Abstract

Imidafenacin, a novel anticholinergic agent with low side effects, shows equivalent efficacy to solifenacin in overactive bladder patients —GAP (Global Assessment Study of Anti-cholinergics on Efficacy and Tolerability for Patients with OAB) Study—

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Overactive bladder (OAB) is a bothersome condition affecting the quality of life (QOL) of the patients. Recently, pharmacological treatment for OAB is centered on anticholinergic agents. However, it is well-known that anticholinergic agents can deteriorate the QOL of the OAB patients due to adverse effects such as dry mouth and constipation. Unwillingness and inability of OAB patients to continue anticholinergic agents due to these adverse effects have been the clinical problem especially in the long term therapy. The purpose of this study is to assess the efficacy and tolerability of imidafenacin and solifenacin in Japanese OAB patients, by evaluating the clinical efficacy, dry mouth, and constipation with overactive bladder symptom score (OABSS), dry mouth scale (DMS), and constipation assessment scale (CAS), respectively. There were significant improvements in total score and four sub-scores of OABSS in both groups. There was no significant difference in the changes of total score of OABSS at 12 weeks. Imidafenacin revealed equivalent efficacy to solifenacin. DMS showed low adverse effects in imidafenacin group. OAB patients were satisfied with the treatment of imidafenacin and solifenacin. This study indicated that imidafenacin is well-tolerated and show satisfactory effects in OAB patients.

key words : Imidafenacin, Solifenacin, Comparative study

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原　著

過活動膀胱を中心とした高齢者における健康調査

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要　旨

インターネットによる質問調査で，10歳以上の男性100名，女性199名の合計299名（平均年齢66.9歳）を対象とした過活動膀胱（overactive bladder：OAB）を中心とする健康調査を実施した．過活動膀胱症状質問票（overactive bladder symptom score：OABSS）を用いて調査した結果，OABと判定された患者は全体の約16.7%であった．しかしながら，OABと判定された患者の中で下部尿路機能障害の治療を受けている患者はわずかに11.0%であった．口腔乾燥質問票（dry mouth score：DMS）および便秘評価尺度（constipation assessment scale：CAS）を用いて口腔乾燥および便秘症状を評価した結果，OABと判定された回答者ではそれらのスコアが有意に高かった．以上の結果，高齢者におけるOAB治療は不十分であり，また治療薬である抗コリン薬を処方する場合は，その副作用（口腔乾燥，便秘）に注意が必要と考えられた．

緒　言

過活動膀胱（overactive bladder：OAB）は，2002年国際禁制学会（International Continence Society：ICS）において，「切迫性尿失禁の有無にかかわらず，通常頻尿および夜間頻尿を伴う尿意切迫感で，感染や他の明らかな病的状態は除外する」と定義された比較的新しい症状症候群である．OABは生命予後に関わる疾患ではないものの，患者の生活の質（quality of life：QOL）を著しく低下させる疾患として注目されている．日本排尿機能学会による本邦の疫学調査[1]によると，40歳以上の男女全体の12.1%，約810万人がOABであると推定されており，潜在患者数は糖尿病を上回ると考えられている．OABの薬物療法には，膀胱の過剰収縮を抑える抗コリン薬が主に使用されている．近年，尿意切迫感や頻尿といった症状はOABという病名のつく病気であることが広く認知され，ただ忍耐するだけでなく治療法があることが知られるようになった．さらに，新しい抗コリン薬が発売されたことにより，治療を受ける患者数も増加した．しかしながら，抗コリン薬では全身的な抗コリン作用に基づく口腔乾燥および便秘などの副作用が問題とされている．OABは高齢者に多く，抗コリン薬で口腔乾燥や便秘が高率に認められる理由の1つとして，年齢による唾液分泌ならび消化管機能の変化も関係している可能性がある．そこで，本研究では40歳以上の男女を対象として，OABを中心とした健康に関するアンケート調査を実施した．さらに，OABと口腔乾燥および便秘の関係についても調査した．

方　法

2010年8月16日から2010年8月21日までの5日間でインターネットにより調査を行った．調査の実施は，民間の調査機関（株式会社マクロミル）に委託した．10歳以上の男女を対象に年齢，性別，治療中の疾患，服用中の薬剤とともに，OAB，口腔乾燥および便秘の各

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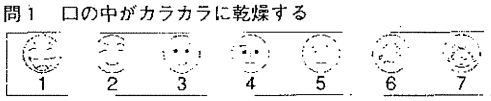
表1　過活動膀胱症状質問票

以下の症状がどれくらいの頻度でありましたか．この1週間のあなたの状態に最も，近いものを，1つだけ選んで，点数の数字を○で囲んでください．

症　状		点　数	
問1	朝起きた時から夜寝る時までに何回くらい尿をしましたか？	0 1 2	7回以下 8～14回 15回以上
問2	夜寝てから朝起きるまでに何回くらい尿をするために起きましたか？	0 1 2 3	0回 1回 2回 3回以上
問3	急に尿がしたくなり我慢が難しいことがありましたか？	0 1 2 3 4 5	なし 週に1回より少ない 週に1回以上 1日1回くらい 1日2～4回 1日5回以上
問4	急に尿がしたくなり我慢できずに尿をもらすことがありましたか？	0 1 2 3 4 5	なし 週に1回より少ない 週に1回以上 1日1回くらい 1日2～4回 1日5回以上
	合計点数		点

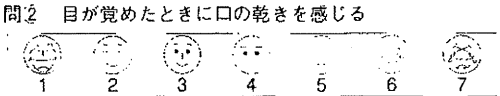
この1週間の状態をお答えください．あなたのお口の状態について，当てはまる番号を○で囲んでください．

問1　口の中がカラカラに乾燥する



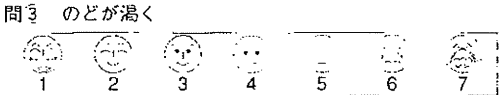
全然感じない　　すごく感じる

問2　目が覚めたときに口の乾きを感じる



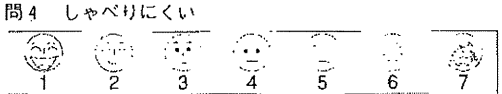
全然感じない　　すごく感じる

問3　のどが渇く



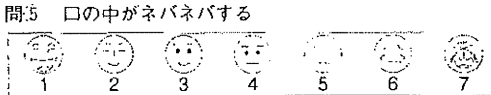
全然感じない　　すごく感じる

問4　しゃべりにくい



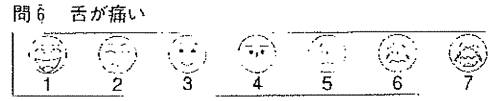
全然感じない　　すごく感じる

問5　口の中がネバネバする



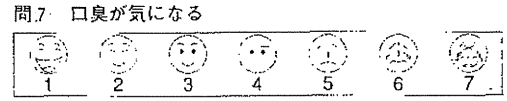
全然感じない　　すごく感じる

問6　舌が痛い



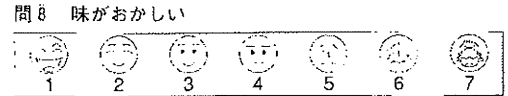
全然感じない　　すごく感じる

問7　口臭が気になる



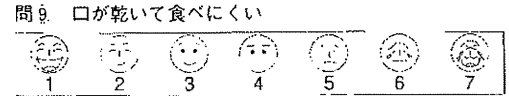
全然感じない　　すごく感じる

問8　味がおかしい



全然感じない　　すごく感じる

問9　口が乾いて食べにくい



全然感じない　　すごく感じる

図1　口腔乾燥質問票

表2　便秘評価尺度日本語版(CAS Ver.2 MT版)

最近1週間の状態で，項目ごとに該当する番号を○で囲んでください．

症　状		点　数	
問①	お腹がはった感じ，ふくれた感じ	ない ときどきある いつもある	0 1 2
問②	排ガス量	ふつうまたは多い ときどき少ない いつも少ない	0 1 2
問③	便の回数	ふつうまたは多い 少ない とても少ない	0 1 2
問④	直腸に内容が充満している感じ	全然ない ときどきある いつもある	0 1 2
問⑤	排便時の肛門の痛み	全然ない ときどきある いつもある	0 1 2
問⑥	便の量	ふつうまたは多い 少ない とても少ない	0 1 2
問⑦	便の排泄状態	らくに出る ときどき出にくい いつも出にくい	0 1 2
問⑧	下痢または水様便	ない ときどきある いつもある	0 1 2
合計点数			点

症状についてアンケートを行った．

OABのアンケートは，過活動膀胱診療ガイドラインにおいて推奨されている過活動膀胱症状質問票[2] (overactive bladder symptom score：OABSS)を用い評価を行った(表1)．OABSSの問3の尿意切迫感スコアが2点以上(尿意切迫感1回以上/週)，かつ合計スコアが3点以上の回答者をOABと判定した．

口腔乾燥のアンケートは，Kamashitaら[3]により信頼性と妥当性が検証された7段階のフェイススケールを用いた口腔乾燥質問票(dry mouth scale：DMS)を用い評価を行った(図1)．質問内容は，①口の中がカラカラに乾燥する，②目が覚めたときに口の乾きを感じる，③のどが渇く，④しゃべりにくい，⑤口の中がネバネバする，⑥舌が痛い，⑦口臭が気になる，⑧味がおかしい，⑨口が乾いて食べにくい，の9問からなる．また，問①②③の合計スコアを「口腔乾燥感スコア」，問④⑤⑨の合計スコアを「口腔乾燥の随伴症状スコア」，問⑥⑦⑧の合計スコアを「その他の症状スコア」とカテゴリー分類を行った．

便秘のアンケートは，日本語版で信頼性と妥当性が検証されている便秘評価尺度[4] (constipation assessment scale：CAS)日本語版Ver. 2 MT版を用いて評価を行った(表2)．

統計解析は，男女別での群間比較，および未治療でOABを有する回答者とOABのない回答者の群間比較にはMann-WhitneyのU検定を用いた．各アンケートのスコアの相関関係にはPearsonの相関係数の有意性検定を用いた．なお，$p<0.05$を統計学的に有意とし，解析は統計ソフトJMP®8.0.2(SAS Institute Japan株式会社)を用いた．

