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Immunology

Potentiating and suppressive IgE-binding factors are expressed by a single cloned gene

(N-linked glycosylation/mutant cDNA/Ia determinant/IgE response)

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ABSTRACT **We have investigated expression of an IgE-binding factor (IgE-BF) cDNA in both COS-7 monkey kidney cells and Chinese hamster ovary cells. Transient expression of the IgE-BF clone in either cell type yielded IgE-BF, which potentiated an *in vitro* IgE response and had an affinity for lentil lectin. In contrast, when the transient expression experiments were carried out in the presence of tunicamycin, the factors no longer bound to lentil lectin. Moreover, IgE-BF expressed under these conditions suppressed an *in vitro* IgE response. IgE-BF lacking affinity for lentil lectin and suppressing the IgE response also resulted from transient expression of the IgE-BF gene in the presence of glycosylation inhibiting factor, a phospholipase inhibitory protein. Thus, IgE-BF that either potentiate or suppress the IgE response can be expressed from a single cloned gene; the difference in biological activities appears to be determined principally by the type of glycosylation of the common polypeptide chain. Previous work showed that IgE-BF bears an antigenic determinant recognized by the anti-Ia monoclonal antibody OX3. IgE-BF produced in the presence of tunicamycin, and IgE-BF expressed from a mutant cDNA lacking one of two carbohydrate-attachment sites, lacked the OX3 determinant. Thus, the OX3 determinant on IgE-BF appears to be associated with a site of N-linked glycosylation.**

Synthesis of an immunoglobulin isotype can be regulated by T-cell-derived factors, which bind specifically to the Fc region of the isotype that they regulate (immunoglobulin binding factors) (1–3). Studies in both human and rodent systems have characterized a family of IgE-binding factors (IgE-BF) from T cells that selectively regulate the IgE response (1, 4). One of these factors potentiates (IgE-potentiating factor, IgE-PF) and another suppresses (IgE-suppressive factor, IgE-SF) IgE responses (1). IgE-SF and IgE-PF from rodent T cells appear to be related in that they are of similar size (60, 30, and 13 kDa) and share antigenic determinants (5). However, these two factors differ in their glycosylation in that IgE-PF has an affinity for lentil lectin and concanavalin A (Con A), while IgE-SF has an affinity for peanut agglutinin but not for lentil lectin or Con A (6). Other studies showed that a single T cell can produce either IgE-PF or IgE-SF (7) and revealed a second T-cell product, glycosylation inhibiting factor (GIF), which regulates this process: induction of IgE-BF production by T cells in the presence of GIF yields IgE-BF with suppressive activity (8, 9). GIF is a T-cell-derived phospholipase inhibitory protein that shares an antigenic determinant with lipocortin (8). These results suggested that IgE-SF and IgE-PF are closely related and may share the same precursor molecules (6, 7).

We have identified cDNA clones that encode rodent IgE-BF by expression in transfected mammalian cells (10). Expression of IgE-BF cDNA clone 8.3 in COS-7 cells yields IgE-BF of 60 and 11 kDa, both of which potentiate an IgE response. The predicted amino acid sequence of the 60-kDa IgE-BF incuded two potential sites for N-linked glycosylation (10). The 60-kDa IgE-BF, like its counterpart from rodent T cells, contains an antigenic determinant recognized by the anti-Ia monoclonal antibody OX3 (11). However, IgE-BF genes are members of a multigene family that is unrelated to the class II genes of the major histocompatibility complex (12). The present experiments demonstrate that a single cloned gene can express both IgE-SF and IgE-PF and that the Ia-like determinant of IgE-BF is associated with a site of N-linked glycosylation.

MATERIALS AND METHODS

Antibodies and Immunoglobulins. Monoclonal rat IgE from the IR 162 immunocytoma, IgG fraction of rabbit antisera specific for rat IgE and IgG2, and goat anti-mouse IgG were described in a previous article (11). Mouse ascites fluid containing the MRC OX3 monoclonal antibody, specific for a polymorphic rat Ia determinant (13), was purchased from Accurate Chemicals (Hicksville, NY). Immunoglobulins were coupled to CL-Sepharose 4B as described (14). Rabbit anti-mouse IgG was purchased from Jackson ImmunoResearch (Avondale, PA).

