HIV-1 gp160 decreases the K^+ voltage-gated current from Jurkat E6.1 T cells by up-phosphorylation

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Abstract HIV-1 gp120/gp160 is known to disturb the activity of $p56^{lck}$, protein kinase C (PKC) and Ca^{2+} homeostasis in T lymphocytes. We found that gp160 decreases the Kv1.3 current of Jurkat E6.1 cells probably by increasing the PKC-dependent phosphorylation of Kv channel protein after 5 days. This decrease is dose-dependent. In contrast, gp160 did not decrease the Kv1.3 current of the JCaM1.6 cell line, a $p56^{lck}$-defective Jurkat cell line. This shows that $p56^{lck}$ was at the beginning of the events which induced the Kv1.3 current decrease. As a consequence of this decrease, Jurkat E6.1 cells were depolarized and exhibited a volume increase.

Key words: Human T cell; Potassium channel; HIV-1 gp160; Protein kinase C; Membrane potential; $p56^{lck}$

1. Introduction

During human immunodeficiency virus (HIV) infection, there is an early impairment of T4 cell functions [1,2] followed by a decrease of the T4 cell number, although only a small number of these cells in the peripheral blood appears to be productively infected [3]. The HIV-1 envelope glycoprotein gp120 is shed from infected cells, and can cross-link the CD4 receptor of T4 uninfected cells [4,5] leading to T4 lymphocyte apoptosis, or anergy, or decreased proliferative response after TCR stimulation [6–9], resulting in their progressive disappearance from the blood [3,10]. Thus, HIV-1 modifies cytokine patterns and disables the transduction signal pathways of T cells [11–13]. HIV-1 induces dysfunctions of Src family protein tyrosine kinase (PTK, $p56^{lck}$), alterations of protein kinase C (PKC) and Ca^{2+} homeostasis [14–17].

PKC and Ca^{2+} are known to regulate the voltage-gated potassium channel activity which is involved in multiple functions of T cells [18,19]. N-type channels (called Kv1.3 channels since the cloning of the corresponding gene) regulate the membrane potential (E_m) of resting cells (−60 mV) and are implicated in the control of the cell volume [20]. After TCR/CD3 cross-linking, the Ca^{2+} cytosolic concentration increases, inhibits Kv1.3 channels and opens K^+-Ca^{2+}-dependent channels, resulting in membrane hyperpolarization (−90 mV [21]). This hyperpolarization, by maintaining a high driving force for Ca^{2+}, is necessary for the activation process [22]. In contrast, the inhibition of Kv1.3 channels depolarizes the cells and reduces the driving force for Ca^{2+}: in response to a mitogenic agent, the proliferation is reduced [23]. Kv1.3 channels can be inhibited by elements of the TCR signal transduction pathways: $p56^{lck}$, PKC and Ca^{2+} [18,19,24,25].

As HIV-1 gp120 disturbs PTKs, PKC and Ca^{2+}, three known inhibitors of Kv1.3 channels, we studied the effects of cell incubation with gp160, the precursor of gp120 on the Kv1.3 current, E_m value and cell volume.

2. Materials and methods

2.1. Cells

Jurkat E6.1 and JCaM1.6 cells ($p56^{lck}$-defective Jurkat cells, both from American Type Culture Collection (ATCC), Rockville, MD, USA), two $CD4^+$ human leukemic T cell lines, were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, L-glutamine (20 mM), penicillin (100 U/ml) and streptomycin (50 μg/ml) (all from Gibco-BRL, Cergy, France) and incubated at 37°C in a humidified atmosphere containing 5% CO_2.

2.2. Solutions and reagents

During patch-clamp experiments, cells were bathed with a Ringer-like saline solution (in mM): 160 NaCl, 4.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$ and 5 HEPES adjusted to pH 7.4 with NaOH. HIV-1 IIIB gp160 (purity 90%, from NIH, Rockville, MD, USA) was kindly provided by Dr. Lenaour (CEA, Fontenay aux Roses). 4-Aminopyridine (4-AP), herbimycin A, staurosporine, 12-*O*-tetradecanoyl phorbol 13-acetate (TPA) and okadaic acid (OA) were from Sigma. All effectors used were added to the bath.

