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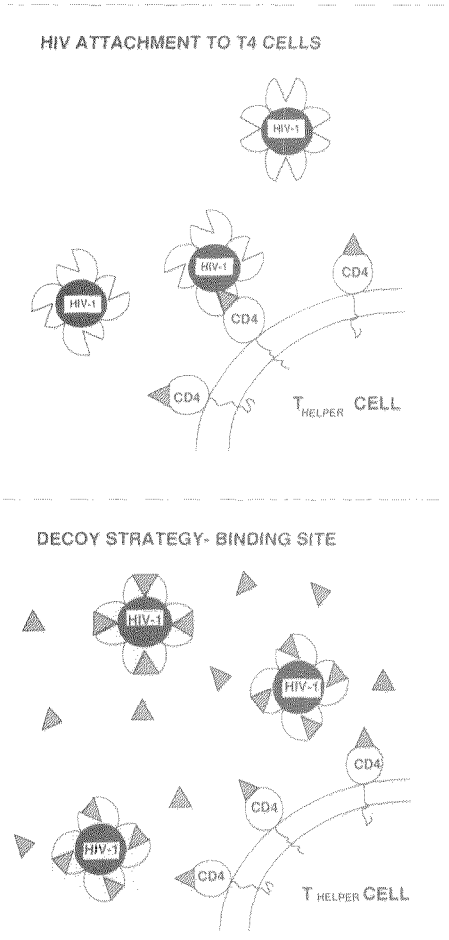


Top: HIV-1 virus infecting a T-cell. The virus recognizes and binds to a specific site (shaded triangle) on the CD4 protein found on the cell's surface. Bottom: A flood of synthetic binding sites overwhelms the virus, tying up all its recognition sites.

A Handle on AIDS

Caltech biologists have found the handle an AIDS (HIV-1) virus must grab in order to infect a cell. The handle, or binding site, is located in a protein on the cell's outside surface. The researchers also created a synthetic version of that binding site which, in a test tube, prevents the virus from infecting cells.

Bradford A. Jameson, research fellow in biology, and Stephen B.H. Kent, senior research associate in biology and group leader for the project, did the work in collaboration with researchers at ORTHO Pharmaceuticals and the University of Alabama. The scientists reported their research in the June 3, 1988, issue of *Science*.

"We do not want to give people false hope. This is not a cure for AIDS," said Kent. "Nevertheless, we believe this to be a significant advance not only in AIDS research but in virology in general. This is the first time that the binding site for *any* virus has been identified with this degree of precision. At best it is one possible step on one possible road that may possibly lead to an AIDS treatment."

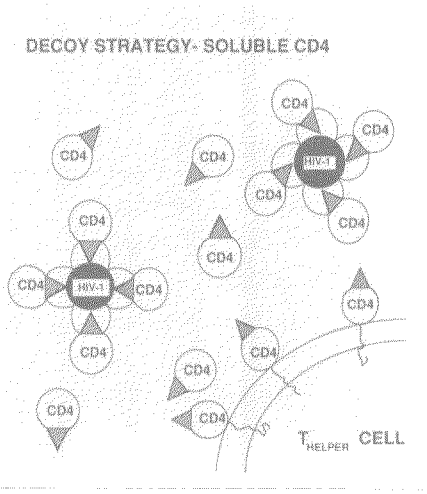
The HIV-1 virus must bind to a susceptible cell in order to infect it. Once infected, a cell may fuse with uninfected cells, killing them. Cell fusion depends on the same binding site that the virus uses to infect the cell in the first place. If researchers could introduce large quantities of a protein fragment containing the binding site into the bloodstream, the fragment might act as a decoy—viruses and infected cells could bind to it instead of infecting other cells. Such a fragment, if linked to a virus-killing drug, could also carry the drug directly to the AIDS virus.

The protein containing the binding site is called CD4, and it appears on the surface of helper T-cells—a type of white blood cell vital to the body's immune system. Scientists know the sequence in which amino acids are strung together to form CD4, so the Caltech researchers synthesized 10 overlapping segments of the molecule, each about 30 amino acids long. ORTHO Pharmaceuticals then provided an assortment of antibodies, each of which binds to a different region of the intact CD4 protein. Some of these antibodies were known to prevent HIV-1 from infecting suceptible cells, presumably by blocking the binding site, while others were ineffective.

