Soluble Envelope Glycoprotein Trimers from a CD4-Independent HIV-1 Elicit Antibody-Dependent Cellular Cytotoxicity-Mediating Antibodies in Guinea Pigs

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CD4-independent HIV variants can infect coreceptor-expressing cells lacking CD4. The envelope (Env) glycoproteins on these HIV-1 variants expose a coreceptor binding site that overlaps some CD4-induced (CD4i) epitopes. Reports have demonstrated that CD4i antibodies mediate antibody-dependent cellular cytotoxicity (ADCC). Here we investigated the immunogenicity of soluble Env trimers (sgp140) from a CD4-independent HIV-1 in guinea pigs and found that the sgp140 elicited ADCC-mediating antibodies. Therefore, these sgp140 might be useful in vaccine regimens aimed at eliciting ADCC responses.

Human immunodeficiency virus type 1 (HIV-1) enters cells by sequential interaction of its surface envelope (Env) glycoprotein with cell surface CD4 and a coreceptor (1–3). Binding to the initial receptor CD4 triggers conformational changes in Env that allow its binding to either chemokine coreceptor CCR5 or CXCR4 (2–5). Receptor binding also induces conformational changes in Env that result in the formation of a six-helix bundle (6–9), which is thought to provide the energy needed to fuse the viral and cell membrane (10–12), resulting in delivery of the viral genome into the cell and initiation of the infection cycle.

Although some HIV-1 strains can infect cells that express only low levels of CD4 (13), for most HIV-1 strains, CD4 is an obligate receptor. However, CD4-independent coreceptor-using HIV-1 isolates have been derived by passage on CD4-negative, coreceptor-positive cells in tissue culture (14–17). CD4-independent HIV-1 strains have been identified only rarely in infected people (18–20), while CD4-independent strains of HIV-2 and simian immunodeficiency virus (SIV) are more commonly encountered in vivo, particularly in body compartments like the brain, where cells expressing high levels of CD4 are limited in number (21–23). The Env glycoproteins of the CD4-independent HIV-1 variants are preactivated and can bind directly to the coreceptor and mediate virus entry. The exposed coreceptor binding sites on Env glycoproteins are conserved and overlap some CD4-induced (CD4i) epitopes (15, 16, 24). Although monoclonal antibodies (MAbs) against CD4i epitopes have limited neutralizing activity against primary HIV-1 isolates because of steric hindrance (25), Zhang et al. reported that soluble Env trimers (sgp140) from an unusual CD4-independent HIV-1 isolate, R2, elicited extensively cross-reactive anti-HIV-1 neutralizing antibodies (NAb) in rabbits (20). It is not clear whether the ability to elicit extensively cross-reactive NABs is a specific property of R2 Env glycoproteins or a general property of CD4-independent Env glycoproteins. Moreover, recent reports have demonstrated that antibodies against some CD4i epitopes are able to mediate antibody-dependent cellular cytotoxicity (ADCC) (26–29). Therefore, we investigated whether Env from a CD4-independent HIV-1 variant is capable of eliciting antibodies that neutralize HIV-1 infection or mediate ADCC.

Solic Env trimers (sgp140) from a CD4-independent HIV-1 ADA variant that contains an N197S mutation (ADA N/S) (16) and sgp140 from the ADA wild-type strain were stably expressed in Chinese hamster ovary (CHO) cells and purified through sequential chromatography with a lentil lectin column (GE Healthcare), a Ni-nitrilotriacetic acid (NTA) column (Qiagen), and a Sepharose 6 size exclusion column (GE Healthcare). The sgp140 bound to lentil lectin and Ni-NTA were eluted by α-d-methylmannoside and imidazole, respectively. The sgp140 were isolated by size exclusion chromatography, and their purity and integrity, respectively, were examined by SDS-PAGE (Fig. 1A) and enzyme-linked immunosorbent assay (ELISA) with selected MAbs (Fig. 1B). The ELISA data showed that, compared to ADA sgp140, the purified CD4-independent ADA N/S sgp140 interacted much more strongly with two MAbs against CD4i epitopes, A32 and 17b (Fig. 1B), which indicated that the CD4i epitopes on the ADA N/S sgp140 were well exposed. On the contrary, ADA sgp140 and ADA N/S sgp140 interacted similarly to a CD4 binding site MAb, VRC01 (Fig. 1B), verifying the integrity of both proteins. Of note,
The Env sgp140 trimers consisted of complete gp120 and gp41 ectodomains and contained mutations in the gp120/gp41 proteolytic cleavage sites.

The sgp140 from ADA and ADA N/S, as well as a phosphate-buffered saline (PBS) control, were then used to immunize guinea pigs (Fig. 2). Antisera collected from the immunized animals were heat inactivated at 56°C for 30 min before analysis. We first measured the Env-specific antibody titers in the sera by ELISA with purified sgp140 from ADA or ADA N/S as the coating antigen. We found that CD4-independent ADA N/S sgp140 elicited higher Env-specific antibody titers than ADA sgp140. Especially for the antisera binding to its corresponding immunogen, ADA N/S sgp140 elicited significantly higher titers of a specific antibody against the ADA N/S antigen than did ADA sgp140 (P < 0.05, unpaired t test) (Table 1). The titers of Env-specific antibodies in the control sera from PBS-immunized animals were all below 80 (data not shown).

