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Dendritic Cells as Sensors of Infection

Minireview

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Introduction

A stable environment and an abundant supply of nutrients make an inviting place for a pathogen. Consequently, complex multicellular organisms have had to evolve defense mechanisms to make their internal environment more hostile to invaders. All immune systems have one feature in common: they respond to infection by switching from a resting to an active state. For example, *Drosophila* flies do not make microbicidal peptides until infected by fungi or bacteria. Similarly, T and B cells are generally resting in the absence of infection although they can be rapidly activated in response to an invading pathogen. Thus, there must be key features of an infectious process that trigger immune responses.

These features are recognized primarily by cells and molecules of the innate immune system. The innate response limits infection and activates antigen-presenting cells (APC) to trigger adaptive immunity, which increases specificity and generates memory. Over the last 25 years, dendritic cells (DC) have emerged as the major APC involved in this process. DC provide T cells with antigens as complexes with MHC or MHC-like molecules and, simultaneously, deliver critical information about the context in which the antigens were encountered. An infectious context promotes DC immunogenicity and the development of immunity while absence of infection fails to do so. DC also convey information about the nature of the infectious agent, favoring the appropriate class of T cell response. This review explores the topic of DC as sensors of infection and its consequences for the adaptive response.

Dendritic Cell Activation

DC comprise a large family of leukocytes with related morphology and the potential to interact with naive T cells (see Banchereau et al., 2000, for a review). Unlike other APC such as macrophages (MØ) or B cells, DC are thought to have no function other than to regulate T and B cell responses. However, just like that of T and B cells, this "effector" activity of DC requires prior activation. As originally defined by Janeway (1989), APC activation referred specifically to upregulation of costimulatory ability with a consequent increase in immunogenicity through increased delivery of signal 2 to T cells. However, DC activation also promotes peptide loading of MHC class II molecules and increases display of MHC:peptide complexes to $CD4^+$ T cells. In many cases, activation also leads to upregulation of chemokine receptors that bring DC into close proximity to T cells. Both of these features suggest that DC activation also leads to an increase in antigen (signal 1) delivery to T cells (see Manickasingham and Reis e Sousa, 2001, for a review). Finally, DC activation can trigger production of cytokines such as IL-12, IL-18, or IL-10, which can polarize emerging T cell responses (signal 3).

Because so many DC properties are affected by activation, it is hard to come up with an all-encompassing definition of the term. Adding to the difficulty, it is not clear that all forms of DC activation necessarily result in increased immunogenicity. For example, some forms of activation could selectively increase delivery of signal 1 without signal 2, giving rise to tolerogenic rather than immunogenic DC (see Manickasingham and Reis e Sousa, 2001, for a discussion). Here, activation is used simply to refer to a change from the resting state. This can refer to any changes that affect the ability of APC to deliver signals 1, 2, and/or 3 to T cells, including changes in expression of surface markers, cytokine production, migratory properties, endocytic activity, morphology, or longevity. This broad definition allows for the possibility that there may be multiple forms of DC activation with different functional consequences.

DC activation is generally seen in response to pathogens or hallmarks of their presence. For example, live infection or injection of rodents with LPS, extracts of microorganisms, double-stranded RNA (dsRNA), or bacterial DNA can all trigger changes in DC expression of MHC, adhesion and costimulatory molecules, and in cytokine production. Similarly, resting DC grown in vitro from mouse or human progenitors can be rapidly activated by exposure to lipopolysaccharide (LPS), inflammatory cytokines, dsRNA, heat-shock proteins (HSPs), or other stimuli. DC activation in this context is thought to lead to increased immunogenicity and may be seen as a physiological response to infection with profound implications for T cell immunity.

