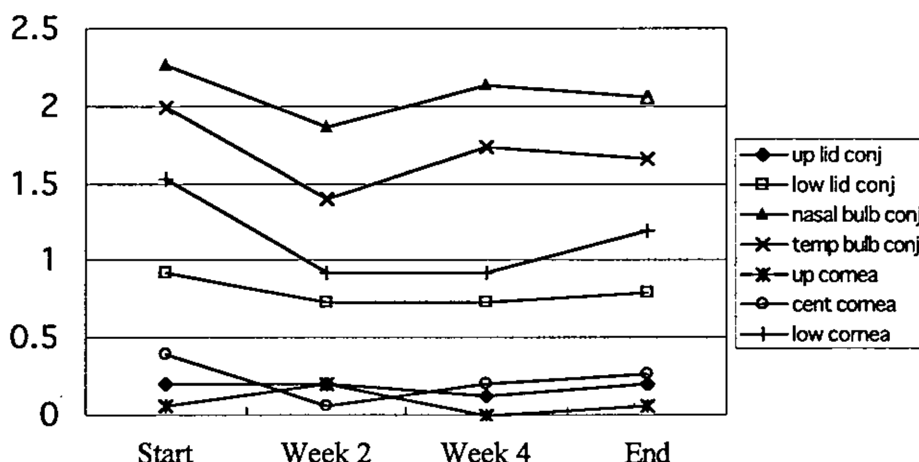

FIGURE 8. Rose bengal staining. Fine detail of rose bengal staining of cevimeline 30 mg three times daily.

encouraging results, further studies are needed to define more clearly the optimum dosage of cevimeline for the treatment of dry eye in patients with SS.

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Effect of preoperative tear function on early functional visual acuity after laser in situ keratomileusis

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Purpose: To assess the effect of preoperative tear function on early changes in functional visual acuity (FVA) after laser in situ keratomileusis (LASIK).

Setting: Minamiaoyama Eye Clinic, Tokyo, Japan.

Methods: This prospective single-center study assessed the effect of preoperative and postoperative tear functions on FVA in 30 eyes of 15 patients who had LASIK. Functional visual acuity was defined as the binocular recognition acuity measured by the FVA tester (Wellsystem) during a 10-second, blink-free period. All patients had a Schirmer test with anesthesia and tear-film breakup time (BUT) measurements preoperatively and 1 day and 1 week after LASIK. Corneal topography and Landolt visual acuity and FVA measurements were performed before surgery and 1 day and 1 week after LASIK. Eyes with a Schirmer test reading less than 5.0 mm and a BUT less than 5 seconds were grouped as definite dry eye (DDE). Eyes with a normal Schirmer test score but a shortened BUT were grouped as probable dry eye (PDE).

Results: In all patients, the best uncorrected Landolt visual acuity was 20/20 or better at the postoperative examination times. In the DDE group, the mean preoperative FVA declined from 1.2 to 0.75 ± 0.16 (SD) at 1 day and increased to 1.2 at 1 week. No change in FVA was observed postoperatively in the PDE group.

Conclusion: Laser in situ keratomileusis patients with low basal tearing and full uncorrected distance Landolt acuity may experience a transient decrease in FVA that returns to baseline within 1 week.

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Dry-eye patients often complain of decreased visual acuity during daily activities such as reading, driving, and video display terminal (VDT) work.[1,2] Many myopic dry-eye patients intolerant to contact lenses resort to visual rehabilitation by refractive surgery. It is reported that 50% of LASIK patients have preoperative dry eye and that the LASIK procedure itself induces aqueous deficiency.[3,4] Laser in situ keratomileusis has also been shown to significantly alter the early postoperative tear stability.[3-5] A stable tear-film layer over the surface of the cornea is essential for clear visual imaging. An irregular corneal surface resulting from aqueous deficiency is reportedly associated with poor quality of vision.[6]

Many LASIK patients complain of the quality of their vision despite optimal uncorrected binocular visual acuity in the early postoperative period. We think this may be related to disturbances in the tear film.

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An assessment of functional visual acuity (FVA) is reportedly useful in the detection of subtle changes in visual quality in dry-eye patients.[1,2,6] In this study, we performed conventional and FVA measurements before and after LASIK and studied the effect of tear functions on early postoperative FVA.

Patients and Methods

Thirty eyes of 15 consecutive patients who had bilateral LASIK for myopia (range −1.25 to −9.0 diopters [D]) were enrolled in this study. All had slitlamp biomicroscopy, corneal topography, and best corrected Landolt visual acuity and FVA measurements before and 1 day and 1 week after LASIK. Tear-function examinations including Schirmer test with anesthesia and tear-film breakup time (BUT) were performed preoperatively and 1 day and 1 week after LASIK. In each patient, the degree of preoperative myopia, amount of myopic correction, and ablation depth were recorded.

Tear Examinations

The standard Schirmer test with topical anesthesia (oxybuprocaine chloride 0.4%) was performed. The standardized strips of filter paper (Alcon Inc.) were placed in the lateral canthus, away from the cornea, and left in place for 5 minutes with the eyes closed. Readings were reported in millimeters of wetting for 5 minutes. A reading of less than 5.0 mm was referred to as dry eye.

For BUT measurements, 2.0 μL of fluorescein 1% was applied to the conjunctival sac by a micropipette. The patients were then instructed to blink several times. The interval between the last blink and the appearance of the first black spot in the central corneal tear film was measured. A BUT value less than or equal to 5 seconds was considered abnormal. The dry-eye patients were divided into 2 groups of "definite" and "probable" dry eyes according to the preoperative tear functions. Eyes with a Schirmer test reading less than 5.0 mm and a BUT less than 5 seconds were grouped as definite dry eye (DDE), and those with a normal Schirmer test score but a shortened BUT were grouped as probable dry eye (PDE).

Functional Visual Acuity Measurements

The FVA tester (Wellsystem) was used to measure the recognition visual acuity continuously. Functional visual acuity was defined as the binocular recognition acuity measured by the FVA tester during a 10-second, blink-free period. The details of the testing procedure have been reported.[1,2] First, visual acuity was measured with no restraints to blinking using this instrument (baseline FVA). Topical anesthesia (oxybuprocaine chloride 0.4%) was administered before the FVA examination to minimize discomfort and prevent reflex tearing and blinking. Five minutes after the topical anesthesia was instilled, patients were instructed not to blink for 10 seconds during the measurement of FVA. The examiner confirmed the absence of blinking during the 10-second period. Patients indicated the orientation of the automatically presented Landolt rings using the joystick. Initially, the 24/20 Landolt ring was shown on the terminal display at 5 m from the patients. The Landolt ring increased in size when the answer was incorrect and decreased in size when it was correct. If the Landolt ring was recognized correctly, the same-size ring was displayed at random again. The result was displayed as a plot graph when the measurement was complete.

Corneal Topography

Corneal topography of each patient was measured using TMS-2 videokeratoscope software (Tomey Corp.). Topography was measured immediately after the eyes were opened and after the 10-second, blink-free period. The surface regularity index (SRI) was measured at 0 and 10 seconds (SRI 0 and SRI 10, respectively).

Statistical Analysis

Data were processed using Graph Pad Software. The paired *t* test was used for analysis of the nonparametric values. The change in SRI from baseline over time was assessed in each group by the 2-way repeated-measures analysis of variation test. A probability level less than 5% was considered statistically significant.

Results

The mean age of the 3 women and 12 men was 37.1 years ± 7.3 (SD) (range 23 to 55 years). Of the 30 eyes, 12 (6 patients) were in the DDE group and 18 (9 patients) in the PDE group based on the preoperative tear functions. The best uncorrected Landolt visual acuity was 20/20 or above in all cases at each post-LASIK examination.

Refractive and Ablation Data

The mean degree of preoperative myopia was −6.5 ± 2.4 D in the DDE group and −5.6 ± 2.3 D in the PDE group. The difference was not significant ($P>.05$). The mean amount of myopic correction was −4.9 ± 2.2 D in the DDE group and −5.8 ± 2.1 D in the PDE group ($P>.05$). The mean depth of ablation was 80.5 ± 30.4 μm and 87.8 ± 33.0 μm, respectively ($P>.05$).

Tear-Function Examinations

The overall mean BUT was 4.42 ± 0.82 seconds in the DDE group and 3.06 ± 0.85 seconds in the PDE group. In the 12 DDE eyes that presented with

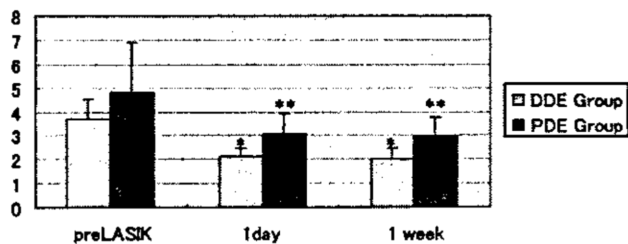

Figure 1. (Tanaka) The change in BUT after LASIK in dry-eye patients.

