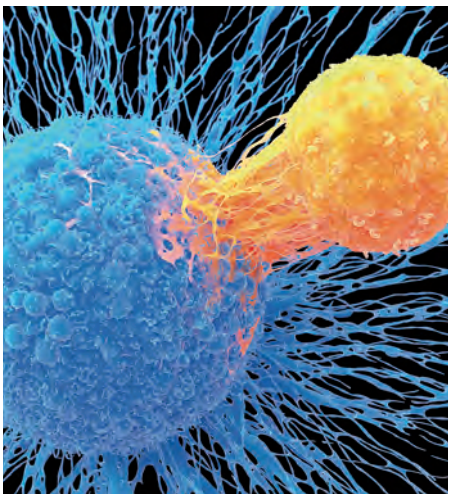
Milestone 2

T cells as killers

The cytotoxic (killing) function of T cells is central to their activity in immune defence — for example in eliminating infected or cancerous cells — but it was not until 1970 that cytotoxic activity was attributed to thymus-derived lymphocytes (T cells). Until the mid-1950s, immunology research was focussed largely on the humoral arm of the immune system mediated by antibodies. Yet there had been accumulating evidence that lymphocytes (rather than antibodies) are the active participants in the rejection of grafts and tumours, and in the resistance to infection. Some early evidence that this involved cell-mediated cytotoxicity came in 1960, with the demonstration by Govaerts that 'cellular antibodies' (that is, lymphocytes, as opposed to sera) from immunized animals destroyed allogeneic targets in vitro. This finding was extended to a variety of contexts in which lymphoid cells from sensitized donors destroyed various antigen-bearing ('target') cells. And in 1968, Kolb and Granger observed the release of a toxic cell-free factor termed lymphotoxin by activated human lymphocytes.

So, in 1970, drawing on the knowledge that neonatally thymectomized animals show impaired cell-mediated immunity (Milestone 1), Cerottini and colleagues explored whether thymus cells contained the precursors of 'sensitized lymphocytes' active in cell-mediated immunity. They transferred thymus-derived or bone marrow-derived cells to irradiated allogeneic recipient mice, and then tested immune spleen cell populations in the recently developed cytotoxic assay involving ^{51}Cr-labelled target cells. They found that the precursors of cytotoxic cells in the immune spleen cells were thymus derived. In a second study published in late 1970, the same authors confirmed that T cells were not only required but also sufficient for cytotoxicity. Knowing that thymus-derived cells express the surface marker θ antigen (later known as Thy1 and CD90), the authors showed that elimination of T cells from sensitized spleen cells by incubation with anti-θ antiserum in the presence of complement abrogated anti-allogeneic cytotoxicity in vitro but had no effect on the alloantibody response.

Thus, by the early 1970s, it was clear that some T cells can autonomously induce target cell death. Capturing this process through microcinematography, Rothstein et al. (1978) observed sensitized lymphocytes approach the target cell, form a conjugate and then leave the cell. The target cell burst after a delay, whereas the effector cell could go on to kill other target cells. Sanderson (1976) looked closely at the morphological changes of cell death using time-lapse imaging and described dying cells targeted by cytotoxic T lymphocytes (CTLs) undergoing violent 'zeiosis' of the cytoplasm (blebbing), which was distinct from the events that occur during complement-mediated lysis, but similar to apoptotic cell death. This suggested that CTLs did not kill the target cells directly but that the targets undergo cell death using their own self-destruction machinery.



"cytotoxic cells in the immune spleen cells were thymus derived"

Around this time, it became clear that there was exquisite specificity to the damage inflicted by CTLs, with few innocent bystanders, suggesting a directional secretory process. In 1982, Henkart and Henkart proposed the exocytosis model (published following the first International Workshop on Mechanisms in Cell-mediated Cytotoxicity, in 1981) showing that the lytic process is highly directed (polarized) towards the target by receptors on the effector cell, and involves lymphocyte reorganization of its granule contents to produce and subsequently to secrete clusters of membrane vesicles bearing lesion-forming molecules. This then results in localized transfer to the target cell membrane of lethal pore-forming material (Milestone 16).

Identifying this pore-forming material was the next step. This began in 1983 with detailed electron microscopy by Podack and Dennert, who saw that membrane lesions arise by tubular complexes that form transmembrane channels that are assembled from subunits during the cytolytic reaction. Henkart et al. (1984) then showed that granules purified from lymphocytes, but not from non-lytic cells, were sufficient to kill target cells through the formation of calcium-dependent ring-like structures. In 1985, Masson and Tschopp isolated from these cytolytic granules the 66kD protein perforin, which they showed polymerized and inserted into lipid bilayers to form tubular structures in the presence of calcium. The key physiological role of perforin in cytotoxicity was later confirmed by the generation of perforin-deficient mice, which showed defective cytotoxicity mediated by T cells and natural killer cells, leading to impaired clearance of virus infection and tumours (Kagi et al. 1994). Similarly, in 1999, perforin gene defects were shown to be responsible for the rare human immune disorder familial haemophagocytic lymphohistiocytosis, in which lymphocytes show defective cytotoxic activity and express little or no perforin in their granules (Stepp et al. 1999).

It is now understood that cytotoxic lymphocytes (which chiefly belong to the CD8$^+$ T cell subpopulation; Milestone 5) kill target cells using pathways other than perforin-mediated cytotoxicity, such as through the FAS pathway and the cytotoxic mediator TNF, highlighting the importance of this feature of the immune response.

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Nature Reviews Immunology

Milestone studies

Cerottini, J. C., Nordin, A. A. & Brunner, K. T. In vitro cytotoxic activity of thymus cells sensitized to alloantigens. *Nature* **227**, 72–73 (1970) | Cerottini, J. C., Nordin, A. A. & Brunner, K. T. Specific in vitro cytotoxicity of thymus-derived lymphocytes sensitized to alloantigens. *Nature* **227**, 1308–1309 (1970)

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