The predictive value of fluctuations in IgM and IgG class anti-dsDNA antibodies for relapses in systemic lupus erythematosus. A prospective long term observation

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Abstract

***Objective*—This study investigated the predictive value of rises in IgM class antibodies against double stranded DNA (anti-dsDNA) for ensuing relapses in systemic lupus erythematosus (SLE) in comparison with rises in IgG class antibodies. In addition, it was analysed whether rises in IgM class anti-dsDNA were associated with specific clinical manifestations of SLE.**

***Methods*—Thirty four of a cohort of 72 SLE patients who were positive for IgM class anti-dsDNA at the start of the study or at the time of a relapse were analysed monthly for class specific anti-dsDNA levels during a median observation period of 19.6 months. Disease activity was scored according to the SLE Disease Activity Index. Anti-dsDNA were measured by IgM and IgG class enzyme linked immunosorbent assay (ELISA) and by Farr assay.**

***Results*—During the study 18 of 34 patients experienced 26 relapses. Twenty two (85%) of the relapses were accompanied by a positive test for IgM class anti-dsDNA by ELISA, 23 (89%) were positive for IgG class anti-dsDNA by ELISA, and 25 (96%) were positive by Farr assay. Patients with rises in IgG class anti-dsDNA by ELISA or in anti-dsDNA by Farr assay had a significantly higher cumulative risk for relapses than patients without those increases (p=0.04 and p=0.03, respectively). This was not the case for rises in IgM class anti-dsDNA (p=0.16). Moreover, a rise in IgM class anti-dsDNA before a relapse was not associated, expressed in terms of odds ratios, with specific clinical manifestations of SLE.**

***Conclusion*—Relapses of SLE are frequently accompanied by IgM class anti-dsDNA. Rises of IgM class anti-dsDNA, in contrast with rises in IgG class anti-dsDNA, are not a sensitive tool for predicting a relapse and are not associated with specific clinical manifestations of SLE.**

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Systemic lupus erythematosus (SLE) is characterised by a multitude of clinical manifestations and the production of various autoantibodies.[1] The latter may react with a variety of nuclear, cytoplasmic and cell surface antigens.[2] Among these, antibodies against double stranded DNA (anti-dsDNA) are highly specific for SLE.[3 4] Anti-dsDNA are found in 40–85% of the patients[5–8] depending on the assay used for their detection,[9] and their presence may precede the diagnosis of SLE by more than one year.[10] The antibodies are thought to be involved in the pathogenesis of the disease. For example, lupus nephritis may result from immune complex deposition or in situ immune complex formation in which nucleosomes and autoantibodies to nucleosomal constituents such as dsDNA are involved.[11] Nucleosomes may bind to basement membranes because of charge interaction between cationic histones and anionic membrane components.[11] Antibodies to dsDNA are of diverse immunoglobulin classes. In most patients with SLE IgG class anti-dsDNA predominate and seem to be the most specific antibodies for the diagnosis of SLE.[4 12] The occurrence of predominantly IgM class anti-dsDNA in serum of SLE patients seems to be associated with a less active disease and a longer survival.[13 14] IgM class anti-dsDNA have, however, also been found in normal persons and in other diseases such as rheumatoid arthritis, mixed connective tissue disease, and chronic active hepatitis.[3 14–16]

Previous studies showed controversial results concerning the predictive value of changes in anti-dsDNA values in relation to disease exacerbations in SLE. Some studies showed no relation between changes in anti-dsDNA and changes in disease activity.[6 17] On the other hand several other studies showed a closer relation between a rise in anti-dsDNA values and ensuing disease activity.[7 8 18–20] In this respect, assessment of anti-dsDNA values by ^{125}I Farr assay proved most sensitive for prediction of relapses when compared with IgG class enzyme linked immunosorbent assay (ELISA) and Crithidia luciliae immunofluorescence test.[7] In 85% of anti-dsDNA positive patients a rise in anti-dsDNA preceding a relapse could be detected by Farr assay, in 74% by IgG class ELISA, and in 63% by Crithidia luciliae assay.[7] The Farr assay is not immunoglobulin class specific and, probably, detects IgG as well as

