

HETEROGENEOUS IgE GLYCOFORMS CHARACTERIZED BY DIFFERENTIAL RECOGNITION OF AN ENDOGENOUS LECTIN (IgE-BINDING PROTEIN)¹

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IgE is highly glycosylated, but the function of the oligosaccharide side chains is largely unknown. The previous discovery of an animal lectin, IgE-binding protein (εBP), affords an opportunity to study potential carbohydrate-dependent effector functions of IgE. εBP is a β-galactoside-specific lectin with binding affinity for IgE and is now known to be equivalent to carbohydrate-binding protein 35 and the Mac-2 Ag; thus, it may have multiple functions in addition to IgE binding. We have previously shown that rat rεBP recognizes sialidase-treated human myeloma IgE to a much greater extent than the untreated IgE. In contrast, human εBP binds essentially equivalently to a monoclonal murine IgE with or without sialidase pretreatment. To validate a possible role for εBP in the IgE system, we investigated the pattern of recognition of εBP for various polyclonal human IgE samples. We show that polyclonal IgE derived from four individuals with hyper-IgE syndrome or atopic dermatitis recognizes εBP and that there is individual variation in the proportion of IgE recognized by εBP, ranging from >60% for one sample to almost undetectable levels in another. We conclude that εBP does indeed recognize polyclonal IgE and that this recognition is modulated by sialylation of IgE oligosaccharides. Furthermore, there exist different IgE glycoforms, varying in the degree of sialylation, and these are distributed in a distinct manner in different individuals.

IgE is a glycoprotein with a high carbohydrate content. Although the carbohydrate structures of one human myeloma IgE were determined many years ago, there has been little progress in the understanding of the significance and function of the carbohydrates on IgE. This report describes our finding of heterogeneity in glycosylation of human polyclonal IgE, as characterized by differential recognition by a lectin that we have previously termed εBP.³

εBP is a β-galactoside-specific lectin with IgE-binding activity that was originally identified from rat basophilic leukemia cells (1, 2). Structural details of the 31-kDa protein have been revealed through molecular cloning studies, including the identification of a unique two-domain polypeptide structure (3, 4). Subsequently, rat εBP was shown to be the species homologue of a murine lectin, carbohydrate-binding protein 35 (5). Studies conducted in other laboratories indicate that εBP is related or identical to proteins designated L-34 (6) and RL-29/HL-29 (7, 8). Recently, the identity of the murine homologue of εBP with the macrophage cell surface marker Mac-2 (9) and the macrophage non-integrin laminin-binding protein (10) has been established.

A spectrum of biologic function for εBP is gradually emerging through compilation of the data from the studies cited above. In addition to IgE-binding activity, this protein has been identified as (i) a possible component of the heterogeneous nuclear ribonucleoprotein complex (11), (ii) being related to neoplastic transformation and the capacity of tumor cells to metastasize (12), and (iii) a cell surface protein in macrophages with a possible role in both lectin-mediated phagocytosis (9) and cell matrix adhesion (10). Therefore, our current view on εBP is that it is multifunctional, with IgE-binding as one of the functions. However, the precise role of this lectin in the IgE system remains an important subject of investigation.

We recently reported the cDNA sequence of human εBP, together with the unexpected finding of differential recognition of IgE glycoforms by εBP (13). Although the binding of εBP from various species, including both rat rεBP and human native εBP, to murine monoclonal IgE has been conclusively established (14), we could not demonstrate the binding of rεBP or native human εBP to human myeloma IgE (myeloma PS). The binding could, however, be exhibited by the myeloma IgE after pretreatment with sialidase. We interpreted these data as an indication of the presence of different IgE glycoforms that vary, at least, in the extent of sialylation. This conclusion is supported by the observation that the natural ligand of εBP lectin is a β-galactoside structure and that ligand binding is diminished when the galactose residue is derivatized with sialic acid (7, 8). Several explanations of our findings were considered, including (i) an inherent species-dependent pattern of sialylation of IgE, i.e., more extensive sialylation of human IgE, compared with rodent IgE, resulting in differential lectin recognition, and (ii) the possibility that human myeloma IgE is aberrantly (i.e., more extensively) sialylated, relative to IgE derived from nontransformed cells. We favored the second explanation and, further, suggested that there may be carbohydrate heterogeneity in IgE, reflected in

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³ Abbreviations used in this paper: εBP, IgE-binding protein; GAHE, goat anti-human IgE; RAGG, rabbit anti-goat γ-globulin; HRF, histamine-releasing factor.

differential recognition by ϵ BP.

In order to clarify the findings described above, in particular the nature of ϵ BP-IgE interaction and whether this interaction exists with polyclonal human IgE, we studied the reactivity of IgE from patients with high levels of IgE. We have characterized polyclonal IgE from three patients with hyper-IgE syndrome and one patient with atopic dermatitis, and we established that polyclonal IgE does indeed bind ϵ BP. Significantly, we uncovered IgE heterogeneity, as reflected by a subpopulation of IgE that is reactive with ϵ BP and variation of the size of such subpopulations between individuals.

MATERIALS AND METHODS

Reagents. ϵ BP was prepared as described (13), and a sample was kindly provided by Dr. L. Frigeri (Scripps Clinic and Research Foundation, La Jolla, CA). PS IgE was kindly provided by Dr. K. Ishizaka (La Jolla Institute for Allergy and Immunology, La Jolla, CA) and other myeloma IgE (DES and DZA) were a generous gift of Dr. H. Spiegelberg (University of California, San Diego, CA). Murine hybridoma IgE (H1-DNP- ϵ -26.82) was prepared as described (15). GAHE was obtained from Dr. D. Katz (Medical Biology Institute, La Jolla, CA) (16). RAGG and human IgG, IgM, and IgA were from Calbiochem, Inc. (La Jolla, CA). Immobilized antibodies and ϵ BP-Sepharose 4B were prepared by conjugation to CNBr-activated Sepharose 4B (Pharmacia, Piscataway, NJ), following the manufacturer's protocol. The following ratios of protein to CNBr-activated Sepharose 4B (dry weight) were used: GAHE-Sepharose 4B, 10 mg/1 g; RAGG-Sepharose 4B, 1 mg/0.2 g; and ϵ BP-Sepharose 4B, 1 mg/0.4 g.

