14. P. D. Markham *et al.*, *Int. J. Cancer* **31**, 413 (1983).
15. M. Popovic *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 5402 (1983).
16. J.-C. Chermann *et al.*, *Antibiot. Chemother.* **32**, in press.
17. S. Chouaib *et al.*, *J. Immunol.* **132**, 1851 (1984).
18. G. F. Burns *et al.*, *Cell. Immunol.* **71**, 12 (1982).
19. D. Zagury *et al.*, *Int. J. Cancer* **31**, 705 (1983).
20. F. W. Ruscetti *et al.*, *ibid.*, p. 171.
21. N. Yamamoto *et al.*, *Nature (London)* **299**, 367 (1982).
22. M. Popovic *et al.*, *Science* **224**, 497 (1984); R. C. Gallo *et al.*, *ibid.*, p. 500.
23. E.L.'s lymphocyte phenotypes and functions were determined in August 1983 and January and February 1984. No lymphopenia was observed and the responses to mitogenic or allogenic stimulation were normal. The percentages of T-cell subsets were, respectively: T3: 70, T4: 33, T8: 27 in August 1983; T3: 52, T4: 5, T8: 52 in January 1984; T3: 65, T4: 14, T8: 50 in February 1984. The fractionation experiment reported here was performed in September 1983.
24. J. L. Virelizier *et al.*, *Arch. Virol.* **50**, 279 (1976).
25. F. B. Bang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **46**, 1065 (1960).
26. D. T. Purtillo *et al.*, *Lancet* **1976-I**, 882 (1976).
27. M. D. Daniel *et al.*, *Science* **223**, 602 (1984).
28. P. A. Marx *et al.*, *ibid.*, p. 1083.
29. L. Montagnier *et al.*, *Ann. Virol. (Inst. Pasteur)*, **135E**, 119 (1984).
30. We thank C. Gazengel for providing the patient's sera, F. Chapuis, F. Rey, C. Axler, J. Gruest, and S. Chamaret for technical assistance, and E. Martin for typing the manuscript. We are indebted to G. Janossy (Royal Free Hospital, London) for information about the cellular affinity chromatography technique. This work was supported by grants from the Ministère de l'Industrie et de la Recherche (contracts 82 L 13.13., 83 C 14.11. and 83 C 14.14), Centre National de la Recherche Scientifique (CNRS), and Ligue Nationale contre le Cancer. F. Barré-Sinoussi, J. C. Chermann, and L. Montagnier are also members of the research team No. 147 of CNRS.

1 May 1984; accepted 29 May 1984

Adaptation of Lymphadenopathy Associated Virus (LAV) to Replication in EBV-Transformed B Lymphoblastoid Cell Lines

Abstract. *A strain of lymphadenopathy associated retrovirus (LAV) passaged in vitro was used to infect a lymphoblastoid cell line obtained by transformation with Epstein-Barr virus of B lymphocytes from a healthy donor. The virus produced from this line (B-LAV) was also able to grow at a high rate in some other lymphoblastoid lines and in a Burkitt lymphoma line. This adapted strain retained the biochemical, ultrastructural, and antigenic characteristics of the original strain, as well as its tropism for normal T4$^+$ lymphocytes. It is thus possible to grow LAV in large quantities that can be used for the preparation of diagnostic reagents. The interaction between such a human retrovirus and Epstein-Barr virus, a DNA virus, may have some implication for the pathology of the acquired immunodeficiency syndrome and related diseases.*

Lymphadenopathy associated virus (LAV) is a human T-cell lymphotropic retrovirus that was first isolated from the cells of a lymph node of a homosexual man with lymphadenopathy syndrome (LAS) (*1*). Similar isolates were made from several patients with the acquired immunodeficiency syndrome (AIDS), including a B hemophiliac (*2, 3*), a Zairian woman (*2*), a Zairian couple (*4*), and a Haitian man (*2*).

