Human cathepsin S, but not cathepsin L, degrades efficiently MHC class II-associated invariant chain in nonprofessional APCs

Jacek Bania*, Evelina Gatti*, Hugues Lelouard*, Alexandre David*, Fanny Cappello*, Ekkehard Weber†, Voahirana Camosseto*, and Philippe Pierre**

*Centre d'Immunologie de Marseille-Luminy, Centre National de la Recherche Scientifique-Institut National de la Santé et de la Recherche Médicale-Université de la Méditerranée, Campus de Luminy, Case 906, 13288 Marseille Cedex 09, France; and †Institute of Physiological Chemistry, Martin Luther University, Hollystrasse 1, 06097 Halle, Germany

Communicated by Ralph M. Steinman, The Rockefeller University, New York, NY, March 19, 2003 (received for review January 14, 2003)

MHC class II-restricted antigen presentation plays a central role in the immune response against exogenous antigens. The association of invariant (Ii) chain with MHC class II dimers is required for proper antigen presentation to CD4⁺ T cells by antigen-presenting cells. MHC class II complexes first traffic through the endocytic pathway to allow Ii chain degradation and antigenic peptide loading before their arrival at the cell surface. In recent years, a considerable effort has been directed toward the identification of proteases responsible for Ii chain degradation. Targeted gene deletion in mice has allowed a precise description of the cysteine proteases involved in the last step of Ii chain degradation. By using nonspecialized cellular models expressing MHC II molecules, we are now exploring the contribution of known cysteine proteases to human Ii chain processing. Surprisingly and contrary to the situation in mouse, cathepsin S was found to be the only human cysteine protease able to efficiently degrade the Ii-p10 fragment in epithelial cells. This selectivity has implications for thymic selection and indicates that differences between man and mice are probably more profound at this level than expected.

antigen presentation | lysosome | protease

Major histocompatibility complex (MHC) class II molecules are heterodimeric cell surface glycoproteins expressed by professional antigen-presenting cells (APCs); MHC class II molecules bind exogenous peptides and present them to CD4⁺ T cells (1). Class II α and β chains are translocated into the endoplasmic reticulum where they form nonamers in association with the invariant chain (Ii chain) (1). The exogenous origin of the presented peptides requires that MHC class II molecules traffic and intersect with the endocytic pathway (2). Ii chain, in addition to its role in MHC class II folding, is responsible for the endosomal targeting of the complexes (3). Ii chain also sterically prevents the loading of antigenic peptides on MHC class II with a 14-aa domain termed "CLIP" (class II-associated invariant chain peptide). Endosomal MHC class II-Ii complexes must therefore be rendered competent for antigenic peptide loading by degrading Ii chain. The removal of CLIP and the editing of immunogenic peptide are facilitated by the endosome-resident molecules HLA-DM and -DO (4). Endosomal proteolysis plays a central role both in Ii chain degradation and antigenic peptide generation (5), and increasing evidence suggests that professional APCs can modulate their activity through the control of Ii chain degradation (6, 7). Ii chain is degraded sequentially in discrete intermediates known as p22 (LIP, 22 kDa), p10 (SLIP, 10 kDa), and CLIP (8). The leupeptin-insensitive protease(s) involved in the generation of p22 and p10 is/are still not determined; however, several cysteine proteases have been shown to convert p10 in CLIP (9, 10). Cathepsin S (Cat S) is an IFN- γ -inducible protease that is mainly expressed in APCs (11). Cat S-deficient APCs strongly accumulate p10 and display a delayed transport of MHC class II molecules to their surface.

