Production of a non-functional nef protein in human immunodeficiency virus type 1-infected CEM cells

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The nef gene product of the human immunodeficiency virus (HIV) is suggested to be a negative factor involved in down-regulating viral expression by a mechanism in which the correct conformation of the nef protein is essential. The nef protein expressed by vaccinia virus recombinants is phosphorylated by protein kinase C. We investigated the synthesis of the nef protein and its state of phosphorylation during HIV-1 infection of a T4 cell line (CEM cells). Maximum synthesis of viral proteins occurred 3 days after infection, when more than 90% of cells were producing viral proteins. The synthesis of the nef protein was detected in parallel with the env and gag proteins. As expected, the nef protein was myristylated but not phosphorylated, and its half-life was less than 1 h. By the use of the polymerase chain reaction technique, we isolated and sequenced the nef gene of this HIV-1 stock. Two significant mutations were observed. Firstly, threonine, at amino acid number 15, the site of phosphorylation by protein kinase C, was mutated into an alanine, and secondly aspartic acid of the tetrapeptide WRFD, which is probably involved in GTP binding, was mutated into an asparagine. The mutated nef gene was expressed in a vaccinia virus system, in which it was not phosphorylated and its half-life was dramatically reduced compared to the wild-type nef gene product. Furthermore, down-regulation of CD4 cell surface expression was no longer affected by the mutated nef gene. These results emphasize that phosphorylation of the nef protein provides an efficient test to monitor its biological activity.

Introduction

The human immunodeficiency virus (HIV) contains several non-structural proteins which are involved in the regulation of virus infection (Ratner et al., 1985; Wain-Hobson et al., 1985; Guyader et al., 1987). Among these proteins is the product of the nef gene, previously referred to as F, 3' orf, orf B or E'. The nef protein is a 27K protein which is myristylated, localized mainly in the cytoplasm and partly associated with membranes (Allan et al., 1986; Franchini et al., 1986). Because of its capacity to down-regulate viral expression, the nef protein has been classified among the regulatory proteins that control HIV expression, possibly acting like a signal transducing protein (Guy et al., 1987; Gallo et al., 1988). In accord with this, recent results from different laboratories suggest that the nef protein acts as a specific repressor of HIV transcription and might be critical for maintenance of HIV-1 latency (Luciw et al., 1987; Ahmad & Venkatesan, 1988; Ahmad et al., 1989; Niederman et al., 1989). In general however, a functional nef gene does not abolish virus expression completely but seems to retard the rate of viral replication (Terwilliger et al., 1986).

By the use of a vaccinia virus recombinant expressing the nef gene, we have recently demonstrated that the nef protein is phosphorylated by protein kinase C (on Thr 15 and another unidentified site), has GTP binding and GTPase activities and is also capable of down-regulating CD4 cell surface expression in vaccinia virus-infected CEM T4 cells (Guy et al., 1987). The mechanism by which the nef protein controls CD4 expression is not yet clear; such a specific effect, however, could be conveniently used to demonstrate its biological activity. In view of these observations, we investigated the synthesis and phosphorylation of the nef protein during HIV-1 infection of T4 cells. HIV-1 (Bru isolate) was passaged 10 times in CEM cells to produce a highly infectious virus stock. Under these experimental conditions, the nef protein was synthesized but was found not to be phosphorylated and had a very short half-life. Here we demonstrate that these properties were due to two
important mutations in the nef gene, one affecting the conformation of the nef protein and the other affecting its phosphorylation. Accordingly, a vaccinia virus recombinant with the mutated nef gene produced a nef protein which was not phosphorylated and had a reduced half-life. Furthermore, such a mutated nef gene product was no longer able to down-regulate CD4 expression in vaccinia virus-infected CEM cells. These results indicate that prolonged culturing of HIV might favour mutations of the nef gene at specific sites required for the expression of a biologically functional protein.

**Methods**

**Viruses and cells.** The HIV-1Br_u isolate of HIV-1 (Montagnier et al., 1984) was used in this study. CEM cells were cultured in suspension medium RPMI-1640 (Gibco-BRL) containing 10% (v/v) foetal calf serum and 2 µg/ml polybrene (Sigma). CEM clone 13 cells are derived from the human lymphoid cell line CEM (ATCC-CCL119) and express the T4 antigen to a high level. About 80 to 90% of the cells synthesize viral proteins and can be recognized by a c.p.e. involving vacuolization of cells and appearance of small syncytia 2 to 3 days post-infection (p.i.) (Laurent et al., 1989). The original HIV-1 stock was prepared on phytohaemagglutinin-stimulated fresh lymphocytes from normal donors. Vaccinia virus was of the Copenhagen strain. Hamster BHK-21 cells were grown in MEM (Gibco) containing 10% (v/v) foetal calf serum.

