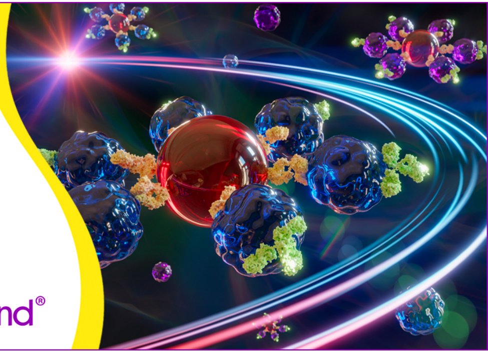
Advance allergy and asthma research by analyzing multiple cytokines with LEGENDplex™

Start multiplexing

BioLegend®



The Journal of Immunology

RESEARCH ARTICLE | APRIL 01 1995

A human lectin, galectin-3 (epsilon bp/Mac-2), stimulates superoxide production by neutrophils. FREE

A Yamaoka; ... et. al

J Immunol (1995) 154 (7): 3479–3487.
https://doi.org/10.4049/jimmunol.154.7.3479

Related Content

Galectin-3 Induces Death of *Candida* Species Expressing Specific β-1,2-Linked Mannans

J Immunol (October,2006)

Activated Rat Macrophages Produce a Galectin-1-Like Protein That Induces Apoptosis of T Cells: Biochemical and Functional Characterization

J Immunol (May,1998)

Activation of the Neutrophil Nicotinamide Adenine Dinucleotide Phosphate Oxidase by Galectin-1

J Immunol (April,2002)

Downloaded from http://journals.aai.org/jimmunol/article-pdf/154/7/3479/1068809/3479.pdf by guest on 18 April 2025

A Human Lectin, Galectin-3 (ϵbp/Mac-2), Stimulates Superoxide Production by Neutrophils[1]

Atsushi Yamaoka, Ichiro Kuwabara, Luciano G. Frigeri, and Fu-Tong Liu[2]

Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037

A family of soluble animal lectins, galectins, with β-galactoside-binding activity, is gaining increased attention. One member of this family, galectin-3, has been previously designated by this group as ϵbp, for its IgE-binding activity. On the basis of the saccharide specificity and other biochemical characteristics of ϵbp, it is possible that this lectin could have an important extracellular modulatory role, functioning through recognition of critical cell surface glycoproteins on many cell types. We present evidence here that recombinant human ϵbp activates human neutrophils in a dose-dependent manner as demonstrated by superoxide production. The observed activity is dependent on the lectin property of ϵbp intrinsic to its carboxyl-terminal domain, as it could be inhibited effectively by lactose, a known saccharide ligand of ϵbp. However, the amino-terminal domain is also necessary for the observed activity, as ϵbp-C (the carboxyl-terminal domain fragment) is devoid of neutrophil-activating activity, even though it retains the carbohydrate-binding property. Affinity purification of lysates from cell surface-radioiodinated neutrophils revealed two major protein bands of M_r 115,000 and M_r 180,000 that are recognized by ϵbp and preliminary data suggested that one of these proteins is NCA-160, a human carcinoembryonic Ag-related glycoprotein. This study thus lends further support to our view of an extracellular function for ϵbp and suggests that this protein has an important role in inflammation and host defense through modulating the function of neutrophils. *The Journal of Immunology,* 1995, 154: 3479–3487.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/154/7/3479/1068809/3479.pdf by guest on 18 April 2025

Animal lectins have been recognized as an important class of proteins that play key roles in a variety of biologic processes. A family of animal lectins, now termed galectins (1, 2), that possess the S-type carbohydrate recognition domain (3) is receiving increased attention, with the most extensively studied members of this family being proteins of 14 kDa and 29 to 31 kDa (reviewed in Refs. 4 and 5). The latter, now known as galectin-3 (1), has been designated as ϵbp by this laboratory for its IgE-binding activity (6–8) and as Cbp35 (9), Mac-2 (10, 11), L-29 (12, 13), and L-34 (14, 15) by various other groups. The amino-terminal half of the protein consists primarily of tandem repeats and the carboxyl-terminal half shares significant sequence similarities with the 14-kDa lectin (4). The amino acid sequences of galectin-3 in various species have been determined (see references cited above) as well as their carbohydrate specificities (16–19). Although the carboxyl-terminal domain of galectin-3 is known to contain the carbohydrate-binding site, the function of the amino-terminal domain is less well defined. However, we and others have shown that galectin-3 has a tendency to self-associate and this property appears to be critically dependent on the amino-terminal domain (20, 21).

Although galectins are well characterized biochemically, much remains to be learned about their functions. Galectin-3 has a wide tissue distribution and is expressed by many different cell types (22–24). It is found predominantly in the cytoplasm of quiescent fibroblasts but is located in the nucleus of proliferating cells (25), a finding that has led to the suggestion that this lectin may be a component of a growth regulating system. Galectin-3 has been identified as a major non-integrin laminin-binding protein on the surface of macrophages, thus suggesting a role in cell adhesion to extracellular matrices (26). Various studies by several laboratories have associated galectin-3 with tumor transformation and metastasis (27–29), although a more definitive role of this protein in these processes awaits further elucidation.

Received for publication August 1, 1994. Accepted for publication December 19, 1994.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] This work was supported by National Institutes of Health Grants AI20985 and AI32834 and a Dermatology Foundation Research Award sponsored by Dermik Laboratories. The General Clinical Research Center at Scripps Clinic is supported by National Institutes of Health Grant MO1 RR00833. This is publication number 8343-MEM of the Department of Molecular and Experimental Medicine.

[2] Address correspondence and reprint requests to Dr. Fu-Tong Liu, Department of Molecular and Experimental Medicine, SBR-4, The Scripps Research Institute, 10666 North Torrey Pines Road, La Jolla, CA 92037.

Copyright © 1995 by The American Association of Immunologists

0022-1767/95/$02.00

Several lines of evidence suggest a role for galectin-3 in inflammation. It was first identified as a macrophage cell surface Ag expressed on thioglycolate-elicited murine macrophages and not on resting macrophages (30). The expression of galectin-3 in human neutrophils was documented in a recent study that also implicated this protein as having a role in IgE-mediated activation of neutrophils (31). Although galectin-3 may have intracellular functions in these inflammatory cells, cumulative data support a possible extracellular role for this protein in inflammation, especially because it is secreted by various cell types (10, 32–34). On the basis of its known saccharide specificity (16, 17), galectin-3 is expected to recognize a spectrum of appropriately glycosylated glycoproteins on the cell surface of a variety of cells. As this lectin behaves bivalently (or multivalently) (20), it has the potential to cross-link these specific cell surface glycoproteins, and thereby modulate the functions of these cells. Because of these known properties of galectin-3, we recently proposed that this protein may be a broad spectrum biologic response modifier (5). In fact, we have shown that galectin-3 recognizes the mast cell high affinity IgE receptor in addition to IgE and that it activates rat basophilic leukemia cells (35).

Neutrophils play a crucial role in host defense through their bactericidal activities. The production of active oxygen species such as the superoxide anion (O_2^-) is an essential event and these reactive oxygen derivatives contribute to microbial killing. To establish a role for galectin-3 in inflammation and to further test the above-mentioned hypothesis, we studied the effects of this protein on the function of neutrophils. As an initial step, O_2^- production by human neutrophils exposed to recombinant human εbp was analyzed. Here we report that εbp is in fact capable of inducing human neutrophils to produce O_2^-. The activity was dependent on the lectin function of εbp because it was inhibited by saccharide ligands of the protein. Moreover, the activity requires also the presence of the amino-terminal domain, as the carboxyl-terminal domain fragment, which contains the lectin part, was inactive.

