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Antigen-Specific Blockade of T Cells In Vivo Using Dimeric MHC Peptide¹

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Ag-specific immune tolerance in clinical organ transplantation is currently an unrealized but critical goal of transplant biology. The specificity and avidity of multimerized MHC-peptide complexes suggests their potential ability to modulate T cell sensitization and effector functions. In this study, we examined the ability of MHC-peptide dimers to modulate T cell function both in vitro and in vivo. Soluble MHC dimers induced modulation of surface TCR expression and inhibited T cell cytolytic activity at nanomolar concentrations in vitro. Furthermore, engagement of TCR by soluble dimers resulted in phosphorylation of the TCR ζ -chain and recruitment and phosphorylation of ζ -associated protein-70 to the signaling complex, the latter of which increased upon dimer cross-linking. Significantly, Ag-specific inhibition of an alloreactive TCR-transgenic T cell population in vivo resulted in consequent outgrowth of an allogeneic tumor. The prolonged Ag-specific suppression of expansion and/or effector function of cognate T cells in vivo suggests that soluble MHC dimers may be a means of inducing sustained Ag-specific T cell unresponsiveness in vivo. *The Journal of Immunology*, 2001, 167: 2555–2560.

Current immunosuppressive therapies are broad based and nonspecific. Induction of peripheral tolerance in an Ag-specific manner is an unrealized clinical goal. It follows that a rational approach to Ag-specific immunomodulation would be to use the specificity of the peptide-MHC-TCR interaction because it dictates the specificity of the T cell arm of the adaptive immune response. Indeed, soluble MHC has been proposed as a tolerogen (1, 2). However, its efficacy in suppressing alloresponses in most transplantation models has been limited (3, 4). One likely explanation for the lack of suppressive effects of soluble MHC is that the inherent low affinity of the MHC-TCR interaction does not result in a sufficient engagement time for effective blockade either by steric hindrance or inhibitory signal transduction. The immune system compensates for this low affinity interaction through multivalent presentation of peptide-MHC ligand (5–7) and rapid serial engagement (8). Such mechanisms are necessary and sufficient to initiate T cell signal transduction. However, it has been demonstrated as well that suboptimal engagement of TCR-CD3 complexes through mere bivalent engagement (9) altered peptide li-

gand engagement (10), or supraoptimal engagement by continued stimulation (11) can result in transduction of a unique signal that leads to immune dysfunction and tolerance induction.

The development of multimerized forms of the MHC dimeric and tetrameric complexes loaded with specific peptides has enabled the direct visualization of Ag-specific T cells during a variety of infections and immunizations (12–14). Moreover, soluble dimers as well as plate- or bead-bound monomers of class I and class II molecules have exhibited T cell activation capabilities in vitro, but their immunotherapeutic potential has yet to be realized in vivo. In the present study, we made use of the 2C TCR-transgenic (Tg)⁵ mouse that expresses a monoclonal T cell population specific for a nanomer peptide (QL9) presented in the context of H-2L^d (15) as well as for a strong agonist peptide (SIY) in the context of K^b (16). The addition of peptide dimer to 2C T cell cultures in vitro resulted in 2C TCR down-regulation and inhibition of cytolytic activity. Early activation events were induced by MHC dimer engagement of TCR, including up-regulation of CD69 expression. Also observed was phosphorylation of TCR ζ -chain and ζ -associated protein-70 (ZAP-70) induced by the dimer even in the absence of cross-linking. Finally, the administration of peptide-MHC dimer profoundly suppressed 2C T cell function in vivo in the presence of alloantigen, suggesting that MHC dimers may be useful for Ag-specific peripheral immunosuppression.

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Materials and Methods

Mice

BALB/c (H-2^d) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Recombination-activating gene (RAG)-2-deficient mice (H-2^b), obtained as a gift from Dr. C. Simon (University of Chicago, Chicago, IL), were bred in house. The 2C TCR-Tg (H-2^b) mice were originally obtained from Dr. D. Loh (Washington University School of Medicine, St. Louis, MO) and intercrossed with RAG-2^{-/-} mice to obtain 2C × RAG-2^{-/-} mice (2C-RAG-knockout (KO) mice).

