HIV TESTING IN HIV-VACCINATED POPULATIONS

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Wednesday, September 16, 1998

Many problems arise in relation to vaccine testing and HIV 1 serology. Among the most complicated of these is the possibility of false HIV positive results appearing on serological tests. Such a result can have a number of possible consequences, including constraints on international travel, blood donation, military service, or health and life insurance. In addition, there can be discrimination from friends, employers and family. Such problems are only likely to become more magnified as increased testing of HIV 1 vaccine candidates moves into further Phase I , II and III trials.

At present there are six NIH-funded Phase I clinical trial sites of candidate HIV vaccines in the United States. These include the University of Rochester, Vanderbilt University, University of Washington, the University of Alabama at Birmingham, St. Louis University, and Johns Hopkins University. These sites are primarily responsible for the Phase I and II trials that take place inside the AIDS Vaccine Evaluation Group (AVEG) of the NIH. These sites have been conducting trials of candidate HIV vaccines since 1998. There have been over 30 clinical trials which have used a variety of different vaccine approaches including subunit vaccines, peptide-based systems, canary pox vectors that encode most of the genes of HIV, Vaccinia vectors which also have encoded multiple genes, DNA vaccines, mucosal vaccines, Salmonella-based vaccines, and multiclade based vaccines. To complicate matters further, these candidate vaccines have been given with over 10 different experimental adjuvants in these trials.

The number of patients tested to date in these Phase I trials is not small. As of October 1998 over 2,600 seronegative volunteers had been enrolled in candidate HIV 1 vaccine trials. The majority of these volunteers have received vaccine and not placebo. Of these vaccines, approximately 2,000 were at low risk and approximately 500 at high risk. However, even low risk patients have some exposure risk. Evaluation in the AVEG reveals HIV 1 seroincidence in these Phase I trials ranging from 0.22% for low risk individuals, up to 1.46% in the high risk individuals. In these trials 32 individuals have become infected with HIV 1. This rate of infection potentially leads to a fairly major problem, that is, during vaccine trials, especially high risk trials, there may be positive serology both from infection and from the vaccine itself. Distinguishing between these two conditions is obviously critically important. What percent of individuals that receive candidate HIV 1 vaccines will actually test positive? The answer to this question lies in the serological tests used.(1)

When extremely specific serological tests are used which include reagents not included in the vaccine, the number of individuals testing positive is extremely low. However, when cell lysates or first or second generation ELISA tests are used, the rate can be fairly high. In initial trials of HIV 1 vaccines conducted in the early 1990's, approximately one half of all recipients of subunit gp160 and gp 120 vaccines tested positive by the Abbot HIV 1 second generation test at the time of their peak neutralization titer. However, now that these test kits are using synthetic peptides, the rate of testing positive has decreased. When tests are used based on the Sanufi kit which includes the immunodominant portions of gp41 and areas of pol, there is essentially little to no cross-reac-

tivity with the gp120 vaccines. All gp160 vaccines that include the immunodominant epitopes of gp41 will tend to be problematic due to the fact that almost all commercially available testing is based on the that immunodominant sequence. In recent vaccine trials using canary pox vectors which encode multiple HIV genes and are boosted with gp120 subunits ("prime-boost"), the number of volunteers testing positive by the Abbot HIV 1/2 EIA is approximately 50% at the time of peak antibody titer. Persistence of these vaccine-induced antibody responses is quite variable. The antibody responses that are induced by subunit vaccines decline quickly over a three month to one year period. However, volunteers from AVEG trial 002, who received Vaccinia products which encoded gp160 and were boosted with subunit gp160 have had persistent antibody responses seen up to 10 years later.

Are there any major implications for the numbers of individuals vaccinated? In small cities such as Rochester and Nashville, the location of two of the AVEG units, it can be estimated that up to 100 people could have been positive due to vaccine trials rather than true infection when using blood bank screening methodologies that use an ELISA. In areas where only 2-3,000 individuals may be HIV positive, this could represent a significant number of the positive tests seen in the testing sites. Therefore, in cities that have vaccine units, close communication between health departments and the vaccine testing site is needed. Initially the number of such sites was relatively limited. They included the six AVEG sites mentioned above and the Phase II/III HIVNET sites in Chicago, San Francisco, New York, Denver, Philadelphia, Boston, Providence and Seattle. However, with the initiation of the VaxGen Phase III efficacy trial of the bivalent gp120 subunit vaccine, the number of potentially affected individuals has markedly increased. This trial will enroll approximately 5,000 individuals in 50-60 centers across the United States. Two out of every three recipients will receive vaccine for a total vaccinated population of 3,333. Since the vaccine includes only the gp120 subunit, it would seem relatively simple to establish that a positive ELISA is not vaccine by use of Western blot. However, in AVEG trials anywhere from 10-20% of individuals have a random falsely positive band at p24. If criteria are employed for interpreting Western blot which include one envelope band and a p24 band, then a large number of individuals that are vaccinated in this trial could eventually test positive if the Western Blot was interpreted by an inexperienced or uninformed interpreter. This trial will be further complicated by the fact that individuals will be receiving booster vaccines over three years and are likely to have high titer gp120 antibody responses during this entire vaccine period. The persistence of these vaccine responses after three years of immunization is not known at present.

