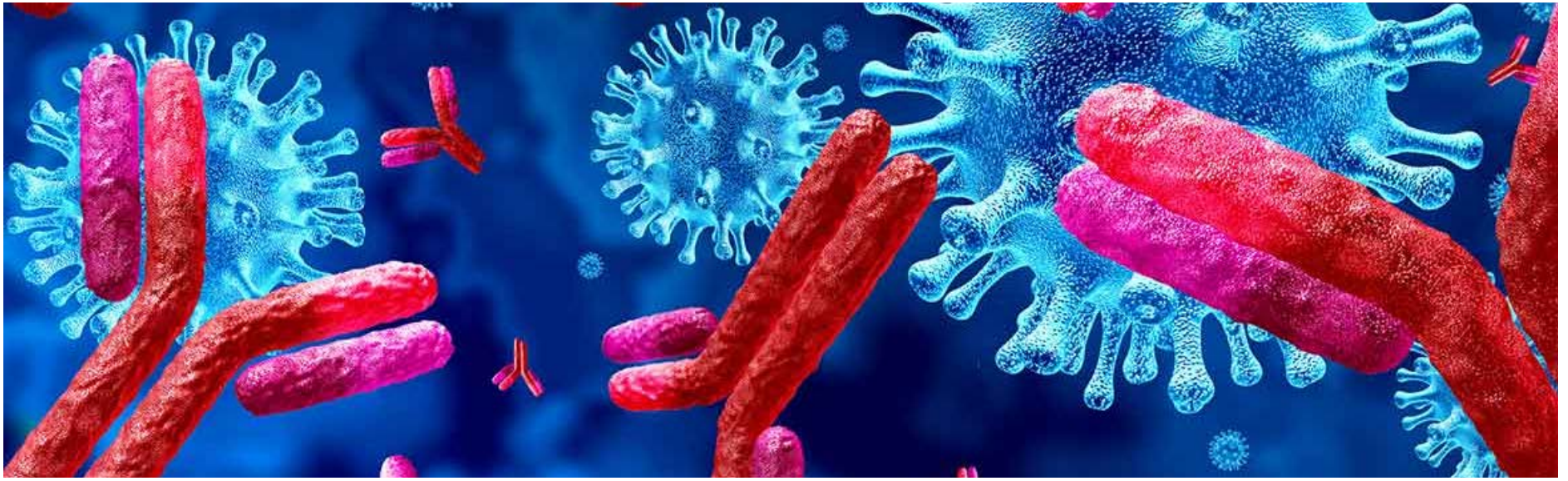

Structural and mechanistic basis of neutralization by a pan-hantavirus protective antibody

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Current situation of hantavirus

Infections by rodent borne hantaviruses are associated with more than 50,000 annually diagnosed cases of zoonotic disease worldwide.

Hantavirus causes three diseases:

- Epidemic Nephropathy (EP) for Old World hantaviruses (OWH)
- Hemorrhagic Fever with Renal Syndrome (HFRS) for OWH
- Cardiopulmonary Syndrome (CPS) for New World hantaviruses (NWH)

Neither the Food and Drug Administration (FDA) nor the European Medicines Agency have approved the use of the vaccines.

The therapy is currently a supportive treatment to provide hemodynamic and oxygen support in patients suffering from more severe clinical forms of these diseases.

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Hantaviral genome and proteins

Hantaviruses are enveloped viruses with a tri-segmented negative strand RNA genome that encode three structural proteins

- RNA dependent RNA polymerase (segment large)
- Surface glycoprotein precursor (segment medium)
- Nucleocapsid protein (segment small)

Surface glycoprotein precursor that is proteolytically processed into N-terminal (Gn) and C-terminal (Gc) subunits.

The Gn/Gc complexes associate to form square-shaped hetero tetrameric [(Gn/Gc)4] spikes

- Mediate all steps in viral entry

Gn/Gc complexes, really complex

Each tetramer is formed by four Gn subunits in the center and four Gc subunits at the periphery

Gn ectodomain comprises two independently folded regions:

- The head (GnH), predicted to interact with cellular receptor
- The base (GnB), which provides tetrameric contacts stabilizing the spike

Gc as a class II fusion protein, folds into three structured β sheet rich domains (I, II and III)

- Mediates lateral contacts between spike tetramers
- Induces the membrane curvature required for budding of nascent virions
- Catalyzes viral fusion with an endosomal membrane

