The many roads to cross-presentation

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Cross-presentation of extracellular antigens by MHC class I molecules is required for priming cytotoxic T lymphocytes (CTLs) at locations remote from the site of infection. Various mechanisms have been proposed to explain cross-presentation. One such mechanism involves the fusion of the endoplasmic reticulum (ER) with the endosomal–phagosomal system, in which the machinery required for peptide loading of MHC class I molecules is introduced directly into the phagosome. Here, we discuss the evidence for and against the ER-phagosome concept as well as other possible mechanisms of cross-presentation.

The scientific community warmly receives data that support new theories addressing major problems in a field. These theories can sometimes become dogma—textbook knowledge—even when based on inconclusive data. Such data is difficult to correct with essentially negative results, and such results equally are difficult to publish. A recent publication by Touret et al. (1) is an exception to this rule. In this study, the concept of ER–phagosome fusion was tested and refuted, leaving open several alternative routes for cross-presentation. Here we discuss recent studies on the biology of cross-presentation and explain why the ER–phagosome mechanism is unlikely to contribute to this process.

Classical and cross-presentation by MHC class I molecules

Cross-presentation is the process by which extracellular antigens, which are normally presented in association with MHC class II molecules, are instead presented by MHC class I molecules. This differs from the classical MHC class I processing pathway in which MHC class I molecules present antigens that are synthesized within the cell. Classical MHC class I antigen presentation begins with the degradation of intracellularly synthesized proteins by the proteasome. Only a fraction of the peptide fragments that result from this degradation survives complete destruction and is transported into the ER by the peptide transporter TAP (transporter associated with antigen presentation) (2). In the ER, the peptides are loaded onto newly synthesized MHC class I molecules, and these complexes are then transported to the cell surface (3). In contrast, the MHC class II processing pathway is dedicated to the presentation of exogenous and self-antigens that are degraded in the endocytic pathway. The proteases involved in endocytic degradation (cathepsins) are different than those used in the MHC class I pathway. Peptides are formed as intermediates during late endosomal protein degradation and are loaded onto MHC class II molecules in a reaction catalyzed by the chaperone protein HLA-DM before transport to the plasma membrane (4). MHC class I and MHC class II molecules thus sample antigenic information from different sources, intracellular and extracellular antigens, respectively. A major exception occurs during cross-presentation.

In vivo, DCs—the major cell type responsible for cross-presentation—acquire endogenous antigens from infected cells in the periphery, and then migrate to the lymph nodes where they display antigenic peptides in association with MHC class I molecules. MHC class I–peptide complexes are recognized by antigen-specific CTLs, which become activated and expand in response to antigen recognition (5). In this scenario, the source of antigens (intracellular, but from a different cell, or extracellular as in vaccination settings) is distinct from that usually sampled by the classical MHC class I antigen presentation pathway (intracellular antigens within the antigen presenting cell). Hence the mechanism of antigen degradation and delivery of the peptide to MHC class I molecules is also likely to be different. The mechanism of cross-presentation has garnered much interest in recent years, in part because cross-presentation is likely to be important in activating CTLs in response to vaccine antigens. But the mechanism (or mechanisms) has yet to be definitively defined.

Deciphering mechanism

There are many difficulties inherent in defining the mechanism of cross-presentation. One is the source of antigen. In vaccine studies, heat shock proteins (such as gp96), apoptotic bodies, the content of late endosomes (exosomes), cell lysates, intact cells, peptides, antibodies, and bead-associated proteins have all been used as sources of antigen (3). Most of these antigens are extracellular but are derived from an intracellular source (the infected cell) and are likely liberated as a result of cell lysis. But intracellular antigens from intact cells can also be cross-presented. One way this could occur is through the swapping of intracellular peptides through gap junctions (6). It has been known for a long time that most tissue cells are electrically coupled with their neighboring cells through gap junctions, small channels that connect the cytosols of adjacent cells. Dendritic cells and activated monocytes can also establish gap junctions with other cells—including infected cells—and thereby acquire antigenic fragments for cross-presentation (6). Notably, tumors usually close their gap junctions, opting to live solitary lives. This may explain why tumors often elicit poor CTL responses. Still, this immunological coupling through gap junctions could explain cross-presentation under conditions in which the an-
A gap-expressing cell does not release the antigen into the extracellular milieu.

