

The Requirement for Proteasome Activity in Class I Major Histocompatibility Complex Antigen Presentation Is Dictated by the Length of Preprocessed Antigen

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Summary

Accumulating evidence has implicated the proteasome in the processing of proteins along the major histocompatibility complex (MHC) class I presentation pathway. The availability of potent proteasome inhibitors provides an opportunity to examine the role of proteasome function in antigen presentation by MHC class I molecules to CD8⁺ cytotoxic T lymphocytes (CTLs). We have investigated the processing and presentation of antigenic epitopes from influenza hemagglutinin in target cells treated with the inhibitor of proteasome activity MG132. In the absence of proteasome activity, the processing and presentation of the full-length hemagglutinin was abolished, suggesting the requirement for proteasome function in the processing and presentation of the hemagglutinin glycoprotein. Epitope-containing translation products as short as 21 amino acids when expressed in target cells required proteasome activity for processing and presentation of the hemagglutinin epitope to CTLs. However, when endogenous peptides of 17 amino acids or shorter were expressed in target cells, the processing and presentation of epitopes contained in these peptides were insensitive to the proteasome inhibitor. Our results support the hypothesis that proteasome activity is required for the generation of peptides presented by MHC class I molecules and that the requirement for proteasome activity is dependent on the size of the translation product expressed in the target cell. The implications of these findings are discussed.

The antigen receptor on CD8⁺ T lymphocytes recognizes short peptide fragments of polypeptides bound to MHC class I molecules (1, and for review see 2). These peptide fragments are derived from polypeptide precursors of self and foreign proteins found primarily in the cell cytoplasm (3). The peptide fragments generated in the cytoplasm are transported from the cytosol to the endoplasmic reticulum (ER)¹ through the action of the ATP-dependent transporter for antigen presentation (TAP) complex, which is encoded by two genes, *TAP-1* and *TAP-2*, located in the MHC locus (4–7).

The characteristics of peptide transport mediated by the TAP complex for presentation to MHC class I molecules have been investigated by a number of groups using isolated microsomes or permeabilized cell systems (8–11). These analyses have provided information on the size and

sequence of peptides optimally transported by the TAP transporter. In contrast, the process of peptide fragmentation in the cytosol and the type and properties of the protease(s) involved in peptide generation in the cytosol are less well understood. Several lines of evidence have implicated the high molecular weight multicatalytic cytosolic protease complex, the proteasome, as a critical enzyme for the processing of protein antigens in the cytosol for presentation along the MHC class I processing pathway (12–14). Indeed, two subunits of the proteasome (LMP-2 and LMP-7 [for latent infection membrane proteins 1 and 2]) are encoded by genes within the MHC locus (15–16).

Because proteasome activity is required for normal cell function and viability, it has not been possible to eliminate permanently proteasome activity in living cells and assess the impact of the proteasome deficiency on antigen processing and CD8⁺ T cell recognition. Recently, Rock et al. (17) described a class of agents that inhibit the proteasome proteolytic pathway in cells. These agents are peptide aldehydes that act selectively to inhibit the major peptidase

¹Abbreviations used in this paper: aa, amino acid; ER, endoplasmic reticulum; HA, hemagglutinin; LMP, latent infection membrane protein; TAP, transporter for antigen presentation; VV, vaccinia virus.

activities of the 20S and 26S proteasome particles and inhibit the degradation of ubiquitinated protein substrates by the 26S particles. With the development of such highly specific proteasome inhibitors (17, 18), the effect of inhibition of proteasome function on antigen processing and CD8⁺ T cell recognition can now be assessed.

In this report, we examined the effect of the proteasome inhibitor MG132 on the processing and presentation to CTLs of endogenously expressed intact influenza hemagglutinin (HA) protein, truncated forms of the HA protein, and minigene products encoding various lengths of HA protein containing CD8⁺ T cell epitopes. We found that the presentation of the full-length HA protein, truncated HA proteins, and minigene products of 21 amino acids (aa) or longer to CTLs were sensitive to the proteasome inhibitor MG132 but that the presentation to CTLs of epitopes in translation products up to 17 aa in length was insensitive to the MG132. These results suggest that proteasome activity is required only for the processing and presentation of protein products longer than 17 aa. The significance of these results for proteasome function and antigen processing is discussed.