Direct Activation of DC by PAMPs

How does infection trigger DC activation? Janeway proposed that APC possess germline-encoded pattern recognition receptors (PRRs) that recognize and are triggered by evolutionarily conserved molecules essential to pathogen function, which are absent from the host (Janeway, 1989). These so-called pathogen associated molecular patterns (PAMPs) are widespread. Bacteria possess an abundance of PAMPs, from cell wall components (LPS, lipoproteins, peptidoglycans, lipoarabinomannan) to DNA containing unmethylated CpG motifs. Yeast and fungal cell walls have PAMPs in the form of mannans and β-glucans, reoviruses have a genome made of dsRNA, and protozoa express several unique glycosylated proteins and lipids. However, bona fide PRRs took a long time to be discovered after their existence was postulated in 1989. Although several receptors on DC and MØ were known to bind microorganisms and mediate their uptake, they did not necessarily trigger APC activation. For example, the mannose receptor is expressed on human DC and can mediate internalization of many microorganisms, including yeasts, *Leishmania*, and some bacteria. However, the mannose receptor is not known for its ability to activate DC. The same is true of related molecules such as langerin, DEC-

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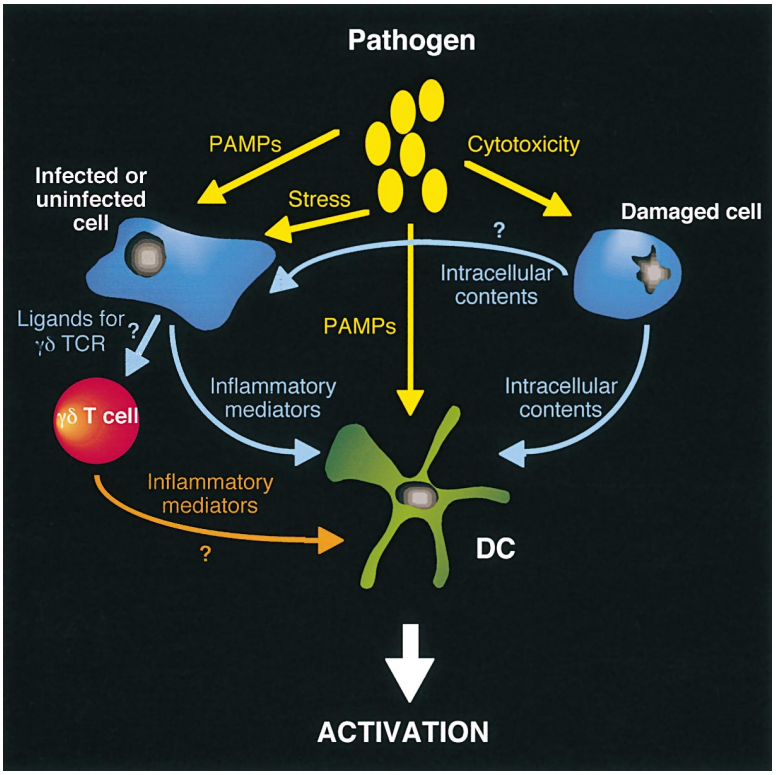

Figure 1. Potential Pathways for DC Activation in Response to Infection

Arrows indicate different stimuli, colour coded according to source. Question marks indicate pathways that are more speculative. For the sake of clarity, receptors are not shown. See text for details.

205, and other DC lectins. Other receptors with some specificity for microorganisms (β-glucan receptor, scavenger receptors) also appear to act primarily as endocytic receptors rather than PRRs.