2.3. Electrophysiology

Experiments were carried out using the whole-cell and the cell-attached recording modes of the patch-clamp [26]. Pipettes, filled with (in mM) 140 KCl, 5 NaCl, 10 EGTA and 5 HEPES, had a 5–10 MΩ resistance. Command potentials were corrected for the junction potential. Membrane currents were recorded with an Axopatch 1-D amplifier (Axon Instruments, Foster City, CA, USA) controlled by the pClamp 6.0 software (Axon Instruments).

K^+ currents were obtained in the whole-cell configuration by applied 1-s pulses from −120 to 0 mV in 30-mV steps. The holding potential was −60 mV. In Fig. 1A, the *I/V* curves were obtained by imposing a 500-ms ramp from −100 to +100 mV.

The E_m value was estimated by the method previously described by Verheugen et al. [27]. A −200 to +100 mV ramp of 500 ms was applied in the cell-attached configuration. The potential value corresponding to nil current was taken as a measure of $-E_m$.

Assuming a constant relation of 1 μF/cm^2, the membrane capacitance compensation allows the indirect measurement of the cell surface area [26].

2.4. Statistical analysis

Values are means ± S.E.M. Student's unpaired *t*-test was performed. $P < 0.05$ was considered significant.

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Abbreviations: HIV-1, human immunodeficiency virus type 1; PKC, protein kinase C; OA, okadaic acid; Stauro, staurosporine; TPA, 12-*O*-tetradecanoyl phorbol 13-acetate; E_m, membrane potential; 4-AP, 4-aminopyridine

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3. Results and discussion

3.1. Kv1.3 current characterization

Under patch-clamp conditions, Jurkat E6.1 and JCaM1.6 cells exhibited characteristics typical for the N-type outward K^+ currents due to the Kv1.3 channels from human T lymphocytes [28,29]. Both cell types showed the same outward K^+ current, which was activated at membrane potentials positive to −40 mV (Fig. 1A), and with an interpulse interval of 30 s (Fig. 1A,B). In the voltage-step protocol (Fig. 1B), the current rapidly increased (within 30 ms), followed by an exponential decrease with a half-inactivation time of 302 ± 9 ms for Jurkat E6.1 cells, and 327 ± 21 ms for JCaM1.6 cells, at 0 mV pulse. These currents were inhibited by 1 mM 4-AP (Fig. 1C), 10 mM TEA-Cl and CsCl (which replaced KCl in the pipette solution) and by using a 2-s interpulse interval (not shown). During the 5 days of the experiment, the peak amplitude of the Kv1.3 current for 0 mV pulse from both non-treated Jurkat E6.1 and JCaM1.6 cells was constant (respectively 292 ± 13 pA, $n = 130$, and 251 ± 63 pA, $n = 20$, Fig. 2A,B).

3.2. HIV-1 gp160 treatment and Kv1.3 current

For Jurkat E6.1 cells, HIV-1 gp160 affected the Kv1.3 peak current in a time- and concentration-dependent manner. gp160 at 25 μg/ml, a concentration close to saturation [30], progressively decreased the Kv1.3 current over 5 days (Fig. 2A). After 6 days, cells became hard to use for patch-clamp experiments, probably due to membrane structure modification. Thus, we analyzed the gp160 effect on Kv1.3 current after 5 days, at different concentrations (Fig. 2C). With concentrations < 0.25 μg/ml, gp160 had no effect on Kv1.3 current. In contrast, concentrations > 0.25 μg/ml decreased the Kv1.3 current by nearly 40% ($P < 0.01$). The calculated inhibition constant was 0.353 μg/ml. A concentration of 25 μg/ml had a saturating effect, and was used in all other experiments. These results indicate that gp160 decreased the Kv1.3 current only in maximal conditions: at a saturating concentration and after 5 days. In blood, where a lower concentration of gp160 can be detected [7], the process could be longer, except in lymphoid organs, where HIV accumulates and replicates actively despite a low viral burden and low to absent viral replication in human peripheral blood mononuclear cells (PBMC) [31]. As shown in Fig. 2D, the Kv1.3 current decrease was not due to a current kinetics modification: the two traces, one from non-treated, the other from gp160-treated cells, are perfectly superimposed after normalization to the peak current. Furthermore, the activation potential (−32 ± 1 mV, $n = 9$ and −34 ± 1 mV, $n = 10$) and the half-inactivation time did not vary (302 ± 9 ms, $n = 9$ and 297 ± 10,

