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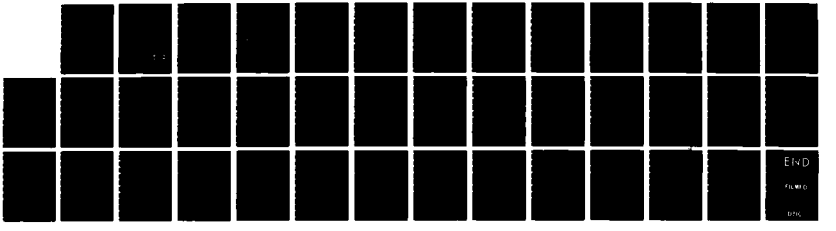

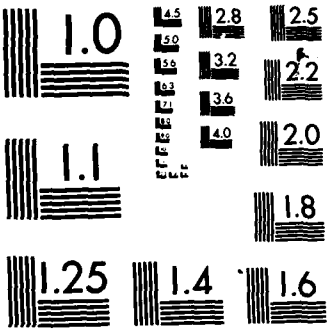

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Clonal Analysis of HLA-DR and -DQ Associated Determinants - Their Contribution to Dw Specificities

Nancy L. Reinsmoen and Fritz H. Bach

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ABSTRACT

In order to investigate the distribution of epitopes recognized by T cell clones directed against HLA class II products, bulk primed cell populations were generated using cells matched for class I determinants but disparate for class II determinants. Cells were cloned by single cell deposition (FACS IV) or limiting dilution (1 cell/3 wells), and assayed for proliferative and cytolytic function with panels of well-characterized cells. All cytolytic clones generated from an anti-DR4/Dw4/DQw3 priming combination or an anti-DR2/Dw2/DQw1 priming combination lysed essentially all targets sharing the same Dw type as the sensitizing cell. In some cases, other targets were also lysed. For instance, some clones were lytic to targets bearing the same DR antigen but another Dw subtype including a few clones lytic to virtually all cells carrying that DR specificity. An occasional target cell expressing a different DR antigen from the sensitizing cell was also lysed by these clones, in some cases to the same extent of lysis seen on the specific target. Monoclonal antibody inhibition studies identified three groups of clones: the DQ directed clones and clones apparently directed at more than one DR product. However, the number of molecules detected for each haplotype remains to be investigated. Our data indicate that determinants detected on both DR and DQ products are associated with the Dw type of the sensitizing cell showing that there is polymorphism recognized by T cells on both DR and DQ that is subtypic to the serologically defined specificities. Thus, it appears that the bulk T cell response is a composite of individual clones recognizing distinct determinants on these class II molecules. The implications of these findings for studies of HLA restricted recognition are discussed.

ABBREVIATIONS

MLC	mixed lymphocyte culture
HTC	homozygous typing cell
PLT	primed lymphocyte test
IL-2	interleukin-2
CML	cell mediated lympholysis
EBV	Epstein Barr Virus
mAb	monoclonal antibody
PBL	peripheral blood lymphocytes
LCL	lymphoblastoid cell line

INTRODUCTION

The complexity of the HLA-D region has long been recognized based on cellular responses to class II products. Understanding of this region has expanded with study of the protein products and genomic analyses. There appear to be three different families of genes encoding the class II products: DR, DQ (previously referred to as DC, DS, MB, LB-E) and DP (formerly known as SB). The DR family is composed of 3 polymorphic β genes and one relatively invariant α gene. Several, perhaps all, of the serologically defined specificities of DR1 through DRw14 appear to be alleles present on one of the DR αβ products ($\alpha\beta_1$ by convention) while DRw53 and w52 are probably, at least in some situations, encoded by alleles and present on a DR $\alpha\beta_2$ product. Expression of a third DR αβ product, and whether the number of DR β products expressed by each haplotype is the same, has not been extensively investigated. The DQ family consists of two α and two β genes with DQw1-3 specificities presumably being encoded by allelic genes. Six specificities have been identified for DP, a family of genes consisting of two α and two β genes. This area has recently been reviewed (1,2).

The cellular methods used to investigate these products are the mixed lymphocyte culture (MLC*) (3-5) utilizing homozygous typing cells (HTCs*) as stimulators (6-9) to define Dw antigenic clusters (10) and the primed lymphocyte culture (PLT*) (11,12) including expansion of these primed cells through cloning methodologies (13). Stimulatory determinants in PLT, which presumably reflect those determinants capable of stimulation in the primary MLC, have been reported as being associated with DR, DQ, DP, (14-34) and the HLA-A,B chromosomal segment (35-39). Dw specificity clusters defined with HTCs are subtypic to the serologically defined DR antigens; for example, several Dw specificities are associated with DR4 (40-42) and DR2 (19,42-48). Dw antigenic clusters (Dw1-19) (10) are not attributable to a single locus,

although the DR product may be immunodominant (20), but rather to the composite responses to stimulation by various stimulatory determinants predominantly associated with class II products. Thus, Dw specificities are "haplotype assignments".

Although there is some evidence that DR and DQ products can stimulate T lymphocytes, it is not clear whether there are determinants associated with both these products that contribute to the Dw haplotype assignment, i.e. have a population distribution corresponding to the subtypic Dw specificity. Our previous studies of class II proteins provided evidence for polymorphism of DR β chains correlating with the Dw subtype of DR4 positive cells (49), a finding which was independently confirmed and extended to DR2 and DQ (50-53). This report provides the first evidence by cellular assays, using panel studies of phenotypically well-characterized cells as well as monoclonal antibody blocking studies, that determinants associated with the DR as well as the DQ product appear to contribute to the definition of a Dw specificity. Thus, the functional polymorphism recognized by T lymphocytes is more extensive than that defined serologically. Further, we show that cloned T cells respond to what are probably two separate DR αβ dimers, although there is no evidence for a further subtype polymorphism of one of these dimers. To the extent that restricting determinants are Dw subtype associated, as has been recently found (54-56), these data indicate that the restricting determinants could be associated with DR, DQ, or both products.

