

Modulating the immune response with dendritic cells and their growth factors

Bali Pulendran, Eugene Maraskovsky, Jacques Banchereau and Charles Maliszewski

Different subsets of dendritic cells (DCs) appear to play a role in determining the specific cytokines secreted by T helper (Th) cells. A model is proposed that links together factors such as the pathogen, microenvironment, DCs and T cells in a mechanism that results in a flexible determination of T-cell polarization.

The adaptive immune system has evolved several strategies that are effective against different pathogens. For example, in response to intracellular microbes, CD4⁺ T helper (Th) cells differentiate into Th1 cells, which secrete interferon γ (IFN- γ); by contrast, extracellular organisms such as helminths induce the development of Th2 cells, whose cytokines [interleukin 4 (IL-4), IL-5 and IL-10] direct IgE and eosinophil-mediated destruction of the pathogens. Although the cytokines that influence the type of Th-cell responses are known¹, the original sources of these cytokines *in vivo* are less clear. Results from several groups suggest a role for distinct subsets of dendritic cells (DCs) in orchestrating this decision-making process. This raises the possibility that cytokines that mobilize one or another DC subset *in vivo* may have a role as vaccine adjuvants, in promoting different types of immune responses.

This article summarizes current knowledge of DC development *in vivo* and reviews the emerging literature suggesting that distinct DC subsets, and their growth factors, can direct Th responses differently. These ideas are discussed in the context of the notion that DC function is not fixed, but is adaptable in response to signals from the microenvironment and the pathogen, thus permitting flexibility during the evolution of the immune response. With the guidance of these perspectives, a model is proposed in which Th-cell polarization is determined at several levels, including: (1) the pathogen; (2) pathogen recognition receptors on DCs; (3) DC subsets; (4) the microenvironment, and (5) cytokines released by T cells and other cells in the vicinity.

DC subsets: lineage versus maturation stage

Since its initial description more than 25 years ago², the DC has assumed center stage as the key initiator of adaptive immunity. We now know that there are several subsets of DCs that differ in phenotype, function and localization in the microenvironment^{3,4}.

The key question is whether this heterogeneity reflects lineage or maturational differences, or both. It has been proposed that mature DCs originate from at least two distinct lineages, the so-called 'lymphoid'⁴⁻⁸ and 'myeloid'⁹⁻¹² lineages. The myeloid lineage has been proposed to consist of two pathways: the Langerhans cell (LC) and the 'interstitial' pathways. Within these pathways, DCs at different maturational stages may differ in phenotype, function and localization.

Although DC biology has been impeded by the rarity of DCs *in vivo*, much has accrued from the study of DCs *in vitro*. However, there is an urgent need to study the corresponding DCs *in vivo*. A recent solution to this problem has been the identification of growth factors, such as Flt3 ligand [Fms-like tyrosine kinase receptor 3 ligand (Flt3L)], granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor (G-CSF), that mobilize DC subsets or their precursors *in vivo*¹³⁻²¹.

Mice

In the secondary lymphoid organs of mice, at least three major subpopulations of DCs have been described: CD8 α ⁺ 'lymphoid' DCs, CD8 α ⁻ 'myeloid' DCs and LC-derived DCs. CD8 α ⁺ DCs in the thymus may develop from a lymphoid precursor population that also yields T cells and natural killer (NK) cells^{6,7}. However, clonal analysis has not been performed to show that lymphoid cells and DCs can arise from the very same precursor cells, thus these DCs are referred to as 'putative lymphoid-related DCs'⁴; this review will refer to such cells as CD8 α ⁺ DCs.

DCs with similar phenotype to CD8 α ⁺ thymic DCs are found in the T-cell-rich areas in the spleen, lymph nodes and Peyer's patches (Table 1)^{13-15,22-29}. Whether these DCs are also lymphoid-related is unknown. By contrast, the CD8 α ⁻ 'myeloid' DCs are localized in the marginal zones of the spleen, the sub-capsular sinuses of the lymph nodes and the sub-epithelial dome of the Peyer's patches^{13-15,22-29}. However, in response to microbial stimuli such as lipopolysaccharide (LPS), or toxoplasma extracts, these CD8 α ⁻ DCs can rapidly migrate to the T-cell areas^{22,28}. A subset of these

Bali Pulendran*
Jacques Banchereau
Baylor Institute for
Immunology, 3434 Live
Oak, Dallas, TX 75204,
USA.
*e-mail: balip@
baylordallas.edu

Eugene Maraskovsky
Charles Maliszewski
Immunex Corporation,
51 University St, Seattle,
WA 98101, USA.

