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SERIES:ADVANCES IN IMMUNOLOGY THAT CLINICIANS SHOULD KNOW(III)

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.

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,[1,2] but they are also involved in the maintenance of tissue homeostasis by regulating tissue repair and regeneration.[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[4] leaves open the possibility of establishing a universal immunobiological model which explains all human disease.[5]

The most widely studied of these sensors are toll-like receptors (TLRs). In recent years, the identification of

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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]

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 glycocalyx 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 overreaction 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)[27,28]; 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 chemotaxis as an initial response to the entry of microbes rather than as a potent inflammatory response.[25] TLR activation in airway epithelial cells induces the release of molecules which drive dendritic cells (DC) to polarise naive T helper (Th) function.[26,29] 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.[8]

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.[30–34]

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[35] and reactive oxygen species,[36] 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.[37] In addition, TLR activation regulates the expression of major histocompatibility complex (MHC) molecules and co-stimulatory molecules,[38] and induces the release of IL-12 and IL-10, cytokines which differentially alert DCs to polarise naive T cells and activate specific adaptive immunity.[39]

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 helminths and are the major effectors of IgE-associated allergic disorders,[40] 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.[41–47] TLR-mediated activation of mast cells induces production of chemokines and Th2 cytokines, which can also be accompanied by degranulation.[46–49] 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.[50]

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.[51–53]

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.[54,55]

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.[56] TLR3 is expressed on the cell surface, where it functions as a receptor independently of lysosomes, whereas TLR7/8 function requires the participation of lysosomes, as do other cell types.[57]

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.[57] 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.[58]

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.[59–62]

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-4 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.[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.[97–100] TLRs expressed on T cells seem to enhance cell proliferation, adhesion, and survival, although they also modulate cytokine production.[98] In conventional human and murine αβT cells, TLR2, TLR5, TLR7, and TLR9 act as co-stimulatory receptors in concert with a T-cell receptor signal, rather than by inducing a direct cellular response. Human alternative γδT cells also express mRNA for various TLR2 and TLR3, and human CD8+ cells express TLR3 as a functional co-receptor.[101] There is also evidence that the naturally occurring Treg cells can be directly regulated by TLR2, TLR5, 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.[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.[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.[97,104] In addition, binding of TLR in B cells stimulates proliferation, the release of immunoglobulin, and the production of chemokines.[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.[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.[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 haematopoiesis, regulate the stem-cell niche, and may participate in the repair of tissue damage inflicted by normal wear and tear, injury, or disease.[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.[3,108,110] Accordingly, modulation of TLRs offers new therapeutical 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.[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-κB in TLR signalling.[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.[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.[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 IκBα, a protein that prevents NF-κB translocation to the nucleus. This mutation is gain-of-function, as it enhances the inhibitory capacity of IκBα which results in impaired NF-κB activation. Clinical manifestations overlap with EDA-ID.[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.[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.[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.[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.[123]

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, bacteraemia, malaria, and tuberculosis[112]; a different TIRAP polymorphism (C558T) is linked with increased susceptibility to meningeal tuberculosis.[124]

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.[125–127] In addition, some viruses have evolved mechanisms to block TIR adaptors, thus limiting TLR signalling and modulating the immune response.[128]

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.[129,130]

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 retention and subsequent posterior modification promotes chronic inflammation.[131] The precise triggers for endothelial damage in atherosclerosis have not been defined, but exposure of the arterial wall to risk factors such as oxidated 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.[132–134]

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.[135] 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.[14,136]

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.[137] 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.[138]

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.[139] 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.[140,141]

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.[26] 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.[142,143] 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.[144]

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.[25,29]

In addition to DCs, other cells that participate in the induction and control of allergic reaction, such as mast cells, mononuclear phagocytes and T and B lymphocytes, also express TLRs that are activated by microbial antigens. In this way, the presence of pathogens can modulate the allergic response.

Therefore, understanding the regulatory role of TLRs in the pathogenesis of allergic inflammation may help to improve inflammation control in allergic patients.[145,146] There is experimental evidence that modulation of DCs by TLR ligands could be used to prevent and cure allergy. Thus, TLR2 has been reported to cooperate with IFN-γ to reverse the Th2 skew in an in vitro allergy model.[89,147] Under certain conditions, TLR stimulation, especially via TLR9, reduces Th2-dependent allergic inflammation through induction of Th1 responses and could prove useful in the treatment of allergic diseases, whereas other TLR ligands appear less attractive.[146] Modulation of DCs to induce a tolerant state mediated by Tregs is currently seen as a useful therapeutic option to avoid this aberrant immune response.[140,148] The potential of TLR ligands as a novel class of pharmaceutical tool for the prevention or treatment of allergic disorders is currently being analysed.[71,149]

TLRs in autoimmunity

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-α induced by endocytosed self-DNA and self-RNA through TLRs is considered a key event in the pathogenesis of SLE.[151]

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.[158–160] 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 immunity[164] and that the increased efficiency of adjunctive treatment with the TLR-7 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.[160,166] Many of these therapies are currently being evaluated in

clinical trials.[96,159,167,168] However, although TLR-based therapies have enormous biological potential and offer promising results, their benefits are not free of risk,[161,163,169–172] 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

