El sistema inmunitario innato desarrolló uno de los mecanismos de reconocimiento frente a las infecciones microbianas y el inicio de defensa del hospedador. Este mecanismo está basado en el reconocimiento de patrones moleculares presentes en los distintos componentes microbianos así como en determinados ligandos endógenos. Estos patrones son reconocidos, entre otros receptores, a través de los llamados receptores Toll-like (TLRs), los cuales son responsables de la activación de reacciones inflamatorias y tienen un papel crítico en el inicio de la respuesta inmunitaria innata. La función de los TLRs en varias enfermedades humanas ha sido investigada a través del estudio de polimorfismos de genes que participan en la señalización a través de TLR. Estos estudios sugieren que varias enfermedades, incluidas enfermedades infecciosas, autoinmunitarias, ateroesclerosis e inflamatorias de las vías respiratorias están afectadas por la función de estos TLR. Los futuros estudios en este campo continuarán aumentando el conocimiento de la patogénesis y etiología de estas enfermedades así como revelarán información sobre las distintas opciones terapéuticas.

PALABRAS CLAVE: Receptores tipo toll / Polimorfismo genético / Enfermedades infecciosas / Autoinmunidad / Ateroesclerosis / Enfermedades inflamatorias de las vías respiratorias.

**ABSTRACT**

The innate immune system has evolved one of the mechanisms of recognition of microbial infection and initiation of host defence. This is based on the recognition of molecular patterns that are present in microbial components or endogenous ligands. These patterns are recognized, among other, through the Toll-like receptors (TLRs), which activate inflammatory reactions and are critical in the initiation of adaptive immune responses. The function of TLRs in various human diseases has been investigated by the study of several polymorphisms in genes that participate in TLR signalling. These studies have suggested that several diseases, including infectious, autoimmune, atherosclerosis and inflammatory airway diseases are affected by the TLR function. The future studies in this field will continue to improve the understanding of the pathogenesis and aetiology of these diseases and they will as well reveal information about therapeutic options.

KEY WORDS: Toll-like receptors / Genetic polymorphism / Infectious diseases / Autoimmunity / Atherosclerosis / Inflammatory airway diseases.
INTRODUCTION
Innate immunity is the first-line of host defence of multicellular organisms that rapidly operates to limit infection upon exposure to infectious agents. Accumulating evidence indicates that activation of the innate immune system is a prerequisite for the induction of acquired immunity, particularly for the induction of a T helper 1 (Th1)-cell response\(^1,2\). Toll-like receptors (TLRs) play a crucial role in innate immunity, through their ability to bind pathogen-associated molecular patterns (PAMPs)\(^3\). The receptors of the innate immune system that recognise PAMPs are called pattern-recognition receptors (PRRs)\(^4\). PRRs are expressed on the cell surface, activate signalling pathways that induce antimicrobial effector responses and inflammation upon recognition of PAMPs. Therefore TLRs control multiple functions and activate signals that are critically involved in the initiation of adaptive immune responses\(^5,6\). Different TLRs appear to recognise specific microbial products (PAMPs), including lipopolysaccharide, bacterial lipoproteins, peptidoglycan and bacterial DNA\(^7\). Numerous studies, conducted mainly in vitro, have led to the identification of a large number of different ligands for each member of the TLR family\(^7,8\). Recognition of a single ligand has been demonstrated for TLR3 in responses to poly(I:C) (double-stranded viral RNA)\(^9\), TLR5 for responses to flagellin and flagellated bacteria\(^10\), and TLR9 for mediating responses to CpG bacterial DNA\(^11\). On the contrary, TLR2 and TLR4 have a broad specificity for the recognition of microbial patterns. TLR2 is responsible for the recognition of Gram-positive bacteria (peptidoglycan, lipoteichoic acid)\(^12\), mycobacterial species\(^13,14\), protozoan parasites\(^15,16\), as well as microbial lipoproteins, glycoproteins, glycolipids, and nonenteric LPS\(^17-21\). TLR4 recognizes LPS\(^22,23\), the LPS mimetic drug Taxol\(^24\), the fusion protein of respiratory syncytical virus (RSV)\(^25\), as well as fungal ligands\(^26,27\). TLRs can also dimerise upon interaction with the proper ligand. Dimers consisting of TLR4-TLR4, TLR2-TLR6 and TLR1-TLR2 have been described\(^28\). The Drosophila protein Toll was originally described as a type I transmembrane receptor that controls the dorsal-ventral patterning of the fly embryo\(^29\). Toll signalling has been identified as an essential element of an effective anti-fungal immune response in the fly\(^30\). Several studies have identified mammalian homologs of Toll, proteins now referred to as Toll-like receptors (TLR)\(^31-33\). TLRs are grouped into the same gene family based on their sequence similarity. Eleven mammalian members have been described, namely TLR1 to TLR11. All of them are type I transmembrane proteins that show peculiar structural features. Several leucine-rich repeats (LRRs) are present in the extracellular domain of the molecule\(^33\). TLRs cross the cytoplasmatic membrane once, and their intracellular portion is extremely similar to the cytoplasmatic domain of the IL-1R and related molecules, which is designated Toll-IL-1R or TIR domain\(^34\). By contrast, the extracellular region of the TLRs and IL-1R differs markedly: the extracellular region of TLRs contains LRRs motifs, whereas the extracelluar region of IL-1R contains three immunoglobulin-like domains.

