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Characterization of a monoclonal anti-capsid antibody that cross-reacts with three major primate lentivirus lineages

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Abstract

Mouse monoclonal antibodies with varying specificities against the Gag capsid of simian and human immunodeficiency virus (SIV/HIV) were generated by immunizing mice with whole inactivated SIVagmTYO-1. Monoclonal antibody AG3.0 showed the broadest reactivity recognizing the Gag capsid protein (p24–27) and Gag precursors p38, p55, and p150 of HIV-1, HIV-2, SIVmac, and SIVagm. Using overlapping peptides, the AG3.0 epitope was mapped in capsid to a sequence (SPRTLNA) conserved among HIV-1, HIV-2, SIVrcm, SIVsm/mac, and SIVagm related viruses. Because of its broad cross-reactivity, AG3.0 was used to develop an antigen capture assay with a lower detection limit of 100 pg/ml HIV-1 Gag p24. Interestingly, AG3.0 was found to have a faster binding on/off rate for SIVagmVer and SIVmac Gag than for SIVagmSab Gag, possibly due to differences outside the SPRTLNA motif. In addition, the ribonucleic acid (RNA) coding for AG3.0 was sequenced to facilitate the development of humanized monoclonal antibodies.

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NUCLEOTIDE SEQUENCE ACCESSION NUMBERS The DNA sequence for AG3.0 has been submitted to GenBank under accession numbers JN252707 (heavy chain CDR) and JN252708 (light chain CDR).

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Keywords

AG3.0; epitope; Gag; capsid; p24; p27; human immunodeficiency virus; simian immunodeficiency virus

INTRODUCTION

Since the discovery of HIV-1 and HIV-2 (Barre-Sinoussi et al., 1983; Clavel et al., 1986; Gallo et al., 1983) an increasing number of related SIVs have been isolated from nonhuman primates. Serological evidence of SIV infection has been shown for at least 40 of the 69 different primate species in Africa and has been confirmed by sequence analysis in 32 (Aghokeng et al., 2010; Locatelli et al., 2008a). Complete SIV genome sequences are available for 20 species (Liegeois et al., 2009; Van Heuverswyn and Peeters, 2007; VandeWoude and Apetrei, 2006). The primate lentiviruses can presently be classified into 10 distinct lineages based upon phylogenetic relationships of full-length sequences: 1) SIVcpz from chimpanzees (*Pan troglodytes*) including HIV-1 and SIVgor from western gorillas *(Gorilla gorilla)* (Santiago et al., 2003; Van Heuverswyn et al., 2007; Van Heuverswyn et al., 2006); 2) SIVsm from sooty mangabeys *(Cercocebus atys)* including HIV-2 and SIVmac from macaques *(Macaca spp.)* (Clavel et al., 1986; Franchini et al., 1987; Hirsch et al., 1989); 3) SIVagm from African green monkeys (members of the *Chlorocebus aethiops* superspecies) (Allan et al., 1991; Hirsch et al., 1993b; Johnson et al., 1990); 4) SIVsyk from Sykes' monkeys *(Cercopithecus albogularis)* (Hirsch et al., 1993a); 5) SIVlho from L'Hoest monkeys *(C. lhoesti)* including SIVsun from sun-tailed monkeys *(C. solatus)* and SIVmnd-1 from mandrills *(Mandrillus sphinx)* (Beer et al., 1999; Hirsch et al., 1999; Tsujimoto et al., 1989); 6) SIVrcm from red-capped mangabeys *(Cercocebus torquatus)* including SIVdrl from drills *(Mandrillus leucophaeus)*, and SIVmnd-2 from mandrills (Beer et al., 2001; Hu et al., 2003); 7) SIVdeb from DeBrazza monkeys *(C. neglectus)* (Bibollet-Ruche et al., 2004) including SIVden from Dent's mona monkeys *(C. mona denti)* (Dazza et al., 2005); 8) SIVgsn from greater spot-nosed monkeys *(C. nictitans)* including SIVmon from mona monkeys *(C. mona)*, and SIVmus-1 and mus-2 from mustached monkeys *(C. cephus)* (Aghokeng et al., 2007; Courgnaud et al., 2003a); 9) SIVtal from talapoin monkeys *(Miopithecus ogouensis)* (Liegeois et al., 2006); and 10) SIVcol from Colobus monkeys (*Colobus guereza*) (Courgnaud et al., 2001). Some lineages, such as SIVcpz, are presumed to be the result of recombination between other lineages (SIVrcm and SIVgsn) (Bailes et al., 2003). Smaller fragments from other SIV-infected African primate species have also been sequenced and may indicate the existence of either separate lineages [SIVbkm from black mangabeys *(Lophocebus aterrimus)* (Takemura et al., 2005), SIV-ASC-Qu from Schmidt's guenon *(C. ascanius schmidti)* (Verschoor et al., 2004)], or reveal genetic relationships to the lineages mentioned above (SIVwrc, SIVolc to the SIVlho lineage) (Courgnaud et al., 2003b; Locatelli et al., 2008b).

For diagnostic purposes, monoclonal antibodies (mAbs) are useful to characterize immunodominant antigenic sites or functional domains on viral components. In addition, they are needed to develop a variety of virological assays, most importantly antigen capture assays for HIV and SIV. Moreover, measurements of SIV antigen in cells and tissues, such

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as kinetic studies, virus titrations, immunohistochemistry, and neutralization tests, require a reliable and fast method for measuring virus production. HIV/SIV capsid protein (p24–27) is the most abundant protein produced during virus replication (Veronese et al., 1988) and is found both inside infected cells and in virus particles released by infected cells forming a protective shell for viral RNA. Antigen-capture enzyme-linked immunosorbent assays (ELISAs) that determine the p24–27 amount in plasma or cell culture samples are frequently used in HIV/SIV research as endpoint for neutralization as well as antiviral assays. Besides antigen capture ELISA, there are other assays available for quantitating HIV/SIV in body or tissue culture fluids, such as viral RNA quantification or the measurements of reverse transcriptase (RT) activity. Compared to the antigen capture ELISA, those other methods have both advantages and disadvantages. The measurement of viral RNA is highly sensitive, but requires exact knowledge of the underlying sequence and necessitates storage of the samples at very low temperatures to prevent degradation of the viral RNA. On the other hand, quantification of RT enzyme activity is less sensitive than that of p24–27 protein determination because Gag is the major structural protein and is present at about 5,000 copies per virion (Briggs et al., 2004). In addition, inappropriate sample storage can also interfere with sensitivity of RT quantitation since enzymes are more sensitive to temperature changes than are proteins. Measurement of RT activity is also not specific for HIV/SIV since all retroviruses code for this enzyme. Compared to viral RNA and RT, p24–27 is a fairly stable protein, is specific for and also antigenically cross-reactive with a wide variety of HIVs and SIVs, and large numbers of samples can be processed on a single ELISA plate. Antigen capture assays for HIV and SIV are commercially available, but are expensive and can have limited utility for detecting a wide range of SIV and HIV types. Neutralization tests or virus titrations usually require processing of high quantities of samples, and thus it is desirable to develop cost-effective in-house assays that incorporate monoclonal antibodies broadly reactive to SIV and HIV. Furthermore, determinations can be made by detecting p24–27 expression in infected cells by means of immunohistochemical staining methodologies or western blot/radioimmunoprecipitation.