⁵ Abbreviations used in this paper: Tg, transgenic; ZAP-70, ζ -associated protein-70; RAG, recombination-activating gene; KO, knockout; MCMV, murine CMV; MFI, mean fluorescence intensity.

Reagents

Anti-CD69-FITC, goat anti-mouse IgG1-PE (Southern Biotechnology Associates, Birmingham, AL), and hamster anti-mouse-TCR-PE H57 (anti-CD3; BD PharMingen, San Diego, CA) were used in FACS (BD Biosciences, Mountain View, CA) analyses. A mAb to TCR ζ -chain (H146-968) (17) and anti-phosphotyrosine 4G10 (Upstate Biotechnology, Lake Placid, NY) were used, respectively, in immunoprecipitations and Western blotting. Rat anti-mouse IgG1 (Southern Biotechnology Associates) was used to cross-link the MHC-peptide dimers. Rat anti-hamster Ab (Southern Biotechnology Associates) was used to cross-link hamster antimurine CD3, 145-2C11. The latter was produced and purified in our laboratory. Peptides were synthesized using F-moc solid-phase technology by the Molecular Genetics Facility at the University of Georgia (Athens, GA). The K^b-binding peptides were SIYRYYGL (SIY) and SIINFEKL (OVA); the L^d-binding peptides were LSPFPFDL (p2Ca), QLSFPFDL (QL9), and YPHFMPTNL (murine CMV (MCMV)); the K^{bm3}-binding peptide was DQYKFYSV (DEV-8). The H-2K^b dimer was engineered as previously described (18). The divalent H-2L^d protein was constructed, expressed, and purified using previously described methods (19). Specifically, the BALB/c H-2L^d cDNA from pL^d.444 (20) was amplified using primers 5'-L^dMluI-AT ACGCGTCGAGATGGGGGCGATGGCTCC and 3'-L^dXhoI-ACCTCGA GTGCGGCCCGCCATCTCAGGGTGAGGGG. The fragment was digested with MluI and XhoI and inserted into the same sites of pX-Ig (18). The sequence was verified. The resulting plasmid was cotransfected by electroporation with a human β_2 -microglobulin expression plasmid into J558L plasmacytoma cells. A clone that secreted relatively high levels of protein, as determined by ELISAs specific for H-2L^d or the IgG1 portion of the molecule, was grown in hybridoma serum-free medium (Life Technologies, Rockville, MD), and protein was purified from supernatants by affinity chromatography to the V region of the Ig portion.

In vitro activation of T cells

Splenocytes were prepared from spleens from 2C-RAG-KO mice. After maceration, RBCs were lysed. Debris was then excluded by filtering the suspension through sterile Nitex mesh (Tetko, Elmsford, NY). BALB/c splenocytes were prepared and irradiated to serve as APCs to present the octapeptide, p2Ca, derived from α -ketoglutarate dehydrogenase, in the context of H-2L^d. Cells were distributed in 24-well plates in culture medium. These cells became the source of "activated" 2C cells for cytolytic assays (day 4 or 5) and for in vivo studies (day 6). Before assays, cells were found to be 98–99% pure as shown by staining with the clonotypic Ab against the 2C TCR, IB2.

FACS analyses

Flow cytometry with dimers was performed with 3×10^5 cells/sample. Dimers were loaded with peptide as previously described (19). Cells were washed in FACS wash buffer containing 0.2% FBS and 0.5% sodium azide. Cells were incubated with dimers for 1 h on ice followed by a single wash in FACS wash buffer. Goat anti-mouse IgG1-PE was then added for 30 min followed by a final wash and then assayed on a FACScan and analyzed using CellQuest software (BD Biosciences). Staining with anti-CD69-FITC or H57-597-PE was performed on 3×10^5 cells on ice for 1 h followed by a wash and analyzed as described above.

