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Chapter 2: Innate Immunity

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Abstract

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.

Keywords

host defense; innate immunity; Toll-like receptors; NOD-like receptors

THE “NEW” SCIENCE OF INNATE IMMUNITY

The integrated human immune response has traditionally been divided into two branches: innate and adaptive (or acquired) immunity. While 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 ‘non-specific immunity’ and now stands as a proud partner with 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!

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While 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 may 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 as a separate Primer chapter is devoted to this important aspect of innate immunity (Chapter 23: Complement Disorders and Hereditary Angioedema. Michael Frank).

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

The human microbial defense system can be simplistically viewed as consisting of three levels: (i) anatomical and physiological barriers; (ii) innate immunity; and (iii) adaptive immunity (Figure 1 and Table 1). Failure in any of these systems will greatly increase susceptibility to infection.

Anatomical and physiological 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 individuals 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 anatomical and physiological barriers.

Innate immunity augments the protection offered by anatomical and physiological barriers.¹ The innate immune system relies upon 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-defense 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 utilized 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 (such as insulin and myelin) are created by the random process of gene rearrangement that generates receptors expressed by T cells 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 following the first exposure to a pathogen. Adaptive immunity relies upon a clonal system with each T cell and B cell expressing its own unique receptor and following the initial encounter with a pathogen, it takes up to five 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 upon T and B lymphocytes, innate immune protection is a task performed by cells of both hematopoietic and non-hematopoietic origin (Figure 1 and Table 1). Hematopoietic cells involved in innate immune responses include macrophages, dendritic cells, mast cell, neutrophils, eosinophils, natural killer (NK) cells and natural killer 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 (LBP), C-reactive protein and other pentraxins, collectins, and anti-microbial peptides including defensins. Circulating innate immune proteins are involved in both in sensing microbes and effector mechanisms to facilitate clearance of the infection. For example, mannose-binding lectin (MBL), 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 while adaptive immunity evolved much later and is only found in jawed fish and all 'higher' vertebrates.² During evolution, adaptive immunity developed in the context of a functioning innate immune system. Consequently, the classic demarcation between innate and adaptive immunity is overly simplistic as many adaptive immune responses build on the foundation of innate immunity. For example, the capacity of neutrophils to kill bacteria is enhanced when the bacteria are opsonized by antibodies produced through the coordinated efforts of T and B cells. In a similar fashion, the C3d fragment that is generated in the course of complement activation acts as a molecular adjuvant to profoundly influence the subsequent adaptive immune response. Specifically, C3d fragments act to bridge innate and adaptive immunity as covalent binding of single or multiple copies of C3d to a foreign antigen generally enhances B cell effector and memory function.³ Another illustrative example of the interdependence of innate and adaptive immunity is the critical role played by antigen-presenting cells of the innate immune system (e.g. dendritic cells) to empower full activation of the T and B cells of the adaptive immune system. Further blurring of the distinction between innate and adaptive immunity is highlighted by the fact that cells of the adaptive immune system, including regulatory T lymphocytes, express Toll-like receptors (TLRs) and other innate immune receptors.⁴ The inter-relatedness of innate and adaptive immunity is most eloquently articulated by Beutler in his observation that "...the roots of adaptive immunity are buried deep in the soil of the innate immune system".⁵

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 upon a limited number of genetically predetermined germline-encoded receptors that recognize either highly conserved structures expressed by large groups of microbes or common biological 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 biological consequences of infection.

At least three broad strategies are used by the innate immune system to recognize invading microorganisms (Table 2). In the first, innate immunity relies upon a limited repertoire of germline-encoded receptors to recognize ‘microbial non-self’ – conserved molecular structures that are expressed by a large variety of microbes. Charles Janeway coined the terms ‘pattern recognition receptors’ (PRRs) to collectively describe these receptors and ‘pathogen-associated molecular patterns’ (PAMPs) to denote the microbial structures recognized by the PRRs.⁶ However, this terminology has been criticized as being vague⁵; 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 immunological ‘danger’ in the form of ‘damage-associated molecular patterns’ (DAMPs). DAMPs represents common metabolic consequences of infection and inflammation.⁷ DAMPs are 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 (HMGB-1) and other endogenous alarmins, heat-shock proteins (HSPs) 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.⁸

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 two illustrative examples – TLRs and NLRs – where our mechanistic understanding has increased considerably in the past 5 years and where the clinical relevance of these systems is beginning to emerge.