Upon ligand binding, TLRs (homo- hetero-) dimerise and undergo a conformational change required for the recruitment of downstream signalling molecules. After TLR ligation, the adaptor molecule myeloid-differentiation primary-response protein 88 (MyD88) is recruited to the cytoplasmatic TIR domain, where it facilitates the association of tumour-necrosis factor (TNF)-receptor-associated factor 6 (TRAF6) to the complex. The IRAK1-TRAF6 complex then disengages from the receptor and interacts with another preformed complex consisting of transforming growth factor-β (TGF-β)-activated kinase (TAK1), TAK1-binding protein 1 (TAB1) and TAB2 or TAB3. Next, IkB kinases (IKKs) are activated, and phosphorylate the inhibitor of NF-κB (IkB). This phosphorylation leads to the degradation of IkB and...
consequently the release of NF-kB to the nucleus\(^\text{35}\). MyD88 is essential for responses against a broad range of microbial components. However, a closer study of MyD88-deficient cells has revealed the existence of MyD88-dependent and –independent pathways, both of which mediate signalling in response to LPS\(^{36}\).

Due to the fact that TLRs are clearly at the top of the immune-system pyramid, great efforts have been made to elucidate their possible role on human diseases. Here, we review some of the findings that have arisen from these investigations, relating to infectious diseases, autoimmunity, atherosclerosis and inflammatory airway diseases (Table I).

### TLRs AND INFECTIOUS DISEASES

Recognition of microbial non-self plays a crucial role in host defence. This recognition strategy is based on the detection of PAMPs that are essential products of microbial physiology, unique to microbes and recognised by PRRs. The TLRs are a part of this innate immune defence. The role of the TLR family in host defence against microbes bearing one or more of these PAMPs has been confirmed by experimental assays with mice bearing spontaneous or genetically engineered defects of various TLR signalling components\(^{38}\). Regarding Gram-negative infection genetic differences in susceptibility to LPS are well established; C3H/HeJ and C57BL/10ScCr mice strains are hypersensitive to LPS. Since the C3H/HeJ mouse strain is exquisitely sensitive to progressive overwhelming infection by Salmonella typhimurium\(^{37}\), extensive studies led to the identification of tlr4 as the gene encoded by Lps, with C3H/HeJ mice harbouring a spontaneous point mutation (Pro712His) in the cytoplasmic signalling domain \(^{38, 39}\). Two other mouse strains bear mutations of tlr4; C57BL10/ScN and its subline C57BL10/ScNCr carry a genomic deletion and do not express TLR4, while motor neuron degeneration (mnd) mutant mice have a large insertion within exon 2 that results in an aberrantly spliced transcript predicting a truncated protein \(^{40}\). Relative to mice bearing a wild-type TLR4, C3H/HeJ and C57BL10/Sc mutant mice have also been shown to develop prolonged bacteremia after experimental infection with Neisseria meningitidis, another Gram-negative bacteria recognized for its exuberant production of LPS\(^{41}\). The role of TLR4 seems clear in response to Gram-negative LPS in mice, and several groups have studied the effect of the two cosegregating missense mutations (Asp299Gly and Thr399Ile) on humans at risk for sepsis. These polymorphisms have been associated with a blunted physiological response to inhaled endotoxin\(^{42}\), for this observation the Asp299Gly allele has been associated with a predisposition with Gram-negative sepsis\(^{43-46}\). The biological significance of the Asp299Gly and Thr399Ile has been demonstrated by transfection of THP-1 cells with either the wild-type or the mutant allele of the TLR4 gene\(^{46}\). Although these data support the relevance of TLR4 to human LPS responsiveness, a retrospective analysis

