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Differential Presentation of the Same MHC Class I Epitopes by Fibroblasts and Dendritic Cells

Eric A. Butz and Michael J. Bevan

Ag is presented to CTL as peptide associated with MHC class I molecules, which are present on most types of cells. We have investigated the presentation of D^b-restricted lymphocytic choriomeningitis virus (LCMV) peptides by a fibroblast line (MC57) and a dendritic cell line (JawsII) to splenocytes from LCMV-immune C57BL/6 mice. We found that when LCMV-infected MC57 were used to restimulate the spleen cells, the resulting CTL line lost its ability to respond to the two dominant epitopes of the immune response to LCMV glycoprotein (gp)33 and nucleoprotein (np)396 but remained strongly lytic for targets coated with the subdominant gp276 epitope. In contrast, when LCMV-infected JawsII cells were used to restimulate the splenocytes, the resulting line continued to target gp33 and np396 but lost reactivity to gp276. When uninfected JawsII or MC57 cells were coated with peptides and used as stimulators, the resulting CTL lines continued to recognize all three epitopes, indicating that costimulatory or other potential innate differences in Ag presentation between the two cell lines are unlikely to account for the selective expansion of CTL specificities. When infected, both cell types produce similar levels of infectious LCMV, have similar levels of the NP and GP proteins from which np396 and gp33 are derived, and can be recognized by CTL specific for each of the three epitopes. These data indicate that in the generation of peptides for MHC-I binding and presentation to CTL, MC57 and JawsII process the same set of virus proteins in quantitatively different ways. *The Journal of Immunology*, 1998, 160: 2139–2144.

Presentation of peptide Ags to CD8⁺ CTL occurs in the context of MHC class I Ags (MHC-I).³ Shortly after their synthesis, MHC-I molecules are loaded with peptide in the ER. Most of these peptides are derived from cytosolic proteins degraded by the multicatalytic proteasome complex and transported into the ER by the TAP proteins (1, 2). While exogenous proteins can also enter the MHC-I presentation pathway under the correct conditions (3) this system is largely specialized for the presentation of intracellular Ags, thus allowing the immune system to screen cells for viral and tumor Ags (4). While the general mechanisms of MHC-I Ag processing and presentation have been extensively studied, we are not aware of detailed studies comparing the generation and presentation of MHC-I-restricted Ags by different kinds of cells.

Efficient induction of CD4⁺ T cell immune responses requires that the T cells must interact with specialized APC that express MHC class II and costimulatory molecules (5). Dendritic cells, macrophages and activated B cells are the principle APC in this context. Although nearly all nucleated cells express MHC-I, naive CTL also require presentation of Ag by bone marrow-derived APC for efficient priming (6–8). Dendritic cells are highly potent inducers of CTL responses (9–11) and are thought to be the principle

APC involved in priming CTL. Macrophages are also capable of inducing CTL responses (10, 12). Once primed, CTL can recognize their cognate Ags on a wide variety of cells and respond by lysing the target cell and/or secreting cytokines.

Lymphocytic choriomeningitis virus (LCMV) is a mouse virus that is the prototypic member of the Arenavirus family. The structural proteins of LCMV (NP, GP1, and GP2) are all encoded on the same ambisense RNA segment (13) with GP1 and GP2 being derived from a single precursor protein, GPC (14). The Armstrong strain of LCMV establishes a noncytolytic infection in a wide variety of cell types in vitro. Intravenous or i.p. inoculation of mice evokes a strong CTL response that clears the infection within 2 wk. In H-2^b haplotype mice there are three principle targets for the anti-LCMV CTL response: gp33–41 (gp33), derived from GP1, gp276–286 (gp276) from GP2, and, np396–405 (np396) from NP (15). All three epitopes are presented by the same MHC-I molecule, D^b.

Here we show that LCMV-infected JawsII dendritic cells and MC57 fibrosarcoma cells preferentially present different LCMV epitopes to a polyclonal pool of anti-LCMV CTL. Our data suggest that different cell types are able to process the same set of intracellular proteins in quantitatively different ways in the generation of MHC-I-restricted epitopes.

