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Antibody Library Display on a Mammalian Virus Vector: Combining the Advantages of Both Phage and Yeast Display Into One Technology

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Abstract: Utilizing a vaccinia virus based library technology, we previously developed an antibody discovery platform that enabled efficient selection of fully functional IgG antibodies from highly diverse immunoglobulin gene libraries expressed on the surface of mammalian cells. Recently, we have further modified this platform to enable efficient expression of a library of fully human antibodies on the surface of vaccinia virus; an enveloped mammalian virus. Similar in concept to phage display, conditions are utilized under which each vaccinia virion expresses a single antibody specificity on its surface. Various panning and magnetic bead based methods have been developed to allow screening of a library of vaccinia-MAb virions and selection of recombinant vaccinia virus encoding specific antibodies. Upon infection of mammalian cells the antibody is not only incorporated into newly produced virus, it is also displayed on the surface of the host cell. Similar to methods utilized in yeast display, the cells displaying vaccinia encoded antibody can also be selected using a combination of magnetic beads and cell sorting, and the virus encoding the specific antibody heavy and light chains readily recovered and analyzed. This technology allows for rapid high throughput selection of vaccinia-MAb virions in a cell free panning system, followed by cell based screening for high specificity and fine selection of optimal antibodies.

Keywords: Expression library, human antibody selection, vaccinia virus.

INTRODUCTION

In vitro methods for screening libraries of antibody fragments (generally scFv or Fab) expressed as fusion proteins on the surface of bacteriophage or yeast cells are well-established [1, 2]. Compared to rodent based immunization, these *in vitro* methods have the significant advantage of not being limited by self-tolerance, allowing for selection of antibodies that would be difficult to select in animals. A limitation of these technologies, however, is that the expressed antibodies do not undergo normal mammalian post-translational modifications. This is particularly the case for bacterial based methods, where the tertiary structure of the antibody repertoire is constrained by the environment of the bacterial cell, and the available antibody repertoire is correspondingly restricted. This is likely to be less of a problem in yeast based approaches, but to what extent the yeast cell environment may filter the available antibody repertoire is still unknown.

IMMUNOGLOBULIN EXPRESSION LIBRARIES CONSTRUCTED IN A POXVIRUS VECTOR FOR EXPRESSION IN MAMMALIAN CELLS

As described above, the predominant large library based platform technologies employed to date have inherent limitations that can interfere with successfully selecting antibodies with the desired specificities or affinities. There was a need, therefore, for an alternative method to identify immunoglobulin molecules from an unbiased immunoglobulin repertoire that can be synthesized, properly glycosylated and correctly assembled in mammalian cells.

The ease of cloning and propagation in a variety of mammalian host cells has led to the widespread use of poxvirus vectors for expression of foreign proteins and as delivery vehicles for vaccine antigens [3]. Vaccinia virus infects most mammalian cells and recombinant proteins expressed by vaccinia virus infected cells undergo normal post-translational modifications and trafficking. The conventional technology for generating recombinant vaccinia virus has been termed homologous recombination. In this method a transfer plasmid containing a vaccinia promoter and the gene of interest is created, such that the expression cassette is flanked by vaccinia DNA to allow for targeting to a non-essential region of the vaccinia genome. The vaccinia *tk* gene is often used as the insertion site so that recombinant virus can be selected for its TK deficient phenotype with BRDU. Recombinant virus is produced by transfecting the transfer plasmid into vaccinia virus infected cells, allowing recombination to occur over the course of several days, and then selecting for recombinants. Because recombination is a rare event, and there is no selection against formation of non-recombinant virus using this method, only approximately 0.1% of the virus produced will be recombinant. This frequency is sufficient for the selection of vaccinia containing a known gene of interest, but it is far too low to permit efficient construction and screening of a diverse cDNA library in vaccinia vector.

We have developed a method termed Trimolecular Recombination that allows for the creation of large and diverse cDNA libraries in a vaccinia virus based vector [4, 5]. The

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vaccinia genome consists of a linear 180,000 bp double stranded DNA. In the Trimolecular Recombination method, purified vaccinia DNA is cut in the tk gene, generating a right and left arm. The viral DNA arms are then co-transfected into cells along with transfer plasmid containing a vaccinia *tk* gene. Vaccinia DNA replication requires an intact genome, and because there is no homology between the two arms to allow for recombination and restoration of replication competent full length genome, infectious vaccinia cannot be generated from the separate input arms. As a result, the background of non-recombinant virus is close to zero. However, if recombination occurs between the two arms and the transfer plasmid, then the transfer plasmid can act as a bridge, and the recombination events result in a full length replication competent vaccinia genome incorporating the gene of interest on the transfer plasmid. Using this method, nearly 100% recombinant virus is produced enabling construction of libraries containing millions of different vaccinia recombinants. This technology makes possible the use of vaccinia virus as a vector for expression cloning in mammalian cells. We have employed this technology to identify antigens recognized by tumor specific T cells and antibodies, and as the basis for new methods to enable screening of large antibody libraries in mammalian cells.

