T Cells on the Mucosal Frontline

Despite their sparse numbers and apparently limited variability, γδ T cells are turning out to have an important role in the front lines of the body's defenses against pathogens

Over a decade ago, a long molecular hunt ended when immunologists finally bagged one of the field's most elusive molecules: the receptor that the T cells of the immune system use to recognize antigens, the distinctive molecules carried by pathogens and other foreign invaders. Until then, they had been stymied in their efforts to understand this key step in the initiation of many immune responses. But the discovery of the receptor almost immediately left researchers confronting another mystery.

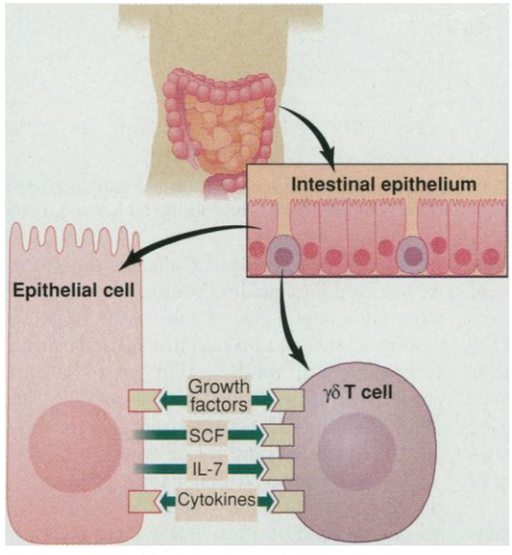
For more information on recent immunology research, please see the special section, "Turning the Immune System Off," which begins on page 237.

They found that T cells actually carry two different types of receptors. The great majority—some 90%—carry so-called αβ receptors and are the classic T cells that circulate around the body, performing such jobs as helping get rid of viruses and triggering antibody production. But a small percentage turned out to carry a different receptor variant—designated the γδ receptor—and no one knew what this maverick T cell population might do. A growing body of evidence now suggests, however, that these γδ cells have a more important role in the body than their relatively sparse numbers might suggest.

Indeed, they may be the first line of defense against invading pathogens, especially those attempting to enter the body through mucosal surfaces such as the lining of the gut. "At these sites γδ cells represent a significant population, and interest in unraveling their role is now compelling," says immunologist Adrian Hayday at Yale University. Already, researchers are learning that γδ cells act in ways that seem consistent with a role in mucosal immunity. "The development of responses is very clearly different in the mucosa compared with the periphery, and it is attracting increasing interest," says immunologist Pierre Vassalli at the University of Geneva.

Among other things, although γδ cells have much less variability in their antigen-recognition sites than αβ cells have, they nonetheless appear capable of dealing with the broad spectrum of antigens they are likely to encounter at mucosal surfaces. What's more, they may be able to go to work without needing to be activated by the same complex series of steps that αβ cells require—a big plus for cells that may need to act quickly on the front lines of pathogen defenses. Other findings are also raising researchers' estimation of γδ cells. Evidence suggests, for example, that they help the body damp down autoimmune reactions and repair tissues damaged by inflammation.

The new respect for γδ cells is a big change from immunologists' view of these cells soon after they were discovered, when there was good reason to wonder whether the cells play any important role in the body. The researchers were intrigued by one finding: Although the cells are a relatively small T cell population—they constitute only about 5% of circulating T cells—they are much more common in certain epithelial tissues. They make up about 50% of the lymphocytes in the intestinal mucosa of mice, for example, and as much as 40% of the lymphocyte population in human colon epithelium. That suggested that they might be frontline sentinels. As Hayday points out, "the γδ T cells are in a unique position to monitor epithelial cells." Counterbalancing that, however, was the apparent lack of variability in what the cells could recognize.



Cross talk. Epithelial cells and γδ T cells may interact through growth factors and cytokines.

The receptors of both αβ and γδ cells consist of two similarly designed protein chains (designated α and β or γ and δ). But although the αβ receptors display an enormous range of structural variation, indicating that they can recognize an equally enormous range of different target antigens, the antigen specificities of the γδ receptors found in individuals appear to be much more limited.

T cells assemble each of the genes for their receptor proteins by combining two to three separate segments of DNA, and the repertoire of these elements is much smaller for the γ and δ genes. In the most extreme case, essentially all the γδ cells in the epithelia of the mouse reproductive tract turned out to use the same genetic elements to construct the antigen-binding portion of their receptor. In the skin, anywhere from 60% to 99% of the T cells share a similar receptor construction. Although the receptor makeup does vary between locations, this lack of structural variation in the γδ receptors at any one site raised questions about whether they would be able to deal with the wide variety of pathogens mammals encounter.

