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Improbability of effective vaccination against human immunodeficiency virus because of its intracellular transmission and rectal portal of entry

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ABSTRACT **The worldwide effort to produce a vaccine against AIDS continues to disregard the fact that even human immunodeficiency virus (HIV)-specific neutralizing antibodies and cell-mediated immunity are ineffective against virus within cells without viral antigens on the cell membrane—and that much of HIV infection is transmitted in this manner. According to a recent report, a simian immunodeficiency virus vaccine that protected monkeys against an intravenous challenge with *cell-free* virus was, as predicted, ineffective against an intravenous challenge with the same amount of virus in infected cells. Moreover, antibody and HIV have been found to coexist in cell-free plasma from asymptomatic and symptomatic patients. Excluding direct introduction of HIV into the bloodstream, the most common and efficient form of transmission of HIV infection is by receptive anal intercourse, and semen contains large numbers of infected cells per milliliter. Recent reports showing that colorectal cells can be persistently infected by HIV and that HIV RNA and cDNA are present in the cells of the colon of dead AIDS patients indicate that either cell-free or intracellular HIV has the capacity to multiply at the portal of entry in the colorectal area without interference from neutralizing antibodies. The available data provide no basis for testing any HIV vaccine in human beings either before or after infection. The main challenge is to find a way to kill cells with chromosomally integrated HIV cDNA without harming normal cells, perhaps by identifying repressor proteins that might be produced by the cells with integrated HIV cDNA and thus could become specific targets for cell-killing drugs.**

The purpose of this communication is to indicate why the worldwide search for a vaccine against AIDS has been and continues to be based on assumptions that fail to take into consideration the most important facts that distinguish this disease caused by the human immunodeficiency virus (HIV) from viral diseases transmitted by cell-free viruses (e.g., polio and measles) for which effective vaccines have been used for decades. Thus, Hamburg and Fauci (1) said that in searching for an anti-HIV vaccine "the aim is to induce neutralizing antibodies as well as cell-mediated responses against HIV and thereby to protect uninfected individuals against subsequent infection if they are exposed to the virus." Almost all published reports on experimental HIV-1 vaccines in chimpanzees (2, 3) or simian immunodeficiency virus (SIV) vaccines in monkeys (4–6) up to the end of 1991 have based their claims of effectiveness on the production of neutralizing antibodies, with or without cell-mediated immune responses, and on the demonstration of a protective effect against intravenous challenge, usually with a very small dose of cell-free virus.

Recently, it was pointed out that challenge with *cell-free* virus was an inadequate test for effectiveness of an HIV-1 vaccine against AIDS, because the two most important vehicles of infection in human beings—semen and blood—contain large numbers of *virus-infected* cells in addition to smaller amounts of cell-free virus (7). Subsequently, reported experiments by Johnson *et al.* (8) showed that an inactivated, whole SIV vaccine that protected pig-tailed macaques against an intravenous challenge with 50 ID_{50} of *cell-free* SIV did *not* protect against *50 ID_{50}* of *SIV-infected* peripheral blood mononuclear cells (PBMC) from rhesus macaques moribund with AIDS.

The recent report by Stott (9) that protective SIV vaccines, prepared from virus grown in human cell lines, produce antibodies against uninfected human cells and that vaccination of monkeys with uninfected humans cells can also protect against challenge with SIV derived from human cells has now been amply confirmed (10–13). Cranage *et al.* (13) reported that 10 monkeys that were vaccinated with human cell-derived SIV were protected from a challenge with 10 monkey 50% infectious doses (MID_{50}) of cell-free SIV_{MAC251} derived from human cells, but not when challenged with 10 MID_{50} of cell-free SIV_{MAC251} grown in rhesus monkey PBMC despite the fact that all had specific SIV antibodies.

In their tests on 7 monkeys that received a vaccine of whole inactivated SIV derived from human cells, Osterhaus *et al.* (12) obtained the same protection with a challenge of 10 MID_{50} of SIV derived from human cells as Cranage *et al.* (13). However, in 8 monkeys that received a vaccine containing both the Gag and Env proteins of SIV grown on human cells and challenged with 10 MID_{50} of rhesus PBMC, 4 were infected and 4 were not—all 4 control monkeys that received inactivated measles vaccine and were challenged with the same dose of rhesus PBMC were infected. Osterhaus *et al.* (12) concluded that "This is the first demonstration in the SIV macaque model that vaccination can protect against challenge with *cell-associated* [my emphasis] SIV." The tabulated data in this report show that all 21 monkeys that received either measles vaccine or SIV vaccine showed similar ELISA antibody titers against rhesus PBMC (log $10^{1.9}$ − log $10^{2.7}$) on the day of challenge. The difference between SIV infection in 4 of 8 vaccinated and 4 of 4 in measles vaccine controls may or may not be significant, but in my judgment does not justify the conclusion of these authors, especially in view of the fact that Cranage *et al.* (13) found no protection against 10 MID_{50} of cell-free virus derived from rhesus PBMC and the contrary results of Johnson *et al.* (8) when 50 MID_{50} SIV-infected rhesus PBMC were used for challenge. Moreover, one must distinguish between "*cell-associated*" SIV or HIV, which can include PBMC with

