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Letter to the Editor

The Need for a Search for a Proximal Pathogenic Principle of Human AIDS

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I would like to comment on the discussions in the scientific community concerning the etiology of AIDS[1] and AIDS-related complex. My comments relate to numerous articles, editorials, news, views, comments, and letters concerning the nature of the agent that is responsible for the causation of AIDS. Some correspondents (*e.g.*, Ref. 6) have presented treatises which, although highly controversial, are not entirely without merit, because they have called attention to a series of outstanding problems in AIDS research. In this letter I will attempt to show that the apparent "inconsistencies" concerning the role of HIV in the causation of human AIDS may be resolved and that there is an urgent need to identify the proximal pathogenic principle(s) of human AIDS.

The link between HIV infection and AIDS is clearly strong and is based on the close correlation between the finding of antibodies to HIV in >90% of AIDS patients but not in 99.5% of healthy individuals (1–5). This close association between HIV and AIDS establishes a credible correlation but not a direct causal relationship, as has been pointed out (6, 7). A series of enigmas (6, 7) in the proposed viral etiology and in the natural history of AIDS should not be shrugged off or disregarded. Paramount among these are: (*a*) the long latency of the disease following virus infection; (*b*) the relatively low penetrance of the disease in cohorts of documented infected individuals; (*c*) the low levels of virus infection (1 in 10^4 or 10^5 lymphocytes) and the lack of viremia or circulating virus particles; (*d*) the progression to disease in individuals who possess neutralizing titers of antiviral antibodies (albeit low neutralizing titers); and (*e*) the lack of satisfaction of Koch's postulates in this apparent viral disease. These and other related questions must be answered if we are not to find in the future that we have spent major efforts studying and combatting this serious disease in the wrong directions.

Genetic Variation among HIV Isolates

Cheng-Mayer *et al.* (8), Hahn *et al.* (9), and Fisher *et al.* (10) have recently shown that there is considerable variability among HIV isolates derived over time from the same infected individuals or from the same individual at any one time. This variability among HIV prototypes resides mostly in the gene encoding the envelope glycoprotein. In two studies (10, 11) as many as 17 distinct but related prototypes of HIV were isolated serially from the same individual. Distinct prototypes were shown to have very different pathogenic (*in vitro*) characteristics (8) and/or intrinsic *in vitro* proliferation capacity (10). Different HIV prototypes possessed characteristic proliferation profiles on various T-cell lines, on activated T-cells, and on macrophages. It is not known which of these HIV prototypes constitute essential, proximal immunosuppressive principles or proximal tumorigenic principles, if any, although Cheng-Mayer *et al.* (8) have shown a correlation between *in vitro* cytopathogenicity of some isolates and virulence in the hosts.

Origin of Heterogeneity among HIV Isolates

The origin of the heterogeneity among field isolates of HIV is a matter of contention (see Ref. 12 for a review).

One hypothetical explanation for this heterogeneity is the possibility that most HIV infections occur with a mixture of related, preexisting prototypes, some of which will predominate early following infection, while the titer of other virus variants may increase later in the infected host. The shift in predominance of HIV prototypes may be influenced by the availability in the infected individual of susceptible host cells, or by the specific immune status of the host, and would present itself in virus isolation experiments as apparent mutations or recombinations of the virus.

Another explanation for the heterogeneity of field isolates of HIV may be found in the possible recombination among proviral sequences, as mentioned by Levy in his recent review (13).

Alternatively the observed heterogeneity among HIV isolates may reflect inherent mutations arising as the result of errors of virus replication [see, *e.g.*, review by Coffin (12)]. One difficulty with this hypothesis is that replication of HIV has not been shown to lead to any considerable degree of sequence heterogeneity. In fact, in spite of numerous suggestions (12) that retrovirus mutants may arise as the result of errors in replication, there appears to be no evidence for the induction of genetic changes of HIV during infection *in vitro* (11, 14).

Evidence for the genetic stability of *in vivo*-passaged retroviruses is also available from work in murine systems. Pathogenic murine leukemia viruses have been shown to be genetically highly stable through numerous *in vivo* and *in vitro* passages that have included uncounted cycles of infection (presumed to be the process during which most of the genetic errors occur). One example in question is the Friend murine leukemia virus, which presumably was separated from the Rauscher murine leukemia virus strain some 25 years ago, during which time both virus stocks have undergone countless *in vivo* and/or *in vitro* passages. In spite of this, the Friend and Rauscher prototype viruses were found to be highly homologous in the envelope gene at the nucleic acid level (15). A similar lack of mutations was documented by Bestwick *et al.* (16) for the replication-defective pathogenic Rauscher and Friend spleen focus-forming virus components of these virus complexes. At least 25 years and countless passages separate these defective spleen focus-forming viruses as well (17). It thus appears reasonable to summarize that error-prone replication is probably not the mechanism by which HIV variants occur.

