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HIV-1 gp120 Envelope Protein Modulates Proliferation of Human Glomerular Epithelial Cells

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Abstract Glomerular epithelial cells (GEC) have been demonstrated to undergo morphological alterations in human immunodeficiency virus (HIV)-associated focal glomerulosclerosis. In the present study, we evaluated the effect of HIV-1 gp120 envelope protein on the growth of cultured human (H) GEC. gp120 protein enhanced ($P < 0.001$) the proliferation of HGEC at lower concentrations. The mitogenic effect of gp120 protein on HGEC was further confirmed by enhanced accumulation of proliferating nuclear cell antigen (PCNA) by gp120 protein-treated cells, as compared with control cells. On the contrary, gp120 protein at higher concentrations suppressed ($P < 0.001$) the growth of HGEC. To evaluate the mechanism of gp120 protein-induced HGEC growth suppression, we examined the effect of gp120 protein on HGEC apoptosis. gp120 protein at higher concentrations promoted the apoptosis of HGEC. At higher concentrations, gp120 protein also enhanced DNA fragmentation of HGEC. Anti-gp120 antibody attenuated the proliferative as well as the apoptotic effects of gp120 protein on HGEC. Because protein kinase C as well as tyrosine kinase inhibitors partially inhibited gp120-induced proliferation, gp120 appears to be activating both the protein kinase C and tyrosine kinase pathways. In addition, gp120 protein at lower concentrations enhanced mRNA expression of c-fos and at higher concentrations promoted mRNA expression of c-jun. We conclude that gp120 has a bimodal effect on proliferation of HGEC. This effect may be mediated through the activation of early growth genes. J. Cell. Biochem. 76:61–70, 1999.

Approximately 10% of patients with human immunodeficiency virus (HIV) infection are reported to develop chronic renal disease [Rao, 1991; Bourgoignie and Pardo, 1991; Vaziri et al., 1985; Rappaport et al., 1994]. Classic HIV-associated nephropathy (HIVAN) is a pan-nephropathy accompanied by abnormalities of glomeruli, tubules, and interstitium [Rao, 1991; Bourgoignie and Pardo, 1991; Vaziri et al., 1985]. In glomeruli, the initial abnormalities have been reported to involve visceral epithelial cells [Cohen and Nast, 1988, 1992]. In these reports, epithelial cells, in one or more segments of glomeruli, were found to be increased in number and were associated with mitotic figures [Cohen and Nast, 1988, 1992]. The affected epithelial cells were enlarged and coarsely vacuolated and contained protein reabsorption droplets. Involved capillary walls were also shown to be either collapsed or wrinkled, or both. These lesions eventually progressed to focal glomerulosclerosis. In addition to these descriptive morphologic abnormalities, there is a paucity of data showing the natural course of glomerulosclerosis in patients with HIV-associated nephropathy [Rao, 1991; Bourgoignie and Pardo, 1991; Vaziri et al., 1985; Rappaport et al., 1994]. Moreover, the role of glomerular epithelial cells in the development of glomerular lesions in HIV patients has not been well delineated [Rao, 1991; Bourgoignie and Pardo, 1991; Vaziri et al., 1985; Rappaport et al., 1994]. Nevertheless, glomerular epithelial cells have been demonstrated to play a pathogenic role in the development of glomerular disease in various experimental animal models [Fries et al., 1989; Ryan and Karnovsky, 1975; Bertani et al., 1982].

Kopp et al. [1992] developed an animal model of HIVAN. In this model, mice transgenic for HIV-1 genes developed glomerular lesions identical to those of HIV patients. Total kidney lysates from these animals showed the presence of a peptide identified by HIV-1 antisera specific to gp160 protein [Dickie et al., 1991];

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furthermore, when vaccinated with gp160 protein, these transgenic mice showed attenuation of the glomerular lesions [Shirai and Klinman, 1993]. The envelope glycoprotein of HIV-1 is produced from the enzymatic cleavage of gp160 into external gp120 and the transmembrane gp41 proteins [Capon and Ward, 1991]. gp120 remains noncovalently bound with gp41 on the outer envelope of the virus and is easily shed from the cell surface [Schneider et al., 1986]. The in vivo importance of the role of gp160 and gp120 proteins in the development of HIV-1-associated complications is supported by the findings that circulating gp120 is found in the sera of HIV-infected subjects [Oh et al., 1992]. Because HIV-1 has been reported to target immune cells through the binding of gp120 protein to CD4 receptors, gp120 protein appears to play a role in the invasion, as well as the progression, of HIV infection; moreover, cell membrane-associated gp120-anti-gp120 complexes have been reported on $CD4^+$ cells of HIV-1-seropositive patients [Amadori et al., 1992; Daniel et al., 1993]. We hypothesize that gp120 protein may also be playing a role in the induction of HGEC injury. This is consistent with the finding of the presence of CD4 receptors in HGEC [Kapasi and Singhal, 1998].

