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Review
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Translating Innate Immunity into Immunological Memory: Implications for Vaccine Development

Bali Pulendran[1,*] and Rafi Ahmed[2,*]
[1] Department of Pathology
[2] Department of Microbiology and Immunology
Emory Vaccine Center, 954 Gatewood Road, Atlanta, GA 30329, USA
*Contact: bpulend@rmy.emory.edu (B.P.); ra@microbio.emory.edu (R.A.)
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Vaccination is the most effective means of preventing infectious diseases. Despite the success of many vaccines, there is presently little knowledge of the immunological mechanisms that mediate their efficacy. Such information will be critical in the design of future vaccines against old and new infectious diseases. Recent advances in immunology are beginning to provide an intellectual framework with which to address fundamental questions about how the innate immune system shapes adaptive immunity. In this review, we summarize current knowledge about how the innate immune system modulates the quantity and quality of long-term T and B cell memory and protective immune responses to pathogens. In addition, we point out unanswered questions and identify critical challenges, the solution of which, we believe, will greatly facilitate the rational design of novel vaccines against a multitude of emerging infections.

Introduction

A hallmark of the immune system is its ability to remember an encounter with a pathogen for several decades, even for a whole lifetime (Kaech et al., 2002). This fundamental property of the immune system is the basis for vaccination, and the goal of a successful vaccine is to induce long-term protective immunity against a given pathogen. A central component of vaccines are immune stimulating agents called adjuvants that enhance both the magnitude and duration of immune responses. The nature of the adjuvant can determine the particular type of immune response, which may be skewed toward cytotoxic T cell (CTL) responses, antibody responses, or particular classes of T helper (Th) responses and antibody isotypes (Pulendran, 2004; Rappuoli, 2004). This is of vital importance since effective protection against different pathogens requires distinct types of immune responses. Despite the common origins of vaccinology and immunology in the pioneering work of Pasteur, Jenner, and others, most of our best vaccines have been empirically derived (Plotkin, 2005; Rappuoli, 2004). Therefore, we are largely ignorant of the immunological mechanisms by which our best vaccines work. Furthermore, the most commonly used empirical adjuvants in clinical practice, alum and the oil-based formulation MF59, while useful for enhancing antibody responses, have no discernible effect on the cellular immune responses. However, recent advances in immunology have triggered research into the mechanisms that underlie the innate and adaptive immune responses against pathogens and vaccines. This information will aid in the development of vaccines against pathogens such as HIV, TB, malaria, and dengue, which have posed difficult challenges.

Emerging concepts in innate immunity suggest that dendritic cells (DCs) play a critical role in sensing pathogens or vaccines, either directly or indirectly, and integrating this information to regulate the quantity, quality, and longevity of the adaptive immune response (Janeway and Medzhitov, 2002; Banchereau et al., 2000; Shortman and Liu, 2002). In this review, we will discuss these advances in innate and adaptive immunity and their relevance to vaccine development. This review is divided into four parts. (1) The first part (Innate Immunity: The Science of Adjuvants) examines how the innate immune system "senses" pathogens and vaccines by recognizing pathogen-associated molecular patterns (PAMPs) via pathogen recognition receptors (PRRs) such as Toll-like receptors (TLRs), which are expressed by DCs (Germain, 2004; Beutler, 2004; Janeway and Medzhitov, 2002; Takeda et al., 2003). This section briefly summarizes the biology of TLRs and other PRRs and then discusses the immunological consequences of triggering distinct PRRs on DCs. (2) The second section (Programming Adaptive Immunity with Innate Immunity) discusses the critical parameters of T and B cell responses that are controlled by the innate immune system. The goal of this section is to explore the functional elements of innate immunity and their roles in shaping the quantity and quality of T and B cell memory (and thus protective immunity) and identify fundamental gaps in our knowledge, which

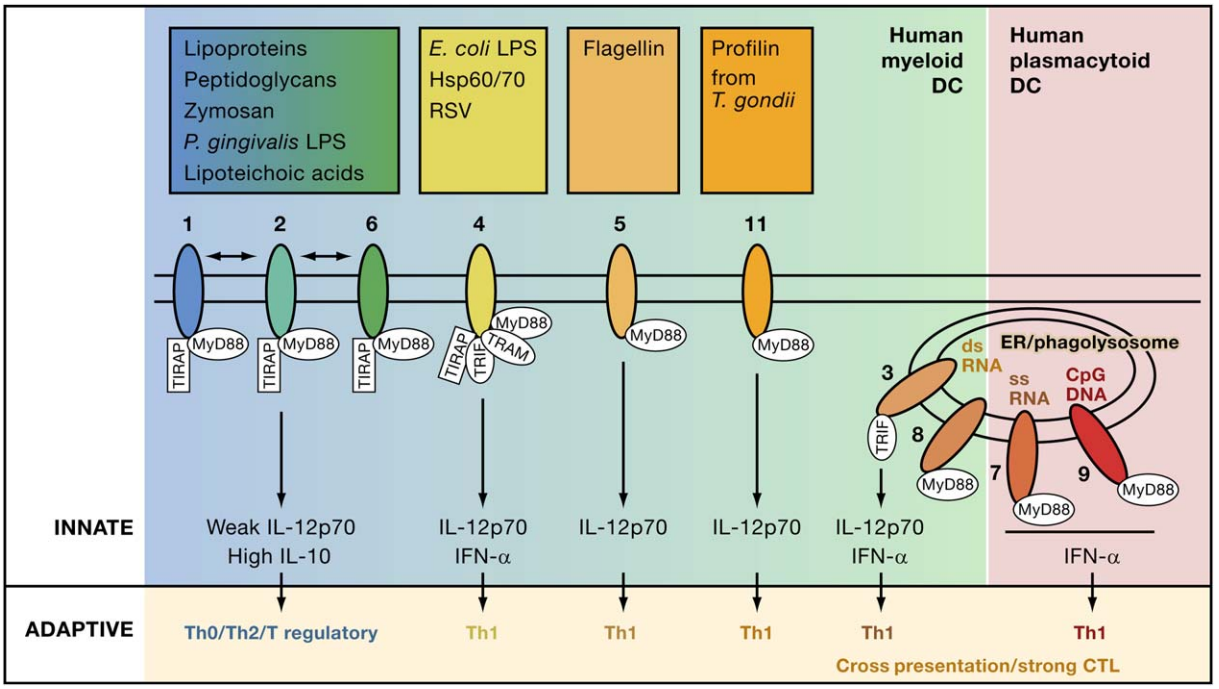

Figure 1. Triggering Distinct TLRs on DCs Elicits Different Cytokine Profiles and Different Immune Responses

Triggering DCs through TLRs 1–10 results in the induction of distinct DC responses and adaptive immunities. In humans, TLRs 9 and 7 are expressed in the ER/phagolysosomes of plasmacytoid DCs, and triggering these yields robust IFN-α, which subsequently biases toward Th1 responses and also favors crosspresentation, $CD8^+$ T cell priming, and CTLs. Human myeloid DCs express TLR2 (heterodimerized with TLRs 1 or 6); TLR3 (in ER/phagolysosomes); and TLRs 4, 5, 8, and 11 on the surface. Stimulation of TLRs 3, 4, 5, or 8 is known to yield robust IL-12p70 and potent Th1 responses. Stimulation of TLRs 3 or 4 is also known to yield some IFN-α and favor crosspresentation and $CD8^+$ T cell priming. However, stimulation of TLR2 can often yield a response characterized by little IL-12p70, robust IL-10, and a bias toward Th2 or T regulatory responses. Interestingly, the TLRs associated with different adaptor proteins, which mediate distinct functions. For example, MyD88 signaling mainly yields proinflammatory cytokines (IL-12, IL-6, and TNF). Signaling via TRIF or TRAM induces robust type 1 interferons and upregulates costimulatory molecules.

represent critical challenges for the future. (3) The third part (Learning Immunology from "Good" Vaccines) discusses the merits of studying empirically derived, "successful" vaccines such as the yellow fever vaccine (YF-17D) to unravel the innate immune receptors and immunological mechanisms by which they mediate protection, with a view to exploiting this knowledge in the design of future vaccines. (4) In the final section (Some Critical Immunological Challenges for Vaccinologists), we conclude by briefly summarizing the critical challenges discussed in the preceding sections that could guide the translation of innate immunity to vaccine-induced protective immunity.

