

Characterization of a second secreted IgE isoform and identification of an asymmetric pathway of IgE assembly

(ϵ heavy chains/carboxyl-terminal cysteine/IgE polymerization)

F. D. BATISTA, D. G. EFREMOV, AND O. R. BURRONE*

International Centre for Genetic Engineering and Biotechnology, Area Science Park, Padriciano 99, I-34012 Trieste, Italy

Communicated by Cesar Milstein, Medical Research Council Laboratory of Molecular Biology, Cambridge, U.K., December 20, 1995 (received for review June 27, 1995)

ABSTRACT A number of alternatively spliced ϵ transcripts have been detected in IgE-producing B cells, in addition to the mRNAs encoding the classical membrane and secreted IgE heavy (H) chains. In a recent study, we examined the protein products of three of these alternatively spliced isoforms and found that they are intracellularly retained and degraded because of their inability to assemble into complete IgE molecules. We have now similarly examined a more recently described ϵ mRNA species that is generated by splicing between a donor splice site immediately upstream of the stop codon in the H-chain constant region exon 4 (CH4) and an acceptor site located in the 3' part of the second membrane exon. We show that this isoform is efficiently secreted by both plasma cells and B lymphocytes and therefore represents a second secreted IgE isoform (ϵ_{S2}). The ϵ_{S2} H chain is only six amino acids longer than the classical secreted IgE H chain (ϵ_{S1}) and contains a C-terminal cysteine, which is a characteristic sequence feature of μ and α H chains. However, unlike IgM and IgA, the ϵ_{S2} C-terminal cysteine (Cys-554) does not induce polymerization of H_2L_2 molecules (where L is light chain), but rather creates a disulfide bond between the two H chains that increases the rate of association into covalently bound H_2L_2 monomers. This C-terminal cysteine also does not function as an intracellular retention element because the ϵ_{S2} isoform was secreted in amounts equal to that of the ϵ_{S1} , both in B lymphocytes and in plasma cells. The ϵ_{S2} H chains secreted by B lymphocytes differed from the ϵ_{S1} H chains in the extent of glycosylation. Interestingly, a difference in glycosylation between B-lymphocytes and plasma cells was also noted for both isoforms. The presence of the Cys-554 also allowed the identification of a distinctive asymmetric pathway of IgE assembly, common to both types of ϵ H chains.

During terminal B-cell differentiation to plasma cell, a switch in the production from membrane-bound to secreted immunoglobulin occurs. This event is regulated by differential splicing and polyadenylation of the primary heavy (H) chain transcripts, which allows the production of two types of immunoglobulin H chains (1–4). These two H-chain isoforms differ only in their C termini where they contain either a hydrophilic (secretory) or a hydrophobic (membrane) sequence (5–11).

A more complex splicing pattern has recently been identified in IgE-secreting cell lines and IgE-producing peripheral blood lymphocytes, which has suggested that more than two IgE isoforms exist (12–16). The alternative splicing occurs between splice sites located in the last two H chain constant region exons (CH3 and CH4) and the two membrane exons (M1' and M2). Consequently, several alternatively spliced ϵ mRNAs, encoding four different ϵ isoforms, are produced together with

the classical membrane (ϵ CH4-M1'-M2) and secreted (ϵ CH4-S) ϵ H-chain mRNAs. Two of the alternatively spliced ϵ transcripts contain either the complete M2 exon (ϵ CH4-M2') or its 3' part (ϵ CH4-M2'') in different reading frames from that of the membrane IgE, while the other two lack portions of the CH4 exon (ϵ CH4'-I and ϵ CH4*).

We have recently investigated the protein products of the ϵ CH4-S, ϵ CH4-M1'-M2, ϵ CH4-M2', ϵ CH4*, and ϵ CH4'-I ϵ mRNA isoforms by expressing them as chimeric mouse/human anti-4-hydroxy-5-iodo-3-nitrophenacetyl (NIP) antibodies in a mouse myeloma cell line (16). All isoforms were found to be efficiently translated in the myeloma cells, but only the classical secreted and membrane IgE were correctly assembled and transported. The ϵ CH4-M2', ϵ CH4*, and ϵ CH4'-I H chains failed to assemble into H_2L_2 dimers and were intracellularly retained and degraded, which suggested that these ϵ mRNA isoforms are aberrantly spliced molecules whose protein products are eliminated by posttranslational events.

We have now extended our studies to the recently identified ϵ CH4-M2'' mRNA species (15). We show that this isoform encodes an ϵ H chain that is efficiently secreted by both myeloma and lymphoma cells. The ϵ CH4-M2'' H chain contains a cysteine at the terminal position, whose role in the assembly and polymerization of IgE molecules was investigated in the present report.

