# A new RIDDle in DC-mediated cross-presentation

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**Hyperactivity of a branch of the unfolded protein response in CD8α$^+$ dendritic cells degrades endoplasmic reticulum–associated mRNAs, which leads to a defect in the cross-presentation of dead cell–derived antigens.**



A subset of mouse dendritic cells (DCs), CD8α$^+$ DCs, which have similarities to CD141$^+$ human DCs, are particularly efficient at internalizing and cross-presenting dead cell–derived antigens to major histocompatibility (MHC) class I molecules for the activation of CD8$^+$ T cells. This process has been proposed to control infection by certain types of cytotoxic viruses and parasites, elicit antitumor immune responses and facilitate central and peripheral tolerance[1]. Therefore, understanding its regulation in both physiological and pathophysiological settings is an important goal. In this issue of *Nature Immunology*, Osorio *et al*. report that hyperactivity of the endoplasmic reticulum (ER) stress-reactive endonuclease IRE-1α causes a defect in the cross-presentation of dead cell–derived antigens by CD8α$^+$ DCs[2]. The mechanism for this seems to involve the previously described process of regulated IRE-1α-dependent degradation (RIDD), whereby hyperactive IRE-1α, which normally functions to selectively process mRNA encoding the ER stress protein XBP-1, exerts its nuclease activity on ER-associated mRNAs other than *Xpb1* mRNA[3].

IRE-1α is an ER-spanning protein that is activated as part of the unfolded protein response (UPR) to ER stress. Its activation involves trans-autophosphorylation, nucleotide binding and homo-oligomerization, which are triggered by the accumulation of unfolded proteins in the ER[4]. In normal physiology, such modifications of IRE-1α activate its cytosolic endo-RNase domain, which selectively splices *Xbp1* mRNA and thereby enables its translation into the transcriptionally active XBP-1 protein. XBP-1 induces many proteins that serve to relieve ER stress, including protein chaperones and proteins that facilitate the degradation of misfolded proteins. Treatment of *Drosophila* cells *in vitro* with drugs that activate a robust UPR results in not only the splicing of *Xbp1* mRNA but also the endonuclease-mediated degradation of various mRNAs undergoing cotranslational translocation across the ER membrane[3]. Such mRNAs contain a consensus sequence in a stem-loop structure sensitive to IRE-1-mediated cleavage. The authors speculate that the RIDD process may work in concert with the translation-suppressing effect of the UPR to relieve ER stress[3].

Studies subsequent to that initial report[3] revealed that RIDD can be activated in cultured mammalian cells treated with ER stressors or chemical reagents that force the autophosphorylation and high-order oligomerization of IRE-1α. Moreover, as exemplified in the study of Osorio *et al*.[2], IRE-1α and RIDD are hyperactivated when XBP-1, which is a negative regulator of IRE-1α, is targeted through genetic engineering[5]. Although the physiological relevance of such models of RIDD activation is uncertain (discussed below), studies of those models can reveal interesting biological effects. For example, in XBP-deficient plasma cells, RIDD cleaves mRNA encoding secretory immunoglobulin μ-chains, which leads to defective production of immunoglobulin M[6], and in certain cell lines treated with experimental ER stress–inducing agents, RIDD-mediated degradation of microRNA has been linked to the activation of caspase-2 and apoptosis[7]. Evidence *in vivo* that RIDD is an important component of the physiological UPR or contributes to chronic UPR-driven disease processes is lacking. However, IRE-1-mediated cleavage of individual mRNAs has been shown to have functional consequences under certain dietary conditions in mice or during development in *Drosophila*[8,9].

Osorio *et al*. find that the combination of high basal IRE-1α activity in CD8α$^+$ DCs plus the added IRE-1α-activating effect of deletion of XBP-1 leads to the hyperactivation of IRE-1α sufficient to activate RIDD[2]. By comparing cells in which XBP-1 is deleted with those in which IRE-1α is deleted (which also cannot generate XBP-1s protein), the investigators conclude that defective cross-presentation of antigens derived from dead cells, which is the main alteration in the phenotype of XBP-1-deficient CD8α$^+$ DCs, is IRE-1α dependent and thus is probably due to RIDD. Interestingly, another alteration in their phenotype, expansion of the ER, is not IRE-1-α dependent and probably reflects a compensatory response to the deficiency in XBP-1-induced ER protein chaperones. Curiously, a published report has shown that DCs from lymphoid chimeric mice lacking XBP-1 have diminished survival[10], but the mechanism for this has not been reported.

