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Alteration of DNA topoisomerase II activity during infection of H9 cells by human immunodeficiency virus type 1 in vitro: a target for potential therapeutic agents

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Summary

Infection of H9 cells with human immunodeficiency virus type 1 (HIV-1) was found to decrease the phosphorylation of DNA topoisomerase II during the initial phase of infection. Simultaneously, with a later overshoot of phosphorylation and the subsequent activation of DNA topoisomerase II, the production of HIV-1 started. Applying three new protein kinase C inhibitors from the class of *O*-alkylglycerophospholipids we demonstrated that inhibition of protein kinase C-mediated phosphorylation of DNA topoisomerase II resulted in an inhibition of HIV-1 production. Based on the differential effect of the two protein kinase C activators, phorbol ester and bryostatin, we conclude that phosphorylation of DNA topoisomerase II is mediated by the form α and γ of protein kinase C. These data suggest that agents which inhibit these two forms of protein kinase C are also potential candidates for an anti-HIV therapy.

Hg cells; HIV-1; DNA topoisomerase II activity

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Introduction

At present the main strategies to develop chemotherapeutic agents to treat patients with acquired immune deficiency syndrome (AIDS) or with AIDS-related complex focus on inhibitors of replication of human immunodeficiency virus (HIV-1) (Sarin, 1988). However, since the discovery that the *tat* protein from HIV-1 can be taken up by cells where it might cause an altered expression of cellular genes (Frankel and Pabo, 1988), it is apparent that different cell-biological approaches intervening with the consequences of the HIV infection are equally promising. For one example, we previously established that the cellular 2′,5′-oligoadenylate/ribonuclease L system is closely coupled with HIV production (Schröder et al., 1989b). A stimulation of this cellular pathway, e.g. by mismatched double-stranded RNA (Montefiori and Mitchell, 1987) results in a delay of HIV release (unpublished data).

Increasing experimental evidence has accumulated that the protein kinase C-mediated signalling pathway is disturbed in HIV-infected cells (Kornfeld et al., 1988; Müller et al., 1989a,b). Moreover, inhibition of protein kinase C activity, e.g. by glycyrrhizin (Ito et al., 1988), 1-5-isoquinolinesulphonyl-2-methylpiperazine (Fields et al., 1988) or alkyl-lysophospholipid analogues (Kucera et al., 1989) proved to inhibit HIV production. The underlying molecular mechanism for this event is not fully understood. At present two possibilities are discussed: (i) inhibition of protein kinase C-mediated phosphorylation of HIV *rev* protein (Hauber et al., 1988), and (ii) inhibition of phosphorylation of DNA topoisomerase II (Müller et al., 1990). In the present study we used synthetic *O*-alkylglycerophospholipid analogues as tools to demonstrate the correlation between protein kinase C activity, topoisomerase II phosphorylation and HIV-1 production.

Materials and Methods

Materials

$H_3{}^{32}PO_4$ (10 Ci/l) was obtained from the Radiochemical Centre Amersham (U.K.); 12-*O*-tetradecanoylphorbol-13-acetate [TPA], 1,2-dioleoyl-rac-glycerol and L-α-phosphatidyl-L-serine from Sigma, St. Louis, MO (U.S.A.).

Bryostatin

Bryostatin was enriched from an extract of *Bugula neritina* as described (Pettit et al., 1987). The carbon tetrachloride fraction was used for the experiments. The animals were collected in Rovinj (Yugoslavia).

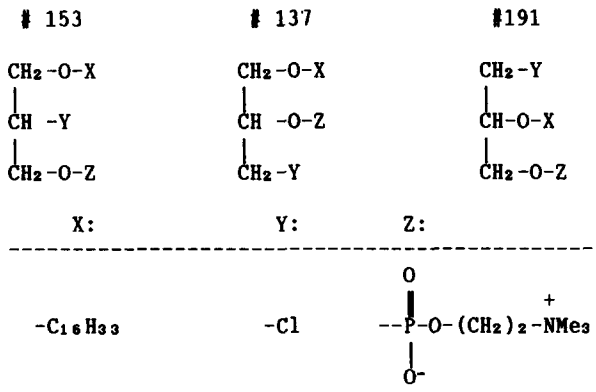
Synthetic O-alkylglycerolipids

The description of the synthesis of compounds Nos. 153, 137 and 191 has been published (Brachwitz et al., 1982, 1984 and 1987); the formulae are shown in Fig. 1.

