Antagonism of Direct Alloreactivity of an HLA-B27-Specific CTL Clone by Altered Peptide Ligands of Its Natural Epitope[1]

Marina García-Peydró,* Alberto Paradela,* Juan P. Albar,† and José A. López de Castro[2]*

Antagonism of allospecific CTL by altered MHC ligands is a potential approach to specific immunomodulation of allogeneic T cell responses in acute graft rejection and graft-vs-host disease. In this study we have analyzed the capacity of peptide analogs of a natural HLA-B27-allospecific CTL epitope to antagonize direct alloreactivity. Alanine scanning demonstrated that positions 4, 5, and 7 of the peptide epitope were critical for allorecognition. A number of relatively conservative substitutions at each of these positions were then tested for their effect on allorecognition and antagonism. All substitutions at position 5 abrogated cytotoxicity. In contrast, a few changes at positions 4 and 7 were tolerated, indicating a limited flexibility of the allospecific CTL in recognition of peptide epitope variants. Most of the substitutions impairing cytotoxicity actually induced antagonism. However, whereas epitope variants with changes at positions 4 and 7 behaved as weak or intermediate antagonists, some of the variants with changes at position 5 antagonized CTL alloreactivity almost completely. The results in this study demonstrate for the first time that antagonism of direct class I-mediated alloreactivity can be achieved by variants of a natural allospecific peptide epitope. ***The Journal of Immunology,*** **2000, 165: 5680–5685.**

CD8$^+$ T cells recognize, via their TCR, antigenic peptides bound to MHC class I molecules on the cell surface. This interaction is highly specific, as subtle changes in the structure of the peptide epitope or the MHC molecule can affect CTL recognition, but endowed with some flexibility (1, 2). Thus, single amino acid substitutions of TCR contact residues in the peptide epitope can generate agonists that are still recognized by the specific CTL. In addition, altered peptide ligands can also act either as partial agonists, eliciting only a subset of CTL effector functions (3–5), or as antagonists, which are recognized by CTL but inhibit their effector response to the antigenic peptide. Indeed, naturally occurring variants of viral epitopes, or epitopes from other intracellular parasites, can antagonize the corresponding CTL responses in vitro (6–9). Antagonists can also be generated by modifications of haptens covalently attached to peptide side chains (10) or by *N*-hydroxylation of the peptidic main chain (11).

The mechanism involved in TCR antagonism remains obscure, and diverse models have been proposed to explain this effect. The kinetic model (12) suggests that a lower time of occupancy of the TCR by the antagonist/MHC complex is crucial. Antagonists may show faster TCR dissociation rates (13) or lower affinity (14), but antagonists with higher affinity for the TCR have also been described (15), which might challenge this hypothesis. Structural models propose that antagonists prevent conformational change in the TCR that would be necessary for intracellular signaling. The similar structure of the same TCR complexed with either an agonist or an antagonist (16) disfavors this possibility. Alternatively, antagonists could prevent formation of supramolecular structures, as observed in class II-restricted systems (17, 18). Finally, the antagonist/MHC complex might compete with the agonist/MHC complex for TCR binding, inhibiting the formation of signal-inducing agonist/MHC/TCR complexes and engaging the TCR in unproductive interactions (10). An important question is whether TCR interaction with an antagonist peptide generates a dominant-negative signal that prevents cell responsiveness. Two recent reports concerning class I-restricted Ags (19, 20) have demonstrated that exposure of a TCR to a specific antagonist does not inhibit a second independent TCR, expressed on the same T cell, from eliciting a cellular response upon recognizing its antigenic peptide. However, the opposite finding has been reported for class II-restricted T cells (21, 22).

The ability of self-restricted CTL to cross-react with peptide epitope variants containing single amino acid substitutions has been extensively explored (4, 23–27), and numerous antagonists have been reported for this type of CTL (4, 6–8, 15, 28, 29). In contrast, equivalent studies in alloreactivity are hampered by the difficulty of identifying the peptides specifically recognized by alloreactive T cell clones (30–36). To circumvent this problem, a recent study used peptide libraries to obtain class II-restricted alloreactive T cell antagonists without knowledge of the natural epitope (37). To our knowledge, only one recent study (38) has reported antagonism of direct alloreactivity for a class I-specific CTL clone by analogs of an allorestricted peptide. The identity of this peptide with the endogenously processed natural allospecific epitope was not established.