Plasma with high IgE levels. Plasma samples from patients with hyper-IgE syndrome (lots 533767, 211067, and 796915) were obtained from Scripps Laboratories (San Diego, CA). The IgE levels of these plasma samples were 25,000 IU/ml. A patient with atopic dermatitis (C. N.) was referred by Dr. D. Mathison (Scripps Clinic, La Jolla, CA), and the plasma used for this study contained 9000 IU/ml IgE. The IgE concentrations were determined with a radioallergosorbent (RAST) kit from Pharmacia.

Preparation of ^{125} I-labeled polyclonal human IgE. Polyclonal human IgE sera (100 μ l) were first mixed with GAHE-Sepharose 4B (50 μ l of beads) for 3 h at 4°C. Thereafter, the beads were washed with 3×1 ml of 1% Triton X-100, 10 mM Tris-HCl, pH 7.5, 5 mM Na₂EDTA, 150 mM NaCl (TEN-1%TX-100), 1×1 ml of PBS, and 2×1 ml of 100 mM phosphate buffer, pH 7.5 (PB). The beads were then suspended in 50 μ l of PB and iodinated by using 1 mCi of Na¹²⁵I by the chloramine T method (17). After washing of the beads with 3×1 ml of TEN-1%TX-100, iodinated protein was eluted with 4×0.2 ml of 0.1 M glycine, pH 2.3, into separate tubes containing 50 μ l of 1 M Tris-HCl, pH 8.8. To the pooled eluate were added 100 μ l of 5% BSA/PBS. Typically, the total radioactivity was 1.9 to 2.9×10^6 cpm/100 μ l. Thereafter, the material was absorbed against RAGG-Sepharose 4B (50 μ l) for 1 h at 4°C, to remove soluble GAHE (detached from the immunoadsorbent). Aliquots were then treated with sialidase, and a portion was incubated with GAHE-Sepharose 4B or ϵ BP-Sepharose 4B, as described below.

Two-cycle affinity purification of IgE. Proteins (250 μ l) bound to GAHE-Sepharose 4B, radioiodinated, and then eluted as described above were mixed with 200 μ l of normal human plasma, and the mixture was exposed to GAHE-Sepharose 4B (50 μ l) for 2 h at 4°C. The beads were washed and the bound protein was eluted as described above.

Radioiodination of myeloma IgE and GAHE. Human myeloma IgE PS and DES and affinity-purified GAHE were radiolabeled with Na¹²⁵I by the chloramine T method (17), by using 10 μ g of the protein and 0.5 mCi of the isotope.

Sialidase treatment of ^{125} I-labeled IgE. To ^{125} I-labeled myeloma IgE PS or purified human polyclonal IgE (200 μ l) was added 0.2 U of sialidase (from *Clostridium perfringens*; Sigma, St. Louis, MO). The mixture was incubated at 37°C for 1 h and immediately processed as described below.

Binding of ^{125} I-IgE to ϵ BP-Sepharose 4B or GAHE-Sepharose 4B. Fifty microliters of ϵ BP-Sepharose 4B or GAHE-Sepharose 4B suspended in 100 μ l of 0.5% BSA/PBS were mixed with ^{125} I-IgE (100 μ l; with and without prior sialidase treatment) and incubated for 3 h at 4°C with gentle mixing. For lactose-inhibition experiments, beads were suspended in 100 μ l of 300 mM lactose before addition of the labeled IgE. After incubation, beads were washed with 3×1 ml of TEN-1%TX-100 and 1×1 ml of 62.5 mM Tris-HCl, pH 6.8, and bound protein was eluted with 100 μ l of 1.5% SDS-PAGE sample buffer (62.5 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 5% 2-mercaptoethanol), by boiling for 3 min. Samples were then analyzed

by γ -counting, SDS-PAGE on a 10% polyacrylamide gel (18), and autoradiography.

Immunoblot analysis of polyclonal human IgE. Purified polyclonal IgE, obtained as described above except without the iodination step, was fractionated by SDS-PAGE and immunoblotted by using ^{125} I-labeled affinity-purified GAHE, essentially as described (19).

Binding of ^{125} I-labeled IgE to GAHE-Sepharose 4B and inhibition of binding by myeloma IgE. ^{125} I-labeled IgE (polyclonal IgE, two-cycle affinity purified on GAHE-Sepharose 4B and then bound to and eluted from ϵ BP-Sepharose 4B) or ^{125} I-labeled myeloma IgE (1300 to 5000 cpm, 20 μ l) was added to (i) 20 μ l of plain Sepharose 4B plus 20 μ l of PBS or (ii) 20 μ l of GAHE-Sepharose 4B/plain Sepharose 4B (1:9) plus 20 μ l of PBS or myeloma IgE or polyclonal IgG, IgA, or IgM in PBS (GAHE-Sepharose 4B was "diluted" with plain Sepharose 4B so that much less myeloma IgE would be consumed in the competitive inhibition experiments). The mixture was incubated at 4°C for 2 h and then centrifuged at $15,600 \times g$ at 4°C for 2 min. The clear supernatants were transferred to fresh tubes and the beads were washed with 50 μ l of TEN-1%TX-100, followed by centrifugation as described above. The pooled supernatants were then counted in an automated γ -counter. The percentage bound was calculated as $[(S_p - S_g)/S_p] \times 100\%$, where S_p = cpm of supernatants from tubes containing plain Sepharose 4B only and S_g = cpm of supernatants from tubes containing GAHE-Sepharose 4B. The percentage of inhibition was calculated as $[(B_0 - B_x)/B_0] \times 100\%$, where B_0 = percentage bound in the absence of any inhibitor and B_x = percentage bound in the presence of an inhibitor. All experiments were done in duplicates.