Cell culture and virus

The culture conditions of the uninfected and HIV-1 [HTLV-IIIB]-infected H9 cells (human T-cell line, clone 9) were as described (Schröder et al., 1989b). H9 cells were maintained in RPMI 1640 medium containing 20% fetal calf serum. HIV-1 was obtained from culture fluids of virus producing H9 cells (Popovic et al., 1984). 5×10^5 H9 cells/ml were infected with 2×10^7 HIV-1 particles and cultivated for up to 4 days. Where mentioned, the compounds were added at day 0 (5 h after addition of the virus) to the uninfected or infected cultures. The compounds were dissolved in dimethyl sulfoxide at a stock concentration of 2 mM; final concentration of dimethyl sulfoxide was less than 0.1%.

The HIV reverse transcriptase activity in the culture fluids was used as a measure for virus production (Sarin et al., 1987). The reverse transcriptase activity in the culture supernatant after an incubation period of infected cells was 6.2×10^5 cpm/ml culture fluid. HIV-1 p17 and p24 protein expression was measured by an immunofluorescence technique using monoclonal antibodies as described (Sarin et al., 1987); the reactivity of the antibodies with HIV-1-infected H9 cells was 25%



Compound #153:

1-O-Alkyl-2-chloro-2-deoxy-rac-glycero-3-phosphocholine

Compound #137:

1-O-Alkyl-3-chloro-3-deoxy-rac-glycero-2-phosphocholine

Compound #191:

2-O-Alkyl-1-chloro-1-deoxy-rac-glycero-3-phosphocholine

Fig. 1. Formulae of the *O*-alkylglycerophospholipid analogues used in this study.

for anti-p17 and 37% for anti-p24. The values measured for reverse transcriptase activity and for p17- and p24-positive cells in the nontreated HIV-1-infected control cultures were set at 100%.

The cell concentration was determined electronically using the Cytocomp Counter (Model Michaelis). The IC_{50} (concentration causing 50% inhibition of cell growth) was estimated by logit regression (Sachs, 1974).

Preparation of nuclei and nuclear matrices

Nuclei were isolated from H9 cells by the method of Blobel and Potter (1966), except that 1 mM PMSF and 5 mM 2-mercaptoethanol were added to all the buffers used. Nuclear matrices were prepared by the described procedure (Comerford et al., 1986); they were stored at −20°C in 50 mM Tris-HCl buffer (pH 7.4; 250 mM sucrose, 5 mM $MgCl_2$, 1 mM EGTA and 1 mM PMSF). Of the matrix protein 118 μg corresponded to 10^6 nuclei.

DNA topoisomerase II and polyclonal antibodies

DNA topoisomerase II was isolated from calf thymus and purified to homogeneity. This preparation was used to raise polyclonal antibodies in rabbits (Rottmann et al., 1987).

Protein kinase C

This enzyme was purified to apparent homogeneity from brains of Sprague-Dawley rats as described (Wolf et al., 1985). The specific activity was determined to be 1250 units/mg at a protein concentration of 10 μg/ml.

The assay (total volume, 300 μl) was performed as described (Schröder et al., 1988). Enzyme activity was measured as phosphorylation of histone III-S (0.5 mg) in the presence of 0.5 mM $CaCl_2$, phosphatidylserine (2 μg) and 1.2-dioleoyl-rac-glycerol (0.1 μg). One and a half units of protein kinase C were added as the last component to the assays; the incubation was performed at 30°C for 5 min.

Phosphorylation of DNA topoisomerase II in intact cells

H9 cells (infected or noninfected) were routinely incubated in the standard assay for 1 h in the presence of $H_3{}^{32}PO_4$ (100 μCi/ml). In one control experiment the cells were preincubated with $H_3{}^{32}PO_4$ for 3 and 6 h. Then nuclei were isolated (Blobel and Potter, 1966) and extracted (4°C; 20 min) with a 50 mM Tris-HCl buffer (pH 7.4; 5 mM $MgCl_2$, 200 mM sucrose, 1 mM EGTA, 1 mM PMSF, 0.2 mM Na-tetrathionate, and 5 mM *N*-ethylmaleimide). The material was pelleted by centrifugation (2000 × *g*, 4°C, 10 min) and the pellet was processed to obtain the nuclear matrix (see above).

