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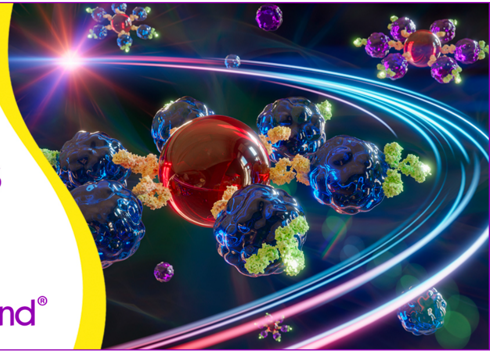

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CONSERVATION AND ALTERATION OF HLA-B27-SPECIFIC T CELL EPITOPES ON MOUSE CELLS

Implications for Peptide-Mediated Alloreactivity[1]

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Alloreactive CTL responses generate a great variety of clonal specificities. Such diversity may be related to recognition of multiple peptides constitutively bound to any given MHC alloantigen. Among human alloreactive CTL, only a fraction of the clones lyse mouse P815 cells expressing class I HLA proteins. In this study the fine specificity of HLA-B27 allorecognition on human or mouse cells by five human HLA-B27-specific CTL clones was comparatively analyzed. This was done to examine what degree of variation in epitope structure is compatible with recognition of HLA Ag on mouse cells. Nine site-specific HLA-B27 mutants were expressed on both human and mouse cells, after DNA-mediated gene transfer, to construct two analogous series of target cells. The reaction patterns of four of the five CTL clones with these cell panels were compatible with conservation of their corresponding epitopes upon expression of HLA-B27 on mouse cells. The reaction pattern of the fifth clone was different with either cell panel, indicating that its epitope was structurally altered on mouse cells. It also suggested a selectively increased expression of the determinant on these cells. The results suggest that most of the epitopes recognized by allospecific CTL clones reacting across species are either independent of any bound peptide or involve identical peptides from both cell types. However, some of these clones recognize alloantigen-bound peptides that are somewhat different in structure depending on the cell type, and may be expressed at the mouse cell surface in greater amounts. Such peptides could arise from related proteins in both species, and be polymorphic as a result of phylogenetic divergence.

Lysis by CTL requires recognition of specific Ag, together with binding of various T cell adhesion proteins to their ligands on the target cell. Besides the TCR, three T cell molecules are involved in CTL adhesion. These are LFA-1, CD2, and CD8. LFA-1 binds to ICAM-1 and ICAM-2 (1, 2), CD2 binds to LFA-3 (3, 4), and CD8 binds to a nonpolymorphic site on the $\alpha 3$ domain of class I HLA molecules (5). LFA-1 binding can be promoted by the TCR and by CD2 through induction of a high avidity state in that molecule, which is mediated by intracellular signaling pathways. TCR- and CD2-mediated induction differ in that the former is transient, whereas the later is long-lived (6–8). Activated CD8 binding does also occur. This may happen through the formation of a trimolecular complex involving a common ligand—the class I HLA molecule—for the TCR and for CD8 (9, 10), or through a metabolically induced increase in CD8 avidity upon TCR binding (11).

Lysis of mouse target cells expressing class I HLA Ag, by human alloreactive CTL, is frequently inefficient (12). P815-HTR, a cell line derived from the murine mastocytoma P815 (13), is significantly more susceptible to lysis by human CTL than other mouse cells, when transfected with class I HLA genes (12, 14, 15). Nevertheless, many human alloreactive CTL clones cannot kill HLA P815 transfectants (16–18). Two factors may contribute to this failure: 1) insufficient avidity of human CTL for mouse target cells, and 2) changes in particular epitopes upon expression on mouse cells.

Human CTL adhesion to mouse targets differs significantly from analogous interaction between human cells. First, CD2/LFA-3 binding does not occur, as mouse cells lack the appropriate ligand for human CD2. Second, although LFA-1 binds to some molecule(s) on mouse cells, it is likely that it does so less efficiently than with its physiologic ligands. Indeed, cotransfection of human ICAM-1 with HLA-DR on mouse L cells was critical for human T cell activation by transfectants with modest levels of HLA expression (19). We have previously shown that, for human alloreactive CTL clones with similar fine specificity, their capacity to lyse P815 transfectants expressing HLA-B27 correlated with CTL avidity (18).

Correlation between fine specificity of alloreactive T cell clones and their capacity to lyse xenogeneic target cells has also been reported (17). An interpretation of this finding involved recognition of endogenous peptides bound to the alloantigen, that would be differentially expressed on human and mouse cells. This would provide a basis for epitope changes upon HLA Ag expression on either cell type. There is increasing evidence that endogenous peptides are constitutively bound to class I MHC molecules (20–23), and that they are involved in allorecognition (24–28). Indeed, tissue-specific peptides may mediate allorecognition in some instances (28–31).

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The above findings imply that only those human CTL of appropriate specificity and sufficient avidity can recognize and kill mouse cells expressing class I HLA molecules. This must impose great restrictions to a possible alteration of the corresponding HLA epitopes upon expression on mouse cells. To examine this question, we have analyzed the fine specificity of various anti-HLA-B27 human CTL clones which cross-reacted with HLA-B27^{+} mouse transfectant cells. The results suggest that most of the CTL clones examined recognized unaltered epitopes on mouse cells. At least one clone probably recognizes an structurally different, but related, epitope that may be expressed at a higher level on mouse transfectants.

MATERIALS AND METHODS

Cell lines. The murine mastocytoma cell line P815-HTR (13), and HMy2.CIR, a class I HLA-deficient mutant derived from the plasma cell leukemia line LICR.LON.HMy2 (32), were used as recipients for transfections. Both cell lines and their transfectants were cultured in Dulbecco's modified Eagle's medium, containing 10% inactivated FCS and 2 mM L-glutamine (all from Flow Laboratories, Middlesex, UK).

HLA-B27 mutants and DNA-mediated gene transfer. The following site-specific mutants from HLA-B*2705 were used: N63, Y74, N77, S77, I80, Y74N77, N77I80, N77A81, and E152. They are designated by the single-letter code of the amino acid(s) introduced, followed by the corresponding position number(s). Their derivation and features have been described in a previous report (33).

