The significance of toll-like receptors in human diseases

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Abstract

Toll-like receptors (TLRs) are a family of transmembrane receptors that have been preserved throughout evolution and which selectively recognize a broad spectrum of microbial components and endogenous molecules released by injured tissue. Identification of these ligands by TLRs triggers signalling pathways which lead to the expression of numerous genes involved in a defensive response. In mammals, the products of these genes initiate inflammation, coordinate the effector functions of innate immunity, instruct and modulate adaptive immunity and initiate tissue repair and regeneration. Different mutations and experimental models which alter TLR function have revealed the significance of these receptors in susceptibility to infection and their involvement in the pathogenesis of a large number of non-infective inflammatory disorders such as cancer, allergy, autoimmunity, inflammatory bowel disease, or atherosclerosis. TLRs are currently viewed as important targets for the development of new vaccines and innovative therapies to prevent and treat human diseases.

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Introduction

Over the last two decades, research has revealed the existence of a network of germline-encoded receptors (termed pattern recognition receptors or PRRs) which recognize microbial molecular motifs (pattern-associated molecular patterns or PAMPs) and endogenous molecules produced by injured tissue. These receptors regulate many aspects of innate immunity and determine the polarisation and function of adaptive immunity,\textsuperscript{1,2} but they are also involved in the maintenance of tissue homeostasis by regulating tissue repair and regeneration.\textsuperscript{3} This multiplicity of functions reflects the existence of a tightly controlled innate receptor network that surveys tissue for alterations in homeostasis, and alerts and drives immunity. The involvement of these receptors in a long list of conditions\textsuperscript{4} leaves open the possibility of establishing a universal immunobiological model which explains all human disease.\textsuperscript{5}

The most widely studied of these sensors are toll-like receptors (TLRs). In recent years, the identification of
several TLR mutations and common polymorphisms has made it possible to determine their role in susceptibility to infection, and they have been associated with many other non-infectious diseases.6–8

In a previous work in this series, we reviewed the main structural and functional features of TLRs, their ligands and signalling pathways, and the importance of cooperation between TLRs in the induction of a specific immune response.7 In this review, we highlight the importance of TLRs in the activation and modulation of inflammation, and examine their role in some of the most frequent human diseases.

**TLRs as initiators of inflammation**

In mammals, proteins and immune cells which participate in host defence are distributed throughout the body and continuously recirculate in blood and lymph. However, when a pathogen gains entry to the host, or if an injury occurs, it is necessary to concentrate them and their products at the site of damage. Cells of the affected tissue and resident immune cells sense pathogens and damage through multiple PRRs that cooperate by activating a cascade of biochemical events which in turn initiates the inflammatory response by allowing exudation of plasma proteins and by driving selective extravasation of leukocytes from the blood into the surrounding tissue. TLRs are the most extensively studied sensors of damage that participate in the initiation of inflammation.

**TLRs on epithelial barriers**

Although the epithelium is considered a protective physicochemical barrier, epithelial cells are also able to sense pathogens and injury through TLRs and induce the production of antimicrobial peptides, cytokines, and chemokines which neutralise pathogens and initiate inflammation.8–10

This recognition is the first step in the host-pathogen interaction and has important implications for immune protection. In addition, PAMPs mediate signals through TLRs to induce a set of non-immune epithelial responses including cell migration, wound repair, proliferation, and survival of primary epithelial cells playing an essential role in the regulation of mucosal homeostasis.11

Expression and activation of epithelial TLRs varies according to their location. In the gut, the mucosal epithelium is continuously exposed to a vast quantity of antigens from food and commensal bacteria, as it is the largest surface of the body in contact with environmental antigens. Mucosa and intestinal microflora constitute a complex and highly regulated ecosystem in which more than 2000 species of microorganisms continuously interact with nutrients and host cells in a symbiosis essential for normal gut function and host health.12 Thus, to maintain a normal intestinal function, the immune system must develop immune tolerance to harmless antigens from food and commensal bacteria, whilst maintaining the ability to develop appropriate immune responses against pathogens.

