Innate immunity

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Recent years have witnessed an explosion of interest in the innate immune system. Questions about how the innate immune system senses infection and empowers a protective immune response are being answered at the molecular level. These basic science discoveries are being translated into a more complete understanding of the central role innate immunity plays in the pathogenesis of many human infectious and inflammatory diseases. It is particularly exciting that we are already seeing a return on these scientific investments with the emergence of novel therapies to harness the power of the innate immune system. In this review we explore the defining characteristics of the innate immune system, and through more detailed examples, we highlight recent breakthroughs that have advanced our understanding of the role of innate immunity in human health and disease. (J Allergy Clin Immunol 2010;125:S24-32.)

Key words: Host defense, innate immunity, Toll-like receptors, nucleotide oligomerization domain–like receptors

THE “NEW” SCIENCE OF INNATE IMMUNITY

The integrated human immune response has traditionally been divided into 2 branches: innate and adaptive (or acquired) immunity. Although appreciation of innate immunity dates back to at least the 1908 Nobel Prize–winning efforts of Ilya Mechnikov, until the last decade, study of innate immunity has been eclipsed by dramatic discoveries in the field of adaptive immunity. However, the recent molecular definition of how the innate immune system senses infection to empower protective immune responses has precipitated a renaissance in the field of innate immunity. Innate immunity has shed its older, disparaging title of “nonspecific immunity” and now stands as a proud partner to the adaptive immune system in protecting human hosts from infectious insults. For any who doubt the impressive protective capacity of the innate immune system, it is instructive to consider that only vertebrates boast the added benefits of an adaptive immune system, leaving most organisms on our planet to survive on innate immunity alone!

Although innate immunity is critical for host defense against infectious challenges, the innate immune system is emerging as a critical regulator of human inflammatory disease. Indeed, innate immune responses have been implicated in the development of asthma and atopy, as well as a variety of autoimmune disorders, including type 1 diabetes, inflammatory bowel disease, and systemic lupus erythematosus.

In this review we examine the basic structure of the innate immune system and how innate immunity interfaces with adaptive immune responses. We explore the role of innate immunity in human health and disease, and we outline how novel therapies can harness the beneficial capacity of the innate immune system. Rather than attempting to comprehensively review this enormously broad topic, our focus is on highlighting common defining mechanisms of innate immunity and illustrating the clinical relevance of innate immunity to human health. We have deliberately avoided a detailed exploration of the complement system because a separate Primer chapter is devoted to this important aspect of innate immunity.

ORGANIZATION OF THE HUMAN IMMUNE SYSTEM: THREE LEVELS OF HOST DEFENSE

The human microbial defense system can be simplistically viewed as consisting of 3 levels: (1) anatomic and physiologic barriers; (2) innate immunity; and (3) adaptive immunity (Fig 1 and Table 1). Failure in any of these systems will greatly increase susceptibility to infection.

Anatomic and physiologic barriers provide the crucial first line of defense against pathogens. These barriers include intact skin, vigorous mucociliary clearance mechanisms, low stomach pH, and bacteriolytic lysozyme in tears, saliva, and other secretions. The extreme susceptibility to infections observed in subjects with severe cutaneous burns or primary ciliary dyskinesia demonstrates that intact innate and adaptive immune systems are not able to compensate for failure of essential anatomic and physiologic barriers.

Abbreviations used

DAMP: Damage-associated molecular pattern
IPAF: IL-1β-converting enzyme (ICE) protease-activating factor
IRAK4: IL-1 receptor–associated kinase 4
MAL: MyD88 adapter-like
MPL: Monophosphoryl lipid A
MyD88: Myeloid differentiation primary response gene 88
NK: Natural killer
NLR: Nucleotide oligomerization domain–like receptor
NLRP3: NLR family, pyrin domain-containing 3
NOD: Nucleotide oligomerization domain
SNP: Single nucleotide polymorphism
TIR: Toll/IL-1 receptor–like domain
TIRAP: Toll/IL-1 receptor–like domain–containing adaptor protein
TLR: Toll-like receptor

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Innate immunity augments the protection offered by anatomic and physiologic barriers. The innate immune system relies on a limited repertoire of receptors to detect invading pathogens but compensates for this limited number of invariant receptors by targeting conserved microbial components that are shared by large groups of pathogens. Speed is a defining characteristic of the innate immune system: within minutes of pathogen exposure, the innate immune system starts generating a protective inflammatory response. Moreover, innate immunity plays a central role in activating the subsequent adaptive immune response.