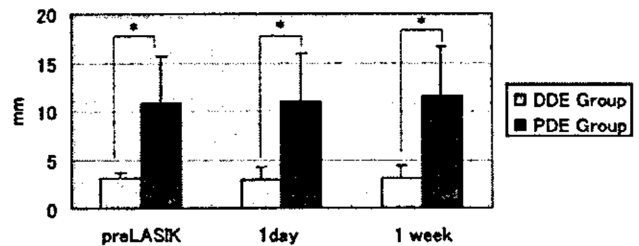

Figure 2. (Tanaka) The change in Schirmer test values after LASIK.

a decrease in 1-day FVA, the preoperative BUT was 3.70 ± 0.82 seconds; it declined to 2.13 ± 0.35 seconds at 1 day ($P<.05$). The BUT remained at 2.00 ± 0.45 seconds at 1 week. In the 18 PDE eyes with stable 1-day FVA, the BUT was 4.80 ± 2.09 seconds preoperatively and 3.06 ± 0.85 seconds at 1 week; the BUT remained at 2.98 ± 0.75 seconds at 1 week, as shown in Figure 1 ($P<.05$). Although the mean pre-LASIK BUT in the DDE group was lower than that in the PDE group, the difference was not statistically significant ($P>.05$). A statistically significant between-group difference in the 1-day BUT values was noted ($P<.05$).

In the 12 DDE eyes that presented with decreased 1-day FVA, the mean preoperative Schirmer test value was 3.05 ± 0.67 mm; this declined to 2.97 ± 1.32 mm at 1 day and 3.07 ± 1.30 mm at 1 week. The differences were not significant ($P>.05$). In the 18 PDE eyes with stable 1-day FVA, the mean Schirmer test value was 10.80 ± 4.80 mm preoperatively and 11.00 ± 4.90 mm at 1 day, as shown in Figure 2 ($P>.05$). The between-group difference in Schirmer test values at each examination time was statistically significant ($P<.05$).

Functional Visual Acuity

The baseline FVA was 24/20 in all patients before LASIK surgery. It decreased to a mean 15/20 in 6 DDE patients at 1 day ($P<.05$). No changes in FVA from baseline values were noted in the PDE group (Figure 3). At 1 week, the FVA recovered to the preoperative level in all patients.

Corneal Topography

At 1 day, the SRI 10 in the 12 eyes in the DDE group with decreased FVA significantly increased compared to the eyes in the PDE with stable FVA ($P<.05$). The SRI 10 was 2.20 ± 0.54 in DDE group and 1.54 ± 0.62 in the PDE group, as shown in Figure 4. At 1 week, no significant between-group differences were observed in the SRI 10 values ($P>.05$). The variation in the SRI 10 from baseline to 1 week was statistically significant in both groups ($P<.05$). At 1 day, the mean SRI was 1.39 ± 0.33 in the DDE group and 1.28 ± 0.55 in the PDE group ($P>.05$). The SRI 10 was 2.20 ± 0.54 and 1.54 ± 0.62, respectively ($P<.05$). The increase in SRI from 0 to 10 seconds was statistically significant ($P<.05$) in the DDE group, as shown in Figure 5.

Discussion

Dry eye is a major reason patients consider LASIK, and it is a common post-LASIK complication. Toda and coauthors[4] report that more than 75% of patients having LASIK have preoperative dry eye; 35.2% have DDE and 41.2% have PDE, according to the modified criteria of the Japanese Dry Eye Association.[7] It has

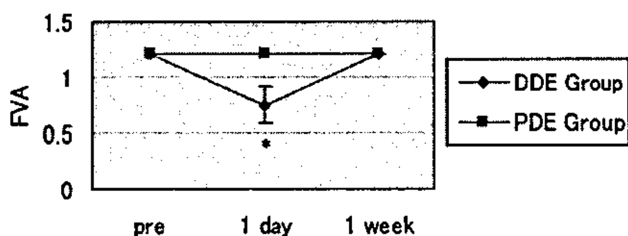

Figure 3. (Tanaka) The change in FVA after LASIK.

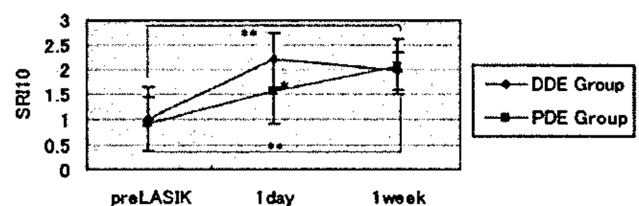

Figure 4. (Tanaka) The change in SRI after LASIK.

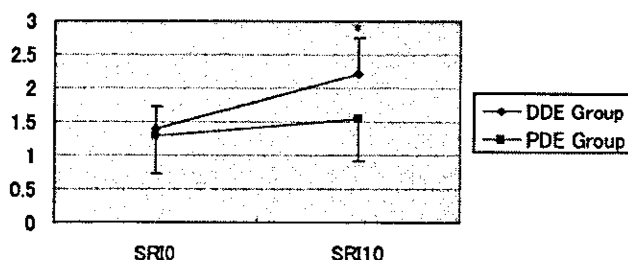

Figure 5. (Tanaka) The change in SRI from 0 to 10 seconds.

been demonstrated that post-LASIK, dry eye develops with compromised tear function for at least 1 month; some patients may have dry eye 1 year after LASIK.[3,4] It is well known that LASIK is associated with significant improvements in uncorrected and best corrected visual acuity, even in dry-eye patients. Although standard visual acuity testing is an excellent measure of 1 aspect of visual function, contrast sensitivity and glare testing provide more important and precise information about specific aspects of visual function.

Recently, FVA testing, described as "functional visual acuity for 10 seconds without blinking," was reported to be an important method of defining "detailed visual function."[1] The method has been shown to be efficient in detecting "masked impairment of visual function" in dry-eye patients who complain of decreased visual acuity despite normal conventional visual acuity test results.[1] The definition of FVA testing has been proposed as an important indication of an individual's performance of certain daily activities such as driving, reading, and VDT work.[2]

In this study, we examined the effects of dry eye on FVA in the first post-LASIK week since the modern LASIK procedure enables most patients to return to daily activities the day after LASIK. All patients attained 20/20 uncorrected conventional visual acuity the day after surgery and the first post-LASIK week. A group of patients complained of difficulties reading and driving and visual fluctuation the day after LASIK. The analyses and findings in the PDE patients (BUT-deficiency type) and DDE patients (BUT- and aqueous-deficiency type) made us realize that it was the DDE patients who had these complaints and who also displayed a significant reduction in FVA 1 day after LASIK despite normal conventional visual acuity.

In corneal topography, the SRI has been shown to reflect the regularity and optical quality of the cornea and also to correlate with the potential visual acuity.[6,8] The SRI values in our patients showed a significant increase from baseline to 1 week in both groups. The DDE patients also had significantly higher SRI 10 values than the PDE patients at 1 day. The increase in the mean SRI value from baseline at 0 second to 10 seconds was also significant in the DDE group compared with the PDE group. An investigation of preoperative refractive differences as well as LASIK parameters such as the amount of myopic correction and the ablation depth showed no significant differences between the 2 groups, suggesting that the SRI changes probably resulted from tear stability differences and minute corneal surface irregularities. Thus, the changes in early FVA in our patients may be explained by poor tear spreading and the surface depression created by LASIK or a greater contribution by the dynamic precorneal tear film to the optical power of the eye after LASIK. A further reduction in post-LASIK BUT scores in the first week might also have contributed. Holladay and coauthors[9] report that finer markers of visual function such as contrast acuity and glare testing revealed marked deterioration on the first day after LASIK. They attributed these changes to microscopic corneal irregularities that do not affect clinical visual performance assessed by conventional methods.

We think investigations into the changes in tear stability and function in dry-eye patients having LASIK, as evaluated by tear-film lipid-layer interferometry or the recently developed tear stability analysis system, which measures blink-free corneal topography changes over 10 seconds, would be very interesting. It would be even more interesting to look into the relationship between these parameters and tests defining finer visual function such as wavefront analysis, glare testing, contrast sensitivity, and FVA.

Our preliminary findings suggest that BUT- and aqueous-deficient dry-eye patients who have LASIK experience a reduction in FVA on the first post-LASIK day; this is associated with complaints of difficulties in driving or reading. It has been shown that blink rates decreased considerably during reading and driving when most patients kept their eyes open for more than 10 seconds.[10] Although we did not measure the blink rate in our patients, the relationship between early post LASIK FVA and blink rate in a large number of patients would provide useful information.

In conclusion, DDE patients having LASIK experience a transient reduction in FVA, which returns to baseline within 1 week. Further research in a large number of patients is therefore essential to clarify the restrictions and define the necessary precautions, if any, in relation to daily activities in DDE patients having LASIK.

