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A bivalent COVID-19 mRNA vaccine combined B-cell and T-cell immunogens elicits strong humoral and cellular immune response in mice

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INTRODUCTION & AIM

The humoral response is the most important component of the immune response against SARS-CoV-2, helping prevent disease. However, the specificity of antibodies to the vaccine strain, which correlates with protection against it, decreases against emerging strains and cannot provide a comparable level of protection. At the same time, virus-specific T cells are often specific to conserved epitopes of various viral proteins and retain their activity against emerging strains. T cell epitopes from conserved regions of viral proteins could become part of a universal vaccine aimed at inducing a broad-spectrum cytotoxic and helper T cell response against COVID-19.

To test the effect of such an immunogen on the development of a specific immune response, we created a bivalent mRNA vaccine, which is a mixture of two mRNAs, one encoding the receptor-binding domain of the SARS-CoV-2 S protein, and the other an artificial multi-epitope immunogen composed of immunodominant T cell epitopes of various SARS-CoV-2 proteins.

The immunogenicity of the developed bivalent mRNA vaccine was studied in a BALB/c mouse model.

METHOD

Design of the immunogens. The RBD immunogen encodes the receptor-binding domain of the S protein of the SARS-CoV-2 virus, which is the main target of neutralizing antibodies, with an attached leader sequence. The BSI immunogen encodes an artificial T cell immunogen capable of inducing T-helper and cytotoxic lymphocyte responses. To create the artificial immunogen, we selected fragments of the S, N, M, and E proteins containing epitopes restricted by the human and mouse MHC I and MHC II systems. T cell epitopes were predicted using NetMHCpan-4.1 and the Immune Epitope Database 2.22 (IEDB 2.22).

Vaccine design. The *rbd* and *bsi* genes were flanked by the 3′- and 5′-UTR's of the human α-globin and the polyA tail. The mRNA vaccines were synthesized using a Biolabmix kit (Russia), with the addition of pseudouridine in the ribonucleotide mixture, as well as a Cap analogue. Lipid nanoparticles were used as a delivery vehicle, using a formula from Moderna.

Immunization. The BALB/c mice were immunized with 10 μg of mRNA-RBD, or with 10 μg of mRNA-BSI, or with a mixture of 10 μg of mRNA-RBD and 10 μg of mRNA-BSI. Intact mice were as negative control. Immunization was performed twice intramuscularly (in the upper part of the hind limb thigh) on days 0 and 21.

Assessment of the humoral immune response. Murine sera were tested in ELISA for specific interaction with recombinant RBD and in VNT for live virus neutralization ability.

Assessment of the cellular immune response. The magnitude of the T-cell immune response in mice was determined by the ELISpot method using the Mouse Interferon gamma ELISPOT Kit (Abcam, USA). Using sandwich ELISA (Abclonal, China), the concentration of cytokines in the culture media in which splenocytes grew was determined. The T cells were stimulated with synthetic virus-specific peptides.

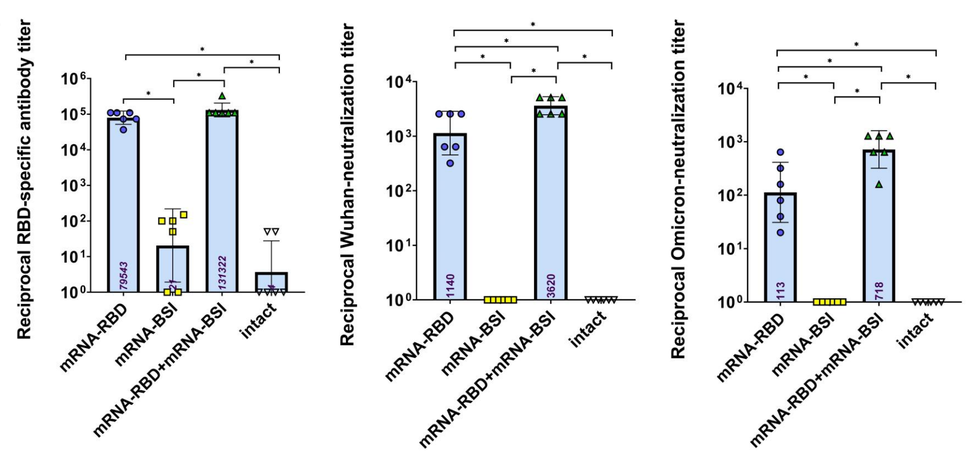
Statistical analysis. Results were processed using the nonparametric Mann-Whitney test using GraphPadPrism 6.0 software.

