IDENTIFICATION OF TWO *cis*-ENCODED HLA-DQ MOLECULES THAT CARRY DISTINCT ALLOANTIGENIC SPECIFICITIES

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The HLA-D region encodes a series of polymorphic differentiation antigens that appear to be involved in the control of cellular interactions in the generation of an immune response (1). Two types of HLA-D region products have been serologically identified, the DR antigens, which are homologous to the murine I-E molecules, and the DQ (formerly DS or DC) antigens, which are homologous to the murine I-A molecules (2, 3). These antigens consist of two noncovalently associated subunits (alpha, 34 kD; beta, 27 kD) and both the DR and DQ molecules carry antigenic specificities defined by alloantisera (4, 5). Within the DQ series, two alpha and two beta chain genes have been identified in the haploid genome, and hybridization data suggest additional genes (6, 7). Evidence also exists for the expression of two distinct I-A-like molecules (8–10). The studies reported here provide evidence not only for the expression of two distinct DQ-like molecules but show that each of these carry distinct alloantigenic specificities that can be defined by monoclonal antibodies.

Materials and Methods

Cells and Antibodies. Lymphoblastoid cell lines (LCL)[1] were produced by Epstein-Barr virus (EBV) transformation of peripheral blood lymphocytes. The families and HLA-Dw homozygous cells were characterized at the Seventh and Eighth International Histocompatibility Workshops or with reagents of the Workshop. The homozygous cells were obtained locally or through exchange. Monoclonal antibodies (MAb) Genox 3.53 (IgG1) (11), L243 (IgG2) (12), and 2.06 (IgG2) (13) were obtained from the American Type Culture Collection, Rockville, MD and MAb S1 and R1 (IgM) (14) and 16.23 (IgG3) (15) were produced in this laboratory. MAb Leu-10 was obtained from Becton, Dickinson & Co., Mountain View, CA. Typing was performed on 3–6-d-old phytohemagglutinin blasts using a single-cell, peroxidase-linked, enzyme-linked immunosorbent assay (ELISA) as previously described (16).

Immunoprecipitation. Cells were radiolabeled using the lactoperoxidase-glucose oxidase procedure (17), lysed in 1% taurocholate (Sigma Chemical Co., St. Louis, MO) in phosphate-buffered saline, pH 8, containing 2 mM benzamidine hydrochloride (EGA Chemie, Steinhiem/Albruch, FRG) and 2 mM phenylmethylsulfonyl fluoride (Fluka A. G., Buchs, Switzerland). Immunoprecipitation was performed by incubating 10^7 cpm for 6–12 h at 4°C with 20 μl protein A–Sepharose (Sigma Chemical Co.) which had

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[1] *Abbreviations used in this paper:* EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; LCL, lymphoblastoid cell line; MAb, monoclonal antibody; SDS-PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

previously been incubated with rabbit anti-mouse Ig (Sigma Chemical Co.) and MAb as culture supernatant. Bound material was eluted by boiling in sodium dodecyl sulfate (SDS) sample buffer and was analyzed by electrophoresis under reducing conditions on 10% SDS-polyacrylamide gel electrophoresis (PAGE) (18). Fixed, dried gels were exposed with an intensifying screen at −70°C to Kodak XAR-5 film.

Results

Monoclonal Antibodies S1, R1, and Genox 3.53 Are Directed Against an Alloantigenic Specificity Similar to DQw1. MAb S1 and R1 detect polymorphic B lymphocyte antigens that segregate with HLA and display biochemical characteristics of Ia-like antigens (14). Analysis of binding with a panel of cells obtained from 68 unrelated individuals revealed a strong association between the binding of these MAb and the presence of the DR antigens 1, 2, or w6 (Table I), a characteristic of the DQw1 (formerly DC1) alloantigen (19). MAb Genox 3.53, which has been shown to detect the DQw1 alloantigen (20), displayed a similar DR antigen association in this panel. Genox 3.53 precipitates DQ (I-A like) molecules that are distinguishable in some cells from the DR (I-E like) molecules in SDS-PAGE (4, 21). As seen in Fig. 1, Ia molecules precipitated from the cell line Sch1 (Dw6 homozygous) by Genox 3.53 (Fig. 1, lane *6*) migrate faster than the HLA-DR-associated molecules precipitated by 16.23 (lane *7*). Both MAb S1 (Fig. 1, lane

TABLE I

Association of Determinants Detected by Monoclonal Antibodies with DR Alloantigens 1, 2, and w6

MAb	+ +*	+ −	− +	− −	n‡	χ^2
S1	33	10	3	22	68	25.87
R1	36	12	0	20	68	28.94
Ge	36	5	0	27	68	46.92

* Binding of MAb, presence of DR 1, 2, or w6.
‡ Number of individuals tested.

