

**Dw/LD-RELATED MOLECULAR POLYMORPHISM
OF DR4 β -CHAINS***

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Antigenic determinants encoded by genes of the HLA-D region have been related to at least three separate molecular complexes, DR, MB (also known as DC and LB-E), and SB. At the cell surface each complex has a dimeric structure composed of a structurally conserved α -chain noncovalently associated with a polymorphic β -chain. The DR and MB series of determinants have been extensively characterized using allospecific serological reagents and, to date, 10 DR and 3 MB allotypes are recognized. Beyond these serologically detectable polymorphisms, HLA-D region products exhibit an additional degree of antigenic complexity revealed by cellular reagents such as homozygous typing cells (HTC). These cells respond to antigenic determinants (Dw/LD determinants) that are phenotypically distinct from those recognized by available antisera. Thus, within a panel of individuals of a single serologically defined DR type, such as DR4, a number of different Dw/LD specificities including Dw4, Dw10, DYT, DB3, and LD40 exist (1).

In this report we have addressed this cellularly detected antigenic complexity from a molecular perspective. Using isoelectric focusing (IEF) to analyze DR molecules immunoprecipitated from a panel of individuals homozygous for DR4, but expressing different Dw/LD determinants, we have observed that (a) between DR4 β -chains, but not α -chains, considerable structural polymorphism exists and (b) the particular DR β -chain structural configuration observed upon IEF is associated with the cell Dw/LD type. These data, thus, provide the first molecular evidence associating the Dw/LD determinants recognized by cellular reagents to the serologically defined DR molecule.

Materials and Methods

Identification and Derivation of DR4 Lymphoblastoid Cell Lines. DR and Dw/LD typing was performed according to previously described methods (2, 3). Whole blood was obtained from healthy DR4, Dw/LD homozygous donors. After centrifugation on a Ficoll-Hypaque cushion, lymphocytes were macrophage depleted by plastic adherence and B cell enriched by rosette-depleting T cells with AET-treated sheep erythrocytes (4). LCL were established by infecting the B cell-enriched lymphocyte population with Epstein-Barr virus according to published procedures (5). LCL were maintained with RPMI 1640 containing 25 mM Hepes buffer and 10% heat-inactivated fetal bovine serum.

Cell Labeling, Immunoprecipitation, and Two-dimensional Gel Analysis. LCL were cell-surface-labeled by lactoperoxidase-catalyzed ^{125}I -iodination and membrane proteins detergent-solubi-

* Paper 326 from the Immunobiology Research Center, Depts. of Laboratory Medicine/Pathology and Surgery, University of Minnesota, Minneapolis. Supported by grant AI/GA17687 from the National Institutes of Health and grant 6-255 from the March of Dimes.

lized as described previously (6). DR complexes were isolated from the detergent lysates by immunoprecipitation with a purified HLA-DR monoclonal antibody, clone L243 (7) (Becton, Dickinson & Co., Mountain View, CA) utilizing *Staphylococcus aureus* Cowan strain I as an immunoabsorbent (8). Immunoprecipitated DR molecules were separated into α - and β -chain component by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The appropriate molecular weight regions of the first dimensional gel were identified with fluoresceinated or dansylated molecular weight markers and areas containing the DR α - and β -chains were excised. Flat-bed IEF was performed essentially as described by Shackelford et al. (9). Samples were cast directly into the IEF gel and sealed in place by polymerizing the gel onto the hydrophilic surface of a GelBond PAG film (FMC Corp., Rockland, ME). LKB ampholines (LKB, Bromma, Sweden) and the mixtures used are detailed in the legend to Fig. 2. Focusing was carried out at 600 V for 1 h and 1,100 V for 7 h. After IEF the gel was fixed, dried, and autoradiographed using Cronex II (DuPont Instruments, Wilmington, DE) intensifying screens and Kodak XAR-5 film at -70°C .

