図 1

「抗 SS-A 抗体陽性女性の妊娠症例の管理方針等に関するアンケート」

抗 SS-A 抗体陽性女性の妊娠症例の貴施設における管理方針等についてお伺い致します。

(1) 管理方針を定めている（いずれかに○）

【 はい いいえ 】

(2)個別の症例管理に際してどの科が関わっていますか（当てはまるものすべてに○）

（ 内科系 産科系 小児科系
循環器科系 皮膚科系 ）

(3)NLE（房室ブロック含む）の発症リスクについて、現在既に十分な情報がある

【 はい いいえ 】

～ご協力どうもありがとうございました。～

厚生労働科学研究費補助金（成育疾患克服等次世代育成基盤研究事業）
分担研究報告書

抗SS-A抗体陽性妊娠症例におけるステロイド剤が妊娠経過ならびに児に及ぼす影響

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研究要旨

抗SS-A抗体陽性女性から出生した児に心ブロック(CHB)が発症するのは稀であるが、重篤な病態である。心ブロックを予防する方法は確立されていないが、母体へのステロイド投与の効果を示唆する報告がある。一方で、胎児がステロイドに暴露することにより子宮内発達遅延などの弊害をもたらず可能性があり、慎重論もある。抗SS-A抗体陽性女性からCHB児出産のリスクは1％前後であるがCHB児出産の既往があるとそれが20%になることがわかっており、現在同定されている唯一のリスク因子である。従って、CHBの発症への薬物治療の有効性を評価するためには、CHB児出産の既往の有無で分けて評価しなければならない。本研究では、研究班員所属施設自験例194例を解析し、ステロイド剤の使用と児のCHB発症と、妊娠結果への影響について、CHB児出産の既往のあるなし、ステロイド剤投与あるなしで4群に分けて解析した。その結果、CHB児出産の既往に関係なく、プレドニゾロン10mg相当量以上のステロイド剤を投与されていた症例でのCHB発症はなかった。この結果を確かなものにするためには全国から集積した644例を対象に再評価が必要である。

A.研究目的

抗SS-A抗体陽性女性から出生した児に心ブロックが発症するのは1%前後といわれ稀であるが、心ブロック児を出産した症例ではその確率は約20%になるといわれている。心ブロックを予防する方法は確立されていないが、母体へのステロイド投与の効果を示唆する報告がある。一方で、胎児がステロイドに暴露することにより子宮内発達遅延などの弊害をもたらず可能性があり、慎重論もある。

本研究では研究班員所属施設自験例194例を解析し、ステロイド剤の使用と児のCHB発症について解析した。

B.研究方法

研究班員所属施設において1999年～2009年3月までに妊娠が終了した抗SS-A抗体陽性妊娠症例194例を対象とした。

後ろ向きにカルテから診療情報を収集し、当研究班作成の調査票に記入した。これらをデータクリーニングした後、解析した。

C.研究結果

CHB児出産の既往がある10例のうち、CHB予防目的でステロイド剤を投与した6例(ベタメサゾン1～2mg)では児にCHBの発症はなく、投与しなかった4例のうち1例で児にCHBの発症を認めた。一方、CHB児出産の既往がなくステロイド剤の投与（目的は問わない）を受けていなかった79例のうち、10例で児にCHBを発症した。CHB児出産の既往がなくステロイド剤の投与（目的は問わない）を受けていた105例のうち、4例で児にCHBを発症した。この4例が服用していたステロイド剤はプレドニゾロンでそれぞれ2.5mg, 3mg, 5mg, 7.5mgであった。（表1）

表1.
CHB児出産歴の有無、妊娠中のステロイド服用の有無とCHB発症の関係

	CHB(−)	CHB(+)
CHB児出産歴の既往あり，steroidあり	6	0
CHB児出産歴の既往あり，steroidなし	3	1
CHB児出産歴の既往なし，steroidなし	69	10
CHB児出産歴の既往なし，steroidあり	101	4

D.考察

抗 SS-A 抗体は生殖年齢女性の約１％にみられるといわれているが、無症候の場合が多く、保有していることを知らずに妊娠を終了している症例が多く、その実態は不明である。一方、抗 SS-A 抗体を保有しているとわかっていて妊娠初期から追跡できる症例は少ないため、その自然歴の把握も難しい。

抗 SS-A 抗体はシェーグレン症候群や全身性エリテマトーデスなど膠原病患者にみられる抗体であり、すでにステロイド剤が投与されていることが多い。また、前回 CHB 児出産の既往のある女性において CHB 発症のリスクは 20%と高いため、ベタメサゾンを含むステロイド剤がプレドニンゾロン換算で 10～20mg 投与されることがある。従って、本研究班ではステロイド剤が CHB 発症を抑えるかどうかを検証するために、CHB 児出産の既往の有無、ステロイド剤投与の有無で４群に分けて比較した。CHB 児出産既往のあるハイリスク群のうちステロイド剤（ベタメサゾン　1～2mg）を投与されていた 6 例すべてで、CHB の発症がなかった。一方、CHB 児出産既往のあるハイリスク群で、ステロイド投与されていなかった 4 例のうち、1 例で CHB を発症した（この症例は CHB 予防目的に血漿交換療法を受けていた）。症例数が少なすぎて統計処理はできないものの、CHB 児出産既往のあるハイリスク群であってもステロイド剤（該当症例はすべてベタメサゾン）で CHB を予防できる可能性を示唆する結果であった。ただし、ベタメサゾンは胎盤移行率が高いことから母体への投与には慎重論が多い。では胎盤移行性の非常に低いプレドニンゾロン（PSL）で CHB が予防できないだろうか。これについては、以前海外のケースシリーズで中等量の PSL が投与されていた妊婦で CHB を発症したことより CHB の発症を予防できないと言い伝えられてきた。今回の研究対象で、CHB 児出産既往のないのは 184 例で、そのうちステロイド剤を服用していたのは 105 例で、そのほとんどは PSL の内服であった。この 105 例のうち CHB 発症は 4 例あったが、いずれも PSL7.5mg 以下と少量であった。一方、CHB 児出産既往がなく、ステロイド剤を服用していない 79 例のうち 10 例に心ブロックが発症していた。これらから、PSL であっても 10mg 以上の内服であれば CHB の予防ができる可能性も示唆される。しかし、今回の研究対象は自験例で、CHB を発症してから研究者所属施設に紹介されてきた症例を含んでいてバイアスがかかっている。PSL での予防効果を検証するためには、CHB 発症してから紹介された症例を除いての解析、さらには前向きコホート研究が必要であろう。

