Safety Assessment of Sublingual Vaccine Using Poly(I:C) Adjuvant: Comparison with Nasal Vaccine in Cynomolgus Macaques and Mouse

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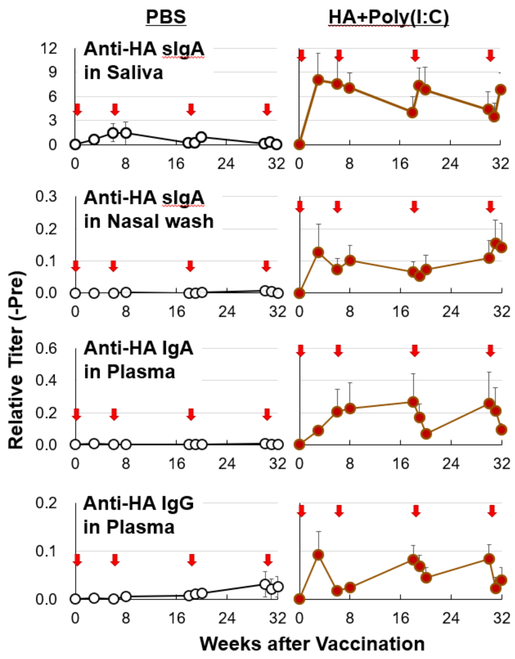
■ Introduction

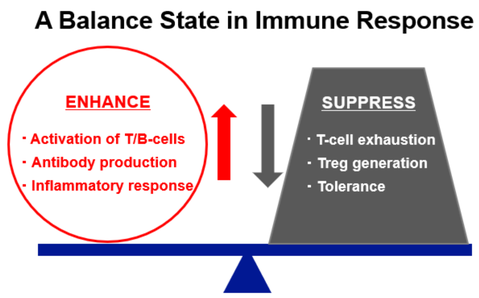
We reported a sublingual vaccine formulation with Poly (I:C) adjuvant and influenza HA antigens induced mucosal and systemic immunological responses, resulting in antigen-specific antibodies in saliva, nasal washes and blood. It elicits a balanced state in immune response.

Poly(I:C) is a double-stranded (ds) RNA adjuvant that activates TLR3-mediated immune responses, but it remains unapproved due to its proinflammatory side effects. However, the safety of Poly(I:C) adjuvant for sublingual vaccine is still unknown.

In the current study, we directly compared the safety of the Poly(I:C) adjuvanted vaccines on sublingual and nasal routes in Cynomolgus macaques and mice.



Regulated Gene Expression Enhancing Immune Response	Regulated Gene Expression Suppressing Immune Response
Upregulated: *CCL7, CCL2, CXCR4, PFKFB3, JUN, KLHL2, PTX3, FADD, ETV6*	Upregulated: *TNFRSF12A, RGS1, SLA, EDN1*
Downregulated: *ITGB5, RGS10, PGLYRP1*	Downregulated: *AQP1, AQP3, GP9, GP1BB, WIPI1, EPAS1, HSPA1B*



