is expected to make a definitive diagnosis or, at least, to arrange investigation so that one can be made as speedily as possible. Having marginalized danger, the generalist is under no such pressure and may employ the technique of review over time, utilizing an already extensive knowledge of an individual's personal background and medical and social history.

There are at least two major ways in which developments in molecular medicine and genetics may adversely affect this process. The first is, once again, through the beguilingly increasing availability of genomic information, whose introduction into the consultation could distort the diagnostic method and could lead to amplification of the need of investigation and referral in many patients whose symptoms, in reality, merely betoken a self-limiting episode of minor illness. The second is the increasing availability of near-patient (office) diagnostic tests, frequently depending on identification of proteins expressed by a range of genes, associated with the presence of specific diseases. In this context the distinction between wanted and unwanted knowledge may be thrown into sharp focus. Instead of making judicious use of time and appropriate technology, primary care physicians may increasingly find themselves under pressure to undertake investigations that may have more significance for the need for further referral and investigation than for the management of self-limiting disorders.

The gatekeeper role of the primary care physician, acting as a referral filter between relatively inexpensive primary care and potentially expensive secondary care, is likely to be of critical importance in sustaining a cost-effective health care system. The characteristic diagnostic methods of the primary care physician, coupled with the appropriate use of time and technology, are important ways in which medical care is contained within the primary care sector. The escape into this sector of seductive technology that has not been properly evaluated may have serious consequences, resulting in escalation of the demand for expensive secondary care referrals and investigations.

It is possible to think of other ways in which an increasing appreciation of both risk and hazard in the general population and of diagnostic information that is neither needed nor wanted in both primary and secondary care sectors could lead to a general lowering of thresholds for consultation and referral. As well as welcoming the immensely important diagnostic and therapeutic advances promised by molecular medicine, health policy-makers and physicians alike will do well to sustain a keen awareness of the potential for disbenefits and adverse effects on precarious systems of health care.

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HIV-1 Tat protein as a potential AIDS vaccine

Intracellular traffic of the HIV-1 transactivator protein Tat appears essential for pathogenesis, and interdiction by immunization-induced antibodies to Tat should prevent AIDS.

GIDEON GOLDSTEIN

The Tat transactivator of HIV-1 is essential for the massive initial output of virus that is thought to enable HIV mutational variants to outpace and overwhelm the immune system[1,2], leading to chronic infection, eventual destruction of the immune system, and progression to AIDS. Tat protein release and cellular uptake are necessary for this process; it is thus proposed here that immunologic interdiction of extracellular Tat protein by prophylactic active immunization should critically reduce explosive replication of the virus and permit effective immune control.

Acute infection with HIV-1 is characterized by early high levels of plasma viremia, which decline as the immune response develops, but then persist through the long period of clinical latency[3]. The early relative sequence homogeneity of the virus, indicative of a single dominant strain, is soon changed by the rapid appearance of viral variants, presumably due to selective pressure of the developing immune response[1,2]. HIV-1 reverse transcriptase is highly error-prone, leading to high mutation rates and corresponding viral diversity, even within a single host[4]. This propensity to multiply rapidly, and to mutate frequently, has confounded conventional therapeutic and vaccine approaches to the treatment and prophylaxis of HIV-1 infection. Thus the developing immune response following acute infection is already exerting selective pressure for variant strains that can evade immune control.

Vaccination has been successful in control of various viral diseases and induces specific cytotoxic T-cell elimination of infected cells displaying viral proteins in association with HLA molecules and/or specific antibody blocking and clearing of free virus. This approach is effective for viruses with stable phenotypes, such as smallpox and measles, and for viruses with limited variation in their antigenic epitopes, such as poliomyelitis. But this mode of vaccination becomes more problematic with viruses such as influenza, for which the predominant epitopes may change from year to year, necessitating the preparation of an annual vaccine for use before the winter flu season.

For HIV-1, the huge diversity in immunogenic viral epitopes and the rapid mutational variations that occur within and between individuals[5,6] have so far prevented successful application of these conventional approaches[6].

