1: O'Brien DR. Eliminating human immunodeficiency virus (HIV) from infected individuals and cells: is it possible? Med Hypotheses. 1992 May;38(1):20-4. PubMed PMID: 1614355.

ELIMINATING HUMAN IMMUNODEFICIENCY VIRUS (HIV) FROM INFECTED INDIVIDUALS AND CELLS: IS IT POSSIBLE?

Denis R. O'Brien. The Central Institute of Technology, School of Pharmacy, Trentham, New Zealand

ABSTRACT

The literature from 1984 to 1991 has been searched for reports of patients who have eliminated human immunodeficiency virus (HIV) from their system. While such reports are scarce, it appears that a small number of HIV-positive patients have reverted to a negative state either spontaneously or following radical immunosuppressive regimens for neoplastic disease. Although no carefully planned animal experiments or clinical trials have been reported, it would appear that bone marrow ablation and replacement may eliminate HIV from healthy, asymptomatic HIV-positive individuals. Although much of the clinical experience to date suggests that radical immunosuppression is not indicated in advanced AIDS patients in whom the virus has likely spread beyond the immune system, such cases do not represent evidence that immunosuppression in not indicated in healthy, HIV-positive individuals.

INTRODUCTION

Finding a cure for acquired immune deficiency syndrome (AIDS) has been the goal of an intense, worldwide research effort since 1984 when the causative agent of AIDS, human immunodeficiency virus (HIV), became available in a form amenable to laboratory study. Between 1984 and 1991 over 26,000 research articles on AIDS and HIV appeared in the medical literature. In spite of this impressive accumulation of knowledge, there is still no treatment which stops and/or reverses the primary disease process -- depletion and debilitation of T4-lymphocytes. At present antibiotics for treating or preventing some opportunistic infections and anti-viral drugs for slowing the progression of the disease represent the only clear clinical dividends from millions of dollars spent on AIDS research. Such strategies, while capable of extending the lives of AIDS patients, obviously do not constitute cures. In fact, the opinion that AIDS is incurable is not infrequently expressed by AIDS researchers. For instance, Lane et al. (1) noted bluntly that "This disease appears to be invariably fatal." Such pessimism is supported by established facts regarding the molecular biology of HIV.

In the earliest stages of infection HIV appears to be restricted to T-cells expressing the CD4 surface protein (2). As the disease progresses, the virus can infect a variety of cell types including glia, microglia, endothelial cells, fibroblasts, enterochromaffin and crypt cells (3,4). With regards to long-term prognosis, the most salient feature of HIV infection is the propensity of the virus to become integrated into the chromosomes of host cells and to reside there, possibly for years, before being

Med H° 1992 May
35(1):20-4

replicated and released. As infected cells undergo mitosis they replicate the viral DNA along with the human DNA. Cell lines carrying the provirus are therefore established *in vivo* and eventually the patient becomes a mosaic of cells, some of which carry the viral DNA in their genomes and some of which do not. Haseltine and Wong-Staal (2) have described the situation with a saturnine outlook characteristic of people working in this field: "..the viral DNA...will be duplicated together with the cell's own genes every time the cell divides. Thus established, infection is permanent."

It is not surprising that this pernicious modus operandi of HIV should be the source of much pessimism. For even if ways could be found to eliminate the free-living virus from infected individuals, it is unlikely that ways will ever be found to remove the viral DNA incorporated into the chromosomes of a plethora of the infected cells. Against this scenario one might reasonably ask, as virtually all AIDS patients do, whether there is any hope of being cured of AIDS. Are there any indications that the virus, particularly the integrated virus, can be eliminated from a patient? A search of the literature to June, 1991 reveals little reason for optimism; however, there are a few reports suggesting that HIV may not necessarily be a permanent denizen of the individuals or cells it infects. Reports of successful, or partially successful, iatrogenic intervention deserve particularly close scrutiny because at present they represent the only glimmer of hope of finding a way of eliminating HIV, and thereby truly curing AIDS.

