nological response, with the severity of the inflammation being attributable to the density of joint innervation and, more specifically, to the release of SP into the joint by peripheral afferent fibers. Conceivably the increase in SP levels in nerves that innervate an inflamed joint is secondary to the immunological mechanisms operating in the joint. The SP would exacerbate the inflammation, and thus may not have the reparative role proposed by Lembeck and Gamse (*16*).

The mechanism by which SP acts is unknown. It is significant that local application of SP produces many of the tissue changes of acute inflammation, including vasodilation, increased vascular permeability (*17*), pavementing of leukocytes in venules, stimulation of phagocytosis by polymorphonuclear leukocytes, and mast cell degranulation (*18*). Thus, SP may directly increase the inflammatory response in arthritic joints. It follows that attempts to diminish SP levels in these joints may prove effective in reducing the inflammation and tissue destruction.

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Transmission of HTLV-III Infection from Human Plasma to Chimpanzees: An Animal Model for AIDS

Abstract. *Two of three chimpanzees given plasma from patients with acquired immune deficiency syndrome (AIDS) or pre-AIDS showed serum antibodies to type III human T-cell leukemia virus (HTLV-III) 10 to 12 weeks after transfusion. One animal also developed lymphadenopathy, transient depression of the ratio of T4 to T8 lymphocytes, and impaired blastogenic responses. No opportunistic infections occurred. Adenopathy persisted for 32 weeks, and antibody to HTLV-III persisted for at least 48 weeks. This transmission of HTLV-III by lymphocyte-poor plasma confirms the potential risk of such plasma or plasma derivatives to recipients. The susceptibility of the chimpanzee to HTLV-III infection and the ability to simulate the human lymphadenopathy syndrome in this animal makes it a valuable model for further study of AIDS.*

Originally identified in male homosexuals and abusers of intravenous drugs (*1*), the acquired immune deficiency syndrome (AIDS) has more recently been recognized as a potential consequence of blood transfusion (*2*). Our investigation, designed to determine whether there was a transmissible agent in human blood capable of inducing AIDS and to establish an animal model in which the pathogenesis, treatment, and prevention of AIDS could be studied, began before the virologic investigations (*3–5*) that linked human AIDS to a type C retrovirus. Retroviruses designated human T-cell leukemia virus type III (HTLV-III) (*3*) and lymphadenopathy-associated virus (LAV) (*5*) have been reproducibly recovered from patients with AIDS and the AIDS-related syndrome. Whether or not HTLV-III and LAV are identical viruses remains to be determined. Common to both these agents is tropism for the T4 lymphocyte, a cell whose depletion is the focal point of the chain of immunologic and clinical events that comprises AIDS (*1*).

In experiments to maximize the potential for AIDS transmission to chimpanzees, each of three study animals (CH132, CH114, and CH133) was sequentially infused with plasma from three different patients selected to represent the spectrum of AIDS-related disorders (specifically, the lymphadenopathy syndrome, Kaposi's sarcoma, and life-threatening opportunistic infection) as defined by the Centers for Disease Control (*6*). One animal served as a control and received 3 units of normal donor plasma. To enhance further the potential for transmission of an agent with a low titer of antibody and to simulate the human transfusion experience, inocula were given in large volume (50 to 150 ml) to each chimpanzee. Plasmas were ABO-compatible, and no adverse reactions were associated with this interspecies transfusion program. The chimpanzees were housed individually in an isolation hut at the Southwest Foundation for Biomedical Research (SFBR) in San Antonio, Texas. Their clinical status was monitored by biweekly physical examination, and their immunologic status was assessed by biweekly determination of absolute lymphocyte counts, the number of T3, T4, and T8 subsets, B cells, natural killer cell activity, lymphocyte mitogen responsiveness, interleukin-2 activity, and reactions in mixed lymphocyte culture by means of established techniques (*7*).

