Brief Definitive Report

STRUCTURAL STUDIES ON THE MURINE Ia ALLOANTIGENS

V. Evidence That the Structural Gene for the I-E/C Beta Polypeptide is Encoded within the *I-A* Subregion*

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The *I* region of the murine major histocompatibility complex (MHC) encodes a group of membrane alloantigens (Ia antigens) which appear intimately associated with immune response (*Ir*) genes mapped to this region (1, 2). Although five *I* subregions have been defined by serological and functional analysis (3), Ia antigens have been demonstrated by immunoprecipitation techniques for only the *A* and *E/C*[1] subregions.

The A and E/C alloantigens are each composed of two nondisulfide bonded polypeptide chains, alpha (31–34,000 daltons) and beta (26–29,000 daltons) (4, 5). Both subregion and haplotype-specific electrophoretic variation in these Ia antigens have been shown by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (5) and two-dimensional gel analysis (2-D gels) (6). By partial NH_2-terminal sequence analysis, the *A* and *E/C* subregion polypeptides are not homologous and only the β-chains display haplotype associated sequence variation (7, 8). Among the E/C allelic products, comparative peptide mapping has demonstrated extensive haplotype associated structural variation (50%) in the β-chains, but only limited (10%) variation in the α-chains (9). A conservative interpretation of the above data is that at least the A and E/C β-polypeptides are *I*-region encoded.

Further insight into which loci control the expression of the E/C alloantigen has recently been provided in an elegant study by Jones et al. (10) by using 2-D gel analysis. Specific anti-E/C immunoprecipitates examined by this technique revealed two electrophoretically distinct species of polypeptides. Subsequent analysis of anti-E/C immunoprecipitates and whole splenic lysates from intra-*I* region recombinants and F_1 hybrids demonstrated that the two E/C polypeptides were controlled by different genes. A locus in E/C encoded one polypeptide; the other polypeptide, designated as A_e, was controlled by the *A* subregion. Thus, strains with the same haplotype in *E/C*, but a different haplotype in *A*, expressed E/C alloantigens with electrophoretically distinct A_e polypeptides; this variation could result from either post-translational modification or primary structural differences.

In the present report, we have isolated the E/C α- and β-subunits from appropriate

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[1] Although Ia.7 was originally mapped to the *I-C* subregion, the notation *I-E/C* will be used as a result of the present inability to distinguish between products encoded by *I-E* and *I-C*.

TABLE I

Reactivity of (B10 × HTI)F_1 Anti-B10.A(5R) Antiserum

	K	I					S	G	D
		A	B	J	E	C			
Donor: B10.A(5R)	b	b	b	k	k	d	d	d	d
Recipient: (B10 × HTI)F_1	b	b	b	b	b	b	b	b	b
	b	b	b	b	b	b	b	?	d

* Potential reactivities of the antiserum are boxed.

I-region recombinants and analyzed them by comparative tryptic peptide mapping. These studies aim to extend the observations of Jones et al. to the E/C α- and β-subunits and also to ascertain whether the electrophoretic variation described for A_e is due to post-translational modification or primary structural differences.

Materials and Methods

Mice. B10.A, B10.A(3R), and B10.A(5R) mice were from our own colony.

Antisera. (B10 × HTI)F_1 anti-B10.A(5R)—designated HTI anti-5R—was obtained from the Research Resources Branch of the National Institutes of Health.

Isolation of Radiolabeled Ia Antigens. Murine splenocytes (4×10^8) were radiolabeled for 8 h with either [^{3}H] or [^{14}C]leucine, lysine, arginine, and tyrosine (9). Cells were lysed with NP40 and the lysates chromatographed on a lentil lectin affinity column (5). The adherent fraction (glycoprotein pool) was concentrated and depleted of Ig and nonspecific material as described (5). Ia antigens were immunoprecipitated with HTI anti-5R and the complexes bound to *Staphylococcus aureus* (*S. aureus*) (5); immune complexes were eluted and the E/C α- and β-polypeptides resolved by SDS-PAGE (5).

Trypsin Digestion and Ion Exchange Chromatography. The [^{3}H] and [^{14}C]labeled α- and β-polypeptides were mixed and digested with trypsin (9). Soluble tryptic peptides were separated by cation exchange chromatography using a pyridine-acetate pH/ionic strength gradient. Fractions were collected into scintillation vials and counted. The ^{3}H and ^{14}C cpm were normalized and corrected for channel spillover (9).

Results

A description of the alloantiserum, HTI anti-5R, is shown in Table I. This antiserum could potentially have reactivity against *I-J*, *I-E/C*, *S*, and *G*; because the S and G products are not expressed on lymphocytes and I-J antigens are expressed only on a small subpopulation of T cells, it is probable that only the I-E/C product (Ia.7) is recognized.