1. TOLL-LIKE RECEPTORS (TLRS)

Overview of TLR Structure and Function—The recent explosion of interest in innate immunity was catalyzed in the mid-1990s when the *Drosophila* protein Toll was shown to be critical for defending fruit flies against fungal infections.⁹ This observation opened the way for the subsequent description of similar proteins, called Toll-like receptors (TLRs), in mammalian cells. The human TLR family consists of 10 receptors that are critically important for innate immunity.^{10, 11} 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 while the cytosolic component, called a Toll/interleukin-1 receptor (TIR) domain, 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 lipopolysaccharide (LPS), TLR5 and

flagellin, TLRs1/2/6 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, 2, 4, 6 and 9, 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 (reviewed in ^{11, 12} and depicted in Figure 2). The emerging model has ligation of microbial products by TLRs culminating in the activation of nuclear factor kappa-B (NF-κB), activator protein-1 (AP-1), interferon-regulatory factor (IRF)-3 and other transcription factors, driving the production of proinflammatory cytokines, maturation of dendritic cells and other immunological responses.

Human Disease Resulting from TLR Defects—Naturally-occurring genetic mutations in humans, causing extreme immunodeficiency phenotypes, present powerful opportunities to determine the relationship between specific immunological defects and human disease processes *in vivo*. Recent description of human primary immunodeficiencies associated with abnormal TLR signaling demonstrate that this pathway is critical for human defense against infection. Empowered by technological advances in genotyping and bioinformatics, we are now beginning to appreciate how common genetic variation and polymorphisms in genes controlling the innate immune response alters infectious susceptibility in a subtle but specific fashion. Importantly, human primary immunodeficiencies associated with abnormal TLR signaling provide unique insights into the immunological pathways vital for host defense and identify candidate genes that may cause subtle immunodeficiencies in the broader population of apparently healthy people¹³.

a) Monogenic primary immunodeficiencies: Interleukin-1 receptor associated kinase 4 (IRAK4) deficiency (OMIM #607676)¹⁴ and myeloid differentiation primary response gene 88 (MyD88) deficiency (OMIM #612260)¹⁵ are novel primary immunodeficiencies specifically affecting TLR function. MyD88 and IRAK4 are binding partners involved in downstream signaling from most TLRs (Figure 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 suffer from recurrent infections caused by pyogenic Gram-positive bacteria, with *Streptococcus pneumoniae* causing invasive infection in all reported cases while *Staphylococcus aureus* and *Pseudomonas aeruginosa* caused 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 as IRAK4-deficient patients are able to control viral infections by TLR3- and TLR4-dependent production of IFNs.¹⁶

Arguably one of the most powerful messages to arise from the recognition of IRAK4- and MyD88-deficiency is the value of studying humans to understand human immune function! While MyD88-deficient patients are susceptible to *Streptococcus 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.

b) 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.¹⁷ 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 is now allowing us to understand how common genetic variants alter human susceptibility to infection.

Although humans are identical at most of the 3 billion base pairs in their genome, inter-individual variation is present in approximately 3 million nucleotides (i.e. 0.1% of the genome).¹⁸ A common type of human genetic variation is the single nucleotide polymorphism (SNP), where two 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 (i.e. non-synonymous) SNPs in TLRs 1, 2 and 5, as well as variants in the adaptor molecule TIR domain-containing adaptor protein (TIRAP, also known as 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's disease caused by the flagellated bacterium, *Legionella pneumophila*.¹⁹ In a similar fashion, polymorphisms in the adaptor molecule MAL/TIRAP which mediates signaling through TLR1, 2, 4, and 6, have been associated with susceptibility to tuberculosis, malaria and pneumococcal disease.²⁰

Given the role of TLRs in sensing the extracellular environment and shaping 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/-159 C to T) which appeared to alter the functional production of CD14, made an excellent candidate to influence susceptibility to asthma and atopy. Initial investigations showed remarkable variation with some studies indicating the T-allele as a risk factor, others the C-allele, and others finding no association.²¹ However, when the level of LPS (or endotoxin) exposure was considered a biologically plausible gene-by-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.²² Through this informative example it is clear that complex interactions between genes and 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 etiology of atopic disease.

