AN HLA-DR5 HOMOZYGOUS CELL LINE EXPRESSES TWO DS (I-A-LIKE) MOLECULES*

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Human la antigens are important in the effective collaboration between immunocompetent cells in the generation of immune responses. The human la antigens are encoded within the HLA complex and are borne on molecules composed of two noncovalently associated glycoproteins of ~34,000 daltons (α chains) and ~28,000 daltons (β chains). Three types of human la molecules have been identified and biochemically characterized: DR, DS, and SB. DR molecules display N-terminal amino acid sequence homology to murine I-E molecules (1–3). Recent evidence indicates that DR homozygous cells express a single DR α chain which associates with either of two distinct DR β chains to form two dimeric DR molecules (3). SB molecules appear to be structurally different from the corresponding DR molecules, although SB α chains, like DR α chains, appear to be homologous to murine I-E α chains (4). DS (or DC) molecules display N-terminal amino acid sequence homology to murine I-A molecules (2, 5). Previous studies indicate that HLA-DR homozygous cells express a single DS molecule (2, 5). The data presented here indicate that an HLA-DR5 homozygous cell line expresses two structurally distinct DS (I-A-like) molecules.

Materials and Methods

Cells. The B lymphoblastoid cell line Swei, HLA-A29, A29; B40, B40; DR5, DR5; MB3; MT2, MT4, was obtained from Dr. John Hansen (Fred Hutchinson Cancer Research Center, Seattle, WA) (6).

Preparation of Radiolabeled Antigens. la antigens were radiolabeled with [35S]methionine (700–1,300 Ci/mM, Amersham Corp., Arlington Heights, IL) and [3H]leucine (130–190 Ci/mM, Amersham Corp.) for two-dimensional (2-D) gel studies or with [3H]leucine for peptide mapping and immunoprecipitated as previously described (7).

Antisera. MGH87B, an anti-MT2 alloserum, and MGH88B, an anti-MT4 serum, were kindly provided by Dr. Tom Fuller (Massachusetts General Hospital, Boston, MA) (7). Rb03 is a rabbit antiserum that detects human la molecules homologous to murine I-A molecules (2). Moreover, Rb03 isolated no DR molecules from multiple cell lines in previous 2-D gel studies (2, 8).

Two-dimensional (2-D) Gel Electrophoresis. 2-D gel electrophoresis was performed according to the method of O’Farrell (9) with modifications as previously described (7).

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Peptide Mapping by HPLC. α and β chains of Ia molecules were isolated by preparative SDS-11% PAGE and the [3H]leucine-labeled tryptic peptides were prepared and separated by high pressure liquid chromatography as previously described (10).

Results

An antigen preparation of the Swei cell line labeled with both [35S]methionine and [3H]leucine was immunoprecipitated directly with sera MGH87B, MGH88B, and Rb03; the precipitated material was analyzed by 2-D gels. The α and β chain portions of these gels and a schematic diagram of each α and β chain spot pattern are shown in Fig. 1. The β chain portions will be discussed first. The β chain pattern of MGH87B consists of a heterogeneous set of 10 spots. We have previously shown that this pattern is produced by at least three structurally distinct β chains referred to as β1, β2, and β3 (11). The β chain pattern of MGH88B (third panel) consists primarily of β1 as we have previously shown (11). In addition, the three spots of β2 are very faintly visible on this MGH88B gel.

![FIGURE 1. Fluorographs of 2-D gels of Ia molecules precipitated from a radiolabeled antigen preparation of the DR5 homozygous cell line, Swei, by the antisera listed at the left. The first three panels show material isolated by direct precipitation with MGH87B, Rb03, and MGH88B, respectively. The three lower panels show sequential immunoprecipitation experiments in which an aliquot of the radiolabeled antigen preparation was first depleted of MGH88B-reactive material and then divided into three equal aliquots before immunoprecipitation with Rb03 (88B/Rb03), MGH87B (88B/87B), and MGH88B (88B/88B), respectively. Schematic representatives of the spots in each α or β chain gel are shown to the right of each gel. In the β chain schematic, β1 is shown as stippled spots, β2 as open spots, β3 as closed spots, and β4 as hatched spots. The three dark spots with <25,000 mol wt at the acidic end of the β chain gels are invariant and are not related to the β chains. The acidic end of each α and β chain gel is the right. The portions of the α and β chain gels shown here correspond to pH 5.0–6.0 and pH 6.0–7.4, respectively.](image-url)
The β chain pattern of Rb03 (second panel) consists of a heterogeneous set of 11 spots. Four of these spots are identical to β₁ isolated by MGH87B and MGH88B, as shown here, and by other alloantisera (7, 11). However, the other seven spots do not correspond to any of the spots of β₂ or β₃ and, therefore, represent a fourth structurally distinct Ia β chain (β₄) on this DR5 homozygous cell line.

