At the largest ever meeting of prion disease researchers last month, there was much new knowledge on display, but still no consensus on whether this mysterious disease agent is acting alone

Prions: A Lone Killer or a Vital Accomplice?

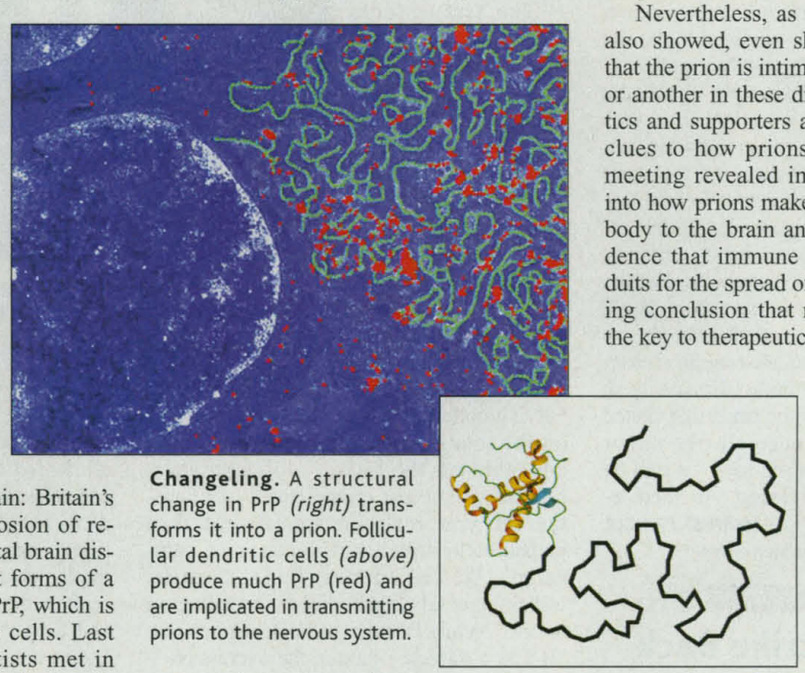
TÜBINGEN, GERMANY—Is it an epidemic, or isn't it? European health officials still are not sure. For more than 3 years, they have been anxiously watching the slow accumulation of cases of variant Creutzfeldt-Jakob disease (vCJD), a fatal neurodegenerative disorder linked to eating beef from cattle infected with bovine spongiform encephalopathy (BSE), or "mad cow disease." As of late September, the toll had risen to 46 cases in the United Kingdom and one in France. So far, earlier concerns that thousands might eventually die have not been realized. But fears of this nightmare scenario continue to generate headlines. As *Science* went to press this week, the long-running government inquiry into the disastrous BSE epidemic in British cattle, chaired by Sir Nicholas Phillips, had just resumed hearings in London, and French officials were adamantly defying a European Union decision to lift an embargo on imports of British beef.

One thing, however, is certain: Britain's BSE crisis has fueled an explosion of research into vCJD and similar fatal brain diseases linked to prions, aberrant forms of a normal cellular protein called PrP, which is particularly plentiful in nerve cells. Last month, more than 400 scientists met in Tübingen* to review the latest advances in prion disease research—the largest such gathering ever. The impressive turnout was proof that the field has come a long way since the early 1980s, when neurologist Stanley Prusiner of the University of California, San Francisco (UCSF), began championing the then-heretical hypothesis that the prion acts essentially alone to infect tissues and reproduce itself—without the benefit of an RNA- or DNA-based genome, thought to be fundamental to all life-forms. Since those days, Prusiner has won a lot of converts to this idea. He also won the 1997 Nobel Prize in Physiology or Medicine, although in the wake of controversy over the award the Nobel committee hastened to clarify that it should not be construed as a blanket endorsement of his ideas (*Science*, 10 October 1997, p. 214).