Materials and Methods

Mice

C57BL/6 mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and used at 8 to 12 wk of age. LMP2^{-/-} mice were the kind gift of Dr. Luc Van Kaer (Vanderbilt University, Nashville, TN) (16).

Virus

The Armstrong 53b strain of LCMV, originally obtained from Dr. Peter J. Southern (University of Minnesota, Minneapolis, MN) was grown on BHK-21 cells (American Type Culture Collection, Bethesda, MD) (ATCC CCL-10) and titered on Vero C1008 cells (ATCC CRL-1586) as described (17). Mice were infected by i.v. injection of 10⁵ plaque-forming units (pfu) of virus.

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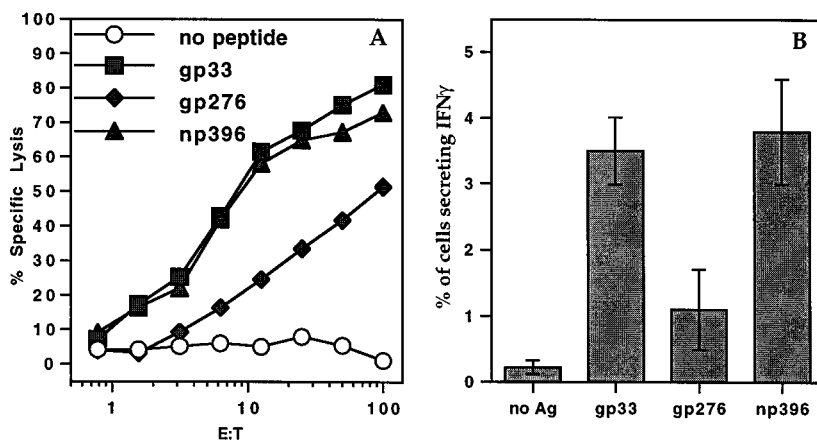
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² Address correspondence and reprint requests to Dr. Michael J. Bevan, HHMI, Department of Immunology, Box 357370, University of Washington, Seattle, WA 98195.

³ Abbreviations used in this paper: MHC-I, major histocompatibility class I antigens; LCMV, lymphocytic choriomeningitis virus; gp33, LCMV glycoprotein 33–41; gp276, LCMV glycoprotein 276–286; np396, LCMV nucleoprotein 396–404; pfu, plaque-forming unit; HRP, horseradish peroxidase; ER, endoplasmic reticulum.

FIGURE 1. The ex vivo CTL response of C57BL/6 mice to LCMV. *A*, Splenocytes from three female C57BL/6 mice infected 8 days earlier with LCMV were used as effectors in an 8-h ^{51}Cr -release assay. The targets were EL4 cells incubated for 1 h before use in PBS alone (circles) or with 1 μM gp33 (squares), gp276 (diamonds), or np396 (triangles). *B*, Splenocytes from groups of C57BL/6 mice on day 8 of LCMV infection were used in an ELISPOT assay to determine the frequency of cells secreting IFN- γ in response to specific LCMV-derived peptides. Effector cells were incubated with EL4 cells alone (no Ag) or with EL4 cells coated with the indicated peptide. These data are representative of five to eight experiments, and error bars indicate the SD of triplicate samples.



Cell lines

The fibrosarcoma cell line MC57 (18) was grown in RPMI 1640 supplemented with 10% heat-inactivated FCS, 2 mM L-glutamine and antibiotics (RP10). JawsII, an immortalized C57BL/6 bone marrow-derived dendritic cell line obtained from Dr. V. L. MacKay (ZymoGenetics, Seattle, WA) (9), was grown in RP10 with 5 ng/ml granulocyte-macrophage-CSF (R&D Systems, Minneapolis, MN). EL4 thymoma cells (ATCC TIB-41) and RMA-S cells were maintained in RP10.