In this study, the generation and characterization of several mouse monoclonal antibodies against SIVagm is presented; one antibody (AG3.0) was broadly reactive to HIV-1, HIV-2/SIVsm/SIVmac and SIVagm by western blotting and radioimmunoprecipitation. The epitope recognized by AG3.0 mapped to a 7-mer peptide within the N-terminal portion of the Gag capsid, a highly conserved region among primate lentiviruses (HIV-1, HIV-2/SIVmac, SIVagm, and SIVrcm). The antibody was donated to the NIH AIDS Research and Reference Reagent Program (ARRRP; www.aidsreagent.org, catalog number 4121) and has been used in experimental studies by a number of investigators (Leblanc, Perez, and Hope, 2008; Potash et al., 1998; Sakuma et al., 2007; Schiavoni et al., 2004). As a next step, a p24–27 antigen capture assay comparable in sensitivity to immunostaining of infected cells was developed. Testing of representative virus strains from six primate lentiviral lineages by antigen capture assay revealed the broad spectrum binding of mAb AG3.0 in recognizing p24–27 species from HIV-1, HIV-2, SIVmac, SIVagm, and SIVrcm, but not SIVlho, SIVsun, and SIVsyk. The lower limit of quantitation of the assay was determined to be 100 pg/ml p24 Gag. Subsequently, both the heavy and light chains of AG3.0 were sequenced and annotated to facilitate humanized anti-p24 antibody development.

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RESULTS

Generation of three monoclonal antibodies that react with SIV Gag

Mouse monoclonal antibodies against whole disrupted SIVagmTYO-1 were produced using a standard mouse-cell hybridoma technique. Hybridomas were screened for reactivity to SIVagmTYO-1 by whole virus ELISA. The specificity of positive hybridomas was further evaluated by immunoblotting and three hybridomas were selected, whose supernatants detected SIV Gag p27. The hybridomas were cloned by single cell endpoint dilution and were designated AG3.0, AG5.0, and AG6.0. All three monoclonal antibodies were determined to be of the immunoglobulin G type 1 (IgG1) isotype. As shown in Fig. 1, AG3.0 showed the broadest reactivity by immunoblotting and detected Gag p24–27 from all four SIVagm isolates (ver, gri, sab, tan) as well as from SIVmac, HIV-2 and HIV-1, AG5.0 reacted with all SIVagm subtypes, and HIV-1 and HIV-2 Gag proteins, but not with SIVmac Gag while AG6.0 only reacted with SIVagm Gag from vervet and tantalus monkeys. Additionally, radioimmunoprecipitation analysis (RIPA) was performed with both cell and virus lysates (Fig. 2). RIPA differs from immunoblot analysis in that radiolabeled native proteins in solution react with antibody whereas in immunoblotting viral proteins are mostly denatured due to the presence of sodium dodecyl sulfate (SDS). The monoclonal antibody AG3.0 reacted with p24–27 and the Gag precursor proteins p38, p55, and p160 from SIV- and HIV-infected cell lysates. Monoclonal antibody AG1.0, recognizing gp120 from SIVagm and SIVmac, and antibody AG4.0, an anti-cellular antibody, served as controls. Overall, AG3.0 was the most broadly cross-reacting monoclonal antibody recognizing the Gag capsid and precursor products of at least three different primate lentivirus lineages.

AG3.0 reacts with a 7-mer Gag peptide that is conserved between several lentivirus lineages

In order to determine the epitope recognized by AG3.0, we initially used a series of overlapping Gag p26 peptides provided by the EU AIDS Vaccine Integrated Project's Programme EVA for epitope mapping. Twenty-two 20-mer peptides with a 10 amino acid overlap were tested for reactivity with AG3.0 (Table 1). AG3.0 reacted with a single peptide spanning amino acid 11 to 30 from SIVmac251Gag (HLPLSPRTLNAWVKLIEEKK), but not with any of the surrounding peptides, indicating that the minimal AG3.0 epitope is located in the center of the peptide at the N-terminus of Gag capsid. Subsequent fine mapping using 13-mers with 12-mer overlaps covering the sequence GGNYVHLPLSPRTLNAWVKLIEEKK (SIVmac251Gag aa 141–165) revealed a minimal epitope with the sequence SPRTLNA. This was concluded from the fact that two of the peptides missing one amino acid at the beginning or the end of the epitope (NYVHLPLSPRTLN and PRTLNAVWKLIEE, Fig. 3) were not recognized by AG3.0. A sequence alignment between representatives of ten primate lentiviral lineages is shown in Table 2A and demonstrates that the epitope SPRTLNA is conserved between most SIVcpz, SIVsm, SIVagm, and SIVrcm related viruses. Only the HIV-1 N group and SIVcpz-ANT differ by one amino acid at the N-terminus (threonine instead of serine). This peptide also differs in the following viruses (conserved amino acids underlined): SIVlho (SPRTIQT), SIVsun (SPRTVQT), SIVmndGB1 (SPRIIQT), SIVcol (SPRTLGA), SIVsyk (PVRTLKT), SIVmon (EPRLLKT), SIVmus-1 (NARILKT), SIVmus-2 (SPRILKT), SIVgsn (NSRILKT),

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SIVdeb (SPRIVKT), SIVden (STRVLKT), and SIVtal (SPRLLKT). Generally, the N-terminus of the epitope was more conserved than the C-terminus among the ten primate lentivirus lineages. The only conserved amino acid among all simian and human immunodeficiency viruses described so far was arginine in position 3.

AG3.0 antigen capture assay for detecting virus from three major lentiviral lineages

Antigen capture assays are useful tools for identifying and quantifying plasma virus levels in infected monkeys and for measuring virus growth in tissue culture. Since AG3.0 recognized three major lineages commonly used in AIDS research, a broadly reactive antigen capture assay was developed, which could detect a wide variety of HIV/SIV capsid antigens. In order to generate pure antibody material, IgG was purified from hybridoma supernatants using Protein G sepharose columns, and the optimal AG3.0 IgG concentration was determined as follows: An ELISA plate coated with twofold dilutions of AG3.0 was reacted with 0.2% Tween20 disrupted SIVagmVER.DE.x.AGM3 supernatant. Captured protein was detected using a polyclonal antibody from an SIV-infected monkey followed by goat anti-human immunoglobulin G-peroxidase conjugate and substrate. The optimal IgG concentration for coating ELISA plates was determined as 0.675 μg/well.

Next, virus supernatant from three different primate lentivirus lineages (HIV-1, HIV-2/SIVsm, and SIVagm) as well the chimeric virus SHIV 89.6 (HIV-1 89.6 *env, tat, rev, vpu* in an SIVmac239 backbone) were serially titrated in C8166 or Molt4clone8 cells (Table 3) and the amount of p24–27 content determined in an AG3.0 antigen capture assay. Wells were considered positive if the optical density exceeded the mean of the negative controls plus three times the standard deviation. In parallel, the virus-exposed cells were subjected to HIV/SIV-specific immunostaining. The mean tissue culture infectious dose ($TCID_{50}$) was calculated using both endpoints, i.e., antigen capture assay optical densities of supernatants and HIV/SIV-specific immunostaining of cells. As shown in Table 3, the results of the antigen capture assay matched the results of the cellular immunostaining within the expected range of assay variability. These results suggested that the antigen capture assay is equivalent to immunostaining as endpoint analysis for virus titrations and virus neutralization assays.

