

# 'Silent' HIV Infection among Wives of Seropositive HIV Carriers in the Ethiopian Community in Israel

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We have previously described the phenomenon of 'silent HIV carriers', i.e. individuals with HIV specific immunity and a positive PCR for HIV-1, yet HIV seronegative. In the present study, we have looked for such 'silent' carriers among wives of individuals infected with HIV in Africa (Ethiopia). In addition to determining HIV serology, peripheral blood mononuclear cells (PBMC) were tested by PCR for HIV-1 and for their ability to generate specific antibodies to HIV upon polyclonal B-cell activation (P-BAT). Out of 16 wives so tested, three were HIV seropositive and among the 13 seronegatives, eight were P-BAT positive and five were both P-BAT and PCR positive. These findings suggest that (1) 'silent' HIV carriers may indeed be present in African populations; (2) interpretation of the 'silent' carrier phenomenon is not clear and will depend on clinical follow-up and the ability to culture virus from such carriers; and (3) results of HIV serology in this population and probably in other African populations should be viewed with caution.

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At present, testing for the presence of HIV in an individual is based on serology [1]. In low risk populations a negative result is interpreted as meaning 'not infected'. In risk groups, such as homosexuals, intravenous drug users, and sexual partners of seropositive individuals, a negative serology to HIV is taken with caution and repeated testing (3-6 months later) is recommended to rule out an infection that has not yet been detected by serum antibodies (the 'window period'), but will shortly cause seroconversion.

Originally the window period was estimated at a few weeks. As more data were gathered, the mean has risen to 3 months, and only 95% of those infected are expected to seroconvert within 6 months [2]. Only a few studies have attempted to assess the duration of time a person can carry the infection prior to seroconversion [2-8]. These studies identified the infection mainly by PCR, and thus were heavily criticized. However, there have been other studies on 'seronegative yet infected' individuals ('silent' carriers), belonging to the different risk groups for HIV (2, 4, 8), as well as children born to HIV seropositive mothers

[9], and sexual partners of seropositive HIV carriers [5, 7, 10, 11]. The infection was identified by PCR, virus isolation or in situ hybridization.

Because PCR is an extremely sensitive tool but has a high rate of possible false positives, we have used an additional test [12, 13]. This test is based on polyclonal activation of B cells in vitro (P-BAT) and generation of HIV specific antibodies in the culture supernatant fluids. The readout of the test utilizes regular ELISA and Western blot (WB) assays. When this test was initially used on a high risk population in a downtown hospital in the USA [13], 16% of the seronegative individuals were found to be P-BAT positive, i.e. had HIV specific B cells in their blood. More than half of these P-BAT positive, HIV seronegative individuals were found HIV PCR positive, by more than one laboratory.

In some African countries, the number of HIV seropositive people in the population is very high [14, 15]. It has been reported [7] that among couples discordant for HIV infection in central Africa, more than half the seronegative partners are actually infected according to PCR data. We

were therefore interested in identifying such 'silent' carriers among the sexual partners of HIV-infected persons belonging to the Ethiopian community who have recently arrived in Israel, and this is the subject of the present report.

## SUBJECTS AND METHODS

**Subjects.** Wives of 16 seropositive carriers were called in for testing, as they are at risk for HIV. Their ages ranged from 25 to 55 years, and they were all in good health.

**Processing of blood.** Twenty millilitres of blood in heparin were drawn. After removing the plasma, the PBMC were isolated on Ficoll, counted and aliquoted for the different assays.

**Polyclonal B-cell activation test.** Cells ( $4 \times 10^6$ ) were cultured in 24-well plates in 2 ml of RPMI supplemented with 10% FCS (heat inactivated), glutamine and antibiotics (penicillin and streptomycin). Pokeweed mitogen (PWM) was added (adjusted for each batch) at 1/400 of the stock (Gibco, Grand Island, NY, USA). The cells were incubated at 37°C in 6% CO<sub>2</sub> for 7 days. On day 8 an aliquot of the culture supernatant fluid (SF) was tested without dilution on an HIV ELISA (Diagnostic Pasteur, France). The ELISA plate was incubated overnight at 4°C prior to developing. The rest of the culture remained sterile in the 37°C incubator. On the following day, samples that tested positive by ELISA were tested undiluted for antibodies to HIV by WB (Diagnostic Pasteur). The results of the WB were analysed and were considered positive if there were bands for a least two major HIV proteins.

