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Arginase 1 is involved in lacrimal hyposecretion in male NOD mice, a model of Sjögren's syndrome, regardless of dacryoadenitis status

Yuta Ohno[1], Keitaro Satoh[1,2], Akiko Shitara[1], Takeshi Into[3] and Masanori Kashimata[1]

[1]*Department of Pharmacology, Asahi University School of Dentistry, 1851-1 Hozumi, Mizuho, Gifu, 501-0296, Japan*
[2]*Department of Pharmacology, Meikai University School of Dentistry, 1-1 Keyakidai, Sakado, Saitama, 350-0283, Japan*
[3]*Department of Oral Microbiology, Asahi University School of Dentistry, 1851-1 Hozumi, Mizuho, Gifu, 501-0296, Japan*

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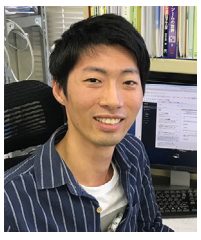
Key points

- Few reports have explored the possibility of involvement of non-inflammatory factors in lacrimal hyposecretion in Sjögren's syndrome (SS).
- RNA-sequencing analysis revealed that only four genes, including arginase 1, were down-regulated in the lacrimal gland of SS model male mice (NOD mice) after onset of lacrimal hyposecretion and dacryoadenitis.
- Even in non-dacryoadenitis-type NOD mice, tear secretion and arginase 1 expression remained low.
- An arginase 1 inhibitor reduced tear secretion and partially reduced saliva secretion in BALB/c mice.
- The results indicate that a non-inflammatory factor, arginase 1, is involved in lacrimal hyposecretion in male NOD mice, regardless of dacryoadenitis status.

Abstract Lacrimal fluid (tears) is important for preservation of the ocular surface, and thus lacrimal hyposecretion in Sjögren's syndrome (SS) leads to reduced quality of life. However, the cause(s) of lacrimal hyposecretion remains unknown, even though many studies have been conducted from the perspective of inflammation. Here, we hypothesized that a non-inflammatory factor induces lacrimal hyposecretion in SS pathology, and to elucidate such a factor, we conducted transcriptome analysis of the lacrimal glands in male non-obese diabetic (NOD) mice as an SS model. The NOD mice showed inflammatory cell infiltration and decreased pilocarpine-induced tear secretion at and after 6 weeks of age compared to age-matched BALB/c mice. RNA-sequencing analysis revealed that only four genes, including arginase 1, were downregulated, whereas many genes relating to inflammation were upregulated, in the lacrimal glands of male NOD mice after

Yuta Ohno is a research associate in the Department of Pharmacology at Asahi University of Dentistry. He graduated from Gifu Pharmaceutical University in 2013 and worked as a pharmacist for several years. During that period, he worked on both basic and clinical research and earned his PhD. In 2018, he started working on exocrine biology at Asahi University, and focused on the mechanisms of lacrimal hyposecretion. His aims are to elucidate the mechanism of exocrine hyposecretion and to develop a drug that will alleviate patients' suffering from dry eye/mouth syndrome due to hyposecretion.



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onset of lacrimal hyposecretion and dacryoadenitis (lacrimal gland inflammation). Changes in the level of arginase 1 expression were confirmed by real-time RT-PCR and western blot analysis. Furthermore, non-dacryoadenitis-type NOD mice were used to investigate the relationships among arginase 1 expression, lacrimal hyposecretion and dacryoadenitis. Interestingly, these NOD mice retained the phenotype of dacryoadenitis with regard to tear secretion and arginase 1 expression level. An arginase 1 inhibitor reduced tear secretion and partially reduced saliva secretion in BALB/c mice. In conclusion, a non-inflammatory factor, arginase 1, is involved in lacrimal hyposecretion in male NOD mice, regardless of dacryoadenitis status. These results shed light on the pathophysiological role of arginase 1 in SS (dry eye).

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Corresponding author Yuta Ohno: 1851-1 Hozumi, Mizuho, Gifu 5010296, Japan. Email: ohnoyuta@dent.asahi-u.ac.jp

Introduction

Lacrimal fluid (tears) is essential for preservation of the ocular surface because of its physiological functions of wetting corneal cells, providing oxygen and nutrition, and protecting against bacteria and foreign substances. Thus, dysfunction of the lacrimal glands, where lacrimal fluid is produced, can lead to dry eye and reduced quality of life. The causes of lacrimal dysfunction include Sjögren's syndrome (SS) (Kuklinski & Asbell, 2017), the use of drugs having anticholinergic effects (Askeroglu *et al.* 2013), radiotherapy for head-and-neck tumours (Bhandare *et al.* 2012), metabolic syndrome (Kawashima *et al.* 2014) and work involving video display terminals (Nakamura *et al.* 2010).

SS is an autoimmune disease accompanied by infiltration of inflammatory cells into exocrine glands. This infiltration has been considered to induce lacrimal and/or salivary hyposecretion by destroying acinar cells in the lacrimal and/or salivary glands of SS patients (Fox, 2005). However, therapy for SS has been limited to symptomatic treatments, such as artificial tears, artificial saliva, application of topical solutions of immunosuppressive agents to the ocular surface, and systemic use of muscarinic agonists (Baer & Walitt, 2018). On the other hand, some studies have found that the severity of inflammatory cell infiltration did not necessarily correlate with the volume of tear or saliva secretion (Paranyuk *et al.* 2001; Jonsson *et al.* 2006). Those findings suggested that as-yet unidentified non-inflammatory factors might be involved in lacrimal or salivary hyposecretion, in both SS and dry eye syndrome. Identification of such factors might help us prevent development of SS and dry eye syndrome.

Non-obese diabetic (NOD) mice are used as a primary SS model because of similarities with humans in the pathology of infiltration of inflammatory cells into the lacrimal and/or salivary glands (Leiter *et al.* 1987). The inflammation rate in the glands of NOD mice is sex-dependent: high in male lacrimal glands and in female salivary glands (Miyagawa *et al.* 1986; Takahashi *et al.* 1997). Inflammatory cell infiltration into the lacrimal glands (dacryoadenitis) of male NOD mice was absent at 4 weeks of age but observed at and after 6–8 weeks of age (Takahashi *et al.* 1997; Hunger *et al.* 1998). On the other hand, the onset of lacrimal hyposecretion in male NOD mice was reported to be at 8 weeks of age (Doyle *et al.* 2007). Again, there have been few studies on non-inflammatory factors in lacrimal hyposecretion in male NOD mice, but many studies on inflammatory factors. The innate immune system seems to be involved in the development of inflammation in exocrine glands (Warner & Núñez, 2013). Deficiency of Myd88, an adaptor molecule for toll-like receptors, in female NOD mice was reported to largely suppress inflammatory cell infiltration into salivary glands compared to that seen in Myd88$^{+/+}$ female NOD mice (Hansen *et al.* 2016; Kiripolsky *et al.* 2017; Into *et al.* 2018).

RNA sequencing (RNA-seq) is a widely used method for understanding the transcriptome; it quantifies gene expression at the RNA level by using deep-sequencing techniques (Wang *et al.* 2009). The results of RNA-seq analysis usually do not provide specific data. For example, RNA-seq data include not only disease-related factors but also strain-specific or growth-specific differences, when comparing different strains, such as a disease model and control mice, or different ages, such as before and after onset of disease. Interpretation of RNA-seq data is complicated, and thus multiple comparisons of RNA-seq data seem to be needed to eliminate potential factors that fluctuate depending on the age or strain of the animal model. To date, RNA-seq analysis of the lacrimal gland has been reported in early-stage disease in SS model mice (Chen *et al.* 2018). Large numbers of genes related to inflammation, innervation and cell survival were, indeed, upregulated or downregulated in the lacrimal glands of the early-phase SS model mice compared with control mice.

Arginase is an enzyme that catalyses hydrolysis of L-arginine to L-ornithine and urea (Wu & Morris, 1998). In mammals, arginase has two isoforms, arginase 1 and 2, which are present in the cytosol and in mitochondria, respectively. Arginase 1 is known to catalyse the terminal

step of the urea cycle in the liver, where it is strongly expressed (Caldwell *et al.* 2018). With regard to exocrine glands, arginase 1 was reported to be expressed in human lacrimal glands (Jäger *et al.* 2013) and mouse salivary glands (Yasuda *et al.* 2004), although its function in those glands remains unknown.

With this background in mind, we hypothesized that a non-inflammatory factor(s) induces lacrimal and/or salivary hyposecretion in SS pathology. First, we investigated the age of onset of lacrimal hyposecretion and dacryoadenitis in male NOD mice. Then, to identify possible non-inflammatory factors, we performed RNA-seq at three age points, i.e. before, and in the early and developing stages of onset of lacrimal hyposecretion and dacryoadenitis, in male NOD mice and age-matched control mice. By statistical comparison of gene expressions between strains at the same age and between ages in the same strain, we focused on factors with a strong possibility of being associated with decreased tear secretion and/or increased inflammation in the lacrimal glands of male NOD mice. In addition, we verified whether the identified factors were non-inflammatory by using Myd88$^{-/-}$ NOD mice as non-inflammatory-type NOD mice, and by administering an inhibitor of arginase 1 to control mice.

Materials and methods

Ethical approval

The investigators understand the ethical principles under which *The Journal of Physiology* operates, and the work conducted in this study complies with the Journal's animal ethics checklist (Grundy, 2015).

All animal studies were approved by Asahi University's Committee on the Ethics of Animal Experiments (Approval Numbers: 18-020, 18-021, 19-003, 19-019, 20-006 and 20-009) and were carried out in accordance with the guidelines issued by that Committee.