Gn/Gc spikes are thus the primary target for neutralizing antibodies (nAbs) capable of blocking one or more steps in hantavirus entry

Viral membrane fusion

Gn interacts cotranslationally with Gc to form a metastable heterodimeric prefusion complex

The acidic endosomal milieu triggers conformational changes in the glycoprotein shell

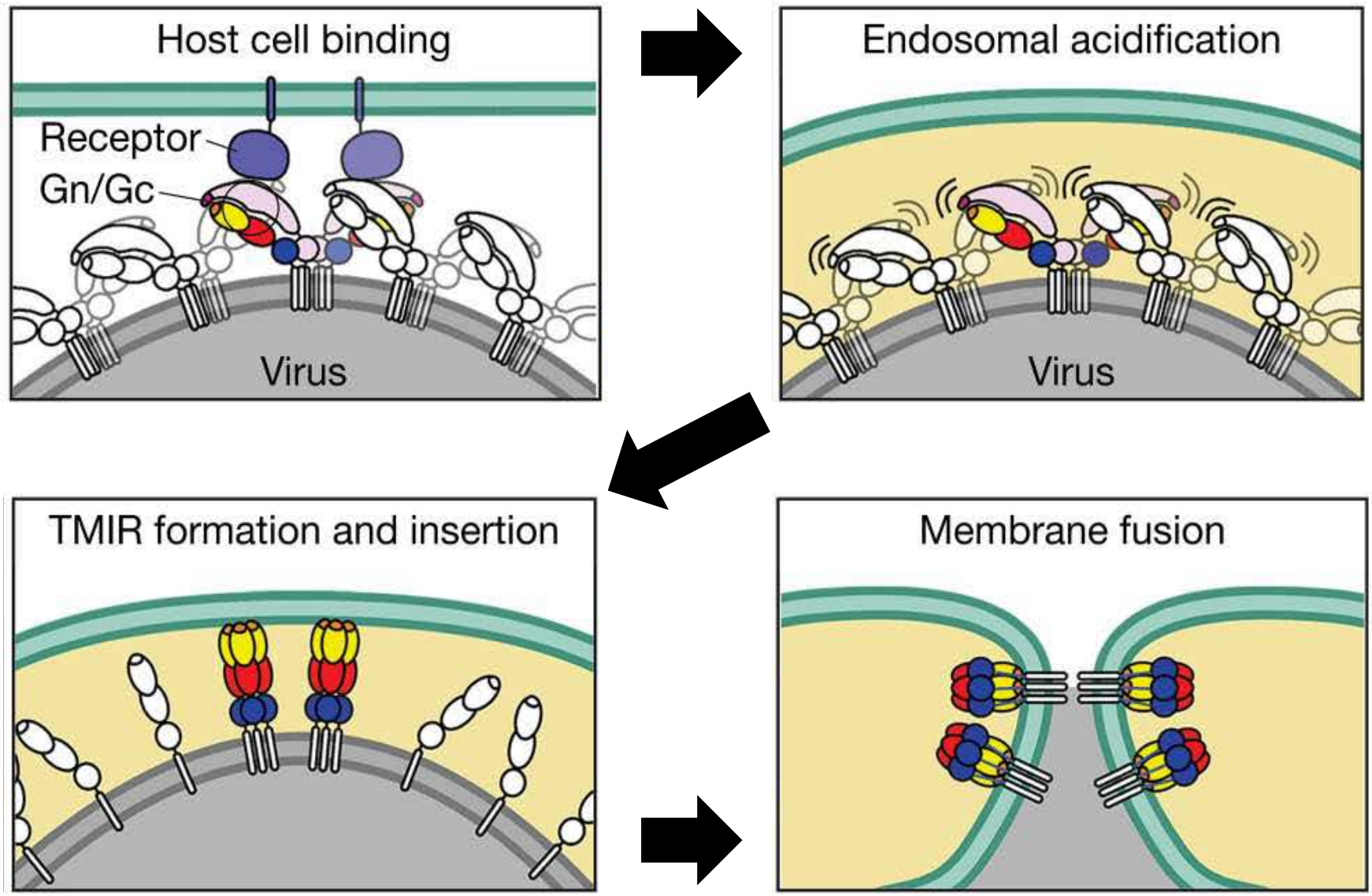
The prefusion complex dissociates as follows

- The domain II reorganizes to form a target membrane interacting region (TMIR) that inserts into the endosomal membrane

