Update TESTING in the BLOOD BANK

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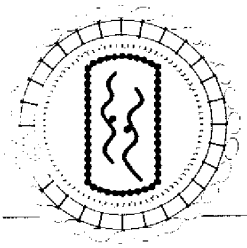

Perspectives on the Development of a Human Immunodeficiency Virus (HIV) Vaccine

by James R. Carlson, Ph.D. †

The AIDS pandemic has made the development of a protective vaccine a critical public health priority.[1,2] HIV vaccines must be developed to protect unexposed individuals, and since no cure currently exists and a high proportion of HIV-infected individuals progress to AIDS, it is also necessary to consider post-exposure immunotherapy.[3] Initial vaccine efforts using novel approaches in laboratories around the world are underway and results have been encouraging; however, it is apparent that considerable research remains to be done. In preparing for this challenge a concerted effort is needed to explore the biological complexities of HIV infections by establishing a systematic approach to vaccine development that is built on a solid scientific basis. A logical first step in this work should be the development and testing of inactivated, whole HIV vaccines accompanied by an HIV typing program. This approach can lead to a better understanding of protective immunity against HIV and guide the formulation of other immunopreventive strategies. Obviously, comparisons will need to be made between the "crude" whole HIV preparations and the more refined genetically engineered subunit vaccine materials. Vaccines made by private industry, by academia or by the government will need to be compared one to another and to a set of known standards using inactivated whole HIV.

Based on past experience with immunization against infectious agents including retrovirus infections of animals, protection appears to correlate best with the presence of neutralizing antibody.[4] Since neutralizing antibody has been shown to be directed at the viral outer envelope, HIV envelope proteins have been selected by many as the major immunogens for preparation of initial candidate HIV vaccines.[5] In fact, recent studies have demonstrated that HIV envelope protein represents a major target antigen for antibodies in AIDS patients[6] and native HTLV-III gp120 has been shown to be immunogenic and capable of inducing neutralizing antibodies in small animals, rhesus and chimpanzees.[7] Although these results are encouraging none of the current HIV envelope immunogens has as yet been shown to induce antibodies cross-reactive in vitro with a spectrum of HIV isolates nor to protect against homologous HIV challenge in chimpanzees.[8] The extensive degree of envelope variation among different HIV isolates and the apparent lack of naturally occurring protective antibodies in infected humans calls into question the significance of raising neutralizing antibodies to HIV envelope as the primary goal of immunization.[9,10] The issue of HIV strain variation needs further investigation, both by sequence analysis and by an HIV typing program based on cross-neutralizations with hyperimmune animal sera. The significance of antibodies to core proteins (p55, p25, p18) also needs careful appraisal. Evidence now exists that maintenance of anti-p25 antibody may be associated with resistance to AIDS in HIV carriers.[2,11]

Although cellular immunity has been relatively unexplored in the initial quest for an HIV vaccine, recent data suggest that cellular immunity to HIV in the form of antibody-dependent cell cytotoxicity (ADCC) and cell-mediated cytotoxicity correlate with resistance to AIDS.[12] There is also precedence in animal retrovirus models for cellular immunity being directed at retroviral core as well as envelope antigens expressed on the surface of infected cells.[13] This provides further evidence that HIV core proteins should also be considered in future HIV vaccine development, either in the form of recombinant protein or as a component of inactivated HIV.

Considering the questions of safety, purity and practicality in modern vaccine development, it is understandable that to date most of the candidate HIV vaccine materials have been made from recombinant HIV proteins expressed in yeast, *E. coli*, mammalian cells or from synthetic peptides.[14] In fact, these approaches have great potential for ultimate success, however, genetically engineered subunit vaccines still have an unproven track record. The only recombinant DNA vaccine in routine use for humans is the

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means following large numbers of seronegative, high-risk individuals over time and periodically screening them for evidence of seroconversion. Large numbers of prevalent seropositive (infected) subjects can be found, but without knowing the duration of infection at time of enrollment, it is more difficult to completely fulfill the objectives stated above.

