6.6

Replication of Human Immunodeficiency Virus Type 1 in Human T-Cells Expressing Antisense RNA

Kazuyasu Mori, Axel Rethwilm, Andreas Schwinn and Ivan Horak
Institut für Virologie und Immunbiologie der Universität Würzburg, Versbacher Str. 7, 8700 Würzburg

Summary

Stable transfected human T-cell lines expressing antisense HIV-1 gag or tat RNAs were tested for their resistance against HIV-1 infection. Quantitative analyzes of p24 and viral RNA expression did not detect any differences between antisense-RNA expressing and control cell lines. The failure of an antisense inhibitory effect might be due to the observed ceassation of the antisense RNA-transcription in HIV-1 infected cells.

The lack of successful treatment of HIV-induced AIDS justify attempts to explore feasibility of gene therapeutic procedures. One of the discussed regimes is the transplantation of bone marrow cells which have been rendered resistant to HIV infection. The unresolved problem is how to achieve and maintain the HIV resistance. Genetically manipulated cells expressing HIV antisense RNA could theoretically be used for this purpose. The first suggestion for therapeutic use of antisense oligonucleotides was in papers from Zamecnik's laboratory. They demonstrated an inhibitory effect of complementary oligodeoxynucleotides on Rous sarcoma virus replication (Zamecnik and Stephenson, 1978). An important modification of this technology was introduced by Izant and Weintraub (1984). They used antisense-RNA expressing vectors which, when integrated into the host genome, constantly supply the cell with antisense RNA. Subsequently, similar approaches have been used successfully to inhibit expression of several cellular and viral genes.

To inhibit HIV replication, thus far only attempts have been using synthetic oligonucleotides (Agraval et al., 1988; Zaia et al., 1988; Shibahara et al., 1989). The problem of this approach are in a requirement for a continuous treatment of cells with high concentrations of antisense oligonucleotides and their cytotoxicity. Here we have examined the use of antisense-RNA expressing vectors to prevent HIV infection of human T-cells.

HIV-1 tat and gag genes were chosen for construction of the antisense vectors from following reasons. They represent relatively more stable parts of the HIV genome (Starcich et al., 1986) and thus the efficiency of their antisense transcripts should be less dependent on the sequence variability of an infecting virus. Furthermore, since gag and tat gene products are essential at the early stages of the virus replication, suppression of their cellular concentration by the antisense RNA should be an effective way to inhibit the virus production.

Human CMV enhancer/promoter, which is known as one of the strongest promoter sequences (Boshart et al., 1985), was cloned in front of reversed HIV-1 gag and tat sequences (Arya et al., 1985; Fisher et al., 1985) to generate the recombinant vectors pgagRneo and ptatRneo (Figure 1). Linearized DNA of these constructs was transfected into H9 and Jurkat cells. Neomycin resistant cell clones were recloned and the presence of the integrated transgene was confirmed by Southern-blot hybridization. Cell lines with no apparent DNA rearrangement were selected and screened for the expression of antisense RNA. Northern-blot hybridizations revealed several cell lines positively hybridizing with gag or tat probes. The exact nature of the hybridizing RNA was proved by S1 nuclease mapping. This analysis confirmed that these RNAs represent the expected antisense transcripts. The two bands seen in Figure 1 correspond to two differently spliced antisense gag RNAs.

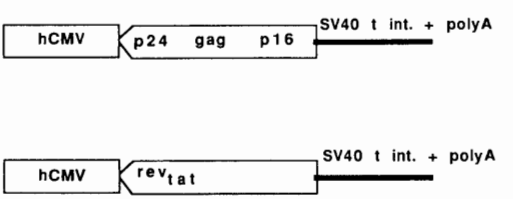

Figure 1: HIV-1 antisense constructs

In order to assess a potential antiviral effect of the antisense RNAs, cells were infected with HIV-1 (m.o.i. 0.01) and the course of the infection was monitored by cell viability counting. These observations indicated a slightly delayed progression of the viral infection in some cell lines (data not shown). However, in order to evaluate quantitatively the effect of the antisense RNA on the HIV-1 replication two problems had to be encountered. Firstly, the expression of the CD4 receptor varies among different cell lines and thus may affect their susceptibility to HIV infection. Therefore, only the cell lines with comparable CD4 receptor expression were used for further studies. Se-

