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This Week's Citation Classic®

Popovic M, Sarngadharan M G, Read E & Gallo R C. Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* 224:497-500, 1984.
[NCI, Bethesda, and Litton Bionetics, Inc., Kensington, MD];
Gallo R C, Salahuddin S Z, Popovic M, Shearer G M, Kaplan M, Haynes B F, Palker T J, Redfield R, Oleske J, Safai B, White G, Foster P & Markham P D. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* 224:500-3, 1984.
[NCI, Bethesda, MD; N. Shore Univ. Hosp., Manhasset, NY; Duke Univ. Sch. Med., Durham, NC; Walter Reed Army Inst. Res., Washington, DC; Univ. Med. and Dent. New Jersey, Newark, NJ; Mem. Sloan Kettering Cancer Ctr., New York, NY; Univ. N. Carolina, Chapel Hill, NC; and Litton Bionetics, Inc., Kensington, MD];
Schüpbach J, Popovic M, Gilden R V, Gonda M A, Sarngadharan M G & Gallo R C. Serological analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. *Science* 224:503-5, 1984.
[NCI, Bethesda; NCI-Frederick Cancer Res. Facility, Frederick; and Litton Bionetics, Inc., Kensington, MD]; and
Sarngadharan M G, Popovic M, Bruch L, Schüpbach J & Gallo R C. Antibodies reactive with human T-lymphotropic retroviruses (HTLV-III) in the serum of patients with AIDS. *Science* 224:506-8, 1984.
[Litton Bionetics, Inc., Kensington, and NCI, Bethesda, MD]

The unusual nature of the topic and circumstances of this group of papers, which all appeared in a single issue of *Science*, May 4, 1984, led to our decision to expand the space normally devoted to a *Citation Classic* commentary. Please note that the work of the Montagnier group in Paris was discussed in *Current Contents®/Life Sciences* 30(8):18, 23 February 1987. (See reference 9.)

We applied techniques that we had developed for the detection and isolation of the first human retroviruses (HTLV-I and -II) to a study of lymphocytes from people with AIDS and in AIDS risk groups to test our hypothesis that AIDS was due to a human T-lymphotropic retrovirus infecting T4 cells. We obtained 48 isolates of a new retrovirus, which we called HTLV-III, from these individuals and none from the peripheral blood of 115 healthy heterosexuals. In these papers we showed that: (1) the isolates are related to each other; (2) they are different from HTLV-I or -II; (3) they are the cause of AIDS; and (4) six of these isolates were continuously produced in a cell line for the first time, leading to the technology that allowed the development of a blood test that has worked toward eliminating blood transfusion AIDS. [The *SCI*® indicates that these papers have been cited in over 1,045, 1,235, 375, and 695 publications, respectively.]

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The research that led to these publications was conceived in early 1982 when I first proposed that AIDS was most likely caused by a T-lymphotropic retrovirus. This idea was mainly derived from earlier experiences and discoveries, in my laboratory and by colleagues, of the first human retroviruses HTLV-I and -II in the late 1970s and early 1980s.[1-4] These viruses cause leukemia, but as we and others had shown, they also produce some impairment of T-cell function. In addition, they are highly T-cell lymphotropic and usually infect T4 cells; they are transmitted by blood, sex, and to the neonate. We thought these characteristics fit what might be expected for the cause of AIDS. Additionally, studies by Myron Essex and coworkers[5] indicated that a retrovirus of cats (feline leukemia virus) caused not only T-cell leukemia, as first demonstrated by William F.H. Jarrett and his colleagues in Glasgow,[6,7] but also that a variant of it was the cause of an AIDS-like illness in cats. Thus, the idea that AIDS was due to a human retrovirus, most likely a variant of HTLV-I or -II, was suggested.

When HTLV-III (later called HIV) was characterized, it became clear that this new human retrovirus differed substantially from HTLV-I and -II, despite the similarities in T-cell tropism and mode of transmission. Our early experiments in late 1982 and 1983 were both exciting and frustrating.[8] We could detect the presence of a retrovirus in primary cultures from at least some AIDS patients by the presence of reverse transcriptase, but specific antibodies to HTLV-I and -II failed to show anything. Moreover, its profound T4-cell-killing properties (not originally appreciated) made many obstacles in producing this virus.

A major breakthrough for us was the first mass production of this virus as reported in the first of these papers that enabled us to (1)

make the first specific reagents to this new virus, (2) demonstrate that our many isolates belonged to one and the same subgroup, (3) obtain sufficient viral proteins to do unequivocal seroepidemiology, and (4) develop the first successful antibody test for application in blood banks for testing donor blood for this virus. These studies led to the first unequivocal evidence that this virus was the cause of AIDS. Later, these reagents were used to show that all of these isolates were of the same virus group as the virus identified by the Pasteur Institute group (originally called simply "an HTLV" and later called LAV).[9] The first published detection of the virus was from a patient with lymph-node enlargement, but tests of sera originally showed less than 20 percent of AIDS patients had antibodies to LAV. No doubt this was due to insufficient virus production. We found and described 48 isolates of HTLV-III (now generically HIV) from patients with AIDS and at risk; none of the 115 nonrisk healthy people yielded such isolates. We think the 1984 *Science* papers are frequently cited because it was the first time anyone stated that they knew the cause of AIDS.

