The EMBO Journal vol.3 no.6 pp.1221 – 1225, 1984

The nucleotide sequence of the murine I-Eβ^b immune response gene: evidence for gene conversion events in class II genes of the major histocompatibility complex

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Communicated by R.A.Flavell

We have determined the DNA sequence of the murine I-Eβ^b immune response gene of the major histocompatibility complex (MHC) of the C57BL/10 mouse and compared it with the sequence of allelic I-E and non-allelic I-A genes from the d and k haplotypes. The polymorphic exon sequences which encode the first extracellular globular domain of the Eβ domain show ~8% nucleotide substitutions between the Eβ^b and Eβ^d alleles compared with only ~2% substitutions for the intron sequences. This suggests that an active mechanism such as micro gene conversion events drive the accumulation of these mutations in the polymorphic exons. The fact that several of the nucleotide changes are clustered supports this hypothesis. The Eβ^b and Eβ^k genes show ~2-fold fewer nucleotide substitutions than the Eβ^d/Eβ^b pair. The Aβ^{bm12}, a mutant I-Aβ^b gene from the C57BL/6 mouse, has been shown to result from three nucleotide changes clustered in a short region of the β1 domain, which suggests that a micro gene conversion event caused this mutation. We show here that the Eβ^b gene is identical to the non-allelic Aβ^{bm12} DNA sequence in the mutated region and suggest, therefore, that the Eβ^b gene was the donor sequence for this intergenic transfer of genetic information. Diversity in class II MHC genes appears therefore to be generated, at least in part, by the same mechanism proposed for class I genes: intergenic transfer of short DNA regions between non-allelic genes.

Key words: major histocompatibility complex/I-Eβ^b immune response gene/class II genes/nucleotide sequence

Introduction

The murine major histocompatibility complex (MHC) encodes a cluster of polymorphic cell surface proteins which are involved in the regulation of the immune response. Encoded in this chromosomal region are the Ia antigens, or class II MHC glycoproteins which are expressed on the surface of B lymphocytes and macrophages. Ia molecules present on the surface of antigen-presenting cells, in concert with the foreign antigen, activate the helper T cells required to induce a B cell antibody response. This T cell activation requires that the T-helper cells derive from a mouse of the same I-region haplotype as the antigen-presenting cells; in this way, helper T cell activation is foreign antigen dependent and I-region restricted (Benacerraf, 1981).

There are two well-characterized murine Ia antigen complexes, A and E. Each consists of an α-chain of mol. wt. ~35 000 and a β-chain of mol. wt. ~29 000; the Aβ, Aα and Eβ chains are encoded within the A region, while the Eα chain maps to the E region of the MHC. I-region restriction of T cell responses is a consequence of polymorphic differences in the amino acid sequence of allelic Ia antigens. Biochemical studies of the isolated proteins show that both Aβ and Eβ chains are highly polymorphic (for review, see Kaufman *et al.*, 1984) and more recent analyses of cDNAs encoding the Aα chains has also shown considerable polymorphism in these chains (Benoist *et al.*, 1983).

The Aβ and Eβ chains consist of two extracellular globular domains, a polymorphic β1 domain, a conserved β2 domain, a transmembrane domain and a short carboxy-terminal cytoplasmic domain. The cloning of the chromosomal α and β genes has led to the sequence determination of the Eα^d gene, (McNicholas *et al.*, 1982; Hyldig-Nielsen *et al.*, 1983) the Aβ genes of several haplotypes (Larhammer *et al.*, 1983; Choi *et al.*, 1983) and the Eβ^d genes (Saito *et al.*, 1983). In addition, the cDNA sequence for the Eβ^k gene was reported recently (Mengle-Gaw and McDevitt, 1983).

The analysis of the DNA sequences of class I genes of the MHC has provided evidence for gene conversion as a mechanism involved in the generation of polymorphic H-2 genes. Using the same approach we show here that gene conversion events can be demonstrated in the class II Eβ gene.

