Cell, Vol. 111, 927–930, December 27, 2002, Copyright ©2002 by Cell Press

Pattern Recognition Receptors: Doubling Up for the Innate Immune Response

Minireview

Siamon Gordon[1]
Sir William Dunn School of Pathology
University of Oxford
South Parks Road
Oxford OX1 3RE
England

Antigen presenting cells (macrophages and dendritic cells) express pattern recognition molecules that are thought to recognize foreign ligands during early phases of the immune response. The best known of these are probably the Toll-like receptors, but a number of other receptors are also involved. Several of these recognize endogenous as well as exogenous ligands, suggesting that they play a dual role in normal tissue function and host defense.

Macrophages and dendritic cells (DC) are specialized phagocytes that play an important role in clearance of effete host cells and molecules, as well as in defense against infection. These antigen presenting cells (APC) are widely dispersed throughout the body, including at portals of entry to microorganisms. They participate in initial capture and processing of potential antigens (innate immunity) and then in activation of specific T and B lymphocyte effector mechanisms (adaptive immunity). These activated cells in turn cooperate with activated macrophages to enhance destruction of intra- and extracellular pathogens. In addition to their efficient endocytic and phagocytic activities, APC are potent secretory cells that induce and regulate local and systemic inflammatory and immune responses.

Recognition of Foreign and Self Structures by APC

APC express a large variety of surface molecules, enabling them to recognize infectious and endogenous ligands prior to engulfment, intracellular signaling, and altered biosynthesis and secretion. Pattern recognition of pathogen-associated molecules has become an important topic in the study of innate immunity. As outlined by Janeway and Medzhitov (2002), this term refers to the concept that germline-encoded receptors for conserved molecular patterns are responsible for recognition of microbial nonself ligands by APC. Implicit is the concept that the potential ligands are essential for microbial survival and, therefore, cannot be substantially altered by innate immune selection pressure. Recognition is followed by uptake and then surface presentation in conjunction with MHC Class I and II molecules. When combined with the enhanced expression of costimulatory molecules also induced by microbial stimuli acting through the same or different receptors, the APC are able to activate adaptive immune responses (Figure 1). Somatic rearrangement of the receptors on T and B lymphocytes that recognize the presented antigens expands the repertoire and specificity of peptide antigens that can initiate a response in these cells. These antigens can derive from host as well as exogenous sources, and powerful mechanisms are in place to prevent inappropriate inflammatory and autoimmune responses to potential self-antigens.

The receptor repertoire of APC is extensive, recognizing a wide range of protein, saccharide, lipid, and nucleic acid ligands of endogenous as well as exogenous origin. (Table 1 lists selected receptors, ligands, and recent references. For a general review of macrophages see Gordon, 1999). These include selected native glycoproteins found in plasma (many lysosomal hydrolases contain terminal saccharide structures recognized by tissue macrophages and therefore undergo rapid clearance), modified lipoproteins, protease-inhibitor complexes, and possibly, stress-induced heat shock proteins. Particulate ligands taken up by phagocytosis include senescent, apoptotic, and necrotic cells, as well as microorganisms. Opsonins, especially specific antibodies and complement, coat targets and contribute to their enhanced uptake and destruction. Immune recognition of self structures on normal, stressed, transformed, or infected cells can also be mediated by NK cells (triggered by missing self and regulated by competing inhibitory and activating receptors) and $\gamma\delta$ T cells, which have restricted repertoires for nonclassical MHC Class IB molecules.

As the characterization of receptors expressed by APC and their ligands grows, it has become necessary to broaden the concept of APC pattern recognition of foreign organisms to include modified self-ligands and to bear in mind the spectrum of pro- and anti-inflammatory and immunogenic and suppressive responses induced within the host (Figure 1). This review summarizes evidence that pattern recognition of microbes is part of a wider homeostatic clearance mechanism that allows multicellular organisms to maintain a constant internal environment.