MATERIALS AND METHODS

Cell Lines and Transfection. The J558L plasmacytoma cells were grown in Dulbecco's modified Eagle's medium (DMEM) 10% fetal calf serum/penicillin (100 units/ml)/streptomycin (100 μ g/ml). The WEHI 231 B-cell lymphoma (from Roberto Sitia, DIBIT-San Raffaele Scientific Institute, Milan, Italy) was maintained in DMEM/5% fetal calf serum/penicillin (100 units/ml) streptomycin (100 μ g/ml)/50 μ M 2-mercaptoethanol. Transfections were done by electroporation as described elsewhere (16), except that G418 at 800 μ g/ml (Geneticin, Life Technologies, Gaithersburg, MD) was used for selection of the WEHI 231 clones. Cells were cloned by limiting dilution, and anti-NIP IgE antibody production was assayed by immunoblot analysis.

Construction of ϵ CH4-M2'' Expression Vector. A PCR fragment containing the 3' part of the CH3 exon, the CH4 exon, and the complete coding region of the M2' exon was obtained by PCR of pUC18 ϵ CH4-S DNA (from ref. 16) with oligonucleotides E3 (5'-CATGCGGTCCACGACCAAGAC-3', from the CH3 exon) and M2'L (5'-TGGGTACCTAGCAGC-CACCCCTCCTCGATGACTCGGGATTACAGACCACC-3'). The M2'L oligonucleotide contains the last 18 nt from the

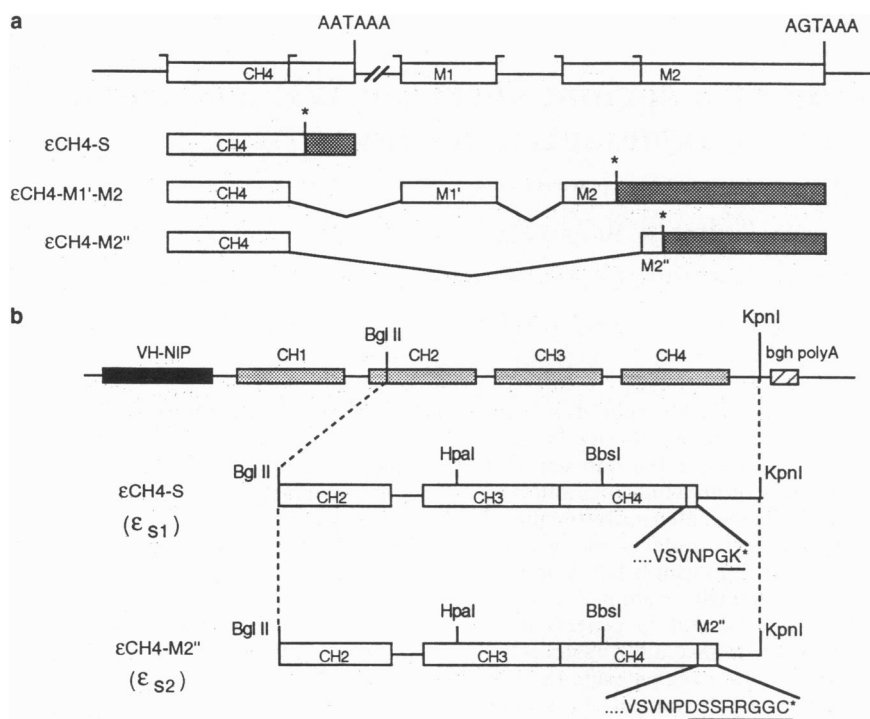


FIG. 1. Diagram of ϵ mRNA isoforms. (a) Schematic representation of the 3' end of the C ϵ gene and the CH4-S, CH4-M1'-M2, and CH4-M2'' ϵ mRNA isoforms. The donor and acceptor splice sites are indicated. Open boxes represent coding sequences; shadowed boxes represent 3' untranslated regions. Stop codons are indicated by asterisks. (b) Diagram of the chimeric mouse VH-NIP/human C ϵ gene constructs. The 3' ends of the two secreted ϵ isoforms were cloned in their cDNA form into pCIG-C ϵ . pCIG-C ϵ contains the mouse immunoglobulin (Ig) enhancer (E-Ig) and promoter (P-Ig) located upstream of the mouse leader (L) and variable exons, the four exons of the human C ϵ gene, and the bovine growth hormone gene poly(A) site (bgh-polyA). The amino acid sequences of the C termini of the two ϵ H-chains are shown from position 542 in the CH4 domain, and the differences between them are underlined. Stop codons are indicated by asterisks.

CH4 exon (underlined), the complete coding region of the M2'' exon and a *Kpn* I site. The PCR fragment was cloned in the *Sma* I site of pUC18, and the *Bbs* I/*Kpn* I fragment was subcloned in pUC18 ϵ CH4-S. The *Bgl* II/*Kpn* I fragment from this plasmid was transferred to the pCIG-C ϵ vector that was used for the transfection. The pCIG-C ϵ was also used to express the ϵ CH4-S, ϵ CH4*, ϵ CH4'-I, ϵ CH4-M2', and ϵ CH4-M1'-M2 isoforms, and its construction is described in detail in ref. 16.

