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Signaling in B cells via Toll-like receptors

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Toll-like receptors (TLRs) and their ligands have emerged as important regulators of immunity, relevant to a wide range of effector responses from vaccination to autoimmunity. The most well-studied ligands of TLRs expressed on B cells include the lipopolysaccharides (for TLR4) and CpG-containing DNAs (for TLR9), which induce and/or co-stimulate B cells to undergo proliferation, class switching and differentiation into antibody-secreting cells. Recent developments in this area include advancements into our understanding of the role of these receptor pathways in B cells, and in particular the relevance of TLR9, which has received substantial attention as both a Th1-like inflammatory immunomodulator and a pathogenic co-stimulator of autoreactive B cell responses.

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Introduction

The Toll-like receptors (TLRs) include at least 11 type I integral membrane glycoproteins, and are members of a larger superfamily that includes the IL-1 receptors [1]. TLRs recognize a diverse array of ligands, including pathogen-associated molecular patterns (PAMPs), such as bacterial lipopolysaccharides (LPS), RNAs and DNAs, which are widely recognized by immune cells, including cells of both adaptive and innate lineages.

In the past few years, several studies have specifically examined the expression of TLRs in B cells, primarily in human tonsillar or peripheral blood populations (Table 1). Although most studies agree that mRNAs corresponding to all TLRs can be detected in B cells, significant expression is only generally agreed upon at this time for TLR1 and TLR6–10, all of which are upregulated during activation; for example, in response to B cell receptor (BCR) or CD40 ligation, CpG oligonucleotide exposure *in vitro*, and as seen in activated B cell subsets *ex vivo*. B cells are now particularly well recognized for their expression of the TLR9 and TLR10, which are induced in response to BCR stimulation and appear to predominate in activated and/or memory populations [2•,3•].

This spectrum of inducible expression of the TLRs has been generally presumed to extend to rodent B cells; however, species differences clearly exist, as naïve murine B cells are known to express TLR4 and undergo proliferation and plasmacytoid differentiation *in vitro* in response to LPS exposure, in contrast to human B cells, which seem to lack significant TLR4 expression, at least in the naïve resting state [4•]. As such, the vast majority of studies involving TLRs in B cells have focused upon TLR9 and its ligands (hypomethylated CpG-containing DNAs); nonetheless, several rodent-based studies have addressed TLR4 and its predominant ligand, LPS.

This review focuses particularly upon recent advances regarding TLR9 signaling in B cells; for an overview of TLRs in general, their ligands and functional importance during global immune responses, the reader is directed to one of many excellent recent reviews (e.g. [1,5,6]).

An overview of the signaling pathways of TLR4 and TLR9

The signaling mechanisms of both the TLR4 and TLR9 pathways have been studied extensively (Figures 1 and 2). TLR4 is expressed on the cell surface in complex with the MD-2 molecule, and this heterodimer participates in LPS recognition to initiate intracellular signaling by at least two major adaptor pathways. These pathways include the TIRAP–MyD88 pathway, which regulates rapid NF-κB activation and related inflammatory cytokine production, and the TRIF–TRAM pathway, which regulates the activation of interferon regulatory factor (IRF)-3 and the subsequent induction of type I interferons and co-stimulatory molecules (reviewed in [1]). By contrast, TLR9 is expressed in the endoplasmic reticulum and is recruited to endosomal/lysosomal compartments after stimulation with CpG DNAs, activating the MyD88 pathway without TIRAP, culminating in NF-κB activation [7••]. Interestingly, some studies have preliminarily suggested that TRIF also provides an additional MyD88-independent pathway for TLR9 signaling [8].

These pathways have primarily been elucidated in macrophages, dendritic cells and/or cultured cell lines. Relatively few investigations have specifically confirmed the details of these pathways in B cells, although they are likely to be relevant as they have been observed in multiple cell lineages. It is worth noting, however, that,

Table 1

Expression of Toll-like receptors on human B cells

			Compared to		
Receptor	Naïve/Resting	Activated	PMN	DC	Mono
TLR1	+	++	+++	ND	ND
TLR2	+/−	+/−	+++	ND	ND
TLR3	+/−	+/−	ND	+	ND
TLR4	+/−	+/−	+++	ND	ND
TLR5	+/−	+/−	ND	++	ND
TLR6	+	+++	+++	ND	ND
TLR7	+	+++	ND	ND	+++
TLR8	+	++	ND	+++	ND
TLR9	+	++++	ND	ND	ND
TLR10	+	++++	ND	ND	ND
TLR11	ND	ND	ND	ND	ND