結　果

男性回答者100名，女性回答者199名，合計299名の調

表3　回答者の背景

回答者数		299
性別例数		
	男性	100
	女性	199
年齢(歳)	平均±標準偏差	66.9±10.8
治療中の主な疾患　例数(%)		
	高血圧症	66(22.1%)
	高脂血症	31(10.1%)
	糖尿病	15(5.0%)
	悪性腫瘍	12(4.0%)
	心疾患	10(3.3%)
飲酒　例数(%)		
	あり	117(39.1%)
	なし	182(60.9%)
喫煙　例数(%)		
	あり	43(14.1%)
	なし	256(85.6%)

査結果を回収した．回答者の平均年齢は66.9±10.8歳（男性平均年齢65.6±11.0歳，女性平均年齢69.7±9.8歳であった．回答者のうち156名（52.2%）で他の疾患を治療中であり，主な治療中の疾患（3%以上）は多い順から高血圧症66名（22.1%），高脂血症31名（10.4%），糖尿病15名（5.0%），悪性腫瘍12名（4.0%），心疾患10名（3.3%）であった．また，常用的に飲酒している回答者および喫煙している回答者は，それぞれ117名（39.1%）および43名（14.1%）であった（表3）．

全回答者のOABSS合計スコアの平均値は2.4±2.3であった（表4）．年齢とOABSSの相関関係について解析した結果，OABSS合計スコア，夜間排尿回数スコアおよび尿意切迫感スコアは年齢とともに増加し，有意な相関関係を示した（図2-1）．OABSSにより OABと判定された回答者（OAB回答者）は50名（16.7%）であった．OAB回答者のOABSS合計スコアの平均値は6.1±2.2であった．OAB回答者では合計スコアおよび各サブスコアはOABと判定されなかった回答者（非OAB回答者）と比較して有意に高かった．合計スコア，尿意切迫感スコアおよび尿失禁スコアは，10歳代から80歳代まですべての年齢層で高値であった（表5）．OAB回答者および非OAB回答者において，夜間排尿回数が増加するにつれてOABSS合計スコアは増加した．非OAB回答者のうち夜間排尿回数が2回以上の回答者は18.5%であったのに対し，OAB回答者で夜間排尿回数が2回以上の回答者は54.0%と多かった（表6）．男女別に解析

表4　全回答者のOABSS，DMSおよびCAS

OABSS	合計スコア	2.4±2.3
DMS	口腔乾燥感スコア（1 2 3）	8.8±4.2
	随伴症状スコア（4 5 9）	6.6±3.4
	その他の症状スコア（6 7 8）	6.1±3.1
	合計スコア	21.5±9.5
CAS	合計スコア	3.4±2.6

平均±SD

した結果，OABSSの平均スコアは男性で2.7±2.5，女性で2.2±2.1であった．OAB回答者は男性19名（19%），女性31名（15.6%）であった．OAB回答者のOABSS合計スコアは，男性で6.5±2.6，女性で5.8±1.8であった．男女間で各アンケートのスコアに有意差は認められなかった（表7）．常用的に飲酒している回答者としていない回答者において，OABSS合計スコアおよびOAB患者数に差は認められなかった（表8）．一方，常用的に喫煙している回答者は14.4%であった．喫煙している回答者と喫煙していない回答者において，OABSS合計スコアに有意な差は認められなかった（表9）．しかしながら，常用的に喫煙していない回答者および喫煙している回答者におけるOAB患者数は，それぞれ38名（11.8%）および12名（27.9%）であり，喫煙していない回答者と比較して喫煙している回答者においてOAB患者率が高い傾向が認められた．

排尿障害で治療を受けている回答者は頻尿7名（2.3%），尿漏れ2名（0.7%）であった．OABと判定された回答者の中でも，排尿障害の治療を受けている回答者は頻尿6名（12.0%），尿漏れ1名（2.0%）と少なかった．

口腔乾燥の指標であるDMSの合計スコアは21.5±9.5であった．そのサブスコアである口腔乾燥感スコア（問1 2 3），口腔乾燥の随伴症状スコア（問4 5 9）およびその他の症状スコア（問6 7 8）は，それぞれ8.8±4.2，6.6±3.4および6.1±3.1であった．一方，便秘の指標であるCASの合計スコアは3.1±2.6であった（表4）．男女間で各スコアに有意差は認められなかった（表7）．

OAB回答者と非OAB回答者について，各アンケートのスコアを比較した．頻尿・尿失禁を治療中の回答者は薬物治療および他の治療法の影響を考慮し，除外した上で解析を行った．OAB回答者はDMSおよびCASのスコアが，非OAB回答者と比較して有意に高かった（表10）．各アンケートの合計スコアの相関関係

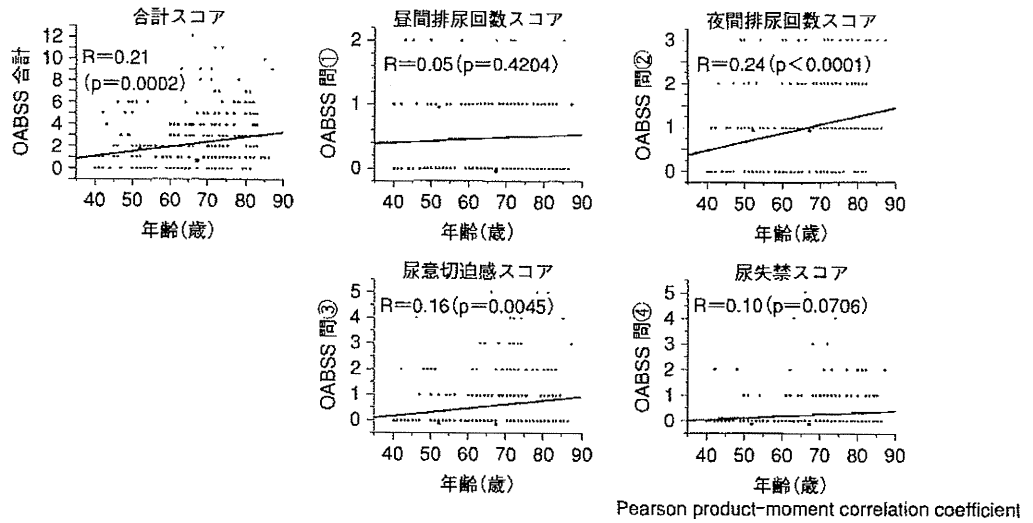


図2-1　OABSS合計スコアおよびサブスコアと年齢の相関(全回答者)

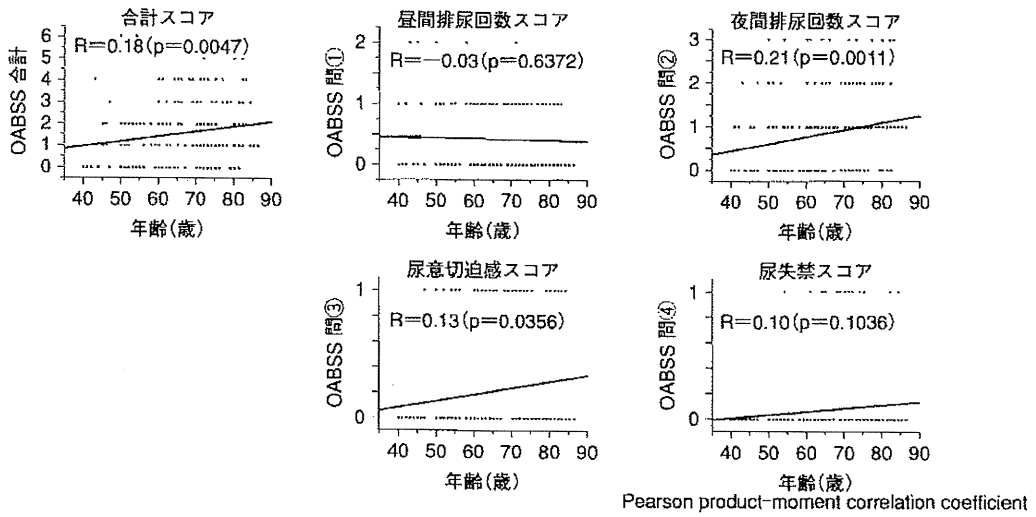


図2-2　OABSS合計スコアおよびサブスコアと年齢の相関(非OAB)

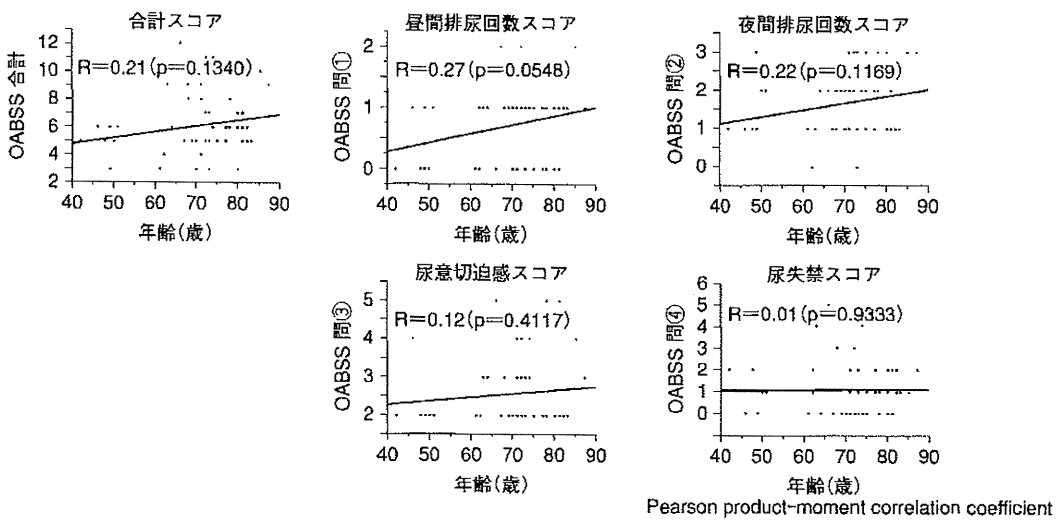


図2-3　OABSS合計スコアおよびサブスコアと年齢の相関(OAB)

