Innate immunity: an integrated overview

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Abstract

The innate immune system with its multiplicity of molecular sensing mechanisms detecting numerous pathogen-derived and self-generated molecular patterns is now known to play a role not only in defence against invading microorganisms such as microbes, parasites, viruses and fungi, but also in promoting disease processes initiated by the release of endogenous danger molecules from damaged or inflamed cells. Causative roles have currently been established in the pathophysiology of cardiovascular disease, ischemic inflammatory injury, lymphocytic leukemia, asthma, rheumatoid arthritis, chronic obstructive pulmonary disease, malignant melanoma, acute pancreatitis, diabetes and even chronic pain. Major mediating mechanisms involve Toll-like receptors, NOD-like receptors, retinoic acid inducible gene receptors, cytosolic DNA receptors and C-type lectin receptors, often in combination. Therapeutically targeting one or more of these sensors or pathways could lead to novel approaches to the treatment of a wide range of common disorders and inflammatory diseases. ■ Heart Metab; 2013;60:34–37

Keywords: C-type lectin receptors; innate immunity; NOD-like receptors; RIG-1-like receptors; Toll-like receptors.

Introduction

Current understanding of regulatory mechanisms underlying innate immunity has increased markedly over the past two decades. No longer are these integrated pathways viewed simply as a first line of defence against invading pathogens, such as bacteria, viruses and fungi, but are now also recognized as important sentinels and mediators of intrinsic pathophysiological events involved in inflammation, autoimmunity and chronic disease [1–10].

Five major groups of highly conserved membrane-bound and soluble receptors (PRR) have so far been identified that can recognize a broad range of characteristic pathogen-specific molecules (PAMP) or endogenous danger molecules released from damaged or dying cells (danger associated molecular patterns, DAMP). These include TLR, NOD-like receptors (nu-cleotide-binding oligomerization domain receptors), retinoic acid inducible gene receptors (RIG-1-like receptors), cytosolic DNA receptors and CLR.

PRR activation leads to the initiation of downstream mechanisms aimed at pathogen destruction and elimination, or initiation of sterile inflammation and autoimmune disease. In this sense the innate immune response may be a double-edged sword that requires careful regulation in order to avoid extensive and progressive autoimmune damage. Mediator molecules include IL-1β and IL-18, which stimulate interferon-gamma (IFNγ) production and initiate the development of T helper type 1 responses. This further amplifies cytokine release and triggers pathogen removal. Other mechanisms include the induction of microbial peptides, pyroptotic (caspase-1-dependent) cell death, phagocyte recruitment and induction of autophagy [11].

ABBREVIATIONS

AIM: absent in melanoma-2; **ASC:** apoptosis-associated speck-like protein containing a CARD; **CARD:** caspase activation and recruitment domain; **CD14:** Cluster of differentiation 14, a co-receptor; **CLR:** c-type lectin receptors; **CpG DNA:** a DNA site, cytosine and guanine separated by one phosphate; **CRD:** conserved carbohydrate recognition domains; **DAI:** DNA-dependent activator of IFN-regulatory factors; **DAMP:** danger associated molecular patterns; **DC:** dendritic cells; **DC-SIGN:** Dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; **DNGR-1:** DCNK lectin group receptor-1; **dsDNA:** double stranded DNA; **ICAM:** intercellular adhesion molecule; **IFN:** interferon; **IKK:** inhibitor of nuclear factor κ-B kinase; **IRF:** interferon regulatory factor; **LGP2:** Laboratory of Genetics and Physiology-2; **LR-RFIP1:** IL-β Leucine-rich repeat flightless-interacting protein 1; **MHC:** major histocompatibility complex; **mincle:** macrophage inducible C type lectin; **NEMO:** NF-κ-B essential modulator; **NF-κ-B:** nuclear factor kappa-B; **NLR:** NOD-like receptors; **PAMP:** pathogen associated molecular patterns; **PRR:** pattern recognition receptors; **STING:** stimulator of IFN genes; **TLR:** Toll-like receptors **TNF:** tumor necrosis factor

Toll-like receptors

TLR were the first and are the most characterized of all PRR so far studied. All are homologues of the *Drosophila* Toll gene, first identified in 1985 as an important factor in embryogenesis, immunity to fungal infections and later in 1997 in mammals as Toll-related protein (TLR4). All TLR (10 in humans) are type 1 transmembrane proteins that share a common structure composing a single membrane-spanning region, an N-terminal extracellular leucine-rich domain and a C-terminal cytoplasmic tail containing a conserved region known as the Toll/IL-1 receptor domain. Receptors have their own individual specificity and often recognize several PAMP. TLR2 is essential for the recognition of a broad range of PAMP, including bacterial lipoproteins, peptidoglycan and lipoteichoic acids, whereas others may be more specific. TLR3 is implicated in virus-derived double stranded RNA recognition. TLR4 is predominantly activated by lipopolysaccharide. TLR5 detects bacterial flagellin while TLR9 is required for response to unmethylated CpG DNA. TLR7 and TLR8 have recently also been shown to recognize small synthetic antiviral molecules. In many instances, TLR require the presence of a coreceptor to initiate the signaling cascade. TLR4, for example, interacts with MD2 and CD14, a protein that exists both in soluble form and as a glycophosphatidylinositol-anchored protein, to induce nuclear factor κB (NFκB) in response to lipopolysaccharide stimulation.