Intestinal epithelial cells are structurally and functionally polarised, with an apical surface facing the intestinal lumen and a basolateral surface facing the adjacent cells in the lamina propria. A continuous layer composed of mucus and the glyocalyx lines the apical side of gut epithelium to reinforce physical protection by trapping germs. Release of mucines, IgA, and antimicrobial peptides prevents microorganisms from coming into close contact with the apical surface of the epithelial cell layer. When bacteria breach this protective barrier, epithelial cells sense it through TLRs and activate an inflammatory response. Intestinal epithelial cells express almost all the TLRs identified, but their expression and activation are strategically regulated to avoid unnecessary inflammation, and they play an essential role in preserving peripheral tolerance.13–16

Prolonged exposure of these epithelial cells to PAMPs from commensal bacteria induces selective down-regulation of the apical expression of TLR2 and TLR4,13,17–19 which are relocated either to intracellular compartments such as the Golgi apparatus or to the basolateral membrane, where they retain their full signalling ability to detect internalised antigens.20,21 TLR5, however, is expressed exclusively on the basolateral surface. This strategic distribution of intestinal TLRs allows the host to detect a pathogen when it crosses the intestinal epithelial barrier, thus preventing an over-reaction to the commensal bacteria present in the intestinal lumen. In the case of crypt epithelial cells, which are not exposed to commensal bacteria, TLR2 and TRL4 are located in the plasma membrane and recognize external ligands on the cell surface.22 In addition, intestinal epithelial cells express relatively high levels of mRNA for TLR3, since viral dsRNA is not a natural ligand of microflora, thus allowing these cells to stimulate an immune response to control viral infection without being detrimental to the host.22

Basolateral TLR9-mediated signals are believed to activate an inflammatory response, whereas apical TLR9 stimulation delivers negative signals that curtail inflammatory responses induced by basolateral stimulation by other TLRs.23

In-vitro studies using intestinal epithelial cells have demonstrated that prolonged incubation with several TLR ligands results in a state of hyporesponsiveness to successive challenges with those ligands, associated with increased expression of TLR antagonists. Thus, functional negative regulatory mechanisms in the gastrointestinal mucosa also seem to prevent inappropriate immune responses to luminal bacterial products.24

Taken together, these findings suggest that luminal bacterial products help to maintain colonic homeostasis and to regulate tolerance and inflammation via activation of specific epithelial TLRs.

Likewise, the epithelial mucosa of the airway is an important component of the innate immune system. It senses microorganisms and damage and initiates a protective inflammatory response, although it too must remain inactive against a long list of innocuous antigens to which it is permanently exposed. The lung epithelium is a major source of neutralising molecules, cytokines, chemokines, and other inflammatory mediators which affect the innate and adaptive immune responses and play an important role in inflammatory lung diseases, including chronic obstructive pulmonary disease and asthma.25,26 The production of these mediators is mainly initiated by TLRs. Research using cell lines or primary cell tissue has revealed that airway epithelial cells express functionally active TLR1-10 (the
most highly expressed are TLR2, TLR3, TLR5, and TLR6$^{7,28}$, however, their exact expression patterns and levels have yet to be elucidated. TLRs expressed on bronchial epithelial cells induce a different cytokine profile from that of macrophages, and seem to be involved in bringing about the recruitment of neutrophils by chemokinesis as an initial response to the entry of microbes rather than as a potent inflammatory response. TLR activation in airway epithelial cells induces the release of molecules which drive dendritic cells (DC) to polarise naïve T helper (Th) function. Furthermore, TLR expression in small airway epithelial cells is regulated by Th1 and Th2 cytokines, and the response of TLRs in the lung epithelium to viral and bacterial infections seems to contribute to exacerbations of lung diseases.

In vascular endothelial cells, TLR activation contributes directly to the inflammatory response of the microvasculature. In these cells, TLRs induce secretion of immune mediators to the bloodstream, participate in leukocyte recruitment, induce angiogenesis, and generate paracrine signalling to local immune cells.

### TLRs on macrophages and mast cells

Tissue-resident macrophages express all TLRs (except TLR3) and are highly responsive to their agonist. In these cells, TLRs are important for each stage of phagocytosis, ranging from engulfment of invading pathogens to antigen processing and presentation of antigenic peptides. TLRs regulate the generation of vasoactive lipids and reactive oxygen species, and lead to the production of cytokines such as tumour necrosis factor (TNF-α) and interleukin (IL-1β), and to the release of chemokines that induce endothelial cell activation and drive inflammatory cell recruitment. In addition, TLR activation regulates the expression of major histocompatibility complex (MHC) molecules and co-stimulatory molecules, and induces the release of IL-12 and IL-10, cytokines which differentially alert DCs to polarise naïve T cells and activate specific adaptive immunity.