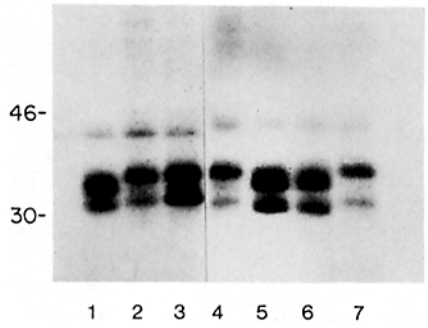

FIGURE 1. Ia-like antigens precipitated from a DRw6 homozygous LCL by MAbs. Immunoprecipitates were prepared from surface-iodinated Sch LCL as described in Materials and Methods and separated on 10% SDS-PAGE under reducing conditions. The numbers at the left indicate the position of molecular weight markers (46 kD, ovalbumin; 30 kD, carbonic anhydrase). Antigens precipitated by MAb R1, S1, and Genox 3.53 are in lanes *1*, *5*, and *6*, respectively. DR-like antigens precipitated by MAb S2 (Drw6-associated), S3 (MT2-like), R3 (MT2-like), and 16.23 (DR3-associated) are in lanes *2*, *3*, *4*, and *7*, respectively. Characterization of MAb S2, R3, and S3 has been presented elsewhere (14).

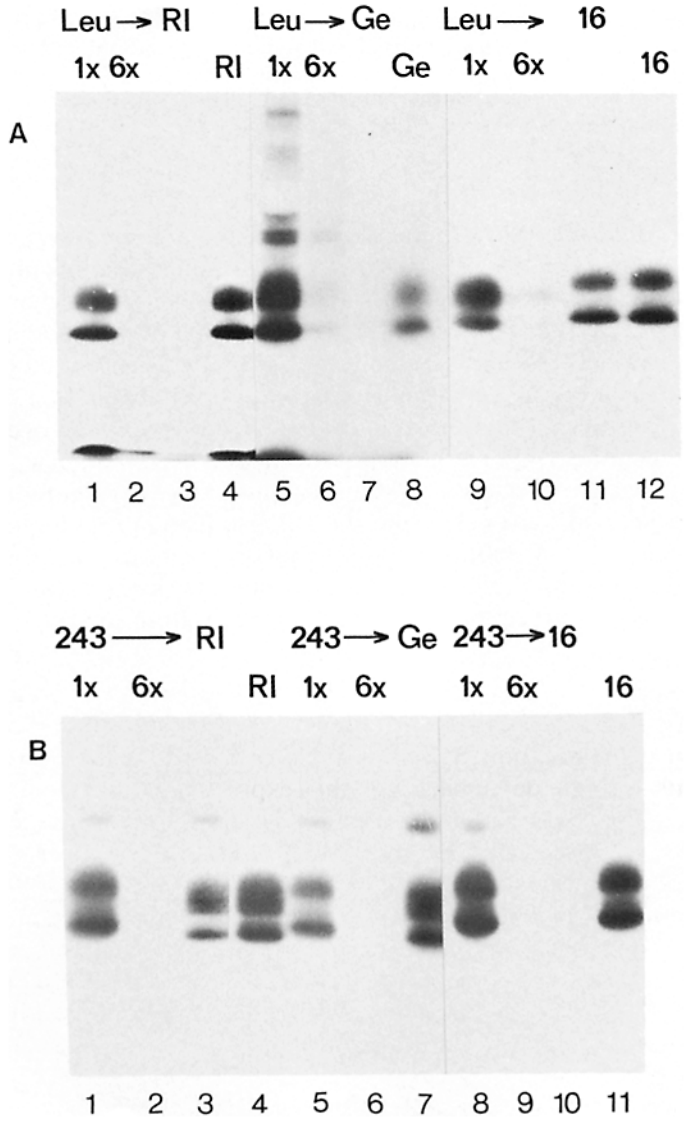

FIGURE 2. Serial immunoprecipitation of Ia antigens from iodinated Chf Jag (HLA-DR 6,6). Immunoprecipitates were prepared as described in the text and separated on 10% SDS-PAGE under reducing conditions. (*a*) Sequential immunoprecipitation using Leu-10 (anti-HLA-DQ). Immunoprecipitates obtained after one round (lanes *1*, *5*, *9*) and six rounds (lanes *2*, *6*, *10*) of incubation with Leu-10. These lysates were then precipitated with MAb R1 (lane *3*), Genox 3.53 (lane *7*), and 16.23 (lane *11*). Control lysates were exposed to six rounds of precipitation with a mouse myeloma and then immunoprecipitated with R1 (lane *4*), Ge (lane *8*), and 16.23 (lane *12*). (*b*) Sequential immunoprecipitation using L243 (anti-HLA-DR). Immunoprecipitates obtained after one round (lanes *1*, *5*, *8*), and six rounds (lanes *2*, *6*, *9*) of incubation with MAb L243. Lysates were then precipitated with MAb R1 (lane *3*), Genox 3.53 (lane *7*), or 16.23 (lane *10*). Control lysates were exposed to six rounds of precipitation with a mouse myeloma and then immunoprecipitated with R1 (lane *4*), or 16.23 (lane *11*).

5) and R1 (lane *1*) precipitated molecules from this cell line that migrated with the characteristics of the DQ (Genox) rather than the DR (16.23) molecules. That the antigens precipitated by R1, S1, and Genox all belong to a population of DQ molecules was directly shown by sequential immunoprecipitation using DR- and DQ-specific antibodies (Fig. 2). MAb Leu-10 detects a determinant common to the DQ molecules of most haplotypes (21). MAb L243 has been shown by analysis on HLA-D region mutants of LCL to be directed against the products of the DR locus (22). Iodinated LCL (HLA-DR 