

**CHARACTERIZATION OF RABBIT MONOCLONAL
ANTIBODIES PRODUCED BY AN HIV-1 VACCINE**

A Major Qualifying Project Report

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ABSTRACT

Study of antibody specificities can help design better vaccines. This project focused on investigating the binding properties of a panel of rabbit monoclonal antibodies (RmAbs) elicited by a novel vaccine consisting of an HIV-1 gp120 AE consensus DNA-prime injection followed by a boost vaccine containing artificial gp120 AE consensus protein. Recombinant gp120 proteins from several isolates in clade AE were produced by transient transfection of 293F cells with DNA plasmids expressing individual AE gp120's. The gp120 proteins were purified by lectin beads, then verified for expression using SDS-PAGE and Western blots before being used as coats for ELISA to examine the reactivity of RmAb to these expressed gp120 proteins. Amino acid sequences of these gp120 proteins were used to map the potential epitopes recognized by the rabbit monoclonal antibodies. The data show that the novel vaccine was successful in producing rabbit monoclonal antibodies against several clade AE HIV-1 gp120 proteins.

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BACKGROUND

I. Human Immunodeficiency Virus

HIV Overview

The Human Immunodeficiency Virus (HIV) is a retrovirus that infects and destroys CD4⁺ lymphocytes. HIV causes a fatal condition known as Acquired Immunodeficiency Syndrome (AIDS), which results from the destruction of the immune system by an HIV infection (Kwong et al., 1998). In 2011, 34.2 million people were living with HIV worldwide, and 1.7 million deaths were caused by HIV. There is no vaccine available, but current treatment include antiretroviral therapy, which does not cure HIV but can improve quality of life and increase life expectancy (UNAIDS, 2013).

HIV Viral Subtypes

There are two types of HIV, HIV-1 and HIV-2. HIV-1 is responsible for the worldwide pandemic, while HIV-2 is found primarily in some West African countries, but is rarely found elsewhere in the world. As HIV-1 is the type responsible for most cases of HIV infection worldwide, this project, much like most HIV vaccine research, focuses on vaccines against HIV-1 (McCutchan, 2006).

HIV-1 is further divided into subgroups M, N, and O. Of these three subgroups, the large majority of HIV-1 infections are in subgroup M. The M group of HIV-1 has been found to contain ten clades (or subtypes) dictated by differences in their genetic sequences, which are termed A, B C, D, E, F, G, H, J, and K. There are also circulating recombinant forms (CRFs) of these clades where two clades combine to form a new clade, for example A and B combine to form AB. In order to be recognized as a CRF, an

identical recombinant virus must be identified in at least three epidemiologically unlinked individuals and fully characterized by genetic sequencing. This project will focus on the recombinant CRF AE, which is a recombinant of HIV-1 A and E. CRF AE is prevalent in Southeast Asia (Buonaguro et al., 2007) (**Figure-1**).



Figure 1: HIV-1 Viral Clades and Their Global Distribution (McCutchan, 2006)

II. HIV-1 Envelope

HIV-1 is an enveloped fusogenic virus, and the envelope region (*env*) of the HIV-1 viron serves a critical role as a receptor. The entry of HIV-1 into the host cell is dependent upon a series of interactions involving viral envelope surface glycoproteins. These envelope glycoproteins are displayed on the surface of the viral envelope as trimeric spikes (**Figure-2**) which interact with the CD4 glycoprotein and chemokine receptors CCR5 and CXCR4 on the surface of the host cell to facilitate cellular entry (Kwong et al., 1998). These trimeric envelope spikes are composed of three copies of an

extracellular glycoprotein termed gp120, and three copies of a transmembrane glycoprotein termed gp41 (Zhou et al., 2007). Each gp120 molecule is non-covalently associated with a gp41 molecule, which anchors the complex to the viral membrane (Kwong et al., 1998).

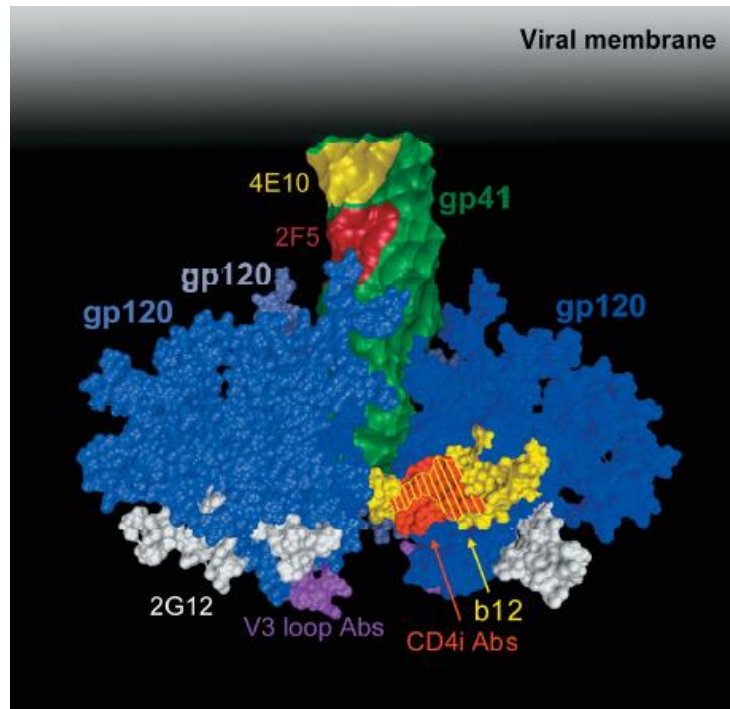


Figure 2: HIV-1 trimeric envelope spike showing gp120, gp41, and the binding sites of known neutralizing antibodies (Burton et al., 2004)

HIV-1 gp41 regulates membrane fusion with the host cell, and contains the domain responsible for trimerization of the glycoprotein spikes. The extracellular gp120 contains domains for receptor binding to CD4⁺ and CCR5/CXCR4 (Dey et al., 2007). The amino acid sequence of the gp120 envelope region contains five variable regions, termed V1-V5, which are dispersed between conserved regions of the sequence. The conserved regions contain the amino acid domains responsible for the viral receptor binding, as well as interactions with gp41 (Kwong et al., 1998). While the HIV-1 *env* is highly diverse due to extensive variable regions in the amino acid sequence, HIV-1 *env*

must maintain some conserved determinants to retain the ability to bind the CD4+ receptor for cellular entry (Zhou et al., 2007). The trimeric envelope spike is the only viral gene product on the surface of HIV-1 and contains some conserved sequences required for host cell entry, making this glycoprotein complex an ideal target for neutralizing antibodies and vaccine development (Dey et al., 2007).

III. HIV-1 Vaccine Progress

HIV-1 is a complex virus, with extreme genetic diversity and conformational hindrance of neutralization attempts by the immune system. It is difficult for one antibody to block HIV-1 isolates with broad diversity. Protection against an HIV-1 infection requires a preventative vaccine. However, candidate HIV-1 vaccines have not been able to elicit potent neutralizing antibodies against different subtypes. It is evident that the production of effective vaccine against HIV-1 will be a challenge, and will likely involve novel methods of vaccine development.

Heterologous Prime-Boost Vaccine

Many vaccines require multiple immunizations to fully protect against disease, and studies have shown that HIV-1 will be no exception. These multiple immunizations are termed prime-boost, and can be homologous (same vaccines) or heterologous (different vaccines). Homologous prime-boost vaccines are multiple immunizations of the same type of vaccines, which applies to vaccines such as live attenuated vaccines, inactivated vaccines, and polysaccharide vaccines. Heterologous prime-boost vaccines are multiple immunizations with the same antigen but delivered by different types of vaccines, which can be applied to more novel vaccines such as DNA or viral vector

vaccines (Lu, 2009). While heterologous prime-boost vaccines are a relatively new development in the vaccine field, in many cases were more effective than homologous prime-boost vaccines (Ramshaw and Ramsey, 2009). A successful HIV-1 vaccine will need to stimulate the production of neutralizing antibodies and generate a cellular T cell immune response. The concept of an HIV-1 heterologous prime-boost vaccine incorporating both of these elements seems more promising than a homologous prime-boost vaccine with just one of the elements. Evidence from recent vaccine studies supports this idea (Lu, 2009).

