CHAPTER 4.5.2

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(1,3)-β-Glucans in Innate Immunity: Mammalian Systems

Gordon D. Brown[1] and David L. Williams[2]

[1]*Institute of Infectious Disease and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa*

[2]*Departments of Surgery and Pharmacology, James H. Quillen College of Medicine, East Tennessee State University, Johnson City, TN, USA*

p0010 The ability of (1,3)-β-glucans to stimulate innate immunity and modulate disease outcome has been known for more than four decades. However, the vast majority of these studies were phenomenological in nature. What was lacking was a mechanistic understanding of how (1,3)-β-glucans modulate innate immunity and alter disease outcome. Recent advances have dramatically increased our understanding of how glucans modulate innate immunity at the cellular and molecular level. The discovery of membrane-associated glucan-specific pattern recognition receptors, such as Dectin-1, has provided insights into how (1,3)-β-glucans are recognised by cells. New light has also been shed on the intracellular signal transduction pathways that are activated by glucans and how modulation of these signalling pathways ameliorates disease. This chapter focuses on recent advances in our understanding of the mechanisms by which (1,3)-β-glucans modulate innate immunity in mammals, and how modulation of innate immunity with glucan alters host response to disease.

AUQ1

I.A. Introduction

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p0020 There is an extensive literature demonstrating the ability of (1,3)-β-glucans to stimulate innate immunity and modulate disease outcome in mammalian systems (Bohn and BeMiller, 1995; Chen and Seviour, 2007; Williams et al., 1996; Williams, 1997; Williams et al., 2004b; Williams et al., 2004a). This literature has been the subject of numerous in-depth reviews (Bohn and BeMiller, 1995; Williams et al., 1996; Williams, 1997; Williams et al., 2004b; Williams et al., 2004a). There are also a number of clinical reports describing the

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prophylactic and/or therapeutic effects of glucans (Babineau et al., 1994a; Babineau et al., 1994b; Browder et al., 1990; Takahashi et al., 2006). However, most of these studies were phenomenological in nature; that is, they reported the effect of glucan administration in a given disease state. What was lacking was a mechanistic understanding of how (1,3)-β-glucans modulate innate immunity and subsequently alter the response to disease. Over the past decade, dramatic advances have been made in our understanding of the mechanisms of (1,3)-β-glucan immunobiology. This chapter focuses on recent advances in our understanding of the mechanisms by which (1,3)-β-glucans modulate innate immunity in mammals, and how modulation of innate immunity by (1,3)-β-glucans alters host response to disease.

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I.A.1. Mammalian receptors for (1,3)-β-glucans

p0030 In contrast to invertebrates, discussed in Chapter 4.1.2, (1,3)-β-glucan recognition in vertebrates occurs primarily through cell surface receptors. These receptors were first identified on human monocytes in the mid-1980s (Czop, 1986) and have subsequently been described on many other immune and non-immune cells, including neutrophils, macrophages, natural killer (NK) cells, eosinophils, alveolar epithelial cells, endothelial cells and fibroblasts (reviewed in Williams, 1997; Brown and Gordon, 2003). (1,3)-β-Glucan recognition by these cells is thought to involve multiple receptors (Battle et al., 1998; Kougias et al., 2001; Mueller et al., 2000), and at least four receptors have been identified, including lactosylceramide, scavenger receptors, complement receptor 3 (CR3) and Dectin-1. Of these molecules, Dectin-1 appears to be the major receptor for (1,3)-β-glucans on leukocytes and is capable of mediating many of the biological activities of these carbohydrates.

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II.A.1. Lactosylceramide

p0040 Lactosylceramide (LacCer, CDw17 or Galβ4Glcβ1Cer) is a glycosphingolipid consisting of a hydrophobic ceramide lipid and a hydrophilic sugar moiety and is found in microdomains on the plasma membranes of many cells. The ability of LacCer to recognise (1,3)-β-glucans was first demonstrated biochemically, using isolated human leukocyte membrane components (Zimmerman et al., 1998). Although relatively little is known about the role of LacCer in (1,3)-β-glucan mediated immunomodulation, the interaction of this glycosphingolipid with these polysaccharides has been shown to induce a variety of cellular responses, such as the production of cytokines including MIP-2 and TNF (Evans et al., 2005; Hahn et al., 2003), activation of NF-κB (Evans et al., 2005), and enhancement of the oxidative burst and anti-microbial functions of leukocytes (Wakshull et al., 1999). LacCer is thought to mediate

the attachment of many microbes and may be an adhesion receptor for pathogens, such as *Candida albicans* (Jimenez-Lucho et al., 1990; Karlsson, 1989). Indeed, LacCer may play an important role in the innate response to these pathogens, especially on non-immune cells (Evans et al., 2005; Hahn et al., 2003; Jimenez-Lucho et al., 1990). How LacCer mediates intracellular signal transduction is unknown, but it may involve clustering and subsequent activation of the src kinase, Lyn (Iwabuchi and Nagaoka, 2002).

s0040 ### *II.A.2. Scavenger receptors*

p0050 Scavenger receptors recognise modified low-density lipoproteins and consist of a structurally heterogenous family of glycoprotein receptors. Although a specific receptor has not been identified, scavenger receptors have been implicated in (1,3)-β-glucan recognition (Dushkin et al., 1996; Rice et al., 2002; Vereschagin et al., 1998). The ability of soluble (1,3)-β-glucans to inhibit the interaction of isolated monocyte membranes with classic scavenger receptor ligands is probably the best supporting evidence (Rice et al., 2002), although the ability of (1,3)-β-glucans to interact with these receptors may be related to their charge, as the affinity of these interactions was found to be affected by the charge of the molecules (Rice et al., 2002). It is notable that at least one invertebrate scavenger receptor (Drosophila SR-CI) recognising these carbohydrates has been identified (Pearson et al., 1995), and this receptor is discussed in Chapter 4.1.1.

s0050 ### *II.A.3. Complement receptor 3 (CR3)*

p0060 Complement receptor 3 (CR3, Mac-1, $\alpha_m\beta_2$) is an integrin composed of two non-covalently linked chains, CD11b and CD18. CD11b is unique to CR3, whereas CD18 is found in all β_2 integrins. CR3 can be up-regulated following cellular activation and has been described on monocytes, macrophages, dendritic cells, neutrophils, eosinophils, NK cells, and on some T and B cells (Ross, 2000). Integrins are involved in immunity and cell–extracellular matrix interactions, in which they play a role in physical attachment as well as in intracellular signalling. The importance of integrins is exemplified by diseases such as leukocyte adhesion deficiency, where the loss of CD18 (and hence all β_2 integrins) causes life-threatening recurrent infections (Ehlers, 2000).

p0070 CR3 recognises a number of endogenous ligands (including intercellular adhesion molecules, extracellular matrix proteins, plasma proteins and proteases), but also acts as an opsonic receptor for the complement component, iC3b, and as a non-opsonic receptor for a variety of exogenous ligands. Importantly, CR3 possesses a distinct lectin domain, that maps to a site

C-terminal to the I-domain, which can recognise selected carbohydrates including (1,3)-β-glucan (Diamond et al., 1993; Ross et al., 1999; Thornton et al., 1996). Like other integrins, CR3 can mediate intracellular signalling following ligand binding, the so-called 'outside-in' signalling, but can also mediate 'inside-out' signalling, in which the affinity of the receptor for its ligand is influenced (Coppolino and Dedhar, 2000; Giancotti and Ruoslahti, 1999; Plow and Zhang, 1997). Signalling via CR3 can induce a number of cellular responses, including cytotoxicity, phagocytosis, adhesion and migration. However, some of these responses, such as cytotoxicity (Xia et al., 1999) and phagocytosis (Wright and Silverstein, 1982), require a second stimulus. Recently, it has been shown that CR3-mediated phagocytosis requires translocation of this receptor into LacCer-enriched lipid rafts, and signalling via Lyn kinase (Nakayama et al., 2007), whereas CR3-mediated cytotoxicity was shown to involve signalling via the Syk phosphatidylinositol 3-kinase (Li et al., 2006).