For the immunoprecipitation analysis, the fraction was precipitated with 10% trichloroacetic acid, extracted with acetone, and the topoisomerase II was immu-

noprecipitated with a rabbit polyclonal antiserum raised against DNA topoisomerase II and with protein A-Sepharose (Pharmacia), essentially as described (Müller et al., 1990). The immunoprecipitates were dissociated in gel sample buffer by boiling for 2 min and analyzed by gel electrophoresis (7% polyacrylamide gel containing 0.1% Na-dodecyl sulfate) and autoradiography.

The phosphorylation was quantitated by integration of the densitometry tracing obtained from the autoradiograms using a Shimadzu (CS-910/C-R1A) integrating densitometer.

Quantitation of DNA topoisomerase II

Nuclear matrix preparations were electrophoresed by Na-dodecyl sulfate-polyacrylamide (7.5%) gel electrophoresis (15 μg/slot). Subsequently, proteins were transferred to nitrocellulose and incubated with antiserum to DNA topoisomerase II. Specific antibody staining was detected by application of the 4-chloro-1-naphthol/hydrogen peroxide procedure (Rottmann et al., 1987; Schröder et al., 1989a). The amounts of immunocomplexes were estimated by scanning with an integrating densitometer (Shimadzu CS-910 (C-R1A)). The amount of the complex at day 0 was arbitrarily set at 1.00.

In control experiments it was established that antiserum which had been pretreated with purified topoisomerase II did not react with the 170 kDa protein band, corresponding to the DNA topoisomerase II (Rottmann et al., 1987; Schröder et al., 1989a) on the Western blot.

DNA topoisomerase II assay

The DNA topoisomerase II activity was assayed by relaxing negatively supercoiled plasmid pBR322 in the presence of ATP (Osheroff et al., 1983). Each reaction mixture (final volume 40 μl) contained 0.6 μg of negatively supercoiled circular pBR322 DNA and 1 mM ATP in a 10 mM Tris-HCl buffer (pH 8.0; 5 mM $MgCl_2$, 50 mM KCl, 50 mM NaCl, 15 μg/ml of bovine serum albumin). After incubation for 0–30 min at 30°C, reactions were terminated as described previously (Schröder et al., 1987) and samples were subjected to electrophoresis on 1% agarose gels. Gels were stained with ethidium bromide and DNA bands visible under UV light were photographed using a Polaroid 665 film. The percentage of relaxed DNA was quantitated by scanning densitometry (Osheroff et al., 1983; Schröder et al., 1987, 1989a).

Routinely, either 3×10^4 nuclei or 5 μg (with respect to protein) of nuclear matrix preparation were added to the standard assay.

Protein was measured as described by Lowry et al. (1951), using bovine serum albumin as a standard.

Results

Alteration of phosphorylation of DNA topoisomerase II

As reported previously the DNA topoisomerase II undergoes phosphorylation in intact cells, a process which can be monitored by incorporation of ^{32}P into the enzyme followed by immunoprecipitation (Rottmann et al., 1987; Müller et al., 1990). Here we applied the same procedure and found that immediately after infection of H9 cells with HIV-1, the extent of phosphorylation of nuclear matrix-associated DNA topoisomerase II decreased; at a later stage, the degree of phosphorylation increased again (Figs. 2, 3). Five days after infection, the extent of phosphorylation of DNA topoisomerase II in HIV-infected cells was determined to be 50% higher than in noninfected cells (Fig. 3). As a control, the antiserum against DNA topoisomerase II was adsorbed with purified DNA topoisomerase II prior to its application for the immunoprecipitation analysis. The results show that this antiserum preparation did not precipitate any material from the nuclear matrix (Fig. 2A; lane c). In a further control experiment, the cells (4 days after infection with HIV) were preincubated for 3 and 6 h (instead for 1 h) with ^{32}P prior to the immunoprecipitation procedure. The results revealed that after a preincubation procedure for 3 h (6 h) with ^{32}P the degree of phosphorylation of DNA topoisomerase II was 115% (105%), if the extent of phosphorylation of DNA topoisomerase II was set at 100%. This finding indicates that after an incubation

