Structural Biology Meets Clinic Question Roster

Short talks: Structural Biology (Host: Tobias Madl)

Questions for Mateusz Sikora & Martin Beck

Q: Would you believe that the S-protein can only bind to ACE2? E.g. Heparin sulfate, tmpssr2, lectin-like receptors + potentially others

Matt: There are other binders, e.g. Heparin sulfate, tmpssr2, lectin-like receptors + potentially others

Q: is it known how many Spike proteins are essential for membrane fusion?

Matt: Not aware of such study.

Q: is on the spike proteins binding sites for Ca2+ or for other ions?

Matt: There seems to be no indication of ions binding to the S.

Q: Can you say anything about the M protein?
Matt: M has three transmembrane helices, long tail than might interact with N-protein/RNA and only very short extraviral tail, which is glycosylated. In the cryoET images M appears as transmembrane stripes that appear rather dense and with a different degree of regularity.

Questions for Christian Löw

Q: Did I understand it correctly that the nanobody libs were just 'there'? You didn't have to make them first? In case you did - how did you diversify them so much?

Christian: The libraries have been developed in the lab of Markus Seeger at the University of Zurich. We use it for selecting binders against different membrane proteins. Details on how the libraries were generated you can find below. To answer your question - yes we have the libraries in the lab and are ready to use for selections.

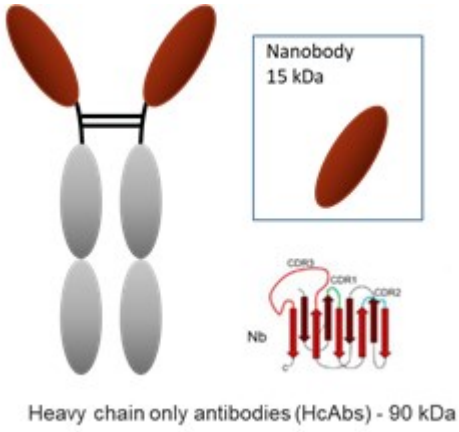
1.) Synthetic single domain antibodies for the conformational trapping of membrane proteins. Elife 2018 - PMID: 29792401
2.) Generation of synthetic nanobodies against delicate proteins.
Nat Protoc. 2020 - PMID: 32269381

Q: How did you screen the big libs, before you went into ELISA plates?

Christian: We do one round of ribosome display and two rounds of phage display (including an off-rate selection). We need the antigen in a biotinylated and free form (ca. 200 ug in total). We follow the enrichment levels by RT-PCR.

Q: I didn't understand the Fc-construct maybe you can explain that again.

Christian:



Left: Fc-nanobody construct: Fc part with 2 nanobodies (avidity effects, but larger)

Right: nanobody only (only one binding site but significantly smaller)

Questions for Chris Oostenbrink

Q: What do you think. which NB-affinities are necessary to overcome the glycan barrier?

Chris: This is maybe also a question for Christian Löw. The nanobodies he talked about were mostly binding to the tip of the RBD, where the glycosylation is least. I don't think it is a matter of designing NB with sufficient binding affinity that they can overcome the glycan barrier but to design or select NBs that bind to Spike in the presence of the glycans.

Christian L: In case selected binders compete with the ACE2 binding site, neutralizing binders need to have an affinity which is higher than RBD-ACE2-binding. In our case glycans did not play a significant role; this might be different for "real antibodies" due to larger binding interfaces.

Q: ACE2 is not primarily a binding site for the virus. Might the deglycosylated form interfere with

the native ACE2 function?

Chris: Very true. There are several points to consider: 1) actually the suggestion is to deglycosylate the soluble version of ACE2 only, such that the membrane bound ACE2 is still functional; 2) the soluble ACE2 is indeed still helping to regulate the blood pressure and is an active enzyme; 3) interestingly, the deglycosylation only affected the enzymatic activity of soluble ACE2 to a small extent (some 75% of the glycosylated version).

Q: if one to seven copies of spike proteins are enough for infection, wouldn' t you need lots of soluble ace protein to neutralize?

Chris: Yes. Basically the same amount as you would need of a neutralizing antibody that works with the same principle (blocking the interaction). This is the reason why enhancing

the affinity of soluble ACE2 compared to the native ACE2 is so important. Even a little improvement can make a difference here.

Q: Is it known whether the characterized antibodies against the spike protein are directed more against protein or against glycan epitopes?

Chris: I think they are mostly directed against the protein. A purely glycan epitope would be very dangerous, as these are intrinsically human glycans, which occur at many other places.

