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The Necessity of Cofactors in the Pathogenesis of AIDS: a Mathematical Model

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Current arguments for the role of cofactors in the initiation of a chronic HIV infection and progression of AIDS are given. The natural history of an HIV infection as affected by cofactors which provide additional stimulatory signals is explored through a mathematical model. The model demonstrates that "antigen load" plays a role in determining susceptibility to an HIV infection. It also suggests that certain individuals may not be able to be infected by small doses of HIV and that the identification and treatment of existing cofactors may be useful in treating early stages of HIV infection. Prevention of cofactor exposures may also protect against HIV infection.

Introduction

It is generally accepted that HIV infection, the interaction of the virus and infected cells with the immune system, and the process by which AIDS develops are complex phenomena. Not surprisingly then, models of various types are used to help uncover consistent collections of assumptions and findings that present testable predictions as to how HIV infection is established and evolves. Here we examine the earliest stage of this process—before and just after HIV exposure—through a mathematical model. As far as we know, all other models of this type have assumed the a priori presence of an HIV infection.

It has been known for nearly a decade that, to quote Peterman *et al.* (1988), "as yet unexplained biologic variation in transmissibility or susceptibility" to human immunodeficiency virus (HIV) exists. This paper explores the role of cofactors in producing that variation. Sources of this variability would necessarily include the nature of the dominant strains (e.g. tropism) of the infecting virus, dose and mode of presentation of the virus, and the susceptibility of the host at the time of exposure to the infectious material presented in that way. Once an infection is established, the nature of the interaction of the host with HIV (primarily through aspects of the immune response) and the changes induced in each, give rise to further variability seen in the rate of progression.

Many lines of evidence suggest that HIV requires immunologically active cofactors to initiate the development of AIDS, and that these cofactors differ from one risk group to another [reviewed in Root-Bernstein (1993)]. The identification of a cofactor does not necessarily specify a mode of action for that factor, rather it indicates an association with either the incidence of or an influence in the natural history of an HIV infection. The range of possible roles of cofactors could include the direct alteration of the immune response to HIV or simply providing an opportune production of an increased number of stimulated T lymphocytes. Cofactors could be present chronically throughout the history of an HIV infection, or just appear at a time when their action (or the host's response to their presence) serves to encourage the progression of HIV. Cofactors associated with increased HIV replication *in vitro* and/or clinical studies showing increased rate of progression to AIDS include: herpes simplex type 2 (HSV-2), human herpes simplex type 6 (HHV-6), cytomegalovirus (CMV), Epstein-Barr virus (EBV),

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human T-cell lymphotropic virus, type I (HTLV-I), mycoplasmas (*M. fermentans* and *M. pirum*), drug use, alcoholism, and exposure to allogeneic stimuli (including blood transfusions, blood clotting factor concentrates, and semen). Malnutrition is also independently predictive of a poor prognosis and increased rate of development of AIDS among HIV-infected people.

The notion that HIV may benefit from the presence of other infections or immunomodulatory agents is not new. As early as 1985, Levy and his colleagues showed that active CMV infection almost invariably precedes HIV infection and concluded that, "we suspect, therefore, that cytomegalovirus infection is an important cofactor in the genesis of the syndrome and that cytomegalovirus infection of lymphocytes may be a necessary precursor for the subsequent full expression of infection by the syndrome-associated virus" (Drew *et al*., 1985, p. 63). In fact, screening blood transfusions for CMV prior to the availability of HIV tests significantly lowered the risk of transfusion-associated AIDS (Brady & Ng, 1987). Subsequent studies in all risk groups have further implicated herpes viruses as frequent cofactors in the development of AIDS (Buimovici-Klein *et al*., 1988; Sonnabend, 1989; Webster *et al*., 1989; Sabin *et al*., 1995; Lauener *et al*., 1995). Gallo has argued that HHV-6 and HTLV-1 both act synergistically with HIV (Lusso *et al*., 1989, 1990; Lusso & Gallo, 1995) and Knox & Carrigan (1996) have shown that HHV-6 can break HIV latency and may, like CMV, precede HIV infection. Lo (Lo *et al*., 1989; 1991a,b) and Montagnier (Lemaitre *et al*., 1990; Blanchard & Montagnier, 1994) have argued that mycoplasma species are also cofactors for HIV in AIDS. Genital ulcer disease caused by various sexually transmitted diseases has long been implicated as an important cofactor in the acquisition and transmission of HIV (Kreiss *et al*., 1989; Torian *et al*., 1995). Continued injected drug use has been clearly implicated as a cofactor in AIDS development (Des Jarlais *et al*., 1988; Squinto *et al*., 1990; Weber *et al*., 1990). Even things as apparently harmless as influenza and tetanus vaccination has been shown to markedly increase HIV replication among HIV-infected individuals, apparently due to stimulation of HIV-infected cells (O'Brien *et al*., 1995; Stanley *et al*., 1996). In fact, standard protocols used by almost every laboratory that grows HIV in culture employ immunologically active drugs (e.g., steroids), T-cells co-infected with herpes viruses or mycoplasmas, or mitogenic stimulation in order to promote HIV replication, infection, and T-cell killing (Lemaitre *et al*., 1990; Sarngadharan & Markham, 1987; Zagury *et al*., 1986). In short, many observations concerning the epidemiology of AIDS, the microbiology and immunology of individual patients, and techniques of cell culture, argue for an important role for cofactors (Sonnabend, 1989; Root-Bernstein, 1990, 1993, 1995).

