A NEW LOOK AT HIV TRANSMISSION FROM SEROPOSITIVE MOTHERS TO THEIR INFANTS: THE FACTS BEYOND SEROLOGY

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ABSTRACT. Once the curtain of maternal antibodies is removed (12–18 months) only a fraction of the infants are seropositive. Some babies from whom virus has been isolated or detected in their cells subsequently become seronegative. What does the negative serology of these children really tell us about exposure to HIV? It is suggested that seroconverting is only one of the ways to respond to an HIV exposure from an infected mother; it is not the only or the best way. Some form of tolerance to HIV, emerging after *in utero* exposure of the fetus, could theoretically lead to a seronegative state despite infection. Based on monkey studies with simian immunodeficiency virus (SIV), this tolerance could offer protection against pathological outcome of the infection. Seronegative yet infected/exposed children of HIV-positive mothers exist, though their number remains unknown. They might hold the key to a protective immunity to HIV.

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Based on serology, the vertical transmission rate of human immunodeficiency virus (HIV) from HIV-positive mothers to their offspring ranges from 13% to 55% depending on the region of the world (1–4). These numbers are based on prospective studies in which the infants were followed for at least 18 months, ensuring that all maternal antibodies have been lost (1). Seronegative children are considered noninfected and, based on the generally accepted axiom that "once infected always infected", it is assumed that they were never exposed to the virus.

Other methods for earlier detection of the HIV infection in these infants are currently being tested in many Western countries (5,6). These include polymerase chain reaction (PRC) for the detection of viral sequences in the cells' genome (7), virus isolation techniques (5), p24 antigen assays (8), the detection of anti-HIV IgA (which does not cross the placenta) (9), and *in vitro* antibody production of HIV-specific antibodies (10,11). Nevertheless, the golden standard for calculating predictive values and specificity for all these experimental methods has been the serological outcome of the infant at age 12–18 months (6).

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Whenever no correlation was found between HIV detecting tests and the final serological state of the infant, the tendency until recently was to trust serology and consider the other findings as false positive.

The purpose of this paper is to suggest a different interpretation of the data accumulated to date, both in HIV and in animal models of HIV, such as simian immunodeficiency virus (SIV), with regard to children born to HIV- or SIV-positive mothers.

THE IGNORED FACTS

SIV in a Sooty Mangabey Colony

The sooty mangabeys (SM) are an African species of monkey that are naturally infected with SIV although the infection does not lead to any pathological sequelae (12). Thus the SM tolerates the SIV. In the Yerkeys Primate Center (which cares for hundreds of SM), approximately 75% of the monkeys are seropositive for SIV. A more detailed analysis shows that 95% of the SM young ones (<2 years) are seronegative (once maternal antibodies are lost), while 95% of the adults are seropositive. Based only on serology it seemed that the majority of the SM become infected via sexual contact, as sexual maturity is reached at 2–2.5 years of age.

In-depth study of the immunity to SIV in the SM colony revealed that not only all the seropositive monkeys but also the seronegative ones had SIV-specific immunity that could be detected by: a) polyclonal B cell activation test (P-BAT) showing that peripheral blood mononuclear cells (PBMC) of the seronegative SM could produce SIV-specific antibodies following stimulation *in vitro* (13,14), b) T cell proliferation in the presence of SIV proteins (15), and c) the presence of CD8+ cells that could suppress SIV replication *in vitro* (16). These suppressor cells develop only following infection (17,18). In view of all these parameters of SIV-specific immunity, the question of possible latent infection with SIV was addressed. PCR revealed SIV-specific sequences in the PBMC of seronegative SM (19), and virus cultures yielded transient reverse transcriptase and p24 peaks (unpublished data), as can be seen in some latent forms of HIV as well (20).

It thus appears that, in SM, serology did not give the true picture of vertical transmission. Most, if not all, SM born to SIV-positive mothers were exposed to the virus (*in utero*?) which they harbor probably in some kind of latent (or different) form. Most of the monkeys remain seronegative until sexual maturation, which probably leads to exposure to other (SIV) sources. The protective immunity seems to remain throughout their lifetime.

