

## Perforin

with

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### What is perforin and how does it work?

**Mark Smyth:** Many cytotoxic lymphocytes including cytotoxic T lymphocytes (CTL) and natural killer cells (NK), utilize contact-dependent mechanisms to kill virus-infected and malignant cells. In particular, the granule-exocytosis mechanism requires calcium and within a few minutes of effector-target cell interaction (conjugation), the vectorial liberation of a granules releases pore-forming protein, perforin, proteoglycans, and a family of serine proteases (granzymes) into the intercellular space (1-3). Most significantly, a multitude of cell death pathways are packaged within the granules, but they all appear to depend on perforin for their effective delivery (4). The cell death pathways triggered operate through cysteine protease (caspase) activation, but some also in the absence of activated caspases (5). The past view has been that ring-like poly-perforin lesions, structurally and functionally similar to complement membrane attack complex (MAC) pores disrupt the target cell membrane, but this view is

now being seriously questioned. There is virtually no evidence for stable transmembrane pore formation *in vivo* and the selectivity of perforin's membrane-disrupting activity has been clearly demonstrated. Granzyme uptake into cells does not require perforin, and is mediated very efficiently and rapidly through receptor-mediated endocytosis. The recent revelations that granzymes and perforin both bind to the target cell surface as part of a single macromolecular complex bound with serglycin (2), further diminishes the feasibility of passive diffusion of granzymes. Rather, it is likely there is a role for perforin in disrupting endosomal trafficking *after* granzyme uptake into the target cell, although no one has been able to demonstrate entry of perforin into the target cell cytoplasm (6). Recently, it was demonstrated that the mannose 6-phosphate receptor (MPR) may act as a receptor for granzyme B uptake (7). Further work is required to illustrate this pathway in a physiological context.

### What is perforin's role in immune responses?

**Mark Smyth:** The role of perforin and granzymes in the pathophysiology of many diseases is now being dissected using gene-targeted mice that lack these molecules, and in humans with perforin mutations. Perforin plays a major role in the immune response against certain viruses and pathogens (eg. lymphocytic choriomeningitis virus (LCMV), Ectromelia and *Listeria monocy-*

*genes*, but also in cancers (spontaneous tumors of B cell origin), certain auto-immune diseases, transplantation, and viral immunopathology [recently reviewed in (4)]. These phenotypes are largely explained by the poor cytolytic capacity of perforin-deficient lymphocytes and the unsuspected roles for perforin in regulating B cell homeostasis and T cell memory.

### Living without perforin: Familial Hemophagocytic Lymphohistiocytosis

**Mark Smyth:** An excessive accumulation of CD8<sup>+</sup> T cells result-

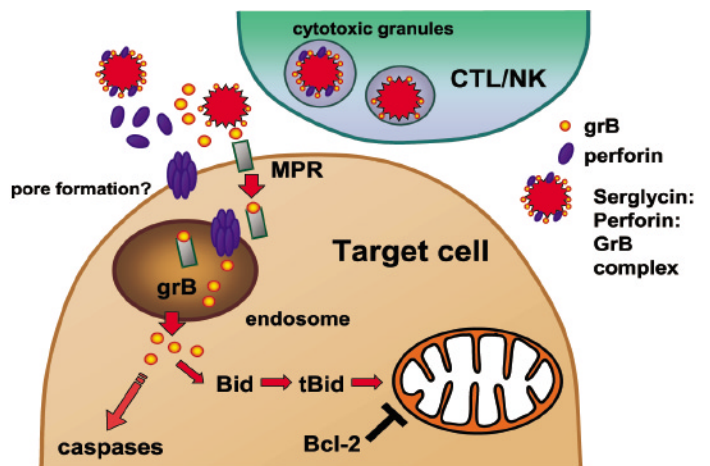
ing in immune-mediated damage in perforin-deficient mice mimics the clinical picture in perforin-deficient children suffering familial hemophagocytic lymphohistiocytosis (FHL) (8). FHL is a rare, rapidly fatal, autosomal recessive immune disorder characterized by uncontrolled activation of T cells and macrophages and overproduction of inflammatory cytokines. FHL occurs in previously healthy infants, and is only curable by BMT. FHL is attributable to any one of at least three loci, including mutated perforin at 10q21-22. Mutations includ-

ing homozygous nonsense mutations, mis-sense mutations and single amino acid deletions established perforin as an important down-regulator of human cellular immune activation. CTL and NK cell activity is markedly reduced or absent in these patients, and in many their cultured lymphocytes revealed little or no perforin in the cytotoxic granules. Although a causative infectious agent for FHL has not yet been defined, it is likely in perforin mutant patients to be a microbe whose control is strictly perforin-dependent.

### What must we learn?

**Mark Smyth:** Only perforin is indispensable for granule-mediated cell death, yet it is remarkable that so little is known about how perforin functions at the molecular level. Clearly, perforin is an excellent target for significantly diminishing CTL-mediated tissue damage in settings such as graft rejection and autoimmune diseases such as diabetes. Obtaining recombinant perforin in useful quantities has not yet been possible and therefore, neither a crystal or NMR structure of perforin or any of its domains is yet available. The pre-

dicted structure and function of its processed carboxy-terminus, modelled on the known structure of synaptotagmin-like molecules (9), has given us some new clues to express the active protein. The inability thus far to assign perforin functions to discrete parts of the perforin molecule represents a major gap in our basic understanding of effector lymphocyte biology, but the future rational design of perforin inhibitors (and perhaps agonists) may become a reality for regulation of CTL/NK effector functions in disease.



Revised model of granule exocytosis-mediated killing - challenging the dogma. (MPR = mannose 6-phosphate receptor; grB = granzyme B). It now remains unclear whether serglycin complexes, free granzymes, and/or free perforin enter target cells and if and how perforin/granzymes are released from complexes.

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