The Adhesive Specificity of the Soluble Human Lectin, IgE-Binding Protein, toward Lipid-Linked Oligosaccharides. Presence of the Blood Group A, B, B-like, and H Monosaccharides Confers a Binding Activity to Tetrasaccharide (Lacto-*N*-tetraose and Lacto-*N*-neotetraose) Backbones†

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ABSTRACT: The immunoglobulin E-binding protein, εBP (also known as CBP35, Mac-2, L-34, and L-29), is a β-galactoside-binding protein of approximately 30 kDa and a member of the animal lectin family termed S-type or S-Lac. Multiple biological activities have been attributed to this lectin such as mediation of IgE binding to the surface of Langerhans cells and activation of mast cells through binding to the high affinity IgE receptor. In order to better understand the cell-binding activity and the proposed role for εBP as a biological response modifier, we have studied the specificity of binding of the radioiodinated εBP to a series of lipid-linked, structurally defined oligosaccharide sequences of the lacto/neolacto family. The results show that the minimum lipid-linked oligosaccharides that can support εBP binding are pentasaccharides of the lacto/neolacto series and that the lectin binds more strongly to oligosaccharides of this family that bear the blood group A, B, or B-like determinants than to those bearing blood group H. This preferential binding of εBP is also manifest with whole cells, as erythrocytes of blood groups A and B are more strongly bound by εBP than those of blood group O. Blood group Le^a and Le^x sequences are not bound by the lectin. The present results in the context of the earlier observations on the specificity and biological activity of εBP raise the possibility that, *in vivo,* its degree of cell association and thresholds for activation of various cells of the immune system, and by inference allergic predispositions, may be influenced by blood group status and other polymorphic carbohydrate antigen systems based on lacto/neolacto backbones.

The IgE-binding protein (εBP)[1] was originally identified in rat basophilic leukemia cells (Liu et al., 1985; Albrandt et al., 1987). Subsequently, it became clear (Laing et al., 1989; Abbott & Feizi, 1989) that it has greater than 85% identity of amino acid sequence with the galactose-binding protein of the mouse, CBP35 (Jia & Wang, 1988) and that it is a β-galactoside-binding protein. This protein has also been studied in other biological settings and designated Mac-2 (Cherayil et al., 1989, 1990), L-29 (Oda et al., 1991), and L-34 (Raz et al., 1991a) in the mouse and human; and L-34 (Raz et al., 1989) and RL-29 (Leffler et al., 1989) in the rat. εBP has two domains (Albrandt et al., 1987; Robertson et al., 1990): an amino-terminal domain containing proline- and glycine-rich tandem repeats, and a carboxyl-terminal carbohydrate-binding domain which is homologous with the soluble 14-kDa β-galactoside-binding protein (Liu, 1990; Abbott & Feizi, 1991) and which is a member of a unique family termed S-type or S-Lac lectins[2] (Drickamer, 1988; Leffler et al., 1989).

Although εBP, like other members of the S-type lectin family, lacks a recognizable signal sequence, there is overwhelming evidence for its occurrence outside cells as well as intracellularly. CBP35 immunoreactivity has been found predominantly in the cytoplasm of quiescent fibroblasts but in increased amounts in the nucleus of proliferating cells (Moutsatsos et al., 1987). These and earlier immunochemical observations on varying levels in the nucleus and cytoplasm of proteins antigenically related to S-type lectins (Childs & Feizi, 1980; Carding et al., 1985) have suggested that CBP35 and related proteins may be components of growth-regulating systems. More recently, a role for CBP35 has been suggested in RNA splicing (Wang et al., 1992). εBP/Mac-2 immunoreactivity has been detected on cell surfaces (Ho & Springer, 1982; Frigeri & Liu, 1992). A role for the lectin in cell adhesion has been proposed (Woo et al., 1990), and changes in its expression have been observed in association with neoplastic transformation and metastasis (Raz et al., 1991b; Irimura et al., 1991; Castronovo et al., 1992; Lotz et al., 1993).

The involvement of εBP in inflammatory processes has been the subject of several investigations. This protein is expressed

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[1] Abbreviations: As-GM_1 and As-GM_2, asialio-GM_1 and -GM_2 gangliosides; BSA, bovine serum albumin; Cer, ceramide; DPPE, L-1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine; DTT, dithiothreitol; εBP, IgE-binding protein; IgE, immunoglobulin E; LNFP, lacto-*N*-fucopentaose; LNT, lacto-*N*-tetraose; LNnT, lacto-*N*-neotetraose; PBS, phosphate-buffered saline; pLNH, paralacto-*N*-hexaose; pLNnH, paralacto-*N*-neohexaose; pLNP, paralacto-*N*-pentaose; S1, 3′-sialyl-LNT; S3, 6′-sialyl-LNnT.

[2] The term "galectin" has now been proposed (Barondes et al., 1994).

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on mast cells (Frigeri & Liu, 1992), neutrophils (Truong et al., 1993) and monocytes/macrophages (Cherayil et al., 1989, 1990). The εBP content of human neutrophils appears to be variable among individual people. This finding, and the evidence that εBP may be involved in neutrophil activation induced by IgE, has raised the possibility that its presence may be related to allergic disease state (Truong et al., 1993). εBP has also been detected on the surface of epidermal Langerhans cells and shown to mediate IgE binding to these cells (Wollenberg et al., 1993). Collectively, from these various observations and the recent finding that εBP binds to the high-affinity IgE receptor on mast cells and is capable of activating mast cells (Frigeri et al., 1993), a picture is emerging that εBP may be a broad-spectrum biological response modifier (Liu, 1993) whose activities are mediated through its interactions with appropriately glycosylated cell surface glycoproteins.

An important requirement for understanding the functions and mechanisms of action of εBP and related proteins is to determine the range of carbohydrate structures that it recognizes. This has been investigated for L-29 by using several oligosaccharides as inhibitors of the binding to asialofetuin (Leffler & Barondes, 1986; Sparrow et al., 1987). The results suggest that L-29 recognizes oligosaccharides longer than disaccharides, a notion also supported by results of quantitative precipitation assays with CBP35 using oligosaccharides coupled to bovine serum albumin (Knibbs et al., 1993). Of special interest is the finding by Leffler and Barondes (1986) that substitution of lactose or lacto-*N*-tetraose with the blood group A monosaccharides renders the resulting oligosaccharides extremely potent inhibitors of L-29.