METHODS

HLA-A, -B, -C, -DR typing

HLA-A, -B, -C typing was performed according to the standard NIH method using approximately 150 antisera. HLA-DR typing for the specificities DR1-8 was performed using a panel of approximately 120 antisera according to the

technique of the 7th International Histocompatibility Workshop (57) on B cells enriched by a nylon wool method (58).

HLA-D typing

HLA-D typing was performed on all cell donors with a panel of approximately 40 homozygous typing cells (HTCs)* representing the specificities Dw1 through 15, LD-5a (44) and FJO (48). MN2 is a previously described HLA-DR2 associated HLA-Dw/LD specificity defined by PLT reagents (43). The MLC technique has been previously described (59-61). Reproducible double normalized relative responses (RR) of 50% or less were considered typing responses.

Target #3 types only as Dw13 by HTCs; however, family studies indicate both haplotypes type as DR4 but only one haplotype carries the Dw13 specificity. Multiple HLA-Dw typings of target #3 and other family members carrying the DR4, Dw blank haplotype demonstrate occasional borderline typing responses to Dw4 HTCs (>40 double normalized relative responses). However, a definite Dw4 assignment could not be given.

Interleukin-2 (IL-2) Production

Peripheral blood lymphocytes were isolated by ficoll-hypaque gradient centrifugation on 10-12 individuals. Cells were pooled, irradiated at 1000 rads, and adjusted to 10 x 10^6/ml. To 75 cm^2 flasks were added 50 x 10^6 cells, 50 µg indomethacin, 0.5 ml pooled human sera (PHS), and 42.5 ml RPMI-1640. The flasks were incubated 48 hours in a 37°C, 5% CO_2 humidified atmosphere. After centrifugation, the supernatant was decanted and stored at -20° C. Lymphocult-T (Biotest 812,810) was used as the source of IL-2 in the latter experiments.

Priming Cell Combinations

Disparate priming specificities are underscored. A) Anti-DR/Dw4 priming: Responder=HLA-A1,2; B8,w44; DR3,w6;w52; Dw3,6; DQw1,2; DPw1,4. Stimulator=

HLA-A1,2; B8,w44; DR3,4; w52,w53; Dw3,4; DQw2,3; DPw1. B) Anti-DR/Dw2 priming: Responder=HLA-A1,3; B8,7; DR3,8; w52,w53; Dw3,8; DQw2,3; DPw4. Stimulator= HLA-A1,3; B8,7; DR2,3; w52; Dw2,3; DQw1; DPw4,6.

Cloning Techniques

Cloned reagents were generated using a method previously described with some modifications (13). Primed cells cultured for 10 days were stimulated with peripheral blood lymphocytes (PBL*) from the original stimulator (3,000 rad) at a responder:stimulator ratio of 1:3 and recultured for three days. The blasts were separated on a 15-38% percoll gradient (Pharmacia) and plated at a concentration of 1 cell per 3 wells in 72 well histo-plates (006-020-0401 Dynatech Inc. Alexandria, VA) with 1 x 10^4 feeder cells (lymphoblastoid cell line (LCL*) of the original stimulator cell and cultured in 20% IL-2, 15% PHS, 65% RPMI-1640. Alternatively, blast cells were submitted to single cell deposition utilizing the fluorescence-activated cell sorter (FACS IV - Becton Dickinson, Mountain View, CA). After 10 days those wells with positive growth were transferred to flat bottom wells (Linbro 76-013-05 with 1 x 10^5 feeder cells and eventually to nickel well plates (Linbro FB-4-TC).

Primed lymphocyte test (PLT*)

The PLT method has been previously described (11, 12, 62). Briefly, cloned cells were added to V bottom plates at a concentration of 10^3 cells per well with 5 x 10^4 irradiated (3000 rad) stimulator cells. After 40 hours incubation at 37°C in a 5% CO_2 humidifed atmosphere, the cultures were labeled for 8 hours with 2 μCi tritiated thymidine (New England Nuclear, Boston, Mass. NEN-0270E, specific activity = 20 Ci/mMol), harvested using a semi-automated cell harvester (SATCH-Knight Inc., Minneapolis, MN) and counted in a LKB 1216 RackBeta β scintillation counter.

Cell Mediated Lympholysis (CML*)

Target Cells: Epstein Barr Virus (EBV*) transformed lymphoblastoid cell lines (LCL) were used as target cells and labeled with 0.25 ml. chromium 51 (.25mCi) for 1 hour; washed 3 times with cold RPMI-1640 containing 20% PHS; and adjusted to 1×10^4 cells ml.

Effectors: The cloned reagent effectors were resuspended in IL-2-free culture medium the day prior to testing. Effectors were adjusted to the appropriate concentrations (50:1, 30:1, 20:1, or 12:1) and 100λ effectors + 100λ targets were added to V bottom microtiter plates, spun at 500 rpm for 5 minutes and incubated in a 37° 5% CO_2 humidified environment for 4 hours. The plates were then spun at 1000 rpm for 10 minutes and 150λ supernatants were aspirated and placed in scintillation vials. Ready-Solv™ HP (Beckman 566436) (2.5 ml.) was added to each vial and the samples were counted in a β scintillation counter. Spontaneous release of ^{51}Cr was assessed by incubating target cells without effector cells and maximum release of the isotope was assessed by incubating target cells with .1% hexadecyltrimethylammonium bromide. Results were calculated as follows:

$$\text{percent cytotoxicity} = \frac{\text{cpm experimental wells} - \text{cpm spontaneous release}}{\text{cpm maximum release} - \text{cpm spontaneous release}} \times 100$$

The percent standard deviation of all results presented did not exceed 10%.