Studies on alloreactive CTL antagonism are of great potential importance in exploring the possibilities to antagonize CTL responses mediating acute allograft rejection and graft-vs-host disease (GVHD)[3] in vivo. A basis for such hope is that, despite the

*Centro de Biología Molecular Severo Ochoa (Consejo Superior de Investigaciones Cientificas), Universidad Autónoma de Madrid, Facultad de Ciencias, Cantoblanco, Madrid, Spain; and †Immunology and Oncology Department, Pharmacia-Consejo Superior de Investigaciones Cientificas, Centro Nacional de Biotecnología, Cantoblanco, Madrid, Spain

Received for publication December 20, 1999. Accepted for publication August 24, 2000.

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[1] This work was supported by Grants SAF97/0182 and SAF99/0055 from the Plan Nacional de Investigación y Desarrollo and Grant 08.3/0022/1998 from the Comunidad Autónoma de Madrid. We thank the Fundación Ramón Areces for an institutional grant to the Centro de Biología Molecular Severo Ochoa.

[2] Address correspondence and reprint requests to Dr. José A. López de Castro, Centro de Biología Molecular Severo Ochoa, Universidad Autónoma de Madrid, Facultad de Ciencias, Cantoblanco, 28049 Madrid, Spain. E-mail address: aldecastro@cbm.uam.es

[3] Abbreviations used in this paper: GVHD, graft-vs-host disease; C1R, HMy2.C1R.

0022-1767/00/$02.00

diversity of alloreactive CTL, it is known that allospecific T cell populations infiltrating human allografts that are being rejected show a highly restricted clonality (39–41). Selective clonal expansions of alloreactive CTL also occur during GVHD (42, 43). Thus, it is conceivable that antagonists of immunodominant epitopes in these harmful responses might effectively modulate acute graft rejection and GVHD. A prerequisite for a rational design of such antagonists is to identify peptide epitopes involved in alloreactivity, and to test the capacity of altered epitope ligands to induce antagonism of specific allo-CTL.

We have previously identified the RRFFPYYV octamer as the natural ligand recognized by the HLA-B27-allospecific CTL clone 27S69 (36). This provided the opportunity to test the capacity of analogs of a natural allospecific epitope to antagonize direct alloreactivity, and to identify the structural features of such antagonists. For this purpose, those residues of the natural peptide epitope that were critical for specific T cell recognition were first identified. Then, the effect of relatively conservative substitutions at these positions on CTL allorecognition and antagonism was analyzed.

Materials and Methods

CTL 27S69

This alloreactive CTL clone was raised against B*2705. Its culture conditions and fine specificity with other HLA-B27 subtypes have been described (44).

HLA-B27 transfectant cell lines

HMy2.C1R (C1R) is a human lymphoid cell line with low expression of its endogenous class I Ags. B*2705-C1R transfectant cells were cultured in DMEM (Life Technologies, Paisley, U.K.) with 5% heat-inactivated FCS. T2 is a TAP-deficient human cell line of lymphoid origin (45). The B*2705-T2 transfectant was a gift from Dr. David Yu (University of California, Los Angeles, CA). It was cultured in DMEM supplemented with 5% FCS. RMA-S is a TAP-deficient murine cell line (46). B*2705-RMA-S transfectant cells were cultured in RPMI 1640 supplemented with 10% FCS. When cultured at 26°C, T2 and RMA-S transfectants express class I molecules presumably devoid of peptides or bound to low affinity ligands (47). These molecules are unstable at 37°C, but their surface expression at this temperature can be stabilized by exogenous peptide ligands.

