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Ultrasensitive retrovirus detection by a reverse transcriptase assay based on product enhancement

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ABSTRACT **Reverse transcriptase (RT) is an indispensable component of infectious retroviruses. We have developed an ultrasensitive RT test in which RNA of bacteriophage MS2 serves as the template for RT-mediated cDNA synthesis. A fragment of the cDNA is selectively amplified by polymerase chain reaction and the amplification product is analyzed by Southern blot hybridization or enzyme immunoassay. The procedure was 10^6 to 10^7 times more sensitive than a conventional RT test and detected as little as 10^{-9} unit of murine leukemia virus RT, which corresponded to 2.1×10^2 molecules, a number present in 3–11 virions. As a screening assay for filterable particle-associated RT, it was positive with supernatants from cell cultures producing human immunodeficiency virus (HIV) type 1 or human T-cell leukemia virus (HTLV) type 1 or 2, but was negative with nonproducer cultures. It was positive with plasma samples from all tested individuals infected with HIV-1, HIV-2, or HTLV-1 and sera from cats infected with feline leukemia virus or feline immunodeficiency virus. Control samples from blood donors or uninfected cats were negative. Density banding experiments with culture supernatants showed that the RT activity was associated with virus particles. The assay should detect all replication-competent retroviruses or similar agents. It may be used as a screening assay for such agents, for quantitation of the viral load, drug susceptibility testing of RT, and control of virus inactivation in biological products.**

Infectious retroviruses are important causative agents of human and animal disease. They all possess a characteristic enzyme, reverse transcriptase (RT), and can thus be detected by assays for this activity (1, 2). However, the current tests are insensitive when compared with virus-specific methods. Detection of human immunodeficiency virus type 1 (HIV-1) by RT assay, for example, is 100 times less sensitive than by antigen (Ag) assay (3). Polymerase chain reaction (PCR) is again orders of magnitude more sensitive (4).

The narrow detection range of sequence-based tests such as PCR is, however, a disadvantage when detection of retroviruses in general is the aim. Attempts to detect uncharacterized retroviruses by the use of PCR with primers from conserved genomic regions have been met with some success but lack sensitivity and, therefore, require prior isolation and multiplication of the virus in a suitable host cell line usually difficult to find (5). Here, we report the development of an ultrasensitive RT assay capable of detecting retroviruses at a sensitivity hitherto reserved to virus-specific sequence amplification.

MATERIALS AND METHODS

Cell Cultures. Uninfected cell line H9, HIV-1 producer H9/HTLV-III$_B$, and the human T-cell leukemia virus type 1 (HTLV-1)-transformed nonproducer C81-66-45 were obtained from R. C. Gallo (National Institutes of Health, Bethesda, MD). HTLV-III$_B$ was also produced in C81-66-45 (hence called C81-66-45/HIV-1). The HTLV-1 producer MT-2 was from I. Miyoshi (Kochi Medical School, Kochi, Japan), the cloned HTLV-2 producer 76D9 was from J. Jendis (our laboratory), and the uninfected monocytoid cell line RC2A was from P. Stoeckbauer (Center for Hematology and Blood Transfusion, Prague). All lines were kept in RPMI 1640 medium plus 10% fetal bovine serum, 100 international units of penicillin G per ml, 100 μg of streptomycin per ml, 2.5 μg of amphotericin B per ml (all from GIBCO), and 5% interleukin 2 (IL-2) (Cellular Products).

