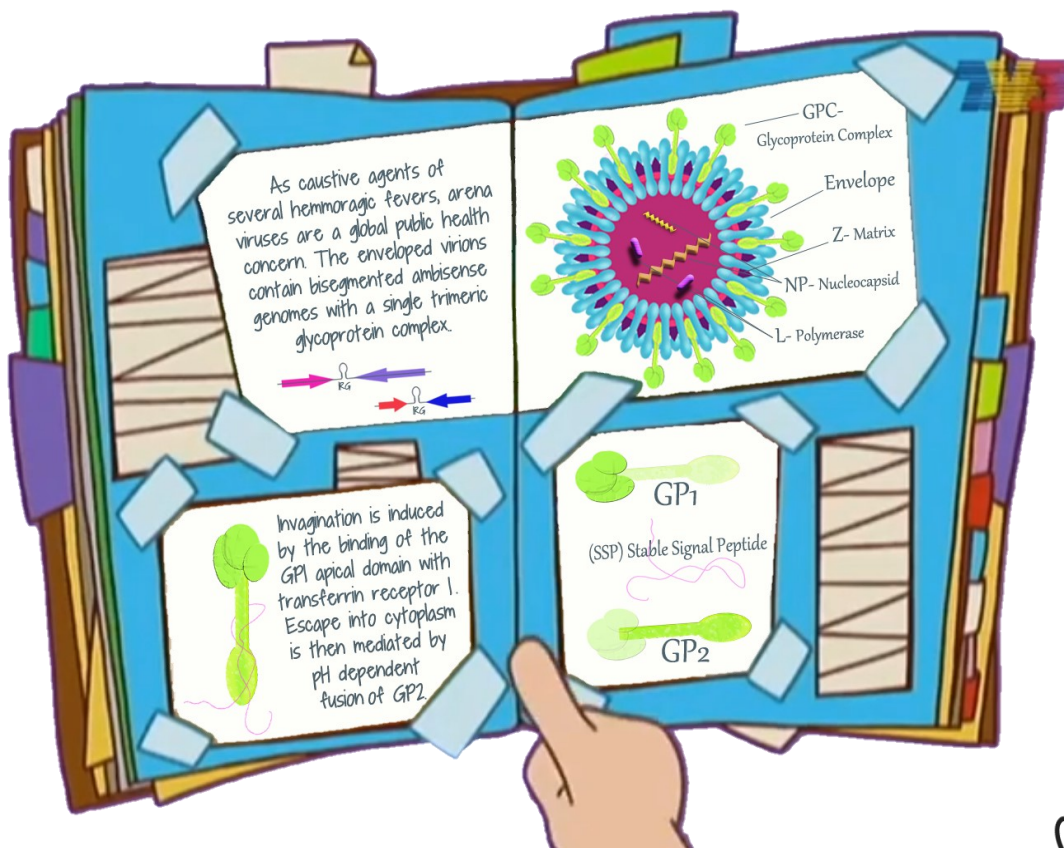


ARENA WHO???

Let's pull out the spotlight and turn on the microphone because arenaviruses can use a bit more attention than the average virology course or textbook frequently provides. We're talking about bi-segmented ambisense genomes here! Now, before you whip out the ever so popular Baltimore classification, or go cross-eyed straining to figure out where those are... STOP! They aren't listed. Actually, they just shoved the ambisense genome in with Group V the (-) ssRNA because of replicative behavior. This week it's important to zoom in a little closer because we're focusing specifically on **New World, clade- B Mammarenaviridae**. From here on forget about Lassa fever it's Old World. Not only does immune response differ in the New World viruses like Machupo (Bolivia), Junín (Argentina), Guanarito (Venezuela), and Sabiá (Brazil) but they also follow a clathrin-dependent endocytic recycling pathway using the transferrin receptor 1. The distinction is paramount to our discussion.

SO WHAT? WHO CARES?



