A wealth of evidence suggests that pathogens may play a role—perhaps even a causal one—in chronic diseases like Alzheimer's or MS. Proving that theory, however, is another matter

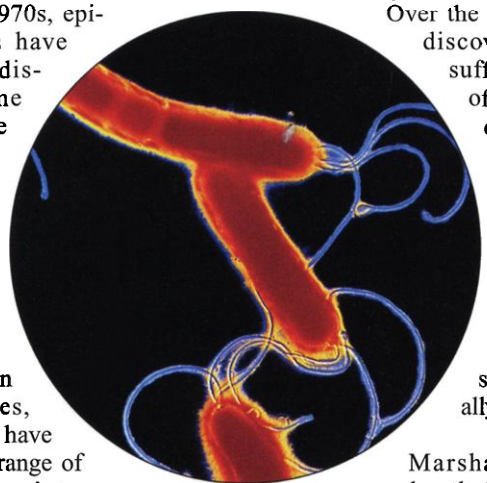
Do Chronic Diseases Have An Infectious Root?

In the 1970s, epidemiologists documented remarkably high rates of multiple sclerosis (MS) on the isolated Faeroe Islands in the North Atlantic. MS is nothing new to medicine, but it was new to the Faeroe Islands: There was no sign of it there before the 1940s. The epidemiologists found that the disease got its start during an outbreak coinciding with the arrival of British soldiers during World War II.

That an influx of visitors can trigger an outbreak is not unusual. But for it to trigger this specific disease was, because MS was generally thought to be a chronic condition brought on by genetic factors and perhaps a defective immune system.

But if MS is caused by a germ, what germ is it? That's a question that scientists have been asking about a growing list of chronic diseases that were once thought to be mainly a matter of genes or lifestyle. Since the 1970s, epidemiological clues have emerged for other diseases, and in some cases, scientists have been able to make a persuasive case for specific bugs.

In the 1980s, for example, researchers discovered that bacteria cause ulcers and that certain viruses trigger cancer. And in the past 2 decades, dozens of pathogens have been implicated in a range of diseases, from Alzheimer's to arthritis. Recently, some biologists have argued that evolutionary theory predicts that all but the rarest chronic diseases must be caused by infections.



Inspirational. The discovery that *H. pylori*—not acid—causes ulcers gave credence to the idea that pathogens lie behind many chronic diseases.

But despite many exciting hints, researchers are a long way from clinching the argument. Alzheimer's disease, MS, and schizophrenia offer three cautionary lessons. Every step of the research is fraught with controversy, from isolating the pathogens to determining how they might cause the disease to sorting out how a host's genetic profile influences the course of disease. And because these illnesses are chronic, scientists have to confront frustrating questions about cause and effect that don't come up with acute illnesses like Ebola or the mumps. Is the pathogen the cause of a particular disease or just a late-coming bystander? And what if two or more pathogens are implicated in the same chronic disease? Are both the cause, or neither? "There is a real chicken-and-egg problem here," says Stephen Reingold, vice president of research at the National Multiple Sclerosis Society.

A humbling lesson

For researchers who suspect that pathogens lie behind many chronic diseases, ulcers are the great inspiration. In 1981, a young Australian gastroenterologist named Barry Marshall learned of a mysterious bacterium lurking in the stomachs of patients. Over the next few years, he discovered that people suffering from ulcers often carried the microbe, which came to be known as *Helicobacter pylori*. Defying decades of conventional wisdom, Marshall speculated that the bacterium—and not acid or stress—might actually cause ulcers.

To test his idea, Marshall swallowed a broth full of *H. pylori*, and sure enough, he soon developed gastritis, the prelude to ulcers. Marshall cured himself with antibiotics, and subsequently, he and his coworkers successfully treated a number of people suffering from ulcers, clearly pinning the bacterium as the culprit. Other researchers have since shown that *H. pylori* infects perhaps one-third of all people, causing not only ulcers but gastric cancers as well.

"All of us have been humbled by the *Helicobacter* story," says Subramaniam Sriram of Vanderbilt University in Nashville, Tennessee. "It's made us look at infectious agents once again." Robert Yolken of John Hopkins University agrees: "The *Helicobacter* model is the big success story." But it was not the only one. At about the same time that Marshall was swigging *H. pylori*, other researchers were finding some of the first compelling evidence that cancers could also be triggered by viruses. Hepatitis B was associated with liver cancer, for example, while human papillomaviruses were linked to cervical cancer.