Materials

Phenol red-impregnated thread (Zone-Quick) was purchased from Ayumi Pharmaceutical Co. (Tokyo, Japan). Medetomidine (Dorbene) was purchased from Kyoritsu Seiyaku Co. (Tokyo, Japan), midazolam from Sandoz K.K. (Tokyo, Japan), butorphanol (Vetorphale) from Meiji Seika Pharma Co., Ltd (Tokyo, Japan), paper plugs (JM paper point) from J. Morita Corp. (Osaka, Japan), Tissue Total RNA Purification Mini Kit from Favorgen (Ping-Tung, Taiwan), DNase I from Nippon Gene (Tokyo, Japan), ReverTra Ace qPCR RT Master Mix from Toyobo (Tokyo, Japan), SYBR Premix Ex Taq II (TB Green Premix Ex Taq II Tli RNaseH Plus) and EmeraldAmp PCR Master Mix from Takara Bio Inc. (Shiga, Japan), RIPA lysis buffer (EzRIPA Lysis kit) from Atto Co. (Tokyo, Japan), skim milk from Morinaga-Nyugyo (Tokyo, Japan), rabbit anti-arginase 1 antibody from GeneTex, Inc. (Irvine, CA, USA), rabbit anti-α-tubulin antibody from Proteintech (Rosemont, IL, USA), anti-rabbit IgG HRP-linked antibody from Beckman Coulter (Fullerton, CA, USA), whole-cell lysates of 293T cells, which overexpress human liver arginase (arginase 1), from Abcam (Cambridge, UK), ECL Western blotting detection reagents from GE Healthcare (Chicago, IL, USA), and CB-1158 dihydrochloride from MedChemExpress Co., Ltd (Monmouth Junction, NJ, USA).

Mice

Male non-obese diabetes/ShiJcl (NOD) mice were purchased from CLEA Japan (Tokyo, Japan). Male BALB/cCrSlc (BALB/c) mice were purchased from Japan SLC (Hamamatsu, Japan). NOD.B6-Myd88$^{-/-}$ mice were generated as previously described (Into *et al.* 2018). Briefly, a congenic strain, NOD.B6-Myd88$^{-/-}$, was generated by crossing NOD with B6-Myd88$^{-/-}$ mice. Heterozygotes were backcrossed with NOD for 11 generations, followed by appropriate sister–brother mating to generate NOD.B6-Myd88$^{-/-}$ mice.

All mice were maintained under controlled conditions (23 $\pm$ 2°C, 50% humidity, 12 h light/dark cycle) in the animal facility at Asahi University School of Dentistry. All mice were given free access to water and standard chow (MF; Oriental Yeast Co., Ltd, Tokyo, Japan).

Measurement of tear and saliva volumes

Tear volume was determined by the cotton thread test using a phenol red-impregnated thread. Mice were anaesthetized via an intraperitoneal (I.P.) injection of an anaesthetic agent mixture (0.75 mg kg^{-1} medetomidine, 4.0 mg kg^{-1} midazolam and 5.0 mg kg^{-1} butorphanol) at a volume of 0.05 mL per 10 g body weight. The anaesthetic agent mixture was decided based on previous reports (Kawai *et al.* 2011; Miwa *et al.* 2019). Pilocarpine was injected I.P. at 0.5 mg kg^{-1}, which was decided based on our earlier study in which we measured the saliva volume (Satoh *et al.* 2013). The volume of tear fluid was measured by carefully placing a phenol red-impregnated thread at the canthus of each eye for 30 s every 2 min after injection. The length of thread that changed colour due to absorption of tear fluid was measured in millimetres. The total tear volume was calculated by summing the length of the colour-changed thread in each 30-s measurement segment from 2 to 20 min after pilocarpine injection. The maximum tear volume value was the longest length of the colour-changed thread in each 30-s measurement segment from 2 to 20 min after pilocarpine injection. Comparison of the maximum tear volume was based on the highest value at any time in one group with the highest value at

any time in another group. All tear volume values were normalized for body weight.

Saliva volume was measured simultaneously with tear volume, in the same mice. Saliva volume was determined by a gravimetric method using paper plugs, as in our earlier study (Satoh *et al.* 2013). With the animal under anaesthesia as described above, pilocarpine was injected I.P. at 0.5 mg kg^{-1}. The secreted saliva was then absorbed into paper plugs inserted into the oral cavity for 30 s every 2 min after injection. The saliva-saturated plugs were weighed and corrected for the original weight of the paper plug. The volume of secreted saliva was calculated as the increase in weight of each paper plug. Total saliva volume was calculated by summing the increase in weight of each paper plug in each 30-s measurement segment from 2 to 20 min after pilocarpine injection. Maximum saliva volume was the greatest increase in the weight of the paper plug in each 30-s measurement segment from 2 to 20 min after pilocarpine injection. All saliva volume values were normalized for body weight.

Table 1. List of RNA integrity number (RIN) of each sample

Sample ID	RIN
NOD_4wk_1	9.8
NOD_4wk_2	9.4
NOD_4wk_3	9.3
NOD_6wk_1	9.2
NOD_6wk_2	9.1
NOD_6wk_3	9.3
NOD_10wk_1	9.5
NOD_10wk_2	9.9
NOD_10wk_3	9.6
BALBc_4wk_1	9.5
BALBc_4wk_2	9.1
BALBc_4wk_3	8.9
BALBc_6wk_1	8.6
BALBc_6wk_2	9.5
BALBc_6wk_3	9.1
BALBc_10wk_1	8.2
BALBc_10wk_2	8.7
BALBc_10wk_3	9.1

Histological analysis

Mice were placed in a chamber containing room air, and killed by exposing them to carbon dioxide gas in a rising concentration (the gas flow rate was ∼20% of chamber volume per minute). Lacrimal glands were isolated, and the glands were then fixed in 10% formalin and paraffin-embedded. For each animal, three sections (5 µm thick) were cut at various depths of the lacrimal gland. The sections were stained with haematoxylin and eosin. Images of tissue sections were obtained with a BX53F microscope and a DP80 digital camera (Olympus, Tokyo, Japan) and processed using ImageJ software (National Institutes of Health, Bethesda, MD, USA). The images were then manually combined to generate representative lacrimal gland images using PowerPoint (Microsoft, Redmond, WA, USA). The degree of inflammation in the lacrimal glands was expressed as (1) a focus score, the total number of foci per 4 mm^2 of a section of lacrimal gland; and (2) the total area of foci. A 'focus' was defined as an infiltration of >50 mononuclear cells in a section. Results were expressed as the mean ± standard deviation (SD) calculated from the mean values of three sections per animal.

Transcriptome analysis

Total RNA was isolated from the lacrimal glands from the mice by using a Tissue Total RNA Purification Mini Kit after eliminating DNA contamination with DNase I. RNA-seq and the analysis were outsourced to Macrogen Japan Corp. (Kyoto, Japan). To check the quality of the RNA, the RNA Integrity Number (RIN) was calculated using a bioanalyser (Agilent Technologies Japan, Ltd, Tokyo, Japan). Each RNA sample satisfied RIN > 8 (Table 1), suggesting the RNA was of high quality. For each RNA sample, cDNA libraries were prepared using the TruSeq Stranded mRNA library prep kit (Illumina, San Diego, CA, USA), and then paired-end sequencing of 100 bp was performed on a NovaSeq 6000 (Illumina). Quality control metrics were checked on raw sequencing reads with FASTQC version 0.11.7. After trimming low-quality bases with Trimmomatic 0.38, reads were mapped to the *Mus musculus* genome (mm10) with HISTAT2 version 2.1.0. At least 96% of the reads in each sample were successfully mapped. StringTie version 1.3.4d was then used for transcript assembly. The expression profile was calculated as fragments per kilobase of transcript per million mapped reads (FPKM). Multidimensional scaling analysis and hierarchical clustering analysis were performed on quantile-normalized $\log_2$-transformed FPKM+1 values. Differentially expressed gene (DEG) analysis was performed using normalized FPKM values. The generated nucleotide sequence data are available in the DDBJ Sequenced Read Archive under accession number DRA010121.

Real-time RT-PCR

Isolation of total RNA with elimination of DNA contamination was performed by the method described above. Single-strand cDNA was synthesized from 0.5 µg total RNA by reverse transcription with random primers and oligo dT primers using ReverTra Ace qPCR RT Master Mix (Toyobo, Tokyo, Japan). Real-time RT-PCR was performed on 25 µl of reaction mixture containing

each primer, template cDNA and SYBR Premix Ex Taq II using a Thermal Cycler Dice Real Time System (Takara Bio Inc.). Reactions were performed in 40 cycles of 95°C for 5 s and 60°C for 30 s after an initial denaturing at 95°C for 30 s. Melting curve data were obtained by increasing the temperature from 60 to 95°C. The primer sets were as follows: Arg1: 5′-TGGAAGAGTCAGTGTGGTGC-3′ (forward) and 5′-TGGTTGTCAGGGGAGTGTTG-3′ (reverse); and Gapdh: 5′-TGTGTCCGTCGTGGATCTGA-3′ (forward) and 5′-TTGCTGTTGAAGTCGCAGGAG-3′ (reverse). Gene expression was quantified by using a standard curve, and then normalized to *Gapdh* and to the corresponding experimental control. Reactions were run in duplicate.

Western blot analysis

Mice were killed with carbon dioxide by the methods described above, and their lacrimal and parotid glands were isolated. These glands were homogenized in ice-cold RIPA lysis buffer (1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS, 150 mM NaCl and 20 mM Hepes) containing protease inhibitors (pepstatin A, aprotinin and leupeptin), phosphatase inhibitors (NaF, sodium orthovanadate and sodium glycerophosphate) and 1 mM phenylmethylsulfonyl fluoride; the homogenates were then incubated on ice for 15 min. The homogenates were spun at 14,000 *g* for 10 min. Supernatants were collected, and the protein concentrations were determined by the method of Bradford (1976). The supernatants were used for Western blotting. Protein samples of 2 μg were separated by SDS-PAGE using a Mini-Protean 3 Cell system (Bio-Rad Laboratories, Hercules, CA, USA). After electrophoresis, the separated proteins were transferred onto a PVDF filter using a Trans-Blot Turbo System (Bio-Rad). The blots were blocked at room temperature for 50 min in skim milk and then probed with a primary antibody, anti-arginase 1 (diluted 1:2000), for 120 min, or α-tubulin (diluted 1:8000), for 90 min. The blots were washed three times with Tris-buffered saline (pH 7.6) containing 0.05% Tween 20, probed for 90 min with anti-rabbit IgG HRP-linked antibody (diluted 1:10,000), and washed again. Whole-cell lysates of 293T cells, which overexpress human liver arginase (arginase 1), were used as a positive control. Immunoreactivity was determined using ECL Western blotting detection reagents. Images were acquired using Light-Capture II (Atto Co.). The intensity of arginase 1 was measured with CS Analyzer 3.0 (Atto Co.).

