Proc. Natl. Acad. Sci. USA
Vol. 83, pp. 6583–6587, September 1986
Immunology

cDNA clones encoding murine IgE-binding factors represent multiple structural variants of intracisternal A-particle genes

(IgE regulators/retroviral proteins)

EDWARD L. KUFF*, JUDY A. MIETZ*, MARY L. TROUNSTINE†, KEVIN W. MOORE†, AND CHRISTINE L. MARTENS†

*Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892; and †DNAX Research Institute of Molecular and Cellular Biology, Palo Alto, CA 94304

Communicated by Maxine Singer, May 5, 1986

ABSTRACT **Previously [Moore, K. W., Jardieu, P., Mietz, J. A., Trounstine, M. L., Kuff, E. L., Ishizaka, K. & Martens, C. L. (1986) *J. Immunol.* 136, 4283–4290], we examined a T-hybridoma-derived cDNA clone, 8.3, that encodes a biologically active murine IgE-binding factor (IgE-BF), and we showed that it was a variant member of the endogenous retroviral gene family related to mouse intracisternal A particles (IAPs). We have now characterized four more IgE-BF cDNA clones by heteroduplex and restriction enzyme analysis and found that they all represent different structural variants of the full-size IAP genomic element. In clones 8.3 and 10.2, which have been fully sequenced, the open reading frames span deletions 3.4 and 1.9 kilobases (kb) long, respectively, and specify different *gag-pol* fusion polypeptides. Clone 9.5 contains a 2.1-kb deletion entirely within the *pol* region. Two other clones (4.2 and 11.7) contain no internal deletion and may represent truncated cDNA copies of full-size (7.2 kb) IAP gene transcripts. Structural variants very similar to clone 10.2 are common in the mouse genome, and clone 9.5 is also probably not a unique gene form. The sequences of clones 8.3 and 10.2 are different in detail, but each is closely homologous to a randomly cloned mouse genomic IAP element throughout the *gag*-related portions of their open reading frames. Antibodies against two oligopeptides specified by the sequence of clone 8.3 immunoprecipitated IAP-related proteins from mouse neuroblastoma and myeloma cells, confirming that the IgE-BF produced by this clone shares sequence with expressed IAP elements in different cell types. Thus, information related to the IgE-BF is an integral part of the murine IAP retrotransposon *gag* gene.**

Soluble factors with affinity for the Fc region of IgE (IgE-binding factors, or IgE-BF) help to regulate IgE synthesis by B lymphocytes (1). IgE-BF are produced by rat, mouse, and human lymphocytes after appropriate antigenic stimulus or exposure to homologous IgE. The rat–mouse T-cell hybridoma 23B6, when exposed to rat IgE, secretes IgE-BF with biochemical, antigenic, and biological properties similar to the factors produced by normal T cells (2). A previous report (3) described the isolation from this hybridoma of several cross-hybridizing cDNA clones that directed the synthesis of IgE-BF when transfected into cultured COS7 monkey cells. The factors produced by two of these clones not only bound to IgE but were biologically active in potentiating IgE production by B lymphocytes in an *in vitro* assay. One such clone, 8.3, was analyzed in detail (4) and shown to be a member of the endogenous retrovirus-like intracisternal A-particle (IAP) gene family of the mouse (5). A sequence relationship between clone 8.3 and a cloned Syrian hamster genomic IAP-related element was noted by Toh *et al.* (6), who suggested on the basis of this heterologous comparison that the clone was a recombinant between an IAP element and another cellular gene. However, our study detected no cellular (non-IAP) sequences in clone 8.3 (4).

In the present work, we have characterized four additional IgE-BF cDNA clones by heteroduplex and restriction enzyme analysis. Each one proved to be a different structural variant of the full-size [7.3-kilobase (kb)] mouse IAP element. Structural, nucleotide sequence, and immunological data indicate that IgE-BF specificity is encoded by sequences closely related to those of the IAP *gag*-encoded structural protein. No evidence for recombination with extraneous cellular sequence elements was found in any of the cDNA clones. Some questions raised by these observations are discussed.

MATERIALS AND METHODS

Cell Lines. The T-cell hybridoma 23B6 was formed by fusion of cells from a rat mesenteric lymph node and the AKR mouse T-lymphoma B5147 (2). The neuroblastoma cell line N4 was derived from the C1300 tumor in an A/J mouse (7).

cDNA Clones. These were prepared in the pCD expression vector (8) from the poly(A)$^+$ RNA of 23B6 cells that had been cultured in the presence of rat IgE to induce secretion of IgE-BF (3). Several clones that induced transient expression of IgE-BF on transfection into COS7 monkey cells (3) were selected for further study.

