Tracking Variation in the AIDS Virus Family

Last month researchers gathered at the Bethesda campus of the National Institutes of Health for a "Conference on Genetic Variation of Immunodeficiency Viruses." Presentations at the meeting showed that these viruses, which include the virus that causes AIDS (acquired immunodeficiency syndrome), exhibit an impressive range of genetic and biological variation. Some conference highlights are reported here. A fuller discussion of AIDS virus variability and what it means will appear in a future Research News report.*

Possible Third Member of AIDS Virus Family Found

First there was the AIDS virus, known now as human immunodeficiency virus 1 (HIV-1). Then came HIV-2, which also causes an AIDS-like disease, although there is still some controversy about whether it is as virulent as HIV-1. Now, according to Guido van der Groen of the Institute of Tropical Medicine in Antwerp, a new HIV variant—possibly an HIV-3—has been found.

Still to be determined is how widespread the putative new virus is and whether it is a significant cause of disease. So far it has been isolated from just two individuals, a married couple, from Cameroon on the West Coast of Africa. The man does not have full-blown AIDS, but has the symptoms of AIDS-related complex, including swollen lymph nodes and chronic diarrhea. His wife, who is pregnant, is currently well. At the very least, however, the discovery of an HIV-3 would mean that that the virus family is larger and even more varied than previously thought.

Van der Groen has been studying the virus isolated from the Cameroonian couple in collaboration with researchers at Innogenetics N.V., a biotechnology company in Antwerp. The investigators soon learned that it has unusual properties. Antibody studies indicated, for example, that the virus is distinct from both HIV-1 and HIV-2. Moreover, the molecular weights of some of its proteins differed slightly from those of the corresponding proteins of the other two HIVs. "These data essentially point out that this virus is different from the existing isolates we already know," van der Groen says.

To confirm that they have isolated a new member of the HIV family, the researchers must show that the nucleotide sequence of the genome of the candidate HIV-3 is significantly different from those of the HIV-1 and HIV-2 genomes. That work is under way at Innogenetics, where a partial sequence has been determined. Van der Groen says, however, that the terms of his working agreement with Innogenetics prohibit him from releasing the sequence data at this time because the company plans to patent the virus. A patent could be valuable if, for example, the viral components ever prove useful for diagnostic purposes or for making a vaccine to protect against AIDS.

Van der Groen was able to tell the meeting participants that the sequence determined so far, which is of the long terminal repeat (LTR) on the 3′ (right-hand) end of the viral genome, differs in about 30% of its bases from the HIV-1 LTR and in more than 50% of its bases from that of HIV-2. During the discussion after van der Groen's talk, Gerald Myers of Los Alamos National Laboratories, who has been tracking genetic variation in the HIVs, noted that the nucleotide sequences of the HIV-1 LTRs differ by about 15%. So finding that 30% difference provides some confirmation that a new HIV strain may have been found.

And yes, it does bother van der Groen that commercial restraints kept him from revealing as much of the data as he would have liked. Situations of this type bother other observers as well, but more such episodes can be no doubt be expected.

A Nonpathogenic AIDS Virus Variant?

In another development at the conference, Simon Wain-Hobson of the Pasteur Institute in Paris described a new virus isolate that may be a nonpathogenic, or at least less virulent, form of the AIDS virus, HIV-1. Having an avirulent HIV-1 variant would be a boon to AIDS researchers because it would help them to pinpoint the precise molecular features that cause the AIDS virus to produce such a devastating illness in the people it infects. Then it might be possible to design more specific drugs to combat the virus's effects.

An avirulent HIV-1 would not, however, be useful for vaccine development, Wain-Hobson says, because of the possibility that it could mutate and become pathogenic. "It can help you understand the disease, but it won't help make a vaccine," he points out.

