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Research Focus

Outside looking in: the inner workings of the crosspresentation pathway within dendritic cells

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The entry of exogenous antigen into the MHC class I-restricted crosspresentation pathway can contribute to CD8$^+$ T cell tolerance and immunity, in particular to peripheral self-antigens or selected viruses and bacteria showing restricted tissue tropism. Dendritic cells are the key crosspresenting cells and, as such, they are thought to carry specialized machinery dedicated to this purpose. Two recent papers describe intracellular components tailored to the dendritic cell crosspresentation pathway.

Dendritic cells and the crosspresentation of exogenous antigen

MHC class I-restricted antigen presentation requires access to the proteasomal processing machinery found in the cell cytosol. Exogenous antigens do not usually access this processing pathway, but there is a mechanism, known as crosspresentation, by which such antigens are shunted into the cytosol [1]. From its inception, crosspresentation has been seen as the exclusive domain of professional antigen-presenting cells such as macrophages and dendritic cells (DCs) [2–4]. Over time, DCs have gained prominence as the dominant crosspresenting cell *in vivo* [5], suggesting that these cells possess specialized machinery required for this purpose. Two recent papers from the laboratories of Cresswell and Amigorena have identified key intracellular players involved in crosspresentation, and have linked these players to exclusive DC functionality [6,7]. Together, these studies provide a detailed picture of the inner workings of this pathway.

The case for endoplasmic reticulum contribution to cross-presentation

The crosspresentation field was galvanized by a proposal from Desjardins and colleagues that phagosome formation in macrophages might involve fusion with the endoplasmic reticulum (ER) [8]. This proposed mechanism implied that antigen entering through the phagocytic pathway could, potentially, find itself in a hybrid ER–phagosomal compartment [9–11]. The formation of these compartments provided a solution to a problem that had puzzled investigators. Namely, it was known that crosspresented peptides were generated largely in the cytosol because crosspresentation requires the action of the proteasome in addition to the transporter associated with antigen processing (TAP), which translocates peptide from the cytosol to the ER lumen [12]. So, how were the endocytosed antigens transferred to the cytosol? It was known that proteins can be retrotranslocated out of the ER through mechanisms associated with the degradation of misfolded ER proteins. Because one of the components of this mechanism, sec61, was also recruited to the proposed ER–phagosome, the formation of this hybrid compartment could, therefore, provide a means by which exogenous antigen is transported to the cytosol [9–11]. Unfortunately, the original proposal for ER–phagosome formation is now mired in controversy. Desjardins' hypothesis was based on the detection of ER-derived components within purified phagosomes [8]. However, Touret *et al.* [13] recently attributed this observation to the presence of ER contaminants in phagosomal preparations, casting doubt over the original proposal.

ER-retrotranslation machinery is involved in dendritic cell crosspresentation

The publication by Ackerman *et al.* [6] provides a reprieve to the ER–phagosome hypothesis and, potentially, reasserts its role in crosspresentation. This study follows previous observations by the Cresswell group that exogenous proteins access the lumen of the ER or an ER-like entity, resulting in crosspresentation [9,14]. In the most recent study, they elegantly articulated this by showing that incubating crosspresenting cells with the herpes simplex virus-derived TAP antagonist ICP47 blocked MHC class I presentation. It was reasoned that endocytosed ICP47 must retrotranslocate to the cytosol because ICP47 inhibits this route by binding to the cytosolic face of TAP. More importantly, Ackerman *et al.* demonstrated a role for sec61 in the retrotranslocation of exogenous antigen by showing that exotoxin A, a known inhibitor of sec61, inhibited crosspresentation [6]. Another element associated with the retrotrotranslation complex, the p97 ATPase, was also implicated in this process. A dominant-negative form of this protein, which interacts with the sec61-translocated peptide, inhibited crosspresentation while leaving direct presentation intact. Finally, these investigators revisited the ER-mediated phagocytosis hypothesis of Desjardins. Although they did not address ER membrane contribution to phagosome formation, they showed that phagosomes were fully capable of retrotranslocation and even glycosylation (a reaction restricted to the ER lumen), consistent with the original hypothesis.

