H-2\textsuperscript{u} haplotype mice are unique among all Ea\textsuperscript{+} strains because they do not provide in heterozygotes an Ea chain that interacts with E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.}.cDNA compared to those of previously analyzed alleles. Although no glaring structural abnormalities were found, we have identified some u-specific residues and suggest which are the most likely to provoke a pairing anomaly.

The murine immune response is governed by loci within the major histocompatibility complex (MHC)\cite{1-3}. Genetic studies have shown that responders to a given foreign antigen differ from nonresponders by their I subregion haplotype. Encoded within the I subregion are the Ia antigens, polymorphic glycoproteins expressed primarily on antigen-presenting cells and B cells. Definitive evidence now exists that the Ia antigens are the mediators of I subregion-linked immune response effects.

Two murine Ia antigen complexes have thus far been characterized: the A complex, consisting of Aa and A\delta, and the E complex, composed of Ea and E\delta. In heterozygotes, haplotype mixing seems to occur\cite{4,5}. That is, when an H-2\textsuperscript{u} mouse is mated with an H-2\textsuperscript{v}, the F\textsubscript{1} offspring may display four A complexes at the cell surface—Aa\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu. and Aa\textalpha.A\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.. Similarly, four E complexes can potentially be assembled and expressed in H-2\textsuperscript{u} x H-2\textsuperscript{v} heterozygotes.

Recently, multiple alleles of all the Ia chains have been sequenced\cite{6}. Aa, A\delta, and E\delta exhibit extensive polymorphism, whereas the few known Ea sequences are much more homogeneous, consistent with pre-existing serologic and biochemical data\cite{7,8}. However, further Ea polymorphism might be revealed when more alleles are examined. In particular, tryptic peptide analysis indicates that Ea\textsuperscript{u} shares fewer peptides with E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.} than they share with each other\cite{9}.

In addition to this sequence heterogeneity, there appears to be a quantitative diversity in the expression of certain Ia alleles\cite{10}. A well-studied example is the unique failure of H-2\textsuperscript{u} haplotype mice among all other Ea\textsuperscript{+} haplotypes to provide in heterozygotes an Ea chain capable of interacting with E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.} to promote responsiveness to pigeon cytochrome c\cite{11,12}. To elucidate the cause of this nonresponsiveness, biochemical and serologic studies were performed on F\textsubscript{1} hybrids between strains bearing Ea\textsuperscript{+}E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.} and those carrying E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.}E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.}. An approximately eightfold deficiency in the Ea\textsuperscript{+}E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.} complex was observed on the surface of spleen cells, as compared with levels of Ea\textsuperscript{+}E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.}\cite{12}. This deficit seemed to be due to a preferential association of Ea\textsuperscript{+} with E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.} and, as a consequence, immune responses predicated on a functional Ea\textsuperscript{+}E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.} complex appeared faulty\cite{11}. It was eventually possible to observe a response, both in vitro and in vivo, but more than 10 times the amount of antigen was required\cite{11}(Kovac and Schwartz, personal communication). The primary defect seemed to be at the level of antigen presentation rather than due to a hole in the T cell repertoire (Kovac and Schwartz, personal communication).

Two hypotheses might explain the difficulty in forming Ea\textsuperscript{+}E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.} complexes. First, Ea\textsuperscript{+} may have an aberrant structure that prevents an efficient pairing with most E\delta chains. Following this reasoning, a compensatory alteration in E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.} must have occurred to allow efficient association of Ea\textsuperscript{+} with E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.}. Secondly, E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.} may have a modified structure which enables it to outcompete all other E\delta chains for an Ea. If the Ea concentration is limiting, the consequences will be a reduced level of Ea\textsuperscript{+}E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.} complexes at the cell surface. In either case, there must have occurred a mutation or mutations that affect \alpha/\beta-chain pairing. As a first step in identifying this modification, we have sequenced Ea\textsuperscript{+} and E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.} and compared their primary structures with those of other known \alpha and \beta alleles.

**MATERIALS AND METHODS**

Mice. B10.PL mice were obtained from our breeding facility at Stanford or from that of Prof. J. Klein, Tubingen, FRG.

