

Innate microbial sensors and their relevance to allergy

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Activity Objectives

1. To identify the innate microbial sensors and their diverse repertoire.
2. To comprehend the structural and functional approaches to microbial recognition.
3. To analyze current evidence for the relevance of the microbial sensors in allergic and inflammatory disorders.

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The innate immune system oversees the gateway to immunity with its microbial sensors. Innate microbial sensors are germ line–encoded receptors with genetically predetermined specificities for microbes. The readiness and effectiveness of the innate immune system to provide immediate and appropriate responses at the host–environment interface is dependent on its sensitive and comprehensive microbial detection systems. The purpose of this review is to provide an overview of innate microbial sensors, our growing understanding of their diverse repertoire, and their elegant structural and functional approaches to microbial recognition. Their relevance to allergic disease is also discussed: the potential recognition and uptake of allergens by some of these receptors, inhibited expression of other microbial sensors by allergic immune responses and inflammation, and their upregulation by microbial exposures in early life that may help to protect against the development of allergic immune responses and disease. (*J Allergy Clin Immunol* 2008;122:846-58.)

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Innate microbial sensors recognize molecular structures that distinguish large groups of microbes from the host. These microbe-specific molecular structures are called pathogen-associated molecular patterns (PAMPs), and the receptors that recognize them are called pattern recognition receptors (PRRs) (Fig 1). PAMP structures, although biochemically distinct, share common features:

1. PAMPs are produced by microbes, not by their hosts.
2. PAMP structures are essential to the integrity and survival of the microbes.
3. PAMPs are highly conserved invariant structures of entire classes of microbes.

Consequently, host organisms can rely on PAMP recognition for microbial sensing because microbes cannot alter PAMPs without endangering their survival.

This innate approach to microbial recognition by PRRs fundamentally differs from the developmental process of microbial recognition by the T and B lymphocytes of the adaptive immune system. T and B lymphocytes develop an extremely diverse repertoire of antigen specificities (approximately 10^{14} different T-lymphocyte receptors and 10^{18} different immunoglobulin receptors, respectively) during receptor diversification processes of receptor gene rearrangement and, in B lymphocytes, hypermutation, from which the useful receptors (ie, those specific for microbial pathogens and not self) are selected for clonal expansion. In the individual organism, useful receptors of adaptive immunity are selected and “learned” over time and are not inherited by their offspring, as are the innate PRRs. Thus, host defense is fully reliant on germ line–encoded, innate PRRs, whose usefulness has been determined through eons of natural selection.

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Abbreviations used

AD:	Atopic dermatitis
APC:	Antigen-presenting cell
CLR:	C-type lectin receptor
CRP:	C-reactive protein
DC-SIGN:	Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin
FCAS:	Familial-cold autoinflammatory syndrome
HBD:	Human β -defensin
LPS:	Lipopolysaccharide
MBL:	Mannose-binding lectin
NF- κ B:	Nuclear factor- κ B
NLR:	Nucleotide-binding oligomerization domain-like receptor
NOD:	Nucleotide-binding oligomerization domain
PAMP:	Pathogen-associated molecular pattern
PRR:	Pattern recognition receptor
SP:	Surfactant protein
TLR:	Toll-like receptor

Innate PRRs can be conceptually organized in several fundamental ways. PRRs can be categorized by their structural and functional similarities (Table I). Microbial recognition by PRRs can be direct, mediated by membrane-bound or cytoplasmic receptors; or indirect, whereby soluble PRRs coat or opsonize the pathogen, thereby enabling recognition by opsonic receptors. Secreted PRRs can also provide host protection by directly killing microbes, by providing a chemoattractive gradient for innate and adaptive immune effector cells, and/or by serving as helper proteins for other microbial sensors (eg, CD14 and LPS binding protein for Toll-like receptor [TLR]-4). Innate microbial sensors are expressed by many innate immune cell types at the forefront of innate immune responses, including macrophages, dendritic cells, monocytes, B lymphocytes, epithelial cells, and mast cells. Immediate microbial sensing is achieved in part by the constitutive expression of some PRRs at the host-environment interface, with rapid upregulation of other PRRs with microbial detection or inflammation.

Antimicrobial peptides

Antimicrobial peptides (AMPs) are small, secreted polypeptide PRRs with great structural diversity between and within species, while sharing a fundamental feature in their molecular structures: clustering hydrophobic, cationic amino acids target the outer bilayer of bacterial membranes that are made of negatively charged phospholipids, an attractive surface for the cationic and hydrophobic domains of AMPs to bind.^{1,2} In contrast, the outer cell membranes of plants and animals are composed of lipids with no net charge. Thus, AMPs distinguish microbes from host cells by their membrane charge, and integrate into microbial outer membranes to form pores and cracks, thereby disrupting microbial membrane integrity and function. AMPs are secreted onto epithelial surfaces to form a microbicidal shield as a first line of host defense, damaging organisms before they can attach and invade the host. AMPs have antimicrobial activity against a broad range of bacteria, fungi, Chlamydiae, and enveloped viruses.³ By expressing multiple AMPs from several different structural classes, epithelial and innate immune cells generally evade the development of microbial resistance to AMPs.

Two main categories of AMPs in human beings are the defensins and cathelicidin (LL-37). In human beings, defensins

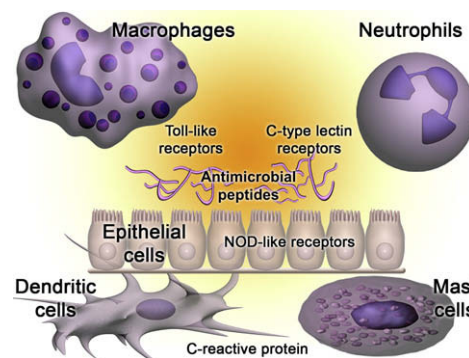


FIG 1. Innate microbial sensors. Schema of the main categories of microbial pattern recognition receptors at the host-environment interface, and the innate immune cell types that express them.

are subcategorized into the α and β classes. α -Defensins are granule proteins of neutrophils (eg, human neutrophil peptides 1 through 4) and are also synthesized by Paneth cells at the base of small intestinal crypts (α -defensins 5 and 6).⁴ Human β -defensins (HBDs) are expressed on all epithelial surfaces, including those of the airways, urinary and gastrointestinal tracts, and skin. Their production can be constitutive (eg, HBD-1) or inducible (HBD-2, HBD-3, HBD-4). HBD-2 expression is induced in epithelial cells by bacterial PAMPs via TLR-2 or TLR-4, and by innate inflammatory cytokines such as IL-1 β and TNF- α .⁵⁻⁷ A genomic search for defensin sequence homology in human beings revealed 5 β -defensin gene clusters with as many as 25 additional defensins.⁸ Other types of AMPs have been described in human beings—for example, dermcidin in sweat.⁹ This suggests that what is currently known about human AMPs may only be a sampling of what is used.