1. Barton GM. A calculated response: control of inflammation by the innate immune system. J Clin Invest. 2008;118:413–20.
2. Joffre O, Nolte MA, Sporri R, Reis e Sousa C. Inflammatory signals in dendritic cell activation and the induction of adaptive immunity. Immunol Rev. 2009;227:234–47.
3. Rakoff-Nahoum S, Medzhitov R. Role of toll-like receptors in tissue repair and tumorigenesis. Biochemistry (Mosc). 2008;73:555–61.
4. Chen K, Huang J, Gong W, Iribarren P, Dunlop NM, Wang JM. Toll-like receptors in inflammation, infection and cancer. Int Immunopharmacol. 2007;7:1271–85.
5. Atkinson TJ. Toll-like receptors, transduction-effector pathways, and disease diversity: evidence of an immunobiological paradigm explaining all human illness? Int Rev Immunol. 2008;27:255–81.
6. Misch EA, Hawn TR. Toll-like receptor polymorphisms and susceptibility to human disease. Clin Sci (Lond). 2008;114:347–60.
7. Montero Vega MT, de Andres Martin A. Toll-like receptors: a family of innate sensors of danger that alert and drive immunity. Allergol Immunopathol (Madr). 2008;36:347–57.
8. Ritter M, Mennerich D, Weith A, Seither P. Characterization of Toll-like receptors in primary lung epithelial cells: strong impact of the TLR3 ligand poly(I:C) on the regulation of Toll-like receptors, adaptor proteins and inflammatory response. J Inflamm (Lond). 2005;2:16.
9. Song J, Abraham SN. TLR-mediated immune responses in the urinary tract. Curr Opin Microbiol. 2008;11:66–73.
10. Gribar SC, Richardson WM, Sodhi CP, Hackam DJ. No longer an innocent bystander: epithelial toll-like receptor signaling in the development of mucosal inflammation. Mol Med. 2008;14:645–59.
11. Fritz JH, Le Bourhis L, Magalhaes JG, Philpott DJ. Innate immune recognition at the epithelial barrier drives adaptive immunity: APCs take the back seat. Trends Immunol. 2008;29:41–9.
12. Neish AS. Microbes in gastrointestinal health and disease. Gastroenterology. 2009;136:65–80.
13. Rakoff-Nahoum S, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. Cell. 2004;118:229–41.
14. Harris G, KuoLee R, Chen W. Role of Toll-like receptors in health and diseases of gastrointestinal tract. World J Gastroenterol. 2006;12:2149–60.
15. Michelsen KS, Arditi M. Toll-like receptors and innate immunity in gut homeostasis and pathology. Curr Opin Hematol. 2007;14:48–54.
16. Stenson WF. Toll-like receptors and intestinal epithelial repair. Curr Opin Gastroenterol. 2008;24:103–7.
17. Cario E, Brown D, McKee M, Lynch-Devaney K, Gerken G, Podolsky DK. Commensal-associated molecular patterns induce selective toll-like receptor-trafficking from apical membrane to cytoplasmic compartments in polarized intestinal epithelium. Am J Pathol. 2002;160:165–73.
18. Ortega-Cava CF, Ishihara S, Rumi MA, Kawashima K, Ishimura N, Kazumori H, et al. Strategic compartmentalization of Toll-like receptor 4 in the mouse gut. J Immunol. 2003;170:3977–85.
19. Abreu MT, Thomas LS, Arnold ET, Lukasek K, Michelsen KS, Arditi M. TLR signaling at the intestinal epithelial interface. J Endotoxin Res. 2003;9:322–30.
20. Hornef MW, Normark BH, Vandewalle A, Normark S. Intracellular recognition of lipopolysaccharide by toll-like receptor 4 in intestinal epithelial cells. J Exp Med. 2003;198:1225–35.
21. Otte JM, Cario E, Podolsky DK. Mechanisms of cross hyporesponsiveness to Toll-like receptor bacterial ligands in intestinal epithelial cells. Gastroenterology. 2004;126:1054–70.
22. Furrie E, Macfarlane S, Thomson G, Macfarlane GT. Toll-like receptors-2, -3 and -4 expression patterns on human colon and their regulation by mucosal-associated bacteria. Immunology. 2005;115:565–74.
23. Lee J, Gonzales-Navajas JM, Raz E. The "polarizing-tolerizing" mechanism of intestinal epithelium: its relevance to colonic homeostasis. Semin Immunopathol. 2008;30:3–9.
24. Shibolet O, Podolsky DK. TLRs in the Gut. IV. Negative regulation of Toll-like receptors and intestinal homeostasis: addition by subtraction. Am J Physiol Gastrointest Liver Physiol. 2007;292:G1469–73.
25. Gon Y. Toll-like receptors and airway inflammation. Allergol Int. 2008;57:33–7.
26. Wang Y, Bai C, Li K, Adler KB, Wang X. Role of airway epithelial cells in development of asthma and allergic rhinitis. Respir Med. 2008.
27. Sha Q, Truong-Tran AQ, Plitt JR, Beck LA, Schleimer RP. Activation of airway epithelial cells by toll-like receptor agonists. Am J Respir Cell Mol Biol. 2004;31:358–64.
28. Koff JL, Shao MX, Ueki IF, Nadel JA. Multiple TLRs activate EGFR via a signaling cascade to produce innate immune responses in airway epithelium. Am J Physiol Lung Cell Mol Physiol. 2008.
29. Holgate ST. The epithelium takes centre stage in asthma and atopic dermatitis. Trends Immunol. 2007;28:248–51.
30. Into T, Kanno Y, Dohkan J, Nakashima M, Inomata M, Shibata K, et al. Pathogen recognition by Toll-like receptor 2 activates

