



9th International Conference on Vaccine R&D (2024,Boston)



# Development of Sublingual Vaccine Formulated with SARS-CoV-2 RBD or Influenza HA Antigen and Poly(I:C) Adjuvant in Nonhuman Primates, Cynomolgus Macaques

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- Sublingual Route, Poly(I:C) Adjuvant
- Development of Poly(I:C)-adjuvanted Vaccine in Nonhuman Primates
- Mechanism Underlying Immune Response Mediated by Sublingual Poly(I:C)-adjuvanted Vaccine
- Safety Assessment of Sublingual Poly(I:C)-adjuvanted Vaccine

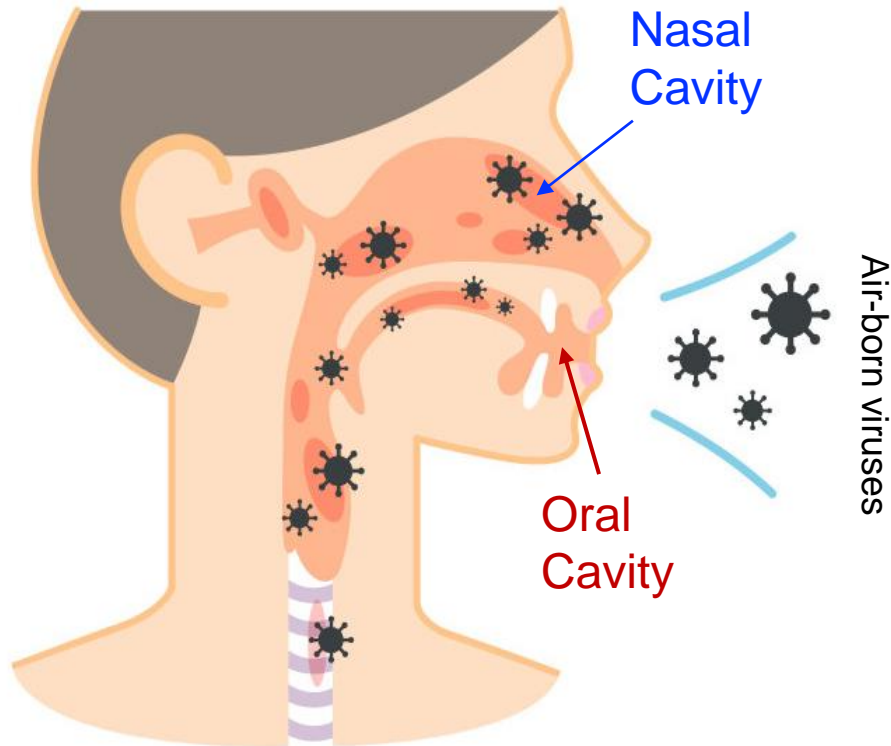
# **Sublingual Route, Poly(I:C) Adjuvant**

Preclinical Studie on Sublingual Poly(I:C) –adjuvanted  
Vaccine in Non-human Primates

Mechanism Underlying Immune Response Mediated by  
Sublingual Poly(I:C)-adjuvanted Vaccine

Safety Evaluation of Sublingual Poly(I:C)-adjuvanted Vaccine

# Sublingual Route



## Oral and Nasal Cavities

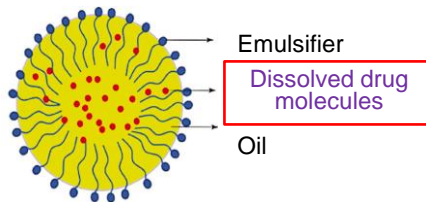
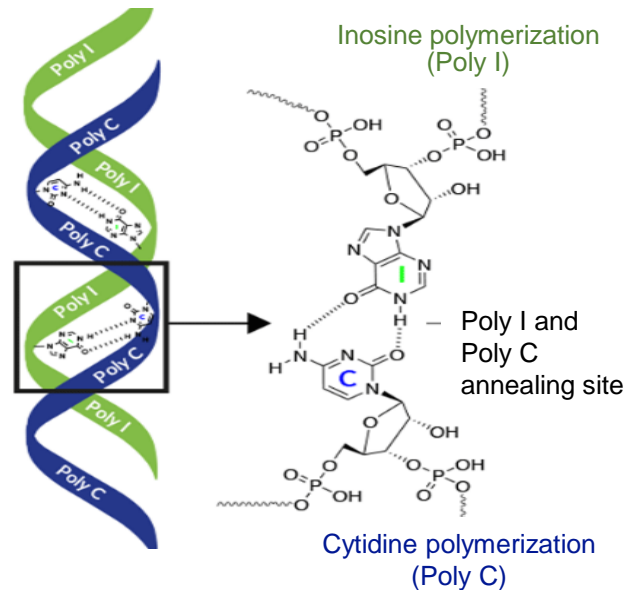
- The first gate to contact with corona and/or influenza viruses.
- mucosal immune system to response against these viruses.

## Sublingual Route for Vaccination

- Vaccination via the intranasal route causes adverse effects on brain, as shows later.

# Poly(I:C) Adjuvant

## Structure of Poly(I:C)



## Polyinosinic:polycytidylic acid (Poly(I:C))

- Ligand for TLR 3 to activate host immune responses.
- Still remains unapproved as adjuvant due to its adverse effects.
- Adverse effects were previously studied in mice and need further examinations in nonhuman primates.

## AddaS03 (AS03 analog)

- AS03 analog and using for preclinical studies.
- Oil-in-water nano-emulsion stimulating Th1/Th2 cytokine.
- AS03 were approved as an adjuvant for intramuscular injected vaccine of influenza.