Mast cells reside in the connective tissue and mucous membranes, and respond rapidly to different stimuli by releasing granules rich in histamine and heparin, along with various hormonal mediators, chemokines, and cytokines which activate the microvasculature to cause vasodilatation and extravasation of fluid, which is responsible for the characteristic signs of acute inflammation. Although these cells are considered essential in host defence against helmints and are the major effectors of IgE-associated allergic disorders, recent works have revealed that they also play a critical role in host defence against bacterial and viral infection. Both human and rodent mast cells can express a wide range of TLRs that are profoundly influenced by the microenvironment. Direct activation of mast cells is mediated through TLR receptors that recognize microorganism-derived components or danger signals similarly to that of other leukocytes, although some responses to traditional TLR ligands rely on signalling through co-receptors. TLR-mediated activation of mast cells induces production of chemokines and Th2 cytokines, which can also be accompanied by degranulation. In addition, the combination of TLRs with the high-affinity IgE receptor synergistically increases the ability of murine mast cells to produce inflammatory cytokines such as TNF-α, IL-12 p70, IL-6, IL-5, IL-13, and eotaxin 2, revealing that direct activation of mast cells via TLRs by their respective microbial ligands contributes to innate immune responses to pathogens. The presence of pathogens can thus modulate the allergic response.

### TLRs in the activation of effector cells

In an inflammatory response, the initial cellular infiltrate consists of effector cells of innate immunity such as phagocytes, eosinophils, and NK cells, all of which express TLRs that drive their effector functions.

Resting neutrophils express mRNA for all TLRs (except TLR3), whereas unstimulated monocytes express higher levels of TLR mRNA (except TLR7). Their agonists directly elicit inflammatory responses (except for cytosine-phosphate-guanine [CpG] motifs, which require pre-treatment with granulocyte macrophage-colony stimulating factor). TLR activation seems to participate in homing and survival of neutrophils and in many of their effector functions, such as the release of antimicrobial peptides, generation of reactive oxygen intermediates, phagocytosis, biosynthesis of vasoactive substances, and secretion of cytokines and chemokines.

Human eosinophils differentially express TLR1, 2, 4, 5, 6, 7, and 9. Ligands such as peptidoglycan (TLR2 ligand), flagellin (TLR5 ligand), and imiquimod R837 (TLR7 ligand) significantly up-regulate cell surface expression of intercellular adhesion molecule (ICAM)-1 and CD18 and induce the release of IL-1β, IL-6, IL-8, growth-related oncogene-α and superoxides. Eosinophil TLR7/8 systems represent a potentially important mechanism in host defence against viral infection. This activation of eosinophils through TLRs supports the idea that microbial infection may lead to the exacerbation of allergic inflammation.

NK/NKT cells can express all known TLR mRNA (TLR1-10), which enables them to recognize pathogens and activate effector functions such as cytotoxic response and cytokine production. TLR3 is expressed on the cell surface, where it functions as a receptor independently of lysosomes, whereas TLR7/8 function requires the participation of lymphocytes, as do other cell types.

NK cells are activated or primed by accessory cell–derived cytokines, and this collaboration sometimes plays an essential role in the activation of effector functions that resolve infection. In addition, when infection occurs, macrophages produce IL-12, which renders NK cells highly responsive to TLR agonists so that they can produce interferon (IFN)-γ and chemokines. These in turn recruit and fully activate macrophages, thus leading to the development of inflammatory foci that are presumably necessary for efficient eradication of microbes.

TLRs are also constitutively expressed on somatic cells such as fibroblasts, adipocytes, and smooth muscle cells, and participate in inflammation. Different mediators released by sentinel and effector cells dramatically increase TLR expression in somatic cells so that they can recognise PAMPs and the endogenous agonists generated at inflammation sites, and respond to them by releasing new mediators which amplify the process.
TLRs as drivers of adaptive response

TLRs in T-cell polarisation

The adaptive immune response generated against a specific antigen is controlled by DCs. These cells are professional antigen-presenting cells with the capacity to stimulate naive T cells and polarise their function, thus acting as a bridge between innate and adaptive immunity.63,64 The naive CD4+ T cell differentiates into a Th1, Th2, Th17, or T regulatory (Treg) cell phenotype, according to the density and nature of the antigenic peptide presented, the class of co-stimulatory molecules expressed by DCs, and the type of polarising signals released.

