On the Track of an Elusive Disease

Chronic Fatigue Syndrome has been shown to have distinctive clinical features, but so far no causative agent—if there is one—has been identified

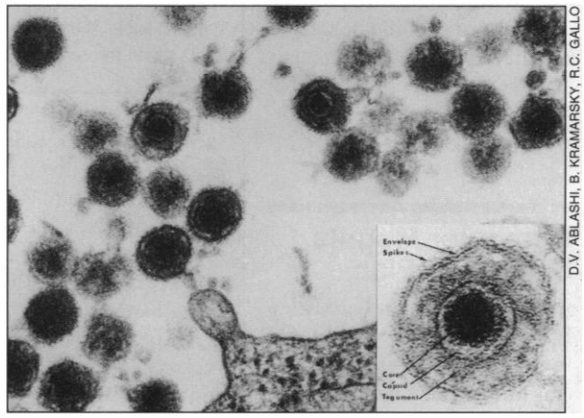
WHEN REPORTS FIRST STARTED COMING IN A few years ago of a strange flu-like malady that struck victims in the prime of their lives, leaving them with chronic, debilitating fatigue, few researchers were willing to pay those reports much attention. The malady was dismissed as the "yuppie flu"—a 1980s version of an old disease called neuromyasthenia—and was widely regarded as merely a psychosomatic disorder that belonged in the realm of psychiatrists, not internists. But in recent months, the disorder, dubbed Chronic Fatigue Syndrome (CFS), has begun to get some scientific respect.

Congress, urged on by effective lobbying from patient-advocate groups, has encouraged the federal government to enhance its spending on CFS considerably—from next to nothing 3 years ago to a little more than $6 million in the coming year—and now the investment is starting to pay off. A small but growing group of virologists, immunologists, and neuroendocrinologists has begun to put together a picture of CFS that seems to set it apart from other psychiatric and medical disorders that share some of the same symptoms, and a handful of groups around the world are feverishly trying to track down the cause of the syndrome. So far, however, they have turned up little more than promising leads.

The Centers for Disease Control (CDC) is also taking the disease seriously. Two years ago it set up four surveillance sites—in Atlanta, Reno, Grand Rapids, and Wichita—to gather data on the syndrome, and the agency now estimates that there are at least 100,000 cases of CFS in the United States. CDC will soon start a national prevalence survey with part of the $2.8 million that Congress put in the agency's budget for 1992.

Walter J. Gunn, who heads CFS program activities at CDC in Atlanta, says research on the syndrome is at a stage similar to the one that AIDS research was at a decade ago. Scientists were then faced with a complex set of medical problems that were related in some unknown way. Until HIV was discovered, no one knew which of the various medical abnormalities was the cause of AIDS, and which were merely opportunistic hangers-on. While no one is suggesting that CFS will turn out to be the public health problem that AIDS has become, Gunn expects that this peculiar syndrome, like AIDS, will present an irresistible challenge that will draw more and more talented researchers into the field. "Success breeds success," says Stephen E. Straus, chief of the laboratory of clinical investigation at the National Institute of Allergy and Infectious Diseases (NIAID). "When one shows that even an amorphous syndrome like this one is amenable to scrutiny—that you can generate reasonable data out of it—other good people start feeling it's worthwhile to venture into the field."

Many researchers nevertheless remain skeptical that the syndrome is anything more than just a neurotic complaint of middle-aged white women, the group most frequently diagnosed with CFS. "If you took a poll today you would still probably find many practicing physicians and many scientists saying 'it hasn't been proved to me that there is a definitive disease with a definitive objective abnormality that defines the disease,'" says Anthony Komaroff, a general internist and clinical epidemiologist at Brigham and Women's Hospital in Boston. Indeed, in the end it may turn out that the medical and psychological components of the disease are so intertwined that they may never be fully teased apart. But Komaroff, who has just been awarded one of three grants from NIAID to establish CFS Cooperative Research Centers, agrees with Straus that that attitude is changing. "The evidence for objective abnormalities is growing stronger."



D.V. ABLASHI, B. KRAMARSKY, R.C. GALLO

Piecing together the CFS jigsaw

The most compelling evidence comes from recent studies of the symptoms of CFS. "The pattern that is emerging is a chronically activated immune system, an immune system engaged in some kind of chronic war against some kind of thing that it perceives as foreign," says Komaroff. There have been reports that natural killer cells and macrophages, two of the body's front-line defenses against invading organisms, are reduced in either number or function in CFS patients. In addition, a subset of white blood cells called CD8 cells are activated, consistent with an immune response to a viral infection. Komaroff and others have begun to see a pattern of neurologic changes that seem to accompany other CFS symptoms.