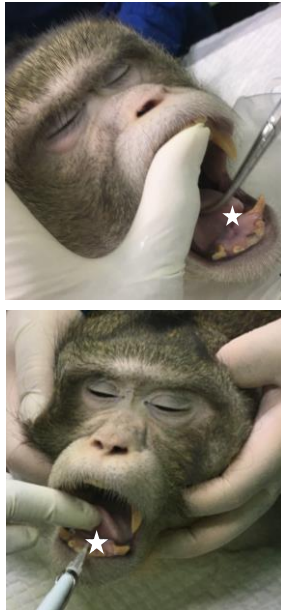
Sublingual Route, Poly(I:C) Adjuvant

# **Development of Sublingual Poly(I:C) -adjuvanted Vaccine in Nonhuman Primates**

Mechanism Underlying Immune Response Mediated by  
Sublingual Poly(I:C)-adjuvanted Vaccine

Safety Evaluation of Sublingual Poly(I:C)-adjuvanted Vaccine

# Outline of Sublingual Administration of Vaccine in Macaques



Sublingual space



Pretreatment with N-acetylcysteine (NAC)  
to dissociate mucin barrier



Wash with PBS

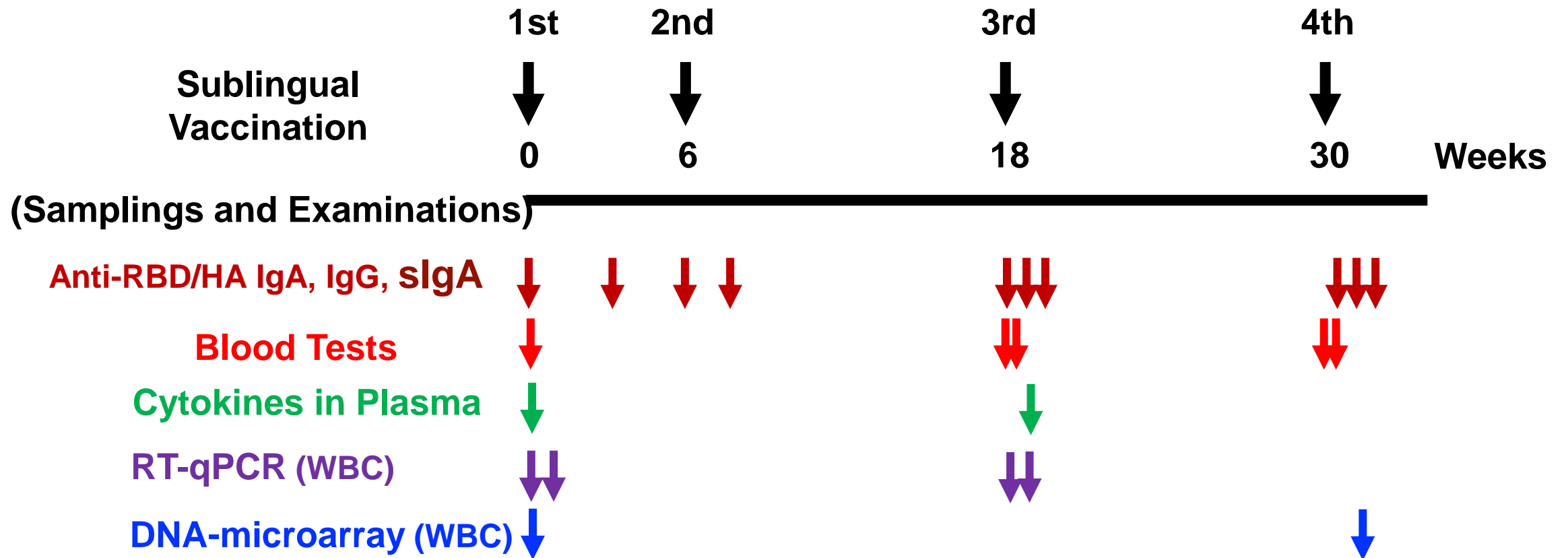


Administration of Poly(I:C)-adjuvanted vaccine

- SARS-CoV-2 Vaccine:  
500  $\mu$ l : 150  $\mu$ g SARS-CoV-2 -RBD antigen and  
400  $\mu$ g Poly(I:C) or 250  $\mu$ l AddaS03 adjuvant
- Influenza Vaccine:  
500  $\mu$ l : 30  $\mu$ g Influenza HA antigen and  
400  $\mu$ g Poly(I:C) adjuvant



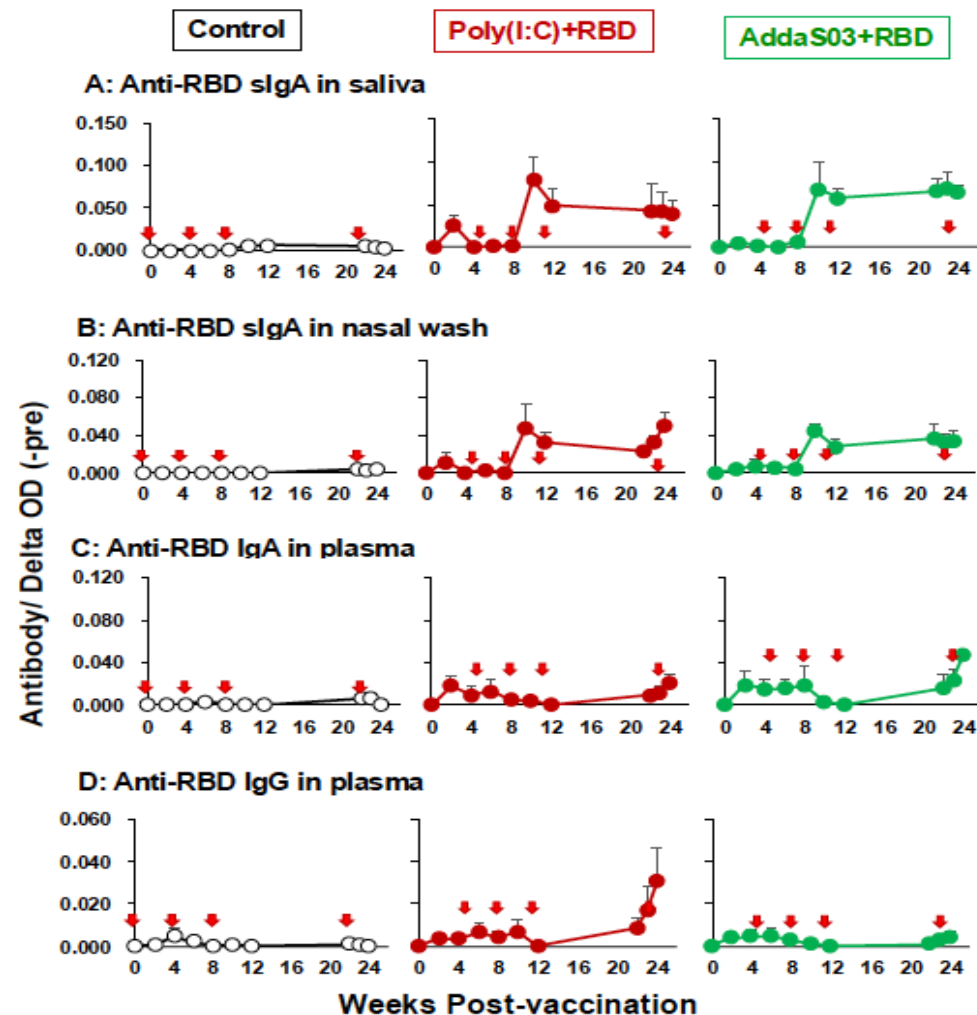
# Vaccinations, Samplings, and Examinations