Coupling of antigen-containing cells and APCs by gap junctions does not, however, explain how extracellular antigens, such as those used in vaccination studies, are cross-presented. Previous studies with a variety of extracellular antigens have demonstrated crucial roles for TAP (7) and the proteasome (8, 9) in cross-presentation. One interpretation of these results is that these antigens (or peptides derived thereof) somehow enter the cytosol of DCs, making them available for proteosomal degradation, transport into the ER, and presentation on MHC class I molecules. Another possibility lies in the observation that MHC class I molecules can be recycled from the cell surface along the endocytic MHC class II pathway and exchange endogenous for exogenous peptides en route (10). Notably, TAP and proteasome activities are both required for surface expression of MHC class I molecules (11), without which the recycling pool cannot exist. Hence, the involvement of TAP or proteasomes in cross-presentation is not necessarily evidence for entry of exogenous antigens into the cytosol of DCs, but it also does not exclude this possibility. Recent studies revealed a role for a putative endocytosis signal in MHC class I (12) and for endosomal proteases (13) in cross-presentation, which support a role for the recycling pathway. In this model, antigens would be degraded by endocytic proteases rather than the proteasome, and thus some antigens that would normally be presented in the classical pathway might not survive to be cross-presented.

Figure 1. Various models of cross-presentation. In the classical MHC class I antigen–presenting pathway, intracellular antigens are degraded by the proteasome and peptidases. A fraction of the resulting peptides associate with TAP in the ER membrane where newly synthesized MHC class I molecules are arrested until loaded with peptide. MHC class I–peptide complexes then leave the ER and are transported to the plasma membrane. Extracellular antigens can enter this pathway in various ways. (A) Gap junctions allow direct transfer of peptides from infected cells into the cytosol of DCs. (B) MHC class I molecules can enter the recycling pathway and exchange peptides. (C) ER components become an integral part of the phagosomal pathway. The ERAD pathway then exports exogenous antigen from the phagosome into the cytosol and phagosomal TAP allows retro-transport of peptides back into the phagosome. (D) Exogenous antigens can be transported over the endosomal membrane. (E) Exosomes secreted by infected cells can bind to DCs for cross-presentation.
Other mechanisms besides the recycling pathway might also result in cross-presentation (Fig. 1). For example, exosomes—vesicles derived from the interior of endocytic structures that are released by many cell types—can also induce CTL responses by cross-presentation (14). Whether simple binding of these small vesicles, which contain MHC class I–peptide complexes, to the plasma membrane of DCs suffices to trigger CTL activation is unclear and the mechanism is still poorly defined. In addition, various experiments have shown that extracellular proteins can be transferred from endosomes into the cytosol of DCs (9), although how this occurs is unclear. It might involve dissolution of the endocytic membrane or specific protein transporters that pump the antigen out of endosomes and/or lysosomes. Note that solubilization of an antigen–containing endocytic structure would liberate endosomal proteases and likely result in the death of the cross-presenting cell.

ER–phagosome model

Recently, at least three papers offered an alternative model of cross-presentation: direct fusion of phagosomes with the ER membrane. In other words, they propose that the phagosomal membrane is formed—entirely or in part—from the ER membrane. As a consequence of this fusion, the enzymatic machinery required for the release of phagosomal proteins into the cytosol (the ER–associated degradation [ERAD] system) and the MHC class I loading machinery become an integral part of the endocytic system (15, 16). The ERAD system, which shuttles misfolded proteins from the ER into the cytosol for proteasomal degradation, would thus become the phagosome–to–cytosol protein transporter mentioned earlier. This model offers a new mechanistic explanation for MHC class I cross-presentation, but has recently been tested and refuted (1). Moreover, we suggest that this model is problematic for other reasons and is thus unlikely to contribute significantly to cross-presentation in vivo.

Early studies of bacteria that live and propagate in phagosomes suggested that the phagosomal membrane was largely derived from the plasma membrane, with a minor contribution from other endocytic structures including late endosomes, lysosomes, and MIICs (vesicles that accumulate MHC class II molecules) (17). But more recent studies—most of which used synthetic beads as a substitute for antigen—found ER–specific proteins such as calnexin, calreticulin, and the ERAD translocon subunit Sec61p in the isolated bead-containing fractions (18). Based on this finding, the authors concluded that the membrane of the bead-containing phagosome (beadosome) was derived, at least in part, from the ER membrane. But these results could also be explained by contamination of the beadosome membrane with ER–derived vesicles during purification.

