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Production of a recombinant monoclonal antibody to Herpes Simplex Virus glycoprotein D for immunoaffinity purification of tagged proteins

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Abstract

We have developed a stable Chinese Hamster Ovary (CHO) cell line for the production of a recombinant monoclonal antibody (mAb) to a short protein sequence derived from the N-terminus of human herpes simplex virus type 1 glycoprotein D (HSV-1 gD). The antibody (designated r34.1) provides a useful tool for the immunoaffinity purification of HSV-1 gD tagged proteins, and provides a generic purification system by which various proteins and peptides can be purified.

Recombinant 34.1 was assembled using cDNA derived from a HSV-1 gD specific murine hybridoma engineered to encode a full-length IgG molecule. Antibody expression cassettes were transfected into CHO-S cells, and a stable cell-line expressing up to 500 mg/L of antibody, isolated. Affinity purified r34.1 exhibited nanomolar affinity for its cognate ligand, and is stable throughout multiple cycles of immunoaffinity purification involving ligand binding at neutral pH, followed by acid elution. The HSV-1 gD tag expression and purification strategy has been used to enhance the secretion and purification of several vaccine immunogens including HIV envelope protein rgp120s, but the protocol has potential for generic application.

Keywords

HIV-1 vaccine; CHO cells; Antibodies; Immunoaffinity chromatography

1. Introduction

Immunoaffinity purification of proteins tagged with short immunogenic sequences such as polyhistidine, c-myc and FLAG (Munro and Pelham, 1984; Evan et al., 1985; Hochuli et al., 1987; Hopp et al., 1988) have long been used as a convenient method to rapidly purify novel recombinant proteins. Early studies demonstrated improved recombinant gp120 expression

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when the endogenous HIV envelope secretory peptide was replaced with a HSV-1 gD signal peptide, and the first 11 amino acids of the mature form of gp120 were replaced with a 27 amino acid tag-sequence from the N-terminus of HSV-1 gD (Lasky et al., 1986; Berman et al., 1988). When the HSV-1 secretory signal/tag epitope sequences are incorporated into a protein, the signal peptide is removed during protein biosynthesis, and the resulting fully-processed protein possesses the N-terminal HSV-1 gD tag KYALADASLKMADPNPFRGKDLPVLDQ. Incorporation of the tag enabled the development of a generic immumoaffinity purification process by which difficult to express rgp120's from multiple genetic clades could be purified. Recombinant gp120s from the IIIB, MN, GNE8 and A244 strains of HIV-1 used in numerous clinical trials were manufactured as HSV-1-gD flag fusion proteins to facilitate expression and purification (Berman et al., 1990; Schwartz et al., 1993; Berman et al., 1994; Belshe et al., 1994; Berman et al., 1996; Berman et al., 1997; Gorse et al., 1999; Berman, 1998; Berman et al., 1999; Pitisuttithum et al., 2004; Flynn et al., 2005; Pitisuttithum et al., 2006; Rerks-Ngarm et al., 2009; Pitisuttithum et al., 2014), but to date, antibodies used in the purification process (Berman, 2015) have not been made widely available. The N-terminal region of HSV-1 gD has biological significance as it contains a fourteen amino acid receptor binding ligand for the herpes virus entry mediator A, (Carfí et al., 2001; Connolly et al., 2003) and is highly immunogenic with multiple overlapping T-cell and B-cell epitopes (Dietzschold et al., 1984; Heber-Katz et al., 1985; Heber-Katz et al., 1988; Cairns et al., 2015). It is also a target for human HSV-1 and HSV-2 neutralizing antibodies (Cairns et al., 2015) and multiple murine monoclonal antibodies (Dietzschold et al., 1984; Cohen et al., 1984; Eisenberg et al., 1985). In clinical trials, vaccination with the AIDSVAX B/E antigens induced antibodies to both HIV and the HSV-1 gD peptide (Tomaras et al., 2013; Gilbert et al., 2017; Wang et al., 2017). While deletion of the first 11 amino acids from the mature form of gp120 prevented rgp120 dimerization, and improved the ability of the AIDSVAX B/E vaccine to induce specific types of HIV antibodies compared with wildtype gp120, insertion of the HSV-1 gD sequences did not impair HIV antibody production (Alam et al., 2013).

Here we describe the production of a recombinant murine HSV-1 gD antibody from a stable CHO-S cell line that is generally applicable for affinity purification of gD tagged proteins. The parental CHO-S cell line is a stable aneuploid cell line established from the ovary of an adult Chinese hamster (Tjio and Puck, 1958), distinguished as a separate subclone from the common CHO K1 cell line. Its history and stability have been extensively described (D'Anna et al., 1996; Deaven and Petersen, 1973; D'Anna et al., 1997).