Acute infection with HIV and clinical outcome

There is now ample evidence that exposure to HIV-1 has various outcomes. Some subjects remain HIV-1 seronegative yet show evidence of specific T-cell immunity to HIV-1. Reports of such cases include health care workers exposed to contaminated blood, infants born of HIV-1-infected mothers, and also subjects repeatedly exposed to HIV-1 from unprotected intercourse, including homosexuals, prostitutes and sexual partners of infected individuals. Thus it has been postulated that an initial encounter with low doses of virus can evoke protective cellular immunity, with little or no subsequent viremia, in the absence of seroconversion[1]. The corollary is that a suitably attenuated infection could protect from further exposure to amounts of infectious HIV-1 that would lead to a more typical progressive clinical course in an unprotected person. Some asymptomatic HIV-1-seropositive subjects progress to overt disease very slowly or not at all, and these subjects have low or absent plasma virus levels[1,3] and low levels of HIV mRNA in peripheral blood mononuclear cells (PBMCs)[7].

Clinical observations suggest that the nature of the initial infection critically determines the various outcomes of HIV-1 infection, in that subjects who seroconvert following a virus-like illness have a worse prognosis and more rapid progression than subjects who seroconvert silently with no obvious acute illness[8]. In keeping with this, post-seroconversion measures of viral burden are powerful prognostic predictors of disease progression[3,7,9]. Asymptomatic HIV-infected subjects have plasma viremia despite evidence of an active anti-HIV-1 cytotoxic T-cell response, a steady state with high viral proliferation and high daily elimination of infected cells. This steady-state balance may shift over time in favor of the virus, leading to clinical progression to AIDS and death.

The critical relation between magnitude of initial infection and clinical outcome is well illustrated by laboratory studies of another potentially chronic virus, lymphocytic choriomeningitis virus (LCMV)[10]. Small inocula give rise to increasing viremia during the first week, until the appearance of LCMV-specific T cells. As these increase, viremia declines, leaving the host free of virus and immune to reinfection. But with a thousandfold larger inoculum, there is early high viremia and only a transient cytotoxic response. High levels of virus appear to induce high-dose tolerance of the immune system. Intermediate doses of LCMV lead to a state reminiscent of viremic asymptomatic HIV-infected subjects: LCMV viremia coexisting with LCMV-specific cytotoxic T cells over a long period[10].

Thus clinical and experimental observations imply that control of viral expansion during the initial acute viral infection is a key point for intervention to negate chronic viremia and clinical progression. For particular viruses this is achieved by conventional vaccination, specific cytotoxic T cells eliminating infected cells more rapidly than infection of fresh cells, while specific antibodies block the cell-to-cell transmission necessary for viral infectivity and expansion. But when this mechanism is thwarted by the high mutability of HIV-1, and the rapid selection of variant strains resistant to the immune response, a different approach is called for.

A novel mode of vaccination proposed for AIDS protection

As will be discussed in detail below, specific immunization with Tat protein, the product of the HIV-1 *tat* gene, affords a radically different strategy for AIDS vaccination, because it is directed to a product of the virus that is essential to pathogenesis rather than to the virion itself, analogous to a toxoid vaccine for diphtheria or tetanus.

Tat protein is a transactivator essential not only for effective transcription and replication of the virus within cells but also for the prodigious output of virus that follows initial infection, because this is largely due to released Tat protein, which passes extracellularly to other cells and renders them susceptible to productive viral infection (Fig. 1). Prior specific vaccination against Tat protein is designed to abolish the intercellular traffic of this transactivator, thereby removing the evidently essential basis for the massive multiplication of virus, which fosters uncontrollable production of viral variants surviving and eventually destroying immune defenses.

Nontranscriptional function of Tat protein

The Tat protein of HIV-1 is a transcriptional activator essential for efficient replication of HIV-1 (ref. 11). Tat is an unusual activator in that it binds to a Tat-responsive RNA element, termed TAR, located 40 base pairs downstream from the transcription initiation site. An additional function(s) for Tat was detected