B*2705 (34), B*2702 (35) and each of the mutant genes were separately transfected in HMy2.CIR and in P815 cells. The construction and features of the human transfectant series have been previously described (33). Murine cells were cotransfected with human β2m (36). Transfection of P815 cells by a calcium phosphate method, and selection with G418 was done exactly as described elsewhere (18). For each of the transfectants, surviving cells in each proliferating well were screened for HLA-B27 and human β2m expression by FMF[3] analysis. Populations showing coexpression of both genes, a homogeneous FMF profile, and expression of high—and as close to one another as possible—levels of HLA-B27 were selected for further use. HLA-B27 expression in the murine transfectant series used in this study is shown in Figure 1. Expression was stable as assessed by periodic FMF analysis.

mAb and FMF analysis. Cell surface expression of HLA-B27 and human β2m was measured with mAb ME1 (anti-HLA-B27 + B7 + Bw22) (37) and BBM.1 (anti-human β2m) (38), respectively. These mAb were used as 1/100 dilution of ascites fluid and as 1/2 dilution of culture supernatant, respectively. FMF analysis was otherwise done as previously described (10). The mAb B9/4 (anti-CD8) (39) and Bear-1 (anti-CD11b) (40) were used, at 1/300 dilution of ascites fluid, in some experiments.

T cell clones and cytotoxicity assays. Five human HLA-B27-specific alloreactive CTL clones showing cross-reactive lysis with HLA-B27^{+} human and mouse cells were used in this study. They were designated as 202DRD, 212DRD, GM7, 5A2, and 64.8P. All five clones were derived from HLA-B27^{-} responder individuals against B*2705 (202DRD, 212DRD, GM7, and 5A2) or B*2704 (64.8P). Details on their obtention and fine specificity have been published elsewhere (33).

^{51}Cr-release cytotoxicity and cold target inhibition assays were done exactly as described elsewhere (10).

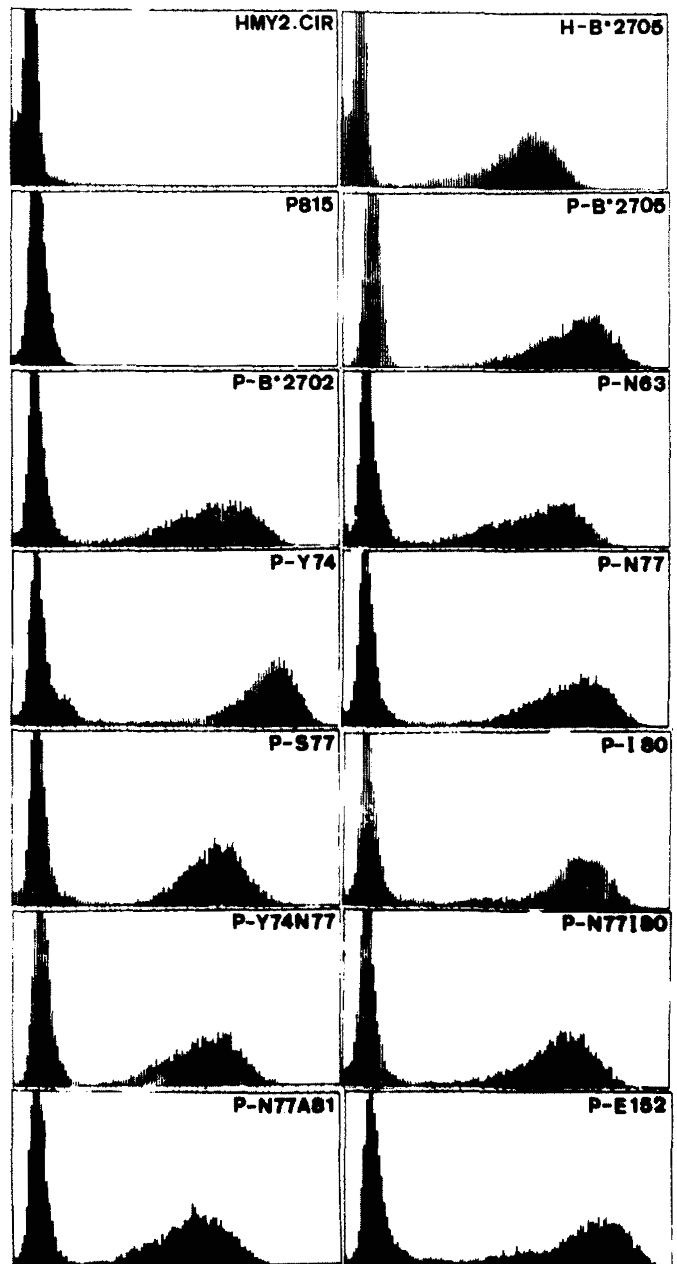

Figure 1. FMF analysis showing the expression of HLA-B27 on mouse cells. Number of cells (y-axis) is plotted vs log fluorescence intensity (x-axis). The ME1 mAb (see *Materials and Methods*) was used. The negative fluorescence peak in each panel was separately measured after incubating cells with only the second antibody. The two upper panels show the FMF profile of HMy2.CIR cells transfected only with the pSV2neo plasmid and of HLA-B27^{+} HMy2.CIR (H-B*2705), as a reference. The third panel shows the FMF profile of untransfected P815 cells. The remaining panels show the FMF profile of P815 cells transfected with B*2705, B*2702, or the various HLA-B27 mutants as indicated in each panel. A similar analysis (data not shown) was carried out with the BBM.1 mAb to establish expression of human β2m in all mouse transfectants.

RESULTS

Recognition of HLA-B27 on human and mouse cells by alloreactive CTL clones. Cytotoxicity of the CTL clones toward B*2705^{+} HMy2.CIR and P815 transfectants is compared in Figure 2 (*A* to *E*). CTL 64.8P, 212DRD, and 5A2 showed similar lytic efficiency toward either transfectant, whereas the cytotoxicity of 202DRD and, especially, GM7 was lower toward mouse cells. In cold target competition assays, human and mouse transfectants showed equally efficient inhibition of hot target cell lysis by CTL 64.8P, 212DRD, and 202DRD (Fig. 2, *F* to *H*). This indicates that recognition of both transfectants by each of these clones was similarly efficient, in spite of the somewhat lower cytotoxicity of CTL 202DRD toward mouse cells. In contrast, lysis of the human transfectant by CTL GM7 was not inhibited by its mouse counterpart (Fig. 2*I*), confirming the more efficient killing of human targets by this CTL clone. For CTL 5A2, inhibition of human hot target lysis by both cell types was obtained, but the mouse transfectant was somewhat more efficient (Fig. 2*J*). Thus, CTL 5A2 might recognize the mouse transfectant slightly better than its human

[3] Abbreviations used in this paper: FMF, flow microfluorimetry.