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Abbreviations: HIV, human immunodeficiency virus; SIV, simian immunodeficiency virus; PBMC, peripheral blood mononuclear cells; MID_{50}, monkey 50% infectious doses; CID_{50}, chimpanzee 50% infectious doses; $TCID_{50}$, tissue culture 50% infectious doses.

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budding virus on the cell membrane, from "*intracellular*" SIV or HIV in which there is no expression of antigens on the cell membrane of infected cells that can have either totally unexpressed chromosomally integrated cDNA or unintegrated DNA or RNA genome.

A similar problem is posed in the recent report by Fultz *et al.* (14), which concludes that the "results show that it is possible to elicit long-lasting immunity against *cell-associated* [my emphasis] HIV-1." The three chimpanzees used in this study had been vaccinated and challenged over a period of 2 years as previously reported (3) and were now challenged intravenously with an estimated 9–22 HIV-infected chimpanzee PBMC. The inoculum was not titrated in chimpanzees because of their high cost and limited availability, and although the one control chimpanzee that received this inoculum was infected, one cannot be certain of the regularity of infection with so few cells and of the significance of the fact that the three vaccinated chimpanzees were not infected. However, as was just pointed out above, cell-associated virus is not the same as intracellular virus or genome that is not expressed on the cell membrane. It is of interest in this respect to note the following statement in this report (14): "PBMC obtained from all three chimpanzees on the day of cell-associated virus challenge were tested *in vitro* for the ability to prevent cell to cell transmission. Although some inhibitory activity was detected, results were inconstant (data not shown), and this assay did not correlate with protection *in vivo*." However, one may interpret the results of the challenge of these chimpanzees with a *small number* of cell-associated HIV from chimpanzee PBMC, they provide no evidence of protection against intracellular virus or genome, such as might be found in a larger inoculum as was used by Johnson *et al.* (8). In regard to the statement in the abstract of the report by Fultz *et al.* (14) that the results provide evidence for "long lasting protective immunity," it is noteworthy that when one of the protected chimpanzees was tested 1 year after the cell-associated virus challenge (without further boosting) with a challenge of *cell-free* HIV, there was no protection. The authors state that "this chimpanzee became infected despite the presence of an apparently stable humoral response." In this connection, the earlier report of these investigators (3) noted that although all three vaccinated chimpanzees, after challenge with *cell-free* virus, showed no evidence of infection for 6 months, one chimpanzee with a prechallenge neutralizing titer of >512 began to yield positive HIV blood cultures at 32 weeks and thereafter and from bone marrow at 37 weeks after challenge. They (3) also noted that the three vaccinated chimpanzees that showed high neutralizing antibody titers against the homologous HIV-1 BRU strain neutralized "*only marginally*" other HIV-1 isolates (RF, SF-2, and MN).

Special Factors in Natural Transmission of HIV

The experimental vaccine studies have overlooked the fact that much or most of natural infection with HIV is transmitted *not* by cell-free virus *but rather by cells* carrying incompletely expressed virus, RNA genome, or unintegrated or integrated DNA provirus (cDNA) (15, 16). Neutralizing antibodies are ineffective against such intracellular virus or genome, and cell-mediated immunity is ineffective against cells in which virus-specific antigens are not expressed on the cell membrane. Moreover, such cells can transmit viral genome by cell-to-cell contact without the intervention of specific HIV receptors. The extensive studies on candidate HIV vaccines or on prototype SIV vaccines have also overlooked the fact that, in HIV-infected persons, semen and blood contain large numbers of infected lymphocytes in which the virus is not expressed on the cell membrane. It has been reported (17) that seminal fluid can have 10^8 infectious doses of HIV-1 per ml, a much higher concentration than is found in the blood (18).

Except for transmission by infected blood as in blood transfusion or by careless intravenous drug addicts, the most effective mode of transmission of HIV is by the introduction of semen into the colorectal area of men and women during receptive anal intercourse (19). It has already been amply demonstrated that naturally occurring or oral vaccine polioviruses multiply extensively in the intestinal tract of human beings who have acquired their antibody either transplacentally or as a result of vaccination with killed poliovirus vaccine (20).

