Abstract

**Background.** FcγRIIa is a low affinity receptor that has two co-dominantly expressed alleles, R131 and H131, which differ in their ability to bind immunoglobulin G (IgG) subclasses. Cells expressing H131 bind more efficiently complexed IgG2 and IgG3 than those expressing the R131 variant. The FcγRIIa polymorphism has been shown to be associated with lupus nephritis. Here we evaluated the relevance of FcγRIIa gene polymorphism in the development of lupus immune complex (IC)-mediated nephritis by comparing the genotype and allelic distribution of this receptor in lupus nephritis to ethnically matched healthy controls in Brazilians.

**Methods.** 119 systemic lupus erythematosus (SLE) patients and 48 healthy volunteers were recruited. FcγRIIa genotyping was performed by PCR with allele-specific primers to distinguish between the two allelic forms (H131 and R131).

**Results.** Comparison of FcγRIIa genotypes distribution in SLE patients with nephritis and in controls showed a significant increase in FcγRIIa-R131 homozygosity (P ≤ 0.02). The genotype distribution in lupus nephritis (45% with FcγRIIa-R/R131, 30% with H/R131 and 25% with H/H131) was distinct from that observed in controls (21% with FcγRIIa-R/R131, 52% with H/R131 and 27% with H/H131). In contrast, there was no difference in the distribution of FcγRIIa genotypes in lupus without nephritis and controls (P = 0.3). Reinforcing the relevance of FcγRIIa polymorphism in IC-mediated nephritis, patients with renal failure had an over-representation of the R131 allele (70%) when compared with normal controls (47%) (P = 0.06).

**Conclusions.** The skewed distribution of FcγRIIa genotypes with the predominance of homozygous R/R131 genotype observed in lupus nephritis emphasizes its importance as a heritable risk factor for IC-mediated renal injury in Brazilian lupus patients.

**Keywords:** autoimmunity; Fc receptors; glomerulonephritis; immunoglobulin; systemic lupus erythematosus

Introduction

Immunoglobulin G (IgG)-containing immune complexes (IC) are involved in the pathogenesis of several nephropathies including systemic lupus erythematosus (SLE) glomerulonephritis (GN) [1]. Abnormal clearance of IgG IC is considered to be an important factor in the pathogenesis of GN, particularly in SLE nephritis. This clearance depends essentially on cellular expression of receptors for IgG (FcγR) [2,3]. Three classes of FcγRs have been identified on human cells of the immune system: a high affinity receptor FcγRI (CD64) and low affinity receptors, FcγRII (CD32) and FcγRIII (CD16). The FcγRII binds only multimeric IgG IC and is predominantly expressed on myeloid cells. Three isoforms, FcγRIIA, FcγRIIB and FcγRIIC, have been recognized and have divergent functions, stimulatory or inhibitory, depending on the presence of immunoreceptor tyrosine-based activation/inhibition motifs (ITAM/ITIM) in their intracytoplasmic domain [3]. The stimulatory FcγRIIA is present on mononuclear phagocytes, neutrophils and platelets and has two co-dominantly expressed alleles, R131 and H131, which differ at amino acid position 131 (arginine or histidine, respectively) in the extracellular domain of the molecule. This amino acid substitution strongly influences their ability to bind human IgG2 [3].
binds IgG2 in humans, whereas R131 binds poorly. As this subclass of immunoglobulin is not a potent activator of the classical complement pathway, FcγRIIa-H131 is essential for an efficient handling of IgG2 IC [4].

Allelic variants of FcγRII are common within the population and can influence, in certain environmental and genetic contexts, susceptibility to IC-mediated diseases, particularly SLE. The possible relevance of the FcγRIIa polymorphism in SLE is further supported by the absence of a skewed distribution of this receptor in other autoimmune diseases such as thyroiditis, multiple sclerosis and primary phospholipid syndrome [5]. Evidence for a role of FcγRIIa polymorphism in susceptibility to SLE nephritis is supported by a number of studies, but a unique allelic variant profile as a risk factor for the development of renal disease is still controversial [6,7]. Decreased H131 allelic and FcγRIIa-H/H131 genotype frequencies have been described in Korean lupus patients, particularly in those with nephritis [8] whereas an over-representation of FcγRIIa-R131 homozygosity in patients with SLE nephritis was found in Dutch and African-American SLE patients compared with healthy controls raising the possibility of ethnic differences [9–11].

The aim of present study was, therefore, to analyse the FcγRIIa genotype and H/R131 allelic distributions in a large population of Brazilian patients with SLE nephritis compared with patients without nephritis.

