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Transcomplementation of Simian Immunodeficiency Virus Rev with Human T-Cell Leukemia Virus Type I Rex

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A molecular clone of the simian immunodeficiency virus SIV_{SMM} isolate PBj14, lacking the ATG initiation codon for Rev protein (PBj-1.5), did not produce virus or large unspliced or singly spliced viral RNA upon transfection of HeLa cells. Low but significant levels of virus and large viral RNA production were observed upon transfection of PBj-1.5 into HeLa Rev cells expressing the *rev* gene of human immunodeficiency virus type 1. Furthermore, abundant virus and large viral RNA production occurred upon transfection of PBj-1.5 into HeLa Rex cells expressing the *rex* gene of human T-cell leukemia virus type I. Virus produced from HeLa Rex and HeLa Rev transfections was infectious, produced large amounts of virus, and was cytopathic for Rex-producing MT-4 cells. In contrast, no or only low levels of virus production were observed upon infection of H9 cells. These studies show that a defective SIV *rev* gene can be transcomplemented with human immunodeficiency virus type 1 Rev and with high efficiency by human T-cell leukemia virus type I Rex, and they suggest that *rev*-defective viruses could serve as a source for production of a live attenuated SIV vaccine.

The genomes of human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) and simian immunodeficiency virus (SIV) contain genes for both structural and nonstructural proteins (10, 25, 30). Of the latter, several are involved in the regulation of viral production and infectivity. Thus, the transactivator (Tat) protein increases both transcription and translation (1, 27). Rev (regulator of expression of virion proteins) is necessary for the transport of mRNA transcripts from nucleus to cytoplasm (16) and for the translation (5) of unspliced or singly spliced mRNAs coding for the viral structural proteins Gag, Pol, and Env. In the absence of Rev, only multiply spliced mRNA molecules that code for the nonstructural proteins are translated (20, 21).

Earlier studies have shown that HIV-1 Rev (Rev_1) can compensate in *trans* for a defective *rev* gene in both HIV-1 and HIV-2. Rev_2, in contrast, compensates for a defective *rev* gene only in HIV-2 (19, 28). Rev_2 may in fact even inhibit the function of Rev_1, possibly because of ineffective and/or aberrant multimerization of the two Rev molecules on the Rev-responsive element (RRE) RRE_1 (12). Furthermore, the Rex protein (Rex_1) of human T-cell leukemia virus type I (HTLV-I) (15) has been shown to compensate a defective Rev_1 gene in HIV-1 (26). Here, we describe results showing that Rex_1 can also compensate for a defective *rev* gene in SIV (Rev_{SIV}). We also observed weak but significant transcomplementation of Rev_{SIV} by Rev_1.

We transfected HeLa cells or HeLa cells constitutively expressing rev_1 (HeLa Rev) or *rex* (HeLa Rex) genes (generous gifts from George Pavlakis, Frederick Cancer Research Institute, Frederick, Md.) with molecular clones of HIV-1 or SIV defective for the *rev* gene. Five million semiconfluent cells were transfected with 5 μg of appropriate DNA (3). At 72 h after transfection, culture supernatants were harvested, cells were spun down, and the supernatant was filtered through a 220-nm-pore-size Millipore filter. The amount of viral p27 core antigen (Abbott, Abbott Park, Ill.) was determined from the supernatants. The extraction of cytoplasmic RNA, formaldehyde gel electrophoresis, and Northern (RNA) blotting were performed as described elsewhere (29). The filters were hybridized for 14 h with SIV (a 0.6-kb *Stu*I fragment from PBj-1.5) or HIV-1 (a 0.5-kb *Bgl*II fragment from HIV-1 HXB2) long terminal repeat probes, radioactively labelled by random priming with [^{32}P]dCTP (29), washed extensively, and exposed for several days at −70°C with an intensifying screen.

We first tested the system using a molecular clone of HIV-1 (pHXB2 107) with a defective *rev* gene (a stop codon at position 6) (27). This clone produced no viral structural proteins or infectious virus when transfected into HeLa cells, but abundant virus production was seen after transfection into HeLa Rev and HeLa Rex cells (data not shown).