IgE-Binding Factors. IgE-BF were concentrated from the culture medium by affinity chromatography on IgE-Sepharose, assayed by inhibition of rosette formation (binding activity), and tested for potentiation of IgE production in cultured rat mesenteric lymph node cells (biological activity) as described (2, 4, 9).

Mouse Genomic IAP Clones. The MIA14 and MIA48 IAP elements were isolated previously (5, 10) from a BALB/c mouse embryo genomic library in λ phage Charon 4A. MIA14 in a "typical" full-size genomic IAP element in the sense that it forms colinear duplexes with other 7.3-kb genomic isolates and with 7.2-kb (35S) IAP RNA (5, 10). For heteroduplexing, we used pMIA14.10-1, a previously described (4) pBR322-derived subclone in which the 7.3-kb MIA14 element is truncated at the *Hin*dIII site at map position 6 (see Fig. 1) but otherwise complete, and pMIA14.10-3, containing the same insert in the opposite orientation. For cloning of 4.2-kb *Hin*dIII fragments derived from deleted IAP genomic elements (see *Results*), 100 μg of *Hin*dIII-digested BALB/c embryo DNA was electrophoresed in a 1% agarose gel and the region corresponding to a 4.2-kb marker was excised.

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

Abbreviations: IgE-BF, IgE-binding factor(s); IAP, intracisternal A particle(s); LTR, long terminal repeat; bp, base pair(s); kb, kilobase(s).

DNA was recovered by electroelution and cloned (10) into the *Hin*dIII site of pBR322. Plasmids that hybridized with a radiolabeled IAP probe (10) were examined for proper insert size and the appearance of a 1.4-kb fragment on *Bam*HI digestion (see Fig. 1). Four plasmids were selected for heteroduplexing (4, 5, 10) and restriction enzyme analysis.

DNA Sequencing. This was done by both the Maxam–Gilbert (11) and the chain-termination (12) method. For the latter, the M13 mp10 and M13 mp11 vectors and the Amersham dideoxy sequencing kit were used. Nucleotide sequence homologies were examined with the NUCALN program (13), using a *k*-tuple number of 3, a window size of 20, and a gap penalty of 4. The complete sequence of clone 10.2 has been determined (M.L.T., unpublished data). A partial sequence, covering the regions shown in Fig. 1, has been determined for MIA14 (J.A.M., unpublished data). Both sequences are available on request from the authors.

RESULTS

Four IgE-BF cDNA clones were characterized earlier in terms of their secretory products (3). Clones 8.3 and 9.5 produced IgE-potentiating factors on transfection into COS7 monkey kidney cells, whereas clones 4.2 and 10.2 coded for IgE-BF that had no apparent biological activity. The products of clone 8.3 were two structurally related glycoproteins of 60 and 11 kDa. Clone 10.2 produced a 60-kDa glycoprotein. The binding proteins of clones 9.5 and 4.2 were not sized. Clone 8.3 was fully sequenced (3, 4): it is 3400 base pairs (bp) long, contains a 556 amino acid open reading frame, and terminates in a sequence that is closely homologous to the long terminal repeats (LTRs) of previously cloned mouse IAP elements (14, 15). Heteroduplex analysis of clone 8.3, the results of which are included in Fig. 1, showed that this gene contained a 3.4-kb deletion with respect to a reference 7.3-kb genomic IAP element, MIA14, and that the open reading frame represented a fusion of coding sequences from both *gag* and *pol* regions of IAP (4).

IgE-BF cDNA Clones Represent Multiple Structural Variants of IAP Genes. Clones 4.2, 9.5, 10.2, and 11.7 (which also produces a biologically inactive IgE-BF) were heteroduplexed against the same pBR322-based subclone of MIA14 that was used for analysis of clone 8.3 (4). The results are shown in Fig. 1. Each clone had a different structural relationship to the genomic IAP element. Clones 10.2 and 9.5 both represented deleted gene forms. Clone 9.5 is particularly interesting, since its deletion includes the *pol*-related regions that contribute to the open reading frames in clones 8.3 and 10.2. Clones 4.2 and 11.7 could represent truncated cDNAs derived from transcripts of full-size IAP elements. Differences in restriction sites establish that they are independent isolates. Clone 11.7 extends to a position near the protein start site in clones 8.3 and 10.2 (open bars, Fig. 1) and, considering the possible error in heteroduplex measurements, could well reach the same initiation codon. Clone 4.2 may initiate its IgE-BF at a position 100–300 bp downstream from the usual start site. Such a protein could retain both of the N-glycosylation sites specified in the sequence of clone 8.3 (Fig. 3).

