# MOLECULAR LOCALIZATION OF HUMAN CLASS II MT2 AND MT3 DETERMINANTS

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The antigens encoded in the *HLA-D* region of the human major histocompatibility complex (MHC)[1] were originally defined with homozygous typing cells by mixed leukocyte culture and, subsequently, by use of human alloantisera reacting with antigens selectively expressed on B cells (1, 2). These antigens were later shown to be the human equivalents of the murine Ia molecules. The major serologically defined specificities, called DR, were found to be closely associated with the specificities defined by HTCs. Later, the development of alloantisera permitted identification of additional serologic specificities such as DC, MB, MT, and Te (3–8) which are not associated with a single DR specificity but, instead, are found associated with two or more DR alleles and are, therefore, called supertypic. For example, MT2 is usually found on DR3-, DR5-, DRw6-, or DRw8-bearing cells while MT3 is usually found on cells from DR4-, DR7-, or DRw9-positive donors. The apparent equivalence of terms used by different investigators is shown in Table I (see also reference 3).

Studies of the structural bases for these serological specificities have focused on three types of molecules that have been described biochemically. In a total Ia isolate, DR molecules are the predominant set of *HLA-D* region antigens and are homologues of the murine I-E molecules (9). These molecules bear the DR serologic specificities, which have been shown to be associated with their polymorphic beta chains (9). A DR homozygous cell line expresses DR molecules that are a set of closely related proteins which appear to share a single alpha chain and possess several structurally distinct beta chains (10, 11). It is not clear if all of these I-E-like species carry the DR serologic determinants that are defined with alloantisera. The DS (DC) antigens are homologues of the murine I-A antigens (12) and bear the MB serologic determinants (13, 14). There may be

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[1] *Abbreviations used in this paper:* FACS, fluorescence-activated cell sorter; MHC, major histocompatibility complex; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

TABLE I
*Supertypic Series of HLA-D Region Antigens*

| MB Series | | | MT Series | | |
|---|---|---|---|---|---|
| MB | Associated DR specificities | Equivalent specificities | MT | Associated DR specificities | Equivalent specificities |
| MB1 | DR1, 2, w6, w10 | DC1, Te21, MT1 | MT1 | DR1, 2, w6, w10 | DC1, Te21, MB1 |
| MB2 | DR3, 7 | DC3, Te24 | MT2 | DR3, 5, w6, w8 | (BR3)* |
| MB3 | DR4, 5, w9 | DC4, Te22, MT4 | MT3 | DR4, 7, w9 | (BR4)* |

* Molecules bearing MT2 and MT3 determinants have been called BR3 and BR4, respectively (20, 24).

two different kinds of DS molecules expressed by a DR homozygous cell line but only one may carry MB determinants (15 and R. G. Giles, R. DeMars, and J. D. Capra, unpublished results). The third set of *HLA-D* region antigens are the SB antigens which are intermediate in homology with the murine I-A and I-E antigens and are distinct from both DR and DS antigens (16–18).

The nature of the *HLA-D* region molecules that bear the MT2 and MT3 supertypic specificities has been controversial. Two-dimensional gel electrophoresis and peptide mapping have identified MT determinants on DR molecules (19–22), on DS molecules (21, 22), and even on a third set of molecules related to, but distinct from, DR and DS, sometimes termed BR (20, 23, 24). In addition, two studies have shown that there may be a sharing of MT serologic determinants between DR and DS molecules (21) and between DR and BR molecules (20). Three different constructs have emerged from these studies to explain the MT2 and MT3 specificities: (*a*) MT2 and MT3 reside on DR molecules, (*b*) MT2 and MT3 reside on DS molecules, and (*c*) MT2 and MT3 reside on molecules that are distinct from DR and DS, and are the products of an additional locus. Since the majority of studies used complex alloantisera in their analyses, additional reactivities directed against specificities other than MT might have added to the number of molecules observed.

Monoclonal antibodies directed against MT2- and MT3-like supertypic specificities were used in this study to define the molecules bearing these specificities from a set of cell lines. MT2- and MT3-bearing molecules have been compared with DR, DS, and, in some instances, SB molecules derived from the same cell line using inhibition of cell surface fluorescence or cytotoxicity, as well as amino acid sequence analysis and peptide mapping. The data show that the major populations of molecules bearing MT2 and MT3 determinants are indistinguishable from DR molecules.

## Materials and Methods

*Monoclonal Antibodies.* The murine monoclonal antibodies with MT-like specificities, I-LR2 (25) and 109d6 (26, 27), have been previously defined. Monoclonal antibody L203 recognizes monomorphic determinants present on all B cell lines. By amino acid sequence analysis, the molecules recognized by L203 are homologous to murine I-E antigens and have been termed DR for this reason (28). Monoclonal antibody L203 blocks many anti-DR alloantisera, suggesting that it recognizes molecules bearing the serologically defined DR specificities (29, 30). The monoclonal antibody IIIE3 recognizes a subset of the L203-reactive antigens which, by sequence analysis of the reactive molecules, has been identified

as DR-like (28). Additional binding studies using HLA deletion mutants suggest that L203 and IIIE3 are directed against framework determinants of DR antigens (31).[2] Monoclonal antibody I-LR1 recognizes SB antigens; however, in a DR5 cell it also binds to DR molecules (C. K. Hurley and S. Shaw, unpublished results). The monoclonal antibody IVD12 is specific for the MB3 supertypic specificity and primarily detects DS molecules on both DR4 and DR5 cell lines (13, 32). SFR3-DR5, a DR5-specific monoclonal antibody, was a gift from Dr. Susan Radka (33). Monoclonal antibody 7.3.19.1, a monoclonal antibody with an MT2-binding pattern, was a gift from Frits Koning (34). Monoclonal antibody 61D2 is directed against HLA-A, B, C antigens (35). Monoclonal antibody L368, directed against $\beta$2-microglobulin, was a gift from Dr. Ron Levy.

*Cell Lines.* 721.127 (DR3) is a B lymphoblastoid cell line bearing only one copy of the MHC because the other copy was physically deleted by means of $\gamma$ irradiation (31). PRIESS (DR4) has been described (36). Cell lines GM3105A (DR5), GM3164 (DR4), and GM3163 (DR7) were from the Human Genetic Mutant Cell Repository, Camden, NJ. 113w7 (DR7) and DHI (DR5) were gifts from Dr. Mary Ann Robinson. YBE (DR3) was a gift from Dr. John Sachs.

*B Cell Panel.* Peripheral blood B cells from members of the tissue-typing laboratory reference panel were screened by indirect immunofluorescence for the presence of I-LR2- or 109d6-reactive cell surface antigens. Reactivity with I-LR2, 109d6, or control monoclonal antibodies was determined by counting positive cells in a fluorescence microscope equipped with epifluorescence optics. The HLA-DR, MB, and MT phenotype of the cells used in the B cell panel had been previously determined by microcytotoxicity using typing reagents from the Eighth International Histocompatibility Workshop, as well as local alloantisera. Likewise, B cells from several informative families were examined by fluorescence for their ability to bind I-LR2 and 109d6.

*Preparation of Fluorescein-conjugated Monoclonal Antibodies.* Monoclonal antibodies I-LR2 and L203 were isolated by ammonium sulfate fractionation followed by DEAE-Sephadex chromatography. This material was then conjugated with fluorescein isothiocyanate and made up to a protein concentration of 1 mg/ml (37).

*Fluorescence Inhibition Experiments.* Cells ($1 \times 10^6$) from the B cell lines DHI, GM3105A, and 721.127 were suspended in 100 $\mu$l of a saturating concentration of ascites (1:50) containing various monoclonal antibodies except SFR3-DR5, which was used as a 5× concentrate of culture supernatant. Except for control antibodies and antibodies with polymorphic specificities, all antibodies were shown to bind to GM3105A, DHI, and/or 721.127 by indirect immunofluorescence. The exceptions were shown by indirect immunofluorescence to bind to the appropriate cells. Briefly, cells were incubated with the monoclonal antibodies, washed, and stained with fluoresceinated rabbit anti-mouse IgG (Cappel Laboratories, Cochranville, PA). The stained cells were examined using the fluorescence-activated cell sorter (FACS III; B-D FACS Systems, Sunnyvale, CA). To test for inhibition, the cells were incubated for 30 min at 4°C with various monoclonal antibodies and washed twice with cold RPMI 1640 containing 0.1% sodium azide. After incubation with 100 $\mu$l of a 1:20 dilution of fluoresceinated I-LR2, the cells were washed twice and analyzed in the FACS.

*Alloantisera.* Alloantisera specific for the specificities MT2 (9w688 from H. Festenstein, 9w689 and 9w699 from D. Woodfield), MT3 (9w733 from M. Fotino, 9w734 from J. Dausset, 9w743 from N. Suciu-Foca), DR4 (9w575 from P. Terasaki), DR7 (9w630), and DR5 (9w597 from H. Betuel and 9w603 from D. Singal) were obtained as part of the Ninth International Workshop serum exchange. All of these sera were tested in our laboratory with a panel of B cells from at least 75 individuals and were found to have high correlation ($r > 0.8$) with their given specificities.