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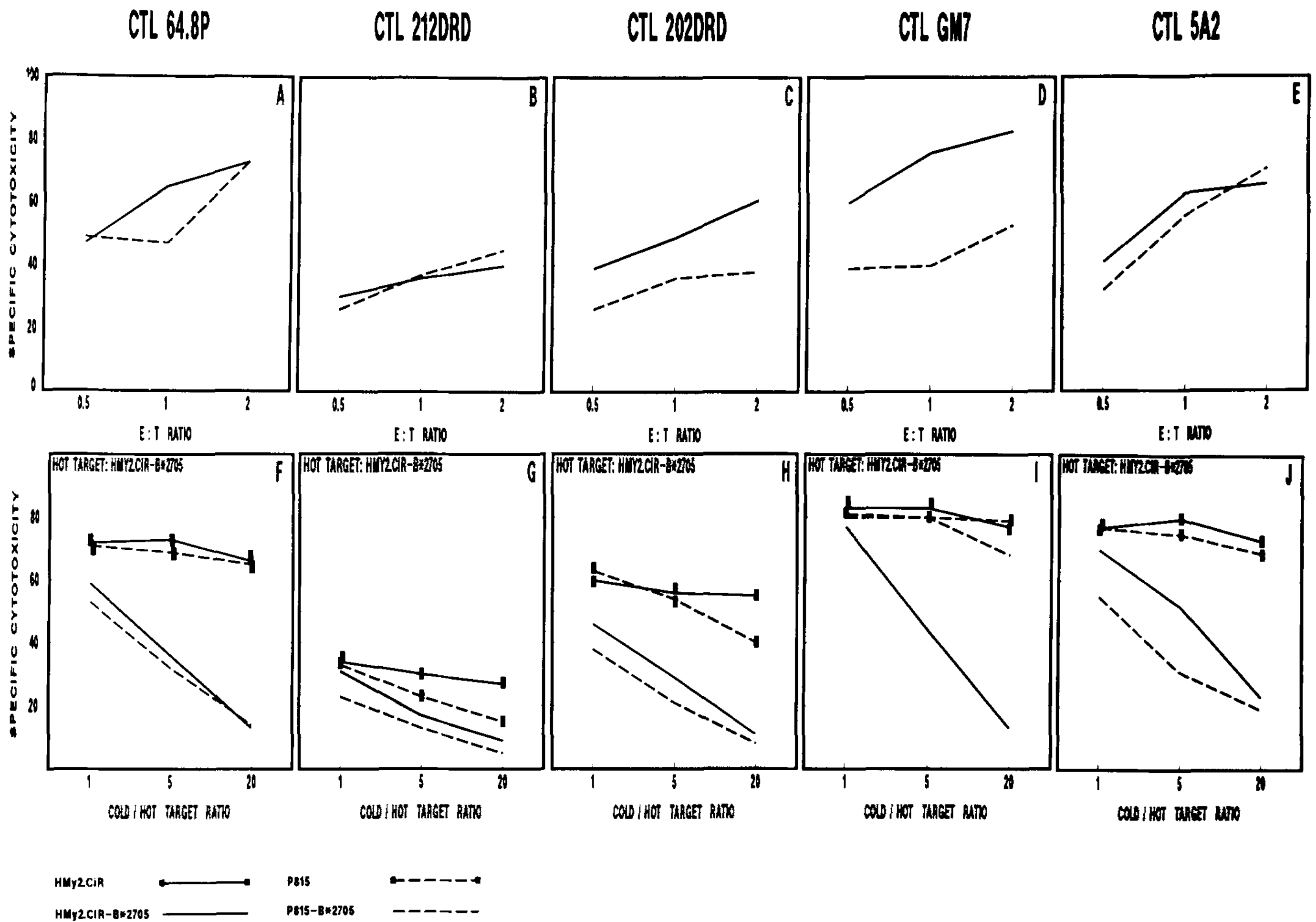

Figure 2. *A* to *E*, Specific cytotoxicity, measured as ^{51}Cr release, of five anti-HLA-B27 human CTL clones against human (HMy2.CIR) and mouse (P815) HLA-B27^{+} transfectants. Results are average values of at least three independent experiments. Lysis of untransfected cells was <7% at E:T ratio 2:1. *F* to *J*, Cold target inhibition of the lysis of labeled B*2705^{+} HMy2.CIR cells by the CTL clones indicated on top, with human and mouse B*2705^{+} transfectants. Effector to hot target ratio was 1:1 in these assays. Results are means from at least two independent experiments, except for CTL 202DRD (*H*) in which only one experiment could be done. Lysis of hot target cells in the absence of competing cells was 65% (*F*), 38% (*G*), 52% (*H*), 86% (*I*), and 66% (*J*), respectively.

counterpart.

Anti-CD8 mAb inhibited the lysis of P815-B*2705, but not that of its human counterpart, by CTL 5A2. Lysis of both human and mouse transfectants by the other CTL clones was totally inhibited by the same antibody (data not shown).

Conservation of allospecific T cell epitopes upon HLA-B27 expression on mouse cells. The fine specificity of the CTL clones toward HLA-B27^{+} human and mouse cells was examined with a panel of HLA-B27 mutants transfected both in HMy2.CIR and in P815 cells. Both transfectant series were used as target cells with each CTL clone. The reaction pattern of these clones with the human transfectants has been previously reported (33). Nevertheless, a new set of experiments was carried out, along with those using the mouse series, to assure the stability of the fine specificity patterns.

The cytotoxicity of CTL 212DRD toward both sets of transfectants was identical except with the I80 mutant (Fig. 3, *A* to *C*). Whereas I80 on P815 was lysed as the wild type, the relative cytotoxicity of the corresponding human transfectant was reduced by about 50% at the three E:T ratios tested. However, in cold target competition assays, the inhibitory capacity of the mutant was identical to that of the wild type for both human and mouse cells (Fig. 3, *D* and *E*). This suggests that the observed differences in cytotoxicity do not reflect any significant change in the way the I80 mutant is recognized in either cell type. Thus, within the resolution of the system used, CTL 212DRD recognizes HLA-B27 on human or mouse cells with the same fine specificity.

The cytotoxicity pattern of CTL 202DRD with both series of transfectants (Fig. 4, *A* to *C*) can be, in general, accounted for by its somewhat lower cytotoxicity toward P815 cells. Most of the mutants, were lysed with similar relative cytotoxicity, as referred to the corresponding wild type. For I80, the situation was similar as with CTL 212DRD in that the lower relative lysis of the human transfectant (Fig. 4) was not substantiated by cold target inhibition experiments (data not shown). Thus, the cytotoxicity pattern of CTL 202DRD strongly suggests that it recognizes HLA-B27 on HMy2.CIR and P815 cells with the same fine specificity.