Cultures of fresh human blood cells were set up from either donor buffy coats obtained from the local Red Cross transfusion center or from known cases of HIV-1 infection. Cultures were kept in RPMI 1640 medium plus 20% fetal bovine serum and antibiotics (basic medium) under one of the following modalities. (*i*) *Stimulated whole blood (WHB) culture*: The washed cell pellet of 1 ml of EDTA blood was cultured in basic medium plus 10% IL-2, 2 μg of phytohemagglutinin-P (PHA) per ml (GIBCO), 1% (vol/vol) pokeweed mitogen (PWM) extract (GIBCO), and 10 μg of lipopolysaccharide (LPS) per ml (Sigma). (*ii*) *Stimulated WHB coculture*: The washed WHB pellet was cocultured with 2×10^6 Ficoll-purified normal peripheral blood mononuclear cells (PBMCs) pretreated with PHA for 3 days and the cells were kept in the medium used for stimulated WHB cultures. (*iii*) *WHB coculture*: The washed WHB pellet was cocultured with 2×10^6 PHA-pretreated normal PBMCs and the cells were kept in basic medium plus 10% IL-2. (*iv*) *PBMC culture*: 10^6 PBMCs were cultured for 3 days in basic medium plus PHA. (*v*) *PBMC coculture*: 10^6 PBMCs were cocultured with 2×10^6 PHA-pretreated normal PBMCs and kept for 14 days in basic medium plus IL-2 or PHA or PWM at the above concentrations.

Plasma and Sera. EDTA plasma samples from patients infected with HIV-1, HIV-2, or HTLV-1 were stored at −70°C. Sera, stored at −20°C, from cats infected with feline leukemia virus (FeLV) or feline immunodeficiency virus (FIV) or from cats bred under specific pathogen-free conditions (SPF cats) were from H. Lutz (University Veterinary Hospital, Zurich).

Sample Pretreatment. One milliliter of plasma, serum, or culture supernatant was centrifuged at 16,000 × *g* for 10 min and filtered through 0.2 μm (Minisart NML, Sartorius). These measures greatly improved specificity of the product-

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Abbreviations: RT, reverse transcriptase; PERT assay, product-enhanced reverse transcriptase assay; HIV, human immunodeficiency virus; HTLV, human T-cell leukemia virus; Ag, antigen; IL-2, interleukin 2; WHB, whole blood; PHA, phytohemagglutinin-P; PWM, pokeweed mitogen; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell; FeLV, feline leukemia virus; FIV, feline immunodeficiency virus; SPF, specific pathogen-free; BSA, bovine serum albumin; MuLV, murine leukemia virus.

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enhanced RT assay (PERT assay), while not significantly affecting sensitivity. The volume was adjusted to 2.25 ml by phosphate-buffered saline (PBS, GIBCO) and particulate material was pelleted at 70,000 × g_{avg} for 90 min. The pellet was resuspended in 30 μl of buffer A consisting of 50 mM KCl, 25 mM Tris·HCl (pH 7.5), 5 mM dithiothreitol, 0.25 mM EDTA, 0.025% Triton X-100, and 50% glycerol (6).

PERT Assay. *Reverse transcription.* Genomic RNA of bacteriophage MS2 (Boehringer Mannheim) was used for template. A synthetic oligonucleotide, RT-1 [5′-d(CATAGGTCAAACCTCCTAGGAATG)-3′], served as primer. Primer/template was freshly prepared for each experiment by mixing, per reaction, 0.3 μg of MS2 RNA (0.28 pmol) with 72 ng of primer RT-1 (9 pmol) in H_2O, heating at 95°C for 5 min, annealing at 37°C for 30 min, and chilling the mixture on ice for 5 min. Per reaction, primer/template in 1.4 μl of H_2O was added to 23.6 μl of buffer containing the other ingredients, resulting in a reaction mixture of 56 mM Tris·HCl (pH 8.3), 56 mM KCl, 9 mM $MgCl_2$, 11.2 mM dithiothreitol, 1 unit of RNase inhibitor (RNasin) per μl (Promega), 0.13 μg of bovine serum albumin (BSA) per μl (Boehringer Mannheim), 0.4% Triton X-100, and 1 mM (each) dNTP. The reaction mixture was overlaid with 50 μl of mineral oil, and 3 μl of pretreated sample or an alternative source of RT in buffer A was added. The reaction was carried out at 37°C for 5 hr and the mixture was heated at 95°C for 7 min to inactivate the RNase inhibitor.

