Trans-activation of the long terminal repeat of human immunodeficiency virus type 1 by the parvovirus B19 NS1 gene product

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Persistent parvovirus B19 infections in human immunodeficiency virus type 1 (HIV-1)-infected patients have been reported. The two viruses could share common target cells. The NS1 protein of B19 regulates B19 expression and we have investigated its possible effect on the long terminal repeat (LTR) of HIV-1. In transient transfection experiments, NS1 trans-activated the expression of reporter genes under the control of the HIV-1 LTR. The effect of NS1 was apparent only in the presence of the HIV-1 Tat protein, and required intact TAR and TATA box sequences.

In the immunocompromised child or adult, particularly in patients with AIDS, parvovirus B19 infection may result in a chronic severe anaemia caused by erythroid bone marrow failure (Bowman et al., 1990; Chrystie et al., 1990; Frickhofen et al., 1990; Kurtzman et al., 1989; Mitchell et al., 1990). The seroprevalence of anti-B19 IgM antibodies in children with AIDS is 40 to 50%, indicating a high incidence of active or recent B19 infections (Nigno et al., 1992). In vivo, B19 parvovirus infection seems restricted to cells of the erythroid lineage of the bone marrow (BFU-E and CFU-E) (Young et al., 1984). However B19 DNA could be detected in macrophages (Morey et al., 1992; Porter et al., 1988), a classical target of the human immunodeficiency virus (HIV) (Meltzer et al., 1990). Megakaryocytes, another potential HIV target (Zucker-Franklin & Cao, 1989) can also be infected in vitro by B19 (Srivastava et al., 1990). Therefore it is possible that cells can be dually infected by B19 and HIV-1 in vivo.

The 3' half of the ssDNA genome of B19 encodes two structural proteins (VP1 and VP2). Its 5' half encodes a non-structural protein (NS1) which is an early gene product (Ozawa et al., 1987) and increases transcription from the B19 promoter (Doerig et al., 1990). The seemingly high incidence of chronic B19 infections in AIDS patients, and the fact that immediate early gene products from several DNA viruses such as herpesviruses or adenovirus (Davis et al., 1987; Gendelman et al., 1986; Laspi et al., 1990) were shown to modulate HIV expression prompted us to test a possible effect of NS1 on the HIV-1 long terminal repeat (LTR).

The NS1 expression vector (pNS1) used in this study consisted of the 5' half of the genome (−320 to +2828 of pEMBL/B19; Doerig et al., 1990), containing the promotor, the NS1 gene and a polyadenylation signal from B19. The production of NS1 by this vector was assessed by transfection into a promonocytic cell line (U937; Sundström & Nilsson, 1976) and immunoprecipitation (data not shown). To test the possible effect of NS1 on the HIV-1 LTR, we used a luciferase (Luc) reporter gene placed under control of U3 and R sequences (XhoI−644 to HindIII+78) from the HIV-11LA isolate (pLTRLuc; Schwartz et al., 1990). U937 cells were transfected by a modified DEAE–dextran procedure (Wang et al., 1987) with 500 ng of pLTRLuc and 50, 100 or 500 ng of pNS1 vector. Cells were lysed 24 h after transfection and the Luc activity was measured (Schwartz et al., 1990) and standardized as a function of the protein concentration in the extract. Under these conditions, the basal level of Luc activity was not increased and we did not observe a significant influence of NS1 on the HIV-1 LTR (Fig. 1).

HIV-1 transcription is a complex process involving an interplay between cis-acting regulatory sequences present in the viral LTR and viral or cellular trans-activators, a dominant factor being the viral protein Tat (for reviews see Cullen, 1990 and Frankel, 1992). To examine the effect of NS1 in the presence of Tat, we used the CMVtatt expression vector (Schwartz et al., 1990) containing the human cytomegalovirus (CMV) immediate early enhancer/promoter. Co-transfection of pLTRLuc (500 ng) with CMVtatt (50 ng) induced on average a threefold increase (range two to four) in Luc activity. This modest trans-activation of the HIV-1 LTR was enhanced by NS1 since co-transfection with 100 ng of pNS1 resulted in an 11-fold increase (range six to 17)
of the basal LTRLuc activity (Fig. 1), which represents a fourfold stimulation above that induced by Tat alone. The effect of NS1 was highly reproducible in four experiments. It was not observed in the presence of higher amounts of CMVTat and did not increase with higher amounts of transfected NS1 plasmid (data not shown). The specificity of the effect was assessed by using an inactivated NS1 mutant (pMNS1), derived from pNS1 by a deletion resulting in a frameshift and translation stop at position 18 of the protein. No increase in Luc activity was observed when the mutant pMNS1 and CMVTat were co-transfected with pLTRLuc (Fig. 1). Moreover a CMVLuc reporter was not trans-activated by pNS1 in U937 cells (data not shown) which ruled out an affect of NS1 on the expression of Tat through activation of the CMV promoter/enhancer.