p0080 CR3 was first identified as a (1,3)-β-glucan receptor more than two decades ago and has been implicated in a number of cellular responses to these polysaccharides (Ross et al., 1985). The involvement of this receptor in the anti-tumourogenic properties of (1,3)-β-glucans is perhaps the best characterized, where the binding of these polysaccharides to the lectin domain primes leukocytes for CR3-dependent cytotoxicity of iC3b-coated target cells (Vetvicka et al., 1996; Xia et al., 1999). CR3 has also been implicated in the β-glucan-mediated enhancement of complement-mediated haematopoietic recovery, neutrophil chemotaxis, adhesion and transendothelial migration (Cramer et al., 2006; Harler et al., 1999; LeBlanc et al., 2006; Tsikitis et al., 2004; Xia et al., 2002; Yan et al., 1999). Interestingly, CR3 is now thought to recognise only low molecular weight (1,3)-β-glucans, generated from high molecular weight (1,3)-β-glucans through the actions of macrophages and other cells (Hong et al., 2004; Li et al., 2007). CR3 was originally proposed to be the principal (1,3)-β-glucan receptor on leukocytes (Ross, 2000), but the identification of Dectin-1 (see Section D) and the ability of CR3-deficient leukocytes to still recognise and respond to (1,3)-β-glucans suggest that CR3 may only play a minor role (Brown et al., 2002; Gantner et al., 2005; Li et al., 2007; Mueller et al., 1996).

s0060 ### II.A.4. Dectin-1

p0090 Dectin-1, or dentritic cell-associated C-type lectin-1 (CLEC7A), was first identified in a dendritic cell line as a receptor for T-cells (Ariizumi et al., 2000), but was subsequently found to be a (1,3)-β-glucan receptor, following functional screening of a macrophage-derived cDNA expression library (Brown and Gordon, 2001). Dectin-1 is a member of the group V C-type

lectin-like receptors (Zelensky and Gready, 2005) and is a type-II trans-membrane glycoprotein possessing a single non-classical C-type lectin-like domain, a stalk region, and a cytoplasmic tail which contains an immunoreceptor tyrosine-based activation-like motif (ITAM). Dectin-1 is alternatively spliced into two major and a number of minor isoforms. The major isoforms differ by the presence or absence of the extracellular stalk domain and appear to have slightly different functionalities, at least in transfected cell lines *in vitro* (Heinsbroek et al., 2006; Willment et al., 2001). One minor human isoform (hDectin-1E), which lacks the stalk and transmembrane domains, was recently shown to be located primarily in the cytoplasm and capable of interacting with a cytoplasmic scaffold protein, RanBPM (Ran Binding Protein in the Microtubule-organizing centre) (Murrin and Talbot, 2007; Xie et al., 2006). Dectin-1 is also N-glycosylated, a post-translation modification which contributes to the surface expression and function of this receptor (Kato et al., 2006).

p0100 Dectin-1 is expressed by a variety of leukocytes, with slight differences in expression between human and mouse. In mouse, the receptor is expressed in many tissues and largely by myeloid cells, including macrophages, monocytes, dendritic cells and neutrophils, although the receptor is also expressed on a subpopulation of T cells (Reid et al., 2004; Taylor et al., 2002). Alveolar macrophages and inflammatory cell populations show the highest levels of Dectin-1 expression (Reid et al., 2004; Taylor et al., 2002); however, Dectin-1 is absent on marginal zone macrophages, which suggests that this receptor is not involved in the trapping and clearance of antigen, nor is the receptor expressed in immune-privileged tissues, such as the eye and the brain. A variety of cytokines and other biological response modifiers, including (1,3)-β-glucans, are able to significantly influence the levels of Dectin-1 expression on leukocytes (Ozment-Skelton et al., 2006; Willment et al., 2003). The human receptor is similarly expressed, but is also found on B-cells, mast cells and eosinophils, and does not appear to be induced under inflammatory conditions (Olynych et al., 2006; Willment et al., 2005).

p0110 Despite its identification as a receptor for an undefined endogenous T-cell ligand (Ariizumi et al., 2000), Dectin-1 is now generally accepted to be a pattern recognition receptor for (1,3)-β-glucans (Brown and Gordon, 2001; Janeway, Jr., 1992). Dectin-1 can recognise both soluble and particulate (1,3)-β-glucans from a variety of sources, including fungi and bacteria (Brown and Gordon, 2001). As mentioned above, CR3 was originally proposed to be the major (1,3)-β-glucan receptor (Thornton et al., 1996), but a variety of approaches, including the use of blocking monoclonal antibodies and cells from receptor deficient mice, have clearly demonstrated that Dectin-1 is the primary receptor for (1,3)-β-glucans on leukocytes (Brown et al., 2002; Taylor et al., 2006; Underhill et al., 2005).

p0120 Dectin-1 specifically recognises (1,3)-linked β-glucans in a metal ion-independent fashion, and does not recognise monomers or polymers with other linkages (Adams et al., 2008; Brown and Gordon, 2001; Palma et al., 2005). Recent evidence indicates that the affinity of Dectin-1 for (1,3)-β-glucans is influenced by the polymer chain length and degree of branching, but that Dectin-1 can have extremely high affinity interactions for certain glucans, including those, such as (1,3)-β-glucan phosphate, which are used as biological response modifiers (Adams et al., 2008). Like the other non-classical C-type lectin-like receptors, Dectin-1 lacks the residues typically involved in calcium coordination and carbohydrate recognition and the mechanism by which this receptor recognises (1,3)-β-glucan ligand is unknown. However, mutational analysis has shown that two residues, Trp^{221} and His^{223}, in the carbohydrate recognition domain are an essential requirement for (1,3)-β-glucan binding (Adachi et al., 2004). The structure of the carbohydrate recognition domain of Dectin-1 has been determined, and these two residues, which are highly conserved in all identified Dectin-1 homologs, flank a shallow groove on the surface of this receptor, which may be the ligand binding site (Brown et al., 2007). Structural analysis of Dectin-1 has also revealed that Dectin-1 can dimerize through a novel interface, creating another groove in which the (1,3)-β-linked trisaccharide (laminaritriose) was found to bind (Brown et al., 2007). However, given that the minimum oligosaccharide that is functionally recognised by Dectin-1 is an octasaccharide, the physiological significance of this potential binding site is unclear (Adams et al., 2008).

p0130 Dectin-1 is able to mediate a number of cellular responses following (1,3)-β-glucan binding, including ligand uptake through phagocytosis and endocytosis (Herre et al., 2004a), PLA_2 and COX activation (Suram et al., 2006), the respiratory burst (Gantner et al., 2005; Underhill et al., 2005) and the induction of a variety of cytokines and chemokines, including TNF-α, MIP-2, IL-23, IL-6, IL-2 and IL-10 (Brown et al., 2003; Gantner et al., 2003; LeibundGut-Landmann et al., 2007; Rogers et al., 2005; Steele et al., 2003). The signalling cascades leading to these various responses are complex and not yet fully understood, but require the cytoplasmic ITAM-like motif of Dectin-1, which becomes tyrosine phosphorylated upon ligand binding (Gantner et al., 2003) presumably by Src family kinases (Olsson and Sundler, 2007). The ITAM-like motif of this receptor is reminiscent of the ITAM motifs found in other activation receptors, such as DAP12 (Lanier et al., 1998) and the Fc receptors (van den Herik-Oudijk et al., 1995). However, unlike these other dual tyrosine-based ITAM-containing receptors, only the membrane proximal tyrosine residue in the cytoplasmic tail of Dectin-1 is required for signalling (Brown et al., 2003; Gantner et al., 2003; Herre et al., 2004a; Rogers et al., 2005; Underhill et al., 2005).

