Natural Killer Cells Help Defend the Body

Immunologists take a new look at immune surveillance and interferon action in the light of natural killer activity

Natural killer (NK) cells are a novel type of cell having the spontaneous ability to destroy tumor cells and also cells infected with viruses. As such, natural killers may play a major role in the body's defenses against cancer and virus infections.

The discovery of these cells about 6 years ago had two major consequences. It breathed new life into the somewhat moribund theory of immune surveillance, which holds that cancer cells raise spontaneously in the body but are recognized and destroyed by cells of the immune system before they can grow into a life-threatening tumor. Because not all of the theory's predictions appeared to be borne out, it had fallen into some disrepute in recent years. The existence of NK cells may have solved at least one of the major problems concerning immune surveillance.

At the same time, research on NK cells is contributing to a better understanding of the action of interferon, a potent, naturally occurring agent that has received widespread publicity because of suggestions that it might be useful in treating cancer and virus infections. Recent research has shown that interferon markedly stimulates the activity of NK cells, a finding which implies that the cells mediate at least some of interferon's actions. The interferon–NK cell link might have implications for the design of experimental cancer therapies.

The discovery of NK cells grew out of work on immune surveillance early in this decade when acceptance of the theory was at its peak. As it was then formulated, the theory emphasized specific killing of tumor cells by T lymphocytes. T cells, so-called because they require a thymus gland for their maturation, may perform any of a number of immune functions, including cell killing. The idea was that cancer cells carried unique antigens that would be recognized as foreign by the immune system, triggering the production of cytotoxic T lymphocytes specifically directed against the antigens.

According to Ronald Herberman of the National Cancer Institute (NCI), investigators studying this specific cell-killing usually obtained lymphocytes from cancer patients or from tumor-bearing animals and compared their effects on the corresponding isolated cancer cells with those of lymphocytes from normal individuals—the negative controls, or so the investigators thought. But the control cells frequently turned out to be more effective tumor cell killers than the lymphocytes from the tumor-bearing patients or animals. These experiments were often disregarded, Herberman notes, because they did not fit the prevailing theory that normal lymphocytes ought not to react.

Nevertheless, a few investigators, among them Herberman, Rolf Kiessling and Hans Wigzell of the Karolinska Institutet, and Mitsuo Takasugi of the University of California Medical School at Los Angeles, pursued the observation. And their investigations led to the conclusion that normal individuals and animals of all the species tested have a type of cell with the apparently spontaneous (or natural) ability to kill tumor cells. This type of cell-killing does not require priming by exposure to a particular tumor antigen, as does that by cytotoxic T lymphocytes, and is not specific for any one kind of tumor. It may be influenced by regulatory factors, however. "In retrospect, the phenomenon was there from the beginning," Herberman says, "but people were ignoring it." They are not ignoring it any longer.

The discovery of NK cells appears to have cleared up the mystery of the normal to low incidence of cancer in nude mice, which was observed by Osias Stutman of the Memorial Sloan-Kettering Cancer Center, among others. Because nude mice are genetic mutants that lack thymus glands and consequently do not have mature T lymphocytes, they would be expected to have a higher than normal cancer incidence, if these cells perform immune surveillance. The fact that they do not has been a major problem for the immune surveillance theory. The same is true of mice whose thymuses were removed at birth. Studies by Herberman and by Kiessling and Wigzell (who is now at Uppsala University in Sweden) have shown that the mice have normal, perhaps even elevated, numbers of NK cells—an intriguing hint that these cells, not mature T lymphocytes, may be performing immune surveillance.

Actually proving that NK cells carry out immune surveillance, especially in humans, will be difficult. Herberman points out, however, that "there is an increasing amount of evidence that they interfere with the growth of tumor grafts in rodents." The animal studies suggest that the cells both retard the development of primary tumors and prevent metastatic spread of the cancer, the most dangerous aspect of the disease.

Researchers have generally shown that when tumor cells are injected into mice with low NK cell activity, they form larger tumors in less time than when they are injected into mice with high NK cell activity. Particularly helpful in this regard is the beige mouse, a mutant found by John Roder of Queen's University in Kingston, Ontario, and Axel Duwe of University College in London to have very low levels of NK cell activity. (Most of the other immune reactions of the animals are normal.) Kiessling and his colleagues showed that beige mice, when injected with leukemia cells, develop tumors faster and at a higher incidence than do mice of the same strain with normal NK activity.