In the present study we have investigated the binding specificity of εBP toward a series of immobilized lipid-linked oligosaccharides. Here we report that the minimum lipid-linked oligosaccharide bound by εBP is a pentasaccharide and that the lectin binds preferentially to oligosaccharides and whole cells expressing the blood group A and B antigens over those expressing blood group H.

MATERIALS AND METHODS

Preparation of Recombinant Human εBP. Recombinant human εBP was expressed in *Escherichia coli*, purified by affinity chromatography as described (Hsu et al., 1992), and stored at −80 °C in phosphate-buffered saline (PBS; 20 mM sodium phosphate, pH 7.4, with 0.15 M NaCl) containing 1 mM dithiothreitol (DTT) and 10% (v/v) glycerol.

Radiolabeling of εBP. εBP was labeled by the chloramine T method (Greenwood et al., 1963); free iodine was removed by filtration on a PD10 Sephadex G25M column (Pharmacia) equilibrated in PBS containing 1 mM DTT, 0.1% w/v bovine serum albumin (BSA) from Sigma, and aprotinin [Sigma; 0.1 trypsin inhibitory unit (TIU)/mL] and eluted in the same solution without BSA. The specific activity of the protein was approximately 30 μCi/μg. In 12% polyacrylamide gel electrophoresis in the presence of SDS and β-mercaptoethanol (Laemmli, 1970), the iodinated protein migrated at the same position as the unlabeled protein, at approximately 30 kDa.

Glycolipids. The glycolipids investigated are shown in Tables 1 and 2. These consisted of lactosylceramide (Sigma); globoside and Forssman glycolipid from BioCarb (Lund, Sweden); ceramide 4 [(Cer 4) paragloboside] derived from myeloid cells (gift of Dr. Bruce Macher); ceramide 5 (Cer 5), ceramide 8 (Cer 8), ceramide 9 (Cer 9), ceramide 10 (Cer 10), ceramide 12 (Cer 12), and ceramide 15 (Cer 15) derived from rabbit erythrocytes (Hanfland et al., 1981; Egge et al., 1985) (gifts of Dr. Peter Hanfland); and blood group B-active glycosylceramide Cer-B6 and Cer BGM_1 (gifts from Dr. Gunnar C. Hansson). The gangliosides GM_1 and GM_2 and their asialo analogues, As-GM_1 and As-GM_2, were from BioCarb.

Neoglycolipids. Neoglycolipids (Tang et al., 1985) were prepared by conjugation of oligosaccharides to the lipid L-1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (DPPE) as described previously (Stoll & Hounsell, 1988) with minor modifications (Feizi et al., 1994). The conjugates were monitored by thin-layer chromatography and liquid secondary ion mass spectrometry (Lawson et al., 1990). The following oligosaccharides were used: lacto-*N*-tetraose (LNT); 3′-sialyl-LNT (S1); lacto-*N*-neotetraose (LNnT); 6′-sialyl-LNnT (S3); lacto-*N*-fucopentaose I (LNFP-I); lacto-*N*-fucopentaose II (LNFP-II); lacto-*N*-fucopentaose III (LNFP-III); blood group A disaccharide (A2); blood group A tri-, tetra-, penta-, hexa-, and heptasaccharides (A3–A7, respectively); urinary oligosaccharides from GM_1 gangliosidosis patients: GM_1-A, GM_1-B, and GM_1-C [for descriptions of these oligosaccharides, see Gooi et al. (1985), Stoll et al. (1988), Childs et al. (1989), and Larkin et al. (1992)]. Paralacto-*N*-hexaose (pLNH) and paralacto-*N*-neohexaose (pLNnH) were from the late Dr. A. Simon R. Donald. Paralacto-*N*-pentaose (pLNP) was generated from pLNnH by digestion with jack bean β-galactosidase (Seikagaku Kogyo Co., Tokyo, Japan) using established procedures (Mizuochi et al., 1982).

For microwell binding assays, neoglycolipids were separated from other reaction mixture components using a 100-mg PBA BondElut column (Analytichem International, U.S.A., purchased from Jones Chromatography, Hengoed, Glamorgan, U.K.) by a method adapted from Stoll and Hounsell (1988). The column was washed with 2 × 1 mL of 0.3% aqueous ammonia, 1 mL of methanol, and 2 × 1 mL of chloroform/methanol (1:1) containing 0.1% ammonia (solvent A). The conjugation mixture (containing 25–100 μg of oligosaccharide) was dried under nitrogen, dissolved in solvent A containing 5% water, and applied to the column, which was then washed with 1 mL solvent A followed by 2 × 1 mL chloroform/methanol/0.1 M aqueous boric acid (30:70:30). Solvents were removed from fractions by sequential additions of 200 μL of 1% methanolic acetic acid and 200 μL of methanol, evaporating under nitrogen between additions. Fractions were dissolved in chloroform/methanol/water (25:25:8), chromatographed by high-performance TLC in chloroform/methanol/water (60:35:8), and quantified with a Shimadzu CS-9000 scanner after staining with orcinol or primulin, using the neoglycolipid derivative of maltopentaose as a standard.

