Expression of Novel Secreted Isoforms of Human Immunoglobulin E Proteins*

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Four human IgE isoforms produced by alternative splicing of the epsilon primary transcript were expressed as chimeric mouse/human anti 5-dimethylaminol-1-naphthalenesulfonyl antibodies in the murine myeloma cell line Sp2/0. The four isoforms include the classic secreted form and three novel isoforms with altered carboxyl termini. All of these isoforms lack the transmembrane region encoded by the M1/M1′ exon and are therefore predicted to be secreted proteins. When expressed in Sp2/0 cells, three of the IgE isoforms are assembled into complete molecules of two Ig heavy chains and two Ig light chains, whereas the fourth isoform is predominately assembled into half-molecules of one Ig heavy chain and one Ig light chain. All four isoforms are secreted with similar kinetics. In contrast, one Ig heavy chain and one Ig light chain. All four isoforms include the secreted terminus at the end of the last constant domain or two downstream exons (M1 and M2) that encode the transmembrane and intracellular amino acids. Splicing to the M exons removes from the transcript the splicing acceptor located within the M2 exon. The reading frame is created by splicing directly to the M2′ exon (IgE grinded) is expressed in the J558L cell line, it is degraded intracellularly, suggesting a cell line-dependent regulation of secretion. These data show that these novel isoforms of human IgE, predicted to occur from in vivo and in vitro mRNA analysis, can be produced and secreted by mammalian cells. The different forms of IgE may have physiologically relevant but distinct roles in human IgE-mediated immune inflammation. The availability of purified recombinant human IgE isoforms makes it possible to analyze the functional differences among them.

Alternative RNA splicing determines the production of secreted versus membrane-bound forms of immunoglobulins (1, 2). This is accomplished in mammals by the alternative usage of either a secreted terminus at the end of the last constant region domain or two downstream exons (M1 and M2) that encode the transmembrane and intracellular amino acids. Splicing to the M exons removes from the transcript the nucleotides that encode the hydrophilic COOH terminus and polyadenylation signal for the smaller, secreted form of the Ig.

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The one functional genomic locus encoding human epsilon heavy chain contains four Ig domain exons (C1 to C4) and the two membrane exons (M1 and M2). We (3–5) and others (6, 7) have previously shown that RNA prepared from the IgE-producing human cell line AF-10 and from fresh B lymphocytes stimulated to make IgE contain a variety of epsilon mRNAs produced by alternative splicing. In contrast to what is observed with other isotypes, the most common form of mRNA encoding membrane IgE is produced by splicing to a novel splice acceptor 156 base pairs upstream of the normal M1 acceptor site (4, 7). The M1′ exon produced using this splice acceptor encodes 52 novel amino acids that are largely hydrophilic followed by the amino acids normally encoded by M1.

Other alternatively spliced epsilon mRNAs are present that encode a series of potentially secreted proteins. The splicing events that generate these mRNAs utilize several novel exons including M2′, M2″, and C5 in addition to the classic secreted form (see Fig. 1A). The M2′ exon is created by splicing directly from C4 to the normal M2 splice acceptor. The omission of M1 results in a frameshift in M2, which creates an open reading frame encoding 136 hydrophilic amino acids (i.e. M2″). M2″ is a short tail (8 amino acids) created by splicing from C4 to a splice acceptor located within the M2′ exon. The reading frame of M2′′ is different from that of M2′ (4, 5).

It is of great interest whether these novel mRNAs encode functional proteins and whether these various forms of IgE play distinct roles in the immune response. Using polyclonal anti-peptide antibodies, we have detected the protein produced of one of these novel splice variants in the supernatant and cytoplasm of AF-10 cells and in serum from a patient with IgE myeloma (3). However, the low level of IgE present in normal serum makes it impossible to isolate sufficient quantities with adequate purity for definitive functional studies. Human IgE is the least abundant Ig with average serum concentrations (125 ng/ml) generally 100,000-fold less than IgG in normal individuals. Purification from serum would be further confounded by the similar molecular size of several of the splice variants. Therefore, we have focused on developing expression systems for the production of each epsilon splice variant.

