How Killer Cells Kill Their Targets

The killer cells of the immune system may destroy their target cells by secreting lethal proteins directly onto them

THE various killer cells of the immune system make a major contribution to the body's defenses against infections and possibly cancer. Loss of their ability to seek out and destroy target cells that have acquired foreign characteristics, perhaps as a result of virus infection or cancerous transformation, can be disastrous. The immune defects of AIDS (acquired immune deficiency syndrome), for example, include depression of some forms of cell-mediated killing, which helps to make the patients more susceptible to infections and some malignancies.

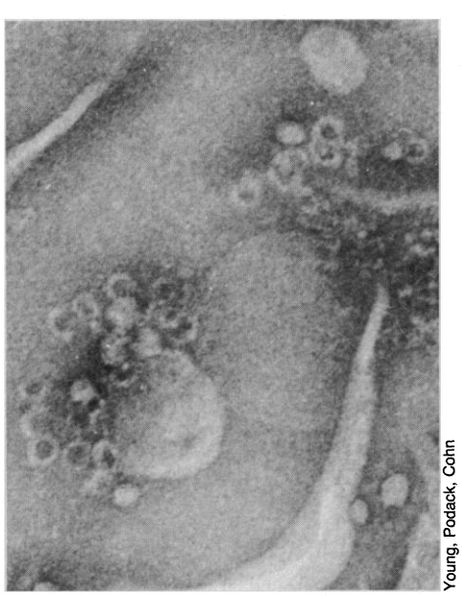
In contrast, stimulation of killer cells may have therapeutic value. A recent case in point arose last December when researchers at the National Cancer Institute (NCI) reported that killer lymphocytes that are activated by exposure to interleukin-2 show promise as a potential therapy for several human cancers.

Nevertheless, understanding of the way or ways in which killer cells actually destroy their targets has been slow to come, although that situation now appears to be changing. The conclusion is not universally accepted, but recent evidence suggests that the cells work by secreting directly onto their targets proteins that inflict lethal damage. Several types of killer cells use similar methods, releasing proteins that poke holes in the target cell membranes.

Immune responses are not only mediated by killer cells but are also brought about by antibodies that act in cooperation with the proteins of the complement system to destroy foreign antigens. The manner of killing being proposed for the killer cells is reminiscent of that used by the complement proteins. A number of gaps need to be filled before a complete picture of killer cell activities can be drawn, but if the current results are borne out by further investigation, it would mean that both the antibody- and cell-mediated branches of the immune system use similar methods of killing.

Some of the early evidence that killer cells damage the membranes of their targets came from Pierre Henkart's laboratory at NCI. For example, Henkart and Pierre Dourmashkin of the Medical Research Council's Clinical Research Center in Harrow, England, showed that circular pores of roughly uniform size form in the membranes of red blood cells that are under attack by either of two types of killer cells, including natural killers, which are so called because they can work without being specifically activated. Eckhard Podack, who is at the New York Medical College in Valhalla, and Gunther Dennert of the Salk Institute in San Diego detected similar ring-shaped lesions in the targets of a third type of cell, the cytotoxic T cell which does require specific activation. The pores formed by the various cells resembled those produced when certain of the complement proteins aggregate in membranes and form tubular lesions. Presumably the killer cells were also releasing proteins that produce similar pores in their target membranes.

A variety of evidence pointed to the granules that are generally present in the cytoplasm of killer cells as the likely source of the pore-forming proteins. For example, Henkart's and Podack's groups showed that isolated granules not only are very effective at killing cells but also produce ring-shaped pores very much like those induced by the killer cells.



Young, Podack, Cohn

Killer cell target

Ring-shaped pores form in the membrane of a red blood cell that has been attacked by a cytotoxic T cell.

Podack, with John Ding-E Young and Zanvil Cohn of Rockefeller University and their colleagues, found that the pores opened in this way are more or less permanent and allow free passage of several physiologically important ions. Such pores could prove lethal by disrupting the normal ionic milieu within the cell. In addition, influx of water could cause the cell to swell and burst.

During the last several months pore-forming proteins have been isolated from the granules of several types of killer cells. Young, Cohn, and Podack have isolated the proteins they call "perforins" from natural killer cells, cytotoxic T cells, eosinophils, and even *Entamoeba histolytica*, a pathogenic microorganism that causes dysentery in humans and is an active killer of cells. "The common denominator of all these proteins is that they make holes in membranes," Young explains. "No matter what triggers the cell to release the protein, the killing phenomenon is the same for all."