MACS investigators in Los Angeles, Chicago, Pittsburgh and Baltimore began recruiting gay/bisexual men between the ages of 18 and 60 in April 1984. Within a year 4,955 participants had been enrolled. At time of enrollment the following seroprevalence was found: Los Angeles 54%, Chicago 45%, Pittsburgh 21% and Baltimore 31%. Since the beginning of the study, there have been approximately 200 cases of AIDS, which all have developed in persons who were seropositive at entry. Furthermore, approximately 220 participants who were seronegative at baseline have seroconverted since entry into the study.

MACS is a large, statistically powerful and potentially extraordinarily informative study. As in most epidemiologic research, especially of a prospective nature, a frustratingly long time period must pass before meaningful results become available. Nonetheless, several papers are now being published from the MACS database. Major findings to date are summarized below.

In a paper published in *Lancet* in February, 1987, we reported on risk factors for HIV seroconversion among those who were seronegative at study entry. The cases were 95 participants who seroconverted in the first six months of the study. The only statistically significant risk factor was receptive anal intercourse; 92 of 95 infections were associated with this single unsafe practice. The other three new infections were associated with insertive anal intercourse; we were unable to demonstrate any risk associated with oral-sex. These findings have obvious importance in designing educational interventions.

We will publish a paper in May, 1987, in the new British journal, *AIDS*, on the acute syndrome associated with HIV seroconversion. Self-reported symptoms significantly associated with seroconversion in 22 prospectively studied participants (compared to seronegative and seropositive controls) were swollen lymph nodes, fever, headache and night sweats. These symptoms generally were mild and transient. Furthermore, they are nonspecific and therefore have weak positive predictive value.

Our first paper on risk factors for AIDS (n = 59) among the prevalent HIV infected participants (n = 1,828) appeared in *The New England Journal of Medicine* in January, 1987 (316;2:61-6). We demonstrated that reduced numbers of CD4 cells (T-helper lymphoctyes) was the single strongest predictor of the development of AIDS. In the multivariate model, four additional variables were statistically significant independent predictors: an increased number of CD8 cells (T-suppressor lymphocytes), an increased level of serum antibody to cytomegalovirus, a decreased level of serum antibody to HIV and a history of having had sex with a person who had or developed AIDS. We suspect that all these predictors are *markers* of progression rather than *determinants* of progression, although the last-mentioned finding may suggest that some strains of HIV are more virulent than others. Future studies of more AIDS cases, especially those that develop in the seroconverters, will clarify and extend these findings. Hopefully, we will identify *determinants* of progression that are potentially mutable, leading to effective interventions to slow the progression to disease among those infected.

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Anti-HIV; the British Approach to Maintaining the Safety of the Blood Supply

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Hepatitis B virus was unusually obliging to transfusion microbiologists; the abundance of surface antigen it produces provided the basis for rapid, simple and effective tests for donor screening. Not surprisingly, human immunodeficiency virus (HIV) does not behave in the same way and a test for antibody to the virus as evidence of past — and persistent — infection, rather than an antigen assay, remains the most appropriate screen test. In Great Britain, before implementation of blood donor screening, three prerequisites were formulated by the Transfusion Service as a whole:[1]

1. A Battery of Confirmatory Tests

Evaluation of available anti-HIV test systems to choose those best suited to the requirements of the Transfusion Services was performed. In conjunction with this, reference laboratories were made available to offer a battery of confirmatory tests on any repeatedly reactive donor samples. It was not anticipated that as many 'unconfirmed' reactions would be reported from the reference laboratories as was the case in the United States. In fact, the use of a battery of tests, rather than the single 'gold-standard' Western Blotting has meant that very few 'reactive' donors have not been classified either as 'confirmed positive' or 'suitable for continued donation'. In practice, the use of a combination of type I, II and III assays (as classified by Mortimer[2]), with

or without a Western Blot, has satisfied transfusion microbiologists in Great Britain and avoided the problem of inventory control associated with 'indeterminate' samples after confirmatory tests by the reference laboratory. Thus an 'antiglobulin' confirmatory test together with a 'competitive' assay and a G-antibody capture assay provide a system for confirmation based on three different principles. In contrast, a Western Blot used to confirm an antiglobulin ELISA relies on two assays based on the same principle.