In the present study, we evaluated the direct effect of HIV-1 gp120 envelope protein on cultured human glomerular epithelial cell proliferation. Because HIV-1 gp120 protein at higher concentrations suppressed the growth of HGEC, we examined the effect of gp120 protein on HGEC apoptosis and necrosis. To confirm the effect of gp120 protein on HGEC growth, we also examined the effect of anti-gp120 antibody on gp120-induced HGEC proliferation. To evaluate the molecular mechanism of gp120 protein-induced HGEC proliferation and apoptosis we studied the effect of gp120 protein on the expression of early growth genes.

MATERIALS AND METHODS

Human Glomerular Epithelial Cells

Human glomerular epithelial cells (HGEC) were harvested as described previously [Ding et al., 1997; Garg et al., 1995]. Glomerular epithelial cells were identified by their characteristic polygonal shape and cobblestone appearance at confluence, sensitivity to low doses of puromycin aminonucleoside, and positive staining for heparan sulfate proteoglycan, vimentin, and WT-1, and negative staining for factor VIII [Ding et al., 1997; Garg et al., 1995]. HGEC were grown in 100-mm plastic Petri dishes containing 5 ml of incubation medium including RPMI 1640 (Gibco, Grand Island, NY), heat-inactivated fetal calf serum (FCS; Gibco), penicillin (50 U/ml, Gibco) and streptomycin sulfate (50 µg/ml, Gibco). Culture dishes were kept in a 95% air, 5% CO_2 environment at 37°C.

HIV-1 gp120 envelope protein and anti-gp120 protein antibody were obtained from Advanced Bioscience Laboratories (Kensington, MD). Orosomucoid gp α-1 acid (human, purified from Cohn fraction VI) was obtained from Sigma Chemical Co. (St. Louis, MO). HTLV-1 peptide envelope-$1^{191–214}$ (residues 191–214 from the HTLV external envelope) was obtained from the National Institutes of Health (NIH) AIDS Research and Reference Reagent Program, Ogden Bioservices Corporation, Rockville, MD). These peptides were further purified and tested for endotoxin contamination. Mouse monoclonal anti-proliferating nuclear cell antigen (PCNA, 36 kDa, cyclin) antibody was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

Proliferation Studies

Equal numbers of HGEC (10,000 cells) were seeded in 24-well plates and incubated in media containing 10% FCS and grown to subconfluence. Subconfluent cells were growth arrested for 48 h by incubating in RPMI containing 1% insulin, selenium, and transferrin (ITS) and 0.5% bovine serum albumin (BSA). Subsequently, cells were washed twice with phosphate-buffered saline (PBS) and reincubated in media containing either vehicle (control), or variable concentrations of HIV-1 gp120 protein (0.0001–0.1 µg/ml) for 48 h. Three to nine sets of experiments were carried out, each set in triplicate.

To confirm the role of gp120 protein in HGEC proliferation, equal numbers of growth-arrested HGEC were incubated in media containing either vehicle (control), gp120 protein (0.001 µg/ml), anti-gp120 protein antibody (5 µg/ml), or anti gp120 protein antibody (5 µg/ml) + gp120 protein (0.001 µg/ml) for 48 h. Four series of experiments were performed, each in triplicate.

To determine whether the effect of gp120 protein was specific or whether other viral or

nonviral proteins may also modulate HGEC proliferation, equal numbers of growth-arrested HGEC were incubated in media containing either vehicle (control), HIV-1 gp120 protein (0.001 μg/ml), HTLV peptide (0.001 μg/ml), or glycoprotein α-1 (0.001 μg/ml) for 48 h. Four sets of experiments were carried out, each in triplicate.

Protein kinase C (PKC) and tyrosine kinases have been demonstrated to play a role in signal transduction for the proliferation of a variety of cultured cells [Ganz et al., 1988]. To determine whether gp120 protein-induced HGEC proliferation is mediated by either PKC or tyrosine kinase, we evaluated the effect of H7 (1-[5-isoquinolinyl sulfonyl]-2-methylpiperazine; Sigma), a PKC inhibitor, as well as genistein (Sigma), a tyrosine kinase inhibitor, on gp-120 protein-induced HGEC proliferation. Equal numbers of growth-arrested HGEC were incubated in media containing either vehicle (control), gp120 protein (0.0001 μg/ml), H7 (10^{-6} M), genistein (10^{-6} M), H7 + gp120, or genistein + gp120 for 48 h. Three sets of experiments were performed, each in triplicate.