Product amplification. Amplification of the MS2 cDNA was carried out by PCR with primer RT-2 [5′-d(TCCTGCTCAACTTCCTGTCGAG)-3′] and primer RT-1. To the RT reaction mixture, 75 μl of a PCR reaction mixture containing 14 pmol of RT-1, 25 pmol of RT-2, 8 ng of RNase A (United States Biochemical), 2.5 units of *Taq* DNA polymerase (Amplitaq, Perkin–Elmer) in 37.5 mM KCl, 10 mM Tris·HCl (pH 8.3), and 0.01% gelatin were added. After 30 min of incubation at 37°C, for template degradation by RNase A, 25 cycles consisting of 94°C/30 sec of denaturation, 55°C/100 sec of annealing, and 72°C/110 sec of synthesis were carried out on a Techne Laboratories (Princeton) PHC-2 thermal block. The amplification produced a 112-bp-long DNA fragment that represented positions 21–132 of the 5′ end of the MS2-RNA (GenBank accession no. J02467).

Conventional RT Assay. The assay was done as described (7). Five micrograms of poly(rA)·oligo(dT) (Pharmacia) was used for primer/template. Virus from pretreated samples was lysed by adding half the volume of a solution containing 1.5 M KCl and 0.9% Triton X-100. Ten microliters of this mixture was assayed; the reaction volume was 100 μl. The final buffer conditions were as those for the RT reaction of the PERT assay and the reaction was carried out at 37°C for 22 hr. The enzymatic activity was assessed by measuring the incorporated tritiated thymidine monophosphate.

RNA PCR. For cDNA synthesis, 3 μl of virus was lysed and reverse-transcribed in 25 μl of a reaction mixture containing 25 pmol of sequence-specific primer HT-X2 [5′-d(TGAGCCGATAACGCGTCCATCGAT)-3′], 50 mM Tris·HCl (pH 8.3), 50 mM KCl, 8 mM $MgCl_2$, 10 mM dithiothreitol, 1 unit of RNase inhibitor per μl, 0.12 μg of BSA per μl, 0.4% Triton X-100, 1 mM (each) dNTP, and 50 units of recombinant murine leukemia virus (MuLV) RT (GIBCO/BRL). The mixture was incubated at 37°C for 90 min. The whole reaction mixture was then added to 75 μl of a PCR mixture containing 25 pmol of primer HT-X1 [5′-d(TTCGGATACCCATGCTACGTGTTTG)-3′] and 2.5 units of *Taq* DNA polymerase in 37.5 mM KCl, 10 mM Tris·HCl (pH 8.3), and 0.01% gelatin. The samples were subjected to 30 cycles of amplification, using the same cycle profile as for the PERT assay.

Detection of Amplified DNA Products. *Southern blot.* Ten microliters of amplified MS2 DNA was separated on a 1.5% agarose gel and blotted to nylon membrane (Hybond-N plus, Amersham) by alkaline transfer, according to the manufacturer's protocols. Probe RT-3 [5′-d(TTAATGTCTTTAGCGAGACGC)-3′], labeled at the 5′ end with ^{32}P, was used for hybridization (8). Membranes were exposed to Fuji RX x-ray films. For quantitation, bands were cut from the membrane and assayed in a scintillation counter.

Nonisotopic detection of amplified DNA products. Amplified HTLV-1 DNA was analyzed by an ELISA-based detection system (9) modified by the use of other oligonucleotide probes. The HTLV-1 DNA product was hybridized with probes HT-X4-DIG [5′-digoxigenin-d(TGGCCACCTGTCCAGAGCATCA)-3′] and HT-X6-Bio [5′-biotin-d(TGTGTACAGGGCGACTGGTG)-3′]. The hybrids were bound to avidin-coated microtiter plates. Unbound material was washed off and bound digoxigenin was detected with a horseradish peroxidase-labeled anti-digoxigenin antibody and 3,3′,5,5′-tetramethylbenzidine as substrate in a 5-min incubation.

