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Generation of an Immunodominant CTL Epitope Is Affected by Proteasome Subunit Composition and Stability of the Antigenic Protein

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Generation of the HLA-A0201 (A2) influenza Matrix 58–66 epitope contained within the full-length Matrix protein is impaired in cells lacking the proteasome subunits low molecular protein 2 (LMP2) and LMP7. This Ag presentation block can be relieved by transfecting the wild-type LMP7 cDNA into LMP7-deficient cells. A mutated form of LMP7, lacking the two threonines at the catalytic active site, was equally capable of relieving the block in presentation of the influenza Matrix A2 epitope. These observations were extended by analyzing whether modification of the influenza Matrix protein could overcome the block in presentation of the A2 Matrix epitope. Expression of either a rapidly degraded form of the full-length Matrix protein or shorter Matrix fragments led to an efficient presentation of the A2 influenza Matrix epitope by LMP7-negative cells. These findings demonstrate two main points: 1) LMP7 incorporation into the proteasome is of greater importance for the generation of the influenza A2 Matrix epitope than the presence of the LMP7’s catalytic site; and 2) the interplay between cytosolic proteases and stability of target proteins is of importance in optimization of Ag presentation. These observations may have relevance to the immunodominance of tumor and viral epitopes and raise the possibility that generation of shorter protein fragments could be a mechanism to ensure optimal Ag presentation by cells expressing low levels of LMP7. The Journal of Immunology, 1999, 163: 6045–6052.

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D8+ CTL recognize intracellular proteins in the form of 8– to 10-aa peptides presented by MHC class I molecules (reviewed in Ref. 1). Endogenously synthesized Ags are degraded into smaller fragments and transported from the cytosol into the lumen of the endoplasmic reticulum by the MHC-encoded TAP complex. Translocated peptides bind to newly synthesized MHC class I molecules and are then transported to the cell surface for recognition by CD8+ CTL.

The identities of the proteases involved in the processing of newly synthesized target proteins are still unclear. Accumulating evidence suggests that a large cytosolic protease, called proteasome, plays an important role in the generation of CTL epitopes. Several reports demonstrate that specific inhibition of the proteasome by either peptidyl aldehydes (2, 3) or lactacystin (4, 5) results in defective Ag presentation of defined CTL epitopes. Furthermore, it was shown that the proteasome is capable of generating optimal length peptides for binding to MHC class I molecules (6–8). Recent studies suggest that the proteasomal processing pathway is not the only proteolytic activity involved in the generation of antigenic peptides for presentation via the MHC class I pathway (9–15).

The catalytic core of the proteasome (20S) is a symmetrical structure made of four stacked heptameric rings. The outer two rings contain structural (α) subunits, while the inner two rings contain catalytic (β) subunits, some of which contain an active amino-terminal threonine. A stepwise assembly process of the 20S structure ensures that the catalytic threonines are not exposed before the whole structure is completed. The β subunits are synthesized initially with an amino-terminal prosequence that is cleaved off by an autocatalytic process during the assembly (16, 17). Mature proteasomes have a broad proteolytic activity, degrading bonds on the carboxyl side of basic, hydrophobic, and acidic residues (18). Two proteasome subunits, called low molecular protein 2 (LMP2) and LMP7, are encoded in the MHC, closely linked to the peptide transporter (TAP) genes (19–21). The close association of LMP2 and LMP7 with TAP1 and TAP2 genes and the finding that all four genes might be playing a role in the Ag presentation pathway. Binding of LMP2 and LMP7 to the proteasome displaces two constitutively expressed proteasome subunits, called MB1 and δ (22, 23). It was shown that displacement of these constitutive proteasome subunits by LMP2 and LMP7 alters the cleavage specificity of purified proteasome (24–30), suggesting that their binding to the proteasome could alter the pool of antigenic peptides presented by MHC class I molecules.

A role for LMP2 and LMP7 in Ag presentation was initially called into question (31–34). Additional experiments conducted both with LMP knockout mice (35, 36) and with LMP-deficient

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4 Abbreviations used in this paper: LMP, low molecular protein; NP, nucleoprotein.
ROLE OF LMP7 IN THE SELECTION OF A VIRAL CTL EPIOTPE

human (37) and mouse cells (38) demonstrated that cells lacking LMP2 and/or LMP7 have a defined defect in Ag presentation. This defect is selective for certain epitopes, while other epitopes are efficiently generated. The mechanisms by which LMP products control the generation of certain CTL epitopes is not known.