Monoclonal antibody (mAb*) inhibition studies

The mAb inhibition studies were performed by preincubating the target cells with a final mAb concentration of 1:80, 1:400, or 1:4000 for 30 minutes. The target cells were then added to the effector cells and the technique completed as usual.

Monoclonal antibodies used:

mAb	Specificity	Contributor	References
L243	DR monomorphic	American Type Culture Collection	63
w6/32	class I	American Type Culture Collection	64
S3/4	DQw1	University of Minnesota	
Genox 3.53	DQw1	American Type Culture Collection	65
B7/21 (FA)	DP monomorphic	Trowbridge	66, 67
Hu-4	DR monomorphic	Aizawa, Sasazuki	68, 69, 70
Hu-30	DR1 and DR2 cells	Aizawa	68, 69, 70
PL3	DRw53	Knowles, Horibe, Dupont	71
Tu22	DQ monomorphic + DR	Wernet	72

RESULTS

Utilizing cells from unrelated donors phenotypically identical for HLA-A and B (see Materials and Methods), two bulk primed reagents were generated: one against DR4, DRw53, Dw4, DQw3 disparities and the other against DR2, Dw2, DQw1 disparities. The bulk primed reagents were "cloned" by single cell deposition using the fluorescence activated cell sorter (FACS IV - Becton Dickinson) or by limiting dilution of one cell per three wells. The resulting "clonal" cell populations were expanded in IL-2-containing medium in the presence of x-irradiated feeder cells (lymphoblastoid cell line (LCL) of the original sensitizing cell). After expansion for three weeks, the clones were tested for proliferation in the PLT assay using a panel of well-characterized PBL stimulator cells, and for lytic reactivity in a micro-CML assay using a panel of LCL target cells.

Panel Studies and Monoclonal Antibody Inhibition Studies of Cytolytic Clones Derived from the Anti-DR4, Dw4, DRw53, DQw3 Priming Combination

A total of 55 clones were tested: six clones demonstrated proliferative response to antigen but not cytolytic reactivity, 11 clones were cytolytic but not proliferative, 4 clones demonstrated both proliferative and cytolytic reactivity while 28 clones were nonreactive. Six clones demonstrated very

weak proliferative or cytolytic reactivity and were eliminated from further analysis. Results are presented for all clones which maintained reactivity and could be expanded to the numbers required for these testings.

Table I illustrates the combined results of three CML testings. The disparate priming specificities of the original stimulator cell that are shared by the designated LCL targets are underscored as well as % cytotoxicity values of 10% or greater. The target cell panel included cells which typed for the five HL.-Dw specificities associated with DR4 (Dw4, Dw10, Dw13, Dw14, and Dw15) as well as DQw3 positive, DR4 negative cells and DQw3 negative, DR4 negative cells. All clones lysed Dw4 positive targets including S (the original stimulator cell). Although target #3 could not be given a definitive assignment of Dw4 (see Materials and Methods), that target was lysed by most but not all clones. Some clones (15, 33, 46, 57) lysed only the Dw4 target cells and target #3; other clones (44, 67, 21, 56, 48, 63) also lysed target cells which typed for other DR4 associated Dw specificities. In addition, certain clones lysed targets which did not type for DR4, Dw4, or DQw3 (targets 13, 14, 15, 17, 18, 22).

Of 10 clones tested in mAb inhibition assays, the cytolytic activity of 9 was inhibited by the DR monomorphic mAb, L243 and Hu-4 (Table II). The anti-DQ mAb, Tu22, also appeared to inhibit cytolysis in some cases by some of the clones; this mAb has been reported to have anti-DR activity (72). The cytolytic activity of clone 21 is not blocked by the anti-DR or -DQ mAb directed at monomorphic determinants at the concentrations shown but is completely blocked (104% inhibition) at a 1:20 dilution of L243 in addition to being significantly inhibited by an anti-DRw53 mAb, PL3. This clone lyses 8 of 8 HLA-DRw53 positive target cells tested as well as 4 targets presumed to be DRw53 positive based on DR and DQ phenotyping (14 to 51% cytotoxicity), but

does not lyse 7 DRw53 negative targets or 3 targets presumed to be DRw53 negative based on DR and DQ phenotyping (Table I) (-20 to 4% cytotoxicity) An intermediate lysis of 9% cytotoxicity was observed with the Dw15 HTC target which does not type as DRw53 with local antisera but does type as DRw53 with 9th International Histocompatibility Workshop antisera.

Panel Studies and Monoclonal Antibody Inhibition Studies of Cytolytic Clones Derived from the Anti-DR2, Dw2, DQw1 Priming Combination

Clones were generated against DR2, Dw2, DQw1 disparate specificities using unrelated cells which typed identically for HLA-A and B and shared the DR3, Dw3, DQw2 and DPw4 specificities. This priming combination was also disparate for DPw6; the 7 DPw6 specific clones generated have, however, been excluded from this analysis. Clones were obtained by single cell deposition (FACS IV) or limiting dilution of one cell per three wells. A total of 123 clones were tested in PLT and CML assays. Twenty-six clones demonstrated proliferative but not cytolytic reactivity; 26 clones were cytolytic but not proliferative; 21 clones demonstrated both functions while 37 were non-reactive. Six additional clones demonstrated very weak and questionable proliferative or cytolytic reactivity and were not included in any of the subsequent experiments. Results are presented for all clones which maintained reactivity and could be expanded to the numbers required for these testings.

Table III illustrates the combined results of three CML testings. All clones tested lysed the Dw2 targets tested. In addition some clones (1-12, 3-84, 3-91) lysed the Dw12 target. Other clones (3-29, 3-27, 1-109) lysed additional DR2 positive targets. Clone 1-17 lysed the Dw2 positive targets as well as the DR2 negative, DQw1 positive targets tested. Clones 3-27 and 1-109 also lysed some DR2 negative, DQw1 negative targets.