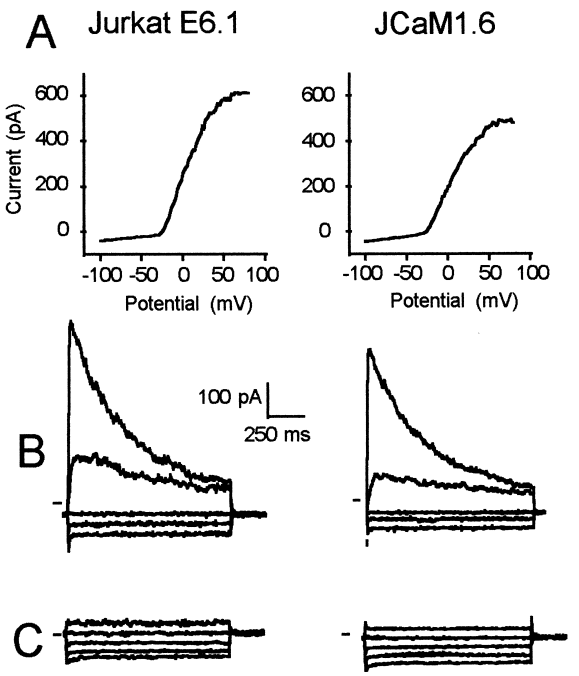

Fig. 1. Typical kinetics of Kv1.3 current from Jurkat E6.1 and JCaM1.6 cells. A: With a ramp protocol, cells exhibited an outward current for potential > −40 mV. B: With a step protocol, cells exhibited A-type current kinetics. C: Application of 4-AP (1 mM) to the bath inhibited the Kv1.3 current.

