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Original works

Identification of anti-HIV-1 antibodies in bloodstains of various ages

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Summary. The persistence of anti-HIV-1 antibodies in bloodstains has been studied by ELISA and Western Blot (WB) analysis. The immunoblot technique was found to be specific and more sensitive and the antibodies could be detected in 0.7 mg of bloodstains for up to 6 months. The authors emphasize the importance of such an investigation on non-genetic markers in individual diagnosis for forensic purposes.

Key words: Bloodstains – Non-genetic markers – HIV-1 antibodies

Zusammenfassung. Die Haltbarkeit von Anti-HIV-1-Antikörpern in Blutspuren wurde vergleichend mit ELISA und mit Westernblot untersucht. Die Immunoblottechnik wies neben ihrer höheren Spezifität auch eine höhere Empfindlichkeit auf und die Antikörper konnten bis zu einer Lagerungszeit von 6 Monaten in 0,7 mg einer getrockneten Blutspur nachgewiesen werden. Die Autoren betonen die Bedeutung einer solchen Untersuchung auf nicht genetische Marker bei individuellen Untersuchungen mit forensischen Zielsetzungen.

Schlüsselwörter: Blutspuren – Nicht-genetische Marker – HIV-1-Antikörper

Introduction

The detection of non-genetic markers in biological stains can be useful in individual diagnosis for forensic purposes. An example of this is the detection of specific antibodies, especially those against infections, due to the stability of immunoglobulins in dried bloodstains [1]. This advantage has been used for many years in epidemiological studies to transmit whole blood from remote areas by collecting on filter paper discs and recently by this means HIV-antibodies were found in eluates for at least 3 months [2].

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In a forensic approach to bloodstain analysis, the possibility to detect anti-HIV in dried stains was reported by Tappero et al. [3] who investigated blood from cadavers stored at room temperature. Using the immunoenzymatic test E.I.A. specific positive results were found for 40 days and more. Also Stiebler et al. [4] tested for anti-HIV in blood stains on cotton obtained from living persons and from cadavers with an enzyme linked immunosorbent assay and immunoblotting. False positives were recorded during the investigation period (7–30 days) with ELISA but not with Western Blot (W.B.) particularly in samples stored at +20°C. Excluding bacterial contamination and the influence of the cotton, this non-specificity was possibly related to the use of whole blood rather than serum. Following the criteria of Biotech/Du Pont HIV-1 Western Blot Kit for the interpretation of results HIV-antibodies could be detected up to 4 months, but a disappearance of some bands was observed.

In this paper the results of a study on persistence of anti-HIV antibodies in bloodstains assayed with screening and confirmatory tests (ELISA and WB respectively) is reported. The influence of different substrates on the methods of detection was also investigated and a case is briefly illustrated.

Materials and methods

Specimens. Blood samples were obtained by venipuncture (using EDTA as anticoagulant) from 6 HIV-1 seronegative and from 6 HIV-1 seropositive subjects.

Blood drops (about 50 µl) were dried on a microplate at room temperature without any sterile conditions. After a period of 0, 7, 14, 21, 28, 40, 60, 90, 180 days, the dried samples approx. 10.4 mg were reconstituted with 150 µl of phosphate buffer solution (PBS) for 24 h at room temperature.

In addition blood from one subject from each group was dried on wool, cotton, silk and polyester. After a few days the stains were cut into pieces and extracted for 24 h in PBS for analysis.

Serological test. Ten µl of extract were analyzed for antibodies to HIV-1 by a commercial ELISA (Wellcome UK) according to the manufacturer's instructions.

All samples were further tested with a commercial Western Blotting assay (Du Pont de Nemours, France). Each strip was in-

Table 1. Results of studies in bloodstains from 6 HIV-1 seropositive subjects tested after different storage times showing the presence of specific antibody detected by ELISA and by Western Blotting (+: positive, −: negative ±: borderline, 0–180: days of storage)

Blood-stains	ELISA									WB								
	0	7	14	21	28	40	60	90	180	0	7	14	21	28	40	60	90	180
1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	±	−	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	±	−	+	+	+	+	+	+	+	+	+

cubated overnight with 1/100 dilution of each sample and the antigen-antibody reaction was detected by a rabbit biotin-avidin system using 4-chloro-1-naphthol as the substrate.