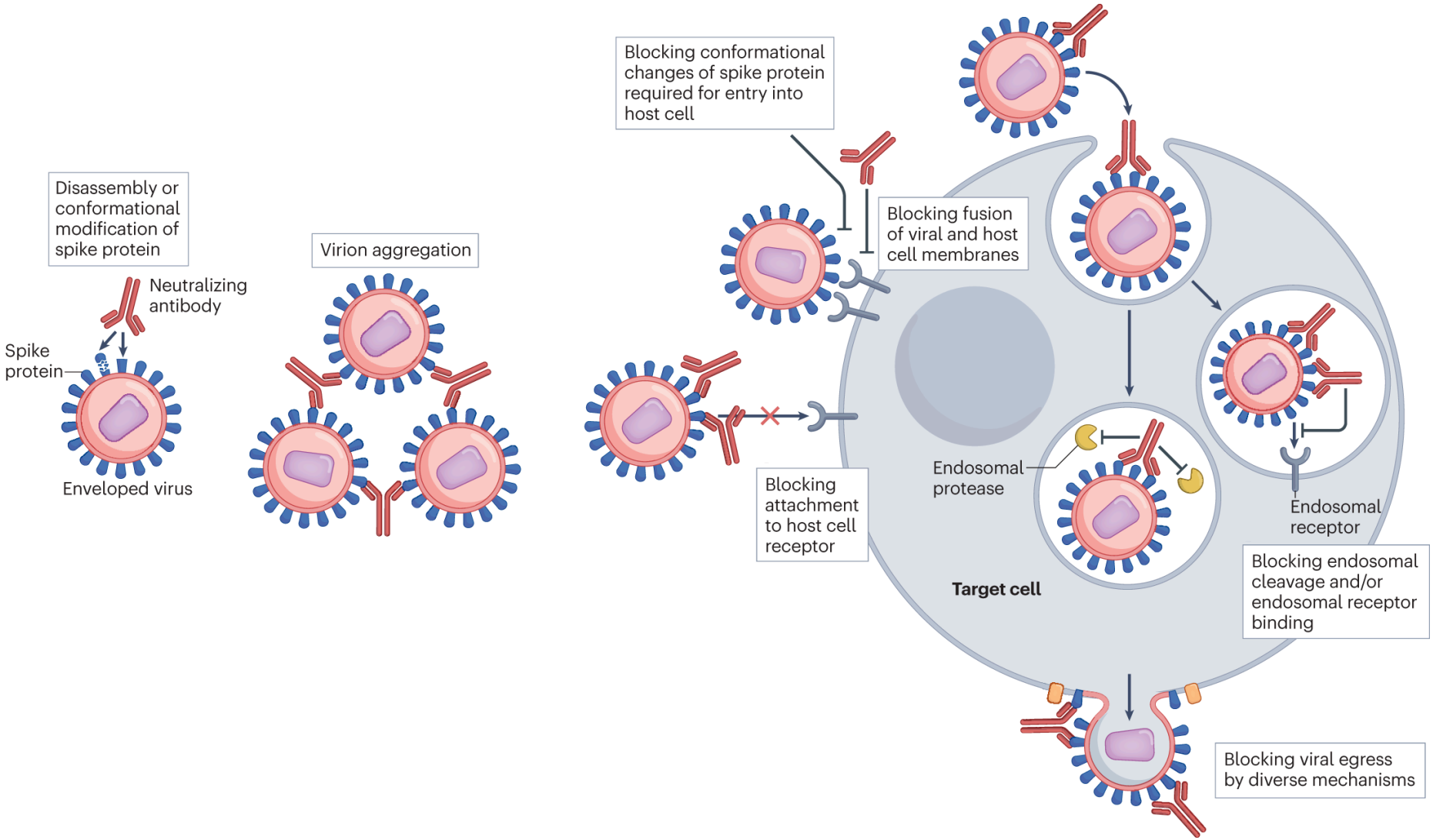
- TMIR brings domain III closer to the endosomal membrane

These structural rearrangements lead to the formation of an energetically favored post fusion trimer while driving the merger of viral and endosomal membranes

Viral membrane fusion



Neutralizing antibodies



ADI-42898

A rare neutralizing antibody (nAb) isolated from a PUUV-convalescent donor

- Recognizing a quaternary epitope spanning Gn and Gc

Neutralized a large panel of divergent OWHs and NWHs

Protected against viral infection in a bank vole reservoir model of PUUV

Protected against infection and lethal disease in an ANDV Syrian hamster model

Candidate for broad antihantavirus prophylaxis and therapy

ADI-42898 prevented capture of rVSV-ANDV-Gn/Gc by a specific hantavirus receptor, protocadherin-1

