

DEMONSTRATION OF A THIRD STRUCTURALLY
DISTINCT HUMAN Ia BETA CHAIN BY
TWO-DIMENSIONAL GEL ELECTROPHORESIS*

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The human Ia or class II antigens are determined by the *HLA-DR* region within the human major histocompatibility complex (MHC) on chromosome six and are borne on molecules composed of two noncovalently associated glycoprotein chains of 33,000–34,000 daltons (α chain) and 28,000–30,000 daltons (β chain). Recent studies (1–4) using both alloantisera and monoclonal antibodies have documented the presence on HLA-DR homozygous cell lines of two α chains and two β chains which combine to form at least two Ia molecules. The data presented here establish the existence of a third structurally distinct Ia β chain on HLA-DR homozygous cell lines.

Materials and Methods

Cells. The following B lymphoblastoid cell lines were obtained from the Human Genetic Mutant Cell Repository, Camden, NJ: PGF(GM3107), HLA-A3,A3; B7,B7; DR2,DR2; MB1; MT1; Te21; and MST1(GM3161), HLA-A3,A3; B7,B7; DR2,DR2; MB1; MT1; Te21. The cell line Swei, HLA-A29,A29; B40,B40; DR5,DR5; MB3; MT2, MT4; Te22, was obtained from Dr. John Hansen. The cell line BK types as HLA-A2, A-unidentified; B49,B51; DR1,DR5; MB1,MB3; MT1,MT2,MT4. The cell line MAR types as HLA-A2,A3; B18,B45; DR2,DR4; MB1,MB3; MT1,MT3. The cells were maintained as previously described (4).

Preparation of Radiolabeled Antigens. Class II antigens were radiolabeled with ³⁵S-methionine (700–1300 Ci/mM, Amersham, Arlington Heights, IL) and immunoprecipitated with alloantisera as previously reported (4).

Antisera. A summary of the serologically and immunochemically detected specificities of the alloantisera, defined as previously described (4), can be found in Table I.

Two-dimensional (2-D) Gel Electrophoresis. 2-D gel electrophoresis was performed according to the method of O'Farrell (5) with some modifications as previously described (4). The first dimension separation by isoelectric focusing (IEF) was performed in 5 × 200-mm cylindrical gels, after which the IEF gels were cut into two pieces that contained the α and β chains, respectively. Each piece was applied to a separate sodium dodecyl sulfate-polyacrylamide slab gel.

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TABLE I

Alloantiserum	Laboratory of origin	Serologically defined specificity	Chemically defined specificity
DA6*	Fuller	DR5 + Te22‡	DR5 + Te22 + MT2
Absorbed DA6§	Fuller	Not tested	DR5
Ia-172	Mann	MT2	MT2
MGH88B	Fuller	MT4	MT4
Banaszak	Duquesnoy	DR2	DR2
Dobbe¶	Duquesnoy	MB1/MT1 + DR1	MB1/MT1 + DR1
MGH72	Fuller	MB1/MT1	MB1/MT1
Ia-715**	Mann	MB1/MT1	MB1/MT1
Pohl	Rodey	DR1 + DR2	DR1 + Te21

* Serum 1225 in the Eighth International Histocompatibility Workshop (8W1225).

‡ At 1:10 dilution.

§ Details of absorption and immunochemical analysis previously reported (4).

|| 7W154.

¶ 8W119.

** 8W567.

Results

2-D Gel Electrophoresis. A ³⁵S-methionine-labeled antigen preparation of the DR1,DR5 heterozygous cell line, BK, was immunoprecipitated with serum DA6, which had been absorbed with DR3 and DR4 cells (absorbed DA6), serum Ia-172, and serum MGH88B; the precipitated material was analyzed by 2-D gels. The β chain portions of these gels are shown in Fig. 1. The β chain patterns of DA6 and Ia-172 consist of a heterogeneous set of nine spots as previously reported (4). The β chain pattern of MGH88B consists of at least four spots (a fifth more basic spot is occasionally seen) which are a subset of the DA6 and Ia-172 β patterns. Based on these β chain patterns, we have previously reported the existence of at least two structurally distinct β chains on DR5 cells; one chain (previously referred to as L2) consisted of the four spots precipitated by MGH88B, whereas the other β chain (previously referred to as L1) appeared to consist of the spots precipitated by DA6 and Ia-172, but not by MGH88B (4). However, the β pattern precipitated by the absorbed DA6 serum reveals the absence of two of the more basic spots precipitated by DA6 and Ia-172, but not by MGH88B. This result indicates that the portion of the β chain pattern that is unique to DA6 and Ia-172 actually consists of two structurally distinct β chains.