RESULTS

IgE binds to solid-phase ϵ BP. Previously, the interaction of IgE with ϵ BP was demonstrated with IgE immobilized on Sepharose 4B (1–3, 5, 13, 14). For the purpose of establishing the reactivity of human polyclonal IgE to ϵ BP, we elected to use as the principal method of analysis the measurement of IgE binding to immobilized ϵ BP, owing, in part, to the practical difficulty of obtaining sufficient quantities of polyclonal IgE. Previous findings established the cross-reactivity of rat ϵ BP with human IgE (when desialylated) (14) and, because of the recent availability of homogeneous rat ϵ BP (13), we chose to use the recombinant lectin to evaluate reactivity with polyclonal human IgE. As shown in Figure 1, ϵ BP presented on the solid-phase maintained the capacity to recognize radio-labeled murine monoclonal IgE in a lactose-inhibitable

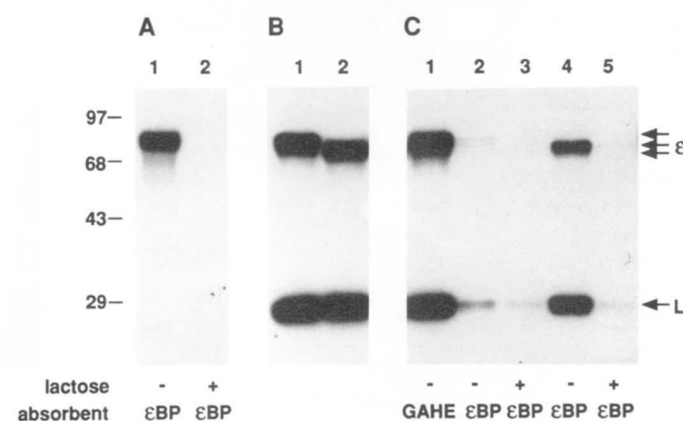


Figure 1. Binding of murine IgE and human myeloma IgE to ϵ BP-Sepharose 4B. Radioiodinated murine monoclonal anti-DNP IgE (H1-DNP- ϵ -26.82) and human myeloma IgE (PS) were bound to ϵ BP-Sepharose 4B in the presence or absence of lactose and, in the case of human IgE, also to GAHE-Sepharose 4B. The bound protein was eluted and analyzed by SDS-PAGE and autoradiography. Human myeloma IgE was also treated with sialidase; both treated and untreated IgE samples were processed as described above. A, murine IgE bound to ϵ BP-Sepharose in the absence (lane 1) or presence (lane 2) of lactose. B, human myeloma IgE PS untreated (lane 1) or treated (lane 2) with sialidase. C, human myeloma IgE PS bound to GAHE-Sepharose 4B (lane 1) or to ϵ BP-Sepharose 4B in the absence (lane 2) or presence (lane 3) of lactose; sialidase-treated human IgE bound to ϵ BP-Sepharose 4B in the absence (lane 4) or presence (lane 5) of lactose. Arrows (right margin), locations of the ϵ -chain and L chain of IgE. Numbers (left margin), m.w. ($\times 10^{-3}$) markers.

manner (Fig. 1A, lane 1 vs lane 2). There was minimal binding of myeloma IgE (PS) to solid-phase rBP (Fig. 1C, lane 2 vs lane 1), a result consistent with previous results obtained by using a somewhat different approach (14). In addition, the substantial binding of sialidase-treated PS IgE to rBP (Fig. 1C, lane 4) and the essentially quantitative inhibition of binding by lactose (Fig. 1C, lane 5) paralleled our earlier findings. The effect of sialidase digestion of IgE was demonstrated by the decrease in size of the IgE H (ϵ) chain, which is known to contain exclusively the carbohydrate content of IgE (20), as shown by SDS-PAGE (Fig. 1B). Interestingly, murine monoclonal IgE was nearly exclusively radioiodinated on the H chain, as evident by the detection of only the ϵ -chain on the autoradiographs (Fig. 1A), whereas both H and L chains of human myeloma IgE PS were labeled.

Polyclonal human IgE is recognized by ϵ BP. We were then poised to address the question of whether human polyclonal IgE is recognized by ϵ BP and to what extent sialidase treatment is required to modulate this recognition. Sera from four patients with hyper-IgE syndrome were studied, and initial experiments suggested reactivity of IgE from some of these patients with ϵ BP. We then focused on one of these sera (patient 533) and did an extensive analysis. IgE present in the sera was affinity purified by using highly specific GAHE conjugated to Sepharose 4B, and the purified IgE was subsequently iodinated. The radiolabeled IgE was then absorbed to either GAHE-Sepharose 4B or rBP-Sepharose 4B, and the specifically bound material was eluted and analyzed by SDS-PAGE and autoradiography.

As shown in Figure 2, proteins purified by this approach exhibited three major bands on SDS-PAGE, M_r 80,000 and 25,000 bands, consistent with the size of ϵ -chain and L chain, respectively, of IgE, and a M_r 54,000

band. The identity of the M_r 54,000 polypeptide is unknown at this time. However, it is not IgG, because this protein was not absorbed by anti-IgG antibodies or protein A (data not shown). By comparing IgE bound to GAHE and ϵ BP adsorbents, it is evident that there was substantial binding of the radiolabeled IgE to ϵ BP (Fig. 2A, lane 2 vs lane 1). Based on densitometric analysis of the autoradiographs in reference to IgE bound to GAHE and ϵ BP, respectively, it was determined that about 60% of IgE was recognized by ϵ BP. The specificity of binding of IgE to ϵ BP was established by the complete inhibition of binding by lactose (Fig. 2A, lane 3). Although not shown, when serum from a normal healthy donor (with a very low level of IgE) was subjected to the same analysis, bands characteristic of IgE were not detected on SDS-PAGE.

