The interface between innate and adaptive immunity

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This focus analyzes some of the ways the innate immune system influences adaptive immune responses. Here the main principles and themes that govern this intricate relationship are discussed.

‘Immunity’ refers to the global ability of the host to resist the predation of microbes that would otherwise destroy it. Immunity has many facets, but the greatest dichotomy separates adaptive immunity (‘acquired immunity’) from innate immunity (‘natural immunity’ or ‘innate resistance’). This dichotomy was not limited to the science but included those who pursued it, and the adaptive and innate immunologists began to part company almost when the science of immunology was born, ultimately finding themselves separated by a deep divide both in their methods and in their objectives. Denizens of the first camp have spent the last few decades trying to understand the generation of receptor diversity, the basis of self tolerance and the interactions that occur between lymphoid cells, once perceived as unitary and now known to be of many types. Meanwhile, much closer to the field of microbial pathogenesis, innate immunologists tried to understand how microbes were recognized in the first example (and most importantly, what receptors recognize them), how leukocytes kill microbes and how leukocytes ‘sound a systemic alarm’ through cytokine mediators.

The discovery of the Toll-like receptors (TLRs) as sensors of microbial molecules transformed the views of discrimination between self and non-self, a key requirement of any immune system. It turns out that much of microbial recognition is served by only a handful of TLRs. But it must not be forgotten that the innate immune system has evolved other means to recognize microbial molecules transformed the views of discrimination between self and non-self, a key requirement of any immune system. It turns out that much of microbial recognition is served by only a handful of TLRs. But it must not be forgotten that the innate immune system has evolved other means to recognize microbial pathogens as well. These include the complement system, the specialized receptors that enable natural killer (NK) cells to sense non-self, ‘missing-self’ and ‘induced-self’, and certain intracellular sensors as well. All of these systems for microbial perception are complementary and are involved in the development of the ensuing adaptive immune response (Fig. 1). In this issue, Carroll, Raulet, Iwasaka and Medzhitov, and Cook and colleagues provide a comprehensive update of the present knowledge of how the adaptive immune response is ‘shaped’ by these various innate immune sensors.

Many challenges must be met if the intricate communication between these fundamentally ‘innate’ sensing systems and the adaptive response that follows are to be understood. But the reward is potentially enormous, not only because of the need to understand what ignites the adaptive immune response in the first place but also because it may be that the seeds of allergic and autoimmune diseases are planted at the interface between innate and adaptive cells.

Microbial recognition and adjuvanticity

The subordinate status of the adaptive immune response has been known since the 1960s, when it was shown that mononuclear phagocytic cells were required for effective lymphoid responses to antigens.1,2 It is now known that adaptive immune dependency on innate immune cells stems from the need for antigen presentation, a function discharged by antigen-presenting cells, including both myeloid dendritic cells (DCs), a specialized derivative of peripheral blood monocytes, and plasmacytoid DCs, which are of unclear ontogenic origin. The initial uptake and phagocytosis of microbes by antigen-presenting cells is facilitated by receptor-mediated recognition of microbial molecules. Both scavenger receptors and receptors of the complement system are important in this process. Concurrent maturation of DCs, a process that entails upregulation of major histocompatibility complex class I and class II molecules, costimulatory molecules such as CD40, CD80 and CD86, and the production of cytokines, allows for the optimal interaction between DCs and naive CD4+ and CD8+ T cells in an antigen-specific way.

Specific molecules of microbial origin, such as lipopolysaccharide (LPS), a component of the outer membrane of Gram-negative bacteria, have long been known to exert an adjuvant effect on the adaptive immune response. Although the receptors that recognized LPS and other microbial molecules remained elusive for some time, their existence was widely discussed, and it was shown by the 1970s that the adjuvant effect of LPS was dependent on the integrity of a single locus known as Lps. In 1998 the Lps locus was identified through positional cloning and was shown to be identical to the TLR4 (Tlr4) locus. The identification of TLRs as receptors for molecules of microbial origin, not only essential for sensing infections at an early stage but also essential for adjuvanticity, has cast new light on the interface between innate and adaptive immunity. So far a total of 13

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TLRs have been identified in mammals, and most are now known to recognize specific, conserved microbially derived molecules or their synthetic counterparts. Many of these ligands, such as lipid A analogs (which activate TLR4), double-stranded RNA (which activates TLR3) and DNA molecules containing unmethylated CpG motifs (which activate TLR9), have been used for their adjuvant effects.