Eight clones were tested in monoclonal antibody inhibition assays using anti-DR monomorphic mAb (L243, Hu-4), anti-DQw1 mAb (S3/4, Genox 3:53), anti-"DR2"-mAb (Hu-30), anti-DP mAb (FA), and anti-class I mAb (w6/32) (Table IV). The cytolytic activity of three clones (3-19, 3-29, 3-27) was inhibited by the anti-DR monomorphic mAb L243 and also Hu-4 where tested. The cytolysis of three additional clones (389, 317, 117) was inhibited by the DQw1 mAb.

The cytolytic activity by clones 1-109 was inhibited at the highest concentration of Hu30; 57% inhibition of clone 1-12 was also observed. All mAb tested were used in ascites form except for Hu-30 which was obtained at a 1:10 dilution of the ascites form; thus the final concentrations of Hu-30 mAb tested were 1:800, 1:4,000 and 1:40,000. Hu-4 did not inhibit the cytolysis of these two clones while there was slight inhibition demonstrated by L243 at the concentration shown.

Table V demonstrates the patterns of target cell lysis by the 3 DR-directed and 3 DQ-directed clones. Clone 3-19 lysed only Dw2 positive targets while clone 3-29 lysed all DR2 positive targets and clone 3-27 lysed some additional DR2 negative targets. Two of the DQ directed clones (3-89 and 3-17) lysed only Dw2 positive targets while clone 1-17 also lysed two additional DQw1 positive cells (targets 7 and 8).

Panel Studies of Proliferative Clones Derived from Both Priming Cell Combinations

The PLT testings of representative proliferative clones are illustrated in Tables VI and VII. Some clones (16, 56, 63) were restimulated by some DR4 positive cells and not by DR4 negative cells tested to date. Other clones (40, 41) were restimulated by some DR4 positive cells as well as some DQw3 positive cells. Clone 16 was restimulated by all DR4 positive cells tested. However, no clones were restimulated exclusively by Dw4 positive cells. Likewise, as illustrated in Table VII, no clones were exclusively restimulated

by Dw2 positive cells. Other clones (1-101, 1-84, 3-22, 3-29, 1-109) were restimulated by several DR2 positive cells. Three clones (3-22, 3-29, 1-109) were also restimulated by a DR2 negative, DQw1 negative cell (#15).

PLT reagents were generated by plating 5 cells per well; these reagents may not be true clones but are derived from a limited number of primed cells. When these reagents were screened for proliferative reactivity (Table VIII), several, as represented by reagents 596, 5178, 5186, and 5210, were restimulated by the Dw2 positive cells with clear discrimination from other DR2 positive or DR2 negative cells.

DISCUSSION

The definition of specificities recognized by T lymphocytes has relied largely on the proliferative response to class II MHC products in the mixed lymphocyte culture assay. The primary MLC has used HTC's as stimulators to define Dw specificities; the secondary MLC, i.e. the PLT assay, has yielded a better understanding of the individual stimulatory components, especially with cloning of the PLT-reactive cells.

We were prompted to investigate individual allodeterminants associated with DR and DQ with cloned T cells with two interrelated questions in mind. First, to establish which class II dimers express polymorphisms associated with the Dw subtypes and thus provide direct evidence for individual class II products regarding the existence of the more extensive polymorphisms recognized by T lymphocytes as compared with the serologically defined DR and DQ segregant series. Second, based on studies in mouse demonstrating that the various class II products may function differentially in regulating immune response (in that I-A restricts recognition of the beta subunit of lactate dehydrogenase for T helper cells and I-E for T suppressor cells (73-75)) to establish the basis for the probing of similar questions in man. The

investigation of allodeterminants is relevant in this regard; we have performed/participated in several studies recently that demonstrate that restricting determinants for several different nominal antigens are subtypic to the serologically defined DR and DQ specificities and may be closely related to the Dw associated allodeterminants (54-56). A detailed dissection of allodeterminants recognized by T lymphocytes is thus essential for any such undertaking.

Bulk primed cell populations were generated using cells matched for class I determinants and disparate for class II determinants. The primed cells were cloned by single cell deposition (FACS IV) or limiting dilution (1 cell/3 wells), and assayed for proliferative and cytolytic function with panels of cells well-characterized for HLA. The results suggest that there are multiple stimulatory determinants on the DR and DQ molecules; many of these determinants are commonly shared with cells which type for the same HLA-Dw specificity as the original sensitizing cell. In addition, these determinants may be occasionally shared by, or highly cross-reactive with, cells which do not express the same Dw, DR or DQ specificities as the sensitizing cell.

Cytolytic clones generated from the anti-DR4/Dw4 priming combination (Table I) or the anti-DR2/Dw2 priming combination (Table III), in general, lysed targets sharing the same Dw type as the sensitizing cell, with some clones lysing additional target cells. Whether the clones which only lyse targets bearing the same Dw specificity as the sensitizing cell are all directed against a single determinant or whether several determinants are frequently found on cells of that Dw type remains to be elucidated.

Monoclonal antibody inhibition studies of those clones directed at "DR" identified most clones derived from the anti-DR4/Dw4/DQw3 priming combination

which were blocked by the mAb directed at DR monomorphic determinants but not by the anti-DRw53 mAb, PL3. One clone (#21) appeared to be directed at a determinant associated or identical with DRw53 in that it lysed 8 of 8 DRw53 positive targets and was inhibited by the mAb PL3, described by Horibe et al. (71). Knowles et al.[1] have performed biochemical analysis of the DRw53 determinant defined by PL3 and have demonstrated that this determinant is carried on a single beta chain which appears to be identical to the previously described BR molecule (76). They found that this beta chain migrates identically in isoelectric focusing (IEF) techniques for all DR4 positive cells regardless of their Dw assignment by HTC typing. In contrast, the other DR beta chains show considerable heterogeneity in their isolectric points, as we initially showed for DR β chain heterogeneity in IEF correlating with DR4 associated Dw subtypes (49). Based on their results and our own panel studies we believe that the reactivity of clone #21 is probably directed against a different DRαβ dimer (referred to as $DR\alpha\beta_2$ (77)) than that recognized by the majority of clones that are strongly blocked by the anti-DR monomorphic mAb (presumably DR β1) and not blocked by PL3.

