

Toll-Like Receptor-Mediated Activation of Mast Cells: Implications for Allergic Disease?

Jean S. Marshall Jeffrey D. McCurdy Tim Olynich

Dalhousie Inflammation Group, Departments of Pathology and Microbiology & Immunology,
Dalhousie University, Halifax, Nova Scotia, Canada

Key Words

Toll-like receptor · Asthma · Allergy A · Atopic dermatitis · Leukotriene · Inflammation · Innate immunity

Abstract

Toll-like receptors have a critical role in innate immunity and host defence. However their role in allergic disease has not been studied in great detail. The presence of these receptors on mast cells opens up new possibilities concerning the role of Toll-like receptors in the pathogenesis of asthma and atopic dermatitis. The current review examines the biology of Toll-like receptors expressed on mast cells. In particular, mast cell expression of Toll-like receptors and the diverse responses observed following Toll-like receptor-mediated activation are considered. Several pathogens such as *Staphylococcus aureus* and respiratory syncytial virus are known to contribute to the development or maintenance of allergic disease and also express potent activators of the Toll-like receptor pathways. The importance of such interactions and the full role of pathogens in chronic allergic disease remain to be elucidated. The unusual ability of Toll-like receptor 2 activators to selectively induce leukotriene production by mast cells opens up new possibilities concerning mechanisms of disease exacerbation during infection.

Copyright © 2003 S. Karger AG, Basel

Overview

The mast cell has traditionally been viewed primarily as an early effector cell in allergic disease. More recently, it has been recognized that these cells also play a critical role in host defense as key effector cells of the innate immune response. A major component of innate immune function is mediated through a class of pattern recognition receptors known as the Toll-like receptors (TLR). Work from a number of groups has recently defined the expression and function of TLR by mast cells. Studies suggest that human mast cells have the ability to selectively produce a number of mediators, including leukotriene C₄ (LTC₄), without degranulation in response to certain pathogen products. These observations may have important implications for understanding the role of mast cells and other immune effector cells in allergic disease, particularly in the context of infection (fig. 1).

Mast Cells and Infection

Studies using rodent models of disease have clearly implicated the mast cell in host defense against bacterial infection. Echtenacher et al. [1] and Malaviya et al. [2] performed groundbreaking studies demonstrating that W/W^V mast cell-deficient mice were highly susceptible to acute septic peritonitis induced by cecal ligation and puncture or *Klebsiella pneumoniae* instillation. Local correction of the mast cell deficiency, in the peritoneal cavi-

KARGER

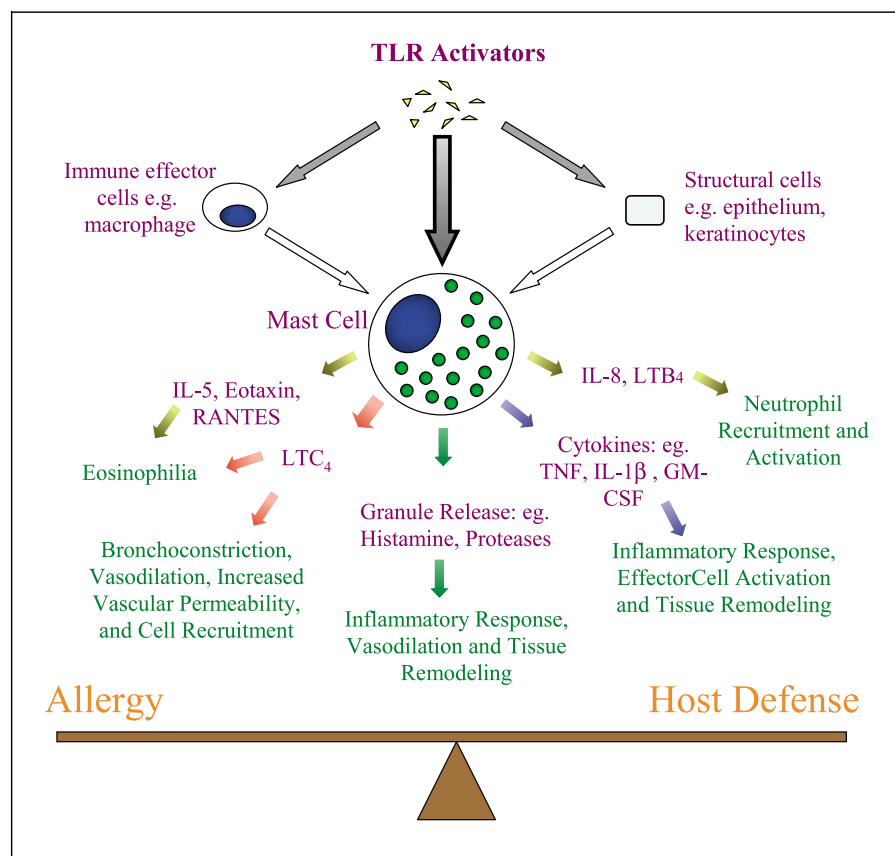
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2003 S. Karger AG, Basel
1018–2438/03/1322–0087\$19.50/0

Accessible online at:
www.karger.com/iaa

Correspondence to: Dr. Jean S. Marshall
Department of Microbiology and Immunology
Sir Charles Tupper Medical Building, Dalhousie University, College Street
Halifax, Nova Scotia, B3H 4H7 (Canada)
Tel. +1 902 494 5118, Fax +1 902 494 5125, E-Mail jean.marshall@dal.ca

Fig. 1. The spectrum of potential mediator production from mast cells following TLR-mediated activation. Mast cells may be activated directly by TLR activators and other pathogen-associated signals. In addition, mast cells may receive cytokine or other signals from TLR-activated cells within the microenvironment. The nature of the TLR-mediated signal in addition to the cytokine microenvironment of the mast cell and additional pathogen or host-defense-related signals will determine the profile of mediators released from mast cells. In many situations the mediators released will likely enhance effective host defense. However, under some circumstances TLR-mediated mast cell activation could lead to a mediator profile that would be expected to induce acute exacerbations of allergic disease or enhance chronic allergic inflammation.



ty, restored the ability of mast cell-deficient mice to survive infection. Mast cell-deficient mice, subjected to bacterial challenge, had a substantial defect in neutrophil recruitment. The production of both tumor necrosis factor (TNF) and leukotriene B₄ (LTB₄) by mast cells, may have a critical role in mobilizing an effective neutrophil response to bacterial challenge. Complement components also appear to be important in mast cell-dependent host responses to bacteria [3, 4].

The ability of mast cells to participate effectively in host responses to viral pathogens is less understood. It has long been recognized that viral infection of the upper respiratory tract can exacerbate the symptoms of asthma [5, 6], which may suggest direct or indirect mast cell-virus interactions. Some viral infections of the respiratory tract have also been associated with increased mast cell density and evidence of mast cell activation [7–9]. However, the role of mast cells in viral responses *in vivo* has not been studied in detail. Recent studies using human cultured mast cells have suggested that mast cell infection with an RNA virus (dengue virus) can lead to substantial production of a number of inflammatory cytokines and chemo-

kines such as IL-6, RANTES, MIP-1 α and MIP-1 β [10, 11]. In contrast, human adenovirus did not induce such mediator production [11]. A number of other virus-associated proteins have also been shown to have the ability to activate human mast cells and may be of interest with respect to immunity to HIV [12]. Viral activation of mast cells may enhance the innate immune response to pathogen challenge. However, the ability of the most common viral pathogens of the respiratory tract to induce mediator responses from human mast cells remains to be evaluated.