Innate Immunity: The Science of Adjuvants

Although adjuvants have long been known to shape the quality and quantity of immune responses, it is only recently that the mechanisms of their action are beginning to be revealed. A key event that triggers the immune response is when the immune system "senses" the vaccine or microbe. Information sensed about the vaccine or microbe is integrated by DCs and translated to antigen-specific T and B cells to modulate the strength, quality, and persistence of the adaptive immune response.

Direct Innate Immune Sensing

Since the review by Akira and colleagues in this issue of *Cell* (Akira et al., 2006) provides a detailed account of this topic, we will only discuss it briefly here. The innate immune system can recognize microbes directly through various PRRs expressed in, and on, DCs. An important family of PRRs are the TLRs (Germain, 2004; Beutler, 2004; Janeway and Medzhitov, 2002; Takeda et al., 2003), which are widely expressed on innate immune cells, including DCs, macrophages, mast cells, neutrophils, endothelial cells, and fibroblasts. TLRs have broad specificity for conserved molecular patterns shared by bacteria, viruses, and parasites (Figure 1). Moreover, different TLRs are expressed by distinct subpopulations of DCs and in distinct cellular compartments. TLR4, for example, is expressed on the surface membrane of human myeloid DCs and monocytes and is essential for the recognition of LPS (Beutler, 2004). In contrast, TLR9 appears to be expressed in the endosomal compartment of plasmacytoid DCs (pDCs) and B cells and is involved in the recognition of viral and intracellular bacterial DNA (Takeda et al., 2003). Signaling via TLRs on innate immune cells often represents the trigger for an adaptive immune response.

Although much research has focused on the TLR family as innate sensing receptors, emerging evidence also points to other families of plasma-membrane and cytoplasmic receptors, including the C type lectins and NOD proteins (see Akira et al., 2006, Geijtenbeek et al., 2004; Inohara et al., 2005). The C type lectins such as DC-SIGN recognize a range of microbial stimuli from pathogens such as HIV, HCV, *Helicobacter pylori*, and *Mycobacterium tuberculosis*. NOD proteins recognize components of intracellular bacteria. Interestingly, mutations in the gene encoding NOD2 have been associated with increased frequencies of inflammatory bowel disease, suggesting a role for these proteins in regulating inflammation (Inohara et al., 2005). In the case of viruses, in addition to TLR-dependent recognition, viral nucleic acids can also signal through TLR-independent mechanisms. For example, RIG-I and melanoma differentiation-associated gene 5 (Mda5; also called helicard), both intracellular RNA helicases, can sense dsRNA (for more information, see Akira et al., 2006).

PRRs and DC Subsets

The adaptive immune system has evolved diverse responses to defend the host against a myriad of different pathogens. For example, immune responses are diverse with respect to the cytokines made by Th cells and the class of antibodies secreted by B cells. Thus, in response to intracellular microbes or viruses, $CD4^+$ Th cells differentiate into Th1 cells, which produce IFN-γ and help the induction of $CD8^+$ cytotoxic T cells (CTLs), which kill the cells infected with the intracellular pathogens; in contrast, helminths induce the differentiation of Th2 cells, whose cytokines (principally IL-4, IL-5, and IL-10) induce IgE and eosinophil-mediated destruction of the pathogens (O'Garra and Robinson, 2004; Mowen and Glimcher, 2004). Furthermore, so-called T regulatory cells that suppress the proliferation and differentiation of Th or cytotoxic T cells serve to limit the potential immunopathology that might be caused by an overexuberant immune response. Interestingly, specific pathogens have evolved mechanisms to induce T regulatory cells, most likely as an immune evasion strategy to suppress host immunity (O'Garra and Vieira, 2004). How does the immune system generate such a diversity of responses? The existence of multiple subsets of DCs raises the question of whether they are functionally specialized to promote distinct immune responses (Pulendran, 2004, 2005; Shortman and Liu, 2002). In mice, for example, the splenic $CD8\alpha^+$ versus $CD8\alpha^-$ DCs can differentially influence the Th1/Th2 balance (Pulendran et al., 1999; Maldonado-Lopez, 1999). Furthermore, plasmacytoid DCs in humans and mice preferentially produce IFN-α (reviewed by Liu, 2005). In part, such functional specialization seems to be achieved by the differential distribution of TLRs on distinct DC subsets—for example, in humans, TLR9 and TLR7, which mediate robust induction of type I IFNs, are preferentially expressed on plasmacytoid DCs (Figure 1). However, despite these examples of functional specializations, DC subsets also exhibit considerable functional plasticity. Thus, activation of a given DC subset via distinct PRRs can differentially program that subset to stimulate distinct types of T cell responses (Pulendran, 2004, 2005).

Indirect Innate Immune Sensing

DCs can also sense pathogens indirectly by detecting inflammatory mediators produced by various cells such as macrophages, NK cells, NK T cells, mast cells, and endothelial cells (Germain, 2004; Beutler, 2004; Janeway and Medzhitov, 2002; Takeda et al., 2003). Such "danger signals" (Matzinger, 1994) include heat-shock proteins (Srivastava, 2002) and uric-acid crystals (Shi et al., 2003). Therefore, DCs are equipped with multiple surveillance mechanisms that can sense pathogens either directly or indirectly and thus represent an important nodal point in which pathogen- or vaccine-associated signals are integrated and transmitted to the adaptive immune system.

Programming Adaptive Immunity with Innate Immunity

Despite the paucity of information on the correlates of protective immunity against pandemics like HIV, TB, and malaria, growing evidence suggests that prophylactic vaccines against such diseases must be capable of stimulating (1) long-lived, antigen-specific plasma cells that produce neutralizing antibody; (2) persisting $CD4^+$ and $CD8^+$ T cells; and (3) migration of pathogen-specific T and B cells to mucosal sites. Exactly what parameters of innate immune activation regulate (1)–(3) is still largely a mystery and will be considered below.

T Cell Immunity: Programming Memory T Cell Generation and Maintenance

Adaptive immune responses are initiated in the T cell-rich areas of the secondary lymphoid organs, where naive T cells encounter antigen-bearing DCs that have migrated there from the site of vaccination. Before vaccination, antigen-specific T cells are present in the host at very low frequencies (Kaech et al., 2002). After vaccination, these "naive" T cells expand ("clonal expansion" phase, 7–10 days) to reach a markedly higher frequency (as much as 100,000-fold higher, in the case of antigen-specific $CD8^+$ T cells responding to many viruses; Murali-Krishna et al., 1998; Butz and Bevan, 1998). Such proliferating cells differentiate into effector T helper cells or cytotoxic T lymphocytes.

After removal of the antigen, there is a phase of numerical reduction of antigen-specific cells ("clonal contraction" phase, 2–4 weeks) whereby a subset of effector T cells survive and further differentiate into long-lasting memory T cells whose numbers are maintained over time ("maintenance of memory" phase) (Kaech et al., 2002). Despite the fact that these three phases have been studied in some detail, the question of whether they occur in different microenvironments has not been thoroughly explored. This is in contrast to the situation with B cells, where microenvironments like germinal centers and plasma foci represent the sites where somatic hypermutation of immunoglobulin genes in developing memory B cells and the generation of short-lived antibody-forming cells occur,

