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Inhibition of HIV-1 envelope-dependent membrane fusion by serum antilymphocyte autoantibodies is associated with low plasma viral load

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ABSTRACT

The HIV-1 envelope protein (Env) mediates the membrane fusion process allowing virus entry to target cells and the efficiency to induce membrane fusion is an important determinant of HIV-1 pathogenicity. In addition to virus receptors, other adhesion/signaling molecules on infected and target cells and virus particles can enhance fusion. The presence of antilymphocyte autoantibodies (ALA) in HIV patients' serum suggests that they may contribute to the inhibition of Env-mediated membrane fusion. Here, sera from 38 HIV-1 infected treatment-naïve men and 30 healthy donors were analyzed for the presence of IgG and IgM able to bind to CD4-negative Jurkat cells. The use of CD4-negative cells precluded the binding of virus-antibody immune complexes, and allowed detection of ALA different from anti-CD4 antibodies. IgG and IgM antibodies binding to Jurkat CD4-negative cells was detected in 74% and 84% of HIV-positive sera, respectively. Then, the activity of sera on fusion of $CD4^+$ with HIV Env^+ Jurkat cells was determined before and after their adsorption on CD4-negative Jurkat cells to remove ALA. Sera inhibited fusion at variable extents, and inhibitory activity decreased in 58% of serum samples after adsorption, indicating that ALA contributed to fusion inhibition in these sera (herein called fusion inhibitory ALA). The contribution of ALA to fusion inhibition in individual sera was highly variable, with an average of 33%. IgG purified from a pool of HIV^+ sera inhibited fusion of primary CD4 T lymphocytes with Jurkat Env^+, and adsorption of IgG on CD4-negative Jurkat cells diminished the fusion inhibitory activity. Thus, the inhibitory activity of sera was related to IgG ALA. Our observations suggest that fusion inhibitory ALA other than anti-CD4 antibodies may contribute significantly to the inhibition of Env-mediated cell-cell fusion. Fusion inhibitory ALA, but not total ALA levels, associated with low plasma viral loads, suggesting that specific ALA may participate in virus containment by inhibiting virus-cell fusion in a significant fraction of HIV-infected patients.

1. Introduction

An increased ability to induce membrane fusion during the entry of HIV-1 into target cells is a feature of highly pathogenic virus strains. The interaction of HIV-1 Env with the CD4 receptor and a co-receptor molecule induces the activation of the gp41 Env subunit, which directs the membrane fusion process [1]. Virus-cell fusion is involved in infection of target cells by cell-free viral particles and in virus cell-to-cell transmission, a highly efficient route of virus spread between cells that is considered an important mode of dissemination [2–6]. In addition, Env fusion capability relates to the induction of multinucleated cells through cell-cell fusion. Multinucleated cells have the potential to express anomalous activation [7], to transfer virus to surrounding cells [8] and to induce lymph node and brain pathologies [9–13]. Finally, fusogenicity is critical for the gp41-mediated apoptosis of bystander cells [14–17]. An increased ability of patient's viruses to induce cell-cell fusion *in vitro* is related to disease progression [16,18–20], perhaps reflecting a higher *in vivo* occurrence of the events described before.

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In addition to CD4 and coreceptors (mainly CCR5 and CXCR4), other cellular surface molecules increase the efficiency of HIV-1-induced membrane fusion. A variety of adhesion and activation molecules are incorporated into the virus membrane during the assembly and budding of virions and have been implicated in the entry process [21–24]. Adhesion molecules favor the close interaction between infected and uninfected cells during the formation of syncytia and during the cell-to-cell transmission of virus [2,25,26]. Other molecules, such as α4β7 integrin, support the access of the virus to CD4 on T memory cells during infection of the gut-associated lymphoid tissue [27,28]. Thus, the involvement of a variety of cell surface molecules in the fusion process implies that in addition to anti-Env antibodies, antilymphocyte autoantibodies (ALA) may participate in the modulation of fusion.

Although the presence of ALA during HIV infection has been described for a long time [29,30], their effect on Env-dependent membrane fusion is scarcely considered in the analysis of the humoral immune response during HIV-1 infection. The effect of ALA on Env-dependent membrane fusion is worth investigating since autoimmune events are related with the induction of antibodies able to neutralize a variety of HIV strains in humans [31,32] and breaking peripheral tolerance promotes the production of HIV-1-neutralizing autoantibodies in mice [33]. Anti-CD4 antibodies have been frequently reported in HIV-1-infected individuals, although it is not clear if they contribute significantly to virus containment [34], and a pathological role for these antibodies has been suggested [35]. However, antibodies against a variety of other lymphocyte surface molecules have been detected in HIV-infected individuals [36–42]. Moreover, it has been reported that healthy and HIV-1 infected individuals contain IgM ALA able to bind CD4, CXCR4 and CCR5, and inhibit HIV infectivity as well as syncytia formation [43]. Thus, evaluation of the effect of ALA on the virus envelope-mediated fusion process may contribute to the understanding of the role of the humoral immune response in virus control.