**Metabolic labelling of cells.** HIV-1-infected CEM cells were incubated in MEM without L-methionine, L-cysteine and serum, but supplemented with 200 µCi/ml L-[35S]methionine (specific activity > 1000 Ci/mmol) and 200 µCi/ml L-[35S]cysteine (specific activity > 1000 Ci/mmol). Incubation time varied. For in vivo phosphorylation, HIV-1-infected CEM cells were incubated in phosphate-free medium containing 1 mCi/ml [32P] (37 MBq/ml) for 2 h. Labeling of infected cells with 200 µCi/ml [3H]myristic acid (50 Ci/mmol) was carried out in myristate-free medium containing 2% lipoprotein serum for 2 h. All radioactive materials were from Amersham. Metabolic labelling of vaccinia virus-infected cells with [32P] or [35S]methionine was carried out as described previously (Guy et al., 1987).

**Preparation of extracts and immunoprecipitation.** Cell and viral extracts were made in lysis buffer containing 100 mM-Tris-HCl pH 8.8, 0.5 M-NaCl, 2% Triton X-100, 100 µg/ml each of soybean trypsin inhibitor and PMSF (Lathe & Hirth, 1980). Immunoprecipitation of the recombinant nef protein was carried out with a specific serum in buffer A (Guy et al., 1987).

**Two-dimensional gel isoelectric focusing.** Two-dimensional gel isoelectric focusing was performed as described by O'Farrell (1975) with the following modification: immunoprecipitated HIV-1 proteins were eluted by boiling in SDS-PAGE sample buffer before dilution in a volume of buffer containing 9.5 M-urea, 8% (v/v) Nonidet P40, 0.5% (v/v) 2-mercaptoethanol, 2.5% (w/v) Ampholines, pH ranges 6.5 to 9, and 2.5% (w/v) Ampholines, pH ranges 5 to 8 (Laurent et al., 1989).

**Polymerase chain reaction (PCR).** The nef gene of the HIV-1 stock was isolated by PCR using the GENEAMP kit (Cetus) and a heating block (Hybaid). The cellular DNA from infected CEM cells was prepared according to Loche & Mach (1988) in a laboratory where the nef gene of the HIV-1Br_u isolate had ever been prepared (in order to avoid contamination). A first amplification was carried out using 10 pmol of two specific primers, TG 2050 (5’GGAAAGGATCTCTATAA-GATGGG 3’) and TG 2051 (5’AGAGGCTCCAGGCTCAAATCTGGY 3’), corresponding to regions immediately upstream and downstream of the nef gene, respectively. A second amplification was performed using primers TG 2050 and TG 2154 (5’ AAGGGGATCTTTATGCAAGCCT 3’), TG 2154 being downstream of TG 2151.

**Cloning and sequencing.** The fragment obtained after two amplifications was digested by BamHI and cloned into an M13 vector. Sequencing of the mutant nef gene was carried out using internal primers by the dideoxynucleotide method (Sanger et al., 1977). Five different clones were sequenced, all of which gave identical sequences.

**Transfer of the nef gene into vaccinia virus.** The nef gene was transferred into the vaccinia virus genome as previously described (Kieny et al., 1984). The vaccinia virus with the mutated nef gene was referred to as VV nef, whereas the virus with the wild-type nef gene was referred to as VVwt (Guy et al., 1987).

**Expression of the nef protein in Escherichia coli.** The mutated nef protein was expressed in E. coli under the control of a thermoinducible lambda PL promoter: the recombinant protein was very unstable.

