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Minireview

Is there enough gp120 in the body fluids of HIV-1-infected individuals to have biologically significant effects?

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Over the past decade, many publications have described experiments in which the recombinant monomeric form of the gp120 surface envelope (Env) glycoprotein of human immunodeficiency virus type 1 (HIV-1) has been added to cells in vitro (Fig. 1). The ensuing cellular responses (e.g., activation of signal transduction pathways resulting in cytokine release, chemotaxis, proliferation, anergy, or apoptosis) are monitored. The outcome is generally that gp120 can kill a target cell or perturb its normal functions, and it is assumed that what is observed in vitro is relevant in vivo. Our intent is to question whether such an extrapolation is reasonable on quantitative grounds, particularly when the presence of antibodies (Abs) in the plasma of HIV-1-infected persons is taken into account. We cite only a small selection from this abundant literature, to illustrate the range of active gp120 concentrations reported.

In the in vitro experiments, the gp120 concentrations vary from 1 pM to 1 μM (ca. 0.12 ng/ml to 120 μg/ml, as 1 nM ≈ 0.12 μg/ml, e.g. Arthos et al., 2002; Chirmule et al., 1990; Davis et al., 1997; Esser et al., 2001; Goldman et al., 1994; Herbein et al., 1998; Hesselgesser et al., 1998; Huang et al., 2001; Kanmogne et al., 2001; Keswani et al., 2003; Kornfeld et al., 1988; Mann et al., 1987; Masci et al., 2003; Munshi et al., 2003; Oyaizu et al., 1990; Schneider-Schaulies et al., 1992; Tamma et al., 1997; Vlahakis et al., 2003; Wahl et al., 1989; Weinhold et al., 1989; Weissman et al., 1997; Yao et al., 2001). Sometimes biological effects occur only at the higher end of the range, although particularly in neuronal cell systems lower gp120 concentrations can be active. In those systems, the primary effects may be partly on microglial cells, which are reported to amplify secondary effects on neurons (cf. Garden, 2002; Kaul and Lipton, 1999; Keswani et al., 2003, reviewed in Kaul et al., 2001).

Historical measurements of plasma gp120 concentrations

Two papers are usually cited to suggest that the gp120 concentrations used in vitro resemble those in body fluids, specifically plasma (Gilbert et al., 1991; Oh et al., 1992). Our impression is that these papers are often either cited incorrectly or misunderstood. What do they, in fact, report? Oh et al. detected gp120 in a majority of AIDS patients' sera in the range 0.1–0.8 nM. No gp120 was found in the sera of HIV-1-infected individuals with AIDS-related complex (ARC). Thus, only in sera from people at the late clinical stages of infection, when HIV-1 antigen levels tend to rise, was free gp120 ever, apparently, detectable. However, gp120 in complex with antibody (Ab) was found in a larger proportion of sera, a point to which we shall return. In contrast, Gilbert et al. (2003) detected gp120 only in the range 2–20 pM, and then only in a minority of sera from p24-antigenemic AIDS and ARC patients. The plasma gp120 concentrations detected by Oh et al. were thus one to two orders of magnitude higher than those described by Gilbert et al. (2003). Hence, the two papers should not be cited as agreeing with each other.

Both papers rely on capture enzyme-immunoassays to quantify gp120. The assay of Gilbert et al. (2003) uses a soluble form of CD4, the primary receptor for gp120 (see Fig. 1), to capture gp120 onto a solid phase. The bound gp120 is then detected with a polyclonal sheep Ab raised against a peptide from the C terminus of gp120 of the T-cell line-adapted isolate IIIB. This antibody, D7324, cross-reacts strongly with gp120s from multiple HIV-1 strains, particularly within subtype B but also outside it (Moore and Jarrett, 1988; Moore et al., 1994b). As soluble CD4 is pan-reactive with properly folded gp120s, the assay used by Gilbert et al. (2003) is relatively little affected by gp120 sequence diversity. In contrast, Oh et al. employed a polyclonal serum to gp120 of the IIIB isolate for capture, with a monoclonal Ab (MAb) to the V3 loop of IIIB gp120 as the detection