*Lymphocytotoxicity Inhibition.* To test the ability of monoclonal antibodies L203, I-LR2, IVD12, and 109d6 to inhibit the cytotoxic activity of specific alloantisera, cells were precoated with monoclonal antibody before performing the cytotoxicity assay. Cells incubated with L368, a monoclonal antibody reactive with $\beta$2-microglobulin, were used

[2] Shaw, S., A. Ziegler, and R. DeMars. Specificity of monoclonal anti-murine and anti-human Ia antibodies analyzed by binding HLA deletion mutant cell lines. Manuscript submitted for publication.

as negative controls. Briefly, cells ($4 \times 10^6$/ml) were incubated with an equal volume of monoclonal antibody (ascites at a 1:100 dilution) for 30 min at room temperature and washed in RPMI 1640 medium. After a 15 min incubation with an $F(ab')_2$ fragment of a sheep anti-mouse IgG antibody (Cappel Laboratories) at room temperature, cells were washed and suspended in RPMI containing 10% fetal calf serum. Cytotoxicity was performed according to the standard NIH complement-dependent microcytotoxicity method using MT- and DR-specific alloantisera. Percent lysis was determined by eosin dye exclusion using an inverted microscope.

*Isolation of Radiolabeled Antigens.* Cells were radiolabeled with $^3$H-amino acids and lysed with Nonidet P-40. A glycoprotein pool was affinity purified on a lentil lectin column as previously described (28). Briefly, the *HLA-D* region antigens were isolated by passage of the glycoprotein pool over antibody affinity columns using either 0.05 M diethylamine or 1.5 M ammonium thiocyanate for elution. For some studies, radiolabeled antigens were isolated by immunoprecipitation. In these experiments, an immunoglobulin-depleted pool was incubated with monoclonal antibody either in the form of an ammonium sulfate fraction or 10 times concentrated culture supernatant. Rabbit or goat anti-mouse immunoglobulin (Cappel Laboratories) was added and the immune complexes removed by centrifugation. Alpha and beta polypeptide chains were isolated from the purified antigens using preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (28).

*Amino Acid Sequence Determination and Peptide Mapping.* Sequential Edman degradation of isolated alpha and beta chains labeled with a single $^3$H-amino acid was performed using Beckman 890C and 890D amino acid sequencers (Beckman Instruments, Inc., Fullerton, CA) (28). The resultant butyl chloride fractions were monitored for radioactivity using a scintillation counter.

For peptide mapping, $\sim 5 \times 10^4$ cpm of an isolated chain plus a protein carrier were dialyzed against water to remove excess SDS, lyophilized to dryness, and redissolved in 0.1 M ammonium bicarbonate. 50 μg of trypsin (TPCK-Trypsin; Worthington Biochemical Corp., Freehold, NJ) were added and the reaction mixture incubated at 37°C for 3 h. Fresh trypsin was added and the digestion continued overnight. The peptides were separated using a Waters high performance liquid chromatograph equipped with a MicroBondapak C18 column (Waters Associates Inc., Milford, MA). A linear gradient of 0.02% trifluoroacetic acid and 70% acetonitrile/30% isopropanol was used to elute the peptides. Aliquots from the gradient were examined for radioactivity as above.

*Affinity Chromatography of Column Eluates.* Several experiments were performed to assess the reactivity of certain antigens with two different monoclonal antibodies. These experiments were designed to determine the degree of "uniqueness" vs. "overlap" of MT and DR specificities. Such experiments were performed with several cell lines (721.127, 113w7, and GM3163) and the I-LR2 (MT2), 109d6 (MT3) and L203 (DR) monoclonal antibodies. A typical experiment comparing L203 and I-LR2 is described. A glycoprotein pool from the cell line 721.127 was chromatographed on a L203 antibody affinity column. Protein carrier was added to the eluate containing the L203-reactive antigens and, after dialysis to remove the elution buffer, the eluate was split into three aliquots. One aliquot was repassed over the L203 column, one aliquot was passed over an I-LR2 affinity column, and the third aliquot was passed over an irrelevant antibody column. The eluates from the three columns were dialyzed and examined by SDS-PAGE. Estimates of counts bound were made by totalling the area under the alpha and beta chain peaks from the gels. The reverse experiment using an I-LR2 eluate was performed as above. In the case of the 109d6/L203 comparisons, estimates of the counts bound were determined by measuring the radioactivity in each column eluate.

## Results

*Monoclonal Antibodies Recognize MT2- or MT3-like Supertypic Specificities.* Monoclonal antibody I-LR2, when tested on homozygous typing cells, exhibited an MT2-specific pattern of binding (25). Further studies using periph-

TABLE II

*Correlation Between HLA-D Region Phenotype and I-LR2 Reactivity from a B Cell Panel of 72 Donors*

| Phenotype | +/+* | +/− | −/+ | −/− | $\chi^2$ | $r$ |
|---|---|---|---|---|---|---|
| DR1 | 11 | 7 | 28 | 26 | 0.167 | 0.048 |
| DR2 | 8 | 13 | 32 | 19 | 2.700 | −0.194 |
| DR3 | 20 | 0 | 19 | 33 | 20.945 | 0.539 |
| DR4 | 13 | 17 | 28 | 14 | 2.992 | −0.203 |
| DR5 | 13 | 0 | 26 | 33 | 11.265 | 0.395 |
| DRw6 | 9 | 0 | 30 | 33 | 6.721 | 0.305 |
| DR7 | 1 | 6 | 38 | 27 | 3.347 | −0.215 |
| DRw8 | 1 | 6 | 39 | 26 | 3.657 | −0.225 |
| DRw9 | 1 | 0 | 38 | 33 | 0.007 | 0.009 |
| DRw10 | 0 | 5 | 40 | 27 | 4.516 | −0.250 |
| MB1 | 22 | 21 | 18 | 11 | 0.451 | −0.079 |
| MB2 | 21 | 6 | 19 | 26 | 7.260 | 0.317 |
| MB3 | 23 | 18 | 17 | 14 | 0.017 | 0.015 |
| MT2 | 40 | 6 | 0 | 26 | 47.408 | 0.811 |
| MT3 | 15 | 18 | 25 | 14 | 1.818 | −0.158 |
| DR3 + 5 + w6 | 38 | 0 | 0 | 34 | 68.043 | 0.972 |

$\chi^2$ with Yates' correction; $r$ values were obtained using the formula $r = \sqrt{\chi^2/n}$ and the positive or negative correlation by the formula ad = bc.

* Presence of *HLA-D* region phenotype/I-LR2 reactivity by immunofluorescence.

eral blood B lymphocytes from a panel of 72 normal individuals confirmed this specificity (Table II). I-LR2 bound to cells bearing DR specificities 3, 5, and w6, which are included in the MT2 supertypic specificity, with a correlation of 0.811. Note that I-LR2 did not react with most DRw8 cells although all of the DRw8 cells had been typed as MT2 positive with alloantisera. If only DR3, 5, and w6 are considered, the combined correlation with I-LR2 reactivity is 0.972 (Table II). A similar specificity pattern has been observed for an independently derived MT2-like monoclonal antibody, 7.3.19.1 (34). Segregation of the I-LR2-reactive determinant with DR3 and DR5 was observed in two potentially informative families. The inheritance of I-LR2 in one of those families is illustrated in Table III. As indicated in one family (Deh, Table III), I-LR2 binding was inherited in parallel with reactivity to DR3 and MT2 alloantisera; in a second family (data not shown), I-LR2 binding was inherited in parallel with reactivity to DR5 and MT2 alloantisera.

The specificity of the monoclonal antibody 109d6 as MT3 has been defined using homozygous typing cells (26, 27, 38). Studies on peripheral blood B lymphocytes from normal and diseased patients supported this assignment (26, 27, 38). Additional panel testing on 20 normal individuals and 38 patients further confirmed the specificity of the antibody (Table IV). Every donor who was typed positive for MT3 reactivity using alloantisera was positive for 109d6 reactivity. Six donors that were MT3 negative also reacted with 109d6. This was due to the binding of 109d6 to DRw10-bearing cells. The correlation of 109d6 reactivity with MT3, therefore, was 0.768. However, when DRw10 binding was added into the calculation (Table IV), the correlation became 0.961. Additional experiments are being performed to determine the nature of the target antigen

