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# Characterization of Three New Intra-*I* Region Recombinant Mouse Strains, B10.ASR7 ($H\text{-}2^{as3}$), B10.BAR4 ($H\text{-}2^{h6}$), and B10.BASR1 ($H\text{-}2^{as4}$)

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The ability to mount immune responses to T-dependent antigens is controlled by four genes that map to the *I* region of the murine major histocompatibility complex (McDevitt et al. 1981, Nagy et al. 1981). These *Ir* genes code for four distinct Ia polypeptide chains, $A_\alpha$, $A_\beta$, $E_\beta$, and $E_\alpha$, which associate to form the I-A ($A_\alpha$; $A_\beta$) and I-E ($E_\alpha$; $E_\beta$) molecules (Uhr et al. 1979, Vitetta and Capra 1979). Restriction endonuclease analysis of the *I* region in *H-2* recombinants with intra-*I*-region crossover events has demonstrated that these recombinatorial events are concentrated in the area between the $E_\beta$ and $E_\alpha$ genes (Steinmetz et al. 1982, Hood et al. 1983). There are three previously described exceptions to this rule. D2.GD ($H\text{-}2^{g2}$) and A.TFR5 ($H\text{-}2^{ap5}$) have intragenic recombinatorial events within the $E_\beta$ gene (Jones 1981, Lafuse et al. 1980), while B10.STA62 has a recombinatorial event that separates the $A_\alpha A_\beta$ duplex from the $E_\beta$ gene (Singh et al. 1981). In this report, we describe two new *H-2* haplotypes that very likely arose by recombination between the $E_\beta$ locus and the $A_\alpha$–$A_\beta$ segment and one haplotype that may have arisen by recombination between the $E_\beta$ and $E_\alpha$ loci.

The current assignments of the *I* region alleles for B10.ASR7 ($H\text{-}2^{as3}$), B10.BAR4 ($H\text{-}2^{h6}$), and B10.BASR1 ($H\text{-}2^{as4}$) were based on cytotoxicity data and indicated that in all three recombinant strains the crossover event occurred between the genes for *H-2K* and $E_\alpha$ (Klein et al. 1983). These three strains were then examined for expression of defined Ia specificities to determine whether the recombinant strain I-A molecule more closely resembled one parent or the other (Table 1). As determined by microcytotoxicity and confirmed by immunofluorescence (data not shown), the I-A molecules of all three strains exhibited monoclonal antibody binding patterns consistent with the *k* haplotype, in that 11.4.1 ($H\text{-}2K^k$, m93), 11.5.2 ($I\text{-}A^k$, m2), 10.2.16 ($I\text{-}A^k$, $I\text{-}A^s$, $I\text{-}A^f$, m27), and 17/227 ($I\text{-}A^k$, $I\text{-}A^b$, $I\text{-}A^d$, m15) bound, but MK-S4 ($I\text{-}A^s$) and 28-16-8S ($I\text{-}A^b$, $I\text{-}A^d$, m8) failed to bind. Competitive inhibition radioimmunoassays performed to evaluate whether the

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**Table 1.** Microcytotoxicity determination of *I*-region alleles for congenic and recombinant mouse strains*

| Strain | Parents | *K* | $A_\beta$ | $A_\alpha$ | $E_\beta$ | $E_\alpha$ | *D* | Percent dead cells† | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | 11.4.1 | 10.2.16 | 14.4.4S | 11.5.2 | 17/227 | MKS4 | 28-16-8S |
| B10.A | *k/d* | *k* | *k* | *k* | *k* | *k* | *d* | 44 | 61 | 48 | 47 | 44 | 8 | 8 |
| B10.S | *s* | *s* | *s* | *s* | *s* | *s* | *s* | 13 | 47 | 14 | 12 | 9 | 47 | 11 |
| C57BL/6 | *b* | *b* | *b* | *b* | *b* | *b* | *b* | 12 | 10 | 11 | 16 | 38 | 5 | 32 |
| B10.ASR7 | *a/s* | *k* | *k* | *k* | *s* | *s* | *s* | 47 | 45 | 6 | 46 | 43 | 7 | 6 |
| B10.BAR4 | *a/b* | *k* | *k* | *k* | *k* | *b* | *b* | 57 | 49 | 10 | 33 | 35 | 11 | 14 |
| B10.BASR1 | *a/h4* | *k* | *k* | *k* | *s* | *s* | *s* | 52 | 62 | 11 | 48 | 38 | 8 | 10 |

* Microcytotoxicity assay was performed using Ficoll-Hypaque-purified lipopolysaccharide blasts in flat-bottomed 96-well plates.

† Percent dead cells was determined by trypan blue dye exclusion. One-hundred cells were counted in each determination. Antisera alone, complement alone, and untreated cell controls demonstrated <12% dead cells. Monoclonal antibodies 11.4.1 (H-$2K^k$), 10.2.16 (I-$A^k$, I-$A^f$, I-$A^s$), 14-4-4S (I-$E^k$, I-$E^k$), 11.5.2 (I-$A^k$), 17/227 (I-$A^k$, I-$A^b$, I-$A^d$), and 28-16-8S (I-$A^b$), obtained from the American Type Culture Collection, and MKS4 (I-$A^s$), obtained from P. Marrack (National Jewish Hospital, Denver, Colorado), were used at a 1 : 40 dilution of culture supernatants.

recombinatorial events in these three strains resulted in altered distributions of Ia specificities on the I-A molecule demonstrated similar binding patterns using monoclonal antibodies 11.5.2, 10.2.16, and 17/227 (data not shown). Furthermore, the absence of 14-4-4S (I-$E^k$, I-$E^d$, m7) binding confirms the original assignments of the $E_\alpha$ alleles to be *s* (B10.ASR7 and B10.BASR1) and *b* (B10.BAR4), both haplotypes which fail to code for a complete $E_\alpha$ gene product. The failure to express I-E molecules in these strains is due to the lack of an $E_\alpha$ gene product and not a defect in the $E_\beta$ gene since $F_1$ crosses created between these strains and A.TFR5 ($E_\alpha^+$; $E_\beta^-$) results in expression of I-E products (data not shown).