Splenocytes from B10.A, B10.A(3R), and B10.A(5R) (haplotypes shown in Table II) were radiolabeled with ^{3}H or ^{14}C amino acids; NP40 lysates were prepared and the glycoprotein pools from the lentil lectin affinity column were precipitated with HTI anti-5R. The E/C α- and β-polypeptides were isolated and appropriate chains were mixed (^{3}H and ^{14}C), digested with trypsin, and compared by cation exchange chromatography.

Fig. 1 compares α-chains of B10.A with 3R (panel A), 5R (panel B), and as a control B10.A (panel C). The E/C α-polypeptides of 3R and 5R are identical to those of B10.A as evidenced by coelution of all peptides.

As shown in Fig. 2, the E/C β-chains from 3R (panel A) and 5R (panel B) are

TABLE II

Haplotypes of Strains Analyzed

Strain	K	I					S	G	D
		A	B	J	E	C			
B10.A	k	k	k	k	k	d	d	d	d
B10.A(3R)	b	b	b	b	k	d	d	d	d
B10.A(5R)	b	b	b	k	k	d	d	d	d

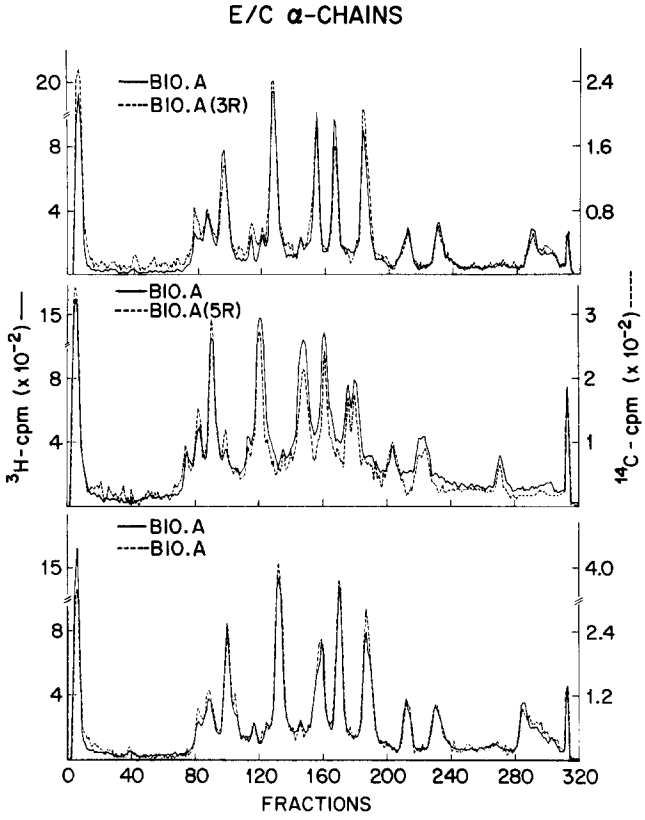

FIG. 1. Ion exchange chromatography of tryptic digests of E/C α-polypeptides. [^{3}H]labeled E/C^k α-chains from B10.A are compared with [^{14}C]labeled E/C^k α-chains from B10.A(3R) (panel A), B10.A(5R) (panel B), and B10.A (panel C).

quite different from the β-chains of B10.A. The most obvious peptide differences are denoted by the four arrows; B10.A β-chains contain at least two peptides not shared by 3R or 5R, and vice versa. Other possible differences are seen just before the first arrow and after the last arrow. The control in panel C (B10.A vs. B10.A) shows no peptide differences. Thus, although all three strains possess the *k/d* allele in the *E/C* subregion (Table II), the E/C β-polypeptides from B10.A are approximately 40%

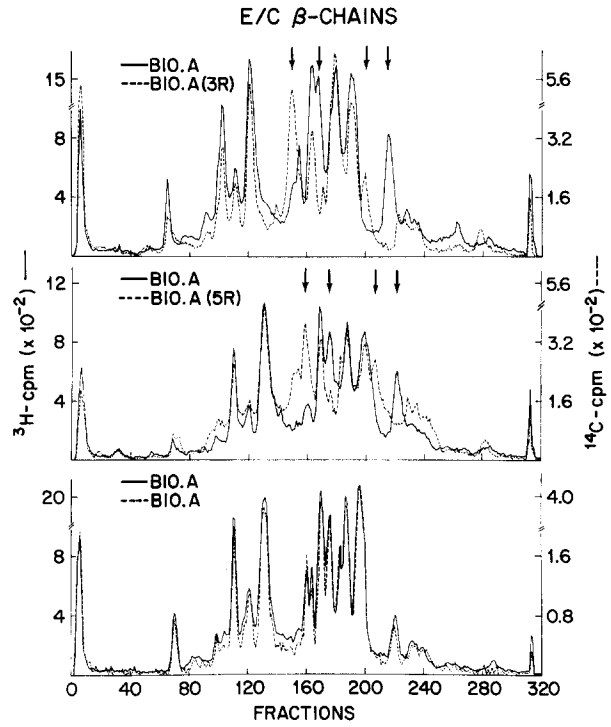

FIG. 2. Ion exchange chromatography of tryptic digests of E/C β-polypeptides. [^{3}H]labeled E/C^k β chains from B10.A are compared with [^{14}C]labeled E/C^k β-chains from B10.A(3R) (panel A), B10.A(5R) (panel B) and B10.A (panel C). Major peptide differences are denoted by arrows.

