IMMUNOLOGY

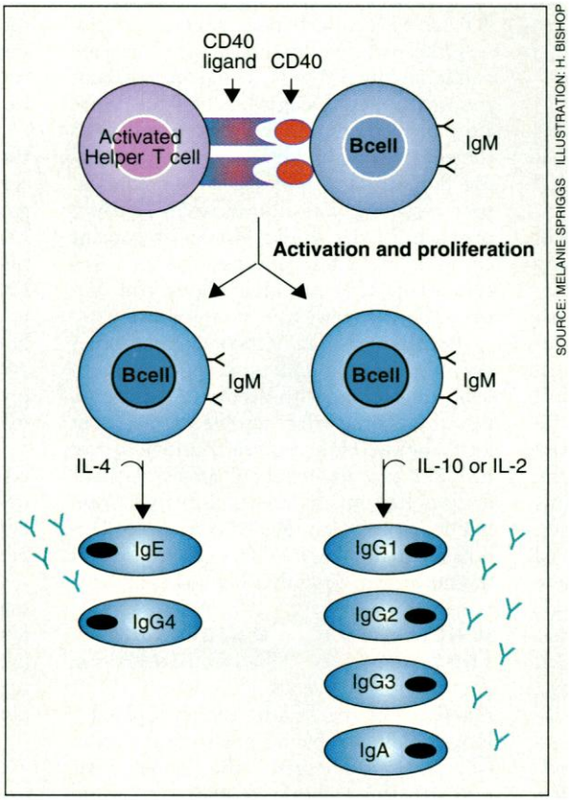
Cell Communication Failure Leads to Immune Disorder

It's tempting to think of communication within the human body as the sole province of the nervous system. But it's not. Other biological systems also have a strong need for communication. Take the immune system, for example. Researchers have long known that immune cells need to talk back and forth with each other for the body's immune defenses to function properly. Now, in a remarkable flurry of recent activity, five independent research teams have identified the gene for an inherited immunodeficiency disease that shows just how crucial that kind of immune-system crosstalk can be. One group, led by Melanie Spriggs of Immunex Corp. in Seattle, Washington, reports their results on page 990 of this issue of *Science*.

The disorder those groups focused on is a rare condition known as X-linked hyper-immunoglobulin M syndrome (HIM). Patients who have HIM fail to produce the full set of antibodies they need to fight off invading pathogens and, as a result, are susceptible to opportunistic infections. The identification of the gene defect provides the first explanation of the antibody deficiency in HIM. The problem might, for example, have been in the immune system's B cells, which actually produce the antibodies. But it's not. Instead, it's in a protein made by the B cells' partners, the T cells, without whose cooperation the antibodies can't be made. "For this interaction, it turns out to be a key molecule, and this genetic defect gives a view of what happens when you don't have it," says clinical immunologist Max Cooper of the University of Alabama School of Medicine in Birmingham.

Identification of the HIM gene defect could lead to better treatments for the disease, perhaps even gene therapies, as well as to better tests for identifying carriers of the bad gene who can pass it on to their offspring. But the implications go far beyond one rare genetic disorder. The new information might be used to devise ways of blocking the undesirable antibody production seen in autoimmune diseases such as rheumatoid arthritis. And by defining the molecules that tell B cells to proliferate and make antibodies, these results open the door to the production of human monoclonal antibodies, a goal long sought by the biotech industry because of their potential for treating disease.

The road to the discovery of the HIM gene began in the mid-1980s, with the identification of a B cell surface protein designated CD40. At the time, researchers already knew B cells need signals from activated "helper T cells" before they can proliferate and begin making antibodies. CD40 was intriguing, because it looked as if it might be one of the receptor proteins through which the B cells picked up T cell signals. The researchers found, for example, that they could duplicate the T cells' effects by treating B cells in culture with monoclonal antibodies that specifically bind to CD40, plus cytokines such as interleukin 4.



SOURCE: MELANIE SPRIGGS ILLUSTRATION: H. BISHOP

Let's talk. Input from helper T cells is needed to get B cells to make the "class switch."