E.結論

本研究では抗 SS-A 抗体陽性女性の妊娠例において、母体に 10mg 以上のプレドニゾロンが投与されていれば CHB を予防できる可能性があることを示すことができた。今後はさらに多くの症例で、精度を高めた解析が必要である。

F.健康危険情報

特記すべき事項なし

G.研究発表

1.論文発表

なし

2. 学会発表

なし

H. 知的財産権の出願・登録状況（予定を含む）

1．特許取得

なし

2．実用新案登録

なし

3．その他

なし

III.　研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Shimane K, Kochi Y, Horita T, Ikari K, Amano H, Hirakata M, Okamoto A, Yamada R, Myouzen K, Suzuki A, Kubo M, Atsumi T, Koike T, Takasaki Y, Momohara S, Yamanaka H, Nakamura Y, Yamamoto K	The association of a nonsynonymous single-nucleotide polymorphism in TNFAIP3 with systemic lupus erythematosus and rheumatoid arthritis in the Japanese population.	Arthritis Rheum	62	574-579	2010
Ito I, Kawasaki A, Ito S, Kondo Y, Sugihara M, Horikoshi M, Hayashi T, Goto D, Matsumoto I, Tsutsumi A, Takasaki Y, Hashimoto H, Matsuta K, Sumida T, Tsuchiya N	Replication of association between FAM167A(C8orf13)-BLK region and rheumatoid arthritis in a Japanese population.	Ann Rheum Dis	69	936-937	2010
Nakano S, Morimoto S, Suzuki S, Watanabe T, Amano H, Takasaki Y	Up-regulation of the endoplasmic reticulum transmembrane protein UNC93B in the B cells of patients with active systemic lupus erythematosus.	Rheumatology	49	876-881	2010
Ando S, Amano H, Amano E, Minowa K, Watanabe T, Nakano S, Nakiri Y, Morimoto S, Tokano Y, Lin Q, Hou R, Ohtsuji M, Tsurui H, Hirose S, Takasaki Y	FTY720 exerts a survival advantage through the prevention of end-stage glomerular inflammation in lupus-prone BXSB mice.	Biochem Biophys Res Commun	94	804-810	2010
Toyama S, Tamura N, Haruta K, Karakida T, Mori S, Watanabe T, Yamori T, Takasaki Y	Inhibitory effects of ZSTK474, a novel phosphoinositide 3-kinase inhibitor, on osteoclasts and collagen-induced arthritis in mice.	Arthritis Res Ther	12	R92 1-11	2010
Kawasaki, A., Ito, S., Furukawa, H., Hayashi, T., Goto, D., Matumoto, I., Ohashi, J., Graham, R.R., Matsuta, K., Behrens, T.W., Tohma, S., Takasaki, Y., Hashimoto, H., Sumida, T., and Tsuchiya, N.	Association of TNFAIP3 interacting protein 1, TNIP1 with systemic lupus erythematosus in a Japanese population: a case-control association study.	Arhritis Reas.Ther.	12	R174	2010
Iizuka, M., Wakamatsu, E., Matsumoto, I., Tsuboi, H., Nakamura, Y., Hayashi, T., Goto, D., Ito, S., and Sumida, T.	Pathogenic role of immune response to M3 muscarinic acethylcholine receptor reactive immune response induces Sjogren's sndrome-like sialoadenitis.	J. Autoimmunity	35	383-389	2010

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Shen, N., Fu, Q., Deng, Y., Qian, X., Zhao, J., Kaufman, K.M., Tang, Y., Chen, J-Y, Yang, W., Wong, M., Kawasaki, A., Tsuchiya, N., Sumida, T., Kawaguchi, Y., Yum C-Y, Takasaki, Y., Hashimoto, H., Harley, J.B., Guthridge, J.M., Grossman, J.M., Cantor, R.M., Song, Y.W., Bae, S, Cehn, S, Hahn, B.H., Lau, Y.L., and Tsao, B.P.	Sex-specific association of X-linked Toll-like receptor 7(TLR7)with male systemic lupus erythematosus	Proc. Natl. Acad. Sci.	107	15838-15843	2010
Tsuboi, H., Matumoto, I., Wakamatsu, E., Iizuka, M., Nakamura, Y., Hayashi, T., Goto, D., Ito, S., and Sumida, T.	New epitopes and function of anti-M3 muscarinic acetylcholine receptor antibodies in patients with Sjogren's syndrome.	Clin. Exp. Immunol.	162	53-61	2010
Sumida, T., Tsuboi, H., Iizuka, M., Nakamura, Y., and Matsumoto, I.	Functionla role of M3 muscarinic acetylcholine receptor (m3R) reactive T cells and anti-M3R autoantibodies in patients with Sjogren's syndrome.	Autoimmunity Reviews	9	615-617	2010
Tashiro, T., Nakagawa, R., Inoue, S., Omori-Miyake, M., Chiba, T., FUjii, S-I, Shimizu, K., Mori, K., Yoshiga, Y., Sumida, T., Watarai, H., and Taniguchi, M.	Induction of Th1-biased cytokine production by a-carba-GalCer, a neoglycolipid ligand for natural killer T cells.	Int. Immunol.	22	319-328	2010
Segawa, S., Goto, D., Yoshiga, Y., Sugihara, M., Hayashi, T., Chino, Y., Matsumoto, I., Ito, S., Ito, S., and Sumida ,T.	Inhibition of TGF-b signaling attenuates IL-18 plus IL-2-induced interstitial lung disease.	Clin. Exp. Immunol.	160	394-402	2010
Chen Q, Muramoto K, Masaaki N, Ding Y, Yang H, Mackey M, Li W, Inoue Y, Ackermann K, Shirota H, Matsumoto I, Spyvee M, Schiller S, Sumida T, Gusovsky F, Lamphier M.	A novel antagonist of the prostaglandin E(2) EP(4) receptor inhibits Th1 differentiation and Th17 expansion and is orally active in arthritis models.	Br. J. Pharmacol.	160	292-310	2010

