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Immunodeficiency and Genetic Defects of Pattern-Recognition Receptors

Mihai G. Netea, M.D., Ph.D., and Jos W.M. van der Meer, M.D., Ph.D.

The susceptibility of adopted children to infection correlates more with the susceptibility of their biologic parents than with that of their adoptive parents. This suggests that genetic factors can increase the risk of infection. These factors are often genetic polymorphisms, but in a few cases monogenic defects underlie vulnerability to repeated infections. Genetic studies of susceptibility to infection have typically focused on defects of antibody production, or a lack of T cells, phagocytes, natural killer cells, or complement, each of which can cause a classic immunodeficiency syndrome. Recently, genetic defects have been identified that impair recognition of pathogens by the innate immune system, increasing susceptibility to specific classes of microorganisms. For example, defects in the interferon-γ pathway have been implicated in recalcitrant infections by intracellular pathogens such as mycobacteria and salmonella.

In this review, we focus on types of immunodeficiencies in which defects in pattern-recognition receptors and their downstream intracellular pathways predominate.

Pattern-Recognition Receptors and Innate Immunity

During an encounter with a pathogen, cells of the innate immune system recognize conserved structures of the microbe called pathogen-associated molecular patterns. Complement factor 1q, C-reactive protein, and fibronectin also recognize microbial structures but are not usually considered pathogen-recognition receptors. Cells of the innate immune system recognize pathogen-associated molecular patterns by means of germline-encoded pattern-recognition receptors, which allow for semispecific recognition of pathogens. An example of such a receptor is the macrophage mannose receptor, discovered in 1990. In 1992, Charles Janeway, Jr., took the field of innate immunity in a new direction with his concept of selective recognition of conserved microbial structures by pattern-recognition receptors.

There are four classes of pattern-recognition receptors: toll-like receptors (TLRs), C-type lectin receptors (CLRs), nucleotide-binding oligomerization domain (NOD) leucine-rich-repeat containing receptors (NLRs), and retinoic acid–inducible gene I protein (RIG-I) helicase receptors. Table 1 summarizes the properties of these receptors and the corresponding pathogen-associated molecular patterns. These patterns are constituents of microbial cell-wall components, microbial nucleic acids, or metabolic products. We use the term pattern-recognition receptor to mean a cellular receptor that causes activation of innate immunity; we do not include molecules that microorganisms use for attachment to and invasion of host cells.
Families of Pattern-Recognition Receptors

Toll-like Receptors
The role of TLRs in antimicrobial defense was described in 1996 by Lemaitre and colleagues, who observed that fruit flies lacking the insect homologue of a toll receptor rapidly die from aspergillosis. Since then, 13 mammalian TLRs have been discovered. All the extracellular domains of these receptors contain leucine-rich repeats that recognize microbial structures; the amino acid sequence of the cytoplasmic domain of the toll–interleukin-1 receptor is highly homologous with the sequences in the interleukin-1 receptor and the interleukin-18 receptor. Ligand recognition by TLRs and intracellular signal transduction by adaptor molecules activate kinase cascades and translocate transcription factors to the nucleus, where they induce gene expression and production of cytokines (Fig. 1). In addition to their role in innate immunity, TLRs and other pattern-recognition receptors activate antigen-presenting cells and bridge innate and adaptive immunity by coordinating responses of T cells and B cells.

C-Type Lectin Receptors
The large family of CLRs includes dectin-1, macrophage mannose receptor, dendritic-cell–specific intercellular adhesion molecule 3–grabbing non-integrin, dectin-2, and the circulating mannose-binding lectin. These receptors have carbohydrate-recognition domains and recognize microbial polysaccharides. They appear to be essential for recognition of fungi and bacteria in the body.

