Differential structure and activity between human and mouse intelectin-1: Human intelectin-1 is a disulfide-linked trimer, whereas mouse homologue is a monomer

Shoutaro Tsuji1,2, Makiko Yamashita2, Akihito Nishiyama2, Tsutomu Shinohara2, Zhongwei Li2, Quentin N. Myrvik3, Donald R. Hoffman4, Ruth Ann Henriksen5, and Yoshimi Shibata6

1Biomedical Sciences, Florida Atlantic University, Boca Raton, FL 33431, USA; 2404 Palmeto Dr, Casswell Beach, NC 28465, USA; 3Pathology and Laboratory Medicine and 4Physiology, Brody School of Medicine at East Carolina University, Greenville, NC 27834, USA

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Human intelectin-1 (hITLN-1) is a 120-kDa lectin recognizing galactofuranosyl residues found in cell walls of various microorganisms but not in mammalian tissues. Although mouse intelectin-1 (mITLN-1) has been identified previously, its biochemical properties and functional characteristics have not been studied. Therefore, we have compared structures and saccharide-binding specificities of hITLN-1 and mITLN-1 using recombinant proteins produced by mammalian cells. Recombinant hITLN-1 is a trimer, disulfide-linked through Cys-31 and Cys-48, and N-glycosylated at Asn-163. Despite 84.9% amino acid identity to hITLN-1, recombinant and intestinal mITLN-1 are unglycosylated 30-kDa monomers. Recombinant hITLN-1, as well as recombinant and intestinal mITLN-1 were purified by Ca2+-dependent adsorption to galactose-Sepharose. In competitive binding studies, hITLN-1 was eluted from galactose-Sepharose by 100 mM 2-deoxygalactose, a galactose-binding specificity. In contrast, mITLN-1 was partially eluted by the galactofuranosyl disaccharide, and only minimally by the other saccharides indicating that the two intelectins have different saccharide-binding specificities. When the N- and C-terminal regions of hITLN-1 were replaced, respectively, with those of mITLN-1, galactose-Sepharose binding was associated with the C-terminal regions. Finally, hITLN-1 binding to galactose-Sepharose was not affected by the substitution of the Cys residues in the N-terminal region that are necessary for oligomer formation, nor was it affected by the removal of the N-linked oligosaccharide at Asn-163. Although both hITLN-1 and mITLN-1 recognize galactofuranosyl residues, our comparative studies, taken together, demonstrate that these intelectins have different quaternary structures and saccharide-binding specificities.

Keywords: galactofuranose/galactose/innate immunity/intelectin/lectin

Introduction

Human intelectin-1 (hITLN-1), a 40- or 120-kDa protein under reducing or nonreducing conditions, respectively, is a Ca2+-dependent galactofuranose-binding lectin, but is not a member of the C-type lectin family (Tsuji et al. 2001). We previously found that hITLN-1 preferentially binds galactose-Sepharose and arabinogalactofuranan of Nocardia rubra (Tsuji et al. 2001). Galactofuranosyl residues, which are not found on mammalian tissues, are recognized as dominant immunogens (Notermans et al. 1988; Daffe et al. 1993; Leitao et al. 2003). Thus, hITLN-1 is expected to recognize not only N. rubra (Daffe et al. 1993), but also Aspergillus fumigatus (Leitao et al. 2003), Mycobacterium tuberculosis (Pedersen and Turco 2003), Streptococcus oralis (Abeygunawardana et al. 1991), Leishmania major, and Trypanosoma cruzi (Suzuki et al. 1997), all of which contain galactofuranosyl residues in the cell walls. Some observations indicate that hITLN-1 and mouse intelectin-1 (mITLN-1) may play an immunological role against selected microorganisms or foreign antigens (Pemberton, Knight, Gamble, et al. 2004; Pemberton, Knight, Wright, et al. 2004; Datta et al. 2005; Kuperman et al. 2005; Wali et al. 2005).