TABLE III

*Segregation of I-LR2 and 109d6 Reactivity with HLA in Families*

| Family member | HLA genotype | | | | Haplotypes | Reactivity | |
|---|---|---|---|---|---|---|---|
| | A, B, C | DR | MB | MT | | I-LR2 | 109d6 |
| Deh Father | 1, w57, w6 | 7 | 2 | 3 | a | + | + |
| | w24, w38, – | 3 | 2 | 2 | b | | |
| Mother | 1, w44, w5 | 4 | 3 | 3 | c | – | + |
| | w24, 7, w7 | 4 | 3 | 3 | d | | |
| Sib 1 | 1, w57, w6 | 7 | 2 | 3 | a | – | + |
| | w24, 7, w7 | 4 | 3 | 3 | d | | |
| Sib 2 | 1, w57, w6 | 7 | 2 | 3 | a | – | + |
| | 1, w44, w5 | 4 | 3 | 3 | c | | |
| Sib 3 | w24, w38, – | 3 | 2 | 2 | b | + | + |
| | 1, w44, w5 | 4 | 3 | 3 | c | | |
| Sib 4 | w24, w38, – | 3 | 2 | 2 | b | + | + |
| | w24, 7, w7 | 4 | 3 | 3 | d | | |
| Her Father | w24, w35, w4 | 5 | 3 | 2 | a | + | – |
| | 28, –, w3 | 5 | 3 | 2 | b | | |
| Mother | w24, 18, – | 5 | 3 | 2 | c | + | + |
| | w24, w61, w3 | 4 | 3 | 3 | d | | |
| Sib 1 | 28, –, w3 | 5 | 3 | 2 | b | + | – |
| | w24, 18, – | 5 | 3 | 2 | c | | |
| Sib 2 | 28, –, w3 | 5 | 3 | 2 | b | + | – |
| | w24, 18, – | 5 | 3 | 2 | c | | |
| Sib 3 | 28, –, w3 | 5 | 3 | 2 | b | + | + |
| | w24, w61, w3 | 4 | 3 | 3 | d | | |
| Sib 4 | 28, –, w3 | 5 | 3 | 2 | b | + | + |
| | w24, w61, w3 | 4 | 3 | 3 | d | | |
| Sib 5 | w24, w35, w4 | 5 | 3 | 2 | a | + | – |
| | w24, 18, – | 5 | 3 | 2 | c | | |

recognized by 109d6 on cells from DRw10-positive donors. The inheritance of 109d6 with HLA haplotypes is shown in Table III.

To further support the conclusion that I-LR2 and 109d6 recognize determinants associated with the MT2 and MT3 supertypic specificities, the ability of the monoclonal antibodies to block cytotoxicity of MT-specific alloantisera was examined with both B cell lines and normal B cells. The results of some of these studies are shown in Tables V, VI, and VII. Table V compares the ability of I-LR2 to block MT2-specific alloantisera to that of the anti-DR monoclonal antibody L203. While L203 was able to block the three MT2 alloantisera in all combinations, I-LR2 was only able to block partially two of the three MT2 sera. I-LR2, in contrast to L203, was unable to block alloantisera directed against DR specificities. IVD12, an anti-DS (MB3) monoclonal antibody, did not block DR- or MT2-directed cytotoxicity.

Tables VI and VII illustrate similar experiments with MT3 alloantisera and monoclonal antibody 109d6. In the DR4 cell line, L203 inhibited both DR4 and MT3 cytotoxicity; 109d6 only inhibited MT3 alloantisera killing in some but not all circumstances. In the DR7 cell line GM3163, L203 blocked cytotoxicity of all MT3 alloantisera. However, 109d6 only weakly blocked one of the three

TABLE IV

*Correlation Between HLA-D Region Phenotype on B Cells and 109d6 Reactivity from a B Cell Panel of 58 Donors*

| Phenotype | +/+* | +/− | −/+ | −/− | $\chi^2$ | $r$ |
|---|---|---|---|---|---|---|
| DR1 | 2 | 1 | 37 | 18 | 0.365 | 0.078 |
| DR2 | 5 | 6 | 33 | 14 | 1.446 | −0.157 |
| DR3 | 5 | 6 | 33 | 14 | 1.446 | −0.157 |
| DR4 | 17 | 0 | 21 | 20 | 10.590 | 0.427 |
| DR5 | 9 | 6 | 29 | 14 | 0.042 | −0.026 |
| DRw6 | 4 | 12 | 34 | 8 | 13.674 | −0.485 |
| DR7 | 12 | 0 | 26 | 20 | 6.155 | 0.325 |
| DRw8 | 3 | 3 | 35 | 17 | 0.152 | −0.051 |
| DRw9 | 3 | 1 | 35 | 19 | 0.017 | 0.017 |
| DRw10 | 6 | 0 | 32 | 20 | 2.025 | 0.186 |
| MB1 | 21 | 17 | 17 | 3 | 3.897 | −0.259 |
| MB2 | 18 | 5 | 20 | 15 | 1.884 | 0.180 |
| MB3 | 19 | 3 | 19 | 17 | 5.412 | 0.305 |
| MT2 | 23 | 20 | 15 | 0 | 8.689 | −0.387 |
| MT3 | 32 | 0 | 6 | 20 | 34.243 | 0.768 |
| DR4 + 7 + w9 + w10 | 38 | 0 | 0 | 20 | 53.658 | 0.961 |

$\chi^2$ with Yates' correction; $r$ values were obtained using the formula $r = \sqrt{\chi^2/n}$ and the positive or negative correlation by the formula ad = bc.

* Presence of *HLA-D* region phenotype/109d6 reactivity by immunofluorescence.

TABLE V

*Inhibition of DR5 and MT2 Alloantisera by Monoclonal Antibodies in DHI (DR5)*

| Blocking antibody | Specificity | Serum dilution | Cytotoxicity scores* | | | | |
|---|---|---|---|---|---|---|---|
| | | | Alloantisera | | | | |
| | | | 9w597 (DR5) | 9w603 (DR5) | 9w688 (MT2) | 9w689 (MT2) | 9w699 (MT2) |
| L368 | β2-microglobulin | Und | 8 | 8 | 8 | 8 | 8 |
| | | 1:2 | 8 | 6 | 8 | 6 | 8 |
| | | 1:4 | 8 | 1 | 8 | 1 | 8 |
| | | 1:8 | 1 | 1 | 1 | 1 | 4 |
| L203 | DR | Und | 1 | 1 | 1 | 1 | 4 |
| | | 1:2 | 1 | 1 | 1 | 1 | 1 |
| | | 1:4 | 1 | 1 | 1 | 1 | 1 |
| IVD12 | MB3 | Und | 8 | 8 | 8 | 8 | 8 |
| | | 1:2 | 8 | 6 | 8 | 6 | 8 |
| | | 1:4 | 8 | 1 | 6 | 1 | 8 |
| | | 1:8 | 1 | 1 | 1 | 1 | 1 |
| I-LR2 | MT2 | Und | 8 | 8 | 8 | 4 | 8 |
| | | 1:2 | 8 | 6 | 6 | 1 | 8 |
| | | 1:4 | 8 | 1 | 1 | 1 | 8 |
| | | 1:8 | 1 | 1 | 1 | 1 | 1 |

* Cytotoxicity reactions were recorded as follows: 8, >81% lysis; 6, 51–80% lysis; 4, 21–50% lysis; 2, 11–20% lysis; 1, <11% lysis.

TABLE VI
*Inhibition of DR4 and MT3 Alloantisera by Monoclonal Antibodies in Priess (DR4)*

| Blocking antibody | Specificity | Serum dilution | Cytotoxicity scores* | | | | |
|---|---|---|---|---|---|---|---|
| | | | Alloantisera | | | | |
| | | | 9w575 (DR4) | 9w630 (DR7) | 9w733 (MT3) | 9w734 (MT3) | 9w743 (MT3) |
| L368 | β2-microglobulin | Und | 8 | 1 | 8 | 8 | 8 |
| | | 1:2 | 8 | 1 | 1 | 8 | 8 |
| | | 1:4 | 8 | 1 | 1 | 8 | 8 |
| | | 1:8 | 1 | 1 | 1 | 8 | 6 |
| | | 1:16 | 1 | 1 | 1 | 1 | 1 |
| L203 | DR | Und | 1 | 1 | 1 | 8 | 1 |
| | | 1:2 | 1 | 1 | 1 | 6 | 1 |
| | | 1:4 | 1 | 1 | 1 | 1 | 1 |
| | | 1:8 | 1 | 1 | 1 | 1 | 1 |
| IVD12 | MB3 | Und | 8 | 1 | 8 | 8 | 8 |
| | | 1:2 | 8 | 1 | 1 | 8 | 8 |
| | | 1:4 | 8 | 1 | 1 | 8 | 8 |
| | | 1:8 | 1 | 1 | 1 | 6 | 1 |
| 109d6 | MT3 | Und | 8 | 1 | 8 | 8 | 2 |
| | | 1:2 | 8 | 1 | 1 | 8 | 1 |
| | | 1:4 | 8 | 1 | 1 | 1 | 1 |
| | | 1:8 | 1 | 1 | 1 | 1 | 1 |

* Cytotoxicity reactions were recorded as follows: 8, >81% lysis; 6, 51–80% lysis; 4, 21–50% lysis; 2, 11–20% lysis; 1, <11% lysis.

MT3 alloantisera. Results with normal B cells were in agreement with these findings (data not shown). Note that while 109d6 was able to block MT3 alloantiserum 9w743 cytotoxicity in the DR4 line, it had no effect in the DR7 line. This suggests that the serum may have some anti-DR7 reactivity or that the relationship of the 109d6 determinant to the MT3 determinant is different in DR4 and DR7 cells. The anti-DS antibody IVD12, again, did not block either DR- or MT3-directed cytotoxicity.

