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Chemical characterization of murine Ia alloantigens determined by the *I-E/I-C* subregions of the *H-2* complex

(immunoprecipitation/two polypeptides/NH_2-terminal sequencing/sequence homology)

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ABSTRACT **Alloantisera directed against the alloantigens determined by the *I-E* and *I-C* subregions of the murine major histocompatibility complex precipitate two components that have molecular weights of 35,000 and 29,000. These components, when analyzed by partial NH_2-terminal sequencing, show no homology to two components of similar size determined by the *I-A* subregion. However, the large chain determined by the murine *I-E* and/or *I-C* subregion is homologous to the large chain of the human *HLA-D* region alloantigen, although the small chains isolated from these two species do not display any such homology.**

The *I* region of the murine major histocompatibility complex (MHC) contains genes that determine a number of immunological functions, including immune responsiveness (1), cell cooperation between T and B cells (2), and cell cooperation between T cells and macrophages (3). In addition, the *I* region determines the expression of cell membrane Ia alloantigens (4) found on all three cell types (4–6). Treatment of various lymphoid cell populations with antiserum against Ia results in alteration of the ability to respond in a variety of *in vitro* systems, suggesting a crucial role for Ia antigens in immune responses (7, 8). Indeed, soluble molecules expressing Ia alloantigenic determinants are involved in a number of cell interaction phenomena that specifically enhance or suppress the immune response (9–12). In addition, subpopulations of lymphocytes performing different functions (e.g., memory B cells, suppressor T cells) preferentially express different Ia alloantigens (13–15), suggesting that such molecules are involved in mediating these functions.

By mapping *Ir* genes and by serologically analyzing Ia alloantigens in various intra-MHC recombinant strains, the *I* region has been subdivided into several subregions, *I-A*, *I-B*, *I-J*, *I-E*, and *I-C* (4, 16). Antigenic specificities determined by different subregions redistribute independently in the membrane (17) and are on separate molecules when isolated by indirect immunoprecipitation (18). Thus, apparently each subregion determines one or more distinct cell surface molecules. Whether Ia alloantigens from the different cell types—B cells, T cells, or macrophages—differ structurally is unknown. However, because of the crucial role that Ia alloantigenic molecules play in regulating the immune response, we are investigating their chemical structure with the intention of elucidating their mechanism of action as well as examining their structural relationships to other cell surface molecules that mediate immune function (i.e., H-2K and H-2D transplantation antigens and immunoglobulins).

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MATERIALS AND METHODS

The method used for the isolation, purification, and sequence analysis of Ia alloantigens from spleen cells is similar to that previously used for the *H-2K* and *H-2D* gene products (19). However, modifications involved the use of spleen cells that were taken from B10.A(5R) mice and biosynthetically labeled simultaneously with six tritiated amino acids (alanine, valine, tyrosine, leucine, proline, and phenylalanine). Ia alloantigens were solubilized with Triton X-100, immunoprecipitated, and purified by sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel electrophoresis. Amino-terminal sequence analysis was performed on a Beckman model 890C automated sequencer. Phenylthiohydantoin (PTH) amino acid derivatives were resolved by high-pressure liquid chromatography.

Antiserum (B10 × HTI)F_1 against B10.A(5R) was prepared by hyperimmunization with a mixture of spleen, lymph node, and thymus cells as described (20). This serum, when tested by direct cytotoxicity and absorption analysis on standard recombinant strains, shows two specificities, Ia.7 and Ia.22, located in the *I-C* and *I-E* subregions, respectively (20). Ia.7 is shared by the $H\text{-}2^k$ and $H\text{-}2^d$ haplotypes, while Ia.22 is associated with the $H\text{-}2^k$ haplotype and absent from the $H\text{-}2^d$. Thus, absorption with $H\text{-}2^k$, $H\text{-}2^a$, and $H\text{-}2^{t5}$ cells completely clears the reactivity in this serum, while absorption with $H\text{-}2^d$ cells leaves residual cytotoxic activity for $H\text{-}2^k$, $H\text{-}2^a$, and $H\text{-}2^{t5}$ (16).

