Clinical and Experimental Immunology ORIGINAL ARTICLE doi:10.1111/j.1365-2249.2008.03646.x

Up-regulated expression of Toll-like receptors mRNAs in peripheral blood mononuclear cells from patients with systemic lupus erythematosus

A. Komatsuda, H. Wakui, K. Iwamoto, M. Ozawa, M. Togashi, R. Masai, N. Maki, T. Hatakeyama and K. Sawada

Third Department of Internal Medicine, Akita University School of Medicine, Akita, Japan

Accepted for publication 20 February 2008

Correspondence: A. Komatsuda, Third Department of Internal Medicine, Akita University School of Medicine, 1-1-Hondo, Akita, Japan. E-mail: komatsud@med.akita-u.ac.jp

Summary

Recent studies in animal models for systemic lupus erythematosus (SLE) have shown that Toll-like receptors (TLR-7 and TLR-9) and interferon (IFN)-α are involved in the pathogenesis of murine lupus. Recent studies using flow cytometry have also shown increased expression of TLR-9 in peripheral blood mononuclear cells (PBMCs) from SLE patients. In this study, we performed quantitative real-time reverse transcription–polymerase chain reaction analyses of PBMCs from 21 SLE patients and 21 healthy subjects, to estimate *TLR2*, *TLR3*, *TLR4*, *TLR5*, *TLR7*, *TLR8*, *TLR9*, *IFN-α* and *LY6E* (a type I IFN-inducible gene) mRNA expression levels. Expression levels of *TLR2*, *TLR7*, *TLR9, IFN-α* and *LY6E* mRNAs in SLE patients were significantly higher than those in healthy controls. Expression levels of *TLR7* and *TLR9* mRNAs correlated with that of *IFN-α* mRNA in SLE patients. These results suggest that up-regulated expression of *TLR7* and *TLR9* mRNAs together with increased expression of *IFN-α* mRNA in PBMCs may also contribute to the pathogenesis of human lupus.

Keywords: interferon-α, peripheral blood mononuclear cells, real-time reverse transcription–polymerase chain reaction, systemic lupus erythematosus, Toll-like receptors

Introduction

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease of generalized autoimmunity characterized by B cell hyperactivity, abnormally activated T cells and defects in the clearance of apoptotic cells and immune complexes [1,2]. Because these immune dysfunctions lead to the production of a wide range of autoantibodies and immune complex deposition in vital organs, autoantibody-producing B cells appear central to the pathogenesis of SLE. Several mechanisms have been proposed to explain the production of autoantibody-producing B cells: impaired survival/apoptosis signals preventing negative selection [3], dysfunctional complement or inhibitory Fc receptors [4,5], loss of peripheral tolerance through activation of myeloid dendritic cells induced by interferon (IFN)-α overproduction [6,7] and activation of Toll-like receptors (TLRs) in response to accumulation of apoptotic bodies [8,9].

The TLR family plays a critical role in the mammalian innate immune system, the first line of host defence against invading pathogens. To date, 12 members of the TLR family have been identified in mammals, and have been shown to bind to a variety of ligands, including lipids, proteins and nucleic acids derived from both microorganisms and endogenous tissues [10]. TLRs are now considered to have an important role in autoimmunity, because numerous *in vitro* studies have documented activation of both autoreactive B cells and plasmacytoid dendritic cells by TLR ligands [8]. Experimental evidence in animal models for SLE suggests a role for TLR-7 (a receptor for single-stranded RNA) and TLR-9 (a receptor for DNA) in the development of murine lupus [8,9]. Two recent studies using flow cytometry showed increased expression of TLR-9 in peripheral blood mononuclear cells (PBMCs) from human lupus patients [11,12]. However, TLR-7 was not examined in these studies.