Results

The Eβ^b gene was cloned from a C57BL/10 (B10) cosmid library as a set of overlapping cosmid clones (G.Widera, unpublished) and subcloned into pUC8 and pUC13 as shown in Figure 1. The DNA sequence of the entire protein coding sequence and the intron sequences flanking the exons was determined using the Maxam-Gilbert procedure with the strategy outlined in Figure 1. The DNA sequence of exon 1 was determined by dideoxy sequencing using a synthetic oligonucleotide as primer (see Materials and methods).

The overall intron-exon organization of the Eβ^b gene is the same as that described for the A$\beta^{b,d,k}$ and Eβ^d genes (Figure 1). The first exon encodes the 5′-untranslated sequence, (5′ UTS), a leader sequence and the first five amino acids of the β1 domain. The second and third exons encode the remainder of β1 and the non-polymorphic β2 domain; the fourth exon encodes a transmembrane sequence and the remaining two exons encode a short segment of the cytoplasmic domain, and the remainder of that domain together with the 3′ UTS, respectively.

The 5′-flanking sequence contains a TATA box and CCAAT box previously described for a large number of eukaryotic genes. If we assume that transcription initiates ~30 nucleotides downstream from the TATA box (see e.g., G.Grosveld *et al.*, 1982) this would predict a 5′ UTS of ~50 nucleotides, which is consistent with the proposed mRNA start for the Eβ^d-gene (Saito *et al.*, 1983).

Comparison of the DNA sequence of the Eβ^b, Eβ^d and Eβ^k genes

There are 35 nucleotide differences (or 3.0% of the nucleotides compared) between the exon sequences of the Eβ^b and Eβ^d genes. Twenty three out of these 35 substitutions occur in

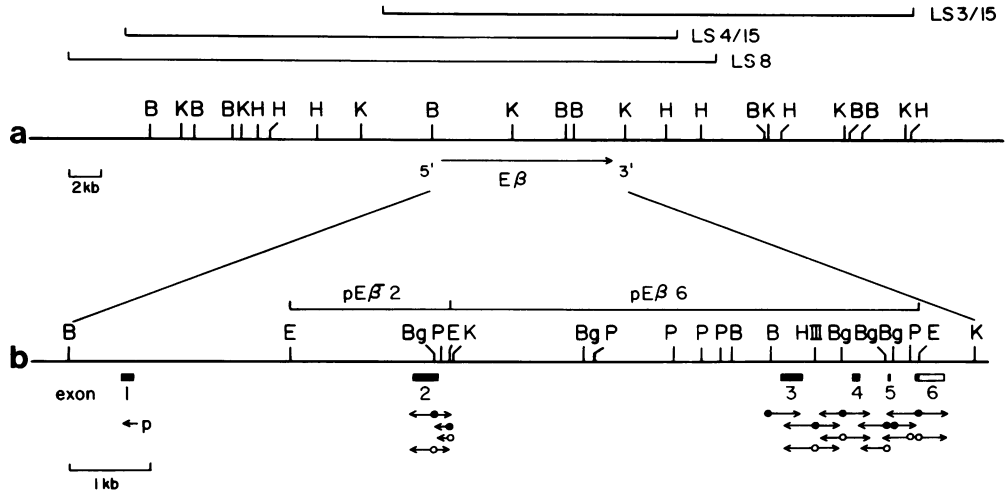

Fig. 1. **(a)** Restriction map of the genomic DNA segment surrounding the $E\beta^b$ gene. For the three overlapping cosmids containing this gene, the restriction sites for B: *Bam*HI, H: *Hpa*I, and K: *Kpn*I are shown. **(b)** Organization of the $E\beta^b$ gene. The exons are shown by filled boxes, the open box in exon 6 represents the 3'-untranslated part of the gene. pEβ2 and pEβ6 show the subclones of the 2-kb and 6.2-kb *Eco*RI fragments, cross-hybridizing with DRβ cDNA, in pUC8 and pUC13 (Vieira and Messing, 1982), respectively. For the subclones, in addition to the cosmids, the restriction sites for Bg: *Bgl*II, E: *Eco*RI, HIII: *Hind*III, and P: *Pst*I are given. The arrows indicate the sequencing strategy: closed circles indicate 5', open circles indicate 3' labelling of the respective restriction sites, P indicates the use of an oligonucleotide primer.

