TWO-DIMENSIONAL GEL ELECTROPHORETIC ANALYSIS
OF THE MT3 AND DR4 MOLECULES FROM THE
DIFFERENT D-TYPED CELLS

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The HLA-D/DR region codes for the human class II antigens that play
important roles in many immune phenomena (1). At the Eighth International
Histocompatibility Workshop, 12 D specificities defined by the mixed lymphocyte
reaction and 10 DR specificities defined by the microcytotoxicity test using
alloantisera have been approved (2). The DR specificity correlates to the D
specificity for the most part, but there are some populations that have the same
DR specificity but different D specificities. In the DR4 population, there are at
least six D specificities (Dw4, Dw10, DYT, DKT2, DB3, and LD40) (3, 4). On
the other hand, it has been shown that there are at least three kinds of human
class II molecules, DR, DC(MB), and SB antigens (5-7). Furthermore, we have
demonstrated that the MT3 molecule exists independently of the DR molecule
in a DR4 homozygous cell line and that the MT3 antigen may belong to the
fourth group of human class II antigen, distinct from the DR, DC, and SB
antigens (8). It is important to clarify which class II molecules are involved in
the dissection of the D specificity. In the analysis of the relationship between
HLA-D and class II molecules, we must consider not only the DR molecule but
also the other class II molecules of which specificities are closely linked to HLA-
DR. In fact, Zeevi et al. (9) have already prepared the primed lymphocyte typing
(PLT) clones whose specificities are associated with DR4, MB3, or MT3, using
DR4-positive cells for the stimulator. In addition, it has been shown that three
D specificities (Dw4, DKT2, DYT) can be distinguished by their differential
reactivities against two kinds of monoclonal antibodies that seem to recognize
the determinants on the MB3 molecule (10, 11), suggesting that the class II
molecule coded for by the DC locus also relates to the differences in the D
specificities. It has also been reported, using monoclonal antibodies against a DR
monomorphic determinant and two-dimensional (2-D)1 gel electrophoresis, that
the DR light chain showed a structural polymorphism among the DR4 homozy-

1 Abbreviations used in this paper: 2-D, two-dimensional.

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gous cell lines and that the polymorphism correlated with the differences of the D specificities (12, 13), though it was not confirmed in these studies whether the class II molecule recognized by the monoclonal antibodies carried the DR4 alloantigenic determinant defined serologically. On the other hand, it is not yet clear whether the MT3 molecule show a structural polymorphism among the DR4 homozygous cell lines with different D types.

In this paper, to analyze which class II molecules mainly cause the dissection of the D specificity among Dw4, DKT2, and DYT, we identify the major class II antigens and compare qualitatively and quantitatively among the cell lines with different D specificities by 2-D gel electrophoresis and alloantisera.

Materials and Methods

Cell Lines and Culture. Epstein-Barr virus transformed B lymphoid cell lines Wa (HLA-Aw24/Aw24, Bw54/Bw54, DYT/DYT, DR4/DR4, MT3/MT3), L-KT13 (HLA-Aw24/Aw31, Bw51/B7, DKT2/DKT2, DR4/DR4, MT3/MT3), and ER (HLA-A2/A2, Bw44/Bw44, Dw4/Dw4, DR4/DR4, MT3/MT3) were kind gifts from Dr. A. Wakisaka, Hokkaido University, Sapporo, Dr. N. Kashiwagi, Kitasato University, Sagamihara, and Dr. T. Sasazuki, Tokyo Medical and Dental University, Tokyo, respectively. All cells were maintained at 2×10⁵ cells/ml in RPMI 1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum.

Antisera. Alloantisera 070402 (anti-DR4), DKA05 (anti-DR4), and DKA11 (anti-MT3) were purchased from Hoechst Japan Co., Tokyo. Alloantisera TD148 (anti-MT3) and S19 (anti-MT3) were kind gifts from Dr. H. Maeda, Tokyo University Hospital, Tokyo and from Saitama Blood Center, Japan Red Cross, Saitama, respectively. Alloantiserum T2297 (anti-DR4) was obtained from the Blood Transfusion Service, Tokyo Women’s Medical College.

Purification of Membrane Glycoproteins and Labeling. The membrane glycoprotein was purified as described previously (14, 15). Briefly, the membrane from 2–6×10⁹ cells were solubilized with 1% Nonidet P-40 in 0.15 M NaCl, 20 mM Tris-HCl, pH 7.8. Glycoproteins were purified from the membrane fraction by lentil lectin chromatography. A small portion of the glycoproteins was labeled with ¹²⁵I by the chloramine T method.

