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Immunology

cDNA clones encoding IgE-binding factors from a rat–mouse T-cell hybridoma

(isotype regulation/pcD vector/transient expression)

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ABSTRACT **cDNA clones encoding rodent IgE-binding factors (IgE-BF) were isolated from cDNA libraries of a rat–mouse T hybridoma that secretes IgE-suppressive factor (IgE-SF) upon incubation with rat IgE. COS7 cells transfected with two of the cDNAs expressed IgE-BF, which selectively potentiate an *in vitro* IgE response. IgE-BF expressed in COS7 cells are glycoproteins of ≈60 and ≈11 kDa. DNA sequence analysis of an IgE-BF cDNA revealed a 556-amino acid (62 kDa) protein coding region. The results suggest that IgE-potentiating and IgE-suppressive factors share common precursor polypeptides and that the 11-kDa IgE-BF is derived from a 60-kDa precursor.**

Synthesis of immunoglobulin by B lymphocytes is regulated at several levels by T lymphocytes and their products. Studies of isotype-specific regulation of immunoglobulin synthesis (1–3) have shown that synthesis of a heavy-chain isotype by B cells can be regulated by T-cell factors that bind specifically to the Fc region of the immunoglobulin isotype that they regulate. These soluble factors are apparently related to the lymphocyte Fc receptor (1, 4) and are called "immunoglobulin-binding factors," or Ig-BF.

IgE-binding factors (IgE-BF) from rodent T lymphocytes that regulate the IgE response have been characterized (3). One of these factors selectively potentiates the IgE response (IgE-potentiating factor, IgE-PF), while another selectively suppresses the IgE response (IgE-suppressive factor, IgE-SF). Some evidence suggests that IgE-PF and IgE-SF are closely related. Both factors are glycoproteins that have affinity for IgE and can be purified by chromatography on IgE-Sepharose. They have similar molecular sizes, 13–15 kDa, and share antigenic determinants (4) but differ in their glycosylation: IgE-PF has affinity for lentil lectin and Con A, while IgE-SF has affinity for peanut agglutinin but not Con A or lentil lectin (5). Several studies suggest that the same T cells have the capacity to form both factors (3, 6). These studies led to the proposal that IgE-PF and IgE-SF are polypeptides of similar primary structure that differ in glycosylation and perhaps other features of their post-translational processing.

The present experiments were undertaken to isolate cDNA clones that encode rodent IgE-BF. Huff *et al.* (7) fused rat lymphocytes with cells of the AKR mouse thymoma BW5147 and established a rat–mouse T hybridoma cell line (23B6) that upon incubation with IgE forms at least three species of IgE–BF. One of these factors has a molecular size of 13 kDa and exhibits IgE-SF activity, while a 26-kDa IgE-BF has neither suppressive nor potentiating activity. A 60-kDa IgE-BF has also been detected in 23B6 culture supernatants (unpublished data). We used 23B6 cells as a source of mRNA encoding IgE-BF. We describe here the isolation and expression of cDNA clones that encode IgE-BF from this hybridoma cell line.

MATERIALS AND METHODS

cDNA Libraries. Total cellular poly(A)$^+$ RNA was isolated (8) from 23B6 cells that had been induced for production of IgE-BF (7). This RNA was used to construct cDNA libraries. A cDNA library was constructed in the pcD expression vector as described (8). A cDNA library of induced 23B6 RNA was also constructed in the λ phage vector λgt10 (gift of T. Huynh) by a procedure adapted from that described by T. St. John (personal communication).

Enriched cDNA Probe. A ^{32}P-labeled single-stranded cDNA probe was prepared essentially according to Davis *et al.* (9). Reverse transcription of 5 μg of poly(A)$^+$ RNA from induced 23B6 cells was followed by a single hybridization to 50 μg of poly(A)$^+$ RNA from BW5147 (c_0t ≈1500) and hydroxylapatite chromatography. Single-stranded [^{32}P]cDNA in the effluent fraction was enriched for RNA sequences more abundant in induced 23B6 cells than in the BW5147 fusion partner.