Cathelicidin LL-37 is released from neutrophils, macrophages, and epithelial cells; one of its most interesting and clinically relevant features is its inducible expression by vitamin D. The gene encoding LL-37 has a vitamin D receptor binding site.^{10,11} In both keratinocytes and macrophages, stimulation of TLR-2 induces the cytochrome P450 enzyme that converts vitamin D to its active form (1,25(OH)₂D₃), which in turn induces LL-37 expression. By this route, vitamin D can influence microbicidal defenses of both skin and circulating phagocytic cells.¹² It is postulated that certain human infections, such as *Mycobacterium tuberculosis*, might be more prevalent among populations with inadequate plasma levels of vitamin D.¹³

In addition to their direct microbicidal activity, AMPs also serve as chemoattractants for innate and adaptive immune cells. Human α -defensin attracts immature dendritic cells and peripheral blood T cells.¹⁴ HBD-2 and HBD-3 are inducible and chemoattractive for immature dendritic cells and memory T lymphocytes via the chemokine receptor CCR6.¹⁴ Recruitment of dendritic cells by AMPs induces their maturation; when combined with T-cell chemoattraction, AMPs instigate the development of antigen-specific adaptive immune responses. Cathelicidin LL-37 attracts neutrophils, monocytes, mast cells, and T lymphocytes via formyl peptide-like receptor 1, a PRR that also binds bacterial formyl peptides (discussed below in “Other microbial PRRs”).¹⁵ The β -defensins and LL-37 are also chemoattractive for mast cells and can induce their degranulation.^{16,17}

To summarize, an array of AMPs form an effective antimicrobial barrier; when breached, AMPs are induced to destroy

TABLE I. Pattern recognition receptors in human beings

Recognizes		Expressed by	Functions
Antimicrobial peptides			
Defensins	Microbial membranes (negatively charged)		Opsonization, microbial cell lysis, immune cell chemoattractant
α-Defensins	Microbial membranes (negatively charged)	Neutrophils, Paneth cells	Opsonization, microbial cell lysis, immune cell chemoattractant
β-Defensins	Microbial membranes (negatively charged)	Epithelium	Opsonization, microbial cell lysis, immune cell chemoattractant
Cathelicidin (LL-37)	Microbial membranes (negatively charged)	Neutrophils, macrophages, epithelium	Opsonization, microbial cell lysis, immune cell chemoattractant
Dermcidin	Microbial membranes (negatively charged)	Sweat glands	Opsonization, microbial cell lysis, immune cell chemoattractant
Toll-like receptors			
TLR-1	Bacterial lipoproteins, lipotechoic acid	Many immune and epithelial cell types	Cell activation, dimerizes with TLR-2
TLR-2	Bacterial lipoproteins, peptidoglycan, lipotechoic acid, lipoarabinomannan, fungal zymosan	Many immune and epithelial cell types	Cell activation, dimerizes with TLR-1 or TLR-6
TLR-3	Viral double-stranded RNA	Many immune and epithelial cell types, cell activation	
TLR-4	Bacterial endotoxin, respiratory syncytial virus coat proteins	Many immune and epithelial cell types, cell activation	
TLR-5	Bacterial flagellin	Many immune and epithelial cell types, cell activation	
TLR-6	Bacterial lipoproteins, lipotechoic acid	Many immune and epithelial cell types, cell activation	Cell activation, dimerizes with TLR-2
TLR-7	Viral single-stranded RNA	Many immune and epithelial cell types, cell activation	
TLR-8	Viral single-stranded RNA	Many immune and epithelial cell types, cell activation	
TLR-9	Bacterial/viral unmethylated CpG DNA	Many immune and epithelial cell types, cell activation	
TLR-10	Unknown	Many immune and epithelial cell types, cell activation	
MD-2	Bacterial endotoxin	Many immune and epithelial cell types	TLR-4 coreceptor
CD14	Bacterial endotoxin	Many immune and epithelial cell types	TLR-4 enhancer
LPS binding protein	Bacterial endotoxin	Liver	TLR-4 enhancer
C-type lectin receptors			
Mannose receptor	Microbial mannan	Macrophages	Cell activation, endocytosis/phagocytosis, mediator production
Dectin-1	β-1,3-Glucan	Macrophages, dendritic cells, neutrophils, lymphocytes	Cell activation, endocytosis/phagocytosis, mediator production
Dectin-2	Hyphal mannose	Macrophages, dendritic cells	Cell activation, endocytosis/phagocytosis, mediator production
DC-SIGN	Hyphal mannose	Immature dendritic cells	Cell activation, endocytosis/phagocytosis, mediator production
Collectins			
MBL	Microbial mannose, fucose, n-acetyl glucosamine	Liver	Complement activation
Surfactant proteins A and D	Microbial oligosaccharides	Alveolar type II cells, nonciliated bronchial epithelium	Opsonization, killing, proinflammatory and anti-inflammatory mediator release
NOD-like receptors			
NOD-1	Peptidoglycans from gram-negative bacteria	Ubiquitous	Cell activation
NOD-2	Bacterial muramyl dipeptides	Macrophages, dendritic cells, Paneth cells, gastrointestinal epithelium	Cell activation
NLRP1	Anthrax lethal toxin		Inflammasome scaffolding protein
NLRP3 (cryopyrin)	Microbial RNA, urate crystals, asbestos/silica	Neutrophils, monocytes	Inflammasome scaffolding protein
NLR4	Bacterial flagellin		Inflammasome scaffolding protein
C-reactive protein	Phosphorylcholine from bacteria and apoptotic/necrotic cells, nuclear histones	Liver	Complement activation, opsonization via Fcγ receptor and C1q binding
Other PRRs			
N-formyl methionine receptor	Bacterial n-formylmethionine	Phagocytes	Phagocytosis
RIG-1 and MDA-5	Viral double-stranded RNA		Immune cell activation

microbes and attract an array of defensive cells to provide defensive support of the breached barrier. Their attraction of professional APCs with T lymphocytes stimulates the development of an adaptive immune response to the invading microbes at the site of injury. Thus, in states of health, the defenses provided by AMPs are by and large clinically silent. Inflammation occurs when the AMP-based defenses have proved inadequate and a robust secondary defensive response involving the effector and adaptive immune systems is mobilized.

