immune responses using model antigens that may not reflect structural features of antigens resulting from co-evolution, or those that use systems where an abnormally high frequency of specific T or B cells exist, may not be representative for understanding immune tolerance or immunity against infections and tumors. In the latter case, antigens generally expose to the immune system only one or few essential antigenic sites or peptides to relatively low frequencies of T and B cells (Fig. 1) (*1*, *13*, *26*). Thus, discrepancies between the immunology of model antigens and immunity against infections may eventually be resolved by more stringent definition of relevant characteristics of the chosen experimental system (*10*, *12*, *26*, *33*). Understanding these critical parameters will enhance our understanding of basic immunology and will not only help predict the rules of why, how, and when the immune system reacts but will also enable us to better explain the pathophysiology of infectious disease. Ultimately, this will vastly improve rationales on how to offer protection through vaccination.

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21. The popular postulate of cross priming assigns to dendritic cells (DC), those cells that carry antigen to lymph nodes, the exclusive and essential capacity to induce helper and cytotoxic T cells. Intracellular antigens are generally presented via MHC class I proteins and phagocytized cell-external antigens via MHC class II, for recognition by lytic $CD8^+$ T cells and nonlytic $CD4^+$ helper T cells, respectively. To induce $CD8^+$ killer T cells against infections that avoid DCs and against extralymphatic peripheral tumours (carcinomas or sarcomas), it has been proposed that DCs are also uniquely capable of activating naïve $CD8^+$ T cells after processing phagocytozed antigens via MHC class I. These processes have been termed cross-presentation and cross-priming (*22*, *23*). However, we suggest that the assumption that only DCs can induce $CD8^+$ T cells have been overstated because antigenic fibroblasts, or epithelial cells that lack conventional second signals, efficiently induce $CD8^+$ T cells if they reach draining lymph nodes or the spleen and if helper T cells are available (*24*, *25*). Therefore, we suggest that cross-priming is not essential and, at best, is inefficient under physiological conditions in vivo. This makes sense because otherwise B cells or DCs picking up soluble antigens may generally become over-susceptible to elimination by cytotoxic T cells, a process that would prematurely stop immune responses. Nevertheless, with some manipulation certain limitations of cross-priming may be overcome and might, perhaps, become exploitable therapeutic setting. Last but not least, our tendency to use experimental animals raised under nonphysiologically clean conditions (so called specific pathogen-free) may skew the specificity of reactivity due to reduced nonspecific, concomitant immunity and unwanted bystander effects.
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VIEWPOINT

Sensing Pathogens and Tuning Immune Responses

Bali Pulendran,* Karolina Palucka, Jacques Banchereau

The immune system is capable of making qualitatively distinct responses against different microbial infections, and recent advances are starting to reveal how it manages this complex task. An integral component of the immune system is a network of cells known as dendritic cells (DCs), which sense different microbial stimuli and convey this information to lymphocytes. A better understanding of DC biology has allowed a model to be constructed in which the type of immune response to an infection is viewed as a function of several determinants, including the subpopulation of DCs, the nature of the microbe, microbe recognition receptors, and the cytokine microenvironment.

When a microbe enters the body, the immune system is faced with a series of challenges. First, a decision needs to be made as to whether to respond to that specific microbe or not. Second, if a response is made, it must be tailored to fight that particular microbe. For example, in response to intracellular microbes, such as viruses and certain bacteria, $CD4^+$ T helper (T_H) cells differentiate into T_H1 cells, which secrete interferon-γ (IFN-γ) and possess a specific range of functions. In contrast, extracellular pathogens such as helminths induce the development of T_H2 cells, whose cytokines [interleukin 4 (IL-4), IL-5, and IL-10] direct immunoglobulin E– and eosinophil-mediated destruction of the pathogens (*1*). Generating the right class of immune response can be a matter of life and death itself. Thus, in leprosy, the tuberculoid form of the disease is characterized by a protective type 1 response, but the lepromatous form induces an often lethal type 2 response.