To further establish that the protein detected in the experiment described above is IgE, additional purification of the radiolabeled IgE was performed. Thus, IgE that was purified by GAHE-Sepharose 4B and radiolabeled was rebound to GAHE-Sepharose 4B a second time, in the presence of unlabeled normal human plasma, with the desire that nonspecific binding components of the radiolabeled proteins (in particular, M_r 54,000 protein) would be diminished by competitive binding of the corresponding unlabeled proteins possibly also present in normal human plasma. The material so purified was then bound to rBP-Sepharose 4B. As shown in Figure 2B, there was a substantial enrichment of IgE (ϵ -chain and L chain), compared with the contaminants.

With the highly purified IgE, we proceeded with experiments to further substantiate the identity of IgE bound to ϵ BP. The protein was mixed with GAHE-Sepharose 4B in the presence of varying concentrations of three different human myeloma IgE proteins, PS, DES, and DZA. All three myeloma IgE inhibited the binding of the radiolabeled protein to GAHE, in a dose-dependent manner, whereas human IgG at comparable doses was unable to inhibit the binding (Fig. 3A). The inhibition curve is qualitatively similar to the inhibition of binding of one of the myeloma IgE (DES) to GAHE by other myeloma IgE (Fig. 3B). Interestingly, in both cases, the myeloma IgE PS, which was the immunogen used in raising the GAHE

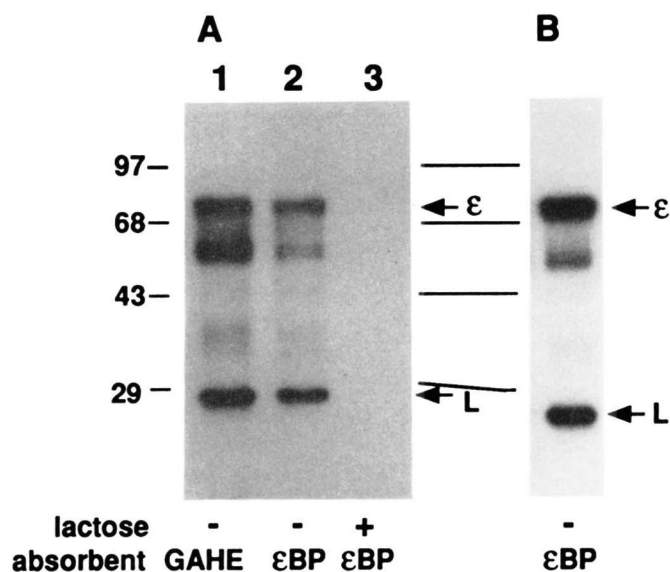


Figure 2. Binding of human polyclonal IgE to rBP-Sepharose 4B. A, plasma from a patient with hyper-IgE syndrome (patient 533) was mixed with GAHE-Sepharose 4B, and the bound protein was radioiodinated, eluted, and rebound to GAHE-Sepharose 4B (lane 1) or rBP-Sepharose 4B in the absence (lane 2) or presence (lane 3) of lactose. The bound protein was eluted and analyzed by SDS-PAGE and autoradiography. B, protein eluted from the first cycle of GAHE-Sepharose 4B described in A was repurified with GAHE-Sepharose 4B in the presence of normal human plasma. The two-cycle-purified IgE was exposed to rBP-Sepharose 4B and the bound and eluted protein was analyzed as in A. Arrows (right margin), locations of the ϵ -chain and L chain of IgE. Numbers (left margin), m.w. ($\times 10^{-3}$) markers.

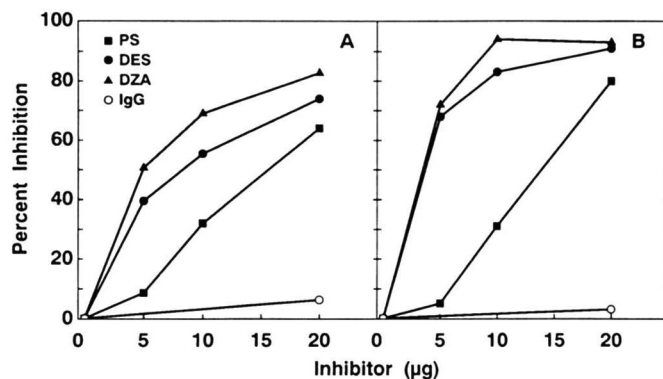


Figure 3. Inhibition of binding of ^{125}I -labeled IgE to GAHE-Sepharose 4B by myeloma IgE. A, ^{125}I -labeled human polyclonal IgE eluted from rBP-Sepharose 4B (Fig. 2B) was bound to GAHE-Sepharose in the presence of 5, 10, or 20 μg of human myeloma IgE PS, DES, or DZA or 20 μg of human polyclonal IgG. The percentage of IgE bound to GAHE-Sepharose 4B and percentage of inhibition by various inhibitors were calculated as described in *Materials and Methods*. In the absence of any inhibitor, 81% of the radiolabeled protein was bound. B, the binding of ^{125}I -labeled human myeloma IgE (DES) to GAHE-Sepharose 4B was analyzed as in A. The symbols used for each inhibitor are as in A. In the absence of any inhibitor, 90% of the radiolabeled protein was bound.

used for this study, was the least active inhibitor, perhaps reflecting a certain degree of denaturation of this IgE protein. Because all three myeloma proteins are highly purified, each exhibiting only the ϵ -chain and L chain on SDS-PAGE (data not shown), these results convincingly established that the protein affinity purified by GAHE-Sepharose and subsequently bound to and eluted from ϵ BP-Sepharose 4B is IgE. In turn, we then concluded that polyclonal human IgE is indeed recognized by ϵ BP.