To determine whether this effect of gp120 protein was specific to HGEC, we evaluated the effect of gp120 protein on kidney fibroblasts (NRK-49F, Cat #CRL 1570; American Type Culture Collection, Rockville, MD). Equal numbers of kidney fibroblasts were incubated in medium containing either vehicle (control) or gp120 protein (0.0001 μg/ml) for 48 h. Three series of experiments were conducted, each in triplicate. At the end of the incubation period, cells were trypsinized and counted in a hemocytometer as described previously [Sharma et al., 1996].

Apoptosis Studies

Because higher concentrations of gp120 protein suppressed the growth of HGEC, we evaluated the effect of higher concentrations of gp120 protein on HGEC apoptosis and necrosis. Morphologic evaluation of apoptosis and necrosis was performed by staining the cells with Hoechst (H)-33342 (Sigma) and propidium iodide (Sigma). H-33342 stains the nuclei of live cells and identifies apoptotic cells by increased fluorescence: whereas propidium iodide costains the necrotic cells (pink color) [Singhal et al., 1998a,b].

Equal numbers of HGEC (10,000 cells/well) were seeded in 24-well plates. Cells were grown to subconfluence. Subsequently, cells were washed with PBS and incubated in media (RPMI + 1% FCS) containing either vehicle (control) or variable concentrations of gp120 protein (0.001–0.1 μg/ml) for 24 h. Four sets of experiments were carried out, each in triplicate.

To confirm the role of gp120 on HGEC apoptosis, equal numbers of HGEC (10,000 cells/well) were plated in 24-well plates and grown to subconfluence. Cells were washed and incubated in media (RPMI + 1% FCS) containing either vehicle (control), gp120 protein (0.01 μg/ml), anti gp120 protein antibody (5 μg/ml), or anti gp120 protein antibody (5 μg/ml) + gp120 protein (0.01 μg/ml) for 24 h. Four series of experiments were performed, each in triplicate.

To evaluate whether the effect of gp120 protein was specific or whether other viral or nonviral proteins may also modulate HGEC apoptosis, equal numbers of HGEC (10,000 cells/well) were plated in 24-well plates containing media (RPMI + 10% FCS) and grown to subconfluence. Subsequently, cells were washed with PBS and incubated in media (RPMI + 1% FCS) containing either vehicle (control), HIV-1 gp120 protein (0.01 μg/ml), HTLV peptide (0.01 μg/ml), or glycoprotein α-1 (0.01 μg/ml) for 24 h. Four sets of experiments were carried out, each in triplicate.

At the end of the incubation period, 1 μg/ml of H-33342 was added to the incubation media for 7 min at 37°C. Subsequently, cells were placed on ice and propidium iodide (final concentration, 1 μg/ml) was added to each well. Cells were incubated with dyes for 10 min on ice, protected from light, and examined under ultraviolet light. Eight random fields were counted in each well and the percentage of live, apoptotic, and necrosed cells was calculated for each field.

DNA Isolation and Gel Electrophoresis

Equal numbers of subconfluent HGECs were incubated in media (RPMI + 1% FCS) containing either buffer (control) or gp120 protein (0.01–0.1 μg/ml) for 24 h. At the end of the incubation period, cells were washed twice with PBS and lysed in DNA lysis buffer. DNA was extracted [Singhal et al., 1998a,b] and run on a 1.8% agarose gel and electrophoresed at 5 V/cm in 0.5 × TE buffer (Tris 10 mM; EDTA 1 mM,

pH 8.0) containing 10 µg/ml ethidium bromide [Singhal et al., 1998a,b].

Protein Extraction and Western Blotting

To determine the effect of gp120 protein on the accumulation of PCNA and to examine the effect of anti-gp120 antibody on gp120-induced PCNA accumulation, subconfluent growth-arrested HGEC were incubated in medium (RPMI + 1% FCS) containing either vehicle (control) or gp120 protein (0.001 µg/ml), anti-gp120 protein antibody (1 µg/ml), or gp120 protein + anti-gp120 antibody for 24 h. At the end of the incubation period, cells were lysed with lysis buffer and protein was assayed using a BCA kit (Pierce, Rockford, IL). A total of 20 µg of protein from each variable was separated on a 4–20% gradient polyacrylamide gel and blotted onto a nitrocellulose membrane using a Bio-Rad Western blotting apparatus (Hercules, CA). Nitrocellulose membranes were then processed for PCNA using mouse monoclonal anti-PCNA antibody (1µg/ml; Santa Cruz Biotechnology, Santa Cruz, CA). Membranes were reprocessed using horseradish peroxidase labeled secondary goat anti-mouse IgG (Santa Cruz Biotech.); blots were developed using enhanced chemiluminescence [ECL](Amersham, Arlington Heights, IL).

