Science -- First draft

Popovic [redacted]

RESCUE AND CONTINUOUS PRODUCTION OF HUMAN T-CELL LYMPHOTROPIC RETROVIRUS (HTLV-III) FROM PATIENTS WITH AIDS

— Way to deal c̄ this LAV - originally

① Lack of cross hybridiz. c̄ I, II

② " " Ag " reaction

③ Relationship to CIA

④ in published serology

When the hell are the figures

ABSTRACT

A susceptible and permissive human neoplastic T-cell population is described for routine isolation of cytopathic variants of human T-cell lymphotropic retroviruses (HTLV-III) which can be 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 therefore enables us to prepare specific viral probes for immunological and nucleic acid studies can be prepared. One cytopathic effect of HTLV-III infection is the induction of multi-nucleated giant cells which can be used as an indicator for the detection of this virus.

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 ( ). 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~~ chronic form of a T-cell variant of hairy cell leukemia ( ). To date, this ~~virus represents~~ is the only reported isolate of HTLV-II obtained from a patient with a 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~~ associated with the acquired immune deficiency syndrome (AIDS) ( ). Here we report development of a system for routine detection & isolation of these and other HTLV variants in patients with AIDS and pre-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~~, absolute lymphopenia and reduced helper T-lymphocyte (OKT4+) subpopulation(s). ~~This results 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 was 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 [illegible] have proposed that a [illegible] 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 virus (   ), ~~is based on the facts that~~ retroviruses of the HTLV family ~~are characterized by~~ T-cell tropism, they 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 can lead to~~ an [illegible] of ~~a specific~~ T-cell function (   ), [illegible] in some cases may result in a selective cell killing (   ). ~~Moreover~~, [illegible] epidemiological [illegible] 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 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 sensitive assays for retroviral reverse transcriptase based on

neoplastic mature T-cells ( ). The viral rescue and transmission of HTLV into permissive cells followed well established procedures 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 enabled preferentially isolation of HTLV types with immortalizing (transforming) capability ( ). HTLV variants which possess "weak" or lack immortalizing properties for normal T-cells from peripheral blood of healthy and exhibit mainly cytopathic effect on them might be more important in the cause of AIDS. In fact such variants were frequently detected but only can only be detected transiently using these normal T-cells as targets 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 obtained chiefly from patients with AIDS or "pre" AIDS. To overcome these obstacles, we performed an extensive survey for a cell population which is highly susceptible to and permissive for cytopathic variants of HTLV and preserve the capacity for permanent growth of the cells after infection with the virus. We report here the establishment and characterization of a new immortalized T-cell population which is susceptible to and permissive for HTLV cytopathic variants and can be used for their routine rescue and continuous production of these viruses from patients with AIDS and pre-AIDS.

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

Mika
You are CRAZY

One of them, however, was positive for herpes-type virus particles, the second one originated from a patient with marine 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 exhibited positivity 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 disrupted HTLV-III ( ) and reacted with p61 of HTLV transformed human T-cells in precipitation assays( ). In order to susceptible and permissive T-cell population for HTLV-III in spite of the cytopathic effect of the virus, and to preserve permanent growth and continuous virus production in the presence of the 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 the infected T-cell clones after 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 virus 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, as determined by

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One was selected for study after initial studies showed that it was negative for HTLV or for any other viral particles by electron microscopy. When it was determined that [illegible] continuous production of HTLV-III.

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. Within 6 days of infection, a cytopathic effect was manifested by a decrease from 10 to 90% of the initial cell number and, in addition, 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) determined by immunofluorescent assay in IFA with the patient's serum (from A.I.D.S patient E.T.) and hyperimmune rabbit serum raised against the whole disrupted HTLV-III virus was in the range from 10% to over 80%. After 14 days of infection, total cell number and the proportion of HTLV-III a portion of IFA positive cells for the viral antigens increased in all 8 clones. The clones with the fastest growth rates highest proliferation was found in clone H/4, H/6; and H/3 and lowest was in clone H/3. The virus positive cultures exhibited consistently large multinucleated giant cells which in Wright-Giemsa staining revealed a high number of nuclei (Fig. 1a). These multinucleated giant cells are similar to those induced by HTLV-I and HTLV-II except that the nuclei exhibit a characteristic ring formation Electron microscopic examinations of the infected cultures showed an abundant number of viral particles (Fig. 1b). that they released considerable amounts of virus

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-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 has been followed for several months. In addition, the viability of the cells was in the range from 65-85% and the doubling time of the H4/HTLV-III, this culture, which is called cell culture was approximately 36-48 hours (data not shown) after 3 weeks of infection. Thus, the data clearly indicate

continuous HTLV-III production by permanently growing T-cell population in long term culture.