Weibel-Palade body exocytosis in human aortic endothelial cells. J Biol Chem. 2007;282:8134–41.
31. Erridge C, Spickett CM, Webb DJ. Non-enterobacterial endotoxins stimulate human coronary artery but not venous endothelial cell activation via Toll-like receptor 2. Cardiovasc Res. 2007;73: 181–9.
32. Breslin JW, Wu MH, Guo M, Reynoso R, Yuan SY. Toll-like receptor 4 contributes to microvascular inflammation and barrier dysfunction in thermal injury. Shock. 2008;29:349–55.
33. Pegu A, Qin S, Fallert Junecko BA, Nisato RE, Pepper MS, Reinhart TA. Human Lymphatic Endothelial Cells Express Multiple Functional TLRs. J Immunol. 2008;180:3399–405.
34. Fitzner N, Clauberg S, Essmann F, Liebmann J, Kolb-Bachofen V. Human skin endothelial cells can express all 10 TLR genes and respond to respective ligands. Clin Vaccine Immunol. 2008;15: 138–46.
35. Qi HY, Shelhamer JH. Toll-like receptor 4 signaling regulates cytosolic phospholipase A2 activation and lipid generation in lipopolysaccharide-stimulated macrophages. J Biol Chem. 2005;280:38969–75.
36. Yang CS, Shin DM, Lee HM, Son JW, Lee SJ, Akira S, et al. ASK1-p38 MAPK-p47phox activation is essential for inflammatory responses during tuberculosis via TLR2-ROS signalling. Cell Microbiol. 2008;10:741–54.
37. Zarember KA, Godowski PJ. Tissue expression of human Toll-like receptors and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. J Immunol. 2002;168:554–61.
38. McCoy CE, O'Neill LA. The role of toll-like receptors in macrophages. Front Biosci. 2008;13:62–70.
39. Ozato K, Tsujimura H, Tamura T. Toll-like receptor signaling and regulation of cytokine gene expression in the immune system. Biotechniques. 2002:66–8 70, 72 passim.
40. Chi DS, Walker ES, Hossler FE, Krishnaswamy G. Bacterial activation of mast cells. Methods Mol Biol. 2006;315:383–92.
41. Supajatura V, Ushio H, Nakao A, Akira S, Okumura K, Ra C, et al. Differential responses of mast cell Toll-like receptors 2 and 4 in allergy and innate immunity. J Clin Invest. 2002;109: 1351–1359.
42. Varadaradjalou S, Feger F, Thieblemont N, Hamouda NB, Pleau JM, Dy M, et al. Toll-like receptor 2 (TLR2) and TLR4 differentially activate human mast cells. Eur J Immunol. 2003;33:899–906.
43. Kulka M, Alexopoulou L, Flavell RA, Metcalfe DD. Activation of mast cells by double-stranded RNA: evidence for activation through Toll-like receptor 3. J Allergy Clin Immunol. 2004;114: 174–82.
44. Orinska Z, Bulanova E, Budagian V, Metz M, Maurer M, Bulfone-Paus S. TLR3-induced activation of mast cells modulates CD8+ T-cell recruitment. Blood. 2005;106:978–87.
45. Dawicki W, Marshall JS. New and emerging roles for mast cells in host defence. Curr Opin Immunol. 2007;19:31–8.
46. Mortaz E, Redegeld FA, Dunsmore K, Odoms K, Wong HR, Nijkamp FP, et al. Stimulation of cysteinyl leukotriene production in mast cells by heat shock and acetylsalicylic acid. Eur J Pharmacol. 2007;561:214–9.
47. Yamashita M, Nakayama T. Progress in allergy signal research on mast cells: regulation of allergic airway inflammation through toll-like receptor 4-mediated modification of mast cell function. J Pharmacol Sci. 2008;106:332–5.
48. Nigo YI, Yamashita M, Hirahara K, Shinnakasu R, Inami M, Kimura M, et al. Regulation of allergic airway inflammation through Toll-like receptor 4-mediated modification of mast cell function. Proc Natl Acad Sci U S A. 2006;103:2286–91.
49. Kikawada E, Bonventre JV, Arm JP. Group V secretory PLA2 regulates TLR2-dependent eicosanoid generation in mouse mast cells through amplification of ERK and cPLA2alpha activation. Blood. 2007;110:561–7.