The cytotoxicity of CTL GM7 toward both transfectant series is compared in Figure 5 (*A* to *C*). The most drastic difference was obtained with Y74. For this mutant, cytotoxicity toward the human target was the same as for the wild type, whereas with the corresponding mouse transfectant it was reduced to background levels. Nevertheless, cold target competition assays (Fig. 5, *D* to *F*) indicated that the human Y74 transfectant was unable to inhibit lysis of the corresponding wild type (Fig. 5*D*)

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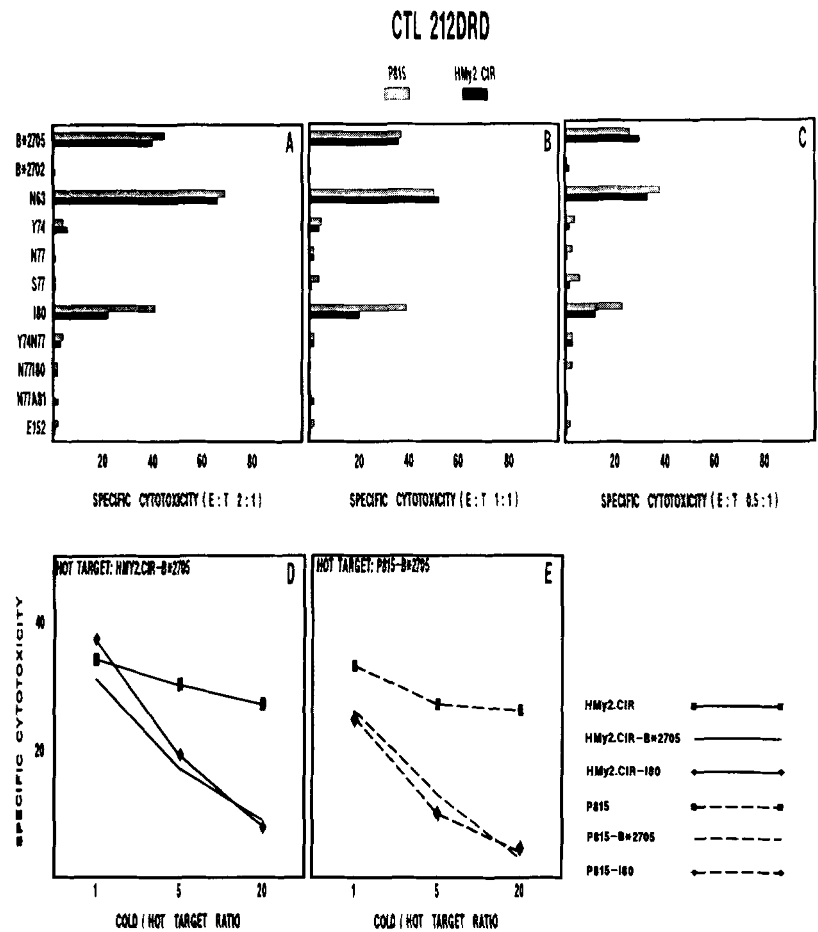

Figure 3. *A* to *C*, Cytotoxicity of CTL 212DRD against P815 and HMy2.CIR transfectants expressing B*2705, B*2702, or the specified HLA-B27 mutants. Results are expressed as percent specific ^{51}Cr release at three E:T ratios. Data are means of at least three independent experiments. Lysis of untransfected P815 and HMy2.CIR cells was 4% and 2%, respectively, at E:T ratio 2:1. *D* and *E*, Cold target inhibition of the lysis of labeled human (*D*) or mouse (*E*) B*2705⁺ transfectants by CTL 212DRD, with the I80 mutant expressed on the same cell type. Inhibition by cold wild-type transfectant and by untransfected cells is also included. Assays were performed at effector to hot target ratio 1:1. Data are means of two independent experiments. Lysis of hot target cells in the absence of competing cells was 38% (*D*) and 47% (*E*), respectively.

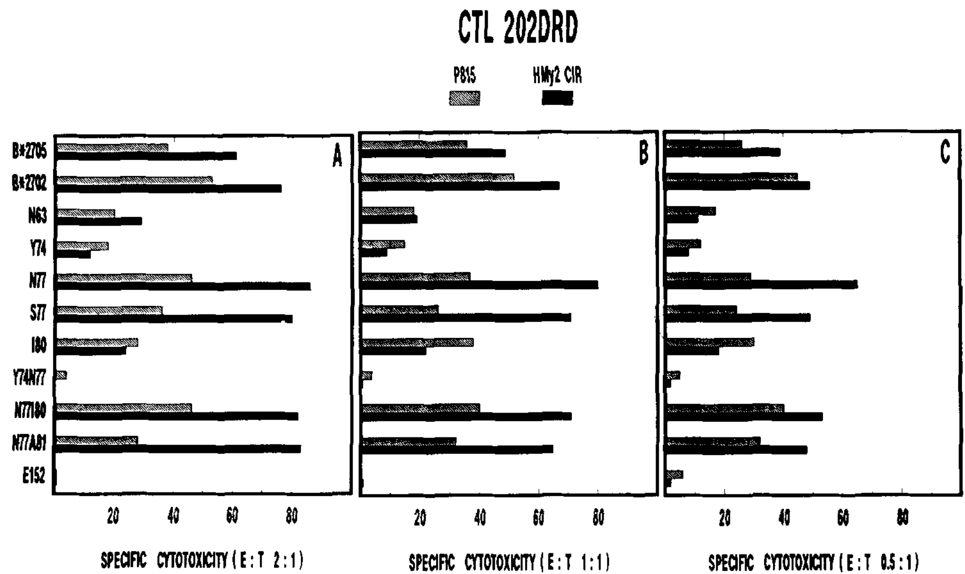

Figure 4. *A* to *C*, Cytotoxicity of CTL 202DRD against P815 and HMy2.CIR transfectants expressing B*2705, B*2702 or the specified HLA-B27 mutants. Results are expressed as percent specific ^{51}Cr release at three E:T ratios. Data are means of at least two independent experiments, except for P815-Y74N77 and P815-E152, where only one experiment could be done. Lysis of untransfected mouse and human cells was 1% and 2%, respectively.

and was less efficient than the wild type in inhibiting lysis of hot HMy2.CIR-Y74 cells (Fig. 5*E*). Differences in surface expression between the mutant and the wild type are very unlikely to account for this effect, as both human transfectants express similar levels of HLA-B27 (33). Thus, these results indicate that the mutation decreases the avidity of this clone for the Y74 transfectant compared with the corresponding wild type. This decrease is not sufficient for altering lytic efficiency toward the human Y74 target, but abrogates lysis of the mouse counterpart. The situation with S77 targets was analogous, but clearly less conspicuous than with Y74, as lysis of the human transfectant was partially affected. As discussed below, these results do not imply a change in the fine specificity of CTL GM7 toward HLA-B27 expressed on human or mouse cells (see *Discussion*).

