Alternative RNA of Epsilon Transcripts Produces mRNAs Encoding Two Membrane and Four Secreted IgE Isoforms

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Key Words

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Abstract

We elucidated a series of alternatively spliced human ϵ mRNAs by employing a human ϵ -specific RT-PCR strategy. Two mRNA A isoforms provide for the expression of distinct membrane IgE proteins, the common form of which contains a novel 56-amino-acid sequence after the last ϵ constant-region domain. Other mRNAs encode, in addition to the classic secreted ϵ chain, at least three novel ϵ secreted proteins that lack a transmembrane domain and possess distinctive C-terminal features. Furthermore, the expression of these ϵ mRNA isoforms is differentially regulated by stimuli such as interleukin-10, Fc ϵ RII cross-linking and aromatic hydrocarbons found in diesel exhaust.

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Introduction

Humans have only one functional epsilon (ϵ) Ig gene (fig. 1) which was expected to code for a single membrane and a single secreted IgE. Northern analysis revealed unpre-dicted bands hybridizing with ϵ membrane sequences and on Western blot analysis, human polyclonal IgE exhibited protein heterogeneity. In fact, we found that a series of alternative 3' ϵ RNA splices encode two membrane and four secreted forms of ϵ protein and that this alternate splicing can be differentially regulated.

Identification and Quantification of ϵ mRNAs

RNA was isolated and reverse transcribed. Pairs of PCR primers were designed to detect different forms of ϵ mRNA containing membrane exon sequences. PCR products were probed, cloned and se-quenced [1,2]. The procedure for quantitating and comparing the levels of different ϵ mRNA isoforms by reverse transcription and PCR amplification are detailed elsewhere [3, 4]. Quantification was validated by employing plasmid cDNAs representing the different ϵ mRNAs and showing a log-linear correlation between the number of starting copies and the yield for each cDNA alone and in combination.

Results

Materials and Methods

Cell Lines and Cell Culture

The human IgE-secreting cell lines used were AF-10, 2C4/F3 and 2C10/F9 [1, 2]. Productive ϵ transcripts were from purified tonsil B cells induced by interleukin (IL)-4 and α CD40. Cells spontaneously producing IgE came from patients with high levels of IgE.

Splicing of CH ϵ 4 to MY and M1 Encodes

Two Membrane IgE Molecules

Analysis of ϵ mRNAs containing ϵ membrane sequence revealed two forms. The common form spliced CH4 to an acceptor site far 5' to the murine M1 site, probably resulting from the AG dinucleotide 9 base pairs upstream of the hu-

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A. Human epsilon locus and the splicing involved in generation of various mRNA species

5' SwMchmu | y Switch epsilon f-

B. Diagram of epsilon mRNAs resulting from alternative splicing C. Nature of the encoded epsilon proteins

mRNA coding for membranes epsilon

Common membrane form, contains an additional 52 aminoacid spacer between CH ϵ 4 and the normal transmembrane coded region

The “predicted” membrane form but mRNA is very rare in humans

mRNA coding for secreted epsilon

The “Classic” secreted epsilon with only 2 aminoacids after CH4

Epsilon chain with the last 37 aminoacids replaced by 27 aa from CH5 making it 10 aa shorter. Probably does not have the normal CH4-CH4 interchain interactions that stabilize the IgE molecule and FcER binding.

Large epsilon chain with an additional 134 C-terminal aminoacids

VDJ CH1

Epsilon chain with 8 novel aminoacids added to CH4 making it 6 aa larger than the classic secreted. Contains a terminal cysteine that could be involved in J chain binding and formation of dimers/multimers.

Fig. 1. Production of alternative spliced human ϵ mRNAs. Panel A shows the human ϵ locus and its RNA splicing patterns. Panel B shows the resulting ϵ mRNAs. Panel C describes properties of the proteins that are encoded by the alternative spliced mRNAs.

man M1 site. An AG in the splice acceptor 5' polypyrimidine tract inhibits its use. The other membrane-coding ϵ mRNA, using the M1 splice acceptor, was very rare. Surface expression of the large membrane ϵ has been detected by using antipeptide antibodies directed against its unique 52-amino-acid (aa) region [5].

Splicing of CH ϵ 4 Directly to M2 Sequences Codes for

Two Secreted IgE Molecules

Analysis of ϵ mRNAs containing M2 and its 3' sequence revealed two ϵ mRNA species resulting from splicing CH4 directly to M2 sequences. One uses the M2 splice acceptor site but the resulting frameshift generates a reading frame for 134 additional C-terminal aa. Polyclonal and antipeptide antisera detected this protein on Western blots of IgE from serum of IgE myeloma patients and subjects with high levels of polyclonal IgE. The other splice from CH4 (CH4-M2'') leads to a small open reading frame inside the M2' exon which codes 8 aa.

