



Dendritic cell lineage, plasticity and cross-regulation

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Dendritic cells (DCs) are professional antigen-presenting cells that have an extraordinary capacity to stimulate naïve T cells and initiate primary immune responses. Here we review progress in understanding the additional functions of DCs in regulating the types of T cell-mediated immune responses and innate immunity to microbes. In addition, evidence for the existence of myeloid and lymphoid DC lineages and their different functions are summarized. We propose that the diverse functions of DCs in immune regulation are dictated by the instructions they received during innate immune responses to different pathogens and from their evolutionary lineage heritage.

Dendritic cells (DCs) are professional antigen-presenting cells within the immune system. They are continuously produced from hematopoietic stem cells in the bone marrow and are widely distributed, as immature DCs, into both lymphoid and nonlymphoid tissues^{1–4}. Immature DCs, including epidermal Langerhan's cells, splenic marginal zone DCs and interstitial DCs within nonlymphoid tissues, continuously sample self-antigen to maintain T cell self-tolerance¹. Immature DCs can also take up foreign antigens. When triggered by pathogens, the pattern-recognition receptors expressed by immature DCs cause them to mature to immunogenic DCs. These mature DCs can initiate primary T cell-mediated immune responses because they express high amounts of cell surface major histocompatibility complexes (MHC) and costimulatory molecules^{1–4}. Some studies suggest that DCs have the capacity to induce different types of T cell-mediated immune responses, depending on their lineage, maturation stage and activation signals.

T_H1- and T_H2-inducing DC subsets

The first experimental evidence suggesting that DCs may direct the type of T cell-mediated immune response came from the observation that DCs could produce the T helper subset 1 (T_H1)-polarizing cytokine interleukin 12 (IL-12)^{5–9}. The question was: if all DCs produce IL-12, how could the T_H2 response ever be induced? One explanation is that DCs are heterogeneous and not all DCs have the same capacity to produce IL-12. In human blood there are two distinct types of DC precursor: these are myeloid monocytes (pre-DC1s) and plasmacytoid DC precursors (pre-DC2s)⁹. In mouse spleen, CD8⁺ “lymphoid” and CD8[–] “myeloid” DC subsets have been identified^{10,11}. Whereas CD40 ligand (CD40L)-activated myeloid DC1s derived from monocytes produce a large amount of

IL-12 and preferentially induce T_H1 development, CD40L-activated lymphoid DC2s derived from plasmacytoid precursors produce lower amounts of IL-12 and preferentially induce T_H2 development^{9,12,13}. Paradoxically, opposite results were observed in mice. Whereas CD8⁺ splenic “lymphoid” DCs produce large amounts of IL-12 and induce T_H1 responses, CD8[–] “myeloid” DCs produce lower amounts of IL-12 and preferentially induce T_H2 responses. Although humans and mice are opposites in terms of the functional classification of DC subsets, both studies showed the existence of a high IL-12-producing DC subset that induces T_H1 responses and a low IL-12-producing subset that induces T_H2 responses (Fig. 1). However, the concept of different DC lineage and function was challenged by the findings that a given DC subset has a remarkable plasticity in directing different types of T cell responses¹⁴.

The functional plasticity of the DC system

In humans, DCs showed different effector functions, which depended on multiple factors, in directing T cell responses (Fig. 1). Whereas DC1s at a mature stage induce T_H1 differentiation and strong cytotoxic T lymphocyte (CTL) responses⁹, DC1s at an immature stage induce IL-10-producing CD4⁺ and CD8⁺ regulatory T cells^{15–17}. Pro-inflammatory and anti-inflammatory factors also affect DC function. Immature DC1s derived from monocytes after 5–7 days of culture with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 induce both T_H1 and T_H2 differentiation¹⁴. Two groups of signals stimulate immature DC1s to induce T_H1 differentiation: (i) lipopolysaccharide (LPS) derived from Gram-negative and Gram-positive bacteria *Staphylococcus aureus* Cowan's antigen (SAC)¹⁴, unmethylated bacterial CpG-containing oligonucleotides^{18–21} and double-stranded viral RNA²² and (ii) T cell signals such as CD40L⁹ and interferon γ (IFN- γ)¹⁴. Many signals stimulate immature DC1s to induce T_H2 differentiation or to inhibit T_H1 differentiation; these include anti-inflammatory molecules such as IL-10, transforming growth factor- β (TGF- β), prostaglandin E₂ (PGE₂) and steroids^{14,23} and OX40 ligand cosignaling^{24,25}. DC2s also show different effector functions depending on the type of differentiation factor. Whereas pre-DC2s cultured with IL-3 preferentially promote T_H2 differentiation, pre-DC2s activated by virus prime naïve T cells to produce IFN- γ and IL-10^{26,27}. Other factors that affect DC effector function include the DC:T cell ratio and the duration of DC activation. In cultures, DC1s induce T_H1 development at high DC:T cell ratios and T_H2 development at low DC:T cell ratios²⁸. Early-activated DCs produce large amounts of IL-12 and induce T_H1 responses. However, DCs that are “exhausted” or “paralyzed”, as a result of prolonged activation, lose the ability to produce IL-12 and preferentially induce T_H2 responses^{29–31}.