表5　年代別のOABSS

	年齢層	例数	OABSS 問① 昼間排尿回数スコア	問② 夜間排尿回数スコア	問③ 尿意切迫感スコア	問④ 尿失禁スコア	合計スコア
全回答者	40歳代	22	0.5±0.7	0.7±0.8	0.6±1.1	0.2±0.6	1.9±2.1
	50歳代	40	0.5±0.6	0.8±0.9	0.3±0.5	0.1±0.3	1.6±1.7
	60歳代	77	0.4±0.5	1.0±0.8	0.6±1.0	0.3±0.9	2.3±2.3
	70歳代	119	0.5±0.5	1.1±0.9	0.7±1.1	0.3±0.6	2.5±2.3
	80歳代	41	0.6±0.5	1.4±1.0	0.9±1.2	0.3±0.7	3.2±2.5
	合　計	299	0.5±0.6	1.0±0.9	0.6±1.0	0.3±0.7	2.4±2.3
非OAB回答者	40歳代	17	0.5±0.7	0.5±0.7*	0.1±0.2*	0.0±0.0*	1.0±1.2*
	50歳代	38	0.5±0.6	0.7±0.8*	0.2±0.4*	0.0±0.2*	1.3±1.4*
	60歳代	66	0.4±0.5	0.9±0.9	0.3±0.4*	0.1±0.3*	1.7±1.4*
	70歳代	98	0.4±0.5*	0.9±0.8*	0.2±0.4*	0.1±0.3*	1.7±1.2*
	80歳代	30	0.5±0.5*	1.2±1.0	0.3±0.5*	0.1±0.3*	2.1±1.6*
	合　計	249	0.4±0.5*	0.9±0.8*	0.2±0.4*	0.1±0.3*	1.6±1.3*
OAB回答者	40歳代	5	0.4±0.6	1.4±0.9	2.4±0.9	0.8±1.1	5.0±1.2
	50歳代	2	0.5±0.7	2.0±0.0	2.0±0.0	1.0±0.0	5.5±0.7
	60歳代	11	0.7±0.7	1.2±0.8	2.6±0.9	1.6±1.9	6.2±2.9
	70歳代	21	0.7±0.6	2.0±0.9	2.7±0.9	1.0±1.2	6.3±2.2
	80歳代	11	0.9±0.5	1.7±0.9	2.6±1.0	1.1±0.8	6.3±2.0
	合　計	50	0.7±0.6	1.7±0.9	2.6±0.9	1.1±1.3	6.1±2.2

平均±SD，Mann-Whitney U-test(v.s. OAB回答者)，*：$p<0.05$.

表6　夜間排尿回数別の例数とOABSS合計スコア

	OABSS問② 夜間排尿回数	例数(%)		OABSS合計スコア (平均± SD)
全回答者	0回	93(31.1%)		0.6±0.9
	1回	133(44.5%)		2.2±1.5
	2回	49(16.4%)		4.5±2.4
	3回以上	24(8.0%)		5.7±2.0
	合　計	299(100.0%)		2.4±2.3
非OAB回答者	0回	90(36.1%)		0.5±0.7
	1回	113(45.4%)		1.7±0.8
	2回	32(12.9%)	18.5%	3.1±0.8
	3回以上	14(5.6%)		4.4±0.8
	合　計	249(100.0%)		1.6±1.3
OAB回答者	0回	3(6.0%)		3.7±0.6
	1回	20(40.0%)		5.0±1.6
	2回	17(34.0%)	54.0%	6.9±2.4
	3回以上	10(20.0%)		7.6±1.5
	合　計	50(100.%)		6.1±2.2

について解析した結果，OABSSとDMSの相関係数は0.38($p<0.0001$)，DMSとCASの相関係数は0.39($p<0.0001$)であり，OABSSとCASの相関係数は0.21($p=0.0003$)であった．いずれも有意な弱い相関関係を示した(図3)．DMSと年齢の相関係数は−0.05($p=0.3710$)，CASと年齢の相関係数は0.05($p=0.3625$)であり，DMSおよびCASは年齢との有意な相関関係は認められなかった(図4)．

表7　全回答者における男女別のOABSS，DMSおよびCAS

OAB回答者	男性(N＝100) 19名(19%)	女性(N＝199) 31名(15.6%)	p値 Mann-Whitney U-test
OABSS			
合計スコア	2.7±2.5	2.2±2.1	n.s.
DMS			
口腔乾燥感スコア	8.4±4.0	8.9±4.3	n.s.
随伴症状スコア	6.2±3.1	6.9±3.6	n.s.
その他の症状スコア	5.8±2.9	6.3±3.2	n.s.
合計スコア	20.4±8.8	22.1±9.8	n.s.
CAS			
合計スコア	3.1±2.5	3.5±2.6	n.s.

平均±SD

表8　飲酒および非飲酒者のOABSS，DMSおよびCAS

		飲酒あり N＝117	飲酒なし N＝182	p値 Mann-Whitney U-test
OABSS	問①	0.4±0.6	0.5±0.5	n.s.
	問②	1.0±0.9	1.0±0.9	n.s.
	問③	0.7±1.1	0.6±1.0	n.s.
	問④	0.3±0.8	0.2±0.6	n.s.
	合計	2.4±2.4	2.3±2.2	n.s.
DMS	問①	2.6±1.5	2.7±1.5	n.s.
	問②	2.9±1.7	3.1±1.7	n.s.
	問③	3.0±1.4	3.2±1.4	n.s.
	問④	2.3±1.4	2.2±1.3	n.s.
	問⑤	2.6±1.4	2.7±1.5	n.s.
	問⑥	1.5±1.0	1.5±1.1	n.s.
	問⑦	2.9±1.6	2.7±1.6	n.s.
	問⑧	1.9±1.3	1.8±1.2	n.s.
	問⑨	1.7±1.1	1.8±1.1	n.s.
	合計	21.4±9.4	21.6±9.5	n.s.
CAS		3.1±2.4	3.5±2.6	n.s.

平均±SD

考　察

40歳以上の男女を対象として，OABを中心とした健康に関するアンケート調査を実施した．回答者299名のうち，他の疾患を治療中であると回答した人は156名(52.2%)であり，半数以上の回答者が他の疾患で治療中であった．

本調査において，OABと判定された回答者は50名(16.7%)であった．本間らの報告[1]によると40歳以上の12.4%がOABと診断され，OABの有症率は本間らの報告とほぼ同じ結果であった．40歳以上において，OABSS合計スコアは年齢とともに増加した．OABSSのサブスコアと年齢との相関を検討した結果，年齢とともに増加するサブスコアは夜間排尿回数スコアおよび尿意切迫感スコアであり，その他のスコアと年齢との間に相関は認められなかった．夜間排尿回数スコアおよび尿意切迫感スコアは，非OAB回答者においても年齢とともに増加した(図2-2)．この結果から，OABの有無とは関係なく夜間排尿回数および尿意切迫感は年齢とともに増加することが明らかとなった．一方，OAB回答者では若年層から夜間排尿回数スコアが高く，夜間排尿回数2回以上の回答者はその54.0%に達した．夜間排尿回数2回以上では骨折リスクの上昇などが知られており[5]，夜間頻尿は下部尿路機能障害の

表9　喫煙および非喫煙者のOABSS，DMSおよびCAS

		喫煙者 N＝43	非喫煙者 N＝256	p値 Mann-Whitney U-test
OABSS	問①	0.5±0.6	0.5±0.6	n.s.
	問②	0.9±0.9	1.0±0.9	n.s.
	問③	1.0±1.4	0.6±0.9	n.s.
	問④	0.2±0.6	0.3±0.7	n.s.
	合計	2.6±2.5	2.3±2.2	n.s.
DMS	問①	3.0±1.4	2.6±1.5	n.s.
	問②	3.6±1.5	2.9±1.7	＜0.05
	問③	3.6±1.3	3.0±1.4	＜0.05
	問④	2.7±1.5	2.1±1.3	＜0.05
	問⑤	3.2±1.6	2.6±1.4	＜0.05
	問⑥	1.7±1.0	1.5±1.0	n.s.
	問⑦	3.3±1.5	2.7±1.6	＜0.05
	問⑧	2.2±1.6	1.8±1.2	n.s.
	問⑨	2.1±1.3	1.7±1.1	＜0.05
	合計	25.2±9.4	20.9±9.4	＜0.05
CAS		3.7±2.4	3.3±2.6	n.s.

平均±SD

表10　OAB回答者(頻尿・尿失禁を治療中の回答者を除く)と非OAB回答者のOABSS，DMSおよびCAS

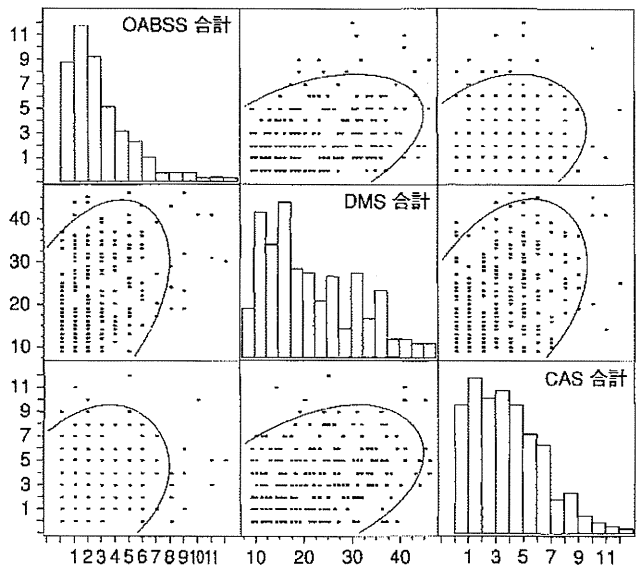
	OAB回答者 (N＝44)	非OAB回答者 (N＝247)	p値 Mann-Whitney U-test
OABSS			
合計スコア	5.8±2.1	1.6±1.3	＜0.05
DMS			
口腔乾燥感スコア	10.9±4.2	8.3±4.0	＜0.05
随伴症状スコア	8.3±3.7	6.3±3.3	＜0.05
その他の症状スコア	7.8±3.5	5.8±3.0	＜0.05
合計スコア	27.1±10.2	20.3±9.0	＜0.05
CAS			
合計スコア	4.3±2.4	3.2±2.6	＜0.05