Yeast Expression Plasmid and Host. The yeast expression plasmid pAAR6 (10) with the alcohol dehydrogenase promoter and the *TRP1* gene as auxotrophic selectable marker was used as a vector for transcription of IgE-BF cDNA in yeast. A tryptophan auxotroph, 20B-12, derived from a protease-deficient yeast strain (11) was the host for this vector. RNA was isolated from yeast cultures by guanidinium thiocyanate lysis of mechanically disrupted cells.

Assay of IgE-BF Activities. IgE-BF were assayed by their ability to inhibit formation of IgE-specific rosettes by $Fc_\varepsilon R^+$ lymphocytes and fixed ox erythrocytes coated with rat IgE (12). Mesenteric lymph node (MLN) cells from a rat infected with *Nippostrongylus brasiliensis* were employed as $Fc_\varepsilon R^+$ cells. The percentage of rosette inhibition was determined in duplicate assays of a single sample and was expressed as the average. Variation between replicate assays of a given sample was less than 10% of the average values.

IgE-BF were fractionated on rat IgE-coupled Sepharose and on lentil lectin Sepharose (Pharmacia) as described (13). Purified IgE-BF were assessed for the ability either to potentiate or to suppress the IgE response by the methods described (14, 15). To determine the potentiating activity, MLN cells of rats immunized with dinitrophenyl derivatives of ovalbumin (DNP-OA) were cultured for 5 days with

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Abbreviations: Ig-BF, immunoglobulin binding factor(s); IgE-BF, IgE-binding factor(s); IgE-SF, IgE-suppressive factor; IgE-PF, IgE-potentiating factor; MLN, mesenteric lymph node; bp, base pair(s); DNP-OA, dinitrophenyl derivative of ovalbumin.

DNP-OA at 0.1 μg/ml in the presence or absence of purified IgE-BF. The suppressive effect of IgE-BF was assessed in a culture system that contained IgE-PF (14). Culture filtrates of MLN cells obtained from *N. brasiliensis*-infected rats were used as a source of IgE-PF (15). Purified IgE-BF to be tested was added to the DNP-OA primed MLN cells together with IgE-PF, and the cells were cultured for 5 days with DNP-OA. In both systems, Ig-forming cell responses were evaluated by enumerating IgE-containing cells and IgG2-containing cells by immunofluorescence.

RESULTS AND DISCUSSION

Identification of IgE-BF cDNA Clones. When 23B6 cells are cultured with IgE, IgE-BF are released into the culture medium, while in the absence of IgE, no factors can be detected (7). RNA isolated from 23B6 cells grown under these different conditions gives analogous results when injected into *Xenopus laevis* oocytes (Table 1). Injection of poly(A)$^+$ RNA from IgE-induced 23B6 cells resulted in appearance in the supernatant of material that inhibited 40–46% of IgE-specific rosettes. All of this rosette-inhibiting activity was absorbed by IgE-coupled Sepharose and could be recovered by elution at pH 3.0. The activity was not absorbed by rat IgG- or bovine serum albumin-coupled Sepharose. In contrast, translation of RNA from uninduced 23B6 cells gave no detectable IgE-BF activity (Table 1). Negative results were also obtained with RNA isolated from the fusion partner, BW5147, which makes neither detectable IgE-BF (7) nor Fc receptor (16).

A 23B6 cDNA library in the λgt10 vector was screened with a single-stranded, enriched ^{32}P-cDNA probe. cDNA clones hybridizing to the probe were picked and pooled and their cDNA inserts (*Eco*RI fragments) were recloned into plasmid pUC8 (17). A pool of 192 pUC8 clones that hybridized to the [^{32}P]cDNA probe was then employed in hybrid-selection (18) and *in vitro* translation experiments to identify cDNA clone fragments that hybridize with mRNAs encoding IgE-BF. As shown in Table 1, supernatants from *X. laevis* oocytes injected with mRNA selected with the pool of 192 clones contained IgE-BF while a control experiment with an unrelated plasmid gave negative results. Pooled and individual pUC8 clones were then tested by hybrid-selection and *in vitro* translation. Three clones were thus identified as cDNA fragments sharing homology with mRNAs encoding IgE-BF. The sizes of their cDNA inserts ranged from 300 base pairs (bp) to 1200 bp (Table 1).

