vations, although the interpretation of O_3 observations will be difficult because of the number of chemical and radiative effects modified by the volcanic cloud. Stolarski and Cicerone (*1*) originally suggested that the direct injection of chlorine into the stratosphere by volcanoes could result in substantial O_3 destruction, but this source of stratospheric chlorine has largely been dismissed in comparison with other sources. The observation that a single, large volcanic event can increase the stratospheric HCl burden by 40 percent over a large part of the globe should lead to a reassessment of the role of volcanoes in stratospheric chlorine chemistry.

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Suramin Protection of T Cells in Vitro Against Infectivity and Cytopathic Effect of HTLV-III

Abstract. *A recently discovered member of the human T-cell leukemia virus (HTLV) family of retroviruses has been etiologically linked to the acquired immune deficiency syndrome (AIDS). This virus, which has been designated HTLV-III, is tropic for OKT4-bearing (helper-inducer) T cells. Moreover, the virus is cytopathic for these cells. Suramin is a drug used in the therapy of Rhodesian trypanosomiasis and onchocerciasis, and it is known to inhibit the reverse transcriptase of a number of retroviruses. Suramin has now been found to block in vitro the infectivity and cytopathic effect of HTLV-III at doses that are clinically attainable in human beings.*

Approximately 3 years ago, an apparently new and unexplained disorder called acquired immune deficiency syndrome (AIDS) was recognized (*1–3*). The disorder is a pandemic immunosuppressive disease that predisposes to life-threatening infections with opportunistic organisms and to certain neoplasms (especially Kaposi's sarcomas and occasionally lymphomas), which may be signs of the underlying immune impairment. Characteristically, AIDS is associated with a progressive depletion of T cells, especially the helper-inducer subset bearing the OKT4 surface marker (*2*). No therapy is known to cure AIDS.

The hypothesis that AIDS is caused by a member of the human T-cell leukemia virus (HTLV) family of retroviruses first received direct empirical support in 1983 (*4, 5*). For example, Essex and Lee and their colleagues discovered the presence of antibodies to cell membrane antigen of HTLV-I–infected T cells in serum samples from more than 40 percent of patients with AIDS (in the most sensitive range of their assay) (*6*). This antigen is now known to be expressed on the envelope of HTLV-I (*7*). At the same time, three observations reinforced the possibility that a then-uncharacterized member of the HTLV family might be playing a role in the pathogenesis of AIDS: (i) The report by Barré-Sinoussi *et al.* (*5*) of a new retrovirus, termed lymphadenopathy-associated virus (LAV), isolated from a homosexual man, who was thought to have a possible prodrome of AIDS. These workers, in the first paper describing their findings, concluded that the new retrovirus belonged in the HTLV family but that it was distinct from the other members then defined. (ii) Detection of AIDS sera containing antibodies to a membrane (HTLV envelope) protein but lacking antibodies to certain internal core structural proteins of HTLV-I and HTLV-II (*4*). (iii) The infrequent isolation of HTLV-I and HTLV-II from AIDS patients, even in the presence of antibodies to HTLV-I envelope determinants (*4*).

In the spring of 1984 several converging lines of investigation linked a cytopathic member of the HTLV family of retroviruses to the pathogenesis of AIDS (*8, 9*). The retrovirus, which is now known to play a role in the etiology of AIDS, is referred to as HTLV-III; this virus preferentially infects and destroys $OKT4^+$ (helper-inducer) T cells (*10*). Detectable viral replication in vivo characterizes the HTLV-III infection seen in AIDS, at least early in the disease. By contrast, only on rare occasions (*11*) has it been possible to detect viral replication in vivo in adult T-cell leukemia, which is caused by HTLV-I, the prototypical member of the HTLV family. The discovery of HTLV-III offers researchers new strategies for the experimental therapy of the disease, including the use of drugs that inhibit reverse transcriptase (*12*).

