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# Characterization of the antibody response elicited by HIV-1 Env glycomutants in rabbits

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**Abstract**

HIV-1 *N*-glycans are known to shield underlying epitopes towards the protective antibody repertoire. We previously described HIV-1 acute infection Env glycomutants designed from 3D-model in which the removal of clustered *N*-glycans did not disturb the envelope antigenicity, but increased the neutralization sensitivity. The potential of such immunogens to elicit neutralizing responses was estimated after rabbit immunizations with a DNA/protein protocol. Maturation of the Env-specific antibody response was confirmed by a change in avidity and conformational dependence. For one immunogen, the neutralizing response was increased with a higher breadth compared to the Wild-Type. Our data suggest that Env selective deglycosylation based on 3D data may represent a valuable strategy to improve elicitation of neutralizing antibodies.


*Keywords:* *N*-Glycans; Env; Neutralizing antibodies

## 1. Introduction

Human immunodeficiency virus type 1 (HIV-1) is a major public health concern facing a WHO estimation of at least 42 million people infected worldwide and the emergence of drug resistant strains. It is now well accepted that vaccine is an urgent need, more especially for developing countries.

An efficient vaccine should target both cellular and humoral arms of the immune system at peripheral as well as mucosal sites. Indeed, recent finding about HIV pathogenesis clearly indicates that viral replication must be controled early, before spreading and massive infection of memory T cells [1]. Thus, antibodies may be critical in reducing viral load during the initial stage of the infection, triggering maturation of cellular response [2]. Administration of protective antibodies prior to viral challenge could protect macaques, chimpanzee and mice from viral infection or diminish the peak of viremia when animals became infected [3–6]. In addition, the presence of high antibody titers to HIV-1 in pregnant women is reported to reduce mother-to-child virus transmission [7].

The HIV-1 envelope glycoprotein (Env) is the major target of neutralizing antibodies and consequently the most appropriate protein to stimulate humoral immunity. The Env glycoprotein is synthetized as a precursor (gp160), which is hydrolyzed into two subunits (gp120 and gp41) in the *trans*-Golgi and exportated at the cell surface as a trimer of gp120/gp41 heterodimers. Gp120 is highly glycosylated (about 50% of its weight) [8]. The interactions between the two subunits are mainly hydrophobic, non-covalent and allow gp120 to shed from functional spikes. Env promotes binding of virus to target cells through CD4/chemokine receptor complex and triggers events leading to fusion between viral and cellular membranes [9].

Env does not elicite efficient neutralizing response in infected people. During the course of natural infection, antibody response develops in a long time frame and it takes months before antibodies of relevant avidity and affinity are induced [10–12].

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Various forms and parts of the Env complex have been tested as vaccine immunogens in animal and human over the past 20 years [13]. Until now, studies based on the gp120 sub-unit, have failed in terms of broad neutralizing response. Indeed, gp120 is able to induce isolate-specific antibody response mainly directed against V3 loop and linear epitopes [14–16]. In addition, neutralizing antibodies induced by candidate vaccines targeted preferentially T-cell line adapted strains (TCLA) and not primary isolates which are remarkably less sensitive to neutralization. Thus, Vaxgen gp120 vaccine trials were inefficient at eliciting protection against HIV-1 [17].

Consequently, current studies are focusing on developing Env immunogens more efficient than gp120 monomers. Indeed, efficiency of an antibody to neutralize HIV-1 appears to be directly correlated with its functional affinity for its epitope in oligomeric Env [18,19]. An effective Env-based vaccine must mimic the antigenic structure of the trimeric complex from the native virion. However, despite some encouraging results, oligomeric immunogens failed to elicit potent broadly reactive neutralizing antibodies against primary isolates [20–23]. Several explanations for this failure could be hypothesized: (i) the complex 3D structure of gp120 and the hetero-oligomerization status of Env spike, (ii) the high degree of genetic variation and (iii) the extensive glycosylation of gp120, continuously restructured by the changing antibody repertoire [24].