This situation changed drastically in 1997 with the discovery of the mammalian Toll-like receptors (TLRs), of which there are at least 10 to date (see Aderem and Ulevitch, 2000, for a review). TLRs are homologs of *Drosophila* Toll and 18-wheeler, two genes involved in the innate response to fungal and bacterial infections in adult flies. TLRs recognize PAMPs and signal through a pathway remarkably conserved between *Drosophila* and vertebrates that leads to activation of NF-κB, a family of transcription factors implicated in many inflammatory responses. It is becoming clear that TLRs not only activate APC in response to PAMPs but can also discriminate between closely related types of pathogens. Mammalian TLR4 mediates activation in response to LPS found in Gram-negative bacteria but fails to respond to lipoteichoic acid and other components of Gram-positive organisms. *Drosophila* Toll and 18-wheeler can distinguish between fungi and bacteria, respectively, to trigger production of the appropriate microbicidal effectors. Although there appear to be too few receptors for the number of potential PAMPs, TLRs can act in combination, as suggested by the reported cooperation of TLR2 and TLR6 in the recognition of yeast cell walls and Gram-positive bacteria (Aderem and Ulevitch, 2000). Interestingly, TLRs may recognize PAMPs primarily in endocytic compartments. TLR9-dependent activation of MØ and DC by CpG-containing DNA requires prior internalization of the PAMP via a DNA-specific receptor into an acidic endosomal compartment (Hacker et al., 1998), and TLR 2 in MØ is recruited to phagosomes containing yeast cell walls (Aderem and Ulevitch, 2000). One of the functions of endocytic receptors such as the mannose receptor may, therefore, be to concentrate PAMPs in endosomes for TLR sampling.

Many PAMPs probably remain to be characterized, especially in metazoan parasites and protozoa. The existence of PAMPs in viruses has been questioned, but it is clear that viral replication in infected cells often involves production of small amounts of dsRNA, even when the virus itself does not have a dsRNA genome (Jacobs and Langland, 1996). dsRNA-activated enzymes such as protein kinase R (PKR) are traditionally associated with their ability to shut off protein synthesis in interferon-activated cells. However, PKR can trigger NF-κB activation, and dsRNA recognition also leads to activation of IRF-3 and IRF-7, two transcription factors that initiate production of interferons (see Mamane et al., 1999, for a review). Thus, some dsRNA-recognition enzymes could also trigger APC activation, fitting the criteria for PRR.

Indirect Activation of DC by Signs of Infection

A natural extension of Janeway's hypothesis is that APC might also recognize PAMPs indirectly (Figure 1). PRRs on APC could be triggered by PAMP surrogates, as in *Drosophila*, where Toll is triggered by Spätzle, a product of a proteolytic cascade initiated by fungal infection (Aderem and Ulevitch, 2000). In addition, APC could be activated by signals made by other cells in response to PAMPs (Figure 1). Many tissues may have their own PRR and produce proinflammatory cytokines and chemokines in response to infection. A classic example is virally infected cells, which produce interferons in response to dsRNA (see above). Interferons can act to activate DC as well as increase viral resistance in neighboring cells. Similarly, keratinocytes can secrete TNFα,