Toll-like receptors

Toll-like receptors (TLRs) have been prototypical innate microbial sensors since their identification as the immunoreceptors that recognize microbe-specific PAMPs such as bacterial endotoxin.^{18–20} TLRs are structurally similar transmembrane PRRs with large leucine-rich extracellular domains and cytoplasmic domains that are notably similar to that of the mammalian IL-1 receptor.²¹ Accordingly, the IL-1 receptor and TLRs share a MyD88-dependent signaling pathway that leads to nuclear factor- κ B (NF- κ B) activation. The immediate cellular responders of the innate immune system (eg, monocytes, macrophages, dendritic cells, epithelial cells, neutrophils) and many other cell types express TLRs.

TLR-4 was the first human TLR identified and senses bacterial endotoxin, a LPS PAMP that makes up most of the outer layer of the lipid bilayer of the outer cell wall of all gram-negative bacteria. TLR-4 specifically recognizes the lipid A moiety of endotoxin, a highly conserved structure that confers much of endotoxin's biological activities.^{22–24} Picogram amounts of endotoxin, estimated to equal ~ 10 LPS molecules per cell, can be immune stimulatory.²⁵ This exquisite sensitivity for endotoxin-mediated cell activation can be partly attributed to the TLR-4-related enhancer proteins, LPS binding protein, and CD14, and on the TLR-4 recognition cofactor, MD-2. LPS binding protein is a soluble protein that is produced by the liver, binds endogenous and exogenous LPS, and shuttles to TLR-4.²⁶ CD14 is a soluble and cell surface receptor that binds LPS and improves TLR-4 sensitivity and detection of endotoxin.²⁷ CD14's crystal structure reveals a large hydrophobic pocket that serves as the binding site for LPS.²⁸ MD-2 is an extracellular adaptor protein that binds both the hydrophobic portion of LPS and the extracellular domain of TLR-4.²⁹ MD-2 is constitutively associated with TLR-4 and is required for TLR-4 recognition of endotoxin.³⁰ Variability in endotoxin sensitivity can be attributed to a variety of factors, including expression levels of these coreceptor/enhancer PRRs, as well as cellular conditions that prime TLR-4-mediated activation such as innate immune cytokines (eg, IFNs), low-level endotoxin exposure, or other PAMP exposures.^{25,31–33} Conversely, high levels of endotoxin exposure suppress TLR-4-mediated activation.³³

Ten human TLRs have been identified and collectively recognize a diverse range of microbial cell wall components, proteins, and nucleic acids: all classic PAMPs that distinguish microbes from their mammalian hosts. Microbial cell wall components are recognized by TLR-4 (endotoxin from gram-negative bacteria), TLR-2 in combination with TLR-1 or TLR-6 (peptidoglycan and lipoproteins from gram-positive bacteria and mycoplasma),³⁴ and TLR-5 (bacterial flagellin).³⁵ Microbial nucleic acids are recognized by TLR-9 (unmethylated CpG sequences characteristic of microbial DNA),³⁶ TLR-7 and TLR-8 (viral single-stranded RNA),³⁷ and TLR-3 (viral

double-stranded RNA).³⁸ One can appreciate fuller reviews of TLRs in recently published articles.^{34,39,40}

C-type lectin receptors

Following the development of innate microbial sensing paradigms from the TLRs, an expansion of scientific understanding of other families of innate microbial sensors is well underway. One such example can be found in the C-type lectin receptor (CLR) superfamily, whose members have 1 or more structurally related C-type lectin-like domains that typically have calcium-dependent functions.⁴¹ Some CLRs are microbial sensors: mannose receptor, dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), dectin-1, dectin-2, and the collectins. They can mediate microbial binding, uptake, and killing, and contribute to the initiation and/or regulation of anti-microbial immune responses. They are believed to be especially relevant to fungal immunity.

The mannose receptor (CD206) has 8 extracellular C-type lectin-like domains that recognize carbohydrates with terminal mannan that are characteristic of a variety of microbes, especially fungi. It is widely expressed on tissue macrophages and some dendritic cells, mediating microbial phagocytosis and inducing NF- κ B activation with proinflammatory cytokine production.^{42,43}

Dectin-1 has a single extracellular carbohydrate recognition domain specific for β -1,3-glucan, a major cell wall component of a wide variety of fungi.^{44,45} When engaged, dectin-1 induces a variety of cellular responses through its intracellular immunoreceptor tyrosine-based activating motif, including the activation of phospholipase A2 and COX-2, endocytosis/phagocytosis, and the production of proinflammatory cytokines and chemokines. It is expressed on macrophages, dendritic cells, neutrophils, and some lymphocytes.

Dectin-2 is widely expressed on macrophages, dendritic cells, and Langerhans cells, and recognizes high mannose structures, preferentially the hyphal forms of fungi.⁴⁶ Its cytoplasmic tail associates with the Fc receptor γ -chain for intracellular signaling.⁴⁷

DC-SIGN (CD209) is primarily expressed on the cell surface of immature dendritic cells and also recognizes high mannose structures.⁴⁸ It recognizes various fungal species and is believed to mediate their uptake, although DC-SIGN-mediated activation induces an IL-10-biased cytokine response and is believed to suppress antifungal responses.^{49,50}

The collectins form a structurally related family of secreted CLRs, so named because they contain a collagenous domain.⁵¹ Three collectins—mannose-binding lectin and surfactant proteins A and D—are PRRs that bind microbe-specific terminal monosaccharides (mannose, fucose, n-acetyl glucosamine) with low affinity, but high avidity when ligands are clustered as they are on microbial surfaces.⁵² Collectins can serve as opsonins by binding to microbial cell walls and flagging them for phagocytosis and recognition by the complement system. Mannose-binding lectin (MBL) is an acute-phase reactant produced by the liver that recognizes terminal mannose residues of carbohydrates on gram-positive and gram-negative bacteria, fungi yeast, and some viruses and parasites.⁴² MBL's structural similarity to the complement component C1q allows it to opsonize microbes for phagocytosis via the C1q receptor, and activate the complement cascade through MBL-associated serine proteases related to C1r and C1s that cleave C4 and C2, leading to complement-mediated opsonization, neutrophil chemoattraction and phagocytosis, and pore formation causing cell lysis.