MAMMALIAN CELL DISPLAY

One embodiment of our antibody technology utilizes co-infection with separate libraries of human heavy chain gamma 1 and light chain immunoglobulin variable genes that have been constructed in vaccinia virus. The gamma 1 heavy chain constant region contains a trans-membrane domain, so that when cells are co-infected with heavy chain and light chain recombinant vaccinia, the antibody is expressed, assembled, and trafficked to the cell surface. Antigen specific antibodies can be isolated by "reverse flow cytometry", where the antibody expressing cells are stained with fluorescently labeled antigen and anti-IgG secondary reagents, and cells expressing recombinant virus are isolated using a combination of magnetic bead selection and cell sorting. Following selection, the specific viral recombinants can be isolated by simply freeze/thawing the selected cells and amplifying the vaccinia virus that is released. After each cycle of selection, an aliquot of selected virus can be used to infect fresh host cells and the cells tested for antigen specific binding by FACS. Once a sufficient level of enrichment has been achieved, which is generally after 1 - 2 cycles of selection, individual vaccinia recombinants can be isolated and characterized.

This method allows for the rapid screening of a large number of antibody combinations, and the affinities of selected antibodies can be driven by varying the antigen concentrations employed for staining and selection. A challenge associated with this method, as with other methods of antibody selection, is the low frequency of antibodies of interest in the primary libraries. This requires large scale screening. In addition, the use of the magnetic beads and FACS selection can result in a significant loss of cells during the different purification steps. We, therefore, employ a redundancy of at least 10X cells per each possible combination of immunoglobulin heavy and light chains in order to ensure recovery of at least one of each antibody of potential interest (e.g. to screen a library with $5x10^6$ different heavy chain recombinants in combination with 10^3 light chain recombinants, we would co-infect at least $5x\ 10^{10}$ cells at a multiplicity of infection (moi) of 1 for both heavy chain and light chain recombinants).

Our initial use of this technology focused on the conversion of mouse antibodies into human antibodies by a procedure termed V gene replacement. This method follows a two step process. The antigen specific mouse heavy chain is engineered so that it is expressed as a chimeric membrane bound gamma 1 heavy chain in vaccinia virus. In the first step, the chimeric heavy chain is used to screen a library of human light chains in order to select human light chains that can replace the original mouse light chain in pairing with the chimeric heavy chain while retaining antigen specific binding. In the second step, the selected light chains are used to screen a library of fully human heavy chains in order to isolate human heavy chains that can replace the original chimeric heavy chain. This results in the selection of fully human antibodies that bind the same antigen as the original mouse antibody. This process has been used successfully for a number of projects, and has been previously described in greater detail [6].

The next step in the evolution of this technology was *de novo* selection of fully human antibodies. Manipulating separate libraries of heavy and light chain recombinants generates significant combinatorial diversity, so that even small libraries of 10^6 Ig-H and 10^6 Ig-L can be paired into 10^{12} combinations. The number of mammalian cells required to perform a screening of this magnitude is clearly unrealistic. In order to keep the screening complexity at a manageable size, and to increase the throughput of heavy chains that are screened, we have developed a selection strategy in which a small number of defined light chains are used to screen for heavy chains [7-9]. Once the initial antibody is selected, the heavy chain is then fixed and used to select new optimal companion chains from a much larger library of light chains. Either or both heavy and ligh chains can be subsequently targeted for mutagenesis and affinity maturation. This approach simplifies the manipulations involved, since only one new chain is isolated a ta time, and permits more rapid screening of very large Ig-H libraries. In addition, the light chains isolated are germline, and the same light chain may be paired with multiple heavy chains to generate different antigen specificities, which can facilitate the construction of bispecific antibodies that require shared light chains.

SELECTION OF C35 SPECIFIC ANTIBODIES

C35 (C17orf 37) is a novel breast cancer associated antigen. To select C35 specific antibodies, a library of Ig-H and two germline Ig-L were co-infected into Hela cells. Following overnight infection the cells were selected for C35 specific binding cells using antigen-coated magnetic beads and cell sorting (MACS, Magnetic Activated Cell Sorting). Following the first cycle of selection, cells were frozen and thawed to release the vaccinia virus, and the virus was amplified and used along with a fresh aliquot of the two Ig-L recombinant virus for a second cycle of selection. Following the second cycle, an aliquot of the selected virus was tested for enrichment of C35 specific binders by flow cytometry.

