A UNIQUE SEQUENCE OF THE NZW I-Eβ CHAIN AND ITS POSSIBLE CONTRIBUTION TO AUTOIMMUNITY IN THE (NZB × NZW)F₁ MOUSE

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Class II molecules are critical in normal immune responses. In association with antigen, class II molecules are recognized by TCRs. This recognition leads to the activation of those T cells and ultimately a response to that antigen. It is also clear that these class II molecules are important in the development of disease both in the human and mouse (1). For example, in the human system, the DQβ chain (the human homologue of mouse I-A) has been implicated in the development of insulin-dependent diabetes mellitus (IDDM) (1, 2). Indeed, it has been suggested that a single amino acid change at position 57 in the DQβ chain can alter the predisposition to IDDM. In addition, the DRβ chain has been implicated in the development of rheumatoid arthritis (1, 3) and the DPβ chain has been implicated in celiac disease (4).

Similarly, in the murine system, the response to certain well-defined antigens is mediated by I-A or I-E. In the nonobese diabetic (NOD) mouse, the animal model for IDDM, a gene linked to the H-2 complex has been shown to contribute to the development of IDDM (5). The I-Aβ chain from the NOD mouse has been studied and contains a unique sequence involving amino acids 57-58 (6). It has been suggested that this unique sequence may contribute to disease susceptibility, possibly by allowing the presentation of self antigens. Finally, it has recently been suggested that genes linked to the H-2 complex may contribute to the development of lupus in mice (7). This H-2 linkage appears to be the case for genes contributed by the NZW mouse (8) to the development of accelerated autoimmunity in (NZB × NZW)F₁ mice (7), as well as genes contributed by SWR in (NZB × SWR)F₁ mice (9).

The observations that nephritis in the (NZB × SWR)F₁ mouse is linked to the I-Aβ locus of the SWR mouse and that antibodies to murine class II molecules have been used to ameliorate disease in (NZB × NZW)F₁ mice (10) suggest that the H-2-linked genes of the NZW that accelerate autoimmunity are the class II genes.
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themselves. We therefore evaluated these genes for a unique sequence that might contribute to autoimmunity. Because the majority of the polymorphism of class II genes lies in the second exons encoding the first domains, we sequenced the second exons of I-A\textgamma, I-A\textbeta, I-E\textalpha, and I-E\textbeta of NZW using the technique of the polymerase chain reaction (11). We report here that the second exons of NZW I-A\textalpha and I-E\textalpha are identical to their counterparts of the previously sequenced u haplotype, and that the second exon of NZW I-E\textbeta is identical to the second exon of I-E\textbeta except for single C\textrightarrow{}G base change at nucleotide position 215 resulting in an arginine for threonine substitution at amino acid position 72. The substituted G and arginine are identical to those found at these positions in the s haplotype.

Materials and Methods

Oligonucleotides. Oligonucleotides corresponding to previously sequenced mouse class II I-A\textgamma, I-A\textbeta, I-E\textalpha, and I-E\textbeta genes were constructed using the ABI DNA synthesizer (Applied Biosystems, Inc., Foster City, CA) (Table I). The primer oligonucleotides corresponded to the first 18–24 and the last 21–24 bases of the second exon of the I-A genes, the first 19 and the last 21 bases of the second exon of I-E\textalpha, the first 17 and the last 21 bases of the second exon of the I-E\textbeta gene, and the most 3’ 21 bases of the first intron and the most 5’ 21 bases of the second intron of the I-E\textbeta gene. The screening oligonucleotides for I-A\textgamma and I-E\textbeta corresponded to an 18–24 base nonpolymorphic region found in the second exon of these genes.