In mice, the functional plasticity of DCs is dependent on the type of pathogen (Fig. 1) and on the tissue microenvironment. At the yeast stage, the fungus *Candida albicans* stimulates DCs to produce IL-12 and induce T_H1 responses. However, at the hyphae stage, *C. albicans* stimulates DCs to produce IL-4 and induce T_H2 responses³². LPS from

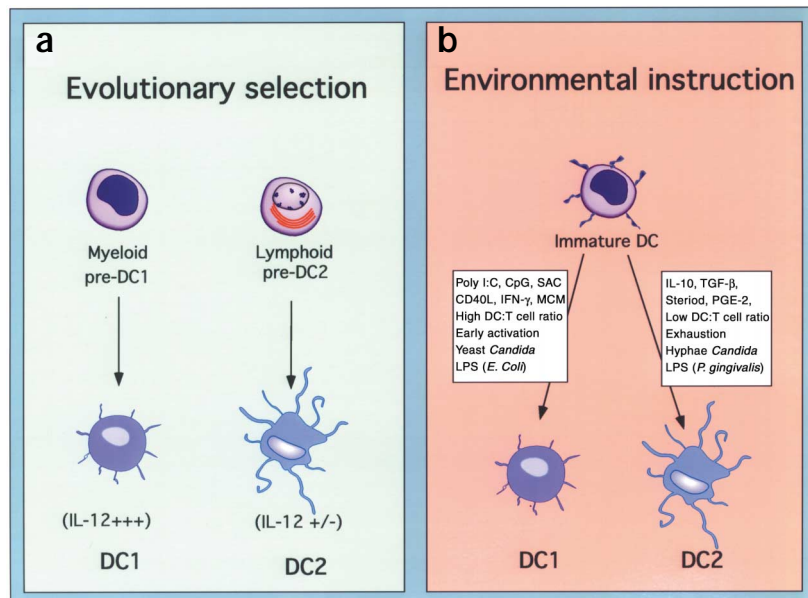


Figure 1. Regulation of T cell-mediated immune responses by DCs. (a) Evolutionary selection: two DC subsets develop via a myeloid and lymphoid pathways. These two DC subsets express different sets of pattern-recognition receptors in order to recognize different antigens. Myeloid DC1s produce a large amount of IL-12 during cognate T cell–DC interaction and preferentially induce T_H1 differentiation. Lymphoid DC2s produce a lower amount of IL-12 during cognate T cell–DC interaction and preferentially induce T_H2 differentiation. (b) Environmental instruction: each DC subset has a certain degree of flexibility in directing T cell responses. This depends on signals from pathogens and the microenvironment. MCM, monocyte conditional medium.

Escherichia coli stimulates $CD8^+$ DCs to produce IL-12 and induces a T_H1 response. However, LPS from *Porphyromonas gingivalis* does not stimulate $CD8^+$ DCs to produce IL-12 and preferentially induces a T_H2 response^{33,34}. In addition, DCs activated by antigen from a nematode worm induce a T_H2 response³⁵. In terms of tissue origin, DCs isolated from Peyer's patches³⁶, respiratory tracts³⁷ and liver³⁸ preferentially induce T_H2 differentiation. In contrast, $CD11c^+$ DCs isolated from the spleen preferentially induce T_H1 differentiation. The functional differences among different tissue DCs may result from differences in the tissue cytokine microenvironment and exposure to particular pathogens, as well as from the lineage origin of different tissue DCs.

Evidence for a lymphoid DC lineage

A lymphoid DC lineage was first proposed when it was shown that the earliest T cell precursors in mouse thymus could give rise to T cells, B cells, natural killer (NK) cells and DCs but not to other cell types³⁹. A B lymphoid-related DC lineage was later proposed when it was shown that mouse $CD19^+$ pro-B cells have the potential to differentiate into DCs in cultures⁴⁰. Interestingly, thymic DCs express CD8; this observation led to the discovery of $CD8^+$ and $CD8^-$ splenic DC subsets in mice^{41,42}. A kinetic population study of splenic DC development by continuous 5-bromodeoxyuridine (BrdU)-labeling experiments showed that both DC subsets can develop independently and do not represent two maturation stages of one DC subset^{43,44}. Because thymic lymphoid DCs express CD8, it was proposed that splenic $CD8^+$ and $CD8^-$ DCs represent lymphoid and myeloid subsets, respectively^{41,42}. This classification became even more attractive after the discovery that $CD8^+$ and $CD8^-$ DC subsets induce T_H1 versus T_H2 responses, respectively^{10,11}.

However, new studies suggest that CD8 is not a lymphoid marker for peripheral DCs. This is because: (i) the thymus is not the origin of splenic

$CD8^+$ DCs⁴⁴; (ii) purified myeloid progenitors can give rise to $CD8^+$ DCs in mice upon adoptive transfer⁴⁵; and (iii) myeloid DCs can be induced to express CD8 *in vitro* by activation⁴⁶. Although CD8 cannot be used as a lymphoid DC marker for peripheral DCs, the evidence for a $CD8^+$ lymphoid DC lineage within the thymus is substantial and CD8 is a valuable marker for the separation of two functionally different DC subsets.

In humans, a lymphoid DC lineage was suggested by the characterization of a unique DC precursor called a plasmacytoid T cell⁴⁷, plasmacytoid monocyte⁴⁸ or a $CD4^+CD11c^-$ pre-DC^{49–57}. Unlike monocytes that differentiate into immature DC1s in response to GM-CSF and IL-4^{58,59}, the differentiation of pre-DCs into immature DC2s requires IL-3^{49–57} or virus²⁶. Several lines of evidence suggest that pre-DC2s are lymphoid in origin. Pre-DC2s lack expression of the myeloid antigens CD11c, CD13 and CD33 and mannose receptors^{49–57} and express the lymphoid markers CD2, CD5 and CD7^{49–57} as well as pre-T cell receptor α chain transcripts⁵⁷. In addition, they show little phagocytic activity and do not differentiate into macrophages after culture with GM-CSF and M-CSF⁵⁹. Finally, development of pre-DC2, T and B cells, but not myeloid DCs, is blocked by ectopic expression of Id2 or Id3⁵⁷.