Table 1. Hybrid-selection and *in vitro* translation of IgE-BF in *X. laevis* oocytes

Source of mRNA*	cDNA on filter†	IgE-BF in supernatant‡, %
23B6 (induced)	—	40–46§
23B6 (uninduced)	—	6
BW5147	—	5
None	—	4
23B6 (induced)	rat IgE cDNA (1400 bp)	6
	192 pUC8 clones, pool	33
	A18 (1200 bp)	35
	6 (300 bp)	32
	55 (350 bp)	19
Yeast carrying plasmid		
8.3 R6-1	—	7¶
8.3 R6-2	—	34¶

*Poly(A)$^+$ RNA was prepared by one cycle of binding to oligo(dT)-cellulose.
†Supernatants from surviving oocytes (10 μl) were collected after 48 hr and assayed for IgE-BF.
‡Hybrid-selected RNA was concentrated by ethanol precipitation and dissolved in H_2O for injection into *X. laevis* oocytes.
§A 1:3 dilution of the supernatant inhibited rosettes by 29%. Rosette-inhibiting activity in the original supernatant was absorbed by IgE-Sepharose and recovered by elution with glycine·HCl buffer, pH 3.0.
¶Supernatants were absorbed with IgE-Sepharose and the beads were eluted at pH 3.0. Rosette-inhibiting activities of the eluates are shown.

The observation that hybrid-selected mRNA could direct expression and secretion of IgE-BF by oocytes suggested that a full-length cDNA clone in the mammalian cell expression vector pcD (8) might function similarly in COS7 (19) monkey kidney cells. Thus, a 23B6 cDNA library in this vector (≈10^5 clones) was screened using the 1200-bp *Eco*RI fragment of A18 (Table 1) as a probe. Plasmid DNA from each of 70 hybridizing clones was introduced into COS7 cells for transient expression of the gene product (20). COS7 supernatants derived from four cDNA clones (4.2, 8.3, 9.5, and 10.2) contained IgE-BF (Table 2) that bound specifically to rat IgE-coupled Sepharose and could be eluted at acid pH. These IgE-BF did not bind to rat IgG-, mouse IgG-, or human IgE-coupled Sepharoses, as expected for a rodent IgE-BF (3, 7, 21).

These results indicated that the cDNA clones we identified encode IgE-BF structural genes. However, an alternative possibility was that transfected cDNA clones induced expression of IgE-BF genes in the host cell. We isolated a 3.1-kilobase *Pst* I restriction fragment from clone 8.3 that contains the entire protein-coding sequence (Fig. 1, see below). This fragment was cloned in both orientations into the *Eco*RI site of yeast expression plasmid pAAR6. Poly(A)$^+$ RNA from yeast cells carrying these plasmids was translated in *X. laevis* oocytes. Supernatants from oocytes were chromatographed on rat IgE-Sepharose and the effluent and eluate fractions were tested for IgE-BF activity. RNA from yeast carrying the cDNA clone in the correct 5′–3′ orientation (8.3R6-2) was translated to give IgE-BF (Table 1), while no activity was detected after injection of RNA derived from the clone having the opposite orientation (8.3R6-1). These results established that cDNA clone 8.3 does encode IgE-BF.

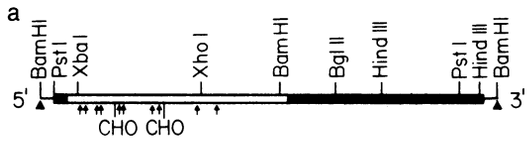
cDNA Clones Isolated from a Cell Making IgE-SF Direct Expression of IgE-PF in COS7 Monkey Cells. IgE-BF in IgE-Sepharose eluates derived from clones 4.2, 8.3, 9.5, and

