Testing for HIV Specific Proteins in Otherwise Western Blot Negative Theiller Albino Mice

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ABSTRACT
Théiller albino mice were used to carry out various experiments in order to check for the presence or absence of HIV specific proteins in Western-Blot negative blood donors and recipient mice. The results of this study have shown one or more HIV specific bands and some indeterminate bands for positive but not complete absence of bands. The most likely explanation is that the mice had antibodies that cross-reacted with one or more of the proteins of HIV.

Key words: HIV, Western Blot, Antibodies, Specificity

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INTRODUCTION
We know that AIDS exist and there is a correlation between AIDS and the antibody tests. And that sex plays a role in the development of a positive antibody test and AIDS. We do however, have misgivings that the HIV antibody proteins in the Western Blot are not specific.

There is no proof that all of the supposed HIV proteins actually come from HIV. The reason is that many attempts at virus isolation have presented numerous insurmountable difficulties. Thus one cannot say for sure that any of the HIV antibody proteins actually come from HIV. It follows therefore that if you cannot know for sure that the proteins in the antibody test come from HIV, then you cannot know for sure whether these proteins are anti-HIV antibodies.

In the Western Blot (WB), the presumed HIV proteins are present separately instead of in a mixture and, after being allowed to react with a blood sample, each protein is capable of giving a visible signal if it has bound an antibody. These tests which are thought to be highly specific are generally used to confirm them. In fact so much weight is put on these Western Blots that a positive result is almost always taken to equal an active HIV infection. In order to realize that this problem exists, for analogy purposes, diagnosing infections using antibodies is serological diagnosis, and is like trying to identify objects from the shadow they cast on the ground. There is a connection but clouds, buildings, trees and so forth may produce shadows that may look the same or similar. The best way therefore is to prove the existence of HIV in every patient by means that are unambiguous for a unique retrovirus. Although many claims are made that the HIV antibody test, have a high specificity, some schools of thought think that this is not the case. A test that is not specific will give a positive reading in the presence of other than those it is supposed to detect. Clearly, if an HIV antibody test is not specific, a positive result is at best ambiguous.

Some groups of people generally make a lot of antibodies because they are exposed to a much greater than the normal amount of diseases and unhealthy conditions. This includes poverty-stricken Africans and intravenous drug users. Reactivity in both Elisa and Western Blot analysis may be nonspecific in Africans.

A study by Kion and Hoffman using alloimmune mice (mice that have been exposed to cells from another murine strain), the mice were shown to make antibodies against gp120 and p24 of human immunodeficiency virus (HIV), and mice of the autoimmune strains MRL-1pr/1pr and MRL-(+)/ made antibodies against gp120. This was surprising because the mice were not exposed to HIV. Furthermore, anti-anti-MHC antibodies (molecules that have shapes similar to those of major histocompartmentibility complex molecules) were detected in both alloimmune sera and MRL mice.

MATERIALS AND METHODS
Western Blot Kit for HIV specific proteins with the following provided:
- R1 HIV-1 Western Blot strips (40)
- C0 Western Blot Negative Control, 1 vial (0.2ml)
- C1 HIV-1 Western Blot Low Positive Control, 1 vial (0.2ml)
- C2 HIV-1 Western Blot High Positive Control, 1 vial (0.2ml)
- R2 Western Blot Specimen Diluent/Wash (5x) 2 bottles (100ml)
- R3 HIV-1 Western Blot Conjugate, 1 bottle (80ml) and R4 Western Blot Color Development Reagent, I bottle (100ml). Vaccutainers ( containing EDTA), Microscope, Aspirator, Rotary platform(50-60 rpm), Antisera, warming lamp.

Animals
Theiller albino mice (25-30 g) of either sex were used. They were housed in standard cages and kept under room temperature appr. (24 ± 7°C); relative humidity ( appr. 60-70%) in a 12 hr light-
dark cycle. The rats were given a standard laboratory diet and water *ad libitum*. Food was withdrawn 12 hr before and during the experimental hours.