For other chronic diseases, however, the evidence is little more than circumstantial. Multiple sclerosis, for example, sometimes strikes its victims more like an epidemic than a genetic disorder, as it did in the Faeroes. Similarly, schizophrenia has signs of being triggered by infections during pregnancy. It is more likely to strike people born in cities than on farms and to strike people born in winter (when the flu and other diseases are common) than other times of the year.

Even without decisive evidence, some biologists argue that pathogens must cause most common chronic diseases. Foremost among these advocates is Paul Ewald, an evolutionary biologist at Amherst College in Massachusetts. Ewald suggests that people with chronic diseases ought to leave fewer children and grandchildren behind to propagate their genes than do healthy individuals. And so genetic disorders should gradually reduce themselves to minuscule levels. (The only exceptions would be disorders that are balanced by some benefit provided by the same genes, as in the case of sickle cell anemia, which is linked to protection from malaria.)

Purely genetic disorders, Ewald contends, can't cause more than about 1 death in 10,000. "That's the point at which you'd be able to barely maintain a genetic disease," he says. "When you get above that, you know that something must be maintaining it."

To Ewald, that something is most likely a

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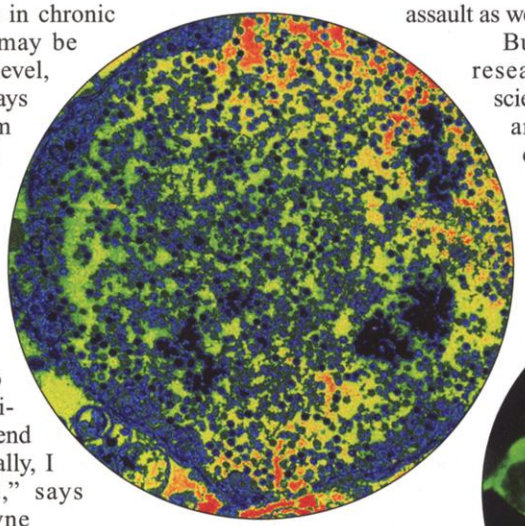
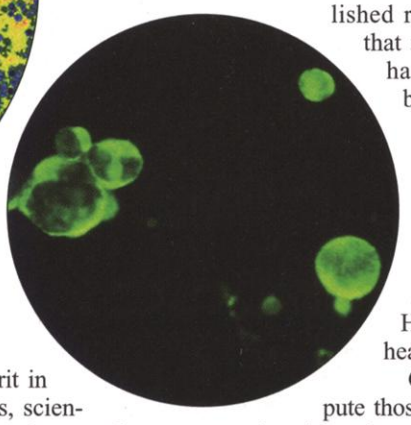
pathogen, because it can cause a chronic disease without paying this evolutionary penalty. As long as the pathogen can escape to new hosts before its own dies, it can continue to create sickness. Humans may evolve better defenses against a parasite, but the parasite can respond in kind, evolving new tricks for getting around them.

Ewald has captured public attention, with features on his work appearing in magazines like *Newsweek* and *Atlantic Monthly*. But he has had a mixed reception among specialists in chronic diseases. "Infection may be important at a broad level, but so is starvation," says Paul Ridker of Brigham and Women's Hospital in Boston. Although he finds the theory intellectually stimulating, Ridker argues that it is no substitute for detailed research. Perhaps not surprisingly, scientists who are investigating possible infectious causes tend to be positive. "Generally, I think Ewald's right," says Alan Hudson of Wayne State University in Detroit, Michigan. But even champions of pathogens like Barry Marshall, now at the University of Western Australia in Crawley, concede that "so far nothing looks as good as *H. pylori*—and most of the leads have been rather weak."





Ubiquitous. Herpes simplex 1 and 2 are widespread viruses that have been implicated in Alzheimer's and schizophrenia, respectively.

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Mysterious MS

Take MS, a disease in which immune system cells attack the insulating sheath of myelin that surrounds neurons in the brain and spine. As the disease progresses, its victims typically lose their muscle coordination, speech control, and eyesight. More than 300,000 people in the United States alone suffer from the disease—many more than evolutionary theory would predict if it were strictly a genetic disorder. Epidemiological studies also hint at a pathogen. People who migrate before age 15 from MS hot spots (such as Australia and Ukraine) to places with low rates are less likely to contract the disease than are those who stay behind. That decline is consistent with a scenario in which MS is caused by an infection that strikes in adolescence.