While a rapidly growing number of genetic association studies suggest that TLR polymorphisms may 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.²³ 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.

2. NUCLEOTIDE OLIGOMERIZATION DOMAIN (NOD)-LIKE RECEPTORS (NLRs)

Overview of NLR Structure and Function—While TLRs are outward looking innate immune receptors detecting microbial signatures either in the extracellular milieu or engulfed in the lumen of endocytic vesicles, nucleotide oligomerization domain (NOD)-like receptors (or NLRs) are a recently appreciated family of receptors that survey the intracellular environment.^{24, 25} 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-1 β and IL-18.²⁶

The human NLR family consists of at least 23 members and can be structurally divided into four subfamily designations N-terminal effector domains.²⁷ The first NLRs reported to have a direct function as intracellular pathogen detectors were NOD1 and NOD2.²⁵ 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. IPAF (for ICE protease-activating factor) is another member of the NLR family known to detect bacterial pathogens.²⁸ IPAF partners with TLR5 to detect infection by flagellated bacteria—TLR5 senses extracellular flagellin while 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).²⁹ When triggered NLRP3 (also called NALP3 or cryopyrin) activates the caspase-1 'inflammasome' leading to interleukin 1 β (IL-1 β) and IL-18 processing (Figure 2). The NLRP3 inflammasome appears to be activated by common metabolic 'danger signals' such as potassium efflux which occurs during inflammation due to disruption of the plasma membrane or increased extracellular ATP released by injured cells. Other clinically relevant NLRP3 activators include uric acid, asbestos, silica and alum.

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.

a) 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 three well-established autoinflammatory diseases are all caused by activating, gain-of-function mutations in *NLRP3*.³⁰ These diseases, collectively known as the cryopyrinopathies, are: (i) familial cold autoinflammatory syndrome (OMIM #120100), which presents with cold-induced fevers, urticaria-like rash, and constitutional symptoms; (ii) Muckle-Wells syndrome (OMIM #191900), which is characterized by fevers, hives, sensorineural hearing loss, and arthritis unrelated to cold exposure; and (iii) neonatal-onset multisystem inflammatory disease (NOMID) [or chronic infantile neurologic cutaneous articular (CINCA) syndrome] (OMIM #607115), which is a devastating neonatal disease presenting with fever, urticaria and chronic aseptic meningitis. In these disorders *NLRP3* mutations affect IL-1 β production, and IL-1 β is upregulated in these diseases.³¹ Appreciation of the role of the IL-1 β axis in these diseases associated with *NLRP3* mutations has allowed the rational use of targeted anti-inflammatory therapy.³² Strikingly, even the most clinically severe cryopyrinopathy, NOMID/CINCA, appears to respond well to the IL-1 receptor antagonist, anakinra.³³

More insight into the clinical relevance of NLRs arose when it was recognized that 30–50% of patients with Crohn's disease in the Western hemisphere carry *NOD2* mutations on at least one allele.^{34, 35} The most common mutations are located in or near the leucine-rich repeat (LRR) domain of *NOD2* and patients homozygous for the *3020insC* mutation, resulting in partial truncation of the LRR, demonstrate a much more severe disease phenotype. It seems paradoxical that while Crohn's disease results in overt inflammation that probably is triggered by normal bacterial flora, the *NOD2* mutations associated with Crohn's disease result in a protein product less capable of responding to the bacterial ligand, muramyl dipeptide (MDP)

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.³⁶ Other evidence suggests that *NOD2* variants are associated with Crohn's 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.³⁷ Nevertheless, the genetic polymorphisms that show a well-established association with Crohn's disease (including *NOD2*) account for only approximately 20% of the genetic variance observed in Crohn's disease, suggesting that significant additional genetic contributions have yet to be discovered.

b) NLR contribution to vaccine responsiveness: Increased understanding of NLRs has allowed us to shed light on the mechanism of action of vaccine adjuvants.⁶ 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 people 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.³⁸⁻⁴⁰ This adjuvancy may occur directly via 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, so 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.

