Insight into the mechanisms regulating immune homeostasis in health and disease

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Summary

Innate and adaptive immune systems consist of cells and molecules that work together in concert to fight against microbial infection and maintain homeostasis. Hosts encounter microbes / exogenous pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs) all the time and they must have proper mechanisms to counteract the danger such that appropriate responses (e.g., degree of inflammation and types of mediators induced) can be mounted in different scenarios. Increasing numbers of endogenous danger signals of host origin are being identified including, for example, uric acid and cholesterol crystals, high mobility group box1 (HMGB1) protein, oxidized LDL, vesicans, heat shock proteins (HSPs) and self DNA. Many of these endogenous ligands have been shown to be associated with inflammation-related diseases like atherosclerosis, gout and type 2 diabetes. Several DAMPs appear to have the ability to interact with more than one receptor. We are now beginning to understand how the immune system can distinguish infection from endogenous ligands elaborated following cellular insults and tissue damage. Appropriate responses to maintain the homeostatic state in health and disease depend largely on the recognition and response to these stimuli by germline encoded pattern-recognition receptors (PRRs) present on both immune and non-immune cells. These receptors are, for example, Toll-like receptors (TLRs), C-type lectin receptors (CLRs) and cytosolic receptors (e.g., RLRs, NLRs and some intracellular DNA sensors). Atypical PRR "danger" receptors, like the receptor for advanced glycation end products (RAGE) and their ligands have been identified. A proper response to maintain homeostasis relies on specific negative regulators and regulatory pathways to dampen its response to tissue injury while maintaining the capacity to eliminate infection and induce proper tissue repair. Moreover, some PRRs (e.g., TLR2,TLR4 and NLRP3) and atypical PRRs can recognize both PAMPs and DAMPs, either as single entities or after forming complexes (e.g., immune complexes, or DNA- HMGB1 and DNA-LL37 complexes), so there must be a mechanism to selectively depress or alleviate the inflammatory response to DAMPs, while leaving that of PAMPs intact. Excessive inflammatory responses can induce considerable tissue damage and can be highly detrimental to the host. For example, CD24 reacting with HMGB1 and HSPs has been implicated to function as negative regulator for RAGE. In this review, I will briefly overview the information on various host and microbial components and bring together the information to synthesize a model to explain how homeostasis can be maintained in states of health and disease. Understanding the molecular mechanisms by which the immune system functions under different scenarios will provide us with ways and means to design appropriate approaches, for example, to prevent or treat autoimmune and inflammatory diseases or the ability to design new drugs or formulate safe chemicals for vaccine adjuvants. *(Asian Pac J Allergy Immunol 2011;29:1-14)*

Key words: *damage-associated molecular pattern, danger signal, homeostasis, immunoregulation, pathogen-associated molecular pattern, pattern-recognition receptor, Toll-like receptor, NLRP*

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Abbreviations:
AIM2 = absent in melanoma
ALR = AIM2-like receptor
APC = antigen presenting cell
CLR = C-type lectin receptor
DAI = DNA-dependent activator of interferon regulatory factors
DAMP = damage-associated molecular pattern
DC = dendritic cell
DC-SIGN = dendritic cell-specific intracellular adhesion molecule-grabbing non-integrin
HMGB1 = high mobility group box1
HSP = heat shock protein
IFN = interferon
IL = interleukin
LPS = lipopolysaccharide
LRR = leucine-rich repeat
ITIM = immunoreceptor tyrosine-based inhibitory motif
MAMP = microbe-associated molecular pattern
MBP = mannose-binding protein
miRNA = microRNA
NALP = Natch domain-, leucine-rich repeat-, and pyrin-containing domain
NLRP3 = NOD-, LRR- and pyrin-domain containing 3
NLR = nucleotide oligomerization domain (NOD)-like receptor
NOD = nucleotide binding and oligomerization domain
PAMP = pathogen-associated molecular pattern
PRR = pattern-recognition receptor
PS = phosphatidylserine
PTP = protein tyrosine phosphatase
RAGE =receptor for advanced glycation endproducts
RIG-I = retinoic acid-inducible gene I
RLR = RIG-I-like receptor
ROS = reactive oxygen species
SHP = Src homology (SH)-2-containing PTP
SHIP = SH2-containing inositol-5-phosphatase
TLR = Toll-like receptor
TRX = thioredoxin
TXNIP = thioredoxin-interactive protein

The immune system is well known for its function in defending a host against microbial invasion. However, in addition to the defensive function, it must also be able to distinguish between infection and tissue damage and know how to respond appropriately to these 2 scenarios. It was not until recently that some clues were available to explain this phenomenon, particularly when sensors for cellular damage and metabolic stress were identified and described. The discrimination between self and non-self paradigm of the immune response has dominated the field of immunology for several decades.[1] This "self-non-self" model, which was originally proposed in the late 50s by Sir Frank MacFarlane Burnet, enhanced our understanding of immune responses and paved the way for him and his colleague Peter Brian Medawar to win the Nobel Prize in Medicine in 1960. However, this original model fails to explain many fundamental immunological phenomena, for instance why the body rejects transplants and not fetuses or tumors, or why it responds poorly to purified inert foreign proteins. Therefore, after its inception in the late 50s, it had been criticized and modified several times to accomodate new findings. A significant major modification was the introduction of antigen-presenting cell (APC) into the model and the observation that the latter must be activated before it could stimulate lymphocytes.[2-4] This major modification, proposed by Charles A. Janeway, Jr. in the late 80s, is known as the "infectious non-self" model.[2] It is well documented now that the activation of APCs depends on the ability of these APCs to recognize and differentiate different groups of microbes using special receptors.[3,4] The recognition of pathogen-associated molecular patterns (PAMPs) or microbe-associated molecular patterns (MAMPs) by pattern-recognition receptors (PRRs) present on the APCs allows these cells to discriminate between "infectious non-self" and "noninfectious self".[5] This proposal, sometime referred to as "PRR" model, has revolutionalized our understanding of immune response and provided us with a novel conceptual framework for the regulation of adaptive immunity by innate immunity.[6,7] Prior to this proposal a pioneering discovery of dendritic cell (DC) was made by Ralph Steinman and a subsequent demonstration of its function as an APC has further advanced our understanding on how the immune response is

initiated and regulated.[8,9] This was followed by the identification and characterization of these receptors and the various signaling pathways by Shizuo Akira,[10-12] Bruce Beutler[13-15] and others.