平均±SD

中でも最も注意すべき症状と思われる．しかしながら，OABと判定された回答者の中で下部尿路機能障害の治療を受けている回答者はわずかに14.0%であった．これらの結果から，今後も，一層QOLに影響を及ぼしている，または骨折リスクにつながるようなOABに焦点を当てて，治療をより積極的に行う必要性があると思われた．加えて，高齢者においてはOABと判定されなくても夜間頻尿とそのリスクについて啓発する必要性が考えられた．

本調査においては常用的に喫煙している回答者は14.4%であった．常用的に喫煙している回答者において，OAB患者率は高い傾向が認められたものの，OABSS合計スコアおよび各スコアは喫煙していない回答者と比較して有意差が認められなかった．喫煙はOABのリスクファクターであるとの報告[6]がある．ニコチンは膀胱を刺激する作用があり[7]，その作用によってOABを誘発するとも考えられるが，喫煙とOABとの関係に関してはより詳細な調査が必要と思われた．なお，常用的な飲酒は特にOABに関係ないと考えられた．

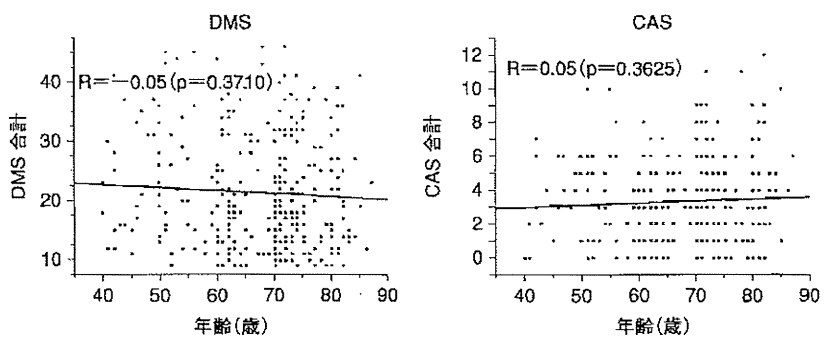
今回，回答者の口腔乾燥症状を評価するため，新たにフェイススケールを用いた質問票(DMS)を作成し



	R	p値
OABSS-DMS	0.38	<0.0001
OABSS-CAS	0.21	0.0003
DMS-CAS	0.39	<0.0001

Pearson product-moment correlation coefficient

図3　OABSS，DMSおよびCASの相関関係



Pearson product-moment correlation coefficient

図4　年齢とDMSおよびCAS合計スコアの相関

た．DMSは口腔乾燥に関連する9項目の質問からなり，口腔乾燥との関連性から質問を3つのカテゴリーに分類した．口腔乾燥に直接関係した症状を質問している項目は質問①②③の3項目であり，この合計スコアは「口腔乾燥感スコア」とした．質問④⑤⑨の質問内容は口腔乾燥に伴う随伴症状と考え，それらの合計スコアは「口腔乾燥の随伴症状スコア」とした．質問⑥⑦⑧の合計スコアを「その他の症状スコア」とした．その結果，DMS合計スコアの平均値は21.5±9.5であった．OAB回答者と非OAB回答者においてDMSの平均値を比較した結果，OABと判定された回答者においてDMS合計スコアおよび3つのサブカテゴリーの合計スコアは有意に高かった．これらの結果から，OAB回答者においては非OAB回答者と比較して口腔乾燥の症状が強いと考えられた．OABの治療には抗コリン薬が使用され，抗コリン薬の副作用として口腔乾燥が高頻度で認められることはよく知られている．しかしながら，OABと口腔乾燥の関係について調べられた報告はほとんどない．本研究の結果，OABと判定された回答者は，抗コリン薬による治療を受ける前から口腔乾燥の症状があることが明らかとなった．OAB回答者で口腔乾燥が高い理由の詳細については不明であるが，その理由の1つとしてストレスが考えられた．OABはQOLに影響を与える疾患で尿意切迫感などの急な尿意は患者に強い緊張感を与えるとともに，尿失禁は恥ずかしいとの感覚から患者に強いストレスを与えると考えられる．このようなことから，OAB患者では常に健常者と比較して高い緊張状態が持続し，その結果交感神経が優位となり唾液分泌量が低下している可能性がある．

一方，回答者における便秘を評価するためCASを用

いた．CASは，抗がん剤の便秘の程度を評価するため海外で開発された質問票である[8]．その後，一般的な便秘の評価にも使えることが証明され，1995年には深井らによって日本語に翻訳された[4]．深井らの報告によると，認知症のない障害のある高齢者(いわゆる寝たきり老人)と健常な高齢者で便秘の症状をCASで比較した結果，障害のある高齢者では有意にCASスコアが高いことが明らかとなった[9]．本調査において，回答者のCAS平均スコアは3.4±2.6であった．さらに，CAS平均スコアは男性および女性において差が認められなかった．OAB回答者と非OAB回答者のCASスコアを比較した結果，CASスコアはOAB回答者において有意に高かった．以上の結果から，高齢者における便秘の程度には男女差がないこと，およびOAB患者では治療前より便秘傾向が強いことが明らかとなった．海外における尿失禁患者に関する疫学調査によって尿失禁に関係する様々なリスクファクターが研究されている．尿失禁に関係する代表的なリスクファクターは年齢および肥満などであるが，便秘もリスクファクターとなる報告が複数存在する[10,11]．本研究でも同様な結果が得られたこととなり，便秘がOABのリスクファクターであると同時に，OAB患者では口腔乾燥と同様，便秘傾向である点については注意が必要と思われた．

OAB治療の中心は抗コリン薬による薬物療法である．抗コリン薬では口腔乾燥および便秘が高頻度で発生する副作用としてよく知られている．特に，口腔乾燥は服薬の継続を困難にするほど患者にとって問題の大きい副作用である．本調査により未治療でOABと判定された患者は，治療前の時点において既に口腔乾燥および便秘症状が強いとの結果が得られた．OAB治療において抗コリン薬は治療の中心を担っており，非OAB回答者よりOAB回答者は口腔乾燥および便秘症状が既に治療前より症状が強いという結果を踏まえ，処方時には唾液腺マッサージなどの口腔乾燥症状緩和の方法，食事内容への指導，緩下薬の併用などを考慮する必要がある．

結　　語

インターネットによるアンケート調査の結果，OABと判定された回答者は16.7%であった．OABSS合計スコアは年齢とともに増加したが，OABSSで年齢と相関のあるサブスコアは夜間排尿回数スコアおよび尿意切迫感スコアであった．OABと判定された回答者の中で治療を受けている回答者はわずかに14.0%であった．口腔乾燥および便秘症状をDMSおよびCASで評価した結果，OABと判定された回答者においてそれらのスコアは有意に高かった．

以上の結果，未治療のOAB患者では口腔乾燥および便秘症状が強いことが明らかとなった．既に口腔乾燥，便秘を有する可能性の高いOAB患者への，抗コリン薬投与は副作用発現に注意し，対処法を患者に提示する必要があると考えられた．

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Th2 and Regulatory Immune Reactions Contribute to IgG4 Production and the Initiation of Mikulicz Disease

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Objective. **Mikulicz disease has been considered to be a subtype of Sjögren's syndrome (SS). However, recent studies have suggested that Mikulicz disease is an IgG4-related disease and is distinguishable from SS. In addition, it has been reported that both interleukin-4 (IL-4) and IL-10 induce IgG4 production and inhibit IgE. This study was undertaken to examine the expression of these cytokines in patients with Mikulicz disease and patients with SS.**

Methods. **Labial salivary gland (LSG) sections from 15 patients with Mikulicz disease and 18 patients with SS were examined for subsets of the infiltrating lymphocytes, expression patterns of messenger RNA (mRNA) for cytokines/chemokines, and relationships between the IgG4:IgG ratio and the expression of mRNA for IL-4 or IL-10.**

Results. **Immunohistochemical analysis showed lymphocyte infiltration of various subsets in the LSGs of SS patients, and the selective infiltration of IgG4-positive plasma cells and Treg cells in the LSGs of Mikulicz disease patients. The levels of mRNA for both Th1 and Th2 cytokines and chemokines in LSGs from patients with SS were significantly higher than in controls, while the expression of both Th2 and Treg cells was significantly higher in the patients with Mikulicz disease than in controls. Furthermore, the expression of IL-4 or IL-10 in the LSGs was correlated with the IgG4:IgG ratio.**

Conclusion. **These results suggest that the pathogenesis of Mikulicz disease is different from that of SS. Mikulicz disease is a unique inflammatory disorder characterized by Th2 and regulatory immune reactions that might play key roles in IgG4 production.**

Mikulicz disease has been considered to be a subtype of Sjögren's syndrome (SS), based on the histopathologic similarities between the two diseases (1). However, Mikulicz disease shows several differences in comparison with typical SS. In Mikulicz disease, enlargement of the lacrimal and salivary glands is persistent, salivary secretion is either normal or moderately dysfunctional, patients have a good response to corticosteroid treatment, and hypergammaglobulinemia and low frequencies of anti-SSA and anti-SSB antibodies are found on serologic analyses. Since Yamamoto et al (1–3) reported that serum IgG4 levels are elevated and IgG4-positive plasma cells infiltrate into the gland tissue in Mikulicz disease, these symptoms have also been recognized in autoimmune pancreatitis (4), primary sclerosing cholangitis (5), tubulointerstitial nephritis (6), interstitial pneumonia (7), Ridel's thyroiditis (8), and Küttner's tumor (9). These diseases are now called "IgG4-related diseases" (2,10). IgG4 is a Th2-dependent Ig and has low affinity for target antigen. Interleukin-4 (IL-4) directs naive human B cells to switch to IgG4 and IgE production (11).