Table 1: Expression of p24 in cells infected with HIV-1 or HIV-2

cell line	a) antisense RNA	b) CD4 (%)	c) p24 (ng/ml) d) HIV-1	HIV-2	ratio p24 HIV-1/HIV-2
HB-6-1	-	26.8	7.5	0.15	50.0
HB-4-5	-	86.5	11.3	0.19	59.5
HB-2-1	gag ++	23.9	9.3	0.16	58.1
HB-1-6	gag +++	71.1	10.0	0.19	52.6
JB-5-5	-	6.9	1.4	0.03	46.6
JC-1	-	22.7	4.6	0.08	57.5
JA-3-2	tat ++	10.1	0.8	0.02	40.0
JA-2-6	tat +	10.2	2.5	0.04	62.5
JA-9-0	tat +	12.3	2.0	0.05	40.0

a) The cell lines derived from the H9 or Jurkat cells are marked with capital H or J, respectively.
b) Relative expression of antisense RNAs was estimated by Northern-blot hybridization.
c) CD4 receptor expression is indicated as % of positive cells stained by a FITC-anti-CD4 antibody analyzed by FACS.
d) p24 expression in HIV-1 or HIV-2 infected cells (m.o.i. 0.01) was determinded 3 days p.i. using a HIV-1 p24 ELISA kit (DuPont).

condly, there are inherent differences among different cell lines to support virus replication. For this reason replica plates were infected with HIV-1 or with HIV-2 and their p24 expression was measured in an ELISA test. Due to considerable sequence differences (Zagury et al., 1988), HIV-2 replication should not be affected by the HIV-1 antisense RNA and thus its p24 expression served as an internal control. The results presented in Table 1 do not show any significant differences in HIV-1 p24 expression between antisense-RNA expressing and control cells.

To confirm the data, we analyzed the expression of viral sense and transgene antisense-RNAs directly in cells infected with HIV-1 with a probe capable of detecting both types of transcripts. Our preliminary data indicate that relatively high levels of antisense transcripts are present in uninfected cells, but their amount decreases after HIV-1 infection concomitantly with increasing viral transcription. Thus it is an additional evidence that despite of the high ratio of antisense to sense-RNA made in early stages of infection, the HIV-1 replication was not significantly affected.

Several reasons might account for the failure of an antisense effect. A general switch off of cellular transcription is excluded, since the transcription of other cellular genes was not affected by the HIV-1 infection. It seems more likely that a competition for common transcription factors (Nabel and Baltimore, 1987) together with a compartmentalization of viral transcription provides a decisive advantage for HIV-1 replication.

Acknowledgements

We thank F. Wong-Staal and R. Gallo for their generous gift of HIV-1 DNA clones.
This work was supported by the Bundesministerium für Forschung und Technologie (BGA II 010-86) and from Fonds of the Chemische Industrie.

References

(1) Agrawal, S., Goodschild, J., Civeira, M. P., Thornton, A. H., Sarin, P. S. and Zamecnik, P. (1988): Oligodeoxynucleoside phosphoramidates and phosphorothioates as inhibitors of human immunodeficiency virus. Proc. Nat. Acad. Sci. USA 85: 7079–7083

(2) Arya, S. K., Guo, C., Josephs, S. F. and Wong-Staal, F. (1985): Trans-activator gene of human T-lymphotropic virus type III (HTLV-III). Science 229: 69–73

(3) Boshart, M., Weber, F., Jahn, G. and Schaffner, W. (1985): A very strong enhancer is located upstream of an immediate early gene of human cytomegalovirus. Cell 41: 521–530

(4) Fisher, A. G., Collalti, E., Ratner, L., Gallo, R. C. and Wong-Staal, F. (1985): A molecular clone of HTLV-III with biological activity. Nature 316: 262–265

(5) Izant, J. G. and Weintraub, H. (1984): Inhibition of thymidine kinase gene expression by anti-sense RNA: A molecular approach to genetic analysis. Cell 36: 1007–1015

(6) Nabel, G. and Baltimore, D. (1987): An inducible transcription factor activates expression of human immunodeficiency virus in T cells. Nature 326: 711–713

(7) Shibahara, S., Mukai, S., Morisawa, H. and Yamamoto, N. (1989): Nucleic Acids Res. 17: 239–252

(8) Starcich, B., Hahn, B., Shaw, G. M. and Wong-Staal, F. (1986): Identification and characterization of conserved and variable regions in the envelope gene of HTLV-III/LAV, the retrovirus of AIDS. Cell 45: 637–648

(9) Zagury, I. F., Franchini, G., Reitz, M. and Gallo, R. (1988): Genetic variability between isolates of human immunodeficiency virus (HIV) type 2 is comparable to the variability among HIV type 1. Proc. Nat. Acad. Sci. USA 85: 5941–5945

(10) Zaia, J. A., Rossi, J. J., Murakawa, G. J. and Cantin, E. M. (1988): Inhibition of human imunodeficiency virus by using an oligonucleoside methylphosphonate targeted to the tat-3 gene. J. Virol. 62: 3914–3917

(11) Zamecnik, P. and Stephenson, M. L. (1978): Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. Proc. Nat. Acad. Sci. USA 75: 280–284