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Antibodies to C1q in systemic lupus erythematosus: Characteristics and relation to FcγRIIA alleles

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Antibodies to C1q in systemic lupus erythematosus: Characteristics and relation to FcγRIIA alleles. Autoantibodies to the collagen-like region of the first complement component (C1qAB) are found in patients with systemic lupus erythematosus (SLE), particularly those with renal disease. In a cohort of 46 SLE patients with diffuse proliferative glomerulonephritis, we found declining C1qAB titers in 77% of treatment responders and in only 38% of treatment non-responders ($P < 0.03$). To further characterize this autoantibody, we tested 240 SLE patients for the presence of C1qAB. Positive titers were found in 44% of patients with renal disease and 18% of patients without renal disease (χ^2 $P < 0.0003$). Analysis of IgG subclass revealed IgG2 C1qAB alone in 34%, IgG1 C1qAB alone in 20%, and both IgG1 and IgG2 in 46% of patients. Fewer than 10% of patients had measurable titers of IgG3 or IgG4 C1qAB. The pathogenic role of these IgG2-skewed C1qAB may relate to impaired immune complex clearance by the mononuclear phagocyte system: IgG2 antibodies are efficiently recognized by only one IgG receptor, the H131 allele of FcγRIIa (FcγRIIa-H131). In contrast, FcγRIIa-R131, which is characterized by minimal IgG2 binding, has recently been associated with lupus nephritis. In our C1qAB positive patients, the presence of FcγRIIA-R131 was associated with an increased risk for renal disease. Autoantibodies to C1q may have pathogenic significance in SLE patients with genetic defects in the ability to clear IgG2 containing immune complexes.

Autoantibodies to the collagen-like region of the first complement component (C1qAB) have been described in patients with a variety of diseases characterized by the presence of circulating immune complexes [1–9]. Systemic lupus erythematosus is the prototypic immune complex disease. In SLE, C1qAB are associated with proliferative renal disease [3, 4, 10]. Rising titers may predict an ensuing relapse of nephritis [11].

There are several different mechanisms by which C1qAB may exert a pathophysiologic role. The autoantibody could act systemically to enlarge circulating immune complexes or up-regulate activation of the classical complement pathway. Alternatively, C1qAB may bind to glomerular immune complex deposits and amplify the potential for complement activation and subsequent tissue injury; renal deposition of C1q is characteristic of proliferative lupus nephritis [12]. Several studies have addressed the subclass distribution of C1qAB [8, 13–15]. While some investigators have found a predominance of IgG2 C1qAB [8], others have detected both IgG2 and IgG3 [14, 15]. Still others have noted an autoantibody subclass distribution similar to that of normal serum [13].

The qualitative nature of the autoantibody response in the context of effector cells may determine pathogenic potential. Among the many phagocyte receptors for immunoglobulin (FcγR), only one type, FcγRIIa, is capable of binding IgG2 [16–19]. FcγRIIA has two codominantly expressed alleles (R131 and H131) that differ at amino acid position 131, and as a consequence differ substantially in their ability to bind human IgG2. No other immunoglobulin subclass exhibits this differential recognition among FcR variants [16]. FcγRIIa-H131 is the only human FcγR that recognizes IgG2 efficiently, and optimal IgG2 handling occurs only in the homozygous state [17–20]. In contrast, the R131 allele, characterized by minimal IgG2 binding, has recently been associated with lupus nephritis [20, 21]. C1qAB may thus manifest enhanced pathogenicity in patients with inherited deficiencies in the removal of IgG2 containing immune complexes. These findings suggest that genetic susceptibility to lupus nephritis may be related to the impaired handling of potentially nephritogenic autoantibodies. To examine this possibility, we used the following approach: we studied the relationship between C1qAB titers and clinical response to treatment of lupus nephritis; we determined the prevalence of C1qAB in a population of 240 SLE patients; we characterized the C1qAB IgG subclass distribution; and we analyzed autoantibody associations with renal disease and serologic markers of disease activity. Our results reveal an IgG2 C1qAB subclass predominance and a relationship between FcγRIIA alleles and C1qAB.

Key words: systemic lupus erythematosus, lupus, antibody, FcγRIIA, complement, IgG2.