p0140 Unexpectedly, this single tyrosine-based ITAM-like motif was shown to recruit Syk kinase (Fuller et al., 2007; Rogers et al., 2005; Underhill et al., 2005), an activity which was previously thought to strictly require the dual tyrosine residues found in traditional ITAM motifs. Although signalling via Syk appears to mediate the majority of the cellular functions ascribed to Dectin-1, including the respiratory burst and cytokine production, some responses, such as phagocytosis in macrophages, do not involve signalling through this kinase (Herre et al., 2004a; LeibundGut-Landmann et al., 2007; Rogers et al., 2005; Underhill et al., 2005). The requirement for Syk may, however, be cell type specific, as phagocytosis in dendritic cells, for example, demonstrated a partial requirement for Syk (Rogers et al., 2005). The Syk-independent signalling pathways are unknown and probably novel. In myeloid cells, downstream signalling from Syk has been shown to involve ERK (Slack et al., 2007), the novel adaptor CARD9, which couples to Bcl10 and regulates Bcl10-Malt1-mediated NF-kappaB activation (Gross et al., 2006), and nuclear factor of activated T-cells (NFAT) (Goodridge et al., 2007).

p0150 While Dectin-1 signalling is sufficient for the induction of certain cytokines, such as IL-10 and IL-23, the induction of proinflammatory cytokines and chemokines requires collaborative signalling from the Toll-like receptors (TLR). Signals from TLR2 (Underhill et al., 1999) and TLR6 (Ozinsky et al., 2000) were shown to be required for the induction of cytokines, such as TNF-α and MIP-2 (Brown et al., 2003; Gantner et al., 2003), in response to zymosan, a yeast cell wall-derived particle which contains branch-on-branch (1,3;1,6)-β-glucans and TLR agonists (Gantner et al., 2003; Ikeda et al., 2008). Using highly purified, receptor-specific reagents, Dectin-1 was recently shown to synergistically enhance TLR-mediated proinflammatory cytokine and chemokine production (Dennehy et al., 2008). This collaborative signalling required both the Dectin-1/Syk and TLR2/MYD88 pathways, and resulted in enhanced translocation of NF*k*B to the nucleus (Dennehy et al., 2008). Furthermore, Dectin-1 could collaborate with multiple MyD88-coupled TLRs (Dennehy et al., 2008). It should be noted, however, that in certain cells, such as alveolar macrophages, Dectin-1 may be able to directly trigger pro-inflammatory cytokine production (Steele et al., 2005).

p0160 How signalling from Dectin-1 integrates with the TLR pathway is unclear. Dectin-1 and TLR2 co-localize upon binding of complex ligands such as zymosan (Herre et al., 2004a) and it is possible that these receptors form a signalling complex. Dectin-1 does associate with tetraspanins, including CD63 and CD37 (Meyer-Wentrup et al., 2007; Underhill et al., 2005), suggesting that it might be part of a tetraspanin-mediated supramolecular signalling complex, such as has been implicated in T- and B-cell receptor signalling (Levy and Shoham, 2005). The association of Dectin-1 with CD37, in particular, has been shown to be involved in the

expression of Dectin-1 at the leukocyte cell surface and the regulation of Dectin-1-mediated IL-6 production (Meyer-Wentrup et al., 2007).

p0170 Dectin-1 probably plays a major role in (1,3)-β-glucan-mediated immunomodulation, although this has not yet been formally demonstrated *in vivo*. As we have seen, signalling via Dectin-1 can induce a variety of cellular responses, many of which have long been known to be induced by (1,3)-β-glucans. It is very likely that beneficial effects of these polysaccharides, particularly their anti-infective activities, stem, at least in part, from the ability of Dectin-1 to enhance TLR-mediated cytokine production (Dennehy et al., 2008). Furthermore, recent results using blocking monoclonal antibodies *in vivo* indicate that Dectin-1 may also be involved in the anti-cancer activity of (1,3)-β-glucans (Ikeda et al., 2007).

p0180 Through its ability to recognise (1,3)-β-glucans, Dectin-1 also plays a major role in the innate recognition of and response to fungal pathogens. Fungal cell walls can consist of more than 50% (1,3)-β-glucan, some of which, such as the (1,3;1,6) side-chain-branched β-glucans, are exposed on the cell surface, although possibly restricted to certain areas, such as the scar tissue remaining at the point of cell duplication (Gantner et al., 2005). Dectin-1 can recognise several fungal species, including *Candida* (Brown et al., 2003; Dennehy and Brown, 2007), *Pneumocystis* (Steele et al., 2003), *Saccharomyces* (Backer et al., 2008; Brown et al., 2003), *Coccidiodes* (Viriyakosol et al., 2005) and *Aspergillus* (Gersuk et al., 2006; Hohl et al., 2005; Steele et al., 2005). Recognition of these fungi by Dectin-1 induces many responses, including fungal uptake and killing, cytokine production and the induction of adaptive immunity (reviewed in Brown, 2006; Netea et al., 2008). The central role of Dectin-1 in immunity to certain fungal species has been demonstrated using Dectin-1 deficient mice (Nakamura et al., 2007; Saijo et al., 2006; Taylor et al., 2006), and the growing evidence that fungal pathogens may mask their (1,3)-β-glucan with mannan or α-glucan to avoid recognition through this receptor (Brown, 2006; Rappleye et al., 2007; Wheeler and Fink, 2006). In addition to fungi, there is also evidence that Dectin-1 may play a role in the immune response to mycobacteria, but as these organisms lack (1,3)-β-glucan, it is unclear how they are recognised by this receptor (Rothfuchs et al., 2007; Yadav and Schorey, 2006). Although Dectin-1 may play a protective and /or beneficial role, as described above, this receptor may also be involved in autoimmunity and disease. Fungal (1,3)-β-glucans, including curdlan, zymosan and intact organisms, were shown to induce autoimmune forms of rheumatoid arthritis in genetically susceptible mice, and inhibition of Dectin-1 function prevented the induction of the disease (Yoshitomi et al., 2005). This suggests that the immune responses triggered by Dectin-1 may induce autoimmune diseases in certain genetic backgrounds. Dectin-1 could also be involved in fungal-induced respiratory disorders, such as allergic bronchopulmonary aspergillosis,

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and although the mechanisms behind these disorders are not firmly established, this receptor is responsible for pulmonary inflammation following exposure to fungi, such as *Aspergillus fumigatus* (Steele et al., 2005).

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I.B. Structure/activity relationships between (1,3)-β-glucans and Dectin-1 in mammalian systems

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As indicated above, the weight of experimental evidence points to Dectin-1 as the primary mammalian receptor for (1,3)-β-glucans (Brown et al., 2002; Brown et al., 2003). Previous reports indicate that the physicochemical properties of glucans (e.g. primary structure, polymer size, surface charge, solution conformation and side-chain branching) may be important for recognition and interaction with pattern recognition receptors in the innate immune system (Mueller et al., 1996; Mueller et al., 2000). Many of the early studies on the structure–activity relationships of glucans employed competitive binding experiments with transformed and/or immortalized cell lines, rather than pure receptor, and in some cases the polysaccharides employed were not critically characterized or they were not homogeneous glucans. However, recent studies have shed new light on how the structure of (1,3)-β-glucans influences recognition by membrane-associated innate immune pattern recognition receptors (Adams et al., 2008; Mueller et al., 1996; Mueller et al., 2000). Using a library of natural product and synthetic (1,3)-, (1,6)- and (1,3;1,6)-β glucans as well as non-glucan polymers, Adams et al. (2008) have demonstrated that recombinant murine Dectin-1 is highly specific for glucans that have a (1,3)-β-glucopyranosyl backbone. Dectin-1 did not recognise non-β-linked polysaccharides such as *Saccharomyces cerevisiae* mannan or the (1,4;1,6)-α-glucan, pullulan, nor did Dectin-1 interact with barley (1,3;1,4)-β-glucan, although there are reports using cell-based assays which suggest that (1,3;1,4) β glucans are recognised (Brown and Gordon, 2001). While Dectin-1 is highly specific for (1,3)-β-glucans, it does not recognise all (1,3)-β-glucans equally. Dectin-1 differentially interacted with (1,3)-β-glucans over a very wide range of binding affinities (2.6 mM to 2.2 pM) (Adams et al., 2008). Indeed, one of the most surprising observations that emerged from this study was the remarkably high affinity interaction of Dectin-1 with certain (1,3)-β-glucans (2.2 pM) (Adams et al., 2008). These authors also reported that Dectin-1 can recognise synthetic oligomeric? (1,3)- and (1,3;1,6)-β-glucan ligands, and that binding affinity increased when the (1,3)-β-oligoglucoside contained a single glucose side-chain branch (Adams et al., 2008).