Materials and Methods

Viral Constructs. The minigene constructs were produced as described (19). The sequence of translation products used in this study is shown in Table 2. The construction of the recombinant vaccinia virus (VV) HAΔR1 has been described previously (20). The HA gene product expressed by this VV has a deletion of 166 aa in the middle of HA, i.e., HA residues 224–356 (20). The Sindbis virus vector THA expresses a truncated A/JAP/57 HA product consisting of HA residues 1–266 (19). All truncated HA products and minigene products were expressed in target cells by use of recombinant Sindbis or Vaccinia vectors as noted in the text.

CTL Clones. The generation, characterization, and maintenance of CTL clones 14-1 and 40-2 are described in detail elsewhere (2).

Virus Infection. Log-phase P815 cells were infected with recombinant VVs at a multiplicity of infection of 10 and with Sindbis viruses at a multiplicity of infection of 20 in medium consisting of 1% FCS in PBS. Infection with influenza A/JAPAN/57 was carried out essentially as described (20), with the following modifications. After 20 min incubation of cells with virus at 37°C, P815 cell samples were split, and the proteasome inhibitor MG132 (Myogenics, Inc., Cambridge, MA) was added to the test sample at a final concentration of 10–50 μM in MEM (GIBCO BRL, Gaithersburg, MD) as noted in the text. The control cell sample received medium without MG132. The cells were incubated at 37°C for 40 min. The two cell samples were then washed once to remove unbound virus, and MG132-containing medium was added to the test sample, followed by 25 μCi of Na⁵¹Cr₂O₄ (DuPont/New England Nuclear, Boston, MA). The target cells were incubated for 3 h at 37°C to allow viral gene expression and ⁵¹Cr uptake. Control P815 cells were infected and ⁵¹Cr labeled in parallel in medium without MG132.

CTL Assay. The *in vitro* cell-mediated cytotoxicity assays were carried out for 6 h as described (20). Since cytotoxicity assays were performed in medium without MG132, brefeldin A (21) at a final concentration of 5 μg/ml was added to the assay medium

to block export of nascent MHC class I molecules to the surface of target cells during the cytotoxicity assay.

FACS[®] Analysis. Log-phase P815 cells were infected at 4°C with A/JAPAN/57 virus as above and further incubated for 20 min at 37°C. Cell samples were washed once and then divided into three groups. Cells were either incubated in medium supplemented with MG132 to a final concentration of 10 or 50 μM or in medium without the inhibitor. After 4 h of incubation at 37°C, the cells were washed and treated with a murine mAb against the A/JAPAN/57 HA, followed by a fluorescein-conjugated rabbit antibody to mouse Ig (Southern Biotechnology Associates, Inc., Birmingham, AL). Flow cytometric analysis was carried out on a FACScan[®] flow cytometer (Becton Dickinson & Co., Mountain View, CA).

Results

Inhibition of Proteasome Activity Blocks HA Presentation. Recently, a class of aldehyde inhibitors has been identified that can selectively inhibit the enzymatic activity of the proteasome complex (17). One of these inhibitors, MG132, is particularly potent, with a K_i of 4 μM (22). Since the proteasome has been implicated in the fragmentation of cytosolic proteins for presentation to MHC class I-restricted CTLs, we wanted to examine the effect of proteasome inhibition by MG132 on the presentation of the A/JAPAN/57 HA to H-2K^d-restricted CTLs. Although the HA is a type I membrane glycoprotein with a signal sequence for ER translocation, processed peptides for presentation to MHC class I-restricted CTLs are normally generated by fragmentation of a cytosolic form of the HA translation product (23).