The diversity of agents that seem to promote HIV activity has made it difficult to determine whether HIV requires any particular cofactor to cause AIDS, and some investigators have even argued that the diversity of putative cofactors itself is proof that they are neither necessary nor important. On the other hand, the diversity of cofactors may implicate a common mechanism of HIV activation by means of increased T-cell production or activation that can be initiated by a wide range of agents. Moreover, the diversity of putative cofactors leads to some interesting and testable hypotheses. The most important is that different groups at risk for AIDS will be exposed to different sorts of cofactors, and at different rates. These differences in types and rates of exposure should be reflected in differences in rates of infection with HIV, and differences in the rates at which HIV progresses to AIDS in each group.

To begin with, there is no doubt that different risk groups who acquire HIV infections are exposed at different rates to these putative cofactors. This fact is, in part, mirrored in the way risk groups are traditionally defined. Gay men have an increased risk of developing anti-semen antibodies that cross-react with their own T-cells [reviewed in Sonnabend (1989) and Root-Bernstein & DeWitt (1995)], unusually high rates of sexually transmitted diseases (STDs), addictive and recreational drug use, and antibiotic use [reviewed in Root-Bernstein (1990, 1993)]. Intravenous drug users (IVDUs) also have very high rates of STDs, drug use, illicit antibiotic use to control needle-associated infections, and malnutrition [reviewed in Root-Bernstein (1990, 1993)]. Blood transfusion patients exposed to HIV have their predisposing medical condition, surgical procedures, anesthetics, opiate analgesics, high dose antibiotics, and various herpes viruses such as CMV and EBV as additional immunosuppressive risks [reviewed in Root-Bernstein (1990, 1993)]. Hemophiliacs, especially severe ones, have their blood clotting concentrate, viral contaminants such as hepatitis viruses, and opiate analgesics, steroids and gold salts for joint injuries as additional immunosuppressive risks, but as a group have no higher risks of STDs than the general population [reviewed in Root-Bernstein (1990, 1993)]. Infants who contract HIV share the risks of their mothers, which often include the rigors of surgery, or exposure to STDs, malnutrition,

and drugs [reviewed in Root-Bernstein (1990, 1993)]. Infants have the additional problem that their HIV infection may be in place prior to the full development of their own immune systems.

Because different risk groups are exposed to different assortments of putative cofactors, each group should develop AIDS at different rates following HIV infection, and Kaplan-Meier analyses of epidemiological data confirm this prediction. Young hemophiliacs develop AIDS an average of about 18 years following infection; gay men and older hemophiliacs about 10 years following infection; blood transfusion patients, about 6 years; organ transplant recipients, about 2 years; and infants with perinatally acquired HIV infections, about 6 months [reviewed in Root-Bernstein (1995)]. Elderly patients (over age 60) also have an increased rate of AIDS development following HIV exposure (Medley *et al*., 1987). Cox proportional regression analyses of rate of progression to AIDS following HIV diagnosis show similarly significant differences. Homosexual men progress at a rate approximately two times that of intravenous drug users, and hemophiliacs and heterosexuals progress at about half the rate of IVDUs, and less than a quarter of the rate of homosexual men (Eskild *et al*., 1994a,b; Multicohort, 1994; Wolfs *et al*., 1989). Within risk groups, severe alcoholism has also been implicated as an independent cause of increased disease progression (Fong *et al*., 1994) as has malnutrition and sustained weight loss (Suttman *et al*., 1995; Chlebowski *et al*., 1995; Sappey *et al*., 1994). These findings are difficult to interpret in terms of theories of AIDS that assume HIV as the sole, direct cause of immune suppression, since there is no reason to believe that different risk groups are infected by HIV strains differing widely in pathogenicity. On the other hand, these findings may be explained if cofactors for HIV variously affect disease progression.

Similarly confounding are data that show that time-to-AIDS has been increasing progressively (Rogers *et al*., 1987). During the period of 1981–1985, it was thought that the average time from HIV infection to AIDS was about 2 or 3 years (Kim *et al*., 1987); in 1986–7, it was thought to be about 5 years (Ebbesen, 1986; Medley *et al*., 1987); by 1988 it was calculated to be 6 years (Moss *et al*., 1988); and now it is an average of about 10 years (Weiss, 1993). This increase in mean time to AIDS is partially due to statistical difficulties in estimating how many are infected and when they were infected. Due to variability in the rate of progression, those that developed the disease more quickly were observed early on, while those that develop more slowly were only to be observed later. The statistical assumption underlying most procedures is that there is only one distribution of time to AIDS which holds for all infected populations. If that was the case, we should have seen estimates which were gradually becoming more precise (more decimal points known) as time progressed and more individuals were added to the base of data. The increasing estimates of mean time to AIDS are easily understood if the HIV-infected people who developed AIDS most quickly were those with the ''most'' cofactors, and those with the ''least'' cofactors progressed much more slowly. Thus each population of infected individuals (as defined by the level and nature of their common cofactors) has its own distribution and mean time to AIDS. Along these lines, it has been suggested that HIV infection in sub-Saharan Africa progresses more rapidly than in Western countries because of increased immune activation due to endemic chronic parasitic infections and other pathogens (Bentwich *et al*., 1995).

