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SPECIAL REPORT

Three Cases of HIV-1 Seroreversion

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Three patients were enrolled, two as hemophiliacs, and one with acute EBV infection. Serial serum samples of each patient were tested with at least 3 different HIV antibody EIA tests, an immunofluorescent test and two western blots (WB). In the third case, PCR and reverse transcriptase enzyme activity measurement were also done. One of the regularly checked serum samples of hemophiliac patients was reactive with different HIV screening and confirmatory assays. Their next blood samples, two weeks and one month later, respectively, were negative with the same tests. In Case 3. two and a half years after the first examination, the EIA tests results changed to negative, but the WB was still indeterminate. In the case of the two hemophiliac patients, the patients may have been exposed to HIV containing blood products (before 1985), but were not infected. Regular treatment with factor VIII concentrate, in which HIV antigens may be present, can boost the immune response and results in transient seropositivity. In the case of the EBV infected patient, the transient HIV seropositivity may be the consequence of EBV induced proliferation of anti-HIV-antibody producing B cell clones. During our ten year HIV confirmatory practice we tested more than 40000 samples, from which transient seropositivity were observed only in the three cases summarized in this paper. (Pathology Oncology Research Vol 3, No 3, 224–228, 1997)

Key words: HIV, transient seropositivity

Introduction

Reversal of HIV-seropositivity due to disappearance of passively acquired maternal antibodies is a common event in non-infected infants born to HIV-infected mothers.[1] Recently it was demonstrated that HIV-1 infection may be cleared concomitantly with the loss of specific antibodies in a part of the infected newborns, too.[2-5] There are, however, only scarce data on transient HIV-1 seropositivity in adults and even these data are controversial. Fardzagedan et al[6] reported on loss of HIV-1 antibodies in four asymptomatic homosexual men from the Multicenter AIDS Cohort Study. Similar findings were observed in hemophiliacs[7] and in the wife of a patient with hemophilia.[8] By contrast, Roy et al[9] failed to find any case of true seroreversion of HIV-1 antibodies while reviewing a large cohort of 4911 seroreactive men of the US Army HIV Data System.

In the present paper we report on three cases of transient seropositivity that occurred in a ten year practice of a national HIV confirmatory laboratory in Hungary. Two of the subjects were hemophiliacs while the third case of transient seropositivity occurred in a young girl without any AIDS risk factor after an EBV infection. At least one serum sample of these subjects fulfilled at least one of the various criteria for confirmed HIV seropositivity.[10] In all three cases, however, HIV antibodies disappeared from the blood of the patients. It seems therefore that other causes than the HIV infection were responsible for the development of the HIV-1 antibodies in these subjects.

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Materials and Methods

Patients

Case 1: 48 year old, male patient with hemophilia A. He has been regularly (yearly) controlled for HIV antibodies since 1985. He was given cryoprecipitate last in December, 1992 before a dental extraction. In February 1993 he got an infection with high temperature and icterus. His liver function tests were positive. At this time he had positive CMV IgG antibody but negative IgA and IgM anti-CMV, IgG and IgM anti-EBV tests. He was HBsAg negative, anti-HBs positive, anti-HBc positive, anti-HBcIgM negative and tested positive for anti-HCV antibodies. His T cell subset (CD3+, CD4+, CD8+) percentage and counts were within the normal limits.

Case 2: 50 year old, male patient with severe hemophilia A. Similarly to case 1, he also was yearly controlled for HIV antibodies since 1985.. He was regularly treated, about once a week, with cryoprecipitate.

Case 3: 16 year old female, with clinical symptoms of infectious mononucleosis which began 2 weeks before the detection of HIV antibodies in December, 1992. No HIV risk factors were found at her: she denied any sexual contact or i.v. drug abuse and did not ever receive blood transfusions or treatment with blood products.