The function of the host defense lectin, collectins, is dependent on oligomeric structures (Super et al. 1992; Kurata et al. 1993; Holmskov et al. 1995). Since intelectin (ITLN) homologues are generally oligomeric (Roberson and Barondes, 1982; Chamow and Hedrick, 1986; Nishihara et al. 1986; Abe et al. 1999; Suzuki et al. 2001; Tsuji et al. 2001; Nagata 2005), this structure may influence their function. Comparison of amino acid sequences shows that most mammalian ITLNs have 10 conserved Cys residues but two of these are not found in the N-terminal regions of mouse and rat ITLNs (supplementary data). We hypothesized that the oligomeric structure and carbohydrate-recognition specificity of mITLN-1 differ from those of hITLN-1 because mITLN-1 lacks the conserved Cys residues, Cys-31 and Cys-48 in hITLN-1.

To test this hypothesis, recombinant mITLN-1 and mITLN-1 isolated from the small intestine of C57BL/6 mice were purified by adsorption to galactose-Sepharose. The properties of recombinant mITLN-1 were compared to those of recombinant hITLN-1. To determine the possible structural role of the conserved Cys residues present in hITLN-1, but not in mITLN-1, mutant forms of hITLN-1 were prepared in which the two Cys residues were replaced with Ser. In addition, the N- and C-terminal regions of hITLN-1 were replaced with the corresponding regions of mITLN-1. Structural features and galactose-Sepharose-binding properties of the mutant and chimeric ITLNs were examined. Despite the 84.9% amino acid identity between these two proteins, we have found significant structural and functional differences.
Results

Comparison of hITLN-1 and mITLN-1 structures
Conserved Cys residues often contribute to the three-dimensional structure of proteins including oligomeric structures. Thus, the absence or mutation of these residues can have significant effects on the structure and function of proteins. As reported previously, for native hITLN-1 (Tsuji et al. 2001), recombinant hITLN-1 was detected as a 120-kDa protein on Western blotting under nonreducing conditions. However, recombinant mITLN-1 was a 30-kDa protein under nonreducing conditions (Figure 1). Under reducing conditions, recombinant hITLN-1 and mITLN-1 appeared as 40- and 34-kDa bands, respectively. Since the predicted molecular weights of mature hITLN-1 and mITLN-1 are about 33 k these results suggested that mITLN-1 is a monomer, in contrast to hITLN-1, a disulfide-linked homooligomer. To confirm that recombinant mITLN-1 is similar in structure to native mITLN-1, intestinal mITLN-1 was isolated from C57BL/6 mice. Intestinal mITLN-1 showed 30- and 34-kDa bands under nonreducing and reducing conditions, respectively, identical to those of recombinant mITLN-1 (Figure 1).

Location of intermolecular disulfide bonds in hITLN-1
To analyze which Cys residues form intermolecular disulfide bonds in hITLN-1, we prepared recombinant chimeric molecules from hITLN-1 and mITLN-1. Figure 2 shows the regions of hITLN-1 and mITLN-1 that were exchanged to form chimeras. Figure 3A shows that the molecular mass for a chimeric molecule consisting of the N-terminus of hITLN-1 and the C-terminus of mITLN-1 (Hu/Mo chimeric ITLN-1) was similar to that of hITLN-1 under nonreducing and reducing conditions (Figure 1). Under reducing conditions, recombinant hITLN-1 and mITLN-1 appeared as 40- and 34-kDa bands, respectively. Since the predicted molecular weights of mature hITLN-1 and mITLN-1 are about 33 k these results suggested that mITLN-1 is a monomer, in contrast to hITLN-1, a disulfide-linked homooligomer. To confirm that recombinant mITLN-1 is similar in structure to native mITLN-1, intestinal mITLN-1 was isolated from C57BL/6 mice. Intestinal mITLN-1 showed 30- and 34-kDa bands under nonreducing and reducing conditions, respectively, identical to those of recombinant mITLN-1 (Figure 1).