Qualitative differences in TLR activation

The adaptive immune response may tend toward tolerance or it may tend toward activation of T helper type 1 (T_H1) or T_H2 cells. Moreover, different responses are appropriate for the elimination of different microbes (intracellular pathogens famously require a robust T_H1 response for clearance). The nature of the response is at least partly influenced by events that occur ‘upstream’ of the T cells themselves, as different invaders tend to produce one outcome or another. For example, helminthic infestation generally causes a T_H2 response, whereas mycobacterial infections tend to cause a T_H1 response when immune function is normal. It is therefore worth taking a ‘top-down’ approach, asking whether distinct combinations of activated TLRs triggered by each microbe might foretell the outcome that occurs.

Can the TLRs indeed function as a ‘bar code reader’?

Although the importance of TLRs in the recognition of pathogens and in the subsequent induction of inflammatory responses is widely appreciated, many questions still remain as to how TLR-ligand interactions occur. For some TLRs, a few supramolecular details are known. For example, LPS activation of TLR4 requires a complex between MD-2, CD14 and TLR4 itself, whereas the scavenger receptor CD36 functions as a coreceptor, acting in conjunction with TLR2, and specifically recognizes lipoteichoic acid as well as mycoplasma-derived lipopeptide (S-MALP-2; data not shown). It is possible that all TLRs operate in concert with multiple binding molecules to acquire maximum sensitivity and specificity. A location at which different TLRs are expressed may influence precisely which molecules they are likely to encounter and may be a key factor in self versus non-self discrimination.

Although some interactions among different TLRs are known to occur and could allow for increased signaling diversity, the inclusion of other proteins in the picture might allow for still more signaling diversity, the data that flow from the TLRs are necessarily compressed. At present, four adaptor proteins, MyD88 (myeloid differentiation factor 88), MAL (MyD88 adapter–like), Trif (Toll receptor–associated activator of interferon) and TRAM (Toll receptor–associated molecule) have been shown to transduce signals via the intracellular TIR (Toll–interleukin 1 receptor–resistance) domains of the various TLRs. Whereas most TLRs activate the MyD88-dependent pathway, resulting in activation of MAP kinases and the transcription factor NF-κB, it is now clear that different TLRs use different (combinations of) adaptor molecules. For example, whereas TLR4 activation results in the recruitment of all four adaptor molecules, TLR3 activation is entirely dependent on Trif.

Further data compression occurs at the level of signaling kinases, as IRAK4 and TBK1 are believed to gate most of the responses to all TIR domain signals. The former kinase channels signals toward the activation of NF-κB, whereas the latter channels signals mainly toward the activation of interferon regulatory factor 3. Even allowing for differences in signal intensity and timing, it is difficult to see how diversity of adaptive immune responses can survive in the face of signaling degeneracy.

The type I interferons are now recognized as key molecules that augment and sustain T cell responses. They are essential for the upregulation of costimulatory molecules on DCs, a prerequisite for the optimal interaction of DCs with naive CD4+ and CD8+ T cells. If a single class of molecules causes upregulation of costimulatory proteins, how is there room
for a ‘decision’ about $T_{H1}$ versus $T_{H2}$ versus tolerance? Do qualitative differences in signals emanating from the various TLRs make a difference in the type of interferon produced? Do specific subsets of DCs mandate the choice that is made? Or is there yet another response pathway that drives the adaptive response toward a $T_{H1}$ or $T_{H2}$ profile? One possible escape from the degeneracy snare is that adaptive response diversity is determined by the type of cell that expresses each set of TLRs and the locations at which these cells reside (reviewed by Iwasaki and Medzhitov). For example, plasmacytoid DCs expressing TLR9 but not TLR3 have been reported to produce large amounts of type I interferon during viral infections, an effect mediated mainly through activation of TLR9. In contrast, myeloid DCs that express TLR3 as well as TLR9 are able to induce type I interferon only via the TLR3 pathway. In the ultimate extension of this model, there may be many types of antigen-presenting cell, with diversity of TLR expression capable of dictating the shape of the adaptive immune response that follows.

Adaptive immunity beyond TLRs
Although it is clear that TLRs expressed on DCs are essential for the initiation of an adaptive response, they are not the only receptors that are influential. One essential response mechanism, reviewed by Raulet, is embodied in the interaction between DCs and NK cells. NK cells express a multitude of receptors that are able to recognize self as well as non-self cell surface proteins. Their main function is to survey the host for cells that are infected by intracellular pathogens. Activation of NK cells can be achieved by the recognition of foreign antigens as well as ‘induced self’ or ‘missing self’: increased or decreased expression, respectively, of target cell surface molecules that are normally recognized by NK inhibitory receptors. NK cells can also be activated by DCs, either directly via specific cell surface molecules or indirectly through cytokines. This in turn can lead to the production of interferon-$\gamma$ by NK cells and to the subsequent maturation of DCs and enhancement of T cell responses. In addition, NK cells have the ability to eliminate immature DCs, thereby inhibiting T cell responses. These NK functions indicate that the interaction between NK cells and DCs represents an intricate balance between enhancement and repression of DC function that could be decisive for the development of tolerance versus a T cell response.