CTL lines were derived from spleen cells of C57BL/6 mice infected 4 to 6 wk earlier with 10^5 pfu of LCMV i.v. Initial restimulation was by addition of 2.5×10^4 MC57 or JawsII cells (infected 3 days earlier at a multiplicity of infection of approximately 1, or peptide coated as indicated) to 5×10^5 LCMV-immune spleen cells in U-bottom 96-well plates. Thereafter, lines were stimulated weekly by the addition of infected or peptide-coated MC57 or JawsII cells and 5×10^5 irradiated (2000 R) C57BL/6 spleen cells. After the second restimulation 5% rat Con A supernatant was added as a source of exogenous IL-2 in the presence of 50 mM methyl- α -D-mannopyranoside (Sigma, St. Louis, MO) to neutralize excess Con A. In some experiments the MC57 and JawsII cells were treated for 30 min with 50 mM mitomycin C (Sigma) to prevent their proliferation in the CTL cultures; in other experiments the cells were irradiated (20,000 R). Results were identical with either treatment.

CTL clones 1A6 (anti-gp33), 1B7 (anti-gp276), and B5A9 (anti-np396) were derived from LCMV-immune C57BL/6 spleen cells by weekly passage on peptide-coated, irradiated C57BL/6 spleen cells and cloned by limiting dilution.

Abs and flow cytometry

For anti-D^b staining 28-14-8S ascites (ATCC HB 27) was used, followed by a polyclonal anti-mouse IgG-FITC (PharMingen, San Diego, CA). Samples stained for flow cytometric analysis also included the anti-Fc γ RII Ab 24G2 at 10 $\mu\text{g}/\text{ml}$ (PharMingen) to reduce nonspecific FcR-mediated binding. Analysis was done on a Becton Dickinson FACScan with Lysis-II software (Becton Dickinson, Mountain View, CA).

The anti-IFN- γ Ab R4-6A2 (ATCC HB-170) was protein G purified from tissue culture supernatants. Biotinylated XMG-1.2, which recognizes a different epitope of murine IFN- γ , was purchased from PharMingen.

Peptides

The D^b-binding LCMV peptides, LCMV gp33–41 (KAVYNFATC), gp276–286 (SGVENPGGYCL), and np396–404 (FQPQNGQFI), were synthesized using an Applied Biosystems Synergy (Foster City, CA) peptide synthesizer. Peptide concentrations were determined using the BCA assay (Pierce Chemical Co., Rockford, IL).

Cytotoxicity assays

Target cells were prepared by incubation for 1 to 2 h with or without peptide in the presence of sodium ^{51}Cr chromate, washed three times in PBS, and resuspended in RP10. For the assay, 10^4 target cells were added to 96-well U-bottom plates along with different numbers of effector cells in a total volume of 200 μl . After 4 h (8 h for assay of primary effector cells directly ex vivo), 100 μl of supernatant was removed and assayed for ^{51}Cr content in a Wallac 1470 Wizard gamma counter (Wallac Oy, Turku, Finland). Specific lysis was calculated as: (experimental release – spontane-

ous release)/(maximum release – spontaneous release). Spontaneous release was determined for target cells in medium alone and maximum release was determined by incubating target cells in 1% Triton X-100. For the 4-h assay spontaneous release was typically less than 10% and never more than 15%; for the 8-h assays it was typically 15 to 25%.

IFN- γ ELISPOT assays

Cells secreting IFN- γ in an Ag-specific manner were detected using a standard ELISPOT assay (19). In brief, EL4 target cells were incubated in PBS with or without 1 μM peptide, as indicated, washed several times, and added at 10^5 per well to graded numbers of effector cells in 96-well Multiscreen-HA plates (Millipore, Bedford MA) precoated with protein G-purified R4-6A2. After 20 to 24 h culture, cells were removed and the plates were extensively washed and then developed by incubation with XMG-1.2-biotin, followed by streptavidin-HRP (Sigma), and diaminobenzidine (Sigma).