There were many obstacles encountered in this work: (1) there was a general opinion against a retrovirus as the cause of AIDS because it was only a few years earlier that we had discovered the first human retrovirus, so that retroviruses were just gaining recognition; (2) most retroviruses are not so cytopathic, therefore a cell-killing and not a cell growth-promoting virus was the logical candidate; (3) as noted above, the extreme T4-cell killing by the virus made for a delay in obtaining clear-cut evidence that it was the cause of the disease; (4) because people with AIDS usually have many infections, it was often stated that it might not be possible to prove any one organism was the cause of AIDS.

Our approach was to follow procedures we had developed for the discovery of HTLV-I, namely, sensitive assays for reverse transcriptase, the unique DNA polymerase of retroviruses, and growth of the patient's T4 cells with T-cell growth factor, interleukin-2. When this virus was detected in a few patients, at first neither we nor anyone else had a way to tell whether any two were the same. But as noted above, after successful mass production of the virus, we obtained specific reagents that enabled us to type them. Mass production was achieved by transmitting HTLV-III from primary peripheral blood T4 cells of an infected person to a cloned population of immortalized human leukemic T4 cells, which we found were relatively resistant to the killing effect of the virus.

We have been very fortunate to receive several scientific awards that, in general, have been awards for research on human retroviruses. More recent awards for these papers on HTLV-III include a citation from the Infectious Disease Society of America, an immunology prize (the Rabbi Shai Shacknai Memorial Prize) from the Hebrew University-Hadassah Medical School in Jerusalem, the Hubert Humphrey Cancer Research Award, the Gairdner Foundation International Award, the Lions Club Humanitarian Award, and the 1987 American Foundation for AIDS Research Award. I have also had the unique honor of receiving a second Lasker Foundation Award (for clinical science, jointly with my colleagues Luc Montagnier and Max Essex).

The work was performed chiefly by the group that I direct at the Laboratory of Tumor Cell Biology, National Cancer Institute of the National Institutes of Health in Bethesda, Maryland. S.Z. Salahuddin, P.D. Markham, and M. Popovic worked on the virus detection and isolation. G.M. Shearer, M. Kaplan, B.F. Haynes, T.J. Palker, R. Redfield, J. Oleske, B. Safai, G. White, and P. Foster gave clinical and immunology support and also provided incisive discussions.

For recent reviews of this topic, see references 10-12.

1. **Essex M.** Feline leukemia and sarcoma viruses. (Klein G, ed.) *Viral oncology*. New York: Raven Press, 1980. p. 205-29.
2. **Gallo R C, Blattner W A, Reitz M S & Ito Y.** HTLV: the virus of adult T-cell leukaemia in Japan and elsewhere. *Lancet* 1:683, 1982.
3. **Gallo R C, Sliski A & Wong-Staal F.** Origin of human T-cell leukaemia-lymphoma virus. *Lancet* 2:962-3, 1983. (Cited 50 times.)
4. **Essex M, McLane M F, Lee T H, Falk L, Howe C W S, Mullins J I, Cabradilla C & Francis D P.** Antibodies to cell membrane antigens associated with human T-cell leukemia virus in patients with AIDS. *Science* 220:859-62, 1983. (Cited 290 times.)
5. **Essex M.** Feline leukemia—a naturally occurring cancer of infectious origin. *Epidemiology Rev.* 4:189-203, 1982.
6. **Jarrett W F H, Martin W B, Crighton G W, Dalton R G & Stewart M F.** Transmission experiments with leukaemia (lymphosarcoma). *Nature* 202:566-7, 1964. (Cited 215 times.)
7. **Jarrett W F H, Crawford E M, Martin W B & Davie F.** A virus-like particle associated with leukaemia (lymphosarcoma). *Nature* 202:567-8, 1964. (Cited 195 times.)
8. **Gallo R C, Sarin P S, Gelmann E P, Robert-Guroff M, Richardson E, Kalyanaraman V S, Mann D, Sidhu G D, Stahl R E, Zola-Panzer S, Leibowitch J & Popovic M.** Isolation of human T-cell leukemia virus in acquired immune deficiency syndrome (AIDS). *Science* 220:865-7, 1983. (Cited 295 times.)
9. **Barré-Sinoussi F, Chermann J C, Rey F, Nugeyre M T, Chamaret S, Gruest J, Dauguet C, Axler-Blin C, Vézinet-Brun F, Rouzioux C, Rozenbaum W & Montagnier L.** Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* 220:868-71, 1983. (Cited 1,300 times.) [See also: **Barré-Sinoussi F, Chermann J-C & Montagnier L.** Citation Classic. *Current Contents/Life Sciences* 30(8):18, 23 February 1987.]
10. **Wong-Staal F & Gallo R C.** Human T-lymphotropic retroviruses. *Nature* 317:395-403, 1985.
11. **Gallo R C.** The first human retrovirus. *Sci. Amer.* 255(12):88-98, 1986.
12. **———.** The AIDS virus. *Sci. Amer.* 256(1):47-56, 1987.

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