ABERRANT $\alpha_e$ (E$\alpha$) Ia POLYPEPTIDE CHAIN IN $H-2^e$ 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 ($A_\omega$) and small ($A_\alpha$) 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, $E_\omega$, 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 $A_\alpha$ (or $E_\alpha$), on the cell surface is dependent on the presence of $E_\omega$ chains in the same cell; synthesis of both $A_\omega$ and $E_\omega$ 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 $A_\omega$, $A_\alpha$, and $A_\epsilon$ polypeptide chains. Recently we observed an abnormal $A_\epsilon$ chain in mice of the recombinant haplotype $H-2^e$ that may help to map the locus coding for this chain. The $H-2^e$ haplotype was derived from a cross between strains DBA/2 ($H-2^d$) and C57BL/6 ($B_6; H-2^b$) in the colony of Dr. Frank Lilly, Albert Einstein College of Medicine, Bronx, N. Y., giving rise to the apparent haplotype $H-2K^d I-A^d I-B^b I-J^b I-C^b S^b H-2D^b$ (5, 6). However, the results presented below show that the $A_\epsilon^e$ chain differs in electrophoretic mobility from both $A_\epsilon^d$ and $A_\epsilon^b$ chains, raising the possibility that the recombination event occurred within the gene coding for the $A_\epsilon$ 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)F1 and (B10.GD × A.TFR5)F1 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 × HT1)F1 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 $[^3S]$-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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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\textsubscript{w} chain in the \textit{H-2}\textsuperscript{g2} haplotype came from initial studies of Ia antigens from strain D2.GD, a DBA/2 congenic carrying the \textit{H-2}\textsuperscript{g2} haplotype. The A\textsubscript{w} chain differed in electrophoretic mobility from A\textsubscript{w} chains of both \textit{d} and \textit{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\textsubscript{w} 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) (\textit{I-A}\textsuperscript{b}, \textit{I-E}\textsuperscript{b}). The D2.GD pattern has A\textsubscript{w} and A\textsubscript{b} spots similar to those of DBA/2 but clearly different from B6, confirming the \textit{d}-haplotype origin of these chains. No E\textsubscript{w} chain is apparent in either the B6 or the D2.GD gels as a result of the apparent failure of the \textit{I-E}\textsuperscript{b} subregion to code for a synthesized E\textsubscript{w} chain (1). The A\textsubscript{w} chain from B6 is absent from the immunoprecipitate; antisera directed against the \textit{I}-regions of the \textit{h}, \textit{k} and \textit{s} haplotypes all fail to immunoprecipitate A\textsubscript{w} chains from strains in which the \textit{I-E} subregion does not code for a detectable E\textsubscript{w} chain (i.e., \textit{I-E}\textsuperscript{b} and \textit{I-E}\textsuperscript{b}). However, a spot in the region of the gel appropriate for A\textsubscript{w} chains is present in the D2.GD pattern. Evidence presented below will confirm that this spot corresponds to the A\textsubscript{w} chain. The anti-H\textsuperscript{2d} 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\textsubscript{w} from DBA/2 and A\textsubscript{b} precipitated by anti-I-E\textsuperscript{b} antibodies from B10.A(3R), thus confirming the earlier observations.

