Cell Suicide in Health and Disease

Cells can—and often do—kill themselves, in a process known as apoptosis. This capacity is essential to the proper functioning of the body; flawed regulation may lie behind many diseases

by Richard C. Duke, David M. Ojcius and John Ding-E Young

As you read this article, millions of your cells are dying. Relax. Most are sacrificing themselves to ensure your survival. Burgeoning research indicates that the health of all multicellular organisms, including humans, depends not only on the body’s ability to produce new cells but on the ability of individual cells to self-destruct when they become superfuous or disordered. This critical process, today called apoptosis, or programmed cell death, was overlooked for decades. But biologists have recently made rapid strides in understanding how cellular suicide is enacted and controlled.

Many investigators are motivated both by scientific curiosity and by a desire to combat some of the world’s most frightening diseases. It turns out that aberrant regulation of apoptosis—leading to too much or too little cell suicide—probably contributes to such varied disorders as cancer, AIDS, Alzheimer’s disease and rheumatoid arthritis.

Researchers who studied embryonic development in the first half of the 20th century were the earliest to realize that cell death is not, as had long been assumed, invariably bad for the body; in fact, it is necessary. By the 1950s, they had shown that multicellular creatures obtain their final form by predictably eliminating selected cells. The tadpole deletes its tail during transformation into a frog; mammals erase countless neurons as the nervous system takes shape. Microscopists had also identified major signposts distinguishing this physiological cell death from accidental destruction, or necrosis.

Necrotic death occurs when a cell is severely injured, by a physical blow or by oxygen deprivation, for example. Swelling is a defining feature. Internal organelles—most obviously the mitochondria (the cell’s power plants)—and the entire cell balloon and rupture. These effects occur because injury prevents the cell from controlling its fluid and ion balance; water and charged particles (especially sodium and calcium ions) that are normally pumped out now stream in. Another hallmark is inflammation: circulating macrophages and other white blood cells of the immune system converge on the necrotic cells and ingest them. Inflammation helps to limit infection and clear away debris, but the activities and secretions of the white cells can also damage normal tissue in the vicinity, sometimes extensively.

Scientists viewing the cell undergoing apoptosis see very different changes. They find no swelling. Instead the dying cell shrinks and pulls away from its neighbors. Soon it appears to boil: blebs form on the surface and disappear, immediately replaced by others. Internal organelles retain their structure, but the nucleus, which is altered little during necrosis, invariably changes dramatically during apoptosis. Most prominently, its usually dispersed chromatin (chromosomal DNA with its associated proteins) condenses into one or more distinct blobs near the nuclear envelope.

At this point, apoptotic cells are often ingested by neighboring cells—including by scavenger cells that reside in all tissues—without inciting an inflammatory response. Dying cells that are not consumed may undergo further changes: typically the nucleus comes apart, and the cells divide into a number of “apoptotic bodies” that can contain a piece or two of the nucleus. As before, these bodies are removed quietly. (Biochemical studies contributed another signature of apoptosis in the late 1970s—the chromatin frequently breaks into fragments that produce a ladderlike pattern when the pieces are sorted by size on laboratory gels.)

Interestingly, certain cells that undergo programmed death are not gobbled up; today we know they persist for a long time or even indefinitely. The lens of the eye, for instance, is made up of the carcasses of cells that replaced most of their cytoplasm with the protein crystallin as they died. In the skin, cells called keratinocytes are generated by precursors in a deep layer; they then migrate to the surface, dying along the way. Instead of crystallin, they replace their contents with a tough protein called keratin and acquire a water-repellent coating. These dead cells constitute the protective outer layer of the skin until they are sloughed off and replaced by other keratinocytes.

Suicide by Slicing

Although most of the observable events that define apoptosis were well documented as early as the 1950s, and its role in embryonic development was understood, the importance of apoptosis to the daily maintenance of the fully formed organism would not gain recognition for another 20 years. The Australian pathologist John F. R. Kerr and his Scottish colleagues Andrew H. Wylie and Alastair R. Currie broke the ground in a paper published in 1972.

