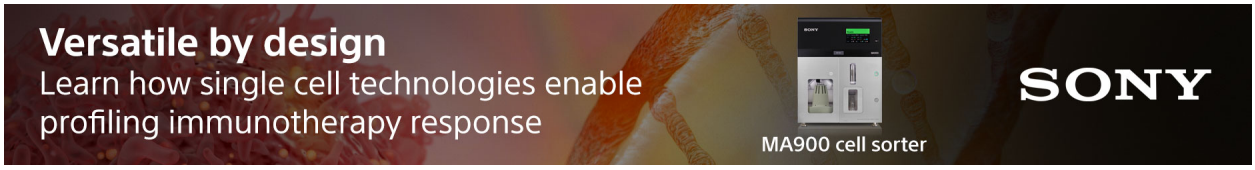

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Development of Th1-Inducing Capacity in Myeloid Dendritic Cells Requires Environmental Instruction[1]

Pedro L. Vieira,* Esther C. de Jong,*† Eddy A. Wierenga,* Martien L. Kapsenberg,[2]*† and Paweł Kaliński[2]*

Dendritic cells (DC) are key initiators of primary immune responses. Myeloid DC can secrete IL-12, a potent Th1-driving factor, and are often viewed as Th1-promoting APC. Here we show that neither a Th1- nor a Th2-inducing function is an intrinsic attribute of human myeloid DC, but both depend on environmental instruction. Uncommitted immature DC require exposure to IFN-γ, at the moment of induction of their maturation or shortly thereafter, to develop the capacity to produce high levels of IL-12p70 upon subsequent contact with naive Th cells. This effect is specific for IFN-γ and is not shared by other IL-12-inducing factors. Type 1-polarized effector DC, matured in the presence of IFN-γ, induce Th1 responses, in contrast to type 2-polarized DC matured in the presence of PGE_2 that induce Th2 responses. Type 1-polarized effector DC are resistant to further modulation, which may facilitate their potential use in immunotherapy. ***The Journal of Immunology*****, 2000, 164: 4507–4512.**

Antigen-specific Th cells control the effector mechanisms of immunity through the differential secretion of cytokines. Committed Th1 cells (high producers of IFN-γ) and Th2 cells (high producers of IL-4 and IL-5) develop from a common pool of naive Th cells (ThN)[3] (1, 2). Depending on the character of the Ag and the route of its entry, already 3 days after primary immunization, immune responses induced in the same lymph nodes may show either the Th1 or the Th2 pattern, resulting in the production of different Ab isotypes (3, 4). Despite an abundance of data on the mechanisms governing Th cell polarization, it is not entirely clear how such an early, pathogen- and tissue-type dependent, polarizing signal can be delivered from peripheral tissues to lymph node-based ThN.

Although the commitment toward either the Th1 or the Th2 phenotype can be influenced by many signals active at the moment of ThN priming, the levels of IL-12p70 (IL-12) produced by APC are of major importance (1, 2). Dendritic cells (DC) are the professional APC for ThN, and thus for the initiation of primary immune responses (5, 6). They produce IL-12 upon interaction between CD40 on the APC and the rapidly induced CD40 ligand (CD40L, CD154) on the activated Th cell (7–10).