Jameson and Kent reasoned that the antibodies which prevented HIV-1 infection were probably attaching themselves at or near the binding site, while the ineffective ones attached elsewhere. When they tested their fragments against the antibodies, they found that the effective antibodies tended to attach themselves to a particular piece of CD4. That piece, the researchers concluded, must contain the HIV-1 binding site.

But what does the binding site look like? A protein's three-dimensional shape determines its properties, and subtle differences in shape can have marked effects. Although the

CD4 protein molecules can be prepared in soluble form. A CD4 solution also overwhelms the virus, leaving the T-cells unmolested.



shape of CD4 itself is still unknown, it belongs to a class of proteins—the immunoglobulin superfamily—all of which have very similar shapes. (Another group of Caltech biologists, led by Leroy E. Hood, has pioneered the study of the immunoglobulin superfamily. Hood, the Bowles Professor of Biology and chairman of the biology division, is a coauthor of the CD4 study.) By drawing a structural analogy, the researchers were able to approximate CD4's shape. Because CD4's amino acid sequence is completely known, they knew where in the protein the active segment appeared; knowing how the protein folds up into three dimensions allowed them to deduce the binding site's general properties and its location on the protein's surface.

The next step was to see if the fragment alone could inhibit HIV-1 infectivity. The fragment, called CD4-derived synthetic peptide 25-58, was synthesized at Caltech and sent to the University of Alabama for infectivity tests. (There are no laboratories at Caltech working with the live AIDS virus.) The Alabama researchers used the most stringent test for HIV-1 infectivity, an *in vitro* (test tube) assay measuring the virus's ability to induce cell fusion. The 25-58 fragment did, in fact, inhibit infectivity in a dose-dependent fashion. Three other CD4 fragments, from regions adjacent to the presumed binding site, were used as controls. They had no inhibitory effect.

Researchers at several laboratories had first synthesized CD4 back in December, 1987, and had found that a CD4 solution prevented cells from being infected by HIV-1 *in vitro*. But for many reasons, including potential difficulties with using large proteins in therapy, many researchers had hoped to identify the specific part of the CD4 molecule to which HIV-1 binds, as this work has now done.

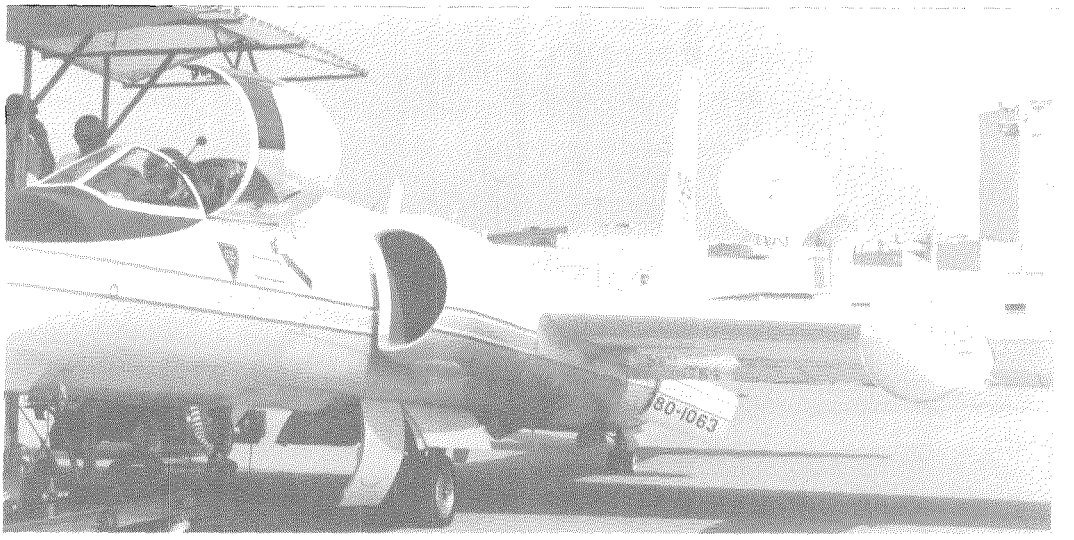
Kent says the next step is to define the binding site's exact shape more precisely, and to determine which specific amino acids are critical to the binding process. Then chemists can begin to synthesize analogs of the binding site, hoping to find one that binds to the virus even more strongly. Other laboratories could then begin clinical trials of the synthetic binding site. "We are going to be conducting intensive explorations into the binding site over the next two years," said Kent. "There's probably a large chance that in two years this work will no longer be thought of as a direct route to an AIDS therapy. Of course, we all hope that this turns out to be the one in a hundred that does work out."□—*DS*