T and B lymphocytes are the main self-defensive weapons of the adaptive immune system, so-named because this system is shaped by antigen exposure. In contrast to the limited number of pathogen receptors used by the innate immune system, the adaptive immune system boasts an extremely diverse, randomly generated repertoire of receptors. The benefit of this receptor diversity is that the adaptive immune system can recognize virtually any antigen, but there is a price for this diversity.

First is the risk of autoimmune disease. Receptors specific for self-proteins (eg, insulin and myelin) are created by means of the random process of gene rearrangement that generates receptors expressed by T and B cells. Consequently, elaborate tolerance mechanisms have evolved to eliminate or regulate self-reactive cells.

Second is the time delay required to generate a protective adaptive immune response after the first exposure to a pathogen. Adaptive immunity relies on a clonal system, with each T and B cell expressing its own unique receptor, and after the initial encounter with a pathogen, it takes up to 5 days for clonal expansion of these rare antigen-specific T and B cells to occur before the adaptive immune response is sufficiently robust to clear the pathogen.

**ELEMENTS OF THE INNATE IMMUNE SYSTEM**

In contrast to the adaptive immune system, which depends on T and B lymphocytes, innate immune protection is a task performed by cells of both hematopoietic and nonhematopoietic origin (Fig 1 and Table I). Hematopoietic cells involved in innate immune responses include macrophages, dendritic cells, mast cells, neutrophils, eosinophils, natural killer (NK) cells, and NK T cells. In addition to hematopoietic cells, innate immune responsiveness is a property of the skin and the epithelial cells lining the respiratory, gastrointestinal, and genitourinary tracts.

To augment these cellular defenses, innate immunity also has a humoral component that includes well-characterized components, such as complement proteins, LPS binding protein, C-reactive protein and other pentraxins, collectins, and antimicrobial peptides, including defensins. Circulating innate immune proteins are involved in both sensing of microbes and effector mechanisms to facilitate clearance of the infection. For example, mannose-binding lectin, a member of the collectin family of receptors, binds mannose-containing carbohydrates on microbes, triggering activation of the complement cascade, which enhances clearance of the pathogen.

**HOST DEFENSE IS ACHIEVED THROUGH INTEGRATION OF INNATE AND ADAPTIVE IMMUNITY**

Innate immunity, an evolutionarily ancient component of host defense, is present in all multicellular organisms, whereas adaptive immunity evolved much later and is only found in jawed fish and all “higher” vertebrates. During evolution, adaptive immunity developed in the context of a functioning innate immune
The innate immune system serves as the initial immune defense against foreign and dangerous material. In the most simplistic view, the innate immune system is hardwired with germline-encoded receptors to recognize "microbial nonself," conserved molecular structures that are expressed by a large variety of microbes. Charles Janeway coined the terms pattern recognition receptors to collectively describe these receptors and pathogen-associated molecular patterns (PAMPs) to denote the microbial structures recognized by the pattern recognition receptors. However, this terminology has been criticized as being vague; therefore in this review we will focus on naming specific receptors and their microbial ligands.

### INNATE IMMUNE RECOGNITION STRATEGIES

The innate immune system serves as the initial immune defense against foreign and dangerous material. In the most simplistic view, the innate immune system is hardwired with germline-encoded receptors for immediate responsiveness. In contrast to adaptive immunity, innate immune responses do not require genetic recombination events or a developmental phase to mediate function.