**Fluorescence-activated cell sorting (FACS) analysis.** Different aliquots of CEM-T4 cells were infected with 50 p.f.u./cell of the various recombinant viruses in 1 ml phosphate-buffered saline (PBS). After 1 h at room temperature, cells were diluted in 10 ml RPMI medium and, 16 h p.i., 10⁶ cells in 100 µl were incubated for 30 min at 4 °C with the following reagents: mouse anti-vaccinia virus polyclonal antibodies, two murine monoclonal anti-CD4 antibodies (OKT4 (Ortho) and Lea3a (Becker-Dickinson)), the 4B4 murine monoclonal antibody (Cooultronics) directed against a 135K cell surface protein (CDW29) not related to the CD4 molecule, and the 110-4 monoclonal antibody directed against the gp110-120 of HIV-1 (Genetic Systems). The cells were washed twice by centrifugation (300 g for 4 min) and resuspended in 100 µl of a fluorescein isothiocyanate-conjugated sheep anti-mouse Ig (Biosys) for 30 min at 4 °C. After two washes, the cells were resuspended in 0.4% formaldehyde in PBS and analysed with the FACS system (Becton Dickinson). All the different washes and serum dilutions were carried out in Ca²⁺- and Mg²⁺-free PBS supplemented with 1% bovine albumin and 0.1% NaN₃.

**Treatment of the nef protein by bacterial alkaline phosphatase.** 35S-labeled cell extracts from HIV-1-infected cells were immunoprecipitated with patients’ serum. Aliquots of the immunoprecipitated HIV-1 proteins were suspended in 50 µl of buffer (20 mM-Tris-HCl pH 7.6, 50 mM-NaCl, 5 mM-MgCl₂, 3 mM-MnCl₂ and 100 units/ml aprotinin) and incubated as such or with 8 units of bacterial alkaline phosphatase (BAP; Sigma) for 90 min at 37 °C. All samples were analysed by two-dimensional gel isoelectric focusing and processed for fluorography (Laurent et al., 1989).
A non-functional nef protein in CEM cells

Results

Detection of the nef protein in HIV-1 infected CEM cells

The production of viral particles in HIV-infected CEM cells can be recognized by a c.p.e. involving vacuolization of cells and appearance of small syncytia 2 to 3 days p.i. At this stage we labelled cells with [35S]cysteine and [35S]methionine and analysed cell extracts by immunoprecipitation. During 45 min of labelling, only a very small amount of 35S-labelled major core protein p25 was produced by cleavage of the 35S-labelled gag precursor p55. Longer periods of labelling resulted in the accumulation of comparatively large quantities of p25 that interfered with the detection of the nef protein. Fig. 1(a) shows that serum A immunoprecipitated the envelope precursor (gp160) and the nef protein (p27), whereas serum B immunoprecipitated both of these proteins and the gag precursor (p55), and its partially cleaved product (p40) and p25.

To confirm that p27 was in fact the nef protein, immunoprecipitation assays were carried out in the absence or presence of unlabelled recombinant nef protein in excess. The immunoprecipitation of 35S-labelled p27 was completely abolished in the presence of the recombinant nef protein whereas immunoprecipitation of gp160 was not affected (Fig. 1a, serum A). The 35S-labelled immune complex preparations obtained with sera A and B were further analysed by two-dimensional gel isoelectric focusing (Fig. 1b). The nef protein was resolved as a single spot with an isoelectric point (pI) of 6.2. Because of their Mr, and pI values, the different subspecies of p25 (a, b, c and d) were used as convenient markers to identify the nef protein (Laurent et al., 1989).

Synthesis and the half-life of the nef protein in HIV-1 infected cells

HIV-1-infected cells were pulse-labelled with [35S]-methionine and [35S]cysteine for 45 min each day p.i. The nef protein became detectable on the third day p.i., in parallel with the envelope precursor (Fig. 2) as well as the gag precursor and its products (not shown). Pulse-chase experiments indicated that the nef protein has a very short half-life, estimated at around 30 min (Fig. 2). This is most probably due to its rapid degradation as the

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**Fig. 1.** (a) Characterization of the nef protein. Lanes 1 and 2, HIV-1-infected cells were labelled with [35S]methionine and [35S]cysteine for 45 min and cell extracts were immunoprecipitated with serum A (lane 1) or serum B (lane 2). Lanes 3 to 5, HIV-1-infected cells were labelled for 2 h and extracts were immunoprecipitated with serum A in the absence (lane 3) or presence (lanes 4 and 5) of recombinant nef protein from E. coli. The recombinant nef proteins (0.5 and 1.0 µg) were first incubated with serum A at 4°C for 90 min before the addition of labelled extracts. The samples containing the washed immune complexes were analysed in a 12.5% polyacrylamide gel electrophoresis. A fluorograph is shown. The positions of different HIV-1 proteins are indicated on the right. (b) Two-dimensional gel isoelectric focusing analysis of the nef protein. 35S-labelled extracts from HIV-1-infected CEM-cells (10⁶ cells) were immunoprecipitated with sera A and B (i and ii). The purified proteins were analysed by two-dimensional gel isoelectric focusing. The pH gradient obtained by isoelectric focusing (1st dimension) was between pH 6.0 and 8.5. In the second dimension, proteins were resolved by SDS-PAGE and fluorographed. The pI value of nef p27 protein is 6.2.
The nef protein of HIV-1 is myristylated but is not phosphorylated