RT-PCR

Isolation of total RNA and synthesis of cDNA were performed by the methods described above. PCR was performed on 10 μl of the reaction mixture containing each primer, template cDNA and EmeraldAmp PCR Master Mix using a TaKaRa PCR Thermal Cycler Dice (Takara Bio Inc.). Reactions were performed for 30 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 30 s after initial denaturing at 95°C for 2 min. The primer sets were as follows: Myd88: 5′-ACCCCACTCGCAGTTTGTTG-3′ (forward) and 5′-TCCTGTTGGACACCTGGAGAC-3′ (reverse); and Gapdh: 5′-ACCACAGTCCATGCCATCAC-3′ (forward) and 5′-TCCACCACCCTGTTGCTGTA-3′ (reverse).

Administration of arginase 1 inhibitor

An arginase 1 inhibitor, CB-1158 dihydrochloride, was dissolved in distilled water. CB-1158 was orally administered at 100 mg kg^{-1} (volume: 0.1 ml per 10 g body weight) 2 h before measurement of tear and/or saliva secretion. The vehicle group was administered the same volume of water.

Statistical analysis

Data are presented as the mean ± standard deviation (SD) (n = sample size). Statistical comparisons were made using a two-tailed Student's t test (Figs 1*E*,*F*, 2*B*,*C*, 4*A*,*C*, 5*C*,*D*, 7*B*,*C* and 8*B*,*C*); one-way ANOVA followed by Tukey's multiple comparisons test (Fig. 6*B*,*D* and *E*); two-way ANOVA followed by Sidak's multiple comparisons test (Figs 1*B*–*D*, 7*A* and 8*A*); or two-way ANOVA followed by Tukey's multiple comparisons test (Fig. 6*C*). Statistical comparisons between two groups in RNA-seq were performed using an independent t test. Values (P) below 0.05 were regarded as statistically significant differences. These statistical analyses were performed using GraphPad Prism7 (GraphPad Software, La Jolla, CA, USA).

Results

Lacrimal fluid in male NOD mice

We used the phenol red-impregnated thread technique to measure the tear volume induced by pilocarpine injection in order to examine the onset of lacrimal hyposecretion in NOD mice (Fig. 1*A*). At 4 weeks of age, tear secretion in the NOD mice showed a similar pattern to that in the BALB/c mice (Fig. 1*B*). At 6 and 10 weeks of age, tear secretion in the NOD mice at some points was significantly decreased compared to in the BALB/c mice (Fig. 1*C* and *D*). Total tear volume after pilocarpine treatment of the NOD mice decreased to about 60% at 6 weeks of age and less than half at 10 weeks of age compared to in BALB/c mice (BALB/c *vs.* NOD = 1.14 ± 0.22 *vs.* 0.64 ± 0.36 mm g^{-1} body weight

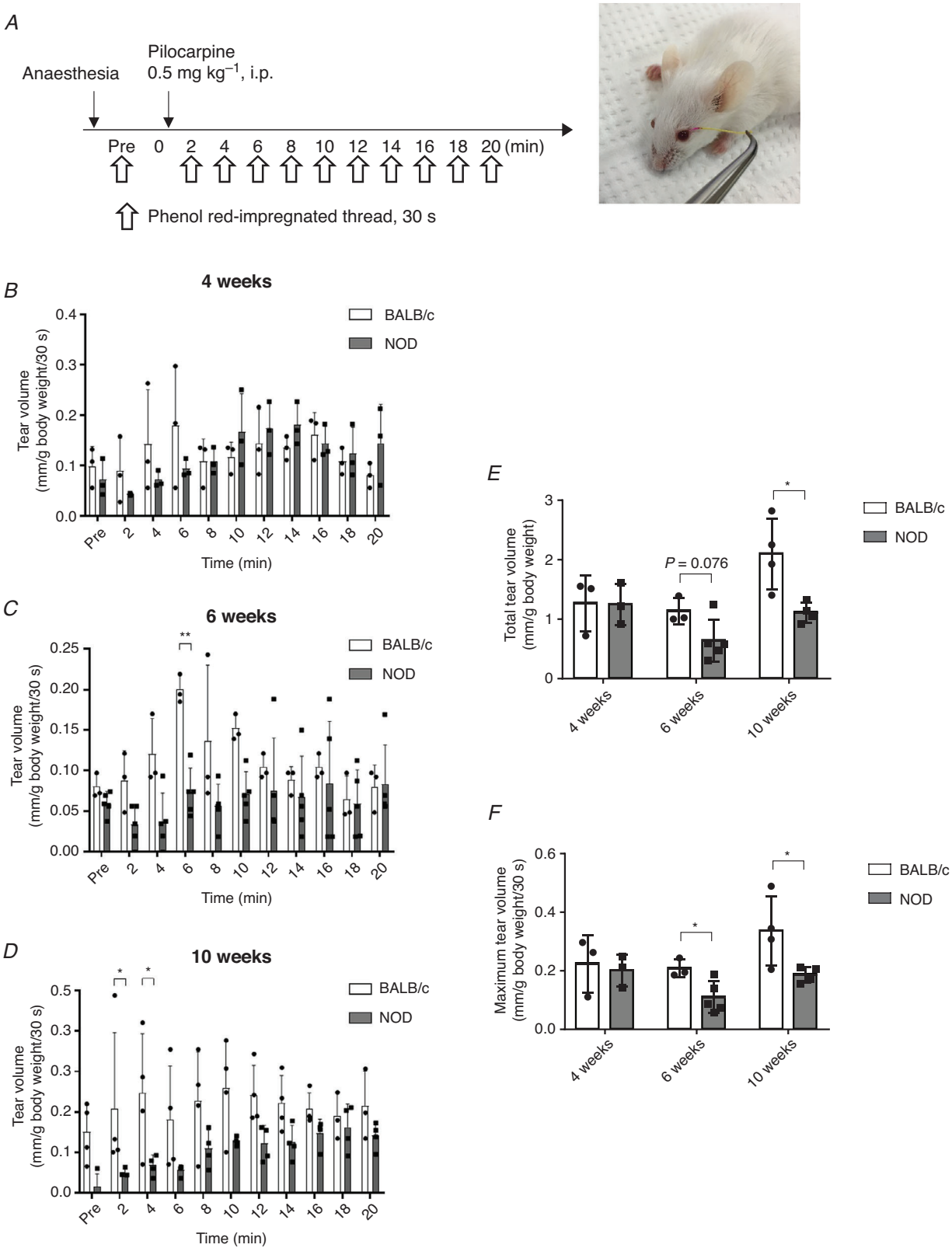

Figure 1. Reduced pilocarpine-induced tear secretion in male NOD mice at and after 6 weeks of age
A, the experimental design for measurement of tear secretion. We measured the length of the colour-changed phenol red-impregnated thread to estimate tear volume. We also weighed the saliva-saturated paper plugs and

corrected for the original plug weight to estimate saliva volume, which was measured simultaneously with tear volume, in the same mice. *B–D*, body weight-adjusted tear volume for 30 s in every 2 min in BALB/c and NOD mice at each time point after pilocarpine treatment, at 4, 6 and 10 weeks of age, respectively. *E*, total tear volume after pilocarpine treatment in male BALB/c and NOD mice at 4, 6 and 10 weeks of age. *F*, maximum tear volume in male BALB/c and NOD mice at 4, 6 and 10 weeks of age. The obtained values are presented as mean ± SD: $^{*}p < 0.05$, $^{**}p < 0.01$ *versus* BALB/c group. All experiments were conducted with 3–5 animals in each group.

at 6 weeks of age; 2.10 ± 0.59 *vs.* 1.11 ± 0.17 mm g^{-1} body weight at 10 weeks of age; Fig. 1*E*). Maximum tear volume showed similar results (BALB/c *vs.* NOD = 0.21 ± 0.03 *vs.* 0.11 ± 0.05 mm g^{-1} body weight/30 s at 6 weeks of age; 0.34 ± 0.12 *vs.* 0.19 ± 0.03 mm g^{-1} body weight/30 s at 10 weeks of age; Fig. 1*F*). These results suggest that the onset of lacrimal hyposecretion in NOD mice is at around 6 weeks of age, and the symptom persists until at least 10 weeks of age.

Dacryoadenitis in male NOD mice

We examined the onset of dacryoadenitis in NOD mice by performing haematoxylin and eosin staining of lacrimal glands removed from NOD and BALB/c mice at 4, 6 and 10 weeks of age. Inflammatory cells infiltrated the lacrimal glands at 6 and 10 weeks of age in the NOD mice, but not at any age in the BALB/c mice (Fig. 2*A*). The focus score and the area of inflammatory cell foci in the lacrimal glands were dramatically increased in the 10-week-old NOD mice (8.80 ± 0.46 and 12.40 ± 6.42%, respectively) compared to the 6-week-old NOD mice (2.90 ± 1.35 and 0.93 ± 0.59%, respectively) (Fig. 2*B* and *C*). These results suggest that dacryoadenitis starts at around 6 weeks of age in NOD mice and gets worse until 10 weeks of age. Here, based on the results shown in Figures 1 and 2, we defined the before, and the early and developing stages of onset of lacrimal hyposecretion and dacryoadenitis as 4, 6 and 10 weeks of age in NOD mice, because those two symptoms manifest almost simultaneously.