Meanwhile, Henkart and his colleagues obtained the pore-forming protein they have named "cytolysin" from the granules of large granular lymphocytes and more recently from cytotoxic T lymphocytes. Cytolysin resembles perforin in that both have molecular weights of 65,000 to 70,000 and comparable pore-forming abilities. Moreover, according to Henkart, antibodies to one cross-react with the other. "We really have two different names for proteins that may not be identical but are certainly related," Henkart says.

One of the more intriguing observations from Young, Cohn, and Podack concerns the apparent resemblance of perforin to the complement protein designated C9, which is a major component of the pore-forming complexes deposited in cell membranes during a complement attack. Perforin and C9 have similar molecular weights and are immunologically related.

In addition, investigators from at least three laboratories have found another possible parallel between killing mediated by cells and that produced by complement. Several of the nine or so proteins that make up the complement system are enzymes that are called serine proteases because they split protein molecules at residues of the amino acid serine. Activation of the system sets off a cascade of reactions in which the serine proteases progressively cut and thereby activate one another, ultimately leading to the activation of the proteins that constitute the pore-forming complex.

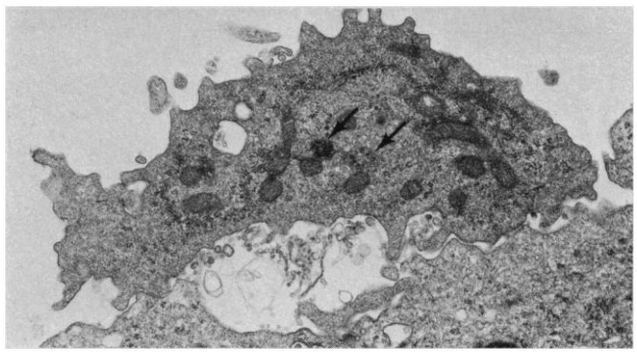
Killer cells also produce serine proteases that may play a role analogous to that of the

complement enzymes. For example, Mark Pasternak and Herman Eisen of the Massachusetts Institute of Technology have found a close correlation between killing ability and serine esterase production in several lines of cells. Moreover, Howard Gershenfeld and Irving Weissman of Stanford University and, independently, Corrine Lobe, R. Christopher Bleackley, and their colleagues at the University of Alberta in Edmonton have independently cloned a total of three genes that are expressed only in cells that have been activated to kill—and encode proteins that have the earmarks of serine proteases. Some 25 to 40 percent of the amino acid sequences of the killer cell proteins are identical to those of known serine proteases. In particular, the critical amino acids in the enzymes' active site are present in appropriate locations in the killer cell proteins, and the surrounding amino acids are also highly conserved.

The pattern of expression of the three genes is consistent with the possibility that they contribute to the lethal effects of killer cells. "There is a direct correlation between expression of the genes and cytotoxicity," Bleackley notes. The genes are turned on after the cells are activated and reach their peak expression between 10 and 24 hours before the cells attain their peak cytotoxicity. The Stanford and Alberta workers speculate that these proteins participate in a serine protease cascade that activates the pore-forming proteins of the killer cells just as the complement proteases activate that system's pore-forming complex.

Killer cells may have other weapons in their arsenal in addition to those of the proposed complement-like system. For example, lymphotoxin, which was independently discovered in the laboratories of Gail Granger at the University of California at Irvine and Nancy Ruddle at Yale University School of Medicine, is produced by lymphocytes and has cytotoxic effects, although the slowness of killing by this protein has raised questions about whether it has a physiological role. Whereas lymphocytes kill in minutes or hours, lymphotoxin by itself requires at least 24 hours to work.

Recently, Ruddle and her colleagues have shown that this time can be shortened to a few hours under conditions in which the target cells are induced to take up the protein internally. This finding, coupled with work from Podack's group indicating that lymphotoxin may be present in the perforin-containing granules, leads Ruddle to hypothesize that lymphotoxin exerts its effects inside the cell after entering through the perforin-induced pores. Her results suggest that these internal effects include causing the breakup of target cell DNA, although it may do this indirectly. Other investigators have found that cell-mediated killing is accompanied by fragmentation of the DNA of the target; this does not happen during killing by complement.



Natural killer cell

Vesicles can be seen in the space between the natural killer cell (top) and its target. The arrows point to fused granules [M. P. Henkart and P. A. Henkart, in Mechanisms of Cell Mediated Cytotoxicity, *W. F. Clark and P. Goldstein, Eds. (Plenum, New York, 1982), p. 237].