In their report, they contended that the same type of cell death evident during development also happens in mature organisms and continues throughout life. They suggested that unlike nec-
rosis, in which a cell is a passive victim, this form of death is active, requiring the cell to expend energy toward its own demise. The researchers further proposed that inappropriate initiation or inhibition of cell suicide could contribute to many diseases, including cancer. And it was they who, on the counsel of a co-worker, adopted the Greek word apoptosis to distinguish this kind of cell demise from necrosis (Greek for “make dead”). In classical Greek, apoptosis means “dropping off,” as in the dropping off of flower petals or falling leaves. (The word is usually pronounced “APP-oh-TOE-sis,” with the second “p” remaining silent.)

Despite the profound insights in the 1972 publication, its observations went largely unnoticed for more than a decade, until the few groups then pursuing apoptosis began to confirm the paper’s predictions. For example, they indeed found signs that apoptosis is ongoing and that its failure can contribute to cancer. The researchers also began to pinpoint some of the molecules involved in apoptosis.

CELL DYING through apoptosis, or cell suicide, undergoes distinctive changes. First it shrinks and pulls away from its neighbors (top right). Then blebs appear on the surface (making the cell appear to boil), and the chromatin (nuclear DNA complexed with proteins) condenses at the edges of the nucleus. Soon the nucleus, and then the cell itself, breaks up, and the cell fragments are quickly ingested by other cells in the vicinity.
that carry out and regulate the process. Countless scientists are now involved in deciphering exactly how and when cells kill themselves. We still have many unanswered questions but have uncovered some core principles. Most, if not all, cells manufacture a set of proteins that can serve as weapons of self-destruction. As long as a cell is useful to the body, it will restrain its death machinery. If, however, the cell becomes infected or malignant or otherwise threatens the health of the organism, the lethal proteins will be unleashed.

Apoptosis may be set in motion by various triggers, including withdrawal from a cell of the chemical signals (known as growth, or survival, factors) through which cells reassure one another of their importance. Death can also be triggered by a cell’s receipt of external or internal messages that override the reassuring ones or by the cell’s receipt of conflicting directives as to whether it should divide.

In some cell types, apoptosis will be triggered predictably; keratinocytes that migrate to the skin surface are dead and gone approximately 21 days after they begin their journey. Yet those same cells, as well as cells that are meant to last a lifetime (such as neurons and skeletal muscle cells), can be convinced to die prematurely if they become problematic. Sunburn, for example, will lead to apoptosis in keratinocytes that have not yet ascended very far through the skin.

In all cell types and in all multicellular organisms studied so far, the suicide weapons consist of various protein-cleaving enzymes known as ICE-like proteases. They are called “ICE-like” because they structurally resemble the first member of the group discovered—interleukin-1 converting enzyme (ICE). The ICE-like proteases that destroy the cell might be thought of as a collection of sharp knives that are normally kept under wraps. When the enzymes are activated (the blades are unsheathed and wielded), they chop various other proteins in ways that lead to destruction of the cell. Some of the cleaving destroys essential structural components of the cell. And some of the cutting leads directly or indirectly to destruction of the cell’s genetic material, thereby preventing the cell from maintaining itself.

In spite of their shared death machinery, cells can differ in the specific signals that induce them to eliminate themselves. The ease and speed with which the death program is activated can also vary from one type of cell to another and from one stage of development to another in a single cell. And a given cell may be sensitive to several different triggers. A major focus of current apoptosis research is specifying the full range of apoptotic inducers and deciphering how they lead to activation of the destructive ICE-like proteases. Scientists know that instructions delivered by the inducers are conveyed to the proteases by a series of intermediaries and that different triggers may use separate intermediaries. But, for the most part, the chains of interactions, or signaling pathways, await full characterization. Investigators have been especially stymied in finding the molecules that directly activate the proteases.

No Shortage of Triggers

A sense of the progress made so far can be obtained from a brief survey of some ways that cells known as T lymphocytes are persuaded to commit suicide at different stages in their life cycle. T cells are central players in the immune response against invading viruses and other microbes.

T cells arise from precursors in the bone marrow. The immature cells migrate to the thymus, where, as so-called thymocytes, they become more specialized. In particular, they begin to display the receptor molecules that later enable mature T cells to detect infection. To be beneficial, T cells must be able to attach through their receptors to microbial antigens (protein markers signaling an invader’s presence). At the same time, they must be blind to substances made by the body itself, because self-reactive T cells can destroy normal tissues. Only those thymocytes that make useful receptors will mature fully and enter the bloodstream to patrol the body.

While still in the thymus, thymocytes that fail to make functional receptors undergo apoptosis, as they are of no use. Thymocytes also kill themselves if their receptors bind strongly to molecules displayed in the thymus. Tight binding is a sign the cells might later target healthy tissue for autoimmune destruction.

