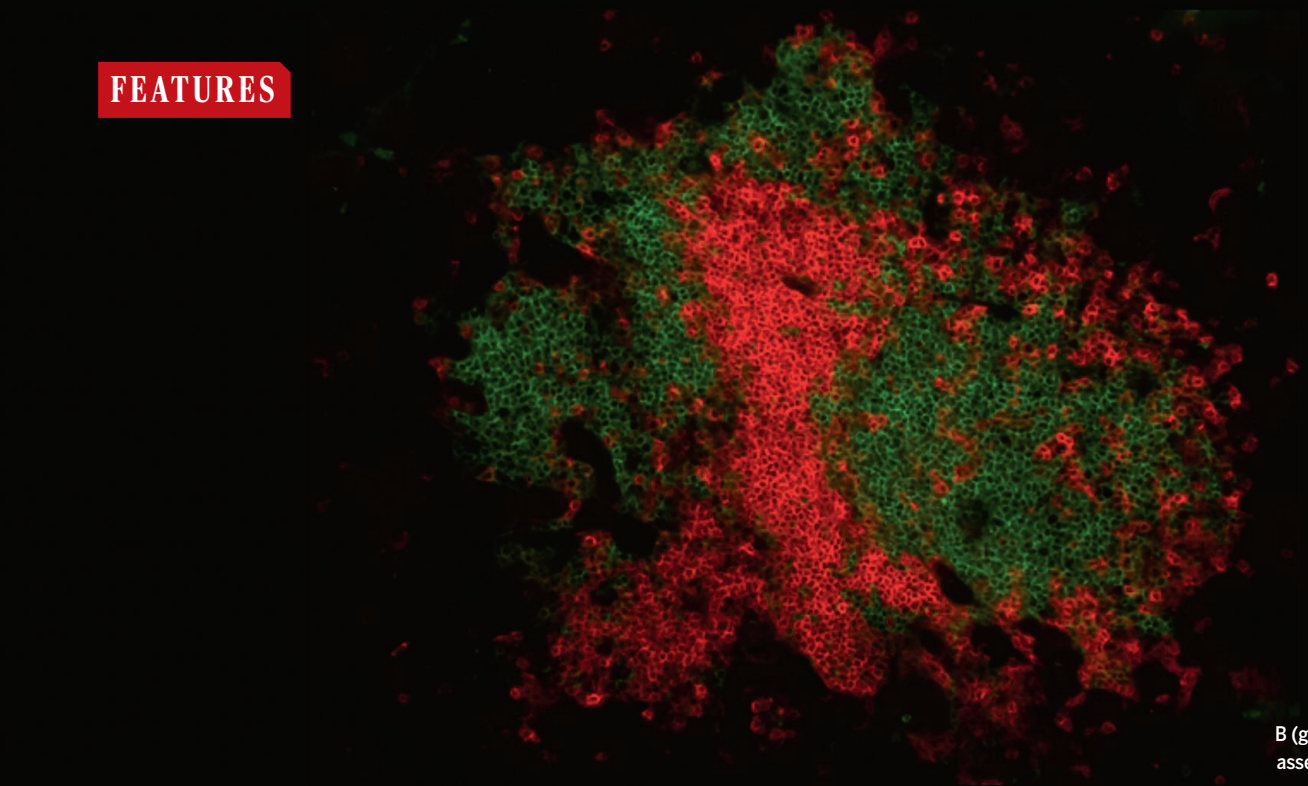


B (green) and T cells (red) assemble inside a tertiary lymphoid structure.

IMAGE: N. RUDDLE *ET AL. PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES* **11**. (13 MARCH 2007)

IMMUNITY GOES LOCAL

The temporary immune command posts that form near infections or inflammation draw clinical interest

By **Mitch Leslie**

Nancy Ruddle just could not make her mice get sick. During the 1990s, the immunologist at Yale University School of Medicine and her colleagues spent months trying to create a strain of diabetic mice. In hopes of inducing the hallmark of type I diabetes—destruction of the insulinmaking β cells by the immune system—they had genetically altered the animals to trigger inflammation in the pancreas, where the cells reside. But the mice—and their β cells—remained stubbornly healthy. "At first we were rather disappointed with them," Ruddle recalls. "We said we'd better find out what was going on."

Peering through a microscope at pancreatic tissue from one of the mice, a colleague of Ruddle's spotted something unexpected. He announced, "this is a lymph node," Ruddle says. She was certain he was wrong because the pancreas doesn't contain these immune system outposts, where cells muster before being activated to fight microbes. Ruddle figured he had just seen one of the loose gatherings of immune cells that sometimes appear at sites of inflammation.