Microwell Binding Assays. For binding assays, neoglycolipids and glycolipids were dissolved in methanol containing 4 μg/mL each of cholesterol and egg lecithin (carrier solution). Round-bottomed wells (Dynatech Immulon 1) were coated in triplicate with glycolipids or neoglycolipids in carrier solution, and binding of εBP was assayed as described previously (Solomon et al., 1991), with the following exception: the iodinated lectin (7×10^4 cpm/well) was used, the solution for blocking the microtiter wells and for diluting the radiolabeled lectin contained aprotonin (0.1 TIU/mL) and BSA (3% w/v) instead of hemoglobin, and binding was at 4 °C for 6 h. Carrier solution alone was used to assess background binding (on average 1% of counts added). In all experiments the lipid-linked hexasaccharide A6 and the lipid-linked disaccharide GN2 were used as a positive and a negative standard, respectively. There was unusual variability in the counts bound to the lipid-linked oligosaccharides in experiments performed on different days. However, within indi-

Table 1: Lipid-Linked Oligosaccharides Bound by ϵBP[a]

Designation	Sequence
pLNnP	GlcNAcß1-3Galß1-4GlcNAcß1-3Galß1-4Glc
pLNnH	Galß1-4GlcNAcß1-3Galß1-4GlcNAcß1-3Galß1-4Glc
pLNH	Galß1-3GlcNAcß1-3Galß1-4GlcNAcß1-3Galß1-4Glc
LNFP-I	Galß1-3GlcNAcß1-3Galß1-4Glc |1,2 Fucα
Cer 5	Galα1-3Galß1-4GlcNAcß1-3Galß1-4Glc
A6	GalNAcα1-3Galß1-3GlcNAcß1-3Galß1-4Glc |1,2 Fucα
Cer B6	Galα1-3Galß1-3GlcNAcß1-3Galß1-4Glc |1,2 Fucα
S1	NeuAcα2-3Galß1-3GlcNAcß1-3Galß1-4Glc
Cer 8	Galß1-4GlcNAcß1-6\ Galß1-4GlcNAcß1-3Galß1-4Glc Galß1-4GlcNAcß1-3/
Cer 10	Galα1-3Galß1-4GlcNAcß1-6\ Galß1-4GlcNAcß1-3Galß1-4Glc Galα1-3Galß1-4GlcNAcß1-3/
Cer 12	Galß1-4GlcNAcß1-6\ Galß1-4GlcNAcß1-6\ Galß1-4GlcNAcß1-3/ Galß1-4GlcNAcß1-3Galß1-4Glc Galß1-4GlcNAcß1-3/
Cer 15	Galα1-3Galß1-4GlcNAcß1-6\ Galß1-4GlcNAcß1-6\ Galα1-3Galß1-4GlcNAcß1-3/ Galß1-4GlcNAcß1-3Galß1-4Glc Galα1-3Galß1-4GlcNAcß1-3/
GM_1-A	Galß1-4GlcNAcß1-2Manα1-6\ Manß1-4GlcNAc Galß1-4GlcNAcß1-2Manα1-3/
GM_1-B	Galß1-4GlcNAcß1-2Manα1-6\ Galß1-4GlcNAcß1-4\ Manß1-4GlcNAc Manα1-3/ Galß1-4GlcNAcß1-2/
GM_1-C	Galß1-4GlcNAcß1-6\ Manα1-6\ Galß1-4GlcNAcß1-2/ Manß1-4GlcNAc Galß1-4GlcNAcß1-4\ / Manα1-3/ Galß1-4GlcNAcß1-2/

[a] Lipid-linked oligosaccharides evaluated for binding by ϵBP. For the binding experiments, the oligosaccharides were assayed as neoglycolipids or, where indicated by the prefix or suffix "Cer", they were assayed as glycosylceramides.

vidual experiments the hierarchy of binding intensities between different immobilized compounds was maintained, and the variability within triplicates was not greater than 15% above or below the mean values. It is possible that the binding of ϵBP [which is known to manifest unusual concentration-dependent cooperativity in ligand binding (Hsu et al., 1992; Massa et al., 1993)] is sensitive to subtle differences in coating patterns and presentation of the lipid-linked oligosaccharides.

Hemagglutination of Human Erythrocytes by ϵBP. ϵBP in PBS containing 0.3% w/v BSA and 1 mM DTT was serially diluted in the wells of a 96-well microtiter plate (V-well from Dynatech, Chantilly, VA), an equal volume of erythrocytes (2% suspension, 25 μL) was added to each well, and the mixtures were incubated at 20 °C for 1 h. The minimum concentrations of ϵBP that gave hemagglutination of erythrocytes of blood groups A, B, or O were determined visually

Table 2: Lipid-Linked Sequences Not Bound or Negligibly Bound by ϵBP

Designation	Sequence
Lac/Lac Cer	Galß1-4Glc
LNT	Galß1-3GlcNAcß1-3Galß1-4Glc
LNnT or Cer 4	Galß1-4GlcNAcß1-3Galß1-4Glc
Cer 9	GlcNAcß1-6 / Galß1-4GlcNAcß1-6 / GlcNAcß1-3 / Galß1-4GlcNAcß1-3Galß1-4Glc / GlcNAcß1-3
LNFP-II	Galß1-3GlcNAcß1-3Galß1-4Glc |1,4 Fucα
LNFP-III	Galß1-4GlcNAcß1-3Galß1-4Glc |1,3 Fucα
LNDFH-I	Galß1-3GlcNAcß1-3Galß1-4Glc |1,2 |1,4 Fucα Fucα
A2	GalNAcα1-3Gal
A3	GalNAcα1-3Gal |1,2 Fucα
A4	GalNAcα1-3Galß1-4Glc |1,2 Fucα
A5	GalNAcα1-3Galß1-4Glc |1,2 |1,3 Fucα Fucα
A7	GalNAcα1-3Galß1-3GlcNAcß1-3Galß1-4Glc |1,2 |1,4 Fucα Fucα
S3	NeuAcα2-6Galß1-4GlcNAcß1-3Galß1-4Glc
As GM_2 Cer	GalNAcß1-4Galß1-4Glc
As GM_1 Cer	Galß1-3GalNAcß1-4Galß1-4Glc
GM_2 Cer	GalNAcß1-4Galß1-4Glc |2,3 NeuAcα
GM_1 Cer	Galß1-3GalNAcß1-4Galß1-4Glc |2,3 NeuAcα
BGM_1 Cer	Galα1-3Galß1-3GalNAcß1-3Galß1-4Glc |1,2 |2,3 Fucα Neucα
Globoside Cer	GalNAcß1-3Galα1-4Galß1-4Glc
Forssman Cer	GalNAcα1-3GalNAcß1-3Galα1-4Galß1-4Glc
GN_2	GlcNAcß1-4GlcNAc

by the settling pattern. For inhibition studies, serial 2-fold dilutions of saccharides (25 μL), lactose, thiodigalactoside, arabinose (all from Sigma), and blood group A tetrasaccharide (BioCarb), were prepared in microtiter wells; 20-μL aliquots of human blood group A erythrocytes were added to the wells followed by 5 μL of the recombinant human ϵBP (16 μg/mL). The plates were incubated at 20 °C for 1 h and the minimum concentrations of saccharide that inhibited ϵBP-induced hemagglutination noted.

