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Mechanisms of Lymphocyte-Mediated Lysis

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Cytotoxic T lymphocytes (CTLs) and natural killer (NK) cells use multiple mechanisms to destroy their target cells. Pore formation resulting in osmotic lysis of the target is one mechanism; the pore-forming protein (perforin) responsible for this activity has been purified. Antigenically and functionally it resembles proteins of the membrane attack complex of complement. The other known mediators of cytotoxicity appear to be closely interrelated. Tumor necrosis factor (TNF), lymphotoxin (LT), and leukalexin are the three members of this group that have been purified, although their mechanisms of action are still unknown. CTLs fragment the DNA of target cells, as do TNF, LT, and leukalexin; this may be one of the mechanisms of action of these mediators. CTLs and NK cells do not self lyse. The basis of this phenomenon is unclear, although recent advances have shed some light on the problem.

Key words: cytotoxic T lymphocytes, natural killer cells, cytotoxins

The immune system must identify and destroy cells that pose a threat to the body, such as cancer cells and cells harboring intracellular pathogens. This task is accomplished in part by two cell types: the cytotoxic T lymphocytes (CTLs) and the natural killer (NK) cells [1].

Lymphocytes are divided into two major subsets: bursa-derived (B) lymphocytes produce antibodies; thymus-derived (T) lymphocytes coordinate and regulate the entire immune system and serve as effector cells for some functions such as cell killing. All T cells express the CD3 (or T3) marker on their surface. CD (cluster of differentiation) is the designation for markers on human leukocytes. The CD3 complex is closely associated with the T-cell receptor (TCR), an immunoglobulin-like protein that specifically recognizes antigens. In addition to the CD3 complex, all mature T cells have either the CD4 (or T4) or the CD8 (or T8) molecules on their cell surfaces. During antigen recognition by T cells, the CD4 and CD8 molecules recognize major histocompatibility complex (MHC) class-II and -I antigens, respectively. MHC molecules and antigens are linked on the surface of target cells, and it is the antigen–MHC complex that is recognized by the T-cell receptor. MHC molecules

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thus determine whether or not the TCR recognizes antigens on the target cell, a phenomenon known as "MHC restriction."

The T cells are functionally subdivided into several groups: helper T cells regulate all classes of immune cells by releasing mediators (lymphokines) that act on specific cell types; suppressor T cells act on other T lymphocytes to turn down the immune response. Helper and suppressor T lymphocytes are regulatory cells. Other T cells are effectors, and CTLs are included among these.

CTLs help defend the body from threats such as tumor cells and virus-infected cells by specifically killing them; they also participate in graft rejection. CTL-mediated lysis of target cells is MHC restricted. Most CTLs are $CD8^+$ and are thus MHC class-I restricted; some are $CD4^+$ and consequently MHC class-II restricted.

NK cells are lymphocytes of uncertain lineage [2] that lack mechanisms to specifically recognize target cells. They possess receptors for the Fc fragment of immunoglobulin and can thus recognize and kill cells coated with antibodies. This antibody-dependent cell cytotoxicity (ADCC) is also a property of macrophages and granulocytes in addition to NK cells [3]. The NK cells appear to correspond morphologically to the large granular lymphocytes (LGLs) found in blood and lymphoid tissues. In LGLs the cytoplasm is abundant relative to the nucleus, which confers a low buoyant density, enabling separation of LGL from other lymphocytes by physical methods [3–6]. Although NK cells are lymphocytes [2], they lack the characteristic markers of T or B cells. Minato et al. [7] obtained a continuous LGL line from hematopoietic stem cells in vitro that was $CD3^+$, $CD4^-$, and $CD8^-$. Southern blots revealed rearrangement of the beta and gamma chains of the T-cell receptor, indicating that the cell line was derived from T-cell precursors. The pattern of cytotoxic activity on a panel of tumor-cell targets was similar, but not identical, to that of splenic NK cells. T lymphocytes therefore may contribute to the NK population, although NK cells in general lack T-cell markers like CD3. CTL lines maintained in vitro in the presence of interleukin-2 (IL-2) lose MHC restriction and acquire an NK-like pattern of activity. This heterogeneity in lineage raises the question of whether the term "NK" should refer to a pattern of activity characterized by MHC nonrestriction and rapid lysis of target cells, rather than to a specific lineage.