There is individual variation in the size of a subpopulation of IgE recognized by ϵ BP, reflecting differential sialylation. Having established the method to analyze the reactivity of human polyclonal IgE to ϵ BP, we proceeded with a comparison of three sera from patients with hyper-IgE syndrome. As shown in Figure 4, the three sera, containing comparable levels of total IgE (25,000 IU/ml), afforded similar quantities of radiolabeled IgE after affinity purification with GAHE-Sepharose 4B (Fig. 4A, lanes 1 to 3). In contrast, the amount of IgE recog-

nized by ϵ BP varied, particularly from the serum of patient 796, which contained a very low level of IgE bindable to ϵ BP (Fig. 4A, lane 6 vs lane 3), whereas serum from patient 211 contained ϵ BP-bindable IgE comparable to that of patient 533 (Fig. 4A, lane 5 vs lane 4). Thus, it appears that the variance detected is a reflection of different proportions of lectin-reactive IgE within each sample.

Similar to the results shown in Figure 2, there are bands in addition to the ϵ -chain and L chain of IgE. To rule out the possibility that these additional bands represent degraded ϵ -chain, we resorted to an immunoblotting approach. Thus, the sera were subjected to sequential affinity purification with GAHE-Sepharose 4B and then ϵ BP-Sepharose 4B, by using the conditions identical to the first approach with the exception that the iodination step was omitted. The proteins eluted from ϵ BP-Sepharose 4B were then analyzed by immunoblotting, by using ^{125} I-labeled specific GAHE antibodies. As shown in Figure 4B, only a single band with the mobility of ϵ -chain was detected from serum of patients 533 and 211. Consistent with the results shown in Figure 4A, a very low level of the ϵ -chain was detected from serum of patient 796. The results support the conclusions made above and further indicate that the additional bands seen in Figure 4A are not degraded ϵ -chain.

We then considered the possibility that differential IgE sialylation, within a population of polyclonal IgE, could account for the observed pattern of lectin reactivity and that removal of putative sialic acid residues would augment the amount of ϵ BP-bindable IgE. Thus, comparison of the binding properties of each polyclonal IgE, with and without sialidase treatment, was investigated. As shown in Figure 5A, for two of the samples tested (211 and 796) a pronounced increase in binding was found (Fig. 5A, lane 4 vs lane 3 and lane 6 vs lane 5) and in a manner qualitatively similar to that found for sialidase-treated myeloma IgE (PS) (Fig. 1C). One sample (533), however, was unique in its maximal capacity to bind ϵ BP without sialidase treatment. It should be noted that the level of ϵ BP-bound IgE from patient 211 without sialidase treatment varied somewhat in different experiments (e.g., data presented in Fig. 5A, compared with Fig. 4), which probably reflects some deterioration of ϵ BP-Sepharose 4B used in the former experiments.

Further characterization of sialidase-treated IgE is shown in Figure 5B. As shown by SDS-PAGE analysis, the binding of sialidase-treated IgE to ϵ BP is inhibited by lactose, suggesting that the desialylated IgE interacts with the lectin by a mechanism similar to that for untreated IgE (Fig. 2). It is interesting that one of the samples (from patient 211) showed only partial inhibition by lactose (Fig. 5B, lane 4 vs lane 3). From consideration of a number of observations made during the course of this study (data not shown), we think the most probable explanation is that serum sample 211 contains IgE antibodies specific for the saccharide component of Sepharose 4B.

From the aforementioned analyses, it was concluded that only a subpopulation of IgE in each individual binds ϵ BP and the size of this subpopulation varies from individual to individual. Further, this variation most likely reflects differences in the extent of sialylation of IgE by a given individual. It is interesting to note that the reactivity of purified IgE with ϵ BP appears to be inversely