Materials and Methods

Reagents

Cytochrome *c* (Type III), cytochalasin B, FMLP, and PMA were obtained from Sigma Chemical Co. (St. Louis, MO). Collagenase (*Achromobacter iophagus*) and SOD[3] were from Boehringer Mannheim (Indianapolis, IN). Recombinant human εbp was produced in *Escherichia coli* and purified as previously described (20) and stored in PBS (10 mM sodium phosphate and 0.15 M NaCl, pH 7.2) containing 10% (v/v) glycerol at −85°C. Endotoxins were not detectable in the concentrated stock of the recombinant protein, by using a commercial kit (*Limulus* amebocyte lysate, Pyrogent, BioWhittaker, Walkersville, MD). On the basis of results obtained with the positive control, it was determined that the endotoxin level was less than 16.8 pg/ml in the solution containing 60 μg/ml εbp, which is the highest concentrations of εbp used in stimulation of neutrophils. The carboxyl-terminal domain fragment of εbp (εbp-C) was prepared by digesting recombinant εbp with collagenase as described previously (20). ^{125}I-Labeled εbp was prepared by using the chloramine-T method (36). The sp. act. of the radiolabeled protein was typically approximately 7×10^8 μCi/mmol.

Preparation of human neutrophils

Human polymorphonuclear leukocytes were purified from peripheral blood of healthy volunteers by dextran sedimentation and Ficoll-Paque (Pharmacia LKB, Uppsala, Sweden) density gradient centrifugation as described (37). The purity of the preparations was routinely >95%, as assessed by Wright-Giemsa staining.

Radioligand binding assay

Binding of radiolabeled εbp to human neutrophils was performed by mixing human neutrophils (5×10^5 cells) with ^{125}I-labeled εbp (0.42 pmol) in 120 μl of binding buffer (RPMI 1640 containing 20 mM HEPES, 0.3% BSA, and 0.1% sodium azide), pH 7.2. In some experiments, various concentrations of saccharides or unlabeled εbp were added. After incubation at 4°C for various time periods, 50-μl aliquots of the mixture were layered on 200 μl of dibutyl phthalate in 400-μl polyethylene microfuge tubes, which were subsequently centrifuged in a Beckman microfuge E (Beckman Instruments, Fullerton, CA) for 2 min. The tips of the microfuge tubes containing the cell pellets were then sliced off and counted in a gamma counter.

Assay of superoxide formation

O_2^- production by neutrophils was monitored by the well-established cytochrome *c* assay (38). This assay is based on the reduction by O_2^- of ferric cytochrome *c* to ferrous cytochrome *c*, resulting in an absorbance change at 550 nm. This reduction is inhibitable by SOD. Two different methods were used and both were performed at 37°C. In the first method, a double-beam spectrophotometer (Uvikon 810, Kontron, Zürich, Switzerland) with an electrically controlled temperature regulator was used. Human neutrophils (2.5×10^6/ml), suspended in 750 μl of PBS containing 0.5 mM $MgCl_2$, 0.8 mM $CaCl_2$, and 7.5 mM glucose, pH 7.4, were placed in both sample and reference cuvettes. Both cuvettes also contained cytochrome *c* (75 μM) and cytochalasin B (5 μg/ml). The reference cuvette also contained 60 μg/ml of SOD. After preincubation for 5 min, the stimulants were added to both cuvettes and the absorbance change at 550 nm was monitored.

In the second method, samples were placed in 96-well plates, the absorbance change was monitored with a kinetic microplate reader (ThermoMax, Molecular Devices, Menlo Park, CA), and the data were analyzed by using enzyme kinetic software (Softmax version 2.01) as described (39). The constituents of each assay mixture were the same as described above, except that the volume of each reaction was reduced to 250 μl. The O_2^--generating activity, expressed as maximal rate, was calculated by using a molar extinction coefficient of 20.5×10^3 M^{-1} cm^{-1} and a light path of 0.6 cm as previously described (39). As the concentrated εbp stock contained glycerol, controls containing appropriate concentrations of glycerol were also included. At the concentrations tested, glycerol did not induce O_2^- release.

For the demonstration of the inhibition of εbp activity by saccharides, aliquots of 0.5 M solutions of lactose, galactose or arabinose were added to both the sample and reference cuvettes to a final concentration of 20 mM after full activation of O_2^- production had been induced by εbp. In other experiments, εbp or FMLP, a known activator of neutrophils was mixed with serially diluted solutions of lactose, galactose, or arabinose and the mixtures were added to the neutrophil suspensions.

Treatment of neutrophils with collagenase-digested εbp

A total of 7.5 μg of εbp was mixed with 1.5 U/ml collagenase and the mixture was either added immediately to the neutrophil suspensions or first incubated at 37°C for 6 h before adding to the neutrophil suspensions. In some experiments, the solution of collagenase (3 U/ml) was denatured by heating at 100°C for 5 min before being added to εbp. The mixture was then further incubated at 37°C for 6 h.

[3] Abbreviations used in this paper: SOD, superoxide dismutase; CEA, carcinoembryonic Ag; εbp-C, carboxyl-terminal domain of εbp; NCA, nonspecific cross-reacting Ag.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/154/7/3479/1068809/3479.pdf by guest on 18 April 2025

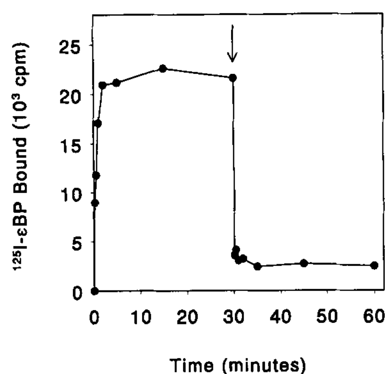


FIGURE 1. Time course of ^{125}I-labeled ϵbp binding to neutrophils. Human neutrophils (5 × 10^5) were incubated with ^{125}I-labeled ϵbp (3.5 nM) for the indicated time periods at 4°C. Bound ^{125}I-labeled ϵbp was determined as described in *Materials and Methods*. The *arrow* denotes the time of the addition of lactose to a final concentration of 25 mM. The values represent the means of duplicate measurements. Similar results were obtained in three separate experiments.

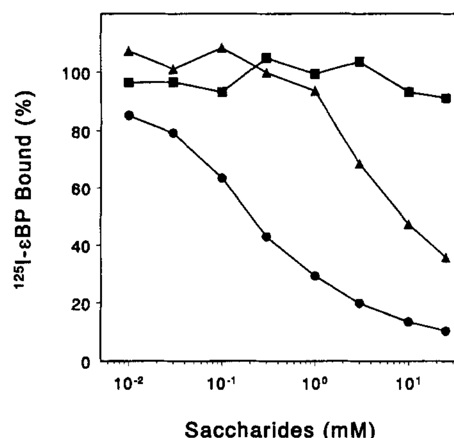


FIGURE 2. Inhibition by saccharides of ^{125}I-labeled ϵbp binding to neutrophils. Human neutrophils (5 × 10^5) were incubated with ^{125}I-labeled ϵbp (3.5 nM) at 4°C for 30 min in the presence of various concentrations of saccharides (lactose, galactose, and arabinose). Bound ^{125}I-labeled ϵbp was determined as described in *Materials and Methods*. The percent bound ^{125}I-labeled ϵbp was calculated on the basis of binding without saccharide as being 100%. The values represent the means of duplicate measurements. Similar results were obtained in two separate experiments. ●—●, lactose; ▲—▲, galactose; ■—■, arabinose.