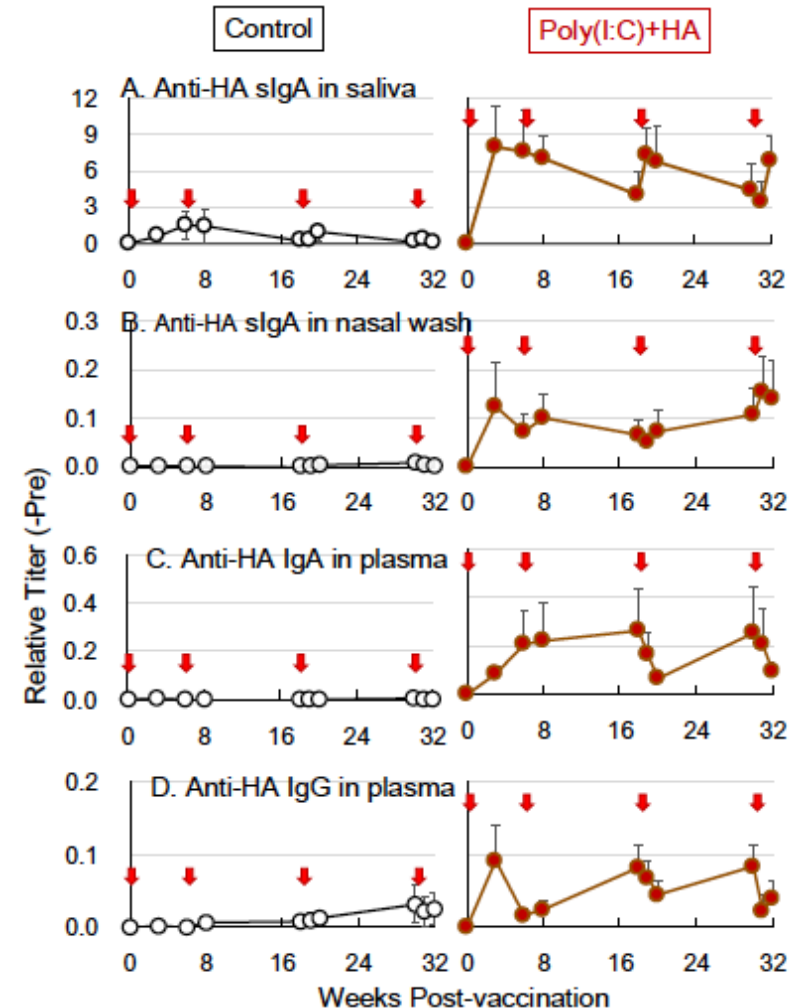


# Sublingual Vaccine-induced Antigen-specific Antibodies in Saliva, Nasal Washes, and Plasma

Vaccination with SARS-CoV-2 RBD  
+ Poly(I:C) or AddaS03 Adjuvant



Vaccination with Influenza HA  
+ Poly(I:C) Adjuvant



Sublingual Route, Poly(I:C) Adjuvant

Development of Sublingual Poly(I:C) -adjuvanted Vaccine  
in Non-human Primates

## **Mechanism Underlying Immune Response Mediated by Sublingual Poly(I:C)-adjuvanted Vaccine**

Safety Evaluation of Sublingual Poly(I:C)-adjuvanted Vaccine

# DNA Microarray for Mechanism Analysis

Peripheral blood white cells

RNA extraction

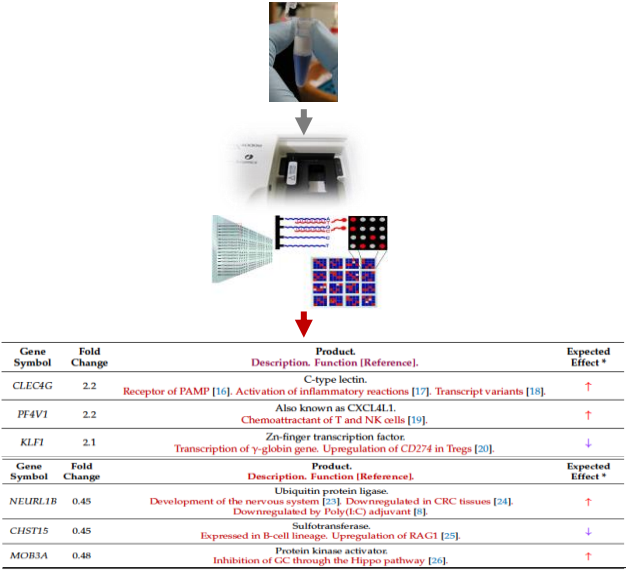
DNA microarray

Selection of target genes

(Immune response-associating genes:  
2-fold upregulated, 1/2-fold downregulate )

Genome informatics

Mechanism events in an immune response elicited  
by sublingual Poly(I:C)-adjuvanted vaccine