Another pathway that is able to modulate B cell and/or T cell responses is described by Carroll and involves activation of the complement system. The complement system comprises a group of more than 30 plasma proteins, the main function of which is the recognition and elimination of microbes. Activation of this system can be triggered either by a target-bound antibody (the classical pathway), recognition of microbial polysaccharide structures (the lectin pathway) or recognition of other foreign surface structures as yet uncharacterized (the alternative or properdin pathway). Complement participates in humoral immunity through the ligation of complement factors C3 with the CD21-CD35 receptor expressed on B cells and follicular DCs, resulting in regulation of B cell responses at multiple stages. In addition, the complement system is essential for the efficient opsonization of microbes and subsequent uptake and antigen processing by DCs, which results in an efficient T cell response. Finally, there are TLR-independent pathways that lead to NF-$\kappa B$ translocation and/or production of type I interferon by DCs. So far at least two intracellular pathways have been described. One pathway entails the activation of NOD (nucleotide-binding oligomerization domain) proteins by Gram-positive peptidoglycan-derived structures. A TLR3-Trif-independent double-stranded RNA response pathway that leads to the activation of type I interferon synthesis and the subsequent maturation of DCs has been reported. A positional cloning approach has demonstrated that polymorphisms of $CARD15$, which encodes NOD2, are genetically linked to the development of Crohn disease, but so far no overt immunodeficiency has been noted in mice that lack this gene. There is also little information bearing on the TLR3-independent double-stranded RNA response pathway, and future studies will need to show which genes are involved and the importance of this pathway in the elimination of viral infections.

Abnormal immune responses and disease
Many of the diseases that afflict mankind are now thought to be the result of dysfunctional innate and/or adaptive immune responses. The detrimental effect of unfettered, systemic innate immune activation (septic shock) has been recognized for some time, and it is now known that most or all of the events in septic shock begin with TLR activation. At the same time, sepsis itself may stem from innate immunodeficiency and the failure to contain a small inoculum of microbes. Increased susceptibility to meningooccal disease is associated with heterozygosity for TLR4 alleles that encode rare missense variants. In other cases, for example, allergic diseases such as asthma, autoimmune diseases such as systemic lupus erythematosus and sterile inflammatory diseases such as rheumatoid arthritis, the main cause of disease is more difficult to pin down. Both rheumatoid arthritis and systemic lupus erythematosus are correlated with the production of inflammatory cytokines (tumor necrosis factor in rheumatoid arthritis and type I interferon in systemic lupus erythematosus) that may be induced by noninfectious and so far elusive endogenous molecules. It would be useful to know whether these endogenous ligands actually exist and, if so, what they are. Equally importantly, it would be useful to know the primary lesions in each disease, which in the end must surely be mutations. Do these mutations cause a failure of the innate immune system to effectively discriminate self from non-self antigens, or is the problem one of regulation rather than recognition itself? Might the problem be still more distal, at the innate-adaptive interface or beyond? Many different lines of inquiry may now be brought to bear in addressing these issues.

Concluding remarks
Little is actually known about innate-adaptive connections. The understanding that interactions between B7 and CD28 family members are important for the transmission of a costimulatory signal for adaptive immune activation is, in the end, one of the few things that is certain. Recent success in the elucidation of TLR function has blinded many to the fact that allograft recognition is an extremely powerful adaptive immune response, yet it is mostly (or perhaps entirely) independent of TIR domain signaling. By extension, graft-versus-host disease might also be likely to be triggered without much involvement of TIR domain signaling pathways. Hence, a largely separate pathway for the upregulation of costimulatory molecules must serve adaptive immune activation when alloreactive cells are the antigen. The key proteins and cellular participants remain to be identified.

Exceptional examples of tolerance to cells that would be expected to provide an allostimulatory signal have likewise defied explanation. Why, for example, does the trophoblast elicit no response? As a full complement of T cells specific for the allograft is available to permit rejection, the explanation must be sought upstream. The nexus of the innate and adaptive systems hides many secrets even now, and both adaptive and innate immunologists are finally united in their desire to uncover them, working across a gap that is ever more narrow.
The authors declare that they have no competing financial interests.