hITLN-1, but not mITLN-1, contains Cys-31 and Cys-48, the Cys residues conserved in mammalian ITLNs (supplementary data and Figure 2). To determine whether these Cys residues form the intermolecular disulfide bonds linking hITLN-1 monomers, each Cys residue was replaced with Ser. As shown in Figure 3A, recombinant hITLN-1 with Ser substituted for Cys-48 (48C>S hITLN-1) showed a distinct 60-kDa band and a faint 32-kDa band under nonreducing conditions. Furthermore, recombinant hITLN-1 with Ser substituted for both Cys-31 and Cys-48 (31,48C>S hITLN-1) showed only a 32-kDa band.
band under nonreducing conditions. Under reducing condition, both mutants were detected as a 40-kDa band, the same as intact hITLN-1. Recombinant hITLN-1 with Ser substituted for Cys-31 (31C>S hITLN-1) showed a distinct 32-kDa band and a faint 60-kDa band under nonreducing conditions (Figure 3B). Thus, the mutation of either Cys-31 or Cys-48 prevented the formation of 120-kDa oligomeric hITLN-1, although the mutants bound to galactose-Sepharose. These results suggest that intermolecular disulfide bonds in hITLN-1 are formed by Cys-31 and Cys-48, with 31,48C>S hITLN-1 yielding a monomeric form, and 48C>S hITLN-1 mainly a dimeric form.

To determine the molecular weights of human and mouse ITLN-1, recombinant hITLN-1 and mITLN-1 were analyzed by matrix-assisted laser dissociation ionization time of flight (MALDI-TOF). Purified, unreduced, hITLN-1 yielded a major ion at an average molecular weight of 105.5. No ion was seen with molecular weight >120,000. Reduced hITLN-1 appeared at 35,500. Since the calculated molecular weight of the mature hITLN-1 monomer is 32,897, native hITLN-1 would correspond to a glycosylated trimer. For mITLN-1, with a calculated molecular weight of 32,982, an ion was detected at approximately 34,000 under nonreducing conditions. There was no ion seen with molecular weight >50,000. These results suggest that mITLN-1 is a monomer. Analysis of the molecular weights of both ITLNs was difficult because purified ITLNs were unstable and tended to become insoluble under many conditions.

**Location of glycosylation site in hITLN-1**

Because the reduced mITLN-1 showed a 34-kDa band in agreement with the predicted molecular weight, it may not be glycosylated despite the presence of a glycosylation site at Asn-154. This was confirmed by the treatment of mITLN-1 with peptide N-glycanase F (PNGase F) which did not alter the electrophoretic migration of proteins (Figure 4). hITLN-1 contains potential N-glycosylation sites at Asn-154 and Asn-163. To determine whether Asn-163 in hITLN-1 is an N-glycosylation site, we prepared recombinant hITLN-1, substituting Lys, the corresponding residue from mITLN-1, for Asn-163 (163N>K hITLN-1). As shown in Figure 4, 163N>K hITLN-1 migrated at 110-kDa under nonreducing conditions. After reduction, only a 34-kDa band was seen, which corresponded to the band of reduced hITLN-1 treated with PNGase F (Figure 4). In our preliminary study, however, Asn-154 mutant protein (154N>K) was not recovered from the cell lysate or culture supernatant (data not shown). These results indicate that hITLN-1, but not mITLN-1, has an N-linked oligosaccharide present at Asn-163 and further suggest that Asn-154 is not glycosylated in either hITLN-1 or mITLN-1.

**Differential saccharide-binding specificities between hITLN-1 and mITLN-1**

To determine the binding specificities of hITLN-1 and mITLN-1 for galactoses, hITLN-1 or mITLN-1 was competitively eluted from galactose-Sepharose with either 100 mM galactose or 2-deoxygalactose in 10 mM Ca^{2+}. As shown in Figure 5A, hITLN-1 was partially eluted by galactose and completely eluted by 2-deoxygalactose, whereas mITLN-1 was poorly eluted by either saccharide. Neither hITLN-1 nor mITLN-1 was eluted by...
6-deoxygalactose. hITLN-1 and mITLN-1 were, respectively, completely and partially (33%) eluted by 2-acetamido-2-deoxy-4-O-beta-D-galactofuranosyl-D-glucopyranose (GalpG). However, neither hITLN-1 nor mITLN-1 was eluted by lactose, melibiose, 2-acetamido-2-deoxy-4-O-beta-D-galactopyranosyl-D-glucopyranose (GalpG), the galactopyranoside isomer of GalpG, or N-acetylgalactosamine (Figure 5A). Additionally, hITLN-1 was eluted by D-ribose, L-ribose, and D-xylose. In contrast, mITLN-1 was poorly eluted by these pentoses (Figure 5B). These results suggest that although both hITLN-1 and mITLN-1 preferentially bind a galactofuranosyl-, compared to a galactopyranosyl-containing saccharide, they have different saccharide-binding specificities from each other.

