学 位 論 文 の 要 旨

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学 位 論 文 名　The Central Role of CD30L/CD30 Interactions in Allergic Rhinitis Pathogenesis in Mice

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論 文 内 容 の 要 旨

INTRODUCTION

CD30 ligand (CD30L), one of the tumor necrosis factor (TNF) superfamily members, is a 40-kDa type II membrane-associated glycoprotein and is expressed on effector $CD4^+$ T cells. The receptor for CD30L is CD30, a member of the TNF receptor superfamily, and is preferentially expressed on effector or memory T cells. A number of lines of evidence suggest that a CD30L/CD30-mediated signal is involved in the development of diseases associated with both T helper (Th) 1 and Th2 cell responses, such as diabetes in young non-obese diabetic mice, mycobacterial infection, and allergic inflammation. Recently, we have found that the CD30L/CD30 signal executed by the T-cell–T-cell interaction plays a critical role in Th1 and Th17 cell differentiation ***in vitro*** and ***in vivo***. Thus, CD30L/CD30 signaling might not be directly correlated with a physiological step for the specific Th cell differentiation subset but might be important for the amplification and/or activation of effector and/or memory Th cells.

In the present study, to examine the role of CD30L in allergic rhinitis, we evaluated an ovalbumin (OVA) model of allergic rhinitis in CD30L knock out (KO) mice. We showed that CD30L positively modulates the allergic symptoms and $CD4^+$ Th2 cell responses during the effector phase. Based on this result, the feasibility of CD30-Ig as an application in the treatment of allergic rhinitis has been confirmed. Implications of these findings for therapeutic application of CD30L/CD30 modulation for allergic rhinitis are discussed.

MATERIALS AND METHODS

Wild type (WT) mice and CD30L KO mice (Balb/c background) were sensitized by intraperitoneal (i.p.) injection with OVA (25 μg) or phosphate buffered saline (PBS) /aluminum hydroxide hydrate gel (alum) three times at 1 week interval, and then, from day 21 to day 35 after the first sensitization with OVA; mice were intranasally (i.n.) challenged with 20 μL of PBS or OVA (20 mg/mL) in the bilateral nostrils. After the i.n. challenge with OVA, the frequencies of sneeze and nasal rubbing in 5 min were counted. OVA-specific IgE and IgG2a productions on days 21, 28 and 35 were determined by enzyme-linked immunosorbent assay (ELISA). Mice were killed 12 h after the final OVA i.n. challenge, and then histologic examination was implemented for counting eosinophils. Mononuclear cells obtained from nasal mucosa, nasopharynx-associated lymphoid tissue (NALT), cervical lymph node (CLN) and spleen were isolated by gentle teasing through stainless steel screens, and flow cytometric analysis was performed. NALT and spleen cells were harvested 12 h after the final OVA i.n. challenge, and then $CD4^+$ T cells (2×10^5) purified by autoMACS were cultured in round-bottom microtiter plates with irradiated spleen cells (1×10^6) and OVA (200 μg/mL) in complete RPMI 1640 medium containing 10% fetal bovine serum (FBS), 5 mM 2-mercaptoethanol (2-ME), 10 U/mL penicillin, and 100 mg/mL streptomycin for 96 h at 37 ℃ in 5% CO_2. The levels of cytokines in the supernatants were determined by ELISA using a Cytokine Development Kit. The secreted CD30-Ig protein by growing NIH 3T3 cells with serum-free medium was purified by HiTrap Protein G HP and analyzed by SDS-PAGE for purity. For ***in vivo*** treatment with soluble CD30-Ig, 20 μL of CD30-Ig or isotype control murine IgG1 (0.5mg/ml) was i.n. introduced into OVA-sensitized WT mice on days 21–35. Counting the frequencies of sneeze and histologic examination of nasal mucosa were implemented. The statistical significance of the data was determined by Student's *t*-test. A value of $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

We assessed the role of CD30L in a mice model of allergic rhinitis. The frequencies of sneeze and nasal rubbing after OVA challenge were significantly reduced in CD30L KO mice than those in WT mice. No sneezing or nasal rubbing occurred in non-sensitized group after either PBS or OVA challenge. Histopathologically, CD30L KO mice showed a mild inflammatory cell infiltration in the mucosa as compared with WT mice 12 h after the final OVA i.n. challenge. Moreover, we found that the accumulation of eosinophils in the nasal mucosa was significantly lower in CD30L KO mice than that in WT mice. The total numbers of cells were significantly lower in the nasal mucosa, NALT, and cervical LNs of CD30L KO mice than those of WT mice. The numbers of $CD4^+$ T cells were significantly lower in these tissues of CD30L

KO mice and the number of B cells was significantly lower in NALT and cervical LNs of CD30L KO mice than WT mice. The numbers of eosinophils ($CD11b^+$ Gr-1dull) and mast cells ($c\text{-}kit^+$ $Fc\varepsilon RI^+$ IgE^+) were significantly lower in the nasal mucosa of CD30L KO mice than WT mice.

On day 21 after the first OVA sensitization, a significant level of OVA-specific IgE was detected in the serum, but a similar level of the OVA-specific IgE was detected both in OVA-sensitized CD30L KO mice and WT mice . The level of OVA-specific IgE was significantly lower in CD30L KO than that in WT mice on days 28 and 35 after the OVA challenge. $CD4^+$ T cells obtained from NALT and cervical LNs of CD30L KO mice produced lower levels of IL-4, IL-5, and IL-13 in response to OVA, as compared with $CD4^+$ T cells obtained from those of WT mice. CD30L KO mice showed impaired Th2 responses in NALT and cervical LNs but not in spleen, especially after i.n. challenge with OVA.

We investigated whether soluble CD30-Ig fusion protein suppresses allergic rhinitis development, which is known to suppress CD30L/CD30 signaling ***in vivo*** as well as ***in vitro***. The numbers of sneeze and nasal rubbing decreased by treatment with CD30-Ig during the effector phase. In the nasal mucosa, eosinophil infiltration decreased by treatment with CD30-Ig during the effector phase. OVA-specific IgE, but not IgG2a, was significantly decreased by the addition of CD30-Ig on day 35. The total numbers of cells were significantly lower in the nasal mucosa, NALT, and cervical LNs of CD30-Ig-treated mice than in control mice. The numbers of $CD4^+$ T cells were significantly lower in these tissues of CD30-Ig-treated mice, and the numbers of B cells were significantly lower in NALT and cervical LNs of CD30-Ig-treated mice than control mice. The numbers of eosinophils and mast cells were significantly lower in the nasal mucosa of CD30-Ig-treated mice than control mice.

Our results obtained by employing CD30L KO mice elucidated that allergic rhinitis was impaired or never fully developed during the effector phase in the absence of CD30L. These findings suggest that blocking of CD30L might be useful for the therapy of allergic rhinitis. CD30-Ig might be useful for treating allergic rhinitis. In fact, in this study, it was shown that CD30-Ig treatment inhibited the development of allergic rhinitis mediated by allergen in mice.

CONCLUSION

CD30L/CD30 signaling is more important in the secondary response at the allergic inflammatory site rather than during the initial phase of antigen priming in the LNs and spleen. The suppression of CD30L/CD30 signaling by soluble CD30-Ig can suppress or regulate rhinitis development and might be useful for the therapy of allergic rhinitis.