1. ACTIVATION OF TLRs AND MODULATION OF ALLERGIC IMMUNE RESPONSE

The interaction of two 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.⁴¹ TLR based therapies in allergy target in particular the dendritic cell interaction with T cells, which is a critical component in shaping the Th2 immune response associated with allergic inflammation. As 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 (IL-12, IFNs, etc) that favors inhibition of Th2 immune response. Thus, TLR based therapies target the innate immune response to consequently inhibit the adaptive Th2 immune response and do not directly target T cells.

Studies have examined whether activation of TLRs can modulate allergic immune responses in pre-clinical 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 mouse model.^{41, 42} Administration of an inhaled TLR9 agonist for approximately 8 months to monkeys allergic to dust mite demonstrated that they had reduced eosinophilic airway inflammation, mucus, airway remodeling, and reduced airway responsiveness.⁴³ The only published studies in human asthmatics were performed in mild asymptomatic asthmatics treated with an inhaled TLR9 agonist prior to allergen challenge.⁴⁴ Although treatment with the inhaled TLR9 agonist

increased expression of IFN-inducible genes, there was no inhibition of the early or late phase decrease in forced expiratory volume in one second (FEV₁), nor a reduction in sputum eosinophils. These studies suggest that either TLR9 based therapies will not be effective in human subjects with asthma, or that different doses, routes of administration (i.e. systemic vs local), or different study populations (symptomatic asthmatics as opposed to allergen challenged asymptomatic asthmatics) need to be evaluated.

In addition to TLR9 agonists, studies predominantly in mouse models have also evaluated the ability of TLR4 and TLR7/8 based therapies to modulate allergic responses. In mouse models of asthma TLR4 ligands either inhibit or potentiate allergic responses depending upon the timing of administration of the TLR4 ligand and associated allergen sensitization or challenge. In human studies in ragweed allergic rhinitis subjects, administration of a topical intranasal TLR4 ligand was safe but did not inhibit allergic responses in asymptomatic subjects challenged intranasally with ragweed allergen.⁴⁵ Studies have also investigated whether administration of a TLR7/8 agonist imiquimod would inhibit asthma responses in pre-clinical models. Imiquimod is an FDA-approved therapy which is used as a topical treatment for genital warts, actinic keratoses, and superficial basal cell cancer. In pre-clinical mouse models the TLR7/8 agonist inhibits asthma responses. At present no human studies in allergy or asthma have been reported with the TLR 7/8 agonist.

2. 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 allergic rhinitis or asthma. Studies in mouse models have demonstrated that a conjugate of a TLR9 agonist and an allergen had a 100-fold enhanced uptake by antigen presenting cells compared to TLR9 ligand alone.^{41, 46} 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 non-conjugated mixture of the TLR9 ligand and allergen. In mouse models, the TLR9 allergen conjugate significantly reduces rhinitis and asthma responses.⁴¹

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 humans have demonstrated mixed results in terms of the effectiveness of the TLR9 ragweed allergen vaccine. Studies in ragweed allergic rhinitis subjects in Canada demonstrated that administration of the TLR9 ragweed allergen vaccine reduced nasal mucosal biopsy eosinophil counts and Th2 cytokines, but did not reduce nasal symptom scores during the ragweed season.⁴⁷ 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.⁴⁸ Subjects treated with the TLR9 ragweed allergy vaccine also used fewer doses of allergy rescue medications during the ragweed season compared to placebo treated subjects. Interestingly, although the study subjects immunized with the TLR9 ragweed vaccine only received six injections of the vaccine prior to 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 pre-clinical models will, or will not, 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 TLR-9 based therapies.

3. TLR-BASED VACCINE ADJUVANTS IN INFECTIOUS DISEASE

Vaccination has proved extremely effective in preventing infectious diseases, but knowledge of the immunological 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 renaissance in innate immune biology is facilitating the rational design of novel vaccine adjuvants.⁴⁹ Characterization of the NLR system has shed light on the mechanism of action of alum adjuvancy, while our understanding of TLR function is accelerating the discovery of safe and effective vaccine adjuvants.