HIV-1-infected cells were labelled with either [35S]-methionine and [35S]cysteine or [3H]myristic acid and extracts were assayed by immunoprecipitation using serum B. As expected, the nef protein was found to be labelled with [3H]myristate, as were the gag precursors (p55 and p40) and the matrix protein p18, which is derived from the N-terminal portion of p55 (Fig. 3a). The nef protein was not found in the culture medium of infected cells (Fig. 3a, lanes V).

To investigate the phosphorylation of the nef protein, infected cells were labelled with 32P on the fourth day p.i. and cell extracts were immunoprecipitated using sera A and B. Although both sera immunoprecipitated the nef protein (as shown in Fig. 1a), no 32P-labelled protein corresponding in position to that of the nef protein was observed (Fig. 3b). The fact that gag products (p55, p40, p25 and p18) were phosphorylated (Veronese et al., 1988; Laurent et al., 1989) in these cells indicated that labelling conditions were optimal for in vivo phosphorylation. These results suggested that the nef protein was not phosphorylated in HIV-1-infected cells. It might be argued, however, that the level of incorporation of 32P into the nef protein was too low to be detectable by our assay conditions. For this reason we investigated the effect of BAP on the pI value of 35S-labelled nef protein. The effect of BAP treatment on the four subspecies (a, b, c and d) of p25 was used as a reference to monitor the dephosphorylation reaction. We have previously shown that, among the four subspecies of p25, b is the phosphorylated form of a, whereas d is the phosphorylated form of c (Laurent et al., 1989). Accordingly, BAP treatment led to a modification in the pattern corresponding to the four spots of p25, i.e. species d and b were dephosphorylated leading to an increased level of species c and a, respectively (as in Fig. 2 in Laurent et al., 1989). Under such dephosphorylation conditions, however, the pI of the nef protein was not modified (data not shown). Thus, these results confirmed that the nef protein produced during HIV-1 infection was not phosphorylated.

The sequence of the HIV-1 nef gene

To investigate the reason for the lack of phosphorylation of the nef protein, we isolated the nef gene of HIV-1 using PCR techniques. The nucleotide sequence of five different clones was found to be identical, thus suggesting that the HIV-1 was probably clonal. At the nucleic acid level, 10 mutations were identified compared to the original HIV-1_Bru isolate (Fig. 4a). These mutations occurred along the length of the nef gene, with the
Fig. 4. The sequence of the HIV-1 nef gene. (a) The sequence of the mutated nef gene (nef') from our HIV-1 stock compared to that of the HIV-1Bru isolate (Wain-Hobson et al., 1985). Only mutations in the nef gene are indicated by spots. (b) The deduced amino acid sequence of the mutated nef gene (nef') compared to that of the HIV-1Bru isolate. The single-letter code is used. The site phosphorylated by protein kinase C and the tetrapeptide sequence are indicated by a rectangle.

Fig. 5. The half-life of the mutated nef protein is reduced. BHK-21 cells were infected with vaccinia viruses containing either the wild-type (black columns) or the mutated (unshaded columns) nef gene. Cells were pulse-labelled (30 min) with [35S]methionine (time, 0 h), 4 h p.i., and the radioactive label was chased for 2 and 4 h (as indicated on the abscissa). Extracts were immunoprecipitated with serum A and samples were analysed by SDS-PAGE. The fluorograph was scanned by densitometry to quantify the band of the nef protein (the band which is myristylated; Guy et al., 1989). The values are expressed in arbitrary units (AU).

Expression of the mutated nef gene in vaccinia virus

We studied the expression of the nef gene by infection of BHK cells with VVnef (mutated) and compared it with that of the wild-type nef gene, also expressed in a vaccinia virus system (VVwt).