Although B and T lymphocytes respond to antigens with high specificity, they alone are not capable of making these complex decisions. These choices are made jointly by the nature of the microbe and by dendritic cells (DCs). DCs are scattered throughout the body, including the various portals of microbe entry, where they reside in an immature form (*2*–*5*). Immature DCs can be considered "immunological sensors," alert for potentially dangerous microbes, and are capable of decoding and integrating such signals. They then ferry this information to naïve T cells in the T cell areas of secondary lymphoid organs, undergoing a maturation process en route. Here, the mature DCs present this information to T cells, thus launching an immune response and immune memory through which the antigenic encounter can be remembered even for a lifetime (*4*). DCs can also tune the immune response by modulating either the amplitude or the class of the response

Baylor Institute for Immunology Research, 3434 Live Oak, Dallas, TX 75204, USA.

*To whom correspondence should be addressed. E-mail: balip@baylordallas.edu

(*2–5*). Different subpopulations of DCs appear to be capable of inducing distinct types of responses (*5–9*), but emerging evidence from several groups suggests that DC function is also modulated by microbes and the microenvironment (*2–5*).

DC Subsets

Like lymphocytes, DCs can be divided into subsets that differ in phenotype, function, and microenvironmental localization (*2–5*). It is not known whether this diversity reflects the existence of distinct lineages of DCs, different maturation stages, or both. In the secondary lymphoid organs of mice, at least three DC subsets are known: $CD8\alpha^-$ "myeloid" DCs; $CD8\alpha^+$ DCs, postulated to be of lymphoid origin (*10*); and Langerhans cell–derived DCs (LCDCs) (*2–5*). $CD8\alpha^+$ DCs are located in the thymic cortex and T cell areas of secondary lymphoid organs, whereas $CD8\alpha^-$ DCs reside in the marginal zones of the spleen, the subcapsular sinuses of the lymph nodes, and the subepithelial dome of Peyer's patches (*2–5*). Langerhans cells (LCs), the precursors of LCDCs, reside in the skin and mucosal epithelia and contain unique structures called Birbeck granules (*2–5*). As LCs migrate to the T cell areas of lymph nodes, they mature into LCDCs.

In human skin, two subsets of immature DCs are found: LCs in the epidermis and interstitial DCs in the dermis (*2–5*). In human blood, two subsets of DCs have been identified: $CD11c^+$ immature DCs, which differentiate into mature $CD11c^+$ DCs in response to inflammatory stimuli, and $CD11c^-$ precursor DCs, which differentiate into plasmacytoid DCs (pDCs) in response to IL-3 (*2–5*). $CD11c^-$ precursors appear to be the principal source of type 1 IFNs in response to viruses and other stimuli (*11*, *12*).

Sensing Microbes

When a microbe infects a tissue, resident immature DCs sense the microbe by recognizing evolutionarily conserved molecular patterns that are integral to microbial carbohydrates, lipids, and nucleic acids. This is achieved through so-called pattern recognition receptors (*13*), of which the recently characterized Toll-like receptors (TLRs) are prime examples (*14–16*). Toll was originally discovered in *Drosophila* as a key mediator of embryogenesis. Later, *Drosophila* was shown to have several genes encoding homologs of Toll, and these were implicated in antimicrobial immunity [reviewed in (*15*)]. Mammalian TLRs, of which 10 have been described, have broad specificity for conserved molecular patterns shared by large groups of pathogens [such as lipopolysaccharides (LPSs) in Gram-negative bacteria and bacterial CpG DNA]. It appears that TLRs offer DCs a means of discriminating between different stimuli. Thus, *Escherichia coli* LPS signals through TLR4; peptidoglycans from *Staphylococcus aureus* and zymosan signal through TLR2; CpG bacterial DNA signals through TLR9; and bacterial flagellin signals through TLR5 (*14–16*).

Once a DC has detected a specific microbe, information about the pathogen is then relayed to naïve T ymphocytes in the draining lymph nodes, in a sequence of events. First, immature DCs capture the microbe or its products by several mechanisms, including the actin-dependent process of phagocytosis (for particulate antigens) and receptor-mediated endocytosis or macropinocytosis (for soluble antigens) (*2*, *3*). Then immature DCs exit the site of infection and migrate toward the T cell areas of the proximal lymph nodes via afferent lymphatics. The migration of epithelial LCs is guided by the chemokines 6Ckine and MIP-3β, which are expressed in the lymphatics and T cell areas of the lymphoid organs (*2*, *3*). These are ligands for the CCR7 receptor, which is up-regulated on LCs as they migrate. During this journey, LCs differentiate into LCDCs, losing their antigen-capturing capacities but acquiring the capacity to process and display peptide antigens on their surface, in conjunction with molecules of the major histocompatibility complex (MHC) (*2*, *3*).