50. Qiao H, Andrade MV, Lisboa FA, Morgan K, Beaven MA. FcepsilonR1 and toll-like receptors mediate synergistic signals to markedly augment production of inflammatory cytokines in murine mast cells. Blood. 2006;107:610–8.
51. Muzio M, Bosisio D, Polentarutti N, D'Amico G, Stoppacciaro A, Mancinelli R, et al. Differential expression and regulation of toll-like receptors (TLR) in human leukocytes: selective expression of TLR3 in dendritic cells. J Immunol. 2000;164: 5998–6004.
52. Parker LC, Whyte MK, Dower SK, Sabroe I. The expression and roles of Toll-like receptors in the biology of the human neutrophil. J Leukoc Biol. 2005;77:886–92.
53. Hattermann K, Picard S, Borgeat M, Leclerc P, Pouliot M, Borgeat P. The Toll-like receptor 7/8-ligand resiquimod (R-848) primes human neutrophils for leukotriene B4, prostaglandin E2 and platelet-activating factor biosynthesis. Faseb J. 2007;21: 1575–85.
54. Nagase H, Okugawa S, Ota Y, Yamaguchi M, Tomizawa H, Matsushima K, et al. Expression and function of Toll-like receptors in eosinophils: activation by Toll-like receptor 7 ligand. J Immunol. 2003;171:3977–82.
55. Wong CK, Cheung PF, Ip WK, Lam CW. Intracellular signaling mechanisms regulating toll-like receptor-mediated activation of eosinophils. Am J Respir Cell Mol Biol. 2007;37:85–96.
56. Lauzon NM, Mian F, MacKenzie R, Ashkar AA. The direct effects of Toll-like receptor ligands on human NK cell cytokine production and cytotoxicity. Cell Immunol. 2006;241:102–12.
57. Hart OM, Athie-Morales V, O'Connor GM, Gardiner CM. TLR7/8-mediated activation of human NK cells results in accessory cell-dependent IFN-gamma production. J Immunol. 2005;175: 1636–42.
58. Sawaki J, Tsutsui H, Hayashi N, Yasuda K, Akira S, Tanizawa T, et al. Type 1 cytokine/chemokine production by mouse NK cells following activation of their TLR/MyD88-mediated pathways. Int Immunol. 2007;19:311–20.
59. Boyd JH, Divangahi M, Yahiaoui L, Gvozdic D, Qureshi S, Petrof BJ. Toll-like receptors differentially regulate CC and CXC chemokines in skeletal muscle via NF-kappaB and calcineurin. Infect Immun. 2006;74:6829–38.
60. Sukkar MB, Xie S, Khorasani NM, Kon OM, Stanbridge R, Issa R, et al. Toll-like receptor 2, 3, and 4 expression and function in human airway smooth muscle. J Allergy Clin Immunol. 2006;118:641–8.
61. Miller LS, Modlin RL. Toll-like receptors in the skin. Semin Immunopathol. 2007;29:15–26.
62. Kopp A, Buechler C, Neumeier M, Weigert J, Aslanidis C, Scholmerich J, et al. Innate Immunity and Adipocyte Function: Ligand-specific Activation of Multiple Toll-like Receptors Modulates Cytokine, Adipokine, and Chemokine Secretion in Adipocytes. Obesity (Silver Spring). 2009;17:648–56.
63. Foti M, Granucci F, Ricciardi-Castagnoli P. Dendritic cell interactions and cytokine production. Ernst Schering Res Found Workshop. 2006:61–80.
64. Steinman RM, Hemmi H. Dendritic cells: translating innate to adaptive immunity. Curr Top Microbiol Immunol. 2006;311: 17–58.
65. Sato K, Fujita S. Dendritic cells: nature and classification. Allergol Int. 2007;56:183–91.
66. Lee HK, Iwasaki A. Innate control of adaptive immunity: dendritic cells and beyond. Semin Immunol. 2007;19:48–55.
67. Iwasaki A, Medzhitov R. Toll-like receptor control of the adaptive immune responses. Nat Immunol. 2004;5:987–95.
68. Duez C, Gosset P, Tonnel AB. Dendritic cells and toll-like receptors in allergy and asthma. Eur J Dermatol. 2006 12–6.
69. Blander JM. Coupling Toll-like receptor signaling with phagocytosis: potentiation of antigen presentation. Trends Immunol. 2007;28:19–25.