*Some Monoclonal Antibodies Block MT2- or MT3-Specific Binding.* A panel of monoclonal antibodies was used to inhibit the binding of fluoresceinated I-LR2 (anti-MT2) to DR3 (721.127) or DR5 (GM3105A and DHI) cell lines. The amounts of this inhibition are presented in Table VIII. When tested on the DR5 cell lines, I-LR2 completely inhibited fluoresceinated I-LR2 while an irrelevant antibody (61D2, an anti-HLA-A, B, C monoclonal antibody) did not inhibit the binding. SFR3-DR5, the anti-DR5 monoclonal antibody, and to a lesser extent, L203, inhibited I-LR2, while IVD12, an anti-DS monoclonal antibody, did not inhibit its binding. In studies using the DR3 cell line, the results obtained with the monoclonal antibodies excluding IVD12 and I-LR1 were similar. IVD12, SFR3-DR5, and I-LR1 do not bind to the DR3 cell line. Monoclonal antibody 7.3.19.1, an independently derived antibody with an MT2-like binding pattern (34), completely blocked I-LR2 binding on the DR3 line but inhibited only

TABLE VII

*Inhibition of MT3 Alloantisera by Monoclonal Antibodies in the B Cell Line GM3163 (DR7)*

| Blocking antibody | Specificity | Serum dilution | Cytotoxicity scores* | | | | |
|---|---|---|---|---|---|---|---|
| | | | Alloantisera | | | | |
| | | | 9w575 (DR4) | 9w630 (DR7) | 9w733 (MT3) | 9w734 (MT3) | 9w743 (MT3) |
| L368 | β2-microglobulin | Und | 1 | 8 | 8 | 8 | 8 |
| | | 1:2 | 1 | 8 | 8 | 8 | 8 |
| | | 1:4 | 1 | 8 | 1 | 8 | 6 |
| | | 1:8 | 1 | 2 | 1 | 1 | 1 |
| L203 | DR | Und | 1 | 1 | 6 | 1 | 1 |
| | | 1:2 | 1 | 1 | 1 | 1 | 1 |
| | | 1:4 | 1 | 1 | 1 | 1 | 1 |
| IVD12 | MB3 | Und | 1 | 8 | 8 | 8 | 8 |
| | | 1:2 | 1 | 8 | 8 | 8 | 8 |
| | | 1:4 | 1 | 8 | 2 | 8 | 8 |
| | | 1:8 | 1 | 2 | 1 | 2 | 1 |
| 109d6 | MT3 | Und | 1 | 8 | 8 | 8 | 8 |
| | | 1:2 | 1 | 8 | 8 | 8 | 8 |
| | | 1:4 | 1 | 8 | 1 | 1 | 8 |
| | | 1:8 | 1 | 1 | 1 | 1 | 1 |

* Cytotoxicity reactions were recorded as follows: 8, >81% lysis; 6, 51–80% lysis; 4, 21–50% lysis; 2, 11–20% lysis; 1, <11% lysis.

weakly in the two DR5 cell lines tested. Strong inhibition was found in the DR5 lines, however, when a second antibody (goat anti-mouse Ig) was used before adding fluoresceinated I-LR2 (data not shown). This suggests that monoclonal antibody 7.3.19.1 detects a determinant on the DR5 lines that is distinct from the I-LR2 determinant but on the same molecule. I-LR1 strongly inhibited the binding of I-LR2 to the DR5 cell line. The inhibition of I-LR2 by I-LR1 in a DR5 line is not entirely unexpected since I-LR1 is known to recognize DR5 molecules in addition to SB2 and SB3 molecules (C. K. Hurley and S. Shaw, unpublished observations).

*MT2 and MT3 Specificities Reside on DR-like Molecules.* The molecules reactive with either I-LR2 (MT2) or 109d6 (MT3) were isolated from DR3, DR4, DR5, or DR7 cell lines. By SDS-PAGE, I-LR2-reactive molecules, like the DR molecules, contained two polypeptide chains, while the 109d6-reactive molecules usually appeared as three peaks on the gel profile (Fig. 1). L203 and IIIE3 were used to isolate DR molecules from these cell lines. Partial amino-terminal amino acid sequences of molecules reactive with all of these monoclonal antibodies were determined (Tables IX and X). Table IX compares the alpha chain sequences of MT and DR molecules from DR3, 4, 5, and 7 cell lines. Table X compares the beta chains from the same preparations. Although only the tyrosine and phenylalanine residues are compared, the predominant sequences of the alpha and beta chains of I-LR2- and 109d6-reactive molecules are identical to those derived using the anti-DR monoclonal antibodies.

TABLE VIII

*FACS Analysis of the Inhibition of Fluorescein-conjugated I-LR2 by Various Monoclonal Antibodies*

| Blocking antibody | Specificity | B cell lines | | | | | |
|---|---|---|---|---|---|---|---|
| | | 721.127 (DR3) | | DHI (DR5) | | GM3105A (DR5) | |
| | | MFI* | %I‡ | MFI | %I | MFI | %I |
| —§ | | 54.8 | 5 | 57.4 | 6 | 44.5 | 0 |
| 61D2 | HLA-A, B, C | 56.9 | 0 | 59.3 | 0 | 43.8 | 0 |
| I-LR2 | MT2 | 19.7 | 100 | 28.7 | 100 | 19.5 | 100 |
| L203 | Monomorphic DR | 49.7 | 27 | 49.5 | 32 | 36.1 | 32 |
| SFR3-DR5 | DR5 | 59.8 | 0 | 35.9 | 76 | 23.6 | 83 |
| 7.3.19.1 | MT2 | 20.8 | 97 | 53.3 | 20 | 38.3 | 23 |
| IVD12 | MB3 | 52.9 | 11 | 63.2 | 0 | 47.7 | 0 |
| I-LR1 | SB2, SB3, DR5 | 56.0 | 2 | 38.7 | 67 | 24.0 | 81 |

* Mean fluorescence intensity (MFI) on $10^4$ cells analyzed on FACS.

‡ Percent inhibition calculated from the following formula: %I = 100 × [1 − (MFI [experimental] − MFI [I-LR2])/(MFI [61D2] − MFI [I-LR2])].

§ RPMI 1640 containing 10% human serum.

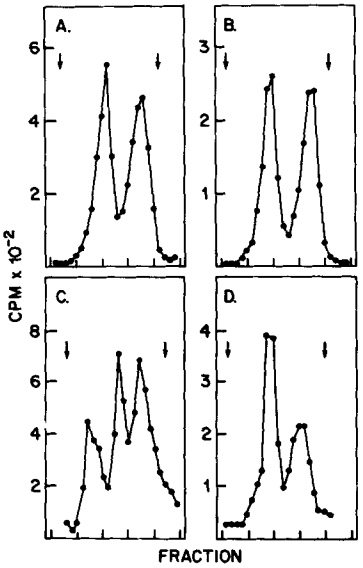


FIGURE 1. SDS-PAGE profiles of molecules isolated from the cell line 721.127 using monoclonal antibodies (*A*) I-LR2 and (*B*) L203/L243, and from the cell line PRIESS using monoclonal antibodies (*C*) 109d6 and (*D*) IIIE3. The arrows indicate the approximate positions of immunoglobulin heavy and light chains.

One of the more striking features of these sequence data is the ease with which one can distinguish each of the DR specificities with just the tyrosine sequences of the beta chains. Two cell lines were studied in each of four DR specificities. Note that both DR3 lines contain tyrosine at positions 10, 26, and 30; both DR5

TABLE IX

*Amino-Terminal Amino Acid Sequence Data: Alpha Chains*

| Cell line | Monoclonal antibody | Position | | | | | |
|---|---|---|---|---|---|---|---|
| | | 12 | 13 | 22 | 24 | 26 | 32 |
| 127 (DR3) | I-LR2 | F | Y | F | F | F | F |
| YBE (DR3) | | | Y | | | | |
| 127 | L203* | F | Y | F | F | F | F |
| YBE | | | Y | | | | |
| 3105 (DR5) | I-LR2 | F | Y | F | F | F | F |
| DHI (DR5) | | | Y | | | | |
| 3105 | L203 | F | Y | F | F | F | F |
| DHI | | | Y | | | | |
| PRIESS (DR4) | 109d6 | F | Y | F | F | F | F |
| 3164 (DR4) | | | Y | | | | |
| PRIESS | L203* | F | Y | F | F | F | F |
| 3164 | | | Y | | | | |
| I13w7 (DR7) | 109d6 | F | Y | F | F | F | F |
| 3163 (DR7) | | | Y | | | | |
| I13w7 | L203 | F | Y | F | F | F | F |
| 3163 | | | Y | | | | |

* Anti-DR monoclonal antibody IIIE3 was used in these experiments as well.

lines contain tyrosine at positions 10, 30, and 32; both DR4 cell lines contain tyrosine at positions 30 and 32; and both DR7 cell lines contain tyrosine at positions 13 and 32. In every instance, the MT-bearing molecule has the identical sequence as the DR molecule.