ADI-42898 blocks viral membrane fusion

Mechanisms

1) ADI-42898 directly blocks viral membrane fusion, or it elutes bound virions from the cell surface

Despite this activity, ADI-42898 could not prevent viral attachment to PCDH1 bearing endothelial cells

2) ADI-42898 recognizes and stabilizes the Gn/Gc prefusion complex, so viral membrane fusion is not carried out

- Inhibition of the acid induced Gn/Gc rearrangements
- Preventing formation of the TMIR
- Preventing insertion of the TMIR into the endosomal membrane

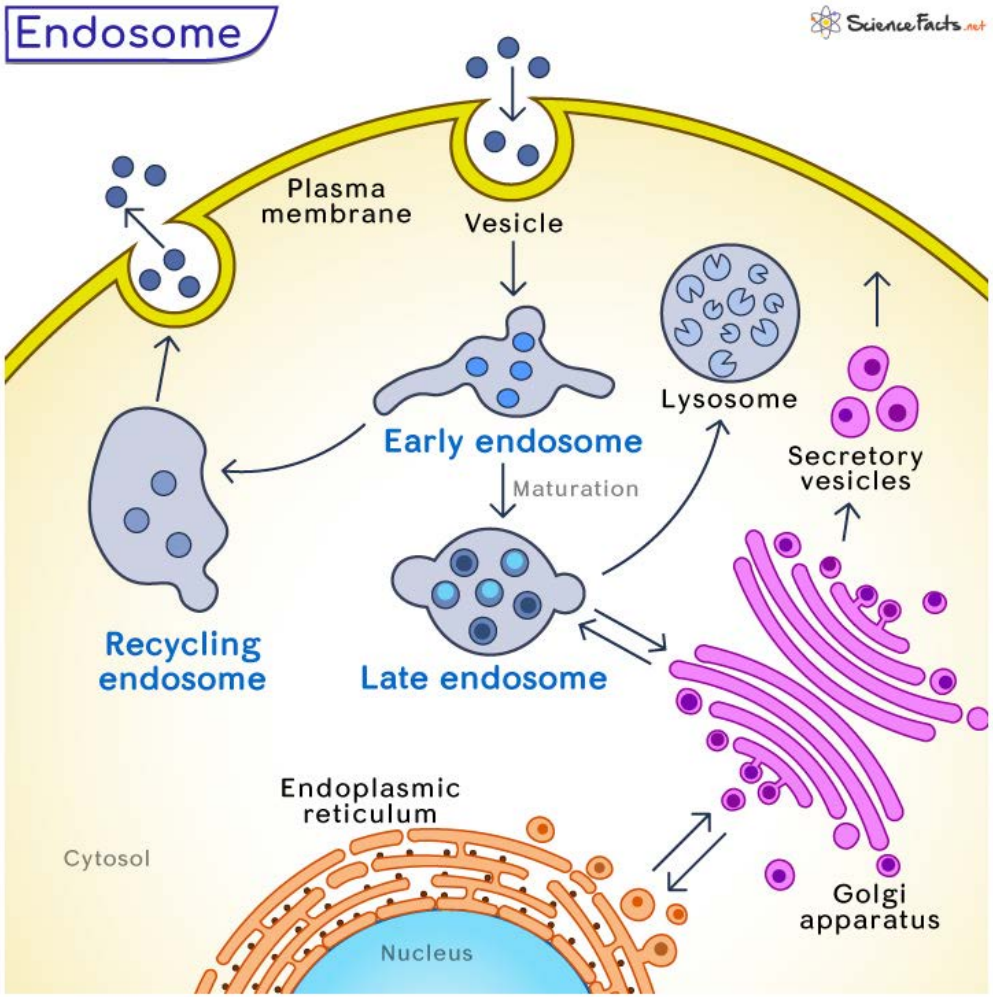
In Syrian hamsters a single 6-mg/kg dose of ADI-42898 was highly protective when administered 3 days after virus exposure

ADI-42898 lability to endosomal pH

Immunocomplexes are labile at the acidic pH of late endosomes (pH 5)

Although both PUUV and ANDV GnH/Gc dissociated, in the ANDV complex was markedly less stable at acidic pH than its PUUV counterpart

