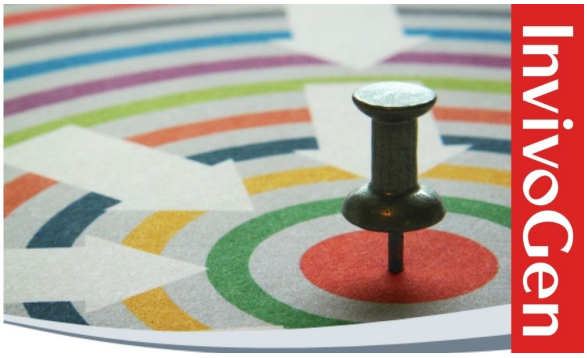

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HLA VARIANTS OF HUMAN LYMPHOBLASTOID CELL LINES AS TARGETS FOR CYTOTOXIC T CELLS: ANALYSIS OF CTL REACTIVITY AGAINST 2 HLA-DR VARIANTS[1]

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To test whether HLA-D-associated determinants can restrict cytotoxic activity, hapten-specific CTL were raised by co-culture of peripheral blood lymphocytes with TNP-modified autologous cells. Effector cells were tested against autologous cells as well as the B lymphoblastoid cell line T5-1 and 2 HLA-DR variants of T5-1 derived by immunoselection. T5-1 has a normal complement of HLA-A, B, C antigens, and expresses the DR1/DR3 specificities. A partial HLA-D loss variant, 816, and a total HLA-D loss variant, 616, were tested along with the parent. T5-1, 616, and 816 were used either as ^{51}Cr-labeled targets or as unlabeled target competitors of the lysis of autologous cells.

The 816 variant, deficient in DR1 expression, was comparable to the parent as a target in cross-reactive lysis. The 616 variant, lacking membrane expression of all HLA-D-associated antigens, was on the average only 70% as susceptible as the parent line. Effector cells from 31 individuals representing HLA-DR types 1 through 7, 10, and 11 were tested. Some individuals showed a much better ability than others to distinguish the parent and 616 variant.

Likewise, in ability to inhibit lysis of autologous T cell blasts, the total HLA-D loss variant (616) was on the average only 60% as effective as the parent cell line. Once again, some individuals were more able than others to distinguish T5-1 from 616, but this ability did not appear to segregate with a particular HLA type. Controls showed that the variant was not resistant to T cell-mediated lysis and that the 3 B lymphoblastoid cell lines possessed equivalent levels of HLA A, B, and C antigens. In addition, they all coupled with approximately 55×10^7 molecules of TNP per cell. These results suggest that full expression of the 29,000/34,000-dalton HLA-D heterodimer is required for maximal lysis and that such specificity may explain some of the widespread cross-reactivity seen with hapten-specific CTL.

The human equivalent of the murine I region, termed HLA-D, has recently been the subject of much biochemical and immunologic study (reviewed in 1). Genes in this region code for the polymorphic specificities expressed on the smaller of 2 chains that comprise a dimer consisting of 29,000 and 34,000 m.w. noncovalently linked proteins. Although in the mouse the equivalent H-2 I region is a rather weak histocompatibility barrier (2), retrospective studies of human skin grafting and cadaveric kidney transplants suggest that the HLA-D region may be an important histocompatibility barrier (3, 4). Furthermore, Feighery and Stastny (5) were able to demonstrate the *in vitro* generation of HLA-DR-specific cytotoxic T lymphocytes (CTL)[2] using HLA A and B-matched, HLA-D-mismatched pairs of responders/stimulators in mixed lymphocyte culture. Although human cytotoxic T cells have been shown to require expression of appropriate HLA A and B antigens in lysis of cells bearing viral, haptenic, or minor histocompatibility antigens, for lysis to occur, the HLA-D antigens have not thus far been shown to function in this manner.

Since the development of an *in vitro* system for generation of proliferative and cytotoxic responses to haptenated autologous cells (6), the extent of HLA restriction of the lysis of TNP-modified targets has yielded conflicting results. Shaw *et al.* (7, 8) demonstrated that approximately 40% of cytotoxic activity was restricted by the polymorphic HLA-A and B determinants. Charmot and Mawas (9), however, found no convincing evidence for HLA restriction of the cytotoxic response. Both studies confirmed the existence of rather widespread cross-reactivity, in that effector cells from 1 individual would lyse the TNP-modified target cells of another individual even where no HLA A and B antigens are shared. In the former study, some evidence was presented suggesting that TNP-specific CTL were restricted by HLA-linked but widely shared specificities not defined by current serologic techniques.