Measurement of HIV antibodies

We tested each serum sample with at least 5 of the following HIV antibody EIA tests: Vironostika a-HTLV III (Organon Teknika BV, Boxtel, Holland.)., Vironostika HIV Uniform II.(Organon Teknika BV, Boxtel, Holland.) Abbott HIV-1/2 third generation test (Abbott Diagnostics Division, Delkenheim, Germany), Abbott Envacor HIV-1 EIA (Abbott Diagnostics Division, Delkenheim, Germany), Detect HIV (BioChem ImmunoSystems Inc. Montreal, Canada), Ortho HIV-1/-2 (Ortho Diagnostic Systems, Neckargemünd, Germany), and a particle agglutination test: Serodia HIV-1(Fujirebio Inc. Tokyo, Japan)

We also used an immunofluorescent test: Fluorognost HIV-1 IFA (Waldheim Pharmazeutika GmbH, Wien, Austria), as well as two western blots: New Lav Blot I (Sanofi Diagnostics Pasteur, Marnes la Coquette, France), and HIV Blot 2,2 (Genelabs Diagnostics Pte Ltd, Singapore). The first sample of each patient was examined for the presence of HIV-1 p24 antigen too (Abbott Diagnostics Division, Delkenheim, Germany) . In the third case Amplicor HIV-1 DNA PCR (Roche Diagnostic Systems, Inc., Branchburg, NJ, USA) measurements were also performed. In case 3, we cultured the peripheral mononuclear blood cells of the patient in the presence of PHA and IL-2 using the method of Gartner and Popovic.[11] HIV-1 production was checked by the reverse transcriptase assay as previously described.[12]

Results

Case 1

Table 1. shows the findings obtained in a 48 year old patient with hemophilia A. His serum sample was first checked for the presence of HIV antibodies in 1985 by the Vironostika a-HTLV III. assay and tested negative. He was found seronegative again at the regular yearly checks. At the serological control performed in February, 1993, however, he was found to be HIV-seropositive by the Abbott 3rd generation assay. The same sample was examined with three other EIA test, one particle agglutination assay (Serodia) and with a Western blot test (HIV Blot 2.2). Positive results were obtained with another EIA (Vironostika a-HTLV III) and the Serodia assay. No core antibodies could be detected by the Envacor test while envelope antibodies were near to the cut-off. Western Blot testing found two bands corresponding to gp120 and gp160 antibodies.

According to the WHO criteria this result should be considered to be positive while according to the American Red Cross and US FDA criteria the Western blot result is indeterminate. No p24 antigen could be detected by the Abbott antigen assay.

We repeated the same examinations in the next blood sample of the same patient obtained two weeks later. All results were negative, and negative results were obtained with two EIA and one Western blot kit in a sample obtained two years later, as well.

***Table 1.* Hemophilia A patient (Case 1). Anti HIV-1/2 EIA test results (OD/cut off)**

	Abbott 3.gen	*Ortho*	*a-HTLV III*	*Serodia*	*Core/env*	*WB*	*p24Ag*
02.17.93	+ (7,6)	–	+ (1,16)	+	-/(1,02)	gp120 gp160	–
02.03.93	–	–	–	–	–	–	
06.06.95	–	–	ND	ND	ND	–	

ND – no data

Table 2. **Hemophilia A patient (Case 2). Anti HIV-1/2 test results (OD/cut off)**

	Abbott3.gen	*Ortho*	*a-HTLV III*	*Serodia*	*Detect HIV*	*WB*	*p24Ag*
04.05.92	ND	ND	ND	–	–	–	–
04.13.93	+ (7,4)	0,92	ND	+	+ (2,01)	p25, p34 gp160	
05.14.93.	–	ND	–	–	ND	p25	
04.21.95.	–	–	ND	–	–	–	

ND - no data

Case 2

The findings obtained in serial serum samples of an other patient with hemophilia A are summarized in *Table 2*. The patient was regularly controlled for the presence of HIV antibodies and always tested negative, last in May 1992. One year later a strong seropositivity was observed in his serum sample with the Abbott 3rd generation assay. One other EIA test (Detect HIV) and the Serodia agglutination assay also gave strongly positive results and O.D. values near the cut-off was obtained in the Ortho EIA test. Three bands (p25, p34 and gp160) were found with the Western blot (Pasteur New Lav Blot I) which should be considered as positive according to the American Red Cross and US FDA criteria and indeterminate according to the criteria of WHO. His next blood sample taken one month later was negative with three EIA and the Serodia test, but in the Western Blot test a weak p25 band could be seen. 23 months later his serum was found to be negative with three different EIA and the particle agglutination assay and no bands were seen in the Western blot.

