Variegation of the Immune Response with Dendritic Cells and Pathogen Recognition Receptors

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Bali Pulendran

One of the most fundamental questions in biology is: “How do cells differentiate in the right place, at the right time, into the right kinds?” Understanding the phenomenon of cell differentiation in its spatial and temporal framework is a prelude to understanding the development and physiology of all multicellular systems, including the immune system. Insights over the past 2300 years, since Aristotle, suggest that biological differentiation is guided by the interplay between genetic programs and specific environmental signals. This is exemplified by the mammalian immune response to pathogens, where qualitatively different types can emerge. Although it is appreciated that this type immunity is critical for optimal defense against different pathogens, the early “decision-making mechanisms” are largely obscure. Recent developments in innate immunity and genomics, especially in the biology of dendritic cells (DCs) and pathogen recognition receptors, have stimulated intense research in understanding the mechanisms guiding the differentiation of Th1, Th2, and T regulatory responses. In this study, I summarize recent findings which suggest that activation of DCs via distinct pathogen recognition receptors stimulate different gene expression programs and signaling networks in DCs that guide the variegation of immune responses. The Journal of Immunology, 2005, 173: 2457–2465.

Decision making in the immune response

The mammalian immune system can be said to have four fundamental properties: 1) a highly diverse repertoire of Ag-binding B and T cell receptors, clonally distributed on lymphocytes (1), that enables the system to recognize virtually any Ag with high specificity; 2) memory, which allows the host to remember the initial antigenic encounter, even for a lifetime (2); 3) immunological tolerance which encompasses a set of checkpoints against self-destructive immune responses (3); and 4) the ability to launch qualitatively different responses against different pathogens (4). Although there is considerable understanding of 1) and increasing awareness of the mechanisms underlying 2) and 3), the factors that determine “decision making” have remained an abiding puzzle. Clearly, the solution of this problem would not only illuminate our conceptual framework of the immune system, but also provide deep insights into a universal biological phenomenon: how cells differentiate into diverse phenotypes, guided by their own genes vs nurture. In addition, such knowledge is likely to be of critical importance in the design of novel vaccines and drugs that can generate optimally effective immune responses against a multitude of emerging and re-emerging infections. Thus, understanding the molecular mechanisms and players that regulate decision making in the immune response might be considered a Holy Grail of 21st century immunology and a grand challenge for biology. Recent advances in the biology of dendritic cells (DCs), TLRs, and other pathogen recognition receptor (PRR) systems are beginning to reveal the secrets of this decision-making process to such an extent that many of the critical molecular players within DCs that regulate Th1/Th2 and T regulatory responses are becoming known (5). The present review will summarize recent discoveries that point to a role for distinct DC subsets and PPRs, such as TLRs and C-type lectins, in orchestrating the type of immunity. Although the microenvironmental milieu is also known to play a major role, this will not be discussed here.

Genetic programming vs plasticity in dendritic cells

The existence of phenotypically distinct subsets of DCs that are localized in distinct microenvironments (4–7) raises the question of whether they too, like distinct subsets of lymphocytes, may have evolved to serve distinct functions. As indicated in Table I, there is evidence for functional specialization of distinct DC subsets, but there is also compelling evidence for functional plasticity of a given DC subset. Distinct DC subsets are known to exhibit intrinsic differences in their ability to: 1) regulate the quality of the Th response (Th1, Th2, or CTL); 2) produce antiviral type I IFNs; and 3) cross-present exogenous Ags to CD8+ T cells.

