C. The first and the most important of the four *Science* Papers said to prove HIV the cause of AIDS. This is the typed draft produced by the Lead Author M. Popovic, with all the handwritten editing and comments made by R. Gallo just 7 days before the manuscript went in for publication. (The cover page unfortunately has faded.)

Science -- First draft

Popovic

RESCUE AND CONTINUOUS PRODUCTION

OF HUMAN T-CELL LYMPHOTROPIC RETROVIRUS (HTLV-III)

FROM PATIENTS WITH AIDS

— way to deal c̄ the LAV - originally

(1) Lack of cross hybridy c̄ I, II

(2) " " Ag " [illegible]

(3) [illegible] to EIA

(4) [illegible] published [illegible]

When the hell are the [illegible]

ABSTRACT

A susceptible and permissive human neoplastic T-cell population is described for cytopathic variants of human T-cell lymphotropic retroviruses (HTLV-III) which are isolated from pre-AIDS or AIDS patients. The infected T-cell population preserves its capacity for permanent in vitro growth and exhibits continuous virus expression. This system is suitable for isolation of cytopathic variants of HTLV from patients with lymphadenopathy (pre-AIDS) and AIDS, and continuous virus production in high amounts enables us to prepare specific viral probes for immunological and nucleic acid studies. The cytopathic effect of HTLV-III on the infected T-cell population is characterized by [illegible] of multi-nucleated giant cells which could be used as an indicator for the detection of the virus production.

This abstract is rather trivial for a putative breakthrough paper for Science.

A family of human T-cell lymphotropic retroviruses (HTLV) comprises two major and well characterized subgroups of human retroviruses, called HTLV-I (   ) and HTLV-II (   ), and recently a new variant of HTLV has been isolated from a patient with lymphadenopathy named also as lymphadenopathy associated virus (LAV) (   ) which is described here as HTLV-III. The most common isolate obtained from patients with mature T-cell malignancies is HTLV-I (   ). Seroepidemiological and nucleic acid hybridization data indicate that HTLV-I, including its new subtype, is etiologically associated with T-cell leukemia/lymphoma of adults (   ). The disease clusters in the south of Japan (   ), the Caribbean (   ), Africa (   ) and can be found in other parts of the world. HTLV of subgroup II (HTLV-II) was first isolated from a patient with a benign form of T-cell variant of hairy cell leukemia (   ). To date, this virus represents the only isolate obtained from a patient with neoplastic disease. However, isolation of retroviruses and seroepidemiological data suggest that HTLV of both subgroups, including new variants from subgroup III, may be involved in the pathogenesis of the acquired immune deficiency syndrome (AIDS) (   ).

Epidemiologic data strongly suggest that AIDS is caused by an infectious agent which is transmitted by intimate contacts or blood products (   ). To date, over 3000 cases of AIDS have been reported in the U.S. (   ). Patients with the disease include mainly homosexuals (   ), intravenous drug users (   ), Haitian immigrants to the U.S. (   ), and hemophiliacs (   ). Recently, an increased number of AIDS cases have been reported in children whose parents have AIDS or intimate contact(s) with a person having the disease (   ). Although the disease in patients is

manifested by opportunistic infections, predominantly Pneumocystis carinii pneumonia and Kaposi's sarcoma, the underlying disorder affects the patient's cell-mediated immunity ( ). ~~The T-cell dysfunction is often marked by an absence of delayed hypersensitivity~~ with absolute lymphopenia and reduced helper T-lymphocyte (OKT4+) subpopulation(s). ~~This is reflected in reverse ratios of helper-to-suppressor T-lymphocyte (OKT4+/OKT8+), poor lymphocyte responsiveness to mitogens (...) In some cases, a decreased natural killer cell activity has been found as well.~~