Table 2. Fractionation of IgE-BF in COS7 cell transfection supernatants on Ig-coupled Sepharoses

cDNA clone	IgE-BF in supernatant, %	Ig used	IgE-BF in effluent/eluate, %
4.2	25	Rat IgE	0/25
8.3	36	Rat IgE	0/31
		Human IgE	33/0
		Rat IgG	30/0
		Mouse IgG	33/0
9.5	20	Rat IgE	0/25
10.2	30	Rat IgE	3/21

Elution of the Sepharose column results in a 1:4 dilution of the original material (13). Therefore, the "supernatant" value was obtained from a 1:4 dilution of the material applied to the immunosorbent. Values are % IgE-specific rosette inhibition. Transient expression (20) supernatants were harvested after 72 hr and assayed for IgE-BF. Purified monoclonal rat IgE (IR162) and human E myeloma protein, PS, were the same preparations as those described in a previous article (21). Mouse and rat IgGs were isolated from normal sera by chromatography on DEAE-cellulose followed by gel filtration through ACA 34 (LKB). Each immunoglobulin was coupled to Sepharose CL-4B (Pharmacia); 8–10 mg of Ig was coupled to 1 ml of activated Sepharose.

a



b

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      10         20         30         40         50         60         70         80         90        100        110        120
GCTAGAGTACCAGTGAGTACAGCTTTACGAGGTAAGTCTGATCTTGAACTTTCTAAGGAAATTCAAGACAGTCTATCAGAAGTAAAGTGGAAAATGGCTTTACAAGTTATGTTTGGCCTT
                                                                                                    METAlaLeuGlnValMETPheGlyLeu

     130        140        150        160        170        180        190        200        210        220        230        240
GAATTTTTTCTAGTGTTAGAAGCCCTTTTGTTCCTTTTCACATGTTATCAAGTGGTTAAGGCAGGGCGGATTCTAGATGAAATTCAGGACAAGCTATCAGAAGTAAAGCGGGGAGAGAGA
GluPheLeuValLeuGluAlaLeuLeuPheLeuPheThrCysTyrGlnValValLysAlaGlyArgIleLeuAspGluIleGlnAspLysLeuSerGluValLysArgGlyGluArg

     250        260        270        280        290        300        310        320        330        340        350        360
GTAGGAACAAAGAGGAAATATGGTACACAAAATAAGTATACAGGCCTTTCCAAGGGTCTTGAACCCGAGCAAAAGTTAAGGTTAGGTAGGAATACCTGGAGAGAGATTAGAAGAAAAAGA
ValGlyThrLysArgLysTyrGlyThrGlnAsnLysTyrThrGlyLeuSerLysGlyLeuGluProGluGluLysLeuArgLeuGlyArgAsnThrTrpArgGluIleArgArgLysArg

     370        380        390        400        410        420        430        440        450        460        470        480
GGAAAAAGGGAAAAGAAGAAAGATCAATTAGCGGAGGTCTCTAGGAAAAGGAGCCTGTGCTCATCGCTGGATGGGCTCGGGAAGCCAGCTCTTAGTAGCTCTGAAGCAGGTGAAGAATCC
GlyLysArgGluLysLysLysAspGlnLeuAlaGluValSerArgLysArgSerLeuCysSerSerLeuAspGlyLeuGlyLysProAlaLeuSerSerSerGluAlaGlyGluGluSer

     490        500        510        520        530        540        550        560        570        580        590        600
TCCTCTGAGGAAACAGACTGGGAGGAAGAAGCAGCCCATTACCAGCCAGCTAATTGGTCAAGAAAAAAGCCAAAAGCGGCTGGCGAAGGCCAGTTTGCTGATTGGCCTCAGGGCAGTCGG
SerSerGluGluThrAspTrpGluGluGluAlaAlaHisTyrGlnProAlaAsnTrpSerArgLysLysProLysAlaAlaGlyGluGlyGlnPheAlaAspTrpProGlnGlySerArg