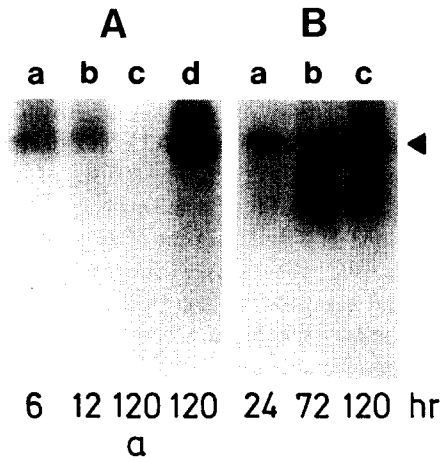


Fig. 2. Phosphorylation of DNA topoisomerase II in HIV-1-infected H9 cells. H9 cells were infected with HIV-1 for 6–120 h (A) or 24–120 h (B). One hour prior to harvesting the cells, $H_3{}^{32}PO_4$ was added to the cultures. Subsequently, nuclear matrix was prepared and DNA topoisomerase II was immunoprecipitated and analyzed by Na-dodecyl sulfate-gel electrophoresis and autoradiography; 1.5 μg of matrix preparation each was applied per slot. a: In one control the topoisomerase II-specific antiserum (0.8 mg/assay) was adsorbed with 10 μg of purified DNA topoisomerase II (5 h; 4°C) prior to its application for the immunoprecipitation analysis (Fig. 2A; lane c). Further details are given in Materials and Methods.

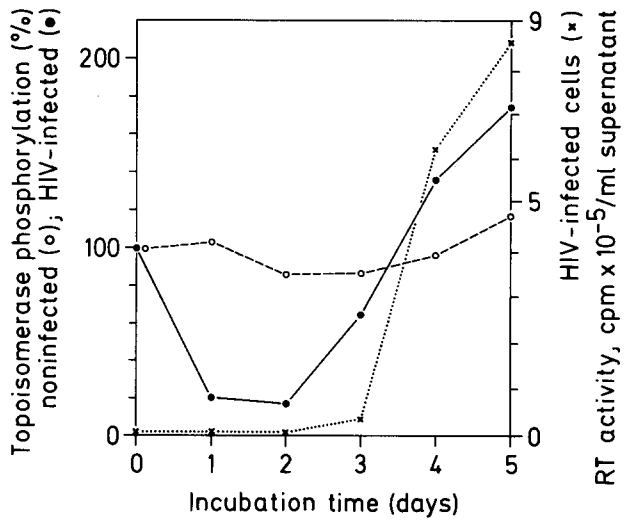


Fig. 3. Time course of phosphorylation of DNA topoisomerase II in noninfected (o---o) and in HIV-1-infected H9 cells (•—•). At different time periods after HIV infection the cells were incubated for 1 h with ^{32}P. Subsequently the nuclear matrix-associated DNA topoisomerase, labelled with ^{32}P, was immunoprecipitated and analyzed by Na-dodecyl sulfate gel electrophoresis and autoradiography as shown in Fig. 2. The extent of phosphorylation was quantitated by densitometric scanning of autoradiographs; the value at day 0 was set at 100%. In separate assays the reverse transcriptase (RT) activity as a measure for virus release from HIV-infected cells was determined in the culture supernatants (×....×). The average values of triplicate samples are plotted; the SD was less than 20%.

period of 1 h the ^{32}P-ATP pool was in an equilibrium.

In order to establish that the measured alterations in the extent of phosphorylation of nuclear matrix associated DNA topoisomerase II in HIV-infected cells are not due to a change in the concentration of the enzyme, the amount of DNA to-

TABLE 1

Determination of DNA topoisomerase II level in HIV-infected H9 cells

Time after infection (day)	Amount of DNA topoisomerase II (arbitrary units)
0	1.00
1	1.26
2	1.42
3	0.93
4	0.86
5	1.25

Nuclear matrices prepared from cells after different times after infection (15 μg of protein/slot) were electrophoresed, and the proteins were transferred to nitrocellulose and incubated with antiserum to DNA topoisomerase II. After staining the immunocomplexes, the band corresponding to DNA topoisomerase II was scanned. Further details are given under Materials and Methods. The means of quadruplicate experiments are presented; the SD varied between 15 and 24%.