DCs can be divided into several subsets on the basis of cell surface marker expression, maturity, and function.65 Although many subtypes arise from different developmental pathways, their phenotype and function are mainly modulated by signals that the cells receive from pathogens, the environment, and other immune cells.66 Under steady-state conditions, tissue-resident DCs are mostly immature, but in infectious processes, immature DCs migrate to the injured region where they detect pathogens and damage via PRRs and receive environmental inflammatory signals that induce their maturation and activation. Many PRRs participate in these processes, and of these, TLRs have been shown to be decisive for the establishment of an adaptive immune response. Pathways activated in DCs through TLRs trigger an array of responses that affect the capture, processing, and presentation of antigens, as well as migratory activities and cell survival. In addition, these pathways induce up-regulation of different surface co-stimulatory molecules and stimulate production of polarising cytokines and chemokines.67-73

In humans, two major subsets of blood-derived DCs have been described: the myeloid DCs (mDC), which derive from monocytes and are found in peripheral tissue, secondary lymphoid organs, and blood, and the less frequent plasmacytoid DCs (pDC), which reside mainly in lymph nodes and around highly endothelial venules. The striking differences in TLR expression between these DC subsets restrict their reactivity to the presence of a specific pathogen.73-75

Human mDCs express TLRs which recognize bacterial components on the cell surface, particularly TLR1, TLR2, TLR4, and TLR6, whereas TLR3 is expressed in putative endosomes.76

Immature mDCs constitutively express the Jagged notch ligand, which promotes antigen-specific CD4+ T cells to differentiate into Treg cells or into Th2 cells.74,77 mDC maturation induced through TLRs by microbial PAMPs reduces the expression of Jagged-1 notch ligand, up-regulates the expression of Delta-4 notch ligand (a co-receptor that induces Th1 polarisation), and induces the production of Th1-polarising cytokines. Binding of bacterial PAMPs to TLRs also generates a potent negative signal which prevents the development of Th2 cells.74,78 In contrast, a number of helminth-derived products interact with TLRs to induce a different programme for the maturation of mDCs, which evolve to a different subset known as DC2. These DC2s are relative immature and in some cases are refractory to subsequent stimulation through TLR activation. DC2s can promote a robust antigen-specific Th2 response.79

The binding of a ligand to a specific TLR can elicit different types of T-cell response, depending on the DC microenvironment and the cadence and route of antigen administration. For example, TLR4-stimulated DCs in the presence of IFN-γ produce high levels of IL-12 p70 and express Delta-4 notch ligand to promote Th1 cell development.80-83 However, in the presence of TGF-β and IL-6, TLR4 ligand favours the release of IL-23 by DCs, thus inducing proliferation and stabilising Th17.84 When histamine and/or thymic stromal lymphopoietin (TSLP) are present at high levels, TLR4-stimulated DCs produce low levels of IL-12 p70 and express Jagged notch ligand, thus promoting Th2 polarisation.85

TLR2 ligands also have divergent effects on polarising DCs. Under the influence of IL-10 and TGF-β, TLR2 ligands stimulate DCs to polarise naive T cells to Treg cells, which in turn also express TLR2, and the binding of specific ligands induces their proliferation.71 Other authors have found that synthetic lipopeptides containing the typical lipid part of the lipoprotein of gram-negative bacteria stimulate a distinct regulatory cytokine pattern and inhibit several Th2 cell-related phenomena. Triggering of TLR2 by these lipopeptides promotes the in vitro differentiation of naive T cells into IL-10 and IFN-γ-producing T cells and suppresses IL-2 production by Th2 cells.86 TLR2 ligand also acts as an adjuvant for the Th1 response by enhancing the presentation of endogenous peptides.87 In addition, activation of TLR2 expressed on T cells directly triggers Th1 effector functions.88 These results would justify the fact that TLR2 ligands inhibit allergen-specific Th2 responses in sensitised individuals.89 However, under certain conditions, some TLR2 ligands drive DC activation to induce a Th2 response or to produce high levels of IL-23, which in turn promote proliferation of Th17 cells.90,91

Human pDCs possess high levels of TLR7 and TLR9 and constitutively express abundant interferon regulatory factor 7.92 This TLR repertoire expression gives these cells the ability to respond to both microbial DNA and to RNA and DNA-containing or RNA-containing immune complexes in the endosome. TLR-activated pDCs produce large amounts of type I and type III IFNs, TNF-α, IL-6, and waves of chemokines, but they do not secrete IL-12 and hardly induce any T-cell proliferation.92-94 Although type I IFNs were first characterized as the major cytokines which confer early protection against viruses and microbes, they also mediate in an array of immunoregulatory functions and directly or indirectly promote Th1 polarisation.92,95 Similarly, oligodeoxynucleotides containing unmethylated CpG motifs are TLR9 ligands that stimulate a strong Th1 response in vivo. Interestingly, some of them have been developed as adjuvants for various vaccines against intracellular pathogens and cancer, and are also considered good candidates for immunotherapy in atopic disorders.96 However, depending on the nature of the stimulus, pDC may also activate a Th2 response under non-IFN-stimulating conditions.92 pDCs have also been involved in the development of B-cell maturation to antibody-secreting plasma cells and in the establishment of immunological memory.92