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Hosokawa S, Takahashi N, Kitajima H, Nakayama M, Kosaki K, Okamoto N.	Brachmann-de Lange syndrome with congenital diaphragmation hernia and NIPBL gege mutation	Congenital Anomalies	50	129-132	2010
Namba F, Hasegawa T, Nakayama M, Hamanaka T, Yamashita T, Nakahira K, Kimoto A, Nozaki M, Nishihara M, Mimura K, Yamada M, Kitajima H, Suehara N, Yanagihara I.	Placental Features of Chorioamnionitis Colonized With Ureaplasma Species in Preterm Delivery	Pediatric Research	67	166-172	2010
Ito Y, Goldschmeding R, Kasuga H, Claessen N, Nakayama M, Yuzawa Y, Sawai A, Matsuo S, Weening J, Aten J.	Expression patterns of connective tissue growth factor and of TGF-β isoforms during glomerular injury recapitulate glomerulogenesis	Am J Physiol Renal Physiol	299	545-558	2010
Katayama Y, Horigome H, Takahashi H, Tanaka K, Yoshinaga M.	Determinants of Blood Rheology in Healthy Adults and Children Using the Microchannel Array Flow Analyzer.	Clinical and Applied Thrombosis/ Hemostasis.	16	414-421	2010
Yoshinaga M, Ichiki T, Tanaka Y, Hazeki D, Horigome H, Takahashi H, Kashima K	Prevalence of childhood obesity from 1978 to 2007 in Japan	Pediatr Int.	52	213-217	2010
Horigome H, Nagashima M, Sumitomo N, Yoshinaga M, Ushinohama H, Iwamoto M, Shiono J, Ichihashi K, Hasegawa S, Yoshikawa T, Matsunaga T, Goto H, Waki K, Arima M, Takasugi H, Tanaka Y, Miura M, Ogawa K, Suzuki H, Yamagishi H, Ikoma M, Suda K, Takagi J, Sato J,	Clinical characteristics and genetic background of congenital long QT syndrome diagnosed in fetal, neonatal and infantile life. A nation-wide questionnaire survey in Japan.	Circ Arrhythm Electrophysiol	3	10-17	2010

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Ban Y, Noma M, Horigome H, Kato H, Tokunaga C, Sakakibara Y,	Kawashima procedure after staged unifocalizations in asplenia with major	Annals of Thoracic Surgery	89	971-973	2010
Kato Y, Horigome H, Takahashi-Igari M, Yoshida K, Aonuma K.	Isolation of pulmonary vein and superior vena cava for paroxysmal atrial fibrillation in a young adult with left ventricular	Europace.	12	1040-1041	2010
鎌倉洋樹、山岸良匡 村島温子	抗SS-A抗体陽性女性の妊娠症例の把握	日本医事新報	4491	62−64	2010
中山雅弘	乳幼児突然死症例・診断の手引き	周産期医学	40	505-509	2010
前野泰樹	胎児不整脈の診断と胎内治療	久留米医学会雑誌	73	7-13	2010
漢　伸彦、前野泰樹	胎児心臓病スクリーニングのコツと落とし穴	産婦人科治療	98	456-461	2010
吉兼由佳子、前野泰樹	先天性心疾患の胎児スクリーニング	産婦人科治療	101	520-525	2010
前野泰樹	最新の胎児心エコー法情報	心エコー	11	1228-1239	2010

IV.　研究成果の刊行物・別刷

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The Association of a Nonsynonymous Single-Nucleotide Polymorphism in *TNFAIP3* With Systemic Lupus Erythematosus and Rheumatoid Arthritis in the Japanese Population

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Objective. **Genome-wide association (GWA) studies in systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) in Caucasian populations have independently identified risk variants in and near the tumor necrosis factor α (TNFα)–induced protein 3 gene (*TNFAIP3*), which is crucial for the regulation of TNF-mediated signaling and Toll-like receptor signaling. The aim of this study was to assess the role of *TNFAIP3* in the development of SLE and RA in Japanese subjects.**

Methods. **We selected 2 single-nucleotide polymorphisms (SNPs) from previous GWA studies. Rs2230926 is a nonsynonymous SNP in *TNFAIP3* and is associated with SLE, while rs10499194 is an intergenic SNP associated with RA. We then performed 2 independent sets of SLE case–control comparisons (717 patients and 1,362 control subjects) and 3 sets of RA case–control comparisons (3,446 patients and 2,344 control subjects) using Japanese subjects. We genotyped SNPs using TaqMan assays.**

Results. **We observed a significant association between rs2230926 and an increased risk of SLE and RA in the Japanese population (for SLE, odds ratio [OR] 1.92, 95% confidence interval [95% CI] 1.53–2.41, $P = 1.9 \times 10^{-8}$; for RA, OR 1.35, 95% CI 1.18–1.56, $P = 2.6 \times 10^{-5}$). The intergenic SNP rs10499194 was also associated with SLE and RA, while the risk allele for RA in Caucasians was protective against the diseases in our population.**

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Dr. Ikari has received speaking fees from Abbott Japan and Mitsubishi Tanabe Pharma (less than $10,000 each). Dr. Momohara has received speaking fees from Astellas Pharma, Chugai Pharmaceutical, Dainippon Sumitomo Pharma, Kaken Pharmaceutical, Mitsubishi Tanabe Pharma, Sanofi-Aventis, Santen Pharmaceutical, Takeda Pharmaceutical, and Wyeth (less than $10,000 each). Dr. Yamanaka has received speaking fees from Abbott Japan, Chugai Pharmaceutical, Eisai, Mitsubishi Tanabe Pharma, Hoffman-LaRoche, Takeda Pharmaceutical, and Wyeth (less than $10,000 each). Dr. Yamamoto has received consulting fees, speaking fees, or honoraria from Astellas Pharma and Chugai Pharmaceutical (less than $10,000 each) and owns stock or stock options in ImmunoFuture.