DC1 and DC2 invariant receptors

Although the experimental evidence for a lymphoid lineage is substantial, one question remains: why does the immune system require more than one DC lineage or

subset when one alone is capable of generating all kinds of appropriate T cell responses, depending on the pathogen type?

The evolutionary advantage of the subdivision of a cell type within the immune system is that it recognizes and presents antigens from pathogens and injured host cells more effectively. For example—unlike conventional B and T cells—B1, $\gamma\delta$ T and NKT cells express restricted and distinct antigen receptors that are capable of recognizing common antigens from bacteria or damaged host cells presented by non-MHC class I or class II antigens⁶⁰.

If myeloid and lymphoid DCs really exist and perform different functions, they should express different evolutionary traits that are manifested in the pattern-recognition and presentation receptors. Indeed, human myeloid pre-DC1s, but not lymphoid pre-DC2s, express toll-like receptor 2 (TLR2) and TLR4. In contrast, lymphoid pre-DC2s, but not myeloid pre-DC1s, express high amounts of TLR7 and TLR9 (N. Kadowaki *et al.*, unpublished data). In accordance with their receptor expression, pre-DC1s rapidly produce large amounts of pro-inflammatory cytokines such as TNF- α , IL-6 and IL-12 in responses to TLR2 and TLR4 ligands such as glycoproteins from mycobacteria and Gram-positive bacteria^{61–64}. In contrast, pre-DC2s do not respond to bacterial TLR2 and TLR4 ligands. Pre-DC2s produce type 1 IFN and differentiate into DCs in response to TLR9 ligand and bacterial DNA rich in unmethylated CpG motifs⁶⁵.

In addition, pre-DC1s and pre-DC2s express different lectin molecules. DC1s but not DC2s express high amounts of mannose receptors and can rapidly uptake large amounts of polysaccharide antigens such as fluorescein isothiocyanate–dextran⁴⁹. In contrast, DC2s specifically express a unique lectin recognized by the monoclonal antibody BDCA2^{66,67}. Pre-DC2s can uptake antigen *via* BDCA2 and process and present antigen to T cells. Binding of antibodies to BDCA2 inhibits pre-

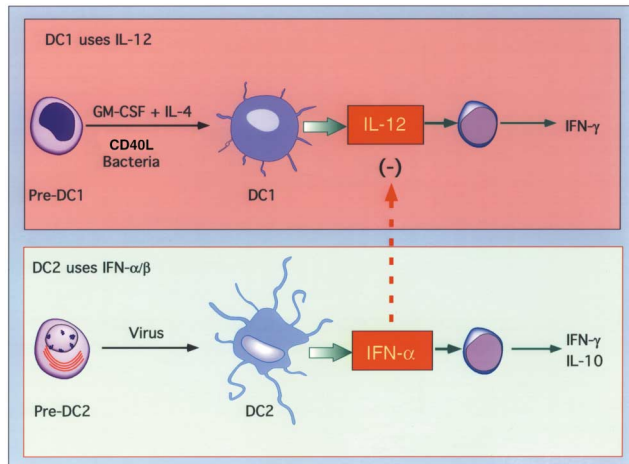


Figure 2. Two independent DC systems induce T cells to produce IFN- γ in humans. DC1s use IL-12, DC2s use IFN- α and IFN- β . IFN- α and IFN- β produced by DC2s can strongly inhibit IL-12 production by DC1s.

DC2 production of type 1 IFN induced by influenza virus⁶⁷. Unlike DC2s, DC1s express MHC class I-like molecules such as CD1a,b,c and d. This indicates that, through CD1, DC1s are able to present lipid antigens to T cells. Indeed, DC1s, but not DC2s, were able to present α -galactoceramide to NKT cells *via* CD1d⁵⁶.

In addition to the expression of different sets of receptors, lymphoid DC2s show poor phagocytosis and macropinocytosis at all maturation stages compared to myeloid DC1s⁴⁹. This suggests that human myeloid DC1s may recognize and present a much broader range of antigens or pathogens. Myeloid DC1s are located at sites of pathogen entry such as skin and mucosal tissues; in contrast, under normal physiological conditions lymphoid DC2s are mainly located within the T cell areas of lymphoid tissues⁴⁹. This suggests that, although lymphoid DC2s can be rapidly recruited to the site of antigen entry, they are not the first to respond to microbial invasion *via* the body surface. Lymphoid DC2s, therefore, may be specialized to recognize self-antigens or blood-borne pathogens.

These data suggest that human myeloid DC1 and lymphoid DC2 lineages have evolved to preferentially recognize different antigens or pathogens and may activate different T cell-mediated immune responses, as appropriate. The expression of invariant receptors to recognize and present different microbial antigens by mouse CD8⁺ and CD8⁻ DC subsets is less well studied. To date, it has been shown that CD8⁺, but not CD8⁻, DCs express CD1d and lectin DEC-205⁴².

The lymphoid DC2 response

Studies have shown that human lymphoid DC2s, isolated by expression of two immunoglobulin-like receptors (ILT3⁺ILT1⁻), produced large amounts of IL-12 in response to LPS or CD40L^{27,68}. This contrasts with the observation that ILT3⁺ILT1⁻ pre-DC2s produce more than 800 pg/ml of IL-12 p75 in response to LPS^{27,68}. Because 10–30% of ILT3⁺ILT1⁻ pre-DC2s do not express high amounts of IL-3R α ^{27,68}, a key marker for human pre-DC2s^{9,50}, their response to LPS might be due to contaminating myeloid DCs or macrophages^{27,68}.