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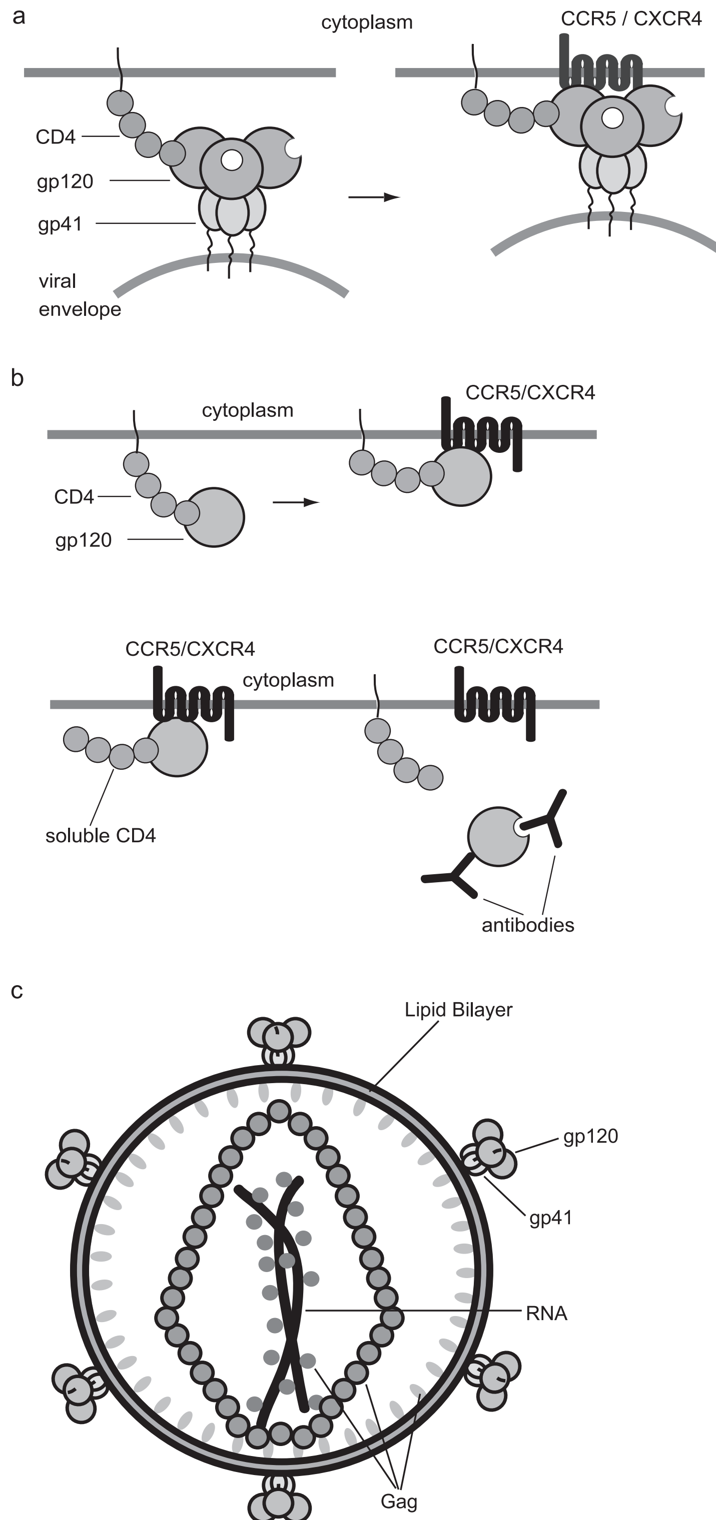
reagent. Details of the specificity of the latter MAb are not provided, but it is stated "to have 10–15% cross-reactivity with other strains". Regardless of whether this value refers to the extent of binding or the proportion of test gp120s that it reacted with, it is now well understood that the recognition of gp120 from primary viruses by IIIB-specific V3-loop MAbs is usually poor. The cross-reactivity capabilities of the assay used by Oh et al., and hence its ability to detect and quantify gp120 in plasma, is, therefore, questionable at best. This assay would be expected to *underestimate* plasma gp120 content by failing to recognize gp120 from the infecting strain. However, its results suggest that gp120 is



present in plasma at surprisingly high concentrations, both relative to what was found by Gilbert et al. (2003) and to viremia, as discussed below.

A related issue, approached by Oh et al. and addressed more directly by Gilbert et al. (2003), is that of interference by plasma antibodies. Gilbert et al. (2003) found that mixing gp120 with control human serum significantly decreased the subsequent signal and that high titers of HIV-1$^+$ sera could abrogate the signal completely. One of us (J.P.M.) observed much the same effect in unpublished experiments many years ago, using a capture enzyme immunoassay based on Ab D7324 and a polyclonal anti-gp120 serum. Thus, when a known amount of gp120 was spiked into different HIV-1$^+$ sera, the anti-gp120 Abs present interfered significantly with gp120 detection, and to an extent that varied greatly between the sera. Indeed, it was impossible to judge from the assay readout what amount of gp120 had been added to the different HIV-1$^+$ sera. Therefore, any estimation of how much gp120 was naturally present in the HIV-1$^+$ sera was clearly problematic. The same concerns apply to p24 antigen quantification in the presence of plasma anti-p24 antibodies: only when immune complexes are dissociated, for example by the use of heat, can p24 concentrations be properly determined (Schupbach and Boni, 1993).

Taken together, the uncertainty about the efficiency of gp120 capture, the extent of cross-reactivity of the detecting Abs with any gp120 present in plasma (at least in the assay used by Oh et al.), and the interference by plasma anti-gp120 Abs, all but preclude any accurate estimate of plasma gp120 concentrations by the methods that have been used to date. Of note is that Gilbert et al. (2003) found no correlation between plasma p24 and gp120 concentrations, which may reflect differences in the extent of Ab complexing with the two antigens. The limitations of the published assays need to be taken into account when these papers are cited, particularly in respect of the high gp120 concentrations reported by Oh et al.

Alternative estimates of plasma gp120 concentrations

What concentrations of gp120 could be expected in HIV-1$^+$ plasma? Plasma concentrations of the viral Gag protein (Fig. 1) p24 provide a useful guide. Most plasma p24 antigen is normally Ab-complexed or virion-associated. But after its release as a free protein, it is detected at concentrations <40 pM (Ledergerber et al., 2000), that is, just above the 2–20 pM reported for free gp120 by Gilbert et al. (2003). If virions were the only source, gp120 concentrations would be 40- to 60- fold lower than those of p24 (Chertova et al., 2002; Layne et al., 1992; Zhu et al., 2003). It can be calculated that a plasma viral load of 10^6 virions/ml—a high level for chronic HIV-1 infection—corresponds to only 0.03–0.07 pM of virion-associated gp120 and 2–3 pM p24. While this concentration of virion-associated p24 is somewhat below the upper range of total p24 in plasma (Ledergerber et al., 2000), this gp120 concentration is between two (Gilbert et al., 1991) and four (Oh et al., 1992) orders of magnitude lower than the often cited values.

Gp120 that is not associated with virions could potentially be derived from infected cells. The envelope glycoprotein complex (Fig. 1) is produced and processed via the secretory pathway, whereas the Gag precursor is synthesized on free ribosomes in the cytoplasm. Although virions incorporate approximately 50-fold fewer Env than Gag molecules when they bud from cellular membranes (see Fig. 1c) (Chertova et al., 2002), we do not know the ratio of Gag to Env in infected cells in vivo. It could be argued that the majority of Env never exits from the secretory pathway, and that significant additional amounts of gp120 is released from dead or moribund cells as "viral debris" (Parren et al., 1997). However, some of this debris would not interact with receptors and such lysed cells would also release p24. Hence, it is hard to explain how gp120 proteins capable of receptor binding could be present at higher concentrations than p24.

