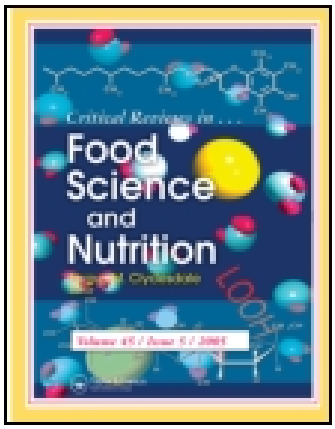

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Cereal-Derived Arabinoxylans as Biological Response Modifiers: Extraction, Molecular Features, and Immune-Stimulating Properties

Shuangyue Zhang, W. Li, C. J. Smith & H. Musa[a]
[a] Department of Food and Tourism Management, Hollings Faculty, Manchester Metropolitan University, Manchester M14 6HR, United Kingdom
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Cereal-Derived Arabinoxylans as Biological Response Modifiers: Extraction, Molecular Features, and Immune-Stimulating Properties

SHUANGYUE ZHANG,W. LI, C. J. SMITH, and H. MUSA

Department of Food and Tourism Management, Hollings Faculty, Manchester Metropolitan University, Manchester M14 6HR, United Kingdom

Arabinoxylans are of significant importance to human health due to their potential to modulate both the adaptive and innate immune systems. Arabinoxylans of various structures and sources have been shown to affect different immune cells to augment a wide range of immune responses in vitro and in vivo in animals and humans. This review article discusses current research on the immune-enhancing activities of arabinoxylans and other cereal polysaccharides in relation to their structural heterogeneity. There are inconsistencies in the literature regarding the relationships between the immunomodulatory effects and the structure and source of arabinoxylans. Possible mechanisms underlying these relationships which might explain the effects of such bioactive polysaccharides are proposed.

Keywords Arabinoxylans, cereal, structure, immune stimulation, dietary fibres

INTRODUCTION

Arabinoxylan, a polysaccharide composed of a β-(1,4)-D-xylopyranose linked xylan backbone with α-L-arabinofuranosyl substitutions at O-3 and/or O-2, is a major dietary fibre component found in a variety of cereals including oat, sorghum, rice, wheat, corn and rye (Izydorczyk and Biliaderis, 1995; Zhou et al., 2010; Broekaert et al., 2011). The manner of arabinofuranosyl substitution differs between arabinoxylans depending on their origin (Pastell et al., 2009). Wheat and rye endosperm arabinoxylans are generally less branched with the Ara/Xylo ratios of 0.5–0.7 and 0.48–0.55, respectively, compared to rice (Ara/Xylo: 0.80) and sorghum (Ara/Xylo: 0.87) (Izydorczyk and Biliaderis, 1995; Izydorczyk et al., 1998b). Hydroxycinnamic acid derivatives (ferulic/coumaric/sinapic/dehydrodiferulic acids) have been reported to form unique ester linkages with the 5-OH group of arabinofuranosyl residues (Izydorczyk and Dexter, 2008; Broekaert et al., 2011) which may promote polysaccharide-polysaccharide (β-glucan/cellulose/galactose/glucose) and polysaccharide-protein cross-linking (Izydorczyk and MacGregor, 2000; Dervilly-Pinel et al., 2001a; Dervilly-Pinel et al., 2004; McCleary et al., 2008). Together, these structural features contribute to the variations in the degree of branching (DB), molecular weight and spatial arrangement of arabinoxylans which then influence their functionality (Saulnier et al., 2007).

Dietary fibre consumption has been shown to have many health benefits such as prebiotic activity, reducing the risk of various diseases, improving lipid and fat metabolism (Lu et al., 2004; Anderson et al., 2009; Kojima et al., 2010; Broekaert et al., 2011; Saeed et al., 2011; Damen et al., 2012; Delcour et al., 2012). More recently, there has been a growing body of research detailing the immune-enhancing ability and anti-tumour activity of arabinoxylans. The human immune system functions through two branches: the innate responses and adaptive responses. During an early innate response, natural killer (NK) cells, granulocytes (neutrophils), monocytes and macrophages are activated to phagocytose pathogens and present antigens to T- and B-lymphocytes. These rapid but non-specific innate responses in turn trigger the adaptive immune system (slow but highly specific) to produce antibodies via B-lymphocytes and to attack foreign molecules via cytotoxic and helper T-lymphocytes. In addition, the presence of memory B cells allows the adaptive immune system to

Address correspondence to Weili Li, Centre for Food, Nutrition & Health Research, Hollings Faculty, Manchester Metropolitan University, Hollings Faculty, Old Hall Lane, Manchester M14 6HR, United Kingdom. E-mail: 10997519@stu.mmu.ac.uk or w.li@mmu.ac.uk

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acquire enhanced and rapid immune responses in case the same antigen infects the body again (Voet and Voet, 2004; Alberts, 2008).

Studies have suggested that enzymatically modified rice bran arabinoxylan (Biobran) with low molecular weight (30–100 kDa) can significantly stimulate both the innate and adaptive immune systems by enhancing NK cell activity, macrophage phagocytosis, dendritic cell maturation, B and T cell function (Ghoneum, 1998a; Ghoneum and Abedi, 2004; Ghoneum and Matsuura, 2004; Ghoneum et al., 2008b; Cholujova et al., 2009). These effects are accompanied by the up-regulation of antigen expression on dentritic cells and elevated levels of cytokine production (Ghoneum and Jewett, 2000). Biobran can also sensitise human cancer cells to daunorubicin (chemotherapeutic agent), apoptosis (Ghoneum and Gollapudi, 2003; Ghoneum et al., 2004; Ghoneum and Gollapudi, 2005b; Gollapudi and Ghoneum, 2008) as well as inhibiting tumour growth in Ehrlich carcinoma-bearing mice (Badr El-Din et al., 2008; Noaman et al., 2008a). Other low molecular weight rice polysaccharides have been shown to promote anti-inflammatory and anti-complementary activities in vitro (Wang et al., 2008; Hoshino et al., 2010). Interestingly, wheat bran arabinoxylans modified via alkali (large molecular weight: 100–200 kDa) or enzyme (low molecular weight) only enhanced immunity and inhibited tumour growth in vivo (Zhou et al., 2010; Cao et al., 2011). Large molecular weight arabinoxylans (200–400 kDa) from banana peel showed some stimulatory effect on macrophage activation whereas low molecular weight corn husk arabinoxylans significantly augmented cytokine production and NK cell activity (Zhang et al., 2004; Ogawa et al., 2005). However, barley arabinoxylans of low molecular weight showed negligible immune-enhancing activities in vitro (Samuelsen et al., 2011).

The discovery of dietary fibre-derived Biological Response Modifiers (BMRs), such as β-1,3-glucan, β-1,6-glucan, and α-1,6-mannan demonstrated that their biological activity may be associated with specific structural confirmations (Brown and Gordon, 2003; Volman et al., 2008; Rieder et al., 2011). So far, there is no consensus as to the main structural features required for arabinoxylans to confer their immune-modulating ability. This inconsistency in the literature may be due to the use of arabinoxylans derived from various sources with different molecular weights and DB. The aim of this review is to give an overview of the immune modulating properties of dietary arabinoxylans in relation to their structural characteristics and also to propose possible mechanisms by which dietary arabinoxylans may work.

STRUCTURAL HETEROGENEITY OF CEREAL ARABINOXYLANS

In cereal arabinoxylans, L-arabinofuranose (Araf) is the main sugar substituent on the linear β-(1,4)D-xylopyranose (Xylp) linked xylan backbone (Saulnier et al., 2007; Broekaert et al., 2011). They are substituted to Xylp residues at O-2 and/or O-3 via α-1, 2 and α-1, 3 glycosidic linkages leading to the conformation of four distinctive structural elements within the polymer: mono-substituted Xylp at O-2 or O-3, di-substituted Xylp at O-2 & O-3, and unsubstituted Xylp (Izydorczyk and Biliaderis, 1995; McCleary et al., 2008). While the majority of Araf substitutions are monomeric, a small proportion of Araf has been reported to form short oligosaccharide side chains composed of two or more 1→2, 1→3 and 1→5 linked Araf residues (Izydorczyk and Dexter, 2008). In addition, less common disaccharide side chains consisting Araf and Xyl/galactopyranosyl residues have also been reported (Saulnier et al., 2007; Izydorczyk and Dexter, 2008; Pastell et al., 2009) in arabinoxylans of various sources. In contrast, glucuronic acids, uronic acids, D-galactose, D-glucose, and acetyl groups are substituted at O-2 and/or O-3 of the xylan backbone in much lower quantities (Broekaert et al., 2011). The presence of hydroxycinnamic acid derivatives is another unique feature of cereal arabinoxylans. Ferulic, *p*-coumaric, sinapic, and dehydrodiferulic acids are able to esterify via O-5 of the Araf residues attached to the Xyl backbone (Izydorczyk and Biliaderis, 1995; Izydorczyk et al., 1998b; Saulnier et al., 2007). The ferulate esters then form dimers via phenoxy radicals leading to covalent cross-linking between arabinoxylan chains and arabinoxylans and other cell wall constituents (Izydorczyk and MacGregor, 2000; Dervilly-Pinel et al., 2001a; Lazaridou and Biliaderis, 2007).