Indeed, while the Tübingen meeting reflected a growing scientific consensus that prions are key players in causing these diseases, a few presentations—as well as the buzz in the corridors—underscored the continuing skepticism of some researchers that the "protein-only hypothesis," as it is often called, can explain all the data. These researchers continue to suspect that some sort of virus or other RNA- or DNA-based organism is also involved (*Science*, 12 July 1996, p. 184). For many participants, these doubts were reinforced when researchers in Edinburgh reported results that might contradict key experiments performed in Prusiner's lab—experiments that many scientists had thought conclusively showed that prion protein alone could cause disease.

Although other researchers, including Prusiner's collaborators, shrug off the Edinburgh group's findings, the controversy shows that the issue is far from settled. "I don't want to believe lock, stock, and barrel in the protein hypothesis until it satisfactorily explains prion biology," comments virologist Jeffrey Almond, a former member of the United Kingdom's Spongiform Encephalopathy Advisory Committee and vice president for research and development in the French operations of drug firm Pasteur Mérieux Connaught.

Nevertheless, as the Tübingen meeting also showed, even skeptics do not dispute that the prion is intimately involved one way or another in these diseases, and both skeptics and supporters are busy gleaning new clues to how prions behave. Talks at the meeting revealed important new insights into how prions make their way through the body to the brain and provided strong evidence that immune system cells are conduits for the spread of infection—a disquieting conclusion that may nevertheless hold the key to therapeutic strategies.



Changeling. A structural change in PrP *(right)* transforms it into a prion. Follicular dendritic cells *(above)* produce much PrP (red) and are implicated in transmitting prions to the nervous system.

CREDITS: (LEFT TO RIGHT) JEFFREY AND MCGOVERN; R. GLOCKSHUBER AND K. WÜTHERICH/*FEBS LETTERS*

Spontaneous generation?

More than a dozen types of prion diseases, also known as spongiform encephalopathies for the spongy appearance of diseased brains, occur in humans and animals. Some are infectious, such as vCJD in humans, BSE in cattle, and scrapie in sheep. Others appear to be inherited, including familial CJD and Gerstmann-Sträussler-Scheinker syndrome (GSS), which are linked to mutations in the gene that codes for PrP. Still another, a form of CJD called sporadic CJD, appears to develop at random and is normally neither transmitted nor inherited.

In 1994, Prusiner's group, including molecular biologist Karen Hsiao Ashe, published findings that many researchers thought clinched the protein-only hypothesis. The team created transgenic mice with multiple copies of a mutant *PrP* gene, referred to as *P102L* in humans and *P101L* in its mouse version, which had earlier been shown to cause GSS in humans. The mutant mice,

* Characterization and Diagnosis of Prion Diseases in Animals and Man, Tübingen, Germany, 23 to 25 September 1999.

which expressed high levels of the mutant PrP, spontaneously developed GSS-like neurological symptoms. Moreover, when certain other healthy animals, including some mice and hamsters, were inoculated with brain extracts from the sick animals, a significant number developed a similar disease. The finding seemed to confirm Prusiner's contention that the mutant protein—with no help from any virus—causes the *de novo* creation of infectious prions. Indeed, research by Prusiner and other scientists has shown that prions exert a bad influence on normal PrP by binding to it and converting it to the abnormal form. Exactly how the abnormal protein causes disease is not well understood, but the clumping of prions together in the brain appears to be responsible for some of the pathological effects.

While many researchers found Prusiner's experiments convincing, others have remained perplexed by some oddities in the findings. For example, although normal wild-type hamsters succumbed to infection by brain extracts from the sick transgenic mice, normal wild-type mice did not. Only other transgenic mice, which expressed low rather than high amounts of mutant PrP and did not spontaneously get sick, were vulnerable to the inoculation. Some researchers suggested that the hamsters might have become infected with hamster scrapie prions that contaminated the laboratory rather than with the mouse brain extracts. Other scientists suspected that Prusiner's transgenic mice—some of which harbored as many as 60 copies of the mutant *PrP* gene and expressed eight times the normal level of the protein—might have become ill because of damage to their genomes from inserting so many foreign genes, or simply because they were producing too much PrP, and not because the mutation was producing prions.