Ozone: The Hole Story

Caltech's Jet Propulsion Laboratory (JPL) and the "hole" in the ozone layer go back a long way together. In 1974, JPL's Mario Molina and F. Sherwood Rowland of U.C. Irvine discovered that chlorofluorocarbons (CFCs)—chlorine-containing compounds used as refrigerants, solvents, fire extinguishing agents, and aerosol propellants—were destroying ozone in the earth's stratosphere. (The U.S. banned CFC use in aerosol cans in 1978 as a direct result of that finding.)

The hole, discovered by British scientists in 1985, isn't really a hole. It's a seasonal decline in the stratospheric ozone concentration over Antarctica—a drop of more than 50 percent compared to the late 1960s. The drop-off begins early in the austral spring (late August to early September), levels off in October, and eventually climbs back to normal in November. The hole may be getting bigger and recovery may be taking longer each year, with alarming implications for the ozone layer worldwide. (Another group, the Ozone Trends Panel, recently determined that average annual ozone concentrations over much of the Northern Hemisphere have decreased

The ER-2 research plane carried 14 automated instruments. The instruments had to be light and compact, yet rugged enough to function unattended for 7 hours at −90° C and air pressure one percent that at sea level. Gary's instrument projects from the pod at right.



by about two percent between 1969 and 1986.)

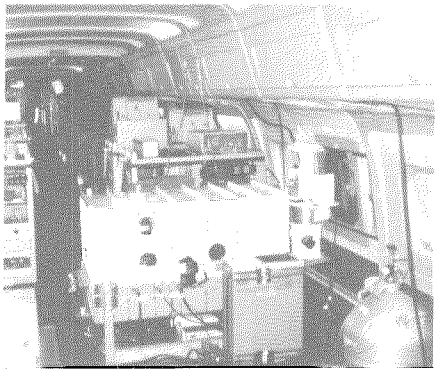
JPL's Barney Farmer and Bruce Gary joined a group of about 150 scientists from four nations who explored the hole in August through September of 1987. The Airborne Antarctic Ozone Experiment (AAOE) was designed to map the hole's chemical and physical properties in detail, shedding light on its origins and providing a database for future research.

Ozone, a form of oxygen containing three oxygen atoms per molecule instead of the usual two, absorbs ultraviolet (UV) light. Small doses of UV light cause suntans. Medium doses can damage DNA, causing skin cancer and spontaneous mutations, and large doses can kill cells outright—meat lockers have UV lamps to keep bacteria down. Five percent of the sun's energy output is in the UV region, and there's not much ozone between us and the sun: if all the stratospheric ozone were brought to the earth's surface at room temperature and pressure, it would form a layer only two to four millimeters thick.

Lab work by Molina and others has shown that chlorine atoms, knocked loose from CFCs by ultraviolet light, can convert ozone into ordinary oxygen molecules. An intermediate step in the reaction forms chlorine monoxide (ClO), which breaks down, regenerating the chlorine to destroy more ozone. But there's more to the story. Nitric oxide (NO), a naturally ocurring trace gas, reacts with ozone to form nitrogen dioxide (NO_2) and ordinary oxygen. Some gases deactivate chlorine; NO_2 and chlorine monoxide form inactive chlorine nitrate ($ClONO_2$). Unfortunately, the Antarctic stratosphere is cold enough that "good" gases can freeze out onto the tiny ice crystals that make up the polar stratospheric clouds, denitrifying the air. Reactions on the crystals' surfaces can break down reservoir gases like $ClONO_2$ and HCl, re-releasing active chlorine.