Immunoprecipitations. Metabolic labeling and immunoprecipitations were done as described (16). Briefly, 5×10^6 cells/ml were labeled with [^{35} S]methionine (Amersham) at 100–250 μ Ci/ml (1 Ci = 37 GBq) and chased with cold methionine as indicated. Cell lysates and supernatants were immunoprecipitated with rabbit immunoglobulins to human IgE (ϵ chains) (Dako, Glostrup, Denmark) and purified by protein A-Sepharose. The samples were analyzed by SDS/PAGE in the presence or absence of mercaptoethanol, as indicated in the figure legends. Treatments of labeled supernatants with recombinant N-glycosidase F (PNGase F) and neuraminidase (Sialidase) were done according to the protocols provided by the manufacturer (New England Biolabs).

NIP-Sepharose Affinity Chromatography and Immunoblot Analysis. Functional immunoglobulin molecules were isolated from cell lysates of transfected cells by immunoadsorption on NIP-Sepharose and separated on SDS/PAGE. Bands were electroblotted to nitrocellulose filters and treated with rabbit immunoglobulins to human IgE (ϵ chains) (Dako) or goat immunoglobulins to mouse λ chain (Pierce). The ϵ chains were detected using alkaline phosphatase-conjugated goat anti-rabbit serum (Dako), whereas λ chains were detected with peroxidase-conjugated rabbit anti-goat polyclonal serum (Dako) and the enhanced chemiluminescence detection system (Amersham).

RESULTS

Expression of ϵ CH4-M2'' Isoform in Plasma Cells. The ϵ CH4-M2'' mRNA species is generated by splicing between a donor splice site immediately upstream of the stop codon in the CH4 exon and an acceptor site located in the 3' part of the second membrane exon (this part of the M2 exon is referred to as the M2'' exon) (Fig. 1a). The splicing eliminates the last two codons from the CH4 exon and adds eight codons from the M2'' exon of which the last one encodes a cysteine. Therefore, the ϵ CH4-M2'' H chain should be only six amino acids longer than the classical secreted ϵ H chain (ϵ CH4-S). This small difference in molecular weight would preclude discrimination of the two H chains in cell lines that produce the corresponding mRNAs. To investigate the properties of the protein product of the ϵ CH4-M2'' transcript, we generated a chimeric mouse/human ϵ -chain gene construct that contained a mouse variable region (V) H segment from an antibody with anti-NIP specificity and the constant region of the ϵ CH4-M2'' mRNA species (Fig. 1b). This chimeric gene was transfected in the J558L mouse myeloma cell line, which produces the L chain of the anti-NIP antibody. Transfectants expressing the ϵ CH4-M2'' H chain were identified by immunoblot analysis of the anti-NIP affinity-purified cellular extracts with anti-IgE polyclonal rabbit antiserum (data not shown). The cells were labeled with [^{35} S]methionine, and the immunoprecipitated IgE molecules were analyzed under reducing conditions in a 10% SDS/PAGE (Fig. 2a). The ϵ CH4-S transfectoma (described in ref. 16) that expresses the classical ϵ H chain was analyzed in parallel as a control. Bands of ≈ 80 kDa, corresponding in size both to the ϵ CH4-S and ϵ CH4-M2'' H chains (indicated as ϵ S1 and ϵ S2 in Fig. 2, respectively), were immunoprecipitated from the cellular extracts and supernatants of both cell lines. The bands in the supernatants were of slightly higher molecular weight, as expected from the terminal glycosylation of secreted