VaxGen AIDSVAX B/E HIV-1 Vaccine Trial

One of the first major HIV-1 vaccine trials was the VaxGen AIDSVAX B/E vaccine trial in Thailand. The major HIV-1 subtypes in Thailand were subtype B among injection drug users, and sexually transmitted subtype E (which would later become CRF AE). The VaxGen vaccine administered in this trial, AIDSVAX B/E, was a homologous prime-boost vaccine containing two recombinant gp120 (rgp120) HIV-1 antigens from the prevalent HIV-1 subtypes. One antigen was a CXCR4-dependent subtype B strain developed in the laboratory, and the other antigen was a CCR-5-dependent subtype AE primary isolate (Pitisuttithum et al., 2006). As the extracellular portion of the trimeric envelope spike, gp120 is capable of triggering natural antibody response in an HIV-1 infection, eliciting both neutralizing and non-neutralizing antibodies. Neutralizing antibodies, which effectively act to neutralize the CD4+ receptor binding function of gp120, are able to recognize variable regions and conserved regions of the gp120 amino acid sequence (Wyatt et al., 1998). A total of 2,546 participants were enrolled in this trial and were immunized with either AIDSVAX B/E at months 0, 1, 6, 12, 18, 24, and 36.

However, this vaccine did not produce efficacy against either HIV-1 subtype B or subtype E, and therefore did not prevent or delay HIV-1 infection (Pitisuttithum et al., 2006).

Merck STEP HIV-1 Vaccine Trial

Following the failure of the VaxGen trial, the focus of HIV vaccine research shifted away from inducing sterilizing immunity and more towards controlling viral load. A vaccine involving T-cell mediated immunity was therefore expected to elicit strong cellular immunity against HIV. This was the basis for the Merck STEP HIV vaccine trial. HIV antigens expressed in replication-deficient adenoviral vector vaccines were found to be capable of eliciting T-cell response in humans. The STEP HIV vaccine trial used a recombinant adenovirus serotype 5 (rAD5)-based vaccine. In this trial, 3,000 uninfected individuals were vaccinated with three rAD5 vectors, each expressing a different HIV gene: Ad5-gag, Ad5-pol, and Ad5-Nef. Each individual was immunized three times over a course of 26 weeks with either the MRKAd5 gag/pol/nef HIV-1 vaccine or the placebo (Selaky, 2008; Buchbinder et al., 2008). The determination of whether an individual received the rAD5 vaccine or the placebo was done in a randomized fashion with a 1:1 ratio. Shockingly, this vaccine trial resulted in an overall HIV-1 acquisition in 5.1% of men that received the vaccine, and 2.3% of men that received the placebo. Only one female participant acquired an HIV infection, so the vaccine efficacy analysis was limited to the male participants. The efficacy analysis following this trial was the first evaluation of a cell-mediated immunity (CMI)-based vaccine. Based on this analysis, it was determined that this vaccine did not protect against HIV-1 infection nor lower the HIV-1 viral load in infected participants (Buchbinder et al., 2008).

RV144 HIV-1 Vaccine Trial

The concept of using a heterologous prime-boost vaccine was used in the first partially successful HIV-1 vaccine trial. The RV144 phase 3 trial in Thailand used a canary pox vector vaccine prime ALVAC-HIV (vCP1521) with subsequent gp120 vaccine AIDSVAX B/E boosts. This trial involved a total of 16,402 participants who were vaccinated at weeks 0, 4, 12, and 24 with ALVAC and boosted with AIDSVAX B/E at weeks 12 and 24. The estimated efficacy of the vaccine regimen against HIV-1 was 31.2% over a period of 42 months. The results of this study showed a statistically significant reduction in HIV-1 infection in the participants that received the vaccine compared to the placebo (Rerks-Ngarm et al., 2009). Further analysis of the results of the RV144 trial showed a key correlation between serum antibody binding and the V2 variable region of the HIV-1 envelope glycoprotein. This correlation provides evidence that antibodies are a necessary component of a successful HIV-1 vaccine (Rolland et al., 2012).

Lu Lab Contribution

The Nucleic Acid Vaccine Lab and its Principal Investigator Shan Lu are working to create a DNA vaccine expressing HIV-1 AE gp120 proteins that will elicit a neutralizing antibody response and protect against HIV-1 infection. Previous studies reported by the Lu Lab have shown that polyvalent Env DNA prime followed by gp120 boost vaccines are capable of generating neutralizing antibodies against HIV-1 (Wang et al., 2005; Wang et al., 2008). However, in previous studies, only polyclonal antibody responses were studied, which is hard to identify specific epitopes within the HIV-1

envelope glycoprotein. The Lu Lab has currently focused their research on utilizing a monoclonal antibody approach elicited by vaccines to dissect the specificity of the antibody repertoire induced by their candidate vaccines.

IV. Human Monoclonal Antibodies against HIV-1

HIV-1 is extremely proficient at avoiding elimination and avoiding control of disease progression by both humoral and cell-mediated immune responses. However, an HIV-1 infection is capable of triggering the human immune system to produce antibodies against the invading virus. Serum antibodies directed against HIV-1 envelope gp120 can neutralize essential viral functions by binding to specific epitopes on the virion (Posner et al., 1991). The formation of neutralizing human monoclonal antibodies (HmAbs) generated as a result of an HIV-1 infection provide a useful tool in HIV-1 vaccine research, as they demonstrate that the human humoral system is capable of neutralizing the HIV-1 virus. As monoclonal antibodies, these HmAbs also allow any binding observations to be linked to the particular epitope recognized by the HmAb. However, neutralizing HmAbs are only found in 2-4% of the HIV-1 infected population after two to three years following the initial infection (Gray et al., 2011). Although HmAbs are an effective tool for studying the neutralization of HIV-1, a tool that is more readily available and cost effective, such as the use of rabbit monoclonal antibodies against HIV-1, would be a much more efficient means for conducting this type of research.

V. Rabbit Monoclonal Antibodies

The Lu Lab has recently developed a new technology of generating rabbit monoclonal antibodies (RmAbs) elicited by an HIV-1 immunogen. Rabbits are an ideal

animal model for antibody studies because rabbit antibodies have a small amount of background reactivity and a large volume of immune sera. Vaccine trials using rabbits are also quicker and less costly than human vaccine trials. Monoclonal antibodies specifically bind to single epitopes, so the development of RmAbs against HIV-1 allow for antibody binding activity by the RmAbs to be mapped to particular sequences on the HIV-1 immunogen. This novel technology will allow for a more definitive study of neutralizing antibody binding epitopes elicited by HIV-1 vaccines.

The Lu Lab designed consensus nucleotide and protein sequences for HIV-1 clade AE antigens, in which every amino acid is the most frequently observed at each position in the alignment of global circulating clade AE isolates. To test the immunogenicity of this gp120 AE consensus vaccine, rabbits were inoculated three times with DNA encoding the gp120 AE consensus protein followed by twice gp120 AE consensus protein immunization. Analysis of immunized polyclonal rabbit sera has shown that this vaccine is able to elicit cross-clade binding antibody (Ab) and neutralizing Abs.