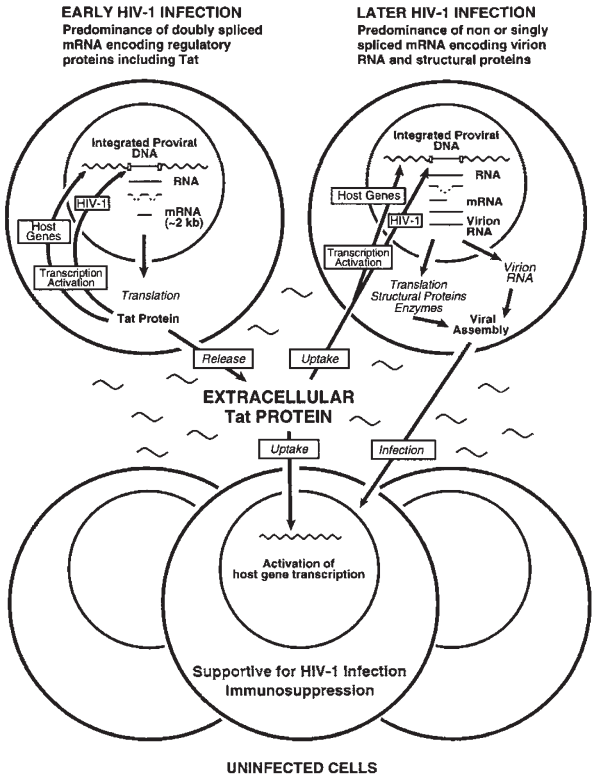

Fig. 1 Diagram emphasizing the essential role of extracellular Tat protein in HIV-1 replication and infection. In early HIV-1-infected cells, doubly spliced mRNAs encoding regulatory proteins, including Tat, predominate, and Tat is released extracellularly. Later in HIV-1 infection, singly or nonspliced mRNAs predominate[34], and these encode enzymes and structural proteins. Extracellular Tat protein is avidly taken up by cells, enhancing HIV-1 replication in infected cells and activating host cellular genes in all cells, rendering them supportive for infection with HIV-1. Furthermore, cell-cycle progression is inhibited in T cells, with increased susceptibility to apoptosis, changes contributing to immunosuppression.

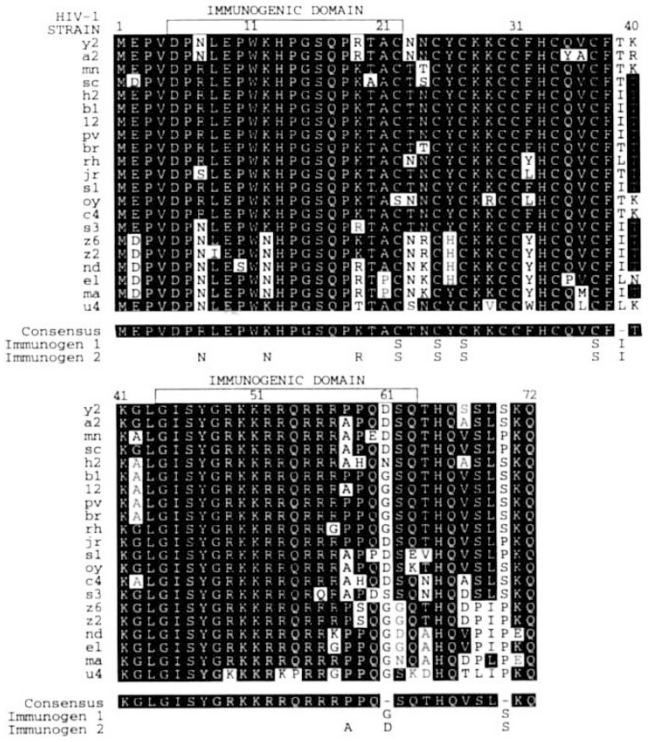

Fig. 2 Complete amino acid (aa) sequences of the first exon of 21 HIV-1 Tat proteins. There is high amino acid sequence conservation, probably due to constraints related to the two other open reading frames in the region of the tat gene. The key immunogenic domains are aa 5 to 22 and aa 44 to 63. Accordingly, the consensus sequence could be used as the primary immunogen, with alternative amino acids in the immunogenic domains incorporated in additional immunogens, as necessary. In a further refinement, Ser for Cys substitutions could be made at positions 22, 25, 27, and/or 37, since these changes would block any potential for transactivation by the immunogen itself and would be unlikely to affect antibody responses to the immunogenic domains.