The general structural description of arabinoxylan (Figure 1) is said to vary significantly among cereal species due to the complexity of tissue components within cereal grains (Izydorczyk and Biliaderis, 1995; Dervilly-Pinel et al., 2001a; Storsley et al., 2003; Adams et al., 2004; Höije et al., 2006). It has been reported in previous works (Maes and Delcour, 2002; Ordaz-Ortiz and Saulnier, 2005), that wheat bran, starchy endosperm and husks contain different quantities of arabinoxylans with various xylose to arabinose ratios when subjected to extraction. The method of extraction can add even more structural diversity (Moers et al., 2005) as there are a wide range of techniques available including hot water extraction (Izydorczyk et al., 1998b), enzymatic extraction (Lu et al., 2005; Van Craeyveld et al., 2009), alkali extraction (Zhou et al., 2010) and solvent extraction (Yamagishi et al., 2003) yielding arabinoxylans of various molecular weight, DB, and tertiary conformation (Izydorczyk and Biliaderis, 1995). These structural characteristics may be crucial to the biological activity of arabinoxylans as the stimulation of the innate immune system relays on the recognition of foreign pathogen associated immunostimulants by receptors expressed on the surface of immune cells (Alberts, 2008).

EXTRACTION OF CEREAL ARABINOXYLANS

The water solubility/extractability of cereal arabinoxylans relays largely on their branching pattern and molecular weight.

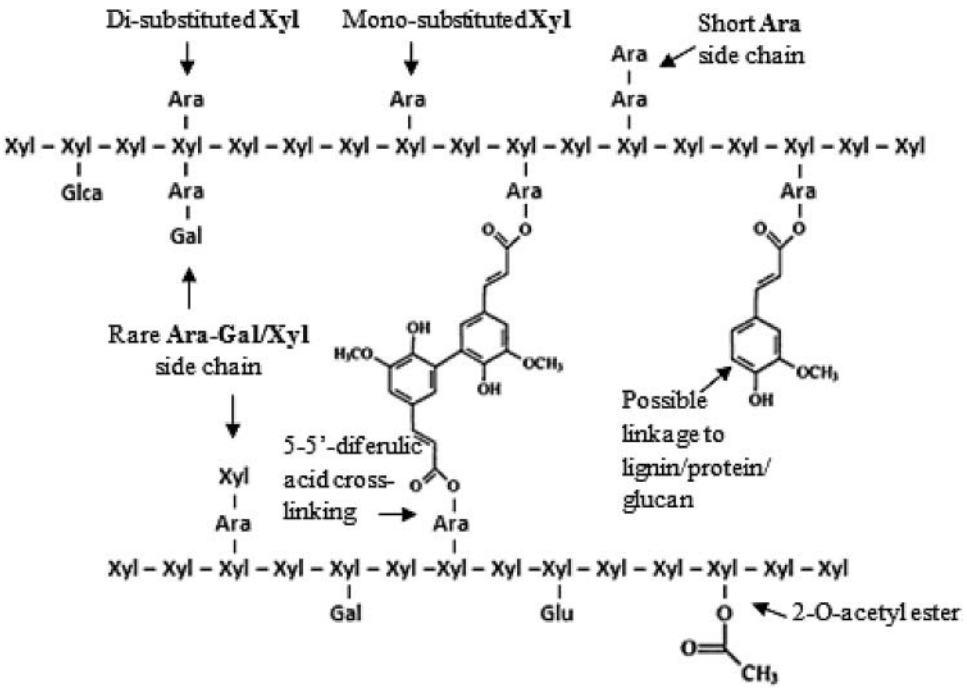

Figure 1 Simplified schematic representation of the main structural features within cereal arabinoxylans. The composition of different substituents shown above may vary between arabinoxylans extracted from various sources (Adams et al., 2004).

The presence of Araf/galactose/glucose side chains can prevent chain–chain interaction which increases water solubility whereas ferulic/glucuroic acids act to promote cross-linkages (Muralikrishna and Subba Rao, 2007; Saulnier et al., 2007; Izydorczyk and Dexter, 2008). In wheat endosperm, 75% of arabinoxylans are water un-extractable despite them having a higher proportion (A/X ratio varied from 0.51 to 0.67 and 0.47 to 0.58 for WU-AX and WE-AX, respectively) of Araf side chains compared to water extractable arabinoxylans (Cleemput et al., 1993; Andersson et al., 1994; Delcour et al., 1999; Ordaz-Ortiz and Saulnier, 2005; Dornez et al., 200911). This is due to their large molecular weight and high ferulic acid content leading to the formation of covalent/non-covalent linkages between arabinoxylan chains and other cell wall components such as proteins, β-glucans, lignin, and cellulose (Beaugrand et al., 2004; Saulnier et al., 2007). Thus, wheat arabinoxylans extracted solely by water tend to have their Araf and ferulic acid side chains well balanced with A/X ratios ranged from 0.5 to 1.30 (Bengtsson et al., 1992; Beaugrand et al., 2004).

Enzymes are added to this aqueous extraction system as a means of improving polymer solubility via structural modifications. Endoxylanase and xylanase are commonly used to cleave the glycosidic bond between 1→4-linked β-D-Xylp units releasing a mixture of oligosaccharides with various molecular weights (Escarnot et al., 2011). They often exhibit substrate selectivity, for example, GH10 xylanase cleaves specifically at sites that are in close proximity to side chain substitutions whereas GH11 xylanase cleaves at sites far away from substituents (Beaugrand et al., 2004; Maslen et al., 2007). Thus, highly branched arabinxoylans are more susceptible to GH10 degradation releasing shorter oligosaccharides carrying substituents at the non-reducing terminal of the β-D-Xylp residue (Vardakou et al., 2003; Izydorczyk and Dexter, 2008; Pastell et al., 2009). Other enzymes including exo-xylosidases (cleaves xylose from reducing end of xylan backbone), arabinofuranosidases (removes Araf), feruloyl esterases (removes ferulic/diferulic acids) and acetyl xylan esterase (removes acetyl groups) can also alter the intra-chain and inter-chain interactions of arabinoxylans leading to increased solubility/extractability (Faulds et al., 2003; Smaali et al., 2006; Pitkanen et al., 2011).

In order to break the ester-linkages and hydrogen bonds between long chains, water un-extractable arabinoxylans are often treated with $Ba(OH)_2$ and NaOH (Roubroeks et al., 2000; Agger et al., 2011). Thus, the DB and tertiary conformation of alkali-extractable arabinoxylans appeared to be different from those treated with water (Dervilly-Pinel et al., 2004; Pastell et al., 2009). In addition, enzymatic extraction using arabinofuranosidases, feruloyl esterases and acetyl xylan esterase also give rise to arabinoxylans with different A/X ratios, branching pattern and molecular weight by releasing approximately 50% of ferulic acid and 30% of Araf side chains (Faulds et al., 2003; Pitkanen et al., 2011). Treatment with endoxylanases, xylanases and exo-xylosidases may not affect the A/X ratio but they can significantly reduce the molecular weight of long polymers (Lu et al., 2005; Escarnot et al., 2011). Various extraction methods are summarised in Table 1.

Table 1 Methods used by previous studies to extract arabinoxylans from various sources

Reference	Extraction	Source	Ara/Xyl	Other substuents
Roubroeks et al. (2000)	Water, ethanol alkali	Rye bran	0.50–0.95	Short arabinose side chains, β-glucans, mannose, glucose, galactose
Izydorczyk and Biliaderis (1995)	Alkali	Rye bran	0.43–0.78	Short arabinose side chains, glucose
Bengtsson et al. (1992)	Water	Rye endosperm	0.48–0.55	Ferulic acid
Zhang et al. (2004)	Alkali	Banana peel	2.6	Galactose, cross-linked arabinogalactan
Zhang et al. (2003)	Alkali	Corn hull	0.63	Galactose, glucose, glucuronic acid
Cholujova et al. (2009)	Water, enzyme	Rice bran	0.41–0.54	Galactose, glucose
Wang et al. (2008)	Water, alkali	Rice bran	0.93–2.00	Galactopyranose, glucose
Shibuya et al. (1985)	Alkali	Rice endosperm	0.80–1.04	Galactose, glucose, mannose, fructose, rhamnose, uronic acid
Dervilly-Pinel et al. (2001a) Dervilly-Pinel et al. (2004)	Water, ethanol	Wheat endosperm	0.37–1.28	Glucose, mannose, galactose, feruli acid, short arabinose side chain
Izydorczyk and Biliaderis (1995); Pastell et al. (2009)	Alkali	Wheat endosperm	0.56–0.60	Short arabinose side chains, glucose
Beaugrand et al. (2004); Hollmann and Lindhauer (2005)	water	Wheat bran	0.53–1.25	Short arabinose side chains Glucose, galactose, ferulic acid, p-coumaric acid, sinapic acid
Höije et al. (2006) Pastell et al. (2009),	Alkali	Barley husk	0.20–0.28	Glucose; galactose, uronic acid, Xylp-Araf side chains
Izydorczyk and Dexter (2008)	Water, Alkali	Barley endosperm	0.46–0.92	Galacose, mannose, glucose, Ferulic/dehydri diferulic acid

DEGREE OF BRANCHING

The Ara/Xyl ratio can be used as an indicator for the DB of cereal arabinoxylans. Generally, the A/X ratio for cereal endosperm arabinoxylans can range from 0.4 to 0.8 and bran arabinoxylans from 0.6 and 1.1, but wider natural variations do exist (Pastell et al., 2009). Studies have revealed that arabinoxylans derived from wheat (Ara/Xyl: 0.50–0.70) and rye endosperm (Ara/Xyl: 0.48–0.55) are less branched compared to rice (Ara/Xly: 0.80) and sorghum (Ara/Xyl: 0.87) (Izydorczyk and Biliaderis, 1995; Izydorczyk et al., 1998a). The proportion of un-substituted, mono-substituted and di-substituted xylose residues found in barley is very similar to wheat (Ordaz-Ortiz and Saulnier, 2005; Izydorczyk and Dexter, 2008). However, the amount of Xyl-O-2 mono-substitution was reported to be very different as only 0.3–1.9% was found in wheat whereas hull-less and hulled barley contained 6–16% (Höije et al., 2006; Izydorczyk and Dexter, 2008). It has also been proposed that arabinoxylans from rice, sorghum, and maize bran display more structural complexity than those from wheat, rye and barley due to the presence of xylopyranose, galactopyranose and glucuronic acid substituents (Izydorczyk and Biliaderis, 1995; Izydorczyk and Dexter, 2008).