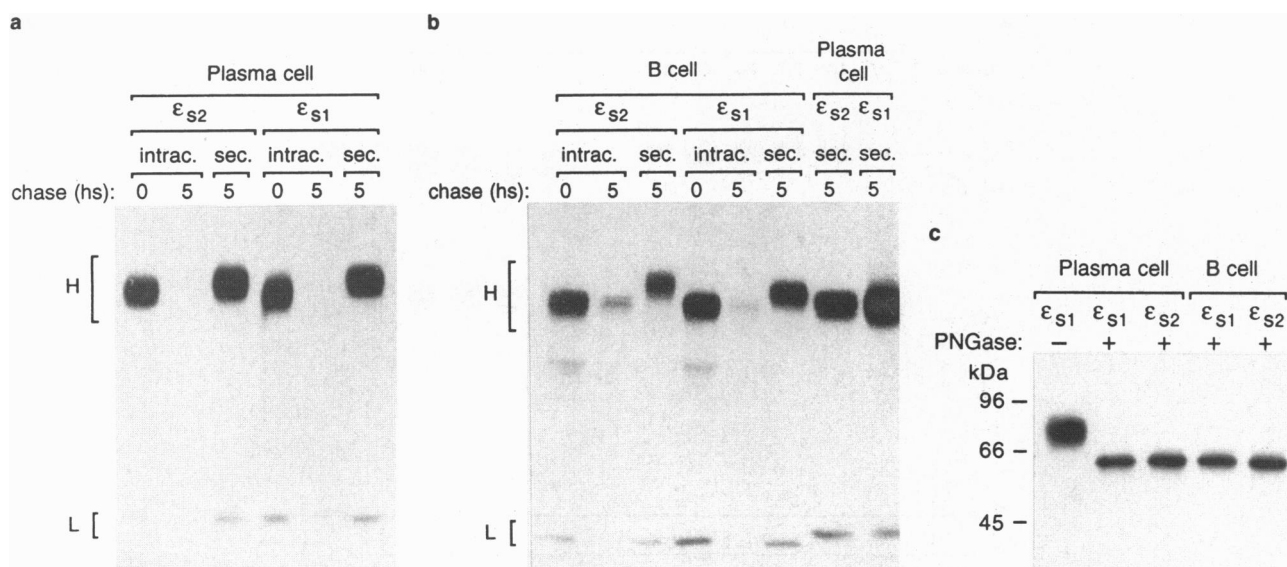


FIG. 2. Expression of the ϵ_{S1} and ϵ_{S2} isoforms in plasma cells and B lymphocytes. Transfectoma cell lines were pulse-labeled with [^{35} S]methionine for 1 hr and chased in the presence of cold methionine for 5 hr. Cell lysates (intrac.) and supernatants (sec.) were immunoprecipitated with an anti-human IgE rabbit antiserum and analyzed on reducing SDS/10% PAGE. The H and L chains produced by the plasma cells are shown in *a*, whereas those produced by the B cells are in *b*. Secreted material immunoprecipitated from the ϵ_{S1} and ϵ_{S2} plasma-cell transfectomas was included in the SDS/PAGE from *b* for comparison. (*c*) PNGase F treatment of secreted ϵ H chains immunoprecipitated from the four cell lines. Untreated intracellular ϵ_{S1} H chains were included as control (ϵ_{S1} -).

proteins. The quantity of protein immunoprecipitated from the supernatants was identical to the amount of protein obtained after the pulse, indicating that there was no intracellular retention or degradation of either isoform. Thus, these experiments showed that both ϵ CH4-S and ϵ CH4-M2^o mRNA species encode ϵ H chains that are efficiently secreted by plasma cells. To simplify the nomenclature, the two secreted ϵ H-chain isoforms will further be referred to as ϵ_{S1} (ϵ CH4-S) and ϵ_{S2} (ϵ CH4-M2^o).

Expression of ϵ_{S1} and ϵ_{S2} Isoforms in B Lymphocytes. B-lymphocytes can secrete IgG and IgE, whereas they retain and degrade the secretory forms of IgM and IgA intracellularly. Studies on IgM and IgA have shown that the C-terminal cysteine of the secretory H chains is implicated in the retention of these immunoglobulins in B lymphocytes (17–19). To investigate whether the ϵ_{S2} isoform, which contains a cysteine in the terminal position, would behave similarly, we independently transfected the mouse B-cell lymphoma WEHI 231 with the ϵ_{S1} and ϵ_{S2} constructs. Pulse-chase experiments were done on positive transfectomas, and the intracellular and secreted IgE molecules were immunoprecipitated and analyzed as described above. As shown in Fig. 2*b*, the two ϵ isoforms were equally secreted by the B cells, demonstrating that the different C-terminal tailpiece of ϵ_{S2} does not affect the secretion of this molecule. Surprisingly, the ϵ H chains secreted by the B lymphocytes were of higher molecular weights than those secreted by the plasma cells, indicating a different pattern of glycosylation in these two cell types. In addition, the secreted ϵ_{S2} H chain was of a higher molecular weight than the secreted ϵ_{S1} H chain. This difference was not observed in the plasma-cell supernatants, indicating that the ϵ_{S1} and ϵ_{S2} isoforms are glycosylated to different extents only in B cells. These results were confirmed by treating the secreted ϵ_{S1} and ϵ_{S2} H chains with PNGase F, which cleaves the high mannose, hybrid, and complex oligosaccharides. Fig. 2*c* shows that treatment with PNGase F completely eliminated the differences in molecular weight between the ϵ H chains secreted by the two cell lines, as well as the difference between the ϵ_{S1} and ϵ_{S2} H chains from the B-cell supernatants. Treatment of the same samples with neuraminidase revealed that these differences in glycosylation are in part due to the different sialic acid content (data not shown).

The presence of a C-Terminal Cysteine in ϵ_{S2} Does Not Lead to IgE Polymerization. The cysteine present in the penultimate position of the IgA and IgM H chains is also involved in the dimerization or polymerization of these molecules, respectively. On the other hand, none of the immunoglobulin isotypes that exist solely as monomers contain a cysteine in their C termini. To investigate the possibility that the ϵ_{S2} C-terminal cysteine is also involved in the polymerization of H₂L₂ molecules, we analyzed under nonreducing conditions the secreted material from the ϵ_{S2} transfectoma. As shown in Fig. 3, no band of a higher molecular weight than that expected for the IgE H₂L₂ monomer was present, demonstrating that the ϵ_{S2} isoform does not associate in a polymer, either in plasma or in B cells. Moreover, the absence of HL monomers, which were

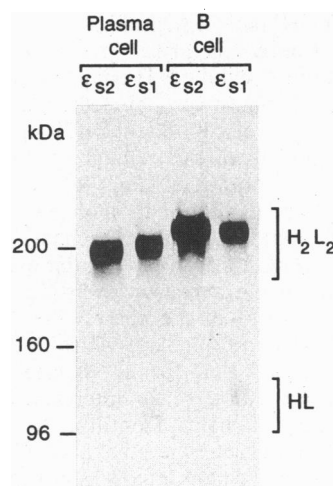


FIG. 3. Analysis of the secreted ϵ_{S1} and ϵ_{S2} IgE molecules under nonreducing conditions. Cells from the four transfectomas were labeled with [^{35} S]methionine for 3 hr, and the secreted IgE antibodies were immunoprecipitated from the cellular supernatants with a rabbit polyclonal antihuman IgE antiserum. Samples were analyzed on a nonreducing SDS/6% PAGE. Localizations of the H₂L₂ and HL molecules are indicated by brackets. Apparent molecular masses are shown at left in kDa.