Results

Fig. 1 is an SDS-PAGE analysis of molecules immunoprecipitated by the anti-DR antibody, L243, from detergent lysates of four DR4 ^{125}I -labeled LCL that represent four different Dw/LD phenotypes. The L243 antibody used for immunoprecipitation in this study has been well characterized and is known to react with a monomorphic DR determinant (7). Furthermore, this antibody does not appear to recognize MB products, and, in most cases, the reactivity includes all known subsets of mature cell surface DR molecules (9–12). From each cell lysate a typical DR α - β -chain dimer with molecular weights of $\sim 34,000$ and $28,000$, respectively, was immunoprecipitated. Of note is the finding that the DR β -chain from the DR4/Dw10 LCL reproducibly migrates slower than the DR β -chain immunoprecipitated from DR4 LCL of other Dw/LD types (Fig. 1, see asterisk). This has been confirmed with another DR4/Dw10 LCL (data not shown) and suggests that the DR4 β -chain associated with the Dw10 phenotype may be larger, or has a different molecular configuration, than the DR4 β -chains associated with other Dw/LD types.

IEF patterns of DR4 β -chains, isolated from the first dimension SDS gels, are shown in Fig. 2. In our analyses, all DR4 β -chains, regardless of Dw/LD type, focused

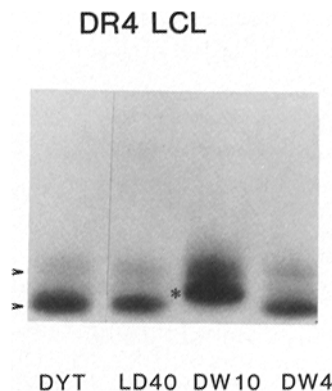


FIG. 1. SDS-PAGE migration patterns of DR4 β -chains from Dw/LD disparate LCL. DR4 chains were immunoprecipitated from lysates of ^{125}I -labeled Dw/LD disparate LCL and analyzed as described in Materials and Methods. In our hands, DR4 α -chains consistently labeled less intensely than the corresponding β -chains using the lactoperoxidase ^{125}I procedure. The reduced mobility of the DR4 β -chains associated with the Dw10 phenotype (see asterisk) was confirmed with another Dw10 LCL. Arrowheads denote position of DR α - and β -chains.

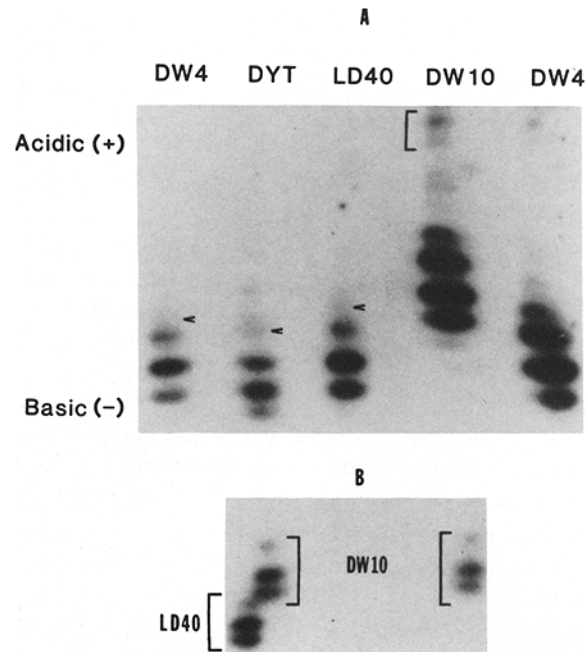


FIG. 2. IEF analysis of DR4 β -chains from Dw/LD disparate LCLs. DR4 β -chains were isolated from the first dimension SDS-PAGE and focused as described in Materials and Methods. (A) Minor DR bands are denoted with arrowheads. Bracketed spots originated from traces of DR4 α -chain present in the first dimension gel slice. Samples were polymerized at the anode and a mixture of pH 3.5-10, 4-6 and 6-8 (1:0.25:0.25) ampholines were used. Conditions of analysis were identical in (B) except that the pH 6-8 ampholine was replaced by pH 3.5-5 ampholine.

