Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 4419–4420, May 1993
Medical Sciences

A proposal for a new approach to a preventive vaccine against human immunodeficiency virus type 1

(simpler retrovirus/more complex retrovirus/co-virus)

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Contributed by Howard M. Temin, February 22, 1993

ABSTRACT **Human immunodeficiency virus type 1 (HIV-1) is a more complex retrovirus, coding for several accessory proteins in addition to the structural proteins (Gag, Pol, and Env) that are found in all retroviruses. More complex retroviruses have not been isolated from birds, and simpler retroviruses have not been isolated from humans. However, the proviruses of many endogenous simpler retroviruses are present in the human genome. These observations suggest that humans can mount a successful protective response against simpler retroviruses, whereas birds cannot. Thus, humans might be able to mount a successful protective response to infection with a simpler HIV-1. As a model, a simpler bovine leukemia virus which is capable of replicating has been constructed; a simpler HIV-1 could be constructed in a similar fashion. I suggest that such a simpler HIV-1 would be a safe and effective vaccine against HIV-1.**

There is as yet no safe and effective vaccine against human immunodeficiency type 1 (HIV-1), the causative agent of AIDS (1–4). Probably the primary reason for this failure is that the nature of a protective immune response against HIV-1 is not known. Clearly, the usual immune response in persons after infection with HIV-1 is not sufficiently protective. In this article, I shall argue that evolution has provided a natural experiment that might direct us to a safe and effective vaccine against HIV-1.

HIV-1 is a lentiretrovirus, one of the more complex retroviruses (5); Hilleman (2) calls it an extraordinary virus. As a more complex retrovirus, HIV-1, like human T-cell leukemia virus type I (HTLV-I), bovine leukemia virus (BLV), and human spumaretrovirus (HSRV), differs from simpler retroviruses in having many genes in addition to *gag*, *pol*, and *env*, which are common to all retroviruses.

All retroviruses contain the *gag*, *pol*, and *env* genes and the cis-acting sequences acted on by the Gag and Pol proteins, as well as the sequences involved in the control of transcription, splicing, and polyadenylylation. In the case of simpler retroviruses, such as murine leukemia virus, the processes of viral transcription, splicing, and polyadenylylation are controlled by cellular proteins. The additional genes in more complex retroviruses code for proteins that act with cellular proteins to control transcription, splicing, and polyadenylylation and enable more complex retroviruses to have more-complex replication cycles (5, 6).

Simpler retroviruses were first found in chickens and have been much studied in chickens and mice. They are also found in vipers, fish, and cats, and there are even a few isolates from monkeys (7). Gibbon ape leukemia virus is a murine leukemia virus-related simpler retrovirus, and Mason–Pfizer monkey virus is a primate type D simpler retrovirus. Study of the human genome indicates that in the past many simpler retroviruses infected our ancestors, as shown by the relic proviruses in human DNA (7–16).

More complex retroviruses were first isolated in horses (equine infectious anemia virus). Since then they have been isolated from many other mammals, including at least five from humans (HIV-1, HIV-2, HTLV-I, HTLV-II, and HSRV). So far, more complex retroviruses have not been isolated from vertebrate families other than mammals, and simpler retroviruses have not been isolated from humans or ungulates. However, more complex retroviruses have been isolated from both humans and ungulates.

I propose that this phylogenetic distribution is not an artifact of virus isolation techniques, but that it reflects the ability of humans and ungulates to respond to infection by simpler retroviruses with a protective response, and the inability of birds and mammals other than humans and ungulates to respond to infection by simpler retroviruses with such a complete protective response.

Under this hypothesis, retroviruses infecting birds were under no selective pressure to evolve into more complex retroviruses, since the simpler retroviruses were already quite successful in infecting birds. On the other hand, in some mammals the immune system and perhaps other host characteristics were more successful in controlling simpler retroviruses, providing selective pressure for the evolution of more complex retroviruses. [Welsh *et al.* (17) describe such a mechanism in human serum for controlling simpler retroviruses.] The presence of genomes of simpler retroviruses in the human genome (endogenous viruses) indicates that, earlier in evolution, ancestors of present-day humans were successfully infected by such simpler retroviruses (7–16).

A conclusion from this line of reasoning is that present-day humans are able to mount a protective response against simpler retroviruses. Since HIV-1 is a more complex retrovirus, this conclusion does not yet help direct us toward methods for making a safe and effective HIV-1 vaccine. However, if we were able to construct a simpler retrovirus that expressed only the Gag, Pol, and Env proteins of HIV-1, this virus should induce in humans a protective response against itself, the simpler HIV-1. If such a protective response occurs against infection by a simpler HIV-1, I further propose that this protective response would protect against wild-type HIV-1 infection, and thus that the simpler HIV-1 would form an effective vaccine against HIV-1, either as a live "attenuated" virus or as an inactivated virus.