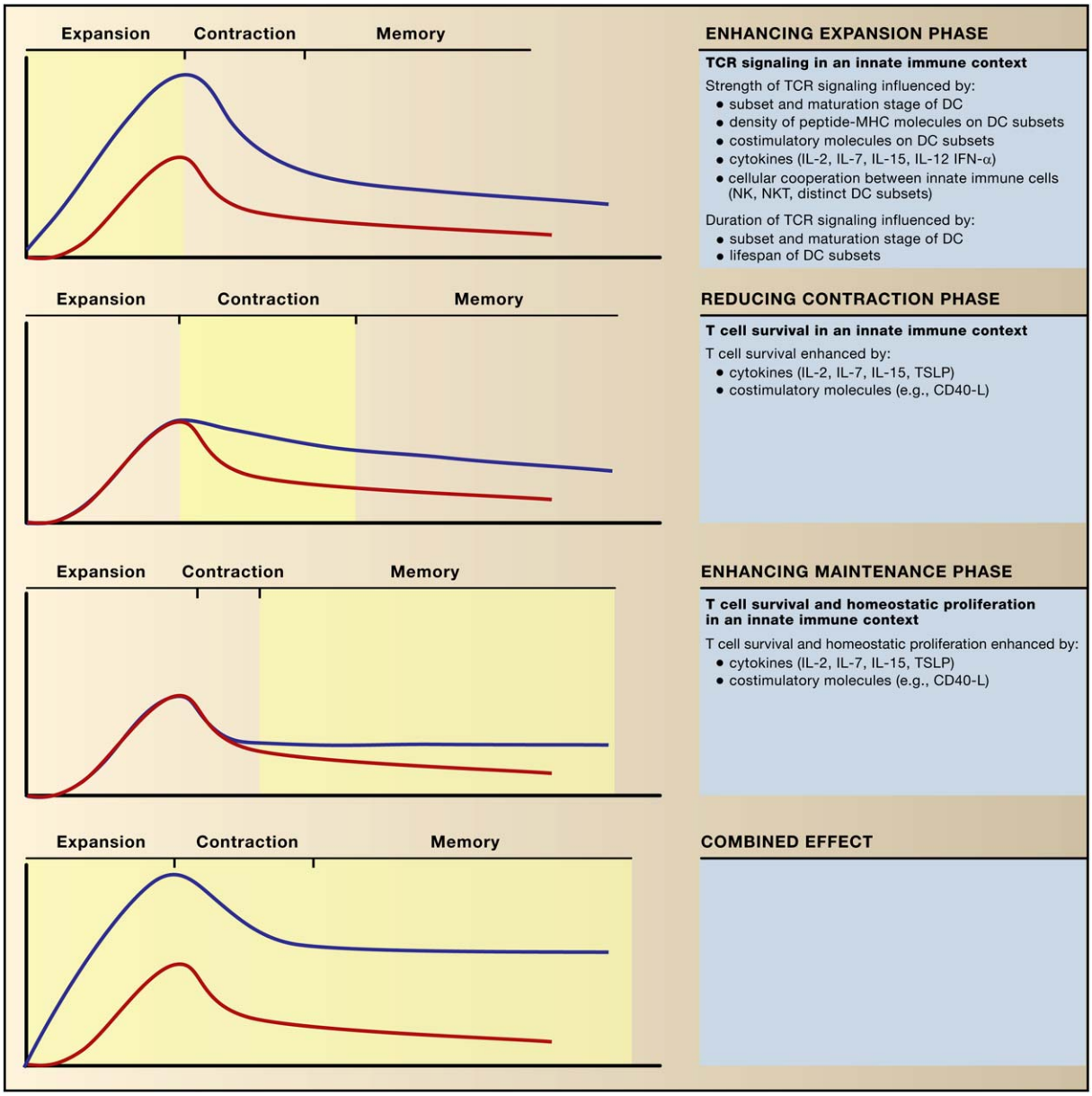

Figure 2. Innate Variations on a Theme of Memory

Multiple ways in which the innate immune system might modulate the clonal expansion, clonal contraction, and maintenance of memory during an antigen-specific T cell response.

respectively (Ahmed and Gray, 1996; McHeyzer-Williams and McHeyzer-Williams, 2005). With T cells also, it is possible that clonal expansion, clonal contraction, and memory-cell maintenance occur in specialized microenvironments under the control of innate immune mechanisms.

Enhancing the Quantity of Memory T Cells. It may be safely assumed that increased numbers of memory T cells result in enhanced protection against a pathogen, although there might be a threshold number of memory T cells above which no further benefits for protection are evident. In principle, the quantity of memory T cells might be regulated by (1) enhancing the expansion phase, (2) reducing the contraction phase, (3) stabilizing the memory phase, or (4) some combination of these strategies (Figure 2).

Enhancing Clonal Expansion. Accelerating the generation of effector $CD8^+$ T cells by vaccination may be crucial to control pathogens that cause rapid disease and morbidity, including biothreat agents like *Bacillus anthracis* and *Yersinia pestis*. The rate of T cell expansion is a function of the number of naive T cells recruited into the response, the rate of T cell proliferation, and the rate of

cell death. These, in turn, are dependent on the "context" in which the T cell recognizes antigen, the abundance of antigen, and the duration of antigen exposure during the expansion phase, all of which can be regulated by innate immune mechanisms.

Within the T cell-rich areas of the lymphoid organs, antigen-bearing DCs and the rare, antigen-specific naive T cells must find each other. Recent estimates suggest that the precursor frequency of naive epitope-specific CD8 T cells is on the order of 1 in 2×10^5. Thus, in an uninfected mouse containing $\sim$2–4 $\times$ 10^7 naive CD8 T cells, there are estimated to be 100–200 epitope-specific cells (Blattman et al., 2002). Therefore, in any given draining lymph node, this is likely to be considerably smaller—perhaps only 10 or 20. Finding one of those T cells in a lymph node may thus seem challenging, especially if DCs have a relatively short half-life. In the apparent absence of microbial stimulation, the rate of migration of tissue DCs into the draining lymph nodes occurs constitutively but at a relatively low level. Such DCs have engulfed dead host cells and present "self-antigens" to naive T cells so as to establish and maintain peripheral tolerance to self (Hawiger et al., 2001). However, in response to signals from pathogens or adjuvants (e.g., TLR ligands), resident immature DCs at the site of infection or vaccination undergo a maturation program characterized by enhanced expression of costimulatory molecules and inflammatory cytokines and migrate to the draining lymph nodes. The increased cytokine and chemokine levels at the site of infection also result in the recruitment of monocytes and DC precursors from the blood. When such precursors enter the site of infection, they rapidly differentiate into DCs and replenish the depletion of DCs from that site (Banchereau et al., 2000; Shortman and Liu, 2002). The precise roles of distinct DC subsets such as Langerhans cells, dermal DCs, and plasmacytoid DCs in this process are largely unknown. Recent work, however, suggests the concerted action of resident DC subsets and DCs that migrate from the periphery in serial waves (Itano et al., 2003), each of which are specialized to perform distinct functions such as ferrying the antigen to the site, crosspresentation to $CD8^+$ T cells, IL-12 secretion, and IFN-α secretion. Under these inflammatory conditions, the T cell areas of the draining lymph nodes receive large numbers of highly stimulatory DCs. This influx results in an increased density of antigen-MHC complexes, costimulatory molecules, and proinflammatory cytokines. Furthermore, mature DCs extend many long dendrites, which can cover a considerable volume, thus enhancing the potential zone of DC-T cell contacts, as demonstrated by elegant studies using two-photon laser scanning microscopy (Miller et al., 2002). It has also been suggested that the chemokines secreted by activated DCs such as thymus and activation-regulated cytokine (TARC) or macrophage-derived chemokine (MDC) attract antigen-specific T cells (Tang and Cyster, 1999; Lanzavecchia and Sallusto, 2001), although this is yet to be clearly demonstrated in vivo by live-tissue imaging studies (Huang et al., 2004).

