Immune recognition of fungal β-glucans

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Summary

The recognition of conserved microbial structures is a key aspect of metazoan immunity, and β-glucans are emerging as a major target for the recognition of fungal pathogens. A number of receptors for these carbohydrates have been identified, which upon recognition, trigger a variety of immune responses. In contrast to many other systems, there is little apparent conservation in these mechanisms between vertebrates and invertebrates. In this review, we will highlight all the known receptors for β-glucans and will discuss the various immune responses they can initiate, with reference to fungal infection, in both vertebrates and invertebrates.

Introduction

The innate ability to detect pathogens is essential for multicellular existence, and has been achieved through the evolution of germ-line encoded receptors which can recognize non-self structures, the so-called ‘pattern recognition receptors’ (PRRs) (Janeway, 1992). The structures recognized by these receptors, termed the pathogen associated molecular patterns (or PAMPs), are not found in the metazoa and are thought to be normally essential for the survival of the microbial pathogen. The most well described PAMPs include lipopolysacharide (LPS), peptidoglycan and lipoteichoic acid (LTA) of bacteria, and β-glucan of fungi, which is the topic of this review. Recognition of these structures triggers responses designed to protect the host from the invading pathogen, forming part of the innate immune system found in all higher organisms.

β-Glucans are a heterogeneous group of glucose polymers found in fungi, plants and some bacteria. They consist of linear β(1→3)-linked backbones with β(1→6)-linked side chains of varying length and distribution, and can form complex tertiary structures stabilized by interchain hydrogen bonds. Although the primary role of β-glucan recognition appears to be the initiation of immune responses for the control of fungal pathogens, the receptors and mechanisms by which this is achieved differ significantly between vertebrates and invertebrates. In vertebrates, the recognition and response to these structures are initiated by cell surface receptors, whereas this process occurs primarily in the haemolymph in invertebrates. We will review here the mechanisms of β-glucan recognition in invertebrates and vertebrates, looking at both the receptors and the immune responses triggered by these carbohydrates.

Effects of β-glucans in vertebrates

Fungal extracts have been known to modulate immune function for millennia, but interest over the last few decades has focused particularly on β-glucans. The administration of purified β-glucans has been shown to have a number of beneficial effects, including protection against tumour development and infections with fungal, bacterial, viral and protozoal pathogens, prompting interest in the pharmaceutical development of these carbohydrates (Ross et al., 1999; Tzianabos, 2000). In certain circumstances, however, β-glucans can have negative effects on the host, including lethal toxicity in combination with certain anti-inflammatory drugs (Takahashi et al., 2001), granuloma formation (Williams et al., 1996) and they may contribute to asthma (Rylander and Lin, 2000). β-Glucans are also long lived in vertebrate systems, which lack the appropriate glucanases, and, depending on their physical properties are either degraded through oxidation in the liver or secreted through glomerular filtration (Suda et al., 1996).

The immune-modulating abilities of β-glucans are thought to stem from their ability to activate leukocytes, but there is some confusion about their precise biological
effects. This has occurred through the use of different β-glucans which vary in their origin, molecular structure and purity; parameters which have been shown to influence their activity (Bohn and BeMiller, 1995). In addition, some soluble glucan polymers have been shown to possess activity in vivo, yet do not show marked in vitro effects (Brown et al., 2003; Williams et al., 2003). It is also likely that the effects of β-glucans obtained in vivo result from both activatory and suppressive effects on immune function (Williams et al., 2004).

Although unclear, there are some generalizations regarding the cellular effects of β-glucans, which can be broadly categorized by their molecular weight. Large molecular weight and/or particulate β-glucans appear to be able to activate leukocytes directly, stimulating their phagocytic, cytotoxic and anti-microbial activities, including reactive intermediates, as well as stimulating the production of pro-inflammatory mediators, cytokines and chemokines, including IL-8, IL-1β, IL-6 and TNF-α (Czop, 1986; Williams et al., 1996). β-Glucans also enhance the recognition and clearance of apoptotic cells (Fadok et al., 2000). The effects of lower molecular weight β-glucans are less clear, although they possess biological activity in vivo. These carbohydrates do not generally appear to activate leukocytes directly, but in some instances have been shown to induce cytokines, such as IL-8 and IL-6, and nuclear transcription factors, such as NFκB and NF-κBIL-6 (Adams et al., 1997; Battle et al., 1998). These β-glucans are thought to modulate cellular responses to secondary challenges, but there are conflicting data as they have been shown to both prime and suppress these responses (Engstad et al., 2002; Nakagawa et al., 2003). These β-glucans also have unusual intracellular trafficking, a property which may be related to their activity (Herre et al., 2004a). The small β-glucans (<5000–10000 MW) are biologically inactive, although they can act as β-glucan receptor antagonists (Brown and Gordon, 2001).

