versity of Cambridge told *Science*, that puzzle is, if anything, growing more acute. When he and his colleagues tested the usual method for estimating stars' ages, they came up with evidence that the technique may actually be understating stellar ages.

Gilmore and his colleagues Ted von Hippel of the National Optical Astronomy Observatories in Tucson, Arizona, and Derek Jones of the University of Cambridge derive their evidence from a star cluster—a clutch of stars that all formed at about the same time. By measuring the temperature of the cluster's white dwarf stars, they found evidence that these stellar cinders have been cooling for longer than the accepted age of the cluster. The disparity suggests, says Gilmore, that "the standard model of the way stars evolve is seriously in error."

Astronomers ordinarily estimate the age of a star cluster by checking the brightness and colors of its stars against stellar evolution models, which predict how those characteristics should change over time. A more direct strategy, though, is to examine a cluster's dimmest white dwarfs. Because white dwarfs no longer produce energy, they cool at a steady rate. And because the oldest dwarfs in a cluster are the cinders of stars that burned only briefly after it formed, their age is a good proxy for the age of the cluster.

But cool white dwarfs are by nature hard to see, so it took the repaired Hubble Space Telescope to pursue this dating strategy, explains Gilmore. From the observed temperatures of faint white dwarf stars in the open cluster NGC 2477, he and his colleagues derived an age of 1.25 billion years. That's more than twice the age astronomers had calculated based on stellar evolution models. Stellar evolution researcher James Kaler of the University of Illinois isn't discouraged, saying, "I think that getting as close as they do between the various ways of measuring ages of stars is quite remarkable. ... Little by little it will all fall together."

But Gilmore and his colleagues think the finding could exacerbate an existing problem: The same stellar evolution models that seem to underestimate the age of NGC 2477 also assign other clusters an age of 12 billion years or so, greater than some recent estimates of the age of the universe as a whole. Based on measurements of how fast the universe is expanding, those estimates have been coming in as low as 8 billion years (*Science*, 28 October 1994, p. 539).

To test the stellar evolution models more directly, Gilmore and his colleagues are now planning to look for white dwarfs in much older clusters. If the white dwarfs push up the age of star clusters even further, a cosmic conflict may turn into a crisis.

–Alexander Hellemans

Alexander Hellemans is a science writer in Amsterdam.

IMMUNOLOGY

Researchers Find Molecules That Muzzle Killer Cells

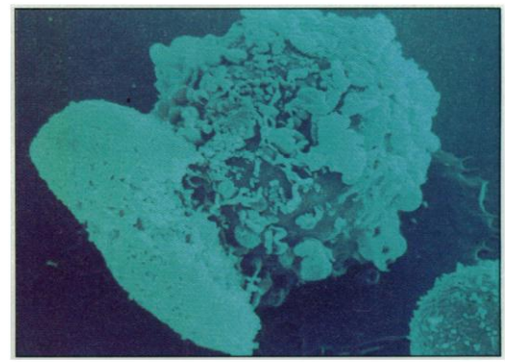
Natural killer (NK) cells are the pit bulls of the immune system. Lab studies have shown that once excited, they can kill all kinds of cells. But in the body, they are more discriminating—they kill tumor cells or virally infected cells, but not normal cells. Now immunologists are getting a clearer—and somewhat surprising—picture of just how the immune system muzzles NK cells to keep them from killing indiscriminately.

Two research groups, one led by Marco Colonna of the Basel Institute for Immunology in Switzerland and the other by Eric Long of the National Institute of Allergy and Infectious Diseases and Alessandro Moretta of the University of Genoa in Italy, have cloned human genes that encode proteins located on NK cells that apparently act as receptors for signals that tell the cell "don't kill." The Basel team reports their results on page 405, and the Long and Moretta results will be published in the May issue of *Immunity*.

What the two groups found has come as a big jolt to the field: The human NK cell receptor proteins are completely unrelated to those previously identified in mice. That implies one of two things: Either mice and humans use totally different genes to perform the same function—which is highly unlikely—or NK cells have two different systems of inhibitory receptors working simultaneously. "Until now we have known almost nothing about NK cells," says University of California (UC), Berkeley, immunologist David Raulet. "Now they seem more complicated than we had thought."

