

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

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>**, and Shin Nakamura<sup>4,5</sup>

1 Innovation Research Center, EPS Holdings, Inc.

2 EP Mediate Co., LTD

3 Research Center, EPS Innovative Medicine Co., Ltd.

4 Intelligence & Technology Lab, Inc.

5 Biomedical Institute, NPO Primate Agora

## 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.

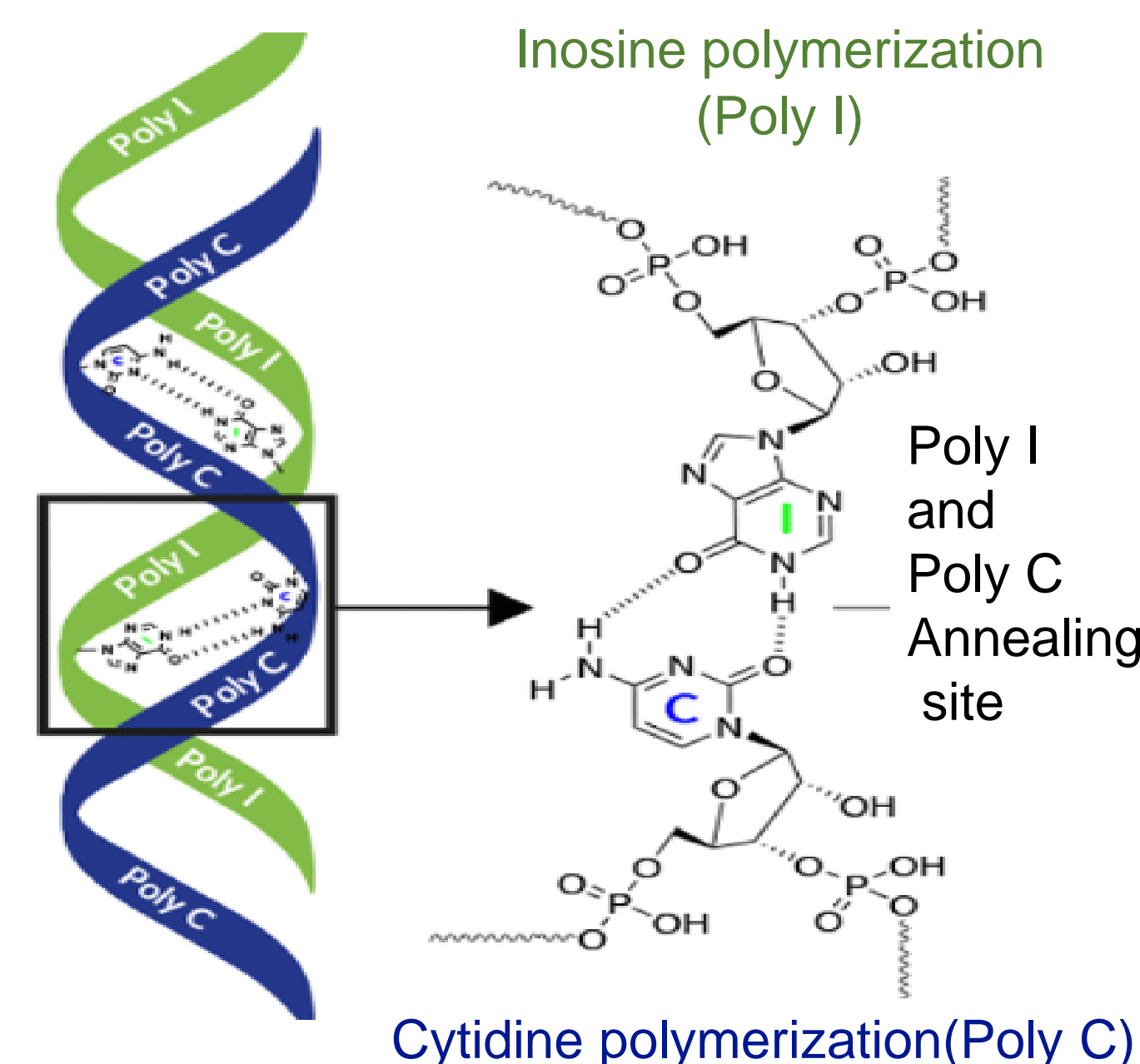
However, sufficient development has not progressed due to the need for the use of adjuvants and the need for evaluation other than IgG production.