But after studying his find and other similar structures in the animals' kidneys and skin, she and her lab members had to admit that the structures looked a lot like lymph nodes. They showed the same segregation of two main kinds of immune cells, B and T cells, and the same distinctive small veins that allow immune cells to enter the nodes. "That was one of the things that convinced me" that the objects weren't just flash mobs of immune cells, Ruddle says.

Two decades later, immunologists are convinced that these structures are far more important to the body's immune defenses, and to strategies for manipulating them, than Ruddle could have imagined. Researchers now call what she and her colleagues observed tertiary lymphoid structures (TLS) or tertiary lymphoid organs: organized congregations of immune tissue that can sprout at sites of inflammation

or infection almost anywhere in the body. They appear to serve as local command centers that instigate immune system counterattacks against pathogens and tumors—and may also promote the self-directed attacks of autoimmune diseases and the rejection of transplanted organs.

These days, research on TLS "is exploding," says immunologist Andreas Habenicht of the University of Munich in Germany. Armed with new understanding of the signals that create these structures, drug companies have even begun testing compounds to block TLS formation in people with autoimmune diseases.

RUDDLE'S LAB WASN'T THE FIRST to discern TLS—and, it turns out, the structures didn't explain the diabetes-free mice. Researchers noted these oddities as far back as the early 1900s but largely ignored them. But her group's rodent findings, published in 1996, made the structures impossible to overlook, others say. "In hindsight you went, 'Wow, that's an amazing observation,'" says immunologist Troy Randall of the University of Alabama, Birmingham.

For decades, scientists thought that the defensive responses of B and T cells began in the lymph nodes, spleen, and related tissues—the so-called secondary lymphoid organs. (The bone marrow and thymus are the primary lymphoid organs.) At these sites, B and T cells meet up with antigens, distinctive molecular bits from invading microbes or tumors that trip the immune system's alarms. These encounters, along with stimulatory signals from other immune cells, switch on T cells' pathogen-fighting capabilities and spur B cells to mature into antibody producers.

Researchers have determined, however, that B and T cells can interact with antigens and receive marching orders within a TLS as well. In a 2009 study, for instance, immunologist Reinhold Förster of Hannover Medical School in Germany and colleagues exposed mice to a virus and then found that T cells that could react to it were activated in TLS. "They are local sites of organization of attack against a virus or other pathogen," says immunologist Bart Lambrecht of Ghent University in Belgium.

Although secondary lymphoid organs assemble only at defined locations during embryonic development, TLS form whenever and wherever they are needed, at trouble spots throughout the body. TLS appear, for example, in the lungs of tuberculosis patients as well as near or inside tumors. Immunologist Jorge Caamaño of the University of Birmingham in the United Kingdom and colleagues reported that fat within the abdominal cavity is riddled with the structures, which may help battle pathogens that escape from the gut. TLS also arise in several autoimmune diseases—in the brains of people with multiple sclerosis and in the joints of people with rheumatoid arthritis—and in transplanted organs being rejected.

Researchers have a general picture of how TLS assemble. The process begins when inflammation draws immune cells to the site of an infection or injury. By producing signals, which can include a molecule called lymphotoxin, those cells in turn stimulate stromal cells, residents of the local tissue, to provide a scaffold for the incipient TLS and lure T and B cells. But many details of TLS formation remain mysterious, including which cells are key to building them. "The challenge is to understand what are the driving cells," says immunologist Simon Jones of Cardiff University in the United Kingdom. "There are a number of candidates."

Although most TLS are microscopic, they can grow much larger—in the rare condition follicular bronchiolitis, they can be big enough to clog the tubes that bring air into the lungs. But unlike secondary lymphoid organs, which are permanent, TLS typically disassemble, sometimes within a few weeks, after inflammation or infection clear.

