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Analysis of Spike-specific immunity induced by IDLV-Spike in mice: comparison between females and males

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Introduction

- Studies on vaccines against several pathogens indicated higher immunoreactivity in women than in men[1].
- Few COVID-19 vaccine studies reported sex-disaggregated vaccine efficacy data from both preclinical and clinical studies[2].
- We showed that Integrase-defective Lentiviral Vector (IDLV) delivering the optimized Wuhan Spike protein with cytoplasmic tail truncation, D614G mutation, double proline substitutions and mutated furin cleavage site, was highly immunogenic in mice[3].
- Here we compared the immunogenicity of IDLV expressing the optimized Spike from Wuhan (IDLV-S-Wu) or Omicron BA.1 VoC (IDLV-S-BA.1) in both female and male mice.

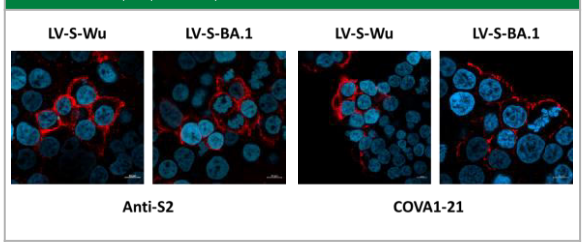
Methods

- Simian Immunodeficiency Virus (SIV)-based IDLV-S-Wu and IDLV-S-BA.1 were produced by co-transfection in 293T.
- IDLVs were characterized *in vitro* for Spike expression and incorporation on vector particles by Confocal Laser Scanner Microscopy (CLSM) and Western Blot (WB).
- 6 female and 6 male BALB/c mice per group were intramuscularly immunized once with IDLV-S-Wu, IDLV-S-BA.1 or IDLV-Mock (control vector).
- Immune response was assessed monthly by ELISA and a neutralization assay based on LV expressing luciferase and pseudotyped with Spike glycoprotein[3,4].

Results

- To evaluate the *in vitro* expression of Spike proteins, 293T cells were transduced with LV expressing the optimized Spike from Wuhan or Omicron BA.1 VoC. After staining with anti-S2 polyclonal Ab or anti-S1 COVA1-21 monoclonal Ab, cells were observed at CLSM **(Fig.1).** Images showed that Spike proteins were efficiently expressed in transduced cells and localized at membrane level, confirming the ability of LV to deliver membrane bound antigens.

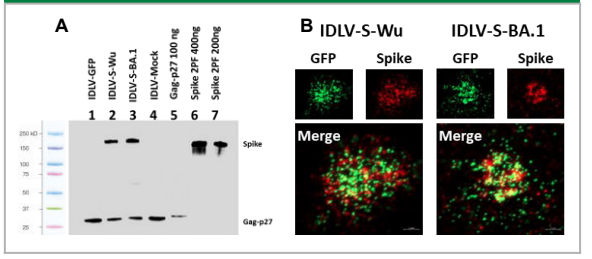
Figure 1. CLSM analysis of 293T cells transduced with LVs expressing Wuhan or Omicron BA.1 Spikes. Cells were stained with anti-S2 or anti-S1 COVA1-21 Abs (red). Nuclei were stained with DAPI (blue). Bars, 10 µm.



Results of 2

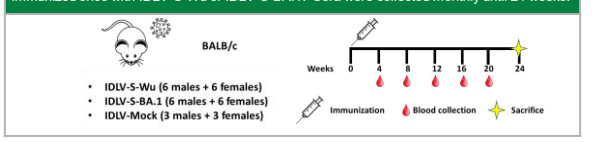
- To confirm the presence of pseudotyping Spike on vector particles, IDLV stocks were normalized for p27 Gag amount and analyzed by WB **(Fig 2A).** Filters were probed with anti-S2 and anti-Gag Abs. IDLV-GFP, IDLV-Mock, Spike 2PF and p27 SIVGag proteins were used as controls. A band corresponding to Gag p27 was detected in both IDLVs expressing Spike antigens as well as in IDLV-GFP and IDLV-Mock. Spike was detected in both IDLV-S-Wu and IDLV-S-BA.1 preparations, confirming that both IDLV-Spike were pseudotyped with Spike protein.
- The presence of pseudotyping Spike proteins on the vector surface was confirmed by CLSM using GFP-labelled SIV-based IDLV-S-Wu or IDLV-S-BA.1 obtained by including pSIVGag-GFP plasmid in the transfection procedure to allow for the incorporation of Gag-GFP fusion protein into IDLV particles. Staining with anti-S1 COVA1-21 Ab showed the co-localization of SIV-GagGFP (green) with Spike proteins (red) visualized as yellow dots in **Fig 2B**.

Figure 2. Spike expression on the vector particles. A) WB of lysates from concentrated IDLV-S stocks using polyclonal anti-S2 and anti-p27 Gag Abs. IDLV-Mock, SIV-Gag p27 and Spike 2PF proteins were included as controls. **B)** CLSM observation of GFP-labeled IDLV-S-Wu (left panel) and IDLV-S-BA.1 (right panel) immuno-stained with anti-S1 nAb. Bars, 5 µm.