CD4+ T helper cells including at least 5 subsets have been identified. Th0, Th1, Th2, Th17, and Treg cells are generally considered to maintain the balance

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and homeostasis of the immune system and possibly to induce various diseases by their impaired regulation. The difference in the functions of Th1 and Th2 cells has been characterized by the patterns of cytokines secreted by these Th cells. Th1 cells induced by IL-12 are mainly responsible for cell-mediated immunity, while Th2 cells induced by IL-4 are responsible for humoral immunity. These Th subsets are then mutually controlled by the cytokine that each produces. Several studies have indicated that many autoimmune diseases or allergic diseases are caused by the collapse of the Th1/Th2 balance. Th0 cells are produced by both Th1 and Th2 cytokines and are considered to be precursors of Th1 and Th2 cells. Treg cells are essential for the maintenance of immunologic self-tolerance and immune homeostasis. Recently, a subset of IL-17–producing T cells (Th17 cells) distinct from Th1 and Th2 cells was described and was shown to play a crucial role in the induction of autoimmunity and allergic inflammation (12). Furthermore, it has been demonstrated that chemokines are intimately involved in the Th1/Th2 balance and immune responses in various diseases, such as rheumatoid arthritis (13), systemic lupus erythematosus (13,14), SS (13,15), systemic sclerosis (13,16), idiopathic inflammatory myopathy (13), and atopic dermatitis (17).

The relationship of Th1/Th2 imbalance to the pathogenesis of SS has been investigated widely, and a polarized Th1 balance has been associated with the immunopathology of the disease (18–20). Numerous interferon-γ (IFNγ)–positive CD4+ T cells are detected in the salivary glands of SS patients, and an intracellular cytokine assay demonstrated subsequent promotion of Th1 cells in SS (21). We have previously shown that SS was initiated and/or maintained by Th1 cytokines and subsequently progressed in association with Th2 cytokines (22). Ogawa et al (15) reported that Th1 chemokines, such as IFNγ-inducible 10-kd protein (IP-10) and monokine induced by IFNγ, are involved in the accumulation of T cell infiltrates in the salivary glands of patients with SS. These findings suggest that Th1 cells play a central role in the pathogenesis of SS.

In contrast, patients with Mikulicz disease frequently have a history of bronchial asthma and allergic rhinitis and show severe eosinophilia and elevated serum IgE levels. We previously reported that peripheral CD4+ T cells from patients with Mikulicz disease revealed deviation of the Th1/Th2 balance to Th2 and elevated the expression of Th2-type cytokines (23,24). Moreover, recent studies have indicated that peripheral blood CD4+ T cells in patients with IgG4-related lacrimal gland enlargement showed a Th2 bias and elevated serum IgE levels (24). Therefore, it is suggested that Mikulicz disease has a Th2-predominant phenotype. The findings of a previous study showing that autoimmune pancreatocholangitis, which is an IgG4-related disease, could also be characterized by the overproduction of Th2 and regulatory cytokines (25) deserve our attention.

To date, pathogenetic differences between immune responses in SS and Mikulicz disease are not well understood. In this study, we identified the expression patterns of cytokines, chemokines, and chemokine receptors in the salivary glands of these diseases to clarify the involvement of characteristic immune responses in the development of Mikulicz disease.

PATIENTS AND METHODS

Patients. Fifteen patients with Mikulicz disease (12 women and 3 men with a mean ± SD age of 56.3 ± 13.0 years) and 18 patients with SS (16 women and 2 men with a mean ± SD age of 54.6 ± 12.8 years) who were referred to the Department of Oral and Maxillofacial Surgery at Kyushu University Hospital were included in the study. Mikulicz disease was diagnosed according to the following criteria (3): persistent symmetrical swelling (lasting longer than 3 months) of >2 lacrimal and major salivary glands, elevated serum levels of IgG4 (>135 mg/dl), and infiltration of IgG4-positive plasma cells in the tissue (ratio of IgG4-positive cells:IgG-positive cells >40%) on immunostaining. SS was diagnosed according to the criteria of both the Research Committee on SS of the Ministry of Health and Welfare of the Japanese Government (1999) (26) and the American–European Consensus Group criteria for SS (27).

All patients exhibited objective evidence of salivary gland involvement based on the presence of subjective xerostomia and a decreased salivary flow rate, abnormal findings on parotid sialography, and focal lymphocytic infiltrates in the labial salivary glands (LSGs) and submandibular glands. All patients with SS had primary SS with strong lymphocytic infiltration in the LSGs, had no other autoimmune diseases, and had never been treated with corticosteroids or any other immunosuppressants. LSG biopsies were performed as described by Greenspan et al (28). As controls, LSGs biopsy specimens were obtained from 18 patients with mucoceles who had no clinical or laboratory evidence of systemic autoimmune disease. These control LSGs were all histologically normal. Written informed consent was obtained from all patients and healthy controls.

Histologic analysis of LSGs. Formalin-fixed and paraffin-embedded sections (4 μm) of LSG specimens were prepared and stained with hematoxylin and eosin for conventional histologic examinations. The degree of lymphocytic infiltration in the specimens was judged by focus scoring (28,29). One standardized score is the number of focal inflammatory cell aggregates containing 50 or more mononuclear cells in each 4-mm^2 area of salivary gland tissue (30). All of the patients with Mikulicz disease and patients with SS

in this study had strong lymphocytic infiltration (focus scores of 10–12).

Immunohistochemical analysis of LSGs. For the immunohistochemical analysis of lymphocyte subsets, 4-μm formalin-fixed and paraffin-embedded sections were prepared and stained by a conventional avidin–biotin complex technique as previously described (31). The mouse monoclonal antibodies used to analyze lymphocyte subsets were anti-CD4 (clone B12; MBL), anti-CD20 (clones L26 and M0755; Dako), and anti-FoxP3 (clone mAbcam 22510; Abcam). The mouse monoclonal antibody and rabbit polyclonal antibody used to analyze IgG4-positive and IgG-positive plasma cells were anti-IgG (A0423; Dako) and anti-IgG4 (The Binding Site). HDP-1 (antidinitrophenyl [anti-DNP] IgG1) was used as a control mouse monoclonal antibody. The polyclonal antibodies used to analyze the cytokines were anti–IL-4 (clone ab9622), anti–IL-10 (clone ab34843), anti–IFNγ (clone ab9657) (all from Abcam), and anti–IL-17 (clone sc-7927; Santa Cruz Biotechnology). SS1 (anti–sheep erythrocyte IgG2a), NS8.1 (anti–sheep erythrocyte IgG2b), and NS4.1 (anti–sheep erythrocyte IgM), were used as control rabbit polyclonal antibodies. The mouse monoclonal antibodies used to analyze the chemokines and chemokine receptors were anti–IP-10 (clone ab73837; Abcam), anti-CXCR3 (clone ab64714; Abcam), anti–thymus and activation–regulated chemokine (anti-TARC) (54015; R&D Systems), anti–macrophage-derived chemokine (anti-MDC) (57203; R&D Systems), and anti-CCR4 (MAB1567; R&D Systems). HDP-1 (anti-DNP IgG1) was used as a control mouse monoclonal antibody. The sections were sequentially incubated with primary antibodies, biotinylated anti-mouse IgG secondary antibodies (Vector Laboratories), avidin–biotin–horseradish peroxidase complex (Vector Laboratories), and 3,3′-diaminobenzidine (Vector Laboratories). Mayer's hematoxylin was used for counterstaining. Photomicrographs were obtained using a light microscope equipped with a digital camera (CoolSNAP; Photometrics). Stained IgG4-positive cells and IgG-positive cells were counted in 1-mm^2 sections from 5 different areas, and the ratio of IgG4-positive cells to IgG-positive cells was calculated.

RNA extraction and complementary DNA (cDNA) synthesis. Total RNA was prepared from the LSG specimens by the acid guanidinium–phenol–chloroform method as previously described (32–34). Three micrograms of the total RNA preparation was then used for the synthesis of cDNA. Briefly, RNA was incubated for 1 hour at 42°C with 20 units of RNasin ribonuclease inhibitor (Promega), 0.5 μg of oligo(dT)$_{12\text{-}18}$ (Pharmacia), 0.5 m*M* of each dNTP (Pharmacia), 10 m*M* of dithiothreitol, and 100 units of RNase H reverse transcriptase (Life Technologies).

Quantitative estimation of messenger RNA (mRNA) by real-time polymerase chain reaction (PCR). Quantitative cDNA amplification was performed according to the recommendations of the manufacturer and as previously described (32–34). The cDNAs for the cytokines, chemokines, and chemokine receptors were analyzed by real-time PCR using LightCycler FastStart DNA Master SYBR Green 1 (Roche Diagnostics) in a LightCycler real-time PCR instrument (version 3.5; Roche Diagnostics). The cytokines, chemokines, and chemokine receptors examined were IL-2, IFNγ, IL-12, IP-10, CXCR3, IL-4, IL-5, TARC, MDC, CCR4, IL-10, transforming growth factor β (TGFβ), FoxP3, IL-17, and IL-6. The markers of lymphocytes examined were IgG and IgG4.

The primer sequences used were as follows: for β-actin (260 bp), forward 5′-GCAAAGACCTG-TACGCCAAC-3′, reverse 5′-CTAGAAGCATTTGCGGTGGA-3′; for CD3δ (184 bp), forward 5′-GATGTCATTGCCACTCTGC-3′, reverse 5′-ACTTGTTCCGAGCCCAGTT-3′; for IL-2 (416 bp), forward 5′-ACTCACCAGGATGCTCACAT-3′, reverse 5′-AGGTAATCCATCTG-TTCAGA-3′; for IFNγ (355 bp), forward 5′-AGTTATATCTTGGCTTTTCA-3′, reverse 5′-ACCGAATAATTAGTCAGCTT-3′; for IL-4 (203 bp), forward 5′-CTGCCTCCAAGAACACAACT-3′, reverse 5′-CACAGGACAGGAATTCAAGC-3′; for IL-5 (104 bp), forward 5′-ATGAGGATGCTTCTGCATTTG-3′, reverse 5′-TCAACTTTCTATTATCCACTCG-3′; for IL-6 (115 bp),

