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Don't mess with ERAAP!

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Abstract

Immunosurveillance monitors subversion of the endoplasmic reticulum aminopeptidase ERAAP. ERAAP-deficient cells are killed by T cells that recognize nonclassical major histocompatibility complex class I Qa-1 molecules presenting peptides generated in the absence of ERAAP.

In a classic example of scientific myopia, the genes encoding the many 'nonclassical' major histocompatibility complex (MHC) class I molecules expressed by vertebrates were once considered a 'junkyard', encoding molecules that could not pass muster as 'classical' class I molecules, which function mainly to present self and foreign peptides to CD8⁺ T cells. Truth, however, eventually comes into focus. 'Junk' genes encoding MHC molecules (like 'junk' DNA) are anything but junk, as they encode critical participants in the recognition of viruses and tumors by natural killer (NK) cells, the presentation of lipid antigens, antibody trafficking, iron metabolism and maternal-fetal tolerance¹. In this issue of *Nature Immunology*, Nagarajan *et al.* add another important function to that list². They report that the mouse nonclassical MHC class I molecule Qa-1 presents a peptide generated when the endoplasmic reticulum aminopeptidase ERAAP (ERAP) is inhibited, which allows a substantial subset of CD8⁺ T cells to monitor the activity of this enzyme dedicated to processing antigens presented by classical MHC class I.

Qa-1 was discovered in 1978 as a lymphocyte marker, but its first distinct function was identified decades later, as a ligand for the NK cell receptor heterodimer CD94-NKG2 (also present on some T cells), on the basis of its presentation of a highly conserved peptide (Qdm³) present in the endoplasmic reticulum–insertion sequence of two of the three classical class I molecules (H-2D and H-2L, but not H-2K)⁴. As the presentation of Qdm requires the entire classical MHC class I pathway (proteasomes, the transporter associated with antigen processing (TAP) and tapasin; Fig. 1), Qa-1 serves as a sentinel that directs NK cells to destroy potentially dangerous cells whose MHC class I pathway has been manipulated by pathogens or oncogenesis. Humans have a nearly exact functional homolog in HLA-E, which interacts with the homologous NK cell receptor by presenting a Qdm-like peptide from the signal sequence of many classical HLA class I molecules. HLA-E is expressed by T cells, B cells, NK cells and endothelial cells and is upregulated by proinflammatory cytokines, which also induce the secretion of a soluble form of HLA-E that protects cells from lysis by NK cells⁵. Qa-1 also presents foreign and non-Qdm self peptides to CD8⁺ T cells⁶. In the case of activated autoreactive CD4⁺ cells, the presentation of self peptide leads to CD8⁺ T cell–mediated suppression of autoimmunity⁷.

Early investigation into the proteolytic processing of TAP-independent antigens delivered to the endoplasmic reticulum showed that cells robustly trim amino termini⁸. Biochemical studies led to the identification and cloning of interferon-responsive genes encoding ERAAP in mice and ERAP1 and ERAP2 in humans^{9–11}. The first ascribed function elucidated for ERAAP involved the shedding of cytokine receptors¹², and ERAAP polymorphism has been linked to human autoimmune diseases¹³. ERAAP-deficient mice present peptides with amino-terminal extensions that diminish their affinity and hence their stability as cell-surface complexes with MHC class I molecules¹⁴. The altered repertoire elicits a robust CD8⁺ T cell response when wild-type mice are immunized with ERAAP-deficient cells¹⁵.

What do those T cells recognize? That seemingly esoteric question has yielded an interesting and important answer. Nagarajan *et al.* have isolated CD8⁺ T cells that recognize Qa-1 presenting a TAP-dependent ligand generated when ERAAP deficiency is induced genetically or chemically². This recognition does not require the expression of classical MHC class I molecules, the sole source of Qdm. To identify antigenic peptides, the authors heroically screen 2,880 plasmid pools comprising a cDNA library derived from ERAAP-deficient splenic dendritic cells. They transfect each pool into Qa-1-expressing L929 mouse fibroblasts and assess its ability to activate a Qa-1-restricted T cell hybridoma that recognizes ERAAP-deficient cells. They identify three positive pools, each containing a plasmid encoding the ‘hypothetical’ protein Fam49b. They use a series of truncation plasmids to identify the nine-residue peptide FYAEATPML (FL9) located at positions 190–198 in Fam49b and find that synthetic FL9 peptide activates T cells in a Qa-1-dependent manner at subnanomolar concentrations.