~~Despite intensive research efforts, the causative agent of AIDS has not yet been identified.~~ Although patients with AIDS are often chronically infected with cytomegalovirus ( ), or hepatitis B virus ( ), we have proposed that a retrovirus causing AIDS is a retrovirus from a family of HTLV. This assumption, besides being a well known precedence of causing immune deficiency in cats by feline leukemia viruses ( ), is based on the facts that retroviruses of the HTLV family are characterized by T-cell tropism, preferentially infect "helper" T-cells (OKT4+), exhibit cytopathic effects on various human and mammalian cells as demonstrated by syncytia induction ( ); and the infection of T-cells by HTLV caused alteration of specific T-cell function ( ) in some cases may result in a selective cell killing ( ). Moreover, HTLV are transmitted by intimate contact and blood products. Seroepidemiological results by M. Essex et al. and their colleagues showed that the presence of antibodies directed to cell membrane antigens of HTLV infected cells is from 30-40% of patients with AIDS ( ). In addition, over 20 HTLV isolates of both subgroups and numerous new variants were obtained from patients with AIDS ( ). The successful detection and isolation of HTLV was made possible by the discovery of TCGF ( ) which enabled selectively to grow different subsets of normal and

and the development of

neoplastic mature T-cells ( ). The viral rescue and transmission of HTLV into permissive cells followed well established procedure worked out in the system of avian sarcoma virus transformed mammalian cells ( ). The cocultivation procedure using cord blood T-cells from newborns as recipient cells for HTLV enabled preferential to obtain with immortalizing (transforming) capability ( ). HTLV variants which possess "weak" or lack the immortalizing properties for normal T-cells and exhibit mainly cytopathic effect on them can only be detected transiently using normal T-cells as target in cocultivation or cell-free transmission experiments. This was our main obstacle for frequent isolation and particularly for detailed biological, immunological and nucleic acid characterization of these cytopathic variants of HTLV. To overcome these obstacles, we performed an extensive survey for a cell population which would be highly susceptible to and permissive for cytopathic variants of HTLV and preserve capacity for permanent growth after infection with the virus. We report here the establishment and characterization of an immortalized T-cell population which is susceptible to and permissive for HTLV cytopathic variants and can be used for their rescue and continuous production.

Several in vitro established permanent cell lines originated from human malignancies were assayed for susceptibility to infection with HTLV as a reference virus (gift from Dr. Montagnier) has been used in the first series of experiments. Two cell lines with characteristics of mature T-cells were susceptible to infection as determined by reverse transcriptase (RT) assays,

One of these, however, was positive for herpesvirus-like particles, the second one established from a patient with adult T-cell leukemia was negative for HTLV infections as well as no viral particles were found by an extensive electron microscopic examination. The infected parental cell line by HTLV-III was positive for particulate reverse transcriptase activity in culture fluids and about 20% of the infected cell population was positive in indirect immune fluorescent assay (IFA) using serum from a hemophiliac patient (E.T.) with lymphadenopathy. The serum of the patient (E.T.) exhibited positivity in IFA, disrupted HTLV-III ( ) and reacted with p61 of HTLV transformed human T-cells in the precipitation assays( ).

To establish a susceptible and highly permissive T-cell population for HTLV-III infection, which would preserve the permanent growth and continuous virus production, extensive cloning of the parental T-cell population was performed. A total of 51 single-cell clones were obtained by both capillary ( ) and limited dilution ( ) techniques and screened for proliferation capability of cells and HTLV-III infection.

A representative example of response to virus infection of 8 T-cell clones which are susceptible to and permissive for HTLV-III is shown in Table 1. In parallel experiments, 2 X 10^6 cells of each T-cell clone were exposed to 0.1 ml of concentrated virus containing 10^5 cpm of reverse transcriptase (RT) activity. Then the cell growth, morphology, positivity of cells for the viral antigen(s) and RT activity in culture fluids were assessed after 6 and 14 days of infection. Although all 8 clones were susceptible to and permissive for the virus,

Redundant

IFA for the presence of viral antigen(s) and RT activity in culture fluids, there were considerable differences between infected clones in capability to proliferate after infection. [illegible] days of infection [illegible] cytopathic effect was manifested by [illegible] 10 [to] 90% of the initial cell number and, [illegible] a high proportion of multinucleated (giant) cells were consistently found in all 8 infected clones. The percentage of T-cells positive for viral antigen(s) in IFA with the patient's serum ([illegible]) and hyperimmune rabbit serum raised against the whole disrupted virus was in the range from 10% to over 80%. After 14 days of infection, total cell number [illegible] IFA positive cells [illegible]gens increased in all 8 clones. The highest proliferation was found in clone H/4, H/6; and H/9 and lowest was in clone H/3. The virus positive cultures exhibited consistently round giant cells which in Wright-Giemsa staining revealed a high number of nuclei (Fig. 1a). Electron microscopic examinations of the infected cultures showed an abundant number of viral particles (Fig. 1b).

