Apoptosis and autoantibodies in systemic lupus erythematosus

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IgG subclass distribution of autoantibodies differs between renal and extra-renal relapses in patients with systemic lupus erythematosus

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Submitted
Summary

IgG subclasses of autoantibodies differ in their potential to induce an inflammatory response as they differentially interact with complement and Fcγ-receptors. IgG subclass distribution of anti-nucleohistone and anti-dsDNA antibodies were analysed longitudinally in SLE patients prior to and at the moment of an extra-renal (n=23) or a renal relapse (n=17). Kidney biopsy specimens of patients with a renal relapse were analysed for IgG subclass deposition. IgG1 anti-nucleohistone and IgG1 anti-dsDNA antibodies were present in plasma of 39 out of 40 patients. At the moment of a relapse, IgG2- and IgG3 anti-nucleohistone and IgG2 anti-dsDNA antibodies were more frequently present in patients with renal disease compared to those with extra-renal disease. Increase in levels of IgG1 anti-dsDNA were observed in 10 out of 11 patients prior to a renal relapse but occurred in only 10 out of 22 patients with an extra-renal relapse (p=0.02). Rises in IgG2 anti-dsDNA occurred at an equally low rate both prior to renal and extra-renal relapses. A rise in IgG2 anti-nucleohistone antibodies preceded a renal relapse in 8 of 11 patients and an extra-renal relapse in only 4 out of 22 patients (p=0.006). In kidney biopsies all IgG subclasses could be detected. IgG1- and IgG2-subclass antibodies to nucleohistone and to dsDNA are the predominant subclasses found in plasma of lupus patients with renal disease. The frequent occurrence of a rise of IgG2 anti-nucleohistone and IgG1 anti-dsDNA in patients prior to a renal relapse, suggest that, besides IgG1 subclass autoantibodies, IgG2-subclass antibodies to nucleohistone have a particular pathophysiological role in lupus nephritis.

Introduction

Systemic lupus erythematosus (SLE) is a multi-system autoimmune disease associated with a multitude of autoantibodies. The kidneys are frequently involved, presumably due to autoantibody deposition at the glomerular basement membrane (GBM) which results in complement activation and attraction and influx of inflammatory cells [1]. Deposition of antibodies at the GBM can occur via trapping of circulating immune complexes within the glomerulus, via nucleosomes that bind to the GBM and act as a substrate for autoantibody binding, or through cross-reactivity of antinuclear antibodies with glomerular structures [1]. Antibodies most closely associated with lupus nephritis are anti-dsDNA antibodies [1]. In addition, there is cumulating evidence for an important role of nucleosomes and anti-nucleosome antibodies in the pathogenesis of SLE. Nucleosome specific antibodies emerge before anti-dsDNA antibodies in lupus-prone mice. Both antibodies to dsDNA as well as to nucleosomes can be eluted from kidney specimens of lupus mice with overt glomerulonephritis [2]. IgG class antibodies seem most relevant as there is a close relation between levels of IgG anti-dsDNA and histologic activity scores in patients with lupus
IgG subclasses in SLE

nephritis [3]. Furthermore, in the majority of patients, a renal relapse is preceded by a significant rise of IgG anti-dsDNA as detected by ELISA [4].

All IgG subclasses can be found in kidney biopsies [5]. Of the different subclasses, IgG1 and IgG3 activate complement more efficiently than IgG2 while IgG4 does not activate complement at all [6]. The IgG subclass distribution of autoantibodies could therefore be of relevance in the pathogenesis of lupus nephritis. To evaluate their possible nephritogenic role, we monitored levels of total IgG and IgG subclasses of antibodies to nucleohistone and to dsDNA in time in patients who suffered a renal relapse. IgG subclass distribution in kidney biopsies of patients with a renal relapse was assessed as well. Serological data obtained from patients with renal relapses were compared with those of lupus patients who developed an extra-renal relapse, in order to evaluate the specificity of the findings for lupus nephritis.