All viral isolates have in common a major, antigenically related protein with a molecular weight of 25,000 (p25), a Mg^{2+}-dependent reverse transcriptase (RT), mature virions with a morphology similar to that of equine infectious anemia virus (EIAV), and D type particles. The p25 of LAV is immunoprecipitated by serum from horses infected with EIAV, suggesting antigenic similarity with the core protein of this virus (*5*). In contrast, the p25 of LAV and LAV-related viruses is not antigenically related to the p24 of human T-cell leukemia virus type I (HTLV-I), the core proteins of Mason-Pfizer virus (*5*) or similar viruses isolated from simian AIDS (*6*), or to other known animal retroviruses (*5*). An important biological feature of members of the LAV family is their specific tropism for activated T4$^+$ (helper) lymphocytes, in which they induce a cytopathic effect without giving rise to immortalized lines (*1, 2, 7*). Initially, we found that LAV could not be grown in B lymphocytes, Raji cells, MOLT/4, or in several established fibroblastic lines (*1*).

We report here the successful adaptation of LAV to persistent infection of several lymphoblastoid cell lines obtained by the transformation of B lymphocytes with Epstein-Barr virus (EBV) as well as in one Burkitt lymphoma cell line. Thus, it is now possible to grow LAV on a large scale in permanent cell lines. The growth of LAV in EBV-transformed cells may have important implications for the pathogenic role of such viruses in AIDS and other diseases.

The first LAV isolate was initially propagated in cord blood lymphocytes and then in lymphocytes from several adult donors whose sera contained no antibody against LAV and whose lym-

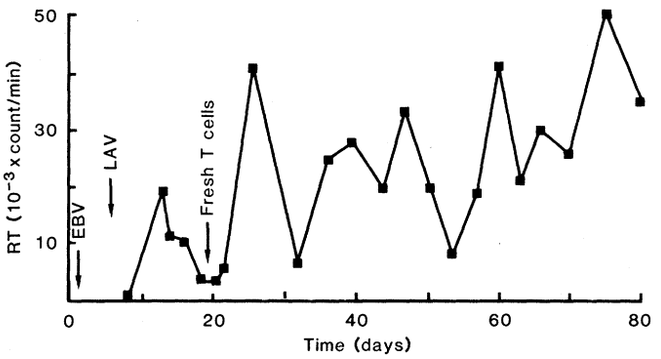

Fig. 1 (above). EBV and LAV co-infection of lymphocytes from a blood donor (F.R.). The culture medium (RPMI 1640 with 10 percent fetal calf serum) included TCGF, antiserum to interferon α, and Polybrene, as described (*1*). Production of LAV was determined by assaying RT activity in 1-ml portions of pelleted virus (*1*).

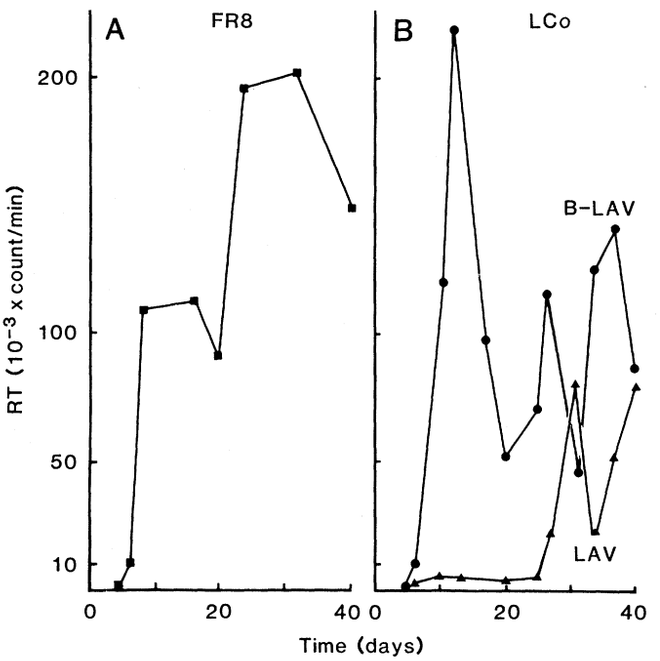

Fig. 2 (right). (A) Virus production by the established FR8 lymphoblastoid line. The cells were infected with LAV at time zero as described (*1*). (B) Virus production by the LCo lymphoblastoid line after infection by LAV and B-LAV. Same medium as in Fig. 1, except that TCGF was omitted.

phocytes did not spontaneously release LAV after activation (*1*). Blood from one particular donor, F.R., was used for the present experiments because of the high and regular yields of LAV obtained from his T lymphocytes upon infection. In these cultures, however, LAV production was transient and was regularly followed by a decline of virus production and cell death.