2,2; DR 2,3; or DR 6,6) were exposed to several rounds of precipitation with L243, Leu-10, or an irrelevant antibody and then precipitated with MAb S1, R1 Genox 3.53, or 16.23. As can be seen in Fig. 2 *a* (Chf Jag; HLA-DR 6,6), MAb Leu-10 removed molecules binding to R1 (lanes *1–4*) and Genox (lanes *5–8*) but not to 16.23 (lanes *9–12*). MAb L243 (Fig. 2 *b*) removed 16.23-binding molecules (lanes *8–11*) but not those binding R1 (lanes *1–4*) or Genox (lanes *5–7*). Identical results were obtained for S1- as well as R1-binding molecules in all three cells examined.

MAb S1, R1, and Genox 3.53 Detect Different Epitopes and Define Two Distinct Alloantigenic Specificities. Although all three MAb appeared to be directed against a DQw1-like alloantigenic specificity, it can be seen from Table I that complete concordance in the binding of the three antibodies was not observed. This suggests that they may detect distinct epitopes denoted here as S1, R1, and Ge. Fifty unrelated individuals whose B lymphocytes expressed at least one of these three epitopes were investigated. While the majority expressed all three determinants, 15 variant individuals were found. Seven expressed only R1 and Ge and eight expressed only S1 and R1. No cells in the panel were found that expressed only a single determinant or that expressed S1 and Ge in the absence of R1. Through segregation analysis in 13 families, haplotypes of three types were identified: $S1^+/R1^+/Ge^+$, $S1^+/R1^+/Ge^-$, and $S1^-/R1^+/Ge^+$. Thus, the three different epitope combinations observed in the unrelated panel were heritable and did not merely reflect variations in the B lymphocyte antigen expression. The number of variant cells (i.e., those individuals expressing only two of the three DQw1 determinants) was too few to indicate an association of the variant phenotype with a given DR antigen. However, when a panel of HLA-D homozygous cells was investigated, an association could be detected. In a study of 41 homozygous cells covering the specificities Dw 1–10, the $S1^+/R1^+/Ge^+$ phenotype was found in all cells expressing the DR antigens 1, 2, or 6 (Fig. 3). The $S1^-/R1^+/Ge^+$ phenotype was not found in this panel but the $S1^+/R1^+/Ge^-$ phenotype was detected in all cells tested that were homozygous for the DR specificities 4 and w8. In the unrelated panel of Table I, the $S1^+/R1^+/Ge^-$ phenotype was found in approximately half of the individuals bearing DR4 or DRw8 antigens.