In barley, arabinoxylans constitute approximately 20–25% and 85% of endosperm and aleurone cell-wall polysaccharides, respectively (Muralikrishna and Subba Rao, 2007). The highest A/X ratio was found in the pericarp tissues of both hull-less (1.15) and hulled barley (1.12) and lower A/X ratios were reported in the aleurone (0.57, 0.66) and endosperm (0.92). The branching pattern consisted of two distinct regions. One of which contains a large proportion of mono/di-substituted xylose residues separated by one or two un-substituted Xyl and the other region contains two or more un-substituted Xyl to connect those highly substituted regions (Muralikrishna and Subba Rao, 2007). Thus, mono-substituted (20–25%), di-substituted (19–26%), and un-substituted (47–65%) Xyl together give rise to the overall branching pattern of barley arabinoxylans (Izydorczyk and Dexter, 2008). Apart from low level substituents including galactose, glucose, mannose, ferulic acid and uronic acid, short side chains consisting 2-O-β-D-xylopyranosyl-α-L-arabinofuranosyl have recently been identified at the O-3 position in barley husk arabinoxylans (Höije et al., 2006; Pastell et al., 2009).

Arabinoxylans comprise almost 70% of wheat endosperm and is often referred to as pentosans due to their high xylose and arabinose composition (Ralet et al., 1990; Saulnier et al., 2007; Toole et al., 2011). The A/X ratio may vary from 0.31 to 1.06 reflecting the wide natural structural heterogeneity within wheat arabinoxylans (Ordaz-Ortiz and Saulnier, 2005). The proportion of uXyl (60–65%), mXyl (12–21%), and dXyl (13–30%) together give rise to a unique branching pattern which consists of a constant, highly branched region and a fairly variable region with less side chains (Andersson et al., 1994; Izydorczyk and Biliaderis, 1995; Delcour et al., 1999). The highly branched region (region A) contains di-substituted Xyl at O-2, 3 separated by one or two un-substituted Xyl and the variable region (region B) may contain some mono/di-substituted Xyl appearing mainly in pairs or isolated by un-substituted Xyl residues (Gruppen et al., 1993; Muralikrishna and Subba Rao, 2007). The variations in the proportion of region A and B are the main factor contributing to the various A/X ratios of wheat arabinoxylans (Gruppen et al., 1993).

Rice endosperm and bran arabinoxylans exhibit great variations in their A/X ratios depending on the method of extraction used. The majority of Araf substitutions are attached via O-2 similar to barley arabinoxylans. In addition, 1→2, 1→3 and

1→5 linked arabinose residues together with non-reducing end xylose and galactose were found in rice bran arabinoxylan whereas rice endosperm contained mostly single monosaccharide side chains (Muralikrishna and Subba Rao, 2007). The complexity in the branching pattern of rice arabinoxylans is extended by the presence of uronic acids linked via O-2 to xylose residues (Shibuya et al., 1985).

TERTIARY CONFORMATION OF ARABINOXYLANS

Rao and Muralikrishna (2004) revealed that X-ray diffraction analysis revealed that arabinoxylans have a 3-fold symmetry forming left-handed helices in solution. In the solid state, they conform into extended chains of ribbon-like strands with a highly un-substituted backbone (Dervilly-Pinel et al., 2001b; Saulnier et al., 2007). The xylan chain confers relative flexibility as the xylose residues are only linked by hydrogen bonds. Thus, it can aggregate into soluble complexes stabilised via intermolecular hydrogen bonding. Despite the intrinsic flexibility of the xylan chain, arabinoxylans are considered rigid molecules due the presence of arabinose side branches which help to maintain the xylan backbone in its more extended conformation. However, in solution, the persistent length which reflects on the rigidity of the polymer is not significantly affected by the A/X ratio. Therefore, the arabinose side chains may only have small or no effect on the conformation of arabinoxylans in solution allowing the long polymers to behave as random coils with semi-flexibility. Furthermore, arabinoxylans in wheat, rice and rye have been confirmed by X-ray diffraction studies to be asymmetric molecules (Yui et al., 1995; Dervilly-Pinel et al., 2001b; Ordaz-Ortiz and Saulnier, 2005).

STRUCTURAL AND IMMUNE-STIMULATING PROPERTIES OF RICE BRAN ARABINOYXLAN (MGN-3/BIOBRAN)

MGN-3/Biobran, developed in 1992 by Dr Hiroaki Maeda, is an enzymatically modified small chain (30–50 kDa) arabinoxylan extracted from rice bran with a main structure consisting of monomeric arabinose side chains attached to a xylose-linked backbone (Ghoneum, 1998a). HPLC analysis revealed small traces of galactose, glucose and β1,3-glucan suggesting that other low molecular weight hemicelluloses are cross-linked with MGN-3 which may affect their branching pattern and tertiary conformation in solution (Ghoneum and Jewett, 2000). Several in vitro and in vivo studies have demonstrated the ability of MGN-3 to enhance the function of various immune cells including macrophages, dendritic cells, T-lymphocytes, NK cells and B cells;(Ghoneum, 1998a; Ghoneum and Brown, 1999; Ghoneum and Jewett, 2000; Ghoneum and Abedi, 2004; Ghoneum and Matsuura, 2004; Ghoneum et al., 2008a; Cholujova et al., 2009). Apart from immune cell lineages, MGN-3 can also induce apoptosis of certain tumour cell lines in vivo and inhibit tumour growth in rats, however, only a limited number of human studies have been conducted (Ghoneum and Brown, 1999; Ghoneum and Gollapudi, 2003; Ghoneum and Gollapudi, 2005b; Ghoneum and Gollapudi, 2005a; Noaman et al., 2008b; Bang et al., 2010). Although the mechanistic basis underlying the immune-modulating effects of MGN-3 is not well understood, it was pointed out that these long un-digestible arabinoxylans must be modified into low molecular weight polysaccharides so that they can easily diffuse through intestinal walls or directly into the blood stream and then be transported to the lymph nodes where different immune cells reside (Ghoneum, 1998a; Ghoneum and Jewett, 2000; Alberts, 2008).

IN VITRO STUDIES

Biobran arabinoxylans can enhance the differentiation of human blood monocytes into immature dendritic cells (iDCs) in vitro in the presence of two cytokine maturation cocktails (CMC1: TNFα, IL1β and IL6; CMC2: LPS, IFNγ) (Cholujova et al., 2009). This enhanced immune response is accomplished by the down-regulation of CD14 monocyte expression marker (65% in CMC1 and 100% in CMC2) at 1,000 μg/mL of MGN-3 (Zhou and Tedder, 1996). Treatment with 400 μg/mL of MGN-3 induced a large increase in CD83, CD80 and CD86 expression on iDCs suggesting that MGN-3 can also stimulate iDC maturation via the up-regulation of co-stimulatory proteins (DC80 and CD86) which in turn send co-stimulatory signals for effector T cell activation (Harris et al., 1997; Poindexter et al., 2004; van Rijt et al., 2004; Cholujova et al., 2009). Furthermore, the ability of untreated monocytes and iDCs to take up FITC-labelled dextran was reduced by approximately 75% and 60%, respectively, in the presence of 1,000 μg/mL of MGN-3 which confirmed the enhancing effect of MGN-3 on DC maturation as low endocytic activity is only associated with mature DCs (Cholujova et al., 2009).

There are other surface expression markers analysed in this study which showed insignificant contributions. CD1a is an antigen presenting molecule with significant heterogeneity in its expression among DCs derived from the myeloid lineage (Alberts, 2008). Studies have shown that both $CD1a^+$ and $CD1a^-$ iDCs and mature DCs can be derived from peripheral monocyte human blood (Ito et al., 1999; Cravens and Lipsky, 2002; Cernadas et al., 2009). Although MGN-3 was able to down-regulate CD1a expression in iDCs but there was no isolation of $CD1a^-$ DCs by flow cytometry, thus CD1a expression alone cannot be used to distinguish iDCs from mature DCs. CD11c (adhesion protein) and CD123 (interleukin 3 receptor protein) were also analysed but again these markers are not specific to iDC or mature DCs. As the surface expression of CD80, CD86 and CD83 on monocytes and iDCs was already enhanced by cytokine treatments alone,

it is only logical to conclude at this stage that MGN-3 has the potential to enhance cytokine-induced iDC differentiation and maturation.