To establish a basis for uniform nomenclature, we will designate the three β chains β_1 , β_2 , and β_3 based on the isoelectric point (pI) of the most acidic spot in each β chain. The β chain whose most acidic spot is most acidic relative to the most acidic spots of the other two β chains will be designated β_1 , whereas the β chain whose most acidic spot is relatively most basic will be designated β_3 . The β chain whose most acidic spot has a pI intermediate between the most acidic spots of β_1 and β_3 will be designated β_2 . Therefore, the β chain precipitated only by MGH88B is β_1 (hatched spots), the β chain precipitated by absorbed DA6, but not by MGH88B, is β_2 (open spots), and the β chain precipitated by DA6 and Ia-172, but not precipitated by absorbed DA6 or MGH88B is β_3 (closed spots). There is some molecular weight heterogeneity among the three β chains; the spots of β_1 have a slightly lower molecular weight than the spots of β_2 . The most basic spot of β_3 has the same pI as the most basic spot of β_1 ; however, the two spots are separable because of a very slight difference in molecular weights. The most acidic spot of β_3 has the same molecular weight as the most basic spot of β_2 ; however, the two spots are separable because of a very slight difference in pI. Examination of the intervals between the spots that

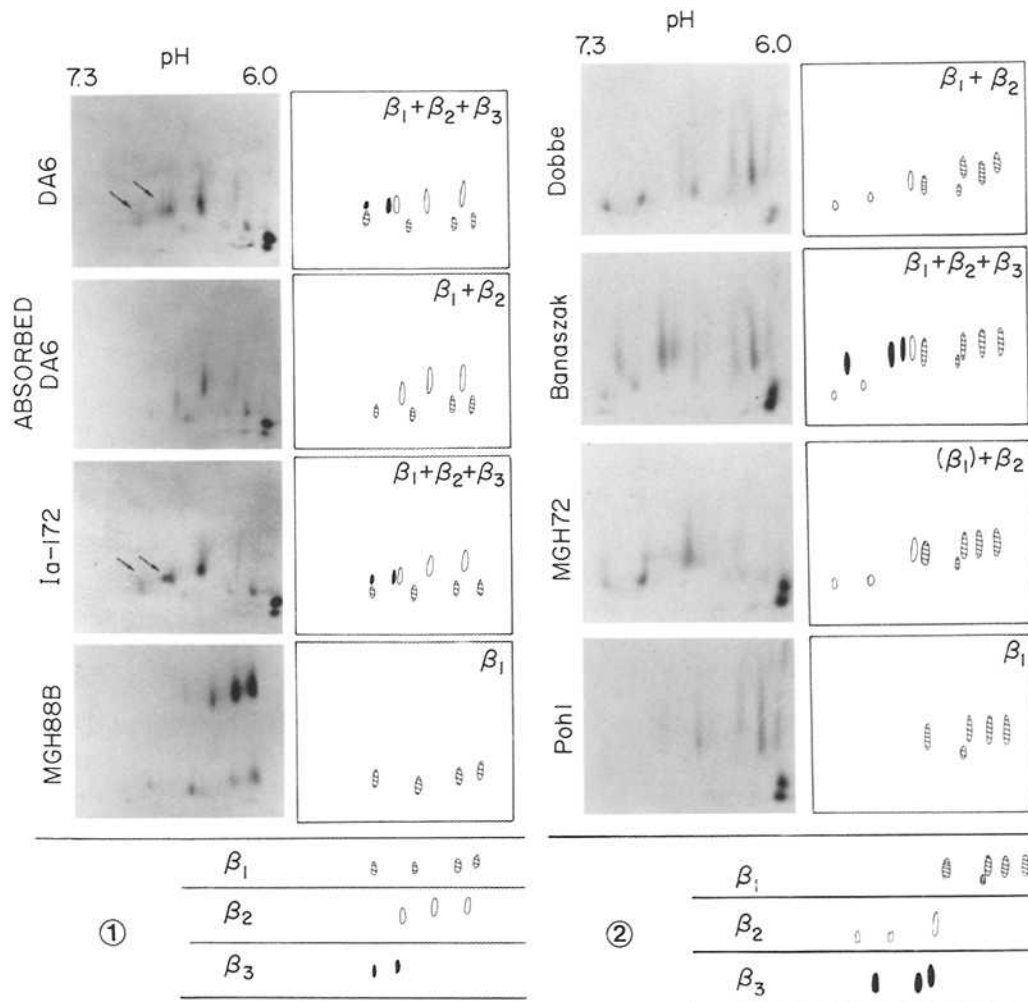


FIG. 1. Fluorographs of 2-D gels of β chains precipitated from a ^{35}S -methionine-labeled antigen preparation of the BK cell line (DR1, DR5) by sera DA6, absorbed DA6, Ia-172, and MGH88B are shown at the left. Schematic representations of the spots in each gel are shown at the right. Schematic representations of the spots that comprise β_1 , β_2 , and β_3 are shown at the bottom. Arrows indicate the two spots of β_3 , which are precipitated by sera DA6 and Ia-172, but not by the absorbed DA6 serum. The two dark spots with $<25,000$ mol wt at the acidic end of some β chain gels are an inconsistent finding and therefore do not appear to be related to the β chain spots. The row of spots at 44,000 daltons with MGH88B most likely represents HLA class I molecules.

FIG. 2. Fluorographs of 2-D gels of β chains immunoprecipitated from a ^{35}S -methionine-labeled antigen preparation of the DR2 homozygous cell line, MST1, by sera Dobbe, Banaszak, MGH72, and Pohl are shown at left and schematic representations of the spots in each gel are shown at right. See legend to Fig. 1.

comprise each β chain provides additional confirmation that the spots of each β chain are more closely related to each other than to the spots of another β chain. The spots of each distinct β chain are separated from each other by some integral multiple of the shortest interval between two spots of that β chain. For example, the interval between the middle spot of β_2 and the most acidic spot of β_2 is the same as the interval

between the middle spot and the most basic spot. The shortest interval, or charge unit, is unique for each of the three β chains.