Leukocytes in blood and spleen can be driven to become cytotoxic in vitro when cultured in the presence of IL-2. These lymphokine-activated killer (LAK) cells, when adoptively transferred to a tumor-bearing host, prevent some solid tumors from metastasizing. Studies in a number of different laboratories suggest that the LAK phenomenon can be attributed to IL-2-stimulated NK cells [8].

MECHANISMS OF KILLING

Excluding phagocytosis, mammalian cells use two general pathways for killing target cells: they either cause structural damage or interfere with vital functions. CTLs and NK cells appear to employ both mechanisms. Structural damage to target-cell membranes leads to osmotic lysis of target cells (Fig. 1). Cytotoxic cells also appear to fragment DNA in target cells, leading to cell death. The relative importance of these mechanisms in different in vivo situations is controversial and is therefore the focus of intensive research.

Several strategies have been used to investigate the mechanisms of cytotoxicity. One of these is to dissect out the different components of the cell machinery by

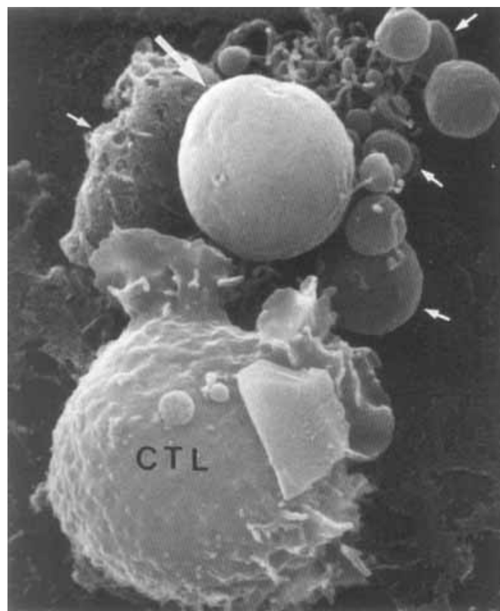

Fig. 1. Destruction of a P815 mastocytoma target cell by a murine CTL. The target-cell membrane has been disrupted and the nucleus (large arrow) and cell debris (small arrows) are all that remain. The CTL is undamaged. (Scanning electron microscopy performed by Dr. Gilla Kaplan of The Rockefeller University.)

purifying them and testing their cytotoxic activity in vitro [9–11]. Another avenue, relying on molecular biology, is to isolate specific transcripts that are expressed only in CTLs, essentially by making cDNA libraries from CTL and other lymphocyte subsets and using subtractive hybridization to clone those messages expressed only in CTLs [12–16]. Research has also focused on the role of the target cell and the possible existence of a genetically determined "suicide" program [17].

The prevalent model for CTL and NK cell killing divides the process into several stages [3,10,11,18]. Recognition of and binding to the target cell is the first stage, which is followed by delivery of the lethal hit. The final stage is that of killer-cell-independent lysis, during which the killer cell may dissociate from the target without interrupting the lytic process. The killer cell may then initiate a new lytic cycle with another target cell.

Binding

The MHC, CD4, and CD8 molecules are responsible for specific recognition and binding of target cells by T cells. Binding is a rapid, magnesium-dependent process, requiring only 2–3 min [19]. Adhesion between T cells and other cells may also involve other surface molecules such as the lymphocyte-function-associated (LFA) antigens. LFA-1 appears to be involved in the binding of CTLs and NK cells to their targets [20–23]. LFA-3 is present on a variety of cell types—erythrocytes, epithelial and endothelial cells, fibroblasts, and many myeloid cells—and may mediate adhesion between these cells and mature lymphocytes, including NK cells [21]. The role of these nonspecific adhesion molecules in killing by CTLs and NK cells is still controversial, although it is clear that the presence of specific antigens on the target

cell (TC) is not in itself sufficient for recognition and lysis [24,25]. Transfection experiments show that cells bearing antigens specifically recognized by CTLs are not lysed unless nonspecific conjugate formation occurs first [25].

Delivery of the Lethal Hit

Both CTLs and NK cells contain numerous granules in their cytoplasm. Binding of the TC is accompanied by polarization of the microtubule-organizing center and the Golgi apparatus toward the TC [26,27], followed by movement of granules toward the area of contact [27–32]. Immunofluorescent staining discloses that in the killer cell both actin and tubulin are polarized toward the target [26], whereas no such reorganization is observed when cells are presented with nonlysable targets. Finally, in a study of CTLs by Nomarski optics cinematography, the nucleus of the killer cell was seen to be displaced away from the target cell soon after target binding, and granules migrated toward the site of cell–cell contact [33].