The effect of plasma antibodies on gp120–receptor interactions

We noted above that plasma anti-gp120 Abs mask the detection and quantification of gp120. The same antibodies have a very significant effect on the receptor interactions of any gp120 that is present in plasma. Abs to gp120 are usually present at high enough concentrations in plasma to bind up most of the gp120 present. The ratio $[Ab]/K_d$ determines their degree of binding to gp120, in accordance with the law of mass action (Klasse and Sattentau, 2002). Anti-gp120 Ab concentrations have been estimated to be in the micromolar range (Binley et al., 1997); so for high-affinity binding (K_d < 10 nM), the occupancy of gp120 by

Fig. 1. (a) The HIV-1 envelope glycoprotein (Env) complex consists of trimers of non-covalently linked heterodimers of an outer, receptor-binding moiety, gp120, anchored to a transmembrane protein gp41, which is involved in the fusion of the viral envelope with the cell membrane. The gp120 moiety is shown (*left*) interacting with the four-domain receptor, CD4. This binding induces a conformational change that facilitates the interaction of gp120 with a coreceptor, CCR5 for R5 virus and CXCR4 for X4 virus (*right*). The interactions of gp120 with CCR5 and CXCR4 are weak in the absence of CD4. (b) A monomer of gp120 is shown to undergo interactions corresponding to those in (a). This scheme is reproduced in many experiments making use of monomeric recombinant gp120. A significant degree of binding and many experimental effects are only obtained at much higher concentrations than what could realistically be present in extracellular fluids in vivo (*top*). A complex between gp120 and soluble CD4 is shown to interact with a coreceptor on the cell surface. In the absence of CD4, the affinity of gp120 for CCR5 or CXCR4 is low (*bottom*, *left*). Specific antibodies prevent gp120 from binding to CD4; this also precludes further, downstream contact with the coreceptor (*bottom, right*). The blocking effect of antibodies is likely to occur in vivo except in certain tissues where their concentration is lower, such as the central nervous system. (c) An HIV-1 virion is shown schematically. The Env trimers of heterodimers (gp120 and gp41) stud the phospholipid bilayer that surrounds the viral Gag proteins and RNA genome. The copy-number ratio of the Gag to Env in virions is ~50.

Abs should approach saturation. And the titers of Abs able to inhibit the binding of gp120 to CD4 (and hence indirectly to CCR5 or CXCR4) are in the range 1:100 to 1:1000 in HIV-1^+ sera (Callahan and Norcross, 1989; Moore et al., 1994a). Thus, in the presence of undiluted HIV-1^+ plasma, as occurs in vivo, there would be effectively no binding of gp120 monomers to CD4 or the co-receptors. This is rarely accounted for in the design and interpretation of in vitro studies with recombinant gp120, but it always should be.

Less complexing of gp120 by Abs would occur in some tissue locales. For example, Abs are present only at low levels in the central nervous system, even when HIV-1 infection causes intrathecal Ab production and blood–brain barrier leakage (Goudsmit et al., 1987; Kaul et al., 2001). In general, Ab concentrations in different tissues are likely to vary considerably from those of gp120 and virus. Predicting the net effects of variations in relative and absolute concentrations of Ab, gp120 and virus is a complex task that we do not attempt here.

Concentration of gp120 in tissues

The putative levels of gp120 measured, or plausibly present, in plasma are far below some of those that have significant effects on cells in vitro. But could the latter concentrations nevertheless be biologically relevant by matching those in compartments other than blood? The gp120 concentrations in, for example, the interstitial spaces of lymph nodes or other solid organs are unknown. Nevertheless, if the greater density of cells, the smaller extracellular space and the possibly slower dilution kinetics were quantitatively factored in, it seems plausible that gp120 could be present within interstitial lymph node spaces at concentrations several orders of magnitude higher than in plasma. Furthermore, if small secluded spaces are created during cell-to-cell transmission of HIV-1 and HTLV-1, the so-called virological synapses (Igakura et al., 2003; Jolly et al., 2004), then viral proteins may be present at high local concentrations in those clefts. In vitro studies involving Env-producing cells may, therefore, be more realistic than those using soluble, recombinant gp120 (Castedo et al., 2001, 2002; Jekle et al., 2003). However, the gp120 concentration gradients produced by such cells are difficult to assess. And membrane-associated Env may differ from soluble gp120 in, for example, its qualitative effects on T-cell activation (Schwartz et al., 1994).

Another relevant complication is that gp120 from X4 viruses, but not R5 viruses, binds to heparan-sulphate glycosoaminoglycan (GAG) moieties of proteoglycans, and thereby can be retained within tissues both in the extracellular matrix and on cell surfaces (Moulard et al., 2000; Ugolini et al., 1999). GAGs are present on the surface of many cell types (Ugolini et al., 1999). An analogy may be drawn between gp120 and chemokines that, in vivo, do not seem to act as free proteins. Chemokines, instead, interact with G-protein-coupled receptors while in the form of surface-bound GAG complexes that establish haptotactic gradients in tissues (Proudfoot et al., 2003). Such potentially modulating effects of the tissue environment complicate the rational design and interpretation of in vitro experiments, which by necessity simulate in vivo conditions imperfectly.

We conclude that the relevant gp120 concentrations in the organism are essentially unknown.

Affinity of gp120 for its receptors and the influence of receptor occupancy

Ultimately, any consequences of local concentrations of gp120 depend on its affinity for the relevant receptors and the degree of binding required for signals to be transduced. Several effects of gp120 are mediated through CD4 binding, either directly or indirectly through subsequent CD4-dependent interactions with a chemokine coreceptor. The K_d of gp120 binding to CD4 is in the range 1–10 nM (Ashkenazi et al., 1990; Ivey-Hoyle et al., 1991; Moebius et al., 1992; Moore, 1990). That is higher even than the *concentrations* reported by Oh et al. and 1000-fold higher than those found by Gilbert et al. (2003). However, gp120 may also bind with high affinity to DC-SIGN and other C-type lectin receptors (Geijtenbeek et al., 2002; Turville et al., 2002), as well as to the GAG moieties of proteoglycans (Moulard et al., 2000; Ugolini et al., 1999). Although the latter interactions of soluble monomeric X4 gp120 are readily reversible (Mondor et al., 1998b), binding to such accessory attachment molecules could raise the effective gp120 concentrations available for other receptor interactions.