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Lowe and colleagues have reported that a linear (1,3)-β-hepta oligoglucoside is the minimum binding subunit for recognition by membrane-associated receptors (Lowe et al., 2001).

This study employed isolated membranes from a human U937 promonocytic cell line in a cell-based binding assay. Brown and Gordon (2001) reported that the minimum binding unit for Dectin-1 is more complex than a linear heptasaccharide. Palma et al. (2005) reported that the minimum (1,3)-β-glucan-binding subunit for Dectin-1 contains 10–11 glucose subunits. AUQ3 They employed neoglycolipid (1,3)-β-glucan ligands, but did not conduct competitive binding analyses or establish affinity, and speculated that the neoglycolipid nature of their ligands may have influenced the interaction such that the minimum (1,3)-β-glucan recognition motif was over-estimated. Adams et al. (2008) reported that Dectin-1 recognition of glucan ligands requires a backbone chain length of at least seven glucose subunits and at least one glucose side-chain branch. Thus, the data of Adams et al. (2008) support and extend the conclusion of Brown and Gordon (2001) that the minimum binding unit for Dectin-1 is more complex than a linear heptasaccharide. It is important to distinguish between the minimum (1,3)-β-glucan polymer size and structure that is required for Dectin-1 recognition and binding versus the minimum structure that is required for induction of biological activity. The reports discussed indicate that the minimum glucan recognition subunit for Dectin-1 is a polymer containing between eight and 11 (1,3)-β-linked glucose subunits with a single side-chain branch. However, (1,3)-β glucans of this size have not been shown to reliably stimulate intracellular signaling or exert biologic effects when administered parenterally. *In vivo* studies in mice suggest that (1,3;1,6)-β-glucans derived from *S. cerevisiae* and composed of >70 glucose subunits are required for induction of intracellular signalling and expression of biological activity (Williams et al., 1991). It has been speculated that, in addition to being recognised and bound by membrane-associated receptors, the (1,3)-β-glucan polymer must be of sufficient size to cross-link receptors on the cell surface as a prerequisite for induction of biological activity; however, this has not been proven unequivocally.

s0080 I.C. *In vivo* pharmacokinetics, pharmacodynamics and bioavailability of glucans

p0210 There are several reports which focus on the pharmacokinetics of individual (1,3)-β-glucans (Miura et al., 1996; Yoshida et al., 1996; Yoshida et al., 1997). Some of these reports are clouded by a failure to fully characterize the carbohydrate used, but nevertheless they tend to support the conclusion that intravenously administered (1,3)-β-glucans have similar half-lives (Miura et al., 1996; Yoshida et al., 1996; Yoshida et al., 1997). It has also been reported that (1,3)-β-glucans are eventually deposited in the liver and spleen (Miura et al., 1996; Yoshida et al., 1996; Yoshida et al., 1997). Glucan derived from *Grifola frondosa* is reported to have a $t_{1/2}$ of 5.4 and 6.4 h following systemic administration in autoimmune-prone and normal mice,

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respectively, with about 70% of the (1,3)-β-glucan recovered in the liver and spleen (Miura et al., 1996). Following intravenous administration in rabbits, Yoshida et al. (1996, 1997) reported a distribution $t_{1/2}$ of less than 5 min for a 92-kDa radiolabelled (1,3)-β-glucan isolated from *Candida albicans*. Intravascular clearance studies suggested that the rapid distribution phase was followed by a prolonged elimination phase of several hours (Yoshida et al., 1996). Most of the radiolabelled (1,3)-β-glucan (>97%) was associated with cell-free plasma, while radioactivity associated with blood cells was initially found in platelets and later (2 h) distributed to polymorphonuclear leukocytes and red blood cells (Yoshida et al., 1996). More than 80% of the material was contained in the liver and 10% in the kidney after 24 h. There is also evidence that uptake into cells may differ not only for individual polysaccharides but also for various cells or tissues. Substantial differences were reported for accumulation of glucuronoxylmannan from *Cryptococcus neoformans* by macrophages and neutrophils (Monari et al., 2003). While neutrophils rapidly ingested polysaccharides, which were expelled or degraded after 1 h, macrophages continued to accumulate glucuronoxylmannan for up to 1 week (Monari et al., 2003). This supports the view that cells may respond differentially to (1,3)-β-glucans and related polysaccharides based on a number of parameters.

p0220 Rice et al. (2004) performed a comparative study of the pharmacokinetics of three well-characterized (1,3)-β-glucans following intravenous administration, i.e. glucan phosphate, laminarin and scleroglucan, which had different molecular sizes, branching frequencies, solution conformations and polymer charge (Rice et al., 2004). The total volume into which the (1,3)-β-glucans distributed in the body (V_Dß) values and distribution compartment as AUQ4 determined $t_{1/2}$ values were similar for all three (1,3)-β-glucans. Volume of distribution (V_D) values with an estimated plasma concentration at time=0 (C_P0) were lowest for glucan phosphate (100±19 mL/kg), compared with values for laminarin (252±99 mL/kg), and scleroglucan (261±130 mg/kg). Thus, all three (1,3)-β-glucans had similar pharmacokinetics, even though their physicochemical properties varied considerably. Rice et al. (2004) concluded that the *in vivo* clearance of glucans from the blood was largely independent of molecular size, branching frequency, polymer charge and solution conformation. This is consistent with observations from other laboratories. Interestingly, there is also evidence that the three (1,3)-β-glucans studied by Rice et al. (2004) differed in bioactivity. Laminarin does not stimulate innate immunity, while (1,3)-β-glucan phosphate and scleroglucan increase immune function (Williams et al., 1999b). However, all three (1,3)-β-glucans are bound and internalized by pattern recognition receptors on a variety of cell types (Mueller et al., 2000). Their data suggest that the bioactivity of (1,3)-β-glucans is not dramatically influenced by *in vivo* pharmacokinetics.

s0090 ### I.C.a. In vivo effect of systemic glucan administration on Dectin-1 levels

p0230 As noted above, Dectin-1 is the primary receptor for (1,3)-β-glucans and mediates the internalization and inflammatory response to free and pathogen-associated (1,3)-β-glucans (Brown et al., 2002; Brown et al., 2003; Brown and Gordon, 2001). *In vitro* cell-based studies have shown that Dectin-1 will bind free (1,3)-β-glucans as well as whole *Candida albicans* and *Saccharomyces cerevisiae* cells in a (1,3)-β-glucan-dependent manner, and upon binding the Dectin-1/glucan complex is rapidly internalized (Ariizumi et al., 2000; Brown et al., 2002; Brown et al., 2003; Brown and Gordon, 2001; Herre et al., 2004a; Taylor et al., 2002). Ozment-Skelton et al., (2006) have reported a similar effect following systemic glucan administration in mice (Ozment-Skelton et al., 2006). Specifically, they examined the effect of intravenous glucan administration on circulating leukocyte surface Dectin-1 levels, and observed that a single intravenous administration of a water-soluble (1,3)-β-glucan phosphate resulted in a significant reduction in leukocyte Dectin-1 levels from 3 h to 7 days. This reduction was due to a loss of Dectin-1 from the surface of blood neutrophils and monocytes, which was likely due to internalization of the Dectin/(1,3)-β glucan complex. The intravenous administration of non-glucan polysaccharides did not decrease the percentage of Dectin-1-positive blood leukocytes, suggesting that this effect was specific for (1,3)-β-glucans. One of the most interesting observations was the prolonged effect of (1,3)-β-glucan administration on circulating leukocyte Dectin-1 levels. This is particularly significant in light of the relatively short half-life of (1,3)-β-glucans in the blood (Rice et al., 2004). Based on these data it is reasonable to speculate that even a single administration of a bioactive (1,3)-β-glucan can have effects that far exceed the plasma/serum clearance of the polysaccharide. This may relate to the rapid internalization of the Dectin-1/(1,3)-β-glucan complex and the activation of multiple intracellular signalling pathways (Wei et al., 2002b; Williams et al., 1999b; Williams et al., 2006).