Table I presents a chronological perspective of some important developments in this area. In the mid-1990s, Shortman and colleagues (8, 9) demonstrated that the CD8α+ve DCs from the spleens of mice were weaker at stimulating CD4+ T cells.
CD8⁺ T cell proliferation compared with CD8α⁻negative DCs (8, 9), thus suggesting that a specific subset of DC might be dedicated for keeping the immune response in check. Contrary to this, in 1997, it was demonstrated that microbial stimuli could induce murine CD8α⁺ DCs to secrete much higher levels of the biologically active form of IL-12p70 relative to the CD8α⁻ subset (10, 11). Given the pivotal role of IL-12p70 in inducing Th1 responses, the question of whether the distinct subsets might differentially stimulate Th1 vs Th2 response arose. In 1999, it was demonstrated that the CD8α⁺ and CD8α⁻ subsets in the spleen differentially primed Th1 vs Th2-biased responses (12, 13). Similar results with distinct human DC subsets in vitro, were obtained shortly thereafter (14). Taken together, these three reports provided evidence for the notion that distinct DC subsets might indeed regulate Th responses differentially. Subsequent work with the equivalent DC subsets from Peyer’s patches confirmed these findings (15). Then, Siegal et al. (16) demonstrated that human plasmacytoid DCs could be induced to make much higher levels of IFN-α than monocyte-derived DCs). This was later confirmed with murine plasmacytoid DCs (17–20). Collectively, these observations suggested that distinct subpopulations of DCs may have some intrinsic biases in their ability to stimulate qualitatively different types of immune responses.

This notion was challenged by a series of reports from various groups showing an impressive degree of flexibility or “plasticity” of DCs in response to different microbial stimuli. For example, various environmental factors such as IL-10 (21), prostaglandins and steroids (22, 23), tissue of DC residence (15, 24), or duration of in vitro culture (25), affect the ability of DCs to produce cytokines and to induce Th1 and Th2 responses. Furthermore, the nature of the microbial stimulus was also shown to exert a potent influence; thus early reports suggested that DCs exposed to helminth products could stimulate a Th2-like response, while the same DCs when exposed to LPS induced a Th1-biased response (26, 27). This was followed by several other reports suggesting that specific subsets of murine or human DCs cultured in vitro with different stimuli or different TLR ligands, responded with great plasticity of gene expression and cytokine secretion (28–37). Furthermore, a specific murine splenic DC subset cultured with different doses of OVA peptide has been shown to induce distinct Th responses (38), and one report claimed that electroporation of RNA into DCs could induce non-plasmacytoid, CD11c⁺ splenic DCs to secrete IFN-α (39).

Clearly, these reports have highlighted the considerable degree of functional plasticity in DCs. However, a central question that must be asked is: How flexible are DCs? Recent work suggests that there might be constraints on DC flexibility. For example, our work suggests that stimuli such as Escherichia coli LPS and Staphylococcus aureus Cowan I bacteria preferentially induce IL-12p70 from the splenic CD8α⁺ subset (10, 33, 37); in contrast, stimuli such as Pam-3-cys induce IL-10, preferentially from the CD8α⁻ subset (33). Moreover, recent work from Reis e Sousa’s group (34, 35) suggests that the CD4⁺ CD8α⁻ CD11b⁺ splenic DC subset appears to be very resistant to IL-12p70 induction by any of the stimuli tested, but a potent producer of IL-10. Finally, a recent report suggests that human papillomavirus type 16 virus-like particles increase transcription of IFN-γ and numerous Th1-related cytokines and chemokines in CD8α⁻ CD11c⁺ DCs, but induce Th2-associated cytokines and chemokines in the CD8α⁻ CD11b⁺ CD11c⁺ DCs.