     610        620        630        640        650        660        670        680        690        700        710        720
CTTCAAGGTCCGCCCTATGCGGAGTCCCCGCCCTGCGTAGTGCGTCAGCAATGCGCAGAGAGATGCGCAGAGAGGCAGTGCGCAGAGAGGCAGTGCGCAGACTCATTCATTCCCAGAGAG
LeuGlnGlyProProTyrAlaGluSerProProCysValValArgGlnGlnCysAlaGluArgCysAlaGluArgGlnCysAlaGluArgGlnCysAlaAspSerPheIleProArgGlu

     730        740        750        760        770        780        790        800        810        820        830        840
GAACAAAGGAAAATACAACAGGCATTTCCGGTCTTTGAAGGAGCCGAGGGTGGGCGTGTCCACGCTCCGGTAGAATACTTACAAATTAAAGAAATTGCCGAGTCGGTTCGTAAATATGGA
GluGlnArgLysIleGlnGlnAlaPheProValPheGluGlyAlaGluGlyGlyArgValHisAlaProValGluTyrLeuGlnIleLysGluIleAlaGluSerValArgLysTyrGly

     850        860        870        880        890        900        910        920        930        940        950        960
ACCAATGCTAATTTTACCTTGGTGCAGTTAGACAGGCTCGCCGGCATGGCACTAACTCCTGCTGACTGGCAAACGGTTGTAAAAGCCGCTCTCCCTAGTATGGGCAAATATATGGAATGG
ThrAsnAlaAsnPheThrLeuValGlnLeuAspArgLeuAlaGlyMETAlaLeuThrProAlaAspTrpGlnThrValValLysAlaAlaLeuProSerMETGlyLysTyrMETGluTrp

     970        980        990       1000       1010       1020       1030       1040       1050       1060       1070       1080
AGAGCGCTTTGGCACGAAGCTGCACAAGCGCAGGCCCGAGCAAACGCAGCTGCTTTGACTCCAGAGCAGAGAGATTGGACTTTTGACTTGTTAACGGGTCAGGGAGCTTATTCTGCTGAT
ArgAlaLeuTrpHisGluAlaAlaGlnAlaGlnAlaArgAlaAsnAlaAlaAlaLeuThrProGluGlnArgAspTrpThrPheAspLeuLeuThrGlyGlnGlyAlaTyrSerAlaAsp

     1090       1100       1110       1120       1130       1140       1150       1160       1170       1180       1190       1200
CAGACAAACTACCATTGGGGAGCTTATGCCCAGATTTCTTCCACGGCTATTAGGCCTGGAAGGCGCTCTCGAGCAGGTGAAACCACTGGTCAGTTAACAAAGATAATCCAGGGACCTCAG
GlnThrAsnTyrHisTrpGlyAlaTyrAlaGlnIleSerSerThrAlaIleArgProGlyArgArgSerArgAlaGlyGluThrThrGlyGlnLeuThrLysIleIleGlnGlyProGln

     1210       1220       1230       1240       1250       1260       1270       1280       1290       1300       1310       1320
GAATCCTTCTCAGATTTTGTGGCCAGAATGACAGAGGCAGCAGAGCGTATTTTTGGAGAGTCAGAGCAAGCTGCGCCTCTGATAGAACAGCTAATCTATGAGCAAGCCACAAAGGAGTGC
GluSerPheSerAspPheValAlaArgMETThrGluAlaAlaGluArgIlePheGlyGluSerGluGlnAlaAlaProLeuIleGluGlnLeuIleTyrGluGlnAlaThrLysGluCys

     1330       1340       1350       1360       1370       1380       1390       1400       1410       1420       1430       1440
CGAGCGGTCCATAGCCCCAAGAAAGAACAAAGGCTTACAAGACTGGCTCAGGGTCTGTCGAGAGCTTGGGGCGAAACCCAGACTCCTTAAGACTGATAATGGACCAGCTTATACGTCTCAA
ArgAlaValHisSerProLysLysGluGlnArgLeuThrArgLeuAlaGlnGlyLeuSerArgAlaTrpGlyLysProArgLeuLeuLysThrAspAsnGlyProAlaTyrThrSerGln