To examine the effect of MG132 on HA presentation, P815 target cells were infected with A/JAP/57 virus for 40 min at 37°C in the presence of this inhibitor and further incubated with Na⁵¹Cr₂O₄ for 3 h more at 37°C to allow viral gene expression during radiolabel uptake. After incubation at 37°C, the infected target cells were suspended in medium without MG132 and tested for recognition by HA-specific CTLs in a standard chromium release cytotoxicity assay. MG132 was excluded from the cytotoxicity assay to avoid potential nonspecific toxicity to the inhibitor on CTL function or target cell viability during the assay. Since MG132 is a reversible inhibitor of proteasome activity, MHC class I molecules containing newly processed HA peptides could be generated in infected target cells by the now active proteasome during the cytotoxicity assay (17). To circumvent this problem, cytotoxicity assays were carried out in the presence of brefeldin A, which blocks the transport of nascent MHC class I molecules from the ER to the cell surface (21).

Fig. 1 shows the effects of MG132 on recognition of processed HA by two K^d-restricted CD8⁺ CTL clones: 14-1, which is directed to the HA 204–212 epitope, and 40-2, which is directed to the HA 210–219 epitope (24). Treatment of the target cells with MG132 during influenza viral gene expression abolished target cell recognition by these HA-specific CTLs. Similar results were obtained when the infection of target cells was carried out with either a recombinant VV expressing the full-length A/JAP/57 HA

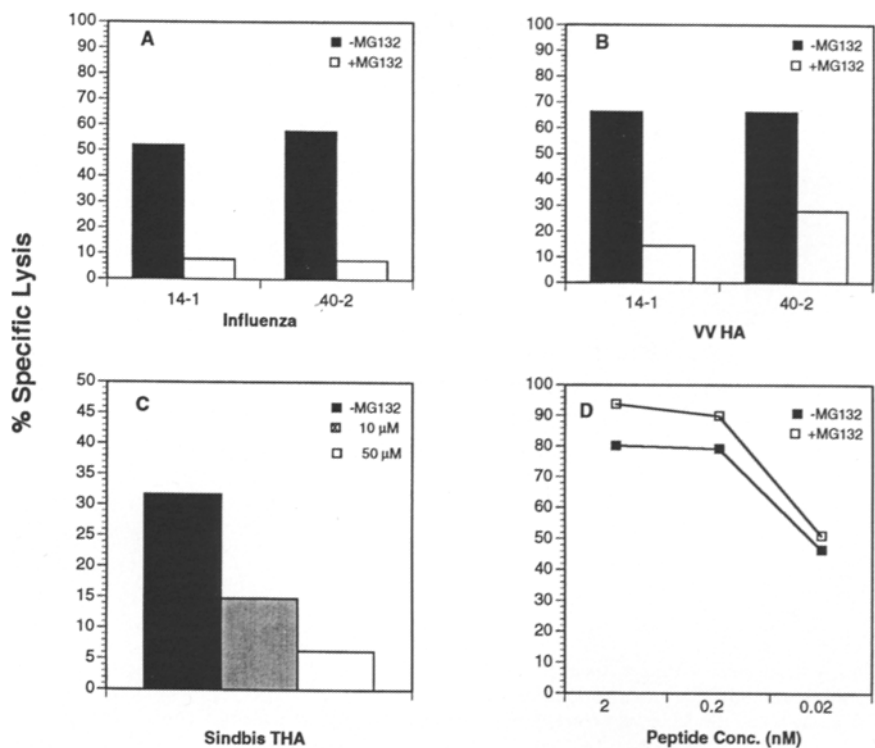


Figure 1. The processing and presentation of influenza HA expressed during viral infection is blocked by the proteasome inhibitor MG132. Log-phase P815 cells were infected, with either influenza virus (A), a recombinant VV expressing full-length HA (B), or a Sindbis virus vector expressing the NH₂-terminal 266 aa of the A/JAPAN/57 HA (C), and incubated in medium supplemented with MG132 to a final concentration of 50 μ M (and 10 μ M in C) as described (see Materials and Methods). In D, uninfected cells were incubated in medium with or without MG132 as described above for infected cells, and the uninfected target cells were then loaded with the HA 204–212 peptide at the peptide concentrations indicated in D. Cytotoxicity assays were carried out for 4–6 h in the presence of 5 μ g/ml brefeldin A. CTL clones 40-2 and 14-1 were used at an E/T ratio of 5:1. Values are the mean ⁵¹Cr release of quadruplicate wells with SEs (<5% of mean values) not displayed on the histograms or lines.