### Table I. Evidence for functional specialization of DC subsets vs flexibility in programming DC function

<table>
<thead>
<tr>
<th>Year</th>
<th>Evidence for Functional Specialization of DC Subsets</th>
<th>Evidence for Functional Plasticity of DCs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1995–1996</td>
<td>Mouse CD8α⁺ DCs in the spleen induce much weaker CD4⁺ and CD8⁺ T cell priming than CD8α⁻ DCs (8, 9)</td>
<td>Resting respiratory tract DCs preferentially stimulate Th2 responses and require obligatory cytokine signals for induction of Th1 immunity (24). Prostaglandin E₂ suppresses IL-12 production from human MDDCs and promotes Th2 responses (22, 23). IL-10 modulates DC function of murine splenic DCs (21).</td>
</tr>
<tr>
<td>1997–1998</td>
<td>Mouse CD8α⁺ DCs can be induced to secrete much higher levels of IL-12 p70 than CD8α⁻ DCs (10, 11)</td>
<td>Murine bone marrow-derived DCs conditioned by helminth components vs LPS to differentially induce Th2 vs Th1 responses (26). Human MD DCs or murine bone marrow-derived DCs can be conditioned by environmental signals or prolonged culture to induce Th2 responses (25, 27, 29).</td>
</tr>
<tr>
<td>1999</td>
<td>Distinct DC subsets in mouse spleen and human blood differentially induce Th1 vs Th2 responses (12–14)</td>
<td>Human MD DCs or murine bone marrow-derived DCs can be conditioned by environmental signals or prolonged culture to induce Th2 responses (25, 27, 29).</td>
</tr>
<tr>
<td>2000</td>
<td>Distinct DC subsets in murine Peyer’s patches differentially bias Th1 vs Th2 responses (15)</td>
<td>Human MD DCs or murine bone marrow-derived DCs can be conditioned by environmental signals or prolonged culture to induce Th2 responses (25, 27, 29).</td>
</tr>
<tr>
<td>2001</td>
<td>E. coli LPS preferentially induces IL-12p70 in mouse CD8α⁺ vs CD8α⁻ DCs (33)</td>
<td>Murine bone marrow-derived DCs are conditioned by SEA to induce Th2 responses (30).</td>
</tr>
<tr>
<td>2002</td>
<td>Murine splenic CD8α⁻ CD4⁺ CD11b⁺ DC subset cannot be induced to produce IL-12p70 by any stimulus tested (34, 35)</td>
<td>Ability of murine CD8α⁺ and CD8α⁻ DCs to cross-present Ags can be modulated by external stimuli (100).</td>
</tr>
<tr>
<td>2003</td>
<td>Different TLR ligands condition human MDDCs to induce distinct Th responses (36)</td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>E. coli LPS induces IL-12p70 from murine CD8α⁺ DCs, whereas Pam-3-cys does not; Pam-3-cys induces IL-10 from the CD8α⁻ subset, whereas LPS does not (37)</td>
<td>Different TLR ligands condition human MDDCs to induce distinct Th responses (36). Electroporation of RNA induces IFN-α from non-plasmacytoid DCs (39).</td>
</tr>
</tbody>
</table>
Taken together, these studies suggest that microbial stimuli induce distinctive responses in different DC subsets. The reader will appreciate that these seemingly contradictory findings have been the source of many a spirited debate in the field! However, DC biologists might well spare ourselves any agony, by remembering that this issue of “genetic programming” vs plasticity is a universal biological theme, whether one considers pattern formation and lineage fate decisions during embryogenesis (41), or functional specialization vs plasticity of different areas of the cerebral cortex (42), or in the learning of language (43). In each case, it appears that developmental fate decisions are governed by mechanisms that rely on a superimposition of a basic genetic program (“hardwiring”) on a dynamic environment.

Pathogen recognition receptors

TLRs in the center stage. For more than 100 years since Ellie Metchinkoff’s pioneering observations of phagocytic cells in starfish larvae (44), most immunologists have relegated the innate immune system to a “second tier” system of nonspecific host responses of limited influence (Table II). Burnet’s clonal selection theory (1), and the subsequent demonstration that bacterial Ags such as flagellin could be directly recognized by Ag receptors on B lymphocytes (45), helped reinforce the perceived supremacy of the adaptive immune system. However, spectacular advances in innate immunity over the past 6 years have established a new paradigm in which the burden of pathogen sensing is now placed on the innate immune system (46–48). It is now clear that the innate immune system recognizes microbial components directly through various specific PRRs expressed on innate immune cells, such as DCs. TLRs constitute an evolutionarily conserved family of PRRs, of which 11 have yet been described and are widely expressed on a variety of innate immune cells, including DCs, macrophages, mast cells, neutrophils, and endothelial cells. Several excellent articles have reviewed their biology extensively (49–52); therefore, in this review this topic will be discussed only briefly.