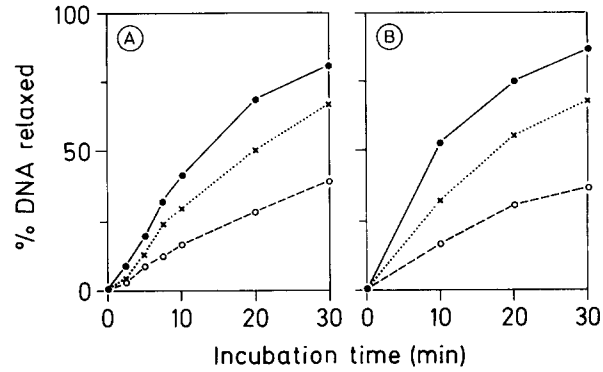


Fig. 4. Time course of relaxation of pBR322 DNA topoisomerase II present in whole nuclei (A) and nuclear matrix (B) from HIV-infected H9 cells. Subcellular fractions from cells infected with HIV for 0 days (×....×), 2 days (○---○) or 4 days (●—●) were used for the determinations. The assays were performed as summarized under Materials and Methods. The results represent means of 3 independent experiments; the SD was less than 10%.

poisomerase II was determined. As summarized in Table 1, the level of DNA topoisomerase II protein did not change substantially.

Time-course experiments revealed (Fig. 3) that the extent of phosphorylation of DNA topoisomerase II in uninfected cells remained unchanged during the entire incubation period of 5 days. However, the phosphorylation of this enzyme decreased drastically in HIV-infected cells at 1 day after infection: 24% of the value at day 0. The extent of phosphorylation increased again at 3 days after infection and reached a value of 175% as compared to the value obtained at day 0.

In parallel assays the reverse transcriptase activity in supernatants from HIV-infected cultures was measured. Almost simultaneously with the increase of phosphorylation of DNA topoisomerase II (at days 3–4), HIV production started (Fig. 3).

TABLE 2

DNA topoisomerase II activity in HIV-infected H9 cells at different periods of time after infection

Time after infection (day)	DNA topoisomerase II activity (arbitrary units)			
	Whole nuclei		Nuclear matrix	
	Untreated	Plus No. 191	Untreated	Plus No. 191
0	1.00	1.00	1.00	1.00
1	0.46	0.87	0.27	0.71
2	0.54	0.82	0.48	0.94
3	0.88	1.05	0.93	1.12
4	1.47	1.17	1.65	0.94
5	1.90	1.13	2.27	1.17

The determination of the enzyme activity was performed after an incubation time of 10 min. In one series of experiments the cells were incubated together with 3 μM of compound No. 191. Details of the experiments are given in the legend to Fig. 4. The means of 5 experiments are given.

Alteration of DNA topoisomerase II activity

It is known from the literature that after phosphorylation of DNA topoisomerase II in intact cells, the activity of this enzyme increases (Ackerman et al., 1985; Rottmann et al., 1987). The assay procedure based on the relaxation of negatively supercoiled plasmid pBR322 DNA was applied to determine the activity of DNA topoisomerase II.

As shown in Fig. 4, during the initial phase (0–10 min) of the enzyme reaction the kinetics are almost linear, while thereafter the curve levels off. If the activity of the enzyme (incubation period, 1 min) from both whole nuclei (Fig. 4A) and nuclear matrix of HIV-infected cells (Fig. 4B) at time 0 post-infection was set at 1.0, the activity of the whole nuclear DNA topoisomerase II dropped to 0.54 (matrix enzyme, 0.48) 2 days post-infection and increased thereupon at day 4 to 1.47 (1.65). These and additional kinetic data are also summarized in Table 2.

