HIV-1 envelope protein, gp120, has no effects on inositol phosphate production and metabolism in the Jurkat T-cell line either in the presence or absence of receptor stimulation

Martina T. Sumner*, Stephen B. Shears

Inositol Lipid Section, Laboratory of Signal Transduction, National Institute of Environmental Health Sciences, P.O. Box 12233, National Institutes of Health, Research Triangle Park, NC 27709, USA

Received 25 June 1997

Abstract We have used HPLC techniques to investigate the effects of gp120 upon inositol phosphate turnover in Jurkat E6-1 CD4$^+$ T-cells, to pursue previous reports that this viral coat protein: (a) inhibits receptor-activated inositol phosphate release; (b) stimulates basal inositol phosphate release; (c) inhibits inositol polyphosphate 5-phosphatase. Treatment of cells with up to 10 μg/ml gp120 from between 10 min and 24 h was without effect upon inositol phosphate turnover in both basal cells, and in C305 and OKT3 stimulated cells. This is the first report that biologically competent gp120 does not affect any aspect of inositol phosphate turnover in either basal or receptor-activated lymphocytes.

Key words: HIV envelope glycoprotein; Signal transduction; CD3/TCR complex; Inositol phosphate metabolism

1. Introduction

Circulating, free gp120 is believed to contribute to the non-infective actions of the HIV virus [1–3]. In particular, there is considerable interest in the possibility that gp120 can perturb the normal functions of peripheral CD4$^+$ T-lymphocytes by modulation of phospholipase C (PLC) activity. The latter enzyme hydrolyzes phosphatidylinositol (4,5)P_2 to yield two intracellular signals: diacylglycerol, which activates protein kinase C, plus Ins(1,4,5)P_3, which mobilizes intracellular [Ca^{2+}] [4,5]. Specifically, it has been reported that gp120 inhibits CD3-mediated production of inositol phosphates in the P28D T cell clone by up to 50% [6]. More recently, it was proposed that the latter effects arose from inhibition of receptor-dependent tyrosine phosphorylation of PLC-γ [7]. Thus, gp120 may contribute to the pathogenesis of HIV infection by decreasing the efficiency with which the occupied receptor activates PLC, thereby diminishing the proliferative response to antigen. To our knowledge, no independent laboratory has described an attempt to pursue the significance of this description of gp120-mediated perturbation of receptor-dependent inositol phosphate signalling. This was therefore our goal, but with the proviso that the most rigorous and sensitive procedures be employed, namely, HPLC separation and individual analysis of the full range of inositol polyphosphates that occur inside T-cells [8]. We chose the Jurkat E6-1 CD4$^+$ cell line for these experiments, as they are a well-characterized model for the study of PLC-dependent cell signalling and have been used extensively to study the interaction of gp120 and CD4 [2,9–13]. In addition, Jurkat cells are very permissive for HIV infection [13]. These cells also display appropriate receptor-mediated interactions of CD4 with the rest of the TCR complex [14].

Other workers have suggested there is an additional target of gp120 in T-cells, namely, inhibition of the inositol polyphosphate 5-phosphatase [15]; such an effect was believed to contribute to the elevated levels of these inositol polyphosphates observed in lymphocytes prepared from HIV-infected individuals, which would be anticipated to result in inappropriate cellular activation [15–17]. These ideas have been highlighted as being of considerable interest [18], but to date they have not been independently confirmed. We therefore investigated if we could detect any effect upon 5-phosphatase following the treatment of T-cells with gp120.

A third means by which gp120 has been reported to perturb inositol phosphate signalling in lymphocytes is by activation of receptor-independent (basal) PLC activity, leading to improper Ins(1,4,5)P_3-mediated Ca^{2+} signalling [19]. However, two other groups have been unable to reproduce these data [9,20]. On the other hand, the value of a description of gp120 failing to elicit a biological effect is critically dependent upon the viability of the protein being unequivocally demonstrated. This problem is illustrated by a report that several preparations of gp120 made available to the research community were biologically inactive [21]. Indeed, it has been a cause of some concern for many years that the extent to which a preparation of gp120 is glycosylated, and the methodology used to purify it, may both impact upon its activity [22,23]. When Kaufmann et al. (1992) reported that gp120 did not affect signalling in T-cells, they indicated that their gp120 still bound to CD4, but others have shown that this property can be retained by biologically inactive gp120 [21]. Our goal was to avoid these problems of interpretation, so when we investigated the effects of gp120 upon inositol phosphate signalling, we used a preparation of recombinant gp120 that has previously been shown to be biologically indistinguishable from viral gp120 [21,24,25]. We also utilized a range of bioassays to verify the activity of our gp120 preparations.