Once the DC has located the antigen-specific T cell and recruited others to the vicinity, several events facilitate productive T cell activation. In vitro studies suggest that at the site of cell-cell contact, the "immunological synapse," proteins segregate into two concentric areas: (1) a central area known as c-SMAC (central supramolecular activation cluster), where the TCR-MHC and additional short molecules such as CD2, CD28, protein kinase C-θ (PKC-θ), Lck, Fyn, CD4, and CD8 cluster, and (2) a peripheral outer ring known as p-SMAC, containing larger molecules such as LFA-1 and CD45, which strengthen the synapse (reviewed by Bromley et al., 2001). Although c-SMAC formation does not appear to be critical to initiate TCR signaling, it is believed that c-SMACs enhance T cell activation by concentrating the TCR and MHC in a single area for sustained periods, thus facilitating a "serial triggering" mechanism, allowing one MHC-peptide complex to trigger repeatedly through as many as 200 TCR molecules (Lanzavecchia and Sallusto, 2001). Thus, differences in timing, spacing, and molecular composition of the synapse can strongly influence the magnitude of the T cell response. An important challenge is to determine whether c-SMAC and p-SMAC structures observed in vitro actually form in vivo. In this context, recent in vivo imaging studies with two-photon microscopy suggest that, for $CD8^+$ T cells, interactions with DCs occur in three successive stages: (1) transient serial encounters during the first 8 hr, in which the T cells progressively decrease their motility and upregulate activation markers; (2) long-lasting (>1 hr) stable conjugates with DCs during the next 12 hr, in which the T cells begin to secrete interleukin-2 and interferon-γ; and (3) T cell migration on the second day, coinciding with the onset of proliferation and reduction of DC contacts (Mempel et al., 2004). Consistent with these kinetics, T cells appear to be able to commit to clonal expansion after less than 24 hr of antigenic stimulation. $CD8^+$ T cells especially appear to begin a program of cell differentiation and proliferation within hours of exposure of the "parental" cell to antigenic stimulus that results in 7–10 cell divisions over the course of the next few days (Kaech and Ahmed, 2001; van Stipdonk et al., 2001; Mercado et al., 2000).

Once synapse formation is underway, commitment of T cells to clonal expansion can be regulated by the strength and duration of TCR signaling, which is dependent on the context in which the T cell sees the antigen—for instance, the subset of DCs, the density of peptide-MHC complexes on the surface of DCs, the duration of DC-T cell interactions, and the life span of DCs (Lanzavecchia and Sallusto, 2001). For example, the induction of the prosurvival molecule bcl-X_L in DCs is known to augment immune responses (Josien et al., 2000). Furthermore, specific molecules expressed on the surface of DCs (so-called costimulatory molecules) can provide additional signals that augment the interaction of TCR with MHC-peptide complexes. For example, molecules of the B7 family such as CD80 and CD86, which are expressed on DCs, bind to their receptor CD28 on T cells and result

in the upregulation of CD40 ligand on T cells. In turn, CD40 ligand binds to CD40 on DCs and "licenses" the DCs to express additional molecules that influence T cell differentiation. Such molecules include cytokines such as IL-12 and IL-18, which are secreted by DCs and induce IFN-γ production by T cells, thus favoring robust Th1 immunity (Lanzavecchia and Sallusto, 2001). In addition, cytokines such as IFN-α secreted by pDCs (Liu, 2005), or proinflammatory cytokines such as TNF-α or IL-6, may act by enhancing costimulatory molecules and immunogenicity of DCs themselves (Le Bon et al., 2003); they may also act directly on T cells to induce robust $CD8^+$ T cell expansion (Kolumam et al., 2005) or bypass suppressor effects of T regulatory cells (Pasare and Medzhitov, 2003). Thus, adjuvants that enhance DC survival, induction of costimulatory molecules, and controlled release of type I IFNs and proinflammatory cytokines may be particularly effective in inducing T cell expansion. However, these factors must be strictly regulated since prolonged antigenic stimulation (Zajac et al., 1998; Moskophidis et al., 1993), chronic stimulation with DCs (Menges et al., 2001), or inappropriate timing of exposure to IFN-α (Nagai et al., 2003) can impair T cell differentiation.

In addition, signals from other innate immune cells, such as NK or NK T cells, can also influence clonal expansion. In contrast to conventional T cells that recognize peptide antigens, NK T cells recognize glycolipids via the CD1d receptor. Activation of NK T cells in mice by injection of α-GalCer, a glycolipid ligand for CD1d, induces rapid release of cytokines and stimulates activation of NK cells, DCs, and T cells. Immune responses to injection of α-GalCer-loaded mature DCs in patients with advanced cancers resulted in a more than 100-fold increase in expansion of several subsets of NK T cells for up to 6 months after vaccination. NK T activation was associated with an increase in serum levels of IL-12 p40 and IP-10, factors associated with robust Th1 responses. In addition, there was an increase in memory $CD8^+$ T cells specific for cytomegalovirus in vivo in response to α-GalCer-loaded DCs (Chang et al., 2005). Furthermore, recent evidence suggests that NK cell-secreted IFN-γ augments Th1 responses and that this occurs via TLR signaling (Martin-Fontecha et al., 2004).

Reducing Clonal Contraction. Emerging evidence suggests that clonal contraction can also be influenced by several cell-extrinsic factors, such as the cytokine environment, costimulatory molecules, and the strength and duration of signaling by antigen-bearing DCs. With respect to cytokines, type I IFNs, as well as members of the IL-2 family (e.g., IL-2, IL-4, IL-7, and IL-15, which share a common γ chain receptor), can enhance T cell survival (Marrack and Kappler, 2004). Consistent with this, newly emerging memory $CD8^+$ T cells express IL-7R (Kaech et al., 2003). In addition to such cytokines, certain costimulatory molecules can regulate cell death. For example, mice deficient in CD40 ligand display enhanced death of $CD8^+$ T cells and 10-fold lessened memory-cell formation after viral infection, but the clonal expansion phase appears to be normal (Whitmire and Ahmed, 2000).

Stabilizing the Memory Phase. In the case of $CD8^+$ T cells, the memory phase seems relatively stable, suggesting that the rate of cell death is equal to the rate of cell division, known as "homeostatic proliferation," which appears to operate in the absence of antigenic stimulation (Murali-Krishna et al., 1999). However, with $CD4^+$ T cells, there appears to be a decline in antigen-specific memory T cells after an initial immune response to lymphocytic choriomeningitis virus (LCMV) in mice (Homann et al., 2001). On the other hand, the numbers seem more stable in humans vaccinated against smallpox (Hammarlund et al., 2003). Clearly, factors that enhance cell division or reduce cell death are important in maintaining the numbers of memory T cells. Cytokines such as IL-15, IL-7, and thymic stromal lymphopoietin (TSLP) may be important in both processes (Soumelis et al., 2002). For example, memory $CD8^+$ cells in bone marrow undergo more vigorous homeostatic proliferation and respond faster to antigen stimulation than those in other tissues (Di Rosa and Santoni, 2002; Becker et al., 2005). Whether such cytokines are constitutively produced by specific cell types in the bone marrow is not known. Furthermore, similar to in recent studies with memory B cells (McHeyzer-Williams and McHeyzer-Williams, 2005), certain subsets of T cells express TLRs (Peng et al., 2005). Although expression of such molecules in memory T cells deserves further study, it is an intriguing concept that continuous TLR signaling in response to low levels of microbial stimuli might affect homeostatic proliferation. As will be appreciated from the above discussion, an important challenge is the elucidation of mechanisms by which innate immunity and adjuvants regulate memory T cell generation and maintenance (critical challenge 1).

A counterpoint to understanding the influence of innate immunity in memory T cell generation is to appreciate how several pathogens, particularly those that cause chronic infections such as HIV, HCV, and TB, can interfere with this process to induce dysfunctional T cells. In this context, our recent data suggest that, in mice chronically infected with LCMV clone 13, antigen-specific $CD8^+$ T cells express very high levels of PD-1, a ligand for PD-L1 (Barber et al., 2006), another member of the B7 family, which, unlike CD80 and CD86 discussed above, is known to be an inhibitory receptor for T cells (Greenwald et al., 2005). Interestingly, blockade of PD-L1/PD-1 interaction in vivo with an antagonistic antibody relieves the inhibitory effect, restores T cell function, and reduces viral load (Barber et al., 2006).