The IL-12-producing capacity of DC is subject to regulation. Several reports have shown that the ability of myeloid DC to produce IL-12 can be stably suppressed by inflammatory mediators such as PGE_2 and IL-10, by glucocorticoids or β_2 agonists, all resulting in DC populations with enhanced Th2-promoting capacity (11–15). In contrast, no studies have addressed the possibility to obtain reciprocally modified myeloid DC with enhanced Th1-promoting capacity. While numerous factors such as IFN-γ, LPS, CD40L, fixed bacteria, bacterial DNA, and dsRNA can induce IL-12 production or up-regulate its level when present at the site of DC-ThN interaction (9, 10, 16–19), it remains unresolved whether high IL-12 production can be also predetermined by the environmental factors immature DC meet in peripheral tissues. Until now, no inflammatory mediators, or pathogen-related products, have been identified that can induce stable effector DC with an increased capacity to produce IL-12 upon a subsequent encounter with ThN in the lymph nodes. The lack of such studies can, at least partially, be explained by the view that human myeloid DC are a Th1-promoting APC type per se, as judged by their intrinsic ability to produce IL-12 upon activation (6). This view was supported by a recent observation that human myeloid DC, in contrast to human $IL\text{-}3R^+$ plasmacytoid DC, can induce the Th1 differentiation pattern in ThN (18). However, that comparative study used an exogenous IL-12 inducer (third-party CD40L-bearing cell line), leaving open the question of whether the interaction of myeloid DC with ThN in neutral conditions would still result in Th1 responses. Accepting the possibility that myeloid DC may not be a Th1-driving APC population per se, in the current study we addressed the question of whether immature DC can be instructed to adopt such a function by environmental factors present at the site of the induction of their maturation. We provide evidence that, in contrast to various other IL-12-inducing factors, IFN-γ has the unique capacity to prime DC for high IL-12 production and strong Th1-promoting capacity.

Materials and Methods

Generation of immature DC from peripheral blood monocytes and induction of their maturation under polarizing conditions

Immature DC were generated from peripheral blood monocytes (10) cultured in IMDM (Life Technologies, Paisley, U.K.) containing 10% FCS (HyClone, Logan, UT), recombinant human (rh)GM-CSF (500 U/ml; Schering-Plough, Uden, The Netherlands), and rhIL-4 (250 U/ml; Pharma

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[3] Abbreviations used in this paper: ThN, naive Th cells; DC, dendritic cells; CD40L, CD40 ligand (CD154); poly(I:C), polyriboinosinic polyribocytidylic acid; J558-CD40L, CD40L-transfected J558 cell line; sCD40LT, soluble trimeric human recombinant CD40L; SEB, *Staphylococcus aureus* enterotoxin B; rh, recombinant human.

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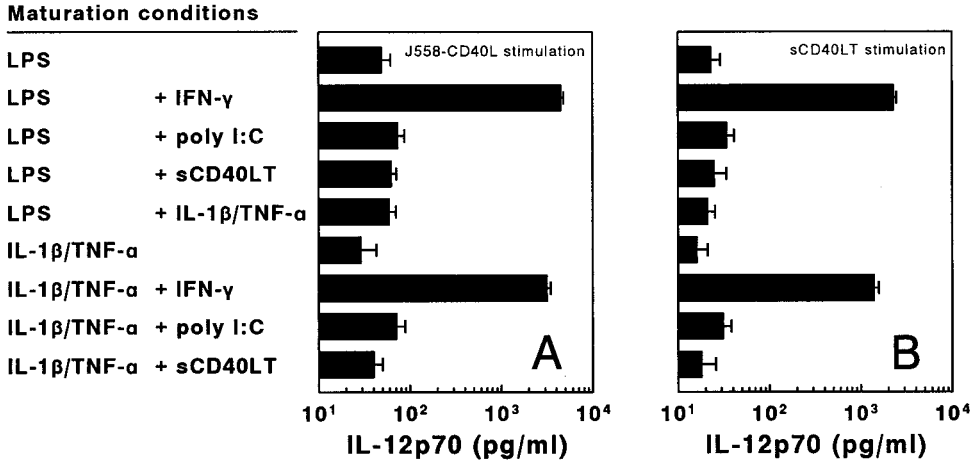

FIGURE 1. IFN-γ enhances the ability of maturing DC to secrete IL-12p70 upon subsequent stimulation. Maturation of DC was induced by either LPS or IL-1β/TNF-α. Both modes of maturation were performed in either the absence of any additions or the presence of IFN-γ, poly I:C, or sCD40LT. Additionally, maturation was induced by a combination of LPS and IL-1β/TNF-α. Differentially matured DC were harvested after 48 h at day 8, washed to remove residual factors, and stimulated in the absence of IFN-γ with either (*A*) J558-CD40L or (*B*) sCD40LT. IL-12p70 concentrations in 24 h supernatants were determined by ELISA. Results, expressed as mean ± SD of triplicate cultures, are from one representative experiment of four. In all groups, no IL-12p70 was detectable in the absence of either J558-CD40L or sCD40LT.