2. Materials and methods

2.1. Chemicals and reagents

[^{3}H]myo-inositol was purchased from American Radiolabelled Company (St. Louis, MO). [^{3}H]thymidine was purchased from New England Nuclear (Boston, MA). FCS and gentamicin were purchased from Gibco BRL Life Technologies (Grand Island, NY). Methionine sulfoximine was obtained from Sigma Chemical Company (St. Louis, MO). OKT3 antibody (an anti-CD3 antibody) was obtained from the

*Corresponding author. Fax: (1) (919) 541-3687.
E-mail: sumner1@niehs.nih.gov

PII S0014-5793(97)00880-6

Division of Cancer Treatment, National Cancer Institute, MD. C305 ascites was a kind gift from Dr. A. Weiss (University of California at San Francisco). C305 is an anti-TCR antibody which is largely dependent upon CD4 compared to OKT3 which is mostly independent of CD4. Human phycoerythrin-conjugated anti-CD3 and anti-CD4 antibodies were purchased from Becton Dickinson (San Jose, CA). Quantum Simply Cellular Microbeads Kit was purchased from Sigma Chemical Co. Adsorbosphere HPLC columns were purchased from Alltech Associates (Deerfield, IL). Ion-exchange resin (Ag 1-X8 200-400 mesh, formate form) was purchased from Bio-Rad (Melville, NY). Media (RPMI-1640, DMEM-S, GMEM-S) was kindly provided by the media preparation team at the National Institute of Environmental Health Sciences.

2.2. Cell culture

Jurkat E6-1 cells, obtained from Advanced Biotechnologies (Columbia, MD), were cultured in RPMI-1640 medium supplemented with 10% FCS and 50 μg/ml gentamicin. ADP240/17.1 Chinese hamster ovary cells, expressing the full length recombinant HIV_{IIIB} (gp120, see ref 25 for details), were obtained from Celltech Therapeutics Limited through the Medical Research Council AIDS Directed Programme Reagent Project (UK). ADP240/17.1 CHO cells were grown in 850 cm^2 roller bottles in GMEM-S media supplemented with 10% FCS and 200 μM methionine sulfoximine. When cells reached confluency, the media was changed to DMEM-S supplemented with 0.5% FCS. After 7 days the media was removed, clarified by 0.22 μm filtration and frozen at −30°C. This process was repeated until a sufficient volume (usually about 2 liters) was available for purification of gp120.

2.3. [^{3}H]-thymidine incorporation

Jurkat cells were plated at 5×10^5 cells/ml in a 24 well plate at a volume of 250 μl in triplicate. [^{3}H]thymidine (1 μCi/ml) was added to the cells and a time course (0, 0.5, 1, 2, 3 h) in the presence and absence of a 23 h pretreatment of 1 μg/ml gp120 was performed. The reaction was terminated by addition of 1 ml 10% TCA. The cells were vacuum filtered and washed 3 times with 5 ml 10% TCA. The radioactivity in the filter was determined by liquid scintillation.

2.4. Flow cytometry

Flow cytometry was performed using a FACStar^{PLUS} Fluorescence-Activated Cell Sorter (Becton Dickinson, San Jose, CA) equipped with a Coherent INNOVA 90 laser tuned to an excitation wavelength of 488 nm and operated at 200 mW. Fluorescence was determined using a 575/26 bandpass filter. Ten thousand events were collected per data file. Jurkat cells were washed in PBS and 10^6 cells were incubated with saturating concentrations of either anti-CD3 or anti-CD4 antibodies. The Quantum Simply Cellular Microbeads kit was used to quantitate the fluorescence.

2.5. Inositol phosphate assay

Jurkat cells were labelled with 50 μCi/ml [^{3}H]inositol for 96 h. At that time, medium was removed and the cells were washed twice in Krebs-Ringer buffer (115 mM NaCl, 5.4 mM KCl, 0.96 mM NaH_2PO_4, 1.35 mM $CaCl_2$, 0.6 mM $MgSO_4$, 11 mM glucose, 25 mM HEPES, pH 7.4) without added [^{3}H]inositol. Cells were further incubated in the presence or absence of OKT3, C305, and gp120 as described in the figures. Incubations were quenched with perchloric acid, neutralized with freon/octylamine and analyzed on an Adsorbosphere SAX HPLC column as described previously [26,27]. Radioactivity in the HPLC eluate was measured on-line [27]. The identification of inositol phosphate peaks was by co-elution with standards and by comparison with the published characteristics of this HPLC system [26,27]. Since cellular InsP_6 was unaffected by any of the experimental procedures employed in this study (Fig. 1 and data not shown), the levels of individual inositol phosphates were frequently normalized to the level of InsP_6 in order to compensate for any sampling variability that may have occurred.