Toll-Like Receptors and Their Activation

Toll-like receptors are a highly conserved family of pattern recognition receptors that play an essential role in the induction of immunity through interaction with microbial products from virtually all classes of pathogenic organisms [13–15]. A receptor, designated Toll, was first described in *Drosophila* where it was shown to have a host defense function. Ten mammalian homologues for these

Table 1. Selected defined activators of TLR

TLR	Activators	References
1	Lipopeptide (Pam ₃ Cys)	44
2	PGN, Zymosan, LTA, Lipopeptides (Pam ₃ cys/Pam ₂ Cys), LPS (<i>P. gingivalis</i> and <i>L. interrogans</i>), HSP 60, bacterial fimbriae, mycobacteria	18, 25, 26, 65, 72, 97–107
3	Poly I:C (dsRNA)	108
4	LPS, Lipid A, RSV protein F, HSP 60, fibrinogen, <i>M. tuberculosis</i>	18, 21, 64, 104, 109–116
5	Flagellin	38, 50
6	PGN, Zymosan, Pam ₂ Cys	37, 117
7	Antiviral compound R-848, S-27609, Imiquimod	39–41
8	Antiviral compound R-848	39
9	Bacterial DNA (CpG DNA), CpG oligonucleotides	34, 35, 118

Note the broad range of pathogen types and the ability of some individual TLR to provide signaling in response to a variety of pathogen products from diverse organisms.

receptors have now been characterized. All are type I membrane proteins with an ecto-domain containing leucine-rich repeats and a highly conserved intracellular domain termed the Toll-interleukin-1 receptor-like domain on the basis of its strong structural and functional similarity to the IL-1 receptor [16].

TLRs generally function as part of a macromolecular complex involving one or more coreceptors. The lipopolysaccharide (LPS) signaling complex is the best studied and involves, in addition to TLR4, LPS binding protein, the GPI-linked protein CD14, an extracellular adaptor molecule known as MD2 and one or more intracellular adaptor molecules such as MyD88 [17–20]. In many cases, it is unclear whether the specific TLR activator, such as LPS, actually binds to the TLR, or rather provides a mechanism for the complex to become active in inducing signaling [21–23]. However, Sato et al. [22] and Iwaki et al. [23] showed that soluble TLR2 cosedimented with zymosan and peptidoglycan (PGN) from *Staphylococcus aureus*, respectively, suggesting physical interaction between TLR2 and specific activators. TLRs generally function within signaling complexes as either homodimers (e.g. TLR4) or as heterodimers. For example, TLR2 is known to form functional dimers with either TLR1 or TLR6 that may have different signaling capacities [24].

A wide range of pathogen-associated and endogenous stimuli are now known to activate cells via a TLR-dependent pathway. These include a number of agents that are associated with the development of allergic disease or its exacerbation, in addition to agents that have been suggested to inhibit the development of atopic responses (see table 1). The majority of LPS types signal through

a TLR4-dependent mechanism, although LPS from some bacterial species such as *Leptospira interrogans* and *Porphyromonas gingivalis* have been shown to act via TLR2 [25, 26]. High levels of LPS exposure during childhood have been implicated in the prevention of allergic disease [27]. These observations may be related to the profound ability of LPS and other TLR activators to enhance the expression of certain type 1 cytokines, such as IL-12, IL-18 and IFN- γ by several cell types [28]. However, such cytokine expression is also highly proinflammatory. Other TLR4 activators include HsP60, and the F-protein of respiratory syncytial virus (RSV). RSV infection is frequently linked to asthma-like symptoms in early childhood, and its link to the development of asthma has been extensively studied [29, 30]. It is not yet known if the changes in airway function that occur as a result of RSV infection are TLR dependent.

Bacterial and viral DNAs can be distinguished from mammalian DNA by their high content of unmethylated CpG motifs. CpG-rich bacterial and viral DNA sequences as well as synthetic CpG-containing oligonucleotides have been demonstrated to enhance the development of a Th1 response and prevent allergic inflammation in several animal models [31–33]. More recently, CpG oligonucleotides have been shown to act through a mechanism highly dependent upon TLR9 to induce a type 1 cytokine response [34, 35]. These features of TLR9 function are currently being exploited not only in vaccine technology but also in the treatment of allergic disease, as reviewed by Krieg [36]. Although, in rodent models, TLR9 is expressed on a variety of cells types, the expression of functional TLR9 in the human may be restricted to plas-

macytoid dendritic cells. CpG motif-mediated activation would then be expected to enhance their maturation and antigen-presenting capacity. CpG administration enhances the induction of effective Th1 responses in several human vaccine studies and may modulate allergic responses through this mechanism.

TLR3 is expressed on a wide variety of cell types found at the host-environment interface including epithelial cells, as well as a variety of immune effector cells. This TLR family member has recently been demonstrated to provide a mechanism by which double-stranded RNA species activate the innate immune response. This response is mimicked by the synthetic analogue poly I:C. Signaling through TLR3 appears not to primarily utilize the MyD88 adapter molecule and leads to the expression of high levels of IFN- γ - and type-1-associated chemokines in a variety of cell types.

One of the functionally more complex TLRs is TLR2, which usually forms heterodimers with either TLR1 or TLR6, although it is capable of signal transduction as a TLR2/TLR2 homodimer. This TLR mediates signals in response to the peptidoglycan associated with the cell walls of many gram-positive bacteria including *S. aureus*. In addition, responses to the yeast cell wall component zymosan as well as a variety of bacterial lipoproteins and lipopeptides are TLR2 dependent. It has been suggested that responses to PGN and zymosan occur predominantly through a TLR2/TLR6 heterodimer [37] while responses to tripalmytoylated lipopeptides such as Pam₃CSK₄ occur through a TLR2/TLR1 heterodimer [24].

TLR5 has recently been shown to bind to bacterial flagellin, but may have additional activators [38]. The biologically relevant ligands to TLR7 and TLR8 have not been fully elucidated. However, a number of synthetic antiviral compounds, such as R-848, S-27609 and Imiquimod are known to activate cells via TLR7 [39–41]. There is a great deal of work remaining to be done to fully understand the range of activators through which this critical family of host defense receptors can be activated.

Expression of TLRs by Structural and Inflammatory Cells

TLRs are widely expressed on both immune effector cells and structural cells throughout the body; however, the range of TLRs expressed by specific cell types is quite variable. Monocytes express a wide range of TLRs including TLR1, TLR2, TLR4, TLR5, TLR6 and TLR8. B cells express TLR1, TLR6, TLR7, TLR10 and possibly TLR9,

while plasmacytoid dendritic cells express TLR1, TLR3, TLR6, TLR7, TLR9 and TLR10 [42–45]. NK cells have also been shown to express TLR4 and TLR3, although they appear to lack expression of TLR2. An initial study by Muzio et al. [46] showed that human neutrophils expressed the RNA for TLR1, 2 and 4 but not TLR3 or 5. However, a more recent and extensive study on neutrophil expression of TLRs by Hayashi et al. [47] showed expression of TLR1, 2, 4, 5, 6, 7, 8, 9 and 10 but not TLR3. TLR-mediated activation leads to the production of IL-8, superoxide generation, L-selectin shedding, increased phagocytosis and respiratory burst [48]. These data are consistent with the pivotal role of neutrophils in host defense against a variety of pathogens.

In the intestine, epithelial cells express low levels of TLR2 and TLR4 near the apical surface [49] while TLR5 was selectively expressed on the basolateral surface of the epithelium [50]. A wide range of pathogens and other stimuli has been shown to enhance the expression of TLRs at this epithelial site [51–53]. In contrast, in the human airways, it has been consistently found that epithelial cells express a number of TLRs and respond to their activators. A study by Becker et al. [54] showed that human tracheobronchial epithelial cells responded to LPS in a CD14-dependent manner to produce β -defensin-2. They also showed that these cells express the RNA for TLRs 1–6 in both the absence and presence of LPS activation [55]. Droemann et al. [56] also showed that alveolar epithelial cells express TLR2. This suggests an important innate immune function for epithelial cells in the lung.