For analysis of MS2 DNA products, hybridization was carried out with probes RT-3-Bio [5′-biotin-d(TTAATGTCTTTAGCGAGACGC)-3′] and RT-5-DIG [5′-digoxigenin-d(ATGGCTATCGCTGTAGGTAGC)-3′]. After binding of the hybrids to avidin-coated wells, bound digoxigenin was detected with alkaline phosphatase-labeled anti-digoxigenin Fab fragments (Boehringer Mannheim) at a dilution of 1:625. The enzymatic reaction was carried out with *p*-nitrophenyl phosphate (Pierce) in diethanolamine buffer at 37°C for 20 min. The system is at least as sensitive as Southern blot.

Sucrose Gradient Density Banding. Culture supernatants were pretreated as above, but the pellets were resuspended in 0.5 ml of TN buffer (50 mM Tris, pH 7.5/50 mM NaCl) instead of buffer A. The samples were layered on 16-ml continuous 10–60% (wt/wt) sucrose in TN buffer gradients and centrifuged in a Kontron (Zurich) TST 28.17 rotor at 70,000 × *g* for 16 hr. Fractions of 0.5 ml were collected from the bottom and their densities were determined in a Zeiss refractometer. For analysis by PERT assay or RNA PCR, the fractions were diluted to 2.0 ml with PBS and virus was pelleted at 4°C/70,000 × g_{avg} for 90 min. The pelleted material was resuspended in 30 μl of buffer A.

RESULTS

The PERT assay uses PCR for the selective enhancement of the MS2 cDNA synthesized by RT activity. The procedure described in *Materials and Methods* represents the results of many experiments performed to define conditions under which the assay would work at the desired high sensitivity and specificity.

In this procedure it was critical to guarantee that the reagents used did not contain amplifiable DNA, that such DNA was not introduced with the sample, and that no cDNA was produced from the RNA template by endogenous RT activity associated with the assay reagents themselves. Genomic RNA of MS2 was chosen for template since the replication of this phage does not involve a DNA intermediate (10). A systematic contamination of the template by amplifiable DNA or inadvertent introduction of such DNA by a sample was thus impossible. As a heteropolymer the MS2 RNA had the further advantage of being much less efficiently used by eukaryotic DNA polymerases than the synthetic homopolymeric templates usually employed for RT tests (7). To prevent degradation by RNases present in the test sample, the template was stabilized by placental RNase inhibitor.

In experiments shown in Fig. 1, the assay reagents were systematically examined for contaminating amplifiable DNA and for endogenous RT activity, using ELISA-based product analysis. In Fig. 1*A*, the reverse transcription step of the procedure was omitted and the assay reagents were directly submitted to PCR. In the absence of MS2 RNA, the reagents

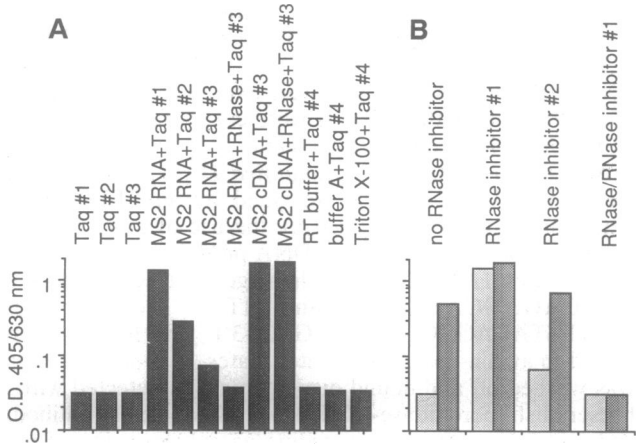

FIG. 1. Identification of sources of endogenous signal generation in the PERT assay. (*A*) Absence of contamination with DNA homologous to MS2 RNA and presence of endogenous RT activity in different batches of *Taq* DNA polymerase. Reagents tested were directly submitted to amplification by 30 cycles of PCR for MS2 DNA. (*B*) Demonstration of endogenous RT activity in two different preparations of RNase inhibitor, tested in the absence (▨) or presence (▩) of a low amount of MuLV RT.

