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.

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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-

trifugation or serial plastic adherence, and depleted of E-rosette$^+$ and surface Ig$^+$ cells (*10*). Two samples, SK8 and SK10, which formerly revealed no significant suppression, now evinced marked inhibition of Ig synthesis (Fig. 1). In no instance did prior incubation of hybrid supernatants with monocytes remove constitutive SSF activity.

In agreement with the effector profile of SSF-AIDS elaborated by T lymphocyte–adherent cell interactions in patients with AIDS or its prodromes (*2, 3*), selected constitutive (SK7) and "induced" (SK8/monocyte) hybrid supernatants were noncytotoxic, as determined by supravital dye exclusion in 6-day PWM-stimulated indicator cultures. With normal target PBMC of divergent HLA-DR allotypes, SSF function proved not to be genetically constrained. Indomethacin (1 to 10 μg/ml), sheep antibody to human α-interferon (Interferon Sciences, Inc.; 1 to 10 units per culture), and a murine monoclonal antibody to human γ-interferon (1 to 10 units per culture) did not perturb SSF activity. In addition, SK7 and SK8/monocyte factors had no influence on a cytopathic effect–inhibition assay for γ-interferon, conducted with vesicular stomatitis virus and WISH cells (*11*).

Dilution curves for SK7 and SK8 supernatants, before and after exposure to monocytes, are given in Fig. 2A. Titers of hybridoma SSF are equivalent to or greater than those obtained with the patient's unstimulated PBMC (titer 1:1000). Figure 2B shows the kinetics of SSF activity. Corresponding to the spontaneous SSF-AIDS product (*2, 3*), SK7 and SK8/monocyte factors depressed PWM-induced Ig formation only if introduced within the first 48 hours of culture initiation. This supports the contention that the SSF is noncytotoxic, and that it is able to disrupt differentiation of B cells to plasmacytes during the critical period at which regulatory cell helper activity is maximal (*12*).

The cellular site of action of SSF was further examined by using B cell–enriched indicator populations activated with EBV; such populations act as helper T cell–independent polyclonal mitogens. Supernatants from SK7, SK8, and SK8/monocyte cultures did not block polyclonal PFC after EBV induction of allogeneic B cells. In contrast, supernatants from PBMC derived from normal and pre-AIDS patients, when activated with Con A, were capable of depressing such synthesis (data not shown). Restriction of SSF activity to the T lymphocyte was supported by absorption protocols. Purified populations of normal E-rosette$^+$ T cells, or non-T cells (B lymphocytes plus adherent cells) were incubated with SK7, SK8, or SK8/monocyte supernatants for 4 hours at 37°C. SSF activity in the SK7 and SK8/monocyte mixtures was diminished by prior incubation with T lymphocytes, whereas its activity did not change after absorption with the other immune cell subsets (Table 1). These absorptions do not definitively establish the presence of specific receptors for SSF-AIDS on T cells. To further address this question, we are attempting to radioactively label purified SSF-AIDS. This experiment does indicate that simple release of a preformed monokine under the influence of SK8 factor is unlikely, because 4 hours of incubation of SK8 supernatant with non-T cells proved inadequate to generate suppressor-effector factor (Table 1), which appears at about 12 hours of incubation and is maximal at 48 to 72 hours.

Table 1. Effect of absorption of T hybridoma factors with immune cell subpopulations on suppressor-effector activity. Supernatants generated as noted in the legend to Fig. 1 were used as sources of factor. They were reacted with adherent cell-depleted E-rosette$^+$ T cells, or non-T cells (B plus adherent cell) subsets, prepared as described (*2, 10*). 2×10^7 cells were incubated with 1 ml of factor at 37°C for 4 hours with constant rocking. Supernatants were collected by centrifugation and then added at a 1:9 final dilution to PWM-induced indicator cultures. Duplicate microwells were harvested on day 6 and the number of PFC determined in a reverse hemolytic plaque assay. KE37.3.2 supernatants were unaffected by these prior absorptions.