As shown in (Fig. **1**), a significant percentage of virus was antigen specific. Individual Ig-H clones were then picked using standard vaccinia methods, amplified, co-infected with Ig-L and tested for binding to C35 by flow cytometry. A number of positive clones were identified. The Ig-H chain genes were isolated from the specific vaccinia clones and cloned into mammalian expression plasmids containing the secreted gamma 1 heavy and kappa light chain constant regions. Antibody was produced as full length soluble IgG1/Kappa by transfection of the plasmids into CHO cells and the secreted antibody was tested for specificity and affinity.

Characterization of these antibodies revealed that at least 10 distinct antibodies were selected. As expected the affinities of these antibodies selected with only two germline light chains are modest ($\geq$ 10 nM), but affinity can be rapidly improved by either fixing the selected VH containing virus and using this to screen a large library of light chains under more stringent conditions, and/or by mutagenizing the selected VH(s) and re-screening under more stringent conditions.

VACCINIA DISPLAY

The advantage of our vaccinia library technology is the ability to express and select antibody libraries in mammalian cells. However, a disadvantage of this technology is that it requires the use of mammalian cells for antibody selection! The use of mammalian cells reduces the screening throughput because of the size and relative difficulty of manipulating mammalian cells. Our challenge was to modify our antibody technology to still allow for expression and production of the antibody library in mammalian cells, but to allow for selection to occur in a cell free system, where the throughput could be increased. To achieve this, we elected to develop a strategy to allow for the expression of antibodies on the surface of the vaccinia virus itself. Because selection can occur in a cell free system, and vaccinia virus can be easily concentrated by standard centrifugation methods, this technology allows for a considerable increase in the number of antibody combinations that can be efficiently screened.

Poxviruses are unique among DNA viruses because their life cycle occurs exclusively in the cytoplasm of the host cell. During its life cycle, two distinct infectious forms of the virus are produced: intracellular mature virion (IMV or MV), and the extracellular enveloped virion (EEV or EV) [10]. Vaccinia virus replication occurs in "viral factories" which are located in a peri-nuclear region. Following DNA replication, the viral genomes are packaged into IMV using a membrane that is derived from the host Endoplasmic Reticulum (ER) [11, 12]. The majority of virus stays inside the cell in this IMV form, and is released upon cell lysis. Some IMV is transported along microtubules and released outside of the cell. During this transport the virus becomes wrapped with a second membrane derived from the Trans-golgi network (TGN). This exported virus is initially tethered to the cell surface as cell associated enveloped virus (CEV). The CEV is cleaved by a vaccinia protein to release the EEV, which is