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Ocular Surface Treatment Before Laser in situ Keratomileusis in Patients With Severe Dry Eye

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ABSTRACT

PURPOSE: To evaluate the efficacy and safety of laser in situ keratomileusis (LASIK) in patients with severe dry eye associated with Sjögren's syndrome.

METHODS: Three patients (six eyes) with Sjögren's syndrome who underwent bilateral LASIK were retrospectively evaluated for visual outcome, intraoperative and postoperative complications, dry eye status (subjective symptoms and objective findings, Schirmer test, vital staining of the ocular surface), and outcome satisfaction by subjective questionnaire. All patients had negative reflex tearing and were treated with topical autologous serum and/or punctal occlusion prior to LASIK to improve the ocular surface. This treatment was continued postoperatively.

RESULTS: Mean attempted correction of six eyes was -8.46 ± 1.55 D (range -7.00 to -10.63 D). One year after LASIK, mean uncorrected visual acuity was 1.07 (range 0.7 to 1.5), mean best spectacle-corrected visual acuity was 1.29 (range 1.2 to 1.5), and mean refraction was -0.19 ± 0.51 D (range -1.00 to +0.50 D). Tear production, rose bengal and fluorescein staining, and dry eye symptoms were not exacerbated after LASIK. No complications, such as intraoperative epithelial defect, diffuse lamellar keratitis, epithelial ingrowth, or recurrent erosion occurred. All three patients were satisfied with the outcome of their surgery.

CONCLUSION: LASIK can be safely and effectively managed in patients with severe dry eye with reduced reflex tearing by preoperative and postoperative treatments consisting of a combination of artificial tears, topical autologous serum, and punctal occlusion. Careful assessment of preoperative and postoperative ocular surface status is mandatory in such patients. [*J Refract Surg* 2004;20:270-275]

Contact lens intolerance due to dry eye conditions is often a motive for refractive surgery.[1,2] Furthermore, if dry eye patients have high myopia, high astigmatism, and/or anisometropia, it is often difficult for them to wear spectacles that sufficiently correct their refractive error. In such cases, refractive surgery may be the only option that achieves satisfactory uncorrected visual acuity. We recently reported that patients who had preoperative dry eye could safely undergo photorefractive keratectomy (PRK) or laser in situ keratomileusis (LASIK) without increased risks of complications or lower predictability for the attempted corrections.[2,3] However, the patients in these studies had mild to moderate dry eye with positive reflex tearing measured by Schirmer test with nasal stimulation.[4]

Sjögren's syndrome is associated with reduced reflex tearing. Reflex tearing plays an important role in the maintenance of ocular surface integrity. When LASIK is performed in such patients, ocular surface management and the control of dry eye-related symptoms are of utmost importance. Ocular surface management is probably most important in the early postoperative period as symptoms and signs of dry eye could temporarily worsen for several weeks postoperatively.[5-8]

Recently, a combination of autologous serum eye drops and punctal occlusion has been used effectively in the treatment of ocular surface disorders associated with severe tear deficiency, including Stevens-Johnson's syndrome, ocular cicatricial

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The authors have no proprietary interest in the materials presented herein.

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Table 1
Dry Eye Patient Data Before LASIK

Patient	Age (yr)	Eye	UCVA	BSCVA	Refraction (D)	Schirmer (mm)	Tear Break-up Time (sec)	Preoperative Treatment	Correction (D)
M.K.	47	R	0.05	1	sphere -6.75 cylinder -1.75	5	0	Serum, artificial tears	sphere -6.75 cylinder -1.75
		L	0.05	1	sphere -6.50 cylinder -1.00	4	0	Serum, artificial tears	sphere -6.50 cylinder -1.00
S.K.	52	R	0.08	1.2	sphere -7.25 cylinder -0.75	3	2	Serum, plugs (4)	sphere -6.75 cylinder -0.50
		L	0.04	1.2	sphere -9.50	3	2	Serum, plugs (4)	sphere -8.50
H.H.	53	R	0.03	1.5	sphere -10.50	1	2	Serum, plugs (4)	sphere -10.00
		L			sphere -9.75 cylinder -1.75	1	1	Serum, plugs (4)	sphere -9.75 cylinder -1.75

Serum=autologous serum eye drop (number of puncta occluded)
**Some plugs lost*

Table 2
Dry Eye Patient Data 1 Year After LASIK

Patient	Age	Eye	Postoperative	UCVA	BSCVA	Refraction (D)	Schirmer (mm)	Tear Break-up Time (sec)	Satisfaction Grade
M.K.	47	R	Serum, plugs (2)	1.5	1.5	sphere 0 cylinder 0	5	4	1
		L	Serum, plugs (2)	1.2	1.5	sphere +0.50 cylinder 0	4	4	1
S.K.	52	R	Serum, plugs (4)*	1.2	1.2	sphere 0 cylinder 0	2	2	2
		L	Serum, plugs (4)*	0.7	1.2	sphere -0.75 cylinder -0.50	4	2	2
H.H.	53	R	Serum, plugs (4)	1.2	1.2	sphere 0 cylinder 0	not done	2	1
		L	Serum, plugs (4)	1.2	1.2	sphere 0 cylinder 0	not done	1	1

Serum=autologous serum eye drop (number of puncta occluded)
**Some plugs lost*

pemphigoid, and Sjögren's syndrome.[9-11] Corneal transplantation used to be contraindicated for patients with severe dry eye, however, by using autologous serum eye drops and punctal occlusion, successful surgical outcomes were achieved in many of these patients. We predicted that LASIK would be safely performed in patients with severe dry eye such as Sjögren's syndrome if the ocular surface was managed by such means. However, for successful outcomes, it is imperative that the ocular surface is improved to optimal condition before surgery, by punctal occlusion and eye drops, and that this treatment is continued after surgery.

We performed LASIK on three patients with severe dry eye associated with Sjögren's syndrome and evaluated its safety and efficacy, focusing in particular on wound-healing-related epithelial complications and dry eye status.

PATIENTS AND METHODS

Three middle-aged females with Sjögren's syndrome were included in this study. Preoperative profiles of these patients are listed in Table 1. All patients were highly myopic and had used hard contact lenses preoperatively but complained of dryness, foreign body sensation, ocular fatigue, discharge, and/or redness that worsened with contact lens wear. These symptoms made contact lens wear impossible for long periods. A complete ophthalmic examination and assessment of dry eye status were performed at baseline. Basic tearing and tear stability determined by Schirmer test with anesthesia and tear break-up time were less than 5 mm and 5 seconds, respectively. Reflex tearing measured with Schirmer test with nasal stimulation[4] was less than 10 mm (negative) in all patients. All four puncta were occluded with silicone punctal plugs (Eagle

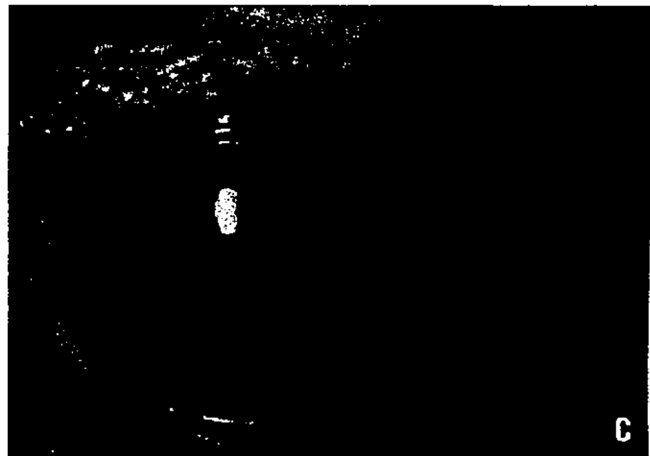

Figure 1. Slit-lamp microcroscopy of the ocular surface in patient H.H; **A)** before commencement of the combination therapy of autologous serum and punctal occlusion; **B)** 1 month after treatment; **C)** 1 month after LASIK. Rose bengal staining improved with the combination therapy.

DISCUSSION

Exacerbation of dry eye symptoms is a common postoperative complication after LASIK.[5-8] Many patients without preoperative dry eye experience dry eye symptoms and decreased tear functions for several months after LASIK. Furthermore, patients with preoperative dry eye exhibited more severe symptoms and ocular surface damage after LASIK compared to patients without preexisting dry eye, even though efficacy and predictability were comparable between these groups.[8] We performed LASIK on severe cases of dry eye associated with Sjögren's syndrome after strict informed consent, and found that with proper management, these patients can be candidates for LASIK.

In patients with severe dry eye with decreased reflex tear secretion, ocular surface dessication has adverse effects on the ocular surface epithelium and insufficient tear components may compromise normal wound healing. Problems with epithelial wound healing may lead to flap dislocation, epithelial ingrowth[15], or diffuse lamellar keratitis.[16] If postoperative LASIK dry eye is extremely severe, epithelial defect or corneal ulcer may appear. In our patients, all of whom had decreased reflex tearing, such problems were not experienced during the 1-year postoperative follow-up. Postoperative refraction and visual acuity were also good in these patients. The good visual outcome and lack of complications is probably mostly attributable to extensive management of dry eye with autologous serum eye drops and punctal plugs. We scheduled LASIK only after ocular surface findings were sufficiently improved.