Another corollary of the cofactor hypothesis is that HIV may not only benefit from cofactor effects, but may actually *require* the presence of cofactors in order to establish an active infection. Again, available data are not unambiguous, but they are suggestive. To begin with, not everyone exposed to HIV, or even exposed repeatedly, contracts an active infection. Some small fraction of those instances seem to be due to mutations which prevent HIV fusion with a host cell (Alkhatib *et al*., 1996). But, many people exposed to HIV in all risk groups develop a strong T-cell mediated immune response that apparently eliminates infection completely without invoking a serum antibody response (Clerici *et al*., 1992; Urnovitz *et al*., 1993; Clerici & Shearer, 1994). In other words, HIV infection is not necessarily chronic [reviewed in Root-Bernstein (1995)]. Stanley *et al*., 1996, have shown that *in vitro*, cells taken from HIV-uninfected individuals are more susceptible to HIV infection if these individuals had been immunized to a common recall antigen (tetanus toxoid).

Other observations that can be interpreted as suggesting the necessity of cofactors for HIV infection comes from analyses of the epidemiology of AIDS. All available data for Western nations indicates that although HIV became an epidemic more than 15 years ago, cases of AIDS have remained, and probably will remain, almost entirely within risk groups defined by their unusual exposure to putative cofactors: gay men engaging in high risk behaviors; intravenous drug abusers; people exposed to blood and blood products; and promiscuous heterosexuals with high rates of STDs who use drugs and/or have high rates of poverty and malnutrition (National

Research Council, 1993). This may in part be due to the different strains of HIV-1 which predominate in different parts of the world. But, even in third world countries, the risk of AIDS is directly associated with high levels of endemic disease (e.g., malaria), high rates of STDs, rampant malnutrition, and administration of blood transfusions that are often not screened for STDs, herpes viruses, hepatitis, or HIV due to lack of money and/or appropriate laboratories [reviewed in Root-Bernstein (1993)]. It could very well be the case that strain differences we observe simply reflect the strains which grow best given the nature of the cofactors most common in those populations. In short, HIV acts as if it may use to its advantage pre-existing endemic disease, immune suppression, and its ability to mutate.

The data summarized above create problems for theories proposing that HIV alone is sufficient to cause AIDS. We have therefore devised a mathematical model of HIV infection and immune function that could compare a number of possible alternative hypotheses:

(1) that HIV is both necessary and sufficient to cause AIDS;
(2) that cofactors affect the rate of progression or degree of HIV infection; and
(3) that HIV requires cofactor stimulation to become a chronic infection (i.e., that HIV is necessary but not sufficient for AIDS).

The results are presented below.

The Model

A simple mathematical model, consisting of a system of nonlinear differential equations, was constructed for the purpose of exploring the effect of "cofactors" on the natural history of an HIV infection. The definition of cofactor used was, operationally, any non-HIV substance which would result in an increase in the activation of the immune response, and through it, increased activation of the predominant host of HIV, $CD4^+$ T lymphocytes. The model is designed to first describe the pre-HIV exposure state, and then the response of the system to a low-level introduction of HIV. Activation of T-cells seems to be required for complete integration of HIV after the virus has bound and successfully entered the cell (Gowda *et al*., 1989; Zach *et al*., 1990). Activation of infected T-cells also marks the beginning of HIV replication (Zach *et al*., 1988; Zagury *et al*., 1986). In assigning immune activation a central role, we are, in spirit, following Ascher & Sheppard (1988) and Ascher *et al*. (1995) where the idea of viewing AIDS as a disease of immune activation was explored—although in that discussion, a central role for cofactors was not considered.

Explorations of the roles of cofactors which altered the immune response to HIV directly would require knowledge of the particular nature of the three-way interaction between the factor, HIV, and the immune response—information not generally available. This has dictated that the investigation here involve this simplest family of cofactors which modulate the stimulated fraction of T-cells. Although the model here is simple, one would expect behavior similar to the type described below even if further complexities were added. In this sense, this model may be the simplest description in which the potential role of cofactors may be observed. As most of the parameters as they appear here have not been measured and many will vary from individual-to-individual, the behavior of the model solutions will be presented in a way that gives general results—true for all reasonable parameter values.

