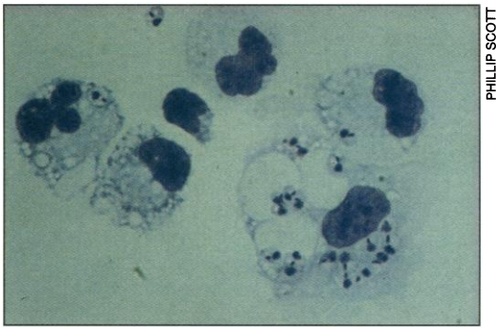
Leishmania Susceptibility Puzzle Gets Another Twist

Immunologists have long been puzzled by the fact that a particular highly inbred strain of mice fails to fight off a parasitic protozoan called *Leishmania major*. It might sound like an arcane conundrum, but it has "a direct clinical relevance to the real world," says immunologist Robert Coffman of DNAX Research Institute of Molecular and Cellular Biology in Palo Alto, California. For one thing, *Leishmania* is a major scourge, afflicting an estimated 12 million people worldwide, causing skin ulcers and other debilitating symptoms. And for another, the parasite is one of many cell-invading pathogens that are normally held in check by so-called cell-mediated responses, carried out by armies of immune cells that kill infected cells. Understanding why this response fails in the mouse strain in question, the so-called BALB/c strain, could therefore help immunologists grasp how the animals, and perhaps humans as well, normally fight off intracellular parasites, including viruses.



PHILLIP SCOTT

Hiding out. It takes cellular immunity to rid mice of *L. major*–infected macrophages like these.

The researchers did in fact appear to be closing in on an answer to this puzzle. But their evidence may have just suffered a heavy blow. It comes, appropriately, from a new line of "knockout" mice, in which the gene for an important immune system regulator known as interleukin-4 (IL-4) was inactivated. Previous work had suggested this cytokine makes BALB/c mice susceptible to *L. major* infections by triggering the animals' immune systems to increase production of T_H2 cells, which mount an antibody attack that is ineffective against intracellular parasites, instead of the T_H1 cells needed for cell-mediated immunity. If so, then knocking out the IL-4 gene in BALB/c mice should make the animals resistant to the infection.

But on page 987, Nancy Noben-Trauth of the Jackson Laboratory in Bar Harbor, Maine, and Pascale Kropf and Ingrid Müller of Notre Dame University in South Bend, Indiana, report that they found just the opposite: The knockouts remained every bit as susceptible as the controls. Immunologist Alan Sher of the National Institute for Allergy and Infectious Diseases describes this result as "very surprising. The whole basis of this phenomenon is that IL-4 is what causes the susceptibility."

The Jackson Lab–Notre Dame team's finding is buttressed, however, by a second set of very different experiments described on page 984. Work by Kenneth Murphy's team at Washington University School of Medicine in St. Louis suggests that BALB/c mice are susceptible to *Leishmania*, not because IL-4 is producing an inappropriate T_H2 response, but because their T cells fail to respond to interleukin-12, the cytokine that normally prods T_H1 cells into action.

Other immunologists, citing results that seem to conflict with Noben-Trauth's, say it's far too soon to dismiss IL-4, however. And that leaves the issue of the cytokine's importance for *Leishmania* resistance up in the air. But researchers will be eager to resolve it. "Understanding why a pathogen stimulates primarily T_H1 or T_H2 responses is extremely important," says Coffman, whose team discovered the two different classes of T_H cells. Indeed, as immunologist Richard Locksley of the University of California, San Francisco, points out, if the animal "makes the wrong choice, it dies." And similarly, human variations in resistance to *Leishmania* and other infectious diseases may also be traced to whether they mount T_H1 or T_H2 responses.

For the current work, Noben-Trauth and Birgit Ledermann of Sandoz Corp. in Basel, Switzerland, while working with the late George S. Köhler, generated a line of genetically pure BALB/c mice in which the IL-4 gene was inactivated by standard gene-targeting techniques. Most mouse strains can successfully fight off *L. major* infections. But when ordinary BALB/c animals are infected by the parasite, their immune system is unable to control the parasite, and it continues to reproduce, forming progressively growing skin lesions. The animals eventually die some 12 to 15 weeks after infection.

