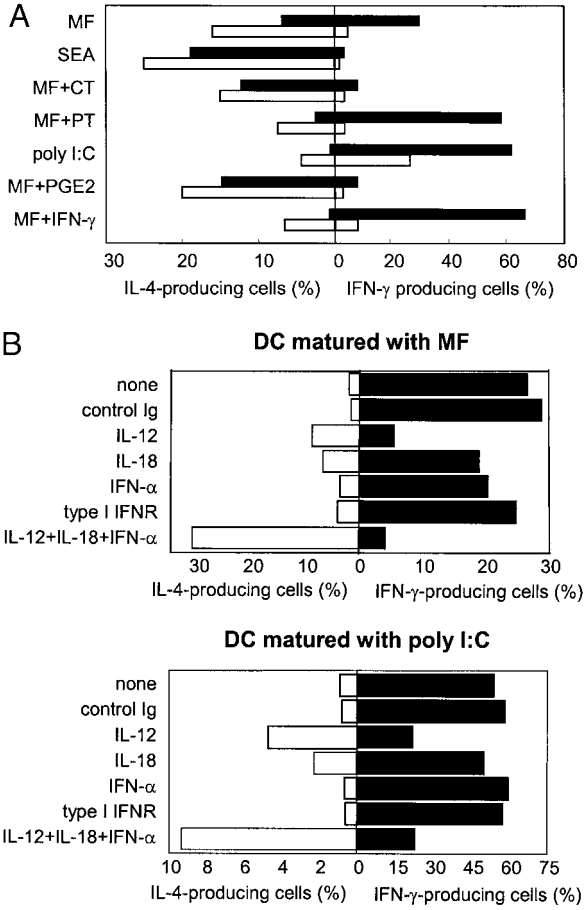

FIGURE 3. Relative contribution of IL-12, IL-18, and type I IFN to the Th1 or Th2 development induced by DCs matured by different microbial compounds. Sentinel, immature DCs were matured and used to stimulate naive Th cells, as indicated in Fig. 1. Stimulation was performed in the absence or presence of neutralizing or blocking Abs in optimal concentrations, as indicated in the figure. *A*, The effect of anti-IL-12 Abs (open bars) with all different types of DCs compared with control Ab (filled bars). *B*, The effects of anti-IL-18, anti-IFN-α, and anti-type I IFNR with DCs matured with MF or poly(I:C). Results from one representative experiment of three.

Role of DC-derived soluble factors in the induction of Th1 orTh2 cells

In an attempt to further define the Th1- and Th2-driving molecules expressed by the various effector DC types, we tested to what extent soluble factors were critical. Supernatants of CD40L-activated DCs (after 24 h) were added to cultures of naive T cells stimulated with anti-CD3 and anti-CD28 Abs. After 10–14 days, the effector Th cells were restimulated, and the IL-4 and IFN-γ expression was determined. Supernatants of MF + IFN-γ-primed DCs strongly promoted the development of Th1 cells, which could be almost completely blocked by IL-12 Ab (Fig. 4). Likewise, the supernatants of the poly(I:C)-primed DCs supported the development of Th1 cells. However, this effect could only be partially blocked by anti-IL-12 Ab, indicating that the Th1-promoting activity of these DCs is mediated by an unknown soluble factor. Supernatants of CD40L-activated DC2s primed with the combination of MF and PGE_2 or with CT exhibited a strong Th2-driving capacity. This activity could not be blocked by neutralizing Abs to IL-4, IL-13, or monocyte chemoattractant protein-1 or by preventing the production of eicosanoids (e.g., PGE_2) by these DC2s (data not shown). In contrast, supernatants of CD40L-stimulated SEA-primed DCs failed to support Th2 development, indicating that these cells exert the Th2 bias via a membrane-bound factor.