Patients and Methods

Patients fulfilling at least four revised American College of Rheumatology (ACR) criteria for the diagnosis of SLE [7] could participate in this study. Patients originated from the cohorts of SLE patients (n=156) who participated in a prospective study at the University Hospitals of Groningen (AZG) and Utrecht (AZU), the Netherlands. Patients belonging to this cohort are seen at the outpatient department at least every 3-4 months for clinical evaluation. At every visit disease activity is scored and the SLE Disease Activity Index (SLEDAI) is calculated [8]. Attention is paid to the occurrence of infections. Of all patients who developed a relapse of the disease in the period between September 1991 till September 1997 (AZG) and September 1991 till January 1995 (AZU) data from their first relapse were included. Criteria for a relapse were pre-defined (table 1). Patients were classified into those with a renal relapse, defined as a biopsy proven WHO class III, IV or Vd lupus nephritis according to the World Health Organization criteria [9], with or without extra-renal symptoms, and those with an extra-renal relapse of the disease. Blood samples were drawn monthly in EDTA (Vacutainer; Becton Dickinson, Mountain View, CA) during the study period. Plasma was stored at -80°C until needed.

Measurement of anti-dsDNA and anti-nucleohistone antibodies

Anti-dsDNA antibodies were detected by Farr-assay using 125I-labeled recombinant ds-DNA (Diagnostic Products Corporation, Los Angeles, USA) which is free of contamination with ssDNA. Farr assay was performed according to the manufacturer's instruction and positive samples were measured at different dilutions to obtain measurements within the range of the assay. Results of this assay were expressed in IU/ml using Wo/80 as the ultimate standard [10]. Normal value of this Farr-assay in our laboratory is < 10 IU/ml; intra- and interassay variations are both less than 10 %. Antibodies to dsDNA were also detected by ELISA as described [11]. For detection of
IgG subclasses, microtiter plates (Nunc-Immuno plate Maxisorb, Nunc, Denmark) were precoated with 100 µl/well protamine sulfate (500 µg/ml in millipore water) at 4°C for 45 minutes. Plates were washed with millipore water. Coating was performed with DNA (10 µg/ml DNA, Sigma, St Louis, USA, in 10 mM Tris, pH 8.0, 0.15 M NaCl) overnight at 4°C.

**Table 1:**

_Criteria for major and minor disease exacerbations in SLE:_

Criteria for major exacerbation: fulfilling **one or more** of the following *:

1. **severe renal disease:**
   a. recent renal biopsy showing active proliferative lupus nephritis (>50% of glomeruli affected, and/or
   b. decrease of creatinin clearance of >25% within 4 months, accompanied by an active sediment (>5 ery's, h.p.f., and/or casts) and by proteinuria of >0.5 gram/day)
2. **severe central nervous system disease:** seizures, cerebral vascular accident, coma, transverse myelitis, psychosis, choreathetosis, central nerve palsy
3. **hematological disease:** hemolytic anemia (Hb<3.8 mmol/l) and/or thrombocytopenia (<50x10⁹ /l)
4. **severe serositis:** pericarditis with (impending) tamponade and/or massive pleural effusion
5. **uveitis and/or retinal vasculitis**
6. **myocarditis with arrhythmia and/or congestive heart failure**
7. **severe myositis with proximal muscle weakness**
8. **lung involvement with hemoptysis**
9. **major vasculitis:** with ulcerations and/or mononeuritis multiplex
10. **miscellaneous:** fever (>38°C rectally), serositis, hemolytic anemia (>3.8 mmol/l) or thrombocytopenia (>50x10⁹ /l), all without improvement after prednisolone in a maximum dosage of 30 mg/day during at least one week

Criteria for minor exacerbation: fulfilling **all** of the following items:

1. increase of activity index by ≥ 2 points within 6 months with a minimal activity score of 3 points accompanied by:
2. the clinically judged necessity to start prednisolone at a dosage of at least 10 mg/day, or to increase the prednisolone dosage with ≥ 5 mg/day, or to start with anti-malarials, or immunosuppressive drugs, and:
3. not fulfilling the criteria for a major exacerbation.

