Killing Mechanisms of Cytotoxic T Lymphocytes

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Cytotoxic T lymphocytes mediate lysis of target cells by various mechanisms, including exocytosis of lytic proteins (perforin, granzymes) and receptor-ligand binding of Fas/APO molecules. Death of target cells is characterized by either necrosis or apoptosis, depending on the killing mechanism used and on the metabolism of the target cell itself.

Cytotoxic T lymphocytes (CTL) are antigen-specific effector cells of the immune system with the ability to lyse target cells such as virus-infected cells, allografted cells, or even parasites in a contact-dependent manner. Like other T lymphocytes, CTL initially differentiate in the thymus, where they acquire the specific T-cell receptor (TCR), a 90-kDa heterodimer composed of an α-chain of 40–50 kDa and a β-chain of 37–45 kDa. The TCR is closely associated with glycoprotein CD3, a complex of three to five invariant proteins that are apparently responsible for signal transduction. The majority of CTL also bears CD8 molecules, thought to bind reversibly to target cell receptor. Other surface molecules (CD2, CD28, intracellular adhesion molecule) appear to play an important role in activation of CTL following antigen recognition via TCR.

CTL are incapable of recognizing free antigens. Antigens are therefore initially absorbed by specific cells such as dendritic cells and Langerhans cells, which process and present the antigen at their surface along with molecules of the class I major histocompatibility complex (MHC I). Thus antigen binding of CTL is always restricted to the correct MHC determinants. Antigen recognition and binding are followed by proliferation and differentiation of CTL, an event which is promoted by various cytokines [interleukin (IL)-1, IL-2, and IL-12]. The antigen-specific activation of CTL is associated with distinct morphological alterations. The cells increase in size, become highly polarized, and form unique lysosomal granules containing a specific pattern of lytic proteins.

The lysis of target cells via CTL proceeds through a sequence of programmed steps, including killer-target cell binding, delivery of the lethal hit, target cell lysis, and killer cell recyle. After recognition of the appropriate antigen, the CTL bind firmly to the surface of the target cell, an event that depends on the presence of Mg<sup>2+</sup> but not Ca<sup>2+</sup>. The subsequent lysis of the target cells is mediated by two different mechanisms: exocytosis of lytic proteins and/or receptor-ligand binding of Fas/APO molecules. The various pathways may result in different types of target cell death: necrosis and apoptosis. Here, the different killing mechanisms of CTL are described in more detail.

Granule exocytosis model

Lysosomes of CTL have a unique morphology that can be seen only by electron microscopy (13). Each granule has an electron-dense core surrounded by small, round vesicles. Within the core, several types of lytic proteins are stored in an inactive form. Upon binding of the effector cell to the target cell, the lysosomes are directed toward the contact area by reorientation of the microtubule organization center. Subsequently, the content of the granules is released into the intercellular space via exocytosis. Cytolysis of target cell is then mediated by two types of lytic proteins, perforin and granzymes, that act either alone or in combination with each other.

Perforin pathway. The perforin pathway is shown in Fig. 1. Perforin is a pore-forming protein of 70 kDa (6). It is encoded by a single copy gene located on chromosome 10 and contains three exons. Mouse and human perforin genes display significant homology, and the transcription of the gene may be controlled in both species by similar multiple cis- and/or trans-acting regulatory factors. Furthermore, distinct sequence homology and immunologic cross-reactivity exist between perforin and complement components; in particular, in the central portion of the molecules (amino acid residues 189–218). Another conserved region located between residues 356 and 366 was found for the epidermal growth fac-
tor precursor type. Experiments using synthetic peptides and recombinant perforin revealed that the NH₂-terminal portion of perforin is the most important domain for the lytic activity.

After synthesis in the rough endoplasmic reticulum, perforin molecules are presumably post-translationally modified by N-glycosylation and are stored in the dense core matrix of CTL lysosomes as monomers. Released perforin monomers are then inserted into the membrane of target cells. Although the receptors postulated for perforin have not yet been found, there is some evidence that phosphorylcholine moieties serve as binding molecules (12). In the next step, membrane-bound perforin monomers polymerize and form pores with an average diameter of 16 nm (10). The pores resemble that of the complement membrane attack complex; the latter, however, are smaller in diameter (~10 nm) and are made by several complement components (C5b, C6, C7, C8, and C9). Death of the target cell via perforin is mediated either by uncontrolled influx of small molecules such as Ca²⁺ from the extracellular fluid or by induction of osmotic stress, which results in colloid osmotic lysis. Furthermore, perforin pores may serve as a conduit for other CTL-killing proteins such as granzymes (8).

