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Competitive Inhibition In Vivo and Skewing of the T Cell Repertoire of Antigen-Specific CTL Priming by an Anti-Peptide-MHC Monoclonal Antibody

Doo Hyun Chung,* Igor M. Belyakov,† Michael A. Derby,† Jian Wang,* Lisa F. Boyd,* Jay A. Berzofsky,† and David H. Margulies2*

We have recently described a mAb, KP15, directed against the MHC-I/peptide molecular complex consisting of H-2Dd and a decamer peptide corresponding to residues 311–320 of the HIV IIIB envelope glycoprotein gp160. When administered at the time of primary immunization with a vaccinia virus vector encoding gp160, the mAb blocks the subsequent appearance of CD8+ CTL with specificity for the immunodominant Ag, P18-I10, presented by H-2Dd. This inhibition is specific for this particular peptide Ag; another H-2Dd-restricted gp160 encoded epitope from a different HIV strain is not affected, and an H-2Ld-restricted epitope encoded by the viral vector is also not affected. Using functional assays and specific immunofluorescent staining with multivalent, labeled H-2Dd/P18-I10 complexes (tetramers), we have enumerated the effects of blocking of priming on the subsequent appearance of the P18-I10-specific CTL population.

With the recognition that some autoimmune diseases might reveal a limited or clonal inflammatory T cell response to self Ags (18), it has become reasonable to explore molecular and cellular methods to intervene specifically at the level of restricted T cell responses. One general approach has been to use peptides to compete for the presentation of the autoimmunity-inducing peptide (19) or to exploit oligomerized peptides with a similar goal (20). Preparations of MHC molecules complexed to a broad repertoire of peptides or prepared with a limited, disease-directed set of peptides are also being evaluated (21, 22). Each of these approaches to immune modulation has its own particular advantages and drawbacks.

One alternative to peptide therapies is to exploit mAbs directed against the specific MHC/peptide complex of the APC in an effort to block the ongoing presentation of a self or chronically expressed Ag. We and others have described various MHC-restricted, peptide-specific mAbs that mimic specific TCRs in their peptide specificity and MHC dependence (23–31). Such mAbs might be expected to compete for TCR interaction with the specific immunogenic MHC/peptide complex and block the ongoing activation of specific T cells. We recently described a mAb, KP15, specific for H-2Dd complexed with an HIV envelope gp160-derived peptide P18-I10, and characterized its fine specificity with respect to synthetic variant peptides (31). Preliminary experiments showed that when given to animals during priming with the envelope glycoprotein expressed by a recombinant vaccinia vector, this mAb blocks the CTL response against the immunodominant antigenic peptide. To further understand the specificity and mechanism by which KP15 modulates the immune response, we have characterized the immunological effects of KP15 during the induction of CTL directed against H-2Dd/P18-I10 in vivo.

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Here, using both functional assays and specific fluorescence staining with multivalent MHC/peptide complexes (tetramers) we demonstrate that KP15 blocks the interaction between TCR and MHC/peptide complexes and affects the early phase of CTL induction. Surprisingly, the inhibition by KP15 in vivo also skews the TCR repertoire of H-2D\textsuperscript{d}/P18-I10 specific CTL, as revealed by significant quantitative changes in the representation of a particular TCR V\textgreek{b} family that emerges from in vitro culture following in vivo priming. Overall, these results suggest that MHC/peptide-specific mAb may be useful as a general approach in the therapeutic modulation of immune responses against infectious pathogens, persistent Ags resulting from allogeneic engraftment, or harmful self Ags.

Materials and Methods

Abs and mice

The following Abs, purchased from PharMingen (San Diego, CA), were used: anti-CD8 (2.43), PE- or FITC-conjugated anti-TCR V\textgreek{b}(RR4-7), anti-TCR V\textgreek{b}(TR310), anti-TCR V\textgreek{b}(1.4D.5), and anti-TCR V\textgreek{b}(3.1B.3). Tri-color-conjugated anti-CD8 mAb (Ly2) was purchased from Caltag (Burlingame, CA). FITC-conjugated F(ab\textsuperscript{9})\textgreek{g} goat anti-mouse IgG mAb was purchased from Jackson ImmunoResearch Laboratories (West Grove, PA). 34-5-8 (anti-\alpha\textgreek{2} of H-2\textdagger) and 34-2-12 (anti-\alpha\textgreek{3} of H-2\textdagger) were obtained from American Type Culture Collection (Manassas, VA). Ab was purified from cell culture supernatant by protein A- or protein G-Sepharose affinity chromatography. KP15 is an IgG1 murine mAb that specifically recognizes this H-2D\textsuperscript{d}/peptide complex (i.e., is MHC restricted and peptide specific), and has been characterized in detail previously (31). BALB/c mice were obtained from the National Cancer Institute production facility.