SPONTANEOUS DISAPPEARANCE OF HIV

Burger et al. (5) described a woman, the wife of an HIV-infected haemophiliac, who was herself sero-positive for antibodies to HIV and had a depressed T4-cell count. Although the husband eventually succumbed to an AIDS related infection, the wife became sero-negative for HIV antibodies four months after her sero-positive results were first recorded. Attempts to isolate HIV from the woman's lymphocytes were negative after she reverted to a sero-negative state; her T4-cell count increased to normal levels. At a 1 year follow-up (6) the patient remained asymptomatic, sero-negative and free of detectable viral DNA. Her T-cell counts remained elevated and in the low normal range. Unfortunately, the patient's original HIV-positive blood samples were not retained for later confirmation and analysis and therefore the possibility that the woman's original diagnosis was erroneous due to a false positive assay or passive transfer of antibodies from her husband cannot be excluded. However, the observed increase in her T4-cell count, which was contemporaneous with the loss of HIV antibodies, is consistent with the interpretation that she had been temporarily infected by HIV.

A second report of apparent spontaneous elimination of HIV came from Farazdegan et al. (8) who undertook a large multi-centre prospective study in which 4954 gay men were examined at 6 month intervals over a period of almost 4 years. Of the 3300 subjects who remained in the study as of the date of its publication, 1000 had become sero-positive for HIV antibodies. All positive ELISA results were confirmed by Western blot analysis. During the course of the study four of the 1000 sero-positive subjects (0.4%) reverted to a sero-negative state. The report is not clear as to the clinical status of three of these men; however, one man was reported to be asymptomatic throughout the study -- both before and after sero-conversion to the negative state. In all 4 cases T4-cell counts increased following loss of p24 antigen and antibodies.

To insure that these patients had not been originally misdiagnosed nor samples mislabelled, the original stored blood samples were re-examined by Western blot, ELISA and polymerase chain reaction (PCR) amplification of HIV DNA. Samples were also checked for genetic identity with the subjects and with original samples. It was estimated that the single probe PCR technique employed, which amplifies a conserved region of the HIV *gag* sequence, could detect viral DNA in as few as 3 cells out of 150,000. HIV was detected in all of the original sero-positive samples. PCR-amplified *gag* sequence could be detected in two of the 4 patients who reverted to sero-negativity, even though

antibodies to p24 could not. In the remaining two patients neither viral DNA nor p24 antibodies were detectable.

An optimistic interpretation of these observations is that HIV had infected these individuals and then subsequently disappeared, at least in two cases. An alternative explanation that the virus was still present in an occult, undetectable state in the patients' blood cells seems barely tenable given the impressive powers of PCR to ferret out and amplify target DNA sequences (9). However, the residual HIV DNA detected in two of the patients may have represented latent, integrated DNA inaccessible to the patients' immune systems. Follow-up studies on these individuals are obviously crucial but have not yet been reported.

Many questions are raised by the above observations. Why do some rare infected individuals become sero-negative and lose all traces of the virus? How far can such a patient progress along the disease continuum before becoming sero-negative? Had the virus spread beyond the immune system before disappearing? Had it integrated into the chromosomes and, if so, did these subjects possess molecular mechanisms that deleted the HIV provirus from the chromosome? Do these individuals share a condition that makes them, like most mammals, resistant to HIV? Where there conditions that facilitated conversion to a negative state, for instance co-infection by another virus, such as human herpesvirus-6 which is known to inhibit HIV replication (10)?

Each of these questions implies a number of hypotheses, any one of which could point the way to a strategy for curing AIDS. Furthermore, confirmation of elimination of HIV from any individual would constitute a refutation of the dogma that HIV infection is invariably permanent and fatal. If follow-up observations to the above reports demonstrate long-lasting remission, the emphasis in AIDS research may shift to attempting to understand the conditions and mechanisms responsible for the elimination of HIV from certain, rare infected individuals.

THE ELIMINATION OF HIV BY BONE MARROW ABLATION AND TRANSPLANTATION

In contrast to these reports of apparently spontaneous loss of HIV, there have been no reports of rational anti-HIV therapeutic interventions successfully eliminating HIV from an individual. While a number of strategies have been suggested and developed for attacking the free virus, preventing its absorbtion to cells or slowing the rate of integration into the genome, there has not been a surfeit of viable suggestions as to how the virus already integrated into the DNA of infected cells might be eliminated. It would appear that without some means of eliminating the provirus, efforts to neutralise or eliminate the free virus, no matter how effective, would be beneficial in the short-term only.

There are only two ways in which the HIV provirus might be eliminated from an infected individual: Either the provirus in the genome of each individual cell must be selectively eliminated, or, alternatively, all of the cells carrying the provirus must be eliminated. Clearly, the prospects for dissecting the HIV provirus out of the chromosomes of all infected cells are virtually nil, notwithstanding the miracles we have come to expect from molecular biology. Therefore, if therapeutic intervention is to be successful in eliminating the provirus from an individual, it must effectively eliminate all infected cells.

