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Alternative Generation of MHC Class II-Restricted Epitopes. Not So Exceptional?

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Abstract

By convention, peptides presented at the cell surface by MHC class II molecules (MHCII) are derived from internalized ("exogenous") antigen that is denatured and fragmented in the endocytic compartment and loaded onto MHC in the late endosome with the assistance of the H2-DM chaperone. Over the past two decades several alternatives to this pathway have been described but the extent to which they contribute to natural $CD4^+$ T cell (T_{CD4+}) responses has not been assessed, mainly because studies have focused primarily on individual epitopes. My laboratory has begun to address this issue in virus infection models and a picture is emerging in which classical presentation plays a relatively minor role, with a number of alternative presentation pathways collectively accounting for the majority of peptide presentation. The potential ramifications for this fundamentally altered view of MHCII peptide supply are discussed.

Introduction

In the late 19th century, seeking to replicate Pasteur's success with rabies, Robert Koch developed tuberculin to serve as a therapeutic vaccine for tuberculosis. While the clinical trials were a disaster and seriously threatened Koch's reputation, the hypersensitivity to tuberculin experienced by infected individuals resulted in a highly reliable test of carrier state that remains the gold standard. Soon thereafter, delayed-type hypersensitivity (DTH) reactions became widely studied, first in the context of other infectious organisms and extracts thereof, and later with more refined material. Thus, in 1929 Dienes demonstrated that purified protein (ovalbumin) could induce DTH and this was followed by similar reports on many other proteins that would eventually comprise the battery of "shelf" antigens that have become quite familiar to cellular immunologists. Ironically, DTH responses, which we now understand to be driven by T_{CD4+} activation, were initially touted as supporting a humoral basis for immunity. By the time the activating principle of DTH responses was reduced to a peptide, a firm relationship between externally provided protein and T_{CD4+} activation had been established (1). Thus, identification of the classical pathway outlined above was intuitively appealing and helped to cement the notion that T_{CD4+} are dedicated to the detection of extracellular (exogenous) antigens. In parallel, a similarly satisfying basis for $CD8^+$ T cell (T_{CD8+}) activation was being elucidated. Involvement of the multicatalytic proteasome and transporter of antigenic peptide (TAP), as well as loading of MHCI in the endoplasmic reticulum were consistent with the observation that killing of target cells required that antigen be delivered to the cytosol, usually via biosynthesis. Inactivation of virus, for example, obviated T_{CD8+} priming in vivo and T_{CD8+} killing in vitro, leading to the

conclusion that T_{CD8+} are dedicated to the detection of intracellular (endogenous) antigens (2).

Intuitive appeal of the classical processing pathway notwithstanding, several fundamentally distinct alternatives have been described over the past two and a half decades. Such alternatives were not readily appreciable with the conventional antigens that descended from the DTH era; typically these were stable, readily available globular proteins that are confined to the endocytic compartment of most cell types when provided exogenously. Viruses, however, provided the opportunity to study antigens that interact with the antigen-presenting cell (APC) in far more dynamic fashion. Indeed, it was not long after such studies were initiated with both measles (3) and influenza (4) viruses that MHCI-like presentation of MHCII-restricted epitopes was described; presentation of the epitope was possible only when the antigen was synthesized by the APC. Over the intervening years there have been many additional examples of endogenous MHCII presentation from the study of additional viral proteins, natural cellular proteins or antigens expressed via transfection or transduction. Three distinct pathways have been mechanistically described: 1) Macroautophagy, in which cytosolic protein, often in form of insoluble aggregates, is surrounded by a double membrane that subsequently fuses with the lysosome (5). 2) Chaperone-mediated autophagy involving directed transport of proteins bearing the KFERQ motif from cytosol to lysosome (6). Participation by the proteasome has been implicated in this pathway. 3) A decidedly MHCI-like pathway in which both the proteasome and TAP are implicated (7). Given some of the preliminary data that will be described below, it seems likely that additional pathways exist, identifiable only when an epitope dependent on that pathway is identified and studied in detail.

