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*J Immunol* 2013; 190:205-210; Prepublished online 26 November 2012;
doi: 10.4049/jimmunol.1201469
http://www.jimmunol.org/content/190/1/205

**Supplementary Material**
http://www.jimmunol.org/content/suppl/2012/11/29/jimmunol.1201469.DC1

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Impact of IgA Constant Domain on HIV-1 Neutralizing Function of Monoclonal Antibody F425A1g8

Xiaocong Yu,*† Mark Duval,* Christopher Lewis,* Melissa A. Gawron,* Rijian Wang,*† Marshall R. Posner,*†,1 and Lisa A. Cavacini*†

With the majority of HIV infections resulting from mucosal transmission, induction of an effective mucosal immune response is thought to be pivotal in preventing transmission. HIV-specific IgA, but not IgG, has been detected in the genital tract, seminal fluid, urethral swabs, urine, and vaginal wash samples of HIV-negative sex workers and HIV-status discordant couples. Purified mucosal and plasma IgA from some individuals with highly exposed, persistently seronegative status can neutralize infection and present cross-clade neutralization activity, though present at low levels. We generated a CD4-induced human mAb, F425A1g8, and characterized the impact of its isotype variants on HIV neutralizing activity. The result showed that, in contrast to little neutralization by the F425A1g8 IgG1 in the absence of sCD4, the IgA1 variant of the Ab displayed significant independent neutralization activity against a range of HIV clade B isolates in the absence of sCD4. Studies of the neutralizing function of IgA isotypes, and the functional relationship between different antigenic epitopes and IgA Abs, may also suggest strategies for the intervention of virus transmission and spread within the mucosa of the host, as well as to serve to inform the design of vaccine strategies that may be more effective at preventing mucosal transmission. This research clearly suggests that IgA isotype, because of its unique molecular structure, may play an important role in HIV neutralization. The Journal of Immunology, 2013, 190: 205–210.

Human immunodeficiency virus infection occurs most often through the mucosal route via hetero- or homosexual contact. As expected, the adaptive immune system responds to HIV infection with production of HIV-specific Abs (1); however, numerous studies have clearly demonstrated the general inability of the humoral immune system to develop functionally effective neutralizing Abs during natural infection or vaccination (2–4). The immune system is confounded by the immunogenicity of the variable loops, which are exposed on the surface of the virus, and tend to elicit strain-specific Abs (5) as well as the transient exposure of specific neutralizing epitopes upon virion binding or engagement with CD4. Therefore, a huge gap in HIV vaccine development has been the inability to generate an immunogen that can elicit effective neutralizing Abs (6).

Research has shown that not all people are equally susceptible to infection by HIV-1. Some individuals may remain HIV-1 seronegative despite repeated viral exposure (7). In a study of these highly exposed, persistently HIV-1 seronegative (HEPS) (8) subjects, HIV-specific IgA responses were detected in the genital tract of female sex workers from Thailand and Kenya (9, 10). HIV-specific IgA, but not IgG, was also present in seminal fluid, urethral swabs, urine, and vaginal wash samples from HIV-1 HEPS heterosexual couples (11, 12). Purified mucosal and plasma IgA from HEPS individuals can neutralize HIV-1 infection (8, 13, 14). Although present at low levels, these IgA demonstrated cross-clade neutralizing activity and were able to inhibit HIV mucosal transcytosis (15, 16). However, with a number of conflicting reports in the literature (17–21), precisely which isotype may protect the mucosa from HIV infection remains unresolved (22). More recently, analysis of the immune correlates to the RV144 vaccine study suggests that Env-specific IgA Abs may mitigate the effects of potentially protective Abs (23). Given that anti-HIV IgA Abs are rare in infected individuals, it has been difficult to characterize how Ab isotype structure and antigenic specificity participate in viral neutralization, which is clearly of significance in the design of novel immunogens to elicit neutralizing Abs.

In this article, we will report on the novel discovery that the IgA isotype switch variant of the CD4-induced (CD4i) Ab, F425A1g8, displays significant neutralizing activity, whereas little neutralization is accomplished by the parental hybrid or any of the IgG subclasses in the absence of sCD4.