RMA-S D^b stabilization assay

The ability of the three LCMV peptides to stabilize D^b on the surface of RMA-S cells was determined essentially as described (20). This cell line has a defect in the TAP peptide transporter that results in the surface expression of relatively large numbers of unstable MHC-I (21). These empty MHC-I can be stabilized by the addition of exogenous MHC-I-binding peptides. We used a spontaneous mutant of RMA-S that fails to express the K^b molecule on its surface (RMA-S K^b-), which we obtained from Dr. Robert Rich (Baylor University, Houston, TX). Briefly, RMA-S K^b- cells were incubated overnight at 31°C, then with the indicated concentrations of gp33, gp276, or np396 peptide for 1 h at 31°C, and shifted to 37°C for 3 h. Cells were stained on ice in buffer containing 0.1% sodium azide using the anti-D^b Ab B22.249 (Cedarlane, Westbury, NY) followed by goat anti-mouse IgG-FITC (Sigma). After analysis on a Becton Dickinson FACScan flow cytometer the mean channel of fluorescence was plotted against the concentration of peptide for each point.

Western blots

JawsII and MC57 cells were cultured with or without LCMV infection (at a multiplicity of infection of about 1) for 3 days. Cells were washed twice in TNE (10 mM Tris-HCl (pH 7.5)/1 mM EDTA/100 mM NaCl) and then lysed in TNE/2% Nonidet P-40. Standard reducing SDS-PAGE gels were run loading 5×10^5 cell equivalents in each lane and then electrophoretically blotted onto nitrocellulose. Blots were stained using a guinea pig anti-LCMV serum (a gift from Dr. Rafi Ahmed, Emory University, Atlanta, GA) followed by donkey anti-guinea pig-HRP (Jackson ImmunoResearch Laboratories, West Grove, PA) and visualized using an enhanced chemiluminescence kit (Amersham, Arlington Heights, IL) and BioMax-MR film (Kodak, Rochester, NY).

Results

The ex vivo CTL response to LCMV

During the C57BL/6 immune response to LCMV the strongest CTL responses are restricted by D^b and directed against the gp33 and np396 epitopes, with a somewhat weaker response against gp276 (Fig. 1A). This pattern of specificity for the three epitopes is

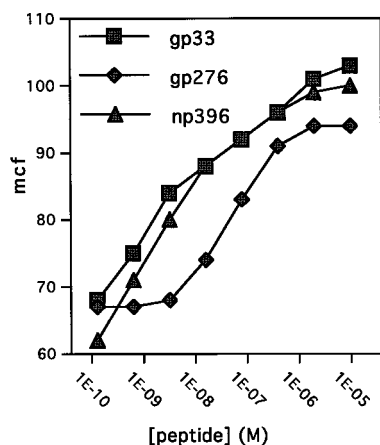


FIGURE 2. Stabilization of D^b on the surface of RMA-S cells by LCMV-derived epitopes. Flow cytometric analysis of RMA-S surface stabilization was performed as described in *Materials and Methods*. The resulting plot of mean channel of fluorescence (mcf) indicates the relative affinities of the three peptides for D^b. In the absence of peptide the mcf was 60.

highly reproducible in this infection. The degree of lysis against each epitope is a reflection of the number of CTL specific for each epitope, which we enumerated using an IFN- γ ELISPOT assay (Fig. 1*B*). This pattern of response is consistent with earlier reports that the hierarchy of CTL responses corresponds to the relative affinities of the epitopes for MHC-I (22–24). In this case, gp33 and np396 have approximately equal abilities to stabilize D^b on the surface of RMA-S cells, while gp276 is about 10-fold less efficacious in this assay (Fig. 2).

Differential maintenance of CTL responses by different cell types

In the process of deriving epitope-specific CTL lines we were surprised to find that, after several rounds of stimulation on LCMV-infected MC57 fibroblasts, an LCMV-immune CTL line lost specificity for gp33 and np396 and became monospecific for gp276. To examine this in more detail LCMV-immune spleen cells were restimulated every 7 days with syngeneic spleen cells plus either LCMV-infected MC57 cells or LCMV-infected JawsII dendritic cells. The ability of these CTL lines to lyse peptide-coated targets was assayed at the time of each restimulation (Fig. 3). When stimulated with infected MC57 cells, the CTL line targeted gp276 efficiently, but progressively lost its ability to lyse first np396-coated-EL4 targets and, later, gp33-coated targets (Fig. 3, A, C, and E). When stimulated with LCMV-infected JawsII cells, the CTL line retained activity against gp33 and np396, but the response to gp276 was lost (Fig. 3, B, D, and F). The same syngeneic spleen cells were added to both restimulation cultures and the same results were obtained whether the stimulator cells were treated with gamma irradiation or with mitomycin C before their addition to the T cell restimulation cultures (data not shown).