RESULTS

The isolation, purification, and microsequencing methods used herein have been used previously to characterize the cell surface products of the MHC (21). In addition, the close agreement between amino acid sequences obtained by this method and those obtained by more conventional sequencing methods validates its reliability (22).

The antiserum used here is putatively directed against the cell surface products of the *I-J*, *I-E*, *I-C*, and *S* subregions of the *H-2* complex by virtue of the fact that the donor [B10.A(5R)] and recipient (B10 × HTI) strains differ solely at these defined regions. However, since the I-J alloantigen is present on only a small subpopulation (less than 10%) of T cells and *S* codes for no detectable cell surface antigens, this antiserum when used with unpurified spleen cells is directed primarily against the *I-E* and *I-C* subregions of B cells. The profile of cell surface proteins immunoprecipitated by this antiserum and subjected to $NaDodSO_4$/polyacrylamide gel electrophoresis under reducing conditions reveals two partially overlapping

Abbreviations: PTH, phenylthiohydantoin; $NaDodSO_4$, sodium dodecyl sulfate; MHC, major histocompatibility complex.

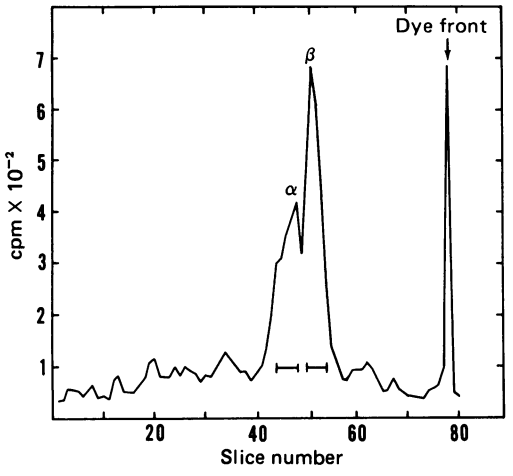

FIG. 1. NaDodSO$_4$/polyacrylamide gel electrophoresis of cell surface proteins immunoprecipitated by antiserum (B10 X HTI)F$_1$ against B10.A(5R). α, Molecular weight 35,000; β, molecular weight 29,000.

peaks with approximate molecular weights of 35,000 (α) and 29,000 (β) (Fig. 1). The two chains are not covalently linked by disulfide bonds, since they migrate in comparable positions in the absence of a reducing agent. To prevent crosscontamination, protein comprising the ascending edge of the α peak and the descending edge of the β peak was used for sequence analysis. The sequence data for the α and β chains, corrected for losses due to manipulations, are shown in Figs. 2 and 3, respectively. Calculated on the basis of net cpm obtained for the phenylalanine residues at positions 12, 24, and 26, a repetitive yield of 94% was obtained for the α chain. Similarly, a repetitive yield of 93% was obtained for the β component. Both values are consistent with values obtained by conventional sequence analysis. In both instances a major sequence may be deduced (Fig. 4).

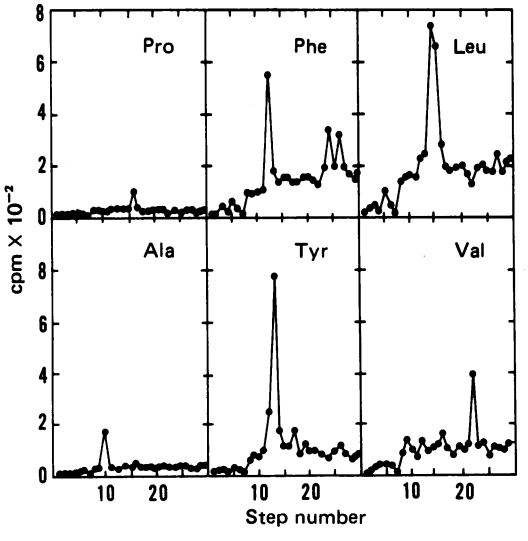

FIG. 2. Sequence data for the α chain. Sequence residues were combined with a mixture of phenylthiohydantoin (PTH) derivatives of proline, phenylalanine, leucine, alanine, tyrosine, and valine. Each of the PTH-amino acids was isolated by high-pressure liquid chromatography and collected individually in a scintillation vial; radioactivity was measured. Background (30 cpm) was subtracted from each sample and losses due to manipulations were corrected against an internal standard of PTH-norleucine.