We here analyzed the processing and presentation of the A2 influenza Matrix epitope in LMP-deficient cells. We previously demonstrated that its presentation was impaired by deletion of the proteasome subunits LMP2 and LMP7. We have now extended these observations and demonstrated that this Ag presentation block can be overcome by either transfecting LMP-deficient cells with a mutated form of LMP7 or by increasing the rate of degradation of the Matrix protein.

Materials and Methods

Cell lines

LCL721.45 (45) and LCL721.174 (174) cells were described previously (39, 40). Cer-43 CTL clone, which recognizes the A2-restricted influenza Matrix epitope GILGFVFTL (corresponding to residues 58–66), was provided by A. Lanzavecchia (Basel Institute for Immunology, Basel, Switzerland). All other CTL clones were generated as previously described (41). 174/TAPs are .174 cells transfected with plasmids containing the TAP1 and TAP2 genes as described previously (37). 174/TAPs/LMP7 (174/TAPs cells transfected with a plasmid containing the LMP7b gene) were described previously (15). 174/TAPs/LMP7 T1A/T2A were .174/TAPs transfected with plasmid prep4-LMP7 T1A/T2A.

Mutagenesis of LMP7

Oligonucleotides GCGGATCCTTATTGATTGGCTTCCCGGTA were used in a primary PCR to amplify the 5'-end of LMP7 with the mutation 171A/2A. The amplified fragment and oligonucleotide GCGGATCCTTATTGATTGGCTTCCCGGTA were used as primers for the secondary PCR, in which the whole of the LMP7 coding sequence was amplified and cloned between the HindIII and BamHI restriction sites of pREP4. The resulting plasmid, pREP4-LMP7 T1A/T2A, was transfected into .174/TAPs, and hygromycin B selection was applied.

Recombinant vaccinia virus

Recombinant viruses were made by homologous recombination into the thymidine kinase gene using the vector pSC1130R.2 as previously described (37). The coding sequence for each fragment was amplified by PCR and from influenza Matrix cDNA cloned into pSC11-mod (42) (provided by J. Yewdell, Bethesda, MD) by BglI and SalI sites (in the case of 28, 36, 49, and 74 fragments) or pSC11.2R30 (37) between the NcoI and BamHI sites (in the case of 74, 105, 164 fragments and Matrix mutants E2, and E6). Primers are named according to the direction from which they begin with a mutated form of LMP7 or by increasing the rate of degradation of the Matrix protein.

Oligonucleotides

The following oligonucleotides were used: 5'-N-Term, 5'-aag cgt cga cca tta gtc ttc ttg aag agg tgg aa cag aac ac-3'; 74-3', 5'-ggg gta ccc tat tgc cta tga gac cga tgc tg-3'; 3'-Matrix, 5'-ggg gta cct tat tgc cta tga gac cga cgc tgc tg-3'; 5'-N-Term and 3'-Matrix as the 3'-primer. The E6 construct was made in a similar way. 3'-E6 was used in place of 3'-E2, and the template was the E2 mutant of Matrix instead of the wild-type Matrix.

Chromium release assay

Target cells were infected with recombinant vaccinia at a multiplicity of infection of five per cell for 90–120 min at 37°C. After washing, cells were incubated overnight in R10 (RPMI 1640 with 10% FCS, 2 mM glutamine, 100 μg/ml streptomycin, and 100 U/ml penicillin) at 37°C (puromycin was used at a concentration of 2 μM; it was added for 30 min and then washed before the overnight incubation). Cells were then counted, labeled with 51Cr for 120 min, and used as targets. Supernatants were collected after 4 or 7 h.

Lactacystin treatment of target cells

Cells were infected with recombinant vaccinia virus at a multiplicity of infection of 10 for 90 min at 37°C, then incubated for 2 h in R10 following a wash in the same medium. Cells were then resuspended at 2 × 10^6/ml in methionine- and cystine-free R10 for 1 h at 37°C. Promix (143 μl; 70% [35S]methionine and 30% [35S]cysteine, Amersham, Arlington Heights, IL) was then added, and the mixture was incubated for 30 min at 37°C. Labeled cells were chased with R10 and excess nonlabeled methionine and cystine. At the indicated time points after the chase cells were washed in ice-cold PBS and resuspended in 0.5 ml of lysis buffer (150 mM NaCl, 50 mM Tris-HCl (pH 7.5), 5 mM EDTA, 0.5% Nonidet P-40, 0.5% Mega 9, 2 mM PMSF, and 5 mM iodoacetamide), followed by centrifugation to pellet nuclei. Lysates were precleared with Pansorbin overnight at 4°C and immunoprecipitated using anti-Matrix Abs M2 2B3 (J. Yewdell, Bethesda, MD) and Protein-A coated Sepharose beads. Following extensive washing, proteins were eluted from the beads using standard SDS-PAGE loading sample buffer and heated at 95°C for 5 min. Ten percent of the sample was removed for SDS-PAGE. The remainder of the samples was cooled and diluted to a 0.1% SDS concentration. MCP21 Abs were precleared with Pansorbin cell extracts, and a second immunoprecipitation was conducted using anti-LMP7 antiserum (17) and protein G-Sepharose beads.

