Human Intelectin Is a Novel Soluble Lectin That Recognizes Galactofuranose in Carbohydrate Chains of Bacterial Cell Wall*

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Galactofuranosyl residues are present in various microorganisms but not in mammals. In this study, we identified a human lectin binding to galactofuranosyl residues and named this protein human intelectin (hIntL). The mature hIntL was a secretory glycoprotein consisting of 295 amino acids and N-linked oligosaccharides, and its basic structural unit was a 120-kDa homotrimer in which 40-kDa polypeptides were bridged by disulfide bonds. The hIntL gene was split into 8 exons on chromosome 1q21.3, and hIntL mRNA was expressed in the heart, small intestine, colon, and thymus. hIntL showed high levels of homology with mouse intelectin, Xenopus laevis cortical granule lectin/oocytes lectin, lamprery serum lectin, and ascidian galactose-specific lectin. These homologues commonly contained no carbohydrate recognition domain, which is a characteristic of C-type lectins, although some of them have been reported as Ca\(^{2+}\)-dependent lectins. Recombinant hIntL revealed affinities to D-pentoses and a D-galactofuranosyl residue in the presence of Ca\(^{2+}\), and recognized the bacterial arabinogalactan of Nocardia containing D-galactofuranosyl residues. These results suggested that hIntL is a new type lectin recognizing galactofuranose, and that hIntL plays a role in the recognition of bacteria-specific components in the host.

In host defense, the recognition of bacterial components is important for induction of immune responses. The cell wall components of pathogens have various biological activities and contain the bacteria-specific carbohydrate chains that do not exist in mammals. The recognition of these carbohydrate chains is useful to induce the cellular responses and fluid-phase immune reactions for elimination of pathogens.

In the innate immune response, the bacterial carbohydrate chains are recognized by the animal lectins that are present on cells as phagocytosis receptors or in plasma as opsonins or agglutinins. As a phagocytosis receptor, the mannose receptor binds materials containing terminal mannose residues such as zymosan and enhances their clearance by phagocytes (1, 2).

The collectins and the ficolins are soluble lectins, and these lectins function as opsonins or agglutinins for bacteria (3–6). In addition, the mannose-binding lectin (MBL), a typical collectin, and ficolin/P32 form complexes with MBL-associated serine proteases in plasma. Binding of these complexes to targets activates the complement system, and complement activation induces opsonization of the targets by phagocytes and the target killing by formation of the membrane attack complex (7–9). This lectin-dependent complement activation pathway is named the lectin pathway and plays important roles in innate immunity (10, 11). These biological defense lectins commonly have affinity to mannose or N-acetylgalactosamine, and binding is sustained by Ca\(^{2+}\) (1–6), although the opposite results have been reported with regard to the Ca\(^{2+}\) dependence of ficolins (5, 6, 12).

On the other hand, animal lectins also include a group of lectins that have affinity to galactose. These galactose-binding lectins generally participate in cell differentiation (13), apoptosis (14, 15), recognition of tumor antigens (16), and the uptake of galactoxygenated glycoproteins such as aged proteins (17, 18). However, there has been no report of galactose-binding lectins of mammals binding to bacterial components, although it has been reported in insects that the Sarcophaga lectin functioned as a host defense lectin (19, 20).

The cell wall skeleton of Mycobacteria or Nocardia acts as an immune response activator and is ingested by phagocytes (21–23). They contain little mannose but possess unique galactans consisting of galactofuranosyl residues, which mammals lack (24). Thus, the specific recognition of these galactans is thought to be useful for their uptake into phagocytes. Although the cell wall skeleton of Mycobacterium contains no mannose, it is ingested by phagocytes (21).

In the present study, we purified a novel human galactose-binding lectin and cloned its cDNA. We demonstrated that this lectin, human intelectin (hIntL), is a new type of Ca\(^{2+}\)-dependent lectin that has affinity to galactofuranosyl residues and recognizes bacterial arabinogalactan.

EXPERIMENTAL PROCEDURES

Galactose-Sepharose—Galactose-Sepharose was produced by incubating epoxy-activated Sepharose 6B (Amersham Pharmacia Biotech) with galactose according to the manufacturer’s instructions.