If some intrinsic aspect of MC57 were to favor the stimulation of gp276-specific CTL or to prevent the stimulation of gp33- or np396-specific CTL, then MC57 coated with a pool of the three peptides should preferentially select gp276-specific CTL. Similarly, if JawsII were somehow unable to support the expansion of gp276-specific CTL, then that specificity should still be lost when peptide-coated JawsII are used to restimulate LCMV-immune CTL. To assess this, uninfected MC57 and JawsII cells were coated exogenously with a pool of the three peptides (1 μ M each), washed thoroughly, and then used to restimulate LCMV-immune

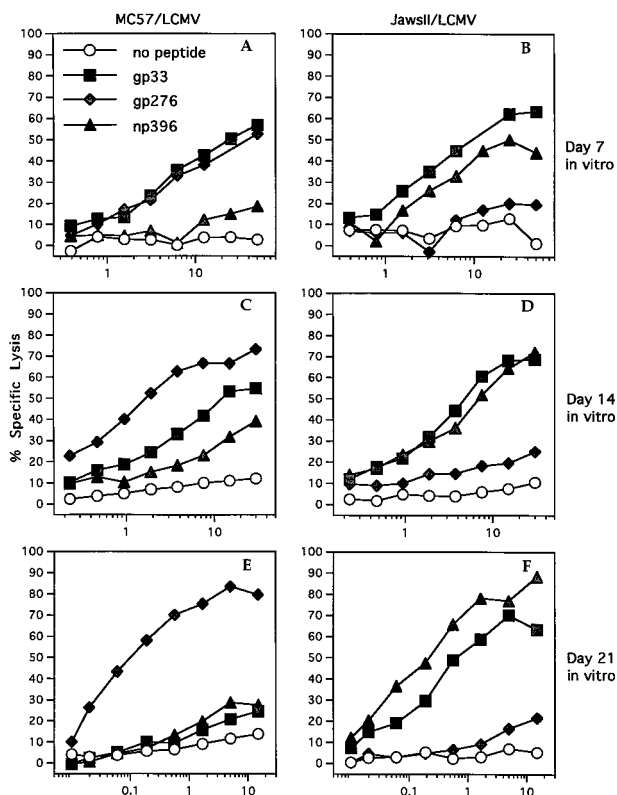


FIGURE 3. LCMV-infected MC57 fibroblasts and JawsII cells select CTL of different specificities from the wild-type C57BL/6 anti-LCMV CTL repertoire. Three weeks after primary LCMV infection with 10^5 pfu LCMV i.v., splenocytes from a C57BL/6 female were divided into two groups. One group (A, C, E) was restimulated with weekly intervals with MC57 cells infected 3 to 4 days previously with LCMV, the other group (B, D, F) was restimulated with JawsII cells infected 3 to 4 days previously with LCMV. Both groups received C57BL/6 spleen cells as feeder cells for each restimulation and, after the second week, the cultures were supplemented with IL-2. At the end of the first (A, B), second (C, D) and third (E, F) weeks of restimulation the epitope specificity of each line was tested using peptide-coated EL4 cells as targets in a standard 4-h CTL assay. The experiment was repeated three times with similar results.

spleen cells at weekly intervals. At the end of the third round of restimulation, both lines were equally lytic for gp33- and np396-coated EL4 cells and only slightly less so for gp276-coated targets (Fig. 4). Therefore, the MC57 cells will support CTL responses to gp33 and np396 when these peptides are added exogenously, and JawsII will likewise support responses to gp276 when it is added exogenously.