In experiments using cynomolgus monkeys, it was confirmed that RBD-specific s-IgA was secreted in nasal secretions in a dose-dependent 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.

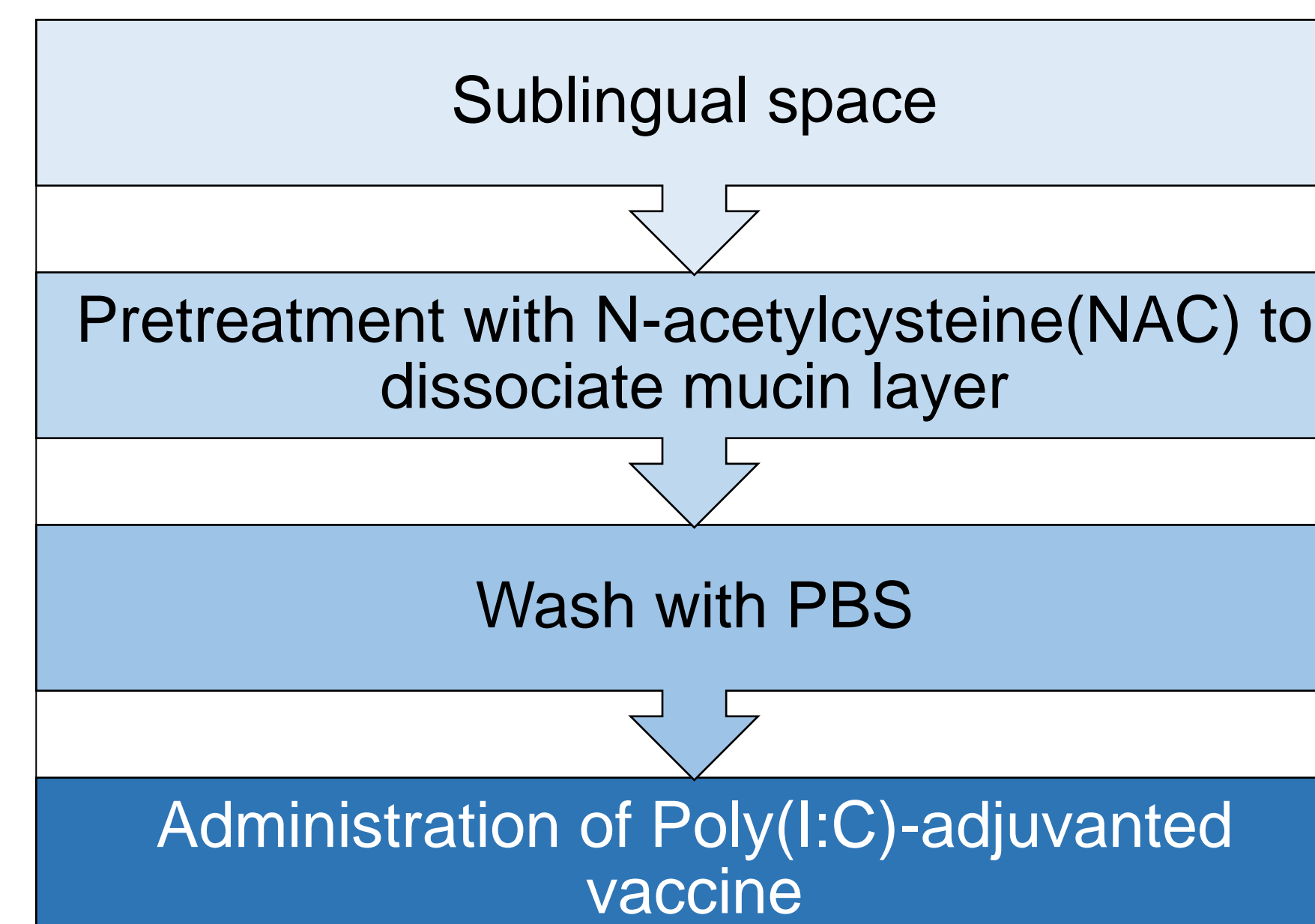