Purification of Human Intelectin—Human placental tissue was obtained from Kobayashi Maternity Hospital, Osaka, Japan. The sync-
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The centa is indicated by the sequence of hIntL purified from the placental extract (accession no. AB036706). The N-terminal amino acid sequence of hIntL was analyzed under non-reducing (NR) or reducing (R) conditions, and detected by SDS-PAGE as described under “Experimental Procedures.” The purified hIntL was treated with N-glycanase and then placed on a galactose-Sepharose column. Molecular size is expressed in kilodaltons.

**Fig. 1.** SDS-PAGE of the placental hIntL and Western blotting of hIntL treated with N-glycanase. A, placental hIntL was purified as described under “Experimental Procedures,” resolved by SDS-PAGE under non-reducing (NR) or reducing (R) conditions, and detected by silver staining. Ext, placental extract applied to galactose-Sepharose (5 μg); Pur, purified placental hIntL (0.1 μg). Molecular size is expressed in kilodaltons. B, the purified placental hIntL (0.1 μg) or rhIntL (0.2 μg) was treated with N-glycanase, resolved by SDS-PAGE under reducing conditions, and detected by Western blotting using rabbit anti-hIntL as a control for hybridization, the blots were washed twice at 37 °C for 30 min in 0.1 SSC (SSC is 15 mM sodium citrate containing 150 mM NaCl) containing 0.1% SDS, and then stained with Coomassie Blue. The blots were washed twice at 37 °C for 30 min in 3% SDS, and twice at 50 °C for 30 min in 3% SSC containing 0.1% SDS, followed by autoradiography. As a control for hybridization, the blots were rehybridized with a β-actin probe.

Preparations of Recombinant hIntL and Polyclonal Antibodies against hIntL—The rabbit kidney cell line RK-13 were used in the present study. The culture supernatants were collected. Approximately 1 mg of recombinant human intelectin (rhIntL) was purified from 700 ml of culture supernatant by galactose-Sepharose chromatography. SDS-PAGE under non-reducing conditions. The purified hIntL was obtained by electro-elution from the 120-kDa band isolated by SDS-PAGE under non-reducing conditions.

cDNA Cloning—The N-terminal amino acid sequence of hIntL was used to search for an expressed sequence tag database, and the full-length cDNA sequence was deduced. PCR primers were designed on the basis of the predicted sequence (5'GCTGAGGAAGAGATCTAACGGATGACCAGGC-3' and 5'GACGTAATACTGAATTTATTGCTCTTTCCGTTGAGCT-3'). cDNA was synthesized from human placental poly(A) RNA using oligo(dT) primer and SuperScript II RNase H reverse transcriptase (Life Technologies, Inc.). A single band was amplified by PCR using these primers and cDNA (30 cycles of 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 1.5 min), and the PCR products were directly sequenced using several internal sequence primers. The primer regions were determined using a 5' or 3' rapid amplification of cDNA ends kit (Marathon cDNA amplification kit, CLONTECH).

**Fig. 2.** cDNA sequences of hIntL. The nucleotide sequence (upper) and the deduced amino acid sequence (lower) are numbered (GenBank® accession no. AB036706). The N-terminal amino acid sequence of hIntL purified from the placenta is indicated by the solid underline. The broken underline indicates a sequence similar to part of the fibrinogen domain. The amino acids in black boxes are potential N-glycosylation sites. The double underline indicates the position of the polyadenylation signal.

**Table 1.** Northern Blotting—The MTN blot membranes containing 2 μg of poly(A) RNA from various human tissues (CLONTECH) were hybridized at 65 °C for 1 h in ExpressHyb hybridization solution (CLONTECH) with 32P-labeled cDNA of open reading frame sequences of hIntL. The blots were washed twice at 37 °C for 30 min in 0.1 SSC (SSC is 15 mM sodium citrate containing 150 mM NaCl) containing 0.1% SDS, and then stained with Coomassie Blue. The blots were washed twice at 37 °C for 30 min in 3% SDS, and twice at 50 °C for 30 min in 3% SSC containing 0.1% SDS, followed by autoradiography. As a control for hybridization, the blots were rehybridized with a β-actin probe.
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Fig. 3. Comparison of amino acid sequences of hntl and its homologues. Amino acid sequences of hntl, Mus musculus mouse intelectin (mIntl), X. laevis cortical granule lectin (xCGL), L. japonica lamprey serum lectin (lSL), and H. roretzi ascidian galactose-specific lectin (aGSL) are shown. The highly homologous amino acids are shown in shaded characters, and identical amino acids in all proteins are marked in the consensus row.