For SIV, we used a putative Rev^- molecular clone from the SIV_{SMM} isolate PBj14 (11). Of several clones obtained from this isolate, one (PBj-1.5) contained a transition at the initiation codon of Rev and showed no reverse transcriptase activity upon transfection into CEM × 174 cells (8). When different target cell lines were transfected with this clone, expression of the three classes of mRNAs (1.8 to 2.2, 4.5, and 9.4 kb) and abundant virus production were obtained from the HeLa Rex cell line, while only low virus production was obtained in the HeLa Rev cells (Fig. 1 and 2). Transfection of HeLa cells failed to show virus production with the *rev*-defective SIV clone (Fig. 2), and in Northern blotting, no large unspliced genomic or singly spliced *env* mRNA was seen (Fig. 1). Control transfections with pBR322 to the same cell lines showed no SIV mRNA in Northern blotting and were negative in the p27 antigen assay.

The production of infectious virus from HeLa Rev_1 and HeLa Rex but not from HeLa transfections was further confirmed by infection of H9 and MT-4 cells with the supernatants from these cultures. The MT-4 cell line is abortively infected with HTLV-I (one integrated copy per cell) and has low or undetectable HTLV-I production (14).

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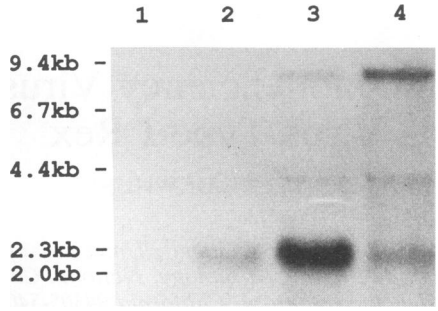

FIG. 1. Northern blot analysis of RNA samples isolated from HeLa, HeLa Rev, and HeLa Rex cells transfected with the *rev*-defective SIV clone PBj-1.5. Lanes: 1, HeLa pBR 322 cells (negative control); 2, HeLa PBj-1.5 cells; 3, HeLa Rev PBj-1.5 cells; 4, HeLa Rex PBj-1.5 cells.

Culture supernatants were collected from the transfection experiments, cleared by centrifugation (900 × *g* for 10 min), and filtered through a 220-nm-pore-size Millipore filter, and 3 ml of supernatant was used to infect 10^7 target cells. Cell cultures were then fed every 3 days. Samples for p27 antigen assay were taken at days 7, 14, and 21 postinfection.

Infection of MT-4 but not of H9 cells with the Rev^- HIV-1 clone pHXB2 107 resulted in continuous and abundant virus production (data not shown). Likewise, abundant p27 core antigen production was seen in MT-4 cultures infected with supernatants from the HeLa Rev or HeLa Rex cells transfected with PBj-1.5, but not from MT-4 cells infected with supernatants from corresponding HeLa cell transfections (Fig. 3). Cytopathic effects evidenced by syncytium formation were also seen in cultures infected with HeLa Rex and HeLa Rev supernatants. In contrast, PBj-1.5 infection of H9 cells (which do not express Rex or Rev) showed only transient low-level p27 core antigen production (Fig. 3). We have thus shown that the HTLV-I Rex protein is capable of complementing in *trans* defective *rev* genes in both HIV-1 and SIV and that Rev_{SIV} can also be transcomplemented with Rev_1.

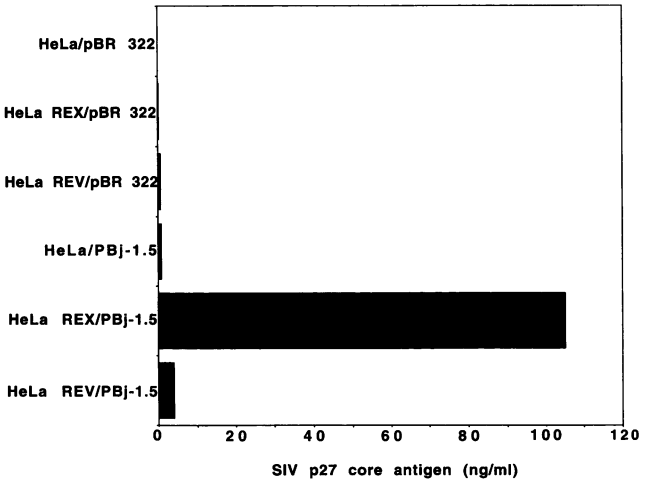

FIG. 2. SIV p27 core antigen in supernatants of HeLa, HeLa Rev, or HeLa Rex cell lines 3 days after they were transfected with the *rev*-defective SIV clone PBj-1.5 or with the control plasmid pBR 322.