Immune Response-associated Genes:  
Poly(I:C)-adjuvanted Vaccines Mediated Their Up- or Down-regulated Expressions,  
Resulting in an Enhanced and/or Suppressed Immune Response

Selection of Immune Response–associating Genes Based on DNA Microarray Analyses

Regulated Gene Expression  
Enhancing Immune Response

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

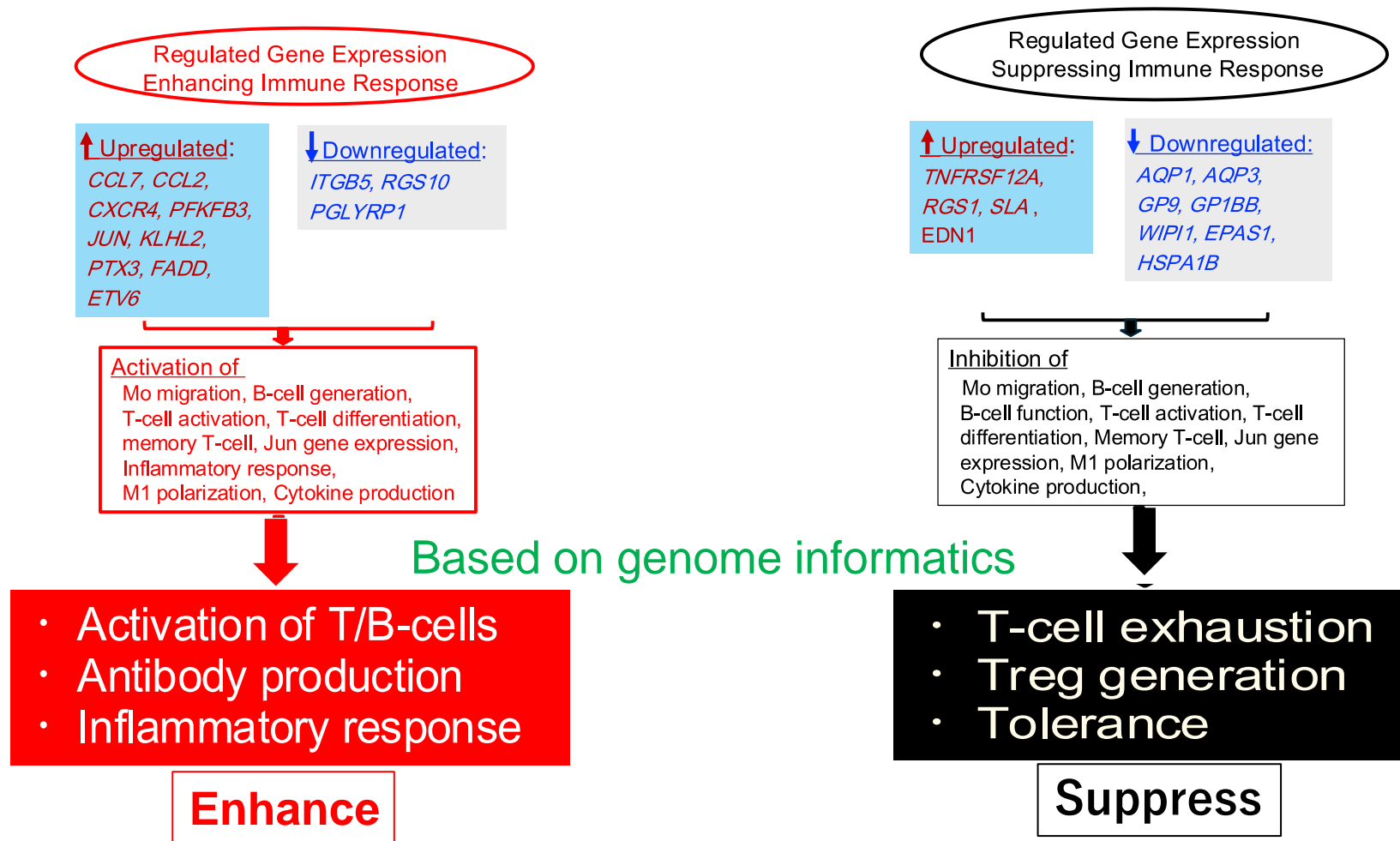
↓ Downregulated:  
*ITGB5, RGS10*  
*PGLYRP1*

Regulated Gene Expression  
Suppressing Immune Response

↑ Upregulated:  
*TNFRSF12A,*  
*RGS1, SLA,*  
*EDN1*

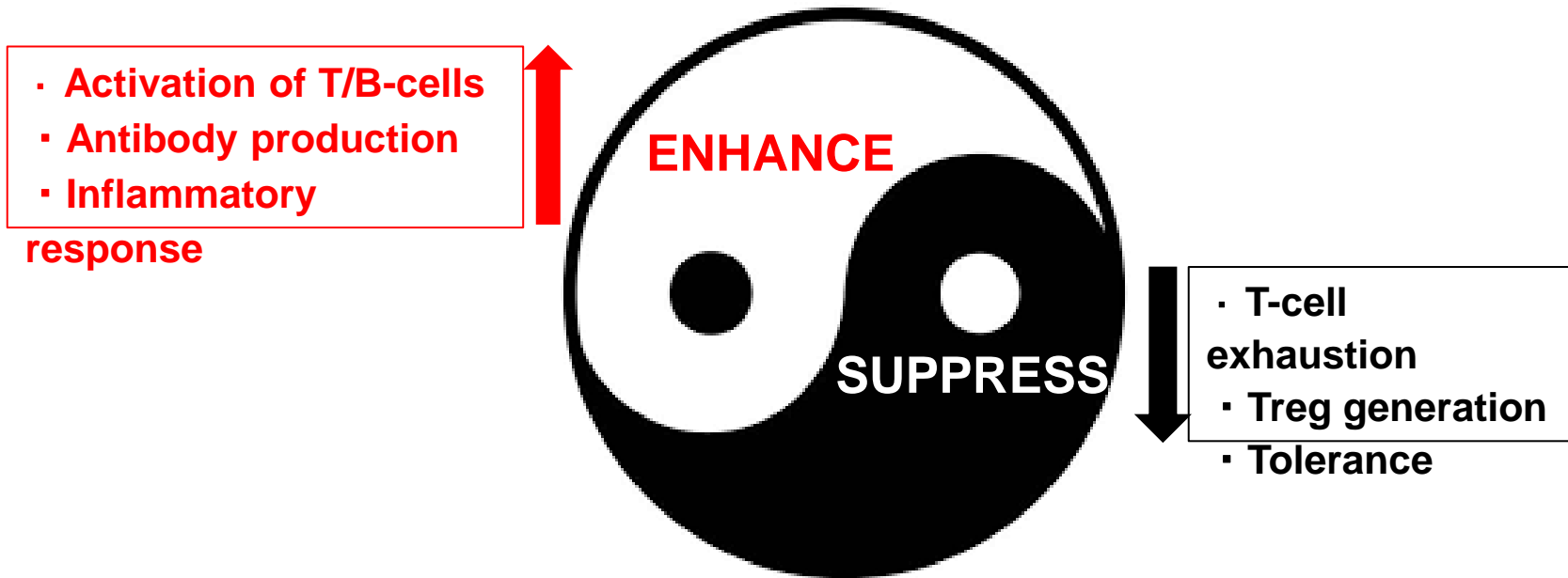
↓ Downregulated:  
*AQP1, AQP3,*  
*GP9, GP1BB,*  
*WIPI1, EPAS1,*  
*HSPA1B*