2. Methods

2.1. Cell lines and culture conditions

Murine P3×63Ag8.653 cells were obtained from the American Type Culture Collection (ATCC). CHO-S cells were purchased from Thermo Fisher, Life Technologies (Carlsbad, CA). Immortalized hybridomas were prepared by fusion of hyper-immune spleen cells from immunized animals and screened a previous study (Morales et al., 2014), to select a wild type gD binding peptide sequence (KYALADASLKMADPNPFRGKDLPVLDQLLE) designated 34.1. The peptide includes a three amino acid spacer sequence included in the

fusion protein (underlined). Monoclonal antibodies were raised according to the guidelines of the Animal Welfare Act, and the protocol approved by the Animal Care and Use Committee at the University of California at Santa Cruz. Static cultures were maintained in cell culture dishes and grown in a Sanyo incubator (Sanyo, Moriguchi, Osaka, Japan) at 37 °C, 8% CO_2, 85% humidity. For protein production, hybridomas were batch-cultured semi-adherent in DMEM supplemented with 5% FBS (Thermo Fisher, Life Technologies, Carlsbad, CA). Tissue culture supernatant was harvested by centrifugation flowed by 0.2 μm filtration (Thermo Fisher, Hampton NH) before affinity purification on a HiTrap protein G column (GE Healthcare, Chicago, Chicago IL). CHO-S cells were maintained in CD-CHO medium (Glutamax (Thermo Fisher, Life Technologies, Carlsbad, CA) supplemented with 8 mM Glutamax. Suspension-adapted cells were maintained in shake flasks (Corning, Corning NY) using a Kuhner ISF1-X shaker incubator (Kuhner, Birsfelden, Switzerland). For normal cell propagation, shake flasks cultures were maintained at 37 °C, 8% CO_2, 85% humidity and a rotation speed of 125 rpm. All cell counts were performed using a TC20™ automated cell counter (BioRad, Hercules, CA) with viability determined by trypan blue (Thermo Fisher, Life Technologies, Carlsbad, CA) exclusion.

2.2. Sequencing and molecular cloning

Total RNA was extracted from 1×10^7 34.1 hybridoma cells with RNAEasyPlus (Qiagen Hilden Germany) and cDNA synthesized by reverse-transcription of template-primed with oligo-dT (Thermo Fisher Scientific Waltham, MA) using M-MuLV reverse-transcriptase (New England Biolabs Ipswich, MA) according to the manufacturers protocol. The light chain hypervariable region amplicon was obtained by Rapid Amplification of cDNA polymerase chain reaction (5' RACE PCR) (Ruberti et al., 1994; Doenecke et al., 1997) of total RNA followed by direct Sanger sequencing of the PCR product at the UC Berkeley Sequencing Facility. Sequence alignment of framework regions demonstrated a light chain kappa sequence. In order to express a complete light chain, a custom synthetic gene was designed to code a murine kappa secretory signal, the hyper-variable 34.1 kappa sequence and the constant region of a murine Ig kappa antibody (Svasti and Milstein, 1972) flanked by EcoR1 and Not1 restriction sites for cloning into an expression vector. The heavy chain hypervariable sequence could not be obtained using the RACE PCR protocol. Instead, a single pair of degenerate primers (Fields et al., 2013) was used to produce the hypervariable heavy chain amplicon which was ligated into a TA shuttle vector pMD20 (Clontech Mountain View, CA) using standard molecular techniques, for sequencing. The resulting hyper-variable region sequences were aligned against the NCBI IgG database (http://www.ncbi.nlm.nih.gov/igblast/) to determine germline gene usage.

A full-length heavy chain antibody was engineered to consist of a murine kappa secretory signal, the hyper-variable 34.1 region sequence and a murine IgG_1 heavy chain Fc sequence (Honjo et al., 1979). Restriction enzyme sites (EcoR1 and XBa1) were included at the 5′ and 3′ ends of the gene respectively, and a Kpn1 site at the Fc junction. Synthetic genes were purchased from Thermo Fisher Life Technologies (Carlsbad CA) and ligated *via* the restriction sites EcoR1 and XBa1 into a pCDNA3.1 (Thermo Fisher Life Technologies Carlsbad CA) derived vector, to construct two independent CMV transcription unit vectors. Plasmid DNA was prepared using the endotoxin free Qiagen Giga Prep purification kit

(Qiagen, Hilden, Germany) and linearized by digestion with Pvu1 (New England Biolabs, Ipswich, MA) prior to electroporation.

2.3. Selection of a CHO-S cell line with stable high-level expression of recombinant 34.1 mAb

Light and heavy chain expression cassettes vectors were transfected into CHO-S cells at a ratio of 2:1 by electroporation using a Maxcyte STX, (MaxCyte Inc., Gaithersburg, MD) according to the manufacturer's instructions. Instead of using limiting dilution to select single cell clones, cells were seeded in methylcellulose CHO-Growth A, containing L-glutamine (Molecular Devices, Sunnyvale, CA), 500 μg/mL of G418 (Thermo Fisher Life Technologies, Carlsbad, CA) and 10 μg/mL of clone detect anti-murine FITC IgG (H and L) conjugate (Molecular Devices, Sunnyvale, CA) in 6-well plates (Greiner Kremsmünster, Austria). The plates were incubated in static culture for 13 days. Clones were then imaged and selected using the ClonePix2 robot. Before each procedure to isolate clones, the robot was sanitized and calibrated according to the manufacturer's instructions to ensure that the selected colonies were accurately isolated, and free from contamination. The plates were then imaged under white light, and also under fluorescence excitation (490/525 nm). Both images were superimposed to reveal an immunoprecipitin "halo" around a proportion of colonies. The colonies were then sorted according to rank of exterior fluorescent intensity "halo" (Lee et al., 2006) and default instrument parameters designed to select well separated compact colonies and exclude non-viable, and irregular ones. Forty-six individual clones with high exterior fluorescent intensity were aspirated with micro-pins controlled by the ClonePix2 system, and dispersed automatically in a 96 well plate (Greiner Kremsmünster, Austria) containing 100 μL of growth media in CD-CHO media supplemented with 8 mM Glutamax and 500 μg/mL G418/well. The selected clones could then be transferred for downstream applications.