The strategy used for immune recognition is the main feature distinguishing innate and adaptive immunity. In contrast to the massive, randomly generated repertoire of antigen receptors expressed by T and B lymphocytes, the innate immune system relies on a limited number of genetically predetermined germline-encoded receptors that recognize either highly conserved structures expressed by large groups of microbes or common biologic consequences of infection. Pathogens can rapidly evolve and, in principle, could avoid detection by the innate immune system by simply altering the targeted microbial molecules. However, the innate immune system has evolved to recognize either microbial components that are essential for the viability and virulence of microbes and are thus less prone to modifications or common biologic consequences of infection.

At least 3 broad strategies are used by the innate immune system to recognize invading microorganisms (Table II). In the first innate immunity relies on a limited repertoire of germline-encoded receptors to recognize "microbial nonself," conserved molecular structures that are expressed by a large variety of microbes. Charles Janeway coined the terms pattern recognition receptors to collectively describe these receptors and pathogen-associated molecular patterns (PAMPs) to denote the microbial structures recognized by the pattern recognition receptors. However, this terminology has been criticized as being vague; therefore in this review we will focus on naming specific receptors and their microbial ligands.

A second approach used by the innate immune system is to detect immunologic danger in the form of damage-associated molecular patterns (DAMPs). DAMPs represent common metabolic consequences of infection and inflammation.
molecules that are upregulated and released during the cell lysis and tissue damage that occurs in the context of both infectious and sterile inflammation. Well-characterized DAMPs include high mobility group box 1 protein and other endogenous alarmins, heat shock proteins, and uric acid.

In the third innate immune recognition strategy, innate immune receptors detect “missing self,” molecules expressed by normal healthy cells but not expressed by infected cells or microbes. Recognition of these signals indicates that all is well, and an inhibitory signal is delivered to prevent activation of the immune response against host tissues. This inhibitory system is well illustrated by NK cells. Inhibitory receptors specific for self–MHC class I molecules play a central role in missing-self recognition by NK cells, ensuring NK cells preferentially attack infected cells that downregulate their MHC class I proteins.9

**ROLE OF THE INNATE IMMUNE SYSTEM IN HEALTH AND DISEASE**

We will now turn our attention to specific components of the innate immune system. We deliberately selected 2 illustrative examples, TLRs and nucleotide oligomerization domain (NOD)–like receptor (NLRs), for which our mechanistic understanding has increased considerably in the past 5 years and for which the clinical relevance of these systems is beginning to emerge.

**TLRs**

**Overview of TLR structure and function.** The recent explosion of interest in innate immunity was catalyzed in the mid-1990s when the *Drosophila* species protein Toll was shown to be critical for defending fruit flies against fungal infections.10 This observation opened the way for the subsequent description of similar proteins, called TLRs, in mammalian cells. The human TLR family consists of 10 receptors that are critically important for innate immunity.11,12 TLRs allow for recognition and response to diverse microbial epitopes on pathogens, enabling the innate immune system to discriminate among groups of pathogens and to induce an appropriate cascade of effector adaptive responses.

TLRs exist as dimeric proteins (either heterodimers or homodimers). The ectodomains of TLRs are composed of leucine-rich repeat motifs, whereas the cytosolic component, called a Toll/IL-1 receptor–like domain (TIR), is involved in signaling. Individual TLRs recognize a distinct but limited repertoire of conserved microbial products; for example, well-characterized receptor-ligand pairs include TLR4 and LPS, TLR5 and flagellin, and TLR1/TLR2/TLR6 and lipoproteins. Collectively, the complete TLR family allows the host to detect infection by most (if not all) types of microbial pathogens. For example, gram-positive organisms, such as *Streptococcus pneumoniae*, are initially recognized by TLR1, TLR2, TLR4, TLR6, and TLR9, which in turn interact with a range of downstream signaling molecules to activate an inflammatory cascade. TLR signaling pathways have been the focus of considerable attention (Fig 2).12,13 The emerging model has ligation of microbial products by TLRs culminating in the activation of nuclear factor κB, activator protein 1, interferon regulatory factor 3, and other transcription factors, driving the production of proinflammatory cytokines, maturation of dendritic cells, and other immunologic responses.