Polymerase Chain Reaction (PCR). For I-A\textgamma and I-E\textbeta and PCR was performed essentially as described in the procedure from the Perkin Elmer Cetus Gene Amp DNA Amplification Reagent kit (see also reference 12) (Perkin Elmer Cetus, Norwalk, CT). Briefly, 1 \mu{}g of NZW genomic DNA was amplified in a solution of reaction buffer (50 mM KCI, 10 mM Tris-Cl, pH 8.3, 1.5 mM MgCl\textsubscript{2}, 0.01% gelatin), 0.20 mM of each dNTP, 1.0 \mu{}M each of 5’ primer and 3’ primer, and Taq polymerase (2.5 U) in a total volume of 100 \mu{}l. The mixture was overlaid with mineral oil and taken through 30 cycles of amplification (1.5 min at 94°C, 2 min at 50–55°C [42°C for intronic primers], 3 min at 72°C). The entire reaction was ligated into Sma I-digested M13mp18, and Escherichia coli strain JM109 was transformed (13) with the ligation mixture. After transformation, plaques were transferred to Nitroplus 2000 (Micron Separations Inc., Westboro, MA) and screened using a third oligonucleotide made to a nonpolymorphic portion of the second exon of the class II gene (Table I). The probe was end-labeled with \gamma-[\textsuperscript{32}P]dATP (14). Filters were prehybridized in 6 \times SSC (1 \times SSC: 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0) (15), 5 \times Denhardt’s solution (1 \times Denhardt’s: 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA), 0.05% sodium pyrophosphate, 100 \mu{}g/ml salmon sperm DNA, 0.5% SDS, and hybridized in 6 \times SSC, 1 \times Denhardt’s, 100 \mu{}g/ml yeast tRNA, 0.05% sodium pyrophosphate, and 5 \times 10\textsuperscript{6} cpm of each probe per filter at 37°C overnight (16). The filters were washed at 37°C in 6 \times SSC, 0.05% sodium pyrophosphate and subjected to autoradiography. Positive plaques were picked and sequenced.

For I-E\textalpha, single-stranded template for sequencing was prepared directly from PCR using a modification of the limiting primer technique (12, 14). Molar primer ratios of 1:15 or 1:20 were used in a PCR reaction solution of 200 or 400 \mu{}l, using the same constituent concentrations as with the I-A\textgamma and I-E\textbeta PCRs. The limiting primer was set at a concentration of 10 ng/0.1 ml reaction mixture, and the PCR was run for 40 cycles, with denaturation at 94°C for 2 min, annealing at 55°C for 3 min, and extension at 72°C for 2 min. Reaction mixtures that showed the major product band to be the expected size on a 3% agarose gel were selected for sequencing.

DNA Sequencing. Positive plaques were sequenced by the Sanger dideoxy chain termination method (17) using [\textsuperscript{35}S]dATP and the Sequenase DNA Sequencing Kit (U.S. Biochemical Corp., Cleveland, OH). The sequencing reactions were run on 6% denaturing polyacrylamide gels, dried, and subjected to autoradiography. Sequence was performed on DNA iso-
lated from at least three independent PCRs for each gene to preclude any sequence errors introduced during the PCR.

**Southern Analysis.** An aliquot of the PCR was electrophoresed on a 3% Nusieve agarose gel (FMC Bio Products, Rockland, ME) and transferred by the method of Southern (18) to Genescreen plus (New England Nuclear, Boston, MA). Preparation of the probe and hybridization of the membrane were as described for screening plaques.

**Results**

**NZW I-Aα.** The PCR was performed on NZW genomic DNA using oligonucleotides corresponding to I-Aα and β chain genes (Table I). The amplified DNA from each PCR was ligated into M13. Plaques were screened with a third oligonucleotide corresponding to a nonpolymorphic portion of the class II gene located within the amplified DNA (Table I). Positive plaques were picked and the PCR insert was sequenced. The sequences obtained for the second exons of both NZW I-Aα and I-Aβ were identical to those of the previously sequenced u haplotype with no nucleotide or amino acid changes. Comparisons between NZW and previously sequenced I-Aα genes and polypeptides are shown in Fig. 1. The isoleucine at position 46 appears to be unique to the I-Aα protein (Fig. 1 B), and represents a conservative substitution for the valine found in most haplotypes.

