Major Histocompatibility Complex Class I–restricted Cross-presentation Is Biased towards High Dose Antigens and Those Released during Cellular Destruction

By Christian Kurts, Jacques F. A. P. Miller, Rathan M. Subramaniam, Francis R. Carbone, and William R. Heath

From the Immunology Division, The Walter and Eliza Hall Institute of Medical Research, Parkville 3050, Victoria, Australia; and The Department of Pathology and Immunology, Monash Medical School, Prahran 3181, Victoria, Australia

Summary

Naive T cells recirculate mainly within the secondary lymphoid compartment, but once activated they can enter peripheral tissues and perform effector functions. To activate naive T cells, foreign antigens must traffic from the site of infection to the draining lymph nodes, where they can be presented by professional antigen presenting cells. For major histocompatibility complex class I–restricted presentation to CD8+ T cells, this can occur via the cross-presentation pathway. Here, we investigated the conditions allowing antigen access to this pathway. We show that the level of antigen expressed by peripheral tissues must be relatively high to facilitate cross-presentation to naive CD8+ T cells. Below this level, peripheral antigens did not stimulate by cross-presentation and were ignored by naive CD8+ T cells, although they could sensitize tissue cells for destruction by activated cytotoxic T lymphocytes (CTLs). Interestingly, CTL-mediated tissue destruction facilitated cross-presentation of low dose antigens for activation of naive CD8+ T cells. This represents the first in vivo evidence that cellular destruction can enhance access of exogenous antigens to the cross-presentation pathway. These data indicate that the cross-presentation pathway focuses on high dose antigens and those released during tissue destruction.

Key words: CD8+ T lymphocytes • T cell activation • antigen presentation • antigen presenting cells • apoptosis

CD8+ CTLs, which recognize antigens presented by class I molecules encoded by the MHC, are important for immunity to viruses and other intracellular pathogens. Access to the class I–restricted presentation pathway generally requires that protein antigens are expressed within the cytoplasmic compartment of the presenting cell (1). This pathway has been described as the endogenous pathway, contrasting it with the class II–restricted pathway, which generally presents exogenous (extracellular) antigens.

Despite the apparent demarcation between the class I– and class II–restricted pathways, Bevan demonstrated over 20 years ago that under certain circumstances exogenous antigens can access the class I–restricted pathway (2). Such “cross-presentation” has now been observed in many tolerogenic as well as immunogenic responses (2–14). It has been proposed that the role of cross-presentation is to protect against tissue-tropic viruses that, in the absence of infection of professional APCs, would not access the class I–restricted pathway of these cells, and would therefore fail to prime naive CTLs (15, 16). In this model, the advantage of the cross-priming APC is that it can capture antigens from virus-infected tissues, process them into the class I pathway, and present them to naive CTLs in the draining LNs.

Recently, we used transgenic expression of the model antigen OVA to provide direct evidence that peripheral tissue antigens can be transported to draining LNs for class I–restricted presentation by a bone marrow–derived APC (10). In this earlier study, transgenic mice expressing membrane-bound OVA (mOVA) under the control of the rat insulin promoter (RIP) in the pancreas, kidney, and thymus were injected with class I–restricted, OVA–specific, CD8+ T cells from the OT-I transgenic line. When responses were assessed several days later, OT-I cells had proliferated and accumulated in nodes draining OVA–expressing tissues. Furthermore, antigen was presented by a bone marrow–derived cell, implying that tissue antigens were captured and then...
processed by a professional APC (the cross-presenting APC).

In this report, we examined the requirements for antigen entry into the cross-presentation pathway, using various transgenic mice expressing the model antigen OVA at different levels in the pancreas. Our studies show that only relatively high dose antigens constitutively gain effective entry into this pathway, but that cellular destruction may enhance access of low dose antigens. The implications of these findings with respect to immunity and tolerance are discussed.

Materials and Methods

Mice. All mice were bred and maintained at the Walter and Eliza Hall Institute for Medical Research. OT-I, RIP-mOVA, and RIP-OVA<sup>lo</sup> transgenic mice have been described previously (10, 17). Note that RIP-OVA<sup>lo</sup> mice were referred to previously as RIP-OVA mice (17). RIP-OVA<sup>hi</sup> mice were generated using the same techniques.