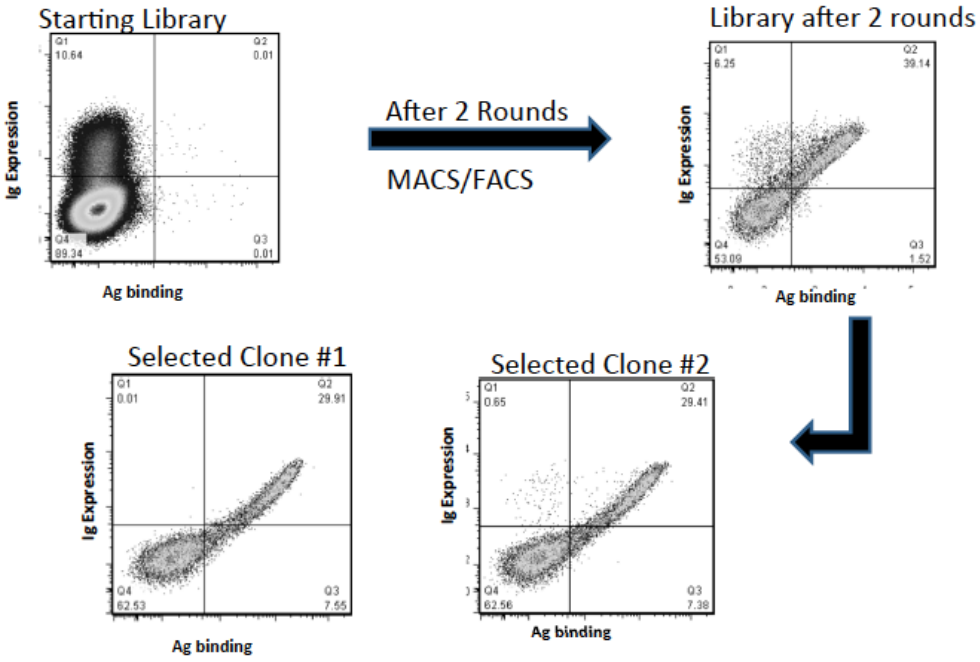

Fig. (1). Hela cells were co-infected with human Ig-H Library and with a cocktail of human light chains. After overnight infection, the cells were harvested and stained with biotinylated C35 and SAV-APC for 30 minutes on ice. Unbound antigen was washed away, and antigen binding cells were isolated using anti-APC microbeads (Miltenyi) and magnetic bead separation. The positively selected cells were then further enriched by cell sorting. Vaccinia virus was recovered from the selected cells by freeze/thaw and amplified on BSC1 cells and titered. The virus from the first cycle was used for a second cycle of infection and selection as described above. After the second cycle an aliquot of the round 2 virus was tested for binding to C35 by flow cytometry, and then, when enrichment was confirmed, vaccinia clones were picked and tested for binding to C35-His + anti-HIS- APC and anti-human IgG Fab-FITC by flow cytometry (negative control antigen + anti-His APC also tested).

responsible for disseminated infection within a host, or for disseminated infection in cell culture.

Our initial decision related to selecting which form of the virus to use as our vector, and how to target the antibody into that form of the virus.

Proteins that are expressed on the cell surface will generally traffic through the trans-golgi network. Our first experiments tested whether cells infected with the virus expressing the normal cell membrane targeted IgG1/kappa would incorporate IgG/K into the EEV or IMV. As shown below (Fig. **2**), the normal cell membrane associated form of immunoglobulin is not incorporated into EEV. This suggests that there may be some vaccinia specific sequences that concentrate a protein into the TGN for incorporation into the virus, or that other specific membrane incorporation signals are required for integration into the EEV membrane.

We reasoned that targeting EEV instead of IMV would likely result in cell surface expression. This cell surface expression would allow for the combining of phage like panning strategies for one or two rounds, followed by cell based selection strategies where the concentration of antigen can be adjusted to fine tune affinity selection and capture matched pairs of recombinant heavy and light chains.

At least four virus-encoded proteins have been reported as components of the EEV envelope; A33R, A34R, A56R, and B5R [13]. There has been one published report of a scFv-A56R fusion protein being incorporated into EEV, and we elected to determine whether far more diverse antibody-A56R fusion proteins would efficiently assemble into the EEV membrane.

PREPARATION OF CH1-A56R FUSION PROTEIN

The vaccinia A56R protein is a haemagglutinin of 314 amino acids with both N and O linked glycosylation [14]. The A56R protein contains an N terminal Ig-domain, and previously it had been shown that this domain could be replaced with a scFv and that this scFv-A56R fusion protein is incorporated into EEV [15].

A vaccinia transfer vector encoding a fusion protein in which the Ig domain of A56R was replaced with the human heavy chain CH1 domain of C gamma 1 was constructed using standard molecular biology methods. This pJEM vector contains a signal peptide as well as restriction endonuclease sites for the insertion of variable heavy chain genes in frame with the signal peptide- CH1-A56R fusion to generate an Ig-A56R protein. Expression of the fusion protein is controlled by a vaccinia promoter, and the entire expression cassette is flanked by vaccinia tk gene sequences, allowing for insertion into vaccinia virus by Trimolecular Recombination. This vector was used to insert heavy chain variable gene sequences of various specificities, and then to create recom-