     1450       1460       1470       1480       1490       1500       1510       1520       1530       1540       1550       1560
AAATTCCAACAGTTCTGCCGTCAGATGGACGTGACCCACCTGACTGGACTTCCATACAACCCTCAAGGACAGGGTATTGTTGAGCGTGCGCATCGCACCCTCAAAGCCTATCTTATAAAA
LysPheGlnGlnPheCysArgGlnMETAspValThrHisLeuThrGlyLeuProTyrAsnProGlnGlyGlnGlyIleValGluArgAlaHisArgThrLeuLysAlaTyrLeuIleLys

     1570       1580       1590       1600       1610       1620       1630       1640       1650       1660       1670       1680
CAGAAGAGGGGAACTTTTGAGGAGACTGTACCCCGAGCACCAAGAGTGTCGGTGTCTTTGGCACTCTTTACACTCAATTTTTTAAATATTGATGCTCATGGCCATACTGCGGCTGAACGT
GlnLysArgGlyThrPheGluGluThrValProArgAlaProArgValSerValSerLeuAlaLeuPheThrLeuAsnPheLeuAsnIleAspAlaHisGlyHisThrAlaAlaGluArg

     1690       1700       1710       1720       1730       1740       1750       1760       1770
CATGTTCAGAGCCAGATAGGCCCAATGAGATGGTTAAATGGAAAAATGTCCTTGATAATAAATGGTATGGCCCGGATCCTATCTTGATAA
HisValGlnSerGlnIleGlyProMETArgTrpLeuAsnGlyLysMETSerLeuIleIleAsnGlyMETAlaArgIleLeuSer . .
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FIG. 1. Structure of IgE-BF cDNA clone 8.3. (*a*) Restriction map. The open bar indicates the putative protein coding region; the dark bar indicates the untranslated sequence; potential N-linked glycosylation sites (CHO) and proteolytic cleavage sites (arrows) are also shown. (*b*) Nucleotide and predicted amino acid sequence of clone 8.3. Sequence data were obtained by both chemical methods and the chain termination method in phage M13 mp10 and mp11. Both strands were completely sequenced. Potential N-linked glycosylation sites are boxed; four potential proteolytic cleavage sites are underlined.

10.2 (Table 2) were tested for the ability to selectively suppress or potentiate an IgE response by DNP-OA primed rat MLN cells *in vitro*. IgE-BF from transient expression of cDNA clones 8.3 and 9.5 enhanced the number of IgE-containing cells in these cultures approximately 4-fold (Fig. 2*a*) without affecting the IgG2 response. Similar results were obtained with a preparation of IgE-PF from MLN cells of a rat infected with *N. brasiliensis*. In contrast, IgE-BF derived from cDNA clones 4.2 and 10.2 failed to enhance the IgE response. Moreover, none of the IgE-BF from the four cDNA clones exhibited IgE-SF activity.

Although IgE-BF derived from expression of cDNA clones 8.3 and 9.5 selectively potentiated the IgE response (Fig. 2*a*), the 23B6 cells from which these clones were isolated make IgE-SF but no detectable IgE-PF, under conditions used to prepare mRNA (7). Earlier studies have shown that a single T cell has the capacity to produce either IgE-PF or IgE-SF. The two factors have common antigenic determinants but differ in their lectin-binding properties (3). These and other results led to the proposal (5, 6) that the biological activity of these factors is determined by the process of glycosylation of the same precursor molecules. The results described in this paper support this hypothesis. While it is possible that distinct IgE-PF mRNAs are present in 23B6 cells but their translation products do not appear in the medium, we believe it more likely that the polypeptides translated from mRNAs represented by these cDNA clones are processed differently by the 23B6 and COS7 cells to give factors with suppressive and potentiating activities, respectively. Experiments that examine switching from formation of IgE-PF to formation of

