Native complex membrane antigen expression on poxvirus for antibody discovery

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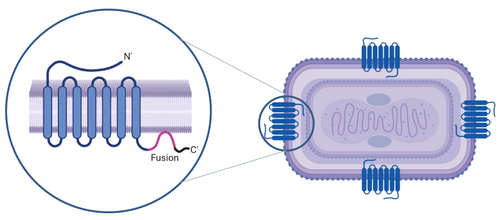
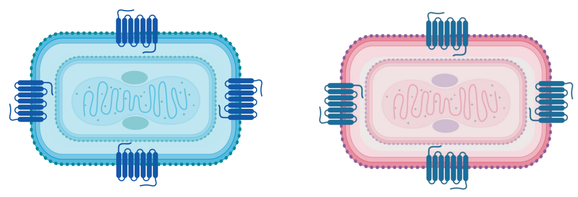
Vaccinex has developed a fusion protein technology to enable the direct incorporation of multi-pass membrane proteins such as GPCRs and ion channels into the membrane of two antigenically distinct poxviruses. The protein of interest is correctly folded and expressed in the cell-derived viral membrane and does not require any detergents or refolding before downstream use. Antigen expressing virus can be readily purified and used for antibody selection using any in vitro display platform where alternating between the two strains eliminates any anti-viral antibodies from being selected.
Antigen virions can also be used for in vivo antibody discovery methods. Immunization with the viral strains produce potent antibody responses. The resulting immune cells can then be used to create an immunized phage library for in vitro panning. Here we describe the discovery of functional antibodies against CXCR5 and P2X2 utilizing our antigen virions post-immunization.

Technology Introduction

Vaccinex fusion protein technology provides for efficient incorporation of multi-pass membrane proteins into the poxvirus membrane. This system ensures native conformations for antibody discovery both *in vitro* and *in vivo*.



The fusion protein is intra-viral and can be fused with fluorescent proteins like GFP for antigen tracking.

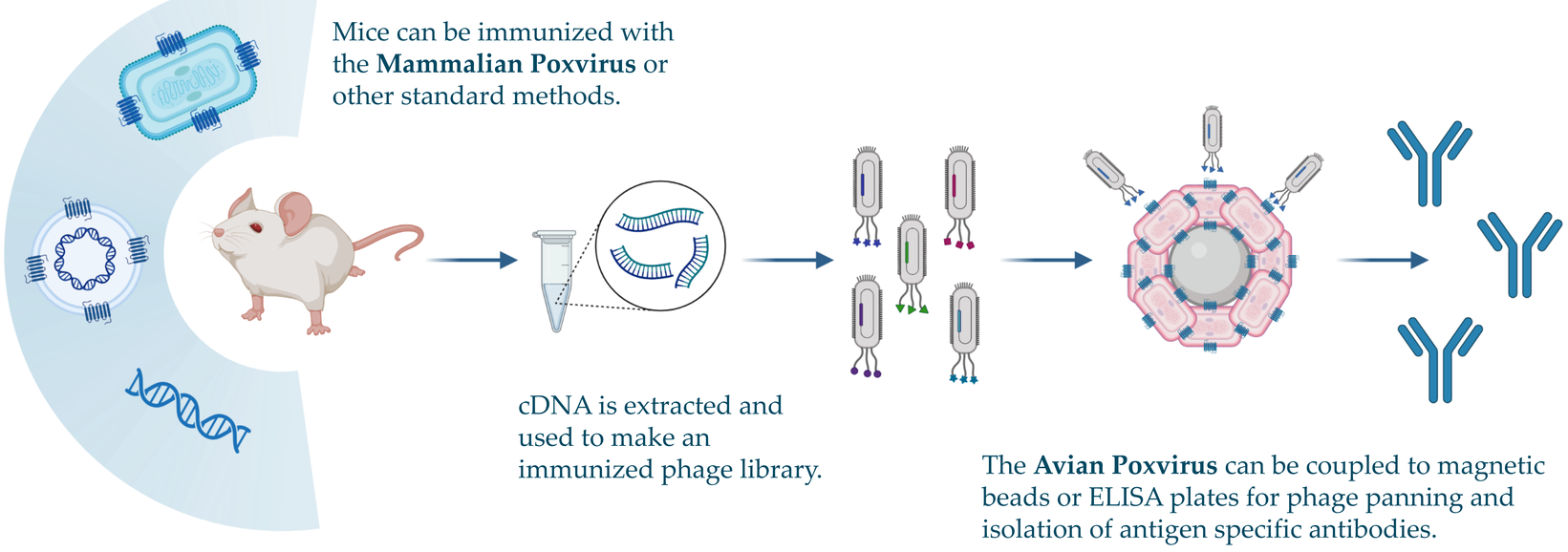


Mammalian Poxvirus | Avian Poxvirus

Antigens are produced in two antigenically distinct, highly attenuated BSL1 poxviruses to **eliminate anti-viral** background. Additionally, the poxvirus membrane has limited protein complexity with 4 known proteins incorporated.