Binding of Fluorescein-Labeled ϵBP to Human Erythrocytes. Fluorescein-labeled ϵBP was prepared according to a published procedure (Colligan et al., 1991) with some modifications. Briefly, recombinant human ϵBP was dialyzed at 4 °C against 50 mM sodium borate and 0.2 M NaCl, pH

8.5, containing 10 mM lactose and 5% (v/v) glycerol. To the dialyzed solution (1 mg/mL ϵBP) was added 20 μL of 100 mg/mL fluorescein isothiocyanate dissolved in dimethyl sulfoxide, and the mixture was incubated at 24 °C for 3 h in the dark. The reaction mixture was then dialyzed against 50 mM Tris-HCl, pH 7.5, 0.2M NaCl, 0.5% (v/v) glycerol, and 0.02% sodium azide and stored at −80 °C until use.

For binding studies, 25-μL suspensions of erythrocytes (1×10^7 cells/mL) were added to 25 μL of PBS with or without 5 mM lactose followed by 10 μL of fluorescein-labeled ϵBP (final concentration 3.5 μg/mL). The mixtures were incubated for 30 min at 4 °C, washed with PBS four times, and resuspended in PBS. Ten thousand erythrocytes were analyzed by flow cytometry (FACScan, Becton Dickinson) and Lysis II program.

RESULTS

Influence of Oligosaccharide Backbone Chain Length on Lectin Binding. Among the β-galactosyl-terminating oligosaccharide backbone sequences tested as glycosphingolipids or neoglycolipids (Tables 1 and 2), the minimum sequences bound by ϵBP were the hexasaccharides pLNnH and pLNH (Figure 1A). There was negligible binding to the disaccharides as in the lactose neoglycolipid and lactosylceramide, and to the tetrasaccharides as in the LNT and LNnT neoglycolipids and ceramide 4 (paragloboside) (Figure 1A,B). However, the pentasaccharide pLNnP obtained by β-galactosidase treatment of pLNnH gave substantial binding, indicating that the presence of GlcNAcβ1–3 on the outer of the two galactose residues of the LNnT sequence serves to present a binding motif to the lectin.

The bi-, tri-, and tetraantennary oligosaccharides of N-glycosidic type, bearing the *N*-acetyllactosamine sequence on each outer chain, clearly supported lectin binding (Figure 1C), showing that a repeated Galβ1–3(4)GlcNAc/Glc sequence is not essential for binding and that the lipid-linked trimannosyl-*N*-acetylglucosaminyl core can effectively display a binding motif for the lectin. It is possible that this is a reflection of a requirement for display of two or more lactosamine units for the lectin binding. However, the presence of two lactosamine units *per se* is not sufficient for binding, as clustered LNT and LNnT neoglycolipids elicited negligible ϵBP binding. The binding strengths to the three N-glycosidic oligosaccharides were comparable.

Influence of Branching of Poly-N-acetyllactosamine Backbone on Lectin Binding. The dibranched ceramide 9 with three terminal *N*-acetylglucosamine residues did not support ϵBP binding; this indicates that the further substitution of a 3-substituted internal galactose by GlcNAcβ1–6 hinders binding. The modest lectin binding to ceramides 8 and 12 (Figure 2A) would therefore seem to be elicited by one or more of the outer Galβ1–4GlcNAc disaccharides.

Influence of Backbone Substitution with Blood Group Determinants or Sialic Acid on Lectin Binding. Substitution of the LNT backbone with the blood group A monosaccharides GalNAcα1–3(Fucα1–2) or the blood group B monosaccharides Galα1–3(Fucα1–2) linked to the outer galactose rendered the resulting lipid-linked hexasaccharides, A6 and Cer B6, strong ligands for ϵBP (Figure 2B). Such an effect was not seen with the blood group A monosaccharides linked to the disaccharide backbone, lactose, as in A4 (Table 2).

The mere presence of the blood group H monosaccharide Fucα1–2, or the blood group B-like monosaccharide Galα1–3 linked to the terminal galactose of the LNT sequence (as in LNFP-I neoglycolipid and ceramide 5, respectively), was sufficient to elicit a binding signal (Figures 1B and 2B,C). The intensity of binding to ceramide 5 was approximately equivalent to that of A6 and Cer B6, while binding to LNFP-I was consistently weaker (Figure 2B,C). In contrast to the blood group H fucose, the fucose residues (Fucα1–4 or Fucα1–3), which confer Le^a or Le^x activity when linked to the *N*-acetylglucosamine of LNT and LNnT as in LNFP-II and LNFP-III, respectively, did not confer lectin reactivity. Moreover, these fucose residues hindered lectin reactivity when they were present as part of the Le^b and ALe^b structures, as, for example, in LNDFH-I and A7 (Figure 2C). The presence of a capping Galα1–3 on the outer galactoses of ceramides 8 and 12, forming ceramides 10 and 15, respectively, gave rise to a marked accentuation of lectin binding (Figure 2A).