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METHODS

Study subjects

African American ($N = 129$) and Caucasian ($N = 111$) SLE patients meeting at least four revised ACR criteria for disease

classification [22], together with ethnically and geographically similar non-SLE controls, were recruited from the following collaborating medical centers: Hospital for Special Surgery/Cornell University Medical Center, New York, New York; National Institutes of Health, Bethesda, Maryland; and SUNY Brooklyn/Downstate Medical Center, New York, New York. Patients included previously reported Caucasians and African Americans [20] in whom sera was available ($N = 190$), and additional patients recruited from these centers over the next year. There were 19 men and 221 women. The mean age was 41 ± 12.9 years, and the mean duration of SLE 11 ± 9.0 years. The mean daily dose of prednisone was 12.5 mg; 20% of patients were receiving additional immunosuppressives (cyclophosphamide, azathioprine, or methotrexate).

Renal disease was defined as the presence of any of the following manifestations: proteinuria, (> 500 mg/24 hr or 3+), cellular casts, or renal biopsy diagnostic of glomerulonephritis. Renal biopsy data were available on 75 patients: 3 patients had WHO Class II lesions, 16 WHO Class III, 47 WHO Class IV, and 9 WHO Class V. Laboratory values recorded at study entrance included recent anti-DNA antibody titers, complement levels, and quantitative or qualitative measurements of proteinuria. Whole blood and sera were stored at −70°C. Patients were entered into the study without regard for disease activity.

For the longitudinal study, sera from ethnically heterogeneous SLE patients ($N = 46$) with WHO class IV lupus nephritis were stored at the time of renal biopsy and at one year following nephritis treatment. Treatment regimens included: (*1*) i.v. cyclophosphamide monthly for six months followed by every three months for a minimum of 2½ years ($N = 21$); (*2*) i.v. methylprednisolone monthly for a minimum of 12 months to a maximum of three years ($N = 13$); and (*3*) the combination of regimens 1 and 2 ($N = 12$). A clinical response to treatment was defined by the presence of all of the following: (*1*) < 10 RBC/HPF, (*2*) absence of cellular casts, (*3*) < 1 gram of proteinuria, and (*4*) failure to double serum creatinine. The effect of treatment assignment on clinical response has been published elsewhere [23].

Indirect ELISA for C1qAB

Both basic and subclass ELISAs for C1qAB were performed using a modification of the methods of Wisnieski and Jones [8]. Whole C1q was purified from human plasma by the method of Tenner, Lesavre and Cooper [24]. Ninety-six well microtiter plates (Immulon 4; Dynatech Laboratories, Chantilly, VA, USA) were coated with C1q at 10 μg/ml in PBS, stored at 4°C overnight, and blocked prior to use with gelatin. Test sera were diluted 1:50 in gelatin-PBS containing 0.75 M NaCl (GPBS-5x), and plated in triplicate. Plates were incubated 60 to 90 minutes at 25° and washed three times with veronal buffered saline (VBS) containing 0.1% Tween 20 (Sigma Chemicals, St. Louis, MO, USA). An alkaline phosphatase-conjugated goat anti-human IgG Fc (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) was added for 60 minutes, followed by repeat washings, and the addition of paranitrophenyl phosphate (Sigma) in a diethenolamine buffer. Color was allowed to develop until dilutions of a positive standard serum run on each plate met pre-defined values along the linear portion of its binding curve (specifically, the 1:800 dilution gave an OD_{405} of 0.2/well). The upper limit of normal, based on the mean ± 5 SD of 30 normals run on three occasions, was chosen to eliminate false positives and low-level true positives which would not be detectable in the subclass assay. Sera with values > 0.17 ODU per well at 1:50 dilution were defined as positive for C1qAB. A decline in titer was defined by a 50% or greater drop in ODU, based on interplate variability.