2. Alternative Test Sites for High-Risk Groups

Alternative test sites where people in high-risk groups could obtain anti-HIV testing and counselling without having to give a potentially infectious blood donation were set up. Confidential testing has therefore been made available at Sexually-transmitted Disease Clinics or via general practitioners. The value of alternative test sites has been clearly established in Scotland.[3] Fifteen percent of individuals attending an alternative test site in Edinburgh admitted that they would otherwise have donated blood to obtain an anti-HIV test. Three of these individuals were anti-HIV positive. In the absence of routine HIV antigen screening and with the advent of new strains of virus, the continuation of efforts to discourage donation by people at risk of contracting AIDS becomes even more important. At the North London Blood Transfusion Centre at Edgware we have extended the facility for self-exclusion by providing a confidential questionnaire similar to that in the New York Blood Center.[4] In the summer of 1984, despite providing blood donors with the initial Department of Health leaflet on AIDS, the questionnaire revealed that homosexual men were still donating blood. At a blood donor clinic in the West End of London, 1.7% of established and 1.3% of first-time male donors admitted to being homosexual.[5] Although 18% were positive for anti-HBc compared with 1-2% of the overall donor population, none was anti-HIV positive. Subsequently, leaflets for donors have been progressively revised to clarify and extend the risk groups.

3. Counselling Centres for Anti-HIV Positive Blood Donors

Counselling centres with trained staff were identified for referral of blood donors found anti-HIV positive. Short- and long-term psychological and, if necessary, medical support would then be available to follow on from two initial counselling sessions at the Transfusion Centre.

Once these three requirements were fulfilled, British Transfusion Centres started simultaneous total donor screening for anti-HIV in October 1985. Most Centres favoured a competitive assay for anti-HIV since this proved rapid, simple, sensitive and highly specific.[6] Although the rates of donors confirmed to be anti-HIV positive were the same whether Centres were using an antiglobulin or a competitive type of assay, the rates of repeatedly reactive donors on the initial screening test were 17 times greater with the antiglobulin assay. The specificity of the competitive assay has been largely responsible for the absence of the confusion associated with the HLA and other cross-reactions seen with virus lysate antiglobulin assays. However, many new and improved assays are becoming available. Cloned or synthetic antigen for antiglobulin assays, monoclonal antibody for competitive assays and novel gelatin particle agglutination assays may all contribute to improvements in anti-HIV screening. Evaluation of the role of recent HIV antigen assays will also be required. The impact that HIV-2 may have on this new technology is also likely to prove very challenging. The encouragement of 'self-exclusion' will assume increasing importance in this context.

To date Great Britain has enjoyed an encouragingly low rate of donors positive for anti-HIV (approximately 1 in 50,000) although there can be as much as twice that prevalence in certain areas, e.g., cosmopolitan regions or pockets of high rates of infection in intravenous drug abusers. This low prevalence correlates at the North London Blood Transfusion Centre with a halving in the rate of donors found positive for HBsAg compared with the rate prior to the AIDS publicity. This low rate has been maintained for the past two years. So far the vast majority of the donors found anti-HIV positive in Great Britain are from recognised risk groups (and mainly from male homosexuals) so that heterosexual transmission appears to play a minor role. Furthermore in the United Kingdom out of 3.5 million donations, only one instance has been recorded of a seronegative donor transmitting HIV and subsequently seroconverting. The situation therefore is under control but there is no room for complacency in the face of future challenges.

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