Vertebrate responses to β-glucans appear to be mediated primarily by cell surface receptors and, although opsonization does contribute to the recognition of particulate glucans, no known plasma molecules recognizing these carbohydrates have been described. β-Glucan receptor activity has been identified on both immune and non-immune cells, including monocytes, macrophages, neutrophils and Langerhans cells, eosinophils, natural killer (NK) cells, endothelial cells, alveolar epithelial cells and fibroblasts (Brown and Gordon, 2003). A number of cellular receptors have been implicated in these activities, including CR3, lactosylceramide, scavenger receptors and Dectin-1 (Fig. 1). Cellular recognition is thought to be mediated by combinations of these receptors (Battle et al., 1998), but only Dectin-1 has been clearly demonstrated to play a role in the cellular responses to these carbohydrates (see below).

The molecular mechanisms behind the cellular responses to β-glucans are not well understood and although some insights into these processes have been gained over the last few years, most of these studies have utilized impure and/or particulate β-glucans, such as zymosan. The Toll-like receptors have been identified as the major signal transducers for inducing inflammatory reactions to PAMPs, and have also been shown to play a role in zymosan (and fungal) mediated signal transduction. TLR-2, in particular, forms functional pairs with TLR-6 to induce cytokine and chemokine production in response to zymosan (Underhill et al., 1999; Ozinsky et al., 2002; Nakagawa et al., 2003).

Fig. 1. Cartoon representation of the β-glucan pattern recognition receptors found in vertebrates and their known effects. Scavenger receptors comprise a group of molecules with heterogeneous structures and although the member(s) capable of β-glucan have not been identified the structure of SR-A is shown. SR-A is a homo-trimeric molecule consisting of an alpha helical coil-coiled (red), a collagenous domain which is responsible for ligand binding (purple), and a scavenger receptor cysteine rich domain (blue). CR3 is heterodimeric molecule composed of the αm (CD11b; blue) and β2 (CD18; green) chains while lactosylceramide (LacCer) consists of a hydrophobic ceramide lipid and a hydrophilic sugar moiety (yellow). Dectin-1 is composed of a carbohydrate-like binding domain (green), a stalk region, and a cytoplasmic tail containing an ITAM-like motif. The intracellular signalling pathways triggered by these receptors are mostly unknown and it should be noted that the production of pro-inflammatory cytokines and chemokines by Dectin-1 also requires signals generated from the TLRs.
et al., 2000). Other downstream components of the TLR signalling pathway have also been shown to be required, including MyD88 and NFκB (Young et al., 2001; Kataoka et al., 2002). Although able to interact with zymosan, the Toll-like receptors do not appear to recognize β-glucan structures, and indeed the recognition and response to these carbohydrates was shown to require the β-glucan receptor, Dectin-1 (Brown et al., 2003; Gantner et al., 2003; Sato et al., 2003). Dectin-1 has also been shown to induce intracellular signalling independently of the Toll-like receptors, and is currently the most likely candidate mediating immune responses to β-glucans.

Vertebrate recognition proteins

Scavenger receptors

Scavenger receptors comprise a heterogeneous group of molecules which recognize modified low-density lipoproteins, selected polyanionic ligands and a variety of microbes (Peiser et al., 2002). These receptors are expressed by myeloid cells and some endothelial cells and have been shown to be involved in both homeostasis and immunity. A number of studies have implicated soluble β-glucans as ligands, but no specific scavenger receptor(s) have been identified (Rice et al., 2002). Although thought to be able to recognize the basic glucan structure, the receptor affinity for these carbohydrates is greatly affected to be able to recognize the basic glucan structure, the glucans (Thornton et al., 2003). CR3 also possesses a carbohydrate binding site, located in the C-terminus of CD11b, which opsonized particles. CR3 also possesses a carbohydrate recognition domain (CRD) and a cytoplasmic tail possessing an immunoreceptor tyrosine-based activation motif (ITAM). This receptor is predominantly expressed on cells of the monocyte/macrophage and neutrophil lineages and its expression can be markedly influenced by cytokines and microbial products (Taylor et al., 2002; Willment et al., 2003). Although the signalling mechanisms behind these activities are unknown, it may involve the activation of Lyn kinase (Iwabuchi and Nagaoka, 2002).

CR3

Complement receptor 3 is a heterodimeric integrin receptor composed of the αm (CD11b) and β2 (CD18) chains. CR3 is expressed on myeloid cells, NK cells and selected lymphocytes and functions as a cellular adhesion molecule and as a phagocytic receptor for a wide variety of opsonized and unopsonized pathogens, including fungi. A number of domains have been identified in CR3, including the I domain, which is responsible for the recognition of a variety of ligands, including the intercellular adhesion molecules (ICAMs), extracellular matrix proteins and iC3b-opsonized particles. CR3 also possesses a carbohydrate binding site, located in the C-terminus of CD11b, which recognizes a number of carbohydrates, including β-glucans (Thornton et al., 1996; Xia and Ross, 1999). β-Glucan recognition by the lectin domain of CR3 has been proposed to affect the transendothelial migration of activated neutrophils and to play a role in CR3 dependent cytotoxicity of iC3b coated tumour cells (Ross, 2000; Tsi-kitis et al., 2004). The exact role of CR3 in responses to β-glucans is unclear, however, as leukocytes lacking CR3 can still bind and respond normally to β-glucans (Brown et al., 2002; Gantner et al., 2003).