To determine whether the inferior ability of DC2s to produce IL-12 was due to “DC exhaustion” after prolonged activation by CD40L, IL-12 production by DC1s and DC2s from the same donor was measured every 6 h over a 48-h period of activation by CD40L. DC1s rapidly produced IL-12 after 12 h (86 pg/ml) of CD40L activation and peaked at

24 h (1256 pg/ml). After 24 h, the ability of DC1s to produce IL-12 was greatly reduced and could not be boosted by a second round of CD40L stimulation. In contrast, DC2s produced a maximum of 5.6 pg/ml of IL-12 at all time-points after CD40L stimulation¹⁷. This study shows that in comparison to DC1s, the inability of DC2s to produce a large amount of IL-12 is due to an intrinsic property of DC2s as opposed to “DC exhaustion”.

Pre-DC2s are natural type 1 IFN-producing cells

Although DC2s have an inferior capacity to produce IL-12 compared with DC1s, human lymphoid pre-DC2s have a remarkable ability to rapidly produce large amounts of type-1 IFN in response to viruses and bacteria^{68,69}. These cells represent the natural type 1 IFN-producing cells (IPCs)⁷⁰. The identification of mouse pre-DC2 as IPCs is based on their capacity to produce large amounts of IFN- α in response to viral activation^{71,72}. Surprisingly, mouse IPCs express CD11c, Gr-1 and B220. This subset of mouse DCs appears to be different from CD8⁺ and CD8⁻ DC subsets. The identification of mouse IPCs will permit the *in vivo* analysis of IPC lineage development and the function of these cells.

Cytokine induction of IFN- γ in DC1s and DC2s

After producing large amounts of type 1 IFN in response to viral stimulation, IPCs differentiate into DCs. This differentiation is mediated by type 1 IFNs that maintain the survival of pre-DC2s and by TNF- α , which induces pre-DC2 differentiation to DC2s in an autocrine manner²⁶. Unlike IL-3-induced DC2s, which promote T_H2 differentiation, virus-induced DC2s induce naïve CD4⁺ T cells to produce large amounts of IFN- γ and IL-10. IFN- γ production by CD4⁺ T cells is dependent on IFN- α and IFN- β produced by virus-induced DC2s but is independent of IL-12²⁶. This confirms data showing that human type 1 IFN can promote IFN- γ production in an IL-12-independent manner⁷³. Therefore, there are two independent antigen-presenting cell types that induce CD4⁺ T cells to produce IFN- γ : DC1s, which produce IL-12, and DC2s, which produce type 1 IFN (Fig. 2). However, a key difference between the two systems is that whereas IL-12-producing DC1s stimulate CD4⁺ T cells to produce IFN- γ , IFN- α -producing DC2s stimulate CD4⁺ T cells to produce both IFN- γ and IL-10²⁶ (Fig. 2).

Unlike in humans, type 1 IFN is not capable of inducing mouse CD4⁺ T cells to produce IFN- γ ^{73,74}. This is because of a mutation in signal transducers and activators of transcription 2 (STAT2) that results in a selective loss of type 1 IFN-induced STAT4 activation in mice⁷³. However, type 1 IFN can promote IFN- γ production by mouse CD8⁺ T cells during viral infection *in vivo*, possibly *via* a STAT4-independent mechanism⁷⁵.

DC2 and DC1 cross-inhibition

The inhibitory effects of IFN- α and IFN- β on IL-12 production were originally discovered in the mouse system⁷⁶. Type 1 IFNs strongly inhibit IL-12 production by mouse splenic cells induced by SAC *in vitro*. In addition, type 1 IFNs induced by lymphocytic choriomeningitis virus (LCMV) infection strongly inhibit IL-12 production *in vivo* after stimulation with LPS. Anti-type 1 IFN up-regulates IL-12 production in LCMV-infected mice⁷⁶. It is probable that type 1 IFNs directly inhibit IL-12 production by mouse CD8⁺ DCs. In humans, the biological effects of type 1 IFN are complicated. Type 1 IFNs inhibit IL-12 production *via* CD40L-activated monocyte-derived DC1s⁷⁷. The ability of type 1 IFNs to down-regulate IL-12 may be responsible for the therapeutic value of using IFN- β to treat multiple sclerosis. However, one study shows that type-1 IFN in the sera of many systemic lupus erythematosus (SLE) patients can strongly induce monocytes to differentiate into DCs, which

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potently activate both T and B lymphocytes⁷⁸. The ability of IFN- α to activate monocytes and DCs may be due to cofactors such as apoptotic cells, nucleosome-antibody complexes and other inflammatory factors within the SLE sera.

Negative-feedback regulation of T_H2 responses

A common feature of T cell cytokine-mediated T_H cell differentiation is the positive autocrine effect. For example, IL-2 promotes differentiation of IL-2-producing T_H0 cells, IL-4 promotes differentiation of IL-4-producing T_H2 cells, IFN- γ promotes differentiation of IFN- γ -producing T_H1 cells, IL-10 promotes differentiation of IL-10-producing regulatory CD4⁺ T cells⁷⁹ and TGF- β induces TGF- β -producing T_H3 cells⁸⁰. Because negative-feedback regulation represents a general mechanism used to maintain homeostasis of all physiological processes, the immune system probably uses a negative-feedback mechanism to balance T_H1 and T_H2 responses.

IL-4, a prototype T_H2 cytokine, kills T_H2 -promoting DC2s cultured with IL-3^{9,53}. In contrast, IL-4 shows two positive effects on T_H1 -promoting DC1s: (i) when cultured with GM-CSF, IL-4 promotes DC1 differentiation^{58,59} and (ii) in response to microbial stimuli and CD40L, IL-4 primes DC1s to produce large amounts of IL-12 p75^{81,82}. These data suggest that positive- as well as negative-feedback regulation of T_H1 - T_H2 responses operate within DCs (Fig. 3). Several disease models in rodents support the theory that such feedback regulation of IL-4 may operate *in vivo*. In experimental autoimmune uveoretinitis in rats, IL-4 promotes IFN- γ production and aggravates the disease⁸³. In addition, IL-4-deficient mice cannot mount a T_H1 response to *C. albicans* at the late stage of infection⁸⁴ and IL-4 exacerbates disease in a T_H1 cell-transfer model of colitis⁸⁵.