Affinity purification of neutrophils cell surface glycoproteins that are recognized by ϵbp

Affinity purification of radioiodinated cell surface proteins. Human neutrophils were radioiodinated on the cell surface as described (35, 40). The cells were lysed and the proteins that are recognized by ϵbp were affinity purified by a previously described procedure (35) with some modifications. Briefly, the lysate (50 μl of 5 × 10^7 cells/ml) was incubated with ϵbp-sepharose beads (50 μl) in the presence or absence of 100 mM lactose for 3 h at 4°C. The bound proteins were eluted by the SDS-PAGE sample buffer and analyzed by 10% PAGE under reducing conditions (41), followed by autoradiography.

Immunoblotting analysis of affinity-purified proteins. Human neutrophils were ruptured by a freeze-thaw cycle and centrifuged, and the pellet was then extracted with a buffer containing 1% Triton X-100 as described (42). The solubilized membrane proteins were added to ϵbp-Sepharose 4B and the bound proteins were subsequently eluted as described above. The isolated proteins were resolved by SDS-PAGE and transferred to nitrocellulose membrane by electroblotting (43). The blots were incubated with polyclonal anti-CEA Ag IgG (Dako Corp., Carperteria, CA; 1:500 dilution) for 3 h at 4°C, followed by the addition of goat anti-rabbit IgG conjugated to alkaline phosphatase (Zymed Laboratories, San Francisco, CA; 1: 2000 dilution) for 30 min at room temperature. The blots were then developed with a chemiluminescence protein detection kit (Western-Light, Tropix, Bedford, MA) by following the manufacturer's instructions.

Results

Binding of ϵbp to human neutrophils

As an initial step in assessing the effects of ϵbp on the function of neutrophils, we tested the binding of recombinant human ϵbp to human neutrophils. Figure 1 shows that when ^{125}I-labeled ϵbp was added to human neutrophils, there was rapid binding of the radiolabel to the cells with the reaction being completed within 2 min. The binding of ϵbp to the neutrophils was dependent on its lectin function, as the binding was nearly completely reversed upon addition of lactose, which is known to bind ϵbp (Fig. 1).

To further assess the nature of the lectin-carbohydrate interaction in the binding of ϵbp to neutrophils, the effects of various saccharides, including lactose, galactose, and arabinose, were evaluated quantitatively. Affinity of these saccharides for ϵbp is known to be lactose > galactose >> arabinose (20, 44). As shown in Figure 2, lactose inhibited the binding of ^{125}I-labeled ϵbp in a dose-dependent manner, with an IC_{50} of 0.2 mM. Galactose was less effective with an IC_{50} of 9.1 mM. The relative inhibitory activity of lactose and galactose approximates their relative affinity for ϵbp (20). As expected, arabinose was ineffective.

The effect of unlabeled ϵbp on the binding of radiolabeled ϵbp was then evaluated. Interestingly, addition of unlabeled ϵbp resulted in dose-dependent increased binding of ^{125}I-labeled ϵbp to the neutrophils instead of inhibition (Fig. 3). Maximal binding of radiolabeled ϵbp was reached with ϵbp at 1 μM and the binding then declined as the concentrations of the unlabeled ϵbp increased. When the data were converted to the total ϵbp bound to neutrophils, a dose-response curve was generated that indicated increased binding as the concentration of ϵbp increases (Fig. 3).

Induction of human neutrophil O_2^- production by ϵbp

Purified human neutrophils were treated with recombinant human ϵbp at 1 μM (30 μg/ml) in the presence or absence of cytochalasin B. Figure 4*A* shows that, in the presence of cytochalasin B, ϵbp caused prompt release of O_2^- without a notable lag time (*curve a*). The initial burst of O_2^- release, which continued for approximately 5 min, was followed by a slower release that lasted for more than an hour

Downloaded from http://journals.aai.org/jimmunol/article-pdf/154/7/3479/1068809/3479.pdf by guest on 18 April 2025

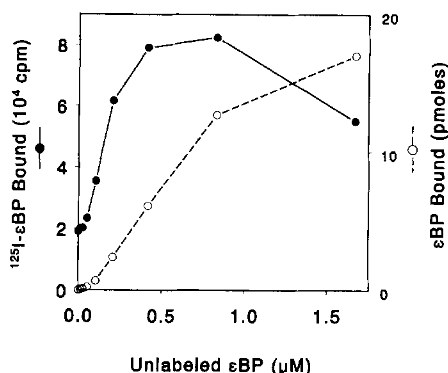


FIGURE 3. Effect of addition of various amounts of unlabeled ϵbp on ^{125}I-labeled ϵbp binding to neutrophils. Human neutrophils (5×10^5) were incubated with ^{125}I-labeled ϵbp (3.5 nM) at 4°C for 30 min in the presence of various amounts of unlabeled ϵbp (0.1 to 1.7 μM). Bound ^{125}I-labeled ϵbp was determined as described in *Materials and Methods* (closed circles). The total amounts of bound ϵbp is also shown (open circles), which was calculated from the percentage of ^{125}I-labeled ϵbp bound and the concentrations of total ϵbp present in the mixture. The values represent the means of duplicate measurements. Similar results were obtained in two separate experiments.

(data not shown). Cytochalasin B was necessary for maximal stimulation, as in its absence there was a lag time of greater than 1 min for the O_2^- release to be observed and the rate of O_2^- release was much slower (Fig. 4*A*, *curve b*). Although not shown, cytochalasin B by itself is incapable of inducing O_2^- production. The adjuvant role of cytochalasin B for the ϵbp activity is consistent with the well documented effect of this agent on neutrophil activation caused by various stimulants (45, 46).

The effect of ϵbp concentration (10 to 60 μg/ml) on O_2^- production by human neutrophils was then tested. As shown in Figure 4*B*, recombinant human ϵbp functioned in a dose-dependent manner. The effect of ϵbp reached a maximal level at a concentration of 1.5 μM (45 μg/ml), and half-maximal effect was observed at 0.7 μM (22 μg/ml). No significant effect was observed at ϵbp concentrations less than 0.33 μM (10 μg/ml). Although not shown, FMLP, a well known activator of neutrophils, was included for comparison and the maximal activity of ϵbp was found to be approximately 25% of that of FMLP (both in the presence of cytochalasin B).

