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Anti-IL-7 receptor-α treatment ameliorates newly established Sjögren's-like exocrinopathy in non-obese diabetic mice☆

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ABSTRACT

The levels of interleukin (IL)-7 and its receptor are elevated in the salivary glands of patients with Sjögren's syndrome (SS). Our previous study indicates that IL-7 plays a critical pathogenic role in the development and onset of SS in a mouse model of this disease. The present study aims at determining whether IL-7 also plays a role in sustaining SS pathologies after the disease onset, by using the non-obese diabetic (NOD) model. Intraperitoneal administration of a blocking antibody against the IL-7 receptor α chain (IL-7Rα) to female NOD mice aged 10 weeks, which exhibited newly onset clinical SS, for the duration of 3 weeks significantly ameliorated characteristic SS pathologies including hyposalivation and leukocyte infiltration of the submandibular glands (SMGs). These changes were accompanied by a decrease in IFN-γ-producing CD4 T- and CD8 T cells, B cells, and lymphocyte chemoattractants CXCL9, −10, −11 and −13 in the SMGs. Anti-IL-7Rα treatment markedly diminished the amount of TNF-α in the SMGs and increased the level of claudin-1 and aquaporin 5, two molecules critical for normal salivary secretion. Furthermore, neutralization of IFN-γ and TNF-α, individually or in combination, considerably improved salivary secretion, reduced leukocyte infiltration and down-regulated CXCL9 and −13 expression in the SMGs. Collectively, the results indicate that endogenous IL-7R signals promote Th1 and Tc1 responses and IFN-γ- and TNF-α production to sustain the persistence of SS-like sialadenitis in NOD mice. These findings suggest that IL-7 and Th1 cytokines could serve as promising therapeutic targets for this prevalent autoimmune disease.

1. Introduction

Sjögren's syndrome (SS) is a prevalent systemic autoimmune disorder that affects millions of Americans, with a female to male ratio of 9:1 [1–4]. It is characterized by lymphocyte infiltration of exocrine salivary and lacrimal glands, impairment in the salivary and tear secretion and elevation of an array of autoantibodies, with the clinical manifestation as xerostomia (dry mouth) and keratoconjunctivitis sicca (dry eyes) [1,2,5,6]. Accumulating evidence has indicated that autoreactive effector T cells play a crucial pathogenic role in SS [7–9]. Multiple cytokines that can affect or mediate effector T cell functions, including IFN-γ, TNF-α, IL-4, IL-17 and type-1 IFNs, critically contribute to the induction and development of this autoimmune inflammatory disease as demonstrated by *in vivo* studies using mouse models of this disease [1,10–14].

IL-7 is a pleiotropic cytokine produced by non-hematopoietic cells, such as stromal and epithelial cells, and provides essential signals for the development and homeostasis of T cells under physiological conditions [15–17]. IL-7 signals through IL-7 receptor (IL-7R), which is composed of the IL-7Rα chain and the common γ chain. Elevated levels of both IL-7 and IL-7R are detected in the inflamed tissues of various autoimmune and inflammatory disease conditions, including experimental autoimmune encephalomyelitis, systemic erythematosus lupus, rheumatoid arthritis, inflammatory bowel's disease and type-1 diabetes [18–23]. Moreover, blocking the IL-7-IL-7R pathway with anti-IL-7Rα antibody can prevent, reverse or attenuate the pathologies and clinical manifestations of these disorders in mouse disease models [19,20,23–25] and such effect is associated with a preferential reduction in T helper 1 (Th1) and T cytotoxic 1 (Tc1) immune responses characterized by the production of IFN-γ [18,23,24].

In SS patients, IL-7 production and IL-7Rα-expressing T cells are markedly increased in salivary gland tissues and the degree of such increase is associated with the severity of tissue immunopathology and the dry mouth symptoms [26,27]. Our previous study, using the C57BL/6.NOD-*Aec1.Aec2* (B6·NOD-*Aec*) model of SS, has demonstrated that exogenous IL-7 administration accelerates, whereas blockade of

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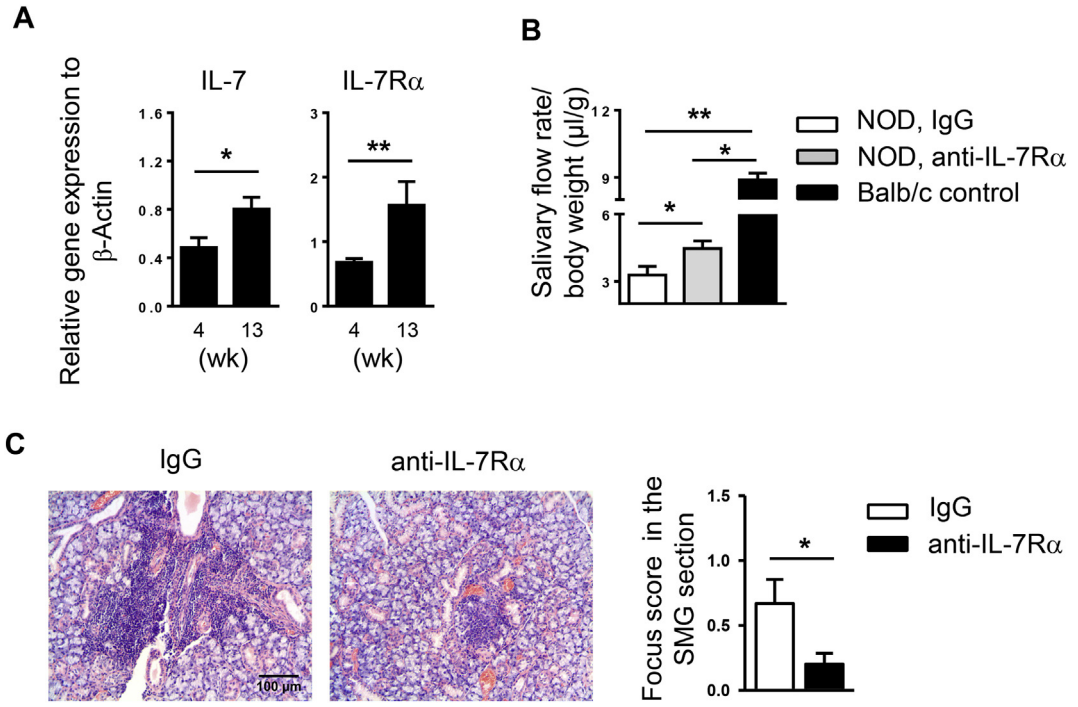