Peptide synthesis and purification

The natural B*2705 ligands RRFFPYYV (36), RRYQKSTEL, and FRYN GLIHR (48), and a set of analogs of the former peptide carrying single amino acid substitutions at residues 1, 4, 5, or 7, were used in this study. Peptide variants were designated with the one-letter code of the amino acid introduced followed by the number of the position changed. All peptides were synthesized using standard fluorenylmethoxycarbonyl chemistry and purified by HPLC. Their correct composition and molecular mass were confirmed by amino acid analysis using a 6300 amino acid analyzer (Beckman Coulter, Fullerton, CA), which also allowed their quantification, and by matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) and electrospray ion/trap mass spectrometry (MS).

Epitope stabilization assay

The quantitative procedure used has been described (49). Briefly, B*2705-RMA-S transfectant cells were incubated at 26°C for 24 h. Then, the synthetic peptide was added at 10^{-4} to 10^{-9} M. Cells were incubated for 1 h at 26°C, then at 37°C, and collected for flow microcytometry analysis after 2 h. Binding of the natural RRFFPYYV octamer was quantitated as the molar concentration of this peptide at 50% of the maximum fluorescence obtained. Binding efficiency of the peptide variants was measured as the molar concentration of these peptides required to obtain half-maximal fluorescence of the RRFFPYYV octamer (EC_{50}). Values of $EC_{50} \leq 10$ μM were considered to reflect high affinity. EC_{50} values between 10 and 50 μM were considered as intermediate affinity, $EC_{50} > 50$ μM indicated low affinity.

Cytotoxicity assays

In assays to test peptide analogs of the CTL 27S69 epitope for agonist activity, B*2705-T2 targets were preincubated in the absence of peptide for 18–20 h at 26°C, ^{51}Cr-labeled, and incubated for 30 min at room temperature with synthetic peptides in RPMI 1640 medium (Life Technologies) with 1% FCS. Effector cells were then added, and incubation at 37°C was conducted for 5 h in the continuous presence of peptide. The procedures have been described in detail elsewhere (36). Recognition of the natural epitope of CTL 27S69 was quantitated as the peptide concentration required to obtain half of the maximum lysis observed with this peptide in the concentration range used. Recognition of epitope variants was measured as the peptide concentration required to obtain half-maximal lysis of the octamer epitope (LC_{50}).

In experiments to determine whether peptide epitope variants were antagonists, peptides were added to ^{51}Cr-labeled B*2705-C1R targets, which constitutively express the natural epitope, and incubated for 30 min at room temperature in RPMI 1640 medium (Life Technologies) with 1% FCS. Effector cells were then added and incubation at 37°C was conducted for 4 h in the continuous presence of peptide. Antagonist activity was calculated as percent inhibition of lysis relative to the specific lysis of the B*2705-C1R targets without adding exogenous peptide.

In addition, the following classical TCR antagonism assay was performed. ^{51}Cr-labeled B*2705-T2 targets were prepulsed with a low dose (10^{-8} M) of the RRFFPYYV epitope in RPMI 1640 medium supplemented with 2% FCS for 20 min at room temperature. Cells were washed twice and then pulsed with various amounts of the octamer analogs in the same conditions. Finally, effector cells were added and incubation was conducted for 4 h. Antagonist activity was calculated as percent inhibition of lysis relative to the specific lysis of the B*2705-T2 targets prepulsed only with the octamer epitope.

Results

Identification of epitope residues critical for recognition by CTL 27S69

Molecular modeling of the B*2705/RRFFPYYV complex (36) predicted that Arg1, Phe4, Pro5, and Tyr7 of the octamer epitope were potentially accessible to the TCR. To assess their contribution to CTL allorecognition, analogs of the octamer with Ala substitutions at these positions were used to sensitize B*2705-T2 targets for lysis by CTL 27S69 (Fig. 1*A*). The only replacement tolerated by this clone was the change at position 1, whereas all other Ala analogs completely abrogated recognition. Efficient binding (EC_{50}, 4–15 μM) of all Ala analogs to B*2705 in an epitope stabilization assay (Fig. 1*B*) indicated that lack of recognition of A4, A5, and A7 was not due to inability to bind B*2705 at the cell surface. These results indicate that recognition of the RRFFPYYV epitope by CTL 27S69 critically depends on the Phe4, Pro5, and Tyr7 side chains, but not on Arg1.