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***Conclusion.* We demonstrated a significant association between the nonsynonymous variant in *TNFAIP3* and the risk for SLE and RA in the Japanese population. *TNFAIP3*, similar to *STAT4* and *IRF5*, may be a common genetic risk factor for SLE and RA that is shared between the Caucasian and Japanese populations.**

Systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) represent multigenic diseases and are considered to be caused by interactions between susceptibility genes and environmental factors that result in an abnormal immune response. In fact, familial and linkage studies have provided strong evidence for the role of multiple genetic factors in the development of SLE and RA (1). In addition, association-based approaches in candidate loci using single-nucleotide polymorphisms (SNPs) have also identified several genes that contribute to these diseases. More recently, genome-wide association (GWA) studies in SLE and RA have revealed many susceptibility genes and pathways that contribute to disease development (2).

Familial and linkage studies have also shown familial aggregation of RA, SLE, and other immune-mediated diseases (1). In fact, several gene polymorphisms, including *PTPN22*, *STAT4*, and *IRF5* variants, have been shown to predispose to SLE and RA. Recent GWA studies in Caucasian populations have also identified the tumor necrosis factor α (TNFα)–induced protein 3 gene (*TNFAIP3*) as another common genetic risk factor for SLE and RA (3–6). *TNFAIP3*, also known as the A20 protein, is a negative regulator of the NF-κB signaling pathway that is essential in the pathogenesis of both SLE and RA (7). The association of *TNFAIP3* with diseases has been independently reported in SLE and RA, and it is of great interest that the peaks in association in the GWA studies are different between SLE and RA. In Caucasian populations, the significantly associated SNP markers for SLE, including the nonsynonymous SNP termed rs2230926, are located in the *TNFAIP3* region, while those for RA are located in the intergenic region between *TNFAIP3* and the oligodendrocyte transcription factor 3 gene (*OLIG3*). In addition to the difference in the diseases themselves, the association between *TNFAIP3* polymorphisms and these diseases in the Asian populations remains unclear (8).

In order to elucidate a genetic role for *TNFAIP3* in the development of SLE and RA in the Japanese population, we investigated 2 independent case–control cohorts of patients with SLE and 3 independent cohorts of patients with RA.

PATIENTS AND METHODS

Subjects. The subjects in the SLE study group comprised 2 cohorts of Japanese patients with SLE and unrelated control subjects. An SLE case–control cohort from the RIKEN (SLE cohort 1) consisted of 376 patients (mean age 43.2 years, 90.3% women) and 934 unrelated control subjects (mean age 52.6 years, 25.0% women). An SLE case–control cohort at Hokkaido University (SLE cohort 2) consisted of 341 patients (mean age 46.2 years, 88.3% women) and 428 unrelated control subjects (mean age 47.7 years, 28.7% female). All patients with SLE fulfilled the 1997 American College of Rheumatology (ACR) revised criteria for SLE (9).

The subjects in the RA component of the study comprised 3 cohorts of Japanese patients with RA and unrelated control subjects. The first cohort of patients with RA from BioBank Japan (RA cohort 1) consisted of 1,112 patients (mean age 60.5 years, 89.7% female, 69.7% positive for rheumatoid factor [RF]), and 934 unrelated control subjects. The second cohort from RIKEN (RA cohort 2) consisted of 830 patients (mean age 64.3 years, 83.7% women, 75.0% RF positive), and 658 unrelated control subjects (mean age 48.6 years, 57.4% women). The 934 unrelated control subjects in the first cohort of RA patients were the same as those used in SLE cohort 1. An RA case–control cohort from the Institute of Rheumatology Rheumatoid Arthritis (IORRA) cohort (RA cohort 3), which is a prospective observational cohort of patients with RA studied at Tokyo Women's Medical University, comprised 1,504 patients (mean age 59.3 years, 84% women, 88% RF positive), and 752 control subjects (mean age 38.4 years, 50% women). All patients with RA met the 1987 ACR (formerly, the American Rheumatism Association) revised criteria for a diagnosis of RA (10).

All subjects entered into this study were self-identified as Japanese and were recruited through several medical institutions located in Japan. DNA samples from the patients in the first cohort of RA patients in BioBank Japan were provided by the Leading Project for Personalized Medicine from the Ministry of Education, Culture, Sports, Science and Technology, Japan (11). All subjects provided informed consent prior to their participation in this study, and the study was preapproved by the ethics committee of each institution.

SNPs. For the selection of SNPs required to genotype in and near *TNFAIP3*, we reviewed previous GWA studies of SLE and RA (3–6). We then selected 2 SNPs, rs2230926 and rs10499194. SNP rs2230926 is a nonsynonymous variant in exon 3 of *TNFAIP3* and was strongly associated with SLE in the GWA study by Musone et al (5). Although the GWA study of SLE by Graham et al indicated that rs5029939, located in intron 2 of the gene, is most significantly associated with a predisposition to SLE (6), there is strong linkage disequilibrium (LD) (r^2 = 0.86) between these SNPs according to HapMap phase II data for Japanese and evidence that rs5029939 may be substituted by rs2230926 (Figure 1). Two previous GWA studies in RA revealed that rs10499194 and rs6920220, which are located between *TNFAIP3* and *OLIG3*, were significantly associated risk variants for RA (3,4). The HapMap data for Japanese individuals indicate that the minor allele frequency (MAF) of rs6920220 is 0.011, and that the MAF for control subjects in RA cohort 3 (IORRA) was <0.01. Results of a recent study in Korean populations also indicated

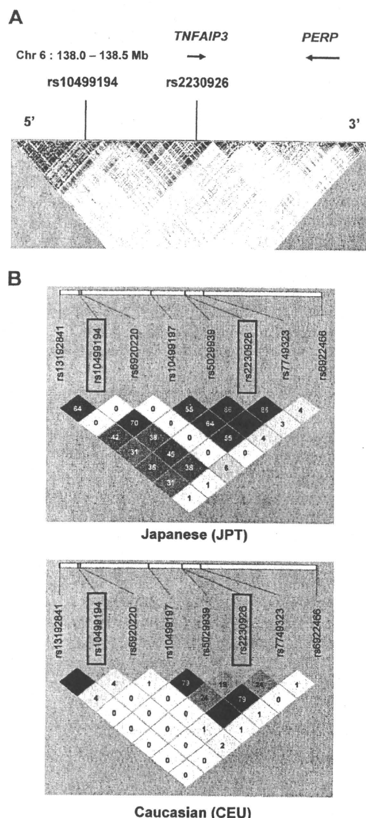

Figure 1. Pairwise linkage disequilibrium (LD) patterns for polymorphisms in the *TNFAIP3* region, according to HapMap phase II data. **A,** Pairwise LD pattern in the expanded *TNFAIP3* region derived from the HapMap data for Japanese patients, with r^2 values. *OLIG3* is located ~370 kbp away from *TNFAIP3* in the 5′ region and is not shown. **B,** Pairwise LD patterns for single-nucleotide polymorphisms (SNPs) in the *TNFAIP3* region that were significantly associated with systemic lupus erythematosus and rheumatoid arthritis in previous genome-wide association studies. The upper and lower panels were constructed using HapMap data for Japanese and Caucasian patients, respectively. The diagram shows pairwise LD values as quantified using the r^2 value. A stronger LD is depicted graphically by the densely shaded boxes. The boxed areas show the 2 SNPs genotyped.

that the variant was too rare (MAF <0.01) to be evaluated for associations (8).