Quite distinct degrees of binding, or occupancies, of cellular receptors may be required to exert the different effects on the target cells that we are discussing. But generally, the occupancy can be estimated from the formula $[[\text{gp120}]/K_d]/[[1 + [\text{gp120}]]/K_d]$ (Klasse and Moore, 1996). Thus, for 99% occupancy, the concentration of gp120 must be >100-fold above K_d. That means 0.1–1 μM for CD4 binding. Indirect effects of gp120 on T-cell activation, mediated by blocking the interactions of antigen-MHC class II with CD4 and the T-cell receptor (Chirmule et al., 1995), would quite plausibly require the binding of gp120 to a large proportion of CD4 molecules. Some effects involving signaling via cell-surface receptors are, in principle, different. Thus, much lower occupancies, produced by gp120 concentrations close to or below K_d (Munshi et al., 2003) could conceivably be effective. Most biological effects would nevertheless require a detectable occupancy. Hence, we face a double conundrum: either active concentrations of gp120 are above K_d for receptor binding, which may not be realistic under in vivo conditions; or they are lower, which makes it difficult to explain how substantial binding could be achieved.

Some effects of gp120 are suggested to occur independently of CD4 (for example, Iyengar et al., 1999). The

affinity of gp120 for CCR5 and CXCR4 in the absence of CD4 is usually found to fall below the limit of detection. Thus, there was no detectable X4 gp120 binding to CXCR4 at concentrations as high as 0.25–0.5 μM (Doranz et al., 1999; Mondor et al., 1998a), and little binding of R5 gp120 to CCR5 at 0.4–0.5 μM (Trkola et al., 1996; Wu et al., 1996). There is, however, one starkly contrasting report of higher-affinity gp120 binding ($K_d \approx 70$ nM) to CXCR4 on CD4-negative, differentiated neuronal cells (Hesselgesser et al., 1997). The binding of soluble-CD4–gp120 complexes to CCR5 has a K_d of ~4 nM (Doranz et al., 1999; Wu et al., 1996), and to CXCR4 of ~200 nM (Babcock et al., 2001). Despite the poor or controversial capacity of gp120 to interact directly with CCR5 or CXCR4, a pathophysiological role for the interaction of gp120 with these molecules on neurons and astrocytes has been proposed (Kaul et al., 2001). If the highest reported gp120–CXCR4 affinity is accurate (Hesselgesser et al., 1997), then the dose dependence of X4 gp120-mediated apoptotic effects via CXCR4 on $CD4^-$ neuronal cells is as expected, that is, a significant and increasing response from 20 nM to 1 μM (Hesselgesser et al., 1998). But whether that extremely high concentration range is relevant in vivo remains to be confirmed. In contrast, much lower concentrations of gp120 (0.1–200 pM) have also been found to be neurotoxic, with and without intermediary effects on Schwann and glial cells (Keswani et al., 2003; Meucci et al., 1998). The occupancy of CXCR4 at gp120 concentrations in the sub-nanomolar range would be immeasurably low (<0.1%), even if we assume that the $K_d \approx 70$ nM (Hesselgesser et al., 1997).

It is possible to investigate whether gp120 is bound to cells from HIV-1-infected individuals, and at what occupancy, ex vivo. The presence of gp120 attached to CD4 on the T-cell surface ex vivo has been inferred, although not directly detected (Amadori et al., 1992). But there is also a converse finding of the failure to detect specific masking of the gp120-binding site on CD4 on T cells from HIV-1-infected individuals (Kunkl et al., 1994). Resolving whether gp120 is detectable on the surface of $CD4^+$ (or $CD4^-$) cells ex vivo would help clarify gp120's pathogenic role.

The outstanding task, then, is to assess and explain the occupancy of receptors by gp120 in vivo and what effects that has on the cells.

Use of virions in vitro

Some in vitro experiments have used virus-like particles or inactivated virions to study HIV-1-induced apoptosis, for comparison with the effects of recombinant soluble gp120 (Esser et al., 2001; Vlahakis et al., 2003; Yao et al., 2001). When virus for this use is concentrated by several orders of magnitude, considerations apply that are similar to those for monomeric gp120: how well does the virion concentration used in vitro reflect what is present in vivo? Can virion densities rise to particularly high levels in certain locales, such as interstitial spaces in lymph nodes, and there exert the effects observed in vitro? The affinity of virions for target cells is unknown but liable to be the net outcome of two opposing influences. The receptor-interactive surfaces on the gp120 subunits are relatively inaccessible in the context of the virion-associated Env trimer, which will reduce the functional affinity of the interaction. Countering this, is the polyvalency effect of multiple trimers interacting with multiple receptors (as partly illustrated for murine leukemia virus; Yu et al., 1995). The binding of X4 virions to heparan sulphate proteoglycans on the cell surface is indeed more avid than that of monomeric gp120 (Mondor et al., 1998b).

Inactivated virus with a content of 0.4 nM of p24 (Esser et al., 2001), or even as high as 4 nM (Vlahakis et al., 2003), has been used in vitro. This corresponds to ~8–80 pM virion-associated gp120. The degree of receptor binding that may ensue at these levels of virion-associated Env cannot be rationally predicted at present. But the maximal virus-induced apoptotic effect could not be mimicked by the corresponding amounts of monomeric gp120 or heat-denatured virions, and it required the presence of MHC class II on the virion (Esser et al., 2001). However, in another experimental system, HIV-1-induced cytolysis occurred regardless of the presence of MHC class II (LaBonte et al., 2003). Cytolysis is an alternative mechanism of cell death to induced by receptors, and cytolysis are alternative mechanisms of cell death, cytolysis requires fusogenic Env protein, and affects only the infected cell (LaBonte et al., 2003).

The relative relevance of the experimental use of soluble Env, inactivated virions and fusogenic replicating virus to the pathogenesis of AIDS needs to be elucidated.