Table 1 Criteria for major and minor relapses of SLE

Major relapse: fulfilling one or more of the following:*
1 *Severe renal disease*
(a) decrease in creatinine clearance > 25% within 4 months, accompanied by an active sediment (> 5 erythrocyte sedimentation rate (mm 1st h), and/or casts), and proteinuria > 0.5 g/day
(b) recent renal biopsy showing active proliferative lupus nephritis (> 50% of glomeruli affected)
2 *Severe central nervous system disease*
seizures, cerebral vascular accident, coma, transverse myelitis, psychosis, choroathetosis, central nerve palsy
3 *Haematological disease*
immune haemolytic anaemia (Hb < 60 g/l) and/or thrombocytopenia (< 50×10^9/l)
4 *Severe serositis*
pericarditis with (impending) tamponade and/or massive pleural effusion
5 *Uveitis and/or retinitis*
6 *Myocarditis with arrhythmia and/or congestive heart failure*
7 *Severe myositis with proximal muscle weakness*
8 *Lung involvement with haemoptysis*
9 *Major vasculitis*
with ulcerations and/or mononeuritis multiplex
10 *Miscellaneous*
fever (> 38° C rectally), serositis, haemolytic anaemia (> 60 g/l), or thrombopenia (> 50×10^9/l), all without improvement after prednisolone at a maximum dose of 30 mg/day for at least one week
Minor relapse: fulfilling all of the following:
1 Increase in SLE-DAI† of ⩾ 2 points within 6 months, with a minimal activity index of 4 points, accompanied by:
2 The need to start prednisolone or immunosupressive drugs based on clinical evidence, and:
3 Not fulfilling the criteria for major relapse

* Only features present within two weeks of the outpatient consultation or relevant admission are taken into account. † SLE disease activity index.

IgM class anti-dsDNA. This may explain why changes in anti-dsDNA values as detected by Farr assay correlate better with changes in disease activity than changes in IgG class anti-dsDNA as detected by ELISA. Studies in which IgM class anti-dsDNA have been measured longitudinally in relation to disease activity are scarce.[13 14] In addition, longitudinal changes of IgM class anti-dsDNA have not been compared with changes of IgG class anti-dsDNA and changes in anti-dsDNA as detected by Farr assay. To determine the relevance of serial measurements of IgM class anti-dsDNA values, we performed this prospective longitudinal study, to investigate, firstly, whether IgM class antibodies against dsDNA are present in plasma samples of patients with SLE without the concomitant presence of IgG class antibodies. Secondly, we analysed the value of rises in IgM class anti-dsDNA values as measured by ELISA for predicting a relapse of the disease. We compared the findings with the predictive value for ensuing relapses of rises in IgG class anti-dsDNA as determined by ELISA and rises in anti-dsDNA values as measured by ^{125}I Farr assay. Thirdly, we analysed whether rises in IgM class anti-dsDNA are associated with specific clinical manifestations.

Methods

PATIENTS

This study concerns a cohort of 72 SLE patients, fulfilling the 1982 revised ACR criteria for the diagnosis of SLE,[21] who participated in a prospective long term clinical follow up study.[7] From this cohort of SLE patients, 34 were selected for further analysis, based on a positive test for IgM class anti-dsDNA as measured by ELISA, either at the start of the study or at the moment of a relapse. All 72 patients were seen at least every three months at the outpatient clinic of the University Hospital in Groningen. At every outpatient clinic visit the SLE disease activity index (SLE-DAI) was calculated from signs and symptoms recorded according to a protocol by one physician (EJterB), and routine laboratory tests were performed. The decision to treat patients was based on clinical symptoms and results of standard laboratory procedures without knowledge of the values of anti-dsDNA. Blood samples were drawn in EDTA monthly and plasma was stored at −80°C until assayed. Relapses were defined as described previously (table 1).[7 8]

STUDY METHODS

For assessment of anti-dsDNA all monthly samples were assayed by IgG and IgM class ELISA and by ^{125}I Farr assay.