In man, where the population is essentially outbred and heterozygous, the highly polymorphic HLA system makes studies of CTL specificity more difficult than in the mouse. Hence, the value of an alternative approach for analysis of those HLA antigens recognized by human CTL is apparent. We present here a study using as targets a B lymphoblastoid cell line and 2 HLA-DR variants derived by immunoselection. The parent line, T5-1, was treated with an anti-DR1 serum plus complement (C), and 2 variants, 616 and 816, were isolated and described (10). T5-1 has the genotype A1, B8, DR3/A2, B27, Cw1, DR1. The 816 variant has lost reactivity with a panel of DR1 sera but has maintained the DR3 determinant. In addition,

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[2] Abbreviations used in this paper: CTL, cytotoxic T lymphocyte; LyCM, lymphocyte-conditioned medium; NK, natural killer (cell); SpA, protein A from Staphylococcus Cowan strain I; TNBS, trinitrobenzenesulfonate; PHS, pooled human serum; brbc, bovine red blood cells; 29/34, refers to the 29,000/34,000 bimolecular complex that carries the HLA-DR and perhaps other closely associated determinants.

it maintains reactivity with a monoclonal anti-human Ia 7.2 (11) and rabbit anti-human B cell-specific sera. The 616 variant has lost reactivity with both the DR1 and DR3 antisera as well as the monoclonal and heterologous antisera. More recent experiments show that T5-1 also possesses the MB-1 and MB-2 determinants (12). The 616 variant has lost both of these, whereas the 816 has lost only the MB-1 (Gladstone and Pious, manuscript in preparation). Since 616 and 816 have lost expression of determinants in addition to those selected against, and because these lesions are associated with the absence from the membrane of the 29,000/34,000 dimer in 616, the deleted antigens will be referred to as 29/34-associated rather than merely HLA-DR. This is meant to include, of course, the HLA-DR determinants. That variants derived in immunoselection have lost more than 1 determinant confirms previous experiments that show that the antibody acts only to select a preexisting variant and hence determines the minimum and not the maximum size of the lesion. The antisera do not themselves induce the observed variation (13).

Hence, these variants are perhaps the closest human equivalent to the congenic strains used in murine specificity studies. They maintain unaltered expression of the HLA A, B, and C determinants and thus allow us to test whether *any* 29/34-associated determinants are required (616) and/or whether the 29/34 determinants associated with the DR1/MB-1 specificities (816) are important in restricting CTL recognition. Our results suggest that in the majority of responders tested, expression of monomorphic determinants associated with the 29,000/34,000 dimer is required for optimal lysis to occur.

MATERIALS AND METHODS

In vitro sensitization. Generation of hapten-specific CTL was performed essentially as described previously (6), using peripheral blood mononuclear cells. Responders were cultured with an equal number of trinitrobenzenesulfonate (TNBS) modified and x-irradiated (1600 R) stimulators for 1 wk, followed by restimulation for an additional 4 to 5 days. Cells were cultured in RPMI 1640 containing 10% heat-inactivated, pooled human sera (PHS) from untransfused males, and antibiotics. Haptenation of cells (20×10^6/ml) was performed with 5mM TNBS for 10 min at 37°C, followed by several washes to remove unreacted TNBS.

Cytotoxicity assay. After boosting in secondary cultures, effector cells were harvested, resuspended in fresh medium, and assayed in round-bottom microtiter plates in a 4-hr assay at various effector:target ratios, using 10^4 targets/well. Targets were either autologous blast cells maintained in lymphocyte-conditioned medium (LyCM) (see below) or lymphoblastoid cell lines. Targets were labeled overnight in medium plus $Na^{51}CrO_4$, washed, and either left uncoupled or were haptenated with 5 mM TNBS as for stimulator cells. At the end of assay, 100 μl supernatant was harvested and counted in a gamma counter. Each effector:target ratio was performed in triplicate.