Case 3

The results obtained in sequential serum samples of case 3 are shown in *Table 3*. The first sample from the patient was found to be reactive and sent for confirmation in December 1992. The sample tested strongly positive with the Abbott 3rd generation and the Vironostika EIA assays while in two other EIA kits and in the Abbott Envacor assay negative results were obtained. In the Western blot assay several bands (strong p18, p34, p40, p52 and p55 as well as weak gp120 and gp160) were found (*Table 4.*). We asked for a new sample which was taken 6 weeks after the first one. The three a-HIV EIA tests were positive in this sample, too. Results similar to the first sample were obtained except that Detect HIV

Table 3. **HIV antibody ELISA results (Case 3)**

	Vironostika a-HTLV III	*Abbott 3.gen*	*Abbott Encavor*	*Detect HIV*	*Ortho*
12.22.92	+	+ (6,7)	–/–	–	–
02.03.93	+	+ (1,6)	–/–	+ (1,14)	–
03.11.93	ND	–	ND	–	–
04.29.93	ND	–	ND	–	–

ND - no data

Table 4. **Western blot bands (New Lav Blot I) (Case 3)**

	p18	*p25*	*p34*	*p40*	*gp41*	*p52*	*p55*	*p68*	*gp120*	*gp160*
12.22.92	+	–	+	+	–	+	+	–	((+))	((+))
02.03.92	+	(+)	+	+	–	+	+	–	+	+
03.11.93	+	–	+	+	–	+	+	–	(+)	(+)
04.29.93	+	–	+	+	–	+	(+)	–	–	–
10.19.93	+	–	+	+	–	+	+	–	–	–
03.30.95	+	–	(+)	(+)	–	(+)	+	–	–	–

(+) – weak band; ((+)) – very weak band

Table 5. **EBV serology IFT (Case 3)**

	EBVCAIgM	*EBVCAIgA*	*EBVCAIgG*	*EBVearly*
12.22.92	+++	++++	pos 1:640	–
02.03.93	+	++++	ND	++
03.11.93	–	++++	ND	++
04.29.93	–	+	pos 1:640	+

EBVAC – Epstein-Barr virus capsid antigen; EBVearly – Epstein-Barr virus early antigen

assay also became positive and in the Western blot new bands including weak p 25 and strong gp120 and gp160 bands could be detected too. This Western blot pattern should be considered as positive according to the WHO, American Red Cross and US FDA criteria. A new sample taken one month later tested negative in three EIA assays including the two previously reactive (Abbott 3rd generation and Detect HIV) tests but the WB was still positive. We obtained similar results in her fourth sample taken six weeks later but gp120 and gp160 bands disappeared and therefore WB became indeterminate. Two and a half years after the occurrence of transient seropositivity her serum sample is still HIV Western Blot indeterminate.

In the first or second sample of the patients other supplementary assays (indirect immunofluorescent assay, p24 antigen test, Amplicor PCR DNA measurement, measurement of the reverse transcriptase activity of the cultivated lymphocytes of the patient) and the other retrovirus antibody assays (a-HTLV I-II) were performed and found to be negative. The EBV infectious mononucleosis diagnosis was serologically proven by detection of EBV CA IgM, IgA, IgG, and EBV EA (*Table 5.*).

Discussion

Three cases of transient HIV-1 seropositivity are demonstrated in the present paper. In all three cases at least one of the serum samples could have been considered as confirmed HIV-1 seropositive: they tested positive in at least two EIA tests and fulfilled the criteria of a positive Western blot assay as determined by the WHO, American Red Cross and/or the USA FDA criteria for the licensed WB kits.[11] Western blot of three sequential serum samples taken in a 10 week interval of case 3 could be considered positive according to the WHO and FDA licensed kit criteria. After that the WB assay became indeterminate and remained indeterminate till the last examination performed two and a half year after observation of the first seropositivity. Case 3 therefore meets the definition of Roy et al[9] for a true seroreverter: "an individual having two reactive samples confirmed by retesting...followed by a non-reactive sample confirmed in the same manner". By contrast, transient seropositivity lasted for a short time, for two and four weeks, respectively, in cases 1 and 2. Serum samples tested later on from these subjects tested negative or indeterminate. Of course, attributional or transcriptional errors found to be the major causes of apparent seroreversion by Roy et al[9] and Holmberg et al[13] can not be definitely excluded in these two cases. Our sample registration and testing protocol, however, renders this possibility highly improbable. In addition, in the second serum sample obtained from case 2 taken four weeks after his first seropositive sample a p24 band was present on the WB which could be detected in the WB of the first sample as well.

Although these three cases apparently meet the criteria of transient seropositivity, it seems that the development of HIV antibodies in high amounts was due to other causes than the HIV infection. In the first two cases seroreversion took place very quickly and in control examinations performed 2 years later, both subjects tested definitely negative. Both subjects were patients with hemophilia A regularly treated with cryoprecipitate. Concentrate treatment was administrated to them 2 months and one week before the detection of transient seropositivity.