Adoptive Transfer and FACS<sup>®</sup> Analysis. Preparation and adoptive transfer of OT-I cells, 5,6-carboxy-succinimidyl-fluorescin-ester (CFSE)-labeling, and analysis on a FACScan<sup>®</sup> (Becton Dickinson, Mountain View, CA) were carried out as described previously (11, 18, 19). PE-conjugated anti-CD8 (YTS 169.4) was from Caltag Labs (South San Francisco, CA). Dead cells were excluded by propidium iodide staining.

Activation of OT-I Cells In Vitro. OT-I cells were activated by culturing 10<sup>7</sup> OT-I spleen cells for 5 d in 30 ml of medium with 10<sup>8</sup> irradiated (1,500 <cGy>) B6 spleen cells previously coated for 30 min at 5 x 10<sup>7</sup> cell/ml with 0.2 mg/ml OVA<sup>257–264</sup> peptide. Stimulator cells were washed once after peptide pulsing. Culture medium consisted of mouse tonicity RPMI1640, 10% fetal calf serum, 2 mM glutamine, 5 x 10<sup>-5</sup> M 2-ME, and antibiotics.

Results

Antigen Dose Affects Cross-presentation. To examine the role of antigen dose in cross-presentation of tissue antigens, two lines of transgenic mice were produced expressing different amounts of secreted OVA in the β cells of the pancreas, under the control of the RIP. These were termed RIP-OVA<sup>lo</sup> and RIP-OVA<sup>hi</sup> mice. RIP-OVA<sup>hi</sup> mice expressed sufficient OVA to show weak OVA-specific staining immunohistologically, whereas OVA expression by RIP-OVA<sup>lo</sup> mice could not be detected by this technique (Fig. 1). However, expression of OVA by the latter line has been shown previously using alternative approaches (reference 17 and see Fig. 3).

To investigate the effect of antigen dose on the access of peripheral tissue antigens to the cross-presentation pathway, naïve CD<sup>8</sup> cells from the OT-I cell line were labeled with the fluorescent dye CFSE (11, 18, 19) and injected intravenously into both RIP-OVA<sup>lo</sup> and RIP-OVA<sup>hi</sup> mice. 3 d later, the pancreatic (draining) and inguinal (nondraining) LNs were examined for proliferating OT-I cells. When CFSE-labeled cells proliferate, their fluorescence is equally distributed between daughter cells resulting in a 2<sup>n</sup>-fold reduction in fluorescence intensity, where n is the number of cell divisions. Adoptive transfer of CFSE-labeled OT-I cells revealed that only RIP-OVA<sup>hi</sup> mice were able to supply sufficient OVA to the cross-presentation pathway for stimulation of naïve OT-I cells in the pancreatic draining LN (Fig. 2). These findings indicated that antigen expres-
Lower Levels of Tissue Antigen Expression May Still Be of Physiological Relevance. To determine whether the lower antigen dose expressed by RIP-\textsuperscript{OVA\textsubscript{lo}} mice was of physiological relevance, we asked whether activated CTLs, which have the capacity to enter tissues, could recognize tissue cells expressing this low dose of OVA. RIP-\textsuperscript{OVA\textsubscript{lo}} mice were injected with 10^7 activated OT-I cells (generated by stimulation with antigen for 5 d in vitro) and then examined for the onset of diabetes. With proliferation was abolished (data not shown). By contrast, CFSE-labeled B6 CD8\textsuperscript{T} cells did not proliferate in the pancreatic LNs in either the presence (Fig. 4, E and F) or absence (data not shown) of activated CTLs. Thus, an antigen-specific response of OT-I cells was observed after the destruction of β cells by activated CTLs. When proliferation was examined in bm1→RIP-mOVA.B6 bone marrow chimeras, where the bone marrow compartment expressed an inappropriate MHC haplotype for presentation of OVA to OT-I cells (10), proliferation was abolished (data not shown). This latter point indicates that a bone marrow-derived APC was responsible for capturing and cross-presenting OVA released by cellular destruction.