Cold target inhibition. This was performed at effector:target ratios of from 20:1 to 50:1. Autologous ^{51}Cr-labeled targets and unlabeled (cold) target (haptenated or unhaptenated) lymphoblastoid cell lines were mixed, followed by addition of effectors. In all these experiments, autologous targets are T cell blasts maintained in LyCM.

Lymphocyte-conditioned medium (LyCM). LyCM was prepared using RPMI 1640 plus 10% FCS, 1% PHA-M, and antibiotics. Mononuclear cells prepared from Ficoll-Hypaque were adjusted to 10^6/ml to which was added, as stimulator, 2×10^5/ml of x-irradiated (5000 R) Daudi lymphoblastoid cell line (14). This technique results in optimal growth-factor production (S. Gillis, personal communication). Supernatants were harvested at 48 hr and used at 20% with RPMI 1640 and 10% FCS for maintenance of lymphoblasts.

Autologous T cell blasts. These were derived from unfractionated peripheral blood and were maintained at 2×10^5 to 10^6/ml by splitting and daily feeding with LyCM. By day 12, these cells were 30 to 50% Ia positive as determined by lysis with a monoclonal anti-Ia reagent, 7.2, plus C (11). Approximately 90% were E rosetting cells. These cells are essentially PHA blasts, which have been maintained during the TNP-sensitization phase for use as targets.

Lymphoblastoid cell lines. Lines used in these experiments are T5-1, a B cell line derived by Epstein-Barr virus transformation of B cells from a normal Caucasian female, and 2 HLA-DR variants of T5-1, 616, and 816 (10). These lines were maintained in RPMI 1640, 10% FCS, plus antibiotics. Target cells were taken from these cultures at stationary phase. Viability was greater than 95%. T5-1 and variants were checked routinely for comparability of cell volume. Fresh stocks of frozen cells were thawed for use every 8 wk. The HLA type of T5-1 is A1, B8, DR3/MB2; A2, B27, Cw1, DR1/MB1. The 616 variant is A1, B8; A2, B27, Cw1; and the 816 is A1, B8, DR3/MB2; A2, B27, Cw1.

Mixed lymphocyte cultures. These were established with peripheral blood mononuclear cells: 10^7 responders in 10 ml RPMI 1640 plus 10% PHS plus 2.5×10^6 x-irradiated (5000 R) T5-1 or 616, or 10^7 normal PBL, x-irradiated (1600 R). Responders were harvested on day 6 and assayed for cytotoxicity against T5-1 and 616. No TNBS was used in these experiments.

Antibodies. Two monoclonal reagents were used in these studies. First, 7.2 is an IgG2b antibody derived from the fusion of a spleen from a mouse immunized with human peripheral blood cells with the NS1 myeloma cell line (11). It precipitates a 29,000/34,000 dimer from B-LCL, is reactive with B cells and monocytes, and is capable of blocking proliferation in mixed lymphocyte culture (W. Newman, unpublished results). This was a kind gift of Drs. John Hansen and Paul Martin. Second, W6/32 is a monoclonal IgG2a antibody specific for the 45,000 m.w. chain of HLA (15). It was purchased from Accurate Chemical and Scientific Corp., Hicksville, NY.

Radioimmunoassay. Radioimmunoassay of binding of the 7.2 and W6/32 monoclonal reagents was performed using ^{125}I Staph-A protein (SpA). Because 7.2 bound SpA poorly, it was necessary to use a goat anti-mouse IgG in a 2-step procedure. 5×10^4 cells were added in triplicate to V-bottom microtiter wells and incubated at 37°C for 1 hr with undiluted culture supernatant of 7.2 (cytotoxicity titer 1/400) or W6/32 at 20 μg antibody/ml. These concentrations were determined to be in antibody excess. Cells were washed twice, and for 7.2, incubated with a 1/50 dilution of goat anti-mouse IgG for ½ hr, 37°C. ^{125}I SpA was then added to the wells and incubated at 37°C for 1 hr, followed by 3 washes. Cells were resuspended and counted on a gamma counter. This procedure has been published in greater detail by Brown *et al.* (16).