Cultures were assayed for HSV-1 gD specific antibody production, then expanded into shake flasks culturing as CHO-S cells with the exception of adding G418 at a maintenance dose of 250 μg/mL. To evaluate stability, cells were continuously passaged and cryopreserved at regular intervals to evaluate antibody production over time. On completion of twenty-four passages, the cells were thawed and assayed for protein production.

2.4. Protein production

A single clone (D8) was scaled up for a 1.5 L (in duplicate) protein production experiment in Thomson 5 L flasks (VWR Radnor, PA). Cells were cultured in CD-OPTI CHO medium (Thermo Fisher Life Technologies, Carlsbad, CA) essentially as for transient antibody production (Steger et al., 2015) that is by growth to a density of 1×10^7 cells/mL under standard conditions, then dropping the temperature to 32 °C, supplementing with 1 mM sodium butyrate, and feeding daily with Maxcyte CHO A Feed (0.5% Yeastolate (BD Franklin Lakes NJ, 2.5% CHO-CD Efficient Feed A, 0.25 mM Glutamax, 2 g/L Glucose (Sigma-Aldrich St. Louis, MO). Supernatant was harvested by pelleting the cells at 250 g for 30 min followed by pre-filtration through Nalgene™ Glass Pre-filters (Thermo Scientific Waltham, MA) and 0.45 μm SFCA filtration Nalgene (Thermo Scientific Waltham, MA), then stored frozen at −20 °C before chromatography purification.

2.5. Antibody specificity determination

Antibody binding to HSV-1 gD peptides was measured by a fluorescent immunoassay (FIA). Fluotrac high binding 96 well plates (Griener Bio-One Kremsmünster, Austria) were coated with 4 μg/mL HSV-gD peptides overnight in PBS, then blocked with 1%BSA/PBS 0.05% tween for 2 h. Three-fold serial dilutions of antibody were added to each well followed by Alexa 488 labelled polyclonal anti-species antibody (Jackson ImmunoResearch, West Grove PA) at a 1/5000 dilution. Incubations were performed for 90 min at room temperature followed by a 4× wash in PBS 0.05% tween buffer unless otherwise noted. Absorbance was read using an EnVision Multilabel Plate Reader (PerkinElmer, Inc. Waltham, MA) using a FITC 353 emission filter and FITC 485 excitation filter, and plotted on Graph Pad Prism 6 for Mac., GraphPad Software, La Jolla, *CA.*

Western blot was performed on dithiothreitol (DTT) reduced or nonreduced tissue culture supernatant (1 μL), or purified protein (50 ng) following electrophoresed on a 4–12% NuPage PAGE SDS gel, in MES buffer (Thermo Scientific Waltham, MA). Protein was transferred to a PDVF membrane using the iBlot 2 Dry Blotting System. The membrane was blocked for 1 h in 5% MILK/PBS then probed for 1 h at room temperature with agitation, with goat anti-mouse H and L chain specific horseradish peroxidase conjugated antibody (Jackson ImmunoResearch, West Grove, PA) at a concentration of 1 μg/mL in 5% milk PBS, and washed three times for 10 min each wash using 100 mL of 0.05%Tween/PBS. The membrane was developed using WesternBright ECL kit (Advanta Menlo Park, CA) and visualized using an Innotech FluoChem2 system (Genetic Technologies Grover, MO).

2.6. Binding affinity of recombinant 34.1 for to rgD-gp120

Kinetic experiments were conducted on a Biacore X100+ instrument (GE Healthcare, Chicago IL.) capturing r34.1 with an anti-murine Fc antibody covalently attached to a CM5 sensor chip at 100–150 RUs. This was accomplished by flowing r34.1(1 μg/mL) over the chip for 30–60 s at a flow rate of 10 μL/min. Then a dilution series of gD tagged recombinant gp120 (analyte) from 100 to 1 μM, was passed over the chip to generate binding affinity data. The biosensor surface was regenerated with a 30s pulse of 10 mM Glycine pH 1.7 between injections (GE Healthcare, Chicago, IL.) BR-1008–38). All sensorgram data were globally fit to a 1:1 biomolecular binding model that included a mass transport term and processed using Biacore Evaluation software X100+ version 2.0.1 (GE Healthcare Chicago, IL.) to determine k_a, k_d, and KD. Chi2 values < 1 and Rmax ≤100 were established as quality cut offs for acceptable data.