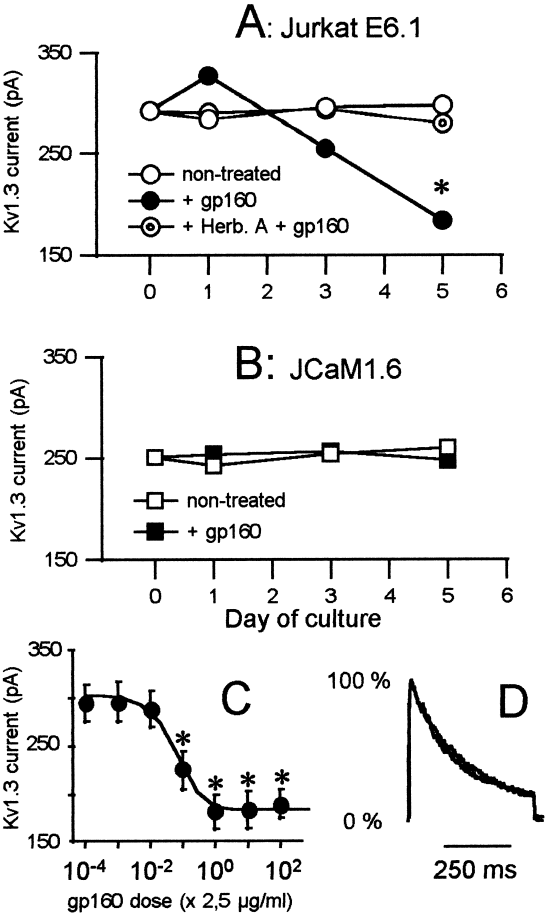

Fig. 2. HIV-1 gp160 effect on Kv1.3 peak current from Jurkat E6.1 and JCaM1.6 cells. A: Time-dependent effect of gp160 (25 μg/ml) without or with pretreatment by herbimycin A (Herb. A) (10 μM) on Kv1.3 peak current of Jurkat E6.1 cells at 0 mV, $20 < n < 30$. S.E.M. were omitted to clarify the figure, but were between 13 and 32 pA. B: Time-dependent effect of gp160 (25 μg/ml) on Kv1.3 peak current of JCaM1.6 cells at 0 mV, $5 < n < 10$, $48 <$ S.E.M. < 70 pA. C: Dose-dependent effect of gp160 on Kv1.3 peak current of Jurkat E6.1 cells at 0 mV after 5 days of culture, $n = 10$ cells for each point. D: Two Kv1.3 current traces for 0 mV membrane potential, one from a non-treated, the other from a gp160-treated Jurkat E6.1 cell, are superimposed after peak normalization. $^*P < 0.05$, significantly different from non-treated cell Kv1.3 peak current.

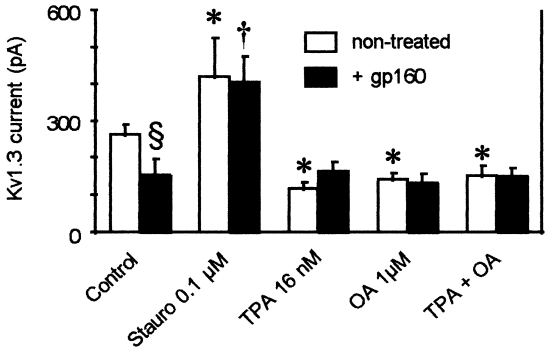

Fig. 3. Effect of phosphorylation-dephosphorylation effectors on non-treated and gp160-treated Jurkat E6.1 cell Kv1.3 peak current at 0 mV after 5 days of culture. Stauro = PKC inhibitor, TPA = PKC activator and OA = protein phosphatase 1 and 2A inhibitor. $n = 10$ for each bar. $^{\S}P < 0.05$, significant difference between non-treated and gp160-treated cell Kv1.3 peak currents in control conditions. $^{*}P < 0.05$, significant difference between corresponding treatment and control non-treated cell Kv1.3 peak currents. $^{\dagger}P < 0.05$, significant difference between corresponding treatment and control gp160-treated cell Kv1.3 peak currents.

$n = 10$, respectively in non-treated and gp160-treated cells). It is not clear why this process takes 5 days to reach the maximal effect. It is interesting to note that during HIV-1 infection, some phenomena take a long time to work: for example, Nokta et al. [17] show a PKC activity increase during 24 h post infection, followed by a net decrease between 4 and 7 days.

In contrast, gp160 (25 μg/ml) did not affect the Kv1.3 peak current from JCaM1.6 cells (Fig. 2B). $p56^{lck}$, a Src family protein tyrosine kinase, is associated with the CD4 receptor and is necessary for the TCR/CD3 signal transduction pathways [32]. Soula et al. [33] observed a rapid activation of $p56^{lck}$ following gp160 binding to CD4 on Jurkat cells. JCaM1.6 cells possess a non-active $p56^{lck}$, and could not be activated by TCR/CD3 or CD4 cross-linking [32]. The absence of an inhibitory effect of gp160 on JCaM1.6 cell Kv1.3 current shows that $p56^{lck}$ is necessary for the action of gp160. To confirm this result, we used herbimycin A, a Src family protein tyrosine kinase inhibitor in pretreatment, before gp160 treatment of Jurkat E6.1 cells. Herbimycin A (10 μM) did not increase the Kv1.3 current of non-treated cells in resting conditions (285 ± 23 pA and 305 ± 28 pA, respectively, before and after treatment, $n = 5$, data not shown); this shows that in resting conditions Kv1.3 channels are not tyrosine-phosphorylated. As shown in Fig. 2A, herbimycin A prevented the Kv1.3 peak current decrease induced by gp160. $P56^{lck}$ is also associated with the Fas receptor [25]. After Fas/Fas ligand interaction, $p56^{lck}$ was shown to be activated and to phosphorylate the Kv1.3 channel proteins, resulting in a Kv1.3 current decrease [25].