Electron microscopy has also been used to show that the phagosomal membrane is formed from the ER. Gagnon et al. showed that the ER membrane and the plasma membrane fused at the site of bead contact (18). However, in that study the content of the ER lumen did not diffuse into the extracellular medium and membrane-like structures that separated the ER from the phagocytic cup were still visible, suggesting the possibility that bona fide fusion did not occur. The authors also noted that an ER–specific enzymatic activity (glucose–6–phosphatase [G6Pase]) was detected in the beadosome. Since then, several new isotypes of G6Pase have been identified, only one of which contains an ER retention motif (19). Thus, it is possible that this enzyme might be more widely localized than it was originally thought to be.

Despite these caveats, the concept that the ER contributes to the phagosomal membrane is highly attractive as it provides a mechanistic explanation for the cross-presentation of extracellular proteins. More recently, two papers were published claiming that this route was operational when antigens were given in association with 3-μm beads (15, 16). Whether other antigen cocktails utilize the same pathway was not addressed. These studies also failed to satisfactorily address the underlying issue of ER contamination, which renders the localization of ER–specific markers (such as Sec61, TAP, tapasin, calreticulin, and Erp57) in purified bead-containing vesicles open to alternative interpretations. Indeed, Guermonprez et al. used cryo–electron microscopy to detect the ER marker calreticulin directly by antibodies (10). They failed to detect these at the phagosomal membrane but only “in close apposition” in the ER.

In the study by Houde et al., the proteasome and undefined polyubiquitinated proteins were coisolated with the beadosome, and this resulted in a rather eccentric model (16). In this model, the bead–associated antigens are pumped from the beadosome into the cytosol by the Sec61–containing ERAD system (20) and are ubiquitinated by beadosome–associated enzymes during retrotranslocation. In a sort of a coupled reaction, the retrotranslocated antigens are degraded by the beadosome–associated proteasome, and the resulting peptide fragments associate exclusively with TAP complexes located in the beadosome. If correct, this suggests that the physical laws for Brownian motion do not apply to bead–derived antigens since they and their degradation products “know” where to be targeted to: beadosome–associated proteasomes and TAP, respectively. Ackerman et al. used another approach to test the feasibility of direct fusion of the ER to bead-containing phagosomes (21). They performed the same type of experiments as discussed above (with similar problems) but also showed that a soluble viral TAP inhibitor (US6) could access a macropinocytic compartment and block cross-presentation of a cointernalized soluble protein. However, a subsequent paper by the same authors showed that exogenous proteins could follow a retrograde transport pathway from endosomes, through the Golgi and back into the ER (22). This suggests that soluble proteins might be cross-pre-
resented as a result of their ability to directly access the MHC class I processing machinery in the ER lumen. Retrograde transport through the Golgi could thus explain how soluble antibodies are able to gain access to the ER and why soluble US6 inhibits cross-presentation. In other words, it does not prove the existence of a mixed ER–phagosome fusion, but rather reveals yet another potential mechanism by which extracellular antigens could be cross-presented.

So does the ER–phagosome exist? As mentioned earlier, experiments using bacterial phagosomes suggested that the plasma membrane around ingested beads or bacteria (1) could be cross-presented. However, no data are available on this point.

One experiment deserves special attention because of its elegance. In this experiment, the biotin-binding protein avidin was expressed with an ER retention signal (KDEL) in a macrophage cell line. Beads coated with biotin were then phagocytosed by the cells, and direct contact between the ER–retained avidin and bead-associated biotin was quantified. No avidin–biotin interactions were observed, casting more doubt on the concept of direct interaction between the ER and phagosomes (1). Touret et al. conclude that the plasma membrane and the endocytic pathway are the major sources for phagosomal membranes, with no significant contribution by the ER (estimated between 0 and 10%) (1).