Minor sequences that correspond to known DS sequences were observed in some I-LR2 and, to a greater extent, 109d6 preparations. Both DS alpha and DS beta chain sequences were observed. In the example shown in Fig. 2, an alpha chain preparation from a DR4 cell line contains two sequences, a predominant DR sequence (tyrosine at position 13) and a minor DS sequence (tyrosine at positions 11, 16, and 19). The DS population represents ~20% of the molecules present in this preparation. The amount of DS sequence obtained with the MT monoclonal antibodies varied from preparation to preparation and represented anywhere from 3 to 30% of the molecules present. In general, when 109d6 was used with DR4 cell lines, approximately an 80:20 ratio of DR/DS molecules was detected, while in DR7 cell lines, the DR/DS ratio was closer to 95:5.

The higher molecular weight peak found in the 109d6 preparations (Fig. 1*C*) was identified as HLA-A/B by amino acid sequence. The preparation contained tyrosines at positions 7 and 27 and sometimes position 9, corresponding to previously characterized class I molecules (39). This suggests that either HLA-

TABLE X
*Amino-Terminal Amino Acid Sequence Data: Beta Chains*

| Cell line | Monoclonal antibody | Position | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 7 | 10 | 13 | 17 | 18 | 26 | 30 | 31 | 32 |
| 127 (DR3) | I-LR2 | F | Y | | F | F | Y | Y | F | |
| YBE (DR3) | | | Y | | | | Y | Y | | |
| 127 | L203* | F | Y | | F | F | Y | Y | F | |
| YBE | | | Y | | | | Y | Y | | |
| 3105 (DR5) | I-LR2 | F | Y | | F | F | F | Y | F | Y |
| DHI (DR5) | | | Y | | | | | Y | | Y |
| 3105 | L203 | F | Y | | F | F | F | Y | F | Y |
| DHI | | | Y | | | | | Y | | Y |
| PRIESS (DR4) | 109d6 | F | | | F | F | F | Y | F | Y |
| 3164 (DR4) | | | | | | | | Y | | Y |
| PRIESS | L203* | F | | | F | F | F | Y | F | Y |
| 3164 | | | | | | | | Y | | Y |
| 113w7 (DR7) | 109d6 | F | | Y | F | F | F | | ND‡ | Y |
| 3163 (DR7) | | | | ND | | | | | | |
| 113w7 | L203 | F | | Y | F | F | F | | F | Y |
| 3163 | | | | Y | | | | | | Y |

* Anti-DR monoclonal antibody IIIE3 was used in these isolations as well.
‡ Not determined.

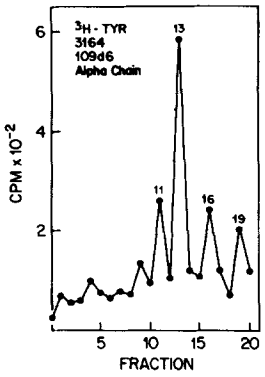


FIGURE 2. Amino acid sequence profile of an [$^3$H]tyrosine-labeled alpha chain isolated from the cell line GM3164 using monoclonal antibody 109d6. The horizontal axis represents position in the protein sequence.

A/B molecules carry a 109d6 determinant or that class I molecules may be complexed with some class II molecules.

*At Least Some Molecules Carry Both DR and MT Specificities.* To examine if I-LR2/109d6 and L203 recognize identical populations of molecules, aliquots of molecules reactive with one of the monoclonal antibodies were passed over the

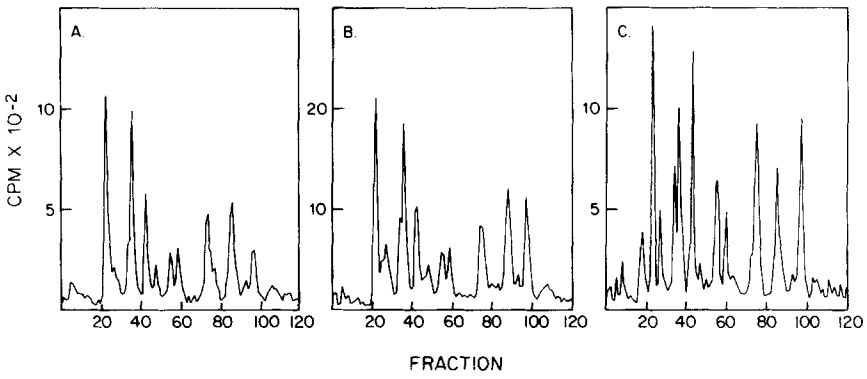


FIGURE 3. Tryptic peptide maps of [$^3$H]tyrosine-labeled beta chains from the cell line 721.127 isolated using monoclonal antibodies (*A*) L203/L243 (*B*) L203/L243 and I-LR2, and (*C*) I-LR2.

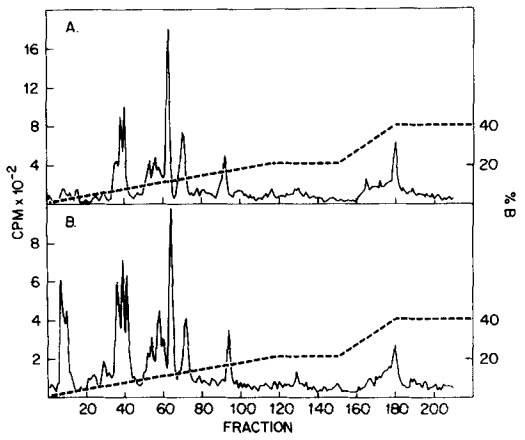


FIGURE 4. Tryptic peptide maps of [$^3$H]phenylalanine-labeled beta chains from the cell line 721.127 isolated using monoclonal antibodies (*A*) I-LR2 and (*B*) L203. The elution gradient is indicated by the dotted line.

same affinity column and the complementary column, and the percentage of bound counts estimated. Although the percentages were variable, it was clear that L203 recognized a large proportion of the molecules reactive with I-LR2 or 109d6 antibodies. In the reverse experiment, although there was some variability, in general less than half of the counts that bound and were eluted from an L203 affinity column could be rebound to either an I-LR2 or 109d6 column. An irrelevant antibody column bound a negligible amount of radioactive material in all cases. These data suggest that at least some molecules carry both DR and MT specificities, but some molecules carry only DR specificities (as defined by L203) and not MT specificities.

*Molecules Bearing the MT2 Specificities Are Indistinguishable from DR Molecules.* The alpha and beta chains of I-LR2-binding molecules from the DR3 cell line 721.127 were compared with L203-binding alpha and beta chains from the same cell line by peptide mapping. Using a tryptic digest of either [$^3$H]tyrosine-

or [$^3$H]phenylalanine-labeled molecules, both the alpha and beta chains were virtually indistinguishable between MT2 and DR preparations. Two of these comparisons are shown in Figs. 3 and 4. Only differences in the ratios of peak heights between the I-LR2 and L203 maps were observed. The additional peak observed around fraction 10 in the L203 preparation in Fig. 4 was not consistently present. Since the MT3 preparations were more complicated because both DR and DS molecules were present, a peptide map comparison was not made.

## Discussion

A number of biochemical studies have had as their goal the molecular localization of *HLA-D* region–associated serologic specificities. Characterization of the molecules bearing these determinants is important in defining the functional relevance of each of the multiple molecules controlled by this genetic region. One set of these serologic specificities resides on the DR- or I-E-like antigens. 10 DR allelic products have been recognized; all of those studied express structurally distinct DR beta chains. Since there are several I-E-like molecules in a homozygous cell (10, 11), it is unclear if all of these closely related molecules bear DR serologic determinants. In addition, it is not known if all cells bearing a particular DR allele express exactly the same set of DR molecules. For instance, in DR4-positive cells, the DR beta chains exhibit variation correlated with HLA-D specificity (40), In general, therefore, a cell has several I-E-like molecules, all or some of which bear DR serologic specificities.

Some of the *HLA-D* serologic specificities have been associated with more than one DR specificity and have, therefore, been termed supertypic specificities. These specificities could represent either public determinants on DR molecules or determinants on unique molecules. The MB (DC) supertypic determinants appear to represent the latter. Population studies had suggested that the MB specificities represented an allelic series in Hardy-Weinberg equilibrium (3). Using monoclonal antibodies and alloantisera directed against MB1 (DC1 or MT1) and MB3 determinants, these specificities were found to reside on molecules distinct from DR (13, 14, 41–43). These molecules, which are homologous to murine I-A, have been termed DS (12). These observations are in accord with evidence that expression of DR1 and MB1 could be separately eliminated from DR1, MB1 cells by means of mutagenic treatment (31, 44). Although MB1, 2, and 3 are alleles, it now appears that variability exists within a set of molecules bearing a particular MB allelic specificity. That is, the DS molecule in DR4 cells may be structurally distinct from the DS molecule in DR5 cells although both molecules bear the MB3 specificity (32). These differences may represent the DS allelic products observed by Goyert and Silver (45). While it is not conclusive that additional MB determinants will not be found on DR molecules, the simplest interpretation of the present data is that the MB supertypic series represents public determinants on DS or I-A-like molecules.