Transcriptome analysis of lacrimal glands in male NOD mice

To assess expression of genes comprehensively, we performed RNA-seq of the lacrimal glands from 4-, 6- and 10-week-old NOD and BALB/c mice (Fig. 3*A*). Multidimensional scaling analysis and heat mapping of one-way hierarchical clustering revealed that the gene expression patterns in lacrimal glands of NOD mice at 6 and 10 weeks were different from at 4 weeks (Fig. 3*B* and *C*). On the other hand, the gene expression pattern in lacrimal glands from 4-week-old NOD mice was relatively similar to that in 4-week-old BALB/c mice (Fig. 3*B* and *C*).

DEG analysis revealed 827 genes that satisfied a fold change of either ≥ 2 or ≤ 1/2, with an independent *t* test *P*-value <0.05 in at least one comparison pair (Fig. 3*C*). At and after 6 weeks of age, 259 genes showed increased expression in the NOD mice compared to the BALB/c mice, whereas there were no significant differences in gene expression in the lacrimal glands of the NOD and BALB/c mice at 4 weeks of age. Immunity-related genes comprised the top 10 genes among them (Table 2). In contrast, only four genes (*Esp23*,*Arg1*, *Obp1a* and *Spc25*; Table 3) showed decreased expression at and after 6 weeks of age in the NOD mice compared to the BALB/c mice, but there were none at 4 weeks of age. We focused on *Arg1* for two reasons: (1) *Esp23* and *Obp1a* are not conserved in humans, and (2) *Arg1* shows higher expression than *Spc25* in mouse lacrimal glands (data not shown), but is more down-regulated than *Spc25* at and after 6 weeks of age in NOD mice.

Arginase 1 expression level in lacrimal glands of male NOD mice

First, we performed quantitative RT-PCR of *Arg1* to validate the RNA-seq data. The RNA expression level of *Arg1* normalized to *Gapdh* in lacrimal glands from NOD mice was significantly decreased at 6 and 10 weeks of age compared to the age-matched BALB/c mice (BALB/c *vs.* NOD = 1.31 ± 0.59 *vs.* 0.18 ± 0.12 at 6 weeks; 0.87 ± 0.14 *vs.* 0.21 ± 0.15 at 10 weeks), but not at 4 weeks of age (BALB/c *vs.* NOD = 1.00 ± 0.07 *vs.* 0.92 ± 0.16) (Fig. 4*A*). These values validated our RNA-seq data.

Next, we performed western blotting to check the protein expression level of arginase 1. In western blot analysis, arginase 1 derived from mouse tissues usually appears as double bands (Swärd *et al.* 2013), whereas arginase 1 derived from human tissues is seen as a single band (Shen *et al.* 2017). We detected double bands reacting with anti-arginase 1 antibody in homogenates of the lacrimal glands from both mouse strains, but only one band in lysates of human arginase 1-overexpressing cells used as a positive control. The intensity of these double bands was significantly decreased in 6- and 10-week-old NOD mice compared to the age-matched BALB/c mice (BALB/c *vs.* NOD = 1.00 ± 0.13 *vs.* 1.38 ± 0.26 at 4 weeks; 1.54 ± 0.08 *vs.* 0.64 ± 0.22 at 6 weeks; 1.48 ± 0.22 *vs.* 0.33 ± 0.09 at 10 weeks) (Fig. 4*B* and *C*). These results

show that male NOD mice have low levels of arginase 1 in their lacrimal glands at and after 6 weeks of age, and suggest that arginase 1 could be involved in development of lacrimal hyposecretion and/or inhibition of inflammation in the lacrimal glands of those mice.

Lacrimal fluid and arginase 1 expression in non-inflammatory type NOD mice

We previously reported that infiltration of inflammatory cells into the submandibular gland was largely suppressed

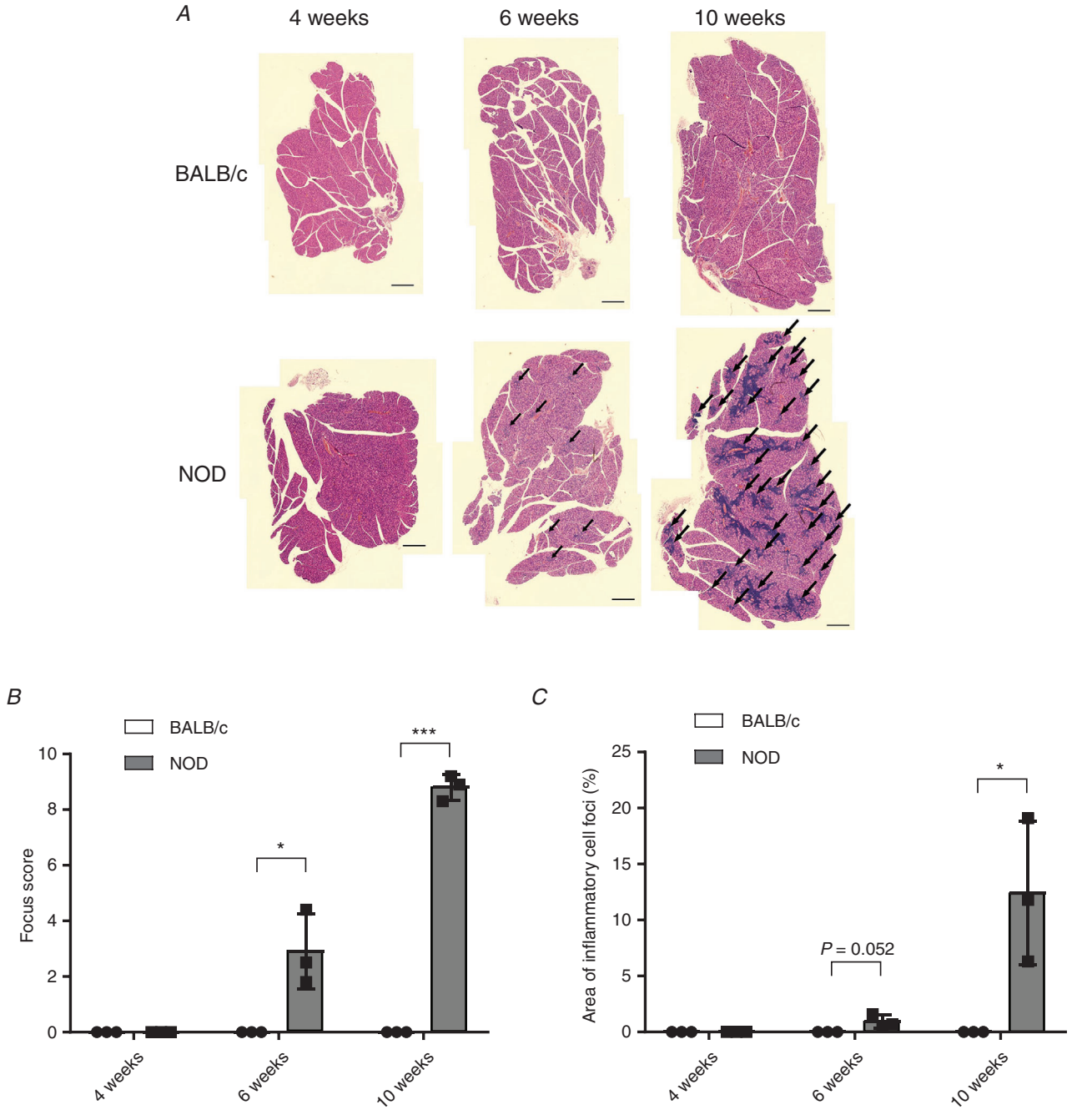

Figure 2. Inflammatory cell infiltration of lacrimal glands of male NOD mice at and after 6 weeks of age
A, representative cross-sections of haematoxylin and eosin-stained lacrimal glands from male BALB/c and NOD mice at 4, 6 and 10 weeks of age. Arrows show infiltration of inflammatory cells. Scale bars represent 0.5 mm. *B* and *C*, the focus score (*B*) and the area of inflammatory cell foci (*C*) were determined in the lacrimal glands of male BALB/c and NOD mice at 4, 6 and 10 weeks of age. The obtained values are presented as the mean ± SD: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *versus* BALB/c group. All experiments were conducted with three animals in each group.

by Myd88 deficiency in female NOD mice (NOD Myd88$^{-/-}$ mice) (Into *et al.* 2018). Here, we confirmed that male NOD Myd88$^{-/-}$ mice do not express the *Myd88* gene in the lacrimal glands and show little or no infiltration of inflammatory cells into the lacrimal glands (Fig. 5*A* and *B*). Quantitative analysis showed that the focus score and the area of inflammatory cell foci in male NOD Myd88$^{-/-}$ mice were greatly suppressed to 0.26 ± 0.44 and 0.15 ± 0.27%, respectively, compared with male NOD Myd88$^{+/+}$ mice (7.10 ± 1.37 and 3.36 ± 0.33%, respectively) (Fig. 5*C* and *D*). Therefore, we regarded male NOD Myd88$^{-/-}$ mice as a non-inflammatory lacrimal gland model in NOD mice.