In the present study, we examined *TLR2*, *TLR3*, *TLR4*, *TLR5*, *TLR7*, *TLR8*, *TLR9*, *IFN-α* and *LY6E* (a type I IFN-inducible gene [13]) mRNA expression levels in PBMCs from SLE patients, by quantitative real-time reverse transcription–polymerase chain reaction (RT–PCR). Expression levels of *TLR2, TLR7, TLR9, IFN-α* and *LY6E* mRNAs in SLE patients were significantly higher than those in healthy subjects. Furthermore, expression levels of *TLR7* and *TLR9* mRNAs correlated with that of *IFNα* mRNA. These findings suggest a possible role for TLR-7, TLR-9 and IFN-α in the development of human lupus, as well as in murine lupus.

Table 1. Characteristics of the studied subjects.

Subjects	SLE ($n = 21$)	Healthy controls ($n = 21$)	P-value
Female/male	(14/7)	(13/8)	n.s.
Age mean (years) (range)	30·4 (13–66)	32·2 (23–55)	n.s.
Race	Japanese	Japanese	
SLEDAI score (range)	16·4 (4–35)		
Renal disease	11		
Central nervous system disease	0		
Serosistis	0		
Haematological disease	18		
Leucopenia	13		
Thrombocytopenia	11		
Polyarthritis	11		
Skin involvement	17		
Vasculitis	4		
Fever	8		
Treatment			
None	21		

SLE, systemic lupus erythematosus; n.s., not significant; SLEDAI, SLE Disease Activity Index.

Materials and methods

Patients

We recruited 21 consecutive untreated patients entering outpatient clinics of Akita University Hospital and its affiliated hospitals who fulfilled the American College of Rheumatology 1997 revised criteria for SLE [14,15] for this study. Twenty-one sex- and age-matched healthy subjects were also included. SLE activity was assessed by the SLE Disease Activity Index (SLEDAI) score [16] at the onset of SLE. The protocol of this study was approved by the ethics committee of the institution involved, and informed consent for genetic studies was obtained from all the subjects. The characteristics of SLE patients and healthy subjects are summarized in Table 1.

Laboratory assessments

We assessed laboratory data including serum levels of complements C3 and C4, CH50, circulating immune complexes determined by a C1q binding assay, and titres of anti-dsDNA, anti-RNP and anti-Sm antibodies determined by enzyme-linked immunosorbent assays in each SLE patient. We also assessed peripheral blood leucocyte, neutrophil, monocyte and lymphocyte counts.

Quantitative real-time RT–PCR

We quantified *TLR2*, *TLR3*, *TLR4*, *TLR5*, *TLR7*, *TLR8*, *TLR9*, *IFN-α*, and *LY6E* mRNA expression levels in PBMCs from SLE patients and healthy subjects. PBMCs were isolated by Ficoll-Conray (GE Healthcare Bio-Science AB, Uppsala, Sweden; Daiichi Seiyaku Corp., Tokyo, Japan) density gradient centrifugation.

Total RNA was prepared with an RNeasy kit (Qiagen, Hilden, Germany) and used for cDNA synthesis with an oligo(dT) primer (Amesham Biosciences, Piscataway, NJ, USA). PCR primers used in this study are shown in Table 2.

Table 2. Primers.

		Tm (°C)
TLR-2-forward	GGC CAG CAA ATT ACC TGT GTG	64
TLR-2-reverse	AGG CGG ACA TCC TGA ACC T	
TLR-3-forward	CCT GGT TTG TTA ATT GGA TTA ACG A	62
TLR-3-reverse	TGA GGT GGA GTG TTG CAA AGG	
TLR-4-forward	CTG CAA TGG ATC AAG GAC CA	64
TLR-4-reverse	TTA TCT GAA GGT GTT GCA CAT TCC	
TLR-5-forward	TGC CTT GAA GCC TTC AGT TAT G	62
TLR-5-reverse	CCA ACC ACC ACC ATG ATG AG	
TLR-7-forward	TTA CCT GGA TGG AAA CCA GCT ACT	64
TLR-7-reverse	TCA AGG CTG AGA AGC TGT AAG CTA	
TLR-8-forward	CAG AAT AGC AGG CGT AAC ACA TCA	62
TLR-8-reverse	TGT CAA GGC GAT TGC CAC TGA	
TLR-9-forward	TGA AGA CTT CAG GCC CAA CTG	64
TLR-9-reverse	TGC ACG GTC ACC AGG TTG T	
IFN-α-forward	TGC TTT ACT GAT GGT CCT GGT	60
IFN-α-reverse	TCA TGT CTG TCC ATC AGA CAG	
LY6E-forward	ATC TTC TTG CCA GTG CTG CT	60
LY6E-reverse	AGT CAC GCA GTA GTT GTC CC	
GAPDH-forward	ATG GCT ATG ATG GAG GTC CAG	60
GAPDH-reverse	TTG TCC TGC ATC TGC TTC AGC	