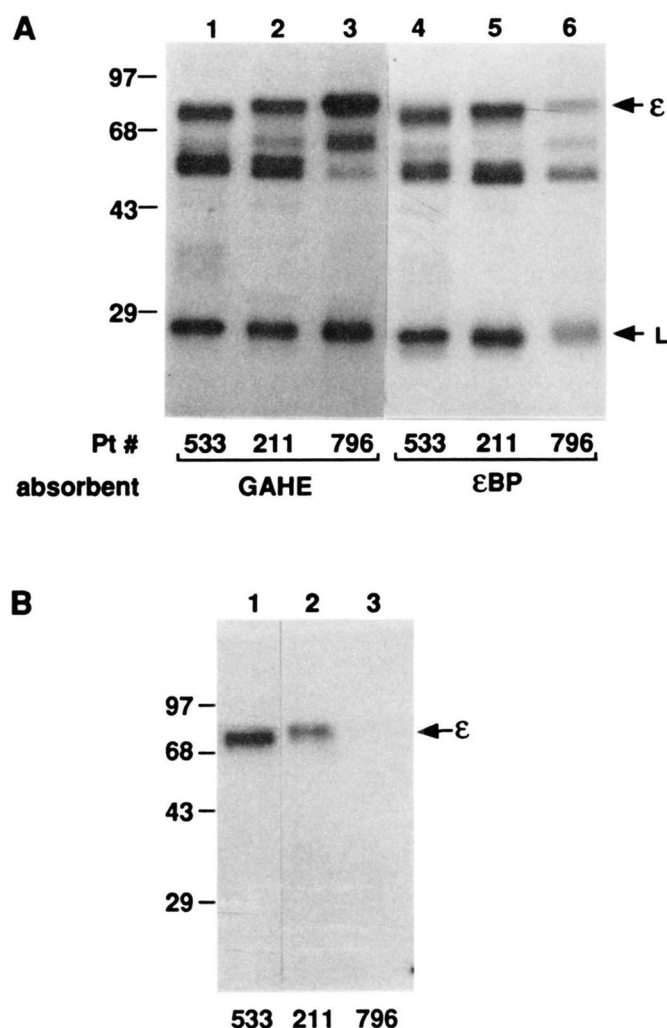


Figure 4. Human polyclonal IgE from different individuals shows variable binding to ϵ BP. **A**, plasma samples from patients with hyper-IgE syndrome were processed as described for Figure 2A. Three patients, patient 533 (lanes 1 and 4), patient 211 (lanes 2 and 5), and patient 796 (lanes 3 and 6), were included. Autoradiographs of SDS-PAGE analysis of proteins bound to GAHE-Sepharose 4B (lanes 1 to 3) and ϵ BP-Sepharose 4B (lanes 4 to 6) are shown. Time of exposure for the autoradiographs was 18 h (lanes 1 to 3) or 44 h (lanes 4 to 6). **B**, plasma samples from patients with hyper-IgE syndrome, 533 (lane 1), 211 (lane 2), and 796 (lane 3), were subjected to sequential affinity purification on GAHE-Sepharose 4B and ϵ BP-Sepharose 4B, and the isolated protein was fractionated by SDS-PAGE and immunoblotted by using ^{125} I-labeled GAHE. The positions of the ϵ -chain (A and B) and L chain (A) of IgE (right margin) and m.w. ($\times 10^{-3}$) markers (left margin) are denoted.

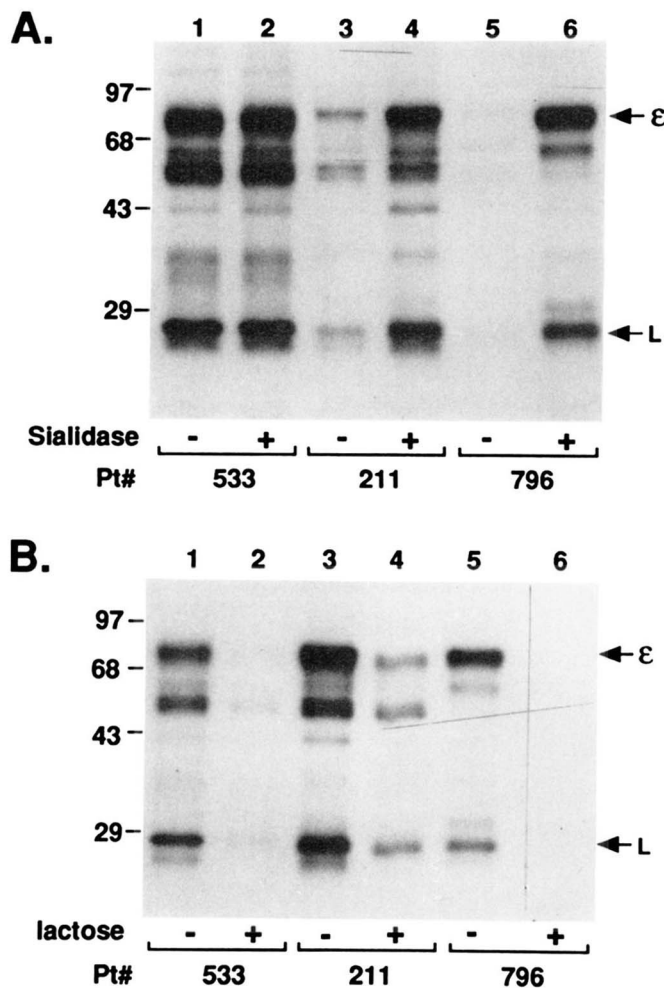


Figure 5. Sialidase-treated human polyclonal IgE shows enhanced binding to ϵ BP. **A.** polyclonal IgE purified from patients with hyper-IgE syndrome, by using GAHE-Sepharose 4B, was either treated with sialidase (lanes 2, 4, and 6) or untreated (lanes 1, 3, and 5) and subsequently mixed with ϵ BP-Sepharose 4B. The bound and eluted protein was then analyzed by SDS-PAGE and autoradiography. Three sera were analyzed, from patient 533 (lanes 1 and 2), patient 211 (lanes 3 and 4), and patient 796 (lanes 5 and 6). **B.** purified polyclonal IgE treated with sialidase, as described in **A.**, was mixed with ϵ BP-Sepharose 4B in the absence (lanes 1, 3, and 5) or presence (lanes 2, 4, and 6) of lactose. The autoradiograph of SDS-PAGE analysis of the bound and eluted protein is shown. Serum numbers (bottom margin), locations of the ϵ -chain and L chain of IgE (right margin), and m.w. ($\times 10^{-3}$) markers (left margin) are denoted.

proportional to the size of the ϵ -chain, i.e., the higher the apparent m.w. of the ϵ -chain, the less the reactivity with ϵ BP (see Fig. 4). This is, again, consistent with the notion that extensive sialylation of IgE (and thus higher m.w.) abolishes its reactivity with ϵ BP.

A subpopulation of polyclonal IgE from an allergic patient is recognized by ϵ BP. In order to further generalize our conclusion that normal human polyclonal IgE is recognized by ϵ BP, we proceeded to analyze IgE from atopic patients. Serum from a patient (C. N.) with atopic dermatitis, containing a high level of IgE (9000 IU/ml), was investigated. In this case, a two-cycle affinity purification procedure was used, similar to that used in the experiment presented in Figure 2B. As shown in Figure 6A, material obtained after the two-cycle purification exhibited primarily the ϵ -chain and L chain on SDS-PAGE (Fig. 6A, lane 1). A small portion of the IgE was found to bind to ϵ BP (Fig. 6B, lane 1), and this binding was inhibitable by lactose (Fig. 6B, lane 2). Based on counts bound to ϵ BP-Sepharose (3090 cpm), compared with those bound to GAHE-Sepharose 4B (9090 cpm), it was calculated that