NOD-like receptors

NLR are intracellular cytoplasmic sensors that recognize a wide variety of PAMP, which enter the cell via phagocytosis or pores, as well as endogenous DAMP released in response to cell stress or damage. NLR are found throughout the animal kingdom in lymphocytes, macrophages and DC as well as some non immune cells, for example epithelium.

Activation of NLR proteins, NLRP3, NLRP1 and NLRC4 and the interferon inductible 200 family member absent in melanoma-2 (AIM2) results in the formation of large protein complexes termed inflammasomes. Once activated NLRP3, NLRP1, NLRC4 and AIM2 undergo a conformational change that allows interaction with an inflammasome-adaptor protein, ASC (PYCARD), which, in turn, interacts with caspase-1. The resulting inflammasome facilitates the autoactivation of caspase-1, which cleaves the pro-forms of IL-1β and IL-18 to active forms. Inflammasome activation is crucial for host defence to pathogens, but recent research has also identified a role in the pathogenesis of several inflammatory diseases such as type 2 diabetes, inflammatory bowel disease and atherosclerosis [12].

C-type lectin family

Soluble C-type (calcium-dependent) and membrane-bound lectin receptors (CLR) are a large family of antifungal innate immunity receptors that recognize a wide range of carbohydrates on pathogen surfaces. Type 1 receptors include DEC-205 and the macrophage mannose receptor, which contain several CRD and are transmembrane proteins. Type 2 receptors in contrast typically carry a single CRD and include Dectin-1, Dectin-2, mincle the DC-specific ICAM3-binding non integrin and DNGR-1, which are important in viral recognition, DC trafficking and the formation of the immunological synapse. Mannose-binding lectin is a soluble CLR that may play important roles in transplant rejection, cardiovascular disease and other secondary consequences of diabetes [13, 14]. CLR activation triggers key signaling path-

ways that induce the expression of specific cytokines or directly activate NFκB, thereby modulating signaling by TLR or triggering complement activation via the lectin pathway *(Figure 1)*. Therapeutically, CLR signaling may have important significance in the development of innovative approaches to vaccine development. Targeting specific CLR may be a powerful method to enhance antigenicity and influence whether antigen is presented in the context of MHC class I or MHC class II molecules. MHC class I presentation is vital for inducing strong CD8 T-cell responses, necessary for immunity to HIV-1. DNGR-1 may have particular significance because of its restricted pattern of expression to DC that may be exploited in cancer therapy [15].