Finally, the reaction pattern of CTL 64.8P was generally conserved with both transfectant series, except for Y74N77 (Fig. 6, *A* to *C*). Cytotoxicity for the human transfectant of this mutant was the same as for the wild type, whereas lysis of its mouse counterpart was very much reduced. This was substantiated by cold target

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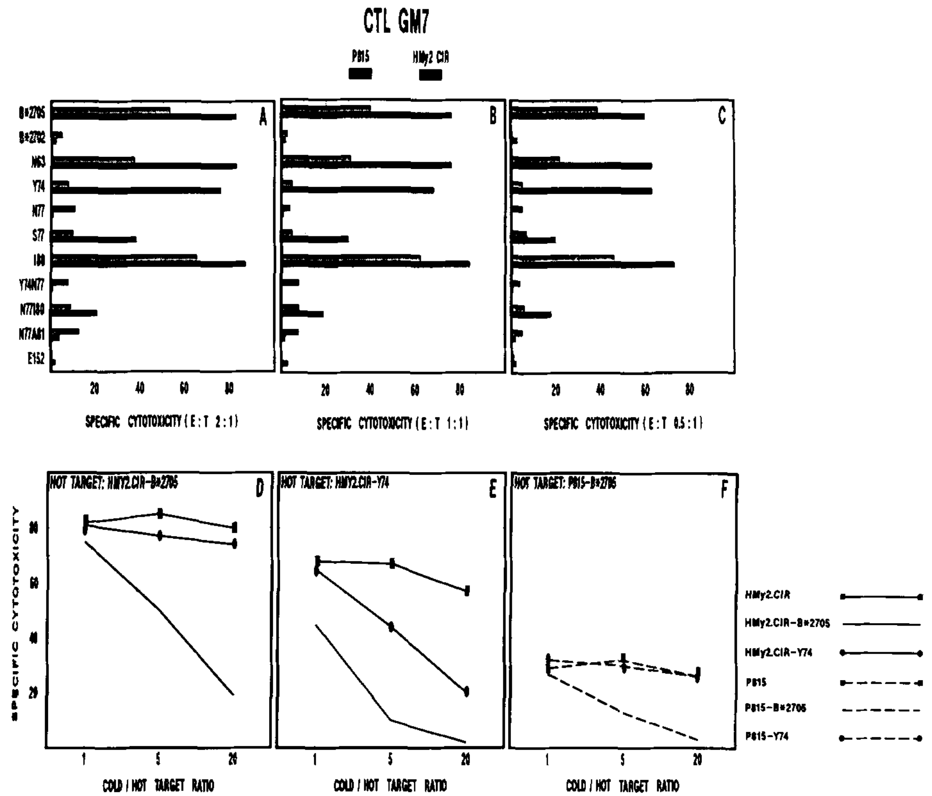

Figure 5. *A* to *C*, Cytotoxicity of CTL GM7 against P815 and HMy2.CIR transfectants expressing B*2705, B*2702 or the specified HLA-B27 mutants. Results are expressed as percent specific ^{51}Cr release at three E:T ratios. Data are means of at least two independent experiments. Lysis of untransfected mouse and human cells was 6% and 4%, respectively. *D* to *F*, Cold target inhibition of the lysis of indicated hot targets by CTL GM7 with the Y74 mutant expressed on the same cell type. Inhibition by cold wild-type transfectant and untransfected target cells is also included. Assays were performed at effector to hot target ratio 1:1. Data are means of two independent experiments. Lysis of hot targets in the absence of competing cells was 83% (*D*), 70% (*E*), and 37% (*F*), respectively.

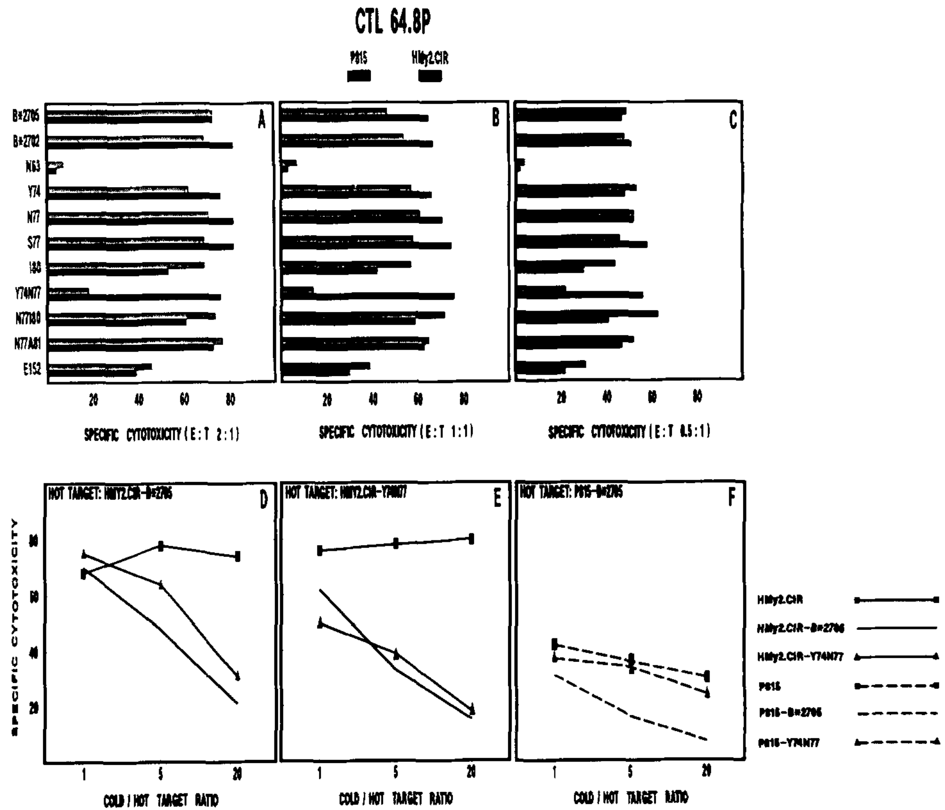

Figure 6. *A* to *C*, Cytotoxicity of CTL 64.8P against P815 and HMy2.CIR transfectants expressing B*2705, B*2702 or the specified HLA-B27 mutants. Results are expressed as percent specific ^{51}Cr release at three E:T ratios. Data are means of at least three independent experiments. Lysis of untransfected mouse and human cells was 5% and 4%, respectively. *D* to *F*, Cold target inhibition of the lysis of indicated hot targets by CTL 64.8P with the Y74N77 mutant expressed on the same cell type. Inhibition by cold wild-type transfectant and untransfected target cells is also included. Assays were performed at effector to hot target ratio 1:1. Data are means of two independent experiments. Lysis of hot targets in the absence of competing cells was 74% (*D*), 74% (*E*), and 55% (*F*), respectively.

competition assays (Fig. 6, *D* to *F*), in which the mutant on human cells showed little, if any, difference from the wild-type in its inhibitory capacity of hot human transfectants (Fig. 6, *D* and *E*). In contrast, P815-Y74N77 was unable to inhibit lysis of labeled P815-B*2705 (Fig. 6*F*). These results indicate that there is a difference in the relative avidity of Y74N77 recognition on either human or mouse cells, as referred to their corresponding wild-type transfectants. As with CTL GM7, this does not necessarily mean different fine specificity of CTL 64.8P toward HLA-B27 on either cell type (see *Discussion*).