MODEL DESCRIPTION

The quantity of the totality of cofactors present will be considered the "non-HIV antigen load" or simply "antigen load." The antigen load at time t, $F(t)$, is actually a weighted sum of the concentration of all stimulatory cofactors present. A weighted sum is necessary as not all cofactors are necessarily equal in their ability to assist the progression of the HIV infection through stimulation. One of the simplifications is to assume that changes in the antigen load as a function of t can be described through a single dynamic equation as opposed to a quantity requiring tracking each of the factors individually.

Other quantities in the model are $U(t)$, the number of unstimulated non-HIV infected $CD4^+$ T-cells per ml, $S(t)$, the number of stimulated non-HIV infected $CD4^+$ T-cells per ml, and $I(t)$, the number of infected $CD4^+$ T-cells per ml. The meaning of the parameters follows the equations below. A more complete description of the model and its behavior will be presented elsewhere. The system (1–4) involves four differential equations involving ten different non-negative parameters:

$$F'(t) = c - (c_1 + c_2 S(t))F(t) \tag{1}$$

$$U'(t) = c_3 - c_4 F(t)U(t) - c_5 U(t) + c_6 S(t) \tag{2}$$

$$S'(t) = c_4 F(t)U(t) - c_6' S(t) - c_7 S(t)(F(t)I(t)) \tag{3}$$

$$I'(t) = -c_4' f(t)I(t) - c_5 I(t) + c_7 S(t)(F(t)I(t)). \tag{4}$$

In the first equation, the parameters c and c_1 involve the rate at which the antigen load enters from

the external world and is removed by nonspecific means, generally though immune scavenger cells (macrophages, monocytes, and polymorphonuclear cells). Depending on the cofactor, this nonspecific removal could also be through the action of the kidney, liver, or blood serum components (such as the complement system). The parameter c_2 describes the removal of antigen through specific means (rate of removal being proportional to the level of activation of the immune system). To be true to the physical system, the parameter c should be a random quantity changing in time to reflect the changing and random nature of antigen exposure. Usually the behavior of such stochastic systems is best approximated by a deterministic system using the time-average of that random term. This is the formal justification for the constant antigen-load source.

The second equation describes the population dynamics of the unstimulated mature $CD4^+$ T-cells. There is a source of these cells (from bone marrow and periphery) at rate c_3. The unstimulated cells die at rate c_5 and expand their population as stimulated cells lose that stimulation at rate c_6. The rate at which unstimulated cells become stimulated (and leave the unstimulated population) is proportional to the level of the antigen load and the number of unstimulated cells (with constant of proportionality c_4). Instead of using a "source" to maintain this unstimulated population, studies were also done using the division of peripheral cells as the primary source. The basic results were the same, illustrating that only the equilibrium value before HIV introduction was critical (data not shown). For this reason, the algebraically simpler constant source term is used here.

The stimulated T cell population has input from the unstimulated population (the first term) and loses population as cells revert back to unstimulated small T cells (rate c_6), die, or enter a terminal differentiation state. Stimulated T-cells can also divide. We have included the base line reproduction rate in the parameter c_3, so that the rate included below is that part of the reproduction rate that exceeds the base line reproduction rate. The parameter c_6', then, includes the following aspects

$$c_6' = c_6 + [\text{death rate} + (\text{terminal differentiation rate}) - \text{excess reproduction rate}].$$

It will be assumed in all of the theorems that the sum of the rates in brackets above is non-negative, that is, that $c_6' \geqslant c_6$.

The last term in this equation has a somewhat unusual form, involving the product of F and I. This product is proportional to the rate at which infected cells are "stimulated" and begin producing virus. As the infective half-life in serum is relatively short (Layne *et al*., 1992), this product is also (approximately) proportional to the infective free virus concentration at time t. The triple product in last term, then, is proportional to (with constant of proportionality c_7) the rate at which stimulated cells interact with free virus and become infected. The form of this term comes from a "quasi-steady state" assumption on the following equation of infectious free virus, $V(t)$:

$$V'(t) = s_1 FI - s_2 V.$$

In this equation, free virus is produced at a rate proportional to the non-HIV antigen load and the number of infected cells and infectivity is lost exponentially. Assuming that this process takes place on a different time-scale from the other processes represented by the other equations, except for negligible time, $V' \approx 0$, and as a result,

$$V = \frac{s_1}{s_2} FI$$

for most of the process. The role of HIV itself in this process suggests that a better formulation may be

$$V'(t) = s_1(F + V)I - s_2 V$$

to indicate the potential HIV contribution to the stimulation of infected cells. If the viremia stage of an HIV infection was to be modeled, this extra term would be necessary. Here we are interested in the very early stages of the process, and as a result, the first virus equation was used.

The final equation involves the (eventual) death of infected cells as they are stimulated (rate c_4') and the natural death of infected cells (not directly due to HIV)—rate c_5. The last term again describes the rate of infection of stimulated T cells. We have used the rate coefficients with the primes to indicate parameters with the same kinetic role as the unprimed version. The relationship between c_4 and c_4' depends on whether infected and uninfected T cells are stimulated at the same rate when presented with the same stimuli.