Degranulation accompanies delivery of the lethal hit. Calcium appears to play a crucial role in the early events associated with killing. Binding of the target cell leads to an influx of calcium into the CTLs [34,35]. Degranulation is calcium-dependent [19,36,37], and agents that reduce calcium levels inhibit killing of target cells. Other drugs that interfere with exocytosis also inhibit killer cell activity. They include colchicine, a disrupter of microtubule organization; chloroquine, a weak base that localizes selectively in lysosomes and endosomes, increasing their pH [38]; and monensin, which interrupts membrane traffic through the Golgi apparatus [39,40]. Granules contain, in addition to serine esterases [41,42], various cytotoxic proteins [41,43,44]. Numerous lines of evidence attest to the importance of granules in cell killing. Granules isolated by differential centrifugation are cytolytic when tested against a variety of targets [43].

Perforin and Osmotic Lysis

One of the cytotoxic mediators in granules is a 70-kD pore-forming protein (PFP or perforin), which has been purified from human and murine NK cells and CTLs [44–46] and shown to be cytolytic [41,43,44, 47–49]. Perforin-like activity is also present in LAK cells [50]. The pore-forming protein from CTLs and NK cells appears to be identical, based on their identical molecular mass and functional and structural properties. Formation of pores on the target cell is strictly calcium dependent [19]. Electron microscopy reveals pores on the membranes of target cells that have been lysed by 1) CTLs or NK cells, 2) granule-enriched fractions from CTLs or NK cells, and 3) purified perforin. In all three cases (Fig. 2) the pores formed are cylindrical structures that appear as rings on cross section. The diameter of the pores varies between 10 and 15 nm [46,51]. Purified perforin polymerizes in the presence of calcium, forming macromolecular complexes that resemble the pores [52]. These pores are also formed in artificial lipid bilayers (Fig. 3), providing a convenient model for studying their characteristics [46,49]. Experiments with lipid bilayers have revealed that the pores are high-conductance, voltage-resistant channels permeable to all ionic and nonionic species [46,49] with Stokes diameters smaller than 9 nm. Pore formation occurs only after insertion of perforin into target-cell membranes, where it polymerizes to create pores. A polymer composed of as few as four molecules of perforin appears to be sufficient for functional pore formation. Production of electron-microscopically visible pores is not necessary for killing and seems to represent

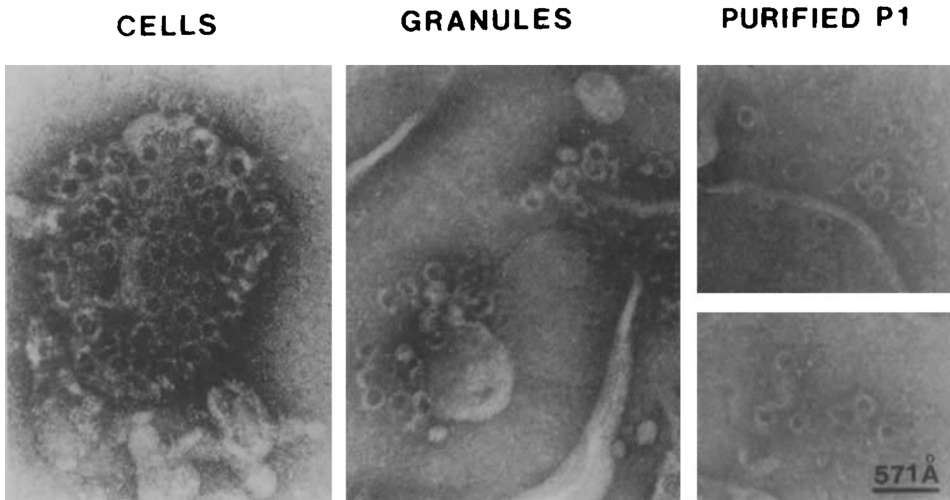

Fig. 2. Ultrastructural comparison of perforin complexes assembled by intact CTL (left), cytolytic granules (center), and purified perforin (right) in the presence of $CaCl_2$. Reproduced from Podack et al. [114] with permission of the National Academy of Sciences.

excess polymerization [53]. The current model of pore formation calls for the barrel stavelike insertion of perforin monomers around a central pore, which grows in diameter with the addition of each monomer. How the initial lesion forms is still not clear.