Modulating the Quality of Effector and Memory T Cells with Innate Immunity. As discussed above, a hallmark of adaptive immunity is the existence of qualitatively different types of responses, such as Th1, Th2, and T regulatory responses (O'Garra and Robinson, 2004; Mowen and Glimcher, 2004; O'Garra and Vieira, 2004). In addition, differentiating memory $CD4^+$ and $CD8^+$ T cells can be subdivided into the central memory and effector memory

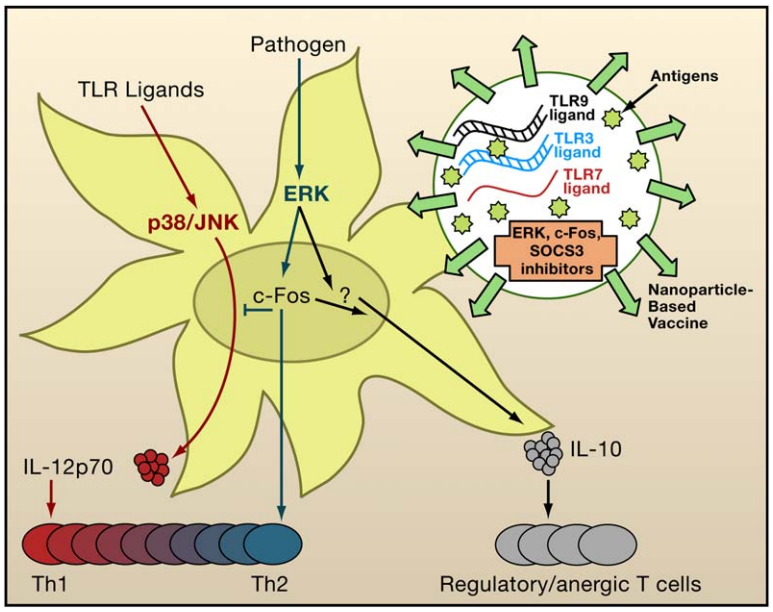

Figure 3. Dendritic-Cell Signaling Pathways that Induce Th2 or T Regulatory Responses, and Possible Strategies to Overcome Such Pathways

Activation of the p38 and JNK MAPKs in DCs mediates robust IL-12p70 and Th1 responses. However, activation of the ERK MAPK results in IL-10 production and impairment of IL-12p70 in part via the AP-1 transcription factor c-Fos, a repressor of IL-12 (Dillon et al., 2004; 2006). Since IL-10 is known to exert potent regulatory influences on DCs and T cells, it is conceivable that such pathways might operate in DCs in chronic infections or tumors. Thus, vaccination strategies that encompass inhibitors of the ERK/c-Fos pathway together with TLR ligands might serve to "ease the regulatory brakes" while "stepping on the accelerator" via TLR activation. Such strategies might include nanoparticles into which inhibitors of the ERK/c-Fos pathway or other regulatory proteins such as STAT3 or SOCS1 are encapsulated, together with the relevant antigen and multiple TLR ligands.

subsets, which differ in their phenotype, function, and homing properties (Sallusto et al., 2004). A central problem is how the innate immune system regulates the generation of qualitatively distinct types of adaptive immune responses. This is considered below.

Innate Immune Control of T Helper Differentiation. Several studies suggest that distinct subsets of DCs differentially modulate T helper responses (Maldonado-Lopez, 1999; Pulendran et al., 1999; Rissoan et al., 1999). However, the nature of the microbial stimulus and local microenvironmental cues (Shortman and Liu, 2002; Pulendran, 2005) also play important roles in tuning the T helper response. For example, viruses stimulate IFN-α-mediated differentiation of plasmacytoid DCs into Th1-inducing DCs (Liu, 2005), whereas IL-3 induces such DCs to differentiate into Th2-inducing DCs (Rissoan et al., 1999). Different forms of the fungus *Candida albicans* instruct DCs to induce either Th1 or Th2 responses, and *E.coli* LPS induces IL-12(p70) in DCs, which elicits Th1 responses, while *P. gingivalis* LPS, schistosome egg antigens (SEA), filarial nematode-secreted products, or cholera toxin fail to induce IL-12(p70) and stimulate Th2-like responses (reviewed in Pulendran, 2005). The cytokines and chemokines in the local microenvironment can also modulate DC function and immune responses. For example, Peyer's patch or respiratory-tract DCs prime Th2 responses, while total spleen DCs prime Th1/Th0 responses (Iwasaki and Kelsall, 2001). Furthermore, human epithelial cells trigger DC-mediated allergic inflammation by producing TSLP (Soumelis et al., 2002). These results are consistent with observations that DCs in distinct microenvironments induce different Th responses.

The nature of the PRRs that modulate DC cytokines and the ensuing immune response is an area of active study. Most TLRs induce strong IL-12(p70) from DCs, which subsequently stimulate Th1 responses (Brightbill et al., 1999). However, only a subset of the TLRs (TLRs 3, 4, 7, and 9) can induce type 1 interferons, which are important for antiviral defense (Liu, 2005). Emerging evidence suggests that TLR2 ligands can stimulate Th2 or T regulatory responses (Agrawal et al., 2003b; Dillon et al., 2004, 2006; Redecke et al., 2004). The molecular mechanism by which specific TLR2 ligands favor a Th2 bias remains to be established, although recent work suggests that the robust and sustained phosphorylation of ERK MAP kinase results in phosphorylation of the AP-1 transcription factor c-Fos in DCs, which in turn suppresses expression of the Th1-defining cytokine IL-12, thus favoring a Th2 bias (Agrawal et al., 2003b; Dillon et al., 2004) (Figure 3).

In the case of tolerogenic T cell responses, the yeast cell-wall particle zymosan, which consists of several carbohydrates, signals through both TLR2 and the C type lectin dectin-1 and activates DCs to secrete the anti-inflammatory cytokine IL-10 via a mechanism dependent on the induction of ERK MAP kinase. Zymosan also induces macrophages in the splenic red pulp to secrete TGF-β, another anti-inflammatory cytokine (Dillon et al., 2006). Consistent with these effects on antigen-presenting cells (APCs), injection of zymosan plus antigen into mice results in suppression of the T cell response via a mechanism dependent on IL-10 and TGF-β (Dillon et al., 2006). These data suggest several targets for pharmacological modulation of immune responses in various clinical settings (Figure 3). It is conceivable that such regulatory innate immune networks are induced by pathogens that cause chronic infections or by tumors and result in induction of T regulatory cells and suppression of immunity against the pathogens or tumors (Figure 3) (O'Garra and Vieira, 2004). Thus, an attractive therapeutic strategy might be to inhibit regulatory signaling networks within DCs, in addition to providing

adjuvant signals that enhance the immune response. For example, such a strategy might consist of vaccine nanoparticles containing inhibitors of the ERK/c-Fos pathway together with specific combinations of TLR ligands or inhibitors of other molecules such as the Janus kinase STAT3 (Nefedova et al., 2004) or suppressor cytokine signaling 1 (SOCS1) (Shen et al., 2004), which have also been shown to exert regulatory functions in DCs (Figure 3). Such strategies may well simultaneously release the "intrinsic breaks" associated with chronic infections or tumors while providing strong stimulatory signals via the TLR ligands. Thus, elucidation of the signaling networks within DCs that regulate Th2 or T regulatory responses and discovery of strategies to relieve such regulatory pathways are needed (critical challenge 2).

Innate Immune Control of Central Memory versus Effector Memory Differentiation. Central memory (TCM) cells express CD62L and CCR7, reside in the T cell areas of lymphoid organs, and respond to antigenic restimulation by proliferating and rapidly differentiating into effector cells. In contrast, effector memory (TEM) cells do not express CD62L and CCR7 but express receptors for migration into inflamed tissues and display immediate effector functions (Sallusto et al., 2004). Based on these observations, a model was proposed in which tissue-homing CCR7-negative effector memory cells serve a sentinel role in the rapid control of invading pathogens. In contrast, the lymph-node-homing CCR7-positive central memory cells would be available in secondary lymphoid organs as a reserve pool of memory cells from which effector cells can develop in case of a future infection (Sallusto et al., 2004). Although this concept was based on data generated in vitro, recent in vivo studies suggest that antigen-specific memory T cells persist in nonlymphoid compartments long after vaccination (Masopust et al., 2001), which suggests that memory cells are indeed capable of homing to such sites in vivo. Consistent with this, recent studies suggest that CCR7 is required for T cell exit from peripheral tissues (Debes et al., 2005). Clearly, an important question is whether innate immune signals regulate the differentiation of central versus effector memory T cells and, if so, what such signals are. Again, as with effector cells, cytokines and the strength and duration of antigenic stimulation appear to play important roles (Manjunath et al., 2001). Recent studies suggest that central memory T cells proliferate in response to IL-2, IL-7, and IL-15 and differentiate into effector T cells (Sallusto et al., 2004). In addition, the strength of the TCR signaling may influence the decision to form central versus effector memory cells (Gett et al., 2003). For example, during the early stages of an immune response, the mass influx of highly stimulatory DCs might deliver strong TCR signals, thus favoring effector T cell differentiation; however, at a later stage, when the influx of highly stimulatory DCs begins to diminish, a milder form of T cell stimulation may favor the generation of central memory T cells. An alternative view is that distinct subsets of DCs or different TLR ligands may differentially induce central versus effector memory cells.