Depletion of Fc receptor-positive cells. This was performed using a rabbit anti-bovine rbc IgG fraction. Bovine rbc (brbc) were washed 3 times with PBS and then incubated as a 2% suspension with antiserum at 1/10 for 1 hr at 4°C, followed by 3 washes to form antibody-coated erythrocytes (EA). Effector cells at 4×10^6/ml in 10% FCS, brbc adsorbed, were mixed with an equal volume of a 1.5% suspension of EA, spun at 200 × G for 5 min, and kept 4°C for 1 hr. Cells were gently resuspended

and layered over Ficoll-Hypaque, then centrifuged 400 × G for ½ hr. Nonrosetting cells were collected at the interface, washed 2 times, and resuspended in medium for cytotoxicity assay. This procedure resulted in a population >95% depleted of Fc receptor-positive cells as determined by analytical re-rosetting of the EA-depleted population.

Quantitation of cell surface TNP. This was performed as described by Inbar *et al.* (17). Briefly, 80 × 10^6 cells were washed free of serum in PBS and coupled at 37°C for 10 min with 5 mM TNBS, pH 7.4, 20 × 10^6 cells/ml. The cells were washed extensively with RPMI 1640, then 5 × 10^7 cells were incubated in PBS with 0.5% Nonidet P-40 for 15 min at room temperature. After centrifugation to remove debris, the supernatants were clarified by addition of Na- deoxycholate (1%), and absorbancy at 348 nM was determined. Cells not treated with TNBS were used as a blank.

Statistical analysis. Statistical analysis was performed using Student's paired *t*-test.

RESULTS

To test whether anti-TNP cytotoxic T cells can distinguish parent cells from Ia-negative variant, effector cells were tested in cross-reactive lysis. Shown in Figure 1 is the lytic activity of HR anti HR-TNP effector cells on the T5-1_{TNP} and 616_{TNP} targets. HR's HLA type is Aw24, Aw31, B13, B40 and DRw6, DR7 and hence shares no serologically defined HLA antigens with T5-1. As can be seen in Figure 1*a*, there is considerably less lysis of the 29/34-negative 616 variant than of the parent. At an effector:target ratio of 20:1, the 616 is only 49% as susceptible to lysis as is the parent. In 2 repeated testings of the same individual, values of 29% and 40% were obtained. To address the possibility that the 616 may be relatively insusceptible to lysis, CTL from the same responder were generated by co-culture for 5 days with irradiated T5-1 or 616 (unhaptenated) and tested on the parent and variant in a standard 4-hr Cr release assay. As seen in Figures 1*b* and 1*c*, CTL raised against T5-1 lyse T5-1 and 616 comparably, and vice versa. Hence the 616 variant is not inherently insusceptible nor is the T5-1 especially susceptible to lysis. T5-1 is relatively insusceptible to lysis by natural killer (NK) cells (18, and data not shown). Also, the ability of anti-TNP effector cells to distinguish the parent and variant was unaffected by prior depletion of Fc receptor-positive cells using antibody-coated bovine erythrocytes (data not shown).

It is of interest to note that with donor HR above, no HLA DR antigens are shared with T5-1, suggesting that it is not HLA-DR, but rather other 29/34-associated determinants that are responsible for a substantial portion of the lysis of T5-1_{TNP} targets.

To try and extend the results with HR, a total of 31 individuals representing DR types 1 through 7, 10, and 11 have been tested with T5-1_{TNP} and 616_{TNP} in cross-reactive lysis. A summary of these results is presented in Figure 2, along with data from 9 individuals on a second DR variant, 816, deficient in the expression of D region antigens of one haplotype (DR1/MB1). Results are expressed as a ratio (r) of lysis of the variant to the parent at an effector:target ratio of 20:1. A value of 1.0 would indicate no difference. For the 616 variant, the mean r value was 0.70 (±0.032 SEM, $p<0.0005$), and for the 816 variant, the mean r value was 0.98 (±0.117 SEM, $p>0.05$). In all of these experiments, unhaptenated T5-1 was poorly lysed.

As was noted for individual HR (Fig. 1), many donors were identified who did not share the T5-1 DR1/DR3 antigens but who nevertheless showed reduced lysis with anti-TNP effector cells on the 616 variant. Also, in lysis of the DR1/MB1-negative 816 variant by effector cells from 9 different individuals, the mean value showed no significant reduction. These results indicate that in cross-reactive lysis by anti-TNP effector CTL, widely shared 29/34-associated determinants, rather than the polymorphic DR alloantigenic determinants, are important CTL targets.