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■ Methods

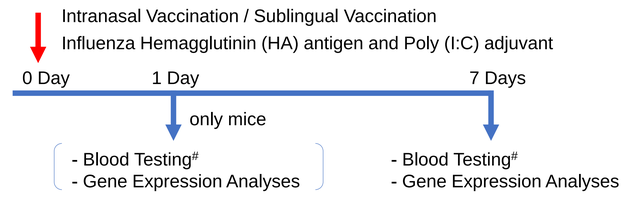


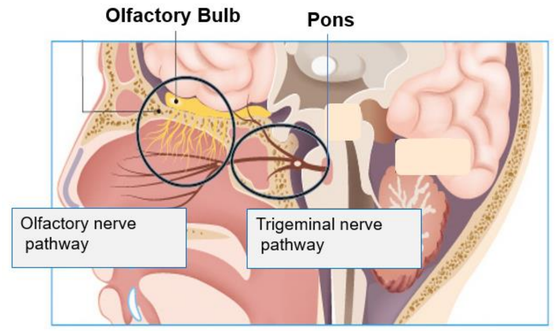
Table Gene Information

Symbol	Product; Description; Function
Saa3	Serum amyloid A 3; acute response protein
Tnf	Tumor necrosis factor; inflammatory cytokine
IL6	interleukin 6; immune-inflammatory response
IL1b	interleukin 1 beta; inflammatory cytokine
Ccl2	C-C motif chemokine ligand 2(MCP1); chemokine
Timp1	Tissue inhibitor of metalloproteinase 1; tissue repairing protein
C2	Complement component 2; opsonic function; phagocytic cell activation
Ifi47	interferon gamma inducible protein 47; pathogen defense protein
Aif1	Allograft inflammatory factor 1; microglial marker
Omp	Olfactory marker protein; odor detection/signal transduction
Nos2	Nitric oxide synthase 2, inducible, iNos
Gzmb	Granzyme-B; NK cell protease; apoptosis induction

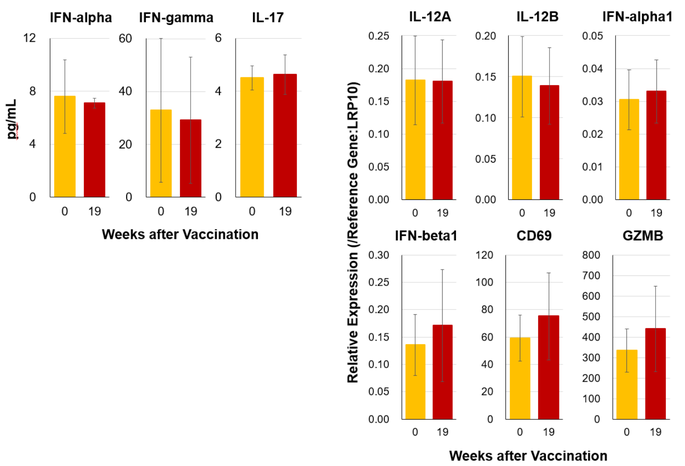
■ Results

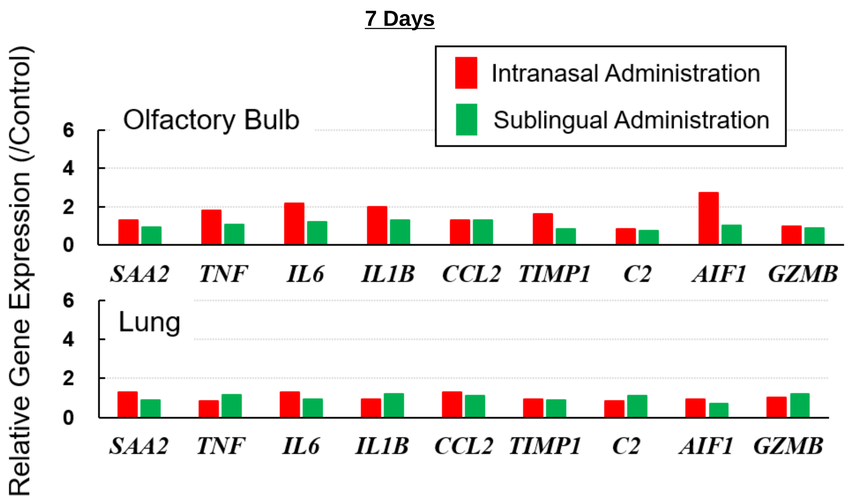
Monkeys;
- Biochemical blood tests and complete blood counts were observed minimal differences between control and vaccination.
- The levels of inflammatory cytokines (IFN-alpha, IFN-gamma, and IL-17) in plasma did not increase after the third vaccination.
- The gene expression levels of inflammatory cytokines (IL-12A, IL-12B, IFN-alpha1, IFN-beta1, CD69 and GZMB) did not increase.
- In the olfactory bulb, the intranasal vaccine upregulated inflammatory-related genes after 7days post-vaccination.