Because LAV can induce T-cell fusion (*2*, *7*) and because EBV is known to have fusion activity in B cells (*8*), we performed co-infection experiments of unfractionated lymphocytes (B and T) with both viruses. It was hoped that stable hybrids of LAV-infected T cells and of EBV-transformed B cells would be formed and that such hybrids would be able to continuously produce LAV.

Several regimens were tried. The one that gave rise to continuous productive infection of LAV was the following. Whole lymphocytes of F.R. were first stimulated for 24 hours with Protein A and then infected (*9*) with an EBV strain, M81, derived from a nasopharyngeal carcinoma (*10*). Five days later, half of this culture was infected with LAV as described (*1*) and then divided in two subcultures: one was cultured in medium lacking T-cell growth factor (TCGF; interleukin-2), the other in medium containing TCGF. As expected, the TCGF-fed culture produced LAV as detected by a peak of RT activity appearing between day 12 (day 6 after LAV infection) and day 21 in the supernatant. In contrast, the cells cultured in the absence of TCGF did not yield any detectable RT. This shows that at that time LAV was produced only by activated T cells maintained in TCGF, and not by B cells. On day 19, at the time of decline of LAV production, a subculture of the TCGF-fed cells received fresh T cells from the same donor; these T cells had been activated for 3 days with phytohemagglutinin (PHA) in order to provide new targets for the virus. Six days later (day 25), a new peak of RT appeared, but contrary to the first infection, it was not transient. It was followed by continuous but fluctuating production (Fig. 1) that subsequently increased to high amounts, greater than 100,000 count/min per milliliter of supernatant.

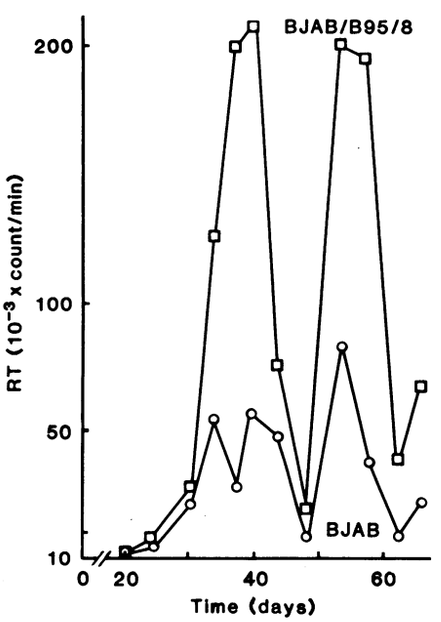

Fig. 3. Infection of the BJAB Burkitt lymphoma cell line and of its EBV-transformed derivative (BJAB/B95/8) with B-LAV. Same culture conditions as in Fig. 2.

At the time of the second LAV infection, large cells transformed by EBV could be readily seen in this culture, as well as in the control culture not infected with LAV, indicating that immortalization of B cells by EBV had already occurred. The immortalized B-cell line was termed FR8. Continuous LAV production could thus be accounted for by a prolonged survival of infected T cells in the presence of the EBV-transformed lymphoblastoid line, or alternatively by B cells of the newly established lymphoblastoid line, or by T-B cell hybrids. To distinguish between these possibilities, the control, uninfected culture of EBV-transformed cells, which had not received TCGF, was exposed to infected cells from the same LAV passage as those used at the beginning of the experiment. The culture was thereafter maintained in the absence of TCGF. It was expected that, under these conditions, the T cells would rapidly decline. Indeed, we determined, by using the monoclonal antibody OKT3 (Ortho), that no $T3^+$ cell remained in the culture by day 49 and that the culture was composed only of B cells as determined by the presence of surface membrane immunoglobulins. LAV production began in this culture without any lag period (Fig. 2a). This experiment clearly established that the virus could grow on B lymphocytes but only after they had been transformed by EBV. This new property of LAV was probably acquired during passage in vitro before the co-infection experiment, since an earlier passage of LAV, from July 1983, could not grow in the FR8 lymphoblastoid line.

Sequence comparison of proviral DNA's will help our understanding of the molecular basis of this change in tropism. Recent work on animal retroviruses suggests that the long terminal repeat sequences rather than the envelope (*env*) gene may play a crucial role in the virus tropism and pathogenesis (*11*).

