Plant Systems Biology
A VIB-UGENT DEPARTMENT

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Engineering chimeric antibodies aimed for passive mucosal immunization against HRSV

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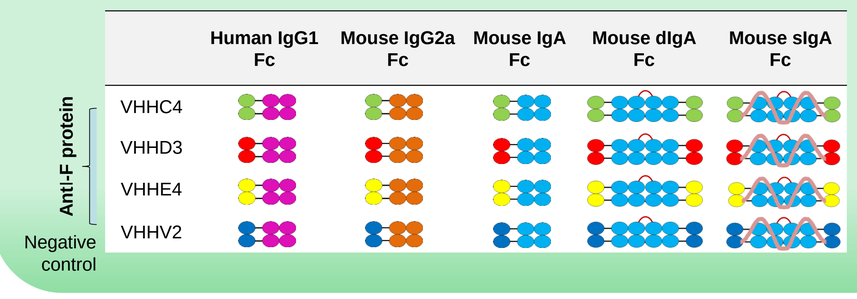
Human Respiratory Syncytial Virus

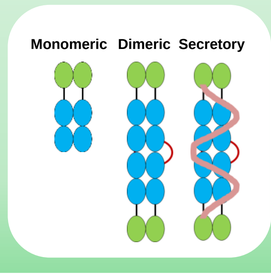
Human Respiratory Syncytial Virus (HRSV) is the leading cause of acute lower respiratory tract infection in infants and frequently causes severe disease in the elderly. There is no licensed HRSV vaccine. As an alternative, the prophylactic treatment with an HRSV fusion protein targeting monoclonal antibody (mAb) is often recommended for infants that are at risk for developing HRSV infection. However, the high cost that is associated with the current mammalian cell-based mAb manufacturing systems hampers the broad implementation of this therapy. Also, it is possible that IgA type antibodies against HRSV may contribute even better to protection. Therefore, we aim to compare the effectiveness of IgG with secretory IgA based passive immunization against HRSV. In this context, we choose for the transient expression platform in plants that permits rapid small scale production of IgG and IgA based antibody versions [1].

Our Approach: We have genetically fused three different single domain antibodies [1] that are specific for the HRSV fusion protein F to the fragment crystallizable part (Fc) of different murine and human monomeric IgA and IgG antibodies. In order to obtain all different permutations we took advantage of the GoldenBraid2.0 [2] cloning system.



A total of 15 different versions of chimeric antibodies against HRSV and 5 negative controls have been engineered and produced in *Nicotiana benthamiana* via *Agrobacterium tumefaciens* – mediated transient expression.

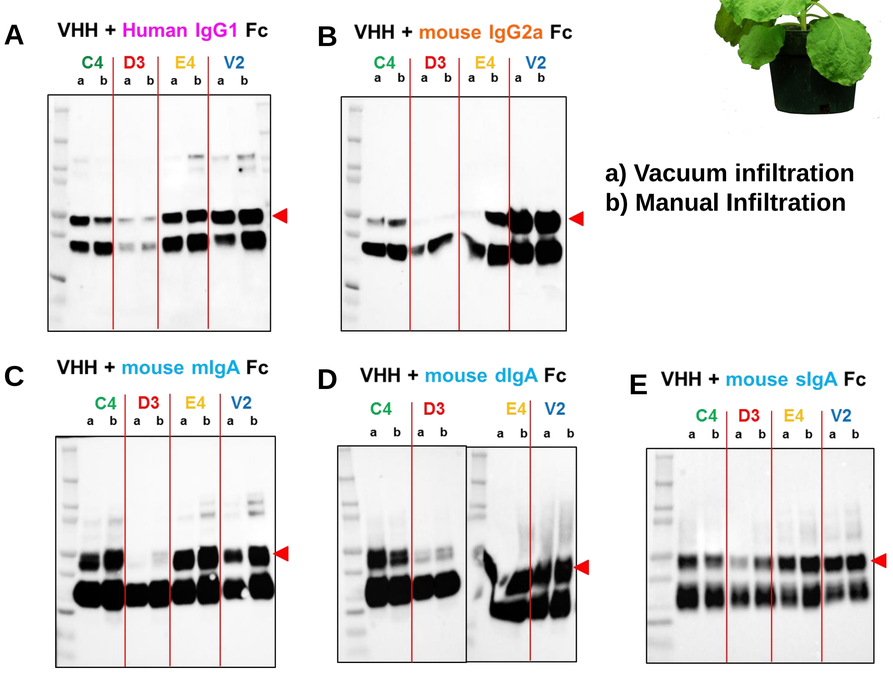
Since secretory IgA are the predominant antibodies in mucosal surfaces, they might be more effective than monomeric IgA and IgG in virus neutralization; therefore, IgA based antibodies will also be tested in their dimeric and secretory forms.