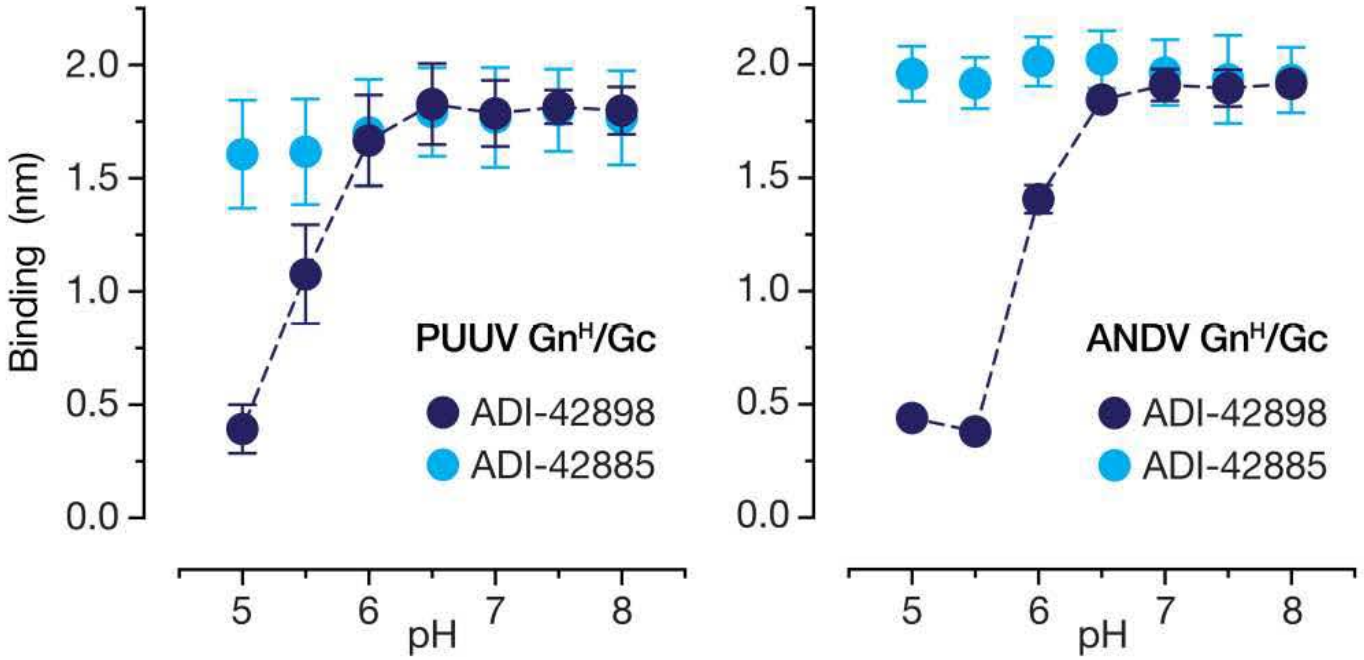
The potency of ADI-42898 against ANDV is greatly diminished relative to PUUV because a larger proportion of the antibody molecules bound to ANDV virions (affinity)



ADI-42898 lability to endosomal pH

ADI-42885 is a neutralizing Gc directed monoclonal antibody (mAb)

The acidic environment of the ANDV endosome increases the dissociation of ADI-42898 from the Gn/Gc tetramer



How the affinity of ADI-42898 was improved

They generated a panel of 1020 Fabs carrying single CDR mutations

- Twelve clones bearing eight distinct amino acid exchanges showed improved off-rates to ANDV GnH/Gc

- 26 progeny Fabs with improved and parent-like binding affinity for ANDV and PUUV GnH/Gc

Of these, ADI-65533 and ADI-65534 were selected because they displayed a great enhancements over ADI- 42898 in neutralization of Gn/Gc of ANDV

Both mAbs showed a lack of polyreactivity in human serum, there was no accelerated clearance or off-target binding.