Inhibition of protein kinase C activity by O-alkylglycerophospholipid analogues

It was tempting to speculate that inhibitors of phosphorylation of DNA topoisomerase II might also serve as inhibitors of HIV production. More precisely, in light of earlier experiments which revealed that protein kinase C is involved in the phosphorylation of DNA topoisomerase II (Rottmann et al., 1987; Schröder et al., 1989a), inhibitors of this enzyme might be expected to serve as inhibitors of HIV production. The *O*-alkylglycerophospholipid analogues Nos. 153, 137 and 191 (see Fig. 1) were all found to achieve a dose-dependent inhibition of protein kinase C activity; at a final concentration of 19 μM the enzyme activity was inhibited by 74% (compound No. 153), by 86% (No. 137) and by 78% (No. 191), respectively (Table 3).

In parallel experiments, DNA topoisomerase II activity in HIV-infected cells

TABLE 3

Effect of *O*-alkylglycerolipids on protein kinase C activity

Compound (conc.)	Protein kinase C activity (nmoles ^{32}P incorporated)
None	7.2 ± 0.9
No. 153; 1.9 μM	5.7 ± 0.7
5.8	4.1 ± 0.5
19.0	1.9 ± 0.2
No. 137; 1.9 μM	6.4 ± 0.7
5.8	3.8 ± 0.5
19.0	0.9 ± 0.1
No. 191; 1.9 μM	3.1 ± 0.4
5.8	2.4 ± 0.3
19.0	0.7 ± 0.1

The assay conditions are described under Materials and Methods; the means (±SD) of 5 independent experiments are given.

(both whole nuclear activity and nuclear matrix activity) which had been incubated in the presence of 3 μM of compound No. 191 was determined (Table 2). The results revealed that the pronounced alteration of DNA topoisomerase II activity in response to HIV-1 infection did not occur in cells incubated with compound No. 191.

Effect of O-alkylglycerophospholipid analogues on HIV/H9 cells

The cytostatic activity of the *O*-alkylglycerophospholipid analogues was relatively low: the IC_{50} value varied between 28.8 μM (compound No. 191) and 44.7 μM (No. 153). In comparison, the inhibitory activity of the activators of protein kinase C, TPA and bryostatin was more pronounced (Table 4).

All three protein kinase C inhibitors tested (compounds Nos. 153, 137 and 191) displayed a significant anti-HIV activity (Table 4). At the optimal concentration (3 μM) the protective effect of the compounds was greater than 84%, the cells grew to a density of $>18.9 \times 10^5$ cells/ml (as compared to 22.4×10^5 for noninfected controls and 5.9×10^5 for HIV-infected controls). This effect is also reflected by (i) the strong inhibition of virus production (measured by reverse transcriptase activity) and (ii) the reduction of HIV-1 protein expression by the three compounds. In comparison, the protein kinase C activator TPA stimulated virus production,

TABLE 4

Effect of protein kinase C activators (12-*O*-tetradecanoylphorbol-13-acetate [TPA] and bryostatin) and inhibitors (compounds Nos. 153, 137 and 191) on HIV-1-infected H9 cells

Compound	IC_{50} (μM or μg/ml)	Compound concentration (μM or μg/ml)	HIV-1 added	Cell number (cells × 10^5/ml)	Percentage of		
					RT	p17	p24
None	–	–	–	22.4 ± 3.7	0	0	0
None	–	–	+	5.9 ± 1.2	100	100	100
TPA	0.43±0.04 μM	0.003 μM	+	5.1 ± 0.8	244	178	194
		0.01	+	6.2 ± 1.1	185	152	187
Bryostatin	1.94±0.18 μg/ml	0.01 μg/ml	+	5.7 ± 0.9	94	115	109
		0.1	+	5.3 ± 0.9	118	107	118
		0.3	+	6.4 ± 1.0	111	121	114
No. 153	44.7 ±4.9 μM	1 μM	+	11.2 ± 1.9	43	64	74
		3	+	19.3 ± 3.2	11	15	24
		10	+	8.6 ± 1.4	39	35	38
No. 137	36.1 ±3.4 μM	1 μM	+	7.4 ± 1.2	24	47	69
		3	+	18.9 ± 3.3	17	18	28
		10	+	13.3 ± 1.4	19	22	24
No. 191	28.8 ±2.6 μM	1 μM	+	12.4 ± 2.0	29	38	43
		3	+	21.3 ± 3.7	14	14	15
		10	+	17.8 ± 2.8	26	19	12