Summary and future perspectives

The remarkable functional plasticity of a given DC subset endows the immune system with the flexibility to mount appropriate T cell responses in response to the invading pathogens. Although the expression of a limited set of pattern-recognition receptors by a particular DC subset may restrict its flexibility, it confers DCs with an evolutionary memory that allows them to respond to a particular pathogen more rapidly and efficiently. Therefore, the type of T cell-mediated immune response to a particular pathogen or antigen may be dictated by its lineage origin and the innate response by a DC to that particular pathogen or antigen.

The finding that CD8 is not a reliable lymphoid marker for mouse DCs, together with the identification of mouse IPCs, may explain the disparate lineage and function data obtained from human and mouse DC studies. The discovery that human IPCs do not express TLR4 and do not respond to LPS may explain differing opinions on the ability of pre-DC2s or DC2s to produce IL-12 and also emphasizes the importance of ensuring cell purity when studies are carried out.

One of the key areas in DC research will be to determine the expression and function of pattern-recognition receptors on DCs. Following the completion of the human genome project, ten human TLRs were identified and now three key questions need to be addressed. What are the internal ligands as well as the microbial ligands for all the TLRs? How does each TLR instruct DCs to initiate appropriate innate and adaptive immune responses? How do receptor expression patterns explain the different cytokine production profiles of DC subsets?

Pre-DC2s are the professional type 1 IFN-producing cells in antiviral innate immunity; we believe this justifies the designation of these cells as

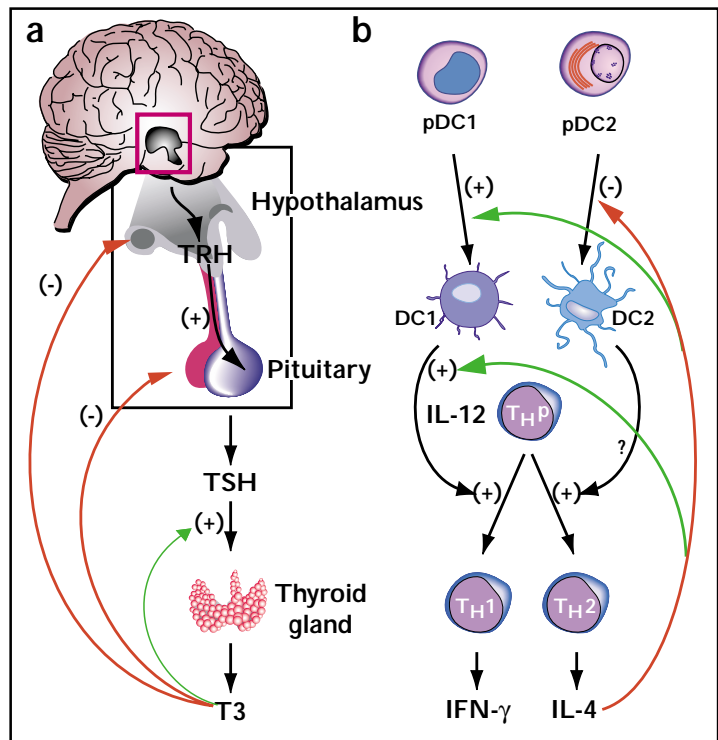


Figure 3. Negative-feedback regulation via the neuron-endocrine and the immune systems. (a) Thyroid hormone inhibits its own secretion by inhibiting secretion of two hormones: thyroid-stimulating hormone (TSH) from the pituitary gland and thyroid-releasing hormone (TRH) from the hypothalamus. (b) The T_H2 cytokine, IL-4, inhibits T_H2 development by promoting DC1 maturation and IL-12 production and blocking DC2 development by inducing apoptosis of pre-DC2s. T_H1 , T helper precursor cell.

a new member of the hematopoietic cell family. However, several important questions concerning the basic biology of pre-DC2s remain. What, in addition to the Fms-like tyrosine kinase type 3 ligand (FLT-3 ligand), regulates the developmental of pre-DC2s from hematopoietic stem cells⁸⁶? What are the molecular mechanisms that underlie robust IFN- α production by pre-DC2s in response to viruses and bacteria? Do pre-DC2s play a critical role in controlling viral infections *in vivo*, in particular in response to HIV and hepatitis C virus (HCV) *in vivo*?

In response to IL-3 or viral stimulation *in vitro*, pre-DC2s can differentiate into DC2s and differently regulate T cell-mediated immune responses. Two studies provided indirect evidence that DC2s might be involved in the inhibition of graft-versus-host disease induced by G-CSF-immobilized blood transplantation¹³ and in allergen-induced rhinitis⁸⁷. Further research must be done to determine the function and fate of pre-DC2 and/or IPCs *in vivo* when there is no immune response and during immune responses to microbial antigens, allergens or alloantigens. In addition, the molecular mechanisms by which mature DC2s induce IL-4- and IL-10-producing T cells must be determined.

DCs can change their effector functions depending on their maturation stages and activation signals from pathogens and cytokines. The new challenge will be to determine how to manipulate the effector functions of DCs *in vivo* and to provide more effective vaccines for treating tumors, infectious diseases and autoimmune diseases.