An illustrative example is development of the novel adjuvant, monophosphoryl lipid A (MPL).⁵⁰ The TLR4 ligand LPS is a potent adjuvant, but its toxicity prevents use in humans. 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 virus⁵¹, and as a hepatitis B vaccine for patients with advanced renal disease.⁵² 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 as 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 now translate this basic mechanistic understanding into a more complete appreciation of the role of innate immunity in health and disease.

ABBREVIATIONS

CINCA	Chronic infantile neurological, cutaneous, and articular syndrome
DAMP	Damage-associated molecular pattern
HMGB-1	High mobility group box 1
HSP	Heat-shock protein
IPAF	ICE protease-activating factor
IRAK4	Interleukin-1 receptor associated kinase 4
KIR	Killer-cell immunoglobulin-like receptors
LBP	LPS binding protein
LPS	Lipopolysaccharide
LRR	Leucine-rich repeat
MAL	MyD88 adapter-like
MBL	Mannose-binding lectin
MDP	Muramyl dipeptide

MPL	Monophosphoryl lipid A
MyD88	Myeloid differentiation primary response gene 88
NLR	nucleotide oligomerization domain (NOD)-like receptors
NOMID	Neonatal-onset multisystem inflammatory disease
PAMP	Pathogen-associated molecular pattern
PRR	Pattern recognition receptor
RAGE	Receptor of advance glycation end product
SNP	Single nucleotide polymorphism
TIR	Toll/interleukin-1 receptor-like domain
TIRAP	Toll-interleukin 1 receptor (TIR) domain-containing adaptor protein
TLR	Toll-like receptor

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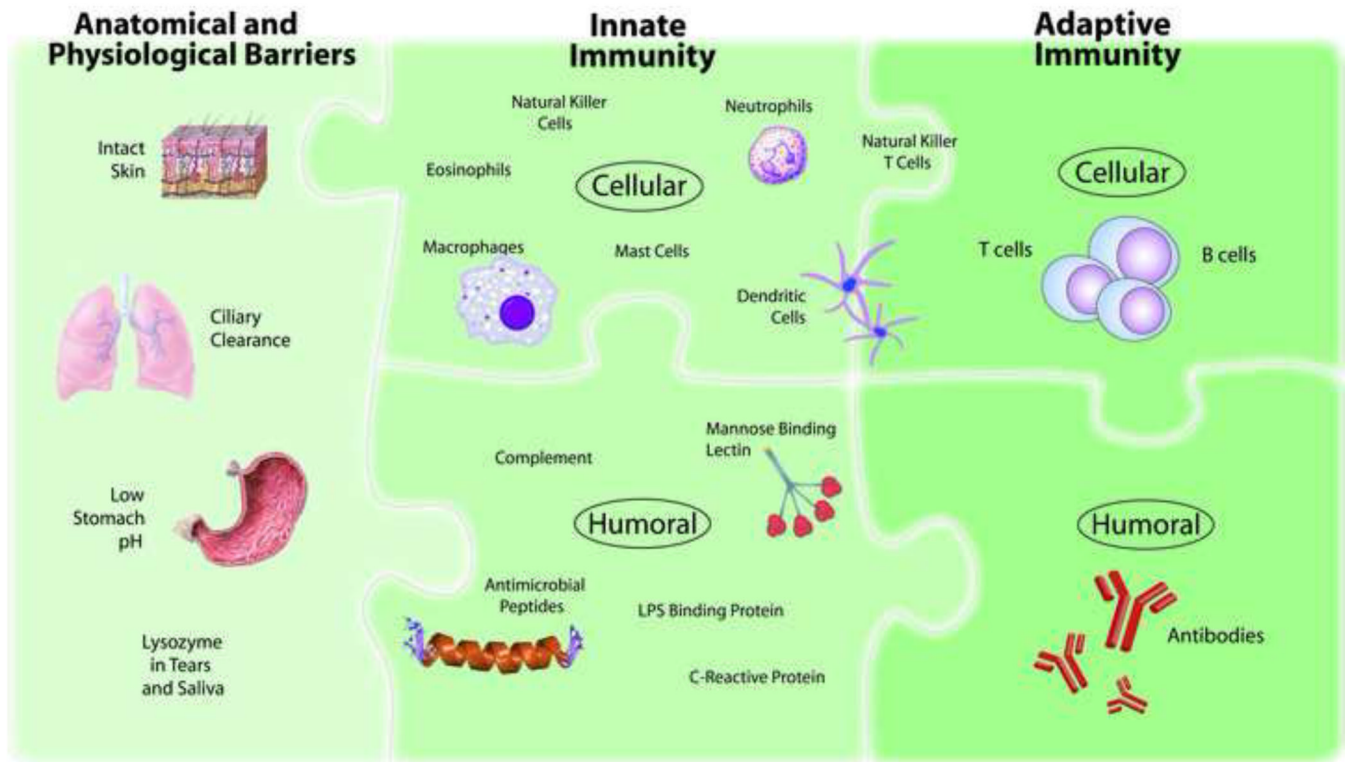