Here, we aimed to assess the contribution of ALA to the inhibition of HIV-1 Env-mediated lymphocyte membrane fusion by testing the effect of sera from ART-naïve HIV-1 infected men on a flow cytometry HIV-1-envelope-dependent Jurkat cell-cell fusion assay [44,45]. Jurkat cells are a well-characterized model that has been widely used for T cell signaling and HIV infection studies [46]. Previously, the effect of sera from a different cohort of 49 HIV patients was tested on this assay [47], showing that sera inhibited fusion at variable extents. Inhibition of fusion correlated with the asymptomatic stage of human HIV infection, whereas sera that had no effect or enhanced fusion were associated with AIDS. Removal of IgG or IgM from sera reduced or eliminated inhibition and enhancing activities, respectively. The inhibitory effect of IgG on fusion could be achieved by antibodies against the HIV envelope proteins and/or against lymphocyte antigens. In this work, we determined the effect of sera on fusion before and after the adsorption on CD4-negative Jurkat cells to remove ALA. Adsorption on CD4-negative cells prevented the elimination of other serum components that may interfere with fusion, such as CD4-binding virus particles and virus-IgG immune complexes. This procedure does not remove anti-CD4 antibodies. Thus, any change in the activity of the adsorbed sera on fusion should reflect the removal of ALA (except anti-CD4 antibodies). We determined if the presence of fusion inhibitory ALA (detected as a diminished fusion inhibition activity of sera after adsorption on Jurkat CD4-negative cells) correlated with total serum IgG and IgM ALA levels, as well as with the patient's viral load and CD4 cell count.

2. Methods

2.1. Study subjects

Patients analyzed attended the Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán in Mexico City from May 2014 to August 2016 (Table 1). Antiretroviral treatment-naïve subjects without opportunistic infections, neoplastic illness or wasting syndrome were included after having signed an informed consent form. Healthy donors were negative for HBV, HCV, VDRL, HIV-1 and HIV-2. The study was approved by the Research Ethics Committee of Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán.

Table 1
HIV-1 patients and healthy controls characteristics.

HIV patients	n = 38	range
Age (years)	35[a]	18–60
Male	100%	
ART naïve	100%	
Viral load (copies/ml)	272,479	1,560–2,908,948
$CD4^+$ T-cell count (cells/μl)	562	12–829
Healthy donors	n = 30	
Age	31.5	18–45
Male	100%	

[a] Averages or percentage values are shown.

2.2. Cells

The Jurkat cell line HXBc2(4) transfected with the *env* and *rev* genes from the HIV-1 HXBc2 strain [48] and the non-transfected Jurkat T $CD4^+$ cell line (E6-1) were obtained through the AIDS Research and Reference Reagent Program (AIDSRRRP). Jurkat clone D1.1, lacking CD4 expression (CD4-negative cells), was obtained from ATCC. HXBc2(4) cells were grown in RPMI medium (Gibco BRL, MD) containing 10% FBS (RPMI-10), 200 μg/ml of G418, 200 μg/ml of hygromycin, and 1 μg/ml of tetracycline. $CD4^+$ and CD4-negative Jurkat cells were grown in L-glutamine-rich RPMI-10 containing penicillin/streptomycin. Primary CD4 T lymphocytes were purified by negative selection using antibodies coupled to magnetic beads ($CD4^+$ T cells Isolation Kit human, Miltenyi Biotec, Auburn, CA), as indicated by the manufacturer. Cells were maintained in RPMI medium containing 10% FBS and used for fusion experiments the next day.

2.3. Binding of IgG and IgM from HIV-positive patients to Jurkat cells

Jurkat cells (5×10^5) were incubated with HIV^+ or control serum at a final dilution of 1/10 in PBS containing 0.1% azide and 2% FBS for 40 min at 4 °C. After washing twice with PBS-azide, FITC-anti-human IgG or APC-anti-human IgM antibodies (HP6017 and MHM-88 clones, respectively, Biolegend, CA) were added and incubated for 45 min on ice. After washing, the cells were fixed with 4% PFA in PBS for 20 min, washed and resuspended in 1 mL of PBS before FACS analyses using an Attune Cytometer System (Applied Biosystems, CA). A serum sample was considered positive for IgG or IgM binding to Jurkat cells when the percentage of stained cells was above the mean plus two standard deviations of the figures obtained for sera from healthy controls.

2.4. Adsorption of serum on CD4-negative T cells

One hundred and fifty microliters of each serum were incubated with 7.5×10^6 $CD4^-$ Jurkat cells in 600 μL of serum-free AIM-V media and mixed for 40 min at 4 °C using a rotating mixer. The cells were pelleted by centrifugation, and the adsorbed sera were recovered. Pooled sera and purified IgG were adsorbed in the same conditions.