Vertical Transmission of SIV in Rhesus Macaques

When rhesus macaque (RM) monkeys (from Asia) are experimentally infected with SIV from SM (in the proper infectious doses) they seroconvert, and after an asymptomatic period (1–2 years) they develop AIDS-like syndromes and eventually die (21). In this model of HIV infection the vertical transmission of SIV was also studied (22). In one of the experiments 15 female RM were infected at different stages of pregnancy. Of the 12 live births only 2 remained seropositive after the loss of maternal antibodies (~3 months). Yet, seven more were P-BAT positive, indicating the presence of SIV-specific immunity. Some of these seronegative infants were found to be PCR positive for SIV (23). One of these seronegative yet PCR positive P-BAT positive infants eventually seroconverted at the age of 15 months. Two of the seropositive babies died (<2 years) and the third had a low CD4 count. The seronegative ones are doing well with no signs of SIV pathology (24). Another reported interesting phenomenon is that while four mothers had SIV isolated from their milk only two of their infants ever seroconverted. Thus although two of the infants were exposed to SIV not only *in utero* or during delivery but also repeatedly through maternal milk, they remained seronegative (24).

Transmission of HIV from Mother to Child

There have been occasional reports of detection of HIV infection (or the presence of its sequences) in seronegative children born to HIV-positive mothers. A study of 100 children over 15 months of age found that 19 were seropositive and another 5 were antibody negative but presumed to be infected because of viral isolation or antigen detection (25). Other studies were performed specifically on seronegative children of HIV-positive mothers. In one study 9 of 11 had HIV-1 detected by *in situ* hybridization and one had p24 antigen (26).

Another study detected HIV sequences in 5 of 10 seronegative children (2–5 years old) by PCR (27). Rogers et al. (28) reported that of 22 seronegative children (6 months old) 5 of 6 tested were either culture or PCR positive. Viewing HIV serology as the golden standard, most of these reports were not given enough attention.

The European Collaborative Study, which has been following over 600 infants born to HIV-positive mothers, recently reported that a few seronegative children persistently gave PCR positive results, and virus could occasionally be isolated from them (6). These children are already 4–5 years old, seronegative, healthy and still virus positive to date. In a short communication, Baur et al. (29) reported on continuous clearance of HIV in a vertically infected infant. High levels of virus were isolated from her PBMC at birth, and lower levels at 12 months of age. By 18 months, no virus was isolated and PCR could not detect any HIV sequences. Thus a once-infected child is presently seronegative and the state of her infection remains unclear (silent? latent? gone?)

HIV IN CHILDREN BORN TO SEROPOSITIVE MOTHERS IN ISRAEL

The prevalence of HIV infection in Israel is still very low. It more than doubled following the immigration of Ethiopian Jews (30). This immigration presents a unique situation — namely an African HIV infection transferred to a Western environment. Using serology, several tens of HIV-seropositive women at childbearing ages were identified. The HIV state of their children was studied using methods other than serology. The P-BAT assay was used in an attempt to detect HIV-specific B cells (11,31), and the proliferation in the presence of HIV-specific peptides indicated the presence of HIV-specific T cells. HIV-specific immunity was detected both at the B and T cell level in some of the seronegative children (Jehuda-Cohen et al., submitted for publication). In some of these children with a "silent immunity", HIV-specific sequences were detected by PCR.

DISCUSSION

The first indications that serology might not be telling us the whole story of HIV infection were apparent following the development of methods for detecting viral sequences by PCR, *in situ* hybridization, and improvements in the HIV virus isolation technique. Yet discrepancies between serology and PCR, for example, led to a call for caution in relying on PCR data alone, owing to the many false positive results it gives. The fact that virus isolation from these seronegative children was not always successful, coupled with the generally healthy state of seronegative yet infected children, contributed to the medical community ignoring the phenomenon of exposed/infected yet seronegative children. There are at least two findings that should lead us to reconsider our axioms and the current golden standard (i.e., serology): a) the reports of repeated virus isolation from a few of these children (1,29); and b) the development of tests for detecting HIV-specific immunity, independent of serology (13,14,31–33). Both these findings seem to suggest the possibility of a seronegative HIV infection). The studies in HIV-specific immunity and the reports of clearing HIV from the PBMC might indicate even more than that: under a certain type of immune response the HIV can be cleared to the point of no detection by our current methods (29). Thus some individuals may have had only a transient HIV infection.