In vitro stimulation and immunoprecipitation

For stimulation, purified 2C splenic T cells were obtained. Cells (13×10^6) were first incubated with 0.5 μ g dimer in 0.1 ml PBS for 15 min on ice. To initiate in vitro stimulation, dimer-bound cells were then incubated in an equal volume of prewarmed (37°C) PBS or with cross-linking Abs, rat anti-mouse IgG1 (10 μ g final) or goat anti-hamster H and L chain. After a 2-min incubation, cells were immediately transferred to ice. Immunoprecipitations were performed on lysates prepared in lysis buffer (0.5% Triton X-100, 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 5 mM EDTA (pH 8.0), 1 mM sodium vanadate, 10 μ g/ml leupeptin, 10 μ M aprotinin, and 1 mM PMSF) and centrifuged at 14,000 rpm to remove detergent-insoluble material. TCR ζ was immunoprecipitated overnight (4°C) using protein A beads precoated with H146-968. Immune complexes were washed four times with lysis buffer, resolved on 12% SDS-PAGE, and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA). Blotting was performed with an anti-phosphotyrosine mAb, 4G10, and an HRP-conjugated sheep anti-mouse secondary Ab. Blots were developed using SuperSignal substrate (Pierce, Rockford, IL).

Cytolysis assay

Target cells, T2-L^d or T2-K^{bm3}, were loaded with ⁵¹Cr at 37°C for 1 h followed by three washes. Cells were then incubated with 10 nM peptide

at room temperature for 2 h, counted, and added to 96-well plates (5000/well). Effectors were incubated in the presence of dimer at room temperature for 1 h at the concentrations indicated and then added to the targets in triplicate wells at the ratios indicated. Plates were incubated at 37°C (4 h) and harvested on a 1470 Wizard automatic gamma counter (PerkinElmer Wallac, Gaithersburg, MD). Results were calculated as percentage of cytotoxicity using the following equation: (experimental release – spontaneous release)/(maximum release – spontaneous release) \times 100. Maximum release refers to the amount of ⁵¹Cr released from target cells alone resulting from lysis by 10% Triton X-100, and spontaneous release refers to the amount of ⁵¹Cr released from untreated cells alone.

In vivo suppression assay

Spleen cells from 2C-RAG-KO mice were stimulated and harvested at day 6 as described above. Cells were washed three times in PBS and administered directly to the retro-orbital blood sinus of RAG2^{-/-} mice (10^5 cells/animal). The following day (day 0), PBS-washed cells from the highly transfectable variant of the murine mastocytoma P815 (H-2^d; referred to hereafter as P1.HTR) were administered to the left flank s.c. (1×10^6 /animal), and dimer (50 μ g/dose) or PBS was administered i.p. on alternate days for a total of four injections. Animals were palpated for tumor beginning on day 7, and growth was quantified with a Vernier caliper by measuring two perpendicular axes. Two measurements were taken at 90° to each other, and the square root of their product was calculated to give an estimate of mean tumor diameter. The measuring was performed blindly.

Results

Sensitivity of class I MHC dimers for cognate TCR

Peptide-loaded MHC dimers supported by a scaffold of whole Ig are specific for T cells bearing cognate ligand (19). To analyze the sensitivity of peptide-loaded class I dimers for cognate TCR, primed T cells (day 5) were stained with various concentrations of dimer. The L^d dimer loaded with peptide cognate for 2C TCR, QL9, showed \sim 300-fold greater mean fluorescence intensity (MFI) above background when 2 ng (0.4 nM final concentration) was added to 3×10^5 cells (Fig. 1A). In contrast, a 3-log higher concentration of L^d dimer with noncognate peptide did not stain the cells above background. High doses of L^d dimer, 40 nM, were associated with lower MFI, as is often seen with mAb. This is likely due to weaker, monomeric interactions between a single arm of an MHC dimer and TCR, as is commonly seen with excess dimeric reagent. Similar staining patterns were observed with syngeneic MHC (K^b) dimer loaded with the strong agonist peptide, SIYRYYGL (SIY) (16), with no staining observed with a noncognate peptide, SIINFEKL (OVA; data not shown).

The peptide-MHC dimers were subjected to size exclusion chromatography to assay for the presence of aggregates and to test their activity. The analysis demonstrated that the majority of MHC dimer was in the single-dimer form of 250 kDa (molecular formula, pepMHC₂Ig). When this fraction was isolated and incubated at 4°C for 24 h and again analyzed using the same size exclusion column, only the single-dimer peak was seen. Material from this peak was the only fraction that bound T cells detectably as observed in flow cytometry, whereas higher molecular mass fractions, suggestive of aggregates (molecular formula, npepMHC₂Ig), did not bind T cells (data not shown).