2.5. Effect of sera on fusion assay

Labeling of cells and cytofluorometric fusion assay were performed as previously described [44,45]. Jurkat $CD4^+$ or primary CD4 T cells were stained with the lipophilic red fluorescent DiI dye (1,1′-dioctadecyl-3,3,3′,3-tetramethylindocarbocyanine perchlorate), and Env^+ Jurkat cells with the green fluorescent DiO dye (3,3′-dioctadecyloxacarbocyanine perchlorate) (Molecular Probes,Eugene, OR). Labeled

cells were cocultured for 5 h, at 37 °C with 5% CO_2 in 96-well plates. Double fluorescent cells were quantified in 10,000 events with an Attune Cytometer System (Applied Biosystems, California) and Attune Cytometry software version 1.2.5 (Applied Biosystems). Loosely aggregated cells were dissociated by gently pipetting immediately before analysis. To test the effect of sera on fusion, DiI-labeled $CD4^+$ cells were preincubated with either adsorbed or non-adsorbed sera or IgG from patients or healthy donors control sera at the final dilution indicated in 100 µl of serum-free medium (AIM-V medium, Gibco BRL) for 40 min at 37 °C with 5% CO_2 in 96-well plates. Then, 2×10^5 DiO-labeled Env^+ cells were added to the wells and gently mixed in a total volume of 200 µl. Co-cultures were incubated for 5 h at 37 °C in 5% CO_2. The assays were performed in duplicate. The cells were collected from the plates, washed with 2 ml of PBS and resuspended in 300 µl of 2% paraformaldehyde in PBS. We performed flow cytometry immediately after fixation. Fusion activity (FA) was defined as $1-(F_i/F_0)$ x 100, where F_0 and F_i are the fusion values obtained in the absence and presence of serum, respectively.

2.6. Purification of IgG from HIV^+ sera

IgG was purified from HIV^+ and healthy serum pools by G protein affinity chromatography on protein G-Sepharose (Proteus Protein G Midi spin column Kit, BioRad, Kidlington, UK), as indicated by the manufacturer.

2.7. Antinuclear antibody (ANA) determination

Antinuclear antibodies (ANA-IgG) were detected in serum samples by indirect immunofluorescence using the HEp-2 cell line as indicated (INOVA Diagnostics, CA) and AutoCyte Image Titer software (Tripath Imaging Inc, NC). The results were positive if any pattern was above the values obtained for healthy Mexican individuals, according to the international consensus on ANA patterns (ICAP) [49].

2.8. Statistical analysis

Comparisons between groups were performed by unpaired U-Mann-Whitney and Kruskal-Wallis tests. Spearman's ranked correlation coefficients were used to determine the correlation between variables. Statistical analyses were performed using Statistica v.7 software.

3. Results

3.1. Inhibition of HIV envelope-dependent cell fusion by sera from HIV-positive individuals

Sera from 38 HIV-positive treatment-naïve and 30 healthy men were tested for activity in an HIV-1-envelope dependent cell-cell fusion assay between $CD4^+$ and Env^+ Jurkat cells. The accurate quantification of fusion events by the flow cytometry assay was previously established [44,50].

Jurkat $CD4^+$ cells were pre-incubated with sera for 40 min at 37 °C before coculture with Jurkat Env^+ cells, and fusion was analyzed after 5 h. HIV-positive sera inhibited fusion to variable extents, and inhibition was dilution-dependent, with mean fusion-inhibiting activities of 32.3% ± 2.8, 23.3% ± 2.9, and 14.9% ± 3.2 for the 1:10, 1:20 and 1:50 dilutions, respectively (Fig. 1**a**). A high correlation between the inhibition values obtained with the three dilutions of sera was observed (Fig. 1**b**) HIV-negative sera caused small levels of inhibition or enhanced fusion (Fig. 1**a**). The fusion-inhibiting activity of HIV-positive sera did not associate with either patient viral load or $CD4^+$ T cell count ($p = 0.3$). Interference with fusion could be due to anti-Env antibodies, ALA, virus particles or virus-antibody immune complexes.

3.2. Binding of serum antibodies to Jurkat $CD4^+$ cells

Inhibition of the Env-mediated fusion of Jurkat cells by HIV-positive sera may be partially due to the binding of antilymphocyte antibodies (ALA) to the cell membrane. Fig. 2 shows the percentages of stained Jurkat cells by IgM and IgG from individual sera of HIV patients and HIV-negative controls. Eighteen out of 19 (94%) and 32/38 (84%) of HIV^+ patient sera were positive for IgM and IgG antibodies binding to Jurkat cells, respectively. Intriguingly, the binding of IgG from HIV patients to $CD4^+$ Jurkat cells revealed two main groups of sera that showed high and low binding (Fig. 2**b**). However, there was no correlation between the level of IgG binding to $CD4^+$ Jurkat cells and the fusion-inhibiting activity of the sera, the viral load or the CD4 T cell count (Supplementary Data 1A in Research Data section). It should be noted that the secondary anti-human Fc antibodies used for detection might have also bound virus-antibody immune complexes bound to CD4 [51,52] in addition to bona fide ALA, and thus the assessment of ALA may be overestimated. We also observed IgM able to bind Jurkat cells in sera from many HIV-negative individuals, while this is not the case for IgG. This is in agreement with previous observations of a high frequency of natural IgM-ALA in healthy donors and proposed an anti-inflammatory and anti-HIV role for this immunoglobulins [43,53].

