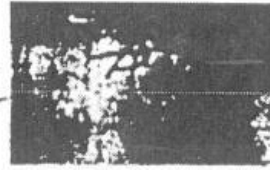


C. The first and the most important of the four *Science* Papers said to prove HIV the cause of AIDS. This is the typed draft produced by the Lead Author M. Popovic, with all the handwritten editing and comments made by R. Gallo just 7 days before the manuscript went in for publication. (The cover page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

- ① Lack of cross reactivity: I, II
- ② " " Ag. reaction
- ③ Resistance to CIA
- ④ inactivated factor

When the  
hell is the  
hell

ABSTRACT

A ~~susceptible~~ <sup>and</sup> permissive human neoplastic T-cell population is described for <sup>routine isolation of</sup> cytopathic variants of human T-cell lymphotropic retroviruses (HTLV-III) ~~isolated~~ <sup>isolated</sup> from pre-AIDS or AIDS patients. The infected T-cell population preserves its capacity for permanent in vitro growth <sup>and</sup> exhibits continuous virus expression. ~~HTLV-III is suitable~~ <sup>is suitable</sup> for isolation of cytopathic variants of HTLV from patients with lymphadenopathy (pre-AIDS) and AIDS. ~~and combination of~~ <sup>and combination of</sup> virus production in high amounts <sup>enables us to prepare</sup> specific viral probes for immunological and nucleic acid studies. <sup>can be prepared. One</sup> The cytopathic effect of HTLV-III ~~infection~~ <sup>is its induction</sup> of multi-nucleated giant cells which ~~can~~ <sup>can</sup> be used as an indicator for the detection of ~~the~~ <sup>this</sup> virus production.

This abstract is rather trivial for ~~our~~ <sup>our</sup> 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 (LAY) ( ) 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 chronic form of a 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 II, may be associated with 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

*human T-cell leukemia/lymphoma virus*

*has been isolated from a patient with lymphadenopathy named also as lymphadenopathy associated virus (LAY) ( ) which is described here as HTLV-III.*

*including its new subtype*

*chronic*

*is reported of HTLV-II*

*associated with the pathogenesis of*

*Here we report development of a system for routine detection of HTLV in patients*

*and large scale application for detailed characterization*

*II*

*Just*

*don't*

*believe it.*

*100%*

*one*

*oh my god*

*infectious*

*AIDS*

*with*

*as AIDS*

*THE*

*major*

*of the*

*isolates*

*belong to*

*a*

*subgroup*

*which*

*is called*

*HTLV-III*

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,~~ <sup>with</sup> absolute lymphopenia and reduced helper T-lymphocyte (OKT4+) subpopulation(s). ~~There is also a~~ <sup>reverse</sup> ~~ratio of helper-to-suppressor T-lymphocyte (OKT4/OKT8), poor lymphocyte responsiveness to mitogens ( ),~~ <sup>in some cases, a decreased</sup> ~~natural killer cell activity was found in~~ <sup>with</sup> ~~the~~ <sup>1</sup>

~~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 ~~the~~ <sup>the</sup> ~~agent~~ <sup>causing</sup> AIDS is a ~~retrovirus~~ <sup>retrovirus</sup> from a family of HTLV. ~~This assumption, besides being a well known precedence of causing immune deficiency in cats (feline leukemia virus) ( ),~~ <sup>is supported by</sup> ~~the facts that retroviruses of the HTLV family are characterized by T-cell tropism,~~ <sup>and</sup> ~~preferentially infect "helper" T-cells (OKT4+),~~ <sup>cytopathic effects on various human and mammalian cells as demonstrated by</sup> ~~cytopathic effects on various human and mammalian cells as demonstrated by~~ <sup>induces cell fusion</sup> ~~syncytia formation ( ),~~ <sup>and the infection of T-cells by HTLV can lead</sup> ~~to an~~ <sup>alter</sup> ~~inhibition of specific T-cell function ( ),~~ <sup>in some</sup> ~~cases may result in a selective cell killing ( ),~~ <sup>and</sup> ~~are transmitted by intimate contact and blood products.~~ <sup>epidemiologically</sup> ~~epidemiologically~~ <sup>showed that the presence of antibodies directed to cell membrane antigens of HTLV infected cells is from 30-40% of patients with</sup> ~~showed that the presence of antibodies directed to cell membrane antigens of HTLV infected cells is from 30-40% of patients with~~ <sup>AIDS ( ).</sup> ~~In addition, over 20 HTLV isolates of both subgroups and~~ <sup>new</sup> ~~new~~ <sup>variants were obtained from patients with AIDS ( ).</sup> ~~The successful~~ <sup>detection and isolation of HTLV was made possible by</sup> ~~detection and isolation of HTLV was made possible by~~ <sup>the</sup> ~~discovery of~~ <sup>TCGF which enabled selective</sup> ~~TCGF which enabled selective~~ <sup>to grow different subsets of normal and</sup> ~~to grow different subsets of normal and~~