<table>
<thead>
<tr>
<th>TLR</th>
<th>SNP</th>
<th>Disease</th>
<th>Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>TLR2</td>
<td>Arg753Gln</td>
<td>Staphylococcal, Mycobacterium leprae and Mycobacterium Tuberculosis infection</td>
<td>Yes (^{53-56})</td>
</tr>
<tr>
<td></td>
<td>-16934 A/T</td>
<td>Asthma and allergies</td>
<td>Yes (^{131})</td>
</tr>
<tr>
<td></td>
<td>-196 to –174 del,</td>
<td>Asthma</td>
<td>No (^{135})</td>
</tr>
<tr>
<td></td>
<td>-191 G/A, 597 T/C,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1350 T/C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR3</td>
<td>-7 A/C, +71 C/A,</td>
<td>Asthma</td>
<td>No (^{135})</td>
</tr>
<tr>
<td></td>
<td>Leu412Phe, 1377 C/T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR4</td>
<td>Asp299Gly</td>
<td>Response to inhaled endotoxin, Gram negative sepsis, severe RSV bronchiolitis, rheumatoid arthritis, diabetic neuropathy, Crohn’s disease, ulcerative colitis, atherosclerosis</td>
<td>Yes (^{42-46, 60, 80, 92, 95, 110, 111})</td>
</tr>
<tr>
<td></td>
<td>Thr399Ile</td>
<td>Meningococcal disease, rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, atherosclerosis, asthma</td>
<td>No (^{8, 47, 81, 82, 101, 112, 113, 130})</td>
</tr>
<tr>
<td>TLR5</td>
<td>392 Stop</td>
<td>Legionella pneumophila infection</td>
<td>Yes (^{58})</td>
</tr>
<tr>
<td>TLR6</td>
<td>Ser249Pro</td>
<td>Asthma</td>
<td>Yes (^{132})</td>
</tr>
<tr>
<td>TLR9</td>
<td>-1237 C/T</td>
<td>Crohn’s disease, asthma</td>
<td>Yes (^{97, 133})</td>
</tr>
<tr>
<td>TLR10</td>
<td>+1031 G/A, +2322 G/A</td>
<td>Asthma</td>
<td>Yes (^{134})</td>
</tr>
</tbody>
</table>

### Table I. Polymorphisms in TLRs genes and their relationship with human pathologies

- **TLR2 SNP**: Arg753Gln
  - Disease: Staphylococcal, Mycobacterium leprae and Mycobacterium Tuberculosis infection
  - Association: Yes \(^{53-56}\)
- **TLR3 SNP**: -7 A/C, +71 C/A, Leu412Phe, 1377 C/T
  - Disease: Asthma
  - Association: No \(^{135}\)
- **TLR4 SNP**: Asp299Gly
  - Disease: Response to inhaled endotoxin, Gram negative sepsis, severe RSV bronchiolitis, rheumatoid arthritis, diabetic neuropathy, Crohn’s disease, ulcerative colitis, atherosclerosis
  - Association: Yes \(^{42-46, 60, 80, 92, 95, 110, 111}\)
- **TLR5 SNP**: 392 Stop
  - Disease: Legionella pneumophila infection
  - Association: Yes \(^{58}\)
- **TLR6 SNP**: Ser249Pro
  - Disease: Asthma
  - Association: Yes \(^{132}\)
- **TLR9 SNP**: -1237 C/T
  - Disease: Crohn’s disease, asthma
  - Association: Yes \(^{97, 133}\)
- **TLR10 SNP**: +1031 G/A, +2322 G/A
  - Disease: Asthma
  - Association: Yes \(^{134}\)
of the Asp299Gly mutation failed to demonstrate an association of this polymorphism with the frequency or severity of documented meningococcal disease\textsuperscript{6, 47}. On the other hand a critical role for TLRs in host defence against Gram-positive bacteria was strongly suggested by several experiments in which TLR2-deficient mice were unable to respond to peptidoglycan and lipoproteins derived from these organisms (Staphylococcus aureus)\textsuperscript{48, 49}. Extensive in vitro data strongly suggest that TLR2 participates in mycobacterial infection\textsuperscript{50, 52}. Several studies have suggested that a mutation in the TLR2 gene (Arg753Gln) may predispose individuals to staphylococcal, Mycobacterium leprae and Mycobacterium tuberculosis infections\textsuperscript{53-56}. Underhill et al.\textsuperscript{52} reported that TLR2 is the principal mediator of macrophage activation in response to mycobacteria and might cause defective mycobacterial phagocytosis by macrophages, resulting in an impaired Th1 response characteristic of patients with lepromatous leprosy. These findings suggest that this TLR2 polymorphism plays an important role in defence against Gram-positive infections.