the polymorphic β1 domain of the molecules and cause 14 amino acid substitutions (Figure 3). Clustered changes can be seen between residues 4 and 13 (four substitutions), between residues 24 and 35 (five substitutions) and 68 and 75 (four changes) in this domain. In the rest of the molecule only one nucleotide exchange gives an amino acid substitution (residue 140 in the β2 domain: Lys→Glu); seven out of the 12 nucleotide substitutions outside the β1 domain are located in the 3'-untranslated part of the gene. The compared intron sequences show with 98.2% a higher overall homology than the compared exon sequences with 97.0%. More importantly, the divergence of the sequences encoding the β1 domain is 4-fold greater than the divergence of the introns.

The $E\beta^b$ DNA exon sequences show a much higher degree of homology to the sequence published recently for an $E\beta^k$ cDNA (Mengle-Gaw and McDevitt, 1983). From a total of 18 nucleotide changes, 11 occur in the β1 domain and cause seven amino acid substitutions between $E\beta^b$ and $E\beta^k$, all in this domain. Six of the amino acid substitutions are located outside the loop created by the disulfide bonds between residues 16 and 80, so that the polymorphic differences between these two alleles in this domain are mainly restricted to the amino- and carboxy-terminal extremities of this domain. Comparison of the $E\beta^k$ with the $E\beta^d$ alleles shows an even higher degree of polymorphism between these two haplotypes than between $E\beta^b$ and $E\beta^d$ with 33 nucleotide and 21 amino acid substitutions in the β1 domain. The similarity of the β1 domains between the b and k haplotypes does not extend to the Aα and Aβ genes [20 kb and 35 kb to the 5' side of the Eβ gene (Steinmetz *et al.*, 1982; G.Widera, unpublished)] nor to the Eα gene. The Aα cDNA (Benoist *et al.*, 1983) and Aβ genomic sequences (Choi *et al.*, 1983) have been compared for these two haplotypes recently and these show 14 nucleotide substitutions for the Aα-alleles, which cause 10 amino acid changes and 31 nucleotide substitutions for the Aβ-alleles, which cause 15 amino acid substitutions in the corresponding allelic domains. The Eα genes of the two haplotypes differ in that the $E\alpha^b$ gene has an ~650-bp deletion at its 5' end (Mathis *et al.*, 1983; Hyldig-Nielsen *et al.*, 1983).

Sequence comparison of the Eβ and Aβ genes: a conserved region in the β1 domain of Ia-β genes

We have also compared the β1 domain of the $E\beta^b$ gene with that of the non-allelic $A\beta^b$ and $A\beta^d$ genes (Figure 3). Numerous differences exist between these non-allelic sequences except in the region between amino acid residues 42 and 85 (underlined in Figure 3), where only six amino acids differ between both alleles ($E\beta^b/E\beta^d$) and the non-alleles ($E\beta^b/A\beta^b$; $E\beta^b/A\beta^d$; $E\beta^k/A\beta^d$: five differences). This high degree of homology which holds also for other known mouse and human sequences of this domain (Kaufmann *et al.*, 1984) suggests a conserved function for this region of the I-Eβ and I-Aβ chains.

A gene conversion event between the $A\beta^b$ and $E\beta^b$ generated the $A\beta^{bm12}$ mutant class II gene

The immune response of the laboratory mouse to the A-chain loop of sheep insulin is I-E^k restricted. C57BL/6 mice (H-2^b) which do not express an E molecule are non-responders, while C57BL/6 H-2^{bm12} mutant mice derived from C57BL6 wild-type are responders; the mutant mice carry the $A\beta^{bm12}$ gene. The restriction element for this immune response has therefore transferred from the I-E to the I-A molecule (Hochman and Huber, personal communication). McIntyre and Seidman (1984) have recently shown that the $A\beta^{bm12}$ gene differs from the $A\beta^b$ gene by three nucleotides which cause three amino acid substitutions at amino acid residues 68, 71 and 72. Since these changes are closely clustered, it is likely that they are caused by a gene conversion event. Inspection of the DNA sequence of the $A\beta^b$ and $E\beta^b$ genes shows that the mutations in the bm12 mouse are found within the region of extreme conservation between the Aβ and Eβ genes. In fact, between the codons for amino acids 42 to 85 there are only eight nucleotide differences between these non-allelic genes, causing six amino acid substitutions. More importantly, the DNA sequence of the $E\beta^b$ gene is identical to the $A\beta^{bm12}$ mutant at this site (Figure 4) which shows that the $E\beta^b$ gene was probably the donor for this intergenic sequence transfer. Inspection of Figure 4 shows that (assuming a single genetic event) the minimum size of the genetic exchange would be