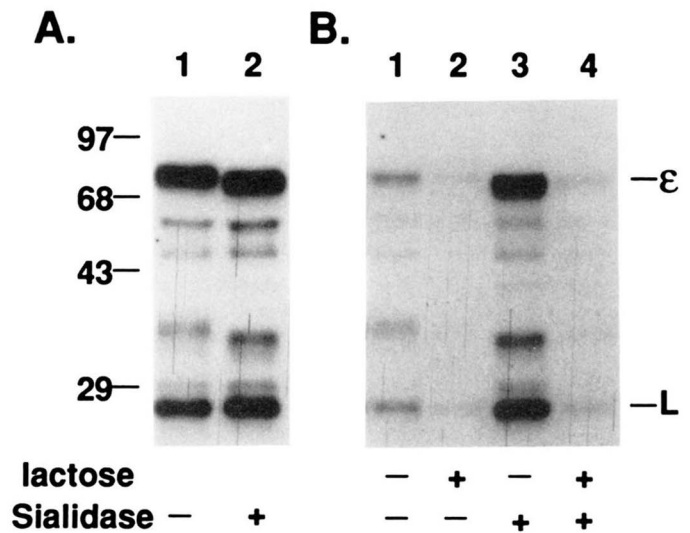


Figure 6. IgE from an atopic patient binds ϵ BP. **A.** IgE from a patient (C. N.) with atopic dermatitis was purified by two-cycle affinity purification, as described in *Materials and Methods*. The protein, with (lane 2) and without (lane 1) sialidase treatment, was analyzed by SDS-PAGE and autoradiography. **B.** the IgE shown in **A.** was mixed with ϵ BP-Sepharose 4B in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of lactose. The bound and subsequently eluted protein was analyzed by SDS-PAGE and autoradiography. Lanes 1 and 2, untreated IgE; lanes 3 and 4, sialidase-treated IgE. The locations of the ϵ -chain and L chain of IgE (right margin) and m.w. ($\times 10^{-3}$) markers (left margin) are denoted.

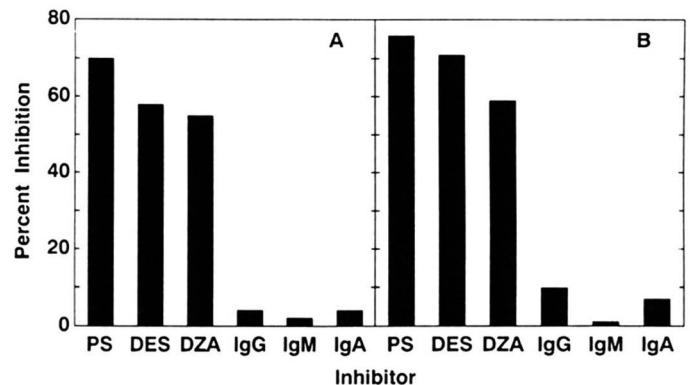


Figure 7. Inhibition of binding of ^{125} I-labeled polyclonal IgE to GAHE by myeloma IgE but not by IgG, IgM, or IgA. **A.** purified polyclonal IgE from patient C. N., bound and eluted from ϵ BP-Sepharose (corresponding to Fig. 6B, lane 1), was then bound to GAHE-Sepharose 4B in the presence of 20 μ g of human myeloma IgE PS, DES, or DZA or polyclonal human IgG, IgM, or IgA. The percentage of binding inhibition by the various inhibitors was calculated as described in *Materials and Methods*. In the absence of any inhibitor, 53% of the radiolabeled protein was bound. **B.** identical to **A.**, except IgE pretreated with sialidase (corresponding to Fig. 6B, lane 3) was used. In the absence of any inhibitor, 71% of the radiolabeled protein was bound.

34% of the IgE was bindable to ϵ BP. Similar to other samples, the IgE treated with sialidase (Fig. 6A, lane 2) showed substantially increased binding to ϵ BP (Fig. 6B, lane 3), and this binding too was nearly quantitatively inhibitable by lactose (Fig. 6B, lane 4). Similar results were obtained with a serum sample from a patient with chronic pulmonary aspergillosis (data not shown). Additional evidence confirming the identity of the purified protein as IgE was obtained by subjecting the final ϵ BP-bound and eluted protein to an additional round of binding to GAHE-Sepharose 4B and showing that the binding could be largely inhibited by three myeloma IgE but not by IgG, IgM, and IgA. This was demonstrated for both the native IgE (Fig. 7A) and sialidase-treated IgE (Fig. 7B). In addition, the comparable levels of inhibition observed for the sialidase-treated IgE and the untreated IgE suggest

that desialylation of IgE has not significantly perturbed the conformational stability of this protein.

DISCUSSION

The major conclusions drawn from this study are threefold. Firstly, human polyclonal IgE is recognized by the lectin ϵ BP. This was shown for IgE from patients with hyper-IgE syndrome and atopy. Secondly, only a subpopulation of IgE from an individual reacts with ϵ BP. Finally, there is individual variation in the proportion of IgE recognized by ϵ BP, from >60% for one sample (patient 533) to amounts barely detectable in another (patient 796). Based on our knowledge of carbohydrate specificity of ϵ BP (7, 8, 13), we interpreted the finding that only a subpopulation of IgE reacts with ϵ BP as a reflection of the heterogeneous sialylation of IgE. Specifically, IgE containing the appropriate terminal galactose residues is recognized by ϵ BP and sialylation of these galactose residues results in a loss of recognition by ϵ BP. The fact that IgE treated with sialidase exhibited enhanced reactivity to ϵ BP supports this analysis, because sialidase would cleave any sialic acid residues from IgE. Thus, we conclude that there exist different IgE glycoforms, varying in the degree of sialylation, and these are distributed in a distinct manner in different individuals.