GAPDH, glyceraldehyde-3-phosphate dehydrogenase; IFN, interferon; TLR, Toll-like receptor; LY6E, lymphocyte antigen 6 complex, locus E.

Table 3. Relative quantification of Toll-like receptors (TLRs) mRNA expression [control *versus* systemic lupus erythematosus (SLE) patients].

	Control	SLE	*P*-value*
TLR-2	0·082 ± 0·045	0·119 ± 0·058	0·019
TLR-3	1·801 ± 2·137	2·609 ± 2·069	0·089
TLR-4	3·035 ± 1·505	4·407 ± 2·239	0·051
TLR-5	0·243 ± 0·184	0·269 ± 0·132	0·279
TLR-7	1·365 ± 1·171	1·872 ± 0·756	0·011
TLR-8	3·658 ± 1·854	3·473 ± 1·985	0·741
TLR-9	1·477 ± 1·883	2·791 ± 1·914	0·007
IFN-α	0·037 ± 0·045	0·069 ± 0·059	0·033
LY6E	0·094 ± 0·044	0·343 ± 0·163	0·0000067

**P*-value was estimated by Mann–Whitney *U*-test. LY6E, lymphocyte antigen 6 complex, locus E; IFN, interferon.

Real-time RT–PCR reaction was carried out in a final volume of 20 μl containing 10 μl DNA Master Hybridization Probe 2× (Qiagen, Hilden, Germany), 1 μl of 10 pmol forward and reverse primers, 1 μl of cDNA and 7 μl of water, according to the manufacturer's instructions. After an initial denaturation step at 95°C for 900 s, temperature cycling was initiated. Each cycle consisted of denaturation at 95°C for 15 s, hybridization at suitable temperatures (Table 2) for 20 s and elongation at 72°C for 20 s, using a LightCycler (Roche Diagnostics, Mannheim, Germany). A total of 45 cycles were performed. Each sample was run in triplicate.

Quantitative real-time RT–PCR curves were analysed by LightCycler 3·5 software (Roche Diagnostics). For relative quantification of *TLRs*, *IFN-α* and *LY6E* mRNA expressions, the mRNA expression of *GAPDH* was used as a control.

Statistics

Differences in the mean age and sex distribution between SLE patients and healthy subjects were determined using the χ^2 test. We compared *TLRs*, *IFN-α* and *LY6E* mRNA expression levels in PBMCs in SLE patients and healthy subjects, using the Mann–Whitney *U*-test. Relations between *TLRs* mRNA expression levels and *IFN-α* mRNA expression level, the SLEDAI scores and laboratory parameters were examined by the Spearman's correlation coefficient rank test. All analyses were performed using an Excel Statistical Software (Igakutosho Shuppan Corp, Tokyo, Japan). Data are expressed as mean ± standard deviation. A *P*-value less than 0·05 was regarded as statistically significant.

Results

Patients

The characteristics of 21 SLE patients are shown in Table 1. The SLEDAI score ranged from 4 to 35, with a mean of 16·4. Because all patients had more than four SLEDAI scores they were considered to have mild to very high activity [17]. Haematological disease, including leucopenia and thrombocytopenia, was the most common manifestation, which was found in 18 patients. Skin rash was observed in 17 patients. Renal disease, polyarthritis, fever and vasculitis were found in 11, 11, eight and four patients respectively.