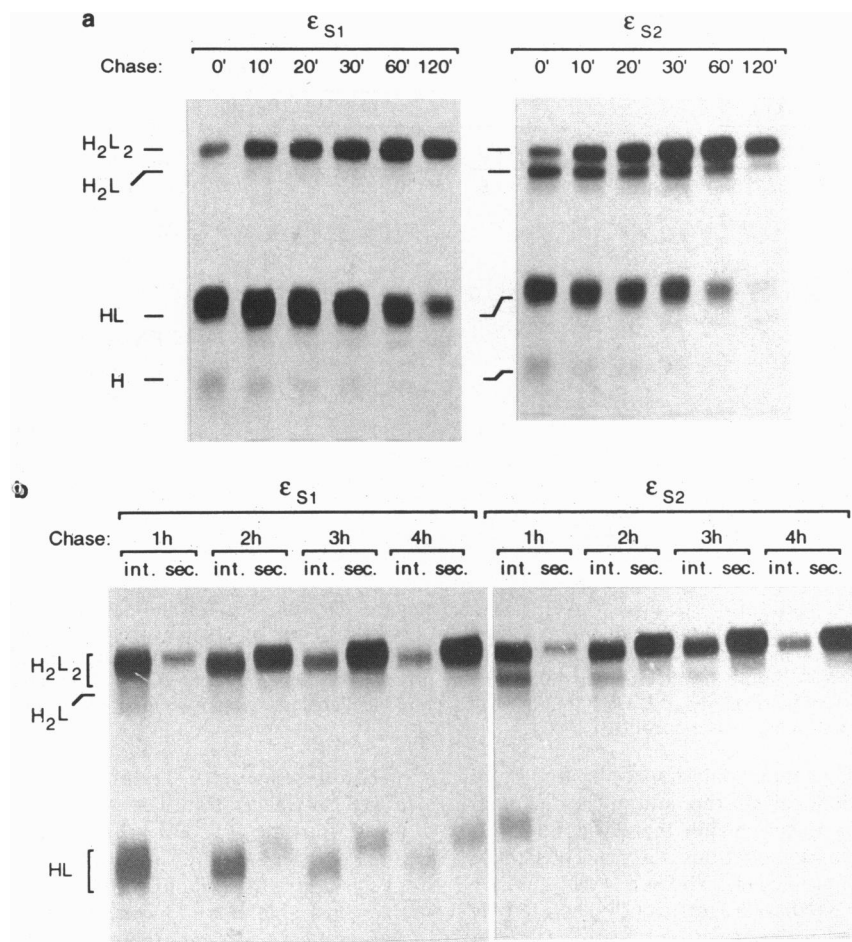


FIG. 4. Assembly of ϵ_{S1} and ϵ_{S2} H-chains. Plasma-cell transfectomas expressing ϵ_{S1} or ϵ_{S2} H chains were pulsed for 10 min with [^{35} S]methionine and chased for the indicated times in the presence of excess cold methionine before lysis. The anti- ϵ immunoprecipitates were resolved on nonreducing SDS/6% PAGE. (a) Cellular extracts. (b) Cellular extracts (int.) and supernatants (sec.).

present in the supernatants of the ϵ_{S1} transfectoma, suggested that the ϵ_{S2} terminal cysteine is involved in an interchain disulfide bond between the two H chains of the same IgE molecule, which could stabilize the H_2L_2 monomers and/or increases the rate of their association.

The latter possibility was further investigated by comparing the assembly of the ϵ_{S1} and ϵ_{S2} H chains. Cells from the two plasma-cell transfectomas were labeled with [^{35} S]methionine and chased for 0, 10, 20, 30, 60, and 120 min. The IgE was immunoprecipitated from the cellular lysates and analyzed under nonreducing conditions (Fig. 4a). Two bands corresponding in size to HL and H_2L_2 molecules were observed after immunoprecipitation of the ϵ_{S1} H chains. However, in the immunoprecipitations of the ϵ_{S2} cellular extracts, an H_2L intermediate was also present which was clearly converted to H_2L_2 during the course of the chase. The presence of this covalently linked H_2L intermediate, which was not observed in the ϵ_{S1} , demonstrated that the ϵ_{S2} C-terminal cysteine is involved in the formation of an interchain disulfide bond between the two ϵ_{S2} H chains. Densitometric quantitation of the HL/H_2L_2 ratios at each time-point showed a higher rate of association of the ϵ_{S2} H chains into H_2L_2 molecules, suggesting that the presence of the covalently linked H_2L intermediate allows more rapid assembly into complete IgE molecules. To determine whether the higher rate of assembly affected the rate of IgE transport, we examined the kinetics of IgE secretion in the ϵ_{S1} and ϵ_{S2} plasmacytoma cell lines. Cellular extracts and supernatants were analyzed essentially as described above, except that longer chase periods were used to allow time for secretion. As shown in Fig. 4b, the ratio of intracellular vs.