Time-lapse cinematography shows that the TC swells before lysis. Electron microscopy extends these observations to reveal blebbing of the target-cell membrane during the lytic process. Henney [36,37] demonstrated that the efflux of large cytoplasmic markers and membrane destruction can be prevented by the addition of exogenous high molecular weight dextrans. Dextrans of molecular weight of less than 40,000 fail to protect against lysis. Pore formation allows rapid equilibration of small molecules and ions down their electrochemical gradients. On the other hand, large intracellular molecules cannot pass through these pores, resulting in an increased osmotic pressure in the TC compared to its surroundings. Water rushes into the TC under the influence of this osmotic gradient, leading to cell swelling and consequent rupture. The protective effect of large dextrans is largely due to their ability to balance out the osmotic pressure across cell membranes.

An important unresolved problem is whether exocytosis is absolutely required for cell killing. Recently, Ostergaard et al. [54] and Trenn et al. [55] showed that in the absence of extracellular calcium, killing of target cells occurred without the release of serine esterases, which were used as markers of cytotoxic granules. These observations challenge the concept that degranulation is a prerequisite for killing. Another subject of debate is the physiological role of perforin. Long-term, IL-2-stimulated cultures of CTLs and NK cells, hardly representative of in vivo circumstances, were used in most past studies on perforin. In fact, perforin was not detected in resident murine lymphocytes of primary cytotoxic cells in mixed lymphocyte cultures [56]. Splenic lymphocytes acquire hemolytic activity only if stimulated with IL-2 [50,57]. Berke [58,59] showed that peritoneal exudate lymphocytes (PELs) produce perforin only when stimulated with IL-2, although other groups have detected

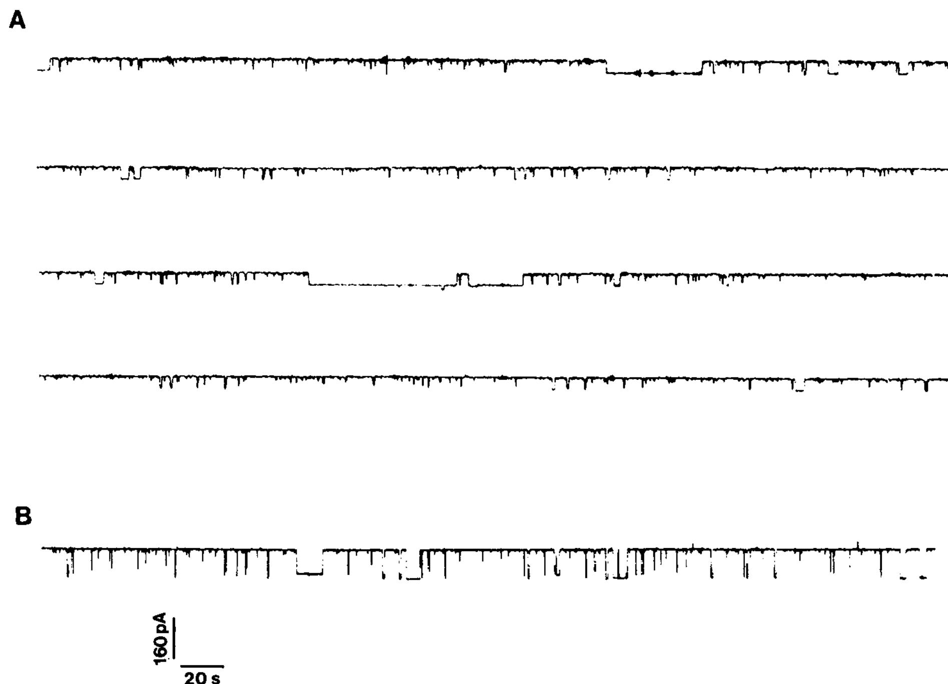

Fig. 3. (A) Single channel fluctuation associated with perforin. A lipid bilayer was exposed to purified perforin. The trace shows a continuous recording from upper left to lower right, obtained for 10 min after addition of perforin, with the bilayer clamped at +120 mV. The upward deflections represent channel openings. (B) Perforin was reconstituted into lipid vesicles and incorporated into a planar bilayer. The current trace was obtained 15 min after perfusion of bilayer chamber with urea free buffer. Reproduced from Young et al. [52] by copyright permission of The Rockefeller University Press.

perforin in alloimmune primary PELs [60]. It is clear that CTLs maintained in culture gradually lose MHC restriction [61–64], though they retain their T-cell markers. Perforin may therefore be a marker of MHC-unrestricted killer cells, irrespective of whether such cells derive from NK cell or CTL populations. When relatively high and sustained local concentrations of IL-2 develop, as might occur in a focus of inflammation or tumor growth, it would be advantageous to have cells with the enhanced cytolytic capability conferred by perforin. The decreased specificity of recognition by such cells, which MHC restriction would otherwise bestow, may be an acceptable trade-off.