(Fig. 1 b) or a recombinant Sindbis virus vector (SIN THA) expressing a truncated HA gene encoding HA residues 1–266 (Fig. 1 c) (19). Inhibition of CTL recognition by MG132 was dependent on the dose of inhibitor used to block proteasome activity; MG132 at a final concentration of 50 μ M was found to produce maximum inhibition (Fig. 1 c).

MG132 treatment did not have a nonspecific effect of the susceptibility of target cells to lysis by CD8⁺ CTLs. As Fig. 1 d shows, MG132-treated target cells were as efficiently lysed as untreated target cells after pulsing with a synthetic peptide corresponding to the HA 204–212 epitope. Also, to examine the possibility that MG132 affects the expression of the HA gene in influenza-infected target cells, the level of HA protein expression on the surface of treated or untreated influenza-infected P815 cells was determined by flow cytometric analysis. As Table 1 shows, cell surface HA expression of A/JAP/57-infected P815 cells was comparable both in control infected target cells and in cells infected and maintained in either 10 μ M or 50 μ M MG132.

Endogenous Presentation of Minimal Epitopes Is Insensitive to MG132. If MG132 inhibits CTL recognition of target cells by blocking proteasome-dependent fragmentation of the HA, then target cells that express a preprocessed HA epitope should not be sensitive to inhibition by MG132. To examine this, we constructed recombinant Sindbis viruses (Table 2, SIN204(10) and SIN210(10) expressing minigenes encoding 10 aa peptides corresponding to the minimal HA epitopes recognized by clone 14-1, i.e., HA 204–212, and by clone 40-2, i.e., HA 210–219. As Fig. 2 demonstrates, MG132 did not inhibit the presentation of these minimal minigene products when expressed in the cytoplasm of infected cells.

MG132 Inhibition Is Dependent on the Length of the Endogenous Peptide Product. Since MG132 inhibited the endogenous presentation of the full-length HA translation product but not the minimal CTL epitope, it was of interest to determine whether MG132 sensitivity was dependent on the size of the translation product containing the HA epitope. Since the SIN THA virus tested above (see Fig. 1 c) expressed a truncated form of the A/JAP/57 HA and MG132 inhibited the recognition of target cells infected with the SIN THA virus by CTLs, expression of a nonnative HA translation product per se was not sufficient to overcome the inhibitory effect of MG132. A similar result was obtained when we examined the effect of MG132 on presentation of another truncated HA gene product, HA Δ R1 when it was expressed by a recombinant vaccinia vector (Fig. 3 a). The HA Δ R1 gene construct has an in-frame de-

Table 1. Influenza HA Expression in MG132-treated Cells

Treatment*	Mean Fluorescence [‡]
Uninfected	2
Influenza infected	44.7
Influenza + 10 μ M MG132	60.2
Influenza + 50 μ M MG132	62.3

*P815 cells were infected and treated with MG132 cells as described (Materials and Methods). After 4 h incubation at 37°C in medium with or without MG132, cell surface HA expression was determined by flow cytometry.

[‡]Values are mean of 5 \times 10⁴ cells sampled in arbitrary units.

Table 2. *Viral Expression Vector*

Virus and vector*	HA sequence [‡]	Length (aa)
SIN 210(10)	<i>M A Y S V G T S T L</i>	10
SIN 204(10)	<i>M Y Q N V G T Y V S</i>	10
SIN 204(13)	<i>M S T L Q N V G T Y V S</i>	13
SIN 204(17)	<i>M L Y Q N V G T Y V S V G T S T L</i>	17
SIN 204(21)	<i>M E T E Q R T L Y Q N V G T Y V S V G T Z</i>	21
SIN 204(28)	<i>M E T E Q R T L Y Q N V G T Y V S V G T H M E Q N G A Z</i>	28
SIN 204/210(34)	<i>M E T E Q R T L Y Q N V G T Y V S V G T S T L N K R I L D A T P Q Z</i>	34
SIN 204/210(46)	<i>M E T E Q R T L Y Q N V G T Y V S V G T S T L N K R D P R R Y A P M I R P A K L D V L P R N Z</i>	46
VV 204/210(46)	<i>M E T E Q R T L Y Q N V G T Y V S V G T S T L N K R D P R R Y A P M I R P A K L D V L P R N Z</i>	46
SIN THA	NH ₂ -terminal 266 aa	266
VV HAΔRI	Internal deletion of 165 aa	396
Influenza	Full-length HA	561
VV HA	Full-length HA	561

*Vector designation indicates viral vector. SIN, Sindbis; VV, vaccinia. Number designations 204 and 210 indicate presence of coding sequence for 204–212 or 210–219 epitopes. Values in parentheses correspond to length of translation product.