For productive immunity to occur, DCs must present not only peptide-MHC complexes but also additional costimulatory signals (such as molecules of the B7 family, including CD80 and CD86) to T cells. The interaction between CD86 and its corresponding ligand CD28 on T cells results in the up-regulation of CD40 ligand on T cells. The T cells may then engage CD40 on DCs and trigger a burst of cytokine expression, including IL-12, which induces IFN-γ in T cells (*2*, *3*). Signaling through CD40 also up-regulates numerous other costimulatory molecules, which may play distinctive roles in tuning the immune response.

Tuning the Response

The cytokines produced in the local microenvironment are key in determining the type of T_H response generated. For example, IL-12 and IL-4 induce T_H1 and T_H2 cells, respectively (*1*). But as discussed below, the initial commitment to make T_H1 or T_H2 cytokines appears to depend on several parameters.

Different DC subsets can induce distinct T_H responses. In mice, freshly isolated $CD8\alpha^+$ and $CD8\alpha^-$ DCs from spleens (*6*, *7*) or Peyer's patches (*9*) induce T_H1 and T_H2 responses, respectively. $CD8\alpha^+$ DCs can be induced to secrete IL-12, which is essential for their ability to induce T_H1 immunity (*6*, *9*, *17*, *18*). Consistent with this differential skewing, cytokines, which differentially expand these DC subsets in vivo, promote different responses. Thus, granulocyte-macrophage colony-stimulating factor, which preferentially expands $CD8\alpha^-$ DCs, elicits T_H2 responses; whereas Flt3 ligand (Flt3-L), which expands both DC subsets, elicits both T_H1 and T_H2 responses (*7*). In humans, monocyte-derived DCs (MDDCs) and pxxxxx DCs (pDCs) can induce T_H1 and T_H2 responses in vitro, respectively (*8*). However the extent of polarization by these cells may differ according to their method of isolation and maturation (*12*), the ratio of DCs to T cells (*19*), or the duration of DC activation (*20*). As with mice, IL-12 secretion by MDDCs seems essential for their T_H1 induction (*8*).

Certain characteristics of the microbe also play an important role in tuning the response. For example, viruses stimulate IFN-α from $CD11c^-$ precursors (*11*, *12*) and induce their differentiation into DCs that elicit IFN-γ– and IL-10–producing T cells (*21*); however, IL-3 induces their differentiation into T_H2-inducing pDCs (*8*). Different forms of the fungus *Candida albicans* instruct a murine DC cell line to induce either T_H1 or T_H2 responses (*22*). As stated above, the immune system can discriminate between different microbial stimuli through receptors such as TLRs. This is reminiscent of the situation in *Drosophila*, where fungi and bacteria signal through Toll and its homolog 18-Wheeler, respectively, to elicit distinct antimicrobial peptides (*15*). In mammals, it is unknown whether signaling through different TLRs leads to different types of adaptive immune responses. We have recently found that the TLR-4–dependent *E. coli* LPS induces a T_H1 response, but LPS from the oral bacterium *Porphorymonas gingivalis*, which signals through a TLR4-independent pathway (*23*), induces a T_H2-like response. Consistent with this, *E. coli* LPS, but not *P. gingivalis* LPS, induces IL-12 in splenic $CD8\alpha^+$ DCs (*24*).

Finally, cytokines secreted by activated T cells can also modulate DC function. Thus, T_H1-inducing DCs, when exposed to IL-10 or TGF-β, induce T_H2-like responses [reviewed in (*25*)]. Conversely, IFN-γ can instruct DCs to acquire some T_H1-inducing capacity (*25*). These results are consistent with observations that DCs in distinct microenvironments induce different T_H responses. For example, Peyer's patches or respiratory tract DCs prime T_H2 responses, whereas total spleen DCs prime T_H1/T_H0 responses (*9*, *26*). These observations may also explain why the route of antigen entry is a crucial determinant of the type of immunity; inhaled antigens induce a T_H2 response, whereas antigens injected subcutaneously induce a T_H1 response.

DCs: Deterministic Dictators or Passive Brokers?