Effect of saccharides on ϵbp-induced O_2^- production

We next tested whether the activity of ϵbp is dependent on its lectin function. As before, three saccharides (lactose, galactose and arabinose) were used in an inhibition assay. In the first set of experiments, human neutrophils were exposed to 1 μM (30 μg/ml) of ϵbp for approximately 2 min, until the maximum rate of O_2^- generation was achieved, and then lactose or arabinose was added to a

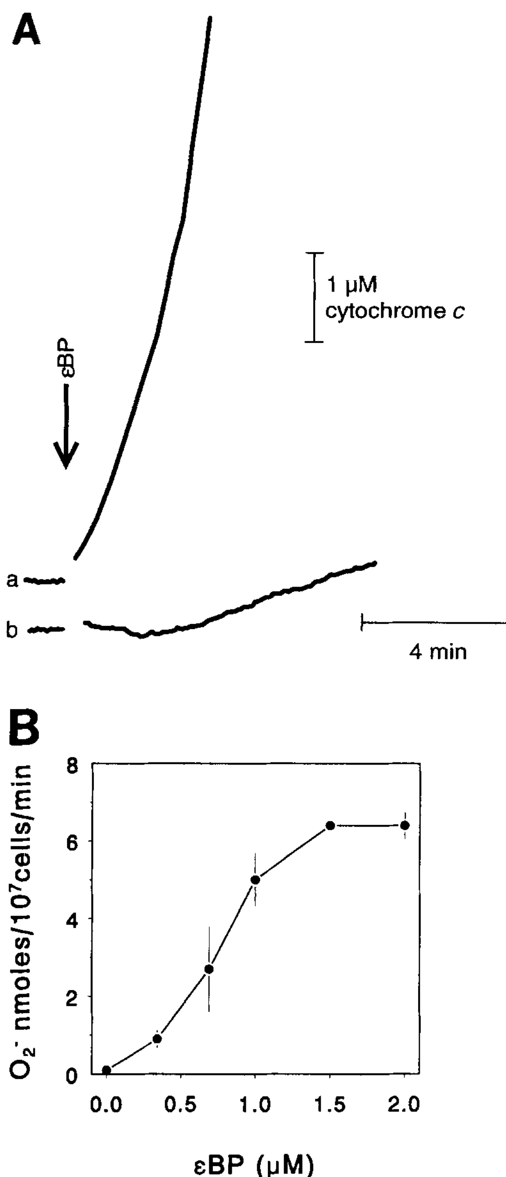


FIGURE 4. *A*: The release of superoxide from human neutrophils induced by ϵbp. Human neutrophils (2.5×10^6/ml) were suspended in 0.75 ml of PBS (pH 7.4) containing 0.5 mM $MgCl_2$, 0.8 mM $CaCl_2$, and 7.5 mM glucose and cytochrome *c* in the presence or absence of cytochalasin B. SOD was added to the reference cuvette. The mixture was preincubated at 37°C for 5 min before the addition of ϵbp (final concentration of 1 μM; 30 μg/ml). The absorbance change at 550 nm was recorded with a double-beam spectrophotometer. *Curve a*, with cytochalasin B; *curve b*, without cytochalasin B. *B*: Effect of ϵbp concentration on superoxide production by human neutrophils. Neutrophils (2.5×10^6/ml) in 0.25 ml of PBS (pH 7.4) containing 5 μg/ml cytochalasin B, 75 μM cytochrome *c*, 0.5 mM $MgCl_2$, 0.8 mM $CaCl_2$, and 7.5 mM glucose were preincubated for 5 min at 37°C in 96-well plates. The control samples also contained SOD. Serially diluted solutions of ϵbp were added to each well, the absorbance change at 550 nm was monitored with a kinetic microplate reader, and the readings were converted to the rate of O_2^- generation. The data points represent means ± SD of three experiments.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/154/7/3479/1068809/3479.pdf by guest on 18 April 2025

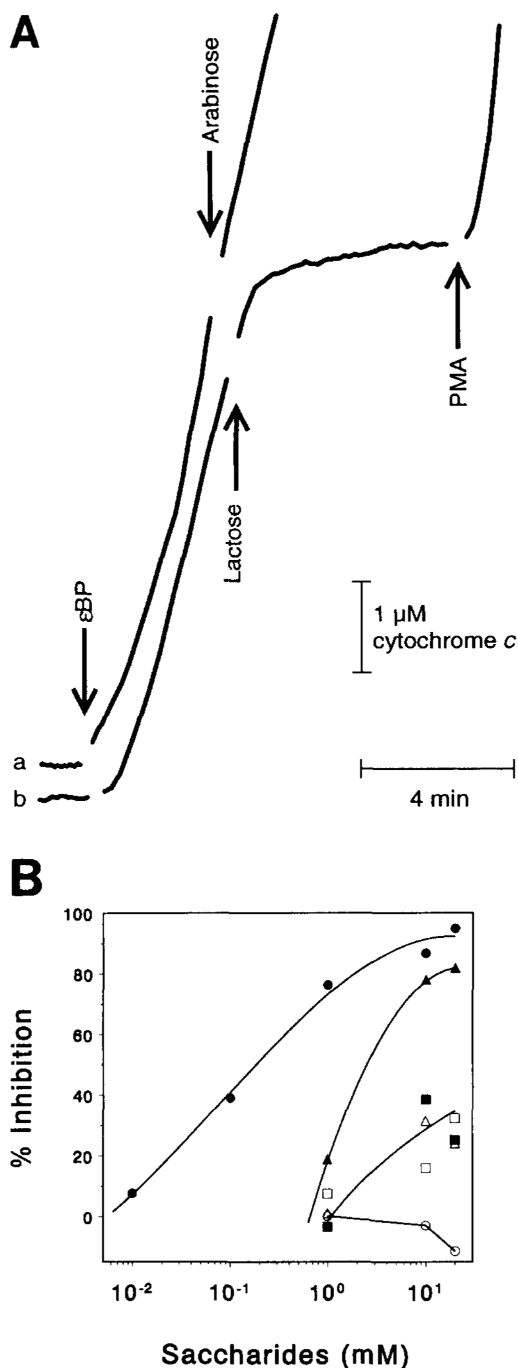


FIGURE 5. *A*: Inhibition of εbp-induced superoxide production by saccharides. Following the addition of εbp to neutrophils as described in Figure 4*A*, lactose or arabinose was added, to a final concentration of 20 mM, at the time points indicated by *arrows*. To the samples that received lactose, PMA was subsequently added to a final concentration of 0.2 μg/ml, and the absorbance change at 550 nm was recorded. Similar results were obtained in three separate experiments. *B*: Effect of saccharide concentration on εbp-induced superoxide production by human neutrophils. The indicated concentrations of saccharides were added to the neutrophil suspensions prepared as described in Figure 4*B*. The mixtures were incubated for 5 min at 37°C in 96-well plates. Subsequently, εbp or FMLP was added to a final concentration of 1 μM (30 μg/ml) and 0.1 μM, respectively, and the absorbance change was recorded and used to calculate the percent inhibition. The data points represent means of three experiments. ● and ○, lactose; ▲ and △, galactose; ■ and □, arabinose; closed symbols, εbp; open symbols, FMLP.

final concentration of 20 mM. As shown in Figure 5*A*, O_2^- release diminished within 1 min after addition of lactose but was not affected by addition of arabinose. To the group that received lactose, subsequent addition of PMA induced rapid O_2^- release, indicating that lactose did not affect the O_2^--producing NADPH oxidase or neutrophil cellular function. The fact that arabinose had no effect indicates that lactose inhibition was mediated by inhibition of εbp and was not due to nonspecific effects such as osmolarity changes.