**NZW I-Aβ.** The DNA and protein sequence for the first domain of I-Aβ are shown in Fig. 2. The leucine at position 8 and the tyrosine at position 81 are unique to the I-Aβ protein and are not found in any other known sequences. The leucine at position 8 represents a conservative change from valine present in most other haplotypes. The tyrosine at 81 is a nonconservative change from the histidine found in most other haplotypes. Of interest, the glutamine at position 85 and valine at position 88 seen in I-Aβ and NZW I-Aβ are also both found in the NOD I-Aβ molecule (6) and are therefore unique to the u and NOD haplotypes.

**NZW I-Eα.** The DNA and protein sequences of I-Eα second exons of NZW and haplotypes u,k, and d are shown in Fig. 3. The I-Eα second exons of NZW and u are identical, but differ from the other known haplotypes by two changes, as previously reported (19). The tyrosine for phenylalanine substitution at position 22 is a conservative one, while the lysine for glutamic acid substitution at position 75 is nonconservative.

**NZW I-Eβ.** PCR was performed on NZW genomic DNA using oligonucleotides corresponding to known I-Eβ sequences of several haplotypes (Table I). Because of initial difficulties in isolating amplified DNA corresponding to the NZW I-Eβ second exon, an additional methodological step was introduced to ensure that the NZW I-Eβ second exon was actually being amplified. A portion of each PCR was electrophoresed through an agarose gel and blotted onto nitrocellulose; the filter was hybridized with a third oligonucleotide corresponding to the nonpolymorphic nucleotides 40–60 of I-Eβ (Table I). B10.BR genomic DNA (k haplotype) and oligonucleotides to the k haplotype were used as a positive control. A band at ~270 bp corresponding to amplified I-Eβ from the k haplotype was seen. Also present, but to a lesser extent, was amplified DNA corresponding to NZW I-Eβ (data not shown). With this assurance, the amplified DNA from the latter PCR was ligated into M13. Plaques were again screened with the nonpolymorphic I-Eβ oligonucleotide 40–60, positive plaques were picked, and the PCR insert was sequenced with the nonpol-
### Table 1

Oligonucleotides Used for Amplification and Screening of the Second Exon of NZW Class II DNA

| Genes | 5' primer | 3' primer | Screening of M13 plaques |
|-------|-----------|-----------|-------------------------|
| I-Aa  | GCC GAC CAC GTA GGC TCC TAT GGT* | ATT GGT AGC TGG GGT GGA ATT TGA* | TTC TAT GTG GAC TTG GAT AAG AAG* |
|       | AC        | A         |                         |
|       | GT        |           |                         |
| I-Ab  | CAT TTC GTG CAC CAG TTC* | ACC TCC CTG CCG CGG CTT GAA* | TGC TAC TTC ACC AAC GGG* |
|       | T         | G         |                         |
|       | GT        |           |                         |
|       | GC        |           |                         |
| I-Ea  | C AAA GAG GAA CAC ACC ATC* | ATT GGC ATC TGG AGT GTT GTT* | None required (see text) |
|       | T         | A         | C                       |
| I-Eb  | CA GGG TTT TTG GGA TAT* | AAC TCT CCG CGG CAC AAG GAA* | TCT GAG TGT CAT TTC TAC AAC* |
|       | C         | C         |                         |

* Oligonucleotides from second exon.
† Oligonucleotides from intronic sequences flanking the second exon.
**A**

Nucleotide sequences of the second exon of I-Act from NZW and u (26). The oligonucleotides used in the PCR are underlined. The sequences are numbered from the first nucleotide of the second exon.

**B**

Predicted protein sequences for the first domains of I-Act from NZW and haplotypes u, k, d, b, f, and q (26-29). The sequences are numbered from the first amino acid of the mature protein. The boundary between the first and second exons in the first domain is shown.