Some cDNA Structural Variants Are Not Unique. *Hin*dIII cleaves cDNA clones 9.5 and 10.2 at sites in the 5′ LTR and at map position 6 (Fig. 1) to generate fragments of 3.9 and 4 kb, respectively. When hybridized with an IAP probe, Southern blots of *Hin*dIII-digested mouse genomic DNA present a prominent band at about 4.2 kb, in addition to the principal 6.0-kb band generated by cleavage at the corresponding sites within the 7.3-kb IAP elements (5). We cloned the 4.2-kb, agarose gel-fractionated *Hin*dIII fragments from mouse DNA directly into pBR322 and, from a number of IAP-related recombinants, selected four that yielded 1.4-kb

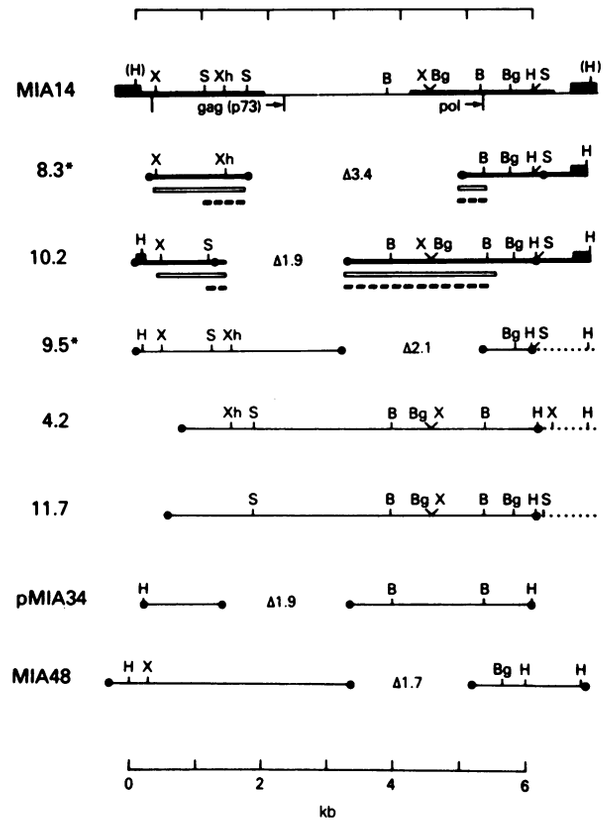

FIG. 1. Physical maps showing the structural relationships between five IgE-BF cDNA clones (8.3, 10.2, 9.5, 4.2, and 11.7) and three mouse genomic IAP elements (MIA14, MIA48, and pMIA34). Heavy solid lines indicate regions where nucleotide sequence has been determined; open bars show open reading frames as determined by sequence; heavy broken lines show regions of nucleotide and amino acid homology between these IAP elements and the cloned IAP-related sequence element H18 from Syrian hamster (6, 16). Sequenced long terminal repeats (LTRs) are shown as filled boxes. Filled circles delimit the regions of homology between MIA14 and the other clones as determined by heteroduplex analysis. The subclone of MIA14 in pBR322, pMIA14.10-1, used for heteroduplex analysis, was truncated at the *Hin*dIII site at map position 6 (4). Dotted lines indicate regions of cDNA clones revealed by restriction enzyme analysis but not visualized on heteroduplexes or determined by sequence analysis. Restriction sites: H, *Hin*dIII; X, *Xba* I; S, *Sst* I; Xh, *Xho* I; B, *Bam*HI; Bg, *Bgl* II. pMIA34 was tested with *Hin*dIII and *Bam*HI only; MIA48 was tested with these enzymes plus *Bgl* II and *Xho* I. The LTRs in MIA14 are unusual in lacking a *Hin*dIII site; this results from a single base change in the enzyme recognition site.

fragments on digestion with *Bam*HI (see Fig. 1). Heteroduplex analysis revealed that all of them had the structure and dimensions exemplified by pMIA34 (Fig. 1). The deletions in these genomic clones measured from 1.90 to 2.01 kb; the conserved 5′ and 3′ regions averaged 1.26 kb (range 1.15–1.32) and 2.87 kb (range 2.70–3.01), respectively. The corresponding values for cDNA clone 10.2 were 1.89 kb (deletion), 1.22 kb (5′), and 2.89 kb (3′).