Role of OX40L in the induction of Th2 cells

In the search for the identity of the Th2-driving, membrane-bound factor on the SEA-primed DC, we focused on OX40L, a factor known to be expressed by part of the peripheral blood DC and involved in Th2 cell development (10). Interestingly, OX40L was detectable only in DC2s and not on any of the DC1 types (Fig. 5*A*). Even after CD40 ligation, which has been described to strongly up-regulate OX40L expression, only a small minority of the cells expressed OX40L, whereas all DC2 cells showed enhanced OX40L expression (Fig. 5*A*).

To determine the role of OX40L-OX40 interaction in Th2 development, blocking OX40L mAb was added during the coculture of type 2 DCs with naive Th cells. Clearly, anti-OX40L mAb affected the cytokine balance only of the effector T cells generated by SEA-primed DCs. Blocking of OX40L expressed on SEA DCs resulted in a strongly reduced development of IL-4-producing Th cells from naive precursors, whereas when MF + PGE_2 DCs (or MF + CT DCs, data not shown) were used to stimulate naive Th cells, no difference was observed in the development of IL-4- or IFN-γ-producing Th cells (Fig. 5*B*).

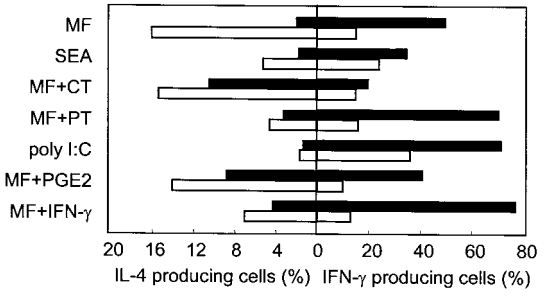

FIGURE 4. Not only soluble DC-derived factors are involved in T cell polarization. Sentinel immature DCs were matured as described in Fig. 1. After 48 h, the cells were thoroughly washed and stimulated (4×10^4 cell/well) with the CD40L-expressing mouse fibroblast cell line J558 (4×10^4 cell/well). Supernatants were harvested after 24 h and added to naive $CD4^+CD45RA^+$ Th cells that were activated with plate-bound anti-CD3 and anti-CD28, with neutralizing Abs directed against IL-12 (open bars) or with control Ab (filled bars). After 14 days, responding Th cells were analyzed as described in Fig. 1. Results from one representative experiment of four.