In principle, two opposite mechanisms could mediate distinct immune responses

through different TLRs. First, a single DC subset may have the potential to induce virtually any T_H response, depending on the microbial stimulus and the TLR triggered by the stimulus (this is the Instruction Model). If so, why evolve so many functionally different subsets? Perhaps distinct DC subsets, with genetically preprogrammed T_H induction potentials, may express different repertoires of TLRs. Thus, recognition of a particular product by a given DC subset will select a particular response, distinct from that induced by another product activating a different subset (this is the Selection Model). These two models probably represent two extreme situations, and as discussed below (Fig. 1), elements of both models may operate.

In the alternative model shown in Fig. 1, DC subsets may express broadly distinct repertoires of TLRs and recognize different microbial stimuli. Thus, at the site of an infection, microbial stimuli 1 and 2 may preferentially activate immature DC1s and DC2s, which express different TLRs and which have genetic propensities to generate T_H1 and T_H2 responses, respectively. However if this were the only mechanism, then there would be no flexibility for the T_H response to adapt to the changing dynamics of the infection. Therefore, DCs display some functional plasticity: Stimulus 1 may prompt DC2s somewhat toward a T_H1-inducing mode, and stimulus 2 may prompt DC1s somewhat toward a T_H2-inducing mode. A further level of regulation may occur in the draining lymph node during the early stages of the response. Here, T_H1 (IFN-γ) and T_H2 (IL-10) cytokines made by T cells may suppress DC2s and DC1s, respectively, so as to amplify a given response. However, later in the response, T_H2 cytokines may enhance the T_H1 induction by DCs (*27*), to prevent an uncontrolled T_H2 response. In this model, therefore, the immune response is a function of the type of microbe, the DC subsets, the microbe-recognition receptors, and the cytokine microenvironment.

Turning Down the Volume

An immune response that continues unabated may cause overproduction of cytokines that activate other T cells, specific to the body's antigens, leading to autoimmunity. Therefore, DCs may also play crucial roles in down-regulating immune responses. For instance, DCs may express molecules that inhibit T cell expansion. B7 molecules on DCs engage CTLA-4 on activated T cells and inhibit their proliferation; and B7-H1 molecules on antigen-presenting cells engage the inducible costimulator receptor [reviewed in (*28*)] on activated T cells and induce IL-10, which dampens T cell activation. In principle, these molecules may be up-regulated on the same DCs that initially primed the T cells, or they may be constitutively expressed on a specialized subset of DCs dedicated for switching off T cells (*29*, *30*). Thus, these regulatory DCs may capture and present antigens from live or apoptotic stimulatory DCs to terminate a T cell response. Indeed, immature DCs that capture apoptotic cells do not stimulate T cells efficiently and may induce immunological tolerance (*30*, *31*). Consistent with this idea, a discrete population of DCs in rat Peyer's patches have been shown to transport apoptotic cells from the intestinal epithelium to the lymph nodes, suggesting a possible mechanism through which oral tolerance may occur (*32*).

From Their Plagues to Our Vaccines

Cells that play such crucial roles in the immune response must also be the prime targets of many conspirators wishing to manipulate the immune system. This appears to be the case with many pathogens, and at least a few immunologists. For example, parasites such as *Plasmodium falciparum* (*33*) or measles viruses (*34*) abort DC maturation, thus impairing T cell activation. *Schistosoma mansoni* suppresses LC migration from the epidermis (*35*), and HIV uses a "Trojan horse" strategy to infect $CD4^+$ T cells in the lymph