Indeed, there were indications that γδ cells might be nothing more than remnants of some evolutionarily primitive antigen recognition system in mucosa. Researchers found, for example, that γδ cells have a simpler, more direct way of recognizing antigens. The αβ cells can recognize antigens only after the target proteins have been "processed," broken down into small peptides, which are then displayed on the surface of "antigen-presenting cells" in conjunction with proteins encoded by genes within the major histocompatibility complex (MHC).

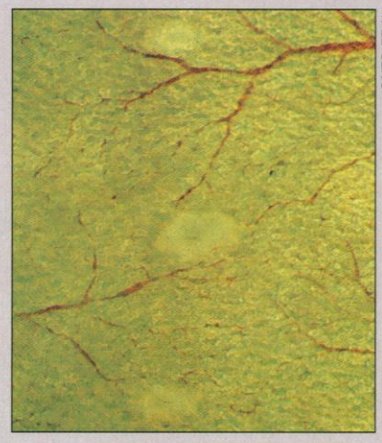
In contrast, γδ cells in the epithelia appear to recognize and respond to some MHC proteins directly without added peptides, eliminating the protein-processing step. The small γδ cell population in circulation also recognizes some bacterial antigens directly without any processing. In this way, γδ receptors seemed to behave more like antibodies than like αβ receptors, an idea that received additional support earlier this year. In an x-ray crystallography study that appeared in the 29 January issue of *Nature*, Roy Mariuzza's group at the University of Maryland Biotechnology Institute in Rockville found that the structure of the δ chain's antigen-recognizing segment resembles that of antibody chains.

Another indication that γδ cells are more

Pinpointing the Source of Intestinal T Cells

Studies of γδ T cells are not only toppling narrow notions about how the immune system defends the body (see main text); they are also challenging the classical view that T cells must pass through the thymus gland to develop properly. Over the past few years, many studies on mice have suggested that some T cells found in the gut, including γδ T cells, can develop even when the mice lack a functional thymus. Just where these cells develop if not in the thymus has been much less clear, however. But a team of Japanese researchers led by Hiromichi Ishikawa of the Keio University School of Medicine in Tokyo may have solved the problem.

Although the gut T cells, like those found elsewhere in the body are born in the bone marrow, the Keio team's work, which is described on page 275, indicates that the cells mature in the cryptopatches, clusters of cells located just under the layer of epithelial cells lining the gut. If so, cryptopatches would give the intestinal lining its own homegrown supply of T cells to combat the large array of pathogens and antigens that an animal constantly ingests.



SAITO ET AL.

T cell source? Intestinal T cells may mature in cryptopatches (light areas).

Two years ago, the Keio group found that the patches consist primarily of cells that could be immature T cells, a few hundred per patch. The team found, for example, that the cryptopatch cells carry on their surfaces two proteins, one called c-kit and the other the receptor for the immune signaling molecule interleukin-7, that are hallmarks of immature T cells. That suggested that the cryptopatches might be the key site in which stem cells from the bone marrow develop to provide mature intestinal T cells.

In the current work, the team set out to see whether that is in fact the case. They did this by removing tiny fragments of gut containing the cryptopatches and transferring cells from the gut fragments into the blood system of immunodeficient mice lacking T cells. As predicted, the recipient animals developed mature T cells in their gut. In contrast, when the team injected cells from pieces of lymph nodes removed from the gut region, no T cells developed at epithelial sites in the gut. "It's strong evidence pointing to a role for the cryptopatches," says immunologist John Klein at the University of Tulsa. –N.W.

primitive came from studies reported 7 years ago in mice, in which researchers showed that these cells appear in the fetus well before the first αβ cells. That could be of some benefit, Hayday says: "The greatest exposure to new antigens for a mammal occurs at birth and γδ T cells may be a vital defense at this time. There's good evidence the αβ cells are not yet working at full clip." But whether they had a role in the adult was unclear, especially as Susumu Tonegawa of the Massachusetts Institute of Technology and his colleagues found no obvious immunological defects in mice that were unable to make γδ T cells because the researchers had knocked out the δ chain gene.

But although most of these early findings hinted that γδ cells are a minor player in immunity, at least in adults, more recent results are reviving interest in the cells. Driving much of this revival is evidence that the cells may have a role in mucosal immunity, a critical component of the body's defenses because most invading pathogens have to cross a mucosal surface to enter the body. One indication of that is, of course, their relative abundance in those surfaces.