RK-13 (5 × 10^7 cells) were injected with complete Freund's adjuvant (Difco Laboratories) into a rabbit every week (25). Three days after the fourth immunization, antiserum was collected, and the polyclonal antibodies were purified by precipitation with ammonium sulfate at 33% saturation. The specific anti-hntl antibodies were isolated from the antiserum by precipitation with ammonium sulfate at 33% saturation. The specific anti-hntl antibodies were isolated from the antiserum by precipitation with ammonium sulfate at 33% saturation.

Digestion by N-Glycanase Treatment and Western Blotting—rhntl (0.2 μg) or placental hntl (0.1 μg) was dissolved in 20 μl of 200 mM Tris-HCl (pH 8.0) containing 0.1% SDS, 50 mM 2-mercaptoethanol, and 50 mM EDTA, and was denatured by boiling for 5 min. Each solution was divided into two aliquots to which 5 μl of 7.5% Nonidet P-40 and 2% bovine serum albumin. The plates were incubated for 3 h with 100 μl of the culture supernatant of hntl-transfected RK-13, washed five times with TBS containing 0.05% Tween 20 and 2 mM CaCl2, and then incubated for 1 h with horseradish peroxidase-conjugated goat anti-rabbit IgG, developed with o-phenylenediamine and H2O2, and the absorbance at 492 nm was determined.

RESULTS

Purification of Ca2+-dependent Galactose-binding Protein—To identify Ca2+-dependent galactose-binding protein, we employed an affinity chromatography method using galactose-Sepharose and protein analysis by SDS-PAGE, as described under "Experimental Procedures." Human placental tissue was used as a protein source, since it produces a variety of lectins and host defense proteins. The N-terminal amino acid sequence of this 120-kDa protein (Fig. 2, box underline) was deduced. Based on the deduced amino acid sequences of several proteins that showed affinity to galactose-Sepharose were sequenced and an unknown sequence was obtained from the GenBank database or the literature as follows: M. musculus mouse intelectin, AB016496; X. laevis cortical granule lectin, X82626; L. japonica lamprey serum lectin, AB055981; H. roretzi ascidian galactose-specific lectin, see Ref. 27. The highly homologous amino acids are shown in shaded characters, and identical amino acids in all proteins are marked in the consensus row.

Binding of rhntl to a Bacterial Carbohydrate Chain—The arabinogalactan of Nocardia rubra (a gift from Prof. I. Azuma, Hokkaido University, Sapporo, Japan) was covalently bound to universal binding gel (Bio-Rad) covalently bound to purified rhntl.

Protein Structure of 120-kDa Protein—We searched the expressed sequence tag data base for the N-terminal amino acid sequence of this 120-kDa protein (Fig. 2, solid underline), and the full-length sequence was deduced. Based on the deduced sequence, PCR primers were designed and used for reverse transcription-PCR using human placental mRNA. The PCR...
products were directly sequenced, and the primer regions were sequenced by 5’ or 3’ rapid amplification of cDNA ends. As shown in Fig. 2, the cDNA sequence of the 120-kDa protein (GenBank® accession no. AB036706) contained a ATG translational initiation codon in accordance with Kozak’s rule, a 939-base pair open reading frame encoding 313 amino acids, and a polyadenylation signal, AATAAA, followed by a poly(A) tail in the 3’-untranslated sequence. The transcript of this 120-kDa protein cDNA revealed a high degree of homology with the hypothetical protein mouse intelectin (26). Hence, we named this protein human intelectin (hIntL). The N-terminal amino acid of the purified placental hIntL was Thr-19, and analysis of the cDNA predicted the presence of a N-terminal hydrophobic signal sequence (amino acids 1–18). In addition, hIntL did not contain a significant transmembrane domain. These observations suggested that mature hIntL is a secretory protein.