The products of both the wild-type and the mutated nef genes were found to be myristylated (data not shown). However, there were significant differences between the two proteins. Firstly, the half-life of the mutated nef gene product was 30 to 60 min whereas that of the wild-type nef protein was more than 4 h (Fig. 5); secondly, the wild-type nef was excreted whereas the mutated nef was not (data not shown); thirdly, in infected cells, the wild-type nef was phosphorylated and such phosphorylation was enhanced in the presence of the protein kinase C activator (Gould et al., 1985) 12-O-tetradecanoylphorbol-13-acetate (TPA), whereas no phosphorylation was observed for the mutated nef, either in the absence or presence of TPA (Fig. 6), indicating that protein kinase C phosphorylation was affected. These results confirm that the lack of phosphorylation and the short half-life of the nef protein observed during HIV-1 infection were due to the specific mutations (Fig. 4) of the nef gene.
The mutated nef protein is not phosphorylated by protein kinase C. BHK-21 cells were infected with vaccinia virus (VV) recombinants, either VVwt (lanes 1 and 3) or VVnef (lanes 2 and 4). Some cells were stimulated with 10 μg/ml TPA for 10 min (lanes 3 and 4) before labelling (2 h) with 32P. Other cells were not stimulated (lanes 1 and 2). All extracts were immunoprecipitated using serum A and analysed by SDS-PAGE and autoradiography. A doublet can be seen. The lower band corresponds to the protein kinase C-catalysed phosphorylation of the nef protein, whereas the upper band corresponds to that catalysed by autophosphorylation (Guy et al., 1987). The numbers on the right indicate Mr markers.

The mutated nef protein does not modify the expression of the CD4 antigen

FACS was employed in vaccinia virus-infected CEM cells to investigate the effect of the nef protein on the expression of the CD4 antigen (Guy et al., 1987). As we have shown previously, cell surface expression of the CD4 antigen was significantly reduced by the wild-type nef protein, whereas no modification occurred with the mutated nef gene product (Fig. 7). These results therefore illustrated that the mutated nef protein was devoid of a specific biological activity.

Synthesis of the nef protein in CEM cells infected with the original HIV-1 preparation

CEM cells were infected with the original HIV-1 preparation which was used to prepare our stock virus preparation. About 90% of cells were positive (by immunoenzymic staining assay) for the synthesis of viral proteins 6 days p.i. (Laurent et al., 1989). Cell death occurred on the seventh day p.i. The synthesis of nef protein was detectable on the fourth day p.i. in parallel with env and gag gene products (data not shown). Interestingly, the half-life of this nef protein was estimated to be 4 h (data not shown), identical to that of the wild-type recombinant nef protein expressed in the vaccinia virus system (Fig. 5). Furthermore, analysis of 35S-labelled nef protein by two-dimensional gel isoelectric focusing suggested that it was phosphorylated. The nef protein of the original HIV-1 preparation was resolved as two subspecies with slightly different pI values (data not shown).

Discussion

A highly cytopathogenic HIV-1 stock was generated by several consecutive passages of the original HIV-1_Bru isolate (Montagnier et al., 1984) on CEM T4 cells. Here, we report the biochemical and biological characterization of the nef protein synthesized during infection of CEM cells with this HIV-1. We show that the nef gene contains two important mutations, affecting phosphorylation of the phosphorylation site by protein kinase C and the putative GTP binding site which is responsible for proper folding of the nef protein. As a consequence of these mutations, the nef protein was not phosphorylated and its half-life was reduced. In addition, the mutated nef
protein was unable to down-regulate the expression of CD4 on the surface of CEM cells. These results indicate that some naturally acquired mutations of the nef gene produce a biologically inactive product. By such a strategy, a latent HIV might become virulent in seropositive individuals, but this latter possibility remains to be demonstrated. As several factors are involved in the regulation of HIV infection (Cullen & Greene, 1989), it should be emphasized that mutations of the nef gene could represent one of the ways by which HIV infection could be controlled; our results do not exclude modifications in the HIV-1 sequence besides those of the nef gene. Recently, Spire et al. (1989) have described a highly cytopathic strain of HIV-1 (HIV-1 NDK), the sequence of which is only slightly different from that of the Bru isolate. Interestingly, the nef gene of HIV-1 NDK contains exactly the same amino acid substitutions at the protein kinase C phosphorylation and putative GTP binding sites as we show here.