Genomic clone pMIA34 (in pBR322) was heteroduplexed against clone 10.2 [in Okayama–Berg vector (8)] with the result shown in Fig. 2. The IAP homology region (continuous lines in diagram) measured 4.0 ± 0.1 kb, in good agreement with the distance of 4.12 kb between the relevant *Hin*dIII sites in clone 10.2 as determined by sequence analysis. Homology was continuous throughout this region at the resolution of the electron microscopy (probably about 50 bp).

These results indicate that the mouse genome contains multiple IAP variants with close resemblance to cDNA 10.2. In an earlier study, we happened to clone a genomic IAP

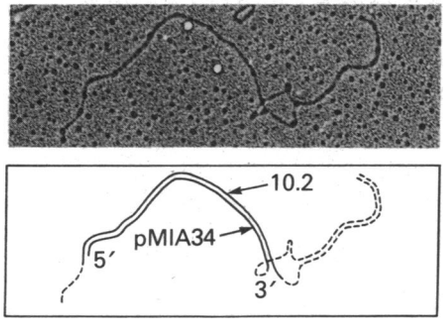

FIG. 2. (*Upper*) Electron micrograph of a heteroduplex between the IgE-BF cDNA clone 10.2 and pMIA34, a recombinant containing a randomly cloned 4-kb *Hin*dIII fragment derived from a mouse genomic IAP element. The cDNA clone is in the pBR/simian virus 40 (SV40)-derived vector pcD (8), and pMIA34 is in unmodified pBR322. (*Lower*) Vector sequences are shown by broken lines in the diagrammatic representation. 5′ and 3′ indicate the limits and orientation of the continuous homology region between the IAP-related sequences (solid lines).

element, MIA48, that contains a 1.7-kb deletion that spans both *Bam*HI sites (Fig. 1). This element resembles cDNA clone 9.5 in general form. We have already noted the possible correspondence between the cDNA clones 4.2 and 11.7 and the full-sized IAP elements. Genomic IAP elements resembling clone 8.3 have not been encountered; this clone may represent a low-copy-number or unique structural variant.

Nucleotide Sequence and Amino Acid Homologies. Clone 10.2 has been completely sequenced (M.L.T., unpublished data): it is 4984 nucleotides long, terminating in an LTR sequence similar to but not identical with that of clone 8.3. Clone 10.2 contains a 3225-bp open reading frame that begins at the same ATG as in clone 8.3 and spans the 1.9-kb deletion (Fig. 1). This open reading frame specifies a *gag-pol* fusion protein of 1075 amino acids, or about 116 kDa, although the IgE-BF secreted by cells transfected with clone 10.2 is only 60 kDa.

The nucleotide sequence of MIA14 was determined over the regions shown in Fig. 1. In regions where comparable data are available, cDNA clones 8.3 and 10.2 share 90–96% homology with MIA14 (data not shown).

We focused attention on the *gag*-related portion of the cDNA reading frames because this was the only known or potential coding region common to all five cDNAs (Fig. 1). Fig. 3 shows the translated amino acid sequences for the *gag* coding regions of clones 8.3 and 10.2 (i.e., the regions upstream of the deletion points). The nucleotide sequence of MIA14 was translated to obtain a putative amino acid sequence for the corresponding region of this randomly selected 7.3-kb genomic IAP element. Also shown in Fig. 3 is the amino acid sequence of Syrian hamster IAP clone H18 (6, 16) from the point where nucleotide homology with the murine elements begins (Fig. 1).

Both cDNA clones show extensive amino acid homology over their entire *gag*-related regions with the derived sequence of mouse genomic clone MIA14. There is no evidence for the break in homology seen on comparison with the heterologous hamster IAP element. Clone 8.3 contains an additional nucleotide at amino acid codon 421 as compared to both MIA14 and H18 (vertical arrow, Fig. 3). As a result, 8.3 is translated in an alternative reading frame for 21 amino acids before the junction with *pol* sequence. The *gag*-related region of clone 10.2 protein is 108 amino acids shorter than that of clone 8.3 and contains a number of substitutions and other small differences that could be related to the lack of biological activity of the 10.2-derived IgE-BF. However, both of the N-glycosylation sites found in clone 8.3 (Fig. 3, asterisks) are