**Human disease resulting from TLR defects.** Naturally occurring genetic mutations in human subjects causing extreme immunodeficiency phenotypes present powerful opportunities to determine the relationship between specific immunologic defects and human disease processes in vivo. Recent description of human primary immunodeficiencies associated with abnormal TLR signaling demonstrates that this pathway is critical for human defense against infection. Empowered by technologic advances in genotyping and bioinformatics, we are now beginning to appreciate how common genetic variation and polymorphisms in genes controlling the innate immune response alter infectious susceptibility in a subtle but specific fashion. Importantly, human primary immunodeficiencies associated with abnormal TLR signaling provide unique insights into the immunologic pathways vital for host defense and identify candidate genes that might cause subtle immunodeficiencies in the broader population of apparently healthy persons.14

**Monogenic primary immunodeficiencies.** IL-1 receptor–associated kinase 4 (IRAK4) deficiency (OMIM #607676)15 and myeloid differentiation primary response gene 88 (MyD88) deficiency (OMIM #612260)16 are novel primary immunodeficiencies specifically affecting TLR function. MyD88 and IRAK4 are binding partners involved in downstream signaling from most TLRs (Fig 2); hence the clinical and laboratory phenotypes of IRAK4 and MyD88 deficiencies are identical. The narrow spectrum of infections experienced by affected individuals is striking in light of their profound impairment of TLR function and pathogen sensing. IRAK4- and MyD88-deficient patients predominantly experience recurrent infections caused by pyogenic gram-positive

### Table II. Common innate immune recognition strategies

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**RAGE**, Receptor of advanced glycation end product; **HMGB1**, high mobility group box 1.
bacteria, with *Streptococcus pneumoniae* causing invasive infection in all reported cases and *Staphylococcus aureus* and *Pseudomonas aeruginosa* causing infections in about half the patients. The surprising clinical observation that IRAK4-deficient patients are resistant to viral infections was recently explained at a molecular level because IRAK4-deficient patients are able to control viral infections by means of TLR3- and TLR4-dependent production of interferons.\(^\text{17}\)

**FIG 2.** Overview of TLR signaling and the NLRP3 inflammasome. TLR ligation initiates a signaling cascade that culminates in the translocation of the transcription factor nuclear factor κB (NF-κB) and others to the nucleus, generating an acute inflammatory response. The NLRP3 (or NALP3) inflammasome is triggered by a wide variety of stimuli, culminating in the activation of caspase 1, which will then cleave pro–IL-1β and pro–IL-18 to drive an inflammatory response. Human mutations and polymorphisms in many of the genes encoding elements of these pathways appear to alter susceptibility to infectious and inflammatory diseases. TRAF6, TNF receptor-associated factor 6; TAK1, Transforming growth factor-beta-activated kinase 1; IKK, I-kappa-B kinase; ASC, Apoptosis-associated speck-like protein containing a card.
Arguably one of the most powerful messages to arise from the recognition of IRAK4 and MyD88 deficiency is the value of studying human subjects to understand human immune function. Although MyD88-deficient patients are susceptible to *S. pneumoniae* and a limited number of pyogenic bacteria, they are able to resist infection by most common bacteria, viruses, fungi, and parasites. In contrast, MyD88 deficiency renders mice profoundly susceptible to most pathogens tested.

**Contribution of TLR polymorphisms to human disease.** At the population level, susceptibility to common diseases, such as infections, seldom follows the simple pattern of Mendelian inheritance seen in IRAK4 and MyD88 deficiency. Most infections follow a complex mode of inheritance, with disease arising from an intricate interplay between environmental and genetic factors. The complexity of common infectious diseases has made them, until very recently, largely impervious to genetic analysis. However, advances in high-throughput genotyping techniques and bioinformatics are now allowing us to understand how common genetic variants alter human susceptibility to infection.