s0100 ### I.C.b. In vivo effects of (1,3)-β-glucans in animal models of disease

p0240 Over the past four decades literally hundreds of published works have appeared which describe the anti-infective, anti-tumour and immunobiological activities of many glucan preparations in a variety of disease states. This literature has been the subject of numerous reviews (Bohn and BeMiller, 1995; Brown and Gordon, 2003; Dennehy and Brown, 2007; Di Luzio et al., 1985; Herre et al., 2004b; Suzuki et al., 1996; Williams et al., 1996; Williams, 1997; Williams et al., 2003b; Williams et al., 2004b; Williams and Di Luzio, 1985a). However, the mechanisms associated with (1,3)-β-glucan-induced protection are only emerging. What follows is a brief

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review of our current knowledge of the cellular and molecular mechanisms of (1,3)-β-glucan-induced protection in animal models of disease.

s0110 *I.C.b.1. Bacterial infections*

p0250 (1,3)-β-Glucans have been reported to increase resistance to a variety of bacterial pathogens, in a variety of animal models (Gualde et al., 1985; Kournikakis et al., 2003; Kupfahl et al., 2007; Liang et al., 1998; Persson et al., 2003; Rice et al., 2005; Williams et al., 1978; Williams et al., 1983; Williams et al., 1999b; Williams and Di Luzio, 1980; Williams and DiLuzio, 1980). The beneficial effect of (1,3)-β-glucan in sepsis have been attributed to a diverse array of effects, including stimulation of innate immunity, increased bacterial clearance, increased bactericidal activity, modulation of cytokine production and other non-specific effects (Liang et al., 1998; Williams et al., 1996). Wakshull and colleagues reported that the microbicidal activity of a PGG-(1,3)-β-glucan involves increased leukocyte numbers, enhanced oxidative burst and microbicidal activity as well as stimulation of neutrophil NFκB activation (Liang et al., 1998; Wakshull et al., 1999). However, the precise cellular and molecular mechanisms associated with (1,3)-β-glucan-induced protection against infectious diseases have not been fully elucidated. Furthermore, while the data discussed above suggest potential mechanisms of action, they also present an interesting paradox in light of recent results. By way of example, it has also been shown that (1,3)-β-glucans will decrease morbidity, maintain cardiovascular function and increase survival outcome in murine models of fulminating polymicrobial sepsis (Ha et al., 2006; Williams et al., 1999b). The pathophysiology of this experimental infection is known to involve the host overexpressing inflammatory mediators which result in a systemic inflammatory response that culminates in severe shock, multi-organ failure and death (Williams et al., 1999a; Williams et al., 1999b). Thus, a major question was how a pro-inflammatory agent, even a mild pro-inflammatory agent such as a (1,3)-β-glucan, might ameliorate a disease that has a significant inflammatory component. Indeed, agents such as (1,3)-β-glucan would seem to be contraindicated in diseases that have a significant inflammatory process. Recent data have shed new light on this paradox and in doing so have identified a novel mechanism of action for (1,3)-β-glucans in certain infectious and inflammatory diseases. In the normal host, (1,3)-β-glucan binding to its cognate receptors activates intracellular signalling pathways which are associated with stimulation of innate immunity (Battle et al., 1998; Williams et al., 1999b; Williams et al., 2000; Williams et al., 2003a). In striking contrast, administration of (1,3)-β-glucan ligands in the presence of an inflammatory and/or septic insult results in blunting of early increases in tissue transcription factor activity and cytokine transcription that are associated with the host inflammatory response to the injury (Ha et al., 2006; Williams et al., 1999b; Williams et al., 2000).

Preventing early activation of pro-inflammatory transcription factors, such as NFκB and NF-IL6, positively correlates with maintenance of cardiac function and improved survival outcome in septic shock (Ha et al., 2006; Williams et al., 1999b). It is important to note that (1,3)-β-glucans did not suppress NFκB or NF-IL6 levels, but rather prevented the dramatic increase in transcription factor activation that is observed in septic mice.

p0260 The mechanism by which (1,3)-β-glucans attenuate pro-inflammatory responses may relate to their ability to stimulate the phophoinositide-3-kinase/Akt (PI3k/Akt)-dependent signalling pathways (Li et al., 2003; Williams et al., 2004a; Williams et al., 2006). The phosphoinositide 3-kinases (PI3Ks) are a conserved family of signal transduction enzymes which are involved in regulating cellular proliferation and survival (Cantley, 2002; Fruman and Cantley, 2002). More specifically, the PI3Ks and the downstream serine/threonine kinase Akt (also known as protein kinase B (PKB) regulate cellular activation, inflammatory responses, chemotaxis and apoptosis (Cantley, 2002). Williams et al., (2004a) reported that (1,3)-β-glucan-phosphate-induced protection in murine polymicrobial sepsis is mediated through a PI3k/Akt-dependent mechanism. They treated mice with the PI3K inhibitor wortmannin or LY294002 in the presence and absence of (1,3)-β-glucan and sepsis. PI3K inhibition completely eliminated the protective effect of (1,3)-β-glucans, indicating that protection against septic mortality was mediated through PI3K (Williams et al., 2004a). Thus, manipulation of the endogenous PI3k/Akt signaling pathway by (1,3)-β-glucans may represent a new therapeutic approach to the management of important diseases.

s0120 *I.C.b.2. Fungal infections*

p0270 The discovery of Dectin-1 as the primary (1,3)-β-glucan pattern recognition receptor in mammalian systems has dramatically advanced our knowledge of how the innate immune system recognizes and interacts with fungal (1,3)-β-glucans (Brown and Gordon, 2003; Brown and Gordon, 2005; Herre et al., 2004b). It has been reported that (1,3)-β-glucan administration will increase resistance to fungal infections (Rice et al., 2005; Williams et al., 1978). These findings have led many investigators to speculate that Dectin-1 plays a central role in the innate immune response to infection, specifically fungal infections (Dennehy and Brown, 2007). These data also imply that (1,3)-β-glucan mediates its anti-infective activity through a Dectin-1-dependent mechanism. However, the role of Dectin-1 in the *in vivo* response to fungal infections is controversial. Taylor et al. (2006) have reported that mice which are genetically deficient in Dectin-1 show increased mortality following *Candida albicans* infection. In striking contrast, Saijo et al. (2006) reported that survival during *C. albicans* infection is similar in Dectin-1 knock-out and wild-type mice. However, Saijo et al. (2006) also reported

that Dectin-1 plays a role in the response to *Pneumocystis carinii* infection. Interestingly, the increase in susceptibility of Dectin-1 knock-out mice to *P. carinii* was observed only at early time periods, and the authors concluded that Dectin-1 was not required for 'chronic infection by *P. carinii*'. The precise reason for the discrepancies between the reports of (Taylor et al., 2006) and (Saijo et al., 2006) are not clear at this time. One possible explanation relates to the genetic background of the Dectin-1-deficient mice. Three different genetic backgrounds were employed to generate the Dectin-1-deficient mice (Saijo et al., 2006; Taylor et al., 2006). Taylor et al. (2006) employed a 129/Sv background for their studies whereas Saijo et al. (2006) employed Dectin-1-deficient mice on the Balb/c background for the *P. carinii* studies, and a C57Bl/6J background was used for the *C. albicans* studies. In addition, different strains and challenge doses of *C. albicans* were employed (Saijo et al., 2006; Taylor et al., 2006). Nakamura et al. (2007) have recently reported that Dectin-1 is not required for innate host defense against *Cryptococcus neoformans*. These investigators employed a Dectin-1-deficient mouse strain on the C57Bl/6J and provide an equivocal picture of the role of Dectin-1 in the *in vivo* response to fungal infection.

