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Defining cross presentation for a wider audience

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Cross presentation is the process of production of peptide-MHC Class I complexes by cells in which the antigen that is the source of peptide is not translated. The majority of recent studies have described many facets of the classical TAP-dependent cross presentation pathway, but numerous pathways for transfer of antigenic material from a donor to a recipient cell followed by subsequent MHC-I-restricted presentation have been established, including transfer of protein antigen, peptide, RNA, DNA or even peptide-MHC-I complexes. The extent to which each of these pathways generates overlapping or unique peptide repertoires is unknown, as is the contribution of each of these pathways to generation of protective CD8+ T cells during infection or anti-tumor immune responses.

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Introduction

The discovery of cross priming predated both the observation that peptide antigens were presented in complex with MHC molecules, and the subsequent establishment of the dogma that peptide derived from antigens that are endogenously synthesized within a cell are presented in complex with MHC Class I (MHC-I) and those derived from exogenous antigen are presented in complex with MHC Class II (MHC-II). It has become clear that the dogma that was established is misleading, as both endogenous and exogenous antigens can be presented in complex with either MHC-I or MHC-II [1]. Numerous pathways of transfer of antigenic material from a donor to a recipient cell following by subsequent MHC-I-restricted presentation have been established, including transfer of protein antigen, peptide, RNA, DNA or even peptide-MHC-I complexes (p-MHC-I) [2]. However, as the terms 'cross presentation' or 'cross priming' have entered the lexicon of immunologists and vaccinologists around the world, the meanings of the terms have become warped, and in more and more cases are applied to direct presentation of endogenous antigens or even to MHC-II-restricted presentation. Therefore we seek here to define cross presentation in terms of the possible routes of presentation of exogenous or endogenous antigens.

The need to define cross presentation is not just a semantic exercise. The repertoire of peptides generated, and the efficiency with which they are generated by each possible pathway can differ massively. Therefore, describing any process as a part of cross presentation can be misleading if terminology is not well defined. Failure to accurately distinguish between antigen presentation pathways can lead to an over or underestimation of the efficiency of any given presentation event, and could lead to inaccurate design of vaccine strategies to generate protective CD8+ T cells (T_{CD8+}). The extent to which cross presentation contributes to initiation of primary T_{CD8+} responses or expansion of memory T_{CD8+} responses remains unknown, although it is clear that the number of p-MHC-I generated from endogenous sources can significantly exceed those that are cross presented from exogenous sources [3••,4]. One might assume that the entire repertoire of directly presented peptides from endogenous sources should be able to access the cross presentation pathway to ensure that all potential T_{CD8+} reactivities are triggered even in the presence of effective strategies to block the MHC-I pathway. However, until all potential pathways of cross presentation are considered as a whole, the entire breadth of the repertoire of cross presented peptides cannot be defined.

Definition of cross presentation

For the purposes of this review we define cross presentation as presentation of p-MHC-I by cells in which translation of the antigen being presented has not occurred. Examples of such presentation would include professional APC (pAPC)-mediated presentation of virus-encoded antigen when the virus does not infect pAPC, when the virus encodes an effective blocker of the endogenous MHC Class I pathway, or of tumor-encoded antigen (when the tumor is not of a pAPC origin). In a therapeutic setting this will include targeting of exogenous antigen to receptors present on subsets of pAPC to induce protective T_{CD8+}, a fashionable approach that has yet to yield notable results in the clinic [5].

Endogenous presentation

Endogenous presentation or direct presentation is the presentation of p-MHC-I by cells in which translation of the antigen is occurring. Presentation requires newly

synthesized MHC-I and antigen [6,7], as long-lived antigen enters the presentation pathway very poorly [8–10], and presentation is not dependent upon the half-life of intact protein antigen. The direct presentation pathway is not the focus of this review, but is included here to contrast the repertoire of peptides that are directly presented with those presented via each potential cross presentation pathway. The direct presentation pathway uses rapidly degraded proteins [6,7], or products of nonsense-mediated decay of the pioneer round of translation as substrates [11] and so can present peptides derived from cryptic and non-conventional translational products including 5′ and 3′ untranslated regions, intron, and intron/exon junctions [12–14,15•,16•]. Therefore, it is the task of the cross presentation pathways to faithfully reproduce this peptide repertoire unless strategies used by pathogens and tumors to block the endogenous MHC-I processing pathway are to be effective against the population as a whole.

