Control of functional T helper cell polarization by dendritic cells
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CHAPTER 9

Dendritic Cells Interface Innate and Adaptive Immunity

General Discussion

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Introduction

The innate immune system has evolved to recognize a broad spectrum of pathogens and is often extremely successful as a first line of defense. Regardless of their success, innate immune cells also provide specific immune cells with information required for a most effective second line of immunity, which is indispensable when innate immunity fails (reviewed in refs. 1, 2).

Dendritic cells (DC) share many features with mononuclear phagocytes of the innate immune system, such as the expression of pathogen recognition receptors, but act uniquely as highly specialized antigen (Ag)-presenting cells that initiate specific immune responses in the T cell areas of the secondary lymphoid organs (3). Different pathogens require different classes of protective immunity, which are mediated by different types of T helper (Th) cells. It has been suggested that DC can carry from peripheral tissues to the lymph nodes signals that inform T cells not only on the type of pathogen but also on the most adequate type of T cell response (reviewed in ref. 1). This procedure allows DC that have been activated by intracellular pathogens or their compounds, to commit CD4\(^+\) Th cells to become protective IFN-\(\gamma\)-producing effector Th1 cells that activate macrophages, cytotoxic CD8\(^+\) T cells and natural killer (NK) cells. Alternatively, helminths may induce DC that
drive the development of protective IL-4–, IL-5–, IL-13–producing effector Th2 cells that induce IgE production in B cells and the activation and recruitment of eosinophils and mast cells (1, 2, 4, 5). The T cell response is not only matched to the nature of the pathogen, but is also aimed at reducing the risk of collateral damage to the infected tissue. For instance, the tissues of the central nervous system and the anterior chamber of the eye do not allow for rapid inflammatory reactions, as they cannot be repaired after damage.

**DC carry three types of signals for T cells**

Immature DC are highly skilled sentinels that sample at the potential sites of pathogen entry, such as lung, gut, and skin epithelia (3). DC become activated upon engagement with pathogens, or their compounds, and/or certain inflammatory mediators produced by the host tissue cells in reaction to the invading pathogen. As a result, DC are committed to undergo maturation while migrating towards the draining lymph nodes. This cellular differentiation process includes the change from an Ag-capturing mode into an Ag-presenting mode, involving the increase in expression of molecules involved in T cell adhesion and costimulation (e.g. DC-SIGN, LFA-1/3, ICAM-1, B7 family members, and CD40) (Fig. 1). Having reached the T cell areas of the lymph nodes, DC provide naive Th cells with several pathogen-related signals (1, 2). Signal 1 is an Ag-specific signal reflecting the structure of the pathogen and is delivered to the Ag-specific T cell receptor (TCR) by pathogen-derived peptides presented in the context of major histocompatibility complex (MHC) molecules. Signal 2 is a costimulatory signal reflecting the pathogenicity of the pathogen and determines not only whether or not an immune response will occur, but also the strength of this immune response (6). Signal 2 can be mediated by different molecules, which may be redundant and act at different maturation stages of the T cells. Costimulation, for instance, is provided by CD80 (B7-1) and CD86 (B7-2) that ligate CD28, B7-related protein 1 (B7RP-1) ligating inducible costimulator (ICOS) and OX40 ligand (L) ligating OX40. In Chapter 4 it is shown that B7RP-1, the third member of the B7 family of costimulatory molecules, delivers an indispensable signal for optimal production of signature cytokines not only by resting naive and memory Th cells but also by effector Th1 and Th2 cells.
Together, signals 1 and 2 allow for the Ag-specific activation of primary Th cells which results in the early production of IL-2 that sustains clonal expansion in an autocrine fashion, and allows further differentiation and acquisition of effector function (4-7). We have recently proposed that the commitment of naive Th cells into effector Th1 or Th2 cells is controlled by a signal 3 from the effector DC (8). In Chapters 2 and 3 it is further discussed that signal 3 reflects the way sentinel DC are activated by pathogens or by inflammatory mediators produced by tissue cells in reaction to pathogens. Indeed, 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 pathogen-invaded tissue. Like for signal 2, signal 3 can be mediated by several molecules.

**Figure 1.** Schematic representation of cell surface molecules involved in the interaction between APC and T cells. Ag-specific MHC/TCR interaction, adhesion molecules and costimulatory molecules and their ligands are depicted.

**DC-derived Th1-polarizing factors**

IL-12 is a dominant Th1-driving factor readily produced by DC upon engagement with various microbial compounds (9-16). The levels of IL-12 expressed by effector DC upon engagement of naive T cells in the lymph nodes, and, consequently, the level of Th1 development may vary with the conditions of DC maturation. In
Chapter 2 we show that IFN-γ, which can be produced by NK cells during viral infections (17) contributes to enhanced development of type-1–polarized DC (DC1).

