

Innate immunity: sensing the environment and regulating the regulators

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Purpose of review

Innate immune cells act as sensors for environmental pathogens and key regulators of pathogen-specific T-cell effector responses. The expression of Toll-like receptors is essential for these functions. Innate immunity is a rapidly evolving field. Great progress has been made in the past year in characterizing the signaling pathways downstream of Toll-like receptors, and the role of Toll-like receptors in the regulation of pathogen responses.

Recent findings

The ability to control the activation of regulatory T cells has emerged as a key function of innate immunity. Regulatory T cells are involved in the induction of tolerance and the prevention of harmful immune pathology, particularly T-cell responses directed against self-antigens. Toll-like receptor-mediated interactions between pathogen-stimulated innate immune cells and regulatory T cells result in the release of suppression by regulatory T cells, thus allowing pathogen-specific responses. However, pathogenic microorganisms may exploit this interaction to evade the host's response. In addition, recent studies raise the possibility that regulatory T cells may express some Toll-like receptor family members, thereby responding directly to pathogens.

Summary

A deeper understanding of the complex role of innate immune cells as sensors of the environment and regulators of pathogen responses will probably influence the current models of immune regulation, particularly those centered on the role of the environment in shaping immune responses.

Keywords

dendritic cells, innate immunity, regulatory T cells, Toll-like receptors

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Abbreviations

DC dendritic cell
TLR Toll-like receptor
Th T helper
Treg T regulatory cell

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Introduction

The selective pressure imposed by infections has driven multi-cellular organisms to develop immune defense mechanisms that protect the host by destroying the invading microbes or neutralizing the factors responsible for their virulence [1]. Immune responses hinge upon the functional integration between two components: one innate and non-antigen specific, the other adaptive and antigen specific. The complex interplay between the two is perhaps the most distinctive feature of immune processes.

Innate immunity uses a family of non-clonal pattern recognition receptors (the Toll-like receptors; TLRs) to act as an interface with both the external and the internal milieu. On the one hand, innate immune cells sense the signals provided by environmental pathogens, which alert the system to the presence of potentially dangerous infectious intruders. Through the release of inflammatory cytokines and the expression of co-stimulatory molecules, innate immune cells (typically, dendritic cells; DCs) then talk to CD4 T cells, instructing them to move along differentiation programmes dictated by the nature of the microbial threat [1–3].

Innate immunity is a rapidly evolving field. Over the past year, our understanding of the signaling pathways downstream of TLRs has been enriched by the discovery and characterization of new components. Among these, TIRAP/Mal [4] mediates TLR2/4 but not TLR9, TLR5 and TLR7 signaling, thereby providing some degree of specificity to these pathways [5,6]. IRAK-4 [7] and IRAK-M [8], both downstream of MyD88, have been found to regulate TLR-dependent functions positively or negatively, respectively. The key role played by IRAK-4 *in vivo* has been eloquently highlighted by the discovery that IRAK-4 deficiency results in enhanced susceptibility to pyogenic bacterial infections [9]. The traditional boundaries between adaptive and innate immune responses have been made more blurred by the discovery that SOCS-1, a molecule known to act as a negative regulator of STAT/cytokine receptor signaling, also negatively regulates lipopolysaccharide responses [10,11]. Finally, the observation that TLR4 is monoallelically expressed [12] is likely to be only a prelude to genetic and functional studies that may redefine TLR-mediated events.

All these data are novel and undoubtedly important. However, yet another theme emerged recently whose implications may reach even further. Cells and receptors of innate immunity have been found to control the function of CD4 T regulatory cells (Tregs), currently the rising star in the immunological firmament. Because of their biological significance, the interactions between innate immunity and T-cell regulation will be the focus of the rest of this article.

No activation without regulation

The recent explosion of interest in, and information on, Tregs is almost unprecedented in immunology. The features of these cells have been extensively reviewed [13–17]. For the purpose of our discussion, suffice it to say that Tregs arise spontaneously in the thymus, and constitute only a small proportion (5–10%) of the total CD4 T-cell population. Tregs express the CD25 activation marker often, but not invariably, such that positive identification of this subset is only achieved using functional suppression assays. A new tool towards Treg identification may be provided by the demonstration that these cells selectively express the transcription factor Foxp3 [18,19•]. This molecule appears to be more than just a marker, because the ectopic expression of Foxp3 was sufficient to confer regulatory properties to CD4+CD25– cells. Of note is the fact that an aggressive, fatal, autoimmune disease, the immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, has been mapped to multiple mutations in human Foxp3, which abrogate Treg function [20].

The cardinal feature of Tregs lies in their ability to induce tolerance and prevent harmful immune pathology, particularly T-cell responses directed against self-antigens such as in autoimmunity and transplantation. The specificity of Tregs is still an open issue. Although there is experimental evidence for antigen specificity in suppression, such specificity applies to the afferent limb of the response, namely sensitization, but not to the efferent limb, namely actual suppressive effects. In fact, suppression can be extended to immune responses other than those directed against the initial antigen, which has resulted in the concept of bystander suppression. The mechanisms underlying Treg-mediated suppression of T-cell effector activity are still unclear, but are thought to require cell–cell contact, and result in the inhibition of IL-2 transcription in responder T cells. The secretion of IL-10 and transforming growth factor beta, and possibly membrane expression of transforming growth factor beta [21], are the hallmark of Treg activity. It remains to be seen whether Tregs are just one population, or rather include subsets (e.g. CD4+CD25+, TH3, TR1) with different functional specialization.