Production of MHC class I tetramers

A cDNA construct encoding the extracellular domains of the H-2\textsuperscript{d} under control of the T7 promoter was engineered to include sequences encoding the BirA biotinylation signal (32) at the carboxyl terminus of the protein and was provided by K. Natarajan (National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, MD). H-2\textsuperscript{d}BirA was expressed in Escherichia coli, refolded in vitro in the presence of human \beta\textgreek{2}-microglobulin and either peptide P18-I10 (RPGGRAFVTI (110)) (33-35) or motif peptide (AGPARAAAL) (36) according to previously published methods (37, 38), and purified by size exclusion chromatography on a Superdex-75 Gel filtration column (Amerham Pharmacia Biotech, Piscataway, NJ). The purified MHC/peptide complexes were then biotinylated using biotin ligase in the presence of free biotin (Avidity, Denver, CO) at 25°C for 2 h. After removal of free biotin by dialysis, tetramers were produced by mixing the biotinylated H-2\textsuperscript{d}/peptide complex with streptavidin-R-PE (BioSource International, Camarillo, CA) at a molar ratio of 8:1. The specificity of the H-2\textsuperscript{d}/P18-I10 tetramer was confirmed using the B4.2.3 T cell hybridoma (39). The H-2\textsuperscript{d}/motif tetramer was tested on S167, an Ly49A-transfected Chinese hamster ovary cell line (gift from W. Yokoyama, Washington University, St. Louis, MO). A control H-2\textsuperscript{L}/\textgreek{a}CMV tetramer was made by a similar procedure using an H-2\textsuperscript{L} BirA vector provided by J. Altman (Emory University, Atlanta, GA), and the \textgreek{a}CMV peptide (YPHRMPFNL). (We designate these multivalent preparations tetramers based on the known valency of streptavidin and the conditions under which multimerization was performed. It is possible that our preparations contain monomers, dimers, and trimers as well as tetramers and may also contain higher order multimers.)

FACS analysis

LKD8, a TAP-defective cell line expressing transfected H-2\textsuperscript{d} (40), was incubated with 10 \textmu g/ml of either MN-T10 (IGPGRAFYTT) or P18-I10 (RPGGRAFVTI) peptide for 2 h at 37°C and washed with PBS before staining. These cells were analyzed by indirect immunofluorescence using purified 34-5-8 or KP15 followed by FITC-conjugated anti-mouse IgG mAbs. Cells from immunized mice were analyzed for TCR expression and H-2\textsuperscript{d}/P18-I10 tetramer binding by direct immunofluorescence using FITC-conjugated anti-TCR, PE-conjugated tetramer, and Tri-color-conjugated anti-CD8. The percentage of TCR- and tetramer-positive cells was analyzed among gated CD8\textsuperscript{+} T cells, using CellQuest software.