In a recent study, Batista et al. (8) report the expression in J558L murine myeloma cells of five constructs that encode individual splice variants of IgE (8). The conclusions of this study were that only one form of IgE is detectable on the surface of the transfected cells and only one form of soluble IgE is secreted by myeloma cells. These forms were found to correspond to the CH4-M1′ membrane-bound and the classic secreted (CH4-S) forms of IgE, respectively. Although Western blotting of protein secreted by the cell line U266 revealed heterogeneity of epsilon chains, Batista et al. (8) conclude that this is the result of differential glycosylation. They further conclude that the novel epsilon isoforms produced by alternatively spliced mRNAs are degraded intracellularly...
and therefore cannot constitute functionally relevant forms of IgE.

In the present study, we demonstrate the expression and secretion of four soluble isoforms of human IgE by the murine myeloma cell line Sp2/0. The isoforms examined are classic secreted (CH4-S), CH4-M2′, CH4-M2″, and CH4-CH5. We designate these proteins as IgE-globular, IgE-grandé, IgE-tailpiece (IgEtp), and IgE chimeric CH4 (IgE-chCH4), respectively. All four isoforms show similar kinetics and efficiency of assembly and secretion, although one of the isoforms (i.e. IgE-chCH4) is secreted predominately as HL.1 half-molecules. Furthermore, the production of secreted protein is shown to depend on the murine myeloma cell line used for expression.

**EXPERIMENTAL PROCEDURES**

Vectors and Proteins—To create isofrom-specific-specifon constructs, a portion of the human epsilon gene encoding C41 through C44 was fused in-frame to three RT-PCR clones. The RT-PCR clones span the downstream sequences resulting from alternative splicing events as well as a portion of C44 (see Fig. 1B), RT-PCR cloning of 3′ portions of the various epsilon mRNAs has been reported (4, 5). Briefly, total RNA was isolated from both the IgE-producing human myeloma AF-10 and from purified B cells stimulated to produce IgE with interleukin-4 and CD-40 monoclonal antibody. The RNA was then reverse-transcribed using oligo(dT)15 primer (Boehringer Mannheim) and mouse Moloney leukemia virus reverse transcriptase (Life Technologies, Inc.). cDNA was then used as substrate for PCR reactions using upstream primers located within C44 at Asp497-Glu504 (IgE-chCH4) or at Glu411-Val549 (IgE grandé and IgEtp) (5) and a downstream primer located 21–42 nucleotides 3′ of the M2′ exon (4). PCR reaction mixtures contained 10% MeSO, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2.5 mM MgCl2, and 100 μM nucleoside-free bovine serum albumin. Primer concentration was 0.5 μM. Reactions were carried out for 40 cycles at the following temperatures: melting for 1 min at 94°C, annealing for 1 min at 72°C, and extension for 1 min at 72°C.

Isoform-specific epsilon chain genes were first created using PCR mutagenesis to create novel Xbal or restriction sites within the C44 coding region of each of these RT-PCR clones and an EcoRI site at the 3′ end of each clone (before the polyadenylation addition signal). PCR mutagenesis was then used to create the identical mutations within the C44 exon of the human genomic epsilon gene. The Xbal site was created at arginine 520; the NheI site was added at alanine 543. In all cases introduction of the restriction sites did not alter the amino acid sequence. DNA for NheI sites was then used to fuse the various downstream sequences with the epsilon gene. The 3′-untranslated region (UTR) from human γ3 heavy chain gene had been provided with an EcoRI site 5′ of the polyadenylation addition signal (9) and was substituted for the 3′-UTR of the human epsilon gene by ligation at the EcoRI sites created at the 3′ ends of the RT-PCR clones. The genes encoding the different epsilon isoforms were then cloned into pSV2 gpt containing the coding sequence for a heavy chain variable domain specific for the hapten dansyl chloride under control of the IgG heavy chain promoter and IgG heavy chain enhancer (9). This was done by ligation at the BamHI site at the 3′ end of the 3′-UTR (see Fig. 1C) and by ligation of the XhoI site immediately 5′ of the C41 exon with a Sall site 3′ of the Ig heavy chain enhancer in the expression vector. The Sall and XhoI sites were destroyed in the ligation and are indicated by a • in Fig. 1C. The anti-dansyl light chain used is a chimeric kappa chain consisting of a murine variable (Vκ) domain and human C κ domain. (10).