Toll-Like Receptors (TLR)

APC express a range of TLR transmembrane proteins implicated in differential recognition of microbial and foreign molecular targets (for a recent review see Takeuchi and Akira, 2002). While undoubtedly important in downstream signaling responses and NF-kB activation, the TLR have not been shown to bind directly to ligands such as lipopolysaccharide (LPS) or their complexes with LPS binding protein (LBP) (Tobias, 2002). Instead, these bind to CD14, also implicated in apoptotic cell, heat shock protein, and fibrinogen clearance (see Table 1). The ligands identified so far that signal through TLR are mainly exogenous, but there are reports of endogenous ligands and of intracellular rather than surface engagement through which selected TLR act. Although the discovery of the importance of TLR in signaling pathways induced by different bacteria and fungi owed a great deal to studies on *Drosophila melanogaster* (see review by Imler and Hoffmann, 2001), only one *Drosophila* Toll has been implicated in innate immune recognition.

Similar questions arise in regard to peptidoglycan recognition proteins (PGRPs), recently described in *Dro-*

[1]Correspondence: christine.holt@path.ox.ac.uk

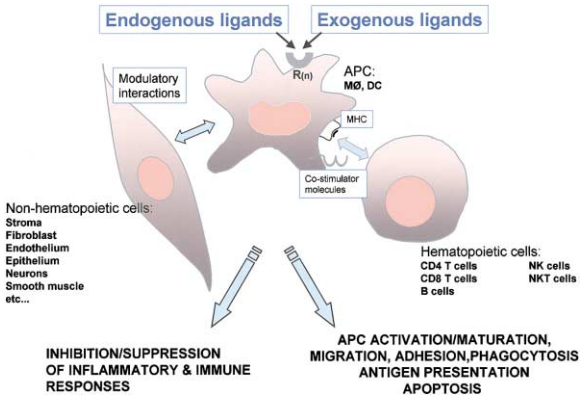

Figure 1. Antigen Presenting Cells in the Immune Response

Antigen presenting cells (APC) integrate signals from endogenous and exogenous ligands with cellular interactions to generate diverse responses. The thesis of this review is that many pattern recognition receptors are able to interact with both endogenous (host derived) and exogenous ligands.

sophila and higher species (see Gottar et al., 2002, for example). These molecules are implicated in downstream responses to Gram negative as well as Gram positive bacterial infection and, based on transmembrane domain predictions, may be secreted or membrane proteins.

Type 3 Complement Receptor (CR3)

CR3 is a myeloid cell phagocytic receptor for complement opsonised particles, irrespective of host or microbial origin, and also for direct interactions with pathogens such as *Mycobacterium tuberculosis* and yeast-derived zymosan as well as host ligands (reviewed by Ross, 2000). It is a β2 integrin also known as CD18/CD11b, and it plays a key role in myelomonocytic cell recruitment to sites of inflammation. CR3 expression by tissue macrophages is selective, e.g., by microglia, but not resident alveolar macrophages. It binds a promiscuous range of ligands, including I-CAM-1, selected clotting components, senescent platelets, and possibly, denatured proteins. CR3 contributes to clearance of apoptotic cells and may provide a relatively silent means of entry to macrophages for selected pathogens. The opsonic phagocytic mechanism differs from that mediated by Fc receptors, and CR3-mediated uptake by macrophages does not trigger release of arachidonate or reactive oxygen metabolites. Structural studies have proven difficult, and the signaling pathways involved remain poorly defined.

Scavenger Receptors (SR)

This has become a loose term to cover a wide range of structurally unrelated membrane molecules expressed by macrophages and selected endothelia with broad specificity for polyanionic ligands. The endocytosis of modified low-density lipoprotein (LDL) by Class A SR is the best characterized, and stems from the original observation by Brown and Goldstein on foam cell formation and structural studies by Krieger, Kodama, and their colleagues (for references see Krieger and Stern, 2001, and subsequent series of review articles). An SRCR-type domain is found in a range of adhesion and endocytic

