Seronegativity and HIV infection

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SUMMARY An African girl born to an HIV seropositive mother was seropositive during the neonatal period, became seronegative, and was again found to be seropositive at 18 and 20 months of age. We suggest that seronegative children born to seropositive mothers should be followed up for months or even years before HIV infection can be ruled out.

The diagnosis of human immunodeficiency virus (HIV) infection is usually confirmed by the detection of antibodies against HIV in serum. In children less than 15 months old born to HIV seropositive mothers, the presence of specific clinical signs and immunological abnormalities are needed in addition to the presence of HIV antibodies, to exclude a passive mother to child transfer of antibodies during pregnancy.¹ Among the serological tests, the enzyme linked immunosorbent assay (ELISA) is the commonest test used throughout the world because of its satisfactory sensitivity and specificity, its feasibility, and its low price. Nevertheless, HIV infected adults and children with negative ELISAs, and sometimes with negative confirmation tests such as western blot and indirect immunofluorescence as well, have been reported.²⁻⁵

We describe a girl born to an HIV seropositive mother who was seropositive during the neonatal period, became seronegative, and was found to be seropositive again at 18 and 20 months of age.

Methods

HIV antibodies were detected by a commercial

ELISA (Vironostika, Organon Teknika) and by a commercial indirect immunofluorescent assay (IFA, JLC Allaman). In addition, all serum samples were tested by a western blot technique to search for IgG and IgM antibodies to HIV (Biotech Du Pont de Nemours). This latter test was performed after absorption of rheumatoid factor (RF absorbent, Behring). HIV antigen was tested using a commercial ELISA (Innogenetics), and serum immunoglobulins were quantitated by radial immunodiffusion (Nor-Partigen, Behring).

Case report

This girl has been followed up clinically (at least every month) and serologically since birth because her mother presented with persistent generalised lymphadenopathy and was diagnosed as HIV seropositive by ELISA and western blot (positive for all bands) during pregnancy.

The infant was born at full term, but was small for gestational age (birth weight 2300 g). She was 24 months old at the time of writing and had only had common minor infections such as acute diarrhoea and upper respiratory tract infections. Although she

Table Sequential detection of HIV antibodies and HIV antigen and quantitation of serum immunoglobulins in a Rwandese child from birth to 20 months of age

Age	ELISA	Indirect immunofluorescent assay	IgG (western blot)	IgM (western blot)	HIV antigen	IgG (g/l)	IgA (g/l)	IgM (g/l)
15 days	Positive	Positive	Positive	Negative	Negative	10.2	0.42	1.38
7 months	Negative	Negative	Weak positive	Negative	Negative	16.9	1.01	2.99
12 months	Negative	Negative	Negative (p24 only)	Negative	Negative	28.1	0.72	2.71
18 months	Positive	Positive	Positive	Negative	Negative	27.3	0.93	2.70
20 months	Positive	Positive	Positive	Negative	Negative	33.2	13.50	2.90

All values except IgG and IgA at 15 days old are above the upper limit of normal.

was growing steadily, her weight was only 8200 g and her height 78 cm at 24 months of age. Her clinical examination remained completely normal; she never showed any signs of persistent generalised lymphadenopathy, parotitis, or chronic otitis media. Her psychomotor development followed normal milestones. The chest radiograph was normal at 12 and 24 months of age. She was breastfed for 17 months. Except for routine immunisation, she never received any injections.

Five serum samples were available for immunological and serological analysis (table). The serum sample from 15 days after birth was positive for HIV antibodies by ELISA, indirect immunofluorescence and western blot (table). Two subsequent samples at 7 and 12 months were negative by ELISA and indirect immunofluorescence, and showed a reduction in reactivity on western blot (figure). The two

latter samples (at 18 and 20 months) were reactive again by ELISA, indirect immunofluorescence, and western blot. At 15 days of life, IgM subclass hyperimmunoglobulinaemia was noted. After the 7 month sample the hyperimmunoglobulinaemia was also present for IgG and IgA.

Discussion

The progressive loss of antibodies to HIV in a child in good general health is usually considered to be a good prognostic factor—that is, it indicates that the infant had acquired antibodies passively from his mother and was not infected perinatally.¹ The case presented here, as well as three others briefly described from Italy,⁶ show that some infected children after being seropositive in the neonatal period may present for rather a long period without detectable antibodies in their serum. The occurrence of seronegative but virus positive subjects has been acknowledged among both adults and children.²⁻⁵

A child with epidemiological, clinical, and immunological evidence of AIDS, but without HIV antibodies from 8 months until her death at 14 months of age, has been described.⁵ Nevertheless serum samples from the same child, when evaluated with more sensitive reagents, were found to be strongly positive for IgG antibodies to HIV.⁷ Borkowsky *et al*³ reported nine of 85 HIV infected children, six of whom were tested only once, who lacked HIV antibodies when measured by a commercial ELISA. Only four of these nine cases negative on ELISA had detectable antibodies on western blot analysis, or on ELISA with recombinant HIV antigens or both. All nine children had detectable levels of HIV antigen.³ In the case reported here HIV antigen was never detected. This is not surprising because the antigen is only sporadically detected in the early stages of HIV infection in infants, and because of the comparative rarity of HIV antigen recovery by ELISA in HIV infected patients from Africa.⁸ In some series^{2,4} (but not all,^{3,5} including our case), negative HIV antibodies seemed to be associated with hypogammaglobulinaemia. In contrast, in our case, severe hypergammaglobulinaemia was noticed when the child was 7 months of age, that is when she was seronegative. Thus hypergammaglobulinaemia in children less than 15 months old born to seropositive women could be an early immunological sign of HIV infection.

It is most likely that this child was infected transplacentally or during delivery, but she may have acquired the HIV infection postnatally. Indeed, HIV has been isolated from human milk,⁹ and postnatal transmission through breast milk has been strongly suggested in four recent cases.¹⁰⁻¹² Postnatal

Figure HIV IgG western blot pattern in sequential serum samples from a child born to a mother infected with HIV. Lane A=serum sample collected at 15 days of life; lane B=serum sample collected at 7 months; lane C=serum sample collected at 12 months; lane D=serum sample collected at 18 months; and lane E=serum sample collected at 20 months. Molecular weights expressed as kilodaltons.

infection could explain the delayed appearance of HIV antibodies in our patient.

Large prospective studies are needed to show what proportion of children born to HIV infected mothers become temporarily seronegative. Seronegative children born to seropositive mothers should be followed up for months or years before HIV infection can be completely ruled out, even if their clinical examination and psychomotor development remains normal. In addition, serum samples from these seronegative children should be tested by more sensitive assays than commercial ELISA, indirect immunofluorescence, and western blot.

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