To examine the kinetics of cross-presentation after cellular destruction, RIP-\textsuperscript{OVA\textsubscript{hi}} mice were treated with activated CTLs to cause islet damage and then injected with CFSE-labeled naive OT-I cells either 1 or 4 d later. In previous studies we had shown that it takes 24 h for OT-I cells to begin proliferating after exposure to cross-presented antigen (11). Thus, Figure 4.(351,519),(588,900) Activated OT-I cells cause diabetes when adoptively transferred into RIP-OVA\textsuperscript{lo} mice or RIP-OVA\textsuperscript{hi} mice. OT-I cells were activated with antigen in vitro for 5 d and then 10^7 cells were adoptively transferred intravenously into adult RIP-OVA\textsuperscript{hi} (solid line) or RIP-OVA\textsuperscript{lo} (dashed line) mice. These mice were then monitored daily for induction of diabetes as measured by glucosuria. Islet destruction was confirmed by histological examination of pancreatic tissue sections from several of these mice. These data represent the compilation of five experiments.
roughly estimate the minimum time necessary for antigen to appear in LNs after OT-I cell-induced β cell damage. Simply, since it takes at least 24 h for the cells to start proliferating after exposure to cross-presented antigen (11), the antigen must reach the LNs no earlier than day 3 after induction of tissue damage based on the lack of proliferation found in Fig. 4 G, and no later than day 6 given the proliferation shown in Fig. 4 C.

Discussion

As previously reported, antigens expressed in peripheral tissues can be captured by bone marrow-derived APCs, processed via the cross-presentation pathway, and then presented in the draining LNs to naive CD8⁺ T cells (10). In this report, we show that peripheral tissue antigens must be expressed at relatively high levels to be cross-presented constitutively. When antigens were expressed at high doses, as in RIP-OVA² mice (Fig. 2), they were cross-presented in the draining LNs and caused activation and proliferation of naive CD8⁺ T cells. Antigen expression levels below a certain threshold, as seen in RIP-OVA⁰ mice, were ignored by naive CD8⁺ T cells, which failed to proliferate in the draining LNs (Fig. 2).

Here, we also show that antigen access to the cross-presentation pathway can result after cellular destruction by CTL (Fig. 4). How CTL lysis allows antigen entry into this pathway is unknown, but it has recently been reported that apoptotic cells can be captured and presented by myeloid dendritic cells (20). This raises the possibility that apoptosis of target cells by activated CTLs may facilitate access to the cross-presentation pathway.

One of the critical observations of this report is that although lower dose antigens were unable to stimulate via the cross-presentation pathway, they still directly sensitized tissue (islet) cells, in which they were expressed, for recognition by activated CTLs. However, the direct recognition pathway is only available for recognition by activated CTLs, since only activated CTLs will have the capacity to leave the secondary lymphoid compartment and interact with these tissues. Thus, there appear to be three categories into which peripheral tissue antigens fall: category 1, those that are expressed at high levels and can be both cross-presented to naive CTLs and directly seen by activated CTLs; category 2, those that are expressed at lower concentrations and are nonstimulatory via the cross-presentation pathway, but can sensitize peripheral tissue cells for direct recognition by activated CTLs; and category 3, those antigens that can never be seen by CTLs.

The importance of these categories becomes apparent when we consider the physiological roles of the cross-presentation pathway. Recently, we provided evidence that cross-presentation of self-antigens leads to peripheral deletion of autoreactive CD8⁺ T cells (11). We showed that adoptively transferred OT-I cells were activated by cross-presented tissue antigens in RIP-mOVA mice, leading to proliferation and then deletion of the responding OT-I cells. If, as we have shown (11), self-tolerance is a consequence of cross-presentation of self-antigens, then only those antigens expressed at levels sufficiently high to be cross-presented will induce such tolerance. Thus, the idea that peripheral antigens are ignored (21–23), may only apply to antigens that do not stimulate by cross-presentation, i.e., category 2 antigens. High dose self-antigens (category 1) would induce deletional tolerance via the cross-presentation pathway, whereas those lower dose antigens in category 2 would be ignored by naive CTLs but still able to sensitize peripheral tissues for recognition by activated CTLs. Perhaps this explains why some model self-antigens expressed by islet β cells were reported to induce tolerance (11, 24, 25), whereas others were ignored (21–23). Analysis of the effect of antigen dose on peripheral tolerance will be the subject of a later report (Kurts, C., J.F.A.P. Miller, F.R. Carbone, and W.R. Heath, manuscript in preparation).