# Sublingual Poly(I:C)-adjuvanted Vaccine Mediated Enhance or Suppress of Immune Response through the Up- or Down-regulated Gene Expressions



# Summary for Molecular Mechanism Underlying Immune Response Mediated by Poly(I:C)-adjuvanted Vaccine

**Sublingual Poly (I:C)-adjuvanted Vaccine Elicited a “Yin/Suppress and Yang/Enhance”-like Balance State in Immune Responses**



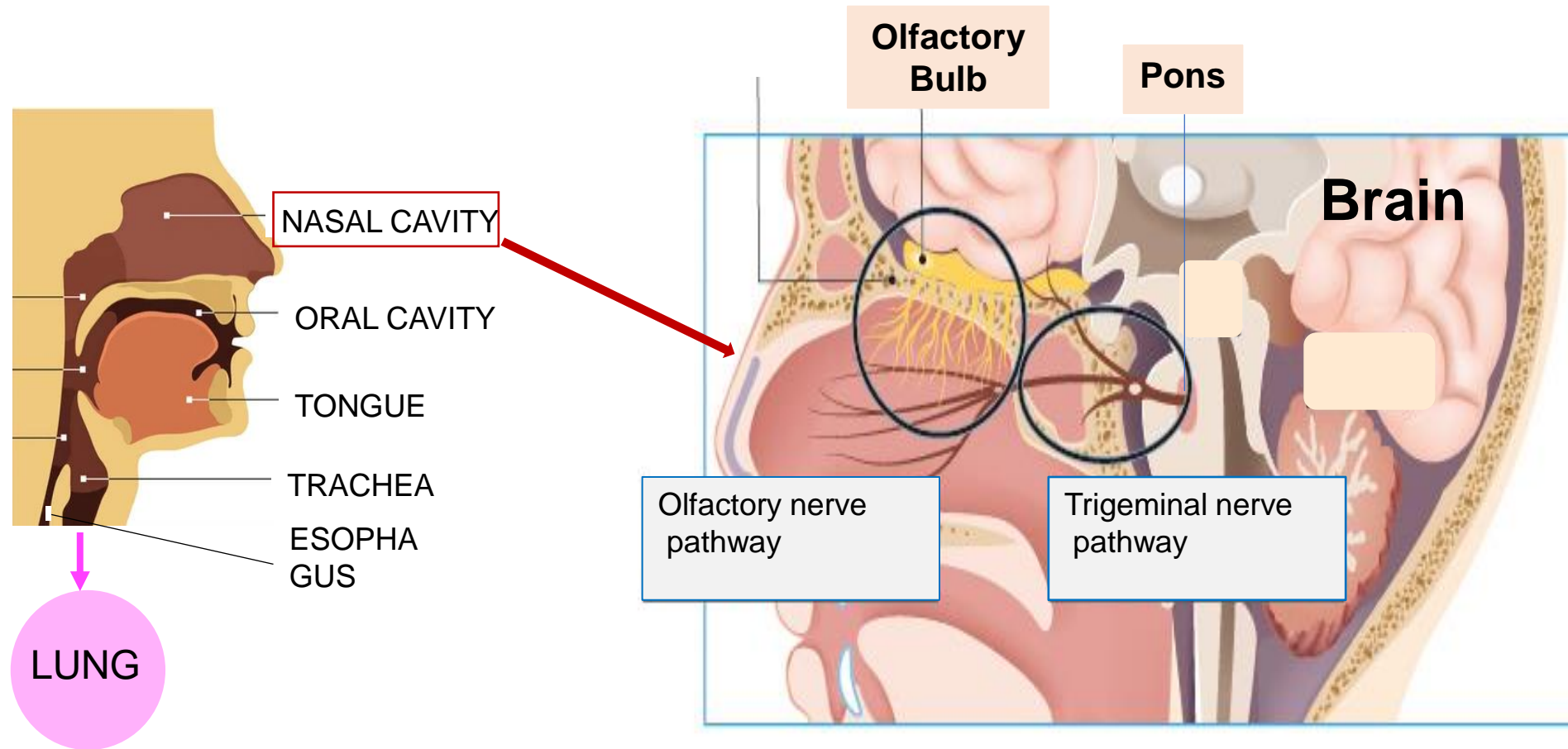
Sublingual Route, Poly(I:C) Adjuvant

Development of Sublingual Poly(I:C) -adjuvanted Vaccine  
in Non-human Primates

Mechanism Underlying Immune Response Mediated by  
Sublingual Poly(I:C)-adjuvanted Vaccine