CTL 27S69 recognizes epitope variants substituted at positions 4 and 7, but not 5

To further investigate the molecular interactions of the 27S69 TCR with Phe4, Pro5, and Tyr7 of the octamer epitope, a panel of peptide variants carrying relatively conservative substitutions (in size and/or polarity) at these three positions were synthesized. As shown in Table I, none of these substitutions significantly affected peptide binding to B*2705 (EC_{50}, 2–9 μM). The ability of CTL 27S69 to recognize these analogs was tested in peptide sensitization assays using B*2705-T2 target cells (Fig. 2 and Table I). Among the substitutions at position 4 (Fig. 2*A*), the change of Phe to Tyr decreased recognition by CTL 27S69 only about 10-fold relative to the natural epitope. Other peptide variants substituted at this position were recognized much less efficiently; recognition of W4 was reduced about 10^4-fold, L4 and V4 about 10^6 to 10^7-fold, and I4 was not recognized. In addition, all the substitutions of Pro5 tested (Fig. 2*B*) abolished allorecognition. Among analogs substituted at position 7 (Fig. 2*C*), recognition of H7 and L7 was reduced only about 20-fold, recognition of F7 about 300-fold, and the remaining substitutions were either marginally (W7 and V7) or not recognized (T7).

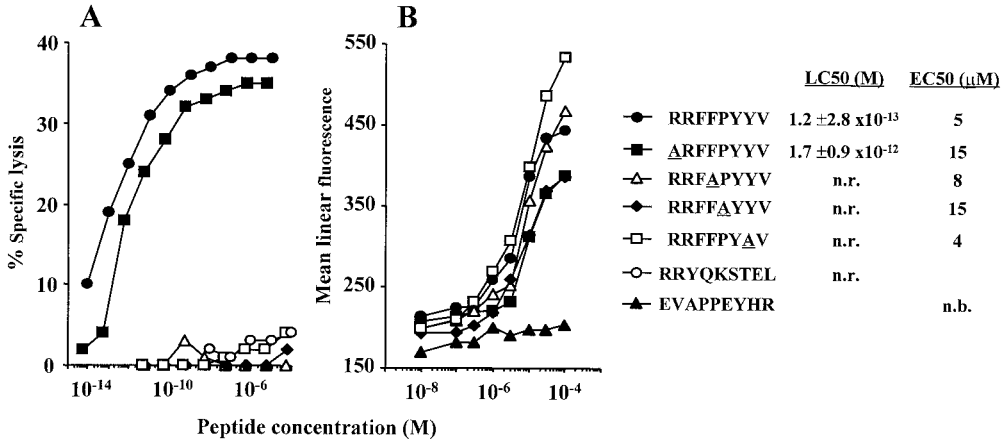

FIGURE 1. *A*, Lysis of B*2705-T2 transfectant cells sensitized with the natural RRFFPYYV octamer epitope or monosubstituted Ala variants by CTL 27S69. The natural B*2705 ligand RRYQKSTEL was used as negative control. LC_{50} values (the peptide concentration required to obtain half-maximal lysis of the octamer epitope) for RRFFPYYV and ARRFFPYYV are indicated. Other analogs were not recognized (n.r.) in this assay. The E:T ratio used was 2.5:1. Data are means of three independent experiments. *B*, Epitope stabilization assay showing binding of Ala-monosubstituted RRFFPYYV analogs to HLA-B*2705 on RMA-S transfectant cells. EC_{50} values (see *Materials and Methods*) are indicated. Data are means of two experiments.

These results indicate that none of the RRFFPYYV variants tested can fully mimic the CTL 27S69 epitope. However, efficient recognition of Y4, H7, and L7 revealed a limited flexibility of this epitope at positions 4 and 7. In contrast, CTL 27S69 exhibited an apparently exquisite specificity for Pro5.