during analysis of a series of genetically altered HIV-1 proviruses[11]. As expected, replication was disabled if either Tat or TAR were rendered nonfunctional. However, replication was restored in *tat*-defective mutants by incorporating Tax, the corresponding transcriptional activator in the related retrovirus HTLV-1, because Tax also can activate transcription via the HIV-1 long terminal repeat (LTR), utilizing the NF-κB motifs. Defective replication in *tat*-negative or TAR-negative mutants could also be repaired by insertion of cDNA for a Gal4-VP16 fusion protein and four Gal4 binding sites, providing a distinct transactivator in these mutants. Replication was restored in all constructs that contained appropriate binding and activator modules. Interestingly, infectivity in tissue culture did not parallel complementation of defective transcriptional activation. Only constructs in which *tat* was intact and producing Tat protein were infective, even if Tat was not involved in transcriptional activation because TAR was disabled. It was concluded that Tat protein must have a novel nontranscriptional function in virion infectivity[11]. Other studies point to the extracellular release of Tat , as well as uptake by both infected and uninfected cells, as a key element by which Tat protein facilitates the infectivity of HIV-1 (see below).

Tat protein intercellular passage is critical for HIV infectivity

Tat protein is released from infected cells by a process that does not involve cell death and appears to involve nonclassical pathways, since Tat contains no amino-terminal hydrophobic leader sequence that could direct secretion into the endoplasmic reticulum. *In vitro* release is extensive[12,13], and biologically significant levels of Tat have been detected in the serum of HIV-1-infected subjects[14], reinforcing the clinical relevance of the *in vitro* studies. Tat binds to and is avidly taken up by cells, with eventual translocation to the nucleus[15,16]. Amino acids 49–57 of Tat (Tat_{49-57}), containing the basic domain, is essential for uptake, and this sequence also contains a nuclear localization signal (GRKKR)[17]. Tat uptake not only enhances HIV-1 transcription in infected cells, it also affects a range of host cell genes in both infected and uninfected cells. This includes activation of tumor necrosis factor (TNF), interleukin-6 (IL-6), CD-4, IL-2, IL-2Rα, IL-10, transforming growth factor-β1 (TGF-β1), and CD95 ligand[18–22]. Other cellular genes such as p53 and Mn-superoxide dismutase are downregulated[23,24].

A particularly noteworthy effect of Tat is the modification of cell-cycle-dependent protein kinase (Cdk) activity[25]. Enhanced total Cdk activation rendered Tat-treated cells susceptible to apoptosis, changes that are strikingly similar to those found in lymphoid cells from HIV-1-infected subjects[26]. These effects of Tat protein on uninfected cells, and the similar apoptosis-sensitizing effects of CD95 ligand gene overexpression[14], could represent major contributions by Tat to the immunosuppression of HIV-1 infection.

Antibodies to Tat block cellular uptake

Both monoclonal and polyclonal antibodies to Tat protein have been readily produced in animals and shown to block uptake of Tat *in vitro*. Two major immunogenic regions were identified from monoclonal antibody binding patterns on synthetic peptides, and these corresponded to the proline-rich domain toward the N-terminus (amino acids 5–22) and the basic domain (amino acids 44–62). Approximately half the reactive antibodies to either immunodominant domain inhibited Tat uptake, as measured by inhibition of Tat-mediated transactivation of an HIV-1 LTR reporter construct[27]. The N-terminal domain of Tat also appears to be immunodominant in humans, as judged by the binding patterns of antibodies found in HIV-1-infected subjects[28]. The blocking of Tat protein uptake by antibodies has also been demonstrated by the use of inhibition of Tat-induced growth stimulation of AIDS-Kaposi's sarcoma cells, and Tat-induced production of TGF-β1 (ref. 22).

Most important, monoclonal or polyclonal antibodies to Tat added to tissue culture medium attenuated HIV-1 infection *in vitro*[29,30], reaffirming the essential role of extracellular Tat for the amplification of HIV-1 infection. Antibody to Tat caused a significant reduction and a consistent delay in HIV-1 replication with PBMCs from HIV-1-infected subjects cocultivated with activated normal PBMCs (ref. 30), in keeping with the proposed application of prophylactic immunization for the attenuation of acute infection. Additionally, antibodies to Tat inhibited HIV-1 replication in chronically infected cell lines[29,30], supporting a potential application of immunization

for the treatment of HIV-1-infected subjects early in the course of infection. Antibodies to Tat occur both naturally and in response to HIV-1 infection, and there is a correlation of low or absent antibodies with progression to AIDS (ref. 30–32), further supporting the notion that extracellular Tat interdiction by antibodies can contribute to the control of HIV-1 infection.