Table 1. Murine DC subsets in secondary lymphoid organs^a

Postulated lineage	Lymphoid	Myeloid	
	CD8 α ⁺ DC	CD8 α ⁻ DC	LC-derived DC
Phenotype ^{13-15,23-29}	CD11c ⁺ MHC class II ⁺ CD8 α ⁺ DEC205 ⁺ CD11b ^{dull/-} 33D1 ⁻ CD4 ⁻ CD86 ⁺ CD40 ⁺ Birbeck granule ⁻ Lag ⁻	CD11c ⁺ MHC class II ⁺ CD8 α ⁻ DEC205 ⁻ CD11b ⁺ 33D1 ⁺ (subset) ¹⁴ CD4 ⁺ (subset) ^{14,30} CD86 ⁺ CD40 ⁺ Birbeck granule ⁻ Lag ⁻	CD11c ⁺ MHC class II ⁺ CD8 α ^{+/-} (?) DEC205 ⁺ CD11b ⁺ 33D1 ⁻ CD4 ⁺ CD86 ⁺ CD40 ⁺ Birbeck granule ⁺ Lag ⁺
Localization ^{14,15,22-25,28}	T-cell zones of lymphoid organs; thymic cortex	Marginal zones of spleen (move to T-cell zones, when activated with LPS or toxoplasma); sub-capsular sinus of lymph nodes?; sub-epithelial dome of Peyer's patches	Immature LCs in epithelia; LC-derived DCs in T-cell zones of lymph nodes
Function			
Ag capture ^{14,20,29}	+	++	++
Ag processing ²⁰	++	++	++
IL-12 secretion ^{14,28,42}	++++	+/-	++++
IFN- γ secretion ⁴⁷	++++	-	?
CD4 ⁺ T-cell priming ^{17,20,42,45}	++++ (Th1)	++++ (Th0/Th2)	?
CD8 ⁺ T-cell priming ^{20,46}	+++	++++	++++

^aAbbreviations: Ag, antigen; DC, dendritic cell; IFN- γ , interferon γ ; LC, Langerhans cell; LPS, lipopolysaccharide; MHC, major histocompatibility complex; Th, T helper.

CD8 α ⁻ DCs also express CD4 and 33D1 (Table 1)^{14,30}. For the sake of simplicity, the CD8 α ⁻ DC subset will not be further subdivided in the present discussion, as the relationship between CD4 expression and lineage development or function is yet to be determined.

LCs express distinctive markers such as Birbeck granules, Langerin and E-cadherin³¹⁻³⁴ and are localized in the epithelia. Pathogen entry at this site can induce the maturation of LCs, and their migration to the T-cell areas of the draining lymph nodes. The lineage origins of LCs in mice is controversial. Some skin-sensitization studies indicate that mature LC-derived DCs in the lymph nodes have the phenotype CD11c⁺ CD11b⁺ DEC205⁺ CD8 α ^{dull/-} (Table 1)²⁹, but other similar studies suggest that a population of cells expressing significant levels of CD8 α in the lymph nodes may develop from LCs (Ref. 27). It is not clear whether this population represent the true CD8 α ⁺ 'putative lymphoid-related' DC.

Humans

In humans, although knowledge has been gathered from *in vitro* studies, the biology, tissue distribution and function of DC subsets *in vivo* are only beginning to be explored. At least three subsets of human DCs are known: (1) myeloid DCs (also known as interstitial or dermal DCs); (2) LC-derived DCs; and (3) plasmacytoid DCs with the phenotype CD11c⁻, CD1a⁻, IL-3 receptor (IL-3R)⁺, (Refs 3, 5).

Myeloid DCs and LC-derived DCs can both develop from CD11c⁺ HLA-DR⁺ precursors in the blood, under different conditions³¹⁻³³. Myeloid DCs differ from LC-derived DCs in that they do not express Lag antigens, Langerin or Birbeck granules (Table 2)³⁴. Myeloid DCs are closely related to monocytes. For example, when cultured with GM-CSF and IL-4, monocytes generate myeloid DCs (Refs 11, 12). Conversely, immature myeloid DCs when cultured with M-CSF differentiate to the macrophage phenotype^{34,35}. *In vivo*, the choice of whether a monocyte becomes a DC or a macrophage may in part be influenced by the endothelium. Monocytes that reverse transmigrate the endothelium in the abluminal-to-luminal direction (as would occur during entry into lymphatics) become DCs; those that remain in the tissues become macrophages³⁶. A subset of DCs found within germinal centers (GCs) in humans, the so-called GCDCs (Ref. 37), have a phenotype similar to myeloid DCs. It is thus tempting to speculate that interstitial DCs migrate into the lymphoid follicles, where they become known as GCDCs.

CD11c⁻ plasmacytoid DCs can be derived from the CD11c⁻ IL-3R⁺ precursors in the blood (Table 2)³⁸, which were originally described by pathologists as the plasmacytoid T cells or plasmacytoid monocytes. These cells express few myeloid markers and spontaneously die in culture, but can be rescued by IL-3 and CD40L (Ref. 38). A phenotypically similar population of cells has been