RATIONAL DESIGN OF UNIVERSAL IMMUNOTHERAPY FOR TFR1-TROPIC ARENAVIRUSES

In the words of Scrooge McDuck “work smarter, not harder.” Which is exactly what the *Rational design of universal immunotherapy for Tfr1-tropic arenaviruses* has set out to do. All of the New World (NW) clade- B pathogens have a similar transmembrane glycoprotein and use transferrin-receptor 1 (Tfr1) entry. Therefore because neutralizing monoclonal antibodies (mAbs) have saved animals from lethal doses of Junín virus (JUNV) and there have been favorable outcomes treating Machupo virus (MACV) with anti-JUNV antibodies it seems possible, one treatment may be developed for all NW pathogens. Before we press on, I do feel it's important to note, the cross-reactivity mentioned has only been seen between JUNV and MACV not other NW viruses. To my knowledge, that extends throughout clinical practice and other research, it is entirely likely there is a unique connection between these two viruses.

True, emerging and re-emerging pathogenic New World arenaviruses are causative agents of viral hemorrhagic fever and defined as category A pathogens of bioterrorism concern but they also have a lot in common with other viruses. These highly infectious and lethal viruses are spread via aerosolized particles, food contamination and direct contact; nosocomial infection has been documented. With exception to Junín, there are no approved prophylactic nor therapeutic options. Currently, major preventative measures exist in forms such as controlling wild rodent populations. However, with climate shifts and destruction to habitat these methods are likely to become less effective.

Borrowing a tactic explored for HIV they set out to engineer a molecule fusing the constant region (Fc) of immunoglobulin IgG1 with a more effective Tfr1 ortholog to outcompete human cellular receptors. This should neutralize a viral particle additionally enlisting complement, cytotoxic and phagocytic immune functions providing proactive treatment in a cost-effective manner. If this works, there are remarkable potentials for a variety of other zoonotic viruses. Using rodent derived orthologs as a foundation in broad-spectrum design does present limitations such as immunogenicity. However, in acute life-threatening viral disease, prolonged use is not needed and natural immunity may be developed against future exposure.

GO AHEAD, THIS TIME YOU CAN CHEAT!

(ADCC) Antibody-dependent cellular cytotoxicity

(Arenacept LALA) Two Fc mutations causing reduced affinity to Fc γ

(Arenacept- M1) Mutation to avoid binding problems with an N-acetylglucosamine attached at Asn204

(Arenacept- M3) Mutation intended to increase hexamer formation to activate complement

(CD) Circular dichroism

(CDC) Complement-dependent cytotoxicity

(EC₅₀) Half-maximal effective concentration

(GPC) Glycoprotein complex

(GTOV) Guanarito virus

(hTfR1) Human-transferrin receptor 1

(IC₅₀) Half-maximal inhibitory concentration

(JUNV) Junin virus

(K_d) Dissociation constant

(LASV) Lassa virus: an old-world arena virus

(mAbs) Monoclonal antibodies

(MACV) Machupo virus

(MLV) Murine leukemia virus

(NW) New World

(P₄,22) Tetragonal space group

(PRNT) Plaque reduction neutralization test

(sAD) Stand-alone apical domain

(SBAV) Sabiá virus

(SDS-PAGE) Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

(SPR) Surface plasmon resonance

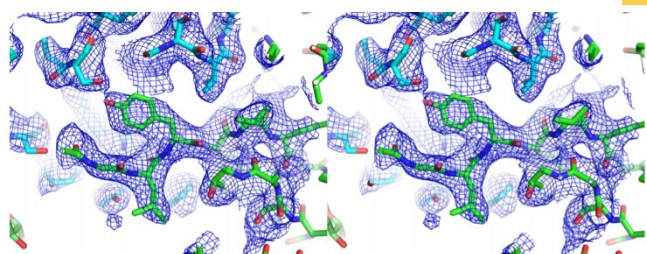
(TfR1) Transferrin receptor 1

(WWAV) Whitewater Arroyo virus

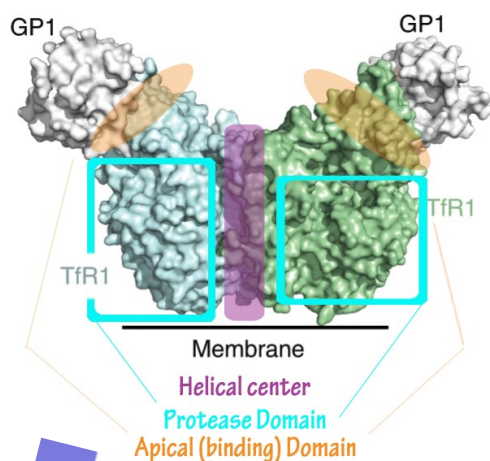
(wwTfR1) White-throated woodrat transferrin receptor 1