used in the amplification step, including three different batches of *Taq* DNA polymerase (Taq 1–3), yielded each an OD of 0.032, which was equal to the substrate background. This indicated that the step 2 reagents were free of contaminating amplifiable DNA. The same batches of *Taq* DNA polymerase, when used in combination with a constant amount of MS2 RNA, yielded positive signals of different intensities. This suggested the presence of RT activity in *Taq* DNA polymerase preparations, as reported by others (11).† This interpretation was corroborated by the demonstration that degradation of the MS2 RNA by 8 ng of RNase A prior to amplification reduced the signal to the background level. The following controls showed that the signal reduction exerted by RNase A was not due to inhibition of amplification: direct amplification of MS2 cDNA by Taq 3 yielded the same signal, independent of whether or not RNase A was added. This result also excluded a contamination of MS2 RNA or RNase A by MS2 cDNA. Amplification of RT buffer, buffer A, or Triton X-100 with still another batch of *Taq* DNA polymerase (Taq 4) showed in addition that the reagents used for reverse transcription were free of contaminating DNA.

Using the complete PERT assay procedure, the reagents of the reverse transcription step were tested for product generation in the absence or presence of a low activity of MuLV RT (Fig. 1*B*). In the absence of RNase inhibitor, the assay showed the expected results: absence of signal in the absence of RT and good signal with RT, respectively. However, with RNase inhibitor 1, purified from human placenta, a strong signal was produced and the added RT activity could no longer be distinguished from the background. Less endogenous activity was found with the recombinant RNase inhibitor 2 (the human placenta protein expressed in *E. coli*). No product was generated when the template was digested by RNase A prior to incubation with RNase inhibitor 1, indicating that neither RNase A nor the RNase inhibitor was contaminated by amplifiable DNA.

In conclusion, these experiments showed that the assay reagents were free of amplifiable DNA, but that the RNase inhibitor and the *Taq* DNA polymerase both contained RT activity, which raised the background and, consequently, the detection limit of the assay. Therefore, all batches of RNase inhibitor were screened for RT activity and only those with low activity were used. In addition, the template was routinely digested by RNase A prior to amplification. These measures guaranteed a background sufficiently low to permit detection of very low RT activities.

The sensitivity of this system was assessed in a model experiment in which serial dilutions of recombinant MuLV RT were tested in parallel by conventional RT test and PERT assay (Fig. 2*A*). The detection limit of the RT test was between 10^{-2} and 10^{-3} unit. By contrast, the PERT assay clearly detected 10^{-9} unit, while 10^{-10} unit was within background. Thus, the detection limit of the PERT assay was 10^6 to 10^7 times lower than that of the conventional test.

The dilution experiment also showed that below 10^{-7} unit the signal obtained with the PERT assay was proportional to the RT activity and that thus, in this range, the test could be used for quantitation. The detection limit and quantitative range both depended on the number of amplification cycles (Fig. 2*B*): 10 cycles rendered RT detection 100 times more sensitive than the conventional procedure. An increase to 25 cycles resulted in an additional 10^5-fold increase in sensitivity. With >25 cycles, the increasing background precluded a further gain in sensitivity (not shown).

The capability of the assay to identify retroviruses present at numbers too low to be detected by conventional RT assay was assessed in a sucrose gradient density banding experiment performed with supernatants from HTLV-1-producing MT-2 cells and a PBMC coculture control originating from two normal blood donors, respectively. The MT-2 supernatant was diluted 1:1000 into the PBMC supernatant prior to sample pretreatment and banding, while the PBMC supernatant was used undiluted. Gradient fractions were analyzed by PERT assay (Fig. 3*A*). No PERT activity was detected in any of the fractions of the PBMC coculture. In MT-2 supernatant, the activity banded at a single peak at a density of 1.14 g/ml. RNA PCR confirmed that this peak corresponded to HTLV-1 particles (Fig. 3*B*). Conventional RT assay performed with fractions of the diluted MT-2 supernatant indi-