Although human subjects are identical at most of the 3 billion base pairs in their genome, interindividual variation is present in approximately 3 million nucleotides (ie, 0.1% of the genome). A common type of human genetic variation is the single nucleotide polymorphism (SNP), in which 2 alternative bases occur at appreciable frequency (>1%) in the population. There is convincing evidence that common TLR SNPs regulate cellular signaling events, cytokine production, and susceptibility to infection based on the specific pathogens recognized by the TLR. Arguably the best evidence implicates amino acid–changing (ie, nonsynonymous) SNPs in TLR1, TLR2, and TLR5, as well as variants in the adaptor molecule TIR-containing adaptor protein (TIRAP, also known as MyD88 adaptor-like [MAL]). This genetic variation in the population results in some individuals having a subtle but specific immunodeficiency. For example, a common TLR5 polymorphism in the ligand-binding domain of TLR5 (392STOP) abolishes flagellin signaling and is associated with increased susceptibility to Legionnaire disease caused by the flagellated bacterium *Legionella pneumophila*. In a similar fashion, polymorphisms in the adaptor molecule MAL/TIRAP, which mediates signaling through TLR1, TLR2, TLR4, and TLR6, have been associated with susceptibility to tuberculosis, malaria, and pneumococcal disease.

Given the role of TLRs in sensing the extracellular environment and shaping the inflammatory response, the TLR pathway has been hypothesized to influence the development of atopy and asthma. The best-studied example is CD14. CD14 is encoded on chromosome 5q31.1 in a region linked to atopy and asthma, and CD14 partners with TLR4 to recognize LPS. Therefore a SNP in this gene (*CD14* T159 C to T), which appeared to alter the functional production of CD14, made an excellent candidate to influence susceptibility to atopy and asthma. Initial investigations showed remarkable variation, with some studies indicating the T allele as a risk factor, others indicating the C allele, and others finding no association. However, when the level of LPS (or endotoxin) exposure was considered, a biologically plausible gene-environment interaction was revealed, with data suggesting that the C allele is a risk factor for allergic phenotypes at low levels of exposure, whereas the T allele is a risk factor at high levels of exposure. Through this informative example, it is clear that complex interactions between genes and the environment determine asthma-related outcomes. Consequently, if we fail to integrate genetic and environmental factors in our study of asthma and allergy, we will only generate an impoverished appreciation of the cause of atopie disease.

Although a rapidly growing number of genetic association studies suggest that TLR polymorphisms might be associated with susceptibility to different infectious and immunologically mediated diseases, very few of these studies have been replicated in a convincing fashion. For example, the initial association reported between MAL/TIRAP and susceptibility to tuberculosis was not replicated in another large study. As this field advances and expands to include genome-wide association studies, it is essential to appreciate that the best studies will include large sample sizes, statistical adjustments for multiple comparison, replication of findings with independent cohorts, multiple study designs (including case-control and family-based studies), adjustment of the analysis for population admixture, consideration of environmental variables, and detailed molecular and cellular analyses to determine whether a polymorphism alters function.

**NLRs**

**Overview of NLR structure and function.** Although TLRs are outward-looking innate immune receptors detecting microbial signatures either in the extracellular milieu or engulfed in the lumen of endocytic vesicles, NLRs are a recently appreciated family of receptors that survey the intracellular environment. In common with other innate immune receptor systems, the NLRs have ancient origins, being structurally reminiscent of plant R-proteins that mediate plant cell defense against pathogenic bacteria. NLRs sense microbial products and metabolic stress, driving inflammation through the formation of an inflammasome: a large cytoplasmic complex that activates inflammatory caspases and the production of the cytokines IL-1β and IL-18.