How can all the data be put together in harmony and logic? Let us assume, for the sake of this exercise, that all data, even if conflicting with serology, are true and real. The simple deductions that can then be made are that:

- Not all vertically transmitted HIV infections lead to seroconversion, i.e., the active and chronic secretion of anti-HIV antibodies.
- Some HIV infections in these maternally infected infants can be transient, the virus being cleared or removed to a point of no detection. This past infection/exposure to HIV has been either documented and measured (29) or only hinted at by the presence of HIV- or SIV-specific B cells and T cells (13–16, Jehuda-Cohen et al., submitted for publication).
- Based on the few reports in the literature and the information gathered from the monkey model of AIDS, the prospects of those children who did not seroconvert, in spite of their exposure/infection, are much better. For all we know, and in view of the AIDS model of SIV in SM, they might never develop HIV-related symptoms.

There are many factors that could affect the outcome of a vertically transmitted viral infection — the time of maternal infection, the state of her immune system, the viral load, and the type and level of specific immunity formed by the mother against that virus. Also important are the time of infection of the fetus/infant, and the other constraints on his/her immune system during, and following, the infection.

One could build a hypothetical model in which, under certain circumstances, the exposure of the fetus to HIV could lead to a form of tolerance to it. This tolerance would lead to suppression of the response against the virus, leading to a seronegative yet infected/exposed state. A nonresponsive immune system might offer the host an advantage on at least three levels: preserving the CD4+ cells, slowing viral replication, and avoiding autoimmune antibodies. The depletion of CD4+ cells is attributed mainly to the immune response against the HIV and not to the direct cytopathic effect of the virus itself (34). A tolerant system will avoid not only the production of anti-HIV antibodies but also the cascade of autoantibodies that follows a seropositive HIV infection (35). Since HIV replicates only in activated cells, under these tolerating conditions the virus might: a) remain in low numbers, undetected or rarely detected by us, providing the constant presence of low levels of antigen to keep the state of tolerance (36); b) it could settle in defined sequestered areas and not stimulate the immune system; or c) it could be cleared out completely by other mechanisms.

Another possible explanation of these findings is that except for an immune response that leads to a massive antibody response, the immune system has other courses of action. One such course would be to enhance the cellular immunity while keeping the humoral arm suppressed. A similar approach has been suggested by Shearer and coworkers (37). They proposed that the balance between a TH1 (T cell+/antibody-) and a TH2 (T cell-/antibody+) immunity is critical for the outcome of an HIV infection. While the first offers protective immunity, a shift to the second (namely seroconversion) leads to a lack of protection (37). If so, then seroconversion is not a sign of exposure/infection but of a deterioration to nonprotective (or even destructive) immunity. Some of the destructive roles that HIV-induced antibodies have been reported to exhibit (34) only lend more weight to the argument that the system might do better without seroconversion.

It is noteworthy that several recent works have suggested that some immunosuppressive therapy might be applied early to infected individuals in order to avoid initiation and development of autoimmune disorders (35,38). Studies in chimpanzees infected with HIV showed that stimulation of the immune system (e.g., through immunization) enhanced the HIV expression *in vivo* (39). All these data should lead to reevaluation of vaccine design and immunotherapy in AIDS.

Whatever the mechanism of remaining seronegative following exposure to HIV, an important mes-

sage can be learned from these children. They encountered the virus and had a better outcome than most of the HIV-infected population that seroconverted. They might hold the key to a protective immunity to HIV, an immunity that does not lead to self-destruction, and an immunity that might be able to lead to clearance of the virus or to coexistence with it. Such an immunity could be the model that we need to strive for in vaccine development. The immunity beyond serology deserves further exploration.

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