In vivo Antibody Discovery Methods

Antigen virions are excellent immunogens. With two antigenically distinct poxvirus strains available, we can immunize with one strain and perform downstream applications with the other to avoid anti-virus background.



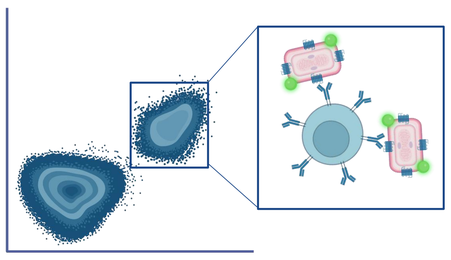
Mice can be immunized with the **Mammalian Poxvirus** or other standard methods.

cDNA is extracted and used to make an immunized phage library.

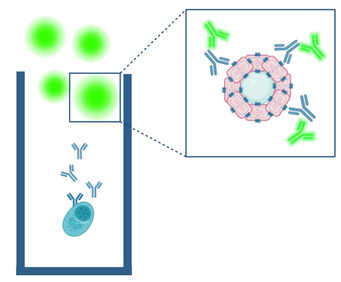
The **Avian Poxvirus** can be coupled to magnetic beads or ELISA plates for phage panning and isolation of antigen specific antibodies.

Additional downstream applications of antigen virions

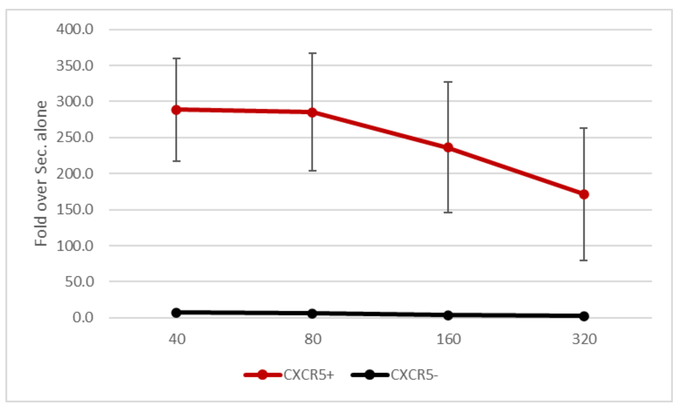
The antigen virions can be labelled with **biotin** or **fluorescent** beads to allow for **Memory B cell sorting** from mice or other species.



The antigen virions can be coupled to polystyrene beads for **plasma cell** analysis using single cell instruments.



Example: Antibody Discovery for CXCR5

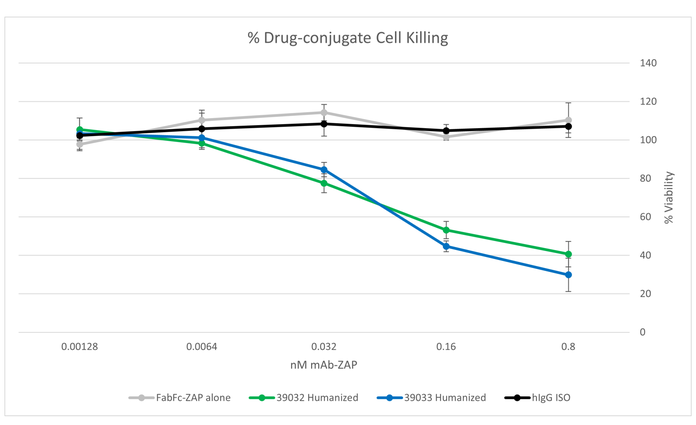


Sera titers for mice immunized with CXCR5 antigen virions.

Balb/c mice were immunized with CXCR5 antigen virions and mice with sufficient anti-CXCR5 titer were sacrificed and their v-genes extracted for an immunized phage library that was used to pan on CXCR5 antigen virus.