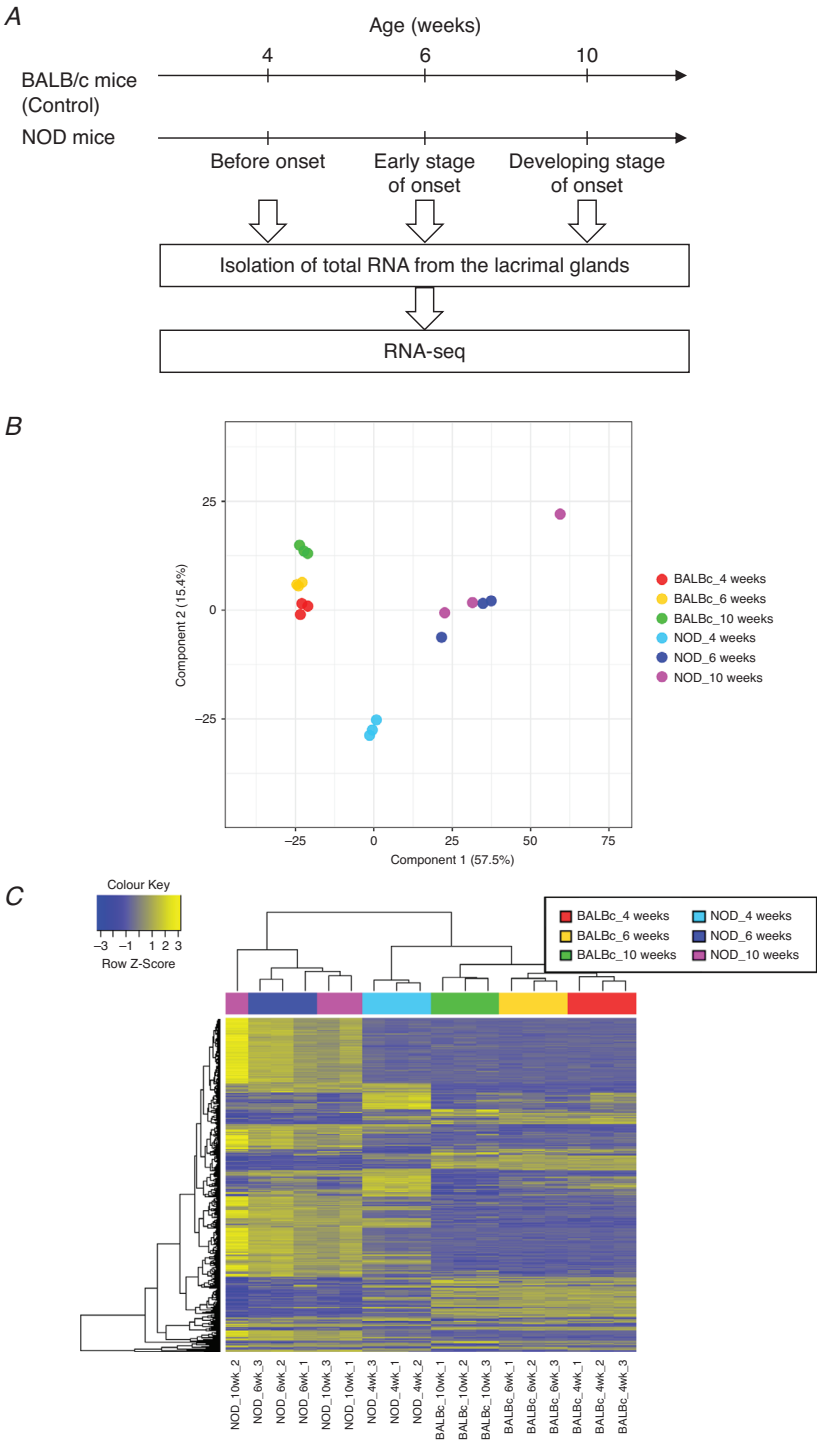

Figure 3. Changes in gene expression in lacrimal glands from male NOD mice at and after 6 weeks of age as revealed by transcriptome analysis
A, experimental design of transcriptome analysis. Total RNA was extracted from lacrimal glands from male BALB/c and NOD mice at 4, 6 and 10 weeks of age, and then RNA-seq was performed. The experiment was conducted with three animals in each group. *B*, multidimensional scaling plot, showing similarity of the gene expression patterns. *C*, heat map of one-way hierarchical clustering using Z-score for normalized values (log_2-based). This graphically represents the similarity of expression patterns between samples and genes.

Table 2. Top 10 genes that were significantly increased in the lacrimal glands of male NOD mice at and after 6 weeks of age; data from RNA-seq, showing fold change

Gene ID	Gene symbol	Description	NOD_4wk/ BALBc_4wk	NOD_6wk/ BALBc_6wk	NOD_10wk/ BALBc_10wk	NOD_6wk/ NOD_4wk	NOD_10wk/ NOD_6wk	BALBc_6wk/ BALBc_4wk	BALBc_10wk/ BALBc_6wk
		Satisfying	−2 <, < 2	2 <, ascending order	2 <	2 <			
23833	*Cd52*	CD52 antigen	1.13	16.03	14.94	14.04	1.26	−1.01	1.35
20304	*Ccl5*	chemokine (C-C motif) ligand 5	1.08	12.59	10.09	10.57	−1.36	−1.10	−1.09
14969	*H2-Eb1*	histocompatibility 2, class II antigen E beta	1.30	11.63	12.12	13.42	1.18	1.51	1.13
13040	*Ctss*	cathepsin S	1.58	11.28	9.88	7.22	1.02	1.01	1.17
110454	*Ly6a*	lymphocyte antigen 6 complex, locus A	1.95	10.43	13.51	5.40	−1.12	1.01	−1.45
14960	*H2-Aa*	histocompatibility 2, class II antigen A, alpha	1.14	9.91	9.72	11.72	1.19	1.35	1.21
16149	*Cd74*	CD74 antigen (invariant polypeptide of major histocompatibility complex, class II antigen-associated)	−1.00	9.78	10.60	12.73	1.31	1.30	1.21
107350	*AW112010*	expressed sequence AW112010	1.27	9.26	8.97	8.07	−1.12	1.10	−1.09
55985	*Cxcl13*	chemokine (C–X–C motif) ligand 13	1.52	9.03	22.31	6.05	2.55	1.02	1.03
14469	*Gbp2*	guanylate binding protein 2	1.23	8.91	7.57	6.67	1.03	−1.08	1.21

Table 3. All genes (four in total) that were significantly decreased in the lacrimal glands of male NOD mice at and after 6 weeks of age; data from RNA-seq, showing fold change

Gene ID	Gene symbol	Description	NOD_4wk/ BALBc_4wk	NOD_6wk/ BALBc_6wk	NOD_10wk/ BALBc_10wk	NOD_6wk/ NOD_4wk	NOD_10wk/ NOD_6wk	BALBc_6wk/ BALBc_4wk	BALBc_10wk/ BALBc_6wk
		Satisfying	−2 <, < 2	<−2, descending order	<−2	<−2			
100126779	*Esp23*	exocrine gland secreted peptide 23	−1.65	−8.44	−11.43	−5.68	−1.74	−1.11	−1.28
11846	*Arg1*	arginase, liver	−1.46	−4.09	−3.57	−2.51	−1.08	1.12	−1.24
18249	*Obp1a*	odorant binding protein IA	−1.41	−3.81	−4.12	−2.71	−1.20	1.00	−1.11
66442	*Spc25*	SPC25, NDC80 kinetochore complex component, homologue (*S. cerevisiae*)	1.05	−2.15	−2.30	−2.46	−1.30	−1.09	−1.21

To examine the relationships among inflammation, lacrimal fluid secretion and arginase 1 expression, we investigated the arginase 1 expression level and pilocarpine-induced tear volume in male NOD Myd88$^{-/-}$ mice. Western blot analysis (Fig. 6*A* and *B*) found that, for the NOD mice, the double bands reacting with anti-arginase 1 antibody showed similar intensity, with and without lacrimal gland inflammation in the NOD

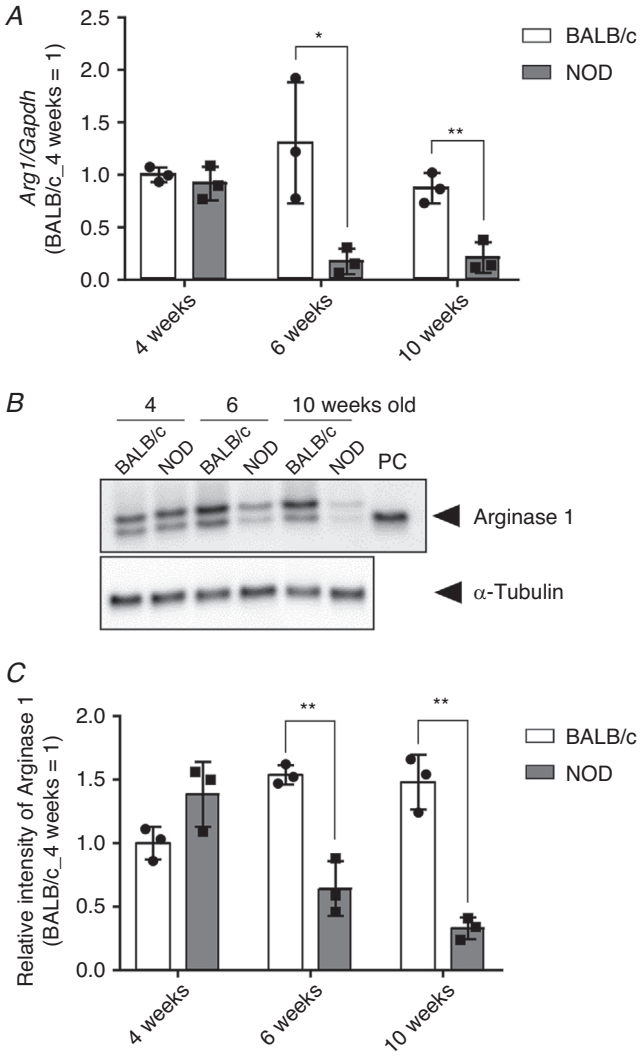

Figure 4. Decreased expression level of arginase 1 in lacrimal glands from male NOD mice at and after 6 weeks of age
A, RNA expression level of *Arg1* in lacrimal glands from male BALB/c and NOD mice at 4, 6 and 10 weeks of age. The expression level of *Arg1* was measured using quantitative real-time RT-PCR and normalized to *Gapdh*. *B*, representative immunoreactive bands showing arginase 1 protein expression in lacrimal glands from male BALB/c and NOD mice at 4, 6 and 10 weeks of age. α-Tubulin was used as an internal control. PC: positive control. *C*, the intensity of the immunoreactive bands of arginase 1. The obtained values are presented as the mean ± SD: $^{*}p < 0.05$, $^{**}p < 0.01$ *versus* BALB/c group. All experiments were conducted with three animals in each group.