Source of IgE-BF. IgE-BF cDNA clone 8.3 (10) was expressed transiently in COS-7 cells as described (11). This cDNA clone was also expressed transiently in Chinese hamster ovary (CHO) fibroblasts (generously provided by J. Rothman) using a similar procedure, except that MEM-α medium (GIBCO) was substituted for Dulbecco's minimal essential medium (DMEM) at all steps, and 40 μg of plasmid DNA was added to each plate. CHO cells were treated with 20% (vol/vol) glycerol in Dulbecco's phosphate-buffered saline (DPBS; GIBCO) for 1 min following the 4-hr incubation with DNA. Cells were cultured in MEM-α medium containing 10% fetal calf serum, and culture supernatants were harvested after 60 hr and assayed for IgE-BF.

In some experiments, rodent IgE-BF were obtained by incubation of a rat–mouse T-cell hybridoma 23B6 (14) for 24 hr with rat IgE at 10 μg/ml. Culture supernatants were filtered through Diaflo YM 100 membranes (Amicon, Danvers, MA), and IgE-BF was recovered from the filtrates.

Source of GIF. GIF was obtained from culture filtrates of the 23A4 hybridoma (14). Hybridoma cells were cultured for 3–4 days as described (15). The culture supernatant was filtered through an Amicon XM 50 membrane and concen-

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Abbreviations: IgE-BF, immunoglobulin E binding factor(s); IgE-SF, IgE-suppressive factor; IgE-PF, IgE-potentiating factor; GIF, glycosylation inhibiting factor; CHO, Chinese hamster ovary.