Two of the 4 pulmonary surfactant proteins (SPs), SP-A and SP-D, are collectins with similar structures: they share carbohydrate domains that bind oligosaccharide PAMPs such as bacterial LPS, tubercle lipoarabinomannan, and a variety of other bacterial lipids and viral proteins (eg, influenza hemagglutinin and neuraminidase envelope glycoproteins, respiratory syncytial virus G-fusion and F-fusion proteins).⁵³ SP-A and SP-D are secreted by alveolar type II cells and nonciliated bronchial epithelial cells. They aggregate and opsonize microbes for phagocytosis by a variety of immune cells, including alveolar macrophages, monocytes, neutrophils, and dendritic cells. They induce TLR-mediated NF- κ B activation and cytokine production via TLR-4 and TLR-2. SP-A induces the expression of scavenger and mannose receptors on phagocytes, thereby improving phagocytosis. SP-A and SP-D have direct bactericidal⁵⁴ and fungicidal⁵⁵ properties, and also help to dampen inflammatory responses by enhancing the uptake of proinflammatory apoptotic cells by macrophages and suppressing TNF- α release and the expression of MHC class II and costimulatory molecules by dendritic cells.^{56,57} One can appreciate a fuller review of lung collectins in a concurrently published article by Haczku.⁵⁸

Nucleotide-binding oligomerization domain-like receptors

The nucleotide-binding oligomerization domain (NOD)-like receptor (NLR) family of cytosolic PRRs are structurally similar, having leucine-rich repeat regions that bind and recognize PAMPs, and NODs that facilitate self-oligomerization during activation.⁵⁹ NLRs structurally resemble plant R-proteins that mediate antipathogen responses in plants. As cytosolic PRRs, they recognize microbial PAMPs that find their way into cellular cytoplasm.

The human NLR family has 23 members that can be conceptually organized in 2 groups. The best characterized, NOD-1 and NOD-2, recognize different core motifs of bacterial peptidoglycans, and their binding results in mitogen-activated protein kinase and NF- κ B activation.⁶⁰ NOD-1 is ubiquitously expressed and specific for a core motif of peptidoglycans of gram-negative, but only a few strains of gram-positive, bacteria.^{61,62} In comparison, NOD-2 detects the peptidoglycan muramyl dipeptide in all gram-positive and gram-negative bacteria.^{63,64} NOD-2 is produced in macrophages, dendritic cells, and Paneth cells under resting conditions, and is induced by TNF- α and IFN- γ in gastrointestinal epithelial cells.⁶⁵ NOD-2 has been of particular interest in Crohn disease because, in human beings, mutations in the NOD-2 gene are strongly associated with an increased risk for this disorder.⁶⁶

A different set of NLRs (including NLRP1, NLRP3, and NLRC4) form a protein scaffold for a signaling platform, a so-called "inflammasome," for caspase-1 activation and the processing and maturation of proinflammatory IL-1 β and IL-18.⁵⁹ These NLRs recognize a diverse range of microbial PAMPs that find their way into cellular cytoplasm, including anthrax lethal toxin (NLRP1),⁶⁷ bacterial flagellin (NLRC4),⁶⁸ bacterial and viral RNA (NLRP3),^{69,70} and bacterial pore-forming toxins such as nigericin and maitotoxin (NLRP3).⁷¹ As well, nonmicrobial cell stressors activate inflammasomes via NLRP3: urate crystals in gout,⁷² and reactive oxygen species induced by asbestos and silica exposure.⁷³

NLRP3 (also known as cryopyrin) has been of particular interest because it forms the genetic basis of 2 autosomal-

dominant cold autoinflammatory disorders, familial-cold autoinflammatory syndrome (FCAS) and Muckle-Wells syndrome.⁷⁴ A third disorder, neonatal-onset multisystem inflammatory disease, is also linked to mutations in the cryopyrin gene; neonatal-onset multisystem inflammatory disease joins FCAS and Muckle-Wells syndrome as variants of 1 disease now referred to as "cryopyrin-associated periodic syndrome."⁷⁵ These conditions are characterized by recurrent episodes of systemic inflammation in the absence of infection. In FCAS, minimal generalized cold exposure is the stimulus of the characteristic 1-day to 2-day episodes of fever, urticaria, and arthralgia; for the other related conditions, the inciting stimulus is unknown.

C-reactive protein

C-reactive protein (CRP), the prototypical acute-phase reactant to infections and inflammation in human beings, has been used as a clinical indicator of acute infections and response to treatment, and to assess inflammatory status in chronic diseases. Considering its rapid release from the liver in response to IL-6 and IL-1 β , it is perhaps logical that CRP is an innate PRR.⁷⁶ CRP is a pentraxin protein family member, with 5 identical protomer subunits in a cyclical arrangement. Its crystal structure reveals binding sites for 2 calcium molecules and 1 phosphorylcholine molecule per protomer.^{77,78} CRP specifically recognizes bacterial membrane phosphorylcholine. CRP binds activating Fc γ receptors (Fc γ RI and Fc γ RIIa) as well as inhibitory Fc γ RIIb because it has regions that are homologous to IgG's binding region. CRP can also activate the classic complement cascade by binding the complement factor C1q that can subsequently bind C1q receptors on phagocytes. CRP opsonizes bacteria by binding C1q and Fc γ receptors on phagocytes, promoting phagocytosis.

Interestingly, CRP also binds self-antigens: membrane phosphorylcholine that is not normally exposed on cell surfaces unless damaged, apoptotic, or necrotic. CRP also binds nuclear histone antigens, especially H1 and H2A.⁷⁹ This suggests that CRP may prevent autoimmune responses by masking and enhancing the clearance of self-antigens while promoting anti-inflammatory responses.⁸⁰ Although CRP can be antimicrobial, proinflammatory, and anti-inflammatory, current research suggests that its primary role might be the regulation of acute inflammation.⁷⁶

Other microbial PRRs

Other microbial PRRs that do not fall into the structure-function families described include the N-formylmethionine receptor. The sequence N-formylmethionine initiates all bacterial proteins, but only mitochondrial proteins in mammalian cells.⁸¹ Their engagement by the N-formylmethionine receptor recruits phagocytes and activates them for phagocytosis and killing. The MHC class I-b molecule of mice is a specific binding receptor for microbial n-formyl peptides, thereby forming an intriguing link between MHC class I and microbial PRR-type recognition.⁸² Other known cytoplasmic PRRs include retinoic acid-inducible gene-1 and melanoma differentiation-associated gene-5, helicases that recognize viral double-stranded RNA and mediate antiviral immune responses to different double-stranded RNA viruses.⁸³