To determine whether HTLV-III is continuously producd by the infected T-cells in long term cultures, both the virus production and cell viability of the HTLV-IIIA infected clone H4 were followed for several months. As shown in Figure 2a, there was a fluctuation in the amount of virus production, however, culture fluids harvested from the H4/HTLV-III cell cultures at approximately 14 day intervals consistently exhibited particulate RT activity which was been followed for [illegible] months. In addition, the viability of the cells was in the range from 65-85% and the doubling time of the H4/HTLV-III cell culture was approximately 36-48 hours (data not shown) after 3 weeks of infection. Thus, the data clearly indicate a

[Handwritten margin note:] These multinucleated giant cells are similar to those induced by HTLV-I and HTLV-II except that the nuclei exhibit a characteristic ring formation

that this

con[illegible] permanently growing T-cell population can continuously produce HTLV-III in long term culture.

[illegible], The yield of the virus produced by H4/HTLV-III cells was assessed by purification of concentrated culture fluids through a sucrose density gradient and assays of particulate RT activity [illegible] in each fraction collected from the gradient. As shown in Figure 2b, similar to other retroviruses, the highest RT activity was found at density 1.16g/ml. Electron microscopic (EM) examinations of the aliquots from the fractions with highest RT activity revealed that the banded virus particles [illegible] were highly purified. An approximate estimation (   ) of the number of viral particles determined by EM and RT activity suggests that the viral yield from [illegible] culture fluid is about 10^{11} virus particles per L of culture fluid. Therefore, the established T-cell clones are susceptible to and highly permissive for cytopathic variants of HTLV; all of them preserve proliferation capacity after infection, and in addition, as demonstrated in the case of H4/HTLV-III [illegible], some of them can proliferate and continuously produce large amounts of HTLV-III in long term culture.

We have used two clones, H/4 and H/9, for the rescue of cytopathic variants of HTLV from patients with lymphadenopathy (pre-AIDS) or AIDS. Examples [illegible]

As shown in Table 2, [illegible] cocultivation [illegible] cell-free [illegible] infection, were effective for virus [illegible] HTLV-III isolates have been successfully obtained in these systems by cocultivation (4 patients) and by cell-free infection of T-cell clones (1 patient) [illegible]. Are summarized in Table [illegible]

In all five cases, the virus release into culture fluids was found by RT assay and extracellular virus particles were detected [illegible] cases so far.

[illegible] more than — additional isolate or detection of HTLV-III have now been obtained in our laboratory [illegible]

all those detected by other techniques will now be adopted to these T-cell clones for long-term production and detailed analysis.

analyzed by using experimental rabbit serum against HTLV-III as well as the patients ... both sera reacted with acetone-fixed cells and

and the positivity was between 5 and 80%. Thus, the data indicate that the T-cell clones are suitable for HTLV-III rescue either by cocultivation or by cell-free infection. The transient expression of cytopathic variants of HTLV in cells from AIDS patients and the previous lack of a proliferative cell system which could maintain growth and still be susceptible to and permissive for the virus represented a major obstacle in detection, isolation and elucidation of the precise causative agent of this disease. The establishment of T-cell populations described here, which, after infection, continuously grow and produce virus, provides the possibility for detailed biological, immunological and nucleic acid studies of this agent. has opened the way to routinely detection of the highly cytopathic variants of HTLV in AIDS and provides the first opportunity for detailed molecular immunological analyses.

In all cases where this has already been done the ... HT and H/9 clones

CONCLUSION NOT COMPLETED

REFERENCES NOT DONE

(per Mika)

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End of Popovic's Draft.