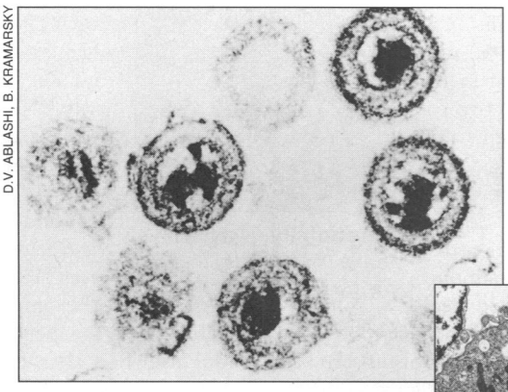
The latest published results also indicate that CFS patients have altered levels of certain brain hormones, compared with control subjects. NIAID's Straus says he and colleagues at the National Institute of Mental Health and the University of Michigan decided to look at the hypothalamic-pituitary-adrenal (HPA) axis based on findings that that axis was altered in patients with major clinical depression. If CFS patients showed a similar alteration, it would add weight to the argument that CFS was merely an elaboration of a psychiatric disorder.

After nearly 4 years of gathering data, researchers from the three institutions published a paper earlier this month in the *Journal of Endocrinology and Metabolism* confirming that the HPA-axis in CFS was indeed altered, but not the way researchers expected. Although the differences from normal controls were modest, CFS patients consistently showed decreased levels of the steroid hormone cortisol, and increased levels of the pituitary hormone ACTH (adrenocorticotropin hormone), exactly the opposite of the changes seen with depression. This finding, says Straus, "provides an alternative explanation for some of the immune findings in the syndrome. Instead of presuming that there's

a chronic infection which is driving some of the abnormalities, there may simply be a lack of steroid restraint on the immune system which lets the immune system run at an abnormal level unchecked." Straus says the next step will be to look at other neuroendocrine pathways in CFS to see whether these are altered as well.

No smoking gun

But while the symptoms of CFS are becoming clearer, the cause remains murky. For the past year, virologists at CDC and elsewhere have been focusing on work by Elaine DeFreitas of the Wistar Institute in Philadelphia, who found evidence that a human retrovirus similar to HTLV-II had infected CFS patients but not a group of healthy control subjects (see *Science*, 14 September, 1990). Patient advocate groups seized on DeFreitas' work as the ultimate proof that CFS was not a psychiatric illness but one with a known cause. DeFreitas' results were impressive enough to prompt CDC to try to replicate the work, and to ask several other labs to do likewise. But today, despite a year of efforts, no one can report success. "We haven't yet been able to confirm her findings," says CDC's Gunn. "But we haven't refuted her findings either."



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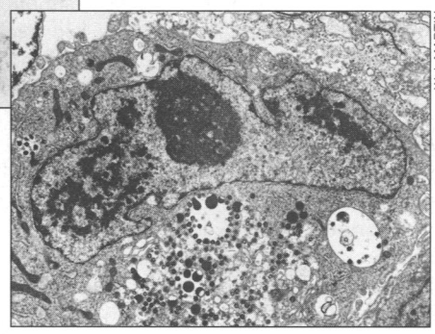

W.J. MARTIN

Onetime suspects. *Human herpes virus-6* (far left) *and Epstein-Barr virus* (left) *have been cleared; a spumavirus* (below) *is still under active investigation.*

Thomas Folks, a retrovirologist at CDC, says the problem with replicating DeFreitas' work is that it is based on polymerase chain reaction (PCR), amplification of tiny amounts of DNA in cells. PCR can be a tricky technique in the best of circumstances, and in this case, the DNA sequences being sought are very similar to sequences found in normal, uninfected cells. So if you increase the sensitivity of the assay to look for what is certain to be a rare viral sequence, you lose specificity and run the risk of a false positive result. "You're working at the hairy edge of what's possible in PCR," says Gunn. But, he adds, "there is every likelihood that [DeFreitas' finding] will be confirmed eventually."

Some virologists think Gunn is overly optimistic, however. "There are a lot of people who are frustrated" trying to confirm DeFreitas, says Jay A. Levy, a virologist at the University of California at San Francisco. So far, Levy himself has drawn a blank, but he says he's just received some new reagents from her and will try again. Peter O. Behan, professor of clinical neurology at the University of Glasgow in Scotland, also tried to replicate DeFreitas' work without success. He now thinks she is seeing a naturally occurring sequence. Despite repeated attempts, *Science* could not reach DeFreitas for comment.