2.7. Anti-HSV-1 gD tag affinity column preparation and stability and quality testing

Recombinant 34.1 was coupled to Aminolink beads (Thermo Fisher Scientific, Waltham MA) according to the manufacturers instructions. Five mL Omnifit (Sigma Aldrich St. Louis MO.) columns were prepared for small-scale protein purification. For stability testing, multiple cycles of binding and elution of rgD-gp120 from the MGAT1$^-$ A244_N332 line (O'Rourke et al., 2018) were completed. Each cycle compared binding of 1 mg of rgD-gp120 per mg of antibody coupled beads using an ACTA column chromatography system (GE Healthcare Chicago IL). The binding and elution conditions were as follows; protein was captured from supernatant in 0.5 M NaCl, 0.1 M TMAC, 50 mM Tris, pH = 7.4, after a

ten column volume wash with binding buffer, bound protein was eluted with 0.1 M NaOAc, pH = 3.0 before neutralizing with 1 M Tris-HCL pH 8.00, and concentration buffer exchange with a 30,000kD cutoff Amicon filter (Millipore, Burlington MA). Protein purified using the r34.1 affinity column was tested for CD4-IgG binding by direct binding ELISA as previously described (Morales et al., 2014; Doran et al., 2014) replacing bN-mAbs with the entry inhibitor CD4-IgG Capon (Capon et al., 1989).

3. Results and discussion

Recombinant gp120s, including Env proteins from the IIIB and A244 strains of HIV-1 used in numerous clinical trials, were manufactured as HSV-1-gD flag fusion proteins to facilitate expression and purification. These proteins were purified by a generic process that involved initial immunoaffinity purification, followed by a size exclusion chromatography polishing step. The CRF01AE A244 rgD-gp120 and IIIB clinical trial proteins were produced using a cGMP compliant affinity chromatography resin containing anti-gD monoclonal (5B6) mapping to the N-terminus of HSV-gD (Berman, 2015). The 5B6 antibody has subsequently been used to purify numerous rgp120s (Yu et al., 2012; Doran et al., 2018), including clade C viruses (Smith et al., 2010) and domain-fragments of gp120 (Morales et al., 2014; Nakamura et al., 1993; Nakamura et al., 2012) that were tested as immunogens, and used in mAb mapping studies. Since this antibody is not widely available, we produced an antibody with similar antigenic specificity to 5B6, similarly compatible with a HSV-1 gD tag purification system. HSV-1 gD is highly immunogenic, and multiple anti-gD antibodies have been described. Indeed, a robust anti-HSV gD response was raised during the RV144 clinical trial, which resulted in isolation of anti-gD monoclonal antibodies (Wang et al., 2017) and a follow up-study to determine if the response might protect against HSV-2 infection, was carried out (Gilbert et al., 2017). We previously reported selection of a murine hybridoma raised against a gD-tagged rgp140 that bound the wild type N terminal HSV-1 gD peptide sequence for use in immunoassays (Morales et al., 2014), but despite multiple sub-cloning attempts, the line was unstable in culture. Sequencing hyper-variable region light and heavy chain cDNA from the hybridoma, we expressed a recombinant IgG_1 using a stable transgenic CHO-S cell line. The recombinant antibody was purified from culture supernatant by standard chromatography methods, then covalently coupled to sepharose beads. Many monoclonal antibodies are acid labile, but r34.1 coupled beads were demonstrated to have minimal loss of affinity for ligand through multiple rounds of acid incubation followed by regeneration, a key requirement for an immunoaffinity substrate for a tag expression and purification system. Recombinant tagged envelope proteins rgD-gp120's purified on an r34.1 affinity column retain functional conformation as determined by the ability to bind the CD4 receptor.

3.1. Antibody reconstruction

Detailed sequence analysis revealed that the heavy chain hyper variable specificity determination sequences were quite short (GenBank Accession numbers MH813474 and MH813474). The heavy chain CDR1 and CDR2 are both eight amino acids long, and the CDR3 is 7 amino acids long. The light chain hyper-variable CDR1 was eleven amino acids long, the CDR2 three amino acids, and the CDR3 nine amino acids long. The highest

homology matches in the IGBLAST database (Ye et al., 2013) were obtained against murine germline kappa-chain genes IGKV1–117*01, IGKJ1*01, and IGKJ1*02, and murine heavy chain germline IGHV1–2*02, IGHD6–13*01, and IGHJ1*01 genes. Aligning the framework regions, it was possible to design synthetic DNA sequences to re-engineer the full-length light and heavy chains from available data (Table 1).

Although the original 34.1 mAb was determined to be isotype IgG_1, the antibody was reconstructed as an IgG_1 with three rather than two, inter-heavy chain disulfide bonds. Genes were expressed on two separate plasmids under the control of a CMV mammalian expression cassette based on the pcDNA3.1 vector (Thermo Fisher Life technologies, Carlsbad), modified by the addition of a chimeric intron between the start of transcription and the start of translation.

3.2. Stable CHO-S r34.1 clone selection

Stable G418 resistant CHO-S lines were generated using a ClonePix2 (Molecular devices Sunnyvale, CA) robot, following electroporation of CHO-S cells with linearized vector light and heavy chain, and selection using G418. Colonies were visualized using white light. When illuminated by 490 nm wavelength light, colonies actively secreting IgG have a green precipitin "halo" visible at 525 nm (Fig. 1 A and B). The mean exterior fluorescence intensity of the halos was ranked, and those with the highest intensity (forty-two colonies) robotically picked. Selected colonies were propagated and assayed by ELISA and western blot. Of these, a single clone (D8) in Fig. 1C, was chosen based on a combination of expression, secreted light: heavy chain ratio, and cell line growth, and designated CHO-S r34.

