cent of patients with AIDS were found to react with it (38). In contrast, HTLV-III is found to HTLV-I and II (39, 40) and, by allelic criteria, this virus belongs to the HTLV family of retroviruses. In addition, more than 85 percent of serum samples from AIDS patients are reactive with proteins of HTLV in AIDS patients and provides the first opportunity for detailed immunological characterization of LAV because the virus has not yet been transmitted to a permanently growing cell line for true isolation and elucidation of the precise causative agent of AIDS. The isolation of LAV from cells in AIDS patients and the previous lack of a cell system that could maintain growth and still be susceptible to and permissive for the virus represented a major obstacle in detection, isolation, and elucidation of the HTLV-III and LAV variants of HTLV in cells from AIDS patients. Also at risk are heterosexuals, intravenous drug users, hemophiliacs, and, more recently, homosexual or bisexual males (about 70 percent of reported cases). Also at risk are hetero-

The transient expression of cytopathic variants of HTLV in cells from AIDS patients and the previous lack of a cell system that 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 AIDS. The isolation of T-cell populations that continuously grow and produce virus after infection opens the way to the routine detection of cytopathic variants of HTLV in AIDS patients and provides the first opportunity for detailed immunological (31, 33) and molecular analyses of these viruses.

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33. March 1984; accepted 19 April 1984

Frequent Detection and Isolation of Cytopathic Retroviruses (HTLV-III) from Patients with AIDS and at Risk for AIDS

Abstract. Peripheral blood lymphocytes from patients with the acquired immunodeficiency syndrome (AIDS) or with signs or symptoms that frequently precede AIDS (pre-AIDS) were cultured in vitro with added T-cell growth factor and assayed for the expression and release of human T-hemotropic retroviruses (HTLV). Retroviruses belonging to the HTLV family and collectively designated HTLV-III were isolated from a total of 48 subjects including 18 of 21 patients with AIDS, three of four clinically normal mothers of juveniles with AIDS, 26 of 72 adult and juvenile pat-
ents with AIDS, and from one of 22 normal male homosexual subjects. No HTLV-III was detected in or isolated from 115 normal heterosexual subjects. The number of HTLV-III isolates reported here underestimates the true prevalence of the virus since many specimens were received in unsatisfactory condition. Other data show that serum samples from a high proportion of AIDS patients contain antibodies to HTLV-III. That these new isolates are members of the HTLV family but differ from the previous isolates known as HTLV-I and HTLV-II is indicated by their morphological, biological, and immunological characteristics. These results and those reported elsewhere in this issue suggest that HTLV-III may be the primary cause of AIDS.

The acquired immunodeficiency syn-
drome known as AIDS was initially recog-

1. Groups reported to be at risk for AIDS include homosexual or bisexual males (about 70 percent of reported cases), intravenous drug users (about 17 percent of cases), and Haitian immigrants to the United States (about 5 percent of cases). Also at risk are hetero-

2. In some cases, the number of helper T lymphocytes may be reduced by a virus-related T-cell proliferation (pro-AIDS). This include

3. The increasing incidence of this disease, the types of patients affected, and other epidemiological data suggest the existence of an infectious etiologic agent that...
can be transmitted by intimate contact or by whole blood or separated blood components (2). As indicated by Popovic et al. (3), we and others have suggested that specific human T-lymphotropic retroviruses (HTLV) cause AIDS (4, 5). Many properties of HTLV are consistent with this idea (6).

An association of members of the HTLV family with T lymphocytes from some AIDS or pre-AIDS patients was reported previously. For example, the first subgroup of HTLV to be characterized, HTLV-I, was isolated recently from T cells from about 10 percent of AIDS patients, and a virus related to HTLV-II was isolated from one AIDS patient (4). Another HTLV isolate was obtained from the lymph nodes of a patient with lymphadenopathy and at risk for AIDS (7). This isolate has been difficult to grow in quantities sufficient to permit its characterization. HTLV proviral DNA was detected in T lymphocytes from two additional AIDS patients (8) and HTLV-related antigens were found in another two patients (4). Studies in which disrupted HTLV-I or the purified structural proteins (p24 or p19) were used to detect antibodies in serum samples from patients with AIDS and pre-AIDS indicated that 10 to 15 percent of the patients had been exposed to HTLV-I (9). Essex and his co-workers, using HTLV-infected T-lymphocyte cultures to detect antibody in serum samples from patients with AIDS and pre-AIDS, indicated that 10 to 15 percent of the patients had been exposed to HTLV-I (9). Essex and his co-workers, using HTLV-infected T-lymphocyte cultures to detect antibody in serum samples from patients with AIDS and pre-AIDS had been exposed to HTLV-I (9). Essex and his co-workers, using HTLV-infected T-lymphocyte cultures to detect antibody in serum samples from patients with AIDS and pre-AIDS had been exposed to HTLV-I (9). Further studies suggested that at least some of the antigens detected in this system were products of the genome of a member of the HTLV family (10), but it was not known whether the antibodies were specifically against HTLV-I, HTLV-II, or a virus of a different subgroup.