Quantification of expression levels of *TLRs, IFN-α* and *LY6E* mRNAs in PBMCs from SLE patients and control subjects by real-time RT–PCR

We examined *TLRs*, *IFN-α* and *LY6E* mRNA expression levels in PBMCs from 21 untreated SLE patients and 21 control subjects by real-time RT–PCR. The results showed that relative *TLR2*, *TLR7*, *TLR9*, *IFN-α* and *LY6E* mRNA expression levels (*TLR2*/*GAPDH*, *TLR7*/*GAPDH*, *TRR9*/*GAPDH*, *IFN-α*/*GAPDH* and *LY6E*/*GAPDH* mRNAs) were significantly higher in SLE patients than in control subjects (Table 3). We compared *TLRs* mRNA expression levels with *IFN-α* and *LY6E* mRNA expression levels, SLEDAI scores and laboratory parameters in SLE patients. The results revealed that expression levels of *IFN-α* mRNA correlated significantly with those of *TLR3*, *TLR5*, *TLR7*, *TLR8* and *TLR9* mRNAs (Table 4). On the other hand, expression levels of *LY6E* mRNA did not correlate with those of *TLRs* and *IFN-α* mRNAs. Titres of anti-dsDNA antibodies correlated inversely with expression levels of *TLR9* and *IFN-α* mRNAs (Figs 1 and 2). No significant differences were found between *TLRs* mRNA expression levels and SLEDAI scores, circulating immune complex levels or other laboratory parameters in SLE patients.

Discussion

In this study, we have demonstrated that *TLR2*, *TLR7*, *TLR9*, *IFN-α* and *LY6E* mRNA expression levels in PBMCs from SLE patients were significantly higher than those in healthy controls, using quantitative real-time RT–PCR. Expression levels of *TLR7* and *TLR9* mRNAs correlated significantly with that of *IFN-α* mRNA. Furthermore, titres of anti-

Table 4. Correlation between *IFN-α* and *TLRs* mRNA expression levels in peripheral blood mononuclear cells from systemic lupus erythematosus patients.

	Results of the Spearman's correlation coefficient rank test	
	P-value	*r*
TLR-2	0·089	0·381
TLR-3	0·0000445	0·770
TLR-4	0·129	0·342
TLR-5	0·002	0·626
TLR-7	0·014	0·528
TLR-8	0·005	0·584
TLR-9	0·001	0·645

TLR, Toll-like receptor.

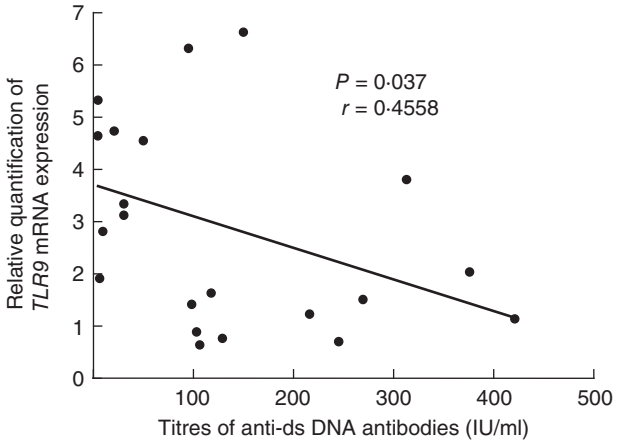

Fig. 1. Inverse correlation between expression level of *TLR9* mRNA in peripheral blood mononuclear cells and titres of anti-dsDNA antibodies for all systemic lupus erythematosus patients. TLR, Toll-like receptor.

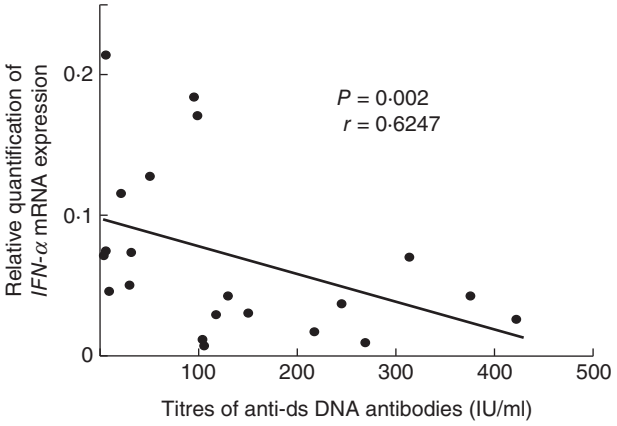

Fig. 2. Inverse correlation between expression level of *IFN-α* mRNA in peripheral blood mononuclear cells and titres of anti-dsDNA antibodies for all systemic lupus erythematosus patients. IFN, interferon.

dsDNA antibodies correlated inversely with expression levels of *TLR9* and *IFN-α* mRNAs. Table 5 summarizes previously reported data of TLR expressions [11,12] and our results in SLE patients.