Although the Janeway's "infectious non-self" model proposed more than 20 years ago allowed us to solve many previous problems that the original "self-non-self" model faced, there are still some unsolved issues, including aspects of autoimmune phenomenon, sterile inflammation or why transplants are rejected by the recipient's immune system.[6] The two hypotheses proposed by Sir MacFarlane Burnet[1] and by Charles Janeway[2] have one common principle and that is both suggesting that "non-self" triggers the immune response, while "self" does not. It was not until a few years later that a totally novel idea was introduced by Polly Matzinger and this new proposal is now known as "danger" model.[16] This novel proposal bravely challenged the long-standing "self-non-self" hypothesis and caused a great deal of controversy in immunology. By adding another layer of cells into the hypothesis, this new proposal offered an explanation as to how the immune response is triggered in different scenarios (e.g., infection-induced *vs.* sterile inflammation) and how it ends.[17-19] At the time of its proposal there was, relatively speaking, no experimental evidence to support it. However, substantial evidence is now available to support this idea and the model is now well accepted by contemporary immunologists worldwide. In brief, this model hypothesizes that the APCs are activated not by the infectious non-self (PAMPs/MAMPs), but by danger or alarm signals generated from injured host cells, damaged tissues or metabolic stress.[16,17] Matzinger's viewpoint is that the primary function of the immune system is to detect and protect host against danger and that the initiation of immune response is not the foreignness or stranger microbes (non-self), but is the alarm signals generated from injured or damaged cells and tissues. To conform to the term "pathogen-associated molecular pattern", the term "damage-associated molecular pattern" (DAMP) was subsequently coined for these endogenous danger signals. The identification of these stimuli and putative receptors has provided significant insight into the initiation and regulation of innate immune response.[20,21]

Altogether, it now appears that immunity is concerned with the recognition of danger and damage and not with foreignness that has prevailed as our belief for several decades. The immune response is therefore the process the body uses to restore homeostasis after encountering an enemy from outside or endogenous danger from cellular insult and tissue damage. Matzinger subsequently extended the original version of her "danger" model by suggesting that DAMPs could not only activate the APCs, but could also educate them in such a way as to provide the host with the type of immune response that would best fit that particular scenario (e.g., the immune response in mucosal tissues).[19] In this communication, I will briefly overview only the pertinent points and attempt to elucidate the possible mechanism whereby the host may discriminate PAMPs/MAMPs (exogenous/stranger/non-sterile) from DAMPs (endogenous/altered self/ danger/damage). With the data currently available, I will attempt to explain how the same receptors can generate different outcomes when they encounter stimuli of exogenous or endogenous origin. More extensive and excellent reviews on cellular receptors and ligands by different experts are available and presented elsewhere.

Exogenous and endogenous ligands for pattern-recognition receptors (PRRs).

It is not difficult to comprehend the meaning of PAMPs or MAMPs, as they represent molecular components that are invariably present on groups of pathogens or microbes and are distinct from those found in the host. These conserved microbial components, largely carbohydrates and glycolipids in nature, are needed for their own survival.[17,18] Those that make up the microbial cell envelope (e.g., lipopolysaccharide and peptidoglycan) can be readily detected by the host cell surface PRRs (e.g., TLRs and CLRs), while the intracellularly located components (e.g., bacterial and viral nucleic acids) can be recognized by cytosolic PPRs (e.g., RLRs and NLRs) after host cell invasion or after being phagocytosed by phagocytes.[10-12,14,15] A large majority of endogenous DAMPs are normally sequestered intracellularly and are not exposed to the immune system under normal physiological conditions but are released as a result of cell injury or cell death

Table1. Representative DAMPs and their putative receptors

Extracellular DAMPs	Putative receptors	Intracellular DAMPs	Putative receptors
Hyaluronic acid	TLR2, TLR4	Uric acid crystals	NLRP3
Heparan sulfate	CD44, TLR4, TLR2	Cholesterol crystals	NLRP3
Defensins	TLR4	HSPs	TLR2,TLR4
Cathelicidins LL37	TLR4	DNA	AIM2, DAI, IFI16, TLR9
HMGB1	RAGE, TLR2,TLR4	RNA	TLR3
Oxidized LDL	TLR4, CD36	TXNIP	NLRP3
Fibronectin	Integrin	K^+efflux	NLRP3
Laminin	Integrin		
Collagen derived peptides	CXCR2		
Chromatin and ribonucleoprotein complexs	TLR7,TLR9, CLRs		
β-amyloid	RAGE,CD36, NLRP3		
Extracellular ATP	NLRP3 (via P2RX7)		

(Table 1).[19,20] Intracellular stores of proinflammatory cytokines (e.g., IL-1 and TNF-α and chemokines can also be released upon cell lysis and function as DAMPs.[21] In addition to the components of intracellular origin, there are a number of extracellular DAMPs that have been identified.[20,21] The latter are generally released as a breakdown product of extracellular matrix and include, for example, heparin sulfate, biglycan and hyaluronan. In addition to these components, inflammatory signals can also be generated by uric acid released from necrotic cells.[20-23] Self DNA can be recognized by DNA sensors when it is altered or inappropriately introduced into the cytosol by diseases or trauma (Figures 1 and 2)[24-26]. It is worth mentioning here that a nuclear protein known as HMGB1 (high mobility group box 1) appears to be an important DAMP that has received considerable attention in recent years, as it is known to be secreted from living cells as well as being released from dying cells.[24,27] It can interact directly with RAGE or can form complexes with DNA or nucleosomes and this complex then reacts with DNA sensors like TLR9.[10-12,24] In fact, it has been proposed that the HMGB1 protein acts as the universal sensor of cytoplasmic nucleic acids that leads to activation of downstream receptors (e.g., TLRs, RLRs and ALRs).[24-27] It is quite obvious from the above discussion that although endogenously generated DAMPs are structurally highly diverse and appear to be unrelated, the outcome of stimulation is similar,