Immunization and injection of KP15 mAb in vivo and cytotoxic T lymphocyte assay

BALB/c mice were immunized i.p. with 5 \times 10\textsuperscript{6} PFU recombinant vaccinia virus expressing either HIV-1 IIIB envelope gp160 (vPE16) (41) or HIV-1 MN envelope gp160 (vMN) (42). To block CTL induction against H-2D\textsuperscript{d}/P18-I10 complexes in vivo, BALB/c mice were injected with various concentrations of KP15 divided into seven doses given 4 h before, coincident with, and 4 h following injection of vPE16 as well as on days 1, 2, 3, and 4 following immunization. At each time point, BALB/c mice were simultaneously injected with equal doses i.p. and i.v. or i.p. or i.v. alone with KP15. (Pilot experiments confirmed that either route of administration of Ab was effective as well as the combination of the two, and that i.v. administration produced the greatest inhibition of priming relative to the dose of mAb.) Three weeks later, cells from the spleen were taken and cultured at 5 \times 10\textsuperscript{5} ml in 24-well culture plates in complete T cell medium: RPMI 1640 containing 10% FBS, 10% rat T-Stim (Collaborative Biomedical Products, Bedford, MA), 2 mM l-glutamine, penicillin (100 U/ml), streptomycin (100 \mu g/ml), sodium pyruvate, MEM nonessential amino acids, and 5 \times 10\textsuperscript{-5} M 2-ME. Spleen cells from immunized mice were mixed in vitro with P18-I10 peptide for 7 days before assay. In some cases the synthetic MN peptide (MN-T10, IGPGRAFYTT) or the H-2\textsuperscript{L}-restricted peptide derived from \beta2-microglobulin (Yew21, TPHPARIGL) (43) was used in parallel. Such in vivo primed, in vitro restimulated spleen cells were used as effecter cells for cytolyis or for staining for expression of TCR and MHC tetramer. Cytolytic activity of CTL was measured in a 4-h assay using either \textsuperscript{51}Cr- or europium chelator-labeled targets. P815 targets were tested in the presence or the absence of P18-I10 peptide at the concentrations indicated in the figure legends. For testing the peptide specificity of CTL, either \textsuperscript{51}Cr- or europium chelator-labeled P815 targets were pulsed for 2 h with peptide at the beginning of the assay. \textsuperscript{51}Cr release assays were conducted by standard procedures as described previously (44, 45). The use of europium as a tracer for cytolyis is described elsewhere (63).

Surface plasmon resonance (SPR) binding analysis

Competition by mAb KP15 for the binding of H-2D\textsuperscript{d}/P18-I10 complexes to a cognate single-chain TCR (scTCR) was analyzed by SPR using a BIAcore 2000 (BIAcore, Piscataway, NJ). An H-2D\textsuperscript{d}/P18-I10-specific three-domain scTCR (46) was covalently coupled to the carboxymethyl dextran CM-5 biosensor surface through free amino groups at pH 4.5 according to the conventional method described previously (47, 48). All binding experiments were performed at 25°C. Bacterially expressed and in vitro refolded soluble H-2D\textsuperscript{d}/P18-I10 prepared as previously described (37) was used as the solution phase ligand, and purified KP15 was used as a competitor at the concentrations indicated in the figure legends.

Results

KP15 specifically blocks CTL induction by H-2D\textsuperscript{d}/P18-I10 in vivo

In our previous characterization of two mAbs that recognize the H-2D\textsuperscript{d}/P18-I10 complex with peptide specificity mimicking that of a TCR (31), we observed that injection of one of these mAbs, KP15, could block the induction of specific CTL against H-2D\textsuperscript{d}/P18-I10 in BALB/c mice immunized with vaccinia virus (vPE16) expressing the gp160 envelope glycoprotein derived from HIV-1 IIIB isolate. This inhibition of specific CTL priming was dose dependent. To explore the nature of the specificity of KP15 in its ability to block the priming of CTL in vivo, we compared this MHC/peptide-specific mAb with two other mAbs directed against distinct domains of H-2\textsuperscript{d}: 1) 34-5-8, which binds to an epitope on the \alpha\textgreek{2} domain that is dependent on bound peptide for its conformation but is not specific for any particular peptide (40); and 2) 34-2-12, which is specific for the \alpha\textgreek{3} domain of H-2\textsuperscript{d}, independent of peptide binding (Fig. 1) (49, 50).

Confirming our previous results, mAb KP15 blocked the in vivo priming of CTL by vPE16 (Fig. 1A). A control isotype-matched

3 Abbreviations used in this paper: SPR, surface plasmon resonance; scTCR, single-chain TCR.
To explore further the specificity of KP15 in such experiments in vivo, we studied its effects on the induction of CTL against a peptide/H-2D\(^d\) complex that KP15 was unable to bind. The MN-T10 peptide represents an H-2D\(^d\)-restricted immunogenic epitope that differs from P18-I10 in sequence and TCR specificity (42). T cells raised against the HIV-1 MN gp160 are H-2D\(^d\) restricted and do not cross-react with cells pulsed with P18-I10. LKD8, a TAP-defective cell line, was used to test whether KP15 binds MN-T10 peptide-loaded H-2D\(^d\) molecules. Flow cytometric analysis using the conformation-sensitive mAb, 34-5-8, demonstrated that the expression of H-2D\(^d\) on LKD8 cells was increased by exposure of cells to either P18-I10 or MN-T10 peptide, indicating that both P18-I10 and MN-T10 peptides bind to H-2D\(^d\) (Fig. 1B, upper panel.