Cells—Sp2/0 and J558. Murine myeloma cells and various transfectants thereof were carried in Iscove’s modified Dulbecco’s medium (Irvine Scientific, Santa Ana, CA) containing 5% bovine calf serum (HyClone, Logan, UT). A stable anti-dansyl light chain-producing line (TWS) was established from Sp2/0 cells as described previously (9). Stable IgE-producing cell lines were created by electroporation of the isoform-specific heavy chain in the pSV2 gpt expression vector into TWS (5). Briefly, 10 μg of DNA linearized at the BamHI site was added to 107 TWS cells in 0.5 ml phosphate-buffered saline, and the cells were pulsed at 200 V with 960 microfarads in a 0.4-cm electrode gap cuvette (Bio-Rad). Stable transfectants were selected for growth in medium containing 42 μg/ml hypoxanthine, 1 μg/ml xanthine, and 2.5 μg/ml mycophenolic acid. Clones producing IgE were identified by ELISA using plates coated with dansyl chloride-bovine serum albumin. Supernatants from clones were added to wells, and bound protein was detected using goat anti-human kappa chain conjugated to alkaline phosphatase. Nonsecreting transfectants of 1558L were identified by lysis of 5 × 104 cells in lysis buffer (lysis buffer — 0.5% Nonidet P-40, 50 mM Tris, pH 7.0, 150 mM NaCl, and 5 mM EDTA) and analysis of lysates by ELISA using plates coated with the anti-human IgE monoclonal antibody CIA 7.12 (see ELISA section below). Bound protein was detected using goat anti-human epsilon chain conjugated to alkaline phosphatase (Sigma).

Isoform-specific ELISA—The anti-IgE monoclonal antibody CIA-7.12 that recognizes an epitope at the Cε2/Cε3 boundary was used as the coating reagent as described (11). After blocking, the cell culture supernatants containing expressed IgE isoforms were incubated for 2 h at room temperature. CIA-7.12 or a 1:500 dilution of α-2331, a rabbit antiserum generated against a peptide corresponding to the COOH-terminal 10 amino acids of IgE grandé (3) was then added to the microplate and incubated for 2 h at room temperature followed by a 2-h incubation with goat anti IgG conjugated to alkaline phosphatase (Sigma).

Western Blotting—Western blotting analysis had been previously described (5–7). Briefly, the expressed IgE isoforms were immunoprecipitated from the cell culture supernatants with the monoclonal antibody CIA-7.12 coupled to Sepharose 4B (Pharmacia Biotech Inc.). After transfer, the nylon membrane was blocked by 4% bovine serum albumin/phosphate-buffered saline/Tween for 4 h at room temperature. For α protein detection, the blot was probed with goat anti-human IgE (ε chain-specific) conjugated to alkaline phosphatase (Kirkgaard and Perry Laboratories, Inc., Gaithersburg, MD). For detection of IgE grandé, the blot was incubated with 1:200 diluted α-2331 overnight at 4°C followed by a 2-h incubation at room temperature with anti-IgG conjugated to alkaline phosphatase (Sigma). Color development was performed with an alkaline phosphatase conjugate substrate kit (Bio-Rad) as described by the manufacturer.

**Protein Labeling, and SDS-Polyacrylamide Gel Electrophoresis—** 4–10% transfigected cells were washed twice in 2 ml of methionine-deficient Dulbecco’s modified Eagle’s medium (Mediatech, Herndon, VA), resuspended in 1 ml of methionine-deficient Dulbecco’s modified Eagle’s medium containing [35S]methionine (15 μCi/106 cells) (ICN, Irvine, CA), and incubated at 37°C for 6–18 h. Supernatant from clones were added to wells, and bound protein was detected using goat anti-humankappachain conjugated to alkaline phosphatase (Sigma) and monoclonal antibody CIA 7.12. Bound protein was then washed in sample loading buffer containing 5% NDET (100 mM Tris, pH 7.4, 100 mM NaCl, 2 M urea, 4% SDS, 10% glycerol, and 0.05% bromphenol blue) and boiled for 2 min. The samples were run on SDS-polyacrylamide gels (12). For two-dimensional gel analysis, samples were first electrophoresed on 5% polyacrylamide gels (12), and the lane containing the sample of interest was excised and incubated in sample loading buffer containing 5% dithiothreitol (Boehringer Mannheim) for 20 min at room temperature. The lane was then embedded in 12.5% polyacrylamide. Electrophoresis in the second dimension was performed at 20 mA with a 1 cm × 1 cm gel (12).