Cytoplasmic Pattern-Recognition Receptors
In addition to the mainly membrane-bound TLRs and CLRs, there are cytoplasmic pattern-recognition receptors — RIG-I helicase receptors and NLRs — that pathogens activate when they invade a cell. RIG-I helicase receptors are receptors mainly for the nucleic acids of viruses. NLRs recognize the peptidoglycans of bacterial cell walls and can activate inflammasomes, which are multiprotein complexes that convert inactive pro–interleukin-1β and pro–interleukin-18 into active cytokines (Fig. 1). The NOD-containing receptors 1 and 2 (NOD1 and NOD2), members of the NLR family, recognize the muramyl peptide moieties of the peptidoglycans of gram-negative and gram-positive bacteria, respectively. Interactions between the different pattern-recognition–receptor related pathways are essential for optimal antipathogen responses. Synergy between TLRs and NOD2, for example, is crucial for activation of the defense against mycobacteria and staphylococci, and cross-talk between TLRs and CLRs is needed for antifungal responses of the innate immune system.

Defects in Pattern-Recognition Receptors
Polymorphisms in genes encoding pattern-recognition receptors can affect susceptibility to infection. In addition, genetic defects in these receptors or the allied downstream pathways can cause immunodeficiency. We know of defects in three of the four classes of pattern-recognition receptors: TLRs, CLRs and NLRs (Table 2). To our knowledge, no deficiency of the RIG-I helicase receptors has been found.

Defects of TLRs
MyD88 and IRAK-4
Myeloid differentiation factor 88 (MyD88) is an adaptor molecule that transduces signals from TLR receptors (with the exception of TLR3) and from the receptors for interleukin-1 and interleukin-18. The signaling involves a cascade of protein kinases, including the serine–threonine kinase IRAK-4. Studies in the past decade have identified germline mutations in IRAK and MYD88 in patients with increased susceptibility to pyogenic bacteria and pseudomonas species. These defects in the TLR–interleukin-1 receptor pathway were found in young patients with recurrent, severe pneumococcal infections. The lymphocytes of one of the first reported patients had a poor response, in vitro, to stimulation with endotoxin and interleukin-1. The invasive infections associated with these mutations are usually meningitis and septicemia caused by Streptococcus pneumoniae and Staphylococcus aureus and less frequently by Pseudomonas aeruginosa and salmonella species. S. aureus is the main cause of localized infection, but P. aeruginosa and S. pneumoniae can also cause this complication.

Invasive infections begin to appear in infancy, with a cumulative mortality of 30 to 40%. Children with an MyD88 or IRAK-4 deficiency, by contrast, do not have major infections when they reach adulthood. This difference suggests that the development of protective T-cell and B-cell
Table 1. Overview of Pattern-Recognition Receptors (PRRs) and Their Respective Pathogen-Associated Molecular Pattern (PAMP) Ligands, According to PRR Class.*