Sequential immunoprecipitation experiments were also performed on the same antigen preparation to more clearly define the Ia molecules expressed by this DR5 homozygous cell line. In these studies, an aliquot of the antigen preparation was depleted of MGH88B-reactive material and was then divided into three equal aliquots that were immunoprecipitated with MGH88B, Rb03, and MGH87B, respectively. Immunoprecipitation of the antigen preparation with MGH88B after initial removal of MGH88B-reactive material (88B/88B, bottom panel) isolates no Ia molecules. This result shows that all of the MGH88B-reactive material has been removed in the initial precipitation. Immunoprecipitation of the MGH88B-depleted antigen preparation with Rb03 (88B/Rb03, fourth panel) isolates a β chain pattern of seven spots which corresponds to β₄ isolated by Rb03 in the second panel. In comparison with the direct Rb03 precipitate in the second panel, pretreatment with MGH88B in the sequential has removed the α chain. This result shows that β₄ is a distinct entity that can be separated from β₁. Confirmation that β₁ and β₄ were distinct polypeptides was obtained by comparative tryptic peptide mapping (not shown). Immunoprecipitation of the MGH88B-depleted antigen preparation with MGH87B (88B/87B) isolates primarily the β₂ and β₃ chains without β₁ or β₄. Based on the previously described specificity of Rb03, these data strongly suggest that this cell line expresses two DS β chains (β₁ and β₄) and, therefore, two DS molecules.

Turning to the α chain portions of the gels (Fig. 1), the α chain pattern of MGH87B consists of a heterogeneous pattern of multiple spots. We have previously shown that this pattern consists of at least two structurally distinct Ia α chains, α₁ and α₂ (7). The MGH88B α chain pattern (third panel) consists of a complex, heterogeneous set of spots that correspond to α₂. The Rb03 α chain pattern is very similar to, although more intense than, the α₂ chain isolated by MGH88B.

The α chain pattern in the 88B/Rb03 sequential experiment is very similar qualitatively to the α chain patterns in the individual Rb03 and MGH88B gels. This is as expected if a single DS α chain can associate with either of the two DS β chains and if the determinant(s) recognized by MGH88B is located on the β₁ chain of the α₂β₁ molecule. Therefore, pretreatment with MGH88B in the sequential has removed the α₂β₁ molecule and precipitation of the depleted antigen preparation by Rb03 isolated the α₂β₄ molecule. The 88B/87B α chain pattern is very similar to the MGH87B α pattern. This is not unexpected because of the inclusion of the less intense α₂ spots within the area of the more intense α₁ spots. The 88B/88B panel documents complete removal of MGH88B-reactive material in the initial precipitation. Therefore, it appears that the Swei cell line expresses a single DS α chain (α₂) that can be found in association with either of the two DS β chains.

The 2-D gel data suggest that the α chains of Ia molecules isolated by MGH88B and Rb03 are very similar. In order to better define the relationship between
FIGURE 2. Comparative tryptic peptide maps of $[^{3}H]$leucine-labeled $\alpha$ chains of $\lambda$ molecules isolated by Rb03 (dashed line) and MGH88B (solid line).

These two $\lambda$ $\alpha$ chains, they were isolated by preparative SDS-PAGE and analyzed by tryptic peptide mapping using high pressure liquid chromatography. The peptide maps were run separately, but are plotted on the same graph for comparison (Fig. 2). Seven major leucine-labeled peptides are resolved in each case and co-elute. These data indicate that the $\alpha$ chains isolated by Rb03 and MGH88B are identical, and therefore, that $\alpha_2$ is the DS $\alpha$ chain. Thus the 2-D gel and tryptic peptide mapping data indicate that the DS $\alpha$ chain ($\alpha_2$) can be found in association with either of two distinct DS $\beta$ chains ($\beta_1$ and $\beta_4$).