2.6. gp120 purification

The gp120 was purified exactly as described by Moore et al., [25]: the frozen media containing gp120 was thawed and then loaded onto an affinity-purified sheep anti-human immunodeficiency gp120-I column. The column was washed using TBS (25 mM Tris, 2.7 mM KCL, 136 mM NaCl, pH 7.5). The bound glycoprotein was eluted with 2 M $MgCl_2$, gp120 was concentrated using Centriplus 30 concentraters and buffer exchanged with 20 mM Tris pH 7.5. Mg^{2+} concentration in the final gp120 buffer exchange was determined by using the fluorescent indicator dye mag-fura-2 and was found to be less than 35 mM (data not shown). Protein was stored at 4°C and used in experiments within one month. Purity was determined by SDS-PAGE analysis, visualized with diamine silver staining [10]. After transfer of proteins to Immobilon-P membrane, the blot was reacted with the D7324 Ab (an affinity purified sheep anti human immunodeficiency gp120 Ab) to detect the gp120. No degradation products were observed and gp120 migrated at approximately 120 kda (data not shown). The concentration of purified gp120 was determined by a Bradford assay and by an ELISA.

2.7. 5-Phosphatase assay on Jurkat T-cells

10^5 and 2×10^5 Jurkat cells were incubated in 100 μl of 0.2 mg/ml saponin, 20 mM HEPES, 100 mM KCl, 10 mM NaCl, 5 mM $MgSO_4$, 1 mM EDTA with or without OKT3 and/or gp120 for 5,10, 15 min at 37°C with 50 μM Ins(1,4,5)P_3 and 10000 dpm of [^{3}H] Ins(1,4,5)P_3. Reaction was stopped by addition of 30 μl of 2 M $HClO_4$ plus 1 mg/ml InsP_6, neutralized with 1.2 M KOH/75 mM HEPES/60 mM EDTA, and analyzed by gravity-fed ion-exchange chromatography as described [28].

3. Results

3.1. Biological activity of gp120

For our studies the species of gp120 that we have used is identical to that which several other groups have rigorously defined to be biologically indistinguishable from viral gp120 [21,24,25]. The recombinant and native proteins have similar affinities for CD4 [25] and similar effects on cell physiology [1,24]. We purified the gp120 exactly as first described by Moore et al., [25]. These workers previously verified that these purification procedures did not lead to any significant degradation of gp120, as assayed by SDS-PAGE and Western blots [25]. We used the same procedures to determine that the purity of our gp120 [[10], data not shown] was identical to that of Moore et al. [25].

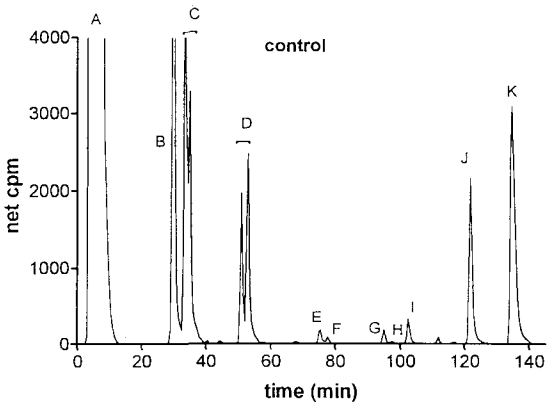
In order to optimize the biological activity of our prepara-

Table 1
Effect of acute treatment with gp120 upon inositol phosphate levels in control and antibody-stimulated Jurkat cells

	Ratio (+gp120/-gp120)							
	InsP_1	InsP_2	Ins(1,3,4)P_3	Ins(1,4,5)P_3	Ins(1,3,4,6)P_4	Ins(1,3,4,5)P_4	Ins(3,4,5,6)P_4	Ins(1,3,4,5,6)P_5
Control	0.99 ± 0.04	0.92 ± 0.06	0.89 ± 0.07	0.92 ± 0.07	1.23 ± 0.12	0.83 ± 0.07	1.05 ± 0.11	1.05 ± 0.03
C305	1.19 ± 0.14	0.95 ± 0.03	1.40 ± 0.48	0.68 ± 0.46	1.09 ± 0.03	1.01 ± 0.08	1.02 ± 0.05	1.01 ± 0.05
OKT3	1.02 ± 0.02	0.95 ± 0.06	1.03 ± 0.04	1.11 ± 0.06	1.06 ± 0.06	1.11 ± 0.07	1.15 ± 0.05	1.04 ± 0.02

Cells were labelled with [^{3}H]inositol for 96 h as described in the Methods (Section 2), except that either gp120 (1 μg/ml) or vehicle were present for the last 24 h of labelling. The culture medium was then replaced with HEPES-buffered Krebs solution, and fresh gp120 was added to the gp120-treated cells. After a further 10 min, cells were treated with OKT3 or C305 of vehicle for 10 min. Then the levels of inositol phosphates were determined as described in the Methods (Section 2). The results represent the ratios obtained in the presence vs the absence of gp120 (means ± standard errors) from four independent experiments (two in the case of C305).