s0130 ### *I.C.b.3. Viral infections*

p0280 There are a limited number of reports on the anti-viral effect of various glucan preparations (Jung et al., 2004; Marchetti et al., 1996; Schaeffer and Krylov, 2000; Wang et al., 2007; Williams and Di Luzio, 1985b; Williams and DiLuzio, 1980). Most anti-viral (1,3)-β-glucan studies have focused on sulfated homopolysaccharides and heteropolysaccharides (Schaeffer and Krylov, 2000; Wang et al., 2007). Schaeffer and Krylov have reviewed the anti-viral effects of various natural products (Schaeffer and Krylov, 2000). They concluded that sulfated homopolysaccharides are more potent that sulfated heteropolysaccharides (Schaeffer and Krylov, 2000). With respect to (1,3)-β-glucans, most of the anti-viral research has focused on curdlan sulfate (Aoki et al., 1991; Aoki et al., 1992; Gordon et al., 1994; Gordon et al., 1997; Schaeffer and Krylov, 2000; Uryu, 1993; Wang et al., 2007). Curdlan sulfate has been reported to have anti-HIV activity (Schaeffer and Krylov, 2000; Wang et al., 2007). This has been attributed to binding of the virion by curdlan sulfate, interference with viral attachment to the cell, decreased viral penetration into the cell, as well as delaying the events that precede reverse transcription and/or beta-chemokine and cytokine production (Jagodzinski et al., 1994). However, the literature suggests that the curdlan is not that critical to the anti-viral activity. The presence of the sulfate is thought to be essential for anti-viral activity and the potency of the polysaccharide is thought to increase with the degree of sulfation (Jagodzinski et al., 1994). Indeed, non-β-linked glucan homopolysaccharides have also been reported to exhibit anti-viral activity. As an example, Qiu et al. have reported that sulfated

α-glucans exert anti-dengue virus activity (Qiu et al., 2007), while Davis and colleagues have reported that orally administered oat (1,3;1,4)-β-glucan will decrease the morbidity and mortality associated with herpes simplex virus 1 (HSV-1) and exercise stress (Davis et al., 2005). There are a few reports indicating that non-sulfated (1,3)-β-glucans can exert anti-viral activity both *in vitro* and *in vivo* (Davis et al., 2005; Jung et al., 2004; Marchetti et al., 1996; Williams and Di Luzio, 1985b; Williams and DiLuzio, 1980). As an example, Marchetti et al. (1996) have reported that a neutral (1,3)-β-glucan, isolated from *Sclerotium glucanicum*, inhibits replication of HSV-1 in cultured cells.. The relative dearth of information regarding the anti-viral effects of (1,3)-β-glucans suggest that this is an area ripe for investigation. The discovery of Dectin-1 as a specific (1,3)-β-glucan receptor may provide new avenues of investigation regarding potential anti-viral effects of non-sulfated glucans.

s0140 *I.C.b.4. Anti-tumor activity of glucans*

p0290 The ability of glucans to inhibit tumour growth in a variety of experimental tumour models is well established (Adachi et al., 1989; Chihara, 1994; Di Luzio et al., 1979; Mansell and Di Luzio, 1976; Mayberry and Lane, 1993; Stone and Clarke, 1992; Zakany et al., 1980). In most cases, the (1,3)-β-glucan was administered prophylactically and the end-points were tumour growth, tumour volume, degree of metastases and/or survival (Chihara, 1994; Stone and Clarke, 1992; Zakany et al., 1980). The anti-tumour efficacy of (1,3)-β-glucans appears to relate to the type of tumour, the genetic background of the host animal, the dose of (1,3)-β-glucans, the route and timing of (1,3)-β-glucan administration, as well as the tumour load. This work has been the subject of several in-depth reviews (Chen and Seviour, 2007; Chihara, 1994; Stone and Clarke, 1992; Yan et al., 2005). However, interpretation of these data is complicated by the fact that many of the published works do not provide details about the nature or homogeneity of the (1,3)-β-glucans being evaluated. In addition, most of the studies were phenomenological in nature and did not address mechanisms. In some cases the anti-tumour effect of the (1,3)-β-glucans was attributed to modulation of macrophage, lymphocyte, neutrophil and/or NK cell activity (Cook et al., 1978; Di Renzo et al., 1991; Hamuro and Chihara, 1985; Hong et al., 2004). The most significant body of work on the anti-tumour mechanisms of glucans was done by the late Gordon Ross (Ross et al., 1985; Ross et al., 1999). Ross and colleagues identified the complement receptor 3 (CR3, Mac-1, $\alpha_m\beta_2$) as a receptor for (1,3)-β-glucans more than two decades ago (Ross et al., 1985; Ross et al., 1999). The role of CR3 as a glucan receptor was discussed earlier in this chapter. With respect to glucan-induced anti-tumour activity, Ross and colleagues identified β-glucans as 'specific' biological response modifiers that use endogenous antibodies to target tumours for cytotoxicity by neutrophils and complement (Yan et al., 1999). More specifically, these

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investigators proposed that (1,3)-β-glucans could "override the normal resistance of iC3b-opsonized tumors to the cytotoxic activations of phagocytes…", thus allowing effector mechanisms to mediate tumoricidal activity (Ross et al., 1999). Hong and colleagues have recently advanced the concept that orally administered glucans will exert an adjuvant effect when combined with exogenously administered anti-tumour antibodies that activate complement (Hong et al., 2003; Hong et al., 2004). (1,3)-β-Glucans are known to exert adjuvant activity (Mohagheghpour et al., 1995; Ormstad et al., 2000; Williams et al., 1989). In addition, recent evidence points to at least two mechanisms for the absorption of (1,3)-β-glucans from the gastrointestinal tract (Hong et al., 2004; Rice et al., 2005). Thus, this approach may have merit. However, as noted above, the ability of CR3-deficient cells to recognise (1,3)-β-glucans through a Dectin-1-dependent mechanism leaves open the possibility that Dectin-1 may play a role in the anti-tumour efficacy of glucans. In support of this concept, Ikeda et al. (2007) have recently reported that anti-Dectin-1 antibodies will inhibit the anti-tumour efficacy of schizophyllan (SPG) glucan in a murine sarcoma 180 tumor model. These investigators examined differences in tumour size over a 21-day period as their endpoint, but they did not provide any data regarding the effect of anti-Dectin-1 antibodies on survival outcome in SPG-treated tumor-bearing mice. Nevertheless, these data suggest that SPG interaction with Dectin-1 plays some role in the anti-tumor efficacy of this glucan.