When the peptide-specific, H-2D\(^d\)-restricted mAb KP15 was used (Fig. 1B, lower panel), it detected H-2D\(^d\) peptide complexes on P18-I10-pulsed LKD8 cells, but not on MN-T10-pulsed-LKD8 cells, indicating that KP15 lacks cross-reactivity with H-2D\(^d\)/MN-T10 complexes. In vivo immunization with a recombinant vaccinia virus expressing the MN gp160 protein (vMN) was effective at inducing H-2D\(^d\) restricted MN-T10 specific CTL in BALB/c mice. However, KP15 failed to block CTL induction by H-2D\(^d\)/MN-T10 complexes (Fig. 1C). These data confirm that KP15 Abs specifically bind H-2D\(^d\)/P18-I10 complexes and block the induction of CTL directed against H-2D\(^d\)/P18-I10 complexes in vivo.

**FIGURE 1.** KP15 is specific for H-2D\(^d\)/P18-I10 complexes in blocking CTL induction in vivo. A, BALB/c mice were immunized with vPE16 and injected with KP15, 34-5-8, and 34-2-12 (0.3 mg/dose) according to the schedule in *Materials and Methods*. Splenectomies were cultured with 0.1 mM P18-I10 in vitro for 1 wk and used as effector cells in a cytolytic assay. ● and ○, mice injected with isotype-matched mouse IgG; □ and △, mice injected with the indicated mAbs; □ and △, control P815 target cells; ● and □, P815 target cells pulsed with P18-I10. B, LKD8 cells were pulsed with MN-T10 or P18-I10 at 10 \(\mu\)g/ml and stained with 34-5-8 and KP15. The dotted line indicates cells stained with FITC-conjugated anti-mouse IgG, and the solid line indicates cells stained with either KP15 or 34-5-8 followed by FITC-conjugated anti-mouse IgG. C, BALB/c mice were immunized with vaccinia virus expressing envelope gp160 (vMN) and injected with KP15 (0.3 mg/ml/dose); ● and □ or control IgG1 (● and ○). Splenocytes were cultured with 0.1 mM MN-T10 in vitro for 1 wk, and these cells were used as effector cells. MN-T10 (0.1 \(\mu\)M)-pulsed P815 target cells (● and ○) or unpulsed control P815 cells (□ and ○) were used for target cells.

Ab had no effect. Note that in our previous experiments we demonstrated that high doses of the mAb could completely block priming. Here, to analyze the cells that escape KP15 inhibition, we have used a dose that inhibits the response by about 50%. We observed that either of the two anti-H-2D\(^d\) mAbs failed to block the priming of specific CTL directed against H-2D\(^d\)/P18-I10 by vPE16. This was particularly surprising with respect to 34-5-8, because this mAb effectively blocks the interaction between H-2D\(^d\) and TCR in direct binding assays and also inhibits the activation of a T cell hybridoma specific for H-2D\(^d\)/P18-I10 in vitro (31). Nevertheless, 34-5-8 failed to inhibit CTL induction against H-2D\(^d\)/P18-I10 complexes. It is likely that because of the large reservoir of H-2D\(^d\) expressed on virtually all tissues of the mouse, the concentration of 34-5-8 injected was insufficient to saturate and thus block the interaction between TCR and H-2D\(^d\)/P18-I10 complexes in vivo, whereas KP15, specific only for H-2D\(^d\) molecules to which P18-I10 was bound, was able to saturate a smaller cell surface pool consisting of a subset of H-2D\(^d\) molecules bound to an appropriate peptide.
I10 complexes on APC. To test directly whether mAb KP15 physically blocks the interaction of a specific TCR with the H-2Dd/P18-I10 complex, we examined binding and competition with recombinant reagents and SPR as a detection method (Fig. 2B). As reported previously (46), H-2Dd/P18-I10 complexes bind to a recombinant scTCR derived from the B4.2.3 hybridoma. In the presence of graded concentrations of KP15, keeping the concentration of H-2Dd/P18-I10 constant, inhibition of the interaction with the scTCR is clearly seen (Fig. 2B). This result indicates that KP15 competes for the same site on H-2Dd/P18-I10 that binds the scTCR, because binding at a distinct site would not prevent the interaction with the solid phase ligand and would be expected to give an augmented, rather than a diminished, signal. Thus, it is likely that KP15 functions by physically blocking the interaction between TCR and H-2Dd/P18-I10 in vivo rather than by depleting APC expressing H-2Dd/P18-I10 or by eliciting inhibitory factors from the APC.