**PCR.** Cells ( $2 \times 10^6$ ) were washed twice in PBS and the pellet was sent to specialized PCR laboratories for HIV PCR. The primers used were SK 68-69. The probe used was SK 70. Great care was taken to avoid cross-contamination of the samples. The PCR was done using a previously published method [16] with slight modification.

**Serology for HIV.** All plasma samples collected were assayed for HIV-1 antibodies using the Diagnostic Pasteur HIV-1 ELISA and confirmed by WB.

## RESULTS

Of the 16 wives tested, three were found to be seropositive and were also P-BAT and PCR positive (Table I). Of the other 13, who repeatedly tested negative by serology, only two were also negative by P-BAT and PCR. Three of the seronegative wives had a positive PCR test but no antibodies to HIV were detected in their culture SF. All the other eight made HIV specific antibodies after their PBMC were stimulated polyclonally. In five of them PCR confirmed the presence of HIV in their PBMC.

TABLE I. HIV infection among wives of HIV-1 seropositive Ethiopians

Sample no.	Serology	P-BAT		PCR
		ELISA	Western blot	
1	-	-	ND	+
2	-	+low*	ND	+
3	-	+	+	?†
4	-	-	-	-
5	-	-	ND	+
6	-	-	ND	+
7	-	-	-	-
8	-	+	+	+
9	-	+	ND	-
10	-	+	+	-
11	-	+	+	+
12	+	+	ND‡	ND
13	+	+	ND‡	+
14	-	+low	+	+
15	+	+	ND‡	+
16	-	+	+	+

\* OD readings in the 'grey zone', i.e. 0.2-0.3.

† Weak or inconsistent reactivity.

‡ In seropositives there was no need to run a Western blot on the SF.

ND, not done.

## DISCUSSION

Two important findings have come out of this study: (1) a high proportion of HIV seronegative wives of HIV seropositive carriers have HIV specific B cells in their peripheral blood; (2) at least half of these women also have evidence of HIV presence in their peripheral blood by PCR. The presence of HIV specific B cells certainly reflects the immune response after exposure to HIV. Why no antibodies are found in the serum of these individuals and how long such a state continues is not clear. It may reflect a potent protective immunity, especially in the three cases where P-BAT was positive and PCR negative. It may also relate to an abortive or inefficient type of HIV infection. It may, of course, reflect a long incubation-pre-seroconversion period after HIV infection.

A PCR negative result can be interpreted as either 'no current infection' or 'low level of infection', i.e. the frequency of infected cells is below the sensitivity of the PCR. Considering the fact that most of the HIV can be found in the lymph nodes, etc. and not in the peripheral blood, a low level, or an inactive latent infection, could appear as PCR negative. The P-BAT method was originally developed in the SIV model of AIDS,

where the data accumulated over the past 4 years seem to indicate that all P-BAT positives are infected, though with a lower virus load (unpublished data).

We do not know the true condition of the three wives who tested positive only by PCR. We are currently attempting to procure another blood sample in order to repeat the P-BAT assay (as any contamination in the culture can result in a negative result) and the PCR, to try to rule out false positive data.

The finding of positive PCR in over 50% of the seronegative wives is in full accordance with the previous work published from Africa. The fact that similar studies in Europe and North America (based on PCR) [16] have reported only very few such cases may indicate that the epidemic, the mode of transmission and the course of infection are different in Africa from that observed in the West.

It is not known for how long these silent carriers have been in that state (their husbands have been seropositive for at least a year). We intend to try to follow them as part of a prospective study to see whether they do seroconvert and, if so, when. It will also be of interest to try to isolate and characterize the virus from the silent carriers and from their husbands, and to determine if the infection takes a different course in the seronegative versus the seropositive hosts. We will also monitor the levels of HIV specific antibodies made in vitro by the seronegative wives, since an increase in levels may be a strong predictor for seroconversion, as has been found in the SIV model (unpublished data).

PCR is still an expensive and cumbersome technique, requiring highly trained technical personnel, and its readout is, to date, unfortunately questionable. In this report we are offering an alternative that is relatively simple, reliable and much cheaper.

Although the full implications of this study await further data and larger and longer-term studies, it should at least caution us not to base our diagnosis solely on serology. This is especially true for Africa, of whose HIV we know so little, and doubly so among individuals at high risk for contracting HIV. To fully assess the prevalence of the silent carrier phenomenon in Africa, similar studies using the P-BAT assay and the PCR should be carried out in different countries in Africa. Until then, this phenomenon should certainly not be ignored.

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