Biotechnologie Hannover, Hannover, Germany) (20). At day 6, the maturation of DC was induced by a 2-day exposure to either LPS (250 ng/ml; Difco, Detroit, MI) or a combination of the cytokines rhIL-1β (10 ng/ml, sp. act. 2×10^8 U/mg; Pharma Biotechnologie Hannover), and rhTNF-α (50 ng/ml, sp. act. 1×10^8 U/mg; Pharma Biotechnologie Hannover) (12, 20). Maturation was induced in the absence or in the presence of IFN-γ (10^3 U/ml, unless stated otherwise; a gift of Dr. P. H. van der Meide, Biomedical Primate Research Center, Rijswijk, The Netherlands), PGE_2 (10^{-6} M, unless stated otherwise; Sigma, St. Louis, MA), polyriboinosinic polyribocytidylic acid (poly I:C) (20 μg/ml; Sigma), soluble trimeric rhCD40L (sCD40LT) (1 μg/ml; Immunex, Seattle, WA), or additionally by a combination of LPS and IL-1β/TNF-α. Where indicated, DC were kept in nonmaturing conditions during 2 days. All subsequent tests were performed after harvesting the cells at day 8 and after removal of GM-CSF, IL-4, IL-1β, TNF-α, LPS, IFN-γ, PGE_2, poly I:C, and sCD40LT by extensive washing.

Induction of IL-12p70 secretion by differentially matured DC

At day 8, DC were harvested, washed extensively (four times in 10 ml of culture medium), and 2×10^4 cells/well were stimulated in 96-well flat-bottom culture plates (Costar, Cambridge, MA) in IMDM containing 10% FCS in a final volume of 200 μl. The following stimuli were used: CD40L-transfected J558 cell line (J558-CD40L; a gift of Dr. P. Lane, University of Birmingham, Birmingham, U.K.; 5×10^4 cells/200 μl), which has been shown to induce IL-12p70 in an IFN-γ-independent way (7), or sCD40LT (1 μg/ml). DC stimulation was performed in the absence or in the presence of either IFN-γ (10^3 U/ml, unless stated otherwise) or PGE_2 (10^{-6} M). Supernatants were harvested after 24 h, and the concentrations of IL-12p70 were measured by ELISA (11). No IL-12p70 production was detected (detection limit 3 pg/ml) in any population of unstimulated DC.

Isolation of $CD4^+$ $CD45RA^+$ $CD45RO^-$ ThN, cocultures with autologous DC, and induction of memory-type cytokines in maturing Th cells

ThN were isolated from peripheral blood leukocytes with the negative selection human $CD4^+$/$CD45RO^-$ column kit (R&D Systems, Minneapolis, MN). This method yielded highly purified (>98%) $CD4^+$ $CD45RA^+$ $CD45RO^-$ ThN as assessed by flow cytometry (data not shown). ThN (5×10^4 cells/200 μl) were cocultured in 96-well flat-bottom culture plates (Costar) with autologous DC (2×10^4 cells/200 μl) matured under the influence of LPS or of LPS in the presence of either IFN-γ (10^3 U/ml) or PGE_2 (10^{-6} M) and coated with superantigen (*Staphylococcus aureus* enterotoxin B; SEB) (1 ng/ml; Sigma). Where indicated, IFN-γ (10^3 U/ml) was added to the cocultures. Culture supernatants were harvested after 24 h, and the concentrations of IL-12p70 were measured by ELISA. Alternatively, T cells were allowed to expand for 14 days. On day 5, IL-2 (10 U/ml; Cetus, Emeryville, CA) was added and the cultures were further expanded for another 9 days. On day 14, resting memory Th cells were harvested, washed, and restimulated with CD3 mAb (CLB-T3/3; Central Laboratory of the Netherlands, Amsterdam, The Netherlands) and CD28 mAb (CLB-CD28/1; Red Cross Blood Transfusion Service) (11). The concentrations of IFN-γ and IL-4 in 24-h supernatants were measured by ELISA (detection limit 100 pg/ml and 60 pg/ml, respectively) (11).