In human and murine DCs, TLR3 and TLR4 act in potent synergy with TLR7, TLR8, and TLR9 in the induction of a selected set of genes. This synergic TLR stimulation increases production of IL-12 and IL-23, as well as the
Delta-4/Jagged-1 ratio, leading to DCs with enhanced and sustained Th1 polarising capacity.\textsuperscript{80}

**TLRs as regulators of adaptive response**

One of the most intriguing recent observations is that T and B lymphocytes also express TLRs, and their respective ligands activate processes that modulate their function.\textsuperscript{97–100} TLRs expressed on T cells seem to enhance cell proliferation, adhesion, and survival, although they also modulate cytokine production.\textsuperscript{98} In conventional human and murine $\alpha\beta$T cells, TLR2, TLR5, TLR7, and TLR9 act as costimulatory receptors in concert with a T-cell receptor signal, rather than by inducing a direct cellular response. Human alternative $\gamma\delta$T cells also express mRNA for various TLR2 and TLR3, and human CD8+ cells express TLR3 as a functional co-receptor.\textsuperscript{101} There is also evidence that the naturally occurring Treg cells can be directly regulated by TLR2, TLR3, and TLR8. These receptors are able to repress or enhance their suppressive activity, although the exact relationship between microbial stimulation of the TLR pathway and Treg cells is still unclear.\textsuperscript{100,102} Treg cells are also indirectly regulated by TLRs, since mature DCs activated through different TLRs produce IL-6, which renders responder T cells refractory to the suppressive effect of Treg cells.\textsuperscript{103}

Activation of naive B cells requires the sequential integration of signals mediated by antigen receptor cross-linking and by antigen presentation to specific Th cells through immune synapse, although it also seems to be critically dependent on innate stimuli acting on TLR expressed by B cells, or indirectly via cytokines provided by TLR-activated DCs.\textsuperscript{97,104} In addition, binding of TLR in B cells stimulates proliferation, the release of immunoglobulin, and the production of chemokines.\textsuperscript{105,106}

**TLRs in tissue repair and regeneration**

Following acute tissue injury, many cells die by necrosis and release their intracellular content. In addition, matrix turnover leads to the production of many breakdown subproducts. Over the last few years, different studies have revealed that these endogenous molecules act as “danger molecules” that signal through TLRs and stimulate the innate immune system by promoting inflammation.\textsuperscript{107} Interestingly, recent findings suggest that by recognizing microbes and endogenous harmful stimuli, TLRs induce the expression of several genes involved in the wound healing response and in tissue regeneration to recover the structural and functional integrity of injured organs.\textsuperscript{3,108} In this line of research, TLRs and their ligands have recently been shown to control mesenchymal stem cell functions. These cells can be induced to differentiate into mesodermal cell lineages, support and regulate haemopoiesis, regulate the stem-cell niche, and may participate in the repair of tissue damage inflicted by normal wear and tear, injury, or disease.\textsuperscript{109}

It is well known that chronic inflammation due to infection or sterile injury evokes a perpetuating wound healing response that promotes the development of fibrosis, organ failure, and cancer. These dysfunctions are now associated with alterations in signals mediated by TLRs.\textsuperscript{3,108,110} Accordingly, modulation of TLRs offers new therapeutic perspectives in the recovery of tissues after injury and in the control of conditions mediated by an excessive reparative response such as fibrosis or cancer.

