Preclinical Studies on Sublingual Vaccine Using Poly(I:C) Adjuvant in Nonhuman Primate, Cynomolgus Macaque

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Introduction

Secretory IgA (s-IgA) in the oral mucosa plays an important role in preventing infection by SARS-CoV-2 and influenza viruses. Increasing the amount of s-IgA is considered a potential method for preventing infection, and sublingual administration has attracted attention as an effective approach.

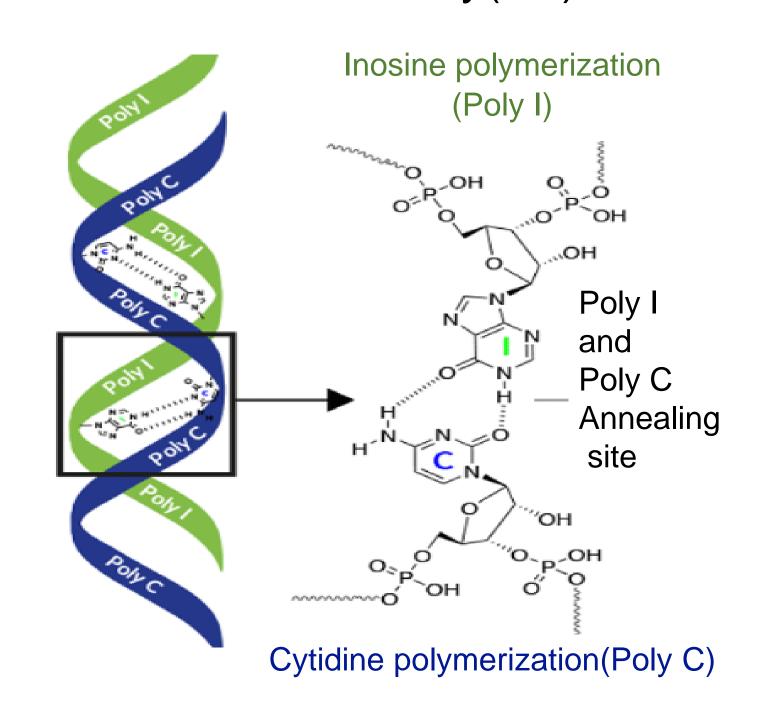
However, sufficient development has not progressed due to the need for the use of adjuvants and the need for evaluation other than IgG production.

In experiments using cynomolgus monkeys, it was confirmed that RBD-specific s-IgA was secreted in nasal secretions in a dosedependent manner after a single administration of SARS-CoV-2 RBD antigen and Poly(I:C).

Therefore, we changed the type of antigen, the type of adjuvant, and the administration method, and investigated the effects in detail, which we will introduce here.

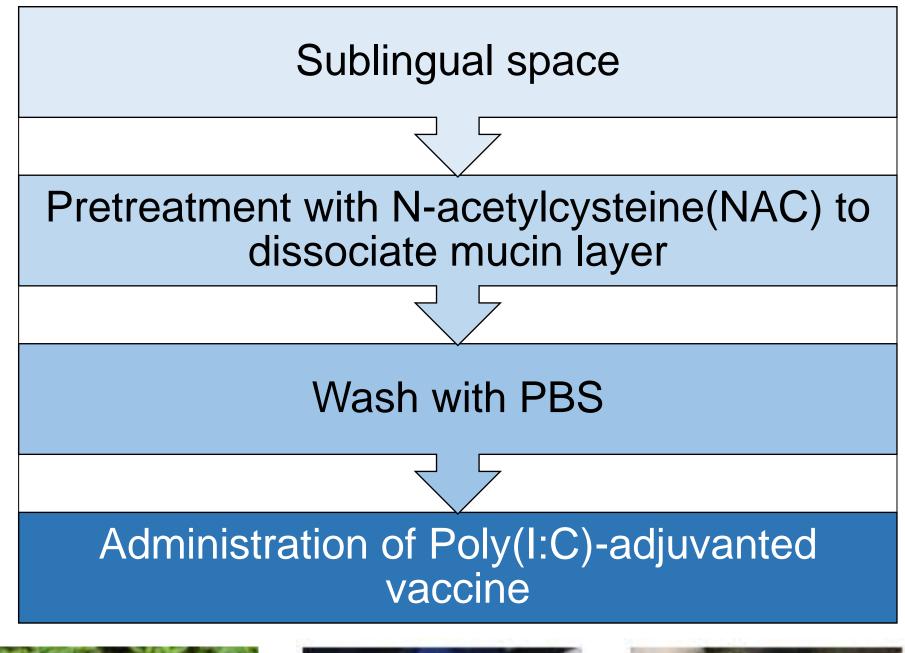
■ Poly(I:C) Adjuvant

Structure of Poly(I:C)



- Ligand for TLR 3 that activates immunological and proinflammatory responses.
- Mimics viral infections and elicits host immune responses.