We also measured whether the antibodies elicited were able to neutralize infectious viral particles by the standard TZM-bl assay described previously (30). The neutralization assay was first performed with neutralization-sensitive (MN.3, MW965) and neutralization-resistant (JR-FL) Env-pseudotyped viruses. Compared to ADA sgp140, CD4-independent ADA N/S sgp140 elicited significantly higher titers of a specific antibody against the ADA N/S antigen than did ADA sgp140 (P < 0.01; ID_{50}, P < 0.001) (Table 1). However, when we expanded the neutralization assay to a panel of tier 2 pseudoviruses (31, 32) for serum number 15, which had the highest neutralization titers against the selected pseudoviruses, no neutralization activity above the background was detected (data not shown). As expected, the control sera from PBS-immunized animals exhibited no neutralization activity in this assay.

Previous studies demonstrated that MAbs and human serum containing antibodies against CD4i epitopes can mediate ADCC (26–29). We therefore examined whether the serum raised by ADA N/S sgp140 also possessed this capacity. We first examined the capacity of serum to interact with cell surface Env by flow cytometry. Env was presented on the target cells by either HIV-1 infection or direct coating with recombinant gp120 (YU2 strain) as previously described (33). For both HIV-infected and gp120-coated cells, the sera from ADA N/S sgp140-immunized animals bound to the cell surface Env significantly more strongly than the sera from ADA sgp140- or PBS-immunized animals (Fig. 3A). When incubated for 30 min at room temperature with gp120-coated cells, sera from ADA sgp140- and PBS-immunized animals exhibited similar poor reactivity with Env on the cell surface (Fig. 3A). However, when assessed for ADCC-mediated killing of the gp120-coated target cells by a fluorescence-activated cell sorting-based method described previously (26, 27, 33), sera (1:2,500 dilution) from

![FIG 1](A) SDS-PAGE of the purified sgp140 from wild-type and N197S mutant ADA (ADA N/S). (B) Interaction of purified sgp140 with the MAbs indicated. Purified sgp140 from wild-type and N/S mutant ADA were used to coat ELISA plates that were then incubated with various concentrations of the MAb indicated. The MAb bound to sgp140 was detected by peroxidase-conjugated anti-human IgG secondary antibodies. OD450, optical density at 450 nm.

![FIG 2](Schematic diagram of the guinea pig immunization protocol used in this study. Groups of six guinea pigs were immunized with each immunogen. Preimmune serum was collected from each animal 1 week prior to the start of immunization. A 25-μg sample of purified sgp140 was emulsified with AS02A adjuvant in a final volume of 0.5 ml and used to inoculate each animal intramuscularly. Booster inoculations were given 6, 10, and 14 weeks after the initial inoculation. Blood samples were collected from each animal 10 days after the last inoculation.)
both ADA sgp140- and ADA N/S sgp140-immunized animals mediated ADCC; this could be due to the fact that in this assay, gp120-coated cells were in the presence of the sera for the complete duration of the assay (4 h at 37°C) and therefore the antibodies had more time to interact with gp120-coated cells. Importantly, however, the sera from ADA N/S sgp140-immunized animals exhibited a significantly higher level of ADCC activity than those from ADA sgp140-immunized animals (*P < 0.01) (Fig. 3B).

As the sera from ADA N/S sgp140-immunized animals exhibited ADCC activities, we examined whether the sgp140 elicited antibodies against CD4i epitopes by competition ELISA. The results showed that the sera from ADA N/S sgp140-immunized animals, at a 20-fold dilution, inhibited over 40% of the binding of the ADCC-mediating CD4i A32 MAb to Env, while the sera from ADA sgp140-immunized animals showed no inhibition, similar to the control sera from PBS-immunized animals (Fig. 4). The results indicated that CD4-independent ADA N/S sgp140 elicited significantly larger amounts of antibodies to CD4i epitopes (*P < 0.0001).

Env from CD4-independent HIV-1 exposes conserved CD4i epitopes and can elicit ADCC-mediating antibodies. Our study demonstrated that soluble Env trimers from a CD4-independent HIV-1 variant were more immunogenic in eliciting Env-specific antibodies than Env trimers from the parental virus, and although the antibodies elicited by the soluble Env trimers from the CD4-independent virus showed very limited improvement in neutralizing HIV-1 infection, they promoted potent ADCC activities. Therefore, these soluble Env trimers might be useful in vaccine regimens aimed at eliciting ADCC responses in humans.

### Table 1 ELISA titers and ID₅₀ and ID₈₀ neutralization titers of sera from immunized animals

<table>
<thead>
<tr>
<th>Immunogen and guinea pig ID</th>
<th>ELISA titer with plated antigen:</th>
<th>ID₅₀</th>
<th>ID₈₀</th>
</tr>
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<tr>
<td>ADA sgp140</td>
<td>ADA sgp140</td>
<td>MN.3, tier 1, clade B</td>
<td>MW965, tier 1, clade C</td>
</tr>
<tr>
<td>6</td>
<td>160,000</td>
<td>3,798</td>
<td>&lt;20</td>
</tr>
<tr>
<td>7</td>
<td>320,000</td>
<td>&gt;43,740</td>
<td>&lt;20</td>
</tr>
<tr>
<td>8</td>
<td>320,000</td>
<td>7,067</td>
<td>&lt;20</td>
</tr>
<tr>
<td>9</td>
<td>160,000</td>
<td>707</td>
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<tr>
<td>10</td>
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<td>372</td>
<td>&lt;20</td>
</tr>
<tr>
<td>11</td>
<td>1,280,000</td>
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<td>&lt;20</td>
</tr>
<tr>
<td>ADA N197S sgp140</td>
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<td>36</td>
</tr>
<tr>
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<tr>
<td>17</td>
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</table>

*Values are the serum dilutions at which there were 50% (ID₅₀) or 80% (ID₈₀) less relative luminescence units than in virus control wells (no test sample).
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