## Poly(I:C) Adjuvant

### Structure of Poly(I:C)



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

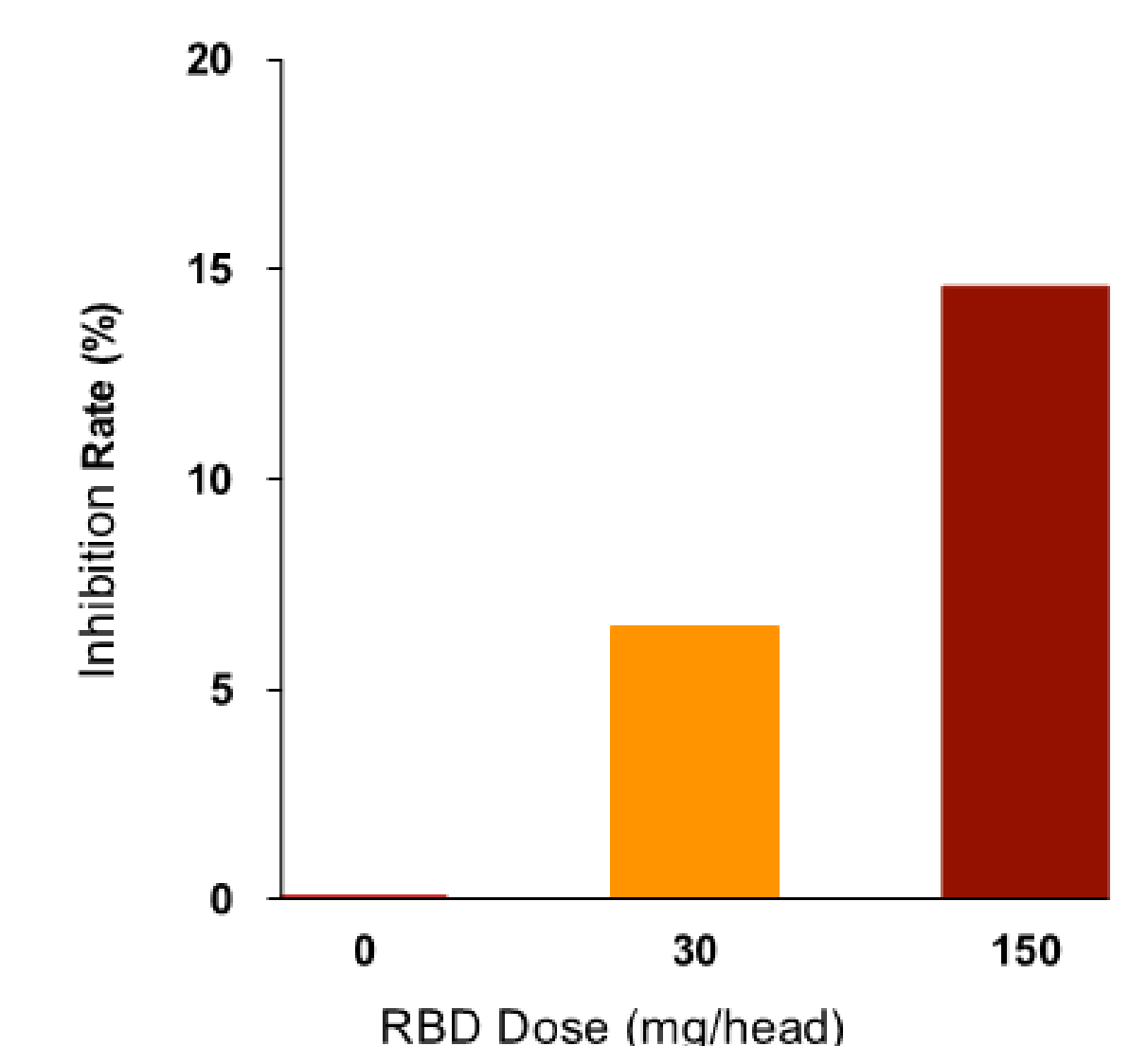
## Vaccine Administration



- SARS-CoV-2 Vaccine: 500  $\mu$ 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