Purified hITLN-1 aggregates in the presence of Ca\(^{2+}\) with loss of saccharide binding (Tsuij et al. 2001), which has limited the determination of binding affinities for saccharides. To further characterize the relative binding specificities of hITLN-1 and mITLN-1 to immobilized galactose, the galactose concentration dependence for competitive inhibition of binding was investigated (Figure 6A). The concentrations of soluble galactose inhibiting 50% binding of hITLN-1 and mITLN-1 were 21 and 33 mM, respectively. To investigate whether the oligomeric structure of hITLN-1 affects the binding to galactose-Sepharose, the binding of site-specific mutants prepared above was examined by competitive elution with 100 mM galactose or 2-deoxygalactose. Like trimeric intact hITLN-1, both dimeric 48C>S hITLN-1 and monomeric 31,48C>S hITLN-1 were completely eluted from galactose-Sepharose by 2-deoxygalactose and partially by galactose (Figure 6B). These results suggest that the oligomeric structure of hITLN-1 does not contribute to the binding specificity for these saccharides. Relatively less monomeric mITLN-1 was eluted by these monosaccharides (Figure 6B) with a higher affinity of mITLN-1 for galactose-Sepharose.

To determine which regions of hITLN-1 and mITLN-1 are associated with the differential binding to saccharides, chimeras formed between amino and carboxyl-terminal domains of hITLN-1 and mITLN-1 were prepared, and the competitive elution from galactose-Sepharose was measured for selected saccharides, D-ribose, D-xylose, 2-deoxygalactose, GalpG, and galactose. As shown in Figure 7, the Mo/Hu chimeric ITLN-1 had an elution pattern similar to that of hITLN-1, while the elution pattern for Hu/Mo chimeric ITLN-1 was similar to that for mITLN-1. These results suggest that the C-terminal regions (residues 168–313) of hITLN-1 and mITLN-1 predominate in determining binding specificity for saccharides.

**Discussion**

In mammals, homologous proteins frequently have similar oligomeric structures. However, our study indicates significant differences in the structures and activities of the mammalian lectins hITLN-1 and mITLN-1. These are as follows: (i) hITLN-1 is a 106-kDa disulfide-linked, and N-glycosylated homotrimer, while mITLN-1 is a 34-kDa non-glycosylated monomer, both by MALDI-TOF analysis; (ii) the disulfide bonds linking hITLN-1 monomers appear to be formed by Cys-31 and Cys-48 residues which are not conserved in mITLN-1; (iii) hITLN-1, but not mITLN-1, has one N-linked oligosaccharide at Asn-163; (iv) although both hITLN-1 and mITLN-1 are eluted from galactose-Sepharose by galactofuranosyl residues, detailed saccharide-binding specificities differ between hITLN-1 and mITLN-1; (v) the distinctive binding is attributed to the amino acid sequences of the C-terminal regions, but not to the oligomeric structure of hITLN-1.
Although the N-terminal regions of ITLN homologues have less amino acid sequence identity, most have two or more non-conserved Cys in this region without a predicted signal sequence (supplementary data; Roberson and Barondes 1982; Chamow and Hedrick 1986; Nishihara et al. 1986; Lee et al. 1997, 2001, 2004; Komiya et al. 1998; Abe et al. 1999; Suzuki et al. 2001; Tsuji et al. 2001; Nagata et al. 2003, 2005; Chang et al. 2004; Pemberton, Knight, Gamble, et al. 2004; Schaffler et al. 2005). These non-conserved Cys may form the basis of oligomeric structures. Some ITLN homologues, hITLN-1 (Suzuki et al. 2001; Tsuji et al. 2001), cortical granule lectin (Roberson and Barondes 1982; Chamow and Hedrick 1986; Nishihara et al. 1986), Xenopus embryonic epidermal lectin (XEEL) (Nagata 2005), and ascidian galactose-specific lectin (Abe et al. 1999), are disulfide-linked oligomers, although the location of the intermolecular disulfide bonds has not been reported. Rat, mouse, grass carp, and zebrafish ITLNs and Xenopus laevis serum lectin type 2 have no Cys in the N-terminal regions, which follow predicted signal sequences (supplementary data). Thus, in rodents and fish, ITLN homologues are predicted to be monomeric.