No apparent DQ directed clones were detected from the clones generated in this priming cell combination, although the responder and sensitizing cells were disparate for the DQw3 specificity. Fewer clones were initially tested from this priming combination than for the anti-DR2/Dw2/DQw1 priming combination (55 versus 112 respectively) where DQ directed clones were observed; DQ directed clones may have been detected in this priming combination had more clones been expanded to the quantities needed for testing. These findings are consistent with our previous suggestion that in

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[1]Knowles, R.W., Flomenberg, N., Horibe, K., Winchester, R., Radka, S.F. and Dupont, B., manuscript submitted.

some combinations DR associated determinants may be immunodominant (20).

Some clones from the anti-DR2/Dw2/DQw1 priming combination appeared to be DR directed while others appeared to be DQ directed based on blocking of cytolytic reactivity with mAb. Certain of the DR directed as well as the DQ directed groups of clones lysed only target cells bearing the same Dw specificity as the sensitizing cell. These results demonstrate that some clones directed against the DR or DQ molecules recognize determinants that are associated with the same Dw specificity as the sensitizing cell. Thus, it appears that determinants on both the DR (presumably DRαβ1) and DQ molecules contribute to the definition of a Dw subtype specificity assignment for a given cell. Neither the existence of Dw subtypes (given the complexity of determinants that make up Dw and the rather loose definition of a Dw specificity) nor the protein polymorphism discussed above, allows strict interpretation with regard to polymorphism of individual products of DR or DQ as seen by T lymphocytes. The question, thus, arises as to the extent of polymorphism detected by T lymphocytes associated with a single class II product that is subtypic to one or more serologically defined specificities of that product. Our demonstration in this study of subtypic determinants of DR and DQ with cloned T cells allows more rigorous interpretation. It would appear that there is a subtype polymorphism in at least some haplotypes for DRαβ1 and DQ defined by T lymphocytes as compared with the serologically defined polymorphism. We have recently speculated on the evolutionary aspects of these polymorphisms based on attempts to find restriction fragment length polymorphisms associated with the Dw subtypes (1,78).

Other clones appeared to recognize determinants on DR molecules from cells which typed for the same DR, but different Dw, specificities; occasionally a target cell which did not bear the same DR type as the

sensitizing cell was lysed to as great a degree as the sensitizing cell. These results indicate that clones are recognizing several different determinants on the DR molecules, some of which may be shared or highly crossreactive with determinants on DR molecules from cells of a different DR type.

The cytolytic activity of two clones (1-12, 1-109) obtained from the anti-DR2/Dw2/DQw1 priming combination was not blocked by the anti-DR monomorphic, -DQ, -DP, or -class I mAb. Cytolysis by one of these clones (1-109) was, and by a second clone (1-12) may have been blocked by the mAb, Hu-30. HU-30 did not block reactivity of a clone (3-27) that was blocked by L243 (DR monomorphic mAb). Certainly L243 or Hu-4 may bind to a different part of the same molecule to which Hu-30 also binds, such that recognition of either determinant is not interfered with by the binding of the other mAb. However, an equally plausible interpretation of these results is that Hu-30 may detect a DR molecule distinct from the DR molecule presumably expressing the DR1-14 alleles (1).

The mAb Hu-30 was initially defined as detecting a polymorphic determinant common to DR1 and DR2 positive cells (68) and has recently been described by Nishimura et al. (69) and Sone et al. (70) to detect a DR product common to Dw2 and Dw12 positive cells which they interpreted to be the DR β chain expressing DR2. They identified another DR product different between Dw2 and Dw12 positive cells which they designated as a novel class II molecule. We believe our results, and theirs as well, are consistent with an alternate interpretation. The DR product detected by Hu-30 and shared by Dw2 and Dw12 cells may be encoded by the "allele" of DRw52 and DRw53, i.e. a specificity which might be called DRw51, which has been previously undefined for DR2 and DR1 positive cells.

In addition, these clones may also be informative to classify cells which cannot be assuredly assigned to a Dw specificity by conventional HTC typing. Target cell #3 (Table III) was lysed by 10 of 14 anti-DR4-Dw4 clones and thus is the most frequently lysed target besides the known Dw4 positive target cells in these experiments. Family studies indicate target cell #3 is DR4 homozygous but heterozygous Dw13, Dw blank. This cell, as well as other family members' cells carrying the DR4, Dw blank haplotype occasionally give double normalized relative responses of 40-50% to stimulation by Dw4 HTCs but not other DR4 HTCs. This cell may carry a DR molecule which is similar to the conventional DR4 molecule contributing to the Dw4 phenotype yet does not share some of the other determinants commonly found with Dw4. Thus, there may be cells for which the Dw type may never be assigned by HTC typing (i.e. Dw blank assignment) but testing with PLT clones will help define specific class II products of that haplotype.

