

Review

PENDING PROBLEM OF "SILENT" HUMAN IMMUNODEFICIENCY VIRUS INFECTION

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Abstract - The problem of "silent" HIV infection is reviewed. Overall, the number of proven "silent" infection in several at-risk populations, including HIV exposed health-care workers, homosexuals, IV drug addicts and children born to HIV-infected mothers, has been very low. Contrary to these observations, we describe a very high prevalence of HIV specific immunity and positive HIV specific PCR signals in an Ethiopian immigrant population recently arrived in Israel. The interpretation of these findings is not entirely clear but we suggest that host immunity and probably different handling of the infection may account for the longer persistence of viral components in the body. Further studies are required to determine the amount and nature of these viral elements and, most importantly, whether they are still infectious.

Key words: "Silent" HIV infection, HIV specific immunity, HIV risk groups, Ethiopian immigrants

THE GENERAL PROBLEM

The human immunodeficiency virus (HIV) is recognized as the etiologic agent of the acquired immunodeficiency syndrome (AIDS). The diagnosis of HIV infection is based primarily on the presence of antibodies to the viral antigens in the serum and is used for screening of infected individuals and of blood for transfusion. Detection of viral antigens (p24) or of virus by culture and isolation and more recently detection of viral sequences by the polymerase chain reaction (PCR) have also been used for the diagnosis of HIV infection. Antibodies to HIV-1 usually develop within 3 to 12 weeks following HIV-1 infection (Gaines *et al.*, 1987). The duration of the so-called "window" period, i.e., seronegative yet infected with the virus, is somewhat variable and depends also on the route of infection.

The period of such latent seronegative HIV-1 infection may be 1-2 months in blood-borne infections (Fincher *et al.*, 1989; Vittecog *et al.*, 1986), while periods of more than 6 months have been reported in sexually transmitted HIV infection (Ranki *et al.*, 1987). The cause for these variations is not clear but may be due to differences in the virulence, infectious dose, mode of transmission, host response and frequency of exposure.

THE "SILENT" INFECTION

The existence of individuals with "silent infection" i.e. with no detectable HIV antibodies yet proven to be infected with HIV for a prolonged period has been reported since 1984 (Groopman *et al.*, 1985; Nagao *et al.*, 1991; Ranki *et al.*, 1987; Salahuddin *et*

al., 1984; Vaira *et al.*, 1990). The first report published by Salahuddin *et al.* (1984) described four individuals, three of whom were symptom free and one with lymphadenopathy, who were sexual partners of patients with AIDS or AIDS-related complex (ARC) and though found to be HIV seronegative. Virus was isolated from their peripheral blood cells. Groopman *et al.* (1985) reported on two individuals, one with AIDS and one with ARC who were HIV antibody negative but culture positive. Since then, more cases of the same kind have been reported in the United States or elsewhere (Aiuti *et al.*, 1993; Imagawa *et al.*, 1989; Nagao *et al.*, 1991; Ranki *et al.*, 1987; Vaira *et al.*, 1990; Wolinsky *et al.*, 1989). Ranki *et al.* (1987) reported that HIV antigen and/or low-titer antibodies to recombinant HIV proteins were seen in serum samples 6-14 months before overt seroconversion in 9 subjects who were initially ELISA negative and 7 subjects who had not seroconverted for 7 to 34 months since the first detection of HIV antigen or low-level antibodies against HIV core protein in serum samples. Imagawa *et al.* (1989) reported on isolation of HIV from blood samples obtained from 31 of 133 seronegative homosexual men who continued to be involved in high-risk sexual activity, and only 4 of the 31 men had seroconverted after 11 to 17 months of the initial isolation of the virus. The remaining 27 men did not seroconvert 28 to 36 months after HIV-1 isolation. Moreover, they demonstrated that HIV-1 provirus had been present in cryopreserved lymphocytes 23 to 35 months before seroconversion in 3 of the 4 seroconverted men by using PCR. Wolinsky *et al.* (1989) described HIV-1 DNA sequences detected in peripheral blood mononuclear cells at least 6 months and as long as 42 months (median, 18 months) before seroconversion, in 20 homosexual men. HIV-1 was also isolated from peripheral blood mononuclear cells 6 to 18 months before seroconversion in three of them. More recently, Aiuti *et al.* (1993) reported that 12 of 65 high-risk HIV-1-antibody-negative individuals were shown by PCR to be carriers of HIV-1 proviral sequences. Of these carriers, seven remained seronegative for a period of 10 to 14 months and two were seronegative for 16 and 18 months after the first positive PCR.

