DISRUPTIVE IN VIVO RATIONAL ANTIBODY DISCOVERY:

A SUCCES CASE IN HIGH-HOMOLOGY PEPTIDE TARGET

AUTHORS

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ABSTRACT

The generation of monoclonal antibodies against low immunogenic antigens, remains a challenging task in biotechnology. Here, we applied the RedMabs platform to a human neuro-amino acid peptide of 28 residues, which is highly conserved in mice, to overcome these challenges. Using two groups of ten Balb/c mice, we compared the effectiveness of RedMabs immunization with standard protocols. Our results showed a dramatic improvement, with RedMabs producing 42 positive clones compared to only 2 from the standard method, a 21-fold increase. Additionally, 7 of these RedMabs-derived clones demonstrated high-affinity constants above 10^-8. This study highlights the RedMabs platform's capability to enhance immune responses and generate high-quality monoclonal antibodies, presenting significant implications for the development of new diagnostics and therapies.

Introduction

Low molecular weight proteins, such as peptides, are commonly low immunogenic antigens. This is why obtaining monoclonals with standard immunization protocols is usually inefficient. The RedMabs platform was applied to a human neuro-amino acid peptide of 28 residues in Balb/c mice. This peptide was also highly conserved in mice, making it a perfect candidate for RedMabs. In 10 standard immunizations, only two monoclonals were obtained, with a very low-affinity constant. However, 42 clones were obtained with RedMabs, 7 of which were found to have a high-affinity constant above 10^-8.

Materials and methods

Two groups of ten 8-week-old Balb/c mice were immunized using different protocols. The immunogen for RedMabs included a 28-mer peptide, BioClonal adjuvant, and immunomodulator, inoculated in five intraperitoneal rounds. For the standard immunization, Complete Freund's Adjuvant (CFA) was used in the first round, followed by Incomplete Freund's Adjuvant (IFA). Serum samples were collected post-immunization to measure antibody titers via 96-well ELISA. After the fifth round, spleens were harvested, and cells were fused with SP2/0-Ag14 myeloma cells using PEG 1450. Hybridomas were selected with HAT medium and screened for antibody production via ELISA. Selected clones were further analyzed for koff using a 96-well plate ELISA, with UREA-treated supernatants compared against a commercial monoclonal antibody.

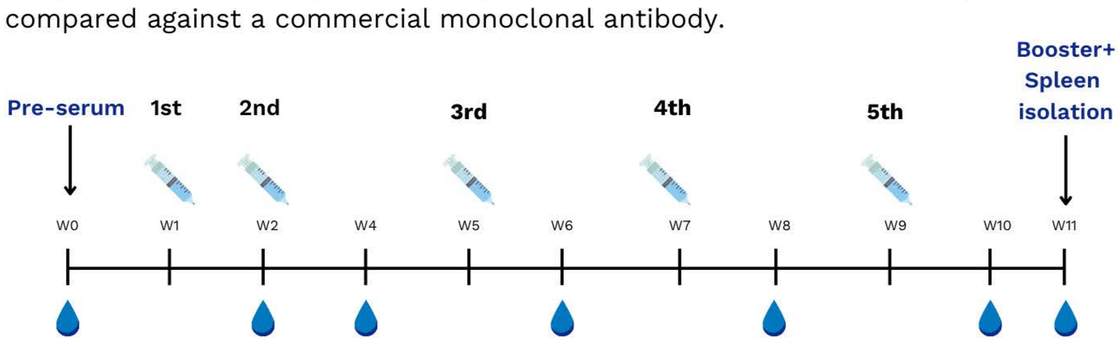

Figure 1. Immunization protocol. Serum was extracted one week after every shoot.