The predicted molecular size of the mature hIntL was calculated to be about 33 k, but the actual size of the placental hIntL was shown to be about 40 kDa on SDS-PAGE under reducing conditions (Fig. 1A). hIntL contained two potential N-glycosylation sites at Asn-154 and Asn-163 (Fig. 2). Following N-glycanase treatment, the molecular sizes of both placental and recombinant hIntL were decreased to 34 kDa, which nearly matched the predicted molecular size (Fig. 1B). Thus, the difference of molecular size appeared to be largely due to the presence of N-linked oligosaccharides.

As shown in Fig. 3, the amino acid sequence of hIntL was 81.5% identical to mouse intelectin (26) and 59.4% identical to *Xenopus laevis* cortical granule lectin (GenBank accession no. X82626), the sequence of which was the same as that of *X. laevis* oocyte lectin (27) except for 5 amino acids. The highly homologous region (residues 37–313) of these proteins showed partial homology with the C-terminal part of *Lampetra japonica* lamprey serum lectin (GenBank® accession no. AB055981) (residues 53–333, 58.1%) or *Halocynthia roretzi* ascidian galactose-specific lectin (28) (residues 69–348, 43.0%). Thus, hIntL is likely to be a human homologue of these molecules. Placental hIntL showed Ca2+-dependent saccharide binding activity as similarly to cortical granule lectin/oocyte lectin, but hIntL did...
not contain the carbohydrate recognition domain, which is a characteristic of C-type lectins. Moreover, hIntL did not contain any known functional domains, except the region from Pro-38 to Val-82 that was similar to part of the fibrinogen domain.

Genomic Structure of the Human Intelectin Gene—A human genome sequence data base search indicated that the human intelectin gene (Itln) was contained in a chromosome 1 clone (GenBank accession no. AL354714). The exon-intron junctions were determined by comparing the genomic sequence with that of hIntL cDNA. As shown in Fig. 4, human Itln was split into 8 exons and the open reading frame was located within exons 2–8. The nucleotide sequences of all exon-intron junctions fulfilled the GT-AG rule. Analysis of some sequences (GenBank accession nos. AL162592, AL354714, AC068728, and AL121985) indicated that Itln was located between junction adhesion molecule and Ly-9, which was followed by CD48. Since these molecules were reported to be located on chromosome 1q21.3–22 (29–31), Itln would be located on chromosome 1q21.3–22.

Northern Blotting of Human Intelectin mRNA—Northern blotting analysis of hIntL mRNA showed that the transcript size was 1.35 kilobase pairs (Fig. 5). It has been reported that mouse intelectin is specifically expressed in the small intestine (26), but hIntL mRNA was also expressed in the heart, small intestine, colon, and thymus, and to a lesser degree in the uterus and spleen. hIntL was purified and cloned from the placenta, but placental hIntL mRNA was barely detected on Northern blotting. Therefore, hIntL appeared not to be a major product in the placenta.

Structure and Activity of Recombinant hIntL—For biochemical analysis of hIntL, the hIntL cDNA was cloned into the vector pEF-BOS and transfected into the rabbit kidney cell line RK-13. The recombinant hIntL (rhIntL) was affinity-purified from the cell lysates and the culture supernatants with galactose-Sepharose in the presence of Ca\(^{2+}\) and was eluted with EDTA. As shown in Fig. 6, rhIntL was purified from both lysates and culture supernatants of hIntL-transfected cells, but not those of mock cells transfected with the empty vector. The rhIntL was the same molecular size as native placental hIntL under non-reducing or reducing conditions (Fig. 1A). Moreover, the molecular sizes of rhIntL and native placental hIntL were similarly decreased by N-glycanase treatment (Fig. 1B). These results indicated that rhIntL has Ca\(^{2+}\)-dependent galactose binding activity, homotrimeric structure via disulfide bonds,
and N-linked oligosaccharides, similarly to the native placental hIntL. The hIntL was purified as a soluble protein from the culture supernatant of hIntL-transfected cells, but the purified and concentrated rhIntL tended to become insoluble and inactive in the presence of Ca\(^{2+}\) (data not shown).

