Brief Definitive Report

ABERRANT A_e (E_β) Ia POLYPEPTIDE CHAIN IN $H\text{-}2^{g2}$ HAPLOTYPE MICE

Possible Result of an Intragenic Recombination or Mutation*

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Two classes of Ia antigens can be found on the surfaces of murine B lymphocytes, one class recognized by antibodies to the *I-A* subregion of the *H-2* complex, the second class recognized by antibodies to the *I-E* subregion. Polymorphisms in both the large (A_α) and small (A_β) subunits of the *I-A*-controlled complex indicate that these proteins are encoded by loci in the *I-A* subregion (1). Whereas the large subunit of the anti-*I-E* subregion-precipitated complex, E_α, appears to be coded for by a locus in the *I-E* subregion, recent studies by this laboratory (2) and others (3, 4) have shown that the small subunit is controlled by a locus in the *I-A* subregion. The expression of this chain, called A_e (or E_β), on the cell surface is dependent on the presence of E_α chains in the same cell; synthesis of both A_e and E_α chains is variable from haplotype to haplotype (2).[1]

Until now, no information has been available concerning the map order within the *I-A* subregion of the loci coding for the A_α, A_β, and A_e polypeptide chains. Recently we observed an abnormal A_e chain in mice of the recombinant haplotype $H\text{-}2^{g2}$ that may help to map the locus coding for this chain. The $H\text{-}2^{g2}$ haplotype was derived from a cross between strains DBA/2($H\text{-}2^d$) and C57BL/6 (B6; $H\text{-}2^b$) in the colony of Dr. Frank Lilly, Albert Einstein College of Medicine, Bronx, N. Y., giving rise to the apparent haplotype $H\text{-}2K^d$ $I\text{-}A^d$ $I\text{-}B^b$ $I\text{-}J^b$ $I\text{-}E^b$ $I\text{-}C^b$ S^b $H\text{-}2D^b$ (5, 6). However, the results presented below show that the A_e^{g2} chain differs in electrophoretic mobility from both A_e^d and A_e^b chains, raising the possibility that the recombination event occurred within the gene coding for the A_e chain itself.

Materials and Methods

Mice. DBA/2, B6, and D2.GD mice were obtained from Dr. Frank Lilly. B10.GD mice were provided by Dr. Donald Shreffler, Washington University School of Medicine, St. Louis, Mo. C57BL/10(B10), B10.D2, and B10.A(3R) mice were obtained from Dr. H. O. McDevitt, Stanford University School of Medicine, Stanford, Calif. (B10.GD × B10.D2)F_1 and (B10.GD × A.TFR5)F_1 mice were bred in our animal facility.

Antisera. The antiserum B10 anti-B10.D2 was obtained from Dr. S. Nimelstein, Stanford University School of Medicine. The antisera BALB/b anti-BALB/c, (B10 × HTI)F_1 anti-B10.A(5R), and C3H anti-C3H.SW were generously provided by Dr. H. O. McDevitt.

Biochemical Characterization of Ia Antigens. Labeling of mouse splenic lymphocytes with [^{35}S]-methionine, immunoprecipitation of Ia antigens, and two-dimensional (2-D) polyacrylamide gel electrophoresis were done as previously published (2, 7). Immunoprecipitated proteins were

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[1] Jones, P. P., D. B. Murphy, and H. O. McDevitt. Variable synthesis and expression of E_α and A_e (E_β) Ia polypeptide chains in mice of different *H-2* haplotypes. Manuscript submitted for publication.

separated by charge in the first dimension using nonequilibrium pH gradient electrophoresis (NEPHGE), which resolves proteins with isoelectric points between pH 4.5 and 9. Proteins in NP-40 extracts were separated on isoelectric focusing (IEF) first-dimension gels, which resolve proteins with isoelectric points between pH 4.5 and 7. The second dimension size separation was done on 10% acrylamide sodium dodecyl sulfate slab gels. Positions of the separated proteins were determined by autoradiography.