Lactosylceramide

Lactosylceramide (LacCer; CDw17) is a glycosphingolipid found in many cell types and consists of a hydrophobic ceramide lipid and a hydrophilic sugar moiety which form microdomains in the plasma membrane. Lactosylceramide has been shown to recognize a variety of microbes (Karlsson, 1989), and was identified as a receptor for β-glucans from a biochemical screen with a radiolabelled high molecular weight β-glucan (PGG-glucan) (Zimmerman et al., 1998). The interaction of β-glucan with this receptor was subsequently shown to enhance the neutrophil oxidative burst and anti-microbial functions and to induce the activation of NFκB and the production of macrophage inflammatory protein (MIP)-2 in alveolar epithelial cells (Wakshull et al., 1999; Hahn et al., 2003). Although the signalling mechanisms behind these activities are unknown, it may involve the activation of Lyn kinase (Iwabuchi and Nagaoka, 2002).

Dectin-1

Dectin-1 is a type II transmembrane glycoprotein which possesses a single extracellular non-classical C-type carbohydrate recognition domain (CRD) and a cytoplasmic tail possessing an immunoreceptor tyrosine-based activation motif (ITAM). This receptor is predominantly expressed on cells of the monocyte/macrophage and neutrophil lineages and its expression can be markedly influenced by cytokines and microbial products (Taylor et al., 2002; Willment et al., 2003). Although the expression of this receptor is heterogeneous on these cells in tissues, its distribution is consistent with a role in pathogen surveillance (Reid et al., 2004). Dectin-1 recognizes soluble and particulate β-(1–3)- and/or β-(1–6)-linked glucans, including intact fungal particles, as well as an unidentified ligand on T-cells (Arizumi et al., 2000; Brown and Gordon, 2001; Steele et al., 2003). Two amino acids (Trp221 and His225) in the CRD have been shown to be critical for the interactions with β-glucan (Adachi et al., 2004), although the mechanism of carbohydrate recognition by the non-classical C-type lectin-like domain of Dectin-1 is still unclear. Human Dectin-1 differs from its murine counterpart in that it is alternatively spliced, in a cell-specific manner, giving rise to several isoforms of which only two are functional for β-glucan recognition (Willment et al., 2001).

Dectin-1 appears to be a primary leukocyte receptor for soluble and particulate β-glucans, including yeast, and can contribute to the recognition of opsonized β-glucan-containing particles (Brown et al., 2002; Steele et al., 2003). This receptor mediates a variety of cellular responses to β-glucans, including phagocytosis, endocytosis and the oxidative burst and can induce the produc-
tion of pro-inflammatory cytokines and chemokines, including TNF-α, MIP-2 and IL-12, although this requires a collaboration with the Toll-like receptors, discussed above (Brown et al., 2003; Gantner et al., 2003; Herre et al., 2004a). These responses are triggered through the cytoplasmic ITAM-like motif of this receptor, utilizing novel signalling pathways which are independent of Syk kinase. Dectin-1 may also associate with other molecules during the recognition of β-glucans, including the tetraspanin receptor, CD63, and Pentraxin 3 (Diniz et al., 2004; Mantegazza et al., 2004).

Role of β-glucans in vertebrate responses to fungal infections

The innate recognition of fungi in vertebrates occurs through both opsonic and non-opsonic mechanisms. Although little is known about the non-opsonic recognition mechanisms, a number of PRRs have been implicated, including those that recognize the major carbohydrate constituents of fungal cell walls (Romani, 2004). The recognition of the β-glucan components in intact fungi, in particular, by CR3, lactosylceramide and Dectin-1, has been shown to be important both in clearance of these organisms and in the triggering of appropriate immune responses (Brown and Gordon, 2003). It has been suggested that fungal pathogens may mask their β-glucan to avoid immune recognition and indeed certain pathogens do not appear to have readily exposed β-glucans (Herre et al., 2004b). Soluble glucans are also released into the plasma during fungal infections, providing a useful diagnostic marker, but their effects on immune function, if any, are unknown.