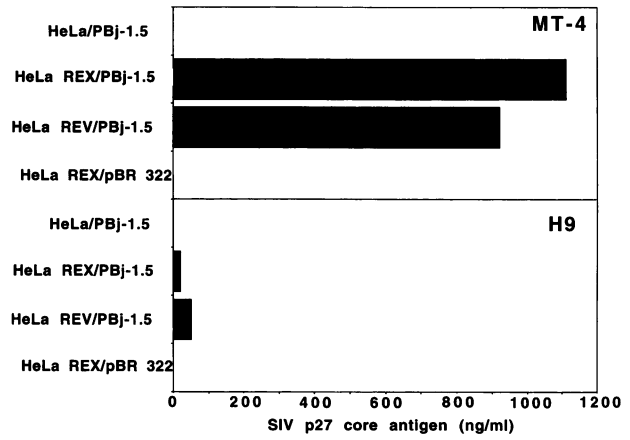

FIG. 3. SIV p27 core antigen produced as a result of infection of human T-cell lines H9 and MT-4 with supernatants containing the *rev*-defective PBj-1.5 virus. Supernatants were harvested at day 14 postinfection. Supernatant from pBR 322 transfection served as a negative control.

The action of Rev is mediated by binding to the RREs within the unspliced (*gag* or *pol*) and singly spliced (*env*) mRNAs (6, 9, 33). This binding is mediated by an arginine-rich region within the Rev protein (23). Comparison of the amino acid sequences of Rev_1, Rev_2, and Rev_{SIV} reveals a high homology among these three proteins within a 10-amino-acid-long sequence, as demonstrated in Table 1. The corresponding amino acid sequence within Rex also reveals a substantial degree of homology. Lazinski and coworkers (17) have analyzed the arginine-rich motifs in a variety of RNA-binding proteins and have suggested a consensus sequence (Table 1). Based on the Lazinski sequence and the corresponding sequences in Rev_1, Rev_2, Rev_{SIV}, and Rex, we suggest a general consensus sequence, rxRRrxRRrxn (see Table 1 for a description of the symbols used), capable of interacting with the Rev- or Rex-responsive elements in HIV, SIV, or HTLV-I. Theoretically, this sequence can form an amphipathic alpha helix (13), which could bind to a B form of double-stranded RNA within the stem-bulge structures in the RRE.

The existence of cellular proteins that may contain the above-described promiscuous consensus sequence may explain the transient low-level virus production from the H9 cell line. As shown in Fig. 3, infection of H9 cells with supernatants containing PBj-1.5 virus resulted in p27 core

TABLE 1. Comparison of the arginine-rich sequences in Rev_1, Rev_2, Rev_{SIV}, and HTLV-I Rex

Protein	Sequence[a]
Rev_1 consensus	QARRNRRRRWR
Rev_2 consensus	SQRRNRRRRWK
Rev_{SIV} consensus	NQRRQRRRRWR
HTLV-I Rex	KTRRRPRRSQR
Promiscuous RRE binding	rxRRrxRRrxn[b]
Lazinski consensus	bobRbjRRzzb[c]

[a] Based on 20 Rev_1, 9 Rev_2, and 11 Rev_{SIV} sequences and on the Rex sequence in the SwissProt protein sequence data base.

[b] Amino acid class covering pattern as in the 1992 human retroviruses and AIDS data base, Los Alamos, N.Mex. r, H, N, D, E, Q, K, R, S, or T; x, anything; n, K or R.

[c] b, basic; o, nonbasic polar; z, charged; j, acidic; x, anything.

antigen production that was 10 to 20 times lower than that in the MT-4 cultures. In contrast, no detectable virus production was seen when HeLa CD4 cells were either transfected with the PBj-1.5 clone or infected with supernatants containing PBj-1.5 virus (data not shown). The lack of virus production by PBj-1.5 in the HeLa CD4 cells rules out the possibility that the low-level virus production in H9 cells would be the result of initiation of translation from the ACG codon, as has been described for parvoviruses (2).

Our findings that *rev*-defective SIV clones can be transcomplemented with the Rex protein to produce replication-deficient, infectious SIV virions may have relevance for the production of live, attenuated SIV and/or HIV vaccines. Recent results indicate that the protective effect against SIV infection observed in monkeys vaccinated with killed SIV preparations (22) was due to an immune response toward cellular products derived from the human cell lines used to grow SIV (31, 32) and that no protection was observed against a challenge virus derived from monkey cells (4, 18). In contrast, protection against SIV_{mac} grown in monkey lymphocytes in animals that were previously infected with an attenuated SIV of low pathogenicity has been demonstrated (7). Similarly, cynomolgus monkeys were protected against the disease caused by SIV_{mac} if they had previously been infected with the non-disease-inducing HIV-2 (24). These results stress the importance of studies with live, attenuated SIV vaccines in order to elucidate mechanisms that could be employed in the development of HIV vaccines.