GENERIC MODEL BEHAVIOR

The basic behavior of solutions to (1–4) is described through the following theorems [proofs given in Merrill *et al*. (1997b)] and illustrative simulations. All simulations employed MAPLE V (MapleSoft, Waterloo, ON). The first theorem describes the overall behavior of all solutions. It basically states that solutions to system (1–4) are "reasonable" and that

if no HIV-infected cells are present at time zero, and there is no process that introduces them, there will be no infected cells at later times.

Theorem 1

For system (1–4) *with all parameters positive and* $c_6' \geqslant c_6$, *all solutions with non-negative initial conditions at time* $t = 0$

1. *Exist for all* $t > 0$, *and*
2. *Remain bounded for all* $t > 0$.

Moreover, if there are no HIV-infected cells at time 0 (*and as a result, the rate of infection by HIV is zero*), $I(0) = 0$, *then* $I(t) = 0$ *for all* $t > 0$.

The boundedness of the solutions of the system under study correctly predicts that humans do not contain unlimited numbers of T-cells. This boundedness result requires the condition $c_6' \geqslant c_6$, reinforcing the reasonableness of that assumption.

The second result concerns the natural or "virgin" equilibrium between the quantities describing antigen load, unstimulated cells, and stimulated cells in the absence of an HIV infection. This result also describes the relationship between the equilibrium value of the stimulated population and the value of c, the rate of introduction of new antigen. It suggests that the equilibrium number of stimulated cells per ml will be larger if the rate at which the cofactor enters the system is larger.

Theorem 2

System (1–4) *with all parameters positive has a unique equilibrium having I coordinate zero*, $(F_0, U_0, S_0, 0)$, in the non-negative orthant. As a function of the parameter c (the rate of new antigen exposure), S_0, *the equilibrium value of the stimulated cell population, is an increasing function*.

In order to describe the susceptibility of the system under study to an exposure of HIV, we determine the response of this equilibrium to a perturbation in the I direction. The eigenvalue λ_0 corresponding to that direction provides the relative decay or growth rate depending on the sign. An asymptotically stable equilibrium ($\lambda_0 < 0$) would indicate that a small number of infected cells would be eliminated over time. An unstable equilibrium ($\lambda_0 > 0$) will indicate that a small number of infected cells would be expected to grow in numbers and not be eliminated.

Theorem 3

The equilibrium $(F_0, U_0, S_0, 0)$ *of system* (1–4) *with* $c_6' \leqslant c_6$ *has at least a three dimensional stable manifold contained in* $\{(F, U, S, I) | I = 0\}$ *for all non-negative parameter choices. Moreover, this equilibrium is asymptotically stable if*

$$\lambda_0 = -c_4' f_0 - c_5 + c_7 S_0 F_0 < 0$$

and unstable if $\lambda_0 > 0$.

The final result establishes the conditions under which one is or is *not susceptible* to an HIV infection through a small initial dose in terms of the parameter c, the rate of introduction of the cofactor, the rate of virus production from stimulated infected cells, c_7, specific elimination rate of the cofactor, c_2, and the death rate of unstimulated cells, c_5. Asymptotic stability of the equilibrium means biologically that one is not susceptible to an introduction of a small number of infected cells. If one is susceptible to infection after introduction of a small number of infected cells, the equilibrium will be unstable. Biologically this implies that in that case, a small number of infected cells will establish a chronic HIV infection.

Theorem 4

If $cc_7 - c_5 c_2 \leqslant 0$, *the equilibrium* $(F_0, U_0, S_0, 0)$ *is always asymptotically stable. Moreover, for c sufficiently large and* $c_3/c_6' - c_6 > c_4'/c_7$, $(F_0, U_0, S_0, 0)$ *is unstable*.

The parameter c is the rate at which cofactors (as measured through antigen load) enter. This last theorem gives evidence that susceptibility to HIV infection may well be determined by antigen load for some individuals.

SIMULATION RESULTS

There are only a few cases needed to illustrate the dynamic behavior of solutions to system (1–4) given the results above:

(1) the dynamics when $I(0) = 0$ (Fig. 1);
(2) the dynamics when $I(0) > 0$ and $\lambda_0 < 0$ (Fig. 2);
(3) the equilibrium dynamics when $I(0) > 0$ and $\lambda_0 > 0$ (Fig. 3); and
(4) the dynamics of $I(t)$ when $I(0) > 0$ and $\lambda_0 > 0$ (Fig. 4).