RNA Extraction and Northern Blotting

To determine the effect of gp120 protein on mRNA expression of c-fos and c-jun, equal numbers of growth-arrested HGEC were incubated in media (serum free) containing either vehicle (control) or variable concentrations of gp120 protein (0.001–0.01 µg/ml) for 10–20 min. At the end of the incubation period, cells were lysed and total RNA was extracted [Singhal et al., 1998a,b]. Northern blots were generated and probed with cDNA specific for c-fos and c-jun and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). Densitometric analysis was performed and the ratio of c-fos/c-jun: GAPDH was determined.

Statistical Analysis

Comparison of HGEC proliferation and apoptosis between control and experimental conditions was carried out by an unpaired Student's *t*-test. When more than two groups were involved, intergroup comparisons were performed by analysis of variance (ANOVA). A Newman-Keuls multiple range test was used to calculate a *q*-value. Results are represented as means ± SEM. The difference was considered statistically significant at $P < 0.05$.

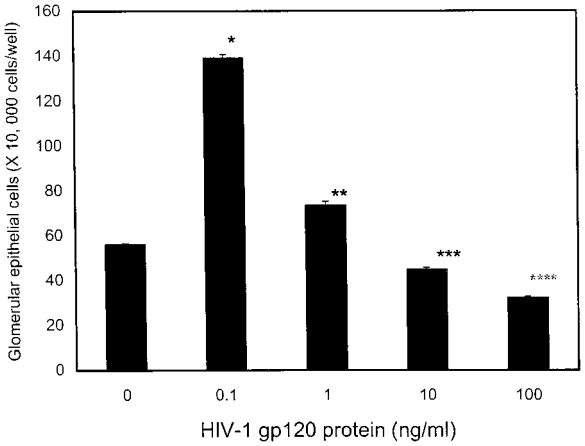

Fig. 1. Effect of gp120 protein on human glomerular epithelial cell (HGEC) proliferation. Equal numbers of growth-arrested HGEC were incubated in media (RPMI + 1% FCS) containing variable concentrations of HIV-1 gp120 protein (0–100 ng/well) for 48 h. At the end of the incubation period, cells were trypsinized and counted in a hemocytometer. Results (mean ± SEM) are from 3–9 sets of experiments, each carried out in triplicate. Intergroup comparisons were performed by analysis of variance. A Newman-Keuls multiple range test was used to calculate a *q*-value. *$P < 0.001$, compared with control (gp120 protein, 0 ng/ml) and 1.0–100 ng/ml; **$P < 0.001$ compared with control (gp120, 0 ng/ml) and gp120 protein 10–100 ng/ml; ***$P < 0.001$ compared with control (0 ng/ml) and gp120 protein, 100 ng/ml; ****$P < 0.001$, compared with control (0 ng/ml) and gp120, 0.1–10 ng/ml.

RESULTS

Proliferation Studies

The effect of gp120 protein on HGEC proliferation is shown in Figures 1 and 2. gp120 protein at lower concentrations (0.1–1 ng/ml) enhanced ($P < 0.001$) the proliferation of HGEC (control, 56.1 ± 0.4; gp120 protein, 0.1 ng/ml, 139.3 ± 1.4; gp120 protein, 1.0 ng/ml, 73.5 ± 1.8 × 10^4 cells/well). By contrast, gp120 protein at higher concentrations (10–100 ng/ml) suppressed ($P < 0.001$) the growth of HGEC (control, 57.6 ± 0.3; gp120 protein, 10 ng/ml, 44.9 ± 0.8; gp120 protein, 100 ng/ml, 32.1 ± 0.6 × 10^4 cells/well). These results suggest that gp120 protein has a bimodal effect on HGEC proliferation.

To confirm the role of gp120 protein on HGEC proliferation, we evaluated the effect of anti-gp120 antibody on gp120 protein-induced HGEC proliferation. As shown in Figure 3, anti-gp120 antibody inhibited ($P < 0.001$) the effect of