Experimental studies likewise hint that a pathogen could cause MS if its own proteins resembled myelin. Once the immune system became primed to attack the invader, it might inadvertently ravage the myelin as well. Indeed, in the July 2001 issue of the *Journal of Clinical Investigation*, a team of immunologists at Northwestern University Medical School in Chicago described creating symptoms resembling MS in a mouse by using an infectious agent. They injected the mouse with a normally harmless virus—but to which they had added a gene from a bacterium, *Haemophilus influenzae*, that makes a myelinlike protein. The mouse's immune system quickly became primed to attack the engineered virus; within 2 weeks its myelin was under assault as well.

But this sort of research enables scientists to create animal models of MS—not to identify the actual culprit in humans. Over the years, scientists have prowled for pathogens that could cause this sort of mimicry in association with MS, and they've found no shortage of candidates, 17 microorganisms in all. But today researchers are focusing the hunt for an MS agent on two ubiquitous pathogens: a virus and a bacterium that were both discovered just 15 years ago.

Human herpesvirus 6 (HHV-6) usually infects people when they are just a few months old, causing a sizable fraction of the fevers experienced by babies. After causing a brief illness in its host, the virus goes into hiding and may lie dormant for the rest of the host's life. But researchers have found that it sometimes becomes active again. Patients who receive bone marrow or organ transplants are often plagued by reactivated HHV-6, possibly because their immune systems are compromised.

Researchers studied this connection in 1995 at the Pathogenesis Corp. in Seattle, Washington (now part of Chiron), finding evidence of HHV-6 in the brains of several dozen people with MS. In these patients, the viruses were producing proteins, and they lurked close to the myelin of their hosts. In people who did not suffer from MS, the virus was there, but researchers found little evidence that it was active.

Since then, several groups have pursued this lead with distinctly mixed results. Donald Carrigan and Konstance Knox of the Institute for Viral Pathogenesis in Milwaukee, Wisconsin, published results last year showing that 56% of patients with MS had active HHV-6 in their brains, whereas healthy subjects had none. But several other teams have failed to find the virus in people with MS, while a paper this January in the *Journal of Medical Virology* found HHV-6 in one-third of healthy people's brains.

Carrigan and Knox dispute those negative findings, arguing that other researchers did not check carefully enough to see whether the virus was active or dormant in the brains. That hasn't been enough to sway many herpes experts, however. "I am frankly rather skeptical about the possible link between HHV-6 and MS," says Steven Dewhurst of the University of Rochester in New York. Just this May, he had more reason for doubt: At the annual meeting of the American Academy of Neurology, a Swedish team reported treating people with MS with valacyclovir, an antiviral medication. It produced no

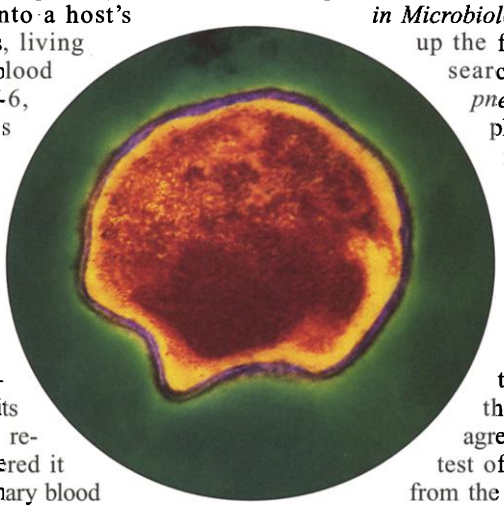
CONFLICTING EVIDENCE

Selected studies reporting the presence of Chlamydia pneumoniae *in brain tissue from people with multiple sclerosis and controls*

Lead author/Journal	MS	Control
S. Sriram *et al.*, *Ann. Neurol.* **46**, 6 (1999)	36/37 (97%)	5/27 (18%)
J. Boman *et al.*, *Neurology* **54**, 265 (2000)	0/48 (0%)	0/51 (0%)
M. R. Hammerschlag, *J. Clin. Microbiol.* **38**, 4274 (2000)	0/25 (0%)	0/16 (0%)
G. Layh-Schmitt *et al.*, *Ann. Neurol.* **47**, 652 (2000)	7/30 (23%)	0/56 (0%)
S. Morre *et al.*, *Ann. Neurol.* **48**, 399 (2000)	0/27 (0%)	0/36 (0%)
Pucci *et al.*, *Ann. Neurol.* **48**, 399 (2000)	0/29 (0%)	0/7 (0%)
J. Treib *et al.*, *Ann. Neurol.* **47**, 408 (2000)	8/22 (36%)	*no control*
T. Derfuss *et al.*, *Brain* **124**, 1325 (2001)	11/46 (24%)	3/61 (5%)

change in the symptoms.