Two necessary conditions must certainly be of concern if therapeutic strategies to eliminate HIV-infected cells are to be successful. First, the therapeutic intervention must occur before the virus has spread from T4-cells to a plurality of cell types. Second, the therapeutic intervention must be sufficiently extreme to insure removal of all infected cells from the system. These conditions are analogous to those faced in the treatment of neoplastic diseases. In fact, the goal of eliminating lymphocytes bearing HIV-contaminated chromosomes in AIDS patients is comparable to the goal of

eliminating haemopoietic cells bearing the Philadelphia chromosome in patients with chronic meylogenous leukemia (CML). Given this point of view, might one not anticipate positive results by treating AIDS patients with the same techniques used to eliminate cells bearing the Philadelphia chromosome in CML patients; i.e., radical ablation of the immune system followed by bone marrow transplantation (BMT) ?

Immunosuppression of AIDS patients is not a novel idea and there are a number of reports indicating moderate and transient clinical improvement following relatively mild immunosuppression. For instance, Andrieu et al. (12) found that cyclosporin-A increased T4-cell counts and eliminated lymphadenopathy in patients who were in the early stages of AIDS. Cyclosporin had little or no beneficial effect in patients who had more advanced AIDS. Other investigators (13) have reported that cyclosporin produced little or no beneficial effect but rather induced a deterioration in the clinical status of AIDS patients who were symptomatic at the time therapy began.

Bone marrow transplantation and/or leukocyte transfusion have also been attempted as AIDS treatments with short-term benefits observed in some instances (1,14,15) and no benefits observed in others (16,17,24). There has been no evidence of long-lasting remission of AIDS. However, lack of long-term benefit is not unexpected in these studies since the patients were in an advanced, symptomatic stage and only relatively mild immunosuppression was attempted prior to BMT. Carrying out BMT or leukocyte transfusion in advanced patients with disseminated HIV would do nothing more than provide fresh leukocytes for residual viruses to infect. In order for such strategies to have any benefit, all reservoirs of the virus must first be purged prior to reconstitution of the immune system. A lack of appreciation of this point explains why some authors have underestimated the potential of BMT as a treatment of AIDS patients (7).

How early in the course of the disease would such radical approaches have to be employed? Baltimore (18) has suggested that a dropping T-cell count may indicate an immune system that is beyond repair. Although there are no data, one might reasonably presume that by the time opportunistic infections have appeared HIV has spread widely throughout the immune system and possibly beyond. It is the 'beyond' -- platelets, epithelial cells, endothelial cells, microglial cells -- that is of particular concern. If the virus has established itself in cells that are not affected by immune suppression, it will certainly re-establish itself in the re-constituted immune system. In order for marrow ablation to be fully effective, intensive chemotherapy and radiotherapy must be applied as soon as possible after HIV infection is detected in order to destroy infected cells while they are sequestered within the immune system. As of the present, virtually all published reports of attempts to suppress and re-constitute the immune system in hopes of eliminating HIV can be justifiably criticised on the basis of doing 'too little, too late'.

There appears to be only one full report of radical immune suppression employed in a symptom-free patient with HIV infection. Holland et al. (19) reported their experience with marrow ablation and allogenic BMT in treating a 41 year old patient with non-Hodgkin's lymphoma. Although HIV-1 could be isolated from the patient's peripheral blood mononuclear cells (PBMC's) prior to therapy, the patient was asymptomatic for AIDS and his blood and CSF were negative for p24 antigens. He had no history of opportunistic infections. It is not clear whether the lymphoma was secondary to the HIV infection but in the absence of other clinical signs of HIV infection, this is unlikely.

Therapy for the lymphoma included methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, decadron and cytosine arabinoside followed by total body irradiation (TBI) of 300 Gy/day for 4 days. Fourteen days prior to TBI the patient was started on IV zidovudine, 5 mg/kg/4 times a day. From 4 days prior to TBI, acyclovir was added at a dose of 500 mg/m^2 of body surface IV every 8 hours. Following TBI an allogenic marrow transplant was carried out and zidovudine and acyclovir were continued.