Altered fine specificity of CTL 5A2 toward HLA-B27 expressed on mouse cells. The cytotoxicity of CTL 5A2 toward human and mouse transfectants is compared in Figure 7. Three different situations were observed: 1) for Y74N77 and N77I80 recognition of the human transfec-

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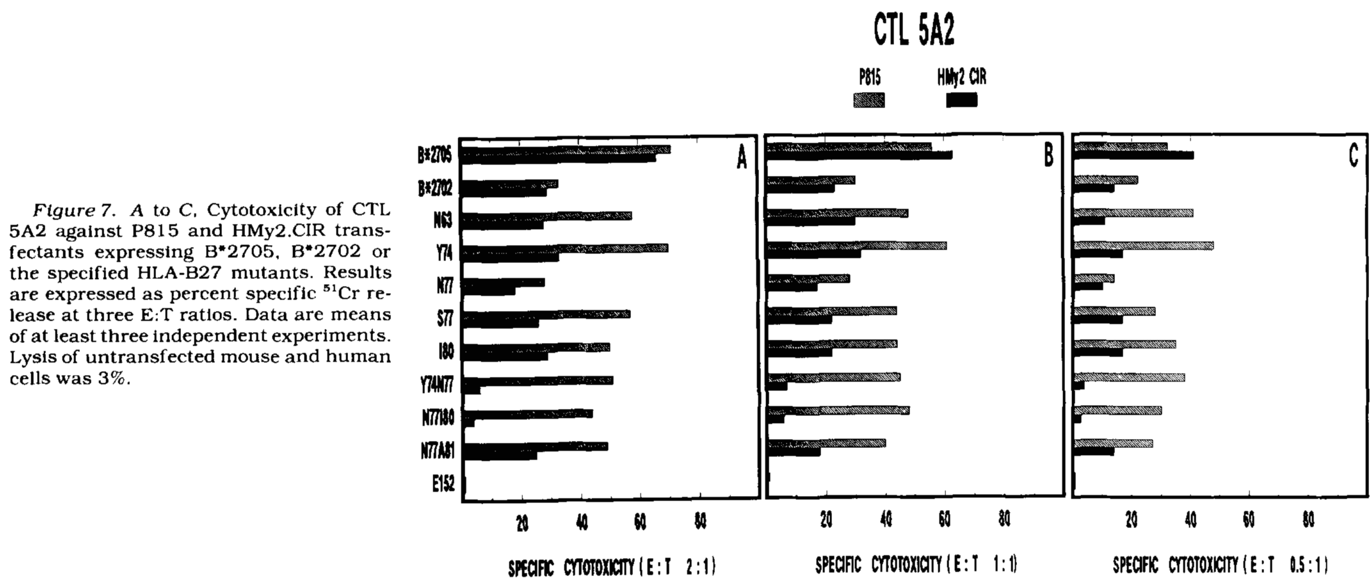

Figure 7. *A* to *C*, Cytotoxicity of CTL 5A2 against P815 and HMy2.CIR transfectants expressing B*2705, B*2702 or the specified HLA-B27 mutants. Results are expressed as percent specific ^{51}Cr release at three E:T ratios. Data are means of at least three independent experiments. Lysis of untransfected mouse and human cells was 3%.

tants was abrogated, whereas lysis of their mouse counterparts was little or not affected; 2) for N63, Y74, S77, I80, and N77A81 relative lysis of the human targets was significantly decreased (ranging from 27 to 52%, depending on the mutant and E:T ratio), whereas that of their mouse counterparts was little or not reduced (ranging from 69 to 100%); and 3) for B*2705, B*2702, N77 and E152 lytic efficiency toward the mouse or human transfectant was very similar.

To further explore the cytotoxicity differences seen with Y74N77, cold target competition assays were carried out (Fig. 8, *A* to *F*). With mouse transfectants, the mutant was able to inhibit lysis of both the wild type (Fig. 8*A*) and itself (Fig. 8*B*), although somewhat less efficiently than cold wild-type transfectants. In contrast, HMy2.CIR-Y74N77 was not inhibitory for its wild-type counterpart (Fig. 8*C*). More strikingly, this mutant transfected on human cells was unable to inhibit the lysis of mouse transfectants expressing either B*2705 or the Y74N77 mutant (Fig. 8, *D* and *E*), whereas P815-Y74N77 significantly inhibited HMy2.CIR-B*2705 (Fig. 8*F*). The same results were obtained in analogous experiments with N77I80 (data not shown). These results indicate that the Y74N77 and N77I80 mutations abrogate recognition of HLA-B27 by CTL 5A2 when expressed on human cells but have little or no effect on lysis if the mutant is expressed on P815 cells.

Cold target competition assays were also carried out for N63 and Y74 (Fig. 8, *G* to *J*). These mutants, expressed on either human or mouse cells, significantly inhibited the lysis of both wild-type and mutant hot targets (only inhibition of Y74 hot target cells is shown, Fig. 8, *I* and *J*). Inhibition was less efficient, particularly for Y74, than that obtained with the corresponding wild type. This indicates that at least the Y74 mutation decreases the avidity of recognition by CTL 5A2. This decrease is sufficiently small on P815 cells as to have no effect on cytotoxicity. Paradoxically, on human cells, it results in lower lytic efficiency toward the mutant targets.

DISCUSSION

In a previous study, fine specificity analysis using a panel of site-specific HLA-B27 mutants transfected into human cells revealed an extreme diversity of epitope specificities among alloreactive CTL clones generated in anti-HLA-B27 responses (33). The TCR β-chains from some of these CTL, including 4 of the 5 clones in this study, differed greatly in sequence and length at their junctional regions (41). These results suggested that HLA-B27 allospecific T cell epitopes could involve multiple endogenous peptides bound to the alloantigen. They also demonstrated the high discriminative capacity of site-directed mutants for detecting fine specificity differences.