The cell line CHO-S r34.1 (D8) was and assayed for protein production (Fig. 2) by seeding duplicate 1500 mL cultures at 5×10^5 cells/mL at 37 °C. After four days of growth, sodium butyrate was added (1 mM), and the temperature dropped to 32 °C. The culture was fed daily as previously described. A viable cell density of between 0.5 and 1×10^7 cells /mL was maintained for 13 days (Fig. 2A) with a percentage viability of > 80% (Fig. 2B). The culture yielded 360–380 mg/L of antibody after 13 days (Fig. 2C). Antibody was clearly the dominant protein accumulating in conditioned media from day 7 of culture (Fig. 2D), facilitating subsequent protein purification.

Protein productivity fell from in the region of 480 mg to 350 mg of antibody per liter determined by quantitative immunoassay over the course of 24 passages (Fig. 3A), comparable to that observed previously (Svasti and Milstein, 1972) for CMV driven protein expression in CHO-S cells with G418 maintenance pressure. The doubling time remained constant at approximately 24 h (Fig. 3B), which is slightly slower than the parental CHO-S line (approximately 20 h).

Antibody was purified using standard Protein G affinity chromatography, followed by size exclusion gel chromatography on an Superdex 200 (GE Heathcare, Chicago IL.) then concentrated with an 30,000 kDa cutoff filter (Millipore, Burlington, MA.) and stored at −20 °C. A gel filtration analysis of the protein G-purified material is shown in Fig. S1.

3.3. Anti-HSV-1 flag antibody gD binding specificity

Critical amino acids in the r34.1 mAb epitope were mapped using a set of HSV-gD peptides (Table 2). Peptide mapping revealed a role for the sequence "KDL" and possibly "LV" corresponding to amino acids 20–22 and 24–25 of the HSV-gD sequence (Fig. 4 and Table 2).

The r34.1 mAb binds wild type HSV-gD peptide, and N and C terminal deletion mutants with an EC50 of 0.39–0.42 μg/mL (in the nM range) in direct binding immunoassay. In the absence of "KDL" and "LV" amino acids, all HSV-gD binding is lost. Proline at position 23 is not required.

To verify production of an antibody that binds the gD flag in the context of a fusion protein, we measured the kinetics of r34.1 binding to a rgD-gp120 using surface flow plasmon resonance. We determined the K_D to be 16 nM with an on-rate of approximately 2.5×10^5 M^{-1} s^{-1} and an off-rate of 3.5×10^{-3} s^{-1} (Fig. 5). Although the antibody has a high affinity for its ligand, the antibody/ligand complex is weak or unstable as indicated by the fast off rate, an ideal characteristic for an immunoaffinity substrate.

3.4. Anti-HSV-1 gD tag affinity column preparation and stability testing

The choice of coupling chemistry is critical in the preparation of an affinity column, and typically determined empirically. We tested two resins; coupling recombinant antibody to a cyanogen bromide-activated resin resulted in an affinity column with low stability that leached antibody after 11 cycles (Fig. S2) whereas r34.1 captured on aldehyde cross-linked beads retained 80% binding capacity through 18 cycles of protein capture followed by acid elution at pH 3.00 and re-equilibration (Fig. 6A). An area under the curve analysis of the chromatograms is given in Fig. 6B. Gel electrophoresis of starting material, unbound flow through, and purified protein is given in Fig. 6C with 5B6 purified protein for comparison. The aldehyde-activated cross-linked agarose bound r34.1 to a capacity of 4 mg of antibody to each mL of beads following coupling performed according to the manufacturer's protocol, and had a maximum ligand-binding dynamic capacity of 5 mg/mL (Fig. 6).

3.5. HSV-1 gD-tag purification with r34.1

In summary, we recovered the hypervarible-region genes that code specificity to herpes simplex virus type 1 glycoprotein D from a mouse hybridoma. The genes were used them to engineer a stable CHO-S line that secretes high levels of this antibody. Subsequent studies have demonstrated that the cell line maintains an acceptable level of productivity for > 20 passages, and that the antibody is stable to repeated cycles of acid elution and regeneration required for immunoaffinity chromatography purification of HSV-1gD-tagged proteins. Recombinant mAb 34.1 is a murine IgG1 that binds to gD-tagged fusion proteins with nanomolar affinity. Although we did not definitively map the r34.1 epitope, it appears to bind a short linear epitope (KDL) that overlaps with the human CH43 mAb epitope derived from an individual immunized with rgD-gp120 during the course of the RV44 HIV trial (Wang et al., 2017). We have recently used the generic r34.1 affinity/size exclusion process to purify rgD-gp120 protein antigens (Yu et al., 2012) Byrne et al., 2018, #5209; O'Rourke et al., 2018, #24921; Doran et al., 2018, #78045} and several rgp120 fragments currently

under evaluation as new immunogens. Proteins purified using 5B6 and r34.1 affinity columns have identical binding to rCD4-IgG which is a surrogate measure of protein folding (Fig. 7).