Of our three patients, patient H.H. was most successfully treated with punctal plugs with no plug loss. The ocular surface showed no staining with fluorescein and rose bengal immediately prior to LASIK and dry eye symptoms were dramatically improved. On the other hand, patient M.K., who had plugs placed only in her upper puncta, and patient S.K., who experienced frequent loss of punctal plugs, complained of dry eye symptoms with moderate staining of the ocular surface. These results may suggest that the management of dry eye before surgery, especially with complete occlusion of lacrimal puncta, is a key for preventing severe postoperative LASIK dry eye and reducing subjective symptoms. Punctal occlusion is an effective treatment for dry eye, however, plug loss is a problem.[17] Surgical punctal occlusion may be advocated in some cases.

Autologous serum eye drops have been used successfully in many severe corneal epithelial disorders.[9,18] Autologous serum is considered to supply essential components that are necessary for epithelial wound healing, such as epithelial growth hormone and vitamin A, and positively promotes

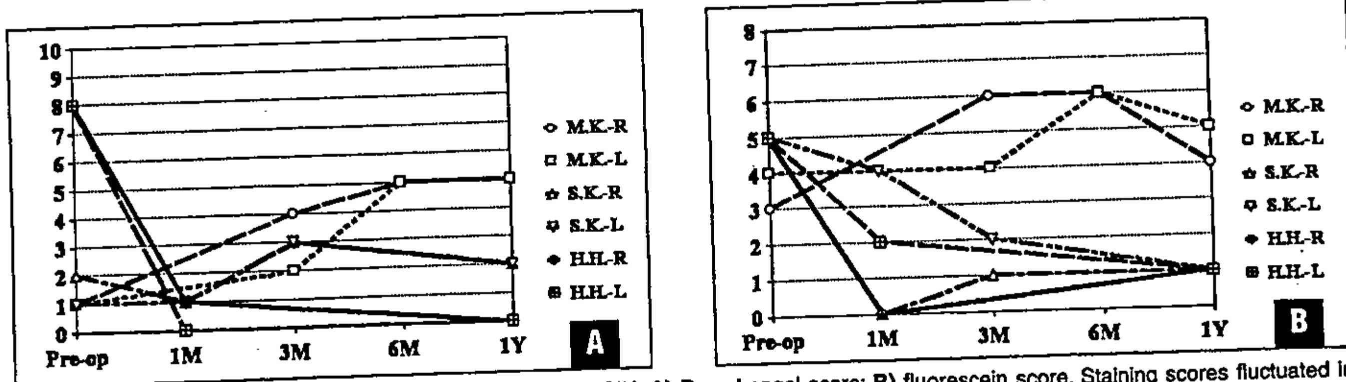

Figure 2. Vital staining of ocular surface before and after LASIK. **A)** Rose bengal score; **B)** fluorescein score. Staining scores fluctuated in patients M.K. and S.K., whereas the scores were continuously low in patient H.H. after LASIK. (Two of three patients had been diagnosed with Sjögren's syndrome and treated with artificial tears for some time before examination at our clinic, hence their rose bengal score was relatively low.)

epithelialization in these patients.[11,19] Autologous serum significantly improved ocular surface abnormality of severe dry eye with Sjögren's syndrome.[11] We suspect that combination of autologous serum eye drops, which supply defective tear components, and punctal occlusion, which prolongs the effects of these factors on the ocular surface, may make LASIK possible in patients with severe dry eye.

In Sjögren's syndrome, lacrimal glands are progressively destroyed with lymphocytic infiltration, leading to a decrease in tear secretion.[20,21] Although the degree of destruction of the lacrimal gland and decrease in tear secretion do not always parallel each other, it is expected that no tears can be produced if healthy acini and ducts in the glands are completely destroyed. We sometimes encounter patients whose ocular surface damage does not respond to extensive treatment of dry eye, even though they have been managed effectively by the same treatment previously. These patients usually have a long history of Sjögren's syndrome, possibly with very little residual healthy lacrimal gland components. Such patients with "absolute" dry eye are probably not candidates for LASIK. Punctal plugs are not expected to be effective because of very little residual tears. Clear lens extraction or phakic intraocular lens may alternatively be indicated for these patients. We decided that LASIK was indicated for our patients, who were not diagnosed with absolute dry eye, because ocular surface damage was improved with punctal plugs. Thus, it is imperative that dry eye treatment with autologous serum and punctal plugs be commenced before surgery; LASIK should not be performed until the ocular surface damage is greatly reduced.

Although we report only three cases, our results suggest that LASIK can be safely and effectively performed in severe dry eye patients associated with Sjögren's syndrome when extensive preoperative and postoperative dry eye management by a combination of autologous serum eye drops and punctal plugs is performed. However, careful attention must be paid to preoperative dry eye status and treatment compliance of the patients when patient selection is made. LASIK should be scheduled only after the ocular surface findings have sufficiently improved. Patients should be educated to use autologous serum eye drops every 2 hours at least for 3 months postoperatively, when dry eye is expected to worsen from LASIK. Thorough informed consent concerning possible complications of LASIK in patients with severe dry eye should be obtained. Careful assessment of preoperative and postoperative ocular surface status is mandatory. Also, it may be appropriate to inform these patients that clear lens extraction or phakic intraocular lens may be an alternative procedure to correct refractive errors.

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Development of Autoimmune Exocrinopathy Resembling Sjögren's Syndrome in Adoptively Transferred Mice With Autoreactive CD4+ T Cells

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Objective. **The pathologic mechanisms responsible for organ-specific tissue damage in primary Sjögren's syndrome (SS) remain unclear, but it has been suggested that the pathology is mediated by autoreactive CD4+ T cells infiltrating the salivary and lacrimal glands. This study was undertaken to investigate whether α-fodrin autoantigen–specific autoreactive CD4+ T cells are capable of inducing autoimmune lesions.**

Methods. **A total of 45 synthetic α-fodrin peptides designed to be 20 amino acid residues in length were generated. To establish an autoreactive T cell line, limiting dilution analysis (LDA) was performed on lymph node cells (LNCs) in the presence of α-fodrin peptides. The effects of adoptive transfer of autoreactive CD4+ T cells into normal syngeneic recipients were investigated.**

Results. **Autoreactive CD4+ T cell lines that recognize synthetic α-fodrin peptide, which produced Th1 cytokines and showed cytotoxic activities, were established in a murine model for SS. T cell receptor V_β usage and third complementarity-determining region (CDR3) sequences indicated that in some cases $V_\beta 6$-CDR3 genes matched between the tissue-infiltrating T cells and the autoreactive T cell lines. Adoptive transfer of the autoreactive CD4+ T cells into normal syngeneic recipients induced autoimmune lesions quite similar to those of SS.**

Conclusion. **Our data help to elucidate the pathogenic mechanisms responsible for tissue destruction in autoimmune exocrinopathy and indicate that autoreactive CD4+ T cells play a pivotal role in the development of murine SS.**

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Primary Sjögren's syndrome (SS) is a T cell–mediated autoimmune disease, and autoreactive T cells bearing the CD4 molecule may recognize unknown self antigen, triggering autoimmunity in the salivary and lacrimal glands and leading to clinical symptoms of dryness of the mouth and eyes (sicca syndrome) (1,2). Previously, we identified involvement of a 120-kd α-fodrin autoantigen in the pathogenesis of primary SS in humans and rodents (3,4), but the mechanisms for tissue destruction in target organs remain unclear.

Although an important role for T cells in the development of organ-sepcific autoimmune disease has been suggested, it is not known whether disease is initiated by a restricted inflammatory reaction to an organ-specific autoantigen. In most cases, antigenic challenge results in the establishment of immunologic memory, a state in which the immune system is maintained to respond effectively upon recurrent antigenic exposure. Autoreactive T cells generally respond to a limited number of immunodominant epitopes in self antigenic proteins, including myelin basic protein, thyroglobulin, and glutamic acid decarboxylase (5–8). Thymectomy on day 3 after birth (3d-Tx) is followed by the development of organ-specific autoimmune diseases (9,10). The *sld* mutation in NFS/N mice (NFS/*sld*, H-2D^q) is involved in the mucous cell differentiation of the sublingual gland (11). Using 3d-Tx NFS/*sld* mutant

mice, we have established and analyzed a murine model of primary SS in which the animals spontaneously develop a disease with many of the characteristics of human SS (12–14). The T cell receptor (TCR) $V_\beta8$ and $V_\beta6$ genes are preferentially used in these lesions from the onset of disease in the 3d-Tx NFS/*sld* mouse model (12). However, little is known about the events triggering T cell invasion of the the salivary and lacrimal glands in prelude to the development of autoimmune lesions.