Supernatant added	Prior absorption with	Polyclonal Ig synthesis (PFC per 10^6 B cells)	Inhibition of PFC (%)
Medium		9975	
Medium/monocyte		9310	6.7
SK7		0	100.0
SK7	Non-T cells	0	100.0
SK7	T cells	7382	26.0
SK8		6650	33.3
SK8	Non-T cells	7980	20.0
SK8	T cells	7714	22.7
SK8/monocyte		0	100.0
SK8/monocyte	Non-T cells	0	100.0
SK8/monocyte	T cells	3990	60.0

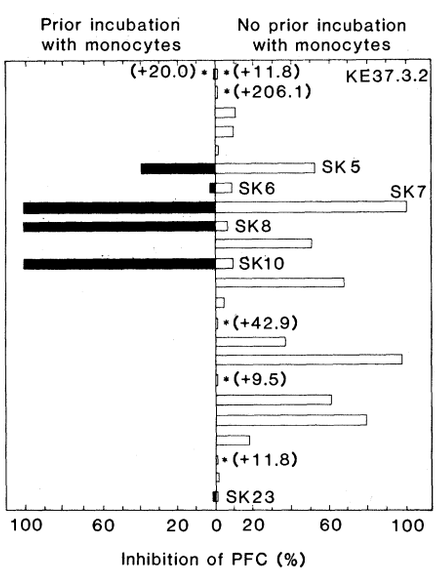

Fig. 1. Effect of T hybridoma factors on PWM-induced Ig production. Supernatants were harvested after 24 hours from cultures of 1×10^6 T hybrids maintained in 1 ml of RPMI 1640 medium containing 10 percent fetal bovine serum (medium) at 37°C in a 5 percent humidified CO_2 atmosphere. They were added, at a 1:9 final dilution (volume/volume), to 3×10^5 normal PBMC per microwell in 0.3 ml of medium, together with a 1:150 dilution of PWM. The contents of duplicate wells were harvested on day 6 of culture, and PFC determined by a reverse hemolytic plaque assay (*2, 3*). Some supernatants were reexamined after 48 hours of incubation at a 1:2 dilution in medium with 1.5×10^5 monocytes per milliliter, prepared as previously outlined (*10*). Data are expressed as the percent inhibition of PFC in the absence of factors.

Lectin-free supernatants from patients with AIDS or its prodromes also inhibit antigen-induced T-cell proliferation (*2, 3*) and the blastogenic activity of normal T lymphocytes in response to various mitogens (*13*). Both SK7 and SK8/monocyte molecules were capable of depressing tetanus toxoid–mediated T-cell mitogenesis of PBMC from presensitized normal individuals (*2, 3*), as well as T-cell responses to optimal concentrations of Con A, PWM, and phytohemagglutinin [75 percent inhibition at a 1:100 dilution (volume to volume)].

The physicochemical properties of SSF-AIDS that are shared with the T hybrid factor include partial inactivation upon heating for 60 minutes at 56°C, or with addition of $3 \times 10^{-5} M$ 2-mercaptoethanol on initiation of PWM-induced target cultures. Both products were resistant to 50 m*M* L-rhamnose and 50 m*M* *n*-acetyl-D-glucosamine, two carbohydrates reported to reverse the inhibitory effects of suppressor factors produced by Con A–activated PBMC and acting, respectively, on Ig production at the B-cell level (*14*) or on mitogen- and antigen-

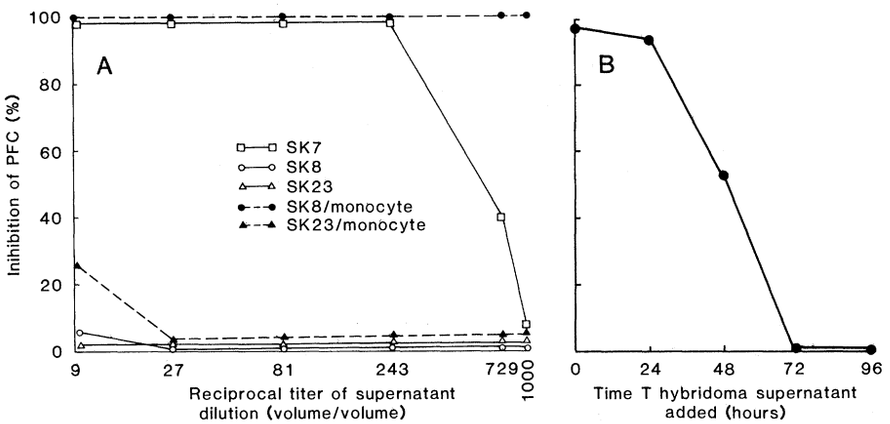

Fig. 2. (A) Dose-response curves for T hybridoma factors. The effects of varying dilutions of T hybrid supernatants, before and after incubation with monocytes, are illustrated. Data are expressed as the percent inhibition of PFC in 6-day PWM-induced indicator cultures. The dilution of SK8/monocyte factor capable of inhibiting PFC by 50 percent varied between 1:750 and 1:1500, depending on the target culture used. (B) Kinetics of T hybridoma factor suppression of T cell–dependent polyclonal Ig synthesis. In this representative experiment, T hybrid supernatants were added at a 1:9 final dilution (volume/volume) to PWM-induced indicator cultures at varying times after culture initiation. The contents of each culture were harvested on day 6 and evaluated in a reverse hemolytic plaque assay, with results expressed as in (A).

induced T-cell proliferation (*15*). The apparent size of SSF was determined by molecular sieve chromatography on Sephadex S-200. A major peak of suppressor activity for PWM-induced PFC was detected at approximately 47,000 daltons, migrating between ovalbumin (45,000) and albumin (67,000).