This inability to process Ii chain properly profoundly affects the immune system of Cat S^{-/-} mice (5, 12–14). Several additional cysteine proteases, such as Cat L, Cat F, and Cat K, have been shown to be also expressed in murine APCs and to degrade Ii chain (at least *in vitro*) (15–17). Cat L-deficient mice have abnormalities in positive thymic selection, due at least partially to the accumulation of p10 and defective MHC class II peptide loading in thymic epithelial cells (TEC; ref. 18). Although a precise picture of mouse Ii chain degradation has emerged from these observations, little information is available on human Ii chain. The existence of a close homologue of Cat L, the human thymus-specific Cat V (or Cat L2), is also sufficiently intriguing to investigate the conditions of human Ii chain degradation (19, 20). By using two different cell systems expressing Ii chain and HLA-DR, we demonstrate that Cat S is the only cysteine protease able to efficiently process the human p10 fragment *in vivo*. Interestingly, Cat L and Cat V/L2 were shown not to participate in Ii-p10 processing. Our findings suggest that, in contrast to mice, human Cat S is likely to play a unique role in Ii chain degradation and consequently also in human thymic epithelial cells.

Materials and Methods

Cell Culture. HeLa cells and MeJuSo cells were grown in DMEM containing 5% FCS and 2 mM glutamine. IFN- γ (PeproTech, Rocky Hill, NJ) treatment was performed at 300 ng/ml. *N*-morpholinurea-leucine-homophenylalanine-vinylsulphone-phenyl (LHVS; H. Ploegh, Harvard University, Cambridge, MA) was used at 5 nM. The P1.4D6 human thymic epithelial cell line from M. Toribio (Centro de Biología Molecular Severo Ochoa, Madrid) was grown as described (21).

Antibodies. Rabbit polyclonal anti-Cat S was from M. Brown (Boehringer Ingelheim, Ridgefield, CT). Mouse mAb anti-human Ii chain (PIN-1), mouse mAb anti-human MHC class II (XD-5), mouse mAb anti-human MHC class II (DA6.147), and rabbit polyclonal anti-human DR were the kind gifts of P. Cresswell (Yale University School of Medicine, New Haven, CT). Mouse mAb anti-human Cat L2 (30D10) was from C. Lopez-Otin (Universidad de Oviedo, Oviedo, Spain) and anti-human Cat K (CT3021) was from S. Weiss (University of Michigan, Ann Arbor). Other anti-cathepsin antibodies were provided by E.W. Immunoblots and immunofluorescence microscopy were performed as described (22).

Abbreviations: APC, antigen-presenting cell; Ii chain, invariant chain; CLIP, class II-associated Ii chain peptide; CIITA, MHC class II transactivator; Cat, cathepsin; AEP, asparaginyl endopeptidase; LHVS, *N*-morpholinurea-leucine-homophenylalanine-vinylsulfonyl; TEC, thymic epithelial cells.

*To whom correspondence should be addressed. E-mail: pierre@ciml.univ-mrs.fr.

Preparation of Membrane Fraction. Harvested cells were homogenized in a ball-bearing homogenizer in 10 mM triethanolamine (pH 7.4), 250 mM sucrose, and 1 mM EDTA. After centrifugation at $1,000 \times g$, the post-nuclear supernatant was centrifuged at $18,000 \times g$ for 30 min. Membranes were used for digestion reactions or solubilized in sample buffer containing 0.5% SDS and 25 mM DTT, and were resolved by SDS/PAGE.

Ii Chain Degradation Assay. Equal amounts of HeLa-MHC class II transactivator (CIITA) membrane were incubated for 2 h at 37°C in 100 mM citric acid-phosphate buffer (pH 5) with 3 mM cysteine and 0.1% 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) with Cat L purified from human liver (Calbiochem) or recombinant human Cat S from M. Brown. Resulting samples were separated on 12% SDS/PAGE gel and immunodetected with PIN 1.

Pulse-Chase Labeling and Site-Directed Labeling. HeLa-CIITA cells were pulsed for 30 min with 0.5 mCi (1 Ci = 37 GBq)/ml [^{35}S]methionine/cysteine and chased as described (22). MHC class II was precipitated for 2 h with anti-human DR DA6.147 coupled to protein A Sepharose. Cells were incubated for 1 h with CBZ- ^{125}I Tyr-Ala-CN $_2$ (ESP, Livermore, CA) and washed twice with PBS before lysis in 50 mM Tris-HCl (pH 7.4), 150 mM NaCl, and 1% Triton X-100 followed by SDS/PAGE analysis. The identity of the iodinated proteases was confirmed by immunoprecipitation and labeling of purified cathepsins. Transfections were performed by using lipofectamin; human Cat S and p41 cDNA were from M. Brown and P. Benaroch (Institut Curie, INSERM, Paris).