70. Watts C, Zaru R, Prescott AR, Wallin RP, West MA. Proximal effects of Toll-like receptor activation in dendritic cells. Curr Opin Immunol. 2007;19:73–8.
71. Goldman M. Translational mini-review series on Toll-like receptors: Toll-like receptor ligands as novel pharmaceuticals for allergic disorders. Clin Exp Immunol. 2007;147:208–16.
72. van Vliet SJ, den Dunnen J, Gringhuis SI, Geijtenbeek TB, van Kooyk Y. Innate signaling and regulation of Dendritic cell immunity. Curr Opin Immunol. 2007;19:435–40.
73. Hochrein H, O'Keeffe M. Dendritic cell subsets and toll-like receptors. Handb Exp Pharmacol. 2008:153–79.
74. Liotta F, Frosali F, Querci V, Mantei A, Fili L, Maggi L, et al. Human immature myeloid dendritic cells trigger a TH2-polarizing program via Jagged-1/Notch interaction. J Allergy Clin Immunol. 2008;121:1000–5 e8.
75. Benko S, Magyarics Z, Szabo A, Rajnavolgyi E. Dendritic cell subtypes as primary targets of vaccines: the emerging role and cross-talk of pattern recognition receptors. Biol Chem. 2008.
76. Visintin A, Mazzoni A, Spitzer JH, Wyllie DH, Dower SK, Segal DM. Regulation of Toll-like receptors in human monocytes and dendritic cells. J Immunol. 2001;166:249–55.
77. Hoyne GF, Le Roux I, Corsin-Jimenez M, Tan K, Dunne J, Forsyth LM, et al. Serrate1-induced notch signalling regulates the decision between immunity and tolerance made by peripheral CD4(+) T cells. Int Immunol. 2000;12:177–85.
78. Pearce EJ, Kane CM, Sun J. Regulation of dendritic cell function by pathogen-derived molecules plays a key role in dictating the outcome of the adaptive immune response. Chem Immunol Allergy. 2006;90:82–90.
79. Perrigoue JG, Marshall F, Artis D. On the hunt for helminths: Innate immune cells in the recognition and response to helminth parasites. Cell Microbiol. 2008.
80. Napolitani G, Rinaldi A, Bertoni F, Sallusto F, Lanzavecchia A. Selected Toll-like receptor agonist combinations synergistically trigger a T helper type 1-polarizing program in dendritic cells. Nat Immunol. 2005;6:769–76.
81. Skokos D, Nussenzweig MC. CD8- DCs induce IL-12-independent Th1 differentiation through Delta 4 Notch-like ligand in response to bacterial LPS. J Exp Med. 2007;204:1525–31.
82. Raymond T, Schaller M, Hogaboam CM, Lukacs NW, Rochford R, Kunkel SL. Toll-like receptors, Notch ligands, and cytokines drive the chronicity of lung inflammation. Proc Am Thorac Soc. 2007;4:635–41.
83. Sun J, Krawczyk CJ, Pearce EJ. Suppression of Th2 cell development by Notch ligands Delta1 and Delta4. J Immunol. 2008;180:1655–61.
84. Weaver CT, Harrington LE, Mangan PR, Gavrieli M, Murphy KM. Th17: an effector CD4T cell lineage with regulatory T cell ties. Immunity. 2006;24:677–88.
85. Kato A, Favoreto Jr S, Avila PC, Schleimer RP. TLR3- and Th2 cytokine-dependent production of thymic stromal lymphopoietin in human airway epithelial cells. J Immunol. 2007;179:1080–7.
86. Akdis CA, Kussebi F, Pulendran B, Akdis M, Lauener RP, Schmidt-Weber CB, et al. Inhibition of T helper 2-type responses, IgE production and eosinophilia by synthetic lipopeptides. Eur J Immunol. 2003;33:2717–26.
87. Sieling PA, Chung W, Duong BT, Godowski PJ, Modlin RL. Toll-like receptor 2 ligands as adjuvants for human Th1 responses. J Immunol. 2003;170:194–200.
88. Imanishi T, Hara H, Suzuki S, Suzuki N, Akira S, Saito T. Cutting edge: TLR2 directly triggers Th1 effector functions. J Immunol. 2007;178:6715–9.
89. Taylor RC, Richmond P, Upham JW. Toll-like receptor 2 ligands inhibit TH2 responses to mite allergen. J Allergy Clin Immunol. 2006;117:1148–54.