[‡]The sequences corresponding to HA 204–212 are underlined, and the sequences corresponding to HA 210–219 are italicized.

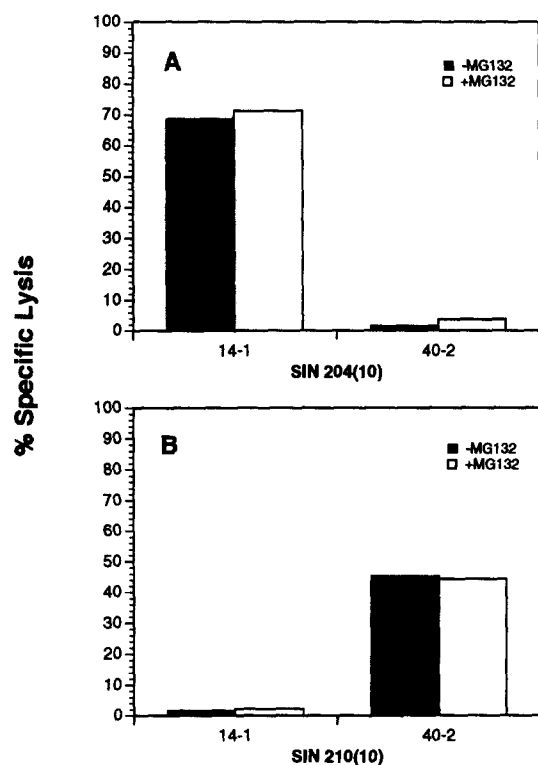


Figure 2. MG132 does not inhibit the processing and presentation of minimal minigene products. Log-phase P815 cells were infected with Sindbis virus vectors expressing 10-mer peptides corresponding to the minimal HA 204–212 (A) or HA 210–219 (B) epitopes recognized by CTL clones 14-1 and 40-2, respectively. Infected cells were treated with 50 μ M MG132 and assayed for lysis as described in Fig. 1. CTL clones were used at an E/T ratio of 5:1. The HA protein sequences encoded by the SIN 204(10) and the SIN 210(10) virus vectors are shown in Table 2.

letion in the A/JAP/HA cDNA that eliminates the coding sequence for the middle third of the A/JAP/HA, i.e., HA 224–356 (19), but retains the HA 204–212 and HA 210–219 sites. As Fig. 3 a shows, MG132 inhibits presentation of the HA epitopes expressed from the VV HAΔR1 vector.

We previously reported that the 46-aa product of a minigene encoding the HA residues encompassing the HA 204–212 and HA 210–219 sites can be preprocessed and presented to CTLs (25). In view of our finding that target cells expressing the minimal epitopes were insensitive to inhibition by MG132, it was of interest to determine whether an endogenous translation product of intermediate length, i.e., 46 aa, would be insensitive to this inhibitor of proteasome activity. This was not the case (Fig. 3, b and c). The presentation of the 46-aa minigene product expressed in target cells by either a Sindbis or vaccinia expression vector showed as sensitive an inhibition by MG132 as that in cells expressing the longer translation product.