After identification of CD40, a second milestone was achieved in 1989 when Brian Seed's group at Harvard Medical School succeeded in cloning the CD40 gene. That feat enabled researchers to address the next big question: If CD40 is the B cell molecule that receives signals from T cells, what is the molecule that sends them? Cloning the gene made it easier to answer that question, since researchers could use it to make the CD40 receptor protein. Because the receptor binds specifically to the sending molecule—the so-called CD40 ligand—it could then be used to construct a probe for identifying the ligand.

By September 1991, Spriggs and William Fanslow and Richard Armitage, also at Immunex, and their colleagues had cloned the mouse gene for the CD40 ligand. This was followed a few months later by the cloning of the human gene by the Immunex group and by a second team including Alejandro Aruffo, Diane Hollenbaugh, and Jeffrey Ledbetter of the Bristol-Myers Squibb Pharmaceutical Research Institute in Seattle and Randy Noelle of Dartmouth Medical School.

A third team also cloned the human gene for the CD40 ligand—but they came to it by a different route. Ulf Korthäuer, Richard Kroczek, and their colleagues at the Max Planck Institute for Clinical Immunology in Erlangen, Germany, had set out to identify genes that get turned on when T cells are activated. One looked particularly interesting, Korthäuer says, because it is turned on very rapidly and the protein it makes resembles the cytokine called tumor necrosis factor. The Max Planck workers thought the protein might be a secreted cytokine, although it also had the features of a membrane protein. They didn't know its precise identity until July last year, when they got a sequence match—with the mouse CD40 ligand—in the European Molecular Biology Laboratory database.

Once the CD40 ligand gene had been cloned, the research moved quickly, Spriggs says. "One thing right after another just fell right into place. It's been really lovely." Everyone involved in the research realized that the CD40 ligand's role in transmitting signals from T cells to B cells meant that defects in its gene might cause hereditary antibody deficiencies. But which one? There are more than a dozen such disorders.

One clue came when the Immunex team mapped the mouse gene to the X chromosome. "I said, 'Holy cow,' " recalls Spriggs, "if it's on the mouse X, it's usually on the X in humans." That focused attention on X-linked antibody disorders, and HIM was a good candidate since its symptoms are exactly what would be expected from a failure of the interaction between CD40 and its ligand. The Korthäuer-Kroczek group, which has moved to the Robert Koch Institute in Berlin, provided additional evidence for this idea in September by mapping the human gene to position q26.3-q27.1 on the X chromosome—just where the HIM gene is located.

Since then five groups have shown that the gene for the CD40 ligand is indeed defective in HIM patients. In addition to the Immunex group, these are: the Bristol-Myers

Tyrosine Kinase Defect Also Causes Immunodeficiency

The immunodeficiency disease that goes by the jawbreaking name of X-linked agammaglobulinaemia (XLA) was the first hereditary antibody deficiency to be described. So it's only fitting that the disease has just become the subject of another first. Two research teams, one in Europe and the other in the United States, have identified the gene at fault—and the protein it encodes turns out to be a new tyrosine kinase. Although cell biologists have discovered more than 40 of these enzymes (and amassed a great deal of evidence showing that they are important regulators of cell growth and differentiation), never before had a tyrosine kinase defect been shown to cause a human hereditary disease.

The discovery of the gene may open the way to gene therapy for XLA, as well as to more accurate tests for detecting carriers of the gene defect and affected fetuses. It should also help immunologists understand the nature of the defect underlying this disorder and perhaps of other immunodeficiencies.

In XLA, an early stage of development in the B cell lineage is blocked, preventing the conversion of the "pre-B cells" in the bone marrow to B cells. As a result, XLA patients have no mature B cells circulating in the bloodstream and no antibodies to fight off infections. But genes closely related to the XLA gene are also active in other types of cells and, says immunologist Roger Perlmutter of the University of Washington Medical School, these relatives are "almost certainly" involved in controlling the "lineage commitments" that determine the fates of embryonic cells. Understanding how the XLA gene defect disrupts B cell formation may therefore help in deciphering other developmental abnormalities, says Perlmutter.

The two groups that discovered the XLA gene found it by different routes. The European team, including David Vetrie of Guy's and St. Thomas' Hospitals in London and Igor Vorechovsky of Karolinska Institute's Center for BioTechnology in Huddinge, Sweden, set out to find the gene by "positional cloning," an approach that's becoming standard for investigators looking for disease genes. The first step in positional cloning is to find the gene's chromosomal location by linking it to genetic "marker" sequences whose locations have already been mapped. After that, the region containing the target gene is cloned and this DNA is used to identify whatever genes from the region are active in cells affected by the disease—B cells in the case of XLA. These genes are screened to see if they are mutated in patients with the disease. Following this path, the researchers ultimately reached the new tyrosine kinase gene; they published their findings in the 21 January *Nature*.