MHC dimers modulate TCR expression

As a first approach toward examining the functional effects of soluble MHC dimers on T cell function, TCR expression was assessed on preactivated 2C T cells cultured with various concentrations of peptide-loaded L^d dimers in vitro for 20 h (Fig. 1B). There was an order of magnitude reduction in MFI of the 2C T cells at dimer concentrations of 20 nM. Incubation of the Tg T cells with as little as 2 nM dimer induced an \sim 50% reduction in TCR expression (Fig. 1B, right panel). In contrast, no evidence of modulation of TCR expression was observed using MHC dimers loaded with noncognate peptide, MCMV (Fig. 1B, left panel).

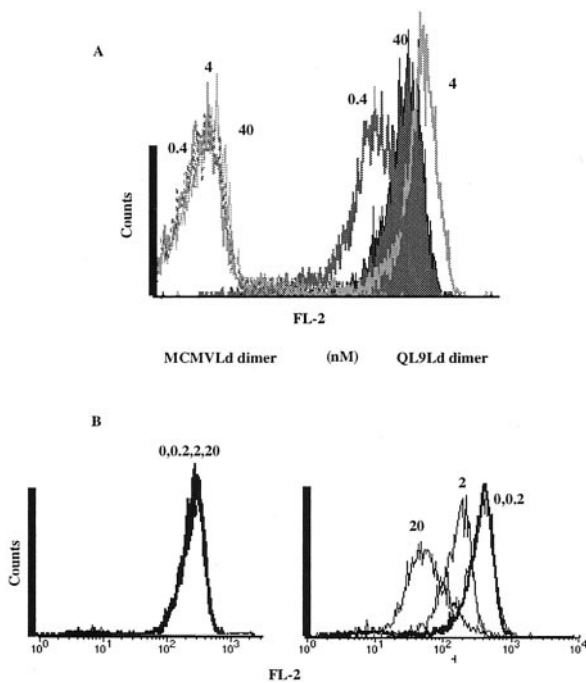


FIGURE 1. Peptide-loaded dimers stain and modulate expression of 2C TCR. *A*, The 2C cells were activated *in vitro* with irradiated BALB/c splenocytes as described in *Materials and Methods*. On day 5, 3×10^5 were stained with peptide-loaded MHC dimers at the concentrations indicated for 1 h on ice followed by a wash and a secondary stain for 30 min with goat anti-mouse IgG1-PE. Values refer to the final concentration of dimer during staining (nanomolar). *B*, Activated 2C cells (day 5) were incubated with the indicated concentrations of QL9 or MCMV dimer (20 h at 37°C) followed by staining with anti-TCR (hamster anti-mouse TCR-PE) for 1 h on ice. Dimer-TCR binding does not sterically hinder this anti-TCR Ab. Values refer to concentration of dimer (nanomolar).

Similar down-regulation of TCR was observed using K^b dimer loaded with SIY but not with K^b loaded with OVA peptide (data not shown).

MHC dimers induce early signaling events

The fact that dimers induce TCR down-regulation (Fig. 1) indicates that their inhibition of CTL cytolytic activity may involve active functional events and not merely receptor/ligand blockade. Indeed, incubation of the TCR-Tg T cells with 20 nM of QL9-loaded L^d dimer induced a 10-fold increase in the expression of the early activation marker, CD69 (Fig. 2A). The control dimer, MCMV-loaded L^d , had no effect on CD69 expression. To obtain a direct assessment of the early signaling events mediated by the QL9-loaded dimer, the cells were stimulated with the dimer loaded with the cognate peptide (2 min) and analyzed for early biochemical changes by probing anti- ζ -chain immunoprecipitates, separated on SDS PAGE, with a phosphotyrosine-specific mAb. As seen in Fig. 2B, QL9-loaded L^d dimer alone induced phosphorylation of TCR ζ -chain as evidenced by an increase in the p23:p21 ratio. When the dimer was cross-linked, the p23:p21 ratio showed little change, whereas the level of coprecipitated ZAP-70 increased 3-fold (Fig. 2, B and C). These results were consistently different from those produced by the non-cross-linked vs cross-linked 2C11, in which case p23:p21 ratios increased 3-fold upon cross-linking and ZAP-70 expression increases were greater (5-fold vs 3-fold; Fig. 2, B and C). Thus, MHC dimer alone does indeed trigger early signal-transduction events. Both naive and previously activated 2C

cells produced the same phosphorylation patterns, and soluble dimer with bulk peptide, with or without cross-linking, consistently gave no signal above the unstimulated control.