We next tested the dose dependency of various saccharides. In these experiments, the effects of these sugars on O_2^- induction by FMLP were also included. As shown in Figure 5*B*, lactose inhibited the activity of εbp in a dose-dependent fashion with an IC_{50} of 0.18 mM. The specificity of lactose inhibition in this case was supported by its lack of any effect on FMLP-induced O_2^- release. Galactose was much less effective than lactose with an apparent IC_{50} of 2.5 mM. However, galactose at 10 mM also inhibited FMLP-induced O_2^- production, indicating that this saccharide might have a nonspecific inhibitory activity on neutrophils. Arabinose also inhibited εbp-induced O_2^- release at 10 mM, although it did not show any inhibition at 1 mM. Similarly to galactose, the inhibitory activity of this saccharide at high concentrations appears to be nonspecific as it also inhibited FMLP-induced O_2^- production. It is to be noted also that the partial inhibition by arabinose is unique to this experimental condition, as no inhibition was detected in the experiment described in Figure 5*A*, in which the saccharide was added after a maximal rate of O_2^- production was already reached.

Amino-terminal domain requirement for εbp activity

We and several other groups have previously shown that the amino-terminal domain of εbp can be digested by collagenase (14, 20, 21, 47, 48). Because, in our view, the amino-terminal portion is critical for the function of εbp (see *Discussion*), we tested the effect of collagenase digestion on the activity of εbp by incubating εbp with collagenase as described in *Materials and Methods*, before addition to the neutrophils. Table I shows that εbp was nearly quantitatively inactivated after the collagenase treatment. Incubation of εbp in the buffer alone or in the presence of heat-denatured collagenase did not have a significant effect. To exclude the possibility that the effect of collagenase treatment is due to the action of the collagenase directly on neutrophils, a mixture of εbp and collagenase was added to the neutrophils without previous

Downloaded from http://journals.aai.org/jimmunol/article-pdf/154/7/3479/1068809/3479.pdf by guest on 18 April 2025

Table I. *Effect of collagenase on O_2^--inducing activity of ϵBP and FMLP*[a]

Treatment	Superoxide Production (% of Control)	
	ϵbp (1 μM)	FMLP (0.1 μM)
None	100	100
Stimulant incubated with PBS	92	84
Stimulant incubated with collagenase	4	93
Stimulant incubated with heat-denatured collagenase	94	89
Mixture of stimulant and collagenase without preincubation	100	99
Stimulant heated at 100°C	3	94

[a] ϵBP and FMLP were treated with collagenase as described in *Materials and Methods* and tested for O_2^- induced as described in Figure 4. The results are representative of three experiments.

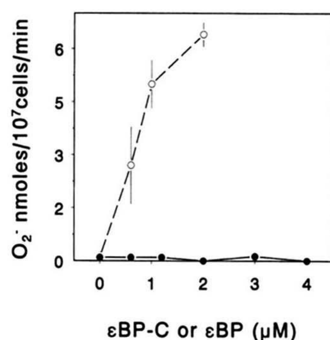


FIGURE 6. Comparison between ϵbp and ϵbp-C on induction of superoxide production by neutrophils. To human neutrophil suspensions prepared as described in Figure 4*B* were added various concentrations of ϵbp or ϵbp-C. The rate of O_2^- generation was estimated by measuring the absorbance change at 550 nm as described in Figure 4*B*. The data points represent means of three experiments. ○- - -○, ϵbp; ●—●, ϵbp-C.

incubation. The initial rate of O_2^- production induced by ϵbp was not affected by such a treatment. As a specificity control, the effect of collagenase treatment on the O_2^--inducing activity of FMLP was analyzed. As shown in Table I, collagenase treatment did not have any effect on the activity of FMLP.

To definitively prove that the ϵbp activation of neutrophils requires the intact amino-terminal domain, the carboxyl-terminal domain of ϵbp (ϵbp-C) was tested for superoxide production exactly as described above. As shown in Figure 6, ϵbp-C in the concentration range of 0.6 to 4 μM (10 to 60 μg/ml) did not induce neutrophil activation, whereas ϵbp in the same experiment was active between 0.5 and 2 μM.

Identification of neutrophil cell surface glycoproteins that are recognized by ϵbp

We next proceeded to identify neutrophil cell surface proteins that are recognized by ϵbp and thus are potentially involved in the ϵbp-induced neutrophil activation. Human neutrophils were cell surface radioiodinated, the cell lysate was mixed with ϵbp-Sepharose 4B, and the affinity-purified proteins were analyzed by SDS-PAGE. *Lane 1* of Figure 7*A* is the profile of the total cell surface radioiodinated proteins, which showed several major bands accompanied by many minor bands. In contrast, a much simpler pattern consisting of two major bands, of M_r 115,000 and 180,000, and some minor bands, were found in the affinity-purified material (*lane 2*). The lectin function of ϵbp is critical for its binding of these proteins, as the binding was diminished in the presence of lactose (*lane 3*).

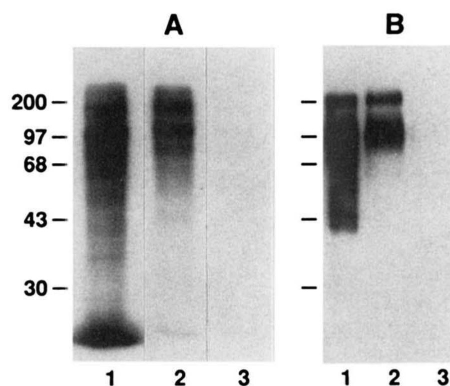


FIGURE 7. Analysis of human neutrophil cell surface proteins that are recognized by ϵbp. *A*: SDS-PAGE analysis of ^{125}I-labeled protein. Human neutrophils were radioiodinated, the cell lysates were mixed with ϵbp-Sepharose 4B and the bound proteins were eluted and analyzed by 10% PAGE and autoradiography. *Lane 1*, total cell lysate; *lane 2*, proteins bound to ϵbp-Sepharose 4B; *lane 3*, proteins bound to ϵbp-Sepharose 4B in the presence of lactose. The numbers on the left margin denote the positions of m.w. markers ($\times 10^{-3}$). *B*: Immunoblotting analysis of neutrophil membrane proteins recognized by ϵbp. Human neutrophil membrane proteins were extracted and mixed with ϵbp-Sepharose 4B. The bound proteins were subjected to immunoblotting analysis with anti-CEA Abs. *Lane 1*, total membrane protein extract; *lane 2*, proteins bound to ϵbp-Sepharose 4B; *lane 3*, proteins bound to ϵbp-Sepharose 4B in the presence of lactose.

Existing information in the literature suggests that neutrophil CEA-related NCA-160 (CD66) carries an oligosaccharide sequence well recognized by ϵbp (see *Discussion*). We therefore tested whether NCA-160 is one of the major proteins recognized by ϵbp, by immunoblotting analysis with anti-CEA Abs which are known to cross-react with NCA-160. Consistent with a previous report (49), the Abs detected a number of proteins in the neutrophil cell lysate (Fig. 7*B*, *lane 1*). In contrast, only two bands were detected in the protein material recovered from ϵbp-Sepharose 4B, an upper band of M_r 180,000 and a lower broad band of M_r 105,000 (*lane 2*). Based on the apparent m.w. and the reactivity to anti-CEA Abs, we concluded that the

Downloaded from http://journals.aai.org/jimmunol/article-pdf/154/7/3479/1068809/3479.pdf by guest on 18 April 2025

upper band could be NCA-160. The binding of these proteins by ϵbp is also dependent on lectin-carbohydrate interactions, as it was inhibited by lactose (*lane 3*).