Third, the reactivity of AG3.0 to HIVs and SIVs from five primate lentivirus lineages was tested by antigen capture ELISA. As shown in Fig. 4, the AG3.0-based antigen capture assay was able to detect Gag capsid from HIV-1, HIV-2, SIVmac, SIVagm, and SIVrcm, but not from SIVsun, SIVlho, and SIVsyk. For this experiment, two-fold dilutions of virus supernatants were assayed, and the optical densities decreased proportionally to the virus dilutions. At about a 100-fold dilution of supernatant, the signal became undetectable. Overall, the cross-reactivity of the AG3.0 antibody was consistent with the amino acid conservation of the Gag epitope (see Table 2A). As described above and shown in Table 2A, the epitope SPRTLNA is mostly conserved among the HIV-1/SIVcpz, HIV-2/SIVsmm, and SIVagm lineages, and SIVrcm. In SIVsyk, SIVsun, and SIVlho, it differs by 3–4 amino acids, which prohibit binding to the monoclonal antibody. Viruses from the SIVdeb, SIVgsn/mon/mus, and SIVtal lineages were not tested, but the epitope differs by 2–5 amino acids from SPRTLNA, and it would be unlikely that the AG3.0 antibody cross-reacts with

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Gag from these viruses. In contrast, the conservation of the epitope in SIVdrl and SIVmnd-2, which are phylogenetically related to SIVrcm. likely predicts cross-reactivity.

Affinity determination of the interaction between AG3.0 and Gag proteins from SIVagm and SIVmac

Surface plasmon resonance (SPR) technology was used to evaluate the binding affinity of the monoclonal antibody AG3.0 to different SIV Gag proteins. Although the calculated maximal response (Rmax) was 100 response units (RU), the observed Rmax was more than double, likely due to multimerization of the Gag protein (data not shown). The highest affinity was obtained using SVagmVer (3.7 nM) while the affinities for SIVmac Gag and SIVagmSab Gag were lower but similar with 20 nM and 35 nM, respectively (Fig. 5A). Interestingly, while the dissociation of SIVagmVer Gag and SIVmac Gag was fairly rapid, the binding between the AG3.0 mAb and SIVagmSab Gag was found to be very stable, with very little dissociation detectable (Fig. 5B).

Sequencing of the AG3.0 antigen binding site

cDNAs generated from RNA coding for the variable fragments of the AG3.0 heavy and light chains were sequenced and analysed using the Vbase2 application (http://www.vbase2.org) to identify the conserved framework regions (FR1–FR4) and variable complementarity determining regions (CDR1–CDR3) according to the International ImMunoGeneTics (IMGT) nomenclature (Fig 6). The three variable heavy chain regions were found to consist of 8, 8 and 16 amino acids for CDR1, CDR2 and CDR3, respectively, whereas the light chain had corresponding CDR lengths of 11, 3, and 9.

DISCUSSION

Monoclonal antibodies against SIVagm Gag p27 were developed to allow the Gag capsid expression in African green monkeys and in pathogenic macaque models of SIV infection to be compared. Gag is an important structural protein and is often referred to as a precursor because it is subject to cleavage by the viral protease, which yields the internal structural proteins of the mature virion (Freed, 1998; Swanstrom and Wills, 1997; Vogt, 1997; Wills and Craven, 1991). Three of the Gag cleavage products, matrix, capsid, and nucleocapsid, are common to all retroviruses and are always arranged in this order within the Gag precursor, with matrix being at the N-terminus. Additionally, the Gag precursors of HIV and SIV possess a C-terminal domain termed p6 that is unique to primate lentiviruses, as well as two “spacer” regions that separate capsid from nucleocapsid, and nucleocapsid from p6 (Henderson et al., 1992; Mervis et al., 1988).

Capsid, which directly follows matrix in the Gag precursor, has crucial roles in particle assembly and after entry into a new target cell. However, the function of capsid in the early phase of the replication cycle is not well understood. In the mature virion, capsid forms the shell of the core, which is occasionally tubular but most often conical, a feature that distinguishes lentiviruses, such as HIV-1, from most other retroviruses (Gelderblom, 1991). Capsid has two predominantly α-helical domains that are connected through a flexible linker region (Gamble et al., 1997; Gitti et al., 1996; Momany et al., 1996) and have different roles

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in virus morphogenesis. The N-terminal domain, which comprises two thirds of HIV-1 capsid, is required for the formation of the mature core but is dispensable for the assembly of immature virus particles (Borsetti, Ohagen, and Gottlinger, 1998; Dorfman et al., 1994; Reicin et al., 1996; Reicin et al., 1995; Srinivasakumar, Hammarskjold, and Rekosh, 1995; Wang and Barklis, 1993). In contrast, the C-terminal capsid domain is crucial both for particle assembly and for core formation (Dorfman et al., 1994; Mammano et al., 1994; McDermott et al., 1996; Reicin et al., 1995). The N-terminal domain of HIV-1 capsid interacts with the human peptidylprolyl cis-trans isomerase cyclophilin A (CyPA), which leads to the specific incorporation of this ubiquitous cytosolic host protein into virions (Franke, Yuan, and Luban, 1994; Luban et al., 1993; Thali et al., 1994). This feature is unique for HIV-1 and absent in other primate lentiviruses and HIV-2. Based on the regular appearance of the synthetic cones, Sundquist et al. propose that retroviral cores are composed of hexagonal lattices that are closed through the incorporation of a total of 12 pentagons (Ganser et al., 2003).

In the study presented here, three monoclonal antibodies against SIVagm from the vervet subspecies (SIVagmTYO-1) were generated and characterized. AG6.0 cross-reacted with two subspecies of African green monkeys (vervet and tantalus), whereas AG5.0 showed a broader cross-reactivity, reacting with SIVagm Gag p26 from vervet, grivet, sabaeus, and tantalus, HIV-1 and HIV-2. Interestingly, although HIV-2 and SIVmac have similar ancestries, having both arisen by transmission from sooty mangabeys (SIVsm), only the p26 from HIV-2ST was recognized. AG3.0 showed the broadest cross-reactivity and recognized not only the SIVagm lineage, but also members of the HIV-2 lineage (HIV-2, SIVmac), HIV-1 lineage, and SIVrcm. In addition, AG3.0 reacted with the Gag precursors p55 and p38 and the Gag-Pol precursor in infected cell lysates. This broadly cross-reactive antibody (AG3.0) is available from the NIH ARRRP as “Monoclonal antibody to HIV-1 p24” under catalog no. 4121 (Simm et al., 1995). In addition, the AG3.0 producing hybridoma cells are also available. A number of monoclonal antibodies specific for HIV and SIV Gag are deposited in the NIH ARRRP but, with the exception of AG3.0, none react with SIVagm or is broadly cross-reactive with SIV and HIV. So far, only Otteken et al. (1992) successfully produced monoclonal antibodies to SIVagm Gag p27 (strain TYO-7). However, these monoclonal antibodies (1.17.3, 1A7 and 1F6) are primarily type-specific, reacting with only certain types of SIVagm and the HIV-2 lineage, but not with HIV-1 or SIVmndGB1. These monoclonal antibodies mapped to a peptide spanning amino acids 152 to 172 in SIVmac251, which is conserved in the HIV-2 lineage, but not in SIVagmTYO-1, HIV-1 IIIB, and SIVmndGB1. One particular monoclonal antibody, FM18, generated against SIVmac, also reacted with SIVagm, but not with HIV-1 (Lairmore et al., 1993). Since this antibody has not been mapped, the full extent of cross-reactivity is unknown. However, our broadest reactive monoclonal antibody, AG3.0, is able to detect p24–27 from all of the main primate lentivirus lineages including HIV-1, SIVmac/HIV-2, SIVagm, and SIVrcm.