Effect of Antibodies Against HIV in Infected Persons

In 1989, Nathanson and Gonzalez-Scarano (21) called attention to the fact that "Persons who have acquired infection as a result of blood transfusion have received considerable amount of plasma containing anti-HIV antibodies; yet they become infected . . . . Likewise children infected *in utero* usually are infected during the third trimester in spite of the benefit of prior acquisition of passive antibody during the first two trimesters. At the very least, these observations document the coexistence of antibody and infectious cells. They further suggest that the presence of antibody prior to, or from the time of, infection fails to provide passive protection." In 1992, Krasinski *et al.* (22) reported that they found no correlation between the concentration of HIV-1 neutralizing antibodies among 28 HIV-1-infected mothers at delivery and the six HIV-1-infected infants to whom they gave birth. In 1989, Ho *et al.* (18) reported the isolation of HIV-1 from *cell-free plasma* (often in high titer) and from PBMC from *each* of 54 *seropositive* persons (16 asymptomatic, 18 AIDS-related complex, and 20 AIDS), indicating that not only the intracellular HIV in the PBMC but also *cell-free* HIV regularly coexisted with antibody. Ho *et al.* (18) concluded that "The finding of high titers of infectious virus in plasma clearly shows that circulating antibodies are insufficient to *neutralize* [my emphasis] HIV-1 *in vivo*." Although Ho *et al.* (18) did not test their blood specimens from the *seropositive* persons for *neutralizing* antibodies, Prince *et al.* (23) earlier reported that tests on seropositive male homosexuals showed that 92.7% had *neutralizing* antibodies with a median titer of about 1:50.

The limited effect of neutralizing antibodies against HIV-1, even when the same strain of cell-free virus is used for the *in vitro* neutralization and the intravenous challenge in chimpanzees, is evident in the remarkable experiments reported by Prince *et al.* (23). In 1988, they (23) perfused two chimpanzees (*Pan troglodytes*) with gamma globulin (IgG) prepared specially for intravenous injection from the plasma of many healthy HIV-1-infected persons. The neutralizing antibody titer of this IgG preparation for the HTLV-IIIB strain varied between 500 and 3200. The first intravenous dose was 10 ml/kg, the equivalent of 700 ml of the anti-HIV-1 IgG for a 70 kg person, administered 1 day before the intravenous challenge with 100 chimpanzee 50% infectious doses (CID_{50}). Just before this challenge, the plasma of these chimpanzees had HIV-1 neutralizing antibody titers of 178 and 354, respectively, and HIV-1 antibody-dependent cell-mediated cytotoxicity titers of >1:20,000. At 5 and 9 weeks after the first dose of HIV-1 IgG, these chimpanzees received additional infusions of 1 ml/kg of the same IgG preparation. These chimpanzees were not protected against infection. Subsequently, Prince *et al.* (24) showed that when a 10 times smaller dose of the stock of the HTLV-IIIB strain of cell-free HIV-1 (i.e., 10 CID_{50}) was used for intravenous challenge the same IgG preparation at *1 ml/kg*, which yielded a prechallenge neutralizing antibody titer of 40 in the chimpanzee's circulating blood, *failed to prevent infection*, whereas *10*

ml/kg, which yielded a titer of 640 in the chimpanzee circulating blood, prevented infection.

It is difficult to understand why neutralizing antibody at a titer of 1:40 against 100 tissue culture 50% infectious doses ($TCID_{50}$) of the virus *in vitro* should fail to prevent infection with only 10 CID_{50} (about 34 $TCID_{50}$) of the same virus *in vivo*, unless the cell-free virus combines much more rapidly with the HIV-1 cell receptors *in vivo* than with the neutralizing antibody.

In regard to the predicted (7) ineffectiveness of neutralizing antibodies against cell-free HIV-1 or SIV during infection at certain mucosal surfaces (e.g., the intestinal tract), it is noteworthy that Gardner and coworkers reported at a recent meeting† that "The same inactivated whole virus and modified live virus (SIV) vaccines that protected [rhesus monkeys] against iv [intravenous] infection apparently did not protect against a few animal infectious doses of *cell-free* virus given by the intact genital mucosa." In this case, the absence of antibodies at the mucosal surface may have permitted primary multiplication in the cells of the vaginal or endocervical epithelium (25) with subsequent entry into the regional lymph nodes and then into the general circulation as *intracellular virus* that would not be accessible to neutralization.

A recently published study (26) on the postmortem distribution of viral genome and infectious virus in four monkeys that died at different times after SIV infection showed "the consistent high viral burden throughout the entire gastrointestinal tract [in the lamina propria and gut-associated lymphoid tissue]." It is noteworthy that in 1989 Fox *et al.* (27) reported the detection of HIV-1 RNA in the intestinal lamina propria of patients with AIDS and gastrointestinal disease. I have as yet seen no reports of studies on macacus monkeys infected with either cell-free or intracellular SIV by the rectal route.‡ Such experiments in monkeys together with serial studies on the location of viral genome and virus at different times after infection could shed much light on the rectal route of infection with HIV in human beings.