In our hands, only a minority of anti-HLA-B27 human CTL clones can kill P815 cells transfected with HLA-B27. The aim of the present study was to compare the structure of individual HLA-B27 allospecific T cell epitopes, expressed on either human or murine P815 cells and recognized by these cross-reactive CTL clones. This was done to explore: 1) what degree, if any, of structural variability is compatible with xenogeneic lysis of HLA transfectants, and 2) whether such variability may imply recognition of peptides by HLA-B27-specific CTL clones.

Comparative fine specificity analysis with analogous series of human and mouse transfectants expressing HLA-B27 mutants was used as a sensitive criterion for conservation or alteration of allospecific T cell epitopes upon expression on mouse cells. The two assays used, direct cytotoxicity and cold target competition, provide complementary information. For each CTL clone, the former depends on the integrity and expression level of the corresponding antigenic determinant, as well as on the presence of ligands for T cell adhesion molecules. The second depends on the relative avidity of competing target cells.

A first group of clones included CTL 212DRD, 202DRD, GM7, and 64.8P. These showed either no significant difference in their recognition of any given mutant on human or mouse cells, or decreased lysis toward the murine transfectant. Equivalent lytic efficiency toward a mutant on both cell types was taken as evidence for a same effect (or lack of effect) of the mutation on epitope structure, regardless of the target cell in which the mutant was expressed.

For interpreting a decreased lysis toward murine vs human transfectants, as observed most conspicuously with CTL 64.8P and Y74N77, and with CTL GM7 and

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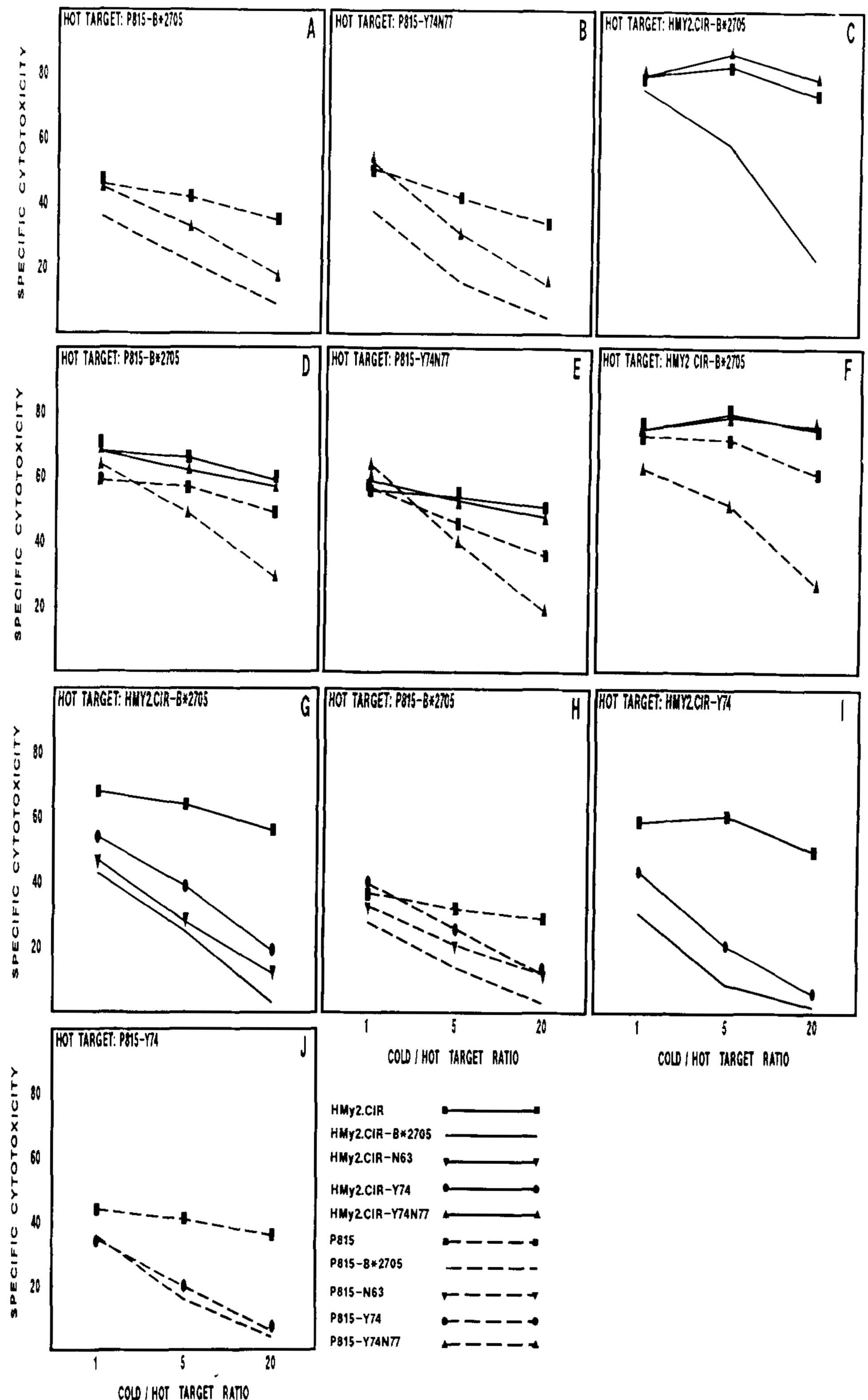

Figure 8. Cold target inhibition of lysis by CTL 5A2. Assays were done at effector to hot target ratio 1:1. Hot target cells are specified in each panel. *A* and *B* show the inhibition of P815-B*2705 (*A*) or P815-Y74N77 (*B*) by both of these cold cells. *C* shows the inhibition of HMy2.CIR-B*2705 by the human B*2705 and Y74N77 transfectants. *D* to *F* show the inhibition of the specified hot cell lysis by human and mouse Y74N77 transfectants. *G* and *H* show the inhibition of hot B*2705$^+$ human (*G*) and mouse (*H*) transfectants by the Y74 and N63 mutants expressed on the same cell type. *I* and *J* show the inhibition of hot Y74$^+$ human (*I*) and mouse (*J*) transfectants by B*2705 or Y74 expressed on the same cell type. In all experiments, inhibition by relevant untransfected cells was included as a control. Data are means of two to four experiments, except in *I*, where only one experiment could be done. Specific lysis of hot targets in the absence of competing cells was 59% (*A*), 44% (*B*), 69% (*C*), 83% (*D*), 48% (*E*), 58% (*F*), 78% (*G*), 44% (*H*), 51% (*I*), and 58% (*J*).