Invertebrate responses to β-glucans

Fungal β-glucans can induce all of the major anti-microbial immune mechanisms found in invertebrates, including the humoral, cellular and phenoloxidase responses. The majority of these responses rely on protease cascades which are initiated by PAMP recognition in the haemolymph (Fig. 2). In most cases, the components of these cascades and the mechanism by which they are activated by the appropriate PRR is unknown. Activation of these cascades triggers responses either in the haemolymph, such as coagulation, or in immune competent cells, such as anti-microbial peptide production. The recognition of β-glucans can also lead to uptake by phagocytosis in certain haemocytes (Brennan and Anderson, 2004). Given the ease of genetic manipulation, many of

![Fig. 2. β-Glucan induced responses and protease cascades in invertebrates. The β-glucan receptors are highlighted in yellow. (PA, phenoloxidase activating enzyme).](image-url)
these mechanisms have been studied in Drosophila (Brennan and Anderson, 2004), but a great deal of information, especially regarding β-glucan recognition, has also been obtained through more classical approaches in other organisms, including Anopheles sp., Maduca sexta and Bombyx mori.

β-Glucans have been shown to induce at least two types of humoral responses in invertebrates, coagulation and anti-microbial peptide production. Although less well described in insects, the coagulation cascade in response to PAMPs has been defined in other arthropods, particularly the horseshoe crab, and is thought to function in wound healing and to restrict the movement of microbes (Theopold et al., 2002; Muta and Iwanaga, 1996). β-Glucans, in particular, are recognized by PRR, such as Factor G, which induces a series of sequential proteolysis events culminating in the coagulation of clottable proteins, such as Coagulin (Fig. 2). The components of this cascade, which are released by haemocytes, are strictly regulated by protease inhibitors, such as serpins, and may be influenced by the phenoloxidase system (Muta and Iwanaga, 1996; Li et al., 2002).

The study of anti-microbial peptide production, particularly in Drosophila, has contributed to some of the most significant findings in modern vertebrate immunology. The discovery of the involvement of the Toll receptors in immunity was first made in Drosophila mutants in response to fungal infection (Lemaitre et al., 1996). Subsequent studies have revealed that, in contrast to the vertebrate receptors, invertebrate Toll receptors do not directly recognize PAMPs, but are rather part of the signalling pathway resulting in gene transcription and anti-microbial peptide production in the fat body (Fig. 2, and Hoffmann, 2003; Ferrandon et al., 2004 for recent reviews). The PRRs involved in sensing and triggering these pathways have only recently been identified, but many of the intermediate components remain unknown. Although the PRR(s) involved in β-glucan recognition via the Toll pathway have not been formally identified, they are likely to be members of the β-glucan recognition protein (βGRP) family (Ferrandon et al., 2004). Recognition results in the production of peptides with anti-fungal activity, including Drosomycin, and may also trigger cellular responses (Hultmark, 2003).

β-Glucan recognition may also trigger the second, imd/relish, anti-microbial pathway, probably through recognition by membrane bound peptidoglycan recognition proteins (PGRPs) (Hedengren et al., 1999; Hultmark, 2003).

Activation of phenoloxidase in invertebrates leads to melanin formation and deposition and the generation of toxic metabolites, both of which have anti-microbial functions (Soderhall and Cerenius, 1998). Phenoloxidase is synthesized as an inactive precursor and becomes activated following proteolysis as the last step in a serine protease cascade which is initiated following PAMP recognition, although the components of this cascade are only partly characterized (Fig. 2). The recognition of β-glucan by a number of PRRs, including βGRPs and PGRPs, have been shown to induce this response, but the molecular mechanism by which this occurs is unknown (Cerenius and Soderhall, 2004).

The cellular responses of haemocytes are important for invertebrate immunity and three types of such cells have been identified in Drosophila, the plasmatocytes, lamellocytes and crystal cells (Meister, 2004). Crystal cells are thought to function as storage cells for prophenoloxidase, while lamellocytes are involved in encapsulation of larger parasites. Plasmatocytes, are phagocytically active cells involved in the clearance of apoptotic cells and microbes, which may also play a role in humoral responses. These cells express β-glucan receptors and are likely to be involved in the recognition and uptake of fungal particles, although this has not been formally demonstrated, and it is possible that the recognition of β-glucan particles can occur indirectly, through opsonization by soluble receptors. Relatively little is known about the ability of these cells to recognize and respond to pathogens.

**Invertebrate recognition proteins**

**Apolipoporin III**

Apolipoporin III (apoLp-III) is a lipid transport molecule found in the haemolymph of insects and is structurally and functionally similar to mammalian apolipoprotein E (apoE). In addition to its role in homeostasis, apoLp-III has been shown to be involved in innate immune responses, acting as a pattern recognition receptor for LPS, LTA, Gram-positive bacteria as well as particulate β-glucan and fungal conidia (Whitten et al., 2004). ApoLp-III has been shown to increase the anti-microbial activity of both the haemolymph and blood cells (Niere et al., 1999; Whitten et al., 2004). Although most of these activities require lipid-induced conformational changes in ApoLp-III, these changes were not required for β-glucan binding (Niere et al., 2001; Whitten et al., 2004). Activated apoLp-III is recognized and endocytosed by subpopulation of haemocytes (Dettloff et al., 2001) but the mechanism by which it influences immune reactions is unclear.