Sialylation at position 3 of the outer galactose (as in S1), but not at position 6 (as in S3) of the LNT or LNnT backbone, conferred moderate lectin-binding activity (Figure 2B). Sialylation at position 3 of the inner galactose residue on the

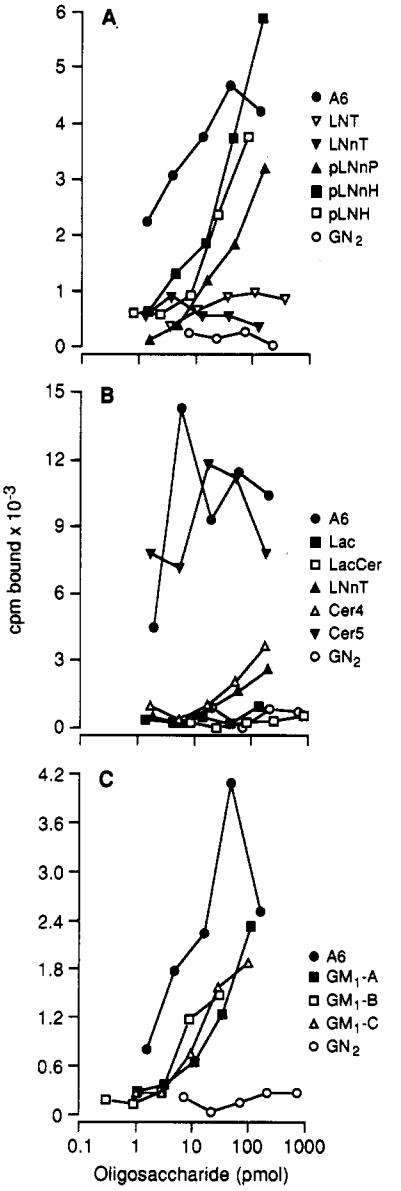

FIGURE 1: Binding of ^{125}I-labeled ϵBP to lipid-linked oligosaccharides immobilized in microwells of plastic plates. Microwell binding assays were performed as described in the Materials and Methods section. Each panel, A–C, shows the results of a separate experiment. Unless otherwise indicated by a prefix "Cer" (to indicate a glycosylceramide), the saccharides were tested as neoglycolipids.

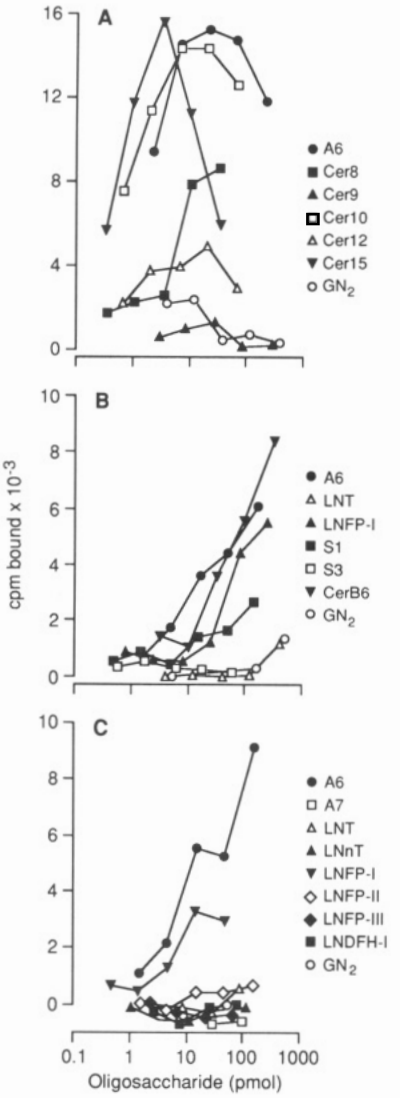

FIGURE 2: Binding of ^{125}I-labeled ϵBP to lipid-linked oligosaccharides immobilized in microwells of plastic plates. Microwell binding assays were performed as described in the Materials and Methods section. Each panel, A–C, shows the results of a separate experiment. Designations of glycosylceramides and neoglycolipids are as in Figure 1.

blood group B-related glycolipid, BGM_1 Cer, abolished lectin-binding activity (Table 2).

Several other lipid-linked oligosaccharides tested, including GM_1 and GM_2 gangliosides and their asialo forms, globoside, and Forssman glycolipids, did not support ϵBP binding.

Preferential Binding of ϵBP to Human Erythrocytes of Blood Groups A and B. In accord with the greater binding to lipid-linked A6 and Cer B6 sequences than to LNFP-I, human erythrocytes of blood groups A and B were more strongly agglutinated by ϵBP than those of blood group O; minimum concentrations of the lectin that gave hemagglutination of group A and group B erythrocytes, 7.4 ± 0.6 and 8.7 ± 0.7 μg/mL, respectively, were significantly lower than for group O erythrocytes, 18.6 ± 1.8 μg/mL ($P < 0.01$) (Figure 3). The agglutination of group A erythrocytes was inhibited by lactose, by thiodigalactoside and by the blood group A tetrasaccharide (A4), their relative inhibitory activities being 1, 6.5, and 12.5, respectively (Table 3).

The preferential ϵBP binding to group A and B erythrocytes relative to blood group O was also clearly shown by flow cytometry using the fluorescein-labeled lectin. The fluorescence intensities for the three cell types were 412, 347, and 105 respectively (Table 4). In each case binding was inhibited in the presence of 5 mM lactose.

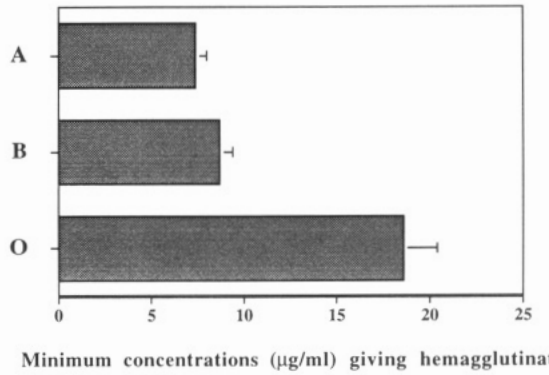

FIGURE 3: Hemagglutination of human erythrocytes by ϵBP. Serial dilutions of the recombinant ϵBP were tested for hemagglutination of erythrocytes of blood groups A, B, or O, as described in the Materials and Methods section. Results are means and standard errors of 15 experiments that were performed in duplicate.