The human NLR family consists of at least 23 members and can be structurally divided into 4 subfamilies based on N-terminal effector domains. The first NLRs reported to have a direct function as intracellular pathogen detectors were NOD1 and NOD2. Both NOD proteins detect distinct substructures generated during the synthesis, degradation, and remodeling of bacterial peptidoglycan, ensuring the recognition of peptidoglycan from both gram-positive and gram-negative bacteria. IL-1β-converting enzyme (ICE) protease-activating factor (IPAF) is another member of the NLR family known to detect bacterial pathogens. IPAF partners with TLR5 to detect infection by flagellated bacteria: TLR5 senses extracellular flagellin, whereas IPAF focuses on intracellular flagellin. In addition to sensing microbial products, NLRs can sense metabolic stress related to infection and sterile inflammation. This sensing capacity is best demonstrated by NLRP3 (NLR family, pyrin domain-containing 3). When triggered, NLRP3 (also called NALP3 or cryopyrin) activates the caspase 1 inflammasome, leading to IL-1β and IL-18 processing (Fig 2). The NLRP3 inflammasome appears to be activated by common metabolic danger signals, such as potassium efflux, which occurs during inflammation because of disruption of the plasma membrane or increased extracellular ATP released by injured cells. Other clinically relevant NLRP3 activators include uric acid, asbestos, silica, and alun.

**Role of NLRs in human health and disease.** Although our molecular appreciation of NLRs is very recent, this class of innate immune receptors plays a central role in several human
inflammatory diseases and mediates the adjuvant effect of a common vaccine component, alum.

**NLR defects associated with inflammatory diseases.**

The convergence of clinically defined autoinflammatory disease with the biology of innate immunity and NLRs came with the discovery that 3 well-established autoinflammatory diseases are all caused by activating, gain-of-function mutations in NLRP3. These diseases, collectively known as the cryopyrinopathies, are (1) familial cold autoinflammatory syndrome (OMIM #120100), which presents with cold-induced fevers, urticaria-like rash, and constitutional symptoms; (2) Muckle-Wells syndrome (OMIM #191900), which is characterized by fevers, hives, sensorineural hearing loss, and arthritis unrelated to cold exposure; and (3) neonatal-onset multisystem inflammatory disease (NOMID) (or chronic infantile neurologic, cutaneous, and articular syndrome [CINCA]; OMIM #607115), which is a devastating neonatal disease presenting with fever, urticaria, and chronic aseptic meningitis. In these disorders NLRP3 mutations affect IL-1β production, and IL-1β is upregulated in these diseases. Appreciation of the role of the IL-1β axis in these diseases associated with NLRP3 mutations has allowed the rational use of targeted anti-inflammatory therapy. Strikingly, even the most clinically severe cryopyrinopathy, NOMID/CINCA, appears to respond well to the IL-1 receptor antagonist anakinra.

More insight into the clinical relevance of NLRs arose when it was recognized that 30% to 50% of patients with Crohn disease in the Western hemisphere carry NOD2 mutations on at least 1 allele. The most common mutations are located in or near the leucine-rich repeat domain of NOD2, and patients homozygous for the 3020insC mutation, resulting in partial truncation of the leucine-rich repeat, demonstrate a much more severe disease phenotype. It seems paradoxical that although Crohn disease results in overt inflammation that is triggered by normal bacterial flora, the NOD2 mutations associated with Crohn disease result in a protein product less capable of responding to the bacterial ligand muramyl dipeptide, which is a component of peptidoglycan. A unifying paradigm addressing this paradox is that NOD2 appears to provide homeostatic signals to maintain the gut environment in a state that is tolerant of its flora and cells with NOD2 mutations are deficient in their production of IL-10, an immunomodulatory and tolerogenic cytokine. Other evidence suggests that NOD2 variants are associated with Crohn disease because they lead to a decrease in the negative regulation of TLR responses occurring in the normal gut and thus a pathologic increase in responses to the normal flora. Nevertheless, the genetic polymorphisms that show a well-established association with Crohn disease (including NOD2) account for only approximately 20% of the genetic variance observed in patients with Crohn disease, suggesting that significant additional genetic contributions have yet to be discovered.

**NLR contribution to vaccine responsiveness.** Increased understanding of NLRs has allowed us to shed light on the mechanism of action of vaccine adjuvants. Aluminum-containing adjuvants (alum) have historically served as immunopotentiators in vaccines and continue to be the most widely used clinical adjuvants. Despite the fact that most persons reading this review have received vaccines containing alum, it is only very recently that we have begun to fully appreciate the molecular mechanism of alum adjuvancy. Studies published in 2008 demonstrated that the NLRP3 (NALP3) inflammasome is involved in mediating the adjuvant effects of alum. This adjuvancy might occur directly through the triggering of the NALP3 inflammasome by alum crystals or indirectly through release of the endogenous danger signal uric acid, which subsequently activates NLRP3.