Ghoneum and Matsuura (2004) investigated the effect of MGN-3 on macrophages. The percentage of attachment and phagocytosis of yeasts by U937 (human macrophage cell line) and P-MΦ (peritoneal macrophages) cells in the presence MGN-3 (100 and 500 μg/mL) showed different patterns as 100 μg/mL of MGN-3 significantly enhanced yeast attachment and phagocytosis by U937 (approximately 190% increase compared to untreated cells) whereas 500 μg/mL was more effective for P-MΦ cells (150% increase compared to untreated cells). The ability of MGN-3 to augment the functional activity of macrophages was further demonstrated by an elevated level of TNF and IL6 secretion by RAW264.7 (murine macrophage cell line), U937 and P-MΦ cells. Moreover, the level of NO released (marker for cytotoxic activity) by RAW264.7 cells also increased drastically from 0 to 59 μM in the presence of 1,000 μg/mL of MGN-3. This response was not seen in murine P-MΦ cells (100 μg/mL of MGN-3 enhanced the highest increase from 0 to 11 μM) suggesting that various cells may respond differently to MGN-3 treatments.

Two earlier studies have examined the stimulatory effect of MGN-3 on human NK cell activity and the possible mechanism underlined. Ghoneum et al. (1998a,b) showed that 100 μg/mL of MGN-3 significantly enhanced NK cytotoxicity by 150% against two cell line targets: human tumour cell line K562 and Raji which is a Burkitt cell lymphoma highly resistant to NK activity. The level of IFNγ secreted by peripheral blood lymphocytes also rose rapidly in the presence of 25, 50 and 100 μg/mL of MGN-3 from 100 pg/mL (cells alone) to 340, 390, and 580 pg/mL, respectively. This increase in TNFγ is most likely to be associated with the increased NK cell activity but other effector cells could be involved such as cytotoxic T cells which also secrete TNFγ upon activation for macrophage recruitment (Voet and Voet, 2004).

Ghoneum and Jewett (2000) confirmed the stimulatory effect of MGN-3 on NK cell activity by testing NK cells isolated from the peripheral blood lymphocyte population. The results indicated that the enhancement in NK cytotoxicity can be maximised in the presence IL-2 (U/mL) and that this response is associated with increased TNFα and IFNγ secretion by purified NK cells (highest increase at 1,000 μg/mL of MGN-3). Furthermore, MGN-3 significantly up-regulated NK cell activation marker CD69 in peripheral blood cells together with CD54 (ICAM-1 adhesion molecule) and CD25 (IL-2 receptor). This suggests suggesting that the mechanism by which MGN-3 modulates human NK cell activation and boosts cytotoxicity involves the induction of IFNγ, TNFα and CD54 (IL-2-activated PBLs often express high levels of CD54 which explains why the stimulatory effect was maximised when combine IL-2 with MGN-3).

Several studies have investigated the anti-tumour property of MGN-3 using cancer cell lines. The number of apoptotic HUT78 cells (leukemia cell line) increased by 200% in the presence anti-CD95 antibody after pre-treatment with 1,000 μg/mL of MGN-3 when compared with cells treated with anti-CD95 antibody alone (20% apoptotic cells). This augmentation was shown to be mediated by an increase in the activation of caspase 8, caspase 9, caspase 3 together with depolarised mitochondrial membrane potential and suppressed Bcl-2 (anti-apoptotic protein) expression. However, MGN-3 treatments alone showed no effect on HUT78 cells suggesting that MGN-3 functions by sensitizing cancer cells to undergo apoptosis induced by death ligands upon binding with receptors such as CD95 (Ghoneum and Gollapudi, 2003).

Apart from HUT78 cells, two studies used human breast cancer cells to assess the anti-tumour potential of MGN-3 in relation to yeast-induced apoptosis (Ghoneum and Gollapudi, 2004; Ghoneum et al., 2004) and chemotherapy. Ghoneum and Gollapudi (2004) demonstrated that 500 μg/mL of MGN-3 was the most effective concentration to be used to enhance the percentage of yeast-induced apoptosis in three human breast cancer cell lines (MCF-7, ZR75 and HCC70). The number of apoptotic cancer cells post yeast phagocytosis increased by 100% (MCF-7 cells), 140% (ZR75 cells) and 67% (HCC70 cells) when compared with cancer cells treated with yeasts alone. These results were associated with increased caspase activity in MCF-7 (32% increase in caspase 8 and 20% in caspase 9) and HCC70 (82% increase in caspase 9 and 26% increase in caspase 3) cells suggesting that MGN-3 may exert its effect through activating events upstream of caspase activation. Surprisingly, ZR75 cells which showed the highest increase in apoptotic cancer cell phagocytosis were not tested for caspases activity. More recently, Gollapudi and Ghoneum (2008) used in vitro MTT assay and flow cytometry to show that MGN-3 increased susceptibility of MCF-7 and HCC70 cells to chemotherapeutic agent daunorubicin by 5.5 fold and 2.5 fold, respectively, as compared to cells treated with daunorubicin alone. This sensitizing effect of was maximised at 1,000 μg/mL of MGN-3 and was accomplished by augmented daunorubicin accumulation in MCF-7 and HCC70 cells. In vitro assays used to test MGN-3 against different cells types are summarised in Table 2.

IN VIVO STUDIES

10 mg/mL of MGN-3 was administered orally or via intraperitoneal (IP) injection daily to two groups of mice (C75BL/6 and C3H). The peritoneal and splenic NK cell activity was measured using ^{51}Cr-release assay at day 2, 5, and 14 in the presence or absence of MGN-3 treatments. For both groups of mice, IP injection of MGN-3 resulted in augmented peritoneum NK cell activity at day 2 and kept rising through day 14 compared to saline-treated mice. However, bone marrow and splenic NK cell activity remained unchanged after MGN-3 treatments at day 2, 5 and 14 (Ghoneum and Abedi, 2004). In contrast, orally administered MGN-3 did not cause any

Table 2 In vitro studies of MGN-3 using various cell types

Reference	In vitro assays	Cell types
(Ghoneum 1998b)	^{51}Cr - release assay, IFN-γ ELISA assay	Human peripheral blood lymphocytes (PBLs)
(Ghoneum and Jewett 2000)	TNF-α ELISA assay, IFN-γ ELISA assay, ^{51}Cr - release assay Cell surface staining (flow cytometry)	Human PBLs and NK cells isolated from PBLs NK cells isolated from PBLs
(Ghoneum and Gollapudi 2003)	Apoptosis assay, Activity of caspases, Mitochondrial potential, CD95 and Bcl-2 surface expression	Leukemia cell line (HUT78)
(Ghoneum and Matsuura 2004)	Phagocytic assay IL-6 and TNF-α secretion assay	Human U937 cell line and murine P-Mφ cells Human U937, murine P-Mφ and RAW264.7 cells Murine P-Mφ and RAW264.7 cells
(Ghoneum and Gollapudi 2005a; Ghoneum and Gollapudi 2005b)	Phagocytic assay, Yeast attachment and uptake, Tumour survival and apoptosis, Activity of caspases	Human breast cancer cell lines (MCF-7 and HCC70)
(Gollapudi and Ghoneum 2008)	Drug sensitivity assay and Daunorubicin accumulation	Breast cancer cell lines (MCF-7 and HCC70)
(Cholujova et al., 2009)	Endocytosis assay with FITC-dextran, dentritic cell differentiation and maturation	Peripheral blood monocytes

significant change in peritoneum NK cell activity whereas a drastic 200% increase was observed in splenic NK cells at day 14. Flow cytometry analysis showed insignificant changes in the number of peritoneum NK cells between mice treated with MGN-3 (16% P-NK) and saline (14% P-NK). In addition, YAC-1 tumour cell line was used as a target to assess the ability of peritoneum NK cells to form conjugates. MGN-3 (100 μg/mL) treated mice demonstrated a 26% increase in conjugate formation compared to control (13%) indicating the potential of MGN-3 having anti-tumour effects by enhancing NK cell cytotoxicity (Ghoneum and Abedi, 2004).

Recently, two papers investigated the anti-tumour effect of MGN-3 using Ehrlich carcinoma bearing mice. Badr El-Din et al., (2008) reported that both IP and intra-tumoral (IT) injections of MGN-3 (40mg/kg body weight) delayed tumour growth as compared to controls. This inhibitory effect became significant as early as Day 14 post-IP treatment and elevated through Day 35 reaching approximately 63% and 45% reduction in tumour volume and tumour weight, respectively. In comparison, the delay in tumour growth only became significant at Day 36 post-IM treatment and elevated through Day 45 reaching a 45% decrease in tumour volume. Moreover, the anti-tumour effect exerted by MGN-3 was accompanied by an increase in the percentage of apoptotic cells (76% increase), NK cell activity (2-fold increase), TNF-α (11% increase) and IFNγ (154% increase) secretion as well as suppressed IL-10 production (2-fold decrease) compared to controls. Results here indicated that MGN-3 may exert its anti-tumour effect by acting as a potent inducer of TNF-α and IFNγ, a finding that has been previously reported in vitro using human blood peripheral lymphocytes, human macrophages, murine macrophage cell line (Ghoneum and Jewett, 2000; Ghoneum and Matsuura, 2004; Badr El-Din et al., 2008).