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REFERENCES

1. **Arya, S., C. Guo, S. Josephs, and F. Wong-Staal.** 1985. Trans-activator gene of human T-lymphotrophic virus type III (HTLV-III). Science **229:**69–73.
2. **Becerra, S. P., F. Koczot, P. Fabisch, and J. A. Rose.** 1988. Synthesis of adeno-associated virus structural proteins requires both alternative mRNA splicing and alternative initiations from a single transcript. J. Virol. **62:**2745–2754.
3. **Chen, C. A., and H. Okayama.** 1988. Calcium phosphate-mediated gene transfer: a highly efficient transfection system for stably transforming cells with plasmid DNA. BioTechniques **6:**632–638.
4. **Cranage, M. P., L. A. E. Ashworth, P. J. Greenaway, M. Murphey-Corb, and R. C. Desrosiers.** 1992. AIDS vaccine developments. Nature (London) **355:**685–696.
5. **D'Agostino, D. M., B. K. Felber, J. E. Harrison, and G. N. Pavlakis.** 1992. The Rev protein of human immunodeficiency virus type 1 promotes polysomal association and translation of *gag/pol* and *vpu/env* mRNAs. Mol. Cell. Biol. **12:**1375–1386.
6. **Daly, T., K. Cook, G. Gray, T. Maione, and J. Rusche.** 1989. Specific binding of HIV-1 recombinant REV protein to the REV-responsive element *in vitro*. Nature (London) **342:**816–817.
7. **Daniel, M. D., F. Kirchhoff, S. C. Czajak, P. K. Seghal, and R. C. Desrosiers.** 1992. Protective effects of a live attenuated SIV vaccine with a deletion in the nef gene. Science **258:**1938–1941.
8. **Dewhurst, S., J. Embretson, D. Anderson, J. Mullins, and P. Fultz.** 1989. Sequence analysis and acute pathogenicity of molecularly cloned SIV. Nature (London) **345:**636–640.
9. **Dillon, P. J., P. Nelbock, A. Perkins, and C. A. Rosen.** 1990. Function of the human immunodeficiency virus types 1 and 2 Rev proteins is dependent on their ability to interact with a structured region present in *env* gene mRNA. J. Virol. **64:**4428–4437.
10. **Fultz, P. N., H. M. McClure, D. C. Anderson, R. B. Swenson, R. Anand, and A. Srinivasan.** 1986. Isolation of a T-lymphotropic retrovirus from naturally infected sooty mangabey monkeys (Cercocebus atys). Proc. Natl. Acad. Sci. USA **83:**5286–5290.
11. **Fultz, P. N., H. M. McClure, D. C. Anderson, and W. M. Switzer.** 1989. Identification and biologic characterization of an acutely lethal variant of simian immunodeficiency virus from sooty mangabeys (SIV/SMM). AIDS Res. Hum. Retroviruses **5:**397–409.
12. **Garrett, E. D., and B. R. Cullen.** 1992. Comparative analysis of Rev function in human immunodeficiency virus types 1 and 2. J. Virol. **66:**4288–4294.
13. **Giniger, E., and M. Ptashne.** 1987. Transcription in yeast activated by a putative amphipathic alpha helix linked to a DNA binding unit. Nature (London) **330:**670–672.
14. **Harada, S., Y. Koyanagi, and N. Yamamoto.** 1985. Infection of HTLV-III/LAV in HTLV-I-carrying cells MT-2 and MT-4 and application in a plaque assay. Science **229:**563–566.
15. **Hidaka, M., J. Inoue, M. Yoshida, and M. Seiki.** 1988. Post-transcriptional regulator (rex) of HTLV-I initiates expression of viral structural proteins but suppresses expression of regulatory proteins. EMBO J. **2:**519–523.
16. **Knight, D., F. Flomerfelt, and J. Ghrayeb.** 1987. Expression of the art/trs protein of HIV and study of its role in viral envelope synthesis. Science **236:**8237–8240.
17. **Lazinski, D., E. Grzadzielska, and A. Das.** 1989. Sequence-specific recognition of RNA hairpins by bacteriophage antiterminators requires a conserved arginine-rich motif. Cell **59:**207–218.