Binding of CD4-IgG to rgD-gp120 purified on either 5B6 or r34.1 immunoaffinity columns, was measured by capture ELISA, immobilizing rgD-gp120 on r34.1 coated wells.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

rgD-gp120	recombinant gD tagged HIV-1 Env protein gp120
CHO-S	suspension adapted Chinese Hamster Ovary cells
HSV	Human Herpes Simplex Virus
cGMP	current Good Manufacturing Practice

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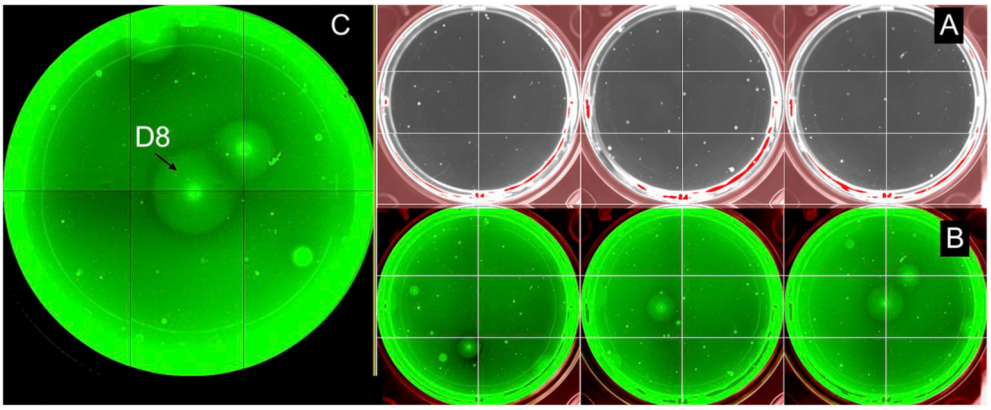

Fig. 1.

Selection of colonies expressing murine IgG antibodies using the ClonePix2 robot. A G418 selected colonies visible in 35mm well illuminated with white light after 10 days in culture. B The same wells illuminated with 490 nm wavelength light. Colonies actively secreting IgG have a green "halo" visible at 525 nm. C well 3 from image B, with white and FITC images superimposed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

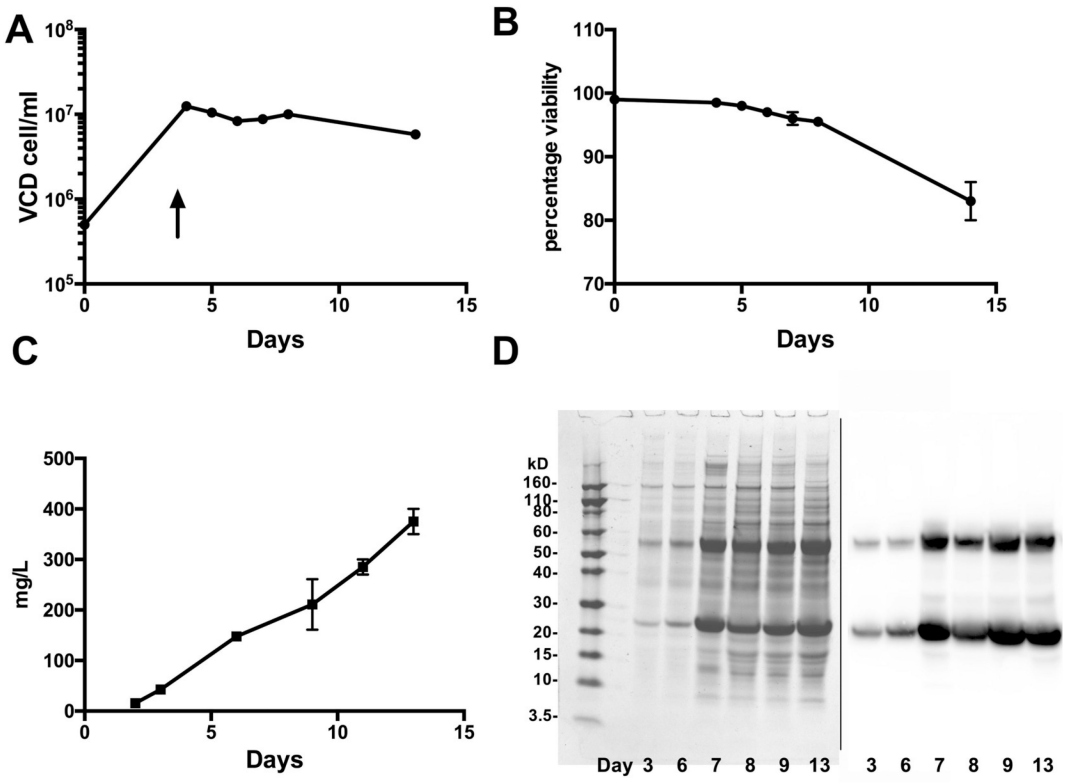

Fig. 2.

