Short Communication

Human immunodeficiency virus 1 (HIV-1) envelope-dependent cell–cell fusion modulation by HIV-positive sera is related to disease progression

L. Huerta,1 G. Gómez-Icazbalceta,1 L. Soto-Ramírez,2 M. Viveros-Rogel,2 R. Rodríguez,2 L. Fuentes,2 E. Lamoyi1 and C. Larralde1

1Department of Immunology, Instituto de Investigaciones Biomédicas, Universidad Nacional Autónoma de México, Apartado Postal 70228, Distrito Federal, CP 04510, México
2Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Secretaría de Salud, Distrito Federal, México

Fusion of CD4+ cells by HIV-1 envelope proteins (Env) is a mechanism of virus spread and cell damage. Production of antibodies able to influence cell–cell fusion in vivo may affect the course of the infection. The effect of sera from 49 HIV-1-positive patients was tested on an in vitro fusion assay using Env-expressing and normal Jurkat T cells labelled with DiI and DiO dyes, and flow cytometry for quantification of cell–cell fusion. Sera varied in their activity on fusion: 69.4 % inhibited, 24.5 % had no effect and 6.1 % enhanced cell fusion. Fusion activity correlated positively with the CD4+ T-cell count and inversely with the viral load. Removal of IgG or IgM from sera reduced or eliminated inhibition and enhancing activities, respectively. Antibodies with inhibitory activity predominate in early and intermediate stages of infection, whereas loss of inhibition or enhancement of fusion correlates with progression to AIDS.

Human immunodeficiency virus type 1 (HIV-1) frequently induces cell–cell fusion with syncytia formation in cultures of CD4+ T cells. Syncytia formation result from the interaction between infected cells expressing the virus envelope glycoprotein (Env) on their surface and neighbouring uninfected CD4+ cells (Lifson et al., 1986; Sodroski et al., 1986). Cell–cell fusion may have an important pathogenic role in vivo. HIV-1-infected giant multinucleated cells are frequently detected in the lymph nodes of asymptomatic and AIDS patients, and have been proposed as virus reservoirs (Budka 1986; Frankel et al., 1996; Koenig et al., 1986). Syncytium-inducing viruses (usually requiring CXCR4 as a co-receptor) associate with an increased rate of CD4+ T-lymphocyte depletion in patients and progression to AIDS (Blaak et al., 2000; Connor et al., 1993; Miedema et al., 1994). Peptides that interfere with virus–cell fusion decrease viral loads in humans (Kilby et al., 1998; Lazzarin et al., 2003). Furthermore, the membrane fusing ability of Env from a simian–human immunodeficiency virus chimera was determinant for CD4+ T-cell depletion in macaques (Etemad-Moghadam et al., 2001). Cell fusion is a significant cause of cell death in vitro, mainly by apoptosis triggered by aberrant initiation of mitosis (Castedo et al., 2002; Ferri et al., 2000; Laurent-Crawford et al., 1991). The magnitude of CD4+ cell–cell fusion events can be significant in lymphoid tissues, where there is close contact between cells (Amendola et al., 1996). On the other hand, cell-to-cell HIV-1 transmission without formation of syncytia, likely involving localized fusion events at cell contact points was proposed as a mechanism of transmission between individuals, and an important route of viral spread (Gupta et al., 1989; Phillips, 1994; Sato et al., 1992). Recently, it was shown that virus strains highly efficient in cell-to-cell transmission and with a syncitium-inducing phenotype are selected in an in vitro model of the rapid T-cell turnover known to occur during HIV-1 infection (Gumurulu et al., 2000).