Immunoprecipitation and 2-D Gel Electrophoresis. Immunoprecipitation was performed as described previously (16). Briefly, 5–10 µl labeled glycoproteins were incubated with 30 µl alloantisera at 4°C overnight. Then 20 µl protein A-Sepharose CL-4B was added to the reaction mixture. The antigen-antibody complex thus isolated was analyzed by 2-D gel electrophoresis, performed as described previously (17). The ¹²⁵I-labeled glycoproteins were detected on the 2-D gel by autoradiography and unlabeled glycoproteins were detected by Coomassie Brilliant Blue staining.

Results

To reveal the quantitative and qualitative differences of the class II antigens expressed on B cells, we first analyzed and compared the membrane glycoproteins from three DR4 homozygous cell lines with different D types (Dw4, DKT2, DYT) by 2-D gel electrophoresis and Coomassie Brilliant Blue staining. Fig. 1B and C show that under these conditions, one heavy chain (H1) and two sets of light chains of class II-like molecules are detected in ER (Dw4) and L-KT13 (DKT2) cells. There is a large difference in the amounts of the two different sets of the light chains. We tentatively designated the set of light chains in a larger amount, L1, and the set of the light chains in a lesser amount, L2. In Wa (DYT) cells, only one set of heavy and light chains (H1, L1) is detected (Fig. 1A). This result is probably due to less expression of L2 in Wa cells than in the other two
FIGURE 1. 2-D gel analysis of the membrane glycoproteins from Wa (DYT) (A), ER (Dw4) (B), and L-KT13 (DKT2) (C) cells. Approximately 150 μg of the membrane glycoproteins from Wa and ER and about 200 μg of the membrane glycoproteins from L-KT13 were subjected to the electrophoresis. Spots were detected by Coomassie Brilliant Blue staining. The basic end of the NEPHGE gel is on the left and the direction of sodium dodecyl sulfate-polyacrylamide gel electrophoresis is from top to bottom. The portion of the gels shown corresponds to the region between 25,000 and 50,000 daltons.

cell lines. The comparison of the 2-D gel pattern among the three cell lines revealed that the electrophoretic mobility of L1 is different from one another. The pI of L1 from Wa (DYT) is the most basic, followed in order by those from ER (Dw4) and L-KT13 (DKT2). On the contrary, significant differences in the electrophoretic mobilities are not observed between L2 from ER and L-KT13 and among the heavy chains (H1) from the three cell lines.

To clarify what alloantigenic determinants reside in these two sets of class II-like molecules, the 125I-labeled membrane glycoproteins from these cell lines were precipitated with three kinds of anti-DR4 typing alloantisera and three kinds of anti-MT3 alloantisera. Fig. 2A shows the 2-D gel pattern of the precipitates with anti-DR4 alloantiserum T2297 from Wa, ER, and L-KT13. The anti-DR4 alloantiserum T2297 precipitates only one set each of heavy and light chains in these cell lines. The other two anti-DR4 alloantisera (070402,
DKA05) also precipitated the same heavy and light chains. In contrast, normal human serum did not precipitate these heavy and light chains (data not shown). Therefore, these heavy and light chains are considered the DR4 heavy and light chains in each cell line. Although the heavy chains of the DR4 molecules from these three cell lines show the same electrophoretic mobility, the relative position of the light chain of the DR4 molecules are different from one another among the three cell lines. The pI of the DR4 light chain from Wa (DYT) is the most basic, and that from L-KT13 (DKT2), the most acidic. Since the electrophoretic mobility of the DR4 light chain in Fig. 2A and of L1 in Fig. 1 are identical in each of the three cell lines, L1 is considered the DR4 light chain.

Fig. 2B shows the 2-D gel pattern of the precipitates with anti-MT3 alloantiserum TD148 from Wa, ER, and L-KT13. The heavy chain precipitated with TD148 is indistinguishable from the DR heavy chain in the three cell lines. Minor light chain spots in Wa and L-KT13 in Fig. 2B show the same electrophoretic mobility as that of the DR4 light chain in Fig. 2A in each cell line. The minor spots are considered to represent the DR4 light chains which are precipitated with a contaminated antibody against DR4. We have already shown (8) that anti-MT3 alloantisera often contain the antibody against DR and that the DR4 molecule does not carry the MT3 antigenic determinant. The major light

![Figure 2](image-url)