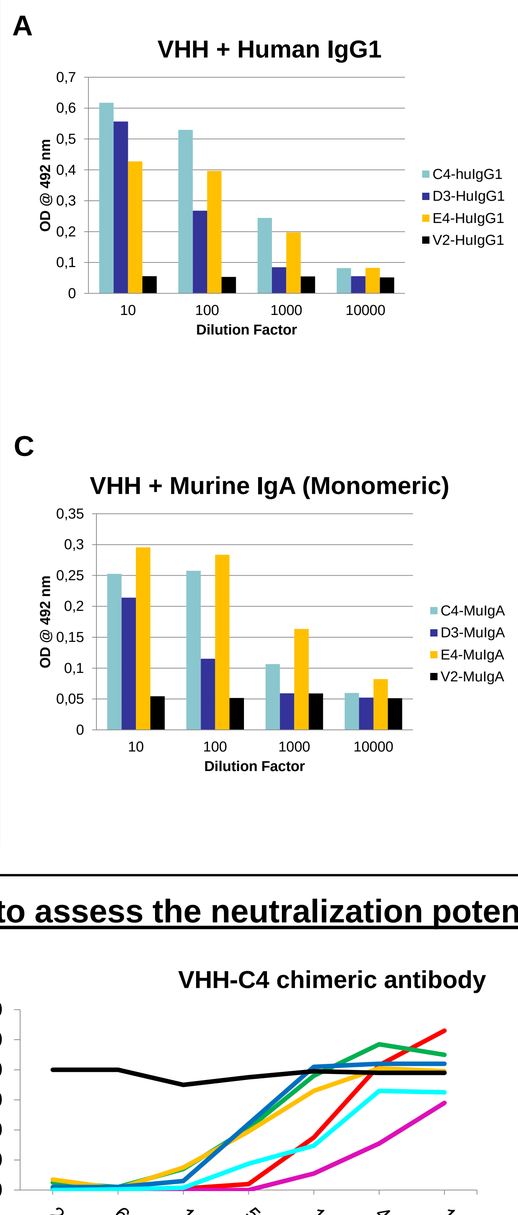
Expression of the chimeric antibodies by agro-infiltration in *N. benthamiana* plants

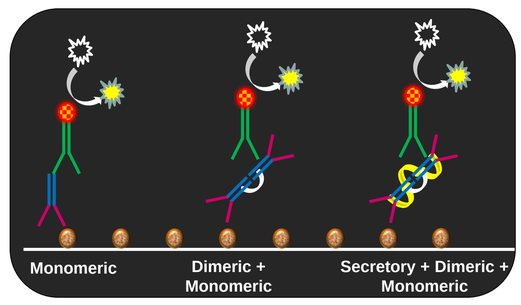
By means of combinatorial approaches using novel synthetic biology tools, it is possible to transiently express 20 different chimeric antibodies against RSV in *N. benthamiana*,.