18. **Le Grand, R., B. Vaslin, G. Vogt, P. Roques, M. Humbert, D. Dormont, and A. M. Aubertin.** 1992. AIDS vaccine developments. Nature (London) **355:**684.
19. **Malim, M., S. Böhnlein, R. Fenrick, S.-Y. Le, J. Maizel, and B. Cullen.** 1989. Functional comparison of the Rev trans-activators Dauber by different primate immunodeficiency virus species. Proc. Natl. Acad. Sci. USA **86:**8222–8226.
20. **Malim, M., J. Hauber, R. Fenrick, and B. Cullen.** 1988. Immunodeficiency virus rev trans-activator modulates the expression of the viral regulatory genes. Nature (London) **335:**181–183.
21. **Malim, M., J. Hauber, S.-Y. Le, J. Maizel, and B. Cullen.** 1989. The HIV-1 rev trans-activator acts through a structured target sequence to activate nuclear export of unspliced viral mRNA. Nature (London) **338:**254–257.
22. **Murphy-Corb, M., L. Martin, B. Davison-Fairburn, R. C. Montelaro, M. Miller, M. West, S. Ohkawa, G. B. Baskin, J.-Y. Zhang, S. D. Putney, A. C. Allison, and D. A. Eppstein.** 1989. A formalin-inactivated whole SIV vaccine confers protection in macaques. Science **246:**1293–1297.
23. **Olsen, H. S., A. W. Cochrane, P. J. Dillon, C. M. Nalin, and C. A. Rosen.** 1990. Interaction of the human immunodeficiency virus type 1 REV protein with a structured region in env mRNA is dependent on the multimer formation mediated through a basic stretch of amino acid. Genes Dev. **4:**1357–1364.
24. **Putkonen, P., R. Thorstensson, J. Albert, K. Hild, E. Norrby, P. Biberfeld, and G. Biberfeld.** 1990. Infection of cynomolgus monkeys with HIV-2 protects against pathogenic consequences of a subsequent simian immunodeficiency virus infection. AIDS **4:**783–789.
25. **Ratner, L., W. A. Haseltine, R. Patrarca, K. J. Livak, B. Starcich, S. J. Josephs, E. K. Doran, J. A. Rafalski, E. A. Whitehorn, K. Baumeister, L. Ivanoff, S. R. Petteway, Jr., M. L. Pearson, J. A. Lautenberger, T. S. Papas, J. Ghrayeb, N. J. Chang, R. C. Gallo, and F. Wong-Staal.** 1985. Complete nucleotide sequence of AIDS virus, HTLV-III. Nature (London) **313:**277–284.
26. **Rimsky, L., J. Hauber, M. Dukovich, M. Malim, A. Langlois, B. Cullen, and W. Greene.** 1988. Functional replacement of the HIV-1 rev protein by the HTLV-I rex protein. Nature (London) **335:**738–740.
27. **Sadaie, R., T. Benter, and F. Wong-Staal.** 1988. Site-directed mutagenesis of two trans-regulatory genes (tat-III, trs) of HIV-1. Science **239:**910–913.
28. **Sakai, H., R. Shibata, T. Miura, M. Hayami, K. Ogawa, T.**

Kiyomasu, A. Ishimoto, and A. Adachi. 1990. Complementation of the *rev* gene mutation among human and simian lentiviruses. J. Virol. **64:**2202–2207.

29. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
30. **Sanchez-Pescador, R., M. D. Power, P. J. Barr, K. S. Steimer, M. M. Stempien, S. L. Brown-Schimer, W. W. Gee, A. Renard, A. Randolph, J. A. Levy, D. Dina, and P. Luciw.** 1985. Nucleotide sequence and expression of an AIDS-associated retrovirus (ARV-2). Science **227:**484–492.
31. **Stott, E. J.** 1991. Anti-cell antibody in macaques. Nature (London) **353:**393.
32. **Stott, E. J., W. L. Chan, K. H. G. Mills, M. Page, F. Taffs, M. Cranage, P. Greenaway, and P. Kitchin.** 1990. Preliminary report: protection of cynomolgus macaques against simian immunodeficiency virus by fixed infected-cell vaccine. Lancet **336:**1538–1541.
33. **Zapp, M., and M. Green.** 1989. Sequence-specific RNA binding by the HIV-1 Rev protein. Nature (London) **342:**714–716.