*Commentary*

# Antigen Presentation to Cytotoxic T Lymphocytes In Vivo

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The elucidation of the separate pathways of antigen presentation by MHC class II molecules to $CD4^+$ T cells and by MHC class I molecules to $CD8^+$ T cells has provided an understanding of how $CD4^+$ helper responses can be directed at extracellular antigens while $CD8^+$ cytotoxic responses can be directed against intracellular antigens. Both class I and class II molecules are made in the endoplasmic reticulum (ER). Class II molecules travel to a post-Golgi compartment of the cell before their peptide-binding groove is charged with a tight-binding peptide. The peptides that load onto class II MHC molecules are produced in lysosomal compartments and are derived from proteins that have been endocytosed from the extracellular medium or that exist in the endosomal membrane. Newly synthesized MHC class I molecules, on the other hand, are loaded with peptides before they leave the ER. In this case, the bulk of the peptides come from cytosolic proteins that have been processed by proteasomes and transported into the ER via the specialized transporter associated with antigen processing (TAP) (1, 2).

While immunologists have been aware for many years of the requirement for "specialized" or "professional" APCs to take up an extracellular antigen, degrade it, and present it to $CD4^+$ T cells in association with MHC class II, for the most part, the requirement for such APC function in MHC class I–restricted responses has not been appreciated. There are several reasons for this lapse. MHC class I–associated peptide antigens mark the presenting cell for a lethal attack by CTLs. To avoid tissue destruction during an immune response, it is important that such marker peptides should bind almost irreversibly to the class I molecule and not be shed onto bystander cells. A second block in our understanding of antigen presentation to CTL is the difficulty in imagining how an APC can introduce foreign proteins from an infected cell into its own class I pathway. This is because there is no established pathway by which a macrophage or dendritic cell can translocate extracellular antigens into the cytosolic processing machinery that leads to class I presentation. This leads to a major conceptual difficulty in understanding the initiaton of CTL responses to intracellular antigens. Thus, if only the pathogen-infected cell or the mutated tumor cell can present class I–associated antigen, then naive $CD8^+$ T cells are limited to meeting antigens only at the peripheral site of infection or tumor growth. Since naive T cells do not extravasate into peripheral nonlymphoid tissues, and since many nonhematopoietic cells lack much of the costimulator function essential for T cell priming, it is clear that some mechanism must exist for presenting MHC class I–associated antigens on professional APC in the lymphoid organs. Recently, a great deal of attention has focused on the priming of class I–restricted CTL in vivo and the need for professional APCs. The existence of such a presentation pathway is highlighted in the case of a colon carcinoma cell line expressing a model CTL antigen (3). After subcutaneous inoculation of the tumor cells, only host presentation to $CD8^+$ T cells was shown to occur. Surprisingly, there was no direct contribution by the tumor cells to the presentation of antigen for T cell priming. Presently, there is general agreement that professional antigen presentation must occur for class I–restricted responses, and a number of different forms of professional presentation have been suggested, including (*a*) phagocytes may possess an ill-defined pathway to shunt protein from the phagosome into the cytosol, where it would enter the normal MHC class I–associated pathway of antigen processing; (*b*) phagocytes may digest ingested material in lysosomes and regurgitate peptides to load onto surface MHC class I; and (*c*) heat shock proteins (HSPs), which may normally be part of a relay team carrying proteasome-derived peptides to MHC class I, are released by dying infected or tumor cells, and these peptide carriers can access the cytosol of professional APCs. A paper in this issue by Arnold et al. (4) shows that a preparation of purified gp96 is able to prime a $CD8^+$ T cell response to a defined CTL epitope (4). This commentary will discuss each of these mechanisms.

The realization that cell-associated antigens may be presented to the immune system by professional APCs grew out of an apparent contradiction between the rules of MHC restriction of CTL recognition and the older transplantation literature. The antigens presented by target cells expressing $MHC^A$ alleles are different and non–cross-reactive with the antigens presented by cells expressing $MHC^B$. Yet it was known from transplantation studies that MHC-mismatched grafts could prime host animals for second-set rejection of test grafts from MHC-matched donors if the priming and test grafts shared the same minor histocompatibility differences from the host (5, 6). Although it was unclear from such skin graft studies that class I–restricted $CD8^+$ T cell priming was responsible for the rapid rejection, work in short-term CTL assays established that MHC-mismatched grafts could prime $CD8^+$ T cells specific for minor histocompatibility antigens (7), H-Y antigen (8), and SV-40 T antigen (9). The priming effect was

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not merely an indirect effect of enhancing the CD4+ T cell response (10). This priming of CTL precursors in vivo, independent of the MHC type of the immunogenic cells, was referred to as "cross-priming." Since it would be of no advantage to have all endocytosed antigens presented to CD8+ T cells and to preserve the logic of the two pathways of antigen processing, I suggested that only particulate antigens, in the form of damaged cells, would be processed by phagocytic APCs for class I presentation (11).