Morphology of perforin-mediated killing is characterized by necrosis of target cells (Figs. 2a and 3). Membrane destabilization, which can be identified by either light microscopy with dye exclusion tests (Fig. 2a) or transmission electron microscopy (Fig. 3), is the initial indication of target cell death. Swelling of organelles and disruption of cytoskeleton are the next stages of necrotic death, but the nucleus remains intact for a prolonged time. Finally, the target cell dissolves completely, forming cell debris with residual organelles and membrane fragments.

Recent studies with perforin-deficient (knockout) mice confirmed the important role of perforin for cell-mediated cytolysis both in vitro and in vivo (4,7). CTL and natural killer cells of perforin-deficient mice are not able to lyse allogenic target cells and tumor cells. Furthermore, these animals cannot clear infections of lymphocytic choriomeningitis virus and they are unable to reject allo- and xenografted tumor cells. These results show unambiguously that the perforin mechanism is also active under in vivo conditions. However, perforin-deficient CTL are not completely inactive, but they show residual lytic capacity that may be mediated by other pathways.

**Granzymes.** Granzymes are a group of serine esterases with trypsin- and asparaginase-like activity, respectively (8). There are at least three types of granzymes (A, B, and H) in human CTL with a molecular mass varying between 27 and 60 kDa. They are highly glycosylated and antigenetically related, as demonstrated by their high degree of cross-reactivity. The various types of granzymes are stored within the granular matrix of CTL lysosomes, together with perforin, and they are secreted via exocytosis in the direction of the target cell in CTL-mediated killing. Within the target cells, the granzymes induce fragmentation of DNA at the internucleosomal level, resulting in apoptotic cell death (3).
The initial morphological alterations of CTL-mediated apoptosis occur within the nucleus (Figs. 2b and 4a), which is characterized by condensation of heterochromatin into spot- or crescentlike deposits along the nuclear envelope, whereas cell organelles appear unchanged. Furthermore, multiple membrane blebs are detectable at the cell surface, which may be released into the intercellular space (Fig. 4b). The subsequent steps of apoptotic cell death are morphologically defined by fragmentation of the nucleus into small round or oval bodies followed by complete destruction of the target cell (Fig. 2c).

In contrast to the classical form of apoptosis, which, e.g., occurs as programmed cell death during prenatal differentiation, the CTL-mediated DNA fragmentation progresses rapidly, i.e., within minutes, and is independent of synthesis of new DNA.
proteins in the target cells. The pathway for granzyme-mediated killing is not completely known. Granzymes themselves are not effective in the target cell killing, as shown by transfection experiments and in studies using purified granzyme A or B. It is therefore suggested that granzymes enter the target cell via perforin pores and are only then able to act intracellularly. Within the target cells, they may promote DNA degradation by cleaving histones, resulting in easier access of deoxyribonucleases to the nucleus. However, more and more evidence indicates that granzymes are also indirectly involved in DNA degradation by either binding to nuclear proteins such as nucleolin, which subsequently cleaves DNA, or activating the IL-1-converting enzyme (ICE) pathway up- or downstream (11). If the latter is the case, then granzymes would trigger a similar intracellular apoptotic pathway as is postulated for receptor ligand-mediated CTL killing.

Receptor-ligand mediated cytolysis (Fas/APO pathway)

Activated CTL bear a specific Fas/APO ligand (Fas/APO-L), also called CD95-ligand, which is expressed following antigen recognition either by de novo synthesis or by transformation of an inactive to an active form (1). Fas/APO-L is a 40-kDa type II transmembrane molecule. It belongs to the tumor necrosis factor (TNF) superfamily, which also includes TNF-α, lymphotoxin (LT)-α, LT-β, OX40-L, CD40-L, CD27-L, and CD30-L. These molecules exist as trimeric membrane-bound proteins and are characterized by partial homology of the COOH-terminal amino acid region within the extracellular space. The corresponding Fas/APO receptor (Fas/APO-R, CD95-R) is a transmembrane 48-kDa glycoprotein. It is expressed on a variety of cells and is upregulated in rapidly proliferating cells, e.g., lymphocytes and tumor cells. The Fas/APO-R has a cytoplasmic 65-amino
The combination of perforin and granzymes significantly increases the lytic ability of CTL.

References