To explain the observed Kv1.3 current decrease of the 5 day-treated Jurkat E6.1 cells by gp160, we hypothesized first that this reduction was due to a $p56^{lck}$-dependent phosphorylation of Kv1.3 channels. In these conditions, when herbimycin A (10 μM) was added for 0.5–1 h before recordings, we favor tyrosine phosphatase activity, and we did not observe an increase of Kv1.3 peak current (184 ± 20 pA and 196 ± 25 pA, respectively, before and after treatment, $n = 5$, data not shown). Thus, the reduction of Kv1.3 current is probably not due to $p56^{lck}$ phosphorylation of Kv1.3 channels.

The putative synthesis of a new protein like β subunit is improbable to explain the Kv1.3 current decrease [34]. Even if Kv1.3 expressed in *Xenopus* oocytes were shown to be able to bind these proteins [34], this binding induces modifications of the current kinetics characteristics, which was not the case in the gp160 effect.

A Ca^{2+} inhibition of the Kv1.3 current also seems improbable, for two reasons: it should modify the current kinetics characteristics [18] and we never observed a Kv current run-up after the whole-cell configuration establishment, such as during Ca^{2+} chelation by the pipette EGTA.

The reduction of the physical number of Kv channels could be an explanation. During T lymphocyte maturation (thymocytes to active T lymphocyte states), the Kv channel number could vary from less than 100 to 700 unity [35]. This variation was not linked to the Kv1.3 mRNA level: in T lymphocytes, TCR/CD3 cross-link induced a Kv1.3 current increase and a Kv1.3 mRNA decrease [29]. In Jurkat E6.1 cells, this cross-link induced a decrease of the Kv1.3 current and the mRNA rate [29]. This reduction did not modify the current kinetics.

An alternative explanation could be the reduction of the number of active channels. The protein kinase-dependent phosphorylation could play a key role in the long-term modulation of ionic channels observed in various cells, with inhibition or activation effects according to the cell type [36,37]. PKC, whose activity is modified by gp160 pretreatment [17], was shown to reduce the N-type current of Jurkat cells without modification of the current kinetics [19]. Furthermore, JCaM1.6 cells, which are Jurkat cells with defective $p56^{lck}$, were not affected by gp160 treatment (Fig. 2B). Thus, gp160 needed an active signal transduction pathways to reduce the Kv1.3 current and did probably not directly affect the channel proteins.

Cai and Douglass [24] showed that PKC or a PKC-like

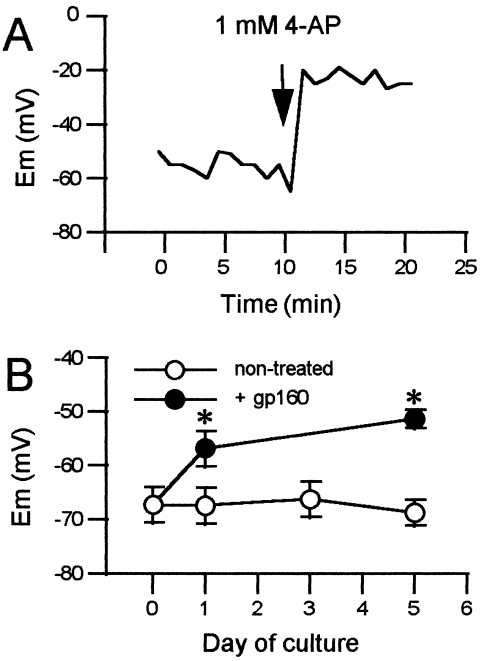

Fig. 4. Jurkat E6.1 cells membrane depolarization after 4-AP treatment (A) or HIV-1 gp160 25 μg/ml (B, $n = 15$ for each point). $^{*}P < 0.05$, significantly different from non-treated cell E_m.