**Pulse-Chase—** 2–10% transfigected cells/time point were washed twice in 2 ml of methionine-deficient Dulbecco’s modified Eagle’s medium. Washed cells were then incubated in 2 ml of methionine-deficient Dulbecco’s modified Eagle’s medium for 1 h at 37°C to deplete the cells of intracellular methionine. Cells were pulsed with [35S]methionine (3.36 μCi/ml) for 1 h (at 37°C) and then chased with 8 ml of chase medium that had been prewarmed to 37°C (chase medium = Iscove’s modified Dulbecco’s medium (Irvine Scientific) + 10% bovine calf serum (HyClone) + 3.36 mg/ml unlabeled methionine (Schwarz/Mann, Orangeburg, NY)). At various time points, 1 ml aliquots were removed to tubes containing an equal volume of prechilled phosphate-buffered saline on ice. Trichloroacetic acid precipitable radioactivity was determined at various time points to confirm that the amount of nonradioactive methionine added was sufficient to prevent continued [35S]methionine incorporation during the 3-h chase.
Cells were separated from the supernatant by centrifugation for 5 min at 225 × g at 4 °C. Cell lysates were prepared by resuspending the cell pellet in 0.5 ml of NDET, centrifuging at 4 °C for 15 min at 15,000 × g, and discarding the pellet. IgE was precipitated from supernatants and cell lysates with a mixture of rabbit anti-human Fab (R27 antiseraum) and rabbit anti-human epsilon (ICN) as described above.

For densitometry of IgE assembly intermediates, nonreducing gels from pulse-chase experiments were visualized by autoradiography and scanned on a Hewlett-Packard ScanJet IIcx scanner. The images were analyzed at 600 dots/inch using the NIH Image software package.

RESULTS

Production of Genetically Determined IgE Isoform-specific Transfectomas—Fig. 1B illustrates the strategy used for generating constructs encoding specific isoforms of human IgE.
Expression of Novel Human IgE Proteins

RT-PCR products encompassing the 3’ portion of Cε4 and sequences downstream were mutagenized by PCR to introduce either an XbaI site or an NheI site within the Cε4 portion of the RT-PCR clone without altering the amino acid sequence of the encoded protein. The identical restriction sites were also engineered in a separate construct containing the Cε1 through Cε4 exons. Mutagenesis was also used to introduce an EcoRI site after the termination codons of the RT-PCR clones. The RT-PCR clones containing 3’ coding sequences were fused to the sequences for Cε1 through Cε4 at the XbaI or NheI site. A polyadenylation addition signal was provided by ligation to an EcoRI site previously engineered immediately upstream of the polyadenylation signal in the 3’-untranslated region from human IgG3 (γ3 3’-UT in Fig. 1). The resulting constructs were subcloned into pSV2 gpt containing an exon encoding a variable heavy domain specific for the hapten dansyl chloride (see Fig. 1C). The expression vectors were then transfected into an Sp2/0-derived cell line previously transfected with a chimeric). The expression vectors were then transfected into an Cε heavy domain specific for the hapten dansyl chloride (see subcloned into pSV2 gpt containing an exon encoding a vari-

