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EDITORIAL

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The innate and adaptive immune systems in allergy: a two-way street

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The lipopolysaccharide debate in allergy

It is generally accepted that allergy is the result of a dysregulated adaptive immune response. How that regulation is lost and, in particular, how the innate immune system participates in this dysregulation is an area of intense research and active debate. One of the rogue players in the atopic adaptive immune response is a T-helper type 2 (Th2) cell. Data from numerous animal and human studies support the hypothesis that the central effector cell in allergy is the Th2 cell, which produces IL-4, IL-5 and IL-13; these cytokines are necessary and sufficient to induce the pathology observed in atopic asthma models [1]. The antigens these T cells respond to are typically inert environmental proteins – antigens that the immune system should ignore. Why and how the immune system responds to these antigens is a question that necessitates introduction of regulatory (or potentially rogue) members of the innate immune system.

Only fully activated antigen-presenting cells (APCs) can prime naïve T cells - the first step in generating an adaptive immune response. Following the discovery of toll-like receptors (TLRs) in the mid-1990s, we now know that one of the primary means of inducing APC maturation is with TLR ligation [2, 3]. Thus, adaptive T cell responses require signals from the innate immune system. This has been established for Th1 responses but remains an area of debate regarding Th2 immunity [4]. Our laboratory and others have shown that priming of Th2 cells in animal models of asthma does not occur in the absence of TLR4 stimulation by lipopolysaccharide (LPS, otherwise known as endotoxin). In contrast, numerous groups have shown that, depending on the timing of exposure, LPS can inhibit the development of allergy (see [5] for a review). Some of this discrepancy may be attributable to differences in the dose of LPS used (in our model, higher doses of LPS favour Th1 and lower doses favour Th2) [6]. Epidemiological studies in humans do not clarify the issue – endotoxin has been found to both inhibit and facilitate allergic disease; the recent identification of numerous TLR polymorphisms in allergic cohorts has similarly failed to confirm one hypothesis or the other [7–10]. In either case, it is clear that dysregulated innate immunity can lead to dysregulated adaptive immunity. But does the adaptive immune system have any way of regulating itself and/or the innate immune system?

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Interleukin-4 in the innate immune system

IL-4 is the main differentiation factor for Th2 cells and signals, along with IL-13, through the transcription factor STAT6 to induce many of its cellular effects [1]. In the current issue of *Clinical & Experimental Allergy*, Fiset et al. [11] report on a new role for IL-4 in the orchestration of allergy – regulation of TLR4 expression on monocytes – and add further complexity to our understanding of the adaptive–innate immune system interplay. The authors used a monocytic cell line that expresses TLR4 to study the effect of IL-4 on LPS-induced nuclear factor-κB (NF-κB) activation, surface expression of TLR4, and transcription of the *TLR4* gene. Interestingly, all of these parameters were transiently suppressed by IL-4 in a STAT6-dependent manner. The authors based the current studies on their previous findings that nasal mucosa explants from atopic adult patients demonstrate reduced LPS responsiveness and the hypothesis that Th2 effector cytokines may play a role in this inhibition [12].

A significant body of literature exists to support this hypothesis. Prior to the identification of TLRs, IL-4 was found to reduce surface expression of CD14, a co-receptor for LPS that is now known to act in concert with TLR4 [13]. IL-4 has been shown previously to down-regulate surface expression of TLR4 and to inhibit LPS-induced cytokine production from human monocytes [14, 15]. Lentsch et al. [16] later demonstrated that STAT6 is normally activated during endotoxemia (intravenous challenge with LPS); in the absence of STAT6, NF-κB activation, TNF-α production and mortality are all increased. In fact, Th2 cytokines have been persistently

mislabeled as 'anti-inflammatory' because of their regulatory role in Th1-mediated disease models and their effectiveness in blocking endotoxic shock [17, 18]. Perhaps these early studies showing Th2 cytokine abrogation of endotoxic shock may be explained by the current study's observation that IL-4 suppresses TLR4 surface expression. Together, these studies suggest that IL-4 negatively regulates the uncontrolled release of highly pro-inflammatory cytokines such as TNF-α, IL-1 and IL-12 following TLR4 detection of Gram-negative bacteria.

Clonal antigen-presenting cells

The proposed teleological role of type 2 immune responses is to combat parasitic infections. One can easily imagine a situation in which both parasitic and bacterial antigens are encountered simultaneously (for example, in the gastrointestinal tract). Typically, the latter type of infection is targeted with a Th1-type response. However, it may not be advantageous for an APC to present both types of antigens (parasitic and bacterial) and induce simultaneous Th1 and Th2 responses, as the two would require distinct differentiation factors that would ultimately inhibit each other. It is possible that the effect of IL-4 noted by Fiest et al., and others represents one way in which the Th2 cell can target a parasite with the appropriate immune response. Th2 cells, responding to a parasitic infection, would produce IL-4, which could act on local APCs to down-regulate surface expression of TLR4 thereby reducing their responsiveness to LPS from co-infecting gram-negative bacteria. This might prevent the APCs from producing cytokines or cell surface molecules that would favour Th1 differentiation. Just as T and B cells have clonal responses, this 'clonal' APC would be directed by the Th2 cell to limit its activity to Th2 in nature. IL-4 may therefore act to encourage clonal monocyte responses by regulating their ability to sense other TLR ligands. But how would this 'immunofocusing' role of IL-4 apply to allergy?