TLRs may be expressed as homodimers or heterodimers (TLR1 plus TLR2 or TLR6 plus TLR2) and have broad specificity for conserved molecular structures of pathogens (reviewed in Refs. 49–52). For example, LPS from E. coli signals through TLR4, whereas TLR2 appears to have several ligands, including peptidoglycan of Gram-positive bacteria, lipoproteins from Mycobacterium tuberculosis, and certain components of Saccharomyces cerevisiae zymosan, as well as highly purified Porphyromonas gingivalis LPS. TLR3 recognizes dsRNA, TLR5 recognizes flagellin, TLR7 can be triggered by the synthetic compounds imidazoquinolines, as well as ssRNA, and may thus be important for viral recognition; TLR9 recognizes certain types of CpG-rich DNA found in bacteria and some viruses (49–52). Variegating immunity with TLRs and adaptors. Although initial studies suggested that all TLRs signal via a common downstream signaling pathway, resulting in a common biologic response, it is now amply clear that triggering different TLRs result in distinct but overlapping signaling networks and biological responses (Fig. 1). For example, signaling through...

<table>
<thead>
<tr>
<th>PRR Family</th>
<th>PRRs</th>
<th>Ligand</th>
<th>DC or Macrophage Cytokine Response</th>
<th>Adaptive Immune Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLRs</td>
<td>TLR2 (heterodimer with TLR1 or 6)</td>
<td>Lipopeptides Pam-3-cts (TLR 2/1) MALP (TLR 2/6) dsRNA</td>
<td>Low IL-12p70 High IL-10 (36, 77) IL-6 (33, 36, 37, 75–81) Th1 (74) Th2 (33, 36, 37) T regulatory (79–81)</td>
<td>Th1 (33, 36, 37) Th2 (33, 36, 37) T regulatory (79–81)</td>
</tr>
<tr>
<td>TLR3</td>
<td>E. coli LPS</td>
<td>High IL-12p70 Intermediate IL-10 IL-6 (33, 36, 37, 76)</td>
<td>Th1 (33, 36, 37, 76)</td>
<td>Th1 (33, 36, 37, 76)</td>
</tr>
<tr>
<td>TLR5</td>
<td>Flagellin</td>
<td>High IL-12p70 (36, 102) Low IL-12p70 (103, 104)</td>
<td>Th1 (36, 102) Th2 (103, 104)</td>
<td>Th1 (36, 102) Th2 (103, 104)</td>
</tr>
<tr>
<td>TLR7/8</td>
<td>ssRNA Imidazoquinolines</td>
<td>High IL-12p70 IFN-α IL-6 (48–52, 105)</td>
<td>Th1 (48–52, 105)</td>
<td>Th1 (48–52, 105)</td>
</tr>
<tr>
<td>TLR 9</td>
<td>Cpg DNA</td>
<td>High IL-12p70 Low IL-10 IL-6 IFN-α (48–52, 106)</td>
<td>Th1 (48–52, 106)</td>
<td>Th1 (48–52, 106)</td>
</tr>
<tr>
<td>TLR10</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>C-type lectins</td>
<td>DC-SIGN</td>
<td>Env of HIV; core protein of HCV; Components of M. tuberculosis; H. pylori Lewis Ag</td>
<td>H. pylori Lewis Ag Suppresses IL-12p70 (95) Suppression of TLR signaling in DCs (95, 96)</td>
<td>Th2 (95) T regulatory (96)</td>
</tr>
<tr>
<td>NOD</td>
<td>NOD2</td>
<td>Muramyl dipeptide of peptidoglycan</td>
<td>Induces IL-10 in DCs (97) Weak T cell response (tolerogenic?)</td>
<td>Weak T cell response (tolerogenic?)</td>
</tr>
<tr>
<td>Mannose receptor</td>
<td>Mannose receptor</td>
<td>Mannosylated lipoarabinomannans from bacillus Calmette-Guerin and M. tuberculosis</td>
<td>Suppression of IL-12 and TLR signaling in DCs (98)</td>
<td>Weak T cell response (tolerogenic?)</td>
</tr>
</tbody>
</table>
any TLR results in the recruitment of IL-1R1-associated protein kinases 1 and 4 to the TLR complex and their phosphorylation. This process results in an association with the TNF receptor-associated factor 6, leading to the activation of the NF-κB and MAPK signaling pathways, which mediate certain core aspects of immune activation, including the induction of inflammatory cytokines such as TNF-α and IL-6. However, the induction of other responses such as type I IFNs and Th2 cytokines appears to be mediated by a specific subset of TLRs. Moreover, even signaling via a single TLR can result in distinct biological responses, depending on the dose of ligand used (53). Understanding the molecular basis for this diversity of biological responses is in its infancy, but as discussed below, already some insights are evident.