**Figure 2.** 2-D gel analysis of the immunoprecipitated DR4 (A) and MT3 (B) molecules from Wa (DYT), ER (Dw4), and L-KT13 (DKT2) cells. 125I-labeled membrane glycoproteins precipitated with anti-DR4 alloantiserum (T2297) (A) and anti-MT3 alloantiserum (TD148) (B). The upward arrowhead indicates the DR4 heavy chain and the downward arrowheads show the DR4 light chain. The upward and downward arrows indicate the MT3 heavy and light chains, respectively. Gel orientation is the same as in Fig. 1.
chain precipitated with TD148 is different from the DR4 light chain in each of Wa and L-KT13 cells. TD148 precipitates only one set of heavy and light chain in ER cells and the light chain is distinct from the DR4 light chain. To illustrate the difference in the relative position of the MT3 alloantisera-specific light chain and the DR4 light chain in ER cells, the 2-D gel pattern of the precipitate with DKA11 (anti-MT3) from this cell line is presented in Fig. 3B, because DKA11 precipitates a small amount of the DR4 light chain in addition to the same heavy and light chains precipitated with TD148. Furthermore, the mixed experiment shows that the MT3 alloantisera-specific light chains from these three cell lines are indistinguishable from one another (Fig. 3A). We have already shown (8) that the heavy chain and the MT3 alloantisera-specific light chain precipitated with TD148 are the components of the MT3 molecule in Wa cells. Since the heavy chain and the MT3 alloantisera-specific light chain precipitated with TD148 from ER and L-KT13 cells are identical to the MT3 heavy and light chains of Wa cells, these heavy and light chains are components of the MT3 molecule in ER and L-KT13 cells. The other two anti-MT3 alloantisera (DKA11, S19) gave the same results. These data indicate that the three cell lines possess the same MT3 molecule, which consists of a heavy chain indistinguishable from the DR heavy chain and the light chain specific for the MT3. Since the MT3 light chain and L2 in Fig. 1 show the same electrophoretic mobility, L2 are considered the MT3 light chains in ER and L-KT13 cells. These results indicate

Figure 3. (A) Comparison of the MT3 molecules from Dw4, DKT2, and DYT homozygous cell lines. One third of TD148 immunoprecipitates from Wa (DYT), ER (Dw4), and L-KT13 (DKT2) were mixed and coelectrophoresed. (B) 2-D gel analysis of the immunoprecipitate with DKA11 (anti-MT3) from ER (Dw4) cells. The indication and gel orientation are the same as in Fig. 2.
that the MT3 molecule is less expressed than the DR4 molecule on the three cell lines.

Discussion

The D specificity is likely to be decided by the sum of the differences of class II molecules. To decide which class II molecule mainly causes the dissection of the D specificity, we must analyze both the qualitative differences of class II antigens among the cells with different D specificities and the quantitative differences of the class II molecules in each cell. In this paper, we have demonstrated directly, using 2-D gel electrophoresis and anti-DR4 alloantisera, that the light chains that carry the DR4 alloantigenic determinant show a structural polymorphism among the DR4 homozygous cell lines with different D specificities (Dw4, DKT2, DYT) (Fig. 2A). We have also shown, by the electrophoretic analysis of the membrane glycoproteins, that most of the class II light chains consist of the DR light chain (Fig. 1). On the contrary, both the heavy and light chains of the MT3 molecules are identical among the DR4 homozygous cell lines with the different D specificities (Figs. 2B, 3). Even if the class II molecules encoded by the DC locus are different from one another among the three cell lines, the differences seem to play only a minor role in the dissection of the D specificity, because the DC locus product expresses little compared with the DR locus product (Fig. 1). These results indicate that the dissection of the D specificity among Dw4, DKT2, and DYT is mainly caused by the differences of the DR4 molecules and that the MT3 molecule does not participate in the dissection.

We have shown in a previous paper (8) and in this paper that the light chain of the MT3 molecule is specific for the MT3 but that the heavy chain of the MT3 molecule is indistinguishable from that of the DR. We have also shown that three different DR4 homozygous cell lines with different D specificities have the identical MT3 molecule. Furthermore, our recent data (manuscript submitted for publication) show no electrophoretic heterogeneity in the MT3 molecule among the DR4, DR7, and DRw9 homozygous cell lines. These observations suggest that the MT3 molecule has no structural polymorphism among the DR4, DR7, and DRw9-positive populations. On the other hand, the class II molecules carrying other supertypic specificities (MB1, MB3) show both serological and structural polymorphisms (7, 11, 18, 19). Our recent analysis (unpublished data) of the MT2 molecule from DR3, 5, w6, and w8 homozygous cells by 2-D gel electrophoresis also suggests that the MT2 molecule is structurally polymorphic. The frequencies of the MT3 antigen are 0.690 in the oriental population and 0.491 in the Caucasian population (20). If the MT3 molecules have no heterogeneity at the population level, the MT3 antigen is one of the most common specificities among the class II antigen specificities. These findings may indicate that the MT3 gene is the prototype of the genes of the human class II antigens.

Summary

This report demonstrates directly, using two-dimensional gel electrophoresis and alloantisera, the following: (a) The DR4 light chains show a structural
polymorphism among the Dw4, DKT2, and DYT cells. (b) Most of the class II light chains consist of the DR light chain. (c) The MT3 molecule is distinct from the DR4 molecule in the Dw4, DKT2, and DYT cells. (d) The MT3 molecule does not show any structural heterogeneity among the Dw4, DKT2, and DYT cells. These results suggest that the dissection of the D specificity among Dw4, DKT2, and DYT is mainly caused by the differences of the DR4 molecules.

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