* Only features occurring within 2 weeks of the outclinic visit or admission under consideration are taken into account
IgG subclasses in SLE

After washing with washing buffer (0.01 M Tris, pH=8.0, 0.15 M NaCl, 0.05% Tween 20) plasma samples (diluted 1:60 in phosphate buffered saline (PBS, pH 8.0) with 0.05% Tween 20, 0.2% BSA) were added and plates were incubated for 1 hour at 37°C and subsequently during 2 hours at 4°C. Plates were washed and mouse anti-human monoclonal antibodies in dilution buffer were added (anti-IgG 1:6000, clone HP6017, anti-IgG1 1:1000, clone 8c/6-39, anti-IgG2 1:6000, clone HP6014, anti-IgG3 1:5000, clone HP6050, anti-IgG4 1:6000, clone HP6025; Sigma, St Louis, USA). After incubation for 1 hour at room temperature plates were washed and alkaline-phosphatase labeled sheep anti-mouse IgG (Sigma; 1:3000 dilution buffer) was added. After final incubation for 1 hour at room temperature plates were washed and para-nitrophenyl-phosphate (Sigma nr104-40T, 1 tablet suspended in 40 ml 10% (w/v) diethanolamin, buffer pH 9.8) was added for development. After 30 minutes incubation in the dark at room temperature the reaction was stopped with NaOH.

Total IgG and IgG subclasses of anti-nucleohistone antibodies were also detected by ELISA. Microtiter plates (Nunc Maxisorb) were coated with 10 µg/ml nucleohistone (Sigma; in 0.15 M NaCl, 0.15 M trisodiumcitrate, pH 7.0) for 1 hour. This nucleohistone preparation contains only the core histone subcomponents and H1 [12]. After washing plasma was added (diluted 1:60 in PBS, 0.05% Tween 20, 0.2% BSA) for incubation at room temperature during 1 hour. Further development of the ELISA was similar to the anti-DNA ELISA as described.

For both assays normal values were below the detection threshold. Values were expressed as arbitrary units. To prevent interassay variation serial samples of individual patients as well as all exacerbation samples were analyzed in one assay. Intra-assay variation was less than 10%. A rise in antibody level of at least 25% within a period of maximal 4 months was arbitrarily considered significant.

Biopsies

Renal biopsy specimens from lupus patients with a renal relapse obtained in the study period were used. From 10 patients with a renal relapse tissue specimens were available. Cryosections (4 µm) were defrosted and fixed in 100 % acetone. After preincubation with 10% v/v normal goat serum (DAKO, Denmark) in PBS, sections were incubated for 3 hours at room temperature with the same monoclonal mouse anti-human antibodies as used in the ELISA. Anti-human total IgG (1:3000), anti-human IgG1 (1:600), anti-human IgG2 (1:600), anti-human IgG3 (1:250) and anti-human IgG4 (1:1000), were diluted in PBS. Specimens of lupus nephritis class IV were used as positive control. Renal biopsy specimens from patients with minimal change nephropathy and acute transplant rejection served as negative control. Possible endogenous peroxidase activity was blocked by incubation for 15 minutes with 0.1% NaN₃ and 0.3% H₂O₂ in PBS. After washing sections were incubated with HRP-conjugated goat-anti-mouse IgG1 (Southern Biotechnology Associates, Birmingham, AL, USA) or HRP-conjugated goat-anti-mouse IgG2a (SBA). All goat-anti-mouse monoclonal antibodies were used at a 1:100 dilution in PBS enriched
with 10% normal human serum (CLB, Amsterdam) to block crossreactivity with human IgG in the biopsies. HRP activity was detected by the addition of 3-amino-ethyl-carbazole. Finally, specimens were counterstained with hematoxyline. Staining was scored by two independent observers on a semiquantitative scale of 0 (no staining), 1+ (weak or scarce, but unmistakenly present) till 6+ (diffuse and global, strong intensity). Discrepancies between observers were resolved by scoring specimens together, thereby reaching consensus.

Statistics
GraphPad Instat (GraphPad Software, San Diego, CA) was used for statistical calculations. Differences in parameters between groups were evaluated with unpaired t-test when normal distribution could be assumed, otherwise the Mann-Whitney U test was applied. Fisher’s exact test was used for comparison of differences in prevalence. Spearman's test was applied for detecting correlations between different study parameters. A p value <0.05 was considered significant.

Results
In the study period, 40 relapses were encountered. Characteristics of the patients and the relapses are shown in tables 2 and 3. Seventeen relapses were classified as renal, all with biopsy proven proliferative lupus nephritis (WHO class III, IV or Vd).