Do such false positive misinterpretations occur? A number of these have occurred within the AVEG. One was published in the Lancet a year ago by Dr. David Schwartz from Johns Hopkins University.(2) In addition, a patient volunteer at another site who received a gp160 Vaccinia based vaccine with a gp160 boost had a Western blot interpreted as positive in a well known research laboratory in the United States. He had an RNA PCR done which was also interpreted as positive. Follow-up at the site revealed his Western blot had not changed since vaccination. Two further RNA PCRs, a DNA PCR, and a CD8+ co-culture revealed that this individual was not infected. However, he behaved as if he had been infected for over a six month period. The cost of this workup was many thousands of dollars. It should be understood that this workup occurred approximately six years after initial vaccination. Thus, the potential problems from false positives are truly real. Basing tests on RNA PCR is also somewhat problematic. This is due to the fact that in some of the best surveys at present approximately 1-2% of seronegative individuals may test falsely positive by RNA

PCR. Although these false positives tend to have low absolute numbers (<10,000 copies), they can be problematic. For example, a physician may interpret an early ELISA or Western blot as potentially positive. An RNA PCR could be done which is also falsely positive. If that individual was quickly placed on very highly active antiretroviral therapy (HAART), as is now recommended, this could lead to a confusing situation. There may be no further seroconversion, negative follow-up PCR, and the physician or health care worker may inappropriately interpret the person as a transient infection and leave the patient on potentially toxic antiretroviral therapy. Such a scenario is not only somewhat likely but even probable with the number of individuals that will enter vaccine trials.

What are the solutions to some of these problems? The AVEG has used a photo identification card and an 800 telephone number. If a volunteer experiences social harm or problems, the number is called and an intervention can occur. We have been successful at notifying insurance companies, employers, and other parties who have falsely interpreted vaccine-induced positive serology as being indicative of HIV 1 infection. The NIH has also stated that it will provide follow-up in NIH-sponsored vaccine trials as long as the individual tests seropositive by any conventional test used and licensed by the FDA. However, this may be more problematic because as individual pharmaceutical companies begin to undertake trials, the long-term follow-up of individuals in these company based trials is not entirely clear. This situation is under consideration by the CDC, and appropriate guidelines will need to be developed.

It is also clear that as trials move forward, thought must be given to the methodology used to differentiate between seroconversion from the vaccine and from true infection. In the NIH-sponsored AVEG trials, an infection algorithm has been established, and can be accessed on the World Wide Web at www.emmes.com/avctn. This algorithm is then adapted specifically for each protocol depending upon the vaccine candidate that is tested. For some vaccines a serological test can be a useful screen. There are a number of ELISA kits that are based merely on the gp41 subunit. Most of these are not presently licensed in the United States but can be used for research purposes. For example if the Sanufi kit based on the gp41 and pol peptides is used, the number of serological false positives tend to be very low. Other kits made by other manufacturers that do not include pol are also quite useful. However, the use of full length envelope vaccines that encode gp41, or the use of gp160 subunits can complicate the use of such serological testing. As previously stated, using the AVEG algorithms no identification of HIV 1 infection is ever made based on a single nucleic acid test. The belief is that the false positive rate is too high to take any chances on a single test. In addition, the FDA has not approved any nucleic acid based test as a diagnostic test for HIV 1.

Lastly, the AVEG and NIH are working in conjunction with CDC to test a number of rapid testing kits from different manufacturers around the world using sera from different vaccine trials. We hope to come up with specific algorithms that may work in the future. Dr. Robert Belshe and Robert Stein, Esq. of the AVEG have taken the lead in suggesting to the FDA language which is to be placed in the packaging of test kits. This exact language is now found in almost all test kits licensed in the United States. It is as follows: "A person who has antibodies to HIV 1 is assumed to be infected with the virus, except for a person who has participated in an HIV vaccine study may develop antibodies to the vaccine, and may or may not be infected with HIV. Clinical correlation is indicated with appropriate counseling, medical evaluation, and additional testing to decide whether a diagnosis of HIV infection is accurate". This FDA language is included in the Sanufi kit, the Abbott kit, the Home Access kit, and the Murex SUDS kit.

The use of multiple rapid testing mechanisms that are based on peptide based sequences will also be extremely useful in algorithms for evaluating vaccine induced responses. It is also hoped that language similar to the above statement would be included in any algorithms adopted by the United States or the Association of Public Health Laboratories (APHL). Further information concerning the complex interaction between HIV candidate vaccines and serological testing can be found at the EMMES Corporation web site at www.emmes.com and at the NIH information number for serological testing, Ms. Mary Allen, 301-402-0846.

In summary, when interpreting potential positive responses of HIV 1 serology, there is a need to remember the large number of vaccine recipients in the United States. The possibility for vaccine induced false positive responses will increase as further trials are undertaken in the United States.

References

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