Rough and fine mapping revealed the minimal epitope recognized by AG3.0 to be SPRTLNA (Table 1, Fig. 3). This epitope is conserved throughout the HIV-1 (including SIVrcm), HIV-2 and SIVagm lineages, and is partially conserved throughout the other SIV lineages (SIVlho, SIVsyk, SIVcol, SIVdeb, SIVtal, SIVgsn), although it is completely absent in non-primate lentiviruses. However, despite this partial conservation, AG3.0 does

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not recognize SIVlho, SIVsun, or SIVsyk Gag, indirectly confirming the minimal epitope needed for binding (Fig. 4). Von Schwedler et al. showed that viruses with a mutated arginine and asparagine in the SPRTLNA motif failed to form a cone-shaped core and were not infectious, indirectly demonstrating the importance of this epitope for viral assembly. The Los Alamos Immunology database contains 21 HIV-1 cytotoxic T-lymphocyte (CTL) epitopes restricted by HLA-A, -B, and -C haplotypes spanning the SPRTLNA peptide (http://www.hiv.lanl.gov/content/immunology/ctl_search) suggesting immunogenic dominance of the region. For example, a dominant CTL epitope ISPRTLNAW has been detected in HIV-1 positive individuals having the protective haplotype HLA B*5701 (Bailey et al., 2006), which is found in a very high frequency in HIV-1 infected non-progressors (11/13 or 85% versus 19/200 or 9.5% of progressors). Non-progressors tended to have an immune response that was highly focused on four p24 epitopes that were presented by B*5701, ISPRTLNAW, KAFSPEVIPMF, TSTLQEQIGW, and QASQEVKNW (Migueles and Connors, 2001). THE SPRTLNA epitope is also part of three human CD4+ T helper cell HIV-1 epitopes and two mouse monoclonal antibody HIV-1 epitopes (AISPRTLNAW for the F5-2 antibody and VHQAISPRTLNAWVK for the ID8F6 antibody) (http://www.hiv.lanl.gov/content/immunology/ctl_search).

After mapping the AG3.0 epitope, we sought to develop an antigen capture ELISA using the AG3.0 monoclonal antibody since it showed the broadest cross-reactivity against HIV/SIV (Binninger-Schinzel et al., 2008). Currently, there is only one commercially available antigen capture assay that cross-reacts with SIVmac, SIVagm, SIVdrl, and SIVmnd-2 (from ZeptoMetrix Corp.) (Hu et al., 2003; Pandrea et al., 2006). However this assay is expensive and the epitope recognized by the monoclonal antibody that forms the basis of the test is not public knowledge. Therefore, the degree of cross-reactivity with different HIV/SIV isolates can only be determined empirically. Most in-house antigen capture assays using monoclonal antibodies developed to date are either optimized for the HIV-1/SIVcpz or HIV-2/SIVmac/SIVsm lineage (Lohman et al., 1991; Thorstensson et al., 1991; Wehrly and Chesebro, 1997). Only one assay, based on a polyclonal sandwich system, was also able to detect SIVmnd to an appreciable degree (Beirnaert et al., 1998). Our AG3.0 in-house antigen capture assay had a lower limit of detection of 100 pg/ml, comparable to that of Zeptometrix's SIV p27 antigen ELISA (62.5 pg/ml). Optimization of the reagents used in the AG3.0 antigen capture assay should further enhance its sensitivity.

The binding affinity of AG3.0 to SIVagm and SIVmac Gag purified from native virus was determined using SPR technology. SIVagmSab2 Gag was found to bind slowly to AG3.0, whereas the overall binding affinity was more similar to SIVmac than to SIVagmVer. In addition, the dissociation curve was markedly different from both SIVmac and SIVagmVer and indicated a very stable complex between SIVagmSab2 and AG3.0. The same results were obtained with two different Gag concentrations (data not shown). Although all three Gag proteins share the same minimal epitope, the binding structures could be influenced by residues flanking the SPRTLNA epitope, resulting in different tertiary structures of the whole Gag protein and therefore different binding affinities to AG3.0 (Table 2B). To our knowledge, crystallization of the SIV capsid protein, needed to address this hypothesis, has not yet been achieved. Finally, the AG3.0 monoclonal antibody RNA was sequenced and annotated to facilitate creating a human- or monkey-adapted monoclonal antibody specific

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for the SPRTLNA epitope. This would allow anti-capsid antibodies for modulation of SIV/HIV infection to be evaluated.

In summary, we generated three mouse monoclonal antibodies against SIVagmTYO-1 (Ver) Gag capsid, each with varying degrees of cross-reactivity to SIV/HIV. The broadest cross-reactivity was found with AG3.0, which recognized p24–27 from the three major primate lentivirus lineages (HIV-1/SIVcpz/SIVrcm, HIV-2/SIVsm/SIVmac, and SIVagm) and mapped to a conserved 7 amino acid motif in Gag, SPRTLNA (aa 156–162 of SIVagmTYO-1 Gag). An antigen capture assay was developed, which could detect as little as 100 pg/ml of p24 Gag.

MATERIALS AND METHODS

Monoclonal antibody production

BALB/c mice were immunized by intraperitoneal injection of 10 μg sucrose gradient purified SIVagm(TYO-1) in phosphate buffered saline (days 0 and 21) in addition to 10 μg of 1% Triton X-100-treated SIVagm(TYO-1) (day 14). The mice were sacrificed at day 24 and the spleen removed for fusion of splenic cells with SP-2 mouse myeloma cells. The hybridomas were grown in 96-well plates using DMEM (Life Technologies) supplemented with 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 20% fetal bovine serum, with 10 mM hypoxanthine and 2 μg/ml of azaserine (Sigma-Aldrich) for selection. Supernatant fluid (100 μl) from each well showing visible hybridoma growth was evaluated for reactivity to SIVagm(TYO-1) by whole virus ELISA and positive samples screened by western blotting. Clones of interest were subcloned twice. The isotype of each monoclonal antibody was determined with the Sigma ImmunoType™ Kit according to the manufacturer's instructions.

Virus purification

Culture supernatant fluid (5–10 liters) from SIVagm(TYO-1) infected MOLT4clone8 cells was concentrated 50–100 fold with a Minitan ultrafiltration system (Millipore) containing 5 filters with a 300,000 molecular weight pore size. The retentate (50–100 ml) was layered onto 20/70% discontinuous sucrose gradients, and the virus was recovered from the interface after centrifugation for 2 hours at 71,500 × g. The virus fraction was diluted 2× with TEN (0.05 M Tris-HCl pH 7.2, 0.15 M NaCl, and 1mM Ethylenediaminetetraacetic acid (EDTA)), layered over a 20–70% continuous sucrose gradient and centrifuged overnight at 71,500 × g. One milliliter fractions were assessed with a refractometer, the virus fractions (1.16–1.18 g/cm^3) diluted 5-fold with TEN buffer, and the virus pelleted by centrifugation at 71,500 × g for 2 hours at 4°C. The virus pellets were resuspended in a volume of 1.0 ml phosphate buffered saline (PBS), and the protein concentration determined using the Bio-Rad Protein Assay according to the manufacturer's instructions (Bio-Rad Life Sciences). The virus was further analyzed for purity by SDS-PAGE with the protein bands visualized by immunoblotting.

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Whole virus ELISA

Purified SIVagm(TYO-1) was diluted to 2 μg/ml in borate buffered saline (BBS) and used to coat Immulon II plates (Dynex Technologies Inc., Chantilly, VA) overnight at 4°C. Wells were blocked with 10% normal goat serum in BBS for 30 min. at 37°C, and the plates washed 3 times with 1% Tween20 in phosphate buffered saline. One hundred microliters of hybridoma supernatant fluid were then added, the plates incubated for 60 min. at 37°C, and washed three times prior to addition of biotinylated anti-mouse antibody. Binding was determined colorimetrically by incubation with streptavidinhorseradish peroxidase (Amersham) before washing and developing with 2,2'-Azinobis(3-ethylbenzthiazoline-sulfonic acid) (ABTS).