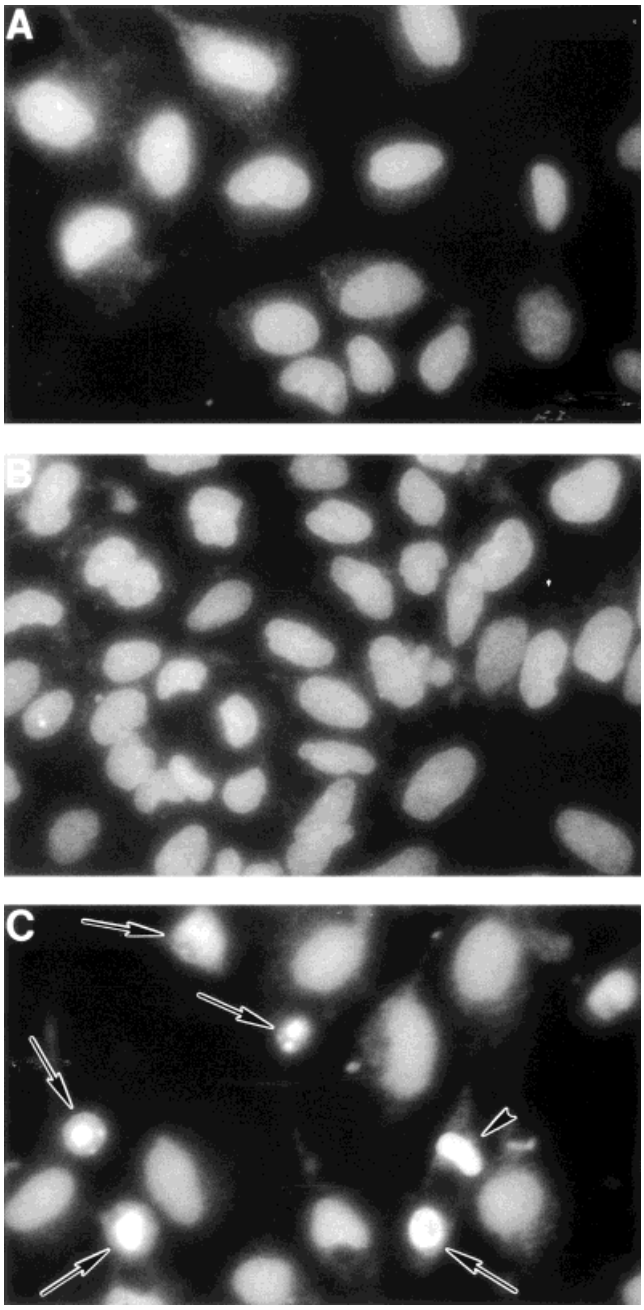

Fig. 2. Effect of gp120 protein on human glomerular epithelial cell (HGEC) proliferation and apoptosis. **A:** Control HGEC. **B:** A lower concentration (0.0001 μg/ml) of gp120 protein treatment enhanced the proliferation of HGEC. **C:** A higher concentration (0.1 μg/ml) of gp120 protein treatment promoted apoptosis (condensed or fragmented nuclei indicated by arrows) and necrosis (arrowhead) of HGEC. ×200.

gp120 protein on HGEC proliferation (control, 54.8 ± 0.5; gp120, 0.001 μg/ml, 74.8 ± 0.6; anti-gp120 antibody + gp120 protein, 55.3 ± 1.3×10^4 cells/well).

As shown in Figure 4, gp120 protein-treated HGEC showed a twofold increase in PCNA production, as compared with control cells. On the contrary, anti-gp120 antibody attenuated gp120-induced accumulation of PCNA by HGEC. These results further confirm the mitogenic effect of gp120 protein on HGEC proliferation.

To evaluate whether gp120 protein has a specific effect on HGEC proliferation we examined the effect of other viral and nonviral proteins on HGEC proliferation. HTLV peptide and nonviral α-glycoprotein did not modulate

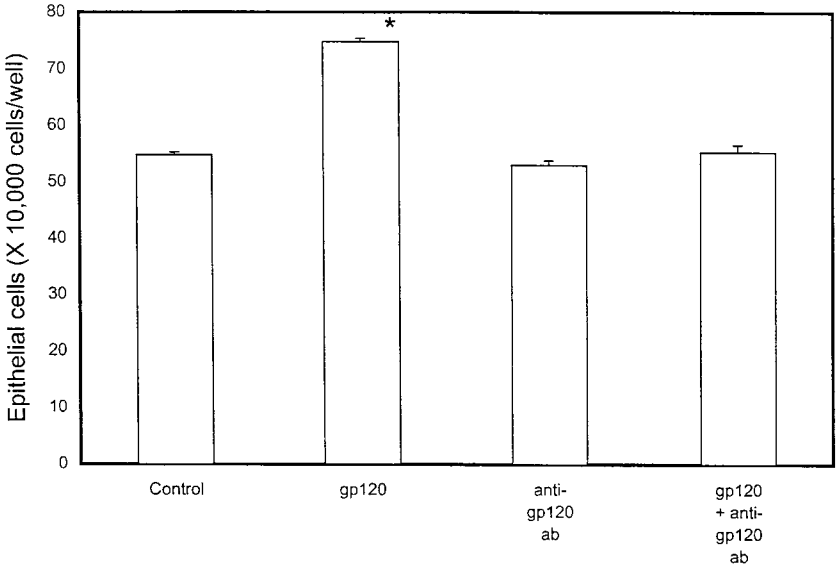

Fig. 3. Effect of anti-gp120 antibody on gp120 protein-induced HGEC proliferation. Equal numbers of growth-arrested human glomerular epithelial cells (HGEC) were incubated in media containing either vehicle (control), gp120 protein (0.001 μg/ml), anti gp120 protein antibody (5 μg/ml), or anti gp120 protein antibody (5 μg/ml) + gp120 protein (0.001 μg/ml) for 48 h. At the end of the incubation period, cells were trypsinized and counted in a hemocytometer. Results are from four series of experiments, each carried out in triplicate. Intergroup comparisons were performed by analysis of variance. A Newman-Keuls multiple range test was used to calculate a q-value. $*P < 0.001$ compared with control, anti gp120 ab, gp120 + anti gp120 ab.