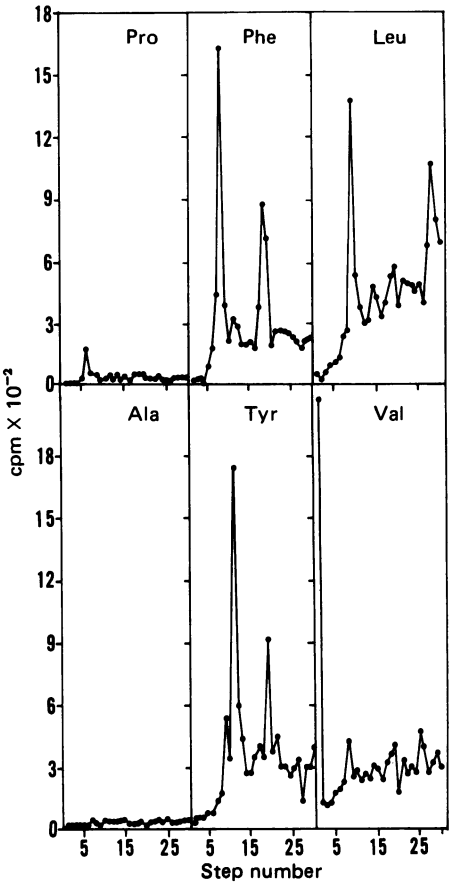

FIG. 3. Sequence data for the β chain. Losses due to manipulations were corrected as in Fig. 2.

DISCUSSION

Antisera directed against the *I-E* and *I-C* subregion alloantigens precipitate two components of different molecular weights. We can provide three possible explanations for this observation. (*i*) The expression of one of these components is controlled by the *I-E* subregion, while the expression of the other component is controlled by the *I-C* subregion. Therefore, the antiserum used in these studies would contain two populations of antibodies, one directed against the *I-E* subregion product and the other directed against the *I-C* subregion product. (*ii*) The *I-E* and *I-C* subregions each controls the expression of two chains, Eα and Eβ and Cα and Cβ, in which the α as well as the β chains isolated from both regions have similar molecular weights. Since a relatively homogeneous sequence is obtained for both the α and β chains, this explanation would require that the respective *I-E* and *I-C* subregion chains are mutually homologous. Either these α and β chains might be noncovalently associated with one another, as is the case for the K(D) products and β_2-microglobulin of the MHC (24), or alternatively the α and β chains might each be antigenic and each be immunoprecipitated by different antibodies. In the latter case, the antiserum would contain four populations of antibodies, one directed against each of the two chains of both the *I-E* and *I-C* subregions. (*iii*) The antiserum and methods used isolated only products from one of the two subregions, either *I-E* or *I-C*. This subregion, in turn, controls the expression of the two polypeptide chains which may or may not be associated with one another.

Recent observations indicate that the *HLA-D* region of the human MHC controls the expression of two components of different molecular weights, one of 34,000 and the other of

