Novel Genetic Immunization for the Development of Antibodies Targeting Conformational and Native Antigens for Diagnostic and Therapy: Application to SARS-Cov-2

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Abstract

The recent COVID-19 pandemic shows the limitation of the conventional vaccination which uses an antigen such as peptide, recombinant protein or attenuated virus… The genetic immunization based on in vivo transfection of RNA or DNA gave a strong and consistent response. These simple and efficient approaches share a common point, the antigen is displayed conformationally to the immune system due to the in-vivo expression of native antigens. Since few years, Covalab has developed its own technology for DNA immunization which was applied to tumor antigens for a proof of concept (1). Then improved technology was used to develop antibodies against SARS-CoV-2 proteins (S, RBD, N, M and E). The easy-to-use method do not require any specific material such as gene gun or electroporator allowing its application to various animals (mouse, rat, rabbit, ship, goat, lama, …). In Different comparative immunization studies using DNA versus recombinant protein (standard method), the DNA immunization showed a faster immune response, a better ratio IgG/IgM and high affinity and specificity of the antibodies. Thanks to its novel DNA-immunization technology Covalab has now one potent neutralizing antibody (nAb) which is currently in preclinical validation against all the variants

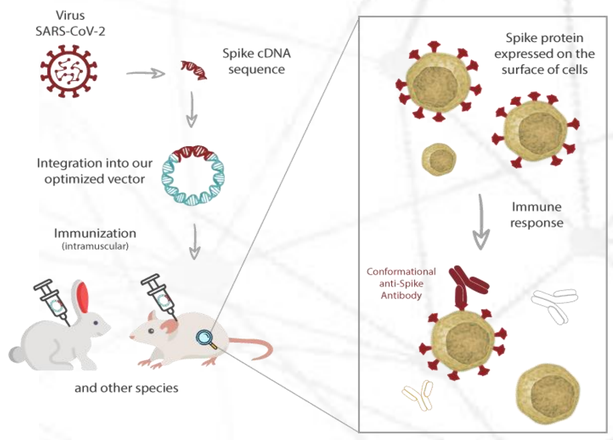
(1). Dubuisson et al. Generation and characterization of novel anti-DR4 and anti-DR5 antibodies developed by genetic immunization. Cell Death Dis. 2019 Feb 4)

Introduction

DNA immunization is more useful than traditional approaches to generating mAbs against conformational and more difficult targets, especially membrane proteins.

Traditional protein-based immunization approaches have difficulty producing full-length protein immunogens by the recombinant protein method if the proteins are naturally expressed in a membrane-associated format, such as Spike protein. The DNA immunization approach can circumvent these problems because full-length proteins can be expressed *in vivo* when they are delivered in the form of DNA vaccines. Furthermore, it is well known that the structural integrity of proteins is critical for the induction of functional mAbs. Expressing intact immunogens *in vivo* by DNA immunization appears to have the best chance of inducing mAbs with the desired biological activities.

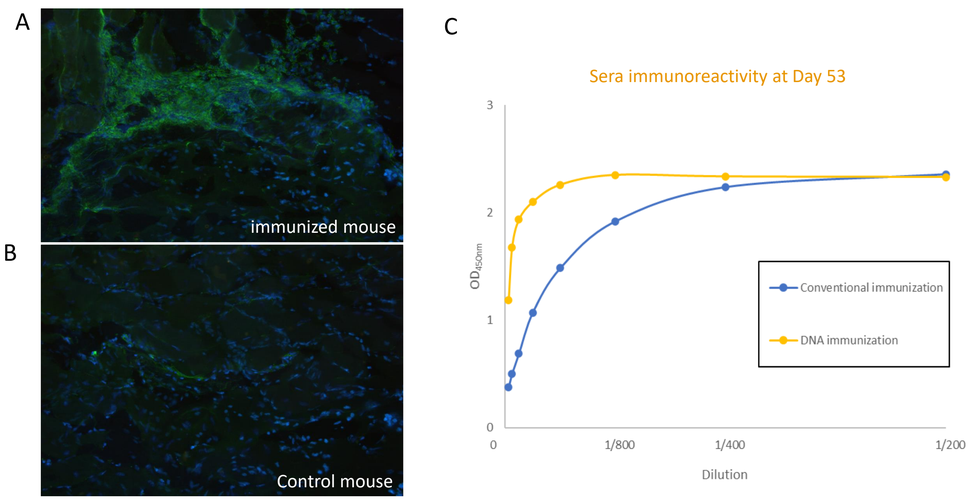
In this exemple, optimised plasmid contaning cDNA of SARS-Cov-2 proteins (full S, S1, RBD and N) were constructed and used to immunize host animals (mouse & rabbit). Critical factors such as immunogen/plasmid design, delivery approach, immunization schedule, use of immune modulators (adjuvants) were studied to improve our technology to be unique and powerful approach.



Plasmid construction & Immunization

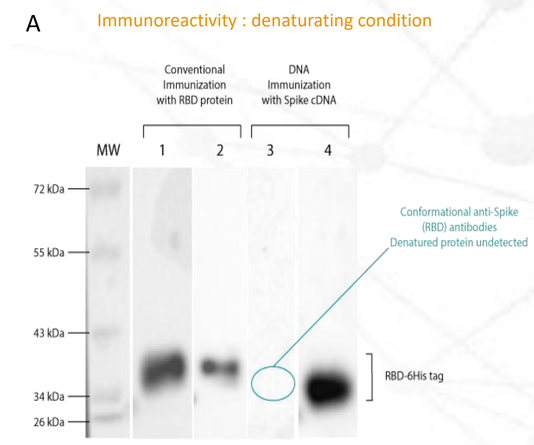
IHC-F ***(Novotec-Lyon)***

IHC-F analysis of SARS-Cov-2 full lenght Spike protein expression *In vivo* using anti SARS-CoV-2 Spike antibody: (A) Immunzed mouse (intramusculaire). (B) control mouse. (C) Mice immune-response followed by ELISA, comparing conventional immunization (recombinant protein, blue curve) and DNA immuinzation (yellow curve), 53 days post primo injection.