The other key paper, Badr El-Din et al., (2008) investigated the antioxidant potential of MGN-3 in relation to its oncostatic effect against solid Ehrlich carcinoma bearing mice. Several studies have reported that the rate of cancer cell proliferation may be directly linked with changes in antioxidant activity (Navarro et al., 1999; Wenger et al., 2001; Badr El-Din, 2004; Gupta et al., 2004; El-Din et al., 2008). Intra-peritoneally injected MGN-3 (25mg/kg) effectively delayed tumour growth in Ehrlich carcinoma bearing mice, normalised lipid peroxidation level and elevated glutathione (GSH) content in both blood (39% increase) and liver (41% increase) after 4 days post-treatment. In addition, the activity of antioxidant scavenger enzymes including superoxide dismutase (SOD), glutathione perioxidase (GPx), catalase (CAT) and glutathione-S-transferase (GST) in blood, liver and tumour tissue all appeared to have increased which was then confirmed by the up-regulation of GPx, SOD1 and CAT mRNA. These responses were more significant during early treatments of MGN-3 (Day 4) compared to later treatments (Day 11). Thus, a possible alternative mechanism by which MGN-3 exerts its anti-tumour effect may involve the activation of various endogenous antioxidant enzymes and lipid peroxidation depletion.

Chemotherapy is considered one of the most effective treatments for many types of cancer even though most chemotherapeutic agents exhibit dose-limiting toxicities such as congestive heart failure, neutropenia, diarrhea, myalgias, neurotoxicity, immune suppression as well as mutagenic and carcinogenic effects (Gollapudi and Ghoneum, 2008). Therefore, it is of great interest to scientists to find therapeutic approaches that could reduce chemotherapeutic-induced toxicity. Two studies evaluated the beneficial effects of MGN-3 on cisplatin and doxorubicin treated rats. These studies showed an improvement in weight gain in cisplatin and doxorubicin injected rats post oral administration of MGN-3 throughout a period of 11 days (Jacoby et al., 2000). Other beneficial effects of MGN-3 observed in cisplatin treated mice include reduced incidences of diarrhoea (60% decrease post MGN-3 treatment at 50mg/kg) and gross gastrointestinal mucosal pathology (43% decrease post MGN-3 treatment at 5mg/kg). In contrast, 5mg/kg of MGN-3 completely diminished incidences of diarrhoea caused by doxorubicin and reduced gross

gastrointestinal mucosal pathology in doxorubicin treated rats drastically by 80%. (Endo and Kanbayashi, 2003) also demonstrated the ability of MGN-3 to accelerate protection against weight loss in mice due to cisplatin treatment and that this effect was not influence by the method of administration. Intriguingly, using polyclonal antibodies against modified rice bran and ELISA assay, they have shown for the first time that immuno-reactive components of MGN-3 are absorbed from the gut into the blood after oral administration by rats. In vivo assays used to test MGN-3 are summarised in Table 3.

HUMAN STUDIES

Effects of MGN-3 have been examined in various in vitro studies and in several animal models, while only a few human studies have been conducted. The first human study was carried out by Ghoneum (1999) using twenty-four healthy individuals (15 females and 9 males, aged 20–46 yrs). Oral administration of MGN-3 over a period of two months enhanced NK cell activity against two tumour cell line targets (Raji and K562). This effect was shown in a dose dependent manner with the most significant response observed at 30–46mg/kg of MGN-3 per day. Flow cytometry analysis revealed that MGN-3 treatments alone did not affect the population of NK cells in blood suggesting that MGN-3 is only responsible for enhancing cellular immune responses. Furthermore, MGN-3 (45mg/kg/day) treatments after one month also caused a 310% increase in the binding capacity of NK cells against K562 target cells which further confirms the immuno-modulatory effect of MGN-3 on NK cell cytotoxicity against tumour cell lines.

Ghoneum and Brown (1999) then examined the effect of MGN-3 on NK cell activity, T and B lymphocyte proliferation in 32 cancer patients post chemotherapy. Oral administration of MGN-3 (3g/day) as a food supplement to patients with leukemia, multiple myeloma, breast and prostate cancer for a month significantly enhanced NK cell cytotoxicity (up to 10-fold increase) in peripheral blood. This response was observed for all four types of cancer. As expected, NK cells purified from peripheral blood lymphocytes of cancer patients demonstrated a drastic increase in granular content at Week 1 post-MGN-3 treatment together with augmented binding and killing capacity against tumour cell line targets compared to untreated patients. Their findings also showed that MGN-3 enhanced T and B cell function in all cancer patients by measuring the proliferation response of T and B cells against different mitogens. These preliminary results are encouraging as MGN-3 is non-toxic and showed no hypo-responsiveness in patients followed up for up to four years. Hence, MGN-3 can possibly be used in conjunction with chemotherapy to reduce the effect of immune-suppression.

STRUCTURAL AND IMMUNE-MODULATING PROPERTIES OF RICE POLYSACCHARIDES

Several studies, primarily in vitro, have indicated that various polysaccharides extracted from rice can augment anti-complementary, anti-inflammatory and anti-leukemia immunity (Yamagishi et al., 2003; Liao et al., 2006; Wang et al., 2008; Yamagishi et al., 2008; Hoshino et al., 2010). Rice (Oryza Sativa) endosperm treated with ethanol and ether, a polysaccharide with the carbohydrate composition of 24.1% galactose, 18.3% mannose, 15.1% arabinose, 11.2% glucose, 8.1% xylose, 14.9% uronic acid was obtained (Yamagishi et al., 2003). In the same study, two rice bran proteoglycans (Bm and A1) were also extracted using ammonium sulphate which contained similar sugars (rhamnose, arabinose xylose, mannose, galactose and glucose) to that observed previously (Yamagishi et al., 1975; Yamagishi et al., 1976; Mod et al., 1978; Mod et al., 1979). However, the composition of these sugars appeared to be different between the two proteoglycans as Bm is composed mainly of arabinose and galactose whereas

Table 3 *In vivo* studies of MGN-3 using different animal models

Reference	In vivo assays	Cell types/animal models
Ghoneum and Brown (1999)	^{51}Cr - release assay ^{51}Cr - release assay Target cell conjugate assay	Human peripheral blood lymphocytes(PBLs) Human PBLs against K562 and Raji cell lines Human PBLs against K562
Jacoby et al. (2000)	Body weight, incidence of diarrhoea and gross gastro-intestinal mucosal pathology	Albino male rats
Endo and Kanbayashi (2003)	Body weight	BALB/c female mice
Ghoneum and Abedi (2004)	^{51}Cr - release assay BLT-esterase release assay Conjugate formation assay	Peritoneal and bone marrow NK cells from C57BL/6 and C3H mice Peritoneal NK cells from C57BL/6 mice Peritoneal NK cells from C57BL/6 mice against YAC-1
Noaman et al. (2008b)	Anti-tumour activity, Body weight, Antioxidant activity assays, Gene expression	Adult female Swiss albino mice
El-Din et al. (2008)	Body weight, Tumour apoptosis, Plasma TNF-α, IFN-γ and IL-10 ELISA ^{51}Cr - release assay, Conjugate formation assay	Adult female Swiss albino mice Splenic NK cells from adult female Swiss albino mice

A1 is composed of arabinose and xylose (Yamagishi et al., 2003). Rice endosperm polysaccharide and Bm proteoglycan demonstrated similar anti-complementary potency when compared to polysaccharides extracted from Angelica acutiloba (a well known medicinal herb used as control) whereas A1 showed significantly higher anti-complementary activity compared to the other three (Yamada et al., 1984; Yamada et al., 1985; Kiyohara et al., 1989; Yamagishi et al., 2008).

The anti-complementary activity of all three polysaccharides were then confirmed to be due to their carbohydrate moiety rather than protein, ferulic acid and polyphenol moieties (Yamagishi et al., 2003). Since galactose, arabinose and xylose were the main components, it is possible that the active ingredient is related to a specific structure containing arabinogalactan or arabinoxylan moiety (Fincher et al., 1983; Yamada et al., 1984; Yamada et al., 1990; Ellis et al., 2010). The molecular weight of these polysaccharides was not determined, hence, one possible explanation for the difference in their anti-complementary activity could be the presence of arabinoxylan in A1. However, other studies have pointed out that the wide structural heterogeneity in these polysaccharides would make it very difficult to establish a correlation between structure and anti-complementary activity (Smestad Paulsen, 2002; Yamagishi et al., 2003). Moreover, the activation of Complement demands high specificity as both steric and structural recognitions are required (Yamagishi et al., 2003; Voet and Voet, 2004). Thus, apart from structural specifications, the geometry of the molecule would also play an important role which is not necessary when activating innate immune responses (Alberts, 2008).