Variegating immunity with TLR expression patterns.

One theoretical possibility of generating immune diversity appears to be by segregated expression of distinct TLRs in functionally different subsets of DCs. Thus, the expression of TLR9 and TLR7 in the endosomes of human plasmacytoid DCs, but not on human monocyte-derived DC (MDDCs) (54, 55), might facilitate the generation of antiviral type I IFNs in response to intracellular pathogens such as viruses. In contrast, TLR4 appears not to be expressed on human plasmacytoid DCs, but is expressed on the surface of human MDDCs, consistent with the induction of IL-12 in such DCs by LPS, perhaps important in generating Th1 responses during bacterial infections. Although this is an interesting hypothesis, it is based on TLR expression patterns in DC subsets, which are in the resting state (not activated) and is not supported by TLR expression patterns in the equivalent DC subsets in mice.

Variegating immunity with different adaptor proteins.

The next conceivable method of generating diversity in TLR signaling appears to be with a limited number of proximal adaptor proteins which themselves contain the Toll/IL-1R (TIR) domain that facilitates their association with TLRs (Fig. 1). In this study, at least three possible mechanisms can be envisioned—mechanism 1: different adaptor proteins that mediate distinct signaling pathways and function might be associated with different TLRs; mechanism 2: a single TLR might be associated with multiple adaptor proteins with bifurcating signaling pathways. Precisely which pathway is actually triggered might be determined by several factors such as the affinity/avidity of ligand binding to the TLR and the accessory receptors involved; and mechanism 3: different adaptor proteins might be segregated spatially, either in different subsets of DCs (e.g., splenic CD8α+ vs CD8α− DCs), or DCs present in distinct microenvironments such as the gut or spleen, or in DCs in different stages of an immune response.

TIR domain-containing adaptor protein (TIRAP), another TIR domain-containing protein, is known to associate with TLR4 and TLR2, but not with other TLRs (59, 60). Initial studies suggested that overexpression of a dominant negative form of TIRAP was shown to inhibit NF-κB activation and up-regulation of costimulatory molecules in DCs (59, 60). Furthermore, an inhibitory peptide was shown to suppress IFN-γ-inducible protein 10 (IP-10) and IFN-γ-related gene induction by LPS (61). These observations led to the belief that TIRAP may be part of a MyD88-independent pathway. However, more recent studies using TIRAP-deficient mice (62, 63) have not yet revealed any significant differences in the biological functions mediated by TIRAP or MyD88. As with MyD88−/− mice, DCs from TIRAP-deficient mice are able to up-regulate costimulatory molecules in response to TLR4 and TLR2 ligands, and cells from TIRAP−/− mice do not appear to have any defects in the production of IP-10 or IFN-αβ-related proteins (62, 63). Taken together, these data suggest that both the MyD88 and TIRAP adaptor proteins may lie in the same pathway downstream of TLR2 and TLR4. What unique biological functions it might mediate is yet to be discovered.
TIR domain-containing adaptor-inducing IFN-β (TRIF) (64) or TIR-containing adaptor molecule 1 (TRAM) is an adaptor protein that appears to mediate unique biological functions different from those of MyD88 or TIRAP. Studies with TRIF-deficient mice (66) or with Lps2 mice, which have a distal frameshift mutation in the *Trif* gene (67), revealed strong deficiencies in both TLR3- and TLR4-mediated expression of IFN-β and activation of IFN regulatory factor 3 and type I IFNs and hypersusceptibility to mouse CMV infections. In fact, none of the effects of poly(IC) could be detected in TRIF−/− mice, suggesting that TRIF is vital for TLR3 signaling. In the case of LPS, however, there was normal activation of IL-1R-associated kinase 1, NF-κB, and MAPK, indicating that TRIF is not involved in the LPS-induced activation of the MyD88-dependent signaling. Surprisingly, inflammatory cytokine production (TNF, IL-6, and IL-12p40) in response to the TLR4 ligand but not to other TLR ligands was impaired in TRIF-deficient macrophages (66). Thus, there is likely some cooperation between the MyD88 and TRIF pathways. Mice deficient in both MyD88 and TRIF showed complete loss of NF-κB activation in response to TLR4 stimulation (66). These studies suggest that TRIF is selectively expressed downstream of TLR3 and TLR4 and that it mediates certain unique functions, such as up-regulation of costimulatory molecules on APCs and the induction of IFN-α/β-related genes. Because TRIF is not associated with TLR2, or TLR9 or TLR7, which also induce type I IFNs, and because MyD88 and TIRAP do not mediate up-regulation of costimulatory molecules, there must be additional adaptors to mediate these processes.