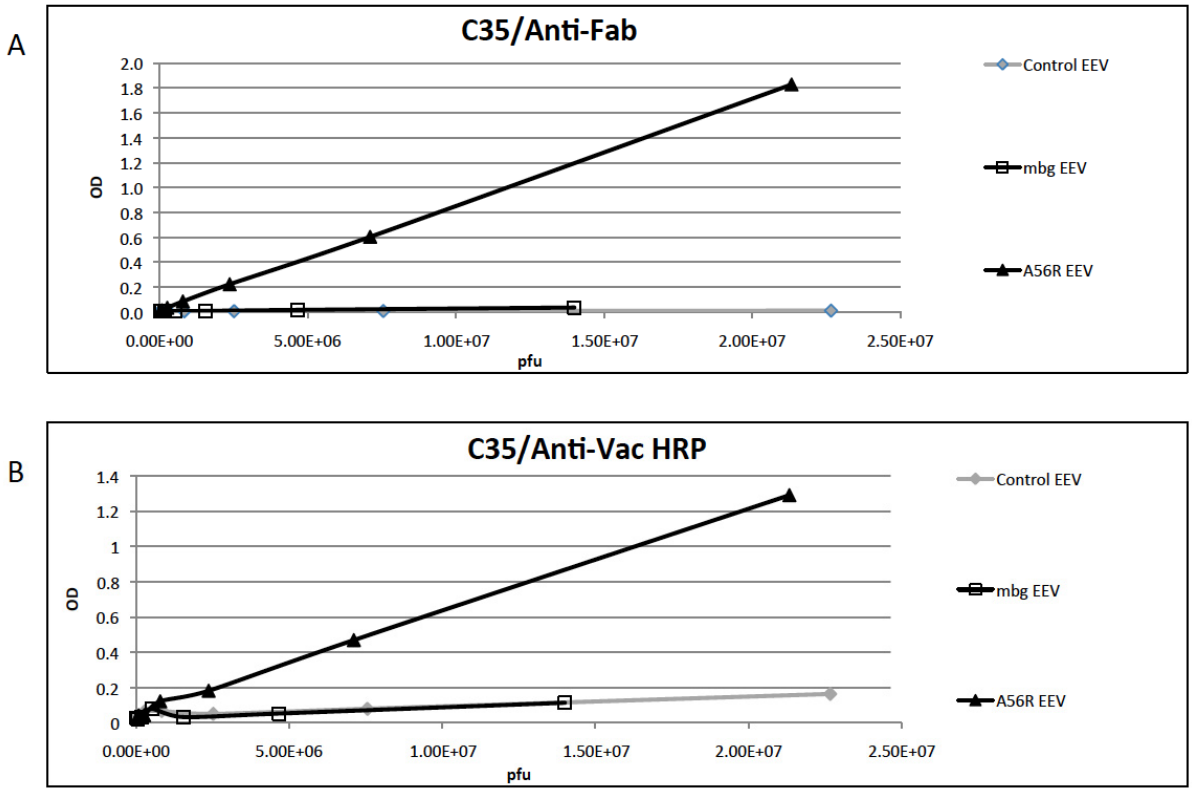

Fig. (2). Purified C35 protein was coated on a 96-well ELISA plate using standard ELISA conditions. EEV harvested from the supernatant of cells 48 hours after infection was then added to the plate, diluted in 1 x PBS/10% FBS/0.05% Tween-20, into designated wells and allowed to bind. Unbound virus was washed off using standard ELISA methods. Bound viral particles were detected using Rabbit anti-Vaccinia–HRP conjugated antibody or using Goat anti-human IgG $F(ab')_2$-HRP conjugated polyclonal antibodies. The results demonstrate that Ig-A56R is incorporated into vaccinia EEV and able to bind to antigen.

binant vaccinia virus. Cells co-infected with such heavy chain constructs and the appropriate light chain, express antigen specific antibodies. Table **1** shows the list of antibody sequences and specificities described here-in. This vector can also be used to clone libraries of VH genes (described in more detail below).

Table 1. Lists the Specificities of the Various Antibodies Described Below.

Antibody Number	VH Number	VK number	Specificity
2408	H2124-A56R	L517	C17orf37 (C35) [16]
8000	H8000-A56R	L8000	Her2/neu
2368	H2090-A56R	L512	CD100 [17]
7000	H7000-A56R	L7000	VEGF

EXPRESSION OF A56R FUSION PROTEIN ON SURFACE OF HELA CELLS

The first step in showing that the fusion proteins were functional was to confirm expression and antigen binding on the surface of infected cells. HeLa cells were co-infected with recombinant vaccinia virus expressing immunoglobulin fusion constructs of various specificities. Antigen specific staining of cells infected with recombinant vaccinia virus was determined by FACS analysis. These results showed that the IgH-A56R fusion proteins are expressed on the cell surface and able to bind in an antigen specific fashion (Data not shown). This suggested that the proteins were trafficking through the TGN, and should be incorporated onto the EEV.

To confirm incorporation of the antibody-A56R fusion proteins into EEV, EEV isolated from supernatant of infected cells was tested by ELISA on antigen coated wells. EEV that incorporated specific antibody was bound to antigen coated wells and could be detected with secondary antibodies specific for vaccinia virus and for immunoglobulin Fab. Results are shown in (Fig. **2**). Wells that were positive with both anti-vaccinia and anti-Fab secondary antibodies indicate that the antibody construct was expressed on the vaccinia virion. EEV containing the C35 specific fusion protein (labeled "A56R EEV") bound to C35, while a control, non-C35 binding EEV ("ControlEEV"), did not bind and EEV containing C35 specific antibody in standard cell membrane associated IgG1 format ("mbg EEV") also did not bind. The data demonstrate antigen specific binding of EEV when the antibody heavy chain is expressed as a fusion protein with A56R, but not when expressed as a normal cell membrane associated IgG1 antibody.