s0150 *I.C.b.5. Ischemia/reperfusion injury*

p0300 A growing body of evidence suggests that innate immune and inflammatory pathways participate in myocardial ischaemia/reperfusion (I/R) injury and congestive heart failure (Barry, 1994; Bozkurt et al., 1998; Bryant et al., 1998; Kapadia et al., 1998; Kiechl et al., 2001; Oral et al., 1995; Torre-Amione et al., 1996). Several recent papers have reported that (1,3)-β-glucan administration will ameliorate organ injury following I/R (Araujo-Filho et al., 2006; Bayrak et al., 2008; Bolcal et al., 2007; Li et al., 2001; Medeiros et al., 2006; Sandvik et al., 2007). Orally administered glucan has been reported to protect the kidney from I/R injury (Bayrak et al., 2008). In this study, (1,3)-β-glucan blunted the I/R-induced increase in serum urea and creatinine that was seen in the control I/R group (Bayrak et al., 2008). Medeiros et al. (2006), and Araujo-Filho et al. (2006) reported that parenteral (1,3)-β-glucan administration decreases bacterial translocation in small bowel I/R injury. They also reported that the muscosal damage associated with small bowel I/R was ameliorated in (1,3)-β-glucan-treated animals (Medeiros et al., 2006). Aarsaether et al. (2006) have translated these basic experimental observations to the clinical setting by pre-treating patients undergoing coronary artery bypass grafting with oral administration of a (1,3;1,6)-β-glucan.. They observed that the compound was well tolerated and that it lowered creatinine kinase isoenzyme MB in the

study group, which suggests decreased injury to the myocardium. Li et al. (2001) reported that (1,3:1,6)-β-glucan phosphate, a water-soluble derivative of *S. cerevisiae* (Li et al., 2003), will ameliorate cardiac I/R injury in either a prophylactic or therapeutic regimen. Pre-treatment of rats with (1,3;1,6)-β-glucan phosphate significantly reduced infarct size/area at risk (47%) when compared with the control group. Similarly, infarct size/area at risk was significantly reduced in (1,3;1,6)-β-glucan phosphate pre-treated animals compared with controls (Li et al., 2003). Of greater clinical importance was their finding that intravenous administration of (1,3;1,6)-β-glucan phosphate 5 min after the initiation of ischaemia significantly reduced infarct size/area at risk (Li et al., 2003). These authors also investigated the cellular mechanisms associated with glucan-induced cardioprotection in I/R injury and found that (1,3;1,6)-β-glucan phosphate administration blunts TLR4-mediated NFκB activation in the ischemic myocardium (Li et al., 2003). They also reported that the (1,3;1,6)-β-glucan phosphate induces a concomitant activation of PI3K/Akt signalling in rat hearts subjected to I/R (Li et al., 2003).

s0160 *I.C.b.6. Wound repair*

p0310 A number of reports indicate that topical or systemic (1,3)-β-glucan administration enhances wound healing in skin and intestinal anastomoses (Chen, 1992; Compton et al., 1996; Leibovich and Danon, 1980; Portera et al., 1997; Wolk and Danon, 1985). Berdal et al. have reported that (1,3)-β-glucan improves wound healing in spontaneously diabetic mice (Berdal et al., 2007). Lyuksutova et al. (2005) reported that (1,3;1,6)-β-glucan phosphate treatment attenuates burn-induced inflammation and increases resistance to *P. aeruginosa* burn wound infection in an experimental model of burn injury. The mechanisms by which (1,3;1,6)-β-glucan phosphate enhances the reparative process have been attributed to increased macrophage activation and infiltration into the wound milieu, stimulating tissue granulation, collagen deposition, re-epithelialization and increased wound tensile strength (Chen, 1992; Compton et al., 1996; Leibovich and Danon, 1980; Portera et al., 1997; Wolk and Danon, 1985). The presence of (1,3)-β-glucan receptors on primary cultures of human fibroblasts has been reported (Kougias et al., 2001). (1,3)-β-Glucan stimulates primary human dermal fibroblast collagen biosynthesis through a nuclear factor-1 (NF-1)-dependent mechanism (Wei et al., 2002b). Additionally, (1,3)-β-glucan stimulates fibroblast AP-1 and Sp1 activation in a time-dependent manner, although the temporal kinetics varied between the two transcription factors; that is, AP-1 binding activity was increased at early time intervals (<12 h), while Sp1 nuclear binding activity was increased at later time intervals (Wei et al., 2002a). Further, (Wei et al., 2002a) reported that (1,3)-β-glucan stimulates human fibroblast expression of neurotrophin 3 (NT-3), platelet-derived growth factor A (PDGF-A), platelet-derived growth factor B (PDGF-B), acidic fibroblast growth factor (aFGF), basic fibroblast growth factor (bFGF),

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transforming growth factor alpha (TGFalpha), transforming growth factor beta (TGFbeta) and vascular endothelial growth factor (VEGF) mRNA. Lowe et al. (2002) found two (1,3)-β-glucan binding sites on primary human vascular endothelial cells. In endothelial cells, (1,3)-β-glucan stimulated NFκB nuclear binding activity and IL-8 expression, but not vascular endothelial growth factor, in a time-dependent manner (Lowe et al., 2002). These data indicate that (1,3)-β-glucans directly and/or indirectly modulate the activity of diverse cells and growth factors that are central to the reparative process. Delatte et al. (2001) have translated these experimental data to the clinical setting. They reported on the effectiveness of a (1,3)-β-glucan/collagen preparation in the treatment of partial thickness burns in a retrospective study of 225 paediatric burn patients, 43 of whom were treated with glucan/collagen. They concluded that paediatric burns can be effectively treated with (1,3)-β-glucan/collagen mixtures, and that this preparation markedly simplified wound care and significantly decreased post-injury pain (Delatte et al., 2001).

s0170 *I.C.b.7. Anti-inflammatory activity*

p0320 The weight of evidence indicates that (1,3)-β-glucans will stimulate innate immunity and activate pro-inflammatory responses (Brown and Gordon, 2003; Williams et al., 2004b). However, there is a growing body of evidence which suggests that (1,3)-β-glucans can elicit an anti-inflammatory response in certain clinically relevant models of disease (Li et al., 2003; Williams et al., 1999b; Williams et al., 2004a). As was noted above, Williams et al. (1999b) and Li et al. (2003) have shown that (1,3)-β-glucan administration can attenuate early activation of pro-inflammatory transcription factors, such as NFκB and NF-IL6. The 'blunting' of pro-inflammatory transcription factor activation positively correlates with reduced morbidity and improved outcome in septic shock (Williams et al., 1999b) and I/R injury (Li et al., 2003). Initially, the anti-inflammatory effect of (1,3)-β-glucans was thought to be mediated via inhibition of the alternative pathway of complement (Stone and Clarke, 1992) This is not a primary reference. However, recent studies have identified activation of the PI3k/Akt signalling pathway as a potential mechanism of glucan-mediated anti-inflammatory responses (Li et al., 2003; Williams et al., 2004a). PI3K is an endogenous compensatory mechanism that suppresses pro-inflammatory and apoptotic processes in response to inflammatory injury (Fukao and Koyasu, 2003; Guha and Mackman, 2002; Li et al., 2003; Williams et al., 2004a). The PI3K/Akt pathway is also thought to play a pivotal role in the maintenance of homeostasis and integrity of the immune response during inflammatory injury (Fukao and Koyasu, 2003; Guha and Mackman, 2002; Li et al., 2003; Williams et al., 2004a). The data suggest that (1,3)-β-glucan activation of the PI3K pathway may be an effective approach for the prevention and/or treatment of septic and inflammatory sequelae.

p0330 The anti-inflammatory effect of (1,3)-β-glucans has also been reported in environmental settings. Rylander and colleagues have studied the relationship of glucan exposure to indoor air related symptoms, allergy and asthma (Rylander et al., 1992; Rylander, 1996; Rylander and Lin, 2000; Thorn et al., 2001). Their results indicate that the response to inhaled (1,3)-β-glucans in humans is quite different from that observed following inhalation of bacterial endotoxin (Thorn et al., 2001). By way of example, endotoxin causes a predominately neutrophilic inflammatory response, while (1,3)-β-glucans does not induce neutrophil recruitment. Indeed, Rylander and Lin, (2000) speculate that (1,3)-β-glucans may prime airway cells for simultaneous or subsequent exposure to another agent. They reported that (1,3)-β-glucans potentiated ovalbumin-induced eosinophilia and IgE responses (Rylander and Lin, 2000). At the same time, some inflammatory responses were shown to be down-regulated (Rylander and Lin, 2000). Specifically, TNFα production is decreased under certain conditions (Rylander and Lin, 2000).