Table 3: Inhibition of ϵBP-Induced Hemagglutination of Blood Group A Erythrocytes by Saccharides

saccharides	concentration (mM)[a]	relative activity
lactose	0.39	1
thiodigalactoside	0.06	6.5
A-tetrasaccharide (A4)	0.03	12.5
arabinose	>50	

[a] Minimum concentrations of saccharides that result in complete inhibition or ϵBP-induced hemagglutination.

Table 4: Binding of Fluorescein-Labeled Human ϵBP to Human Erythrocytes of Blood Groups A, B, or O in the Absence or Presence of 5 mM Lactose

	mean fluorescence intensity[a]	
blood group	without lactose	with lactose
A	412 ± 41	54 ± 5
B	347 ± 24	51 ± 4
O	105 ± 11[b]	47 ± 3

[a] Each value represents the mean ± standard error from 10 samples. [b] The mean fluorescence intensity for O is significantly different from that for the other blood groups ($P < 0.01$).

DISCUSSION

This study differs from earlier investigations focused on inhibition of binding (Leffler & Barondes, 1986; Sparrow et al., 1987) and precipitation (Knibbs et al., 1993), in that binding to immobilized oligosaccharide sequences has been evaluated with special reference to lipid-linked oligosaccharides. Our main conclusions regarding the binding specificity of ϵBP toward oligosaccharides linked to lipid are the following: First, that in order to support lectin binding, the minimum oligosaccharide recognition unit, Galβ1–4(or 3)-GlcNAc, as well as the more potent recognition unit, Galβ1–4(or 3)GlcNAcβ1-3Galβ1–4GlcNAc, must occur at the nonreducing regions of longer oligosaccharide chains. These oligosaccharide chains may be of poly-*N*-acetyllactosamine type, in which case the minimum (lipid-linked) β-galactosyl-terminating oligosaccharides that elicit detectable lectin binding are the hexasaccharides pLNnH or pLNH (where the tetrasaccharide ligand is joined to a lactosyl core), or they may be complex-type chains in which the disaccharide is joined directly to the trimannosyl core. Second, ϵBP-binding activity is conferred if the outer galactose of the lipid-linked tet-

Table 5: Three Categories of Interactions among Lipid-Linked, Blood Group-Related Oligosaccharides with εBP

(1) strong	A6, Cer B6, Cer 5, Cer 10, Cer 15
(2) moderate	pLNnP, pLNnH, pLNH, Cer 8, Cer 12, LNFP-I, S1, GM_1-A, GM_1-B, GM_1-C
(3) weak/inactive	LNT, LNnT, Cer 4, Lac, Lac Cer, Cer 9, A2, A3, A4, A5, A7, LNFP-II, LNFP-III, LNDFH-I, BGM_1 Cer

rasaccharide backbone is substituted with one or two of several different monosaccharides, including those that confer blood group A, B, and H antigenicities. In contrast, when there is substitution of the subterminal *N*-acetylglucosamine with 3- or 4-linked fucose as in the Le^x and Le^a antigen structures, εBP is not bound.

The several substituents at the outer galactose which confer εBP-binding activity to the lipid-linked LNT or LNnT sequence are as follows: GlcNAcβ1–3; Galβ1–4(or 3)-GlcNAcβ1–3; NeuAcα2–3 (but not NeuAcα2–6); Fucα1–2, blood group H; Galα1–3, blood group B-like; GalNAcα1–3(Fucα1–2), blood group A; Galα1–3(Fucα1–2), blood group B. The binding is strongest if the B-like or the blood group A or blood group B antigen structures are present. The preferential εBP binding to the blood group A- or B-active sequences compared with the H sequence is also manifest with whole cells, as erythrocytes of blood groups A and B are more strongly bound by εBP than those of blood group O. Overall, three categories of interactions can be discerned among the oligosaccharide sequences examined: strong, medium, and weak/inactive (Table 5). It will be interesting to determine whether the combining site of εBP recognizes a motif additional to the LNT/LNnT sequence, or whether the blood group-related and other substituents elicit εBP binding by rendering the conformation or orientation of the backbone more suitable for lectin binding.

For εBP binding, the tetrasaccharide recognition unit may be internally located, with the outer galactose 3-substituted or 3,2-disubstituted as described above. However, when there is 3,6-disubstitution as in the branched I-antigen-type backbones, e.g., Cer 9, binding is not elicited; this contrasts with the strong binding to the linear, i-antigen-type sequence, pLNP. These findings provide a biochemical explanation for previous observations that this lectin has a particular affinity for murine laminin (Woo et al., 1990; Massa et al., 1993) which contains not only linear poly-*N*-acetyllactosamine sequences, but also the B-like, Galα1–3, termini (Knibbs et al., 1989).

εBP differs from the 14-kDa β-galactoside-binding lectin which has been similarly investigated for binding to lipid-linked oligosaccharides (Solomon et al., 1991) and for which the recognition unit appears to be no longer than a disaccharide. With the 14-kDa lectin, binding is elicited provided that the disaccharide ligand is joined to lipid via an intervening lactosyl core as in LNT or LNnT, or joined to the trimannosyl core of complex-type chains. Thus it is clear from the present and the earlier study that when linked to lipid (as in glycosylceramides or neoglycolipids), the disaccharide ligand for the 14-kDa lectin and the tetrasaccharide ligand for εBP do not support binding unless presented in continuity with a disaccharide or longer saccharide core. Both lectins bind to the backbone LNT in the presence of the blood group H fucose and the blood group B-like Galα1–3, but in accord with earlier observations (Leffler & Barondes, 1986), only εBP binds to the blood group A sequence. Thus, although both proteins can be inhibited by the disaccharides Galβ1–4(or 3)GlcNAc or their tetrasaccharide analogues, their binding specificities toward blood group-related oligosaccharides differ.