**TLRs in human disease**

**TLRs in immunodeficiency and in susceptibility to infection**

Several authors associate human primary immunodeficiencies with abnormal TLR signalling, thus demonstrating the importance of this pathway in the immune response.\textsuperscript{111,112} The first diseases affecting TLR function were human immunodeficiencies associated with mutations in the gene encoding NEMO, a protein required for the activation of the transcription factor NF-$\kappa$B in TLR signalling.\textsuperscript{7} Loss-of-function mutations in NEMO cause familial incontinentia pigmenti, a genodermatosis that segregates as an X-linked dominant disorder and that is usually lethal in the male foetus. In affected females, it causes highly variable abnormalities and produces severe skin inflammation.\textsuperscript{113,114} Hypomorphic mutations in NEMO are viable and give rise to X-linked anhidrotic ectodermal dysplasia with immunodeficiency (EDA-ID), with differing degrees of severity.\textsuperscript{115,116} In addition to developmental disorders, NEMO-mutated patients present recurrent invasive pyogenic bacterial infections early in life, and later frequently develop atypical mycobacterial disease. An autosomal-dominant form of EDA-ID is associated with a heterozygous missense mutation in the gene encoding I$k$B$x$, a protein that prevents NF-$\kappa$B translocation to the nucleus. This mutation is gain-of-function, as it enhances the inhibitory capacity of I$k$B$x$ which results in impaired NF-$\kappa$B activation. Clinical manifestations overlap with EDA-ID.\textsuperscript{117}

Mutations that affect IRAK4, a member of the IL-1 receptor–associated kinase family involved in TLR signalling, determine immunodeficiency associated with recurrent pyogenic bacterial infections and a poor inflammatory response, but do not present developmental abnormalities.\textsuperscript{118–122} In this immunodeficiency, susceptibility to infection decreases with age, probably due to the development of adaptive immunity. These patients are particularly susceptible to pathogens such as *Streptococcus pneumoniae* or *Staphylococcus aureus*, but are resistant to viral infections (probably through TLR3 and TLR4 production of IFNs). Therefore, the IRAK4–mediated signal is crucial for immunity against specific bacteria, but is redundant against most other microorganisms.\textsuperscript{118}

Studies on the incidence of infectious diseases in people with single-nucleotide polymorphisms (SNPs) in TLRs reveal that these minor alterations can produce a subtle but specific distorted response and underline the role that TLRs play in human susceptibility to infection.\textsuperscript{111} The importance of TLRs in protection against sepsis has been demonstrated in humans exhibiting polymorphisms in TLR genes and in genetically modified mouse strains, thus opening new perspectives in the search for an efficient therapy against this disease.\textsuperscript{113}

Other SNPs affect cytosolic adaptor proteins that TLRs recruit to initiate the inflammatory cascade: in the case of
the TIR domain–containing adaptor protein (or TIRAP), the polymorphism (S180L) is associated with a protective effect against invasive pneumococcal disease, bacteremia, malaria, and tuberculosis; a different TIRAP polymorphism (C558T) is linked with increased susceptibility to meningeal tuberculosis.

Another interesting field worthy of study in susceptibility to infection is the ability developed by many virulent strains of pathogens to evade immunity through TLRs. Such is the case of the bacteria Mycobacterium tuberculosis, Yersinia enterocolitica, Yersinia pestis, Yersinia pseudotuberculosis, and fungi such as Candida albicans, and Aspergillus fumigatus, which activate a TLR2-mediated mechanism to induce an anti-inflammatory cytokine pattern that down-modulates the microbicidal function of leukocytes. In addition, some viruses have evolved mechanisms to block TIR adaptors, thus limiting TLR signalling and modulating the immune response.

**TLRs in atherosclerosis**

Structural cells of the cardiovascular system (eg, endothelial cells, vascular smooth muscle cells, and cardiac myocytes) express functional TLRs that sense PAMPs and danger signals in order to maintain cardiovascular health. Recent reports have suggested their involvement in the development of atherosclerosis and other cardiovascular diseases.

Atherosclerosis is considered an excessive inflammatory-fibroproliferative response to numerous sources of injury to the endothelium and smooth muscle cells of the artery wall. The endothelial response to the injury seems to play an essential role in the initiation of atherosclerosis, whereas the presence of apo-B lipoproteins in the intima, their subsequent posterior modification promotes chronic inflammation. The precise triggers for endothelial damage in atherosclerosis have not been defined, but exposure of the arterial wall to risk factors such as oxidized low-density lipoprotein, mechanical stress, homocysteine, and local or distant infections by viruses and bacteria is associated with the development of lesions. Some of these risk factors are potential inducers of TLR activation, and mechanical stress is associated with up-expression of TLRs. In addition, a mechanism for hyperlipidaemic initiation of sterile inflammation can be postulated, because oxidised lipoproteins or their component oxidised lipids have been identified as TLR ligands. The idea that TLRs participate in the initiation and development of atherosclerosis has been supported by some clinical and experimental studies.