CHO-S r34.1 (clone D8) growth. Cell culture is described in section 2.4. Temperature shift (to 32 °C) and the addition of 1mM sodium butyrate, is indicated by an arrow. A Timecourse graph of viable cell densities (VCD) determined by trypan-blue exclusion. B Time course of cell viabilities determined by trypan-blue exclusion. C Time course of r34.1 production determined by quantitative immunoassay. D SDS PAGE of DTT reduced conditioned media stained with Coomassie blue (left) or western blotted detecting with rabbit anti-murine (H and L) antibody (right) as described in Section 2.5. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

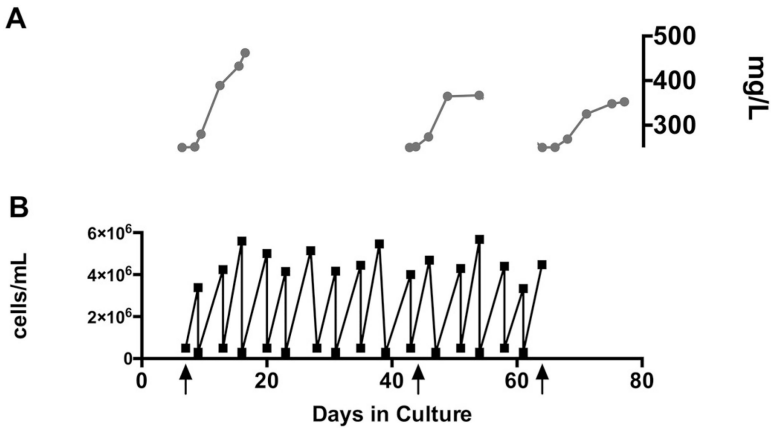

Fig. 3.

Stability testing of CHO-S r34.1 (clone D8). A Protein productivity of cells isolated at three time points over the course of a 24 passage culture. B Viable cell density. Cells were continuously cultured re-seeding a density of 3×10^5 or 5×10^5 cells per mL at 4 and 3 day intervals counting prior to each split to record growth.

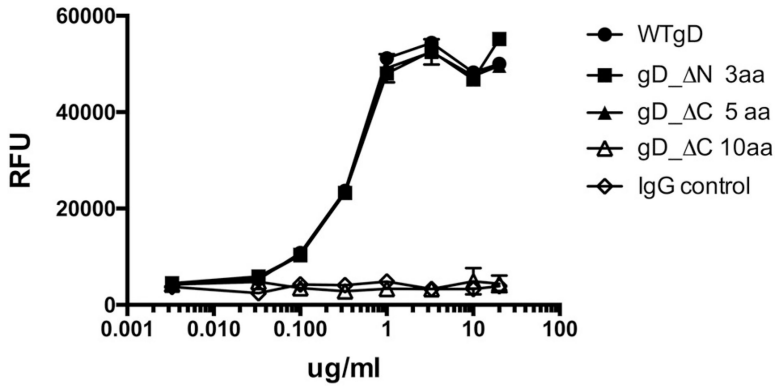

Fig. 4.

Epitope mapping using a series of HSV-1gD peptides. Antibody binding was assayed by direct binding of the recombinant antibody to HSV-1 gD peptide coated plates as described in Section 2.5 and Table 2.

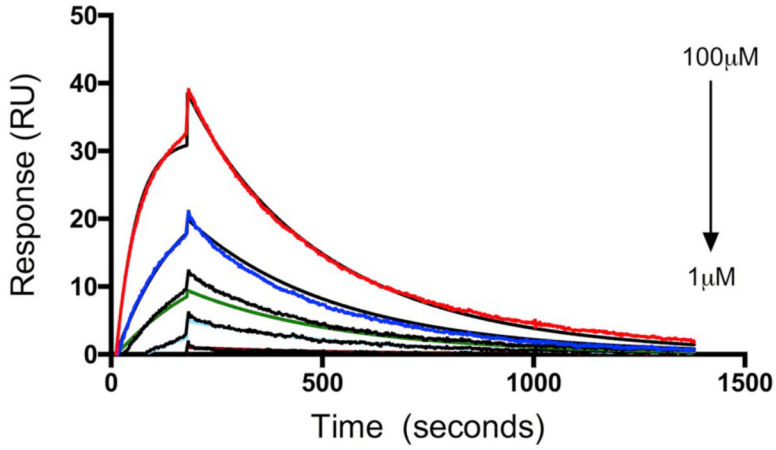

Fig. 5.

Sensogram of gD-rgp120 binding to immobilized recombinant 34.1 antibody. A concentration range of 1–100 μM was assayed (Section 2.6) and data evaluated using Biacore Evaluation software (GE Health Sciences).