These skeptics now see some vindication in new work presented at the Tübingen meeting by molecular biologist Jean Manson of the Institute of Animal Health's Neuropathogenesis Unit (NPU) in Edinburgh. Manson repeated some of Prusiner's experiments, but this time using a new technique, called double replacement gene targeting, that she and her Edinburgh colleagues developed. Manson removed the normal *PrP* mouse gene, replaced it with a marker gene, and then replaced the marker gene with a *PrP* gene containing the human GSS mutation. These transgenic mice harbor only two copies—one on each duplicate chromosome—of the mutant *P101L* gene, which are still in their correct locations in the genome. Unlike Prusiner's transgenic animals, which expressed high PrP levels, the Edinburgh mice did not spontaneously develop disease during their lifetimes, about 900 days. When, however, the *P101L* mice were inoculated with human brain GSS extracts, they did become ill after an average of about 280 days.

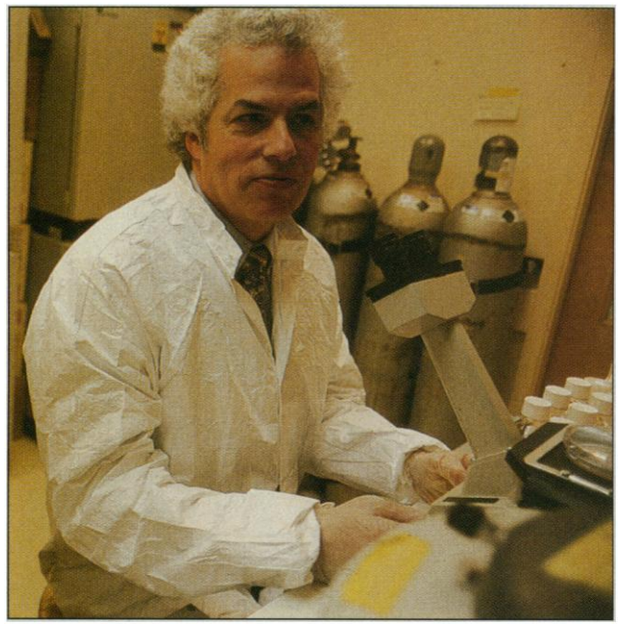
Manson interprets these results, which are currently in press at *EMBO Journal*, as meaning that the GSS mutation itself does not create prions, but rather makes the mice much more susceptible to an external infectious agent, whether it is a prion alone, a prion with an accomplice, or something entirely different. That susceptibility may result from the production of PrP proteins that are more or less easily transformed into abnormal forms by external prions. "Jean Manson's data appear to invalidate those of [Prusiner's group] and demonstrate that the GSS mutation does not by itself produce disease or infectivity," says viral immunologist Bruce Chesebro of the Rocky Mountain Laboratories in Hamilton, Montana. "She has replaced the normal *PrP* gene at precisely the correct position in the DNA, so there is no possible effect from integration at an unusual site or from integration of multiple copies."

Prusiner was not available for comment, but a longtime collaborator at UCSF, biophysicist Fred Cohen, argues that Manson's results do not contradict Prusiner's. The new work is "entirely consistent" with the original experiments, Cohen says, in which "animals that have a high copy number of the *P101L* transgene get a spontaneous disease, while those that express a low copy number do not." Cohen argues that since humans with the mutant prion gene develop GSS, whereas the Edinburgh group's transgenic mice do not, "the Manson experiment is not as clean a model as they suggest."

Indeed, many researchers argue that Manson's results do not disprove the protein-only hypothesis, because an external source of prions could still be acting alone to cause disease in humans or animals made susceptible by the mutation. And supporters of Prusiner's ideas are not prepared to see them overturned by the Edinburgh findings. "The one conclusion you may not draw," states molecular biologist Charles Weissman at St. Mary's Hospital in London, "is that [Manson's experiment] has disproved Prusiner's contention that this mutation can cause a prion disease." Neurologist John Collinge, also at St. Mary's, agrees, saying that while the Edinburgh work is "intriguing," it should not be the takeoff point to launch a search for a virus or other microbe. "The evidence against that is pretty overwhelming."