RIG-1-like receptors

RNA helicase RIG-1 receptors (RIG-like receptors, RLR) are proteins that in general specifically recognize viral RNA and act as sensors of viral replication within the cytoplasm of human cells. They include the cytosolic RNA sensors RIG-1, MDA5 and LGP2 (encoded by the gene DHX58 and termed Laboratory of Genetics and Physiology 2). RIG-1 and MDA5 possess the ability to induce a cellular response via a so-called N-terminal caspase recruitment domain (CARD domain) when viral dsRNA is detected. Whereas LGP2, the remaining RLR, lacks the ability to induce signaling on its own (due to the absence of a CARD domain), it has recently been shown to be a potential coreceptor necessary for effective RIG-1 and MDA5-medi-
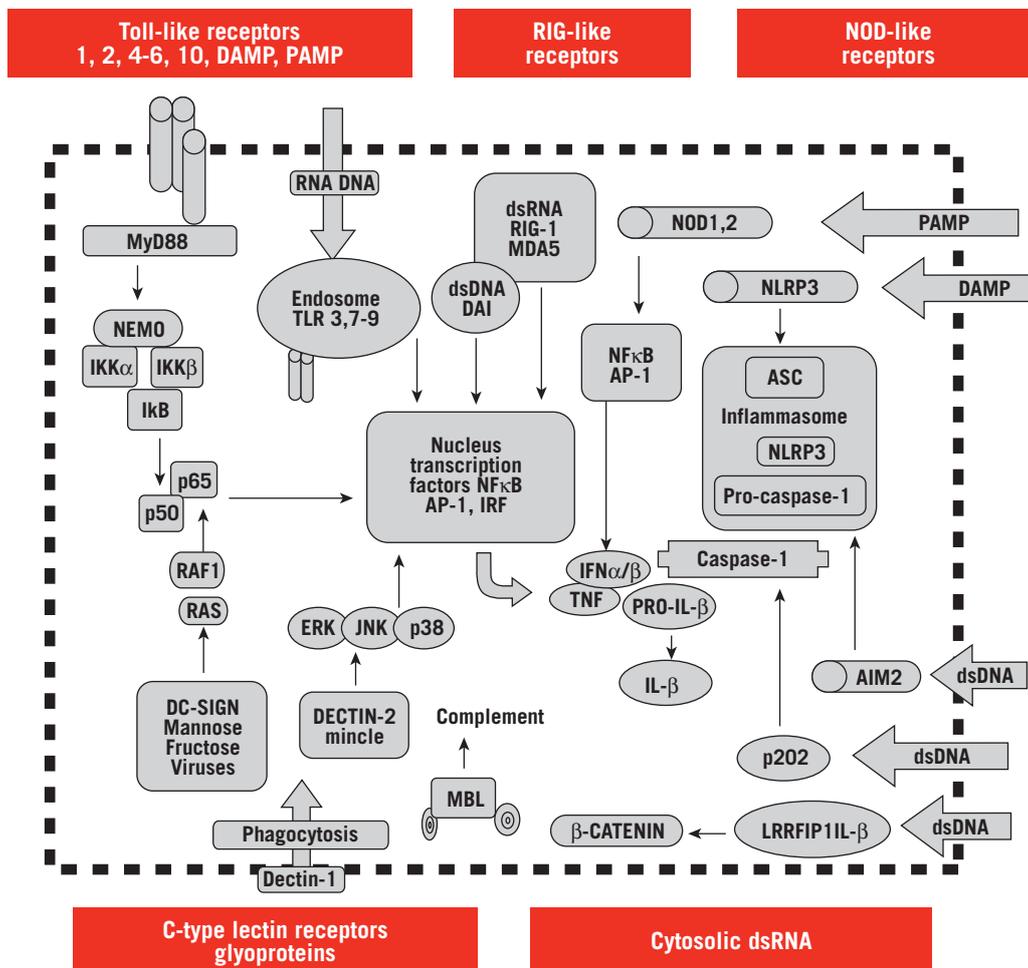

Fig. 1 *Schematic overview of the major molecular pattern-sensing mechanisms and downstream signaling cascades of the innate immune system. AIM2, absent in melanoma-2; AP-1, activator protein 1; ASC, apoptopsis-associated speck-like protein containing a CARD; DAI, DNA-dependent activator of IFN-regulatory factors; DAMP, danger associated molecular pattern; DC-SIGN, DC-specific ICAM3-binding non integrin; ERK, extracellular signal-related kinase; IFN, interferon; Ik, inhibitor of nuclear factor κ; IKK, inhibitor of nuclear factor κ kinase; IRF, interferon regulatory factor; JNK, c-jun N-terminal kinase; LRRFIP, leucine-rich repeat flightless-interacting protein; MBL, mannose-binding lectin; NEMO, NFκB essential modulator; NFκB, nuclear factor κB; NOD-like receptor, nucleotide-binding oligomerization domain receptor; PAMP, pathogen associated molecular pattern; RIG-like receptor, retinoic acid inducible gene receptor; TLR, Toll-like receptor; TNF, tumor necrosis factor.*

ated antiviral responses to certain ligands. Abberant RLR signaling or dysregulated RLR expression has been implicated in the development of autoimmune diseases, therefore RLR-targeted therapeutics may be useful for antiviral and immune-modifying applications [16].

Cytosolic dsDNA sensors

While the recognition of extracellular DNA involves mainly TLR9, recognition of cytosolic DNA involves a complex array of sensors including DNA-dependent activator of IFN-regulatory factors (DAI) and leucine-rich repeat flightless-interacting protein (LRRFIP1), encoded by the *LRRFIP1* gene that trigger different signaling pathways in a cell-specific manner.

The first identified cytosolic DNA sensor, termed DAI, binds cytosolic dsDNA and leads to the production of type I interferon. Furthermore, the DNA sensor IFI16 (gamma-interferon-inducible protein I), part of a larger protein family termed the pyrin and HIN domain (PYHIN) family, has been found to recruit STING, an endoplasmic-resident transmembrane protein induced by an IFN-inducible ligase, to activate a TANK-binding kinase/interferon regulatory factor-dependent pathway to IFN-β induction.