mice (0.36 ± 0.07 and 0.34 ± 0.20, respectively), but the intensity of both bands was significantly decreased compared to in BALB/c mice (1.00 ± 0.18), suggesting that male NOD mice have low levels of arginase 1 in their lacrimal glands regardless of dacryoadenitis. Tear volume showed a similar pattern with and without inflammation of the lacrimal glands in the NOD mice, but it was significantly decreased compared with the BALB/c mice (Fig. 6*C*). Total tear volume after pilocarpine treatment was significantly decreased regardless of dacryoadenitis (0.70 ± 0.03 and 1.15 ± 0.31 mm g^{-1} body weight, with and without inflammation in the lacrimal glands) in the NOD mice compared with the BALB/c mice (2.10 ± 0.60 mm g^{-1} body weight) (Fig. 6*D*). Maximum tear volume showed a similar tendency (0.09 ± 0.01 and 0.18 ± 0.05 mm g^{-1} body weight/30 s, with and

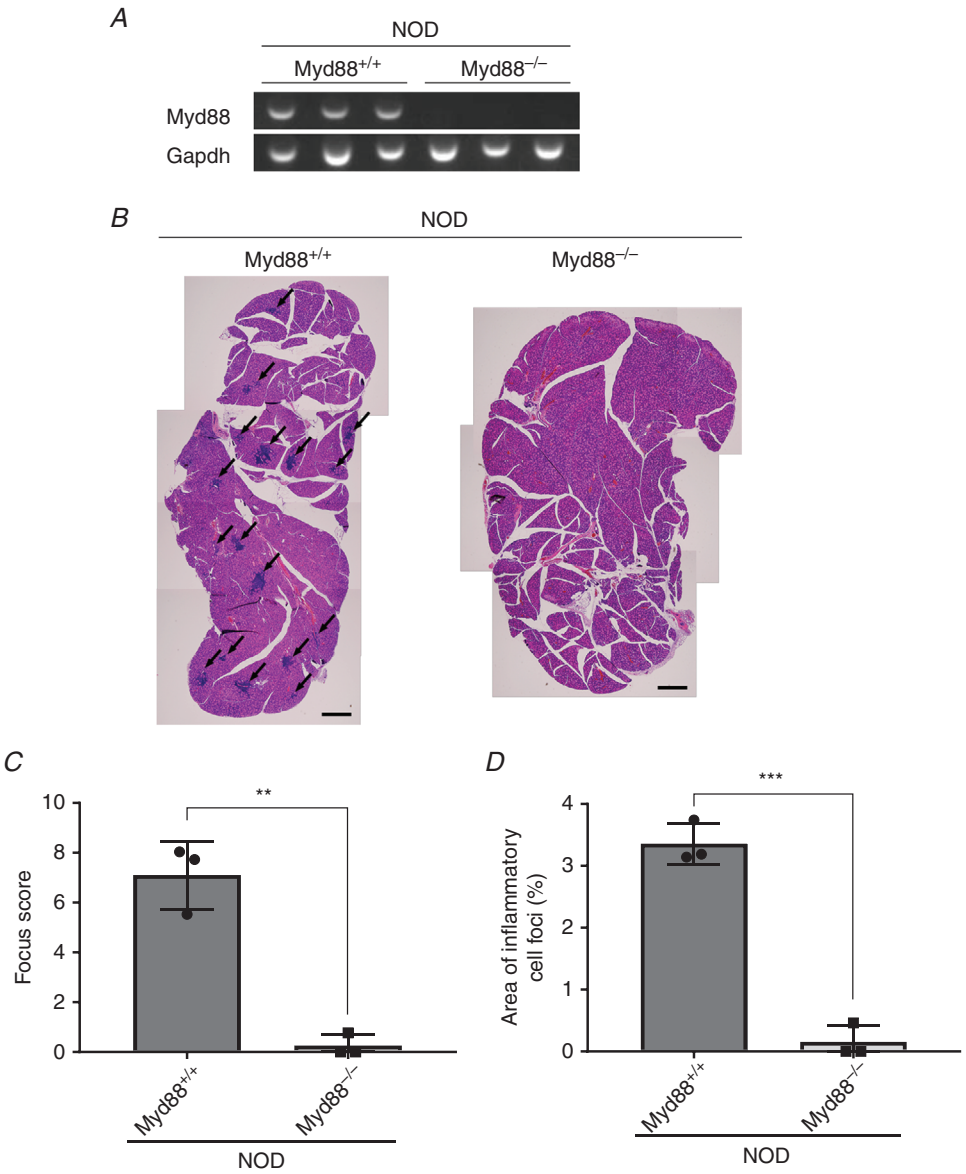

Figure 5. Suppressed dacryoadenitis in Myd88$^{-/-}$ NOD mice
A, Myd88 deficiency in a lacrimal gland from a male Myd88$^{-/-}$ NOD mouse. Myd88 deficiency in the gland was confirmed by RT-PCR. *Gapdh* was used as an internal control. *B*, representative cross-sections of haematoxylin and eosin-stained lacrimal glands from male Myd88$^{+/+}$ and Myd88$^{-/-}$ NOD mice. Arrows show infiltration of inflammatory cells. Scale bars represent 0.5 mm. *C* and *D*, the focus score (*C*) and the area of inflammatory cell foci (*D*) were determined in lacrimal glands from male Myd88$^{+/+}$ and Myd88$^{-/-}$ NOD mice. The obtained values are presented as the mean ± SD: $^{**}p < 0.01$, $^{***}p < 0.001$ *versus* Myd88$^{+/+}$ NOD group. All experiments were conducted with three animals in each group.

without lacrimal gland inflammation) in the NOD mice compared with the BALB/c mice (0.34 ± 0.12 mm g^{-1} body weight/30 s) (Fig. 6*E*). Together, these results show that both tear volume and arginase 1 expression level are decreased in NOD mice, regardless of dacryoadenitis. It is highly likely that lacrimal gland inflammation in NOD mice has little effect on the development of lacrimal hyposecretion, suggesting that the symptom is due to the low level of arginase 1 in the lacrimal glands in those mice.

Effect of arginase 1 inhibitor on lacrimal and salivary fluid in BALB/c mice

We examined the involvement of arginase 1 on tear volume by orally administering CB-1158, an arginase 1 inhibitor, to 10-week-old BALB/c mice. The tear volume induced by pilocarpine treatment was significantly decreased at 4 and 6 min after pilocarpine injection in those mice compared to the control BALB/c mice administered water (Fig. 7*A*). After pilocarpine treatment,

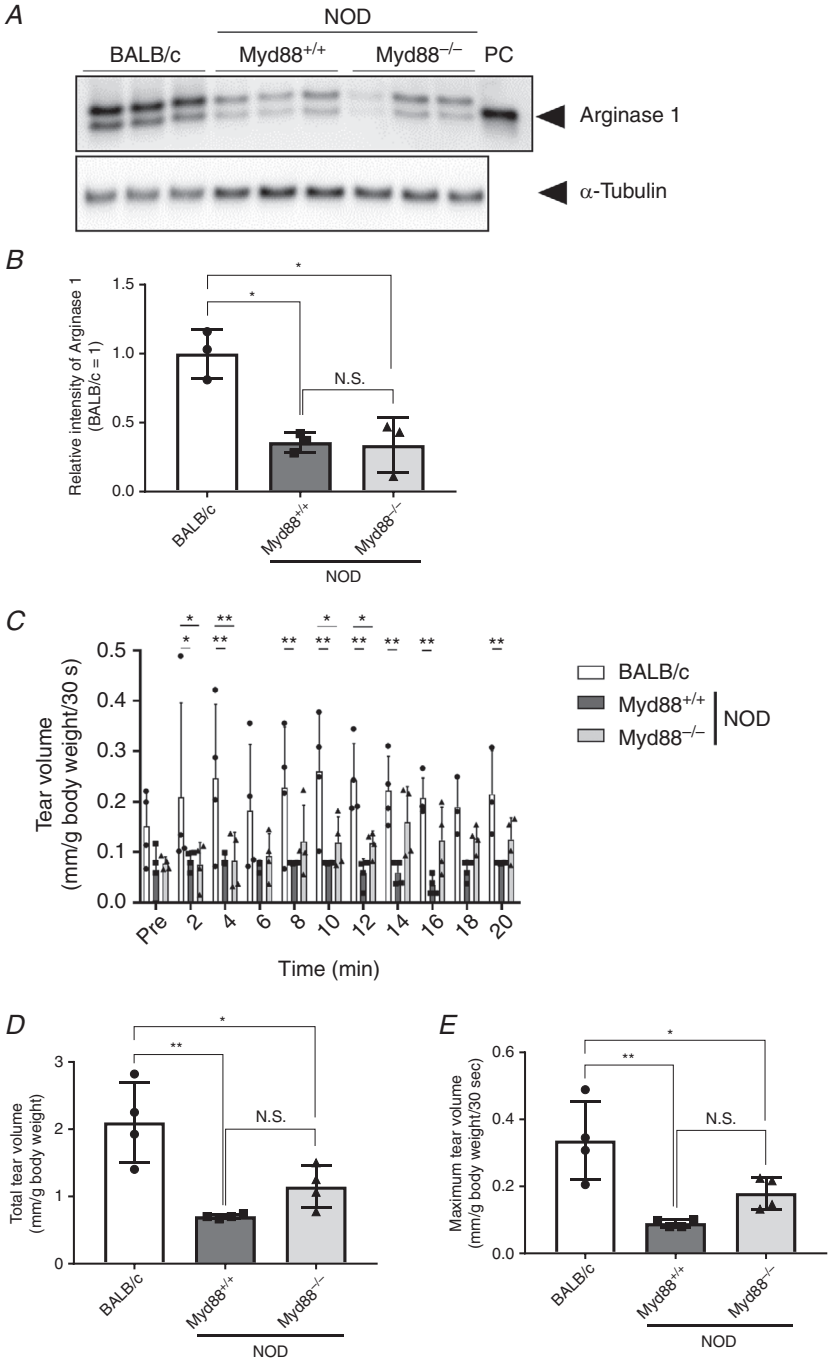

Figure 6. Low levels of tears and arginase 1 expression in Myd88$^{-/-}$ NOD mice
A, representative immunoreactive bands showing arginase 1 protein expression in lacrimal glands from male BALB/c mice, and Myd88$^{+/+}$ and Myd88$^{-/-}$ NOD mice. α-Tubulin was used as an internal control. PC: positive control. *B*, the intensity of the immunoreactive bands of arginase 1. *C*, body weight-adjusted tear volume in male BALB/c and Myd88$^{+/+}$ and Myd88$^{-/-}$ NOD mice at each time point after pilocarpine treatment. *D*, total tear volume after pilocarpine treatment. *E*, maximum tear volume. The obtained values are presented as the mean ± SD: $^{*}p < 0.05$, $^{**}p < 0.01$ *versus* BALB/c group. All experiments were conducted with three or four animals in each group.

total tear volume and the maximum tear volume decreased to 1.45 ± 0.49 mm g^{-1} body weight and 0.26 ± 0.09 mm g^{-1} body weight/30 s, respectively, in the CB-1158-administered BALB/c mice compared with the control BALB/c mice (2.82 ± 0.45 mm g^{-1} body weight and 0.47 ± 0.12 mm g^{-1} body weight/30 s) (Fig. 7*B* and *C*). These results suggest that arginase 1 drives fluid secretion in the lacrimal gland of mice.