tion of gp120, we frequently used it within one week of its purification, and we routinely discarded material that were more than one month old. Additionally, we employed several independent bioassays to confirm the viability of our preparations. We established that our gp120 reproduced (with similar potency) the stimulation, by other active gp120 preparations, of potassium channel activity in the plasma membrane of primary rat astrocytes [[24]; D.J. Benos, personal communication]. Additionally, our gp120 reproduced the results of a study by Dawson et al. [29], in that it had dose-dependent toxic effects upon mixed neocortical neuronal-glial cell cultures [L.-Y. Kong, personal communication]. Consistent with the idea that the latter effect is specifically neurotoxic, up to 23 h pre-incubation with 1 μg/ml gp120 has no appreciable effect on the DNA synthesis of Jurkat cells, as measured by [^{3}H]thymidine incorporation (data not shown). Finally, our gp120 increased the auto-phosphorylation of the protein



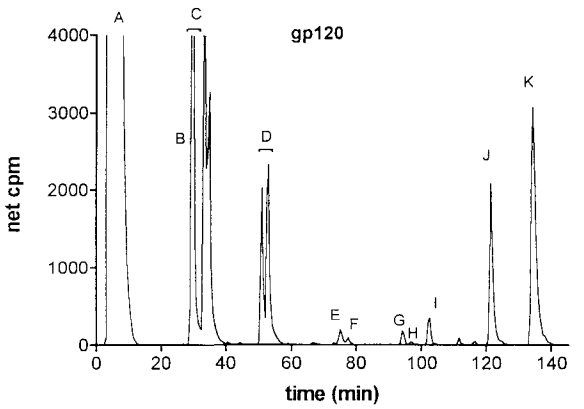


Fig. 1. HPLC elution of [^{3}H]inositol phosphates from basal Jurkat cells incubated either in the presence or absence of gp120. [^{3}H]inositol-labelled Jurkat T-cells were incubated with either 0.5 μg/ml gp120 or vehicle for 30 min, and then the intracellular inositol phosphates were analyzed by HPLC as described in the Methods (Section 2). The integrated c.p.m. for each peak is as follows (these are given in pairs, first for control cells (upper panel) followed by the data for gp120-treated cells (lower panel): peak A, inositol = 578406/580805; peak B, glycerophosphoinositol = 13721/13604; peak C, InsP_1 = 18166/18565; peak D, InsP_2 = 9083/9546; peak E, Ins(1,3,4)P_3 = 359/341; peak F, Ins(1,4,5)P_3 = 91/90; peak G, Ins(1,3,4,6)P_4 = 365/370; peak H, Ins(1,3,4,5)P_4 = 39/42; peak I, Ins(3,4,5,6)P_4 = 717/752; peak J, Ins(1,3,4,5,6)P_5 = 4882/4865; peak K, InsP_6 = 10796/10768.

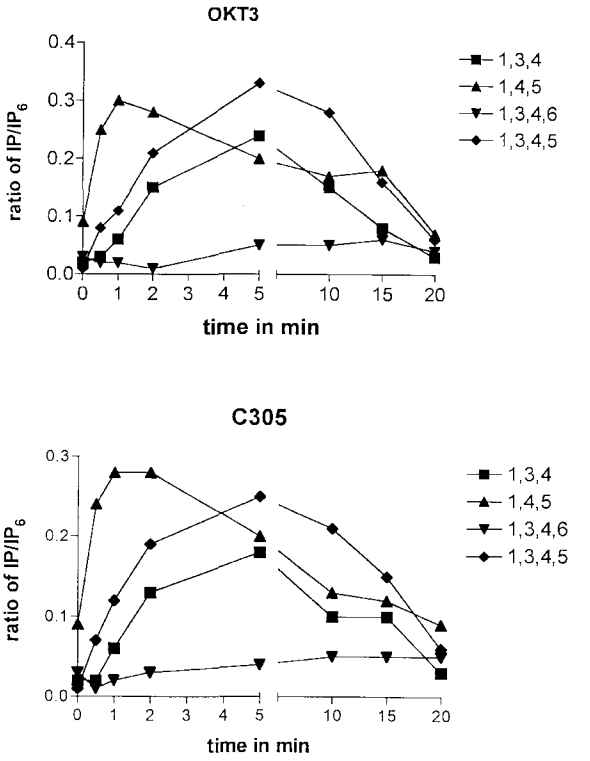


Fig. 2. Time course of inositol polyphosphate production in response to anti-CD3 antibodies OKT3 and C305 in Jurkat cells. Levels of inositol phosphates were determined as described in the legend to Fig. 1, for cells treated for the indicated time with either 10 μg/ml OKT3 (upper panel) or 1:1000 C305 ascites dilution (lower panel). Data shown are from a single data set for Ins(1,3,4)P_3 (■), Ins(1,4,5)P_3 (▲), Ins(1,3,4,5)P_4 (▼) and Ins(1,3,4,6)P_4 (◆).

tyrosine kinase p56^{lck} in a human monocyte cell line [10], in confirmation of several other reports [7,11,30]. We therefore conclude that our gp120 preparations have inherent biological activity, even though no single specific interaction appears to explain these various phenomena.