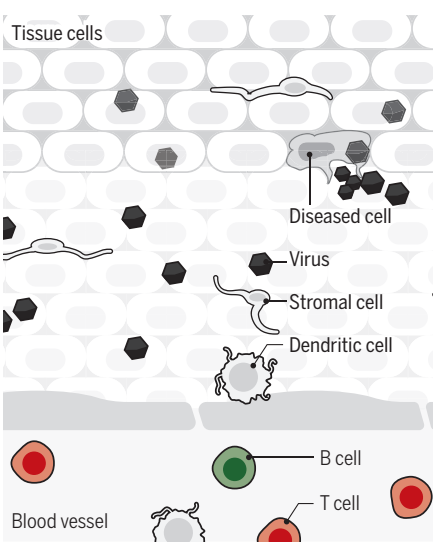
Positioning TLS on the battlefields where immune cells are fighting pathogens or tumors could provide some strategic advantages. "It's nice to have a factory nearby," where immune defenses, such as antibodies, are forged, Lambrecht says. Local command posts could make it easier for roused immune cells to track down their

Immunity's MASH units

Tertiary lymphoid structures (TLS) organize on-the-spot immune responses.

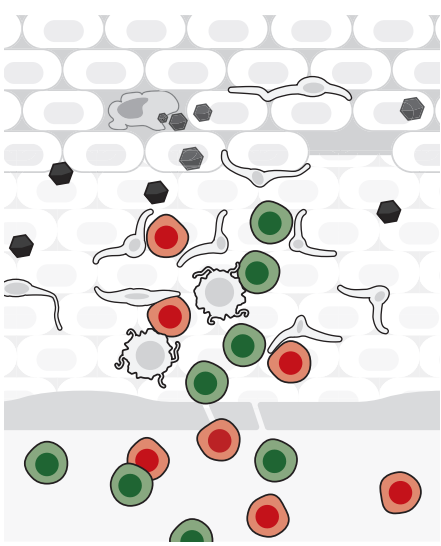
1 Infection begins

A virus has begun invading and killing cells in one part of the body. Their presence alerts dendritic cells and other immune sentinels.



2 Cells congregate

Immune cells swarm into the site of the infection. They interact with local stromal cells that will help organize the TLS.



3 Structure forms

A completed TLS contains activated T cells that can recognize the virus (inset). B cells that matured there have begun releasing antibodies.

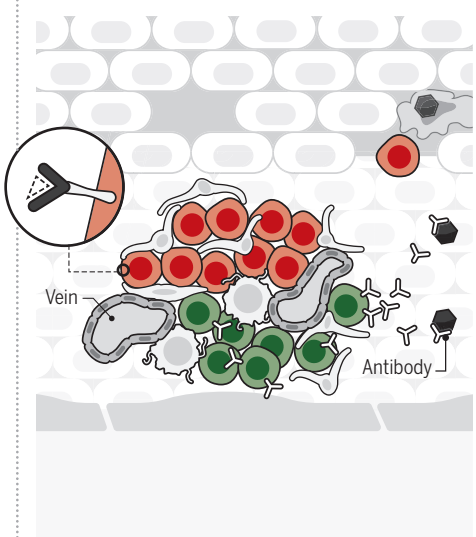


ILLUSTRATION: V. ALTOUNIAN/*SCIENCE*

targets, says immunologist Walter Storkus of the University of Pittsburgh in Pennsylvania. "The cells are being activated in the environment in which they are supposed to act." TLS may also bolster defenses for parts of the body, such as the lungs, that are regularly exposed to pathogens but don't have a lot of secondary lymphoid organs, Förster says.

BUT THOSE ADVANTAGES are far from certain. Indeed, one of the most important questions about TLS is how much they contribute to immune responses. The hundreds of lymph nodes in our bodies are perfectly good at arranging liaisons between antigens and B and T cells, so what do TLS add?

One direct test demonstrated that, at least in mice, TLS can substitute for secondary lymphoid organs. In a 2006 study, Randall and colleagues surgically removed the spleens of mice that had been genetically modified to lack lymph nodes. They then infected the mice with the flu. Randall expected that a lack of secondary lymphoid organs would leave them defenseless. But the mice survived. "In fact, they did way better than the normal mice," he says.

Randall recalls he was taken aback by the findings and suspected a mix-up. "I'll bet the cages got switched," he told his offended lab technician, insisting that she redo the experiment. Three months later she returned in triumph with the same results. The mice could still generate TLS—which were enough to sustain a strong immune defense. "The immune response was amazingly functional with just [TLS]," Randall says.

Although similar experiments aren't possible in humans, indirect evidence suggests that TLS take part in our immune defenses as well. Studies in cancer patients have typically shown that the more TLS they have in or near their tumors, the higher their odds of survival. "I liken them to MASH units that take up positions at the border of the tumor," Storkus says. In people with autoimmune diseases, on the other hand, increased numbers of TLS signal more severe tissue damage, several studies indicate.