Antibodies to HTLV-III were determined in a solid-phase ELISA (enzyme-linked immunosorbent assay) with the H9 HTLV-III clone (*3*) as described (*8*). Assays were performed in duplicate, and

results were expressed as a ratio of the absorbance of the test sample compared to the average of four negative controls. A ratio greater than 6.0 was considered as indicating the presence of antibody to HTLV-III. Positive samples in the screening assay were subsequently titered, and selected samples were tested for the presence of immunoglobulin M (IgM) antibodies to HTLV-III (*6*). All samples were tested under code.

Before inoculation, all chimpanzees were seronegative for antibodies to HTLV-III and had normal T4 numbers, T4:T8 ratios, and mitogen responses. The first animal (CH132, Fig. 1A) received 150 ml of plasma from each of three donors (P_6, P_7, and P_8), who were seropositive for antibodies to HTLV-III (titers were more than 1:1,000,000 for P_6, 1:770,000 for P_7, and 1:73,000 for P_8). The early appearance of antibodies to HTLV-III was followed by a continuous decline to baseline by week 28 after inoculation (Fig. 1A). This is a pattern of passive transfer of antibody from donor to recipient and does not indicate HTLV-III infection. In view of the susceptibility to HTLV-III infection of other chimpanzees in this study (see below), the absence of infection in CH132 implies that some AIDS patients positive for antibodies to HTLV-III are not infectious or that susceptibility to AIDS is governed by factors other than the presence or absence of humoral antibody to HTLV-III (or both).

The next animal (CH114, Fig. 1B) received 150 ml of plasma from each of three donors (P_1, P_2, and P_3) over a 3-day period. Donor P_1 had lymphadenopathy and immunologic abnormalities consistent with AIDS but was asymptomatic and remains well 1 year after initiation of this study. Donor P_2 had Kaposi's sarcoma, and donor P_3 had life-threatening opportunistic infections; both died within 6 months of their apheresis procedure. All three donors had antibody to HTLV-III with titers of 1:215,000, 1:48,000, and 1:930,000, respectively.

Antibodies to HTLV-III in CH114 were detectable at a titer of 1:7300 in the first sample obtained 2 weeks after inoculation (Table 1 and Fig. 1B). Antibody titers then diminished in each biweekly sample, reproducing the pattern of passive transfer observed in CH132 (Fig. 1A). During week 10, however, the titer of antibodies to HTLV-III began to rise steeply to 1:7900 and then reached a plateau with fluctuation to a peak at 1:9700. Antibody then diminished but has persisted at titers of 1:4000 to 1:6000 throughout 1 year of follow-up examinations. The specificity of this antibody for HTLV-III was confirmed by Western blot analysis (*9*) and by blocking the ELISA reactivity with HTLV-III–specific sheep antiserum (*10*). At week 24, 14