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**Figure 1.** Nucleotide and predicted protein sequences of I-A<alpha>. Dashed lines indicate identity with the NZW sequence. The NZW and u sequences are identical. (A) Nucleotide sequences of the second exon of I-Act from NZW and u (26). The oligonucleotides used in the PCR are underlined. The sequences are numbered from the first nucleotide of the second exon. (B) Predicted protein sequences for the first domains of I-Act from NZW and haplotypes u, k, d, b, f, and q (26-29). The sequences are numbered from the first amino acid of the mature protein. The boundary between the first and second exons in the first domain is shown.
morphic I-\(\beta\) oligonucleotide 40–60, positive plaques were picked, and the PCR insert was sequenced. Fig. 4 shows a comparison between I-\(\beta\) nucleotide and amino acid sequences, respectively, from various haplotypes. The sequence of the second exon of the NZW I-\(\beta\) gene corresponds to the previously sequenced u haplotype except for a single nucleotide change (C→G) at base 215. This change was confirmed by sequencing 12 independent clones isolated from five independent PCRs representing both orientations. Two of these clones were isolated from PCRs in which only intronic oligonucleotides flanking the second exon were used as primers. This change results in a nonconservative amino acid substitution at position 72 where a threonine is replaced by an arginine. Of note, both the substituted G and arginine are also present in the I-\(\beta\) gene and polypeptide, respectively, at the corresponding positions.

### Discussion

In this study we demonstrate that within the limits of the PCR and choice of primers, the sequences of the second exons of NZW I-A\(\alpha\), I-A\(\beta\), and I-E\(\alpha\) are all identical to those of the previously sequenced u haplotype, while the second exon of NZW I-\(\beta\) is identical to that of the u haplotype except for a change at amino acid 72. Because the PCR amplifies the oligonucleotides used as primers for the amplification of the second exon, it is possible that a single base change present in the region of the second exon annealing to the primer could be missed. For this reason we cannot exclude a single base difference (potentially resulting in an amino acid difference) between u and NZW in these regions of I-A\(\alpha\), I-A\(\beta\), and I-E\(\alpha\). However, the use

![Partial Table](image.png)
**FIGURE 2.** Nucleotide and predicted protein sequences of I-\(\alpha\)\(\beta\). Dashed lines indicate identity with the NZW sequence. Asterisks indicate gaps relative to the q haplotype sequence. The NZW and u sequences are identical (A) Nucleotide sequences of the first domain of I-\(\alpha\)\(\beta\) chains from NZW, and haplotypes u, q, k, s, f and NOD (27, 28, 30–34). The oligonucleotides used in the PCR are underlined. The sequences are numbered from the first base present in the coding region of the u haplotype. The beginning of the second exon is indicated by the vertical bar. (B) Predicted protein sequences of the first domains of I-\(\alpha\)\(\beta\) chains from NZW and haplotypes u, q, k, s, f, d, b, and NOD (27, 28, 30–34). The sequences are numbered from the first amino acid of the mature protein.

of oligonucleotides corresponding to the intronic sequences flanking the second exon of NZW I-\(\alpha\)\(\beta\) confirm that the sequences of I-\(\alpha\)\(\beta\) and NZW I-\(\alpha\)\(\beta\) second exons are identical except for the single base change.