The possibility that cross-linkage of NCA on the neutrophil cell surface can induce superoxide production was tested. Rabbit anti-CEA antiserum or control serum (both at 1:50 dilution) was added to human neutrophils and the superoxide production was determined in the same manner as that described for ϵbp. In the presence of cytochalasin B, anti-CEA antiserum caused significantly higher superoxide production as compared with the control serum (mean $\pm$ SD from a triplicate experiment: 3.78 ± 0.24 vs 0.2 ± 0.02 nmol/10^7 cells/min). As in the case for ϵbp, the effect of the antiserum is critically dependent on cytochalasin B, as in its absence, the antiserum induced negligible superoxide production.

Discussion

A major conclusion of this work is that human ϵbp, a β-galactoside-binding lectin, is capable of binding to and activating human neutrophils. First, we showed that ϵbp bound rapidly to the surfaces of human neutrophils and the binding was dependent on the lectin function of this protein, as it could be inhibited completely by its known saccharide ligands. Second, we demonstrated that ϵbp induced O_2^- production by neutrophils in a dose-dependent manner. The dose-response curve of ϵbp-induced O_2^- production paralleled that of the binding of ϵbp to neutrophils (Fig. 3 vs Fig. 4*B*). The O_2^--inducing activity of ϵbp is also critically dependent on its lectin function as shown by the saccharide inhibition study. Furthermore, the amino-terminal domain of ϵbp is essential, as previous treatment with collagenase, which is known to digest the amino-terminal portion of ϵbp, abolished its O_2^--inducing activity. In addition, ϵbp-C, the carboxyl-terminal domain fragment that contains the lectin part, is inactive. Third, our initial effort has identified two major ϵbp-binding cell surface glycoproteins. Finally, the fact that the O_2^--inducing activity could be inhibited by various saccharides in a fashion that matches the known affinity of these agents to ϵbp and the fact that the activity was sensitive to the collagenase treatment strongly support the idea that the observed activity is intrinsic to ϵbp and not due to any possible contamination.

We also found that the binding of radiolabeled ϵbp was potentiated by the addition of increased concentrations of unlabeled ϵbp. This result is not surprising as we and others have previously noted cooperativity in binding of ϵbp to IgE (20) and laminin (21). We have proposed that ϵbp has a tendency to form noncovalent dimers or oligomers, through self-association, when present above a threshold concentration (20). Furthermore, this property is dependent on the amino-terminal domain of the protein (20). The ϵbp-C also bound to human neutrophils in a carbohydrate-dependent manner but did not exhibit the cooperative binding noted for ϵbp (data not shown). The fact that the dose-response curve of O_2^- induction by ϵbp matches that of ϵbp binding, and that ϵbp-C is inactive in O_2^- induction, strongly suggests that ϵbp self-association is also pertinent to the observed neutrophil activation. This in turn implies that cross-linkage of appropriately glycosylated cell surface glycoconjugates by ϵbp may be involved in neutrophil activation induced by this lectin. The involvement of cross-linkage of cell surface receptors in cellular activation is in keeping with current views on the initiation of transmembrane signaling in leukocytes (50).

As the activity of ϵbp is dependent on its lectin function, it is likely to be related to this protein's recognition of cell surface glycoconjugates that may be involved in neutrophil activation pathways. Our results are consistent with the concept that activation of neutrophils can be achieved through protein-carbohydrate interactions, as previously supported by the activity of Con A in stimulating O_2^- production by neutrophils (51). Importantly, however, unlike Con A, ϵbp is an endogenous animal lectin and therefore may well play a role in the activation of neutrophils. At the concentrations that stimulate superoxide production, ϵbp induces negligible myeloperoxidase release (less than 5% of that induced by FMLP; data not shown).

The glycoproteins on the cell surface of neutrophils that are recognized by ϵbp need to be more fully characterized. We have suspected that ϵbp might bind to NCA-160, a CEA-related glycoprotein, on the basis of the following information: NCA-160 carries carbohydrate groups Lewis^x and sialyl Lewis^x (49) and sialyl Lewis^x-containing lactosaminoglycan isolated from human neutrophils also carries paralacto-*N*-neohexaose (52), an oligosaccharide sequence well recognized by ϵbp (19). We have shown that one of the major ϵbp-binding glycoproteins is NCA-160 and have obtained preliminary data suggesting that cross-linkage of NCA by anti-CEA Abs on neutrophils, in the presence of cytochalasin B, can indeed lead to superoxide production. These two observations suggest that aggregation of NCA-160 by ϵbp may be important for neutrophil activation. However, additional investigations are necessary for firmly establishing the involvement of NCA-160 in ϵbp's induction of superoxide production by neutrophils. It has been reported that cross-linking of NCA on neutrophils with anti-CEA Abs induce cell aggregation but not NADPH oxidase activation (53). However, those experiments were performed in the absence of cytochalasin B and oxygen consumption was measured instead of superoxide production. It should also be noted that NCA seem to appear on the surface of neutrophils after the cells have degranulated. Therefore, ϵbp's activation of neutrophils through these Ags may occur only in those cells that are undergoing degranulation, perhaps triggered by other stimulants.

Recently, Sato and Hughes (54) reported that ϵbp recognizes three major glycoproteins of molecular masses of

Downloaded from http://journals.aai.org/jimmunol/article-pdf/154/7/3479/1068809/3479.pdf by guest on 18 April 2025

92, 125, and 180 kDa in thioglycolate-elicited mouse peritoneal macrophages. Whether any of these proteins are species homologues of those we identified on neutrophils is unknown at this time. Previously, Rosenberg et al. (55) identified two glycoproteins (98 kDa and 70 kDa) as the major protein ligands recognized by ϵbp in human colon carcinoma cells. One of the proteins was subsequently found to contain a domain that is highly homologous to the macrophage scavenger receptor cysteine-rich domain (56). We have also shown that on the cell surface of rat mast cells, the high affinity IgE receptor is the major glycoprotein recognized by ϵbp (35). Therefore, it appears that ϵbp is likely to recognize different sets of glycoproteins in different cell types. Consequently, it becomes increasingly apparent that ϵbp may have a broad spectrum of functions mediated by recognition of a wide range of glycoproteins.

Our findings suggest an extracellular function for ϵbp that may be either physiologic or pathologic. The requirement for relatively high concentrations of ϵbp, however, deserves further comment. Although ϵbp lacks a classical signal sequence, exocytosis of this lectin from a number of cell types has been well documented (10, 32, 33). Furthermore, ϵbp is known to be present at relatively high concentrations (e.g., >135 μg/ml (21)) in the cytosol of various cell types. Therefore, it is possible that under the influence of certain specific stimuli, or in the event of cell injury, a burst release of cytosolic ϵbp may occur, resulting in a transient high local concentration of this lectin.

Recently, Truong et al. (31) reported that human neutrophils express ϵbp and that IgE immune complexes activate neutrophils, possibly through ϵbp expressed on the cell surface, because the process can be inhibited by a monoclonal anti-ϵbp Ab. Whether IgE-mediated activation of neutrophils involves the same pathway as does the direct activation of neutrophils by ϵbp reported herein remains to be determined. The fact that neutrophils contain ϵbp and may also release this protein suggests that ϵbp may activate this cell type in an autocrine or paracrine fashion. Moreover, ϵbp is expressed by a variety of cell types including many inflammatory cells (5, 11, 31, 57). Therefore, situations in which this lectin, derived from a particular cell type, may act on neighboring neutrophils could be envisioned. As neutrophils are a key cell type involved in inflammation and host defense, it is possible that endogenous soluble lectins, such as ϵbp, may play an important role by modulating the function of neutrophils. We have previously shown that ϵbp can activate mast cells, probably through cross-linking of the IgE receptor (35). The finding that ϵbp can also activate neutrophils suggests that ϵbp may have wide-ranging effects in a variety of physiologic and pathologic processes.