RRFFPYYV analogs can antagonize recognition of this epitope by CTL 27S69

We next examined whether peptide epitope variants could act as antagonists of CTL 27S69 by testing their ability to inhibit lysis of B*2705-C1R target cells, which express the endogenous epitope (Fig. 3 and Table II). Four analogs containing substitutions of Phe4 that were poorly (L4, V4) or not recognized (A4, I4) by CTL 27S69 were tested in this assay (Fig. 3*A*). Significantly reduced lysis (>50% inhibition) was obtained with A4 and V4. While A4 inhibited lysis in a dose-dependent manner, maximal inhibition by V4 was observed at intermediate concentrations and progressively declined at higher ones. L4 behaved as a weak antagonist (about 30% maximal inhibition), and I4 failed to antagonize lysis at any concentration tested. All four variants substituted at position 5 (A5, I5, L5, V5) inhibited CTL 27S69-mediated lysis in a dose-dependent manner by >50% (Fig. 3*B*). L5 and I5 were the most powerful antagonists because they almost completely abolished recognition at the highest concentration used. V5 and A5 inhibited lysis up to ~60%. Finally, the three epitope variants carrying substitutions of Tyr7 (A7, T7, and V7) showed various degrees of dose-dependent inhibition of lysis ranging from ~30% (T7) to 65% (V7) maximal inhibition. The possibility that the decreased lysis observed with most analogs was due to displacement of the endogenous B*2705-bound RRFFPYYV epitope was excluded, as no significant inhibition was induced by an unrelated ligand, FRYNGLIHR, whose binding affinity to B*2705 (EC_{50}, 4 μM) (49) is similar to those of the epitope variants, or by I4, which was also used as an internal control in these experiments. In addition, antagonism of V4 at intermediate, but not higher, concentrations (Fig. 3*A*) further excludes this possibility. The pattern shown by V4 has also been observed with other CTL antagonists (4).

These data demonstrate that multiple substitutions in the allospecific RRFFPYYV epitope that largely decrease or abrogate recognition by CTL 27S69 induce antagonism of this CTL clone.

Allospecific epitope variants are not "superantagonists"

Because the previous antagonism assays involved endogenously presented alloantigen, they did not allow us to estimate the concentration of octamer variants, relative to the natural epitope, required to inhibit recognition by CTL 27S69. Thus, the inhibitory effect of the most powerful antagonists, I5 and L5, was tested on B*2705-T2 targets prepulsed with a suboptimal concentration of the RRFFPYYV epitope (Fig. 4). I5 and L5 significantly inhibited lysis only at 1000-fold or higher molar excess over the octamer, and almost completely abrogated recognition at the maximal antagonist/agonist ratio tested. These results confirm the antagonist function of the two altered epitope ligands and further indicate that a large excess over the natural allospecific peptide is required. Therefore, I5 and L5 do not behave as the "superantagonists" reported in some self-restricted responses (6, 7), which are able to

Table I. *Effect of substitutions at residues 4, 5, and 7 of the RRFFPYYV epitope on recognition by CTL 27S69 and B*2705 binding*

Peptide	Sequence	LC_{50} (M)[a]	EC_{50} (μM)[b]
Natural epitope	RRFFPYYV	$1.4 \pm 4.2 \times 10^{-11}$	5
Y4	RRF**Y**PYYV	$1.4 \pm 6.0 \times 10^{-10}$	6
W4	RRF**W**PYYV	$1.0 \pm 4.7 \times 10^{-7}$	9
L4	RRF**L**PYYV	$3.0 \pm 5.0 \times 10^{-5}$	4
V4	RRF**V**PYYV	$>10^{-4}$	6
I4	RRF**I**PYYV	NR[c]	4
V5	RRFF**V**YYV	NR	6
I5	RRFF**I**YYV	NR	2
L5	RRFF**L**YYV	NR	3
H7	RRFFPY**H**V	$3.3 \pm 3.7 \times 10^{-10}$	6
L7	RRFFPY**L**V	$2.7 \pm 2.3 \times 10^{-10}$	5
F7	RRFFPY**F**V	$4.2 \pm 2.9 \times 10^{-9}$	8
W7	RRFFPY**W**V	$3.5 \pm 4.5 \times 10^{-6}$	5
H7	RRFFPY**V**V	$>10^{-4}$	5
T7	RRFFPY**T**V	NR	8

[a] CTL recognition of peptide variants was calculated from the data in Fig. 2 and is expressed as $LC_{50} \pm$ SD (see *Materials and Methods*).