Results

IFN-γ instructs maturing myeloid DC to produce enhanced levels of IL-12 upon subsequent stimulation

To analyze whether DC can be instructed to adopt an enhanced Th1-promoting capacity, myeloid DC were induced to mature by LPS or by a combination of inflammatory cytokines (IL-1β plus TNF-α) either in the absence or in the presence of IFN-γ. IFN-γ profoundly increased the ability of maturing DC to produce IL-12 upon subsequent stimulation in the absence of IFN-γ with either J558-CD40L (Fig. 1*A*) or sCD40LT (Fig. 1*B*). This effect was observed irrespectively of the mode of induction of DC maturation. Although several immune mediators and pathogen-derived products are able to induce or up-regulate IL-12 production (9, 10, 16, 17, 19), the IL-12-priming effect of IFN-γ appeared to be unique. None of the other IL-12-inducing factors, i.e., LPS, synthetic dsRNA poly I:C, or sCD40LT could replace IFN-γ in priming DC for enhanced IL-12 production upon subsequent CD40L activation (Fig. 1, *A* and *B*).

In contrast to the profound modulation of the IL-12-producing capacity, IFN-γ did not affect the maturation-associated phenotypical changes, neither elevating nor inhibiting the expression of the mature DC marker CD83, the costimulatory molecules CD40, CD80, and CD86, and the class II MHC Ag-presenting molecule HLA-DR (data not shown).

The IL-12-priming effect of IFN-γ was dose dependent and evident even at concentrations as low as 1 U/ml (Fig. 2*A*), suggesting that locally produced IFN-γ in peripheral tissues, e.g., produced by rapidly recruited NK cells at the site of viral infections (21), can instruct migrating DC to secrete increased levels of IL-12 upon subsequent activation in the lymph nodes. In accord with previous observations (11), in sharp contrast to IFN-γ, the presence of PGE_2 during maturation of DC dose-dependently suppressed their ability to secrete IL-12. IFN-γ and PGE_2 reciprocally regulated the

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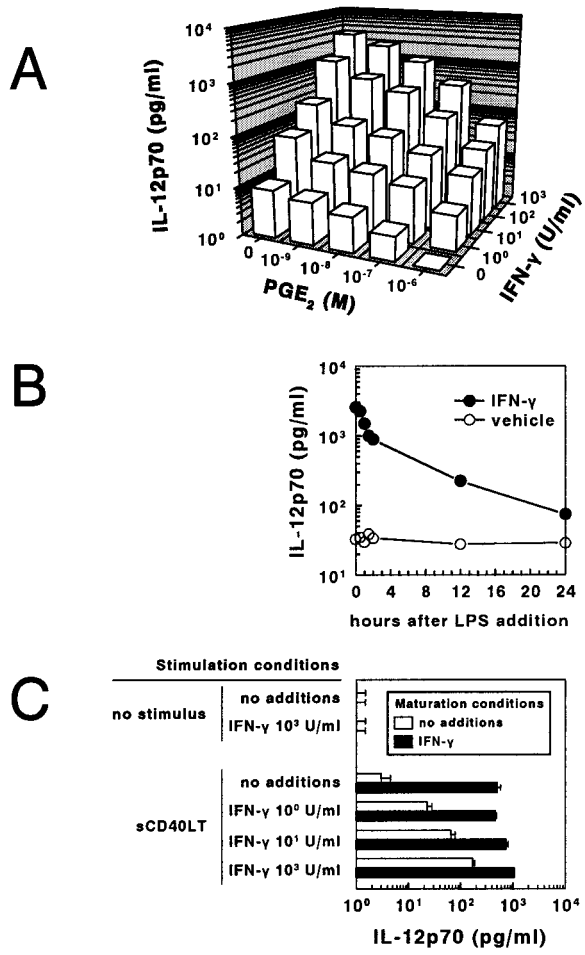