If DeFreitas' virus falls by the research wayside, it wouldn't be the first to meet that fate in the search for a cause of CFS. Five years ago, Epstein-Barr virus (EBV), one of a class of human herpes viruses, looked like a good candidate, since most if not all patients initially diagnosed with the syndrome had EBV antibodies (see *Science*, 31 October 1986, p. 541). Indeed, CFS was once called chronic EBV infection. But subsequent groups of patients failed to show evidence of EBV infection, and most researchers ruled out the virus. Another herpes virus, HHV-6, also enjoyed a brief spell in the limelight as the etiologic agent of CFS, but it turns out to be nearly equally prevalent in healthy controls as it is in CFS patients. In one recent study by Levy and his colleagues (*Lancet*, 21 September, p. 707), all 63 CFS patients had detectable antibodies to HHV-6, but so did 39 of 40 healthy controls. In addition to HHV-6, Levy looked for any substantial differences between CFS patients and controls in the production of antibodies to a panoply of viruses, including cytomegalovirus, EBV, rubeola, adenovirus, Coxsackie B4, and Papovavirus BK. He found none. Significantly, neither group showed any evidence of exposure to the three known classes of human retrovirus, including HTLV-I/HTLV-II, HIV-1/HIV-2 and human spumavirus—a type of retrovirus also known as a foamy virus because of the "foamy" appearance of the cytoplasm of infected cells.

The negative results on spumavirus present a particular puzzle, given recent research by W. John Martin, a pathologist and virologist at the University of Southern California. Martin is claiming that he has recently cultured a spumavirus from more than 200 CFS patients, and that he has obtained some sequence data. These claims are being treated with some skepticism, however.

Martin first pointed the finger at spumaviruses in February 1990 when he found viral particles in the cerebrospinal fluid of a woman who was suffering from speech and motor problems as well as extreme fatigue. Electron microscopy pictures convinced him that he was seeing a spumavirus. But when Martin presented these electron micrographs at a meeting sponsored by the National Chronic Fatigue Syndrome Advisory Council held at the CDC in Atlanta this September, those present were underwhelmed. Levy said in an interview following the meeting that he couldn't make out what they showed. Komaroff agreed that the data Martin presented in Atlanta were inconclusive. And one virologist, who was asked to review a paper Martin submitted for publication, told *Science* that "most of what he showed was negative or uninterpretable."

For his part, Martin insists he was confronted by a hostile, unreceptive audience at the CDC, whose members were unwilling to listen to new ideas. "I would have expected some courtesy," he said in a telephone interview. Many researchers contacted by *Science* said they were upset that Martin has taken his research results to the news media before publishing it in the scientific literature. News of Martin's spumavirus has appeared in *Newsweek*, *The New York Times*, and *The Wall Street Journal*, but not in any refereed journal.

Since that meeting, Martin has been busy culturing the virus from CFS patients, and he says he's about to submit his new results to a peer-reviewed journal. He's still facing an unsympathetic audience, however: "I've seen every shred of data he's willing to share," says Komaroff, and he is unconvinced that a spumavirus is involved. "We looked for the only currently known human spumavirus 3 years ago and found absolutely no evidence of that," he says. But even Komaroff isn't yet ready to shut the door completely on these rare retroviruses. "If there were a new spumavirus, we could have missed that," he admits.

Komaroff isn't bothered by the failure so far to find the smoking gun causing CFS. "My view of the illness, which I suspect is probably the prevalent view among scien-

tists and clinicians studying it, is that it's very unlikely that there is a single etiologic agent," he says. "It's a syndrome that probably has multiple triggers." But CDC's Gunn doesn't agree: "I have trouble buying this thing about multiple causes, because when you look for them they're not there." Gunn believes that researchers will ultimately find a single, major causative factor, and he would not be surprised if it turned out to be a retrovirus, since that class of virus has already been shown to attack both the immune system and the brain.

Skepticism persists

The failure to nail down the cause of CFS is, however, fueling the skepticism of researchers who argue that it would be more profitable to focus on the psyche than the immune system of CFS patients. "I don't think that there is any solid, reliable, reproducible evidence that patients with chronic fatigue syndrome are in any way immunologically, virologically, or biochemically different from any other patients with a chief complaint of chronic fatigue," says Peter Manu, an internist at the University of Connecticut. Manu says that at the time they are diagnosed with the syndrome, at least three-quarters of all CFS patients have a psychiatric disorder that is a sufficient explanation for their symptoms. "I am not saying that the psychiatric disorder that all of us have noted in these people is the sole cause of Chronic Fatigue Syndrome or of chronic fatigue," he says. "But it is the only diagnosable one."