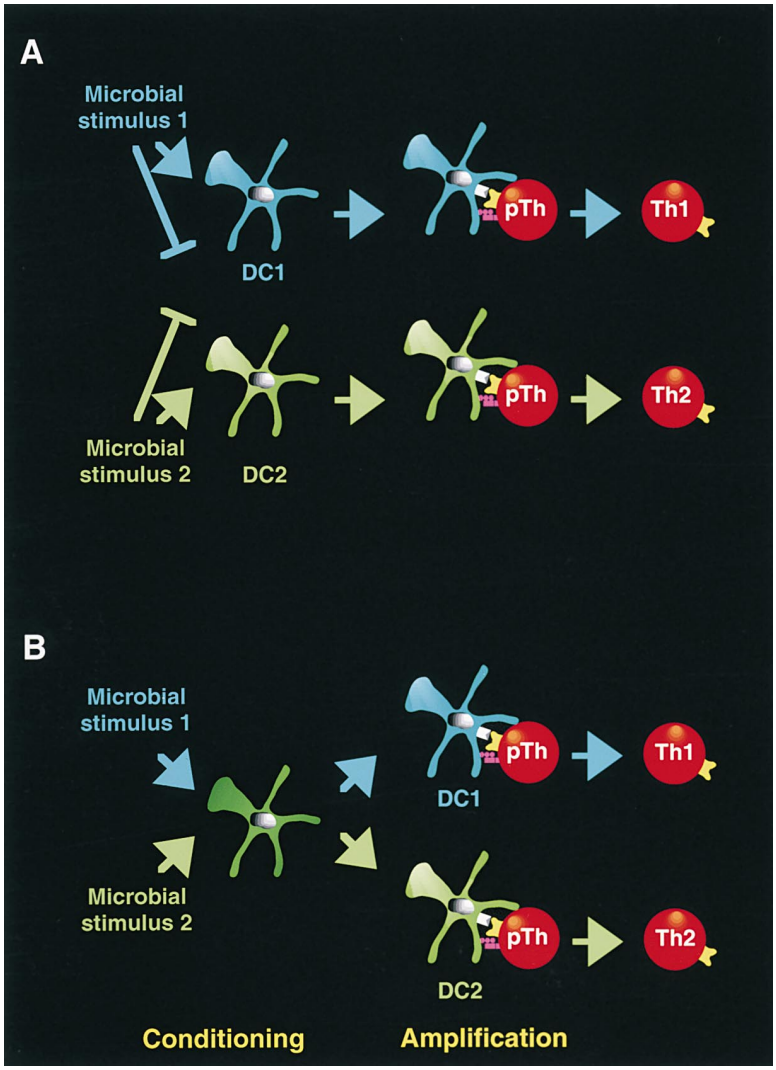

Figure 2. DC Regulation of T Cell Effector Class

(A) Depicts a model in which preexisting DC1 and DC2 are triggered by different infectious stimuli to elicit type 1 and type 2 responses. (B) Depicts a model in which the nature of the stimulus conditions bi-potential DC to become DC1 or DC2. White and gray rectangle indicates MHC loaded with pathogen-derived peptides, yellow indicates the TCR. Purple circles denote DC accessory molecules (e.g., CD80, CD86, CD40) and purple squares denote counterparts on T cells (e.g., CD28, CD40L).

IL-1, and IL-18, all of which activate Langerhans cells, a type of skin and mucosal DC. Finally, in response to *Toxoplasma gondii* antigens, endothelial cells produce MIP-1α and MIP-1β, which can trigger IL-12 production by some murine DC (Aliberti et al., 2000).

However, not all signs of infection are necessarily related to PAMP recognition. Matzinger has proposed that APC activation does not need to involve PRRs but, instead, receptors for self-molecules normally sequestered in intracellular compartments of healthy cells but that are released in situation of "danger," when cells are stressed or die by nonapoptotic means (Figure 1; Matzinger, 1994). Evidence for this notion has accumulated recently with the discovery that mammalian heat-shock proteins (HSPs) can act as DC activators and that these proteins are released from necrotic but not from apoptotic cells (Basu et al., 2000). However, HSPs are highly conserved and bacterial HSPs can also activate DC. Activation by some mammalian HSPs may even involve TLRs (Ohashi et al., 2000). Thus, HSPs may turn out to be both PAMPs and "danger" signals. Other intracellular components shown to activate DC include nucleotides such as ATP, which act through purinergic receptors (Schnurr et al., 2000).

Receptors for stress molecules need not be present on the APC itself. Healthy cells that recognize signals from neighboring stressed cells could produce a range of inflammatory mediators that lead to DC activation. This form of indirect recognition might even involve a third party to convey tissue recognition of stress to the APC. For example, it is increasingly clear that tissues may increase expression of nonclassical MHC molecules such as MICA and MICB upon stress. These, as well as some microbial products, can serve as ligands to trigger $\gamma\delta$ T cells, a type of lymphocyte found in many peripheral tissues (Groh et al., 1998). $\gamma\delta$ T cells have been shown to produce TNFα, GM-CSF, MIP-1α, MIP-1β, and RANTES, and it is conceivable that they could act to activate DC (Figure 1).

One well-known DC activator stands out as apparently unrelated to infection. CD40 ligation is a potent means to activate DC, yet CD40L is expressed by many cells, including platelets, mast cells, basophils, and activated T cells. This ubiquitous expression pattern fails to reveal an obvious link to infection. However, CD40 signaling in DC may be under the control of innate signals. In vivo studies show that DC cannot be activated through CD40

to make IL-12 or upregulate CD80 and CD86 until exposed to PAMPs from certain infectious organisms (Schulz et al., 2000). This suggests that CD40L (and probably other T cell feedback signals) merely amplifies the activation of DC previously conditioned by infection-related stimuli, rather than initiate de novo activation.