The sensitivity of endogenous 46-aa peptide to inhibition by MG132 was striking in view of the insensitivity of endogenously expressed minimal peptide epitopes to inhibition by MG132 (see Fig. 2). This raised the possibility that in the absence of proteasome activity, only a peptide corresponding to the minimal CTL epitope could be presented. It was therefore of interest to determine whether endogenous peptides of lengths longer than the minimal epitope were inhibited by MG132. To examine this, we constructed synthetic minigenes encoding the minimal HA 204–212 epitope flanked by native A/JAP/57 HA residues with total product lengths of 13, 17, 21, 28, or 34 aa. Fig. 4 shows the effect of MG132 on the recognition of target cells expressing these minigene products by use of recom-

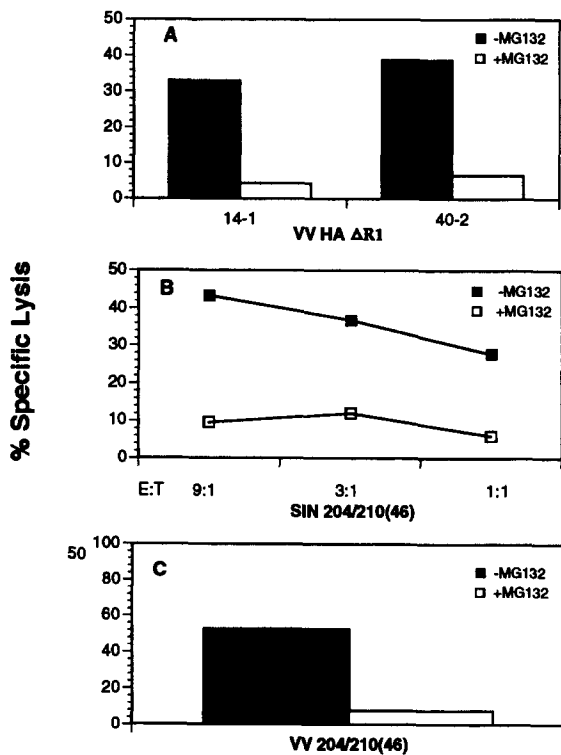


Figure 3. The presentation of truncated HA proteins and large minigene products is sensitive to proteasome inhibition. Log-phase P815 cells were infected with the recombinant VV HA Δ R1 (A), or with a Sindbis virus (B) or VV vector (C) expressing a peptide of 46 aa containing the HA 204–212 and HA 210–219 epitopes. MG132 treatment (50 μ M final concentration) and CTL assays were carried out as in Fig. 1 with the HA 210–219-specific clone 40-2 (A) or the HA 204–212-specific clone 14-1 (A–C). E/T ratios were 5:1, except where noted in B. The sequence of the 46 aa HA peptide is listed in Table 2.

binant Sindbis virus vectors. Although in the absence of MG132 translation products of 13 and 17 aa were not recognized as efficiently as the minimal HA 204–212 epitope, MG132 did not inhibit presentation of these poorly presented minigene products. In contrast, presentation of endogenous peptides of 21, 28, or 34 aa to clone 14-1 was inhibited by MG132. The 34-aa product also contained the intact HA 210–219 epitope. Presentation of this epitope when expressed as part of the 34-aa translation product was also inhibited by MG132 (data not shown). These results indicate that only endogenous peptide products of 21 aa or longer are sensitive to inhibition by MG132.

The alternative interpretation for our observation might be that minigene products of any length with three or fewer NH₂-terminal residues to a minimal epitope do not require proteasome function, whereas translation products with seven or more NH₂-terminal flanking residues require proteasome-dependent proteolysis for processing. To test this possibility, we carried out the experiment using the minigene construct where the HA 204–212 epitope is contained within 44-aa translation product and is flanked by three NH₂-terminal residues (Met, Arg, Thr). MG132 inhibited the processing and presentation of the HA 204–212 epitope to CTLs (data not shown). This suggests that MG132 sensitivity is not related to the number of NH₂-terminal residues.

Discussion

In this report we have examined the effect of the protease inhibitor MG132 on the processing and presentation of the influenza HA to CD8⁺ CTLs. We found that this