Table 1. Selected Macrophage Pattern Recognition Receptors and Ligands

Receptor	Endogenous Ligand	Exogenous Ligand	Reference
CR3 (direct and opsonic)	iC3b, ICAM-1/2, factor X, fibrinogen, heparan sulfate, CD16/CD23, JAM-3	Zymosan (β-glucan)	Ross, 2000
CD14	LPS binding protein, PGRP, apoptotic cells, HDL, HSP, fibrinogen	LPS, PGN	Tobias, 2002
TLR (selected)	HSP-60 (?), PGRP, fibrinogen	LPS, PGN, LTA, flagellin, PAM3 Cys,CpG, lipoprotein, dsRNA, imiquimod, lipoarabinomannan	Takeuchi and Akira, 2002; Gottar et al., 2002
SR-A I/II	Mod. LDL, AGE-mod proteins, apoptotic cells	Lipid A, LTA, *N. meningitidis,* asbestos	Krieger and Stern, 2001; Peiser et al., 2002
MARCO	Mod LDL (?)	Some G^-/G^+, LPS, environmental particles	Sankala et al., 2002
CD36	Thrombospondin, modified lipids, retinal photoreceptor outer segments	Plasmodium falciparum-malaria parasitized erythrocytes	Krieger and Stern, 2001
$\alpha v\beta_3$	MFG-E8 (secreted by MØ), Vitronectin, apoptotic cells	*T.cruzi*	Hanayama et al., 2002
βGR	Selected T cells (sugar independent)	Yeasts (β-glucan)	Brown and Gordon, 2002
MR C-lectin domains	Mannosyl, fucosyl glycoconjugates, lysosomal hydrolases, thyroglobulin, MPO, amylase, TPA, L-selectin	*Klebsiella* LPS, *Sc Pneumoniae* CPS, *Pn. carinii*, Lipoarabinomannan, *Schisto. mansoni*, *Cryptococcus neoformans*	Zamze et al, 2002; East and Isacke, 2002
Cysteine-rich domains	Lutropin, TSH, Sialoadhesin, CD45, others, sulfated Gal-saccharide	HIV gp120	McKenzie et al, 2002
DC-SIGN	Mannosyl, fucosyl, ICAM-3,2	HIV gp120	Geijtenbeek et al., 2002
CD1	αGal-ceramide	Mycobacterial glycolipids	Bendelac and Medzhitov, 2002

Several endogenous ligands can serve as opsonin for microbial uptake. Abbreviations: βGR, β-glucan receptor; CPS, capsular polysaccharide; CR3, type 3 complement receptor; HSP, heat shock protein; LDL, low-density lipoprotein; LTA, lipoteichoic acid; MFG-E8, milk fat globule-EGF-factor 8/opsonin for apoptotic cells; MPO, myeloperoxidase; MR, mannose receptor; PGN, peptidoglycan; PGRP, peptidoglycan recognition protein; SR-A, scavenger receptor, class A; TLR, Toll-like receptor; TSH, thyroid stimulatory hormone.

References cited in Table 1 only: Bendelac, A., and Medzhitov, R. (2002). J. Exp. Med. *195*, F19–F23; Hanayama, R., Tanaka, M., Miwa, K., Shinohara, A., Iwamatsu, A., and Nagata, S. (2002). Nature *417*, 182–187; Sankala, M., Brännström, A., Schulthess, T., Bergmann, U., Morgunova, E., Engel, J., Tryggvason, K., and Pikkarainen, T. (2002). J. Biol. Chem. *277*, 33378–33385.

molecules on hematopoietic and nonhematopoietic cells. MARCO, a related collagenous Class A SR, is encoded by a distinct gene. Artificial generic ligands such as acetylated LDL have proved useful experimentally, but there is still surprisingly little known about natural ligands for these molecules. The availability of knockout mice for various SR will help to clarify their role in innate and acquired defense and, more generally, in homeostasis. Best known so far is the contribution of SR-A to phagocytic uptake of apoptotic thymocytes and to the uptake of unopsonized *Neisseria meningitidis* (Peiser et al., 2002). Lipopolysaccharide, a previously known ligand of SR-A, is not required for Neisserial uptake by macrophages, but is responsible for TLR-4-mediated induction of pro-inflammatory responses. SR-A contributes both to resistance to Gram positive microbial infection in vivo and to susceptibility to atherogenesis, but its role in both is variable and complex. SR-A knockout mice are more susceptible to LPS-induced shock after they have been primed by the mycobacterial vaccine Bacillé Calmette Guerin (BCG) to activate their macrophages. This is accompanied by overproduction of TNFα and other pro-inflammatory mediators, perhaps due to an imbalance between SR-A-dependent clearance of LPS and TLR-4-dependent pathways of secretory stimulation. The signaling pathways induced by SR-A in vitro are poorly defined, as the ligands utilized in many studies can bind to a range of SR molecules.

In relation to the dual recognition properties highlighted in this review, the SR-A has several interesting features. It is able to function as an adhesion molecule in vitro, but can also mediate endocytosis and phagocytosis of modified-host components and of exogenous ligands (LPS, lipoteichoic acid, others). The structural basis for receptor-ligand interactions remains unclear, although there are some clues from studies with both SR-A and MARCO.