We have sought to confirm these results using the lymphoblastoid cell lines as cold target competitors of the lysis of autologous TNP targets. The ^{51}Cr-labeled targets were 90% T cell blasts from 12 days of growth in PHA-containing LyCM. Thirty to 50% expressed Ia antigens, as determined by antibody and C-mediated cytotoxicity using monoclonal antibody 7.2 (11). A summary of these results with T5-1_{TNP} and 616_{TNP} is presented in Figure 3 for the 12 individuals tested. As can be seen, unhaptenated T5-1 was poorly inhibitory, and usually gave less than 5 to 10% inhibition relative to no cold targets. When haptenated, the parent and variants gave the results

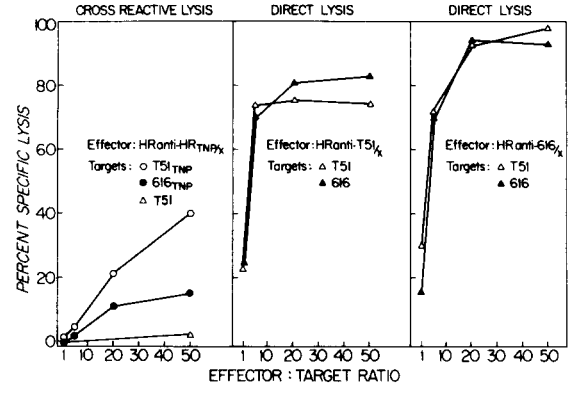

Figure 1. Left, cross-reactive lysis of haptenated T5-1 and 616, by HR effectors raised against TNP-modified autologous cells. HR has the HLA phenotype Aw24, Aw31, B13, B40, Dw6, Dw7. *Center and right,* HR effectors raised against x-irradiated (5000R) T5-1 or 616, and tested in criss-cross fashion.

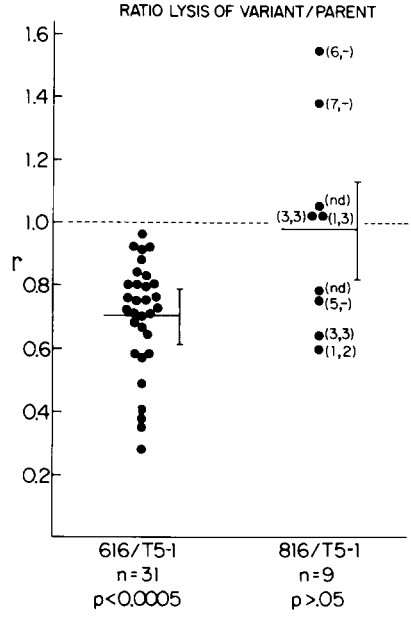

Figure 2. Each point represents the ratio (*r*) of lysis by anti-autologous TNP effector cells of the variant 616 (*left*) or 816 (*right*) relative to the parent (T5-1) at a 20:1 effector to target ratio. Horizontal bar represents the mean *r* value ± SEM. The dashed line at 1.0 represents no differences observed between lysis of variant and lysis of parent.

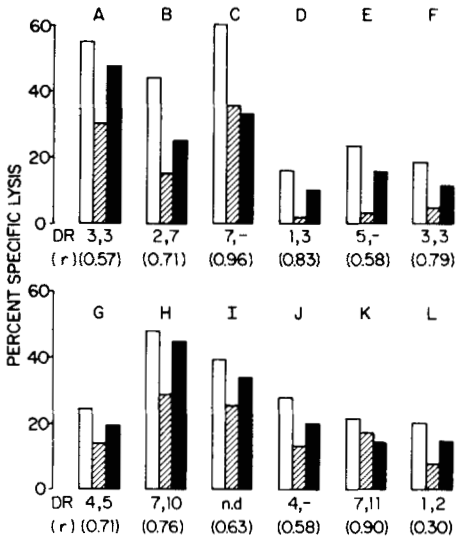

Figure 3. Each set of 3 bars represents the cold target inhibition of the lysis of TNP-autologous blasts by anti-TNP effector cells at a cold to hot ratio of 5:1. Results from the 12 individuals tested (A-L) are presented. Cold targets were unlabeled T5-1 (□), T5-1_{TNP} (▨), or 616_{TNP} (■). Shown beneath the set of values for each individual are the DR types, and in parentheses, the *r* values taken from Figure 2.