The problem of variation, mutation and escape

The major impediment to conventional vaccination against HIV-1 is the great diversity among viral strains of epitopes of the structural proteins (toward which the protective immune response is directed) and their potential to change rapidly under selective pressures because of high viral replication and mutation rates. This high variability in viral antigenic epitopes involves regional, individual-to-individual and within-individual differences. In contrast to this perspective, Tat protein offers a far more attractive vaccine target.

First, the two immunodominant domains in the essential first exon of Tat (amino acids 1–72) are relatively conserved (Fig. 2 and ref. 4), no doubt because of restraints imposed by overlapping reading frames[33]. Antibodies to each of two separate immunogenic domains can inhibit cellular uptake, and the polyclonal response in a Tat-vaccinated subject should yield a variety of antibody binding patterns with a high probability of uptake-blocking antibodies to either or both of the immunodominant domains in the majority of extracellular Tat proteins.

Second, specific antibody interdiction of Tat in the extracellular fluid would inhibit the replication of all HIV-1 quasispecies indiscriminantly and should thus exert no pressure for the selection of variants producing particular Tat proteins. Therefore, selective development of quasispecies producing antibody unreactive to Tat proteins should not occur within a given individual.

Third, prevention of massive virus replication must minimize opportunities for the development of mutant viruses with variant structural proteins permissive for evasion of the earlier antiviral immune response. A further corollary of this analysis is that, to be successful, antibody interdiction of the predominant populations rather than all variants of Tat proteins should suffice to minimize the initial rapid burst of viral expansion.

Some practical considerations

(1) An HIV-1 Tat protein vaccine could potentially employ a variety of approaches to immunogen selection, and synthetic or recombinant Tat_{1-72} has proved immunogenic. In this instance, a vaccine selectively inducing antibodies rather than a cellular immune response would be desirable. The cysteine-rich domain is not immunogenic[27], but disulfide bridging within it is essential for transactivational activity. Any potential for uptake of the immunogen leading to unwanted cellular activation could be obviated by substituting other amino acids, such as serine, for selected cysteine residues during polypeptide or cDNA synthesis (Fig. 2).

(2) Anti-Tat antibodies would not be reactive with the HIV-1 surface proteins used to detect seroconversion after infection. Thus, subjects vaccinated with Tat would not be stigmatized with false-positive tests for HIV-1 infection, and it would remain possible to detect seroconversion if vaccinees did become infected with HIV-1.

(3) The sequence of HIV-2 Tat protein is distinct, and more closely related to SIV than to HIV-1. Nevertheless, the basic organization and function of HIV-2 Tat are similar to those of HIV-1. Thus, distinct immunogens would need to be incorporated into a vaccine to protect against HIV-2 in addition to HIV-1.

(4) Prophylactic immunization would clearly provide the optimal opportunity to block the initial burst of viral expansion and to prevent chronic viremia and progression to AIDS. Nevertheless, consideration should also be given to immunizing asymptomatic HIV-1-infected subjects with viremia, because extracellular Tat likely contributes to the persistent infection and immune abnormalities that are present at this stage of HIV-1 infection. Interdiction of extracellular Tat by antibodies following such immunization could well lead to reduction of viremia with more effective immune control, and result in delay or prevention of progression to AIDS.

(5) The efficacy of Tat vaccination in reducing *in vivo* viral replication and the development of disease is under preliminary evaluation in primates and will eventually need to be tested in humans. If successful, it is envisaged that such a Tat vaccine would be used alone, in a manner analogous to toxoid vaccination for tetanus and diphtheria.

Conclusions

A triad of features, exceptionally rapid high viremia, exceptionally high and rapid mutation of structural virion genes, and direct attack on the immune system, marks HIV-1 as unique among lethal human viruses. Hence there is a need for a novel strategy to combat this particular infection and to find a sufficiently early target of vulnerability other than the virion itself. From the present evidence, these terms of reference appear to be met by specific vaccination against the Tat transcriptional activator, a free protein product of the virus that passes from cell to cell and is responsible for the massive replication and output of virus. Without this protein, chronic infection, leading to immune failure and progression to AIDS, should be contained.

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