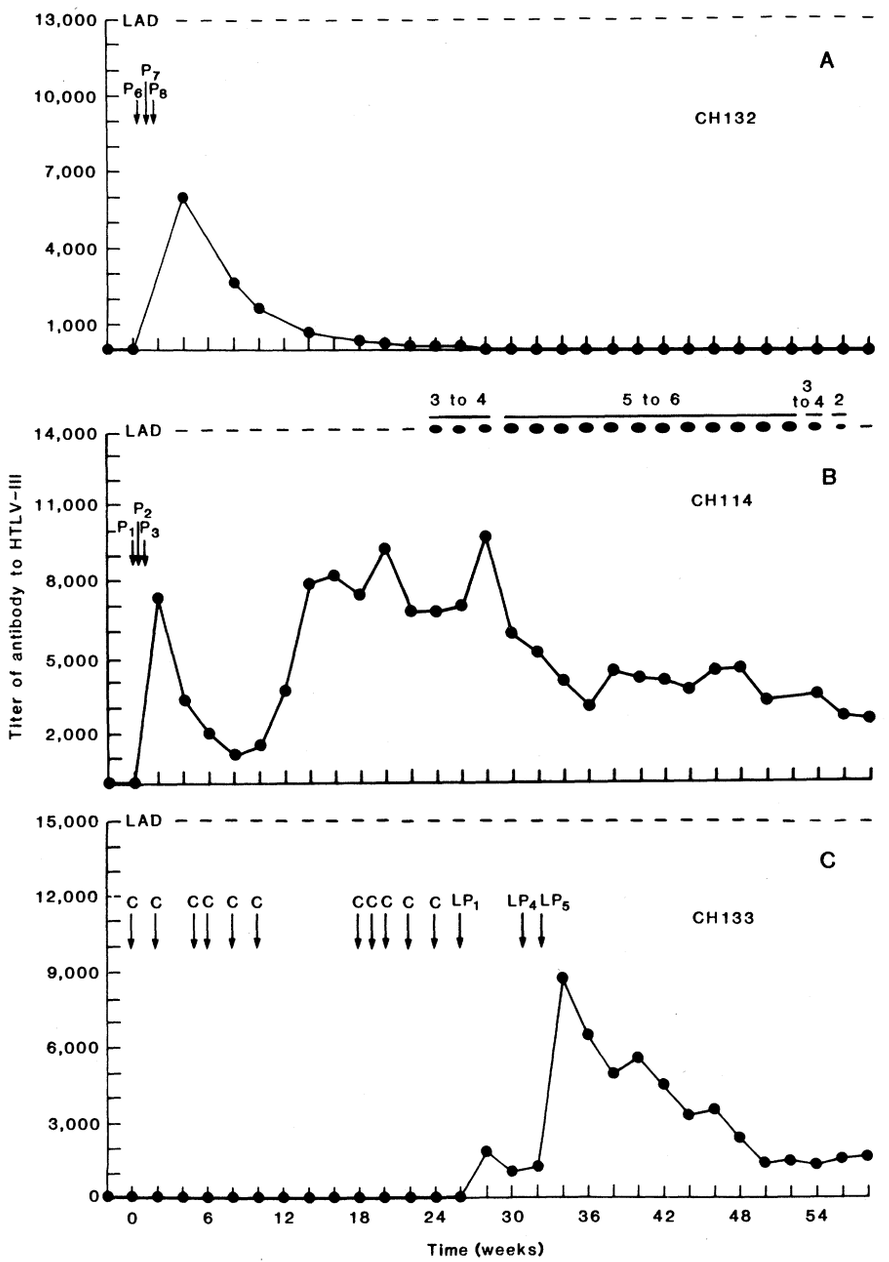

Fig. 1. Clinical and serologic events in chimpanzee 132 (A), 114 (B), and 133 (C). Antibody to HTLV-III was measured in an ELISA and is expressed as the reciprocal titer. LAD relates to the degree of lymphadenopathy expressed either as none (−) or as a figure representative of the relative size (in centimeters) of the largest node palpated. Arrows indicate the date of transfusion; P represents plasma (150 ml), LP represents lymphocyte-rich plasma (150 ml), and C represents single donor cryoprecipitate (each arrow represents 3 to 4 units, each unit being the amount of cryoprecipitate derived from 300 ml of plasma). Donor P_1 and LP_1 were the same donor with the lymphadenopathy syndrome. All other donors were different AIDS patients. (A) CH132 (50 kg in weight, 9 years of age) had antibodies to HTLV-III in the first specimen after inoculation, and the antibody level declined progressively to zero, a pattern consistent with the decay of passively transfused antibody. No evidence of HTLV-III infection was noted despite inoculation of the animal with large volumes of plasma from donors positive for antibodies to HTLV-III. (B) In CH114 (51 kg in weight, 8 years of age) a similar pattern of passive antibody transfer was noted but with active production of antibody beginning at weeks 10 to 12. Antibodies to HTLV-III persisted throughout the course of follow-up examinations. Twelve weeks after the appearance of antibody, the chimpanzee developed substantial lymphadenopathy which persisted for 32 weeks. (C) CH133 (43 kg in weight, 7 years of age) showed no serologic response to repeated infusions of cryoprecipitate. The presence of active antibody to HTLV-III is indicated by the plateau of antibody titer beginning at week 50, a pattern inconsistent with the continuous decay of passively acquired antibody. In addition, IgM antibody to HTLV-III was detected at weeks 32 to 40 (see text). CH133 did not develop adenopathy or clinical illness.