ELISA

This technique used calf thymus DNA (Sigma, St Louis, USA) as a substrate. To achieve coating of DNA (10 µg/ml DNA, 10 mM TRIS

Table 2 Clinical characteristics of 34 patients with SLE at the start of the study

Age (y)	*Range*	*17–75*
	Median	36
Sex	M/F	3/31
Disease duration (y)	Range	2–31
	Median	12
Race	Whites	30
	Orientals	4
	Blacks	0
ACR criterion[22]	*Number*	*%*
1 Malar rash	14	41
2 Discoid rash	6	18
3 Photosensitivity	16	47
4 Oral ulcers	2	6
5 Arthritis	25	74
6 Pleuritis	13	38
Pericarditis	12	35
7 Proteinuria	17	50
Cellular casts	15	44
8 Convulsions	2	6
Psychosis	4	12
9 Haemolytic anaemia	9	26
Leucocytopenia	19	56
Lymphocytopenia	14	41
Thrombocytopenia	18	53
10 Anti-DNA antibodies	34	100
Anti-Sm antibodies	3	9
11 ANA	34	100

The presence of the LE cell phenomenon and/or a false positive lues reaction is not included in the table as neither classification factor was routinely analysed in our follow up of SLE patients.

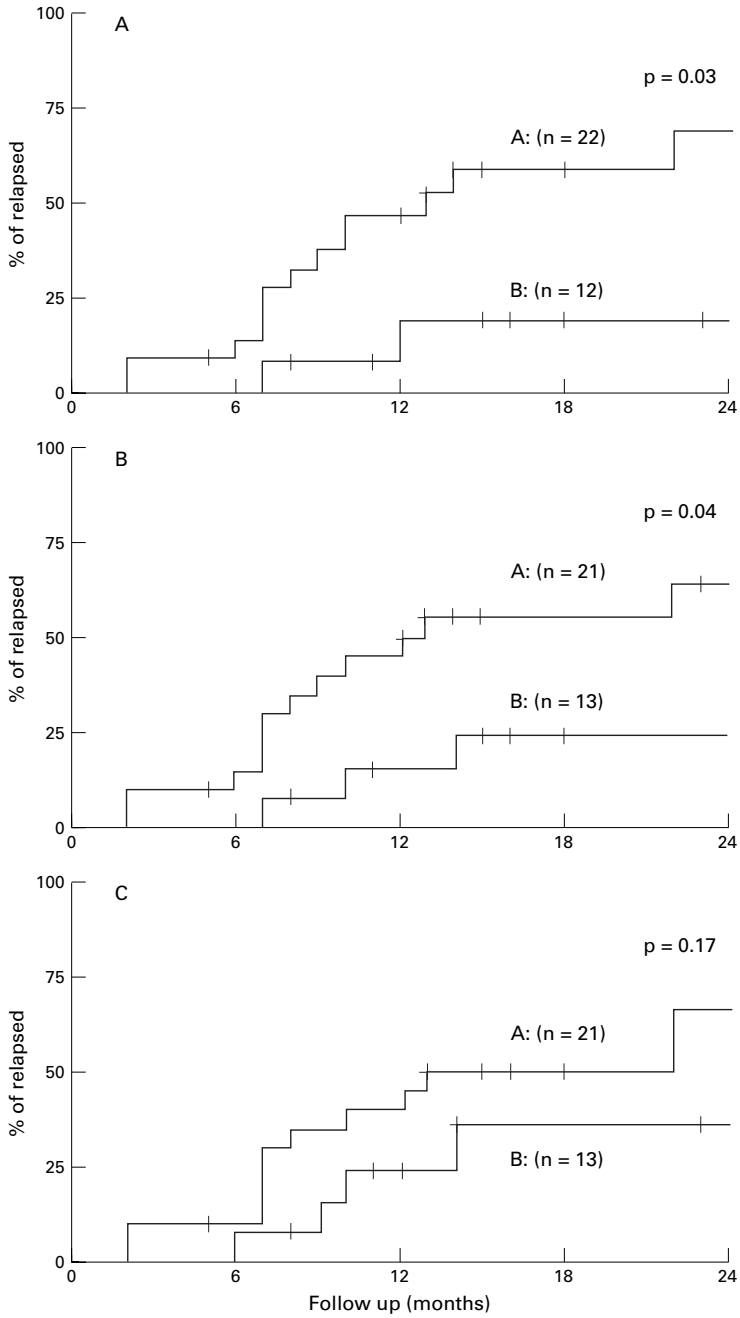

Figure 1 Cumulative risk of relapses in SLE patients with a rise in anti-dsDNA (line A) and without a rise in anti-dsDNA (line B), determined by Farr assay (A), by IgG class ELISA (B), and by IgM class ELISA (C). The p values were calculated by the Gehan-Wilcoxon method.

0.15 M NaCl, pH 8.0, overnight 4°C) on the plates, a precoating was performed with protamine sulphate (Sigma, 500 µg/ml in aquadest, 45 minutes at 4°C).[9] Plasma samples were incubated in serial threefold dilutions, starting at 1:100 to 1:2700, for one hour at 37°C and subsequently during two hours at 4°C in 0.01 M TRIS/HCL, pH 8.0, 0.15 M NaCl, 0.05 % TWEEN 20, 1% bovine serum albumin. Horseradish peroxidase labelled goat anti-human IgG or IgM (Kallestad, Chaska, USA) was used as a conjugate (1:2500, 30 minutes, 37°C). Normal value of IgG class anti-dsDNA level by ELISA was ≤7 IU/ml, of IgM class anti-dsDNA ≤17 U/ml (mean (3SD) of 50 normal controls). The Wo/80 reference preparation was used as standard for IgG class anti-dsDNA.[22] The values obtained for IgM class anti-dsDNA were calculated in comparison to a standard positive sample and expressed in U/ml. Both intra-assay and inter-assay variation of anti-dsDNA were less than 10% for IgG and IgM class anti-dsDNA.