3.3. Effect of serum adsorption on CD4-negative Jurkat cells on HIV envelope-mediated cell fusion

To assess the contribution of HIV patient serum ALA to the inhibition of Env-dependent cell-cell fusion, sera were adsorbed on CD4-negative Jurkat cells and their effect on fusion was compared with their activity before adsorption, tested in parallel. Adsorption on CD4-negative cells avoided the removal of CD4-binding virus particles, virus-antibody immune complexes [51,52] and anti-CD4 antibodies from the sera. Thus, any change in the activity of adsorbed sera on fusion should reflect the removal of ALA other than anti-CD4 antibodies.

IgG and IgM binding to Jurkat CD4-negative cells was detected in 28/38 (74%) and 32/38 (84%) of HIV-positive sera, respectively, and adsorption significantly removed this activity (Fig. 3). Levels of Ig binding to CD4-negative cells were lower than to $CD4^+$ cells, likely reflecting the detection of anti-CD4 antibodies and virus-antibody immune complexes in the latter. We did not observe a correlation between the level of IgG binding to CD4-negative Jurkat cells and the fusion-inhibiting activity of sera, the viral load or the CD4 T cell count (Supplementary Data 1B in Research Data section). The change of fusion inhibitoy activity after adsorption is quite clear for most sera even though ALA are still detected in some samples after their thorough adsorption with CD4-negative cells. This remaining antibodies may be a reflection of a very variable concentration of ALA in sera from HIV patients.

Adsorption decreased the fusion inhibition activity of HIV^+ sera, whereas it had a negligible effect on the activity of HIV-negative sera (Fig. 4**a**). Fig. 4**b** shows the effect of adsorption of HIV^+ sera grouped according to their activity on fusion inhibition. Twelve HIV^+ sera (31.6%) had increased fusion inhibition capacity after adsorption, with a mean 31% difference respective to the non-adsorbed sample (Fig. 4**c**). The opposite effect was observed for 22 sera (58%), which had decreased fusion inhibition capacity respective to the non-adsorbed sample (Fig. 4**b**). A mean decrease of 33% of the fusion inhibition activity was removed by adsorption (Fig. 4**c**). Thereafter, the term "fusion inhibitory ALA" will be used when a decrease of the fusion inhibitory capacity of sera was observed after having adsorbed the serum on Jurkat CD4-negative cells. Finally, adsorption did not modify the effect of the sera in 10.5% of cases (Fig. 4**b**). These observations indicate that serum ALA other than anti-CD4 antibodies contribute significantly to inhibition of HIV Env-mediated membrane fusion.

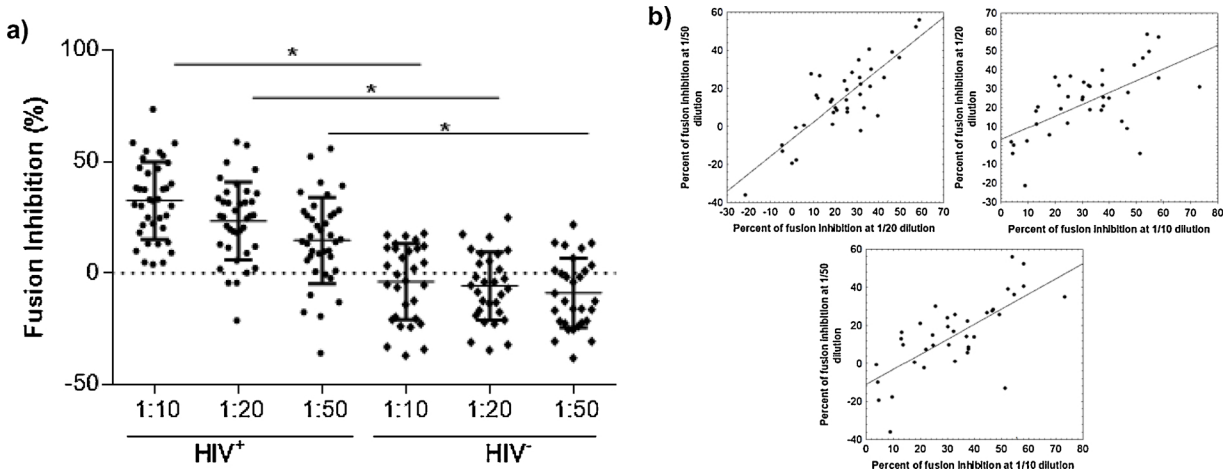

Fig. 1. (**a**) Activity of sera from 38 HIV + individuals and 30 healthy donors on the fusion of Env + with CD4 + Jurkat cells. CD4 + Jurkat cells were pre-incubated with the indicated dilutions of sera before the addition of Jurkat Env + cells. Coculture and flow cytometric analyses of fusion were performed as described in the Materials and Methods section. The percentage of fusion inhibition was calculated respective to the fusion obtained in the absence of serum. Positive and negative values indicate fusion inhibition and enhancement, respectively. Means are indicated. *p < 0.05. (**b**) Correlation between the effect of different dilutions of HIV-positive sera on Env-mediated cell-cell fusion. p < .005 for each plot.