Table 2. Human DC subsets and their blood precursors^a

Postulated lineage	Lymphoid	Myeloid	
	Plasmacytoid DC	Interstitial DC	LC-derived DC
Blood precursors ^{18,19,21,31-33,37-40}			
Phenotype	CD11c ⁻ CD1a ⁻ IL-3R ⁺	CD11c ⁺ CD1a ⁺ IL-3R ⁻	CD11c ⁺ CD1a ⁺ IL-3R ⁻
IFN- α production ^{49,62}	++++	–	–
Mature DCs			
Phenotype ^{18,19,21,31-33,37-40}	CD11c ⁻ IL-3R ⁺ MHC class II ⁺ CD11b ⁻ CD13 ⁻ CD33 ⁻ CD4 ⁺⁺ CD1a ⁻ Birbeck granule ⁻ Langerin ⁻ CD86 ⁺ CD40 ⁺ DC-LAMP ⁺	CD11c ⁺ IL-3R ⁻ MHC class II ⁺ CD11b ⁺ CD13 ⁺ CD33 ⁺ CD4 ⁺ CD1a ⁻ Birbeck granule ⁻ Langerin ⁻ CD86 ⁺ CD40 ⁺ DC-LAMP ⁺	CD11c ⁺ IL-3R ⁻ MHC class II ⁺ CD11b ⁺ CD13 ⁺ CD33 ⁺ CD4 ⁺ CD1a ⁺ Birbeck granule ⁺ Langerin ⁺ CD86 ⁺ CD40 ⁺ DC-LAMP ⁺
Localization ^{5,37,38,49}	T-cell zones of lymphoid organs; DC precursors in blood	T-cell zones of lymphoid organs; germinal centers (GCDCs)?; DC precursors in blood; immature cells in tissue interstices (lungs, heart, kidney)	T-cell zones of lymph nodes; DC precursors in blood; immature cells in epithelia
Function			
IL-12 secretion ⁴⁸	+/-	++++	++++
IL-10 secretion ⁶⁸	–	++++	+/-
CD4 ⁺ T-cell priming ^{18,19,20,31-34,37,38,48,49}	++	++++	++++
CD8 ⁺ T-cell priming ³²	++	+++	++++
DC-B-cell interaction ⁶⁹	?	++++	+

^aAbbreviations: DC, dendritic cell; IFN- α , interferon α ; IL, interleukin; LC, Langerhans cell; MHC, major histocompatibility complex; R, receptor.

described in the adult blood; these cells express RNA for pre-T α and recombinase-activating gene 1 (RAG-1), and contain precursors of mature CD4⁺ TCR $\alpha\beta$ ⁺ cells³⁹. Furthermore, a similar CD11c⁻ pre-T α ⁺ population has been identified in the human thymus, and these can develop into mature DCs upon culture with IL-3 and CD40L (Ref. 40). These thymic DC precursors differ from those found in the blood in that they do not express RAG-1 mRNA and fail to develop into T cells in the appropriate assays. Taken together, these data suggest that the CD11c⁻ IL-3R⁺ DCs may be of lymphoid origin. However, there is no clonal evidence to suggest that the CD11c⁻ DCs arise from the same precursor cells that yield T cells. Further evidence for a lymphoid DC in humans comes from studies⁸ in which a subset of CD34⁺ CD10⁺ lineage negative stem cells in adult bone marrow could differentiate into either lymphocytes or DCs.

Despite much progress, our knowledge of DCs *in vivo* has been limited by their rarity. It is only recently that potent *in vivo* DC growth factors have been identified, such as Flt3L, GM-CSF and G-CSF (Refs 13–21). These cytokines and their effects on expanding DCs *in vivo* are considered below.

Flt3L, GM-CSF and G-CSF: *in vivo* DC growth factors

Flt3L as a DC growth factor in mice

Flt3L stimulates the proliferation of stem and progenitor cells through binding to the Flt3L receptor, which is a type III receptor tyrosine kinase member of the platelet-derived growth factor (PDGF) family⁴¹. Although Flt3L is abundantly expressed, the receptor is mostly restricted to hematopoietic progenitors. Administration of soluble Flt3L to mice results in expansion of hematopoietic progenitors in the bone marrow and spleen, and potent mobilization of stem and progenitor cells into the circulation⁴¹. Examination of the tissues in Flt3L-treated mice has led to the observation that Flt3L induces a large increase in the number of DCs in several sites including the spleen, lymph nodes, thymus and Peyer's patches, as well as in the circulation, lungs and liver¹³.

The CD8 α ⁺ and CD8 α ⁻ DC subsets generated in Flt3L-treated mice resemble the corresponding DC subsets identified in normal mice in terms of phenotype, function and microanatomic localization^{13–15}. Both DC subsets express high levels of CD11c and major histocompatibility complex (MHC) class II, and significant levels of CD86 and CD40, but differ in the expression of several other markers (Table 1)^{13–15}. The CD8 α ⁺ DCs express

DEC205, while the CD8 α ⁻ DCs express high levels of CD11b (Table 1). The former are localized in the T-cell zones, while the latter are in the marginal zones of the spleen^{14,15}. Both DC subsets can take up and process soluble proteins efficiently, and prime antigen-specific CD4⁺ and CD8⁺ T cells *in vitro*²⁰. However, the CD8 α ⁺ DCs can be induced to secrete much higher levels of IL-12 (Refs 14, 28, 42). This finding was counterintuitive to the idea that CD8 α ⁺ DCs may serve to limit T-cell proliferation *in vitro*, by inducing apoptosis in a fraction of the CD4⁺ T cells that they activated⁴³, or by failing to support cytokine secretion in CD8⁺ T cells⁴⁴. Therefore, a series of experiments aimed at assessing the functional capacities of these subpopulations *in vivo* was performed and, as discussed later, these subpopulations appear to direct different classes of immune responses.

Flt3L as a DC growth factor in humans

Recent evidence suggests that injection of Flt3L into healthy human volunteers elicits a profound increase in the numbers of immature DCs or precursor DCs in the peripheral blood^{18,21}. Both the CD11c⁺ immature DCs and the CD11c⁻ plasmacytoid DC precursors (CD11c⁻ pre-DCs) are expanded^{18,21}. Whether mature DCs are also expanded in the secondary lymphoid organs is not known. It now remains to be established whether this dramatic increase in DC numbers *in vivo* can enhance immune responses to vaccine antigens, as observed in mice¹⁶.