The results of this intervention are somewhat difficult to interpret due to lack of statistical analysis and because values for all relevant time points were not reported. However, HIV antibodies appeared to decline 3 weeks following transplantation and continued to decline even though lymphocyte numbers increased. At 25 days after transplantation the authors detected HIV RNA in the patient's bone marrow using PCR techniques but the virus could not be cultured from the patient's PBMC's. At 32 days post-transplantation the HIV RNA had decreased in the patient's bone marrow and was not detectable in PBMC's. At 45 and 47 days HIV RNA was no longer detectable in bone marrow. There were strong indications that the marrow engraftment was successful in spite of the addition of zidovudine and acyclovir to the regimen. Although PCR-detectable HIV appeared to have been eliminated by the radical immune suppression, the lymphoma persisted as shown by biopsy at 39 days post-transplantation. The patient refused further therapy and died from respiratory failure at 47 days post-transplantation. At autopsy the authors attempted to detect HIV in a variety of tissues known to be susceptible to HIV infection. These results were also negative.

This paper is significant in that it appears to represent the only reported case in which therapeutic intervention has eliminated HIV. The authors stress that the aspect of their regimen that promoted the elimination of detectable virus was the combination of aggressive marrow ablation and anti-viral therapy. While this factor should not be underestimated, neither should the fact that the patient was free of AIDS symptoms when the therapy was initiated. Early intervention and the aggressiveness of the immunosuppression set this case apart from other attempted interventions so far reported in the literature.

CONCLUSIONS

The literature contains a number of reports of failed attempts to use immunosuppression to eliminate HIV or to improve the clinical status of AIDS patients. Such negative experiences have led some authors to conclude that immunosuppression is not effective, may be deleterious and should not be used (7,13,24,25). However, careful examination of these conclusions reveals that they are based upon experiences with advanced, symptomatic AIDS patients or on reports in which the status of the patient is not discussed at all. For instance, in one recent negative report the authors fail to provide any indication of the clinical status of the AIDS patient prior to immunosuppression (24). Such reports are essentially unintelligible and no conclusions can be drawn therefrom regarding the efficacy of radical immunosuppression in asymptomatic AIDS patients.

The potential for eliminating HIV with radical immunosuppression and BMT warrants serious consideration. Recent animal studies have established the efficacy of TBI in eradicating viral infections (20), including infections due to retroviruses (21). Success has also been reported in using cyclophosphamide, TBI and BMT to eliminate adult T cell leukemia virus in humans (22). Of particular significance is the use of radical immune suppression and BMT to eradicate cells carrying the Philadelphia chromosome (11). Such observations, together with those described above, indicate that individuals who are sero-positive for AIDS might well be candidates for radical marrow ablation and BMT, provided they are treated early enough.

The difficult dilemma presented by this proposal will be recognised immediately. Radical immunosuppression and BMT carry significant short-term risks related to toxicity, graft-versus-host-disease and opportunistic infections. Fatalities due to toxicity in non-AIDS lymphoma patients treated with intensive chemotherapy, but not radiotherapy, were reported to be about 7% (25). Survival rates following immunosuppression and BMT in patients with neoplastic diseases are on the order of 25% - 50% (23). Where the short-term prognosis is poor, as in lymphoma and leukemia, such risks may be justified. But the person with a positive HIV test can expect many years of good health. Does one ask a patient to gamble these useful years of life against the possibility of totally eliminating HIV and being

cured of AIDS?

At present it would be premature to enter into debate over such difficult questions. Although the risks in using radical immunosuppression in cancer patients are now appreciated, we don't know the extent of the risk when such regimens are applied to healthy HIV-infected individuals. Certainly the experiences of Holland et al. (19) suggest that healthy HIV-positive individuals may tolerate radical immunosuppression well and that marrow engraftment is not jeopardised by concurrent zidovudine therapy. However, animal studies are needed to evaluate both the risks and the benefits before courageous, healthy HIV-positive individuals are asked to undergo such treatment.

BIBLIOGRAPHY

1. Lane HC, Masur H, Longo DL. Partial immune reconstitution in a patient with the acquired immunedeficiency syndrome. N. Engl. J. Med. 311: 1099, 1984.

2. Haseltine WA, Wong-Staal F. The molecular biology of the AIDS virus. Sci. Am. 259: 34, 1988.

3. Jordan CA, Watkins BA, Kufta C, et al. Infection of brain microglial cells by human immundeficiency virus type 1 is CD4 dependent. J. Virol. 65: 736, 1991.