The chimeric molecules and site-specific mutants used in this study contain the conserved Cys residues present in all ITLN homologues (Figure 2 and supplementary data) and retain the capacity to bind galactose-Sepharose. Thus, these molecules have the intramolecular disulfide bonds associated with the monomeric structure of ITLN. Replacement of Cys-48 in hITLN-1 with Ser produced mainly a 60-kDa homodimer (Figure 3B), suggesting that hITLN-1 monomer could form a stable Cys-31–Cys-31 disulfide-linked homodimer. A small amount of homodimer was formed when Cys-31 was replaced by Ser. However, wild-type hITLN-1 was detected as a trimer by MALDI-TOF consistent with Cys-31–Cys-48 intersubunit disulfide bond formation.

The recombinant oligosaccharide-deficient hITLN-1 secreted into culture media also bound galactose-Sepharose (Figure 4). Furthermore, Mo/Hu chimeric ITLN-1, which lacks the N-linked glycosylation site at Asn-163, had the same molecular mass as mITLN-1 (Figure 3A), and a saccharide binding specificity similar to hITLN-1 (Figure 7). Thus, the N-linked oligosaccharide of hITLN-1 is not required for protein secretion or galactose-Sepharose binding. In contrast, XEEL, an ITLN homologue in X. laevis, apparently requires glycosylation for secretion (Nagata 2005). This may be a consequence of the substituted amino acid: for XEEL, the glycosylated Asn was replaced by Lys as is present in mITLN-1. Oligosaccharides of glycoproteins may also serve as ligands for binding to other lectin molecules. An oligosaccharide of cortical granule lectin, an ITLN homologue forming the fertilization envelope in X. laevis (Gerton and Hedrick 1986; Quill and Hedrick 1996), is necessary for ligation to galectin-VIla (Shoji et al. 2005), and the blood-group-B-active trisaccharides are related to cell–cell adhesion (Nomura et al. 1998). However, a specific role for the oligosaccharide in hITLN-1 has not been identified.

Pemberton, Knight, Gamble, et al. (2004) reported that both Balb/c and 129/SvEv strains of mice have mITLN-1 and mITLN-2, an mITLN-1 paralog. In contrast, both C57BL/6 and C57BL/10 mice have only the gene for mITLN-1. Despite significant intestinal mITLN-1 mRNA expression in untreated and Trichinella spiralis-infected C57BL/10 mice, mITLN-1 protein levels were undetectable in samples isolated from these mice (Pemberton, Knight, Gamble, et al. 2004). However, the present study has clearly demonstrated that mITLN-1, detected by our anti-ITLN-1 antibodies, can be isolated from the small intestines of normal C57BL/6 mice by galactose-Sepharose affinity adsorption.

The hITLN-1 mRNA level increases in airway epithelial cells from individuals with asthma (Kuperman et al. 2005) or in primary mesothelial cells following SV40 infection and asbestos exposure (Wali et al. 2005). Others have shown that infection with T. spiralis (Pemberton, Knight, Gamble, et al. 2004; Pemberton, Knight, Wright, et al. 2004) or Trichinurus muris (Datta et al. 2005) induced mITLN-1 and mITLN-2 mRNA expression. These observations and the galactofuranose binding by hITLN-1 and mITLN-1 suggest that ITLN homologues may have an immunoregulatory role in host defense. For instance, the serum ITLN homologue of ascidian (galactose-specific lectin) functions as an opsonin (Abe et al. 1999). However, a recent report indicates no significant modification of immune responses or clearance of microorganisms in mITLN transgenic mice (Voehringer et al. 2007). Although hITLN-1 is a soluble intestinal protein (Tsuji et al. 2001), hITLN-1 has also been reported as a glycosyl/phosphatidylinositol-anchored intestinal lactoferrin receptor (Suzuki et al. 2001), HL-1 of vascular endothelial cells (Lee et al. 2001), or omentin of adipose tissue (Schaffler et al. 2005) and human visceral fat (unpublished data). However, we have not detected mITLN-1 mRNA in cardiovascular tissues or visceral fat of C57BL/6 mice (unpublished data). Because of these differences in expression and the described differences in structure and ligand specificity, the physiological function of mITLN-1 may differ from that of hITLN-1. Recently, it was reported that swine ITLN, which is expected to be oligomeric (supplementary data), is associated with lipid rafts on the enterocyte brush border (Wrackmeyer et al. 2006). It is possible that hITLN-1 is similarly located. Additional investigation will be required to further establish the physiological function of ITLN-1 in both humans and mice.