Calculating the odds of ER–phagosome fusion

The feasibility of this model may be deduced by “number crunching” according to Yewdallian philosophy (25). An estimation of the numbers of MHC class I molecules entering a 3-μm bead-induced phagosome (15, 16) from the ER and the numbers of molecules required to initiate cross-presentation may help reveal whether the ER–phagosome fusion mechanism is plausible. If ER–derived membrane constituted 10% of the phagosomal membrane, would this suffice for efficient cross-presentation? If one approximates that an average rounded cell has a radius of \(~10~\mu m\) and that the plasma membrane is contributed to the membrane of the 3-μm bead-induced phagosome (radius of \(\sim 1.5~\mu m\)). The ER contributes \(~60\%\) of cellular lipids (compared with the plasma membrane’s \(5\%\)) (26), which means that \(0.16\%\) (\(2 \cdot 10^{-2} \times \frac{5}{60}\)) of the ER membrane would be donated to each beadosome, if the beadosome membrane was composed entirely of ER–derived membrane.

MHC class I molecules have a half-life of over 12 h (although this varies somewhat in different cell types) but are available for peptide loading in the ER for 30 min or less (27). Even with this conservative estimation, this implies that at every moment less than 100,000 peptide-receptive MHC class I molecules would be located in the ER of a DC that contains a total of 2 million MHC class I molecules. Of these, 16 (100,000 \(\times 2 \cdot 10^{-2} \times \frac{5}{60}\)) \(\times 0.1\) MHC class I molecules would enter one 3-μm phagosome at a 10% contribution of the ER and 160 molecules at a 100% contribution. These numbers would decrease ninefold for a 1-μm bead and even more for soluble antigens and immune complexes.

A minimum of 40 and 400 MHC–peptide complexes is reported to be required for stimulation of primed and naive T cells, respectively (28). Based on our calculations, T cell activation would thus occur only if nearly all the peptides delivered into the cytosol from bead-derived phagosomes found their way back into phagosomes without any competition from endogenous peptides. Given that endogenous peptides are present in the cytoplasm even before exogenous antigens are degraded (2), beadosome-derived antigenic peptides would likely be outcompeted by endogenous peptides and would thus return to the beadosomes too late to load the few ER–derived MHC class I molecules at that location. However, if the ER translocon protein Sec61, which also inserts polypeptides into the ER lumen as they are translated, is introduced in the phagosome, as suggested by previous studies (15, 16, 18), translation of novel proteins could continue at the phagosomal membrane and result in the deposition of de novo–translated proteins directly into the phagosome. The phagosome would thus mimic the ER by allowing the introduction of nascent proteins (including MHC class I molecules), but no data are available on this point.

Our own electron microscopy analysis shows that typical ribosomal structures can be found associated to the ER membrane, but no such structures were localized to the beadosome membrane (Fig. 2). Bead-induced cross-presentation therefore must rely on the few ER–derived MHC class I molecules, or on cell surface–derived MHC class I molecules (2 million \(\times 2 \cdot 10^{-2} = 40,000\) surface MHC class I molecules in a 3-μm beadosome) for cross-presentation of phagosomal antigens. Surface MHC class I molecules can efficiently exchange peptides between pH 4.5 and 5.5 (10), suggesting that peptide loading could take place in the phagosomal environment, although the loading would be considerably less efficient than in the “specialized” MHC class I–loading complex in the ER. However, inefficiency would not be a major issue in the recycling pathway as 40,000 surface–derived MHC
Figure 2. Beodosomes, ER, ribosomes, and mitochondria in a DC. 1-μm latex beads were endocytosed by human monocyte-derived DCs for 30 min before processing for electron microscopy. The cells were fixed with a mixture of formaldehyde and glutaraldehyde and embedded in epon for thin sectioning. The bead, mitochondria (Mt), and two ER profiles are indicated. The arrowheads point to ribosomes in association to the ER membrane. No such structures are observed on the beadosomal membrane. The box denotes a position in the section with lower resolution where the ER is apposite to the mitochondrion. Bar, 200 nm.

class I molecules would be available to bind peptides, whereas only 160 MHC class I molecules would be available if the beadosome membrane were derived entirely from the ER.