A second group of supertypic specificities is the MT series. Two members of the MT series appear to be identical to previously described MB specificities on the basis of their DR-associated distribution. MT1 appears to be indistinguishable from MB1 (DC1), and MT4 appears to be indistinguishable from MB3 (DC4) (See Table I). Thus, when $\gamma$ rays were used to eliminate expression of DR1 from

a DR1, MB1, MT1 lymphoblastoid cell line, those mutants that continued to express MB1 also expressed MT1 (44). MT2 and MT3 have clearly distinct distributions from the remainder of the MB specificities and represent unique serologic determinants. It is unclear whether MT2 and MT3 are allelic or represent unrelated (i.e., nonallelic) serologic specificities.

In the population studies presented here, MT2 and MT3 alloantisera reactivity paralleled the associated DR specificities. That is, all DR3, 5, and w6 individuals tested were MT2 positive, and almost all DR4, 7, and w9 individuals were MT3 positive. The sole exception is DRw9, in which one of four individuals tested was MT3 negative. This is in contrast to the MB3 supertypic specificity, which is also associated with DR5. Not all DR5 individuals are MB3 positive, as some exhibit other MB specificities. These observations could be explained if MT were a public determinant on DR molecules, in contrast to MB3, which is located on a molecule distinct from DR.

To analyze the biochemical bases of the MT2 and MT3 serologic specificities, two monoclonal antibodies with MT specificity were identified by panel testing. Neither monoclonal antibody displayed a reactivity profile that exactly paralleled that obtained with standard alloantisera. Correlation of I-LR2 and MT2 alloantisera was high although I-LR2 did not bind most MT2-positive DRw8 cells. The correlation of 109d6 binding with MT3 reactivity was not perfect due to reactivity of this monoclonal antibody with DRw10-positive cells. This indicates that the reactivity of this monoclonal antibody was not identical with most of the usual MT3 alloantisera, further emphasizing the complexity of determinants detected on *HLA-D* region products. Excluding this, every panel member who was MT3 positive was 109d6 positive. The reactivity with DRw10 cells, which are negative when tested by MT3 alloantisera, suggests that the 109d6 determinant is distinct from the MT3 determinant but that its inheritance is tightly linked to the inheritance of the MT3 epitope, as would be expected for determinants on the same molecule or on molecules encoded by closely linked genes as are the *HLA-D* region molecule. Based on panel testing and family studies, therefore, I-LR2 and 109d6 recognize MT2- and MT3-like determinants with only a few discrepancies.

A number of serologic determinants may comprise an MT specificity defined by alloantisera. Since the monoclonal antibodies recognize a single serologic determinant, it is possible that by using monoclonal antibodies, one is examining a subset of the MT-bearing molecules. An example of this is illustrated by I-LR2, which fails to recognize MT2-positive DRw8 cells. Alloantisera with this same specificity would be called "MT2 short." In addition, the determinant recognized by the monoclonal antibodies may be closely linked but not identical to those reactive with MT alloantisera. This may be the case for the 109d6 determinant, as suggested by its DRw10 reactivity. These questions were examined by using the I-LR2 and 109d6 monoclonal antibodies to block MT-specific alloantisera. Since I-LR2 partially blocks some MT2 alloantisera, I-LR2 appears to recognize a secondary MT2 determinant. This determinant is absent in DRw8 cells.

MT3 alloantisera were not inhibited by 109d6 on the DR7 B cell line but two of the three MT3 alloantisera were inhibited by 109d6 on the DR4 cell line.

These results suggest that (*a*) some MT3 alloantisera contain additional alloantibodies (such as DR7) that are not inhibited by 109d6 on the DR7 cell line, and/or (*b*) the relationship of the 109d6 determinant to the MT3 determinant is different in DR4 and DR7 cell lines. For example, MT3 alloantisera may detect additional MT3-bearing molecules on the DR7 cell line, not recognized by 109d6, and these additional molecules are not present on the DR4 cell line. The 109d6-blocking data, coupled with the population studies, suggests that 109d6 recognizes a determinant adjacent to, but not identical to, the MT3 determinant recognized by alloantisera. L203 was able to block all DR and MT alloantisera tested, suggesting that molecules bearing MT and DR determinants also carry L203 determinants. In our studies, the vast majority of these molecules are I-E-like. These studies will be expanded upon and discussed further in a future publication.

Both I-LR2- and 109d6-reactive DR molecules appear to represent only a portion of the L203-reactive DR pool. This has been suggested by experiments in which eluates from an antibody affinity column are repassed over the same and complementary columns. A similar result has been observed using another MT2-like monoclonal antibody by Koning et al. (34).

A number of biochemical studies have attempted to define the structural bases of the MT2 and MT3 determinants. Using alloantisera and two-dimensional gel analysis and peptide mapping, MT2 has been localized on DR molecules (19, 20, 22) and on a molecule distinct from DR (20, 23). In one study, the new molecule, BR, was found to be closely related to the DR molecule, with a DR alpha chain and a similar beta chain (20). The definition of "non-DR" molecules by many workers who use alloantisera is often based on "extra spots" seen on two-dimensional gels distinct from the spots defined by DR alloantisera. All of these molecules may be I-E-like and many have L203 epitopes on them. In the present study, a monoclonal antibody was used to isolate molecules bearing an MT2 determinant. Amino acid sequence analysis and peptide mapping of these molecules revealed no differences between MT2-bearing molecules and DR- or L203-reactive molecules. Since L203 strongly inhibits MT2 alloantisera and it has been shown to recognize DR-like molecules (28), molecules carrying MT2 determinants are obviously DR-like, but it is also possible that these I-E-like molecules carrying MT2 determinants do not all carry DR allospecificities. At this point, we do not know precisely which of the many DR-like molecules in a cell carry the DR allospecificities. It is possible that some of these may carry MT2-unique allodeterminants and not DR allodeterminants. Since multiple serologic determinants can make up the MT2 specificity, this study can not determine if another MT2 determinant resides on non-DR molecules. A simplified model that explains much of the data is presented in Fig. 5.

Previous studies have localized the MT3 epitope to DR (21), DS (21), or to a non-DR molecule, either undefined (27, 46, 47) or BR (24, 48). In our study, using a monoclonal antibody with a MT3-like distribution identical to that used by Toguchi et al. (27), MT3-like determinants were found to reside primarily on DR molecules although DS molecules also bore the determinant. A similar result was observed by Goyert et al. (21) and Goyert and Silver (45). Sharing of serologic determinants between the products of different loci (*I-A* and *I-E*) has

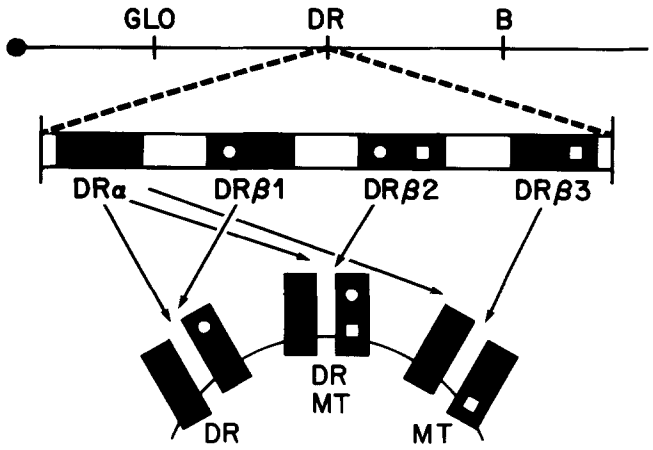


FIGURE 5. Schematic diagram of the possible genetic organization of the HLA-DR subregion. Evidence presented to date suggests that the DR or I-E-like molecules are encoded by a single alpha chain gene and multiple I-E-like beta chain genes within a haplotype. This study suggests that some but not all of these I-E-like species bear MT serologic determinants. The results of this and other studies suggest that some of the I-E-like molecules also bear DR serologic determinants. All three of these subsets of I-E-like molecules may not exist in all cells.

been previously observed in the murine system (49). Interestingly, one of the monoclonal antibodies directed against murine I-A and I-E antigens reacts with human cells in an MT-specific pattern (50). Sharing of determinants by different gene products of human class II antigens has also been observed using other monoclonal antibodies such as I-LR1 (SB and DR) (16, 17) and CC11.23 and SG171 (DR and DS) (21, 31). Recently, Finn et al. (51) have described the sharing of a common antigenic determinant between human DR and the invariant chain.