There is conclusive evidence of a role for TLR-9 in the pathogenesis of murine lupus models [8,9]. Recently, two groups examined the expression of TLRs in PBMCs from SLE patients by flow cytometry [11,12]. Papadimitraki *et al.* [11] demonstrated that the proportion of plasma cells and memory B cells expressing TLR-9 was increased in active SLE patients. Increased percentages of TLR-9-expressing B cells, but not monocytes, correlated with the presence of anti-dsDNA antibodies in their patients. Migita *et al.* [12] also showed that TLR-9 expression levels of $CD19^+$ B cells were elevated significantly in SLE patients. There was no significant correlation between TLR-9 expression levels and clinical markers such as SLEDAI scores, CH50 and titres of anti-dsDNA antibodies in their patients.

Our results of real-time RT–PCR showed increased expression of TLR-9 in PBMCs from SLE patients. This supports the previous observations by Papadimitraki *et al.* [11] and Migita *et al.* [12]. In our patients, there was an inverse correlation between expression levels of *TLR9* mRNA in PBMCs and titres of anti-dsDNA antibodies. Our finding is inconsistent with observations by Papadimitraki *et al.* [11]. This controversy might be due to differences in experimental procedures and/or background of patients. In studies by Papadimitraki *et al.* [11], B cells expressing TLR-9 were examined. Approximately one-third of their patients were receiving corticosteroids and approximately one-third of patients were receiving hydroxychloroquine, a potent inhibitor of TLR-9 signalling. Although they showed a correlation between increased percentages of TLR-9-expressing B cells and the presence of anti-dsDNA antibodies, they did not examine the relationship between percentages of TLR-9-expressing B cells and titres of anti-dsDNA antibodies. On the other hand, we used whole PBMCs including B cells, T cells and monocytes from patients before treatment in our study. In recent studies of murine lupus, there were also opposite findings regarding the role of TLR-9 in anti-DNA antibody formation [18–20]. Lartigue *et al.* [20] suggested that the discrepancy might be due to the different genetic background of mice used in these studies. The exact role of TLR9 in the pathogenesis of anti-DNA responses remains to be determined.

In our patients, there was an inverse correlation between expression levels of *IFN-α* mRNA in PBMCs and titres of anti-dsDNA antibodies. Christensen and Shlomchik [9] proposed a model for immune activation in SLE. Endogenous DNA- and RNA-containing antigens released by apoptotic cells activate TLRs within autoreactive B cells, allowing differentiation to autoantibody-secreting cells. Autoantibody immune complexes then activate plasmacytoid dendritic cells, inducing production of IFN-α. We consider that a large amount of DNA-containing antigens could be neutralized by anti-DNA antibodies and removed from the circulation in

Table 5. Expression of Toll-like receptors (TLRs) in peripheral blood mononuclear cells from patients.

References	Methods	TLR-2	TLR-3	TLR-4	TLR-5	TLR-7	TLR-8	TLR-9
[11]	Flow cytometry	→	→	→	n.d.	n.d.	n.d.	↑
[12]	Flow cytometry	↓	n.d.	→	n.d.	n.d.	n.d.	↑
Present study	Real-time RT–PCR	↑	→	→	→	↑	→	↑

n.d., Not determined; RT–PCR, reverse transcription–polymerase chain reaction.

patients having high titres of the antibodies. As a result, it is possible that expression levels of *IFN-α* mRNA in PBMCs could be lower in such patients than in patients having low titres of anti-DNA antibodies.