To exclude the possibility that immune complexes contribute to the C1q binding measured in this assay, we performed experiments with two types of immune complexes (bovine IgG-rabbit anti-bovine IgG and cryoglobulinemic sera, IgG-IgM). Immune complexes of bovine IgG and rabbit anti-bovine IgG were tested for solid phase C1q binding at 0.15 M, 0.75 M, and 1.2 M NaCl. C1q binding was robust at 0.15 M, undetectable or barely detectable at 0.75 M [< 10% of the positive control serum, which was a mid-range positive serum from a patient with the hypocomplementemic urticarial vasculitic syndrome (HUVS)] and undetectable at 1.2 M NaCl. There was no decrease in binding of average HUVS serum to C1q at 1.2 M versus 0.75 M NaCl. We also tested for solid-phase C1q binding using sera from three patients with cryoglobulinemia, one type II and two type III. The sera were tested at dilutions of 1:10 and 1:40 with binding simultaneously measured at 0.15 M and 0.75 M NaCl. In 0.15 M NaCl, there was measurable C1q binding by IgM-IgG complexes in 1:10 dilution of these sera which decreased by approximately 50% at a 1:40 dilution. In 0.75 M NaCl, C1q binding decreased by 90% relative to binding at 0.15 M NaCl for both dilutions of sera. Concurrently, C1q binding of an HUVS sera (1:200 and 1:800 dilutions) decreased by only 50 to 60%, and substantial binding activity remained at 0.75 M NaCl. These observations argue against immune complex Fc-mediated binding as a sigificant contributor to the C1q binding.

ELISA for IgG subclasses of C1qAB

The subclass ELISA was adapted from the method of Wisnieski and Jones [8]. Each of four 96-well plates (Dynatech) was coated with triplicate standard dilutions of a single myeloma protein (IgG1, IgG2, IgG3, or IgG4; The Binding Site, Birmingham, UK), and incubated five hours at 38 to 42°C to allow adherence to the plate. The remaining wells were coated with whole C1q(10 μg/ml) at room temperature, and the plates stored at 4°C overnight. Prior to use, plates were blocked for two to three hours with GPBS-5x. Sera were diluted 1:20 in GPBS-5X, plated in triplicate, and incubated 60 to 90 minutes. After washing three times with VBS containing 0.1% Tween 20 (Sigma), plates were incubated with the relevant peroxidase-conjugated polyclonal sheep anti-human subclass reagent (The Binding Site). In preliminary experiments, each antibody was titrated to yield a consistent standard curve over the dilutions of myeloma protein used. There was no cross-reactivity in subclass recognition within this range. Plates were developed using o-phenylenediamine (Sigma) with H_2O_2 in a citrate buffer. The reaction was stopped at 30 minutes with 2 N H_2SO_4, and the OD_{492} recorded. Results are reported as nanograms/well of each subclass extrapolated from the linear portion of the corresponding myeloma standard curve (linear range: IgG1, IgG2, IgG4 0.25 to 2.5 μg/ml; IgG3 0.03 to 0.25 μg/ml). High titer sera were subjected to serial dilution.

Radial immunodiffusion for total serum IgG1 and IgG2

Radial immunodiffusion was performed using pre-prepared plates containing monospecific antiserum to IgG1 or IgG2 (The Binding Site). Each plate contained both a subclass specific calibrator and standard control serum. Serum samples (5 μl)

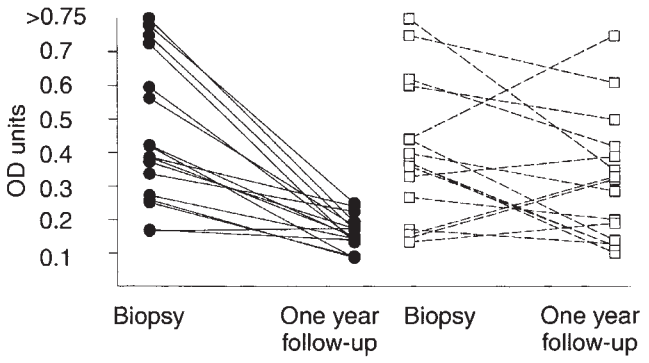

Fig. 1. Correlation of declining C1qAB titers with clinical response to treatment of DPGN. Clinical response was defined in the **Methods** section. A decline in titer was defined as a 50% change in OD units. Responders (●) had declining C1qAB titers more often than non-responders (□); $P < 0.03$.

diluted 1:10 in 7% BSA were pipetted into test wells. Diffusion was allowed to progress for 72 to 96 hours, at which point each calibrator reached a pre-defined diameter. Precipitin ring diameters of test samples were read to 0.1 mm using an eye piece and converted to mg/ml of immunoglobulin using established tables. IgG subclass values were used to calculate percentage of subclass C1qAB relative to total serum subclass.