weeks after antibody seroconversion, CH114 was first noted to develop bilateral inguinal lymphadenopathy (4 by 2 cm) and a lesser degree of cervical adenopathy. Inguinal nodes progressed in size to 6 by 3 cm and persisted at this size for 22 weeks before gradually diminishing; the total duration of adenopathy was 32 weeks. This degree of adenopathy had never been observed in any of the chimpanzees housed at SFBR (*11*). An inguinal lymph node biopsy specimen obtained at week 33 showed severe lymphoid hyperplasia (Fig. 2). An inguinal node biopsy done before the inoculation had not revealed these hyperplastic changes. Special stains, including Giemsa, periodic acid–Schiff, methenamine silver, and Fite, did not reveal mycobacteria, fungi, *Pneumocystis carinii*, or other microorganisms. Also, CH114 did not show antibody seroconversion for the cytomegalovirus or Epstein-Barr virus.

Coincident with the adenopathy, the percentage of T4 cells, the T4:T8 ratio, and lymphocyte response to mitogens (particularly phytohemagglutinin) all decreased (Table 1). The decrease in the percentage of T4 cells and in the T4:T8 ratio was transient but was observed over a 4-week interval (weeks 30 to 34), which coincided with the early period of peak adenopathy. Thereafter, immunologic factors returned to baseline levels. Despite the development of antibodies to HTLV-III, the adenopathy, and the immune defects, CH114 remained clinically well throughout 1 year of follow-up studies. In essence, this chimpanzee developed a syndrome similar to that of donor P_1 (asymptomatic lymphadenopathy syndrome) except that the immune defects were more transient.

The last animal (CH133, Fig. 1C) was used in a two-phase experiment. First, repeated inoculations of single-donor cryoprecipitate were given to determine whether the immunologic abnormalities frequently observed in hemophiliac patients (*12*) could be induced in this manner and whether stimulation with foreign protein might enhance susceptibility to subsequent doses of plasma or lymphocytes contaminated with the AIDS agent. The intermittent administration of 39 units of single-donor cryoprecipitate did not result in depression of the T4:T8 ratio or in the development of antibodies to HTLV-III (Fig. 1C). In the second phase of the study, beginning at week 26, CH133 received 150 ml of lymphocyte-rich plasma (LP) from donor LP_1 (the same donor whose plasma was given to CH114). On week 31, CH133 received lymphocyte-rich plasma from donor LP_4, a patient with Kaposi's sarcoma (HTLV-III antibody titer, 1:24,000). On week 32, plasma and lymphocytes from donor LP_5, a patient with an extensive lymphadenopathic form of Kaposi's sarcoma (HTLV-III antibody titer, >1:1,000,000), were administered.

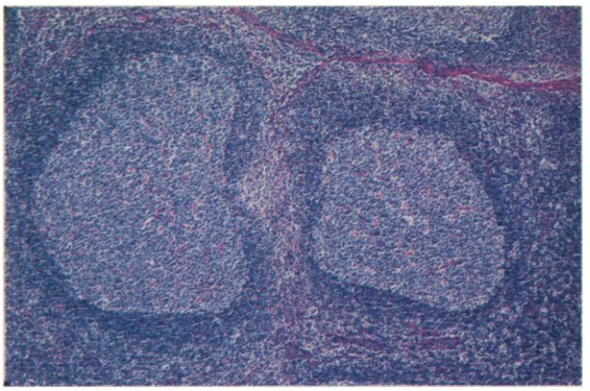

Fig. 2. Photomicrograph of a lymph node biopsy specimen from CH114 showing severe lymphoid hyperplasia with markedly enlarged germinal centers populated by cells with large vesicular nuclei and an indistinct eosinophilic cytoplasm. Mitotic figures are numerous. A biopsy done before inoculation did not show these hyperplastic changes.