**GNBP/βGRPs**

The Gram-negative binding proteins (GNBPs)/βGRPs are perhaps one of the best characterized family of PRRs in invertebrates. These PRRs contain a C-terminal domain similar to bacterial β-glucanas, but lack enzymatic activity because of a number of amino acid substitution in the active site (Royet, 2004). In most insects, β-glucan recognition is mediated by an N-terminal extension of about 100
amino acids, which has also been shown to have immune modulating activity (Fabrick et al., 2004). In addition to purified β-glucan, these proteins recognize LPS, LTA and intact bacteria and yeast (Royet, 2004). Most of these proteins are secreted into the haemolymph, but at least one may be membrane bound via a GPI-linked anchor (Kim et al., 2000). Expression of some of these proteins can be induced upon infection with yeast or bacteria (Jiang et al., 2004). Although these proteins have been implicated in a variety of immune responses in invertebrates, including the activation of the prophenoloxidase cascade and anti-microbial peptide production (Ferrandon et al., 2004; Royet, 2004), the mechanism by which binding of β-glucan triggers these responses is unknown. Mutants in two of the three GNBP genes have been generated in Drosophila, but only one (GNBP3hades) results in susceptibility to fungal infections (Gobert et al., 2003; Ferrandon et al., 2004).

**Hd-PGRP**

The PGRPs are a large family of proteins possessing domains homologous to N-acetylmuramyl-L-alanine amidases found in bacteria and bacteriophages. Although members of this family are primarily involved in the recognition of peptidoglycan, two plasma PGRPs from *Holotrichia diomphalia* (Hd-PGRP-1 and Hd-PGRP-2) have been shown to be capable of recognizing β-glucan. Hd-PGRP-1 is able to induce the prophenoloxidase cascade in the presence of β-glucan, suggesting that it may play a direct role in anti-fungal responses (Lee et al., 2004).

**Factor G**

Horseshoe crab Factor G is a non-covalently linked heterodimer found in the haemolymph which responds specifically to β-glucan. The α subunit, which contains domains similar to bacterial glucanases and carbohydrate binding proteins, mediates the recognition of β-glucans, while the β subunit is a protease zymogen (Takaki et al., 2002). In response to β-glucan, Factor G becomes activated by undergoing autocatalytic proteolysis and initiates activation of the proclotting enzyme and the coagulation cascade, leading to haemolymph clot formation (Muta et al., 1995). The specificity of the interaction of this enzyme with β-glucan has led to its use as a diagnostic reagent for the detection of fungal infections in humans.

**SR-CI**

SR-CI is a class C scavenger receptor that was identified and cloned from *Drosophila* haemocytes (Pearson et al., 1995). This receptor was shown to recognize typical scavenger receptor ligands, described above, as well as intact Gram-positive and Gram-negative bacteria. Although able to recognize the soluble β-glucan, laminarin, the functional significance of this interaction is unclear as the receptor does not recognize intact fungal particles (Pearson et al., 1995; Ramet et al., 2001).

**Role of β-glucans in invertebrate responses to fungal infections**

The recognition and response to β-glucans is an essential component of immunity to fungal pathogens in invertebrates, as they appear to lack the mannose-based recognition systems which contribute to the recognition of these pathogens in vertebrates. The most compelling evidence for this is the extreme sensitivity to fungal infections displayed by organisms harbouring mutations in the β-glucan sensing molecules or pathways, such as Toll or JGBP (Lemaître et al., 1996; Ferrandon et al., 2004). The phenoloxidase and cellular responses are also important in controlling these pathogens, as has been demonstrated in Drosophila mutants lacking these systems (Braun et al., 1998). Indeed, the susceptibility of these mutants has led to their use as models to study virulence characteristics of human fungal pathogens (Alarco et al., 2004).

**Conclusions**

The recognition of β-glucans plays an important role in anti-fungal immunity in both vertebrates and invertebrates, although they have evolved distinct strategies to recognize and respond to these carbohydrates. It is hoped that future studies will more clearly delineate the role of these receptors in mammalian systems and explore the mechanisms by which the receptors trigger immune responses in invertebrates.

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**References**

Adachi, Y., Ishii, T., Ikeda, Y., Hoshino, A., Tamura, H., Aketagawa, J., et al. (2004) Characterization of beta-Glucan recognition site on C-Type lectin, Dectin 1. *Infect Immun* 72: 4159–4171.