Control of T Cell Effector Class by DC

The encounter with an infectious organism requires not only that an immune response be initiated but that it be of the appropriate class. For example, Th2-dominated responses play a role in immunity to helminths whereas Th1 responses are critical to eliminate many intracellular pathogens such as *Listeria* or *Toxoplasma gondii*. Thus, it is essential that APC both alert T cells to the presence of infection and also transmit information about the nature of the infectious organism. Much of this information is transmitted as signal 3 cytokines and other molecules that skew emerging T cell responses.

It is clear that DC populations produce different cytokines in response to different activating stimuli (see Moser and Murphy, 2000, for a review). However, many of these populations are heterogeneous and there is much controversy about whether different classes of immunity are elicited by different DC types responding to different PAMPs or whether a single DC subset has the potential to deliver distinct signals 3 depending on the activating stimulus (Figure 2). Consistent with the former hypothesis (Figure 2A), immunization with murine DC populations containing mostly CD8α^+ DC preferentially induces development of IFN-γ-producing Th1 cells, suggesting that these DC could be precommitted "DC1." The converse is seen with populations enriched for CD8α^- DC, which might therefore be considered "DC2" destined to prime Th2 responses (see Moser and Murphy, 2000, for a review). In agreement with this notion, CD8α^- also produce much less IL-12 than CD8α^+ DC in response to a variety of stimuli (Moser and Murphy, 2000; Schulz et al., 2000). However, other evidence suggests that a single DC type can make different cytokines depending on the activation stimulus (Figure 2B). For example, a mouse clonal DC line can produce distinct cytokines in response to yeasts versus hyphae of the fungus *Candida albicans* that drive Th differentiation along opposing pathways (d'Ostiani et al., 2000). Human monocyte-derived DC also display significant plasticity in their ability to skew Th development (see Kalinski et al., 1999, for a review), and human plasmacytoid DC, that were originally defined as DC2, have recently been shown to produce large amounts of IFN-α after activation by viruses and efficiently induce Th1 development (Cella et al., 2000). Perhaps DC subsets are somewhat specialized to induce different classes of immunity but retain enough plasticity to adjust their response to pathogen signals.

Concluding Remarks

There are many different signals that induce DC activation, from PAMPs to intracellular self-components. Why so much redundancy? It may be argued that all these signals have in common an evolutionary link to infection. In the race against a replicating pathogen, early recognition of infection provides a competitive advantage. Primitive immune systems presumably learned to recognize any signal that correlated with infection, even if only weakly. The reverse of the coin is that some signals may now also activate DC to become immunogenic in the absence of infection. Does this matter? Over time, tolerogenic APC and/or tissues can purge the peripheral repertoire of most self-reactive T cells (see Matzinger, 1994, for a discussion). Thus, provided it does not become so prevalent as to prevent peripheral tolerance, it is better to err on the side of excess APC activation and cry wolf (or, in this case, pathogen) even when there is none around. In this context, it is worth remembering that if innate recognition of infection was absolute, DC could be endowed with destructive function and replace lymphocytes. DC do not have the exquisite antigen discrimination ability conferred by clonally distributed receptors and can only act as advisors, providing T cells with information of evolutionary value. T cells are the ultimate decision makers and can follow or ignore this advice based on their history of antigen exposure.

Many questions remain to be answered in the field. For example, why do we recognize PAMPs but are tolerant of commensal bacteria in gut and skin? Can we correlate particular forms of DC activation with given categories of stimuli? What are the consequences of each form of DC activation for the immune response? Can our understanding of signals for DC activation be used to design better adjuvants for vaccination and immunotherapy? These and other questions are likely to keep DC in the limelight for many years to come, which is perhaps appropriate for a star-shaped APC.

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