[b] Binding to HLA-B*2705 was measured in an epitope stabilization assay and is expressed as EC_{50} (see *Materials and Methods*). Data are means of two experiments.

[c] NR, Not recognized.

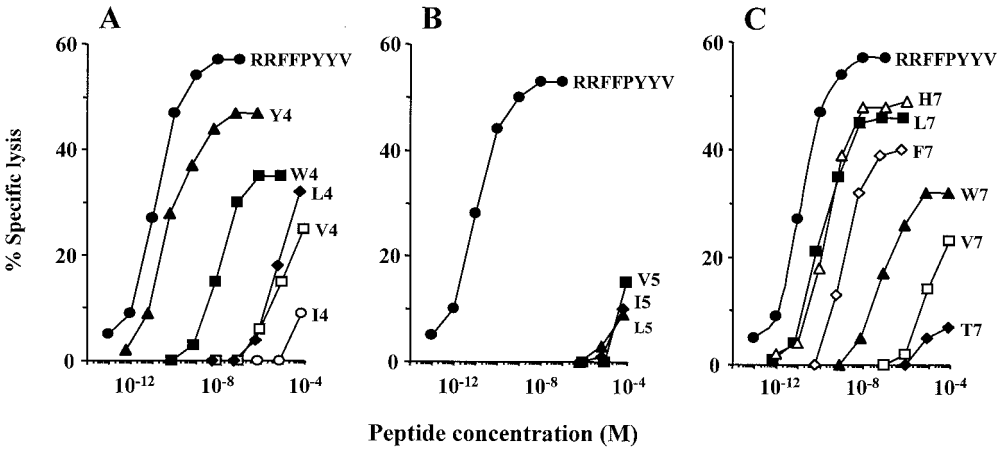

FIGURE 2. Cytotoxicity of CTL 27S69 against B*2705-T2 targets incubated with RRFF PYYV and analogs of this peptide with single amino acid changes at positions 4 (*A*), 5 (*B*), or 7 (*C*). Data are means of three experiments.

inhibit CTL effector function at amounts well below that of the natural epitope.

Discussion

A rational design of peptidic or nonpeptidic antagonists of alloreactive CTL requires sufficient knowledge about the role of peptide residues in allospecific T cell recognition and the flexibility of alloreactive CTL in the recognition of epitope variants. Numerous studies have addressed these issues for self-restricted CTL. However, similar studies in alloreactivity are hampered by the few natural class I MHC ligands known to be allospecific peptide epitopes (30–32, 34–36) and the great difficulties of identifying them.

It is by no means obvious that the role of peptide residues in T cell recognition or antagonism is the same in self-restriction and alloreactivity. Lack of selection against allo-MHC molecules during thymic development raises the possibility that a more significant role of MHC residues in alloreactivity may limit the capacity of allospecific T-cells to discriminate among subtle peptide changes. This has actually been reported for a class II-specific T cell clone, for which recognition of two residues of its allospecific epitope was much more degenerate than for a self-restricted peptide also recognized by the same T cell (50). However, it has also been reported that self-restricted and alloreactive T cell clones are comparably dependent on their interaction with MHC class I residues (51), suggesting that self-restricted and allospecific T cell epitopes may have similar structural features.

Knowledge of a natural ligand of HLA-B27 that is an allospecific T cell epitope allowed us to analyze the flexibility of a class I-directed alloreactive CTL clone in the recognition of peptide epitope variants and their capacity to act as antagonists. The results in this study indicate that CTL 27S69 behaves similarly to self-restricted CTL in 1) the critical involvement of nonanchor peptide positions in the allospecific epitope, and 2) the limited flexibility of this CTL clone for recognizing epitope variants with changes at these positions. That removal of Pro5 always abrogated lysis suggests that this is the most critical residue of the allospecific peptide epitope. This may be for its implication in direct contacts with the TCR, for its role in maintaining the conformation of the epitope, or both. A critical conformational role of Pro5 is likely because this residue imposes stronger stereochemical constraints than any other amino acid due to its rigid structure and unique ability to form stable *cis* peptide bonds. Phe4 was the second most restricted residue, as only the conservative Tyr4 was largely tolerated. A somewhat larger permissiveness of CTL 27S69 for changes at position 7 was suggested by the significant cross-reaction with the H7, L7, and F7 analogs.