With the availability of large quantities of HTLV-III (3), it became possible to develop specific immunological reagents that would facilitate its characterization. HTLV-III was found to share many properties with other HTLV isolates (6), but it was morphologically, biologically, and antigenically distinguishable (3, 11). Here we describe the detection and isolation of HTLV-III from a large number of patients with AIDS and pre-AIDS.

For these studies we used cell culture conditions previously developed in our laboratory for the establishment of T lymphocytes in culture and for the detection and isolation of HTLV-I and HTLV-II from leukemic donors (12). Evidence for the presence of HTLV-III included: (i) viral reverse transcriptase (RT) activity (12) in supernatant fluids; (ii) transmission of virus by coculturing T cells with irradiated donor cells or with cell-free fluids (3, 13); (iii) observation of virus by electron microscopy (12, 13); and (iv) the expression of viral antigens in indirect immune fluorescence assays using serum from a patient positive for antibodies to HTLV-III as described (5, 11), or antisera prepared against purified, whole disrupted HTLV-III (11). Cells and supernatant fluids were also monitored for the expression of HTLV-I and HTLV-II by using antibodies to the viral structural proteins p19 and p24 and by indirect immune fluorescence and radioimmunoprecipitation procedures (14).

As summarized in Table 1, we found...
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HTLV-III in 18 of 21 samples from patients with pre-AIDS, from three of four clinically normal mothers of juvenile AIDS patients, three of eight juvenile AIDS patients, 13 of 43 of adult AIDS patients with Kaposi's sarcoma, and 10 of 21 adult AIDS patients with opportunistic infections. Virus was detected in only one of 22 samples from clinically normal, nonpromiscuous homosexual males believed to be at moderate risk for AIDS. It is interesting, however, that 6 months after these tests were conducted the one positive normal homosexual subject developed AIDS. In no instance, 0 of 115, was virus detected in or isolated from cells of the normal volunteers. Samples from 15 of these were tested under rigorously controlled conditions, which included addition of antibody to α-interferon.

Primary cells from patients usually produce virus for 2 to 3 weeks (Fig. 1). After this time the production of virus declines even though the culture may contain actively replicating cells that can be maintained for long periods in the presence of added T-cell growth factor (TCGF). In some instances virus release can be reinitiated by the addition of antibody to α-interferon (Fig. 1). The HTLV-III-producing cell cultures were characterized by established immunological procedures (13). They were predominantly T lymphocytes (E rosette receptor-positive, OKT3+ and Leu1+) with a helper-inducer phenotype (OKT4+ and Leu3+). The uniformly morphological appearance of HTLV-III is shown in Fig. 2. The diameter of the virus is 100 to 120 nm, and it is produced in high numbers from infected cells by budding from the cell membrane. A possibly unique feature of this virus is the cylindrical shaped core observed in many mature virions.

The incidence of virus isolation reported here probably underestimates its true incidence since many tissue specimens were not received or handled under what we now recognize as optimal conditions (15). This is particularly so for the samples received from late-stage AIDS patients. Such samples usually contain many dying cells and very few viable T4 lymphocytes. However, a high proportion of patients with AIDS and pre-AIDS have circulating antibody to HTLV-III (11).

The HTLV-III produced by cultured T cells from patients with AIDS and pre-AIDS is highly infectious and can be readily transmitted to fresh umbilical cord blood and adult peripheral blood or bone marrow lymphocytes. The production of HTLV-III by these cells is transient, often declining to undetectable levels by 2 to 3 weeks after infection (data not shown). The transmission of HTLV-III to an established T-cell line (3), however, makes possible its production in large quantities for detailed analyses and for development of reagents for its detection (3, 11).

That the viruses we have named HTLV-III belong to the HTLV family is indicated by their T-cell tropism, Mgp-dependent RT of high molecular weight, antigenic cross-reactivity with HTLV-I and -II (11), cytopathic effects on T lymphocytes (3), and their morphological appearance in the electron micrograph. HTLV-III also contains some structural proteins similar in size to those of other members of the HTLV family (11).

These studies of HTLV-III isolates from patients with AIDS and pre-AIDS and from some healthy individuals at risk for AIDS provide strong evidence of a causative involvement of the virus in AIDS.