Figure 1. Integrated Human Immune System

The human microbial defense system can be simplistically viewed as consisting of three levels: (i) anatomical and physiological barriers; (ii) innate immunity; and (iii) adaptive immunity. In common with many classification systems, some elements are difficult to categorize. For example, NKT cells and DCs could be classified as being on the cusp of innate and adaptive immunity rather than being firmly in one camp.

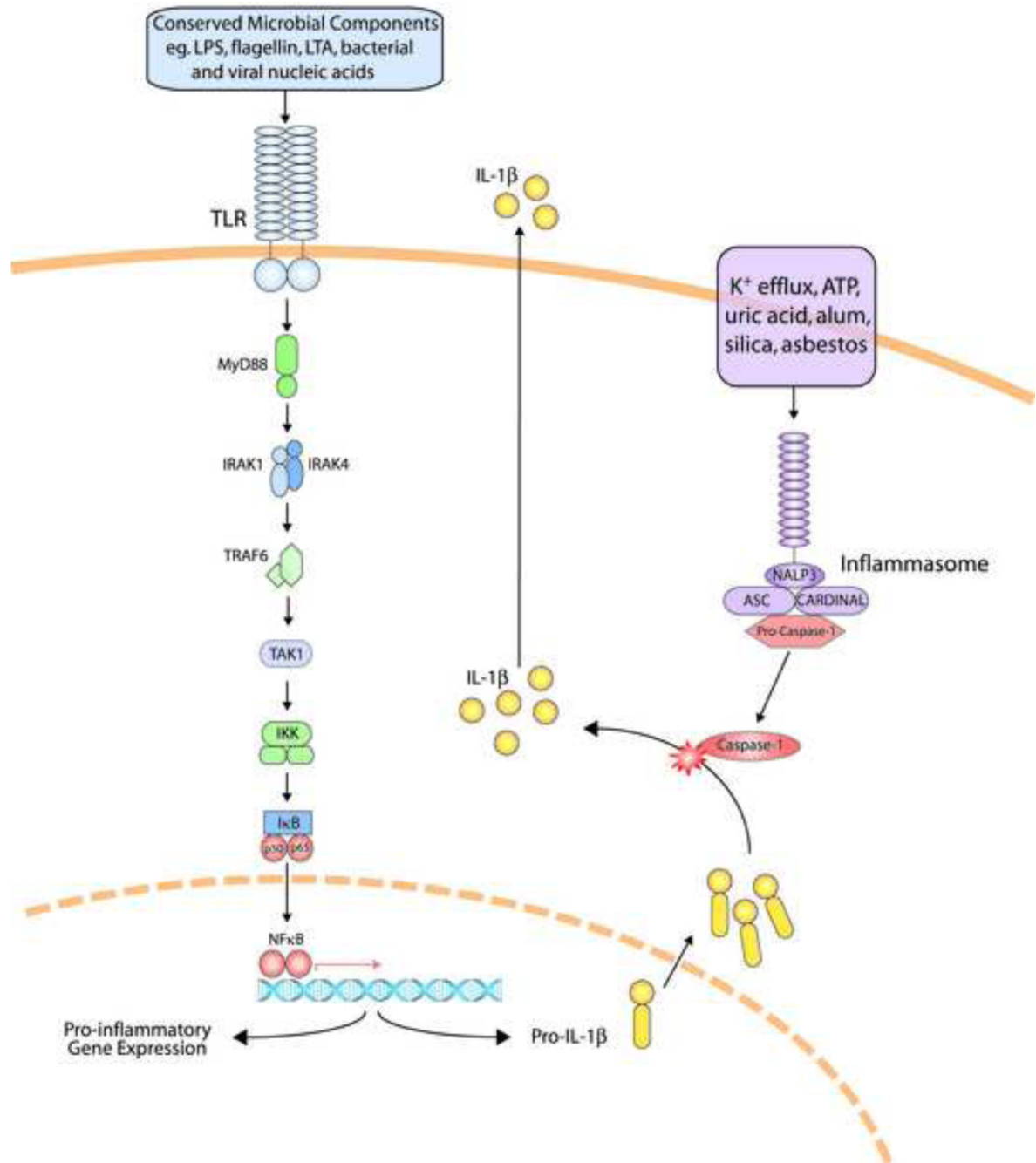


Figure 2. Overview of TLR Signaling and the NLRP3 Inflammasome

TLR ligation initiates a signaling cascade that culminates in the translocation of the transcription factors, NF- κ B 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-IL1 β and pro-IL18 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.

Table 1
Overview of Defining Features of Innate and Adaptive Immunity

Comparing and contrasting some of the defining features of the innate and adaptive immune systems. Adapted from [1].

	Innate Immune System	Adaptive Immune System
Cellular elements	Hematopoietic cells: macrophages, dendritic cells, mast cells, neutrophils, eosinophils, natural killer cells & natural killer T cells. Non-hematopoietic cells: epithelial cells (e.g. skin, airways, gastrointestinal tract).	Hematopoietic cells: T & B lymphocytes.
Humoral elements	Large arsenal of components: Complement proteins, LPS binding protein, C-reactive protein and other acute phase reactants, anti-microbial peptides, mannose-binding lectin.	Immunoglobulins secreted by B cells.
Receptor characteristics	Invariant, germline encoded. All cells of a class express identical receptors (i.e. non-clonal).	Generated by random somatic gene-segment rearrangement. All cells of a class express a single type of receptor with unique specificity (i.e. clonal).