Furthermore, they isolated HIV-1 from cell-free plasma in two of 10 PCR-positive, antibody-negative subjects, who remained seronegative 10 months after the determination of plasma viremia. In another study (Jehuda-Cohen *et al.*, 1990) the "silent HIV infection" was detected first by a test that was designed to detect HIV specific B-cells, *in vitro*, following polyclonal B cell activation (PBAT). Of 165 seronegative individuals at risk for HIV, 30 made HIV specific antibodies *in vitro*. Approximately half of these were found to be PCR-positive too. A more recent study of seronegative wives of seropositive Ethiopian men, found 7/13 of these women to be PCR-positive for HIV (Jehuda-Cohen *et al.*, 1992).

THE RISKS

There is however a considerable number of other studies that suggest that such "silent" infections are very rare among high-risk individuals who remain seronegative for prolonged periods. Horsburgh *et al.* (1990) investigated 208 individuals at high risk for HIV-1 infection who remained seronegative, HIV-1 DNA was detected by PCR in only seven of the 208 samples. Of these seven PCR-positive, antibody-negative individuals, four were seronegative when retested 5 to 21 weeks later and two were considered false-positive PCR because follow-up PCRs were all HIV-1 negative. Pan *et al.* (1991) reported that only one out of 59 individuals seronegative men practising high- or medium-risk sexual behavior was identified as HIV positive by PCR. Other reports reached similar results and concluded that these silent states were extremely rare (Gupta *et al.*, 1992; Lifson *et al.*, 1990; Mariotti *et al.*, 1990; Shepparol *et al.*, 1991).

The existence of HIV-1 positive, seronegative individuals for a prolonged period provoked serious concern about the safety of blood transfusion by the screened blood. Indeed, some cases were reported that became infected with HIV-1 after receiving blood or organs from donors who were screened as seronegative at the time of donation (Cohen *et al.*,

1989; Conley *et al.*, 1992; Donahue *et al.*, 1990; Irani *et al.*, 1991; Kleiman *et al.*, 1988; Simonds *et al.*, 1992; Ward *et al.*, 1988).

The estimation of the risk of HIV-1 transmission by transfusion from a unit of blood collection was performed in the United States (Alter *et al.*, 1990; Busch *et al.*, 1990, 1991; Cumming *et al.*, 1989; Tessman, 1991). The estimation of the risk of transmission of HIV-1 from a unit of screened blood collection was as follows: 1:153,000 per unit transfused in over 17 million donated bloods screened for HIV by the American Red Cross (Cumming *et al.*, 1989), 1:68,000 per unit in 739,700 units collected in Los Angeles and Orange Counties (Kleiman *et al.*, 1988), 1:36,282 per unit in 36,282 units transfused for cardiac surgery in Baltimore and Houston (Busch *et al.*, 1991) and 1:61,171 per unit in 71,800 donations in San Francisco (Alter *et al.*, 1990). In view of these results, the chance of HIV-1 transmission from transfusion of blood or blood product which have been screened for antibodies to HIV-1 is indeed very small. Though there are still few who interpret the accumulated data differently and claim that based on the statistical limits of the above studies the risk could be as high as 1 in 10,695 units (Tessman, 1991). The attempt to use HIV-1 p24 antigen detection in screening of blood donors for improvement of the transfusion safety has not revealed any earlier detection (Alter *et al.*, 1990; Busch *et al.*, 1990).