RNA isolation and cDNA cloning. Poly A RNA were isolated from spleen tissue and were analyzed as described\cite{13}. cDNA libraries, carried in the phage \lambda gt 10, were constructed and screened by standard methods\cite{14}, employing either an Ea\textsuperscript{+} (15) or E\textgamma{\textsuperscript{u}.\textalpha.\textgamma.\textdelta.\textepsilon.\textomega.\textkappa.\textlambda.\textmu.} cDNA.
probe. The largest cDNA inserts were purified on sucrose gradients and were cloned into the EcoRI site of M13mp8, pJN 121, or pBR322. pEAU1 and pEBU25 derive from mice bred in Stanford; pEAU2 comes from Tubingen-bred mice.

DNA sequencing. The cDNA inserts, or subclones thereof, were sequenced by the method of Sanger et al. (17) and/or that of Maxam and Gilbert (18). The sequencing strategy is depicted in Figure 1.

RESULTS

We sought to determine whether Eα is an aberrant α-chain by examining its primary structure. To this end, we isolated two long cDNA copies of the Eα mRNA (pEAU1 and pEBU2) and determined their nucleotide sequence (Figs. 1 and 2). The derived amino acid sequences indicate a typical la antigen composed of two external folded domains (D1 and D2), a connecting peptide, transmembrane region, and cytoplasmic tail. The Eα protein sequence is compared with that of Eα and Eβ in Figure 3. There is substantially less polymorphism among these polypeptides than is usual for alleles of Eβ, Aα, or Aβ (8). The limited diversity that does exist is scattered throughout the molecule. There are four u-specific residues: two in D1 and two in D2.

The Eβ sequence was also determined in order to reveal any obvious structural particularities. A long cDNA clone (pEBU25) was isolated and was sequenced (Figs. 1 and 4). The derived amino acid sequence disclosed a normal Eβ organization, consisting of D1, D2, connecting peptide, transmembrane region, and cytoplasmic tail. The protein sequence is compared with that of Eβ, Eβ, and Eβ in Figure 5. As has been described in detail (16, 21, 22), Eβ chains are highly polymorphic and exhibit four allelically hypervariable regions in D1: residues 1–

![](https://via.placeholder.com/150)

Figure 1. Sequencing strategy for Eα and Eβ. The EACU1 (Eα) and pEBU25 (Eβ) cDNA inserts are illustrated, with a few of the restriction enzyme sites situated as landmarks (Bst = BstEII). The thick portion of the line represents the sequences coding for the mature protein, while the thin portion signifies the untranslated region of the mRNA. This latter region is stippled for Eα (and the corresponding sequences are absent from Fig. 2) because the sequencing information was limited and irrelevant to this report. Arrows beneath the line indicate the direction and extent of the individual sequence runs.

![Figure 2](https://via.placeholder.com/150)

Figure 2. Sequence of the Eα cDNA. The sequence of the coding strand for Eα is a composite of the sequences obtained for pEAU1 and pEAU2. The two isolates had identical sequences, except at position 259, where a T occurs in pEAU1. This T creates a stop codon at amino acid 88 in D2, and we believe that it results from a cloning artifact. The sequence starts at the beginning of D1 and extends to the translation stop codon (boxed).

![Figure 3](https://via.placeholder.com/150)

Figure 3. Eα protein sequence. The Eα protein sequence, deduced from the nucleotide sequence of Figure 2, is shown in full. It is compared with other sequenced alleles (19, 20), which are represented as dashes except where they differ from Eα. u-specific residues are boxed.

![Figure 4](https://via.placeholder.com/150)

Figure 4. Sequence of the Eβ cDNA. The sequence of the coding strand of the Eβ cDNA is shown, as obtained from clone pEBU25. The sequence starts at the beginning of D1 and extends slightly beyond the polyadenylation signal (underlined). The translation stop codon is boxed.

13, 24–35, 68–75, and 87–93. Eα variability is also largely clustered in these regions. In total, there are eight u-specific residues (although two at the amino terminus are provisional because of a paucity of sequence data). Of these, four occur within the D1 hypervariable regions, three reside in conserved stretches of D1, and one is situated in D2.