The ability of KP15 to block CTL priming when given during the first week of immunization is consistent with the above data indicating that it blocks engagement of MHC/peptide complexes by TCR. If the mAb elicited some generalized inhibitory effect, it might be expected to function during any phase of the immunization. To explore whether KP15 is capable of blocking CTL induction in a later in vivo phase, BALB/c mice were given KP15 at any of three phases of the in vivo priming with vPE16: the first week (the initial phase of immunization), the second week (mid phase) following immunization, and the third week (late phase) after immunization. Mice that received KP15 during the initial phase showed a reduced CTL response against H-2Dd/P18-I10 on target cells, but mice injected during the second or third weeks showed a CTL response of the same level as that in control mice (Fig. 3). These data confirm that the blocking effect of KP15 takes place in the initial phase of CTL induction rather than at a later time.

KP15 reduces the number of CTL precursors reactive against H-2Dd/P18-I10 rather than permitting a selective expansion of low avidity CTL

The inhibition of CTL priming by blocking the engagement of the TCR with MHC/peptide complexes on the surface of APC might result in the expansion of populations of CTL of apparent avidity distinct from that of the unblocked population. When high avidity CTL are stimulated with high density MHC/peptide on APC, they die by apoptosis (51, 52). Thus, the selective expansion of high avidity cells results from stimulation with a low concentration of antigenic peptide, and high concentrations of Ag can preferentially expand low avidity clones (51). To evaluate the effects of KP15 on the avidity of the CTL that develop following exposure to the Ab, we cultured in vivo primed cells in the presence of either low (0.0005 μM) or high (50 μM) doses of P18-I10. The cells expanded under these extreme conditions were then evaluated for their apparent avidity by testing their cytolytic activity as a function of the concentration of the peptide needed for sensitization (Fig. 4A). The dose of peptide that sensitizes CTL for half-maximal cytolysis is an accurate indicator of the average avidity of cells specific for a particular MHC/peptide complex (52). Using this parameter, we observed that cells expanded in low dose peptide (Fig. 4A, □) were of ~100-fold greater avidity than those raised in high dose peptide (○). That is, the dose of peptide that sensitized targets for half-maximal lysis was ~5 × 10² pM for the high avidity population and ~5 × 10⁴ pM for the low avidity cells). In addition, using the maximal percent specific lysis as an indicator of the proportion of cells in the culture with specificity for the H-2Dd/P18-I10 complex, we conclude that specific cells account for a greater proportion of the total in the high avidity culture than in the low avidity culture. In vivo blockade of priming resulted in cells with a lower extent of specific lysis, but no major difference in their apparent avidity (compare □ with ■ and ○ with ○). The differences in maximal percent lysis most likely reflect differences in frequency of specific CTL, and thus indicate that KP15 blockade...
H-2Dd/P18-I10 tetramers were used to stain CTL resulting from in vitro priming and in vitro restimulation with low dose (0.0005 μM) KP15 for 30 min and used as target cells without additional washing steps. A and B, Cytolysis activity of CTL was measured in a 3-h assay using europium as described in Materials and Methods.

**FIGURE 4.** KP15 blocks the induction of high and low avidity CTL in vivo. A, BALB/c mice were immunized with vPE16 and injected i.v. with KP15 (0.3 mg/ml/dose). Three weeks later splenocytes from immunized mice were stimulated with either 50 μM P18-I10 or 0.0005 μM P18-I10 in vitro for 1 wk and used as effector cells for a cytolysis assay. B, Splenocytes from immunized mice stimulated with either 20 or 0.0002 μM P18-I10 in vitro were used as effector cells. P815 cells pulsed with P18-I10 were preincubated with various concentrations of KP15 for 30 min and were used as target cells without additional washing steps. A and B, Cytolysis activity of CTL was measured in a 3-h assay using europium as described in Materials and Methods.

reduces the number of specific CTL precursors. However, the inhibition of high avidity CTL may be greater than that of low avidity CTL, as indicated by the differences in the plateau levels of lysis (Fig. 4A).