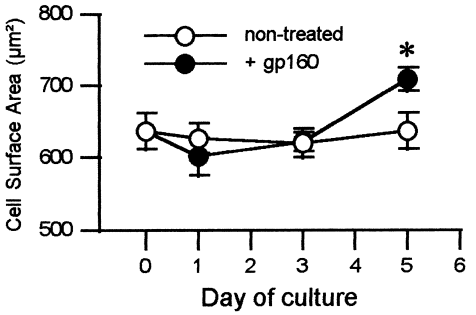

Fig. 5. HIV-1 gp160-induced cell swelling. $n = 30$ for each point. $^*P < 0.05$, significantly different from non-treated cell surface area.

enzyme could phosphorylate the Kv1.3 proteins, by using anti-Kv1.3 antibodies. Unfortunately, the presence of significant levels of endogenous PKC partly phosphorylated Kv1.3 proteins. Thus, they observed that a PKC activity increase, by addition of exogenous PKC, had little effect on Kv1.3 phosphorylation detected by anti-Kv1.3 antibodies. In our case, we used the patch-clamp technique to study the effect of PKC activity change on Kv1.3 current from non-treated and gp160-treated Jurkat E6.1 cells (25 μg/ml, 5 days).

In non-treated cells, staurosporine, a PKC inhibitor [19], increased the Kv1.3 current by 60% (Fig. 3). Staurosporine allowed the complete dephosphorylation of the channel protein [38]. Thus, in control conditions, one third of the Kv1.3 channels was 'disconnected'. In gp160-treated cells, staurosporine increased the Kv1.3 current by 164% and to an amplitude similar to that obtained with non-treated cells.

This result suggests that gp160-treated cells possess the same number of Kv1.3 channels as non-treated cells (same current amplitude after dephosphorylation) and that the observed current difference is due to an increase of the channel phosphorylation rate. This increase may be due to a more active phosphorylation process, or a less active dephosphorylation process. Staurosporine, by inhibiting the PKC activity, makes it possible to observe the phosphatase activity. The fact that staurosporine treatment results in the same current amplitude in non-treated and gp160-treated cells leads to the supposition that the dephosphorylation activity is intact. Thus, in gp160-treated cells, two thirds of Kv1.3 channels are phosphorylated.

TPA, a PKC activator [19], half-decreased the non-treated cells Kv1.3 current, to an amplitude closed to the gp160-treated amplitude current (Fig. 3). OA, a phosphatase inhibitor, did likewise (Fig. 3), which favors that phosphorylation of channel protein by PKC decreased the current at the same rate. In contrast, TPA and OA had no effect on Kv1.3 current from treated cells (Fig. 3). These results suggest that in gp160-treated cells, the Kv1.3 channels could be up-phosphorylated by PKC in comparison with non-treated cells. These results are partly in opposition with a previous work which described a PKC activity increase after 24 h of HIV infection, followed by a decrease after a longer time in lymphocytes [17]. Our experiments were done without infection, and we may hypothesize that other viral proteins could modulate PKC activity or induce a reduction of the protein number. Thus, a HIV-1 gp41 derivative polypeptide was shown to inhibit PKC activity and PKC-dependent immune function in human PBMC [39].

3.3. Membrane potential value and cell size variation of Jurkat E6.1 following HIV-1 gp160 treatment

To verify that Kv1.3 channels are implicated in E_m regulation, we tested the effect of 4-AP on E_m value. 4-AP, a voltage-dependent K^+ channel blocker [40], induced a net depolarization of Jurkat E6.1 cells ($\Delta E_m = -40$ mV, Fig. 4A) and we show that it completely inhibited the Kv1.3 current (Fig. 1C). These results confirm that the Kv1.3 current is implicated in E_m value control. Without reagent, the E_m value was constant during the 5 days of the experiments (Fig. 4B). This value (mean $= -67.3 \pm 3.2$ mV, $n = 23$) is in agreement with those obtained on T lymphocytes with the same technique [27] and with fluorescent probes [21,23]. Addition of gp160 to the cell culture significantly decreased the E_m value ($\Delta E_m = 10.6$ mV, $P < 0.05$) after 24 h (Fig. 4B). After the following 5 days, the depolarization increased slightly ($\Delta E_m = 16.1$ mV, $P < 0.01$). The fact that the observed depolarization appeared rapidly shows that in gp160-treated cells, Kv1.3 channels were not the sole E_m value controller. Thus, the opening of anionic channels is known to be depolarizing [20]. The proliferative response is largely dependent on the E_m value. Like charybdotoxin-induced depolarization [23], the observed gp160-induced depolarization could result in a decreased proliferation after TCR/CD3 cross-linking, by reducing the Ca^{2+} driving force.