Manu goes on to argue that the search for a single viral agent is pointless. "I do not think there's any reason for going for such an arcane etiology," he says. "I cannot see any infection that will predominantly affect middle-aged, college-educated white women. It just doesn't make any epidemiologic sense to me."

While Gunn admits that the majority of patients the CDC is seeing are white women, he says the experience in other countries—notably Australia—suggests that CFS is equally distributed between men and women. As for the suggestion that a psychiatric disorder explains most CFS symptoms, Gunn says that at least half of the patients referred to CDC neither have a diagnosable psychiatric disorder at the time they are diagnosed with CFS, nor do they subsequently develop one.

There will clearly be plenty of room for doubts about the nature of CFS unless a causative agent is identified—just as there was in the early days of AIDS. But even if a CFS virus is found, there will likely be many questions left unresolved. Like AIDS, it could be years before all the pieces of the CFS puzzle fall into place. ■ JOSEPH PALCA

A "Jumping Gene" Caught in the Act

Researchers have identified a transposon before and after it jumped; they now have a working transposon to study

WHEN GENETICIST HAIG KAZAZIAN and his colleagues at Johns Hopkins University School of Medicine analyzed the factor VIII genes of a large number of hemophilic children in the late 1980s, they got a surprise. Sitting in the middle of the gene in two patients, and disrupting its ability to produce the blood-clotting factor VIII was a stretch of foreign DNA that looked suspiciously like a transposon—a so-called jumping gene. Transposons had been widely observed to jump from place to place in the genomes of lower organisms, but nobody had witnessed one moving around in a mammalian genome before.

Serendipity. *Haig Kazazian wasn't strictly looking for transposons.*

Now, beginning on page 1805 of this issue of *Science*, Kazazian, director of the Center for Medical Genetics, and his collaborators report compelling evidence that they have indeed caught a jumping gene in the act. They have found where the transposon disrupting the factor VIII gene of one of the patients came from. The progenitor appears to be a type of transposon known as a LINE on chromosome 22 of the child's parents. "This is the first demonstration that transposable elements can cause human genetic disease," says yeast geneticist Jef Boeke, one of Kazazian's collaborators. And that's not all. A second Johns Hopkins team working in collaboration with Kazazian, headed by molecular biologist Abram Gabriel, reports that the transposon contains a functioning copy of a gene that codes for reverse transcriptase—an enzyme that plays a key role in the process of transposition.

Researchers have long been anxious to get their hands on an active human transposon, with all its protein coding sequences intact, in order to understand how these strange genetic elements work. There are reckoned to be 100,000 LINEs scattered around the human genome, though all but about 4000 have massive deletion mutations and most of the full-length ones appear to be inactivated by point mutations. Nobody knows how they got into the genome in the first place—though some have speculated that they are remnants of retroviruses—how they move about, how frequently they jump, how often they cause disease, and how they may have shaped evolution. "They are obviously shaping our genomes in some way," says Kazazian. "But are they useful?"

Kazazian's discovery comes upon the heels of reports published in *Nature* (31 October) and the *Proceedings of the National Academy of Sciences* (15 December) that another mysterious molecular inhabitant of the genome, called an *Alu* sequence, had disrupted a neurofibromatosis type 1 gene and a cholinesterase gene. Like LINEs, *Alu* sequences are known to move about in the genome, but they lack the machinery to do it by themselves. One possibility, strengthened by the Hopkins work, is that the reverse transcriptase coded by LINEs may help *Alu* sequences relocate in the genome.

By identifying a LINE before and after it jumped, Kazazian's research may help shed some light on these questions. Ironically, however, he had not been searching for transposons. He had wanted to find out what causes new mutations. The factor VIII gene was a logical place to search, because it resides on the X chromosome. Males have only one copy of the X chromosome, which means that male carriers of defective factor VIII genes are inevitably hemophiliac. In the past, few became fathers. Therefore, compared with, say, mutant hemoglobin genes, defective factor VIII genes are less frequently handed down over generations like some cursed heirloom. About one-third of cases of hemophilia A are caused by fresh mutations, says Kazazian.

Once they had identified the cause of the mutation as a LINE insertion, Kazazian's group then set out to find its source. The fact that they were able to distinguish the progenitor from the other 100,000 LINEs in the genome is no mean feat. Kazazian's colleague geneticist Alan Scott had developed a "consensus sequence" for the human LINE by comparing the sequences of 20