Improving the design of experiments using gp120

How could the design of in vitro studies using monomeric gp120 be improved (see box)? The possible presence of biologically active contaminants, including endotoxins in gp120 preparations from commercial and other sources should always be considered. The use of anti-gp120 MAbs specifically to prevent gp120-CD4 or -coreceptor binding is a prudent control. As noted above, HIV-1^+ serum antibodies will have much the same effect as the specific MAbs, and their presence in vivo must be taken into account. Gp120 point mutants defective for CD4 or coreceptor binding provide further controls. Thereby one can at least determine whether the consequences of sprinkling gp120 on mammalian cells depend on receptor binding, or whether they are merely attributable to contaminants in the protein preparation.

Conclusions

We do not argue that gp120 could never have a biological effect on cells in vivo via receptor-mediated interactions. Nor is it impossible that virions could influence cellular

processes in vivo independently of receptor-mediated fusion events.

We do, however, argue that it is not an adequate mimic of in vivo biology simply to add free gp120 (or virions) to target cells in vitro in amounts that are apparently several orders of magnitude greater than in body fluids. Moreover, it is not appropriate to justify the amounts of gp120 used by reference to the two decade-old papers that purport to measure free gp120 in the plasma of HIV-1-infected people. These papers are not consistent with each other, and the more frequently cited study, by Oh et al., has serious design flaws that may cast doubt on the gp120 concentrations it promulgates. The much lower gp120 concentrations recorded by Gilbert et al. (2003) are likely to be closer to true levels. And the presence of plasma anti-gp120 Abs that block receptor binding should inform the design of in vitro experiments (see Box 1). Controls for gp120 purity and for the specificity of the interactions with CD4, chemokine receptors and GAGs should also be included in experimental protocols. Some of these considerations apply, of course, to other studies of similar design that use high concentrations of other HIV-1 proteins, such as Tat and Vpr, in vitro, in the hope that this is relevant to pathogenesis.

Box 1. *Criteria for establishing the biological relevance of experiments using gp120 in vitro*

1. Experimental concentration ranges shown to be relevant to the particular tissue compartment modeled.
2. Specificity of the receptor interactions demonstrated by use of gp120 deletion mutants or Abs blocking receptor binding.
3. Demonstration that the requisite receptor occupancy can be obtained under experimental conditions.
4. Inclusion of anti-gp120 Abs with a binding capacity (concentration and affinity) corresponding to that in the relevant tissue compartment.
5. Comparison of effects of recombinant gp120 with those of realistic levels of virions.

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References

Amadori, A., De Silvestro, G., Zamarchi, R., Veronese, M.L., Mazza, M.R., Schiavo, G., Panozzo, M., De Rossi, A., Ometto, L., Mous, J., et al., 1992. CD4 epitope masking by gp120/anti-gp120 antibody complexes. A potential mechanism for CD4+ cell function downregulation in AIDS patients. J. Immunol. 148 (9), 2709–2716.

Arthos, J., Cicala, C., Selig, S.M., White, A.A., Ravindranath, H.M., Van Ryk, D., Steenbeke, T.D., Machado, E., Khazanie, P., Hanback, M.S., Hanback, D.B., Rabin, R.L., Fauci, A.S., 2002. The role of the CD4 receptor versus HIV coreceptors in envelope-mediated apoptosis in peripheral blood mononuclear cells. Virology 292 (1), 98–106.

Ashkenazi, A., Presta, L.G., Marsters, S.A., Camerato, T.R., Rosenthal, K.A., Fendly, B.M., Capon, D.J., 1990. Mapping the CD4 binding site for human immunodeficiency virus by alanine-scanning mutagenesis. Proc. Natl. Acad. Sci. U.S.A. 87 (18), 7150–7154.

Babcock, G.J., Mirzabekov, T., Wojtowicz, W., Sodroski, J., 2001. Ligand binding characteristics of CXCR4 incorporated into paramagnetic proteoliposomes. J. Biol. Chem. 276 (42), 38433–38440.

Binley, J.M., Klasse, P.J., Cao, Y., Jones, I., Markowitz, M., Ho, D.D., Moore, J.P., 1997. Differential regulation of the antibody responses to Gag and Env proteins of human immunodeficiency virus type 1. J. Virol. 71 (4), 2799–2809.

Callahan, L.N., Norcross, M.A., 1989. Inhibition of soluble CD4 therapy by antibodies to HIV. Lancet 2 (8665), 734–735.

Castedo, M., Ferri, K.F., Blanco, J., Roumier, T., Larochette, N., Barretina, J., Amendola, A., Nardacci, R., Metivier, D., Este, J.A., Piacentini, M., Kroemer, G., 2001. Human immunodeficiency virus 1 envelope glycoprotein complex-induced apoptosis involves mammalian target of rapamycin/FKBP12-rapamycin-associated protein-mediated p53 phosphorylation. J. Exp. Med. 194 (8), 1097–1110.

Castedo, M., Roumier, T., Blanco, J., Ferri, K.F., Barretina, J., Tintignac, L.A., Andreau, K., Perfettini, J.L., Amendola, A., Nardacci, R., Leduc, P., Ingber, D.E., Druillennec, S., Roques, B., Leibovitch, S.A., Vilella-Bach, M., Chen, J., Este, J.A., Modjtahedi, N., Piacentini, M., Kroemer, G., 2002. Sequential involvement of Cdk1, mTOR and p53 in apoptosis induced by the HIV-1 envelope. EMBO J. 21 (15), 4070–4080.

Chertova, E., Bess Jr., J.W., Crise Jr., B.J., Sowder, I.R., Schaden, T.M., Hilburn, J.M., Hoxie, J.A., Benveniste, R.E., Lifson, J.D., Henderson, L.E., Arthur, L.O., 2002. Envelope glycoprotein incorporation, not shedding of surface envelope glycoprotein (gp120/SU), is the primary determinant of SU content of purified human immunodeficiency virus type 1 and simian immunodeficiency virus. J. Virol. 76 (11), 5315–5325.

Chirmule, N., Kalyanaraman, V.S., Oyaizu, N., Slade, H.B., Pahwa, S., 1990. Inhibition of functional properties of tetanus antigen-specific T-cell clones by envelope glycoprotein GP120 of human immunodeficiency virus. Blood 75 (1), 152–159.