The cytostatic activity (IC_{50}), as well as the inhibitory effect on HIV-1 production, was measured by the following parameters: cytoprotection (cell concentration at the end of the 4 day incubation period), percentage of reverse transcriptase (RT) activity and percentage of cells expressing p17 and p24 *gag* proteins. The total incubation time was 4 days. The means of 5 parallel experiments are given.

an observation already reported earlier (Harada et al., 1988), while the other activator, bryostatin, did not affect virus release (Table 4).

Discussion

After binding of HIV-1 proteins to lymphocytes, the production of the second messengers inositol trisphosphate and diacylglycerol is drastically reduced during the first 30–60 s (Müller et al., 1989) followed by a rise of these molecules after 10 min (Kornfeld et al., 1988). From these findings it could be deduced that the activity of the diacylglycerol-dependent protein kinase C also changes in response to HIV infection. This enzyme is known to phosphorylate a series of molecules involved in the regulation of the responsiveness of cells to their environment (Sibley et al., 1987), i.e. the DNA topoisomerase II (Rottmann et al., 1987).

In the present study we focused our interest on the extent of phosphorylation of DNA topoisomerase II, a major component of the nuclear matrix (Berrios et al., 1985). For the enzyme studies we used the DNA relaxation assay. This assay can be used for measurement of both DNA topoisomerase I and II activity. Considering (a) the fact that nuclear matrix preparations contain only very low amounts of DNA topoisomerase I activity (Schröder et al., 1987) and (b) the major alterations in DNA topoisomerase activity in response to HIV infection (this paper) we did not see the reason to apply a more specific DNA topoisomerase II assay (i.e. decatenation/catenation assay). The results of the experiments, summarized in the present study, show that in the early phase of the infection cycle, up to 2–3 days post-infection the phosphorylation of DNA topoisomerase II is very low. However, from day 3 onwards, a strong rise of the phosphorylation of this enzyme is observed which parallels the production of HIV-1.

Since phosphorylation of DNA topoisomerase results in an activation of this enzyme (Rottmann et al., 1987), the activity of DNA topoisomerase II was determined during the course of HIV infection. The experiments demonstrated that the activity of this enzyme correlates well with HIV production. When DNA topoisomerase II activity was highest (4–5 days after infection), HIV production reached its maximum.

We then examined whether inhibition of the phosphorylation, and hence inactivation of DNA topoisomerase II, results in a reduction of HIV production. For these studies we used three synthetic *O*-alkylglycerophospholipid analogues (compounds Nos. 153, 137 and 191), previously also found to display cytostatic activity (Brachwitz et al., 1987). These compounds are potent inhibitors of protein kinase C, as shown in the present report. It was found that concentrations of these compounds (3 μM) which are one tenth of those required for 50% inhibition of cell growth achieved a greater than 80% reduction of HIV growth. Moreover, the overshoot of DNA topoisomerase II activity, which parallels HIV production, was prevented by these compounds. Since the three test compounds are not inhibitors of DNA topoisomerase II (to be published), it is tempting to speculate that prevention of protein kinase C-mediated phosphorylation of this enzyme resulted in

an inhibition of virus growth. On the other hand, inhibitors of DNA topoisomerase II such as coumermycin A1, are inhibitory to HIV replication in vitro (Baba et al., 1989), and this is also the case for arctigenin and trachelogenin (Schröder et al., submitted).

Next, we investigated whether activators of protein kinase C may enhance HIV growth. In accord with the published data (Harada et al., 1988) it was found that the tumor promoter TPA, which binds to all three forms of protein kinase C (Kraft et al., 1988), augmented HIV production. However, bryostatin which binds predominantly to the α form and γ form, but much weaker the β form of protein kinase C (Kraft et al., 1988), displayed no modulating effect on HIV growth. These results indicate a possible role for the protein kinase C (γ form)-mediated phosphorylation of DNA topoisomerase II in the control of HIV growth and thus identify a potential target for antiviral therapy.

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