Another suspect in MS is the bacterial scourge *Chlamydia pneumoniae* (see table). Its cousin, *C. trachomatis*, is notorious as a sexually transmitted disease and this year was implicated in cervical cancer. *C. pneumoniae* invades the lungs, where it sometimes causes respiratory diseases. It can then settle into a host's body for decades, living quietly in white blood cells. Like HHV-6, *C. pneumoniae* is practically universal. Just about everyone becomes its victim at some point.



Multifaceted. Some studies suggest that *C. pneumoniae* is involved in both MS and heart disease; others refute that.

C. pneumoniae was first suspected to play a role in heart disease. Shortly after its discovery in 1986, researchers encountered it lurking in the coronary blood vessels. Several teams also found that people with heart disease were more likely to have antibodies to *C. pneumoniae* than were healthy individuals. Later research has shown that the bacteria actually live in the lesions associated with atherosclerosis. In 1999, scientists reported that a protein made by *C. pneumoniae* closely resembles one found in heart muscle. As it tries to attack the bacteria, the immune system may attack the heart as well, creating the inflammation that may cause atherosclerosis (*Science*, 26 February 1999, p. 1335). Two clinical trials are now under way to see whether antibiotics can lessen further damage in people with heart disease. But many researchers still doubt that the connection is real. "The data are actually weaker than people think," says Ridker.

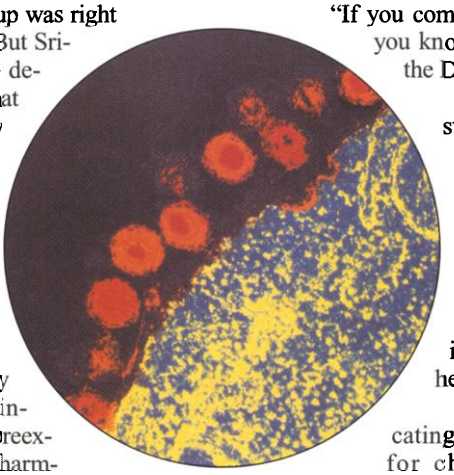
In 1998, Vanderbilt's Sriram reported that he had found *C. pneumoniae* in yet another part of the body: in the cerebrospinal fluid of a man with MS. Sriram subsequently looked at other people with MS and reported that genetic profiling revealed that 97% of them had the DNA of *C. pneumoniae* in the fluid, while only 18% of the controls did.

Sriram's results raised the possibility that *C. pneumoniae* could trick the immune system into attacking myelin just as it attacked heart tissue, or at least make a bad situation worse by aggravating the inflammation. Animal experiments suggest there might be something to this idea. In the August 2001 *Journal of Immunology*, Hudson of Wayne State and his colleagues reported that when they injected a protein from *C. pneumoniae* into the brains of rats, it produced remarkably MS-like symptoms. "His is a very seminal paper," says Sriram.

But when other researchers tried to confirm Sriram's results, many of them failed. Skepticism has been running high, and in April 2001, the title of a review in *Trends in Microbiology* bluntly summed up the feeling of many researchers: "*Chlamydia pneumoniae* and multiple sclerosis; no significant association."

Sriram disputes this finding, noting that other labs used different methods than his and might have missed the bacteria. To resolve the issue, he and the other researchers agreed to conduct a blind test of cerebrospinal fluid from the same set of MS patients and controls. Sriram found *Chlamydia* in 73% of the people with MS and 23% of those without. The other three labs found no evidence of *Chlamydia* at all.

Ewald is among Sriram's defenders, arguing that his methods are more sensitive than those of other labs. "A scientific response to the test would be, 'Well, it looks like the Vanderbilt group was right after all!' " he claims. But Sriram concedes that the debate is open: "I hope that physicians will view this as a debate that's ongoing and not an observation that's being finalized." If the association is real, he notes, it's possible that the bacteria only arrive in the brain after MS has already begun. "Having this infection on top of the preexisting damage may be harmful," he suggests. "The ultimate answer would be a successful clinical trial showing that when you eliminate the agent, you eliminate the disease." As a small step in that direction, Sriram is running a trial on MS patients with antibiotics.