The NW mammarenaviruses bind in the apical domain of TfR1. The domain is not a major player for binding transferrin nor the regulatory competitive binding of hereditary hemochromatosis protein so there shouldn't be much functional TfR1 interference by using it alone. Having previously found the White-throated woodrat TfR1 showed higher binding affinity to the glycoprotein complex than does human TfR1 and that it functions as an entry receptor for other mammarenaviruses it was used as a model to design the immunoadhesin. In order to create a stand-alone protein, they remodeled the apical domain by mutating hydrophobic residues between the apical and protease domains to increase solubility as well as removing a long loop. Two terminal cysteines were finally used to increase stability. The structure was verified for characteristic folding and uniform size via circular dichroism and size exclusion chromatography. Thermostability and solubility were additionally checked and established therapeutically safe.

Verifying this new stand-alone apical domain would bind with the glycoprotein binding subunit GP1 for neutralization was established by measuring the disassociation constants. The viral GP1s were fused onto the C' termini of an antibody Fc region and immobilized on a protein coated sensor chip. The stand-alone apical domain was then injected at increasing concentrations. Surface plasmon resonance then monitors binding shifts over time through electron resonance by reflection of light source through a prism off the back of the chip. One of the benefits of the process is it allows observation without interference like may occur with a fluorescent tag. The binding mode was further observed through the crystallization of Machupo viral GP1 and the stand-alone apical domain as a complex. While there were some observed structural differences due to the mutations made during construction overall, integrity was maintained.



Supplementary Figure 2 | Quality of electron density. A wall-eyed stereo image of the interface between sAD (green) and GP1_{MACV} (cyan) in a 2FoFc map (blue mesh) at $\sigma=1$. Clear electron density for all the side chains at the interface is visible.



The stand-alone apical domain was fused at the C' terminus with an IgG1 Fc linking the domains in a way to allow eager and simultaneous binding. Confocal microscopy was then finally used to verify binding uniqueness when using the native glycoprotein complexes. Unlike Lassa fever virus, Machupo, Junín, Guanarito, Sabiá and Whitewater Arroyo viruses all showed affinity proving immunoadhesin Arenacept is unique to Tfr1 specific viruses.

DOES IT WORK??

Congratulations! Let's all give ourselves a pat on the back. We've built something that binds to a receptor!!! Because that's never been done... exactly. Cool yes, but does it work? The problem with pushing boundaries with extremely deadly viruses means you have to be careful every step of the way. In 1963 when Machupo virus was first isolated, four of the scientists were infected, two of which then transmitted it to their wives. Talk about a surprising "hi, honey, I'm home!"

So, safety is a big limitation in BSL-4 research, one precaution this group took was starting with a murine leukemia based gammaretroviral-pseudotyped virus. Yup, that's a mouthful. If you haven't been studying up on your gene therapy lately, here's the premise. They took a mouse retroviral genome, placed it inside the spike studded Z- protein shell. "The great big book of everything" can help with the visual. When the virus infects a cell, the genome integrates along with luciferase which then can be detected by its blue-green luminescence. HEK293T cells overexpressing Tfr1 were simply monitored for infection in the presence of Arenacept with a favorable conclusion. In a separate analysis, mutating an Arenacept critical binding place notably increased infection emphasizing structural avidity. The question then became, can Arenacept outcompete Tfr1? Keeping things consistent the process was again performed in triplicate, this time using the IgG1 Fc fused to human Tfr1. When they compared the two, the result was so good they decided it was time to revisit whether or not there was going to be a problem