Cells and viruses

All human CD4+ T cell lines were maintained in complete medium consisting of RPMI 1640 supplemented with 15% fetal bovine serum (Life Technologies), HEPES, 2 mM L-glutamine, 50 μg/ml streptomycin and 50 U/ml of penicillin. SIVagm strains VER.KE.x.TYO1, GRI_677, SAB.SN.x.SAB1C, SAB.SN.x.SAB2, SAB.SN.x.SAB4, TAN.UG.x.TAN1, and HIV-2ST (Broussard et al., 2001; Fomsgaard et al., 1991; Jin et al., 1994; Kumar et al., 1990; Shibata et al., 1990; Soares et al., 1997) were grown in Molt4clone8 cells, SIVagm(Ver-1) (Jin et al., 1994) in SupT1 cells, SIVmac251 in Hut78 cells, and HIV-1/IIIB in Molt3 cells.

Western blot analysis

Western blotting was performed as previously described (Allan et al., 1991). Virus in culture supernatants taken from infected cell lines at 48–72 hours was purified through a 20% sucrose cushion, separated by SDS-polyacrylamide gel electrophoresis, and then blotted onto nitrocellulose sheets. The nitrocellulose paper was blocked with 3% bovine serum albumin and subsequently incubated with a 1:8 dilution of monoclonal antibody supernatant fluid or a 1:80 dilution of seropositive and seronegative controls. Viral proteins were detected using the streptavidin-biotin system (Amersham) with diaminobenzidine as the substrate for color development.

Radioimmunoprecipitation analysis

Radioimmunoprecipitation analysis (RIPA) of virus lysates was conducted as described previously (Allan et al., 1992). Infected cells were metabolically labeled for 16 hours with 35-S cysteine and methionine (Translabel, ICN Biomedicals, Inc., Costa Mesa, CA) at 1 mCi/10^7 cells in cysteine-methionine free RPMI 1640 with 15% FBS. The cells were resuspended in RIPA buffer (0.08 M Tris-HCl pH 7.4, 0.15 M NaCl) containing 0.1% SDS, 1% Triton X-100, and 1% deoxycholate with protease inhibitors (1.0 mM phenylmethyl sulfonyl fluoride (Pierce) and 2 μg/ml of leupeptin (Sigma-Aldrich)) and centrifuged at 100,000 × g to remove protein aggregates. Proteins were then immunoprecipitated with monoclonal antibodies bound to protein A sepharose. The complexes were washed 5 times with complete RIPA lysing buffer, the viral proteins solubilized by resuspension in electrophoresis sample buffer (0.08 M Tris-HCl pH 6.8, 2% SDS, 0.1 M dithiothreitol, 10% glycerol, and 1 mg/ml of bromophenol blue), and the viral proteins separated by 11.3%

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SDS-PAGE. Proteins were visualized by impregnating the gel with EnHance (DuPont NEN, Boston, MA) before drying and exposure to Kodak SB5 radiographic film (Eastman Kodak).

Epitope mapping

A panel of overlapping SIVmac Gag synthetic peptides (20-mers, 10 overlap; kindly provided by the European AIDS Reagent Programme, reference ARP714.1-22) were used in a standard ELISA for rough epitope mapping of the AG3.0 monoclonal antibody. For fine epitope mapping, a series of 13-mer synthetic peptides with an overlap of 12 aa covering the sequence GGNYVHLPLSPRTLNAWVKLIEEKK (SIVmac251 Gag 141–165) were synthesized directly onto a cellulose membrane (Jerini Bio Tools GmbH, Berlin, Germany). Briefly, the membrane was washed 3 times in Tris-buffered saline containing 0.05% Tween20 (T-TBS) and blocked overnight at 4°C with T-TBS plus 2% milk powder and 10% sucrose (blocking buffer). After washing 3 times with T-TBS, the membrane was incubated overnight at 4°C with a 1:50 dilution of purified AG3.0 mAb in blocking buffer. The membrane was then washed and incubated for one hour at room temperature with a 1:10,000 dilution of goat anti-mouse IgG peroxidase conjugate (Sigma-Aldrich). After three final washes, spots were visualized using the enhanced chemiluminescence kit (Amersham), followed by exposure to photographic film (RPN 2103H Hyperfilm, Amersham).

Antigen capture ELISA

Protein G sepharose column (Pharmacia) 4 Fast Flow (25 ml) was used to purify IgG from cell culture supernatants according to the manufacturer's instructions. For the antigen capture ELISA, 50 μl of a 13.5 μg/ml AG3.0 solution in distilled water were dried onto 96-well flat-bottom polystyrene assay plates (PRO BIND, BD Biosciences) overnight at 37°C. The next day, the monoclonal antibody layer was blocked with 100 μl PBS/2% nonfat milk powder for 1 hour and subsequently washed 3 times with PBS/0.05% Tween20. SIV/HIV supernatant or SIV/HIV infected cells were lysed with 0.2 % Tween20 for 30 min., 100 μl lysate plus 50 μl PBS/2% nonfat milk powder/0.05% Tween20 added to the AG3.0 coated wells, and the plates incubated overnight at 4°C and the next day for two hours at 37°C. The plates were washed 3 times with PBS/0.05% Tween20, 50 μl of specific anti-SIV plasma from an infected monkey or plasma pool from HIV-1 infected individuals, diluted 1:1,000 in PBS/2% nonfat milk powder/0.05% Tween20, were added to the wells, and the plates incubated at 37°C for 1 hour. The plates were then washed 5 times with PBS/0.05% Tween20 and under the stream of tab water for five seconds. Finally, 10 μg o-phenylenediaminedihydrochloride (Sigma-Aldrich) in 50 μl PBS pH 6.0 and 0.012 μl H_2O_2 were added as substrate, and the reaction stopped with 20 μl 2.5 M H_2SO_4. The optical density of each well was read at 492 nm using a microplate reader.

Immunostaining of cells

Flat bottom 96-well plates were incubated with 5 μg of poly-L-lysine (Sigma) in 100 μl of distilled water overnight at 4°C. Plates were washed twice with PBS, and 200 μl of cell suspension were added to the wells. Plates were incubated for 1 h at 37°C to allow cells to settle, and then the supernatant was removed. Plates were submerged in −20°C methanol and fixed for 30 min. at −20°C before carefully washing three times with PBS and blocking with 100 μl of PBS/2% nonfat milk powder for 1 h at room temperature. The blocking solution

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was then removed, and 50 μl of the appropriate anti-SIV/HIV plasma diluted in PBS/2% nonfat milk powder added for 1 h at 37°C. Wells were washed five times with PBS and incubated with 50 μl of 1:500 anti-human IgG (Sigma-Aldrich) in PBS/2% nonfat milk powder for 1 h at 37°C. Wells were then washed again five times with PBS and finally the substrate added (10 μg 3-amino-9-ethylcarbazol (Sigma-Aldrich), 2.5 μl dimethylformamide, 47.5 μl 20 mM sodium acetate, pH 5.0, and 0.025 μl H_2O_2 per well). After 10 to 20 min of incubation with the substrate, SIV/HIV infected cells were stained red. The wells were washed once with PBS, and the substrate reaction stopped with 20% glycerol.