All retroviruses (including HTLV-III) require the enzyme called reverse transcriptase in their natural cycle of replication (*13*). The reverse transcriptases of retroviruses infecting humans and animals have similar amino acid sequences (*14*). In 1979, de Clercq reported that suramin (molecular weight, 1429)—a drug used to treat Rhodesian trypanosomiasis and onchocerciasis—was a potent competitive inhibitor of the reverse transcriptase of a number of animal retroviruses (*15*). Since the first paper by Gallo and his colleagues describing HTLV-I in adult T-cell leukemia was not published until 1980 (*16*), this finding generated little clinical interest. Moreover, in this human retrovirus–associated disease, a role for an inhibitor of reverse transcriptase is not easy to envision, since the virus induces a monoclonal transformation (*17*). By the time frank malignancy is evident, there would appear to be no further need for viral replication, and therefore, for reverse transcriptase activity, in the disease process. However, the situation in AIDS appears very different. Retroviral infection of many cells appears to be necessary for the development of this disease. Therefore, inhibitors of reverse transcriptase are worth exploring as new modalities of therapy.

We now report that suramin can block the in vitro infectivity of HTLV-III (in

the form of cell-free virions) when assayed in the H9 target population, a line which expresses mature T-cell markers but is permissive for HTLV-III replication and only partially susceptible to its cytopathic effect (*8*). Also, the drug blocked the cytopathic effect of HTLV-III against a normal OKT4$^+$ (helper-inducer) T-cell clone cultured together with HTLV-III–bearing H9 cells at concentrations of 50 μg/ml (3.5×10^{-5} mol) or greater, levels that are clinically attainable in human beings (*18*). Our results with suramin might lead to an in vitro model for screening other pharmaceuticals with potential activity against HTLV-III.

In our first experiments (Fig. 1), we used clone H9 cells to investigate the inhibitory effect of suramin on the infectivity of the HTLV-III. H9 cells are permissive for the replication of HTLV-III, as described in detail earlier (*8*). When the target H9 cells were exposed to the HTLV-III$_B$ isolate (in the form of cell-free virions) and cultured for 4 to 6 days in the absence of suramin, 72 to 76 percent of the target H9 cell population became infected and began to produce the virus, as determined by an indirect immunofluorescence assay in which rabbit antibodies were used to detect expression of the HTLV-III$_B$ p24 *gag* protein. A modest, but temporary, protective effect was observed when the H9 cells were cultured in the presence of suramin at a concentration of 10 μg/ml. However, at concentrations of 100 and of 1000 μg/ml, a striking protective effect was observed throughout the 6-day interval of culture. In addition, at these higher doses, the H9 population did not develop multiple-nucleated giant cells with ring formation. The latter is a characteristic feature of HTLV-III infection in these target cells (*8*).

We then asked whether suramin could block the cytopathic effect of HTLV-III$_B$ against a normal helper-inducer T-cell growth factor (TCGF)–dependent T-cell clone, cultured with H9 cells producing HTLV-III$_B$. The normal T-cell clone (YTA1) used for this purpose has been described (*19*) and displays the following surface membrane phenotype: OKT3$^+$, OKT4$^+$, HLA-DR$^+$, Tac-antigen$^+$, and OKT8$^-$. In addition, YTA1 produces substantial quantities of TCGF and undergoes a proliferative reaction in response to antigen in the presence of appropriate accessory cells. Moreover, YTA1 shows helper activity for immunoglobulin production by normal B cells (*20*).

The results shown in Fig. 2 illustrate the protective effect of suramin on the survival and growth of a cloned normal T-cell population exposed to HTLV-III$_B$ in vitro. The YTA1 target cells were exposed to various doses of suramin for 12 hours and then cultured with irradiated (10,000 rad) HTLV-III$_B$–bearing H9 cells, with the dose of suramin kept constant during the entire time in culture. (Irradiation at this dose eliminates proliferation of H9 cells but not the infectivity of HTLV-III virions.) As a control, uninfected irradiated H9 cells were added to YTA1 cells under the same conditions. All cultures contained 15 percent (by volume) lectin-free TCGF. We determined the total numbers of viable YTA1 cells as a function of time in culture. In the absence of suramin, the