<table>
<thead>
<tr>
<th>Recognized PAMP</th>
<th>Microorganism in Which PAMP Is Found</th>
<th>Signaling Molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>TLRs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLR2-1</td>
<td>Triacyl lipopeptides</td>
<td>Bacteria</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MyD88–TIRAP</td>
</tr>
<tr>
<td>TLR2-2</td>
<td>Peptidoglycan</td>
<td>Bacteria</td>
</tr>
<tr>
<td></td>
<td>Lipoarabinomannan</td>
<td>Mycobacteria</td>
</tr>
<tr>
<td></td>
<td>Phospholipomannan</td>
<td>Candida</td>
</tr>
<tr>
<td></td>
<td>Glycosylphosphatidylinositol</td>
<td>Trypanosoma</td>
</tr>
<tr>
<td>TLR2-6</td>
<td>Diacyl lipopeptides</td>
<td>Mycoplasma</td>
</tr>
<tr>
<td></td>
<td>Lipoteichoic acid</td>
<td>Streptococcus</td>
</tr>
<tr>
<td></td>
<td>Zymosan</td>
<td>Saccharomyces</td>
</tr>
<tr>
<td>TLR3</td>
<td>ssRNA virus</td>
<td>West Nile virus</td>
</tr>
<tr>
<td></td>
<td>dsRNA virus</td>
<td>Reovirus</td>
</tr>
<tr>
<td>TLR4</td>
<td>Lipopolysaccharide</td>
<td>Gram-negative bacteria</td>
</tr>
<tr>
<td></td>
<td>Fungal mannans</td>
<td>Candida</td>
</tr>
<tr>
<td></td>
<td>Envelope proteins</td>
<td>Respiratory syncytial virus</td>
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<tr>
<td>TLR5</td>
<td>Flagellin</td>
<td>Flagellated bacteria</td>
</tr>
<tr>
<td>TLR7 and TLR8</td>
<td>ssRNA viruses</td>
<td>Influenza virus, vesicular stomatitis virus</td>
</tr>
<tr>
<td>TLR9</td>
<td>dsDNA viruses</td>
<td>Herpes simplex virus</td>
</tr>
<tr>
<td></td>
<td>Cpg motifs</td>
<td>Bacterial and fungal DNA</td>
</tr>
<tr>
<td><strong>CLRs</strong></td>
<td></td>
<td></td>
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<tr>
<td>Mannose receptor</td>
<td>Fungal mannans</td>
<td>Candida</td>
</tr>
<tr>
<td>Dectin-1</td>
<td>Beta-1,3-glucans</td>
<td>Fungi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SYK–CARD9, RAF1</td>
</tr>
<tr>
<td>Dectin-2–FcRγ</td>
<td>Mannans</td>
<td>Candida hyphae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SYK–CARD9</td>
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<tr>
<td>MINCLE–FcRγ</td>
<td>Mannans</td>
<td>Candida</td>
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<tr>
<td></td>
<td>Mycobacterial cord factor</td>
<td>Mycobacteria</td>
</tr>
<tr>
<td>Mannose-binding lectin</td>
<td>Repetitive oligosaccharides</td>
<td>Bacteria and fungi</td>
</tr>
<tr>
<td><strong>NLRs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NOD1</td>
<td>Muramyl tripeptide peptidoglycans</td>
<td>Gram-negative bacteria</td>
</tr>
<tr>
<td>NOD2</td>
<td>Muramyl dipeptide peptidoglycans</td>
<td>Gram-positive bacteria</td>
</tr>
<tr>
<td>NLRP1</td>
<td>Anthrax toxin</td>
<td>Bacillus anthracis</td>
</tr>
<tr>
<td>NLRP3</td>
<td>Peptidoglycans</td>
<td>Bacteria</td>
</tr>
<tr>
<td></td>
<td>Bacterial toxins</td>
<td>Listeria, staphylococcus</td>
</tr>
<tr>
<td>NLRC4</td>
<td>Flagellin</td>
<td>Shigella, salmonella, legionella</td>
</tr>
<tr>
<td>AIM2</td>
<td>dsDNA</td>
<td>Francisella tularensis</td>
</tr>
</tbody>
</table>

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mediated immunity after infancy compensates for the defective TLR–interleukin-1 receptor pathway.40,44

**TLR5**
TLR5 is a receptor for flagellin, a protein that forms the pathogen-associated molecular pattern of the flagellum in flagellated bacteria.47 Hawn and colleagues44 described a polymorphism of TLR5 (consisting of a stop codon at position 392) that impairs recognition of flagellin and increases susceptibility to legionella pneumonia. The phenotype associated with this polymorphism is mild and affects the control of only certain flagellated pathogens. The frequency of this stop-codon allele in European populations is as high as 10%; carriers of the allele are susceptible to infection with *Legionella pneumophila*48 and to recurrent cystitis,49 but not to infection with the flagellated bacterium *Salmonella enterica serotype Typhi*, the agent of typhoid fever.50 Protective effects of this TLR5 polymorphism against systemic lupus erythematosus and Crohn's disease have been reported.51,52 The high and variable population frequencies of the polymorphism suggest that it has a redundant role in host defense.53

**Defects of CLR**
CLRs form a large family that specifically recognizes carbohydrate structures of microorganisms and endogenous ligands.15 They have a role in the recognition of fungal pathogens and mycobacteria.