FcγRIIA genotyping

The method used in our laboratory for FcγRIIA genotyping has been published previously [25]. Briefly, DNA was isolated from peripheral blood leukocytes using Puregene reagents (Gentra Systems, Minneapolis, MN, USA). A 1 kB portion of the FcγRIIA gene containing exon 4 and 5 was amplified by polymerase chain reaction in a gene-specific fashion using primers described by Osborne et al [25]. The PCR products were denatured and applied to a Hybond-N in duplicate using a Bio-Dot apparatus (Bio-Rad Laboratories, Hercules, CA, USA). The blots were probed with allele-specific oligonucleotides 3′-end labeled with digoxigenin, immuno-detected with an alkaline phosphatase-conjugated anti-digoxigenin antibody, and visualized with a colormetric substrate system (Boehringer-Manheim, Indianapolis, IN, USA). In 22 of 22 subjects tested, phenotypes determined by quantitative flow cytometry were confirmed to be concordant with genotypes.

Statistical analysis

Comparisons between groups were made using the Chi square, Mann-Whitney U, Fisher's exact, or *t*-tests as appropriate. A probability of less than 0.05 was used to reject the null hypothesis. Statistical analysis was performed with the aid of the Research and Methodology Core of the Hospital for Special Surgery.

RESULTS

C1qAB longitudinal study

Sera from SLE patients ($N = 46$) with diffuse proliferative lupus nephritis were stored at the time of renal biopsy and at one year following initiation of nephritis treatment. Thirty-three patients [clinical responders ($N = 17$) and non-responders ($N = 16$)] had at least one positive test for C1qAB (Fig. 1). Responders and non-responders did not differ with respect to race, age, serum creatinine, or degree of quantitative proteinuria at study entry. Among clinical responders, 77% demonstrated declining C1qAB titers. In contrast, only 38% of non-responders exhibited significant declines in titer ($P < 0.03$, Fisher's exact test). Non-responders more often had rising titers after one year of treatment ($P = 0.62$). There was no significant difference in treatment regimens between patients with declining C1qAB and those with rising or unchanged titers (cyclophosphamide containing regimens vs. methylprednisolone alone, $P = 0.17$). The association between C1qAB and clinical response to nephritis treatment suggests that these autoantibodies may play a role in disease pathogenesis.

Table 1. Demographics and laboratory parameters of C1qAB(+) and C1qAB(−) patients

Data[a]	C1qAB(+)	C1qAB(−)	*P* value
Mean age in years[b] ($N = 232$)	36.6 ± 13.2	42.9 ± 12.2	0.0004
Mean duration SLE in years ($N = 226$)	10.2 ± 9.3	11.4 ± 8.8	0.134
% ↓ C3 ($N = 186$)	52%	32%	0.01
% ↓ C4 ($N = 169$)	52%	23%	0.002
% Anti-DNA + ($N = 157$)	57%	30%	0.001

[a] A total of 240 patients were tested for C1qAB. Numbers in parentheses indicate the population on whom information was available. Laboratories were obtained within three months of testing for C1qAB.
[b] Results are expressed as the mean ± SD

C1qAB cross sectional study

To characterize the association between C1qAB and renal disease in our population and to identify patients for subclass analysis, we performed a multicenter cross-sectional study on 240 SLE patients. Both renal ($N = 139$) and non-renal ($N = 101$) patients were tested for IgG C1qAB by indirect ELISA. Overall, autoantibodies to C1q were found in 33% of patients. C1qAB were found in 44% of patients with renal disease and 18% of patients without renal disease, a difference that was highly significant (χ^2, $P < 0.0003$). Rates of antibody positivity were similar between African American and Caucasian patients (30% vs. 40% respectively, $P = 0.17$). Among all patients with C1qAB ($N = 78$), 77% had renal manifestations of SLE. The positive predictive value of C1qAB for the presence of renal disease was therefore 77%, and the negative predictive value 51%.

The demographic and laboratory parameters of the C1qAB positive and negative patients are summarized in Table 1. Patients with the autoantibody were younger; they were more likely to be hypocomplementemic, and to test positive for antibodies to DNA. The magnitude of proteinuria, graded as 1 to 4+, was positively associated with the presence of C1qAB ($P < 0.001$, Mann Whitney U). Mean prednisone dose was higher in the group with C1qAB (20 mg q.d. vs. 8.8 mg q.d., $P < 0.001$, Mann-Whitney U test) but the use of additional immunosuppressives was not associated with autoantibody positivity. There was no relationship between SLE disease duration and the presence of C1qAB.