In a minority of patients the renal relapse was accompanied by other manifestations of the disease, among which hematological abnormalities were most frequently seen (table 3). The relapses without renal involvement were classified as extra-renal. Seven patients were included at the moment of relapse (6 renal, 1 extra-renal); therefore antibody levels of these patients could not be monitored prior to the relapse.

Plasma anti-nucleohistone antibodies
Total IgG as well as IgG1-subclass antibodies to nucleohistone could be detected in all but one of the patients (table 2). Antibodies of the IgG2 subclass were more frequently present in patients with a renal relapse than in those with extra-renal relapses (p=0.01). Also, IgG3 anti-nucleohistone antibodies could be detected at a higher frequency in patients with a renal relapse (p=0.009).

A weak correlation was found between levels of total IgG anti-nucleohistone antibodies and anti-dsDNA as measured by Farr assay (r=0.44, p=0.02). Furthermore, a correlation was found between levels of total IgG anti-nucleohistone antibodies and anti-nucleohistone antibody levels of the IgG1 (r=0.87, p<0.001) and the IgG2 subclass (r=0.69, p<0.001).
IgG subclasses in SLE

The presence of the various anti-nucleohistone IgG subclasses during specific disease manifestations is shown in table 4. No specific association with particular disease manifestations could be demonstrated, although IgG2 anti-nucleohistone antibodies were present in most patients with nephritis, serositis as well as hematological abnormalities. Serial plasma samples were available in 11 patients with a renal and in 22 patients with an extra-renal relapse. A rise in IgG2 anti-nucleohistone antibodies occurred more frequently in patients with a renal relapse than in those with an extra-renal relapse (p=0.006). With respect to the other subclasses, no differences could be found (table 5).

Table 2:

Clinical and serological characteristics of 40 SLE patients at the moment of relapse.

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>Renal relapse</th>
<th>Extra-renal relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>Male/Female</td>
<td>3/14</td>
<td>1/22</td>
</tr>
<tr>
<td>SLEDAI score</td>
<td>15.5 (6-25)***</td>
<td>9.6 (3-16)</td>
</tr>
<tr>
<td>Farr assay (IU/l)</td>
<td>339 (10-1701)</td>
<td>155 (1-726)</td>
</tr>
</tbody>
</table>

Anti-nucleohistone antibodies: presence (+) or absence (-) of IgG subclasses

- IgG1 (+/-) 17/0 22/1
- IgG2 (+/-) 13/4# 8/15
- IgG3 (+/-) 5/12## 0/23
- IgG4 (+/-) 1/16 0/23

Anti-dsDNA antibodies: presence (+) or absence (-) of IgG subclasses

- IgG1 (+/-) 17/0 22/1
- IgG2 (+/-) 13/4# 10/13
- IgG3 (+/-) 7/10 4/19
- IgG4 (+/-) 0/17 0/23

Data are expressed as mean (range); *** p<0.001, unpaired t-test; # p<0.05, ## p<0.01 Fisher’s exact test.(+) denotes presence, (-) denotes absence of resp. subclass at the moment of relapse.

Plasma anti-dsDNA antibodies

In patients with renal relapses, levels of antibodies to dsDNA as measured by Farr assay tended to be higher compared to those in patients with an extra-renal relapse (p=0.08, table 2). Levels of IgG-class antibodies to dsDNA as measured by ELISA showed a similar trend (p=0.07) and correlated with the results of the Farr assay (r=0.63, p<0.0001). All but one of the patients had antibodies to dsDNA of the IgG1 subclass. The patient without IgG1 antibodies had an extra-renal relapse. Antibodies to dsDNA of the IgG2 subclass were found in increased frequency in patients with a renal relapse compared to those with an extra-renal relapse (13 of 17 versus 9 of 23, respectively, p=0.02). In less than half of the patients, anti-dsDNA antibodies of the IgG3 subclass were present (table 2), showing a similar trend of a higher frequency in patients with renal disease (p=0.07). None of the patients had IgG4 anti-dsDNA antibodies.
Table 3:

Clinical characteristics of 40 relapses

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>Renal relapses (n = 17)</th>
<th>Extra-renal relapses (n = 23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal</td>
<td>17 (100)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Serositis</td>
<td>0 (0)</td>
<td>6 (26)</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>2 (12)</td>
<td>11 (48)</td>
</tr>
<tr>
<td>Cerebral</td>
<td>2 (12)</td>
<td>4 (17)</td>
</tr>
<tr>
<td>Haematological</td>
<td>5 (29)</td>
<td>5 (22)</td>
</tr>
<tr>
<td>Skin</td>
<td>1 (6)</td>
<td>6 (26)</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>1 (6)</td>
<td>6 (26)</td>
</tr>
<tr>
<td>Fever</td>
<td>1 (6)</td>
<td>6 (26)</td>
</tr>
</tbody>
</table>

Number and percentage (%) of patients in subgroup

Levels of total IgG anti-dsDNA correlated with that of the IgG1 subclass (r=0.88, p<0.0001) as well as with that of IgG2 anti-dsDNA (r=0.86, p<0.0001). Longitudinal studies revealed that 9 out of 11 (82%) of the patients with a renal relapse and 13 out of 22 (67%) with an extra-renal relapse had a significant rise of antibodies to dsDNA by Farr assay preceding the clinical relapse by a median period of 3 months (range 1-5).

Table 4:

Clinical manifestations of relapses and presence of IgG subclasses of anti-nucleohistone antibodies

<table>
<thead>
<tr>
<th>Clinical manifestation</th>
<th>IgG1</th>
<th>IgG2</th>
<th>IgG3</th>
<th>IgG4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal (n=17)</td>
<td>17 (100)</td>
<td>13 (76)</td>
<td>5 (29)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>Serositis (n=6)</td>
<td>6 (100)</td>
<td>5 (83)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Musculoskeletal (n=13)</td>
<td>13 (100)</td>
<td>7 (54)</td>
<td>2 (15)</td>
<td>1 (11)</td>
</tr>
<tr>
<td>Cerebral (n=6)</td>
<td>13 (100)</td>
<td>2 (33)</td>
<td>1 (17)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Haematological (n=10)</td>
<td>10 (100)</td>
<td>8 (80)</td>
<td>1 (10)</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Skin (n=7)</td>
<td>6 (86)</td>
<td>3 (43)</td>
<td>1 (14)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Vasculitis (n=6)</td>
<td>6 (100)</td>
<td>4 (67)</td>
<td>1 (17)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Fever (n=7)</td>
<td>7 (100)</td>
<td>4 (57)</td>
<td>1 (14)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

Number of relapses and percentage (%) of manifestation with a particular IgG subclass being positive for anti-nucleohistone antibodies.

Table 5 shows the presence of significant rises of total IgG and IgG subclasses of anti-dsDNA as measured by ELISA in relation to relapses. The majority (91%) of renal relapses were preceded by a significant rise in antibodies to dsDNA of the IgG1 subclass.
IgG subclasses in SLE

In contrast, only 45% of patients with an extra-renal relapse had a significant rise of IgG1 anti-dsDNA antibodies preceding their relapse ($p=0.02$). Significant rises of the other IgG subclasses of anti-dsDNA before disease exacerbation occurred in a minority of patients only.

Table 5:

*Significant rises of anti-nucleohistone and anti-dsDNA antibodies occurring in a period of 6 months prior to a relapse of the disease*

<table>
<thead>
<tr>
<th></th>
<th>Renal relapse</th>
<th>Extra-renal relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td><strong>Anti-nucleohistone</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total IgG</td>
<td>9 (82)$^#$</td>
<td>2 (18)</td>
</tr>
<tr>
<td>IgG1</td>
<td>8 (73)</td>
<td>3 (27)</td>
</tr>
<tr>
<td>IgG2</td>
<td>8 (73)$^{##}$</td>
<td>3 (27)</td>
</tr>
<tr>
<td>IgG3</td>
<td>2 (18)</td>
<td>9 (72)</td>
</tr>
<tr>
<td><strong>Anti-dsDNA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>total IgG</td>
<td>9 (82)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>IgG1</td>
<td>10 (91)$^#$</td>
<td>1 (9)</td>
</tr>
<tr>
<td>IgG2</td>
<td>4 (36)</td>
<td>7 (64)</td>
</tr>
<tr>
<td>IgG3</td>
<td>3 (27)</td>
<td>8 (73)</td>
</tr>
</tbody>
</table>

*Number and percentage (%) of patients in subgroup; $^\# p<0.05$, $^{##} p<0.01$, Fisher’s exact test*

Renal deposition of total IgG and IgG subclasses

In all biopsies immunoglobulins of each IgG subclass could be detected (figure 1). Notably, in contrast to subclass analysis of plasma samples, even IgG3 and IgG4 were detected in every kidney specimen. No correlation was found between the relative amount of any of the IgG subclasses in the kidney biopsy and plasma levels of IgG antibody subclasses to anti-dsDNA or anti-nucleohistone (data not shown).