ing IgE classic, IgE grande´, and IgEtp produce a protein of approximately 190 kDa (Fig. 2A) that reduces upon treatment with 2-mercaptoethanol to a heavy chain of approximately 75 kDa and a light chain of approximately 25 kDa (Fig. 2B). This indicates that these isoforms are secreted as fully assembled H2L2 molecules. As expected, IgE classic and IgEtp migrate with identical mobilities, whereas IgE grande´ migrates slightly slower. The transfectoma expressing IgE ψCH4 secretes HL, which migrates as a broad band that is 75–90 kDa as well as light chain of approximately 25 kDa under nonreducing conditions (Fig. 2A). A minor component migrating at approximately 150 kDa that is apparently H2L is detectable in only some experiments (Figs. 2A and 3H and data not shown). The heavy chain of IgE ψCH4 migrates somewhat faster than the heavy chain of the other three isoforms (Fig. 2B). This difference is not due to glycosylation because it is still evident when the transfectomas are labeled in the presence of tunicamycin, an inhibitor of N-linked glycosylation (data not shown). However, the diffuse migration of the 75–90-kDa species does indicate heterogeneous glycosylation of the epsilon heavy chain because it is no longer evident when the transfectoma is labeled in the presence of tunicamycin. A panel of ten transfectomas expressing IgE ψCH4 (data not shown) all showed this pattern of assembly. The identities of the 75–90- and 150-kDa species as HL and H2L, respectively, are supported by two-dimensional SDS-polyacrylamide gel electrophoresis analysis (data not shown). The species that migrate as 75–90 and 150 kDa in the first dimension (nonreducing conditions) dissociate into heavy and light chains when run reduced in the second dimension. Furthermore, the 75–90-kDa species both immunoprecipitates and reacts in Western blots with epsilon-specific monoclonal and polyclonal reagents (data not shown). In addition to fully assembled H2L2 molecules, all four cell lines secrete free light chain (L) and light chain dimers (L2), as is frequently observed in this expression system (Fig. 2A). Secretion of light chain dimer is not evident for the IgE ψCH4-producing transfectoma in Fig. 2A but can be seen occasionally (e.g. Fig. 3H).

Because the protein secreted by the cell line producing IgE grande´ does not migrate as slowly as one would predict based on amino acid translation, we undertook experiments to verify that the protein was indeed complete and intact. Initially, the expression construct for IgE grande´ was subjected to extensive restriction analysis, which showed that the entire coding region had been retained in the construct (data not shown). Due to concern that the novel 136-amino acid tail of the IgE grande´ could be post-translationally cleaved, the protein was analyzed by ELISA and Western blotting using antibodies specific for the Cε2/Cε3 boundary (CIA-7.12) and for the COOH-terminal ten amino acids of IgE grande´ (α-2331). As expected, both IgE classic and IgE grande´ were recognized by CIA-7.12, whereas α-2331 recognized IgE grande´ but failed to recognize classic secreted IgE (Table I and data not shown). Neither CIA-7.12 nor α-2331 recognized an IgG control. Recognition of IgE grande´ by α-2331 indicates that the large secreted terminus of IgE grande´ is not removed by proteolytic processing. In addition, recognition of IgE grande´ by monoclonal antibody CIA-7.12 indicates that the Cε2/Cε3 interface (the epitope that CIA-7.12 recognizes) is intact; the ability of IgE grande´ to bind antigen confirms that it is present.

IgE Isoform Assembly and Secretion—To dissect the assembly pathways of the various forms of IgE and to determine the efficiency of secretion, we conducted pulse-chase experiments. As shown in Fig. 3A, the classic secreted form of IgE is assembled first to an HL intermediate that is abundant as early as 5 min post-chase. It is later assembled into the H2L2 form that is detectable by 10 min post-chase and abundant within the cells by 20 min post-chase. Very similar kinetics are observed for the assembly of IgE grande´ (Fig. 3C) and IgEtp (Fig. 3E). IgE
FIG. 3. **Intracellular assembly and secretion of IgE isoforms.** 2–8 × 10⁶ cells/time point were incubated in methionine-deficient medium for 1 h to deplete intracellular methionine. Cells were pulsed with [³⁵S]methionine (15 μCi/10⁶ cells) for 5 min at 37 °C and then chased with a 100-fold excess of unlabeled methionine. Samples were taken at various time points following addition of the chase. Epsilon and kappa chains were then immunoprecipitated from the cytoplasms and secretions at each time point and run on 5% gels under nonreducing conditions. A, IgE expression of novel human IgE proteins.
grandé and IgEtp also assemble through an HL intermediate. In addition, for IgEtp, small quantities of H2L are secreted as H2L2 during the course of the experiment. The efficiency of secretion of the two forms is shown in Fig. 4. No free heavy chain is seen in the secretions starting at 60 min post-chase (Fig. 3, B, D, and F). Pulse-chase experiments conducted with IgEΔCH4 show the only detectable species in the cytoplasm to be light chain and species that migrate as HL half-molecules of IgEΔCH4 (Fig. 3G). No free heavy chain is visible, and there is very little assembly to the H2L2 form.