A more recent study has discovered another novel rice bran heteropolysaccharide (RBPS2a) with anti-complementary activity using hot water extraction and ethanol precipitation (Wang et al., 2008). RBPS2a has the average molecular weight of 90 kDa and contains 86.7% polysaccharide which is similar to the rice endosperm polysaccharide obtained by the previous study (Yamagishi et al., 2003; Wang et al., 2008). Gas chromatography indicated that RBPS2a is composed of arabinose, xylose, glucose and galactose in the molar ratio of 4:2:1:4 which correlates well with the theory that the active structure is related to an arabino-galactan moiety (Cummings et al., 1992). RBPS2a was able to significantly induce the in vitro complement consumption and degree of red blood cell lysis by residual complement activity in a dose-dependent manner with its most activity shown at 1,000 μg/mL (Wang et al., 2008).

One study adopted a similar extraction and modification method (longer treatment with carbohydrate hydrolysing enzymes) used for MGN-3 to obtain a different rice bran arabinoxylan (HRB) with similar structure but low molecular weight compared to MGN-3 (Hoshino et al., 2010). Mast cells express high affinity receptors (FcεRI) that cross-links with IgE upon antigen recognition to stimulate the activation and phosphorylation of various protein tyrosine kinases leading to several events including inflammatory mediator secretion and membrane fusion (Guo et al., 1998; Paumet et al., 2000; Blank and Rivera, 2004; Abramson et al., 2005; Galli et al., 2005; Stow et al., 2006). In this study, bone marrow derived mast cells treated with HRB (0–3 mg/mL) showed significant depletion of β-hexosaminidase release after antigen stimulation in a dose-dependent manner. Similarly, the secretion of cytokines (TNFα and IL4) by BMMCs was also inhibited upon HRB pre-treatment suggesting that HRB is able to suppress both degranulation and cytokine release in mast cells after FcεRI ligation (Hoshino et al., 2010).

Interestingly, pre-treatment with HRB (3 mg/mL) had no effect on intra-molecular [Ca^{2+}] release and Akt phosphorylation in BMMCs indicating that HRB has no influence on the early signal transduction events during mast cell activation (Hoshino et al., 2010). However, HRB did manage to suppress NF-κB and MAP kinase activation (events downstream of [Ca^{2+}]i release and PKC activation) as well as membrane fusion between liposomes linking membrane fusion suppression and transcription activation with decreased granulation and cytokine secretion. These results suggest that HRB may function by residing in the cytoplasm of mast cells and inhibits not only intracellular signalling activation but also fusogenic activity of plasma and granule membranes (Hoshino et al., 2010). It is also possible that cytokine secretion by mast cells is modulated via the suppression of NF-κB and MAP kinase activation. Hence, HRB has the potential to be used as an effective treatment for mast cell mediated allergic diseases. Furthermore, a human study conducted using 50 elderly people (aged between 70–95yrs) had shown that the oral administration of HRB (500mg/day) for a period of 6 weeks can shorten the duration of the symptoms caused by common cold syndrome and reduce the physical burden of acute respiratory tract infection as a result of its immunomodulatory function (Maeda et al., 2004). In vitro assays used to test rice polysaccharides against various cell types are summarised in Table 4.

STRUCTURAL AND IMMUNE-MODULATING PROPERTIES OF WHEAT BRAN ARABINOXYLANS

Two recent studies have assessed the immune-stimulating potential of alkaline and xylanase extracted arabinoxylans from wheat bran using various assays including delayed-type hypersensitivity reaction (in vivo), splenocyte proliferation (in vivo and in vitro), macrophage phagocytosis and NK cell activity (both in vivo) (Zhou et al., 2010; Cao et al., 2011). The results demonstrated that arabinoxylans can exhibit significant stimulating effect on both innate and acquired immunity following oral administration to BALB/c mice at 200mg/kg compared to intra-peritoneal treatment (Zhou et al., 2010). A study using S180 tumour bearing mice showed the ability of alkaline-extracted arabinoxylans (AXA) to inhibit tumour growth and interleukin 2 production at a wider range of concentrations (100–400mg/kg) with the most effective result seen at 400mg/kg (Cao et al., 2011). In addition, an increase

Table 4 In vitro studies of immunomodulatory rice polysaccharides using various cell types

Reference	In vitro assays Cell types	
Yamagishi et al. (2003)	Anti-complementary activity assay	Red blood cells
Liao et al. (2006)	Cell growth and differentiation, Superoxide production assay, Phagocytosis assay, Agglutination activity, TNF-α and IFN-γ ELISA	Human peripheral blood mononuclear cells and U937 cells
Wang et al. (2008)	Anti-complementary activity assay	Red blood cells
Hoshino et al. (2010)	De-granulation assay, TNF-α and IL-4 ELISA, Binding of IgE to FcεRI, Membrane fusion assay, $[Ca^{2+}]$i assay, Western blot	Bone marrow derived mast cells from BALB/c mice

in peripheral leukocyte count and bone marrow cellularity were also observed after treatment with AX_A which have never been shown before, suggesting there is a possibility that AX_A is involved in the enhancement of stem cell proliferation (Cao et al., 2011).

In comparison, xylanase-derived arabinoxylans (AX_E) stimulated a higher rate of macrophage phagocytosis and delayed hypersensitivity reaction than alkaline derived arabinoxylans (Zhou et al., 2010). HPLC analysis revealed that AX_E have a lower molecular weight, 32.5 kDa (similar to MGN-3) than AX_A, 352 kDa. Moreover, alkaline treatment have reduced the amount of ferulic acid to negligible levels compared to untreated wheat bran, whereas AX_E still contained low levels of ferulic acid (43.5 mg/100g) allowing them to exhibit a different tertiary conformation with more cross-linakges between adjacent arabinoxylan chains (Zhou et al., 2010). Xylanase treatment however resulted in a larger decrease in glucose (dropped by 94%) and A/X ratio (from 0.86 to 0.56) in contrast to alkaline treatment (82% decrease in glucose with A/X ratio of 0.83) (Zhou et al., 2010) suggesting that these variations in structure between AX_A and AX_E together could be the cause for the variations in their immune-enhancing activities. Surprisingly, both arabinoxylans demonstrated similar enhancing effect on lymphocyte proliferation in vivo but none in vitro (Zhou et al., 2010), thus the mechanism underlines that arabinoxylans function may also be related to the method of administration.

STRUCTURAL AND IMMUNE-MODULATIN PROPERTIES OF AX FROM OTHER SOURCES

Other potential sources of arabinoxylans include corn hulls and banana peels. Using sodium hydroxide corn hull arabinoxylans (506 kDa) and banana peel hemicelluloses (288 kDa) were extracted (Zhang et al., 2004). The corn hull arabinoxylan contained D-galactose and α-D-glucuronic acid side chains at O-2 (Saulnier et al., 1995; Saulnier et al., 2009) whereas banana peel hemicellulose contained a β-D-xylan chain with D-xylosyl and L-arabinoxyl branches linked to arabinogalactans (Zhang et al., 2004). The in vitro macrophage activation assay showed that both polysaccharides (25 μg/mL alone) can stimulate DCF-DA oxidation in macrophages compared to control and that a similar effect was observed in LPS treated cells. Arabinoxylans derived from corn husks also demonstrated immunological function in vivo (Ogawa et al., 2005). Hot water extraction and xylanase treatments gave rise to a partially hydrolysed corn husk arabinoxylan (53 kDa) with 46.9% xylose, 35.2% arabinose, 6.7% galactose, 6.3% glucose, and 4.0% glucuronic acid. In mice models, orally administered corn husk AX (50 mg/kg/day) augmented IL-2, IFNγ, and IL-4 secretion by isolated spleen cells in the presence of ConA compared to control. However, the increase in IL-4 secretion (18% increase) was less significant compared to IL-2 (190% increase) and IFNγ (180% increase). This enhancing effect was not associated with changes in the number of NK^+, $CD3^+$, $CD4^+CD8^-$, and $CD4^-CD8^+$ cells in the spleen or blood. Furthermore, corn husk AX suppressed tumour growth in mice during early stages (Day 7) post tumour transplantation which is accompanied by increased NK cell activity up to 2 fold and TNF-α secretion by spleen cells in the presence of ConA and LPS (Ogawa et al., 2005).

Samuelsen et al. (2011) recently investigated the in vitro immunomodulatory activity of arabinoxylans and mixed linked β-glucans from barley which showed contradictory results to other studies. Four samples (WSM-TPX, WUM-BS-LA, WSM-TP, and WUM-BS) were extracted from barley and tested against intestinal epithelial cells lines (Caco-2 and HT-29) for their ability to enhance cell proliferation and IL-8 secretion. Results indicated that all four showed negligible effect on IL-8 secretion and proliferation of Caco-2 and HT-29 compared to controls. They also had insignificant effect on inducing anti-complement and NF-κB activity in U937–3κB-LUC cells (a monocytic cell line) suggesting that these polysaccharides cannot stimulate inflammatory and anti-complementary responses. These four samples exhibit different monosaccharide composition and molecular weight. Sample WSM-TP (a mixed linked β-glucan with 10% arabinoxylan) has a large molecular weight, 1090 kDa, with 91% glucose, 5.8% xylose and 3.3% arabinose. WSM-TPX (a mixed linked β-glucan) also has a large molecular weight, 886 kDa, with 96% glucose, 1.9% xylose, and 1.6% arabinose. In comparison, WUM-BS and WUM-BS-LA are mainly composed of arabinoxylans with medium and small molecular weight, 412 kDa and 156 kDa, respectively. WUM-BS-LA contains 66% xylose and 30.4% arabinose with glucose (2%) and mannose (1.6%) side chains whereas WUM-BS (52.7% xylose and 17.3% arabinose) is cross-linked with 30% β-glucan. Table 5 summarises the cell/animal models used to test the

Table 5 Cell/animal models used to test the immune-modulating activity of AX from various sources

Reference	Source of AX	Assays Cell line/animal models	
Zhang et al. (2004)	Corn hull, Banana peel	Macrophage activation assay	Murine macrophage RAW264.7 cell line
Ogawa et al. (2005)	Corn husk	Tumour growth, IL-2, IFN-γ and TNF-α ELISA,	Female BALB/c mice
Zhou et al. (2010)	Wheat bran	Splenocyte proliferation assay Splenocyte proliferation assay, DTH b1y foot-pad measurement, phagocytosis assay,	Mice splenic lymphocytes Female BALB/c mice
Cao et al. (2011)	Wheat bran	Anti-tumour assay Tumour growth, DTH reaction, phagocytosis assay, NK cell activity assay, lymphocyte proliferation assay, IL-2 ELISA	K562 and HL-60 cells ICR male mice
Samuelsen et al., (2011)	Barley	Cell proliferation, IL-8 ELISA	Caco-2 and HT-29

immunomodulatory activity of AX from wheat, corn, barley, and banana peel.