Adams, D.S., Pero, S.C., Petro, J.B., Nathans, R., Mackin, W.M., and Wakshull, E. (1997) PGG-Glucan activates NF-kappaB-like and NF-IL-6-like transcription factor complexes in a murine monocytic cell line. *J Leukoc Biol* 62: 865–873.

Alarco, A.M., Marcil, A., Chen, J., Suter, B., Thomas, D., and Whiteway, M. (2004) Immune-deficient Drosophila melanogaster: a model for the innate immune response to human fungal pathogens. *J Immunol* 172: 5622–5628.

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Ariizumi, K., Shen, G.L., Shikano, S., Xu, S., Ritter, R., 3rd, Kumamoto, T., et al. (2000) Identification of a novel, dendritic cell-associated molecule, dectin-1, by subtractive cDNA cloning. J Biol Chem 275: 20157–20167.

Battle, J., Ha, T., Li, C., Delia Beffa, V., Rice, P., Kalbfleisch, J., et al. (1998) Ligand binding to the (1→3)-beta-D-glucan receptor stimulates NFkappB activation, but not apoptosis in U937 cells. Biochem Biophys Res Commun 249: 498–504.

Bohn, J.A., and BeMiller, J.N. (1995) (1→3)-beta-D-glucans as biological response modifiers: a review of structure-functional activity relationships. Carbohydr Polymers 28: 3–14.

Braun, A., Hoffmann, J.A., and Meister, M. (1998) Analysis of the Drosophila host defense in domino mutant larvae, which are devoid of hemocytes. Proc Natl Acad Sci USA 95: 14337–14342.

Brennan, C.A., and Anderson, K.V. (2004) Drosophila: the genetics of innate immune recognition and response. Annu Rev Immunol 22: 457–483.

Brown, G.D., and Gordon, S. (2003) Immune recognition: a new receptor for beta-glucans. Nature 413: 36–37.

Brown, G.D., and Gordon, S. (2003) Fungal beta-glucans and mammalian immunity. Immunity 19: 311–315.

Brown, G.D., Herre, J., Williams, D.L., Willment, J.A., Marshall, A.S.J., and Gordon, S. (2003) Dectin-1 mediates the biological effects of beta-glucan. J Exp Med 197: 1119–1124.

Brown, G.D., Taylor, P.R., Reid, D.M., Willment, J.A., Williams, D.L., Martinez-Pomares, L., et al. (2002) Dectin-1 is a major beta-glucan receptor on macrophages. J Exp Med 296: 407–412.

Cerini, L., and Soderhall, K. (2004) The prophenoloxidase-activating system in invertebrates. Immunol Rev 198: 116–126.

Czop, J.K. (1986) The role of beta-glucan receptors on blood and tissue leukocytes in phagocytosis and metabolic activation. Pathol Immunopathol Res 5: 286–296.

Dettloff, M., Kaiser, B., and Wiesner, A. (2001) Localization of injected apolipophorin III in vivo – new insights into the immune activation process directed by this protein. J Insect Physiol 47: 789–797.

Diniz, S.N., Nomizo, R., Csaiapino, P.S., Teixeira, M.M., Brown, G.D., Mantovani, A., et al. (2004) PTX3 function as an ospon in the dectin-1-dependent internalization of zymosan by macrophages. J Leukoc Biol 76: 649–656.

Engstad, C.S., Engstad, R.E., Olsen, J.O., and Osterud, B. (2002) The effect of soluble beta-1,3-glucan and lipopolysaccharide on cytokine production and coagulation activation in whole blood. Int Immunopharmacol 2: 1585–1597.

Fabrick, J.A., Baker, J.E., and Kanost, M.R. (2004) Innate immunity in a pyralid moth: functional evaluation of domains from a beta-1,3-glucan recognition protein. J Biol Chem 279: 26605–26611.

Fadok, V.A., Bratton, D.L., Rose, D.M., Pearson, A., Ezekewitz, R.A., and Henson, P.M. (2000) A receptor for phosphatidylserine-specific clearance of apoptotic cells. Nature 405: 85–90.

Ferrandon, D., Imler, J.L., and Hoffmann, J.A. (2004) Sensing infection in Drosophila: Toll and beyond. Semin Immunol 16: 43–53.

Gantner, B.N., Simmons, R.M., Canavera, S.J., Akira, S., and Underhill, D.M. (2003) Collaborative induction of inflammasory responses by Dectin-1 and Toll-like receptor 2. J Exp Med 197: 1107–1117.

Gobert, V., Gottar, M., Matskevich, A.A., Rutschmann, S., Royet, J., Belvin, M., et al. (2003) Dual activation of the Drosophila toll pathway by two pattern recognition receptors. Science 302: 2126–2130.

Hahn, P.Y., Evans, S.E., Kottom, T.J., Standing, J.E., Pagano, R.E., and Limper, A.H. (2003) Pneumocystis carinii cell wall beta-glucan induces release of macrophage inflammatory protein-2 from alveolar epithelial cells via a lactosylceramide-mediated mechanism. J Biol Chem 278: 2043–2050.