Further support for the role of NK cells in limiting tumor growth in mice comes from James Talmadge, who is currently at the Frederick Cancer Research Center in Maryland. In work done at Washington State University, Talmadge found that tumor cells that are highly susceptible to NK attack in culture grow poorly in normal mice but grow quickly and produce many metastases in beige mice. In contrast, tumor cells that are not killed by NK cells in culture grow equally well in both types of mice. A treatment that increased the animals' NK cell activity produced a decrease in the tumor growth rate and metastatic frequency of both NK-susceptible and NK-resistant cells. The decrease was seen even in beige mice, which apparently have cells capable of being stimulated.

A final indication of the possible importance of NK cells in immune surveillance is the observation from Herberman's laboratory that urethane, a chemical carcinogen, depresses the activity of NK cells from mice susceptible to the carcinogenic effects of the agent but not of NK cells from resistant mice. The re-

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lation between carcinogens and NK cells will no doubt be energetically explored.

Despite the relative abundance of evidence that NK cells might be performing immune surveillance in mice, there is much less evidence that they play an analogous role in humans. Since the mouse experiments cannot be duplicated in human subjects, investigators have had to content themselves with such expedients as comparing the NK cell activity of cancer patients with that of healthy individuals. In one such survey, which included several hundred patients with advanced cancer, Hugh Pross of Queen's University found that the NK cell activity is far below normal in the patients. As he points out, however, "We do not know which came first, the low NK activity or the cancer."

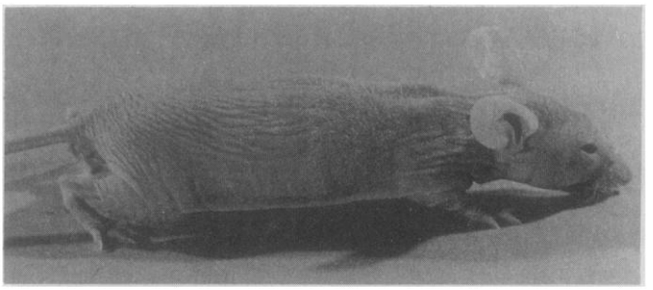
Some indication that low NK activity might predispose to cancers comes from studies of individuals with either of two rare genetic disorders. While investigating a family with a hereditary form of melanoma, Peter Hersey of Kanamatsu University in Sydney, Australia, observed that even family members who did not have the cancer had somewhat depressed NK cell activity. And Herberman, Roder, and Anthony Fauci of the National Institute of Allergy and Infectious Diseases found that patients with Chediak-Higashi disease have little or no NK cell activity. These individuals usually die from infections or from a lymphoma-like condition.

In most studies of NK cell activity the targets used have been tumor cell lines derived from lymphomas or leukemias, which are relatively rare human cancers, accounting for perhaps 10 percent of the total. Solid tumors, especially carcinomas, are far more common, and NK cells would be much less interesting if they did not attack cancer cells from the solid forms. Fortunately, these cells do turn out to be susceptible to natural killing, according to Stutman, who has looked mainly at sarcomas, but finds that carcinoma cells are also attacked.

The natural cytotoxic cells Stutman is studying are not quite identical to classic NK cells, however. He says, "We find a cell that is similar to the classic NK cell, but enough different to put it in its own category. . . . We think natural killer cells will turn out to be a very heterogeneous population." Other investigators also have evidence that the NK cell population consists of related but not identical cells. In addition, other types of immune cells, including specific cytotoxic T lymphocytes and macrophages, are known to attack tumor cells. Sorting out the activities of all these cells in the body will not be easy for researchers.

A nude mouse

These animals are hairless and have no thymus gland and no mature T lymphocytes. [Jackson Laboratory Photo]



The suggestion that NK cells are involved in the body's defenses against cancer would be adequate to ensure their close scrutiny by immunologists. But added to that is the evidence suggesting that interferon's actions against viruses and cancer cells are mediated at least partly by NK cells. According to Giorgio Trinchieri of the Wistar Institute, all three types of interferon (leukocyte, fibroblast, and immune) enhance the cytotoxic effects of NK cells on cultured target cells. The targets may be tumor, virus-infected, or even normal cells.

In what at first glance appears to be a paradoxical effect, interferon also makes some target cells resistant to killing by NK cells. But these observations are not as inconsistent as they seem. Trinchieri explains, "Although the interferon appears to be working against itself, when you treat both the effector [NK] and the target cells, the system actually becomes selective."