In the study of children born to HIV positive mothers we have preliminary evidence that exposure and infection indeed occur and later clear without seroconversion (Bentwich and Bar-Yehuda, 1994). It may very well fit with some of the questionable previous observations that were difficult to reproduce and repeat (Imagawa *et al.*, 1989). It would also fit with the recent observations by Rowland-Jones *et al.* (1993) on HIV specific CTL in such children and with observations made by Clerici *et al.* (1993) on HIV specific cellular immunity in children born to HIV positive mothers. More recent studies by Shearer's and other groups including our own, have established the presence of HIV specific immunity in exposed yet seronegative individuals (Beretta *et*

al., 1994; Clerici *et al.*, 1992; Kelker *et al.*, 1992; Pinto *et al.*, 1994; Rowland-Jones *et al.*, 1995). In these studies mostly T-cell response to HIV envelope peptides was determined, but also HIV specific CTL response was used. Interestingly, health care workers who were exposed to the virus were also found to have such immunity up to 12 months post exposure (Pinto *et al.*, 1994). Altogether PCR determination in the various groups of immunity positive seronegative individuals studied, was mostly negative except in children born to HIV seropositive mothers, where 13% of the children had positive signals (Clerici *et al.*, 1993) and in only a few isolated cases where late seroconversion was observed several months after HIV specific immunity was detected (Clerici *et al.*, 1992).

THE ETHIOPIAN IMMIGRANTS

On this background, we have made some recent interesting observations in a population of Ethiopian new immigrants to Israel that seem to be somewhat different and may shed further light on this problem. Using both the PBAT assay, and the generation of IL-2 following exposure of PBMC to HIV envelope peptides, i.e. B and T-cell specific response assays respectively, we have found a surprisingly high prevalence of HIV specific immunity in non-exposed Ethiopians. Thus, 31 out of 60 of these individuals (51%), had such immune responses without any clear history of prior exposure to HIV (Bentwich and Bar-Yehuda, 1994). Furthermore, we were able to study more thoroughly 24 of them and repeat the study one year later. In the first determination, the T-cell response was positive in 70% while the B cell response (PBAT) was positive in only 37%. One year later, the B cell response became positive in 62% of the group and the T-cell response found in 87% of them, though they were still all HIV seronegative. At the same time, the number of individuals having a positive PCR signal for HIV sequences was only six, and in none of them was virus cultured. The interpretation of these findings is not quite clear and is now under intensive study. Due to the combined presence of B and T-cell HIV

specific response to several HIV antigens, it is difficult to ascribe such phenomena to anything else but to a true immune response to HIV antigens. Thus, it is quite plausible that the increasing signs of specific immunity to HIV, are evidence for previous exposure to HIV probably in Ethiopia, with continuous antigenic stimulation and drive. It may signify that HIV antigens - "defective", "digested", or true infectious viral particles are present in lymph nodes or macrophages. The small number of individuals in whom PCR was found positive may probably reflect the extremely low number of these viral particles. However, further work to rule out the possibility of some cross reactive antigens in these circumstances is also needed (Mutch *et al.*, 1994).

Why do we observe such findings in this population that have not been observed so far in any western populations? Is there something unique in the setting or background of the Ethiopian population that makes it different in the way it handles and disposes of HIV infection? The answer to these questions is still not known and remains to be fully investigated. However, we favor the idea that the high endemic infections load and mostly the helminthic infections present in Ethiopia and probably in other African countries plays a central role in making the African pattern of AIDS very different from the Western pattern of the epidemic (Bentwich *et al.*, 1995). We have suggested that activation of the immune system by those infections makes the host more susceptible to HIV infection and less capable to cope with it. By the same token, we suggest that the immune activated and non HIV infections' burdened individual is not able to dispose and clear the HIV infection in the same way as an individual who is not similarly infected. Thus, the background of Africa and probably of other developing countries, may also give rise to a higher prevalence of "silent" HIV infection than that observed in the developed western countries. The meaning of such "silent" infection remains to be characterized much better, especially as regards its potential for live infection, let alone the nature and amount of viral antigens. We certainly agree with the suggestions made earlier by Koup and Ho (1991) that such findings should be viewed

with extreme caution and that PCR and culture contamination should be rigorously excluded. However, the importance and implications of such a possibility cannot be overemphasized. Careful longitudinal studies and better definition of the circumstances and causes for "silent" infection are needed. More importantly, readiness to pursue and seek the resolution of this extremely controversial, dangerous and not so popular subject but yet of profound implication should be encouraged, especially in Africa and in the developing countries.

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