Recently a study has demonstrated that TLR5 recognizes the flagellin protein, a potent inflammatory stimulus present in the flagellar structure of many bacteria\textsuperscript{57, 58}. The author also suggested that a common TLR5 stop codon polymorphism is associated with susceptibility to infection with Legionella pneumophila, a flagellated bacterium\textsuperscript{59}.

Regarding fungal infection, there is relatively little evidence that implicate mammalian TLRs in antifungal host defence. In vitro assays have shown that TLR2 recognizes zymosan, a yeast cell wall particle, and that TLR4 plays a role in the induction of cytokines and intracellular signalling in response to stimulation with Aspergillus fumigatus and Cryptococcal polysaccharide capsule, respectively\textsuperscript{60}.

There is evidence for the participation of TLR in host defence against viral infection. Some studies using a model of RSV infection of tlrl-deficient C57BL/10ScNcr mice implicated TLR4 and CD14 in the recognition of RSV fusion (F) protein\textsuperscript{25, 59}. TLR4 mutations (Asp299Gly, Thr391le) are associated with an increased risk of severe RSV bronchiolitis\textsuperscript{60}. TLR3 is another candidate for viral recognition, arising from studies using poly (I:C), a synthetic double-stranded RNA (dsRNA) analogue that is a molecular pattern associated with viral infection\textsuperscript{61}. Recent reports also suggest that mouse TLR7 and human TLR7 and TLR8 may participate in antiviral host defence\textsuperscript{61, 62}.

**TLRs AND AUTOIMMUNITY**

Autoimmune disease is an attack on self-tissues by the adaptive immune system\textsuperscript{63}. Systemic autoimmune diseases such as rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) are characterized by the production of autoantibodies, including rheumatoid factor (RF), antinuclear antibodies and antibodies to DNA\textsuperscript{64}. The production of circulating antibodies in response to many pathogens depends on recognition of those pathogens by both T and B cells. This constraint greatly reduces the risk of autoantibody production, as T and B cells would have to break their tolerance to the same self-antigen simultaneously. Some antigens from pathogens are T-cell-independent, being lipopolysaccharide (LPS) the best understood of them. LPS is recognized by TLR4, present on all mouse B cells\textsuperscript{68} but not human B cells\textsuperscript{66}. High concentrations of LPS stimulate the proliferation of mouse B cells. But at lower concentrations, the simultaneous recognition of LPS by antigen receptors and by TLR4 causes a synergistic signal, which provokes proliferation and production of circulating antibodies selectively by bacteria-specific B cells\textsuperscript{66, 67}.