**THERAPEUTIC MODULATION OF INNATE IMMUNITY**

With increased appreciation of the contribution of innate immunity to human health and disease, attention quickly shifted to the possibility of therapeutic modulation of innate immunity. This is an area of active investigation, and therefore rather than attempting to survey the field broadly, we will focus our review on recent attempts to harness the TLR system to modulate infectious and allergic diseases.

**Activation of TLRs and modulation of allergic immune response**

The interaction of 2 fields of research in the 1990s, epidemiologic investigations of the hygiene hypothesis in allergy and asthma and basic research in the field of TLRs, provided the impetus to investigate whether activating TLRs might represent a novel therapeutic option for the treatment and prevention of allergy and asthma. TLR-based therapies in patients with allergy target in particular the dendritic cell interaction with T cells, which is a critical component in shaping the Th1 immune response associated with allergic inflammation. Because TLRs are highly expressed on dendritic cells but not on T cells, the goal of TLR-based therapies in allergy and asthma is to activate dendritic cells to produce a cytokine milieu (eg, IL-12 and interferons) that favors inhibition of the Th1 immune response. Thus TLR-based therapies target the innate immune response to consequently inhibit the adaptive Th1 immune response and do not directly target T cells.

Studies have examined whether activation of TLRs can modulate allergic immune responses in preclinical animal models of allergy and asthma, as well as in more limited studies in human subjects. The majority of studies have evaluated TLR9 agonists, but additional studies have also examined TLR4 agonists and a TLR7/8 agonist. Studies of the TLR9 agonist CpG DNA have demonstrated that it inhibits eosinophilic airway inflammation, Th2 cytokine responses, mucus expression, airway remodeling, and airway responsiveness in a murine model. Administration of an inhaled TLR9 agonist for approximately 8 months to monkeys allergic to dustmite demonstrated that they had reduced eosinophilic airway inflammation, mucus, airway remodeling, and reduced airway responsiveness. The only published studies in human asthmatic subjects were performed in patients with mild asymptomatic asthma treated with an inhaled TLR9 agonist before allergen challenge. Although treatment with the inhaled TLR9 agonist increased expression of interferon-inducible genes, there was no inhibition of the early- or late-phase decrease in FEV1 or a reduction in sputum eosinophil counts. These studies suggest that either TLR9-based therapies will not be effective in human subjects with asthma or that different doses, routes of administration (ie, systemic vs local), or study populations (symptomatic asthmatic subjects as opposed to allergen-challenged asymptomatic asthmatic subjects) need to be evaluated.

In addition to TLR9 agonists, studies predominantly in murine models have also evaluated the ability of TLR4- and TLR7/8-
based therapies to modulate allergic responses. In murine models of asthma, TLR4 ligands either inhibit or potentiate allergic responses depending on the timing of administration of the TLR4 ligand and associated allergen sensitization or challenge. In human studies in subjects with ragweed-induced allergic rhinitis, administration of a topical intranasal TLR4 ligand was safe but did not inhibit allergic responses in asymptomatic subjects challenged intranasally with ragweed allergen. Studies have also investigated whether administration of a TLR7/8 agonist, imiquimod, would inhibit asthma responses in preclinical models. Imiquimod is a US Food and Drug Administration–approved therapy that is used as a topical treatment for genital warts, actinic keratoses, and superficial basal cell cancer. In preclinical murine models the TLR7/8 agonist inhibits asthma responses. At present, no human studies in patients with allergy or asthma have been reported with the TLR7/8 agonist.