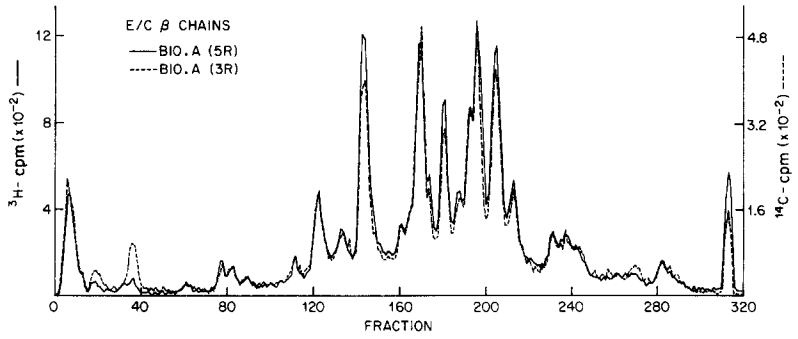

FIG. 3. Ion exchange chromatography of tryptic digests of E/C β-polypeptides. [^{3}H]labeled E/C^k β-chains from B10.A(5R) are compared with [^{14}C]labeled E/C^k β-chains from B10.A(3R).

different from those of 3R or 5R. The β-chains from 3R and 5R have identical peptide maps (Fig. 3) as would be predicted, because both are *b* haplotype in the *I-A* subregion.

Discussion

These data confirm the recent findings of Jones et al. (10) and, in addition, demonstrate that the molecules controlled by the *E/C* and *A* subregions observed by

2-D gel analysis are the E/C α- and β-subunits, respectively. By tryptic peptide mapping, we have found approximately 40% structural variation between the E/C β-chains of 3R (or 5R) and B10.A; post-translational modification (e.g., glycosylation) could not account for such extensive structural variation, and, in addition, tryptic Ia glycopeptides have been detected only in the first (fall through) peptide peak from the ion exchange column (R. G. Cook, unpublished observations). Thus the structural gene for the E/C β-polypeptide lies outside of *E/C* and to the left of *I-J*. We infer from the observations of Jones et al. (10) that the locus resides in the *A* subregion.

The E/C α-polypeptides from B10.A, 3R, and 5R were identical as assessed by tryptic peptide mapping. This implies that the α-subunit is encoded by the *E/C* subregion and that the allospecificity Ia.7 is a determinant(s) controlled by this chain. The β-polypeptide may exert a conformational influence on this allodeterminant, but cannot contain the determinant(s) because serological ambiguities would have been observed in the assignment of Ia.7 to *E/C*.

We have previously examined four allelic E/C alloantigens (*k*, *r*, *p*, and *d*) by comparative tryptic mapping (9). The α-polypeptides, which are encoded by the *E/C* subregion, were approximately 90% identical. The β-chains, on the other hand, displayed only 48–69% homology; this degree of variation is similar to that found in the present study for the E/C β-chains of B10.A and 3R or 5R. Thus, although the serological polymorphism of the E/C alloantigens is apparently controlled by the *E/C* subregion, the extensive structural variation is controlled by the *A* subregion. In addition to encoding the E/C β-polypeptide, the *I-A* subregion also encodes a structurally distinct alloantigen with α- and β-subunits. Whether or not these *A* subregion alloantigens are under a similar two gene control, is under investigation.

This report demonstrates that the structural genes for both the α- and β-subunits of the E/C alloantigen are encoded within the *I*-region of the MHC. For the human DR alloantigens, which are homologous to the murine E/C antigen by NH_2-terminal sequence (11), the evidence suggests that at least α-chain equivalent is encoded within the MHC (12, 13). The combinatorial association of two structurally variable *I*-region encoded polypeptides to form the E/C alloantigen provides a structural basis for understanding the functional requirement for complementation between two *Ir* genes in certain immune responses (14), and has the potential to augment the repetoire of reactivities of these putative receptor molecules.

Summary

The E/C α- and β-subunits of intra-*I*-region recombinants were analyzed for primary structural variation by comparative tryptic peptide mapping. The E/C α-polypeptides from B10.A, B10.A (3R) and B10.A (5R) showed complete coincident elution of peptides; the E/C β-chains from B10.A and 3R (or 5R) were approximately 40% different. This suggests that the structural gene for the E/C β-polypeptide is within the *I-A* subregion.

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