In summary, serum MGH88B precipitated only β_1 . The β chain pattern of absorbed DA6 contains β_1 and β_2 and the β chain patterns of unabsorbed DA6 and Ia-172 include β_1 , β_2 , and β_3 . These relationships are depicted schematically in Fig. 1. Identical β chain patterns were observed when these sera were used to isolate Ia molecules from an antigen preparation of the DR5 homozygous cell line, Swei. The α chain patterns precipitated from DR5 antigen preparations by serum DA6 and serum Ia-172 (4) and by the absorbed DA6 serum (unpublished observations) consist of both α_1 and α_2 (previously referred to as H1 and H2, respectively), whereas the α chain pattern of serum MGH88B consists only of α_2 (4).

To determine whether the demonstration of a third human Ia β chain could be generalized, a ^{35}S -methionine-labeled antigen preparation of the DR2 homozygous cell line, MST1, was immunoprecipitated with sera Dobbe, Banaszak, MGH72, and Pohl; the precipitated material was then compared by 2-D gels (Fig. 2). Serum Banaszak precipitated a complex β chain pattern of eleven spots. Serum Dobbe precipitated only eight of the same spots precipitated by Banaszak, whereas Pohl precipitated a more restricted subset of the Banaszak β pattern. MGH72 precipitated a pattern that was qualitatively the same as the Dobbe pattern. These complex β chain patterns can be explained by postulating no fewer than three structurally distinct β chains, which we have labeled β_1 , β_2 , and β_3 using the same criteria as described above. β_1 (five hatched spots) is precipitated by all four sera; however, Pohl isolates β_1 alone. β_2 (three open spots) is precipitated by Dobbe, Banaszak, and MGH72, but not by Pohl. β_3 is precipitated only by Banaszak. Compared with the three β chains from DR5 cells, there is less molecular weight heterogeneity among the three β chains from DR2 cells.

According to this construct, the complex β chain pattern precipitated by Banaszak includes β_1 , β_2 , and β_3 . The Dobbe β chain pattern contains β_1 and β_2 , but lacks β_3 . Quantitatively similar amounts of β_1 are precipitated by both Dobbe and Banaszak; however, Dobbe precipitated more β_2 than Banaszak. Serum Ia-715 precipitated a β chain pattern identical to Dobbe (data not shown). Serum MGH72 precipitated primarily β_2 , but also a small amount of β_1 . Serum Pohl precipitated only β_1 . These relationships are depicted schematically in Fig. 2. Again, examination of the intervals between the spots that comprise each β chain confirms that the spots of each β chain are more closely related to each other than to the spots of the other β chains; the shortest interval is unique for each of the three β chains. Identical β chain patterns were observed when these sera were used to isolate Ia molecules from antigen preparations of another DR2 homozygous cell line, PGF, and a DR2 heterozygous cell line, MAR (data not shown). The α chain patterns precipitated by Banaszak, Dobbe, MGH72, and Pohl consisted of α_1 and variable amounts of α_2 (data not shown)

Discussion

The data presented here document for the first time the existence of a third structurally distinct human Ia β chain. The use of alloantisera, which contain antibodies to polymorphic determinants on Ia molecules, provided a significant advantage in these studies because we were able to investigate the same antigens on

both DR heterozygous and homozygous cell lines. In contrast, most anti-Ia monoclonal antibodies recognize monomorphic determinants and, therefore, cannot be used to isolate individual Ia molecules from DR heterozygous cell lines.