Discussion

In this study we cross-sectionally as well as longitudinally analysed plasma IgG subclass levels of antibodies to nucleohistone and to dsDNA as well as the IgG subclass distribution in kidney biopsies from SLE patients. We performed this study in order to obtain more insight in the differential role of these autoantibodies and their IgG subclasses in the pathogenesis of SLE. Antibodies to nucleohistone as well as to dsDNA were found in plasma of 39 of 40 patients. These antibodies were mainly of the IgG1 subclass, and
less frequently of the IgG2 subclass. Antibodies of the IgG3 subclass directed to nucleohistone or to dsDNA could be detected in less than half of the patients.

Figure 1: Semi-quantitative analysis of IgG subclass deposition in kidney biopsy specimens from patients with proliferative lupus nephritis. Staining was scored by two independent observers on a semi-quantitative scale from 0 (no staining), 1+ (weak, but unmistakably present) till 6+ (diffuse and global, strong intensity). Horizontal lines denote the median.

Finally, antibodies of the IgG4 subclass directed to nucleohistone were present in only 1 out of 40 patients analysed, whereas those directed to dsDNA were not found at all.

We found that anti-nucleohistone antibodies can be detected in most of our lupus patients. Interestingly, IgG2 and IgG3 anti-nucleohistone antibodies were present more often in lupus patients with a renal relapse compared to patients with an extra-renal manifestation of the disease only. Furthermore, a significant rise in IgG2 anti-nucleohistone preceded 78% of the renal relapses, in contrast to the extra-renal relapses in which only 18% were preceded by a significant rise of IgG2 anti-nucleohistone antibodies. These data support the hypothesis that antibodies to nucleohistone are involved in the pathogenesis of lupus nephritis. In addition, our results suggest that anti-nucleohistone antibodies of the IgG2 subclass play a particular role. The predominance of IgG2 in autoantibody subclass distribution has been described for other autoantibodies in lupus patients. Indeed, antibodies of the IgG2 subclass to C1q are overrepresented in lupus nephritis patients [13 14]. Furthermore, we observed that antibodies to dsDNA of the IgG2 subclass were overrepresented in nephritis patients as well. It should be stated that our nucleohistone ELISA not only measures antibodies specific for the conformational structure of nucleohistones but, probably, also antibodies to the individual components of nucleohistones, that is DNA and histones. As such, it is conceivable that the subclass
IgG subclasses in SLE

distribution of anti-dsDNA antibodies was comparable with that of anti-nucleohistone antibodies, that is, IgG1 being present in the majority of patients, both with renal and extra-renal relapses, and a skewed distribution to IgG2 in patients with a renal relapse. In cross-sectional studies, others also reported a restriction in subclass distribution of antibodies to dsDNA. In accordance with the present study IgG1 has been demonstrated to be the anti-dsDNA antibody subclass most frequently encountered [15-19]. In addition, in support of a pathophysiological role for the IgG1 subclass, we observed that a significant rise of IgG1 anti-dsDNA occurred in 91% of the patients prior to a renal relapse. In contrast to our results, next to IgG1, the IgG3 isotype of anti-dsDNA as well as of anti-nucleosome has been described to be present most frequently in patients with renal disease [18-20]. These discrepancies might, at least in part, be explained by differences in the affinity of the monoclonal antibodies to IgG subclasses used in the various studies [21].