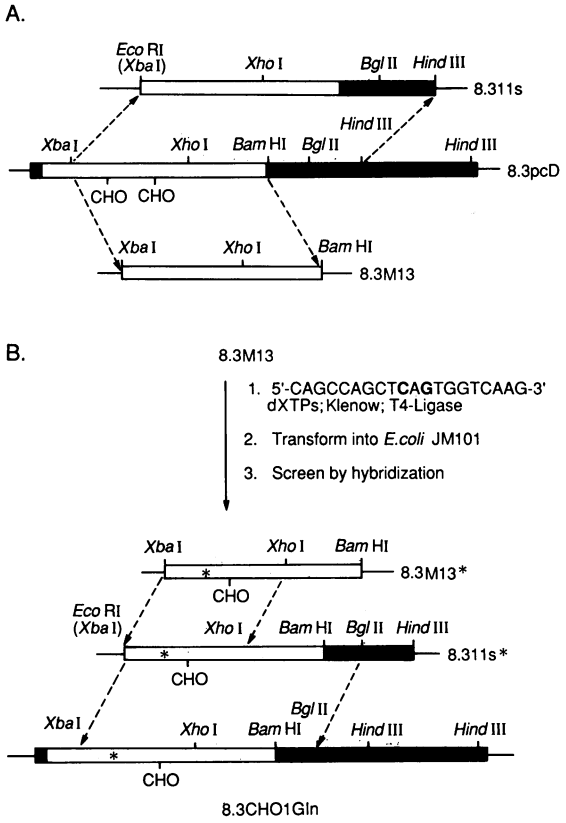

FIG. 1. Construction of carbohydrate-attachment site mutant of IgE-BF cDNA clone 8.3. (*A*) Restriction maps of 8.311s (the intermediate used in reconstruction of mutant IgE-BF genes), the cDNA insert of the original 8.3 clone, and the 1.5-kilobase *Xba* I/*Bam*HI template fragment subcloned into M13mp10. cDNA clone 8.3 was cleaved with *Xba* I and treated with DNA polymerase I large (Klenow) fragment in the presence of the four deoxynucleoside triphosphates. The resulting DNA was allowed to ligate to a 30-fold molar excess of the synthetic *Eco*RI linker 5′ AGGAATTCCT 3′. Use of this linker allowed regeneration of the *Xba* I site. Following complete digestion with *Eco*RI and *Hin*dIII, the 2.2-kilobase fragment was isolated by agarose gel electrophoresis and subcloned into *Eco*RI/*Hin*dIII-digested vector pMT11s (H. Huang and K.W.M., unpublished data), to give plasmid 8.311s. To generate the template for mutagenesis, clone 8.3 was cleaved with *Xba* I and *Bam*HI, and the 1.5-kilobase *Xba* I/*Bam*HI fragment (8.3M13) (10, 12) was isolated by agarose gel electrophoresis for subcloning into *Xba* I/*Bam*HI-digested M13mp10. Recombinant phage carrying the desired insert were used to prepare single-stranded DNA as template for mutagenesis reactions. (*B*) *In vitro* mutagenesis of the carbohydrate attachment site and reconstruction of mutant IgE-BF cDNA clone. The 900-base-pair *Xba* I/*Xho* I fragment was isolated from the plasmid-like replicative form of the mutant M13 clone and cloned into the large *Xba* I/*Xho* I (vector) fragment of clone 8.311s. The 1.9-kilobase *Xba* I/*Bgl* II fragment from the mutant 8.311s plasmid was then cloned into the large *Xba* I/*Bgl* II (vector) fragment of the original 8.3 cDNA. The presence of the desired mutation in the final reconstruction was verified by hybridization of the ^{32}P-labeled primer used in mutagenesis to restriction digests of the plasmid DNA as described above. Plasmid and M13 vector sequences are indicated by thin lines. IgE-BF coding sequence (10) is indicated by open bars; untranslated sequence is indicated by solid bars. N-linked carbohydrate attachment site sequences are indicated by CHO. Plasmid and phage clones bearing mutant sequences are indicated by an asterisk (e.g., 8.311s*).

trated on a YM 5 membrane. GIF in this filtrate was then affinity-purified using the anti-GIF monoclonal antibody 141B9 (9) as described (15). Affinity-purified GIF was stored as a ×100 concentrate relative to the original culture volume and was used at a 1:100 dilution.

Transient Expression of IgE-BF in the Presence of GIF.

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                    8.3pcD
...TAC CAG CCA GCT AAT TGG TCA AGA AAA AAG CCA...
...Tyr Gln Pro Ala Asn Trp Ser Arg Lys Lys Pro...
                       ↓
...CAG CCA GCT CAG TGG TCA AGA AAA AAG...
...Gln Pro Ala Gln Trp Ser Arg Lys Lys...
                  8.3CHO1Gln
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FIG. 2. Sequence of the altered glycosylation site in the IgE-BF coding region. The original 8.3 sequence is shown at the top, with the N-glycosylation site underlined. The CHO1Gln mutant contains a 2-base change mutation (A→C; T→G).

Transfection of COS-7 cells was carried out as described (11) except that a 1:100 dilution of the affinity-purified GIF preparation was included at all stages of the procedure. CHO cells were cultured for 2 hr in the presence of GIF prior to transfection. Supernatants were harvested after 60–72 hr and used for isolation of IgE-BF.

Transient Expression of IgE-BF cDNA in the Presence of Tunicamycin. Transient expression experiments were carried out as described above. After cells were incubated with the DNA/DEAE-dextran mixture for 4 hr, they were rinsed as described above, and DMEM containing purified tunicamycin homologues A1 or B2 (final concentration, 1 ng/ml) (Boehringer Mannheim, Indianapolis, IN) was added. Supernatants were harvested 72 hr later.

Detection of IgE-BF. IgE-BF in transfection supernatants were detected by their ability to inhibit IgE-specific rosette formation of $Fc_\varepsilon R^+$ cells with IgE-coated ox erythrocytes as described (16). Mesenteric lymph node cells from a rat infected with *Nippostrongylus brasiliensis* were used as a source of $Fc_\varepsilon R^+$ cells. The percentage rosette inhibition was determined for duplicate samples and was expressed as the average; experimental variation between replicate samples was <10% of the average value.

Fractionation of IgE-BF. Fractionation of IgE-BF by adsorption to and elution from rat IgE-coupled Sepharose, lentil lectin-Sepharose, or peanut agglutinin-Sepharose (Pharmacia) was described in earlier reports (6, 17). The affinity of the anti-Ia monoclonal antibody OX3 for IgE-BF was determined by incubating a preparation of affinity-purified IgE-BF with a 1:50 dilution of the ascites fluid containing the monoclonal antibody overnight at 4°C. The mixtures were then absorbed with rabbit anti-mouse IgG Sepharose as described (11) and the effluent from the absorbent was tested for IgE-BF activity.