RESULTS & DISCUSSION

Vaccine formulation. The bivalent mRNA vaccine against COVID-19 is a mixture of mRNAs encoding an artificial multi-epitope T-cell immunogen (mRNA-BSI) and the receptor-binding domain of the SARS-CoV-2 S protein (mRNA-RBD). Lipid nanoparticles were used as vaccine delivery system.

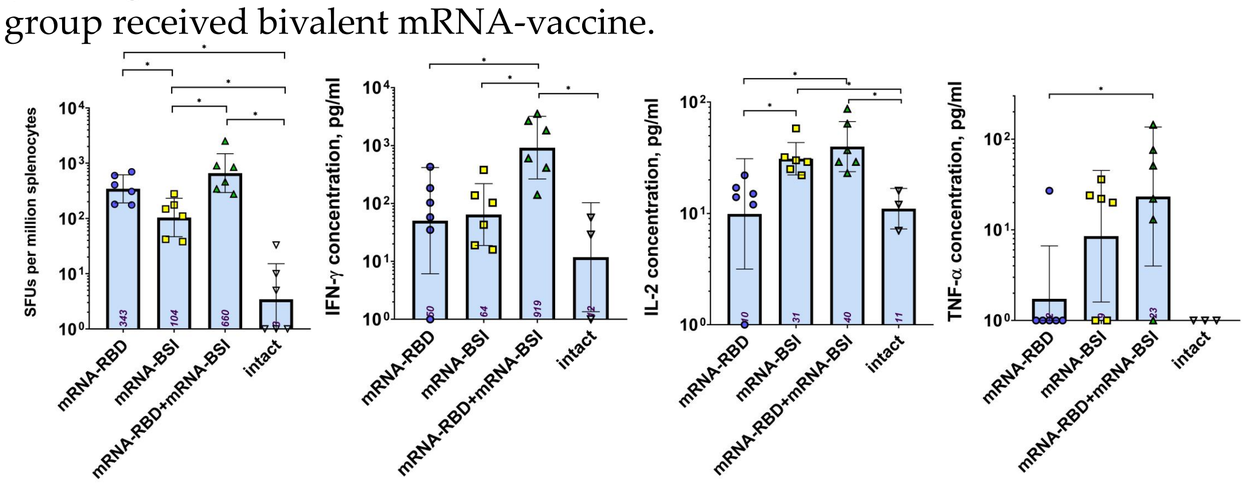
Humoral immune response. An ELISA of serum samples revealed that the group immunized with the bivalent vaccine developed a stronger RBD-specific immune response compared to the group immunized with mRNA-RBD alone. This may be due to the additional T-helper cell support provided by the artificial T-cell immunogen. No RBD-specific antibodies were detected in the group immunized with this immunogen alone.

A live virus neutralization test of the Wuhan and Omicron BA strains demonstrated that sera from mice immunized with the bivalent vaccine neutralized the virus at higher dilutions. The difference compared to the group immunized with mRNA-RBD alone was 3.5-4 times.



Cellular immune response. IFN-γ-ELISpot data showed that T cell immunity was established in all experimental groups of mice two weeks after the second immunization. The group immunized with the bivalent vaccine demonstrated a synergistic response, higher than that observed in the groups immunized with individual mRNAs.

Sandwich-ELISA determined increased cellular secretion of IFN-γ, a slight increase in IL-2 and TNF-α, and no IL-4 secretion in the group received bivalent mRNA-vaccine.



CONCLUSION

A bivalent mRNA vaccine against COVID-19, consisting of a mixture of mRNAs encoding an artificial multi-epitope T cell immunogen and the receptor-binding domain of the SARS-CoV-2 S protein, induces high levels of virus-specific humoral and cellular immunity.

FUTURE WORK / REFERENCES

The B cell component of the vaccine, namely the mRNA-RBD, can be modified to match the actual circulating strain, allowing this platform to provide protection according to epidemiological relevance.