Fig. 1. Anti-IL-7Rα treatment improves salivary secretion and reduces leukocyte infiltration of submandibular glands (SMGs) in NOD mice. (A) Real-time PCR analysis of IL-7 and IL-7Rα levels in the SMGs of NOD mice aged 4 and 13 weeks. The results are presented relative to that of β-actin. Data are the average of analyses of 4–7 mice each group. Error bars represent the SEM. (B) Anti-IL-7Rα antibody or control IgG was *i.p.*-administered to 10-week-old female NOD mice 3 times weekly for 3 weeks. The stimulated salivary flow rate normalized to body weight is shown. (C) H&E staining of SMG sections from mice described in B (scale bar = 100 μm). Bar graph shows the mean leukocyte focus score. Data are representative or the average of 7 mice each group.

endogenous IL-7Rα signals prevents the development and onset of SS [28]. The pathogenic effect of IL-7 at the pre-disease stage is chiefly underlain by enhanced Th1 and Tc1 responses in the target submandibular glands (SMGs) and the augmented production of IFN-γ and TNF-α, two cytokines that are essentially required for the induction and development of SS pathologies [28,29]. Hence, IL-7 and its target Th1 cytokines play a critical pathogenic role in the developmental phase of SS disease.

In the present study, we sought to determine whether the endogenous IL-7R-mediated signaling plays a role in sustaining the persistence of SS pathologies after the disease onset and whether blockade of this pathway can reverse or attenuate the newly established SS disease, by using the non-obese diabetic (NOD) mouse strain, a spontaneous model of human SS [30–32]. By using anti-IL-7Rα antibody-mediated blockade of endogenous IL-7R signaling in these mice, our study demonstrated an indispensable role of this pathway in sustaining the persistence of SS.

2. Materials and methods

2.1. Mice

Female non-obese diabetic (NOD) mice were purchased from the Jackson Laboratory and were housed in the specific pathogen-free animal facility at the Forsyth Institute. All the experimental protocols were approved by the Institutional Animal Care and Use Committee of the Forsyth Institute and all the procedures were implemented in compliance with the National Institutes of Health guidelines for the care and use of laboratory animals.

2.2. Antibodies

Purified monoclonal rat-anti-mouse IL-7Rα (A7R34) and its isotype control rat-IgG2a (2A3) used for injection were obtained from BioXCell. For flow cytometry, fluorescence conjugated anti-CD4, anti-CD8, anti-CD19, anti-IFN-γ, anti-PD-1, anti-IL-17 and anti-CD16/32 antibodies were purchased from BioLegend, and fluorescence conjugated anti-Foxp3 antibody was obtained from eBioscience. For Immunohistochemical staining, biotin-conjugated anti-CD4 and anti-CD8 antibodies were obtained from eBiosience and biotin-conjugated anti-B220 antibody was obtained from BioLegend; anti-TNF-α, anti-claudin-1 and anti-claudin-2 antibodies were purchased from Abcam. For immunofluorescence staining, anti-aquaporin-5 (AQP5) antibody and Alexa Fluor647-conjugated anti-rabbit IgG were purchased from Abcam.

2.3. In vivo administration of anti-IL-7Rα, anti-TNF-α and anti-IFN-γ antibodies

10 week-old female NOD mice received intraperitoneal (*i.p.*) administration of 200 μg anti-mouse IL-7Rα, anti-mouse TNF-α, anti-mouse IFN-γ, anti-TNF-α plus anti-IFN-γ antibodies or the corresponding isotype control IgG 3 times weekly for 3 weeks. All the analyses were performed 2 days after the last injection.

2.4. Histological analysis

SMG tissues were fixed in 4% paraformaldehyde, embedded in paraffin and sectioned to 5 μm thickness. Routine histology was carried out on these sections with hematoxylin and eosin (H&E) to determine the degree of inflammation. The focus score, defined as the number of leukocytic foci (comprising more than 50 mononuclear cells) per 4 mm^2 area of tissues, in each of the three non-consecutive sections of SMG samples were determined, and the average focus score of the three sections was used for further statistical analysis.