Discussion

Several important conclusions follow from these results. One is the suggestion that any agent(s) that increase the baseline level of T lymphocyte stimulation can act as "cofactors" as defined here. As there are many agents that have that potential, there need not be, and probably cannot be, a single cofactor for HIV. Although it is impossible to convert the

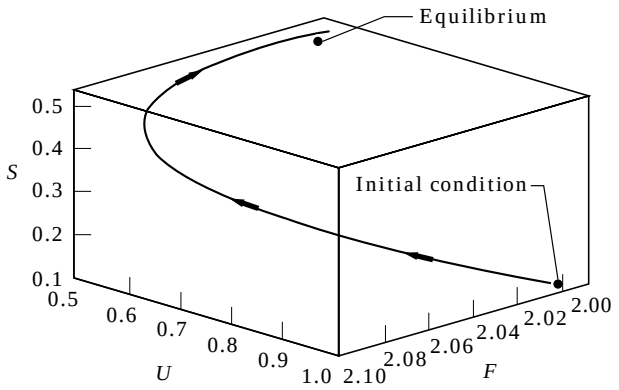

FIG. 1. Typical solution in time for the case that the parameter c (the rate at which new antigen load is introduced) is small and $I(0) = 0$ (no HIV is present). The solution quickly approaches its unique equilibrium value. In biological terms, the immune system controls infection, adjusting the level of stimulated T-cells to accomplish this. Here $\lambda_0 < 0$, but a similar picture results for any value of λ_0.

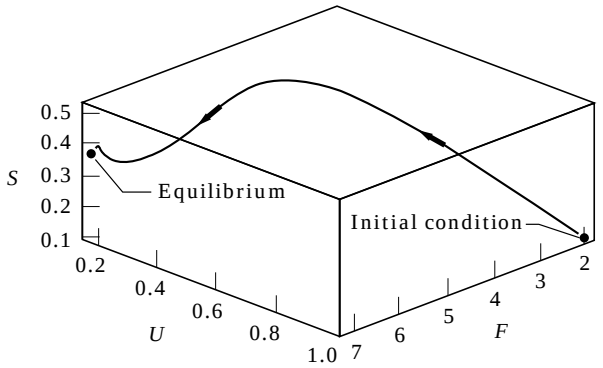

FIG. 3. The equilibrium behavior of immune function when we are at a critical antigen load, $\lambda_0 > 0$, and HIV infection is introduced, $I(0) > 0$. The dynamics for the variable $I(t)$ is shown over the same time interval in Fig. 4. Note that the number of unstimulated cells drops, stimulated cells rise and antigen load at the new equilibrium has increased.

numbers from our equations into specific numbers of cofactor stimuli, it is nonetheless evident that multiple stimuli are more likely to lead to an active HIV infection than a single one (as the level of baseline stimulation will likely be higher). Thus, the finding that most HIV-infected people are multiply-infected with herpes viruses, HTLVs, hepatitis viruses, mycoplasmas and other bacteria, and often have lymphocytotoxic autoantibodies prior to serious immune suppression (Buimovici-Klein *et al*., 1988; Root-Bernstein, 1993), may be highly relevant to understanding why they became infected with HIV in the first place. Indeed, there is no evidence that anyone infected with HIV is free of such cofactor stimuli. AIDS, unlike measles or syphilis for example, does not appear to develop in immunologically healthy individuals.

Notably, previous modelling of AIDS epidemiology by Weyer and Eggers has shown that it is not possible accurately to mimic the progress of AIDS in Europe without assuming the presence of at least one co-epidemic cofactor for HIV (Weyer & Eggers, 1990; Stewart, 1995). It should be noted that the

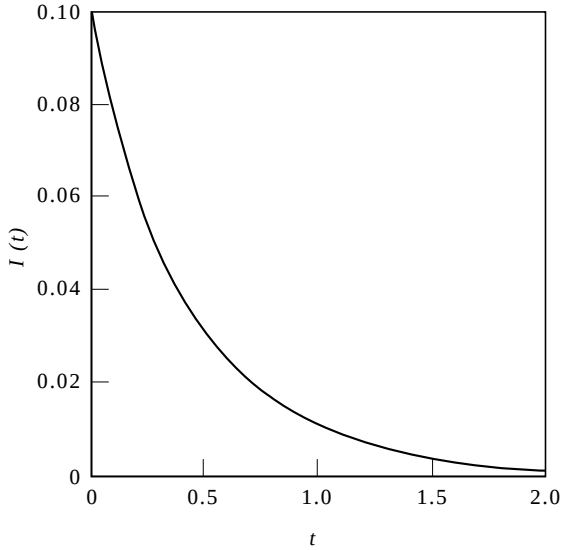

FIG. 2. Same parameter values as in Fig. 1, with a small HIV infection introduced, $I(0) = 0.1 > 0$. The HIV-infected cell population, $I(t)$ goes to zero with time. In biological terms, when $\lambda_0 < 0$, the immune system is able to mount an effective immune response to eliminate HIV (Clerici *et al*., 1992; Clerici & Shearer, 1994).