Acknowledgments

The authors thank Drs. John Apgar and Robert Rubin for critically reviewing the manuscript, Paul Hill for assistance in preparation of the recombinant ϵbp and for critical reading of the manuscript, and Velda Comstock for excellent assistance in preparation of the manuscript.

References

1. Barondes, S. H., V. Castronovo, D. N. W. Cooper, R. D. Cummings, K. Drickamer, T. Feizi, M. A. Gitt, J. Hirabayashi, C. Hughes, K. Kasai, H. Leffler, F.-T. Liu, R. Lotan, A. M. Mercurio, M. Monsigny, S. Pillai, F. Poirer, A. Raz, P. W. J. Rigby, J. M. Rini, and J. L. Wang. 1994. Galectins: a family of animal β-galactoside-binding lectins (Letter to the Editor). *Cell 76:597.*
2. Barondes, S. H., D. N. W. Cooper, M. A. Gitt, and H. Leffler. 1994. Galectins: structure and function of a large family of animal lectins. *J. Biol. Chem. 269:20807.*
3. Drickamer, K. 1988. Two distinct classes of carbohydrate-recognition domains in animal lectins. *J. Biol. Chem. 263:9557.*
4. Liu, F.-T. 1990. Molecular biology of IgE-binding protein, IgE-binding factors and IgE receptors. *CRC Crit. Rev. Immunol. 10:289.*
5. Liu, F.-T. 1993. S-type mammalian lectins in allergic inflammation. *Immunol. Today 14:486.*
6. Liu, F.-T., K. Albrandt, E. Mendel, A. Kulczycki Jr, and N. K. Orida. 1985. Identification of an IgE-binding protein by molecular cloning. *Proc. Natl. Acad. Sci. USA 82:4100.*
7. Albrandt, K., N. K. Orida, and F.-T. Liu. 1987. An IgE-binding protein with a distinctive repetitive sequence and homology with an IgG receptor. *Proc. Natl. Acad. Sci. USA 84:6859.*
8. Robertson, M. W., K. Albrandt, D. Keller, and F.-T. Liu. 1990. Human IgE-binding protein: a soluble lectin exhibiting a highly conserved interspecies sequence and differential recognition of IgE glycoforms. *Biochemistry 29:8093.*
9. Jia, S., and J. L. Wang. 1988. Carbohydrate binding protein 35. Complementary DNA sequence reveals homology with proteins of the heterogeneous nuclear RNP. *J. Biol. Chem. 263:6009.*
10. Cherayil, B. J., S. J. Weiner, and S. Pillai. 1989. The Mac-2 antigen is a galactose-specific lectin that binds IgE. *J. Exp. Med. 170:1959.*
11. Cherayil, B. J, S. Chaitovitz, C. Wong, and S. Pillai. 1990. Molecular cloning of a human macrophage lectin specific for galactose. *Proc. Natl. Acad. Sci. USA 87:7324.*
12. Leffler, H., F. R. Masiarz, and S. H. Barondes. 1989. Soluble lactose-binding vertebrate lectins: a growing family. *Biochem. 28:9222.*
13. Oda, Y., H. Leffler, Y. Sakakura, K. Kasai, and S. H. Barondes. 1991. Human breast carcinoma cDNA encoding a galactoside-binding lectin homologous to mouse Mac-2 antigen. *Gene 99:279.*
14. Raz, A., G. Pazerini, and P. Carmi. 1989. Identification of the metastasis-associated, galactoside-binding lectin as a chimeric gene product with homology to an IgE-binding protein. *Cancer Res. 49:3489.*
15. Raz, A., P. Carmi, T. Raz, V. Hogan, A. Mohamed, and S. R. Wolman. 1991. Molecular cloning and chromosomal mapping of a human galactoside-binding protein. *Cancer Res. 51:2173.*
16. Leffler, H., and S. H. Barondes. 1986. Specificity of binding of three soluble rat lung lectins to substituted and unsubstituted mammalian β-galactosides. *J. Biol. Chem. 261:10119.*
17. Sparrow, C. P., H. Leffler, and S. H. Barondes. 1987. Multiple soluble β-galactoside-binding lectins from human lung. *J. Biol. Chem. 262:7383.*
18. Knibbs, R. N., N. Agrwal, J. L. Wang, and I. J. Goldstein. 1993. Carbohydrate-binding protein 35. II. Analysis of the interaction of the recombinant polypeptide with saccharides. *J. Biol. Chem. 268:14940.*
19. Feizi, T., J. C. Solomon, C.-T. Yuen, K. C. G. Jeng, L. G. Frigeri, D. K. Hsu, and F.-T. Liu. 1994. Adhesive specificity of the soluble human lectin, IgE-binding protein (ϵbp), towards lipid-linked oligosaccharides: presence of the blood group A, B, B-like and H monosaccharides confers a binding activity to tetrasaccharide (lacto-*N*- and lacto-*N*-neo-tetraose) backbones. *Biochemistry 33:6342.*
20. Hsu, D. K., R. Zuberi, and F.-T. Liu. 1992. Biochemical and biophysical characterization of human recombinant IgE-binding protein, an S-type animal lectin. *J. Biol. Chem. 267:14167.*

Downloaded from http://journals.aai.org/jimmunol/article-pdf/154/7/3479/1068809/3479.pdf by guest on 18 April 2025