Vaccine Administration



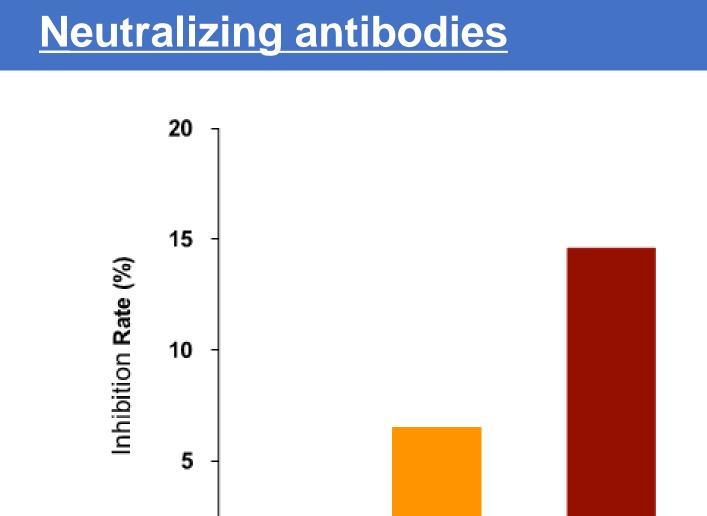






- > SARS-CoV-2 Vaccine:500 μl 150 μg SARS-CoV-2 -RBD antigen and 400 μg Poly(I:C) or 250 μl AddaS03 adjuvant
- Influenza Vaccine:500 μl 30 μg Influenza HA antigen and400 μg Poly(I:C) adjuvant

Results



Neutralizing antibodies in plasma were detected semi-dose dependently with anti-RBD IgA antibody titer.

RBD Dose (mg/head)

- Although little neutralizing activity in the saliva or nasal washings was detected (data not shown)
- These are probably caused by existing of an inhibitor factor, soluble form of ACE2, in saliva or nasal washings.

Antigen specific antibodies Control Poly(I:C)+RBD Poly(I:C)+HA Control AddaS03+RBD A: Anti-RBD slgA in saliva A. Anti-HA sIgA in saliva 0.150 0.100 0.050 B. Anti-HA slgA in nasal wash B: Anti-RBD slgA in nasal wash 0.120 0.0800.040 C: Anti-RBD IgA in plasma C. Anti-HA IgA in plasma 0.120 0.0800.040 D: Anti-RBD IgG in plasma D. Anti-HA IgG in plasma 0.0600.040 0.020 + + + Weeks Post-vaccination Weeks Post-vaccination

- RBD-specific s-IgA were detected in saliva and nasal wash fluid after the third vaccination.
- RBD-specific IgA and IgG in plasma were detected after the fourth vaccination.
- HA-specific s-IgA were detected in saliva and nasal wash fluid after the initial administration.
- HA-specific IgA and IgG in plasma were detected after the initial administration.

DNA Microarray

- Sublingual vaccines affected the transcriptional regulation of many genes that enhance or suppress immune responses.
- Sublingual vaccines appeared to balance the enhancement and suppression of immune responses by regulating gene expression.

Regulated Gene Expression Enhancing Immune Response

↑ Upregulated: CCL7, CCL2, CXCR4, PFKFB3, JUN, KLHL2, PTX3, FADD, ETV6

↓ Downregulated: ITGB5, RGS10 PGLYRP1

Downregulated: ↑ Upregulated: AQP1, AQP3, GP9, GP1BB, WIPI1, EPAS1, HSPA1B

TNFRSF12A, RGS1, SLA, EDN1

Activation of

Mo migration, B-cell generation, T-cell activation, T-cell differentiation, memory T-cell, Jun gene expression, Inflammatory response, M1 polarization, Cytokine production

- **AntibodyActivation of T/B-cells**
- production
- Inflammatory response

Inhibition of Mo migration, B-cell generation, B-cell function, T-cell activation, T-cell differentiation, Memory T-cell, Jun gene expression, M1 polarization, Cytokine production,

Regulated Gene Expression

Suppressing Immune Response

- T-cell exhaustion
- Treg generation
- Tolerance

Discussions & Conclusion

- Oral administration activates not only mucosal immunity but also systemic immunity, regardless of the type of antigen or adjuvants.
- According to microarray results, Sublingual Route has a balance of enhancing and suppressing effects.
- Sublingual vaccines are expected to offer "Wide range of effectiveness from infection prevention to suppression of severity" and "Easy taking by oneself."