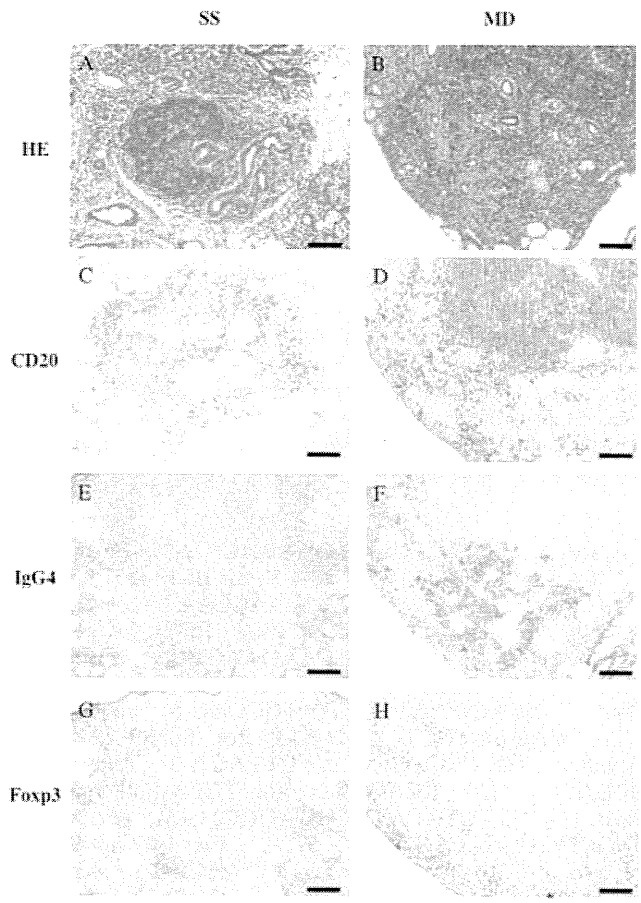

Figure 1. Selective infiltration of IgG4-positive plasma cells and FoxP3-positive Treg cells in the labial salivary glands (LSGs) of patients with Mikulicz disease (MD). Sections from the LSGs of a representative patient with Sjögren's syndrome (SS) and a representative patient with Mikulicz disease were immunostained with hematoxylin and eosin (H&E) (**A** and **B**) and anti-CD20 (**C** and **D**), anti-IgG4 (**E** and **F**), and anti-FoxP3 (**G** and **H**) monoclonal antibodies. Counterstaining with Mayer's hematoxylin was subsequently performed. Bars = 100 mm; original magnification × 100.

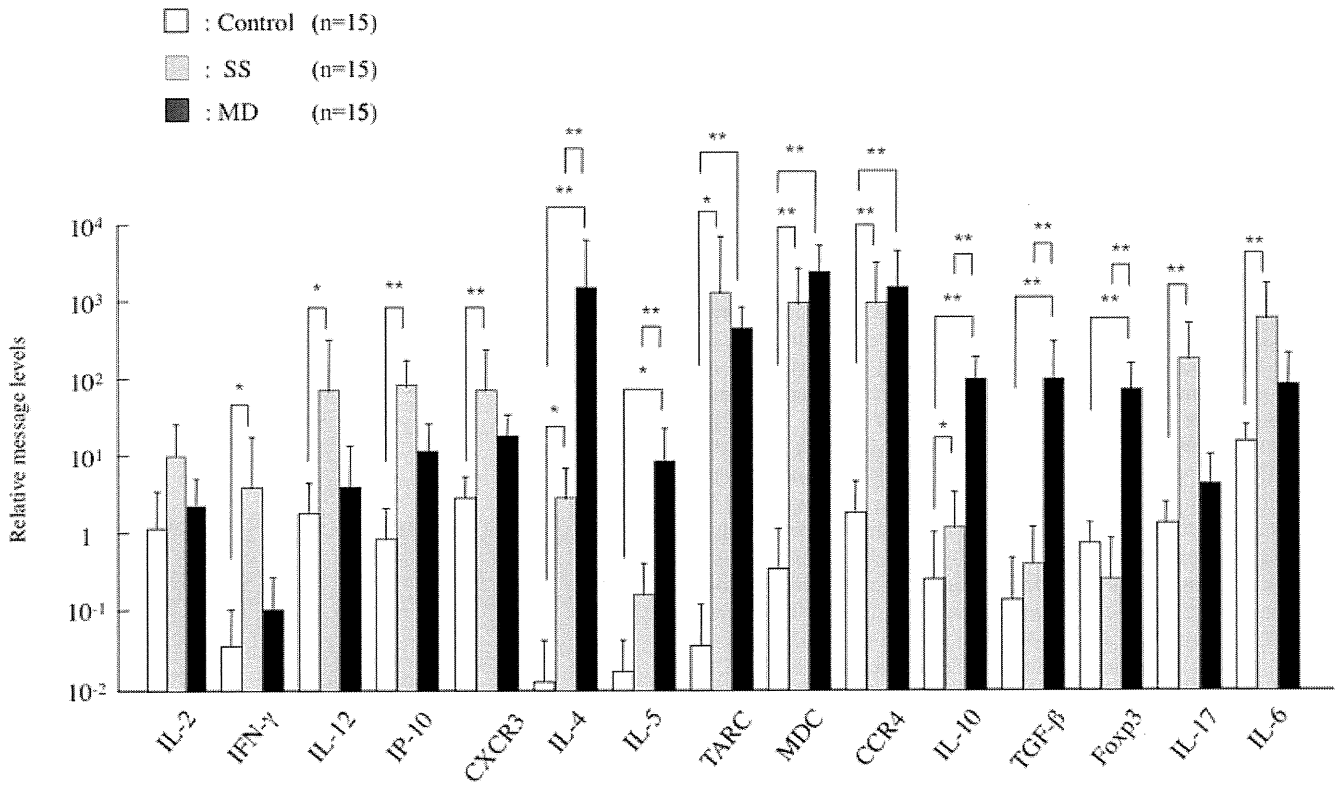

Figure 2. Expression patterns of mRNA for cytokines, chemokines, and chemokine receptors in LSGs from controls, patients with SS, and patients with Mikulicz disease. Levels of interferon-γ (IFNγ), interleukin-2 (IL-2), IL-12, IFNγ-inducible 10-kd protein (IP-10), and CXCR3 (Th1 type); IL-4, IL-5, thymus and activation–regulated chemokine (TARC), macrophage-derived chemokine (MDC), and CCR4 (Th2 type); IL-10, transforming growth factor β (TGFβ), and FoxP3 (Treg cell type); and IL-6 and IL-17 (Th17 type) were quantitatively estimated as described in Patients and Methods. Levels of mRNA in LSGs from SS patients and patients with Mikulicz disease were compared with those in control LSGs. Bars show the mean ± SD. * = $P < 0.05$; ** = $P < 0.01$ by Mann-Whitney U test. See Figure 1 for other definitions.

forward 5′-GGCACTGGCAGAAAACAA-3′, reverse 5′-CTCCAAAAGACCAGTGATGA-3′; for IL-10 (351 bp), forward 5′-ATGCCCCAAGCTGAGAACCAA-3′, reverse 5′-TCTCAAGGGGCTGGGTCAGCTA-3′; for IL-12 (187 bp), forward 5′-CCTGACCCACCCAAGAACTT-3′, reverse 5′-GTGGCTGAGGTCTTGTCCGT-3′; for IL-17 (186 bp), forward 5′-GCAGGAATCACAATCCCAC-3′, reverse 5′-TCTCTCAGGGTCCTCATTGC-3′; for FoxP3 (207 bp), forward 5′-CCCCTTGCCCCACTTACA-3′, reverse 5′-GCCACGTTGATCCCAGGT-3′; for TGFβ (142 bp), forward 5′-GCCCCTACATTTGGAGCCTG-3′, reverse 5′-TTGCGGCCCACGTAGTACAC-3′; for IgG (129 bp), forward 5′-CAAGTGCAAGGTCTCCAACA-3′, reverse 5′-TGGTTCTTGGTCAGCTCATC-3′; for IgG4 (132 bp), forward 5′-ACTCTACTCCCTCAGCAGCG-3′, reverse 5′-GGGGGACCATATTTGGAC-3′; for IP-10 (288 bp), forward 5′-CCTTAAAACCAGAGGGGAGC-3′, reverse 5′-AGCAGGGTCAGAACATCCAC-3′; for CXCR3 (184 bp), forward 5′-CTGGTGGTGCTGGTGGACAT-3′, reverse 5′-AGAGCAGCATCCACATCCG-3′; for MDC (253 bp), forward 5′-CGCGTGGTGAAACACTTCTA-3′, reverse 5′-GAATGCAGAGAGTTGGCACA-3′; for TARC (140 bp), forward 5′-TAGAAAGCTGAAGACGTGGT-3′, reverse 5′-GGCTTTGCAGGTATTTAACT-3′; for CCR4 (214 bp), forward 5′-GTGCTCTGCCAATACTGTGG-3′, reverse 5′-CTTCCTCCTGACACTGGCTC-3′; and for CD3α (184 bp), forward 5′-GATGTCATTGCCACTCTGC-3′, reverse 5′-ACTTGTTCCGAGCCCAGTT-3′.

In order to provide a meaningful comparison between different individuals or samples, we calculated the relative amounts of the PCR products of these molecules to the amounts of the PCR products of CD3δ (for the standardization of T cell mRNA) or the PCR products of β-actin (for the standardization of total cellular mRNA) in each sample, as previously described (22,23,35,36). The CD3δ PCR product levels were used for T cell–specific molecules, such as IL-2, IL-5, IL-12, and IFNγ, while the β-actin PCR product levels were used for T cell–nonspecific molecules, such as IL-4, IL-6, IL-10, IL-17, and chemokines, which were produced by a variety of cell types.

Statistical analysis. The statistical significance of the differences between the groups was determined by the Mann-Whitney U test and Spearman's rank correlation. All statistical analyses in this study were performed using JMP software, version 8 (SAS Institute). *P* values less than 0.05 were considered significant.