A fourth TIR domain-containing adaptor, TRIF-related adaptor molecule (TRAM), has been discovered recently (68). TRAM-deficient mice show defects in cytokine production in response to the TLR4 ligand but not to other TLR ligands. TLR4- but not TLR3-mediated MyD88-independent IFN-β production and activation of signaling cascades are impaired in TRAM-deficient cells. An independent study using dominant negative and short-interfering RNA approaches confirmed that TRAM is restricted to the TLR4 pathway (69). These studies suggest that TRIF and TRAM both function in LPS-TLR4 signaling to regulate the MyD88-independent pathway. However, the question of what adaptor proteins mediate the induction of type I IFN proteins in response to TLR7/8 or 9 triggering remains to be determined.

It would thus appear that segregation of different adaptor proteins with distinct TLRs (mechanism 1) facilitates the generation of unique gene expression programs and functional responses. It is also likely that signaling via a single TLR might generate distinct functional responses, depending on the dose of the ligand, or strength of signal used (mechanism 2). Thus, recent work suggests that although low doses of *E. coli* LPS induce Th2 responses, high doses favor Th1 responses in vivo (53). Consistent with such a model, our recent data suggest that different synthetic derivatives of the LPS of *Neisseria meningitidis*, all of which signal via TLR4, are able to differentially induce type 1 IFNs (TRIF pathway) or proinflammatory cytokines (MyD88 pathway). There is currently no evidence for the geographical segregation of different adaptor proteins in distinct DC subsets, or DCs in different microenvironments (mechanism 3). However, given the strikingly different responsiveness of distinct DC subsets to the same PAMP (e.g., *E. coli* LPS induces much higher levels of IL-12p70 from murine CD8α+ DCs than from CD8α– DCs (33, 37); Pam-3-cys induces much higher levels of IL-10 from CD8α– CD11b+ DCs than from CD8α+ DCs (37); DCs in the Peyer’s patch secrete much lower levels of IL-12p70 than splenic DCs (15)). It is possible that mechanism 3 might be operating.