Other classes of SR (eg CD36) contribute to lipoprotein homeostasis and apoptotic cell clearance yet can interact with pathogens (e.g., *Plasmodium falciparum*) and in selected cases to Gram positive and Gram negative bacteria. *Trypanosoma cruzi* can exploit the down-regulation and deactivation of MØ via $\alpha v\beta_3$/thrombospondin, linked to CD36-mediated entry.

Dectin-1 (β Glucan Receptor)

Macrophages express a range of C type lectin-like receptors, similar to those found on NK cells, but mostly with unknown ligands and functions. We have recently found that a previously described molecule, murine dectin-1, is a major receptor of macrophages for β-glucan structures in zymosan and other yeast-derived particles (Brown and Gordon, 2002). It is also found in neutrophils and DC, and mediates attachment and ingestion of zymosan, requiring an ITAM motif for uptake. Although many fungi contain β-glucan in their wall, pathogenic strains such as *Candida albicans* do not express much ligand on their surface when alive, only after heat killing. Dectin-1 also interacts with selected T cells in a β-glucan-independent manner. This receptor therefore provides a good example of dual specificity for exogenous and endogenous ligands, presumably mediated by distinct ligand binding sites.

Mannose Receptor (MR) and Other C Type Lectins

This well-characterized endocytic receptor is expressed by macrophages, DC, and some endothelial cells. It is a multilectin, which binds mannosy/fucosyl or GlcNAc-glycoconjugate ligands through its Ca^{2+}-dependent carbohydrate recognition domains (CRD) (Reviewed by East and Isacke, 2002). The ligands are present on a range of bacteria, fungi, virus-infected cells, and parasites. In a recent study, CRD4-7 of the MR was shown to bind directly to purified lipopolysaccharides from *Klebsiella pneumoniae* and to capsular polysaccharide of *Streptococcus pneumoniae* (Zamze et al., 2002). Endogenous self-ligands for the CRD include L-selectin, implicated in cell migration and a number of mannose-terminal lysosomal hydrolases, (Lee et al., 2002). In addition, the MR contains an N-terminal, cysteine-rich (CR) domain; studies with a chimaeric CR-Fc fusion protein have revealed novel sulfated saccharide ligands in peripheral lymphoid organs (marginal zone metallophilic macrophages in spleen, subcapsular sinus macrophages in lymph nodes), and inducible binding to follicular dendritic cells (reviewed by McKenzie et al., 2002). The anterior pituitary hormones lutropin and TSH express similar sulfated ligands responsible for MR-mediated clearance by liver sinusoidal cells. Macrophages release a soluble form of the MR into plasma constitutively. A novel antigen transport function has been postulated for the MR, whereby glycoconjugate antigens captured by the CRD (soluble or cell-associated forms of the MR) are targeted to peripheral lymphoid organs for clearance.

The MR therefore exemplifies the dual ligand binding properties of a pattern recognition receptor, with an unexpected role in clearance of potential auto-antigens and injurious self molecules from plasma and extracellular compartments. The multivalent CRD may favor high-avidity binding of selected saccharide ligands and their phagocytosis. It should be noted that only some glycoproteins naturally express the appropriate terminal saccharide residues recognized by the MR.

Several MR-like molecules are now known, including Dec205 and a phospholipase A2 receptor-like molecule, but neither endogenous nor exogenous natural ligands for these molecules have yet been reported. The acute phase protein, mannose binding lectin (MBL), made by hepatocytes, has similar sugar binding specificity as the MR. The collectins, surfactant proteins A and D, also bind exogenous and endogenous ligands through oligomeric, Ca^{2+}-dependent binding and may, in turn, bind to still poorly characterized macrophage receptors. Monomeric or dimeric C type lectin receptors of APC such as DC-Sign, have distinct sugar-specific and sugar-independent binding sites, for example for HIV-1gp 120 and I-CAM-3. (Geijtenbeek et al., 2002).

The C type lectins, including the MR, participate in complex functions related to clearance, homeostasis, immunomodulation, and host defense. As the list of mannosyl recognition molecules continues to grow, it will be important to use specific probes, rather than less specific ligands, to study their particular contributions.