Many of the biological effects of gp120 arise from interaction of the viral coat protein with CD4. We therefore chose the CD4$^+$ Jurkat E6-1 cell line for our experiments. Jurkat cells have previously been used as model systems for studying both the gp120/CD4 interaction and HIV infection [13]. In addition, we used flow cytometry to assay the relative amounts of CD3 and CD4 receptor numbers (see the Methods in Section 2 for details). We estimated that our Jurkat cells expressed 28000 CD3 receptors/cell and about 6000 CD4 receptors/cell as assayed by antibody binding (data not shown; see the Methods in Section 2 for details).

3.2. *The effects of gp120 upon basal phospholipase C activity*

The most physiologically relevant assay for PLC involves the measurement of turnover of Ins(1,4,5)P_3 and its metabolites in intact cells that have been equilibrium-labelled with [^{3}H]inositol. Li$^+$ is widely used as a metabolic inhibitor to 'trap' newly-released inositol phosphates. Unfortunately, Li$^+$ has non-specific effects on signal transduction, including attenuation of Ins(1,4,5)P_3 signals, and elevation of basal levels of inositol phosphates [8]. The use of Li$^+$ in earlier studies [6,9,19] may have contributed to the inconsistent data on the effects of gp120 upon basal inositol phosphate metabolism

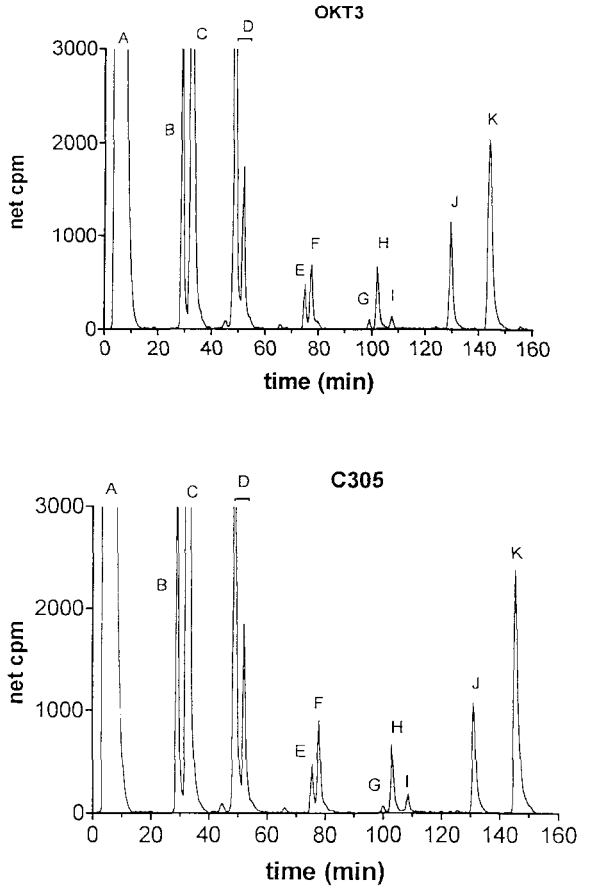


Fig. 3. HPLC elution of [^{3}H]inositol phosphates from Jurkat cells stimulated with either OKT3 or C305. Jurkat cells were labelled with [^{3}H]inositol as described in the legend to Fig. 1 and then stimulated with either OKT3 (10 μg/ml for 2 min) or C305 ascites (1:1000 dilution for 2 min). Inositol phosphates were analyzed by hplc as described in the Methods (Section 2). The integrated c.p.m. for each peak is as follows (these are given in pairs, first for OKT3 (upper panel) followed by the data for C305 (lower panel): peak A, inositol = 429 057/429 277; peak B, glycerophosphoinositol = 7530/7551; peak C, InsP_1 = 17 808/19 960; peak D, InsP_2 = 16 087/15 841; peak E, Ins(1,3,4)P_3 = 939/961; peak F, Ins(1,4,5)P_3 = 1953/2165; peak G, Ins(1,3,4,6)P_4 = 107/130; peak H, Ins(1,3,4,5)P_4 = 1557/1477; peak I, Ins(1/3,4,5,6)P_4 = 301/306; peak J, Ins(1,3,4,5,6)P_5 = 3152/3311; peak M, InsP_6 = 8298/8566.

(see Introduction). Rather than use Li^+, we improved assay sensitivity by high-specific activity labelling with [^{3}H]inositol. The concentration of free gp120 in HIV infected individuals can be as high as 1 μg/ml [31]. We investigated the effects of a variety of gp120 concentrations (0.05 μg/ml to 10 μg/ml) for relatively short times (10, 30, and 60 min, $n = 4$). In none of these experiments did we detect any significant effect of gp120 upon the levels of any inositol phosphate (Fig. 1 and data not shown). We also investigated the effect of incubating Jurkat cells with 1 μg/ml gp120 for 24 h ($n = 4$), and in this case the coat protein was added to the culture medium during the final 24 h of the labelling with [^{3}H]myo-inositol. Again, there was no significant effect upon the inositol phosphate profile (Table 1).