Analogies Between Perforin and Complement

Perforin and the membrane attack complex (MAC) of complement share functional and antigenic similarities [reviewed in 65,66]. Complement is the collective name for a group of at least 15 serum proteins that are prominent in the acute inflammatory response. Complement activation is an archetypal cascade process, and the sequential mobilization of components ensures that even small stimuli result in highly amplified responses. Release of inflammatory mediators and chemotactic factors that recruit neutrophils to the site of injury is one consequence of complement activation. Complement also coats the surface of an invading organism, leading to either its lysis or to opsonization, whereby phagocytosis of the invading organism is

facilitated because phagocytes have receptors for complement. Both pathways of complement activation—classical and alternate—lead to a common membrane attack sequence wherein complement factors C5b, C6, C7, C8, and C9 successively insert into the target membrane, producing a pore. The pores lead to eventual lysis of the target cell by a mechanism resembling that of perforin [11,67]. The pores formed by perforin and complement appear similar on electron microscopy and in their functional characteristics, even though perforin pores are homopolymers and complement pores are now thought to be heteropolymeric, i.e., they are formed by a combination of C5b-6, C7, C8, and C9. Complement MAC factors and perforin share antigenic epitopes. Thus, immunoblots with human C5b, C6, C7, C8, and C9 and mouse perforin show cross-reactivity, but only under disulfide-reducing conditions and using antisera raised against reduced immunogen. These results suggest that the cross-reactive regions are normally masked by disulfide bridges [53]. Moreover, antibodies raised against membranes damaged by lymphocytes react with antigens expressed on lesions produced by the MAC. Whether this cross-reactivity between lesions is due to unmasking of shared epitopes during pore assembly is not clear. The MAC components have now been cloned, and their sequences are homologous, with amino acid sequence identities in the range of 20–30% [68–71]. A preliminary report of the sequence of perforin shows a similar degree of homology with C9 [72]. Considering the divergence of the humoral and the cellular immune responses in terms of function, the similarity of these effector molecules is surprising. Unlike complement, there is no evidence to suggest a cascade-type activation of perforin; intriguingly, many of the complement factors activate the next protein in the cascade by proteolytic cleavage, and the presence of serine esterases in perforin-containing granules is well established, although their function is unknown. Whether perforin is activated by serine esterases is an unanswered question. One significant difference between complement- and cell-mediated lysis is that fragmentation of DNA is seen only in the latter case, which raises the question of whether pore formation suffices to explain all aspects of CTL and NK cell killing.

Perforin-Independent Killing

Recent observations in several laboratories, including ours, have established that CTL cell lines can kill target cells in the absence of calcium and perforin [54,55,73,74]. Granules from CTLs are lytic even in the presence of EGTA. Moreover, several CTL lines that do not produce perforin retain cytolytic activity. It is now evident that a family of cytolytic proteins with functional and immunological relationships to tumor necrosis factor (TNF) exists [75]. In addition to TNF itself, the family includes lymphotoxin (LT), leukalexin, natural killer cytotoxic factor (NKCF), and possibly other proteins.

Lymphotoxin was first described by Granger and Kolb [76] as a soluble mediator of cytotoxicity produced by antigen- or mitogen-stimulated CTLs. Antibodies against LT block CTL-mediated cytotoxicity [77,78], but in view of the extensive cross-reactivity between the TNF-like factors, the significance of these observations is not clear. Analysis of a CTL cell line showed three different types of LT: one was the classical LT described by Granger and Kolb, a second form (LT-2) was antigenically related to TNF and LT and had a cytolytic spectrum similar to NK cells, and the third form (LT-3) was antigenically related to both TNF and LT and resembled LT in its lytic spectrum [79].