RT-PCR. Five micrograms of Trizol-prepared total RNA was used for reverse transcription with oligo(dT) primers. The cDNAs of cathepsins were detected after 25 cycles of PCR at 95°C for 30 s, 56°C for 30 s, and 72°C for 60 s. Common antisense primer for Cat L designed to the conserved region of cathepsin gene was CCCCAGCTGTTYTT, and sense primer was GGGCCTCAAGGCAATGAA (406 bp). Sense primer for Cat S was TCAACTGAAAAATATGGAA, and antisense CCTTCTTCACAAAGTTGTGGCC (390 bp). Sense primer for Cat F was GACAAGAGTGGACAAGGCC, and antisense primer CCGGAGAGGGCGGGAGATCCC (300 bp). Sense primer for Cat V was CTTACAGTGGTCGCACCTGG, and antisense TGCTCCTTCAAAGCCGTAGC (196 bp). Sense primer for Cat K was GATGTGGGGACTCAAGGTT, and antisense TCACATCTTAGGGAAGCTGG (1131 bp). Sense primer for Cat Z was TGYGGCTCCTGCTGGGCC, and antisense ACCCATGAATTCCGGAC (530 bp). Sense primer for asparaginyl endopeptidase (AEP) was GCCCTCATGATCGTGA, and antisense TAGCGACCGAGGCACA (353 bp). All PCR products were sequenced or resolved in 2% agarose gels.

Results

Ii-p10 Accumulates in HeLa Cells Transfected with CIITA and MelJuSo Cells. The transactivator CIITA drives the coordinated expression of MHC class II molecules and Ii chain in APCs (23). As expected, stable expression of CIITA in the epithelial cell line HeLa (HeLa-CIITA) induced the strong expression of HLA-DR, Ii, and DM (Fig. 1*a*). HLA-DR and Ii chain were both detected in the lysosomes of these cells. We anticipated the presence of HLA-DR in lysosomes (24), but Ii chain accumulation in these organelles was unexpected. A radioactive pulse and chase labeling followed by the immunoprecipitation of MHC-Ii chain complexes revealed that processing of p10 was extremely inefficient in these cells (Fig. 1*b*). p10 was still strongly associated with DR molecules 6 h after synthesis, a time of chase well documented to allow full Ii degradation in professional

APCs (12). At the steady state, high levels of p10 and also p22 were detected by immunoblotting in HeLa-CIITA membranes (Fig. 1*c*), further demonstrating the inefficiency of Ii chain degradation in these cells. A similar Ii chain accumulation was also observed in MelJuSo cells (Fig. 1*c*), which have often been used as a model for MHC class II transport to and out of the lysosomes (25). Ii-p10 accumulation in MelJuSo and HeLa-CIITA cells suggested the existence of a strong link between inefficient Ii chain degradation and MHC class II lysosomal retention (6). HeLa-CIITA and MelJuSo therefore constitute interesting models of nonprofessional APCs for the study of human Ii chain processing *in vivo*.

IFN- γ Stimulation Promotes p10 and p22 Degradation. The expression of CIITA is not sufficient to promote full Ii chain processing, which clearly depends on some degree of APC specialization. We therefore tested the effect of IFN- γ on p10 accumulation in HeLa-CIITA (Fig. 1*c*). The total disappearance of p10 and p22 was observed after 24 h of IFN- γ treatment, whereas intact Ii chain and DR β chain levels remained unchanged (Fig. 1*c*). To establish whether proteolysis was responsible for p10 and p22 disappearance, we incubated IFN- γ -treated cells with 5 nM of the Cat S-specific inhibitor LHVS (11). LHVS completely prevented p10 and p22 degradation (Fig. 1*c*), suggesting that IFN- γ -induced Cat S was responsible for the degradation of the two fragments. Interestingly, MelJuSo cells (expressing the DR3 allele), unlike HeLa-CIITA, did not accumulate p22, which is detected only on leupeptin treatment (Fig. 1*c*). In addition, p10 levels were not affected by leupeptin, indicating that only an extremely limited degradation of this fragment was mediated by the cysteine proteases of MelJuSo cells. This result contrasts sharply with the strong effect of leupeptin on p10 accumulation as observed previously in B cells (26).