PLATE BASED AND SOLUTION BASED FUSION PROTEIN SELECTION

In order to enumerate the percentage of EEV that successfully incorporated Antibody-A56R fusion proteins, recombinant vaccinia virus expressing A56R fusions with known C35 or VEGF specific antibodies were tested for binding to target antigen using a plate based panning assay. Recombinant EEV expressing immunoglobulin molecules were expressed and harvested from infected cells and added to sterile antigen coated ELISA plates, followed by washing and the addition of monkey kidney BSC1 cells to each well. Virus that was bound to the plate was able to infect the BSC1 cells and after overnight incubation formed visible plaques. Each plaque represents the presence of one antigen-specific EEV bound to the plate. Plaque formation was detected by staining the wells with crystal violet. Fig. (**3**) shows the plaque assay plate results for C35 binding. These results show that the A56R fusion proteins are expressed at the surface of the vaccinia virions as antigen-binding molecules in association with light chains. Furthermore, the results showed that specific binding segments produced using A56R fusions expressed on EEV were able to bind to their

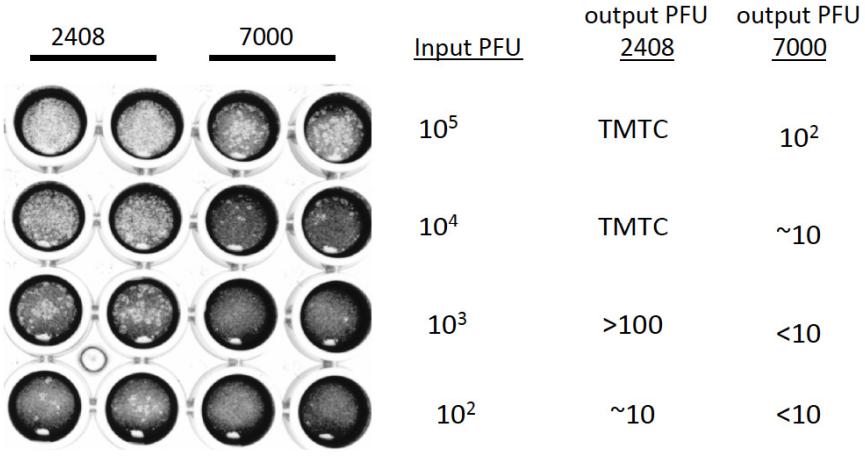

Fig. (3). Sterile 96-well ELISA plates were coated with C35. EEV containing supernatant starting at 10^6 pfu/ml was serially diluted. The EEV was added to designated wells and binding was allowed to proceed overnight at room temperature. The cells were washed 10 times with PBS to remove unbound EEV and then approximately 25,000 BSC1 cells were added to each well, and the plates were incubated at 37º C overnight. Plaque formation was visualized by staining the plate with 0.1% crystal violet. The approximate number of plaques per well are indicated in the figure. This data demonstrates antigen specific binding of the C35 specific Ig-EEV. TMTC = Too many to count.

specific targets, and that a significant percentage of antibody expressing EEV was functional, with the C35 specific 2408-A56R producing >100 plaques after an input of 1,000 plaque forming units (pfu)/C35 coated well, and the VEGF specific control producing 100 plaques in wells C35 coated plates only after an input of 100,000 pfu. A more sensitive method for enumerating functional antibody expressing EEV is described below.

Antibody library selection based on binding to plates is feasible, but we wished to determine whether binding in solution with selection based on magnetic beads would prove more efficient. To evaluate this, a series of bead based selection methods were developed using Streptavidin beads, Protein G beads or Tosylactivated beads to determine how efficiently EEV that incorporate specific antibody-A56R fusion (Ig-EEV) could be selected. A summary of these magnetic bead based selections is shown in Table **2**. In all cases the indicated recombinant antigen was conjugated to the magnetic beads following the manufacturers recommendations (Invitrogen). Ig-EEV (Specific and control) was incubated with the antigen coated beads for 2 hours, unbound virus was removed, and the percentage of bound virus was determined by plaque titration of bound and unbound virus.

These data demonstrate the specific enrichment of Ig-EEV using each of these methods, and that a very high percentage of EEV (>50%) contained functional Ig. The frequency of EEV with functional Ig modestly exceeds expectations of a Poisson distribution for two independent hits of heavy and light chain recombinant virus at multiplicity of infection (moi) = 1 (39.7%). This suggests that moi with either or both heavy and light chain recombinant virus may have exceeded 1 or that EEV with fully assembled Ig have some maturation advantage.

SPIKING EXPERIMENTS

Spiking experiments were performed in which HeLa cells were coinfected with vaccinia expressing C35 specific L517 light chain and a mix of vaccinia expressing C35 specific H2124-A56R heavy chain diluted to 1:10^4 or 1:10^5 with VEGF specific H7000-A56R heavy chain in all cases with light chain and heavy chain recombinants each set at moi=1. EEV were harvested 48 hours after infection and selected with Biotin-C35 and SAV beads as described above. Bound virus was amplified on BSC1 in T75 flasks for two days, then harvested and titered.