Old and colleagues observed that sera from animals treated with *Mycobacterium bovis* and bacterial endotoxin contain a factor causing hemorrhagic necrosis of transplanted tumors in mice, which they called "tumor necrosis factor" [80]. It was independently observed that rabbits infected with *Trypanosoma brucei* were severely cachectic, despite the presence of remarkably low parasite loads. A host factor, cachectin, responsible for this effect was purified. Further analysis and DNA sequencing have revealed that TNF and cachectin are the same protein [81].

Macrophages are the primary producers of TNF, although other cell types, including NK cells, may also produce it. When tested on different cell lines in vitro, TNF may have a cytotoxic or cytostatic effect, or no detectable effect on the target, depending on the cell line tested; indeed, it enhances the growth of normal diploid fibroblasts [82]. Interferon gamma can potentiate lysis by TNF [83], whereas proteinase inhibitors are known to abrogate TNF activity [84,85]. Somewhat surprisingly, possession of TNF receptors does not correlate with susceptibility to lysis by TNF [84]. Whether receptor-bearing but TNF-resistant cells fail to transduce signals properly is unknown. In NIH 3T3 fibroblasts transformed by adenovirus, expression of the E1A oncogene induces susceptibility to TNF lysis, suggesting that TNF lyses tumor cells preferentially [86]. Expression of adenovirus E1A in transformed cells protects against lysis by NK cells, but not against lysis by LAK cells [87]. Further work to clarify these observations may offer crucial insights into the role of TNF and TNF-like proteins in NK-cell-mediated killing. As of now unequivocal proof of the involvement of TNF or LT in killing by CTLs of NK cells is unavailable. The production of TNF by several cell types, the variability of its effect on different cell types, and its synergy with other growth factors all suggest that TNF has the wider role of an intercellular communication mediator, like the interleukins, rather than a cytotoxin.

TNF and LT have been cloned and sequenced [88–93] and are homologous, sharing 36% identity in their amino acid sequences. TNF induces DNA fragmentation [83] in susceptible cells, an effect that CTLs also display. Messenger RNA encoding TNF, and the mature protein itself, are seen in NK cells [94]. Aside from their putative roles in cell killing, TNF and LT induce proliferation of some cell types. LT, for instance, promotes B lymphocyte proliferation [95].

Leukalexin is a calcium-independent cytolytic mediator [74] with an apparent M_r of 50 kD under reducing and 70 kD under nonreducing conditions. Leukalexin lyses target cells more slowly than perforin (Fig. 4). Antibodies to TNF and LT cross-react with leukalexin, though it differs from them in its lytic spectrum and in other biochemical properties. It is present in the granules of CTLs and has been purified from a murine CTL line by affinity chromatography with antibodies raised against TNF. However, Northern blot analysis of poly(A)$^+$ RNA from two CTL cell lines failed to reveal messages for either TNF or LT, suggesting that although leukalexin is related immunologically to TNF/LT, they may represent distinct molecules [74]. Leukalexin also induces DNA fragmentation (Fig. 5). A cytotoxic protein purified from mast cells [96] may be related to leukalexin. The mechanism of action of leukalexin remains unclear at present, as do those of all members of the TNF family.

Other, as yet poorly characterized, TNF-like factors include natural killer cytotoxic factor (NKCF) [97]. NKCF activity may reflect the actions of multiple cytotoxins. It is immunologically related to both TNF [98–100] and LT [98] and perhaps to other as yet unidentified cytotoxins [101]. TNF and LT share the same

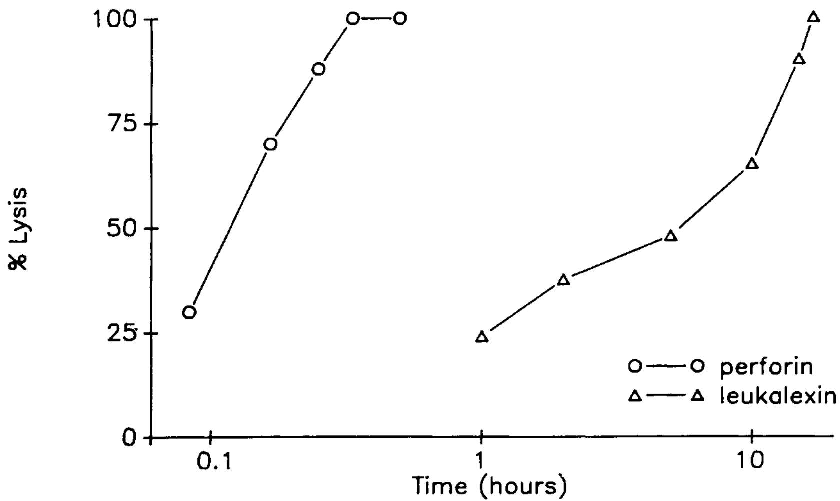

Fig. 4. Time course of perforin- and leukalexin-dependent killing. Lysis of P815 mastocytoma cells by perforin was monitored by OD_{700}. Cytotoxicity of affinity-purified leukalexin on WEHI-164 sarcoma cells was monitored by MTT assay.