The isolation of naturally processed peptides by HPLC shows that FL9 elutes together with the natural peptide, whose recovery requires the presence of Qa-1. To identify cells that recognize complexes of Qa-1 and FL9 (Qa-1–FL9), Nagarajan *et al.* find that in contrast to the staining of NK cells by Qa-1^b–Qdm tetramers, NK cells are not stained by Qa-1–FL9 tetramers². Instead, Qa-1–FL9 tetramers stain ~20% of interferon- γ -secreting CD8⁺ T cells induced by the stimulation of splenocytes with ERAAP-deficient cells, which demonstrates its immunodominant status but also indicates the recognition of other potential peptide ligands. Remarkably, the authors identify CD8⁺ T cells stained with Qa-1–FL9, most expressing the memory markers CD44 and CD122, in naive C57BL/6 spleens at relatively high frequency. Indicative of their probable function, CD8⁺ T cells specific for Qa-1–FL9 efficiently kill ERAAP-deficient cells or tumor cells treated with ERAAP inhibitors.

Nagarajan *et al.* clearly show that immuno-surveillance monitors ERAAP activity via the presentation by Qa-1 of at least one peptide whose presentation is eradicated by ERAAP. Although their findings remain to be extended to humans and other vertebrates, several aspects of this system support the proposal of its generality. First, Qa-1 and HLA-E demonstrate considerable functional and structural homology¹⁶ despite their limited amino acid homology. Second, the gene encoding Fam49 is highly conserved among vertebrates, and BLAST analysis shows that the FL9 peptide is completely conserved in vertebrates as diverse as monotremes, birds, reptiles, amphibians and fish. Nagarajan *et al.* suggest that ERAAP monitoring is used for eradicating tumor cells that downregulate ERAAP to avoid the presentation of immunogenic tumor-specific peptides and cells infected with human cytomegalovirus², which encodes a microRNA that targets ERAP1 to diminish viral peptide presentation¹⁷.

As is typical, this groundbreaking study raises many intriguing questions. Is FL9 actually degraded by ERAAP, or is it regulated by a less-direct mechanism? Are the genes that encode Qa-1, Fam49a and ERAAP regulated coordinately in various tissues to coordinate immunosurveillance? Given the involvement of Qa-1 in the suppression of autoimmune

CD4⁺ T cells (and the link between ERAAP polymorphism and autoimmunity), the latter question is particularly relevant for various CD4⁺ T cell subsets. What are the other peptide ligands presented by Qa-1, and how do they increase the functionality of the system? Why are activated Qa-1–FL9–specific CD8⁺ T cells present in specific pathogen–free mice? Do they control commensal organisms? Are they involved in nontraditional functions, such as organ development or tissue remodeling, by eradicating unwanted (‘junk’) cells that signal their status via ERAAP modulation? Could ERAAP inhibitors be used clinically to recruit Qa-1–FL9–specific CD8⁺ T cells to eliminate tumor cells or autoimmune T cells?

Finally, in this era of increasing direction of research funding toward practical ends, this remarkable study provides a vivid reminder that curiosity-driven research into ‘esoteric’ questions predictably leads to unpredictable translational applications. Now, that is classic!

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Figure 1.

Model of ERAAP immunosurveillance. Like most MHC class I peptide ligands, a peptide of amino acids (AA) 190–198 of Fam49b (Fam49b(190–198)), or an extended precursor) is generated in the cytosol and transported into the endoplasmic reticulum by TAP in ERAAP-sufficient (ERAAP-WT) cells (left). Cells that synthesize the MHC class I molecule Qa-1 do not present Fam49b(190–198) when ERAAP is expressed, either because of its possible destruction by ERAAP in the endoplasmic reticulum, as shown here, or because of a less direct mechanism (for example, losing in a competition with ERAAP-generated peptides). In the absence of ERAAP (ERAAP-deficient (ERAAP-KO) cells; right), Fam49b(190–198) is delivered to Qa-1 and the Qa-1–FL9 complex is delivered to the cell surface, where it activates specific CD8⁺ T cells that kill the cell. This prevents pathogens or tumor cells from interfering with ERAAP function to avoid immunosurveillance. It might also be used for other purposes by the immune system, as memory T cells are present in young, specific pathogen-free mice. QFL, Qa-1–FL9; β_2m , β_2 -microglobulin.