MHC dimers inhibit cytolytic activity *in vitro*

Dimers loaded with a defined peptide allow for specific targeting to Ag-reactive CTL and quantitative analysis of the inhibitory activity. The effects of L^d and K^b dimers loaded with various peptides on cytolytic activity mediated by 2C cells *in vitro* was compared using target cells bearing an allogeneic peptide-MHC combination, dEV-8- K^{bm3} or p2Ca-Ld (Fig. 3). L^d dimers loaded with QL9 inhibited CTL activity over the entire range tested. The IC_{50} was ~ 8 nM at an E:T of 10:1 (Fig. 3, *left panel*). In contrast, MCMV-bearing L^d did not inhibit target cell lysis even at a final concentration of 100 nM (Fig. 3, *left panel*). Syngeneic dimer, K^b , loaded with SIY inhibited lysis with an IC_{50} of ~ 20 nM at an E:T of 3:1, whereas OVA-loaded K^b did not effect CTL activity at any concentration tested. Dimers (L^d and K^b) with bulk undefined peptide showed the same ineffectiveness at inhibiting lysis (data not shown) as dimer with defined, noncognate peptide (MCMV and OVA; Fig. 3). The suppressive effect of the MHC-peptide dimers on CTL activity was observed at concentrations well below the concentration necessary to visualize dimer binding by staining. These results suggest that the threshold concentration for modulating T cell activity is lower than can be observed by flow cytometry, suggestive of a mechanism beyond steric hindrance that may involve signaling events discussed above.

Prolonged suppression of Ag-driven CTL responses *in vivo*

The suppression of cytolytic activity by the MHC dimer *in vitro* combined with the signal observed biochemically suggests that Ag-driven CTL responses *in vivo* may be suppressed by signals induced by MHC dimer. To study the effects of the dimers *in vivo*, an adoptive transfer model was developed. Resting 2C T cells were transferred (*i.v.*) to RAG2 $^{-/-}$ recipients followed by *s.c.* administration of P815 tumor cells that express the H-2 L^d alloantigen naturally bearing the peptide derived from α -ketoglutarate dehydrogenase, p2Ca (21). Previous studies have shown that the transfer of 2C T cells in RAG-deficient animals results in tumor rejection (22). In these studies, we used the highly transfectable variant of P815, P1.HTR, which forms a solid tumor when administered *s.c.* and continually expresses the target Ag for at least 69 days *in vivo*.

Simultaneous administration of the allogeneic QL9-loaded L^d dimer significantly inhibited the rejection of the tumor by 2C, allowing for robust tumor growth (Fig. 4, *left panel*). The rate of tumor growth in these animals resembled the unabated rate of growth of tumor in animals that did not receive an adoptive transfer of 2C CTL. Surprisingly, MCMV-loaded allogeneic L^d dimer mediated suppression equivalent to that mediated by QL9-loaded L^d . Suppression of tumor rejection was similarly observed in mice treated with the syngeneic SIY-loaded K^b (Fig. 4, *right panel*). Bulk peptide-containing K^b consistently mediated an intermediate level of suppression ($\sim 70\%$ of that mediated by SIY-loaded K^b). In marked contrast, tumor rejection by the 2C T cells was unaffected in mice treated with vesicular stomatitis virus-loaded K^b (noncognate for 2C T cells; data not shown). These results demonstrate that class I MHC dimers are effective at protecting Ag-bearing tissue by inhibiting previously activated CTL *in vivo*.