Western blotting

Lysed cells were separated by SDS-PAGE. The gel was blotted onto a Hybond-C membrane and placed overnight in blocking solution (5% powdered milk and 0.2% Tween in PBS) on a rocking platform. Anti-LMP7 antiserum (17) was diluted 1/1000 in blocking solution and incubated with the membrane for 1 h. The membrane was then washed extensively in 0.2% Tween in PBS containing 5% milk proteins. A second 1-h incubation of the membrane in 1/2000 dilution of HRP-conjugated goat anti-rabbit Ab (P448 Dako) in blocking solution followed. The membrane was then washed as described above and treated with ECL reagents (Amersham) as described by the manufacturer. The gel was then exposed to x-ray film for autoradiography.

Results

Role of IFN-γ-inducible proteasome subunits LMP7 in the presentation of the influenza Matrix A2 epitope

The cell line .174 bears a deletion in the MHC that includes both TAP1 and TAP2 genes and the LMP2 and LMP7 genes. We have previously shown that presentation of the influenza Matrix A2-restricted epitope is defective in .174 cells (39). This Ag presentation block was not relieved by transfecting the TAP1 and TAP2 genes (174/TAPs) (37), leading to the hypothesis that its lack of presentation was due to the lack of expression of either LMP2 or LMP7. Consistent with this hypothesis we observed a small, but significant, effect of vaccinia-encoded LMP7 molecules on the presentation of the Matrix A2 epitope (37). We have now extended...
these results by transfecting LMP7 cDNA into .174/TAP cells (Fig. 1D). Transfected LMP7 incorporates into the proteasome, and its amino-terminal pro sequence is removed, exposing the amino-terminal threonines (Fig. 2B). Incorporation of LMP7 into the .174/TAPs proteasome restores presentation of the Matrix A2 epitope (Fig. 1, A–C).

The effect of LMP7 in the generation of the Matrix A2 epitope could result from a direct role of LMP7 in the processing of the Matrix epitope. Alternatively, binding of LMP7 to the proteasome, rather than its cleavage specificity, may allow generation of the Matrix A2 epitope. To distinguish between these two possibilities we transfected .174/TAP cells with a mutated form of LMP7, in which the two amino-terminal threonines were substituted with alanines (LMP7 T1A/T2A). Mutated LMP7 was capable of binding to the proteasome, but, unlike wild-type LMP7, had a lower electrophoretic mobility due to incomplete processing of the amino-terminal prosequence (Fig. 2B). This finding was consistent with a defective LMP7 catalytic site, resulting from the mutation of the amino-terminal threonines. Surprisingly, we demonstrated that .174/TAPs cells transfected with LMP7 T1A/T2A were capable of efficiently presenting the Matrix epitope 58–66 (Fig. 2A). Hence, binding of the inactive LMP7 to the proteasome was capable of rescuing the generation of the Matrix epitope 58–66. We note, however, that presentation of the Matrix A2 epitope in cells containing the mutated LMP7 does not equal the presentation in cells containing the wild-type LMP7.

To establish whether the lack of presentation of the Matrix 58–66 epitope by .174/TAPs was due to an active destruction of the epitope by the proteasome, we studied the effect of the proteasome inhibitor lactacystin on .174/TAPs cells. It was previously shown that treatment of the B cells C1R/A2 with the proteasome inhibitor lactacystin was capable of enhancing the presentation of the Matrix A2 epitope (43). Our results confirmed these findings, as addition of the proteasome inhibitor lactacystin to .174/TAPs cells relieved the block in presentation of the Matrix A2 epitope (Fig. 1C), possibly because maximum presentation had already been reached.