GM-CSF as a DC growth factor in mice

GM-CSF in combination with tumor necrosis factor α (TNF- α) or IL-4 and other cytokines is a potent growth factor for murine and human myeloid DCs *in vitro*⁹⁻¹². GM-CSF has also been used as a vaccine adjuvant in several studies, although the mechanism of its action is not known. Consistent with the ability of GM-CSF to favor myeloid DC development *in vitro*, treatment of mice with pegylated GM-CSF (which exhibits an extended half-life, compared with underivatized GM-CSF) preferentially expands the CD8 α ⁻ 'myeloid' DCs in the spleen^{17,20}. This CD8 α ⁻ DC subset appears to be phenotypically similar to that generated in Flt3L-treated mice.

G-CSF: a novel growth factor for CD11c⁻ pre-DCs in humans

G-CSF does not appear to expand DC subsets in mice¹³, but recent work suggests that G-CSF injections into healthy humans results in a significant increase in the numbers of plasmacytoid CD11c⁻ pre-DCs in the peripheral blood (see Table 2)^{18,19}. However, unlike Flt3L-treated donors, the numbers of CD11c⁺ immature DCs were not increased in G-CSF donors. Thus, these studies suggest that different cytokines can expand distinct DCs or their precursors, both in mice and in humans. As will be

discussed below, Flt3L and GM-CSF appear to regulate immune responses differently in mice. Whether and how this regulation applies to humans who have been injected with such cytokines, remains to be determined.

Modulating the class of immunity with DCs

Distinct DC subsets elicit distinct Th responses

DCs have long been recognized as potent initiators of immune responses, but now several avenues of work ascribe an important role for them in regulating the immune response. This article focuses on the capacity of DCs to modulate the class of the immune response.

In mice, while both CD8 α ⁺ and CD8 α ⁻ DCs can capture soluble antigens, process them and prime antigen-specific CD4⁺ and CD8⁺ T cells efficiently *in vivo*^{17,42,45,46} and *in vitro*^{20,46}, they induce distinct classes of antigen-specific Th responses *in vivo*^{17,42}. CD8 α ⁺ DCs elicit Th1 responses, while CD8 α ⁻ DCs favor Th2 responses^{17,42}. Furthermore, repeated injections of either the CD8 α ⁺ or CD8 α ⁻ DCs yield strongly polarized Th1 and Th2 responses, respectively (B. Pulendran *et al.*, unpublished). Similar results have recently been observed with sorted CD8 α ⁺ and CD8 α ⁻ DCs from Peyer's patches (A. Iwasaki and B. Kelsall, pers. commun.). CD8 α ⁺ DCs can be induced to secrete IL-12 (Refs 14, 28, 42), which appears essential for their Th1 induction⁴². The DC molecules that induce Th2 responses are unknown, although IL-10 is a good candidate.

The Th1/Th2 skewing by the DC subsets seems to result in a relevant skewing of the antibody response. In comparison with phosphate buffered saline (PBS)-treated mice, both Flt3L- and GM-CSF-treated mice displayed profound increases in antigen-specific antibody titers to an injected protein antigen, but the isotype profiles were dependent on the cytokines used. While Flt3L induced a dramatic increase in ovalbumin-specific IgG2a and a more modest increase in IgG1 titers, GM-CSF treatment favored an IgG1 response, with little increases in IgG2a levels¹⁷. These observations are consistent with a report showing that CD8 α ⁺ DCs, rather than CD8 α ⁻ DCs, are induced to secrete IFN- γ by IL-12 (Ref. 47). Both DC subsets can efficiently prime antigen-specific CD8⁺ T cells and induce cytotoxic T lymphocyte activity *in vivo*^{20,46}. However, it is not known whether such DCs induce distinct cytokine profiles in CD8⁺ T cells.

In humans, monocyte-derived myeloid CD11c⁺ DCs promote Th1 responses, while DCs derived from the CD11c⁻ pre-DCs induce Th2 responses⁴⁸. However, the degree to which Th polarization occurs *in vitro* may differ according to the maturation state of the DCs (Refs 18, 49), and the ratio of DCs to T cells⁵⁰. In our study, plasmacytoid CD11c⁻ DCs derived from CD11c⁻ pre-DCs mobilized by either Flt3L or G-CSF induced higher levels of IL-10, but similar levels of IFN- γ and IL-4 compared with