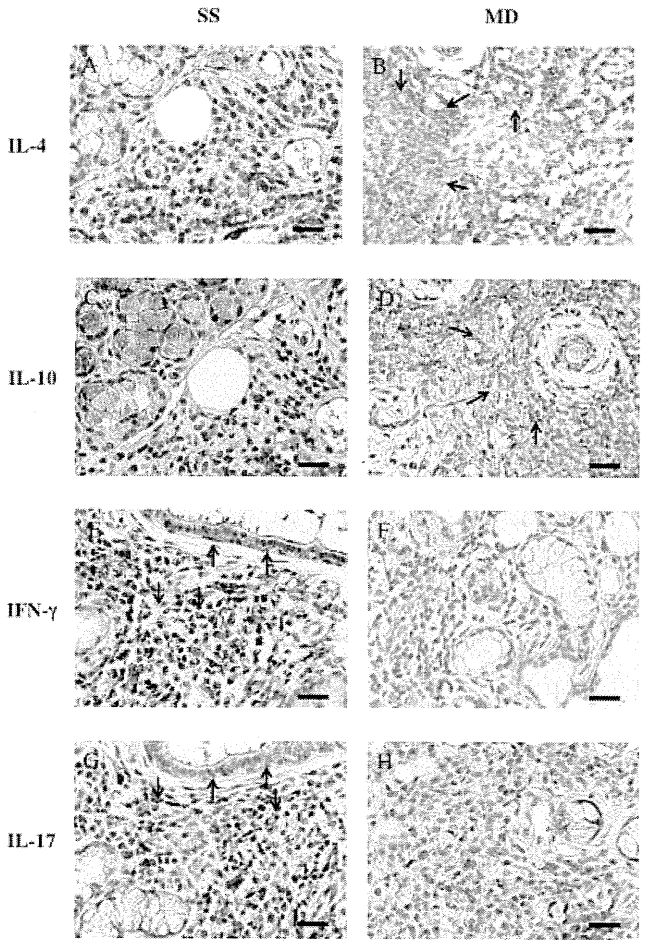

Figure 3. Expression of interleukin-10 (IL-10), IL-4, interferon-γ (IFNγ), and IL-17 in LSG specimens from patients with SS and patients with Mikulicz disease. IL-10 and IL-4 were prominently expressed around the germinal centers in LSG specimens from patients with Mikulicz disease. Sections from LSGs of a representative patient with SS and a representative patient with Mikulicz disease were immunostained with anti–IL-10 (**A** and **B**), anti–IL-4 (**C** and **D**), anti-IFNγ (**E** and **F**), and anti–IL-17 (**G** and **H**) polyclonal antibodies. Counterstaining with Mayer's hematoxylin was subsequently performed. **Arrows** indicate key features of infiltrating cells. Bars = 50 mm; original magnification × 400. See Figure 1 for other definitions.

RESULTS

Results of histologic analysis of lymphocyte subsets in the LSGs. Representative sections showing histologic findings and lymphocyte subsets in LSG specimens from patients with Mikulicz disease and patients with SS are shown in Figure 1. Specimens from patients with SS showed lymphocytic infiltration of various subsets with atrophy or severe destruction of the acini, while specimens from patients with Mikulicz disease showed selective infiltration of IgG4-positive plasma cells and FoxP3-positive Treg cells around the acinar and ductal cells with a lot of lymphoid follicles and mild destruction of the acini.

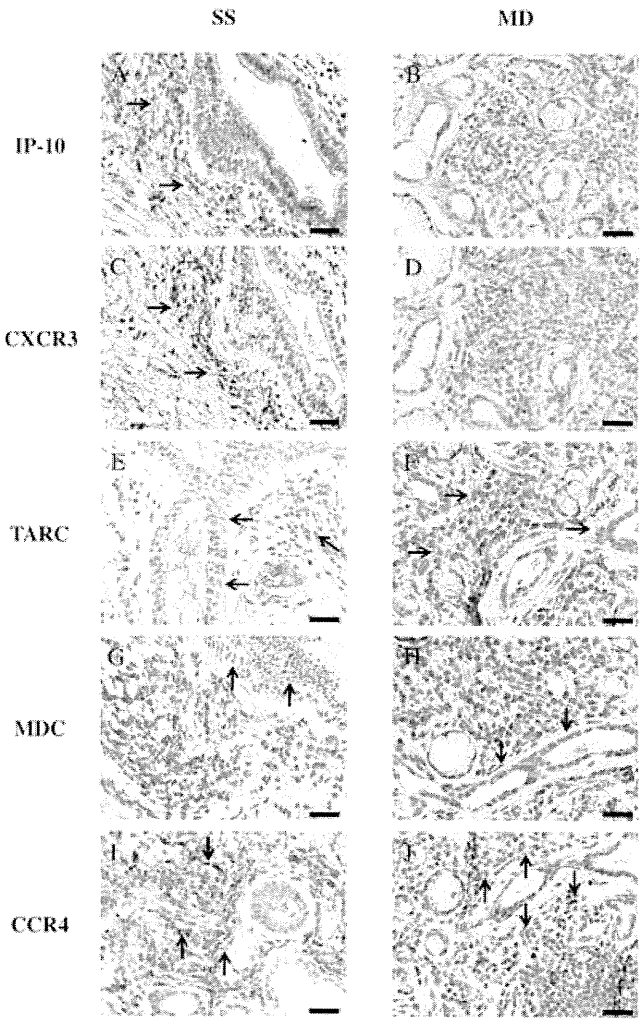

Figure 4. Expression of interferon-γ–inducible 10-kd protein (IP-10), CXCR3, thymus and activation–regulated chemokine (TARC), macrophage-derived chemokine (MDC), and CCR4 in LSG specimens from patients with SS and patients with Mikulicz disease. TARC and MDC were strongly expressed around the germinal centers in LSG specimens from patients with Mikulicz disease. Sections from LSGs of a representative patient with SS and a representative patient with Mikulicz disease were immunostained with anti–IP-10 (**A** and **B**), anti-CXCR3 (**C** and **D**), anti-TARC (**E** and **F**), anti-MDC (**G** and **H**), and anti-CCR4 (**I** and **J**) monoclonal antibodies. Counterstaining with Mayer's hematoxylin was subsequently performed. **Arrows** indicate key features of infiltrating cells. Bars = 50 mm; original magnification × 400. See Figure 1 for other definitions.

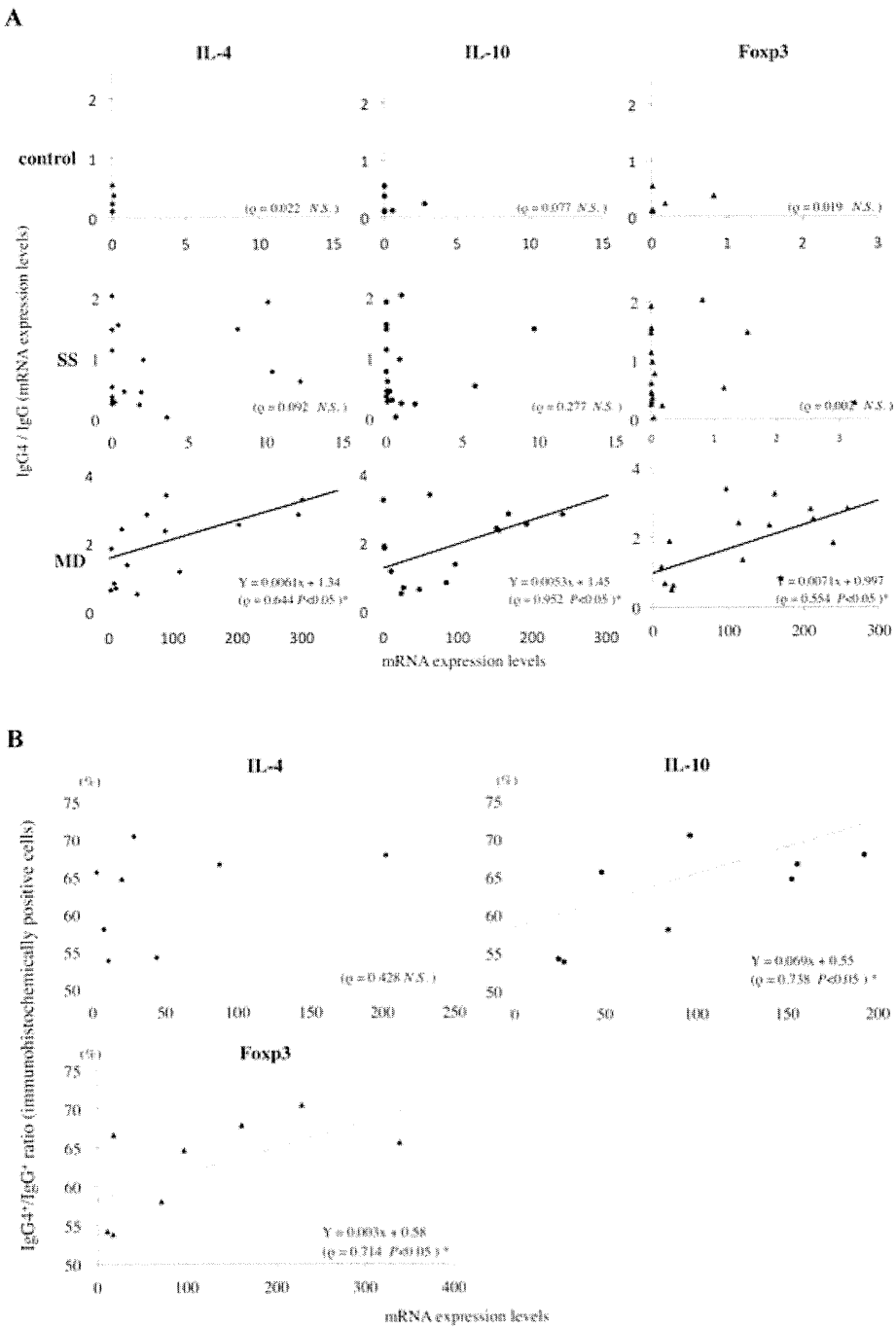

Figure 5. Correlation of the production of IgG4 with the production of interleukin-4 (IL-4), IL-10, and FoxP3 in LSGs from patients with Mikulicz disease. **A,** Correlations between the ratio of IgG4 mRNA to IgG mRNA and the expression of mRNA for IL-4, IL-10, and FoxP3 in LSGs from patients with SS, patients with Mikulicz disease, and healthy controls. Real-time polymerase chain reaction products for IL-4, IL-10, FoxP3, IgG, and IgG4 were quantitatively estimated as described in Patients and Methods. The ratio was calculated as IgG4-positive cells (%) = IgG4-positive cells/IgG-positive cells × 100. The counts were obtained in 1-mm^2 sections from 5 different areas. **B,** Correlations between the frequencies of IgG4-positive cells and the expression of mRNA for IL-4, IL-10, and FoxP3 in LSGs from patients with Mikulicz disease. * = $P < 0.05$ by Spearman's rank correlation. NS = not significant (see Figure 1 for other definitions).