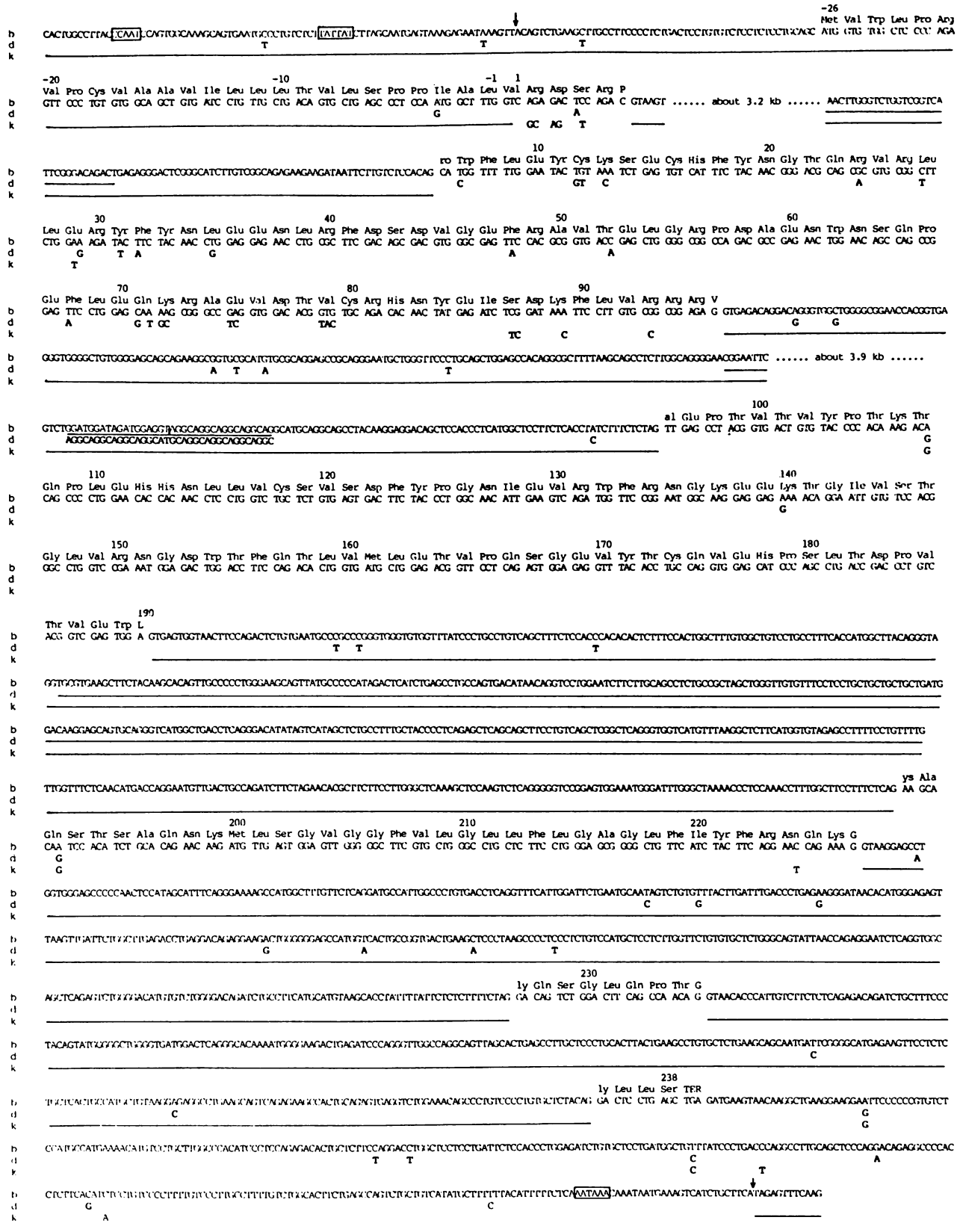

Fig. 2. The DNA sequence of the Eβ^{b} gene is shown and compared with the available sequences of the Eβ^{d} gene and Eβ^{k} cDNA. Only differences in the sequence of the latter two genes to the Eβ^{b} gene are indicated. Lines indicate where sequences were not available for comparison. The 5′ CAAT and TATA boxes and the AATAAA polyadenylation sequence are shown within boxes. The start and the end of the mRNA are shown by arrows and were derived from the Eβ^{d} and Eβ^{k} genes, respectively. The predicted amino acid sequence of the Eβ^{b} chain is shown above the DNA sequence. The six exons encode the following protein domains: exon 1 (L): leader, N-terminal part of the β1 domain; exon 2: β1 domain; exon 3: β2 domain; exon 4 (TM): transmembrane region; exon 5 and 6 (CP, 3′ UTS): cytoplasmic tail and 3′-untranslated sequence of the mRNA.

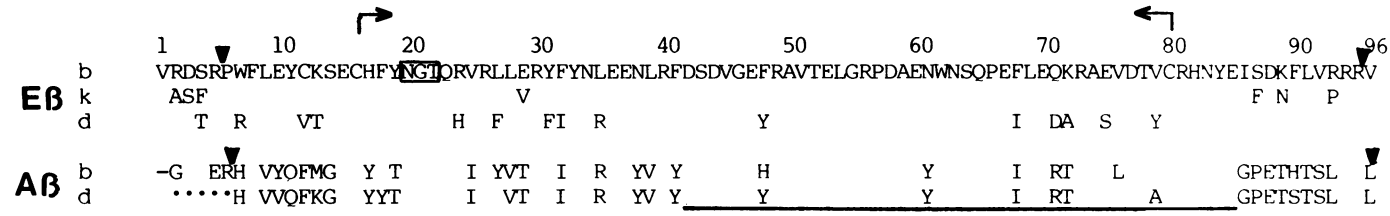

Fig. 3. Amino acid sequence comparison of the β1 domains of I-Eβ and I-Aβ chains. The sequences for the I-Eβ were predicted from the nucleotide sequences of Figure 2, the sequences for I-Aβ were taken from Larhammar *et al.* (1983) and Choi *et al.* (1983). The first amino acid of the $E\beta^k$ chain could not be predicted from the cDNA sequence and was taken from the amino acid determination by Crook *et al.* (1979). The first five residues of the I-$A\beta^k$ chain are not available for comparison as indicated by dots. The I-Aβ chains are starting with residue number 2 in this comparison. Only amino acids different to the sequence of the I-$E\beta^b$ β1 domain are shown. The exon boundaries are indicated above the sequence by an arrowhead. The potential glycosylation site is boxed; the cysteines which presumably form the disulfide bonds in this domain are indicated by arrows. The region of 86% homology between all domains is underlined. The amino acids are given in the one-letter code.