Detection of IgE-PF and IgE-SF. The effect of affinity-purified IgE-BF on *in vitro* IgE responses was determined in cultures of mesenteric lymph node cells of rats primed with dinitrophenyl derivatives of ovalbumin as described (18, 19). The suppressive effect of purified IgE-BF on the IgE response was assessed in a culture system that contained IgE-PF (19). In both systems, the number of IgE- and IgG_2-containing plasma cells that developed in culture was determined by immunofluorescence.

Carbohydrate-Attachment Site Mutant of IgE-BF. Site-specific oligonucleotide-directed mutagenesis of IgE-BF cDNA clone 8.3 was performed with procedures similar to those described by Zoller and Smith (20). A restriction fragment containing the desired region of cDNA clone 8.3 was subcloned into the M13mp10 vector (Pharmacia), and a synthetic oligonucleotide encoding the desired mutation was used as a primer for complementary strand synthesis using DNA polymerase I large fragment (Amersham). The mutant construction scheme is shown in Fig. 1.

A mutation was introduced in the sequence that specifies the NH_2 terminus proximal carbohydrate attachment site of clone 8.3 (10, 12) by altering the asparagine at amino acid 147 to a glutamine residue, as shown in Fig. 2. This alteration

Table 1. Carbohydrate alterations of IgE-BF in supernatants of transfected cells

Cells transfected with clone 8.3	Fractionation of IgE-BF on Sepharose coupled with	
	Lentil lectin effluent/eluate*	Peanut agglutinin effluent/eluate*
CHO	0/23	
CHO + GIF	33/0	
COS-7	0/39	35/0
COS-7 + GIF	32/0	0/38
COS-7 + tunicamycin A1†	28/0	
COS-7 + tunicamycin B2†	36/0	
COS-7 + Me_2SO control†	4/44	

*IgE-BF in supernatants were purified on IgE-Sepharose and then fractionated on either lentil lectin- or peanut agglutinin-Sepharose. Numbers represent % inhibition of IgE-specific rosettes by the effluent/eluate fractions from lectin-coupled Sepharose.
†Tunicamycin homologues were dissolved in dimethyl sulfoxide (Me_2SO) before adding to the culture medium.

destroyed the Asn-Xaa-(Thr or Ser) consensus sequence (21) required for N-linked glycosylation to occur at this position. Mutants were identified by hybridization to ^{32}P phosphorylated oligonucleotide used in the mutagenesis reaction followed by washing under conditions of appropriate stringency (20); the correct sequence at and in the vicinity of the mutant site was verified by sequencing of the mutant phage.

RESULTS

Expression of IgE-BF cDNA in the Presence of GIF or Tunicamycin. IgE-BF cDNA clone 8.3 was transiently expressed in COS-7 cells and in CHO cells. IgE-BF in supernatants were affinity-purified using rat IgE-Sepharose and their lectin affinities were determined. Table 1 shows that all IgE-BF activity in the transfection supernatants from both cell types bound to lentil lectin-Sepharose.

Clone 8.3 was transiently expressed in COS-7 and CHO cells in the presence of GIF. The data in Table 1 show that IgE-BF produced by transfection of clone 8.3 in the presence of GIF no longer had affinity for lentil lectin, but instead bound to peanut agglutinin.

Clone 8.3 was also expressed in COS-7 cells in the presence of the N-linked glycosylation inhibitor tunicamycin. As shown in Table 1, IgE-BF produced by transfection of the cloned gene in the presence of either tunicamycin homologue lacked affinity for lentil lectin.

Transfection experiments were repeated to determine biological activities of expressed IgE-BF. cDNA clone 8.3 was expressed in COS-7 cells in the presence or absence of either GIF or tunicamycin B2. IgE-BF in culture supernatants were affinity-purified and assessed for potentiating or suppressive effects on *in vitro* IgE responses. As reported previously (10), IgE-BF obtained by transfection of COS-7 cells potentiated the IgE response with no concomitant effect on the IgG response (Fig. 3). In contrast, IgE-BF obtained by transfection of COS-7 cells in the presence of GIF or tunicamycin selectively suppressed the IgE response (Fig. 3).

Expression of Mutant 8.3 cDNA in COS-7 Cells. The N-linked glycosylation attachment site mutant cDNA CHO1-Gln (Fig. 2) was expressed transiently in COS-7 cells, and resulting IgE-BF were purified on IgE-Sepharose and assayed as described above. As observed with the original clone 8.3, IgE-BF of both 60 and 11 kDa were obtained in supernatants of transfected cells (data not shown). Moreover, the 60- and 11-kDa species from CHO1Gln IgE-BF retained affinity for lentil lectin (Table 2) and potentiated an *in vitro* IgE response (Fig. 4).