Cathepsin S Is Not Expressed in HeLa-CIITA and MelJuSo Cells. p10 accumulation led us to determine which cysteine proteases are produced by HeLa cells during CIITA expression or INF- γ stimulation. Semiquantitative RT-PCR was performed on mRNA extracted from HeLa, HeLa-CIITA, and MelJuSo cells after INF- γ stimulation (Fig. 2*a*). In addition to ubiquitously expressed Cat L, Cat Z, Cat C, and Cat F, additional transcripts for tissue-specific Cat V/L2 (thymus, testis; refs. 19 and 20) and Cat K (osteoclast; ref. 16) were detected in HeLa cells. Transcription levels were found identical in the parental HeLa cell line and the HeLa-CIITA, as well as the MelJuSo cells. Among all cysteine proteases tested, the transcript for Cat S was the only one not to be detectable by PCR, but Cat S transcription is strongly induced in both INF- γ -treated HeLa-CIITA and MelJuSo cells (27).

Cat S translation in INF- γ -stimulated cells was demonstrated by immunoblot and immunofluorescence microscopy (Fig. 3*a–b*). Cat S was detected only in the lysosomes of stimulated cells, confirming that INF- γ induction of human Cat S is not mediated through CIITA (27). Because most lysosomal cathepsins seem to be relatively insensitive to CIITA expression and/or INF- γ stimulation, we also investigated the fate of AEP, which is involved in antigen degradation (28). Interestingly, AEP has variable expression among cell types, and its transcription is enhanced by INF- γ stimulation (Fig. 2*b*). Cat S and AEP are therefore the only two clearly INF- γ -inducible cysteine proteases detected in HeLa-CIITA. The absence of Cat S in our model cell lines and its induction by INF- γ suggest that it is the main enzyme responsible for efficient human p10 degradation.

Human Cat S cDNA was transfected in HeLa CIITA or MelJuSo cells, and Ii chain degradation was monitored by confocal microscopy (Fig. 3*c*). Cat S expression alone considerably reduced lysosomal Ii chain accumulation in both cell types, demonstrating that Cat S is responsible for p10 degradation.

Fig. 1. Invariant chain degradation. (a) Immunofluorescence microscopy of HeLa-CIITA stained for HLA-DR (Left, red) and Ii chain (Center, green). Colocalization is shown in the merged picture (Right, yellow). (b) [35 S]Met pulse-chase experiment was performed on HeLa-CIITA before HLA-DR immunoprecipitation. (c) HeLa-CIITA membrane (Left) and MelJuSo cells (Right) were immunoblotted for invariant chain. INF- γ stimulation (24 h) induces total Ii chain fragment disappearance, a phenomenon inhibited with 5 nM LHSV. INF- γ treatment does not increase MHC class II expression in HeLa-CIITA (β chain, Bottom), whereas incubation of MelJuSo cells with leupeptin induces p22 accumulation without affecting p10.

tion without participation of any other INF- γ -inducible molecule. The putative function of AEP in Ii chain degradation is still a matter of debate; nevertheless, its high level of transcription in MelJuSo cells and its leupeptin insensitivity argue against a major role in human p22 or p10 degradation.

Interestingly, p22 degradation seemed to vary from cell type to cell type. In MelJuSo cells, p22 was degraded normally in absence of Cat S, a situation reminiscent of the result obtained after LHSV treatment of the DR1-expressing HOM2 cells (11), but different from HeLa-CIITA, which accumulate p22. Cat S can therefore mediate human p22 degradation under specific conditions (HLA polymorphism?) (8), but clearly this activity, at the difference of p10 degradation, can also be performed by a nonspecialized leupeptin-sensitive enzyme.