Innate apoptotic sensors: Variations on a theme

Macrophages exert a fundamental homeostatic process of controlling inflammation through their ability to ingest and clear

apoptotic cells rapidly, recently termed *efferoctosis*.^{84,85} Apoptotic cells would inevitably release cytotoxic, proinflammatory constituents into their environment without this anti-inflammatory process of apoptotic cell recognition and uptake. Macrophages recognize apoptotic cells via molecular pattern recognition reminiscent of microbial recognition by innate immune cells. The plasma membrane of viable cells actively maintains an asymmetric phospholipid distribution such that phosphatidylserine is kept on the inner side of the membrane bilayer. Apoptosis perturbs this asymmetry and rapidly exposes phosphatidylserine on the cell's outer surface, leading to recognition.⁸⁶ Apoptosis also leads to the expression of calreticulin (a chaperone in endoplasmic reticulum) on the surface of apoptotic cells. Macrophages have phosphatidylserine receptors and surface low-density lipoprotein receptor-related proteins (CD91) that recognize phosphatidylserine and calreticulin, respectively, on the surface of apoptotic cells.⁸⁷⁻⁸⁹ On ingestion of apoptotic cells, macrophages release anti-inflammatory IL-10, prostaglandin E₂, and TGF- β to complete the task of maintaining immune homeostasis.⁹⁰

C-reactive protein, the collectins (mannose binding lectin, SP-A, SP-D), and the complement component C1q also bind to apoptotic cells and can mediate their clearance.^{56,87} This exemplifies common themes and multifunctional pathways in the innate immune system for both microbial and apoptotic cell sensing and clearance. The innate immune system relies on PRRs to recognize both microbial PAMPs and distinct "apoptotic cell-associated molecular patterns."^{87,91,92}

INNATE MICROBIAL SENSORS AND ALLERGY

Innate microbial sensors, front-line responders that mediate immune responses to microbes, may also mediate immune responses to nonpathogenic allergens. There is developing evidence that, like microbial antigens, allergens can engage innate PRRs and are processed through innate immune pathways that lead to pathogenic, allergic immune responses. Although the circumstances leading to allergic immunity in human beings are not clear, existing evidence suggests that allergic immune responses can originate in the innate immune system.

Innate sensing of allergens

Some of the innate PRRs have been shown to bind specific allergens and mediate their uptake and processing by innate immune cells. The uptake and presentation of the major dust mite allergen Der p 1 by myeloid dendritic cells is mannose receptor-dependent.⁹³ Grass pollen allergen is naturally contained in starch granules that are phagocytosed by alveolar macrophages in a CLR-dependent manner.⁹⁴ SP-D can also bind, aggregate, and enhance the phagocytosis of grass pollen starch granules by alveolar macrophages.⁹⁵ Developing research in mice implicates dectin-2 as the receptor on myeloid dendritic cells that recognizes glycans in common allergen extracts of *Dermatophagoides farinae*, *Dermatophagoides pteronyssinus*, and *Aspergillus fumigatus*, and induces the production of cysteinyl leukotrienes (Barrett NA, personal communication, August 2008). It is tempting to theorize that CLRs that bind a variety of allergens and are linked to such mediator pathways could induce allergic instead of antimicrobial or regulatory immune responses, perhaps when the concurrent engagement of cooperative microbial sensors is lacking.

Fungal allergens, grass pollen, and dust mite allergens have serine protease activity that can activate protease-activated

receptors expressed by epithelial cells, macrophages, monocytes, mast cells, and inflammatory cells.⁹⁶ House dust mite proteases Der p 1, Der p 3, and Der p 9 stimulate proinflammatory cytokine release by airway epithelial cells via protease-activated receptor 2.^{97,98}

Many naturally occurring allergens and allergenic extracts have been associated with microbial PAMPs. Commercial cockroach, dust mite (especially *D farinae*), house dust, and cat allergen extracts are high in endotoxin content; grass and ragweed pollen also have measurable endotoxin.⁹⁹ In contrast, grass and ragweed pollen and cat allergen extracts are high in β -1,3-glucan content when compared with dust mite extracts.¹⁰⁰ Allergen-associated endotoxin and β -glucan could come from extract-contaminating microbes (eg, bacteria or fungi in allergen sources or cultures) or inherent qualities of the allergens themselves; they might be covalently linked to the allergens. In the case of dust mites, the notably higher levels of endotoxin in *D farinae* versus *D pteronyssinus* cultures might be attributable to a greater burden of gram-negative bacteria (ie, *Bartonella* species) in the microflora of *D farinae*.¹⁰¹ Allergens that are covalently linked to microbial PAMPs could be guided to efficient TLR-mediated allergen processing and presentation in conventional immune pathways, leading to immune memory.¹⁰²

Inhibition of antimicrobial peptides by allergic inflammation in the skin

Antimicrobial peptides in the skin form a protective shield for primary host protection from bacterial and viral pathogens. The main skin AMPs, cathelicidin and human β -defensins 2 and 3, are reduced in atopic dermatitis (AD) lesional skin, their production suppressed by IL-4 and IL-13, products of the atopic immune response in AD skin.¹⁰³⁻¹⁰⁵ This reduction in cathelicidin and HBDs renders AD skin susceptible to herpesviruses and may account for the high burden of *Staphylococcus aureus* seen with severe AD.^{104,106-108} Reducing atopic inflammation reduces *S aureus* burden in AD skin¹⁰⁹ and may help to restore AMP production. Recent research revealed that vitamin D is activated in the skin in a TLR-2-dependent manner, which then promotes cathelicidin production by engaging a vitamin D response element in the cathelicidin promoter.¹⁰⁻¹³ One can appreciate a fuller review of skin AMPs in a recently published article by Schaubert and Gallo.¹¹⁰

TLR stimulation and allergy/asthma prevention in mouse to man

The potential of microbial TLR ligands to lessen allergy and asthma is consistent with the hygiene hypothesis (see reviews^{111,112}). In laboratory and clinical investigations of this theory, bacterial endotoxin has been the prototypical microbial PAMP. Experimental allergen (ovalbumin) without endotoxin (ie, adjuvant) does not induce a persistent immune response; ovalbumin with low-level endotoxin induces IgE-mediated allergic sensitization and a T_H2-type allergic inflammatory response.¹¹³ In contrast, higher levels of endotoxin with ovalbumin lead to nonpathogenic IgG and T_H1-type immune responses specific to the allergen.¹¹³ *In vitro*, endotoxin in higher concentrations induces dendritic cells to produce IL-12 and to costimulate T cells to become effector T_H1 cells.¹¹⁴⁻¹¹⁶ In rodent models, the timing of endotoxin exposure relative to allergen exposure

also alters allergy/asthma outcomes. Endotoxin before or within the first few days of allergen exposure induces a nonpathogenic IgG-specific response.¹¹⁷ In contrast, endotoxin exposure in the later stages of the response amplifies the pathogenic allergic immune response.