On the other hand, genetic susceptibility to infectious prion disease is already well established in sheep, which vary widely in their vulnerability to scrapie depending on the makeup of their *PrP* genes. "These mouse results indicate that familial prion diseases in humans might be analogous to scrapie infection, where differences in susceptibility have been linked to genetic variations," says Glasgow University veterinary pathologist Alun Williams. And just such a pattern may already be showing up in vCJD: All of the victims so far are homozygous for the amino acid methionine at position 129 of their *PrP* genes—that is, the *PrP* genes on both of their duplicate chromosomes contain methionine at this position, whereas the population at large can also be homozygous for valine or heterozygous for methionine and valine.

Thus Manson's work is sure to keep the debate—and the doubts of skeptics—alive for some time to come. "These experiments go to the heart of the prion hypothesis," says Almond. "Is this phenomenon of spontaneous production of infectivity real? We are now having doubts because of [Manson's] results."



Former heretic. Nobel laureate Stanley Prusiner's prion hypothesis remains controversial, but it's still the only game in town.

CREDIT: D. POWERS

Follow that prion!

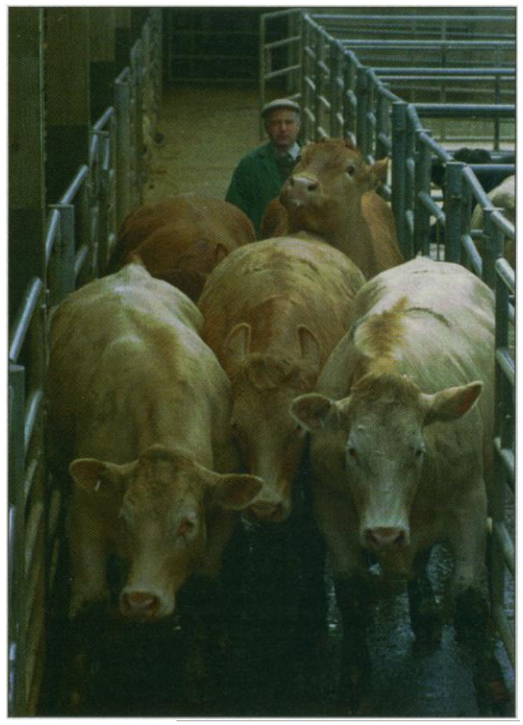
While the Edinburgh results have brought the debate over the protein-only hypothesis back to center stage, the prion is still the target of most studies. The failure to find a virus or other organism, combined with convincing

evidence that PrP must be present for disease to occur—especially experimental findings showing that mice whose *PrP* genes have been "knocked out" cannot be infected—have led to general agreement that prions are, at the very least, surrogate markers for disease. Indeed, the focus on prions has now led to the discovery that there might be other forms as well: In the 1 October issue of the *Journal of Molecular Biology*, a team led by molecular biologists Richard Moore of UCSF and Inyoul Lee of the University of Washington in Seattle, report finding a second prionlike protein in mice, which is encoded by a gene they call *doppel* and appears to also cause serious brain damage in the animals. (A Perspective in next week's issue will discuss this finding.)

Much recent research has focused on understanding how prions propagate through the body and eventually attack the nervous system. A particularly elegant study of this nefarious process was presented in Tübingen by veterinary pathologist Lucien van Keulen of the Institute for Animal Science and Health in Lelystad, the Netherlands. Van Keulen and his colleagues used a biochemical technique called immunohistochemistry—which can detect PrP in tissues—to trace the course of scrapie infection in sheep. In a dramatic series of pathology slides, van Keulen showed that when an infected sheep is about 5 months old, prions begin to accumulate in the lymphoid tissues of its immune system as well as in nerve fibers in its small intestine. By 10 months, infection spreads to nerves serving the visceral organs, and after about 17 months the spinal cord comes under attack. Finally, the prions reach the medulla oblongata, the part of the brain that is just above the spinal cord and is responsible for involuntary functions, and by 26 months the brain is fully infected and the animal enters the terminal stages of the disease.