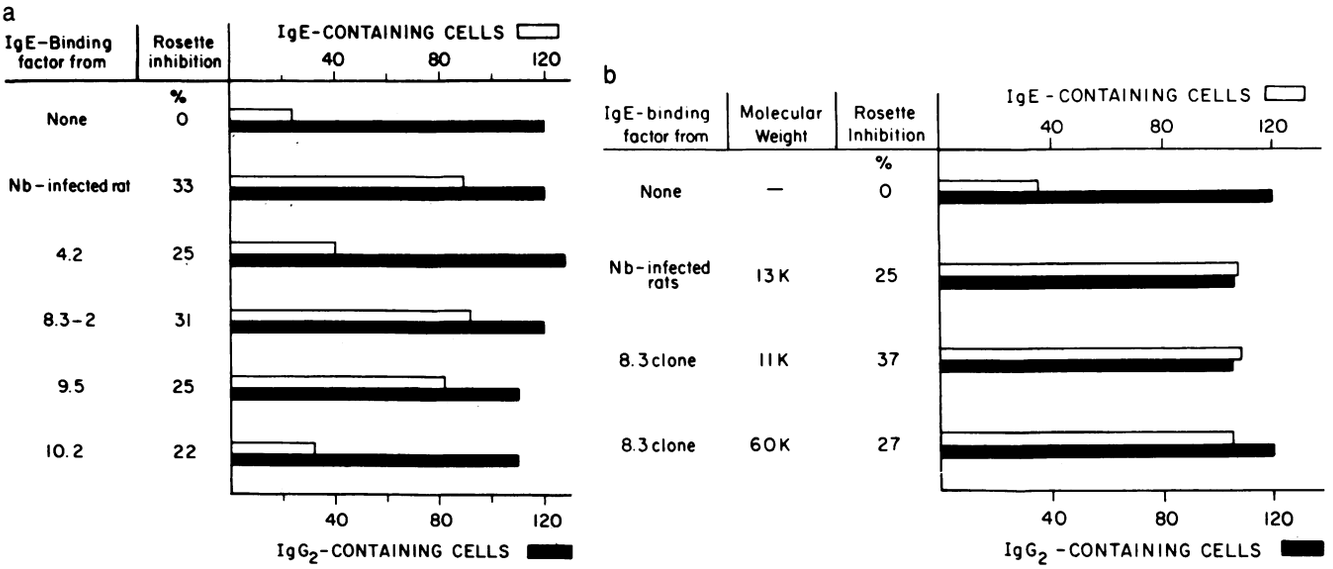

FIG. 2. (*a*) cDNA clones 8.3 and 9.5 direct expression of IgE-potentiating factors in COS7 monkey cells. (*b*) Both the 60-kDa and the 11-kDa IgE-BF from clone 8.3 selectively potentiate and IgE response. □, IgE-containing cells per 10^6 cells; ■, IgG2-containing cells per 10^6 cells. 8.3-2 is an individual preparation of cDNA clone 8.3. Rabbit antisera specific for rat IgE or rat IgG2 were the preparations described earlier (15). Nb, *N. brasiliensis*.

IgE-SF in cells transfected with a single cloned cDNA will be described elsewhere.

cDNA clones 4.2 and 10.2 encode IgE-BF that do not exhibit IgE-SF or IgE-PF activity *in vitro*. 23B6 cells (7) and rat T lymphocytes (3, 14) also form IgE-BF lacking activity in these assays. The relation, if any, of the 4.2 and 10.2 IgE-BF to the inactive factor from 23B6 remains unknown.

IgE-BF Expressed in COS7 Cells Are Glycoproteins of 60 and 11 kDa. The molecular size of IgE-BF expressed in COS7 monkey cells was determined by gel filtration of IgE-Sepharose eluates on Sephadex G-75 (Fig. 3). Transfection of cDNA clone 10.2 into COS7 cells yields IgE-BF that elutes near the void volume with an estimated molecular size of about 60 kDa. Transfection of cDNA clone 8.3 results in two peaks of IgE-BF activity that elute at ≈60 kDa and ≈11 kDa. IgE-BF derived from cDNA clones 8.3 and 10.2 all have affinity for lentil lectin (Table 3) but not peanut agglutinin. Both the 60-kDa and the 11-kDa IgE-BF from clone 8.3 selectively potentiate the IgE response (Fig. 2*b*). Thus, the lectin binding properties and biological activities of the 11-kDa IgE-PF from clone 8.3 are similar to those of the 13-kDa IgE-PF from rodent T cells.