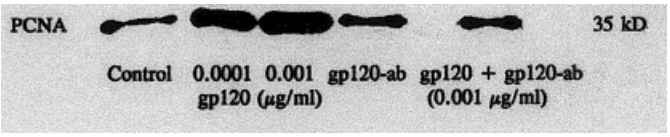

Fig. 4. Effect of gp120 protein on human glomerular epithelial cell (HGEC) accumulation of proliferating nuclear cell antigen (PCNA). Equal numbers of growth-arrested HGEC were incubated in media (RPMI + 1% FCS) containing either vehicle (control), gp120 protein (0.001 μg/ml), anti-gp120 protein antibody (anti-gp120 ab), and gp120 protein + anti-gp120 ab for 24 h. At the end of the incubation period, cells were lysed in lysis buffer, protein extracted, and probed with anti-PCNA antibody. gp120 protein enhanced the accumulation of PCNA. anti-gp120 antibody attenuated the effect of gp120 protein on HGEC PCNA accumulation.

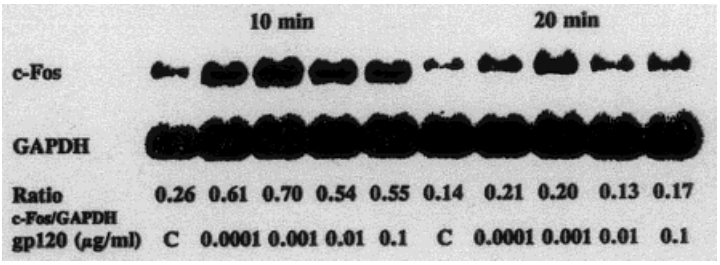

Fig. 5. Effect of gp120 protein on c-fos expression. The upper lane shows mRNA expression of c-fos by control and gp120 protein-treated cells at 10 and 20 min. The lower lane shows expression of GAPDH under identical conditions.

the proliferation of HGEC (control, 57.0 ± 0.1; gp120, 70.5 ± 0.5; HTLV peptide, 57.7 ± 0.41; α-glycoprotein, 57.5 ± 0.3 × 10^4 cells/well).

As shown in Figure 5, gp120 protein at lower concentrations (0.0001 and 0.001 μg/ml) promoted mRNA expression of c-fos by 2.3- to 2.7-fold (0.0001 μg/ml, 2.3- fold; 0.001 μg/ml, 2.7-fold), as compared with control cells at 10 min. These results suggest that c-fos may be playing a role in gp120-induced HGEC proliferation.

H7, a PKC inhibitor, as well as genistein (tyrosine kinase inhibitor) partly inhibited gp120 protein-induced HGEC proliferation (53.5 ± 0.8; gp120 protein, 130.3 ± 2.2; H7, 51.5 ± 0.6; H7 ± gp120, 75.6 ± 0.2; genestein, 53.4 ± 0.3; genestein + gp120, 71.0 ± 1.3 × 10^4 cells/well). These results suggest that gp120 protein-induced HGEC proliferation may be mediated through both PKC and tyrosine kinase pathways.

gp120 protein did not modulate the growth of kidney fibroblasts (control-HGEC, 52 ± 0.8; gp120-treated HGEC, 125.8 ± 0.8; control-kidney fibroblasts, 52.3 ± 0.4; gp120-treated

TABLE I. Effect of gp120 Envelope Protein on HGEC Apoptosis and Necrosis†

		gp120 protein (μg/ml)		
	Control	0.001	0.01	0.1
% apoptotic cells/field	0.83 ± 0.27	1.37 ± 0.67	12.32 ± 0.8*	21.96 ± 0.6*,**
% necrotic cells/field	0.82 ± 0.43	1.42 ± 0.57	4.07 ± 1.3	21.12 ± 2.1***

†Equal numbers of human glomerular epithelial cells (HGEC) were grown to subconfluence in 24-well plates. Subsequently, cells were washed and incubated in media (RPMI + 1% FCS) containing either vehicle (control) or variable concentrations of gp120 protein (0.001–0.1 μg/ml) for 24 h. At the end of the incubation period, cells were stained with H-34442 dye and propidium iodide. Live, apoptotic, and necrosed cells were counted in eight random fields under ultraviolet light. Results (mean ± SEM) are from four sets of experiments, each carried out in triplicate. Intergroup comparisons were performed by analysis of variance (ANOVA). A Newman-Keuls multiple range test was used to calculate a q-value.
*$P < 0.001$, compared with control and gp120 protein 0.001 μg/ml. **$P < 0.001$, compared with gp120 protein, 0.01 μg/ml. ***$P < 0.001$, compared with respective control and gp120 protein 0.001, 0.01 μg/ml.

kidney fibroblasts, 51.8 ± 0.6 × 10^4 cells/well). Because fibroblasts do not carry CD4 receptors, gp120 may not be able to trigger the downstream events.