90. Mandron M, Aries MF, Brehm RD, Tranter HS, Acharya KR, Charveron M, et al. Human dendritic cells conditioned with Staphylococcus aureus enterotoxin B promote TH2 cell polarization. J Allergy Clin Immunol. 2006;117:1141–7.
91. Vanden Eijnden S, Goriely S, De Wit D, Goldman M, Willems F. Preferential production of the IL-12(p40)/IL-23(p19) heterodimer by dendritic cells from human newborns. Eur J Immunol. 2006;36:21–6.
92. Fitzgerald-Bocarsly P, Dai J, Singh S. Plasmacytoid dendritic cells and type I IFN: 50 years of convergent history. Cytokine Growth Factor Rev. 2008;19:3–19.
93. Ito T, Kanzler H, Duramad O, Cao W, Liu YJ. Specialization, kinetics, and repertoire of type 1 interferon responses by human plasmacytoid predendritic cells. Blood. 2006;107:2423–31.
94. Cao W, Liu YJ. Innate immune functions of plasmacytoid dendritic cells. Curr Opin Immunol. 2007;19:24–30.
95. Biron CA. Interferons alpha and beta as immune regulators – a new look. Immunity. 2001;14:661–4.
96. Dorn A, Kippenberger S. Clinical application of CpG-, non-CpG-, and antisense oligodeoxynucleotides as immunomodulators. Curr Opin Mol Ther. 2008;10:10–20.
97. Fillatreau S, Manz RA. Tolls for B cells. Eur J Immunol. 2006;36:798–801.
98. Kabelitz D. Expression and function of Toll-like receptors in T lymphocytes. Curr Opin Immunol. 2007;19:39–45.
99. LaRosa DF, Stumhofer JS, Gelman AE, Rahman AH, Taylor DK, Hunter CA, et al. T cell expression of MyD88 is required for resistance to Toxoplasma gondii. Proc Natl Acad Sci U S A. 2008;105:3855–60.
100. van Maren WW, Jacobs JF, de Vries IJ, Nierkens S, Adema GJ. Toll-like receptor signalling on Tregs: to suppress or not to suppress? Immunology. 2008;124:445–52.
101. Tabiasco J, Devevre E, Rufer N, Salaun B, Cerottini JC, Speiser D, et al. Human effector CD8+ T lymphocytes express TLR3 as a functional coreceptor. J Immunol. 2006;177:8708–13.
102. Kabelitz D, Wesch D, Oberg HH. Regulation of regulatory T cells: role of dendritic cells and toll-like receptors. Crit Rev Immunol. 2006;26:291–306.
103. Pasare C, Medzhitov R. Toll-like receptors and acquired immunity. Semin Immunol. 2004;16:23–6.
104. Ruprecht CR, Lanzavecchia A. Toll-like receptor stimulation as a third signal required for activation of human naive B cells. Eur J Immunol. 2006;36:810–6.
105. Bernasconi NL, Onai N, Lanzavecchia A. A role for Toll-like receptors in acquired immunity: up-regulation of TLR9 by BCR triggering in naive B cells and constitutive expression in memory B cells. Blood. 2003;101:4500–4.
106. Bourke E, Bosisio D, Golay J, Polentarutti N, Mantovani A. The toll-like receptor repertoire of human B lymphocytes: inducible and selective expression of TLR9 and TLR10 in normal and transformed cells. Blood. 2003;102:956–63.
107. Miyake K. Innate immune sensing of pathogens and danger signals by cell surface Toll-like receptors. Semin Immunol. 2007;19:3–10.
108. Kluwe J, Mencin A, Schwabe RF. Toll-like receptors, wound healing, and carcinogenesis. J Mol Med. 2009;87:125–38.
109. Pevsner-Fischer M, Morad V, Cohen-Sfady M, Rousso-Noori L, Zanin-Zhorov A, Cohen S, et al. Toll-like receptors and their ligands control mesenchymal stem cell functions. Blood. 2007;109:1422–32.
110. Kim S, Takahashi H, Lin WW, Descargues P, Grivennikov S, Kim Y, et al. Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. Nature. 2009;457:102–6.
111. Turvey SE, Hawn TR. Towards subtlety: understanding the role of Toll-like receptor signaling in susceptibility to human infections. Clin Immunol. 2006;120:1–9.
112. Khor CC, Chapman SJ, Vannberg FO, Dunne A, Murphy C, Ling EY, et al. A Mal functional variant is associated with protection