predicted from cross-reactive lysis. Namely, the parent line was either as good or more effective in blocking lysis of autologous haptenated T cell blasts than was the 29/34-negative 616 variant. Shown for each individual as well are the r values from cross-reactive lysis shown in Figure 2. At a cold:hot target ratio of 5:1, the mean inhibitory difference between T5-1_{TNP} and 616_{TNP} was 7.8% lysis (SEM ± 1.7, $p<0.0005$) for the 12 individuals shown. This represents an average 41% decrease in the relative inhibiting power of the 616 line compared with T5-1. These same results were evident at a 15:1 cold:hot target ratio. Also shown in Figure 3 are the DR types, which reveal that individuals not sharing the DR1 and DR3 antigens of T5-1 nevertheless can distinguish the T5-1/616 pair in cold target inhibition studies. Hence, other 29/34-associated determinants besides the DR antigens appear to limit the recognition of hapten by cytotoxic effectors. More important, perhaps, than the average response to the T5-1/616 pair is the finding that there were individual differences in the ability to distinguish this pair.

Results with 3 individuals were chosen as representative of the differing ability to distinguish T5-1_{TNP}/616_{TNP} in cross-reactive lysis and cold target inhibition. Full curves on these are presented in Figure 4 together with their mean r values from repeat experiments at 20:1 effector:target ratio, and their HLA-DR types. Individual A (r = 0.57) was well able to distinguish the pair of lines, individual B (r = 0.71) was intermediate, and individual C (r = 0.96) was essentially unable to distinguish the T5-1_{TNP}/616_{TNP} pair here and on 2 repeated testings for a total of 3 experiments. As can be seen, these results are confirmed by the cold target inhibition data in the lower half of Figure 4.

Also shown in Figure 4 for individuals A and C are the cross-reactive lysis data for the DR1 negative 816_{TNP} variant. Note that although individual C shows no reduced lysis on the 816_{TNP} variant, individual A, although not DR1, does show reduced lysis with 816, although not as pronounced as for 616. As stated earlier, the 816 has lost as well the DR1-associated MB1 determinant as well as DR1. More extensive testing of the 816 variant on individuals differing at the DR1 and MB1 loci will be necessary to confirm whether these determinants can restrict T cell recognition of hapten. It is noteworthy that individual A (HLA A 1,1; B 8,8, DR 3,3) and individual B (HLA A 1,2; B 7,8, DR 2,7) share 4 of 4 or 3 of 4 HLA A/B antigens with T5-1/616, respectively. Despite this sharing, loss of the 29/34 antigens in the 616 line coincided with a substantial decrease in lytic susceptibility.

To be certain that the observed difference between haptenated T5-1 and variants was not due to quantitative differences in the amounts of hapten on the membrane, the lines were haptenated, membranes were solubilized in Nonidet P-40, and the number of molecules of TNP/cell was determined spectrophotometrically (17). As seen in Table I, T5-1, 616, and 816 all took up 50 to 60 × 10^7 molecules of TNP/cell.

T5-1, 816, and 616 have been shown in antibody-mediated cytotoxicity to be indistinguishable in their expression of the HLA A, B, and C determinants (10). Moreover, heterologous antisera raised against T5-1 and absorbed with 616 was B cell specific (10), indicating that the major and perhaps only difference between T5-1 and 616 is in the deficiency of Ia antigens in 616. However, it was essential to show that the observed alternations in target susceptibility or cold target inhibiting ability were not due to quantitative alterations of the HLA antigens. For these experiments, we performed a radioimmunoassay with ^{125}I SpA binding to cells treated with monoclonal antibody to HLA heavy chain (W6/32), which reacts with a monomorphic determinant shared by the HLA A, B, and C gene products. Also shown are experiments with the anti-Ia monoclonal antibody 7.2. Both reagents were used in antibody excess. These results are shown in Table II and indicate that there was no substantial increase or decrease in the amount of HLA 45,000 m.w. chain on 616 and 816 relative to T5-1. However, using a monoclonal reagent specific for a framework determinant of the 29/34 dimer, 616 was totally deficient, whereas 816 bound the same number of counts as did the parent T5-1.