Expression of mRNA for cytokines, chemokines, and chemokine receptors in the LSGs. In order to compare the expression of mRNA for cytokines, chemokines, and chemokine receptors in LSGs from patients with Mikulicz disease and LSGs from patients with SS, the relative expression compared to CD3δ was estimated and compared for cytokines and chemokine receptors primarily expressed by T cells, and the relative expression compared to β-actin was estimated for cytokines and chemokines produced by a variety of cell types.

The expression of mRNA for IFNγ, IL-12, IP-10, CXCR3, IL-4, TARC, MDC, CCR4, IL-10, IL-17, and IL-6 in LSGs from SS patients were higher than those in control LSGs (Figure 2). The expression of mRNA for IL-4, IL-5, TARC, MDC, CCR4, IL-10, TGFβ, and FoxP3 in LSGs from patients with Mikulicz disease were higher than those in control LSGs (Figure 2). In addition, the levels of expression of mRNA for IL-4, IL-5, IL-10, TGFβ, and FoxP3 in LSGs from patients with Mikulicz disease were higher than in LSGs from patients with SS (Figure 2).

Protein levels of cytokines, chemokines, and chemokine receptors in the LSGs. The specimens were immunohistochemically examined to evaluate the distributions of these proteins in LSGs from patients with SS and patients with Mikulicz disease. The Th1-type cytokine IFNγ and Th17-type cytokine IL-17 were strongly expressed and detected in and around the ductal epithelial cells in LSGs from SS patients only (Figures 3E and G). Although IL-10 and IL-4 were detected in LSGs from both patients with Mikulicz disease and patients with SS, they were prominently expressed around germinal centers in LSGs from Mikulicz disease patients but not in LSGs from SS patients (Figures 3B and D). In LSGs from patients with SS, IP-10 and CXCR3 were detected in a higher number of infiltrating lymphocytes than in LSGs from patients with Mikulicz disease (Figures 4A and C). In LSGs from patients with Mikulicz disease, TARC and MDC were strongly expressed, especially around germinal centers (Figures 4F and H). In LSGs from both patients with SS and patients with Mikulicz disease, CCR4 was detected in high numbers of infiltrating lymphocytes (Figures 4I and J).

Relationship between IgG4 production and cytokine expression in the LSGs. The relationships between IgG4 production and the expression of mRNA for IL-4, IL-10, and FoxP3 in the LSGs were examined. These molecules were all positively correlated with the ratio of IgG4 mRNA to IgG mRNA in LSGs from patients with Mikulicz disease, but no relationships were confirmed in those from SS patients (Figure 5A). Furthermore, IL-10 mRNA and FoxP3 mRNA in LSGs from patients with Mikulicz disease were correlated with the ratio of IgG4 to IgG in immunohistochemically positive cells (Figure 5B).

DISCUSSION

Mikulicz disease presents with bilateral and persistent swelling of the lacrimal and salivary glands, and it has been considered to be part of primary SS or a subtype of primary SS since the findings by Morgan and Castleman were published in 1953 (37). However, Yamamoto et al (38,39) reported differences in the clinical and histopathologic findings between Mikulicz disease and SS. Serologically, Mikulicz disease patients show hypergammaglobulinemia, hypocomplementemia, and high levels of serum IgG4, but are negative for anti-SSA and anti-SSB antibodies. Immunohistologic analysis of samples from patients with Mikulicz disease revealed the selective infiltration of IgG4-positive plasma cells, which was not observed near acinar and ductal cells. In contrast, similar specimens from SS patients showed no IgG4-positive plasma cells (38,39). In this study, samples from patients with Mikulicz disease showed selective infiltration of IgG4-positive plasma cells and FoxP3-positive cells around acinar and ductal cells with mild destruction of the acini, while samples from patients with SS showed no infiltration of IgG4-positive plasma cells and FoxP3-positive cells, and had atrophy or severe destruction of acini (Figure 1).

In order to examine the differences in infiltrating lymphocytes between LSGs from patients with SS and LSGs from patients with Mikulicz disease, we analyzed the levels of cytokines, chemokines, and chemokine receptors. The levels of Th1-, Th2-, and Th17-type molecules in LSGs from SS patients were significantly higher than those in LSGs from controls. The levels of Th2 and Treg-type molecules in LSGs from patients with Mikulicz disease were significantly higher than those in LSGs from controls. Furthermore, immunohistochemical staining indicated that IFNγ and IL-17 were strongly detected in and around ductal epithelial cells in LSGs from SS patients only, while IL-4 and IL-10 were detected in LSGs from both patients with SS and patients with Mikulicz disease. In particular, these cytokines were prominently expressed around germinal centers in specimens from patients with Mikulicz disease but not in specimens from patients with SS.

It is generally accepted that CD4+ Th cells play a crucial role in the pathogenesis of SS. Several studies of autoimmune diseases have demonstrated pathoge-

netic roles for Th1 cells and the possible protective role for Th2 cells (40,41). Our previous studies of SS suggested that the mutual stimulation of Th1 cells and their target organs via the production of various cytokines plays a key role in the induction and maintenance of SS and results in the eventual destruction of the target organ (22,42,43). Subsequently, additional Th2 cells then stimulate B cells to differentiate, proliferate, and produce immunoglobulins and, thus, play a role in the lymphoaggressiveness of SS. Regarding the possible roles of Th2 cells in the induction of B cell abnormalities, these cells might have an important association with the progression of SS. In contract, Zen et al (25) reported that autoimmune pancreatocholangitis, an IgG4-related disease, is characterized by immune reactions that are predominantly mediated by Th2 cells and Treg cells.

The results of the present study concerning the levels of cytokines, chemokines, and chemokine receptors in the LSGs are consistent with the model of SS and Mikulicz disease as distinct diseases. Immunohistochemical staining indicated that MDC and TARC were detectable in and around the ductal epithelial cells and germinal centers, while CCR4 was expressed on the infiltrating lymphocytes in the LSGs in both SS patients and patients with Mikulicz disease. The interactions of CCR4 with MDC and TARC are suggested to play a critical role in the accumulation of Th2 cells and, consequently, the progression of SS and Mikulicz disease. TARC and MDC are natural ligands for CCR4 on Th2 cells (44,45). In contrast, IP-10 was detected in and around the ductal epithelial cells, while CXCR3 was expressed on the infiltrating lymphocytes in the LSGs in SS patients only. IP-10 is a natural ligand for CXCR3 on Th1 cells (15).

It is well known that allergic immune responses are the development of allergen-specific Th2-type cytokines IL-4 and IL-13, which are responsible for IgG4 and IgE induced by B cells (46). In our previous studies, we demonstrated that Th2 immune reactions contributed to Mikulicz disease and IgG4-related tubulointerstitial nephritis (23,24,35). The expression profile of cytokines demonstrated in this study suggested that Mikulicz disease was characterized by an intense expression of Th2 and regulatory cytokines (Figure 2). In addition, recent studies have shown that class switching of IgG4 is caused by costimulation with IL-4 and IL-10, and that IL-10 decreased IL-4–induced IgE switching but elevated IL-4-induced IgG4 production (47).

Treg cells exert their effects through the modulation of both T and B cell responses, and two subsets of

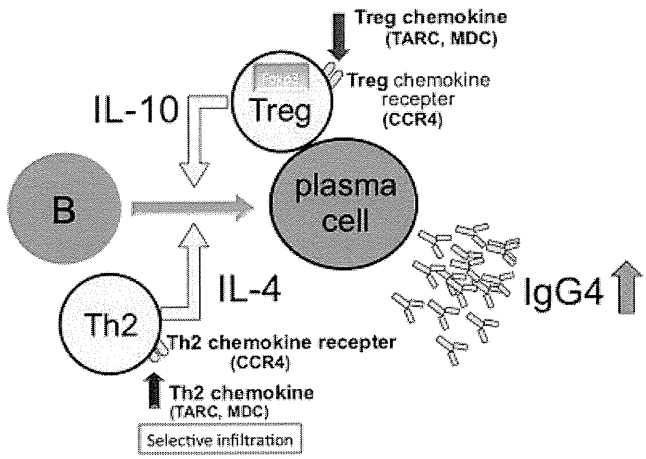

Figure 6. Schematic model of the mechanisms underlying IgG4 production. TARC = thymus and activation–regulated chemokine; MDC = macrophage-derived chemokine; IL-10 = interleukin-10.

Treg cells, CD4+CD25+FoxP3+ Treg cells (48) and IL-10–producing Tr1 cells (49), are crucial in regulating effector T cell function. CD4+CD25+FoxP3+ Treg cells are known to affect the pathogenesis of cases of autoimmune hepatitis and primary biliary cirrhosis (50). Miyoshi et al (51) showed a positive correlation between the number of mature Treg cells ($CD4+CD25^{high}$ Treg cells) and IgG4. These results indicated that increased numbers of $CD4+CD25^{high}$ Treg cells may influence IgG4 production in autoimmune pancreatocholangitis, whereas decreased numbers of naive Treg cells (CD4+CD25+CD45RA+) may be involved in the pathogenesis of the disease (51). Therefore, we examined the relationships between IgG4 and IL-4, IL-10, and FoxP3.

We found that IL-4, IL-10, and FoxP3 were positively correlated with the ratio of IgG4 mRNA to IgG mRNA in samples from patients with Mikulicz disease analyzed by real-time PCR and comparison with the IgG4 to IgG ratio of immunohistochemically positive cells. In particular, IL-10 and FoxP3 levels were strongly correlated with IgG4 production. These results suggested that Th2 and Treg cells might be involved in the pathogenesis of Mikulicz disease. The findings of the present study provided additional support for the model of Mikulicz disease as distinct from SS (Figure 6). However, accumulation of case reports and further examinations are required to elucidate the pathogenesis of the disease.

In this study, we clarified the pathogenesis of Mikulicz disease and found that it is a unique IgG4-related disease, characterized by Th2 and regulatory

immune reactions, which apparently differs from SS. A more thorough understanding of the complex mechanisms of the disease might lead to pharmacologic strategies to interrupt the interactions between chemokines and chemokine receptors or to disrupt the cytokine network as a further means of inhibiting the initiation and/or progression of Mikulicz disease.

AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Nakamura had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Tanaka, Moriyama, Nakashima, Miyake, Nakamura.

Acquisition of data. Tanaka, Moriyama, Hayashida, Maehara, Shinozaki, Kubo.

Analysis and interpretation of data. Tanaka, Moriyama, Nakashima.

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