Farr assay

In this method ^{125}I-labelled recombinant dsDNA (Diagnostic Products Corporation (DPC), Los Angeles, USA) was used. The Farr assay was performed according to the manufacturer's instructions. Positive samples were measured at different dilutions to obtain measurements within the range of the assay. A sample was considered positive for anti-dsDNA when its value exceeded 10 IU/ml (mean (3SD) of 50 normal controls). Both intra-assay and interassay variations were less than 10%. Farr assay values were expressed in IU/ml using Wo/80 as the ultimate standard.[22]

CRITERIA FOR A RISE IN VALUES OF ANTI-DSDNA

A rise in anti-dsDNA was defined as an increase of 25% of the value in a previous sample, which increase had to amount to at least three standard deviations of the values of 50 normal controls (≥6 IU/ml for IgG class ELISA, ≥13 U/ml for IgM class ELISA, ≥15 IU/ml for ^{125}I Farr assay). The rise had to occur within a four month period. To exclude rises resulting from interassay variation, pairs of samples were retested within one assay to confirm the rise, and another sample was obtained as soon as possible after the rise was established and analysed simultaneously with the two samples in which the rise was detected.

STATISTICS

Analysis was done using the SPSS 4.1 statistical package. By using logistic regression analysis we determined the odds ratio with 95% confidence intervals for different clinical characteristics of the first relapse that occurred after a significant rise in IgM class and IgG class anti-dsDNA values and a rise in anti-dsDNA values as detected by Farr assay. The time until the occurrence of the first relapse after a significant rise in anti-dsDNA values was analysed by Kaplan-Meier plots, expressed as cumulative risk. Differences between curves of patients with and without a significant rise in anti-dsDNA were evaluated with the Gehan-Wilcoxon test. p Values <0.05 were considered significant.