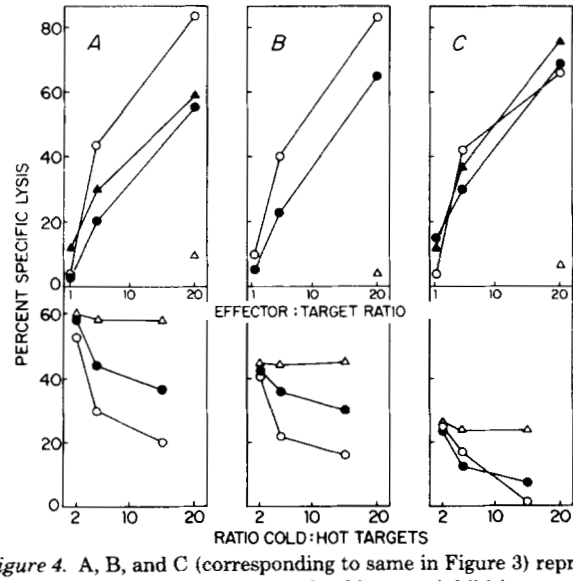

Figure 4. A, B, and C (corresponding to same in Figure 3) represent the complete cross-reactive lysis and cold target inhibition curves of three individuals representing high, intermediate, and negligible abilities, respectively, to distinguish the T5-1/616 pair in cross-reactive lysis (*upper*) or cold target inhibition (*lower*) with autologous haptenated blasts as labeled targets. T5-1 no TNP (△); T5-1_{TNP} (○); 616_{TNP} (●); 816_{TNP} (▲). The HLA types of the responders are as follows: *A*, A 1,1; B 8,8; DR 3,3; *B*, A 9,23; B 5,13; DR 5,-; *C*, A 3,11; B 39,40; DR 7,11.

TABLE I

TNP quantitation, T5-1 and variants

	Molecules TNP/Cell (±SE) $\times 10^{-7}$
T5-1 (parent)[a]	56 ± 9
616 (Ia loss variant)	60 ± 10
816 (DR 1 loss variant)	64 ± 15

[a] Cells were taken from stationary phase and derivatized as described in *Materials and Methods*. Numbers represent the mean ± SE of three separate determinations.

TABLE II

Radiobinding assay, T5-1, and variants

Antibody	T5-1 (Parent)	616 (Ia Loss)	816 (DR 1 Loss)
None	146 ± 36[a]	139 ± 10	146 ± 30
Anti-Ia monoclonal (7.2)	5,228 ± 473	142 ± 18	5,614 ± 277
Anti-HLA monoclonal (W6/32)	17,487 ± 753	17,050 ± 183	18,255 ± 781

[a] Counts bound per 2 min, mean ± SE of 2 separate determinations.

DISCUSSION

We have shown that in most cases CTL derived by co-culture of peripheral blood lymphocytes with autologous haptenated stimulator cells require the presence on target cells of both hapten and monomorphic determinants associated with the HLA-DR 29/34 bimolecular complex for full cytotoxic activity. This conclusion is based on the following observations: 1) Cross-reactive lysis seen on the B cell line T5-1 is significantly diminished when tested on the total Ia-negative variant 616 and is not significantly diminished overall when tested on the DR1 loss variant 816. These differences seen with the 616 line were found in individuals of DR types 1 through 7, 10, and 11, and are not limited to those individuals sharing DR1 and DR3 with the parent line. 2) cold target inhibition analysis of lysis of autologous T cell blasts with parent and variants confirmed that the lesion in 616 coincided with a mean 41% diminution in inhibiting capacity relative to the parent line. 3) controls excluded that the reduced activity of 616 was due to decreased expression of HLA-A, B, C antigens, insufficient uptake of TNP, or inherent insusceptibility to lysis of the 616 line.

Despite loss of the 29/34 dimer, the 616 variant retained substantial lytic susceptibility and cold target inhibiting capacity. Preliminary inhibition experiments with the W6/32 antibody against HLA A, B, C framework determinants reveal that most but not all of this susceptibility is inhibitable. Additional variants of 616, defective at both A or both B loci, may also be helpful in addressing the reasons for the remaining resusceptibility to lysis.