More recent work by Tom Spies and his colleagues at the Fred Hutchinson Cancer Research Center in Seattle suggests how γδ cells overcome the apparent limitation of their sparse receptor variability. They may respond to two cell surface molecules that are related to the main family of MHC molecules, known as MICA and MICB, that epithelial cells may display when stressed—say, by an infection (*Science*, 13 March, p. 1737). In experiments performed on epithelial cells in lab culture, the Spies team found that γδ T cells can recognize these molecules and kill the cells bearing them. "There is a real possibility these γδ T cells respond to these molecules in the body," says Vassalli. "The data are very compelling."

If so, these findings would help explain how γδ cells could be effective at immune surveillance despite the narrow repertoire of their receptors. If the same molecules, such as MICA, are expressed in response to a range of pathogen infections or other sorts of stress, then this mechanism could be highly effective at eliminating infected or damaged cells. "If γδ T cells worked in this way, it would make sense," says immunologist Delphine Guy-Grand at the Necker Hospital in Paris.

The idea is also consistent with a recent finding by Hayday and his colleagues. They looked at the γδ receptors in knockout mice lacking a gene segment commonly used in the construction of a typical receptor in one particular tissue, the skin. They found that the mice were able to circumvent the loss of this particular gene by co-opting another gene segment to create a receptor of similar structure and specificity (*Science*, 13 March, p. 1729). This suggests, Hayday says, that the final γδ receptor repertoire, however narrow, is driven by the need to recognize specific molecules in the part of the body where the T cells reside. If cells in which one γ or δ gene segment is knocked out can use another to create a receptor with the same specificity, the Hayday team's result may also help explain why these knockouts don't show obvious effects.

In addition to recognizing MICA and MICB on epithelial cell surfaces, γδ cells apparently receive signals of another kind from epithelial cells—signals that may help maintain γδ cell populations. They have receptors for growth factors, including stem cell factor and interleukin-7 (IL-7), that epithelial cells secrete. Evidence that these receptors are functionally important comes from knockout studies in which researchers have found that mice lacking the gene for the IL-7 receptor also lack γδ T cells in the gut epithelium, whereas the αβ T cell population is only slightly reduced.

Researchers have also found that γδ cells produce growth factors that may play a role in healing epithelia damaged by infection or inflammation by promoting cell growth there. "These are two complex players, and it's important to understand how they communicate with each other," says mucosal immunologist John Klein at the University of Tulsa.

Beside killing infected cells directly, γδ T cells may also help protect the mucosa by drawing other immune cells in to help combat an infection. Work by several researchers has shown, for example, that the cells release immune cell messengers called cytokines that

may activate αβ cells and attract inflammatory cells useful in cleaning up damaged cells.

In some cases, though, γδ T cells may help damp down immune responses that might damage epithelia. Immunologist Martin Kagnoff at the University of California, San Diego, says that areas of the bowel affected by celiac disease, an autoimmune condition resulting from an adverse reaction to cereal proteins, contain higher than normal numbers of the cells. Although their role in the pathology of the disease is not known, the numbers of γδ T cells are high during "silent" periods of the disease, when the pathology is mildest, suggesting that the cells may help suppress the autoimmune reactions.

Similarly, other studies found that γδ knockout mice develop a more serious disease, with more damage to the intestinal epithelium, than controls do when infected with the common protozoan *Eimeria*. Because animals lacking normal T cells don't develop this damage, the finding suggests that γδ T cells damp down the αβ T cell response to the parasite.

With evidence for their importance now accumulating, γδ T cells are no longer a scorned minority, and the number of researchers looking at ways of modulating immune responses via the mucosal route is growing sharply. Far from being a backwater of the immune system, "everyone is agreed mucosal immunity is an important field," says Vassalli.

–Nigel Williams

PHYSICS

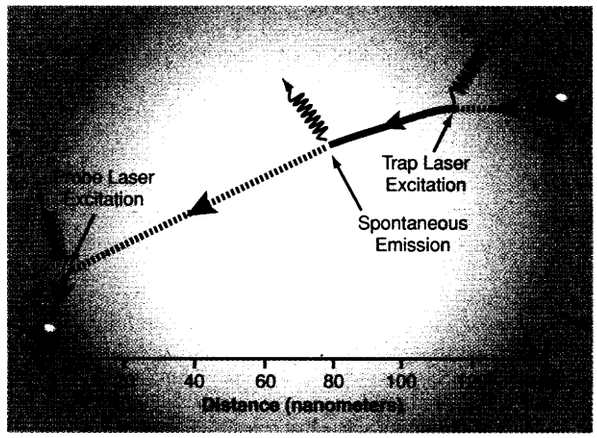
The Subtle Flirtation of Ultracold Atoms