Classical TAP-dependent cross presentation

The classical route of cross presentation involves transfer of long-lived substrates [17–20] from a donor cell to a pAPC where it is internalized and relocated to the cytosol, perhaps via retrotranslocation through the SEC61 translocon [21]. The long-lived nature of the protein substrate is likely required to allow time for transfer between cells and there is now strong evidence to support a reduction of the degradative environment in the endosomal compartments of pAPC that prolongs availability of antigen for the classical cross presentation pathway [22••,23,24•]. Once translocated from an endosomal compartment into the cytosol the antigen is degraded by cytosolic proteases (primarily the proteasome) and the resulting peptides transported via the TAP complex to a compartment where peptide loading occurs in a manner similar to that during direct presentation [25,26]. Initially this compartment was thought to be the endoplasmic reticulum, but phagosomes recruit all of the components of the peptide loading complex, raising the possibility that a protein is translocated from an endosomal compartment into the cytosol, degraded and the resulting peptides pumped back into the endosomal compartment for peptide loading [27–29].

Although some studies have indicated that cell death via certain mechanisms (such as apoptosis or necrosis) is required in order to efficiently target the cross presentation pathway, such reliance would readily allow evasion by pathogens [30–32]. Indeed, many viruses are known to inhibit induction of apotosis or cell death via a variety of mechanisms, and this would allow them to evade cross presentation [33–35]. Indeed, likely any requirement for specific function on the part of the donor cell could be readily subverted by pathogens infecting those cells. Therefore, it is likely that antigen donation is a passive event, at least when generation of p-MHC-I is measured. Indeed, cross presentation can readily occur from live donor cells, even when infected with viruses known to inhibit apoptosis [36], indicating that cell death is likely not required for cross presentation.

The observation that long-lived substrates are the primary substrate for the 'classical' route of cross presentation, whereas direct endogenous presentation primarily occurs from rapidly degraded substrates or from the pioneer round of translation makes it clear that the peptide repertoires produced will only partially overlap, and presented peptides derived exclusively from endogenous antigens will not be uncommon.

Endosomal TAP-independent cross presentation

Endosomal cross presentation involves the uptake of protein but, instead of the translocation to the cytosol observed during classical cross presentation, antigen is directly degraded in an endosomal compartment in a manner independent of proteasomal degradation and TAP-mediated transport [37–43]. Inhibitors of endosomal acidification block this pathway, and Cathepsin S has been implicated in at least one example of presentation via this route [44]. The context of an epitope within a protein and the lipid moieties that contain the antigen likely modulate entry to the endosomal cross presentation pathway, but a number of pressing questions remain. First, the physical characteristics of the protein substrate remain unexplored, as does characterization of the cells capable of supporting presentation via this pathway *in vivo*. More importantly, the extent that the endosomal pathway contributes to the repertoire of cross presented peptides *in vivo* is unclear. This deficit stems mostly from studies using peptides mapped from proteasome-generated endogenous determinants that are directly presented. With some notable exceptions (such as the ovalbumin SIINFEKL determinant in certain contexts [37,38,44]), the chances of endosomal proteases and cytosolic proteases, such as the proteasome, generating identical epitopes are likely slim. Therefore the study of proteasome-generated epitopes will likely substantially underestimate the extent to which the endosomal TAP-independent pathway is used *in vivo*. Indeed, the endosomal cross presentation pathway may generate a repertoire of peptides that overlaps only marginally with the repertoire generated by direct presentation. Does this imply that the peptides may generate a mis-targeted and potentially useless T cell response? The implication during virus infection or anti-tumor responses is that this may be the case. However, the peptides generated may allow T_{CD8+} targeting of cells infected with intracellular bacteria or parasites that do not penetrate the cytosol [43,45•,46–48], thus removing the 'safe haven' of such pathogens.

Endogenous MHC-II

The dogma that peptides derived from exogenous antigens are presented exclusively on MHC-II is clearly