*Handwritten notes:*  
- *the* (circled)  
- *causing AIDS is a retrovirus from a family of HTLV*  
- *This assumption, besides being a well known precedence of causing immune deficiency in cats (feline leukemia virus) ( ),*  
- *the facts that retroviruses of the HTLV family are characterized by T-cell tropism,*  
- *preferentially infect "helper" T-cells (OKT4+),*  
- *cytopathic effects on various human and mammalian cells as demonstrated by*  
- *induces cell fusion*  
- *syncytia formation ( ),*  
- *and the infection of T-cells by HTLV can lead*  
- *to an*  
- *alter*  
- *inhibition of specific T-cell function ( ),*  
- *in some*  
- *cases may result in a selective cell killing ( ),*  
- *and*  
- *are transmitted by intimate contact and blood products.*  
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- *the*  
- *discovery of*  
- *TCGF which enabled selective*  
- *to grow different subsets of normal and*

*highly T-suppressor*

and the development of structural assays for HTLV reverse transcription

neoplastic mature T-cells ( ). The viral rescue and transmission of HTLV into permissive cells followed a well established procedure first 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<sub>1</sub> ~~was~~ enabled preferential ~~to obtain~~ <sup>isolation of HTLV types</sup> ~~HTLV<sub>1</sub> variants~~ with immortalizing (transforming) capability ( ). HTLV

variants, which possess "weak" or lack the immortalizing properties for normal T-cells ~~from~~ <sup>peripheral blood</sup> ~~and exhibit~~ <sup>might be more important in cell culture</sup> mainly cytopathic effect on them ~~can only be detected~~ <sup>transiently using</sup> ~~these normal T-cells~~ as target, in cocultivation or cell-free transmission experiments.

This ~~was~~ <sup>was</sup> ~~the~~ <sup>our</sup> main obstacle for frequent isolation and particularly for detailed biological, immunological and nucleic acid characterization of ~~these~~ <sup>obtained</sup> cytopathic variants of HTLV<sub>1</sub>. To overcome these obstacles,

we ~~have~~ performed an extensive survey for a cell population which ~~was~~ <sup>is</sup> highly susceptible to and permissive for cytopathic variants of HTLV and ~~can~~ <sup>preserve</sup> 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~~ <sup>this cell line can be used</sup> for the rescue and continuous production of ~~these variants~~ <sup>viruses</sup> from patients with AIDS and pre-AIDS.

Several in vitro established permanent cell lines originated from human malignancies were ~~initially~~ <sup>initially</sup> assayed for susceptibility to infection with ~~cytopathic~~ HTLV<sub>1</sub> ~~as a reference virus~~ <sup>as a reference virus</sup> (HTLV from Dr. Montagnier) ~~has~~ <sup>was</sup> been used in the first series of experiments. Two cell lines with characteristics of mature T-cells ~~were~~ <sup>were</sup> susceptible to ~~HTLV<sub>1</sub> infection~~ <sup>infection</sup> as determined by reverse transcriptase (RT) assays,

*(circled text)* HTLV<sub>1</sub> as a reference virus (HTLV from Dr. Montagnier) has been used in the first series of experiments. Two cell lines with characteristics of mature T-cells were susceptible to HTLV<sub>1</sub> infection as determined by reverse transcriptase (RT) assays,