Results and Discussion

The first indication of an abnormal A_e chain in the $H\text{-}2^{g2}$ haplotype came from initial studies of Ia antigens from strain D2.GD, a DBA/2 congenic carrying the $H\text{-}2^{g2}$ haplotype. The A_e chain differed in electrophoretic mobility from A_e chains of both *d* and *b* haplotype mice. Because of the possibility that Dr. Lilly's DBA/2 or C57BL/6 stocks from which the recombinant was derived carried a mutation in the gene coding for the A_e chain, mice of these strains were obtained from his colony.

Fig. 1 shows the 2-D gel patterns generated by Ia antigens from these mice and from strain B10.A(3R) ($I\text{-}A^b$, $I\text{-}E^k$). The D2.GD pattern has A_α and A_β spots similar to those of DBA/2 but clearly different from B6, confirming the *d*-haplotype origin of these chains. No E_α chain is apparent in either the B6 or the D2.GD gels as a result of the apparent failure of the $I\text{-}E^b$ subregion to code for a synthesized E_α chain (2).[1] The A_e^b chain from B6 is absent from the immunoprecipitate; antisera directed against the *I*-regions of the *b*, *k*, and *s* haplotypes all fail to immunoprecipitate A_e chains from strains in which the *I-E* subregion does not code for a detectable E_α chain (i.e., $I\text{-}E^b$ and $I\text{-}E^s$).[1] However, a spot in the region of the gel appropriate for A_e chains is present in the D2.GD pattern. Evidence presented below will confirm that this spot corresponds to the A_e^{g2} chain. The anti-$H\text{-}2^d$ antisera apparently contain antibodies reactive with this protein. By comparison with panels e and g it can be seen that the indicated spot differs from both A_e^d from DBA/2 and A_e^b precipitated by anti-$I\text{-}E^k$ antibodies from B10.A(3R), thus confirming the earlier observations.

A second approach for detecting mobility differences between A_e molecules of different haplotypes is to examine the positions of A_e spots in autoradiograms of 2-D gels of total NP-40 extractable proteins. This approach has been used previously to demonstrate that cells from B10 and B10.A(4R) ($I\text{-}A^k$, $I\text{-}E^b$) mice do in fact synthesize A_e chains, although they do not express them on the cell surface as a result of an absence of E_α chains, and that A_e^b and A_e^k chains have distinct electrophoretic mobilities (2). Few non-*H-2* encoded spots differ between gels from mice of the same genetic background. Fig. 2 shows the Ia regions of such gels, obtained using IEF for the first dimension charge separation as it provides greater separation between spots than does NEPHGE. The positions of Ia spots have been verified by comparison with immunoprecipitated Ia antigens electrophoresed on IEF first-dimension gels. The spot indicated to be A_e in the D2.GD gel clearly differs in position from both A_e^d from DBA/2 and A_e^b from B10.A(3R) and B6, consistent with the results in Fig. 1.

To verify that the spot in question corresponds to an A_e chain, experiments were done to determine whether or not this chain is capable of associating with E_α chains which are absent in cells of the *g2* haplotype. The association with E_α chains allows A_e molecules to become more highly glycosylated and expressed on the cell surface, and also to be precipitated by anti-I-E antibodies (2).[1] B10.GD mice, carrying $H\text{-}2^{g2}$ on the B10 background, were crossed with B10.D2 ($H\text{-}2^d$) and A.TFR5 ($H\text{-}2^{ap5}$: $H\text{-}2K^f$ $I\text{-}A^f$ $I\text{-}B^f$ $I\text{-}J^f$ $I\text{-}E^k$ $I\text{-}C^k$ S^k $H\text{-}2D^d$) (9). If the A_e^{g2} chain associates with the E_α^d chain in the (B10.GD × B10.D2)F_1 cells, the immunoprecipitate should produce spots