An alternative means of assessing the relative avidity of a population of CTL is to titrate the concentration of the target MHC/peptide complex by adding graded amounts of the MHC/peptide-specific mAb (Fig. 4B). For this experiment, a population of low avidity CTL was raised by in vitro culture with high dose (20 μM) P18-I10, and high avidity CTL were raised with a low dose (0.0002 μM; Fig. 4B). The high avidity CTL (Fig. 4B, right panel) were inhibited over a narrower range of KP15 concentration (from 40 to 400 nM) than low avidity CTL (left panel; from 2 to 400 nM), suggesting that low avidity CTL is a population with a broader range of avidities. Although it is tempting to conclude that the apparent avidity of a CTL population is a direct consequence of the intrinsic affinity of the TCR for their MHC/peptide ligand, recent work indicates that additional factors, including but not limited to CD8 density, contribute to the functional apparent avidity (see Footnote 3).

To examine directly whether the injection of KP15 decreases the total number of CTL expressing TCR reactive for H-2Dd/P18-I10, H-2Dd/P18-I10 tetramers were used to stain CTL resulting from in vivo priming and in vitro restimulation with low dose (0.0005 μM) peptide (Fig. 5). Under these conditions, specific H-2Dd/P18-I10 tetramer-positive cells accounted for >80% of the CD8+ cells in these cultures (Fig. 5A, upper left panel, and Fig. 5B). The proportion of specific tetramer-positive cells was inhibited markedly, in a dose-dependent manner, by blocking with KP15 during priming (center and right panels of Fig. 5). The specific staining contrasts markedly with that using control tetramers, H-2Dd/motif and H-2Ld/motif H-2Ld/pMCMV. These data indicate that the injection of KP15 inhibited CTL induction by decreasing the number of precursors of CTL specific for H-2Dd/P18-I10.

**KP15 injection in vivo skews TCR repertoire of H-2Dd/P18-I10 specific CTL**

As with many MHC-restricted Ags, P18-I10 evokes a limited repertoire of TCR Vβ usage in a number of different mouse strains (53). In an attempt to assess the effect of KP15 inhibition on the TCR repertoire of H-2Dd/P18-I10-specific CTL, CTL from KP15-injected mice immunized with vPE16 and restimulated with P18-I10 in vitro were stained with anti-CD8 and anti-TCR Vβ lineage mAbs (Fig. 6). The Vβ families studied were those previously shown to be preferentially used by CTL specific for H-2Dd/P18-I10 (53). CD8+ CTL from mice immunized with vPE16 and blocked with KP15 exhibited a remarkably reduced percentage of CTL expressing Vβ7 TCR compared with those from mice immunized with vPE16 alone. However, the percentages of CTL expressing Vβ6, Vβ8.1,2, and Vβ8.3 in the same animals were similar to those of control vPE16-immunized mice. This result held true whether the CTL were stimulated in vitro with 50 μM peptide to induce low avidity CTL (Fig. 6, left panel) or with 0.0005 μM peptide to expand high avidity CTL (Fig. 6, right panel). These data suggested that the injection of KP15 skewed the priming of CTL in vivo, resulting in a selective lack of induction of CTL bearing Vβ7. However, from these data, it is not clear whether the skewed priming was simply due to the fact that most anti-H-2Dd/P18-I10 CTL expressed Vβ7 over CTL with other Ag-specific Vβ-chains. To address this question, CTL from mice immunized either with vPE16 alone or those immunized and injected with KP15 were stained in a three-color analysis with FITC-conjugated anti-Vβ TCR and CyChrome-conjugated anti-CD8 mAbs along with PE-conjugated H-2Dd/P18-I10 tetramer (Fig. 7). Relatively few of the CD8+ cells stained positively with the control H-2Dd/motif tetramer (3.9 and 3.8% of the vPE16-immunized and vPE16-immunized/blocked, respectively; Fig. 7A, left panels). Specific H-2Dd/P18-I10 tetramers are shown to be preferentially used by CTL specific for H-2Dd/P18-I10.
P18-I10-stained cells accounted for 56.9% of the CD8^+ cells, and this decreased to 16.5% in those cultures derived from mAb KP15-treated animals. The same CD8^+ cells were also examined simultaneously for their expression of specific V^b families and for staining with the H-2D^d/P18-I10 tetramer (Fig. 7B). For each of the V^b families analyzed, the H-2D^d/P18-I10 tetramer-positive cells (upper right quadrant) represented from 3.8% (V^b8.3) to 32.8% (V^b8.1, 2) of the CD8^+ tetramer-positive cells analyzed in those animals immunized with vPE16 alone (Fig. 7B). The presence of KP15 during in vivo priming resulted in a marked decrease (from 28.1 to 8.8%) in the percentage of tetramer-positive, V^b7-positive cells as a fraction of the CD8^+ tetramer-positive cells. The other three V^b populations studied showed increases of different magnitudes, presumably reflecting the compensation for the decrease in V^b7. This indicates the clear lack of expansion of V^b7-positive, tetramer-positive, CD8^+ cells during priming, consistent with specific inhibition of Ag-specific CTL of the V^b7 family. This result confirms and extends the observation of the inhibition detected by the functional experiment described in Fig. 6. These data indicate that the injection of KP15 into mice around the time of immunization with vPE16 preferentially blocked the induction of CTL expressing V^b7 and specific for the H-2D^d/P18-I10 complex, as evaluated with the H-2D^d/P18-I10 tetramer. CTL with the same MHC/peptide specificity expressing other V^b types were not inhibited by the Ab.