Work by Eric Delaporte and George Roelants at the Center for International Medical Research in Franceville, Gabon, originally raised suspicions that people in that country might be harboring an unusual HIV-1 variant. The Franceville workers had found that 2 to 3% of the persons they surveyed tested positive for the AIDS virus when screened by the ELISA method, which detects antibodies to the virus. However, follow-up studies with the more specific Western blot test showed that only about one-third of these individuals had antibodies to all the viral proteins. The other two-thirds had antibodies to just the viral polymerase and the glycoprotein of the viral core. These people did not have symptoms of AIDS.

Attempts to isolate an HIV from the individuals with the atypical Western blots turned up an unusual variant that grows poorly in culture, compared to HIV-1, and does not have the cell-killing effects of the typical AIDS virus. At that point, Wain-Hobson cloned the unusual variant and compared it molecularly to HIV-1. At first examination, they appeared to be indistinguishable. "Here we have a virus that grows poorly yet seems to have all the properties of an HIV-1," Wain-Hobson says.

After more detailed study, Wain-Hobson detected an interesting change in the *tat* gene of the new variant. A codon for the amino acid cysteine had mutated to encode serine. The mutation abolished the function of the *tat* product, which is to stimulate the expression of the genes encoding the viral proteins. Loss of the *tat* function might account for the poor growth of the Gabon virus.

Moreover, Wain-Hobson could show that replacing the serine codon with one for cysteine could restore the *tat* activity of the Gabon virus. Conversely, changing the *tat* cysteine to a serine in a standard HIV-1 strain destroys its *tat* activity. "A single point mutation in the *tat* gene wipes it out in the assay we use," Wain-Hobson says. "How does this virus grow?" One possibility is that a "helper" virus can supply the missing *tat* function for the HIV-1 variant.

However the HIV-1 variant grows, it may be fairly widespread, Wain-Hobson says. There are indications of its presence in Cameroon, the Congo, and the Central African Republic, as well as in Gabon. The Pasteur researcher and his colleagues want to follow up on the current work, to see,

*The conference was sponsored by the AIDS Program of the National Institute of Allergy and Infectious Diseases and held on 19 and 20 July.

among other things, whether the virus is as nonpathogenic as it currently appears.

How the AIDS Virus May Hide in Macrophages

One of the major puzzles about the AIDS virus concerns the way in which it manages to hide in the body, often escaping destruction by the immune system for many years before it causes disease. Howard Gendelman of the Walter Reed Army Institute of Research in Washington, D.C., presented data that may help explain how it does this.

Within the past year or so, the immune cells known as macrophages or monocytes have come to the forefront as the likely first cell type to be infected by the AIDS virus and also the probable reservoir of the virus in the body. Recently, Gendelman and his colleagues have learned how to maintain macrophages in culture for long periods.

While using these cultures to isolate HIV-1 from AIDS patients, they noted a paradox. Although 60 to 90% of the cultured macrophages that became infected with the AIDS virus make viral messenger RNAs, which indicates that the virus replicates in the cells, little virus was present in the culture fluids.

Electron microscopic studies then revealed, Gendelman says, that "the virus was budding and accumulating into cytoplasmic vacuoles instead of budding from the cell surface." In particular, the virus was accumulating in vacuoles associated with the Golgi apparatus, an internal system of membranous disks in which many cell proteins mature and are sorted for transport to their final destinations in or out of the cell. The low virus concentrations in the culture fluid suggests that the macrophages secrete some of the virus particles to the exterior, although most apparently remain inside.

The presence of the particles in the cytoplasmic vacuoles does not appear to have adverse effects on the macrophages. "At least morphologically and by simple criteria they look normal," Gendelman remarks. The Walter Reed workers have also found that macrophages in brain tissue from an AIDS patient contain internal accumulations of virus particles.