Chirmule, N., McCloskey, T.W., Hu, R., Kalyanaraman, V.S., Pahwa, S., 1995. HIV gp120 inhibits T cell activation by interfering with expression of costimulatory molecules CD40 ligand and CD80 (B71). J. Immunol. 155 (2), 917–924.

Davis, C.B., Dikic, I., Unutmaz, D., Hill, C.M., Arthos, J., Siani, M.A., Thompson, D.A., Schlessinger, J., Littman, D.R., 1997. Signal transduction due to HIV-1 envelope interactions with chemokine receptors CXCR4 or CCR5. J. Exp. Med. 186 (10), 1793–1798.

Doranz, B.J., Orsini, M.J., Turner, J.D., Hoffman, T.L., Berson, J.F., Hoxie, J.A., Peiper, S.C., Brass, L.F., Doms, R.W., 1999. Identification of CXCR4 domains that support coreceptor and chemokine receptor functions. J. Virol. 73 (4), 2752–2761.

Esser, M.T., Bess Jr., J.W., Suryanarayana, K., Chertova, E., Marti, D., Carrington, M., Arthur, L.O., Lifson, J.D., 2001. Partial activation and induction of apoptosis in CD4(+) and CD8(+) T lymphocytes by conformationally authentic noninfectious human immunodeficiency virus type 1. J. Virol. 75 (3), 1152–1164.

Garden, G.A., 2002. Microglia in human immunodeficiency virus-associated neurodegeneration. Glia 40 (2), 240–251.

Geijtenbeek, T.B., Engering, A., Van Kooyk, Y., 2002. DC-SIGN, a C-type lectin on dendritic cells that unveils many aspects of dendritic cell biology. J. Leukoc. Biol. 71 (6), 921–931.

Gilbert, M., Kirihara, J., Mills, J., 1991. Enzyme-linked immunoassay for human immunodeficiency virus type 1 envelope glycoprotein 120. J. Clin. Microbiol. 29 (1), 142–147.

Gilbert, P.B., Chiu, Y.L., Allen, M., Lawrence, D.N., Chapdu, C., Israel, H., Holman, D., Keefer, M.C., Wolff, M., Frey, S.E., 2003. Long-term safety analysis of preventive HIV-1 vaccines evaluated in AIDS vaccine evaluation group NIAID-sponsored Phase I and II clinical trials. Vaccine 21 (21–22), 2933–2947.

Goldman, F., Jensen, W.A., Johnson, G.L., Heasley, L., Cambier, J.C., 1994. gp120 ligation of CD4 induces p56lck activation and TCR desensitization independent of TCR tyrosine phosphorylation. J. Immunol. 153 (7), 2905–2917.

Goudsmit, J., Epstein, L.G., Paul, D.A., van der Helm, H.J., Dawson, G.J., Asher, D.M., Yanagihara, R., Wolff, A.V., Gibbs Jr., C.J., Gajdusek, D.C., 1987. Intra-blood–brain barrier synthesis of human immunodeficiency virus antigen and antibody in humans and chimpanzees. Proc. Natl. Acad. Sci. U.S.A. 84 (11), 3876–3880.

Herbein, G., Mahlknecht, U., Batliwalla, F., Gregersen, P., Pappas, T., Butler, J., O'Brien, W.A., Verdin, E., 1998. Apoptosis of CD8+ T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. Nature 395 (6698), 189–194.

Hesselgesser, J., Halks-Miller, M., DelVecchio, V., Peiper, S.C., Hoxie, J., Kolson, D.L., Taub, D., Horuk, R., 1997. CD4-independent association between HIV-1 gp120 and CXCR4: functional chemokine receptors are expressed in human neurons. Curr. Biol. 7 (2), 112–121.

Hesselgesser, J., Taub, D., Baskar, P., Greenberg, M., Hoxie, J., Kolson, D.L., Horuk, R., 1998. Neuronal apoptosis induced by HIV-1 gp120 and the chemokine SDF-1 alpha is mediated by the chemokine receptor CXCR4. Curr. Biol. 8 (10), 595–598.

Huang, M.B., Khan, M., Garcia-Barrio, M., Powell, M., Bond, V.C., 2001. Apoptotic effects in primary human umbilical vein endothelial cell cultures caused by exposure to virion-associated and cell membrane-associated HIV-1 gp120. J. Acquir. Immune Defic. Syndr. 27 (3), 213–221.

Igakura, T., Stinchcombe, J.C., Goon, P.K., Taylor, G.P., Weber, J.N., Griffiths, G.M., Tanaka, Y., Osame, M., Bangham, C.R., 2003. Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. Science 299 (5613), 1713–1716.

Ivey-Hoyle, M., Culp, J.S., Chaikin, M.A., Hellmig, B.D., Matthews, T.J., Sweet, R.W., Rosenberg, M., 1991. Envelope glycoproteins from biologically diverse isolates of immunodeficiency viruses have widely different affinities for CD4. Proc. Natl. Acad. Sci. U.S.A. 88 (2), 512–516.

Iyengar, S., Schwartz, D.H., Hildreth, J.E., 1999. T cell-tropic HIV gp120 mediates CD4 and CD8 cell chemotaxis through CXCR4 independent of CD4: implications for HIV pathogenesis. J. Immunol. 162 (10), 6263–6267.

Jekle, A., Keppler, O.T., De Clercq, E., Schols, D., Weinstein, M., Goldsmith, M.A., 2003. In vivo evolution of human immunodeficiency virus type 1 toward increased pathogenicity through CXCR4-mediated killing of uninfected CD4 T cells. J. Virol. 77 (10), 5846–5854.

Jolly, C., Kashefi, K., Hollinshead, M., Sattentau, Q.J., 2004. HIV-1 cell-to-cell transfer across an Env-induced, actin-dependent synapse. J. Exp. Med. 199 (2), 283–293.

Kanmogne, G.D., Kennedy, R.C., Grammas, P., 2001. Analysis of human lung endothelial cells for susceptibility to HIV type 1 infection, coreceptor expression, and cytotoxicity of gp120 protein. AIDS Res. Hum. Retrovir. 17 (1), 45–53.