Innate Immune Control of Migration Patterns of Effector T Cells. There is emerging evidence that T cells with distinct phenotypes can home to different tissues (Sallusto et al., 2004). For example, expression of the gut-homing receptors, namely the integrin $\alpha4\beta7$ and the chemokine receptor CCR9, is essential for preferential homing to the gut (Hamann et al., 1994; Kantele et al., 1999; Zabel et al., 1999; Svensson et al., 2002). Retinoic acid enhances the expression of $\alpha4\beta7$ and CCR9 on T cells and imprints them with a gut tropism (Iwata et al., 2004). Since many infections occur via mucosal transmission, a protective $CD8^+$ T cell-based vaccine must elicit memory $CD8^+$ T cells that either are present at the sites of virus entry prior to infection or can promptly migrate to these sites after infection. The expression of mucosal homing receptors is likely to be a key factor in determining the homing behavior of these cells in the genital and rectal mucosae. It was recently shown that the expression of $\alpha4\beta7$ integrin predicts the rapid localization of adoptively transferred antigen-specific $CD8^+$ T cells to mucosal sites (Masopust et al., 2004). Whereas the $\alpha4\beta7$-negative TEM cells migrate preferentially to the spleen, the $\alpha4\beta7^+$ TEM cells are capable of migrating to the mucosal effector tissues (Masopust et al., 2004). It is important to note that these $\alpha4\beta7$-positive effector $CD8^+$ T cells lose $\alpha4\beta7$ expression upon their arrival in the mucosa, where they can survive for long periods of time (up to 1 year). This long-term persistence is associated with high levels of bcl-2 expression and thus appears to be related to long survival rather than to in situ proliferation (Grayson et al., 2000). Thus, experiments that assess the ability of adjuvants to induce prompt migration of antigen-specific $CD8^+$ T cells to mucosal tissues represent a key area for future research (critical challenge 1).

B Cell Immunity: Programming B Cell Differentiation and Antibody Formation

Preexisting antibodies in the circulation and at the mucosa provide the first line of defense against reinfection by extracellular as well as intracellular pathogens. The differentiation of B cells to antibody-producing plasma cells can occur via three distinct pathways. The first two pathways consist of very rapid IgM and IgA antibody production to T cell-independent antigens, and the third is involved in slower IgG antibody production, affinity maturation, and memory B cell formation to T cell-dependent antigens (Figure 4). These are considered below.

Programming B-1 B Cells to Rapidly Generate Neutralizing Antibodies and Shape "Natural Memory." B-1 B cells are primarily found in the peritoneal and pleural cavities but also occur less frequently in the spleen and lymph nodes and differ from conventional (follicular) B cells with respect to localization, phenotype, activation status, and antibody V gene usage (Baumgarth et al., 2005). They appear to differentiate, even in the apparent absence of antigenic stimulation, to produce IgM and IgA or "natural" antibodies (Baumgarth et al., 2005). Such B-1 B cells are also involved in the initial IgM response to T cell-independent antigens. Even though their V gene repertoire is

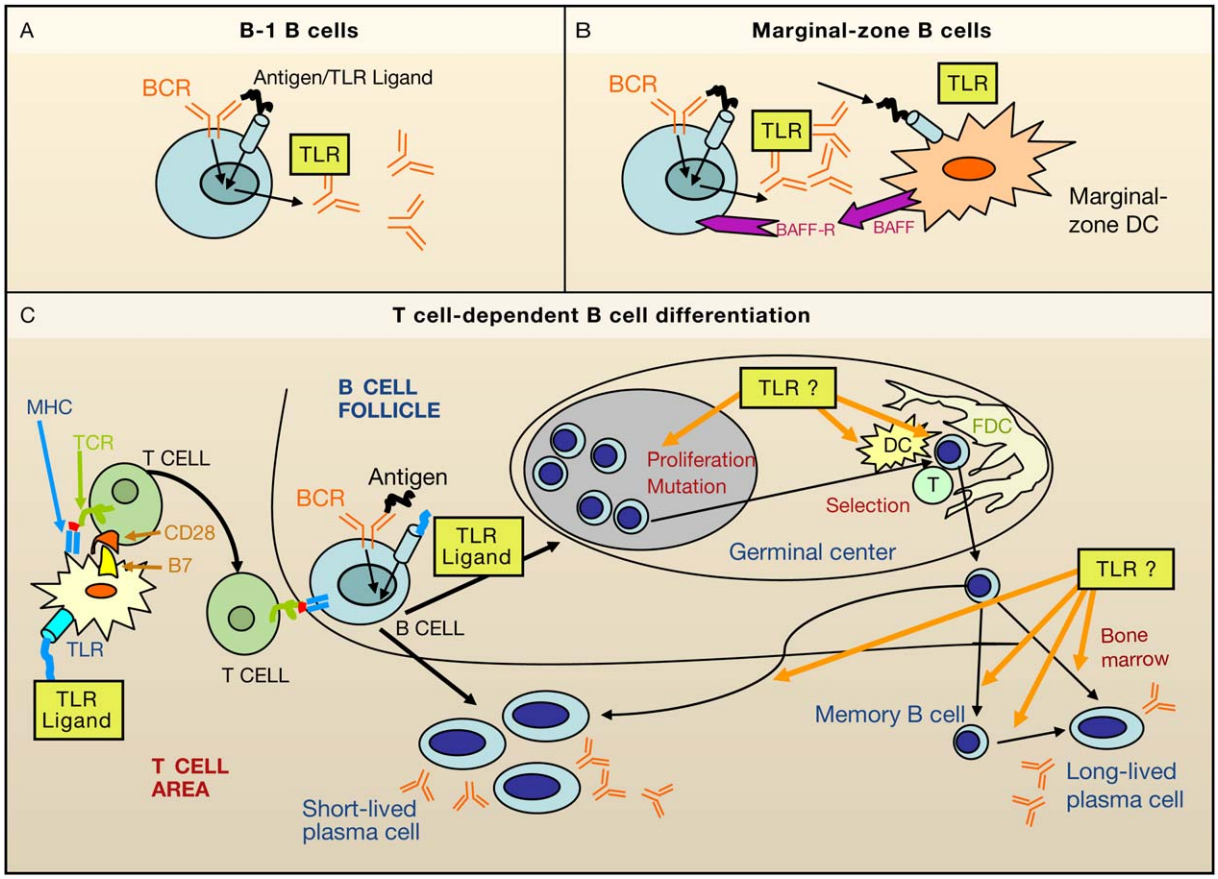

Figure 4. Programming B Cell Responses with Innate Immunity

(A) B-1 B cells are primarily found in the gut and pleural cavities and appear to differentiate even in the apparent absence of antigenic stimulation to produce IgM and IgA "natural antibodies."

(B) Marginal-zone B cells are strategically located in the marginal zone of the spleen, where they play a dominant role in the early IgM response to blood-borne pathogens. Recent studies suggest that immature DCs capture and transport bacteria to the spleen, provide survival signals in the form of BAFF to marginal-zone B cells, and induce their differentiation to plasma cells. Both B-1 B cells (A) and marginal-zone B cells (B) appear to exist in a persistent state of activation. This property might be exploited to generate persistent levels of broadly reactive neutralizing IgM and IgA antibodies reactive to pathogens such as HIV. This might be accomplished using conjugate prophylactic vaccines comprised of the appropriate TLR ligands conjugated with the specific antigens (e.g., HIV gp120) and administered (orally or intravenously) periodically to target the B-1 B cells or marginal zone B cells and to induce persistent antibodies against pathogens.