If high-energy accelerators make the rap music of physics—with their whirling particles and rapid-fire smashups—then collisions between ultracold atoms are its Wagnerian opera. They are a spectacle of slow atomic duets in which subtle forces and rare internal states hardly ever seen in the everyday world can emerge. Trapped in cages of light and magnetic fields and cooled almost to absolute zero, these atoms can form fragile molecules far larger and more tenuously bound than could survive in our room-temperature world. Their interactions can be fine-tuned with lasers and magnetic fields. And rare, spontaneous changes of the atoms' internal states can take place as a collision unfolds, transforming a gentle encounter into a rollicking escape from the cage.

In the past few months, physicists have also discovered new links between the cold, slow-motion world of these collisions and other, better known phenomena. At even lower temperatures, clouds of atoms can form a collective state—in effect, a giant atom—called a Bose-Einstein condensate or BEC (*Science*, 14 July 1995, p. 152; 25 August 1995, p. 1047). The stability of a BEC depends almost entirely on how the atoms making it up interact one-on-one, and ultracold collisions provide a glimpse of these interactions. And in a first-ever detection, a group led by Pierre Pillet of the Laboratoire Aimé Cotton at the Université Paris-Sud has made new strides by getting cesium atoms to form ordinary molecules chilled to 300 millionths of a kelvin—something only seen before with atoms. That breakthrough could be the first step toward studying complex materials in the world of the ultracold.

"This ultracold collision business is a very exciting thing," says William Phillips of the National Institute of Standards and Technology (NIST) in Gaithersburg, Maryland, who won the Nobel Prize last year for his part in the development of laser cooling and trapping. "The more people work on it, the more astounding things we're going to learn."

Magneto-optical traps slow atoms, cooling them, by bathing them in laser light. The cooling works because photons of light carry momentum, and atoms can absorb photons that have specific frequencies. If a laser's frequency is tuned just below one of those frequencies, an atom will "feel" the light only when it is moving into the beam, as the Doppler or train-whistle effect raises the light's apparent frequency. By combining laser cooling with magnetic fields, which can cause light to trap certain atoms once they are moving slowly enough, researchers can capture cold atoms for many minutes at a stretch.



SOURCE: P. GOULD

Excitation and attraction. One laser pulse briefly excites an atom, causing it to be attracted to another atom. A second, probe laser clocks how long it takes for the atoms to converge.

These caged atoms can interact in ways never seen in our hotter, faster paced world. Extending ideas first put forth by William Stwalley of the University of Connecticut, Storrs, NIST's Paul Julienne, and others, Phillip Gould, a physicist who is also at Connecticut, focused last year on the attractions that the trap laser can create by distorting the normally symmetric electron clouds around atoms. The excitation creates a charge pattern in one atom that causes it to attract another one over huge distances. This "photoassociation" can begin dragging two atoms slowly together over distances of 100 nanometers—forming, in effect, a nascent diatomic molecule several hundred times larger than could exist at room temperature.

This acceleration is so gradual, however, that the asymmetry decays in midcollision, after about 30 nanoseconds, causing the excited atom to emit a photon and return to the lower energy, symmetric state (see graphic). "The atoms are still moving toward each other," says Gould, although now that the attraction has been turned off, they are coasting. Still, that coasting speed can be enough to "spit atoms out of the trap," ejecting them before the lasers can turn them back, says Stwalley. He adds that this phenomenon "is something you'd like to completely understand" to minimize the leakage of atoms from optical traps.

Gould and Connecticut colleague Steven Gensemer have been measuring the strength of this photoassociative force by first turning on the trap laser to get the atoms moving toward each other, then hitting them with a second, probe laser, which revives the attraction. If the atoms are close together at that point, the result is a collision violent enough to throw the atoms out of the trap immediately. By varying the interval between the pump and the probe lasers and counting the leaked atoms, Gould can gauge how fast the photoassociated atoms were moving together—and how strong and sustained was the original force pulling them together.

The work, which Gould and Gensemer describe in the 2 February issue of *Physical Review Letters* (*PRL*), might help atom trappers turn off the attraction or avoid it. But Pillet is courting it—and creating ultracold ordinary molecules in the process.

Until now, says Randall Hulet of Rice University in Houston, "nobody's been able to detect ultracold molecules." Directly cooling and trapping ordinary molecules doesn't work, as