Implications of Dual-Specific APC Receptors

As more examples are identified, we need to rationalize the apparent paradox of dual recognition, its molecular basis and differential cellular responses, and consider

its evolutionary origin. A broader concept of pattern recognition should have predictive value, where ligands for receptors may be present within the host as well as on exogenous sources. For a multicellular organism, there is obvious value in having motile, widely dispersed, phagocytic and endocytic cells. This mononuclear system may well be linked to developmental remodeling as well as to innate defense. It is not clear which came first, the pressure of the microbial environment (trophic and symbiotic as well as pathogenic) or the need for increased cell diversification and endogenous cellular interactions. However, it is likely that innate recognition of self and foreign ligands by macrophages and, in parallel, the alternative pathway of complement, long predates the evolution of lymphoid gene rearrangement and MHC diversification.

In truth, there are still few examples in which individual cell surface molecules, or domains, have been shown to bind directly to defined exogenous and endogenous ligands. It is possible that similar receptors differ in glycosylation, for example, or exist as isoforms with distinct binding properties. Biacore and imaging techniques are now available to study isolated receptors and more complex cellular interactions in which several receptors can engage with their ligands simultaneously. The chemical and physical properties of ligands should be matched with receptor structure and specificity. Several different receptors can together engage a complex ligand, such as a bacterium, and individual receptors can interact with multiple ligands of different affinity.

How can we then explain differential signaling by similar ligands and how can we sort out the intracellular recruitment and interactions of different signaling components? Do recognition receptors precede and relay specific information (for example, yeast versus bacterium) to preferred partners, thus entering a pro- or anti-inflammatory pathway? The context in which recognition occurs is clearly important, promoting differentiation and functional maturation of cells like DC. APC differ in their receptor profile as well as in signal transduction pathways and gene expression. When, where, and with what associated molecules do these dynamic and versatile cells respond to their microenvironmental ligands? The nature, degradability, and persistence of a ligand will influence its localization and fate within the cell.

Finally, another useful feature is that it is possible to extend the range of ligands from bacterial (foreign), to viral (mixed origin), parasitic (complex host and foreign), and tumorigenic (mostly modified host). The ability to discriminate between foreign and self should therefore be seen as a continuous spectrum, rather than polar opposites.

Selected Reading

Brown, G., and Gordon, S. (2002). J. Exp. Med. *196*, 407–412.

East, L., and Isacke, C.M. (2002). Biochim. Biophys. Acta *1572*, 364–386.

Geijtenbeek, T.B.H., Engering, A., and Van Kooyk, Y. (2002). J. Leukoc. Biol. *71*, 921–931.

Gordon, S. (1999). In Fundamental Immunology, Fourth Edition (Philadelphia: Lippincott-Raven), pp. 533–545.

Gottar, M., Gobert, V., Michel, T., Belvin, M., Duyk, G., Hoffmann, J.A., Ferrandon, D., and Royet, J. (2002). Nature *416*, 640–644.

Krieger, M., and Stern, D.M. (2001). J. Clin. Invest. *108*, 645–647.

Imler, J.-L., and Hoffmann, J. (2001). Trends Cell Biol. *11*, 304–311.

Janeway, C.A., Jr., and Medzhitov, R. (2002). Annu. Rev. Immunol. *20*, 197–216.

Lee, S.J., Evers, S., Roeder, D., Parlow, A.F., Ristell, L., Lee, Y.-C., Feizi, T., Langen, H., and Nussenzweig, M.-C. (2002). Science *295*, 1898–1901.

McKenzie, E.J., Su, Y.-P., and Martinez-Pomares, L. (2002). In Trends in Glycoscience and Glycotechnology, Volume 14, 269–279.

Peiser, L., Mukhopadhyay, S., and Gordon, S. (2002). Curr. Opin. Immunol. *14*, 123–128.

Ross, G. (2000). Crit. Rev. Immunol. *20*, 197–222.

Takeuchi, O., and Akira, S. (2002). Microbes Infect. *4*, 887–895.

Tobias, P.A. (2002). In Innate Immunity (Totowa, NJ: Humana Press), pp. 255–265.

Zamze, S., Martinez-Pomares, L., Jones, H., Taylor, P.R., Stillion, R.J., Gordon, S., and Wong, S.Y.C. (2002). J. Biol. Chem. *277*, 41613–41623.