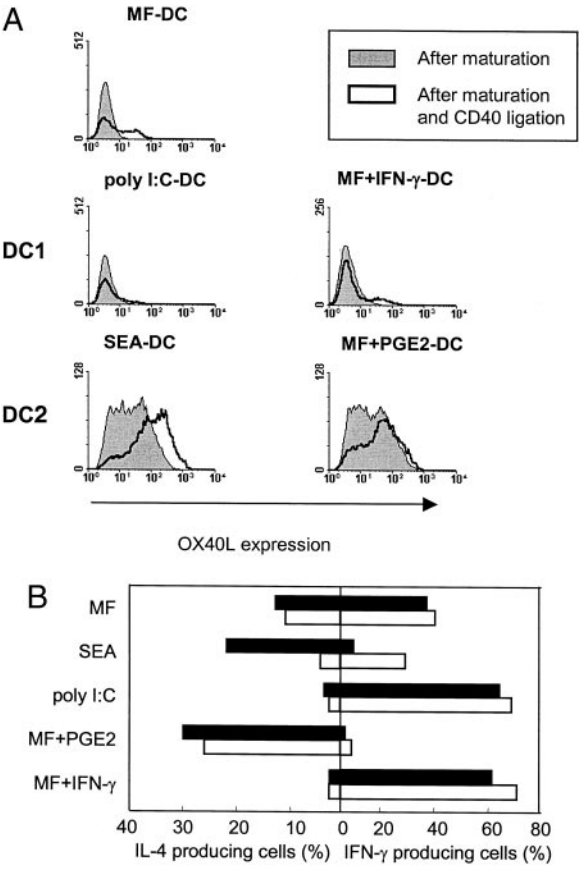

FIGURE 5. Role of OX40L expression by DCs matured by different microbial compounds. Sentinel immature DCs were matured as described in Fig. 1. Subsequently, the DCs either were or were not activated with CD40L. *A*, After 24 h, DCs were harvested and stained with mAb-directed OX40L. The expression was determined by FACS. *B*, SEB-loaded differentially matured DCs (5×10^3 cells/well) were cocultured with $CD4^+CD45RA^+$ naive Th cells (2×10^4 cells/well) in the presence of control Ab (filled bars) or neutralizing anti-OX40L Ab (open bars) in optimal concentration. After 14 days, responding Th cells were analyzed as described in Fig. 1. Results from one representative experiment of three.

DC1 and DC2 have stable IL-12 secretion profiles

To study the stability of the functional phenotype of the various types of effector DCs, we tested whether IL-12 production was altered after stimulation by microbial compounds or cytokines that polarize immature DCs in the opposite direction. Because the pathogenic factors, except for poly(I:C), modulate the cytokine production of immature DCs but do not induce cytokine by themselves (data not shown), the effector DCs were activated with CD40L in the absence or presence of SEA, CT, PGE_2, or IFN-γ or with poly(I:C) alone. As shown in Fig. 6, the IL-12 levels of the various DC1s and DC2s are largely preserved in any condition of stimulation. In general, in the presence of compounds that prime for high IL-12, the IL-12 production was enhanced in DC1s but hardly or not at all in DC2s. In the presence of down-regulators of IL-12 production, the IL-12 levels were unchanged or only marginally decreased.

Discussion

It has been long recognized that immune responses to different types of pathogens are associated with different types of effector

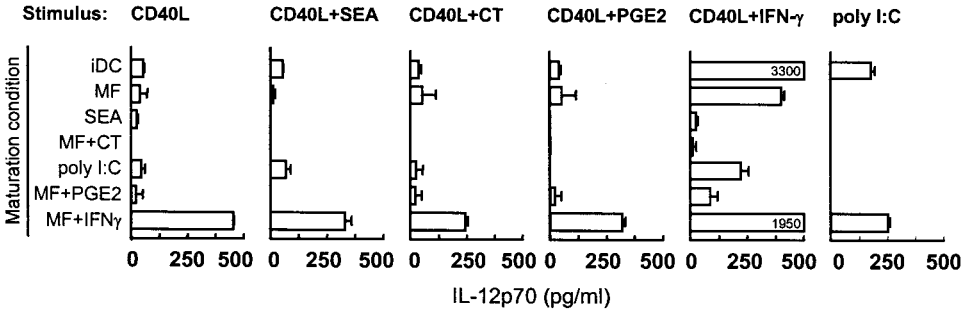

FIGURE 6. DC1 and DC2 subsets are stable and unresponsive to further modulation. Immature DCs and effector DC1s and DC2s (4×10^4 cells/well) were stimulated with J558-CD40L (4×10^4 cells/well) in the absence or presence of SEA (30 μg/ml), CT (1 μg/ml), PGE_2 (10^{-6} M), or IFN-γ (1000 U/ml), or with poly(I:C) (20 μg/ml). After 24 h, supernatants were collected and IL-12p70 was determined by ELISA. Concentrations that were out of range of the axes are given in the respective bars. Results from one representative experiment of four.

responses directed by polarized Th1 or Th2 cell subsets. Here, we demonstrate that microbial compounds induce Th cell polarization via the polarization of sentinel DCs into effector DC1s and DC2s. We and others have shown earlier that maturation of DCs by LPS or MF (19, 20) in vitro primes for a decreased ability to produce IL-12 and that these DCs have the potency to induce mixed populations of Th1 and Th2 cells. The balance between Th1 and Th2 cells strongly depends on the model system used, because it varies with Ag levels (21) as well as with number (22) and source (23) of APCs and is subject to significant donor variability. Our data corroborate previous studies, both in mouse and human (1–4), indicating that pathogens or their signature molecules can induce biased immune responses by direct priming of DCs. Surprisingly, the critical Th1-driving molecules of the DC1 types and the Th2 driving-molecules of the DC2 types differ depending on the pathogen, suggesting a complex network of immune polarization and a multifaceted diversity in signal 3.