Alpha-fodrin is an actin-binding protein that is found at the periphery of chromaffin cells and may be involved in secretion (15). The stimulation of secretion in parotid acinar cells is associated with dramatic rearrangements of the sub-plasmalemmal cytoskelton of α-fodrin (16). In the present study we established α-fodrin–reactive T cell lines capable of inducing autoimmune lesions similar to those found in SS. Moreover, the TCR V_β usage and the third complementarity-determining region (CDR3) sequences of the autoreactive T cell lines were determined.

MATERIALS AND METHODS

Mice. NFS/N mice carrying the mutant gene *sld* (11) were raised in our specific pathogen–free mouse colony. Thymectomy was performed on day 3 after birth.

Histologic and immunohistologic analysis. All organs were removed from the mice, and the sections were stained with hematoxylin and eosin. Histologic grading of the inflammatory lesions was done determined as described previously (17). Immunohistologic analysis was performed by the avidin–biotin–immunoperoxidase method utilizing ABC reagent (Vector, Burlingame, CA). Monoclonal antibodies used were as follows: biotinylated rat monoclonal antibodies to CD4 and CD8 (Cedarlane, Hornby, Ontario, Canada), Mac-1 (Becton Dickinson, Burlingame, CA), and B220, $V_\beta8$, $V_\beta6$, interleukin-2 (IL-2), IL-4, and interferon-γ (IFNγ) (all from PharMingen, San Diego, CA).

Recombinant α-fodrin autoantigen. Recombinant α-fodrin protein, the complementary DNA (cDNA) encoding human α-fodrin (JS-1: 1–1784 bp, 2.7A: 2258–4884 bp, 3′DA: 3963–7083 bp) (18) was constructed by inserting cDNA into the *Eco* RI site of pGEX-4T 1, 2, and 3. The mouse sequences of α-fodrin are identical to the human sequences.

Assessment of proliferative T cell response. Single cell suspensions of spleen cells and lymph node cells (LNCs) were cultured in 96-well flat-bottomed microtiter plates (5×10^5 cells/well) in RPMI 1640 containing 10% fetal calf serum (FCS), penicillin/streptomycin, and β-mercaptoethanol. Cells were cultured with each recombinant α-fodrin protein (JS-1, 2.7A, and 3′DA) (5 μg/ml). During the last 8 hours of the 72-hour culture period, 1 μCi of ^{3}H-thymidine was added per well, and the incorporated radioactivity was determined using an automated beta liquid scintillation counter. We isolated tissue-infiltrating mononuclear cells from affected salivary glands as described previously (14). Infiltrating T cells were purified using nylon wool (Wako, Osaka, Japan).

TCR V_β usage and CDR3 sequencing of polymerase chain reaction (PCR) products. To investigate the comparison of clonotypes of infiltrating T cells in vivo and autoreactive T cell lines, reverse transcriptase PCR (RT-PCR) was used to discriminate the diversities in the D, J, and N regions. Total RNA was prepared with Isogen (Nippon Gene, Tokyo, Japan), and amplification was performed with *Taq* polymerase with 5′ primer specific for the TCR $V_\beta6$ and $V_\beta8$ genes and a 3′ primer specific for the TCR C_β gene. The diluted sample (2 μl) was electrophoresed in nondenaturing 5% polyacrylamide gels containing 10% glycerol. After electrophoresis, the DNA was transferred to Immobilon-S (Millipore, Intertech, Bedford, MA) and hybridized with biotinylated C_β probe, streptavidin, biotinylated alkaline phosphatase, and a chemiluminescent substrate system (Millipore Intertech). The inserted TCR genes were sequenced with dye-labeled primers and AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA), using a 373A automated DNA sequencing system (Applied Biosystems).

Synthetic peptides. Peptides were synthesized using TBOC chemistry with a model 430A peptide synthesizer (Applied Biosystems, CA). A total of 45 synthetic peptides designed to be 20 amino acid residues in length, overlapping by 5 amino acid residues, were generated.

Autoreactive T cell line. To establish an α-fodrin peptide–specific T cell line, limiting dilution analysis (LDA) was performed as described previously (19,20), on LNCs in the presence of α-fodrin peptides and irradiated syngeneic spleen cells. LNCs from 3d-Tx NFS/*sld* mice (5 weeks old) were cultured with α-fodrin peptides (10 μg/ml) in RPMI 1640 supplemented with 10% FCS, 10 m*M* HEPES, and 100 units (100 μg/ml) 100 penicillin/streptomycin in 96-well plates at 1×10^6 cells/well. On day 3, IL-2 (Genzyme, Cambridge, MA) was added; cells were fed with media containing 0.5 ng/ml IL-2 every 3 days. On day 14, an aliquot was analyzed for reactivity to α-fodrin peptides. Cells (1×10^4) from each cell line were cocultured with 1×10^4 irradiated autologous splenocytes in duplicate for 72 hours. Alpha-fodrin–specific T cell lines (stimulation index >3) were retested using synthetic peptides and were maintained by restimulation at 10–14–day intervals with α-fodrin peptide pulse-irradiated splenocytes. Following a third round of stimulation, LDA revealed cloned T cell lines. Autoreactive T cell lines were maintained by stimulation with IL-2 and feeder cells at 7–10–day intervals.

Flow cytometric analysis. Single cell suspensions were stained with antibodies conjugated to phycoerythrin (PE) (anti-CD3 [Gibco BRL, Grand Island, NY]; anti-CD4, B220) or anti-CD4; fluorescein isothiocyanate (FITC) (anti-CD8, Thy1.2, anti-CD44, anti-CD45RB, anti–Mel-14 [the latter 4 from PharMingen]), and analyzed on an EPICS counter (Coulter, Hialeah, FL). For analysis of intracellular cytokines by flow cytometry, cells (10^6/ml) were activated with immobilized anti-CD3 monoclonal antibody (Cedarlane) for 4 hours. Monensin (Wako) was added at 2 m*M*, and 2 hours later cells were collected, washed, and permeabilized with 0.1% saponin in phosphate buffered saline at 4°C for 10 minutes. Cells were incubated with FITC-conjugated anti-IL-2 (8 μg/ml), PE-conjugated anti–IL-4 (5 μg/ml), and FITC-conjugated anti-IFNγ (1 μg/ml) and analyzed on an EPICS counter.

Cytotoxicity assay. Cytotoxicity assays were performed as described previously (21), using peptide-pulsed (10 μg/ml) mouse salivary gland (MSG) cells labeled with ^{51}Cr sodium chromate as target at a 1:50 target:effector ratio.

Cell transfer. To examine whether autoreactive CD4+ T cells induce autoimmune lesions, cells from T cell line 21–1 (1×10^6) were injected intraperitoneally into irradiated (7.5 Gy) normal NFS/*sld* mice at 4 weeks, and analyses performed at 8 weeks (n = 7) and 12 weeks (n = 8) after the injection. As controls, 1×10^6 splenic CD4+ T cells nonpulsed or pulsed and with fibronectin fragment peptide (5 μg/ml; Sigma, St. Louis, MO) from syngeneic mice were injected intraperitoneally into irradiated (7.5 Gy) NFS/*sld* mice and analyzed in the same manner (n = 5 for each).

Measurement of fluid secretion. Measurement of tear and saliva volume in the transferred NFS/*sld* mice was performed by modification of previously described methods (14,21,22).

Western blot analysis. To detect serum autoantibodies against 120-kd α-fodrin antigen (3), samples were solublized by heating, and separated by 10% sodium dodecyl sulfate–polyacrylamide gel electrophoresis. The autoantigen was electrotransferred to nitrocellulose, which was then quenched with 1% powdered milk in borate buffered saline. Nitrocellulose membranes were incubated with testing serum at a 1:200 dilution in borate buffered saline, and then incubated with peroxidase-conjugated horse anti-mouse IgG (Vector) at a 1:1,000 dilution.