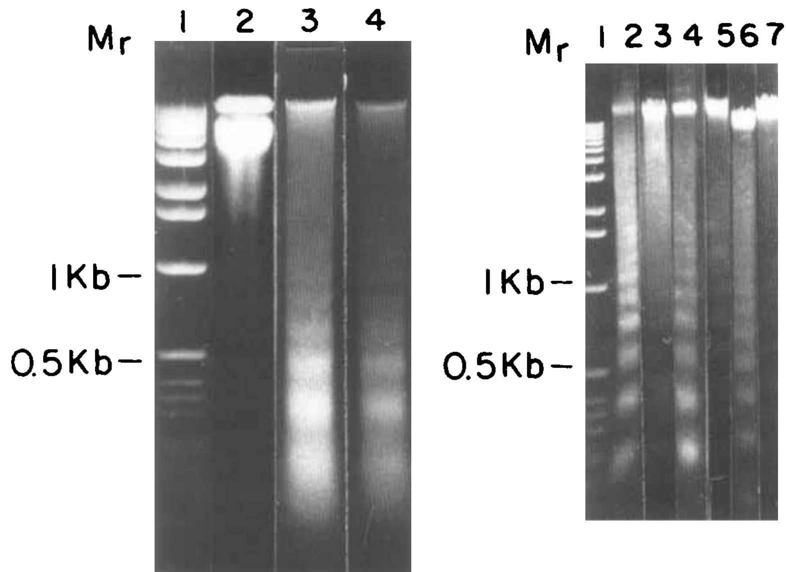

Fig. 5. DNA fragmentation induced by CTL cytotoxin and rTNF. **Left:** Perforin-depleted granule extracts from CTLs were added to WEHI 164 cells for 24 h, after which DNA ws extracted and run in a 1% agarose gel in the presence of ethidium bromide and visualized under UV. **Lane 1,** 1-kb DNA ladder; **lane 2,** untreated cells; **lane 3,** cells treated with rhTNF; **lane 4,** cells treated with CTL extracts. **Right:** EL-4 T leukemia (**lanes 2, 3**), P815 mastocytoma (**lanes 4, 5**), and R1.1 lymphoma (**lanes 6, 7**) cells. Lanes 2, 4, and 6, cells treated with perforin-depleted CTL granule extract; lanes 3, 5, and 7, control cells; **lane 1,** 1-kb DNA ladder. Reproduced from Liu et al. [74] by copyright permission of Cell Press.

receptor on target cells [102], which may also be shared by other members of the family of TNF-related cytotoxins, although there is no data suggesting the latter possibility.

Recent work by Ucker [17] suggests that a single element of an endogenous suicide program can be triggered by different effectors. In a thymoma mutant line resistant to the lethal and DNA-fragmenting effects of both glucocorticoids and CTLs, a single step reversion can restore sensitivity to both. This result strongly argues in favor of a role for target cells in their own destruction and forms in part the basis for the "induced suicide" model for CTL-mediated killing [103].

An area under active investigation is the role of serine esterases in cell killing. As discussed earlier, serine esterase activity is used as a marker for the granules from cytolytic cells. Whether serine esterases activate perforin in any way, thereby forming a cytolytic cascade, is poorly understood. The well-known serine protease activity of complement components fuels speculation that the analogy between perforin and complement can be carried further than previously supposed.

PROTECTION

How do killer cells avoid self lysis? The question is more easily asked than answered, and the answers available are preliminary and incomplete. One key fact is clear: the question is valid, because cytolytic cells do not kill themselves. Initial observations showed that CTLs could lyse many target cells sequentially, without self damage [9,104]. It was later shown that under certain circumstances CTLs can be lysed by other CTLs, suggesting that both lysis and its antithesis, protection from lysis, were vectorial processes [105–107]. These results were obtained with heterogeneous cell populations, which precluded clear interpretation. Luciani et al. [108] have extended these findings by testing CTL cell lines in a lectin-dependent cell cytotoxicity assay (LDCC). To eliminate the problems of specific recognition and MHC restriction, the target cells are coated with the lectin concanavalin A (Con A). CTL lines are refractory to lysis by themselves in the LDCC, but can be lysed by unrelated CTL clones.