The mechanisms that lead to autoantibody production remain relatively obscure, but a report by Leadbetter et al points to a similar mechanism in which B cells seem to produce RF independently of T cells\textsuperscript{66}. In a mouse model of systemic autoimmune disease, the simultaneous activation of cell-surface antigen receptors and another toll-like receptor, TLR9, causes a particular subclass of self-immunoglobulin (IgG2a) to be recognized by B cells as if it were a pathogen. This triggers T-cell-independent proliferation of B cells. In the bloodstream of the autoimmune mice, self-IgG2a accumulates in complexes with self-DNA. This DNA fails to be cleared from the bloodstream for unknown reasons. TLR9 detects bacterial DNA\textsuperscript{11}. Bacterial and vertebrate DNAs differ by the absence of CpG methylation in bacteria. The immune system uses TLR9 to detect the presence of unmethylated CpG dinucleotides as a signal of infection. In fact, vertebrate DNA is not completely methylated: only 70-80% of the CpG dinucleotides in vertebrate genomes are actually methylated. It seems possible that those unmethylated CpGs might be causing the observed effects. Interestingly, DNA methylation is decreased in cells from autoimmune mice and humans\textsuperscript{69}, which supports the hypothesis that unmethylated self-DNA may actually be a pathogenic factor in autoimmunity\textsuperscript{70}. In addition, drugs that can induce SLE in humans inhibit CpG methylation\textsuperscript{71}, strengthening the link between hypomethylated self-DNA and autoimmunity. Although we do not know how rheumatoid factors are involved in diseases, a high titre at diagnosis correlates with the severity of both later RA and disease in tissues other than joints, such as blood vessels\textsuperscript{72}. The finding that mammalian DNA can stimulate TLR9 when present in immune complexes was corroborated by Viglianti et al\textsuperscript{73}.

...
Although TLRs are usually thought to be crucial for the recognition of pathogens, they can also bind to self-antigens, such as heat shock proteins, fibrinogen and others\(75\), released by cells undergoing stress, damage or necrotic death. So, B cells may simultaneously recognize these self-antigens through cell-surface antigen receptors and TLRs, provoking other autoantibodies to nuclear antigens such as self-DNA itself, in autoimmune diseases that are characterized by prominent tissue damage.

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Recently, another point of view about the role of TLRs on autoimmunity has been reported\(76\). In this work, it is proposed that in healthy individuals, tolerance to self-antigens is maintained by the balanced crosstalk between C-type lectin receptors (CLRs), which sample specific carbohydrate structures on self-antigens and induce tolerance\(77,78\), and TLRs, which sense danger signals by PAMPs and induce immune activation. The normally balanced CLR-TLR crosstalk can be disturbed by overstimulation of TLRs and/or understimulation of CLRs, leading to dendritic cell (DC) maturation, and ultimately autoimmunity. They hypothesize that disturbed glycosilation of self-antigens might set the stage for autoimmunity by weakening the capacity of CLRs to buffer «danger signals» through TLRs.

Next, we will review some of the most relevant findings concerning the role of TLRs in some common autoimmune diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), type 1 diabetes (T1D), inflammatory bowel disease (IBD) and multiple sclerosis (MS).

Activation of the innate immune system triggers the release of proinflammatory cytokines and chemokines (including TNF-\(\alpha\)) that can contribute to the initiation and/or perpetuation of inflammatory responses, such as those seen in RA. Active infection of the joint stimulates a strong innate immune response, and such infection commonly culminates in a destructive arthritis. Although the etiology of RA is largely unknown, we know that antigen-presenting cells (APCs) such as macrophages and DCs play an important role in disease pathogenesis\(77-79\). APCs are well equipped with several receptors involved in antigen uptake and processing, including TLRs. TLR4 is the most investigated TLR in RA. This receptor interacts with endogenous ligands released by cells undergoing stress, damage or necrotic death, which are present in inflamed synovial joints of patients with RA, activating immune cells to produce factors that are involved in the breakdown of the cartilage. The most studied SNPs within the TLR4 gene are Asp299Gly and Thr399Ile, but their role on RA susceptibility is unclear. However, Radstake et al. found an association between Asp299Gly polymorphism and RA\(80\), while other reports show no association\(81,82\). It is worth noting that the frequency of TLR4 Asp299Gly heterozygous is of 17% in Radstake’s manuscript, which is considerably higher than the reported in most studies.