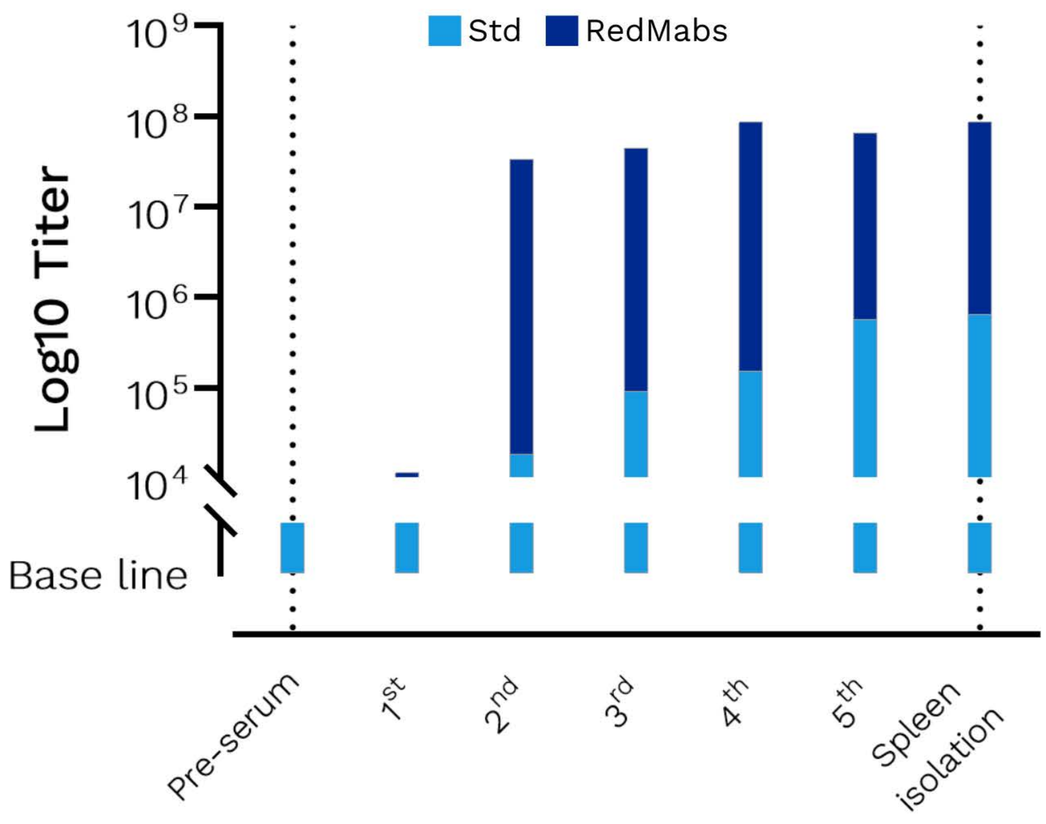

Figure 2. Antibody titers obtained againts peptide sequence after every shot. The positivie tendence and the difference in absolute value is clear, showing a tipical T dependent shape.

Results

Twenty mice were immunized using two different protocols, with five mice per group. All spleens were isolated after the fifth round of immunization. After fusion with SP2/0 cells, culture plates were screened against the peptide used as the immunogen. In the standard immunization, only 2 positive clones were found, while 42 clones were found using the RedMabs immunization protocol. This represents an increase in the number of positive clones by a factor of 21 with RedMabs. Using the same supernatant of the culture from this first screening, the koff was estimated against a commercial murine monoclonal with a known koff of 10^-4. Seven clones were found to have a lower koff than the commercial one, while none of the clones from the common immunization were lower. This demonstrates a significant improvement in both the quantity and quality of antibodies generated using the RedMabs platform.

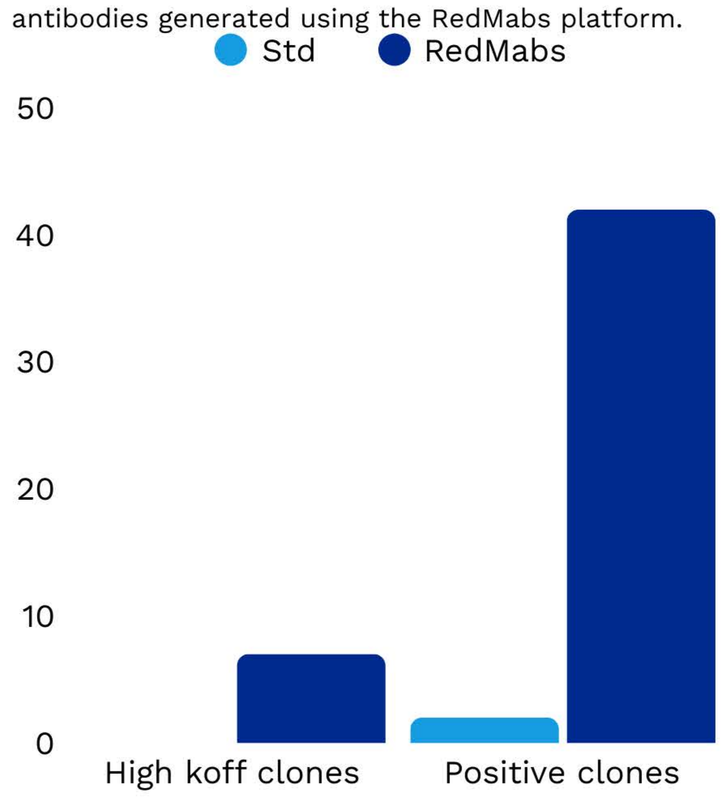

Figure 3. Analysys of positive clones. After ten fusion, Redmabs protocol shows 42 postive clones againts 2 in standar immunization. Besides, seven of this clones in case of Redmabs show high affinity constant. No high affinity clones were obteined

Conclusion

Our study demonstrates that the RedMabs platform significantly enhances the generation of monoclonal antibodies against low immunogenic peptides compared to standard immunization protocols. By incorporating our proprietary adjuvants and immunomodulators, we achieved a substantial increase in the number of positive clones, with a 21-fold improvement over traditional methods. Moreover, a significant portion of these clones exhibited high-affinity constants, highlighting the efficacy of the RedMabs platform in overcoming immunization barriers and producing high-quality monoclonal antibodies. These findings underscore the potential of RedMabs to advance the development of therapeutics and diagnostics for challenging targets.

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