Such a simpler HIV-1 is fundamentally different from vector- or DNA-expressed HIV-1 structural proteins, which cannot replicate in the vaccine recipient (18–21). The simpler HIV-1 would replicate in the vaccine recipient, and it would continue to replicate until a complete protective response was stimulated in the recipient. Furthermore, since the

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Abbreviations: BLV, bovine leukemia virus; HIV, human immunodeficiency virus; HSRV, human spumaretrovirus; HTLV, human T-cell leukemia virus; LTR, long terminal repeat; SNV, spleen necrosis virus.

simpler HIV-1 replicates as a retrovirus using the HIV-1 reverse transcriptase, any vaccine preparation of the simpler HIV-1 would be a swarm containing different nucleotide sequences, not a monosequence as occurs from a cloned DNA preparation. In addition, the simpler HIV-1 would vary as it replicates in the vaccine recipient and would induce a polytypic response, not the monotypic response of the vector- or DNA-expressed proteins.

The simpler HIV-1 would not be HIV-1; it would not have the additional HIV-1 genes that make HIV-1 a more complex retrovirus. Thus, the simpler HIV-1 should not cause AIDS (22). Furthermore, the simpler HIV-1 would replicate less well than HIV-1, further reducing its possible pathogenicity (23). [The simpler HIV-1 would also differ fundamentally from partially deleted HIV-1 (4), since there would be many fewer HIV-1 sequences in the simpler HIV-1.] However, it is possible that such a simpler HIV-1 used as a live virus vaccine might result in some low incidence of leukemia as a result of insertional activation of protooncogenes (24). Such leukemogenesis would be rare, if it occurred at all (25), and any leukemias induced by the simpler HIV-1 would appear only after a long latent period. Also, the possibility of leukemia induction by a simpler retrovirus can be drastically reduced by making the promoter/enhancer sequences of the simpler HIV-1 inefficient.

A simpler BLV with spleen necrosis virus (SNV) long terminal repeat (LTR) sequences has been constructed and shown to replicate (K. Boris-Lawrie and H.M.T., unpublished work). As a further safety feature, the BLV *gag–pol* genes and the BLV *env* gene were expressed from different simpler BLV constructs—that is, as co-viruses—and the co-viruses were shown to replicate as a chimeric BLV/SNV virus. Expression from the BLV/SNV LTR allows functional *env* expression in the absence of other BLV proteins.

A simpler HIV-1 could be constructed in a similar fashion by taking the HIV-1 *gag*, *pol*, and *env* genes and the cis-acting sequences acted on by the Gag and Pol proteins of HIV-1 (*att*, *pbs*, *E*, *ppt*) and placing them in the partially deleted LTRs of a simpler retrovirus. In particular, as for BLV, this means substituting simpler retrovirus LTR sequences for all of the HIV-1 LTR sequences except for the internal *att* sequences. [Such a substitution would also delete the HIV transactivation response (TAR) sequence.]

A simpler HIV-1 construct with only HIV-1 *gag*, *pol*, and *env* genes might not replicate. Rev protein may be necessary for *env* expression unless its cis-acting sequences are mutated (26). Assembly of Gag, Pol, and Env proteins into virions appears to proceed in the absence of accessory proteins (18–22). If not, Vif or other accessory proteins may also need to be present. They could be added back into the constructs.

The utility of such constructs could first be validated in simpler HIV-1/chimpanzee and simian immunodeficiency virus/macaque model systems (see refs. 4 and 27). If there is concern about insertional activation increasing the possibility of leukemia, a killed vaccine made from the simpler HIV-1 could first be tested. However, if it appeared that only a live virus vaccine would be adequate to prevent HIV-1 infection and insertional activation by the simpler HIV-1 appeared to be a problem, the simpler HIV-1 could be further crippled by mutating promoter and enhancer sequences or adding a gene that can be selected against (a suicide gene) expressed from a picornaviral internal ribosome entry site (28–30).

The seriousness of the AIDS pandemic and the so-far low effectiveness of other immunodeficiency virus vaccines, except possibly *nef*$^-$ simian immunodeficiency virus (27), makes other approaches like this one worthy of consideration.

I thank D. Bolognesi, K. Boris-Lawrie, D. Burns, G. Pulsinelli, and B. Sugden for comments. The research in my laboratory is supported by Public Health Service Grants CA22443 and CA07175 from the National Cancer Institute. I am an American Cancer Society research professor.

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