Mice;
- Biochemical blood tests exhibited minimal variation in both sublingually and intranasally vaccination.
- In the olfactory bulb, the intranasal vaccine upregulated inflammatory-related genes after 7days post-vaccination.

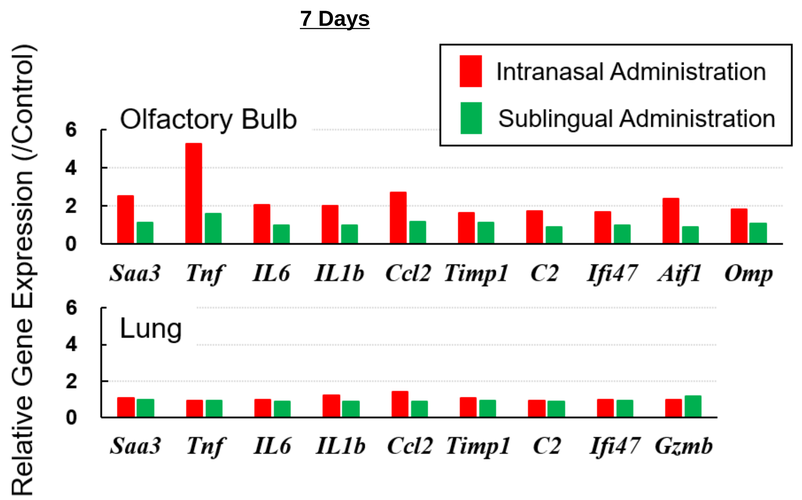


Monkeys

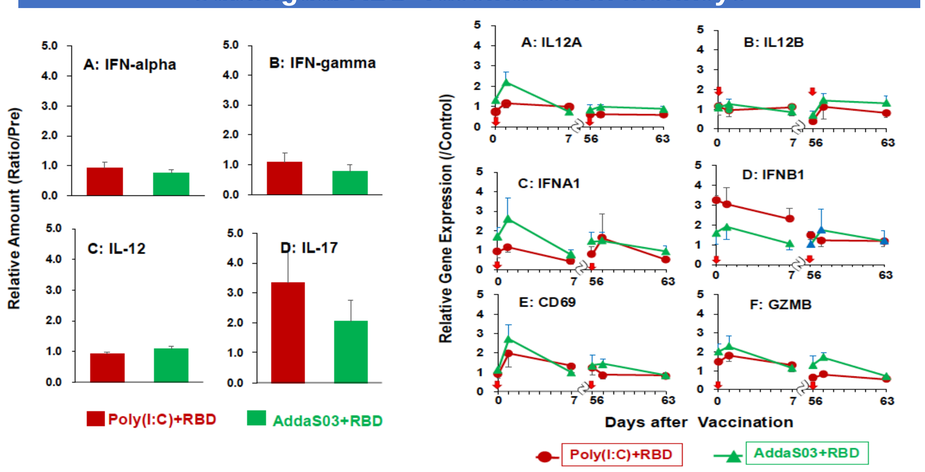




Mice



Sublingual RBD Vaccination in Monkeys



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■ Discussions & Conclusion

We previously reported the sublingual SARS-CoV-2 RBD vaccine with Poly(I:C) or AddaS03 did not increase in the levels of inflammatory cytokines. The vaccine with Poly(I:C) were observed slightly lower than the AddaS03 vaccine in the gene expression levels of inflammatory cytokines. These suggest that the vaccine with Poly(I:C) has a milder effect on immuno-proinflammatory factor than AddaS03.

The sublingual influenza HA vaccine with Poly(I:C) had fewer proinflammatory side effects than those of the nasal vaccine, both in macaques and mice. On nasal administration, the side effects of the Poly(I:C) adjuvanted vaccine were markedly more significant in mice than in macaques. The side effects of Poly(I:C) adjuvanted vaccine are probably overestimated by the results in mice, because of nasal structure and function.

We are hopeful the sublingual Poly(I:C) adjuvanted vaccine be vaccines of the future.

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