**Saccharide Binding Activity of rhIntL**—The rhIntL that was absorbed to galactose-Sepharose was eluted by various monosaccharides in the presence of Ca\(^{2+}\) (Fig. 7A). The absorbed rhIntL was completely eluted by 10 mM EDTA (data not shown). Approximately 50% of the absorbed rhIntL was eluted by buffers containing 100 mM galactose, 100 mM N-acetylgalactosamine, or 100 mM fructose. At the same concentration of monosaccharides, rhIntL was hardly eluted by other hexoses or derived saccharides (mannose, glucose, N-acetylmannosamine, N-acetylglucosamine, sorbose, D-fucose, L-fucose, L-rhamnose, and 2-deoxy-D-glucose). rhIntL was not eluted by cellobiase, maltose, trehalose, sucrose, or raffinose (data not shown). It has been reported that *X. laevis* cortical granule lectin/oocyte lectin has affinity to melibiose and lactose that contain a fixed galactopyranosyl residue, respectively (27, 32, 33). hIntL is likely to be a human homologue of cortical granule lectin/oocyte lectin because hIntL showed a high degree of homology to *X. laevis* cortical granule lectin (Fig. 3). Thus, rhIntL was expected to have affinity to melibiose and lactose. However, rhIntL was not eluted from galactose-Sepharose by melibiose or lactose. Surprisingly, rhIntL was effectively eluted by D-pentoses. D-Xylose, D-ribose, and 2-deoxy-D-ribose completely eluted the absorbed rhIntL, and D-lyxose eluted 50% of the rhIntL. D-\(\alpha\)-Arabinose and L-xylose eluted <20% of the rhIntL.

hIntL had affinity to galactose, but not melibiose or lactose, which contain a galactopyranosyl residue. These results suggested that hIntL recognized furanosides more effectively than pyranosides. Moreover, the high affinities of hIntL to pentoses supported this suggestion, since pentoses more effectively form furanoside rings than hexoses in the aqueous phase. As shown in Fig. 7B, rhIntL absorbed to galactose-Sepharose was completely eluted by 100 mM GalFG containing a fixed galactofuranosyl residue, similarly to EDTA or D-ribose. On the other hand, <30% of the absorbed rhIntL was eluted by 100 mM 2-acetamido-2-deoxy-4-O-\(\beta\)- galactopyranosyl-D-glucopyranose, the galactopyranoside isomer of GalFG, and rhIntL was hardly eluted by lactose or melibiose each containing a fixed galactopyranosyl residue. These results suggested that hIntL is a Ca\(^{2+}\)-dependent lectin that has affinity to furanosides such as the galactofuranosyl residue.

**Binding of hIntL to Bacterial Carbohydrate Chains**—Bacterial carbohydrate chains such as arabinogalactan of cell wall of *Mycobacterium* or *Nocardia* contain galactofuranosyl residues (24). We investigated whether hIntL bound to the arabinogalactan purified from *N. rubra*. As shown in Fig. 8, rhIntL bound to the arabinogalactan of *N. rubra* covalently fixed on the plate. The binding was completely inhibited by EDTA, D-ribose, D-galactose, and D-arabinose, but not D-glucose. Table I lists the saccharides tested as inhibitors of rhIntL binding to arabinogalactan and the concentration of each required to give 50% inhibition of binding. Pentoses (D-ribose, D-lyxose, L-xylose, and D-arabinose) and D-galactose inhibited the binding of rhIntL to arabinogalactan more effectively than hexoses (D-mannose and D-glucose). Moreover, GalFG containing a galactofuranosyl residue efficiently inhibited the binding of rhIntL, but not lactose or melibiose each containing a galactopyranosyl residue. These results suggested that the natural ligands of hIntL are bacterial carbohydrate chains such as arabinogalactan of *N. rubra*, and that hIntL recognizes the furanosyl residues contained in these oligosaccharides in a Ca\(^{2+}\)-dependent manner.

**DISCUSSION**

In host defense, the recognition of specific structures of pathogens is important for induction of immune responses. Some animal lectins induce responses of the innate immune system by recognition of the carbohydrate chains that host cells lack. In this study, we demonstrated that hIntL is a new type of lectin that recognizes galactofuranosyl residues, which hardly exist in mammals. This is the first report of an animal lectin recognizing galactofuranose.

Galactofuranose is contained in the carbohydrate chains of various microorganisms (24, 34–37). The cell wall skeleton of *Mycobacteria* or *Nocardia* has biological activities as an immune activator (21–23) and contains galactofuranose as a constituent of arabinogalactan (24). In a previous study, we found that galactose effectively induced phagocytosis of the cell wall skeleton of *Mycobacterium bovis* by dendritic cells, but not mannose or N-acetylgalactosamine.2 This result allowed us to presume existence of a receptor or an opsonin that recognizes galactofuranosyl residues. hIntL is different from the lectins that induced phagocytosis of the cell wall skeleton of *M. bovis*.