Mind-boggling. Controversial evidence links a common herpesvirus, HHV-6, with schizophrenia.

Analyzing Alzheimer's

C. pneumoniae plays an equally controversial role in the debate over Alzheimer's disease. Theoretically, *Chlamydia* is a compelling candidate for a causal agent. In Alzheimer's patients, protein clumps appear in the brain and neurons become tangled; researchers suspect that inflammation is a key ingredient in this recipe. "One of the things *Chlamydia* does better than anything is elicit inflammation," says Hudson. "If they are in the brain, they are causing inflammation."

In 1998, Hudson and his colleagues reported genetic evidence of *C. pneumoniae* in the brains of 17 out of 19 Alzheimer's patients. Meanwhile, 18 out of 19 healthy people tested negative. When the researchers examined the diseased brain tissue, they found evidence of the bacteria in the very regions of the brain that had been damaged.

Once again, other labs tried to confirm Hudson's results. Two failed to find any bacteria, and a third obtained results that were ambiguous at best. "The interest in pursuing associations between *Chlamydia* and Alzheimer's has lost a great deal of steam," claims Robert Ring of Wyeth-Ayerst Neurosciences in Princeton, New Jersey, one of the researchers who failed to find a link.

Hudson, however, is suspicious of the methods used in the studies. In two out of three cases, the scientists tried to find *Chlamydia* in brains preserved in paraffin instead of fresh tissue. "It's pretty erratic getting stuff from paraffin-fixed samples," says Hudson. And he also maintains that the bacteria exist at low levels that can be missed if researchers don't run enough tests. "If you come up negative, how do you know you didn't just miss the DNA?" he asks.

New research bolsters his case. At an international *Chlamydia* meeting last August in Helsinki, two teams reported finding *Chlamydia* in fresh tissue of numerous Alzheimer brains and in almost none of the healthy brains.

One of the complicating factors in the search for chronic bugs is that many of the best candidates, like *Chlamydia* and HHV-6, are widespread. Far more people carry them than develop the diseases in question, which suggests that other factors, such as the genes of their hosts, must play a role. Ruth Itzhaki of the University of Manchester Institute of Science and Technology in the U.K. is exploring the pathogen-gene relationship in her work on Alzheimer's.

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Itzhaki has found preliminary evidence linking another herpesvirus, herpes simplex virus type 1 (HSV1), to Alzheimer's. As with HHV-6 and *C. pneumoniae*, most people become infected with HSV1 at some point. The virus lurks primarily in the nerves surrounding the mouth, and in 20% to 40% of its hosts, it causes occasional cold sores. Among young people, HSV1 is entirely absent from the brain, Itzhaki's team has found. But it is often present in the brains of elderly people. Itzhaki suspects that the virus sneaks into the brain as the immune system declines with age. Itzhaki's team has found that 63% of elderly people carry the virus, while 74% of elderly people with Alzheimer's do.

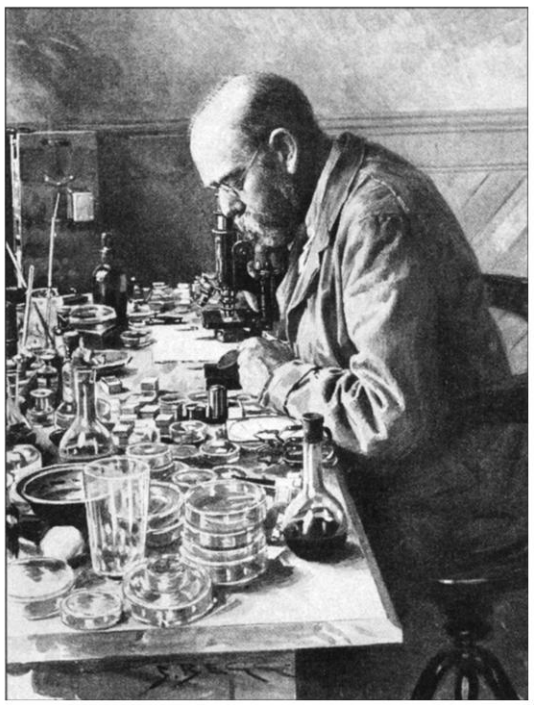
This small difference between the two groups might suggest that HSV1 is a minor risk factor for Alzheimer's. But the genetic evidence suggests otherwise, says Itzhaki. Those of her subjects who carried a gene variant called *ApoE4*, a known risk factor for Alzheimer's, as well as the herpesvirus, were much more likely to have Alzheimer's than were people with either the gene or the virus alone (*Science*, 15 May 1998, p. 1002). She concluded that the combined risk accounts for the disease in 60% of the 61 cases her team has examined.