To better dissect the quality of the Ab response to the consensus vaccine and protein boost, the Lu Lab used the technology of rabbit monoclonal antibodies (RmAbs) against HIV-1 gp120 protein. A panel of twenty-two RmAbs was generated from the sera of two different rabbits, 813 and 612. Rabbit 813 was immunized with the above gp120AE consensus vaccination regimen. Rabbit 612 was immunized with the same type of vaccination regimen, but received an HIV-1 JR-FL gp120 DNA prime vaccine (instead of the gp120AE consensus) and an HIV-1 JR-FL gp120 recombinant protein boost vaccine. The spleens from these two rabbits were used for the production of hybridoma cells to produce the RmAbs. Of the ten RmAbs that were used in the current study, two

of the RmAbs, 53 and 56, were from rabbit 612, and eight were from rabbit 813. These ten 813 RmAbs were used in this project to analyze their binding with a panel of clade AE HIV-1 gp120 proteins. This panel of RmAbs had diverse epitopes, including the C1, V3, C4, and C5 regions on the gp120 protein. This project will test whether these RmAbs of different specificity generated from a vaccine based on “artificial” consensus sequence of clade AE gp120 will also binds to gp120 proteins cloned from circulating clade AE virus.

PROJECT PURPOSE

The overall objective of this project was to assist with the Lu Lab's ongoing research in developing vaccines against HIV-1 by focusing on determining the binding properties of a panel of rabbit monoclonal antibodies elicited by a novel vaccine approach. The Lu Lab vaccine in rabbits used three injections with a consensus gp120 AE consensus DNA vaccine followed by two injections of a gp120 AE consensus protein boost vaccine. The binding properties and possible epitope locations of the rabbit mAbs were tested in ELISAs using various expressed gp-120 clade AE HIV-1 gp120 proteins as coats.

METHODS

The four main sections of this project were gp120 cell transfections, gp120 protein purifications, ELISA assays using gp-120 proteins as coat, and RmAb sequence analysis. Transfections of 293F cells with gp120 DNA plasmids produced clade AE HIV-1 gp120 proteins. The gp120 proteins were purified using lectin beads, and the success of the purification was confirmed by SDS-PAGE and Western blots. The binding profiles of the twelve RmAbs produced by the Lu Lab were tested by ELISA using the transfected gp120 proteins as the ELISA coating antigens. Then finally, an analysis was performed to compare HIV-1 AE gp120 protein sequence variations with the binding profiles of the RmAbs tested by ELISAs.

gp120 DNA Preparations

Nine full length *env* genes of clade AE were obtained from chronically HIV-1 infected individuals from Duke University. The gene segments encoding the gp120 region were produced by PCR from parental DNA, and were further subcloned into the expression vector. gp120 DNA plasmid was produced in the HB101 strain of *E. coli*, then purified using the Qiagen Plasmid Mega Kit.

gp120 Transfections

The transfections were performed using human embryonic kidney 293F cells. The 293F cells were cultured to about one million cells per mL. 250 µg of gp120 DNA was mixed with OPTIMEM media for each individual protein. Ten clade AE gp120 proteins were transfected: 620345.c01, 620345.c10, 703357.c02, 427299.c12, 816763.c02,

356272.c02, R2184.c04, R3265.c06, ChinaAE consensus, and AE consensus.

Additionally, one clade B protein, JR-FL, was also transfected by the same method. The gp120 DNA was incubated in the media for five minutes, then 750 μg PEI was added to each sample and incubated for thirty minutes. After incubation, the DNA suspension for each individual gp120 protein was added to 293F cells. The supernatants containing each gp120 protein were harvested 72 hours after the transfection.

gp120 Protein Purification

Transfected cells were cultured to allow the expressed gp120 proteins to be secreted into the medium. The eleven gp120 proteins were purified using lentil lectin Sepharose 4B beads. 20 mL of supernatant containing the secreted gp120 protein from the 293F transfection was concentrated to 1 mL using Millipore Centrifugal Filter Units for each protein. The solutions were incubated for 2 hours with lectin beads, then the proteins eluted with elution buffer. The concentrations of the purified gp120 proteins were determined by Nano-Drop. The purification of gp120 protein was confirmed by SDS-PAGE and Western blots.

SDS-PAGE

SDS-PAGE was used to confirm the purification of the gp120 antigens from the supernatant of the transfected 293F cells. The purified gp120 proteins from the transfected 293F cells were denatured with 6X SDS and loaded onto the gel at a concentration of 5 $\mu\text{g}/\text{mL}$. Protein marker p77095 was included in the gel for reference. The SDS-PAGE gels were stained with Coomassie blue.

Western Blot

A Western blot was performed to confirm the expression of the purified gp120 proteins from the supernatant of the transfected 293F cells. The purified gp120 proteins were blotted at a concentration of 0.1 µg/mL using SDS-PAGE and a PDVF membrane. The rabbit mAb R16 was used as primary antibody for the Western. Protein marker p77095 was included in the blot for reference. Membrane blocking was done using 0.2% I-Block. The membrane was incubated for 1 hour with rabbit serum at 1:500 dilution from RmAb 16 isolated from rabbit 813 immunized with gp120 consensus AE DNA prime protein boost. Following this incubation, the membrane was washed with blocking buffer every 10 minutes for 1 hour. The membrane was then reacted with AP-conjugated goat anti-rabbit IgG at a 1:5000 dilution for 1 hour, and then washed again with blocking buffer every 10 minutes for 1 hour. Western-light substrate was applied to the membrane for 5 minutes, and then the membrane was dried. To develop the membrane, X-ray films were exposed to the membrane and a Kodak processor was used to develop the image.

Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA assays were used to evaluate the binding of twelve RmAbs with all eleven expressed gp120 proteins. These ELISA assays were performed using 96-well microtiter plates, and were washed with buffer using a plate washing machine between each step. The plates were first incubated with ConA for 1 hour. Then, the plates were coated with each purified gp120 antigen in duplicate from the transfected 293F cells at a concentration of 1 µg/mL. After 1 hour, the wells were blocked with 200 µl/well of blocking buffer, and stored at 4°C to block overnight. The following day, 100 µl of rabbit sera with an RmAb concentration of 1 µg/mL was added in duplicate wells to each gp120

antigen and incubated for 1 hour. Then, a coat of biotinylated anti-rabbit IgG at a 1:1000 dilution was added to the wells as a secondary antibody for 1 hour. Horseradish-peroxidase (HRP)-conjugated streptavidin at a 1:2000 dilution was then added to the wells, which binds to the biotin on the secondary antibody, and the reaction was incubated for 1 hour. Freshly-made TMB substrate was added to the wells in the final step of the assay, and incubated for 3.5 minutes to change the color of solution in the wells blue. The intensity of the blue color is dependent on how much binding of the RmAb to the gp120 coat had occurred within the well. The reaction was stopped by adding 50 μ l of 2 M H₂SO₄. This color change was quantified by an ELISA plate reader at the optical density (OD) of 450 nm (OD₄₅₀). The OD₄₅₀ values for the duplicate wells were averaged for the final data.

Sequence Analysis

The sequences of all eleven expressed gp120 proteins were aligned using Mac Vector to allow for the sequence comparison of the gp120 AE consensus protein versus ten other gp120 proteins. The binding sites of all the RmAbs with known possible epitopes were located within the sequences to allow for analysis of correlations between gp120 protein sequence variations and the binding profiles of RmAbs.

RESULTS

The results of this project were organized into four main sections. These sections are separated as: gp120 purification data, western blot data, ELISA data, and gp120 sequence alignment data. The sections of ELISA data and gp120 sequence alignment data were further analyzed by RmAb epitopes to examine possible correlations between gp120 sequence variations and RmAb binding profiles. The overall result of this project involving all four sections of data is that a gp120 consensus AE DNA-prime protein-boost vaccine regiment was successful in producing rabbit monoclonal antibodies against clade AE HIV-1 gp120 proteins.

gp120 Purification Data

A panel of eleven different expression plasmids encoding eleven different gp120 proteins was transfected into 293F cells as described in Methods. The gp120 proteins of clade AE included in the analysis were as follows: 620345.c01, 620345.c10, 703357.c02, 427299.c12, 816763.c02, 356272.c02, R2184.c04, R3265.c06, China AE consensus (China AE con.), gp120 AE consensus (AE con.). A clade B gp120 protein, JR-FL, was also included. The transfected cells were cultured to allow secretion of the gp120 proteins into the culture supernatant, then the supernatants were harvested to be used as ELISA coating antigens. Purification of gp120 from the supernatant of transfected 293F cells was performed to determine the level of expression of all eleven gp120 proteins, and to use the purified gp120's as coats in ELISAs. This purification was performed using the protocol outlined in the Materials and Methods section. A lectin column was used to

isolate gp120 from each supernatant. The concentration of gp120 protein in each of the samples was determined by Nano-Drop.

