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Dipping into the Cytosol to Broaden the MHC Class II Peptide Repertoire

A new study by Zhou et al. (2005) in this issue of *Immunity* explores how cytoplasmic autoantigens gain access to the MHC class II antigen-processing pathway. Their experiments show that hsc70 and Lamp-2a mediate antigen transport into endosomal compartments for presentation by MHC class II.

The immune system employs a "divide and conquer" approach to deal with the distinct challenges posed by bacterial and viral infections. Viral proteins produced in the cytoplasm (endogenous antigens) are degraded by proteasomes into peptides that are shuttled into the ER and loaded onto waiting MHC class I (MHCI) molecules for presentation to $CD8^+$ T cells at the cell surface. Engulfed bacteria and soluble antigens (exogenous antigens), on the other hand, are directed into the endosomal/lysosomal pathway for degradation. The late endosomal compartments of the cell have been coopted for use by the MHC class II (MHCII) antigen-processing system. Protein fragments derived from the bacterial proteins interact with MHCII molecules and are further trimmed into peptides for presentation to $CD4^+$ T cells. This segregation of labor is not simply a matter of convenience but rather a fundamental aspect of the T cell response; MHCI-restricted $CD8^+$ T cells have the machinery to directly kill virally infected cells, whereas MHCII-restricted $CD4^+$ T cells are wired to help B cells generate neutralizing antibodies against invading bacteria. The synchrony of the system is an intellectually pleasing aspect of an otherwise rather chaotic adaptive immune system.

For over a decade, however, it has been recognized that peptides derived from cytoplasmic and even nuclear antigens can find their way onto MHCII molecules (Chicz et al., 1993). At first, these findings were regarded simply as "leakiness" in the experimental system, perhaps due to endosomal uptake of cytoplasmic antigens from apoptotic cells or cellular debris in the cultures. Accumulating data, however, showing specific $CD4^+$ T cell responses against viral antigens as well as against cytoplasmic tumor antigens have forced immunologists to consider an alternate pathway(s) for the generation of MHCII peptide ligands. One of the appealing aspects of antigen presentation is that, both for MHCI and MHCII, the process follows an orderly pathway involving specialized accessory proteins that facilitate antigen fragmentation, transport, and/or loading. The details of a specific alternative pathway for generating MHCII peptide complexes from cytosolic antigens, however, have remained obscure.

The first clues to the critical players came from breakthrough findings from Fred Dice's laboratory, which described a process called chaperone-mediated autophagy (CMA) (Chiang et al., 1989; Cuervo and Dice, 1996). CMA results in the transport of cytosolic proteins directly into late endosomal and lysosomal compartments (Majeski and Dice, 2004). CMA substrates residing in the cytoplasm of cells are recognized and bound by a molecular chaperone complex that includes the constitutive form of the heat shock 70 kDa protein (hsc70) complexed with several other cytoplasmic heat shock and heat shock-related proteins. The substrate protein and chaperone complex are recognized by the lysosome-associated protein type 2a (Lamp-2a) and pulled into the lysosomal lumen with the aid of lysosomal hsc70 for subsequent degradation by resident proteases. In humans, there are two different isoforms of Lamp-2 (Lamp-2a and Lamp-2b), which arise by differential mRNA splicing. The differences are limited to the transmembrane region and cytosolic tails; importantly, only Lamp-2a mediates CMA. Remarkably, estimates indicate that this pathway may be responsible for the degradation of ~30% of all cytosolic proteins.

In studies presented in this issue of *Immunity*, Blum and colleagues asked if the CMA pathway might also have a role in the adaptive immune response (Zhou et al., 2005). They reasoned that delivery of cytosolic antigens into late endosomal and lysosomal compartments by CMA might be crucial for presentation of these antigens by MHCII, which also resides within these compartments. In these studies, the processing and presentation of the cytosolic autoantigens glutamate decarboxylase (GAD) and a mutant form of Ig κ (SMA) were followed after expression of the autoantigens in transfected Epstein-Barr virus-transformed human B cell lines. Processing and presentation was detected with HLA-DR4-restricted T cell hybridomas specific for epitopes from GAD and SMA.