The exact mechanism of SSF effector function is obscure. As both SSF-AIDS and the T hybrid products must be present during the initial 48 hours of the PWM-induced PFC system, the period during which maximal helper factor activity is observed (*16*), SSF may block lymphokine synthesis, secretion, or receptor function. Supernatant from SK7 had no effect on the synthesis of interleukin-2 by a clone of the Jurkat human T-cell leukemia line, on the utilization of this lymphokine by an interleukin-2–dependent T-cell clone, or on peroxide-mediated macrophage cytotoxicity (*17*). The immunosuppressive effect is not directly related to LAV virion production because at least the constitutive hybridoma does not express virus by reverse transcriptase assay (*9*).

Data with SK8 support a two-step mechanism for the generation of the suppressor-effector molecules. It appears that certain T hybrid clones, such as T lymphocytes from AIDS patients, may recruit a second population of cells with the characteristics of monocytes in the inhibition of T cell–dependent immune responses. The afferent limb of suppressor activity in human diseases and in experimental animal systems may involve several pathways linked by T cell–monocyte collaborations. Production of nonspecific inhibitory factors by T cells from mice (*18*) and man (19) with systemic mycoses is dependent on substances liberated from monocytes of normal or infected individuals. Certain retroviruses, such as murine sarcoma virus, stimulate infected non-T cells to secrete potent soluble suppressors of lectin-mediated thymocyte proliferation and antibody synthesis (*20*). Induction of these factors is independent of virus particle production (*21*). Pierce and co-workers have recovered soluble immune response suppressor (SIRS) from several murine T hybridomas, and have demonstrated the feasibility of utilizing a continuous macrophage cell line to process or generate SIRS under the direction of T lymphokines (*22, 23*). At least two SIRS-like products have been identified in man (*14, 15*), one of which has been produced by a continuous T-cell line (*15*). SSF-AIDS and its related T hybrid products are distinguishable from these substances by the restriction of activity to T cell–dependent processes and the lack of susceptibility to specific sugars.

The question of long-term stability of our T hybridomas requires continued assessment. Repetitive recloning with selection of suitable clones, together with the maintenance of frozen stocks of cells, minimizes the problem of functional loss related to chromosomal attrition or other phenomena. SK7 and SK8 have remained in continuous culture, with two subclonings, for 10 months, with persistence of activity. (SK10 no longer expresses an active lymphokine, despite repetitive cloning.) These T hybrids may thus be exploited as potentially unlimited sources of immunoregulatory molecules. Correlations between the presence of SSF-AIDS in individuals with unexplained lymphadenopathy and fever, circulating antibody to LAV (*24*), and the subsequent development of AIDS (*2, 3, 24*) have been uncovered. The production of SSF by LAV-infected $T4^+$ cells, either alone or in collaboration with monocytes, would be analogous to the activation and release of helper and inducer lymphokines by normal cells infected with HTLV-I (*25*). We also attempted to detect SSF-AIDS in the sera of individuals with AIDS or its prodromes. No direct relation between the elaboration of this factor in vitro and the capacity of a given serum sample to depress T cell–dependent immune reactivities could be identified. This may be secondary to the binding of such molecules to T lymphocytes or other cellular receptors, with consequent depletion from the circulation, or to the simultaneous presence of helper or other factors which effectively block SSF detection in whole serum. Indeed, although inhibition of T-cell mitogenesis in vitro by serum from AIDS patients has been reported (*26*), such activity was not found by another group (*27*), and certain normal sera express inhibitory substances (*28*). However, SK7, SK8, and SK8/monocyte supernatants do have the capacity to induce an immunodeficiency state in vivo. Marked suppression of antigen-specific IgM antibody production was noted in BALB/c mice administered these factors intraperitoneally (*29*). If reproduced in vivo for other types of immune response known to be defective in AIDS, this may serve as a useful animal model for the disorder. In addition, by comparison with a variety of T lymphokines now being tested clinically in an attempt to augment immunologic function of patients with AIDS and other immune deficiencies (*30*), our hybrid factors may be of therapeutic significance in the management of disorders requiring immunosuppression. Several groups have achieved in vitro the translation of murine antigen-specific (*31*) and SIRS (*32*) T hybridoma suppressor factors, and we have tentatively isolated SSF-AIDS activity from a rabbit reticulocyte lysate system using messenger RNA's derived from SK7 and SK8.