The possibility of modulating alloreactive T cell responses through the use of antagonists depends, in the very first place, on the ease with which alterations of allospecific peptide epitopes lead to antagonism. For self class I-restricted CTL, an extensive study (4) demonstrated that as many as 40% of 64 peptide epitope variants with changes at individual TCR contact positions behaved as antagonists for three CTL clones specific for the same peptide. Significantly, changes in some positions led to antagonism much more easily than in others. For instance, 13 of 16 variants in position 4 of the peptide epitope in that study were antagonists. The relative ease with which antagonist of self-restricted CTL can be generated explains that antagonism is used as a mechanism of subversion of CTL responses by viruses or other intracellular pathogens through mutation of relevant epitopes (6–9, 28).

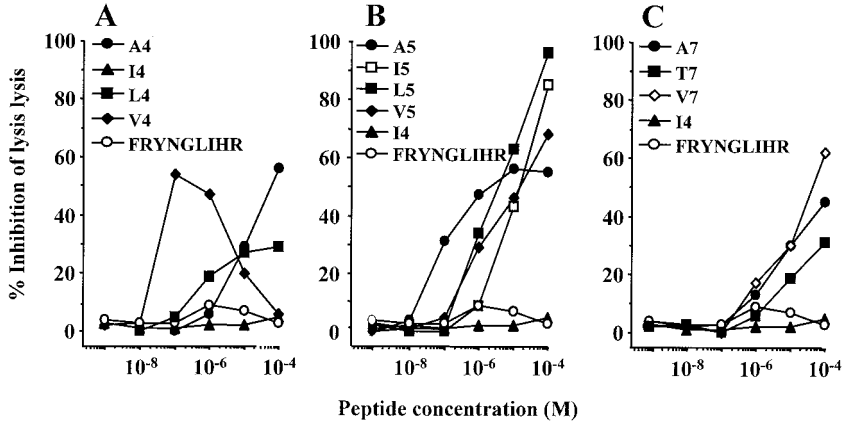

FIGURE 3. Inhibition of CTL 27S69-mediated lysis of B*2705-C1R target cells by RRFFPYYV epitope variants with single substitutions at residues 4 (*A*), 5 (*B*), and 7 (*C*). The B*2705 natural ligand FRYNGLIHR was used as negative control. Cells were preincubated with various amounts of the octamer analogs as described in *Materials and Methods*. Specific lysis of B*2705-C1R targets in the absence of exogenous peptide variants, at the E:T ratio used (0.8:1), was 52%. Data are means of three to five experiments.

Table II. *Inhibition of CTL 27S69-mediated lysis of B*2705-C1R target cells by RRFFPYYV epitope variants*

Peptide Antagonist	Sequence	Maximal Inhibition (% ± SD)
A4	RRF**A**PYYV	56 ± 9.9
L4	RRF**L**PYYV	29 ± 6.3
V4	RRF**V**PYYV	54 ± 8.7
A5	RRFF**A**YYV	55 ± 6.1
I5	RRFF**I**YYV	85 ± 9.0
L5	RRFF**L**YYV	96 ± 5.7
V5	RRFF**V**YYV	68 ± 4.8
A7	RRFFPY**A**V	45 ± 2.9
T7	RRFFPY**T**V	31 ± 2.2
V7	RRFFPY**V**V	62 ± 4.7

That peptide antagonism was more readily detected in recognition of syngeneic than allogeneic peptide-MHC complexes by a clonal CTL line showing such double specificity (15) raised the possibility that allo- and self-restricted CTL recognition might differ in their susceptibility to antagonism. This view could be consistent with reported observations that TCR affinity tends to be higher for allogeneic than syngeneic peptide-MHC complexes (52, 53) and also with the view that contribution of the peptide, relative to the MHC molecule to the binding energy of TCR-peptide-MHC interactions, may be lower in alloreactivity than in self-restricted recognition (54).