Virus titrations

To determine the $TCID_{50}$ of virus stocks, supernatants were initially diluted 1:10 and then 11 dilutions steps with 1:3 dilutions were performed. Row 12 served as negative control (medium only). Fifty microliters of virus dilutions or media were added to 2×10^3 C8166 or Molt4clone8 cells in 8 replicates. The plates were incubated for 7 days, and the SIV/HIV replication was monitored by antigen capture assay or cellular immunostaining.

Surface plasmon resonance (SPR) analysis

To determine the binding affinity of Gag proteins derived from the different SIVs to the monoclonal AG3.0 mAb, samples were analyzed in duplicate with a BIAcore ×100 instrument (GE Healthcare) at 25°C in HBS-EP+ buffer (10 mM Hepes, 150 mM NaCl, 3 mM EDTA, 0.05% Surfactant P20, pH 7.4) using the mouse antibody capture kit (GE Healthcare). The final immobilization level of anti-mouse IgG antibody was 8,500 RU on both flow cells (Fc1 and Fc2) as recommended by the manufacturer. The antibody was captured at a final concentration of 20 μg/ml for 20 sec at a flow rate of 10 μl/min and a constant level of 500 RU and 120 RU for measuring SIV Gag binding. The different analytes were injected under the following conditions: The association/dissociation times for SIVagmVer Gag and SIVmac Gag were 720/1,200 sec and for SIVagmSab Gag 1,080/5,400 sec. Furthermore, association and dissociation curves were estimated for all 3 Gag proteins using an injection period of 1,080 sec and a dissociation time of 3,000 sec at a flow rate of 5 μl/min. The regeneration buffer consisted of 10 mM glycine/HCl, pH 1.7. The apparent kD values were determined using the BiaEvaluation software (GE Healthcare). All three Gag preparations (SIVagmVer, SIVagmSab, and SIVmac) were produced from native purified virus.

Sequencing of the AG3.0 heavy and light chain variable regions

RNA was isolated from 1×10^7 AG3.0 hybridoma cells using the RNeasy plus kit (Qiagen) according to the manufacturer's instructions and eluted in 30 μl DEPC-treated H_2O. cDNA was generated with the Cloned AMV cDNA synthesis kit (Invitrogen) using oligodT primers and a temperature of 50°C for 1h. Amplification of variable region fragments was performed with 1.25U Pfu DNA polymerase (Fermentas) in the supplied PCR buffer containing 2 mM $MgSO_4$, 0.2 mM dNTPs, and 25 pmol of each primer per 50 μl reaction. Forward and reverse primers from a mouse IgG library primer set (Progen) were used in all possible combinations for light and heavy chain fragments. Amplification cycles were as follows: initial denaturation for 5 min at 95°C, followed by 35 cycles of 30 sec/95°C, 30sec/55°C,

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60sec/72°C and a final elongation of 10 min at 72°C. Amplified PCR products of approximately 400kb were purified using a PCR purification kit (Fermentas) or, if more than one product was generated, separated on a 1.5% agarose gel and subsequently extracted using a gel extraction kit (Qiagen). Sequencing was performed on an ABI 3500DX sequencer. Sequences obtained were analysed using Vbase2 (http://www.vbase2.org) to identify the complementarity determining regions of the heavy and light chain variable fragments.

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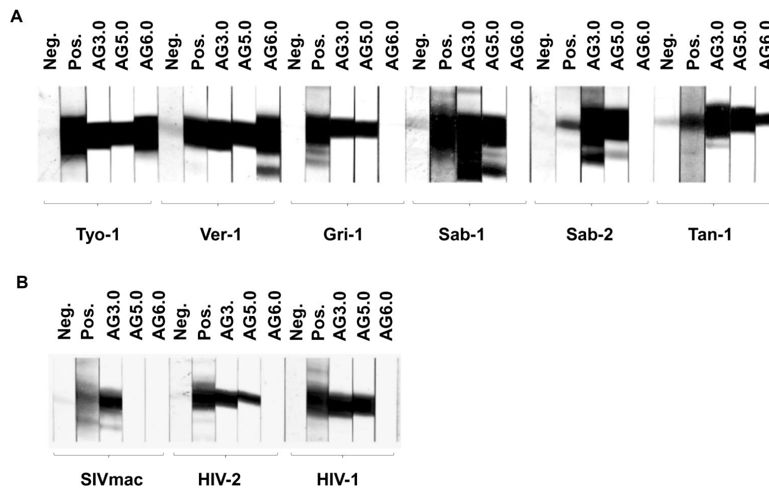

Figure 1. Western blot analysis

Reactivity of different Gag-specific monoclonal antibodies against various purified isolates of SIV and HIV in western blot performed as described in the materials and methods. A: Isolates from the four subspecies of African green monkeys. B: Isolates from rhesus macaques and humans. Neg: Negative control sera from uninfected individuals of the relevant species. Pos: Positive polyclonal control sera from individuals of the relevant species infected with the corresponding virus.

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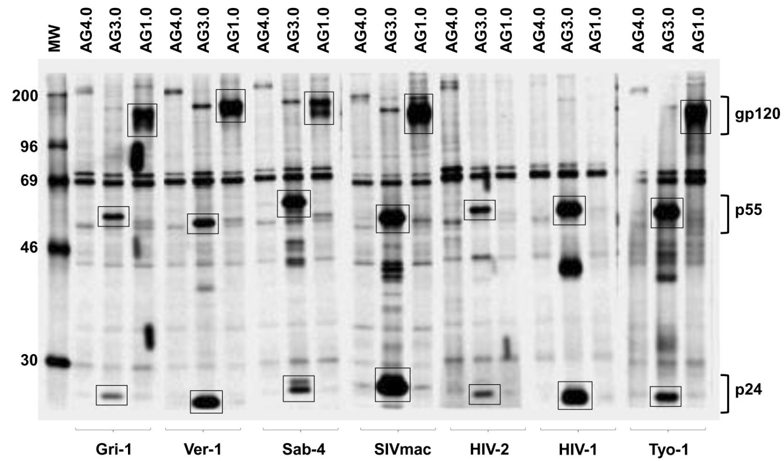

Figure 2. Radioimmunoprecipitation analysis

Binding of the AG3.0 monoclonal antibody to radiolabeled Gag protein from cells infected with various SIV and HIV isolates. The monoclonal antibodies AG1.0, recognizing gp120 from SIVagm and SIVmac, and AG4.0, recognizing a cell protein, were included as controls. Bands showing reactivity with p24–27, p55, and gp120 are marked with a box.

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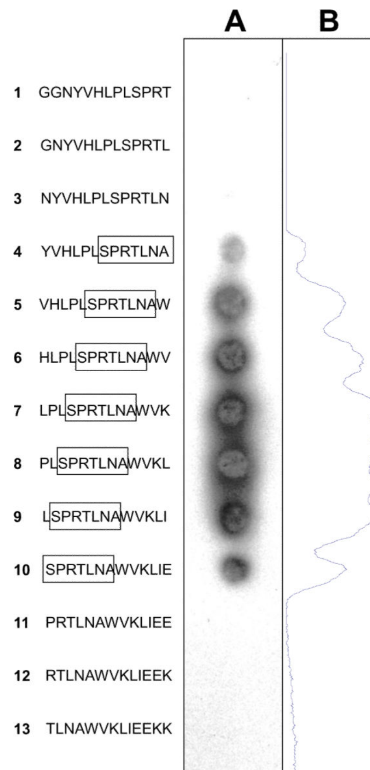

Figure 3. Fine epitope mapping of SIVmac Gag

Binding of the AG3.0 monoclonal antibody to synthetic peptides spanning the putative epitope in Gag within the sequence GGNYVHLPLSPRTLNAWVKLIEEKK (SIVmac251 Gag aa 141–165). The peptides, 13-mers with a 12 amino acid overlap were synthesized directly onto a cellulose membrane and binding of AG3.0 visualized by enhanced chemiluminescence (A). A density plot along the membrane was carried out to aid analysis or reactivity (B).