The ability to mount a T-cell proliferative response to the terpolymer glutamine$^{53}$-lysine$^{38}$-phenylalanine$^{9}$ (GLPhe) is dependent upon the expression of an $E_\alpha^k$; $E_\beta^s$, or $E_\alpha^k$; $E_\beta^b$ molecule (Schwartz et al. 1976). To determine the $E_\beta$ allele in these three recombinant strains, we created $F_1$ crosses between these strains and A.TL. These $F_1$ crosses were then assayed for their ability to respond to GLPhe by T-cell proliferation (Table 2). Whereas none of the parental strains mounted a significant T-cell proliferative response to GLPhe, (A.TL × B10.ASR7)$F_1$ and (A.TL × B10.BASR1)$F_1$ hybrids generated a T-cell proliferative response, indicating that an $E_\alpha^k$; $E_\beta^s$ molecule was formed by transcomplementation. The (A.TL × B10.BAR4)$F_1$ hybrid, on the other hand, failed to generate such a response, suggesting that B10.BAR4 expresses an $E_\beta^k$ chain. These results localize the crossover events in these strains to the region between *H-2K* and $E_\beta$ (B10.ASR7 and B10.BASR1) and between $E_\alpha$ and $E_\beta$ in B10.BAR4.

In an effort to assess whether the recombinatorial event in these three strains resulted in an altered immune response pattern, the ability of these recombinants to generate T-cell proliferative responses to a panel of I-A molecule-restricted antigens was tested (Table 3). Consistent with the monoclonal antibody binding data, these three strains respond to glutamine$^{60}$-alanine$^{30}$-tyrosine$^{10}$ (GAT), chicken oval-

**Table 2.** Determination of the $E_\beta$ allele by the ability to mount a T-cell proliferative response to GLPhe*

| Strain | Haplotype | | | | | | Cpm (S. I.)† |
|---|---|---|---|---|---|---|---|
| | *K* | $A_\beta$ | $A_\alpha$ | $E_\beta$ | $E_\alpha$ | *D* | |
| A.TL | *s* | *k* | *k* | *k* | *k* | *d* | 2827 (1.25) |
| B10.ASR7 | *k* | *k* | *k* | *s* | *s* | *s* | 1114 (0.98) |
| B10.BASR1 | *k* | *k* | *k* | *s* | *s* | *s* | 560 (1.09) |
| B10.BAR4 | *k* | *k* | *k* | *k* | *b* | *b* | 377 (1.61) |
| A.TL × B10.ASR7) | *s/k* | *k/k* | *k/k* | *k/s* | *k/s* | *d/s* | 21887 (13.1) |
| (A.TL × B10.BASR1) | *s/k* | *k/k* | *k/k* | *k/s* | *k/s* | *d/s* | 23154 (7.5) |
| (A.TL × B10.BAR4) | *s/k* | *k/k* | *k/k* | *k/k* | *k/b* | *d/b* | 1595 (0.6) |

* T-cell proliferations were performed as previously described (Arkin 1978). Final concentration of GLPhe in the cultures was 100 μg/ml.
† Stimulation index.

**Table 3.** T-cell proliferative responses of B10.ASR7, B10.BASR1, and B10.BAR4 to a panel of I-A-restricted antigens*

| Antigen | Cpm (S. I.)† | | | | |
|---|---|---|---|---|---|
| | B10.ASR7 | B10.BASR1 | B10.BAR4 | B10.A(4R) | B10.S |
| $Glu^{60}Ala^{30}Tyr^{10}$ | 29697 (10.8) | 36522 (10.3) | 9063 (7.5) | 40806 (11.3) | 3034 (1.8) |
| c-OVA | 8323 (4.3) | 45126 (18.7) | 17909 (13.9) | 34962 (5.7) | 21302 (9.7) |
| Bovine type II collagen | 2208 (1.6) | 2257 (0.98) | 1247 (0.89) | 5248 (1.4) | 39197 (29.8) |

* T-cell proliferations were performed as described (Arkin 1978). Final concentrations of antigens in the cultures were 125 μg/ml GAT, 125 μg/ml c-OVA, and 50 μg/ml collagen. Collagen was prepared as previously described (Rosenwasser et al. 1980).
† Stimulation index.

bumin (OVA), and denatured beef type II collagen in a manner identical with that of mice expressing $I\text{-}A^k$ and not $I\text{-}A^s$ molecules.

The description of these three haplotype recombinatorial events adds to the growing evidence that suggests that the diversity of the immune response can be created by crossover events occurring *intergenically* [B10.STA62, B10.A(4R), etc.] or *intragenically* (B10.GD, B10.TFR5, etc.). We have recently described one haplotype with a possible intragenic recombinatorial event which results in an altered T-cell response to one I-A-restricted antigen (Gutmann et al. 1983). The examination of such recombinant mouse strains may elucidate the mechanisms operative in the generation of immune response diversity through alterations in Ia restriction molecules and not the T-cell repertoire.

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