against invasive pneumococcal disease, bacteremia, malaria and tuberculosis. Nat Genet. 2007;39:523–8.
113. Sebban H, Courtois G. NF-kappaB and inflammation in genetic disease. Biochem Pharmacol. 2006;72:1153–60.
114. Smahi A, Courtois G, Vabres P, Yamaoka S, Heuertz S, Munnich A, et al. The International Incontinentia Pigmenti (IP) Consortium. Genomic rearrangement in NEMO impairs NF-kappaB activation and is a cause of incontinentia pigmenti. Nature. 2000;405:466–72.
115. Zonana J, Elder ME, Schneider LC, Orlow SJ, Moss C, Golabi M, et al. A novel X-linked disorder of immune deficiency and hypohidrotic ectodermal dysplasia is allelic to incontinentia pigmenti and due to mutations in IKK-gamma (NEMO). Am J Hum Genet. 2000;67:1555–62.
116. Doffinger R, Smahi A, Bessia C, Geissmann F, Feinberg J, Durandy A, et al. X-linked anhidrotic ectodermal dysplasia with immunodeficiency is caused by impaired NF-kappaB signaling. Nat Genet. 2001;27:277–85.
117. Courtois G, Smahi A, Reichenbach J, Doffinger R, Cancrini C, Bonnet M, et al. A hypermorphic IkappaBalpha mutation is associated with autosomal dominant anhidrotic ectodermal dysplasia and T cell immunodeficiency. J Clin Invest. 2003;112: 1108–15.
118. Picard C, Puel A, Bonnet M, Ku CL, Bustamante J, Yang K, et al. Pyogenic bacterial infections in humans with IRAK-4 deficiency. Science. 2003;299:2076–9.
119. Day N, Tangsinmankong N, Ochs H, Rucker R, Picard C, Casanova JL, et al. Interleukin receptor-associated kinase (IRAK-4) deficiency associated with bacterial infections and failure to sustain antibody responses. J Pediatr. 2004;144: 524–6.
120. Enders A, Pannicke U, Berner R, Henneke P, Radlinger K, Schwarz K, et al. Two siblings with lethal pneumococcal meningitis in a family with a mutation in Interleukin-1 receptor-associated kinase 4. J Pediatr. 2004;145:698–700.
121. Chapel H, Puel A, von Bernuth H, Picard C, Casanova JL. Shigella sonnei meningitis due to interleukin-1 receptor-associated kinase-4 deficiency: first association with a primary immune deficiency. Clin Infect Dis. 2005;40:1227–31.
122. Ku CL, Picard C, Erdos M, Jeurissen A, Bustamante J, Puel A, et al. IRAK4 and NEMO mutations in otherwise healthy children with recurrent invasive pneumococcal disease. J Med Genet. 2007;44:16–23.
123. Weighardt H, Holzmann B. Role of Toll-like receptor responses for sepsis pathogenesis. Immunobiology. 2007;212:715–22.
124. Hawn TR, Dunstan SJ, Thwaites GE, Simmons CP, Thuong NT, Lan NT, et al. A polymorphism in Toll-interleukin 1 receptor domain containing adaptor protein is associated with susceptibility to meningeal tuberculosis. J Infect Dis. 2006;194: 1127–34.
125. Netea MG, van der Graaf C, Van der Meer JW, Kullberg BJ. Toll-like receptors and the host defense against microbial pathogens: bringing specificity to the innate-immune system. J Leukoc Biol. 2004;75:749–55.
126. Gehring AJ, Rojas RE, Canaday DH, Lakey DL, Harding CV, Boom WH. The Mycobacterium tuberculosis 19-kilodalton lipoprotein inhibits gamma interferon-regulated HLA-DR and Fc gamma R1 on human macrophages through Toll-like receptor 2. Infect Immun. 2003;71:4487–97.
127. Netea MG, Sutmuller R, Hermann C, Van der Graaf CA, Van der Meer JW, van Krieken JH, et al. Toll-like receptor 2 suppresses immunity against Candida albicans through induction of IL-10 and regulatory T cells. J Immunol. 2004;172: 3712–8.
128. DiPerna G, Stack J, Bowie AG, Boyd A, Kotwal G, Zhang Z, et al. Poxvirus protein N1L targets the I-kappaB kinase complex, inhibits signaling to NF-kappaB by the tumor necrosis actor superfamily of receptors, and inhibits NF-kappaB and IRF3 signaling by toll-like receptors. J Biol Chem. 2004;279: 36570–36578.
129. Mitchell JA, Ryffel B, Quesniaux VF, Cartwright N, Paul-Clark M. Role of pattern-recognition receptors in cardiovascular health and disease. Biochem Soc Trans. 2007;35:1449–52.
130. Frantz S, Ertl G, Bauersachs J. Mechanisms of disease: Toll-like receptors in cardiovascular disease. Nat Clin Pract Cardiovasc Med. 2007;4:444–54.
131. Tabas I, Williams KJ, Boren J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. Circulation. 2007;116:1832–44.
132. Tobias PS, Curtiss LK. Toll-like receptors in atherosclerosis. Biochem Soc Trans. 2007;35:1453–5.
133. Schoneveld AH, Hoefer I, Sluijter JP, Laman JD, de Kleijn DP, Pasterkamp G. Atherosclerotic lesion development and Toll like receptor 2 and 4 responsiveness. Atherosclerosis. 2008;197:95–104.
134. Curtiss LK, Tobias PS. Emerging role of toll-like receptors in atherosclerosis. J Lipid Res. 2008.
135. Strober W. Immunology. Unraveling gut inflammation. Science. 2006;313:1052–4.
136. Yamamoto-Furusho JK, Podolsky DK. Innate immunity in inflammatory bowel disease. World J Gastroenterol. 2007;13:5577–80.
137. Pierik M, Joossens S, Van Steen K, Van Schuerbeek N, Vlietinck R, Rutgeerts P, et al. Toll-like receptor-1, -2, and -6 polymorphisms influence disease extension in inflammatory bowel diseases. Inflamm Bowel Dis. 2006;12:1–8.
138. Obermeier F, Dunger N, Strauch UG, Hofmann C, Bleich A, Grunwald N, et al. CpG motifs of bacterial DNA essentially contribute to the perpetuation of chronic intestinal inflammation. Gastroenterology. 2005;129:913–27.
139. Borm ME, van Bodegraven AA, Mulder CJ, Kraal G, Bouma G. The effect of NOD2 activation on TLR2-mediated cytokine responses is dependent on activation dose and NOD2 genotype. Genes Immun. 2008;9:274–8.
140. Larche M. Regulatory T cells in allergy and asthma. Chest. 2007;132:1007–14.
141. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy. J Allergy Clin Immunol. 2007;119:780–91.
142. Prioult G, Nagler-Anderson C. Mucosal immunity and allergic responses: lack of regulation and/or lack of microbial stimulation? Immunol Rev. 2005;206:204–18.
143. Renz H, Blumer N, Virna S, Sel S, Garn H. The immunological basis of the hygiene hypothesis. Chem Immunol Allergy. 2006;91:30–48.
144. Maggi L, Santarlasci V, Liotta F, Frosali F, Angeli R, Cosmi L, et al. Demonstration of circulating allergen-specific CD4+CD25high-Foxp3+ T-regulatory cells in both nonatopic and atopic individuals. J Allergy Clin Immunol. 2007;120:429–36.
145. Horner AA. Update on toll-like receptor ligands and allergy: implications for immunotherapy. Curr Allergy Asthma Rep. 2006;6:395–401.
146. Iwamura C, Nakayama T. Toll-like receptors in the respiratory system: their roles in inflammation. Curr Allergy Asthma Rep. 2008;8:7–13.
147. Weigt H, Muhlradt PF, Larbig M, Krug N, Braun A. The Toll-like receptor-2/6 agonist macrophage-activating lipopeptide-2 cooperates with IFN-gamma to reverse the Th2 skew in an in vitro allergy model. J Immunol. 2004;172:6080–6.
148. Vigouroux S, Yvon E, Biagi E, Brenner MK. Antigen-induced regulatory T cells. Blood. 2004;104:26–33.
149. Kuipers H, Lambrecht BN. Modification of dendritic cell function as a tool to prevent and treat allergic asthma. Vaccine. 2005;23:4577–88.
150. Wagner H. Endogenous TLR ligands and autoimmunity. Adv Immunol. 2006;91:159–73.
151. Marshak-Rothstein A. Toll-like receptors in systemic autoimmune disease. Nat Rev Immunol. 2006;6:823–35.