*Dectin-1–CARD9 Pathway*
Dectin-1 is the major pattern-recognition receptor for beta-1,3-glucan in the fungal cell wall.54,55

**Table 1. (Continued.)**

<table>
<thead>
<tr>
<th>Recognized PAMP</th>
<th>Microorganism in Which PAMP Is Found</th>
<th>Signaling Molecule</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIG-I</td>
<td></td>
<td>IPS1</td>
</tr>
<tr>
<td>Short dsRNA</td>
<td>Paramyxoviruses, orthomyxoviruses, rhabdoviruses, flaviviruses</td>
<td>IPS1</td>
</tr>
<tr>
<td>5' Triphosphate ssRNA</td>
<td>Paramyxoviruses, orthomyxoviruses, rhabdoviruses, flaviviruses</td>
<td>IPS1</td>
</tr>
<tr>
<td>MDA5</td>
<td></td>
<td>IPS1</td>
</tr>
<tr>
<td>Long dsRNA</td>
<td>Picornaviruses, reoviruses, flaviviruses</td>
<td>IPS1</td>
</tr>
</tbody>
</table>

*AIM2 denotes absent in melanoma 2 protein, ASC apoptosis-associated speck-like protein containing a CARD, CARD9 caspase recruitment domain-containing protein 9, CLR C-type lectin receptor, CpG cytosine phosphate guanine, ds double-stranded, FeRγ Fc receptor IgE high-affinity I gamma polypeptide, IPS1 interferon-β promoter stimulator protein 1, MDAS melanoma differentiation-associated protein 5, MINCLE macrophage-inducible C-type lectin, MyD88 myeloid differentiation factor 88, NAIP5 NLR family apoptosis inhibitory protein 5, NLR nucleotide-binding oligomerization domain (NOD) leucine-rich-repeat–containing receptors, NLRC4 NLR family CARD-domain–containing protein 4 (also known as IPAF), NLRP NOD leucine-rich-repeat and pyrin domain–containing protein, RAF1 raf proto-oncogene serine–threonine protein kinase, RIG-I retinoic acid–inducible gene I protein, RIP2 receptor-interacting protein 2, ss single-stranded, SYK spleen tyrosine kinase, TIRAP toll-like–receptor adaptor protein, TLR toll-like receptor, TLR2-1 TLR2–TLR1 heterodimers, TLR2-2 TLR2–TLR2 heterodimers, TLR2-6 TLR2–TLR6 heterodimers, TRAM TRIF-related adaptor molecule, and TRIF toll-like receptor–adaptor molecule.41|

**TLR3–UNC93B Pathway**
Two classes of intracellular pattern-recognition receptors, the RIG-I helicase receptors and TLRs, recognize viruses. The intracellular receptors TLR3, TLR7, TLR8, and TLR9 bind to microbial nucleic acids.41 To our knowledge, no defects in the RIG-I helicase-receptor family are known. Patients with mutations in TLR342 or in UNC93B1, a protein in the TLR3 pathway,43 are prone to recurrent encephalitis caused by herpesvirus. The disease occurs mainly in early childhood (from 3 months to 6 years of age) during an initial infection with herpes simplex virus type 1.40,44

TLR3 deficiency seems to be associated only with encephalitis caused by a herpes simplex virus; children with the deficiency have normal resistance to other pathogens. Recurrences have been documented in two patients, suggesting a role for TLR3 in herpesvirus latency.40 A decreased capacity to release type I interferons was found in one patient's fibroblasts; blocking TLR3-dependent induction of interferons in vitro enhanced viral replication and caused cell death, effects that were reversed by recombinant interferon-β.42 The role of inadequate levels of type I interferons in susceptibility to herpesvirus encephalitis was also shown in a child who was deficient in the signal transducer and activator of transcription 1 protein,45,46 a signaling molecule in the type I interferon pathway.