FIGURE 1. Impaired presentation of the influenza Matrix 58–66 in LMP-deficient cells can be restored by expression of LMP7 or treatment with lactacystin. CTL clones specific for the Matrix 58–66 epitope were used against the following infected targets: .45 (A), the LMP2- and LMP7-negative cell line .174 transfected with TAP1 and TAP2 (.174/TAPs; B), or .174 transfected with both TAP genes and LMP7 (.174/TAPs/LMP7; C). Cells were infected with recombinant vaccinia virus containing the influenza Matrix protein (M1 vac). Cells were either treated (open symbols) or mock treated (filled symbols) with lactacystin. A Western blot analysis (D) shows the presence or the absence of LMP7 in the three cell lines.

FIGURE 2. Binding of a mutated form of LMP7 into the proteasome can rescue presentation of the influenza Matrix 58–66 epitope. .174/ TAPs cells (column 1) and .174/TAPs transfected with either wild-type LMP7 (3 and 5) or mutated LMP7 T1A/T2A (columns 2 and 4) were infected with recombinant vaccinia encoding Matrix (columns 1–3) or a control vaccinia (WR vac, columns 4 and 5). CTL specific for the Matrix 58–66 epitope were used against the infected target cells. B, Proteasomes were immunoprecipitated from extracts of radiolabeled cells using the Ab MCP21. LMP7 subunits were then precipitated from these proteasomes with anti-LMP7 antiserum. Cell extracts were made from .45 cells (lane 1), .174/TAPs cells (lane 2), .174/TAPs/LMP7 1TA/2TA cells (lane 3), and .174/TAPs/LMP7 (lane 4).
Rapid degradation of the full-length Matrix protein overcomes Ag presentation block

The stability of cytosolic proteins may influence their ability to sensitize target cells for lysis (44–46) and induce a stronger immune response (44). It is not known, however, whether rapid degradation of a cytosolic protein may overcome an Ag presentation block that is dependent on the presence of IFN-γ-inducible proteasome subunits. Modifications of protein Ags may result in their rapid degradation by distinct degradation pathways, leading to a more efficient Ag presentation. To address this possibility we decided to study whether the lack of presentation of the Matrix A2 epitope by LMP-negative cells could be overcome by increasing the rate of degradation of the full-length Matrix protein.

The three-dimensional structure of the first 164 amino-terminal residues (of 252 residues) of the influenza Matrix protein has been solved as two domains linked by a flexible hinge (47). The amino-terminal domain is an antiparallel four-α-helix bundle held together by a central hydrophobic core. Because hydrophobic interactions in folded proteins play a major role in holding their tertiary structure together, we reasoned that mutations disrupting these interactions may lead to the protein unfolding, resulting in its rapid degradation (48–50). This reasoning led to the analysis of the rate of degradation of the full-length Matrix protein in which the hydrophobic residues contributing to the hydrophobic core of the influenza Matrix α helix bundle were mutated. In the mutant E6 (Fig. 3) the residues isoleucine 24, leucine 28, leucine 39, leucine 42, methionine 43, and leucine 46 were replaced by negatively charged glutamic acid residues, while in the mutant called E2 (Fig. 3) residues isoleucine 24 and leucine 28 were replaced by glutamic acid residues.

The half-lives of E2 and E6 Matrix proteins were significantly shortened compared with the half-life of the wild-type Matrix protein (Fig. 3C). This result suggests that the presence of negatively charged residues in a hydrophobic region may have altered the folding of the Matrix protein, resulting in its rapid degradation. Although the nature of the protease responsible for the degradation of short-lived E2 and E6 proteins is not known, pulse-chase experiments established that a large proportion of newly synthesized E2 and E6 proteins were degraded by a lactacystin-resistant protease, while degradation of a short-lived form of the influenza nucleoprotein (ubiquitin-Arg-nucleoprotein (NP)) was blocked by lactacystin (data not shown). These results demonstrate that degradation of two distinct short-lived cytosolic proteins can be controlled by distinct proteolytic activities.

We then addressed the hypothesis of whether a rapidly degraded full-length Matrix protein was capable of sensitizing LMP-deficient cells for lysis by A2-restricted Matrix-specific CTL. The results of these experiments demonstrated that targeting the full-length Matrix protein for rapid degradation was capable of relieving the processing block of the LMP-deficient cells, leading to an efficient presentation of the Matrix A2 epitope (Fig. 3, A and B).