Table 2. Examples of membrane-associated receptors and their putative ligands

Receptors	Ligands	
	Exogenous (non-self) PAMPs/MAMPs	Endogenous (self) DAMPs
Cell surface		
TLR1-TLR2	Triacylated lipopeptide (bacteria)	Lipoprotein
TLR2-TLR6	Diacylated lipopeptide (bacteria)	Lipoprotein
TLR4	LPS (bacteria) Taxol (plant)	HMGB1, HSPs, fibronectin Oxidized phospholipids Oxidized LDL Heparan sulfate
TLR5	Flagellin (bacteria)	
Dectin 1, 2	β-glucan, mannan (fungi)	
MINGLE	Nuclear protein (SAP130)	SAP130 (nuclear protein from damaged apoptotic cells)
MR	Mannose (microbes)	
RAGE		Advanced glycation end products, HMGB1, β-amyloid
CD36		Oxidized LDL, apoptotic cell
Complement receptors		C degradation products
FcR		Fc fragment of Igs and immune complexes
Endosome		
TLR3	dsRNA (viruses)	self RNA
TLR7	ssRNA (viruses, bacteria)	self DNA
TLR9	DNA (bacteria, viruses, parasite) CpG DNA	self DNA

and that they all induce inflammatory responses. Table1 shows representative examples of DAMPs together with their putative receptors.[10-12,15,,20,21,27] In addition to the stimuli originating from cellular insults just mentioned, exogenous ligands of non-microbial origin have also been identified. These include, for example, environmental irritants (e.g., silica, asbestos and ultraviolet light), skin irritants (e.g., trinitrochlorobenzene sulfonate), plant extract (e.g., taxol) and vaccine adjuvant like alum.

Pattern recognition receptors PRRs.

These germline-encoded sensing receptors can be classified into cell-associated (predominantly present on APCs, e.g., DCs and macrophages) and secreted forms. Some of these cell-associated receptors are found either on the cell plasma membrane (e.g., TLR1, TLR2, TLR4, TLR5 and

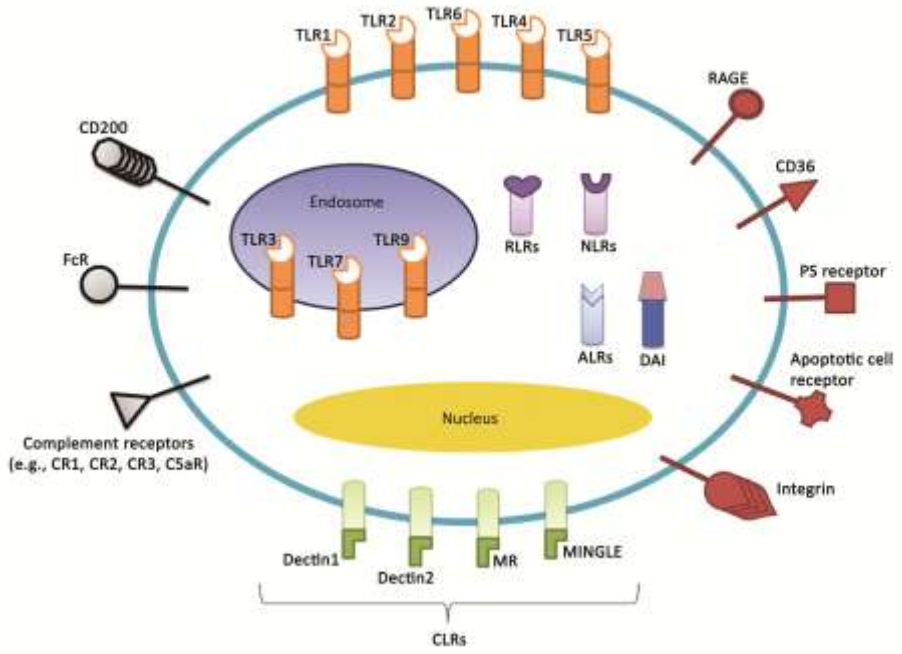

Figure 1. Pattern-recognition receptors (PRRs) and atypical PRRs on a hypothetical mammalian cell. Only those referred to in the discussion are shown in the diagram. Abbreviations: CLRs, C-type lectin receptors; TLR, toll-like receptor; PS, phosphatidylserine; RAGE, receptor for advanced glycation endproducts.

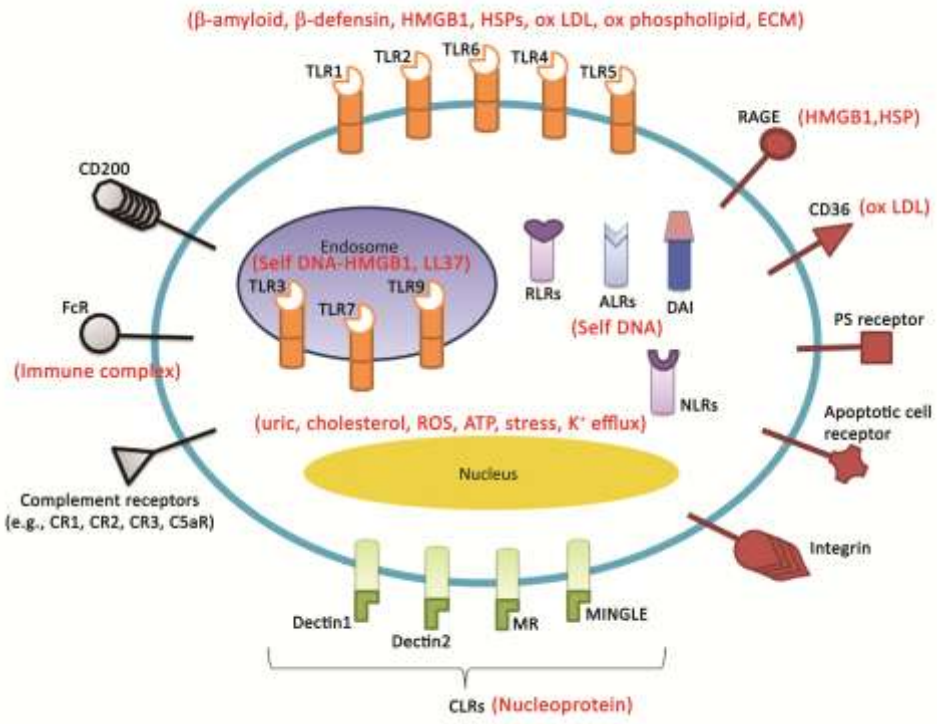

Figure 2. Endogenous ligands suspected to react with host cell receptors. The putative ligands are shown in parenthesis.