We simultaneously examined for possible involvement of arginase 1 in saliva secretion. Saliva volume was significantly decreased at 10 min after pilocarpine injection in the CB-1158-administered BALB/c mice compared to the control (water) BALB/c mice (Fig. 8*A*). Maximum saliva volume decreased to 0.34 ± 0.05 mg g^{-1} body weight/30 s in the CB-1158-administered BALB/c mice compared to the control BALB/c mice (0.43 ± 0.04 mg g^{-1} body weight/30 s) (Fig. 8*C*). However, total saliva volume showed no significant difference between the CB-1158-treated and control groups (2.12 ± 0.36 and 2.35 ± 0.36 mg g^{-1} body weight, respectively) (Fig. 8*B*). These results suggest that arginase 1 partially drives fluid secretion in the salivary gland of mice.

Discussion

In this study, we investigated tear volume in male NOD mice and performed histological analysis of the lacrimal gland. We found that lacrimal hyposecretion and dacryoadenitis occur simultaneously. In addition, transcriptome analysis followed by RT-qPCR and western blot analysis found that the expression level of arginase 1 was decreased in the lacrimal gland at and after 6 weeks of age in male NOD mice compared to age-matched BALB/c mice. In non-inflammatory-type NOD mice, tear volume remained low, with a low arginase 1 expression level in the lacrimal gland. BALB/c mice administered an inhibitor of arginase 1 showed decreased tear volume. Together, these findings indicate that a non-inflammatory factor, arginase 1, is involved in lacrimal hyposecretion in male NOD mice, regardless of dacryoadenitis status (as illustrated schematically in Fig. 9). In addition, the arginase inhibitor partially decreased saliva volume in BALB/c mice. Consequently, arginase 1 would appear to drive tear and saliva secretion.

Lacrimal hyposecretion due to a low level of arginase 1 in lacrimal gland

We hypothesized that a non-inflammatory factor induces lacrimal and/or salivary hyposecretion in SS based on reports that found no correlation between the severity of inflammatory cell infiltration and tear and/or saliva volume (Paranyuk *et al.* 2001; Jonsson *et al.* 2006). Our transcriptome analysis followed by RT-qPCR and western blot analysis identified an enzyme, arginase 1, as a potential factor involved in lacrimal hyposecretion

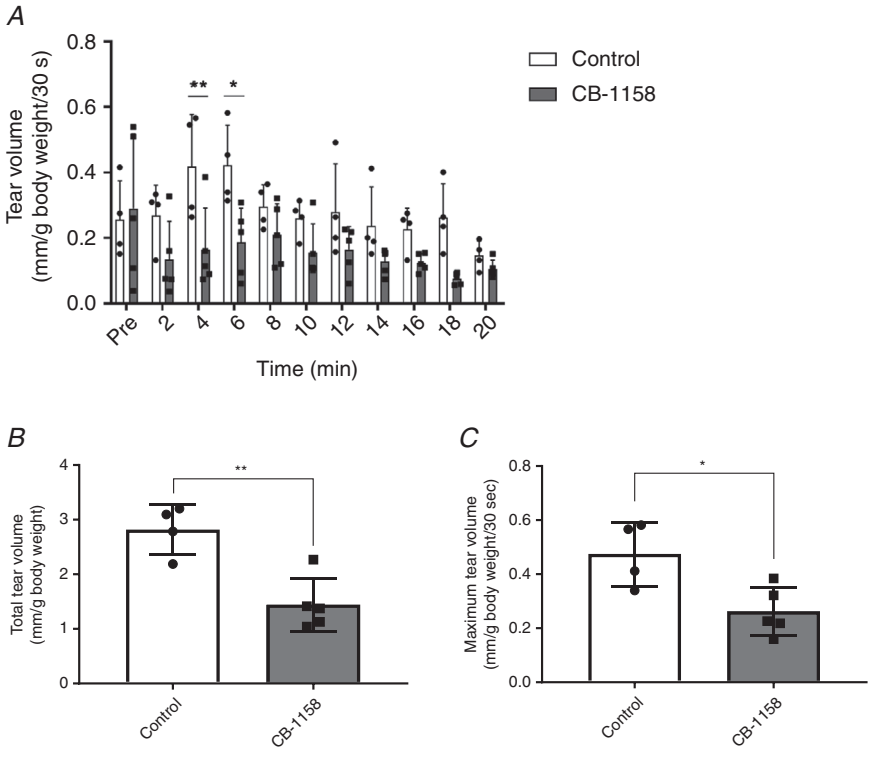

Figure 7. Arginase 1 inhibitor inhibits pilocarpine-induced tear secretion
A, body weight-adjusted tear volume in male BALB/c mice administered an arginase 1 inhibitor. BALB/c mice were orally administered an arginase 1 inhibitor, CB-1158, at 100 mg kg^{-1}, or the control (water), 2 h before pilocarpine treatment. *B*, total tear volume after pilocarpine treatment. *C*, maximum tear volume. The obtained values are presented as the mean ± SD: $^{*}p < 0.05$, $^{**}p < 0.01$ *versus* control group. All experiments were conducted with four or five animals in each group.

and/or inhibition of lacrimal gland inflammation in male NOD mice (Table 3 and Fig. 4). An experiment using non-inflammatory-type NOD mice found that, although there was almost no inflammatory cell infiltration of the lacrimal gland, tear volume remained low, with a low arginase 1 expression level in the lacrimal gland (Fig. 6). These results support our hypothesis that lacrimal hyposecretion is due to a low level of a non-inflammatory factor, arginase 1, in the lacrimal gland, rather than inflammation.

Arginase 1 as a potential driving factor of fluid secretion in the lacrimal and salivary glands

Arginase 1 is an enzyme that catalyses hydrolysis of L-arginine to L-ornithine and urea (Wu & Morris, 1998). CB-1158 is a potent arginase 1 inhibitor via binding to the active site of arginase 1 (Grobben *et al.* 2020). Single administration of CB-1158 at 100 mg kg^{-1}, p.o., to mice was reported to elevate the concentration of L-arginine (Steggerda *et al.* 2017), and this dosage is assumed to have a sufficient inhibitory effect *in vivo*. In addition, even twice-daily oral administration of that dosage was well tolerated by mice for at least 40 days (Steggerda *et al.* 2017). Therefore, we orally administered CB-1158 at the same dosage to BALB/c mice and then measured tear induction by pilocarpine at 2 h after administration of CB-1158.

We found that CB-1158 significantly decreased tear volume in BALB/c mice (Fig. 7). Interestingly, patients with dry eye syndrome, excluding SS patients, showed lower urea concentrations in tears than the healthy controls (Jäger *et al.* 2013). This strongly supports our hypothesis that arginase 1 drives tear secretion by the lacrimal gland, because decreased expression and/or activity of arginase 1 is considered to lead to a low urea concentration in the tears of patients with dry eye syndrome.

Furthermore, simultaneous measurement of saliva secretion following administration of CB-1158 showed similar results with the decreased maximum saliva volume (Fig. 8*C*). In mice, arginase 1 was reported to be highly expressed in the parotid gland compared to the submandibular and sublingual glands (Yasuda *et al.* 2004), suggesting that it is abundant in glands that respond strongly to stimuli. This indicates that arginase 1 may contribute to fluid secretion not only in the lacrimal gland but also in the salivary gland, especially in response to parasympathetic stimulation.

Mechanism of hyposecretion due to low level/activity of arginase 1

The primary fluid secretion process in acinar cells of the lacrimal and salivary glands is explained by transport of ions such as Na^+, K^+ and Cl^- to the acinar lumen,