Because endotoxin is only 1 of many microbial PAMPs that exist in naturally occurring house dust, other microbial PAMPs have also been tested in murine models of allergic asthma. Synthetic oligonucleotide analogs to microbial DNA (ie, rich in unmethylated CpG TLR-9 ligands) prevented the development of atopic immune responses and allergic asthma or allergic rhinitis.^{118,119} In contrast, peptidoglycan (TLR-2 ligand) promoted an allergic asthmatic response.¹¹⁸ To assess the effect of a fuller burden of microbial PAMP exposures that better represents what occurs naturally, sterile but unpurified house dust extract given daily and at low doses was potentially effective in preventing allergic asthma development, similar to that observed with endotoxin alone.^{120,121} TLR-4-deficient mice unable to respond to endotoxin were well protected by sterile house dust extract.¹²¹ These rodent model experiments demonstrate the variable influence that TLR stimulation of the innate immune system can have on allergic outcomes. It can be a key stimulus to a pathogenic allergic immune response when exposure is low or when given after the allergic immune response is ongoing. Yet greater TLR stimulation, especially with multiple PAMP types invoking cooperative microbial PRR activation, and before an allergen-specific immune response has become established, might strongly promote a nonpathogenic response.

Murine models also suggest that endotoxin-mediated TLR stimulation may modify allergic responses to foods. The source of TLR ligands in this circumstance is the natural gut microflora. In murine studies, IgE-mediated peanut anaphylaxis could be induced in 3 strains of TLR-4-deficient mice, or in TLR-4-sufficient mice by administering antibiotics in early life to eliminate gut microflora.¹²² TLR-4-deficient mice could be protected against the development of peanut allergy by coadministration of CpG DNA to provide TLR-9 stimulation to substitute for their TLR-4 deficiency. The immunologic hallmark of the TLR-4-sufficient mice and the TLR-9 costimulated mice that were protected from developing peanut allergy was a peanut-specific IFN- γ (ie, T_H1), but not a T_H2 cytokine response *in vitro*. In this model of peanut allergy, the findings seem similar to the rodent models of allergic asthma: TLR stimulation by gut microflora or CpG DNA in early life converted an intragastric stimulus capable of inducing peanut allergy into a nonpathogenic T_H1 immune response.

Human epidemiologic studies of endotoxin exposure and allergy have generally revealed a picture that is consistent with the basic science and animal model evidence for a preventive influence of microbial PAMP exposure in early life. An early study associated higher house dust levels of endotoxin with lack of allergic sensitization in infants (including food allergen sensitization) and higher proportions of T_H1 cells in peripheral blood samples.¹²³ Since then, higher dust endotoxin levels have been associated with less atopic dermatitis,¹²⁴⁻¹²⁶ less inhalant allergen sensitization, less allergic rhinitis, and less atopy-associated asthma^{127,128} in infancy and childhood. Because this has been observed in multiple longitudinal birth cohort studies,^{124-126,127} as well as a large European rural farm/nonfarm study in which a dose-response relationship between endotoxin exposure and less atopy has been shown,¹²⁸ the strength of the current evidence

for a causal relationship is substantial. Numerous studies have also associated early-life animal exposure—especially to dogs, cows, and pigs, and animal farming or rural living lifestyles—with less atopic dermatitis, allergic sensitization, and/or asthma; some of these studies are also of a longitudinal, prospective study design, stronger for inferences of causation.¹³⁰⁻¹³⁵ These animals/lifestyles have been associated with higher levels of endotoxin in house dust and ambient air samples.^{129,136-139}

Other microbial PAMP exposures may also protect against the development of allergy and/or asthma. In addition to endotoxin, β -1,3-glucan, a fungal PAMP and dectin-1 ligand, was associated with less aeroallergen sensitization in toddler-age children.¹⁴⁰ Similarly, higher levels of fungal polysaccharides from *Penicillium* and *Aspergillus* in house dust have been associated with less doctor-diagnosed asthma in young children.¹⁴¹ A microbial component specific for bacterial peptidoglycan, *N*-acetyl-muramic acid, a TLR-2 and NOD-2 PAMP found in higher levels in farm homes, is associated with less wheezing in school-age children, although not associated with less allergic sensitization.¹⁴² DNA from dust samples from farm homes, farm barns, and rural homes in India was found to have a higher proportion of microbial DNA that was presumably rich in TLR-9 PAMPs.¹⁴³ When combined with a small amount of endotoxin, farm barn DNA augmented IL-10 and IL-12 production by PBMCs *in vitro*.

The protective farming lifestyle in European children has also been associated with higher peripheral blood expression levels of relevant microbial sensors: CD14, TLR-1, TLR-2, TLR-4, TLR-7, and TLR-8.^{133,144} Of particular interest, higher PBMC expression levels of CD14, TLR-2, and TLR-4 in farm children have been associated with maternal exposure during pregnancy to increasing numbers of farm animal species in barn stables, in somewhat of a dose-response relation; current exposures had little or no effect.¹⁴⁵ Accordingly, an attractive hypothesis for the protective effect of proximal living to animals is one of greater burden and diversity of microbial PAMP exposures in early life that heighten the expression of relevant microbial sensors as they shape the readiness of the innate and adaptive immune systems to respond to their environment in the most healthful manner, without allergy or asthma.

In contrast with these protective influences, higher endotoxin levels have been associated with more wheezing in infants and in nonatopic children, often in the same studies in which a protective effect on atopy was observed.^{124,126,128,146,147} This suggests endotoxin-sensitive wheezing phenotypes or asthma in infants and children, similar to endotoxin-sensitive asthma.¹⁴⁸⁻¹⁵⁰ Endotoxin augments allergic inflammatory responses in human lungs and noses¹⁵¹⁻¹⁵³ and induces bronchial obstruction and hyperresponsiveness in subjects with asthma.^{149,150} Accordingly, asthma severity and declines in lung function are associated with endotoxin exposure in common households¹⁵⁴⁻¹⁵⁶ and in many occupational settings.^{157,158}

TLR gene-environment interactions

An important corollary to these observations is that polymorphisms in endotoxin PRR genes might alter the likelihood and/or severity of atopy and allergic disease. In human beings, 2 substitution mutations in TLR-4 reduce airways and systemic responsiveness to inhaled endotoxin.^{159,160} In an investigation of 2 North American cohorts, these and other polymorphisms