The interpretation of HTLV-III antibody response in CH133 is confounded by the intervals between transfusions and the large volume of passively administered antibody. Nonetheless, it appears that this animal developed an active HTLV-III antibody response on the basis of (i) the plateau of antibody titer beginning at week 50, a pattern inconsistent with the continuous decay curve of passively administered antibody as seen in CH132 (Fig. 1A), and (ii) the initial appearance of IgM antibodies to HTLV-III at week 32, which persisted through

Table 1. Clinical, serologic, and immunologic events in chimpanzee 114. Week 0 is a 3-day period during which CH114 received sequential plasma transfusions (150 ml each) from three human donors (P_1, with lymphadenopathy; P_2, with Kaposi's sarcoma; and P_3, with opportunistic infection). LAD is lymphadenopathy: (−) none, (+) 1 cm, (++) 2 cm, (+++) 3 to 4 cm, and (++++) 5 to 6 cm. T4:T8 is the ratio of the number of T4 helper lymphocytes to T8 suppressor lymphocytes (the normal number of T4 is 43.5 ± 7.5, and the normal ratio is 0.96 ± 0.27). Phytohemagglutinin (PHA) and pokeweed mitogen (PWM) responses are expressed as the stimulatory ratio (*13*); note that this ratio consistently diminished during the period of peak adenopathy. Titers of antibody to HTLV-III were measured by serial dilutions in the ELISA as described (*6*). Absorbance and dilution data were fitted with a least-squares procedure, and an end point was determined by the value of a normal control serum diluted 1:20. N.D., not done.

Week	LAD	Reciprocal titer of antibody to HTLV-III	Total lymph (per cubic millimeter)	T4 (%)	T8 (%)	T4:T8	Mitogen response	
							PHA	PWM
−2	−	<40	3696	55.0	55.0	1.00	1.0	0.4
0	−	<40	5016	52.2	44.6	1.17	0.8	1.3
2	−	7300	6579	29.5	43.1	0.68	N.D.	N.D.
4	−	3300	4928	42.8	44.7	0.95	1.5	2.1
6	−	2000	4416	36.0	47.6	0.76	0.2	0.7
8	−	1100	4015	41.7	50.4	0.83	1.5	1.4
10	−	1500	4355	51.0	44.8	1.14	0.7	1.2
12	−	3600	3835	51.0	45.8	1.11	1.0	0.9
14	−	7900	4480	40.8	45.3	0.90	N.D.	N.D.
16	−	8100	4029	49.0	45.3	1.08	2.0	2.9
18	−	7100	6228	44.7	47.2	0.94	0.3	0.2
20	−	9200	5580	38.6	45.2	0.85	0.7	1.2
22	−	6700	4154	38.1	45.3	0.84	1.4	2.1
24	+++	6700	5544	28.2	50.9	0.55	0.4	1.1
26	+++	7000	3102	39.8	44.6	0.89	0.3	0.5
28	+++	9700	6790	39.3	45.1	0.87	0.2	0.3
30	++++	5900	4620	19.3	44.8	0.43	0.5	0.6
32	++++	5120	5440	26.4	52.0	0.51	0.6	0.4
34	++++	4000	6003	28.2	44.0	0.64	0.1	0.2
36	++++	3000	3330	41.5	43.6	0.95	0.4	0.6
38	++++	4400	5280	38.1	42.3	0.90	0.3	0.6
40	++++	4100	3672	34.6	45.9	0.75	0.4	1.3
42	++++	4000	5360	36.6	40.4	0.91	0.5	0.3
44	++++	3600	5076	36.5	48.0	0.76	0.6	0.5
46	++++	4400	4275	26.6	46.4	0.57	0.5	1.2
48	++++	4500	3689	43.1	47.0	0.92	2.5	2.4
50	++++	3100	5593	44.9	36.9	1.22	0.4	1.3
54	+++	3480	4599	32.4	48.9	0.66	0.3	0.2
56	++	2650	5005	30.1	52.9	0.57	1.2	0.8
58	−	2550	4465	34.9	46.8	0.75	1.6	1.1

week 40 when last tested. An IgM antibody to HTLV-III was not observed in CH132 (which showed only passive transfer of antibody) but was detected early in the course of HTLV-III antibody production in HTLV-III–infected CH114. CH133 manifested no clinical or immunologic evidence of AIDS.

The control animal, CH140, received plasma from three normal donors negative for antibodies to HTLV-III. No clinical or immunologic evidence of AIDS was noted, and no specific HTLV-III antibody response developed. As additional controls, 15 uninoculated chimpanzees housed at SFBR were tested, and all were found to be negative for antibody to HTLV-III.

