

Endosomes and Toll-Like Receptors-Role in Autoimmunity

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Commentary

Endosomes are membrane-delimited intracellular transport carriers which form at the plasma membrane or Golgi apparatus [1]. Three main endosomal compartments termed early, late and recycling have been characterized [2]. In general terms, endosomes function to provide a molecular/cellular mechanism for sorting out and re-exporting internalized membrane components [3]. Endosomes belonging to the recycling type are transported to the plasma membrane where they exist as a sub-compartment of early endosomes whereas early endosomes mature into late endosomes which can then fuse with lysosomes. The accumulated evidence indicates that delivery of endocytosed macromolecules to lysosomes was facilitated by the direct fusion of late endosomes with lysosomes [4].

Recent advances have also provided evidence for a tight relationship between potential endosomal dysfunction and autoimmunity. Thus, aberrations in endosomal recognition and endosomal activity may underlie, in part, the pathobiology of such autoimmune disorders as systemic lupus erythematosus (SLE) [5], rheumatoid arthritis (RA) [6], psoriasis, and Sjögren's disease [7].

Over the past decade more attention has been paid to the role of endosomal-based Toll-like receptors (TLRs) in the pathogenesis of autoimmune diseases such as SLE, RA and systemic sclerosis (SSc) [8-10]. TLRs belong to a group of evolutionarily-conserved innate immune pattern recognition receptors. Ten human TLR forms have been identified [6]. From a functional perspective, TLRs recognize pathogen-associated-molecular-patterns which emanate from various types of infectious organisms which include bacteria, viruses and fungi as well as specific molecular structures contained in for example, lipopolysaccharide, flagellin or double-stranded DNA. Another function of the TLR family is their capacity to respond to damage-associated-molecular-patterns comprising many of the endogenous molecules which are associated with the inflammatory response and tissue damage. Importantly in the context of autoimmunity, TLR ligands are capable of activating dendritic cells (DC), macrophages, T-cells and B-cells and other antigen-presenting cells [7]. However, unlike the TLRs which are typically located on cell surfaces and recognize bacterial products, a specific group of TLRs including TLR-3, TLR-7 and TLR-9 are found on endosomes [9]. These endosomal TLRs are fundamentally important for the recognition of double-stranded RNA, single-stranded RNA and hypomethylated double-stranded DNA, respectively, making these TLRs particularly crucial in the pathogenesis of autoimmune diseases. This viewpoint takes on further significance especially as it pertains to lupus by noting that TLR-3, -7, -9 are capable of recognizing nucleic acids, either foreign or self [11]. Thus, in lupus, self-nucleic acids may evade the various mechanisms that normally prevent the interaction between TLR ligands and endosomal TLRs [12]. This is likely to occur as a result of defects in nuclease activity or nuclease/autoantibody

complexes as well as the elevated proportion of autoreactive B-cells which have specificity for nucleic acids, nucleoproteins, and related immune complexes [12].

A group of 5 adaptor proteins are intimately involved in the downstream signal transduction pathways activated by TLR ligands (Table 1) [7].

Adaptor Protein	A Few Functions	Reference(s)
Myeloid Differentiation Response Gene 88 (MyD88)	Mouse: MyD88 interacts with almost all TLRs except TLR3; Human: Activation of NF-κB; MyD88 is an essential signal transducer in the interleukin-1 and Toll-like receptor signaling pathways	[13] (Mice) [14] (Human)
TIR-Domain-Containing Adaptor Protein (TIRAP)/MyD 88 Adaptor-Like Protein (MAL)	Recruits MyD88 to TLR2 and TLR4 which enables MyD88 to signal via IRAK	[15]
TIR-Domain-Containing Adaptor-Inducing Interferon-β (TRIF)	Mediates 2 TLR-associated signaling pathways one of which is MyD88-dependent.	[16]
TRIF-Related Adaptor Protein (TRAM)	Localizes with TLR4; Essential for responses to lipopolysaccharide Critical TIR-adaptor protein regulating mammalian cell apoptosis	[17,18]
Sterile α and Armadillo Motif-Containing Protein (SARM)	Inhibits NF-κB and interferon-regulatory factor-3-mediated TLR3 and TLR4 signaling via AP-1; Mitochondrial SARM is a proapoptotic protein via upregulation of Bcl-2 and suppression of Bcl-xL and ERK phosphorylation	[19,20]

Table 1: Adaptor proteins involved in TLR-mediated downstream signaling.

These downstream signaling pathways involve the participation of many disparate kinases, including IRAK, TAK1, MAPK, PI3K as well as, IRFs and NF-κB [8] which regulate the synthesis of pro-inflammatory cytokines such as IFN-α, IFN-β, IFN-γ, IL-6, the activity of which are critical in perpetuating inflammation. In this regard, TLR activation was shown to be involved in causing a heightened cytokine response in early autoimmune disease states. This was exemplified by the elevated level of cytokine production found, for example, in early cutaneous SSc. Thus, stimulation of DC with TLR-2, -3 or -4-reactive ligands resulted in the increased production of interleukin-1 (IL-1), IL-6 and tumor necrosis factor-α (TNF-α) when compared to DC

from patients in the later stages of cutaneous SSc or compared to healthy controls [10].

Therefore, because TLRs are the major sensing receptors responsible for recognizing microbial pathogens in the form of microbial nucleic acids as well as “self-molecules” these TLR responses are likely to constitute the basis for innate and adaptive immune, autoimmune and inflammatory host responses [11,12].