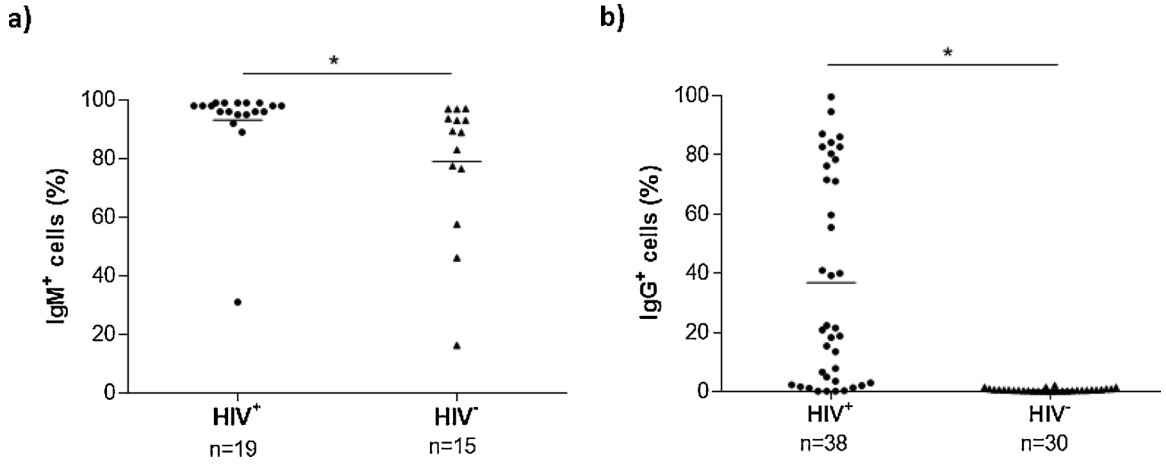

Fig. 2. Binding of serum IgM (**a**) and IgG (**b**) from HIV^+ individuals and HIV^- healthy donors to the surface of Jurkat $CD4^+$ T cells. Cells were incubated with sera and then with an anti-IgG or an anti-IgM fluorochrome-conjugated secondary antibody. The cells were analyzed by flow cytometry as described in the Materials and Methods section. Means are indicated. *p < 0.05.

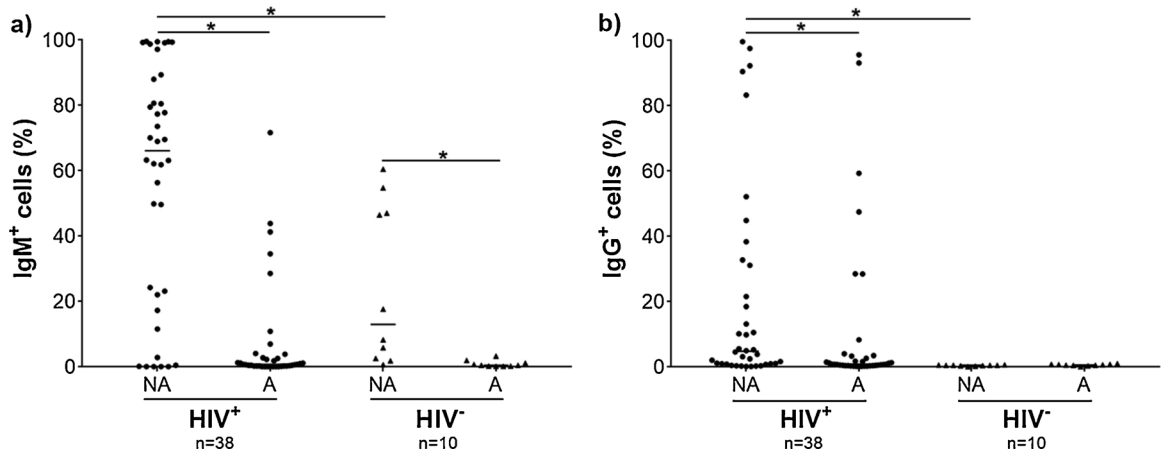

Fig. 3. Binding of IgM (**a**) and IgG (**b**) from HIV^+ and HIV^- non-adsorbed (NA) and adsorbed (A) sera on CD4-negative Jurkat cells. Binding was determined as described in Fig. 2. Medians are indicated. *P < 0.05.