The AAOE flew 21 experiments on two aircraft based in Punta Arenas, Chile. Each plane flew about a dozen missions into the hole. Long hours were the norm—after each flight returned, enough data from it had to be analyzed to determine whether the upcoming flight plan should change. One craft, an ER-2 —the civilian version of the U-2 spy plane—flew six- to eight-hour missions at altitudes of 60,000 to 65,000 feet, where ozone is most depleted. The ER-2 is a small, unpressurized aircraft with barely enough room for the pilot and 14 automated instruments. The other airplane, a converted DC-8 passenger plane, flew missions in excess of 12 hours at lower altitudes corresponding

Farmer's FTIR records infrared spectra through a specially modified window. The DC-8's pressurized passenger cabin accommodated experimenters as well as experiments.



to the bottom of the ozone-depleted layer. The final DC-8 flight traversed the continent, landing in Christchurch, New Zealand on the way home. The DC-8's pressurized passenger compartment allowed the investigators to accompany their instruments.

Farmer's instrument, a Fourier transform infrared spectrometer, or FTIR, flew on the DC-8. Aimed at the sun, the FTIR measured trace-gas concentrations between the sun and the aircraft by recording the gases' absorption of infrared sunlight in the 2 to 16 micron region. The scans were repeated at two-minute intervals, or approximately every 10 to 20 kilometers. One million data points per scan were stored in an onboard computer for Fourier analysis later. About 100 good spectra were obtained per flight. The method identified HCl, $ClONO_2$, N_2O, CFCs, NO, NO_2, N_2O_5, HOCl, ozone, and other species, some in concentrations as low as 0.1 part per billion. The great sensitivity was no scientific luxury: two gases of particular interest, HOCl and ClO, are present at one part per billion at best. The accumulated data show how chemical distributions change as the hole forms. (The other six experiments measured some of the same chemicals by other methods, so each verified the others; air samples were collected as well.)

Gary's instrument, a microwave temperature profiler, rode in the ER-2. (Other devices measured particle sizes, chemical constituents, air temperature and pressure, and collected air samples.) The profiler measured air temperatures by sensing thermal emission from oxygen molecules at two frequencies in the microwave range, 57.3 and 58.8 GHz. The instrument scanned through an arc from −50° to +60° relative to the plane's horizon, producing readings at 15 altitudes within an 8,000-foot slab of air centered on the aircraft. A complete set of readings was taken every three kilometers. The data were plotted as potential temperature surfaces—sheets of air that would have the same temperature if they were at 1,000 millibars pressure. The plots look like a cross section through the rumpled sheets on an unmade bed. The rumples are waves associated with vertically oscillating airmasses. Mountains on the Palmer Peninsula produce very large waves with amplitudes as large as 1,200 meters. These waves propagate into the ozone hole, where they may initiate cloud formation by elevating air parcels to colder altitudes. The larger (10 micron) ice particles fall out, taking with them any nitrogen compounds they may have collected. These mountain waves may thus be responsible for the denitrification and dehydration observed within the hole.

The hole forms within the polar vortex, a self-contained, Antarctic-sized body of air that swirls around the South Pole every winter. The vortex acts like a giant thermos bottle, keeping interfering nitrogen compounds on ice while the returning sun's UV light creates active chlorine. The vortex's boundary can be found by plotting potential vorticity, derived from wind data and Gary's potential temperature readings. The boundary plot, superimposed on chemical distribution data, correlates meteorology and chemistry.

The AAOE scientists have been digesting their data since their return from Chile. They met in Snowmass, Colorado, in mid-May to share their findings. The results agreed with those of a ground-based study at McMurdo Station in 1986, but in much more detail and over a much larger area. The compositional changes found inside the hole included strong evidence for denitrification and dehydration—allowing the chlorine chemistry to proceed unimpeded—and for reservoir gases freezing out on ice crystals. According to Farmer, "We found very strong evidence that the hole is caused by chlorine from man-made chemicals, as had been suspected; aided and abetted by natural meteorology—polar stratospheric clouds containing ice crystals where the perturbed chemistry can occur."□—*DS*