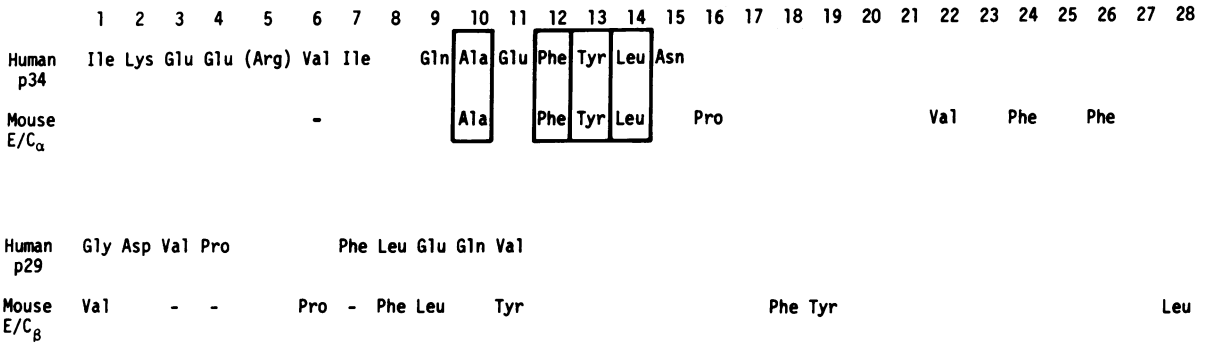

FIG. 4. Partial amino-terminal sequences of human *HLA-D* region (23) and murine *I-E/I-C* subregion alloantigens. The proline residue at position 16 in the α chain was confirmed in a subsequent experiment.

28,000, which are noncovalently associated (23, 25, 26). The role of the *D* region in inducing mixed lymphocyte reactions and its genetic linkage to the MHC (27) suggest that the human *HLA-D* region and the murine *I* region may be analogous. In addition, the tissue distribution of the *HLA-D* region cell-surface products (28) as well as the structure of molecules immunoprecipitated by antisera against *HLA-D* region (23) are very similar to those of the murine Ia alloantigens. Therefore, it is not surprising that the NH_2-terminal amino acid sequence of the α chains isolated from the *I-E* and/or *I-C* subregion of the mouse is strikingly homologous to that of the larger polypeptide (p34) of the human *HLA-D* region alloantigens (29) (Fig. 4). These observations confirm and extend previous findings obtained in analysis of the $E\text{-}C^k_\alpha$ component (30). Of the five residues that can be compared directly on chains isolated from the two species, four are identical. This degree of sequence identity (80%) is comparable to that found between the major transplantation antigens of mice and humans (53%) (21).

Surprisingly, however, the β chain does not display any degree of homology with the smaller polypeptide (p29) of the human *HLA-D* region alloantigen (Fig. 4). At least four explanations seem possible for this observation. (*i*) The *HLA-D* region of the human MHC codes for multiple components, as do the *I-E* and *I-C* subregions of the murine MHC. During the process of isolation only a subpopulation of all the molecules controlled by these respective regions were isolated and fortuitously only one of the two molecules isolated from each species was identical. Thus, we would ultimately expect to isolate the "missing" small chain from each of the other species. (*ii*) The two polypeptide chains are derived by a specific proteolytic cleavage from a larger precursor molecule. This appears to be the case for the three chains of the C4 component of complement, which is linked to the MHC (31, 32); the chains are derived from proteolytic cleavages of a large precursor chain (33). Therefore, it is possible that the precursor molecule of the human *HLA-D* region alloantigens and the murine *I-E* and/or *I-C* alloantigens are proteolytically cleaved at slightly different points, resulting in the larger chains displaying homology with each other and an apparent lack of homology in the smaller chains. However, sequence analysis of internal portions of the smaller chains of the human and mouse alloantigens would be expected to reveal sequence homologies. (*iii*) The larger chain of the human *HLA-D* region alloantigen and murine *I-E* and/or *I-C* alloantigen is determined by the MHC, while the smaller chain represents one of a group of molecules that may be noncovalently associated with the larger chain and is determined by genes not linked to the MHC. The apparent noncovalent association of both chains of the human *HLA-D* region alloantigens and the lack of any linkage between the gene controlling the expression of the smaller component and the MHC (34) support the third interpretation and is analogous to the noncovalent association of the murine K(D) and human A(B) products of the MHC with β_2-microglobulin (21), an immunoglobulin-like molecule whose determining gene is not linked to the MHC (35). One can test this interpretation by isolating $I\text{-}E^k$ and/or $I\text{-}C^d$ subregion alloantigens from mice of different genetic backgrounds and analyzing their NH_2-terminal sequences. (*iv*) The *I-E* and *I-C* subregions each controls the expression of a two-chain component which consists of the same α chain but a different β chain. However, the sequence of only one of the β chains was determined because either the antiserum used binds only one of the subregion products, or one of the two β chains has a slightly higher molecular weight and therefore migrates between the α and β chain regions on $NaDodSO_4$ gels and that portion was not used for sequence analysis. In either case, further analysis may uncover a molecule homologous to the small chain of human *HLA-D* region alloantigen. Moreover, additional analysis of human MHC-linked alloantigens may lead to the discovery of a human analogue of the murine *I-E* and/or *I-C* subregion β chain.