A single band (Fig. 3, A and E) probably representing IgE at various stages of glycosylation, as the H2L2 form in the secretions migrates as a single band (Fig. 3, B, D, E, and F). Pulse-chase experiments conducted with IgEΔCH4 show the only detectable species in the cytoplasm to be light chain and species that migrate as HL half-molecules of IgEΔCH4 (Fig. 3G). No free heavy chain is visible, and there is very little assembly to the H2L2 form.

H2L2 is secreted in varying amounts for each of the isoforms examined, with approximately 40% of the total Ig produced during the 5-min pulse secreted as H2L2 during the course of the experiment (Fig. 4A). Following reduction, the secreted proteins migrate as heavy and light chains of approximately 75 and 25 kDa (Figs. 2B and 5 and data not shown). IgEtp is the most efficiently secreted of the isoforms examined, with approximately 40% of the Ig secreted as H2L2 during the course of the experiment (Fig. 4A). HL is secreted in varying amounts for each of the isoforms examined (Fig. 3, B, D, F, and H). Secretion of small amounts of HL was also reported for IgE classic in the recent study by Batista et al. (8). In the case of IgEΔCH4 (Fig. 3H), the HL form constitutes the majority of IgE secreted by the transfectoma, with a smaller amount secreted as H2L. The efficiency of secretion of these two forms is shown in Fig. 4B and indicates that by 3 h after the pulse, approximately 20% of the labeled Ig is secreted in HL form, whereas only ~10% is secreted as H2L. All four transfectedants synthesize light chain that is secreted as free L and as L2 dimers (Fig. 3, B, D, F, and H).

Two species of 50 and 200 kDa (closed and open arrows in Fig. 3C) co-precipitate with intracellular IgE grande and (to a lesser extent) IgEtp (Fig. 3E). The mobility of these two co-precipitating proteins is not affected by treatment with 2-mercaptoethanol (Fig. 5, cytoplasm). It is also noteworthy that the 200-kDa species is present at zero time. From these data, we conclude that the 200- and 50-kDa proteins are not assembly intermediates of IgE but instead represent non-IgE proteins that are coprecipitated. The 200-kDa protein is not secreted, although a band at ~50 kDa is seen in the secretions (Fig. 3, B, D, and F). However, the latter is no longer detectable after treatment with 2-mercaptoethanol (Fig. 5, secretion) and most probably represents light chain dimers (L2). Thus, the co-precipitating 50-kDa cytoplasmic protein, like the 200-kDa protein, does not appear to be secreted.

These findings are in marked contrast to those of Batista et al. (8), who concluded that the novel isoforms IgE grande (CH4-M2) and IgEΔCH4 (also designated CH4-CH5 and CH4-I) are not secreted by plasma cells. One difference between the present and earlier studies is the murine myeloma cell line used for expression. To determine if the cell lines could account for the different results, we transfected our expression vector for IgE grande into J558L, the murine myeloma cell line used by Batista et al. No positive clones were identified when cell culture supernatants of several hundred selection-resistant transfecotomas were screened by ELISA (data not shown). However, several clones demonstrating high levels of intracellular epsilon protein and are below the level of detection in Fig. 2. In all experiments conducted with IgE classic, IgE grande, and IgEtp, the intracellular H2L2 form migrates as a doublet (Fig. 3, A, C, and D). The data shown are the average results of duplicate wells.

Secondary antibodies were conjugated to alkaline phosphatase. The substrate was 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitroblue tetrazolium (NBT). Rabbit antiserum generated against a peptide corresponding to the COOH-terminal 10 amino acids of IgE grande (Batista et al. (8)) co-precipitats with intracellular IgE grande and (to a lesser extent) IgEtp (Fig. 3, A and E). The data shown are the average results of duplicate wells.

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IgE isoforms.

Cell lines can vary in their ability to assemble and secrete the expressed in J558L. These results demonstrate that different cell clones were positive for intracellular epsilon chain, but screened by ELISA of cell lysates, a large percentage (50%) of non-secreted detectable amount of IgE. Therefore, the intracellular degradation of IgE grande appears to occur whenever it is expressed in J558L. These results demonstrate that different cell lines can vary in their ability to assemble and secrete the IgE isoforms.