The correct folding of the nef protein probably involves two major putative nucleotide-binding domains, a glycine-rich sequence (KGLEG, amino acids 94 to 99) and a tetrapeptide sequence (WR/KFD, amino acids 183 to 186) related to the GXXXXGK and NKXD domains of the G proteins (Gilman, 1987; Guy et al., 1987, 1990a, b). Any mutations within these domains would modify the tertiary structure of the nef protein and thus impair its activity. The tetrapeptide sequence WRFD seems to be one of the sites susceptible to natural mutation, as aspartate was changed to an asparagine residue (Fig. 4). A similarly specific mutation has also been observed for the oncogene ras product p21 and the initiation factor EFTu (Lochrie & Simon, 1988; Hwang & Miller, 1987). In these cases, a 100-fold reduction in GTP binding activity and a change in nucleotide specificity have been reported with the mutated products.

The wild-type nef protein is phosphorylated by two distinct protein kinase activities at different sites: a site containing a threonine residue (amino acid 15), possibly phosphorylated by protein kinase C, and sites containing serine, phosphorylated by an autophosphorylation process (Guy et al., 1987, 1990a, b). The function of these phosphorylations of the nef protein is not yet clear. In vitro, replacement of the codon for threonine 15 by a codon for alanine abolishes phosphorylation by protein kinase C but does not affect the second phosphorylation (Guy et al., 1987). In the case of HIV-1, threonine 15 was found to be mutated into alanine (Fig. 4). This mutated protein was not phosphorylated by protein kinase C and it was also not phosphorylated at the serine sites (Fig. 6). As the mutated protein contained another important mutation in the tetrapeptide sequence, the site responsible for the optimal structure of the nef protein, then the lack of phosphorylation might be due to conformational changes in its structure. The mutated nef protein manifested no changes in its serine residues and it may therefore be considered that the mutation of threonine 15 is the most important of those affecting the phosphorylation sites of the nef protein. In accord with this, the nef protein of our HIV stock was found to be naturally mutated at threonine 15 into alanine, a substitution which was previously observed in other HIV isolates (Ratner et al., 1985; Alizon et al., 1986; Spire et al., 1989).

In HIV-1, 10 mutations of the nef gene were observed at the nucleic acid level (Fig. 4). These mutations occurred along the length of the nef gene, with the exception of a cluster of four mutations within a 22 bp region at nucleotide positions 414 to 465. The other significant mutations, from A to G (nucleotide 43) and from G to A (nucleotide 556), account for the changes at the amino acid level from threonine to alanine and from aspartate to asparagine, respectively. The cluster of four mutations has no significant effect at the amino acid level. However, these mutations are located within the hypothetical binding sites of factors which become associated with the nef protein. Preliminary results have indicated that mutations introduced within this region in vitro modify the binding of factors to the cis element responsive to nef (Ahmad & Venkatesan, 1988), within the negative regulatory element domain of the HIV long terminal repeat (LTR). Although the location of the nef cis-responding element of the HIV LTR is not yet clearly established (Niederman et al., 1989), our working hypothesis is that under latent infection conditions, transcription is blocked by a constitutive repressor which is displaced by activating factors appearing after stimulation of cells. The role of nef is to bind to these activating factors and thus maintain the repression of transcription (Guy et al., 1990b).

Several reports have suggested that the nef protein is a transcriptional silencer because of its capacity to down-regulate viral expression (Terwilliger et al., 1986; Luciw et al., 1987; Ahmad & Venkatesan, 1988; Niederman et al., 1989). In accordance with this, the replication of some strains of HIV-1 and HIV-2 was shown to be suppressed in cell lines expressing the nef protein (Cheng-Mayer et al., 1989). On the other hand, some contradictory results have been reported in which the expression of the nef protein was not correlated with a negative regulatory effect (Hammes et al., 1989; Kim et al., 1989). These contradictory results may be a consequence of differences between the in vitro assays performed and variation between the different cell lines employed. In addition our results point out that perhaps the most important criterion to be studied is the biological activity of the nef protein. Thus, any study on
the function of the nef gene should be carried out in parallel with a detailed study on the biochemical characterization of the nef protein. Such an investigation could be accompanied by study of the biological activity regulation of CD4 expression.

Attempts to isolate HIV from the same patient over a period of time might give isolates with increased virulence (Cheng-Mayer et al., 1988). This effect might be due, at least in part, to mutations of the nef gene. As we have demonstrated to be the case in cell cultures infected with HIV-1, consecutive culturing favours the selection of HIV clones which become adapted to optimal conditions of virus infection and production in vitro. Thus, by such a selection for the mutated nef protein, highly cytopathic viruses might be generated, a phenomenon which is indirectly in favour of a negative role of the nef protein in HIV replication.

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References


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