Work by Locksley's team and several others had strongly suggested that IL-4 knockouts would resist *L. major*. These groups showed that when IL-4 activity in BALB/c mice is blocked with antibodies that bind to the cytokine, the lesions quickly heal and the animals survive. "We've repeated that [experiment], and so have many other people. It works every time," says Phillip Scott of the University of Pennsylvania School of Medicine. But the Jackson Lab–Notre Dame team found no sign of such resistance in the knockout mice, which lacked IL-4 completely. Although the growth of the lesions leveled off somewhat about 30 days after infection, they did not heal, even in animals kept as long as 100 days. "IL-4 is definitely not important for disease progression in BALB/c mice," Noben-Trauth concludes.

If IL-4 is not important, what is? That's where the Murphy group's results may come in. Rather than looking at knockout mice, these researchers used a lab culture system to compare the behavior of T cells from BALB/c mice with that of T cells from another strain (B10.D2) that is naturally resistant to *L. major*. Murphy and his colleagues found that when they cultured the cells under conditions that allow them to develop along their natural "default" pathways, the BALB/c cells, but not the B10.D2 cells, became unresponsive to IL-12 over a period of about a week. If the same thing happens in the animals, it would leave their T cells incapable of developing into the T_H1 cells needed to fight off a *Leishmania* infection.

This change, the researchers found, isn't due to some difference in the cytokines—among them IL-4—that the cells secrete, which could be feeding back on the cells to influence their responsiveness: The effect persisted even when the two kinds of cells were cultured together, exposing them to each other's cytokines. Instead, it seems that whatever determines responsiveness to IL-12 is an intrinsic property of the cells.

The Murphy team is now trying to find out what this property is. They have preliminary evidence suggesting that the B10 cells are more sensitive than the BALB/c cells to another cytokine, interferon γ, which induces production of the receptor through which IL-12 exerts its effects. Other immunologists find this proposal intriguing. "His [Murphy's] work is very provocative and interesting," says Sher. "If you want to forget about IL-4, this gives you a different model."

Still, immunologists note that Murphy's work, which is all done with cultured cells, hasn't directly shown that the differences in IL-12 responsiveness seen by his team cause the different *Leishmania* susceptibilities of the two strains. "The studies are excellent," says immunologist Manfred Kopf of the Basel Institute for Immunology in Switzerland, "but the title [of the paper] is misleading, since he doesn't address *Leishmania*."

And then there's the large amount of antibody and other data implicating IL-4 in

Leishmania susceptibility. "There's a major apparent discrepancy that needs to be resolved. I wouldn't care to decide on the basis of the published data," says Coffman.

Complicating matters even more, other teams, including Kopf's and that of Steven Reiner of the University of Chicago, have been studying resistance to *L. major* in IL-4 knockouts and, in as yet unpublished work, have come up with opposite results: The mice did become resistant. For most of these animals, there may be a good explanation. The knockouts were created in the 129 strain, which is naturally resistant to the parasite, then backcrossed with BALB/c mice up to 10 times to introduce the knocked-out gene into the BALB/c genome. The problem is that a lot of the genes flanking the IL-4 gene in the resistant mice may also end up in the BALB/c mice, and some of these might be responsible for their resistance, rather than the IL-4 knockout itself. Murphy's team, for example, has come up with an intriguing candidate: a gene acting in the pathway by which interferon γ signals are transmitted into the cell interior.

Still, one contradictory result is harder to explain away. Kopf has also conducted *L. major* infection experiments with the same knockouts made by Noben-Trauth and Ledermann and found that they are resistant. (These results have not yet been published since Köhler gave Noben-Trauth first rights to the *Leishmania* experiments.) The results of the two groups, he says, are "not easy to reconcile." He does suggest, however, that the explanation might lie in the different *L. major* strains the two groups used, and he is currently repeating the experiment with the same parasite strain used by Müller.

Until these issues are resolved, immunologists looking for the prime controller of *Leishmania* susceptibility won't know whether IL-4 is down for the count—or ready to spring to its feet again.