Meanwhile, the U.S. team, led by Owen Witte of the Howard Hughes Medical Institute at the University of California, Los Angeles, came up with the same gene serendipitously. (Their results appeared in the 29 January *Cell*.) "We weren't working on the disease at all," Witte explains, "but on genes that regulate B cell development in the mouse." In particular, they had their eyes on tyrosine kinase genes, because of the known involvement of those enzymes in the development of B cells and other cells.

One gene they found was particularly interesting, Witte says, because its sequence was different from those of other known kinases. It got even more interesting when they mapped it to the X chromosome, where the XLA gene was known to be located. Witte and his colleagues went on to show that the gene is mutated in cells from XLA patients. They also found that the activity of the tyrosine kinase is greatly reduced in B cells from patients, but not in cells from carriers or normal controls—a further indication that a defect in the tyrosine kinase gene causes XLA. "We came at it from one vantage point, and they came at it from another," Witte says. "Taken together the two sets of results provide strong evidence that we've got the right gene."

The next step, in addition to seeing whether the gene can be used diagnostically or for XLA therapy, is to try to define the other B cell molecules that interact with the new tyrosine kinase in regulating B cell development. And that should be made easier since the XLA "experiment of nature" has already told researchers exactly what happens when the gene doesn't function correctly.

–J. M.

group, which describes their results in the 29 January *Cell*; the Korthäuer-Kroczek team and another including James DiSanto and Genevieve de Saint Basile of the Hopital Necker in Paris, both of which have papers in the 11 February issue of *Nature*; and in work not yet published, a team led by Raif Geha of Children's Hospital in Boston, Massachusetts, has identified mutations in the CD40 ligand gene in three additional HIM patients. The mutations are of various types—"point mutations" that change a single amino acid, deletions, and "frameshift mutations" that garble the coding sequence for the remainder of the gene; and they are spread throughout the gene. Indeed, says Ledbetter, "so far, it looks like we've all got different mutations." But it seems clear that all these mutations can cause HIM, since studies in lab cultures show that the mutated CD40 ligand proteins cause the same type of immune failure seen in HIM itself.

That failure is an inability to stimulate what is known to immunologists as "class switching." In their resting state, B cells make proteins called immunoglobulins of the M class (IgMs). When B cells are activated by T cells, however, they normally switch and start making one of the other three immunoglobulin classes—A, E, and G—which form the antibodies that actually go out on search-and-destroy missions. The B cells of HIM patients, though, can't carry off this class switch: They make higher than normal amounts of IgM and little or none of the other three types.

The most immediate clinical application of the discovery of the gene responsible for this switching failure is likely to be in a diagnostic test to identify carriers of the defect. Ordinarily these are women, who don't become immunodeficient because the defect is recessive and women have two X chromosomes—one of which will have a good copy of the gene. Any son who inherits the defective gene will come down with HIM, however, since males have only a single X chromosome. Eventually, it may also be possible to correct the gene defect by giving HIM patients a good copy of the gene. Current therapies, which rely on gamma globulin injections to replace the missing antibodies, may not alleviate all the patients' problems. For one thing, they often develop a condition in which there's excessive proliferation of their lymphocytes, which infiltrate and damage vital organs, including lungs and liver. When that happens, Geha says, the individuals may die in their second or third decade.

Also under investigation is the possibility of using antibodies that can block CD40 activation, such as one made by Dartmouth's Noelle, to treat autoimmune diseases. But the most immediate application of the better understanding of cooperation between B and T cells may be in making human monoclonal antibodies. Because researchers have identified the molecules that make B cells proliferate and secrete antibodies, they can now grow the cells in long-term culture, something that was long an obstacle to making human monoclonals. More work will be needed to get the cultured cells to make antibodies of the desired specificity that might be used to help destroy cancer cells, for example. But that prospect is sufficiently inviting that scientists are not likely to stop talking about T and B cell communication any time soon.

–Jean Marx