In line with this suggestion, it was shown that model protein antigens (such as hen OVA or *Escherichia coli* β-galactosidase) could prime CTL responses in vivo much more efficiently as cell-associated rather than soluble antigens (12). In addition, lipid-encapsulated antigens (13), antigens on beads (14), or aggregated antigens (15–17) were effective in priming CD8+ T cells in vivo. In some cases, it was shown that compromising macrophage or dendritic cell function could abrogate the immunizing effect (18, 19). Rock and colleagues have presented evidence that a subset of phagocytic cells is capable of presenting peptides from exogenous bead-bound OVA in association with MHC class I molecules (20). Using peritoneal exudate cells from TAP-1 knockout mice, this group has shown a requirement for TAP function in this form of presentation in vitro, strongly implicating a phagosome to cytosol pathway. Using macrophage cell lines in this assay with various inhibitors further implicated the need for intact proteasome function and nascent class I molecules (20).

In contrast to TAP-dependent presentation of particulate antigens to CD8+ T cells stands the description of a novel vacuolar class I processing pathway for exogenous phagocytosed antigens (21). According to this scheme, peptides produced from phagocytosed material could load onto recycling class I molecules in vacuoles or may spew out of vacuoles to load onto the class I molecules on the surface of the same cell or a neighboring cell. Most of the data supporting this scenario have come from in vitro studies using recombinant *E. coli* or *Salmonella* expressing large amounts of a model antigen as a phagocytic substrate (21). Other support for the notion of macrophages passing on antigen to dendritic cells has also come from in vivo studies with liposome-encapsulated OVA (19). It seems quite unlikely that this mechanism has any relevance to the presentation of typical cellular antigens to CTL precursors. The normal processing pathway for producing peptides that bind stably to MHC class I molecules begins in the cytosol with proteasome cleavages, and may continue in the ER when peptides are trimmed to the perfect size for stabilizing class I molecules. It is unlikely (though not impossible) that vacuolar processing could mimic this. The perfect "natural" peptides found in class I grooves can target cells for CTL recognition when present at picomolar levels, while longer or shorter versions of the peptide require $10^3$- to $10^6$-fold higher concentrations to bind class I for recognition by T cells (22). For antigenic protein substrates that exist in virus-infected or tumor cells at 1 to 0.01% of the total cellular protein, these levels of peptide would not be normally achieved by a vacuolar processing system.

Another fascinating angle on how CTLs may meet antigens in vivo has emerged from studies that began analyzing cellular immunity to chemically transformed tumors in inbred mice. Animals could develop tumor-specific immunity by vaccination with irradiated tumor cells (23). Painstaking efforts to identify the protective tumor antigen eventually focused attention on HSPs, including gp96, hsp90, and hsp70 (24–27). However, the sequence of the tumor-derived gp96 did not differ from that of healthy tissues, suggesting a possible role of HSPs as peptide carriers. None of the tumor-specific antigenic peptides are known in these instances of immunity to methylcholanthrene-induced fibrosarcomas. What Arnold et al. have done is to extend the findings using purified gp96 as an immunogen to include the CTL response to a β-galactosidase peptide (4). Thus, the lumenal gp96 purified from β-galactosidase–transfected cells was shown to be capable of priming a CD8+ CTL response to β-galactosidase and to unknown minor histocompatibility antigens. Remarkably, 30 μg of soluble gp96, presumably associated with a vast complexity of cellular peptides, is able to prime the CD8+ response upon injection intraperitoneally into mice in the absence of an adjuvant. Previous analysis of the cellular requirements for the induction of tumor-specific immunity by HSP has shown that CD8+ cells are primed, and that previous depletion of phagocytic activity by carrageenan injection prevents the priming. Srivastava and colleagues have suggested that HSP carrying their chaperoned peptides are released by dying tumors or infected cells and bind to unknown receptors on the surface of macrophages (28). They are then taken up into the cell to be routed to the macrophage nascent MHC class I molecules. The peptides are further processed during this translocation by the macrophage's own class I processing machinery. The work summarized above shows that HSPs purified from antigenic cells have the capacity to prime class I–restricted CTL responses on the basis of the bound peptides. There are no data available yet to explain whether interfering with this released HSP-macrophage receptor translocation system would prevent the ability of APC to present antigen to CD8+ CTL in vivo. Nor are there any data about the in vitro processing of HSP-bound antigen for class I presentation. The suggestion that HSP, which may participate in the normal transfer of peptides to MHC class I, could also be involved in efficient professional APC presentation to CTL, however, preserves the logic of what type of antigens should be shunted into the class I pathway.

The mononuclear cell type most frequently touted as both a professional APC and a phagocyte is the macrophage, while mature dendritic cells isolated in vitro have the reputation of being nonphagocytic. Since dendritic cells are so efficient in the presentation of antigen to T cells, however, they should not be ruled out for class I–restricted presentation (29). Under some circumstances, dendritic cells as interdigitating cells in central lymphoid organs and their precursors in the tissues, such as Langerhans cells in the skin, have been shown to be actively phagocytic. Fossum and Rolstad (30) showed that in situations where NK cells are lysing allogeneic lymphocytes, the interdigitating cells are very active in phagocytosing the donor cells. Futhermore, bone marrow cultures stimulated with GM-CSF contain aggregates of dividing dendritic cells that are actively phagocytic (31). In addition, Langerhans

cells have also been shown to be phagocytic (32). Thus, in the case of a local viral infection, for example, conditions may exist for Langerhans cells themselves to phagocytose infected cells, to shunt material into the class I processing pathway, and to migrate to the draining LN to present antigens to CTL.

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