Results

Preliminary assays showed that hemoglobin and its derivatives did not interfere with the serological test (data not shown) so that sample extracts were not pretreated to remove such substances.

Results obtained with dried bloodstains from seronegative samples reconstituted at different time intervals showed no specific antibody to HIV-1 both by ELISA and Western Blotting.

On the other hand, ELISA was able to detect antibody to HIV-1 in all samples from seropositive blood samples (Table 1) with no significant variation in optical density (O.D.) from 0–60 days after drying. A decreased level of positivity was observed by ELISA after 90 days in 2 samples (nos 2 and 6) and a complete negative reaction after 180 days in 3 samples (nos 2, 4 and 6).

A variation of patterns was recorded by WB analysis in aging stains with the disappearance of some bands but no false negatives were found when comparing the presence of antibody against at least 2 viral proteins codified by different genes as a criterion of positive diagnosis.

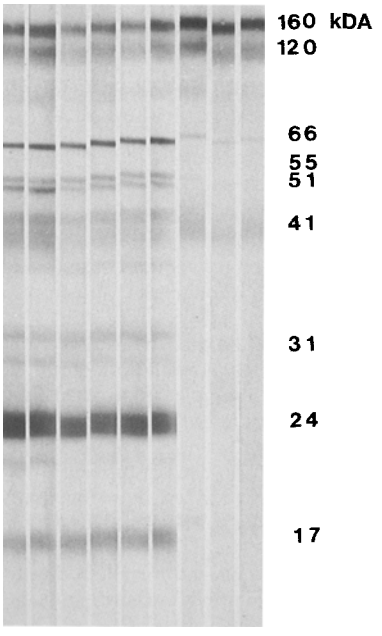

Fig. 1. Immunoblotting analysis of bloodstains after different storage times (from left to right 0, 7, 14, 21, 28, 40, 60, 90 and 180 days)

The immunoblotting pattern of samples rehydrated after 0–40 days showed the presence of antibodies against all the viral proteins detected under these experimental conditions (gp160, gp120, p66, p55, p51, gp41, p31, p24 and p17). Samples from 60 days and onwards (nos 2, 4 and 6) showed IgG antibodies reacting with HIV-1 polypeptides of 160, 120 and 41 kDa with weak reactivity against 66, 55 and 51 kDa.

An example of antibody response against individual HIV-1 polypeptides performed by W.B. is shown in Fig. 1.

Case report

During this study we were involved in a criminal case concerning the examination of a small number of very small human bloodstains, revealed by chromatographical and immunological assays, on the clothes of a young drug addict. The man, known to be HIV-1 seropositive and accused of having murdered his aunt with a knife, hanged himself in prison a few days after the crime. Blood samples obtained from both corpses were grouped in the ABO, Rh, MN, Kell, PGM1 and Hp systems but although differences between the 2 persons could be demonstrated it proved difficult to obtain conclusive results from the stains.

As a preliminary analysis by ELISA and WB assay showed antibodies against HIV-1 only in the blood sample obtained from the suspect, further investigations on the stains were performed for the presence of an immunological reaction against HIV-1.

Pieces of stained cloth (and substrate alone as control) were extracted overnight with saline at 4°C (about 200–400 µl for 0.5–1 cm^2) and 10 µl of the supernatant collected after centrifugation (10.000 rpm) was tested by WB.

All the samples gave positive results except for the negative controls.

