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Infrequent Detection of HIV-1 Components in Tears Compared to Blood of HIV-1-Infected Persons*

Summary: Beside the risk of infection via HIV-1-contaminated blood, ophthalmologists are especially interested in the possibility of HIV-1 infection via tears. Therefore we tried to isolate HIV-1 from tears of 50 HIV-1-infected persons in different stages of disease by reverse transcriptase (RT) and by p24-antigen (p24-AG) in the cultures. Simultaneously we tried to isolate HIV-1 in the supernatant from peripheral blood lymphocytes (PBL), which was successful in 32 of the 50 examined specimens. HIV-1 could not be isolated from the tears of these persons. In addition, polymerasechain-reaction (PCR) was performed to detect proviral sequences (gag, pol, env) of HIV-1 in tears and blood of ten HIV-1-infected patients. While in all the examined patients gag, pol and env could be detected in the blood samples, only one tear sample was found positive for gag and pol DNA fragments. These results indicate that tears of HIV-1-positives contain extremely low quantities of tissue culture infectious doses (TCID) of HIV-1 in contrast to PBL. HIV-1 infection via tears therefore appears to be unlikely.

Zusammenfassung: *Verminderter Nachweis von Komponenten des humanen Immunschwäche Virus-1 in der Tränenflüssigkeit verglichen mit Blutlymphozyten HIV-1-Infizierter.* Neben der Infektionsgefahr durch HIV-haltiges Blut interessiert Ophthalmologen vor allem auch die Möglichkeit einer Infektion über die Tränenflüssigkeit. Wir haben deshalb versucht, HIV-1 aus der Tränenflüssigkeit von 50 HIV-1-infizierten Personen in unterschiedlichem Stadium der Erkrankung zu isolieren und über Reverse Transkriptase (RT) sowie p24-Antigen (p24-AG) im Kulturüberstand nachzuweisen. Parallel hierzu wurde aus dem Kulturüberstand peripherer Blutlymphozyten (PBL) der jeweiligen Personen eine Virusisolierung versucht. Dies gelang in 32 der 50 Fälle. Dagegen konnte in keinem Fall HIV-1 aus den Tränen der entsprechenden Personen isoliert werden. Zusätzlich wurde versucht, bei 10 HIV-1-infizierten Patienten in Tränenflüssigkeit und PBL provirale Sequenzen (gag, pol, env) von HIV-1 mittels der Polymerase-Ketten-Reaktion (PCR) nachzuweisen. Während dies in allen 10 Fällen mit PBL gelang, konnte nur in einem Fall HIV-DNA über gag- und pol-Amplifikate in der Tränenflüssigkeit nachgewiesen werden. Diese Ergebnisse sprechen für einen niedrigen Gehalt der Tränenflüssigkeit an gewebeinfektiösen Einheiten von HIV-1 im Vergleich mit dem Blut HIV-1-infizierter Personen. Damit scheint eine Infektion über den Kontakt mit Tränenflüssigkeit HIV-1-Infizierter weiter unwahrscheinlich.

Introduction

Since the detection of HIV by *Montagnier* and *Gallo* [1–4] different ways of infection with HIV have been discussed besides the transmission of HIV by blood. Soon thereafter HIV was detected in other body fluids, e.g. semen [5,6], saliva [7,8], cerebrospinal fluid [9], colostrum [10] and vaginal/cervical secretions [11,12]. Today infection via sexual contact as well as parenteral inoculation of HIV-containing blood or blood components are epidemiologically of great importance. However, in accordance with their profession, ophthalmologists are especially interested in the possibility of HIV transmission by contact with tears of HIV-seropositive persons.

In 1985 and 1986 *Fujikawa* et al. [13,14] reported the detection of HTLV-III in the tears of HTLV-III-positive patients. HTLV-III is now called HIV-1. Seven patients (six with AIDS and one with ARC) were examined and tears were obtained with sterile filter-strips (Schirmer). The specimens were directly added to lymphocyte cultures. As a control, five samples were taken from healthy subjects. The authors tried to determine HIV growth by reverse transcriptase (RT) activity in the culture supernatant and the presence of p15-antigen (p15-AG) and p24-antigen (p24-AG) in the cultured lymphocytes by immunofluorescence assays [13,14]. In one case (AIDS) RT was definitely determined and p15-AG and p24-AG were found by immunofluorescence in 4 and 5%, respectively, of the cells examined. The other samples of the HIV-infected persons as well as those from the healthy subjects were found to be negative. The authors concluded

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Table 1: Distribution of patients according to Walter Reed stages.

Walter Reed stage	1	2	3	4	5	6
No. of patients	10	5	2	9	9	15

that infectious HIV is present in tears of HIV-infected patients. *Tervo* et al. [15] isolated HIV-1 from contact lenses worn by HIV-positives, but not from their tears. *Baudouin* et al. [16] could not isolate HIV from tears of seven asymptomatic HIV-positive persons, but found RT activity in the tear cultures of two persons with AIDS-related complex.