We also attempted to grow LAV on other lymphoid cell lines of the B lineage. Such lines were obtained in our laboratory by in vitro transformation of unfractionated lymphocytes from adult blood donors or umbilical cord blood with the M81 or the B95/8 strains of EBV. Only one other line from an adult donor and one from umbilical cord blood could be productively infected by the virus (Table 1), after a lag period of 3 weeks. This selective infectibility, observed in three of the cell lines, suggests that LAV tropism may be restricted by a polymorphism expressed at the transformed B-cell membrane. Whether this polymorphism is related to HLA class I or class II determinants or to the expression of other membrane molecules is not known. It was interesting that the virus derived from the FR8 lymphoblastoid line could grow in the same lines with higher RT activity and with a shorter lag period (Fig. 2b). This suggests that after its passage in FR8 cells, LAV underwent a secondary adaptation to B cells. This adapted strain was termed B-LAV.

Several established lines from Burkitt

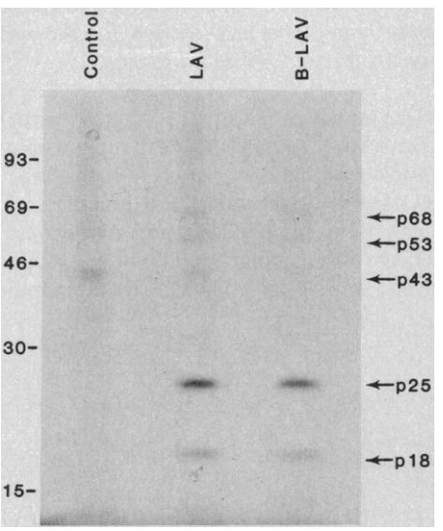

Fig. 4. Major proteins of LAV and B-LAV. LCo cells infected with LAV or B-LAV were labeled for 4 hours in RPMI 1640 medium lacking amino acids but containing 10 percent fetal calf serum in the presence of a mixture of ^{14}C-labeled amino acids (C.E.A., 40 to 50 mCi/mmol, 20 μCi/ml). The supernatant was centrifuged at 10,000*g* for 20 minutes and then at 45,000*g* for 20 minutes in a SW55 Beckman rotor. The viral pellet was resuspended in NTE buffer (*5*) and centrifuged to equilibrium in a Nycodenz gradient as described (*5*). Fractions of the virus-containing band were pooled and recentrifuged, and the pellet was dissolved in denaturing buffer, heated for 3 minutes at 100°C as described (*1*), and subjected to electrophoresis. The control is the supernatant of an uninfected LCo culture, processed exactly as described for the infected cultures.

lymphoma were also tested for their susceptibility to LAV and B-LAV infection. The BJAB (EBV negative) line could be infected by B-LAV, but not by LAV (Fig. 3). There was a 30-day lag period between exposure to infected cells and virus production. Upon its transformation in vitro by the B95/8 strain of EBV, BJAB could also be productively infected with B-LAV. This infected line showed the same lag (Table 1) and then produced four times as much virus as the original EBV negative BJAB line. Other B lymphoma lines did not show evidence of virus production, as assayed by RT activity at intervals for more than 2 months after infection. Negative results were also obtained with the T lymphoma line MOLT/4 and the HL60 myeloid line.

It was important to check whether or not the virus produced by the B-cell line retained the characteristics of the original LAV. The virus produced by LCo, a cell line derived from umbilical cord lymphocytes, was metabolically labeled with [^{35}S]methionine or ^{14}C-labeled amino acids and purified on a Nycodenz gradient (*5*). Figure 4 shows the results of a comparison of polyacrylamide gel electrophoresis under denaturing conditions between B-LAV proteins and LAV proteins. The patterns were identical, showing a prominent p25, a p18, a low molecular weight protein at the bottom of the gel (p12), and three proteins of high molecular weight (43,000, 53,000, 68,000). The band at 43,000 may include a component of cellular origin, since it was also found in a similar preparation made from the control uninfected cells.

The p25 of B-LAV showed the same antigenicity as the p25 of LAV, since it was immunoprecipitated by the same sera. No differences between the two strains could be found with regard to the ionic requirements of their RT (*1, 12*) and their ultrastructural morphology (Fig. 5) [see (*1, 5*)]. The virus produced by FR8, but not that produced by LCo, had a slightly higher density in sucrose gradient than LAV (1.18 instead of 1.17).