Splicing of CH4' to a Cryptic Exon, CH5

Additional mRNAs were detected which contained a sequence from the intron between CH4 and M1. Sequencing showed these to result from the use of a novel splice donor site within CH4, revealing the presence of a cryptic exon, CH5. All these CH4'-CH5-X mRNAs encode a single protein, because there is a stop codon in the CH5 exon.

The Splicing Pattern Generating ϵ mRNAs is

Differentially Regulated

Quantitative PCR studies revealed that the relative amounts of the different ϵ mRNAs expressed in IL-4+ CD40-stimulated peripheral blood lymphocytes are similar between individuals. IL-10 increased total ϵ mRNA and

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altered the ratio of the ϵ mRNA isoforms with a relative increase in the M2' secreted form and a marked decrease in the CH4'-CH5 forms. A similar pattern change was observed with aromatic hydrocarbons extracted from diesel particles [4]. In contrast, cross-linking Fc ϵ RII decreased IgE with a dramatic decrease in the M2' splice variant.

Discussion

Alternative splicing enables different IgE proteins to be generated from a single functional human ϵ gene, a feature of all human IgE-secreting cells examined. Studies by us [1, 2] and others [5,6] have now described nine ϵ mRNAs which code for six different IgE proteins (fig. 1). The common membrane form (CH4-M1-M2) has a proline-rich spacer between the hydrophobic transmembrane residues and the closest Ig domain. This spacer is a potential anti-genic target unique to IgE-bearing B cells as it would not be present on cells that have acquired IgE passively.

The other ϵ mRNAs encode four forms of secreted ϵ chain because they lack the hydrophobic M1 and M1 trans-membrane domain and vary between 10 aa less to 134 aa more than classic secreted ϵ . In addition, Hellman [6] has reported an ϵ mRNA that codes an ϵ protein whose localization is difficult to predict. The ϵ mRNA species (CH4-M2'') produced by splicing CH4 to a splice acceptor site in-

side the M2' exon encodes a secreted ϵ 6 aa longer than the classic secreted protein including a C-terminal cysteine. C-terminal cysteines may associate with the J chain and be involved in the formation of dimers or polymers of IgM and IgA1. A large ϵ protein encoded by splicing that skips the M1 and M1 acceptor sites and splices CH4 directly to M2 has a 134-aa terminal addition which may interfere with Fc ϵ R binding by disturbing the interchain CH4-CH4 interactions that maintain the CH2-CH3-CH4 structure or by directly blocking the CH3 binding site. All CH4'-CH5-X ϵ RNAs encode the same protein in which the C-terminal 37 residues of CH4 are replaced with 27 aa encoded by CH5. Such a protein will probably not fold to form the

usual β -pleated structure of an Ig domain, nor provide for the normal CH4-CH4 interchain interactions.

Finally, we found that the amounts of the different ϵ mRNA isoforms are differentially regulated in vivo and in vitro [3,4]. This may allow varying amounts of the different ϵ chains to be expressed under disease conditions and in response to exogenous stimuli.

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References

Zhang K, Saxon A, Max EE: Two unusual forms of human immunoglobulin E encoded by alternative RNA splicing of ϵ heavy chain membrane exons. *J Exp Med* 1992;176:233-243.

Zhang K, Max EE, Cheah HK, Saxon A: Complex alternative RNA splicing of ϵ immunoglobulin transcripts produces mRNAs encoding four potential secreted protein isoforms. *J Biol Chem* 1994;269:456¹⁶².

Diaz-Sanchez D, Dotson AR, Takenaka H, Saxon A: Diesel exhaust particles induce local IgE production in vivo in humans and alter the pattern of IgE mRNA isoforms. *J Clin Invest*, in press.

Takenaka H, Zhang K, Diaz-Sanchez D, Tsien A, Saxon A: Enhanced human IgE results from exposure to the aromatic hydrocarbons in diesel exhaust: Direct effects on B cell IgE production. *J Allergy Clin Immunol*, 1995;95:103-115.

Peng C, Davis FM, Sun LK, Liou RS, Kim V, Chang TW: A new isoform of human membrane bound IgE. *J Immunol* 1992; 148:129-136.

Hellman L: Characterization of four novel ϵ chain mRNA and a comparative analysis of genes for immunoglobulin E in rodents and man. *Eur J Immunol* 1993;23:159-167.