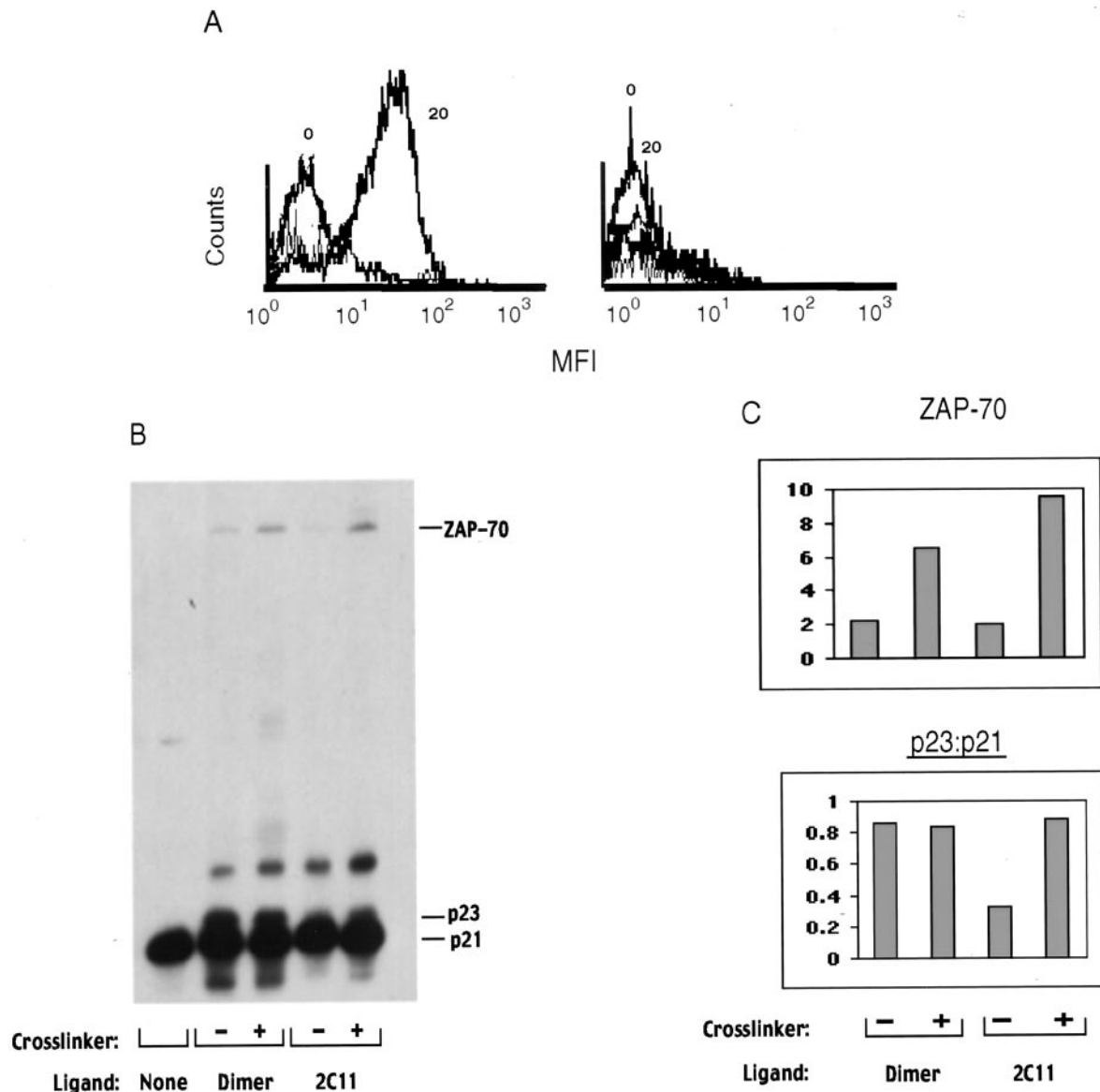


FIGURE 2. MHC dimer induces CD69 expression and transduction of early signaling events. *A*, The 2C cells activated as described (see Fig. 1 legend) were incubated on day 5 with 20 nM QL9 or MCMV (20 h at 37°C) followed by staining with anti-CD69 (rat anti-mouse CD69-FITC). Results were similar in three independent experiments. *B*, Naive purified 2C splenic cells were stimulated for 2 min at 37°C with dimer or 2C11 incubated in the presence or absence of cross-linking Ab followed by immunoprecipitation of TCR ζ -chain with H146-968 and blotting with anti-phosphotyrosine mAb 4G10.