p0340 It should be noted that the term 'anti-inflammatory' may be a misnomer, when applied to (1,3)-β-glucans. The data support the concept of (1,3)-β-glucans eliciting an anti-inflammatory response, but it is not a classic anti-inflammatory response such as might be observed with corticosteroids. Indeed, the effect of (1,3)-β-glucans appears to be quite specific and can be linked to individual signalling pathways, i.e. PI3k/Akt (Li et al., 2003; Williams et al., 2004a). Furthermore, the response is not immunosuppressive, since normal or above normal levels of these important transcription factors are maintained (Williams et al., 1999b). In fact, immune competence is increased, as demonstrated by clinical observations that (1,3)-β-glucan administration stimulates conversion from anergy (immunosuppression) in trauma patients (Browder et al., 1990).

s0180 *I.C.b.8. Stimulation of innate immunity following oral glucan administration*

p0350 Most *in vivo* studies with (1,3)-β-glucans have focused on parenteral administration via the intravenous, subcutaneous or intraperitoneal routes. However, as noted above there is a small, but growing literature which indicates that (1,3)-β-glucans may be orally effective as well. Hong et al. (2004) and Cheung et al. (2002) have reported that orally administered (1,3)-β-glucans function as potent anti-tumour adjuvants when combined with antibodies that recognise epitopes on tumours. Suzuki et al. (1989) reported that a branched water-soluble (1,3)-β-glucan increased splenocyte mitogenic response and NK cell activity following five daily oral administrations. In addition, oral supplementation with the (1,3)-β-glucan inhibited tumour growth of syngeneic Meth A fibrosarcoma, IMC carcinoma and Lewis lung carcinoma (Suzuki et al., 1989). In another study, Suzuki et al. (1990) reported that oral administration

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of glucan to CDF_1 mice increased peritoneal macrophage functional activity as denoted by increased acid phosphatase activity, increased phagocytic activity, increased killing of *Candida* and increased interleukin-1 (IL-1) production. These investigators also reported the oral efficacy of (1,3)-β-glucans in C3H/HeJ mice which are endotoxin hyporesponsive, indicating the effect is not due to increased uptake of endotoxin from the gut following oral (1,3)-β-glucan administration (Suzuki et al., 1989; Suzuki et al., 1990). The immunoadjuvant activity following oral administration (Nicoletti et al., 1992). Orally administered (1,3)-β-glucans increased serum levels of IL-12 in mice (Rice et al., 2005). Interestingly, these authors reported that IL-6 was elevated at 8 hours following oral administration of water insoluble, particulate increase survival in mice challenged with *Staphylococcus aureus* or *Candida albicans* (Rice et al., 2005)., even though there was no evidence for oral absorption of the insoluble glucan (Rice et al., 2005). Baran et al. (2007) have reported that orally administered whole yeast (1,3)-β-glucan particles converts non-protective Th2 responses to a more protective Th1 phenotype in a murine model of mammary carcinoma. Oral (1,3)-β-glucans are also approved by the FDA for lowering serum cholesterol. Oral (1,3)-β-glucans has also been reported to decrease post-prandial glucose surge in Type II diabetes (Braaten et al., 1994; Wuersch and Pi-Sunyer, 1997).

p0360 Not all reports have demonstrated biological activity following oral (1,3)-β-glucan administration. Chihara, (1985), Ohno et al., (1984), Wu et al., (1998),and Dritz et al., (1995) reported that dietary supplementation with (1,3)-β-glucans derived from various sources did not exert any significant immunomodulatory effects. Dritz et al. (1995). reported on the effect of dietary supplementation with (1,3)-β-glucan in weanling pigs. Part of the rationale for this study was to determine whether oral (1,3)-β-glucan administration could effectively modulate innate immunity in domestic animals, such that they are more resistant to opportunistic infections, thus reducing the need for exogenous antibiotics. However, this investigation revealed a "complex interaction" between oral (1,3)-β-glucan, growth performance and susceptibility to *Streptococcus suis.* While the pigs receiving (1,3)-β-glucan showed greater weight gain, they also showed an increased susceptibility to *Streptococcus suis* infection. Wu et al. (1998) reported that dietary supplementation with protein-bound (1,3)-β-glucan did not enhance immune function, suggesting that the complex of glucan and protein may not be biologically active.

p0370 An important caveat is that virtually every study discussed above employed a different oral (1,3)-β-glucan preparation. In some instances, details about the physical state, chemical nature and/or homogeneity of the (1,3)-β-glucans employed were provided, but this was not true in all cases. The variety of (1,3)-β-glucan preparations employed makes it difficult to

determine why some preparations were bioactive after oral administration and others were not. Nevertheless, the weight of evidence suggests that some orally administered (1,3)-β-glucans can interact with intestinal cells and cross the gastrointestinal barrier, where they can exert systemic effects on innate immunity.

p0380 Based on the emerging interest in orally administered (1,3)-β-glucans, Rice et al.(2005) have examined the comparative pharmacokinetics of three water-soluble (1,3)-β-glucans (1,3)-β-glucans with varying physicochemical characteristics following oral administration. They reported that maximum plasma concentrations for the three (1,3)-β-glucans varied considerably with peak plasma levels occurring between 0.5 to 12 hrs. At 24 hrs after oral administration scleroglucan was completely eliminated from the serum, while approximately 25% of the glucan phosphate and laminarin remained in the serum at 24 hrs. It is interesting to note that the largest glucan studied, scleroglucan, had a Mw of $\sim 1 \times 10^6$ g/mol. Despite its relatively large size it was completely eliminated from the serum in 24 hrs, while (1,3)-β-glucans with smaller Mw remained (Rice et al., 2005). This suggests that absorption/transport of glucans across the gastrointestinal tract is complex. In all cases, bioavailability of the orally administered (1,3)-β-glucans was relatively low (0.5 to 4.9%). Interestingly, the liver did not significantly contribute to clearance of plasma (1,3)-β-glucans. Thus, these (1,3)-β-glucans do not appear to be removed from the plasma via a first pass effect in the liver. It was noted that orally administered (1,3)-β-glucans were bound and internalized by a sub-population of intestinal epithelial cells and gut-associated lymphoid tissue (GALT) cells. Internalization of (1,3)-β-glucans by intestinal epithelial cells was not Dectin-1 dependent (Rice et al., 2005), although gut associated lymphoid tissue (GALT) expression of Dectin-1 and TLR2, but not TLR4, increased following oral administration of glucan. These investigators also examined the fate of water insoluble, particulate glucan (1,3)-β-glucan after oral administration (Rice et al., 2005)and found that levels of (1,3)-β-glucan were undetectable suggesting that only soluble (1,3)-β-glucans are directly absorbed into the circulation. However, Hong et al.(2004). have reported that particulate (1,3)-β-glucans glucans in the gastrointestinal tract are internalized by macrophages, which transport the glucan to various sites throughout the body, and slowly degrade the particulate (1,3)-β-glucans, releasing a bioactive soluble (1,3)-β-glucan product.

p0390 When considered as a whole, these data suggest that (1,3)-β-glucans can be taken up from the gastrointestinal tract and that orally administered (1,3)-β-glucans can modulate innate immunity. The data also suggest that the physical state of the (1,3)-β-glucan may dictate the mechanism by which the (1,3)-β-glucan is transported across the gastrointestinal barrier into the systemic circulation.

s0190

I.C. Conclusions

p0400 The ability of (1,3)-β-glucans to stimulate innate immunity has been known for more than four decades. However, recent advances in our knowledge in particular the discovery that Dectin is the primary (1,3)-β-glucan recognition protein have significantly increased our understanding of how (1,3)-β-glucans modulate innate immunity at the cellular and molecular level. These findings will be important in advancing our knowledge of the role that glucan plays as a pathogen associated molecular pattern and they may also be useful in the development of (1,3)-β-glucan based immunotherapeutics.

s0200

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Author Queries

{AUQ1} We followed the heading levels as per MS. Please confirm the style.
{AUQ2} AU: oligomeric? Should question mark stay?
{AUQ3} AU: Check sense here, as text was missing.
{AUQ4} AU: '...distribution compartment as determined....' – please check sense of this sentence.
{AUQ5} Is a Chung. Hua. Cheng. Hsing. Shao. Shang. is a journal name? Please confirm.
{AUQ6} AU: Please provide volume number and page range.