21. Massa, S. M., D. N. W. Cooper, H. Leffler, and S. H. Barondes. 1993. L-29, an endogenous lectin, binds to glycoconjugate ligands with positive cooperativity. *Biochemistry 32:260.*
22. Gritzmacher, C. A., M. W. Robertson, and F.-T. Liu. 1988. IgE-binding protein: subcellular location and gene expression in many murine tissues and cells. *J. Immunol. 141:2801.*
23. Crittenden, S. L., C. F. Roff, and J. L. Wang. 1984. Carbohydrate-binding protein 35: identification of the galactose-specific lectin in various tissues of mice. *Mol. Cell. Biol. 4:1252.*
24. Flotte, T. J., T. A. Springer, and G. J. Thorbecke. 1983. Dendritic cell and macrophage staining by monoclonal antibodies in tissue sections epidermal sheets. *Am. J. Pathol. 111:112.*
25. Moutsatsos, I. K., M. Wade, M. Schindler, and J. L. Wang. 1987. Endogenous lectins from cultured cells: nuclear localization of carbohydrate-binding protein 35 in proliferating 3T3 fibroblasts. *Proc. Natl. Acad. Sci. USA 84:6452.*
26. Woo, H. J., L. M. Shaw, J. M. Messier, and A. M. Mercurio. 1990. The major non-integrin laminin binding protein of macrophages is identical to carbohydrate binding protein 35 (Mac-2). *J. Biol. Chem. 265:7097.*
27. Raz, A., D. Zhu, V. Hogan, N. Shah, T. Raz, R. Karkash, G. Pazerini, and P. Carmi. 1991. Evidence for the role of 34-kDa galactoside-binding lectin in transformation and metastasis. *Int. J. Cancer 46: 871.*
28. Castronovo, V., E. Campo, F. A. van den Brûle, A. P. Claysmith, V. Cioce, F.-T. Liu, P. L. Fernandez, and M. E. Sobel. 1992. Inverse modulation of steady state mRNA levels of two non-integrin laminin binding proteins in human colon carcinoma. *J. Natl. Cancer Inst. 84:1161.*
29. Lotz, M. M., C. W. Andrews, Jr., C. A. Korzelius, E. C. Lee, G. D. Steele, Jr., A. Clarke, and A. M. Mercurio. 1993. Decreased expression of Mac-2 (carbohydrate binding protein 35) and loss of its nuclear localization are associated with the neoplastic progression of colon carcinoma. *Proc. Natl. Acad. Sci. USA 90:3466.*
30. Ho, M.-K., and T. A. Springer. 1982. Mac-2, a novel 32,000 M_r mouse macrophage subpopulation-specific antigen defined by monoclonal antibodies. *J. Immunol. 128:1221.*
31. Truong, M.-T., V. Gruart, J.-P. Kusnierz, J.-P. Papin, S. Loiseau, A. Capron, and M. Capron. 1993. Human neutrophils express immunoglobulin E (IgE)-binding proteins (Mac-2/εbp) of the S-type lectin family: role in IgE-dependent activation. *J. Exp. Med. 177: 243.*
32. Lindstedt, R., G. Apodaca, S. H. Barondes, K. E. Mostov, and H. Leffler. 1993. Apical secretion of a cytosolic protein by Madin-Darby canine kidney cells: evidence for polarized release of an endogenous lectin by a nonclassical secretory pathway. *J. Biol. Chem. 268:11750.*
33. Sato, S., I. Burdett, and R. C. Hughes. 1993. Secretion of the baby hamster kidney 30-kDa galactose-binding lectin from polarized and nonpolarized cells: a pathway independent of the endoplasmic reticulum-Golgi complex. *Exp. Cell Res. 207:8.*
34. Wollenberg, A., H. De la Salle, D. Hanau, F.-T. Liu, and T. Bieber. 1993. Human keratinocytes release the endogenous β-galactoside-binding soluble lectin εbp (IgE-binding protein) which binds to Langerhans cells where it modulates their binding capacity for IgE glycoform. *J. Exp. Med. 178:777.*
35. Frigeri, L. G., R. I. Zuberi, and F.-T. Liu. 1993. εbp, a β-galactoside-binding animal lectin, recognizes IgE receptor (FcεRI) and activates mast cells. *Biochemistry 32:7644.*
36. McConahey, P. J., and F. J. Dixon. 1966. A method of trace iodination of proteins for immunological studies. *Int. Arch. Allergy Appl. Immunol. 29:185.*
37. Böyum, A. 1968. Isolation of mononuclear cells and granulocytes from human blood: isolation of mononuclear cells by one centrifugation and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand. J. Clin. Lab. Invest. 21(Suppl. 97):77.*
38. Curnutte, J. T., R. S. Kipnes, and B. M. Babior. 1975. Defect in pyridine nucleotide dependent superoxide production by a particulate fraction from the granulocytes of patients with chronic granulomatous disease. *N. Engl. J. Med. 293:628.*
39. Mayo, L. A., and J. T. Curnutte. 1990. Kinetic microplate assay for superoxide production by neutrophils and other phagocytic cells. In *Methods in Enzymology,* Vol. 186. Academic Press, New York. p. 567.
40. Morrison, M. 1974. The determination of the exposed proteins in membranes by the use of lactoperoxidase. In *Methods in Enzymology,* Vol. 32. S. R. Colowick and N. O. Kaplan, eds. Academic Press, New York, p. 103.
41. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature 227:680.*
42. Stocks, S. C., M. Albrechtsen, and M. A. Kerr. 1990. Expression of the CD15 differentiation antigen (3-fucosyl-*N*-acetyl-lactosamine, Le^x) on putative neutrophil adhesion molecules CR3 and NCA-160. *Biochem. J. 268:275.*
43. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA 76: 4350.*
44. Frigeri, L. G., M. W. Robertson, and F.-T. Liu. 1990. Expression of biologically active recombinant rat IgE-binding protein in *Escherichia coli. J. Biol. Chem. 265:20763.*
45. Cohen, M. S., J. A. Metcalf, and R. K. Root. 1980. Regulation of oxygen metabolism in human granulocytes: relationship between stimulus binding and oxidative response using plant lectins as probes. *Blood 55:1003.*
46. Zabucchi, G., G. Berton, and M. R. Soranzo. 1981. Mechanism of the potentiating effect of cytochalasin B on the respiratory burst induced by concanavalin A in leucocytes. *FEBS Lett. 125:165.*
47. Ochieng, J., D. Platt, L. Tait, V. Hogan, T. Raz, P. Carmi, and A. Raz. 1993. Structure-function relationship of a recombinant human galactoside-binding protein. *Biochemistry 32:4455.*
48. Agrwal, N., Q. Sun, S.-Y. Wang, and J. L. Wang. 1993. Carbohydrate-binding protein 35. I. Properties of the recombinant polypeptide and the individuality of the domains. *J. Biol. Chem. 268:14932.*
49. Stocks, S. C., and M. A. Kerr. 1993. Neutrophil NCA-160 (CD66) is the major protein carrier of selectin binding carbohydrate groups $Lewis^x$ and sialyl $Lewis^x$. *Biochem. Biophys. Res. Commun. 195: 478.*
50. Metzger, H. 1992. Transmembrane signaling: the joy of aggregation. *J. Immunol. 149:1477.*
51. Cohen, H. J., M. E. Chovaniec, M. K. Wilson, and P. E. Newburger. 1982. Con-A-stimulated superoxide production by granulocytes: reversible activation of NADPH oxidase. *Blood 60:1188.*
52. Fukuda, M., E. Spooncer, J. E. Oates, A. Dell, and J. C. Klock. 1984. Structure of sialylated fucosyl lactosaminoglycan isolated from human granulocytes. *J. Biol. Chem. 259:10925.*
53. Kuijpers, T. W., C. E. van der Schoot, M. Hoogerwerf, and D. Roos. 1993. Cross-linking of the carcinoembryonic antigen-like glycoproteins CD66 and CD67 induces neutrophil aggregation. *J. Immunol. 151:4934.*
54. Sato, S., and R. C. Hughes. 1994. Regulation of secretion and surface expression of Mac-2, a galactoside-binding protein of macrophages. *J. Biol. Chem. 269:4424.*
55. Rosenberg, I., B. J. Cherayil, K. J. Isselbacher, and S. Pillai. 1991. Mac-2-binding glycoproteins: putative ligands for a cytosolic β-galactoside lectin. *J. Biol. Chem. 26:18731.*
56. Koths, K., E. Taylor, R. Halenbeck, C. Casipit, and A. Wang. 1993. Cloning and characterization of a human Mac-2-binding protein, a new member of the superfamily defined by the macrophage scavenger receptor cysteine-rich domain. *J. Biol. Chem. 268:14245.*
57. Truong, M.-J., V. Gruart, F.-T. Liu, L. Prin, A. Capron, and M. Capron. 1993. Human eosinophils express functional IgE-binding molecules (Mac-2/εbp) of the S-type lectin family. *Eur. J. Immunol. 23:3230.*

Downloaded from http://journals.aai.org/jimmunol/article-pdf/154/7/3479/1068809/3479.pdf by guest on 18 April 2025