Kaul, M., Lipton, S.A., 1999. Chemokines and activated macrophages in HIV gp120-induced neuronal apoptosis. Proc. Natl. Acad. Sci. U.S.A. 96 (14), 8212–8216.

Kaul, M., Garden, G.A., Lipton, S.A., 2001. Pathways to neuronal injury and apoptosis in HIV-associated dementia. Nature 410 (6831), 988–994.

Keswani, S.C., Polley, M., Pardo, C.A., Griffin, J.W., McArthur, J.C., Hoke, A., 2003. Schwann cell chemokine receptors mediate HIV-1 gp120 toxicity to sensory neurons. Ann. Neurol. 54 (3), 287–296.

Klasse, P.J., Moore, J.P., 1996. Quantitative model of antibody- and soluble CD4-mediated neutralization of primary isolates and T-cell line-adapted strains of human immunodeficiency virus type 1. J. Virol. 70 (6), 3668–3677.

Klasse, P.J., Sattentau, Q.J., 2002. Occupancy and mechanism in antibody-mediated neutralization of animal viruses. J. Gen. Virol. 83 (Pt 9), 2091–2108.

Kornfeld, H., Cruikshank, W.W., Pyle, S.W., Berman, J.S., Center, D.M., 1988. Lymphocyte activation by HIV-1 envelope glycoprotein. Nature 335 (6189), 445–448.

Kunkl, A., Valle, M.T., Fenoglio, D., Dodi, F., Morandi, N., Rizzo, F., Manca, F., 1994. Detection of T cell CD4 epitopes in HIV-infected individuals. Eur. J. Histochem. 38 (Suppl. 1), 41–46.

LaBonte, J.A., Madani, N., Sodroski, J., 2003. Cytolysis by CCR5—using human immunodeficiency virus type 1 envelope glycoproteins is dependent on membrane fusion and can be inhibited by high levels of CD4 expression. J. Virol. 77 (12), 6645–6659.

Layne, S.P., Merges, M.J., Dembo, M., Spouge, J.L., Conley, S.R., Moore, J.P., Raina, J.L., Renz, H., Gelderblom, H.R., Nara, P.L., 1992. Factors underlying spontaneous inactivation and susceptibility to neutralization of human immunodeficiency virus. Virology 189 (2), 695–714.

Ledergerber, B., Flepp, M., Boni, J., Tomasik, Z., Cone, R.W., Luthy, R., Schupbach, J., 2000. Human immunodeficiency virus type 1 p24 concentration measured by boosted ELISA of heat-denatured plasma correlates with decline in CD4 cells, progression to AIDS, and survival: comparison with viral RNA measurement. J. Infect. Dis. 181 (4), 1280–1288.

Mann, D.L., Lasane, F., Popovic, M., Arthur, L.O., Robey, W.G., Blattner, W.A., Newman, M.J., 1987. HTLV-III large envelope protein (gp120) suppresses PHA-induced lymphocyte blastogenesis. J. Immunol. 138 (8), 2640–2644.

Masci, A.M., Galgani, M., Cassano, S., De Simone, S., Gallo, A., De Rosa, V., Zappacosta, S., Racioppi, L., 2003. HIV-1 gp120 induces anergy in naive T lymphocytes through CD4-independent protein kinase-A-mediated signaling. J. Leukoc. Biol. 74 (6), 1117–1124.

Meucci, O., Fatatis, A., Simen, A.A., Bushell, T.J., Gray, P.W., Miller, R.J., 1998. Chemokines regulate hippocampal neuronal signaling and gp120 neurotoxicity. Proc. Natl. Acad. Sci. U.S.A. 95 (24), 14500–14505.

Moebius, U., Clayton, L.K., Abraham, S., Harrison, S.C., Reinherz, E.L., 1992. The human immunodeficiency virus gp120 binding site on CD4: delineation by quantitative equilibrium and kinetic binding studies of mutants in conjunction with a high-resolution CD4 atomic structure. J. Exp. Med. 176 (2), 507–517.

Mondor, I., Moulard, M., Ugolini, S., Klasse, P.J., Hoxie, J., Amara, A., Delaunay, T., Wyatt, R., Sodroski, J., Sattentau, Q.J., 1998a. Interactions among HIV gp120, CD4, and CXCR4: dependence on CD4 expression level, gp120 viral origin, conservation of the gp120 COOH- and NH2-termini and V1/V2 and V3 loops, and sensitivity to neutralizing antibodies. Virology 248 (2), 394–405.

Mondor, I., Ugolini, S., Sattentau, Q.J., 1998b. Human immunodeficiency virus type 1 attachment to HeLa CD4 cells is CD4 independent and gp120 dependent and requires cell surface heparans. J. Virol. 72 (5), 3623–3634.

Moore, J.P., 1990. Simple methods for monitoring HIV-1 and HIV-2 gp120 binding to soluble CD4 by enzyme-linked immunosorbent assay: HIV-2 has a 25-fold lower affinity than HIV-1 for soluble CD4. Aids 4 (4), 297–305.

Moore, J.P., Jarrett, R.F., 1988. Sensitive ELISA for the gp120 and gp160 surface glycoproteins of HIV-1. AIDS Res. Hum. Retrovir. 4 (5), 369–379.

Moore, J.P., Cao, Y., Ho, D.D., Koup, R.A., 1994a. Development of the anti-gp120 antibody response during seroconversion to human immunodeficiency virus type 1. J. Virol. 68 (8), 5142–5155.

Moore, J.P., McCutchan, F.E., Poon, S.W., Mascola, J., Liu, J., Cao, Y., Ho, D.D., 1994b. Exploration of antigenic variation in gp120 from clades A through F of human immunodeficiency virus type 1 by using monoclonal antibodies. J. Virol. 68 (12), 8350–8364.

Moulard, M., Lortat-Jacob, H., Mondor, I., Roca, G., Wyatt, R., Sodroski, J., Zhao, L., Olson, W., Kwong, P.D., Sattentau, Q.J., 2000. Selective interactions of polyanions with basic surfaces on human immunodeficiency virus type 1 gp120. J. Virol. 74 (4), 1948–1960.