The proliferative clones (Tables VI and VII), like the cytolytic clones, were, for the most part, restimulated by cells which type for the same Dw specificity as the sensitizing cell. Although most clones were not restimulated exclusively by cells with the same Dw type as the sensitizing cell, reagents generated from plating five cells per well frequently demonstrated strong proliferative reactivity associated with restimulation by cells with the same Dw specificity, e.g. by Dw2 positive cells with clear discrimination from other DR2 positive or DR2 negative cells (Table VIII). These reagents are presumably composed of clones that are, in part, directed against different determinants on the DR molecule. If each determinant is common to the Dw2 cells but only occasionally found on other cells, and the various determinants are found on different non-Dw2 cells, the Dw2 positive cells would restimulate each clone comprising the 5 cell/well reagent

resulting in strong proliferation. If a Dw2 negative cell also carried an epitope detected by one of the clones in the reagent, the restimulation of one of potentially five clones would be observed as much weaker restimulation easily distinguished from restimulation by the Dw2 cells. Such reagents started from 5 cell/well may thus be potentially better typing reagents for the Dw specificities, but less useful for understanding the individual determinants involved.

We accept as a working model that the extensive polymorphism for DR (DR1 through DRw14) is associated with an αβ1 dimer and DRw53 with αβ2. We would suggest that much, or perhaps essentially all, of the polymorphism seen by clones blocked with L243 and Hu4 may be also associated with αβ1 for both DR4 and DR2. In fact, although we have only two clones putatively reactive with a determinant associated with DRw53 for DR4 haplotypes (blocked by PL3) and with the potential DR αβ2 dimer of DR2 haplotypes (blocked by Hu30), these clones do not detect a polymorphism subtypic to the DR specificity of the αβ2 dimer (DRw53 and the presumed "DRw51"). There is increasing, albeit limited, evidence supporting the concept that the DRβ2 gene/protein is relatively conserved on haplotypes carrying a single serologically defined specificity such as DR4 or DR2. First, several investigators have found one DR β spot in IEF that is relatively constant within the subtypes of DR2 (68) and DR4[2] (79); in the terminology we are using, this would be the DR β2 protein. Second, the two clones (21 and 1-109) described in the present study as potentially reactive with a DR αβ2 dimer both react with essentially all the DR4 and DR2 positive cells, respectively (although one clone that may be blocked by Hu30 reacts only with Dw2 positive cells).

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[2]Knowles, R.W., Flomenberg, N., Horibe, K., Winchester, R., Radka, S.F. and Dupont, B., manuscript submitted.

Thus, it may be that the more extensive T lymphocyte recognized polymorphism is associated primarily with DRαβ1 and DQ and that DRαβ2 is relatively conserved.

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TABLE I Cytolytic Clones - Anti DR/Dw4 Priming*

	Targets										
	S1	1	2	3	4	5	6	7	8	9	10
DR	3,4	4,4	4,4	4,4	4,4	4,4	4,4	4,4	4,4	4,4	4,4
DRw	53	(53)	(53)	53	(53)	(53)	53	53	-	53	53
Dw	3,4	4,4	4,4	13,4	13,13	13,13	14,14	14,14	15,15	10,10	10,10
DQ	3	3	3	(3)	(3)	3	3	3	-	3	3
DP	1	4	3,4	NT	NT	5	2,3	3,6	5	NT	4
clone #											
15	18	34	25	15	3	1	-2	-19	2	-2	3
33	43	62	46	34	1	8	8	-10	2	-4	1
46	16	62	37	28	9	3	5	-7	8	10	7
57	37	57	25	27	2	5	2	-13	3	1	3
9	13	56	17	-2	-4	-8	1	NT	-4	-3	4
44	9	56	16	-1	-2	-5	-1	NT	4	-1	5
45	11	56	39	1	-1	-3	12	NT	21	-2	2
12	55	65	60	37	6	0	3	-18	6	-4	5
67	35	59	44	46	-1	43	-1	-19	3	1	68
37	21	86	58	16	-2	5	16	NT	3	-6	1
21	14	46	24	22	17	14	26	17	9	17	71
56	31	64	35	32	62	24	55	NT	25	-4	3
48	14	46	25	27	34	5	62	64	19	10	3
63	34	70	42	11	50	53	32	NT	16	22	67

	Targets											
	11	12	13	14	15	16	17	18	19	20	21	22
DR	5,5	5,5	3,3	2,2	1,9	2,6	2,2	1,1	2,3	3,3	6,6	7,7
	52	NT	52	NT	53	52	-	-	52	NT	52	53
Dw	5,5	5,5	3,3	2,2	1,BSK	6,MN2	2,2	1,1	2,3	3,3	6,6	7,7
DQ	3	NT	2	NT	1	1	1	1	1	NT	1	2
DP	4	4	3,4	4,5	NT	NT	2,5	3,4	4,6	1,3	2,4	4
clone #												
15	-5	-4	6	1	3	-4	-2	-5	-5	-18	-9	-7
33	-6	-7	2	-2	4	-6	-6	3	-4	-2	-3	-4
46	-7	-7	4	-1	3	-5	-4	-2	4	-8	5	-8
57	-5	-4	3	-1	5	-5	-4	-1	3	-10	1	1
9	NT	NT	NT	NT	NT	NT	NT	NT	-3	NT	NT	NT
44	-6	-5	17	18	36	-5	-1	16	-4	-20	-7	-6
45	-5	-4	29	24	21	-3	-3	-3	2	-17	-8	-9
12	-4	-5	16	10	8	-4	-1	-7	1	-16	-6	-9
67	2	6	10	NT	NT	-2	16	2	7	-4	-2	-1
37	-4	4	17	6	13	-4	-1	-5	-4	-21	-9	-11
21	-3	-5	-5	4	51	-2	-4	-2	2	-20	-7	29
56	-4	-5	12	8	11	-4	-4	-5	-7	-20	-8	-10
48	-7	-6	4	9	49	-5	-6	55	4	-12	-8	-5
63	-7	-4	15	7	27	-3	1	-4	-7	-21	-9	3

*clones 56, 63, and 46 also demonstrate proliferative reactivity

Specificities indicated within parenthesis have not been tested but are as indicated based on the phenotyping data.