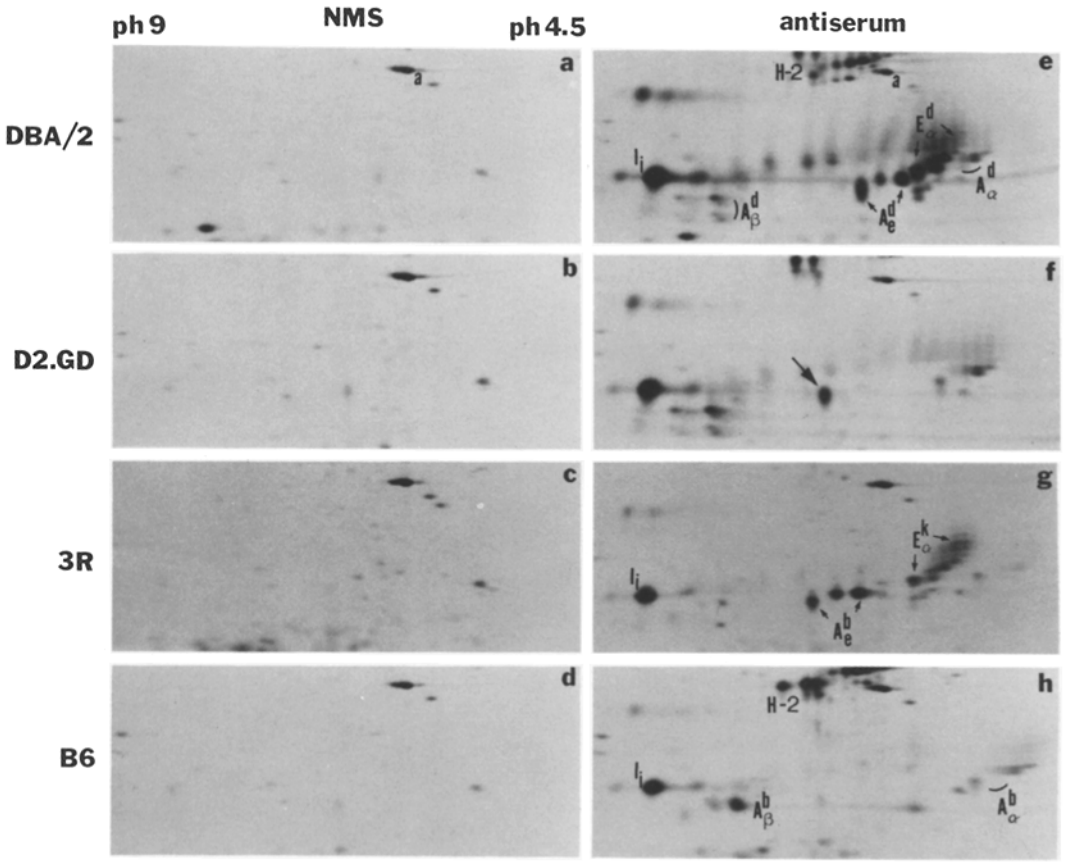

FIG. 1. Detection of an abnormal Ia polypeptide chain in recombinant strain D2.GD. The sera used for immunoprecipitation were: a–d, normal mouse serum; e and f, B10 anti-B10.D2 (anti-H-2^d); g, (B10 × HTI)F_1 anti-B10.A(5R) (anti-I-E^k); h, C3H anti-C3H.SW (anti-H-2^b). Immunoprecipitated proteins were separated on NEPHGE first dimension gels; the pH of the acidic and basic ends are shown above panel a. The portion of the gel shown includes proteins with molecular weights ranging from 25,000 to 45,000 dalton. The spots corresponding to actin (marked by the letter a), H-2 antigens, and the Ia polypeptide chains A_α, A_β, E_α, A_e, and I_i, the invariant Iaassociated chain (8), are indicated in panels e, g, and h. In panel f the arrow marks the unusual Ia polypeptide chain. NMS, normal mouse serum.

corresponding to both cytoplasmic and cell surface forms of A_e^{g2} that are not found in B10.D2 immunoprecipitates. For the (B10.GD × A.TFR5)F_1 immunoprecipitate, the only A_e spots should be from the A_e^{g2} chain because A.TFR5 has $I\text{-}A^f$, which does not code for a detectable A_e polypeptide chain (9).[1] Anti-I-E antibodies precipitate E_α^k chains from A.TFR5 with no A_e chains (9).