FIGURE 2. High-level IL-12 production by mature DC is mainly determined by exposure of DC to IFN-γ during maturation. *A*, Maturation of DC was induced by LPS, by LPS in the presence of increasing doses of IFN-γ, by LPS in the presence of increasing doses of PGE_2, or by LPS in the presence of combinations of IFN-γ and PGE_2. Differentially matured DC were harvested after 48 h at day 8, washed to remove residual factors, and stimulated in the absence of IFN-γ with sCD40LT. IL-12p70 concentrations in 24 h supernatants were determined by ELISA. Results, expressed as mean of triplicate cultures (in all conditions SD ≤ 5% of the mean), are from one representative experiment of three. *B*, Maturation of DC was induced by LPS at day 6 ($t = 0$ h). Either IFN-γ or vehicle was added to the maturation cultures at $t = 0$, 0.5, 1, 1.5, 2, 12, and 24 h. Differentially matured DC were harvested at day 8 ($t = 48$ h), washed to remove residual factors, and stimulated in the absence of IFN-γ with sCD40LT. IL-12p70 concentrations in 24-h supernatants were determined by ELISA. Results, expressed as mean ± SD of triplicate cultures, are from one representative experiment of three. *C*, Maturation of DC was induced by LPS either in the absence or in the presence of IFN-γ. Differentially matured DC were stimulated after 48 h at day 8 with sCD40LT either in the absence or in the presence of increasing concentrations of IFN-γ. IL-12p70 concentrations in 24-h supernatants were determined by ELISA. Results, expressed as mean ± SD of triplicate cultures, are from one representative experiment of four.

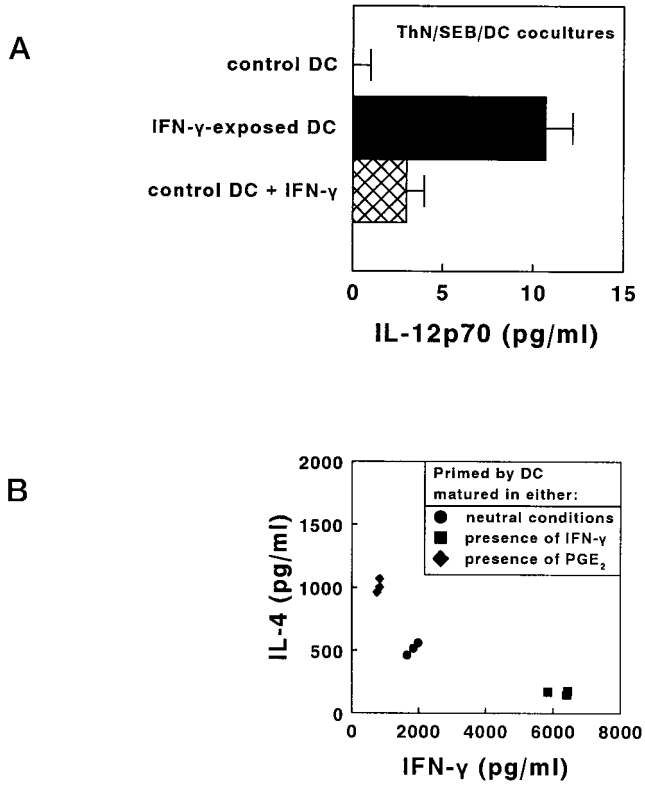

FIGURE 3. Exposure of maturing DC to IFN-γ enhances their capacity to secrete IL-12p70 during their subsequent interaction with ThN and results in their Th1-promoting function. *A*, Maturation of DC was induced by LPS either in the absence or in the presence of IFN-γ. ThN were cocultured with SEB-coated control DC in the absence (□) or in the presence of IFN-γ (▨). Alternatively, ThN were cocultured (in the absence of IFN-γ) with SEB-coated DC that had matured in the presence of IFN-γ (■). No IL-12p70 was produced in the absence of either ThN or SEB. IL-12p70 concentrations in 24-h supernatants were determined by ELISA. Results, expressed as mean ± SD of triplicate cultures, are from one representative experiment of three. *B*, ThN were primed in the presence of SEB by DC matured with LPS under neutral conditions (●) or with DC matured with LPS in the presence of either 10^3 U/ml of IFN-γ (■) or 10^{-6} M of PGE_2 (◆). Th cells were restimulated on day 14 with anti-CD3 mAb and anti-CD28 mAb, and the concentrations of IFN-γ and IL-4 in 24-h supernatants were determined by ELISA. Results from triplicate cultures are from one representative experiment of four.