There are several additional aspects of endosome function and TLR activity that appear to be pertinent to autoimmunity. For example, recycling endosomes regulate T-cell receptor transport to the plasma membrane as well as the internalization of T-cell receptors and their recycling to the cell surface, a determinant of T-cell activation [5]. B-cell activity also appears to be endosome-dependent. Thus, Waite et al. [21] were among the first to demonstrate the presence of serum autoantibodies to early endosome antigen-1 (EEA1) in 13% of the RA, but not the SLE patients they studied. These results also showed that the autoantibodies to EEA1 were associated with early endosomes and internalization of transferrin receptors. Several years later, B-cell epitopes capable of reacting with EEA1 were identified in patients with neurological and other autoimmune conditions [22]. This finding supported the view that autoantibodies to EEA1 were a fundamental component of these disorders. More recently, Kono et al. [23] showed that endosomal TLRs were required for the optimal production of IgG autoantibodies and IgM rheumatoid factor in the lupus-prone/B6-Fas mouse and BXSB mouse each of which develop a lupus-like disease. The authors [23] suggested that nucleic acid-sensing TLRs could be considered the “Achilles heel” in susceptible mouse strains (and perhaps in humans) in which nucleic-acid-containing antigen tolerance is breached resulting in autoimmune responses. The results of this study also posited that whereas endosomal TLRs were considered relatively unimportant for humoral responses to foreign antigen proteins, endosomal TLRs played a critical role in autoantibody production to nucleic acids and nucleic acid “materials” as well as IgM rheumatoid factor role in the pathogenesis of lupus in these 2 susceptible mouse strains [23].

Of note, human TLR7 processing by furin-like proprotein convertases was also shown to be required for functional responses to TLR7 agonists such as R837 or influenza virus [24]. Because endosomal self-RNA can activate TLR7 and trigger autoimmune responses, furin-like proprotein convertases might become a potential target for intervention in autoimmune pathological conditions.

It is well-known that T-cell activation is largely dependent on the generation of fragments of antigen-proteins bound to Major Histocompatibility Complex (MHC) molecules displayed on cell surfaces. However, in essence, it was known for almost 20 years that MHC class II proteins identify processed antigens from within endosomal compartments [25]. Moreover, it is now appreciated more than ever that the processing of both cytoplasmic and endocytized foreign protein antigens which was once believed to involve separate pathways, one for MHC class I and the other for class II peptides do in actuality overlap [26]. This conclusion was based on the discovery that endosomal proteases exist at sites which cross-over between MHC class I and MHC class II. Thus, individual proteases within a given set of endosomal-based enzymes have gradually emerged both as a target for microbial sensing in view of the MHC class II-dependency perspective as well as the fact that these proteases behave as integral components required for generating autoimmune responses. In addition, Barrat and Coffman [27] proposed activation of TLR7/TLR9 via endogenous RNA and DNA and transport of activated TLR7/TLR9

to endosomes in the form of immune complexes or non-covalently associated with cationic peptides as an important mechanism in SLE and psoriasis, thus making inhibition of TLR7/TLR9 activation a relevant target for intervention in these diseases.

Anti-malarial drugs [28] and imidazoquinolines [29] are known to be employed in the treatment of patients with various autoimmune diseases. In that regard, Kuznik et al. [29] reported that imidazoquinolines acting as endosomal TLR7/8 agonists can inhibit TLR9 and TLR3 even in the absence of TLR7 or TLR8. Thus, the mechanism of TLR inhibition by imidazoquinolines was found to be similar to the anti-malarial drugs.

From a clinical perspective the decision to develop small molecule endosomal TLR antagonists for potential therapy of SLE and RA as well as other autoimmune disorders [10, 19] and has taken on greater focus in recent drug development. Thus, a small molecular weight poly-TLR antagonist, CPG-52364, derived from quinazoline [30] was originally developed as a treatment for SLE and may also be potentially useful for treating RA and psoriasis going forward. At the cellular level, it was reported that CPG-52364 inhibited downstream signaling following stimulation of TLR 7, -8 or 9 in peripheral blood mononuclear cells [7].

Several TLR immunomodulatory agents, with variable specificities towards TLR-2, -4, -7 and -9 are also in development [31]. However, not all of these agents designed to potentially treat acute and chronic inflammatory conditions such as intestinal bowel disease (IBD), RA and multiple sclerosis belong to the small molecule inhibitor class. For example, TLR antibodies, virally-derived peptides, CpG oligonucleotide and a DNA-based compound are in various stages of pre-clinical drug development. One of these, DIMS0150 was designed as a dual immunomodulatory agent since it targeted both TLR-9 and nuclear factor kappaB1 (NF- κ B1) activity [31]. Currently, DIMS0150 is in the post-pre-clinical stage of development for the treatment of refractory ulcerative colitis [32]. DIMS0150 is also being evaluated as a topical therapy in a phase III ulcerative colitis clinical trial (EudraCT No.: 2011-003130-14) [33].

Finally, 2 TLR-antagonists, one with specificity towards TLR 7,-8, -9 (i.e. IMO-8400) and another to TLR-7, -9 (i.e. IMO-3100) are in various stages of pre-clinical development for the treatment of psoriasis and other diseases with similar pathologies [34]. Both anti-TLR agents were shown to decrease the expression of IL-17A, to normalize IL-17-induced genes (e.g. β -defensin, CXCL1) while also normalizing keratin expression. Moreover, these anti-TLR agents significantly modulated skin-related genes in a model of skin inflammation in C57BL/6 mice induced by IL-23 thus providing the impetus for considering using TLR-7, -8, -9 antagonists to block the signaling pathways which cause psoriasis vulgaris and other immune-mediated skin diseases.

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