Early work by Plotz et al. [57] demonstrated a CD14-dependent response of peripheral blood eosinophils to LPS. Eosinophil expression of mRNA for CD14, TLR2 and low levels of TLR 4 were also observed. A marked increase in the release of TNF and eosinophil cationic protein was observed following activation with either LPS or lipid A, which are thought to act via TLR4. However, not all studies of eosinophils have led to similar conclusions [58].

A number of structural cells also express TLRs. Human endothelial cells express TLR2 and TLR4 [59] as do human fibroblasts [60]. Of potential importance to host responses in the skin, Keratinocytes express TLR1, TLR2 and TLR5 [61]. In many cell types, TLRs are expressed at relatively low levels on the cell surface, which might limit their detection by flow cytometry or immunohistochemical techniques. Since the major function of some of these molecules has been shown to be intracellular [62], the lack of surface expression does not rule out an important role for these molecules.

TLR Expression and Function on Mast Cells – Mouse Studies

In view of the strategic location, importance in host defense and role in allergic disease of TLRs, a number of groups have examined the expression of TLR by mast cells and their ability to respond to TLR activators. Murine mast cells have been demonstrated to express TLR1, TLR2, TLR6, and TLR8 but not TLR5 [63]. Initial functional studies focused on murine mast cells and mast cell lines. The availability of mouse strains deficient in TLR4 function [64] as well as TLR2 knockout animals [65] made it possible to assess the specific role of TLR4 and TLR2 on murine mast cells quite conclusively. It has been recognized for a number of years that rodent mast cells can respond to LPS. Such activation leads to the production of a range of cytokines, such as IL-6, without concurrent degranulation [66]. The specific role of TLR4 in this response was not examined until 2001. Supajatura et al. [67] and McCurdy et al. [63] separately reported that inflammatory cytokine production by mast cells following LPS activation was TLR4 dependent using cells derived from C3H/HeJ and C57Bl/10ScNcr mice and appropriate controls. In addition, Supajatura et al. [67] reported that W/W^V mice reconstituted with TLR4 mutated mast cells had significantly higher mortality than those reconstituted with normal mast cells following cecal ligation and puncture. The increased mortality was associated with defective neutrophil recruitment and production of pro-inflammatory cytokines. Supajatura et al. [67] extended these studies to compare the roles of TLR2 and TLR4 on mouse mast cells. PGN derived from *S. aureus* was demonstrated to induce the expression of a number of cytokines from mouse mast cells including TNF, IL-4, IL-5, IL-6 and IL-13, while LPS activation did not induce IL-4 or IL-5 production [68]. Studies of intradermal injection sites suggested that PGN could induce some features of allergic inflammation, possibly through TLR2 dependent action as a degranulating agent, although the latter observation remains controversial [69].

Somewhat contrasting data, concerning the mast cell cytokine profiles induced by LPS, were obtained from another group [70]. They observed substantial TH2 cytokine production by LPS-activated mast cells including IL-5, IL-13 and IL-10. Such cytokine expression was highly dependent upon the activation of the MAPK cJun N terminal kinase with an alternate MAPK, P38, playing an additional role possibly at the posttranscriptional level. These observations led to the suggestion that mast cell responses to LPS could contribute to exacerbations of

allergic inflammation during bacterial infection. In more recent studies of murine mast cell responses to TLR2 and TLR4 activators, degranulation-independent cytokine responses to both PGN and LPS were observed. In addition, both LPS and PGN were shown to induce the expression of pro matrix metalloprotease 9 [69]. This protease has been observed to have profound effects on cell recruitment as well as a critical role in the angiogenic process [71].

Another potentially important aspect of TLR function is the ability of TLR-mediated activation to regulate apoptosis. A number of studies have demonstrated that TLR-mediated signals can induce apoptosis in immune effector cells such as monocytes [72, 73]. However, a recent study using a mouse mast cell line has suggested that under some circumstances TLR4-mediated activation of mast cells might protect against apoptosis in the face of growth factor withdrawal [74]. This type of response could have wider implications for the regulation of mast cell populations at sites of TLR-mediated activation, but will require further study both in vivo and in human mast cells.

Overall, such animal studies demonstrate the important function of TLR on mast cells and suggest there could be important implications of such activation not only for the local cytokine milieu in host defense and allergic disease but also for cell recruitment and tissue-remodeling events. Mouse models also provide important opportunities to examine key signaling events in these pathways especially through the use of mast cells derived from embryonic stem cells [75]. However, important differences between species have been noted in TLR expression and function, which make human studies essential before conclusions are drawn regarding the clinical implications of such findings.

Expression and Function of TLR by Human Mast Cells

Understanding human mast cell TLR expression and function is critical to evaluate their potential role in disease. We have reported that human cord blood-derived mast cells (CBMC), derived under endotoxin-free conditions, express a number of TLR including TLR1, TLR2, and TLR6 in addition to the adaptor molecules MD2 and MyD88. However, these cells, did not express detectable levels of TLR4 mRNA. In keeping with observed responses in other cell types, following PGN activation, such CBMC produced substantial levels of the NF6B-dependent cytokines IL-1 β and GM-CSF [76], while there

Table 2. Human mast cell (CBMC) mediator production in response to TLR2 and TLR4 activators

Activator	NF- κ B regulated cytokines	LTC ₄	Degranulation
LPS	–	–	–
LPS IL-4/IFN treated	+	ND	–
PGN	++	+++	–/+ ¹
Zymosan	++	+++	–
Pam ₃ Cys	+	–	+

In studies by McCurdy et al. [76], CBMC did not express TLR4 and were nonresponsive to LPS. However, in more recent studies, treatment of CBMC with either IL-4 or IFN and serum induced the expression of TLR4 and mediator production from mast cells in response to LPS. NF- κ B-regulated cytokines were produced where indicated (+) (e.g. GM-CSF, IL-1 β [McCurdy]), TNF, and IL-10. LTC₄ production was not assessed in IL-4-treated CBMC.

¹ Histamine release was observed by Varadaradjalou et al. [80] in response to PGN; however, only insignificant levels of β -hexosaminidase were observed in resting CBMC treated with PGN [76].

was no such response to *Escherichia coli*-derived LPS, a classical TLR4 activator. Degranulation of human mast cells in response to PGN or other TLR2 activators could have a critical role in exacerbating allergic disease. The response to *S. aureus*-derived PGN was of particular interest in view of reports that a high proportion of subjects with atopic dermatitis have concurrent *S. aureus* infection [77–79]. Using β -hexosaminidase release over 20 min as a measure of degranulation we observed no significant response compared with CBMC treated in parallel with media as a control. Similarly, the yeast cell wall component zymosan, which is thought to also use a TLR2/TLR6 dimer for signaling [24, 44] failed to induce CBMC degranulation. In marked contrast, the synthetic lipopeptide Pam₃CSK₄ induced a significant degranulation response in CBMC derived from multiple donors. While the extent of degranulation observed in response to lipopeptide was only 10–15%, there is little doubt that it could be sufficient to elicit or enhance symptoms in susceptible individuals if mast cells were in contact with bacterial lipopeptide during infection. The reasons why this TLR activator had such different effects from either PGN or zymosan remain to be elucidated but may be related to different TLR heterodimer usage.

The most exciting observations regarding the ability of TLR activators to elicit responses from human mast cells came from studies of leukotriene generation. In general, TLR-mediated activation has not been associated with

lipid mediator production, although COX-2 upregulation in response to LPS is well documented. As previously mentioned, LTB₄ is an important inducer of neutrophil chemotaxis. However, the predominant leukotriene produced by human mast cells, and the one most relevant to allergic disease, in view of its critical roles as a bronchoconstrictor and inducer of inflammatory cell migration, is LTC₄.