Results

From the cohort of 72 SLE patients, 61 patients tested positive at the start of the study by Farr assay, 37 patients by IgG class ELISA, and 32 patients by IgM class ELISA. During follow up, two patients became positive for IgM class anti-dsDNA at the time of a relapse. A total of 34 patients thus tested positive for IgM class anti-dsDNA and were further analysed. In this group of 34 patients the median age at the start of the study was 36 years (range 17–75). SLE was diagnosed a median time of

*Table 3 Clinical characteristics of 18 first relapses in SLE patients related to preceding rises in anti-dsDNA values measured by IgM class and IgG class ELISA and by Farr assay**

Rise in anti-dsDNA by	*Total*	*Renal*		*Retinitis*		*CNS*		*Skin*		*Haemocytology*		*Arthritis*		*Myositis*		*Serositis*		*Vasculitis*		*Miscellaneous*	
		+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−
IgM class ELISA																					
+	8	5	3	2	6	2	6	4	4	4	4	0	8	1	7	1	7	2	6	6	2
−	10	5	5	0	10	0	10	4	6	3	7	4	6	0	10	0	10	2	8	2	8
OR		1.67		—		—		1.50		2.33		—		—		0.57		1.33		1.33	
95% CI		(0.25, 11.07)		—		—		(0.23, 9.80)		(0.34, 16.18)		—		—		(0.04, 7.47)		(0.14, 12.37)		(0.14, 12.37)	
IgG class ELISA																					
+	14	8	6	1	13	1	13	6	8	6	8	3	11	1	13	3	11	3	11	3	11
−	4	2	2	1	13	1	13	2	2	1	3	1	3	0	4	0	4	1	3	1	3
OR		1.33		0.23		0.23		0.75		2.25		0.82		—		—		0.82		0.82	
95% CI		(0.14, 12.37)		(0.01, 4.48)		(0.01, 4.48)		(0.08, 6.96)		(0.18, 27.37)		(0.06, 11.0)		—		—		(0.06, 11.0)		(0.06, 11.0)	
Farr assay																					
+	14	9	5	1	13	1	13	6	8	5	9	4	10	0	14	3	11	2	12	3	11
−	4	1	3	1	13	1	13	2	2	2	2	0	4	1	3	0	4	2	12	1	3
OR		5.40		0.23		0.23		0.75		0.56		—		—		—		0.17		0.82	
95% CI		(0.44, 66.67)		(0.01, 4.84)		(0.01, 4.84)		(0.08, 6.96)		(0.06, 5.24)		—		—		—		(0.01, 1.96)		(0.06, 11.0)	

* By cross tables and regression analysis as odds ratio (OR) with 95% confidence intervals (95% CI).

12 years (2–31) before the start of the study. Table 2 shows the cumulative patient characteristics. Patients were followed up for a median of 19.6 months (range 4.6–37.8). Of these 34 patients, 18 patients developed 26 relapses (17 major with a median SLE-DAI score of 16.9 (range 6–28) and nine minor relapses with a median SLE-DAI score of 8.0 (range 4–16)) during the study period. The first relapse was followed by a second in three patients, by a third in one, and a fourth in another patient. The number of collected plasma samples in the 34 patients was 673.

PREVALENCE OF POSITIVE TEST RESULTS FOR ANTI-DSDNA AT THE START OF THE STUDY AND AT THE MOMENT OF A RELAPSE

At the start of the study, plasma samples of 32 of 34 patients were positive for anti-dsDNA by IgM class ELISA, 27 of 34 patients were anti-dsDNA positive by IgG class ELISA, and 27 patients were positive by Farr assay. Four patients were positive for IgM class anti-dsDNA only and another three were positive for IgM class anti-dsDNA, negative for IgG class anti-dsDNA, but positive for anti-dsDNA by Farr assay. These last three patients were persistently negative for IgG class anti-dsDNA during the study. Three patients were IgM class anti-dsDNA positive at the start of the study and became negative during a relapse. For IgG class anti-dsDNA a comparable switch from positive at the start of the study to negative during a relapse was observed in one patient. It was not observed in patients positive by Farr assay at the start of the study.