Pending the arrival of data to support the proposal of the physiological relevance of RIDD, findings obtained with experimental RIDD can be exploited to delineate various mechanisms of cell biology. In the present study, the selectivity of the defect in the cross-presentation of dead cell–derived antigens versus the cross-presentation of soluble antigens or presentation of endogenous antigens on MHC class I is very interesting. DCs can acquire dead cell–derived antigens by phagocytosis of apoptotic cells through a process called 'efferocytosis', which involves the recognition of apoptotic cells by cell-surface

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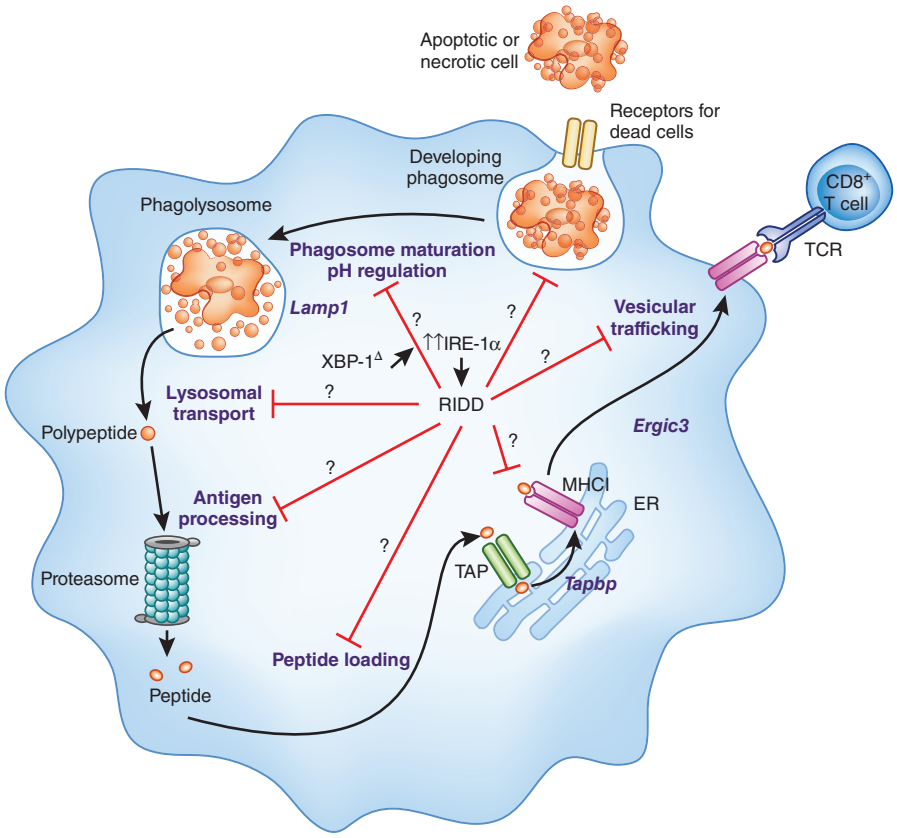


**Figure 1** Possible mechanisms by which RIDD compromises the cross-presentation of dead cell–derived antigens in CD8α+ DCs. Deletion of XBP-1 (XBP-1Δ) in CD8α+ DCs leads to hyperactivation of IRE-1α, which leads to the cleavage of multiple mRNAs by endonucleases (RIDD). The main consequence observed by Osorio *et al.* is defective cross-presentation of dead cell–derived antigens[2]. In theory, one or more mRNAs encoding proteins involved in the various stages of antigen cross-presentation (bold purple font) could be relevant targets of RIDD. Osorio *et al.* have identified several mRNA substrates of RIDD that could be involved[2], such as *Lamp1, Tapbp* and *Ergic3*, but the roles of their products remain to be demonstrated. Moreover, the specificity for dead cell–derived antigens rather than soluble or endogenous antigens raises the possibility that one or more molecules involved in the uptake and/or processing of dead cells may be the relevant targets of RIDD. TCR, T cell antigen receptor; MHCI, MHC class I.