In animal models, Berland *et al.* [21] reported TLR-7-dependent loss of B cell tolerance in pathogenic autoantibody knock-out mice. Christensen *et al.* [22] demonstrated that TLR-7-deficient lupus-prone mice had ameliorated disease, decreased lymphocyte activation and decreased serum IgG. Furthermore, mice with the Y-linked autoimmune accelerating locus mutation, leading a twofold increase in the expression of several genes including TLR-7, develop a severe form of murine lupus [23]. These findings suggest strongly that TLR-7 has an important role in the pathogenesis of murine lupus models. In the present study, we found up-regulated expression of *TLR7* mRNA in PBMCs from SLE patients for the first time. This suggests that TLR-7 expression in PBMCs is also involved in the pathogenesis of human lupus.

It is known that repeated administration of recombinant IFN-α to patients with various malignancies or chronic viral infections could lead to the production of autoantibodies, sometimes to the development of clinical symptoms associated with SLE or other autoimmune diseases [24,25]. Recent studies have identified the IFN gene expression signature in PBMCs from active SLE patients [13,26,27]. These findings suggest that IFN-α can contribute to the pathogenesis of SLE through various mechanisms, including effects on antigen-presenting cells, B cells and T cells [8,9]. For example, Barrat *et al.* [28] showed that mammalian RNA and DNA, in the form of immune complexes, are potent self-antigens for TLR-7 and TLR-9 respectively, and induce IFN-α production by plasmacytoid dendritic cells. They suggested that TLR-7 and TLR-9 have a critical role in the promotion of lupus through the induction of INF-α by plasmacytoid dendritic cells. Our observations support this possibility. Expression levels of *INF-α* RNA in PBMCs from SLE patients were significantly higher than those from healthy controls. There was a correlation between expression level of *INF-α* RNA in PBMCs and those of *TLR7* and *TLR9* mRNAs in our patients.

Previous studies showed up-regulation of various IFN-inducible genes in PBMCs from SLE patients by microarray analyses, although they failed to show increased expression of *IFN-α* mRNA [27,28]. We examined mRNA expression levels of *LY6E*, a type I IFN-inducible gene that is known to be up-regulated in SLE [13], in PBMCs from our subjects, and confirmed its significantly higher expression in SLE patients than in healthy controls. In our patients, expression level of *LY6E* mRNA did not correlate with those of *TLRs* and *IFN-α* mRNAs. Perhaps *LY6E* mRNA is expressed at a higher level in PBMCs compared with *TLRs* and *IFN-α* mRNAs. The discrepancy regarding *IFN-α* mRNA expression in PBMCs from SLE patients between previous microarray data [27,28] and our real-time RT–PCR data might be due to differences in experimental procedures and/or background of patients.

In conclusion, our real-time RT–PCR analyses confirmed the previous flow cytometry finding of increased expression of TLR-9 in PBMCs from SLE patients [11,12]. We also showed up-regulated expression of *TLR7* mRNA together with increased expression of *IFN-α* mRNA in PBMCs from SLE patients. These results support the findings observed in murine lupus models [8,9], and suggest that TLR-7, TLR-9 and IFN-α may also contribute to the pathogenesis of human lupus.