Based on HapMap data for Japanese individuals, pairwise LD patterns for the SNPs in and near *TNFAIP3*, which were significantly associated with SLE and RA in the previous GWA studies, are presented in Figure 1 (for SLE, rs13192841, rs10499197, rs5029939, rs2230926, rs7749323, and rs6922466; for RA, rs10499194 and rs6920220).

Genotyping. We genotyped SNPs using TaqMan assays. For the selected SNPs, predesigned TaqMan SNP genotyping assays were used (probe ID: rs2230926, C.770116_10; rs10499194, C.1575581_10; Applied Biosystems, Foster City, CA). Fluorescence was detected using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). Genotyping assessment was performed on >98% of the samples, for all of the polymorphisms genotyped. All of the SNPs were in Hardy-Weinberg equilibrium in control subjects, according to chi-square statistics ($P > 0.01$).

Case–control association tests. We first performed allele frequency comparisons of rs2230926 and rs10499194 in SLE cohort 1 and RA cohort 1. Then, further case–control association studies were conducted using SLE cohort 2 and RA cohorts 2 and 3, to validate the associations in the first cohorts. In the replication studies, we genotyped the SNPs with a *P* value less than 0.05 in either SLE cohort 1 or RA cohort 1 (the *P* value was determined after correction for conditional logistic analysis, as described below).

Measurement of autoantibodies. Sera from 1,104 patients in RA cohort 1 were available for the measurement of anti–cyclic citrullinated peptide (anti-CCP) antibodies and RF. Anti-CCP antibodies were measured using the Mesacup CCP test (Medical and Biological Laboratories, Woburn, MA), and RF was measured by enzyme-linked immunosorbent assay.

Statistical analysis. The case–control association of each SNP was tested with the Cochran-Armitage trend test. The genotype and allele frequencies for patients and control subjects were used to calculate the odds ratios (ORs) and 95% confidence interval (95% CIs) using Woolf's method. For the combined analysis, we used the Mantel-Haenszel test. We performed conditional logistic regression analysis to evaluate the effect of each polymorphism conditional on the remaining polymorphisms, using Statistica software (StatSoft, Tulsa, OK). We calculated pairwise LD indices between pairs of SNPs (the r^2 value), using HaploView software, version 4.0 (http://www.broad.mit.edu/haploview/ haploview). We calculated the population attributable risk (PAR) using the following formula: $PAR = f(OR - 1)/(1 + f[OR - 1])$, where f is the allele frequency in the control subjects. PAR is defined as the reduction in incidence that would be achieved if the population had been entirely unexposed. We calculated the statistical power of association using the R software program (http://www.r-project.org).

RESULTS

Our results revealed a significant association between rs2230926 and both SLE and RA when comparing allele frequency in the patients and control subjects in the first cohort (for SLE, OR 1.92, 95% CI

Table 1. Association study of rs2230926 and rs10499194 with SLE in Japanese subjects*

dbSNP number, major/minor allele	No. of patients	No. of controls	Minor allele frequency		OR (95% CI)	*P*
			Patients	Controls		
rs2230926, G/T						
SLE 1	376	934	0.113	0.062	1.92 (1.43–2.58)	1.2×10^{-5}
SLE 2	341	428	0.116	0.064	1.91 (1.33–2.73)	3.0×10^{-4}
Combined analysis†	717	1,362	0.114	0.063	1.92 (1.53–2.41)	1.9×10^{-8}
rs10499194, T/C						
SLE 1	376	933	0.084	0.061	1.42 (1.03–1.95)	0.030

* SLE = systemic lupus erythematosus; dbSNP = Database of Single-Nucleotide Polymorphisms; OR = odds ratio; 95% CI = 95% confidence interval.
† By the Mantel-Haenszel method.

1.43–2.58, $P = 1.2 \times 10^{-5}$; for RA, OR 1.52, 95% CI 1.20–1.92, $P = 5.6 \times 10^{-4}$) (Tables 1 and 2). We also observed an association between rs10499194 and SLE patients in cohort 1 (OR 1.42, 95% CI 1.03–1.95, $P =$ 0.030) (Table 1). However, the T allele appeared to represent a susceptibility allele in the SLE and RA patients in cohort 1, whereas the C allele appeared to be a risk allele for RA in Caucasians (3). We speculated that this association could be secondary to the moderate LD between rs2230926 and rs10499194 ($r^2 = 0.14$) according to data on control subjects in SLE cohort 1, and we subsequently performed a conditional logistic regression analysis to evaluate the effects of each polymorphism conditional on the remaining polymorphisms. The results of this analysis indicated that rs10499194 did not retain the statistically significant association when conditionally evaluated on rs2230926 ($P = 0.73$), while rs2230926 retained the significant association when conditionally evaluated on rs10499194 ($P = 3.4 \times 10^{-4}$). We concluded that rs2230926 was primarily associated with SLE located at this locus, and therefore genotyped only rs2230926 for replication studies in SLE (3–6).