capacity of maturing DC to secrete IL-12, without any clear dominance of either factor. These results suggest that the actual IL-12-producing capacity of DC originating from particular environments reflects the ratio of IFN-γ to PGE_2 concentrations (possibly being influenced also by other factors present locally).

The IL-12-priming effect of IFN-γ was maximal when IFN-γ was added at the moment of induction of DC maturation (Fig. 2*B*). It was strongly pronounced if IFN-γ was added within the first 2 h after the induction of maturation, but still clearly visible if IFN-γ addition was delayed for 12 h. This indicates that the capacity of mature DC to secrete IL-12 upon subsequent encounter with ThN is determined mainly by the conditions present at the site of induction of DC maturation. As expected (9–11), the presence of exogenous IFN-γ during the CD40L-mediated stimulation of mature DC was a prerequisite for high-level IL-12 production by control mature DC (Fig. 2*C*, open bars). In contrast, DC matured in the presence of IFN-γ (Fig. 2*C*, filled bars) acquired the capacity to produce large amounts of IL-12, even in the absence of IFN-γ during their subsequent stimulation. Moreover, although control DC dose-dependently responded to IFN-γ when added at the moment of stimulation, the levels of IL-12 produced by these DC never reached those produced by IFN-γ-preexposed DC, remaining at least 10-fold lower. These results are consistent with the decreased responsiveness of mature DC to IFN-γ and their reduced expression of the IFN-γR (20). These observations indicate that high-level IL-12 production by mature DC depends mainly on the presence of IFN-γ at an earlier stage, i.e., during the induction of

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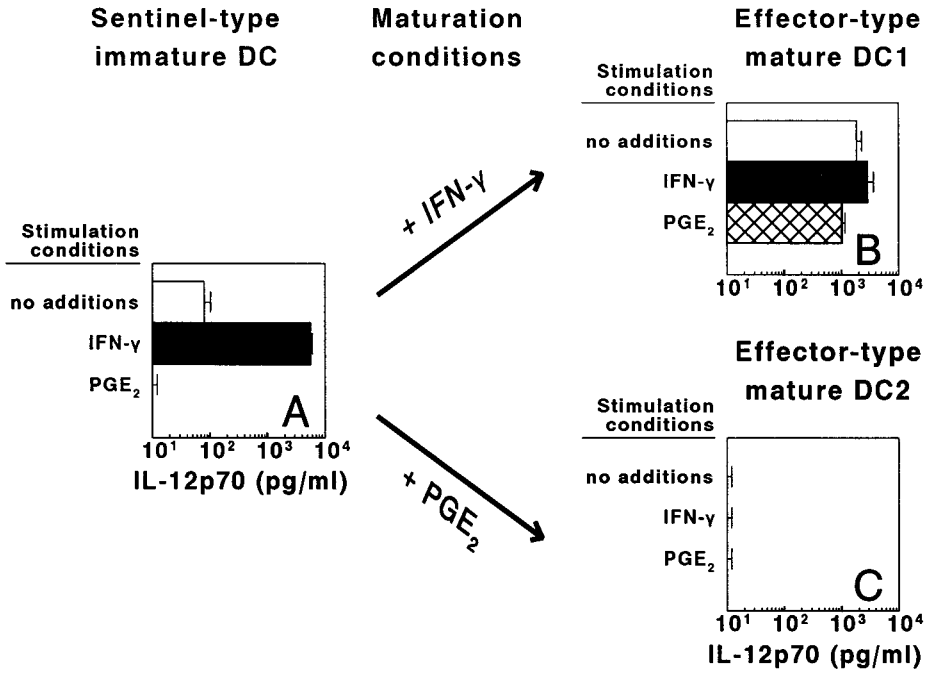