DISCUSSION

la antigens occur as heteromorphic complexes at the surface. It is not yet known what structural features govern αβ-chain pairing, but both D2 and D1 have been implicated. The D2 region of each chain is probably folded very much like a disulphide-bridged immunoglobulin domain. Many of the critical residues dictating an immunoglobulin fold are present, just as they are in several other cell surface molecules, including Thy-1, the T cell receptor, β-2 microglobulin, and the MHC class I heavy chain (25–27). It has been hypothesized that many immunologically important cell surface complexes result from the association of paired immunoglobulin-like domains; for example, β2-microglobulin may interact with the heavy chain D3 region to form class I antigens. By analogy, D2α and D2β could be important for la αβ-chain pairing. On the other hand, recent evidence demonstrates that the D1 domain may be crucial for efficient associa-
very drastic. There is a charge change, arginine acid\textsuperscript{75} that might affect pairing because there is a high degree of conservation within the immunoglobulin fold.

Experiments map this incompatibility to the D\textsubscript{1} region of the \(\alpha\)- and \(\beta\)-chains of the I\(\alpha\) A complex (28). Certain combinations of \(\alpha\) and \(\beta\) pair poorly in transfected L cells, e.g., \(\alpha\text{a}\alpha\cdot\alpha\text{b}^\beta\) or \(\alpha\text{a}\cdot\alpha\text{b}^\alpha\). Exon shuffling experiments map this incompatibility to the D\textsubscript{1} region of \(\alpha\beta\).

\textit{Ea}\textsuperscript{\textsubscript{a}} exhibits allele-specific residues both in D\textsubscript{1} and D\textsubscript{2}. In D\textsubscript{1}, the phenylalanine \(\rightarrow\) tyrosine change at position 22 is chemically quite conservative, whereas the glutamic acid\textsubscript{3} \(\rightarrow\) lysine\textsubscript{2} alteration reverses the charge. Although these differences do not appear very striking, it may be worth noting that the k and d alleles exhibit perfect conservation of D\textsubscript{1}. The dissimilarities in D\textsubscript{2} are also not very drastic. There is a charge change, arginine \(\rightarrow\) glutamin e at position 126, as well as a conservative substitution, isoleucine\textsubscript{116} \(\rightarrow\) valine\textsubscript{116}. Although the latter seems mild on the basis of amino acid chemistry, it does occur adjacent to a cysteine important in maintaining the immunoglobulin fold.

For \(\textit{Ea}^\alpha\), it is particularly difficult to identify mutations that might affect pairing because there is a high degree of allelic polymorphism. The problem is somewhat simplified (oversimplified?) if one concentrates on u-specific residues located within conservative regions. In D\textsubscript{1}, there is a relatively mild aspartic to glutamic acid change at position 58 and a seemingly more disruptive asparagine\textsubscript{38} leucine\textsubscript{99} \(\rightarrow\) tryptophan\textsubscript{38} valine\textsubscript{99} alteration. In D\textsubscript{2}, glycine replaces serine at position 168. This last substitution is noteworthy because of the very high sequence conservation within D\textsubscript{2} of all \(E\beta\) chains; there are only two variable amino acids in the five alleles thus far sequenced.

We conclude, therefore, that the mutation or mutations in \(\textit{Ea}^\alpha\) and/or \(\textit{Ea}^\beta\) which affect \(\alpha\text{a}\cdot\beta\) chain pairing are rather subtle. Although one can hazard guesses, it is not possible to identify them on the basis of sequence analysis. Perhaps this is not surprising in light of recent experiments demonstrating that pairwise combinations of \(\alpha\)- and \(\beta\)-chain alleles exhibit a whole range in their efficiency of association, a range which can be manifested as a differential ability to provoke T cell activation (29, 30). The \(\textit{Ia}\text{a}\) anomaly may just represent the extreme case of a continuum. In addition, it is possible that pairing requires a specific tertiary structure whose perturbation is not evident on the basis of sequence analysis.

Localization of the residues on \(\textit{Ea}\) and \(E\beta\) chains which affect pairing must await exon shuffling and site-specific mutagenesis studies. We have now provided the data base on which to design such experiments.

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REFERENCES

15. Mathis, D. J., C. O. Benoist, V. E. Williams II, M. R. Kanter, and H.
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