The longitudinal data on IgG subclass distribution of anti-nucleohistone and anti-dsDNA antibodies as observed in this study are unique and are therefore difficult to compare with previous studies. Several others reported stable isotype profiles of anti-dsDNA as well as anti-nucleohistone antibodies independent of antibody fluctuations as measured by the Farr assay and independent of disease activity [17,22]. In contrast, Devey et al. found an increase of the IgG1- and IgG3-subclass, but not of the IgG2 anti-dsDNA antibody subclass with increased disease severity in patients with renal disease [19]. Since, in all of the aforementioned studies, time intervals between blood sampling were much longer than the monthly period that we consistently have used, the possibility cannot be excluded that rises in levels of IgG subclasses prior to a relapse have been missed in these studies. We found a subclass-specific rise in levels of anti-nucleohistone and anti-dsDNA occurring over a period ranging from 1 to 5 months preceding a relapse, which is comparable with a previous study from our group in which total levels of anti-dsDNA were evaluated [11].

Also in other studies, all IgG subclasses could be detected in renal biopsies from lupus patients [5 23 24]. Imai et al. analysed glomerular immunofluorescence intensity of IgG subclasses in patients with membranoproliferative glomerulonephritis, membranous nephropathy and lupus nephritis and demonstrated increased intraglomerular deposition of IgG1 and IgG2 in lupus nephritis [5]. This might reflect the predominance of the IgG1 and IgG2 subclasses of anti-nucleohistone and anti-dsDNA antibodies that we found in plasma of patients with a renal relapse. Others, however, found IgG3 to be the dominant isotype in kidney biopsies of patients with class IV lupus nephritis [24]. Interestingly, IgG4 could be detected in all kidney specimens of our patients with a renal relapse, while, in plasma, IgG4 anti-nucleohistone antibodies were found in one patient only. The lack of correlation between levels of the measured autoantibodies in the serum and their deposition in renal tissue suggest that the autoantibodies deposited in the kidney may include other antigenic specificities than the ones measured in the sera. The deposition of antibodies might also be dependent on other factors than their serum concentration such as their affinity to their
respective antigen, the degree of presence of the antigen along the basement membrane
and epitope specificity.
For many years, attention has been paid to IgG subclasses in SLE. Each IgG isotype has
different biological and functional properties. Subclass distribution might therefore
influence the course of SLE [6]. Recently, the role of Fc-gamma-receptors (FcγR) has
renewed the interest in IgG subclasses. First, the important role of FcγR in the mediation
of an inflammatory reaction by immune complexes has been shown in NZB/NZW mice.
These mice spontaneously develop glomerulonephritis. Deficiency of the γ chain of the
FcγR protected them from severe nephritis although immune complex deposition and
complement activation were unaltered [25]. Secondly, polymorphisms of FcγR can
influence the interaction with IgG subclasses, in particular with IgG2 [26]. This is highly
relevant for the second Fcγ-receptor (FcγRIIa) in which the presence of either arginin or
histidin at position 131 (FcγRIIa-R131 and FcγRII-H131, respectively) determines the
interaction with IgG2 and, to a lesser extent, IgG3. In contrast to FcγRIIa-H131, the
FcγRIIa-R131 isoform can not interact with IgG2 and has less affinity for IgG3. It has
been suggested that patients homozygous for FcγRIIa-R131 will be prone to the
development of lupus nephritis due to decreased clearance of complexed IgG2 and IgG3-
subclass antibodies. Indeed, in several studies the FcγRIIa-R/R131 genotype was sig-
nificantly more present in patients with proliferative lupus nephritis. However, there is
still controversy, as in most studies no skewing of FcγRIIa-R131 was observed in lupus
patients with compared to those without nephritis [27].
In conclusion, IgG1- and IgG2-subclass antibodies to nucleohistone and to dsDNA are the
predominant subclasses found in plasma of lupus patients with renal disease. The frequent
occurrence of a rise of IgG2 anti-nucleohistone and IgG1 anti-dsDNA in patients prior to
a renal relapse, suggest that, besides IgG1 subclass autoantibodies, IgG2-subclass
antibodies to nucleohistone have a particular pathophysiological role in lupus nephritis.

References

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before anti-dsDNA and/or antihistone antibodies in serum of MRL-Mp lpr/lpr and +/- mice,
and are present in kidney eluates of lupus mice with proteinuria. Arthritis Rheum
assay (ELISA) for antibodies to double stranded and single stranded DNA in patients with
IgG subclasses in SLE