Two Distinct DQw1-like Alloantigens Are Encoded on One Haplotype. The observations above provide evidence that the MAbs described define two distinct DQw1-like alloantigens, one of which is defined by the epitopes S1 and R1, and one of which is defined by the epitopes S1, R1, and Ge. To investigate the relationship between these alloantigens, LCL from four heterozygous individuals where the three epitopes could be assigned to the same chromosome through segregation analysis were analyzed using sequential immunoprecipitation. In-

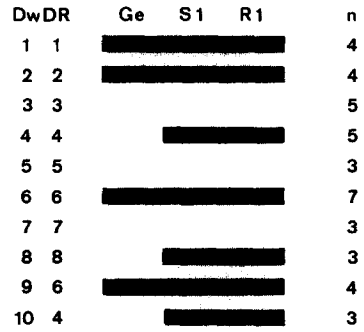

FIGURE 3. Distribution of epitopes detected by monoclonal antibodies Ge, S1, and R1 in HLA-D/DR homozygous cells. The DR and Dw antigens are listed on the left and the number of cells of each specificity that were tested (*n*) on the right. A black bar indicates presence of the epitope and a blank space indicates its absence. Presence of the epitope was determined using a single-cell ELISA.

cluded were haplotypes bearing DR1 (one cell), DR2 (two cells), and DRw6 (one cell). Lysates from surface-iodinated LCL were subjected to five to seven rounds of precipitation with the first MAb and then exposed to the second MAb. Finally, the lysates were incubated with MAb 16.23 or 2.06, which precipitate DR-like antigens. Fig. 4 presents the data from one of the two DR2 cells. Identical results were obtained from all four cells examined. The molecules precipitated by Genox 3.53 and those precipitated by either S1 or R1 appear to be independent. Thus, complete removal of R1-reactive (Fig. 4 *a*, lanes *1–3*) or S1-reactive (Fig. 4 *b*, lanes *1–3*) molecules did not deplete Genox 3.53–reactive molecules (lane *4*, Fig. 4, *a* and *b*). Complete removal of Genox 3.53–reactive molecules (lanes *6–8*, Fig. 4, *a* and *b*) did not remove R1-reactive molecules (Fig. 4 *a*, lane *9*) nor S1-reactive molecules (Fig. 4 *b*, lane *9*). The R1- and S1-bearing molecules on the other hand, appear to be related, since removal of the S1-bearing molecules depleted also the R1-reactive molecules (Fig. 4 *c*, lanes *1–5*) and vice versa (Fig. 4 *c*, lanes *6–10*). (In Fig. 4 *c*, the films were deliberately overexposed to allow detection of remaining $R1^+S1^-$ and $R1^-S1^+$ molecules.) Results obtained on an $S1^+/R1^+/Ge^-$ cell (Va Schw, HLA-DR 4,5) indicate that S1 and R1 epitopes are located on the same molecules in these cells as well (data not shown).

Discussion

The Ia-like antigen epitopes defined by MAb S1, R1, and Genox 3.53 show a strong association with the DR specificities 1, 2, and w6, suggesting that they all detect a DQw1-like specificity (19). The molecules precipitated by all three antibodies are removed by prior exposure to an anti-DQ MAb (Leu-10) but not an anti-DR MAb (L243), indicating that all are DQ rather than DR molecules. Two observations, however, indicate that these antibodies actually define two distinct alloantigenic specificities. Haplotypes encoding R1 and S1 molecules but lacking Ge molecules were observed, and analysis of a panel of homozygous typing cells showed an association of this pattern with DR4/Dw4 and DR8/Dw8 cells. Reciprocal sequential immunoprecipitation using cells in which the R1, S1, and Ge epitopes were encoded by the same chromosome showed that two distinct