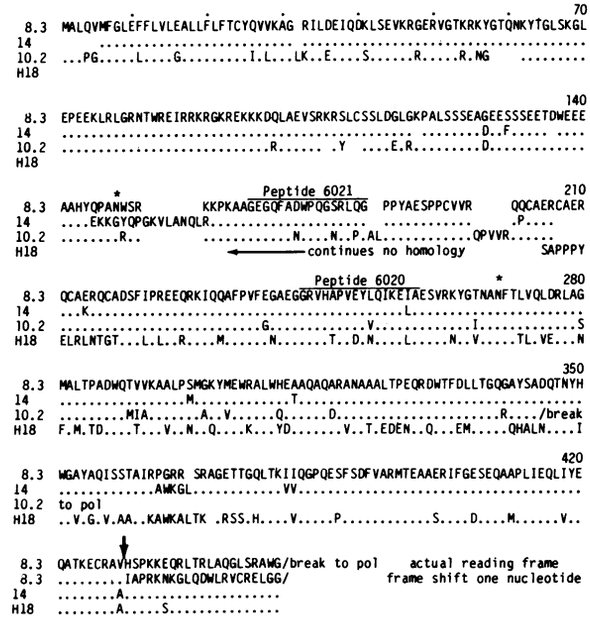

FIG. 3. Amino acid sequences (standard one-letter abbreviations) derived from the nucleotide sequences of cDNA clones 8.3 and 10.2, the mouse genomic IAP element MIA14, and a Syrian hamster IAP-related element, H18 (6, 16). Letters indicate differences between the sequence of 8.3 and those of the other clones. Asterisks show the positions of two potential N-glycosylation sites common to clones 8.3 and 10.2. Oligopeptides 6020 and 6021, used for antibody preparation, are indicated. Vertical arrow shows the position of an additional base pair in the sequence of 8.3 as compared to those of MIA14 and H18. The actual amino acid sequence of clone 8.3 is shown as well as the sequence that is obtained by reading 8.3 in a frame shifted by one nucleotide at amino acid position 421. Homology of this hypothetical sequence with those of MIA14 and H18 is evident.

retained. N-glycosylation is essential for potentiating activity of IgE-BF (1).

The sequences of 8.3 and 10.2 code for NH_2-terminal hydrophobic signal sequences of 29 and 28 amino acids, respectively, and a similar (although incomplete) region is specified by the genomic IAP clone 14. These sequences are consistent with secretion of the IgE-BF. However, the IAP *gag* protein, p73, is not known to be secreted.

Antibodies to cDNA-Specified Oligopeptides Precipitate IAP-Related Proteins in an Unrelated Cell Type. Earlier we showed (4) that rabbit antisera prepared against purified 73-kDa IAP *gag* core protein (p73) were able to bind both the 60-kDa and the 11-kDa IgE-BF of clone 8.3, as well as the IgE-BF produced by hybridoma 23B6 and by normal rat mesenteric lymph node cells. Oligopeptide 6021 prepared from the sequence of clone 8.3 (Fig. 3) was found to block the interaction of anti-p73 serum with the 11-kDa IgE-BF of this clone, while oligopeptide 6020 did not. Antisera prepared against ovalbumin conjugates of both 6020 and 6021 could bind IgE-BF produced by the 23B6 hybridoma. Both antisera also reacted with p73 when it was used as antigen in solid-phase ELISA (4).

The p73 antigen was obtained from IAPs isolated from mouse myeloma MOPC-104E (17). We considered the possibility that IAP protein derived from a mouse myeloma might share some special antigenic relationship with IgE-BF produced by another type of mouse lymphoid cell. Accordingly, antisera against both oligopeptide-ovalbumin conjugates were tested for reactivity toward IAP-related proteins produced by a different cell type, a cultured mouse neuroblastoma line (18). Fig. 4*A* shows the patterns of protein immunoprecipitated with these antisera from extracts of