Materials and methods

Purification of intestinal mITLN-1

Small intestines of mice were isolated from female 8-week-old C57BL/6 mice (Harlan Laboratory, Indianapolis, IN). The following purification procedures were carried out at 4°C. The inside of a small intestine (approximately 0.8 g) was washed with 5 mL saline, minced with scissors, and homogenized with a Teflon homogenizer in 10 mL 20 mM Tris-buffered saline (TBS; pH 7.5) containing 1% Nonidet P-40, 10 mM CaCl₂, and protease inhibitor cocktail for use with mammalian cell and tissue extracts (Sigma, St. Louis, MO). The homogenate was incubated for 1 h and centrifuged (1800 × g, 10 min). The supernatants (10 mL) were mixed with 10 µL of galactose-Sepharose, prepared by incubating epoxy-activated Sepharose 6B (Amersham Pharmacia Biotech, Piscataway, NJ) with galactose for 18 h. After washing four times with TBS containing 1% Nonidet P-40 and 10 mM CaCl₂, mITLN-1 was eluted with 40 µL TBS containing 10 mM ethylenediamine tetra-acetic acid (EDTA).

cDNA cloning of hITLN-1 and mITLN-1

cDNA encoding the open reading frame of mITLN-1 was cloned by reverse transcription-polymerase chain reaction (PCR).
Primers were designed on the basis of a reported mITLN-1 sequence (GenBank accession no. AB016496) (5'-ATG ACC CAA CTG GGA TTC CTG-3' and 5'-TCA GCG ATA AAA CAG AAC CAC G-3') (Komiya et al. 1998). cDNA was synthesized from small intestinal total RNA of C57BL/6 mice using an oligo dT primer and SuperScript II Reverse Transcriptase (Invitrogen, Carlsbad, CA). A single band was amplified by PCR using these primers, cDNA, and Ampli-Taq Gold (Applied Biosystems, Foster City, CA) (25 cycles, 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s). The PCR products were cloned and sequenced. The mITLN-1 sequence of C57BL/6 mice contained several silent mutations compared to the reported sequence, but was the same as the sequence reported by Pemberton, Knight, Gamble, et al. (2004). cDNA of hITLN-1 was prepared as described previously (Tsuji et al. 2001). Point mutations of hITLN-1 were constructed by using GeneTailor Site-Directed Mutagenesis System (Invitrogen). cDNAs for hITLN-1/mITLN-1 chimeras were made by exchange into an open reading frame at a Kpn I site.

Preparation of recombinant ITLN-1

RK-13 cells, a rabbit kidney cell line, were cultured in Eagle’s minimum essential medium containing 5% fetal bovine serum. cDNAs of ITLN-1 were inserted into mammalian cell expression vector pEF-BOS (Mizushima and Nagata 1990), and transfected into RK-13 cells with Lipofectamine 2000 (Invitrogen). The transfected RK-13 cells were incubated for 72 h at 37°C. Recombinant ITLN-1 was purified from the culture supernatants of transfected cells using galactose-Sepharose as described above.

Deglycosylation

To determine whether hITLN-1 and mITLN-1 have N-linked oligosaccharides, the recombinant ITLN-1 preparations were digested by PNGase F (New England Biolabs, Ipswich, MA) according to the manufacturer’s instructions.

Cross-reactive polyclonal antibodies against hITLN-1 and mITLN-1 (anti-ITLN-1)

For preparation of anti-ITLN-1, a New Zealand White (NZW) rabbit was immunized subcutaneously with 2 x 10^6 RK-13 cells transfected with mITLN-1 in complete Freund’s adjuvant. After boosting twice with the same cells in incomplete Freund’s adjuvant, polyclonal antibodies were isolated from serum by precipitation with 33% saturated ammonium sulfate. Anti-ITLN-1 was further purified by affinity chromatography on Affi-gel 10 (Bio-Rad, Hercules, CA) covalently bound to purified recombinant hITLN-1 and elution with 3.5 M NaSCN. The eluate was dialyzed against TBS. The cross-reactivity of anti-ITLN-1 against hITLN-1 and mITLN-1 was confirmed by Western blotting (Figures 1, 3, and 4) and enzyme-linked immunosorbent assay (data not shown).