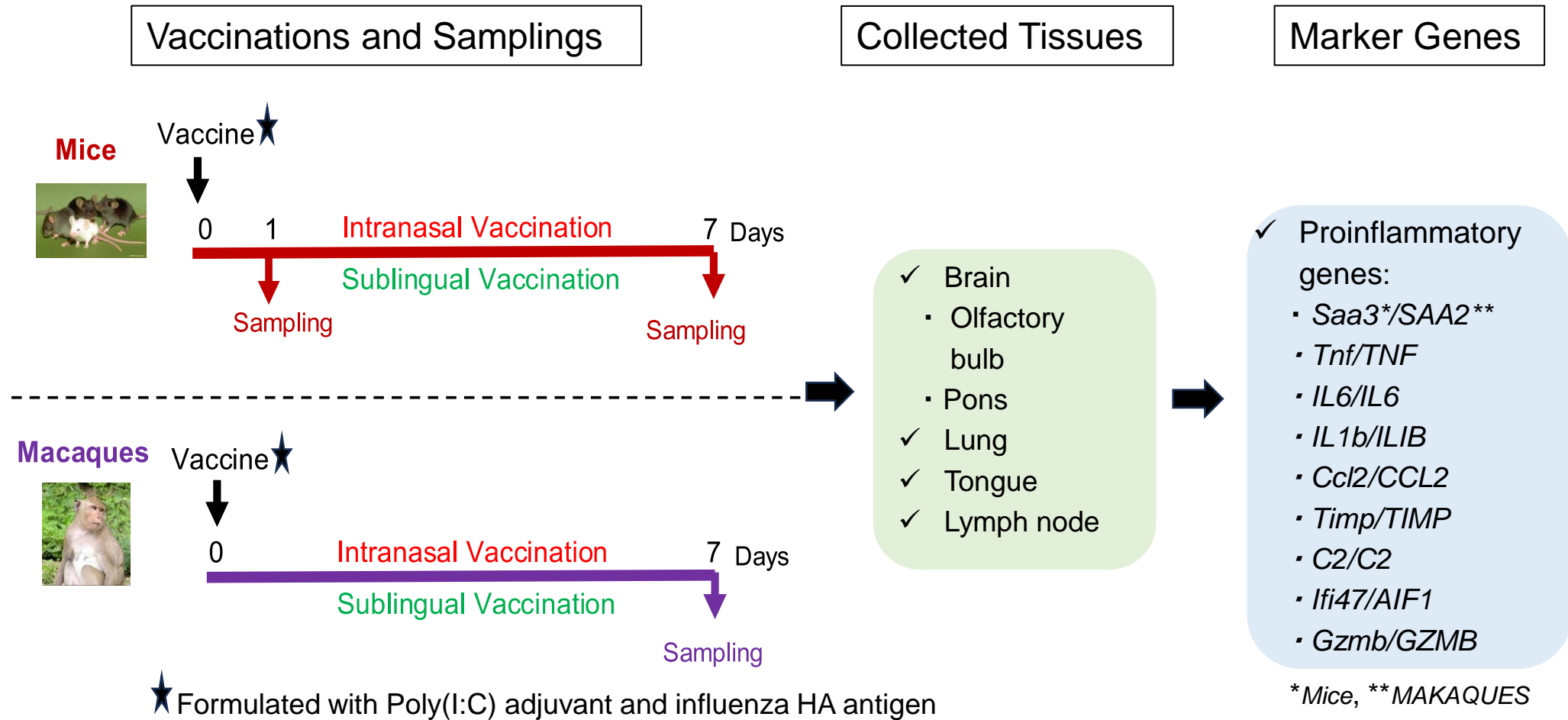
# **Safety Assessment of Sublingual Poly(I:C)-adjuvanted Vaccine, in Comparative Studies on Intranasal Vaccine**

## Safety Assessment of Sublingual Poly (I:C)-adjuvanted Vaccines (2) : Anatomical Characteristics of Oral Cavity (Sublingual) and Nasal Cavity (Intranasal)





# Safety Assessment of Sublingual Poly(I:C)-adjuvanted Vaccine (3): Comparisons with the Intranasal Vaccine in Mice and Macaques

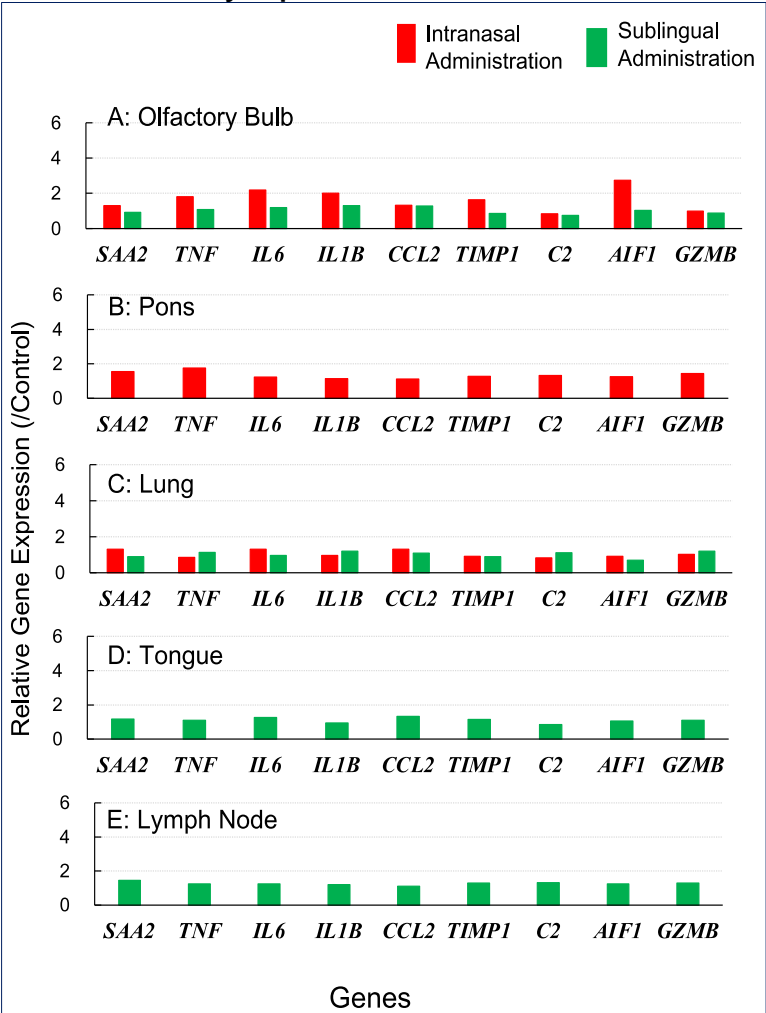


# Safety Assessment of Sublingual Poly(I:C)-adjuvanted Vaccine (4): Comparisons with the Intranasal Vaccine in Gene Expression of Proinflammatory Genes in Several Tissues of Macaques and Mice