And the surprises don't end there. On page 403, Lewis Lanier of DNAX Research Institute in Palo Alto, California, and his co-workers report that they have found members of the same family of human receptor proteins on the surface of another killing cell, the cytotoxic T cell, and that the receptors seem to inhibit the T cells' drive to kill. This finding adds a new and unexpected level of control to these cells, which, like NK cells, play important roles in defending the body against viral infections and cancer. The discovery, says Harvard University immunologist Jack Strominger, suggests that there may be "a modulation, a fine-tuning going on," in T cells that could rein them in from killing normal cells. Failure of the fine-tuning could perhaps contribute to autoimmunity.

This similarity between NK cells and cytotoxic T cells is all the more surprising considering where the search for the NK receptor began: a discovery made 9 years ago by Klas Kärre of the Karolinska Institute in Stockholm, Sweden, that highlighted the fundamental differences between the two cell types. For T cells, a group of cell surface proteins known as the major histocompatibility complex (MHC) class I proteins is key to immune recognition. Present on virtually all cells, the class I proteins have the job of "presenting" fragments of foreign proteins to cytotoxic T cells, thereby triggering them to kill virus-infected or cancer cells. But Kärre found that NK cells selectively kill tumor cells that are missing class I molecules. That meant NK cells might fill an important niche: Some tumor cells and virus-infected cells shut down their production of class I protein and so might slip through T cell surveillance. "The NK cells would act as a backup," says Lanier, "because they would then be able to detect" those cells.



Delicate balance. Inhibitory, as well as stimulatory, receptors regulate killer T cells, like this one attacking a cancer cell.

Several labs subsequently showed that MHC class I proteins disarm NK cells by binding to specific protein receptors on the NK cells' surfaces. In 1992, Wayne Yokoyama, then at UC San Francisco, and his colleagues got the first lead on what those inhibitory receptors might be. Their work with mouse NK cells suggested that the inhibitory receptors belong to a family of proteins called Ly-49, which binds to class I molecules on potential target cells. The Ly-49 proteins are lectins, proteins that bind to the sugar molecules found on many proteins, including the class I molecules. Following the Ly-49 discovery, immunologists expected that the inhibitory receptors on the surface of human NK cells would turn out to be members of the lectin family as

well. And that's why the new findings came as such a shock.

Colonna and Jacqueline Samaridis report that they have cloned a set of four related genes that appear to encode the inhibitory receptors of human NK cells, and these proteins are not lectins. They belong instead to the immunoglobulin superfamily, which also includes antibody proteins and the receptors by which T cells recognize their targets.

Colonna and Samaridis began their search for the inhibitory receptor genes by looking for either immunoglobulin superfamily or lectin genes that are active specifically in NK cells, and then used a variety of methods to confirm that they had the receptor genes. The Long and Moretta group took a different tack, using monoclonal antibodies directed against the receptors as a tool to get their clones. But they came to the same conclusion, identifying five related genes that encode immunoglobulin superfamily members. The teams have not yet compared their genes to see if any of them are the same.

Such new findings are usually a cause for celebration, but Berkeley's Raulet says that

Natural killer cells "seem more complicated than we had thought."

—David Raulet

when he first heard Nicolai Wagtmann, from the Long lab, present that team's results at a meeting in Sicily last May, he was "surprised and dismayed" to find that the new human proteins aren't related to Ly-49, as immunologists had expected. The finding seemed to be a biological paradox—how could a pair of species as closely related as humans and mice, whose immune systems are otherwise so very similar, have such totally different genes performing the same function?

Raulet says his dismay at the time came from the gut feeling that one of the findings must be wrong, although he now believes the new findings are solid. The Colonna and Long groups haven't proven directly that the proteins made by the genes they have cloned bind to class I proteins, as the Ly-49 proteins do, but they have shown that the proteins are recognized by antibodies known to block class I inhibition. That, says Raulet, makes "a reasonable case that these in fact are inhibitory molecules."