Figure 2 shows the immunoblotting results of the serum samples taken from the HIV-1 seronegative victim (lane 1) and from the HIV-1 seropositive subject (lane 2) as well as extracts from stained (lanes 3–4) and unstained (lanes 5–6) areas from the clothes of the latter. The immunoblotting pattern from the corpse of the suspect showed the presence of antibodies against all the viral proteins detected under these experimental conditions

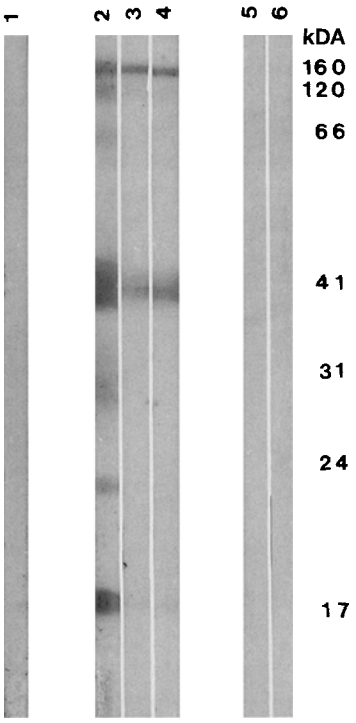

Fig. 2. Immunoblotting results of serum samples from the HIV-1 seronegative victim (lane 1), from the HIV-1 seropositive suspect (lane 2) and of the extract from 2 bloodstains on his clothes (lanes 3 and 4). Lanes 5 and 6 are samples from unstained areas of the same clothes as control

(gp160, gp120, p66, p55, p51, gp41, p31, p24 and p17), while diluted specimens from cloth stains showed only antibody to glycoproteins of 160 K dalton (kDa) and 41 kDa with a weak reaction against another polypeptide of 17 kDa.

These results suggest that all the bloodstains on the clothes came from the suspect.

Discussion

The results obtained demonstrate that the occurrence of specific antibody against HIV-1 can be detected even in small amounts of bloodstains after storage for several months. Assuming the presence of at least 2 proteins codified by 2 different viral genes as criteria for positive diagnosis by WB, 10 µl of reconstituted stain (equivalent for approx, 0.7 mg of the original stain) showed clear positivity, even after 180 days.

Hemoglobin and its derivatives do not influence the reactivity and this agrees with the results of other investigations [5]. The substrates assayed failed to interfere even though such controls must be included in case work.

No false positives were observed with either method. This does not agree with the findings of Stiebler et al. [4]. The delayed non-specificity (7–30 days) described in the paper of these authors is different from false positives in ELISA found by Kringsholm et al. [6] with equal frequency in living and autopsied drug addicts possibly due to antibodies against cellular antigens and was not observed by Tappero et al. [3] who investigated samples of cadaveric blood stored at room temperature over the same period (up to 40 days), using an immunoenzymatic method.

Contrary to Stiebler et al. [4] a difference in positive reactions was seen between ELISA and WB. It is indeed significant that after 180 days half of the seropositive samples did not react with ELISA but were still positive in WB.

The immunoblot profiles of HIV-1 positive stains reconstituted within 60 days showed specific immunoglobulin reactions against all the viral proteins with a decrease in the intensity of the reaction which directly correlated with the length of storage.

Moreover, in agreement with Stiebler et al. [4], disappearance of bands was observed in samples assayed after 60 days and especially those of intermediate-sized proteins.

A high degree of reactivity against glycoproteins, the major target antigen for antibodies, was seen throughout the observation period, in all dried samples extracted at various time intervals confirming that glycoproteins encoded by envelope genes are the antigens most consistently recognized by antibody present in the serum of HIV-1 infected individuals.

A reduced number of bands, irrespective of the time of infection may be due to dilution of the samples. Serial dilutions (1/100, 1/200, 1/400, 1/800) (data not shown) revealed that the reactivity of antibodies against glycoproteins was more persistent than antibodies against other viral polypeptides such as those encoded by gag and pol genes.

This was the situation in the case report where, due to dilution of samples, the immunoblot profile of the stains was weaker than the corresponding serum obtained from the corpse.

In conclusion, anti-HIV-1 antibodies can be detected in bloodstains as small as 0.7 mg and at least 6 months old. For forensic purposes immunoblot must be preferred because it is not only more specific but also has a higher degree of sensitivity.

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