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Lymphadenopathy Associated Virus Infection of a Blood Donor–Recipient Pair with Acquired Immunodeficiency Syndrome

Abstract. *A retrovirus isolated from three patients with the acquired immunodeficiency syndrome (AIDS) in the United States was morphologically and antigenically identical to lymphadenopathy associated virus isolated in France. Two of these isolates were from a blood donor–recipient pair, each of whom developed AIDS. Lymphadenopathy associated virus was isolated from the blood donor's lymphocytes 12 months after his onset of AIDS symptoms and from the blood recipient's lymphocytes 1 month after her onset of AIDS symptoms. Two isolates from the blood donor–recipient pair and an isolate from an epidemiologically unrelated homosexual man were examined by competitive radioimmunoassay to determine their antigenic relatedness to each other and to other human retroviruses. The major core proteins (p25) of the isolates were antigenically identical and all three isolates were identical to prototype lymphadenopathy associated virus isolated in France.*

Human retroviruses have been implicated as etiologic agents of the acquired immunodeficiency syndrome (AIDS). Evidence to support this causal association includes (i) the detection in blood from patients with AIDS and lymphadenopathy syndrome (LAS) of antibodies to membrane antigens of human T-cell lymphotropic virus, HTLV (*1*); (ii) the isolation in France from lymphocytes from a homosexual man with lymphadenopathy of a retrovirus called lymphadenopathy associated virus (LAV) (*2*); (iii) the detection of antibodies to membrane antigens of HTLV in serum from donors who gave blood to patients who subsequently developed transfusion-associated AIDS (*3*); and (iv) the documentation of infection in patients with AIDS, AIDS-related diseases, and their contacts by a retrovirus called HTLV-III (*4*). We now report the isolation and characterization of a retrovirus from blood from three AIDS patients and compare its antigenic relatedness to HTLV-I, HTLV-II, and LAV.

Whole blood for virus isolation and serologic testing was collected from a blood donor–recipient pair, each member of which had developed AIDS. The blood recipient was a 38-year-old woman who was well until she developed uterine bleeding necessitating surgery. With surgery she received 2 units of packed red blood cells obtained from two separate donors. Two weeks after surgery she developed a mononucleosis-like syndrome, which gradually disappeared. Two months after surgery one of the two blood donors to this woman was identified as a 24-year-old homosexual man who had been hospitalized with oral thrush and *Pneumocystis carinii* pneumonia. His ratio of T-helper to T-suppressor cells at that time was 0.02. The second blood donor to this woman was a healthy man with no risk factors for AIDS.

After the donor's history became known, the cellular immune status of the recipient was tested. She was found to have lymphopenia and a decreased ratio of T-helper to T-suppressor cells 7 months after surgery. Thirteen months after surgery she was hospitalized with *Pneumocystis carinii* pneumonia; her ratio of T-helper to T-suppressor cells was 0.46. She had no other known risk factors for AIDS and denied intravenous drug use, sexual contact with any members of groups with an increased incidence of AIDS, and other exposures to blood or blood products within the preceding 5 years.

The blood specimens used in this study were drawn 12 months after the onset of AIDS symptoms in the blood donor and 1 month after the onset of AIDS symptoms in the recipient. Blood was obtained from a third AIDS patient 20 months after the onset of symptoms; this patient was a homosexual man who had had generalized lymphadenopathy for 20 months before the onset of multiple severe opportunistic infections. He was not epidemiologically connected to the blood donor–recipient pair.

Two virus isolation techniques were used on specimens from these patients: (i) passage of cell-free supernatant fluids from primary patient lymphocyte cultures and (ii) cocultivation of patient lymphocytes with normal fetal cord blood lymphocytes. In both techniques lymphocytes were separated from fresh whole blood on Ficoll-Hypaque gradients and placed into culture with phytohemagglutinin (PHA). For cocultivation, fetal cord blood lymphocytes were added weekly to the patient's PHA-stimulated primary lymphocyte cultures to which interleukin-2 had been added. For the cell-free transmission assays, supernatant fluids were removed from the patient's PHA-stimulated primary lymphocyte cultures and added to cultures of fetal cord blood lymphocytes. Lymphocyte cultures were monitored for virus