That leaves the issue of whether humans and mice use unrelated inhibitory receptors, an idea most immunologists reject. "I'm still convinced that we will find in man the Ly-49 genes, and probably in mouse we will find this other set of genes," says Lanier, "and they will be serving redundant functions." That raises "fascinating questions," says Raulet. "If you have two independent inhibitory receptors, how do you coordinate specificity, and why isn't one enough?"

It's not only the NK cells that are raising fascinating questions. In their paper, Lanier's group reports that human T cells carry on their surfaces the same family of inhibitory receptors that have been identified on human NK cells. This follows up on a report last year in the *European Journal of Immunology* by a team led by Lorenzo Moretta of the University of Turin, Italy, that a small subset of T cells have such an inhibitory receptor. But the Lanier group took their analysis further, showing that most T cells that have been activated by an encounter with foreign proteins seem to have one or more forms of the NK receptors, and that the receptors respond to class I molecules by restraining the T cells from attacking cells they would otherwise kill.

"I was very surprised that T cells have these NK receptors," says Harvard's Strominger. Indeed, at first glance, the receptors seem to be working at cross purposes to the T cells' main job, which is to kill cells that present foreign peptides. "The T cell is recognizing the class I–peptide complex on the antigen-presenting cell, but at the same time, the class I molecule is binding to an inhibitory receptor on the T cell and down-regulating it," Strominger says.

Such fine-tuning might mean that a foreign peptide–MHC protein combination that is a perfect fit for a T cell receptor will send an activating signal that is more powerful than the signal going through the inhibitory receptor, causing the T cell to kill its target. But any peptide–MHC protein complexes that don't fit snugly in the T cell receptor might send weaker activating signals that would be overwhelmed by the inhibitory signal. In such a model, the inhibitory receptor "would serve as a failsafe to make sure your activated T cells will only kill things which really provide a strong signal," Lanier says, adding that autoimmune conditions could result from failure of the inhibitory receptors to "tame down or regulate the activated T cell."

That kind of counterbalancing is likely to come into play on the NK cells, which also must be triggered to go into killing mode. But little is known about how NK cells are activated. It would be much better to study the inhibitory signal in T cells, where activation is well understood, says Raulet, "because you could say where along this [activating] pathway does the inhibitory signal intervene." As researchers tackle that question, they will likely reveal more about how the muzzle actually tames the bite of the NK cells.

–Marcia Barinaga

AIDS THERAPY

New Hope Against Blindness

"I know I'm going to die, but I don't want to go blind." Retina specialist William Freeman of the University of California, San Diego (UCSD), says he "uniformly" hears that distressing plea from his AIDS patients. Yet, despite the best efforts of Freeman and his medical colleagues, many of those patients will lose some vision; a few will lose it all. The reason is retinitis, an inflammation of the retina caused by cytomegalovirus, or CMV. This damaging condition, which may affect up to 40% of AIDS patients in the final stages of their disease, is not easy to treat because the available drugs are toxic, expensive, and work best only when given intravenously every day.

Now, however, Freeman and his peers may finally be able to offer their patients a ray of hope: Three new forms of "local" therapy—treatments that involve delivering drugs directly to the eye rather than intravenously—have shown great promise in early human trials. "Local treatment is coming into its own," says ophthalmologist James O'Donnell of the University of California, San Francisco (UCSF), who heads the school's Studies of the Ocular Complications of AIDS (SOCA), a network of 11 clinics sponsored by the National Eye Institute. "It might be that a combination of systemic [IV therapy] and local treatment will be the way of the future."

CMV retinitis usually affects AIDS patients in the terminal stages of the disease, when damaged immune systems no longer suppress this member of the herpesvirus family. Although CMV, which normally is harmless, can cause everything from pneumonia to gastrointestinal bleeding, some of its worst damage is done in the retina. The best proven treatments are the anti-viral drugs ganciclovir and foscarnet, administered through an indwelling catheter. But not only is the catheter site vulnerable to sepsis; ganciclovir and foscarnet damage blood cells and kidneys respectively. So far the only alternative to the IV therapy is a less potent oral form of ganciclovir that was approved by the Food and Drug Administration (FDA) just this past December.

The local treatment closest to market is a tiny ocular implant being developed by Chiron Vision in Irvine, California, a division of Chiron Corp. The implant, which releases ganciclovir for 8 months, requires a