The amplified Bound samples for each spiking experiment were tested for enrichment by flow cytometry. The results from the spiking experiments showed a clear enrichment after just one round (Fig. **4**). The virus from the first round was co-infected along with fresh L517 to make EEV that were subjected to a second round of selection. Testing by flow cytometry showed significant enrichment for antigen binding virus (Fig. **4**).

Collectively, the bead binding and spiking data demonstrated that the selection of antigen specific Ig-EEV is a very robust process with low background.

IG-A56R FUSION PROTEIN LIBRARY CREATION

Libraries of immunoglobulin VH genes from any desired source can be cloned into the A56R transfer plasmid for construction of recombinant vaccinia virus. We have constructed libraries using a combination of bone marrow RNA that was purchased from a commercial supplier (Life Technologies) representing a pool of more than 100 donors, as well as libraries containing synthetic VH sequences. For library selection, the heavy chain library can either be paired with a limited collection of defined germline Ig-L, or can be paired with a much larger random library of light chains. We are currently constructing libraries which co-express the heavy chain-A56R and Light chain in the same virus. Fig. (**5**) shows a schematic for the selection of fully human antibodies using this method.

We have used these libraries and the vaccinia display methods described here to select high affinity antibodies specific for a number of different antigens. The specific results of these selections will be described elsewhere (E. Smith *et al.* manuscript in preparation).

SUMMARY

We have developed a vaccinia virus based library technology in which antibodies are displayed on the surface of

Table 2. Ig-EEV with the Indicated Specificities was Selected with the Indicated Antigen/Magnetic Bead Combinations as Described in the Text. All Combinations Demonstrated Antigen Specific Enrichment of Ig-EEV.

Virus	Selection	% Bound
MAb 7000 anti-VEGF	Biotin-C35 + SAV Beads	0.1%
MAb 2408 anti-C35	Biotin-C35 + SAV Beads	50%
MAb 2408 anti-C35	CD100-Fc + ProG Beads	0.36%
MAb 2368 anti-CD100	CD100-Fc + ProG Beads	58%
MAb 2408 anti-C35	C35-his+Tosylactivated beads	61%
MAb 2368 anti-CD100	C35-his+Tosylactivated beads	0.6%
MAb 2408 anti-C35	CD100-His + Tosylactivated beads	0.1%
MAb 2368 anti-CD100	CD100-His + Tosylactivated beads	46.7%

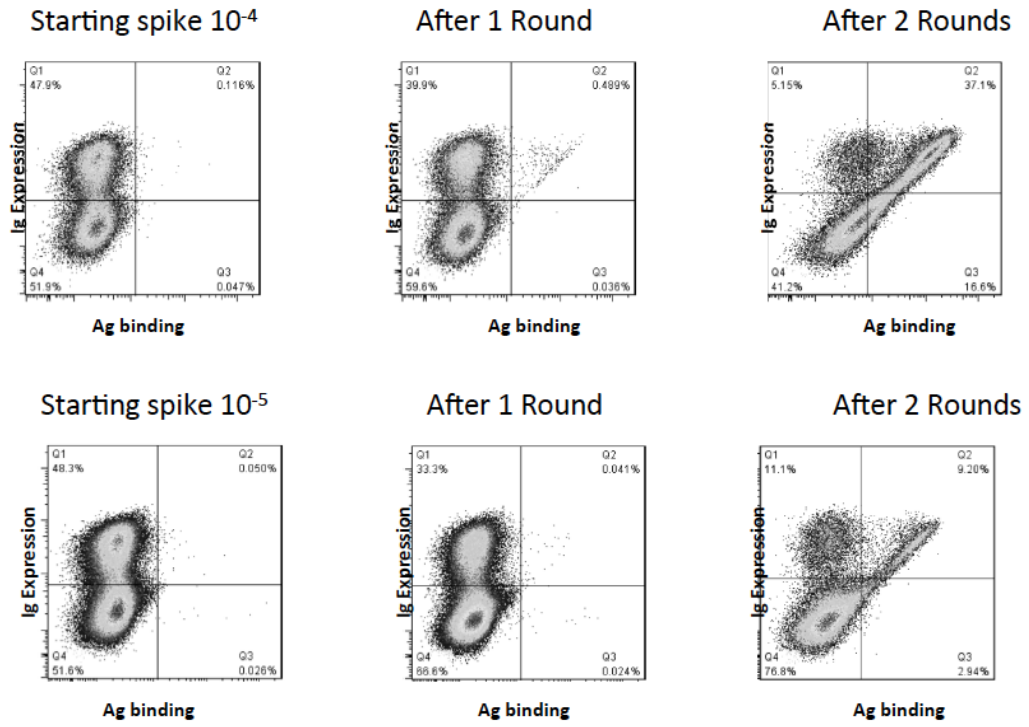

Fig. (4). Various spiking conditions of antigen specific and control virus were generated. These virus populations were selected with antigen coated beads as described in the text. The collected Bound and amplified samples for each spiking experiment were tested for enrichment by flow cytometry as described in figure 1. The results from the spiking experiments at 10^{-4} and 10^{-5} showed a clear enrichment with beads.