Inhibition of pathogenic cell–cell fusion processes by the host immune response could play a significant role in retarding disease progression. In principle, such inhibition would be attainable by HIV-1-specific neutralizing antibodies. However, antibodies detected by cell-free virus neutralization assays are not necessarily effective in inhibiting cell–cell fusion. Blocking HIV-1 Env-dependent cell–cell fusion often requires higher concentration of antibodies or soluble CD4 than is necessary for virus neutralization (Allaway et al., 1993; McKeating et al., 1996; Posner et al., 1991; Trkola et al., 1998; Zwick et al., 2001). Furthermore, some Env-specific antibodies that effectively inhibited infection by cell-free virus did not have an effect on cell-to-cell virus transmission (Gupta et al., 1989; Pantaleo et al., 1995). The amount of gp120/gp41 on the cytoplasmic membrane of infected cells (Connor & Ho, 1994) and env-transfected cells is higher than in viral membrane. Also, close contact of the cytoplasmic membranes of cells provides a more extensive area for the establishment of a higher
number of effective Env–receptor interactions potentiated by other membrane-associated molecules (Hioe et al., 1998). As a result, cell–cell fusion may differ in quantitative and qualitative requirements from virus–cell fusion. Thus, although neutralizing antibodies can restrict infections by free virus particles, they could be less effective in the containment of cell-to-cell virus spread and cell death mediated by extensive fusion activity.

To our knowledge, the effect of sera from HIV-1-infected individuals on cell–cell fusion and its relation to disease markers has not been explored. This is necessary to get a comprehensive understanding of the potential of the humoral immune response to influence the various ways of HIV-1 spreading and cell damage. Therefore, we determined the activity of sera from HIV-1-infected subjects on Env-mediated cell–cell fusion using Jurkat cells stably transfected with a plasmid bearing the env gene from the HIV-1 syncytium-inducing virus HXBc2 (HXBc2 cells) and untransfected Jurkat cells as the CD4+ fusion partner (Cao et al., 1996). The use of cells expressing Env from a highly fusogenic prototypic clade B virus, the most prevalent clade in Mexico and the Western world (Gudino & Soler, 2000; WHO, 2001) allows a fair comparison between individuals affected by different virus variants.

An accurate quantification of fusion events was attained by a recently designed flow cytometry assay (Huerta et al., 2002), involving the labelling of fusion partner cells with the fluorescent carbocyanines DiI and DiO (Molecular Probes). Fig. 1(a) and (b) show typical fluorescence dot plots of fusion between DiO-HXBc2 and DiI-E6 labelled Jurkat cells after 5 h of co-culture; a 14–18% of fused double fluorescent particles was consistently obtained. Dependence of the cell–cell fusion on Env and CD4 is shown by the inhibition caused by an anti-CD4 mAb (clone RPA-T4; BD Pharmingen) and by three HIV-1 neutralizing mAbs (Fig. 1c). Fusion was potently inhibited by the anti-CD4 mAb (IC50 = 4 μg ml−1). Approximately 50% inhibition was obtained with 52 and 64 μg ml−1 of 2G12 anti-gp20 and 2F5 anti-gp41 mAbs, respectively. Less than 50% inhibition was obtained with 50 μg ml−1 of the F105 mAb. These anti-Env mAb concentrations are several to 100-fold higher than those reported to inhibit the infection of cells by HXBc2 and related strains (Posner et al., 1991; McKeating et al., 1996; Trkola et al., 1998; Zwick et al., 2001) but similar to those used by others to inhibit Env-dependent cell–cell fusion (Allaway et al., 1993).

A cross-sectional study of the effect of serum from 49 HIV-1-infected subjects on the Jurkat Env-mediated fusion was subsequently performed. Sera were obtained from patients attending the Infectology Department at the Instituto Nacional de Ciencias Médicas y Nutrición ‘Salvador Zubirán’ in Mexico City, from March to October of 2002. Except one case (perinatal transmission in a 12 year old), all were adults infected by sexual transmission. Only subjects not having received treatment were included in the study after written informed consent. Sera from eight healthy HIV-1-negative individuals used as controls generally caused a slight enhancement of fusion (6% mean increase) when compared with fusion assays without serum. Therefore, activity of sera from HIV-1-infected patients on cell fusion (fusion activity, FA) was defined as enhancing.