Materials and Methods

mAbs, virus, and cell lines

The neutralizing Ab F425A1g8 was generated in our laboratory, as previously described (24), and was shown to bind to the CD4i site of gp120 (data not shown). The Ig expression vectors pLC-HuC1, pHC-HuC1, and pHC-HuC1 were obtained from Dr. Gary McLean (University of Texas Health Sciences Center, Houston, TX). They contained the human Ig L chain and H chain γ1, as well as α1 constant regions, respectively. The CHO-K1 cells were from American Type Culture Collection. The following reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health: SF162 (R5) from Dr. Jay Levy; 89.6 (R5 × 4) from Dr. Ronald Collman; BaL (R5) from Dr. Suzanne Gartner, Dr. Mikulas Popovic, and Dr. Robert Gallo; 93MW960 (clade C, R5) from Dr. Robert Bollinger and the UNAIDS Network for...
HPV; and JR-FL (R5) from Dr. Irvin Chen. Isolate 67970 (CXCR4) was from Dr. David Montefiori. TZM-bl cells were from Dr. John C. Kappes, Dr. Xiaoyun Wu, and Transzyme.

**Construction, production, and purification of F425A1g8 variants**

F425A1g8 VH and VL were PCR amplified, respectively, from the F425A1g8 hybridoma cell line, using the specific primers (Table I), which introduced restriction enzymes sites (5’ NheI and 3’ HindIII for VH; 5’ NheI and 3’ NotI for VL). The VH fragment was cloned into the expression vectors pHCMuC1y and pHChuC1 individually. The VL was cloned into vector pLC-huC1. Paired purified plasmids encoding the F425A1g8 L chain versus IgG1 H chain, and F425A1g8 L chain versus IgA1 H chain, were cotransfected into CHO-K1 cells in equimolar amounts in six-well plates using lipofectamine LTX reagent (Invitrogen Life Technologies). Selection with G418 (800 \( \mu \)g/ml) and puromycin (10 \( \mu \)g/ml) were added after 24 h. Cells were plated in 96-well plates with selection, and wells were screened at 24 h, using IgG and IgA capture ELISA. Positive wells were cloned by limiting dilution until a stable, producing cell line was isolated. Ab was purified from culture supernatant, using Protein G (IgG1) or SSL7 (IgA1) chromatography according to the manufacturer’s instructions (GE Healthcare or Invivogen, respectively).

**SDS-PAGE**

Purified F425A1g8 Ab variants were mixed with 2\( \times \) sample loading buffer (0.12 M Tris; 5% SDS; pH 6.8) with or without DTT (40 mM) and 1/10 volume of tracking dye to final concentration of 100 \( \mu \)g/ml, boiled 3–5 min prior to resolving on a 4–20% gradient gel (Pierce Precise Gel), with 20 \( \mu \)l samples loaded per lane. The gel was stained using GelCode Blue (Pierce), and bands were compared with the m.w. standard (Cell Signaling Technology).

**Immunoreactivity of recombinant F425A1g8 variants**

Live-cell ELISA assay was performed to determine the immunoreactivity of F425A1g8 IgG1 and IgA1 variants to the CD4 binding site. SF2-infected cells (1 \( \times \)10^6) were incubated with Ab at 20, 10, 5, 2.5 \( \mu \)g/ml for 30 min, followed by washing and incubation with HRP-conjugated goat anti-human IgG or IgA (Southern Biotechnology Associates). The human monoclonal b12 IgG1 or IgA1, which were generated as described (25), were run at 20 \( \mu \)g/ml as a standard to determine relative reactivity of the F425A1g8 variants with HIV. After washing, cells were resuspended in 100 \( \mu \)l tetramethylbenzidine substrate and incubated for 10 min. Reaction was stopped by adding 100 \( \mu \)l 1 M phosphoric acid, and samples were read on a plate reader at 450 nm.