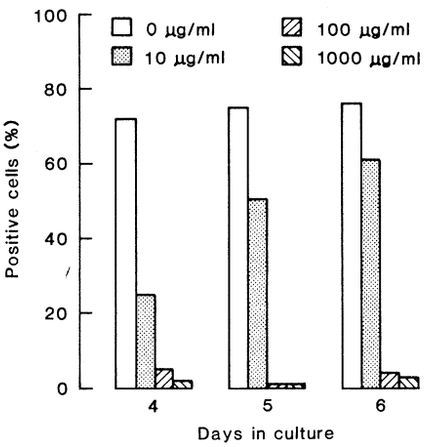

Fig. 1. Inhibition of HTLV-III$_B$ infectivity in H9 cells by suramin. Infection of H9 cells by HTLV-III$_B$ was performed as follows: The target H9 cells were exposed to suramin (10, 100, or 1000 μg/ml) for 2 hours, then to polybrene (2 μg/ml) for 30 minutes before HTLV-III$_B$ infection; control H9 cells were treated similarly but were not exposed to the drug. The H9 cells were then centrifuged (800*g*) and exposed to HTLV-III$_B$ virus (0.5 ml containing 7.5×10^7 viral particles) for 45 minutes and resuspended in fresh culture medium [RPMI 1640 supplemented with 20 percent heat-inactivated fetal calf serum, 4 m*M* L-glutamine, penicillin (50 unit/ml), and streptomycin (50 μg/ml)] and cultured in flasks at 37°C in humidified air containing 5 percent CO_2. The cells were continuously exposed to suramin at each concentration. On days 4, 5, and 6 in culture, the percentage of the target H9 cells containing p24 *gag* protein of HTLV-III$_B$ was determined by indirect immunofluorescence microscopy (*8*). Cells were washed with phosphate-buffered saline (PBS) and suspended in the same buffer at a concentration of 10^6 cells per milliliter. Approximately 50 μl of cell suspension was placed on a slide, air-dried, and fixed in acetone for 10 minutes at room temperature. Slides were stored at −20°C until use. Twenty microliters of rabbit antiserum to the p24 *gag* protein of HTLV-III (diluted 1:2000 in PBS) were applied to these preparations and incubated for 50 minutes at 37°C. Then fluorescein-conjugated goat antiserum to rabbit immunoglobulin G (Cappel) was diluted and applied to the fixed cells for 30 minutes at room temperature. Slides were then washed extensively before microscopic examination under ultraviolet illumination (Nikon; HBO 100 W; filter 450 nm NCB10; magnification ×320). Suramin was obtained from Mobay Chemical Corporation FBA Pharmaceuticals, lot No. FL 551J.

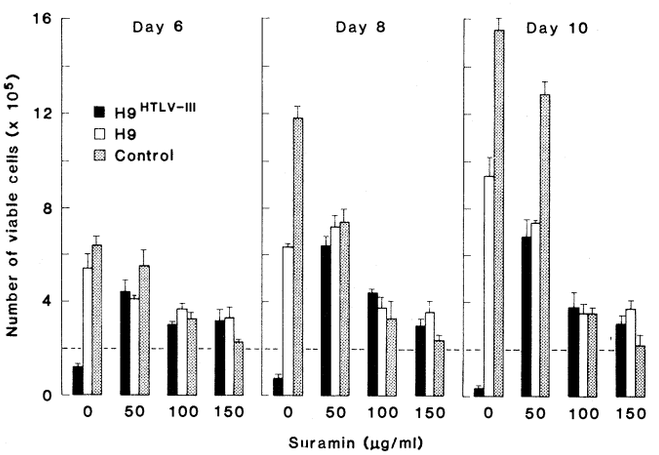

Fig. 2. Inhibition of cytopathic effect exerted by HTLV-III$_B$-bearing H9 cells against a normal helper-inducer T-cell clone (YTA1) by suramin. YTA1 cells (2×10^5) were exposed to suramin at various concentrations (0 to 150 μg/ml) for 12 hours in culture tubes (Falcon 3033) containing 2 ml of 15 percent (by volume) TCGF (Cellular Products) in the culture medium [RPMI 1640 supplemented with 15 percent heat-inactivated fetal calf serum, 4 m*M* L-glutamine, penicillin (50 unit/ml), and streptomycin (50 μg/ml)]. Culture tubes were kept at 37°C in humidified air containing 5 percent CO_2. Then these YTA1 cells were added with the equal number of irradiated (10,000 rad) HTLV-III$_B$-bearing H9 or uninfected H9 cells. Control cells were cultured without any cells added. Cells were continuously exposed to suramin and TCGF. The assays were all performed in duplicate. On days 6, 8, and 10, the viable cells were counted in a hemacytometer under the microscope by the trypan blue exclusion method. When cultured alone in the presence of TCGF, none of irradiated HTLV-III$_B$–bearing H9 or uninfected H9 cells were alive on day 6 in culture. Normal YTA1 cells could be readily distinguished from neoplastic H9 cells by morphology. The YTA1 target cells had been stimulated by soluble tetanus toxoid antigen plus irradiated (4000 rad) autologous accessory cells 6 days before these experiments as part of a cycle of restimulations. Each bar represents the mean number of viable cells (±1 standard deviation) of duplicate determinations. The dashed horizontal line shows the starting number of YTA1 cells.