3.3. Receptor-mediated stimulation of PLC activity in Jurkat T-cells

An important goal of our study was to investigate whether gp120 would impede normal receptor-dependent activation of phospholipase C in T-cells, since this has previously been reported to be a property of the viral coat protein [6]. Therefore, we first checked that our Jurkat T-cells would exhibit the expected response to OKT3 antibody alone. We followed the protocol adopted by other workers who have studied inositol phosphate turnover in Jurkat T-cells [12,32,33] namely, we stimulated the cells with a saturating concentration of OKT3 antibody within 30 min of their transfer from the culture medium into serum-free medium. In such experiments we observed a characteristic 2-fold increase in intracellular levels of Ins(1,4,5)P_3, followed by 24-fold and 9-fold elevations in levels of its downstream metabolites, Ins(1,3,4,5)P_4 and Ins(1,3,4)P_3, respectively (Figs. 2 and 3). The degree of this stimulation compares favorably with earlier studies [12,32,33] and as such is testimony to the metabolic integrity of our cells. However, it was notable that the incubation of Jurkat cells for more prolonged times in the absence of serum, before the addition of OKT3, led to a decline in the ensuing response of PLC. After incubation for 1 h in the absence of serum the PLC response declined to about 50% of its initial, maximal value. Another factor that tends to desensitize the response of Jurkat cells to receptor-activation is internalization of the ligand-bound TCR receptor [12]. We minimized the consequences of these effects by generally completing all our experiments within 30 min of the removal of cells from the culture medium, and by always comparing incubations that contained gp120 with time-matched controls.

The OKT3 antibody recognizes epitopes on CD3-epsilon [34]. Other workers have shown that T-cell responses to anti-CD3-epsilon antibodies were virtually unaffected by anti-CD4 antibodies [32]. We therefore also used the anti-TCR antibody C305 as an alternative stimulus, since this is largely dependent upon CD4 [32]. When a saturating concentration of C305 was added to the Jurkat cells, the pattern of inositol phosphates produced was not significantly different from that initiated by OKT3, either in degree or in the time-course (Figs. 2 and 3). Note also that the prominent nature of the C305 response confirms that an appropriate number of functional CD3/CD4 co-receptor complexes were available on the cell surface.

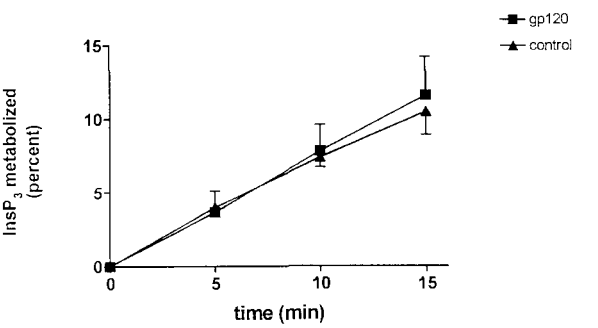


Fig. 4. Effect of gp120 treatment of Jurkat cells upon Ins(1,4,5)P_3 5-phosphatase activity. Jurkat cells were incubated in cell culture media in the presence (■) and absence (▲) of 1 μg/ml gp120 overnight, then washed three times in reaction buffer and incubated with 50 μM Ins(1,4,5)P_3 and a trace amount of [^{3}H]Ins(1,4,5)P_3. Inositol phosphates were separated by gravity-fed ion-exchange chromatography as described in Section 2.

3.4. The effects of gp120 upon receptor-activated phospholipase C activity

One group has reported that gp120 inhibits receptor-activated phospholipase C activity in lymphocytes [6,7]; as far as we are aware, this provocative result has not been pursued by other laboratories. We treated cells with a variety of gp120 concentrations (0.05 μg/ml to 10 μg/ml) for 10, 30, 60, and 120 min, and for 24 h, and we never observed a significant effect of gp120 upon either the C305-mediated or the OKT3-mediated increases in levels of inositol phosphates (Table 1). Note that the data in Table 1 were obtained after 10 min activation of PLC. We also did not detect any effects of gp120 upon the levels of inositol phosphates when we monitored the time-course of PLC activation from 30 s to 20 min (data not shown). The concentrations of gp120 that we used are within the range that Cefai et al. [6] previously proposed was sufficient to inhibit both PLC and the proliferative response to antigen in T-cells.