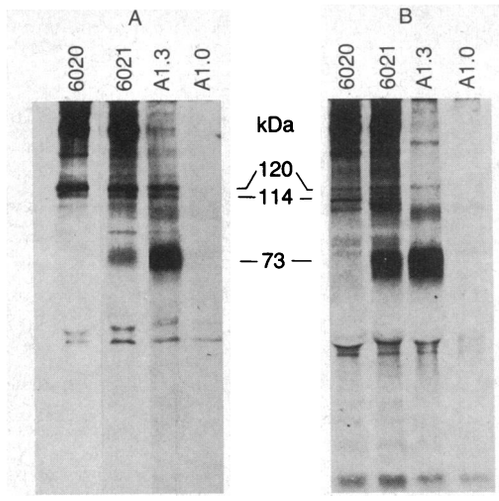

FIG. 4. Immunoprecipitation of IAP-related proteins from the N4 neuroblastoma cell line (*A*) and MOPC-104E myeloma ascites cells (*B*), each metabolically labeled with [^{35}S]methionine for 4 hr. Portions of labeled extracts containing 25 μg of cell protein were immunoprecipitated with 3 μl of rabbit antiserum A1.3 (directed against the major IAP *gag* structural protein p73) or A1.0 (preimmunization serum from the same rabbit) or with 10 μl each of rabbit antisera against ovalbumin conjugates of oligopeptides 6020 and 6021 (see Fig. 3). The immunoprecipitated proteins were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and autoradiography. Apparent molecular sizes were determined by reference to protein size markers (not shown).

[^{35}S]methionine-labeled neuroblastoma cells, compared to the pattern obtained with A1.3, a standard antiserum raised against p73 from myeloma IAPs. Immunoprecipitation reactions of these antisera with MOPC-104E proteins are shown in Fig. 4*B*.

The anti-6020 serum did not immunoprecipitate p73 from either source. Anti-6021 serum reacted strongly with MOPC-104E p73 and to a lesser extent with the neuroblastoma-derived protein. Both oligopeptide antisera showed a clear preference for two larger IAP-related neuroblastoma proteins, of 114 and 120 kDa, and for multiple components of similar size in MOPC-104E. Proteins of 114–120 kDa are produced in many mouse tumor cells and in normal thymus (18) where they are thought to represent *gag-pol* fusion products encoded by a 5.4-kb species of IAP RNA (see *Discussion*). The results in Fig. 4 confirm that peptide structure specified by cDNA clone 8.3 is present in IAP-related proteins produced by other cell types. They also point to hitherto unrecognized antigenic distinctions between the p114–120 and p73 classes of proteins that could be related to the primary sequences of their respective *gag*-related portions or to restrictions in epitope accessibility in p73 when it is presented to the anti-peptide antibodies in a soluble form.

DISCUSSION

This study shows that each of the characterized IgE-BF cDNA clones from a rat–mouse T-cell hybridoma represents a different structural and/or sequence variant of the endogenous IAP retrovirus-like gene family. We found no evidence for major blocks of non-IAP-related DNA at either the electron microscopic or nucleotide sequence levels of analysis. About 1000 IAP elements are found per haploid genome in *Mus musculus* (19), and 300–400 in the rat (20). The gene families of the two species are widely divergent from one another, as evidenced by heteroduplex analysis and low thermal stability of hybrids (20). Although the cDNA clones were everywhere closely homologous to mouse IAP elements and therefore clearly of mouse origin, we cannot eliminate the possible contribution of some sequence from very highly conserved (essential) rat IAP-related genes. The abrupt discontinuity in homology between the *gag* portion of clone 8.3 and the *gag* region of a cloned Syrian hamster IAP gene (6) was not observed when the cDNA clone was compared to homologous mouse IAP elements.

It is paradoxical that the IgE-BF cDNA clones obtained from hybridoma 23B6 are of mouse origin, since the original fusion (2) was intended to perpetuate IgE-BF produced by the immunized rat mesenteric T cells. We have no ready explanation for this phenomenon. Rat IgE-BF genes may also be activated in the hybridoma, but at too low a level to be detected against the predominant mouse response. Possibly the rat partner conferred some necessary glycosylation pattern on the hybrid cells. If so, this pattern is not unique, since fully active (glycosylated) IgE-BF are secreted by COS7 cells transfected with the cDNA clones.

IgE-binding capacity is probably specified by sequences in the *gag*-related portions of the cDNA clones, since (*i*) clones 8.3, 9.5, and 10.2 all encode IgE-BF yet differ entirely in their contiguous downstream sequences, and (*ii*) the active 11-kDa subfragment of the 60-kDa IgE-BF encoded by clone 8.3 is derived from the *gag* region of the larger molecule and is bound by antibody raised against the IAP *gag* protein p73 (4). Furthermore, the IgE-BF produced by hybridoma 23B6 are also bound by antisera against p73 (4). IgE-BF are glycoproteins whose capacity for suppression or enhancement of IgE production is governed by the extent and nature of their carbohydrate side-chains (1). Two potential sites for N-glycosylation are found in the *gag*-related coding sequences of both clones 8.3 and 10.2 (asterisks, Fig. 3). Other sequences uniquely required for IgE-binding and biological activity will need to be determined by analysis of additional cDNA clones and by site-directed mutagenesis.