It seems reasonable to suppose that these patients were exposed to HIV-containing blood products but were not infected before 1985 when untreated factor VIII and IX concentrates were still in usage in Hungary. Previously we have found 28/85 hemophiliacs treated with virus contaminated concentrate to remain HIV-seronegative.[14] Some minute amounts of HIV antigens which may be present even in virus-inactivated and safe concentrates can boost the immune response and result in a transient seropositivity. Interestingly enough Tennenbaum et al[7] also observed transient HIV seropositivity in six patients with hemophilia. In case 1, however, an infection with an non-identified infectious agent could also have been responsible for the transient HIV.

As for case 3, development of transient seropositivity was associated in time with a serologically confirmed EBV infection. It is well known that EBV infects B lymphocytes and this infection can lead to polyclonal B cell activation. Interestingly enough, to our best knowledge, false positive HIV serological tests were not reported to occur after EBV infection. Most probably, however, transient seropositivity observed by us was the consequence of EBV induced proliferation of anti-HIV antibody producing B cell clones.

Our laboratory has been engaged in the confirmation of HIV screening tests since 1986. During this 10 year period, 41511 ELISA reactive samples were tested, out of which 327 samples were confirmed as true seropositive, 3647 samples were found to be false positive while in 299 samples undeterminate results were obtained. Transient confirmed seropositivity was observed only in the three

cases summarized in the present paper, indicating that it is a very rare event which, however, may occur in exceptional circumstances. Such an event may cause real problems and anxiety both in the laboratory and to the clinician who takes care for the patient.

References

1. *Lallemant M, Lapointe N, et al:* Perinatal HIV Transmission. In AIDS in the World Edited by Mann J., Tarantola D.J.M., Netter T.W. Harvard University Press 629-645, 1992.
2. *Bryson YJ:* HIV clearence in infants - a continuing saga. AIDS 9:1373-1375, 1995.
3. *Roques PA, Gras G, Parnet-Mathieu F et al:* Clearance of HIV infection in 12 perinatally infected children: clinical, virological and immunological data. AIDS 9:F19-F26, 1995.
4. *Simpson BJ, Andiman WA:* Difficulties in assigning HIV-1 infection and seroreversion status in a cohort of HIV-exposed children using serologic criteria established by the Centers for Disease Control and Prevention. Pediatrics 93:840-842, 1994.
5. *Chantry CJ, Cooper ER, Pelton SI, et al.:* Seroreversion in human immunodeficiency virus-exposed but uninfected infants. Pediatr Infect Dis J 14:382-387, 1995.
6. *Farzadegan H, Polis MA, Wolinsky SM, et al.:* Loss of human immunodeficiency virus Type 1 (HIV-1) antibodies with evidence of viral infection in asymptomatic homosexual men. Ann Intern Med 108:785-790, 1988.
7. *Tenenbaum SA, Leissinger CA, Garry RF:* Absence of seroconversion of HIV-1 antibody in seroreactive Individuals. JAMA 270:2178-2179, 1993.
8. *Burger H, Weiser B, Robinson WS, et al:* Transient antibody to lymphadenopathy-associated virus/Human T-Lymphotropic Virus Type III and T-lymphocyte abnormalities in the wife of a man who developed the acquired immunodeficiency syndrome. Ann Intern Med 103:545-547, 1985.
9. *Roy MJ, Damato JJ, Burke DS:* Absence of true seroreversion of HIV-1 antibody in seroreactive individuals. JAMA 270:2178-2179, 1993.
10. Morb Mortal Weekly Report 38,1989.
11. *Gartner S, Popovic M:* Technics in HIV Research. Ed. by Aldovini A, Walker BD, Stockton Press, 1990.
12. *Somogyi PA, Gyuris Á, Földes I:* A solid phase reverse transcriptase-micro assay for the detection of Human Immunodefficiency Virus and other Retroviruses in cell culture supernatants. J Virol Methods 27:269-276, 1990.
13. *Holmberg S.D., Horsburgh C.R., Byers R.H.* Errors in reporting seropositivity for infection with human immunodeficiency virus (HIV). Ann Intern Med 107:679-680, 1988.
14. *Újhelyi E., Králl G., Füst G.,et al.* Prevalence of HIV-antibodies in patients with hemophilia In Hungary. Haematologia. 21:83-89, 1988.