3.5. 5-Phosphatase assay on Jurkats

A specific 5-phosphatase which metabolizes Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 to physiologically inactive derivatives, is an important signaling 'off switch' and so its activity is tightly regulated [8,34]. Nye et al. [15,17] suggested that lymphocyte 5-phosphatase might be inhibited in HIV infected individuals, and they further indicated that this effect was brought about by exposure of T-cells to free gp120. If such an effect had occurred in our experiments, we would have anticipated detecting higher intracellular levels of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4 in gp120-treated Jurkat cells; no such effect was observed (Fig. 1; Table 1). Nevertheless, we also checked the effect of gp120 upon 5-phosphatase directly, by incubating Jurkat T-lymphocytes in the presence and absence of 1 μg/ml gp120 (2 or 24 h preincubation in cell culture media), either with or without 10 μg/ml OKT3 for 10 min ($n=4$). The cells were then rapidly permeabilized with detergent, and 5-phosphatase activity was immediately measured. We never observed an effect of gp120 upon 5-phosphatase activity, both when the concentration of Ins(1,4,5)P_3 was 50 μM in order to saturate the enzyme (Fig. 4) and when 5 or 15 μM Ins(1,4,5)P_3 was used (data not shown), which is below and at the K_m value for 5-phosphatase, respectively [8].

4. Discussion

Kornfeld et al. [19] reported that 2 μg/ml gp120 stimulated basal PLC activity in T-cells, thereby elevating InsP_3 levels 50-fold at 10 min. In contrast, we have demonstrated that gp120 has no significant effect upon inositol phosphate production and metabolism in the human Jurkat E6-1 cell line. Our conclusion that gp120 does not alter basal phospholipase C activity in lymphocytes (Fig. 1; Table 1) is in agreement with two other reports [9,20]. However, the new feature of our data that is of particular value is that we have used a preparation of gp120 that was rigorously demonstrated to possess inherent biological activity. The reason that we have been unable to reproduce the data published by Kornfeld et al. [19] may be because it was not gp120 that stimulated basal PLC activity in T-cells in their study, but a contaminant in their original preparation of coat protein. In this respect, it should be noted that the preparations used by Kornfeld et al. [19] were only 50% gp120, whereas our protein was purified to homogeneity [10]. Our data (Figs. 1 and 3; Table 1) also fail to provide any support for the proposal [15,17] that exposure of T-cells to free gp120 leads to an inhibition of the inositol polyphosphate 5-phosphatase. In view of our finding, we believe it is now of particular importance to consider alternative explanations to account for the observation [15,17] that T-cells from HIV-positive individuals may contain elevated levels of Ins(1,4,5)P_3 and Ins(1,3,4,5)P_4. This is not only relevant from the point of view of understanding the molecular mechanisms that underlie pathological actions of HIV, but in addition the reported effects upon 5-phosphatase were said to be of diagnostic value [15,17].

We have also shown that the incubation of T-cells for up to 24 h with gp120 has no effect upon receptor-activated PLC activity. This is a particularly important result because it is apparently the first to contradict an early report that a similar treatment with gp120 inhibited receptor-activated PLC activity in T-cells [6]. At present we cannot account for this difference in results. The authors of this earlier study [6] have recently further indicated that 16 h exposure to gp120 also inhibits receptor-dependent phosphorylation of PLC [7]. However, to achieve the latter effect, Hubert et al. [7] used supra-pathological concentrations of gp120 (30 μg/ml), considerably in excess of those employed in their earlier work (1–4 μg/ml) [6]. Even then, the estimated degree of the effect upon PLC phosphorylation was only 30% [7]. While the data by Hubert et al. [7] are consistent with reports by several laboratories, including our own, that $p56^{lck}$ kinase activity is perturbed by extracellular gp120 [10,11,30], our new data underscore the importance of conducting studies with pathologically-relevant concentrations of the coat protein. In conclusion, our study into the effects of biologically active gp120 makes a strong case that inositol phosphate signalling in T-cells is not generally targeted by this coat protein. We believe this is an important step forward towards the ultimate goal of understanding the contributions that gp120 makes towards the immunopathogenesis of HIV infection.

Acknowledgements: We are grateful to Dr. Marie Iannone at Glaxo-Wellcome for the FACS analysis.