MECHANISMS UNDERLYING THE EFFECT OF DIETARY POLYSACCHARIDES

Although a considerable body of research has been published detailing the immune-stimulating effect of dietary arabinoxylans, little has been done to understand the molecular mechanisms underlining these effects. Other BMRs, for example, dietary β-glucans, have been extensively studied for their receptors and effector immune cells which may help to shed some light on the subject of matter. Biologically active β-glucans have been identified to be composed of a (1→3)-linked β-D-glucopyranosyl backbone with (1→6) linked glucan side chains of varying length and distribution attached (Volman et al., 2008). Like arabinoxylans, β-glucans from various sources exhibit different molecular characteristics. Bacterial β-glucans are mainly consisted of linear β-(1→3) linked chains whereas cereal β-glucans consist of linear β-(1→3) mixed with (1→4) linked glucan chains (Figure 3). Nonetheless, yeast, fungi, cereal and bacteria-derived β-glucans have all been shown to possess immune-modulating properties both in vitro and in vivo (Bohn and BeMiller, 1995; Brown and Gordon, 2003; Rieder et al., 2011).

Several structural models of β-glucan have been proposed and it has been suggested that these structures are responsible for the biological activity. β-glucans derived from lentinan and schizophyllan both contain a single β-D-glucopyranosul branch at O-6 of the backbone with the DB value of 0.33 (Sasaki and Takasuka, 1976; Tabata et al., 1981) whereas β-glucans derived from PGG and CI-6P exhibited higher and lower DB values (0.60 and 0.04–0.08, respectively) (Jamas et al., 1991; Blaschek et al., 1992; Kiho et al., 1992; Bohn and BeMiller, 1995). The degree of branching is considered a possible determinant for their functionality as β-glcuans with DB values between 0.2 and −0.33 appearing to be the most biologically active (Misaki et al., 1981; Bohn and BeMiller 1995). Other studies suggested that the molecular weight may also be important depending on the tertiary structure and branching pattern (Izydorczyk and Dexter, 2008). β-glucans with high molecular weights (100,000–500,000) showed more activity compared to lower molecular weight molecules (5,000–10,000) (Fabre et al., 1984; Blaschek et al., 1992). This may be due to the ability of larger molecules to form highly ordered triple helices which then confer the immune-modulating effects (Hamuro et al., 1971; Norisuye 1985; Ohno et al., 1987; Maeda et al., 1988). However, low molecular weight (<20,000) β-glucans were also shown to exhibit significant anti-tumor activity when their DB value is less than 0.25 (Bohn and BeMiller, 1995). Thus, the structure–activity relationship of β-glucans may rely on a combination of factors including molecular weight, DB and tertiary confirmation. This could apply to arabinoxylans due to the structural similarities between β-glucans and arabinoxylans (Figure 2).

POSSIBLE ARABINOXYLAN RECEPTORS

Since arabinoxylans have been shown to modulate innate immune responses, it is reasonable to speculate that they may function by acting like pathogen associated molecular pattern (PAMPs). Interestingly, arabinoxylans derived from rice bran and corn husk do seem to show some resemblances when compared to LPS derived from Gram-negative bacteria in terms of structure and molecular weight (30–100 kDa). (Otterlei et al., 1993; Ghoneum and Ogura, 1999; Ogawa et al., 2005; Alberts, 2008). For example, they all contain sugar molecules such as glucose and galactose as well as C-3 branched sugar polysaccharides. Thus, TLRs expressed on the surface of professional phagocytes may act as receptors for arabinoxylans to signal the activation of various immune cells. Upon recognition through TLRs, a series of reactions often take place including cytokine secretion, pathogen phagocytosis and oxidative burst which have been demonstrated in macrophages, dendritic cells, NK cells, lymphocytes and mast cells after treatment with arabinoxylans from various sources (Tables 2–5). Although arabinoxylan receptors have not yet been identified, TLR4 appears to be a possible candidate as it specifically

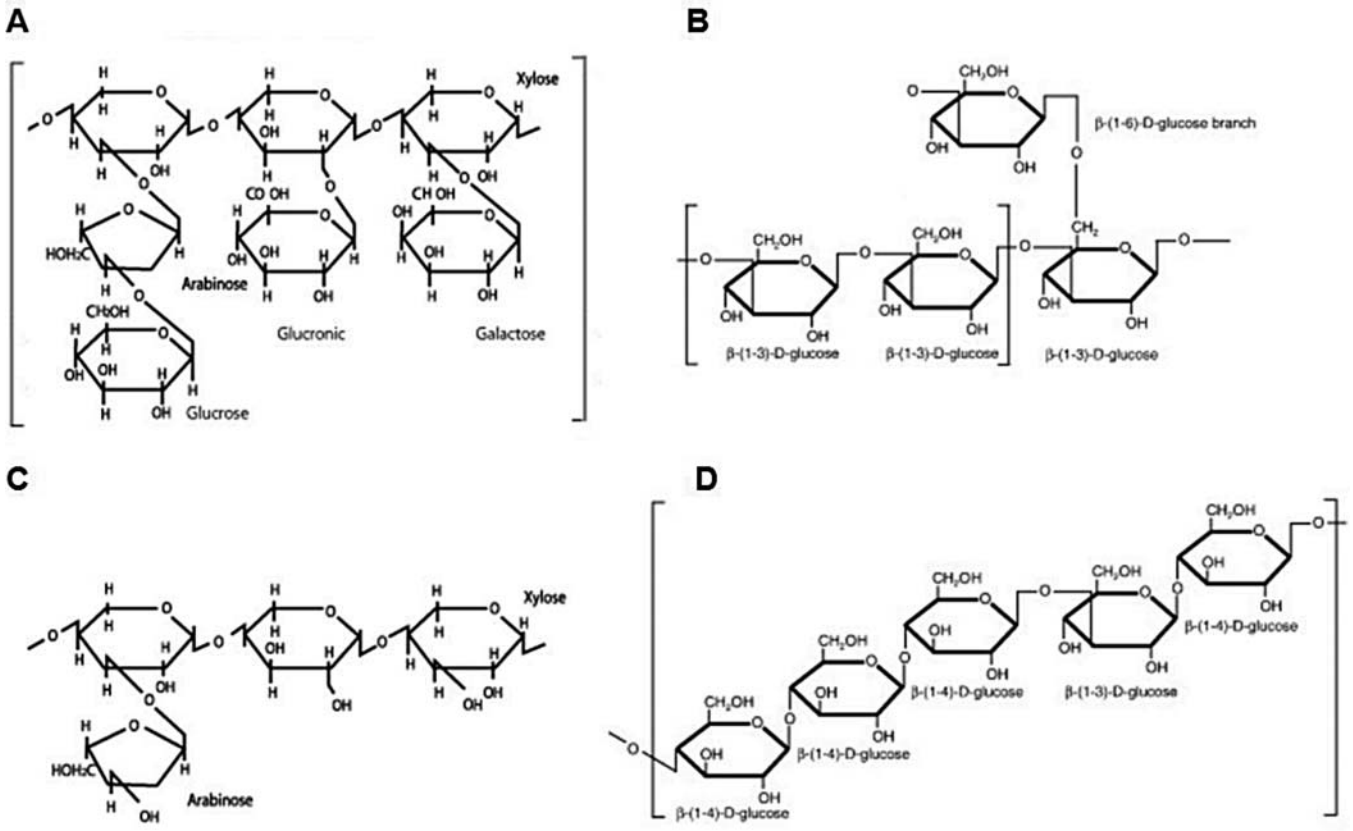

Figure 2 Structure of arabinoxylan and β-glucan of various sources. (A) Rice bran arabinoxylan before any chemical/enzymatic modification. (B) Yeast β-glucan consisted of a β-(1,3)-D-glycopyranosyl polymer with a single β-(1,6)-D-glycopyranosyl side chain attached, one of most active structure (500 kDa). (C) The main structure of MGN-3 derived from structure A via enzymatic modifications. **D**: Cereal β-glucan consisted of a long chain of β-(1,4)-D-glycopyranosyl units separated by single β-(1,3)-D-glycopyranosyl units.

binds to lipopolysaccharides and is abundantly present in all the phagocytes that are responsive to arabinoxylan treatments (Alberts, 2008). Other TLRs such as TLR2 and TLR6 may also be involved as they have shown to be essential for inducing cytokine production upon β-glucan recognition (Volman et al., 2008).