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2 S. Tsuji, J. Uehori, M. Matsumoto, and T. Seya, unpublished data.
80% culture supernatants of hIntL-transfected cells containing various concentrations of saccharides were added to the plate binding arabinoagalactan of N. rubra, and the amount of rhIntL was measured by enzyme-linked immunosorbent assay as described under “Experimental Procedures.” Each saccharide was used at the concentrations of 5–200 μM. The inhibition curves were obtained from the mean of duplicate determinations, and the saccharide concentrations for 50% inhibition of rhIntL binding were calculated from these results. This experiment was repeated twice with similar results.

| Saccharide     | Concentration for 50% inhibition (μM) |
|----------------|--------------------------------------|
| D-Ribose       | <5                                   |
| GalG           | 9                                    |
| D-Xylose       | 17                                   |
| D-Lyxoose      | 55                                   |
| D-Galactose    | 66                                   |
| D-Arabinose    | 70                                   |
| D-Mannose      | 105                                  |
| D-Glucose      | >200                                 |
| Lactose        | >200                                 |
| Melibiose      | >200                                 |

because the results of saccharide affinity of hIntL were not similar to the results of phagocytosis inhibition. However, hIntL bound to the arabinoagalactan that was purified as a pathogen constituent from N. rubra. Thus, hIntL would bind to various pathogens containing galactofuranosyl residues such as Nocardia, Mycobacteria (24), Streptococcus (34), and Leishmania and Trypanosoma (35–37).

The soluble biological defense lectins, MBL and ficolin/P35, function as agglutinins. The agglutination activity of lectin is important to lock the targets into a point. hIntL also forms a complex structure. It has been reported whether the lectin bound to constituents of pathogens. In the present study, we demonstrated that hIntL binds to the arabinoagalactan of N. rubra, and the amount of rhIntL was measured by enzyme-linked immunosorbent assay as described under “Experimental Procedures.” Each saccharide was used at the concentrations of 5–200 μM. The inhibition curves were obtained from the mean of duplicate determinations, and the saccharide concentrations for 50% inhibition of rhIntL binding were calculated from these results. This experiment was repeated twice with similar results.

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| D-Mannose  | 105                                  |
| D-Glucose  | >200                                 |
| Lactose    | >200                                 |
| Melibiose  | >200                                 |

shown to participate in formation of the fertilization envelope that blocks sperm entry (38, 39), hIntL may also participate in fertilization. However, hIntL was also expressed on various tissues other than oocytes, and it has been reported that the other homologues are also present in various tissues (26, 28, 40). Thus, hIntL and its homologues may not only participate in formation of the fertilization envelope but also have other physiological functions.

The result of Northern blotting analysis suggested that hIntL was plentifully expressed in the heart (Fig. 5). It is known that viridans streptococci invading into blood attack heart and cause subacute infectious endocarditis, and the cell surface polysaccharide of Streptococcus oralis, a viridans streptococci, contains galactofuranosyl residues (34). Thus, hIntL may function in heart as a defense protein against these pathogens. It is also possible that hIntL may be an acceptor molecule of viridans streptococci in heart. These possibilities could be tested using galactofuranoside-modified Streptococcus.

Mouse intelectin was reported as the first intelectin homologue in mammals, and the gene was specifically expressed in intestinal paneth cells (26). However, the biochemical and physiological functions were not analyzed in the study because the recombinant mouse intelectin produced in Escherichia coli had toxic activity in bacteria. Although the ascidian galactose-specific plasma lectin, an ascidian homologue of hIntL, was reported as a lectin that bound to galactose and enhanced the agglutycsin of sheep erythrocytes (28), it has not been clarified whether the lectin bound to constituents of pathogens. In the present study, we demonstrated that hIntL has affinity to galactofuranosyl residues and recognizes bacterial carbohydrate chains. The galactofuranosyl residue is a constituent of pathogens and a dominant immunogen (24, 41). Thus, our results suggested that a biological function of hIntL is the specific recognition of pathogens and bacterial components containing galactofuranosyl residues.

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