Like the E_m value, gp160 treatment of Jurkat cells modifies their size. The non-treated cells had a cell surface of 627 ± 21 μm^2 ($n = 130$), and were constant in size during the 5 days of measuring (Fig. 5). The gp160-treated cell size increased significantly by 25% only after 5 days, corresponding to a 40% volume increase, and seemed to follow the Kv1.3 current decrease (Fig. 5). In fact, cell volume is an equilibrium between shrinking and swelling [41]. Thus, the two process are concomitant. Kv and Cl channels, by their opening, allow the cell to shrink, by a net loss of KCl and water [20]. We hypothesize that gp160, by reducing the Kv current, does not allow the necessary efflux of K^+ and the reduction of cell volume.

In conclusion, we show that HIV-1, without infection and only by the interaction of its envelope glycoprotein gp160 with the CD4 receptor on T cell surface, can induce 'membrane disorder' by up-phosphorylation of the Kv1.3 channel proteins and membrane depolarization, in addition to 'cytoplasmic disorders' (modified PKC activity, altered Ca^{2+} concentration variation). It seems that gp160 acts in a different way on Jurkat E6.1 cells compared to the effect of gp120 on astrocytes [42], even if the result in both cases is a progressive depolarization. In astrocytes, this depolarization leads to the opening of Ca^{2+} voltage-gated channels and results in cell death by Ca^{2+} neurotoxicity [42]. In contrast, in Jurkat E6.1 cells, this depolarization probably reduces the Ca^{2+} driving force and the proliferation.

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References

[1] Miedema, F., Petit, A.J., Terpstra, F.G., Schattenkerk, J.K., de Wolf, F., Al, B.J., Roos, M., Lange, J.M., Danner, S.A. and Goudsmit, J. (1988) J. Clin. Invest. 82, 1908–1914.