Discussion

mAbs with specificity for MHC/peptide complexes have been developed in recent years with a primary goal of investigating the steps involved in Ag processing and presentation. Here we have demonstrated that such mAbs can also be used to block specific immune responses, not only resulting in a decrease in the CD8^+ CTL response, but also producing a change in the proportion of MHC/peptide-specific T cells represented by different TCR families. We describe the use of one such MHC-restricted peptide-specific mAb, KP15, in the regulation of priming of BALB/c animals by a vaccinia virus-delivered, H-2D^d-restricted, immunodominant peptide derived from the HIV-1 envelope glycoprotein. The injection of KP15 into mice immunized with a vaccinia virus vector encoding gp160 (vPE16) specifically inhibited the induction of CTL against H-2D^d/P18-I10. These inhibitory effects are due to physical blocking of the TCR on CTL interaction with H-2D^d/P18-I10 complexes on APC and do not seem to be related to the depletion of specific APC or the production or secretion of inhibitory molecules by the APC. Surprisingly, injection of KP15 into mice immunized with vPE16 skewed the TCR repertoire of CTL reactive for H-2D^d/P18-I10. Flow cytometric analysis demonstrated that CTL expressing V^b7 TCR were remarkably decreased as a percentage of the H-2D^d/P18-I10 tetramer-positive CD8^+ CTL from such Ab-treated mice. These data suggest that KP15 preferentially binds a
site of H-2D^d/P18-I10 where V\beta TCR binds and that the mAb sterically competes for the interaction between V\beta TCR on CTL and H-2D^d/P18-I10 on APC in vivo. Such competition was readily demonstrated in in vitro binding experiments. We considered two primary explanations for the preferential inhibition of CTL expressing V\beta TCR. First, that V\beta TCR might as a group have higher avidity for H-2D^d/P18-I10 complexes than other V\beta TCR. We reasoned that if this were the case, the percentage of CTL expressing V\beta TCR would be higher among high avidity CTL populations than in lower avidity CTL. However, the percentage of CTL expressing the particular V\beta TCR tested was similar among both high and low avidity CTL, and the percentage of CTL expressing V\beta TCR was reduced in both high and low avidity CTL from mice injected with KP15.