Previous work has demonstrated that the contribution of NZW to accelerated autoimmunity in the (NZB × NZW)F\(_1\) mouse is linked to the H-2 complex (7). Our
A unique sequence of the NZW I-E\(\beta\) chain may offer a possible explanation for this finding. In a related model, the (NZB × SWR)\(F_1\) mouse, it has been shown that the contribution of the SWR strain (q haplotype) to the development of nephritis is linked to the I-A\(\beta\) locus (as well as the TCR \(\beta\) chain gene) (9). Furthermore, results demonstrating an amino acid unique to the I-A\(\beta\) chain in the NOD (6) mouse and the importance of amino acid 57 in the human DQ\(\beta\) chain contribution to diabetes (1, 2) suggest that single amino acid changes in class II molecules may contribute to the development of autoimmunity. In this context, it is possible that the arginine at position 72 that differentiates the NZW I-E\(\beta\) sequence from the I-E\(\alpha\) sequence from the I-Eu sequence may contribute to the accelerated autoimmunity seen in the (NZB × NZW)\(F_1\). This hypothesis is supported by the postulated class II three-dimensional structure (20) that places this arginine on the \(\alpha\)-helix, where is may influence interaction with antigen or TCR. In addition, five amino acids encoded by the second exon are unique to the NZW (u) I-E\(\beta\) chain (i.e., not present in any other known haplotypes). Many of the amino acids unique to the NZW I-E\(\beta\) chain are located at positions, which based on the hypothetical model of the three-dimensional structure of a class II molecule (20), have the potential to interact with antigen and/or TCR.
A comparison of the known class II sequences from the “autoimmune” haplotypes (q, NOD, and NZW) suggests that other NZW class II molecules may also be important in contributing to autoimmunity. As mentioned in Results, the isoleucine at position 46 in I-Aα and the leucine at position 8 and tyrosine at position 81 in I-Ab are unique to the NZW (u) haplotype. According to the three-dimensional
computer model for class II molecules (20), I-A\alpha isoleucine 46 falls outside the antigen binding groove and would not be directly accessible to either antigen or the TCR. However, isoleucine could cause conformational changes in other parts of the molecule. The I-A\beta leucine 8 is located on the \beta-pleated sheet under the \alpha-helix, and could also potentially influence conformation. The I-A\beta tyrosine 81 is located on the \alpha-helix where it probably does not interact with antigen, but may interact with the TCR (20).

A comparison of the NZW and q haplotypes reveals that the valine at position 15 in the I-A\alpha chain is uniquely shared by both. Similarly, a comparison of the I-A\beta chains from NZW and NOD demonstrates that the glutamine at position 85 and the valine at position 88 are uniquely shared by both. In addition, the I-A\beta genes from NZW and NOD have identical nucleotide sequences in this region from bases 253-267 (Fig. 2), suggesting that a gene conversion event involving these two haplotypes may be one explanation for this region of shared sequence. Based on the hypothetical structure of a class II molecule (20), it is unclear what role valine at position 15 in the \alpha chain, or glutamine 85 or valine 88 in the \beta chain may play in antigen presentation, as they do not appear likely to interact with antigen. However, tertiary structural changes induced by these amino acids may be responsible for the contribution of these sequences to autoimmunity.

Although the sequence of NZW I-E\alpha is identical at the second exon to that of the u haplotype, this sequence still codes two unique amino acid residues relative to other known E\alpha haplotypes, and may play a role in (NZB \times NZW)F1 autoimmunity. The lysine at position 75 is unique to the NZW and the u haplotypes relative to k and d, but the site lies outside the antigen binding groove of the constructed computer model for the class II molecule. The unique tyrosine residue at position 22, however, is near the floor of the groove to one side where one \alpha-helix of E\beta juxtaposes against the E\alpha molecule, and could interact with bound antigen. Furthermore, H-2u haplotype mice have been shown to have an E\alpha molecule that does not consistently associate well with non-u E\beta molecules in heterozygote F1 hybrid crosses, interfering with certain E-restricted immune responses (19). This finding could be significant in the (NZB \times NZW)F1 model also, in light of the NZW identity with the second exon of I-E\alpha.

While the unique sequence of the NZW I-E\beta chain provides one explanation for the contribution of NZW to accelerated autoimmunity, several other explanations exist. It is possible that there may be trans-association between individual class II chains determined by NZB (H-2u) and NZW to form “hybrid” molecules. This hypothesis is supported by results suggesting that administration of anti-I-A\delta antibodies to (NZB \times NZW)F1 mice improved their survival (10).