RESULTS

Establishment of autoreactive T cell lines. We have previously identified a cleavage product of 120-kd α-fodrin as an important autoantigen in the pathogenesis of primary SS in both humans and rodents (3,4). To determine whether an immune response in mice with experimental SS could be mounted against recombinant α-fodrin protein, the cDNA encoding human α-fodrin (JS-1, 2.7A, and 3'DA) were constructed by inserting cDNA into the *Eco* RI site of pGEX-4T 1, 2, and 3. When we compared, in parallel, the proliferative T cell responses with individual recombinant α-fodrin fusion protein, we detected a significantly increased proliferation in SS mouse LNCs, spleen cells, and tissue-infiltrating T cells stimulated with JS-1 protein (N-terminal portion of α-fodrin) (data not shown). By LDA, we succeeded in isolating 3 strongly proliferative autoreactive T cell lines (clones 21–1, 21–2, and 21–3) from JS-1 peptide (p21)–stimulated LNCs (Figure 1A), but not from control peptide–stimulated cells. The majority of autoreactive T cells were CD4+ cells bearing $V_\beta6$ and containing Th1 cytokines such as IL-2 and IFNγ, but not IL-4 (Figure 1B). We confirmed that the autoreactive CD4+ T cell lines had significant cytotoxicity, against MSG cells from NFS/*sld* mice when tested in a ^{51}Cr-release

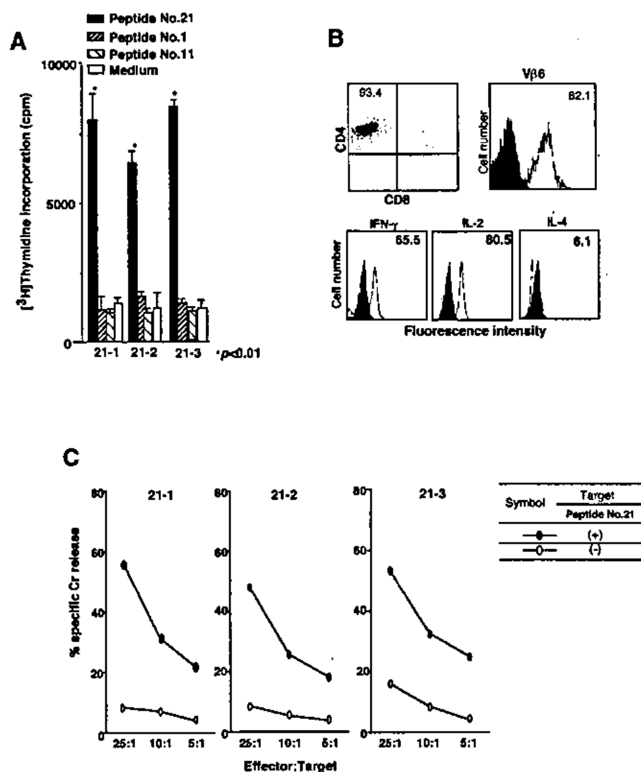


Figure 1. Establishment of autoreactive T cell lines. **A,** Three strongly proliferative T cell lines (21–1, 21–2, 21–3) from JS-1 peptide p21–stimulated lymph node cells were isolated and ^{3}H-thymidine incorporated measured. Values are the mean and SEM. **B,** Flow cytometric analysis, showing that the majority of these T cells were CD4+ and $V_\beta6$+ and contained interleukin-2 (IL-2) and interferon-γ (IFNγ), but not IL-4. **C,** Results of ^{51}Cr-release assay, demonstrating that the autoreactive CD4+ T cell lines had significant cytotoxicity when tested against mouse salivary gland cells. Values are the mean from triplicate studies.

assay (Figure 1C). In contrast, the autoreactive T cells did not kill major histocompatibility complex (MHC)–matched targets of newborn keratinocytes (data not shown).

The TCR V_β usage and the CDR3 sequences of 3 autoreactive T cell lines were determined by RT-PCR amplification and sequencing of the PCR products. Notably, in some cases these sequences (SISAETL and SMQN) were homologous to V–D–J β sequences of the T cells from affected glands of mice with experimental SS at 8 weeks (Figure 2).

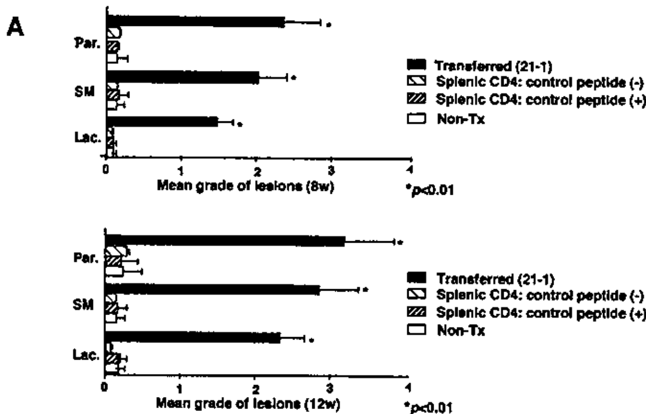
Development of autoimmune lesions after adoptive transfer of autoreactive CD4+ T cells. To analyze whether the autoreactive T cells cause autoimmune lesions, cells from CD4+ T cell line 21–1 (1×10^6) were transferred intraperitoneally into irradiated (7.5 Gy) normal NFS/*sld* mice at 4 weeks. Organ-specific auto-

	$V_{\beta}6$	N-D-N	J_{β}		Frequency
T cells infiltrated in salivary gland					
	TGTGCCAGC	AGTTATGCAGAA	TTCGGTCCCGGCACCAGGCTCGGTTTT	2.1	
	C A S	S N Q E	F G P G T R L G F		
	TGTGCCAGC	AGTATAAGTGCAGAAACGCTG	TATTTTGGTGCGGGCACCCGACTATCGGTG	2.3	
	C A S	S I S A E T L	Y F G A G T R L S V		
Autoreactive T cell lines					
21-1	TGTGCCAGC	AGTATAAGTGCAGAAACGCTG	TATTTTGGTGCGGGCACCCGACTATCGGTG	2.3	6/10
	C A S	S I S A E T L	Y F G A G T R L S V		
	TGTGCCAGC	AGTATGACAGTA	TTCGGTCCCGGCACCAGGCTCGGTTTT	2.1	1/10
	C A S	S M T V	F G P G T R L G F		
	TGTGCCAGC	AGTATAGGGGAGAGATATGAACAG	TACTTCGGTCCCGGCACCAGGCTCACTGTT	2.6	1/10
	C A S	S I G E R Y E Q	Y F G P G T R L T V		
	TGTGCCAGC	AGCCCCGCAGAAACGCTG	TATTTTGGCCCCGGNACCAGACTGACTGTT	2.6	1/10
	C A S	S P A E T L	Y F G P X T R L T V		
21-2	TGTGCCAGC	AGTATAAGTGCAGAAACGCTG	TATTTTGGTGCGGGCACCCGACTATCGGTG	2.3	7/10
	C A S	S I S A E T L	Y F G A G T R L S V		
	TGTGCCAGC	AGTATTTGGGTTGAACAG	TACTTCGGTCCCGGCACCAGGCTCACGGTT	2.5	1/10
	C A S	S I W V E Q	Y F G P G T R L T V		
	TGTGCCAGT	AGTACCGGNCTGGGGGTTCAAGACACCCAG	TACTTTGGCCCAGGCACTCGGCTCCTCGTG	2.5	1/10
	C A S	S T G L G V Q D T Q	Y F G P G T R L L V		
	TGTGCCAGC	AGCCCGCAGAAACGCTGT	ATTTTGGCCTTGGNACCAGACTGACTGTT	2.6	1/10
	C A S	S P A E T L	Y F G L X T R L T V		
21-3	TGTGCCAGC	AGTTATGCAGAA	TTCGGTCCCGGCACCAGGCTCGGTTTT	2.1	7/10
	C A S	S N Q N	F G P G T R L G F		
	TGTGCCAGC	AGTATAAGTGCAGAAACGCTG	TATTTTGGTGCGGGCACCCGACTATCGGTG	2.3	1/10
	C A S	S I S A E T L	Y F G A G T R L S V		
	TGTGCCAGT	AGTACCGGNCTGGGGGTTCAAGACACCCAG	TACTTTGGCCCAGGCACTCGGCTCCTCGTG	2.5	1/10
	C A S	S T G L G V Q D T Q	Y F G P G T R L L V		
	TGTGCCAGC	AGTCGGGACGGATATGAACAG	TACTTCGGTCCCGGCACCAGGCTCACGGTT	2.6	1/10
	C A S	S R D G Y E Q	Y F G P G T R L T V		

Figure 2. T cell receptor V_{β} gene usage and third complementarity-determining region (CDR3) sequences of infiltrating T cells in NFS/*sld* mice that had undergone thymectomy on day 3 after birth and of α-fodrin p21–specific lines (21–2, 21–2, 21–3), determined by reverse transcriptase–polymerase chain reaction amplification and sequencing of the polymerase chain reaction products. The $V_{\beta}6$-CDR3 sequences were homologous between the T cells infiltrating salivary glands and 3 autoreactive T cell lines (underlined).

immune lesions developed exclusively in the salivary and lacrimal glands at 8 weeks (n = 7) and 12 weeks (n = 8) after the injection with autoreactive T cell line 21–1, while a transfer of splenic CD4+ T cells pulsed with fibronectin fragment peptide did not induce any lesions (Figure 3A). Histopathologic examination revealed no inflammatory lesions in other organs including the liver, pancreas, adrenal glands, and reproductive organs of mice treated with the autoreactive T cell line. This suggests that α-fodrin–reactive CD4+ T cells are pathogenic in vivo.