Fig. 1. Quantification of HIV-1-envelope dependent cell–cell fusion by flow cytometry. DiO-HXBc2 Jurkat cells were incubated with DiI-E6 Jurkat cells for 5 h in serum-free AIM-V medium (Invitrogen) in the absence (a) or presence (b) of 33 μg anti-CD4 mAb ml−1. Percentage of dual-fluorescent cells is indicated in the upper right quadrant. (c) Fusion inhibition obtained with anti-CD4, anti-gp41 (2F5) and two anti-gp120 (F105 and 2G12) mAbs. Data are expressed as percentage of fusion obtained in the absence of antibodies.
inhibiting or not effective with respect to the mean of fusion obtained with three dilutions of HIV-1-negative sera ± 2 standard deviations (SD) (−6.167 ± 19.87). On this basis, 34 of 49 HIV-1-positive sera (69.4%) were inhibitors, 12 (24.5%) were not effective and 3 (6.1%) were enhancers of fusion (Fig. 2a). A high correlation between results from the three dilutions tested was found ($r^2 = 0.9394, P < 0.00001$).

To evaluate the participation of IgG and IgM in the inhibitory and enhancing FAs of the sera, immunoglobulins were removed by adsorption with protein G or anti-human IgM coupled to agarose beads (Sigma) and the FA values were estimated before and after adsorption. As shown in Fig. 2(b), adsorption of IgG completely eliminated the FA of all of 12 inhibitory sera, whereas removal of IgM reduced it partially to about 47.7%, on average. Both IgG and IgM seem to participate in the enhancing effect, since removal of each isotype reduced the activity of the two enhancing sera.

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**Fig. 2.** (a) Serum activity upon cell–cell fusion. Three dilutions of each serum were preincubated with DiO-HXBc2 cells for 40 min at 37 °C before the addition of DiI-E6 cells. After 5 h co-culture, cells were washed, fixed and analysed by flow cytometry. FA of each serum was calculated with respect to the fusion obtained in the absence of serum. FA is $(1 - F_i / F_o) \times 100$, where $F_i$ and $F_o$ are the percentages of fusion obtained in the presence and absence of serum, respectively. Patient sera were classified by comparison with HIV-1-negative sera as: I, inhibitors; E, enhancers; N, non-effective. Area between dotted lines represents the mean FA of eight non-infected individuals ± 2SD. Positive values indicate inhibition of fusion, whereas negative values indicate enhancement. (b) Effect of the removal of IgG and IgM from sera.
tested, although adsorption of IgM produced a greater reduction than adsorption of IgG (44% and 100%, and 19 and 73%, respectively). Thus, the FA of inhibiting sera was due mainly to IgG whereas enhancing was related to IgM. Reduction of the inhibitory effect after adsorbing IgM could result from the removal of inhibitory IgG in the form of immune complexes comprising viral particles and both HIV-1-specific IgG and IgM. Possible explanations for the differential activities of IgG and IgM would be: (i) different avidity due to antibody valence; and (ii) different target epitopes on Env protein, among others.

The effect of sera on Env-dependent cell–cell fusion is in significant correlation with the number of CD4+ cells and viral load in circulation of HIV-1-infected individuals as well as with clinical progression of the disease (Fig. 3). Sera from patients with more than 200 CD4+ cells per mm3 inhibited fusion significantly above the sera from patients with the lower numbers of CD4+ lymphocytes (P=0.0054) (Fig. 3a). Likewise, the inhibitory activity of serum is significantly higher for patients with viral loads <75 000 HIV-1 RNA copies ml−1. Inhibitory activity significantly decreases when plasma viral load exceeded that of 75 000 RNA copies ml−1 (P=0.0366). (Fig. 3b). FA of sera showed significant differences between groups in distinct clinical status (Fig. 3c). FA of sera from patients with AIDS was significantly lower than those with non-AIDS conditions (P=0.0277). Although the mean FA of HIV-1-negative sera did not differ from that of sera from patients with advanced disease, the sera from patients with more advanced infection produced the widest range of FA values. Consistent with these data, a positive correlation between FA and the CD4+ cell count (P=0.0272), and a negative correlation between FA and viral load (P=0.0362) were found significant. The three parameters considered (CD4+ T-cell count, viral load and clinical status) were available for 25 of 49 patients studied. For these patients, FA of sera was significantly inhibitory for patients with no disease progression (viral loads <75 000 RNA copies ml−1, CD4+ T-cell count >200 cells per mm3 and asymptomatic or with non-AIDS related conditions) compared with that of patients with advanced disease [viral loads >75 000 RNA copies ml−1, CD4+ T-cell count <200 cells per mm3 and AIDS condition (P=0.0494)].