Replication-competent HIV Subtypes (HXB2, LAV-MAL, ARV2) as "Helper Viruses"

Not all members of the heterogeneous group of HIV prototypes that are found in infectious hosts are, by necessity, pathogenic. The natural history of AIDS suggests to us that a

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[1] The abbreviations used are: AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus; MCF, mink cell focus-inducing; p30, M_r 30,000 protein; gp70, M_r 70,000 glycoprotein.

minority, possibly even a very limited number of HIV subtypes, would be capable of inducing one or another pathogenic response (see the example of murine "AIDS" below). Different pathogenic responses may be mediated by distinct HIV subtypes, as suggested by the heterogeneous responses of individuals infected with HIV. The most prevalent prototypes of HIV (*e.g.*, strains HXB2, LAV-MAL, or ARV2) may well have the properties of helper viruses, which, although necessary in the infectious process and apparently essential in the induction of pathogenesis, would not be sufficient by themselves to induce pathogenesis, because they may lack inherent pathogenic functions of their own.

Source of Pathogenic HIV Subtypes

Assuming that the prevalent, high-titer strains of HIV are indeed helper-type retroviruses that are unable to induce pathogenesis directly, of their own, what would be the origin of the pathogenic principle(s)? [We would assume that these would be defective viruses or at best poorly replicative strains in analogy with the murine and feline models (see *e.g.*, Refs. 18 to 20).] Two sources for the origin of the pathogenic principles come to mind. (*a*) The pathogens may be preexisting and present in the infecting virus pool at the time of initial infection. They would be pseudotypes possessing the coat of the helper virus but might otherwise be genetically quite different, specifically in the gene encoding the envelope glycoprotein (11). These preexisting pathogenic principles may be present at various titers and may even be absent from some infectious "field" virus pools. This situation would bring about the infection of some hosts without concomitant pathogenesis (antibodies to HIV would be induced in the majority of hosts infected with this HIV "helper" virus strain) and appears to be compatible with the epidemiology of AIDS; about one-half of HIV-infected individuals appear not to develop any pathology. While we do not propose to preclude host factors in the development of the pathology of AIDS, from a virologist's point of view, invoking the helper/pseudotype model is sufficient to account for the lack of disease in some of the individuals infected with HIV. (*b*) The proposed pathogenic HIV prototypes need not preexist as discussed in *a*. The pathogenic principle(s) in HIV-infected hosts with symptoms may derive from virus-like cellular sequences with which the helper virus recombines. In rodents this is one of the ways by which pathogenic retroviruses originate [mice harbor more than 50 partial proviral gene sequences which are targets for recombination with infecting retroviruses (21–23)], and there appears to be little reason why a similar mechanism would not be operative in humans. (This mechanism is not mutually exclusive with *a*.) Recombination of retroviruses with envelope gene-like sequences in the host genome is of course one of the hallmarks of retrovirology (24), and the hypothetical need for recombination of HIV with endogenous retroviral *env*-like gene sequences in the generation of pathogenic virus would explain the variable latency between infection with HIV and the development of pathogenesis. The human genome appears to harbor many sequences that hybridize with low stringency with specific polymerase chain reaction-amplified regions of HIV, although this subject has not been exhaustively investigated thus far.

The Proximal Pathogenic HIV May Not Yet Have Been Isolated

The natural history of HIV-induced AIDS and AIDS-related complex and the virology of HIV do not exclude the possibility that the proximal pathogenic principle(s) that is responsible for the causation of these diseases with all their varied pathogenic symptoms has not yet been isolated and/or described. This hypothesis is compatible with the epidemiological data strongly linking HIV with AIDS and answers a host of difficult, open questions about the disease and its characteristics (Ref. 6, and see 5 points above). This hypothesis is also strongly supported by a murine AIDS-like model system.

A Retroviral Murine AIDS Model

In the late 1970s we studied the immunopathology of a disease in mice that was induced by a culture-grown murine retrovirus complex, designated "BM5 virus" (25–29). We grew this virus complex in culture, starting with the "Duplan" strain of the radiation leukemia virus (30). The virus proliferated in culture in the macrophage/fibroblastoid moiety of the hematopoietic organs of infected mice and induced in C57BL/6 mice a polyclonal lymphoproliferative disease that is noninvasive and is associated with severe "wasting" disease (a lethal, progressive immunosuppressive syndrome). The BM5 virus-induced syndrome is both rapid and surprisingly potent in C57BL/6 mice (25, 29). Others have since shown that the disease that is induced by the BM5 virus complex closely resembles human AIDS in many although not all of its characteristics (31–33).