It was previously reported that the Ia alloantigens of the *I-A* subregion of the murine MHC consist of two polypeptide chains, α and β, that have respective molecular weights of 37,000 and 28,000 (36). These two chains are not linked by disulfide bonds although noncovalent association has not been excluded. The similarity in the number and size of the subunits between the *I-A* subregion alloantigens and the *I-E* and/or *I-C* subregion alloantigens as well as the reported serological crossreactivities between these two sets of Ia alloantigens (37) suggest some sequence homology between them. However, no NH_2-terminal sequence homology is apparent, although homology at internal portions of these molecules may exist. Thus, there appear to be two sets of Ia alloantigens with dramatically different sequences but similar subunit sizes and structures. Because the human and mouse MHC appear to be so strikingly similar not only in their genetic organization, but also in the biochemical structure of their gene products, it is likely that the human equivalent of the murine *I-A* subregion Ia alloantigen exists. These genetic products may be determined by genes proximal to the *A* locus of the human MHC since this region determines B cell alloantigens (38) and also contains alloantigens that induce the mixed lympocyte reaction (39), a property common to *I* region determinants. Further immunochemical studies of other cell surface products of various regions of the human MHC complex may lead to its identification. Thus, the murine MHC exhibits the fascinating property of controlling the expression of several families of cell surface molecules, the *K(D)* region products, the *I-A* subregion products, and the *I-E* and/or *I-C* subregion products, which are

composed of subunits and which on the surface appear to be evolutionarily and structurally unrelated. Additional structural work may reveal the true nature of similarities and/or differences among these cell surface proteins.