**MECHANISMS OF DISEASE**

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and in unknown components of Mycobacterium tuberculosis. Genetic analyses of members of a family with recurrent vulvovaginal candidiasis and onychomycosis identified an early stop codon in CLEC7A (the gene encoding dectin-1), causing a loss of 10 amino acids from the extracellular carbohydrate-recognition domain of the protein. In consequence, the cell cannot display dectin-1 on its membrane, thereby negating the ability of monocytes to bind beta-glucans. This defect impairs the production of interleukin-6, tumor necrosis factor, and especially interleukin-17. In
contrast, neutrophils from affected persons can ingest and kill *Candida albicans* normally, indicating redundancy of dectin-1 in the phagocytosis and killing of yeast. Defective cytokine release most likely explains the phenotype. Defective dectin-1 signaling in epithelial cells and intraepithelial gamma–delta T cells may also contribute to the clinical picture, especially since these cells express dectin-1 and produce cytokines and antimicrobial peptides on activation.58,59

We should be cautious in concluding that genetic variants of the gene encoding dectin-1 have a role in disease. About 6 to 8% of Europeans are heterozygous for a disabling variant of the gene, which implies that approximately 1 in 400 Europeans has a deficiency of the protein; they do not, however, have an apparent immunodeficiency. In some African populations, such as the San people of southern Africa, the allele frequency reaches almost 40%.57 The phenotype of patients with dectin-1 deficiency is relatively mild and less severe than that of patients with classic chronic mucocutaneous candidiasis.60,61 Heterozygous carriers of the dectin-1 polymorphism are more likely to be colonized with *C. albicans* when undergoing stem-cell transplantation, and need antifungal therapy more often, than noncarriers.62 For all these reasons, dectin-1 deficiency resembles a genetic polymorphism, which in some people or under some circumstances is associated with susceptibility to fungi but not with severe immunodeficiency.

**CARD9**

Several members of a family with increased susceptibility to infection with fungi,63 mucocutaneous fungal infections in particular, have been shown to have mutations in caspase recruitment domain–containing protein 9 (CARD9), the adaptor molecule that mediates signaling induced by dectin-1. These clinical findings support the role of the beta-glucan recognition pathway in antifungal defense. A severe defect of interleukin-17 production has been reported in patients with CARD9 mutations.53 The more severe phenotype of CARD9 deficiency is most likely due to mechanisms that are independent of dectin-1.64-66

**MANNOSE-BINDING LECTIN**

Some components of the complement system that have the capacity to interact with microbial polysaccharides can function as recognition re-
ceptors. The complement molecule that most closely resembles a pattern-recognition receptor is mannose-binding lectin, which binds carbohydrate structures of microorganisms and activates the complement system. Deficiency in mannose-binding lectin was initially reported in an infant with recurrent bacterial infections and has been associated with bacterial infections (especially with *Neisseria meningitidis*), in addition to fungal and viral infections. Subsequent studies showed, however, that polymorphisms in the mannose-binding lectin gene result in low levels of mannose-binding lectin in approximately 40% of whites, with very low levels in about 8%. Most people in the general population with low or high levels of mannose-binding lectin do not have obvious clinical consequences. Thus, deficiency of mannose-binding lectin should be considered a risk factor for infection rather than an outright immunodeficiency.

Other leukocyte molecules that recognize microbial polysaccharides are the β2-integrins, such as complement receptor 3 (CD11b–CD18), which is known to function as a neutrophil beta-glucan receptor as well as playing a part in leukocyte adhesion. The immunologic defects in patients with β2-integrin deficiency (leukocyte adhesion deficiency I) are mainly due to defective leukocyte adhesion. Neither complement factors nor β2-integrins are considered classic pattern-recognition receptors.