DISCUSSION

Secreted IgE functions via its ability to bind to specific IgE receptors. These receptors make it possible for IgE to act as a very sensitive trigger for initiating both afferent and efferent immune reactivity in the presence of low doses of antigen. Three such “receptors” have been identified; the high affinity IgE receptor (FcεRI), the low affinity IgE receptor (FcεRII or CD23), and galectin 3 (formerly known as epsilon-binding protein) (13).

IgE mediates immediate type hypersensitivity primarily through its association with the high affinity IgE receptor present on the surface of mast cells and basophils. These cells release a variety of soluble mediators upon cross-linking of their receptor-bound IgE by a cognate antigen. There is also evidence that IgE bound to the high affinity receptor on mast cells can be cross-linked by member(s) of a broad class of IgE-dependent histamine releasing factors (14). Studies involving histamine releasing factors have led to the suggestion of a functional heterogeneity of IgE. The basis for this heterogeneity is not understood but has been speculated to be the result of differential glycosylation (14). IgE has also been suggested to participate in a variety of other immune processes such as antigen recognition, antibody-dependent cellular cytotoxicity, and B cell growth via binding to the high affinity IgE receptor (FcεR I), the low affinity IgE receptor (FcεR II), or galectin 3. The existence of splice variants of IgE provide an additional possible explanation for the functional heterogeneity of IgE and suggests that IgE may differ in its primary protein structure as well as glycosylation. Splice variants have also been observed in the mRNA of human IgA (15) and avian IgY (16).

The low serum levels of IgE and the similar molecular size predicted for many putative splice variants make purification of the individual protein isoforms of IgE from serum problematic. Our approach using recombinant DNA transfection has the advantage that vectors can be constructed that encode a single isoform of IgE, guaranteeing the homogeneity of the isoforms produced. In the current study, we have constructed vectors to express four isoforms of IgE in an Sp2/0-derived murine cell line. The kinetics and efficiency of the assembly and secretion are similar for each of these isoforms. The results indicate that the four secreted isoforms examined, IgE classic, IgE grande, and IgEtp are fully assembled by the Sp2/0 cells, whereas IgEϕCH4 is secreted predominately as HL half-molecules. The incomplete assembly of IgEϕCH4 is not entirely unexpected given that the constant regions of Igs are stabilized by noncovalent interactions between the COOH-terminal domains of both heavy chains. In the IgEϕCH4 isoform, the 3′ portion of the Cε4 exon is removed by splicing from a cryptic splice donor within that exon and replaced by sequence from the Cε5 cryptic exon. It is noteworthy that among the residues.
removed from IgE ωCH4 is a cysteine that is universally conserved in immunoglobulin domains (5). The domain structure of IgE ωCH4 is therefore likely to be disrupted. Speculation as to the physiological relevance of this structural variation must await a detailed functional comparison of these IgE isoforms.

In previous studies, we have reported that IgE grandé is detectable in the supernatant of the IgE-producing cell line AF-10, an IgE-stable, mycoplasma-free subclone of U266 (4) in the serum of a patient with an IgE myeloma and in the serum of highly atopic persons with very high serum levels of IgE (3, 4). Because IgE grandé is the only known isoform with an M, sufficiently different from IgE classic to resolve by SDS-polyacrylamide gel electrophoresis, it is impossible to ascertain from the previous data whether the other described isoforms were also present in the U266/AF-10 supernatant or the serum IgE from myeloma or highly atopic patients, although heterogeneity of bands in the appropriate size range was evident (3). In a recent study by Batista et al. (8), Western analysis and immunoprecipitation of the supernatant of U266 showed two species similar in size to the epsilon chain. However, treatment with glycosidase PNGase F caused the two bands to be reduced in size and comigrate as a single species, and it was concluded that only one isoform of IgE (i.e., the “classic secreted” isoform) is secreted by B cells (8). The same study had found that when the murine myeloma cell line J558L was transfected with expression vectors encoding individual splice variants of epsilon heavy chain, the recombinant epsilon chains could be detected in the cytosol of the transfectants but not in the secretions.