secreted material was almost identical for both cell lines at all time points, indicating that the rate of secretion is not dependent on the kinetics of disulfide bond formation. Similar results were obtained from identical analysis of the B-cell transfectomas (data not shown).

Characterization of IgE Assembly Pathway. The detection of covalently linked HL and H_2L intermediates, together with the absence of an H_2 intermediate in the ϵ_{S2} cellular extract, demonstrated that the ϵ_{S2} H chains, or at least a substantial part of them, assemble along a $H + L \rightarrow HL + H \rightarrow H_2L + L \rightarrow H_2L_2$ pathway. The ϵ_{S1} H chains could also assemble through an H_2L intermediate, but this intermediate would not be detected in the nonreducing SDS/PAGE if its H chains were noncovalently associated. This could occur if the two cysteines from the CH2 domain were oxidized at a lower rate than the C-terminal cysteine of ϵ_{S2} , which could result in slower formation of disulfide bridges between the two H chains from the ϵ_{S1} H_2L intermediate. This possibility was examined by NIP-affinity purification of the cellular extracts from both cell lines followed by immunoblot analysis of the purified immunoglobulin molecules with anti- ϵ H-chain and anti- λ L-chain antibodies. We reasoned that if noncovalently linked H_2L intermediates existed in the ϵ_{S1} cell line, they would be detected in the nonreducing SDS/PAGE as H and HL molecules that have dissociated from intermediates with NIP-binding affinity. This hypothesis was confirmed by the experiment shown in Fig. 5. Analysis of the NIP-affinity-purified material under nonreducing conditions with the anti- ϵ H chain antibody showed that H , HL , and H_2L_2 molecules are present in the ϵ_{S1} sample (Fig. 5 Left lane 1). Immunoblot analysis with

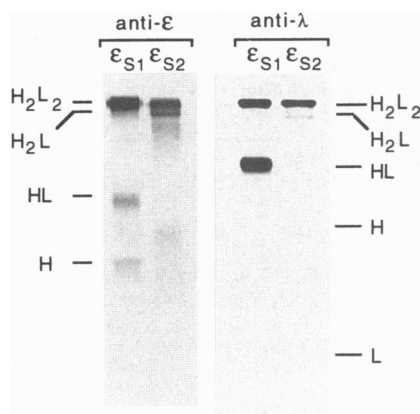


FIG. 5. Identification of a noncovalently linked ϵ_{S1} H_2L intermediate. NIP-Sepharose-purified antibody molecules from cell lysates of the two plasma-cell transfectomas were separated under nonreducing conditions in an 8% (Left) or in a 7–14% SDS/PAGE (Right). ϵ chains were detected with rabbit polyclonal anti-human IgE and alkaline phosphatase-conjugated goat anti-rabbit serum. λ chains were detected with goat anti-mouse λ -chain antibodies, and developed with peroxidase-conjugated rabbit anti-goat polyclonal serum and the enhanced chemiluminescence detection system.

an anti- λ chain antibody showed that free L chains are absent in this sample, thus excluding the possibility that the free H chains have dissociated from noncovalently linked HL molecules.

DISCUSSION

The work reported in this paper describes the characterization of a second secreted human IgE isoform. The ϵ_{S2} H chain is encoded by one of the several alternatively spliced ϵ transcripts previously identified in IgE-producing B lymphocytes and myeloma cell lines. However, unlike the other alternatively spliced ϵ isoforms, which are intracellularly retained and degraded, the ϵ_{S2} isoform is efficiently assembled and secreted by both B lymphocytes and plasma cells.

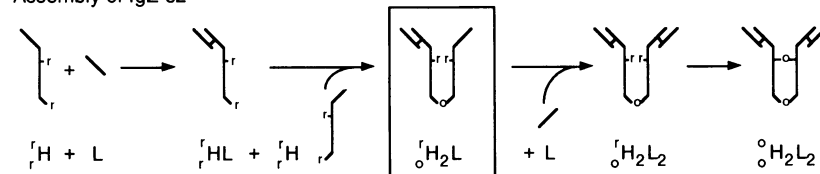
The ϵ_{S2} H chain is extremely similar to the classical ϵ_{S1} H chain, both in size and sequence. The most striking difference is that it contains a cysteine at the C terminus, which is also found at a similar position in IgM and IgA H chains. The penultimate cysteine of the α and μ chains is involved in the covalent assembly of IgA and IgM monomers into dimers and polymers, respectively (17–19). We now show that the presence

of a C-terminal cysteine in ϵ_{S2} is insufficient to induce polymerization of H_2L_2 molecules because only monomers of IgE were detected in the cell line expressing this isoform. However, pulse-chase experiments with this cell line showed that the C-terminal cysteines from the two H chains of the same IgE molecule form an interchain disulfide bond between them, which stabilizes the H_2L_2 monomers. This different reactivity of the ϵ_{S2} C-terminal cysteine could be due to its different sequence context and/or the general structure of the CH4 domain, which could promote the interaction between the C-terminal cysteines from the IgE monomer. Interestingly, this interaction also led to a more rapid formation of disulfide bonds in the H_2L_2 molecules, although this was not associated with a higher secretion rate.