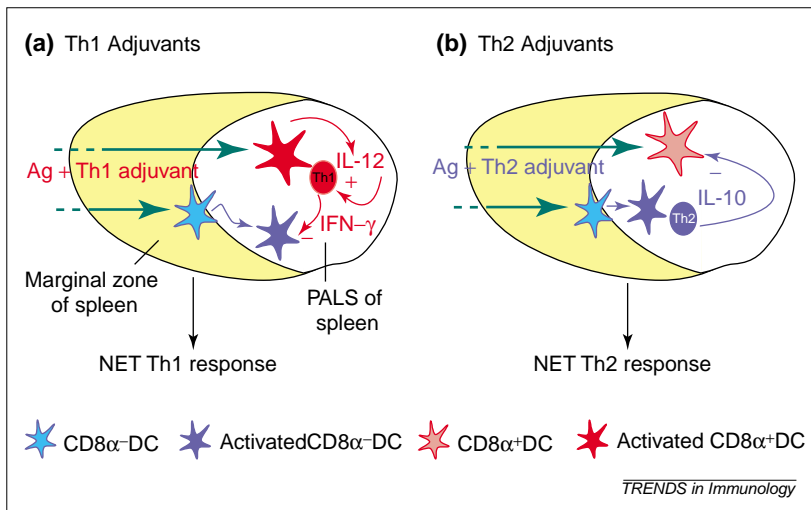


Fig. 1. Determinants of Th immunity: pathogens, pathogen receptors, DC subsets, microenvironment and cytokines. The decision to mount a Th1, Th2 or ThN response is likely to be determined at different levels. (a) A Th1 adjuvant (e.g. *Escherichia coli* LPS) that drains to the spleen may activate both the CD8 α^- and CD8 α^+ DC subsets via a given receptor (e.g. TLR4). CD8 α^- DCs are induced to migrate to the T-cell areas²², but only the CD8 α^+ DCs secrete IL-12 (B. Pulendran, unpublished), resulting in Th1 priming. IFN- γ secreted by the Th1 cells may suppress the Th2-induction potential of CD8 α^- DCs (M. Moser, pers. commun.); thus, the net effect is a Th1 response. (b) In the case of a Th2 adjuvant, both DCs would be activated through a different receptor. This would not induce, or may suppress, IL-12 secretion by the CD8 α^+ DCs, resulting in Th2 priming. IL-10 secreted by Th2 cells may suppress the Th1-inducing potential of CD8 α^+ DCs, the net effect being a Th2 response. Abbreviations: Ag, antigen; DC, dendritic cell; IFN- γ , interferon γ ; IL-12, interleukin 12; LPS, lipopolysaccharide; PALS, periarteriolar lymphoid sheaths; Th, T helper; TLR4, Toll-like receptor 4.

CD11c⁺ DCs generated from CD11c⁺ precursors (Ref. 18). It is conceivable that the induction of IL-10 exerts a regulatory effect on T-cell proliferation, ultimately resulting in T-cell anergy⁵¹. In G-CSF-treated donors, where this CD11c⁺ subset is preferentially increased, this may result in a dampening of T-cell responses. This is consistent with the well-known anti-inflammatory effects of G-CSF in suppressing T-cell proliferation⁵².

Environmental instruction of DCs

The realization that distinct DC subsets can regulate immune responses differently has been accompanied by the equally challenging, but opposing, revelation that DC function can be altered by the microenvironment or by pathogens⁵³. For instance, immature human DCs or murine macrophages treated with IL-10, transforming growth factor β (TGF- β) or prostaglandins have been shown to induce Th2 responses or limit their allostimulatory capacity *in vitro*^{53–56}. However, mature human DCs were not susceptible to modulation⁵³. Thus, immature DCs or macrophages that encounter antigen in an environment enriched in TGF- β or IL-10 (for example, the eye or Peyer's patches), may direct immune responses towards the Th2 pathway, or limit T-cell proliferation.

These *in vitro* studies describing environmental influences on DC function are consistent with several recent studies. In one study, rat respiratory tract DCs (RTDCs), after ovalbumin-pulsing and adoptive transfer, preferentially stimulated Th2

cytokines and isotypes of antibodies⁵⁷. However, pre-culture of these cells with GM-CSF induced production of both Th1 and Th2 responses. A similar study suggests that murine Peyer's patch DCs elicit Th2 responses, while splenic DCs elicit Th1 responses⁵⁸. Paradoxically, sorted CD8 α^+ and CD8 α^- DCs from Peyer's patches induce Th1 and Th2 responses, respectively (A. Iwasaki and B. Kelsall, unpublished); however, the CD8 α^- Peyer's patch DCs induce stronger Th2 responses than the corresponding CD8 α^- DCs in the spleen. This may account for the differences observed with the unsorted DCs in Peyer's patches versus spleen. This, in turn, may hold the key to classical observations correlating the routes of antigen administration with different immunological outcomes. For example, while adjuvants such as LPS or Flt3L are able to enhance immunity and abrogate peripheral tolerance to systemic injections of antigens^{16,59}, they have quite the opposite effect on orally administered antigens^{60,61}.

Emerging evidence suggests that pathogens or their products can also modulate DC function. For instance, DCs derived from the human CD11c⁺ pre-DCs, with IL-3 and CD40L, can induce Th2 responses⁴⁸, but viruses can stimulate IFN- α production from CD11c⁺ pre-DCs (Ref. 62), and induce their maturation into DCs that elicit IFN- γ -producing T cells⁶³. Furthermore, different forms of the fungus *Candida albicans* can instruct an immature, murine cell line to induce either Th1 or Th2 responses, by stimulating the secretion of either IL-12 or IL-4 by the DCs themselves⁶⁴. Finally, our recent work suggests that LPS from two different bacteria can induce different classes of immune responses, probably by differentially activating DCs. Thus, *Escherichia coli* LPS, which signals through the Toll-like receptor 4 (TLR4)⁶⁵, induces Th1 responses, while *Porphyromonas gingivalis* LPS, which is reported to be less dependent on TLR4 signaling⁶⁶, tilts the balance towards Th2 (B. Pulendran, unpublished). Interestingly, *E. coli* LPS, but not *P. gingivalis* LPS, induces IL-12 in the CD8 α^+ DCs (B. Pulendran, unpublished) although both LPSs do activate both sets of DCs.