TLR6, and CLRs) or in association with the membrane of endosome, endoplasmic reticulum or endolysosome (e.g., TLR3, TLR7 and TLR9).[10-12] There are also cell-associated PRRs that are present intracellularly in the cytosol (e.g.,RLRs and NLRs). Representative examples of these membrane-associated PRRs are shown in Figure 1 and Table 2.[10-12,15,23,25-28] Many secreted soluble PRRs have been identified; they are commonly found as components of the complement system [e.g., C1q and mannose binding protein (MBP), and acute phase proteins]. In general, following interaction with appropriate ligands, the PRRs activate certain transcription factors which then translocate to the nucleus to turn on and upregulate genes involved in host defenses and inflammation [e.g., type I interferons (IFNs) and proinflammatory cytokines (tumor necrosis factor-α, IL-1, IL-6, IL-12)].[10-12,14,15] Excessive responses of PRRs can facilitate the development of autoimmunity and the induction of sepsis.[28] In addition to inducing the production of these mediators, some PRRs may also induce the expression of microRNAs (miRNAs) which further fine tune other responses.[29] More than 100 different miRNAs have been identified and some of them have been shown to be associated with certain pathological conditions of the immune system, e.g., autoimmunity (rheumatoid arthritis, multiple sclerosis and experimental autoimmune encephalitis) and cancer.[29] In this regard, proper regulation of miRNA expression could be an important approach for preventing these diseases.

Cell-associated receptors.

There are several excellent reviews describing the biology and chemistry of these PRRs together with the signaling pathways leading to the generation of a diverse array of effector molecules involved in host defenses and regulation of homeostasis. Activation of these receptors usually induces an inflammatory and antimicrobial responses.[10-15] In the present communication, I will overview only the pertinent points that are relevant to the theme of my discussion. In addition to those that detect microbial molecular patterns, the PRRs that recognize endogenous host components will also be mentioned even though their ligands are not strictly of a molecular pattern similar to those found with microbes.

1 Toll-like receptors (TLRs)

TLRs are trans-membrane receptors characterized by the extracellular leucine-rich repeat (LRR) domain and the cytoplasmic Toll/IL-1R (TIR) domain. Based on cellular localization and respective ligands, TLRs can be divided into 2 groups. The group expressed on cell surfaces including TLR1, TLR2, TLR4, TLR5 and TLR6 recognize microbial components, mainly the bacterial envelop. TLR3, TLR7, TLR8 and TLR9 represent another group of membrane-associated PRRs that is found in intracellular vesicles (Figure 1 and Table 2). The lysosomal TLRs largely recognize microbial nucleic acids.[10,11,14,15] The distribution of TLRs differs from one cell type to another. These receptors generate cell type-specific signals via distinct signaling pathways for gene activation in the nucleus.

2 Non-TLR cell surface receptors

This group of receptors is made up of C-type lectin receptors (CLRs) which include, for instance, dectin1, dectin2, MINGLE, DC-SIGN and mannose receptor, scavenger receptor (e.g., CD36 and SR-A) and others (Figure 1 and Table 2). Although these receptors on the APCs mainly function as endocytosed receptors, capturing microbes and antigens for processing and presenting to lymphocytes, they can also induce the production of proinflammatory cytokines. Some CLRs recognize endogenous DAMPs and can contribute to immune homeostasis. On the other hand, changes in the glycosylation of the endogenous ligands of these CLRs are known to be associated with some serious diseases, e.g., cancer. Moreover, CLR and TLR can interact and synergistically activate the inflammatory response, e.g., against fungal infections.

3 Non-TLR cytosolic receptors

These intracellular receptors can be subdivided into 2 groups, RLRs and NLRs (Figure 1 and Table 3). The RIG-1 like receptors (RLRs) are RNA helicases that recognize viral RNAs and induce generation of anti-viral responses, i.e., type I IFNs.[14,15] The NLRs encompass a large family of proteins, consisting of a nucleotide-binding domain and a leucine-rich repeat domain. As many as 20 NLR family members have been genetically identified in humans. One subgroup of NLRs, Nod1 and Nod2, detects PAMPs derived from bacterial cell walls and elicit responses that are distinct from those of TLR responses. The other

Table3. Examples of intracellular (cytoplasmic) receptors and their putative ligands

Receptors	Ligands	
	Exogenous (non-self) PAMPs/MAMPs	Endogenous (self) DAMPs
NLRs		
NOD1	Diaminpimelic acid (gram-negative bacteria)	
NOD2	Muramyl dipeptide (bacteria)	
NLRP1	Anthrax lethal toxin	
IPAF	Flagellin (bacteria)	
NLRP3	*Candida albicans*	Uric acid crystals
	Legionella pneumophila	Cholesterol crystals
	Listeria monocytogenes	ATP
	Staphylococcus aureus	K^+ efflux
	Malarial hemozoin	ROS
	Alum	Metabolic stress
	Silica, asbestos	Glucose
RLRs		
RIG-I	Nucleic acids (viruses)	
MDA-5	Nucleic acids (viruses)	
ALRs		
AIM2	dsDNA (bacteria, fungi and viruses)	self DNA
IFI16	dsDNA (viruses, bacteria)	self DNA
Others		
DAI	dsDNA (virus)	self DNA

NLR subgroup is made up of a large family of molecular complexes known as "inflammasome".[23,30-32] Many inflammasomes have been described. One such protein complex in this family, namely NLRP3, should be singled out in more detail, as different lines of evidence suggest that it may function as a metabolic sensor and therefore play an important role in regulating homeostasis in health and disease.[23,32] The NLRP3 inflammasome can be activated by stimuli that are structurally very diverse (Figures 2 and 3, and Table 3). These include those from different bacteria, viruses, malarial parasites and environmental irritant non-PAMP compounds like alum, silica and asbestos particles.[30-33] In addition to those of exogenous origin, a large array of endogenous host components are known to possess the ability to activate NLRP3, particularly those that are in a transitional form, from the soluble to insoluble state, like uric acid and cholesterol crystals (Table 1).[34-37] Other endogenous DAMPs reacting with NLRP3 include extracellular ATP, hyaluronan, glucose or even β-amyloid.[30,31,38] Structurally, it is unlikely that these diverse stimuli can trigger NLRP3 by direct binding to the NLRP3 complex. It has been postulated therefore that these stimuli activate the NLRP3 indirectly by interacting with other common cellular component or inducing process like increasing K^+ efflux, lysosomal membrane perturbation or disruption, or the dissociation of reactive thioredoxin-interactive protein (TXNIP) from thioredoxin (TRX) in an ROS-dependent manner.[21,30,31,38,39] The TXNIP dissociated from its inhibitor TRX has been suggested to be a true activator of NLRP3.[39] Among the responses induced by NLRP3, the generation of active caspase-1 from procaspase-1 is of particular interest, as it is the enzyme involved in conversion of inactive proIL-1β to active IL-1β. The latter is endowed with strong inflammatory activity known to be associated with a number of autoimmune and inflammatory diseases. It should be mentioned that the biologically active caspase-1 may use other cellular components as a substrate and give rise to responses which may be detrimental to the host. In addition to the RLRs and NLRs, in recent years, several cytosolic DNA sensors have been identified (Figure 2 and Table 3) and reported to function in both host defense and autoimmunity.[25,25a,26] The biology and functions of these receptors, i.e., AIM2, DAI and IFI16, will be described and discussed in a separate section.