CBMC treated with either PGN or zymosan produced LTC₄ in a dose-dependent manner within 20 min. On a per cell basis, the amounts of LTC₄ produced were substantial and of an order of magnitude similar to the amounts induced by IgE-mediated activation. In contrast, however, synthetic lipopeptide did not induce LTC₄ production from CBMC. The ability of selected TLR2 activators to induce LTC₄ production by mast cells opens up the possibility that a range of pathogens and their products could directly induce leukotriene generation from mast cells. In the allergic airway, mast cell numbers may be increased, the integrity of the epithelial barrier compromised, and the local host response to infection inhibited by the ongoing allergic inflammation. The opportunity for pathogen-induced leukotriene generation would therefore be enhanced. Changes in airway responsiveness, associated with asthma, may then amplify the consequences of even limited pathogen-induced LTC₄ generation in terms of airway function. The ability of other TLR activators to induce LTC₄ generation from mast cells remains to be determined; however, it is notable that similar treatment of murine BMMC with TLR activators does not induce detectable cysteinyl leukotriene release [Olynych, unpubl.]. This further suggests that there are critical species differences in the nature of these responses. The induction of LTC₄ generation in the absence of significant mast cell degranulation following PGN or zymosan treatment is particularly unusual in that in response to other activators leukotriene generation and degranulation occur together (table 2). The mechanism of LTC₄ generation by mast cells in response to TLR activators is under active investigation. However, it should be noted that it has not yet been formally demonstrated that such responses are TLR2 dependent. The ability of traditional mast cell-stabilizing agents, such as disodium cromoglycate, to block leukotriene generation in response to TLR2 activators has also not yet been determined.

Human mast cell responses to TLR activators have recently been confirmed by other groups. Varadaradjalou et al. [80] reported that CBMC produced TNF, IL-5, IL-10 and IL-13 in response to either PGN or LPS. Following IL-4 pretreatment of CBMC, they observed increased levels of TLR4 expression. Concordantly, cytokine responses

to LPS were observed in such IL-4-primed cells. More recently, Okumura et al. [81] reported substantial TLR4 surface expression by peripheral blood progenitor derived human mast cells following IFN- γ treatment. Use of gene chip technology demonstrated that LPS-activated, IFN- γ -pretreated mast cells produced a distinct profile of cytokines compared with IgE-activated mast cells. Both IgE-receptor- and LPS-activated mast cells expressed a range of cytokines associated with NF- κ B activation such as GM-CSF, CXCL8 (IL-8), CCL3 (MIP-1 α), CCL4 (MIP-1 β) and TNF. However, LPS-activated mast cells specifically expressed a number of genes that might be associated with the development of an effective host response to pathogen invasion including IL-12, CCL5, CXCL10 and CXCL11 as well as a number of anti-viral response genes. These data add further weight to the concept that TLR-mediated activation could be critical for exacerbations of allergic disease associated with infection. The ability of TLR activators to induce high levels of type 2 cytokines from mast cells, and the upregulation of TLR4 expression following exposure to cytokine environments associated with atopy (IL-4) or viral infection (IFN- γ), may be of particular importance. Given the variability of expression of TLR4 in different mast cell culture systems, studies examining the expression of TLR4 by human mast cells in situ will be of particular importance in understanding the role of this receptor on mast cells in disease.

In addition to their unusual production of LTC₄ in response to certain TLR activators human mast cells might also be capable of inducing a distinct cytokine profile from other effector cells of the innate immune response. For example, in the study by Okumura et al. [81] IFN- γ -pretreated human mast cells were compared with peripheral blood-derived mononuclear cells for their cytokine expression following LPS activation. While a number of common genes were activated, mast cells specifically expressed IL-5, CCL1, CCL5, CCL8 as well as a number of receptors such as Fc γ RIIa and TLR1. The selective expression of IL-5, CCL1 and CCL5 might be particularly important in the mast cell-dependent recruitment of Th2 cells and eosinophils to mast cell-rich sites following TLR-mediated activation.

Interactions between TLR-Mediated Pathways and Other Mechanisms of Mast Cell Activation

There are many other potential mechanisms by which pathogens can activate mast cells, although, unlike interactions via TLR, most of these depend upon, or are

enhanced by, the presence of preexisting antibody. Many TLR-mediated mast cell responses to pathogens are likely to occur in the context of other routes of mast cell activation by pathogens and their products. Complement-mediated mechanisms involving opsonization of bacteria and more direct cellular activation via the anaphylotoxins C3a and C5a have been shown to be important in several models [3, 82, 83]. Reduced mast cell activation and neutrophil recruitment was observed in iC3b receptor (CR3, Mac-1)-deficient mice subjected to cecal ligation and puncture [4]. However, expression of receptors for complement products can be quite variable on mast cells. For example, it has been reported that human skin mast cells but not human lung mast cells carry the CD88 receptor for C5a [84, 85].

Fc-receptor-mediated interactions with pathogens also play a key role in mast cell activation in individuals previously exposed to a specific pathogen. Several reports have documented that patients with peptic ulcers and atopic dermatitis have specific IgE antibodies against *Helicobacter pylori* and *S. aureus*, respectively [86, 87]. Fc γ receptors may also play a key role. For example *Borrelia burgdorferi*, the causative agent of Lyme disease, induces mast cell activation via an Fc γ -receptor-dependent mechanism [88]. According to recent reports, expression of a full range of functional Fc γ receptors by human mast cells following cytokine exposure [89] adds to the potential of these cells to participate effectively in host defense against a wider range of pathogens. Nonclassical immunoglobulin interactions may also play a role in mast cell activation including interactions with a number of immunoglobulin superantigens, such as protein A from *S. aureus* and protein L from *Peptostreptococcus magnus* [89]. While it is likely that Fc-receptor-mediated and TLR-mediated mast cell activation processes would be complementary in terms of host defense, there remains the possibility that IgE-mediated mast cell activation combined with TLR-mediated activation may have consequences which lead to enhanced allergic inflammation or a reduction in effectiveness of host defense mechanisms. These are areas of active investigation.

Another important receptor in response to bacteria may be the CD48 molecule. In rodent models, the interactions between murine mast cells and a number of bacteria including *E. coli*, *Enterobacter cloacae* and *Serratia marcescens* are dependent on the expression of type 1 fimbriae by these bacteria [90]. The critical component for mast cell bacterial interactions is the mannose binding lectin denoted FimH [91–93]. The receptor for FimH on mast cells has been shown to be CD48 and blockade of CD48

function inhibits mast cell responses [94]. CD48 is a GPI-anchored protein, which does not cross the plasma membrane; therefore, it is likely that CD48 is functioning as part of a macromolecular complex with another receptor system, analogous to the role of CD14 in LPS signaling. The role of TLRs in such CD48-dependent signaling remains to be determined.

Opportunities Provided by Our Understanding of TLR-Mediated Mast Cell Activation

Studies of TLR-mediated mast cell activation have been illuminating for many reasons. The ability of different TLR activators to selectively induce specific profiles of cytokines and chemokines, underlines the versatility of this cell type and its ability to selectively enhance specific aspects of host defense. Since mast cells are long-term resident cells in the tissue, TLR-mediated activation in the context of chronic infection, or responses to normal flora could play an important role in defining the local cytokine milieu, particularly at mucosal surfaces. The nature of a mast cell response might be determined, to a large extent, by its expression of TLRs, which, in turn, are regulated by previous cytokine exposure.

In atopic dermatitis, the mast cell interaction with products from *S. aureus* or other bacterial infections could be critical in the local enhancement of chronic inflammation at specific skin sites. Both cytokines and leukotriene responses would be predicted from mast cells. These could lead to increased cell recruitment as well as changes in vascular permeability that may not respond to traditional methods of reducing mast cell mediator release. It is already recognized that bacterial infection can be an important component of the disease process in atopic dermatitis [95]. The consequences of specific TLR blockade remain to be evaluated. In this and other chronic allergic diseases, the precise role of the mast cell is still unclear. However, understanding the potential complexity of mast cell responses and the role of TLRs may provide new approaches to therapy.