The experimental animals were divided in three groups as follows:
One group of five for the negative control, the test group consisted of two groups of ten mice. One group of ten mice to act as donors and another group of ten mice for the recipients. There were no animals in the positive control group because injection of (HIV-1) subcutaneously in the mice provided difficulties for ethical reasons, hence it was necessary to use the positive control provided by Bio-Rad WB manufacturers.

**Withdrawal of blood.**
In all the experiments, blood was withdrawn by lateral tail vein venipuncture and squeezing the blood into vacutainers with the aid of a warming lamp to dilate the tail. Both the negative and test group animals were tested for HIV specific proteins using the WB Method of Electro-blotting at 0, 1 hour and 1 week. All the mice were found to be WB negative before the experiments began (zero-time)

**Determination of Full Blood Picture**
All the animals were tested for full blood picture using a computer assisted Beckman Coulter A\(^c\).T. Diff. 2 Counter.

**Determination of Blood Group**
Twenty mice (10 donor mice and 10 recipient mice) were tested as it was important that both the donor and the recipient be carefully blood grouped. To do this, a drop of blood was mixed with human anti-A antiserum, one with anti-B antiserum and one with anti-D antiserum, because attempts to get mice blood grouping failed with both the Internet and Veterinary Surgeons.

**Cross matching of blood**
The test was done in 20 mice, 10 donors and 10 recipient mice. The saline method was used with the aid of a microscope. After blood grouping and cross-match in the test groups, 0.5ml of the HIV negative blood was administered subcutaneously from donor to recipient mice. The recipient blood was then taken from the mice and re-tested using the Western Blot (WB) Bio-Rad for HIV specific proteins after a period of one hour and one week. Re-testing was done in all test animals including the negative control group.
RESULTS AND DISCUSSION
Figure 1 shows a photograph of low and high positive controls provided by Bio-Rad Western Blot manufacturer.
BLOOD GROUPING

Theiller albino mice showed neither, can they be grouped using the ABO blood grouping criteria for humans, nor did they show any incompatibility on cross matching between donors and recipients.

Full blood picture
The results of this study showed that the mean WBC was 13.18x10^9/L (range 8.0-20.0x10^9/L), RBC was 6.9x10^{12}/L (range 6.61-7.36x10^{12}/L), Hgb was 11.2 g/dL (range 10.1-12.3g/dL) and the Hct was 35.3% (range 35-40%) There was an exceedingly high count of lymphocytes (mean 18.4 x 10^9 )/L and blood platelets( mean 1283 x 10^9/L). There are many differentials for lymphocytosis including: Infection by bacteria, helminths and viruses. The monocyte count of the mice was within the normal human limits. Lastly no comments can be made on these findings because the normal values in the mice could not be found in literature.

After blood grouping and cross-match, the HIV negative blood from donor to recipient mice showed bands corresponding to gp120 and gp160 were observed in nine mice. Two mice had both gp120 and gp160 bands and one unidentified peak, two showed only one band gp160 and five had weak gp160 bands the so called indeterminate bands for positive but not complete absence of bands.
The western blot (WB) is a general laboratory technique for visualizing individual protein/antibody reactions (bands). One would think that if there really were HIV proteins, and that the HIV antibodies were truly specific, then just having one band light up would be proof that HIV is present. But according to experts, that is not the case, you need more than one\(^3\). The intriguing thing is, even if one or two bands are not sufficient to diagnose HIV infection, there must still be a reason why they are there. In fact cross-reaction is the explanation given by all HIV experts for “non-infected” Western Blots\(^3\). But if one or two bands in the WB can be caused by non-HIV, cross reacting antibodies why cant three or four or five or all of the ten bands be caused by cross reacting non-HIV antibodies.

Around the world different combinations of two or three or four bands of the possible ten bands are deemed proof of infection. In Africa you need only two, envelop proteins without gag or pol to prove HIV infection, (WHO Criteria). FDA and Red Cross rules you need three bands.

We conclude therefore that the HIV antibody proteins in the Western Blot antibody test are not specific. These findings are similar to those obtained by Kion and Hoffman.

REFERENCES