The 60-kDa IgE-BF from the mutant cDNA was tested for reactivity with monoclonal antibody OX3. Unlike the 60-kDa IgE-BF from clone 8.3, which bears the OX3 antigenic determinant (11), the 60-kDa IgE-BF from the mutant cDNA was not absorbed by this reagent (Table 2). Furthermore, 60-kDa IgE-BF from clone 8.3 expressed in the presence of tunicamycin also lacked the OX3 determinant (Table 2). In addition, we tested whether tunicamycin affected the OX3 antigenic determinant in IgE-BF produced by the T-cell hybridoma 23B6. The hybridoma cells were cultured with rat IgE for 24 hr in the presence or absence of tunicamycin B2 (1 ng/ml), and the resulting IgE-BF were fractionated by gel filtration into the 60-, 30-, and 15-kDa species (11). All of the 60- and 30-kDa IgE-BF produced in the absence of tunicamycin bound to the OX3 monoclonal antibody, as found previously (11), but neither species of IgE-BF synthesized in the presence of tunicamycin was absorbed by the antibody (Table 2). Thus, tunicamycin prevented expression of the

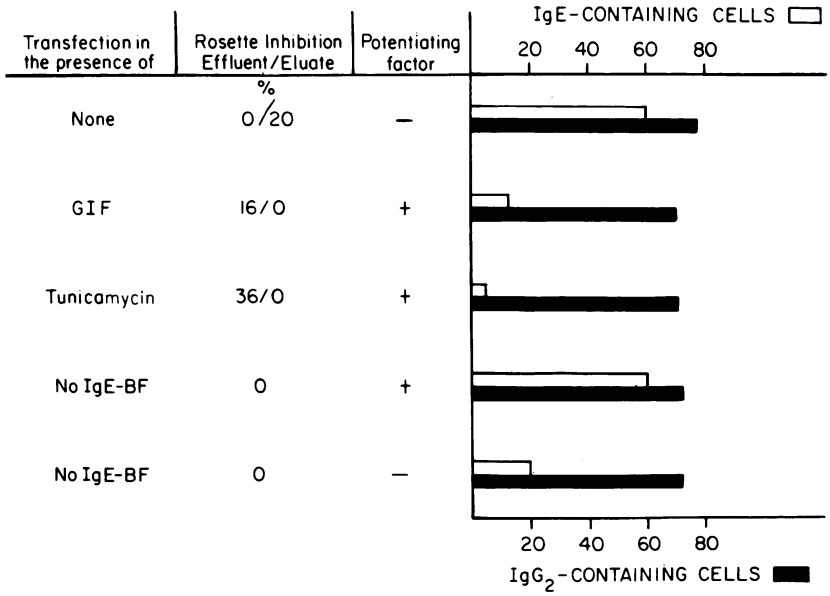

FIG. 3. IgE-BF expressed in the presence of tunicamycin and GIF suppress the IgE response. □, IgE-containing cells per 10^6 cells; ■, IgG_2-containing cells per 10^6 cells. IgE-PF added to cultures were derived from mesenteric lymph node cells of rats infected with *N. brasiliensis*.

Table 2. Requirement of N-linked oligosaccharide 1 in IgE-BF for OX3 determinant

Source of IgE-BF	Molecular size, kDa	IgE-BF on lentil lectin-Sepharose effluent/eluate*	IgE-BF absorbed with	
			OX3 + anti-MGG†	anti-MGG
CHO1-Gln-COS-7	60	0/32	33	32
	11	0/23	ND	ND
8.3-COS-7	60	0/40	4	42
	11	0/30	31	31
8.3-COS-7 + tunicamycin	60	56/0	53	54
	11	35/0	35	35
23B6 + IgE	60	58/0	4	48
	13	35/0	35	30
23B6 + IgE + tunicamycin	60	47/0	47	47
	13	40/0	31	38

Numbers represent % inhibition of IgE-specific rosettes by IgE-BF. ND, not done.
*IgE-BF were fractionated on lentil lectin-Sepharose (cf. footnote to Table 1).
†Samples were incubated with anti-Ia antibody OK3, and immune complexes were removed by passage over Sepharose-coupled rabbit anti-mouse IgG (MGG) as described in *Materials and Methods*. Effluents were assessed for the presence of IgE-BF by rosette inhibition.