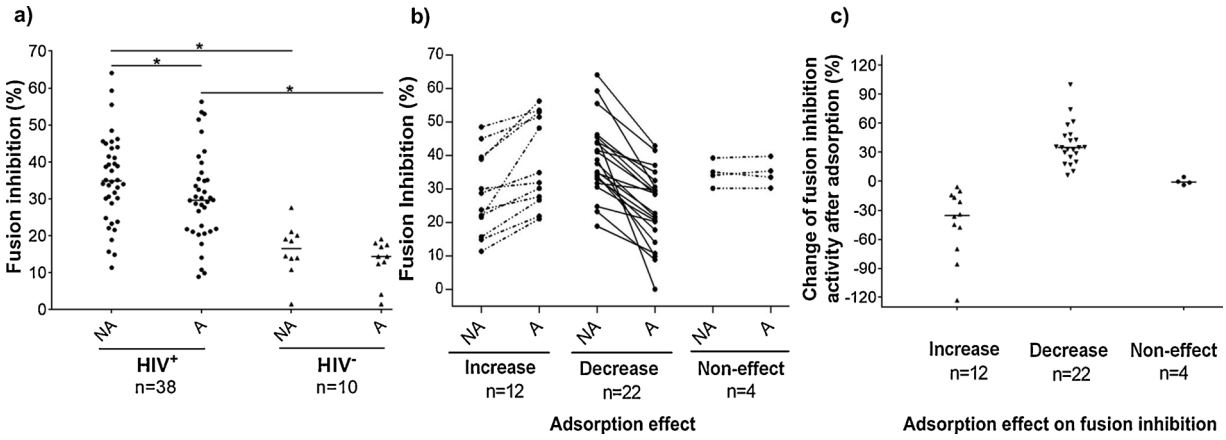

Fig. 4. Activity of non-adsorbed (NA) and adsorbed (A) sera on the fusion of Env$^+$ with CD4$^+$ Jurkat cells. Adsorption was performed on CD4-negative Jurkat cells. Sera activity on fusion was assayed as described in Fig. 1, using a 1:10 serum dilution. The percentage of fusion inhibition was calculated respective to the fusion obtained in the absence of serum. **a**) The effect of adsorption of sera from 38 HIV$^+$ and 10 HIV$^-$ individuals on fusion inhibition. **b**) HIV$^+$ sera are grouped according with the effect of adsorption on fusion inhibition: increase, decrease, and non-effect. **c**) ALA fusion-inhibiting activity (as measured as the percent of change of fusion inhibition after adsorption) for the groups shown in b. Medians are indicated. *p < 0.05.

3.4. Relationship between levels of cell fusion inhibitory ALA and viral load

Table 2 shows that sera containing fusion inhibitory ALA resulted in greater inhibition of fusion by the complete (non-adsorbed) sera than those that did not contain this activity. Accordingly, a significant correlation between the effect of ALA on fusion and that of the complete (non-adsorbed) sera was obtained (Fig. 5**a**). Table 2 also shows that levels of binding of IgG and IgM to Jurkat CD4-negative cells were not different between sera containing and not containing fusion inhibitory ALA, so that the total levels of ALA measured by binding to cells were not indicative of their ability to inhibit fusion. Importantly, viral load was significantly lower in sera containing fusion inhibitory **ALA** (Table 2). Accordingly, we observed a weak trend associating the level of fusion inhibitory ALA, as measured by the change in fusion inhibition after adsorption, and the plasma viral load ($p = 0.091$) the (Fig. 5**b**).

There is an association between immune dysfunction in patients with HIV and AIDS and the development of autoimmune events [54]. Early reports indicated the detection of antinuclear antibodies (ANAs) in 0–23% [55,56] or more [57] of HIV-infected individuals, and they were not always associated with clinical autoimmune disease [56]. We detected ANAs in 52% of HIV$^+$ patients and 10% of healthy individuals (Supplementary Data 2 in Research Data section). ANAs were detected in 50% of sera containing fusion inhibitory ALA, suggesting that ANAs does not correlate with fusion inhibition.

3.5. Effect of IgG adsorption on CD4-negative Jurkat cells on the HIV envelope-mediated fusion of primary CD4 T lymphocytes

In order to be sure that IgG participates in inhibition of fusion, IgG was purified from a pool of 9 HIV$^+$ samples showing both a high inhibitory fusion activity and IgG binding to Jurkat CD4-negative cells. IgG was also purified from a pool of 10 sera from healthy donors as control. The effect of pooled sera and purified IgG on cell fusion was tested before and after their adsorption on Jurkat CD4-negative cells. In addition, the effect of the pooled sera of IgG on the fusion of primary CD4 T lymphocytes with Jurkat Env$^+$ cells was also determined.

Fig. 6 shows that both sera and IgG purified from HIV$^+$ individuals inhibited fusion of Jurkat Env$^+$ with Jurkat CD4$^+$ or with primary CD4 T lymphocytes. Adsorption of sera or IgG on CD4-negative Jurkat cells diminished the corresponding fusion inhibitory activity. Adsorption of sera or IgG from healthy donors had no effect. Thus, the inhibitory activity of serum was related to IgG ALA.