SDS-PAGE gels were used to confirm successful purification of each gp120 protein (**Figure 3**), and confirmed the presence of purified gp120 in all eleven of the samples at 120 kD.

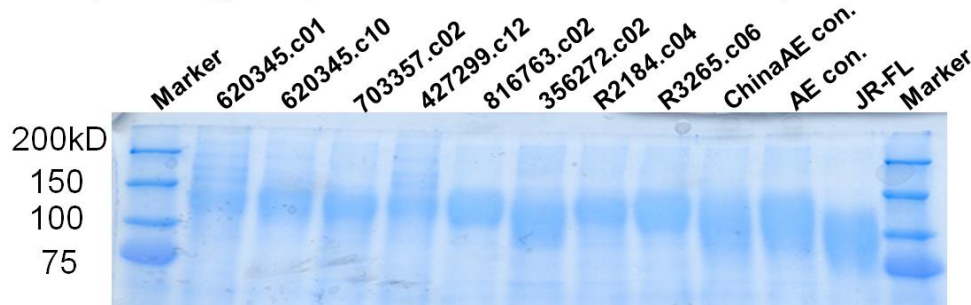


Figure 3: SDS-PAGE Gel to Confirm gp120 Yield. The gel confirmed the presence of gp120 in all eleven of the purified samples run at a protein concentration of 5 $\mu\text{g/mL}$.

Western Blot Data

The levels of purified gp120 proteins were further evaluated by Western blot (**Figure 4**), which indicated strong signals at 120 kDa for all nine gp120 isolates and two gp120 consensus proteins.

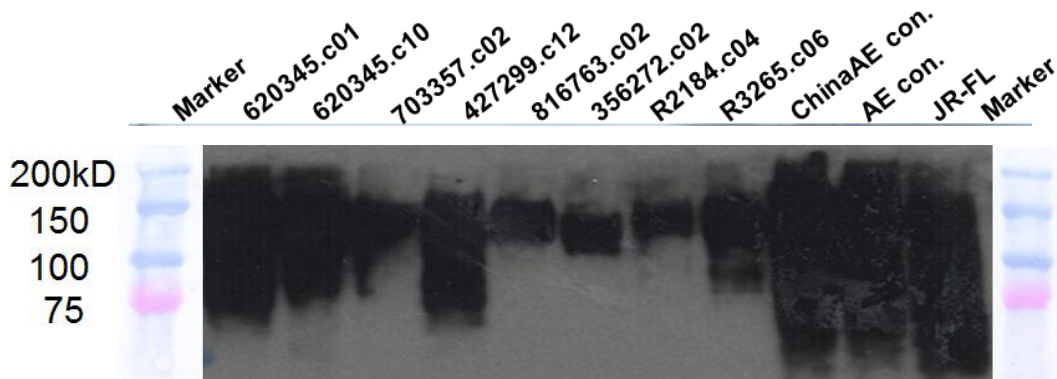


Figure 4: Western Blot to Confirm the gp120 Protein Levels. The western confirmed the presence of gp120 in all eleven of the purified samples using RmAb 16 as primary antibody for detection.

ELISA and Sequence Analysis Data

ELISA assays were performed to test the binding affinity of the ten RmAbs developed in the Lu Lab against all eleven purified gp120 proteins. The results for all twelve RmAbs are shown in **Table 1**, and these results will be further analyzed by epitopes in later sections. gp120 proteins JR-FL, ChinaAE consensus, and gp120AE consensus had high OD₄₅₀ values (red, high mAb binding) for most of the RmAbs. Since the gp120 AE consensus was used to initially immunize the rabbit receiving the clade AE DNA vaccine, it was expected that the RmAbs produced by the clade AE vaccine would bind very well to the gp120 AE consensus protein. Both of the clade-B RmAbs, 53 and 56, also bound very well to gp120 AE consensus protein, despite being produced by the clade B JR-FL vaccine. This clade B JR-FL protein elicited strong binding from both clade B and clade AE RmAbs. These results suggest that RmAbs able to recognize cross-clade gp120 proteins were produced.

| Epitope | RmAb | 620345.c01 | 620345.c10 | 703357.c02 | 427299.c12 | 816763.c02 | 356272.c02 | R2184.c04 | R3265.c06 | JR-FL | ChinaAE con. | AE con. | PBS |
|---------|------|------------|------------|------------|------------|------------|------------|-----------|-----------|-------|--------------|---------|------|
| C1 | 80 | 0.38 | 0.44 | 0.45 | 0.22 | 0.53 | 0.46 | 0.49 | 0.38 | 0.76 | 0.62 | 0.63 | 0.05 |
| | 47 | 0.45 | 0.61 | 0.54 | 0.55 | 0.60 | 0.62 | 0.51 | 0.56 | 0.80 | 0.76 | 0.75 | 0.05 |
| V3 | 56 | 0.62 | 0.69 | 0.45 | 0.09 | 0.06 | 0.25 | 0.06 | 0.05 | 0.76 | 0.79 | 0.82 | 0.04 |
| | 16 | 0.79 | 0.78 | 0.71 | 0.69 | 0.57 | 0.40 | 0.70 | 0.69 | 0.81 | 0.81 | 0.83 | 0.05 |
| | 73 | 0.72 | 0.69 | 0.33 | 0.57 | 0.58 | 0.08 | 0.53 | 0.49 | 0.80 | 0.81 | 0.81 | 0.05 |
| | 28 | 0.76 | 0.51 | 0.09 | 0.58 | 0.52 | 0.39 | 0.71 | 0.72 | 0.79 | 0.80 | 0.80 | 0.05 |
| | 64 | 0.69 | 0.49 | 0.10 | 0.56 | 0.23 | 0.07 | 0.31 | 0.35 | 0.82 | 0.80 | 0.79 | 0.05 |
| C4 | 53 | 0.25 | 0.66 | 0.12 | 0.15 | 0.44 | 0.43 | 0.67 | 0.55 | 0.70 | 0.72 | 0.80 | 0.05 |
| C5 | 76 | 0.69 | 0.66 | 0.63 | 0.64 | 0.76 | 0.36 | 0.75 | 0.75 | 0.78 | 0.79 | 0.63 | 0.05 |
| Unknown | 31 | 0.58 | 0.68 | 0.65 | 0.60 | 0.72 | 0.57 | 0.70 | 0.60 | 0.78 | 0.71 | 0.78 | 0.05 |

Table 1: Average ELISA OD₄₅₀ Values for all 10 RmAbs Against All 11 Purified gp120 Proteins. Also shown is the data for a PBS negative control. OD₄₅₀ values representing strong binding (≥ 0.60) are highlighted in red and OD₄₅₀ values representing weak to no binding (< 0.39) are highlighted in blue.

gp120 Sequence Alignment Data

Amino acid sequences of these gp120 proteins were used to map the potential epitopes recognized by the RmAbs. The sequences of the eleven gp120 proteins were aligned using Mac Vector, and the known RmAb epitopes were identified in the gp120 AE consensus sequence (**Table 2**). The sera from which the majority of the RmAbs were isolated came from the rabbit vaccinated with the gp120 AE consensus, so the RmAbs were initially formed against this particular antigen. Therefore, the RmAb epitopes were located within the sequence of gp120 AE consensus, and this antigen will be used as a basis for comparison with all ten of the other gp120 proteins. All mutations that differ from the sequence of gp120 AE consensus are labeled in red, and the four known RmAb epitopes were highlighted and color coded. In the next few sections, these epitopes were analyzed in detail with the ELISA data representing the RmAbs that bind each epitope in order to identify any possible correlation between gp120 epitope sequence variations and RmAb binding affinity.