**Detection of binding affinity of recombinant F425A1g8 variants using Biacore**

Surface plasmon resonance was used to compare the binding affinity of the F425A1g8 variants to gp120/CD4 complexes, using a Biacore 3000 instrument. The full-length single-chain (FLSC) gp120/CD4 complex was kindly provided by Dr. George Lewis (Institute of Human Virology, Baltimore, MD). Abs were immobilized onto the surface of sensor chip CM-5 (BR100012; GE Life Sciences), using amine coupling. The process involves activation of carboxymethyl groups on a dextran-coated chip by reaction with N-hydroxysuccinimide, followed by covalent bonding of the ligand to the chip surface via amide linkages and blockage of excess activated carboxyls with ethanolamine. Reference surfaces were prepared in the same manner, except that all carboxyls were blocked without added ligand. Purified FLSC gp120/CD4 complex was allowed to flow over the immobilized ligand surface, and the binding response of analyte to ligand was recorded. The maximum resonance unit with each analyte indicates the level of interaction and reflects comparative binding affinity.

**Direct viral neutralization**

The neutralization activity of F425A1g8 variants was determined in vitro using a TZM-bl assay with a panel of three isolates, including an SF162, JR-FL, and 67970. Primary isolate virus was grown in PHA-stimulated PBMCs, as previously described (24, 26, 27), and titred on TZM-bl cells (28) to determine 50% tissue culture-infective dose (TCID50). Serial 2-fold dilutions of F425A1g8 variants were incubated with virus stock diluted to 100 TCID50 for 1 h, at 37°C, prior to the addition of TZM-bl cells (1 \( \times \)10^6 cells per well). With \( \beta \)-galactosidase reagent (Promega) as an indicator of HIV replication, plates were incubated for 48 h, at 37°C and 5% CO2, prior to the measurement of \( \beta \)-galactosidase activity. Percent neutralization was determined on the basis of control wells of virus and media, and IC50 and IC90 values were calculated by regression analysis.

**Ab-dependent cell-mediated viral inhibition**

Ab-dependent cell-mediated viral inhibition (ADCVI) activity was measured using HIV grown in PHA-stimulated PBMCs, as previously described (29). Neutrophils were obtained from peripheral blood of seronegative donors by Ficoll-Hypaque gradient centrifugation. Abs were titered in 96-well, round-bottom plates in 50 \( \mu \)l media containing 20% heat-inactivated FBS. Target cells were PBMCs productively infected with HIV-1 4 d prior to use, as previously described (30), and 1 \( \times \)10^6 infected cells were added per well in 50 \( \mu \)l volume. Within 10 min of the combination of Ab and infected cells, neutrophils were added to the wells at 1 \( \times \)10^6 effector cells per well in 100 \( \mu \)l volume, resulting in an E:T ratio of 10:1. After 4 h, to measure the surviving infectious virus, PHA-stimulated PBMCs were added as indicator cells (1 \( \times \)10^5 cells per well). This indicator PBMCs were incubated for 7 d in the presence of IL-2, at which time the supernatant was quantitated for p24 by a p24-specific ELISA (31). IC50 values were determined by linear regression analysis, and significance was ascertained by the Student t test. Control wells included irrelevant Ab, no effectors, or no targets to determine background release of virus, maximal production of virus, or whether PMNs alone were infected, respectively. Viral inhibition was calculated based on the p24 amount from an irrelevant Ab control. Experiments were repeated three to five times.

**Results**

**The immunoreactivity of F425A1g8 IgG1 and IgA1 variants**

Prior to using the Ab variants in any assays, the Abs were subjected to SDS-PAGE analysis and were determined to be monomeric with no aggregation (Supplemental Fig. 1). To determine the immu-
noreactivity of F425A1g8 variants with the CD4i epitope on HIV-infected cells, a live cell ELISA assay was used. Because HRP-conjugated secondary Abs directly binding to the L chain may be competed with by Ag, IgG isotype- or IgA isotype-specific secondary Abs had to be used. Therefore, b12 IgG1 and IgA1 were used to establish relative reactivity by comparing the absorbance (OD) obtained from F425A1g8 variants with that obtained from b12 controls. The results are expressed as a relative “b12 unit” (OD F425A1g8/OD b12). As shown in Fig. 1, the reactivity of F425A1g8 IgG1 and IgA1 with HIV was retained. Of interest, the IgG1 variant of F425A1g8 had more relative binding than that observed for the IgA1 variant.