Itzhaki speculates that people with *ApoE4* may not be able to repair cell damage caused as the virus triggers inflammation in the brain. Stopping the virus from getting into the brain might be one way to fight the disease. "Vaccines against HSV1 might prevent Alzheimer's, at least in some cases," says Itzhaki. But so far, no one has tested that proposition. Still, Itzhaki has earned some admirers. "I think there's significant merit in her work," says Keith Crutcher of the University of Cincinnati. "This type of work is notoriously difficult, and I think her studies have been carefully conducted."

Genetic interplay

In some diseases, the line between pathogens and the genes of their hosts may be nearly indistinguishable. Certain kinds of viruses, known as endogenous retroviruses, paste their DNA into their host cells. If one of them should infect a cell destined to become a sperm or egg, the virus will infect every cell in the body of the person it gives rise to. It will also be handed down to subsequent generations. Endogenous retroviruses make up an estimated 1% of the human genome, but most of their sequences have mutated into harmless nonsense. Still, some endogenous retroviruses may be able to come back to life (often during fetal development), harnessing their host's genes to make new viruses that can invade new cells.

Hopkins's Yolken and his colleagues have been exploring whether reawakened retroviruses might somehow be involved in schizophrenia, as some are known to cause brain damage. They searched for retroviral DNA in the cerebrospinal fluid of 35 people who had recently developed schizophrenia. As they reported in the 10 April *Proceedings of the National Academy of Sciences*, genetic material from one type of retrovirus, HERV-W, turned up in 29% of schizophrenics, whereas none was found in the cerebrospinal fluid of healthy people or even people with other neurological disorders. One hypothesis that Yolken and his colleagues are now pursuing is that these retroviruses are unleashed in certain individuals before they are born, altering the development of their brains in ways that don't become clear until adulthood.



Gold standard. Some researchers argue that Robert Koch's postulates, articulated in 1882, are too rigorous for determining the infectious basis of chronic diseases.

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In a report to appear in the November *Archives of General Psychiatry*, Yolken and his colleagues report on a potential trigger for these retroviruses. The researchers sifted through the records of a study known as the Collaborative Perinatal Project, in which thousands of pregnancies were monitored between 1959 and 1966. During the project, blood samples were taken from the mothers, and the health of their children was followed for 7 years. The group tracked down 27 subjects who had developed schizophrenia and other psychotic illnesses as adults. They revisited their mothers' blood samples, measuring levels of antibodies to various pathogens. They then measured the same antibodies from mothers of healthy subjects, using two controls for each psychotic subject who were born during the same time of the year and were of the same race and gender.

For five out of six pathogens, the researchers found no significant association with psychosis. But one did pass the test: HSV2—the sexually transmitted form of HSV, which causes genital sores. Women with signs of infection with the virus when they were pregnant were more likely to give birth to children who would later develop schizophrenia and other forms of psychosis.

Yolken points out that HSV2 is a compelling candidate for triggering schizophrenia—and a treatable one at that: "We know they're capable of activating retroviruses, we know they're capable of replicating in the brain, and we know that there are treatments that are available."

Yolken concedes that even with a study that spans 40 years in molecular detail, he is far from proving that a particular pathogen causes schizophrenia. As with other chronic diseases, it defies the classic standards for recognizing infectious diseases articulated by Robert Koch in 1882: showing that the pathogen is present in all victims suffering from a specific disease but not in healthy people, for example, and that an isolated pathogen can cause the same disease in a new host. "We've solved the easy problems"—identifying the agents that cause many acute infectious diseases—says Ewald. He argues that for uncovering the pathogens that may lie at the root of chronic diseases, Koch's postulates should not be the guiding factor. "We're just not going to get the kind of evidence for causation as we do for acute infections. There's just no way. If you're dealing with a disease where the symptoms take 5 decades to develop, how are you going to get an animal model of that?"

But Yolken and other researchers trying to show a link generally believe that they have to come as close to the classical methods as possible if they are to convince their medical colleagues. Says Yolken: "In this day and age, when we have good treatments, you have to show that when you remove the agent, you get a change in the disease to have people to believe it."

—CARL ZIMMER

Carl Zimmer is the author of *Evolution: Triumph of an Idea.*