Unmodified mRNA Compared to Modified mRNA Induces Optimal CD8+ T-Cell Responses in Rhesus Macaques

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BACKGROUND

CD8+ T cells play a pivotal role in controlling replication of HIV-1/SIV *in vivo*. Vaccine-elicited CD8+ T cell responses have been associated with reduced peak viremia and viral set points. Nucleoside-modified mRNA ionizable lipid nanoparticle (LNP) vaccines have been shown to induce particularly strong CD4+ T follicular helper cell and neutralizing antibody responses, whereas unmodified mRNA vaccines induce higher CD8+ T cell responses. In the present study, we compared the induction of CD8+ T cell responses in rhesus monkeys immunized with different ratios of unmodified vs modified mRNA-LNPs encoding a T cell immunogen, SIVconsv239, SIV_{239} analog of the most conserved regions of HIV proteome.

METHODS

We immunized four groups of NHPs (n=5/group) intramuscularly with 50μg of mRNA-SIVconsv239 in LNPs on weeks 0, 4 and 8 as follows: 100% 1-methylpseudouridine modified mRNA (Group 1), 70% modified mRNA with 30% unmodified mRNA (Group 2), 30% modified mRNA with 70% unmodified mRNA (Group 3) and 100% unmodified mRNA (Group 4). Monkeys in Group 5 received ChAdOx1.SIVconsv239 at weeks 0 and 4 as a positive control. We analyzed CD8+ T cell responses by SIV gag p11C tetramer staining of whole blood of Mamu-A*01+ monkeys, and tested PBMC in ELISpot and intracellular cytokine assays using an immunogen-specific peptide pool (M3).

Table 1. Immunization schedule

Groups (n=5 per group)	Immunizations
100% 1-methylpseudouridine modified mRNA	Wks 0, 4, 8
70% 1-methylpseudouridine with 30% unmodified mRNA	Wks 0, 4, 8
30% 1-methylpseudouridine with 70% unmodified mRNA	Wks 0, 4, 8
100% unmodified mRNA	Wks 0, 4, 8
Chimp AdOx1	Wks 0, 4

Optimal ratios of ***unmodified*** vs ***modified*** mRNA elicit T cell responses

Magnitude of mRNA-elicited T cell responses are ***comparable*** to those elicited by live vector ChAdOX1

Responses from mRNA immunizations more ***durable*** than ChAdOX1 immunizations

RESULTS

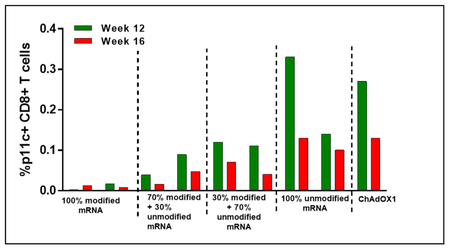


Figure 1. SIV Gag p11c tetramer binding to CD8+ T cells in whole blood of the immunized monkeys. Whole blood from *Mamu-A*01+* monkeys (n=2/group) were stained with p11c epitope-specific tetramer and analyzed by flow cytometry. Percentages of p11c-specific CD8+ T cells are shown. Green bars show responses across groups on week 12 and Red bars show those from Week 16 of the study.

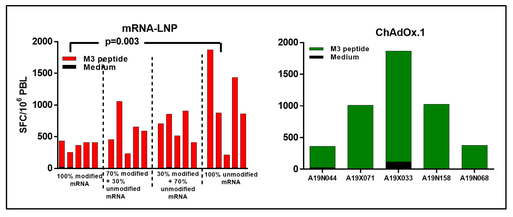


Figure 2. IFN-γ ELISpot responses in the immunized monkeys. PBMC from the immunized monkeys were stimulated with peptide pool spanning the M3 immunogen in the assay. **(A)** Red bars represent M3-specific responses from 2 weeks post 3rd mRNA immunization **(B)** Green bars represent M3-specific responses from 4 weeks post 2nd ChAdOX1 immunization; blacks bars represent background responses in both A and B

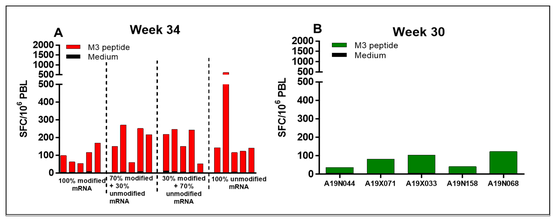
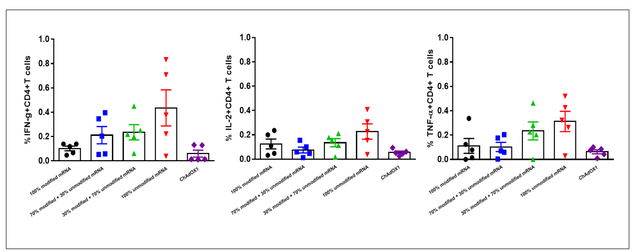
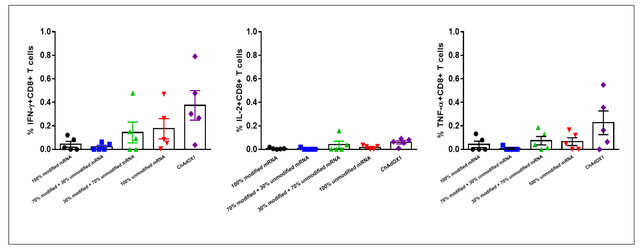


Figure 3. IFN-γ ELISpot responses 22 weeks post 3rd mRNA immunizations (A) and 26 weeks post 2nd ChAdOX1 (B) immunizations. (A) Red bars represent M3-specific responses **(B)** Green bars represent M3-specific responses; blacks bars represent background responses in both A and B

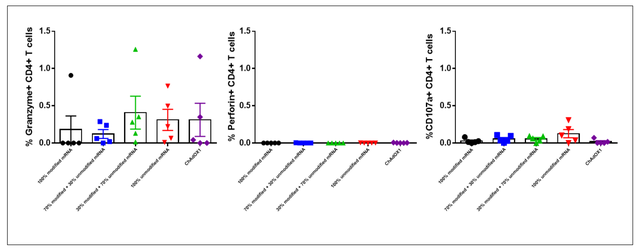
A



B



C



D

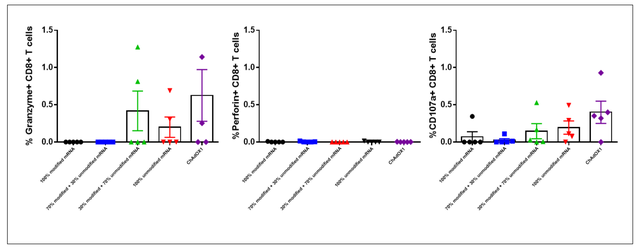


Figure 4. Intracellular cytokine and degranulation molecules releases by CD4+ and CD8+ T cells in peripheral blood. Percentages of CD4+ and CD8+ T cells secreting cytokines and degranulation molecules are shown for each group as Mean ± SEM.

CONCLUSIONS

The present NHP study shows that immunizations with optimal ratios of unmodified versus modified mRNA elicit T cell responses comparable to that with live vector, ChAdOX1. Moreover, mRNA immunizations elicited more durable T cell responses than ChAdOX1.

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