OX3 determinant on IgE-BF produced by the hybridoma cell line as well as by transient expression of cDNA clone 8.3.

DISCUSSION

The data presented here indicate that alterations in glycosylation of IgE-BF expressed from a single cloned gene result in different biological activities of the factors. Expression of IgE-BF cDNA in the presence of either tunicamycin or GIF yielded IgE-BF with lectin affinities different from those expressed by untreated COS-7 or CHO cells. These carbohydrate-deficient IgE-BF suppressed an *in vitro* IgE response, while those from untreated cells potentiated the IgE response. These results demonstrate that expression of a single cloned gene can result in IgE-BF with either potentiating or suppressive activity and suggest that the polypeptide encoded by the cDNA is processed differently by the host cell in the presence and absence of tunicamycin or GIF to yield IgE-SF and IgE-PF, respectively. Thus, alternative types of posttranslational modification of a single polypeptide may confer quite different immunoregulatory activities on the resulting protein. These observations substantiate the earlier hypothesis (6–9) that IgE-PF and IgE-SF share common precursors.

The principal difference between IgE-PF and IgE-SF appears to reside in the extent or pattern of glycosylation. The 15-kDa IgE-PF contains both N-linked and O-linked oligosaccharides, while IgE-SF may contain mostly O-linked and little N-linked sugar (6). Our observations that, in the absence of GIF, IgE-BF cDNA clones isolated from a T-cell hybridoma making only IgE-SF express IgE-PF in both COS-7 monkey kidney cells (10) and CHO cells suggest that the glycosylation pattern of IgE-PF represents the usual function of the cellular glycosylation apparatus. No glycosidase activity has been associated with GIF (8, 22), and incubation of IgE-PF with GIF did not change the affinity of the former molecule for lentil lectin. Several results support the idea that an important aspect of GIF activity could be to alter glycosylation pathways or enzyme activities within the target cell. First, the 60-kDa IgE-SF produced by 23B6 hybridoma cells, which also produce GIF (9), has the OX3 determinant (11). However, 60-kDa IgE-BF produced by these cells, or expressed in COS-7 cells, in the presence of tunicamycin has IgE-SF activity (Fig. 3) but *lacks* the OX3 determinant (Table 2). Second, as discussed in detail below, we have found that the OX3 determinant is associated with the NH_2 terminus proximal N-glycosylation site of IgE-BF (Table 2). Taken together, these results imply that the 60-kDa IgE-SF produced in the presence of GIF does not completely lack N-linked oligosaccharide. Thus, the mechanism of action of GIF may be to affect processing of N-linked carbohydrate rather than to interfere with addition of carbohydrate to N-linked sites.

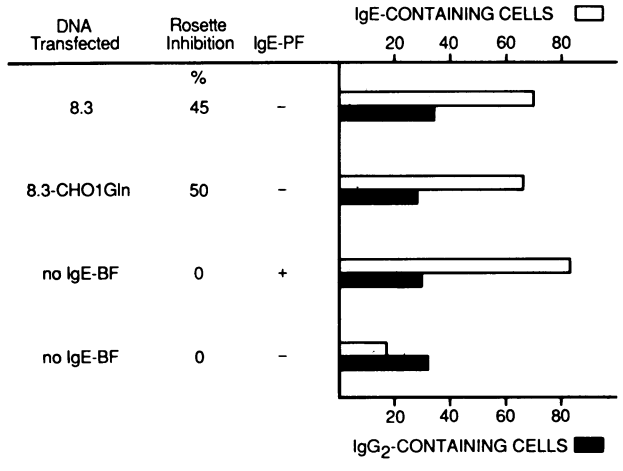

FIG. 4. The IgE-BF carbohydrate attachment site mutant, CHO1Gln, has IgE-potentiating factor activity. IgE-PF added to cultures was the same preparation as that shown in Fig. 3.

The ability of rodent GIF to effect a change in the properties of IgE-BF was defined originally with rodent T cells. The present results show that monkey kidney cells (COS-7) and hamster ovary fibroblasts (CHO) transfected with an IgE-BF cDNA clone can be similarly regulated by this factor. In addition, rabbit lipocortin inhibits the glycosylation of IgE-BF produced by rat lymphocytes (8). These results suggest that this activity of GIF is not species or cell-type specific.