The autoradiograms shown in Fig. 3 confirm the presence of both cytoplasmic and cell surface forms of A_e^{g2} chains in immunoprecipitates from both heterozygotes. In the (B10.GD × B10.D2)F_1 gel, several spots can be seen that are absent from the B10.D2 gel. These same spots appear to be the only A_e molecules in the (B10.GD × A.TFR5)F_1 gel in panel h. The most basic of the A_e^{g2} spots in panels g and h corresponds to the single A_e spot found in both the B10.GD (panel f) and D2.GD (Fig. 1 f) gels. This spot represents a cytoplasmic form of the A_e chain (2). By analogy with other haplotypes, the more acidic A_e^{g2} spots visible in the gels from the

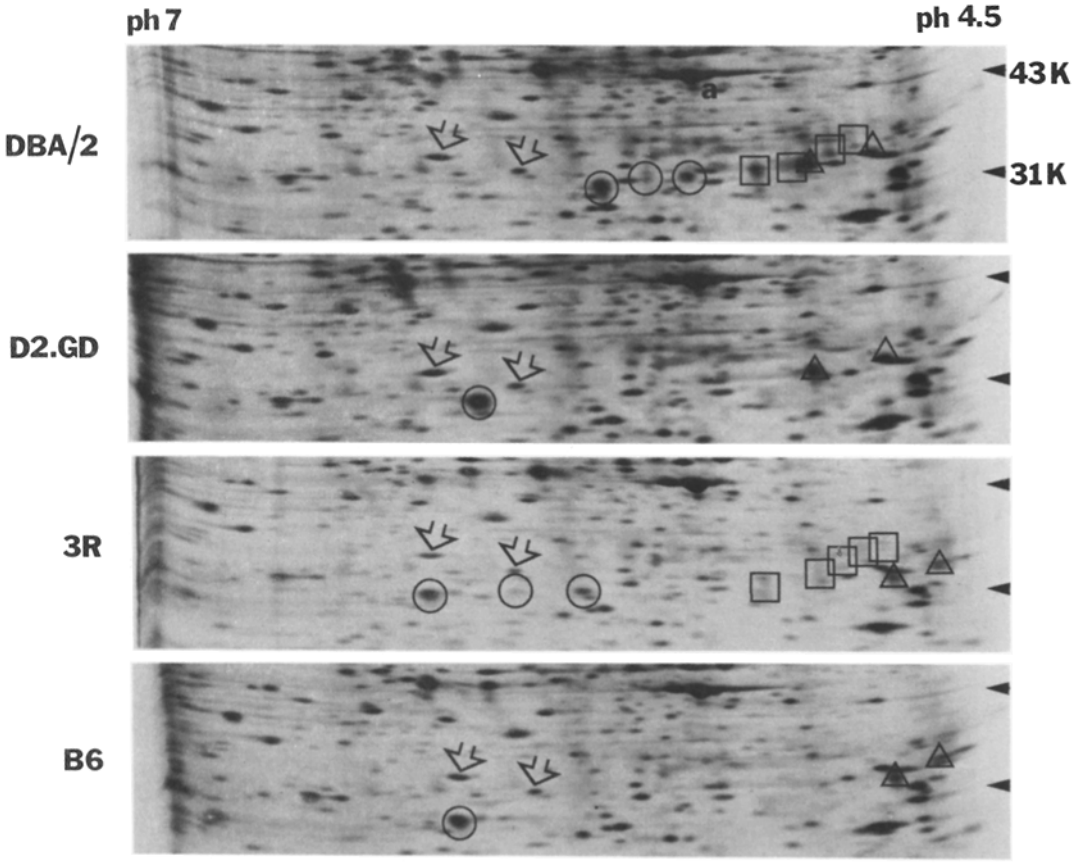

FIG. 2. Ia antigens visualized in gels of total NP-40 extractable proteins. Samples containing 500,000 cpm were electrophoresed using IEF first-dimension gels; the pH range of the gradient is shown. The positions of 43,000 and 31,000 dalton markers are shown on the right. Spots corresponding to Ia polypeptide chains are enclosed by symbols: A_α, △; E_α, □; and A_e, ○. The open arrows mark two spots that are useful as references for comparing the positions of the A_e chains.

heterozygotes represent cell surface forms of this chain, indicating the association between A_e^{g2} and E_α chains (2). The existence of E_α:A_e^{g2} complexes was also shown by the ability of anti-I-E antisera, which do not precipitate A_e^{g2} molecules from B10.GD, to precipitate both A_e^{g2} and E_α chains from the heterozygotes (data not shown).

It is evident from the data presented that in both B10.GD and D2.GD the A_e^{g2} molecules have an unusual electrophoretic mobility, distinct from the mobilities of both A_e^d and A_e^b. The simplest explanation for this phenomenon is that the recombination event creating the *g2* haplotype occurred within the structural gene for the A_e chain, producing a hybrid protein. A mutation in the A_e gene also could have altered the molecular properties of the chain. However, because the DBA/2 and B6 lines from which the *g2* recombinant was derived have normal A_e^d and A_e^b chains, respectively, the mutation would have had to occur subsequent to the recombinational event and to have been established in the homozygous state in strain D2.GD, which was derived by backcrossing $H\text{-}2^{g2}$ to DBA/2.

Another explanation for the altered mobility of the A_e^{g2} chain is that it is a consequence of abnormal glycosylation of the A_e^d chain. This model would require