The Proximal Pathogenic Murine AIDS Virus May Not Yet Have Been Isolated

While working at the Salk Institute between 1980 and 1983, we made a serious effort to isolate the proximal pathogenic/immunosuppressive virus from the BM5 virus complex. Using biological cloning we repeatedly isolated from the BM5 complex both ecotropic (mouse-tropic; B-type) and MCF (B-type) virus clones. Multiple isolations done in mouse, mink, human, and dog cells gave rise to these two classes of virus isolates. The viruses also were molecularly cloned and restriction maps were derived. We were quite surprised that among multiple BM5-derived virus isolates neither the ecotropic nor the MCF virus isolates were pathogenic in mice in extensive, carefully conceived experiments.

We then surmised that the murine AIDS-like disease that is induced rapidly by the BM5 virus complex might be harbored by an ecotropic pseudotype of MCF virus (34). We thus constructed virus pseudotypes using methodologies that ensured the production of both virus types from the same cell (repeated cloning of infected cells and assay by immunoprecipitation ascertained that indeed virus pseudotypes were being produced[2]). Pseudotype virus progeny of 8 independent experiments were tested *in vivo* for the induction of BM5-like pathology. None was found, although ample virus proliferation *in vivo* could be shown. These experiments suggested that the proximal pathogenic principle in the BM5 virus complex does not reside in the predominant MCF prototype virus or in the

[2] The following very extensive strategy was used to ascertain the production by the same cell of both the ecotropic and the MCF isolates. Mink and dog cell lines were infected with cloned MCF virus and single-cell clones were grown out 16 h postinfection. The infected status of these clones was assayed for reverse transcriptase and by immunoprecipitation with anti-p30 and anti-gp70 sera. MCF-infected cell clones were then superinfected with several ecotropic virus isolates and recloned, and clones were assayed for double infection by immunoprecipitation of metabolically labeled cells (the $Pr^{75^{env}}$ and $Pr^{80^{env}}$ precursor glycoproteins to viral gp70 of ecotropic and MCF virus, respectively, can be easily distinguished). The progeny virus from pseudotypes thus constructed were used in *in vivo* infection experiments.

ecotropic isolate of the BM5 complex or its pseudotype.

Similar experiments have since been done on the BM5 model by others with identical results. Therefore, the following assumption can be made.

The Mouse AIDS-like Proximal Pathogen Probably Is a Defective Virus

Molecular cloning experiments of BM5 proviral sequences[3] gave rise to MCF viral genomes that were defective for replication by virtue of one or another deletion that was evident from the detailed restriction maps of the isolates. The defective BM5-derived virus isolates were variants of the associated MCF isolate, inasmuch as they contained MCF-specific envelope sequences. These defective genomes might be the proximal pathogenic principles of BM5 in analogy with the replication-defective variants of feline leukemia virus as described by Overbaugh *et al.* (20) and the Friend spleen focus-forming virus genome studied by Wolff and Ruscetti (19). The properties of the replication-defective BM5 variants have not as yet been further studied.

Lessons to Be Learned from the Murine AIDS Model

In the murine BM5 AIDS model we can study a rapidly pathogenic immunodeficiency syndrome. All (100%) C57BL/6 mice respond to the virus. The viruses can be grown and manipulated with ease in the laboratory, since assay systems are routine in the murine leukemia virus field. Thus the isolation of the proximal pathogenic/immunosuppressive virus from the BM5 complex should be possible. Two groups have tried and have missed the pathogenic virus in their first, serious trials, possibly because the assumption was made that the MCF virus or its pseudotype(s) would be the coveted pathogenic principle. In analogy with the murine BM5 model we may have missed the proximal pathogenic/immunosuppressive component(s) of the HIV system.

Both the feline and the murine AIDS models mentioned above involve non-lentiretroviruses. There have been reports of feline AIDS that is induced by a lentiretrovirus isolate (35). HIV too belongs to the *Lentivirinae* subfamily of the retroviruses. Lentiviruses [*e.g.*, visna virus (36, 37)] have been reported to undergo rapid antigenic drift through mutation in the envelope gene, which is a mechanism through which these viruses escape the host's immunological control (38, 39). Whether lentiviruses undergo mutations/recombinations that result in the generation of pathogenic defective subtypes as in the murine and feline systems has not been reported to date.

Summary

Some of the unexplained aspects of HIV/AIDS epidemiology, virology, and pathology (6, 13) may thus be due to a lack of knowledge of the basic virology of the virus complex and its pathological interaction with cells of the host. There may be no need to call upon anomalous immune responses, vicious transactivating activities, or new virological or biological principles if we can first do the basic virology of the disease-inducing viruses. While we do not want to propose that working out the virology will by necessity be easy on all counts, it may lead us all to more equitable immunological, pathological, and cell biological approaches to this formidable problem. A fine beginning has been made by Fisher *et al.* (10), Saag *et al.* (11), and others. To retrovirologists the problems appear not to be insurmountable.

[3] M. Haas and M. Vogt, unpublished observations.

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