The question: functional commitment or environmental instruction?

Recent developments reveal a role for distinct DC subsets in differentially directing immune responses *in vivo*. However, these developments should be viewed in the context that DC function is not immutable, but is regulated by its microenvironment and by the pathogen. It is perhaps not surprising that nature has evolved multiple ways to manipulate the immune response; in this case, via both functionally distinct DC subsets and some plasticity in DC function. A system in which DC function was immutable would not allow the flexibility to evolve a

Th response in the course of the changing dynamics of the infection⁶⁸. On the other hand, a system where DC function relied solely on environmental instruction would be too chaotic, and would not permit a rapid deployment of the appropriate class of immunity. The optimal situation would be one where functionally different DC subsets are geographically segregated and poised to mount a given Th response rapidly in response to a given pathogen product. However, as the response evolves, the function of these DCs may be modified either directly by the pathogen itself, or by cytokines released by neighboring cells or T cells.

In the model (Fig. 1), a Th1 adjuvant (e.g. *E. coli* LPS) that reaches the spleen may activate both CD8 α^+ and CD8 α^- DCs by signaling through TLR4. However, it would only induce IL-12 in the CD8 α^+ subset, which would result in Th1 priming. The IFN- γ secreted by the Th1 cells would in turn inhibit the

Th2-inducing potential of the CD8 α^- DCs (M. Moser, pers. commun.). Thus, the net effect would be a Th1 response. In contrast to this scenario, a Th2 adjuvant that reaches the spleen may also activate both DC subsets by signaling through a different TLR, but fail to induce, or suppress, IL-12 induction in the CD8 α^+ subset. This may result in Th2 priming and the resulting IL-10 may inhibit the Th1-inducing potential of CD8 α^+ DCs, thus producing a net Th2 response. In this model, therefore, the final Th response is determined by: (1) the microbial product or adjuvant, (2) the receptor on the DC through which the adjuvant signals, (3) the DC subset itself, (4) the local microenvironment and (5) cytokines released by neighboring T cells and other cells. Knowing which DC subsets induce which responses, and which cytokines mobilize which subsets, should permit us to use these tools to manipulate the immune response in various clinical settings.

Acknowledgements

Supported by grants from NIH DK57655-01 and Baylor Health Care Foundation (BP).