Concluding considerations

Various studies have reached diametrically opposing conclusions regarding the origin of the phagosomal membrane—a crucial question underlying the mechanism of cross-presentation. Gagnon et al. showed an ER contribution to the phagosomal membrane in macrophages, but not in other cells such as neutrophils (18). These experiments could also not be confirmed in macrophages and DCs in the recent study by Touret et al. (1). Although the studies that argue for the ER–phagosomal fusion mechanism received much attention, they did not address the mechanism of cross-presentation of physiological antigens, including antibody-bound antigens (29), soluble antigens (30), and intracellular antigens (6), which might all follow distinct pathways of degradation inside the cell. Furthermore, a simple calculation predicts that cross-presentation via the ER–phagosomal pathway would be highly inefficient, if at all possible.

Apart from being an interesting biological question, cross-presentation has direct consequences for vaccination strategies aimed at inducing CTL responses. These vaccines should be able to induce potent CTL responses and T cell memory, and the specificity of the CTL response will result from the cross-presentation of antigenic fragments. Understanding the mechanism(s) of cross-presentation will help rationalize vaccine development and improve the chances to arrive at successful antiviral and antitumor vaccines.

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REFERENCES

1. Touret, N., P. Paroutis, M. Terbizuk, R. Harrison, S. Troumbeta, M. Pypaert, A. Chow, A. Jiang, J. Shaw, C. Yip, et al. 2005. Quantitative and dynamic assessment of the contribution of the endoplasmic reticulum to phagosome formation. Cell. 123:157–170.
2. Reits, E., A. Griebskpor, J. Neijssen, T. Groothuis, K. Jalink, P. van Veelen, H. Janssen, J. Calafat, J.W. Drijfhout, and J. Neefjes. 2003. Peptide diffusion, protection, and degradation in nuclear and cytoplasmic compartments before antigen presentation by MHC class I. Immunity. 18:97–108.
3. Groothuis, T.A., A.C. Griebskpor, J.J. Neijssen, C.A. Herberts, and J.J. Neefjes. 2005. MHC class I alleles and their exploration of the antigen-processing machinery. Immunol. Rev. 207:60–76.
4. Bryant, P.W., A.M. Lennon-Dunedd, E. Fiebig, C. Lagaundres-Gedert, and H.L. Ploog. 2002. Proteolysis and antigen presentation by MHC class II molecules. Adv. Immunol. 80:71–114.
5. Heath, W.R., G.T. Belz, G.M. Behrens, C.M. Smith, S.P. Forehan, I.A. Parish, G.M. Davey, N.S. Wilson, F.R. Carbone, and J.A. Villadangos. 2004. Cross-presentation, dendritic cell subsets, and the generation of immunity to cellular antigens. Immunity. 19:9–26.
6. Neijssen, J.C. Herberts, J.W. Drijfhout, E. Reits, L. Janssen, and J. Neefjes. 2005. Cross-presentation by intercellular peptide transfer through gap junctions. Nature. 434:83–88.
7. Huang, A.Y., A.T. Bruce, D.M. Pardoll, and H.I. Levinsky. 1996. In vivo cross-priming of MHC class I-restricted antigens requires the TAP transporter. Immunity. 4:349–355.
8. Ruedl, C., T. Storni, F. Lehner, T. Bach, and M.F. Bachmann. 2002. Cross-presentation of virus-like particles by skin-derived CD8(–) dendritic cells: a dispensable role for TAP. Eur. J. Immunol. 32:818–825.
9. Norbury, C.C., L.J. Hewlett, A.R. Prescott, N. Shastri, and C. Watts. 1995. Class I MHC presentation of exogenous soluble antigen via macrophocytosis in bone marrow macrophages. Immunity. 3:783–791.
10. Gromme, M., F.G. Uytdehag, H. Janssen, J. Calafat, R.S. van Binnendijk, M.J. Kenter, A. Tulp, D. Verwoerd, and J. Neefjes. 1999. Recyling MHC class I molecules and endosomal peptide loading. Proc. Natl. Acad. Sci. USA. 96:10326–10331.
11. Chefalo, P.J., A.G. Grande III, L. Van Kaer, and C.V. Harding. 2003. Tapanin–/– and TAP1–/– macrophages are deficient in vacuolar alternate class I MHC (MHC-I) processing due to decreased MHC-I stability at phagolysosomal pH. J. Immunol. 170:5825–5833.
12. Lizee, G., G. Basha, J. Tiong, J.P. Julien, M. Tian, K.E. Biron, and W.A. Jeffress. 2003. Control of dendritic cell cross-presentation by the major histocompatibility complex class I cytoplasmic domain. Nat. Immunol. 4:1063–1073.
13. Shen, L., L.J. Sigal, M. Boes, and K.L. Rock. 2004. Important role of cathepsin S in generating peptides for TAP-independent MHC class I crosspresentation in vivo. Immunity. 21:155–165.
14. Wolfsr, J., A. Lozier, G. Raposo, A. Regnault, C. Thery, C. Maurier, C. Flamign, S. Pouzieux, F. Faure, T. Turz, et al. 2001. Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. Nat. Med. 7:297–303.
15. Guernempez, P., L. Saveau, M. Kleijnme, J. Davoust, P. Van Endert, and S. Amigorena. 2003. ER-phagosome fusion defines an MHC class I cross-presentation compart-
ment in dendritic cells. Nature. 425:397–402.