The binding affinity of F425A1g8 IgG1 and IgA1 variants

To further determine binding affinity of the Abs, we obtained a single-chain polypeptide encoding HIV-1 BaL and the D1D2 domain of CD4 linked by a 20-aa linker (an FLSC) and that presents as a natural gp120-CD4 configuration (32). We detected the binding affinity of F425A1g8 IgG1 and IgA1 variants with FLSC gp120-CD4 complex, using Biacore. Given the structure of the FLSC, the CD4 binding site is unavailable for b12 binding; therefore, b12 was used as a negative Ab control in these studies, and gp120 monomer was used as a negative control for the Ag. It was determined that the binding affinities of F425A1g8 IgG1 and IgA1 with FLSC gp120-CD4 complex were very similar (Fig. 2). The $K_d$ of F425A1g8 IgG1 was $1.24 \times 10^{-11}$ M and of F425A1g8 IgA1 was $2.64 \times 10^{-12}$ M. Both F425A1g8 variants failed to bind to gp120 monomer (data not shown). Thus, it is clearly shown that the recombinant F425A1g8 IgG1 and IgA1 retain similar immunoreactivity with CD4i epitope activity.

The neutralizing activity against HIV-1 by IgG1 and IgA1 variants of F425A1g8

Neutralization of HIV was tested using TZM-bl cells and three clade B primary isolate viruses (SF162, JR-FL, and 67970) grown in PBMCs. Serial dilutions of Ab were tested, and IC$_{50}$ values for JR-FL and 67970 and IC$_{90}$ values for SF162 were determined by linear regression. In contrast to minimal neutralization by F425A1g8 IgG1 in the absence of sCD4, the IgA1 variant of the Ab displayed significant neutralization activity against a number of HIV clade B isolates in the absence of sCD4, as shown in Table II and Fig. 3. Even though the F425A1g8 IgG1 neutralized the SF162 isolate, the IgA1 variant of F425A1g8 displayed significantly increased neutralization. This differential neutralization was confirmed in studies using tier 1 and reference panel virus (n = 7, including BaL and SF162) grown in 293T cells (M. Seaman, personal communication). Increased neutralization mediated by IgA1 occurs despite relatively decreased immunoreactivity of the IgA1 to SF2-infected cells, as compared with the IgG1.

Functional activity of F425A1g8 switched variants in mediating ADCVI

We also investigated the impact of the switch constant domain between IgG1 and IgA1 of F425A1g8 on functional ability of ADCVI for HIV and HIV-infected cells. HIV-1–binding Abs mediate ADCVI through an interaction with specific Fc receptors on effector cells, resulting in effector cell–mediated destruction of infected cells with Ab-bound Ag (33). Therefore, ADCVI would be a useful assay for determining the ability of the isotype variants of specific Abs to mediate effector cell destruction of or to inhibit HIV replication in an infected target cell population in vivo. Polymorphonuclear leukocytes (PMNs) or neutrophils are the predominant (60–70%) type of WBC in the circulation and play a key role in phagocytosis and killing of infected cells. Therefore, we measured the ability of the IgG1 and IgA1 variants of F425A1g8 to mediate ADCVI.

Table II. Neutralization of HIV-1 by F425A1g8 IgG1 and IgA1 variants

<table>
<thead>
<tr>
<th></th>
<th>JR-FL Clade B</th>
<th>67970 Clade B</th>
<th>SF162 Clade B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(IC$_{50}$)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(IC$_{50}$)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>(IC$_{50}$)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgG1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>2.3 ± 1.4</td>
</tr>
<tr>
<td>IgA1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.73 ± 0.2</td>
<td>23.3 ± 14.3</td>
<td>1.7 ± 1.0</td>
</tr>
</tbody>
</table>

The results were the mean of triplicate wells and were representative of at least three independent experiments.

<sup>a</sup>IC$_{50}$ or IC$_{90}$ concentration (µg/ml) of Ab required for 50% or 90% inhibition of HIV, respectively.

<sup>b</sup>F425A1g8 IgG1 variant expressed from CHO-K1 cells.

<sup>c</sup>F425A1g8 IgA1 variant expressed from CHO-K1 cells.
a critical role in innate immunity against infections. PMNs consistently express multiple receptors for IgG, including FcγRIIa (CD32), FcγRIIIa (CD16), and FcγRIIIb. They also express FcγR1 (CD64) following induction with G-CSF. In addition to Fc receptors for IgG, PMNs also express Fc receptors for IgA (FcaR, CD89). Cross-linking Fcy receptors as well as cross-linking of the IgA receptor on PMNs by mAbs have been shown to be critical in inducing Ab-dependent cellular cytotoxicity against tumor cells (34, 35). Therefore, although traditional ADCVI (or Ab-dependent cellular cytotoxicity) assays are based on mononuclear cell populations, we propose to use neutrophils as effectors.