It has been reported that synovial cells express low levels of both TLR2 and TLR9\(83\). The former is up-regulated by exposure to peptidoglycans (PG), which is consistent with this PAMP contributing to the development of RA. Synovial fibroblasts from RA patients cultured with PGSs increased their expression of intercellular adhesion molecule 1 (ICAM-1) and various metalloproteinases, and produced the proinflammatory cytokines IL-6 and IL-8. This is consistent with the evidence that PG injected intraarticularly induces inflammation\(84,85\). Recently, it has been reported that the nominal antigen-independent, polyclonal activation of preactivated T cells via TLR2 has a pivotal role in the initiation and perpetuation of pattern-induced chronic inflammatory joint disease\(86\).

Dong et al. demonstrated that intraarticular injection of bacterial DNA induces an inflammatory arthritis\(86,87\). They showed that CpG DNA, recognised by TLR9, triggers TNF\(\alpha\) production, facilitating the development of CpG-mediated arthritis. This was followed by a study by Zeuner et al. documenting that suppressive oligodeoxynucleotides specifically inhibited the immune activation induced by CpG DNA, blocked production of TNF\(\alpha\), and thereby prevented development of RA\(88\).

A typical characteristic of SLE is the presence of autoantibodies against self-antigens, including nucleic acids. Signalling through TLR9 might be responsible for that\(89\). This hypothesis has been supported by Anders et al. who found that CpG-oligodeoxynucleotides (CpG-ODN) or E. coli DNA aggravate renal disease in MRL\(^{pr}pr\) mice, a murine model for SLE\(90,91\). CpG-DNA increased serum levels of DNA autoantibodies of the IgG2a isotype in the MRL\(^{pr}pr\) mice, caused massive proteinuria, and worsened renal pathology. Injected exogenous DNA or ODN bound to TLR9-positive macrophages and DC in glomeruli and renal interstitium of MRL\(^{pr}pr\) mice caused enhanced renal chemokine and chemokine receptor expression. Thus activation of TLR9 by unmethylated synthetic or E. coli DNA triggers exacerbation of autoimmune lupus nephritis.

TLR4 is believed to recognize, in addition to microbial constituents, host-derived molecules\(92\). A recent publication followed this idea by showing that cell surface-expressed GP (a paralogue of the human HSP90, which has been linked to active SLE) as a ligand of TLR2 and TLR4 leads to a MyD88-dependent development of lupus-like autoimmune disease in mice, independent from lymphocyte activity\(93\). Polymorphisms of TLR4 and TLR2 have been studied concerning SLE predisposition and severity, but no association has been found\(91\).
Recently, the important role of TLR3 in the development of T1D has been shown\(^\text{(93)}\). Wen et al found that treatment with poly (I:C), a synthetic dsRNA that can stimulate immune responses similar to those produced by viral infections, plus immunization with whole insulin-protein induced a high incidence of diabetes in B6/RIP-B7.1 mice. This effect is performed through TLR3 recognition of poly (I:C). When they investigated TLR expression in pancreatic islets in humans, TLR3 showed the highest expression level in all the individuals studied.

Of note, Asp299Gly and Thr399Ile polymorphisms of the TLR4 gene have been shown association with reduced prevalence of diabetic neuropathy in patients with T2D\(^\text{(92)}\).

Primary human intestinal epithelial cells (IECs) of normal mucosa constitutively express TLR2, TLR3, TLR4 and TLR5 and their expression is selectively altered in patients with IBD\(^\text{(94)}\). TLR4 is strongly up-regulated in both Crohn’s disease and ulcerative colitis, while the expression of TLR2 and TLR5 remains unchanged. Furthermore, the expression of TLR3 in the intestinal epithelium is down-regulated in active Crohn’s disease, but not in ulcerative colitis. These results suggest that IBD may be associated with distinctive changes in selective TLR expression in the intestinal epithelium. Interestingly, a recent study by Kobayashi et al., using multiple genetic manipulations of the myeloid cell-specific deletion of Stat3 mouse model, showed that enterocolitis is significantly improved in TLR4/Stat3 double-deficient mice\(^\text{(90)}\).

Furthermore, polymorphisms in TLR genes have shown association with IBD. TLR4 Asp299Gly polymorphism is associated with both Crohn’s disease and ulcerative colitis\(^\text{(95)}\); Thr399Ile mutation in the TLR4 gene is associated with ulcerative colitis\(^\text{(96)}\) and a promoter SNP in the TLR9 gene is associated with Crohn’s disease\(^\text{(97)}\).