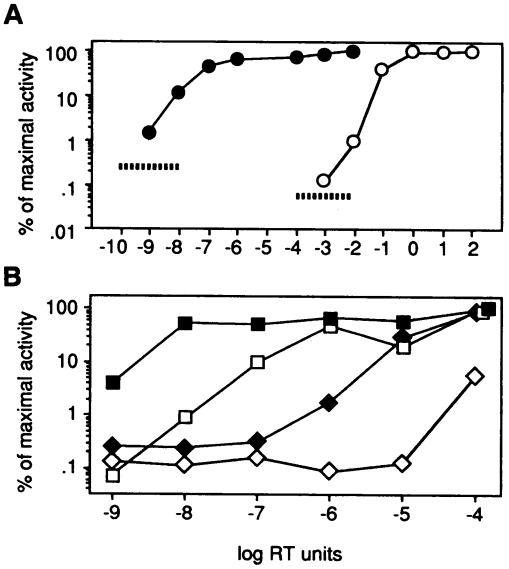

FIG. 2. Detection of RT activity by PERT assay. Southern blot was used for product analysis. (*A*) Comparison of RT activity by PERT assay with 25 cycles of amplification (●) and a conventional RT test (○) in serial dilutions of recombinant MuLV RT. Horizontal broken lines indicate backgrounds. (*B*) Detection level and range of quantitative detection of the PERT assay in dependence of the number of amplification cycles: ◇, 10; ◆, 15; □, 20; ■, 25 cycles.

†Activity was particularly high with *Taq* 1. Boiling partially removed activity indicating contamination by at least one heat-sensitive *Escherichia coli* polymerase. The remaining activity was, though, heat-resistant and thus exerted by *Taq* DNA polymerase itself.

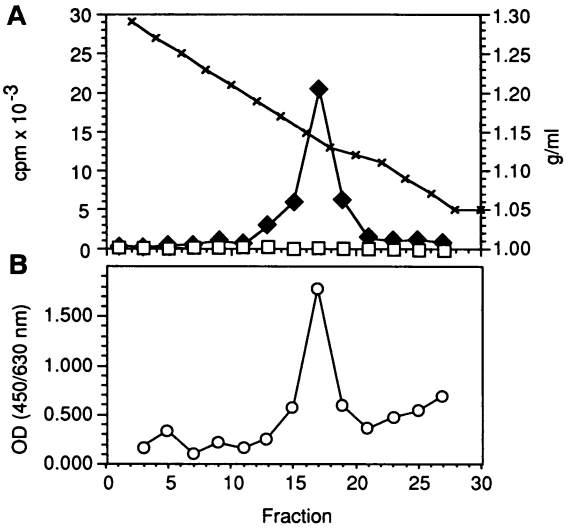

FIG. 3. Sucrose density banding of supernatants from HTLV-1-producing MT-2 cells and from a coculture of normal PBMCs and analysis by PERT assay and RNA PCR. (*A*) PERT analysis. The axis on the left indicates cpm of ^{32}P-labeled probe RT-3 bound to amplified MS2 DNA after Southern blot hybridization. The axis on the right indicates density of the gradient (x). □, Undiluted supernatant of the PBMC coculture; ◆, supernatant of MT-2 diluted 1:1000 in the supernatant of the PBMC coculture. (*B*) Analysis of HTLV-1 RNA (○) by RNA PCR.