Q: Is there a link between glycan and protein dynamics ?

Chris: Certainly. It is important to realize how big the glycans actually are with respect to the proteins. Attaching such a big molecule to a protein will always affect the protein dynamics. Still, the intrinsic flexibility of the glycans is usually much bigger than the flexibility of a typically folded protein.

Q: Do the soluble ACE2 proteins still have enzymatic activity (angiotensin conversion) and might this interfere into human physiology (inflammation, ..) other than only neutralizing?

Chris: See also above, let me still add: this is actually considered to be an advantage of soluble ACE2.

Short talks: Medical/Clinical Research (Host: Peter Schemmer)

Questions for Christian Gruber & Karl Gruber

Q: Why was the 'halo' study done without glycans? Do you think it would make sense to generate ACE2-analog libraries as a response to RBD/Spike mutations that are known now?

Christian: We compared the positions of known glycan sites on the spike with our model of the spike/ACE2 complex. In our opinion, we could neglect the effect of the glycans in this region, which we were most interested in near the flexible RBD loop around S477. Nevertheless, we think the glycans are important, but since we also use (in the lab) the unglycosylated ACE2, we decided to use the simplest possible system.

Q: If computing power would be enormous, would you still need experimental methods to validate if you are correct?

Christian: The final validation will always require an experimental proof. However, predictions can dramatically decrease the number of experiments that have to be carried out.

Q: Which will be the next steps to go for you?

Christian: The most promising candidates will now undergo further pre-clinical testing. Since the number of (preliminary) approved drugs is still small, we continue screening and carry out lead further optimization for several candidate drugs.

Questions for Vanessa Stadlbauer

Q: Chronic liver diseases are often associated with overweight and obesity. Many of these patients are using Metformin. Is there anything known about that drug and Covid-19?

Vanessa: On the one hand, patients with diabetes have a higher risk for a more severe disease course. On the other hand metformin use was associated with decreased mortality in an uncontrolled retrospective study. Several molecular mechanisms have been proposed, however, it is unclear yet whether metformin has beneficial, neutral or detrimental effects in Covid-19 disease.

Q: How does structural biology affecting your research?

Vanessa: Hopefully by developing therapies that I can use in my patients in clinical and translational studies.

Q: Is it known if the half-life of sACE2 in the gastrointestinal tract is long enough that oral administration could be used to help systemic treatment/treatment of the symptoms you described

Vanessa: I am not aware of any studies where sACE was applied orally to humans (or animals), so unfortunately I do not know

Chris O.: hrsACE2 was administered to several patients, but intravenously. See e.g. https://doi.org/10.1016/S2213-2600(20)30418-5

Gustav: Thanks! But I guess the naive question is - if you can take it orally and it survives long enough, do you get it to where it should go? Would be way easier to distribute and administer.

Chris O.: Absolutely. I am not a pharmacist and don't know too much about formulation, but I would be skeptical, that it survives the stomach.

Gustav: I agree. But could a version be engineered that would survive long enough. Anyway, I don't know if it would go where it is supposed to go.

Q: Do gut and liver cells have the same (ACE2) receptors as the cells in the lungs? In the same concentration?

Vanessa: As far as I know the ACE2 receptor in intestinal cells is the same as in the lung and its expression is equal in lungs and colon, and higher in small intestine. See e.g. PMID 32345362

Q: Which other illnesses are there which have a combination of lung and digestive symptoms? Why ?

Vanessa: Several viral infections (e.g. other coronaviruses, rotavirus, influenza) as well as bacterial infections (legionella) and other non-infectious diseases can cause both lung and digestive synptomes. Like in Covid-19, it can be a direct effect of the viruses or bacteria that bind certain receptors or it can be an indirect effect via inflammation.

Questions for Horst Olschewski

Q: In light of the findings that endothelial damage is a very important factor, what do you think about ventilation ?
Horst: Mechanical ventilation is mostly necessary because patients get exhausted due to highly elevated ventilation.

Q: Are there any negative effects known for ventilation ?
Horst: Positive pressure ventilation is beneficial in case of capillary leakage.

Q: Do the endothelial cells have more ACE2 receptors than others? Is there a difference between endothelial cells in lungs and gut?
Horst: It seems that endothelial cells do not possess the ACE2 receptor.

Q: What can you say about the connection of COVID-19 to Asthma?
Horst: Asthma is not a risk factor for death from COVID.

Q: Is an endothelial damage in severe asthma also observed?
Horst: No.