[2] Fauci, A.S., Pantaleo, G., Stanley, S. and Weissman, D. (1996) Ann. Intern. Med. 124, 654–663.
[3] Brinchman, J.E., Albert, J. and Vartdal, F. (1991) J. Virol. 65, 2019–2023.
[4] Kieny, M.P., Rautman, G., Schmitt, D., Dott, K., WainHobson, S., Alizon, M., Girard, M., Chamaret, S., Laurent, A., Montagnier, L. and Lecocq, J.P. (1986) BioTechnology 4, 790.
[5] McKeating, J.A., McKnight, A. and Moore, J.P. (1991) J. Virol. 65, 852–860.
[6] Ameisen, J.C. (1992) Immunol. Today 13, 388–391.
[7] Banda, N.K., Bernier, J., Kurahara, D.K., Kurrle, R., Haigwood, N., Sékaly, R.P. and Finkel, T.H. (1992) J. Exp. Med. 176, 1099–1106.
[8] Laurent-Crawford, A.G., Krust, B., Rivière, Y., Desgranges, C., Muller, S., Kieny, M.P., Dauget, C. and Hovenessian, A.G. (1993) AIDS Res. Human Retroviruses 9, 761–773.
[9] Hubert, P., Bismuth, G., Körner, M. and Debré, P. (1995) Eur. J. Immunol. 25, 1417–1425.
[10] Schnittman, S.M., Psallidopoulos, M.C., Lane, H.C., Thompson, L., Baseler, M., Massari, F., Fox, C.H., Salzman, N.P. and Fauci, A.S. (1989) Science 245, 305–308.
[11] Clerici, M. and Shearer, G.M. (1994) Immunol. Today 15, 575–581.
[12] Weinfold, K.J., Lyerly, H.K., Stanley, S.D., Austin, A.A., Matthews, T.J. and Bolognesi, D.P. (1989) J. Immunol. 142, 3091.
[13] Oyaizu, N., Chirmule, N., Kalyanaraman, V.S., Hall, W.W., Good, R.A. and Pahwa, S. (1990) Proc. Natl. Acad. Sci. USA 87, 2379–2383.
[14] Cayota, A., Vuillier, F., Siciliano, J. and Dighiero, G. (1994) Int. Immunol. 6, 611–621.
[15] Guntermann, C., Dye, J. and Nye, K.E. (1997) J. AIDS Hum. Retrovirol. 14, 204–212.
[16] Morio, T., Chatila, T. and Geha, R.S. (1997) Int. Immunol. 9, 53–64.
[17] Nokta, M.A., Hassan, M.I., Morgan, J.A., Loesch, K.A. and Pollard, R.B. (1994) Proc. Soc. Exp. Biol. Med. 207, 284–291.
[18] Bregestovski, P., Redkozubov, A. and Alexeev, A. (1986) Nature 319, 776–778.
[19] Payet, M.D. and Dupuis, G. (1992) J. Biol. Chem. 267, 4324–4328.
[20] Lewis, R.S. and Cahalan, M.D. (1995) Annu. Rev. Immunol. 13, 623–653.
[21] Wilson, H.A. and Chused, T.M. (1985) J. Cell. Physiol. 125, 72–81.
[22] Grinstein, S. and Smith, J.D. (1989) Am. J. Physiol. 257, C197–C206.
[23] Leonard, R.J., Garcia, M.L., Slaughter, R.S. and Reuben, J.P. (1992) Proc. Natl. Acad. Sci. USA 89, 10094–10098.
[24] Cai, Y.C. and Douglass, J. (1993) J. Biol. Chem. 268, 23720–23727.
[25] Szabo, I., Gulbins, E., Apfel, H., Zhang, X., Barth, P., Busch, A.E., Scholttman, K., Pongs, O. and Lang, F. (1996) J. Biol. Chem. 34, 20465–20469.
[26] Hamill, O., Marty, A., Neher, E., Sakmann, B. and Sigworth, F.G. (1981) Pflügers Arch. 391, 85–100.
[27] Verheugen, J.A., Vijverberg, H.P., Oortgiesen, M. and Cahalan, M.D. (1995) J. Gen. Physiol. 105, 765–794.
[28] DeCoursey, C., Chandy, K.G., Gupta, S. and Cahalan, M.D. (1984) Nature 307, 465–468.
[29] Attali, B., Romey, G., Honoré, E., Schmid-Alliana, A., Mattéi, M.G., Lesage, F., Ricard, Ph., Barhanin, J. and Ladzunski, M. (1992) J. Biol. Chem. 267, 8650–8657.
[30] Goldman, F., Crabtree, J., Hollenback, C. and Koretzky, G. (1997) J. Immunol. 158, 2017–2024.
[31] Pantaleo, G., Graziosi, C., Demarest, J.F., Butini, L., Montroni, M., Fox, C.H., Orenstein, J.M., Kotler, D.P. and Fauci, A.S. (1993) Nature 362, 355–358.
[32] Strauss, D.B. and Weiss, A. (1992) Cell 70, 585–593.
[33] Soula, M., Fagard, R. and Fischer, S. (1992) Int. Immunol. 4, 295–299.
[34] Robertson, B. (1997) Trends Pharmacol. Sci. 18, 474–483.
[35] Lewis, R.S. and Cahalan, M.D. (1988) Trends Neurosci. 11, 214–218.
[36] Catterall, W.A. (1988) Science 242, 50–61.
[37] Levitan, I.B. (1995) Annu. Rev. Physiol. 56, 177–190.
[38] Fischer, E.H., Charbonneau, H. and Tonks, N.K. (1991) Science 253, 401–406.
[39] Ruegg, C.L. and Strand, M. (1990) J. Immunol. 144, 3928–3935.
[40] Hille, B. (1992) Ionic Channels of Excitable Membranes, 2nd edn., Sinauer, Sunderland, MA.
[41] Grinstein, S. and Foskett, J.K. (1990) Annu. Rev. Physiol. 52, 399–414.
[42] Bubien, J.K., Benveniste, E.N. and Benos, D.J. (1995) Am. J. Physiol. 268, C1440–C1449.