This happens in two ways. First, normal cells are well protected by the interferon, whereas virus-infected and most tumor cells are not. Second, in Trinchieri's assay system, NK cells are normally inactivated by their interaction with susceptible target cells. Like bees, they can sting only once. They are not inactivated by cells that have been made resistant by interferon treatment. "The effect," says Trinchieri, "is to concentrate the effects of the NK cells on pathological cell types."

Moreover, Trinchieri finds that tumor and virus-infected, but not normal, cells induce interferon production by NK cells, an indication that production of the agent may be stimulated when it is needed. Overall, the picture developed by the Wistar investigator suggests that the NK-interferon system is an inducible defense mechanism against virus-infected and tumor cells. Interferon does protect some types of tumor cells against killing, and this might be one way in which cancer cells can escape immune surveillance.

Most of Trinchieri's work has been done with cultured cells, but there is evidence that interferon modulates NK cell activity in the living animal. For example, several investigators have shown that stimulation of interferon production, by deliberately infecting animals with a virus, for example, also stimulates NK cell activity in experimental animals.

In humans, NK cell activity increases during naturally occurring viral infections, according to Pross. He finds that the activity changes very little in an individual, although it may vary from person to person. If the individual comes down with the flu or some other viral infection, however, the NK cell activity shoots up, possibly in response to stimulation of interferon production by the virus.

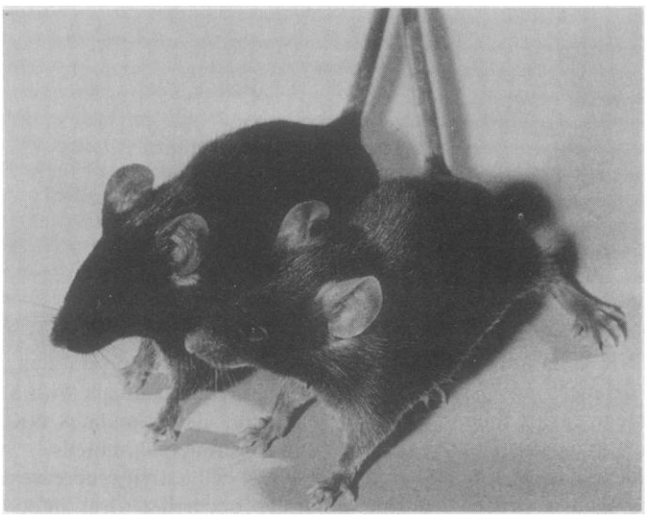
Another indication that NK cells help ward off viral infections comes from Carlos Lopez of the Sloan-Kettering Institute for Cancer Research. He has identified strains of mice that are genetically resistant or susceptible to infection by herpes simplex virus I (HSV I). The resistance is apparently mediated by NK cells.

In addition, Lopez has observed low NK cell activity in several patients suffering from severe viral infections, including recurrent HSV I infections. This finding contrasts with the elevated NK cell activity usually seen in people with virus infections and leads Lopez to hypothesize that the low activity might contribute to the patients' problems.

Persistent viral infections have been suggested as the cause of serious human diseases such as multiple sclerosis and systemic lupus erythematosus. The idea is that the virus somehow escapes complete elimination by the immune system and takes up long-term residence in cells, where it may trigger harmful immune attack on the body's own tissues.

Barry Bloom of the Albert Einstein College of Medicine has been looking into the role of interferon and NK cells in the development of persistent infections, although his results are also pertinent to immune surveillance. "The question," says Bloom, "is whether a defect in the interferon-NK cell system can contribute to the development of persistent infections in humans."

He finds that tumor cells, including baby hamster kidney (BHK) cells and HeLa cells, which are derived from a hu-



Beige mouse

The beige mouse, shown here with its normal littermate (left), is a mutant in which the NK cell activity is highly depressed. [Jackson Laboratory Photo]

man cervical cancer, usually produce fatal tumors when they are injected into nude mice. As few as 10 to 100 cells per animal will do the job. If these cells are persistently infected with mumps or measles virus, however, either they do not grow at all or they form small noninvasive nodules, even when up to 10 million cells are injected per animal.