Our results show that (i) the chimpanzee is susceptible to HTLV-III infection and can thus serve as an infectivity model for the study of AIDS; (ii) in addition to HTLV-III antibody seroconversion, the chimpanzee can develop a clinical syndrome of lymphadenopathy and immunologic impairment providing a disease model that simulates the human AIDS-related lymphadenopathy syndrome; and (iii) HTLV-III infection can be transmitted by lymphocyte-poor plasma, substantiating the potential AIDS risk of noncellular blood components such as pooled clotting-factor concentrates. The susceptibility of the chimpanzee to HTLV-III infection will provide an animal model of AIDS in which to assess antiviral agents and biologic response modifiers and, most importantly, in which to test the safety, immunogenicity, and efficacy of future vaccines.

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13. The stimulatory ratio was calculated as follows. A stimulatory index, representing the amount of label (in counts per minute) of cells plus mitogen divided by that of cells plus medium, was calculated in each test for each animal. The stimulatory ratio is that of the index for CH114 to the index for CH140 (a control animal), the index of each being determined simultaneously in the identical assay.
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Oncogene-Induced Transformation of C3H 10T1/2 Cells Is Enhanced by Tumor Promoters

Abstract. *The tumor promoters 12-O-tetradecanoyl-phorbol-13-acetate and teleocidin markedly enhanced the transformation of C3H 10T1/2 mouse fibroblasts when these cells were transfected with the cloned human bladder cancer c-ras^H oncogene. Transfection studies with the drug resistance marker* gpt *and time course studies indicate that this enhancement is not simply an effect on the process of DNA transfection. These findings, together with parallel studies with NIH 3T3 fibroblasts, also indicate that the competence of animal cells for DNA transfection is a function of the recipient cell line, the transfected marker, and the growth conditions. Our findings suggest that during multistage carcinogenesis tumor promoters may complement the function of activated cellular oncogenes.*

Development of a fully malignant tumor involves complex interactions between environmental and endogenous factors. In addition, carcinogenesis often proceeds through several discernible stages (initiation, promotion, progression) (*1*). DNA transfection studies with the NIH 3T3 cell line have revealed activated oncogenes in a number of human tumors and tumor cell lines (*2–5*). This approach does not in itself, however, indicate the types of interactions that might occur between environmental and endogenous factors in the de novo transformation of normal cells. We are intrigued by the possibility that, during the multistage carcinogenic process, tumor promoters might interact synergistically with cellular oncogenes, since promoters can induce mimicry of transformation, modulate differentiation, and enhance the transformation of cells previously exposed to chemical carcinogens, radiation, or certain DNA viruses (*1, 6–9*). It is of interest, therefore, to determine whether tumor promoters enhance the transformation of cell cultures transfected with an activated oncogene.

In examining possible synergistic interactions between tumor promoters and a cloned oncogene, we used C3H 10T1/2 cells as recipients since they have a more uniform fibroblastic morphology and a lower saturation density than NIH 3T3 cells. In addition, although they are aneuploid, they have an extremely low incidence of spontaneous transformation and are not tumorigenic in nude mice (*10*). They are also particularly well suited for studies of the action of phorbol ester tumor promoters, since they contain an abundance of high-affinity receptors for these and related compounds (*11*) and display striking changes in cell morphology and membrane-related properties in response to these agents (*11, 12*). Furthermore, these tumor promoters markedly enhance the outgrowth of transformed foci in C3H 10T1/2 cultures previously initiated by exposure to several types of chemical carcinogens (*6*) or radiation (*7*), thus mimicking the process of two-stage carcinogenesis on mouse skin.

We first assessed the general competence of C3H 10T1/2 cells for DNA-mediated transfection. For this purpose we chose the dominant drug resistance marker *gpt*, linked to the early region of SV40 to enhance its transcription (*13*). When C3H 10T1/2 cells were transfected with pSV2-*gpt* plasmid DNA (1 μg per plate) by the standard calcium phosphate precipitation technique and then selected