In addition to alternative endogenous antigen processing, there is a variation on classical exogenous processing that deserves mention. In this case, peptides are loaded in the early endosome onto MHCII that appear to traffic to that compartment from the cell surface, with at least some epitopes not requiring participation of the H-2DM chaperone. Fitting into this category is an epitope, S3, within the stalk region of the influenza hemagglutinin glycoprotein. When influenza in internalized, the hemagglutinin undergoes a profound conformational change in response to acidification, resulting in fusion of viral and endosomal membranes and delivery of the genome containing core to the cytosol – an example of the dynamics that are not embodied by nominal antigens. This conformational change includes a complete disordering of the S3-containing region, allowing for direct binding to the open-ended MHCII molecule. Presentation of the epitope occurs independently of H-2M expression and is substantially elevated by the endosomal protease inhibitor, leupeptin (8). This latter observation is compatible with the notion that unfolding renders the epitope susceptible to proteolysis, with most of it being destroyed before the opportunity to load onto MHCII and, consequently, achieving a protease resistant state. If further suggests the raison d'etre for recycling MHCII; very little, if any of the epitope would remain intact if loading were not possible until delivery to the late endosome, the residence of nascent MHCII.

Current Status

Historically, antigen processing has been studied on an epitope by epitope basis. Thus, despite general acceptance of all of these alternative pathways and the likelihood that there are others to be discovered, their relative contributions to the overall T_{CD4+} response to complex antigens have not been quantified. For the past several years my laboratory has been interested in this question. One trivial reason for this pursuit is that we wanted to be sure we were not spending our time at the fringes. A more substantial reason is that the answers could have substantial clinical consequences as discussed below. Our initial

approach was on a global scale; mice were immunized with live influenza and the resulting T_{CD4+} were restimulated in vitro with influenza-infected APC with or without proteasome inhibitor. The differences in spot counts suggested that 30–40% of T_{CD4+} response to influenza was directed at epitopes that depended upon proteasomal activity for their generation. Similar results were obtained with siRNA treatment that targeted ubiquitin-dependent proteasomal degradation. However, such global analyses are shrouded by anonymity. Results could reflect a large response to a single dominant, proteasome-dependent epitope or lesser responses to several epitopes. We imagined that more traction would be gained if we were able to point to the actual epitopes involved. Therefore, we developed a strategy in which we probed the entire response with an overlapping synthetic peptide library. T_{CD4+} from C57Bl/6 mice infected intranasally with mouse-adapted influenza reproducibly demonstrated 13 different specificities distributed among a variety of the influenza proteins. Immunization via the same route with very large amounts of inactivated virus was surprising. According to the conventional model, since nearly all of the proteins encoded by the influenza genome are incorporated in the virion, one might expect most of the specificities to be elicited. Instead, only three specificities are elicited by inactivated virus. Several controls eliminated the possibility that the missing specificities are prevented from being presented due to the lack of infectivity and the resultant innate signaling. The results suggest that very little presentation of influenza-derived peptides results from endosomal processing of influenza virions. Perhaps this is because whole virions are relatively indigestible compared to the individual proteins that are produced during infection. Thus, there are two possibilities for an infection requirement: 1) The epitopes may be generated primarily or exclusively via endogenous processing. 2) Proteins are released from infected cells (influenza is cytolytic) and transferred to professional APCs. We have generated T cell hybridomas with specificity to several of the epitopes and are currently testing these two possibilities.

We were also surprised when we immunized H-2M$^{-/-}$ mice with infectious influenza. H-2M-independent presentation can be readily observed when antigen is denatured but few examples of H-2M-independent presentation from native antigen have been described. Yet, nearly half (six of thirteen) of the specificities are elicited in H-2M$^{-/-}$ mice. Here too we are currently attempting to reproduce the finding in vitro with T hybridomas. Notably, three of the H-2M-independent specificities are those elicited by inactivated virus. Thus, if one were to go by the strictest definition of classical presentation – processing of exogenously provided virions in the endosomal compartment with H-2M-dependent loading – then none of the thirteen epitopes is a pure classical epitope. This is not to suggest that there is no classical processing with respect to these three epitopes, but to indicate that other pathways contribute substantially to their total numbers at the cell surface.