Relative expression levels of the indicated Toll-like receptors on human B cells are indicated. +++, strong expression; ++, moderate expression; +, low but definite expression; +/−, expression at a barely detectable or functionally controversial level. Naïve/Resting, $CD19^{+}CD27^{-}$ or high-density peripheral blood or tonsillar B cells; activated, memory ($CD19^{+}CD27^{+}$) or intermediate-density peripheral blood or tonsillar B cells. Where data are available, expression levels are compared with neutrophils (PMN), dendritic cells (DC) or monocytes/macrophages (Mono). Summarized from references [2–4,29,42,43]. ND, not determined.

unlike macrophages and dendritic cells, B cells also utilize a heterodimer consisting of RP105 and MD-1, which are structurally related to TLR4 and MD-2, to recognize and respond to LPS (reviewed in [9]). Although B cells deficient in either TLR4 or MD-2 do not respond to LPS at all, cells deficient in either RP105 or MD-1 are hyporesponsive to LPS, suggesting that the RP105–MD-1 heterodimer plays a uniquely important role in B cells by enhancing TLR4-dependent LPS responses. Indeed, patients with systemic lupus erythematosus, dematomyositis or Sjögren's syndrome possess an unusually high percentages of RP105-negative B cell populations, which are associated with activation and accentuated production of antibodies and autoantibodies [9,10•]. As such, it is interesting to consider that, in addition to such apparently lineage-specific LPS signaling features as RP105 and MD-1, B cells might possess additional lineage-specific signaling molecules that participate in the recognition of LPS and/or other PAMPs, such as CpG, which might play specific roles in pathogenic states of humoral and/or B cell related immunity.

Effects of TLR9 signaling in B cells

The TLR9 pathway continues to receive growing attention as a consequence of the potential relevance of unmethylated CpG-containing DNAs to the pathogenesis of autoimmune diseases, as well as in immunomodulatory therapeutic strategies [11].

TLR9 signaling in B cell autoimmunity

There now exists strong evidence that TLR9 activation can co-stimulate autoreactive B cells, thereby breaking tolerance. For instance, anti-DNA IgG autoantibodies can form complexes with hypomethylated CpG-containing DNAs, and then bind both to rheumatoid factor (anti-IgG) B cells through their BCR via direct IgG binding, and to TLR9 via binding of the co-complexed DNA, resulting in activation [12••]. These hypomethylated CpG DNAs have traditionally been thought to derive from environmental pathogens, suggesting a need for a non-self trigger for autoreactive B cell activation; however, mammalian DNAs contain rare hypomethylated CpG regions that are capable of co-stimulating autoreactive B cells. Therefore, sources of self DNA, such as apoptotic cells or necrotic debris, can potentially function in this situation as truly autoreactive stimuli in such immune complexes [13•]. Furthermore, anti-DNA B cells themselves, by being able to bind DNAs through both their BCR and TLR9, might be directly co-stimulated by hypomethylated CpG DNA motifs without a need for immune complexes (Figure 2). In this sense, hypomethylated CpG motifs, whether or not in the form of immune complexes, can initiate and/or promote systemic humoral autoimmunity by preventing or promoting the breakage of tolerance of autoreactive B cells.

It is, however, important to note that, at least in the anti-lysozyme transgenic model of B cell tolerance (in which anti-lysozyme transgenic B cells are rendered anergic by transgenic soluble lysozyme), CpG DNAs can break tolerance, inducing proliferation of, and immunoglobulin secretion by, autoreactive cells *in vitro*, but do not appear to be capable of inducing pathogenic disease [14••,15]. This insufficiency has been attributed to the observation that anergic B cells have uncoupled the BCR from a calcineurin-dependent signaling pathway, leading to continuous ERK signaling, which inhibits CpG-induced plasma cell differentiation [14••]. As such, additional stimuli, which could simply consist of excess antigen [15], are probably required to cooperate with CpG DNAs to break tolerance fully and result in pathogenic humoral immunity. Perhaps this tolerance breakdown, in part, involves the physically close coupling of BCR–TLR9 interactions that could be conferred by IgG–CpG DNA-containing immune complexes upon rheumatoid factor B cells, or by CpG DNAs alone upon anti-DNA B cells, resulting in a supramolecular activation complex. Alternatively, such immune complexes could also activate complement and/or Fc receptors, providing additional co-stimulation to the B cells, although there is less evidence for such mechanisms [12••]. Thus, it remains unclear if TLR9 signals are necessary or single-handedly sufficient to both induce autoimmunity and propagate the response to full-blown pathogenic humoral immunity *in vivo*, although their ability to promote autoreactive B cell responses, at least to some degree, seems clear.

Immunomodulatory effects of TLR9 signaling in B cells

CpG DNAs can furthermore skew immune responses towards a Th1-like phenotype, enhancing, for example,