Aside from this study, to our knowledge only one very recent report has described antagonism of the direct alloreactivity of class I-directed CTL by analogs of its allospecific epitope (38). In this previous study, only 8 of 61 (16%) epitope variants substituted at any of three putative TCR contact residues showed >50% antagonism of the CTL activity. This is a significantly lower number of antagonists than reported for self-restricted CTL (4). In contrast, our results showed that 7 of 11 peptide epitope variants (64%) antagonized CTL alloreactivity by >50%. This substantially higher percentage might be simply due to the fact that we have restricted our screening of potential antagonists to relatively conservative substitutions rather than performed a systematic screening of amino acid changes at each position. However, an additional difference that might be relevant to antagonism by altered peptide ligands is that, in contrast to our case, the allospecific epitope in that study (38) was derived by homology with a cross-reactive viral epitope and matching with human proteins (25), and therefore was not necessarily the natural endogenous ligand recognized by the allospecific CTL.

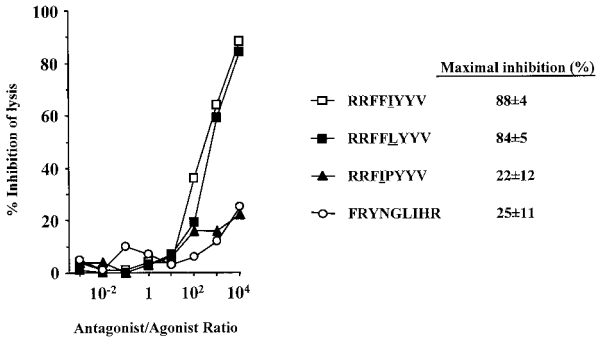

FIGURE 4. Inhibitory effect of octamer analogs on recognition of B*2705-T2 targets prepulsed with the natural epitope by CTL 27S69. Cells were first incubated with 10^{-8} M of RRFFPYYV, washed, and then pulsed with various amounts of the I5 or L5 epitope variants (see *Materials and Methods*). The B*2705 natural ligand FRYNGLIHR and the I4 analog were used as negative controls. Specific lysis of target cells incubated only with the octamer, at the E:T ratio used (2:1), was 48%. Data are means of three independent experiments. Percent maximal inhibition of lysis ± SD is indicated for each peptide.

In two important aspects our results are coincident with those of Burrows et al. (38) and with analogous studies on self-restricted CTL (21). First, it is possible to obtain very potent clonal antagonists (>80% antagonistic activity: I5, L5) of alloreactive CTL by peptide epitope ligands altered at positions involved in TCR contact. Second, changes in one of these positions led to strong antagonism (>50%) much more frequently (in our case, with 4 of 4 changes at position 5). Therefore, it can be concluded that class I-directed CTL alloreactivity can be inhibited by antagonistic peptide epitope variants, just as self-restricted CTL, and that, with some antagonists, this inhibition can be virtually complete for individual CTL clones. Although some peptide antagonists in antiviral T cell responses have been reported that inhibit CTL recognition at molar equivalence or even at 1000-fold lower concentration than the natural peptide epitope (6, 7), a high molar excess of the antagonists is more frequently required to antagonize T cells, as found in our study.

These conclusions raise hopes about the potential use of alloreactive CTL antagonists in the modulation of allogeneic responses in vivo because 1) the restricted clonal heterogeneity often observed in allograft infiltrates and GVHD (39–43) might help to overcome the problem of clonal diversity in alloreactivity; and 2) the relative ease with which certain structural alterations of the allospecific peptide epitope led to antagonism suggests the feasibility of designing nonnatural MHC ligands (11, 55, 56) with enhanced biostability for immunomodulation of alloreactive responses in vivo.

Acknowledgments

We thank Jesus Vazquez and Samuel Ogueta (Protein Chemistry Laboratory, Centro de Biología Molecular Severo Ochoa) for their help in mass spectrometry, Francisco Gavilanes (Universidad Complutense de Madrid) for help in amino acid analyses, and David Yu (University of California at Los Angeles, Los Angeles, CA) for the B*2705-T2 cell line.

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