Hedengren, M., Asling, B., Dushay, M.S., Ando, I., Ekengren, S., Whiborg, M., and Hultmark, D. (1999) Relish, a central factor in the control of hemoral but not cellular immunity in Drosophila. Mol Cell 4: 827–837.

Herre, J., Marshall, A.J., Caron, E., Edwards, A.D., Williams, D.L., Schweighoffer, E., et al. (2004a) Dectin-1 utilizes novel mechanisms for yeast phagocytosis in macrophages. Blood 104: 4038–4045.

Herre, J., Gordon, S., and Brown, G.D. (2004b) Dectin-1 and its role in the recognition of beta-glucans by macrophages. Mol Immunol 40: 869–876.

Hoffmann, J.A. (2003) The immune response of Drosophila. Nature 426: 33–38.

Hultmark, D. (2003) Drosophila immunity: paths and patterns. Curr Opin Immunol 15: 12–19.

Iwabuchi, K., and Nagaoka, I. (2002) Lactosylceramide-enriched glycosphingolipid signaling domain mediates superoxide generation from human neutrophils. Blood 100: 1454–1464.

Janeway, C.A., Jr. (1992) The immune system evolved to discriminate infectious self from noninfected self. Immunol Today 13: 11–16.

Jiang, H., Ma, C., Lu, Z.Q., and Kanost, M.R. (2004) Beta-1,3-glucan recognition protein-2 (betaGRP-2) from Manduca sexta; an acute-phase protein that binds beta-1,3-glucan and lipoteichoic acid to aggregate fungi and bacteria and stimulate prophenoloxidase activation. Insect Biochem Mol Biol 34: 89–100.

Karlsson, K.A. (1989) Animal glycosphingolipids as membrane attachment sites for bacteria. Annu Rev Biochem 58: 309–350.

Kataoka, K., Muta, T., Yamazaki, S., and Takeshige, K. (2002) Activation of macrophages by linear (1,3)-beta-D-glucans. J Biol Chem 277: 36825–36831.

Kim, Y.S., Ryu, J.H., Han, S.J., Choi, K.H., Nam, K.B., Jang, I.H., et al. (2000) Gram-negative bacteria-binding protein, a pattern recognition receptor for lipopolysaccharide and beta-1,3-glucan that mediates the signaling for the induction of innate immune genes in Drosophila melanogaster cells. J Biol Chem 275: 32721–32727.

Lee, M.H., Osaki, T., Lee, J.Y., Baek, M.J., Zhang, R., Park, J.W., et al. (2004) Peptidoglycan recognition proteins involved in 1,3-beta-D-glucan-dependent prophenoloxidase activation system of insect. J Biol Chem 279: 3218–3227.

Lemaitre, B., Nicolas, E., Michaut, L., Reichhart, J.M., and Hoffmann, J.A. (1996) The dorsalventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 86: 973–983.

Li, D., Scherfer, C., Korayem, A.M., Zhao, Z., Schmidt, O., and Theopold, U. (2002) Insect hemolymph clotting: evidence for interaction between the coagulation system and
the prophenoloxidase activating cascade. *Insect Biochem Mol Biol* 32: 919–928.

Mantegazza, A.R., Barrio, M.M., Moutel, S., Bover, L., Weck, M., Brossart, P., et al. (2004) CD63 Tetranspin slows down cell migration and translocates to the endosomal/lysosomal/MICs route after extracellular stimuli in human immature dendritic cells. *Blood* 104: 1183–1190.

Meister, M. (2004) Blood cells of Drosophila: cell lineages and role in host defence. *Curr Opin Immunol* 16: 10–15.

Muta, T., and Iwanaga, S. (1996) The role of hemolymph coagulation in innate immunity. *Curr Opin Immunol* 8: 41–47.

Muta, T., Seki, N., Takaki, Y., Hashimoto, R., Oda, T., Iwanaga, A., et al. (1995) Purified horseshoe crab factor G. Reconstitution and characterization of the (1–3)-beta-D-glucan-sensitive serine protease cascade. *J Biol Chem* 270: 892–897.

Nakagawa, Y., Ohno, N., and Murai, T. (2003) Suppression by Candida albicans beta-glucan of cytokine release from activated human monocytes and from T cells in the presence of monocytes. *J Infect Dis* 187: 710–713.

Niere, M., Dettloff, M., Maier, T., Ziegler, M., and Wiesner, A. (2001) Insect immune activation by apolipoporphin III is correlated with the lipid-binding properties of this protein. *Biochemistry* 40: 11502–11508.

Niere, M., Meisslitzer, C., Dettloff, M., Weise, C., Ziegler, M., and Wiesner, A. (1999) Insect immune activation by recombinant Galleria mellonella apolipoporphin III (1). *Biochim Biophys Acta* 1433: 16–26.