FIGURE 4. Inflammatory mediators modulate the IL-12 production in sentinel-type immature DC and promote the development of stable, polarized, effector DC (DC1 and DC2), resistant to further modulation. Immature DC (*A*) or DC matured with LPS in the presence of either 10^3 U/ml of IFN-γ (*B*) or 10^{-6} M of PGE_2 (*C*) were stimulated with sCD40LT in the absence of any additions (□) or with sCD40LT in the presence of either 10^3 U/ml of IFN-γ (■) or 10^{-6} M of PGE_2 (▨). IL-12p70 concentrations in 24-h supernatants were determined by ELISA. Results, expressed as mean ± SD of triplicate cultures, are from one representative experiment of three.

their maturation, rather than on the presence of IFN-γ during their subsequent stimulation.

Type 1- and type 2-polarized myeloid effector DC induce different Th cell responses

To test whether the presence of IFN-γ during the maturation of DC instructs them to adopt a Th1-inducing function, we used a superantigen model (9–11, 20) that, similar to TCR transgenic animal models (22), allows a significant proportion of human Th cells to be activated by APC and to induce early IL-12 production in a CD40L-dependent mechanism (9, 11). As expected (9, 10), the induction of detectable IL-12 production in control DC by ThN required the additional presence of exogenous IFN-γ (Fig. 3*A*, crosshatched bar). In contrast, the interaction of ThN with DC exposed to IFN-γ during maturation (Fig. 3*A*, filled bar) resulted in substantial IL-12 production independently of any additions. DC matured under neutral conditions and DC exposed to IFN-γ or PGE_2 during maturation all expressed similar levels of HLA-DR and costimulatory molecules and induced similar proliferation in responding ThN, resulting in a similar Th cell yield (data not shown and Ref. 11). However, while the priming of ThN with control DC induced memory Th0-type cells, secreting moderate levels of both IFN-γ and IL-4 after restimulation (Fig. 3*B*, circles), DC exposed to IFN-γ induced a strong bias toward Th1 (Fig. 3*B*, squares). Conversely, DC exposed to PGE_2 promoted a Th2 pattern of differentiation in ThN (Fig. 3*B*, diamonds). In summary, these data demonstrate that the presence of a different set of immune mediators at the site of the induction of DC maturation can instruct maturing DC to adopt reciprocal Th1- vs Th2-inducing functional phenotypes.

Stability of polarized myeloid effector DC

The current observations indicate that mediators of nonspecific immunity, such as a product of activated NK cells, IFN-γ (21), and a common inflammatory mediator, PGE_2, can modulate the production of IL-12 in DC in two different fashions (Fig. 4). First, they can directly modulate the levels of IL-12 produced by sentinel-type immature DC in peripheral tissues (Fig. 4*A*). Probably more important, however, the presence of these mediators at the site of activation of immature DC can drive their maturation toward Th1- or Th2-promoting effector DC types that cannot be repolarized at a later time point. Type 1 effector DC, matured in the presence of IFN-γ, produce high levels of IL-12 upon subsequent CD40 triggering. This high-level IL-12 production does not depend on the presence of IFN-γ at this stage and can no longer be suppressed by PGE_2 (Fig. 4*B*). On the other hand, type 2 effector DC, matured in the presence of PGE_2 (Fig. 4*C*), are deficient in IL-12 production. Also this type of effector DC is stable and resistant to repolarization by IFN-γ. In addition, the differences in IL-12-producing capacities established during the maturation are relatively stable in time (up to 24 h, data not shown and Ref. 11). The possibility to obtain stable type 1-promoting effector DC has interesting clinical implications. Such cells may be candidates for Ag-specific induction of therapeutic Th1 responses in cancer and in chronic infections with intracellular pathogens.