An additional possibility is that the NZW and H-2u class II molecules may be immunodominant, and in this way contribute to accelerated autoimmunity. Several observations support this hypothesis. PL/J is u haplotype (21, 22), and is involved in experimental allergic encephalomyelitis (EAE), the animal model of human multiple sclerosis (23, 24). The NH2-terminal 37 amino acids of rat myelin basic protein (MBP) can induce EAE in PL/J mice, while the COOH-terminal 89-169 amino acid peptide is encephalitogenic in SJL/J mice (s haplotype). However, the (PL/J \times SJL)F1 mouse develops EAE only in response to the NH2-terminal peptide of MBP (23, 24), suggesting that the u haplotype is immunodominant in this autoimmune
response. This hypothesis is supported by the observation that of 16 I-A-restricted T cell clones recognizing MBP, only one is I-A$ restricted (23, 24). An analogous situation may exist in (NZB × NZW)F1 lupus mice. It is possible that the I-A$ molecule contributed by the NZW mouse is immunodominant and may thereby predispose to self recognition.

One possible mechanism for such immunodominance derives from the concept of preferential expression of one haplotype over another. In support of this concept, it has been demonstrated that in (d × u)F1 mice there is a poor expression of I-Ed molecules. This poor expression is apparently due to a preferential association of E$β chains with the Eα chains present (25). It is possible that a similar phenomenon is occurring in (NZB × NZW)F1 mice.

Because both NZW and PL/J (H-2u) mice have been associated with autoimmunity, it would be of interest to evaluate (NZB × PL/J)F1 mice for the presence of a lupus-like disease, and if it is present, to compare disease severity between (NZB × NZW)F1 and (NZB × PL/J)F1 mice. To our knowledge, the (NZB × PL/J)F1 mice have not yet been produced. A similar degree of disease severity might suggest the u haplotype per se was contributing to autoimmunity. Absence of disease or minimally severe disease in the (NZB × PL/J)F1 compared with the (NZB × NZW)F1 would suggest that the substitution at position 72 in the I-Eβ chain and/or NZW background genes are critical for the development of accelerated autoimmunity. However, differences in background genes between PL/J and NZW would preclude any definitive conclusions.

It is clear that the NZW mouse is not the only strain that contributes to accelerated autoimmunity, as the SWR (q) mouse when crossed to NZB produces a similar picture. It is therefore likely that no one class II haplotype is uniquely involved in the development of autoimmunity, and that several, if not all, haplotypes may encode class II molecules permissive for the development of disease. Study of additional class II sequences should further elucidate the mechanisms by which specific changes in sequence may predispose to disease and autoimmunity.

Summary

The (NZB × NZW)F1 mouse strain develops a syndrome of accelerated autoimmunity including severe renal disease and early death. Evidence suggests that class II molecules play a central role in this process. Previous studies have suggested that the NZW strain contributes at least one gene to the development of accelerated autoimmunity that is linked to the H-2 complex, and antibodies to murine class II molecules have been used to ameliorate disease in (NZB × NZW)F1 mice. We therefore wished to sequence the class II molecules from NZW mice to identify any unique sequences that may contribute to disease development. We constructed oligonucleotide primers corresponding to the 5' and 3' regions of the second exon of class II genes from a variety of haplotypes, and used these primers in a polymerase chain reaction to sequence the second exon of the NZW I-Aα, I-Aβ, and I-Eβ genes. We report that the second exons of NZW I-Aα, I-Aβ, and I-Eα are identical to their counterparts of the previously sequenced u haplotype, and that the second exon of NZW I-Eβ is identical to its counterpart from u except for a single base change that results in a substitution of arginine for threonine at amino acid 72. This base and amino acid are identical to those found at the same positions in the s haplotype.
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