receptors, followed by the phagocytosis of those apoptotic cells and their degradation in phagolysosomes. DCs can also internalize cells that have undergone post-apoptotic necrosis or primary necrosis, but the mechanisms are usually different from those of efferocytosis. The proposal of the functional importance of DC-mediated cross-presentation of dead cell–derived antigens in host defense is supported by a variety of *in vitro* and *in vivo* studies, but definitive evidence of a molecular-genetic cause obtained by actual *in vivo* infection models is limited. Progress has been made in this area through the identification of a receptor, DNGR-1, that recognizes necrotic cells[11] and a multiprotein complex of the receptor tyrosine kinase Axl, the lipoprotein receptor–related protein LRP1 (CD91) and the GTP-binding protein RanBP9 that mediates efferocytosis by CD8α+ DCs *in vivo*[12]. In both cases, virus-infected mice lacking those molecules show defective cross-presentation of viral antigen and greater susceptibility to viral infection.

Osorio *et al.* study the uptake, by DCs, of mouse embryonic fibroblasts (MEFs) treated with ultraviolet irradiation[2], which causes apoptosis or necrosis depending on the time of exposure. That distinction may have mechanistic implications for how RIDD functions in CD8α+ DCs, because if the defect were relatively specific for efferocytosis, one set of candidates for RIDD substrates would be mRNAs encoding efferocytosis receptors and/or their adaptors. However, if cross-presentation of antigens from both apoptotic and necrotic cells were affected, the focus would be on molecules common to both processes, such as phagocytosis, or on molecules involved in the key downstream steps of cross-presentation, which include antigen processing, loading onto MHC class I and transport of antigen–MHC class I complexes to the cell surface[13].

Investigators have proposed that DCs can use two cross-presentation pathways depending on the source of antigen, DC subtype and immunological setting. In the 'cytosolic' pathway, internalized and partially degraded antigens are exported to the cytosol, followed by proteasomal processing and loading onto MHC class I in the ER or in the endosomes (**Fig. 1**). Transport into the ER may be mediated by TAP ('transporter associated with antigen processing'), which effects this function in the endogenous MHC class I pathway. In the 'vacuolar' (or 'endocytic') pathway, both antigen processing and loading on MHC class I occur within endosomes or phagosomes in a proteasome- and TAP-independent manner, with MHC class I recycled from the plasma membrane. In reality, this dichotomy is simplistic, and 'hybrid' pathways have been observed in certain experimental settings.

In this context, Osorio *et al.* first show that the export of an endocytosed, soluble reporter molecule to the cytosol is intact[2], but it remains unknown what would happen if that molecule were delivered to the DC inside a dead cell; i.e., the condition in which cross-presentation becomes defective in XBP-1-deficient CD8α+ DCs. In a promising step forward, the authors find mRNA substrates for RIDD in CD8α+ DCs that encode proteins involved in antigen uptake, lysosomal processing and transport (**Fig. 1**). Examples include the cell-surface antigen transporter CD207 (langerin), the lysosomal protein LAMP-1, and TAP-binding protein, which mediates the interaction between TAP and MHC class I. Future studies of molecular-genetic causes will be needed to determine if loss of those or any other of the many mRNA substrates for RIDD identified in XBP-1-deficient CD8α+ DCs encode molecules with a role in defective cross-presentation. If molecules that mediate the uptake of dead cells are not the relevant substrates of RIDD in XBP-1-deficient CD8α+ DCs (as discussed above), future studies should determine why these cells have a selective defect in the cross-presentation of antigens derived from dead cells, because the candidate molecules mentioned by Osorio *et al.* have roles in the acquisition and cross-presentation of antigens in general[2]. Another important issue is whether the coexisting XBP-1-dependent and RIDD-independent ER-expansion phenotype functions in concert with RIDD to effect defective cross-presentation.

In closing, we return to the most important issue surrounding the RIDD field in general, and the study by Osorio *et al.*[2] in particular: the physiological or pathophysiological relevance of RIDD in natural settings *in vivo*. It is essential that future studies of RIDD address this issue, perhaps by

investigating disease processes characterized by chronic, robust ER stress, such as certain types of neurodegenerative disease and advanced atherosclerosis. The type of hyperactivation of IRE-1α necessary for RIDD may be present in such settings. Moreover, it is possible that RIDD is activated in cells infected with certain types of viruses, in which IRE-1α is activated but the transcriptional activity of XBP-1 is blocked[14]. If future studies do identify a role for RIDD in natural disease settings *in vivo*, innovative work on strategies that disable RIDD in favor of *Xbp1* mRNA splicing[15] could have therapeutic benefit, including bolstering CD8α$^+$ DC–mediated host defense based on the work of Osorio *et al.*[2] presented here.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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# VE-cadherin phosphorylation decides: vascular permeability or diapedesis