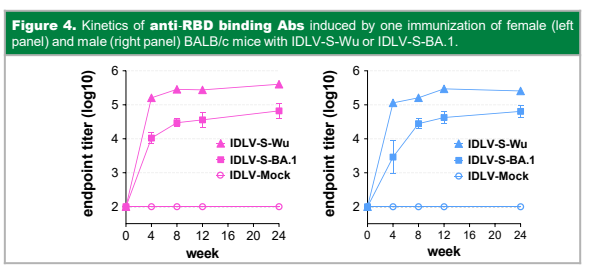
- To evaluate sex-related differences in immune responses, male and female BALB/c mice were immunized once intramuscularly, according the schedule shown in **Fig. 3.**

Figure 3. Immunization schedule. Six female and six BALB/c mice per group were immunized once with IDLV-S-Wu or IDLV-S-BA.1. Sera were collected monthly until 24 weeks.



- Immunization with IDLV-S-Wu or IDLV-S-BA.1 elicited specific and persistent anti-RBD Abs with similar kinetic in males and females **(Fig.4)**. Mice vaccinated with IDLV-S-BA.1 showed lower IgG titers than IDLV-S-Wu immunized mice, regardless of sex. This was expected since we used the RBD from Wuhan as coating protein in the ELISA assay.

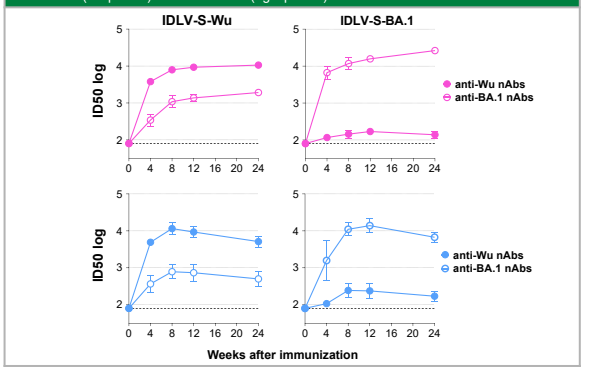
Figure 4. Kinetics of **anti-RBD binding Abs** induced by one immunization of female (left panel) and male (right panel) BALB/c mice with IDLV-S-Wu or IDLV-S-BA.1.



Results of 3

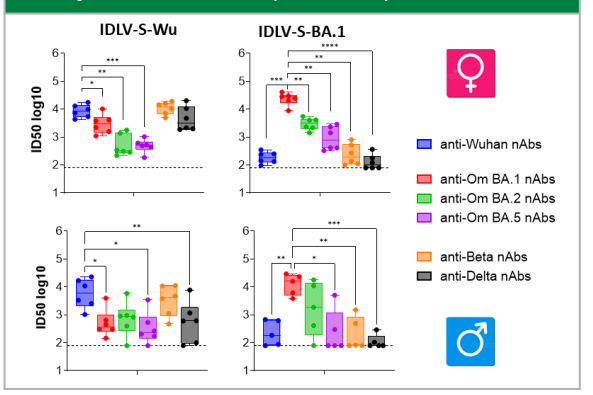
- Sera from vaccinated mice were also tested for the presence of nAbs **(Fig.5)**. All animals developed homologous nAbs that increased up to 6 months in females, while decreased after the peak response in males, regardless of the vector used for immunization. Furthermore, anti-BA.1 nAbs were detected in mice vaccinated with IDLV-S-Wu although at lower titers than homologous Abs, at any tested time-point and regardless of sex. Conversely, immunization with IDLV-S-BA.1 showed strongly reduced cross-neutralization against ancestral virus.

Figure 5. Neutralization activity against the Wuhan strain and the Omicron BA.1 VoC of SARS-CoV-2 in female (upper panels) and male (bottom panels) mice vaccinated either with IDLV-S-Wu (left panels) or IDLV-S-BA.1 (right panels).



- IDLV-S-Wu induced cross-nAbs against Delta, Beta, Omicron BA.2 and BA.4/5 VoCs. On the contrary, mice immunized with IDLV-S-BA.1 developed cross-nAbs only against the Omicron VoCs **(Fig. 6).** Importantly, females developed higher cross-nAb titers than males regardless of the vector used as a vaccine.

Figure 6. Analysis of cross-neutralization in female (upper panels) or male (bottom panels) mice vaccinated with either IDLV-S-Wu (left panels) or IDLV-S-BA.1 (right panels). Asterisks indicate a statistical significant differences between ID50 against the vaccine strain and ID50 against the other VoC, measured by mixed effect analysis.



Conclusion

- IDLV delivering a rationally designed pseudotyping Spike is an efficient vaccine platform for inducing long-lasting functional immune response.
- IDLV-S-Wu induced a better cross-neutralizing response than IDLV-S-BA.1, as observed in humans vaccinated with mRNA.
- nAbs, but not anti-RBD binding IgG, are significantly different between females and males.

References

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