Different levels of inhibitory activity of the HIV-1-positive sera on fusion may reflect the temporal changes in the infected person’s serum levels of antibodies against Env proteins, the bulk of which usually increases early in infection and decays at later times, when CD4+ T cells also decrease and viral load increases (Cecilia et al., 1999; Montefiori et al., 1996; Ngo-Giang-Huong et al., 2001; Pilgrim et al., 1997; Robert-Guroff et al., 1988). In the initial phases of infection, a high level of cell-fusion inhibitory activity in the sera of an HIV-1-infected individual could be interpreted as a sign of effective immune containment of disease progression. In later stages of infection, when serum levels of effective anti-Env antibodies have declined and/or the viral particles are in excess with respect to antibodies, they are sequestered by the virus and there would be no inhibition of fusion. Since this study was performed using the Env protein from a prototype HIV-1 strain, the effect of patient’s serum on fusion is likely due to antibodies reacting with conserved epitopes essential for CD4 and co-receptor–gp120 interactions, and those participating in gp41 activation. However, the participation of autoantibodies specific for cellular molecules in the effect of patient’s sera cannot be ruled out since a variety of autoantibodies including some directed to CD4, lymphocyte molecules and phospholipids are present in a great proportion of HIV-1-positive individuals (Asherson & Shoenfeld, 2003; Muller et al., 1994; Sekigawa et al., 1991).

The comparison between clinical groups with respect to their sera activities on cell-fusion (Fig. 3c) provided an explanation for the results large variance (Fig. 3). The sera of

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**Fig. 3.** Relationship of sera activity on fusion with CD4+ lymphocyte count (a), viral load (b) and clinical status (c). P values for significant differences are shown. Clinical status was defined according to the 1993 CDC Classification System for HIV-1 infection (Gastro et al., 1993). Dilution of sera was 1:50.
HIV-1-positive non-AIDS patients are significantly more inhibitory (mean = 19.07, SD = 20.49) than the sera of healthy persons (mean = −3.65, SD = 8.69) and none of them was enhancing. In AIDS cases, the mean of fusion values falls back to control levels (2-65) and 30% of the sera enhance fusion, one of them well beyond the level of sera from healthy people, resulting in a wide distribution of FAs (SD = 28.38). Enhancement of fusion was also more frequent in the higher (5 of 17, 29%) than in the lower viral load group (1 of 23, 4%). Thus, if there are two functional kinds of fusion-active immunoglobulins in the serum, one that inhibits cell fusion (mainly IgG) and another that enhances it (IgM), then their relative effectiveness and concentrations would determine the net degree of cell fusion along the course of the infection.

Besides the prognostic value of the quantitative cell-FA in the sera of HIV-1-infected persons, the identification of the viral or cell epitopes involved in the inhibition and enhancement of cell-cell fusion may lead to immune interventions for preventing or controlling the progression of HIV-1 disease by way of interfering with Env-mediated cell-cell fusion. The sensitive flow cytometry assay we have developed, may facilitate the regular exploration of the potential of HIV-1-infected patient’s immune response to confront HIV-1 Env-mediated cell fusion.

Given the failure of neutralizing antibodies to control the established infection (Poignard et al., 1999) this work emphasizes that, besides neutralizing antibodies, the effect of sera on syncytium formation should be considered for a comprehensive understanding of the role of immune factors in HIV-1 spreading and cell damage. Identification of the epitopes inducing the production of fusion inhibitory antibodies could be of therapeutic and preventive value. Elucidation of the factors enhancing fusion may provide new insights in the pathogenesis of HIV-1 infection.

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