Because the binding of F425A1g8 differed with strains of virions, a total of five isolates—including clade B, representing R5, R5 × 4, and ×4 isolates, and clade C isolate (R5)—were tested in this variant of the neutralization assay. Ab-mediated destruction of HIV and HIV-infected cells is determined by testing the inhibition of subsequent HIV replication or p24 levels. The results of these assays are summarized in Table III as well as in Fig. 4, as represented by the JR-FL strain. The F425A1g8 IgA1 showed significant ADCVI activity for both clade B isolates and a single clade C isolate. For two of four clade B isolates (SF162 and JR-FL), F425A1g8 IgA1 failed to mediate ADCVI activity, whereas significant activity was observed for F425A1g8 A1 (Table III), with p values ranging from 0.0008 to 0.05 for multiple experiments. Two clade B strains, BaL (R5) and 89.6 (R5 × 4), failed to be inhibited by either isotype variant at the concentrations tested. Of importance, both isotype variants inhibited the clade C isolate, 93MW960. Of interest, the IgG1 isotype had greater activity against the clade C isolate than did IgA1 (p value from 0.0012 to 0.0598). This variation in impact of isotype in ADCVI may result from affinity and/or binding specificity of the Fc fragment of IgG1 and IgA1 subclasses with Fc receptors on the surface of neutrophils. In contrast, the Ag density and epitope orientation may result in differences in outcome. Because only one clade C strain was tested for ADCVI in this project, it would be valuable to explore the impact of IgA1 isotype on clade C. No viral inhibition was observed in mock control wells, which contained Ab, target cells, or indicator cells without neutrophils. Viral replication was similar for control wells containing effector cells, target cells without Ab, and target cells alone (data not shown).

### Table III. ADCVI activity of HIV-1 by F425A1g8 IgG1 and IgA1 variants

<table>
<thead>
<tr>
<th>Isotype</th>
<th>BaL Clade B, R5 (n = 6)</th>
<th>JR-FL Clade B, R5 (n = 6)</th>
<th>93MW960 Clade C R5 (n = 5)</th>
<th>89.6 Clade B, R5 × 4 (n = 3)</th>
<th>SF162 Clade B, R5 (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1</td>
<td>&gt;40</td>
<td>&gt;40</td>
<td>9.5 ± 7.9</td>
<td>&gt;40</td>
<td>&gt;40</td>
</tr>
<tr>
<td>IgA1</td>
<td>&gt;40</td>
<td>16.6 ± 5.1</td>
<td>18.3 ± 13.4</td>
<td>&gt;40</td>
<td>6.1 ± 5.9</td>
</tr>
</tbody>
</table>

n, Repeat times of ADCVI assay.

*The ADCVI activity was determined by IC₅₀, which represents concentration (µg/ml) of Ab required for 50% inhibition of HIV.

**FIGURE 4.** Inhibition of JR-FL by F425A1g8 IgG1 and IgA1 variants measured using ADCVI. ADCVI mediated by F425A1g8 IgG1 (●) and IgA1 (○) Abs and neutrophils. F425A1g8 variants were incubated with JR-FL–infected PBMCs just prior to adding neutrophils at an E:T ratio of 10:1. After 4 h, PHA-stimulated PBMCs were added as indicator cells, and p24 was quantitated by ELISA after 1 wk. Percent inhibition was determined by the following formula: [(p24 control − p24 test)/p24 control] × 100.

### Discussion

We generated and characterized the isotype switch variants of the CD4i Ab F425A1g8. Study on the property of recombinant IgG1 and IgA1 variants has shown that they were monomeric Ab molecules and retained similar high binding affinity with the CD4i epitope. The IgA1 variant of F425A1g8 displayed significant neutralization activity alone. In contrast, little neutralization by the parental hybrid or IgG1 subclass variant in the absence of sCD4 was observed. Combined with epidemiological data, these data suggest that HIV-specific IgA Abs may play an important independent role in providing protective immunity against HIV infection in mucosal surfaces. However, the relationship of IgA structure to functional neutralization of HIV, as well as why the functional IgA Abs could not be induced in the most natural HIV-infected populations, remains to be fully resolved. Exploration of these questions may yield information that may guide vaccine design.