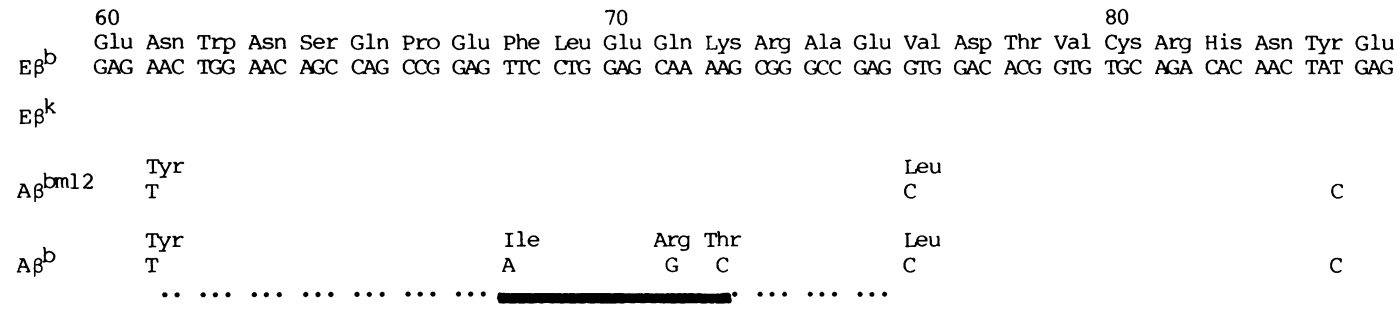

Fig. 4. Sequence comparison of the center part of the β1 domains of $E\beta^b$,$E\beta^k$ and $A\beta^{bm12}$,$A\beta^b$ genes. Only nucleotides and amino acids different to the $E\beta^b$ sequence are shown. The sequence underlined represents the minimum extent of the gene conversion and the dotted line represents the maximum extent of this event.

14 bp (i.e., the limit of the mutations) and the maximum size would be 44 nucleotides – beyond this point the sequences of the $A\beta^{bm12}$ and the $E\beta^b$ gene diverge. Finally, the $E\beta^b$ sequence is identical to the $E\beta^k$ sequence in this region of the sequence which explains the immune response phenomenon.

Discussion

The conclusions that emerge from the comparison of the two allelic Eβ chromosomal genes described here are very similar to those we made previously for class I H-2 genes (Weiss *et al.*, 1983a, 1983b). In both cases the polymorphic exon sequences have diverged to a considerably greater extent than the intron sequences (Table I) which is the converse of the normal situation, seen for example in globin genes (van den Berg *et al.*, 1979; Efstratiadis *et al.*, 1980).

We have previously argued from studies with class I genes that this unusual phenomenon must result from an active process causing the accumulation of mutations in exons. A passive process would cause the same number of changes to be fixed both in introns and exons (Flavell *et al.*, 1982a, 1982b; Weiss *et al.*, 1983a, 1983b). We and others have independently proposed that gene conversion-like events may generate polymorphism in class I genes. The finding that the nucleotides altered in mutant and polymorphic wild-type alleles are clustered has led us to propose that intergenic transfer of short DNA segments (called by us micro gene conversions) is that active process. There is some evidence for such clustering in the $E\beta^d/E\beta^b$ comparison and, more strikingly, in the $E\beta^b/E\beta^k$ comparison where clusters are seen at the C-terminal end of exon 1 (which encodes the first residues of the β1 domain) and the C-terminal end of exon 2, respectively. Moreover, the sequence comparison of the $E\beta^b$ gene with the $A\beta^b$ and $A\beta^{bm12}$ genes (Figure 4) shows that such a micro gene conversion presumably occurred between the $E\beta^b$ and $A\beta^b$ genes to generate the $A\beta^{bm12}$ gene.

Table I. Comparison of $E\beta^{b,d,k}$ nucleotide sequences

	Compared lengths (bp)	Nucleotide differences between haplotypes b/d	b/k	k/d
5′-flanking	82	2		
exon 1 (b/d)	146	3; coding: 1		
(b/k;k/d)	14		5; coding: 3	6; coding: 3
intron 1	61	0		35; coding: 21
exon 2	270	22; coding: 14	5; coding: 4	27; coding: 18
intron 2	265	8[a]		
exon 3	282	2; coding: 1	1; coding: 0	1; coding: 1
intron 3	193	3		
exon 4	111	1; coding: 0	2; coding: 0	1; coding: 0
intron 4	390	8		
exon 5	24	0	0	0
intron 5	294	2		
exon 6	336	7	4	7
intron total	1279	23 = 1.8%		
exon total (b/d)	1169	35 = 3%		
exon total (b/k;k/d)	1037		18 = 1.7%	42 = 4%

[a]A 36-bp deletion in the b-haplotype was counted as a single event irrespective of its length. As none of the four pEβ6 subclones examined, nor the cosmids contain the 36 deleted bp, as determined by *Bam*HI/*Ras*I digestion, we believe that this deletion is not a cloning artefact.

It is clear from the sequence comparisons of the Eβ genes that the $E\beta^b$ and $E\beta^k$ genes are more closely related than the $E\beta^b$ and $E\beta^d$ genes (18 *versus* 35 nucleotide substitutions). This could result from one of the following explanations.