**TLRs in inflammatory bowel disease**

Inflammatory bowel disease (IBD), broadly classified as Crohn’s disease or ulcerative colitis, is caused by a dysregulated mucosal immune response to a luminal antigen, possibly a bacterium or a food, in a genetically predisposed host. Thus, TLR mutations and dysfunction may be contributing factors in the predisposition to and maintenance of IBD, and an increasing amount of clinical and experimental data reveal TLR deregulation in patients with IBD. In active IBD, the expression of TLR3 and TLR4 is differentially modulated in the intestinal epithelium. TLR3 is significantly down-regulated in active Crohn’s disease but not in ulcerative colitis. In contrast, TLR4 is strongly up-regulated in both conditions. TLR5 expression remains unchanged in IBD, but the presence of high titers of flagellin-specific antibodies in the serum of patients with Crohn’s disease also implies the participation of this receptor in the disease.

Polymorphisms of human TLR4 (Asp299Gly and Thr399Ile) have been associated with the development of Crohn’s disease and ulcerative colitis in Caucasian populations. In patients with ulcerative colitis, Pierik et al observed an association between the polymorphisms TLR1 R80T and TLR2 R753G and pancolitis, and a negative relationship between TLR6 S249P and proctitis. These results suggest that TLR2 and its co-receptors TLR1 and TLR6 are involved in the initial immune response to bacteria in the pathogenesis of IBD. An important immune stimulatory effect mediated by TLR9 is induced by non-methylated CpG motifs found in bacterial DNA. In animal models of colitis, administration of CpG was able to perpetue disease activity.

Recently, TLRs were reported to contribute to the pathogenesis of IBD in cooperation with NOD2, a member of the nucleotide-binding oligomerisation domain (NOD)—like receptor family. Although that study supports the idea that alterations in gastrointestinal TLR functions are the underlying mechanisms leading to Crohn’s disease and ulcerative colitis, TLR dysfunction could also be a pathological consequence of chronic inflammation induced by other, unknown factors.

**TLRs in allergy**

Allergic diseases are caused mainly by aberrant Th2 immune responses to innocuous antigens in susceptible individuals. The hygiene hypothesis proposed that, in developed countries, the low microbial stimulation of immunity in early life could lead to a weak Th1 response and a stronger Th2 response to allergens. Today, allergy is viewed as the result of an improper balance between peripheral tolerance and immunity. Although the aetiology of allergy is not completely understood, differential activation of TLRs on DCs and in the epithelium are associated with the prevalence of allergic diseases. It is clear that DCs play an essential role both in the sensitisation phase and in the maintenance of disease, mainly through excessive polarisation to Th2 cells and/or deficient generation of Treg cells. It has been proposed that all types of microbial stimulation (polarising both Th1 and Th2) induce Treg cells that control excessive immune responsiveness and, as a consequence of the reduction in contact with microorganisms, production of Treg diminishes. This leads to a failure in the inhibition of a T-specific response against innocuous antigens such as allergens. However, other authors have recently found functionally active Der p 1–specific Treg cells in both non-atopic and Der p 1–sensitive atopic individuals, thus advising caution when interpreting allergic disorders as simply resulting from defective Treg cell activity.

TLRs also participate in the production of thymic stromal lymphopoietin (TSLP), a recently described cytokine produced by the skin and airway epithelium, capable of...
instructing DCs to polarise naive T cells toward the Th2 subset. In addition, TSLP can interact directly with mast cells to initiate Th2 cytokine production and mediate its pro-allergic effects by a non–T-cell route. TLR-mediated release of TSLP provides an important new link between innate immunity and allergic disease, and opens new therapeutic possibilities in allergy.140,148 The mediated by Tregs is currently seen as a useful therapeutic option to avoid this aberrant immune response.140,148 The identification and characterization of endogenous ligands capable of stimulating immunity through PRRs has provided new perspectives in the study of the aetiology of autoimmune diseases. It has been proposed that, in certain autoimmune disorders, recognition of endogenous ligands by TLRs drives sterile inflammation sustained by innate immune cells that contributes to a loss of tolerance.150 Similarly, it must be emphasized that many autoantigens are generated by tissue injury and are able to stimulate innate immunity through TLRs. This supports the idea that many of them are autoantigens, because they act as autoadjuvants which directly activate innate immunity to induce a self-directed immune response.151,152 For example, different studies in vivo and in vitro have revealed that endosomally translocated self-DNA or self-RNA have, respectively, a TLR9- or TLR7-dependent potential to stimulate pDCs in a similar way to microbial nucleic acid. Activation of pDCs through these TLRs induces release of type I IFN. Because repeated administration of recombinant IFN to patients with tumours or chronic viral infections induces systemic lupus erythematosus (SLE), aberrant production of IFN-γ induced by endocytosed self-DNA and self-RNA through TLRs is considered a key event in the pathogenesis of SLE.151