Separate to any controversy about the origin of the early phagosome, the Ackerman study provides compelling

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evidence for the involvement of an ER-based retrotranslation mechanism during crosspresentation. However, it also raises several interesting issues. First, the sec61 channel seems too narrow to enable the passage of native proteins [15], yet antigens of this type are efficiently crosspresented [3,4]. More intriguing was the finding that the successful crosspresentation of exogenous antigen through this mechanism seemed to be unique to DCs – macrophages were deficient in this respect. Although this DC exclusivity coincides with the cell bias seen with crosspresentation *in vivo*, it is clear that any ability to crosspresent cannot be explained by the presence of ER-based translocation machinery alone. The implicated sec61 provides a general means by which misfolded proteins can be eliminated from the ER compartment. So, if this machinery is ubiquitous, why can only DCs use it for class I-restricted cross-presentation?

DCs regulate phagosomal pH to facilitate crosspresentation of exogenous antigen

One possible explanation for the DC bias in retrotranslocation is the inefficient acquisition of antigens by other cells. This seems improbable because the study also examined macrophages, which are adept at taking up exogenous antigens. Alternatively, DCs might possess a special mechanism for antigen handling not found in other cells. The study by Amigorena's group identified one such mechanism [7]. Although DCs can degrade exogenous antigen, it seems that this process is moderated to a certain extent – at least when compared with cells adapted for microbe destruction, such as macrophages [16]. This attenuation is achieved, at least in part, by limiting endosome acidification through decreased activity of the H^+-pump ATPase, vacuolar ATPase (V-ATPase) [17] and, therefore, the activity of lysosomal proteases [16]. Savina