The majority of tissue-infiltrating cells in the salivary and lacrimal glands of mice that underwent adoptive transfer of autoreactive CD4+ T cells were positive for CD4 and $V_{\beta}6$, but not for CD8 or $V_{\beta}8$ (Figure 3B). Very few B220+ B cells were present in inflammatory lesions (results not shown). A large proportion of Th1 cytokine–positive cells (IL-2, IFNγ), but not IL-4–positive cells, was detected in the salivary glands from autoreactive T cell line–treated mice (Figure 4A). Isotype-matched controls were all negative. Moreover, the autoimmune lesions were accompanied by significantly decreased secretion of saliva and tears (Figure 4B). Serum autoantibody production against JS-1 protein could not be detected in adoptively transferred mice (Figure 4C). T cell line–treated mice showed a significant increase of autoantigen (JS-1 and p21)–specific T cell proliferation in the spleen cells, while no responses against ovalbumin or lysozyme were observed (Figure 5A). The activation markers $CD44^{high}$, $CD45RB^{low}$, and $Mel\text{-}14^{low}$ were significantly upregulated in LNCs gated on CD4 from adoptively transferred mice (Figure 5B). Moreover, we found that CD4+ T cells isolated from LNCs of the treated mice had significant cytotoxicity when tested against MSG



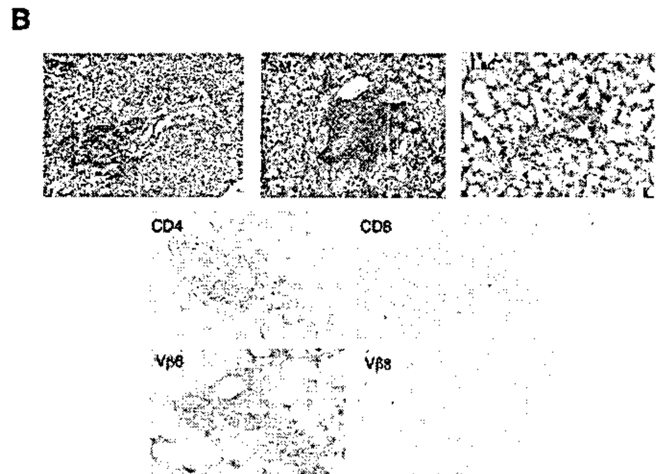


Figure 3. Adoptive transfer of autoreactive T cell line 21–1 into normal syngeneic recipients. **A**, Autoimmune lesions in the salivary and lacrimal (Lac.) glands developed at 8 weeks (n = 7) and 12 weeks (n = 8) after intraperitoneal injection with cells from T cell line 21–1 (1×10^6) into irradiated (7.5 Gy) normal NFS/*sld* mice, but not in controls. Values are the mean and SEM. Par. = parotid gland; SM = submandubular gland; Non-Tx = nonthymectomized. **B**, Representative histologic features in adoptively transferred mice at 12 weeks. The majority of infiltrating lymphocytes were positive for CD4 and $V_{\beta}6$, but not for CD8 or $V_{\beta}8$.

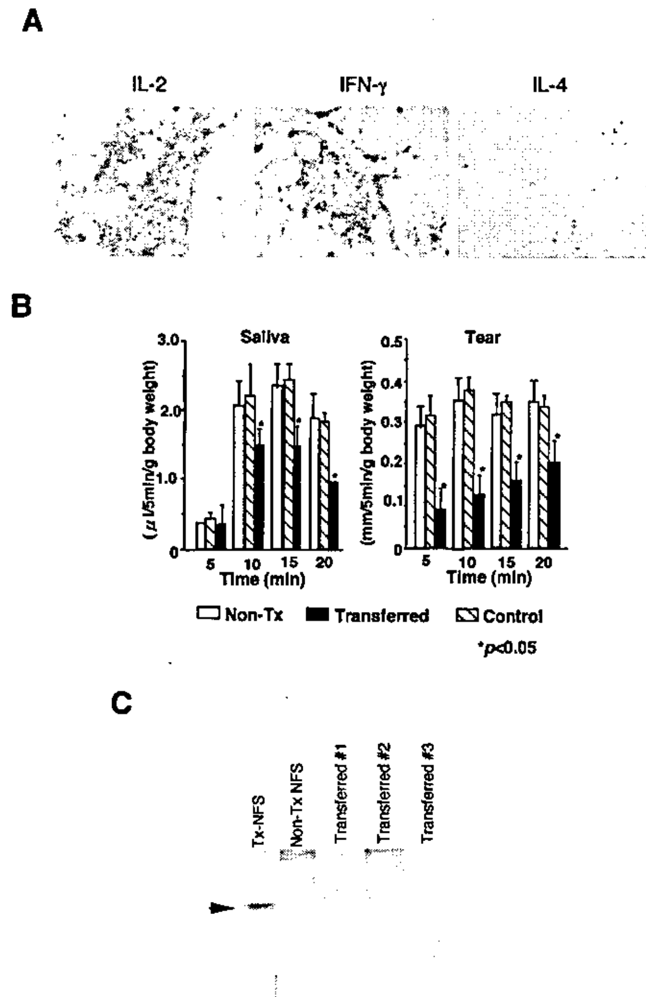


Figure 4. **A,** Immunohistologic features in adoptively transferred mice. A large proportion of infiltrating cells in the salivary glands were positive for Th1 cytokines such as interleukin-2 (IL-2) and interferon-γ (IFNγ), but not IL-4. **B,** Mean and SEM saliva and tear volume (n = 5 per group) in adoptively transferred mice, control-treated mice, and nonthymectomized (Non-Tx) mice. **C,** Western blot analysis. Production of autoantibodies to 120-kD α-fodrin was not detected in sera from 3 different adoptively transferred mice.

cells from NFS/*sld* mice in a ^{51}Cr-release assay (Figure 5C).

DISCUSSION

Autoreactive T cells are conventionally regarded to be eliminated by negative selection in the thymus or by the induction of peripheral tolerance (23,24). The results described here demonstrate that α-fodrin–reactive CD4+ T cells can induce autoimmune exocrinopathy in normal syngeneic mice. Although the specificity of cytotoxic T lympyocyte (CTL) function has been an important issue in organ-specific autoimmune response, the mechanisms responsible for tissue destruction have not been elucidated. In type 1 diabetes mellitus, the role of environmental factors (25,26), the nature of the initiating inflammatory cell (27,28), and the identity of the inciting antigen(s) (29,30) have all been vigorously debated. We have previously identified a cleavage product of 120-kd α-fodrin as an important autoantigen in the pathogenesis of primary SS in both humans and rodents (3,4). We detected significantly increased proliferation of lymph node cells, spleen cells, and tissue-infiltrating T cells from model mice (3d-Tx NFS/*sld*) stimulated with JS-1 protein (N-terminal portion of α-fodrin).

We succeeded in isolating 3 strongly proliferative autoreactive T cell lines (21–1, 21–2, and 21–3) from JS-1 peptide (p21)–stimulated LNCs. The majority of autoreactive T cells were CD4+ T cells bearing $V_\beta 6$ and containing Th1 cytokines such as IL-2 and IFNγ, but not IL-4. Of importance is that the autoreactive CD4+ T cell lines had significant cytotoxicity when tested against MSG cells in a ^{51}Cr-release assay. Furthermore, the TCR V_β usage and the CDR3 sequences of 3 autoreactive T cell lines were homologous to VDJ_β sequences of the T cells from affected glands of mice with experimental SS. (Figure 2).

Previous studies have suggested that clonally expanded T cell populations with restricted usage of TCR gene segments may be essential for the development of autoimmune diseases including SS (31,32). However, the basis for TCR repertoire selection initiating autoimmunity has not yet been fully understood. It should be noted that in this study, infiltrating T cell sequences that are similar to, and in some cases match, the sequences of the autoreactive T cell lines were found (underlined in Figure 2). Previous work has demonstrated that dual TCR T cells may rescue autoreactive T cells from negative selection in the thymus (33). Our data imply that the established autoreactive T cells are found in the common TCR repertoire (V_β6-CDR3: SISAETL). This notion is supported by work by Basu et al (34) which demonstrates the binding of 2 separate ligands, a self peptide (arthritic peptide), and a foreign epitope, on distinct MHC areas by T cells bearing a single TCR.