Members of the large family of intracellular NLRs have two major functions: NOD1 and NOD2 recognize bacterial peptidoglycans, and NLR leucine-rich-repeat and pyrin domain–containing proteins (NLRPs) participate in the formation of inflammasomes and the processing of interleukin-1β–interleukin-18 (Fig. 1). One exception is deficiency of mannose-binding lectin (MBL), which leads to defective complement activation. IRF3 denotes interferon regulatory factor 3, NF-κB nuclear factor-κB, NOD2 NOD-containing receptor 2, RIP2 receptor-interacting protein 2, and SYK spleen tyrosine kinase.
cleotide-binding domain of NOD2. Missense mutations in the NACHT domain of NOD2 in several families with Blau’s syndrome caused a gain-of-function phenotype with increased basal activation of the transcription factor nuclear factor-κB and uncontrolled inflammation.

Does the NOD2 defect cause the inflammation in patients with Crohn’s disease? Most of the data indicate a loss-of-function mutation in NOD2, resulting in decreased production of defensins in the gut mucosa and an unregulated cytokine response. These abnormalities could impair the elimination of invading microorganisms in the mucosa and thereby cause reactive granulomatous inflammation. Studies of autophagy genes (ATG16L1 and IRGM), which encode proteins that regulate the breakdown by the cell of its own constituents, add weight to the hypothesis that genetic defects affecting innate immunity lead to Crohn’s disease. Neutrophil dysfunction and unregulated cytokine responses are also found in patients with Crohn’s disease, leading some researchers to suggest that the disease is a primary immunodeficiency syndrome of gut mucosal immunity.

NLRP3

The inflammasome is a protein platform that participates in activating caspase-1 and the proinflammatory cytokines interleukin-1β and interleukin-18. Members of the NLR family are components of inflammasomes. The most extensively studied member of the NLR family is NLRP3. The NLRP3 gene encodes cryopyrin, which, when activated, assembles with other proteins into an inflammasome. Mutations in NLRP3 occur in the cryopyrin-associated periodic syndromes, such as the familial cold autoinflammatory syndrome, the Muckle–Wells syndrome, and neonatal-onset multisystem inflammatory disorder (NOMID, also known as the chronic infantile neurologic, cutaneous, and articular syndrome). Features of the familial cold autoinflammatory syndrome are cold-induced fever, urticaria, and constitutional symptoms; characteristics of the Muckle–Wells syndrome are fever, hives, sensorineural hearing loss, and arthritis unrelated to cold exposure; and NOMID is characterized by fever, urticaria, epiphyseal overgrowth of the long bones, and chronic aseptic meningitis. In these syndromes, the high level of interleukin-1β, due to increased production, causes most of the symptoms. However, a secondary role of interleukin-18 and interleukin-33 cannot be ruled out because the inflammasome also processes these cytokines. Immunodeficiency is not a feature of this group of diseases, which are autoinflammatory syndromes. Deficiency of NLRP12, a protein that inhibits inflammation mediated by activated monocytes, has recently been found in a form of autoinflammatory syndrome.

CONCLUSIONS

The clinical features of defects in pattern-recognition receptors are usually due to disturbed cytokine homeostasis (Fig. 2) — either decreased cytokine production and increased susceptibility to infections (in the case of TLR3 or MyD88 deficiency) or overwhelming release of cytokines (in the case of NLRP3 defects). The clinical presentation ranges from severe (in cases of MyD88 and IRAK-4 deficiencies) to mild (in cases of mannose-binding lectin and TLR5 deficiencies). The clinical severity is greatest in infancy but decreases thereafter, perhaps because adaptive immune responses that develop after infancy compensate for the ineffective innate immunity. By contrast, the level of severity of classic primary immunodeficiencies usually persists throughout life.

In 2007, Casanova and Abel predicted that the field of primary immunodeficiency would shift from research on rare familial defects in the adaptive immune response to studies of sporadic and selective disorders of innate immunity that are more common than the classic immunodeficiencies. The defects in pattern-recognition receptors are an instructive example of this shift. The uncovering of these defects has given clinical relevance to immunologic pathways, which until now have been studied exclusively in the laboratory or in experimental models of infection.

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REFERENCES


41. Akira S, Hemmi H. Recognition of pathogen-associated molecular patterns


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