Clinical implications

In those with chronic allergies, hyperactive Th2 immunity might have a profound effect on the innate immune system. According to the hypothesis presented by Fiset et al. [11], one would predict that patients with asthma or allergies should have a blunted response to LPS exposure, as they would have a chronically suppressed level of TLR4. In fact, the opposite is true - asthmatics have an exaggerated bronchoconstrictive and systemic cytokine response to inhaled LPS [7, 19]. One explanation might be that IL-4 selectively regulates TLR4 only on monocytes and does not affect other LPS-responsive cells in the lung; however, this kind of selectivity has not been established. This discrepancy raises two issues with the current study. First, the *in vivo* relevance of an *in vitro* cell line subjected to possibly artificial concentrations of IL-4 is difficult to assess (although IL-4 has been shown to reduce LPS responsiveness in animal models of endotoxemia). More importantly, is a reduction of TLR4 surface expression by 20–30% physiologically relevant *in vivo*? In other words, would a relatively modest change in monocyte TLR4 expression affect its ability to control LPS responses in endotoxemia or to activate or inhibit T cells in allergy? Heightened LPS responsiveness in asthmatics suggests that it may not. Indeed, the authors recognize that IL-4 may instead 'fine-tune' LPS responsiveness but will not turn it completely off. What impact might APC fine-tuning have on the progression of atopy?

Before addressing this question, it is necessary to point out that IL-4 affects more than just TLR4 surface expression on APCs; it has been shown to down-regulate FcγRIII on monocytes, but up-regulate MHC class II on these same cells and B7 costimulatory molecules on B cells [13, 20]. Therefore, despite reduced LPS responsiveness, IL-4-treated monocytes may be potent T cell activators. In addition, regulatory T cells (T_{reg}) also express TLR4 and are activated by LPS [21]. Therefore, aberrant allergic Th2 responses may avoid regulation if IL-4 can similarly reduce T_{reg} responsiveness to LPS.

These findings suggest a potential for a self-perpetuating, deregulated Th2 response, which might manifest as increased allergy and reduced Th1 immunity (Fig. 1). Once established, Th2 responses could be resistant to LPS down-regulation if IL-4 acts as a negative regulator of

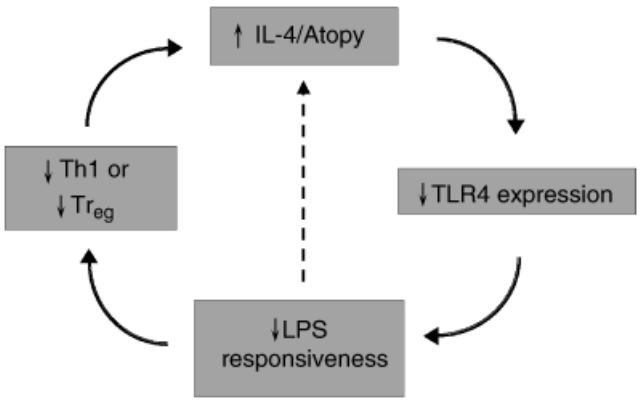


Fig. 1. A self-fulfilling prophecy: IL-4 restricts toll-like receptor 4 (TLR4) expression on monocytes thereby reducing lipopolysaccharide (LPS) responsiveness. This, in turn, might skew immune responses away from either T-helper type 1 (Th1) or regulatory T cells (T_{reg}) cells and release ongoing T-helper type 2 (Th2) responses from these regulatory influences. The dashed central arrow indicates a possible direct effect of reduced LPS responsiveness in creating optimal Th2 priming conditions; high-level LPS exposure could be perceived as a low dose of LPS and favour further Th2 priming over what might normally be a Th1 response. This model predicts what is clinically evident: once a Th2 process is initiated it may be difficult to overcome, even with very high doses of LPS.

TLR4. This may explain why it is difficult to break the allergic cycle once initiated and why the timing of LPS exposure is critical. In fact, we would hypothesize, based on previous work, that reduced LPS responsiveness might promote further Th2 priming [6]. Exposure to a significant dose of LPS in the presence of enough IL-4 to affect TLR4 expression might make the APC respond as if a low dose of LPS (i.e., a pro-Th2 priming dose) was encountered (Fig. 1, dashed arrow). With the additional direct effect of IL-4 in up-regulating MHC class II and possibly co-stimulatory molecules on the monocytes and promoting Th2 differentiation of naïve T cells, a potent environment for further Th2 priming arises. Where would this IL-4 come from in a naïve individual? Perhaps initially from naïve T cells themselves, particularly in a genetically prone individual [22]. These speculations require further studies, however, the idea of adaptive immunity regulating innate immunity poses some intriguing possibilities that could have serious consequences for allergic disease.

Future direction

The regulation of TLR4 by IL-4 may not be unidirectional. Although IL-4 down-regulates TLR4 expression on human monocytes, it has also been shown to up-regulate TLR4 on B cells [14]. What this means for the orchestration of an immune response is unclear. In addition, the mechanism by which IL-4 and STAT6 regulate TLR4 expression needs to be elucidated to better understand how innate immune receptors are regulated. And what of other TLRs? Is this a selective process with only certain TLRs regulated by IL-4 or a global effect on pattern recognition receptors in general? The answers to these questions and others raised earlier will shed light on how Th2 immunity inhibits innate immune responses and potentially propagates maladaptive allergic responses.

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