The results of a case–control association study in SLE cohort 2 confirmed the significant association between rs2230926 and the risk of SLE (OR 1.91, 95% CI 1.33–2.73, $P = 3.0 \times 10^{-4}$). A combined analysis also confirmed a significant association (OR 1.92, 95% CI 1.53–2.41, $P = 1.9 \times 10^{-8}$, PAR = 0.055). In RA cohort 2 a statistically significant association between rs2230926 and a predisposition for RA was also replicated; however, this was not replicated in RA cohort 3 (for cohort 2, OR 1.39, 95% CI 1.07–1.81, $P = 0.013$; for cohort 3, OR 1.19, 95% CI 0.94–1.50, $P = 0.15$) (Table 2). In RA cohort 3, the statistical power required to detect an association at rs2230926 was 0.54 at a significance level of $\alpha = 0.05$ when we presumed that the OR for RA was 1.4 (the combined OR for RA cohorts 1 and 2 was 1.46). It was possible that the statistical power for RA cohort 3 may have been insufficient. A combined analysis on these data suggested a significant association (OR 1.35, 95% CI 1.18–1.56, $P = 2.6 \times 10^{-5}$, PAR = 0.024).

We observed no significant association of rs10499194 in RA cohort 1, but the statistical power to detect the association in this study was insufficient (1 –

Table 2. Association study of rs2230926 and rs10499194 with RA in Japanese subjects*

dbSNP number, minor/major allele	No. of Patients	No. of controls	Minor allele frequency		OR (95% CI)	*P*
			Patients	Controls		
rs2230926, G/T						
RA cohort 1	1,112	934	0.091	0.062	1.52 (1.20–1.92)	5.6×10^{-4}
RA cohort 2	825	655	0.100	0.074	1.39 (1.07–1.81)	0.013
RA cohort 3	1,478	747	0.087	0.075	1.19 (0.94–1.50)	0.15
Combined analysis†	3,415	2,326	0.092	0.069	1.35 (1.18–1.56)	2.6×10^{-5}
rs10499194, T/C						0.26
RA cohort 1	1,112	933	0.069	0.061	1.15 (0.90–1.48)	0.26
RA cohort 2	827	650	0.072	0.048	1.52 (1.11–2.08)	0.0090
RA cohort 3	1,472	716	0.073	0.059	1.32 (1.02–1.73)	0.038
Combined analysis†	3,411	2,299	0.071	0.056	1.30 (1.11–1.53)	8.4×10^{-4}

* RA = rheumatoid arthritis; dsSNP = Database of Single-Nucleotide Polymorphisms; OR = odds ratio; 95% CI = 95% confidence interval.
† By the Mantel-Haenszel method.

β = 0.31) considering the previously reported OR of 0.75 and a significance level of α = 0.05 (3). Therefore, we genotyped rs10499194 in RA cohorts 2 and 3 for confirmation. Unlike in RA cohort 1, a significant association of rs10499194 was observed in RA cohorts 2 and 3 (for cohort 2, OR 1.52, 95% CI 1.11–2.08, P = 0.0090; for cohort 3, OR 1.32, 95% CI 1.02–1.73, P = 0.038) (Table 2). However, the risk allele for Caucasian patients with RA was protective against RA in our population, just as was observed in SLE cohort 1. The combined analysis showed a significant association of rs10499194 with RA (OR 1.30, 95% CI 1.11–1.53, $P = 8.4 \times 10^{-4}$).

We stratified patients in RA cohorts 1 and 3 according to the presence of anti-CCP antibodies and RF and examined for the association between *TNFAIP3* polymorphisms (rs2230926 and rs10499194) and RA susceptibility (see Supplementary Table 1, available in the online version of this article at http://www3.interscience.wiley.com/journal/76509746/home). When the patients were stratified according to anti-CCP antibody status, the G allele of rs2230926 was found to confer increased risk for RA in anti-CCP antibody–positive patients relative to anti-CCP antibody–negative patients (for anti-CCP antibody–positive patients, OR 1.36, 95% CI 1.15–1.62, $P = 4.0 \times 10^{-4}$; for anti-CCP–negative patients, OR 1.16, 95% CI 0.83–1.61, P = 0.39 in the combined analysis). A similar trend was observed when patients were stratified according to RF status. A stratified analysis on rs10499194 also showed that the disease susceptibility allele in Japanese patients with RA (the T allele) conferred higher risk in autoantibody-positive patients than in autoantibody-negative patients.

DISCUSSION

In the current study, rs2230926, located in exon 3 of *TNFAIP3,* was shown to be significantly associated with a predisposition to both SLE and RA in 2 and 3 independent cohorts of subjects, respectively. Our results confirmed that *TNFAIP3* is one of the common genetic risk factors for both SLE and RA, similar to *STAT4* and *IRF5*, in the Japanese and Caucasian populations (2). In addition, recent studies in Caucasian patients with RA have demonstrated that the *TNFAIP3* variant conferred an increased risk of RA in anti-CCP antibody– and RF-positive patients compared with anti-CCP antibody– and RF-negative patients (12,13). Our analysis stratified according to the autoantibodies confirmed this observation in Japanese patients with RA.

TNFAIP3 encodes a cytoplasmic zinc finger protein that is also known as the A20 protein. The A20 protein is required for negative regulation of the NF-κB signaling pathway, which is mediated by innate immune receptors such as TNF receptors and Toll-like receptors, and it prevents overstimulation of the innate immune response (7,14). The disease-associated variant, rs2230926 (T/G), is a nonsynonymous variant that results in a phenylalanine-to-cysteine change at residue 127 of the A20 protein (5). The risk allele is known to be the G allele that encodes Cys. Musone et al have reported that Cys^{127} A20 protein was only modestly, but consistently, less effective at inhibiting TNF-induced NF-κB activity than the Phe^{127} protein (5). This result suggests that reduced negative regulatory activity of A20 protein may allow excessive immune activity, leading to enhanced autoreactivity.

GWA studies of SLE patients in Caucasian populations have suggested that several polymorphisms in the *TNFAIP3* region, including the nonsynonymous SNP rs2230926, are associated with a predisposition to the disease. The genetic significance of rs2230926 was evident in the Japanese patients with SLE or RA entered into our study, although its precise role in Caucasian patients with RA remains unclear. The intergenic SNP rs10499194 is one of the landmark polymorphisms identified in Caucasian patients with RA (3,15), although the significant association with RA could not be replicated in several Caucasian populations (3,12). Because rs10499194 is also associated with RA susceptibility and autoantibody status in our population, rs10499194 could be a landmark for disease causal variants in Japanese patients with RA. However, considering the inverted susceptibility allele of rs10499194 between Japanese patients (T allele) and Caucasian patients (C allele), this association of rs10499194 would appear to be secondary, as a result of LD between rs10499194 and the disease causal variants. This finding is further supported by the lack of independent association at rs10499194 in SLE when conditioned with the rs2230926 genotype, suggesting that the association observed in rs10499194 may be partially influenced by rs2230926.