Exacerbations of allergic disease, associated with infection, are probably best described in the context of asthma. Certain viral infections, such as RSV, are associated with the development of asthma in childhood [29, 30], while many other viral and bacterial infections can lead to exacerbations of established disease. The recognition that mast cells can produce large amounts of leukotrienes as well as proinflammatory cytokines in response to TLR activators may be particularly important in this context.

Human mast cells have been demonstrated to increase their expression of certain TLR, such as TLR-4, following exposure to IFN- γ [81]. Such a cytokine milieu would likely occur in the context of acute viral infection. Thus mast cells would then be 'armed' to respond to a wider spectrum of TLR activators, and possibly to normal bacterial flora. It remains to be seen whether cytokine preexposure similarly increases the potential of human mast cells to produce lipid mediators, such as LTC₄ following exposure to TLR2 activators. Similarly, since most of the work of TLR to date has focused on bacterial pathogens, we do not yet fully understand the nature of TLR-mediated activation that occurs directly as a result the viral infections most commonly associated with asthma exacerbations. Further exploration of the role of TLR3, which binds double-stranded RNA and the ability of viral proteins to activate TLR2 and TLR4 are of particular interest. The poor correlation between mast cell responses in rodent and human models, unfortunately limits the usefulness of animal models to examine some of these concepts. However, as more reagents become available that will selectively modulate TLR function in the human, some of these issues can be more directly addressed.

A great deal of work, over recent years, has focused on strategies to inhibit the development and maintenance of type 2 cytokine responses, through enhancement of type 1 responses as a strategy to prevent or treat allergic disease. Agents such as LPS and its derivatives and CpG oligonucleotides have since been shown to function through TLRs [34, 35]. There is currently no evidence to suggest that human mast cells express functional TLR9, although CpG-ODN can induce cytokine production in murine mast cells [96]. Potentially, mast cells might be a good target cell to locally alter cytokine microenvironments in the context of allergic disease or in adjuvant systems, through selective activation of TLR pathways. However, particularly in the context of infection, there is a theoretical potential for Tol-activator-based therapies to exacerbate allergic disease through inducing mast cell mediator production. Better understanding of the molecular basis for the human mast cell's complex and varied responses to TLR activation will be critical to overcoming such potential pitfalls so that the mast cells' ability to regulate its microenvironment can be effectively harnessed. In this context, the variety of cytokine, chemokine, degranulation and lipid mediator responses make this cell type an excellent model to better understand the complex regulation of early innate immune responses.

References

- Echtenacher B, Mannel DN, Hultner L: Critical protective role of mast cells in a model of acute septic peritonitis (see comments). *Nature* 1996;381:75–77.
- Malaviya R, Ikeda T, Ross E, Abraham SN: Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. *Nature* 1996;381:77–80.
- Prodeus AP, Zhou X, Maurer M, Galli SJ, Carroll MC: Impaired mast cell-dependent natural immunity in complement C3-deficient mice. *Nature* 1997;390:172–175.
- Rosenkranz AR, Coxon A, Maurer M, Gurish MF, Austen KF, Friend DS, Galli SJ, Mayadas TN: Impaired mast cell development and innate immunity in Mac-1 (CD11b/CD18, CR3)-deficient mice. *J Immunol* 1998;161:6463–6467.
- Corne JM, Holgate ST: Mechanisms of virus induced exacerbations of asthma. *Thorax* 1997;52:380–389.
- Johnston SL: Bronchial hyperresponsiveness and cytokines in virus-induced asthma exacerbations. *Clin Exp Allergy* 1997;27:7–9.
- Sorden SD, Castleman WL: Virus-induced increases in bronchiolar mast cells in Brown Norway rats are associated with both local mast cell proliferation and increases in blood mast cell precursors. *Lab Invest* 1995;73:197–204.
- Sorden SD, Castleman WL: Virus-induced increases in airway mast cells in brown Norway rats are associated with enhanced pulmonary viral replication and persisting lymphocytic infiltration. *Exp Lung Res* 1995;21:197–213.
- Everard ML, Fox G, Walls AF, Quint D, Field R, Walters C, Swarbrick A, Milner AD: Tryptase and IgE concentrations in the respiratory tract of infants with acute bronchiolitis. *Arch Dis Child* 1995;72:64–69.
- King CA, Marshall JS, Alshurafa H, Anderson R: Release of vasoactive cytokines by antibody enhanced dengue virus infection of a human mast cell/basophil line. *J Virol* 2000;74:7146–7150.
- King CA, Anderson R, Marshall JS: Dengue virus selectively induces human mast cell chemokine production. *J Virol* 2002;76:8408–8419.
- Marone G, Florio G, Petraroli A, Triggiani M, de Paulis A: Human mast cells and basophils in HIV-1 infection. *Trends Immunol* 2001;22:229–232.
- Akira S, Hoshino K, Kaisho T: The role of Toll-like receptors and MyD88 in innate immune responses. *J Endotoxin Res* 2000;6:383–387.