Munshi, N., Balasubramanian, A., Koziel, M., Ganju, R.K., Groopman, J.E., 2003. Hepatitis C and human immunodeficiency virus envelope proteins cooperatively induce hepatocytic apoptosis via an innocent bystander mechanism. J. Infect. Dis. 188 (8), 1192–1204.

Oh, S.K., Cruikshank, W.W., Raina, J., Blanchard, G.C., Adler, W.H., Walker, J., Kornfeld, H., 1992. Identification of HIV-1 envelope glycoprotein in the serum of AIDS and ARC patients. J. Acquir. Immune Defic. Syndr. 5 (3), 251–256.

Oyaizu, N., Chirmule, N., Kalyanaraman, V.S., Hall, W.W., Pahwa, R., Shuster, M., Pahwa, S., 1990. Human immunodeficiency virus type 1 envelope glycoprotein gp120 produces immune defects in CD4+ T lymphocytes by inhibiting interleukin 2 mRNA. Proc. Natl. Acad. Sci. U.S.A. 87 (6), 2379–2383.

Parren, P.W., Sattentau, Q.J., Burton, D.R., 1997. HIV-1 antibody-debris or virion? Nat. Med. 3 (4), 366–367.

Proudfoot, A.E., Handel, T.M., Johnson, Z., Lau, E.K., LiWang, P., Clark-Lewis, I., Borlat, F., Wells, T.N., Kosco-Vilbois, M.H., 2003. Glycosaminoglycan binding and oligomerization are essential for the in vivo activity of certain chemokines. Proc. Natl. Acad. Sci. U.S.A. 100 (4), 1885–1890.

Schneider-Schaulies, J., Schneider-Schaulies, S., Brinkmann, R., Tas, P., Halbrugge, M., Walter, U., Holmes, H.C., Ter Meulen, V., 1992. HIV-1 gp120 receptor on CD4-negative brain cells activates a tyrosine kinase. Virology 191 (2), 765–772.

Schupbach, J., Boni, J., 1993. Quantitative and sensitive detection of immune-complexed and free HIV antigen after boiling of serum. J. Virol. Methods 43 (2), 247–256.

Schwartz, O., Alizon, M., Heard, J.M., Danos, O., 1994. Impairment of T cell receptor-dependent stimulation in CD4+ lymphocytes after contact with membrane-bound HIV-1 envelope glycoprotein. Virology 198 (1), 360–365.

Tamma, S.M., Chirmule, N., McCloskey, T.W., Oyaizu, N., Kalyanaraman, V.S., Pahwa, S., 1997. Signals transduced through the CD4 molecule interfere with TCR/CD3-mediated ras activation leading to T cell anergy/apoptosis. Clin. Immunol. Immunopathol. 85 (2), 195–201.

Trkola, A., Dragic, T., Arthos, J., Binley, J.M., Olson, W.C., Allaway, G.P., Cheng-Mayer, C., Robinson, J., Maddon, P.J., Moore, J.P., 1996. CD4-dependent, antibody-sensitive interactions between HIV-1 and its coreceptor CCR-5. Nature 384 (6605), 184–187.

Turville, S.G., Cameron, P.U., Handley, A., Lin, G., Pohlmann, S., Doms, R.W., Cunningham, A.L., 2002. Diversity of receptors binding HIV on dendritic cell subsets. Nat. Immunol. 3 (10), 975–983.

Ugolini, S., Mondor, I., Sattentau, Q.J., 1999. HIV-1 attachment: another look. Trends Microbiol. 7 (4), 144–149.

Vlahakis, S.R., Villasis-Keever, A., Gomez, T.S., Bren, G.D., Paya, C.V., 2003. Human immunodeficiency virus-induced apoptosis of human hepatocytes via CXCR4. J. Infect. Dis. 188 (10), 1455–1460.

Wahl, S.M., Allen, J.B., Gartner, S., Orenstein, J.M., Popovic, M., Chenoweth, D.E., Arthur, L.O., Farrar, W.L., Wahl, L.M., 1989. HIV-1 and its envelope glycoprotein down-regulate chemotactic ligand receptors and chemotactic function of peripheral blood monocytes. J. Immunol. 142 (10), 3553–3559.

Weinhold, K.J., Lyerly, H.K., Stanley, S.D., Austin, A.A., Matthews, T.J., Bolognesi, D.P., 1989. HIV-1 GP120-mediated immune suppression and lymphocyte destruction in the absence of viral infection. J. Immunol. 142 (9), 3091–3097.

Weissman, D., Rabin, R.L., Arthos, J., Rubbert, A., Dybul, M., Swofford, R., Venkatesan, S., Farber, J.M., Fauci, A.S., 1997. Macrophage-tropic HIV and SIV envelope proteins induce a signal through the CCR5 chemokine receptor. Nature 389 (6654), 981–985.

Wu, L., Gerard, N.P., Wyatt, R., Choe, H., Parolin, C., Ruffing, N., Borsetti, A., Cardoso, A.A., Desjardin, E., Newman, W., Gerard, C., Sodroski, J., 1996. CD4-induced interaction of primary HIV-1 gp120 glycoproteins with the chemokine receptor CCR-5. Nature 384 (6605), 179–183.

Yao, Q., Compans, R.W., Chen, C., 2001. HIV envelope proteins differentially utilize CXCR4 and CCR5 coreceptors for induction of apoptosis. Virology 285 (1), 128–137.

Yu, H., Soong, N., Anderson, W.F., 1995. Binding kinetics of ecotropic (Moloney) murine leukemia retrovirus with NIH 3T3 cells. J. Virol. 69 (10), 6557–6562.

Zhu, P., Chertova, E., Bess Jr., J., Lifson, J.D., Arthur, L.O., Liu, J., Taylor, K.A., Roux, K.H., 2003. Electron tomography analysis of envelope glycoprotein trimers on HIV and simian immunodeficiency virus virions. Proc. Natl. Acad. Sci. U.S.A. 100 (26), 15812–15817.