### Role of TLRs in modulating Th1, Th2, T regulatory, and CTL responses

Initial studies established the importance of TLRs in inducing Th1 responses (Table III). For example, the TLR4 ligand LPS (70), CpG DNA, poly(IC), and TLR7 ligands induce IL-12p70 and IFN-α from DCs and stimulate Th1 responses (4–7, 33, 36, 47–52). Certain TLR2 ligands also induce weak IL-12p70, and MyD88−/− mice appear to have a selective defect in Th1 responses (58, 71–74), suggesting that all TLRs preferentially mediate Th1 responses and not Th2 responses. However, there is increasing evidence that certain TLR ligands may also mediate Th2 responses (Table III). For example, while *E. coli* LPS (a TLR4 ligand) induces a Th1 response, highly purified LPS preparations from *P. gingivalis* LPS, a putative TLR2 ligand (75), favors a Th2 response (33). *Escherichia coli* LPS but not *P. gingivalis* LPS induces IL-12p70 in the splenic CD8α+ DCs (33). This finding is consistent with other work that suggests that *P. gingivalis* LPS fail to induce IL-12p70 in murine macrophages (75) and human MDDCs in vitro (76). Similar results have been obtained with the synthetic TLR2 ligand...
Pam-3-cys (36, 37). Thus, Pam-3-cys, E. coli LPS, flagellin, and schistosome egg Ags (SEA) activate human MDDCs to express enhanced levels of costimulatory molecules, but differ in the cytokines they induce. Although E. coli LPS and flagellin induce abundant IL-12p70, Pam-3-cys and SEA do not do so but can induce the Th2-inducing or regulatory cytokine IL-10. Although E. coli LPS and flagellin induce strong Th1 responses, Pam-3-cys and SEA favor a Th2 bias (36). In the mouse system, almost identical results are evident; Pam-3-cys induces very little IL-12p70 in splenic CD8α+ DCs compared with E. coli LPS (37). In contrast, it induces much higher levels of IL-10 in the CD8α+ DCs, relative to E. coli LPS (37). Interestingly, the induction of IL-10 was largely abrogated in DCs from MyD88−/− mice (37). Consistent with their differential cytokine induction in DCs, Pam-3-cys and E. coli LPS induced Th2- and Th1-biased responses, respectively (36, 37). These studies are supported by several other reports, which suggest that signaling via TLR2 may result in Th2 or T regulatory responses (77–81).

Specific TLRs may also induce cross-presentation and CTLs. Thus, signaling via TLR3, 7, and 9 induces cross-presentation, whereas TLR2 and TLR4 ligands do not (82). Interestingly, TLR3, 7, and 9 also induce abundant type I IFNs, which are contrast, certain TLR2 ligands, as well as the classic Th2 stimulus SEA, induce sustained and enhanced phosphorylation of ERK, which stabilizes c-Fos, which suppresses the production of IL-12p70. As a result, the balance is shifted toward the Th2 end of the spectrum. Potential therapeutic targets and approaches to modulating the Th balance are highlighted (36, 37).

Thus, signaling via TLR3, 7, and 9 induces cross-presentation, whereas TLR2 and TLR4 ligands do not (82). Interestingly, the induction of IL-10 was largely abrogated in DCs from MyD88−/− mice (37). Consistent with their differential cytokine induction in DCs, Pam-3-cys and E. coli LPS induced Th2- and Th1-biased responses, respectively (36, 37). These studies are supported by several other reports, which suggest that signaling via TLR2 may result in Th2 or T regulatory responses (77–81).

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A signaling pathway for generating Th2 responses

Given that certain TLRs might bias toward the Th2 pathway (Fig. 2), by what signaling mechanisms do they act? Our recent work suggests that Pam-3-cys and SEA induce enhanced duration and magnitude of ERK signaling in DCs (36, 37). Interestingly, DCs from ERK1−/− mice produced enhanced levels of IL-12p70 and greatly diminished levels of IL-10 in response to Pam-3-cys (37), and human MDDCs treated with a synthetic inhibitor of MEK1/2 produced enhanced levels of IL-12p70 in response to TLR4, 5, or 2 stimulation (36). This is consistent with previous reports that ERK suppresses the induction of IL-12 and enhances IL-10 induction (84), as well as with a subsequent report that SEA induces enhanced ERK signaling in DCs (85).

By what mechanism does enhanced ERK signaling impair IL-12 induction? We were struck by a report which suggested that sustained duration and magnitude of ERK signaling results in phosphorylation and stabilization of the early growth transcription factor c-Fos in fibroblasts (86). We thus investigated this question and indeed c-Fos was found to be expressed at enhanced levels, and in a phosphorylated form, in DCs stimulated by TLR2 ligands and SEA, relative to DCs stimulated with E. coli LPS or flagellin (36, 37). Furthermore, c-Fos appears to control the induction of IL-12p70 and IL-10 in DCs, because DCs from c-Fos−/− mice have a dramatic impairment of IL-12p70 and an enhancement of IL-10 (37). Consistently, human MDDCs pretreated with short-interfering RNA against c-Fos and then stimulated with SEA produced high levels of IL-12p70 (36). Thus, the impairment of c-Fos, a single transcription factor within DCs, was sufficient to convert a classic Th2 stimulus into a Th1 stimulus. This raises the possibility that targeting specific transcription factors within DCs might provide novel avenues for controlling the quality of immunity. Indeed, there is now emerging evidence for other transcription factors in playing important roles in DC1/DC2 polarization. Thus, T-bet (87) and NF-κB (88), and c-Rel (89) are required for optimal induction of Th1 responses by DCs, whereas c-Maf appears to suppress IL-12 and enhance IL-10 in macrophages (90). Finally, two recent reports suggest that different Notch receptors on DCs can instruct distinct Th responses (91, 92). Thus, Amsen et al. (91) suggest that DCs can be induced to express Jagged or Delta by Th1-inducing stimuli (LPS) and Th2-inducing stimuli (cholera toxin), respectively, and that the ligands mediate the induction Th1 vs Th2 responses. Precisely how such ligands interact with the ERK-c-Fos pathway described above is yet to be determined.