Which Human Cathepsin Is Responsible for p10 Degradation? We focused on the cysteine proteases most likely to be involved in p10 degradation (8, 17). Based on available mouse data, ubiquitously expressed exopeptidase Cat H, as well as Cat O and the T/NK cell-specific Cat W, were not evaluated (29). RT-PCR experiments indicate that all of the cathepsin genes tested, with the exception of Cat S, were actively transcribed in HeLa-CIITA cells (including Cat C and Cat E, not shown). p10 accumulation in these human cells suggests that, contrary to mouse, Cat S might be the only enzyme able to degrade this Ii chain-processing intermediate efficiently. Therefore, we evaluated expression levels of Cat L, Cat V/L2, and Cat K to determine whether they are truly expressed as active proteases in HeLa-CIITA. Mature forms of these proteases were all detected by immunoblot (Fig. 4a), indicating that they are likely to be transported and activated

normally. Cat L was also shown by confocal microscopy to colocalize with DR in the lysosomes of HeLa-CIITA cells (Fig. 4b), a situation also true for Cat K (not shown). Finally, the active site directed probe CBZ-[125 I]Tyr-Ala-CN $_2$ was used to determine the activity levels of Cat B, Cat L, and Cat S in the endocytic pathway of HeLa-CIITA (Fig. 4c). Radioactive labeling of Cat L and B, and Cat S after INF- γ treatment, further demonstrates that these proteases are fully active in the endocytic pathway of the HeLa-CIITA cells.

Recent observations have linked Cat L stability to a chaperone-like activity of the Ii chain alternatively spliced product p41 (30). p41 was detected neither in HeLa-CIITA cells (INF- γ -treated or not), nor in MelJuSo cells (Fig. 2c). Ii chain alternative splicing is therefore dependent on factors different from CIITA and other INF- γ -inducible molecules. The absence of p41 could explain partially p10 accumulation in absence of Cat S by also reducing levels of active Cat L. Therefore, we transfected human p41 Ii chain cDNA in HeLa-CIITA and monitored Ii chain degradation as well as Cat L activity (Fig. 4d-e). The presence of human p41 did not affect p10 accumulation nor Cat L labeling with CBZ-[125 I]Tyr-Ala-CN $_2$, suggesting that p41 activity was not required to stabilize lysosomal Cat L in these cells.

To fully confirm our observations, we developed an *in vitro* p10 degradation assay to evaluate human Cat L activity (Fig. 5a). Membranes from HeLa CIITA cells were incubated for 2 h at pH 5 with increasing amounts of purified Cat L or purified Cat S. p10 could be resolved as two distinct products that were almost fully degraded by Cat S. Although Cat L was perfectly active on control mouse IgG, intact Ii chain and p10 were not hydrolyzed by the enzyme, even at concentrations 10-fold higher than those used to get efficient degradation by Cat S (11). Only high

Fig. 2. Transcription of cysteine proteases. (a) RT-PCR with primers designed to amplify human Cat K, V/L2, L, Z, S, and F was performed on HeLa and MelJuSo cells treated or not with INF- γ . (b) RT-PCR for the human AEP was performed on HeLa-CIITA and MelJuSo cells.

concentrations of Cat L (4 μ M) could induce partial degradation (likely to be nonspecific) of intact Ii chain and of the highest form of the Ii-p10 doublet. Human Cat L is therefore not able to degrade p10 efficiently either in cell-free assays or within cells.

p10 accumulation in the absence of Cat S could also be explained by a physical segregation in the lysosomes, preventing mature proteases from meeting with Ii chain. Detergent treatment of the membranes during the *in vitro* degradation assay should have abrogated any existing segregation among endogenous proteases and p10 molecules. The fact that p10 proteolysis *in vitro* is not observed in the absence of added exogenous Cat S suggests that the human cathepsins (Cat L, Cat V/L2, Cat K, and probably Cat F) present in HeLa CIITA intracellular compartments are unable efficiently to degrade p10. Human Cat S remains, therefore, the only protease able to perform this function in epithelial cells and probably other cell types.