*(handwritten notes)* and... initially... HTLV... AIDS... CRAZY

was very good  
your problems

One of them, however, was positive for herpes virus particles, the second one, isolated from a patient with hairy T-cell lymphoma, was negative for HTLV infections as well as no viral particles were found by an extensive electron microscope examination. The infected parental cell line by HTLV-III <sup>infectible</sup> ~~was~~ <sup>is</sup> ~~not~~ <sup>permissive</sup> 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 hemophilic patient <sup>(patient)</sup> ~~with~~ <sup>had antibodies to proteins of</sup> E.T. with lymphadenopathy. The serum of the patient (E.T.) exhibited <sup>specific</sup> ~~positive~~ <sup>reactivity</sup> with HTLV-III ( <sup>I and HTLV-II</sup> ) and reacted with p61 of HTLV transformed human T-cells in precipitation assays ( <sup>p61 is an envelope precursor of HTLV-I</sup> ).

need name of cell line

one was selected for study after initial studies showed that it was negative for HTLV or for any other virus particles by electron microscopy. When it was

<sup>improved</sup> ~~to~~ <sup>to</sup> ~~be~~ <sup>more</sup> ~~permissive~~ <sup>permissive</sup> and ~~highly~~ <sup>highly</sup> permissive T-cell population for HTLV-III <sup>in spite of the cytopathic effects of the virus,</sup> would preserve ~~the~~ <sup>the</sup> permanent growth and continuous virus production. <sup>in</sup> ~~the~~ <sup>the</sup> extensive cloning of the parental T-cell population was performed. A total of 51 single-cell clones were obtained by both capillary ( <sup>the clones were then compared</sup> ) and limited dilution ( <sup>and</sup> ) techniques ~~and~~ <sup>and</sup> ~~performed~~ <sup>performed</sup> for proliferation capabilities of ~~the~~ <sup>the</sup> clones after HTLV-III infection.

and 6

A representative example of a response to ~~the~~ <sup>the</sup> virus infection of 8 T-cell clones which are susceptible to and permissive for HTLV-III is shown in Table 1. In parallel experiments,  $2 \times 10^6$  cells of each T-cell clone were exposed to 0.1 ml of concentrated virus <sup>we isolate</sup> ~~containing~~ <sup>containing</sup>  $10^5$  cpm of reverse transcriptase (RT) activity. <sup>meanings without conditions + total</sup> ~~Then~~ <sup>Then</sup> the cell growth, morphology, <sup>expression</sup> ~~positivity~~ <sup>positivity</sup> 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, ~~by~~ <sup>by</sup> ~~detected~~ <sup>detected</sup> by

but continued protein synthesis of HTLV-III. Considerable amount of virus was found in the culture fluids. ~~by~~ <sup>by</sup> ~~detected~~ <sup>detected</sup> by

~~the~~ <sup>the</sup> ~~fact~~ <sup>fact</sup> ~~that~~ <sup>that</sup> ~~the~~ <sup>the</sup> ~~parental~~ <sup>parental</sup> ~~cell~~ <sup>cell</sup> ~~was~~ <sup>was</sup> ~~not~~ <sup>not</sup> ~~reactive~~ <sup>reactive</sup> with ~~the~~ <sup>the</sup> ~~core~~ <sup>core</sup> ~~protein~~ <sup>protein</sup> and ~~with~~ <sup>with</sup> ~~the~~ <sup>the</sup> ~~envelope~~ <sup>envelope</sup> ~~determinants~~ <sup>determinants</sup> ~~of~~ <sup>of</sup> HTLV-III suggest common ~~of~~ <sup>of</sup> envelope determinants ~~exist~~ <sup>exist</sup> in HTLV-I, II, & III.

Redundant

2597

RIA for the presence of viral antigen(s) and RT activity in culture fluids,

there were considerable differences <sup>in each in other</sup> between infected clones in capability to proliferate after infection. <sup>within</sup> 1-3 days of infection

cytopathic effect was manifested by <sup>an</sup> ~~average~~ <sup>increase to</sup> 10-90% of the initial cell number and, <sup>in addition,</sup> 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) <sup>determined by immunofluorescent assay</sup> in RIA with the patient's serum <sup>from A.T.D.S patient (G.T.)</sup> and hyperimmune rabbit serum raised against the whole dis-

rupted <sup>HTLV-III</sup> virus <sup>was</sup> the range from 10% to over 80%. After 14 days of infection, <sup>the</sup> total cell number <sup>and the proportion of HTLV-III</sup> ~~was~~ <sup>positive cells</sup> ~~was~~ <sup>the</sup> ~~was~~ <sup>increased</sup> in all 8 clones. The highest proliferation <sup>was</sup> ~~was~~ <sup>found</sup> in clone H/4, H/6; <sup>and</sup> ~~and~~ <sup>lowest</sup> was in clone H/3. The virus

positive cultures exhibited consistently <sup>show</sup> round giant <sup>multinucleated</sup> cells which in Wright-Giemsa staining <sup>contained numerous</sup> ~~revealed a high number~~ <sup>of</sup> nuclei (Fig. 1a). Electron

microscopic examinations of the infected cultures showed ~~an abundant number~~ <sup>that they released considerable amounts of virus</sup> of viral particles (Fig. 1b).