Soluble PRRs

These are proteins with characteristics of PRRs because they recognize specific molecular patterns just like the TLRs. Many of the proteins belonging to the complement system (e.g., C1q, MBP, properdin, pentraxins, collectins, galectins and ficolins) and acute phase proteins (e.g., C-reactive protein) fall into this group. When binding to their targets (e.g., microbes and infected or transformed cells), these result in target cell destruction by phagocytosis or complement activation. Some of these soluble PRRs, e.g., galectins, function not only as PRRs, but also as potential DAMPs when released from damaged cells. I would like to mention in more detail about complement, as different lines of evidence suggest that it is also a key system for immune surveillance and homeostasis, acting by eliminating not only microbes but also damaged and altered host cells.[40] At the same time, healthy host cells are preserved. By eliminating cellular debris, it minimizes the occurrence of autoimmune disorders like SLE. Components of the complement system can also interact with other innate receptors like TLR which, depending on situation and location, may be either beneficial or detrimental to the host. Moreover, complement is also known to interact with the coagulation,

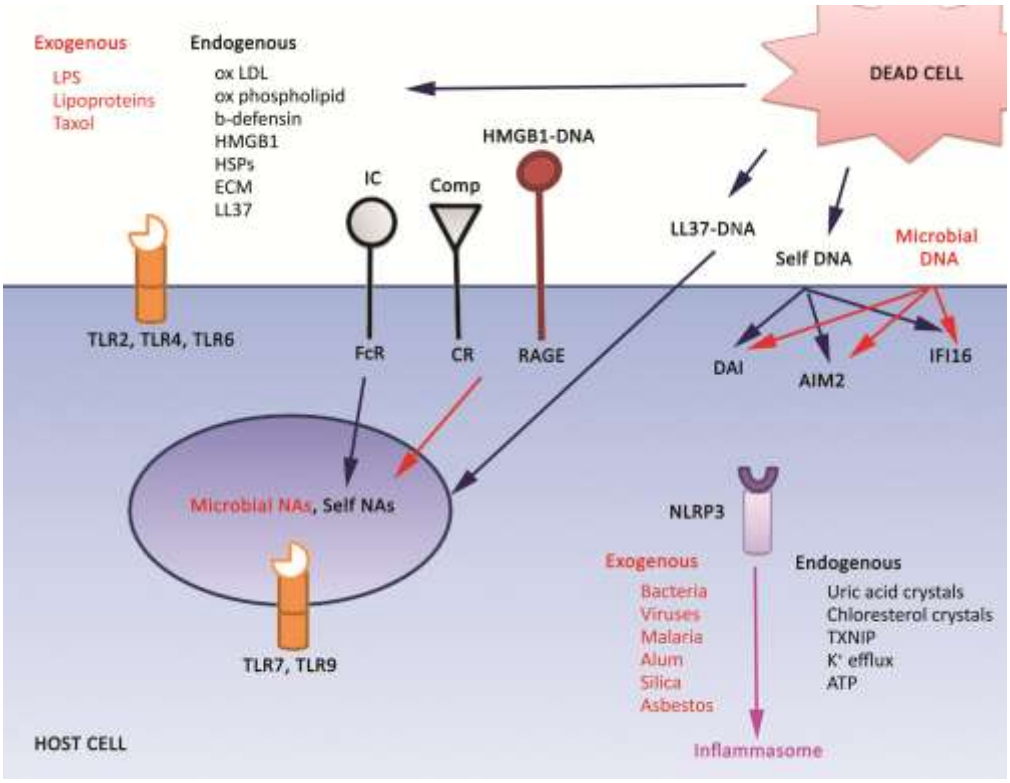

Figure 3. Examples of host cell receptors reported to react with both PAMPs and

kinin and fibrinolytic systems. These 'crosstalks' may substantially contribute to resolution of inflammation by, for instance, promoting elimination of damaged apoptotic cells and immune complexes. On the other hand, the crosstalk (e.g., between C5aR and TLR2) associated with some bacterial infection is known to impair macrophage function, possibly by interfering with the generation of nitric oxide.[40]

Atypical PRRs

There is another group of receptors that can react with PAMPs, DAMPs and ligands with structurally do not resemble a molecular pattern, for examples, HMGB1 proteins and HSPs. These receptors can be referred to as "danger" receptors (Figure 1). Although, strictly speaking, these receptors are not typical PRRs, I include them in this review because they are involved in the main theme of my discussion, i.e., regulating homeostasis. To name just a few, these receptors include RAGE [receptor for advanced glycation endproducts (AGEs)], scavenger receptors CD36 and SR-A [recognizing oxidized LDL (oxLDL) and β-amyloid], and complement receptors like CR1, CR3, C5aR, etc (Figures 1 and 2, Tables 2 and 3). This group of receptors is predominantly membrane- associated and most are found on the cell surface. RAGE is a particular important receptor in this group, because it is suspected of being one of the major receptors involved in regulating homeostasis.[11,12,20,21] It is expressed by several cell types including, for example, immune cells, endothelial cells, cardiomyocytes and neurons. Its ligands include HMGB1 protein, HSPs, β-amyloid, S100 protiens and AGEs. The latter are formed by glycation and oxidation of proteins and lipids which accumulate when cells are under oxidative stress, such as in chronic inflammation states found in association with diabetes and atherosclerosis.[20] In addition to the receptors just mentioned, there are also DNA sensors that are located intracellularly in the cell cytosol which can recognize both self and nonself DNAs (Figures 2 and 3, and Table 3). This group of receptors therefore functionally acts as "danger" signal detectors for the host. By cooperating with other innate immune receptors, many of these receptors may have their specificities and signaling activities altered which may be either beneficial or detrimental to the host. In a worst case scenario, when activation and regulation are markedly altered or out of control, it can lead to sepsis and death.