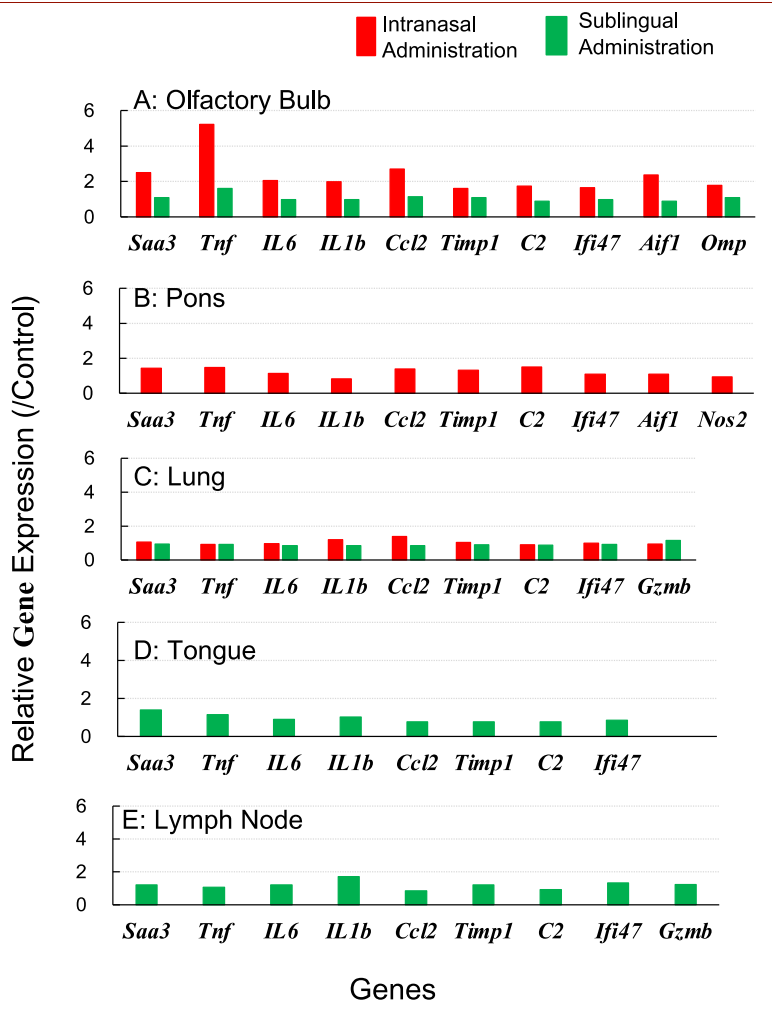
## Macaques

7 days post vaccination



## Mice

7 days post vaccination

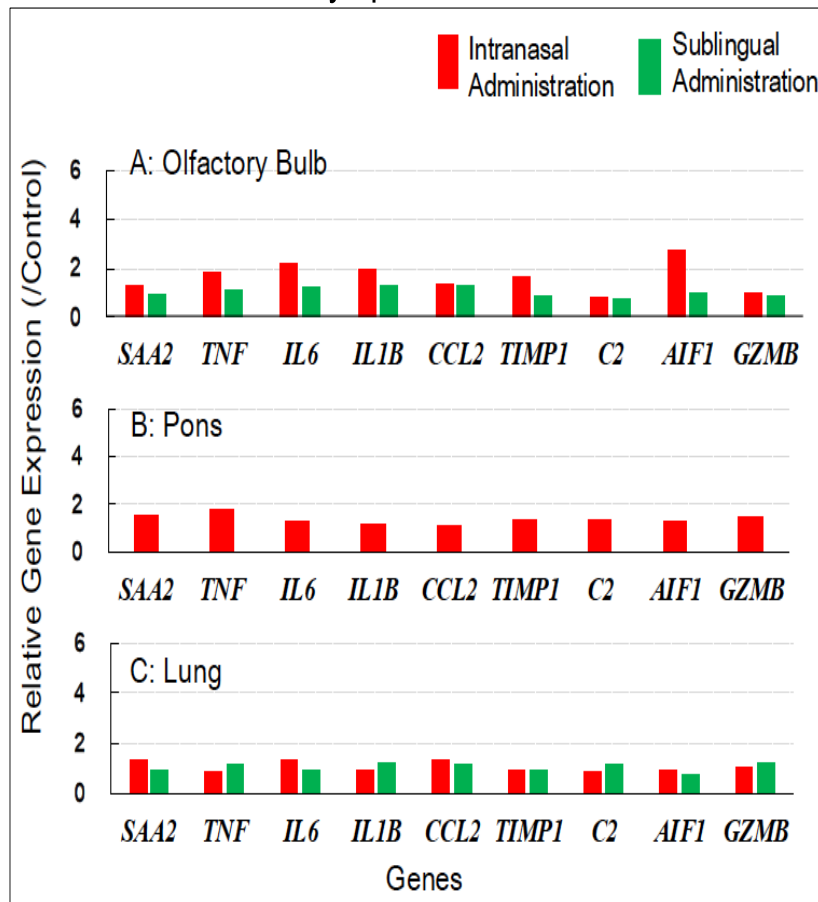


# Safety Assessment of Sublingual Poly(I:C) -adjuvanted Vaccine (5): Intranasal Vaccine Resulted in Marked Upregulated Expression of Proinflammatory Genes in Brain, Olfactory Bulb, in Mice