TABLE II Dissection of Determinants Associated with Different DR Dimers Anti-DR4/Dw4/DQw3 Priming Combination

	Anti-DR mAb						Anti-DRw53 mAb			Anti-DQ mAb		
	L243			Hu4			PL3			Tu22		
Clones	80**	400	4000	80	400	4000	80	400	4000	80	400	4000
15	113*	104	60	121	98	54		NT		14	25	31
37	121	129	121	111	79	57		NT		43	32	67
48	116	106	66	91	81	6	29	-29	-43	-3	9	3
67	111	89	38	117	109	57	24	17	24	-13	-11	2
63	97	32	38	145	104	31	-25	-150	0	38	45	62
33	71	20	21	71	29	17	-8	-10	12	8	29	32
56	100	97	33		NT			NT			NT	
45	100	100	100		NT			NT			NT	
44	97	100	88		NT			NT			NT	
21	7	8	14	20	2	10	101	104	76	14	5	29

* results expressed as % inhibition
value > 50% are underscored
NT = not tested

** reciprocal mAb dilutions

TABLE III Cytolytic Clones - Anti DR2/Dw2 Priming*

	Targets																	
	S1	S2	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
DR	2,3	2,3	2,2	2,2	2,2	2,2	2,2	2,2	6,6	1,1	4,4	4,4	4,4	4,4	3,3	3,3	7,7	5,5
Dw	2,3	2,3	2,2	2,2	2,2	12,12	FJ0	LD-5a	6,6	1,1	4,4	10,10	14,14	13,13	3,3	3,3	7,7	5,5
DQ	1,2	1,2	1	1	NT	1	1	3	1	1	3	3	3	NT	NT	2	2	3
DP	4,6	4	4,5	2,5	NT	NT	NT	NT	2,4	3,4	6,2	4	6,3	NT	1,3	3,4	4	4
clone #																		
3-89	19.1	38.3	5.2	23.3	41.4	7.8	1.3	-2.8	-7.8	-7.5	2.4	-.1	-.7	.1	6.4	-16.5	-1.2	.2
3-17	23.8	25,5	19.4	28.7	29.0	3.5	1.9	-2.8	-3.6	-8.0	3.6	-.6	-.9	-.7	4.2	-19.1	1.7	2.9
3-19	14.8	27.9	13.6	40.1	54.6	4.3	.2	-2.0	-1.8	-5.0	7.4	-.1	-.5	-1.7	4.9	-16.9	.4	-.1
1-12	27.7	43.5	35.1	54.0	40.4	19.8	-.5	-.6	-5.5	2.0	-.9	-1.4	-2.1	-2.7	-.6	-17.3	-3.5	-.7
3-84	11.1	21.5	13.8	43.5	40.4	30.4	4.1	-2.6	-6.9	-4.9	3.3	-.2	-.5	-1.5	1.4	-16.3	-1.2	4.5
3-91	10.5	6.3	14.4	13.5	19.0	17.7	5.3	4.9	-8.0	-6.7	7.2	-1.1	-1.3	-2.4	1.7	-19.3	-.6	1.5
3-29	26.8	27.0	21.6	40.9	30.7	29.2	46.6	42.8	-8.3	-7.1	8.1	-.7	-2.8	-4.2	12.5	-16.3	-2.0	-1.2
1-17	11.2	20.1	22.4	20.0	24.1	.5	4.7	-3.1	51.0	26.2	.7	-.7	-2.0	-2.9	-.6	-17.1	-1.3	-.6
3-27	27.2	44.9	17.6	53.1	49.8	35.9	17.7	-1.3	46.9	-4.8	4.4	51.0	-.2	-1.3	1.8	-15.3	2.0	3.9
1-109	14.6	56.7	33.6	55.0	44.0	8.8	16.1	44.8	12.6	-8.2	14.3	37.4	-3.0	-3.0	5.6	-18.2	22.9	4.3

*clone 3-19, 1-84, 3-91, 3-29, 3-27, 1-109 also demonstrated strong proliferative reactivity (>10,000 cpm) and clones 3-89, 3-17, and 1-17 demonstrated weaker proliferative reactivity

TABLE IV Dissection of Cloned Cytolytic T Cell Reactivity with Monoclonal Antibodies
Anti-DR2/Dw2/DQw1 Priming Combination

	Anti-DR mAb									Anti-DQw1 mAb						Anti-DP			Anti-Class I		
	L243			Hu4			Hu30			S 3/4			Genox 3.53			FA			w632		
Clones	80**	400	4000	80	400	4000	800	4000	40000	80	400	4000	80	400	4000	80	400	4000	80	400	4000
3-19	97*	67	24		NT			NT		12	6	6	15	12	21		NT			NT	
3-29	108	62	0		NT			NT		15	23	8	31	8	46		NT			NT	
3-27	89	53	42	92	97	47	12	15	15	47	37	53	47	26	32	27	64	18	25	35	20
3-89	-8	10	6	-52	-52	-34	0	3	3	115	100	64	49	26	21		NT		0	7	14
3-17	-69	-50	50		NT			NT		138	106	106	113	81	50		NT			NT	
1-17	-57	-86	-29		NT			NT		157	200	200	143	143	114		NT			NT	
1-12	32	-4	20	-34	-42	-49	57	20	3	24	20	8	24	32	40	33	41	26	20	26	20
1-109	46	15	31	-69	-56	-100	81	19	-194	-8	8	-8	15	23	46	36	18	64	6	19	-19

* results expressed as % inhibition

values $\geq$ 50% are underscored

NT = not tested

** reciprocal mAb dilution

TABLE V HLA-DR and DQ Determinants Comprising a "Dw Specificity"