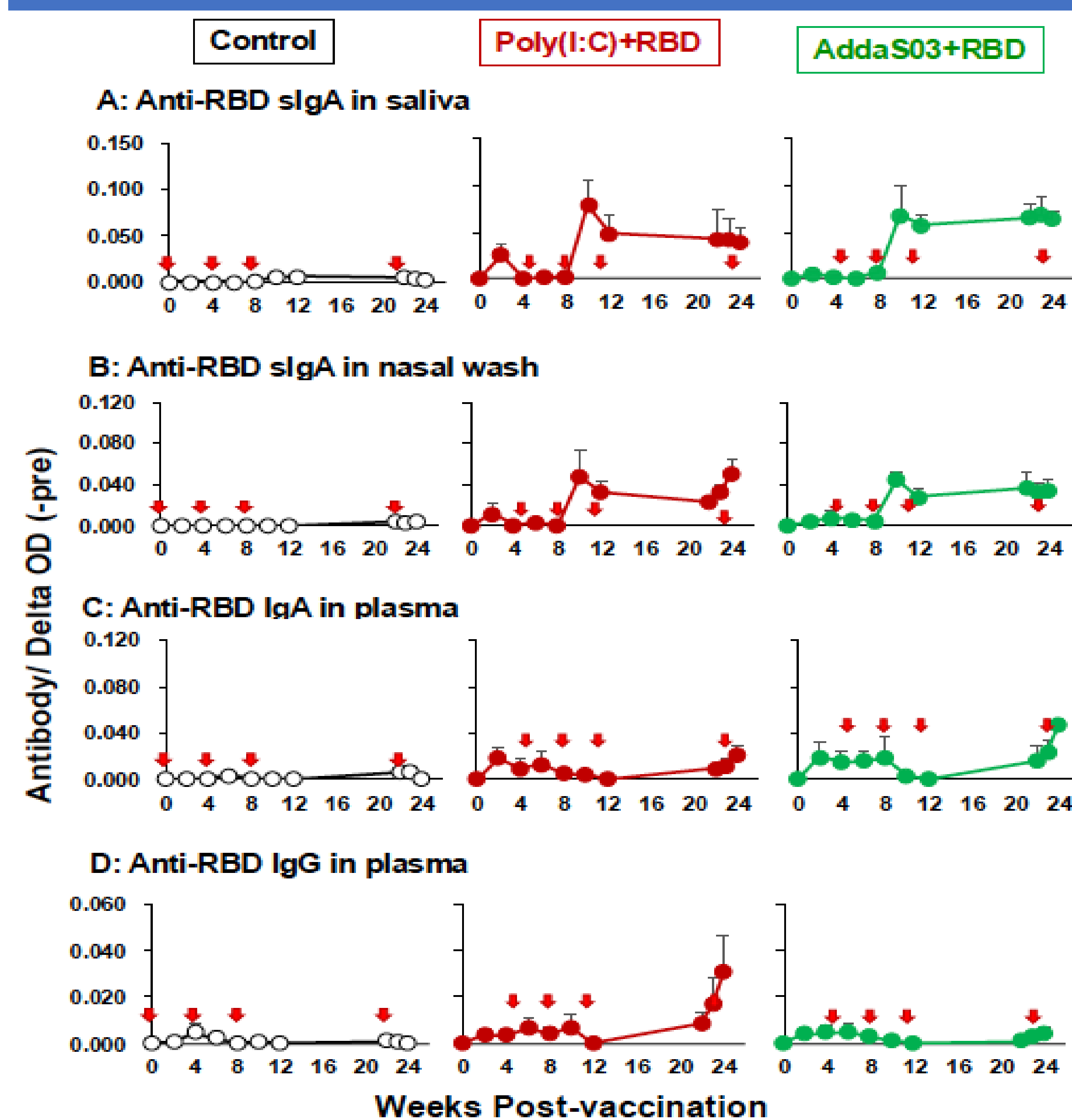
## Results

### Neutralizing antibodies

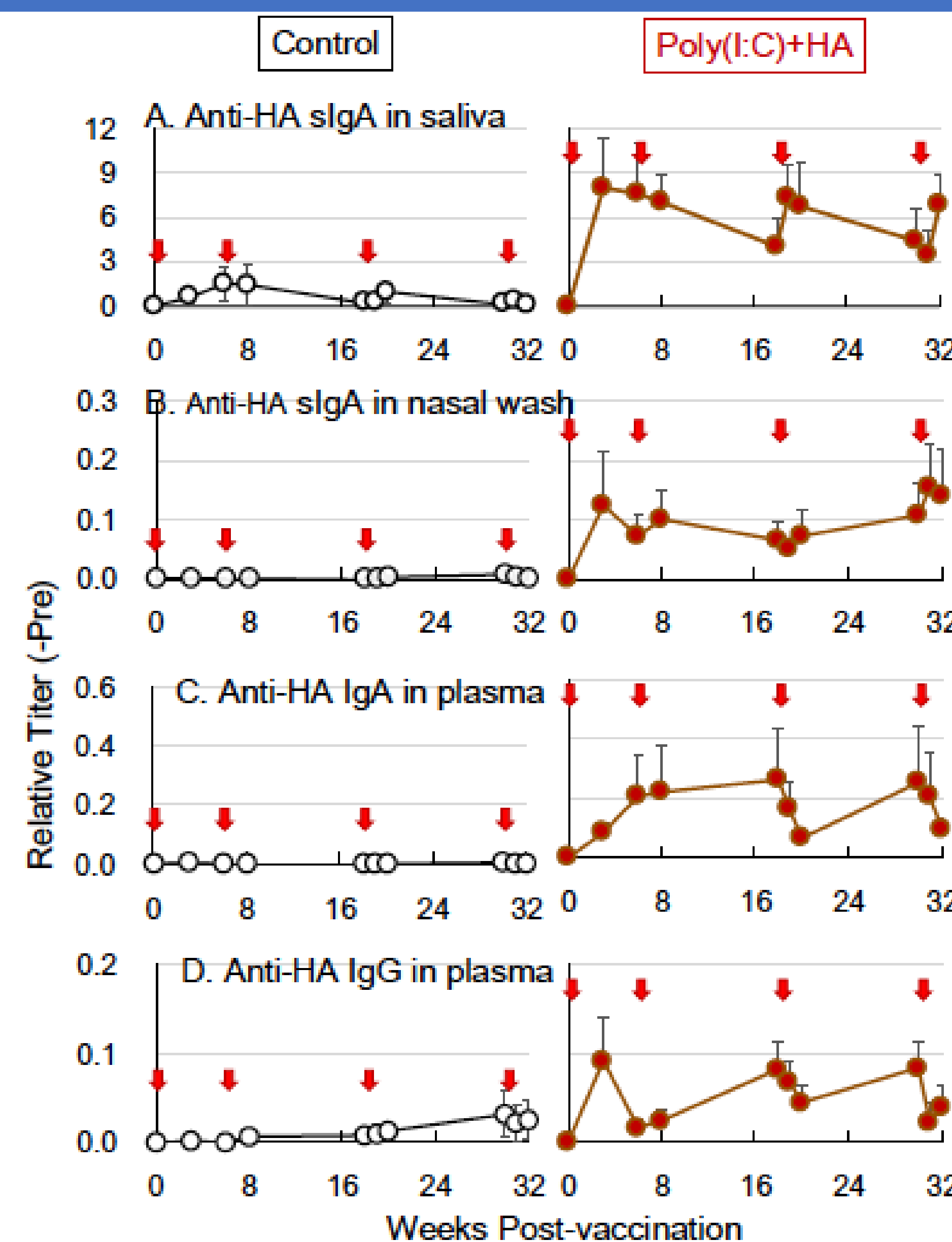


- Neutralizing antibodies in plasma were detected semi-dose dependently with anti-RBD IgA antibody titer.
- Although little neutralizing activity in the saliva or nasal washings was detected (data not shown)
- These are probably caused by existing of an inhibitor factor, soluble form of ACE2, in saliva or nasal washings.