Intracellular carbohydrate ligands for the S-type lectins have not yet been identified. So far, the range of oligosaccharides shown to be bound by these lectins in the present and several previous investigations (Leffler & Barondes, 1986; Sparrow et al., 1987; Abbott et al., 1989; Solomon et al., 1991; Sato & Hughes, 1992; Sato et al., 1993) include oligosaccharide sequences of the type that occur at the surface of cells and in extracellular matrices. For the 14-kDa lectins, these are unsubstituted backbones of lactosamine or poly-*N*-acetyllactosamine type and their sialyl α2–3, galactosyl α1–3, and fucosyl α1–2 (blood group H) analogues; for εBP, these include poly-*N*-acetyllactosamine analogues of these same oligosaccharide sequences, but their blood group A, B, and B-like forms are strongly preferred. Thus, the possibility is raised that εBP and related proteins may be among the much-sought carbohydrate-binding proteins that are linked to functions of blood group antigens (Feizi, 1985, 1989).

Each differentiated cell type has a different repertoire of glycosylated proteins with various functions. Moreover, the glycosylation phenotype and hence the lectin-binding property of a particular glycoprotein will differ at various stages of cellular differentiation of the cell by which it is produced. One of the challenges in cell biology has been to determine the influence of glycosylation on bioactivities of glycoproteins. A possibility that we have considered is that oligosaccharides of cell surface glycoproteins and glycolipids and lectins recognizing them may constitute networks of functionally coupled receptor systems that transmit signals within and across cell membranes [Feizi (1989) and references therein]. On the basis of the observation that a monoclonal anti-blood group A antibody elicited autophosphorylation of the receptor for epidermal growth factor (EGF) isolated from the epidermal carcinoma cell line A431, the possibility has been raised (Feizi & Childs, 1985, 1987; Feizi, 1989) that on the cell membrane an endogenous blood group A-specific lectin may bind and serve to tune the state of activation of the growth factor receptor, which in this cell line is decorated with blood group A (Childs et al., 1984). εBP would seem a good candidate for such a modulatory role.

On mast cells, the IgE receptor, FcεR1, is one of the major glycoproteins bound by εBP through a lectin–carbohydrate interaction. It has been shown that this lectin, when added to the cultured rat basophilic leukemia cells, activates these cells to release serotonin (Frigeri et al., 1993) probably as a result of lectin-induced cross-linking of FcεR1. Interactions of εBP are likely to encompass lymphocytes also, as oligosaccharides of poly-*N*-acetyllactosamine type occur on glycoproteins of lymphocytes such as the T200 molecule (CD45) (Childs et al., 1983) and blood group A, B, and H antigens are expressed, albeit at low levels, on lymphocytes (adsorbed in the form of glycolipids from serum) in accordance with blood group and secretor status (Mollicone et al., 1988). This knowledge and the observations on mast cell activation (Frigeri et al., 1993) together with results from the present study raise the intriguing possibility that levels of susceptibilities to activation of various cells of the immune system and, by inference, allergic diatheses may be influenced by polymorphisms of carbohydrate antigens, particularly the blood group antigens. Knowledge of the repertoire of ligands for εBP will now facilitate the testing of these hypotheses.