(C) T cell-dependent B cell responses begin in the T cell-rich areas of the lymphoid organs, where DCs present antigen to antigen-specific T cells in the context of MHC and costimulatory molecules such as the B7 molecules. The activated T cells then migrate to the border of the T cell area and B cell follicles. Similarly, antigen-specific B cells in the B cell follicles that have "seen" antigen and perhaps received TLR stimulation also migrate to the border of the T cell area and B cell follicles. Here, interactions between the antigen-specific T and B cells results in a B cell differentiation program characterized by the formation of specialized microenvironments called germinal centers. Germinal centers are the sites of somatic hypermutation of the immunoglobulin genes of the B cell receptors and the subsequent selection of high-affinity mutants by competition for antigen-antibody complexes on the follicular dendritic cells (FDCs). The germinal center "reaction" results in long-lived memory B cells and long-lived plasma cells that secrete high-affinity, neutralizing antibody. The precise roles played by TLRs and DCs in the germinal-center reaction and in the generation of memory B cells and long-lived plasma cells are poorly understood. DCs and TLRs may operate at multiple levels, including TLR-mediated activation of DCs in the T cell-rich areas; TLR triggering of B cells directly; by influencing the decision of whether an activated B cell should enter a germinal center or the plasma foci; by regulating the processes of cell division, somatic hypermutation, and selection of high-affinity B cells within the germinal center; and by regulating the decision as to whether to become a long-lived memory B cell or a long-lived plasma cell or return to the foci to become a short-lived plasma cell. In addition, TLRs might continuously trigger long-lived memory B cells to maintain "serological memory" (Bernasconi et al., 2002).

restricted, their antibody may constitute a dominant fraction of serum IgM and up to half of IgA molecules in the gut and is often reactive to host molecules. The roles played by such antibodies are largely unknown, although they likely protect against infections in infancy and against gut flora. The role of the innate immune system in regulating B-1 B cells is poorly understood, although recent studies suggest that complement receptors CD21/CD35 are important in the selection, activation, and expansion of the B-1 B cells (Reid et al., 2002). It is also conceivable

that triggering TLRs on B-1 B cells by PAMPs in the gut flora, in concert with BCR signaling and complement-receptor triggering, results in synergistic and persistent activation of such cells and "natural antibody" production.

Programming Marginal-Zone B Cells to Rapidly Generate Neutralizing Antibodies and "Natural Memory." A subset of B cells that has functional similarities to the B-1 B cells are the marginal-zone B cells, which are strategically located in the marginal zones of the spleens. Like B-1 B cells, they also play a dominant role in the early IgM response to blood-borne pathogens, toxins, and viruses that drain the spleen (Lopez-Cavalho and Kearney, 2004). The level of expression of the costimulatory molecules B7.1 and B7.2 on marginal-zone B cells is higher than on recirculating follicular B cells, suggesting a persistent state of activation, like B-1 B cells. A recent study suggests that blood-derived neutrophils and $CD11c^{low}$ immature DCs are the main cells that capture and transport bacteria to the spleen, provide critical survival signals in the form of TNF superfamily members BAFF and APRIL, and promote their differentiation into plasma cells (Lopez-Cavalho and Kearney, 2004).

The propensity of B-1 B cells to proliferate and differentiate continuously in vivo and the preactivated state of the marginal-zone B cells might be exploited to generate persistent levels of broadly reactive neutralizing IgM and IgA antibodies reactive to pathogens such as HIV (Haynes et al., 2005). Furthermore, it is conceivable that such strategies might be used in vaccination campaigns aimed at "preprogramming" the repertoire of B-1 B cells and marginal-zone B cells toward pathogen specificities that are likely to be encountered in disease-endemic areas. This might be accomplished using conjugate prophylactic vaccines comprised of the appropriate TLR ligands conjugated with the specific antigens (e.g., HIV gp120) plus complement and administered (orally or intravenously) periodically to target the B-1 B cells in the gut or marginal-zone B cells and to induce persistent antibodies against pathogens. Alternatively, the low threshold of activation of B-1 B and marginal-zone B cells might be exploited to induce an accelerated antibody response to pathogens that can cause rapid disease, like *Bacillus anthracis* or Ebola or Lassa viruses. Thus, learning how to optimally trigger B-1 B cells and marginal-zone B cells in order to rapidly elicit neutralizing antibodies against pathogens is a critical challenge (critical challenge 3).

Programming the Development of Long-Lived Memory B Cells and Long-Lived Plasma Cells with Innate Immunity. The pathways described above can generate rapid IgM and IgA antibody production but do not result in high-affinity antibodies, nor do they generate immunological memory in B cells. Clearly the ability of B cells to remember their past antigenic encounter and to generate long-lived neutralizing antibodies is one of the hallmarks of most successful vaccines. An important question is the role that antigen plays in the maintenance of B cell memory. Despite earlier reports that antigen, in the form of immune complexes trapped on follicular dendritic cells, is required for memory B cell turnover (Gray and Skarvall, 1988), recent evidence suggests that memory B cells can survive for a long period, even for several years, without seeing antigen (Lam et al., 1997; Maruyama et al., 2000).

Activation of naive B cells occurs at the margins of the T cell-rich areas and B cell follicles (McHeyzer-Williams and McHeyzer-Williams, 2005). The activated B cells can then (1) remain in the T cell-rich areas and differentiate into short-lived plasma cells in areas called plasma foci (Kelsoe, 2000; MacLennan et al., 2003; McHeyzer-Williams and McHeyzer-Williams, 2005) or (2) migrate into B cell follicles and, with CD4 T cell help, initiate a germinal center (GC) reaction. During the GC reaction, B cells undergo somatic hypermutation, resulting in the generation of high-affinity B cells, a process known as affinity maturation (Kelsoe, 2000; MacLennan et al., 2003; McHeyzer-Williams and McHeyzer-Williams, 2005). Such positively selected high-affinity B cells may migrate to the plasma foci and become antibody-producing cells, migrate to the bone marrow to become a long-lived plasma cell, or differentiate into long-lived memory B cells (Ahmed and Gray, 1996; Slifka et al., 1998). Memory B cell responses are different from primary B cell responses in three ways: (1) they are faster; (2) they produce more antibody, particularly of the IgG, IgA, and IgE isotypes; and (3) they produce higher-affinity antibody.

What role does the innate immune system play in (1) generating short-lived plasma cells in the plasma foci, (2) inducing robust germinal-center responses and affinity maturation, (3) generating long-lived memory B cells, or (4) generating long-lived plasma cells? There is considerable understanding of the roles played by one aspect of the innate immune system, namely the complement system, in regulating these processes (Fearon and Carroll, 2000). In particular, experiments using mice deficient in complement proteins C3 or C4 or the complement receptors CD21/CD25 suggest that such receptors are involved in regulating B cell differentiation at multiple levels, including activation of naive B cells, survival of germinal-center B cells, selection of memory B cells, and persistence of antibody secretion. Importantly, when mice were immunized with a recombinant model antigen, hen egg lysozyme (HEL), fused to murine complement protein C3d, this fusion product was up to 10,000-fold more immunogenic than HEL alone (Dempsey et al., 1996). Thus, C3d is a molecular adjuvant of innate immunity that profoundly influences an acquired immune response.

There is, however, only limited information about the role of TLRs, C type lectins, other PRRs, or DC subsets in B cell differentiation. Furthermore, putative roles of innate cytokines such as IL-15, IL-7, or TNF family members (which regulate effector and memory T cell differentiation and maintenance) are poorly understood. Recently, it has been demonstrated that the TNF family member BAFF/BlyS promotes the survival of naive B cells in the periphery (Mackay et al., 2003) as well as marginal-zone B cells (Lopez-Cavalho and Kearney, 2004). In addition, human myeloid DCs can express BAFF, can directly activate

memory B cells in vitro, and also promote the survival of plasmablasts derived from memory B cells (Banchereau et al., 2000). Furthermore, recent work suggests that plasmacytoid DCs are critical for generation of plasma cells and anti-viral antibodies from memory B cells via a mechanism involving IFN-α (Jego et al., 2003).