- Akira S, Takeda K, Kaisho T: Toll-like receptors: Critical proteins linking innate and acquired immunity. *Nat Immunol* 2001;2:675–680.
- Qureshi ST, Medzhitov R: Toll-like receptors and their role in experimental models of microbial infection. *Genes Immun* 2003;4:87–94.
- O'Neill LA, Dinarello CA: The IL-1 receptor/toll-like receptor superfamily: Crucial receptors for inflammation and host defense. *Immunol Today* 2000;21:206–209.
- Akashi S, Shimazu R, Ogata H, Nagai Y, Takeda K, Kimoto M, Miyake K: Cutting edge: Cell surface expression and lipopolysaccharide signaling via the toll-like receptor 4-MD-2 complex on mouse peritoneal macrophages. *J Immunol* 2000;164:3471–3475.
- Chow JC, Young DW, Golenbock DT, Christ WJ, Gusovsky F: Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J Biol Chem* 1999;274:10689–10692.
- Hailman E, Lichenstein HS, Wurfel MM, Miller DS, Johnson DA, Kelley M, Busse LA, Zukowski MM, Wright SD: Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. *J Exp Med* 1994;179:269–277.
- Kawai T, Adachi O, Ogawa T, Takeda K, Akira S: Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity* 1999;11:115–122.
- Poltorak A, Ricciardi-Castagnoli P, Citterio S, Beutler B: Physical contact between lipopolysaccharide and toll-like receptor 4 revealed by genetic complementation. *Proc Natl Acad Sci USA* 2000;97:2163–2167.
- Sato M, Sano H, Iwaki D, Kudo K, Konishi M, Takahashi H, Takahashi T, Imaizumi H, Asai Y, Kuroki Y: Direct binding of Toll-like receptor 2 to zymosan, and zymosan-induced NF-kappaB activation and TNF-alpha secretion are down-regulated by lung collectin surfactant protein A. *J Immunol* 2003;171:417–425.
- Iwaki D, Mitsuzawa H, Murakami S, Sano H, Konishi M, Akino T, Kuroki Y: The extracellular toll-like receptor 2 domain directly binds peptidoglycan derived from *Staphylococcus aureus*. *J Biol Chem* 2002;277:24315–24320.
- Wyllie DH, Kiss-Toth E, Visintin A, Smith SC, Boussouf S, Segal DM, Duff GW, Dower SK: Evidence for an accessory protein function for Toll-like receptor 1 in anti-bacterial responses. *J Immunol* 2000;165:7125–7132.
- Werts C, Tapping RI, Mathison JC, Chuang TH, Kravchenko V, Saint Girons I, Haake DA, Godowski PJ, Hayashi F, Ozinsky A, et al: Lepidospiral lipopolysaccharide activates cells through a TLR2-dependent mechanism. *Nat Immunol* 2001;2:346–352.
- Hirschfeld M, Weis JJ, Toshchakov V, Salkowski CA, Cody MJ, Ward DC, Qureshi N, Michalek SM, Vogel SN: Signaling by toll-like receptor 2 and 4 agonists results in differential gene expression in murine macrophages. *Infect Immun* 2001;69:1477–1482.
- Lau S, Nickel R, Niggemann B, Gruber C, Sommerfeld C, Illi S, Kulig M, Forster J, Wahn U, Groeger M, et al: The development of childhood asthma: Lessons from the German Multicentre Allergy Study (MAS). *Paediatr Respir Rev* 2002;3:265–272.
- Beutler B: Endotoxin, toll-like receptor 4, and the afferent limb of innate immunity. *Curr Opin Microbiol* 2000;3:23–28.
- Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B: Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med* 2000;161:1501–1507.
- Sigurs N, Bjarnason R, Sigurbergsson F, Kjellman B, Bjorksten B: Asthma and immunoglobulin E antibodies after respiratory syncytial virus bronchiolitis: A prospective cohort study with matched controls. *Pediatrics* 1995;95:500–505.
- Kline JN, Waldschmidt TJ, Businga TR, Lemish JE, Weinstock JV, Thorne PS, Krieg AM: Modulation of airway inflammation by CpG oligodeoxynucleotides in a murine model of asthma. *J Immunol* 1998;160:2555–2559.
- Serebrisky D, Teper AA, Huang CK, Lee SY, Zhang TF, Schofield BH, Kattan M, Sampson HA, Li XM: CpG oligodeoxynucleotides can reverse Th2-associated allergic airway responses and alter the B7.1/B7.2 expression in a murine model of asthma. *J Immunol* 2000;165:5906–5912.
- Jain VV, Kitagaki K, Businga T, Hussain I, George C, O'Shaughnessy P, Kline JN: CpG oligodeoxynucleotides inhibit airway remodeling in a murine model of chronic asthma. *J Allergy Clin Immunol* 2002;110:867–872.
- Takeshita F, Leifer CA, Gursel I, Ishii KJ, Takeshita S, Gursel M, Klinman DM: Cutting edge: Role of Toll-like receptor 9 in CpG DNA-induced activation of human cells. *J Immunol* 2001;167:3555–3558.
- Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, et al: A Toll-like receptor recognizes bacterial DNA. *Nature* 2000;408:740–745.
- Krieg AM: From A to Z on CpG. *Trends Immunol* 2002;23:64–65.
- Takeuchi O, Kawai T, Muhlradt PF, Morr M, Radolf JD, Zychlinsky A, Takeda K, Akira S: Discrimination of bacterial lipoproteins by Toll-like receptor 6. *Int Immunol* 2001;13:933–940.
- Hayashi F, Smith KD, Ozinsky A, Hawn TR, Yi EC, Goodlett DR, Eng JK, Akira S, Underhill DM, Aderem A: The innate immune response to bacterial flagellin is mediated by Toll-like receptor 5. *Nature* 2001;410:1099–1103.
- Jurk M, Heil F, Vollmer J, Schetter C, Krieg AM, Wagner H, Lipford G, Bauer S: Human TLR7 or TLR8 independently confer responsiveness to the antiviral compound R-848. *Nat Immunol* 2002;3:499.
- Doxsee CL, Riter TR, Reiter MJ, Gibson SJ, Vasilakos JP, Kedl RM: The immune response modifier and Toll-like receptor 7 agonist S-27609 selectively induces IL-12 and TNF-alpha production in CD11c(+) CD11b(+) CD8(-) dendritic cells. *J Immunol* 2003;171:1156–1163.
- Skinner RB Jr: Imiquimod. *Dermatol Clin* 2003;21:291–300.
- Zarembek KA, Godowski PJ: Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. *J Immunol* 2002;168:554–561.