Ozinsky, A., Underhill, D.M., Fontenot, J.D., Hajjar, A.M., Smith, K.D., Wilson, C.B., et al. (2000) The repertoire for pattern recognition of pathogens by the innate immune system is defined by cooperation between toll-like receptors. *Proc Natl Acad Sci USA* 97: 13766–13771.

Pearson, A., Lux, A., and Krieger, M. (1995) Expression cloning of dSR-CI, a class C macrophage-specific scavenger receptor from Drosophila melanogaster. *Proc Natl Acad Sci USA* 92: 4056–4060.

Peiser, L., Mukhopadhyay, S., and Gordon, S. (2002) Scavenger receptors in innate immunity. *Curr Opin Immunol* 14: 123–128.

Ramey, P., Arsenault, L., Riddell, R., Li, X., Koziel, H., Gobel, V., et al. (2001) Drosophila scavenger receptor CI is a pattern recognition receptor for bacteria. *Immunology* 155: 1027–1038.

Reid, D.M., Montoya, M., Taylor, P.R., Borrow, P., Gordon, S., Brown, G.D., and Wong, S.Y. (2004) Expression of the [beta]-glucan receptor, Dectin-1, on murine leukocytes in situ correlates with its function in pathogen recognition and reveals potential roles in leukocyte interactions. *J Leukoc Biol* 76: 86–94.

Rice, P.J., Kelley, J.L., Kogan, G., Ensley, H.E., Kalbfleisch, J.H., Browder, I.W., and Williams, D.L. (2002) Human monocyte scavenger receptors are pattern recognition receptors for (1–3)-beta-D-glucans. *J Leukoc Biol* 72: 140–146.

Romani, L. (2004) Immunity to fungal infections. *Nat Rev Immunol* 4: 1–23.

Ross, G.D. (2000) Regulation of the adhesion versus cytotoxic functions of the Mac-1/CR3/alphaMbeta2-integrin glycoprotein. *Crit Rev Immunol* 20: 197–222.

Ross, G.D., Vetvicka, V., Yan, J., Xia, Y., and Vetvickova, J. (1999) Therapeutic intervention with complement and beta-glucan in cancer. *Immunopharmacology* 42: 61–74.
golipid beta-(1,3)-glucan receptor. Immunopharmacology 41: 89–107.
Whitten, M.M., Tew, I.F., Lee, B.L., and Ratcliffe, N.A. (2004) A novel role for an insect apolipoprotein (apolipophorin III) in beta-1,3-glucan pattern recognition and cellular encapsulation reactions. J Immunol 172: 2177–2185.
Williams, D.L., Li, C., Ha, T., Ozment-Skelton, T., Kalbfleish, J.H., Preiszner, J., et al. (2004) Modulation of the phosphoinositide 3-kinase pathway alters innate resistance to polymicrobial sepsis. J Immunol 172: 449–456.
Williams, D.L., Mueller, A., and Browder, W. (1996) Glucan-based macrophage stimulators. Clin Immunother 5: 392–399.
Williams, D.L., Rice, P., Herre, J., Willment, J.A., Taylor, P.R., Gordon, S., and Brown, G.D. (2003) Recognition of fungal glucans by pattern recognition receptors. In Recent Developments in Carbohydrate Research. Gordon, S. (ed.). Trivandrum: Transworld research network, pp. 49–66.
Willment, J.A., Gordon, S., and Brown, G.D. (2001) Characterisation of the human [beta]-glucan receptor and its alternatively spliced isoforms. J Biol Chem 276: 43818–43823.
Willment, J.A., Lin, H.H., Reid, D.M., Taylor, P.R., Williams, D.L., Wong, S.Y.C., et al. (2003) Dectin-1 expression and function is enhanced on alternatively activated and GM-CSF treated macrophages and negatively regulated by IL-10, dexamethasone and LPS. J Immunol 171: 4569–4573.
Xia, Y., and Ross, G.D. (1999) Generation of recombinant fragments of CD11b expressing the functional beta-glucan-binding lectin site of CR3 (CD11b/CD18). J Immunol 162: 7285–7293.
Young, S.H., Ye, J., Frazer, D.G., Shi, X., and Castranova, V. (2001) Molecular mechanism of tumor necrosis factor-alpha production in 1->3-beta-glucan (zymosan)-activated macrophages. J Biol Chem 276: 20781–20787.
Zimmerman, J.W., Lindermuth, J., Fish, P.A., Palace, G.P., Stevenson, T.T., and DeMong, D.E. (1998) A novel carbohydrate–glycosphingolipid interaction between a beta-(1-3)-glucan immunomodulator, PGG-glucan, and lactosylceramide of human leukocytes. J Biol Chem 273: 22014–22020.