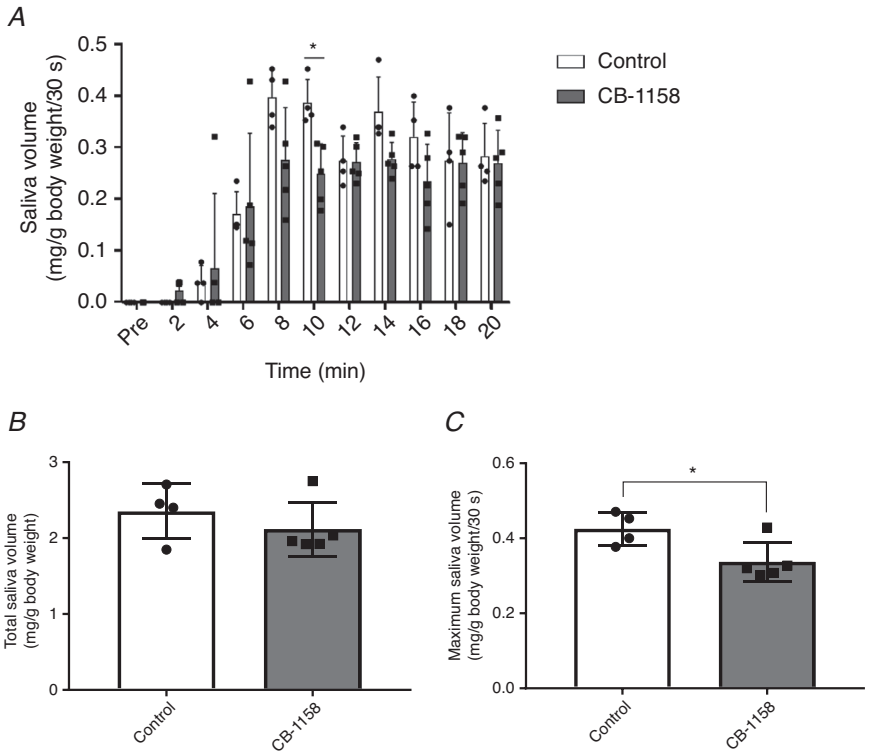

Figure 8. Partial inhibition of pilocarpine-induced saliva secretion by oral administration of an arginase 1 inhibitor
A, body weight-adjusted saliva volume in male BALB/c mice administered an arginase 1 inhibitor. BALB/c mice were orally administered an arginase 1 inhibitor, CB-1158, at 100 mg kg^{-1}, or the control (water), 2 h before pilocarpine treatment. *B*, total saliva volume after pilocarpine treatment. *C*, maximum saliva volume. The obtained values are presented as the mean ± SD: $^*p < 0.05$ *versus* control group. All experiments were conducted with four or five animals in each group.

followed by transcellular or paracellular water transport dependent on an osmotic gradient (Mircheff, 1989; Catalán *et al.* 2009). ATP plays an important role because Na^+–K^+ ATPase indirectly drives that ion transport (Dartt *et al.* 1981; Mircheff, 1989; Catalán *et al.* 2009). Recently, arginase 1 was reported to be involved in aerobic glycolysis, which produces ATP (Monticelli *et al.* 2016). In addition, acinar cells in 1-month-old male NOD mice were reported to show morphological abnormalities of the mitochondria (Ding *et al.* 2006), where ATP is produced under aerobic conditions. These reports provide mechanistic insight into fluid hyposecretion due to a cellular bioenergetics abnormality that results from low level/activity of arginase 1, although further study is needed to confirm this.

Another possible role of arginase 1 in the lacrimal gland in mice

Another known function of arginase 1, expressed in immunosuppressive cells such as M2 macrophages, is immunosuppression due to depletion of extracellular L-arginine, which is necessary for T-cell proliferation (Rodriguez *et al.* 2017). Thus, low expression of arginase 1 in the lacrimal gland may contribute to exacerbation of inflammatory cell infiltration in male NOD mice. Moreover, long-term administration of an arginase 1 inhibitor may cause inflammatory cell infiltration in male BALB/c mice, although localization of arginase 1 in the lacrimal gland was not confirmed in this study. Nevertheless, it may be that a single administration of an arginase 1 inhibitor does not induce inflammatory cell infiltration in the acute phase (2 h after administration); rather, the lacrimal hyposecretion seems to be due to involvement of arginase 1 in fluid secretion.

Potential genes associated with dacryoadenitis and/or lacrimal hyposecretion revealed by multi-intergroup comparisons in the transcriptome

Here, we performed multiple comparisons of RNA-seq data to eliminate potential factors that fluctuate depending on age. We focused on candidates with a high likelihood of being related to factors that seem to be associated with decreased tear secretion and/or increased inflammation in the lacrimal gland of NOD mice. Indeed, the top 10 genes upregulated after the onset of lacrimal hyposecretion and dacryoadenitis in NOD mice (Table 2) were inflammatory-related factors, and some of them correspond to previous reports. For instance, the increases in chemokine (C-C motif) ligand 5 (*Ccl5*) and cathepsin S (*Ctss*) seen in this study fully correspond to previous reports that investigated these factors in the lacrimal gland

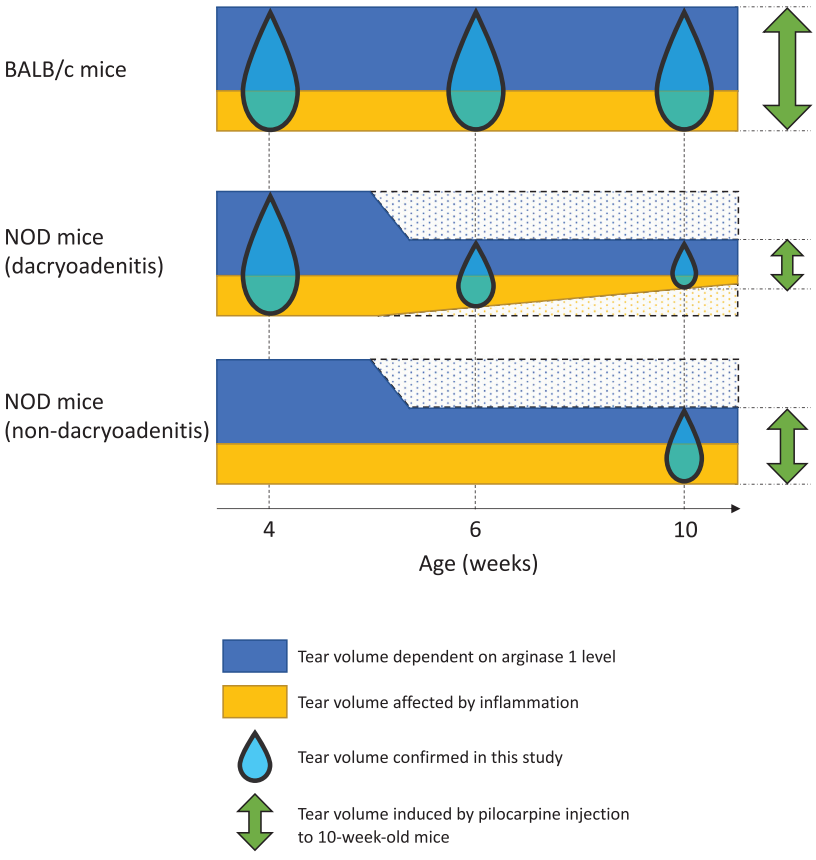

Figure 9. Overview of lacrimal hyposecretion in male NOD mice
Tear secretion induced by pilocarpine treatment occurs in at least two phases, one phase dependent on the arginase 1 expression level and the other phase affected by inflammation (the blue and yellow areas, respectively). At 4 weeks of age, tear secretion in NOD mice, both with and without dacryoadenitis, is similar to in BALB/c mice. At 6 weeks of age, both NOD mice show a decrease in tear secretion as a function of arginase 1. Inflammation of low severity might be a factor in the tear volume decrease in NOD mice with dacryoadenitis. At 10 weeks of age, both NOD mice still show a decrease in tear secretion as a function of arginase 1. In addition, lacrimal gland inflammation significantly affects the tear volume decrease in NOD mice with dacryoadenitis. Therefore, arginase 1 is a key factor for tear volume decrease in NOD mice. Moreover, lacrimal gland inflammation may worsen a decrease in tear secretion.

of male NOD mice (Törnwall *et al.* 1999; Li *et al.* 2010). The expression level of chemokine (C–X–C motif) ligand 13 (*Cxcl13*) was also reported to increase in the lacrimal gland of male NOD mice with age/disease progression (Fava *et al.* 2011). Therefore, the RNA-seq analysis in this study seems to have been successfully performed. Similarly, four genes, including *Arg1*, that were downregulated after onset of lacrimal hyposecretion and dacryoadenitis in NOD mice (Table 3) were speculated to be candidates related to tear secretion and/or inflammation suppression, although those genes have not been reported to be involved in exocrine secretion to date.

Difference in onset of lacrimal hyposecretion compared with a previous report

The onset of lacrimal hyposecretion in male NOD mice in this study was at 6 weeks of age, whereas Doyle *et al.* (2007) reported that the onset was at 8 weeks of age. This difference was surmised to be due to the use of different pilocarpine dosages: Doyle *et al.* administered pilocarpine at 4.5 mg kg^{-1}, which was nine times higher than our dosage (0.5 mg kg^{-1}). We think that our tear measurement method is closer to the actual physiological state and more accurate/sensitive than Doyle *et al.*'s method.

Translational perspective of this study

In summary, our findings suggest that arginase 1 plays an important role in driving tear and saliva secretion, especially in response to parasympathetic stimulation. As far as we know, no similar findings have been reported to date, and it will be necessary to investigate the detailed mechanism. Even though the mechanism remains unknown, our findings are an important step forward to elucidate the physiology of fluid secretion and pathology of SS and dry eye/mouth syndrome. Fluid hyposecretion in SS and dry eye/mouth syndrome may depend on reduced arginase 1 activity or expression levels, and/or genetic variations that cause loss of function. Arginase 1 may be a target for prevention and/or therapy of fluid hyposecretion.

Conclusion

Our findings are the first to show that a non-inflammatory factor, arginase 1, is involved in lacrimal hyposecretion in male NOD mice, regardless of the dacryoadenitis status. Furthermore, while the mechanism by which arginase 1 drives fluid secretion must await further investigation, our results shed light on the pathophysiological role of arginase 1 in SS and dry eye/mouth syndrome.

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Additional information

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing financial interests.

Author contributions

K.S. and M.K. contributed to initiation of the research. Y.O. and K.S. designed the study. Y.O. and K.S. performed experiments on measurements of tear and saliva volumes, respectively. Y.O. extracted RNA from lacrimal glands and performed quantitative RT-PCR. Y.O. and K.S. performed western blotting, data analysis and interpretation. A.S. and M.K. performed data analysis and interpretation. T.I. contributed to the generation of NOD.B6-Myd88$^{-/-}$ mice, and data analysis and interpretation using the mice. Y.O. wrote the paper, which was modified by K.S., A.S. and M.K. All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated and listed as authors qualify for authorship.

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Keywords

arginase 1, dacryoadenitis, dry eye, exocrine, lacrimal gland, NOD mice, salivary gland, Sjögren's syndrome

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Statistical Summary Document