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AE con.      170 IKQACPKISFDPIPIHYCTPAGYAILKCNDKNFNGTGPCRNVSVQCTHGIKFVVSTQLL 229
R3265.c06   170 IKQACPKISFDPIPIHYCTPAGYAILKCNDKNFNGTGPCRNVSVQCTHGIKFVVSTQLL 229
R2184.c04   175 IKQACPKISFDPIPIHYCTPAGYAILKCNEKNFNGTGPCRNVSVQCTHGIKFVVSTQLL 234
356272.c02  169 IKQACPKISFDPIPIHYCTPAGYAILKCKNFNGTGPCRNVSVQCTHGIKFVVSTQLL 228
816763.c02  169 IKQACPRMSFDPIPIHYCTPAGYAILKCKNFNGTGPCRNVSVQCTHGIKFVVSTQLL 228
427299.c12  176 IKQACPRVSFDPIPIHYCTPAGYAILKCNDKNFNGTGPCRNVSVQCTHGIKFVVSTQLL 235
703357.c02  180 IKQACPKISFDPIPIHYCTPAGYAILKCNDKFNGTGPCRNVSVQCTHGIKFVISTQLL 239
620345.c10  169 IKQACPRVSFDPIPIHYCTPAGYAILKCNDKNFNGTGPCRNVSVQCTHGIKFVVSTHLL 228
620345.c01  169 IKQACPRVSFDPIPIHYCTPAGYAILKCNDKNFNGTGPCRNVSVQCTHGIKFVVSTHLL 228
ChinaAE con. 169 IKQACPKISFDPIPIHYCTPAGYAILKCNDKNFNGTGPCRNVSVQCTHGIKFVVSTQLL 228
JR-FL       166 ITQACPKISFEPIPIHYCAPAGFAILKCNDKTFNGKGGPCRNVSVQCTHGIRFVVSTQLL 225
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V3

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AE con.      230 LNGSLAEEEIIRSENLTNNAKTIIVHLNKSVEINCTRPSNNTRTSITIGPGQ-VFYRTG 288
R3265.c06   230 LNGSLAEDEIVIRSENITNNAKTIIVHLNKSVEIKCIRPSNNTRTSMTIGPGQ-VFYRTG 288
R2184.c04   235 LNGSLAEDEIIIRSENLTDNTKTIIVHLNKSVEINCTRPSNNTRTSMTIGPGQ-VFYRTG 293
356272.c02  229 LNGSLAEEEIIRSENLTNNAKTIIVHLNETVEINCTRPSDNKRTSVHMGPGQ-VLYRTG 287
816763.c02  229 LNGSLAEEEIIRSENLTMNVKTIIVHLNKSVEINCTRPSNNTRTSISMGPGQ-VFYRPG 287
427299.c12  236 LNGSLAEDEIIIRSENLTDNSKNIIVHLNESVVINCTRPSNNTVKSIRIGPGQ-TFYRTG 294
703357.c02  240 LNGSIAEEKIIRSENITNNAKTIIVHLNRTIIINCTRPSNNTRTSINIGPGQMLFYKPG 299
620345.c10  229 LNGSLAEEEIIRSENLTNNAKTIIVHLNKSVEINCTRPSNNTRTSTRIGPGQ-VFYRTG 287
620345.c01  229 LNGSLAEEEIVIRSEDITNTKTIIVHLNKSVEINCTRPSNNTRTSVRIGPGQ-VFYRTG 287
ChinaAE con. 229 LNGSLAEEEIIRSENLTDNAKTIIVHLNKSVEINCTRPSNNTRTSITIGPGQ-VFYRTG 287
JR-FL       226 LNGSLAEEEVVIRSEDINFTNNAKTIIVQLKESVEINCTRPSNNTRKSIHIGPGR-AFYTTG 284
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C3

V4

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AE con.      289 DIIIGDIRKAYCEINGTKWNEVLEQVTGKLEKHEFNKTIIFQ-PPSGGDLEITMHHFNCRG 347
R3265.c06   289 DIIIGDIRQAYCEINQTKWNETLRQVAGKLEKHEFN-RTIIFR-PPSGGDLEITMHHFNCRG 346
R2184.c04   294 DIVGNIRKAYCVINGTIWNKVLEQVADKLEKHEFN-RTIVFQ-PPSGGDLEITMHHFNCRG 351
356272.c02  288 EITGDIRKAHCLINGTKWNKVLTQVAGKLSERFNKSKIIIFQ-PPSGGDLEITMHHFNCRG 346
816763.c02  288 EITGDIRRAYCEINGTKWNETLRQVVGKLEKHEFN-RTIIFQ-PSSGGDLEITMHHFNCRG 345
427299.c12  295 DIIIGDIRQAYCNVNGTKWYEVLRNVTKLEKHEFNKTIIVFQQPPGGDLEITTHHFNCRG 354
703357.c02  300 DIIIGDIRKAYCEINGTKWKKTLDQVKRELEEHFNKTIIFQ-PSSGGDPEITMHHFNCRG 358
620345.c10  288 EVITSIRKAYCEINGTKWNETLRQVAKKLEKHEFNKTIIFQ-PPSGGDLEVTTHHFNCRG 346
620345.c01  288 EVITSIRKAYCEINGTKWNETLRQVAKKLEKHEFNKTIIFQ-PPSGGDLEVTTHHFNCRG 346
ChinaAE con. 288 DIIIGDIRKAYCEINGTKWNKTLEQVTGKLEKHEFN-RTIIFQ-PPSGGDLEITMHHFNCRG 345
JR-FL       285 EIIIGDIRQAHCNISRAKWNDTLEQIVIKLEQFENKTIIVFN-HSSGGDPEIVMHSFNCGG 343
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mAb Binding Site Key:

- Pink** = 47, 75, 80
- Blue** = 73, 16, 28, 56, 64
- Green** = 53
- Yellow** = 76