Firstly, the rate of fixation of mutations in class II genes is

a non-random process and, therefore, fluctuations will occur in natural Eβ alleles; in other words, there is no significance to the difference. Secondly, it is possible that the fixation of mutations in the Eβ gene requires that the gene be expressed for phenotypic selection to occur. Since the Eα^b gene is not expressed, the Eβ gene product does not appear on the cell surface and hence does not influence the phenotype of mice of the H-2^b haplotype. Thus, fixation of mutations would be slower than in haplotypes with an 'expressed' Eβ gene. Quantitatively we would expect the number of nucleotide differences to be about one half of the number between two 'expressed' Eβ genes (since both will diverge), which is close to what we observe. Thirdly, it is possible that a DNA correction has occurred in the history of the mouse or between an Eβ^k-like gene and the progenitor of the Eβ^b gene to generate the modern-day Eβ^b gene. A long-range gene conversion as observed for the human γ-globin genes (Slightom *et al.*, 1980) is a mechanism that could generate such a correction. In this regard, it is interesting to note that b-haplotype (C57) and k-haplotype (C58) mice were derived from a common stock when inbred strains of mice were being isolated earlier this century (Klein, 1975).

Conclusions

These data extend our conclusions on the mechanism for the generation of diversity in class I H-2 genes (Weiss *et al.*, 1983a, 1983b; Mellor *et al.*, 1983) to the class II genes. In both cases, differences between 'wild' allelic genes show clustered nucleotide changes and greater divergence of exon sequences than intron sequences (Weiss *et al.*, 1983b; this paper). Finally, mutants exist in both systems which show clusters of multiple nucleotide substitutions (Weiss *et al.*, 1983a; Mellor *et al.*, 1983; McIntyre and Seidman, 1984; this paper). The amount of diversity that can be generated by this system depends on the number of donor genes. Although there are only two expressed immune response genes encoding β chains (Aβ and Eβ) there are, in fact, two other gene segments described, Aβ2 (Larhammer *et al.*, 1983) and Eβ2 (Steinmetz *et al.*, 1982) and we have recently identified two other β-like genes, Aβ3 and Eβ3, in the MHC of the C57BL/10 MHC mouse (G.Widera and C.Wake, unpublished). It is possible, therefore, that these additional genes serve as donors for other intergenic sequence transfers. Further DNA sequence studies on class II genes should help to elucidate this.

Materials and methods

Isolation of cosmids containing the Eβ^b gene

The LS cosmid library was constructed as described (F.Grosveld *et al.*, 1982) using partially *Sau*3A-digested C57BL/10 liver DNA as insert and pTCF as vector. The library was screened and clones were picked as described (Grosveld *et al.*, 1981). Chromosome walking from cosmid Iβ-101 containing the Aβ^b-gene (Larhammar *et al.*, 1983), gave after two steps cosmid LS8, where a 2-kb and a 6.2-kb *Eco*RI fragment could be detected by Southern hybridization (Southern, 1975) using a DRβ cDNA (Wake *et al.*, 1982) as probe. These fragments were described for the Eβ^b gene by Steinmetz *et al.* (1982). The 2-kb *Eco*RI fragment was used as probe for the isolation of cosmids LS3/15 and LS4/15.

Nucleotide sequencing

Subcloning and sequencing of the Eβ^b-gene was performed as outlined in Figure 1. Except for exon 1, the method of Maxam and Gilbert (1980) was used. To sequence exon 1, an oligonucleotide primer (18 mer), synthesized complementary to the intron sequence starting 10 nucleotides 3′ from this exon in the Eβ^d-gene (Saito *et al.*, 1983), was used following the protocol described by Wallace *et al.* (1981).

Acknowledgements

We are grateful to Drs. Brigitte Huber and Claire Wake for helpful discussions and M.Nathanson for preparing the manuscript. We would also like to thank K.McIntyre and J.Seidman for providing access to their data prior to publication. G.W. was supported by a fellowship from the Deutsche Forschungsgemeinschaft. This work was supported by Biogen N.V.

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Received on 22 March 1984