So far, however, "we have a correlation" between TLS abundance and the severity of various conditions but no understanding of mechanism, Randall says. Instead of driving tissue destruction in autoimmune diseases, he notes, TLS could represent the body's attempt to stop it. "The evidence on whether they are good or bad is still very much debated," says immunologist Francesca Barone of the University of Birmingham in the United Kingdom.

Two recent studies underscore that uncertainty. When cancer biologist Eli Pikarsky of Hebrew University-Hadassah Medical School in Jerusalem and colleagues tested 82 people who'd had surgery for liver cancer, they discovered to their surprise that larger numbers of TLS indicated an increased likelihood of tumor recurrence and a greater risk of dying. The researchers suspected that the TLS were somehow aiding tumor growth.

To find out how, the team genetically modified mice to trigger inflammation in their livers. Inflammation drives liver cancer in people, and it spurred the mice to develop tumors in the organ—and to produce TLS there as well. The researchers reported in *Nature Immunology* last year that instead of fighting cancer cells, TLS in the rodents served as cradles, or niches, where precancerous cells could develop into tumors. "You can actually see the tumors bud out of the niche—I don't know if it's beautiful or sad," Pikarsky says.

> ***"In hindsight you went, 'Wow, that's an amazing observation.'"***
>
> **Troy Randall**, University of Alabama, Birmingham

The second study, published in *Immunity* last year, challenged the conventional wisdom about the role of TLS in atherosclerosis. Because the structures may worsen inflammation in autoimmune diseases, researchers assumed that they promote atherosclerosis, in which vessels become inflamed as well as clogged. But when Habenicht and colleagues disrupted TLS in mouse arteries by engineering smooth muscle cells in the vessels to lack the lymphotoxin receptor, the animals developed more severe atherosclerosis. "We've discovered that in the arteries they seem to be protective" at least under certain conditions, Habenicht says. He and his colleagues speculate that TLS inhibit atherosclerosis because they host regulatory T cells, which dial down inflammation.

DESPITE THE UNCERTAINTIES about TLS effects, researchers and several drug companies have been trying to develop ways to manipulate these structures for disease treatments. The first drug tailored to suppress TLS to reach clinical trials is baminercept, developed by the pharmaceutical company Biogen. The drug, designed to prevent activation of the lymphotoxin receptor, has already undergone two phase II clinical trials to gauge its effectiveness against rheumatoid arthritis and Sjögren syndrome, an autoimmune disease in which immune cells attack the salivary glands and the glands that produce tears.

So far the drug, which fuses an antibody to the lymphotoxin receptor, has been a disappointment. Baminercept didn't help rheumatoid arthritis patients. And in a recent trial in people with Sjögren syndrome, it didn't increase the production of saliva, the study's standard of effectiveness. Rheumatologist E. William St. Clair of Duke University in Durham, North Carolina, one of the leaders of the trial, says that he and his colleagues are trying to determine why by testing salivary gland biopsies from the patients.

Other drugs aimed at inhibiting TLS could soon be ready for testing. VX5, an antibody designed to block a molecule that helps stimulate TLS growth, is undergoing animal studies sponsored by the biotech firm Vaccinex. Some scientists are also testing whether approved drugs, such as the B cell–killing antibody rituximab, curb TLS formation in people with autoimmune diseases, such as Sjögren syndrome.

Researchers are also investigating whether inducing TLS could be beneficial in some diseases. Immunologist Yang-Xin Fu of the University of Texas Southwestern Medical Center in Dallas and colleagues have evidence that stimulating TLS could improve the response to a set of powerful new anticancer drugs called checkpoint inhibitors. These molecules thwart a mechanism that protects tumors from T cell attacks, but only 20% to 30% of patients benefit from them.

In a study published in March in *Cancer Cell,* Fu and colleagues hitched a protein known as LIGHT, which stimulates the lymphotoxin receptor and triggers TLS creation, to an antibody that latches onto certain cancer cells. Injected into mice with cancer, the combination homed in on the tumors and induced the growth of structures that resembled TLS. The T cells that swarmed into the tumors to produce these structures ultimately eliminated the cancerous growths—something not seen in mice treated with the checkpoint inhibitor alone. If researchers can deliver LIGHT to human tumors, it might boost the percentage of patients who benefit from checkpoint inhibitors to 70% or 80%, Fu says.

Whether the efforts to harness TLS for treatments will pay off isn't clear. But interest in the structures continues to expand, and Ruddle, who is now an emeritus professor, says she is surprised at how much research her group's 20-year-old finding has spawned. After all, she and her colleagues only wanted to understand why their mice didn't get sick. ■