1. Banchereau, J. & Steinman, R. M. Dendritic cells and the control of immunity. *Nature* **392**, 245–252 (1998).
2. Cella, M., Sallusto, F. & Lanzavecchia, A. Origin, maturation and antigen presenting function of dendritic cells. *Curr. Opin. Immunol.* **9**, 10–16 (1997).



3. Reis e Sousa, C., Sher, A. & Kaye, P. The role of dendritic cells in the induction and regulation of immunity to microbial infection. *Curr. Opin. Immunol.* **11**, 392–399 (1999).
4. Moser, M. & Murphy, K. M. Dendritic cell regulation of T_H1 - T_H2 development. *Nature Immunol.* **1**, 199–205 (2001).
5. Macatonia, S. E. et al. Dendritic cells produce IL-12 and direct the development of T_H1 cells from naive $CD4^+$ T cells. *J. Immunol.* **154**, 5071–5079 (1995).
6. Cella, M. et al. Ligation of CD40 on dendritic cells triggers production of high levels of interleukin-12 and enhances T cell stimulatory capacity: T-T help via APC activation. *J. Exp. Med.* **184**, 747–752 (1996).
7. Koch, F. et al. High level IL-12 production by murine dendritic cells: upregulation by MHC class II and CD40 molecules and downregulation by IL-4 and IL-10. *J. Exp. Med.* **184**, 741–746 (1996).
8. Sousa, C. R. et al. 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 (1997).
9. Rissoan, M.-C. et al. Reciprocal control of T helper cell and dendritic cell differentiation. *Science* **283**, 1183–1186 (1999).
10. Pulendran, B. et al. Distinct dendritic cell subsets differentially regulate the class of immune response in vivo. *Proc. Natl Acad. Sci. USA* **96**, 1036–1041 (1999).
11. Maldonado-Lopez, R. et al. CD8 α - and CD8 α - subclasses of dendritic cells direct the development of distinct T helper cells in vivo. *J. Exp. Med.* **189**, 587–592 (1999).
12. Ito, T. et al. Differential regulation of human blood dendritic cell subsets by IFNs. *J. Immunol.* **166**, 2961–2969 (2001).
13. Arpinati, M., Green, C. L., Heimfeld, S., Heuser, J. E. & Anasetti, C. Granulocyte-colony stimulating factor mobilizes T helper 2-inducing dendritic cells. *Blood* **95**, 2484–2490 (2000).
14. Kalinski, P., Hilkens, C. M., Wierenga, E. A. & Kapsenberg, M. L. T-cell priming by type-1 and type-2 polarized dendritic cells: the concept of a third signal. *Immunol. Today* **20**, 561–567 (1999).
15. Jonuleit, H., Schmitt, E., Schuler, G., Knop, J. & Enk, A. H. Induction of interleukin 10-producing, nonproliferating $CD4^+$ T cells with regulatory properties by repetitive stimulation with allogeneic immature human dendritic cells. *J. Exp. Med.* **192**, 1213–1222 (2000).
16. Dhodapkar, M. V., Steinman, R. M., Krasovsky, J., Munz, C. & Bhardwaj, N. Antigen-specific inhibition of effector T cell function in humans after injection of immature dendritic cells. *J. Exp. med.* **193**, 233–238 (2001).
17. Gilliet, M. & Liu, Y.-J. Generating IL-10-producing CD8 $^+$ T suppressor cells by DC2. *Nature Immunol.* (submitted, 2001).
18. Hartmann, G., Weiner, G. J. & Krieg, A. M. CpG DNA: a potent signal for growth, activation, and maturation of human dendritic cells. *Proc. Natl Acad. Sci. USA* **96**, 9305–9310 (1999).
19. Jakob, T. et al. Bacterial DNA and CpG-containing oligodeoxynucleotides activate cutaneous dendritic cells and induce IL-12 production: implications for the augmentation of T_H1 responses. *Int. Arch. Allergy Immunol.* **118**, 457–461 (1999).
20. Sparwasser, T. et al. Bacterial DNA and immunostimulatory CpG oligonucleotides trigger maturation and activation of murine dendritic cells. *Eur. J. Immunol.* **28**, 2045–2054 (1998).
21. Jakob, T., Walker, P. S., Krieg, A. M., Udey, M. C. & Vogel, J. C. Activation of cutaneous dendritic cells by CpG-containing oligodeoxynucleotides: a role for dendritic cells in the augmentation of T_H1 responses by immunostimulatory DNA. *J. Immunol.* **161**, 3042–3049 (1998).
22. Verdijk, R. M. et al. Polyribonucleoside polyriboctydidic acid (poly(I:C)) induces stable maturation of functionally active human dendritic cells. *J. Immunol.* **163**, 57–61 (1999).
23. King, C. et al. TGF- β 1 alters APC preference, polarizing islet antigen responses toward a T_H2 phenotype. *Immunity* **8**, 601–613 (1998).
24. Akiba, H. et al. Critical contribution of OX40 ligand to T helper cell type 2 differentiation in experimental leishmaniasis. *J. Exp. Med.* **191**, 375–380 (2000).
25. Delespesse, G., Ohshima, Y., Yang, L. P., Demeure, C. & Sarfati, M. OX40-Mediated cosignal enhances the maturation of naive human $CD4^+$ T cells into high IL-4-producing effectors. *Int. Arch. Allergy Immunol.* **118**, 384–386 (1999).
26. Kadowaki, N., Antonenko, S., Lau, J. Y. & Liu, Y. J. Natural interferon α/β -producing cells link innate and adaptive immunity. *J. Exp. Med.* **192**, 219–226 (2000).
27. Cella, M., Facchetti, F., Lanzavecchia, A. & Colonna, M. Plasmacytoid dendritic cells activated by influenza virus and CD40 ligand drive a potent T_H1 polarization. *Nature Immunol.* **5**, 919–923 (2001).