Apoptosis Studies

The effect of gp120 protein on HGEC apoptosis is shown in Table I and Figure 2c. gp120 protein at higher concentrations (0.01–0.1 μg/ml) promoted ($P < 0.001$) HGEC apoptosis (control, 0.8 ± 0.3%; gp120, 0.01 μg/ml, 12.3 ± 0.8%; gp120, 0.1 μg/ml, 21.9 ± 0.6% apoptotic cells/field). gp120 protein at a concentration of 0.1 μg/ml also enhanced ($P < 0.001$) necrosis of HGEC (control, 0.8 ± 0.4%; gp120, 0.01 μg/ml, 4.0 ± 1.3%; gp120 protein, 0.1 μg/ml, 21.1 ± 2.1% necrotic cells/field, Table I).

Anti-gp120 antibody attenuated ($P < 0.001$) the effect of gp120 on HGEC apoptosis (control, 1.4 ± 0.6%; gp120, 0.01 μg/ml, 10.6 ± 0.9%; anti-gp120 antibody, 1.10 ± 0.66%; anti-gp120 antibody + gp120, 3.1 ± 0.6% apoptotic cells/field). These results further confirm the effect of gp120 on HGEC apoptosis.

To determine whether the effect of gp120 protein on HGEC apoptosis is specific, we studied the effect of other viral and nonviral proteins on HGEC apoptosis. HTLV peptide and glycoprotein α-1 did not modulate HGEC apoptosis, as compared with control cells (control, 0.90 ± 0.64%; gp120, 10.36 ± 1.1%; HTLV peptide, 2.24 ± 0.72%; glycoprotein α-1, 1.9 ± 0.29% apoptotic cells/field).

To confirm the effect of gp120 protein on HGEC apoptosis we evaluated the effect of gp120 protein on DNA fragmentation of HGEC. As shown in Figure 6, gp120 protein-treated HGEC showed integer multiples of 180 base pairs (bp)(ladder pattern). These results further confirm the effect of gp120 protein on

Fig. 6. Effect of gp120 on human glomerular epithelial cell (HGEC) DNA fragmentation. Agarose gel electrophoresis showing a molecular marker (**lane 1**) and control cells (**lane 2**). **Lanes 3–5**, gp120 protein-treated cells. gp120 protein at a lower concentration (0.001 μg/ml) did not show any DNA fragmentation (**lane 3**). gp120 protein at a higher concentration showed multiple integers of 180 base pairs (**lane 4**, gp120 protein, 0.01 μg/ml; **lane 5**, gp120 protein, 0.1 μg/ml).

HGEC apoptosis. As shown in Figure 7, gp120 protein at higher concentrations (0.01 and 0.1 μg/ml) promoted mRNA expression of c-jun by 4.3-fold (gp120, μg/ml) to 14-fold (gp120, 0.1 μg/ml) at 20 min, as compared with control cells.

DISCUSSION

The present study demonstrates that gp120 protein at lower concentrations directly stimulates the proliferation of HGEC. By contrast, gp120 protein at higher concentrations promotes apoptosis of HGEC. gp120 protein at higher concentrations also enhances HGEC DNA fragmentation. Anti-gp120 antibody attenuated both the proliferative and apoptotic

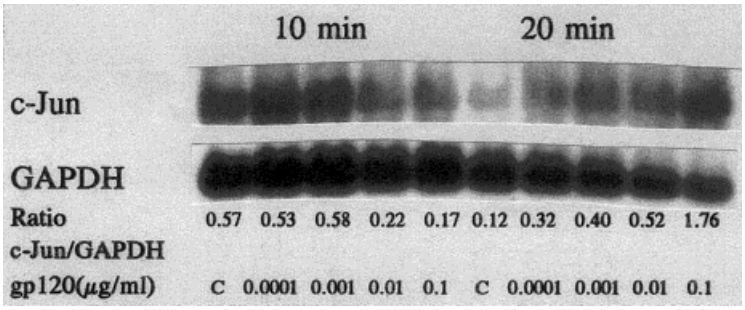

Fig. 7. Effect of gp120 protein on c-jun expression. The upper lane shows mRNA expression of c-jun by control and gp120 protein-treated cells. The lower lane shows expression of GAPDH under identical conditions.

effects of gp120 protein on HGEC, thus further confirming the bimodal effect of gp120 protein on HGEC growth. gp120 protein at lower concentrations enhanced the expression of c-fos and at higher concentrations promoted expression of c-jun.