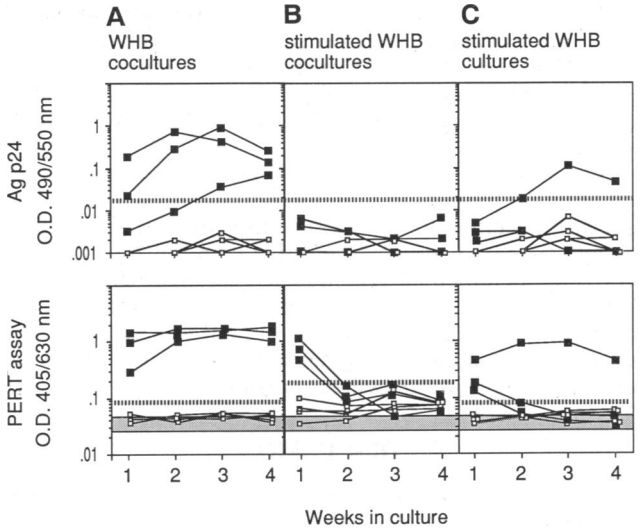

FIG. 5. Ag p24 and RT activity in supernatants of WHB cultures or cocultures from three individuals infected with HIV-1 (■) and four HIV-negative controls (□). The cutoff of positivity for the Ag assay was at an OD of 0.020, which corresponds to about 2 pg/ml (broken horizontal line). PERT assay was performed with 25 cycles of amplification and ELISA-based product analysis. Cutoffs of positivity are shown as horizontal broken lines and the method background (mean ± 1 SD) is shown as a shaded area.

cated absence of activity, while positive and negative controls showed the expected results (data not shown).

Based on these results, a procedure for the detection of filterable particle-associated RT was developed, to be used as a screening assay for retroviruses. Sample fluids were precleared by high-speed centrifugation and filtered. Particulate material was then pelleted by ultracentrifugation and analyzed by PERT assay. In Southern blot analysis (Fig. 4*A*), strong signals were obtained with supernatants from cell lines producing HIV-1, HTLV-1, or HTLV-2, while with the chosen film exposure no signals were detected with undiluted supernatants from nonproducers C81-66-45 and RC2A. Undiluted supernatants from unstimulated PBMC or PHA-stimulated PBMC cultures were also negative, while the PBMC coculture of an HIV-1-infected patient was positive. After longer film exposure, however, faint reactions were also detected with cultures considered uninfected, in particular with stimulated or unstimulated PBMC cultures (data not shown). Distinct signals were found when plasma samples from patients infected with HIV-1, HIV-2, or HTLV-1 or serum samples from cats infected with FeLV or FIV were assayed (Fig. 4*B*). By contrast, a blood donor plasma and the serum of a SPF cat were negative. RNA PCR of virus pelleted from plasma of the HTLV-1-infected patient gave a weak-positive result (not shown), in concordance with the relatively weak signal obtained in the PERT assay. All of five additional plasma samples from unselected blood donors were negative by PERT assay while all of five additional plasma samples from HIV-1-infected patients were strongly positive (Fig. 4*C*).

Since cellular DNA polymerases represent a possible source of false positivity, their interference with the PERT assay was assessed in cell cultures established from whole blood of healthy donors. Cultures from HIV-1-infected individuals were used as positive controls. Fig. 5 shows a representative experiment in which WHB cells from four donors and three HIV-1 positives were maintained under the various culture conditions described in *Materials and Methods*, including WHB cocultures (Fig. 5*A*), stimulated WHB cocultures (Fig. 5*B*), and stimulated WHB cultures (Fig. 5*C*). Supernatants were tested weekly for HIV-1 Ag (upper panels) and for RT activity by PERT assay using ELISA-based product analysis (lower panels). Fig. 5 shows that even under conditions of harsh stimulation leading to extensive cell death, as demonstrated microscopically in the case of stimulated WHB cocultures (data not shown), the RT activities in the blood donor cultures were only minimally (up to four times) higher than the method background.