a) Vacuum infiltration
b) Manual Infiltration

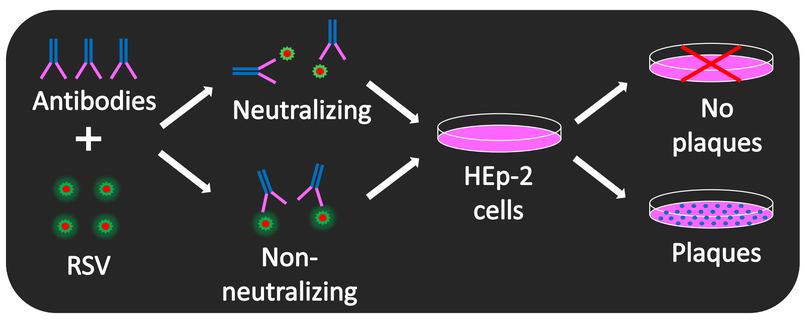


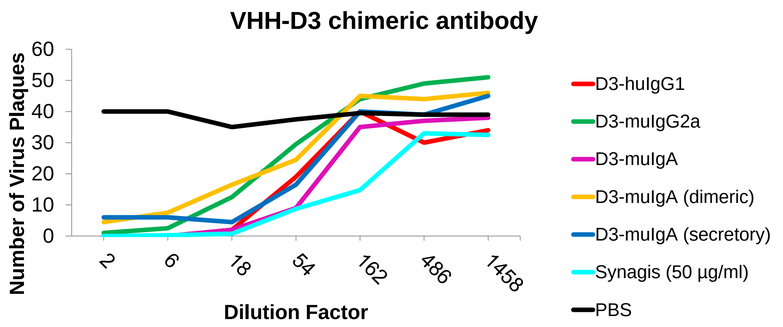
All the RSV specific antibodies showed binding activity for Fusion protein.

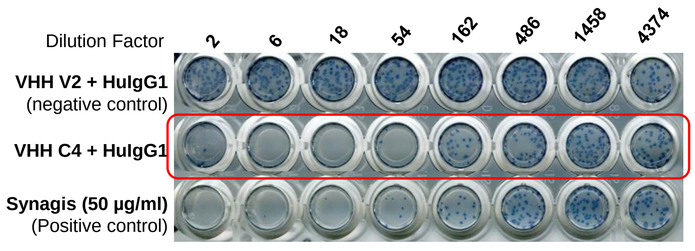


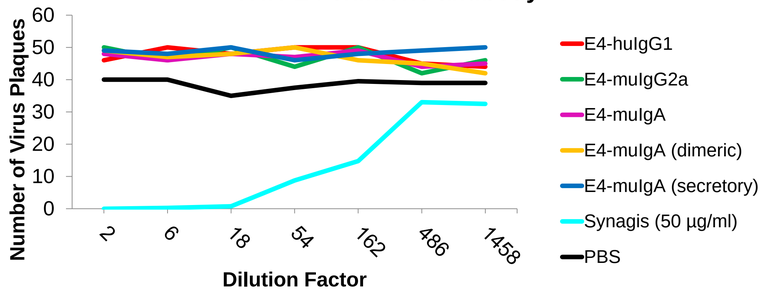


Plaque reduction assay to assess the neutralization potency of RSV specific chimeric antibodies









Conclusions

- E4 nanobodies with the highest binding activity levels do not neutralize RSV infection.
- A high binding activity of a given Nanobody® does not translate to a high neutralizing activity.

Future Perspectives:

- Protein quantification to compare efficiency of our chimeric antibodies with commercially available antibodies.
- Fc effector function: *In vivo* (mouse models) comparisons will be done to test the effect of Fc tails. E4 antibodies (binding, but not neutralizing) will be useful for this purpose.
- Production of the most efficient neutralizing antibody in Arabidopsis seeds and challenge experiments in animal models.

1. Schepens, B., et al. (2011). "Nanobodies(R) specific for respiratory syncytial virus fusion protein protect against infection by inhibition of fusion." J Infect Dis **204**(11): 1692-1701.
2. Sarrion-Perdigones, A., et al. (2011). "GoldenBraid: an iterative cloning system for standardized assembly of reusable genetic modules." PLoS ONE **6**(7): e21622.

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