It may be expected that effector DC produce high levels of IL-12 after their maturation in response to intracellular bacteria and viruses that require protective Th1 responses. Indeed, mouse models have indicated that IL-12 is pivotal in the protection against, for instance, mycobacteria, Leishmania major, and Toxoplasma gondii in certain mouse strains (18-21). In addition, humans with a mutated and dysfunctional β1 chain of the IL-12 receptor (R) may suffer from chronic infection with endosomal pathogens, such as mycobacteria and Salmonella sp. (22). Interestingly, until now, only a small number of inflammatory mediators or pathogen-related products have been identified that can induce stable effector DC with an increased capacity to produce IL-12. Although many other factors, including viral and bacterial products (e.g. dsRNA and LPS) may induce or enhance IL-12 production in immature monocyte-derived DC (11-15, 23), it is shown in Chapters 2 and 3 that such factors do not necessarily share the unique capacity of IFN-γ to induce stably polarized effector DC with an enhanced IL-12–producing capacity. Only pertussis toxin exhibits a priming effect comparable to that of IFN-γ. However, since the neutralization of toxin requires an antibody response, which does not improve with strong Th1 polarization, this type of response may represent one of the mechanisms of immune evasion employed by the Bordetella pertussis bacterium.

It has been suggested that the IL-12–producing capacity of DC is subject to exhaustion (24) during maturation induced by LPS. Consequently, only DC matured for a limited period (12 h) induce IL-12–dependent Th1 polarization, whereas fully matured DC cannot produce IL-12, and, therefore, induce Th2 cells. This temporal restriction would strongly compromise the effectiveness of the immune response. In fact, this finding implies that the class of the immune response would, amongst others, be influenced by the physical distance between the invaded area and the draining lymph node. In Chapters 2 and 3 it is shown, using a more sophisticated read-out model, that effector DC matured for 48 h in response to LPS still produce some IL-12 and, therefore, induce, similarly to control DC matured with maturation factors, a considerable frequency of Th1 cells.

Enhanced Th1 polarization not always depends on abundant production of IL-12. For instance, IL-12–deficient mice are able to mount potent Th1 responses upon
infection with mouse hepatitis virus (25). In addition, patients with a functional mutation of the IL-12R and suffering from chronic infections with various endosomal microorganisms do not suffer from viral infections (22). These findings stress that Th1 development can be alternatively regulated by APC. For instance, IFN-α is involved in DC activation and maturation (15) and may contribute directly to Th1 polarization through direct activation of STAT4 (for signal transducer and activator of transcription 4) (26) or upregulation of IL-12Rβ2 chain expression (27). Indeed, IFN-α can be induced in mouse DC lines, human IL-3Rα plasmacytoid DC (PDC) and monocyte-derived DC upon herpes simplex virus and influenza virus infection (15, 26, 28-30), although we could not detect IFN-γ production by myeloid DC in response to dsRNA (Chapter 3). IFN-α may, nevertheless, counteract the ability of certain viruses, such as rhinoviruses, measles virus, or human immunodeficiency virus to suppress IL-12 production and evade Th1 responses (31-33).

### DC-derived Th2-polarizing factors

It has been argued that the absence of IL-12 is a default condition for DC-induced Th2 polarization (reviewed in Ref. 34). Indeed, Th2 polarization is promoted exclusively by factors that induce the development of effector DC with a stably suppressed IL-12-producing capacity. These factors include certain helminthal compounds, such as phosphorylcholine-containing protein (e.g. ES-62 secreted by the filarial nematode *Acanthochelinonea viteae*) (35), or enzymes involved in prostaglandin (PG) biosynthesis (e.g. the glutathione S-transferase PG-H E-isomerase by *Ascaridia galli*) (36) or lipids eliciting IL-10 (e.g. egg lipids of *Schistosoma mansoni*) (37). Moreover, the IL-12-producing capacity of DC is inhibited by their maturation in the presence of agents with a cAMP-elevating potential, such as cholera toxin, PGE₂, β₂-agonists, and histamine (38-43) (Chapters 2, 3, and 5). It should be noted that cholera toxin (44) and histamine (45) might harbor alternative activities promoting Th2 cell development. Another group of factors inducing IL-12-deficient Th2-promoting DC includes the anti-inflammatory mediators IL-10, vitamin D₃, and the anti-inflammatory drugs, glucocorticoids and glatiramer acetate (38, 42, 46-48) (Chapters 6, 7, and 8; de Jong et al., unpublished). However, the DC generated in the presence of these factors may also
have a reduced T cell stimulatory capacity (38) and induce tolerance in naive Th cells (46, 49).

The nature of the putative DC-derived Th2-polarizing factors has been a subject of debate. In Chapter 3 we provide evidence that regulation of the Th2-promoting capacity of DC is more complex than merely the inhibition of the IL-12–producing capacity and relies on the regulation of molecules actively involved in polarization. For instance, crosslinking of the TNF-family member OX40 on Th cells has been shown to costimulate both proliferation and cytokine production in mouse (50) and man (51, 52). However, although all DC0 and DC2 express OX40L involved in the proliferation of the Th1 and Th2 cells, only OX40L expressed by the DC2, matured upon exposure to protein extract from the eggs of *S. mansoni*, contributes to the Th2-polarizing capacity (Chapter 3). In contrast, DC exposed to cholera toxin or PGE2 promote Th2 polarization, using as yet unknown soluble factor(s) (Chapter 3). Therefore, similar to the induction of Th1 responses, DC also regulate Th2 polarization in an active fashion, arguing against the current concept that Th2 immune responses are induced by default.