mAb 9 and 31 binding sites are unknown

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AE con.      348 EFFYCNITKLENNTCIG----NETMEG-CNG-TIILPCKIRQIIC4 401
R3265.c06    347 EFFYCNITQLFNSTWIGNETIGNETMKGGNG-TIILPCKIRQIINMWQGVGQAMYAPPIS 406
R2184.c04    352 EFFYCNITKLENNTCINDTRN-NETEEE-CQN-NIILPCKIRQIINMWQGVGQAMYAPPIS 409
356272.c02   347 EFFYCDIAEMENSTYEN----QIMQHDD-IMVLPCKIRQIINMWQAGQAMYAPPIS 399
816763.c02   346 EFFYCNITKLENNSTWRENE--TIEHNGTNG-TIILPCKIRQIINMWQGVGQAMYAPPIS 402
427299.c12   355 EFFYCNITLENNTCVNET--INNGTEGWCKG-DIILPCKIRQIINLWQGVGQAMYAPPIS 412
703357.c02   359 EFFYCNITQLFNMTQLNET-----LNE-TIILPCKIRQIINMWQGVGQAMYAPPIS 408
620345.c10   347 EFFYCDIAQLFNSTWRGNE--TKEERNGTSD-IIIILPCKIRQIVRMWQGVGQSMYAPPIS 404
620345.c01   347 EFFYCDIAQLFNSTWRGNE--TKEERNGTSD-IIIILPCKIRQIVRMWQGVGQSMYAPPIS 404
ChinaAE con. 346 EFFYCNITKLENNTCIG----NTSETG-CNG-TIILPCKIRQIINMWQGVGQAMYAPPIS 399
JR-FL        344 EFFYCNSTQLFNSTWNN----NTEGSNNTEGNTIILPCKIRQIINMWQGVGQAMYAPPIS 399
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AE con.      402 GRINCVSNITGILLTRDGGAN--NTANETFRPGGGNKIDNWRSELYKYKVVQIEPLGIA 458
R3265.c06    407 GNISCAENITGILLTRDGGDD--GATNETFRPVGGNKIDNWRSELYKYKVVQIEPLGIA 463
R2184.c04    410 GSINCVSNITGILLTRDGGTNT--SKNNETFRPGGGNKIDNWRSELYKYKVVQIEPLGIA 467
356272.c02   400 GIINCRSNITGILLTRDGGGGA--NNTNETFRPGGGNKIDNWRSELYKYKVVQIEPLGIA 457
816763.c02   403 GKINCSNITGILLTRDGGHN--TSNETFRPGGGNKIDNWRSELYKYKVVQIEPLGIA 458
427299.c12   413 QIRCSNITGILLTRDGGNGTLMNETFRPGGGNKIDNWRSELYKYKVVQIEPLGIA 472
703357.c02   409 GHINCVSNITGILLTRDGGAN--ETNGTETFRPEGGNIDNWRSELYKYKVVQIEPLGVA 466
620345.c10   405 GIINCVSNITGILLSDGGGGP--TADNETFRPAGGDMRDNWRSELYKYKVVQIEPLGIA 462
620345.c01   405 GIINCVSNITGILLSDGGGGP--TADNETFRPAGGDMRDNWRSELYKYKVVQIEPLGIA 462
ChinaAE con. 400 GRINCVSNITGILLTRDGGAN--NNTNETFRPGGGNKIDNWRSELYKYKVVQIEPLGIA 456
JR-FL        400 QIRCSNITGILLTRDGGIN--ENGTEIFRPGGGDMRDNWRSELYKYKVVQIEPLGVA 456
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AE con.      459 PTRAKRRVVE--GS----- 470
R3265.c06    464 PTRAKRRVVE--REKRAVIGAMIFGF LGAAGSTMGAASITLTVQARQLLSGIVQQOSNL 521
R2184.c04    468 PTRAKRRVVE--REKRAVIGAMIFGF LGAAGSTMGAASITLTVQARQLLSGIVQQOSNL 525
356272.c02   458 PTRAKRRVVE--KEKRAAGIGAMIFGF LGAAGSTMGAASITLTVQARQLLSGIVQQOSNL 515
816763.c02   459 PTRAKRRVVE--REKRAVIGAMIFGF LGAAGSTMGAASITLTVQARQLLSGIVQQOSNL 516
427299.c12   473 PSRAKRRVVEMKREKRAVIGAMIFGF LGAAGSTMGAASITLTVQARQLLSGIVQQOSNL 532
703357.c02   467 PTRAKRRVVE--RQKRAVIGAMIFGF LGAAGSTMGAASITLTVRARQLLSGIVQQOSNL 524
620345.c10   463 PTRAKRRVVD--REKRAVIGAMIFGF LGAAGSTMGAASITLTVQARQLLSGIVQQOSNL 520
620345.c01   463 PTRAKRRVVD--REKRAVIGAMIFGF LGAAGSTMGAASITLTVQARQLLSGIVQQOSNL 520
ChinaAE con. 457 PTKAKRRVVE-- 466
JR-FL        457 PTKAKRRVVD----- 466
* * * * *

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mAb Binding Site Key:
Pink = 47, 75, 80
Blue = 73, 16, 28, 56, 64
Green = 53
Yellow = 76
mAb 9 and 31 binding sites are unknown

C1-Specific RmAb ELISA and Sequence Analysis

RmAbs 47 and 80 (**Table 3**) are gp120 C1-specific RmAbs produced by the clade AE vaccine. Of these two RmAbs, RmAb 47 bound the strongest to several of the gp120 proteins. RmAb 80 had reduced or no binding with most of the gp120 proteins.

| RmAb | 620345.c01 | 620345.c10 | 703357.c02 | 427299.c12 | 816763.c02 | 356272.c02 | R2184.c04 | R3265.c06 | JR-FL | ChinaAE con. | AE con. | PBS |
|------|------------|------------|------------|------------|------------|------------|-----------|-----------|-------|--------------|---------|------|
| 80 | 0.38 | 0.44 | 0.45 | 0.22 | 0.53 | 0.46 | 0.49 | 0.38 | 0.76 | 0.62 | 0.63 | 0.05 |
| 47 | 0.45 | 0.61 | 0.54 | 0.55 | 0.60 | 0.62 | 0.51 | 0.56 | 0.80 | 0.76 | 0.75 | 0.05 |

Table 3: Average ELISA OD₄₅₀ values for C1-specific RmAbs 47 and 80 against with all 11 gp120 proteins and a PBS negative control. OD₄₅₀ values representing strong binding (≥ 0.60) are highlighted in red and OD₄₅₀ values representing weak to no binding (< 0.39) are highlighted in blue.

Analyzing the sequence of the C1 epitope, all of the gp120 proteins had identical amino acid sequences except for 620345.c01 and 703357.c02 (**Figure 5**). Both proteins had the point mutation V87I, and 703357.c02 had an additional point mutation Q72H. Both RmAb 47 and 80 had slightly reduced binding with 620345.c01, so it is possible that that the mutation V87I may have interfered with the binding of these RmAbs. RmAb 80 also had significantly lower binding with 427299.c12 compared to the other gp120 proteins, which is an interesting result as 427299.c12 had no mutations in this epitope.

| | | | | | | |
|--------------|----|-----------------|---------------|---------------|------|----|
| AE con. | 61 | NMWKNNMV | EQMQEDV | ISLWDQSLKPCV | KLTP | 92 |
| R3265.c06 | 61 | NMWKNNMAEQMQEDV | ISLWDQSLKPCV | RLTP | 92 | |
| R2184.c04 | 61 | NMWKNNMVEQMQEDV | ISLWDQSLKPCV | KLTP | 92 | |
| 356272.c02 | 61 | NMWTNKMAEQMQEDV | ISLWDQSLKPCV | KLTP | 92 | |
| 816763.c02 | 61 | DMWKNNMVEQMQEDV | ISLWDQSLKPCV | KLTP | 92 | |
| 427299.c12 | 61 | NMWKNNMAEQMQEDV | ISLWDQSLKPCV | KLTP | 92 | |
| 703357.c02 | 61 | NMWKNNMVEQMH | EDV | ISLWDQSLKPCIK | VTP | 92 |
| 620345.c10 | 61 | NMWQNKMAEQMQEDV | ISLWDQSLKPCV | KLTP | 92 | |
| 620345.c01 | 61 | NMWQNKMAEQMQEDV | ISLWDQSLKPCIK | KLTP | 92 | |
| ChinaAE con. | 61 | NMWKNNMVEQMQEDV | ISLWDQSLKPCV | KLTP | 92 | |
| JR-FL | 61 | NMWKNNMVEQMQEDI | ISLWDQSLKPCV | KLTP | 92 | |

Figure 5: gp120 protein sequence alignment of C1-specific binding site shown as pink. Mutations from the original AE con. sequence are shown as red residues.