4. Levy JA. Pathogenesis of human immunodeficiency virus infection. Ann. N. Y. Acad. Sci. 567: 58, 1989.

5. Burger H, Weiser B, Robinson WS, et al. Transient antibody to lymphadenopathy-associated viurs/human T-lymphotropic virus type III and T-lymphocyte abnormalities in the wife of a man who developed acquired immune deficiency syndrome. Ann. Intern. Med. 103: 545, 1985.

6. Burger H, Weiser B. Transient antibody to human immunodeficiency virus [Letter]. Ann. Intern. Med. 105: 464, 1986.

7. Klatzmann D, Montagnier L. Approaches to AIDS therapy. Nature 319: 10, 1986.

8. Farazdegan H, Polis MA, Wolinsky SM, et al. Loss of human immunodeficiency virus type 1 (HIV-1) antibodies with evidence of viral infection in asymptomatic homosexual men. Ann. Intern. Med. 108: 785, 1988.

9. OU C-Y, Kwok S, Mitchell SW, et al. DNA amplification and detection of HIV-1 in DNA of peripheral blood mononuclear cells. Science 239: 295, 1988.

10. Carrigan DR, Knox KK, Tapper MA. Suppression of human immunodeficiency virus type 1 by human herpesvirus-6. J. Infect. Dis. 162: 844, 1990.

11. Beard ME. Bone marrow transplantation. N. Z. Med. J. 1987; 100: 468, 1987.

12. Andrieu JM, Even P, Venet A et al. Effects of cyclosporin on T-cell subsets in human immunodeficiency virus disease. Clin. Immunol. Immunopathol. 47: 181, 1988.

13. Phillips A, Fanning M, Wainberg MA, et al. Cyclosporin-induced deterioration in patients with AIDS. Can. Med. Assoc. J. 140: 1456, 1989.

14. Hassett JM, Zaroulis CG, Greenberg ML. Bone marrow transplantation in AIDS [letter]. N. Engl. J. Med. 309: 665, 1983.

15. Vilmer E, Rhodes-Feuillette A, Rabin C et al. Clinical and immunological restoration in patients with AIDS after marrow transplantation, using lymphocute transfusions from the marrow donor. Transplantation 44: 25, 1987.

16. Verdonck LF, de Gast GC, Lange JM et al. Syngenic leukocytes together with suramin failed to improve immunodeficiency in a case of transfusion-associated AIDS after syngenic bone marrow stansplant. Blood 71: 666, 1988.

17. Lane HC, Zunich KM, Wilson W et al. Syngenic bone marrow transplantation and adoptive transfer of peripheral blood lymphocytes combined with zidovudine in human immunodeficiency virus (HIV) infection. Ann. Intern. Med. 113: 512, 1990.

18. Baltimore D, Feinberg MB. HIV revealed: toward a natural history of the infection [editorial]. N. Engl. J. Med. 321: 1673, 1989.

19. Holland HK, Saral R, Rossi JJ, et al. Allogenic bone marrow transplantation, zidovuine and human immunodeficiency birus type 1 (HIV-1) infection. Studies in a patient with non-Hodgkin lymphoma. Ann. Intern. Med. 111: 973, 1989.

20. Shen R-N, Lu L, Broxmeyer HE. New therapeutic strategies in the treatment of murine diseases induced by virus and solid tumors: biology and implications for the potential treatment of human leukemia, AIDS and solid tumors. Crit. Rev. Oncol. Hematol. 10: 253, 1990.

21. Shen R-N, Hornback NB, Lu L, et al. Low dose total body irradiation: a potent anti-retroviral agent in vivo. Int. J. Rad. Onco. Biol. Phys. 16: 165, 1989.

22. Soube R, Yamauchim T, Miyamura K, et al. Treatment of adult T cell leukemia with mega-dose cyclophosphamide and total body irradiation followed by allogenic bone marrow transplantation. Bone Marrow Transplant 1987; 2: 441, 1987.

23. Santos GW, Yeager AM, Jones RL. Autologous bone marrow tranplantation. Ann. Rev. Med. 40: 99, 1989.

24. Aboulafia DM, Mitsuasu, Miles SA. Syngenic bone-marrow tansplantation and failure to eradicate HIV. AIDS 5: 344, 1991.

25. Schneider AM, Straus DJ, Schuluger AE, et al. Treatment results with an aggressive chemotherapeutic regimen (MACOP-B) for intermediate- and some high-grade non-Hodgkin's lymphomas. J. Clin. Oncol. 8: 94, 1990.