Both cell types synthesize viral proteins

To confirm that the two cell lines were infected and producing virus and the full complement of virus proteins, the viral titer in the supernatants of the day 3 LCMV-infected MC57 and JawsII cells was examined by plaque assay on Vero cells. Titers were found to be similar, $10^{6.5}$ for the MC57 and $10^{6.0}$ for the JawsII. This suggests that both cell lines produced the LCMV structural proteins from which the three epitopes are derived in similarly large amounts. To directly address the levels of virus proteins present in LCMV-infected MC57 and JawsII cells, we made extracts of uninfected and d3 LCMV-infected MC57 and JawsII cells and examined them by Western blotting. The infected MC57 and JawsII cells both contained high levels of NP and had similar levels of GP2 (Fig. 5). MC57 cells seemed to have somewhat higher steady

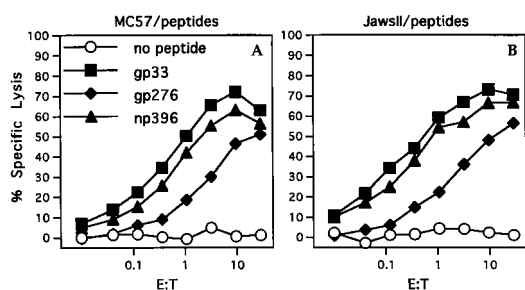


FIGURE 4. Peptide-coated, uninfected MC57 and JawsII cells select a similar repertoire of CTL. Three weeks after primary LCMV infection with 10^5 pfu LCMV i.v. spleen cells from a C57BL/6 female were restimulated weekly with either peptide-coated MC57 cells (A) or peptide coated JawsII cells (B) as described in *Materials and Methods*. After three rounds of restimulation the epitope specificity of each line was tested using peptide-coated EL4 cells as targets in a standard 4-h CTL assay. The experiment was repeated with similar results.

state levels of GPC, the precursor to both GP1 and GP2. Since the guinea pig antiserum reacts with conformation-dependent sites on GP1 and does not stain GP1 in Western blots (25), the relative levels of GP1 could not be assessed directly.

Both cell types present all three epitopes

To determine whether the epitopes derived from these proteins were present on the surfaces of the infected MC57 and JawsII cells we used them as targets for CTL clones specific for each of the epitopes. Both cell types were lysed by all three CTL clones (Table I). Therefore, both the infected MC57 and the infected JawsII cells have all three D^b -restricted epitopes on their surfaces. Each of the CTL clones is sensitive to low levels of peptide (half-maximal lysis in the presence of 10 to 100 pM free peptides) (data not shown) although free peptide concentrations cannot be equated to number of epitopes per cell.

LMP2 is not necessary for generation of the LCMV epitopes

The multicomponent, multicatalytic proteasome is known to be a major site of Ag processing and might differ among cells of different types. Its composition can also change when APC become activated, including the use of the LMP2 and other subunits (2). We obtained LMP2^{-/-} mice to test the hypothesis that their response to LCMV might differ from the response of C57BL/6 mice. Splenocytes were prepared from three female day 8 LCMV-infected LMP2^{-/-} mice (on a C57BL/6 background) and used as effectors in an 8-h ⁵¹Cr-release assay using peptide-pulsed EL4 target cells. The CTL response of these cells to the three epitopes (data not shown) was indistinguishable from that of C57BL/6 splenocytes (Fig. 1A); we conclude that LMP2 does not play a unique roll in the generation of these epitopes.

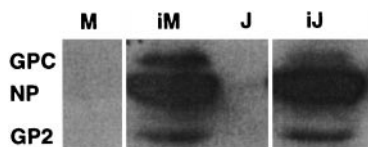


FIGURE 5. Western blot analysis shows that LCMV-infected MC57 and JawsII cells contain similar levels of NP and GP2. Reactions of guinea pig anti-LCMV serum to 5×10^5 cell equivalents of uninfected MC57 (lane 1), day 3 LCMV-infected MC57 (lane 2), uninfected Jaws II (lane 3) and day 3 LCMV-infected JawsII (lane 4). The positions of GPC, NP, and GP2 are indicated (guinea pig serum does not react with GP1 in Western blots).