Earlier studies had suggested that cells of the immune system might drive this process by passing prions to the nervous system. The findings led to proposals that the diseases might be detected early, for example, in biopsies of tonsils (*Science*, 22 January 1999, p. 469). Studies also held out promise of future therapies, since the immune system would likely be a more accessible target for antiprion drugs than nerve cells would. But they also suggested the worrisome possibility that prion diseases might be spread through blood transfusions.

In December 1997, for example, neuropathologist Adriano Aguzzi and immunologist Rolf Zinkernagel at the University of Zurich published findings in *Nature* that suggested a crucial role for B cells, which circulate in the blood and produce antibodies. The Zurich team found that mice carrying mutations that interfere with B cell development or function resist infection when they are inoculated with scrapie. The team also tested another type of immune cell previously suspected in the transmission of prions—follicular dendritic cells (FDCs), which work together with B cells to mount an immune response. FDCs were a less worrisome candidate because these cells normally remain in the spleen and lymph nodes and do not circulate in the blood. Yet these cells appeared to be cut out of the loop, because mice without functional FDCs could still be infected.



Unfit for consumption. Thousands of British BSE-infected cattle have had to be destroyed.

Besides setting off alarm bells among public health officials, the Zurich findings led to speculations about just how the B cells might be spreading infection. They carry significant amounts of PrP in their membranes, and the lymphoid tissues, where they normally reside when not in the bloodstream, are heavily invested with nerve fibers that could pick up abnormal forms of the protein from them. Late last year, however, new results from the Zurich group revised that picture. The team found that immunodeficient mice that lack B lymphocytes—and therefore cannot be infected with scrapie—became susceptible again when they received donor B lymphocytes from PrP knockout mice; that is, even B cells that lacked PrP could apparently play a role in disease. Although Aguzzi and his co-workers argued that the cells might transport prions to the central nervous system via some mechanism not linked to PrP expression, these results left many prion researchers scratching their heads.

However, a series of new experiments presented in Tübingen by Moira Bruce of the NPU in Edinburgh may help point to a solution of the riddle—one that implicates both B lymphocytes and FDCs in the spread of prions. Using a combination of gene knockout techniques and grafts of bone marrow—where the precursors of many immune cells develop—Bruce and her colleagues succeeded in creating two groups of chimeric mice with mismatches in the PrP status of their immune cells. One group had PrP-positive FDCs but PrP-negative B lymphocytes, whereas the other had PrP-negative FDCs but PrP-positive lymphocytes. When inoculated with scrapie, only the FDC-positive group could be infected. Bruce interprets these findings, which are in press at *Nature Medicine*, to mean that PrP-expressing FDCs are essential for the spread of prion infection after all.

In discussions with *Science*, some scientists suggested that the discrepancy between Bruce's and Aguzzi's results could be due to differences in the strains of scrapie used in the two experiments. Whatever the case, many researchers now believe that both B lymphocytes and FDCs are essential players. Collinge, for example, suggests that B lymphocytes might be important because they foster maturation of FDCs. Weissman agrees this is the most reasonable scenario: "My interpretation of the data is that B cells are required for maturation of FDCs, but FDCs are responsible for making prions."

If this view is correct, it could allay concerns about blood safety, because the circulating B lymphocytes would not necessarily be direct carriers of infectivity. And researchers say that figuring out how prions replicate in the immune system during the early stages of disease might open the door to therapies that would block this process. Indeed, a talk at the meeting by Dominique Dormont of the French Atomic Energy Commission's neurovirology service in Fontenay-aux-Roses raised just that possibility. He presented findings that a number of drugs known to interact with immune system cells can increase the survival time of rodents experimentally infected with scrapie or BSE.

"The idea of therapy when the infection is still in the periphery is not a pipedream," says Chesebro. Almond adds: "You have to have hope in something like this, especially if some of the more dire forecasts for vCJD in the U.K. are real." —MICHAEL BALTER

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