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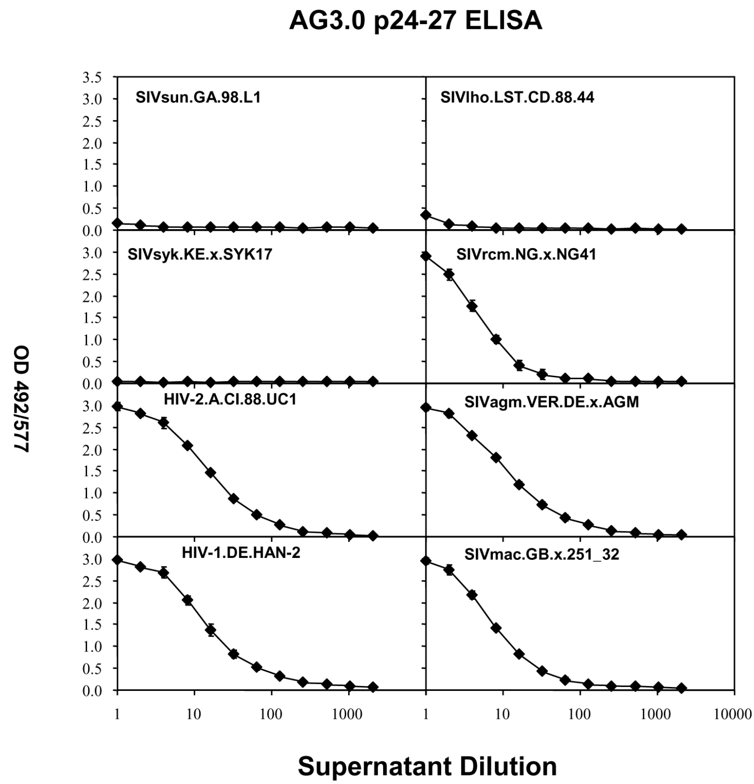
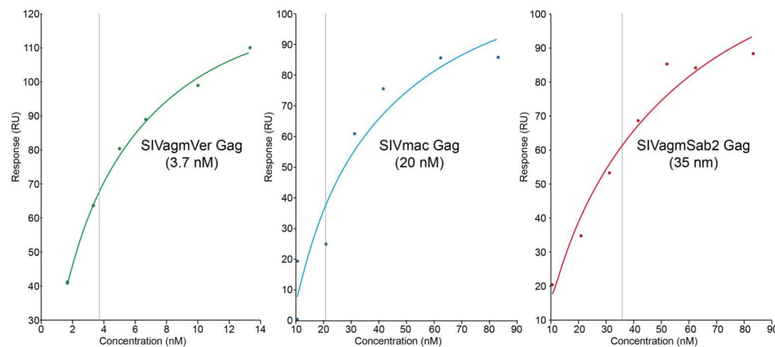


Figure 4. Antigen capture ELISA using various isolates

Supernatants from cells infected with various isolates of SIV and HIV were tested at different dilutions for reactivity in the AG3.0 based antigen capture assay as described in the material and methods.



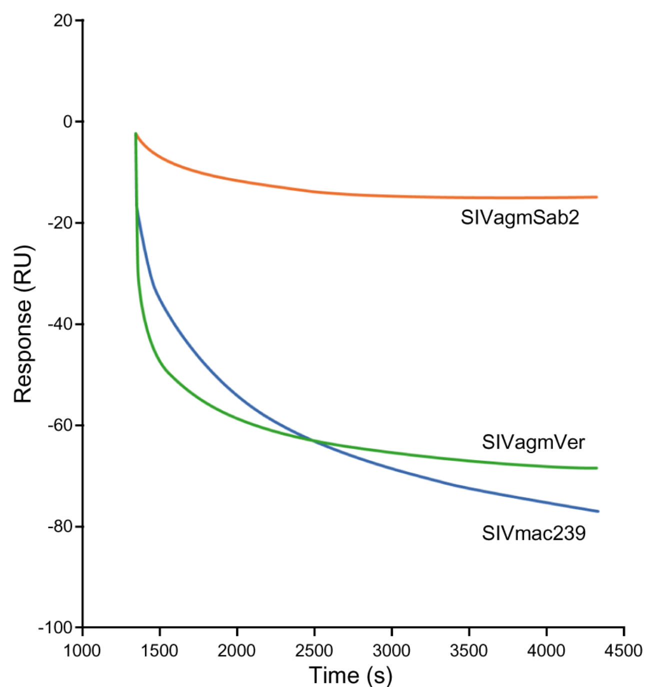


Figure 5. Surface plasmon resonance (SPR) analysis

A: Binding affinity profiles of Gag proteins derived from different SIVs to AG3.0 measured by BIAcore as described in the materials and methods. Sensor chips coated in AG3.0 were exposed to purified preparations of the different Gag proteins at various concentrations. Binding was measured as a change in RU at equilibrium. The apparent kD values were determined using the BiaEvaluation software (GE Healthcare). B: Dissociation curves for the different Gag proteins (1.5 μg/ml) using an injection period of 1,080 sec, a dissociation time of 3,000 sec, and a flow rate of 5 μl/min.

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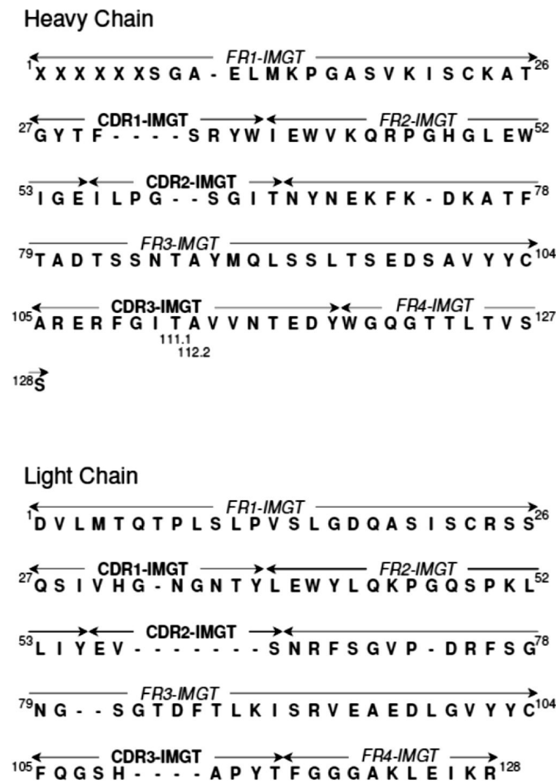

Figure 6. Amino acid sequences of the heavy and light chains of the AG3.0 monoclonal antibody

The four conserved framework regions (FR1–FR4) and the three hypervariable complementarity determining regions (CDR1–CDR3) are displayed according to the IMGT nomenclature (Lefranc, 2007). Unoccupied amino acid positions are marked with a dash (–). X = positions in the conserved FR1 region of the heavy chain, at which the identity of the amino acid could not be unequivocally determined from the sequence.