Table I. Epitope-specific CTL clones recognize their epitopes on both iMC57 and iJaws cells^a

Clone	Specificity	% Specific Lysis at E:T of 20:1			
		MC57	iMC57	JawsII	iJawsII
1AF6	gp33	2	45	-5	36
1B7	gp276	-6	38	-1	31
B5A9	np396	0	55	3	49

^a CTL clones specific for each of the D^b -restricted LCMV epitopes were incubated with MC57 and JawsII targets cells infected with LCMV 3 days previously (prefix "i") or with uninfected target cells in standard 4-hr ⁵¹Cr release assays. The results are representative of two to five experiments.

Discussion

Although bone marrow-derived APC are required to efficiently prime CTL responses (6–8), activated CTL are readily able to recognize and respond to Ag presented by a wide variety of cells. It has been shown that different epitopes can be generated within the same cell by different proteolytic processes (26) and with different efficiencies (27, 28) but it is not clear that all cells process proteins into peptides for presentation in MHC-I in the same way. Given that proteolytic activities vary greatly among various kinds of cells there is the potential for Ag to be processed in different ways. Here we have presented evidence that infected fibroblasts and dendritic cells can process the same set of LCMV proteins into MHC-I epitopes in quantitatively different ways that affect CTL responses.

LCMV infection of C57BL/6 mice results in strong CTL responses that focus on three epitopes: gp33, np396 and, to a somewhat lesser extent, gp276 (Fig. 1) (15). The cytolytic activity targeting each epitope is proportional to the frequency of cells that secrete IFN- γ in response to that epitope (Fig. 1) and correlates with the ability of each peptide to stabilize D^b , the restricting MHC-I for each of the peptides (Fig. 2). This is in agreement with earlier reports that the epitopes for CTL are selected during the immune response based on their affinity for MHC-I (22–24). However, when we restimulated LCMV-immune C57BL/6 splenocytes with either LCMV-infected MC57 fibrosarcoma or JawsII dendritic cells, the resulting CTL lines developed very different specificities (Fig. 3). The line restimulated with infected MC57 cells rapidly lost specificity for np396 (Fig. 3A) and gradually lost its ability to target gp33 (Fig. 3, A, C, and E). Remarkably, using the infected fibroblasts as stimulators, the initially weakest response to gp276 became the only surviving specificity. The line restimulated with JawsII retained its ability to lyse gp33- and np396-coated targets, but lost some of its ability to target gp276 (compare Fig. 1 with Fig. 3, B, D, and F).

When uninfected MC57 and JawsII cells were coated with an equimolar mixture of the three peptides, extensively washed, and used to restimulate LCMV-immune splenocytes, the resulting CTL lines were indistinguishable (Fig. 4) and recognized the epitopes in a manner that would be predicted based on their relative affinity for D^b . While we cannot exclude the possibility that costimulation is Ag dose dependent, since both MC57 and JawsII can restimulate CTL specific for each of the epitopes when the peptides are exogenously loaded, the differences in their abilities to restimulate LCMV-immune CTL do not appear to be due to differential expression of costimulator or adhesion molecules. It suggests that LCMV-infected MC57 fail to restimulate gp33- and np396-specific CTL because they do not generate sufficient surface D^b /peptide to do so. Conversely, JawsII must generate relatively little D^b /gp276 for presentation to CTL.

Since the titer of LCMV in the supernatant of the MC57 and JawsII cultures were similar, both of the infected cell lines must have produced large amounts of the three viral structural proteins from which the epitopes were derived. Also, a Western blot of infected MC57 and JawsII cells showed that both cell types contained similar amounts of the NP and GP2 proteins (Fig. 5).