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REFERENCES

Abbott, W. M., & Feizi, T. (1989) *Biochem. J. 259*, 291.
Abbott, W. M., & Feizi, T. (1991) *J. Biol. Chem. 266*, 5552.
Abbott, W. M., Mellor, A., Edwards, Y., & Feizi, T. (1989) *Biochem. J. 259*, 283.
Albrandt, K., Orida, N. K., & Liu, F.-T. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6859.
Barondes, S. H., Castronovo, V., Cooper, D. N. W., Cummings, R. D., Drickamer, K., Feizi, T., Gitt, M. A., Hirabayashi, J., Hughes, C. R., Kasai, K., Leffler, H., Liu, F.-T., Lotan, R., Mercurio, A. M., Monsigny, M., Pillai, S., Poirer, F., Raz, A., Rigby, P. W. J., Rini, J. M., & Wang, J. L. (1994) *Cell 76*, 597.
Carding, S. R., Thorpe, S. J., Thorpe, R., & Feizi, T. (1985) *Biochem. Biophys. Res. Commun. 127*, 680.
Castronovo, V., Campo, E., van den Brûle, F. A., Claysmith, A. P., Cioce, V., Liu, F.-T., Fernandez, P. L., & Sobel, M. E. (1992) *J. Natl. Cancer Inst. 84*, 1161.
Cherayil, B. J., Weiner, S. J., & Pillai, S. (1989) *J. Exp. Med. 170*, 1959.
Cherayil, B. J., Chaitovitz, S., Wong, C., & Pillai, S. (1990) *Proc. Natl. Acad. Sci. U.S.A. 87*, 7324.
Childs, R. A., & Feizi, T. (1980) *Cell Biol. Int. Rep. 4*, 775.
Childs, R. A., Dalchau, R., Scudder, P., Hounsell, E. F., Fabre, J. W., & Feizi, T. (1983) *Biochem. Biophys. Res. Commun. 110*, 424.
Childs, R. A., Gregoriou, M., Scudder, P., Thorpe, S. J., Rees, A. R., & Feizi, T. (1984) *EMBO J. 3*, 2227.
Childs, R. A., Drickamer, K., Kawasaki, T., Thiel, S., Mizuochi, T., & Feizi, T. (1989) *Biochem. J. 262*, 131.
Colligan, J. E., Kruisbeck, A. M., Margulies, D. H., Shevach, E. M., & Strober, W. S. (1991) *Curr. Protocols Immunol. 5.3.2.*
Drickamer, K. (1988) *J. Biol. Chem. 263*, 9557.
Egge, H., Kordowicz, W., Pater-Katalinic, J., & Hanfland, P. (1985) *J. Biol. Chem. 260*, 4927.
Feizi, T. (1985) *Nature 314*, 53.
Feizi, T. (1989) in *Carbohydrate Recognition in Cellular Function* (Bock, G., & Harnett, S., Eds.) Ciba Foundation Symposium 145, pp 62–79, Wiley, Chichester.
Feizi, T., & Childs, R. A. (1985) *Trends Biochem. Sci. 10*, 24.
Feizi, T., & Childs, R. A. (1987) *Biochem. J. 245*, 1.
Feizi, T., Stoll, M. S., Yuen, C.-T., Chai, W., & Lawson, A. M. (1994) *Methods Enzymol. 230*, 484.
Frigeri, L. G., & Liu, F.-T. (1992) *J. Immunol. 148*, 861.
Frigeri, L. G., Zuberi, R. I., & Liu, F.-T. (1993) *Biochemistry 32*, 7644.
Gooi, H. C., Hounsell, E. F., Picard, J. K., Lowe, A., Voak, D., Lennox, E., & Feizi, T. (1985) *J. Biol. Chem. 260*, 13218.
Greenwood, F. C., Hunter, W. M., & Glover, J. S. (1963) *Biochem. J. 89*, 114.
Hanfland, P., Egge, H., Dabrowski, U., Kuhn, S., Roelcke, D., & Dabrowski, J. (1981) *Biochemistry 20*, 5310.
Ho, M.-K., & Springer, T. A. (1982) *J. Immunol. 128*, 1221.
Hsu, D. K., Zuberi, R., & Liu, F.-T. (1992) *J. Biol. Chem. 267*, 14167.
Irimura, T., Matsushita, Y., Sutton, R. C., Carralero, D., Ohannesian, D. W., Cleary, K. R., Ota, D. M., Nicolson, G. L., & Lotan, R. (1991) *Cancer Res. 51*, 387.
Jia, S., & Wang, J. L. (1988) *J. Biol. Chem. 263*, 6009.
Knibbs, R. N., Perini, F., & Goldstein, I. J. (1989) *Biochemistry 28*, 6379.
Knibbs, R. N., Agrwal, N., Wang, J. L., & Goldstein, I. J. (1993) *J. Biol. Chem. 268*, 14940.
Laemmli, U. K. (1970) *Nature 227*, 680.
Laing, J. G., Robertson, M. W., Gritzmacher, C. A., Wang, J. L., & Liu, F.-T. (1989) *J. Biol. Chem. 264*, 1907.
Larkin, M., Ahern, T. J., Stoll, M. S., Shaffer, M., Sako, D., O'Brien, J., Yuen, C.-T., Lawson, A. M., Childs, R. A., Barone, K. M., Langer-Safer, P. R., Hasegawa, A., Kiso, M., Larsen, G. R., & Feizi, T. (1992) *J. Biol. Chem. 267*, 13667.
Lawson, A. M., Chai, W., Cashmore, G. C., Stoll, M. S., Hounsell, E. F., & Feizi, T. (1990) *Carbohydr. Res. 200*, 47.
Leffler, H., & Barondes, S. H. (1986) *J. Biol. Chem. 261*, 10119.
Leffler, H., Masiarz, F. R., & Barondes, S. H. (1989) *Biochemistry 28*, 9222.
Liu, F.-T. (1990) *CRC Crit. Rev. Immunol. 10*, 289.
Liu, F.-T. (1993) *Immunol. Today 14*, 486.
Liu, F.-T., Albrandt, K., Mendel, E., Kulczycki, A., Jr., & Orida, N. K. (1985) *Proc. Natl. Acad. Sci. U.S.A. 82*, 4100.
Lotz, M. M., Andrews, C. W., Jr., Korzelius, C. A., Lee, E. C., Steele, G. D., Jr., Clarke, A., & Mercurio, A. M. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 3466.
Massa, S. M., Cooper, D. N. W., Leffler, H., & Barondes, S. H. (1993) *Biochemistry 32*, 260.
Mizuochi, T., Taniguchi, T., Shimizu, A., & Kobata, A. (1982) *J. Immunol. 129*, 2016.
Mollicone, R., Calliard, T., Le Pendu, J., Francois, A., Sansonetti, N., Villarroya, H., & Oriol, R. (1988) *Blood 71*, 1113.
Moutsatsos, I. K., Wade, M., Schindler, M., & Wang, J. L. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 6452.
Oda, Y., Leffler, H., Sakakura, Y., Kasai, K., & Barondes, S. H. (1991) *Gene 99*, 279.
Raz, A., Pazerini, G., & Carmi, P. (1989) *Cancer Res. 49*, 3489.
Raz, A., Carmi, P., Raz, T., Hogan, V., Mohamed, A., & Wolman, S. R. (1991a) *Cancer Res. 51*, 2173.
Raz, A., Zhu, D., Hogan, V., Shah, N., Raz, T., Karkash, R., Pazerini, G., & Carmi, P. (1991b) *Int. J. Cancer 46*, 871.
Robertson, M. W., Albrandt, K., Keller, D., & Liu, F.-T. (1990) *Biochemistry 29*, 8093.
Sato, S., & Hughes, R. C. (1992) *J. Biol. Chem. 267*, 6983.
Sato, S., Burdett, I., & Hughes, R. C. (1993) *Exp. Cell Res. 207*, 8.
Solomon, J. C., Stoll, M. S., Penfold, P., Abbott, W. M., Childs, R. A., Hanfland, P., & Feizi, T. (1991) *Carbohydr. Res. 213*, 293.
Sparrow, C. P., Leffler, H., & Barondes, S. H. (1987) *J. Biol. Chem. 262*, 7383.
Stoll, M. S., & Hounsell, E. F. (1988) *Biomed. Chromatogr. 2*, 249.
Stoll, M. S., Mizuochi, T., Childs, R. A., & Feizi, T. (1988) *Biochem. J. 256*, 661.
Tang, P. W., Gooi, H. C., Hardy, M., Lee, Y. C., & Feizi, T. (1985) *Biochem. Biophys. Res. Commun. 132*, 474.
Truong, M.-T., Gruart, V., Kusnierz, J.-P., Papin, J.-P., Loiseau, S., Capron, A., & Capron, M. (1993) *J. Exp. Med. 177*, 243.
Wang, J. L., Werner, E. A., Laing, J. G., & Patterson, R. J. (1992) *Biochem. Soc. Trans. 20*, 269.
Wollenberg, A., De la Salle, H., Hanau, D., Liu, F.-T., & Bieber, T. (1993) *J. Exp. Med. 178*, 777.
Woo, H. J., Shaw, L. M., Messier, J. M., & Mercurio, A. M. (1990) *J. Biol. Chem. 265*, 7097.