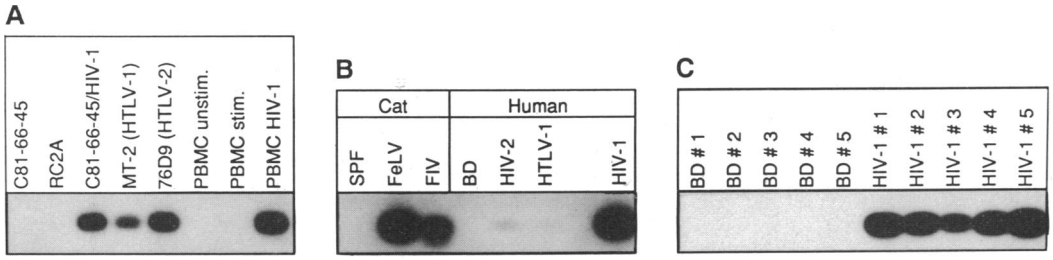

FIG. 4. Detection of filterable particle-associated RT by PERT assay. Southern blot was used for product analysis. Film exposure was 24 hr for *A* and *B* and 4 hr for *C*. (*A*) RT activity in cell cultures. Supernatants from C81-66-45, RC2A, MT-2, unstimulated or stimulated PBMCs, and PBMCs producing HIV-1 were tested undiluted and those from C81-66-45/HIV-1 and 76D9 were at 10^{-3} and 10^{-4}, respectively. (*B*) RT activity in serum or plasma of retrovirus-infected cats or humans (BD, blood donor). (*C*) Absence of RT activity from all of five additional blood donor samples and presence in all of five additional unselected samples from HIV-1-infected individuals of different clinical stages.

Based on these findings, cutoffs of positivity (mean + 5 SD) were calculated and used for interpretation of cultures from HIV-1 positives. All were, at least initially, PERT-positive. By contrast, Ag p24 assay completely failed to detect virus in stimulated WHB cocultures and in two of three stimulated WHB cultures.

Similar results were seen with PBMC cocultures from three other HIV-1 patients and three other blood donors (data not shown). Together, these experiments showed that the PERT assay was definitely more sensitive than the Ag assay and correctly identified all HIV-infected cultures. On the other hand, the cocultures from a total of 14 individuals (2 per coculture) considered uninfected showed very little activity, even under conditions of extensive cell death and, consequently, the release of cellular polymerases.

DISCUSSION

Association with filterable particle-associated RT activity is a hallmark of infectious retroviruses and of diagnostic importance. The PERT assay now adds the high sensitivity of PCR to the broad detection range of the RT assay. The result is an assay capable of detecting, at unprecedented sensitivity and specificity, RT and thus a wide variety of human and animal retroviruses that may have but little sequence homology in common (Fig. 4).

The low background (Fig. 1) permitted 25 rounds of cDNA amplification resulting in the detection of $<10^{-9}$ unit of RT activity (Fig. 2*A*). Given the specific activity of >40,000 units/mg and the M_r of 71,000 of the MuLV RT used, the 10^{-9} unit represents 2.1×10^2 RT molecules, which corresponds to 3–11 virions based on estimates of 20–70 RT molecules per virion in avian and mammalian type C retroviruses (12–14). Direct comparative testing of serial dilutions of HIV-1 by PERT assay and RNA PCR indicated a detection limit of about 10 virions for both methods (unpublished data).

The high specificity of the PERT assay is documented by the demonstration that negative controls yielded negative results (Fig. 4) and control cell supernatants showed minimal activity even under massive stimulation by alloantigen, PHA, PWM, and LPS, which resulted in extensive cell death (Fig. 5), and by gradient analysis of MT-2 or PBMC coculture supernatants showing absence of particle-associated activity from fractions other than those containing virions (Fig. 3). Furthermore, testing of culture supernatants with elevated activity in the conventional RT test confirmed that the PERT assay is considerably more specific: of 29 samples with RT activity ≥20,000 cpm (i.e., 10–15 times the method background), 27 were negative by PERT assay and the 2 positives were also positive for HIV-1 Ag (not shown).

The high sensitivity, specificity, and broad range of agents to which it is sensitive predispose the PERT assay for use as a universal screening assay for all agents associated with RT activity. The procedure permits the direct detection of such agents in primary materials, thereby obviating the need for virus cultivation. In addition to the known agents, the test may identify hitherto unrecognized retroviruses. Further studies will need to show whether other retroelements—e.g., hepatitis B virus—can also be detected. The PERT assay may further be used for quantitation of virus load, for evaluation of virus inactivation procedures, or to determine the susceptibility of a virus to RT inhibitors.

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