Human Cat S mRNA Is Transcribed in TEC. The consequences of our findings with regard to human thymic selection are important. Mouse Cat L, which is closely homologous to both human Cat L and Cat V/L2, has been implicated in Ii chain degradation and T cell-positive selection by TEC (18). Our results argue against

Fig. 3. Cat S is induced by INF- γ . (a) Immunofluorescence microscopy of HeLa-CIITA stained for Cat S with (Right) or without (Left) INF- γ treatment. (b) Cat S was immunodetected in HeLa-CIITA membranes. (c) Confocal microscopy of HeLa-CIITA (Upper) and MelJuSo (Lower) cells transfected with human Cat S cDNA and stained for Cat S (Right) and Ii chain (Left). Neighboring nontransfected cells provide the control for Ii chain levels.

an involvement of human Cat L and Cat V/L2 (described to be mainly expressed in the thymus) with Ii degradation in epithelial cells. We therefore evaluated the possibility that Cat S could be expressed in human TEC. We analyzed the mRNA content of a human TEC line (P1.5D6) derived from cortical epithelium and expressing HLA-DR (Fig. 5b; ref. 21). Semiquantitative RT-PCR performed on P1.5D6 demonstrated the presence of Cat S mRNA, suggesting that the protease could be expressed in human TEC. This observation was confirmed in an explanted cortical thymic epithelial tumor in which Cat S transcripts were detected at a level comparable to PALA B cells (Fig. 5b). Independently of these experiments, transcription analysis by Affymetrix (Santa Clara, CA) GeneChip technology of highly purified human thymus cells indicates that Cat S transcription could be detected in all types of thymic cells, in particular medullary and cortical TECs (B. Kyewski, DKFZ Heidelberg, personal communication). These findings suggest that Cat S could be expressed in human thymic epithelium and thus facilitate Ii chain degradation independently from Cat L or Cat V/L2.

Fig. 4. Cathepsins are active in HeLa-CIITA. (a) Immunodetection of Cat K, V/L2, and L in HeLa-CIITA. (b) Confocal microscopy of HeLa-CIITA stained for Cat L (green) and HLA-DR (red). Colocalization is shown in the merged picture (yellow). (c) CBZ-[¹²⁵I]Tyr-Ala-CN₂ was used to label active Cat L, Cat B, and Cat S in the endocytic pathway of HeLa-CIITA cells. Labeling of purified Cat L and Cat S use in the *in vitro* assay is also shown. (d) Transfected human Ii chain p41 is immunodetected in HeLa-CIITA membranes. (e) HeLa-CIITA transfected with p41 cDNA were labeled with CBZ-[¹²⁵I]Tyr-Ala-CN₂ to monitor Cat L activity.

Discussion

Mouse Ii chain degradation has been studied in detail, and several cysteine proteases have been implicated in this process. Mouse Cat S is principally responsible for the last step of Ii chain processing, which cleaves the intermediate p10 fragment into CLIP. However, data from knockout mice indicate that proteases such as Cat L and Cat F can perform this function in different cell types or *in vitro* (10). We chose two human cell lines (HeLa-CIITA and MeJuSo), with minimal APC specialization and which strongly accumulate p10, to study human Ii chain processing. These cell lines were shown to express most cysteine proteases, with the exception of Cat S. Surprisingly, in addition to ubiquitous proteases such as Cat L, tissue-specific molecules like the thymus/testis-specific CAT V/L2 and the osteoclast-specific Cat K are also expressed in HeLa cells. Whether or not Cat V or Cat K expression results, from cell transformation or their distribution, are as specific as previously believed remains to be determined.

p10 accumulation in the presence of these active cathepsins suggests that the processing of Ii chain in human epithelial cells