HTLV-III$_B$–producing H9 cells had exerted a substantial cytopathic effect on the YTA1 population by day 6 in culture, and by day 10 the YTA1 population was almost completely killed. The addition of suramin at concentrations of 50 μg/ml or greater enabled the YTA1 target population to survive and grow in the presence of HTLV-III$_B$. (At concentrations of 100 μg/ml or higher, the drug clearly blocked the cytopathic effect of the virus but did exert an inhibitory effect on the normal target T-cell growth.) Thus, suramin can block the replication of HTLV-III$_B$ in permissive H9 cells and can also inhibit the cytopathic effect of HTLV-III$_B$ on an OKT4$^+$ T-cell clone.

Most of what is known about the clinical pharmacology of suramin derives from its use in the therapy of trypanosomiasis and onchocerciasis. After intravenous injection, the drug combines with serum proteins, and much of it circulates in the blood (*18*). It persists in the bloodstream for very long periods [for example, Hawking (*18*) has suggested that it may be detected up to 6 months], and its excretion in the urine is very slow. The therapeutic regimens in use result in considerable accumulation in the plasma (frequently in excess of 100 μg/ml) (*18, 21*). Indeed, concentrations as high as 340 μg/ml may be reached under some conditions (*18*). These concentrations exceed those found to block the infectivity (Fig. 1) and cytopathic effect (Fig. 2) of HTLV-III in the studies reported here.

The capability of suramin to inhibit the reverse transcriptases of animal retroviruses was reported in 1979 (*15*), and we recently confirmed that this agent can inhibit the reverse transcriptases of diverse retroviruses including that of HTLV-III (*22*). However, the drug is not widely viewed as an agent with antiviral therapeutic potential.

We believe the current results provide a rationale for a carefully monitored experimental trial of suramin in patients with AIDS to determine whether the drug can inhibit HTLV-III replication in vivo, and if so, whether clinical improvement takes place. However, we wish to stress several points. First, suramin has a number of recognized toxicities in human beings (*18, 21*). Renal damage is the most common toxicity, but the drug may be associated with other acute life-threatening reactions, including shock and coma. Second, it is possible that the administration of suramin to patients with AIDS could lead to side effects not currently known, or to unexpectedly severe forms of the known side effects. Third, it is possible that interfering with HTLV-III infection will not substantially benefit patients with late-stage AIDS, since at that point the damage to the immune system might be irreversible. Therefore, there may be a rationale for using this agent for experimental trials early in the disease. Finally, we believe that the administration of this agent should be undertaken only in a research facility capable of monitoring the patient's clinical, immunologic, and virologic status.

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Optical Measurements of Extracellular Calcium Depletion During a Single Heartbeat

Abstract. *The impermeant dye antipyrylazo III was used to measure depletion of extracellular calcium and net influx of calcium through the sarcolemma during the cardiac action potential. It was found that calcium entry occurs continuously during the action potential and is under direct control of the membrane potential. The inotropic action of epinephrine is accompanied by increased influx of calcium, while strophanthidin enhances the twitch without altering calcium influx during the action potential.*

Calcium for activation of contraction in the frog heart is thought to be transported from the extracellular space across the membrane under direct control of the membrane potential (*1–3*). Although a Ca^{2+} current has been identified that contributes to Ca^{2+} transport, its magnitude and its time and voltage dependence do not correspond fully to those of the tension generated (*4, 5*). It has been suggested, therefore, that activator Ca^{2+} is in part released from intracellular pools (*6*) or that it enters the cell through coupled transport systems (*7–9*). To examine these possibilities we used an impermeant Ca^{2+}-sensitive dye, antipyrylazo III (*10*), to measure depletion of Ca^{2+} from the extracellular space. Depletion of Ca^{2+} is assumed to represent net influx of the ion, since equilibration of the extracellular space with the perfusate is a slow process (time constant, 60 to 90 seconds) (*11, 12*). We found that Ca^{2+} enters the cell continuously during the action potential and does so in sufficient quantity to generate tension. The rapid onset of the depletion signal and its enhancement during the inotropic action of epinephrine suggest that Ca^{2+} influx occurs, in part, through the Ca^{2+} chan-