How the affinity of ADI-42898 was improved

ADI-65534 has three amino acid exchanges in

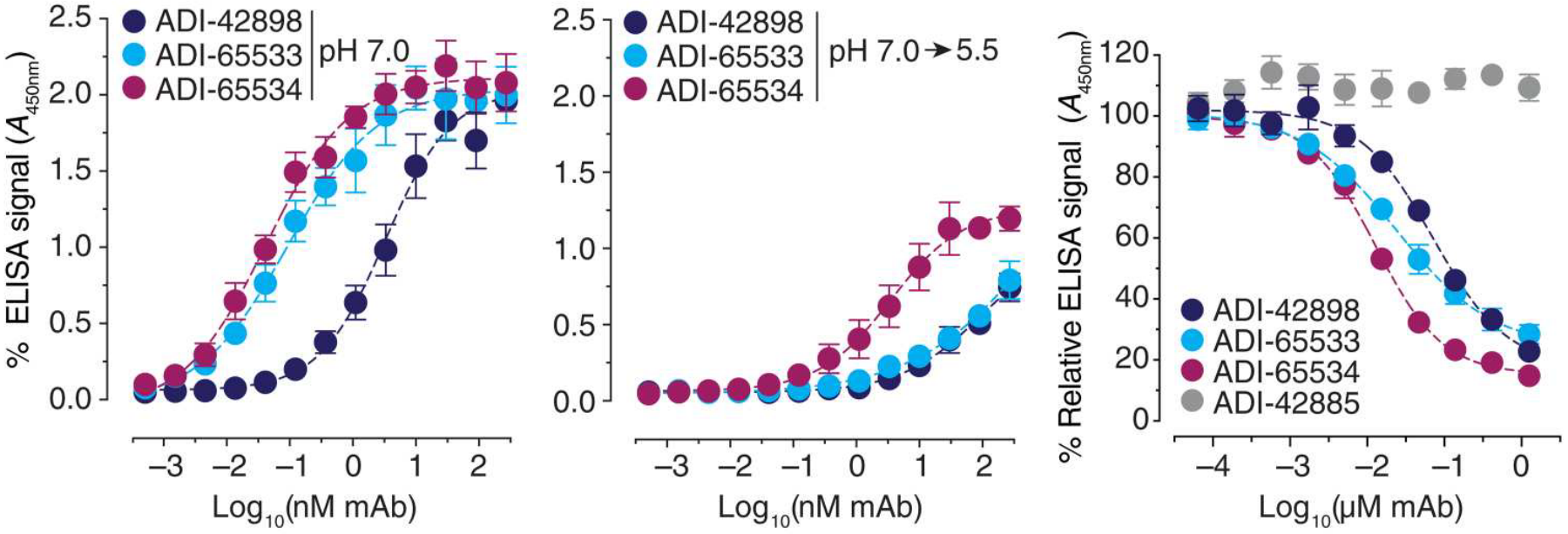
- G100a(H3)V
- C32(L1)V
- T28E in the CDR-H1.

The introduction of a V at this position might allow the formation of additional van der Waals interactions with residue Q768 in ANDV Gn/Gc

The C32 does not contact Gn/Gc but is part of a cluster of hydrophobic amino acids that includes F100d in CDR-H3 and thus may indirectly stabilize the conformation of that loop.

The T28E mutation drives the formation of two Van der Waals bonds, making ANDV interactions stronger.

ADI-65534, better affinity than ADI-42898



So, is ADI-65534 better?

With a 2-mg/kg dose:

- ADI-42898 provided only 50% protection, but ADI-65534 afforded about 85% protection of lethal ANDV in Syrian hamsters, in a pre-exposure

Affinity maturation of ADI-42898 to ADI-65534 did not compromise efficacy against PUUV, because both antibodies afforded equivalent protection in a **pre-exposure** setting in a bank vole model of PUUV

So, is ADI-65534 better?

In a follow-up study, Hamsters are inoculated intramuscularly with 200 plaque-forming units (PFU) of ANDV, and viral titers are measured in lung tissue and serum daily.

Hamsters were treated with a single dose of 25 mg/kg of ADI-65534 on day 6 or 7 after ANDV challenge (when viral replication was approaching its peak).

All animals in the vehicle group succumbed to viral challenge

ADI-65534 protected more than 80% of the hamsters treated on day 6 or 7

Take-away, Food for thought

There is no FDA approved therapy against Hantavirus

ANDV is the only hantavirus that spreads from human to human.

Potential therapeutical strategies involve vaccines, mAb, and proteins that block viral entry.

This article proposes a neutralizing antibody that prevents viral membrane fusion: (ADI-65534).

ADI 65534 is useful against ANDV (New World Hantavirus) and PUUV (Old World Hantavirus), for pre-exposure and postexposure prophylaxis.

RVPVE

Red de Vigilancia de Patógenos Virales Emergentes

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