Antibody	Affinity (nM)	% Migration blocking	% Ligand blocking
39027 mouse IgG2a	0.04	36%	48%
39032 mouse IgG1	0.53	44%	74%
39033 mouse IgG2a	0.34	45%	62%
39034 mouse IgG2a	0.35	53%	62%
39029 mouse IgG1	1.66	29%	8%
39030 mouse IgG1	0.89	49%	15%

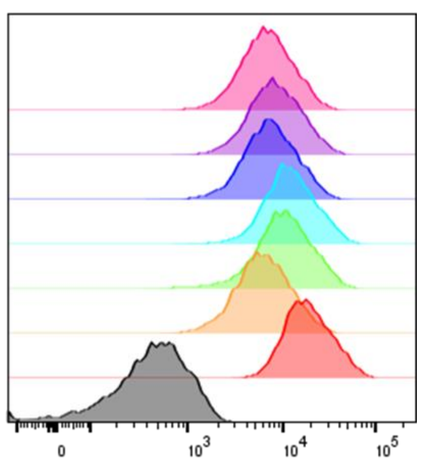
Antibodies exhibited functionality in the ability to prevent migration of CXCR5 expressing cells toward hCXCL13 and blockade of CXCL13 binding. Antibody affinity was determined by flow cytometry using a modified Scatchard method.



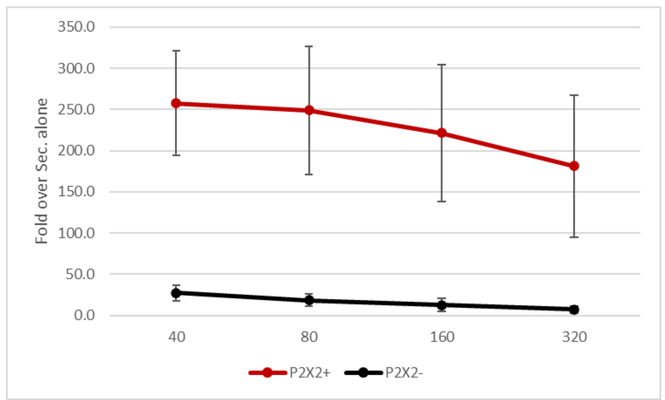
Two antibodies were humanized and then tested for drug-conjugated cell killing using FabFc-ZAP as compared to controls. Both exhibited titratable cell killing of PreB-CXCR5 cells.

Example: Antibody Discovery for P2X2

Balb/c and C57Bl/6 mice were immunized with P2X2 antigen virions. Mice with sufficient anti-P2X2 titer were sacrificed and their v-genes extracted for an immunized phage library.

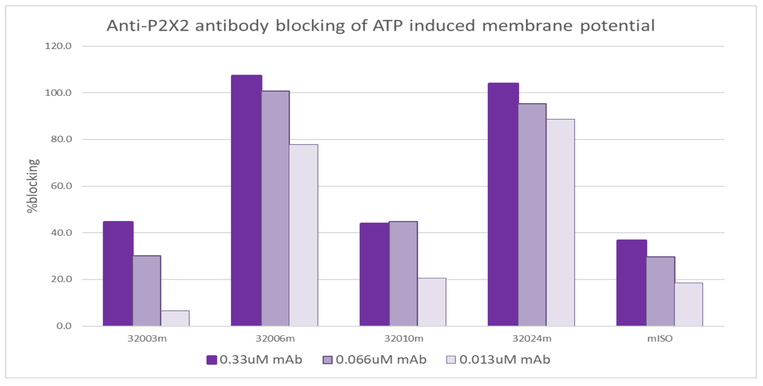


Histograms for individual P2X2 antibodies as CHO supernatants tested at 111 ng/ml as compared to P2X2 negative cells (black).



Sera titers for mice immunized with P2X2 antigen virions.

CHO clones were screened after panning on antigen virions for P2X2 binding and specificity. Select antibodies were purified and tested for affinity and their ability to block ATP induced membrane potential changes.



mAb	Affinity (nM)
32003 mouse IgG1	0.3
32006 mouse IgG2a	0.4
32009 mouse IgG2a	0.9
32010 mouse IgG2a	1.2
32024 mouse IgG2a	0.7

Conclusions

Poxvirus display of complex membrane antigens, including GPCRs, Ion Channels and ECDs in their native conformation is a versatile tool for antibody discovery. Immunization with recombinant poxvirus elicits specific anti-antigen antibodies in mice and can additionally be used for discovery methods such as immunized phage display libraries, single B cell analysis or plasma cell analysis. The use of two antigenically distinct poxviruses with limited membrane diversity facilitates successful antibody selection post-immunization by eliminating any background anti-viral antibodies from the mice. Selected antibodies show high specificity, affinity and functionality for difficult targets such as CXCR5 and P2X2.

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