V3-Specific RmAb ELISA and Sequence Analysis

RmAbs 56, 16, 73, 28, and 64 are all V3-specific RmAbs. One of these RmAbs, 56, was produced by the clade B JR-FL vaccine, and the other four were produced by the clade AE vaccine. All five of these RmAbs bound very well with JR-FL, China AE consensus, and gp120 AE consensus (**Table 4**). Interestingly, although RmAb 56 was produced by the clade B JR-FL vaccine, this RmAb binds equally as well to both of the gp120 AE consensus proteins. Likewise, RmAbs 16, 73, 28, and 64 were all produced by the clade AE vaccine, but they all bind equally as well to the clade B JR-FL protein. The results suggest the potential for cross-clade RmAbs. However, the clade B RmAb 56 did not binding very well with most of the clade AE gp120 proteins. Additionally, these five RmAbs all had reduced or no measurable binding with 356272.c02. RmAbs 73, 28, and 64 also had reduced to no binding with 703357.c02.

| RmAb | 620345.c01 | 620345.c10 | 703357.c02 | 427299.c12 | 816763.c02 | 356272.c02 | R2184.c04 | R3265.c06 | JR-FL | ChinaAE con. | AE con. | PBS |
|------|------------|------------|------------|------------|------------|------------|-----------|-----------|-------|--------------|---------|------|
| 56 | 0.62 | 0.69 | 0.45 | 0.09 | 0.06 | 0.25 | 0.06 | 0.05 | 0.76 | 0.79 | 0.82 | 0.04 |
| 16 | 0.79 | 0.78 | 0.71 | 0.69 | 0.57 | 0.40 | 0.70 | 0.69 | 0.81 | 0.81 | 0.83 | 0.05 |
| 73 | 0.72 | 0.69 | 0.33 | 0.57 | 0.58 | 0.08 | 0.53 | 0.49 | 0.80 | 0.81 | 0.81 | 0.05 |
| 28 | 0.76 | 0.51 | 0.09 | 0.58 | 0.52 | 0.39 | 0.71 | 0.72 | 0.79 | 0.80 | 0.80 | 0.05 |
| 64 | 0.69 | 0.49 | 0.10 | 0.56 | 0.23 | 0.07 | 0.31 | 0.35 | 0.82 | 0.80 | 0.79 | 0.05 |

Table 4: Average ELISA OD₄₅₀ values for V3-specific RmAbs 56, 16, 73, 28, and 64 against all 11 gp120 proteins and a PBS negative control. OD₄₅₀ values representing strong binding (≥ 0.60) are highlighted in red, and OD₄₅₀ values representing weak to no binding (< 0.39) are highlighted in blue.

As discovered by the sequence analysis (**Figure 6**), 703357.c02 and 356272.c02 had one common point mutation S259T, suggesting that this mutation could be interfering with RmAb binding. This mutation is a mutation of the first amino acid of the epitope. R2184.c04 only has one point mutation I275M, and RmAbs 56 and 16 have little to no measurable binding with this protein. This suggests that the mutation I275M is

likely interfering with the binding of RmAbs 56 and 16. R3265.c06 has this same point mutation, I275M, and RmAbs 56, 73, and 16 have reduced to no binding with this gp120 protein.

| | | V3 | |
|--------------|-----|---|-----|
| AE con. | 250 | AKTIIIVHLNKSVEINCTRPSNNTRTSITIGPGQ-VFYRTG | 293 |
| R3265.c06 | 250 | AKTIIIVHLNKSVEIKCIRPSNNTRTSMITIGPGQ-VFYRTGDIIGD | 293 |
| R2184.c04 | 255 | TKTIIIVHLNKSVEINCTRPSNNTRTSMITIGPGQ-VFYRTGDIVGN | 298 |
| 356272.c02 | 249 | AKTIIIVHLNKTVEINCTRPSDNKRTSVHMGPGQ-VLYRTGEITGD | 292 |
| 816763.c02 | 249 | VKTIIIVHLNKSVEINCTRPSNNTRTSISMGPQ-VFYRPEGITGD | 292 |
| 427299.c12 | 256 | SKNIIIVHLNESVVINCTRPSNNTVKSIRIGPGQ-TFYRTGDIIGD | 299 |
| 703357.c02 | 260 | AKTIIIVHLNRTIINCTRPSNNTRTSINIGPGQMLFYKPGDIIGD | 304 |
| 620345.c10 | 249 | TKTIIIVHLNKSVEINCTRPSNNTRTSTRIGPGQ-VFYRTGEVITS | 292 |
| 620345.c01 | 249 | TKTIIIVHLNKSVEINCTRPSNNTRTSVRIGPGQ-VFYRTGEVITS | 292 |
| ChinaAE con. | 249 | AKTIIIVHLNKSVEINCTRPSNNTRTSITIGPGQ-VFYRTGDIIGD | 292 |
| JR-FL | 246 | AKTIIIVQLKESVEINCTRPNNTRKSIHIGPGR-AFYTTGEIIGD | 289 |

Figure 6: gp120 protein sequence alignment of V3-specific binding site shown as blue highlighted residues. Mutations from the original AE con. sequence are shown as red residues.

C4-Specific RmAb ELISA and Sequence Analysis

There was only one C4-specific RmAb 53 included in this analysis (**Table 5**). This RmAb was produced by the clade B vaccine, and it therefore bound well with the clade B JR-FL protein. RmAb 53 also presented with some cross-clade binding, as it bound very well with gp120 AE consensus and a few of the individual gp120 proteins. RmAb 53 had little measurable binding with 703357.c02 and 427299.c12.

| RmAb | 620345.c01 | 620345.c10 | 703357.c02 | 427299.c12 | 816763.c02 | 356272.c02 | R2184.c04 | R3265.c06 | JR-FL | ChinaAE con. | AE con. | PBS |
|------|------------|------------|------------|------------|------------|------------|-----------|-----------|-------|--------------|---------|------|
| 53 | 0.25 | 0.66 | 0.12 | 0.15 | 0.44 | 0.43 | 0.67 | 0.55 | 0.70 | 0.72 | 0.80 | 0.05 |

Table 5: Average ELISA OD₄₅₀ values for C4-specific RmAb 53 against all 11 gp120 proteins and a PBS negative control. OD₄₅₀ values representing strong binding (≥ 0.60) are highlighted in red, and OD₄₅₀ values representing weak to no binding (< 0.39) are highlighted in blue.

From the sequence analysis (**Figure 7**), both proteins 703357.c02 and 427299.c12 had a number of mutations with this epitope, so it would be difficult to determine exactly which mutations could be affecting the binding of RmAb 53. 427299.c12 has one mutation location unique to this protein which is M387L. 703357.c02 also only had one mutation location unique to it at A404N. It is possible that these mutations are affecting RmAb 53 binding; however it would be impossible to determine this for sure based on this data alone.

| | | C4 | |
|--------------|-----|--------------------------------|-----|
| AE con. | 381 | IKQIINMWQGVGQAMYAPPISGRINCVSNI | 415 |
| R3265.c06 | 386 | IKQIINMWQGVGQAMYAPPISGNISCA | 420 |
| R2184.c04 | 389 | IKQIINMWQRVGQAMYAPPIRGS | 423 |
| 356272.c02 | 379 | IKQIINMWQAGQAMYAPPVSGIINCR | 413 |
| 816763.c02 | 382 | IKQIINMWQGVGQAMYAPPISGKINCI | 416 |
| 427299.c12 | 392 | IKQIINLWQEVGQAMYAPPVSGQIRCI | 426 |
| 703357.c02 | 388 | IKQIINMWQRVGQAMYNPPVQGH | 422 |
| 620345.c10 | 384 | IKQIVRMWQGVGQSMYAPPISGI | 418 |
| 620345.c01 | 384 | IKQIVRMWQGVGQSMYAPPISGI | 418 |
| ChinaAE con. | 379 | IKQIINMWQGVGQAMYAPPISGRINCVSNI | 413 |
| JR-FL | 379 | IKQIINMWQEVGKAMYAPPIRGQIRCS | 413 |

Figure 7: gp120 protein sequence alignment of C4-specific binding site shown as green highlighted residues. Mutations from the original AE con. sequence are shown as red residues.