WB & Flow Cytometry

Comparative study of the immunoreactivity of the antibodies developed by DNA and conventional immunizations: (A) Western-Blot analysis using RBD protein in denaturation conditions. (B) FACS analysis of the reactivity of the antibody anti-S protein using hACE2 expressed in CHO cells and S1-subunit to form the natural complex.



Lane 1 & 3: *SARS-CoV-2 Spike RBD reveled with Mouse serum (anti-RBD antibody)*
Lane 2 & 4*: SARS-CoV-2 Spike RBD reveled with an anti-His-tag antibody*

Affinity Study (JG Delcros CRCL-Lyon)

Affinity of Mabs from DNA immunisation was evaluated by bilayer interferometry (BLI) method using SARS Cov 2 RBD protein (n=3).

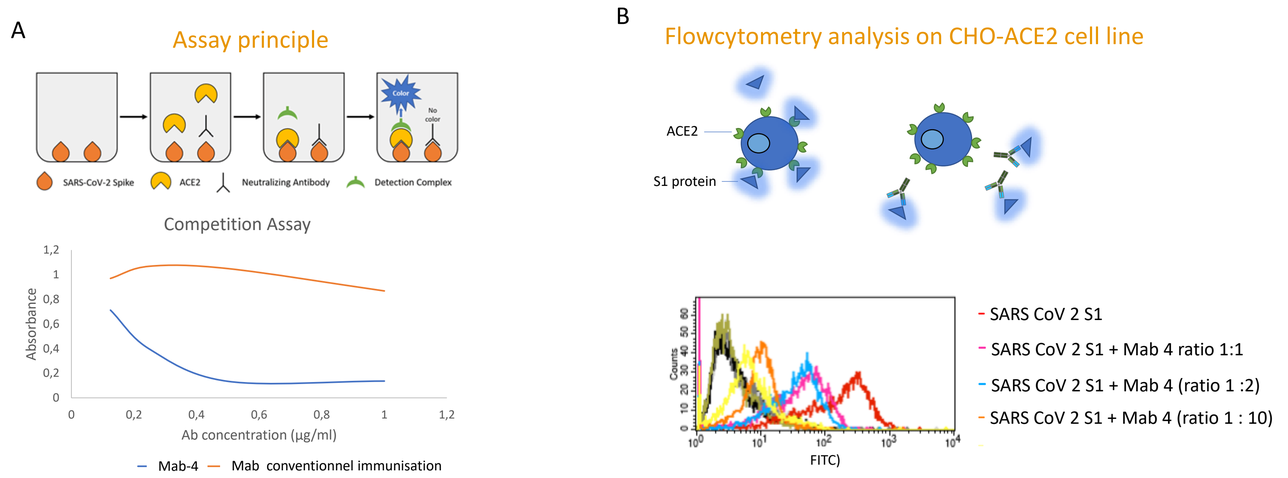
Antibodies affinity

Antibody	Kd (nM)
Mab-1	0,411±0,179
Mab-2	0,266±0,064
Mab-3	5,54±0,97
Mab-4	0,048±0,037

Competition assays

A: Blocking potency comparison for both immunizations method by competitive ELISA assay using coated SARS-Cov-2 S1-subunit and revealed by HRP labelled hACE2 protein.

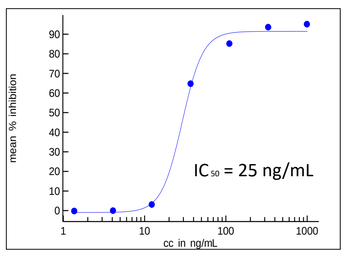
B: Determination of the antibody blocking effect by FACS using 6 His-SARS-Cov-2 S1 subunit. The residual fixation of 6 His-SARS-Cov-2 S1 subunit was revealed by anti 6 his-FITC. Various molar ratio of protein-antibody were pre incubated.



In vitro Ab-neutralization

(Oncodesign-Dijon)

A



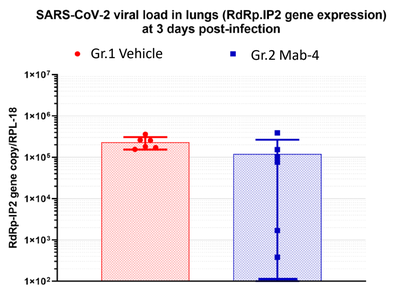
A: EC_{50} determination for the ability of Mab-4 to neutralize viral-induced vero cell death in the CPE assay.

B: Antiviral efficacy assessment of one treatment (Mab-4) in Syrian Golden Hamster intranasally infected with SARS CoV 2 by genes copy quantification (RdRp.IP2). Mean +/- SEM (n=6)

In vivo Ab neutralization

(Voxcan-Lyon)

B



Conclusion

The developed method of “DNA-designed antibodies” is time and cost effective, does not require protein purification and enables generation of antibodies targeting membrane-anchored and glycosylated proteins.

In the current pandemic context of SARS-Cov-2, there is a great demand for effective therapies for the prevention and treatment of COVID-19. The advances of antibody technologies have greatly accelerated the discovery of SARS-Cov-2 neutralizing antibodies (nAb). Thanks to its novel and validate DNA-immunization technology Covalab has now one potent nAb which is currently in preclinical validation against all the variants.