We have initiated similar studies with a virus, ectromelia, (ECTV) that is substantially different from influenza. The cause of murine smallpox, ECTV is DNA-based and several fold larger. In addition, the ECTV virion is considerably more sturdy. Thus, while influenza is rapidly inactivated when exposed to air, poxviruses can survive desiccation and rehydration, an attribute that was instrumental in the eradication of smallpox. We obtained a partial peptide library from the Sette laboratory, covering approximately 20% of the proteome. From this library sixteen different specificities have been mapped. Remarkably, infectivity of the virus is required to elicit responses to every single one of them. It is possible that a more comprehensive analysis would yield an exception, but our speculation is that the ECTV virion is even more indigestible than influenza. What is more, only one of these specificities is elicited in an H-2M$^{-/-}$ mouse, a much lower fraction than was observed with influenza. Further work will be needed before we would venture a guess as to the basis for this dichotomy.

Future Perspectives

The area of MHCI antigen processing has been transformed by the paradigm-disrupting discovery of cross-presentation in which antigens are released from infected and transformed cells and taken up exogenously by the dendritic cell. From there, several pathways appear to function in the generation of peptide/MHCI complexes (9). Cross-presentation solves the problem of viral tropisms that do not include professional DCs and also provides a means of circumventing the immune evasion and subversion that is so effectively practiced by many viruses. I would like to think that a similar transformation is looming on the MHCII side. I have previously suggested that multiple MHCII pathways exist because the processing power of any one can be limiting. Certainly, our published and preliminary evidence suggest that this is the case. And clinical data suggest that sufficient T_{CD4+} engagement is an important predictor of outcome with several human infectious diseases, including hepatitis A, hepatitis B and influenza. Inactivated viral vaccines are often less potent than live vaccines and this has been generally attributed to differences in antigen dose. However, limited T_{CD4+} activation that compromises the establishment of immunological memory could also be an important factor.

Focus here has been on viral immunity, which may be particularly suited for revealing alternative MHCII pathways. Presumably T_{CD4+} responses to extracellular bacteria rely primarily, if not entirely, upon exogenous processing pathways, although classical processing need not necessarily be dominant. Exploration of many additional infection models will be required for adequate perspective

Several fundamental areas are wide open for exploration. Are there, as already suggested, additional exogenous and endogenous processing pathways to be identified? The approach we have taken may already have provided momentum in this direction. What are the cellular components involved in alternative processing? Chaperone-mediated autophagy was revealed by study of glutamic acid decarboxylase (GAD), an autoantigen that is associated with Type 1 diabetes mellitus (6). Indeed, autoimmune diseases are primarily linked to particular MHCII alleles. If most MHCII-restricted autoantigens are endogenously processed, then identifying the cellular components involved in their generation might provide new approaches to the treatment of autoimmune diseases. Such insights could extend to cancer immunotherapy, which essentially seeks to elicit a highly focused autoimmune response. Finally, what determines whether a particular peptide sequence is generated via one pathway versus another? We have already mentioned the possibility that some proteins may be refractory to processing, particularly when part of a macromolecular structure. There is also the mutually inclusive possibility of proteolytic hypersusceptibility. Since the MHCII peptide binding groove is open, disordering of the relevant segment appears to be the only universally required processing step. Thus, proteases may more often be a destructive force in antigen presentation, a notion supported by our studies of the S3 epitope described above. Some epitopes may not be created via a particular pathway because it is a minefield for certain primary sequences. In light of this possibility, caution should be exercised in coopting well-described classical epitopes to explore alternative pathways. Better perhaps to study epitopes in their natural contexts.

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Highlights

- Classical MHC class II processing entails endosomal proteolysis of exogenous antigen.
- Also by convention, MHC class II loads in the late endosome via the H2-DM chaperone.
- This pathway appears to drive little of the $CD4^+$ T cell response to influenza.
- Several alternative pathways collectively account for most presented peptides.
- This may expand approaches to vaccine design, autoimmunity and cancer immunotherapy.