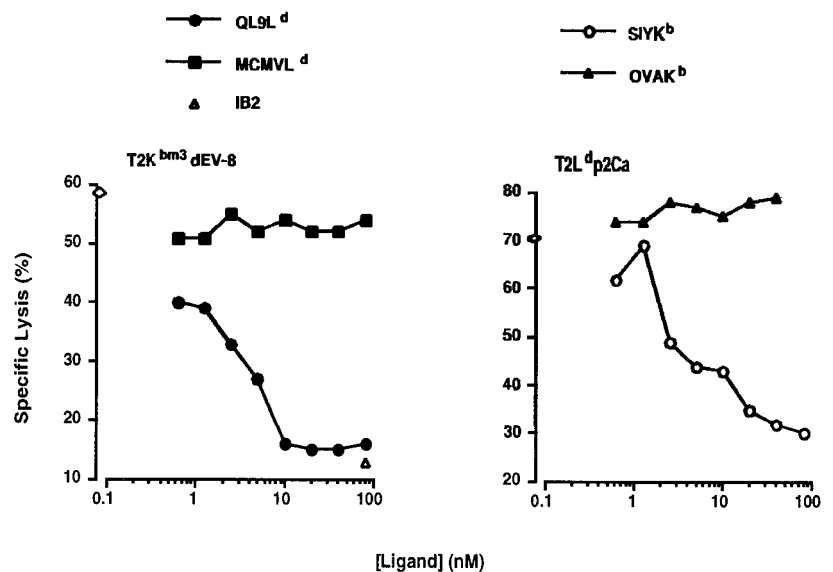
Discussion

The present study was undertaken to test whether MHC dimers presented in a soluble form on a full Ig scaffold can regulate Ag-specific responses of effector CTL in vivo. In this study, we demonstrate that peptide-loaded MHC dimers are specific and effective inhibitors of cell-mediated immunity in vitro and in vivo.

Their potential for in vivo modulation was first indicated by the blockade of CTL activity in vitro at nanomolar concentrations. The inhibition of the cytolytic activity by MHC dimer in vitro was similar to the blocking observed by the anti-clonotypic 2C TCR-specific mAb 1B2 at the highest concentration. The simplest explanation of the inhibitory activity by the peptide-MHC dimers is competitive inhibition of 2C-specific Ag recognition of the target cells by soluble MHC. A total of 1 nM of dimer is theoretically sufficient to give a soluble MHC:cell surface-bound TCR ratio of >200:1. However, additional studies suggested that an active mechanism, including potentially inhibitory signaling events and

TCR down-regulation, may contribute to the diminished cytolytic activity in this 4-h assay. First, 2C-mediated CTL activity was not blocked with a soluble 2C TCR dimer that binds the MHC of target cells (data not shown). Second, the cognate dimers mediated 2C TCR down-regulation upon engagement in vitro (Fig. 1*B*). Finally, TCR ζ -chain phosphorylation and ZAP-70 recruitment and phosphorylation were induced as a consequence of TCR engagement. These results lead to the hypothesis that dimer-mediated suppression of activated CTL may be the net result of mechanisms of suppression similar to those mediated by 2C11-TCR down-modulation and the induction of an apoptotic signal to activated T cells (23, 24). This putative inhibitory signaling outcome is in contrast to a finding in a recent report in which an MHC multimer was used for in vivo activation and enhancement of effector function (25). However, there are several differences in experimental design, including the route of administration (i.p. vs i.v.) and the dosage (alternate days at 50 μ g vs a daily dose of 130 μ g). To distinguish

FIGURE 3. Cytolytic activity of CTL is inhibited by MHC dimer. Day 4 2C cells activated as described (see Fig. legend 1) were incubated in the presence of indicated MHC dimers (1 h) at various concentrations. Without washing the T cells, chromium-loaded T2 target cells bearing allo-MHC K^{bm3} loaded with peptide dEV-8 or allo-MHC L^d loaded with peptide p2Ca were added to give, respectively, a 10:1 or 3:1 E:T ratio, and a 4-h standard chromium-release assay was performed. Maximum lysis mediated by 2C CTL in the absence of dimer is indicated (◆) the on y-axes. Data are representative of three or more independent experiments.



between these mechanisms in vivo, the model system will need to be substantially modified so that sufficient numbers of cells can be recovered for ex vivo analyses.