## Antigen specific antibodies

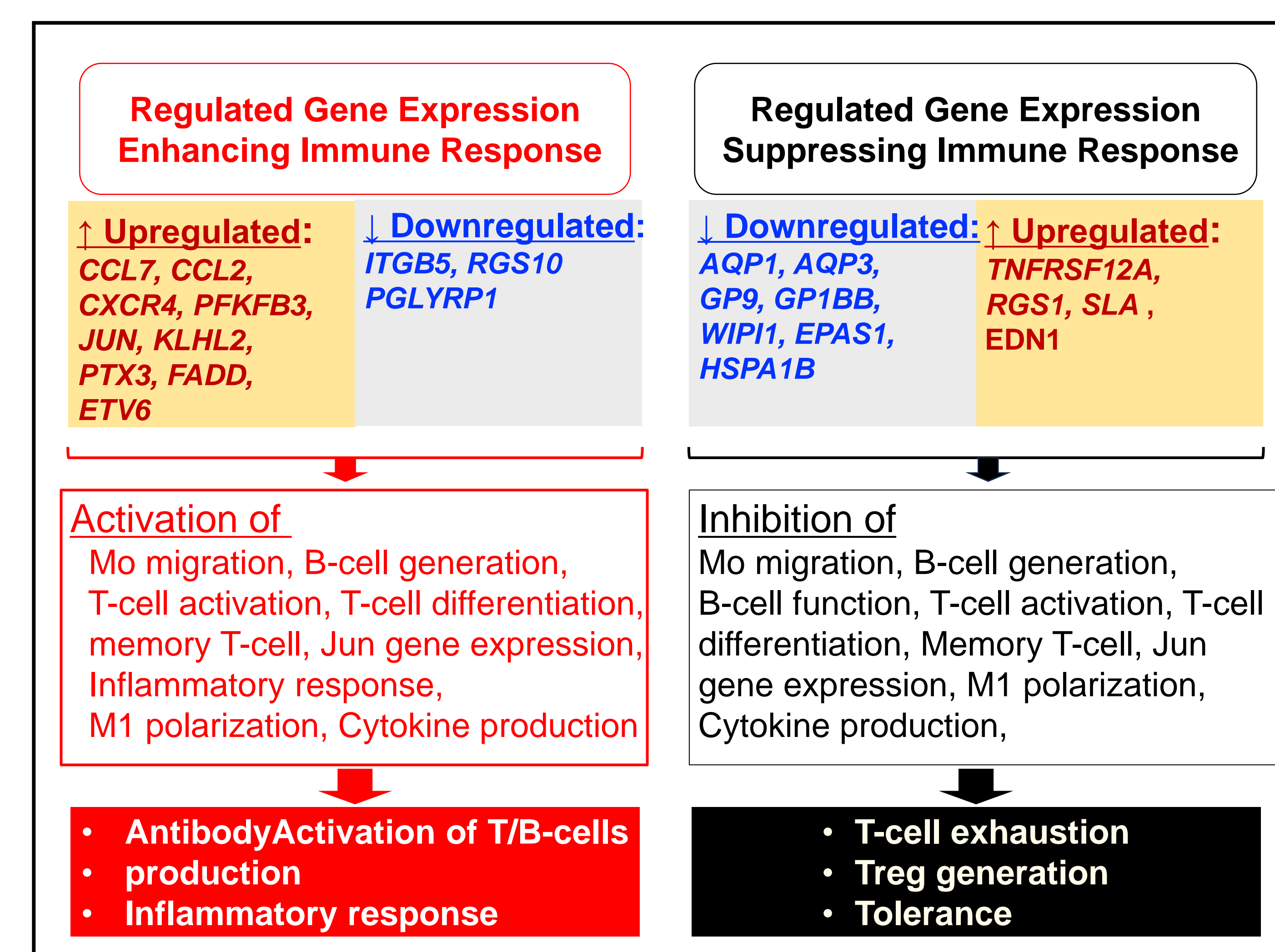


- RBD-specific s-IgA were detected in saliva and nasal wash fluid after the third vaccination .
- RBD-specific IgA and IgG in plasma were detected after the fourth vaccination.
- HA-specific s-IgA were detected in saliva and nasal wash fluid after the initial administration.
- HA-specific IgA and IgG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



## 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."