Presentation of the influenza Matrix A2 epitope contained within shorter fragments

An alternative strategy for destabilizing newly synthesized proteins is by generating protein fragments. Our earlier results showed that fragments of the influenza NP were shorter lived than the full-length NP (45). We sought to address whether the block in presentation of the LMP7-dependent Matrix A2 epitope could be overcome by expressing the epitope 58–66 contained within shorter fragments of the influenza Matrix protein. A panel of vaccinia-encoded Matrix fragments was compared for their ability to sensitize LMP-negative cells for lysis by A2-restricted Matrix-specific CTL (Fig. 4, A and B). The .174/TAP cells were efficiently lysed by Matrix-specific CTL after infection with recombinant vaccinia viruses expressing fragments of the influenza Matrix (Fig. 4B). It is noteworthy that one fragment, spanning the whole of the first two domains of the Matrix protein (fragment 1–164) (47), failed to sensitize for lysis the LMP-negative cells, .174/TAP, thus behaving similarly to the full-length Matrix protein.

These findings suggest that during infection of .174/TAPs cells with vaccinia-encoding wild-type full-length Matrix protein there was no generation of smaller Matrix fragments, capable of being
processed and presented to Matrix-specific A2-restricted CTL. We reasoned that an enhanced generation of influenza Matrix fragments in cells infected with full-length Matrix vaccinia may lead to an efficient generation of the 58–66 epitope by LMP7-negative cells. To address this hypothesis the antibiotic puromycin was used, as puromycin generates short-lived premature termination products from newly synthesized proteins (51–53). We showed that addition of puromycin to .174/TAPs cells for 30 min during infection with Matrix vaccinia sensitized them for lysis by A2 Matrix-specific CTL (Fig. 5).

Discussion

In this paper we analyzed the interplay between proteasomes and influenza Matrix protein for the generation of the A2 Matrix epitope 58–66. Our earlier experiments showed that the LMP-negative cells, .174, transfected with TAP1 and TAP2 cDNA were unable to be lysed by 58–66-specific CTL (37). This Ag presentation block was partially relieved by the expression of vaccinia-encoded LMP7. The minimal effect of the vaccinia-encoded LMP7 was accounted for by the inefficient maturation and incorporation of the vaccinia-encoded LMP7 into the proteasome (37). In this report we have confirmed our previous results and demonstrated that presentation of the influenza Matrix A2 epitope 58–66 is LMP7 dependent (Fig. 1). This is the second CTL epitope presented by human cells shown to be LMP7 dependent (15), which demonstrates that modulation of IFN-γ-inducible proteasome subunits may affect the generation of viral CTL epitopes.

One possibility to account for the positive effect of LMP7 on the generation of the Matrix A2 epitope by .174/TAPs cells is that incorporation of LMP7 into proteasomes introduces a new proteolytic activity, which allows generation of the 58–66 epitope. Alternatively, binding of LMP7 to the proteasome may modify the proteasome’s structure and/or subunit composition, preventing the destruction of the Matrix A2 epitope. Our results are consistent with the latter possibility. Firstly, we showed that transfection of a mutated LMP7, lacking the threonine catalytic active site, was capable of relieving the block in presentation of the Matrix epitope. Secondly, we observed that the proteasome inhibitor lactacystin enhances the killing of .174/TAPs cells by Matrix-specific CTL. The observation that lactacystin had no effect on the presentation of the Matrix A2 epitope by LMP7-transfected .174/TAPs cells and the parental line .45 could be accounted for by maximum killing of LMP7-positive target cells by Matrix-specific CTL. These results are consistent with the report by Luckey et al. (43), in which it was shown that treatment of the B cells C1R with lactacystin enhances presentation of the Matrix A2 epitope and that purified proteasomes destroyed the 58–66 epitope contained within a larger synthetic peptide. The effect of lactacystin on the generation of the Matrix A2 epitope in .174/TAPs cells is different from the effect that we previously observed using a human melanoma cell line (4), in which presentation of the A2 Matrix epitope was inhibited, rather than then enhanced, by lactacystin. It is possible that this discrepancy might be accounted for by a higher sensitivity of the melanoma line to lactacystin, which might have caused a reduced synthesis of the influenza Matrix protein. Although this possibility was not formally ruled out, we considered it unlikely, as the same cells were capable of presenting the HLA-A1 influenza NP epitope if treated with even higher doses of lactacystin (4). An alternative possibility is that distinct processing pathways may dominate proteolysis in different cell types, as shown for the presentation of the HLA-A3 influenza NP epitope by mouse and human cells (54).