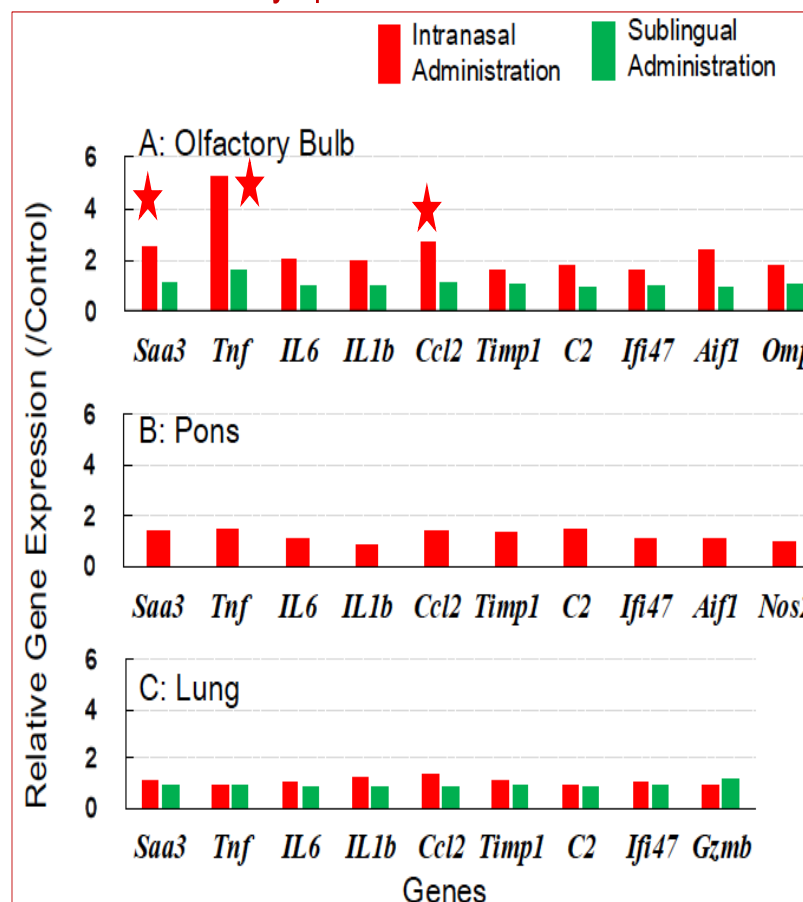
## Macaques

7 days post-vaccination



## Mice

7 days post-vaccination



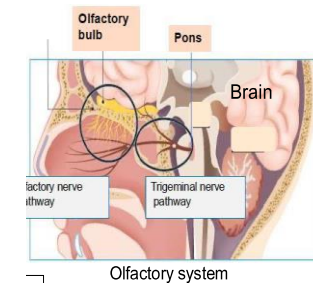
## Highly sensitive response in Mice

Rodents/Mice

Nocturnal habit



- Highly sensitive olfactory system



- In rodents, Poly(I:C) is most effective to activate proinflammatory cytokine pathway

Poly(I:C)-adjuvanted vaccine induced highly upregulated expression of proinflammatory genes in brain, olfactory bulb.

# Summary for Safety Assessment of Sublingual Poly(I:C)-adjuvanted Vaccine

- ✓ The intranasal Poly(I:C)-adjuvanted vaccine had adverse effects to the brain, olfactory bulb, and pons in macaques and mice.
- ✓ The effects to the brain (olfactory bulb) differed by species, with mice responding to the vaccine remarkably severe than macaques.
- ✓ The sublingual Poly(I:C)-adjuvanted vaccine showed no harmful effect to the brain, in both macaques and mice.
- ✓ Therefore, sublingual Poly(I:C)-adjuvanted vaccination appeared to be safe in nonhuman primates.



# CONCLUSION

- Sublingual vaccine formulated with Poly(I:C) adjuvant and SARS-CoV-2 RBD or influenza HA antigen was created in nonhuman primates, *Cynomolgus* macaques.
- Sublingual Poly(I:C)-adjuvanted vaccination elicited “Yin/Suppress and Yang/Enhance-like” balance state in immune responses.
- Sublingual Poly(I:C)-adjuvanted vaccination appears to be safe in primates.



Biology Methods and Protocols, 2023, bpad017  
<https://doi.org/10.1093/biomethods/bpad017>  
Innovations

SARS-CoV-2 sublingual vaccine with RBD antigen and poly(I:C) adjuvant: Preclinical study in cynomolgus macaques

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*Biol Methods Protoc* (2023).  
DOI:10.1093/biomethods/bpad017

fortune  
Journals

Research Article

Mechanism Underlying the Immune Responses of a Sublingual Vaccine for SARS-CoV-2 with RBD Antigen and Adjuvant, Poly(I:C) or AddaS03, in Non-human Primates

Tetsuro Yamamoto<sup>1,2,3</sup>, Fusako Mitsunaga<sup>4,5</sup>, Kunihiro Wasaki<sup>1,2,3</sup>, Atsushi Kotani<sup>1,3</sup>, Kazuki Tajima<sup>1,3</sup>, Masanori Tanji<sup>1,2</sup>, and Shin Nakamura<sup>4,5\*</sup>

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DOI:10.26502/ami.936500113



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Article

Molecular Events in Immune Responses to Sublingual Influenza Vaccine with Hemagglutinin Antigen and Poly(I:C) Adjuvant in Nonhuman Primates, *Cynomolgus* Macaques

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*Vaccines* (2024). doi: 10.3390/vaccines12060643.

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Safety Assessment of a Sublingual Vaccine Formulated with Poly(I:C) Adjuvant and Influenza HA Antigen in Mice and Macaque Monkeys: Comparison with Intranasal Vaccine

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Preprints 2024, <https://doi.org/10.20944/preprints202409.2327.v1>

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## ▪ VRD-P1

[Atsushi Kotani](#)

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



## ▪ VRD-P2

[Kazuki Tajima](#)

Preclinical Studies on Sublingual Vaccine Using  
Poly(I:C) Adjuvant in Non-human Primate, Cynomolgus  
Macaques