Because in each study only small numbers of patients were investigated and in the latter two reports no control cultures were used, in our view the level of infectivity of tears has not yet been fully determined. We therefore performed a prospective study with 50 HIV-positive patients in different stages of the disease to try to answer this question by virus isolation in tissue culture. In addition, we tried to detect HIV-1-nucleic acid in tears and blood of ten HIV-1-positive persons by polymerase chain reaction (PCR), since with this method extremely small amounts of proviral DNA can be determined.

Patients and Methods

Fifty HIV-1-positive patients were examined to yield virus isolation from tears and simultaneously from PBL. All persons were male and their ages ranged from 18 to 47 years with a medium of 32 years (median 32.5 years). Walter Reed stages ranged from one to six (Table 1).

None of the participants had an eye examination the day the specimen was taken, nor was given eye drops. About 10 µl tear fluid was collected under sterile conditions either with sterile microcapillaries or with sterile Schirmer strips, using the method described by *Fujikawa* et al. [13,14]. The strips or the minced capillaries were cocultured with PHA (Wellcome, Burgwedel) stimulated PBL (10^6) in 5 ml medium (RPMI 1640 + 10% fetal bovine serum + antibiotics). The PBL used were mononuclear cells from HIV-negative subjects separated by ficoll-paque centrifugation [18].

Simultaneously 10 ml of heparinized blood of the cubital vein was drawn and PBL isolated by the same procedure as described above: These cells were incubated in 5 ml medium (RPMI 1640 + 10% fetal bovine serum + antibiotics) with 2 x 10^6 acceptor lymphocytes from an HIV-negative donor after addition of 50 µg PHA (Wellcome, Burgwedel) and 10% Interleukin-2 (Biotest, Dreieich). Cultures were monitored twice weekly for the formation of giant cells, for the presence of p24-AG (Abbott, Wiesbaden) and for particle-bound RT [17]. Fresh acceptor lymphocytes were added weekly. Negative cultures were kept for six weeks before deterioration. Cultures were considered positive when p24-AG was positive at least three times with an extinction of > 2.0 and the RT-assay was positive at least once. There was no positive culture without signs of syncytia formation.

It should be noted that this definition of a positive culture is much more stringent and differs from other reports, where the p24-AG-test was also used and a single increase in the extinction or permanent value above the cut-off was considered as a marker of successful HIV isolation [18].

Additionally, PCR was performed in tears and PBL in ten HIV-1-infected patients. In general, the method of *Williams* et al. [19] was followed using double PCR with nested primers. To increase specificity, three primer pairs were used from the gag-, pol- and env-region of the HIV genome. The designation of the primers is given in Table 2. For each PCR 30 cycles were run, one-tenth of the reaction volume of the first PCR was transferred as starter for the second PCR. Final PCR products (amplificates) were visualized by UV-light in agarose-gels in the presence of ethidium bromide.

PCR was performed directly in tears for the detection of HIV-DNA before or after a reverse transcription step of the putative RNA to DNA [20]. The DNA of PBL was liberated by detergent proteinase K digestion, as described by *Kellog* and *Kwok*, and then subjected to the PCR cycles [21].

Table 2: Gag-, pol- and env-primers used in PCR.

First cycle:	
gaga:	CTACT AGTAC CCTTC AGG
gagb:	CGGTC TACAT AGTCT CTAA G
Second cycle:	
sk38:	CCACC TATCC CAGTA GGAGA A [38]
sk39:	CCTTT GGTCC TTGTC TTATG TCCAG AATGC
First cycle:	
pol3:	TGGA AGTTC AATTA GGAAT ACCAC [39]
pol4:	CCTAC ATACA AATCA TCCAT GTATT G
Second cycle:	
pol3n:	TGGAT GTGGG TGATG CATA
pol4n:	AGCAC ATTGT ACTGA TATCT A
First cycle:	
enva:	TGTTC CTTGG GTTCT TG
envb:	GAGTT TTCCA GAGGA ACCCC
Second cycle:	
sk68:	AGCAG CAGGA AGCAC TATGG [38]
sk69:	GCCCC AGACT CTGAG TTGCA ACAG

Table 3: Results of virus isolation (positive p24-AG- and RT-test).

No. of patients	Blood			Tears	
	AB	p24-AG	RT	p24-AG	RT
18	+	–	–	–	–
32	+	+	+	–	–

AB = Antibody-test (ELISA) and Western blot in plasma;
p24-AG = p24-antigen in the culture supernatant;
RT = Particle-bound reverse transcriptase in the culture supernatant;
+ = positive result;
– = negative result.