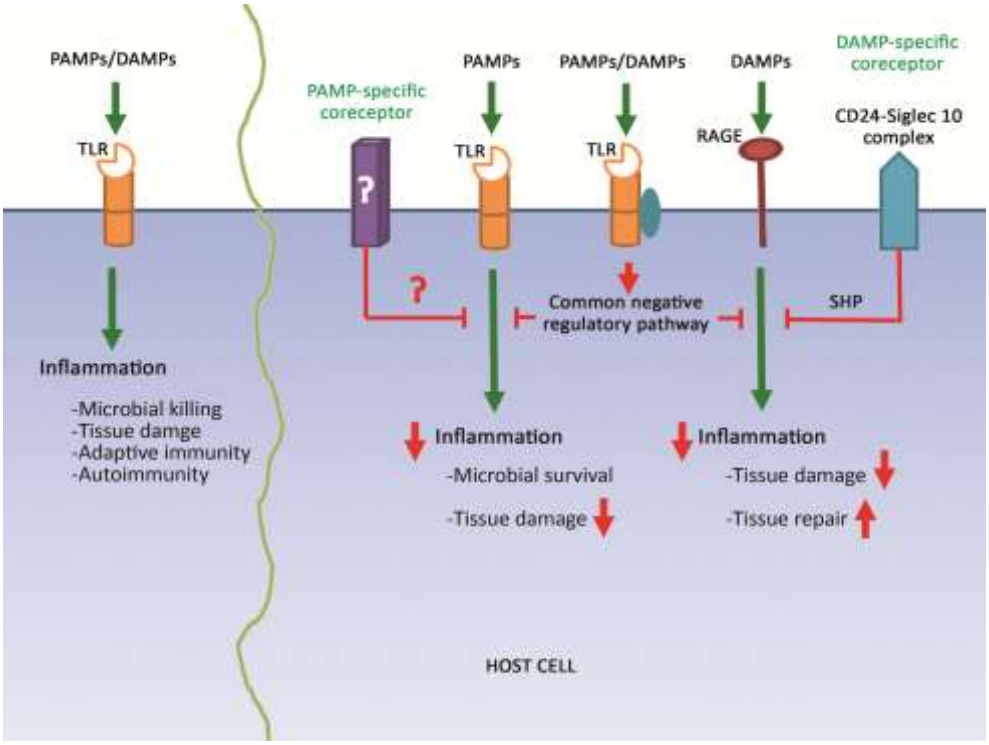

Figure 4. Suspected suppressive pathways regulating and discriminating signals from exogenous and endogenous ligands reacting with the same receptors. Signals generated from specific co-receptor (CD24) can limit the response induced by endogenous ligands reacting with DAMP-specific (RAGE) or common PAMP/DAMP receptors (e.g., TLR 4), thus specifically suppressing host response to endogenous DAMPS.

PRRs with reactivity for both exogenous (PAMPs) and endogenous (DAMPS) ligands

It is now clear that some PRRs can recognize ligands that are highly diverse structurally, this is particularly so for cell-surface receptors, like TLR2 and TLR4 or cytosolic receptors like NLRP3 (Figures 2 and 3, and Table 3). When these receptors were first identified and described, it was thought that they would readily distinguish self from nonself ligands. This is still the case for some receptors like TLR5 which is still shown to react only with bacterial flagellin and to my knowledge no endogenous host ligand has been found to react with this receptor. TLR2 and TLR4, on the other hand, can not only recognize a diverse array of exogenous ligands, but also a number of endogenous ligands like heat shock proteins (HSPs) and HMGB1 protein.[10-12] Moreover, TLR4 has been reported to recognize taxol, a plant extract currently used for the treatment of cancers. Some TLRs interact to form dimers (e.g., TLR2-TLR1 or TLR2-TLR6) that recognize differentPAMPs.[10-12] These dimers recognize not only exogenous PAMPs like bacterial cell envelopes, but also endogenous DAMPs like oxidized LDL, β-defensins, hyaluronic acid fragments, biglycan or versican (a proteoglycan found in tumor cells).[20,21] Some TLRs may also have the ability to collaborate with "co-receptors" on the cell surface, a process which may augment their interaction with PAMPs. On the other hand, these so-called co-receptors may function as negative regulators to damp down the host response to these signals. TLR9 can recognize both microbial and self DNAs, either as single entities or in complexes with other host components like HMGB1 protein.[11,12,23,24] It has also been reported to recognize hemozoin, a protein released from malarial-infected red blood cells. The NLRP3 should be mentioned again in more detail, as it has been reported to respond to an array of both exogenous and endogenous ligands that are structurally and biologically highly diverse, varying from bacterial lethal toxin, vaccine adjuvants (e.g., alum), environmental irritants (e.g., silica and asbestos particles) to endogenous ligands like uric acid and cholesterol crystals and others (Figures 2 and 3).[22,23,28,30-33] It is logical to suspect that these diverse molecules most likely do not interact directly with the NLRP3 itself, but probably sense a common downstream event that then change the microenvironment, which in turn upregulates and triggers the NLRP3.[30,38,39] Such

changes as destabilization or disruption of the cell membrane (which releases cathepsin B into the cytosol), increased K^+ efflux (intracellular K^+ depletion) or dissociation of thioredoxin complex in an ROS dependent manner have been implicated in the activation of NLRP3.[38,39] In the absence of infection, the recognition of endogenous ligands by these PRRs can induce sterile inflammation that leads to induction of autoimmunity and other inflammatory diseases.[11,12,28] It has been documented that hyperactivity of NLRP3 underlies many human diseases.

Many DAMPs that are recognized by these PRRs are formed as a result of microbial infection. Some of these include for example antimicrobial peptide (β-defensin) and oxidized LDL (Figures 2 and 3, and Tables 1 and 3). Infection can also induce apoptosis, which when cells are inappropriately phagocytosed and disposed of, can give rise to degraded self dsDNA that can be recognized by DNA sensors (both TLR9 in the endosome and cytosolic sensors like AIM2, DAI or IFI16.[10-12,25-27,41-43] Moreover, in the presence of autoantibodies (e.g., anti-DNA), the self DNA can form immune complexes that can interact with respective immunoglobulin Fc receptor FcR, then internalize and trigger TLR9 in the endosome.[10-12] The self DNA can also form complexes with cathericidin LL37 prior to reacting with the PRRs.[10-12] Cellular or metabolic stress (e.g., high concentrations of extracellular ATP, glucose and AGEs) can also be recognized directly or indirectly by atypical PRR receptors, serving as stimuli that induce sterile inflammatory response suspected to participate in, for example, atherosclerosis associated with cardiovascular diseases, diabetes or even Alzheimer's disease.