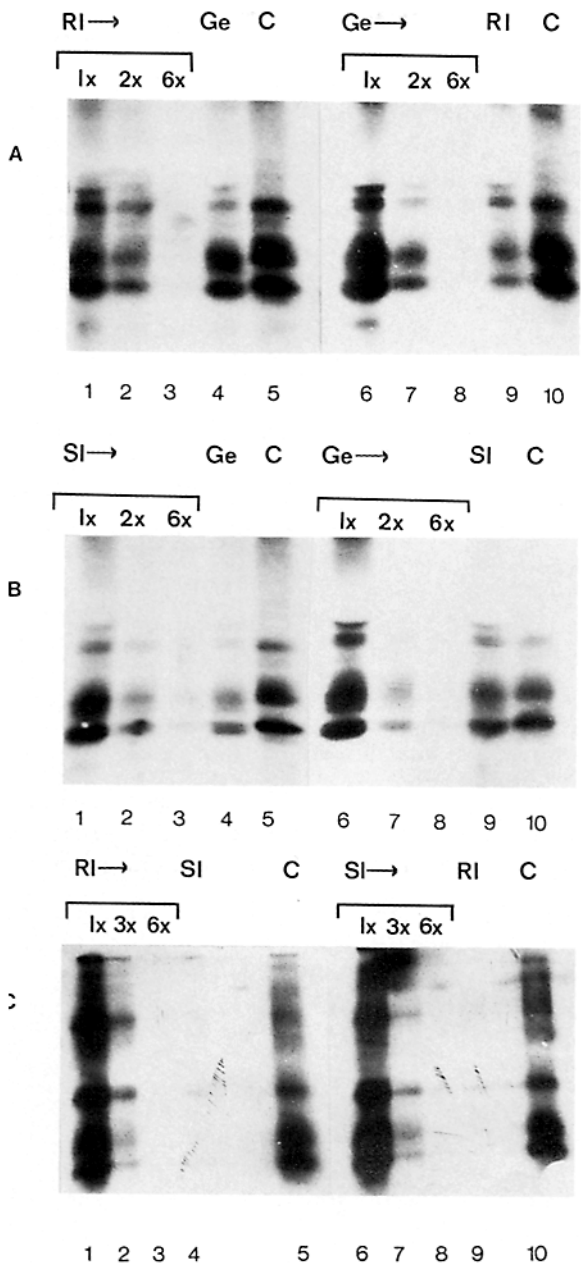

FIGURE 4. Serial immunoprecipitation of DC-like antigens from iodinated LCL of Mo Lea (HLA-DR 2,3). Immunoprecipitates were prepared as described in the text and separated on 10% SDS-PAGE under reducing conditions. R1, S1, and Ge indicate immunoprecipitates prepared with the respective monoclonal antibodies. *C* is an immunoprecipitate prepared at the end of each experiment using the control monoclonal antibody 16.23. (*a*) Sequential immunoprecipitation with MAb R1 and Genox 3.53. Immunoprecipitate obtained after one round (lane *1*), two rounds (lane *2*), and six rounds (lane *3*) of incubation with MAb R1. Precleared lysate was then exposed to Genox 3.53 (lane *4*) and 16.23 (lane *5*). In lanes *6–10*, the lysate was exposed to six rounds of precipitation with Genox 3.53 (lanes *6–8*) and then precipitated with R1 (lane *9*) and 16.23 (lane *10*). (*b*) Sequential immunoprecipitation with MAb S1 and Genox 3.53. The lysate was precleared with MAb S1 (lanes *1–3*) and then exposed to Genox 3.53 (lane *4*) and 16.23 (lane *5*). In lanes *6–10*, the lysate was precleared with Genox 3.53 (lanes *6–8*) and then exposed to S1 (lane *9*) and 16.23 (lane *10*). (*c*) Sequential immunoprecipitation with MAb S1 and R1. The lysate was precleared with MAb R1 (lanes *1–3*) and then exposed to S1 (lane *4*) and 16.23 (lane *5*). In lanes *6–10*, the lysate was precleared with MAb S1 (lanes *6–8*) and then exposed to R1 (lane *9*) and 16.23 (lane *10*).