**TLR-based vaccine adjuvants in allergic disease**

Studies have also examined whether administering a TLR9 agonist conjugated to an allergen would enhance the immunogenicity of the allergen when used as a TLR9-conjugated allergen vaccine in patients with allergic rhinitis or asthma. Studies in murine models have demonstrated that a conjugate of a TLR9 agonist and an allergen had a 100-fold enhanced uptake by antigen-presenting cells compared with TLR9 ligand alone. The ability of a TLR9 ligand to induce a Th1 immune response is also approximately 100-fold greater than that induced by equivalent amounts of a nonconjugated mixture of the TLR9 ligand and allergen. In murine models the TLR9 allergen conjugate significantly reduces rhinitic and asthmatic responses.

Thus based on this enhanced immunogenicity of the TLR9 allergen conjugate, studies have examined whether a TLR9 ragweed allergen conjugate would reduce allergic responses in human subjects with allergic rhinitis. Studies in human subjects have demonstrated mixed results in terms of the effectiveness of the TLR9 ragweed allergen vaccine. Studies in subjects with ragweed-induced allergic rhinitis in Canada demonstrated that administration of the TLR9 ragweed allergen vaccine reduced nasal mucosal biopsy eosinophil counts and Th2 cytokine levels but did not reduce nasal symptom scores during the ragweed season. A second study in Baltimore demonstrated that administration of the same TLR9 ragweed allergen vaccine significantly reduced rhinitis symptom scores in subjects with ragweed-induced allergic rhinitis during the ragweed season.

Subjects treated with the TLR9 ragweed allergy vaccine also used fewer doses of allergy rescue medications during the ragweed season compared with the placebo-treated subjects. Interestingly, although the study subjects immunized with the TLR9 ragweed vaccine only received 6 injections of the vaccine before the first ragweed season, the beneficial reduction in symptoms persisted through the second ragweed season without administration of additional vaccine.

At present, there are limited numbers of published human studies with either administration of TLRs alone or with TLRs conjugated to allergens. Further studies are thus needed to determine whether the interesting observations regarding TLRs in preclinical models will translate into safe and effective therapeutic advances in allergy and asthma. Potential safety concerns of TLR-based therapies in allergy and asthma include the induction of autoimmune disease. However, induction of autoimmune disease has not been observed in the limited number of clinical trials with TLR9-based therapies.

**TLR-based vaccine adjuvants in infectious disease**

Vaccination has proved extremely effective in preventing infectious diseases, but knowledge of the immunologic mechanisms that allow vaccines to be so successful is rather limited. In contrast to live vaccines, subunit vaccines, which consist of specific components of pathogens, have little inherent immunogenicity and need to be supplemented with adjuvants to promote a protective immune response. However, there is a paucity of licensed adjuvants for clinical use, and thus there is a critical need to develop safe and effective adjuvants. The renovation in innate immune biology is facilitating the rational design of novel vaccine adjuvants. Characterization of the NLR system has shed light on the mechanism of action of alum adjuvancy, and our understanding of TLR function is accelerating the discovery of safe and effective vaccine adjuvants.

An illustrative example is the development of the novel adjuvant monophosphoryl lipid A (MPL). The TLR4 ligand LPS is a potent adjuvant, but its toxicity prevents its use in human subjects. However, MPL comes from the cell-wall LPS of gram-negative Salmonella minnesota R595 and is detoxified by mild hydrolytic treatment and purification. MPL lacks the toxicity of LPS but retains the beneficial adjuvant properties. MPL combined with aluminum salt (referred to as the AS04 adjuvant system) shows efficacy in a vaccine against human papilloma virus and as a hepatitis B vaccine for patients with advanced renal disease. Interestingly, this adjuvant combination likely benefits from the immune-enhancing capacity of both the TLR pathway (triggered by MPL) and the NALP3 inflammasome (triggered by alum crystals). Further advances in this area are almost certain because many other TLR ligands are being developed as potential vaccine adjuvants.

**CONCLUSIONS**

In the last decade, we have witnessed exhilarating advances in our understanding of the molecular mechanisms used by the innate immune system to sense infection and trigger a protective immune response. For clinicians and scientists alike, the challenge is to translate this basic mechanistic understanding into a more complete appreciation of the role of innate immunity in health and disease.

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**REFERENCES**