Mature T cells that finally enter the circulation remain at rest unless they encounter the antigens their receptors can recognize. The resting cells, in common with thymocytes and many other cells, are susceptible to additional inducers of suicide: x-rays (such as those delivered during radiation therapy for cancer) and other agents that damage DNA. The damage spurs cells to produce a protein
Apoptotic Cells in the Adult

Virtually all tissues harbor apoptotic cells at one time or another. The cells usually commit suicide for the greater good of the body. The list of examples here is far from exhaustive.

Eye. The lens of the eye, which forms during embryonic development, consists of apoptotic cells that have replaced their inners with the clear protein crystallin.

Intestine. Cells composing the fingerlike projections of the intestinal wall arise at the base of the “fingers” and, over several days, travel to the tip. They die there and are sloughed off.

Skin. Skin cells begin life in the deepest layers and then migrate to the surface, undergoing apoptosis along the way. The dead cells form the skin’s protective outer layer.

Thymus. T lymphocytes—white blood cells that are critical components of the immune system—mature in the thymus. Those that would be ineffectual or would attack the body’s own tissues commit suicide before they have the chance to enter the bloodstream.

Uterus. When the cells of the uterine wall die and are sloughed off during menstruation, they perish by apoptosis.

Other. Cells that become infected by a virus or sustain irreparable genetic mutations often kill themselves. Failure of a genetically altered cell to commit suicide can contribute to cancer.

Cell Suicide in Health and Disease

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I explain why hepatitis viruses can cause viruses to infect relatively few liver cells and cause extensive liver damage even though the viruses are activated by the signal (3). The proteases damage the cell in many ways, including by attacking its structural scaffolding (4) and by activating enzymes that disrupt the nuclear chromatin (5).

A major tactic calls on the subset of T lymphocytes known as cytotoxic, or killer, T cells to eradicate infected cells. After killer cells bind to target cells, they bombard the cells with two kinds of proteins that together inflict a deadly one-two punch. One of the proteins (perforin) inserts itself into the membrane of the infected cell; there it forms a porelike structure that allows uptake of the other proteins—enzymes called granzymes [see “How Killer Cells Kill,” by John Ding-E Young and Zanvil A. Cohn; SCIENTIFIC AMERICAN, January 1988]. Granzymes can activate ICE-like proteases and thus induce apoptosis. But if that approach to killing fails, calcium ions that pass in through the new pores can collaborate with the granzymes to produce a necrotic death.

Less fortunately, the ability of T cells to induce apoptosis in one another and in infected cells can inadvertently doom healthy cells residing near infected tissue. This bystander damage occurs because many cells in the body display Fas, especially when they or their neighbors are infected. When killer cells home to infected cells, the Fas ligand protruding from the T cell surface can dock with Fas on the diseased cells, switching on the apoptotic machinery in the infected cells. That activity is useful and augments other immune tactics for combating infection. But Fas ligand on the T cells can dock with Fas on healthy cells in the vicinity and trigger their suicide as well. Medical researchers have suggested that this bystander effect may explain why hepatitis viruses can cause extensive liver damage even though the viruses infect relatively few liver cells.

**T Cells Die Too Easily in AIDS**

Induction of apoptosis in healthy cells is also believed to contribute to the immune deficiency that plagues AIDS patients. In people who contract the human immunodeficiency virus (HIV), the cause of AIDS, the T lymphocytes known as helper T cells die. As those cells disappear, cytotoxic T cells perish as well, because the cytotoxic cells need growth signals from helper cells in order to forestall apoptosis. When the T cells dwindle, so does the body’s ability to fight disease, especially viral and parasitic infections. Researchers know that many more helper cells succumb than are infected with HIV. It is also evident that a large number of the cells probably die through apoptosis. But no one knows what prompts this self-destruction.

One plausible answer invokes display of too much Fas. Recall that T cells normally make functional Fas only after they have been active for a few days and are ready to die. But helper cells from AIDS patients may display high amounts of functional Fas even before the cells have encountered antigen. This display of Fas would be expected to cause them to undergo apoptosis prematurely, whenever they encounter Fas ligand on other cells (such as on T cells already activated against HIV or other microbes). The primed cells may also trigger their own death, without receiving signals from activated cells, if they encounter the antigen recognized by their receptors. As we mentioned, antigen recognition leads T cells to produce Fas ligand. Ligand on the primed cell can contact the cell’s own Fas molecules and thereby activate the death program. Even worse, such primed, antigen-stimulated T cells, bearing both Fas and Fas ligand, can amplify the premature cell death by inducing suicide in one another [see bottom part of illustration on page 86].