Among the cDNA clones presently studied, only 8.3 seems to have an unusual overall structural arrangement; the others resemble types of IAP elements that have been encountered upon random cloning from the mouse genome and are thus likely to be present in multiple copies. Clone 10.2 is particularly striking in this regard, since it belongs to a class of deleted IAP genetic elements that is abundantly represented in the mouse. Full-size IAP genes produce 7.2-kb transcripts (5) that code for the 73-kDa *gag* structural protein (21). IAP elements lacking 1.9 kb of internal sequence, such as those that gave rise to the *Hin*dIII fragments cloned in pMIA34, -35, -36, and -39, would be expected to provide transcripts of 5.3 or 5.4 kb. This is a prominent-size species of IAP-related poly(A)$^+$ RNA in many transformed cells (18, 21–23), including the 23B6 hybridoma (4), and, among normal tissues, the thymus (18). The 5.4-kb transcripts are deficient in sequence from the 3′ portion of *gag* and the 5′ portion of *pol* and direct the synthesis of 114- to 120-kDa proteins that represent fusion polypeptides encoded by the remaining *gag* and *pol* regions (18). Clone 10.2, which has a 1075 amino acid open reading frame composed of *gag*- and *pol*-related sequences that span a 1.9-kb deletion (Fig. 1), has precisely this structure. The 60-kDa IgE-BF secreted by cells transfected with clone 10.2 (3) could be generated by processing of the putative 116-kDa primary protein product of this gene.

An antigenic relationship has been observed between IgE-BF and the Fc_ε receptors of T and B lymphocytes (24), and the possibility has been raised that the receptor molecules might also be coded by retrovirus-like genes (4). Apparent molecular sizes of 49 kDa and 54 kDa have been observed for low-affinity Fc_ε receptors isolated from mouse B cells (25) and rat macrophages (26), respectively. It may be asked whether the group of 114- to 120-kDa IAP-related proteins includes molecular species that are processed to

lower molecular mass, cell-associated immunoglobulin-binding factors under appropriate stimulus.

The observations reported earlier (4) and in this paper raise many questions about the functional and evolutionary relationships between IgE-BF genes and the IAP retrovirus-like gene family as a whole. The diversity of gene structure associated with products of common function (IgE-binding) is noteworthy. We have shown that most of the structural variants encountered among the cDNA clones are not unique in the normal mouse genome; i.e., there is no evidence to suggest that the binding factor clones are recombinant forms peculiar to the hybridoma cells. Furthermore, rabbit antisera against IAP p73 react with IgE-BF produced by rat and mouse mesenteric node lymphocytes in response to *Nippostrongylus braziliensis* infection (4), confirming that IAP-related genes have a role in the normal IgE immune response. Are IAP elements an evolutionarily distinctive retroviral family in which cellular genetic information for immunoglobulin-binding factor(s) was incorporated into the *gag* coding region and then amplified by retrotransposition or gene conversion? If so, did this happen early in IAP evolution (so as to be widespread among mammalian species) or was it a late event confined to the family Muridae? Could binding-factor information, once incorporated into multiple genetic elements, become further differentiated as to immunoglobulin isotype?

Alternatively, it might be considered that information for the IgE-BF was acquired not by recombination with a cellular gene but by evolution of an original retroviral *gag* protein. However, the presence of a signal sequence in the mouse clones, even though the IAP 73-kDa *gag* protein itself is not secreted, seems more consistent with the acquisition of a cellular secretory-protein gene. Further, the abrupt sequence discontinuity with the hamster element, occurring at the same nucleotide in each IAP clone (Fig. 3), suggests a recombination event in the history of these species in addition to the gradual divergence of a common primordial IAP *gag* sequence. Like the mouse, the Syrian hamster contains about 1000 copies of IAP sequence elements per haploid genome (20, 27). Some of these elements might correspond more closely to the mouse genes if they happen to serve a similar function. The possible occurrence and structure of IgE-BF in the Syrian hamster, not hitherto investigated, would be of interest.

Human IgE-BF closely parallel the rodent IgE-BF in their known properties (4). Cloning of the human genes will show whether they are also associated with retroviral elements. Homologies have been detected between IAP-related sequences and retroviral elements in humans and monkeys (15, 28–31), but these homologies have not extended to the respective 5′-*gag* coding regions.

Rodent IAP may not be unique in incorporating information for cellular genes in their *gag* coding regions. Patarca and Haseltine (32) have pointed out certain similarities in amino acid sequence between an erythroid-potentiating activity (EPA) factor and the *gag* core proteins of Rous sarcoma virus, Moloney murine leukemia virus, bovine leukemia virus, and human T-lymphotropic virus types I–III.