The viral core protein, p24 is commonly found in the circulation during the course of HIV infection [Sydow et al., 1988]. Oh et al. [1992] measured gp120 protein in the serum of AIDS patients and found that it was higher (12–92 ng/ml) than that usually reported for p24 antigen. As gp160/120 has a fivefold higher molecular weight than p24, even if they were shed in equimolar amounts, their serum levels might be many-fold higher than p24. The possible mechanism for the release of a large quantity of gp160/120 into serum may be related to the shedding of gp160/gp120 from the surface of infected cells [Schneider et al., 1986]. In addition, Moore and Jarrett [1988] suggest that CD4 may be causing dissociation of gp120 from the surface virions. Moreover, endogenous antibodies might compete for gp120 from virions and from infected cells. In the in vitro setting as well, cells infected with HIV-1 shed gp120 protein into the medium in quantities greater than that of other viral proteins [Schneider et al., 1986]. In the present study, gp120 protein at a concentration of 1 ng/ml promoted significant proliferation of HGEC. Therefore, it is possible that circulating serum gp120 protein may be inducing a mitogenic effect on HGEC in patients with AIDS.

The common denominator in all forms of focal glomerulosclerosis including collapsing focal glomerulosclerosis, a hallmark of HIVAN, is the primary glomerular epithelial cell injury [D'Agati, 1994; Valeri et al., 1996; Detwiler et al., 1994]. As HIVAN is characterized by glomerular epithelial cell hyperplasia, the present study showing a mitogenic effect on HGEC is consistent with the hypothesis that HIV virus plays an etiologic role in the pathogenesis of HIVAN. Since a PKC inhibitor as well as a tyrosine kinase inhibitor partly inhibited the effect of gp120 on HGEC proliferation, it appears that gp120-induced HGEC proliferation may be mediated through PKC as well as tyrosine kinase pathways.

We previously reported that HGEC carry CD4 receptors, which are the specific target sites for gp120 proteins [Kapasi and Singhal, 1998]. In the present study, both H7 (a PKC inhibitor) and genestein (tyrosine kinase inhibitor) partly inhibited the gp120-induced HGEC proliferation. These results suggest that gp120-induced HGEC proliferation may be mediated by the activation of both PKC and tyrosine kinase pathways. These findings are consistent with the observations of other investigators in other cell lines [Gupta et al., 1994; Baldari et al., 1995; Chirmule and Pahwa, 1996; Kornfeld et al., 1988]. Several investigators have reported that the interaction of gp120 with CD4 receptors on T cells induced activation of nonreceptor tyrosine kinase, which activated downstream effect events, resulting in the activation of Ras [Baldari et al., 1995; Chirmule and Pahwa, 1996]. Besides the phosphorylation of intracellular substrates, addition of gp120 to CD4 triggered an elevation of the cytosolic concentration of calcium and the hydrolysis of phosphatidylinositol to inositol triphosphate and activation of PKC [Gupta et al., 1994; Kornfeld et al., 1988]. We propose that the interaction between CD4 and gp120 protein induces the activation of mitogen-activating protein kinase pathways (MAPKs) through activation of Ras and Raf-1. Both the c-fos and c-jun families of transcription factors are known as activator proteins (AP)-1 [Karin, 1995]. Various agents,

including growth factors, have been reported to activate AP-1 binding to DNA through MAPKs. The MAPKs are quite specific for their transcription factors. MAPKs phosphorylate Elk-1, which subsequently leads to the synthesis of c-fos [Karin, 1996], whereas stress-activated protein kinases (SAPKs) phosphorylate ATF2, another transcription factor, which leads to synthesis of c-jun [Kyriakis and Avruch, 1996]. In addition, gp120 interaction with CD4 may also activate an alternate pathway involving autophosphorylation of lck and an associated increase in its kinase activity, which may lead to increased levels of intracellular calcium and the activation of PKC and NFκB. The present studies indicate that gp120 protein at lower concentrations stimulates the expression of c-fos and at higher concentrations activates the expression of c-jun. We propose that gp120 protein at lower concentrations may be triggering the activation of NFκB and APKs and at higher concentrations may be activating SAPKs.

We conclude that gp120 protein has a bimodal effect on HGEC proliferation. The present study provides a basis for the hypothesis that HIV-1 proteins can directly modulate the function of HGEC and thus contribute to the development and progression of glomerular injury in patients with HIV infection.

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