In summary, serum MGH88B (anti-MT4) precipitated primarily the $\alpha_2$-$\beta_1$ $\lambda$ molecule. We have previously shown that two anti-MB3 allosera also precipitated the $\alpha_2$-$\beta_1$ molecule (7). Serum Rb03 precipitated two $\lambda$ molecules consisting of $\alpha_2$-$\beta_1$ and $\alpha_2$-$\beta_4$. Serum MGH87B (anti-MT2) precipitated three $\lambda$ molecules consisting of $\alpha_1$-$\beta_2$, $\alpha_1$-$\beta_3$, and $\alpha_2$-$\beta_1$. We have previously shown that another anti-MT2 alloserum isolates the same three molecules (11). The monoclonal antibody SG157 that is specific for DR (I-E-like) molecules (1, 2) precipitated the $\alpha_1$-$\beta_2$ and $\alpha_1$-$\beta_3$ molecules (data not shown). Therefore this DR5 homozygous cell line appears to express two distinct DR molecules and two distinct DS molecules.

Discussion

The data presented here document for the first time that an HLA-DR homozygous cell line expresses at least two structurally distinct DS (I-A-like) molecules. We have now identified four distinct $\lambda$ molecules from this DR5 homozygous cell line using two-dimensional gel electrophoresis.

Although human $\lambda$ molecules with N-terminal amino acid sequence homology to murine I-E molecules were identified in 1978 (12), the human equivalents of murine I-A molecules were not identified until more recently (1). The rabbit heteroserum Rb03 is specific for human DS (I-A-like) molecules (2). Both the $\alpha$ and $\beta$ chains of human $\lambda$ molecules isolated with Rb03 have N-terminal amino acid sequence homology to murine I-A $\alpha$ and $\beta$ chains, respectively (2). Bono and Strominger (5) have also identified a human I-A homologue; the $\alpha$ chains of $\lambda$ molecules isolated with the monoclonal antibody Genox 3.53 have N-terminal amino acid sequence homology to murine I-A $\alpha$ chains. However, only one DS molecule was identified in these studies (1, 2, 5). In comparative 2-D gel studies of $\lambda$ molecules isolated by alloantisera and Rb03, we have demonstrated that a DR5 homozygous cell line expresses two DS molecules.

Because our understanding of the complexity of $\lambda$ molecules finds its most
practical application in tissue typing, it is of interest to locate the serologically defined allodeterminants on the various Ia molecules that have been identified. Previous studies indicated that the serologically defined Ia antigens, DC1, MB1, MT1, LB12, and MB3, are borne on DS molecules (2, 5, 13, 14). The location of the MT3 determinant is variable: on DR4 cells, the MT3 determinant resides on DS molecules, while on DR7 cells, the MT3 determinant is found on DR molecules (8). According to the data presented here and previously (14), the MT4 allodeterminant, as well as the MB3 allodeterminant, resides on DS molecules. However, the MT4 and MB3 allodeterminants reside on only one (αβ1) of the two DS molecules identified on this cell line. In addition, the MT2 allodeterminant appears to reside on both DR molecules but on only one DS molecule. The finding that MT2 resides on multiple Ia molecules and is, therefore, a true supertypic determinant is compatible with previous reports of anti-Ia antibodies that recognize epitopes on Ia molecules encoded by different genetic subregions in both the murine and human systems (1, 15, 16). This, then, is the first demonstration that a human Ia allodeterminant resides on both DR and DS molecules from the same cell line. Interestingly, the αβ4 DS molecule was not isolated by any alloantisera that we have tested. These findings emphasize the heterogeneity that exists at the molecular level within the MT system of serologically defined Ia alloantigens (17): the MT1 and MT4 allodeterminants reside only on DS molecules; the MT3 allodeterminant resides on either DR (DR7) or DS (DR4) molecules; the MT2 allodeterminant (as defined by the reagents used in these studies) resides on both DR and DS molecules from the same cell line. Therefore, it is unlikely that all of the serologically defined allospecificities of the MT system are alleles at a single locus.

The methods of molecular biology have recently been able to identify an increasing number of genes that determine Ia α and β chains. Auffray et al. (18) identified a cDNA clone corresponding to a single human I-A-like α chain. This group has also demonstrated polymorphism of the DS α chain genes (19). Long et al. (20) reported the identification of a single I-A-like α chain gene and two I-A-like β chain genes. However, the identification of multiple Ia genes does not prove that all of these genes are expressed and are, therefore, of functional significance in cell-cell interactions. Our findings at the protein level are consistent with the findings at the gene level of Long et al. (20) and indicate that at least two DS β chain genes are expressed by this DR5 homozygous cell line.

Summary

Previous studies have indicated that HLA-DR homozygous cell lines express two DR molecules but only a single DS (I-A-like) molecule. This report demonstrates that an HLA-DR5 homozygous cell line expresses at least two distinct DS molecules. These two DS molecules are formed by the association of a single DS α chain with either of two DS β chains. Four distinct Ia molecules have now been identified from this DR5 homozygous cell line.

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References