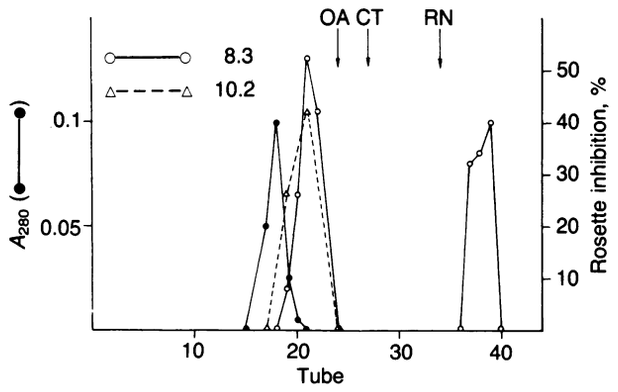

FIG. 3. Molecular sizes of IgE-BF produced by transfected COS7 monkey cells. OA, CT, and RN, elution volumes of molecular size standards: ovalbumin (43 kDa), chymotrypsinogen (25 kDa), and ribonuclease (13 kDa). Supernatants of transfected COS7 cells were concentrated 20-fold, and IgE-BF in the preparation was purified by using rat IgE-Sepharose. Fetal calf serum (0.1 ml) was added to 1 ml of purified IgE-BF and the mixture was applied to a Sephadex G-75 column (1.0 × 90 cm) that had been calibrated with ovalbumin, α-chymotrypsinogen, and ribonuclease. Proteins were eluted with 0.1 M phosphate buffer, pH 7.0. Fractions of the eluate were assayed for inhibition of IgE-specific rosette formation.

DNA sequence analysis of clone 8.3 (Fig. 1*b*) defines a putative protein coding region of 556 amino acids (62 kDa) with a hydrophobic leader region, two potential sites for N-linked glycosylation (Asn-Xaa-[Ser or Thr]) (22), and several potential sites for post-translational proteolytic cleavage (tandem basic residues). Since expression of clone 8.3 in COS7 cells yields IgE-BF of both 60 kDa and 11 kDa, the 11-kDa IgE-BF appears to be a product derived from the 60-kDa precursor. These data suggest that two IgE-BF made by rodent T-lymphocytes (60 kDa and 13 kDa) might also share a precursor/product relationship. We speculate that the smaller species, which must contain one of the sites for *N*-glycosylation, might be generated by proteolytic cleavage at two sites (Arg-Lys; amino acids 246–247; Arg-Arg, 350–351) flanking one of the sites of glycosylation (Asn-253), or at Arg-Lys-Arg (104–106) and Arg-Lys (212–213) flanking the other (Asn-147). Either cleavage would give a polypeptide of about 105 amino acids.

IgE-BF Amino Acid Sequence Is Homologous to Polymerases. The translated amino acid sequence of clone 8.3 was compared to the amino acid sequence data base by R. Doolittle who used a computer search algorithm that identifies local regions of dense homology between sequences. A striking homology was found between amino acids 438–488 of

Table 3. IgE-BF from transfected COS7 monkey cells bind to lentil lectin

cDNA clone	Molecular size, kDa	IgE-BF activity*, %	
		Unfractionated	Effluent/eluate
8.3	60	35	6/32
	11	37	0/34
10.2	60	37	0/40

*Numbers represent % IgE-specific rosette inhibition. IgE-BF from transfected COS7 cells were fractionated on lentil lectin-Sepharose by exactly the same procedures as described (13).

the 8.3 sequence and the polymerases of four retroviruses: human adult T-cell leukemia virus I, HTLV-I [amino acids 715–765 (23)], simian sarcoma virus [70–124 (24)], Moloney murine leukemia virus [974–1029 (25)], and Rous sarcoma virus [692–746 (26)]. The HTLV-I polymerase and the translated 8.3 sequence shared 50% homology in this region. In contrast, the NH_2-terminal 437 amino acids encoded by clone 8.3 shared no significant homology with any other sequence in the data base.

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