1. Ishizaka, K. (1984) *Annu. Rev. Immunol.* **2**, 159–182.
2. Huff, T. F., Uede, T. & Ishizaka, K. (1982) *J. Immunol.* **129**, 509–514.
3. Martens, C. L., Huff, T. F., Jardieu, P., Trounstine, M. L., Coffman, R. L., Ishizaka, K. & Moore, K. W. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 2460–2464.
4. Moore, K. W., Jardieu, P., Mietz, J. A., Trounstine, M. L., Kuff, E. L., Ishizaka, K. & Martens, C. L. (1986) *J. Immunol.* **136**, 4283–4290.
5. Kuff, E. L., Smith, L. A. & Lueders, K. K. (1981) *Mol. Cell. Biol.* **1**, 216–227.
6. Toh, H., Ono, M. & Miyata, J. (1985) *Nature (London)* **318**, 388–389.
7. Amano, T., Richelson, E. & Nirenberg, M. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 258–263.
8. Okayama, H. & Berg, P. (1983) *Mol. Cell. Biol.* **3**, 280–289.
9. Yodoi, J., Hirashima, M. & Ishizaka, K. (1980) *J. Immunol.* **125**, 1436–1441.
10. Lueders, K. K. & Kuff, E. L. (1980) *Proc. Natl. Acad. Sci. USA* **77**, 3571–3575.
11. Maxam, A. M. & Gilbert, W. (1980) *Methods Enzymol.* **65**, 499–560.
12. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463–5467.
13. Wilbur, W. J. & Lipman, D. J. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 726–730.
14. Kuff, E. L., Feenstra, A., Lueders, K., Smith, L., Hawley, R., Hozumi, N. & Shulman, M. (1983) *Proc. Natl. Acad. Sci. USA* **80**, 1992–1996.
15. Christy, R. J., Brown, A. R., Gourlie, B. B. & Huang, R. C. C. (1985) *Nucleic Acids Res.* **13**, 289–302.
16. Ono, M., Toh, H., Miyata, T. & Awaya, T. (1985) *J. Virol.* **55**, 387–394.
17. Kuff, E. L., Wivel, N. A. & Lueders, K. K. (1966) *Cancer Res.* **28**, 2137–2148.
18. Kuff, E. L. & Fewell, J. W. (1985) *Mol. Cell. Biol.* **5**, 474–483.
19. Lueders, K. K. & Kuff, E. L. (1977) *Cell* **12**, 963–972.
20. Lueders, K. K. & Kuff, E. L. (1983) *Nucleic Acids Res.* **11**, 4391–4408.
21. Paterson, B. M., Segal, S., Lueders, K. K. & Kuff, E. L. (1978) *J. Virol.* **27**, 118–126.
22. Hojman-Montes de Oca, F., Dianoux, L., Peries, J. & Emanoil-Ravicovitch, R. (1983) *J. Virol.* **46**, 307–310.
23. Wujcik, K. M., Morgan, R. A. & Huang, R. C. C. (1984) *J. Virol.* **52**, 29–36.
24. Huff, T. F., Yodoi, J., Uede, T. & Ishizaka, K. (1984) *J. Immunol.* **132**, 406–412.
25. Conrad, D. H. & Peterson, L. H. (1984) *J. Immunol.* **132**, 796–803.
26. Finbloom, D. S. & Metzger, H. (1983) *J. Immunol.* **130**, 1489–1491.
27. Suzuki, A., Kitasato, H., Kawakami, M. & Ono, M. (1982) *Nucleic Acids Res.* **10**, 5733–5746.
28. Chiu, I.-M., Callahan, R., Tronick, S. R., Schlom, J. & Aaronson, S. A. (1984) *Science* **223**, 364–370.
29. Callahan, R., Chiu, I.-M., Wong, J. F. H., Tronick, S. R., Roe, B. A., Aaronson, S. A. & Schlom, J. (1985) *Science* **228**, 1208–1211.
30. Chiu, I.-M., Huang, R. C. C. & Aaronson, S. A. (1985) *Virus Res.* **3**, 1–11.
31. Power, M. D., Marx, P. A., Bryant, M. L., Gardner, M. B., Barr, P. J. & Luciw, P. A. (1986) *Science* **231**, 1567–1572.
32. Patarca, R. & Haseltine, W. A. (1985) *Nature (London)* **318**, 390.