Fig. 5. *In vitro* degradation of Ii chain. (a) HeLa-CIITA membranes were incubated alone or with purified human Cat L (1–4 μM) or Cat S (0.4 μM) for 2 h at 37°C. (b) Mouse IgG (detected by immunoblot) was added as a control to demonstrate Cat L activity. In contrast to Ii chain, an IgG fragment is resistant to Cat S activity, whereas it is degraded by Cat L. (c) Human TEC cells were tested by RT-PCR for cathepsin transcription. Cat S transcripts were detected both in freshly isolated tumor cells and in a long-term established TEC cell line (P1.4D6).

is mostly dependent on Cat S. Unfortunately, the quality of the available reagents did not allow us to rule out completely that Cat F could be inactive in these cells, although the high levels of mRNA found in HeLa and MeJuSo cells make this possibility unlikely. Interestingly, and in agreement with our observations, Cat S was recently suggested to be the major enzyme responsible for Ii chain processing in murine macrophages despite the presence of active Cat L and Cat F (31). Unexpectedly, Cat S, under certain conditions, can team up with a yet unidentified leupeptin-sensitive protease to degrade the intermediate p22. Cat S contribution to this process might depend on the HLA haplotype (8). Cat S is principally expressed in bone marrow-derived APCs, but human Cat S expression does not depend on CIITA, and its production *in vivo* could therefore be uncoupled from Ii chain or MHC expression. In the absence of Cat S, human p10 degradation is extremely inefficient, and the transport of MHC class II is probably delayed by a strong retention in the lysosomes. The presence of MHC class II at the surface of Cat S deficient cells could be explained by an extremely weak background degradation activity, resulting in the low steady-state surface levels observed in these cells. Alternatively, a contribution of HLA-DM to p10 removal from MHC class II cannot be excluded.

The detection of Cat S transcripts in human thymic epithelia underlines the differences existing with mice, in which Cat L deletion affects Ii chain degradation in TEC. Cat L clearly has additional functions in thymic selection because its processing activity is required for positive selection independently of degrading Ii chain (32). Our observations support a role for Cat S only in the degradation of p10 in all MHC class II-expressing tissues and suggest that results obtained in mice have to be considered with great care when transposed to human antigen

presentation. However, many other lysosomal proteases are likely to be important for generating antigenic peptides in the thymus of both mice and humans.

This work was supported by grants to P.P. from the Ministère de la Recherche et de la Technologie, the Association pour la Recherche sur le Cancer, and Centre National de la Recherche Scientifique (CNRS). J.B. is supported by CNRS and North Atlantic Treaty Organization fellowships, and E.G. is a European Union Marie Curie and Fondation pour la Recherche Médicale fellow.