We constructed a mutant of the NH_2 terminus proximal glycosylation (CHO1) site [nucleotides 532–540 of the 8.3 sequence (10)] of clone 8.3. The mutation changed the asparagine residue of the Asn-Trp-Ser sequence, to which carbohydrate is bonded, to a glutamine residue, a conservative amino acid change. As observed for 8.3 IgE-BF, transfection of this mutant cDNA yielded 60- and 11-kDa IgE-BF in the culture medium. Both molecular species of CHO1Gln IgE-BF retained affinity for lentil lectin (Table 2), probably because the second carbohydrate attachment site (nucleotides 850–858 of the 8.3 sequence) was intact. As found for 8.3 IgE-BF, mutant factors lacking the CHO1 site exhibited IgE-PF activity (Fig. 4). Since elimination of this carbohydrate attachment site did not alter the biological

activity of expressed IgE-BF, we suggest that it is the NH_2 terminus distal glycosylation site (10, 12) that is involved in determining the biological activity of IgE-BF.

The 60-kDa IgE-BF derived both from transient expression of clone 8.3 in COS-7 cells and from the 23B6 hybridoma bear an antigenic determinant recognized by the anti-rat Ia monoclonal antibody OX3 (11). However, this determinant was not detected in the 60-kDa IgE-BF derived from the CHO1Gln mutant cDNA or from expression of the original 8.3 cDNA in the presence of tunicamycin (Table 2). These results suggest that the Ia-like OX3 determinant of IgE-BF is associated with the CHO1 N-linked glycosylation site of IgE-BF. At least a portion of the OX3 determinant may be composed of carbohydrate, although it is possible that the monoclonal antibody recognizes a conformational determinant, expression of which requires glycosylation at the CHO1 site.

A number of investigators have reported the association of Ig-BF with class II MHC determinants. Suemura *et al.* (23) reported that an IgE-specific suppressive factor ("IgE-TsF") was absorbed by an alloantiserum specific for determinants of the $(KABJE)^d$ region (23). IgG-BF derived from $H\text{-}2^k$ and $H\text{-}2^d$ mouse T cells and an $H\text{-}2^k$ T-cell hybridoma were absorbed by anti-Ia antisera specific for the appropriate Ia haplotype (24, 25). However, the 8.3 IgE-BF sequence shares no homology with class II gene products (10), and genes encoding rodent IgE-BF are members of a multigene family that is not related to genes of the major histocompatibility complex (12). We suggest that the reactivity of IgE-BF with anti-Ia antibodies may reflect a cross-reaction of similar antigenic determinants on otherwise unrelated molecules. The association of the OX3 determinant on IgE-BF with a site of glycosylation is consistent with an earlier report (26) that some Ia determinants are associated with the carbohydrate portions of Ia molecules.

The present data and earlier results (10, 12) suggest the origin of the 11-kDa IgE-BF within the 60-kDa precursor encoded by clone 8.3. This small IgE-BF does not contain the OX3 determinant (11) specified by the CHO1 site, but it does contain a nearby antigenic determinant defined by a synthetic peptide (nucleotides 562–606 of the 8.3 sequence) (12). The NH_2 terminus of the small factor might be defined by a proteolytic cleavage at the Arg-Lys-Lys (nucleotides 541–549) sequence on the COOH-terminal side of the CHO1 site. Since the 11-kDa IgE-PF from clone 8.3 has affinity for lentil lectin, the NH_2 terminus distal N-linked glycosylation site (CHO2) is presumably contained within this factor. While the next conventional proteolytic processing site distal to the CHO2 site (nucleotides 1141–1146) is too far from the CHO2 site to account for the size of the 11-kDa factor, a different site could be utilized. Alternatively, the 11-kDa factor may have an unusually compact structure, since there are five cysteine residues within 25 amino acids in the proposed region. Intrachain disulfide bond formation might thus account for the small apparent size of the 11-kDa IgE-BF. Since mild reduction with dithiothreitol does not alter the size of the 11-kDa IgE-BF (11), we believe it unlikely that the small size of this factor is due to cleavage and loss of an internal segment of the proposed region.

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