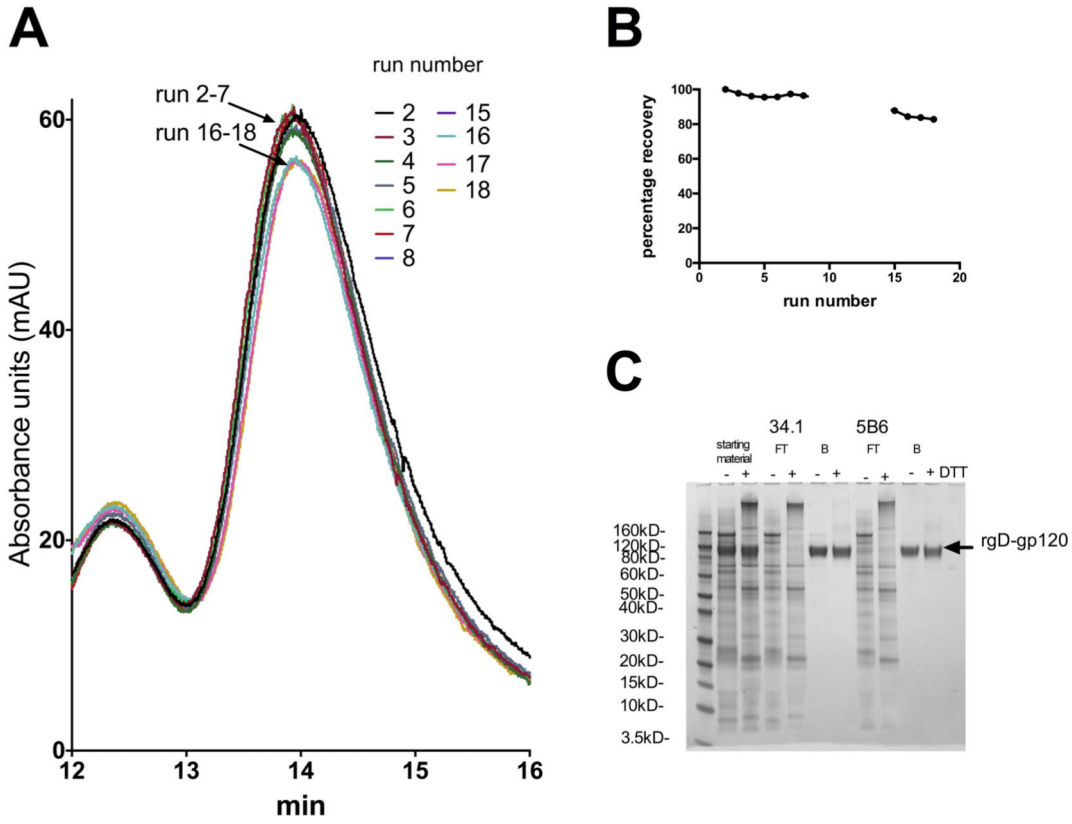

Fig. 6.

Dynamic binding capacity of r34.1 aldehyde coupled agarose affinity column. A Chromatographs of 18 sequential immunoaffinity rgD-gp120 purification experiments superimposed, eluting ligand at pH 3.0, and regenerating the column between runs. C Area under the curve analysis of the data shown in A, to calculate percentage protein recovery through successive column runs. B SDS PAGE electrophoresis of rgD-gp120 purified on an r34.1 or an 5B6 immunoaffinity column. Starting material (conditioned media, FT flow through (non bound) and B bound and eluted, or purified protein, are indicated.

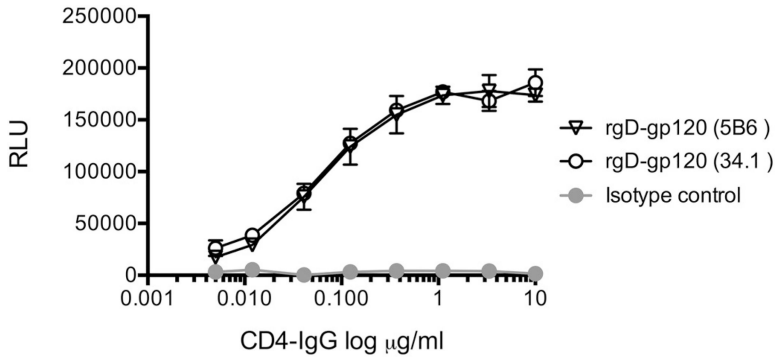

Fig. 7.
CD4-IgG binding to affinity-purified rgD-gp120.

Table 1

Recombinant 34.1mAb light and heavy chain sequences.

> 34.1 light chain
*MGWSCIILFLVATATGVHS***GVVMIQTPLSLPVSLGDQASISCRSGQSLVHNDGDTYLHWYLQKPGQSPKLLIYRVSNRFSGVPDRFSGSGSGTDFTLRISRVEAED
> 34.1 heavy chain
*MGWSCIILFLVATATGVHS***IEVKLQQSGAELVRPGASVKLSCTASGFNIKDDYI** **HWVKQRPEQGLEWIGWIDPENGDTEYASKFQGKATITADTSSNTAYLQLSSLTSEDTAVYYCSKPPWFAYWGQGTLVTVSA**AKTTPPSVYPLAPGSAAQTNSMV

Secretory peptide in italics, 34.1 hypervariable coding region in bold, non-bolded script, constant region sequence.

Table 2

HSV-1gD peptides used in recombinant antibody mapping.

Peptide	Domain	Sequence	r34.1 mAb EC50μg/mL
1. WT gD	Wild type	KYALADASLKMADPNRFRGKDLPVLDQLLE	0.40
2. gD_ΔNterm	3 aa N-terminus deletion	LADASLKMADPNRFRGKDLPVLDQLLE	0.42
3. gD_ΔC5aa	5 aa C -terminus deletion, ΔP	KYALADASLKMADPNRFRGKDLVL	0.39
4. gD_ΔC10aa	10aa C -terminus deletion	KYALADASLKMADPNRFRG	No Binding

Fusion protein linker underlined.

Peptides synthesized by GenScript, Inc. (Piscataway, NJ).

EC50 was calculated from a plot of log (agonist) *vs* response –variable slope (four parameters) on Graph Pad Prism 6 for Mac., GraphPad Software, La Jolla, *CA.*