Detection and response to intracellular nucleic acids

Recognition of the presence of and responses to nucleic acids of both exogenous and endogenous origins has received considerable attention during the last few years. Different lines of evidence suggest that these molecular species, be it RNA, DNA or various intermediates, can readily induce strong innate defenses against viruses and bacteria, as well as inflammation against self nucleic acids that play a role in autoimmune and inflammatory diseases.[25,25a,26,41-43] It is now well documented that recognition and response to exogenous RNAs introduced into the cell, for example, in the endosome where TLR3, TLR7, TLR8 and TLR9 are located, or in the cytosol where RNA helicase RLRs are present, may be involved in the pathogenesis of many diseases. These RNA sensors are available to detect and initiate signals for the generation of type 1 interferons (IFN-α and IFN-β), proinflammatory cytokines and chemokines.[42] In contrast to the situation with the RNAs, the information on recognition of intracellular DNA by cytosolic DNA sensors is currently rather scarce.[25,25a,40-43] Until recently, the detection of both exogenous and endogenous DNAs was believed to be attributable largely to the presence of TLR9 located in the endosome.[10-12,25-26] This receptor recognizes and responds strongly, particularly to unmethylated DNA (e.g., from prokaryotes and CpG oligodeoxynucleotide).

In healthy cells, the cytosol is normally free of DNA. When the cells are infected with DNA viruses or intracellular bacteria like *Listeria monocytogenes*, *Legionella pneumophila* or *Francisella tularensis*, the presence of these exogenous microbial DNA species in the cytosol is unavoidable, therefore, the cytosolic DNA sensors must be available to initiate appropriate innate host defense. During the last few years, there ahave been several reports suggesting the presence of several DNA receptors which can initiate innate IFN type 1 responses to viral and bacterial DNAs and to self DNA derived from damaged apoptotic cells or other cellular insults. Those that have been identified and described include DAI, AIM2 and RNA polymerase III (this last one transcribes the DNA from some DNA viruses to RNA that can be detected by RIG-I of the RLR group of receptors).[41,43] More recently, a new DNA sensor called IFI16 (interferon inducible protein) has been described and characterized, and found to be structurally related to the AIM2 mentioned earlier.[25] These investigators suggested grouping these 2 receptors together into a new group with the name of AIM2-like receptors or ALRs (Figures 1 and 2). However, although the two receptors in this group, namely, AIM2 and IFI16, are similar structurally, they appear to be generating different signals. For example, AIM2 forms inflammasome complexes and induces the production of IL-1 and not type 1 interferon, and *vice versa* for the noninflammasome IFI16 receptor which induces the production of type I IFN.[25,25a] In addition to

responding to microbial DNAs, these DNA sensors have also been shown to respond to self DNA inappropriately introduced into the cell cytosol as, for example, after phagocytosis of damaged apoptotic cells or improperly degraded self DNA. It is logical to speculate that in the presence of a hypothetical disease characterized by destabilization or damage to mitochondria, the mitochondrial DNA may leak into the cytosol and could then be recognized by these sensors, resulting in pathological inflammatory condition and autoimmune diseases.[26] In fact, it was shown very recently that the integrity of autophagy was needed to maintain mitochondrial homeostasis and, when malfunctioning, would induce the release of mitochondrial DNA which triggers the NLRP3 inflammasome.[44] It can be postulated further that the hypomethylation of DNA often noted in some autoimmune diseases like SLE may augment the reactivity of TLR9, which normally reacts best with unmethylated DNA. Thus, the recognition of hypomethylated DNA could signal a stronger innate response and inflammation than normally methylated counterparts present in healthy individuals. The altered self DNA can serve as a strong DAMP that induces responses which, when not properly controlled, can result in hyperactive states like sterile inflammation, sepsis or other serious autoimmune conditions.

How the host distinguishes and regulates signals generated by PAMPs and DAMPs.

From the time when Sir Frank MacFarlane Burnet first proposed the "self-non-self" model of immune response 50 years ago,[1] immunologists have made considerable progress in understanding how the immune response is triggered, in particular with regard to the response to microbial infections. In order to cope with new information, the concept that the immune system discriminates self from non-self and responds to foreignness had to be modified several times. The most significant modification was made by Charles Janeway, who proposed pattern recognition as a basis for initiation of immune response against "infectious non-self".[2] This was shortly followed by a rather controversial hypothesis proposed by Polly Matzinger, who stated that the immune system did not care about discriminating self from non-self or infectious non-self from noninfectious self, but rather about 'dangerous' from 'non-dangerous' signals generated from dying cells or from those undergoing cellular and metabolic stress.[16] In other words, immunity is not designed to combat infection, but is to alert the host to tissue injuries from whatever cause. This concept challenges our original belief and has challenged and revolutionized our thinking about immune response from that time onward.

Although the 'danger model' is now accepted by investigators interested in immunoregulation, there are still some problems that remain unresolved. For example, a large majority of DAMPs that have been identified use the same PRRs sensed by PAMPs (Figure 3, and Tables 2 and 3) and this raises important questions about whether or not the host treats PAMPs and DAMPs equally in terms of the quality and magnitude of the response. It is well recognized that tissue injuries by themselves are not always followed by an adaptive response and autoimmunity and therefore logically there must be mechanisms to regulate the outcome of the response in relationship to different scenarios, so that collateral tissue destruction is kept at minimum while tissue repair is allowed to proceed. Working in the field of immunoregulation, we are all aware that a decision to make a response or the type of effector generated is under the influence of factors operating at different levels; for example, from the time of the positive and negative selection of lymphocytes in the thymus to mature and the development of dendritic cells, whose polarization is known to be influenced by microenvironment, i.e. the types of microbes and the site of activation.[7] In fact, the impact of epithelium on the development of the immune system was recognized many decades ago. However, it was not until very recently that the significance of PRRs and commensals has been recognized and subjected to investigation at a molecular level.[45] Much information is now available on the role of commensals in the stimulation of antimicrobial peptides, induction of different lymphocyte subsets and DC phenotypes and maintaining intestinal physiological functions. The interaction between microbes and non-microbes with epithelial cells results in surface alterations and mediator secretions that impose decisions on the development and response of immune cells (referred to by some investigators as "epimmunome").[45] In this last section, I will focus my discussion on how the host may

differentially regulate its response to PAMPs and DAMPs, so that the response to DAMPs can be selectively and specifically regulated and suppressed, i.e., keeping the protective immune and inflammatory response at a level that will have minimal impact on tissue damage and autoimmunity and at the same time would enhance tissue repair to re-establish a state of homeostasis. In other words, the immune system must provide balance between activation and inhibition to avoid an inappropriate and detrimental inflammatory response.