Activation of APCs through TLR9 is also important in the development of MS\(^\text{(98)}\). TLR4 is necessary for LPS-induced oligodendrocyte injury in the central nervous system\(^\text{(99)}\). In mice, a transcriptional activation of TLR2 has been associated with the clinical course of experimental autoimmune encephalomyelitis\(^\text{(100)}\). Nevertheless, Asp299Gly and Thr399Ile polymorphisms in TLR4 do not predispose to the development of MS\(^\text{(101)}\).

**TLRs AND ATHEROSCLEROSIS**

Atherosclerosis is a consequence of a complicated inflammatory process with immune reactions at the initiation and progression of this disease. Evidence is accumulating that shows expression of TLRs on a variety of cells, including cells of the arterial wall\(^\text{(102)}\). Human endothelial cells in normal arteries express TLR4 and low levels of TLR2\(^\text{(103, 104)}\) and in the atherosclerotic plaque, expression of TLR2 and TLR4 has been described in macrophages and endothelial cells\(^\text{(105)}\). It is now clear that endogenous ligands are also able to activate immune responses through TLRs. The extra domain A of cellular fibronectin (in vivo experiments have demonstrated that this domain is expressed in injured arteries), chlamydial LPS or human heat shock protein 60 (hsp60) are endogenous ligands of TLR4 that have been involved in the induction of atherosclerosis\(^\text{(106-108)}\). The expression of innate immune receptors in healthy arteries and atherosclerotic plaques, and the presence of these ligands in atherosclerotic and injured arteries, suggest that the TLR are involved in the development and progression of atherosclerotic disease\(^\text{(109)}\).

Recently, the Asp299Gly TLR4 polymorphism has been associated with atherosclerosis progression. In a case-control study it was found that subjects with the Asp299Gly allele had lower levels of some of the inflammatory cytokines, acute-phase reactants, soluble adhesion molecules, and other mediators of inflammation; also this allele was associated with a decreased risk of atherosclerosis\(^\text{(110)}\). Another study in patients with acute coronary syndromes confirm previous results, since the Asp299Gly allele was associated with a decreased risk of acute coronary events independently of standard coronary risk factors (OR 0.41; 95% CI 0.18-0.95; P= 0.037)\(^\text{(111)}\). Both results suggest that this TLR4 polymorphism protects against the progression of atherosclerosis disease. But conflicting results have also been reported. A study found no difference in the frequency of TLR4 polymorphisms in patients requiring carotid endarterectomy, compared to controls\(^\text{(112)}\) and another study suggested that predisposition to and progression of coronary artery stenosis are not related to this TLR4 polymorphism\(^\text{(113)}\), although Gly299 allele carriers had a greater reduction in cardiovascular events when treated with pravastatin\(^\text{(114)}\).

The role of TLRs in the cardiac function has also been demonstrated in \emph{thr2}-knockout mice. This study suggests that TLR2 is involved in cardiac remodelling after myocardial infarction\(^\text{(115)}\). The conflicting reports do not exclude, however, involvement of TLR4 in atherosclerosis; the lack of association in some studies provide additional evidence against the importance of TLR4 polymorphism in either cardiac or carotid artery stenosis\(^\text{(112, 116)}\). It is possible that polymorphisms within genes in these pathways, other than TLR4, are important in the pathogenesis of coronary artery disease; other candidate genes have been associated with coronary atherosclerosis or acute coronary syndromes, such as the genes encoding CD14\(^\text{(116, 117)}\), IL-6\(^\text{(118)}\) or MyD88\(^\text{(119)}\).

In conclusion, these data support the concept that an efficient innate immune defence against bacteria and associated
long-term intravascular inflammatory stress are involved in the development of atherosclerosis.