, 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 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 at density 1.16g were highly purified. An approximate estimation ( ) of the number of viral particles determined by EM and RT activity suggests that the viral yield from culture fluids is about 10^{11} virus particles, the data clearly indicate that 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 clones, at least 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. As shown in Table 2, these procedures, cocultivation as well as cell-free infection, were effective for virus rescue. HTLV-III isolates have been successfully obtained by cocultivation from (4 patients) and in one case by cell-free infection of T-cell clones (H/4 and H/9) as target cells. In all five cases, the virus release into culture fluids was found by RT assay and extracellular virus particles were detected in cases so far

More than — additional isolate or detection of HTLV-III have been obtained in our laboratory

all those detected by other techniques will now be adopted to these T-cell clones for [illegible] detailed analysis.

[illegible] both sera reacted with acetone-fixed cells, and

and the positivity was between 5 and 80%. [illegible] 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 a T-cell population described here, which after virus infection can 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 detect the presence of HTLV in AIDS and provides the first opportunity for detailed molecular immunological analyses.

In all cases where this has already been done - the [illegible] H/4 and H/9 clones

CONCLUSION NOT COMPLETED

cytopathic variants

REFERENCES NOT DONE

(per Mika)

Insert - here at end

[redacted]

One [redacted] effect of HTLV-III on other systems is the formation of multiple multinucleated giant cells in a characteristic ring formation in the expected T-cell population. These structures can be used as an indicator to detect HTLV-III in clinical specimens. This system [redacted] opens the way to a rapid, routine detection of HTLV-III and related HTLV cytopathic variants of HTLV

Finally, a [illegible] T-lymphotropic retrovirus different from HTLV-I and II and associated with lymphadenopathy syndrome was detected [illegible] earlier ( ).

We found that this virus, called LAV (provided by L. Montagnier & J.C. Chermann) also grows in H4 and produces similar cytopathic effects on it ~~that [illegible]~~ as HTLV-III.

The LAV isolate was reported to be related to equine infectious anemia virus, and ~~not to [illegible]~~; the sera from ___% of patients with AIDS reacted with it. ~~In contrast,~~ ~~HTLV-III In contr~~ ~~Etc. material~~.

In contrast, HTLV-III is related to HTLV-I + II ~~by nucleic acid homolo~~

([illegible] ___ et al. This

are similar with respect to HTLV-III & LAV. These findings suggest that HTLV-III and LAV may be different. However, it is possible that this is due to the insufficient characterization of the LAV isolate. Due to poor virus production.

The question of the relationship of the various HTLV-III isolates to LAV and to other isolates from AIDS and pre-AIDS patients can now be accomplished.

Table 2. Rescue of HTLV-III from Patients with Lymphadenopathy (pre-AIDS) and AIDS

Patient (Initials)	Diagnosis	Origin	Virus Expression			EM
			RT Activity ($\times 10^4$ cpm)	IFA with Rabbit Serum (% Positive)	IFA with Human Serum (ET) (% Positive)	
RF*	AIDS (heterosexual)	U.S.	6.3	80	33	+
SN*	Hemophiliac (lymphadenopathy)	Haiti	0.25	10	ND	ND
BK*	AIDS (homosexual)	U.S.	0.24	44	5	+
LS*	AIDS (homosexual)	U.S.	0.13	64	19	+
WT**	Hemophiliac (lymphadenopathy)	U.S.	3.2	69	ND	ND

*Cocultivation with H4 target T-cells

**Cell-free infection

IFA is immunofluorescent assays

EM is electron microscopy.

Their assay indicate that
~~HTLV~~ These ~~RD~~ are
also T4 lymphotropic
retroviruses with ~~similar~~ many
properties similar to HTLV-I
and I but ~~not cross~~ including
~~reactive with monoclonal~~
~~antibodies to HTLV-I + II~~,
~~to purified~~ cross reactivity as determined
with hyperimmune sera to
HTLV-II proteins to HTLV-I ~~RBL~~
purified p24 but not
with monoclonal antibodies
to HTLV-I p19 or p24-
(See paper by ——— et al
this issue). ~~Until a full characterization~~
~~we designate these isolates as all of them~~
~~HTLV-III~~ We call all these HTLV-III
~~although~~ it is not yet proven that all are identical.

Now Turn

BACK OVER.

However, ~~another~~ HTLV-I and ~~one~~ HTLV-II have not been routinely ~~found~~ isolated in ~~this disease~~ AIDS or pre-AIDS ~~cases~~. Recent serological studies on the other hand suggest that ~~a~~ these patients have antibo