As a wide range of arabinoxylans have been shown to exert immune-modulating activities (Tables 2–5), it is likely that other receptors apart from TLRs are involved. C-type lectin like receptors can play an important role in the innate immune system by acting like phagocytic pathogen recognition receptors similar to TLRs. These receptors are classified into subtypes according to their functional and structural similarities which allow them to recognise a diversity of carbohydrate structures. For instance, dectin-1 expressed by dendritic cells and macrophages can recognise β-glucans whereas MMRs expressed by immune cells bind specifically to mannose sugars. There is a possibility that arabinoxylans are cross-linked with β-glucans and mannose for structural stability and that these receptors are able to recognise arabinoxylans through their mannose/β-glucan side branches. However, the signalling mechanisms induced by these receptors are unknown but upon ligand-receptor binding macrophages and dendritic cells will become activated to undergo bacterial and fungal phagocytosis.

In addition, some lectin-like receptors can also stimulate T-cell/DC trafficking and cell-cell adhesion between leukocytes and endothelium after binding to endogenous carbohydrate ligands. This helps to explain the ability of MGN-3 to activate cytotoxic T-lymphocytes of the adaptive immune system and also why only orally administered MGN-3 significantly increased NK activity. Humans do not synthesis enzymes that degrade arabinoxylans. Thus, they can only be cleared up through slow oxidation (Brown and Gordon, 2003). Arabinoxylans are often retained in the liver where lectin like receptors expressed by liver sinusoidal endothelial cells will allow them to adhere and then in turn activate resident macrophages (Cambi et al., 2005). Since only orally administered MGN-3 can enter liver endothelial cells, MGN-3 administered via intra-venous injection did not work.

CR3 is a complement receptor expressed by phagocytes, NK cells and small population of B and T cells. Like LLRs, CR3 can function both as an adhesion molecule and a membrane receptor mediating the recognition of various bacterial

Table 6 Biologically active arabinoxylans of various sources and their structural properties

Source	Mw (kDa)	Biological activity	Composition%				
		In vitro In vivo	Ara	Xyl	Gal	Glu	A/X
Rice bran	30–50	Activate DCs, Activate NK, B and Mac and NK; T cells; Anti-tumor Anti-tumor	22–26	48–54	5–7	6	0.50
Rice bran	90	Anti-complement Not tested	40	20	40	10	2.00
Corn husk	53	Not tested Activate NK, IL-2 and IFNγ secretion	35.2	46.9	6.7	6.3	0.95
Wheat bran	352	Not active Inhibit tumor; activate Mac	41.8	50.5	n/a	7.7	0.83
Wheat bran	32.5	Not active Activate Mac	34.8	62.4	n/a	2.8	0.55
Barley	156	Low activity Not tested	30.4	66	n/a	2	0.46
Banana peel	288	Activate Mac Not tested	52.8	25.6	21.6	n/a	2.10

Mw classification: 30–100 kDa (low); 100–200 kDa (medium); 200–400 kDa (large); Ara = arabinose; Xyl = xylose; Gal = galactose; Glu = glucose; A/X = arabinose/xylose ratio; NK = natural killer cells; Mac = macrophages; DCs = dendritic cells.

carbohydrates and lipopolysaccharides. As arabinoxylans have been shown to affect all the cells that express CR3 (Table 6), it is possible for their immune-enhancing activity to be mediated via these complement receptors. Upon binding with CR3, phagocytosis, degranulation and oxidative burst can be induced within different immune cells which were observed when the cells are treated with arabinoxylans, for example, MGN-3 (Figure 3). Studies using rice bran derived polysaccharides to modulate the complement system further demonstrated the possibility of CR3's involvement in arabinoxylan-mediated immune responses (Yamagishi et al., 2003; Wang et al., 2008). It was proposed that the presence of galactose in arabinoxylans is the main structure responsible for their anti-complementary activity. The geometry of the molecules is crucial as the activation of complement requires steric recognition as well as structural specificity. Hence, it would be more difficult to establish the relationship between structure and anti-complement activity.

STRUCTURE-ACTIVITY RELATIONSHIP

Evidence suggests that the activity of these polysaccharides is dependent on their structural properties including molecular weight, DB, and sugar compositions. The most commonly tested arabinoxylans (MGN-3) are small in size with an A/X ratio of 0.5 which is very similar to wheat bran arabinoxylans extracted by enzyme. Both arabinoxylans were able to activate macrophages but MGN-3 appeared to be more active which may be caused by the difference in their sugar composition as MGN-3 contained more galactose and glucose side chains (Table 6). Corn husk arabinoxylans activated NK cell activity and cytokine secretion in vivo. These arabinoxylans also have a similar molecular weight as MGN-3 but are more branched (A/X ratio of 0.95). Thus, the molecular weight of such immune-stimulating polysaccharides may be the more dominant factor compared to the DB when activating immune responses upon binding with the receptors. Barley arabinoxylans with medium molecular weight (156 kDa) showed very

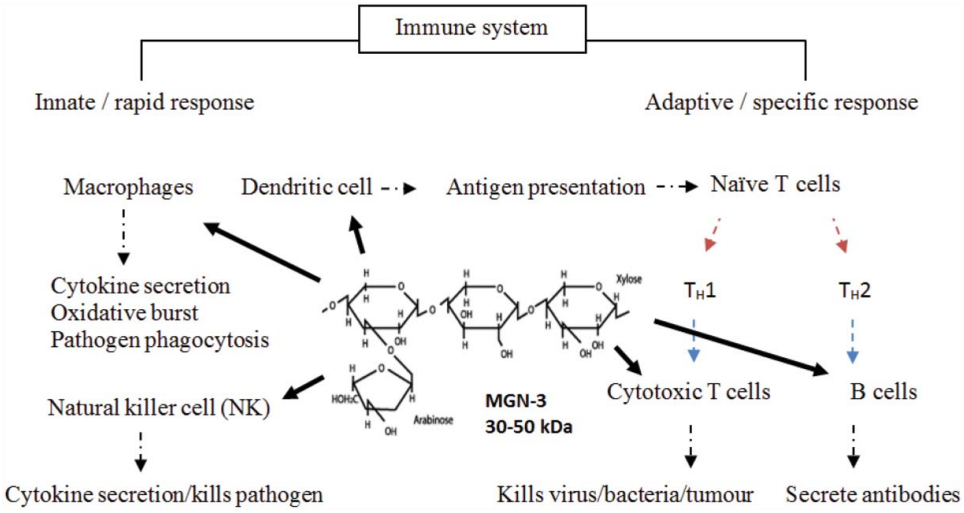

Figure 3 Simplified schematic overview of the main structural features of MGN-3/arabinoxylans and their possible involvement in enhancing immune responses. Key: T_H cells–Helper T lymphocytes, Stimulation, Enhancing cell function, Immune responses, Differentiation.

low activity both in vivo and in vitro with an A/X ratio of 0.49 (similar to MGN-3) which again suggest that the DB may not have any significant effect on triggering their activity. Interestingly, arabinoxylans from banana peel and wheat bran (alkaline extraction) with large molecular weight both showed strong immunomodulatory activity which could be explained by the wide range of possible receptors expressed by different immune cells. The fact that larger arabinoxylans may take weeks to be degraded in mammalian systems means that they can maintain their biological activity for a long period of time which may be an advantage.

If arabinoxylans do function in a similar way as β-glucans, they are most likely to come in contact with the mucosal immune system first after oral administration. Specialized epithelial cells called Microfold (M) cells located in the Peyer's patches in the small intestine are then able to transport arabinoxylans to intestinal intraepithelial lymphocytes and phagocytes. These immune cells can recognise arabinoxylans via receptors proposed previously leading to increased cytokine production and oxidative burst. Furthermore, these phagocytes such as intestinal macrophages can transport arabinoxylans to immune organs throughout the body via the lymphatic system. Within these immune organs including the bone marrow, spleen and lymph nodes macrophages can then take them up via specific receptor recognition and present them to other immune cells from both the innate and adaptive immune systems to stimulate various immune activities.

CONCLUSION

Arabinoxylans can be considered a valuable bioactive food supplement with many health promoting applications due to their immunomodulatory function. Several structural models of arabinoxylans have been studied using a wide range of in vitro and in vivo tests which have sufficiently demonstrated their immune-modulating potential. However, the relationship between their activity and structure is still unclear as these polysaccharides exhibit great structural heterogeneity and were not tested using the same assays. Based on the literature, the only conclusion that can be drawn at this stage is that the biological activity of these immunomodulatory polysaccharides is related to their molecular weight and possibly DB. In addition, the availability of various well-researched extraction methods means that it is now possible to modify a polysaccharide into specific structures so comparisons can be made. Therefore, future research should focus on understanding the structural–activity relationship of these bioactive polysaccharides by deriving specific structures and comparing their activity within the same test system. This structural–activity relationship is also the key to uncover the mechanism by which arabinoxylans and cereal polysaccharides enhance the immune system.

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