A second approach for detecting mobility differences between A\textsubscript{w} molecules of different haplotypes is to examine the positions of A\textsubscript{w} 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) (\textit{I-A}\textsuperscript{b}, \textit{I-E}\textsuperscript{b}) mice do in fact synthesize A\textsubscript{w} chains, although they do not express them on the cell surface as a result of an absence of E\textsubscript{w} chains, and that A\textsubscript{w} \textsuperscript{b} and A\textsubscript{w} \textsuperscript{k} chains have distinct electrophoretic mobilities (2). Few non-H-2 encoded spots differ between gels of 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\textsubscript{w} in the D2.GD gel clearly differs in position from both A\textsubscript{w} \textsuperscript{d} from DBA/2 and A\textsubscript{w} \textsuperscript{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\textsubscript{w} chain, experiments were done to determine whether or not this chain is capable of associating with E\textsubscript{w} chains which are absent in cells of the g2 haplotype. The association with E\textsubscript{w} chains allows A\textsubscript{w} molecules to become more highly glycosylated and expressed on the cell surface, and also to be precipitated by anti-I-E antibodies (2).\textsuperscript{1} B10.GD mice, carrying H\textsuperscript{2d} on the B10 background, were crossed with B10.D2 (H\textsuperscript{2d}) and A.TFR5 (H\textsuperscript{2}\textsuperscript{np5}: H\textsuperscript{2K} \textit{I-A}\textsuperscript{f} \textit{I-B}\textsuperscript{f} \textit{I-E}\textsuperscript{f} \textit{I-C}\textsuperscript{f} \textit{S}\textsuperscript{f} H-2D\textsuperscript{d}) (9). If the A\textsubscript{w} \textsuperscript{g} chain associates with the E\textsubscript{w} \textsuperscript{d} chain in the (B10.GD × B10.D2)F\textsubscript{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'); g, (B10 × HTT)F1 anti-B10.A(5R) (anti-I-E'); h, C3H anti-C3H.SW (anti-H-2'). 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 Aα, Aβ, Eα, Eα, and Iλ, the invariant Ia-associated chain (β), 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 Aα, that are not found in B10.D2 immunoprecipitates. For the (B10.GD × A.TFR5)F1 immunoprecipitate, the only Aα spots should be from the Aα chain because A.TFR5 has I-AF, which does not code for a detectable Aα polypeptide chain (9). Anti-I-E antibodies precipitate Eα chains from A.TFR5 with no Aα chains (9).

The autoradiograms shown in Fig. 3 confirm the presence of both cytoplasmic and cell surface forms of Aα chains in immunoprecipitates from both heterozygotes. In the (B10.GD × B10.D2)F1 gel, several spots can be seen that are absent from the B10.D2 gel. These same spots appear to be the only Aα molecules in the (B10.GD × A.TFR5)F1 gel in panel h. The most basic of the Aα spots in panels g and h corresponds to the single Aα spot found in both the B10.GD (panel f) and D2.GD (Fig. 1 f) gels. This spot represents a cytoplasmic form of the Aα chain (2). By analogy with other haplotypes, the more acidic Aα spots visible in the gels from the
heterozygotes represent cell surface forms of this chain, indicating the association between $\text{A}_e^{g2}$ and $\text{E}_\alpha$ chains (2). The existence of $\text{E}_\alpha: \text{A}_e^{g2}$ complexes was also shown by the ability of anti-I-E antisera, which do not precipitate $\text{A}_\epsilon^{g2}$ molecules from B10.GD, to precipitate both $\text{A}_\epsilon^{g2}$ and $\text{E}_\alpha$ chains from the heterozygotes (data not shown).

It is evident from the data presented that in both B10.GD and D2.GD the $\text{A}_\epsilon^{g2}$ molecules have an unusual electrophoretic mobility, distinct from the mobilities of both $\text{A}_\epsilon^d$ and $\text{A}_\epsilon^b$. The simplest explanation for this phenomenon is that the recombination event creating the $g2$ haplotype occurred within the structural gene for the $\text{A}_\epsilon$ chain, producing a hybrid protein. A mutation in the $\text{A}_\epsilon$ 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 $\text{A}_\epsilon^d$ and $\text{A}_\epsilon^b$ 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 $H^{-2g2}$ to DBA/2.