Tumor necrosis factor (TNF) is still another protein that contributes to killing by cells of the immune system. It is produced by macrophages and is particularly effective against cancer cells. Early clinical trials of TNF for cancer therapy are already beginning in a number of centers. TNF and lymphotoxin are structurally related, according to researchers at Genentech, Inc., in San Francisco, who cloned and sequenced the genes for both proteins. They find that nearly 30 percent of the amino acid sequences are identical in the two. Moreover, lymphotoxin and TNF display a similar range of activities, even though they are produced by different cell types.

Finally, Benjamin Bonavida and Susan Wright of the University of California School of Medicine in Los Angeles are studying "natural killer cytotoxic factor," which, as its name suggests, is produced by natural killer cells. It does not appear to be related to perforin or cytolysin, Bonavida says, but shares some similarities with TNF. According to the UCLA workers, natural killer cells from AIDS patients are deficient in releasing the cytotoxic factor, a circumstance that may contribute to the patients' poor immune responses. At least in culture, treating cells from AIDS patients with interleukin-2 restores their ability to release the factor.

Despite the current glut of immune cell proteins with cytotoxic effects, secretion of toxic substances was long dismissed as a possible mechanism of cell-killing. One reason for this is the need to account for the specificity that is shown by some killers, especially the cytotoxic T lymphocytes, which can only attack targets bearing certain antigens. Cells with the wrong antigens are not affected even though they are nearby while the killing is going on.

In addition, the killer cells themselves can survive the encounters with their targets and go on to kill again. They are homicidal but not generally suicidal. A secreted substance might be expected not to show this discrimination, and, in fact, both the isolated granules and the pore-forming proteins have much wider ranges of activity than the cells from which they are isolated.

For the lethal effects of killer cells to be unleased, however, the cells must make direct contact with their targets. According to proponents of death by secretion, specificity can reside in the recognition event, coupled with a limited secretion of cytotoxic substances directly onto the target at the point of contact. The contact is very tight and the membranes of the two cells fold in on one another. The release of small quantities of the lethal material into such a confined space would make hitting innocent bystander cells very unlikely.

Support for the idea of a localized release comes from Abraham Kupfer and S. Jonathan Singer of the Salk Institute and Dennert, who is now at the University of Southern California School of Medicine in Los Angeles. They find that when natural killer or cytolytic T cells contact their targets two associated structures, the Golgi apparatus and the microtubular organizing complex (MTOC), reorient in the killers toward the targets.

A killer cell destroys one cell at a time even though it may attach to three or four potential targets simultaneously. According to Kupfer, Dennert, and Singer, the Golgi apparatus and MTOC point only at the cell actually being destroyed and then swing to the next. "When a signal is received on one side of a cell, these two organelles move coordinately to face the direction of the signal," Singer says. The functions of Golgi apparatus and MTOC include protein secretion, and the researchers postulate that the reorientation serves to focus the release of the contents of the secretory granule onto the target at the point of contact.

Limiting secretion of the cytotoxic molecules to the point of contact between killer and target may help explain why innocent bystander cells are not killed. It does not readily account for the lack of effect on the killer cells themselves, although Ruddle

notes that lymphotoxin-secreting cells are susceptible to that protein's lethal effects, provided that there are not many potential target cells around to absorb the lymphotoxin.

Gideon Berke of the Weizmann Institute in Rehovot, Israel, has also found that the MTOC of killer cells reorients toward the targets, but he proposes another possible explanation. He points out that the microtubules participate in cell movements as well as in secretion. The reorientation of the MTOC might thus have more to do with the migration of killer cells towards their targets or with the folding of the membrane at the point of contact than with release of cytocidal materials.

Berke is somewhat skeptical about suggestions that killer cells act by secreting proteins onto their targets. "I do not discount the observation that cytocidal material can be extracted from killer cells," he explains, "but there is a need to prove the material is secreted and deposited on [target] cell membranes." Even Henkart concedes that direct proof of a transfer of material from killer to target is lacking, primarily because the experiments are technically difficult.

Moreover, Berke has been unable to detect ring-shaped lesions in killer cell targets like those seen by the other investigators. "I have done an extensive search for those rings and could not detect them in over 2000 targets examined," Berke maintains, even though he could see them in cells under attack by complement. The reason for this discrepancy in results is unclear. However, Henkart suggests that ring formation may not be absolutely necessary for cell-killing. Insertion of the pore-forming proteins into the membrane and lymphotoxin uptake may do sufficient damage even without formation of complete rings.