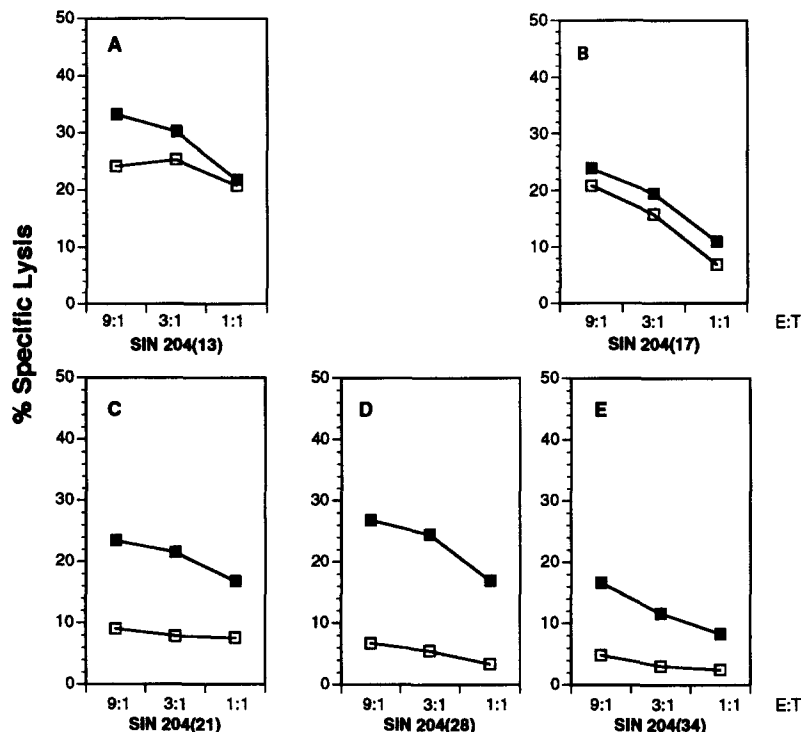


Figure 4. The proteasome dependence of presentation of short translation products. Sindbis virus vectors encoding translation products of 13 aa (A), 17 aa (B), 21 aa (C), 28 aa (D), and 34 aa (E) were used to infect target cells in the presence (open squares) or absence (closed squares) of the MG132 (50 μ M final concentration). Incubation of target cells and assay conditions were as described (Fig. 1). Clone 14-1 was used at the indicated E/T ratios. The sequences of the HA translation products are listed in Table 2.

inhibitor selectively blocked presentation of the full-length HA translation product and T cell epitopes present in translation products of 21 aa or longer, but that MG132 has no effect on the presentation of epitopes contained in translation products of 17 aa or less. These findings suggest that for these viral CTL epitopes, at least, peptide products shorter than 21 aa can be processed and presented to CTLs by a proteasome-independent mechanism. These results further raise the possibility that most peptides longer than 17–20 aa may require proteasome-dependent processing to be presented in association with MHC class I molecules.

Enzyme inhibitors have been used extensively to assess the role and function of proteases in biological processes. An important concern in the use of inhibitors is whether the inhibitor is selective in its action. For example, the proteasome inhibitor MG132 can affect the activity of lysosomal enzymes, e.g., cathepsin B (17), but lysosomal enzymes are unlikely to be important in the processing of the cytosolic proteins examined in this report. Several other lines of evidence in this report suggest that MG132 inhibited antigen processing and presentation by a specific mechanism. First, MG132-mediated inhibition was observed when the HA was expressed either during influenza virus infection or after expression of the HA by VV or Sindbis virus vectors. Since each of these viruses uses a different replication strategy, the effect of MG132 on processing and presentation cannot be easily attributed to MG132-mediated inhibition of a cellular or viral function necessary for HA gene expression in infected target cells. Indeed, the synthesis and cell surface expression of the HA in influenza-infected target cells were unaffected by MG132 treatment. Second, since the inhibitor is not included in the cytolysis assay, suppression of CTL function by MG132 cannot account for the lack of CTL recognition. Also, since exposure of target cells to MG132 did not affect the subsequent loading of synthetic peptides on target cells and the recognition of the target cells by CTL, nonspecific effects of the drug on the susceptibility of target cells to lysis appear unlikely. Finally, when translation products of increasing length were expressed in target cells, inhibition of antigen presentation by MG132 was dependent on the length of the translation product containing the CTL epitope. Taken together, these observations suggest that MG132 is highly selective in its action and that inhibition of proteasome-dependent proteolytic activity likely accounts for the inhibitory effect of this compound on antigen presentation.