The variant 616 has lost reactivity with all sera that define the HLA-D-associated 29,000/34,000 bimolecular complex, including anti-DR sera, a monoclonal anti-Ia (11), and heterologous anti-B cell sera (10). It has also lost expression of the MB1 and MB2 antigens (Gladstone and Pious, manuscript in preparation). It is not clear from our experiments whether the decreased reactivity shown with the 616 variant is due to loss of DR, MB, or as yet other undefined but 29/34-associated determinants. It is unlikely that loss of DR alloantigenic determinants alone is the sole reason for the reduced activity seen, because many non-DR1/DR3 individuals showed reduced lysis on 616, and overall, the DR1 loss variant, 816, showed little reduction in susceptibility. It remains a possibility that loss of MB1 and MB2 is a reason for the reduction in susceptibility to lysis seen here, although MB typing will be necessary to resolve this point.

Of interest was the finding that individuals differed in the degree to which they could distinguish the T5-1/616 cell lines. Although some showed a marked ability to lyse T5-1 better than 616, others showed only a moderate ability to distinguish. Four individuals studied failed to distinguish the T5-1/616 pair. Three of these share 2 or more HLA A and B antigens with 616, and it is possible that the strength of response to these antigens plus hapten masked a response to HLA-D plus TNP determinants. One of these, however—individual C of Figure 4—failed despite lack of shared HLA A, B determinants with T5-1. These results have been performed 3 times. Although no definitive explanation for this minority of responders is available, 2 possibilities can be suggested. First, it may be that the 29/34-associated determinants restricting CTL recognition of TNP are not shared by these nondistinguishers and T5-1, and for lack of these antigens in the priming phase, no diminished response on 616_{TNP} relative to $T5\text{-}1_{TNP}$ would be expected. Possible candidates would be the DC (19) or MB (12) antigens, or perhaps those now being defined by primed lymphocyte typing assay between HLA identical individuals (20).

Perhaps more likely is the possibility that the CTL response to hapten-modified HLA antigens is under HLA-linked control, as has been shown already for the CTL response to influenza-infected autologous cells (21). That is, the nondistinguishers observed here may possess the relevant 29/34-associated determinants recognized by CTL, but recognition of these in association with hapten does not occur. Whether such nonrecognition is under HLA-linked control would require identification and study of a family whose members variously expressed this trait. Some form of genetic regulation of the response to haptenated cells may also explain why some individuals show a much better lysis of the parent than of the Ia-negative variant. It may be that use of the T5-1/616 combination will be the most expeditious manner in which to identify differing (HLA-linked?) abilities to recognize 29/34-associated CTL target antigens.

After initial reports that the HLA-DR/Dw determinants were not targets for CTL (22–24), Feighery and Stastny (5) showed that effectors generated in HLA A, B-matched, DR-mismatched combinations and assayed on Ia-positive monocytes were specific for the HLA DR determinants. Experiments in other laboratories with TNP-specific CTL have not fully addressed the question of whether DR antigens can restrict lysis of hapten-modified targets (7–9). However, in light of our findings with the 616 and 816 cell lines, it does not appear that the allotypic DR specificities are the sole HLA-D-linked determinants to restrict lysis. What is significant in the present findings is that specific loss of the entire 29,000/34,000 heterodimer, including those monomorphic determinants recognized by heterologous and murine monoclonal antisera (616 variant), coincide with a substantial loss in susceptibility to lysis and cold target inhibitory ability. Hence, the serologically defined HLA-DR allotypic determinants do not seem to restrict lysis of haptenated targets even though they do seem to be targets for alloreactive CTL (5).

In murine systems, Starzinski-Powitz *et al.* (25) and Wagner *et al.* (26) have shown that H-2 I-region-restricted, TNP-specific CTL can be generated. However, this specificity represented only 2 to 5% of the lytic activity and was much less substantial than reactivity to TNP in association with the H-2K and H-2D structures. Theirs was a restriction analysis not strictly comparable to the present studies. However, Russell *et al.* (27) and Dennert and Hyman (28) have tested mouse tumor lines devoid

of serologically detectable H-2, and in the TNP system such cells were inefficient both as stimulators of CTL and as targets. They concluded that the serologically defined major histocompatibility complex products were required for ability to stimulate CTL and to serve as targets.

We have extended this technique to the study of human CTL, using variants with limited rather than total loss of HLA antigens. Our results suggest that some of the widespread cross-reactivity seen in the human TNP system may be directed to monomorphic determinants associated with the 29,000/34,000 dimer.

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