The innate immune system is known to be regulated by a large number of negative regulators in order to strike the balance mentioned above. Much information on the negative regulatory pathways that control the outcome of TLR response after PAMP stimulation is currently available and has been the subject of several excellent reviews.[10-12,46] These negative regulators include for example soluble decoy TLRs, transmembrane ITIM proteins (e.g., SIRP-α, Siglecs and FcγRIIB) and intracellular TLR regulators (e.g., SOCS and A20). These negative regulators may have degradative, competitive or dephosphorylation functions. These molecules can be of host or microbial origin. Some of these negative regulators are known to exert their influence on more than one TLR. For example, the intracellular regulator A20 is known to de-ubiquitylates TRAF6 and has been reported to interfere with the function of TLR2, 3, 4 and 9.[46,47] It was proposed further that the A20 might also function to inhibit signaling induced by commensals. It is assumed that these TLR negative regulators are probably not stimulus specific and that they could in theory affect responses to stimulation by both PAMPs and DAMPs (Figure 4). This may be considered to be a common negative regulators / regulatory pathway and functions to suppress responses induced by either PAMPs or DAMPs. Many of these negative regulators with a significant impact on TLR signaling pathways have been identified. The one system that has attracted considerable attention in recent years is the protein tyrosine phosphatase (PTP) system which dephosphorylates, for example, TLR signaling molecules. This includes the well-known SHP-1, SHP-2 and SHIP. Different lines of evidence from experimental animals and patients support the notion that reduction or absence of these negative regulators are associated with enhanced inflammatory response and can cause autoimmune diseases. Very recently, the possible role of miRNAs in fine tuning the response has entered the picture and considerable progress has been made.[29] On the other hand, we should expect that in order to alleviate the magnitude of the response induced by DAMPs while leaving that to PAMPs unaffected, there should be a pathway specifically evolved to regulate the DAMP-specific pathway, for example, like that initiating a response from RAGE or other 'danger' receptors. In fact, there was a recent study showing that this was the case for RAGE.[48,49] It was shown in these studies that there was a negative co-receptor, CD24, that could bind to HMGB1 protein sending a signal via Siglec to activate the SHP phosphatase systems and thereby interfering with the activation of NFκB.[48-52] CD24 is a heat stable host component, expressed as a glycosyl-phosphatidyl-inositol-anchored molecule widely present in several cell types.[50,51] Polymorphism of CD24 is known to be associated with the risk and progression of several autoimmune diseases. Also mice with CD24 deficiency are more susceptible to develop "cytokine storm". The Siglecs, on the other hand, are transmembrane signaling lectins containing the ITIM domain. Many of the Siglec family members bind to sialic acid components present on co-receptors like CD24 and can be considered to be a sialoside-based pattern recognition receptors that confers negative signals.[52]Binding of DAMPs to the CD24-Siglec complex recruits and activates SHP-1, SHP-2 or SHIP, thus generating a suppression activating signal which can, in turn, reduce the level of damage signals. Binding of DAMPs with this co-receptor can also compete directly with the ligand binding to their respective PRRs, thus reducing the chance of triggering of the classical receptors like TLRs and NLRs. By doing so, the CD24-Siglec 10 complex therefore generates a response that can selectively suppress DAMP but not PAMP stimulation. The suppression of the DAMP response initiated from the CD24-Siglec complex might provide us with novel approaches to alleviate local inflammatory response following acute tissue damage or trauma. Moreover, a thorough understanding of negative regulation will provide us with ways and means to amplify local inflammatory response to achieve maximal levels of adaptive immune response for cancer

immunotherapy or to obtain maximum protection after vaccination. Negative regulatory pathways, as diagrammed in Figure 4, represent my overall concept regarding immunoregulation and host discrimination of PAMPs and DAMPs, based on the information currently available. At the rate of progress currently being made, it is not too ambitious to anticipate that many more of these co-receptors and suppressive factors will be identified in the near future and will be available for testing as new drug targets for the manipulation of autoimmune and inflammatory disorders.

Conclusions and future perspectives

During the last few decades, there has been considerable progress in understanding how the immune response is activated and regulated under different scenarios, e.g., infection *vs.* sterile tissue damage, both of which result in inflammation. Data generated from several groups of investigators have provided us with significant insights into the importance of innate immune system in host defense against infection and its ability to fine tune the quality of the adaptive immune response. Synergistic interactions between activating receptors and inhibitory receptors are very important in maintaining healthy homeostatic conditions and so they must be finely tuned and regulated to provide an optimal immune defense against pathogens without initiating severe immunopathological conditions that may end up in serious diseases and death. In general, the activating receptors detect PAMPs or altered self (DAMPs) while the inhibitory receptors and co-receptors detect self or hidden components that are expressed constitutively by the host. In the present communication, I have overviewed the role played by PAMPs and DAMPs in stimulating PRRs and atypical PRRs that lead to the generation of the inflammatory response, which if not properly regulated and controlled will result in pathological conditions like autoimmune and inflammatory diseases, including sepsis and death. Although the host possesses common mechanisms that can suppress inflammatory response generated after recognition of PAMPs and DAMPs, evidence available from recent studies suggest that there are also selective negative regulators and regulatory mechanisms to dampen the response to DAMPs while leaving that to PAMPs intact to ensure that collateral tissue damage and autoimmunity are kept at a minimum while microbial defenses are left intact. Progress on the molecular aspects of novel "danger" receptors and co-receptors for additional endogenous host components to be identified in the future will allow us to more precisely and effectively modulate responses to DAMPs and their outcomes. It should be noted that in this review very little is mentioned about the impact of glycosylation on signaling from the CLRs which may in turn lead to the development of diseases. The development and progress of research in the area of "glycoimmunology", particularly with regard to the role of CLRs in maintenance of homeostasis, has increased rapidly in recent years.[53]

Understanding the complexity of the immune regulatory network will certainly provide us with new ways and means to develop novel methods for preventing and managing more effectively some autoimmune and inflammatory diseases. New targets for drug treatment will be identified. The molecular mechanism regarding the initiation and regulation of the immune response will also allow us to design vaccine adjuvants targeted more optimally for preventing specific microbial and autoimmune diseases, and for cancer prevention and treatment.

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