Other PRRs in the aisle: C-type lectins, NOD, and mannose receptors

Although TLRs have enjoyed center stage, other important candidates are waiting in the aisle. For example, C-type lectins bind carbohydrates from pathogens and from self-glycoproteins, and thus likely are important not only in pathogen sensing, but also in cell adhesion, in migration, and in maintaining self-tolerance (93, 94). DC-specific ICAM3-grabbing nonintegrin (DCSIGN), which is involved in the capture of different pathogens, is expressed by dermal DCs, as well as in the mucosal tissues by interstitial DCs (93, 94). Emerging evidence suggests that several viruses (HIV, CMV, hepatitis C virus (HCV), and dengue), bacteria (Helicobacter pylori, M. tuberculosis ManLAM (lipooligosaccharinmannan) and Klebsiella pneumonia), and yeasts interact with DC-SIGN. A common feature of several of these pathogens is that they cause chronic infections, in which the T regulatory responses are a critical determinant of pathogen persistence. Therefore, many pathogens might specifically target DC-SIGN to suppress DC function and modulate immune responses by inducing Th2 or T regulatory responses that are beneficial for their persistence in the host (95, 96). Thus, learning how to reset the balance toward the Th1 end of the spectrum may offer novel strategies to treat chronic infections such as HIV and HCV.
Another potential intracellular PRR is NOD2, a member of the NOD-leucine-rich repeat protein family, which recognizes the muramyl dipeptide component of peptidoglycans (97). Mutations in CARD15, the gene encoding NOD2, are observed in a significant fraction of patients with inflammatory bowel disease, suggesting that NOD2 might act as an immune brake. Consistent with this, NOD2-/- mice appear to have enhanced Th1 responses to stimulation with certain TLR2 ligands (97). Thus, NOD2 might partly account for the Th2 or T regulatory responses observed previously with TLR2 signaling (33, 36, 37, 75–81). Exactly how NOD2 signaling interacts with the ERK/c-Fos Th2 pathway described above (36, 37) remains to be seen.

Finally, there is some evidence that signaling through the mannose receptor also results in inhibition of TLR signaling. Thus, mannose-capped lipopolysaccharinanns from Mycobacterium bovis, bacillus Calmette-Guérin, and M. tuberculosis inhibit LPS-induced IL-12 production by DCs (98).

**Summary**

The past decade has witnessed a paradigm shift in immunology, triggered by Janeway (46), that places innate immunity at the helm. It is now clear that DCs and PRRs play key instructive roles in the variation of adaptive immunity. Today in the field of DC biology, as there has been in so many other fields, there is a great debate on the respective roles of genetic preprocessing vs functional plasticity. Here, it is perhaps instructive for us to gain a wider biological perspective by considering mechanisms of lineage fate decisions in other biological systems. In such cases, it appears that developmental fate decisions are governed by mechanisms that rely both on genetic hardwiring and dynamic environmental cues. This debate notwithstanding, the sequencing of the human genome, and the ensuing era of “genomics,” have provided an opportune platform to discover the molecular minutia of signaling networks within DCs that control adaptive immunity. However, although these molecular details are of great importance, it is perhaps of even greater import to develop an understanding of the whole “system” and of the fundamental biological processes that shape immunity.

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