- 43 Hornung V, Rothenfusser S, Britsch S, Krug A, Jahrsdorfer B, Giese T, Endres S, Hartmann G: Quantitative expression of toll-like receptor 1–10 mRNA in cellular subsets of human peripheral blood mononuclear cells and sensitivity to CpG oligodeoxynucleotides. *J Immunol* 2002; 168:4531–4537.
- 44 Takeuchi O, Sato S, Horiuchi T, Hoshino K, Takeda K, Dong Z, Modlin RL, Akira S: Cutting edge: Role of Toll-like receptor 1 in mediating immune response to microbial lipoproteins. *J Immunol* 2002;169:10–14.
- 45 Muzio M, Polentarutti N, Bosisio D, Prahaldan MK, Mantovani A: Toll-like receptors: A growing family of immune receptors that are differentially expressed and regulated by different leukocytes. *J Leukoc Biol* 2000;67:450–456.
- 46 Muzio M, Bosisio D, Polentarutti N, D'Amico G, Stoppacciaro A, Mancinelli R, van't Veer C, Penton-Rol G, Ruco LP, Allavena P, et al: Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: Selective expression of TLR3 in dendritic cells. *J Immunol* 2000;164:5998–6004.
- 47 Hayashi F, Means TK, Luster AD: Toll-like receptors stimulate human neutrophil function. *Blood*, in press.
- 48 Sabroe I, Prince LR, Jones EC, Horsburgh MJ, Foster SJ, Vogel SN, Dower SK, Whyte MK: Selective roles for Toll-like receptor (TLR)2 and TLR4 in the regulation of neutrophil activation and life span. *J Immunol* 2003;170: 5268–5275.
- 49 Cario E, Brown D, McKee M, Lynch-Devaney K, Gerken G, Podolsky DK: Commensal-associated molecular patterns induce selective toll-like receptor-trafficking from apical membrane to cytoplasmic compartments in polarized intestinal epithelium. *Am J Pathol* 2002;160: 165–173.
- 50 Gewirtz AT, Navas TA, Lyons S, Godowski PJ, Madara JL: Cutting edge: Bacterial flagellin activates basolaterally expressed TLR5 to induce epithelial proinflammatory gene expression. *J Immunol* 2001;167:1882–1885.
- 51 Cario E, Rosenberg IM, Brandwein SL, Beck PL, Reinecker HC, Podolsky DK: Lipopolysaccharide activates distinct signaling pathways in intestinal epithelial cell lines expressing Toll-like receptors. *J Immunol* 2000;164:966–972.
- 52 Nomura F, Akashi S, Sakao Y, Sato S, Kawai T, Matsumoto M, Nakanishi K, Kimoto M, Miyake K, Takeda K, et al: Cutting edge: Endotoxin tolerance in mouse peritoneal macrophages correlates with down-regulation of surface toll-like receptor 4 expression. *J Immunol* 2000;164:3476–3479.
- 53 Suzuki M, Hisamatsu T, Podolsky DK: Gamma interferon augments the intracellular pathway for lipopolysaccharide (LPS) recognition in human intestinal epithelial cells through coordinated up-regulation of LPS uptake and expression of the intracellular Toll-like receptor 4-MD-2 complex. *Infect Immun* 2003;71: 3503–3511.
- 54 Becker MN, Diamond G, Verghese MW, Randal SH: CD14-dependent lipopolysaccharide-induced beta-defensin-2 expression in human tracheobronchial epithelium. *J Biol Chem* 2000;275:29731–29736.
- 55 Harder J, Meyer-Hoffert U, Teran LM, Schwichtenberg L, Bartels J, Maune S, Schroder JM: Mucoid *Pseudomonas aeruginosa*, TNF-alpha, and IL-1beta, but not IL-6, induce human beta-defensin-2 in respiratory epithelia. *Am J Respir Cell Mol Biol* 2000;22:714–721.
- 56 Droemann D, Goldmann T, Branscheid D, Clark R, Dalhoff K, Zabel P, Vollmer E: Toll-like receptor 2 is expressed by alveolar epithelial cells type II and macrophages in the human lung. *Histochem Cell Biol* 2003;119:103–108.
- 57 Plotz SG, Lentschat A, Behrendt H, Plotz W, Hamann L, Ring J, Rietschel ET, Flad HD, Ulmer AJ: The interaction of human peripheral blood eosinophils with bacterial lipopolysaccharide is CD14 dependent. *Blood* 2001;97: 235–241.
- 58 Sabroe I, Jones EC, Usher LR, Whyte MK, Dower SK: Toll-like receptor (TLR)2 and TLR4 in human peripheral blood granulocytes: A critical role for monocytes in leukocyte lipopolysaccharide responses. *J Immunol* 2002; 168:4701–4710.
- 59 Faure E, Equils O, Sieling PA, Thomas L, Zhang FX, Kirschning CJ, Polentarutti N, Muzio M, Arditi M: Bacterial lipopolysaccharide activates NF-kappaB through toll-like receptor 4 (TLR-4) in cultured human dermal endothelial cells. Differential expression of TLR-4 and TLR-2 in endothelial cells. *J Biol Chem* 2000; 275:11058–11063.
- 60 Hatakeyama J, Tamai R, Sugiyama A, Akashi S, Sugawara S, Takada H: Contrasting responses of human gingival and periodontal ligament fibroblasts to bacterial cell-surface components through the CD14/Toll-like receptor system. *Oral Microbiol Immunol* 2003;18: 14–23.
- 61 Baker BS, Ovigne JM, Powles AV, Corcoran S, Fry L: Normal keratinocytes express Toll-like receptors (TLRs) 1, 2 and 5: Modulation of TLR expression in chronic plaque psoriasis. *Br J Dermatol* 2003;148:670–679.
- 62 Ahmad-Nejad P, Hacker H, Rutz M, Bauer S, Vabulas RM, Wagner H: Bacterial CpG-DNA and lipopolysaccharides activate Toll-like receptors at distinct cellular compartments. *Eur J Immunol* 2002;32:1958–1968.
- 63 McCurdy JD, Lin TJ, Marshall JS: Toll-like receptor 4-mediated activation of murine mast cells. *J Leukoc Biol* 2001;70:977–984.
- 64 Poltorak A, He X, Smirnova I, Liu MY, Huffel CV, Du X, Birdwell D, Alejos E, Silva M, Galanos C, et al: Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: Mutations in Tlr4 gene. *Science* 1998;282:2085–2088.
- 65 Takeuchi O, Hoshino K, Akira S: Cutting Edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to *Staphylococcus aureus* infection. *J Immunol* 2000;165:5392–5396.
- 66 Leal-Berumen I, Conlon P, Marshall JS: IL-6 production by rat peritoneal mast cells is not necessarily preceded by histamine release and can be induced by bacterial lipopolysaccharide. *J Immunol* 1994;152:5468–5476.
- 67 Supajatura V, Ushio H, Nakao A, Okumura K, Ra C, Ogawa H: Protective roles of mast cells against enterobacterial infection are mediated by Toll-like receptor 4. *J Immunol* 2001;167: 2250–2256.
- 68 Supajatura V, Ushio H, Nakao A, Akira S, Okumura K, Ra C, Ogawa H: Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. *J Clin Invest* 2002;109:1351–1359.
- 69 Ikeda T, Funaba M: Altered function of murine mast cells in response to lipopolysaccharide and peptidoglycan. *Immunol Lett* 2003;88:21–26.
- 70 Masuda A, Yoshikawa Y, Aiba K, Matsuguchi T: Th2 cytokine production from mast cells is directly induced by lipopolysaccharide and distinctly regulated by c-Jun N-terminal kinase and p38 pathways. *J Immunol* 2002;169:3801–3810.
- 71 Coussens LM, Raymond WW, Bergers G, Laig-Webster M, Behrendtsen O, Werb Z, Caughey GH, Hanahan D: Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev* 1999;13:1382–1397.
- 72 Aliprantis AO, Yang RB, Mark MR, Suggett S, Devaux B, Radolf JD, Klimpel GR, Godowski P, Zychlinsky A: Cell activation and apoptosis by bacterial lipoproteins through toll-like receptor-2. *Science* 1999;285:736–739.
- 73 Lopez M, Sly LM, Luu Y, Young D, Cooper H, Reiner NE: The 19-kDa *Mycobacterium tuberculosis* protein induces macrophage apoptosis through Toll-like receptor-2. *J Immunol* 2003; 170:2409–2416.
- 74 Yoshikawa H, Tasaka K: Caspase-dependent and -independent apoptosis of mast cells induced by withdrawal of IL-3 is prevented by Toll-like receptor 4-mediated lipopolysaccharide stimulation. *Eur J Immunol* 2003;33: 2149–2159.
- 75 Tsai M, Tam SY, Wedemeyer J, Galli SJ: Mast cells derived from embryonic stem cells: A model system for studying the effects of genetic manipulations on mast cell development, phenotype, and function in vitro and in vivo. *Int J Hematol* 2002;75:345–349.
- 76 McCurdy JD, Olynch TJ, Maher LH, Marshall JS: Cutting edge: Distinct Toll-like receptor 2 activators selectively induce different classes of mediator production from human mast cells. *J Immunol* 2003;170:1625–1629.
- 77 Abeck D, Mempel M: *Staphylococcus aureus* colonization in atopic dermatitis and its therapeutic implications. *Br J Dermatol* 1998; 139(suppl 53):13–16.
- 78 Masenga J, Garbe C, Wagner J, Orfanos CE: *Staphylococcus aureus* in atopic dermatitis and in nonatopic dermatitis. *Int J Dermatol* 1990; 29:579–582.
- 79 Monti G, Tonetto P, Mostert M, Oggero R: *Staphylococcus aureus* skin colonization in infants with atopic dermatitis. *Dermatology* 1996;193:83–87.