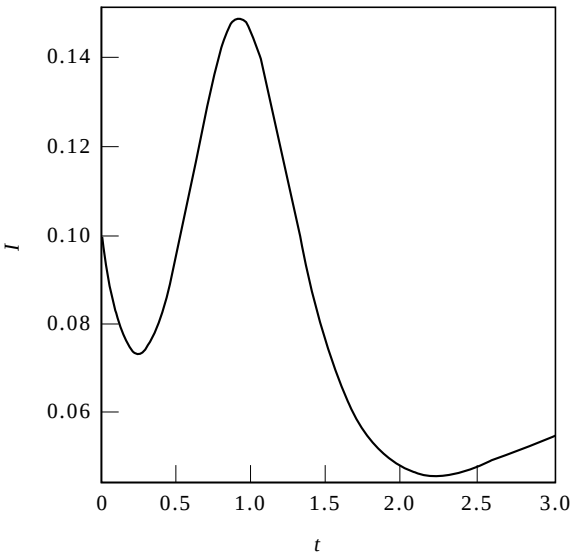

FIG. 4. When the critical antigen load is exceeded, $\lambda_0 > 0$, and HIV infection is introduced, $I(0) > 0$, the concentration of HIV-infected T-cells, $I(t)$, does not go to zero with time. In other words, a chronic HIV infection results.

assumption of a multifactorial etiology for AIDS in which HIV is necessary but not sufficient has accepted precedent. Many cases of infectious diseases caused by synergistic interactions between microbes are known and their etiologies have been proven in laboratory experiments [reviewed in Weyer & Eggers (1990) and Root-Bernstein (1993, 1995)]. It is thus interesting to compare our model with previous work by Merrill *et al*. (1997a) and by Nowak *et al*. (1991) modelling the onset of AIDS due to multiplication of HIV pseudo-species within an individual. At first glance, a purely HIV-induced immune suppression may seem incompatible with one requiring the presence of multiple cofactors. In fact, the two theories are not incompatible.

First, it must be understood that the previous work by Merrill and by Nowak & May assumed the presence of an active HIV infection. The current paper explores what conditions are necessary for such an active HIV infection to take hold and argues that active HIV infection cannot be maintained in the absence of a minimum degree of cofactor stimulation of the immune system. Such activation cannot be achieved by HIV alone, and therefore HIV is, in some sense, an opportunistic infection.

Second, the mathematics underlying both the Merrill and Nowak–May theories do not distinguish between HIV pseudo-species activating individual T-cell clones and such activation occurring due to cofactor infections (Nowak *et al*., 1991; McLean & Nowak, 1992). Thus, cofactor infections may logically replace at least some subsets of HIV pseudo-species in these models, with the same outcome.

Third, and most importantly, both the multiple-pseudo-species and the cofactor theories of AIDS progression are compatible with a single, more inclusive theory of AIDS causation by means of exhaustion of the immune system. It has long been thought, for example, that the immune suppression accompanying Trypanosomiasis and other parasitic diseases is due to chronic polyclonal activation of T cells (Assoku *et al*., 1979; Dineen & Wagland, 1966), and several teams have suggested that a similar mechanism of immune exhaustion produces AIDS (Muller & Taeshita, 1991; Sjamsoedin-Visser *et al*., 1988; Michalany *et al*., 1987; Hood *et al*., 1982). Such immune exhaustion has been shown experimentally to result from over-production of cytokines (Muller & Takeshita, 1991) and to result not only in $CD4^+$ but also $CD8^+$ cell death (Doherty, 1993). Experimentally, immune exhaustion and significant cellular atrophy of the immune system has been produced in mice by means of multiple, concurrent infections (Cayzer & Dobson, 1983) and morphine (Arora *et al*., 1990) and was shown to be a critical factor establishing carrier states for viral infections in otherwise resistent mice (Battegay *et al*., 1994; Mokiphidis *et al*., 1993). Notably, immune exhaustion associated with cytokine over-production has also been seen previously in HIV-negative individuals in two AIDS risk groups: blood transfusion patients (Grob *et al*., 1987) and opiate addicts (Lazzarin *et al*., 1984). These data suggest that multiple-concurrent infections, or other forms of constant immune system activation (as might be found accompanying i.v. drug use, for example) preceding or accompanying HIV infection do have a profound effect on immune function and thereby on the progress of HIV. Our mathematical model, in other words, is not purely theoretical but is based upon well-established immunological observations that have generality beyond AIDS itself.

We also note that the current theory is compatible with several mechanisms, including synergistic interactions of antigens (Sonnabend, 1989), autoimmune mechanisms (Root-Bernstein & Hobbs, 1993), a Th1 to Th2 switch in immune response to HIV (Clerici & Shearer, 1994), and cofactor stimulated retrotransposon activity (Johnson *et al*., 1995), among others.

The theory is testable. Among the testable predictions of the theory are the following. First, an *in vitro* model should be possible. The ability of HIV to infect T-cells, macrophages, and dendritic cells *in culture*, and the rate of HIV replication and cell-killing *in culture*, should be dependent on the presence of cofactor infections, mitogens, or other immunomodulatory agents. Some evidence for such effects already exist (e.g. Lemaitre *et al*., 1990; Lo *et al*., 1991b; Lusso *et al*., 1989, 1990; Knox & Carrigan, 1996), but no attempt to model HIV growth rates has been made.