Another member of the PYHIN family, AIM2, is a cytosolic DNA receptor that forms an inflammasome with ASC, a common adapter of inflammasomes, leading to caspase-1 cleavage and secretion of IL-1β and IL-18. p202 is yet another member of the PYHIN family that binds cytoplasmic dsDNA but, in contrast to AIM2, represses caspase activation *(Figure 1)*.

On the other hand, the cytosolic nucleic acid-binding protein LRRFIP1, on binding dsDNA triggers the production of IFN-β in a β-catenin-dependent manner. β-Catenin binds to the C-terminal domain of IRF3 inducing an increase in IFN-β expression. More recently, the helicase DDX41 has been identified as an additional DNA sensor that depends on STING to sense pathogenic DNA. Therefore, the recognition of cytosolic DNA is considerably more complicated than first anticipated. Clearly, several sensors have been identified that trigger different cell-specific signaling pathways. The general consensus, however, is that yet another unknown cytosolic DNA recognition system may exist. Additional studies to elucidate the complex mechanisms of cytosolic DNA recognition may facilitate the development of new strategies to treat inflammatory diseases [16–18]. ■

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Can infective agents be respectable etiopathogenetic factors for acute coronary syndromes?

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A policeman sees a drunk man searching for something under a streetlight and asks what the drunk has lost. He says he lost his keys and they both look under the streetlight together. After a few minutes the policeman asks if he is sure he lost them here, and the drunk replies, no, that he lost them in the park. The policeman asks why he is searching here, and the drunk replies "this is where the light is".

Atherosclerosis is a multifactorial disease and, among others, inflammation and activation of immune system play well established roles [1, 2]. In ACS, epicardial thrombosis with abrupt vessel occlusion is a crucial final event, initiated at the site of a "vulnerable plaque" [3]. Until recently, plaque rupture was considered predominantly mechanical, occurring at sites of vessel narrowing with turbulent blood flow [4]. However, removal of coronary stenosis has never proved to prevent ACS. On the other hand, exacerbation of inflammatory [5] and specific immune mechanisms has been implicated in platelet function modulation and thrombus formation in ACS [6, 7]. Therefore, pathophysiological pathways underlying the dynamic changes that ultimately cause coronary thrombotic occlusion represent an area of intense interest and research.

Inflammatory response in ACS includes systemic immune activation, local inflammation of the atherosclerotic plaque and immune reactions associated with the thrombotic event itself [8, 9]. Given the profound involvement of immune activation in ACS, infections and other systemic inflammatory reactions have also been proposed to increase the risk of ACS. Indeed, up to 30% of myocardial infarctions occur after upper respiratory tract infections [10], and chronic infectious agents such as *Chlamydia pneumoniae* or oral pathogens, initially linked to atherosclerosis, have been found to increase the risk of ACS [11–13]. In a very recent issue of *Circulation*, Pessi et al [14] assessed bacterial DNA in thrombus aspirates of 101 patients with STEMI and sought to determine the association between bacterial findings and oral pathology. They used real-time quantitative polymerase chain reaction with specific primers and probes to detect bacterial DNA from several oral species and *C. pneumoniae*. Bacterial DNA typical of endodontic infection was identified in 78.2% of thrombi, and periodontal pathogens were measured in 34.7%. In addition, bacteria-like structures (including whole bacteria) and monocyte/macrophage markers for bacteria recognition and inflammation were detected by transmission electron microscopy and immunohistochemistry analysis, respectively. In a subgroup of 30 STEMI patients examined with panoramic tomography, there was a significant association between periapical abscesses and oral viridans streptococci DNA-positive thrombi. The authors concluded that dental infection and oral bacteria, especially viridans streptococci, may be associated with the development of acute coronary thrombosis.

Such results are in line with another recent study, which also showed a lack of association between the severity of coronary atherosclerosis and periodontal

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ABBREVIATIONS

ACS: acute coronary syndrome; **STEMI:** ST-segment elevation myocardial infarction

bacteria [15]. A number of mechanisms that explain an infective etiology of atherosclerosis and ACS, including direct effects on vascular cells, circulating cytokines and inflammatory mediators, as well as initiation of autoimmune reactions have been proposed [16]. Returning to the above-mentioned study, the presence of bacterial DNA together with co-stimulation of immune-specific cells in the thrombus aspirates may suggest that these pathogens disseminate into systemic circulation, migrate to coronary plaques, and cause and/or maintain inflammation of the coronary artery [17].

At present the role of infective agents in ACS is not completely understood. Nonetheless, antimicrobial therapies have already been tested in ACS prevention trials [18–20]. Although treatment results have been contrasting, the objective evidence of bacterial particles in the coronary thrombi should further enhance research in this direction. Indeed, while technological progress has permitted continuous improvement in coronary artery plaque and thrombus removal, this should not prevent us from exploring other, maybe less evident, but probably as relevant causes of ACS. ■

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