The tolerance induced by cross-presented self-antigens contrasts with the immunity seen when foreign antigens were used to induce CTLs by cross-priming (2–5, 7–9, 13, 14). What determines whether tolerance or immunity is
induced is unclear, but CD4+ T cell help appears to be important (12, 14, 26). Since the cross-presentation pathway is involved in both cross-tolerance and cross-priming, the mechanisms by which antigens gain access to this pathway is likely to affect both aspects. The focus of this pathway on high dose antigens may direct CTLs immunity towards cells producing large amounts of viral proteins, whereas the ability to recognize antigens associated with tissue destruction may be important for responses to cytopathic viruses.

In summary, this report provides the first in vivo evidence that cellular destruction can enhance access of peripheral antigens to the cross-presentation pathway. In addition, we show that high dose antigens are preferentially cross-presented, although lower doses may still be of physiological relevance by their ability to be directly presented to activated CTLs entering peripheral tissues. Together, these data lead to the conclusion that the cross-presentation pathway is focused on high dose antigens or those released during cellular destruction.

We thank Jenny Falso, Tatiana Banjanin, Freda Karamalis, and Paula Nathan for their technical assistance.

C. Kurts is supported by a fellowship from the Deutsche Forschungsgemeinschaft (Grant Ku1063/1-2). This work was funded by National Institutes of Health grant AI-29385 and grants from the National Health and Medical Research Council of Australia and the Australian Research Council.

A reference correspondence to William R. Heath, Immunology Division, The Walter and Eliza Hall Institute, P.O. Royal Melbourne Hospital, Parkville 3050, Victoria, Australia. Phone: 61-3-9345-2555; Fax: 61-3-9347-0852; E-mail: heath@wehi.edu.au or to Francis R. Carbone, The Department of Pathology and Immunology, Monash Medical School, Commercial Road, Prahran 3181, Victoria, Australia. Phone: 61-3-9276-2744; Fax: 61-3-9529-6484; E-mail: carbone@cobra.path.monash.edu.au

Received for publication 27 February 1998 and in revised form 1 May 1998.