–Jean Marx

OBESITY RESEARCH

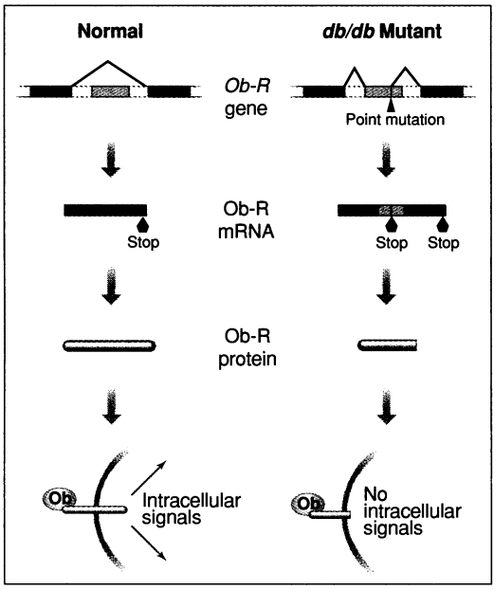
Researchers Nail Down Leptin Receptor

Nowhere has research been moving faster in the past year than in the study of obesity. Just over a year ago, Jeffrey Friedman's team at Rockefeller University cloned the *obese* gene (*ob*), which when mutated causes mice to become grossly fat, and showed that its protein product, leptin, is a key weight-control hormone. Last December, researchers at Millennium Pharmaceuticals, in Cambridge, Massachusetts, took another big step, cloning DNA that appeared to encode the receptor through which leptin exerts its effects. At the time, there was concern that they might not have the right receptor. But now, three groups have confirmed the identification by showing that the leptin receptor is the product of a gene called *db*, which has long been thought to encode the receptor for a weight-controlling hormone.

The findings—published by Louis Tartaglia's team at Millennium in the 9 February issue of *Cell*, by Friedman's group in the 15 February *Nature*, and by a team led by Rudolph Liebel, also of Rockefeller, on page 994 of this issue—prove this is the physiologically important receptor gene, says Bruce Spiegelman, an obesity researcher at Harvard Medical School. "With this new information in hand," he adds, researchers can move forward with confidence to "study how [leptin] signals through [this] receptor." And, as most pathologically obese humans seem to have defects not in *ob*, but in either leptin receptors or the signaling pathways triggered by the receptors, any new understanding of the receptors may aid in the design of new drugs for treating obesity.

When Tartaglia's team first cloned the DNAs encoding the leptin receptor in mice and humans, they had noted an anomaly that raised questions about whether they had the right receptor. Their clone from mice encoded a shorter form of the receptor than the human clone did. The receptor it coded for lacked the part that transmits leptin signals within the cell. They proposed that the short receptor might transport leptin across the blood-brain barrier, and speculated that mice must also make a long form that executes leptin's weight-controlling effects. But it was also possible that their gene simply did not code for the key receptor.

But the Tartaglia team had reason to doubt that was the case—evidence suggesting their receptor gene might be the *db* gene. Researchers had suspected that *db* might code for the leptin receptor, because Jackson Laboratory biochemist Douglas Coleman had shown 20 years ago that *db* mutant mice are fat because they can't respond to the weight-regulating product of the *ob* gene. So when Tartaglia's group mapped its receptor gene to the same genetic region as *db*, they were encouraged. That didn't prove their gene was *db*, however, because the large region they had mapped it to contains dozens of genes.



Cutting it short. The *db* mutation apparently produces a shortened, and probably nonfunctional, leptin receptor.

Now further evidence comes from Liebel's team, which reports additional mapping that seems to place the mouse receptor gene right on top of *db*. They also found that in rats the leptin receptor gene maps to that species' counterpart of *db*, known as *fatty*.

Meanwhile, studies carried out by Friedman's and Tartaglia's groups provided more proof. They've discovered abnormal mRNAs made from the receptor gene in *db* mutant mice that apparently code for a nonfunctional receptor. That is very good evidence that *db* is indeed the receptor gene.

The abnormal RNAs seem to be caused by a mutation that alters the way the noncoding introns are spliced out of the RNA produced by the receptor gene, so that the mRNA which should produce the long form of the receptor is interrupted by an abnormal insert. That extra piece of RNA contains a signal that prematurely stops production of the receptor protein, so that instead of the long form of the receptor, it makes a protein that looks more like the short form and probably lacks signaling ability.

As a result, the *db* mice apparently make none of the functional long form of the receptor, and that would explain why they get so fat. "It is nature's knockout," Tartaglia says. "If this had not turned out to be the case, we would have had to demonstrate the importance of our receptor ... by knocking it out and hoping we got a fat mouse."

But it is only one of several mutations that inactivate *db*. Liebel's team studied another mutant form of the gene, called db^{Pas}, and found that it apparently has a partial duplication, while the *fatty* mutation looks like a deletion. "We are hoping that by identifying the nature of these mutations we will get a better understanding of how the receptor actually works," says Liebel. And that will keep the obesity field moving at a fast pace for the months and years to come.

–Marcia Barinaga