References

- Mosmann, T.R. and Coffman, R.L. (1989) TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* 7, 145–173
- Steinman, R.M. and Cohn, Z.A. (1973) Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. *J. Exp. Med.* 137, 1142–1162
- Banchereau, J. and Steinman, R.M. (1998) Dendritic cells and the control of immunity. *Nature* 392, 245–52
- Shortman, K. *et al.* (1998) The linkage between T-cell and dendritic cell development in the mouse thymus. *Immunol. Rev.* 165, 39–46
- Banchereau, J. *et al.* (2000) Immunobiology of dendritic cells. *Annu. Rev. Immunol.* 18, 767–811
- Ardavin, C. *et al.* (1993) Thymic dendritic cells and T cells develop simultaneously in the thymus from a common precursor population. *Nature* 362, 761–763
- Wu, L. *et al.* (1996) Thymic dendritic cell precursors: relationship to the T lymphocyte lineage and phenotype of the dendritic cell progeny. *J. Exp. Med.* 184, 903–911
- Galy, A. *et al.* (1995) Human T, B, natural killer, and dendritic cells arise from a common bone marrow progenitor cell subset. *Immunity* 3, 459–473
- Inaba, K. *et al.* (1992) Generation of large numbers of dendritic cells from mouse bone marrow cultures supplemented with granulocyte/macrophage colony-stimulating factor. *J. Exp. Med.* 176, 1693–1702
- Caux, C. *et al.* (1992) GM-CSF and TNF- α cooperate in the generation of dendritic Langerhans cells. *Nature* 360, 258–261
- Sallusto, F. and Lanzavecchia, A. (1994) Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor α . *J. Exp. Med.* 179, 1109–1118
- Romani, N. *et al.* (1994) Proliferating dendritic cell progenitors in human blood. *J. Exp. Med.* 180, 83–93
- Maraskovsky, E. *et al.* (1996) Dramatic increase in the numbers of functionally mature dendritic cells in Flt3 ligand-treated mice: multiple dendritic cell subpopulations identified. *J. Exp. Med.* 184, 1953–1962
- Pulendran, B. *et al.* (1997) Developmental pathways of dendritic cells *in vivo*. Distinct function, phenotype, and localization of dendritic cell subsets in Flt3 ligand-treated mice. *J. Immunol.* 159, 2222–2231
- Shurin, M.R. *et al.* (1997) Flt3 ligand induces the generation of functionally active dendritic cells in mice. *Cell. Immunol.* 179, 174–184
- Pulendran, B. *et al.* (1998) Prevention of peripheral tolerance by a dendritic cell growth factor: flt3 ligand as an adjuvant. *J. Exp. Med.* 188, 2075–2082
- Pulendran, B. *et al.* (1999) Distinct dendritic cell subsets differentially regulate the class of immune response *in vivo*. *Proc. Natl. Acad. Sci. U. S. A.* 96, 1036–1041
- Pulendran, B. *et al.* (2000) Flt3-ligand and granulocyte colony-stimulating factor mobilize distinct human dendritic cell subsets *in vivo*. *J. Immunol.* 165, 566–572
- Arpinati, M. *et al.* (2000) Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. *Blood* 95, 2484–2490
- Daro, E. *et al.* (2000) Polyethylene glycol-modified GM-CSF expands CD11b^{high}CD11c^{high} but not CD11b^{low}CD11c^{high} murine dendritic cells *in vivo*. A comparative analysis with Flt3-ligand. *J. Immunol.* 165, 49–58
- Maraskovsky, M. *et al.* (2000) *In vivo* generation of human dendritic cell subsets by flt3 ligand. *Blood* 96, 878–884
- De Smedt, T. *et al.* (1996) Regulation of dendritic cell numbers and maturation by lipopolysaccharide *in vivo*. *J. Exp. Med.* 184, 1413–1424
- Steinman, R.M. *et al.* (1997) Dendritic cells in the T-cell areas of lymphoid organs. *Immunol. Rev.* 156, 25–37
- Leenen, P.J. *et al.* (1998) Heterogeneity of mouse spleen dendritic cells: *in vivo* phagocytic activity, expression of macrophage markers, and subpopulation turnover. *J. Immunol.* 160, 2166–2173
- Kelsall, B. and Strober, W. (1996) Distinct populations of dendritic cells are present in the subepithelial dome and T cell regions of the murine Peyer's patch. *J. Exp. Med.* 183, 237–247
- Vremec, D. and Shortman, K. (1997) Dendritic cell subtypes in mouse lymphoid organs: cross-correlation of surface markers, changes with incubation, and differences among thymus, spleen, and lymph nodes. *J. Immunol.* 159, 565–573
- Anjuere, F. *et al.* (1999) Definition of dendritic cell subpopulations present in the spleen, Peyer's patches, lymph nodes, and skin of the mouse. *Blood* 93, 590–598
- Sousa, C.R. *et al.* (1997) *In vivo* microbial stimulation induces rapid CD40 ligand-independent production of interleukin 12 by dendritic cells and their redistribution to T cell areas. *J. Exp. Med.* 186, 1819–1829
- Salomon, B. *et al.* (1998) Three populations of mouse lymph node dendritic cells with different origins and dynamics. *J. Immunol.* 160, 708–717
- Vremec, D. *et al.* (2000) CD4 and CD8 expression by dendritic cell subtypes in mouse thymus and spleen. *J. Immunol.* 164, 2978–2986
- Strobl, H. *et al.* (1998) Identification of CD68⁺lin[−] peripheral blood cells with dendritic precursor characteristics. *J. Immunol.* 161, 740–748
- Kohrgruber, N. *et al.* (1999) Survival, maturation, and function of CD11c[−] and CD11c⁺ peripheral blood dendritic cells are differentially regulated by cytokines. *J. Immunol.* 163, 3250–3259
- Ito, T. *et al.* (1999) A CD1a⁺/CD11c⁺ subset of human blood dendritic cells is a direct precursor of Langerhans cells. *J. Immunol.* 163, 1409–1419
- Caux, C. *et al.* (1996) CD34⁺ hematopoietic progenitors from human cord blood differentiate along two independent dendritic cell pathways in response to GM-CSF⁺ TNF α . *J. Exp. Med.* 184, 695–706
- Palucka, K.A. *et al.* (1998) Dendritic cells as the terminal stage of monocyte differentiation. *J. Immunol.* 160, 4587–4595
- Randolph, G.J. *et al.* (1998) Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. *Science* 282, 480–483
- Grouard, G. *et al.* (1996) Dendritic cells capable of stimulating T cells in germinal centres. *Nature* 384, 364–367