Evidence that amino-terminal threonines of proteasomes’ β subunits contribute to the activity of the proteasome is compelling (5, 55–57). Site-directed mutagenesis of mouse LMP 2 (56), yeast (57), and Thermoplasma acidophilum (55) proteasome subunits demonstrated the importance of amino-terminal threonines in the activity of the β subunits. Our findings are consistent with this
because the epitope neighboring residues were conserved in all the sequence may account for the ability of shorter lived Matrix protein (Fig. 5).

We consider it unlikely that variations in the proteins’ primary sequence may account for the ability of shorter lived Matrix protein and Matrix fragments to be presented by LMP-deficient cells, because the epitope neighboring residues were conserved in all the full-length Matrix constructs, and no mutations were introduced in the shorter Matrix fragments (see Fig. 4A). It is likely that rapid degradation of mutated full-length Matrix proteins E2 and E6 may result from their misfolding, compared with the folding of the full-length influenza Matrix. Hydrophobic interactions in folded proteins play a major role in holding their tertiary structure (48–50). Hence, introduction of negatively charged residues to the hydrophobic core of the Matrix helix bundles is likely to destabilize folding of the full-length Matrix protein. The half-lives of the Matrix fragments could not be measured, as our Matrix-specific Abs failed to recognize these fragments in Western blot and immunoprecipitation analysis. Surprisingly, fragment 164 (see Fig. 4) failed to sensitize for lysis LMP-negative cells, .174/TAPs, thus behaving similarly to the full-length Matrix protein. Unlike the influenza Matrix fragments that were presented by LMP-deficient cells, the fragment 1–164 spans two whole domains of the influenza Matrix. The 1–164 fragment seems to be stable in vitro even after treatment with acidic conditions (47). It is possible that this fragment is also stable in vivo and that the inability of the fragment 1–164 to sensitize LMP-negative cells for lysis may have resulted from a longer half-life than those of the other fragments.

It has been suggested that a proportion of class I binding peptides is derived from the degradation of defective ribosomal products (61). The lack of killing of .174/TAPs cells infected with either influenza virus or Matrix vaccinia suggests that during expression of the full-length Matrix, there is no generation of shorter fragments capable of sensitizing .174/TAPs cells for lysis by Matrix 58–66-specific CTL. In contrast, treatment of the same cells with the antibiotic puromycin relieves this block and leads to an efficient presentation of the Matrix 58–66 epitope. The effect of puromycin on the generation of the Matrix A2 epitope is likely to result from the generation of Matrix premature termination products. However, we cannot rule out the possibility that the puromycin effect may result from other mechanisms, such as inhibition of puromycin-sensitive aminopeptidases (62, 63).

While these results demonstrate that presentation of a LMP7-dependent epitope can be relieved by increasing the rate of degradation of the target protein, we cannot generalize these findings to other LMP2- and/or LMP7-dependent epitopes encoded within short-lived proteins. We previously showed that presentation of the LMP-dependent influenza NP epitope 366–374 by H-2 Dk molecules cannot be relieved by infecting LMP-deficient cells with a rapidly degraded form of the influenza NP (37). It is becoming clear that the immunogenicity of a protein cannot always be predicted from its degradation rate. Antón et al. demonstrated efficient peptide generation in the presence of doses of proteasome inhibitors sufficient to completely block the target protein degradation (12). An increased rate of degradation of a cytosolic protein would lead to enhanced Ag presentation if the protease(s) responsible for its degradation would also be capable of generating the antigenic peptide. In this respect it is of interest that degradation of the short-lived influenza NP is lactacystin sensitive (4), while degradation of a large proportion of the short-lived form of the Matrix protein is lactacystin resistant (data not shown).

In conclusion, we have investigated the processing events for the generation of a defined CTL epitope contained within the influenza Matrix protein. Our results illustrate two main points: firstly, we show that binding of a mutated form of LMP7 to the proteasome can relieve a defined block in presentation of an immunodominant viral epitope; and secondly, we demonstrate that altering the half-life of a target protein may overcome a block in presentation of an immunoproteasome-dependent CTL epitope. These observations demonstrate the importance of the interplay between immunoproteasomes and protein turnover in the generation of CTL epitopes. Release of IFN-γ by virus- or tumor-specific CTL may result in the modulation of proteasome subunit composition and may alter the processing patterns of viral and tumor Ags. Different target cells may optimize Ag presentation by generating protein fragments that could overcome the lack of expression of IFN-γ-inducible proteasome subunits.

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References