### Table 1. Detection and isolation of HTLV-III from patients with AIDS and pre-AIDS.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number positive for HTLV-III</th>
<th>Number tested</th>
<th>Percent positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>re-AIDS</td>
<td>18</td>
<td>21</td>
<td>85.7</td>
</tr>
<tr>
<td>clinically normal mothers of juvenile AIDS patients</td>
<td>3</td>
<td>4</td>
<td>75.0</td>
</tr>
<tr>
<td>HIV+ AIDS patients</td>
<td>3</td>
<td>8</td>
<td>37.5</td>
</tr>
<tr>
<td>dulant AIDS with Kaposi's sarcoma</td>
<td>13</td>
<td>43</td>
<td>30.2</td>
</tr>
<tr>
<td>dulant AIDS with opportunistic infections</td>
<td>10</td>
<td>21</td>
<td>47.6</td>
</tr>
<tr>
<td>linically normal homosexual donors</td>
<td>10</td>
<td>72</td>
<td>4.3</td>
</tr>
<tr>
<td>linically normal heterosexual donors</td>
<td>0</td>
<td>115</td>
<td>0</td>
</tr>
</tbody>
</table>

With the exception of the normal heterosexual donors and some of the clinically normal mothers of juvenile AIDS patients, all others belong to one of the groups of people identified as being at risk for AIDS: homosexual men, intravenous drug users, intravenous drug users, heterosexual contacts of members of a high-risk group, hemophiliacs treated with pooled blood products, recipients of multiple blood transfusions, and a few of parents born of parents belonging to other groups at risk. Pre-AIDS includes patients with unexplained lymphadenopathy and leukopenia, with an inverted T4/T8 lymphocyte ratio. The clinically normal, nonpromiscuous, homosexual subjects are from Washington, D.C., and are believed to be at moderate risk. The clinically normal heterosexual donors include both male and female subjects living not at risk for AIDS.
Serological Analysis of a Subgroup of Human T-Lymphotropic Retroviruses (HTLV-III) Associated with AIDS

Abstract. The two main subgroups of the family of human T-lymphotropic retroviruses (HTLV) that have previously been characterized are known as HTLV-I and HTLV-II. Both are associated with certain human leukemias and lymphomas. Cell surface antigens (p61 and p65) encoded by HTLV-I are frequently recognized at low titers, by antibodies in the serum of patients with acquired immunodeficiency syndrome (AIDS) or with signs or symptoms that precede AIDS (pre-AIDS). This study reports the discovery of HTLV in these disorders. Another subgroup of HTLV, designated HTLV-III, has not been isolated from patients with AIDS and pre-AIDS. In the studies described here, virus-associated antigens in T-cell clones permanently producing HTLV-III were subjected to biochemical and immunological analyses. Antigens of HTLV-III, specifically detected by antibodies in serum from AIDS or pre-AIDS patients and revealed by Western blot techniques, are similar in size to those found in other subgroups of HTLV. They include at least three serologically unrelated antigenic groups, one of which is associated with group-specific antigens (p55 and p24) and another with envelope-related (p65) proteins, while the antigens in the third group are of unknown affiliation. The data suggest an involvement of HTLV in these disorders. Another subgroup of HTLV, associated with human cells infected by HTLV-III, are specifically recognized by antibodies in serum from AIDS and pre-AIDS patients, and present a preliminary biochemical and immunological analysis of these antigens.

Lysates of two immortalized and infected human T-cell clones, H4-HTLV-III and H17-HTLV-III (17), were tested with samples of human serum in a strip radioimmunoassay (RISA) based on the Western blot technique (18). The sera were from patients with AIDS or pre-AIDS, from contacts of such patients, and from homo- or heterosexual male controls. Sera from the same patients were also tested by the enzyme-linked immunosorbent assay (ELISA) with purified HTLV-III as part of a larger, systematic serologic study of the prevalence of antibodies to HTLV-III in AIDS and pre-AIDS patients (19).

Representative results are shown in Fig. 1. Sera from patients with AIDS or pre-AIDS, and from some homosexuals and heroin-addicts, recognized a number of specific antigens not detected by sera from heterosexual subjects. The most prominent reactions were with antigens of the following molecular weights: 65,000, 60,000, 55,000, 41,000, and 24,000. Antibodies with molecular weights of approximately 88,000, 80,000, 39,000, 32,000, 28,000, and 21,000 gave less prominent reactions. The reaction with the antigen of 55,000 (p55) only occurred in sera that also recognized p24, suggesting a relationship between the two antigens.

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