In a previous study, we suggested that a possible way to bypass these problems would be to analyze, using 3D data, the location of conserved *N*-glycan sites at the surface of gp120 of an acute primary isolate. We considered that, at the acute infection time point, these conserved sites could potentially protect the critical epitopes of gp120 for HIV transmission against immune response. We generated Env glycomutants that we studied in terms of functionality (gp160 cleavage efficiency, gp120 shedding, CD4 and co-receptors binding, cell membrane presentation and pseudovirion infectivity), antigenicity and neutralization sensitivity. We found that some mutants maintained their global antigenicity and functionality despite at least two mutated *N*-glycan sites. In addition these mutants showed an increased sensitivity to neutralizing sera probably due to an enhanced neutralizing epitope exposition [25].

The purpose of the present study was to evaluate the immunogenicity and the neutralizing response of these selected glycomutants in rabbits immunized with DNA encoding gp160 followed by gp140 protein boosts.

## 2. Material and methods

### 2.1. Env constructs used for DNA immunization

Nucleotide sequence encoding the entire gp160 *env* region was generated from DNA PBMC from a patient with an acute HIV-1 infection (patient #133, clade B, homosexual transmission). This sequence was described in a previous study [26]. Wild type 133 *gp160* sequence was cloned into the *Xho*1–*Xba*1 sites of the pCI-*neo* expression vector (Promega; Madison, USA) in which the *neo* gene was replaced by the homologous *rev* gene (pCI-env/r). Several *N*-glycan sites of the *env* gene were silenced by directed mutagenesis as previously described [25].

### 2.2. Antigen purification for rabbit immunizations

PCI-env/r were prepared using the NucleobondPC10000 endonuclease-free kit (Macherey-Nagel, Düren, Germany) and resuspended in PBS 1×. The genes encoding gp140 soluble proteins were cloned in pCI plasmid (Promega, Madison, USA) after mutation of their natural cleavage site and truncation of their gp41 trans-membrane region as previously described [27]. PCI-gp140s were transiently expressed (48 h) using 293FS cells (InVitrogen, Paisley, UK) cultivated in a serum-free medium (Invitrogen). Transfections were performed with 293fectin (Invitrogen). Excreted gp140s were purified on a *Galanthus nivalis* agglutinin (GNA) affinity column (Vector laboratory, Birlingane, USA) from pre-cleared culture supernatants. After adsorption, columns were intensively washed with PBS 1×, eluted with 500 mM of methyl α-D-manno-pyranoside (Sigma–Aldrich, St. Louis, USA) and finally diafiltered on a 30 kDa centricon (Millipore, Bedford, USA) against PBS 1×. The purified proteins were quantified by micro-BCA (Pierce, Rockford, USA), and aliquots were stored at −80 °C before analysis or immunizations. The quality of the purifications was estimated on a 8% SDS-PAGE followed by a Blue-PAGE dying (Fermentas, Burlington, Can.) or an immunoblot revealed with a goat gp120 polyclonal antiserum (BioGenesis, Poole, UK).

### 2.3. Rabbit immunizations

New Zealand White rabbits were housed at Centre Lago (Vonnas, France). Rabbits were first immunized three times intradermally with 1 mg of pCI-env/r (immunizations #1, 2 and 3) and then three times intramuscularly with 50 μg of GNA purified gp140 formulated in complete (immunization #4) or incomplete Freund's adjuvant, (immunizations #5 and 6). Immunizations were performed at 4-week intervals. Rabbits were bled before immunization and 2 weeks after the immunizations #3, 4, 5 and 6 (Fig. 1).

### 2.4. Oligomerization control on blue-native PAGE

GNA purified proteins were diluted in an equal volume of Tris–HCl pH7 100 mM/glycerol 40%/Coomassie blue 0.1% and loaded on a Ready-gel 4–15% Tris–HCl (Bio-Rad, Germany). Electrophoresis was performed in Tris/glycine buffer (Bio-Rad) supplemented with 0.002% Coomassie blue in the cathode buffer compartment. After electrophoresis, the gel was soaked in three successive bathes of Tris/glycine buffer and an adapted Western-blot was performed. Before