Among patients with renal disease who were positive for C1qAB ($N = 61$), biopsy data were available on 41. Two patients had WHO Class II lesions, 9 had WHO Class III, 26 had WHO class IV, and 4 had WHO Class V. Therefore, 85% (35 of 41) of biopsied patients with C1qAB had focal or diffuse proliferative glomerulonephritis.

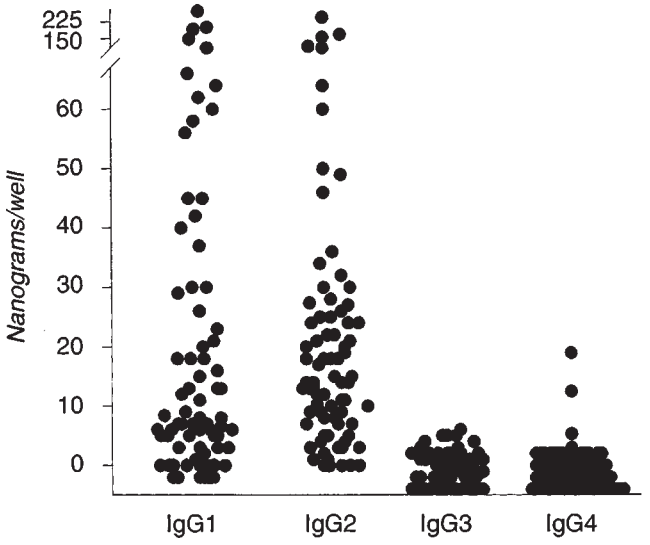

Fig. 2. Subclass distribution of C1qAB. Each symbol (●) represents a single patient within each subclass. Statistical analysis was performed on values greater than 2 SD above the mean of 30 healthy normals (cutoffs: IgG1 = 12.2 ng; IgG2 = 15.6 ng; IgG3 = 2 ng; IgG4 = 5 ng).

C1qAB subclass distribution

Available sera testing positive for IgG C1qAB (N = 77) from Caucasian and African American patients in both the cross-sectional and longitudinal studies were analyzed for C1qAB IgG subclass distribution. Results were expressed as ng/well of C1qAB subclass. The predominant IgG subclasses were IgG2 and IgG1 (Fig. 2). Fifty-six patients had C1qAB subclass values greater than 2 SDs above the mean of 30 healthy normals. Of these, 34% of patients had IgG2 C1qAB only, 20% had IgG1 C1qAB only, and 46% had both IgG1 and IgG2 C1qAB. Therefore, IgG2 C1qAB was present in 80% of patients. The percent of patients with IgG2 C1qAB did not differ between African Americans and Caucasians (80% vs. 82%, respectively). Rare patients had detectable amounts of IgG4 C1qAB. Despite a tenfold increased sensitivity of the IgG3 assay, no patient had detectable nanogram quantities of IgG3 C1qAB.

We considered the possibility that cyclophosphamide therapy may have altered the C1qAB IgG subclass distributions of our patients. However, only five patients with detectable C1qAB in the subclass ELISA were receiving cyclophosphamide therapy. The remainder were begun on therapy after sera were obtained or had received remote treatment. Of these five patients, two had IgG1 C1qAB only, and three had both IgG1 and IgG2 C1qAB, arguing that the IgG2 skewing that we observed is not an artifact of cyclophosphamide treatment.

A subset of sera from patients with IgG1 or IgG2 C1qAB (N = 43) were assayed for total serum IgG1 and IgG2 levels by radial immunodiffusion. The mean total serum IgG1 was 8.8 ± 4.1 mg/ml; the mean total serum IgG2 was 2.8 ± 1.3 mg/ml. These levels did not differ between African Americans (IgG1 9.6 ± 3.7 mg/ml; IgG2 3.1 ± 1.6 mg/ml) and Caucasians (IgG1 8.2 ± 4.3 mg/ml; IgG2 2.6 ± 1.1 mg/ml). The mean ratio of G1/G2 (3.8 ± 2.4 mg/ml) was similar to that reported in the literature for disease free individuals [26]. However, the percentage of IgG2 C1qAB relative to total IgG2 was significantly greater than the percentage of IgG1 C1qAB relative to total IgG1 (0.04 ± 0.06% vs. 0.01 ± 0.02% respectively, $P < 0.005$, t-test). Thus, in our patient population the IgG2 component of the autoantibody response to C1q is disproportionately enriched relative to the overall IgG subclasss distribution, as no alteration in IgG subclass distribution was noted.