References
1. Germain, R.N., and D.H. Margulies. 1993. The biochemistry and cell biology of antigen processing and presentation. Annu. Rev. Immunol. 11:403–450.
2. Bevan, M.J. 1976. Cross-priming for a secondary cytotoxic response to minor H antigens with H-2 congenic cells which do not cross-react in the cytotoxic assay. J. Exp. Med. 143:1283–1288.
3. Bevan, M. J. 1976. Minor H antigens introduced on H-2 different stimulating cells cross-react at the cytotoxic T cell level during in vivo priming. J. Immunol. 117:2233–2238.
4. Gordon, R.D., B.J. Mathieson, L.E. Samelson, E.A. Boyse, and E. Simpson. 1976. The effect of allogeneic presensitization on H-Y graft survival and in vitro cell-mediated responses to H-y antigen. J. Exp. Med. 144:810–820.
5. Gooding, L.R., and C.B. Edwards. 1980. H-2 antigen requirements in the in vitro induction of SV40-specific cytotoxic T lymphocytes. J. Immunol. 124:1258–1262.
6. von Boehmer, H., and K. Hafen. 1986. Minor but not major histocompatibility antigens of thymus epithelium tolerate precursors of cytolytic T cells. Nature 320:626–628.
7. Carbone, F.R., and M.J. Bevan. 1990. Class I–restricted processing and presentation of exogenous cell-associated antigen in vivo. J. Exp. Med. 171:377–387.
8. Huang, A.Y., P. Golumbek, M. Ahmadzadeh, E. Jaffee, D. Pardoll, and H. Levitsky. 1994. Role of bone marrow–derived cells in presenting MHC class I–restricted tumor antigens. Science 264:961–965.
9. Arnold, D., S. Faath, H. Rammensee, and H. Schild. 1995. Cross-priming of minor histocompatibility antigen-specific cytotoxic T cells upon immunization with the heat shock protein gp96. J. Exp. Med. 142:885–889.
10. Kurts, C., W.R. Heath, F.R. Carbone, J. Allison, J.F.A.P. Miller, and H. Kosaka. 1996. Constitutive class I–restricted exogenous presentation of self antigens in vivo. J. Exp. Med. 184:923–930.
11. Kurts, C., H. Kosaka, F.R. Carbone, J.F.A.P. 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.
12. Kurts, C., F.R. Carbone, M. Banden, E. Blanas, J. Allison, W.R. Heath, and J.F.A.P. Miller. 1997. CD4+ T cell help impairs CD8+ T cell deletion induced by cross-presentation of self-antigens and favors autoimmunity. J. Exp. Med. 186:2057–2062.
13. Pulaski, B.A., K.Y. Yeh, N. Shastri, K.M. Maltby, D.P. Penney, E.M. Lord, and J.G. Frelinger. 1996. Interleukin 3 enhances cytotoxic T lymphocyte development and class I major histocompatibility complex “re-presentation” of exogenous antigen by tumor-infiltrating antigen-presenting cells. Proc. Natl. Acad. Sci. USA. 93:3669–3674.
14. Bennett, S.R., F.R. Carbone, F. Karamalis, J.F.A.P. Miller, and W.R. Heath. 1997. Induction of a CD8 cytotoxic T lymphocyte response by cross-priming requires cognate CD4 help. J. Exp. Med. 186:65–70.
15. Bevan, M.J. 1987. Antigen recognition. Class discrimination in the world of immunology. Nature 325:192–194.
16. Rock, K.L. 1996. A new foreign policy: MHC class I molecules monitor the outside world. Immunol. Today 17:131–137.
17. Blanas, E., F.R. Carbone, J. Allison, J.F.A.P. Miller, and W.R. Heath. 1996. Induction of autoimmune diabetes by oral administration of autoantigen. Science. 274:1707–1709.
18. Lyons, A.B., and C.R. Parish. 1994. Determination of lymphocyte division by flow cytometry. J. Immunol. Methods. 171:131–137.
19. Hodgkin, P.D., J.H. Lee, and A.B. Lyons. 1996. B cell differentiation and isotype switching is related to division cycle
20. Albert, M.L., B. Sauter, and N. Bhardwaj. 1998. Dendritic cells acquire antigen from apoptotic cells and induce class I-restricted CTLs. Nature. 392:88–91.
21. Ohashi, P.S., S. Oehen, K. Buerki, H. Pircher, C.T. Ohashi, B. Odermatt, B. Malissen, R.M. Zinkernagel, and H. Hengartner. 1991. Ablation of "tolerance" and induction of diabetes by virus infection in viral antigen transgenic mice. Cell. 65:305–317.
22. Oldstone, M.B., M. Nerenberg, P. Southern, J. Price, and H. Lewicki. 1991. Virus infection triggers insulin-dependent diabetes mellitus in a transgenic model: role of anti-self (virus) immune response. Cell. 65:319–331.
23. Heath, W.R., F. Karamalis, J. Donoghue, and J.F. Miller. 1995. Autoimmunity caused by ignorant CD8+ T cells is transient and depends on avidity. J. Immunol. 155:2339–2349.
24. Lo, D., J. Freedman, S. Hesse, R.D. Palmiter, R.L. Brinster, and L.A. Sherman. 1992. Peripheral tolerance to an islet cell-specific hemagglutinin transgene affects both CD4+ and CD8+ T cells. Eur. J. Immunol. 22:1013–1022.
25. Adams, T.E., S. Alpert, and D. Hanahan. 1987. Non-tolerance and autoantibodies to a transgenic self antigen expressed in pancreatic beta cells. Nature. 325:223–228.
26. Guerder, S., and P. Matzinger. 1992. A fail-safe mechanism for maintaining self-tolerance. J. Exp. Med. 176:553–564.