It is also possible that molecules called oxygen free radicals trigger the suicide of virus-free T cells; these highly reactive substances are produced by inflammatory cells that are drawn to infected lymph nodes in HIV patients. Free radicals can damage DNA and membranes in cells. They will cause necrosis if they do extensive damage but can induce apoptosis if the damage is more subtle. In support of the free radical theory, researchers have found that molecules able to neutralize free radicals will prevent apoptosis in T cells obtained from AIDS patients. Antia apoptotic AIDS therapies are now under study.

**A Role in Autoimmunity**

Although normal helper T cells may be induced to commit suicide by other immune cells in HIV patients, the healthy cells are not technically dying from an autoimmune process. Autoimmunity is said to occur when the antigen receptors on immune cells recognize specific antigens on healthy cells and cause the cells bearing those particular substances to die. But true autoimmune diseases that involve apoptosis do exist.

If the body routinely eliminates self-reactive lymphocytes, how can autoimmunity occur at all? It turns out that the body actually allows some mildly self-reactive lymphocytes to circulate. These
Cells usually do little harm, but they can become overactive through several processes. For instance, if the lymphocytes also recognize some foreign antigen (say, on a microbe or in a food), exposure to that antigen can cause them to become unusually excited; they will then expand their numbers and may attack healthy tissue with gusto.

Autoimmune reactions usually are self-limited; they disappear when the antigens that originally set them off are cleared away. In some instances, however, the autoreactive lymphocytes survive longer than they should and continue to induce apoptosis in normal cells. Some evidence in animals and humans indicates that extended survival of autoreactive cells is implicated in at least two chronic autoimmune syndromes—systemic lupus erythematosus and rheumatoid arthritis. In other words, the lymphocytes undergo too little apoptosis, with the result that normal cells undergo too much.

Medical researchers are looking into the possibility that the autoreactive lymphocytes live too long because they produce molecules that block Fas ligand (protruding from other cells) from docking with Fas on their surface, thereby preventing the ligand from sending a death message into the lymphocytes. Other proposals suggest that the lymphocytes avoid apoptosis by underproducing Fas or overproducing the suicide inhibitor Bcl-2. In any case, increased understanding of how T cells live and die should provide clues to strategies for selectively activating the death program in the specific lymphocytes responsible for autoimmune conditions. For instance, it might be possible to deliver a Fas-activating molecule (perhaps Fas ligand itself) directly into an arthritic joint and thus to prompt the self-annihilation of the overactive immune cells.

Several tissues in the body appear to use Fas ligand to avoid becoming targets of autoimmunity. By displaying Fas ligand, certain cells in the testis, the eye and possibly the brain induce rapid apoptosis in any Fas-bearing activated T cells that come their way. Researchers are hoping to use this discovery to expand organ transplantation. At the moment, the only organs and tissues that can serve as grafts are those whose so-called tissue-typing antigens closely match those on a recipient's tissues. Matches must be close because a poor fit results in immune destruction of the graft. But if donor organs and tissues could be made to display Fas ligand, they might resist immunologic attacks by the host and so become suitable for transplantation.

**Cancer Cells Forget to Die**

In autoimmunity, immune cells fail to die when they are supposed to; in cancer, it is tumor cells that neglect to sacrifice themselves on cue. Indeed, scientists are increasingly describing cancer as a disease involving both excessive proliferation of cells and abandonment of their ability to die [see “How Cancer Arises,” by Robert A. Weinberg; *Scientific American*, September].

Cancer develops after a cell accumulates mutations in several genes that control cell growth and survival. When a mutation seems irreparable, the affected cell usually kills itself rather than risk becoming deranged and potentially dangerous. But if the cell does not die, it or its progeny may live long enough to accumulate mutations that make it possible to divide uncontrollably and to metastasize—to break away from the original tumor and establish masses at distant sites.

In many tumors, genetic damage apparently fails to induce apoptosis because the constituent cells have inactivated the gene that codes for the p53 protein. This protein, it will be recalled, can lead to activation of the cell's apoptotic machinery when DNA is injured. More than half of all solid tumors, including lung, colon and breast, are missing the p53 protein or manufacture a useless version.