References

[1] Habeshaw, J.A., Dalgleish, A.G., Bountiff, L., Newell, A.L., Wilks, D., Walker, L.C. and Manca, F. (1990) Immunol. Today 11, 418–424.
[2] Chirmule, N., Goonewardena, H., Pahwa, S., Pasieka, R., Kalyanaraman, V.S. and Pahwa, S. (1995) J. Biol. Chem. 270, 19364–19369.
[3] Pantaleo, G. and Fauci, A. (1995) Annu. Rev. Immunol. 13, 487–512.
[4] Berridge, M.J. and Irvine, R.F. (1989) Nature 341, 197–205.
[5] Altman, A., Coggeshall, K.M. and Mustelin, T. (1990) Adv. Immunol. 48, 227–360.
[6] Cefai, D., Debre, P., Kaczorek, M., Idziorek, T., Autran, B. and Bismuth, G. (1990) J. Clin. Invest. 86, 2117–2124.
[7] Hubert, P., Bismuth, G., Körner, M. and Debré, P. (1995) Eur. J. Immunol. 25, 1417–1425.
[8] Shears, S.B. (1992) in: Advances in Second Messenger and Phosphoprotein Research (Putney, J.W., Ed.), vol. 26, pp. 63–92, Raven Press, New York.
[9] Kaufmann, R., Laroche, D., Buchner, K., Hucho, F., Rudd, C., Lindschau, C., Ludwig, P., Höer, A., Oberdisse, E., Kopp, J., Körner, I.-J. and Repke, H. (1992) J. AIDS 5, 760–770.
[10] Hui, R., Curtis, J.F., Sumner, M.T., Shears, S.B., Glasgow, W.C. and Eling, T.E. (1995) J. Virol. 69, 8020–8026.

[11] Soula, M., Fagard, R. and Fischer, S. (1992) Int. Immunol. 4, 295–299.
[12] Desai, D.M., Newton, M.E., Kadlecek, T. and Weiss, A. (1990) Nature 348, 66–69.
[13] Cefai, D., Hadida, F., Jung, M., Debre, P., Vernin, J.-G. and Seman, M. (1991) AIDS 5, 1453–1461.
[14] Thome, M., Duplay, P., Guttinger, M. and Acuto, O. (1995) J. Exp. Med. 181, 1997–2006.
[15] Nye, K.E., Knox, K.A. and Pinching, A.J. (1991) AIDS 5, 413–417.
[16] Pinching, A.J. and Nye, K.E. (1990) Immunol. Today 11, 256–259.
[17] Nye, K.E., Riley, G.A. and Pinching, A.J. (1992) Clin. Exp. Immunol. 89, 89–93.
[18] Hughes, P.J. and Michel, R.H. (1993) Curr. Opin. Neurobiol. 3, 383–400.
[19] Kornfeld, H., Cruikshank, W.W., Pyle, S.W., Berman, J.S. and Center, D.M. (1988) Nature 335, 445–448.
[20] Horak, I.D., Popovic, M., Horak, E.M., Lucas, P.J., Gress, R.E., June, C.H. and Bolen, J.B. (1990) Nature 348, 557–560.
[21] Clouse, K.A., Cosentino, L.M., Weih, K.A., Pyle, S.W., Robbins, B., Hochstein, H.D., Natarajan, V. and Farrar, W.L. (1991) J. Immunol. 147, 2892–2901.
[22] Fenouillet, E., Gluckman, J.C. and Jones, I.M. (1994) Trends Biochem. Sci. 19, 65–70.
[23] Scandella, C.J., Kilpatrick, J., Lidster, W., Parker, C., Moore, J.P., Moore, G.K., Mann, K.A., Brown, P., Coates, S., Chapman, B., Masiarz, F.R., Steimer, K.S. and Haigwood L, N. (1993) AIDS Res. Hum. Retroviruses 9, 1233–1244.
[24] Bubien, J.K., Benveniste, E.N. and Benos, D.J. (1995) Am. J. Physiol. 268, C1440–C1449.
[25] Moore, J.P., McKeating, J.A., Jones, I.M., Stephens, P.E., Clements, G., Thomson, S. and Weiss, R.A. (1990) AIDS 4, 307–315.
[26] Glennon, M.C. and Shears, S.B. (1993) Biochem. J. 293, 583–590.
[27] Mennit, I.F., Oliver, K.G., Nogimori, K., Obie, J.F., Shears, S.B. and Putney, J.W. (1990) J. Biol. Chem. 265, 11167–11176.
[28] Shears, S.B. (1994) in: Methods in Toxicology, vol 1B, pp. 328–339, Academic Press.
[29] Dawson, V.L., Dawson, T.M., Uhl, G.R. and Snyder, S.H. (1993) Proc. Natl Acad. Sci. USA 90, 3256–3259.
[30] Hivroz, C., Mazerolles, F., Soula, M., Fagard, R., Graton, S., Meloche, S., Sekaly, R.P. and Fischer, A. (1993) Eur. J. Immunol. 23, 600–607.
[31] Oh, S.-K., Cruikshank, W.W., Raina, J., Blanchard, G.C., Alder, W.H., Walker, J. and Kornfeld, H. (1992) J. AIDS 5, 251–256.
[32] Newell, M.K., Haughn, J., Maroun, C.R. and Julius, M.H. (1990) Nature 347, 286–289.
[33] Guse, A.H. and Emmrich, F. (1991) J. Biol. Chem. 266, 24498–24502.
[34] Salmeson, A., Sanchez-Madrid, F., Ursa, M.A., Fresno, M. and Alarcon, B. (1991) J. Immunol. 147, 3047–3052.