A second explanation would be that V\beta7-bearing TCR have a distinct spatial orientation in their canonical interaction with the H-2D^d/P18-I10 complex, such that the KP15 mAb more effectively blocks the interaction relative to the interaction of the same MHC/peptide complex to TCR of other V\beta families. We tested this possibility for one cloned TCR and demonstrated direct competition between KP15 and TCR binding to H-2D^d/P18-I10. It is conceivable that KP15 binds H-2D^d/P18-I10 at a site that does not overlap the site where the bulk of non-V\beta7-bearing cells interact. However, we have no evidence bearing on this possibility. Structural studies of the KP15/H-2D^d/P18-I10 interaction as well as detailed analysis of other V\beta TCR with H-2D^d/P18-I10 specificity may shed light on this question.

Several other possibilities may be considered as the basis for future experiments. We have demonstrated the effect of KP15 in blocking priming of specific T cells, and we have analyzed the cells with the specific tetramers following priming and in vitro restimulation. It is possible that the requirements for the primary stimulation of V\beta7-bearing reactive cells are different from the recognition requirements for effector function and tetramer staining. Another possible explanation for the differential blocking of V\beta TCR may be related to the degree of cross-reactivity of the non-V\beta TCR for other Ags. Thus, the V\beta7 reactivity may be more focused on H-2D^d/P18-I10 and less cross-reactive than other V\beta families. In the presence of KP15 blockade, the V\beta7 TCR are not stimulated, while cross-reactive TCR of other V\beta families may continue to be stimulated by other Ags. When tested on the H-2D^d/P18-I10 as CTL, or with the H-2D^d/P18-I10 tetramer, these non-V\beta7 TCR continue to be reactive.

Several mAbs against peptide/MHC complexes have been described that have been used to detect endogenously processed peptide/MHC complexes (30), to block the positive selection of thymocytes reactive with particular complexes (54), to treat specific
autoimmune diseases in model systems (23), and in our own studies to inhibit the induction of Ag-specific CTL (31). Although systemic autoimmunity in both human disease and in a number of animal models probably results from the coincidence of several dysregulatory factors, it is clear that T cell recognition of MHC-presented Ags plays an important role in most of these settings. A mAb specific for basic myelin protein/I-A\(^*\) was used in the effective treatment of experimental autoimmune encephalomyelitis of mice (23). The precise mechanism of this therapeutic effect remains unclear. CD8\(^{+}\) and CD4\(^{+}\) T lymphocytes infiltrate the pancreatic islets of NOD mice, and the destruction of cells following adoptive transfer of cells from newly diabetic NOD mice required both CD4\(^{+}\) and CD8\(^{+}\) T cells (55). Thus, regulation of autoreactive CD8\(^{+}\) T cells might be an alternative strategy for treating autoimmune disease. In this paper we have demonstrated that an MHC-restricted, peptide-specific mAb can inhibit the induction of specific CTL against H-2D\(^{b}\)/P18-110 in vivo. Thus, in a situation in which a known peptide/MHC class I complex contributes to the induction of autoreactive CD8\(^{+}\) T cells, mAbs specific against these complexes might be applied to treatment and suppression of such a autoimmune reaction.

It is valuable to consider our experiments along with strategies for developing vaccines directed against viral pathogens. CD8\(^{+}\) and CD4\(^{+}\) T cells play a critical role in reducing viremia and protecting the host from viral infection (56–58). As an example, early SIV clearance during primary infection correlates to the emergence of tetramer-binding CD8\(^{+}\) T lymphocytes, and the in vivo depletion of CD8\(^{+}\) lymphocytes eliminates the ability of infected monkeys to contain SIV replication (59, 60). Thus, the induction of CD8\(^{+}\) CTL reactive for a specific peptide/MHC class I complex is an important consideration in the design of viral vaccines (56, 61, 62). Such immunogenic peptides might not only induce specific CD8\(^{+}\) CTL, but might also induce the production of Abs directed against viral peptide/self MHC complexes in vivo during either viral infection or vaccination. Such Abs might block or skew the CD8\(^{+}\) CTL response. However, the general experience in attempting to raise Abs with such MHC/peptide specificity is that this has been difficult, even in circumstances that would be expected to favor such a response (31). Thus, we would expect the natural elaboration of MHC/peptide-specific Abs to play a minor role in the natural regulation of the CD8\(^{+}\) CTL response.

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References


converting enzyme activity processes a human immunodeficiency virus 1 gp160 peptide for presentation by major histocompatibility complex class 1 molecules. J. Exp. Med. 175:1417.