The C-terminal cysteine of μ and α H chains also functions as an intracellular retention and degradation signal that prevents the secretion of unpolymerized H_2L_2 subunits. The retention is complete for both isotypes in B lymphocytes but differs in plasma cells that can secrete some unpolymerized IgA molecules, presumably due to the presence of a charged amino acid residue upstream of the C-terminal cysteine in the α chain (17). We now show that the C-terminal cysteine of the ϵ_{S2} H chain does not function as an intracellular retention element because monomeric IgE molecules containing ϵ_{S2} H chains were efficiently secreted both by B lymphocytes and plasma cells in almost equal amounts as the ϵ_{S1} IgE. However, it should be noted that the ϵ_{S2} C-terminal cysteine in the IgE monomer is involved in an interchain disulfide bond, which resembles the state of the μ and α C-terminal cysteines in polymerized IgA and IgM subunits. These results further indicate that the C-terminal cysteine-mediated retention of immunoglobulin subunits depends on the presence of a free thiol group, which could interact with putative components of intracellular retention and degradation pathways (20).

The C-terminal cysteine of the ϵ_{S2} H chain also allowed the identification of a distinctive asymmetric pathway of IgE assembly. It is generally believed that complete immunoglobulin molecules are generated by the assembly of two HL intermediates. We now show that at least part of the IgE molecules assemble along a $H + L \rightarrow HL + H \rightarrow H_2L + L \rightarrow H_2L_2$ pathway because H_2L intermediates were detected in the cellular extracts of both the ϵ_{S1} and ϵ_{S2} transfectomas (Fig. 6). The ϵ_{S2} H_2L intermediate was found to be covalently linked, suggesting a high rate of oxidation of the Cys-554, which allows formation of a disulfide bridge between the two H chains, even before the association with the second L chain. On the other hand, no covalently linked ϵ_{S1} H_2L molecules were detected, indicating that the oxidation of the two cysteines from the CH2 domain occurs more slowly and may require association into complete H_2L_2 molecules. This model also provides an expla-

Assembly of IgE-s2



Assembly of IgE-s1

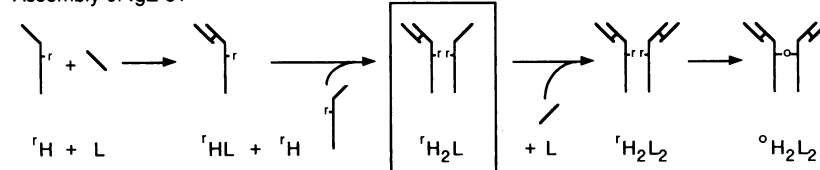


FIG. 6. Schematic representation of the asymmetric pathway of IgE assembly. The oxidation state of the cysteines from the CH2 and CH4 domains is indicated with r (reduced) and o (oxidized). The H_2L intermediate is boxed.

nation for the presence of HL molecules only in the supernatant of the ϵ_{S1} transfectoma, which could result from the dissociation of secreted but noncovalently linked H_2L_2 molecules.

The biological function of the ϵ_{S2} isoform, the regulation of its expression, and its role in atopic diseases are important issues that need to be further studied. However, several characteristics of this isoform already suggest that it might have some properties different from the classical ϵ_{S1} isoform. First of all, the C-terminal cysteine apparently stabilizes the conformation of the IgE molecule by creating a disulfide bond between the two CH4 domains. A stable dimeric conformation of the two ϵ H chains is required for binding to the low-affinity receptor for IgE (Fc ϵ R2) and also increases the binding affinity for the high-affinity receptor (Fc ϵ R1) (21, 22). Secondly, we show that there is a difference in the extent of glycosylation between the two ϵ isoforms in B lymphocytes, as well as a difference in glycosylation between B lymphocytes and plasma cells for both isoforms. Recent studies have shown that the serum of allergic patients contains polyclonal IgE antibodies that differ in the degree of sialylation and possibly in effector functions. Only a portion of these IgE glycoforms bind the ϵ BP, a β -galactoside-binding lectin that has been implicated in the binding of IgE to mast cells, Langerhans cells, and neutrophils (23–25). Recent studies with recombinant human IgE-Fc fragments have shown that the extent of glycosylation also affects the affinity of IgE for the Fc ϵ R2 (26). Finally, the ϵ_{S2} mRNA has been detected in unstimulated lymphocytes from all investigated patients with atopic dermatitis and the hyper-IgE syndrome (15). This species can be induced “*in vivo*” by challenge with diesel exhaust particles and “*in vitro*” by stimulation of PBL with interleukin 4 and anti-CD40 antibody (27, 28). On the other hand, we have recently shown that this isoform is expressed by nonstimulated PBL from only a portion of random blood donors, whereas all of them expressed ϵ_{S1} mRNA (ref. 29 and our unpublished data). This distinct expression pattern suggests a potential different role of the ϵ_{S2} isoform in IgE-mediated hypersensitivity reactions.