Taking into account the biologic impact of rs2230926 demonstrated by Musone et al (5), rs2230926 seems likely to be an important candidate for a causal variant in *TNFAIP3* (5). However, additional polymorphisms that are located in the intergenic region of *OLIG3* and *TNFAIP3* as well as that of *TNFAIP3* and *PERP* may also independently exercise an effect on disease susceptibility, a hypothesis that was previously raised by Musone et al (5) and Graham et al (6). Further mapping of the *TNFAIP3* region in Asian and Caucasian

populations is required for the precise determination of the additional causal polymorphisms present in patients with RA or SLE.

In conclusion, we confirm that *TNFAIP3* is a genetic risk factor for the development of both SLE and RA in the Japanese population. Although the nonsynonymous SNP rs2230926 is a strong causal variant candidate in this region, a search for additional causal variants in *TNFAIP3* is required.

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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. Kochi 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. Shimane, Kochi, Horita, Ikari, Yamada, Atsumi, Koike, Momohara, Yamanaka, Nakamura, Yamamoto.

Acquisition of data. Shimane, Kochi, Horita, Ikari, Amano, Hirakata, Okamoto, Myouzen, Suzuki, Kubo, Takasaki.

Analysis and interpretation of data. Shimane, Kochi, Horita, Ikari, Yamamoto.

REFERENCES

1. Alarcon-Segovia D, Alarcon-Riquelme ME, Cardiel MH, Caeiro F, Massardo L, Villa AR, et al. Familial aggregation of systemic lupus erythematosus, rheumatoid arthritis, and other autoimmune diseases in 1,177 lupus patients from the GLADEL cohort. Arthritis Rheum 2005;52:1138–47.
2. Gregersen PK, Olsson LM. Recent advances in the genetics of autoimmune disease. Annu Rev Immunol 2009;27:363–91.
3. Plenge RM, Cotsapas C, Davies L, Price AL, de Bakker PI, Maller J, et al. Two independent alleles at 6q23 associated with risk of rheumatoid arthritis. Nat Genet 2007;39:1477–82.
4. Thomson W, Barton A, Ke X, Eyre S, Hinks A, Bowes J, et al. Rheumatoid arthritis association at 6q23. Nat Genet 2007;39: 1431–3.
5. Musone SL, Taylor KE, Lu TT, Nititham J, Ferreira RC, Ortmann W, et al. Multiple polymorphisms in the TNFAIP3 region are independently associated with systemic lupus erythematosus. Nat Genet 2008;40:1062–4.
6. Graham RR, Cotsapas C, Davies L, Hackett R, Lessard CJ, Leon JM, et al. Genetic variants near TNFAIP3 on 6q23 are associated with systemic lupus erythematosus. Nat Genet 2008;40:1059–61.
7. Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, et al. Failure to regulate TNF-induced NF-κB and cell death responses in A20-deficient mice. Science 2000;289:2350–4.
8. Lee HS, Korman BD, Le JM, Kastner DL, Remmers EF, Gregersen PK, et al. Genetic risk factors for rheumatoid arthritis differ in caucasian and Korean populations. Arthritis Rheum 2009;60: 364–71.
9. Hochberg MC, for the Diagnostic and Therapeutic Criteria Committee of the American College of Rheumatology. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus [letter]. Arthritis Rheum 1997;40:1725.
10. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–24.
11. Nakamura Y. The BioBank Japan Project. Clin Adv Hematol Oncol 2007;5:696–7.
12. Perdigones N, Lamas JR, Vigo AG, de la Concha EG, Jover JA, Urcelay E, et al. 6q23 polymorphisms in rheumatoid arthritis Spanish patients. Rheumatology (Oxford) 2009;48:618–21.
13. Patsopoulos NA, Ioannidis JP. Susceptibility variants for rheumatoid arthritis in the TRAF1-C5 and 6q23 loci: a meta-analysis. Ann Rheum Dis 2009. E-pub ahead of print.
14. Liu YC, Penninger J, Karin M. Immunity by ubiquitylation: a reversible process of modification. Nat Rev Immunol 2005;5: 941–52.
15. Orozco G, Hinks A, Eyre S, Ke X, Gibbons LJ, Bowes J, et al. Combined effects of three independent SNPs greatly increase the risk estimate for RA at 6q23. Hum Mol Genet 2009;18:2693–9.

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Replication of association between *FAM167A(C8orf13)-BLK* region and rheumatoid arthritis in a Japanese population

Polymorphisms in the genomic region encoding B lymphoid tyrosine kinase (*BLK*) and family with sequence similarity 167, member A (*FAM167A*, also referred to as *C8orf13*) at 8p23.1 have been associated with systemic lupus erythematosus (SLE) in Caucasian[1 2] and Asian[3 4] populations. A recent genome-wide study in a north American population showed new associations with rheumatoid arthritis (RA), among which was a single nucleotide polymorphism (SNP) rs2736340 in the intergenic region of *BLK* and *FAM167A*.[5] In the HapMap Japanese samples (http://www.hapmap.org/index.html.ja), this SNP is in absolute linkage disequilibrium (r^2=1) with rs13277113, previously associated with SLE.[1-4] We have shown that the population frequency of the risk genotype rs13277113A/A and the OR for SLE were substantially higher in the Japanese population than in the Caucasian population.[3]

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Table 1 Association of *BLK* rs13277113 with rheumatoid arthritis (RA) in a Japanese population

		Genotype frequency			Allele frequency		Allelic association	
	n	A/A	A/G	G/G	A	G	p Value	OR (95% CI)
RA	603	308 (0.511)	242 (0.401)	53 (0.088)	858 (0.711)	348 (0.289)	0.018	1.24 (1.04 to 1.49)
Control	492	218 (0.443)	218 (0.443)	56 (0.114)	654 (0.665)	330 (0.335)		

The association was tested by χ^2 analysis using a 2×2 contingency table.

To date, the association of *FAM167A-BLK* region with RA has not been reported in non-Caucasian populations. In this study we examined whether the association between *BLK* and RA was replicated in Japanese subjects.