A second test of the theory is that people exposed to HIV in the absence of its cofactors should have extremely low rates of active infection. This feature of the theory may explain why the incidence of HIV infection following percutaneous exposure among health care workers is less than 4 per 1000 (CDC, 1991; Henry *et al*., 1990). This explanation is testable by examining the virological, bacteriological, autoimmune, and drug status (or simply the degree of T-cell activation) in people exposed to HIV at the time of exposure. Our prediction is that immunologically healthy individuals lacking multiple cofactors will not develop active HIV infections, whereas those who do develop active infections will be found to be immune depressed and/or to have multiple cofactor exposures prior to, at the time of, or immediately

following HIV exposure. In other words, health care workers who seroconvert to HIV will be found to be immunologically different from those who do not. In animal models, chimps and macaques with HIV-1 or baboons with HIV-2, the theory suggests that cofactors would facilitate HIV-infection and AIDS development. The theory suggests that difficulties in obtaining useful animal models of AIDS may be due to the "clean" environment in which the animals are kept.

A third testable prediction is that people who seroconvert to HIV may subsequently serorevert if the cofactor load is sufficiently low. An increasing body of data support the existence of true seroreversions, but so far no one has studied the role of cofactors (or their absence) in such cases [reviewed in Root-Bernstein (1995)]. It is notable, however, that many seroreverters are reported to have had limited risks and/or to have severely modified their risk behaviors following HIV infection [reviewed in Root-Bernstein (1995)]. Once again, monitoring of cofactor exposures may be predictive in such cases, and alteration of risk behaviors and/or treatment of existing cofactors should be beneficial in cases where the immunological load is not too high.

Fourth, the relative antigen load may explain the rate of progression to AIDS and, conversely, the existence of long-term survivors of HIV infection. So far, there is no relevant data on cofactors with regard to long-term HIV survival.

Fifth, our theory may explain the observation that HIV has remained almost completely within high-risk groups identified at the beginning of the epidemic, and has not spread, as was originally predicted, to the general heterosexual population in Western countries (National Research Council, 1993). In the absence of high levels of cofactors in the general population, such spread is not possible. Thus, we predict that there will never be an epidemic of heterosexual AIDS in Western countries as long has general public health measures and nutritional standards are maintained. Rather, HIV and AIDS will always remain within high risk groups characterized by multiple cofactor exposures, such as drug addicts, those exposed to multiple sexually transmitted diseases, people exposed to allogeneic stimuli, immune suppressed people, and the malnourished. These same factors explain the much higher prevalence of HIV in Third World nations (Quinn *et al*., 1987).

A final logical test of the theory, and one that also has important prophylaxis and treatment implications, is that cofactor exposures significantly affect the rate of progression to AIDS is that elimination of such exposures should have the salutary effect of slowing progression to AIDS. On this point the available data are both less abundant and less clear-cut than on the issue of differing rates of progression among differing risk groups, but they are nonetheless suggestive. The most dramatic findings concern the improved immunological profiles of hemophiliacs switched from factor concentrates (which contain significant contaminants, including hepatitis viruses) to ultra-high purity factor products. Long-term studies show that all participants experience a stabilization of T-cell counts and as many as 30% show a marked improvement in T-cell counts (Hilgartner *et al*., 1993; Mannucci *et al*., 1992; Gomperts *et al*., 1992). Similarly, elimination of ongoing intravenous drug use significantly slows progression to AIDS (Groenbladh & Gunne, 1989; Weber *et al*., 1990). Nutritional interventions, in some cases as simple as beta-carotene supplements, have also proven to significantly affect survival for all risk groups (Chlebowski *et al*., 1995; Coutsoudis *et al*., 1995). BCG vaccination against mycobacteria have also been reported to protect against AIDS (Kallenius *et al*., 1989). Significant increases in survival have also been found through the use of Foscarnet for treatment of CMV infections in AIDS (Jacobson, 1994; Markham & Faulds, 1994). Since cofactors may be more amenable to treatment than HIV itself, we believe that AIDS prevention and treatment would benefit from a stronger focus on cofactors.

Concluding Remarks

Although we have avoided discussing specific mechanisms by which cofactors can influence the establishment of a chronic HIV infection, the mathematical model suggests that even simple interactions between cofactors and the immune response can result in a variable susceptibility to HIV. This together with the clinical and *in vitro* observations suggest that trying to understand the complexities seen in HIV infection without also dealing with underlying cofactors is not possible. If indeed cofactors "set the table" for an HIV infection, treating the underlying existing pathogens may be appropriate for early HIV treatment. It follows that prophylaxis against, or effective treatments for, cofactors such as herpes viruses, mycoplasmas, malnutrition, drug use, and other cofactors may be as effective both in preventing and treating AIDS as any direct attack on HIV itself. This statement does not imply that research on HIV is irrelevant to controlling AIDS, but rather that it is only half of a much more complex story. We conclude that HIV and AIDS are problems for the immunologically stressed, both in

Western and under-developed nations. It follows that the spread of HIV, and thus of AIDS itself, can most effectively be regulated by focussing both on HIV *and* on public health measures that address the much more widespread effects of the cofactors that are associated with establishment and progression of HIV infection. Where poverty, malnutrition, endemic disease, and drugs are defeated, HIV and AIDS too will diminish. Conversely, HIV and AIDS will flourish where these other health problems are endemic.

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