The complex array of β chain patterns precipitated from two HLA-DR2 homozygous cell lines and one HLA-DR2 heterozygous cell line by four alloantisera can be explained by the existence of no fewer than three structurally distinct β chains, termed β_1 , β_2 , and β_3 . The two spots of β_3 were precipitated only by serum Banaszak. The existence of a third β chain was also demonstrated on both HLA-DR5 homozygous and heterozygous cell lines when two spots that were precipitated by serum DA6 and serum Ia-172 were not precipitated by the absorbed DA6 serum. The demonstration that identical β_1 , β_2 , and β_3 patterns are precipitated from DR5 homozygous and DR1, DR5 heterozygous cell lines, coupled with the observation that the DR1-associated β chains are distinct from the DR5-associated β chains (data not shown) excludes the possibility that the third β chain is due to the lack of true HLA-DR homozygosity. The designations, β_1 , β_2 and β_3 of β chains isolated from DR2 and DR5 cells were assigned solely based on the pI of the most acidic spot of each β chain; the data presented here do not allow any conclusions about possible homology between, for example, the DR2 β_1 and the DR5 β_1 . There appears to be structural polymorphism of all three β chains since none of the three β chains from DR2 cells are structurally identical to any of the three β chains from DR5 cells.

Several studies using both alloantisera and monoclonal antibodies have recently established the presence of two α chains and two β chains that combined to form two class II molecules on HLA-DR homozygous cells (1-4). Shackelford et al. (1) also used alloantisera and 2-D gels to investigate the Ia molecules expressed by the DR2 homozygous cell line, PGF. They found a total of 10 β chain spots using a combination of sera. A DR2 alloantiserum precipitated a β chain pattern consisting of seven spots. They postulated, but did not demonstrate, that this pattern might represent two β chains. Sera for the specificities MB1, MT1, DC1, and LB12, including serum Ia-715, which was used in the present study, precipitated a β chain pattern of three spots that were distinct from the spots precipitated by the DR2 serum. Although we have detected a similar total number of β chain spots from DR2 antigen preparations, we found that three anti-MB1/MT1 sera (Dobbe, Ia-715, and MGH72) precipitated, with some quantitative variation, a β chain pattern consisting of seven spots (β_1 and β_2). The reason for the differences between our results and those of Shackelford, et al. (1) are unclear, but these observations suggest that there may be considerable heterogeneity among sera that define the same serologic specificity.

An impressive degree of homology has been found between the Ia molecules determined by the murine and human MHC. Until recently, only two α chains, A_α and E_α , and two β chains, A_β and E_β , have been thought to comprise the murine I-A and I-E molecules. However, two studies have suggested that there may be two structurally distinct I-E molecules (and, therefore, presumably three Ia β chains) in some haplotypes (6, 7). Human Ia molecules that appear to be equivalent to the murine I-A and I-E molecules based on amino acid sequence homology have recently been isolated with monoclonal antibodies (3). Additional studies are now in progress to determine which of the three human Ia β chains we have identified with allosera correspond to these human I-A or I-E equivalent molecules.

Previous data (1-4) from several groups have documented that at least two

structurally distinct Ia α and Ia β chains are expressed on HLA-DR homozygous cell lines, whereas a more recent study (8) postulated the existence of at least seven Ia β chains based on partial amino acid sequence data. The data presented in this report demonstrate the existence of a third human Ia β chain. This suggests that at least five separate genes control the expression of class II molecules on HLA-DR homozygous cell lines.

Summary

Previous studies have indicated that HLA-DR homozygous cell lines express two Ia α and Ia β chains that combine to form at least two Ia molecules. This report demonstrates by two-dimensional gel electrophoresis the existence of a third structurally distinct human Ia β chain on DR2 and DR5 cell lines. This suggests that at least five separate genes control the expression of Ia molecules on HLA-DR homozygous cell lines.

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