Although costimulation through ICOS has been implicated to be important in both Th1- and Th2-associated immune responses, in the mouse it is probably more critical in the regulation of Th2 function and expansion (53-58). In Chapter 4 we provide evidence that although costimulation through human ICOS is required for both Th1 and Th2 cytokines, it is selectively necessary for the expansion of Th2 cells. These findings suggest that the strength of Th2 responses may also be dictated by this costimulatory pathway. This has important implications for the understanding of regulation of Th2 polarization.

**The plasticity of DC function**

Different DC subsets secrete different polarizing mediators in response to inflammatory signals (34). In the mouse, CD8α⁺ "lymphoid-like" DC were shown to secrete IL-12 DC and to preferentially support Th1 development as opposed to CD8α⁻ "myeloid-like" DC, that secrete lower levels of IL-12, and support Th2 development (59, 60). There is accumulating evidence showing that CD8α⁻ DC can secrete IL-12, both in vivo, after priming with *Toxoplasma gondii* Ag (61), and in vitro, under the influence of IFN-γ or the absence of IL-10 (62). These examples illustrate
that mouse DC display functional plasticity to adapt to different conditions of activation and suggest that the IL-12–producing capacity of CD8α+ DC may be suppressed in vivo until exposure to the inducing Ag(s).

We have discussed above the plasticity of human monocyte-derived myeloid DC in the polarization of Th responses in opposite directions. Human PDC, that were initially characterized as Th2-promoting DC (63), were recently shown to be able to produce high levels of IL-12p70, in response to bacterial DNA (unmethylated CpG motifs), or IFN-α in response to viruses, resulting in the induction of Th1 responses (16, 26, 30). Together, the murine and the human data indicate that the capacity of DC to induce opposite Th cell polarization is not restricted by lineage-associated differences. This view is further supported by two recent studies. D’Ostiani et al. (64) reported that a murine DC line discriminates between yeasts and hyphae of the fungus *Candida albicans* by secreting IL-12 or IL-4, and promoting Th1 or Th2 development, respectively. Furthermore, Bozza et al. (65) reported an analogous behavior of murine lung DC in response to conidia and hyphae of the respiratory pathogen, the fungus *Aspergillus fumigatus*. This plasticity to adapt to diverse pathogen-derived signals highlights the crucial role of DC in interfacing innate and adaptive immunity.

The plasticity of immature DC is strongly reduced following maturation. This may enable DC to carry “undisturbed” signal 3 from the site of pathogen entry in peripheral tissues to the lymph nodes. DC are susceptible to both polarization and modulation of their stimulatory capacity at the early stages of their development. Interestingly, the susceptibility to polarization is terminated earlier than the susceptibility to modulation of their stimulatory capacity (Chapters 7 and 8). Although glucocorticoids (GC) do not affect the relative stimulatory capacity of immature DC towards memory Th cells, GC suppress the IL-12–producing capacity already at the immature stage (Chapter 7). In contrast, while the impaired T cell stimulatory function induced by the presence of GC during DC maturation can be subsequently reversed, the GC-induced type 2 polarization appears to be irreversible (Chapter 8). This indicates that the ability of DC to mediate a differential signal 2 is subject to continued regulation, even in DC that leave peripheral tissues. In sharp contrast, the tissue-induced ability of DC to provide signal 3 is relatively independent of the conditions DC meet in the lymph nodes.
Figure 2. Functional polarization of dendritic cells. Differences in the ability to induce Th1 or Th2 responses can be observed between DC populations from different tissues. These differences may result from early polarization of DC precursors developing in different environments. Pathogens can further modulate the Th-polarizing capacity of sentinel DC, either directly or indirectly by eliciting distinct inflammatory factors. In analogy to the commitment of Th cells, maturing DC acquire resistance to repolarization that results in the development of polarized effector Th1-inducing DC (DC1) and Th2-inducing DC (DC2).

Concluding remarks
The ability of DC to acquire pathogen-derived and/or tissue type-derived signals is strictly regulated during the maturation of the DC. The Ag uptake capacity of sentinel DC terminates within hours after activation while MHC/peptide complexes become stable and are expressed on the cell surface (66). Upon maturation, DC become resistant to environmental and pathogen-derived factors that modulate sentinel DC (Chapters 2 and 3). These changes confer selectivity to the pathogen and location of entry. The evidence discussed above indicates that the nature of the Th1-driving molecules of the type 1-promoting DC and the Th2 driving-molecules of the type 2-promoting DC can be different and depends on the type of pathogen and the tissue
provoked by the pathogen. It has been proposed that tissue-derived signals instruct the immune system to initiate immune responses as well (67). The data discussed here implicate that both tissue-derived signals and pathogen-related signals, carried by DC, can determine the initial polarization of naive Th cell responses, and hence, the class of the initiated response (Fig. 2). In this model, migrating DC, apart from carrying antigenic and costimulatory signals (signal 1 and signal 2, respectively), are further equipped with the capacity to transmit a third type of signal (signal 3) 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.

References


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