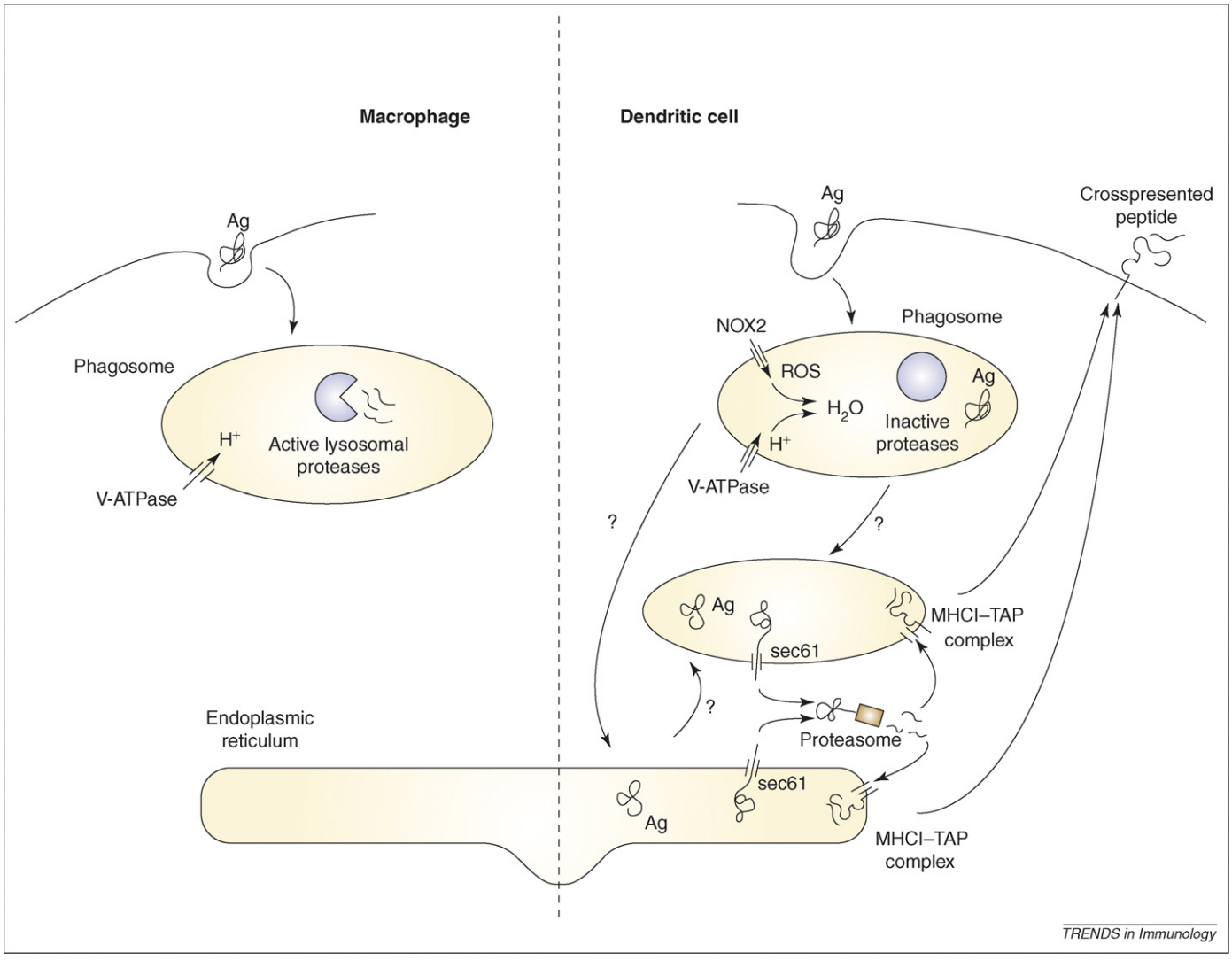

Figure 1. A mechanistic explanation for preferential crosspresentation by DCs. Phagocytosed antigen is handled differently in macrophages and DCs. Macrophages do not transport exogenous antigen into the cytosol through the sec61 retrotranslocation complex [6] and degrade this material more aggressively within the phagocytic compartment, where complete acidification through the V-ATPase results in the full activation of lysosomal enzymes [7]. By contrast, DCs limit phagosomal acidification by means of the NADPH oxidase NOX2, which produces ROS that consume V-ATPase-translocated protons [7]. In addition, exogenous antigen acquired by DCs is exposed uniquely to the sec61 retrotranslocation machinery [6]. Alkalinization and peptide transport are shown to occur within distinct compartments in the DCs, but this does not need to be the case. Finally, Ackerman *et al.* [6] suggest that ER contributes to the phagosome because exogenous material seems to be exposed to ER components. However, the existence of an ER–phagosome compartment is still controversial [13]. We have represented this uncertainty as question marks at the arrows denoting the possible flow between the various intracellular compartments [9,14].

et al. [7] now propose that this dampening of proteolysis also involves active alkalinization and this enables antigen delivery to the cytosol in a form appropriate for cross-presentation. It was known that the NADPH oxidase NOX2 reverses phagosomal acidification in neutrophils as a byproduct of the production of reactive oxygen species (ROS) linked to microbial destruction [18]. In neutrophils, this alkalinization is transient and is ultimately overridden by the normal acidification process. Although DCs produce only small amounts of NOX2 [19], Savina *et al.* [7] suggest that these levels are, nonetheless, important. In their view, NOX2 actively sustains phagosomal pH and is associated with the crosspresentation process. To prove this, they first showed that DC phagosomes have an elevated pH compared with these organelles in macrophages. They proceeded to demonstrate the recruitment of a key component of the NOX2 complex, gp91*phox*, to DC phagosomes and the ablation of alkalinization in DCs deficient in this subunit. More importantly, gp91*phox*-defective DCs showed more-aggressive antigen degradation and, consequently, less efficient levels of crosspresentation.

Concluding remarks

It is still unknown whether the NOX2-mediated regulation of proteolysis is linked to efficient retrotranslocation of exogenous material (as shown in Figure 1), although Ackerman *et al.* [14] had speculated earlier that a mechanism along these lines might be involved. Similarly, it is unclear whether a subset of lysosomal enzymes active at high pH are involved in incomplete antigen degradation and whether such limited proteolysis is important for antigen delivery to the cytosolic compartment, for example, by enabling the passage of large antigens through the size-constrained sec61 pore complex. Interestingly, a commonality in both studies is the DC exclusivity of the described molecular mechanisms. Of course, this is to be expected because it is the DCs that are thought to be the key crosspresenters *in vivo*. Regardless, these new studies highlight the uniqueness of DCs with respect to antigen handling. Indeed, Savina *et al.* [7] speculate that phagosomal pH and, thus, the aggressiveness of antigen degradation define whether a cell is biased to microbial killing (as in the case of macrophages) or skewed to efficient presentation (as in the case of DCs), consistent with previous proposals [16]. Consequently, these studies not only reveal the inner workings of the crosspresentation pathway, but also reinforce the notion that DCs are the most professional of professional antigen presenters, with antigen-handling mechanisms specially geared towards T cell stimulation rather than any other purpose.

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