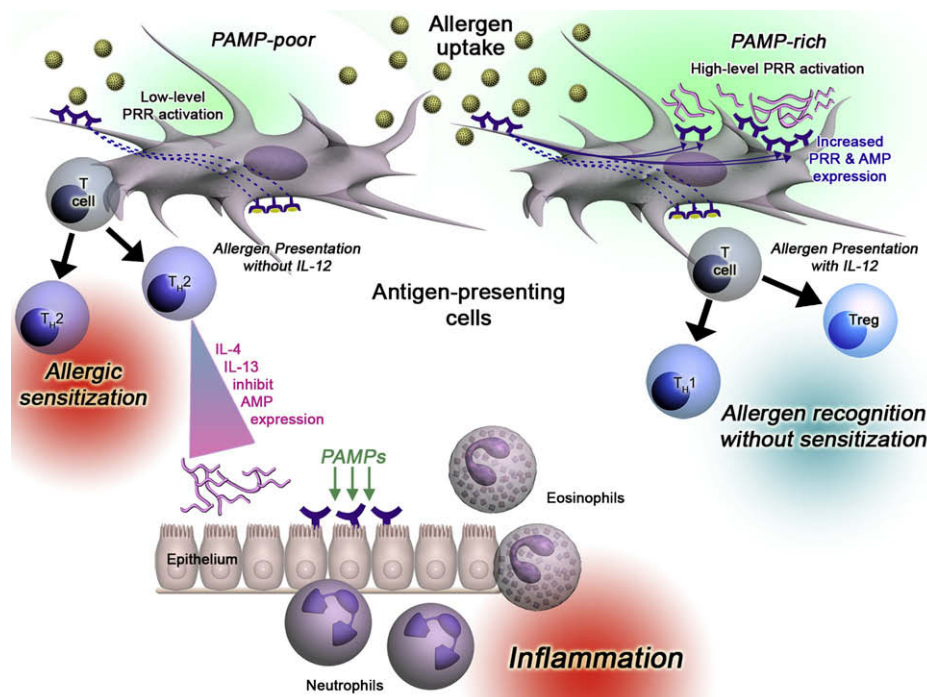


FIG 2. Innate microbial sensors and their relevance to allergy. Microbial PRRs may be relevant to the development of allergy in several ways: (1) some of the innate PRRs (eg, CLRs) can mediate the uptake and presentation of specific allergens (eg, dust mite, grass pollen, fungi) by APCs; (2) low-level activation of microbial PRRs caused by low-level PAMP exposure results in allergen presentation by APCs without IL-12, leading to T_H2 lymphocyte development and allergic sensitization; (3) T_H2 cytokines IL-4 and IL-13 inhibit the production of AMPs such as cathelicidin and human β -defensins in the skin, rendering the skin susceptible to microbial colonization and infection; (4) microbial PRR activation worsens ongoing allergic inflammation, further impairing AMP production, microbial burden, and disease severity; (5) in contrast, a PAMP-rich and PAMP-diverse environment induces high-level PRR activation, optimized by the cooperative activation of multiple PRR types and the induction of PRR expression that further heightens microbial sensing. High-level PRR activation results in allergen presentation with IL-12, leading to T_H1 /T regulatory lymphocyte (Treg) responses to allergen, without allergic sensitization. These differences in innate immune responses and development relative to a PAMP-poor versus PAMP-rich environment, and their impact on allergic outcomes, support the hygiene hypothesis and the atopy-protective effect of animal farming environments in early life.

in the TLR-4 gene were not associated with asthma.¹⁶¹ In a small cohort of Swedish children, 1 of the TLR-4 hyporesponsiveness polymorphisms (Asp299Gly) was associated with a 4-fold higher prevalence of asthma and reduced LPS-induced IL-12p70 and IL-10 release from PBMCs.¹⁶² In a large population study in Europe, the 2 TLR-4 hyporesponsiveness polymorphisms were associated with less bronchial responsiveness and wheeze, but only at moderate levels of exposure.¹⁶³ In the European farm/nonfarm setting, children with high endotoxin exposure had less atopy with only 1 of the 2 TLR-4 hyporesponsiveness polymorphisms; in the same investigation, the prevalence of asthma, atopy, and hay fever was significantly reduced in farm children with presumably high microbial PAMP exposure and a T-allele in a TLR-2 promoter region polymorphism; however, the functional significance of this TLR-2 promoter polymorphism is unknown.¹⁶⁴ Taken together, TLR-4 polymorphisms do not seem to have a major effect on allergy or asthma risk, especially in regard to the TLR-4 hyporesponsiveness mutations, and their effects on risk seem dependent on endotoxin exposure levels.

A common polymorphism in the promoter region (-260C-to-T) of the CD14 gene alters the transcriptional regulation of CD14,¹⁶⁵ such that the C-allele is associated with lower serum levels of

soluble CD14, higher serum IgE levels (in skin test-positive individuals), and more allergic sensitization.^{166,167} In infants and young children in a birth cohort study, only the low-responder CC homozygous CD14/-260 subgroup demonstrated strong dose-response relationships between higher house dust endotoxin levels and less subsequent allergic sensitization to inhalant allergens, as well as significantly less atopic dermatitis and more non-atopic wheeze.¹⁶⁸ In a French cohort in which the farming lifestyle was associated with a reduced risk of nasal allergies and allergic sensitization (odds ratios, 0.54 and 0.47, respectively), those with the high-responder CD14 TT/-260 genotype had an even lower risk of both (odds ratios, 0.26 and 0.21, respectively).¹³⁴ Conversely, in a study of adult farmers with presumably high exposure to endotoxin, those who were high-responder TT at CD14/-260 had significantly lower lung function and a higher prevalence of wheezing.¹⁶⁹

Microbial sensors and allergy: Clinical implications

These studies exemplify the epidemiologic research in human beings that supports a role for microbial PAMP exposures in altering allergy risk, and the beginnings of our understanding of the various roles of innate microbial sensors in allergic

sensitization and disease. Determinants of healthful outcomes from such exposures include early timing, moderate dosing, and genetic polymorphisms that influence innate recognition and response to these exposures. This is consistent with an innate immune paradigm that inadequate activation may not stimulate the full development of effector and homeostatic innate immune responses, resulting in the inadequate development of immune-regulatory processes, heightened adaptive T_H2 responses, and allergic consequences (Fig 2).

Although the structure and functional role of each microbial PRR have largely been characterized in isolation, it is increasingly appreciated that the recognition of intact microbes requires the engagement of many receptors of differing types to induce optimal immune responses.¹⁷⁰ Examples of microbial PRR cooperation include the optimal immune response to live *Candida albicans* that involves cooperative recognition of mannans and glucans by TLR-2, TLR-4, dectin-1, and mannose receptors.⁴³ The potency of sterile house dust extracts (presumably containing a diverse burden of microbial PAMP types) in attenuating allergic asthma, even in TLR-4-deficient mice, supports the relevance of this notion of microbial PRR cooperation to optimize allergy and asthma prevention.¹²¹ The requirement for receptor collaboration for optimal immune responses might explain, at least in part, how allergic instead of nonpathogenic antimicrobial or regulatory immune responses can develop, perhaps when the concurrent engagement of cooperative microbial sensors is lacking.