To establish the effect of NS1 on the HIV-1 LTR in a different experimental context, we have used another reporter vector, pLTRCAT (a gift from Dr M. Boileau-Forget) containing the HIV-1 sequences of the reporter vector, pLTRCAT (a gift from Dr M. Alizon & U. Hazan, unpublished results). It was shown that the terminal

This study indicates that the B19 NS1 gene product can increase expression of the Luc and CAT reporter genes placed under the transcriptional control of the HIV-1 LTR synergistically with the HIV-1 Tat protein. Interestingly, the NS1 protein by itself had no detectable effect. Synergy between Tat and other viral trans-activators has already been shown (Laspia et al., 1990; Gendelman et al., 1986) but unlike NS1 these proteins induced transcription in the absence of Tat. Tat probably acts by binding to an RNA target, the trans-activation responsive element (TAR) located at the 5' end of the HIV mRNAs, and by promoting elongation of these transcripts and/or initiation of novel mRNAs (for reviews see Frankel, 1992 and Cullen, 1990). In addition to the TAR sequences, cis-acting elements located 5' to TAR, such as sequences binding TFII D, Sp1 and NF-κB transcription factors, are necessary for Tat-induced trans-activation (Berkhout & Jeang, 1992; Berkhout et al., 1990; Kamine & Chinnadurai, 1992; Liu et al., 1992). To localize sites critical to the activity of NS1, we used two mutant HIV-1 LTRLuc constructs, one (ATATA) having a deletion of the TATA box (Berkhout et al., 1990) and the other (ATAR) of most of the TAR site (SacI + 32 to HindIII + 78). The Luc activity of these mutants was not affected by co-transfection with CMVTat alone, or CMVTat and pNS1, but they were still responsive to phorbol myristate acetate (PMA, Sigma) (Table 1). These results suggest that trans-activation by NS1 requires the presence of a Tat/TAR RNA complex and TFII D binding sites. B19 NS1 has been found to trans-activate its own promoter (Doerig et al., 1990; N. Sol, F. Morinet, M. Alizon & U. Hazan, unpublished results). It was shown that the terminal
regions of the NS1 protein of paroviruses have structural and functional similarities with acidic transcriptional regulators (Legendre & Rommelaere, 1992). These factors appear to interact with components of the transcription complex (Ptashne, 1988). Moreover the NS1 protein of a related parovirus (the minute virus of mice) was shown to interact with components of the core transcription complex, in particular Spl and TFIID (Ahn et al., 1992). Since Tat probably mediates transcriptional activation through a direct or indirect interaction with the core Spl–TATA promoter (Kamine & Chinnadurai, 1992), the addition of NS1 could induce a modification of the transcription complex important for a more efficient initiation/elongation process.

These results were obtained in U937 cells, considered to be related to cells of the phagocytic lineage that could be infected with B19 or HIV-1 (Meltzer et al., 1990; Morey et al., 1992; Porter et al., 1988). They suggest that HIV-1 gene expression could be affected by the B19 NS1 protein in dually infected cells. The effect of NS1 on the HIV-1 LTR was apparent only in the presence of low amounts of Tat, and could not be observed in chronically HIV-1-infected U937 cells by transfection of pNS1 and pLTRLuc. Nevertheless, in situations of extremely low expression of the HIV-1-LTR, when small amounts of Tat are produced, the synergistic activation of NS1 could facilitate the switch to the acute replication phase of HIV-1, with expression of Rev and structural proteins (Cullen & Greene, 1989). Immediate early proteins from other DNA viruses such as herpesviruses have been shown to trans-activate the HIV LTR but the NS1 protein of B19 seems unique in that it requires the presence of Tat. Some herpesviruses are considered to act as "cofactors" that stimulate the expression of HIV, and possibly participate in the evolution of the disease (Laurence, 1990; Webster et al., 1989). B19 infection could play a similar role in haematopoietic tissue.

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References


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