What these observations might mean for escape of the AIDS virus from immune surveillance and progression of the disease is not yet clear, Gendelman cautions. They nonetheless suggest that the sequestering of HIV-1 particles inside macrophages, where they are presumably safe from immune attack, allows the cells to serve as "Trojan horses" for maintaining and transmitting the AIDS virus in the body. ■ JEAN L. MARX

Bringing Chinese Dragons to the Western World

A big exhibit of Chinese fossils has early mammaliaforms, death assemblages, and film star Michael Douglas explaining cladistics

New York City

THE LARGEST EXHIBITION of Chinese fossil bones ever displayed in the West represents the reawakening of paleontology in the People's Republic of China, as well as providing a reminder of its troubled past. "The Chinese are proving to us that not only can they unearth all these weird-looking creatures, but they can make sense of them, too," says Eugene Gaffney of the American Museum of Natural History in New York, which is putting on the show with colleagues from the Institute of Vertebrate Paleontology and Paleoanthropology in Beijing.

"From the Land of Dragons" displays 42 specimens from China, which offer a glimpse of the richness of the ancient vertebrate presence in Asia and a hint of what is still left to uncover. Discoveries made in China in recent years, which include dinosaurs and early relatives of mammals, rival specimens collected in North America at the turn of the century, when many of the first complete fossils were excavated and many of the shelves in the American Museum were filled.

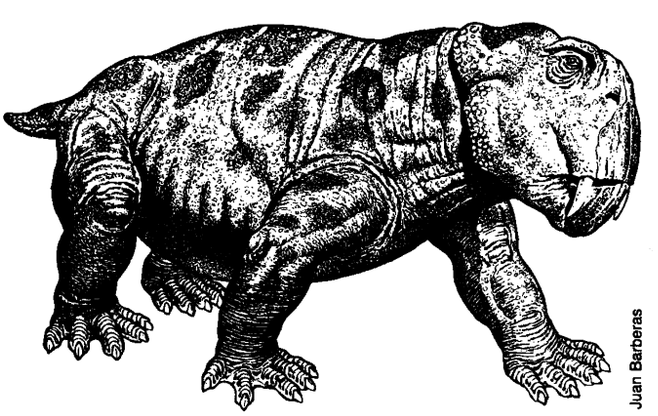
Says James Hopson of the University of Chicago: "The Chinese are finding some terrific stuff." Hopson says that China is exciting to paleontologists because, like North America, it possesses a very diverse and complex geology in which rock from many time periods is exposed.

The exhibition of old bones is remarkable not only for the presence of the Chinese fossils, but because the exhibit takes some risks. It is perhaps the first in this country to try to give museum visitors a real sense of how modern paleontologists use a system called "cladistics" to classify relationships among animals by comparing primitive or derived "characters"—be the characters backbones, jaw hinges, hip sockets, or holes in the head. For example, dinosaurs and birds are more closely related than horses and birds because dinosaurs and birds share a unique kind of hip bone.

Says Gaffney: "The real excitement of paleontology is putting together the puzzle. We wanted to show people that science can make some kind of sense of all these bones we dig up."

These are surely noble goals, but whether loyal patrons of the American Museum, which include armies of screaming, dinosaur-crazed school children, will understand what cladistics and "groups within groups" is all about is uncertain. At times, the going can get rough. But Gaffney and exhibit coordinator Lowell Dingus and designer Willard Whitson certainly give it the old college try. To liven up the show, they even managed to rope film actor Michael Douglas into the project. Accompanied by music from a Mongolian yurt hut, Douglas narrates a snappy 10-minute video, which plays over and over in a corner of the exhibition hall and explains the tenets of cladism and attempts to place the Chinese fossils in an evolutionary context.

Upon entering the hall, the visitor is greeted by a blinking diagram that resembles a family tree but is actually a thinly disguised "cladogram" that asks: "Why



Juan Barberas

Lystrosaurus. *Early relatives of mammals, dicynodonts roamed the supercontinent Pangaea for 60 million years before the dinosaurs took over. The two tusks were the only teeth in their head. They also had a beak.*