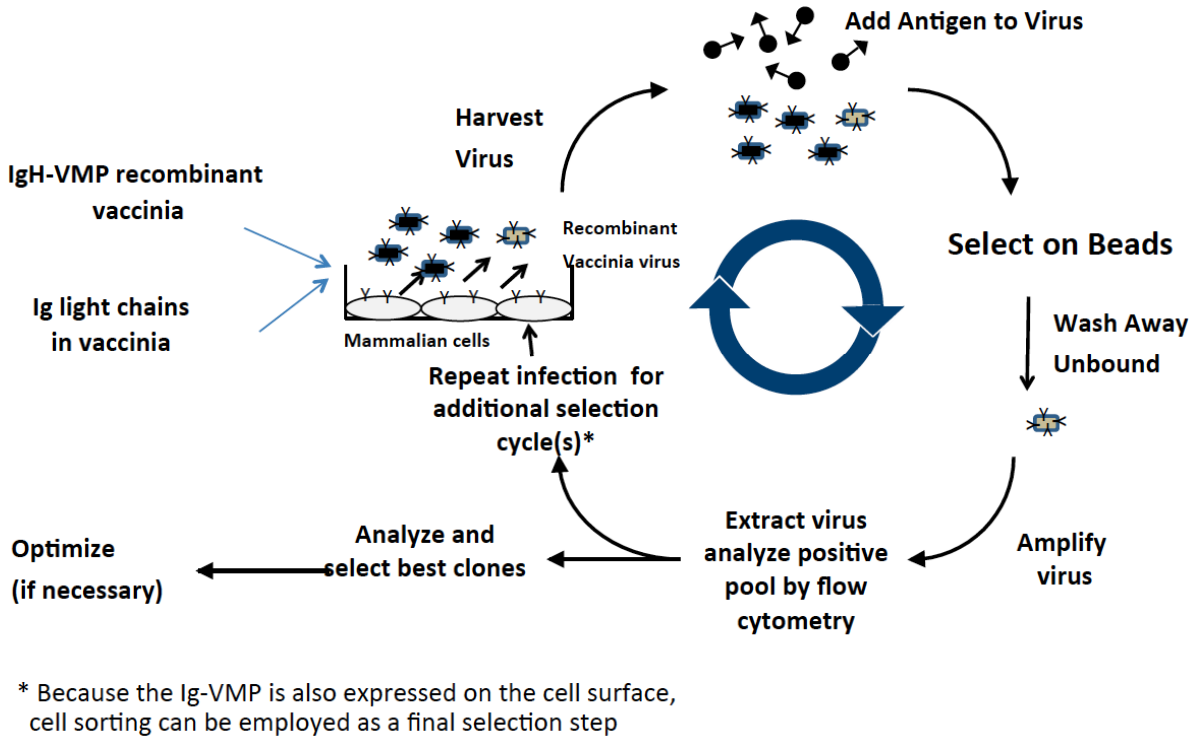

Fig. (5). Vaccinia Display. Mammalian cells are co-infected with a vaccinia library encoding human IgG-VMP (VMP= Vaccinia Membrane Protein) Library and with vaccinia recombinant human light chains at m.o.i.=1. Virus is released from infected cells and concentrated by centrifugation. Virus is then incubated with antigen coated magnetic beads. Unbound virus is washed away, and the recombinant viruses that express a specific antibody are isolated bound to the magnetic beads. The selected virus is rapidly expanded and further enriched by an additional round of selection. The IgG-VMP fusion protein is also expressed on the cell surface, so in a final step, infected cells can be stained with antigen and binders selected by cell sorting. The specific IgG-VMP and Ig-L can be isolated and characterized. If necessary, the selected Ig-H can be used to select optimal Ig-L or targeted for mutagenesis.

enveloped vaccinia virus EEV. This allows for antibody expression to occur within the mammalian cell, but for selection to occur in a cell free system, similar to phage display. The antibodies are also expressed on the mammalian cell surface, and this allows for selection to be refined by cell sorting at different antigen concentrations. This technology can be employed for *de novo* antibody selection, affinity improvement of existing human antibodies, or for the robust conversion of a non-human antibody into a panel of fully human antibodies. In all applications, there is a built in selection for antibodies that are efficiently expressed in mammalian cells.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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