4. Discussion

In agreement with previous observations on a different cohort of HIV patients [47] most of HIV$^+$ sera inhibited the Env-mediated cell-cell fusion with variable efficiency (Fig. 1**a**). Herein we show that part of this effect may have occurred through the participation of ALA other than anti-CD4 antibodies (Fig. 4). Serum from a substantial fraction of HIV patients (approximately 60%) contained fusion-inhibiting activity that could be removed by adsorption of the sera on CD4-negative Jurkat cells (Fig. 4**b**). The change in fusion inhibition after the adsorption of sera on CD4-negative Jurkat cells is compatible with the presence of ALA able to recognize specific lymphocyte surface molecules modulating fusion. The extent of contribution of CD4-negative Jurkat-binding serum components to the effect of sera on fusion was highly variable, with an average of 33% (Fig. 4**c**). In a previous report we showed that fusion inhibition by sera from HIV patients was reduced or eliminated by removal of total IgG using protein G beads [47]. Here we showed that IgG purified from fusion inhibitory sera also inhibited fusion, and that removal of ALA from IgG diminished this effect (Fig. 6). Thus, the inhibitory activity of serum ALA was related to IgG. While adsorption of sera on Jurkat cells does not provide direct evidence that IgG ALA are the only responsible for the change in fusion inhibition, the previous work and present evidence indicates that it plays a major role in fusion inhibition.

Viral load was significantly lower in sera containing than in sera not containing CD4-negative Jurkat-binding serum components (Table 2) and, accordingly, a trend associating the level of fusion inhibitory ALA and the plasma viral load was observed (Fig. 5**b**). This observations suggest that ALA other than anti-CD4 antibodies are capable of inhibiting fusion and may contribute to virus containment *in vivo,* perhaps by blocking the formation of syncytia and the cell-to-cell transmission of virus, in addition to the neutralization of cell-free particles. Identification of cognate cell membrane antigens with the ability to modulate these processes are necessary steps for the full characterization of this activity.

Total IgG or IgM ALA levels did not relate to the ability of sera to inhibit fusion or to viral load, suggesting that fusion inhibition involves the activity of ALA with particular specificities. It is possible that the relative abundance of specific fusion-inhibiting ALA is variable between patients and does not necessary correlate with the level of total ALA produced. Instead, the level of change in fusion inhibitory activity after adsorption, presumably reflecting levels of those ALA that effectively inhibit fusion, correlated with the fusion inhibitory activity of the complete sera and with viral load. We propose that the adsorption assay

Table 2
Comparison between sera from HIV^+ patients containing and non-containing fusion inhibitory ALA[a].

Fusion inhibitory ALA	Fusion inhibition by unadsorbed serum (%)	IgG^+ cells (%)	IgG^+ cells (MFI)	IgM^+ cells (%)	IgM^+ cells (MFI)	Viral load (RNA copies/mL)	Viral load range (RNA copies/mL)	$CD4^+$ cells (cells/μL)	$CD4^+$ cells range (cells/μL)
Yes (n = 22)	39.2 ± 2.3	20.9 ± 8.0	10,258 ± 1,789	53.9 ± 7.3	10,378 ± 2,723	174,183 ± 93,093	1,560 – 201,2391	344 ± 57	28–684
No (n = 16)	29.9 ± 2.8	20.7 ± 6.7	11,447 ± 2,167	60.7 ± 9.9	32,396 ± 10,874	416,647 ± 19,5571	28,201 – 2,908,948	420 ± 51	10–829
p	**0.028**	0.77	0.94	0.62	0.60	**0.042**		0.39	

MFI: mean fluorescence intensity.
Significant *p* values are shown in bold characters.
[a] Average ± SE is shown.

allows the overall detection of fusion inhibitory ALA, which relative abundance may not be related to the total content of ALA in the serum.

The participation of ALA in the inhibition of Env-induced cell-cell fusion may represent a relevant consideration in the assessment of the antiviral humoral immune response during HIV-1 infection. Inhibition of fusion by ALA may be produced by blocking of CXCR4 and CCR5 co-receptors [43]. As our assay involves the CXCR4 coreceptor, antibodies against this molecule may contribute to the inhibition of syncytia formation. This would agree with previous results indicating high levels of anti-CXCR4 antibodies in sera of HIV patients [43]. Likewise, blocking of cellular adhesion molecules [21,22,58], or of components that facilitate the Env-CD4 interaction, such as α4β7 integrin, which is expressed by Jurkat cells [59,60] may also participate in fusion inhibition. Fusion inhibitory ALA may also include antibodies able to bind the viral envelope protein, since cross-reactions between neutralizing Env and self epitopes have been consistently reported [40,41,61–63]. Finally, induction of signaling processes that modify the permissiveness of the cell membrane for fusion by ALA, as shown for anti-CCR5 autoantibodies [37], cannot be excluded. On the other hand, enhancing of the inhibitory activity in 31.6% of sera after adsorption suggests the removal of ALA with the ability to prevent the binding of fusion inhibitory serum components, such as anti-Env antibodies, virus particles, virus-antibody immune complexes, or anti-CD4 antibodies [43].