Y74, the molecular differences in adhesion between human CTL and human or mouse cells must be taken into account. Lack of LFA-3 and of the natural ligands for human LFA-1 on mouse cells makes the avidity of human CTL for mouse targets more critically dependent on TCR and CD8. Thus, a subtle decrease on TCR affinity induced by a mutation may have little effect on global avidity, and little or no influence on lysis, for human target cells but may significantly affect lysis of the corresponding mouse targets. This provides an explanation for the reactivity of CTL 64.8P with Y74N77. We favor this view, but the alternative possibility that such behavior reflects a slight change in the fine specificity of CTL 64.8P for HLA-B27, depending on the cell type in which it is expressed, can-

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not be ruled out. Differences in the relative contribution of TCR to avidity of human CTL toward human or mouse cells is also a likely explanation for the differential lysis of Y74 transfectants by CTL GM7. This is especially so because this clone is less efficient in its recognition of mouse than human HLA-B27⁺ cells. Nevertheless, it is surprising that the apparently large decrease in avidity induced by the Y74 mutation (Fig. 5*D*) has no detectable effects on lysis of the human Y74 transfectant. For instance, this same transfectant showed partially diminished lysis by CTL 5A2, but was able to compete with the wild type in cold target inhibition assays (Fig. 8*G*). This raises the possibility that the decreased affinity of the CTL GM7 TCR for Y74, although insufficient to alter lysis, could impair TCR-induced activation of LFA-1 (6). There is much heterogeneity in the sensitivity of CTL clones to inhibition of lysis by anti-LFA-1 mAb (17). LFA-1 is not required for CTL triggering (42) and sometimes may not be critical for killing. Furthermore, studies on TCR-induced LFA-1 activation have used anti-CD3 antibodies whose binding affinities are much higher than the affinity of TCR-Ag interaction. Little is known about LFA-1 activation by specific Ag and on how affinity differences may modulate it. It has been shown, however, that the degree of TCR cross-linking, which may be influenced by affinity when induced by specific Ag, regulates LFA-1 induction (6). Thus, although failure to optimally activate LFA-1 is a hypothetical explanation for the observed behavior of CTL GM7 with Y74, it should not be ruled out.

With either interpretation, differential effects of affinity changes in killing and global avidity, without implying a change in fine specificity, can account for the reaction pattern of CTL GM7 with the mutants. A possible explanation for the decreased lysis of P815-B*2705 by CTL GM7, compared with its human counterpart, is that the determinant, although unaltered, is expressed at a lower level on mouse cells. This could happen, for example, if a peptide is involved in this determinant and is expressed on the murine cell surface at lower amount than on human cells. Alternatively, CTL GM7 avidity could be sufficient for highly efficient interaction with HLA-B27⁺ human but not mouse targets (18), even at comparable levels of determinant expression.

Thus, comparative fine specificity analysis suggests that CTL 212DRD, 202DRD, GM7, and probably 64.8P recognize HLA-B27 epitopes that remain unaltered upon expression of this molecule on P815 cells. These epitopes must be independent on any peptide bound to HLA-B27, or must involve peptides that are identical in both human and mouse cells. The reactivity of these clones with some of the mutants also suggests that subtle effects of mutations on affinity may be better detected by using mouse transfectants as target cells.

CTL 5A2 presents a different situation in that mouse transfectants were, in general, lysed better than human ones. A possible explanation for the quantitative differences found with many of the mutants, such as Y74 and N63, is that the effect of the mutations is more drastic on human than on mouse transfectants for this CTL clone. This would explain its decreased cytotoxicity toward human targets. The effect could be less obvious in cold target competition assays with human cells, as much contribution to global avidity comes from other adhesion molecules. This would imply a change in the fine specificity of CTL 5A2 toward HLA-B27 depending on the cell type in which it is expressed. Alternatively, the determinant recognized by CTL 5A2 may be expressed at a higher level on mouse cells. In this case, a negative effect on TCR affinity induced by the mutations would affect lysis of human targets, but could be compensated on mouse cells by higher determinant expression. This would mean more TCR binding sites, making increased TCR avidity possible. We favor this interpretation because better lysis of mouse cells was a rather nonselective effect for this CTL clone, being observed with most of the mutations that induced a partial decrease on lysis of human targets.

Differences in the level of epitope expression alone cannot explain the selective lack of reactivity of CTL 5A2 with Y74N77 and N77I80 on human cells. Furthermore, the inability of these human transfectants to inhibit lysis of their murine counterparts (Fig. 8, *D* and *E*) is difficult to explain solely by a quantitative difference in determinant expression. It would imply that increased TCR avidity for the mouse target cell would compensate for the CD2/LFA-3 and LFA-1/ICAM-1 contributions in CTL interaction with the competing human targets. A more plausible explanation is that abrogation of Ag recognition on the human target would also abrogate TCR-induced increase of LFA-1 and CD8 avidity (see the introduction). Thus, the behavior of CTL 5A2 with Y74N77 and N77I80 must reflect a change in fine specificity. This implies that the antigenic determinant recognized on mouse cells is structurally different from that on human cells.

It is likely that structural alteration and increased expression of this determinant occurs upon transfection of HLA-B27 on mouse cells. This interpretation is incompatible with recognition of HLA-B27 alone by CTL 5A2. It implies that a structurally different peptide is bound to HLA-B27 and recognized by CTL 5A2 on either human or mouse cells. This peptide could be expressed on the mouse cell surface at higher levels than on human cells. This could happen as a result of increased synthesis and/or differential processing of its parental protein by the mouse cell, or of increased binding affinity for HLA-B27. Differences in structure between the human peptide and its murine counterpart should be sufficiently small as to be compatible with cross-reactivity. Probably because of this, selective abrogation of lysis was observed with only two of the nine mutants used. There is no obvious candidate for such peptide, as it could arise from any protein synthesized by both HMy2.CIR and P815 cells and be polymorphic as a result, for instance, of phylogenetic divergence of related proteins between both species.

In conclusion, comparative fine specificity analysis of anti-HLA-B27 CTL clones recognizing mouse transfectants suggests that, in most cases, the allospecific epitope is not altered upon expression of HLA-B27 on mouse cells. Decreased lytic efficiency toward mouse cells, as observed for example with CTL GM7, may reflect lower avidity rather than structural alteration of the corresponding epitope. In some cases, however, as with CTL 5A2, the fine specificity of alloantigen recognition on mouse transfectants is altered, likely reflecting a peptide-mediated change in epitope structure.

It remains to be seen to what extent those CTL clones that do not react across species are affected by structurally altered epitopes or simply impaired by insufficient avidity.

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