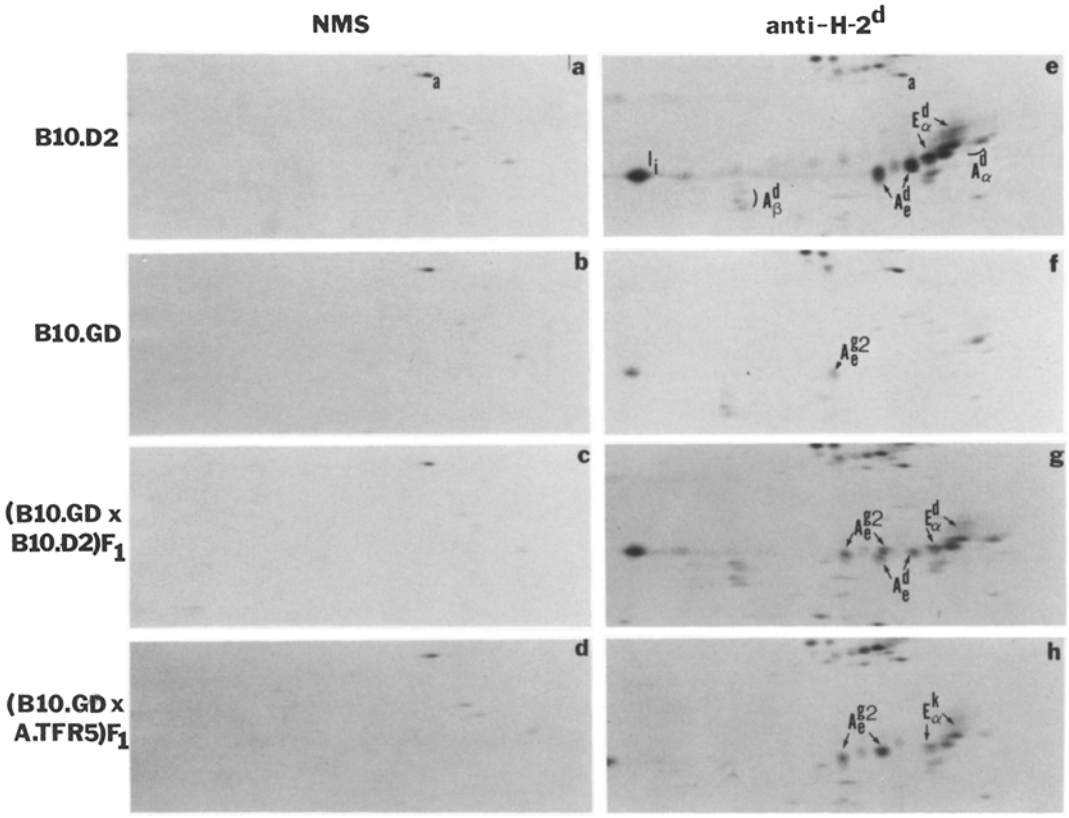

FIG. 3. Association of A_e^{g2} and E_α polypeptide chains in heterozygous mice. The sera used for immunoprecipitation were: a–d, normal mouse serum, e–h, BALB/b anti-BALB/c (anti-H-2^d). The positions of A_e and E_α chains are indicated in panels e–h; the position of A_α^d A_β^d, and I_i chains are indicated only in panel e. NMS, normal mouse serum.

that the new glycosylation pathway is controlled by $H\text{-}2^{g2}$, because the alteration is evident on both the DBA/2 and B10 backgrounds, and because both normal A_e^d and abnormal A_e^{g2} chains are present in (B10.GD × B10.D2)F_1 heterozygotes. Presumably, then, a *b* haplotype-derived locus mapping to the right of the three *I-A* loci encoding Ia polypeptide chains somehow would have to affect glycosyl transferase activity. Because combining the *b* and *d* haplotypes in the *trans* chromosomal position in (*b* × *d*)F_1 mice does not alter the mobility of A_e^b or A_e^d chains (P. P. Jones. Unpublished observations.), the *b* haplotype-derived locus would have to affect the glycosylation of A_e^d in a *cis*-acting manner only, which is very unlikely. Therefore, it seems most reasonable to conclude that the A_e^{g2} chain resulted from either a mutation or an intragenic recombination within the structural gene for this chain. Peptide mapping studies to distinguish between these possibilities are currently under way.