In addition, like LAV, B-LAV retained a selective tropism for the helper-inducer ($T4^+$) T-cell population of lymphocytes from normal donors infected in vitro (*2*). Peripheral blood lymphocytes of normal volunteers were fractionated into monocytes, B lymphocytes, and T lymphocytes. The $T4^+$ and $T8^+$ (suppressor/cytotoxic) fractions were obtained by cellular affinity chromatography. Cells were then cultured in the presence of PHA followed by TCGF. Each fraction was separately infected with virus-containing, cell-free supernatant, and RT was sequentially measured in each infected and uninfected culture. Only $T4^+$ lymphocytes displayed virus production with LAV as well as with B-LAV, under these conditions. Thus far it has not been possible to grow B-LAV in short-term cultures of B cells from F.R., either before or after stimulation of the cells with B-cell mitogens such as Protein A. It is not known whether the virus could grow in long-term cultures such as those maintained in the presence of B-cell growth factor (*13*). Studies of EBV antigen expression did not reveal any significant change in the FR8 and LCo lines after infection with the retrovirus. Both were 100 percent positive for the EBV nuclear antigen (EBNA). Early antigen (EA) expression could be detected in 0.1 percent of LCo cells and 3.5 percent of FR8 cells. No LCo cells were positive for viral capsid antigens (VCA), but 1.5 percent of FR8 cells had VCA. A search for the presence of EBV antigens (EA and VCA) in purified B-LAV preparations yielded negative results. The tests used included immunoprecipitation with polyclonal antibodies against EA and VCA and two monoclonal antibodies to the 52K and 85K components of EA (*14*). The role of EBV in the growth of LAV in B cells is not crucial since an EBV negative Burkitt lymphoma line could be infected by B-LAV. However, LAV production was four times higher in the same line supertransformed by the B95/8 strain of EBV, suggesting that EBV could enhance LAV production. A similar enhancing effect by EBV on animal retrovirus expression was recently described for another BL line, RAMOS (*15*).

Such interaction between a human retrovirus and a herpesvirus may have some bearing on human pathology. It is possible that the adaptation of LAV to B lymphoid cells occurs in some situations in vivo. For example, during a late stage of AIDS, LAV could disseminate through EBV-infected B lymphocytes as well as through the $T4^+$ cell population. LAV-induced depression of the T lymphocyte mediated control over EBV infected B lymphocytes (*16*) could result in an expansion of the latter cells, which would constitute in turn a reservoir for LAV production. This phenomenon may also contribute to the increased frequency of B-cell lymphomas that has been reported in AIDS (*17*).

Our finding is of practical importance because LAV can now be produced continuously by some permanent cell lines

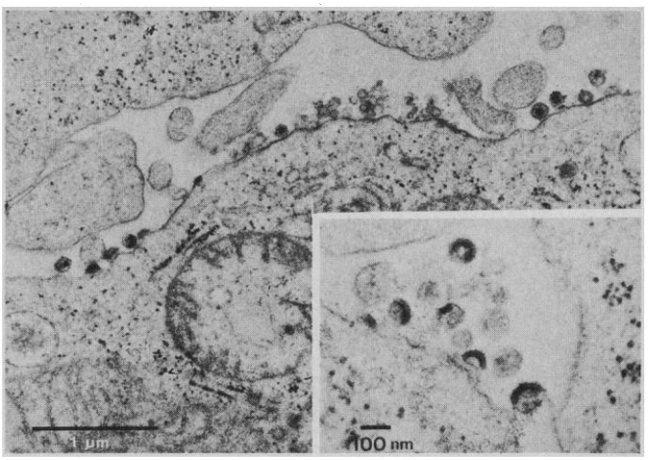

Fig. 5. Electron microscopic examination of a section of an LCo lymphoblastoid cell infected with B-LAV. Note the three typical aspects of LAV: budding particles, immature free particles with a dense crescent, and mature virions with dense eccentric core.

Table 1. Susceptibility and resistance to LAV and B-LAV infection of some established human lymphoid cell lines. Symbols: ++, viral-associated RT production more than 100,000 count/min per milliliter of supernatant (weekly average, two harvests a week); +, RT production between 10,000 and 100,000 count/min. No RT activity could be detected in the supernatant of any of the cell lines before infection with LAV.

