学 位 論 文 の 要 旨

氏 名　山田 高也

学 位 論 文 名　Mucosal Immunity of Nasopharynx: an Experimental Study in TCR-Transgenic (OVA23-3) Mice

発 表 雑 誌 名（巻, 初頁～終頁, 年）　Rhinology (43, 190~198, 2005)

著 者 名　Takaya Yamada, Shingo Kataoka, Keiko Ogasawara, Ryotaro Ishimitsu, Kazuhiro Hashigucci, Tatsuo Suzuki, Hideyuki Kawauchi

論 文 内 容 の 要 旨

INTRODUCTION

The ideal vaccine therapy has been warranted for activation of the mucosal immune response in the upper respiratory tract against various types of microbial infection. However, the precise study in regard to the mucosal route of vaccine administration and its mechanism of action remains to be further investigated. Therefore, to better understand the exact mechanism of nasopharyngeal mucosal immunology, from T-cell aspects, the antigen-specific antibody (Ab) response was investigated in T cell receptor transgenic (OVA23-3) mice (Tg-mice) and wild type BALB/c mice, in comparison, which were stimulated with repeated nasal antigen challenges of ovalbumin (OVA) together with cholera toxin (CT) or OVA alone. In these experiments, antigen-specific IgA and IgG Ab titers in nasal washings and the frequency of Ab-producing B cells in nasopharyngeal-associated lymphoreticular tissue (NALT), nasal passage (NP), cervical lymph node (CLN) and spleen (SP) were respectively determined by enzyme-linked immunosorbent assay (ELISA) and enzyme-linked immunospot (ELISPOT) assays. Furthermore we were interested to see the cytokine profiles of T cells; interferon (IFN)- γ and

interleukin (IL)-4 levels were measured in nasal washings and various Th1 and Th2 type cytokine productions were respectively measured in culture supernatants of NALT, NP, CLN and SP cells.

MATERIALS AND METHODS

Tg-mice express T cell receptor-αβ chain genes, derived from the chicken OVA-specific I-A^d-restricted $CD4^+CD8^-$ T helper cell clone 7-3-7. Mice were intranasally immunized every two days with 2μL of phosphate-buffered saline (PBS) containing a mixture of 100μg OVA and 1μg of CT as a mucosal adjuvant, or OVA alone. OVA-specific IgA and IgG Ab titers in nasal washings and serum samples were determined by ELISA. For an enumeration of OVA-specific immunoglobulin-producing cells, the number of OVA-specific IgA-producing and IgG-producing cells in the NALT, NP, CLN, and SP were respectively determined by ELISPOT assay. Lymphocytes obtained from NALT, NP, CLN and SP ($2.5x10^5$) were cultured with 200μg of OVA for 48 h at 37℃ in 200μL of RPMI-1640 medium in flat-bottomed 96-well culture plates. Commercial ELISA kits were respectively used to measure levels of IFN-γ, IL-4, IL-5, IL-6 and IL-13 in culture supernatants and nasal washings.

RESULTS AND DISCUSSION

As a first step, we examined OVA-specific Ab response in nasal mucosa and sera of BALB/c mice and Tg-mice, following repeated intranasal challenges of OVA with or without CT. As a result, OVA-specific IgA and IgG Abs significantly increased in nasal washings of BALB/c and Tg-mice stimulated with OVA together with CT and even in those of Tg-mice stimulated with OVA without CT. The specific IgG and IgA producing B cells in NALT and NP cells significantly increased in number in BALB/c mice stimulated with OVA and CT and in Tg-mice stimulated with OVA with or without CT. The amount of cytokines was not measurable in nasal washings obtained from BALB/c mice stimulated with OVA with or without CT. On the other hand, IFN-γ and IL-4 was detected in nasal washings of Tg-mice stimulated with OVA

with or without CT. To elucidate the role of T cells in mucosal IgA and IgG response, the cytokine profiles of T cells in NALT, NP, CLN and SP cells were examined. IFN-γ production, and on the other hand IL-4, IL-5, IL-6, and IL-13 production, are respectively noted as a representative of Th1 type and Th2 type cytokines. Those cytokine levels were measured in the culture supernatants harvested from wells containing NALT, NP, CLN and SP cells derived from each group of mice. The lymphocytes obtained from NALT, NP, CLN, and SP of Tg-mice stimulated with OVA together with or without CT, released the highest levels of Th1 type (IFN-γ) and Th2 type (IL-4, IL-5, IL-6, and IL-13) cytokines into the culture supernatants at each interval after intranasal immunization. But Th2 type cytokine production was not detected in the culture supernatants of NALT lymphocytes obtained from BALB/c or Tg-mice stimulated with OVA with or without CT. The important and interesting findings in this study are that antigen-specific IgA Ab-producing B cells in the NALT and NP were induced and that antigen-specific IgA Ab activity was detected in nasal washings, even though Tg-mice were intranasally immunized with OVA without CT. These findings strongly suggest that $CD4^+CD8^-$ helper T cells play an important role in assisting antigen-specific IgA and IgG production of B cells in nasopharyngeal mucosa, by way of Th1 and Th2 cytokine production. From this point of view, our results indicate that the frequency of antigen-specific helper T cells is the key factor to mount the significant local IgA and IgG responses in the nasopharynx.

CONCLUSION

Taking all data into consideration, it can be concluded that helper T cells recruited into the nasal mucosa and/or locally activated in an antigen-specific fashion are essential for mounting antigen-specific IgA and IgG responses.