- 38 Grouard, G. *et al.* (1997) The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J. Exp. Med.* 185, 1101–1111
- 39 Bruno, L. *et al.* (1997) Identification of a committed T cell precursor population in adult human peripheral blood. *J. Exp. Med.* 185, 875–884
- 40 Res, P.C. *et al.* (1999) Expression of pTalpha mRNA in a committed dendritic cell precursor in the human thymus. *Blood* 94, 2647–2657
- 41 Lyman, S.D. and Jacobsen, S.E. (1998) c-kit ligand and Flt3 ligand: stem/progenitor cell factors with overlapping yet distinct activities. *Blood* 91, 1101–1134
- 42 Maldonado-Lopez, R. *et al.* (1999) CD8alpha⁺ and CD8alpha[−] subclasses of dendritic cells direct the development of distinct T helper cells *in vivo*. *J. Exp. Med.* 189, 587–592
- 43 Suss, G. and Shortman, K. (1996) A subclass of dendritic cells kills CD4 T cells via Fas/Fas-ligand-induced apoptosis. *J. Exp. Med.* 183, 1789–1796
- 44 Kronin, V. *et al.* (1996) A subclass of dendritic cells regulates the response of naive CD8 T cells by limiting their IL-2 production. *J. Immunol.* 157, 3819–3827
- 45 Smith, A.L. and de St Groth, B.F. (1999) Antigen-pulsed CD8alpha⁺ dendritic cells generate an immune response after subcutaneous injection without homing to the draining lymph node. *J. Exp. Med.* 189, 593–598
- 46 Ruedl, C. and Bachmann, M.F. (1999) CTL priming by CD8(+) and CD8(-) dendritic cells *in vivo*. *Eur. J. Immunol.* 29, 3762–3767
- 47 Ohteki, T. *et al.* (1999) Interleukin 12-dependent interferon gamma production by CD8alpha⁺ lymphoid dendritic cells. *J. Exp. Med.* 189, 1981–1986
- 48 Risoan, M.C. *et al.* (1999) Reciprocal control of T helper cell and dendritic cell differentiation. *Science* 283, 1183–1186
- 49 Cella, M. *et al.* (1999) Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat. Med.* 5, 919–923
- 50 Tanaka, M. *et al.* (2000) Human monocyte-derived dendritic cells induce naive T cell differentiation into T helper cell type 2 (Th2) or Th1/Th2 effectors. Role of stimulator/responder ratio. *J. Exp. Med.* 192, 405–412
- 51 Groux, H. *et al.* (1996) A CD4⁺ T cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 389, 737–739
- 52 Hartung, T. (1998) Anti-inflammatory effects of granulocyte-colony stimulating factor. *Curr. Opin. Immunol.* 5, 221–230
- 53 Kalinski, P. *et al.* (1999) T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol. Today* 20, 561–567
- 54 Kalinski, P. *et al.* (1998) Prostaglandin E2 induces the final maturation of IL-12-deficient CD1a⁺CD83⁺ dendritic cells: the levels of IL-12 are determined during the final dendritic cell maturation and are resistant to further modulation. *J. Immunol.* 161, 2804–2809
- 55 Steinbrink, K. *et al.* (1997) Induction of tolerance by IL-10-treated dendritic cells. *J. Immunol.* 159, 4772–4780
- 56 Takeuchi, M. *et al.* (1997) On the mechanisms by which transforming growth factor-beta 2 alters antigen-presenting abilities of macrophages on T cell activation. *Eur. J. Immunol.* 27, 1648–1656
- 57 Stumbles, P.A. *et al.* (1998) Resting respiratory tract dendritic cells preferentially stimulate T helper cell type 2 (Th2) responses and require obligatory cytokine signals for induction of Th1 immunity. *J. Exp. Med.* 188, 2019–2031
- 58 Iwasaki, A. and Kelsall, B.L. (1999) Freshly isolated Peyer's patch, but not spleen, dendritic cells produce interleukin 10 and induce the differentiation of T helper type 2 cells. *J. Exp. Med.* 190, 229–239
- 59 Pape, K.A. *et al.* (1997) Inflammatory cytokines enhance the *in vivo* clonal expansion and differentiation of antigen-activated CD4⁺ T cells. *J. Immunol.* 159, 591–598
- 60 Viney, J.L. *et al.* (1998) Expanding dendritic cells *in vivo* enhances the induction of oral tolerance. *J. Immunol.* 160, 5815–5825
- 61 Khoury, S.J. *et al.* (1990) Suppression of experimental autoimmune encephalomyelitis by oral administration of myelin basic protein. III. Synergistic effect of lipopolysaccharide. *Cell. Immunol.* 131, 302–310
- 62 Siegal, F.P. *et al.* (1999) The nature of the principal type 1 interferon-producing cells in human blood. *Science* 284, 1835–1837
- 63 Kadowaki, N. *et al.* (2000) Natural interferon alpha/beta-producing cells link innate and adaptive immunity. *J. Exp. Med.* 192, 219–226
- 64 d'Ostiani, C.F. *et al.* (2000) Dendritic cells discriminate between yeasts and hyphae of the fungus *Candida albicans*. Implications for initiation of T helper cell immunity *in vitro* and *in vivo*. *J. Exp. Med.* 191, 1661–1674
- 65 Poltorak, A. *et al.* (1998) Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282, 2085–2088
- 66 Tanamoto, K. *et al.* (1997) The lipid A moiety of *Porphyromonas gingivalis* lipopolysaccharide specifically mediates the activation of C3H/HeJ mice. *J. Immunol.* 158, 4430–4437
- 67 Doyle, A.G. *et al.* (1999) The activated type 1-polarized CD8(+) T cell population isolated from an effector site contains cells with flexible cytokine profiles. *J. Exp. Med.* 190, 1081–1092
- 68 de Saint-Vis, B. *et al.* (1998) The cytokine profile expressed by human dendritic cells is dependent on cell subtype and mode of activation. *J. Immunol.* 160, 1666–1676
- 69 Dubois, B. *et al.* (1997) Dendritic cells enhance growth and differentiation of CD40-activated B lymphocytes. *J. Exp. Med.* 185, 941–951

The contribution of proteinase inhibitors to immune defense

Peter B. Armstrong

The colonization of a potential host by a parasite requires an ability to cross the integuments and then to escape from the host immune defenses. Proteinases are important virulence factors that assist these processes. Host proteinase inhibitors potentially contribute to immunity by inactivating the proteinase virulence factors of pathogens.

The parasitic life cycle requires the successful execution of several difficult tasks, including invasion across the host integuments, procurement of nutrients for growth and development, and evasion of host immune defenses. Virulence factors

are the unique molecular attributes of the pathogen used to facilitate the colonization and infection of the host and contribute to the production of disease. Proteolytic enzymes are essential virulence factors for prokaryotic and eukaryote parasites during all stages of the infectious process. This review investigates the hypotheses that, by blocking the actions of the proteinases of the infectious cycle, proteinase inhibitors of the host have important roles in limiting parasitic infection. Of particular interest are proteinase inhibitors with a broad reactive