Cell line	Presence of EBV genome	Retrovirus growth	
		LAV	B-LAV
Origin: B lymphocytes from adult donor			
FR8	Yes	++	++
BAR	Yes	+	++
BRA	Yes	–	–
DIER	Yes	–	–
Origin: B lymphocytes from umbilical cord blood			
LCo	Yes	+	++
LC1	Yes	–	–
Origin: Burkitt lymphoma			
Daudi	Yes	–	–
Namalwa	Yes	–	–
Raji	Yes	–	–
Chev	Yes	–	–
BJAB	No	–	+
BJAB/B95/8	Yes	–	++
Origin: T lymphoma			
MOLT/4	No	–	–
Origin: Myeloid leukemia			
HL60	No	–	–

growing in suspension without noticeable cytopathic effects.

Another laboratory has recently described the growth of HTLV type III in a T leukemia cell line (*18*). These independent findings will facilitate comparison between viral isolates, particularly between HTLV-III and LAV, and the development of reagents for serological tests.

L. MONTAGNIER
J. GRUEST
S. CHAMARET
C. DAUGUET
C. AXLER
D. GUÉTARD
M. T. NUGEYRE
F. BARRÉ-SINOUSSI
J.-C. CHERMANN
Unité d'Oncologie Virale, Institut Pasteur, 75724 Paris Cédex 15
J. B. BRUNET
Direction Générale de la Santé, Paris
D. KLATZMANN
J. C. GLUCKMAN
Laboratoire d'Immunologie Néphrologique et de Transplantation, UER Pitié-Salpétrière 75634 Paris Cédex 13

References and Notes

1. F. Barré-Sinoussi *et al.*, *Science* **220**, 868 (1983).
2. L. Montagnier *et al.*, in *Human T Cell Leukemia Lymphoma Viruses*, R. C. Gallo, M. E. Essex, L. Gross, Eds. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., in press).
3. E. Vilmer *et al.*, *Lancet* **1984-I**, 753 (1984).
4. A. Ellrodt *et al.*, *Lancet*, in press.
5. L. Montagnier *et al.*, *Ann. Virol. (Inst. Pasteur)* **135E**, 119 (1984).
6. L. Montagnier *et al.*, unpublished data.
7. D. Klatzmann *et al.*, *Science* **225**, 59 (1984).
8. G. J. Bayliss and H. Wolf, *Nature (London)* **287**, 164 (1980).
9. The conditions used for EBV infection were as follows: 10^7 cells were exposed to 3 ml of crude supernatant from EBV-producing marmoset cells for 1 hour at 37°C and, after centrifugation, were suspended in culture medium at a concentration of 10^6 per milliliter.
10. C. Desgranges *et al.*, *Biomedicine* **25**, 349 (1976).
11. J. Lenz *et al.*, *Nature (London)* **308**, 467 (1984); I. S. Y. Chen, J. McLaughlin, D. W. Golden, *ibid.* **309**, 276 (1984).
12. M. A. Rey *et al.*, *Biochem. Biophys. Res. Commun.*, in press.
13. T. Leanderson *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 7455 (1982).
14. G. R. Pearson *et al.*, *J. Virol.* **47**, 193 (1983).
15. R. D. Lasky and F. A. Troy, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 33 (1984).
16. E. Klein *et al.*, *Cancer Res.* **41**, 4210 (1981).
17. Center for Disease Control, *Mortal. Morbid. Weekly Rep.* **31**, 277 (1982).
18. M. Popovic *et al.*, *Science* **224**, 497 (1984).
19. We thank F. Rey and C. Barreau for technical assistance. This work was supported by grants 83/L14-19 and 83 C 14-11 of the French Ministry of Research and Industry. Members of the Viral Oncology Unit are also members of Research Team 147 of the Centre National de la Recherche Scientifique.