The potential of microbial PRR stimulation to alter allergy has led to some novel therapeutic interventions. Synthetic DNA-based TLR-9 ligands have been developed as immune adjuvants and therapeutic agents to modify the allergic response to allergens.¹⁷¹⁻¹⁷⁴ An immunostimulatory DNA conjugate to ragweed allergen administered as allergen immunotherapy induced long-lasting (3 seasons) suppression of seasonal increases in allergic rhinitis symptoms and allergen-specific IgE.¹⁷⁵ Probiotics have been tested in prevention and therapy trials for atopic dermatitis and allergic sensitization. A recent meta-analysis of 5 published, randomized, controlled prevention trials surmised a preventive effect of prenatal/postnatal supplementation on atopic dermatitis (relative risk, 0.61; 95% CI, 0.49-0.76).¹⁷⁶ In 1 multicenter study of 925 infants over a period of 2 years, probiotics appeared to be safe and associated with less respiratory infections and antibiotic prescriptions.¹⁷⁷ No significant effect from probiotic treatment of ongoing atopic dermatitis was determined from 4 published, randomized, controlled therapy trials.¹⁷⁶ The case for vitamin D supplementation as an intervention to augment cathelicidin production and regulatory immune responses in the skin of patients with atopic dermatitis has been recently demonstrated.¹¹⁰ In a recent controlled clinical study, vitamin D supplementation (4000 IU/d for 21 days) increased cathelicidin expression in lesional AD skin¹⁷⁸; it remains to be determined whether vitamin D supplementation can improve clinical AD outcomes. From these recent findings, we can attempt to normalize the AMP skin barrier and reduce the risk of skin infections in patients with AD by reducing atopic inflammation, improving skin integrity, and providing vitamin D supplementation when needed. The weight of current evidence for the biggest potential benefit from microbial PAMP exposures is in prevention in early life, before disease onset; this has not yet been well tested in human beings at an interventional level.

What do we know?

- The innate immune system senses microbes via a diverse repertoire of microbial pattern recognition receptors (PRRs).
- Microbial PRRs recognize molecular structures called pathogen-associated molecular patterns (PAMPs) that distinguish large groups of microbes from the host.
- Microbial PRRs, such as C-type lectin receptors (CLRs), can bind specific allergens and lead to their uptake by antigen-presenting cells (APCs).
- Endotoxin exposure and animal farming lifestyles in early life are associated with less allergic sensitization and disease.
- The production of antimicrobial peptides (AMPs) in the skin is inhibited by atopic immune responses and inflammation.

What is unknown?

- Does allergen uptake by microbial PRRs play a central role in the development of allergic sensitization in human beings?
- Can allergen uptake by microbial PRRs without sufficient cooperative induction of other microbial PRR pathways lead to allergic sensitization and disease?
- Can intervention with a PAMP-rich exposure in early life reduce allergy risk?
- Can vitamin D induce the production of antimicrobial peptides to improve clinical atopic dermatitis outcomes?
- Are innate microbial sensors relevant to the development of food allergies in human beings?

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Innate microbial sensors and their relevance to allergy

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Learning objectives: "Innate microbial sensors and their relevance to allergy"

1. To identify the innate microbial sensors and their diverse repertoire.
 2. To comprehend the structural and functional approaches to microbial recognition.
 3. To analyze current evidence for the relevance of the microbial sensors in allergic and inflammatory disorders.
-

CME items

Question 1. Pathogen-associated molecular patterns (PAMPs) are produced by —

- A. the host only.
- B. the host and the microbe.
- C. the microbe only.
- D. neither the host or the microbe.

Question 2. Antimicrobial peptides (AMPs) such as defensins and cathelicidin (LL-37) distinguish microbes from hosts based on the microbes' —

- A. tertiary structure of peptides.
- B. molecular weight.
- C. surface receptors.
- D. membrane charge.

Question 3. Which of the following is true regarding Toll-like receptors (TLRs)?

- A. TLRs are prototypical innate microbial sensors present in the cytoplasm of host cells.
- B. TLR4 recognizes unmethylated CpG sequences characteristic of microbial DNA.
- C. TLR signaling is similar to that of the IL-1 receptor in that both signaling pathways can lead to activation of nuclear factor- κ B.
- D. Endotoxin recognition by TLRs leads to host cell activation that can be enhanced by CD16.

Question 4. Bacterial endotoxin is a PAMP recognized by —

- A. TLR1.
- B. TLR2.
- C. TLR4.
- D. TLR6.

Question 5. Which of the following pattern recognition receptors contains an immunoreceptor tyrosine-based activation motif-like motif?

- A. mannose receptor
- B. dectin-1
- C. dectin-2
- D. dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (CD209)

Question 6. Which of the following statements is true?

- A. Collectins are a family of secreted receptors known as C-type lectin receptors that bind microbe-specific terminal monosaccharides and activate complement.
- B. Mannose-binding lectin (MBL) is a protease that cleaves C4 and C2, leading to activation of complement.
- C. MBL-associated serine proteases (MASPs) are acute phase reactants that recognize terminal mannose residues of carbohydrates on gram-positive and gram-negative bacteria, fungi yeast, and some viruses and parasites.
- D. Surfactant proteins A (SP-A) and D (SP-D) may enhance lung inflammatory response.

Question 7. Which one of the following disorders is associated with nucleotide-binding oligomerization domain 2 (*NOD2*) gene mutation?

- A. asthma
- B. diabetes mellitus
- C. thyroiditis
- D. Crohn disease

Question 8. NLRP3 (cryopyrin) mutations are associated with —

- A. sore throat.
- B. diarrhea.
- C. urticaria.
- D. photophobia.

Question 9. C-reactive protein may prevent autoimmune responses —

- A. by binding self-phosphorylcholine in damaged membranes and nuclear histone antigens.
- B. through activation of the classical complement cascade.
- C. through binding bacterial membrane phosphorylcholine.
- D. There is no evidence it can prevent autoimmune responses.

Question 10. Synthetic DNA-based TLR9 ligand has been tried successfully as an immune adjuvant to —

- A. *Der p1*.
- B. *Amb a 1*.
- C. *Phl p 5*.
- D. *Alt a1*.