A complex ménage à trois

Although much of the research on Tregs has focused on their ability to regulate effector T cells, the question that now arises is, who regulates the regulators [15]? More specifically, how is the activity of Tregs controlled such that effective immune responses against pathogens are allowed, but pathological self-reactivity is suppressed? Several independent lines of research indicate that innate immune cells or their receptors play a pivotal and somewhat unexpected role in this process.

It is by now well established that TLRs control the activation of adaptive immune responses by antigen-presenting cells. The binding of conserved pathogen-derived molecules to TLRs expressed on DCs activates co-stimulation and production of inflammatory cytokines [1]. DC maturation is accompanied by migration to the local lymph node, where DCs present antigen to naive T cells, and instruct them to acquire effector functions and a T helper type 1 (Th1) or Th2 phenotype. The group to which we owe the initial characterization of mammalian Toll [22] has now identified a second mechanism of immune induction by TLRs, which is independent of the effects on co-stimulation, and enhances effector T-cell responses by overcoming the suppressive effects of Tregs. In these experiments, microbial induction of the TLR pathway blocked the suppressive effect of CD4+CD25+ Tregs, allowing the activation of pathogen-specific adaptive immune responses. Unexpectedly, this block of suppressor activity was partly dependent on IL-6, which was induced by the TLR-mediated recognition of microbial products [23•]. The fact that TLR-dependent cytokine secretion, but not co-stimulation, was required to overcome suppression suggests that this property of the TLR pathway may be functionally separable from the ability to activate adaptive CD4 T-cell effector responses. Furthermore, and perhaps more importantly, these results demonstrate that the efficient activation of effector T cells in the face of microbial aggression requires overcoming dominant negative regulation by Tregs [15].

Matters are, however, becoming more complicated. The effects of innate pathogen recognition on the interactions between regulatory and effector T cells are not invariably stimulatory. Virulence factors of the pathogen may exploit Tregs to evade the host's immune response. Filamentous hemagglutinin, a virulence factor of *Bordetella pertussis*, thus interacts directly with DCs to induce IL-10 and inhibit lipopolysaccharide-induced IL-12 and inflammatory chemokine production. The DCs generated after interaction with this bacterial product selectively stimulate the induction of Tregs, which inhibit protective Th1 responses against *B. pertussis* *in vitro* and *in vivo* [24•]. Along similar lines, LcrV, the virulence antigen of human pathogenic *Yersinia* species, is

involved in evasion of the host's immune response because it signals in a CD14- and TLR2-dependent fashion, leading to immunosuppression by IL-10 induction [25•]. Pathogen-stimulated Tregs thus contribute to the negative regulation of both inflammatory and protective responses to infectious microbes, suggesting that they may be involved in the 'quality control' of the immune response [13].

In the work discussed so far, pathogen-induced modulation of Treg function occurs at the DC level and is mediated by host-derived factors. A recent provocative report [26•], however, raised the possibility that, at least in the mouse, Tregs may directly respond to pathogen products thanks to their ability to express TLRs. TLR4, TLR5, TLR7, and TLR8 were found to be selectively expressed in murine CD4 T-cell subsets that contain Tregs. Exposure of CD4+CD25+ cells to the TLR4 ligand lipopolysaccharide upregulated several activation markers and enhanced cell survival and proliferation, independent of antigen-presenting cells. Most importantly, lipopolysaccharide treatment significantly increased CD4+CD25+ cell suppressor efficiency and conferred suppressive activity to CD4+CD25- cells [26•]. The finding that the direct engagement of TLRs on Tregs triggers their suppressive activity, whereas pathogen-mediated TLR engagement on DCs releases Treg-dependent suppression, highlights the remarkable complexity of the interactions between innate and regulatory pathways and their often counterintuitive outcomes. It remains to be seen whether TLR-mediated direct activation by bacterial pathogens occurs in human T cells.

Conclusion

We have known for a while that innate immunity is a critical interface between the environment, with its microbial burden and plethora of danger signals, and adaptive responses. Sensing and regulation have been identified as the two key functional properties of this system. Accordingly, major efforts have been directed at identifying the molecular mechanisms underlying these properties. The impact of innate immunity used to be ascribed to its direct regulatory effects on the Th1 and Th2 differentiation programmes. However, it is now becoming clear that this interpretation may not cover the whole biological scope of innate responses, which should be extended to include the modulation of Treg cell function. The implications of this shift in perspective are several and profound. Perhaps the most significant is the advent of yet another, still somewhat enigmatic, player in the regulatory arena, whose interactions with the environment may be either direct or mediated by innate immune cells. The *ménage à trois* between innate immunity, Tregs and effector T cells is perhaps the hottest theme in immunology at present. All of this will

probably influence the current models of immune regulation, particularly those centered on the role of the environment in shaping immune responses. Not by chance, the most recent and sophisticated models of the hygiene hypothesis go beyond the Th1/Th2 paradigm, and focus on the interaction between the environment, innate immunity and Tregs [27,28]. Therefore, now more than ever, innate immunity is the watchful gatekeeper of immune responses.

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