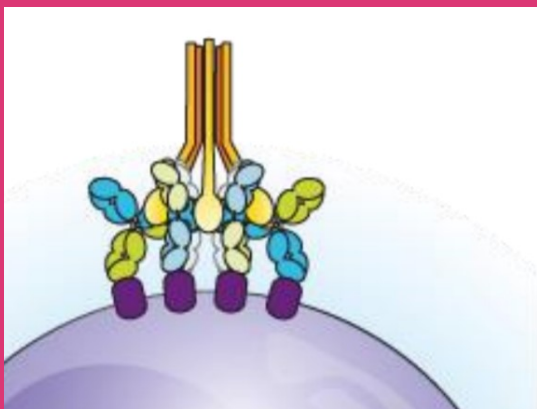
with iron later. Looking at complexed Tfr1-ferritin vs Arenacept-ferritin a few differences were noted. Arenacept contains a side chain that may be in the way and there are two places where polarity just might not be strong enough to hold on. Well, that was promising enough to bust out a few sensor chips and do a little more surface plasmon resonance measurements. They stuck Arenacept and Tfr1-Fc on chips and injected ferritin this time. But there is a small problem. Ferritin is composed of 24 peptide subunits so the set up allows differentiation for the combined binding strengths, meaning it's less sensitive and is only recording larger changes. Measuring like this ferritin immediately and irreversibly binds to human Tfr1, however, it does dissociate from Arenacept. Ultimately what we're paying attention to here is not how precise these values are but the difference between the two. The binding event with Arenacept is much weaker than with the real receptor. The numbers aren't perfect but it is enough to say the likeliness of cellular interference is low enough to stick with this line of research at this point.

LET'S GET DANGEROUS!!

Up until this point, we've been playing it safe but we have a working construct and it's time the gloves came off. Ok, really, it's more like putting on a space suit but whatever. We've got monkey kidney cells, some guinea pig complement and it is ON! The Junín virus plaque reduction neutralization test was set up at different concentrations with and without compliment and incubated for five days prior to read. In the presence of compliment, neutralization values were comparable to those of the corresponding pseudotyped virus. Without... not so much. The authors concluded Arenacept works against the real thing in a way complement can help.

RECALL, one of the attractive reasons for this approach is to engage the immune system. To further explore this action, they mimicked infection by expressing Junín and Machupo glycoprotein complexes on HEL293 cells which were subsequently exposed to immune cells and two different versions of Arenacept—the original and one where the Fc region had been modified so that it was difficult for an immune cell to dock or latch on to it. The anticipated effect was observed, functional Arenacept engages antibody-mediated immune response. The authors did note the result was more pronounced with Junín than Machupo.

You might have heard the phrase “why mess with a good thing?” Yeah, that doesn’t apply here. Instead these people started analyzing crystalline structures! They claim electrons gave them a message about a problem with Asn204 not being quite as good Asn251 and that a little N-acetylglucosamine there could create a binding problem. Electrons are like parents, really picky and always making you do things just right. That’s why Arenacept-M1 got the S206A mutation, it was all about glycosylation sites. Complement was so important to Junín neutralization they made another mutation. When IgG1 binds to a pathogen it does so in clusters, the Fc portions align and C1q is formed. The hexamerization of IgG1 is critical for activating complement. Arenacept-M3 was designed to increase hexamer formation. When they tested these out against Junín virus the results were promising so Machupo got a turn too. Machupo virus neutralized at an EC_{90} value below $1\mu\text{m/ml}$! What is odd though is when they went back to the pseudotyped Machupo virus, tested is with Arenacept-M3 and complement, compliment didn’t make a big difference like it did with Junín. Going back to spike coated HEL293 cells, Arenacept does a good job but Arenacept-M3 does the best, especially with Junín glycoprotein.



[HTTP://WWW.GLOBALENGAGE.CO.UK/BIOLOGICS/DOCS/PARREN.PDF](http://www.GLOBALENGAGE.CO.UK/BIOLOGICS/DOCS/PARREN.PDF)

POINTS TO CONSIDER

- ❑ The pseudotyped virus without complement shows similar results to the real virus with complement.
- ❑ The pseudotyped virus was tested with human cells over expressing TFR1.
- ❑ The real virus was tested with Vero cells.
- ❑ The compliment is from a guinea pig.

- DISCUSSION TIME -

- This study sets out for a broad-spectrum approach to treat mammarenaviruses. How do you feel about the results/ conclusions presented?
 - ◆ - Is this a broad spectrum option? Why or why not?
- Arenaviruses overlap with a lot of other viruses, even the common flu. The bi-segmented ambisense genome is pretty neat yet they are neglected pathogens.
 - ◆ - Keeping in mind now we have a variety of ways to study them and not all are pathogens... How else do you think they might be used or studied?
- Climate change is an concern with arenaviruses. For ex. in Bolivia millions of acres of Amazon have been destroyed by fires in the past year creating a state of emergency. The major way Machupo is prevented is through rodent population control. Now fires threaten their habitat.
 - ◆ - What other pathogen concerns is climate change creating and as scientists how might we rise to the challenge?