Results

HIV-1 was isolated and cultured from the PBL in 32 of the 50 samples tested (Table 3). HIV-1 could not be isolated from any tear cultures from these persons (Table 3).

Using the PCR in all DNA preparations of PBL of the ten persons tested, HIV-1 DNA (gag, pol and env) could be

Table 4: Results of PCR in specimens from blood and tears of ten patients.

Patient	Blood			Tears			WR
	gag	pol	env	gag	pol	env	
1	+	+	+	−	−	−	6
2	+	+	+	+	+	−	6
3	+	+	+	−	−	−	6
4	+	+	+	−	−	−	6
5	+	+	+	−	−	−	6
6	+	+	+	−	−	−	5
7	+	+	+	−	−	−	4
8	+	+	+	−	−	−	3
9	+	+	+	−	−	−	1
10	+	+	+	−	−	−	1

PCR = Polymerase chain reaction;
WR = Walter Reed stage;
+ = positive result;
− = negative result.

detected (Table 4). In contrast, in only one person's tear sample gag- and pol-, but not env-amplificates were determined (Table 4).

Discussion

Epidemiologically relevant ways of infection with HIV are sexual contact and inoculation of HIV-contaminated blood or blood components, e.g. needle sticks or transfusion [22]. However, it is of special interest for ophthalmologists, optometrists and other health-care professionals, dealing with eyes, tears or contact-lenses to know to what extent tears of HIV-positive persons are infectious and what harm may be done during an examination or the fitting of contact lenses.

Infectiousness is a multifaceted process that depends on different factors: virulence of the agent, concentration of (infectious!) HIV in the transferred fluid (semen, blood, saliva, etc.), amount of transferred material, half-life time of the virus, route of inoculation (intravenous, paravasal), local immunologic situation at the point of inoculation (mouth, eye), etc.. Because an estimation of the total number of factors is nearly impossible, infectiousness is frequently equated with the concentration of the infectious agent in the material (to be potentially transferred). The higher the concentration of the infectious agent in the tissue, the more infectious is this tissue considered.

When one has to make assessments on the amount of infectious *virus* in the examined body fluids, it is preferable to distinguish between tissue-culture infectious doses (TCID) and human infectious doses (HID). It should be pointed out that one TCID corresponds to about 10 to 100 HID in macaques [23] or chimpanzees [24]. Therefore the estimation of infectivity of HIV as compared to TCID appears to be realistic.

In our prospective study HIV-1 could not be isolated from tears in any of the 50 HIV-1-infected persons who were in different stages of disease. However, this was possible in the control cultures of the PBL in 32 out of 50 of these patients. This leads to the view that there is a large difference in the amount of infectious HIV-1 in tears compared to that liberated from PBL. However, this does not imply that HIV-1 is not present in tears at all. The amount might be just below the above-mentioned threshold for detecting one TCID.

To be able to determine even small amounts of HIV-1 in tears we performed PCR in the tears of ten HIV-1-seropositive persons and in their PBL. Again the difference was striking. Gag- and pol-, but not env-DNA-fragments could be amplified with the PCR-technique in the tears of only one person, while all three regions – gag, pol and env – of the HIV genome amplificates were detected in all blood samples.

Comparing our results with reports on the isolation of HIV from PBL that documented isolation rates of up to 100% [25], it should be stated that the definition of what is claimed to be a positive culture has not been standardized. Our definition including positive results both in the p-24-AG assay and in the determination of the RT is much more strict. Therefore in 18 of the 50 patients examined (36%) we also obtained a negative isolation result from PBL. This result is in good agreement with other reports on the success of virus isolation, dependent on the clinical stage of immunodeficiency [18].

Nevertheless, compared to the virus load obtained from PBL, the HIV-1 content in tears is essentially lower. The only positive PCR result in the tears of an AIDS patient indicates the presence of HIV-RNA and/or -DNA and therefore might originate from HIV-infected cells shed to this liquid. This does not necessarily indicate the presence of infectious HIV.

Due to the different quantities of HIV-1 found in PBL and tears an infection with HIV-1 via tears seems to be highly unlikely, especially when compared to results obtained from other body fluids [5–16,22,26]. This conclusion is supported by the fact that up to now no case of HIV transmission via tears has been reported. HIV transmission through other ocular tissues has also not been reported [26].

Despite the low viral burden in tears, the general procedure for disinfection should be strictly followed [27–29], as transmission of HIV as well as of other infectious agents cannot be ruled out. Considering the transmission of HIV in contact lens practices, the usual disinfection of trial lens sets appears to be sufficient, *if disinfection and manipulation is performed correctly* [30–37]. After direct contact with all fluids possibly containing HIV, it is important to follow the "Suggestions for Conduct after Contamination with HIV" [27] for safety reasons.

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