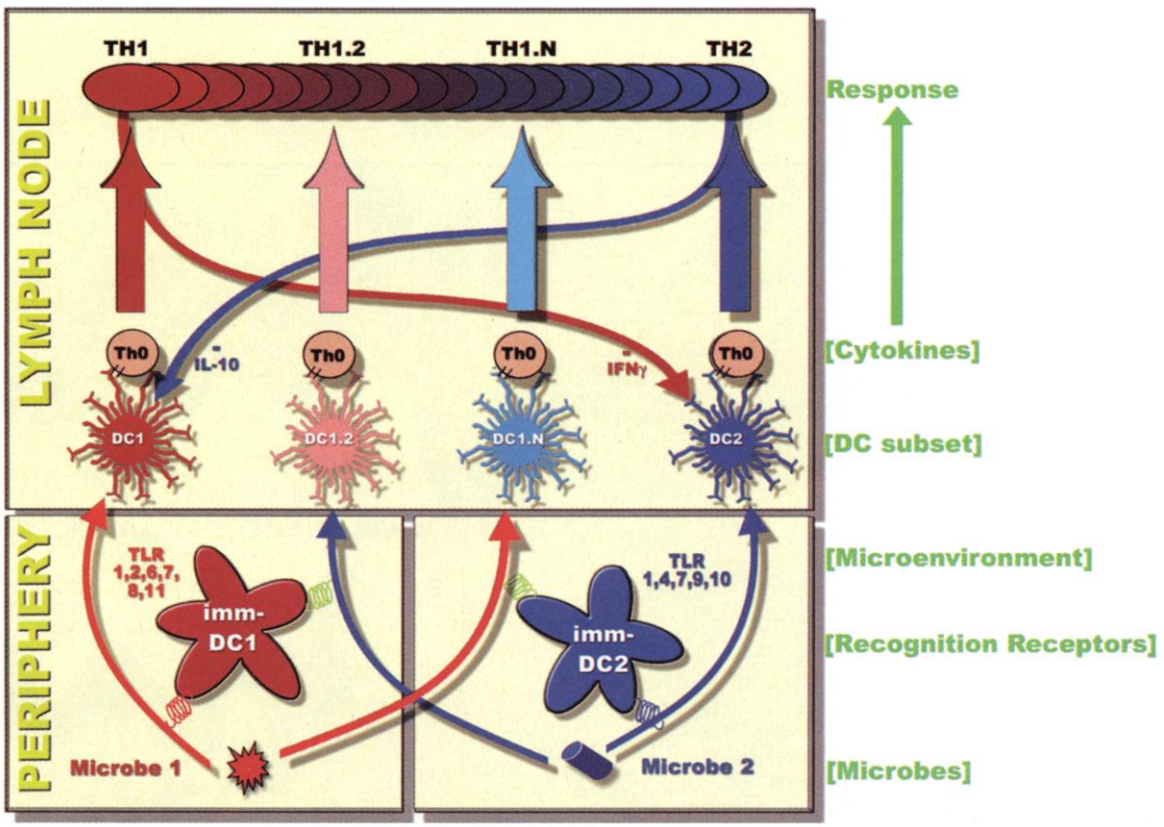

Fig. 1. How DCs tune the adaptive immune response. DCs integrate diverse signals from the environment and their own genes to determine the type of immune response. Different subsets of immature (imm-) DCs at the infection site may express broadly different repertoires of microbe recognition receptors (such as TLRs) and possess some genetically hard-wired differences in their T_H induction potentials. However, microbial stimuli will also exert key influences. For example, stimuli from microbe 1 may be preferentially recognized by immature DC1s to yield a strong T_H1 response, but may also prompt immature DC2s toward T_H1 induction. In the T cell areas of the proximal lymph nodes, cytokines released by the T cells may also regulate DCs. Thus, IL-10 inhibits DC1s and IFN-γ inhibits DC2s. Therefore, the immune response against a microbe can be expressed mathematically, as a complex function, as follows: Immune response = *f*(Microbial stimuli)(DC subset)(Recognition receptor)(Microenvironment)(Cytokines).

nodes by binding to the lectin DC-SIGN on peripheral DCs (*36*).

Like pathogens, immunologists too are learning to exploit DCs in immunotherapy. Antitumor responses can be induced in mice by DCs loaded with tumor antigens (*37*) or by DC in vivo growth factors such as Flt3-L (*2*, *7*). These strategies are currently being tested in cancer patients. The ultimate challenge is to design vaccines that induce optimally effective immunities in different clinical settings by modulating DC function in vivo. The viability of such strategies is clearly demonstrated by pathogens in their tragic experiments in nature: the specter of infectious diseases. Therefore, learning how pathogens manipulate DCs may offer us novel strategies to make the vaccines of the 21st century. Key emerging areas of research are: (i) studying how microbes modulate DC function and gene expression; (ii) determining the DC receptors and signaling pathways through which such microbial stimuli act; (iii) using this information to design small molecules that activate DCs in a particular way, so as to stimulate a given immune response; and (iv) designing vectors that target these small molecules to the appropriate DC subset in vivo. Such strategies may offer vaccines and drugs that stimulate optimally effective immunities against infections or cancers, or those that dampen the response in autoimmunity or transplantation. Microbes have taken hundreds of millions of years to accomplish this feat. We, however, cannot afford to take that long!

References and Notes

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