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**Phosphorylation of the adhesion molecule VE-cadherin at tyrosine residues modulates the opening of endothelial junctions during inflammatory reactions. The replacement of two distinct residues in VE-cadherin shows that Tyr685 regulates vascular permeability and Tyr731 regulates leukocyte diapedesis *in vivo*.**



During an inflammatory response, leukocytes and fluids from the blood must reach the affected tissue. Tight regulation of endothelial junctions of the vascular endothelium is a critical molecular mechanism for controlling such events. VE-cadherin is a vascular endothelial adhesion molecule, specifically and exclusively expressed by endothelial cells, that controls integrity of blood vessels. In this issue of *Nature Immunology,* Wessel *et al.* report constitutive phosphorylation of VE-cadherin at Tyr731 *in vivo* and demonstrate that dephosphorylation of that residue contributes to leukocyte extravasation, whereas mediator-induced phosphorylation of VE-cadherin at Tyr685 results in vascular permeability[1].

VE-cadherin is a type I transmembrane protein with a calcium-dependent adhesive function in vascular cell-cell contacts. It has an extracellular portion that engages in homophilic interactions in *cis* to form dimers. Those dimers interact in *trans* and stabilize adherens junctions between adjacent endothelial cells. The cytoplasmic tail of VE-cadherin is associated with the cell cytoskeleton via proteins of the catenin family. Both the VE-cadherin homophilic interaction and its association with catenins have considerable importance for the integrity of the endothelium.

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Post-translational modifications of VE-cadherin, such as tyrosine phosphorylation or cleavage, are reported to be involved in the induction of vascular permeability and leukocyte transmigration. Several such studies were done *in vitro* and provided controversial data about the specific tyrosine residues involved—thus the urgent need for study of the *in vivo* relevance of VE-cadherin tyrosine phosphorylation. Instrumental for such final proof of different VE-cadherin functions *in vivo* are the new knock-in mice expressing phosphorylation-deficient VE-cadherin mutants with substitution of phenylalanine for tyrosine at position 731 (Y731F) or 685 (Y685F) generated by Wessel *et al.*[1].

Vascular permeability and leukocyte extravasation are very tightly controlled events that occur under physiological and pathophysiological conditions during inflammation. Both events can be mediated by transcellular routes (which pass through the endothelial cell body) and paracellular routes (which pass through cell-cell junctions). Several studies have indicated that the controlled opening of endothelial junctions is necessary for leukocyte transmigration and the egress of solutes from bloodstream to underlying tissues at sites of inflammation. The paracellular route is regulated mainly by proteins of endothelial tight and adherens junctions. Delineating the mechanisms that affect the functions of such proteins during inflammation is of chief importance and would allow the development of new targeted therapies that not only limit edema formation during stroke or myocardial infarction and leukocyte infiltration in inflammatory diseases but also improve drug delivery in solid tumors in which interstitial pressure is substantially elevated because of enhanced vascular permeability.

Therefore, during the past decade, several studies have been aimed at understanding the effects of vascular permeability-increasing factors, such as vascular endothelial growth factor (VEGF), tumor-necrosis factor (TNF), platelet-activating factor, histamine, bradykinin and thrombin, on leukocyte transmigration, vascular permeability and the function of endothelial junctions. Several of those studies have reported a correlation between the phosphorylation of VE-cadherin tyrosine residues and a decrease in the stability of adherens junctions. Five tyrosine residues of the cytoplasmic tail of human VE-cadherin are reported to be phosphorylated in conditions of increasing permeability or leukocyte extravasation and are proposed to participate in endothelial junction destabilization. In Chinese hamster ovarian cells transfected to express mutant VE-cadherin with a simple phosphomimetic tyrosine-to-glutamate substitution or a phosphorylation-deficient tyrosine-to-phenylalanine substitution, phosphorylation of Tyr658 or Tyr731 dissociates p120-catenin and β-catenin from VE-cadherin, which leads to decreased endothelial barrier function[2]. That first *in vitro* study suggested specific roles for Tyr658 and Tyr731 in regulating the stability of endothelial junctions. Human umbilical vein endothelial