Even though MC57 could not effectively restimulate gp33- and np396-specific CTL, these epitopes are present on the surface of the infected cells since CTL clones specific for gp33 and np396 do lyse infected MC57 cells (Table I). Similarly, gp276-specific CTL do lyse infected JawsII cells, even though gp276-specific CTL are lost in the cultures restimulated with infected JawsII. If each of these D^b/peptide complexes are present on both cell types, then why do infected MC57 not restimulate CTL specific for gp33 and np396 in culture, and why do infected JawsII not support CTL specific for gp276? The number of MHC-I/peptide complexes needed to target a cell for lysis by CTL is very small, perhaps fewer than 10 per cell (29, 30). In contrast, the amount of Ag required to stimulate proliferation is much greater than the amount required to elicit cytotoxicity and cytokine release (31). This implies that infected MC57 have sufficient gp33 and np396 to be lysed by CTL specific for those epitopes, but not enough to effectively stimulate CTL proliferation in the restimulation cultures. Likewise, JawsII must have enough gp276 to sensitize them for lysis but not enough to stimulate much CTL proliferation. We have attempted to quantitate numbers of epitopes per cell using acid extracts of infected cells but found that we could not recover gp33 from any of the extracts, and variable efficiencies of recovery for gp276 and np396 made direct comparisons problematic.

As noted previously, peptides tend to be selected as epitopes largely on their relative affinities for MHC-I (22–24). Peptides that are more weakly bound by MHC-I can dominate an immune response when peptides that the MHC-I would bind more strongly are either inefficiently generated (32) or are generated but are homologous to self peptides and a hole exists in the responding CTL repertoire (33). Because the *in vivo* immune response to all three epitopes is robust (Fig. 1) and the same pools of splenocytes were used for restimulation by each cell line there is no hole in the CTL repertoire considered here. Therefore, infected MC57 must inefficiently generate gp33 and np396 for loading into D^b. Where it has been shown that different epitopes from the same protein are processed with different efficiencies, this effect has been attributed to the relative ability of different proteases to generate the appropriate fragments (27, 28). Since GP1 and GP2 are derived from a common precursor, the MC57 must generate gp276 more efficiently than it does gp33. And, as infected MC57 and JawsII have similar levels of NP (Fig. 5), it would appear that MC57 are very inefficient at generating the np396 peptide as well. Indeed, since the gp33 and np396 epitopes have a greater affinity for D^b than the gp276 epitope does (Fig. 2), the MC57 cells must generate considerably more gp276 than gp33 and np396 for the gp276 to out-compete the higher affinity peptides for D^b binding. The MC57 cells may be deficient in the (presumably cytoplasmic) proteolytic activity necessary to generate the gp33 and np396 epitopes or may contain a cytoplasmic or ER protease that destroys the gp33 and np396 epitopes, or one that preferentially favors the generation of the gp276 epitope. More than one of these conditions could also be operative.

In contrast to MC57, infected JawsII must selectively load D^b with gp33 and np396 and does not load sufficient gp276 to be able to restimulate gp276-specific CTL very well. It is likely that JawsII cells generate high levels of the gp33 and np396 peptides that exclude gp276 from D^b by virtue of their higher affinity for the MHC-I molecule.

One potential source of different proteolytic activities within professional APC is the multicomponent proteasome, the inducible LMP-2 subunit of which has been shown to be able to alter anti-viral CTL responses (16). When we infected LMP-2^{-/-} mice with LCMV to see if their response to gp33, gp276, or np396 would differ from that of wild-type mice, we found that they did not (data not shown), excluding an obligate role for LMP-2 in the generation of any of these peptides. The potential role of other proteasome subunits remains to be examined.

One effect of the presentation of different epitopes by different APC could be to ensure the priming of CTL specific for minor epitopes which, while not normally significant elements of immune response, can be important in clearing chronic infections (34). When mice are acutely infected with mouse hepatitis virus, central nervous system infection induces nucleoprotein-specific CTL not seen following peripheral infection (35, 36). These nucleoprotein-specific CTL also play a unique and important role in limiting central nervous system infection (37). To the extent that our results are representative of the function of fibroblasts and dendritic cells *in vivo*, aggregate CTL activity in an immune response may reflect the sum of priming and expansion by multiple APC types, and it may be that the priming and expansion of CTL by specialized APC does not stimulate a repertoire of CTL that are optimal for recognizing all infected cells in the periphery of an infected animal.

Acknowledgments

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