With regards to the long-term production of antibody, two different (although not mutually exclusive) hypotheses have been proposed to explain the longevity of the antibody response in the absence of re-exposure to antigen. First, it has been suggested that antibody levels are maintained by the presence of long-lived plasma cells in the bone marrow, secreting specific antibody for extended periods, possibly several years (Slifka et al., 1998; Manz et al., 1997). The second hypothesis suggests that memory B cells are continually differentiating into plasma cells in an antigen-independent manner due to bystander or polyclonal activation (Bernasconi et al., 2002). In humans, memory B cells constitutively express specific TLRs, including TLR9. Naive B cells do not constitutively express TLR9, but it can be upregulated on naive B cells when stimulated through BCR, thus explaining the differential sensitivity of these subsets to TLR9 triggering. Given that neutralizing antibodies represent a critical line of defense, it is very likely that the immune system has evolved multiple mechanisms to maintain persistent levels of neutralizing antibody. Clearly, several unanswered questions remain. For example, what roles do specific subsets of DCs and specific TLRs or PRRs play in inducing the differentiation of long-lived memory B cells and plasma cells? Is it necessary to trigger TLRs or other PRRs directly on B cells, as well as on DCs, in order to generate long-lived memory B cells and plasma cells? Thus, elucidation of mechanisms by which innate immunity regulates the generation of memory B cells and long-lived plasma cells that secrete neutralizing antibody and home to mucosal sites is a critical challenge (critical challenge 4).

Learning Immunology from "Good" Vaccines

In the past 200-odd years since Jenner, vaccination has controlled the spread of smallpox, diphtheria, tetanus, yellow fever, pertussis, *Haemophilus influenzae* type b, poliomyelitis, measles, mumps, and rubella. In fact, smallpox has been eradicated, and the WHO expects polio to be eradicated soon (Rappuoli. 2004; Plotkin, 2005). In addition, vaccines against hepatitis A, hepatitis B, varicella, and pneumococcal and meningococcal infections have had major impact on the spread of these diseases. Despite their great impact on public health, the majority of successful vaccines have been derived empirically. For example, yellow fever vaccine 17D (YF-17D) is considered to be one of the most effective vaccines available and has been administered to over 400 million people worldwide (Pugachev et al., 2005). In many individuals, neutralizing-antibody titers have been detected for as long as 35 years following a single vaccination. In addition, YF-17D also has been demonstrated to be a potent inducer of cytotoxic T cell responses. Despite its efficacy, its mechanism of action is not understood. Our recent data suggest that YF-17D activates multiple subsets of DCs by signaling through multiple TLRs, including TLRs 2, 7, 8, and 9, resulting in diverse types of adaptive immune responses (Querec et al., 2006). One immunological consequence of this appears to be the generation of immune diversity—distinct TLRs appear to activate specific DC responses that then activate particular T cell responses. Consistent with this, a recent study in nonhuman primates suggests that synthetic ligands that target both TLRs 7 and 8 (and thus activate both myeloid and plasmacytoid DCs) induce stronger and qualitatively different, antigen-specific $CD8^+$ T cell responses than CpG DNA, which only targets TLR9 plasmacytoid DCs (Wille-Reece et al., 2006). Another advantage of triggering multiple TLRs might be to generate immune synergy. Consistent with this, a recent report suggests that combinations of specific TLR ligands display synergistic effects in their ability to induce IL-12p70 from DCs in vitro (Napolitani et al., 2005).

In contrast to highly effective vaccines such as YF-17D, it may also be advantageous to study why some vaccines induce suboptimal immune responses. For example, the currently licensed US anthrax vaccine approved for use in humans (AVA) consists of filtered culture supernatants of an attenuated strain of *B. anthracis* adsorbed to alum. A similar vaccine is available in the United Kingdom. AVA is licensed to be given in a six-dose series at 0, 2, and 4 weeks and 6, 12, and 18 months. It is conceivable that the striking differences in dosing requirements between YF-17D and AVA reflect, amongst other things, major differences in the ability of the two vaccines to stimulate the innate immune system. Indeed, a recent study suggests that the United Kingdom anthrax vaccine is a poor stimulator of DC maturation and subsequent T cell stimulation (Skowera et al., 2005). This may be accounted for by a lack of TLR signals in AVA or by the presence of small amounts of lethal factor (LF), which is known to suppress DC maturation (Agrawal et al., 2003a).

Finally, there is evidence that some vaccine vectors, such as the vaccinia virus, with which billions of individuals were immunized during the smallpox eradication campaign in the 1970s (Crotty et al., 2003), infects DCs, (paradoxically) inhibits DC activation, and causes extensive cell death, resulting in crosspresentation of cellular antigen (Norbury et al., 2002). The mechanism of inhibition of DC activation is poorly understood, although two vaccinia virus ORFs termed A46R and A52R, when expressed in mammalian cells, were shown to interfere specifically with IL-1 signal transduction. A46R partially inhibited IL-1-mediated activation of the transcription factor NF-κB, and A52R potently blocked IL-1-, TLR4-, and TLR3-mediated NF-κB activation (Harte et al., 2003). Furthermore, the roles played by the non-TLR viral sensors, including RIG-1, Mda-5, MAVS, and protein kinase R, in mediating the robust and persistent immunogenicity of such vectors need to be ascertained. Clearly, these data suggest that deconstructing our best empirically derived vaccines to

Table 1. Some Immunological Challenges for Vaccinologists

1	Elucidation of mechanisms by which innate immunity regulates memory T cell generation, maintenance, and migration to mucosal sites
2	Elucidation of the signaling networks within DCs that induce Th2 or T regulatory responses and discovery of strategies to relieve such regulatory networks, particularly in chronic infections and tumors
3	Learning how to optimally trigger B-1 B cells and marginal-zone B cells to rapidly elicit neutralizing antibodies
4	Elucidation of mechanisms by which innate immunity regulates the generation of memory B cells and long-lived plasma cells that secrete neutralizing antibody and home to mucosal sites
5	Understanding the innate immune mechanisms by which the best empirical vaccines induce qualitatively diverse and long-lasting protective immune responses
6	Development of novel vaccine delivery systems (e.g., nanotechnology) to design vaccines that recapitulate or surpass the efficacy of our best empirical vaccines

determine the specific combinations of innate immune receptors and DC subsets that they activate and how this controls specific aspects of adaptive immunity will be instructive in guiding the design of future vaccines. Thus, understanding the innate immune mechanisms by which such vaccines induce qualitatively diverse and long-lasting protective immune responses is a key area for further research (critical challenge 5).

In addition, recent advances are beginning to permit a more "systems biological" approach to understanding the early innate immune signatures of such "good" and "bad" vaccines (Aderem, 2005). The information gathered from such studies should then be used to generate the vaccines of the future—perhaps consisting of multiple TLR ligands that provide both immune synergy (Napolitani et al., 2005) and immune diversity (Querec et al., 2006) in combination with antigen plus or minus synthetic molecules that might regulate signaling within DCs. Thus, the development of novel vaccine delivery systems, perhaps using nanotechnology, to design vaccines that recapitulate the efficacy of our best empirical vaccines is a critical area for further work (critical challenge 6).

Some Critical Immunological Challenges for Vaccinologists

The recent renaissance in innate immunity is beginning to provide answers to fundamental questions of how the immune system generates long-term T and B cell memory and protective immunity against pathogens. A central theme is that critical parameters in the innate immune system, such as the nature of the DC subset or the PRR, exert a profound influence on the strength, duration, and quality of T and B cell responses. In this context, the precise roles played by individual TLRs; specific combinations of TLRs; or, indeed, the growing list of non-TLR PRRs, such as RIG-1, Mda-5, MAVS, and PKR, in the induction of long-term T and B cell responses and memory is likely to be an area for fertile exploration. A related, equally important concept is that this renaissance in innate immunity is providing us with a new vision to understand how our best empirically derived vaccines work. These and the other critical challenge discussed in the present review are summarized in Table 1. Clearly, the successful solution of these challenges will be of great value in the design of future vaccines against a multitude of emerging and reemerging infections.

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