- 80 Varadaradjalou S, Feger F, Thieblemont N, Hamouda NB, Pleau JM, Dy M, Arock M: Toll-like receptor 2 (TLR2) and TLR4 differentially activate human mast cells. *Eur J Immunol* 2003;33:899–906.
- 81 Okumura S, Kashiwakura JI, Tomita H, Matsumoto K, Nakajima T, Saito H, Okayama Y: Identification of specific gene expression profile in human mast cells via Toll-like receptor 4 and FcεRI. *Blood*, in press.
- 82 Fureder W, Agis H, Willheim M, Bankl HC, Maier U, Kishi K, Muller MR, Czerwenka K, Radaszkiewicz T, Butterfield JH, et al: Differential expression of complement receptors on human basophils and mast cells. Evidence for mast cell heterogeneity and CD88/C5aR expression on skin mast cells. *J Immunol* 1995;155:3152–3160.
- 83 Sher A, Hein A, Moser G, Caulfield JP: Complement receptors promote the phagocytosis of bacteria by rat peritoneal mast cells. *Lab Invest* 1979;41:490–499.
- 84 el-Lati SG, Dahinden CA, Church MK: Complement peptides C3a- and C5a-induced mediator release from dissociated human skin mast cells. *J Invest Dermatol* 1994;102:803–806.
- 85 Schulman ES, Post TJ, Henson PM, Giclas PC: Differential effects of the complement peptides, C5a and C5a des Arg on human basophil and lung mast cell histamine release. *J Clin Invest* 1988;81:918–923.
- 86 Abramson JS, Dahl MV, Walsh G, Blumenthal MN, Douglas SD, Quie PG: Antistaphylococcal IgE in patients with atopic dermatitis. *J Am Acad Dermatol* 1982;7:105–110.
- 87 Leung DY, Schneeberger EE, Siraganian RP, Geha RS, Bhan AK: The presence of IgE on macrophages and dendritic cells infiltrating into the skin lesion of atopic dermatitis. *Clin Immunol Immunopathol* 1987;42:328–337.
- 88 Talkington J, Nickell SP: *Borrelia burgdorferi* spirochetes induce mast cell activation and cytokine release. *Infect Immun* 1999;67:1107–1115.
- 89 Woolhiser MR, Okayama Y, Gilfillan AM, Metcalfe DD: IgG-dependent activation of human mast cells following up-regulation of FcγmaRI by IFN-γ. *Eur J Immunol* 2001;31:3298–3307.
- 90 Malaviya R, Ross EA, MacGregor JI, Ikeda T, Little JR, Jakschik BA, Abraham SN: Mast cell phagocytosis of FimH-expressing enterobacteria. *J Immunol* 1994;152:1907–1914.
- 91 Malaviya R, Ross EA, MacGregor JI, Ikeda T, Little JR, Jakschik BA, Abraham SN: Mast cell phagocytosis of FimH-expressing enterobacteria. *J Immunol* 1994;152:1907–1914.
- 92 Malaviya R, Abraham SN: Role of mast cell leukotrienes in neutrophil recruitment and bacterial clearance in infectious peritonitis. *J Leukoc Biol* 2000;67:841–846.
- 93 Malaviya R, Ross E, Jakschik BA, Abraham SN: Mast cell degranulation induced by type 1 fimbriated *Escherichia coli* in mice. *J Clin Invest* 1994;93:1645–1653.
- 94 Malaviya R, Gao Z, Thankavel K, van der Merwe PA, Abraham SN: The mast cell tumor necrosis factor alpha response to FimH-expressing *Escherichia coli* is mediated by the glycosylphosphatidylinositol-anchored molecule CD48. *Proc Natl Acad Sci USA* 1999;96:8110–8115.
- 95 Leung DY: Atopic dermatitis and the immune system: The role of superantigens and bacteria. *J Am Acad Dermatol* 2001;45:S13–16.
- 96 Zhu FG, Marshall JS: CpG-containing oligodeoxynucleotides induce TNF-α and IL-6 production but not degranulation from murine bone marrow-derived mast cells. *J Leukoc Biol* 2001;69:253–262.
- 97 Yoshimura A, Lien E, Ingalls RR, Tuomanen E, Dziarski R, Golenbock D: Cutting edge: Recognition of gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. *J Immunol* 1999;163:1–5.
- 98 Schwandner R, Dziarski R, Wesche H, Rothe M, Kirschning CJ: Peptidoglycan- and lipoteichoic acid-induced cell activation is mediated by toll-like receptor 2. *J Biol Chem* 1999;274:17406–17409.
- 99 Underhill DM, Ozinsky A, Hajjar AM, Stevens A, Wilson CB, Bassetti M, Aderem A: The Toll-like receptor 2 is recruited to macrophage phagosomes and discriminates between pathogens (see comments). *Nature* 1999;401:811–815.
- 100 Lehner MD, Morath S, Michelsen KS, Schumann RR, Hartung T: Induction of cross-tolerance by lipopolysaccharide and highly purified lipoteichoic acid via different Toll-like receptors independent of paracrine mediators. *J Immunol* 2001;166:5161–5167.
- 101 Lien E, Sellati TJ, Yoshimura A, Flo TH, Rawadi G, Finberg RW, Carroll JD, Espevik T, Ingalls RR, Radolf JD, et al: Toll-like receptor 2 functions as a pattern recognition receptor for diverse bacterial products. *J Biol Chem* 1999;274:33419–33425.
- 102 Brightbill HD, Libraty DH, Krutzik SR, Yang RB, Belisle JT, Bleharski JR, Maitland M, Norgard MV, Plevy SE, Smale ST, et al: Host defense mechanisms triggered by microbial lipoproteins through toll-like receptors. *Science* 1999;285:732–736.
- 103 Hirschfeld M, Kirschning CJ, Schwandner R, Wesche H, Weis JH, Wooten RM, Weis JJ: Cutting edge: Inflammatory signaling by *Borrelia burgdorferi* lipoproteins is mediated by toll-like receptor 2. *J Immunol* 1999;163:2382–2386.
- 104 Vabulas RM, Ahmad-Nejad P, da Costa C, Miethke T, Kirschning CJ, Hacker H, Wagner H: Endocytosed HSP60s use toll-like receptor 2 (TLR2) and TLR4 to activate the toll/interleukin-1 receptor signaling pathway in innate immune cells. *J Biol Chem* 2001;276:31332–31339.
- 105 Ogawa T, Asai Y, Hashimoto M, Uchida H: Bacterial fimbriae activate human peripheral blood monocytes utilizing TLR2, CD14 and CD11a/CD18 as cellular receptors. *Eur J Immunol* 2002;32:2543–2550.
- 106 Underhill DM, Ozinsky A, Smith KD, Aderem A: Toll-like receptor-2 mediates mycobacteria-induced proinflammatory signaling in macrophages. *Proc Natl Acad Sci USA* 1999;96:14459–14463.
- 107 Means TK, Wang S, Lien E, Yoshimura A, Golenbock DT, Fenton MJ: Human toll-like receptors mediate cellular activation by *Mycobacterium tuberculosis*. *J Immunol* 1999;163:3920–3927.
- 108 Alexopoulou L, Holt AC, Medzhitov R, Flavell RA: Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. *Nature* 2001;413:732–738.
- 109 Hoshino K, Takeuchi O, Kawai T, Sanjo H, Ogawa T, Takeda Y, Takeda K, Akira S: Cutting edge: Toll-like receptor 4 (TLR4)-deficient mice are hyporesponsive to lipopolysaccharide: Evidence for TLR4 as the Lps gene product. *J Immunol* 1999;162:3749–3752.
- 110 Qureshi ST, Lariviere L, Leveque G, Clermont S, Moore KJ, Gros P, Malo D: Endotoxin-tolerant mice have mutations in Toll-like receptor 4 (Tlr4) (see comments) (published erratum appears in *J Exp Med* 1999 May 3;189:following 1518). *J Exp Med* 1999;189:615–625.
- 111 Hirschfeld M, Ma Y, Weis JH, Vogel SN, Weis JJ: Cutting edge: Repurification of lipopolysaccharide eliminates signaling through both human and murine toll-like receptor 2. *J Immunol* 2000;165:618–622.
- 112 Lien E, Means TK, Heine H, Yoshimura A, Kusumoto S, Fukase K, Fenton MJ, Oikawa M, Qureshi N, Monks B, et al: Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide. *J Clin Invest* 2000;105:497–504.
- 113 Kurt-Jones EA, Popova L, Kwinn L, Haynes LM, Jones LP, Tripp RA, Walsh EE, Freeman MW, Golenbock DT, Anderson LJ, et al: Pattern recognition receptors TLR4 and CD14 mediate response to respiratory syncytial virus. *Nat Immunol* 2000;1:398–401.
- 114 Ohashi K, Burkart V, Flohe S, Kolb H: Cutting edge: Heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. *J Immunol* 2000;164:558–561.
- 115 Smiley ST, King JA, Hancock WW: Fibrinogen stimulates macrophage chemokine secretion through toll-like receptor 4. *J Immunol* 2001;167:2887–2894.
- 116 Means TK, Jones BW, Schromm AB, Shurtleff BA, Smith JA, Keane J, Golenbock DT, Vogel SN, Fenton MJ: Differential effects of a Toll-like receptor antagonist on *Mycobacterium tuberculosis*-induced macrophage responses. *J Immunol* 2001;166:4074–4082.
- 117 Ozinsky A, Underhill DM, Fontenot JD, Hajjar AM, Smith KD, Wilson CB, Schroeder L, Aderem A: The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc Natl Acad Sci USA* 2000;97:13766–13771.
- 118 Bauer S, Kirschning CJ, Hacker H, Redecke V, Hausmann S, Akira S, Wagner H, Lipford GB: Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition. *Proc Natl Acad Sci USA* 2001;98:9237–9242.