Acknowledgements

This study was supported in part by the Global COE Program and a Grant-in Aid for Scientific Research (1759081 to A. K.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1 Mok CC, Lau CS. Pathogenesis of systemic lupus erythematosus. J Clin Pathol 2003; **56**:481–90.
2 D'Cruz DP, Khamashta MA, Hughes GRV. Systemic lupus erythematosus. Lancet 2007; **369**:587–96.
3 Mackay F, Schneider P, Rennert P, Browning J. BAFF and APRIL: a tutorial on B cell survival. Annu Rev Immunol 2003; **21**:231–64.
4 Carroll MC. A protective role for innate immunity in systemic lupus erythematosus. Nat Rev Immunol 2004; **4**:825–31.
5 McGaha TL, Sorrentino B, Ravetch JV. Restoration of tolerance in lupus by targeted inhibitory receptor expression. Science 2005; **307**:590–3.
6 Koutouzov S, Mathian A, Dalloul A. Type-I interferons and systemic lupus erythematosus. Autoimmun Rev 2006; **5**:554–62.
7 Pascual V, Farkas L, Banchereau J. Systemic lupus erythematosus: all roads lead to type I interferons. Curr Opin Immunol 2006; **18**:676–82.
8 Marshak-Rothstein A. Toll-like receptors in systemic autoimmune disease. Nat Rev Immunol 2006; **6**:823–35.
9 Christensen SR, Shlomchik MJ. Regulation of lupus-related autoantibody production and clinical disease by Toll-like receptors. Semin Immunol 2007; **19**:11–23.
10 Akira S, Uematsu S, Takeuchi O. Pathogen reaction and innate immunity. Cell 2006; **124**:783–801.
11 Papadimitraki ED, Choulaki C, Koutala E *et al.* Expansion of toll-like receptor 9-expressing B cells in active systemic lupus erythematosus: implications for the induction and maintenance of the autoimmune process. Arthritis Rheum 2006; **54**:3601–11.
12 Migita K, Miyashita T, Maeda Y *et al.* Toll-like receptor expression in lupus peripheral blood mononuclear cells. J Rheumatol 2007; **34**:493–500.
13 Feng X, Wu H, Grossman JM *et al.* Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. Arthritis Rheum 2006; **54**:2951–62.
14 Tan EM, Cohen AS, Fries JF *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982; **25**:1271–7.

15 Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997; **40**:1725 [Letter].
16 Bombardier C, Gladman DD, Urowitz MB, Caron D, Chang CH. Derivation of the SLEDAI: a disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum 1992; **35**:630–40.
17 Cook RJ, Gladman DD, Pericak D, Urowitz MB. Prediction of short term mortality in systemic lupus erythematosus with time dependent measures of disease activity. J Rheumatol 2000; **27**:1892–5.
18 Christensen SR, Kashgarian M, Alexopoulou L *et al.* Toll-like receptor 9 controls anti-DNA autoantibody production in murine lupus. J Exp Med 2005; **202**:321–31.
19 Wu X, Peng SL. Toll-like receptor 9 signaling protects against murine lupus. Arthritis Rheum 2006; **54**:336–42.
20 Lartigue A, Courville P, Auquit I *et al.* Role of TLR9 in anti-nucleosome and anti-DNA antibody production in *lpr* mutation-induced murine lupus. J Immunol 2006; **177**:1349–54.
21 Berland R, Fernandez L, Kari E *et al.* Toll-like receptor 7-dependent loss of B cell tolerance in pathogenic autoantibody knockin mice. Immunity 2006; **25**:429–40.
22 Christensen SR, Shupe J, Nickerson K, Kashgarian M, Flavell RA, Shlomchik MJ. Toll-like receptor 7 and TLR9 dictate autoantibody specificity and have opposing inflammatory and regulatory roles in a murine model of lupus. Immunity 2006; **25**:417–28.
23 Subramanian S, Tus K, Li Q-Z *et al.* A *Tlr7* translocation accelerates systemic autoimmunity in murine lupus. Proc Natl Acad Sci USA 2006; **103**:9970–5.
24 Rönnblom LE, Alm GV, Oberg KE. Possible induction of systemic lupus erythematosus by interferon-alpha treatment in a patient with a malignant carcinoid tumour. J Intern Med 1990; **227**:207–10.
25 Gota C, Calabrese L. Induction of clinical autoimmune disease by therapeutic interferon-alpha. Autoimmunity 2003; **36**:511–18.
26 Baechler EC, Batliwalla FM, Karypis G *et al.* Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. Proc Natl Acad Sci USA 2003; **100**:2610–15.
27 Bennett L, Palucka AK, Arce E *et al.* Interferon and granulopoiesis signatures in systemic lupus erythematosus blood. J Exp Med 2003; **197**:711–23.
28 Barrat FJ, Meeker T, Gregorio J *et al.* Nucleic acids of mammalian origin can act as endogenous ligands for Toll-like receptors and may promote systemic lupus erythematosus. J Exp Med 2005; **202**:1131–9.