In the analysis of whether the autoreactive T cells cause autoimmune lesions, we found that organ-specific autoimmune lesions developed exclusively in the salivary and lacrimal glands at 8 and 12 weeks after the intra-

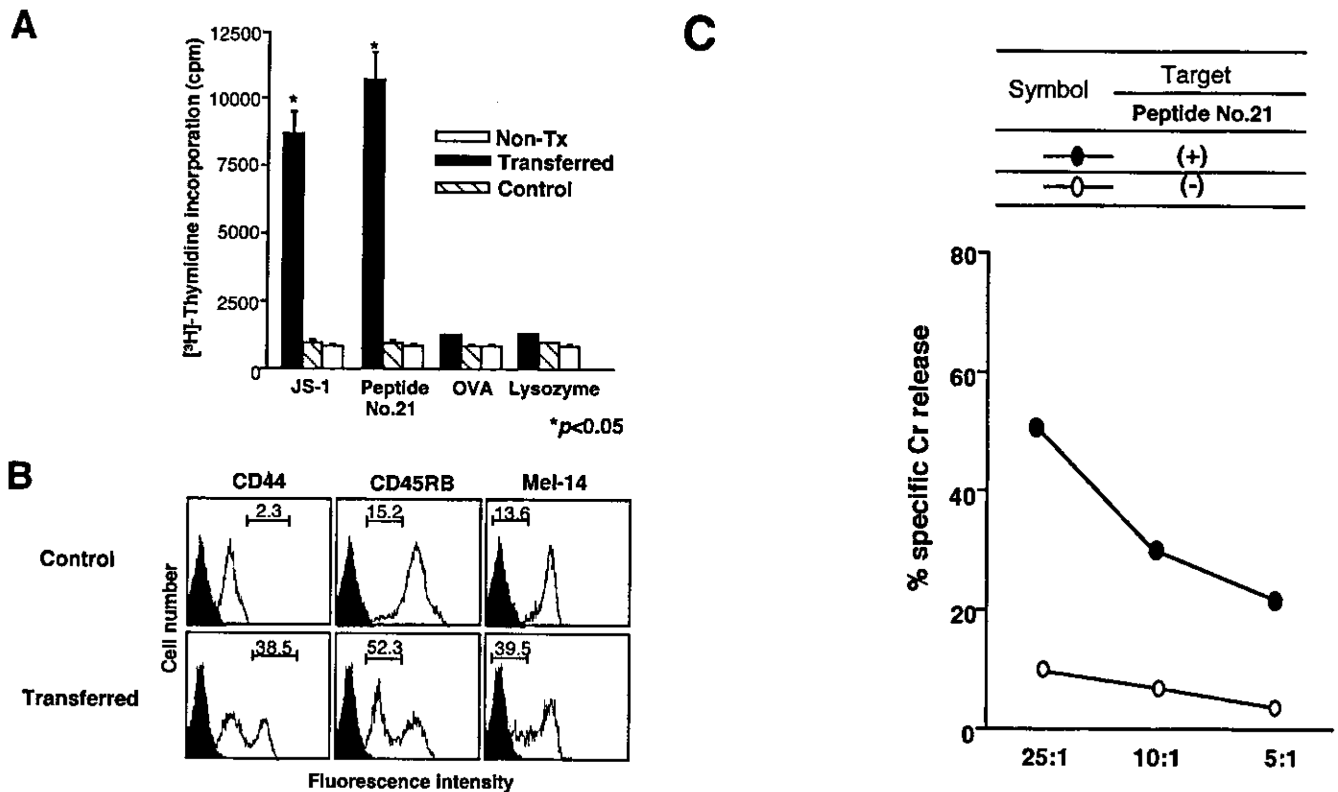


Figure 5. A, T cell proliferation in spleen cells. Adoptively transferred mice showed a significant increase of autoantigen (JS-1 and peptide 21)–specific T cell proliferation, while no responses against ovalbumin (OVA) or lysozyme were observed. Values are the mean and SEM of triplicate experiments. Non-Tx = nonthymectomized. B, Flow cytometric analysis, showing that the activation markers $CD44^{high}$, $CD45RB^{low}$, and $Mel\text{-}14^{low}$ were significantly up-regulated in lymph node cells gated on CD4 from adoptively transferred mice. C, Results of ^{51}Cr-release assay, demonstrating that CD4+ T cells isolated from lymph node cells of adoptively transferred mice had significant cytotoxicity against salivary gland cells from NFS/*sld* mice. Values are the mean from triplicate studies.

peritoneal injection with autoreactive CD4+ T cells. Adoptively transferred mice showed a significant increase of autoantigen-specific T cell proliferation in the spleen cells, while no responses against ovalbumin or lysozyme were observed. The activation markers were significantly up-regulated in LNCs gated on CD4 from adoptively transferred mice, and CD4+ T cells isolated from LNCs of transferred mice had significant cytotoxicity against MSG cells when tested in a ^{51}Cr-release assay. These data indicate that the autoreactive CD4+ T cells recognizing α-fodrin autopeptide are essentially pathogenic for the development of organ-specific autoimmune lesions in murine SS. Since serum production of autoantibodies against α-fodrin autoantigen could not be detected in transferred mice, it is possible that the adoptively transferred disease in these experiments may be entirely dependent on T cell–mediated immune responses. Thus, a critical autoreactive CD4+ T cell function should be operative in the initial stages of the disease, because the T cells in established lesions show strong proliferative activity and secrete Th1 cytokines. Previous investigations have demonstrated the accumulation of antigen-reactive T cells at the site of the inflammation in several human autoimmune diseases as well as in murine models of human autoimmune diseases (35,36).

In conclusion, we have demonstrated that α-fodrin–specific autoreactive CD4+ T cell lines can be established from α-fodrin peptide p21–stimulated LNCs, and that the autoreactive T cells have significant cytotoxicity against MSG cells when tested in a CTL assay. Moreover, we confirmed the development of autoimmune lesions, quite similar to those found in SS, into normal syngeneic recipients, using autoreactive CD4+ T cells. The results of this study provide evidence of an essential role for autoreactive CD4+ T cells specific for a self peptide in the development of organ-specific autoimmune disease in SS.

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Possible Role of Nitric Oxide in Radiation-Induced Salivary Gland Dysfunction

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Takeda, I., Kizu, Y., Okamoto, Y., Saito, I. and Yamane, G. Possible Role of Nitric Oxide in Radiation-Induced Salivary Gland Dysfunction. *Radiat. Res.* 159, 465–470 (2002).

In this study, we developed a murine model of xerostomia to elucidate the mechanism of radiation-induced salivary gland dysfunction and determined the levels of nitric oxide (NO) in the salivary glands to assess its involvement in the salivary dysfunction induced by radiation. In addition, an inhibitor of NO synthesis was administered to the model *in vivo*, and its effect on saliva secretion was investigated. Salivary gland irradiation at a dose of 15 Gy caused a significant decrease in secretion compared to unirradiated salivary glands. There were no marked differences between the irradiated mice and unirradiated mice in water or food consumption or in body weight changes. The NO levels in the cultured salivary gland epithelial cells were increased by treatment with a combination of interferon γ (Ifng), interleukin β (Il1b), and tumor necrosis factor α (Tnfa). Irradiation increased the NO level in the salivary gland tissue. The presence of N^G-monomethyl-L-arginine acetate (L-NMMA), an inhibitor of NO synthesis, caused a decrease in the NO level in cultured salivary gland tissues after irradiation. Administration of L-NMMA to irradiated mice improved saliva secretion. These results suggest that excessive production of NO induced by radiation is involved in the formation of radiation-induced xerostomia. The finding that administration of an inhibitor of NO synthesis ameliorated the dysfunction of irradiated salivary glands indicates that NO plays a role as a mediator of the dry mouth symptoms that occur after irradiation. © 2003 by Radiation Research Society

INTRODUCTION

Radiotherapy is one of the most widely used modalities in the treatment of malignant head and neck tumors, because of the importance of preserving the form and function of organs. Since several vital structures are located in the head and neck region, the radiation field often includes exocrine glands such as the salivary glands, possibly inducing xerostomia as a result of salivary gland dysfunction, a serious complication after irradiation (*1–4*). The direct tissue toxicity of the radiation as well as various factors induced by radiation, such as inflammatory cytokines, has been suggested to be involved in the development of radiation-induced xerostomia (*5–7*). However, a great deal remains unclear concerning the pathogenesis of xerostomia, and no treatment for this condition has been established. This is partly attributable to the fact that no appropriate animal model for studying this condition has been available, as well as because the relationship between the conditions of irradiation and mechanisms of secretory dysfunction have not been investigated sufficiently (*8, 9*).

Nitric oxide (NO) is a radical that has a short half-life and that has been identified as a vascular endothelium-derived relaxing factor (*10–13*). It is generated when the substrate L-arginine is converted into L-citrulline by NO synthase (NOS) (*14*). NO not only exerts physiological actions, such as regulation of the circulation, but also induces the development of various pathological conditions when it is produced in excess. NO has been reported to be an important mediator in the induction of inflammatory reactions through enhancement of vascular permeability and cytotoxic activity (*15–18*). Inducible NOS (iNOS) has been found to have a cytokine reaction site in its promoter region, indicating that its expression is induced by inflammatory cytokines, and interferon γ (Ifng) clearly induces the formation of NO and also enhances the production of NO in inflammatory states through synergistic action with interleukin 1β (Il1b) and tumor necrosis factor α (Tnfa) (*19–21*). Based on these findings, the present study was designed to elucidate the role of NO in secretion of the saliva using a murine model of radiation-induced salivary gland dysfunction.

MATERIALS AND METHODS

Animals

Six-week-old male and female ICR mice (body weight 35–38 g; Clea Japan Inc., Tokyo) were used. The mice were housed in polycarbonate cages in a specific-pathogen-free mouse colony and given food and water *ad libitum*. Each group of animals was used in the study after 1 week of

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