Ligands recognized	Conserved microbial components. Common metabolic or biological consequences of infection (e.g. uric acid, K ⁺ efflux, MHC class I downregulation).	Specific 'details' or epitopes of macromolecules (e.g. proteins, peptides, carbohydrates).
Types of receptors	Activating: TLR, NLR, complement. Inhibitory: Killer cell immunoglobulin-like receptors (KIR).	B cell receptor and T cell receptor.
Response time	Immediate	Delayed by hours to days.
Immunological memory	None. Responses are the same with each exposure. Non-anticipatory immunity	Responsiveness enhanced by repeated antigen exposure. Anticipatory immunity.
Risk of autoreactivity	Low. Self-tolerant receptors selected during evolution	High. Random gene rearrangement generates autoreactive receptors requiring presence of multiple tolerance mechanisms.

Table 2
Common Innate Immune Recognition Strategies

Innate Immune Recognition Strategy	Receptor Families	Specific examples	
		Receptor	Ligand
1. Detecting 'microbial non-self' (i.e. pathogen-associated molecular patterns (PAMPs))	Toll-like receptors	TLR4 TLR5	Lipopolysaccharide Flagellin (extracellular)
	NOD-like receptors	NOD2 IPAF	Muramyl dipeptide Flagellin (intracellular)
	Collectin family	MBP	Microbial terminal mannose residues
2. Detecting common metabolic consequences of cell infection or injury (i.e. damage-associated molecular patterns (DAMPs))	NOD-like receptors	NLRP3 (or NALP3)	Uric acid, K ⁺ efflux, ATP
	RAGE (receptor of advanced glycation end product) family	RAGE	HMGB1, S100
3. Detecting 'missing self'	MHC-class-I-specific inhibitory receptors	KIR	Self MHC class I (inhibitory signal)
		CD94-NKG2A heterodimers	Self MHC class I (inhibitory signal)