152. Krug A. Nucleic acid recognition receptors in autoimmunity. Handb Exp Pharmacol. 2008:129–51.
153. Huang B, Zhao J, Unkeless JC, Feng ZH, Xiong H. TLR signaling by tumor and immune cells: a double-edged sword. Oncogene. 2008;27:218–24.
154. Huang B, Zhao J, Li H, He KL, Chen Y, Chen SH, et al. Toll-like receptors on tumor cells facilitate evasion of immune surveillance. Cancer Res. 2005;65:5009–14.
155. Harmey JH, Bucana CD, Lu W, Byrne AM, McDonnell S, Lynch C, et al. Lipopolysaccharide-induced metastatic growth is associated with increased angiogenesis, vascular permeability and tumor cell invasion. Int J Cancer. 2002;101:415–22.
156. Wang JH, Manning BJ, Wu QD, Blankson S, Bouchier-Hayes D, Redmond HP. Endotoxin/lipopolysaccharide activates NF-kappa B and enhances tumor cell adhesion and invasion through a beta 1 integrin-dependent mechanism. J Immunol. 2003;170: 795–804.
157. Wang RF, Miyahara Y, Wang HY. Toll-like receptors and immune regulation: implications for cancer therapy. Oncogene. 2008;27:181–9.
158. Jurk M, Vollmer J. Therapeutic applications of synthetic CpG oligodeoxynucleotides as TLR9 agonists for immune modulation. BioDrugs. 2007;21:387–401.
159. Stoeter D, de Liguori Carino N, Marshall E, Poston GJ, Wu A. Extensive necrosis of visceral melanoma metastases after immunotherapy. World J Surg Oncol. 2008;6:30.
160. Parkinson T. The future of toll-like receptor therapeutics. Curr Opin Mol Ther. 2008;10:21–31.
161. Killeen SD, Wang JH, Andrews EJ, Redmond HP. Exploitation of the Toll-like receptor system in cancer: a doubled-edged sword? Br J Cancer. 2006;95:247–52.
162. Spaner DE, Foley R, Galipeau J, Bramson J. Obstacles to effective Toll-like receptor agonist therapy for hematologic malignancies. Oncogene. 2008;27:208–17.
163. Melief CJ. Cancer immunotherapy by dendritic cells. Immunity. 2008;29:372–83.
164. Roses RE, Xu M, Koski GK, Czerniecki BJ. Radiation therapy and Toll-like receptor signaling: implications for the treatment of cancer. Oncogene. 2008;27:200–7.
165. Bassukas ID, Gamvroulia C, Zioga A, Nomikos K, Fotika C. Cryosurgery during topical imiquimod: a successful combination modality for lentigo maligna. Int J Dermatol. 2008;47: 519–21.
166. Hong-Geller E, Chaudhary A, Lauer S. Targeting toll-like receptor signaling pathways for design of novel immune therapeutics. Curr Drug Discov Technol. 2008;5:29–38.
167. Krieg AM. Therapeutic potential of Toll-like receptor 9 activation. Nat Rev Drug Discov. 2006;5:471–84.
168. Agrawal S, Kandimalla ER. Synthetic agonists of Toll-like receptors 7, 8 and 9. Biochem Soc Trans. 2007;35:1461–7.
169. Krieg AM, Vollmer J. Toll-like receptors 7, 8, and 9: linking innate immunity to autoimmunity. Immunol Rev. 2007;220: 251–69.
170. Raimondi G, Turner MS, Thomson AW, Morel PA. Naturally occurring regulatory T cells: recent insights in health and disease. Crit Rev Immunol. 2007;27:61–95.
171. Bauer S, Pigisch S, Hangel D, Kaufmann A, Hamm S. Recognition of nucleic acid and nucleic acid analogs by Toll-like receptors 7, 8 and 9. Immunobiology. 2008;213:315–28.
172. Dayan CM, Wraith DC. Preparing for first-in-man studies: the challenges for translational immunology post-TGN1412. Clin Exp Immunol. 2008;151:231–4.