DC1 primed by MF + IFN-γ or by MF + PT drive Th1 cells via high IL-12 production upon CD40 ligation (T cell engagement). Although poly(I:C) induces high levels of IL-12 in sentinel myeloid DC (1) (E. C. de Jong, J. H. N. Schuitemaker, and M. L. Kapsenberg, unpublished observations), when present during maturation it does not prime for high IL-12 production. Instead, poly(I:C)-primed DC1s drive Th1 cells via an unknown soluble factor. Poly(I:C) is used as a model Ag for viral infections. The relatively low importance of IL-12 in response to poly(I:C) is in accordance with the finding by Schijns et al. (24) that IL-12p40/p70-deficient mice still mount potent Th1 responses upon infection with mouse hepatitis virus and the finding that patients with a functional mutation of the IL-12R suffer from infections by various endosomal microorganisms, but not by viruses (25). Liu and coworkers (26) showed that plasmacytoid DCs activated with influenza virus promote the development of Th1 cells via IFN-α. Surprisingly, in myeloid DCs the unknown soluble Th1-driving factor secreted by poly(I:C)-matured DCs is probably not a type I IFN or IL-18. It is also unlikely to be IL-23 because its p40 subunit is not up-regulated in these DCs (data not shown) and polyclonal Abs directed against IL-12 only partially inhibit the Th1-inducing capacity.

Within the DC2s, two types were identified. Although all DC2s express OX40L, only SEA-primed DCs use OX40L to promote the development of Th2 cells. The CT- and PGE_2-primed DCs promote Th2 cells via an unidentified soluble factor that is absent in the SEA-primed cells. Possible candidates for the DC-derived Th2-inducing molecules like IL-4, IL-13, PGE_2, or monocyte chemoattractant protein-1 do not appear to be involved, because they were not produced at detectable levels and neutralization of their activity had no effect. The identification of this factor(s) is an issue of current investigation.

The heterogeneity of effector DCs and, therefore, of signal 3 reflects the different ways in which various microbial compounds or mediators can activate the sentinel DCs. So far, little is known about the molecular cross-talk between DCs and pathogens. The capability of CT and PGE_2 to prime for DC2s can be attributed to their ability to enhance the levels of intracellular cAMP, which block the development of IL-12 responses (27). CT signaling, however, differs from PGE_2 signaling in that PGE_2 by itself is unable to induce maturation (11), whereas CT does induce maturation (4), although not always completely (data not shown). The different mechanisms by which CT and PGE_2 on the one hand and SEA on the other hand prime for DC2s are underscored by the finding that SEA barely up-regulated intracellular cAMP (D. van der Kleij and M. Yazdanbakhsh, unpublished observations).

The capability of PT to prime for DC1 with high IL-12 production, as has also been shown previously in mice in vivo (28), may be explained by the inhibition of Gi protein signaling (29). Thus far, it is unknown how polymerized dsRNA (poly(I:C)) activates DCs to mature into effector DC1s. Possibly it signals through a Toll-like receptor, as has been shown for bacterial DNA motifs and Toll-like receptor 9 (30).

Fully matured effector DCs are resistant to repolarization by microbial stimuli (31) or cytokines (12, 20). This implies that effector DCs primed by a certain microbe are not subject to subsequent cross-modulation by the priming abilities of other pathogens, thereby mediating effective immunity to the first encountered pathogen.

The current findings suggest that, analogous to the development of polarized Th cell subsets from a single precursor population, human DCs are guided by the conditions of their maturation to acquire stable polarized functional effector DC1 or DC2 phenotypes. The present study supports the concept that the type of immune response is optimally adapted to the character of the pathogen via the priming of sentinel DCs into effector DC subsets with unexpectedly diverse functional phenotypes and expression of signal 3.

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