With a panel of cloned CTLs and helper T cells, and target tumor cell lines, Kranz and Eisen [107] approached the problem using another strategy. Only one cloned CTL cell line (clone 2C) was used as the effector, and target recognition relied on coating target cells with a monoclonal antibody against the T-cell receptor on clone 2C. The cytotoxic cell lines, including one helper T cell line with cytotoxic activity, were resistant, whereas the tumor cell lines and noncytolytic helper T cell lines were sensitive to lysis by clone 2C. Defective recognition of cytolytic lines as targets was ruled out by experiments showing that unlabeled cytolytic and noncytolytic cells inhibited lysis of labeled target cells to the same extent. Comparable quantities of serine esterase were released by clone 2C when attacking cytolytic and noncytolytic cell lines. Since serine esterase was taken as a granule marker, and its release a sign of degranulation, these observations suggested that cytolytic cell lines did not protect themselves from lysis by somehow preventing the killer cell from releasing lytic granules. A similar picture emerges from studies conducted in other laboratories [109,110]. Skinner and Marbrook [109] used fluorescein-coated target clones and fluorescein-specific CTL clones and showed that CTLs were resistant to lysis. An intriguing result they obtained was that CTL clones were partially cytotoxic to other

CTL lines and that degree of resistance to lysis and cytotoxic potential went hand in hand. More extensive work, again with clonal populations, by Blakely et al. [110] demonstrated that CTLs were not only more resistant to lysis by other cytolytic cells, but were more resistant to lysis by isolated granules containing perforin. Shinkai et al. [111] showed that mouse CTL lines were resistant to purified perforin and, moreover, that treatment of splenic lymphocytes with perforin led to an increase in NK activity among surviving cells, suggesting that only the cytolytic cells resisted lysis. ATP-depleting agents, such as azide, cyanide, and 2-deoxyglucose, do not increase susceptibility of CTLs to lysis [112], although they potentiate lysis of noncytolytic cells, implying that the protective phenomenon does not involve a rapid removal of lesions or the activation of ion pumps and other energy-dependent processes that might somehow restore electrochemical gradients despite the open pores.

The protective phenomenon appears to be extremely specific. CTLs are fully susceptible to complement-mediated lysis [107], a reminder that in spite of the similarities, important differences exist between the two lytic pathways. Mellitin—a cytolytic, ion channel forming, bee-venom toxin—lyses CTL cell lines and noncytolytic cell lines with equal efficiency [112].

In view of the similarities between complement- and cell-mediated lytic pathways, studies on the protection of body cells against lysis by complement could help in elucidating the mechanisms whereby cytolytic cells avoid self-lysis. Homologous species restriction is a poorly understood phenomenon in which complement lyses erythrocytes less effectively if, and only if, complement and erythrocyte are derived from the same species. The presence of a polypeptide on erythrocyte membranes, homologous restriction factor (HRF) that interferes with channel formation and is immunologically related to C8 and C9 has been reported. A functionally similar protein (protectin) in CTL membranes could, in principle, confer the lysis-resistant phenotype, a possibility we are investigating. Martin et al. [113] recently reported that stimulation of CTLs with anti-CD3 antibodies led to expression of HRF by the CTLs. The presence of HRF on CTL membranes cannot explain the differential resistance to self-lysis and complement-mediated lysis seen in CTL lines by Kranz and Eisen [107]. Perhaps the multiple mechanisms for lysis will be matched by several, and not just one, protective mechanisms. Little is known so far regarding resistance to lysis by perforin-independent mechanisms.

CONCLUSIONS

Clearly, the major cytolytic cells in the body—CTLs and NK cells—use several mechanisms to fulfill their immunological functions. Two well-characterized types of mediators—perforin and TNF-like cytotoxins—are produced by both cell types. A combination of their activities suffices to explain, at least in principle, all known effects of killer cells on their targets. Other cytolytic factors await discovery. Even in the cases of perforin and the TNF-like cytotoxins, their role in vivo is incompletely understood and remains a focus of research and debate. Explaining protection from self-lysis is a challenge yet unmet, though some answers are coming in.

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