It is striking that, in the absence of cross-linking, there was a 3-fold greater p23:p21 ratio between peptide-MHC dimer and 2C11. This greater degree of phosphorylation of the CD3 ζ -chain induced by dimer may reflect the different way each reagent binds TCR. In contrast to 2C11 binding of TCR, the physiologic ligation of TCR by MHC dimer may include CD8 binding to the $\alpha 3$ domain of the MHC and, hence, LCK recruitment to the signaling complex, as indicated by recent reports using tetramers in cross-linking TCR (26). However, the 3-fold greater increase in the amount of phosphorylated ZAP-70 upon cross-linking does provide evidence that multimerizing the dimer does result in a stron-

ger stimulus than dimer alone. This physiologic induction of early biochemical signals suggests that MHC dimers will be useful tools for quantitative studies of TCR engagement with various peptide-MHC combinations. In contrast to APC, studies with MHC dimers can be conducted in the absence of other surface molecules, allowing quantitative assessments of the contribution of MHC alone. The relative contribution of CD8 coligation, for example, can be studied by comparing its role using a variety of ligands: allogeneic, agonist, altered peptide, and positively selecting.

These studies do not rule out a role for costimulatory molecules in the effects of the dimer in vivo. It is possible that the effector cells may be engaging TCR in the absence of CD28 coligation, which could result in a lack of Bcl-xL up-regulation necessary to maintain long-term T cell survival (27).

It is remarkable that MCMV-loaded dimer suppressed the effector activity of 2C cells as well as QL9-loaded dimer. No in vitro result predicted this activity. This is in contrast to bulk peptide-loaded syngeneic K^b, which consistently mediated less suppression (~30%) than K^b loaded with cognate SIY peptide. Although neither bulk peptide-loaded K^b nor MCMV-loaded L^d could be detected in binding 2C, in inducing changes in CD69 or TCR expression or in inducing ζ -chain phosphorylation, they each mediated suppression of 2C effector function in vivo. The role of APC-mediated dimer clustering will have to be studied in vitro to begin to understand the mechanism by which each mediates suppression. The efficient suppression mediated by MCMV-loaded L^d could be explained by a degree of allrecognition of 2C TCR for L^d that is not detectable in in vitro assays. In a similar manner, within the bulk-loaded K^b, there may exist conformers that resemble agonist peptide K^b whose recognition by 2C TCR is below the limits of detection in in vitro assays but avid enough for mediating a signal in vivo.

In summary, peptide-loaded dimers offer a novel approach to the active induction of immunosuppression. Regardless of the mechanism, MHC dimers may be effective in promoting stable peripheral tolerance for Ag-specific T cells. In cases of organ transplantation, a small number of different allogeneic MHC conformers compatible with the graft may be useful early on in promoting graft survival and perhaps also inducing linked suppression. In cases of autoimmunity in which a peptide is implicated, dimers may be useful in suppressing effector functions in an Ag-specific manner.

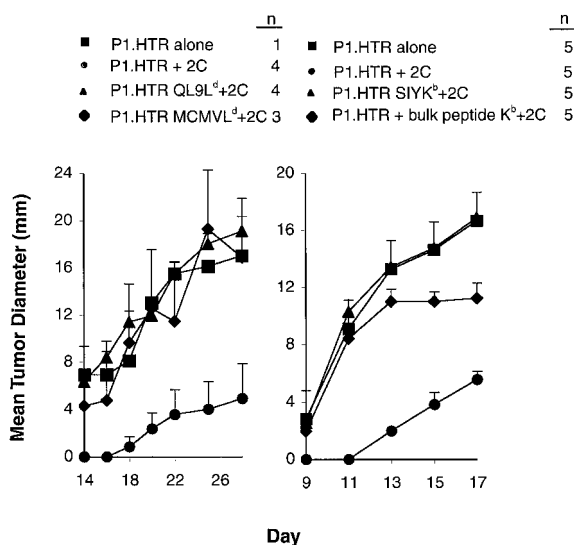


FIGURE 4. Suppression of CTL effector function by MHC dimer in the presence of persistent alloantigen. Mice received a retro-orbital administration of resting 2C T cells (day 6; 1×10^5) and, on the following day, P1.HTR tumor cells (1×10^6) s.c. and dimer (50 μ g, i.p.). Dimer administration continued on alternate days for a total of four injections. Tumors were measured with Vernier calipers on the days indicated, and the square root of the product of two perpendicular measurements taken to give mean tumor diameter. *Left panel*, Suppression mediated by allogeneic dimer, L^d. *Right panel*, Suppression mediated by syngeneic dimer, K^b.

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