More work will be needed to pin down once and for all the role played by protein secretion in cell killing. Investigators are also interested in clarifying the relations among the assorted cytolytic proteins. The gene cloning now being avidly pursued in the various laboratories will help in this regard. Certainly the possible connection between the proteins and cancer and AIDS will not diminish interest in them. ■

JEAN L. MARX

ADDITIONAL READING

P. A. Henkart, "Mechanism of lymphocyte-mediated cytotoxicity," *Ann. Rev. Immunol.* 3, 31 (1985).

E. R. Podack, "The molecular mechanism of lymphocyte-mediated tumor cell lysis," *Immunol. Today* 6, 21 (1985).

N. H. Ruddle, "Lymphotoxin redux," *ibid.*, p. 156.

Weather Balloons at Venus

Starting on page 1407 of this issue, Soviet and French researchers describe how they dropped two instrumented Teflon balloons into the middle of the cloud layer enshrouding Venus and, with the help of American scientists, recovered 46 hours of meteorological data from each of them. Before the arrival of the balloons on 11 and 15 June of last year, the sum total of Venusian meteorological observations came from 15 probes that plunged from the top to the bottom of the atmosphere in an hour or less. From more or less constant altitudes starting at about 54 kilometers, the VEGA balloons provided wind, temperature, and pressure information whose preliminary analysis reveals far more blustery conditions than anticpated. VEGA 2 may even have been thumped by ripples caused by some of Venus's highest mountains.

Earth has nothing like the layer of the atmosphere in which the VEGA balloons flew, but then the Venusian atmosphere bears only a topsy-turvy resemblance to Earth's. Both begin to cool with increasing altitude above the surface, but Earth's atmosphere ends up being hottest where it is thinnest, at its top, whereas Venus's is hottest at the bottom, where carbon dioxide traps solar energy through the greenhouse effect.

Sitting above most of this hot atmosphere are the clouds, ranging between altitudes of about 48 and 60 kilometers. Within them is a planet-wide, 50-kilometer-thick layer traversed by the VEGA balloons that is curiously well mixed. Above and below that layer the atmosphere is stable and tends to resist vertical motion and the resulting turbulent mixing. Before these VEGA missions, researchers guessed that the clouds' absorption of thermal radiation from the underlying 700 K atmosphere led to heating, convection, and stirring that formed the mixed layer, somewhat the way the sun warms Earth's surface and produces puffy mounds of convective, cumulus clouds on a summer day. Alternatively, a sharp enough increase with altitude of the east-to-west global wind, which reaches 360 kilometers per hour at 60 kilometers, might mix the atmosphere through turbulence.

VEGA experimenters may have been looking for a bit of extra vertical motion and turbulence, but the typical vertical winds encountered of 2 to 4 kilometers per hour were more than anyone had bargained for, according to Richard E. Young of the NASA Ames Research Center in Mountain View, California. Calculations using convection theory predicted just such winds, which tends to support a convective origin for the mixed layer, but Young for one did not expect vertical winds as strong as those found only around terrestrial storms to prevail planet-wide on Venus.

Even stronger buffeting awaited VEGA 2 near the end of its active life. As it passed 50 kilometers above Aphrodite, one of the highest mountainous terrains on Venus, VEGA 2 encountered vertical winds as fast as 11 kilometers per hour. VEGA experimenters suspect that winds blowing over the 5-kilometer-high mountains created atmospheric waves above and downstream of the mountains that propagated all the way to the balloon level. Such propagating waves, including those created by convection at the balloon level, can influence the atmosphere far from their origins.

The VEGA balloons also made the first in situ observations of "weather" at Venus. The Pioneer Venus orbiter has returned images of waves propagating through the clouds, but the 6.5°C temperature difference found between the two balloons, whose paths straddled the equator, is the first in situ evidence that eddies as large or larger than those on the daily weather map affect the Venus atmosphere. On Earth, such eddies help carry heat from the equator to the poles. Further analysis of all the VEGA data should help clarify how the heat transport problem is solved on Venus.

A couple of phenomena failed to make an appearance. The cloud particle detector on each balloon's instrument package failed to detect any break in the clouds at that level, although the balloons may simply have remained within the same cloudy air mass throughout. And the lightning sensors found no detectable lightning around or beneath them even when passing over areas suspected of being prone to lightning generation. ■ RICHARD A. KERR