The *HLA-D* region, therefore, encodes at least three distinct molecules: (*a*) DR molecules, the predominant HLA-D molecules, bear homology to murine I-E antigens. Some or all of these I-E-like molecules bear the allele-specific DR serologic determinants as well as MT2- and MT3-like public determinants (see Fig. 5). (*b*) DS molecules, murine I-A homologues, bear MB (DC) determinants and, in some instances, MT3-like determinants. (*c*) SB molecules, distinct from DR and DS and intermediate in structure between I-E and I-A, have not been defined using alloantisera. A fourth set of molecules, termed BR, have been characterized by peptide mapping in other studies (20, 24, 48). By peptide mapping, these molecules exhibit homology with DR molecules and could represent one of the multiple DR subsets. Evidence has been presented that BR molecules do not carry DR determinants, but do bear MT2 or MT3 determinants (20, 24). Further sequence analysis of the BR molecule should establish their relationship to the DR molecules and should definitively establish if a fourth *HLA-D* subregion exists.

The human HLA-D family of molecules presents an interesting example of the divergence of a gene family creating an enormous amount of complexity while maintaining considerable homology. This sharing of specific regions of molecules detected by alloantisera and monoclonal antibody reactivity presents

interesting evolutionary questions. Two distinct issues, evident from the available serological and biochemical studies, almost defy explanation using conventional genetic models. First, at least three DR beta chains are associated with most specificities. The data presented here indicates that these beta chains are structurally distinct from haplotype to haplotype and yet, between alleles, they share public specificities (MT2 and MT3). If, on the one hand, "triplication" of the beta chain loci occurred before the development of allospecificities (for example DR3 and DR5), it is hard to reconcile the presence of three unique beta chains with the sharing of specificities (for example MT2) on these same molecules. On the other hand, if triplication occurred after the development of the allospecificities, one would have to postulate that the triplication occurred as an independent event at least 10 times (to account for DR(w) 1–10). Second, sets of genes must evolve in parallel so that, for example, DR4 and DS4 arise together as unique genes different from DR5 and DS5 (32). Despite this divergence, molecules must maintain regions of homology so that DS4 and DS5 maintain a shared region, measured serologically as MB3. DR3 and DR5 maintain the shared specificity MT2, and DR4 and DS4 maintain the shared specificity MT3. While maintenance of the latter could be evidence of gene conversion, other shared regions may be maintained by selective pressures possibly related to the functional importance of the determinants.

Indeed, MT serologic specificities are not simple serologic anomalies but appear to be important determinants in immune function. Several diseases show a strong association with MT specificities, for example, primary sicca syndrome with MT2 (52) and juvenile onset diabetes and rheumatoid arthritis in Japanese with MT3 (53, 54). In addition, the reactivities of some alloreactive lymphocyte clones have been directed towards MT determinants (55, 56), and some antigen-specific T lymphocyte responses are MT restricted (57, 58). The functional relevance of the HLA-D molecules, therefore, appears to be controlled by the determinants localized on those molecules. Conversely, the molecular localization may affect the impact of those determinants in the immune response. It will be important in future functional studies to ascertain the precise HLA-D determinants involved in antigen and T cell recognition and the molecular context in which those determinants are presented.

## Summary

The specificities of the monoclonal antibodies I-LR2 and 109d6, which recognize MT2- and MT3-like serologic determinants, respectively, have been confirmed by panel testing. In addition, the relationships of these antibodies to other monoclonal antibodies and alloantisera have been studied by means of cell surface fluorescence, complement-dependent cytotoxicity and immunoprecipitation. Using these monoclonal antibodies, molecules encoded by the *HLA-D* region have been isolated and characterized by amino acid sequencing and peptide mapping. By these criteria, the major populations of molecules bearing MT2- and MT3-like determinants are indistinguishable from DR molecules.

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## References

1. Winchester, R. J., S. M. Fu, P. Wernet, H. G. Kunkel, B. DuPont, and C. Jersild. 1975. Recognition by pregnancy serums of non-HL-A alloantigens selectively expressed on B lymphocytes. *J. Exp. Med.* 141:924.
2. van Rood, J. J., A. van Leeuwen, A. Termijtelen, and J. J. Keuning. 1976. B cell antibodies, Ia-like determinants, and their relation to MLC determinants in man. *Transplant. Rev.* 30:122.
3. Duquesnoy, R. J., M. Marrari, and K. Annen. 1979. Identification of an HLA-DR-associated system of B-cell alloantigens. *Transplant. Proc.* 11:1757.
4. Tanikagi, N., and R. Tosi. 1982. The genetic control of human Ia alloantigens: a three-locus model derived from the immunochemical analysis of 'supertypic specificities'. *Immunol. Rev.* 66:5.
5. Gibofsky, A., R. J. Winchester, M. Patarroyo, M. Fotino, and H. G. Kunkel. 1978. Disease associations of the Ia-like human alloantigens. *J. Exp. Med.* 148:1728.
6. Park, M. S., P. I. Terasaki, S. Nakata, and D. Aoki. 1980. Supertypic DR groups: MT1, MT2, and MT3. *In* Histocompatibility Testing 1980. P. I. Terasaki, editor. UCLA Tissue Typing Laboratory, Los Angeles, CA. 854–857.
7. Nakata, S., M. S. Park, and K. Omori. 1980. MT3. *In* Histocompatibility Testing 1980. P. I. Terasaki, editor. UCLA Tissue Typing Laboratory, Los Angeles, CA. 569.
8. Park, M. S., P. I. Terasaki, D. Bernoco, and Y. Iwaki. 1978. Evidence for a second B-cell locus separate from the DR locus. *Transplant. Proc.* 10:823.
9. Shackelfored, D. A., J. F. Kaufman, A. J. Korman, and J. L. Strominger. 1982. HLA-DR antigens: structure, separation of subpopulations, gene cloning and function. *Immunol. Rev.* 66:133.
10. Kratzin, H., C. Yang, H. Gotz, E. Pauly, S. Kolbel, G. Egert, F. P. Thinnes, P. Wernet, P. Altevogt, and N. Hilschmann. 1981. Primarstruktur menschlicher histokompatibilitat santigene der Klasse II. *Hoppe-Seyler's Z. Physiol. Chem.* 362:1665.
11. Andrews, D. W., M. R. Bono, and J. L. Strominger. 1982. A homozygous human cell line contains three subsets of HLA-DR-like antigens distinguishable by amino acid sequencing. *Biochemistry.* 21:6625.
12. Goyert, S. M., J. E. Shively, and J. Silver. 1982. Biochemical characterization of a second family of human Ia molecules, HLA-DS, equivalent to murine I-A subregion molecules. *J. Exp. Med.* 156:550.
13. Giles, R. C., G. Nunez, C. K. Hurley, A. Nunez-Roldan, R. Winchester, P. Stastny, and J. D. Capra. 1983. Structural analysis of a human I-A homologue using a monoclonal antibody that recognizes an MB3-like specificity. *J. Exp. Med.* 157:1461.
14. Bono, M. R., and J. L. Strominger. 1982. Direct evidence of homology between human DC-1 antigen and murine I-A molecules. *Nature (Lond.).* 299:836.
15. Karr, R. W., C. C. Kannapell, R. J. Zangara, S. M. Goyert, J. Silver, and B. D. Schwartz. 1983. An HLA-DR5 homozygous cell line expresses two DS (I-A-like) molecules. *J. Exp. Med.* 158:1374.
16. Hurley, C. K., S. Shaw, L. Nadler, S. Schlossman, and J. D. Capra. 1982. Alpha and beta chains of SB and DR antigens are structurally distinct. *J. Exp. Med.* 156:1557.
17. Shaw, S., R. DeMars, S. F. Schlossman, P. L. Smith, L. A. Lampson, and L. M.

Nadler. 1982. Serologic identification of the human secondary B cell antigens. Correlations between function, genetics, and structure. *J. Exp. Med.* 156:731.