FcγRIIA genotyping analysis

The R131 allele of FcγRIIA has been associated with lupus nephritis in African Americans; a parallel association in Caucasians is controversial [20, 21, 27]. FcγRIIa-R131 is linked to impaired internalization of IgG2 opsonized immune complexes [16–20]. Given the IgG2 predominance of our C1qAB, we questioned whether the presence of R131 confers an additional risk for renal disease in those with known C1qAB. We determined the FcγRIIA genotypes of 78 SLE patients from the cross-sectional study who tested positive for C1qAB from whom DNA was available, and found that 32% of patients were R131 homozygotes, 46% R131/H131 heterozygotes, and 22% H131 homozygotes.

Among Caucasians with C1qAB (N = 41), there were 27% R131 homozygotes, 46% R131/H131 heterozygotes, and 27% H131 homozygotes. Renal disease was present in 87% (26 of 30) of patients with an R131 allele. In contrast, in C1qAB positive patients homozygous for H131, only 55% had renal disease. In this group of C1qAB positive Caucasians, the presence of FcγRIIA-R131 was associated with an increased risk for renal disease [χ^2, P = 0.03; odds ratio for risk of nephritis with R131-containing genotypes 5.4 (95% C.I. 0.9 to 36.3)].

Among African Americans with C1qAB (N = 37), there were 34% R131 homozygotes, 55% R131/H131 heterozygotes, and 16% H131 homozygotes. Renal disease was present in 24 of 31 patients with R131 and 4 of 6 patients homozygous for H131. Due to the small number of H131 homozygotes, we were unable to demonstrate an enhanced risk for renal disease among African Americans with FcγRIIA-R131 versus H131.

A parallel analysis was performed on patients whose sera tested positive for antibodies to double stranded DNA within six months of C1qAB testing (N = 66). Among patients with anti-DNA antibodies, there was no relationship between the presence of R131 and the risk for renal disease (P = 0.6 in both African Americans and Caucasians). These findings were expected given the predominance of IgG1 and IgG3 subclass in antibodies to DNA reported in the literature [28, 29].

DISCUSSION

In 1971, Agnello et al [30] first noted the presence of a low molecular weight reactant in the sera of SLE patients which possessed binding activity in the solid phase C1q binding assay. Subsequently, Uwatoko and Mannik [1] confirmed the existence of monomeric IgG with specificity for the collagen-like region of C1q. C1qAB has since been described in patients with a variety of immune mediated diseases including systemic lupus erythematosus (SLE), hypocomplementemic urticarial vasculitic syndrome (HUVS), idiopathic membranoproliferative nephritis, and rheumatoid arthritis [1–9].

The association of C1qAB with proliferative lupus nephritis is now well established [3, 4, 10, 11]. The mechanisms mediating autoantibody pathogenicity remain unclear. In the present study, we initially evaluated the relationship between C1qAB levels and

clinical response to nephritis treatment. Treatment responders were more likely to demonstrate declining C1qAB, supporting a role for these autoantibodies in disease pathogenesis. It has been proposed that C1qAB may act systemically by up-regulating activation of the classical complement pathway [31]. Alternatively, C1qAB may act locally within the renal glomerulus to enhance tissue injury initiated by immune complex deposition. Indeed, lesions of proliferative lupus nephritis are characterized by prominent deposition of IgG and C1q [12, 32]. Purified C1qAB have been shown to deposit in mouse glomeruli perfused with cationized immune complexes containing human C1q [33].