**TLRs in autoimmunity**

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**TLRs in cancer**

Functional TLRs are expressed in a wide variety of tumours, and evidence suggests that TLR signalling pathways in tumours may be associated with subversion of host defence in favour of the neoplastic process.153 Activation of tumoral TLRs induces the synthesis of proinflammatory factors and immunosuppressive molecules. These enhance the resistance of tumour cells to cytotoxic lymphocyte attack and facilitate their evasion from immune surveillance or, as in the case of multiple myeloma, may promote proliferation and survival of tumour cells by inducing the release of cytokines such as IL-6, IL-13, TNF-α, and other growth factors.154 Moreover, TLRs induce resistance to apoptosis, increase angiogenesis and vascular permeability, and enhance tumour cell invasion by regulating metalloproteinases and integrins.155,156 In addition, alterations in signals mediated by TLRs for tissue regeneration in chronic injury could induce cancer.3,110 This promotion of tumours induced by TLRs justifies the association between multiple chronic inflammatory diseases and infections and the pathogenesis of many cancers.4

These novel functions of TLRs in tumour biology suggest a new class of targets for cancer therapy.153 It has been reported that blockade of the TLR4 pathway reverses tumour-mediated suppression of T-cell proliferation and natural killer cell activity in vitro and in vivo, thus delaying tumour growth and prolonging the survival of tumour-bearing mice.154 However, TLRs also regulate tumour immunity or tolerance through immune responses mediated by Treg, DCs, and other immune cells.157 It has long been noted that some products of microorganisms and several drugs show clinical activity against tumours that could be based on TLR binding to immune cells. Despite the notion that TLRs in tumour cells may benefit tumour progression, several innovative strategies for using TLR agonists in vaccine development have been based on their ability to prime a tolerant immune system to recognize and destroy tumour cells.158–164 However, these immune adjuvants can evoke different host responses by targeting specific TLRs and their associated signalling pathways, and recent studies show that while some immune responses are beneficial, others could be deleterious as anti-cancer therapies.161–163

Under certain conditions, the combination of immunotherapy based on TLR ligands with other approaches may have promising synergistic effects. In this sense, there is evidence that radiation combined with TLR-targeted immunotherapy could enhance tumour-directed immunity164 and that the increased efficiency of adjunctive treatment with the TLR7 agonist imiquimod and cryosurgery could make this a suitable therapeutic strategy for lentigo maligna.165 Thus, it is important not only to carefully select target TLRs by using an optimised mix of TLR agonists, but also to take into account other factors in the tumour microenvironment that modulate innate immunity for a prime adaptive response.

**Conclusions and further perspectives**

TLRs play a crucial role at all stages of the inflammatory response and in tissue repair and regeneration. The possibility of modulating these stages through TLRs has opened an array of opportunities to develop innovative vaccines and therapies for the prevention and treatment of infectious and non-infectious inflammatory disorders.150,166 Many of these therapies are currently being evaluated in
clinical trials. However, although TLR-based therapies have enormous biological potential and offer promising results, their benefits are not free of risk, and more research is required before drugs enter the trial phase and routine clinical practice.

We must remember that TLRs are not the only players in inflammation, and several important questions remain unanswered. For example, we do not know how different TLRs cooperate with each other and communicate with the different PRRs, accessory cells, and microenvironment mediators to elicit the optimal immune response to a specific injury. Accordingly, therapeutic modulation of TLR function could trigger unexpected harmful responses if other simultaneously occurring non-TLR inflammatory signals are not considered. Furthermore, we need a more precise understanding of the role of each TLR in the pathophysiology of the different diseases.

Once a diagnosis has been reached, TLR-based therapy should be prescribed on an individual basis after a thorough evaluation of the patient’s immune status by an expert immunologist. Consequently, the success of these potent biological therapies will require new diagnostic techniques and the efforts of multidisciplinary teams including immunologists with detailed knowledge of potential side effects.

Conflict of interest

The authors have no conflicts of interest to declare.

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References


Toll-like receptors