Human IgE H chain has seven potential N-linked glycosylation sites (21). The human myeloma IgE PS has been determined to be composed of four oligosaccharide units per H chain, of which one is the high mannose type and the other three are sialic acid-containing oligosaccharide chains (22). Two of the sialic acid-containing oligosaccharides contain two termini with the sequence sialic acid $\alpha 2 \rightarrow 6$ Gal $\beta 1 \rightarrow 4$ GlcNAc $\beta 1 \rightarrow 2$ Man $\alpha 1 \rightarrow$, whereas the other has one terminus with the aforementioned structure and another with the sequence of Gal $\beta 1 \rightarrow 4$ GlcNAc $\beta 1 \rightarrow 2$ Man $\alpha 1 \rightarrow$ (22). We have not yet determined the recognition site of ϵ BP on IgE, but the likely candidate is one or more of the N-linked oligosaccharides, although the possibility of O-linked oligosaccharides acting as the ligand for ϵ BP cannot be excluded. From consideration of myeloma IgE oligosaccharide structures, one plausible explanation for our findings is that polyclonal IgE varies in the proportion of oligosaccharide chains that contain the terminal galactose instead of the sialic acid termini.

The functional significance of IgE carbohydrate heterogeneity remains an open question. It is possible that variation in sialylation of IgE among individuals may contribute to the extent of immediate hypersensitivity responses. Thus, a population of IgE with different degrees of sialylation may have different affinities for the IgER (Fc ϵ RI) on mast cells and basophils, resulting in variability in sensitization of these cells. It is interesting to note a previous example of an effect of sialic acids on IgE-Fc ϵ RI interaction; IgE was found to have a higher affinity for IgER on mast cells treated with sialidase (23), a result attributed to the removal of negative charges on the RBL cell surface. Our results should also be viewed in the context of a possible role of ϵ BP in the IgE system. Inasmuch as we know that ϵ BP is present on the surface of mast cells (19), the possibility exists that IgE may be bound simultaneously to Fc ϵ RI and ϵ BP. IgE with different degrees of sialylation may then have different association constants, due to varying affinity for ϵ BP, resulting in differential capacity to sensitize mast cells.

Another attractive possibility takes into consideration the fact that ϵ BP can be secreted from cultured cells (for example by thioglycollate-elicited macrophages) (9) and thus may be considered as a cytokine. Moreover, it is conceivable that ϵ BP has the potential to bind receptor-bound IgE on mast cells or basophils and in a manner that may lead to cross-linking of the receptors (leading to activation of these cells), particularly considering the likelihood that ϵ BP is multivalent (13). In this case, the activity of ϵ BP would be dependent on the extent of sialylation of IgE. This proposed activity would be similar to that reported for HRF (24). In fact, there are striking similarities between our findings of heterogeneous IgE sialylation, as well as differential reactivity of the IgE glycoforms with ϵ BP, and the reported activity of one of the HRF (24). This HRF is IgE dependent and shows activation of basophils sensitized with IgE (IgE⁺) from some individuals but not of those sensitized with IgE (IgE⁻) from other individuals (25). The results have been interpreted as reflecting IgE heterogeneity, possibly as a consequence of carbohydrate differences between the two groups of IgE (24). It is notable that none of the four myeloma IgE tested showed reactivity with ϵ BP (M. W. Robertson and F.-T. Liu, unpublished results), perhaps due to extensive sialylation universal to myeloma IgE; likewise, all 13 myeloma IgE tested by MacDonald and Lichtenstein (24) were found to belong to the IgE⁻ group.

An additional feature of differential IgE sialylation should be addressed. Because the lectin-reactive IgE under consideration would contain fewer sialic acid residues than its unreactive counterpart, it might be far more susceptible to clearance from circulation via hepatic receptors such as the asialoglycoprotein receptor (26). However, it seems reasonable to speculate that an endogenous lectin such as ϵ BP could serve to bind asialo-IgE and thereby "block" the lectin-mediated clearance pathway.

Following the demonstration that ϵ BP does indeed bind polyclonal human IgE, one obvious question is whether the recognition of IgE by ϵ BP, demonstrated *in vitro*, has any physiologic significance. Because ϵ BP is a lectin, one should not expect that it is specific for IgE. Rather, ϵ BP, like many other lectins, is most likely multifunctional (27-29), with IgE recognition being one function falling within the spectrum. It remains to be determined whether IgE binds ϵ BP in the presence of other glycoproteins with similar oligosaccharide structures. However, given that ϵ BP binds IgE, one can envisage unique environments in which ϵ BP-IgE interaction is favored over interactions with other competing glycoconjugates. For instance, ϵ BP is known to reside on the surface of mast cells (19),⁴ and these molecules may be well situated to bind to IgE found on mast cells, by virtue of binding to the high affinity IgER. To derive conclusions on potential roles of ϵ BP in the IgE system requires careful scrutiny of various *in vivo* situations and/or corresponding *in vitro* models.

The phenomenon of carbohydrate heterogeneity of glycoproteins has been well documented. A pertinent example is provided by the extensive variability in glycosylation among monoclonal IgG (30). Of particular relevance to this study are several reports showing that human, rabbit, and murine serum IgG can be either sialylated or nonsialylated (31, 32). Furthermore, there

⁴Frigeri, L. G., and F.-T. Liu. Surface expression of functional IgE binding protein, an endogenous lectin, on mast cells and macrophages. Submitted for publication.

is considerable variability in the sialylation of human myeloma IgG (33), and there is a lower percentage of sialylated structures expressed on IgG secreted by hybridomas, compared with murine serum IgG (30). Although the significance of the carbohydrate heterogeneity to the function of IgG has not been established, clinical relevance of such heterogeneity has been reported (34). For example, total serum IgG from patients with rheumatoid arthritis shows decreased galactosylation (35), and the sialic acid content of rheumatoid factors is lower than that of normal IgG (36).

The identification of biologic importance for endogenous mammalian lectins is gaining momentum through the efforts of many research groups. Evidence is also accumulating that supports a diverse role of ϵ BP in biologic function, and the present study shows that a function of this lectin in the IgE system must be thoroughly considered. In addition, the observation that IgE exhibits carbohydrate heterogeneity opens up a series of questions regarding functional variations of human IgE glycoforms, as a result of differential sialylation, and it is apparent that ϵ BP will be a useful tool to address these questions.

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