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1. Benacerraf, B. & McDevitt, H. O. (1972) *Science* **175,** 273–279.
2. Katz, D. H., Graves, M., Dorf, M. E., Dimuzio, H. & Benacerraf, B. (1975) *J. Exp. Med.* **141,** 263–268.
3. Erb, P. & Feldman, M. (1975) *J. Exp. Med.* **142,** 460–472.
4. Shreffler, D. C. & David, C. S. (1975) *Adv. Immunol.* **20,** 125–195.
5. Frelinger, J. A., Niederhuber, J. E., David, C. S. & Shreffler, D. C. (1974) *J. Exp. Med.* **140,** 1273–1284.
6. Hammerling, G. J., Deak, B. D., Mauve, G., Hammerling, U. & McDevitt, H. O. (1974) *Immunogenetics* **1,** 68–81.
7. Frelinger, J. A., Niederhuber, J. E. & Shreffler, D. C. (1975) *Science* **188,** 268–270.
8. Schwartz, R. H., David, C. S., Sachs, D. H. & Paul, W. E. (1976) *J. Immunol.* **117,** 531–540.
9. Munro, A. J. & Taussig, M. J. (1975) *Nature* **256,** 103–106.
10. Tada, T., Taniguchi, M. & David, C. S. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41,** 119–127.
11. Erb, P., Feldmann, M. & Hogg, N. (1976) *Eur. J. Immunol.* **6,** 365–372.
12. Armerding, D., Sachs, D. H. & Katz, D. H. (1974) *J. Exp. Med.* **140,** 1717–1722.
13. McDevitt, H. O., Delovitch, T. L., Press, J. L. & Murphy, D. B. (1976) *Transplant. Rev.* **30,** 197–235.
14. Murphy, D., Herzenberg, L. A., Okumura, K., Herzenberg, L. A. & McDevitt, H. O. (1976) *J. Exp. Med.* **144,** 699–712.
15. Niederhuber, J. E., Frelinger, J. A., Dine, M. S., Shoffner, P., Dugan, E. & Shreffler, D. C. (1976) *J. Exp. Med.* **143,** 372–381.
16. Shreffler, D. C. David, C. S., Cullen, S. E., Frelinger, J. A. & Niederhuber, J. E. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41,** 477–487.
17. Klein, J. & Hauptfeld, V. (1976) *Transplant. Rev.* **30,** 83–100.
18. Cullen, S. E., Freed, J. H. & Nathenson, S. G. (1976) *Transplant. Rev.* **30,** 236–270.
19. Silver, J. & Hood, L. E. (1976) in *Contemporary Topics in Molecular Immunology,* ed. Reisfeld, R. A. (Plenum Press, New York), pp. 35–68.
20. David, C. S., Cullen, S. E. & Murphy, D. B. (1975) *J. Immunol.* **114,** 1205–1209.
21. Silver, J., Cecka, J. M., McMillan, M. & Hood, L. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41,** 369–377.
22. Silver, J. (1977) in *Methods in Enzymology,* ed. Hirs, C. H. W. (Academic Press, Inc., New York), Vol. 47, pp. 247–260.
23. Klareskog, L., Sandberg-Trägärdh, L., Rask, L., Lindblom, J. B., Curman, B. & Peterson, P. A. (1977) *Nature* **265,** 248–251.
24. Silver, J. & Hood, L. E. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 599–603.
25. Humphreys, R. E., McCune, J. M., Chess, L., Herrman, H. C., Malenka, D. J., Mann, D. L., Parham, P., Schlossman, S. F. & Strominger, J. L. (1976) *J. Exp. Med.* **144,** 98–112.
26. Snary, D., Barnstable, C., Bodmer, W. F., Goodfellow, P. & Crumpton, M. J. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41,** 379–386.
27. Yunis, E. J. & Amos, D. B. (1971) *Proc. Natl. Acad. Sci. USA* **68,** 3031–3035.
28. Thorsby, E., Albrechtsen, D., Hirschberg, H., Kaakinen, A. & Solheim, B. G. (1977) *Transplant. Proc.* **9,** 393–400.
29. Springer, T. A., Kaufman, J. F., Siddoway, L. A., Giphart, M., Mann, D. L., Terhorst, C. & Strominger, J. L. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41,** 387–396.
30. Cecka, M., McMillan, M., Murphy, D., Silver, J., McDevitt, H. & Hood, L. (1977) *Proceedings of the Third Ir Gene Conference,* in press.
31. Meo, T., Krasteff, T. & Shreffler, D. C. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4536–4540.
32. Teisberg, P., Akesson, I., Olaisen, B., Gedde-Dahl, T, Jr. & Thorsby, E. (1976) *Nature* **264,** 253–254.
33. Hall, R. E. & Colten, H. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 1707–1710.
34. Barnstable, C. J., Jones, E. A., Bodmer, W. F., Bodmer, J. G., Arce-Gomez, B., Snary, D. & Crumpton, M. J. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41,** 443–455.
35. Goodfellow, P. N., Jones, E. A., Van Heyningen, V., Solomon, E., Bobrow, M., Miggiano, V. & Bodmer, W. F. (1975) *Nature* **254,** 267–269.
36. Silver, J. (1977) *Transplant. Proc.* **9,** 661–665.
37. Murphy, D. B. (1975) *Fed. Proc.* **34,** 1016.
38. Mann, D. L., Abeloon, L., Harris, S. & Amos, D. B. (1976) *Nature* **259,** 145–146.
39. Suciu-Foca, N. & Dausset, J. (1975) *Immunogenetics* **2,** 389–391.