Another explanation for the altered mobility of the $\text{A}_\epsilon^{g2}$ chain is that it is a consequence of abnormal glycosylation of the $\text{A}_\epsilon^d$ chain. This model would require...
that the new glycosylation pathway is controlled by $H-2^{g2}$, because the alteration is evident on both the DBA/2 and B10 backgrounds, and because both normal $\text{A}^b_e$ and abnormal $\text{A}^d_e$ chains are present in (B10.GD × B10.D2)F1 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 ($b$ × $d$)F1 mice does not alter the mobility of $\text{A}^b_e$ or $\text{A}^d_e$ chains (P. P. Jones. Unpublished observations.), the $b$ haplotype-derived locus would have to affect the glycosylation of $\text{A}^d_e$ in a cis-acting manner only, which is very unlikely. Therefore, it seems most reasonable to conclude that the $\text{A}^d_e$ 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 $\text{A}^e$ chain in $H-2^{g2}$ 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 $E\alpha:A\alpha$ 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 Ae polypeptide chain.

Summary

Mice of strains D2.GD and B10.GD, which carry the recombinant haplotype \(H-2^{e^2}\) (previously typed as \(H-2^{Kd} I-A^d I-B^d I-J^b I-E^b I-C^e S^b H-2^D^d\)), have an \(A_e\) (\(E_d\)) polypeptide chain electrophoretically distinct from the \(A_e\) chains of both \(b\) and \(d\) haplotype mice, including the progenitor strains from which the recombinant \(H-2^{e^2}\) chromosome was derived. The evidence presented suggests that the altered molecular properties of the \(A_e^{e^2}\) 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 \(A_e\) and \(A_d\) chains controlled by \(H-2^{e^2}\) appear to be \(d\) haplotype in origin, this finding would map the gene for \(A_e\) to the right of the loci controlling the \(A_e\) and \(A_d\) chains.

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References

1. Cook, R. G., J. D. Capra, J. L. Bednarczyk, J. W. Uhr, and E. S. Vitetta. 1979. Structural studies on the murine Ia alloantigens. VI. Evidence that both subunits of the I-A alloantigen are encoded by the I-A subregion. J. Immunol. 123:2799.
2. Jones, P. P., D. B. Murphy, and H. O. McDevitt. 1978. Two-gene control of the expression of a murine Ia antigen. J. Exp. Med. 148:925.
3. Cook, R. G., E. S. Vitetta, J. W. Uhr, and J. D. Capra. 1979. 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. J. Exp. Med. 149:981.
4. Silver, J., and W. A. Russell. 1979. Structural polymorphism of I-E subregion antigens determined by a gene in the H-2K to I-B genetic interval. Nature (Lond.). 279:437.
5. Lilly, F., and J. Klein. 1973. An \(H-2^{e^2}\)-like recombinant in the mouse. Transplantation (Baltimore). 16:530.
6. Klein, J., L. Flaherty, J. L. VandeBerg, and D. C. Shreffler. 1978. \(H-2\) haplotypes, genes, regions, and antigens: first listing. Immunogenetics. 6:489.
7. Jones, P. P. 1980. Analysis of radiolabeled lymphocyte proteins by one and two-dimensional polyacrylamide gel electrophoresis. In Selected Methods in Cellular Immunology. B. Mishell and S. Shiigi, editors. W. H. Freeman & Company Publishers. San Francisco. 398.
8. Jones, P. P., D. B. Murphy, D. Hewgill, and H. O. McDevitt. 1979. Detection of a common polypeptide chain in I-A and I-E subregion immunoprecipitates. Mol. Immunol. 16:51.
9. Murphy, D. B., P. P. Jones, M. R. Loken, and H. O. McDevitt. Interaction between I-region loci influences the expression of a cell surface Ia antigen. Proc. Natl. Acad. Sci. U. S. A. In press.
10. Lerner, E. A., L. A. Matis, C. A. Janeway, Jr., P. P. Jones, R. H. Schwartz, and D. B. Murphy. 1980. Monoclonal antibody against an Ir gene product? J. Exp. Med. 152:1085.
11. Schwartz, R. H., C. Chen, and W. E. Paul. Gene complementation in the T-lymphocyte proliferative response to poly (Glu-Lys-Phe)\(_n\). Functional evidence for a restriction element coded for by both the I-A and I-E subregions. Eur. J. Immunol. In press.