Of the 26 relapses that occurred in 18 of 34 patients during the study period, 22 (85%) relapses in 15 patients were accompanied by a positive test for IgM class ELISA, 23 (89%) relapses in 15 patients were positive by IgG class ELISA, whereas 25 (96%) relapses in 17 patients were positive by Farr assay. Three patients were negative for IgG class but positive for IgMclass anti-dsDNA. Two of them were positive by Farr assay. These three patients were persistently negative for IgG class anti-dsDNA during the study.

DIAGNOSTIC VALUE OF A RISE IN ANTI-DSDNA LEVELS FOR PREDICTING A RELAPSE

In patients in whom a rise in anti-dsDNA could be detected, this rise preceded the relapse by several months. The median period between a rise in anti-dsDNA and the occurrence of a relapse was 3.2 (range 0–6) months as determined by IgM class ELISA, 2.3 (range 0–7) months by IgG class ELISA, and 2.1 (range 0–7) months by Farr assay.

Figure 1 shows the diagnostic value expressed in terms of cumulative risk of a first relapse (major or minor) in patients with and without a rise in anti-dsDNA levels. The cumulative risk of a relapse was significantly different for patients with a rise in anti-dsDNA detected by IgG class ELISA or Farr assay in comparison with patients without a rise in anti-dsDNA (Farr assay: p=0.03, fig 1(A); IgG class ELISA: p=0.04, fig 1(B)). By contrast, the difference in cumulative risk of a relapse was

not significant between patients with and without a rise in IgM class anti-dsDNA detected by ELISA (p=0.16, fig 1(C)).

CLINICAL SIGNIFICANCE OF RISES IN IGM CLASS ANTI-DSDNA VALUES, RELATION TO RELAPSES

A rise in anti-dsDNA by IgM class ELISA preceded a relapse in 14 of 26 cases (occurring in 11 patients), by IgG class ELISA in 20 of 26 cases (occurring in 15 patients), and by Farr assay in 21 of 26 cases (occurring in 16 patients). Rises in IgM class anti-dsDNA were accompanied in most cases by rises in anti-dsDNA by IgG class ELISA (11 of 14) and Farr assay (12 of 14). Of the 14 significant rises in IgM class anti-dsDNA followed by a relapse, two rises were not accompanied by rises in anti-dsDNA values by IgG class ELISA or Farr assay. Both patients developed a major relapse. One relapse was characterised by severe vasculitis, the other relapse by malar rash, retinal vasculitis, livedo reticularis, thrombocytopenia, and chorea. In one of the 14 cases a significant rise in IgM class anti-dsDNA was not accompanied by a rise in IgG class anti-dsDNA, but was detected by Farr assay. This patient developed glomerulonephritis. In the 13 cases with predominantly active nephritis during a relapse, a rise in anti-dsDNA preceding the relapse was detected by Farr assay in 85%, by IgG class ELISA in 70%, and by IgM class ELISA in 53%. The two relapses with central nervous system involvement were both preceded by a significant rise in IgM class anti-dsDNA.

Determination of odds ratios of specific clinical manifestations of first relapses (n=18) showed a slightly increased chance of developing haematological, renal, skin, vasculitis, and miscellaneous involvement for patients with rises in IgM class anti-dsDNA in comparison with patients without rises in IgM class anti-dsDNA (table 3). In contrast, a rise in anti-dsDNA measured by Farr assay showed a highly increased odds ratio for developing renal involvement.

The median SLE-DAI score of the 26 relapses was 14 (range 4–28). The median SLE-DAI was comparable for relapses positive for IgM class ELISA in comparison with relapses positive for IgG class ELISA and Farr assay, the range of the SLE-DAI (4–28) being similar for the three tests.