Cells that become cancerous might still be disarmed by other apoptotic triggers. The tendency of normal cells to commit suicide when they are deprived of their usual growth factors or of physical contact with their neighbors is probably a built-in defense against metastasis; prompt activation of apoptosis in tumor cells that leave their native tissue presumably eradicates many metastatic cells before they have a chance to grow.
How Helper T Cells Normally Become Activated...

1 The stage is set for activation of a helper T cell when an antigen-presenting cell ingests a microbe, chops it up and displays the pieces.

2 The T cell becomes activated if its antigen receptor fits onto the displayed antigen, if a CD4 molecule attaches to the antigen complex, and if certain other molecules (at right) link together.

3 Fas could induce death immediately if it met an activated T cell bearing Fas ligand (not shown). But the primed cell could die without such contact if it recognized an antigen and so began to produce Fas ligand.

How Excessive Apoptosis Might Be Triggered in T Cells of HIV Patients

1 Excess suicide of helper T cells in HIV patients may stem from HIV's release of the gp120 protein, which can attach to CD4 molecules.

2 Such binding leads anti-HIV antibodies to link two CD4s together, a union that somehow primes the cell to kill itself, perhaps by inducing it to display functional Fas prematurely.

3 The gp120 protein of the gp120 protein, which can attach to CD4 molecules.

Unfortunately, cancer cells manage at times to become impervious to the apoptotic effects of growth factor deprivation and loss of cell-to-cell contacts.

Other apoptosis-related proteins have been implicated in malignancy. In several cancers, especially certain lymphomas, cell death is blocked by excessive production of the suicide-inhibiting Bcl-2 protein. And there is reason to suspect that some tumors prevent Fas from relaying signals to the death machinery or produce Fas ligand to avoid immunemediated apoptosis.

Oddly enough, certain normal cells make relatively high levels of Bcl-2. This Bcl-2 presumably preserves cells whose loss would be devastating to the body, but the protection comes at a price. Those same cells can be expected to give rise to aggressive tumors when they become cancerous. Protected by Bcl-2, they are less likely than other tumor cells to die; consequently, they may be more able to thrive as metastases in tissues that do not provide the survival factors made by their tissues of origin.

Consider melanocytes. These cells produce the pigment melanin, which darkens other skin cells and thereby helps to prevent them from absorbing lethal, burning doses of sunlight. If the melanocytes perished easily, the other cells would be put at risk. Melanocytes therefore manufacture large amounts of Bcl-2. Yet when melanocytes themselves become genetically damaged, they are less likely to commit suicide than other skin cells and are more likely, when malignant, to form aggressive tumors that spread readily.

Study of apoptosis has also begun to clarify why many tumors are resistant to the killing effects of radiation and chemotherapy. These therapies were once thought to destroy cancers directly, with the malignant cells dying by necrosis. Scientists now know that the cells generally die by apoptosis, often through activation of p53. Cells that lack p53 or that produce high levels of the Bcl-2 inhibitory protein can thus become inured to the effects of anticancer treatments.

Researchers are exploring genetic therapies for overcoming resistance to apoptosis. They are introducing a normal p53 gene into cancers that have damaged forms, with the aim of restoring production of the normal p53 protein. They are also investigating ways to prevent overactive Bcl-2 genes from giving rise to the Bcl-2 protein. Other anticancer approaches aim to block cells from receiving specific growth factors that promote their survival.

Apoptosis in the Heart and Brain

In contrast to cancer, where too little apoptosis occurs, excessive cell suicide accounts for much of the cell death that follows so-called ischemic heart attacks and strokes—those caused by blockage of a blood vessel feeding a segment of the heart muscle or brain. In
3. The activated cell proliferates. Activated T cells also display two molecules—Fas and Fas ligand.

4. In the primed cell, binding of antigen would lead to death, because Fas ligand could link up with the prematurely functional Fas and issue a suicide command. Primed antigen-stimulated cells could also induce suicide in one another.

...And How They Die

3. Activated T cells proliferate. Activated T cells also display two molecules—Fas and Fas ligand.

4. After a few days, Fas becomes able to bind to Fas ligand on the cell's own surface or on that of other immune cells. Fas ligand then instructs the T cell to use its death machinery and self-destruct.

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