An alternative hypothesis is that the fate of C1qAB bound immune complexes is partially dependent on the integrity of the mononuclear phagocyte system. In this model, efficient leukocyte FcγR function would constitute a prerequisite for the optimal clearance of such complexes, placing importance on FcγR specificity and subclass affinity. Human leukocyte receptors for IgG are diverse in structure and function [16]. There are three families of FcγR (FcγRI, FcγRII, and FcγRIII), each containing multiple distinct genes and alternative splicing variants. FcγRIIa, the most ubiquitously expressed FcγR, is present on monocytes, neutrophils, eosinophils, basophils, tissue macrophages, endothelial and dendritic cells. FcγRIIA bears an allelic polymorphism with functional significance: the two codominantly expressed alleles, R131 and H131, differ substantially in their ability to ligate human IgG2. FcγRIIa-H131 is the only human FcγR which recognizes IgG2 efficiently. In contrast, FcγRIIa-R131, characterized by minimal IgG2 binding, is increased in frequency among patients with lupus nephritis [20, 21]. Given the preliminary evidence that C1qAB may demonstrate an IgG2 subclass skewing, we sought to characterize the C1qAB subclass distribution in our large population and examine the relationship between allelic variants of FcγR and the presence of C1qAB.

Our cross-sectional results are consistent with prior studies showing a C1qAB prevalence of 30 to 50% among SLE patients [3, 4]. The relatively low percentage of titers among our renal patients likely reflects the wide variability in nephritis activity within this group at the time of testing. C1qAB were more likely to occur in younger patients, in agreement with the observation of Siegert et al [34]. This may reflect the tendency for renal disease to occur early in the course of SLE or the presence of an older population with remote disease. In our population, C1qAB were associated with antibodies to DNA and low levels of C3 and C4. These findings are consistent with laboratory correlates found in prior studies [3, 4, 35].

The C1qAB in our population were predominantly of IgG2 and IgG1 subclasses. This distribution is consistent with that found by Wisnieski and Jones in a study characterizing C1qAB in patients with SLE ($N = 13$) and hypocomplementemic urticarial vasculitis ($N = 12$) [8], but contrasts with the IgG3 and IgG2 predominance reported by Coremans et al in patients with SLE ($N = 30$) [15]. That this IgG2-skewed autoantibody response occurred despite normal serum levels of total IgG1 and IgG2 demonstrates that it did not reflect an alteration in overall IgG subclass distribution.

Immune complexes composed of IgG2 subclass and antigen activate complement less efficiently than complexes composed of IgG1 or IgG3 and antigen, and are therefore primarily dependent on efficient Fcγ receptor function for adequate internalization by phagocytes. The R131 allele of FcγRIIA, characterized by minimal IgG2 binding, has recently been associated with SLE nephritis in African Americans [20]. In contrast, among Caucasian SLE patients skewing of FcγRIIA alleles was documented in only one of three studies [20, 21, 27]. Accordingly, we explored the possibility that allelic variants of Fcγ receptors may have particular pathogenetic importance in the setting of C1qAB. Among Caucasians with C1qAB, the presence of R131 was associated with an increased risk for renal disease. This contrasts with previous reports showing a lack of FcγRIIA allelic skewing in Caucasian nephritis patients [20, 27], and suggests that, while not sufficient to identify Caucasian patients at risk for renal disease, FcγRIIA-R131 may have predictive value in the setting of pathogenic autoantibodies. It is possible that the association of R131 with renal disease in a Caucasian SLE population from the Netherlands [21] may reflect local differences in C1qAB prevalence.

Among African American patients with C1qAB, H131 was extremely uncommon. This is consistent with previous reports documenting an increased frequency of FcγRIIA-R131 among African Americans with SLE, and suggests that H131 may serve as a protective gene for this ethnicity. Due to the scarcity of H131 homozygotes, we were unable to show a significant association between FcγRIIA-R131 and the presence of renal disease among African Americans with C1qAB. We cannot exclude the possibility that C1qAB and other IgG2 autoantibodies are important contributors to nephritis in this population.

Although few studies have addressed the question of IgG subclass distribution in the glomeruli of patients with lupus nephritis [36–39], a recent series suggests that IgG2 may be a predominant subclass in proliferative lesions [39]. Renal deposition of IgG2, whether indicative of C1qAB or other autoantibodies, supports the relationship between FcγRIIA genotype and predisposition to lupus nephritis. Our data suggest a link between deficiencies in effector cell function and impaired handling of potentially nephritogenic autoantibodies. We anticipate that longitudinal studies will reveal definitive relationships between FcγRIIA alleles and C1qAB.

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