Discussion

In this longitudinal study we analysed the clinical relevance of rises in IgM class anti-dsDNA values in relation to relapses of SLE. We compared the relevance of those rises with that of rises in anti-dsDNA levels as determined by IgG class anti-dsDNA ELISA and Farr assay.

To assess the practical clinical relevance of serial measurements of IgM class anti-dsDNA, we determined the cumulative risk for a relapse after a rise in anti-dsDNA as measured by IgM and IgG class ELISA and Farr assay. Patients with rises in IgG class anti-dsDNA by ELISA or in anti-dsDNA by Farr assay had a significantly higher cumulative risk for relapse than patients without those rises. However, even in our selected group of SLE patients (positive for IgM class anti-dsDNA at the start of the study or during a relapse) we found no statistically significant difference (p=0.17) between the cumulative risk for relapses between patients with and without a rise in IgM class anti-dsDNA. Moreover, only 44% of the cohort of 72 SLE patients tested positive by IgM class anti-dsDNA ELISA at the start of the study. Therefore, the number of patients that might benefit from frequent testing for IgM class anti-dsDNA is limited. Hence, serial measurement of levels of IgM class anti-dsDNA is not suitable for predicting relapses in SLE.

The predominant manifestations of patients positive for IgM class anti-dsDNA during a relapse were: nephritis, vasculitis, haematological (miscellaneous), and skin involvement. Most previous studies concluded that patients with predominantly high IgM class anti-dsDNA have less active disease and a longer survival than patients with predominantly high values of IgG class anti-dsDNA.[14 23 24] In this study we observed also that patients with IgM class antibodies against dsDNA may develop serious relapses such as vasculitis, nephritis, and neurological syndromes. Remarkably, most of the IgM class anti-dsDNA positive relapses were major relapses. These results are in contrast with the general feeling that IgM class anti-dsDNA are associated with a milder course of the disease. It should, however, be noted that in most of our patients both IgG and IgM class antibodies were present.

Although we showed that changes in IgM class anti-dsDNA may precede a relapse the relative contribution of IgM class and IgG class anti-dsDNA to the pathogenesis of the disease remains unclear. In (NZB × NZW)F_1 mice a decline in renal function was observed when the predominant isotype of their anti-DNA antibodies switched from predominantly IgM to IgG2,[25] which process is under control of regulatory cells present in both the thymus and the spleen.[26] In humans with SLE, this switch from IgM to IgG in relation to the development of lupus nephritis is controversial in the medical literature.[14 18 19 23 25] This switch was seen in only three of 18 patients who developed a relapse. In most studies frequent sampling of plasma has not been performed, which makes a critical assessment of isotype switch difficult. In a cross sectional study by Okamura and colleagues[24] on 40 untreated patients with lupus nephritis, a close relation between histological activity scores and IgG anti-dsDNA levels, but not IgM class anti-dsDNA, has been shown. In this study we showed that 53% of the relapses with nephritis as predominant manifestation were preceded by a rise in IgM class anti-dsDNA. Only in one patient who developed nephritis was a rise in IgM class anti-dsDNA before the relapse not accompanied by a rise in IgG class anti-dsDNA. The concomitant occurrence of rises in IgG and IgM class anti-dsDNA values in conjunction with lupus nephritis may suggest a pathogenic role for both immunoglobulin classes of

anti-dsDNA in a substantial number of patients with lupus nephritis.

In conclusion, relapses of SLE are often accompanied by rises in IgM class anti-dsDNA and these changes parallel rises in anti-dsDNA values as tested by IgG class ELISA and Farr assay in most cases. The cumulative risk for a relapse after a rise in IgM class anti-dsDNA is lower than that after a rise in anti-dsDNA values determined by IgG class ELISA or by Farr assay. Rises in IgM class anti-dsDNA are not a sensitive tool for predicting relapses, and are not associated with specific clinical manifestations.

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