as a cluster of three major bands and one or more considerably less intensely labeled bands. These minor bands were frequently not visible under the autoradiographic development conditions as used for the data presented in Fig. 2. Although the extent of this DR β -chain microheterogeneity was essentially identical between Dw/LD disparate LCL, considerable differences were observed in the equilibrium focusing positions of the DR β -chain band clusters. Thus, while the DR β -chains of the three DR4/Dw4 LCL examined (two are shown in Fig. 2 A) showed IEF patterns that were indistinguishable, the DR β -chains from the two DR4/Dw10 LCL, while identical to one another (Fig. 2 B), exhibited markedly more acidic equilibrium focusing positions (Fig. 2 A). Furthermore, the DR4/DYT β -chain cluster is reproducibly more basic as compared to the DR4 β -chains from Dw4, LD-40, and Dw10 LCLs (Fig. 2 A). The DR β -chain cluster of the DR4/LD40 LCL migrated in a manner apparently identical to the DR β -chains from the DR4/Dw4 LCL in several experiments. Neither the use of ampholine mixtures tailored to provide expanded pH ranges nor combining DR immunoprecipitates from DR4/Dw4 and DR4/LD40 LCL could discriminate between these two populations of DR β -chains (data not shown). The DR α -chains from the Dw/LD disparate LCL were indistinguishable from one another by both one and two dimensional gel analyses for all the LCL studied (data not shown).

Discussion

Studies to date have indicated that the β -chain of the DR complex is both serologically and structurally polymorphic in the population, i.e., DR β -chains of

different DR allotypes exhibit different IEF characteristics (13–15). However, structural polymorphism between DR β -chains isolated from cells carrying the same serologically defined DR specificity has not been previously described. Our present study provides firm evidence in this regard: not only is there a minor difference in the migration of the DR β -chains from DR4/Dw10 LCL detectable in first dimension SDS-PAGE, but there are at least three different groups of IEF patterns detectable in the DR β -chains isolated from the seven DR4 LCL examined in this study. There is, thus, clear polymorphism of the DR4 β -chains that can be demonstrated at the molecular level but that is not detected with the currently available alloantisera.

We recognize that DR4, as currently defined serologically, may in the future be “split.” This does not detract from the central focus of these studies, which was to determine whether a molecular corollary for the Dw/LD polymorphism detected among DR4 cells by cellular reagents exists within the DR4 β -chain populations. No conclusions have, in the past, been possible regarding the molecular relationship of DR and Dw/LD specificities despite their close association in population and family studies (16). However, recent studies using mutants of the LCL line, 721 (17, and R. DeMars, C. Chang, and R. Rudersdorf, manuscript in preparation), in combination with “cloned” primed lymphocyte typing (PLT) reagents (18), have suggested a close genetic association between DR and Dw/LD (19).

The findings presented above provide evidence that variation in the structure of the DR4 β -chain correlates with at least three different Dw/LD phenotypes. Although our IEF analyses could not distinguish between the DR4 β -chains from LD40 and Dw4 LCLs, these data do not rule out differences between these molecules. Structural modification of DR β -chains sufficient to cause conformational changes and, hence, presentation of a modified antigenic profile may not necessarily result in overall charge changes. For example, altered complexity of carbohydrate moieties occurring without concomitant changes in the number of terminal sialic acid residues would not be detected by these IEF procedures.

Summary

The results of this study demonstrate the existence of molecular heterogeneity (polymorphism) within DR β -chains isolated from a single serologically defined DR phenotype, DR4. The data are consistent with the possibility that this polymorphism is related to the Dw/LD phenotype as defined with the cellular reagents, homozygous typing cells.

The authors would like to thank N. Reinsmoen, H. Noreen, and the tissue typing laboratories for cellular and serological phenotyping; J. Doughman is acknowledged for excellent technical assistance and J. Gfrerer and N. Johnson for typing the manuscript.

Received for publication 12 January 1983.

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