14 May 1984; accepted 8 June 1984

Immunoregulatory Lymphokines of T Hybridomas from AIDS Patients: Constitutive and Inducible Suppressor Factors

Abstract. *Supernatants derived from peripheral blood mononuclear cell cultures of certain patients with the acquired immunodeficiency syndrome (AIDS) or its prodromes have the capacity to block T cell–dependent immune reactivity in vitro. T cells derived from a patient positive for antibody to the lymphadenopathy associated virus (LAV), and elaborating high titers of these soluble suppressor factors, were fused to a mutagenized clone of the human T lymphoblastoid cell line KE37. Molecules capable of profoundly depressing T cell–dependent polyclonal antibody production and DNA synthetic responses, either directly or after incubation with normal adherent cells, were isolated from stable hybrid clones.*

Patients with the acquired immunodeficiency syndrome (AIDS) have a marked but selective abnormality of immunoregulation, manifest by susceptibility to opportunistic infections and malignancies characteristic of certain genetic and iatrogenic immune deficiencies (*1*). We have previously shown that supernatants from lectin-free cultures of peripheral blood mononuclear cells (PBMC) obtained from homosexual males with AIDS or its prodromes can inhibit spontaneous and pokeweed mitogen (PWM)–induced B lymphocyte differentiation into plasmacytes, and T-cell blastogenic responses to antigen (*2*). These soluble suppressor factors (SSF) are the product of the interaction of T lymphocytes with adherent cells. T cells or T-cell factors from certain AIDS patients, but not from healthy homosexual or heterosexual controls or from heterosexual individuals with Epstein-Barr virus (EBV) or cytomegalovirus mononucleosis, can collaborate with normal adherent cells in the formation of SSF (*2*, *3*). This system provides a model with which to examine cell subsets participating in the induction and expression of immunoregulatory defects in AIDS. The availability of functional T-cell hybrids derived from such patients would facilitate investigation of T lymphokine–mediated inhibitory phenomena, and permit direct comparisons with products isolated in other disorders linked to cell- or factor-mediated immunosuppression.

Human T-cell hybridomas secreting molecules able to interfere with T cell–directed polyclonal immunoglobulin (Ig) synthesis independent of T-lymphocyte mitogenesis (*4*), or with antibody production directly at the level of the B cell (*5*), have been described by others. In those studies the investigators used PBMC from healthy individuals enriched for activated suppressor T cells by exposure to concanavalin A (Con A) in vitro (*5*) or unstimulated T cells from patients with common variable hypogammaglobulinemia (*4*, *6*). We have now immortalized peripheral T lymphocytes obtained from a homosexual male with unexplained generalized lymphadenopathy, persistent fever, malaise, and high titers of SSF-AIDS [see patient Sel. in (*2*)] by fusion with KE37.3.2, a hypoxanthine-guanine phosphoribosyl transferase–deficient mutant of the human T acute lymphoblastic leukemia line KE37, first isolated in our laboratory (*7*). Fusion products were selected in aminopterin and further identified as hybrids by demonstration of shared HLA class I allodeterminants between patient Sel. (HLA-A1,w29/B51,w35/cw4) and KE37.3.2 (HLA-A11,30/B35,44/cw4). These "SK" hybrids retained the membrane antigen profile of the parent line as detected by indirect immunofluorescence: 75 percent Leu-3a$^+$ (helper-inducer T lymphocyte subset); 0 percent Leu-2a$^+$ (suppressor-cytotoxic T subset); 0 percent Leu-4$^+$ (mature pan-T cell marker); >95 percent OKT10$^+$; and 0 percent HLA-DR$^+$ (detected with a murine monoclonal reagent, VG2). Neither the hybrid lines nor KE37.3.2 expressed human T-cell leukemia virus (HTLV) type I or type II products as determined by indirect intracellular immunofluorescence staining with a monoclonal antibody directed against the HTLV core protein p19. However, patient Sel. had serum antibody to LAV, an AIDS-associated retrovirus (*8*), as detected by an enzyme-linked immunosorbent assay (ELISA) for an antibody to the internal antigen p25 (*9*).

Figure 1 shows activities in the supernatants of initially uncloned cells in growth positive wells. Activities were determined by the ability of the supernatants to affect PWM-induced polyclonal Ig production by normal indicator PBMC in a reverse hemolytic plaque-forming cell (PFC) assay. Supernatant from KE37.3.2 had no effect in this system. Nine of 22 SK hybrid supernatants gave significant (>25 percent) constitutive inhibition of PFC; 2 of 22 showed enhancement (>25 percent); and 11 of 22 revealed no activity. Selected supernatants were reassessed after 48 hours of incubation with normal peripheral blood monocytes, isolated by Percoll gradient cen-