Discussion

The ability of DC to adopt reciprocally polarized Th1- and Th2-promoting phenotypes in response to environmental conditions adds to the observation that distinct DC lineages can carry different Th1- or Th2-driving capacities (18, 23, 24). Rissoan et al. (18) showed that human DC derived from peripheral blood monocytes promote the development of Th1 cells from ThN, while $CD4^+$ $CD3^-$ plasmacytoid DC-like cells promote Th2 development. However, data from murine models suggested opposite lineage-specific differences (23, 24) that may either be explained by a difference between mouse and human or, more likely, by differences in the maturational stages of the DC used in either system, reflected in their different abilities to produce IL-12 (20). Although presentation of Ag by separate DC lineages may explain polarization of certain immune responses (e.g., those induced by i.v. Ag administration vs immunization via peripheral tissues), it is less explanatory in cases of peripheral immunization with different Ags that require different types of immunity but both need to be transported from peripheral tissues to the lymph nodes (3, 4). This latter process is a function of myeloid, rather than lymphoid, DC (25). In

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addition, a system employing separate Th1- and Th2-promoting DC lineages may be restricted by its inherent rigidity. If the Th1- or Th2-inducing capacities are programmed within a particular DC lineage, before the cells actually meet a pathogen, the function of such a system depends on selection rather than on adaptation, limiting the scope of its application. It also requires a putative receptor system, efficiently discriminating between Th1- and Th2-inducing pathogens.

The current data indicate that neither a Th1- nor a Th2-inducing capacity is an intrinsic feature of myeloid DC. Both capacities can be acquired by uncommitted immature DC in response to signals delivered by the local microenvironment.

The ability of an individual DC to respond in a flexible fashion to different microenvironments opens the possibility that the tuning of Th cell responses to the type of pathogen and invaded tissue can benefit from the adaptation of DC function to the conditions they encounter in the pathogen-invaded tissue. This hypothesis is supported by an increasing amount of data obtained in vivo. Freshly isolated airway DC and Peyer's patches DC, as opposed to spleen DC, display a Th2-promoting capacity (26–29). These differences can be observed despite similar frequencies of myeloid DC in Peyer's patches and spleen DC populations (29), suggesting a role for tissue-specific DC polarization. Several DC-polarizing factors have been identified, which may be differentially produced in distinct tissues, and the production of which can be differentially regulated by different pathogens. IFN-γ, produced by NK cells during viral infections (21), and probably other as yet unknown factors, may contribute to the development of type 1-polarized DC. Although many other factors, including viral and bacterial products (e.g., dsRNA, bacterial DNA, fixed bacteria, and LPS) may induce or enhance IL-12 production when present at the moment of DC-T cell interaction (9, 10, 16, 17, 19), none of these factors shares the unique capacity of IFN-γ to induce stably polarized effector DC with enhanced IL-12-producing capacity. In contrast, many more factors have been identified that can stably suppress the IL-12-producing capacity of DC. Agents with a cAMP-elevating potential, such as PGE_2, β_2 agonists, and possibly histamine, that inhibit the IL-12-producing capacity of DC (11, 15, 30) and enhance their immunostimulatory function (11), represent a potentially larger group of type 2 DC-polarizing factors. Another set of factors, including IL-10 and glucocorticoids, induces the DC that are similarly IL-12-deficient (11–14, 31), but also have reduced stimulatory capacity (11). This latter type of DC was reported to induce tolerance in ThN (32), while in other models it promotes the induction of Th2-type responses (12, 31). It is noteworthy that tissue environments with high concentrations of IL-10, PGE_2, or TGF-β have been described in the body compartments and several disease states associated with Th2 responses, e.g., the anterior chamber of the eye, certain tumors, chronic disease states, and UV-irradiated skin.

It has been proposed that tissue-derived signals instruct the immune system to initiate immune responses (33). The present data implicate that tissue-derived signals, carried by DC, can also determine the initial polarization of ThN responses and, hence, the class of the initiated response. In this model, migrating DC, apart from carrying antigenic and costimulatory signals ("signal one" and "signal two"), are further equipped with the capacity to transmit a third type of signal that reflects both the nature of the pathogen and of the invaded tissue. This additional signal may allow for a rapid selection of the most appropriate effector mechanisms of immunity, contributing to the effectiveness of the response and reducing the risk of collateral damage to own tissues.

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