C5-Specific RmAb ELISA and Sequence Analysis

There was only one C5-specific RmAb included in the analysis, RmAb 76 (**Table 6**). This RmAb was produced by the clade AE vaccine. Interestingly, this RmAb had strong binding with clade B and clade AE gp120 proteins. However, in general, RmAb bound very well to the entire panel of gp120 proteins. The only reduction in binding was seen with gp120 protein 35272.c02.

| RmAb | 620345.c01 | 620345.c10 | 703357.c02 | 427299.c12 | 816763.c02 | 356272.c02 | R2184.c04 | R3265.c06 | JR-FL | ChinaAE con. | AE con. | PBS |
|------|------------|------------|------------|------------|------------|------------|-----------|-----------|-------|--------------|---------|------|
| 76 | 0.69 | 0.66 | 0.63 | 0.64 | 0.76 | 0.36 | 0.75 | 0.75 | 0.78 | 0.79 | 0.63 | 0.05 |

Table 6: Average ELISA OD₄₅₀ values for C5-specific RmAb 76 against all 11 gp120 proteins and a PBS negative control. OD₄₅₀ values representing strong binding (≥ 0.60) are highlighted in red, and OD₄₅₀ values representing weak to no binding (< 0.39) are highlighted in blue.

The only antigen with which RmAb 76 had reduced binding affinity was 356272.c02. 356272.c02 has two point mutations Q451K and I457L (**Figure 8**). Therefore, it is likely that either of these two mutations, if not both of them, affected the binding of RmAb 76.

| | | | C5 | | |
|--------------|-----|------------|------------------------|----------|-----|
| AE con. | 442 | SELYK | YKVVQIEPLGIAPTRAKRRVVE | 468 | |
| R3265.c06 | 447 | SELYKYKVVQ | IEPLGIAPTRAKRRVVE | 473 | |
| R2184.c04 | 451 | SELYKYKVVQ | IEPLGIAPTRAKRRVVE | 477 | |
| 356272.c02 | 441 | SELYKYKVV | KIEPLGLAPTRAKRRVVE | 467 | |
| 816763.c02 | 442 | SELYKYKVVQ | IEPLGIAPTRAKRRVVE | 468 | |
| 427299.c12 | 459 | SELYKYKVV | EIEPLGIAPSR | 485 | |
| 703357.c02 | 450 | SELYKYKVVQ | IEPLGVAPTRAKRRVVE | 476 | |
| 620345.c10 | 446 | SELYKYKVVQ | IEPLGIAPTRAKRRVVD | 472 | |
| 620345.c01 | 446 | SELYKYKVVQ | IEPLGIAPTRAKRRVVD | 472 | |
| ChinaAE con. | 440 | SELYKYKVVQ | IEPLGIAPT | KAKRRVVE | 466 |
| JR-FL | 440 | SELYKYKVV | KIEPLGVAPT | KAKRRVQ | 466 |

Figure 8: gp120 protein sequence alignment of C5-specific binding site shown as yellow highlighted residues. Mutations from the original AE con sequences are shown as red residues.

Unknown Epitope RmAb ELISA and Sequence Analysis

RmAb 31 has an unknown binding site, so very little site specific analysis can be done for this RmAb (**Table 7**). RmAb 31 was produced by the clade AE vaccine. The ELISA results for RmAb 31 resemble those from RmAbs with epitopes located in

constant regions of the gp120 sequence, so it is likely this RmAb binds within a conformation conserved region as well.

| RmAb | 620345.c01 | 620345.c10 | 703357.c02 | 427299.c12 | 816763.c02 | 356272.c02 | R2184.c04 | R3265.c06 | JR-FL | ChinaAE con. | AE con. | PBS |
|------|------------|------------|------------|------------|------------|------------|-----------|-----------|-------|--------------|---------|------|
| 31 | 0.58 | 0.68 | 0.65 | 0.60 | 0.72 | 0.57 | 0.70 | 0.60 | 0.78 | 0.71 | 0.78 | 0.05 |

Table 7: Average ELISA OD₄₅₀ values for unknown epitope RmAb 31 against all 11 gp120 proteins and a PBS negative control. OD₄₅₀ values representing strong binding (≥ 0.60) are highlighted in red, and OD₄₅₀ values representing weak to no binding (< 0.39) are highlighted in blue.

Overall, the results show that the HIV-1 clade AE gp120 consensus vaccine was successful in producing an effective antibody response which can be further dissected with a panel of monoclonal antibodies in the vaccinated rabbit. Compared to V3-specific RmAbs, RmAbs targeting the conserved region C1, C4, and C5 of HIV-1 gp120 AE consensus tends to be recognized by most, if not all, of gp120 proteins cloned from clade AE viruses. Despite targeting the same epitope within V3 region, RmAbs16, 73, 28, and 64 are able to be recognized by more gp120AE proteins, compared to RmAb 53 elicited by a clade B vaccine. However, the inclusion of the clade B JR-FLgp120 protein demonstrated the possibility of the production of RmAbs able to bind to cross-clade gp120 proteins. The results are further discussed in the Discussion section, along with suggestions for possible further experiments.

DISCUSSION

In this study, eight RmAbs generated from a rabbit immunized with an HIV-1 gp120 AE consensus DNA-prime followed by a gp120 AE consensus protein boost vaccination regimen were analyzed for their binding capability against eight different recombinant isolates of HIV-1 gp120 clade AE isolates. All of the RmAbs tested were able to bind with at least one clade AE gp120 protein, and some of the RmAbs were able to bind with most of, if not all, gp120 proteins. This indicates that the gp120 AE consensus, despite being an artificially designed protein, was able to generate clade AE-specific binding antibody responses. Interestingly, two RmAbs generated from a clade B JR-FL vaccine, RmAbs 56 and 53, showed a lesser degree of cross-reactivity with gp120 AE proteins, compared to the RmAbs from the clade AE vaccine, revealing the antigen specificity produced by the vaccine.

It is evident from the data presented in the results section that the HIV-1 gp120 AE consensus vaccine regimen was successful in producing a broad spectrum of monoclonal antibodies against gp120 in the immunized rabbit, suggesting the potential application of this vaccine design. Although not every RmAb produced was effective at binding with every gp120 protein studied, there was at least one RmAb capable of effectively binding with each of the tested gp120 proteins. Moreover, since these RmAbs elicited from gp120 AE vaccines were generated from one rabbit, it is speculated that the mixture of circulating RmAbs would eventually complement each other and bind with most, if not all, of the gp120 proteins from different clades. There is also evidence

within the results of this project to support the application of this vaccine design for the production of RmAbs able to recognize cross-clade gp120 proteins.

Potential epitopes for one of the RmAb, 31, are not known. While the results from this project may suggest the general locations of these epitopes, further experimentation is needed to identify possible epitopes for this RmAb. In addition to binding to diverse glycoprotein of clade AE viruses, the other biological functions of these RmAbs, such as neutralization activities, will be determined in a future study.

Previously, most of the immunogenicity studies in this field used polyclonal sera, which makes it difficult to link the observed antibody activities with a particular antibody component in a polyclonal serum. Here, the isolation of RmAbs from the vaccine provided a valuable opportunity to assess the specificity of their biological activities. For example, most of the RmAbs were able to recognize epitopes within constant regions of gp120 AE proteins probably due to conservation of the antigen sequence. Some of variable-region specific RmAbs, such as RmAb 64, cannot interact with most of the gp120 AE proteins. Further, when comparing the sequences of different gp120 AE proteins, it helps us to understand, and eventually predict, the binding capability against these diverse antigens.

Genetic diversity and effective evasion of the immune system have made HIV-1 a worldwide epidemic, and complicated all means of treatment and prevention so far. With the promising results of the RVI44 trial as the first partially-successful vaccine trial against HIV, a vaccination method of preventing HIV infection has become a very real possibility. The novel technology developed by the Lu Lab of producing rabbit

monoclonal antibodies against HIV-1 shows much promise for contributing to this field of research.

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