		LCL target cells								
		S	1	2	3	4	5	6	7	8
HLA	DR	2,3	2,2	2,2	2,2	2,2	2,2	2,2	6,6	1,1
	Dw	2,3	2,2	2,2	2,2	12,12	FJO	LD-5a	6,6	1,1
	DQw	1,2	NT	1	NT	1	1	3	1	1
	DPw	4,6	4,5	2,5	NT	NT	NT	NT	2,4	3,4
Anti-DR Clones	3-19	15	14	40	55	4	1	-2	-2	-5
	3-29	27	22	41	31	29	47	43	-8	-7
	3-27	27	18	53	50	36	18	-1	47	-5
Anti-DQ Clones	3-89	19	5	23	41	8	1	-3	-8	-8
	3-17	24	19	29	29	4	2	-3	-4	-8
	1-17	11	22	29	24	1	5	-3	51	26

TABLE VI Proliferative Clones - Anti-DR/Dw4 Priming*

	Secondary Stimulators									
	S	1	2	3	4	5	6	7	8	9
HLA-DR	3,4	4,4	4,7	4,4	4,4	4,6	4,4	4,6	4,4	4,4
Dw	3,4	4,4	4,7	4,4	13,4	14,6	14,14	14,6	14,14	10,10
DQ	3	3	2,3	3	3	1,3	3	1,3	3	3
DP	1	4	4	6	2,4	4	3,6	4	6,4	4
Clones 16	8958	26728	3616	14664	45312	37042	44756	58580	15786	9654
56	21952	36942	12050	28858	27776	2344	27260	15450	25216	998
63	4022	9232	1270	1928	4710	1000	634	348	100	346
40	4244	1654	1732	3874	362	1740	106	116	1262	836
41	5700	8588	2180	5656	7902	280	4350	3722	802	684

	Secondary Stimulators								
	10	11	12	13	14	15	16	17	18
HLA-DR	4,5	4,4	3,5	2,7	2,5	8	1,6	2,3	3,7
Dw	10,5	15,15	3	2,7	2	8	6	2,3	3,7
DQ	3	-	2,3	1,2,3,	1,3	3	1	1,2	2
DP	4	5	2,4	4	4	4	1	1,2	4
Clones 16	17762	22120	524	690	512	812	100	661	100
56	7730	1008	436	408	702	100	100	222	100
63	860	342	116	168	208	100	162	162	248
40	5562	2666	6436	2504	7136	5970	1826	1885	1782
41	1082	9588	498	1744	4370	478	149	100	196

*clones 56 and 63 also demonstrated cytolytic reactivity

TABLE VII Proliferative Clones - Anti-DR2/Dw2 Priming*

	Secondary Stimulators								
	1	2	3	4	5	6	7	8	9
HLA-DR	2,2	2,5	1,2	2,6	1,2	2	2,3	2,5	2
Dw	2,2	2,5	1,2	6,MN2	1,MN2	MN2	12,3	5,-	-
DQ	1	1	1	1	1	1	1,2	NT	NT
DP	4,5	3	4	NT	NT	3,4	-	NT	2,4
Clones 3-70	5184	5860	1574	100	236	1052	576	1152	130
1-101	22344	15732	12758	100	168	100	200	5082	580
1-84	29572	17460	11586	1127	144	9808	812	135	162
3-22	11616	14568	7926	696	1074	2572	7422	7006	100
3-29	18454	28166	14048	422	3312	3470	14750	22674	266
1-109	9422	28080	8226	6126	5642	9840	6798	28814	7790

	Secondary Stimulators								
	10	11	12	13	14	15	16	17	18
HLA-DR	2,4	4,4	4,4	1,1	6,6	4,5	3,3	3,4	7,8
Dw	14,-	4,4	14,14	1,1	6,6	13,5	3,3	3,4	7,8
DQ	1,3	3	3	1	1	3	2	2,3	2
DP	NT	6	6,3	3,4	2,4	4	1,3	3,4	4
Clones 3-70	634	310	248	590	1276	640	280	690	1004
1-101	1712	343	100	116	448	1420	546	174	100
1-84	100	266	432	100	234	434	218	273	196
3-22	6704	100	100	210	250	4950	634	100	131
3-29	13674	230	143	150	100	4708	100	100	120
1-109	8608	722	100	171	8326	5908	112	246	1250

*clones 3-22, 3-29 and 1-109 also demonstrated cytolytic reactivity

TABLE VIII Proliferative LDA Reagents (generated from LDA of 5 cells per well) Anti-DR/Dw2 Priming

	Secondary Stimulators								
	1	2	3	4	5	6	7	8	9
HLA-DR	2,2	2,5	1,2	2,6	1,2	2	2,3	2,5	2
Dw	2,2	2,5	1,2	6,MN2	1,MN2	MN2	12,3	5,-	-
DQ	1	1	1	1	1	1	1,2	NT	NT
DP	4,5	3	4	NT	NT	3,4	-	NT	2,4
Reagent 596	6246	7856	7106	186	100	226	314	458	1280
5178	18565	17346	21498	538	172	408	1692	1606	2224
5186	8918	10840	3570	318	474	140	1032	830	698
5210	4065	4930	6162	362	132	245	738	576	166
5125	4216	13648	6632	390	272	310	5226	278	210
5181	13462	6456	8518	3944	700	1100	15204	10568	441

	Secondary Stimulators								
	10	11	12	13	14	15	16	17	18
HLA-DR	2,4	4,4	4,4	1,1	6,6	4,5	3,3	3,4	7,8
Dw	14	4,4	14,14	1,1	6,6	13,5	3,3	3,4	7,8
DQ	1,3	3	3	1	1	3	2	2,3	2
DP	NT	6	6,3	3,4	2,4	4	1,3	3,4	4
Reagent 596	100	138	100	126	111	100	100	100	364
5178	346	1318	1244	250	1510	964	582	940	1616
5186	770	666	100	238	156	142	100	171	252
5210	294	1288	450	139	750	152	302	100	166
5125	1116	268	180	148	280	100	336	568	366
5181	11200	1170	773	170	254	178	699	628	1768

END

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