A case-control association study was performed for 603 patients and 492 healthy controls. Because the association of *FAM167A-BLK* region with SLE is already established,[1–4] patients with RA complicated with SLE were excluded. All patients fulfilled the American College of Rheumatology classification criteria for RA.[6] The patients and the healthy controls were recruited at Matsuta Clinic, University of Tsukuba, the University of Tokyo and Juntendo University. This study was reviewed and approved by the research ethics committees of University of Tsukuba and other participating institutes. Written informed consent was obtained from all participants, except for some participants before 2001, before the enforcement of the Ethics Guidelines for Human Genome/Gene Analysis Research by the Japanese government. From such participants, oral informed consent had been obtained. In accordance with the guidelines, the latter samples were anonymised in an unlinkable fashion and were included in this study after review and approval by the ethics committee of University of Tsukuba. The genotype of rs13277113 was determined using the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, California, USA).[3] Power calculation based on the risk allele frequency in the Japanese population (0.665) showed that this sample size provides 80% power to detect susceptibility genes with an allelic OR of 1.298. Deviation from Hardy-Weinberg equilibrium was observed neither in the patients nor in the controls.

A significant association with RA was replicated in the Japanese population (table 1). Although the OR was comparable to that in the Caucasian population (1.19 for rs2736340[5]), the risk allele frequency was considerably higher in the Japanese subjects than in the Caucasians (0.273 in cases vs 0.240 in controls for rs2736340[5]). The population attributable risk percentage was estimated to be 22.8% in the Japanese population and 9.3% in the Caucasian population under the dominant model. No significant difference in rs13277113 was observed between *HLA-DRB1* shared epitope positive and negative RA (data not shown).

Our observations indicate that the *FAM167A-BLK* region may be a shared genetic factor for a number of autoimmune diseases in multiple populations, but the genetic contribution may be greater in Asian populations because of the differences in the genetic background.

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Competing interests None.

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REFERENCES

1. **Hom G**, Graham RR, Modrek B, *et al.* Association of systemic lupus erythematosus with C8orf13-BLK and ITGAM-ITGAX. *N Engl J Med* 2008;**358**:900–9.
2. **Harley JB**, Alarcón-Riquelme ME, Criswell LA, *et al*; International Consortium for Systemic Lupus Erythematosus Genetics (SLEGEN). Genome-wide association scan in women with systemic lupus erythematosus identifies susceptibility variants in ITGAM, PXK, KIAA1542 and other loci. *Nat Genet* 2008;**40**:204–10.
3. **Ito I**, Kawasaki A, Ito S, *et al.* Replication of the association between the C8orf13-BLK region and systemic lupus erythematosus in a Japanese population. *Arthritis Rheum* 2009;**60**:553–8.
4. **Yang W**, Ng P, Zhao M, *et al.* Population differences in SLE susceptibility genes: *STAT4* and *BLK*, but not *PXK*, are associated with systemic lupus erythematosus in Hong Kong Chinese. *Genes Immun* 2009;**10**:219–26.
5. **Gregersen PK**, Amos CI, Lee AT, *et al.* REL, encoding a member of the NF-kappaB family of transcription factors, is a newly defined risk locus for rheumatoid arthritis. *Nat Genet* 2009;**41**:820–3.
6. **Arnett FC**, Edworthy SM, Bloch DA, *et al.* The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988;**31**:315–24.

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Concise report

Up-regulation of the endoplasmic reticulum transmembrane protein UNC93B in the B cells of patients with active systemic lupus erythematosus

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Abstract

Objectives. The transmembrane endoplasmic reticulum (ER) protein UNC93B plays an essential role in the normal response to signalling through intracellular Toll-like receptor (TLR)3, TLR7, TLR8 and TLR9. In the current study, we examined the level of UNC93B expression on peripheral B cells from patients with active SLE, and investigated any correlation with SLE pathogenesis.

Methods. Peripheral blood mononuclear cells (PBMCs) and B cells from 43 active SLE patients were analysed by quantitative RT–PCR to determine the precise levels of UNC93B mRNA. We also analysed UNC93B protein expression on B cells from SLE patients using immunoblotting.

Results. The expression of UNC93B mRNA on PBMCs from active SLE patients was significantly higher than that of controls ($P < 0.05$). The intracellular expression level of UNC93B protein on $CD20^+$ B cells from active SLE patients was also higher than in the controls. Moreover, the expression of UNC93B on B cells from lupus patients correlated significantly with high titres of anti-dsDNA antibody ($P < 0.05$).

Conclusions. Up-regulation of the ER membrane protein UNC93B on human lupus B cells suggests that TLR9 and UNC93B play a partial rôle in the pathogenesis of SLE by inducing defective peripheral B-cell tolerance.

Key words: Systemic lupus erythematosus, Innate immunity, Toll-like receptor, Toll-like receptor 7, Toll-like receptor 9, B cell, UNC93B1, Anti-dsDNA antibody, Autoimmune disease, Myeloid differentiation factor 88.

BASIC SCIENCE

Introduction

SLE is a systemic autoimmune disease characterized by the generation of autoantibodies directed against nuclear DNA and nuclear proteins [1, 2]. Although it is generally considered that autoimmunity is related to adaptive immunity, a recent study has demonstrated that abnormalities of the innate immune system may also be related to the pathogenesis of autoimmune disease [3]. Toll-like receptor (TLR) activation initiates the innate immune response by inducing the expression of antimicrobial genes and inflammatory cytokines. Activation of TLR also enhances adaptive immunity via the activation of dendritic cells (DCs). Several mechanisms have been proposed to explain the production of autoantibodies in diseased B cells, including impaired survival or apoptosis signalling that may prevent negative selection, dysfunctional complement or inhibitory Fc receptors, and the activation of TLR in response to the accumulation of apoptotic bodies.

We recently reported that higher expression levels of TLR9 on peripheral blood B cells in active SLE patients correlated with CH50 and SLEDAI, and induced the production of anti-dsDNA antibody and IL-10 synthesis via TLR9–CpG ligation [4]. The response following TLR9 triggering was also found to be dependent on intracellular trafficking of the receptors themselves between the transmembrane endoplasmic reticulum (ER) protein and endosomes [5, 6]. Tabeta *et al.* [7] identified triple D (3d)

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