In cells where Lamp-2 protein levels were knocked down by using antisense cDNA, the presentation of cytoplasmic GAD was significantly decreased. Conversely, in experiments in which Lamp-2a was overexpressed, cytoplasmic GAD and SMA presentation was significantly increased. Overexpression of Lamp-2b, however, had no measurable effect. Importantly, Lamp-2a overexpression did not alter MHCII levels, exogenous antigen presentation, overall cellular morphology, or lysosomal protease maturation or distribution, showing the specificity of Lamp-2a overexpression on cytoplasmic antigen processing. To further implicate the CMA pathway in cytoplasmic antigen processing, the role of hsc70 in cytoplasmic MHCII antigen processing was examined. Similar to the results from the analysis of Lamp-2a, overexpression of cellular hsc70 resulted in a significant increase in GAD presentation, whereas knockdown of hsc70 protein levels resulted in a significant decrease in GAD presentation. These results were unexpected given the high constitutive expression of hsc70 in the cells; and they imply that the levels of this chaperone are nonetheless limiting for the CMA process.

Finally, this manuscript further investigates the nature of the substrate of the hsc70/Lamp-2a pathway for MHCII presentation. Previous studies from the Blum

laboratory examined the role of the proteasome and the cytosolic protease calpain by the use of specific inhibitors (Lich et al., 2000). Presentation of cytoplasmic GAD was selectively blocked by these agents compared to control, nontreated cells, suggesting that GAD was at least partially and perhaps completely degraded into peptides in the cytoplasm prior to transport. In the current study, the need for proteolysis was completely bypassed by delivering the $GAD_{273–285}$ peptide directly into the cytoplasm of cells by electroporation. As was seen after expression of the GAD protein upon transfection, B cells presented the $GAD_{273–285}$ peptide significantly better when overexpressing Lamp-2a, indicating that processed peptides can be a substrate for the hsc70/Lamp-2a pathway. Dice and colleagues, however, have previously shown that proteolysis of the substrate proteins does not occur prior to lysosomal import. This is actually consistent with data from the current paper showing that full-length GAD can be coprecipitated with both hsc70 and Lamp-2. Clearly, the nature of the in vivo substrate for the hsc70/Lamp-2a pathway of MHCII presentation warrants further study.

Recently, other alternative pathways for delivering antigens into the MHCII pathway have been described. For example, presentation of peptides derived from endogenous Epstein-Barr virus was shown to be dependent on autophagy (Paludan et al., 2005), and a proteasome/transporter associated with antigen processing (TAP)-dependent pathway was shown to be important for presentation of peptides from influenza virus (Tewari et al., 2005). Therefore, it is reasonable to propose that presentation of cytosolic and nuclear antigens is an important mechanism for generating $CD4^+$ T cell epitopes. The overall impact of these pathways on immune function would appear to broaden the possible peptide repertoire available for initiating T cell responses. The potential benefits of this are clear for the two systems that describe processing and presentation of viral-derived peptides. The adaptive immune system is always teetering on the edge of autoimmunity, however, and the presentation of additional self-antigens may exacerbate this problem. Indeed, the studies presented here focus on the presentation of a peptide derived from GAD, a major autoantigen for type 1 diabetes. In turn, presentation of altered self-antigens via these pathways may be critical for generating antitumor responses, as has been seen for T cell responses against melanoma antigens (Wang et al., 1999). Finally, it seems likely that similar mechanisms must be in place for intrathymic negative selection that is mediated primarily by dendritic cells and medullary epithelial cells. Because central tolerance is clearly critical for preventing autoimmunity, the greater the breadth of self-antigens that are presented would seem to be of a significant benefit.

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