The entry of HIV-1 into target cells typically requires the sequential binding of the viral exterior envelope glycoprotein, gp120, to CD4 and a chemokine receptor. CD4i Abs recognize the epitope of gp120 structures that are formed or exposed by CD4 binding and can block virus binding to the chemokine receptor. However, CD4i neutralizing Abs demonstrate large conformational requirements for binding in that the site is exposed only upon CD4/gp120 binding, which limits Ab access to the proximal chemokine site (36). The results of many studies have demonstrated that Fabs or single-chain variable fragments of CD4i Abs tend to be more effective at neutralization than the intact molecule, presumably owing to greater access to the epitope (36, 37). The distinct structural properties of IgA provide this Ab isotype some unique functional capabilities. IgA1 molecules have a lengthy hinge region with a 13-aa insertion. Crystal studies have shown that the structure of IgA1 resembles a “T,” whereas the IgG1 molecule has more of a “Y” structure (38). This flexible stretch property of IgA1, compared with IgG1 isotypes, would seem likely to afford a greater reach between its two Ag-binding sites and the potential to decrease steric hindrance (8), allowing improved access to the relatively hidden CD4i epitopes recognized by F425A1g8. This property may be particularly important in an effective neutralizing Ab response to HIV when increasing Ab flexibility could result in cooperative interactions on gp120/gp41 trimers. Increased flexibility of Ab molecules has been shown by our laboratory to increase Ab neutralization activity (26).
It can be suggested that the IgA variant displayed higher ADCVI than did IgG1 because more IgA receptors than IgG receptors are found on neutrophils. However, when comparing IgG1 and IgA variants of other human monoclonal anti-HIV Abs, we generally do not observe a difference in ADCVI activity (data not shown). Regardless, IgG and IgA receptor density is the focus of additional studies.

A number of studies have reported that the IgA Abs may have more advantages than IgG Abs for inhibiting tumor growth and infectious diseases via mediation of immune cell targeting (39). For example, IgA Abs are far more effective than IgG anti-tumor Abs in recruiting neutrophils for destruction of lymphoma and solid-tumor targets (40–42). Specific secretory IgA Ab against Strep-tococcus mutans was effective in preventing recolonization with streptococci, whereas the parental IgG1 Ab was rapidly cleared (43). It has been hypothesized that the long hinge of IgA1 and the FcRn may provide for particularly efficient bridging between Ag on a target cell and FcRn on an effector cell (44). The increase in ADCVI activity observed with the IgA1 construct of F425A1g8 is in our study supports this hypothesis. It may also be possible that failure of IgA to stimulate FCγRIIb resulted in an “inhibitory” response, contributing to more protective activity of IgA compared with IgG (45). Although IgA Abs represent an attractive new candidate for immunotherapy of cancer and infectious diseases (46–53), it has been difficult to determine the importance of such Abs. The authors have no financial conflicts of interest.

The authors thank Dr. Gary McLean (University of Texas Health Sciences Center, Houston, TX) for the generous contributions to this work of Ig expression vector; the AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health, as well as Dr. Jay Levy, Dr. Neal Halsey, Dr. Ronald Collman, Dr. Suzanne Gartner, Dr. Mikulas Popovic, Dr. Robert Gallo, Dr. Robert Bollinger, and Dr. Irvin Chen for supporting HIV strain; Dr. John C. Kappes, Dr. Xiaoyun Wu, and Dr. David Montefiori for providing TZM-bl cells; and Dr. Paula M. Kuzentoski for efforts in editing and revising the manuscript.

Disclosures

The authors have no financial conflicts of interest.

References


Supplement 1: Analysis of purified F425A1g8 IgG1 and IgA1 variants by SDS-PAGE.

**SDS-PAGE:** 4-20% Pierce Precise Gel, Non-reducing samples 100 μg/ml was loaded as 20 μl/lane. Lane 1: b12 dIgA1, lane 2: b12 IgA1, lane 3: F425A1g8 IgA1, lane 4: pre-stained molecular marker.