molecules could be identified. MAb S1 and R1 appear to recognize the same molecules in DQw1-bearing individuals since preclearing with R1 removed all molecules reacting with S1 and vice versa. In contrast, preclearing with R1 or S1 did not remove the molecules binding the Genox 3.53 antibody. Conversely, preclearing with Genox 3.53 did not remove molecules reacting with R1 or S1. Identical results were obtained from four individuals bearing three different DQw1-associated DR allospecificities. Therefore, it appears that DQw1-bearing individuals express two similar but distinct alloantigenic specificities that are located on different molecules. The specificity defined by the MAb Genox 3.53 (called here DQwA.1) has the population distribution of the classic DQw1 alloantigen (i.e., it is expressed in individuals bearing the DR antigens 1, 2, and w6). The other specificity, which we have called DQwB.1, is defined by the antibodies R1 and S1 and has a broader distribution than the DQwA.1, showing an association also with DR4 and DRw8 specificities. The MAb B3T/4, also reported to be directed against the DQw1 antigen (23), shows the same binding pattern as R1 and S1 (21, 24) and is therefore likely to be directed against the DQwB.1 specificity. The DQwA.1 and DQwB.1 specificities are located on distinct DQ-like molecules that are encoded by the same chromosome. This strongly suggests that they are the products of distinct genes (DQA, DQB). The demonstration of $R1^-Ge^+$ as well as $R1^+Ge^-$ molecules at the cell surface, together with the population analysis showing that the R1 and Ge epitopes are HLA-linked polymorphic determinants with distinct DR antigen associations, make it unlikely that the antibodies recognize different modifications of the same gene product. Two genes for DQ-like alpha, as well as DQ-like beta chains have been identified in the haploid genome (6, 7) and two distinct alpha and beta chains have been detected in some homozygous cells (8, 9). In addition, an I-A-like molecule defined by MAb 33.1 has been isolated from several different LCL including DQw1-bearing cells. By amino acid sequence analysis these molecules are distinct from DQw1 molecules defined by Genox 3.53 (10). It is possible that the DQwB.1 allospecificity defined by MAb R1 and S1 (and presumably B3T/4) is located on these I-A-like molecules.

In man as well as in the mouse, structural polymorphism of the I-E (DR) molecules resides almost entirely in the beta subunit, while in the I-A (DQ) molecules, both subunits are extensively polymorphic (13, 25, 26). For the DQ antigens this has been shown by two-dimensional gel electrophoretic analysis of antigens precipitated from homozygous LCL (3, 13, 27) as well as by restriction endonuclease analysis using DQ-specific probes (28). However, it has been noted (6) that the polymorphism observed by restriction endonuclease analysis is limited to one of the two identified alpha and beta chain genes, while the other two appear, thus far, to be invariant. Since the subunit locations of the R1 and Ge epitopes are not yet known, the following models are consistent with the presence of Ge^+R1^- and Ge^-R1^+ molecules in heterozygous cells. Both epitopes could reside on alpha or beta chains or one epitope may reside on an alpha chain and the other on a beta chain. Preliminary Western blotting analyses on immunoprecipitates indicate that Ge^+R1^+ and Ge^+S1^+ molecules exist, which would suggest that the latter alternative may be correct. If this is indeed the case, the observations reported here of independent immunoprecipitates indicate that alpha-beta

complementation (*trans* and/or *cis*) is a regular occurrence in EBV-transformed B lymphocytes. Studies are in progress to directly determine the location of the R1, S1, and Ge epitopes and to analyze their interactions in homozgous as well as heterozygous cells.

Summary

The monoclonal antibodies Genox 3.53, S1, and R1 define polymorphic epitopes localized to DQ molecules. All three antibodies showed a strong association with the HLA-DR antigens 1, 2, and w6 when tested on a panel of 68 unrelated individuals, suggesting that they all recognized the DQw1 allospecificity. However, segregation analysis and binding studies with a panel of HLA-D/DR homozygous cells indicated that these monoclonal antibodies defined two different alloantigens. Cells homozygous for DR 1, 2, or w6 expressed the epitopes defined by all three antibodies (i.e., S1, R1, and Ge) while cells homozygous for DR4 and DRw8 expressed only the S1 and R1 epitopes. Sequential immunoprecipitation analyses in $S1^+/R1^+/Ge^+$ individuals, in which the three epitopes were shown by segregation analysis to be encoded by the same chromosome, revealed two distinct DQ-like molecules. While R1 and S1 appeared to reside on the same molecule, the epitope defined by Genox 3.53 was on a different molecule. Identical results were obtained with DR1-, DR2-, or DRw6-bearing cells. Thus it appears that DQw1-bearing individuals express two *cis*-encoded DQ-like molecules that carry distinct alloantigenic specificities.

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