16. Houde, M., S. Bertholet, E. Gagnon, S. Brunet, G. Goyette, A. Laplante, M.F. Principotta, P. Thibault, D. Sacks, and M. Desjardins. 2003. Phagosomes are competent organelles for antigen cross-presentation. Nature. 425:402–406.

17. Touret, N., P. Paroutis, and S. Grinstein. 2005. The nature of the phagosomal membrane: endoplasmic reticulum versus plasma-lemma. J. Leukoc. Biol. 77:878–885.

18. Gagnon, E., S. Duclos, C. Rondeau, E. Chevet, P.H. Cameron, O. Steele-Mortimer, J. Paiement, J.J. Bergeron, and M. Desjardins. 2002. Endoplasmic reticulum-mediated phagocytosis is a mechanism of entry into macrophages. Cell. 110:119–131.

19. Guionie, O., E. Clottes, K. Stafford, and A. Burchell. 2003. Identification and characterisation of a new human glucose-6-phosphatase isoform. FEBS Lett. 551:159–164.

20. Wiertz, E.J., D. Tortorella, M. Bogyo, J. Yu, W. Mothes, T.R. Jones, T.A. Rapoport, and H.L. Ploegh. 1996. Sec61-mediated transfer of a membrane protein from the endoplasmic reticulum to the proteasome for destruction. Nature. 384:432–438.

21. Ackerman, A.L., C. Kyritsis, R. Tampe, and P. Cresswell. 2003. Early phagosomes in dendritic cells form a cellular compartment sufficient for cross presentation of exogenous antigens. Proc. Natl. Acad. Sci. USA. 100:12889–12894.

22. Ackerman, A.L., C. Kyritsis, R. Tampe, and P. Cresswell. 2005. Access of soluble antigens to the endoplasmic reticulum can explain cross-presentation by dendritic cells. Nat. Immunol. 6:107–113.

23. Russell, D.G. 2003. Phagosomes, fatty acids and tuberculous. Nat. Cell Biol. 5:776–778.

24. Cossart, P., and P.J. Sansonetti. 2004. Bacterial invasion: the paradigms of enteroinvasive pathogens. Science. 304:242–248.

25. Yewdell, J.W. 2001. Not such a dismal science: the economics of protein synthesis, folding, degradation and antigen processing. Trends Cell Biol. 11:294–297.

26. Alberts, B., A. Johnson, J. Lewis, M. Raff, K. Roberts, and P. Walter. 2002. Molecular Biology of the Cell. 4th edition. Garland Science, New York. 661 pp.

27. Neefjes, J.J., G.J. Hammerling, and F. Momburg. 1993. Folding and assembly of major histocompatibility complex class I heterodimers in the endoplasmic reticulum of intact cells precedes the binding of peptide. J. Exp. Med. 178:1971–1980.

28. Kimachi, K., M. Croft, and H.M. Grey. 1997. The minimal number of antigen-major histocompatibility complex class II complexes required for activation of naive and primed T cells. Eur. J. Immunol. 27:3310–3317.

29. den Haan, J.M., and M.J. Bevan. 2002. Constitutive versus activation-dependent cross-presentation of immune complexes by CD8(+) and CD8(−) dendritic cells in vivo. J. Exp. Med. 196:817–827.

30. Kurts, C., H. Kosaka, F.R. Carbone, J.F. Miller, and W.R. Heath. 1997. Class I-restricted cross-presentation of exogenous self-antigens leads to deletion of autoreactive CD8(+) T cells. J. Exp. Med. 186:239–245.