To determine whether HTLV-III is continuously produced by the infected T-cells in long term cultures, both ~~the~~ virus production and cell viability of the ~~HTLV-III~~ 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 <sup>has</sup> ~~has~~ <sup>been</sup> ~~been~~ <sup>followed</sup> for <sup>several</sup> ~~more than~~ months. ~~In addition,~~ The viability of the cells <sup>was</sup> ~~was~~ <sup>in</sup> the range from 65-85% and the doubling time <sup>of this culture, which is called</sup> ~~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

cells are similar to those induced by HTLV-III and HTLV-III except that the nuclei exhibit a characteristic ring formation

can continuously produce HTLV-III <sup>that is</sup> in long term culture. permanently growing T-cell population

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 particulate RT <sup>assays of</sup> activity was determined 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 aliquots from the fractions with highest RT activity revealed that the banded virus particles were highly purified. An approximate estimation ( ) of the number of viral particles determined by EM and RT activity suggests that the total yield from <sup>1 ml</sup> culture fluid is about 10<sup>11</sup> particles. <sup>per ml of culture fluid.</sup> Therefore, the data clearly indicate that the established T-cell clones are susceptible to and highly permissive for cytopathic variants of HTLV; all of them preserved proliferation capacity after infection; and <sup>in addition,</sup> as demonstrated in the case of H4/HTLV-III variants, <sup>at least</sup> some of them can proliferate and continuously produce a large amount 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. <sup>Examples are</sup> as shown in Table 2, <sup>these procedures, cocultivation and cell-free infection,</sup> were effective for virus rescue. HTLV-III isolates have been successfully obtained by cocultivation (4 patients) and <sup>by</sup> cell-free infection of T-cell clones (1 patient) or target cells. <sup>(see summary in Table 2)</sup> In all five cases, the virus release into culture fluids was found by RT assay and extracellular virus particles were detected in 3 cases so far.

~~HTLV-III~~ more than ~~one~~ additional isolate or detection of HTLV-III have been obtained in our laboratory <sup>from primary culture of lymphatic tissue.</sup>



~~with the following~~  
 all those detected by other techniques will now be adopted  
 analyzed in the maximum yield and without HTLV-III as well as  
 the positive was ~~by T-cell clones~~ <sup>by T-cell clones</sup> 5, 80s. ~~the data indicates that~~  
 and the cell clones are susceptible 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 primary lack of a~~ <sup>the primary lack of a</sup> proliferative cell  
 system which would be susceptible to and permissive for the virus repre-  
 sented a major obstacle in detection, isolation, and elucidation of the  
~~precise causative agent of this disease.~~ <sup>precise causative agent of this disease.</sup> The establishment of a T-cell population which,  
~~after virus infection can continuously grow and produce virus.~~ <sup>after virus infection can continuously grow and produce virus.</sup>  
 the possibility for detailed biological, immunological and ~~chemical~~ <sup>biochemical</sup> studies of this agent. ~~has opened~~ <sup>has opened</sup> ~~enables~~ <sup>enables</sup> ~~has opened~~ <sup>has opened</sup> the  
 way to ~~continuously detect this virus~~ <sup>continuously detect this virus</sup> way to ~~continuously~~ <sup>continuously</sup>

In all cases  
 when this has  
 already been  
 done - the  
 above system  
 HTLV and HTLV  
 clones

CONCLUSION NOT COMPLETED

REFERENCES NOT DONE  
(per Mka)

cytopathic  
 HTLV-III variants ~~and~~ <sup>and</sup> HTLV in AIDS  
 and provides  
 the first  
 opportunity  
 for detailed  
 molecular  
 immunological  
 analysis. ~~It~~  
~~also provides~~  
 opportunity

Insert - here at end