Western blotting

ITLN-1 purified by galactose-Sepharose was resolved by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) under nonreducing or reducing conditions and transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore, Billerica, MA). The membranes were blocked with 5% nonfat milk, and then treated for 3 h at 22°C with 1 μg/mL anti-ITLN-1 in 5% nonfat milk. After washing, the membranes were treated with horseradish-peroxidase-conjugated donkey anti-rabbit IgG (Jackson ImmunoResearch, West Glove, PA) and developed with ECL plus (Amersham Pharmacia Biotech).

MALDI-TOF

MALDI-TOF mass spectrometry was performed on an Applied Biosystems Voyager DE pro biospectrometry workstaion. Undigested proteins were crystallized with a matrix of 3,5-dimethoxy-4-hydroxy-cinnamic acid (Sigma) in 0.05% trifluoroacetic acid. Reductions were performed with several concentrations of tris(2-carboxyethyl)-phosphine hydrochloride (Pierce Chemical Co., Rockford, IL). Mass spectrometry conditions were optimized for intact IgG.

Protein digests were performed on reduced proteins, alkylated with iodoacetamide, and digested overnight with Promega (Madison, WI) modified trypsin. The matrix used for MALDI-TOF was alpha-cyano-4-hydroxy-cinnamic acid (Sigma) in 0.05% trifluoroacetic acid.

Competitive binding of ITLN-1 to saccharides

Galactose-Sepharose bound to recombinant hITLN-1, mITLN-1, mutated ITLN-1, or chimeric ITLN-1 was prepared as described above. The bound proteins were eluted with TBS plus 10 mM EDTA or TBS plus 10 mM CaCl_2 and 100 mM selected mono/disaccharides. The saccharides included 2-acetamido-2-deoxy-4-O-beta-D-galactofuranosyl-D-glucopyranose (GalpG, Toronto Research Chemicals, North York, ON, Canada) and those listed in Figure 5 (obtained from Aldrich, Milwaukee, WI). The relative amount of ITLN in the eluate was assessed following the separation of the reduced proteins on 10% SDS-PAGE and staining with Coomassie blue. Densities of the stained bands were measured with image analysis software (Yab GelImage; http://homepage.mac.com/yabyab/).

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Conflict of Information

None declared.

Abbreviations

31C>S hITLN-1, hITLN-1 with Ser substituted for Cys-31; 31,48C>S hITLN-1, hITLN-1 with Ser substituted for both Cys-31 and Cys-48; 48C>S hITLN-1, hITLN-1 with Ser substituted for Cys-48; 163N>K hITLN-1, hITLN-1 with Lys substituted for Asn-163; EDTA, ethylenediamine tetraacetic acid; GalfG, 2-acetamido-2-deoxy-4-O-beta-D-galactofuranosyl-D-glucopyranose; GalpG, 2-acetamido-2-deoxy-4-O-beta-D-galactopyranosyl-D-glucopyranose; hITLN-1, human intelectin-1; Hu/Mo chimeric ITLN-1, a chimeric molecule consisting of the N-terminus of hITLN-1 and the C-terminus of mITLN-1; ITLN, intelectin; MALDI-TOF, matrix-assisted laser dissociation ionization time of flight; mITLN-1, mouse intelectin; M2, mouse intelectin-2; pITLN, porcine intelectin; RKO, rabbit kidney cell line; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TBS, tris-buffered saline; TBS plus 10 mM CaCl 2 ; TBS plus 10 mM EDTA.
intelectin-1; Mo/Hu chimeric ITLN-1, a chimeric molecule composed of the N-terminus of mITLN-1 and the C-terminus of hITLN-1; PCR, polymerase chain reaction; PNGase F, peptide N-glycanase F; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TBS, 20 mM Tris-buffered saline, pH 7.5; XEEL, Xenopus embryonic epidermal lectin.

References


Evol. 208:9–18.


Galetic a binds N-glycans of xenopus laevis galectin-VIIa binds N-glycans of members of the cortical granule lectin family (xCGL and xCGL2). Glyco- biology. 15:709–720.