Other serum components might participate in the effect of sera on fusion, and incubation at 37 °C in the presence of sera may allow cytotoxic reactions due to serum complement or hormones. However, $CD4^+$ T lymphocytes and Jurkat cells are not lysed by complement due to the presence of complement control proteins on their cell membrane [64–66]. Cell death after the incubation period, which can be observed in flow cytometry SSC-H vs. FSC-H dot plots, was not observed during our analyses (data not shown). The cortisol hormone can reach a double-fold concentration in the serum of HIV patients compared with healthy individuals [67], and these concentrations are able to induce lymphocyte apoptosis [68]. However, fusion inhibition was observed using diluted serum, and thus the concentration of cortisol was considerably reduced. Likewise, the concentration of other hormones that can influence the expression of HIV-1 co-receptors *in vitro* [69] is considerably greater than that present in blood [70].

To our knowledge, this is the first study showing an association of IgG fusion inhibitory ALA with low plasma viral loads. Identification of the cell membrane antigens involved by proteomic methods and their mechanism of action deserves further study.

Competing interests

None declared.

Ethical approval

Research Ethics Committee of Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, México. REF. 1192.

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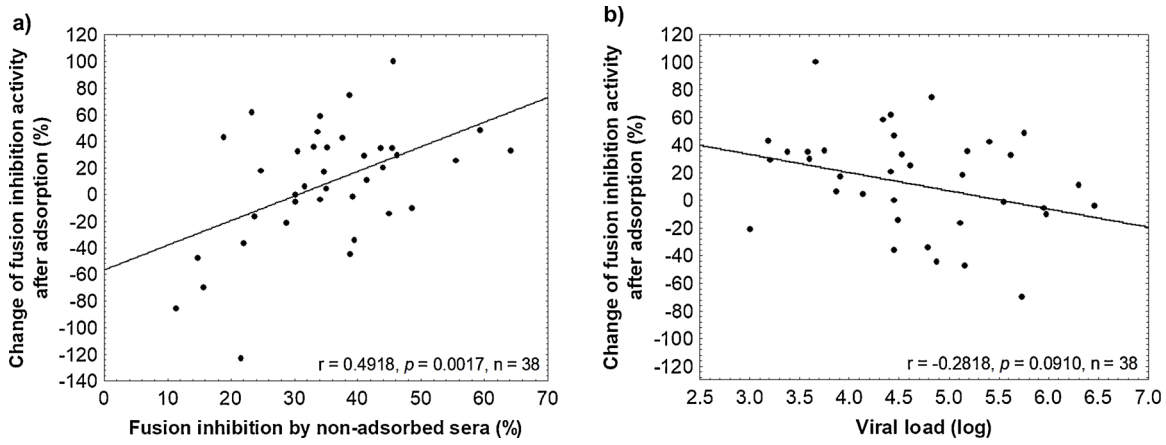

Fig. 5. a) Correlation between sera ALA fusion-inhibiting activity (measured as the percentage of change of fusion inhibition activity after adsorption) and fusion inhibition by non-adsorbed sera. **b**) Correlation between serum ALA fusion-inhibiting activity and viral load.

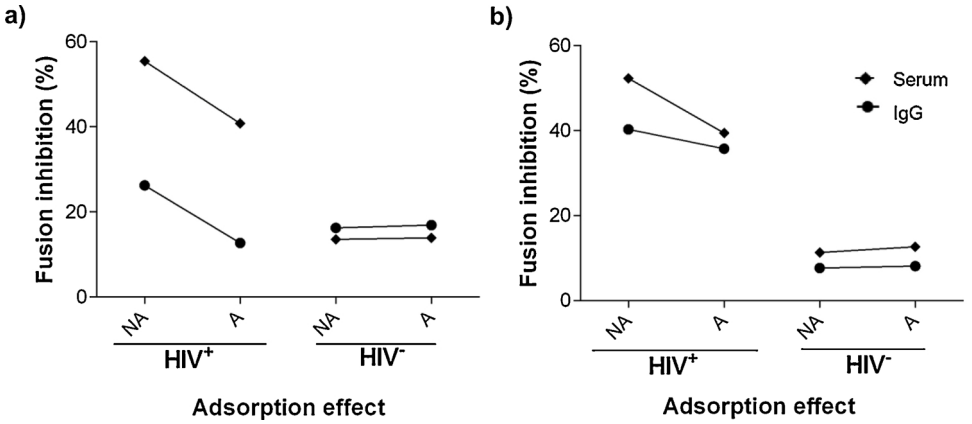

Fig. 6. Effect of sera and purified IgG on fusion of Jurkat Env$^+$ cells with a) Jurkat CD4$^+$ cells (E6-1 cells) and b) primary CD4 T lymphocytes.clear A diminished inhibition of fusion by sera or IgG after adsorption on Jurkat CD4-negative cells is shown. (NA) non-adsorbed, (A) adsorbed sample.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.imlet.2019.05.002.

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