- Hiltbold, E. M. & Roche, P. A. (2002) *Curr. Opin. Immunol.* **14**, 30–35.
- Neeffjes, J. J., Stollorz V., Peters P. J., Geuze H. J. & Ploegh, H. L. (1990) *Cell* **61**, 171–183.
- Stumptner-Cuvelette, P. & Benaroch, P. (2002) *Biochim. Biophys. Acta* **1542**, 1–13.
- Kropshofer, H., Arndt, S. O., Moldenhauer, G., Hammerling, G. J. & Vogt, A. B. (1997) *Immunity* **6**, 293–302.
- Hsieh, C. S., deRoos, P., Honey, K., Beers, C. & Rudensky, A. Y. (2002) *J. Immunol.* **168**, 2618–2625.
- Pierre, P. & Mellman, I. (1998) *Cell* **93**, 1135–1145.
- Lankar, D., Vincent-Schneider, H., Briken, V., Yokozeki, T., Raposo, G. & Bonnerot, C. (2002) *J. Exp. Med.* **195**, 461–472.
- Villadangos, J. A. (2001) *Mol. Immunol.* **38**, 329–346.
- Nakagawa, T. Y. & Rudensky, A. Y. (1999) *Immunol. Rev.* **172**, 121–129.
- Riese, R. J. & Chapman, H. (2000) *Curr. Opin. Immunol.* **12**, 107–113.
- Riese, R. J., Wolf, P. R., Brömme, D., Natkin, L. R., Villadangos, J. A., Ploegh, H. L. & Chapman, H. A. (1996) *Immunity* **4**, 357–366.
- Shi, G.-P., Villadangos, J. A., Dranoff, G., Small, C., Gu, L., Haley, K. J., Riese, R. J., Ploegh, H. L. & Chapman, H. A. (1999) *Immunity* **10**, 197–206.
- Nakagawa, T. Y., Brissette, W. H., Lira, P., Griffiths, D., Petrushova, R. J., Stock, J., McNeish, J. D., Eastman, S. E., Howard, E. D., Clarke, S. R. M., *et al.* (1999) *Immunity* **10**, 207–217.
- Riese, R. J., Shi, G.-P., Villadangos, J., Stetson, D., Driessen, C., Lennon-Dumenil, A. M., Chu, C. L., Naumov, Y., Behar, S. M., Ploegh, H. L., *et al.* (2001) *Immunity* **15**, 909–919.
- Shi, G.-P., Bryant, R. A., Riese, R., Verhelst, S., Driessen, C., Li, Z., Bromme, D., Ploegh, H. L. & Chapman, H. A. (2000) *J. Exp. Med.* **191**, 1177–1186.
- Punturieri, A., Filippov, S., Allen, E., Caras, I., Murray, R., Reddy, V. & Weiss, S. J. (2000) *J. Exp. Med.* **192**, 789–799.
- Watts, C. (2001) *Curr. Opin. Immunol.* **13**, 26–31.
- Nakagawa, T. Y., Roth, W., Wong, P., Nelson, A., Farr, A., Deussing, J., Villadangos, J. A., Ploegh, H. L., Peters, C. & Rudensky, A. Y. (1998) *Science* **280**, 450–453.
- Bromme, D., Li, Z., Barnes, M. & Mehler, E. (1999) *Biochemistry* **38**, 2377–2385.
- Santamaria, I., Velasco, G., Cazorla, M., Fueyo, A., Campo, E. & Lopez-Otin, C. (1998) *Cancer Res.* **58**, 1624–1630.
- Fernandez, E., Vicente, A., Zapata, A., Brera, B., Lozano, J. J., Martinez, C. & Toribio, M. L. (1994) *Blood* **83**, 3245–3254.
- Pierre, P., Denzin, L. K., Hammond, C., Drake, J. R., Amigorena, S., Cresswell, P. & Mellman, I. (1996) *Immunity* **4**, 229–239.
- Ting, J. P. & Trowsdale, J. (2002) *Cell* **109**, S21–S33.
- Karlsson, L., Pélérax, A., Lindstedt, R., Liljedahl, M. & Peterson, P. A. (1994) *Science* **266**, 1569–1573.
- Wubbolts, R., Fernandez-Borja, M., Oomen, L., Verwoerd, D., Janssen, H., Calafat, J., Tulp, A., Dusseljee, S. & Neeffjes, J. (1996) *J. Cell Biol.* **135**, 611–622.
- Brachet, V., Raposo, G., Amigorena, S. & Mellman, I. (1997) *J. Cell Biol.* **137**, 51–65.
- van's Gravesande, K. S., Layne, M. D., Ye, Q., Le, L., Baron, R. M., Perrella, M. A., Santambrogio, L., Silverman E. S. & Riese, R. J. (2002) *J. Immunol.* **168**, 4488–4494.
- Antoniou, A. N., Blackwood, S. L., Mazzeo, D. & Watts, C. (2000) *Immunity* **12**, 391–398.
- Turk, V., Turk, B. & Turk, D. (2001) *EMBO J.* **20**, 4629–4633.
- Lennon-Dumenil, A. M., Roberts, R. A., Valentijn, K., Driessen, C., Overkleef H. S., Erickson, A., Peters, P. J., Bikoff, E., Ploegh, H. L. & Wolf Bryant, P. (2001) *EMBO J.* **20**, 4055–4064.
- Beers, C., Honey, K., Fink, S., Forbush, K. & Rudensky, A. (2003) *J. Exp. Med.* **197**, 169–179.
- Honey, K., Nakagawa, T., Peters, C. & Rudensky, A. (2002) *J. Exp. Med.* **195**, 1349–1358.