National Institute of Allergy and Infectious Diseases

Update

FOR RELEASE
Monday, June 13, 1988

PUBLIC HEALTH SERVICE
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HIV FOUND TO INFECT BONE MARROW CELLS AND TO PERSIST IN MONOCYTES

Scientists have gathered the first direct evidence that human immunodeficiency virus (HIV) can infect bone marrow cells. Although HIV infects and multiplies in at least one type of bone marrow cell, it fails to kill them rapidly or, as is typical of enveloped viruses, bud significant numbers of virus particles from their surfaces. By thus concealing the virus they harbor, these HIV-infected bone marrow cells elude detection by the immune system and thereby serve as havens for the virus. Because bone marrow cells mature into blood cells, this finding could explain the puzzle of why some people infected with HIV exhibit blood-related abnormalities even when little HIV can be detected in their circulating blood.

The results of this study--conducted by Dr. Thomas M. Folks of the National Institute of Allergy and Infectious Diseases (NIAID), Dr. Steven Kessler of The Uniformed Services University of the Health Sciences, Dr. Michelle Cottler-Fox of Georgetown University School of Medicine, Mr. Jesse Justement of NIAID, Dr. Jan Orenstein of the George Washington University School of Medicine, and NIAID Director Dr. Anthony S. Fauci--were presented by Dr. Folks at the IV International Conference on AIDS in Stockholm.

Until now, only indirect clues supported the notion that HIV could infect bone marrow cells. Testing this theory directly had been hampered by the difficulty in obtaining bone marrow samples uncontaminated by other immune system cells known to be HIV targets, such as T4 cells, HIV's primary target, and mature monocytes, roaming scavenger cells.

Using a new technique developed by Dr. Kessler, the researchers overcame this obstacle and obtained nearly pure populations of bone marrow cells containing CD34 surface molecules.

a marker found on bone marrow cells but not on mature monocytes or on the immune system's key players, B cells and T cells. The technique employs a magnetic bead chemically cross-linked to a mouse monoclonal antibody specific for cells carrying the CD34 molecule (CD34+ cells).

By employing this antibody as the pivotal element in a stepwise purification process, the researchers extracted CD34+ cells from bone marrow taken from human cadavers. (Cadavers were used because they could supply the large numbers of bone marrow cells needed for the purification process to work.) When the antibody-coupled magnetic beads were added to the bone marrow sample, CD34+ cells bound to the antibody segments. The CD34+ cells could then be separated from the rest of the bone marrow cell suspension by exposing them to a magnet, resulting in a pure population of CD34+ bone marrow cells. The extracted CD34+ cells were then incubated with highly concentrated HIV for 24 hours and afterwards maintained in culture for 75 days.

One month after the initial infection, the researchers examined the cultures and found evidence that HIV was replicating: reverse transcriptase (RT) activity, an indirect measurement of HIV synthesis, could be detected in infected but not in control cultures. RT activity progressively increased during the culture period. Despite increasing levels of HIV, there was no visual evidence that HIV killed these cells.

At the end of the 75-day period, the scientists harvested the CD34+ cells from the cultures and Dr. Orenstein examined them using a high-resolution electron microscope. Electron microscopy revealed these cells to be bone marrow cells likely destined to develop into monocytes, at this stage also known as "progenitor monocytes". HIV particles were found in more than 75 percent of the these cells, but the amount of virus within each of these cells varied.

The cellular cytoplasm, the living matter inside cells surrounding the nucleus, contained the largest concentration of HIV. A small amount of virus was seen budding from membrane

structures found within the cell. Rarely, HIV particles were seen budding from the plasma membrane, the sheath surrounding the cell.

Despite the near absence of virus budding from the plasma membrane, infected culture supernatants--the liquid left in a culture after the solid material has sifted to the bottom--could infect susceptible target cells, indicating that passageable virus was being produced in these cells.

Because HIV is difficult to detect in mature, circulating monocytes, the scientists believe it is likely that only a small proportion of CD34+ bone marrow progenitor monocytes are infected with HIV in vivo. If true, this would explain why it is relatively uncommon to find unexplained monocyte abnormalities in AIDS patients.

The bone marrow cells could be infected despite having no detectable CD4 markers, the receptor by which HIV usually enters cells. This system thus offers a model of an alternate mechanism by which HIV can infect and propagate in cells. It may also explain why HIV fails to kill these cells despite the fact that they contain high levels of the virus: based on evidence gathered so far, one of the ways that HIV appears to kill cells is through a mechanism whereby CD4 molecules form complexes with HIV's outer membrane, or envelope.

The scientists are now using the magnetically linked antibody method to purify bone marrow samples obtained from patients at various stages of HIV infection. These will be analyzed for any differences among them.

In a related development, NIAID scientist Dr. Guido Poli also reported new experimental data strengthening the theory that immune system scavenger cells are the most important chronic reservoirs of HIV. Dr. Poli and his NIAID colleagues--Drs. Carla Wilson, Thomas M. Folks, Cecil Fox, Ferdinand Massari, and Anthony S. Fauci--have studied the characteristics of HIV infection in circulating monocytes as they mature into tissue macrophages. They have also compared the characteristics of monocytes infected in vitro with monocytes taken from HIV-infected people.

They have found that HIV can persist for prolonged periods inside circulating monocytes and in vitro-derived macrophages without killing these host cells and without the virus itself being killed or disarmed by the immune system. This work supports earlier studies indicating that monocytes/macrophages not only stockpile HIV but also can help spread the virus to susceptible sites throughout the body, for example, to the brain and to cells carrying CD4 molecules, the receptor by which HIV enters cells.

For their study, they took peripheral blood monocytes (PBM) from eight healthy donors and from 16 HIV-infected patients at various stages of disease. The PBMs from healthy people were challenged in the test tube with HIV. It is known that as a monocyte matures into a macrophage, it acquires some new properties--new receptors, for example--and loses others. The researchers investigated if the cells could be infected at various steps of differentiation, or if some stages of development are permissive, or readily infectible, and others are not. To determine this, different monocytes were challenged with HIV at various times. They found that they could infect monocytes during the first 20 days of culture, but not after that. They have not explored whether this is due to an artifact (for example, too few viable cells in the culture), or if it truly represents a characteristic of monocyte/macrophage development.

After HIV challenge, both the laboratory-infected monocytes and those taken from HIV-infected people were maintained under the same laboratory conditions and studied at various times during a 40-day period. In neither group did the investigators find evidence that the virus killed the cells, but in only one of the 16 samples from HIV-infected people did they find evidence of virus propagation. However, both cells and supernatants from the two groups could infect susceptible T cells, indicating that the virus was still viable.

The observation that HIV can persist in these cells for prolonged periods of time adds considerable weight to the evolving theory that monocytes/macrophages are the chief chronic carrier cells of HIV.

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