We thank Drs. Andrew Bradbury and Shubha Anand for critical reading of the manuscript and Dr. Roberto Sitia for providing the WEHI 231 cell line.

1. Brown, L. S. & Morrison, S. L. (1989) *J. Immunol.* **142**, 2070–2080.
2. Peterson, M. L. & Perry, R. P. (1986) *Proc. Natl. Acad. Sci. USA* **83**, 8883–8887.
3. Peterson, M. L. & Perry, R. P. (1989) *Mol. Cell. Biol.* **9**, 726–738.
4. Peterson, M. L., Edward, R. G. & Perry, R. P. (1991) *Mol. Cell Biol.* **11**, 2324–2327.
5. Rogers, J., Early, P., Carter, C., Calame, K., Bond, M., Hood, L. & Wall, R. (1980) *Cell* **20**, 303–312.
6. Rogers, J., Choi, E., Souza, L., Carter, C., Word, C. J., Kuehl, M., Eismberg, D. & Wall, R. (1981) *Cell* **26**, 19–27.
7. Rabbitts, T. H., Foster, A. & Milstein, C. (1981) *Nucleic Acids Res.* **9**, 4509–4524.
8. Word, C. J., Mushinski, J. F. & Tucker, P. W. (1983) *EMBO J.* **2**, 887–898.
9. Cheng, H.-L., Blattner, F. R., Fitzmaurice, L., Mushinski, J. F. & Tucker, P. W. (1982) *Nature (London)* **296**, 410–415.
10. Kikutani, H., Sitia, R. R., Good, A. & Stavnezer, J. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 6436–6440.
11. Early, P., Huang, H., Davis, M., Calame, K. & Hood, L. (1980) *Cell* **19**, 981–992.
12. Hellman, L. (1993) *Eur. J. Immunol.* **23**, 159–167.
13. Peng, C., Davis, F. M., Sun, L. K., Liou, R. S., Kim, Y.-W. & Chang, T. W. (1992) *J. Immunol.* **148**, 129–139.
14. Zhang, K., Saxon, A. & Max, E. E. (1992) *J. Exp. Med.* **176**, 233–243.
15. Zhang, K., Max, E. E., Cheah, H.-K. & Saxon, A. (1994) *J. Biol. Chem.* **269**, 456–462.
16. Batista, F. D., Efremov, D. G. & Burrone, O. R. (1995) *J. Immunol.* **154**, 209–218.
17. Guenzi, S., Fra, A., Sparvoli, A., Bet, P., Rocco, M. & Sitia, R. (1994) *Eur. J. Immunol.* **24**, 2477–2482.
18. Sitia, R., Neuberger, M. S. & Milstein, C. (1987) *EMBO J.* **6**, 3969–3977.
19. Sitia, R., Neuberger, M. S., Alberini, C., Bet, P., Fra, A., Valetti, C., Williams, G. & Milstein, C. (1990) *Cell* **60**, 781–790.
20. Fra, A. M., Fagioli, C., Finazzi, D., Sitia, R. & Alberini, C. M. (1993) *EMBO J.* **12**, 4755–4761.
21. Nissim, A., Helene, J. & Eshhar, Z. (1991) *EMBO J.* **10**, 101–107.
22. Vercelli, D., Helm, B., Marsh, P., Padlan, E., Geha, R. S. & Gould, H. (1989) *Nature (London)* **338**, 649–651.
23. Robertson, M. W. & Liu, F.-T. (1991) *J. Immunol.* **147**, 3024–3030.
24. Truong, M. J., Gruart, V., Kusnier, J. P., Papin, J. P., Loiseau, S., Capron, A. & Capron, M. (1993) *J. Exp. Med.* **177**, 243–248.
25. Wollenberg, A., Salle, H., Hanau, D., Liu, F.-T. & Bieber, T. (1993) *J. Exp. Med.* **178**, 777–785.
26. Young, R. J., Owens, R. J., Mackay, G. A., Chan, C. M., Shi, J., Hide, M., Francis, D. M., Henry, A. J., Sutton, B. J. & Gould, H. J. (1995) *Protein Eng.* **8**, 193–199.
27. Diaz-Sanchez, D., Dotson, A. R., Takenaka, H. & Saxon, A. (1994) *J. Clin. Invest.* **94**, 1417–1425.
28. Diaz-Sanchez, D., Zhang, K., Nutman, T. B. & Saxon, A. (1995) *J. Immunol.* **155**, 1930–1941.
29. Batista, F. D., Efremov, D. G., Tkach, T. & Burrone, O. R. (1995) *Nucleic Acids Res.* **23**, 4805–4811.