Bloom suggests that NK cells may be limiting the growth of the infected cells. Natural killer cells taken from nude mice lyse infected targets in culture, whereas they do not destroy uninfected BHK or HeLa cells. In addition, injection of nude mice with the virus-infected cells greatly augments the NK activity of the animals.

Evidence that interferon cooperates with NK cells in keeping the infected tumor cells in check comes from experiments in which Bloom and Lola Reid, also of Albert Einstein, destroyed the animals' interferon by administering antibodies directed against the agent before injecting the tumor cells. The uninfected cells grew faster than they normally do and formed distant metastases, which they rarely do otherwise. Even the virus-infected cells grew and spread in the absence of interferon.

Interferon is known to directly inhibit the growth of tumor cells, but Bloom does not think that direct inhibition is at work here, partly because interferons are mostly species-specific in their actions. He hypothesizes that the interferon is acting indirectly by stimulating the NK cells of the mice. His results agree with those of other investigators suggesting that the interferon-NK system plays an important role in restricting both local tumor growth and spread of tumor cells to distant tissues.

When Bloom looked at NK cell activity in individuals with multiple sclerosis or systemic lupus erythematosus, he found it to be depressed in one-third to one-half of the patients. About half of the cells with depressed activity taken from the multiple sclerosis patients could be stimulated by interferon; the others could not. This kind of variation in response to the agent may have implications for efforts to use interferon therapeutically, especially in light of the findings that advanced cancer patients may have depressed NK cell activity. Bloom says, "A variety of defects may show up as a lack of cytotoxic activity. It is naïve to think we will correct all of them by giving interferon."

Although most of the research on NK cells is concentrated on their possible role in warding off cancer and viral infections, there are suggestions that they might also be part of the body's normal control mechanisms. As Gustavo Cudkowicz of the State University of New York at Buffalo puts it, "NK cells are so pervasive—they are found in normal individuals of every species studied—that it does not seem reasonable to suppose that these cells are here just to guard against tumors." Cudkowicz has proposed that they help control blood cell formation by the bone marrow.

Studies of bone marrow grafts in mice show that some strains are "naturally resistant" to the grafts. The transplanted cells do not grow in these strains even though they grow well in others. Both Cudkowicz and Kiessling have noted strong similarities between the properties of bone marrow graft resistance and those of NK cell activity. For example, Cudkowicz found that induction of interferon production in mice that normally are weakly resistant, but still able to take the grafts, increases the ability of the mice to resist them. Conversely, antibodies against interferon virtually wipe out the resistance of strains that would otherwise reject bone marrow transplants.

All this suggests to Cudkowicz that there is a population of NK cells that recognize normal blood-forming cells. He thinks that the NK cells do not kill the marrow cells under ordinary circumstances, however; they just keep them from dividing.

As in the case of tumor and virus resistance, there are hints that what holds true for mice is also true for humans. Bone marrow transplantation may be used as a therapy for such life-threatening diseases as leukemia, aplastic anemia (in which production of white and red blood cells by the bone marrow is severely depressed), and severe combined immunodeficiency disease.

But the therapy is severely limited by the fact that many of the patients develop graft versus host disease, in which immune cells produced by the transplanted marrow attack the recipient's tissues. Lopez and Richard O'Reilly, who is also at Sloan-Kettering, have evidence that the patient's own NK cells are somehow involved in triggering the attack. They studied 13 patients before they underwent bone marrow transplantation. Of these, seven had normal and six had very low NK activity. All seven patients with normal activity eventually developed graft versus host disease, whereas none of the other six did. Lopez and O'Reilly propose that NK cell activity may be a good prognostic indicator for determining who is at high risk of developing graft versus host disease. If a high-risk group can be identified, more rigorous preventive measures may be possible for them.

A number of questions remain about NK cells and how they work. For example, little is known about how they recognize and destroy their targets. One of the most vexing questions concerns their cellular nature—whether they are lymphocytes, macrophages, monocytes, or some other kind of immune cell. Many investigators now lean toward the view that they are lymphocytes, perhaps an immature form of T cells, although the issue is not yet settled.

Another question is whether NK cells are subject to any control mechanism in addition to that mediated by interferon. Cudkowicz has evidence for the existence of suppressor cells that decrease NK cell activity, although more work will be needed to confirm this observation. Nevertheless, communication between immune cells seems to be the rule rather than the exception (see the accompanying story on the Nobel Prize for Physiology or Medicine), and there is no reason to believe that NK cells should escape regulation.—JEAN L. MARX