In healthy men, semen contains about 3 million lymphocytes per ml and in infected persons it contains many more. According to Jay Levy (15, 16), the lymphocytes from HIV-infected men are the major source of infection, and Borzy *et al.* (17) reported that seminal fluid may contain 10^8 infectious doses per ml. Moreover, according to Olsen and Shields (28), lymphocytes in human semen can easily pass through the thin, single layer of cells in the rectal mucosa, but not through the multilayered vaginal mucosa, although in the absence of a cervical plug, which occurs on certain occasions, infected cells may also traverse the single-layered mucosa of the cervical canal and uterus.

Of great significance in this connection are the following: (*i*) Cultured colorectal cells can be persistently infected by HIV (29). (*ii*) HIV has been demonstrated in the bowel epithelium of patients with gastrointestinal symptoms, and biopsy specimens from the duodenum and rectum have shown the presence of HIV in epithelial cells of the bowel (30). (*iii*) A recent report by Fantini *et al.* (31) on infection of human colonic epithelial cells with HIV concluded that "epithelial intestinal cells may represent a major site of entry of HIV during receptive anal intercourse." (*iv*) Aoki-Sei and associates from the U.S. National Cancer Institute at a recent meeting§ reported finding HIV RNA and cDNA in the colon of patients who died of AIDS. Thus far, I have seen no reports of tests for cell-free or intracellular HIV in the stools or bowel washings of symptomatic or asymptomatic HIV seropositive persons.

It would be of interest to carry out such tests on macacus monkeys experimentally infected with SIV by the rectal route. Moreover, since extensive primary multiplication of either naturally occurring polioviruses or oral polio vaccine strains in the human intestinal tract gives rise to a local resistance to reinfection without reference to persisting neutralizing antibodies in the blood (20), it would be of special interest if such local resistance to reinfection could be produced in monkeys or chimpanzees rectally infected with *large* doses of live cell-free or intracellular SIV or HIV. Since prolonged *systemic infection* with either HIV or SIV produces no such systemic "intracellular immunity," it also may not occur in the intestinal tract, but it is worth a try in monkeys with SIV and maybe also in chimpanzees with HIV. A live genetically engineered safe deletion mutant of HIV or SIV cannot be expected to do more than the unmodified virus.

According to a recent report by Lifson *et al.* (32), there was no significant difference between HIV-seropositive patients who have remained asymptomatic for many years with normal $CD4^+$ cell counts and patients with symptomatic AIDS as regards either the level of neutralizing antibodies for a standard strain of HIV (mean titer of 1:990 for nonprogressors vs. 1:618 for patients with AIDS) or antibody-dependent cytotoxicity. On the basis of existing information about the natural history of HIV infection, there is no more reason to expect a beneficial effect from HIV vaccines in persons who are already infected than in those who have not yet been infected.

In my judgment, the available data provide no basis for testing any experimental vaccine in human beings or for expecting that any HIV vaccine could be effective in human beings. In view of the fact that the main problem in the natural history of infection with HIV is the cells with chromosomally integrated HIV cDNA, against which presently available drugs have no effect (18), it seems to me that the most important challenge is to find a drug or other procedures that can specifically detect and kill such cells without harming normal cells. In his recently published book *Pietà*, George Klein (33) suggested that chromosomally integrated HIV cDNA probably produces a "repressor" protein, which could serve as a specific point of attack for cell-killing drugs. It seems to me that this hypothesis of George Klein deserves a very high priority for research on a chemotherapeutic agent against AIDS or on a new immunologic approach.

†Gardner, M., Carlson, J., Jennings, M., Luciw, P., Yilma, T., Planelles, V., Giovedoni, L., Marthas, M., Sutjipto, S., Miller, C., Yamamoto, J., Pedersen, N., Steimer, K. & Haigwood, N., Annual Meeting of the Laboratory of Tumor Cell Biology, National Cancer Institute, Sept. 4, 1991, Bethesda, MD.

‡Infection of rhesus macaques by a cell-free SIV by the rectal route was recently reported to require 1500 times more virus than by the intravenous route (34).

§Aoki-Sei, S., Kleiner, D. E., Chandra, R., Yarchoan, R., Husson, R., Pizzo, P. A., Broder, S. & Mitsuya, H., Annual Meeting of the Laboratory of Tumor Cell Biology, National Cancer Institute, Sept. 6, 1991, Bethesda, MD.

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