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Table 1

Rough epitope mapping of AG3.0

SIVmac251 Gag Capsid Sequence					
001	PVQQIGGNYV	**HLPLSPRTLN**	**AWVKLIEEKK**	FGAEVVPGFQ	ALSEGCTPYD
051	INQMLNCVGD	HQAAMQIIRD	IINEEAADWD	LQHPQPAPQQ	GQLREPSGSD
101	IAGTTSSVDE	QIQWMYRQQN	PIPVGNIYRR	WIQLGLQKCV	RMYNPTNILD
151	VKQGPKEPFQ	SYVDRFYKSL	RAEQTDAAVK	NWMTQTLLIQ	NANPDCKLVL
201	KLGLVNPTLE	EMLTACQGVG	GPGQKARL		

Peptide[a]	Sequence	Mean OD	StDev
1	01–20	0.01	*0.00*
2	**11–30**	**0.37**	***0.05***
3	21–40	0.01	*0.00*
4	31–50	0.01	*0.00*
5	41–60	0.01	*0.00*
6	51–70	0.01	*0.00*
7	61–80	0.01	*0.00*
8	71–90	0.01	*0.00*
9	81–100	0.01	*0.00*
10	91–110	0.01	*0.00*
11	101–120	0.01	*0.00*
12	111–130	0.01	*0.00*
13	121–140	0.01	*0.00*
14	131–150	0.01	*0.00*
15	141–160	0.01	*0.00*
16	151–170	0.01	*0.00*
17	161–180	0.01	*0.00*
18	171–190	0.01	*0.00*
19	181–200	0.01	*0.00*
20	191–210	0.01	*0.00*
21	201–220	0.01	*0.00*
22	211–228	0.01	*0.00*

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[a]Peptides were ordered from the European AIDS Reagent Programme, reference ARP714.1–22; peptide 15, 16, and 18 have an additional N-terminal cysteine)

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Table 2A

Alignment of Gag capsid N-terminus (B.FR.83.HXB2_LAI_IIIB aa 160–176)

HIV-1/SIVcpz	GenBank Accession no.	Sequence
A1.RW.92.92RW008	AB253421	IYQSM**SPRTLNA**WVKVI
A2.CD.97.97CDKTB48	AF286238	VHQAV**SPRTLNA**WVKVV
B.US.98.1058_11	AY331295	VHQAI**SPRTLNA**WVKVI
C.IN.95.95IN21068	AF067155	VHQAI**SPRTLNA**WVKVI
D.CD.83.ELI	K03454	VHQAI**SPRTLNA**WVKVI
F1.BE.93.VI850	AF077336	VHQSL**SPRTLNA**WVKVI
F2.CM.95.MP255	AJ249236	VHQAL**SPRTLNA**WVKVI
G.NG.92.92NG083	U88826	IHQAI**SPRTLNA**WVKVV
H.CF.90.056	AF005496	VHQAI**SPRTLNA**WVKVV
J.SE.93.SE7887	AF082394	VHQAL**SPRTLNA**WVKVI
K.CD.97.EQTB11C	AJ249235	VHQAL**SPRTLNA**WVKVI
01_AE.TH.90.CM240	U54771	AHQPL**SPRTLNA**WVKVV
O.BE.87.ANT70	L20587	VHQAI**SPRTLNA**WVKAV
N.CM.02.DJO0131	AY532635	IHQPL**TPRTLNA**WVKVI
CPZ.CD.90.ANT	U42720	RHQPL**TPRTLNA**WVKCV
CPZ.CM.05.SIVcpzMT145	DQ373066	THNPI**SPRTLNA**WVKAV
HIV-2/SIVsmm	**GenBank Accession no.**	**Sequence**
A.CI.88.UC2	U38293	VHVPL**SPRTLNA**WVKLI
B.CI.88.UC1	L07625	VHMPL**SPRTLNA**WVKLV
MAC.US.x.251_1A11	M76764	VHLPL**SPRTLNA**WVKLI
MNE.US.82.MNE_8	M32741	THLPL**SPRTLNA**WVKLI
SMM.US.x.SME543	U72748	VHLPL**SPRTLNA**WVKLV
STM.US.x.STM	M83293	VHLPL**SPRTLNA**WVKLV
Other SIV	**GenBank Accession no.**	**Sequence**
COL.CM.x.CGU1	AF301156	VHQPL**SPRTLGA**WVKCV
DEB.CM.99.CM40	AY523865	VHMPL**SPRIVKT**WINAV
DEN.CD.x.CD1	AJ580407	QHQAL**STRVLKT**WIKEV
DRL.x.x.FAO	AY159321	VHQAI**SPRTLNA**WVKVI
GOR.CM.04.SIVgorCP684con	FJ424871	VHQPL**SPRTLNA**WVKAV
GRV.ET.x.GRI_677	M66437	VHQPL**SPRTLNA**WVKCV
GSN.CM.99.CN166	AF468659	QHQAL**NSRILKT**WVSIV
LST.KE.x.lho7	AF075269	VHTPL**SPRTIQT**WVKIV
MND-1.GA.x.MNDGB1	M27470	QYTPI**SPRIIQT**WVKTV
MND-2.CM.98.CM16	AF367411	VHQGI**SPRTLNA**WVKCI
MON.CM.99.L1	AY340701	QHQAV**EPRLLKT**WVQVI
MUS-1.CM.01.1085	AY340700	QHQAL**NARILKT**WVSIV
MUS-2.CM.01.CM1246	EF070329	QHQSL**SPRLLKT**WVSTV
OLC.CI.97.97CI12	FM165200	EHVPL**ATRTLQS**WVKAL
RCM.NG.x.NG411	AF349680	EHQPI**SPRTLNA**WVKVV
SAB.SN.x.SAB1C	U04005	VHQPL**SPRTLNA**WVKVI
SUN.GA.98.L14	AF131870	IHQPL**SPRTVQT**WVKIV
SYK.KE.x.KE51	AY523867	IHVGV**NTRTLKT**WVEAV
TAL.CM.00.266	AY655744	QHQAL**SPRLLKT**WIATI
TAN.UG.x.TAN1	U58991	VHTPL**SPRTLNA**WVKTV
VER.KE.x.9063	L40990	VHVPL**SPRTLNA**WVKAV
WRC.CI.97.97CI14	AM937062	QWDPL**SPRTIQT**WVKHI

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Table 2B

Alignment of amino acid sequence adjacent to SPRTLNA

```
VER.KE.x.TYO1   AQQQGNAWVHVPLSPRTLNAWVKAVEEKKFGAEIVPM
VER.DE.x.AGM3   --------I----SPRTLNA-----------------
SAB.SN.x.SAB1C  IVSVN-Q---Q--SPRTLNA---VI-----S--V---
MAC.US.x.239    V--I-GNY--L--SPRTLNA---LI--------V--G
```

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Table 3

Comparison of $TCID_{50}$/ml calculations using AG3.0 antigen capture assay or HIV/SIV-specific immunostaining

		Log_{10} Titer ($TCID_{50}$/ml)	
		Endpoint analysis	
Virus	**Titrated in**	**AG3.0 antigen capture assay of supernatants**	**HIV/SIV-specific immunostaining of cells**
SHIV 89.6	C8166 cells	5.52	5.64
HIV-2 UC3	C8166 cells	4.99	4.79
SIVsmmPBj 1.9	C8166 cells	4.87	5.04
SIVagm3	C8166 cells	6.54	6.48
SIVagm3	Molt4clone8 cells	4.81	4.63
SIVmac 32H	C8166 cells	6.71	6.71
HIV-1 LAI	C8166 cells	4.69	4.39