28. Tanaka, H., Demeure, C. E., Rubio, M., Delespesse, G. & Sarfati, M. Human monocyte-derived dendritic cells induce naive T cell differentiation into T helper cell type 2 (T_H2) or T_H1/T_H2 effectors. Role of stimulator/responder ratio. *J. Exp. Med.* **192**, 405–412 (2000).
29. Kalinski, P., Schuttmaker, J. H., Hilkens, C. M., Wierenga, E. A. & Kapsenberg, M. L. Final maturation of dendritic cells is associated with impaired responsiveness to IFN- γ and bacterial IL-12 inducers: decreased ability of mature dendritic cells to produce IL-12 during the interaction with Th cells. *J. Immunol.* **162**, 3231–3236 (1999).
30. Reis e Sousa, C. et al. Paralysis of dendritic cell IL-12 production by microbial products prevents infection-induced immunopathology. *Immunity* **11**, 637–647 (1999).
31. Langenkamp, A., Messi, M., Lanzavecchia, A. & Sallusto, F. Kinetics of dendritic cell activation: impact on priming T_H1 , T_H2 and nonpolarized T cells. *Nature Immunol.* **1**, 311–316 (2001).
32. d'Ostiani, C. F. et al. 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 (2000).
33. Pulendran, B., Maraskovsky, E., Banchereau, J. & Maliszewski, C. R. Modulating the immune response with dendritic cells and their growth factors. *Trends Immunol.* **22**, 41–47 (2001).
34. Hirschfeld, M. et al. Signaling by toll-like receptor 2 and 4 agonists results in differential gene expression in murine macrophages. *Infect. Immun.* **69**, 1477–1482 (2001).
35. Whelan, M. et al. A filarial nematode-secreted product signals dendritic cells to acquire a phenotype that drives development of T_H2 cells. *J. Immunol.* **164**, 6453–6460 (2000).
36. Iwasaki, A. & Kelsall, B. L. 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 (1999).
37. Stumbles, P. A. et al. Resting respiratory tract dendritic cells preferentially stimulate T helper cell type 2 (T_H2) responses and require obligatory cytokine signals for induction of T_H1 immunity. *J. Exp. Med.* **188**, 2019–2031 (1998).
38. Khanna, A. et al. Effects of liver-derived dendritic cell progenitors on T_H1 - and T_H2 -like cytokine responses *in vitro* and *in vivo*. *J. Immunol.* **164**, 1346–1354 (2000).
39. Ardavin, C., Wu, L., Li, C. L. & Shortman, K. Thymic dendritic cells and T cells develop simultaneously in the thymus from a common precursor population. *Nature* **362**, 761–763 (1993).
40. Björck, P. & Kincaid, P. W. $CD19^+$ pro-B cells can give rise to dendritic cells *in vitro*. *J. Immunol.* **161**, 5795–5799 (1998).
41. Vremec, D. & Shortman, K. 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 (1997).
42. Pulendran, B. et al. 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 (1997).
43. Kamath, A. T. et al. The development, maturation, and turnover rate of mouse spleen dendritic cell populations. *J. Immunol.* **165**, 6762–6770 (2000).
44. Shortman, K. Burnet oration: dendritic cells: multiple subtypes, multiple origins, multiple functions. *Immunol. Cell. Biol.* **78**, 161–165 (2000).
45. Traver, D. et al. Development of CD8 α -positive dendritic cells from a common myeloid progenitor. *Science* **290**, 2152–2154 (2000).
46. Brasel, K., De Smedt, T., Smith, J. L. & Maliszewski, C. R. Generation of murine dendritic cells from flt3-ligand-supplemented bone marrow cultures. *Blood* **96**, 3029–3039 (2000).
47. Lennert, K., Kaiserling, E. & Muller-Hermelink, H. K. T-associated plasma-cells. *Lancet* **1**, 1031–1032 (1975).
48. Facchetti, F. et al. Plasmacytoid T cells. Immunohistochemical evidence for their monocyte/macrophage origin. *Am. J. Pathol.* **133**, 15–21 (1988).
49. Grouard, G. et al. The enigmatic plasmacytoid T cells develop into dendritic cells with interleukin (IL)-3 and CD40-ligand. *J. Exp. Med.* **185**, 1101–1111 (1997).
50. Olweus, J. et al. Dendritic cell ontogeny: a human dendritic cell lineage of myeloid origin. *Proc. Natl Acad. Sci. USA* **94**, 12551–12556 (1997).
51. O'Doherty, U. et al. Human blood contains two subsets of dendritic cells, one immunologically mature and the other immature. *Immunology* **82**, 487–493 (1994).
52. Strobl, H. et al. Identification of CD68 $^{lin-}$ peripheral blood cells with dendritic precursor characteristics. *J. Immunol.* **161**, 740–748 (1998).
53. Kohrgruber, N. et al. Survival, maturation, and function of CD11c- and CD11c $^{+}$ peripheral blood dendritic cells are differentially regulated by cytokines. *J. Immunol.* **163**, 3250–3259 (1999).
54. Sorg, R. V., Kogler, G. & Wernet, P. Identification of cord blood dendritic cells as an immature CD11c population. *Blood* **93**, 2302–2307 (1999).
55. Res, P. C., Couwenberg, F., Vyth-Dreese, F. A. & Spits, H. Expression of pT α mRNA in a committed dendritic cell precursor in the human thymus. *Blood* **94**, 2647–2657 (1999).
56. Kadowaki, N. et al. Distinct cytokine profiles of neonatal natural killer T cells after expansion with subsets of dendritic cells. *J. Exp. Med.* **193**, 1213–1220 (2001).