Since the proteasome complex is essential for cell viability and cell cycle progression (22), mutant cell lines deficient in this activity are currently not available. Therefore, the role of this cytosolic protease complex in antigen processing and presentation has been difficult to evaluate. Since two proteasome subunits, LMP-2 and LMP-7, are encoded by genes within the MHC locus (15, 16) in close proximity to the genes encoding the TAP proteins, the proteasome and particularly proteasome complexes containing LMP-2 and LMP-7 have been suggested to play a role in the processing of polypeptides for presentation to MHC class I molecules (12–14). However, cell lines lack-

ing the genes for LMP-2 and LMP-7 have been reported to have normal levels of MHC class I molecules at their cell surfaces and to function normally in antigen presentation by class I molecules (26, 27). Although such results argue against the importance of the proteasome in general and the LMP-2 and LMP-7 proteins in particular in antigen processing, mice that have been made genetically deficient in LMP-2 and LMP-7 function by targeted gene disruption do show abnormalities in the processing of certain antigens (28, 29). To test directly the contribution of the proteasome in antigen presentation, it is necessary to specifically inhibit this protease activity in antigen-presenting cells. The development of inhibitors of proteasome activity now provides investigators with the opportunity to assess the role of the proteasome complex in antigen processing for MHC class I presentation. The results reported here from studies using endogenously expressed antigens and from earlier proteasome inhibition studies by Rock et al. (17) using microinjection of exogenous proteins into target cells directly implicate the proteasome in the processing of proteins for presentation by MHC class I molecules.

The antigenic peptides bound to mature MHC class I molecules are predominately 8–11 aa in length (30). On the basis of this consideration, the processed peptide precursors generated by proteolytic degradation in the cytoplasm might also be expected to be of a similar length. An alternative hypothesis is that the cytosolic precursors of the MHC-bound peptides are considerably longer than the minimal peptide epitopes (31). Accordingly, these long precursors would be transported by the TAP complex into the ER and then undergo extensive trimming in the ER. The observations reported here suggest that processed peptide fragments generated by the proteasome in the cytoplasm are likely to be short, i.e., predominantly 8–17 amino acids in length, and therefore need not undergo extensive modification (trimming) in the ER.

Several reports have examined the effect of peptide length on the efficiency of ATP-dependent peptide transport by the TAP complex (8–10). In general, these studies suggest that peptides of 9–17 aa are most efficiently transported by the TAP transporter. One intriguing explanation for the selectivity of MG132 inhibition for translation products longer than 21 aa is that in the absence of proteasome fragmentation, translation products longer than 21 aa cannot be efficiently transported by the TAP complex. It is tempting to speculate that the proteasome complex involved in antigen presentation primarily generates peptide fragments of a defined length, e.g., 8–17 aa, which would be optimal for TAP-mediated transport from the cytosol to the ER. Consistent with this view are studies on the proteolytic activity of purified proteasomes *in vitro*, which indicate that peptide fragments corresponding to the size of minimal CTL epitopes can be produced from protein precursors by proteasome-dependent proteolysis (32). However, it is equally likely that this multicatalytic protease complex could generate fragments of many different lengths, i.e., from single aa to peptides of >20 aa in length, with varying efficiencies. In this case, the constraints on peptide length imposed

by the TAP complex would favor the transport of peptides that are of optimal length for transport, i.e., 8–17 aa.

It is also noteworthy that among those translation products that can be processed in the presence of the proteasome inhibitor, target cells expressing the minimal translation product were most efficiently recognized. The reasons for this dichotomy are not completely clear and may reflect differences in the efficiency of TAP-dependent peptide transport or perhaps in the efficiency of final trimming of these proteasome-independent peptides in the ER.

In conclusion, our findings support the view that the

proteasome complex plays a central role in antigen processing for MHC class I-associated antigen presentation. These results further suggest that the proteasome activity is required to generate cytosolic peptides of appropriate length from polypeptide precursors for subsequent transport and final processing. If peptides of up to 16–17 aa routinely gain access to the ER as a result of proteasome-mediated fragmentation and TAP-mediated transport, then final trimming of peptides in the ER is likely to be an important step in the formation of stable peptide–MHC complexes in the ER compartment.

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