The aberrant A_e chain in $H\text{-}2^{g2}$ mice may help in efforts to elucidate the molecular mechanisms of *I*-region-controlled functions. Recent studies have provided considerable evidence for a role for the E_α:A_e Ia complex in both *Ir* gene control and genetic restrictions in immune responses (10, 11). The *I-A* subregion of the *g2* haplotype may well determine response patterns different from other strains with the *I-A* subregion

of the *d* haplotype; such altered responses may help to localize responsibility for specific immune processes to the A_e polypeptide chain.

Summary

Mice of strains D2.GD and B10.GD, which carry the recombinant haplotype $H\text{-}2^{g2}$ (previously typed as $H\text{-}2K^d\ I\text{-}A^d \mid I\text{-}B^b\ I\text{-}J^b\ I\text{-}E^b\ I\text{-}C^b\ S^b\ H\text{-}2D^b$), have an A_e (E_β) polypeptide chain electrophoretically distinct from the A_e chains of both *b* and *d* haplotype mice, including the progenitor strains from which the recombinant $H\text{-}2^{g2}$ chromosome was derived. The evidence presented suggests that the altered molecular properties of the A_e^{g2} chain may be a consequence of an intragenic recombination event in the *I-A* subregion within the structural gene for this polypeptide chain. Because the A_α and A_β chains controlled by $H\text{-}2^{g2}$ appear to be *d* haplotype in origin, this finding would map the gene for A_e to the right of the loci controlling the A_α and A_β chains.

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