Secretion of the four isoforms of IgE reported in the present study is clear. In light of the aforementioned report, this raises questions concerning cell line-dependent factors affecting protein expression. Batista et al. (8) expressed their isoform-determined IgE genes in the J558L myeloma cell line using the pRc/CMV expression vector, whereas we expressed the proteins in the Sp2/0 myeloma cell line using the pSV2 gpt expression vector. Because adequate levels of epsilon chain were evident in the cytoplasm of the transfectants in both studies, it is unlikely that the different expression vectors account for the difference in secretion. Indeed, when we expressed IgE grandé in J558L using the same pSV2 gpt-based expression vector, the amount of epsilon heavy chain produced by the J558L transfectant was much greater than that produced by Sp2/0, but the J558L transfectant fails to efficiently assemble and secrete it (Fig. 3, C and D, and Fig. 6, A and B). Quantitation of the epsilon heavy chains on a 12.5% gel under reducing conditions (not shown) indicates that nearly all of the epsilon chain produced by these cells during the 5-min pulse is degraded within 180 min. Although the Sp2/0 IgE grandé transfectomata produce far less epsilon chain than its J558L counterpart, readily detectable levels of IgE (~25% of the epsilon and kappa chains labeled during the 5-min pulse) are secreted in the Hm2 form by 180 min post-chase, and there is no evidence for heavy chain degradation (Fig. 4 and data not shown). Additionally, the kinetics of assembly and secretion of the IgE grandé is very similar to that of IgE classic and IgEtp (Figs. 3 and 4A).

Several factors could cause cell line-dependent variation in protein secretion. One such factor is glycosylation. We have some evidence that IgE grandé is degraded when the producing cell line is labeled in the presence of tunicamycin. Glycosylation-dependent differences in post-translational proteolytic processing have been described for the soluble form of CD23 (17). Also, cell line-dependent variation has been described in the utilization of N-linked carbohydrate addition sites. It is possible that the altered exon usage of some IgE isoforms alters the accessibility of certain addition sites to some glycosylases-glycosidases and that some cell lines are better able to process these carbohydrate addition sites in their altered molecular context. Another possible explanation lies in chaperone proteins. It has been shown that during Ig assembly, lgs interact in a sequential fashion with at least two chaperones, BiP and GRP94 (18). Although we do not observe co-precipitation of either of these two chaperones with IgE under the conditions used, it is interesting to note that both IgE grandé and IgEtp are seen to co-precipitate with two species of ~50 and ~200 kDa (Figs. 3 and 5). We have as yet taken no steps to identify these proteins; however, they have been observed to co-precipitate with other antibody isotypes including IgA.4

We have shown that the protein products of three novel messages for human IgE are translated by the murine myeloma Sp2/0 and are efficiently assembled and secreted. Alternatively spliced mRNAs have been described for one more potentially secreted epsilon protein (5–7) as well as two forms of membrane epsilon chains. It is quite likely that all the described forms of epsilon mRNA are expressed at the protein level.

On the basis of the present and previous studies, it appears that human IgE is comprised of a family of proteins generated by alternative RNA splicing. Individual secreted members of this family may have some unique properties as circulating, cytophilic lgs and may differ in their ability to carry out IgE-mediated functions through binding to FcεRI, FcεRII, or galec 3. Although the contact residues for the former two receptors appear to be intact in the secreted epsilon isoforms, the isoforms may nevertheless function differently because the CH4 domain may be critical in constraining the three-dimensional shape of the IgE constant region. IgE ωCH4 merits special attention because this isoform is secreted primarily as HL half-molecules (Figs. 2A and 3H), although we have not ruled out noncovalent interactions between half-molecules. If IgE ωCH4 binds to FcεRI as a half-molecule, it would be less able to cross-link the receptors. It may therefore be less able to arm mast cells and basophils for antigen-triggered release and may in fact inhibit the release. Our findings gain added significance in that the relative levels of mRNAs encoding various isoforms are altered in allergic and parasitic diseases (3). Additionally, we have demonstrated a cell line-dependent variability in the efficiency of assembly and secretion of IgE grandé, suggesting that additional, as yet uncharacterized regulatory mechanisms of intracellular IgE assembly and/or trafficking may exist. The availability of highly purified IgE protein isoforms will now allow us to determine if the different isoforms have unique functional properties.

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