57. Spits, H., Couwenberg, F., Bakker, A. Q., Weijer, K. & Uttenbogaart, C. H. Id2 and Id3 inhibit development of CD34 $^{+}$ stem cells into predendritic cell (pre-DC)2 but not into pre-DC1. Evidence for a lymphoid origin of pre-DC2. *J. Exp. Med.* **192**, 1775–1784 (2000).
58. Sallusto, F. & Lanzavecchia, A. 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 (1994).
59. Romani, N. et al. Proliferating dendritic cell progenitors in human blood. *J. Exp. Med.* **180**, 83–93 (1994).
60. Benlagha, K. & Bendelac, A. CD1d-restricted mouse $V_{\alpha}14$ and human $V_{\alpha}24$ T cells: lymphocytes of innate immunity. *Semin. Immunol.* **12**, 537–542 (2000).
61. Lien, E. et al. Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *J. Biol. Chem.* **274**, 33419–33425 (1999).
62. Hirschfeld, M. et al. Cutting edge: inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by toll-like receptor 2. *J. Immunol.* **163**, 2382–2386 (1999).
63. Aliprantis, A. O. et al. Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. *Science* **285**, 736–739 (1999).
64. Poltorak, A. et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in *Tlr4* gene. *Science* **282**, 2085–2088 (1998).
65. Hemmi, H. et al. A Toll-like receptor recognizes bacterial DNA. *Nature* **408**, 740–745 (2000).
66. Dzionek, A. et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. *J. Immunol.* **165**, 6037–6046 (2000).
67. Yamaguchi, Y. et al. BDCA-2, a novel plasmacytoid dendritic cell-specific transmembrane protein: molecular cloning and functional characterization. Keystone Symposium: *Dendritic cell: interfaces with immunobiology and medicine*. Abstract 361 (2001).
68. Cella, M. et al. Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nature Med.* **5**, 919–923 (1999).
69. Siegal, F. P. et al. The nature of the principal type 1 interferon-producing cells in human blood. *Science* **284**, 1835–1837 (1999).
70. Fitzgerald-Bocarsly, P. Human natural interferon- α -producing cells. *Pharmacol. Ther.* **60**, 39–62 (1993).
71. Björck, P. Isolation and characterization of murine plasmacytoid dendritic cells. (submitted, 2001).
72. Asselin-Paturel, C., Briere, F. & Trinchieri, G. The IFN- α producing cells in mice are immature CD11c low CD8 α -CD11b $^{+}$ dendritic cells. Keystone Symposium: *Dendritic cell: interfaces with immunobiology and medicine*. Abstract 304 (2001).
73. Farrar, J. D. et al. Selective loss of type 1 interferon-induced STAT4 activation caused by a minisatellite insertion in mouse STAT2. *Nature Immunol.* **1**, 65–69 (2000).
74. Rogge, L. et al. The role of Stat4 in species-specific regulation of Th cell development by type I IFNs. *J. Immunol.* **161**, 6567–6574 (1998).
75. Cousens, L. P. et al. Two roads diverged: interferon α/β - and interleukin 12-mediated pathways in promoting T cell interferon γ responses during viral infection. *J. Exp. Med.* **189**, 1315–1328 (1999).
76. Cousens, L. P., Orange, J. S., Su, H. C. & Biron, C. A. Interferon- α/β inhibition of interleukin 12 and interferon- γ production *in vitro* and endogenously during viral infection. *Proc. Natl Acad. Sci. USA* **94**, 634–639 (1997).
77. McRae, B. L., Semnari, R. T., Hayes, M. P. & van Seventer, G. A. Type I IFNs inhibit human dendritic cell IL-12 production and T_H1 cell development. *J. Immunol.* **160**, 4298–4304 (1998).
78. Blanco, P. et al. Interferon- α and dendritic cells: novel therapeutic targets in systemic lupus erythematosus. (submitted, 2001).
79. Groux, H. et al. A $CD4^+$ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* **389**, 737–742 (1997).
80. Seder, R. A. et al. Factors involved in the differentiation of TGF- β -producing cells from naive $CD4^+$ T cells: IL-4 and IFN- γ have opposing effects, while TGF- β positively regulates its own production. *J. Immunol.* **160**, 5719–5728 (1998).
81. Kalinski, P. et al. IL-4 is a mediator of IL-12p70 induction by human T_H2 cells: reversal of polarized T_H2 phenotype dendritic cells. *J. Immunol.* **165**, 877–881 (2000).
82. Hochrein, H. et al. Interleukin (IL)-4 is a major regulatory cytokine governing bioactive IL-12 production by mouse and human dendritic cells. *J. Exp. Med.* **192**, 823–833 (2000).
83. Ramanathan, S. et al. Recombinant IL-4 aggravates experimental autoimmune uveoretinitis in rats. *J. Immunol.* **157**, 2209–2215 (1996).
84. Menicacci, A. et al. Endogenous interleukin 4 is required for development of protective $CD4^+$ T helper type 1 cell responses to *Candida albicans*. *J. Exp. Med.* **187**, 307–317 (1998).
85. Fort, M. et al. IL-4 exacerbates disease in a T_H1 cell transfer model of colitis. *J. Immunol.* **166**, 2793–2800 (2001).
86. Blom, B., Ho, S., Antonenko, S. & Liu, Y. J. Generation of interferon α -producing predendritic cell (Pre-DC)2 from human $CD34^+$ hematopoietic stem. *J. Exp. Med.* **192**, 1785–1796 (2000).
87. Jahnke, F. L. et al. Experimentally induced recruitment of plasmacytoid (CD123 high) dendritic cells in human nasal allergy. *J. Immunol.* **165**, 4062–4068 (2000).