**TLRs IN INFLAMMATORY AIRWAY DISEASES**

The role of TLR proteins in inflammatory airway diseases, such as asthma and allergy is being intensively studied. These studies are based on two contradictory lines of reasoning. First, exposure to LPS increases the severity of asthma. A study on people sensitive to house dust mite allergen showed that the severity of their asthma correlated more closely with levels of LPS than with those of the allergen itself(120). People with allergic asthma are also more sensitive to the bronchoconstrictive effects of inhaled endotoxin(121) than are non-asthmatics. Thus, LPS can exacerbate asthma, probably by increasing the extent of airway inflammation, through its recognition by TLRs. In contrast to its exacerbating effect on asthma, exposure to LPS and other TLR ligands in early childhood may, paradoxically, decrease the incidence of asthma later in life(122, 123). The essence of the «hygiene hypothesis»(124) is the idea that exposure to specific infections, or perhaps endotoxins, drives the maturing immune system in infancy/childhood towards a Th1 phenotype, and away from the Th2 phenotype associated with atopy. Since TLRs are implicitly involved in responses to many pathogen-related molecules, they may be critical receptor pathways involved in maturation of the normal adult immune system. There is some evidence for this hypothesis, albeit indirect, since polymorphisms in CD14, the correceptor involved in LPS signalling via TLR4, correlate with IgE levels(125). It is clear that LPS can either exacerbate or diminish the severity of asthma, depending on the timing of LPS exposure and whether the disease or its exacerbations result primarily from LPS or allergens. In addition, the type of TLR stimulation during the initial phase of immune activation determines the polarization of the adaptive immune response and may play a role in the initiation of Th2-mediated immune disorders, such as asthma(126). These results may also be explained by the finding that high doses of LPS activate the CD4+CD25+T regulatory (Treg) cells that may prevent the activation of pathogenic T cell clones(127). On the contrary, low doses of LPS activate DCs that in conjunction with IL-6 production, release T cells from the inhibitory effect of the Treg cells(128), thereby leading to the activation of the pathogenic T cell clones.

The importance of TLRs in allergic diseases is less well understood. Studies of polymorphisms in TLR genes have the potential to advance our understanding of the response to endotoxin and the pathogenesis of allergic disease. The best-studied polymorphism is the Asp299Gly amino acid substitution of TLR4. This SNP is associated with differences in LPS responsiveness in humans, demonstrating that gene-sequence changes can alter the ability of the host to respond to environmental stress(42). In addition, Asp299Gly and Thr399Ile polymorphisms in TLR4 gene have been shown to be associated with a modified response to endotoxin in asthmatic patients(129). On the other hand, other studies analysing TLR4 polymorphisms-asthma associations have yielded heterogeneous results. Raby et al.(130) found no evidence that genetic variation in TLR4 contributes to asthma susceptibility. Yang et al.(113) could confirm the previously observed lack of association of TLR4 polymorphisms with asthma. Nevertheless, their findings suggest that genetically determined hypersensitivity to endotoxin may increase atopy severity. These findings are consistent with the known ability of LPS to exacerbate existing asthma and to decrease atopy and suggest that the Asp299Gly polymorphism is predictive of airway and atopic responses in a specific subset of the population.

Polymorphisms mapping to other TLRs genes have been studied in relation to asthma and allergy susceptibility, but not as widely as the TLR4 genetic variants. Regarding to the TLR2 gene, -16934A/T genetic variation is a major determinant of the susceptibility to asthma and allergies in children of farmers (131). Furthermore, SNPs in TLR6, TLR9 and TLR10 genes have shown association with asthma(132-134). Recently, Noguchi et al.(135) screened the 5´ flanking and coding regions of TLR2, TLR3, TLR4 and TLR9 for polymorphisms. They found 16 variants, even though none of the alleles or haplotypes were associated with asthma or total IgE levels in a Japanese population.

**CONCLUDING REMARKS**

TLRs are a family of transmembrane receptors, some of which have been clearly demonstrated to play a key role in innate immunity. The first focus of research was on the TLR signalling pathway. The identified LPS signalling mediators may be important pharmacological targets, and the future identification of all the component of the LPS signalling cascade will be an invaluable platform for designing therapeutical interventions. Also, the effect of various TLR polymorphisms on disease progression becomes better understood, a patient's genotypic profile will comprise an increasingly important factor in the consideration of various therapeutic options. Future studies will need to address the biological importance of the known differences in TLR function, including expression patterns, ligand specificities and the relationships among TLR signalling, genetic polymorphism and human disease.
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TOLL-LIKE RECEPTORS AND HUMAN PATHOLOGY