18. Auffray, C., J. W. Lillie, D. Arnot, D. Grossberger, D. Kappes and J. L. Strominger. 1984. Isotypic and allotypic variation of human class II histocompatibility antigen $\alpha$-chain genes. *Nature (Lond.).* 308:327.
19. Markert, M. L., and P. Cresswell. 1982. Human B cell alloantigens: expression of MB and MT determinants. *J. Immunol.* 128:2004.
20. Tanigaki, N., R. Tosi, K. Sagawa, J. Minowada, and G. B. Ferrarra. 1983. The distribution of DR5, MT2, and MB3 specificities on human Ia subsets. *Immunogenetics.* 17:371.
21. Goyert, S. M., J. Moriuchi, R. Winchester, and J. Silver. 1983. HLA-DS molecules and their relation to supertypic specificities. *Hum. Immunology.* 8:53.
22. Karr, R. W., C. C. Kannapell, J. A. Stein, H. M. Gebel, D. L. Mann, R. J. Dusquesnoy, T. C. Fuller, G. E. Rodey, and B. D. Schwartz. 1982. Molecular relationships of the human B cell alloantigens, MT2, MB3, MT4, and DR5. *J. Immunol.* 128:1809.
23. Markert, M. L., and P. Cresswell. 1980. Polymorphism of human B-cell alloantigens: evidence for three loci within the HLA system. *Proc. Natl. Acad. Sci. USA.* 77:6101.
24. Tankigaki, N., R. Tosi, R. J. Duquesnoy, and G. B. Ferrara. 1983. Three Ia species with different structures and alloantigenic determinants in an HLA-homozygous cell line. *J. Exp. Med.* 157:231.
25. Nadler, L. M., P. Stashenko, R. Hardy, J. M. Pesando, E. J. Yunis, and S. F. Schlossman. 1981. Monoclonal antibodies defining serologically distinct HLA-D/DR-related Ia-like antigens in man. *Hum. Immunol.* 1:77.
26. Winchester, R., T. Toguchi, I. Szer, G. Burmester, J. Cuttner, J. D. Capra, and A. Nunez-Roldan. 1983. Association of susceptibility to certain hematopoietic malignancies with the presence of Ia allodeterminants distinct from the DR series: utility of monoclonal antibody reagents. *Immunol. Rev.* 70:155.
27. Toguchi, T., G. Burmester, A. Nunez-Roldan, P. Gregersen, S. Seremetis, S. Lee, I. Szer, and R. Winchester. 1984. Evidence for the separate molecular expression of four distinct polymorphic Ia epitopes on cells of DR4 homozygous individuals. *Hum. Immunol.* 10:69.
28. Hurley, C. K., G. Nunez, R. Winchester, O. J. Finn, R. Levy, and J. D. Capra. 1982. The human HLA-DR antigens are encoded by multiple beta chain loci. *J. Immunol.* 129:2103.
29. Taylor, C. G., and F. C. Grumet. 1982. HLA-DR antigen grouping by use of monoclonal antibody probes: expansion of known groups, and mechanism of cytotoxicity blocking. *J. Immunol.* 128:1489.
30. Stastny, P., E. Ball, P. Dry, and G. Nunez. 1983. The human immune response region (HLA-D) and disease susceptibility. *Immunol. Rev.* 70:113.
31. DeMars, R., C. C. Chang, and R. A. Rudersdorf. 1983. Dissection of the *D*-region of the human major histocompatibility complex by means of induced mutations in a lymphoblastoid cell line. *Hum. Immunol.* 8:123.
32. Giles, R., C. K. Hurley, and J. D. Capra. 1984. Primary structural variation among serologically indistinguishable DS antigens. The MB3-bearing molecule in DR4 cells differs from the MB3-bearing molecule in DR5 cells. *J. Immunol.* 133:1.
33. Radka, S. F., C. Machamer, P. Cresswell, D. D. Kostyu, F. E. Ward, and D. B. Amos. 1983. SFR3-DR5, a monoclonal antibody with HLA-DR5 specificity. *J. Immunol.* 130:1863.
34. Koning, F., I. Schreuder, M. Giphart, and H. Bruning. 1984. A mouse monoclonal antibody detecting a DR-related MT2-like specificity. *Hum. Immunol.* 9:221.
35. Ugolini, V., G. Nunez, R. G. Smith, P. Stastny, and J. D. Capra. 1980. Initial

characterization of monoclonal antibodies against human monocytes. *Proc. Natl. Acad. Sci. USA.* 77:6764.

36. Thomsen, M., B. Jacobsen, P. Platz, L. P. Ryder, L. S. Nielsen, and A. Svejgaard. 1975. LD typing, polymorphism of MLC determinants. *In* Histocompatibility Testing 1975. F. Kissmeyer-Nielsen, editor. Munksgaard, Copenhagen. 509–518.
37. Ligler, F., E. S. Vitetta, and J. W. Uhr. 1977. Cell surface immunoglobulin. XXII. Reappearance of surface IgM and IgD on murine splenocytes after removal by capping. *J. Immunol.* 119:1545.
38. Nunez-Roldan, A., I. Szer, T. Toguchi, J. Cuttner, and R. Winchester. 1982. Association of certain Ia allotypes with the occurrence of chronic lymphocytic leukemia. Recognition by a monoclonal anti-Ia reagent of a susceptibility determinant not in the DR series. *J. Exp. Med.* 156:1872.
39. Ploegh, H. L., H. T. Orr, and J. L. Strominger. 1981. Major histocompatibility antigens: the human (HLA-A, -B, -C) and murine (H-2K, H-2D) class I molecules. *Cell.* 24:287.
40. Bach, F. H., and A. J. Watson. 1983. Dw/LD-associated molecular polymorphism of DR4 beta chains: intramolecular localization of polymorphic sites. *J. Immunol.* 131:1622.
41. Corte, G., F. Calabi, G. Damiani, A. Bargellesi, R. Tosi, and R. Sorrentino. 1981. Human Ia molecules carrying DC1 determinants differ in both alpha and beta subunits from Ia molecules carrying DR determinants. *Nature (Lond.).* 292:357.
42. Shackelford, D. A., D. L. Mann, J. J. van Rood, G. B. Ferrara, and J. L. Strominger. 1981. Human B-cell alloantigens DC1, MT1, and LB12 are identical to each other but distinct from the HLA-DR antigen. *Proc. Natl. Acad. Sci. USA.* 78:4566.
43. De Kretser, T. A., M. J. Crumpton, J. G. Bodmer, and W. F. Bodmer. 1982. Two-dimensional gel analysis of the polypeptides precipitated by a polymorphic HLA-DR1,2,w6 monoclonal antibody: evidence for a third locus. *Eur. J. Immunol.* 12:600.
44. DeMars, R., C. C. Chang, M. Marrari, R. J. Duquesnoy, H. Noreen, M. Segall, and F. H. Bach. 1983. Dissociation in expression of MB1/MT1 and DR1 alloantigens in mutants of lymphoblastoid cell line. *J. Immunol.* 131:1318.
45. Goyert, S. M., and J. Silver. 1983. Further characterization of HLA-DS molecules: implications for studies assessing the role of human Ia molecules in cell interactions and disease susceptibility. *Proc. Natl. Acad. Sci. USA.* 80:5719.
46. Katagiri, M., H. Ikeda, N. Maruyama, J. Moriuchi, A. Wakisaka, S. Kimura, M. Aizawa, and K. Itakura. 1979. Evidence for two B-cell alloantigen loci in the HLA-D region. *Immunogenetics.* 9:335.
47. Maeda, H., and R. Hirata. 1984. Molecular identifications of HLA-DR4, MT3, and Tb21 antigens on HLA-DR4 homozygous B cell lines. *J. Immunol.* 132:574.
48. Sorrentino, R., G. Corte, F. Calabi, N. Tanigaki, and R. Tosi. 1983. Microfingerprinting analysis of human Ia molecules favors a three loci model. *Mol. Immunol.* 20:333.
49. Pierres, M., C. Devaux, M. Dosseto, and S. Marchetto. 1981. Clonal analysis of B- and T-cell responses to Ia antigens. *Immunogenetics.* 14:481.
50. Pierres, M., P. Mercier, M. Madsen, C. Mawas, and T. Kristensen. 1982. Monoclonal mouse anti-I-$A^K$ and anti-I-$E^K$ antibodies cross-reacting with HLA-DR supertypic and subtypic determinants rather than classical DR allelic specificities. *Tissue Antigens.* 19:289.
51. Finn, O. J., C. J. Stackhouse, and R. S. Metzgar. 1983. Human Ia beta chains and the invariant chain share a common antigenic determinant. *J. Exp. Med.* 158:1344.
52. Moutsopoulos, H., D. Mann, A. Johnson, and T. Chused. 1979. Genetic differences between primary and secondary sicca syndrome. *N. Engl. J. Med.* 301:761.

53. Moriuchi, J., M. Katagiri, A. Wakisaka, N. Matsuura, N. Maruyama, H. Ikeda, M. Aizawa, and K. Itakura. 1980. Association of B cell alloantigen with juvenile onset diabetes mellitus in the Japanese. *Hum. Immunol.* 4:357.
54. Ohta, N., Y. Nishimura, K. Tanimoto, Y. Horiuchi, C. Abe, Y. Shiokawa, T. Abe, M. Katagiri, T. Yoshiki, and T. Sasazuki. 1982. Association between HLA and Japanese patients with rheumatoid arthritis. *Hum. Immunol.* 5:123.
55. Zeevi, A., C. Scheffel, K. Annen, G. Bass, M. Marrari, and R. J. Duquesnoy. 1982. Association of PLT specificity of alloreactive lymphocyte clones with HLA-DR, MB and MT determinants. *Immunogenetics.* 16:209.
56. Zeevi, A., K. Annen, C. Scheffel, and R. J. Duquesnoy. 1983. Detection of non-HLA-DR determinants by alloreactive lymphocyte clones. *Hum. Immunol.* 6:97.
57. Berle, E. J., and E. Thorsby. 1982. Both DR and MT class II HLA molecules may restrict proliferative T-lymphocyte responses to antigen. *Scand. J. Immunol.* 16:543.
58. Ball, E. J., and P. Stastny. 1984. Antigen-specific HLA-restricted human T-cell lines. I. An MT3-like restriction determinant distinct from HLA-DR. *Immunogenetics.* 19:13.