**Subjects and methods**

**Subjects**

Blood samples were collected from 119 Brazilian patients with SLE and 48 healthy individuals. Patients were scored in two pre-defined ethnic groups, Brazilian Caucasians (n = 106) and Afro-South Americans (n = 13). All patients with SLE fulfilled the American College of Rheumatology 1982 revised criteria for the disease and were followed up in the Rheumatology and Nephrology Outpatient Clinics of the University of São Paulo Hospital. SLE nephritis was defined by ≥0.5 g/24 h proteinuria and presence of cellular casts in the absence of infection in urinalysis. Renal flare was defined by appearance of hypocomplementaemia, proteinuria and leukocytes in the urine sediment. Renal failure was defined by serum creatinine levels >1.4 mg/dl. SLE patients with other associated disorders such as essential hypertension, diabetes and unrelated GN were excluded. Kidney biopsy was performed in 33 of 63 SLE patients with nephritis. Histopathologic findings were recorded according to the 1995 World Health Organization (WHO) classification criteria for lupus nephritis. All SLE patients presented renal changes with the predominance (58%) of membranous nephropathy (WHO class V) and 42% had focal or diffuse nephropathy (WHO classes III and IV), a usual recruitment probably due to the hyperspecialized medical care assistance of the University of São Paulo Hospital. This study was approved by the Research Ethics Committee of the University of São Paulo and all patients provided informed consent.

**FcγRIIa genotyping**

FcγRIIa allotypes were assigned in all patients by nested PCR using genomic DNA isolated from the peripheral blood leukocytes (GFXTM Genomic Blood DNA Purification Kit, Amersham Pharmacia Biotech). A 1 kb portion of the FcγRIIa gene, containing exon 4 and part of exon 5, separated by an intron, was amplified by PCR using sense primer P63 (5′-CAAGCCTCTGGTCAAGGTC) and anti-sense primer FcRII-3′ (5′-CAATGACCACAGCCACAA TC). Nested PCR was next performed using the specific sense primers, 494A and 494G (5′-ATTTCTCCCGAGTTTGAGATC), respectively, and P52 as antisense primer (5′-GAAGAGCTGCCCATGCTG) (Figure 1).

PCRg was performed by adding 100 ng of genomic DNA into a 30 ml Tris-buffered PCR reaction mixture containing 1.2 mM MgCl2, 0.2 mM each of 4 dNTPs, 0.5 mM of each primer (P63 and FcRIH3) and 0.1 U of Taq DNA polymerase (Gibco BRL). The amplification procedure consisted of an initial denaturation step at 94°C for 3 min followed by 29 cycles of denaturation at 94°C for 30s, annealing at 60°C for 1 min and extension at 72°C for 40 s, and final extension at 72°C for 5 min. Two microlitres of PCRg amplification products were further subjected to nested-PCR using 0.3 mM of sequence specific primers 494A in a PCR2 and 494G in a PCR5, respectively, mixed with the same amount of antisense P52 primer. Nested-PCRs were performed under the following conditions: 94°C for 3 min followed by 29 cycles of denaturation at 94°C for 30s, annealing at 57°C for 1 min and extension at 72°C for 40 s, before a final extension at 72°C for 5 min. The amplified products were analysed by electrophoresis in a 1.5% agarose gel followed after staining with ethidium bromide.

The genotype of each sample was validated only after three independent PCR assays.

**Statistical analysis**

FcγRIIa genotypes (R/R131, H/H131 and R/H131) and allelic frequencies in patients with or without active nephritis and controls were compared using the χ² test (3 x 2 or 2 x 2 contingency tables). The Fisher’s exact test was used when sample numbers were small. A probability of 0.05 (two-tailed) was used to reject the null hypothesis that there is no difference in the distribution of genotypes between the groups. The proportional comparative test was also used to compare the frequency of FcγRIIa-H/H131 alleles within the groups.

**Results**

The distribution of FcγRIIa polymorphism was analysed in 119 Brazilian patients with SLE and compared with healthy controls (Table 1). An abnormal, but not significant increase in the frequency of the R131 homozygous genotype was observed in SLE patients when compared with the distribution of 48 healthy controls (P = 0.05).

In order to evaluate the possible influence of FcγRIIa genotypes in the development of nephritis, samples from SLE patients with renal involvement were compared with those of the healthy individuals.
A skewed distribution of FcγRIIa genotypes was found with a significant over-representation of R/R131 (45%) in SLE nephritis group (\(n=63\)) compared with normal control (\(P=0.02\)). In contrast, SLE patients without nephritis (\(n=56\)) presented a normal distribution of FcγRIIa genotypes when compared with control (\(P=0.3\)) (Table 1). No significant difference in FcγRIIa genotype frequency was seen between the two ethnic groups, Brazilian Caucasians (\(n=106\)) and Afro-SouthAmericans (\(n=13\)), within the SLE studied population (not shown).

The relative over-representation of FcγRIIa-R131 allele (81%) paralleled by the reduction of FcγRIIa-H131 allele (19%) in patients with \(\geq2\) renal flares in 3 years, although not reaching statistical significance, had a similar tendency to that observed in lupus nephritis patients. Of note, none of the eight patients of the group with \(\geq2\) episodes of renal flares were homozygous for H131 (Table 2). Likewise, lupus patients with renal failure presented a skewed distribution of allelic frequency when compared with normal control, with an increased representation of R131 allele.

### Table 1. Distribution of FcγRIIa genotypes and alleles in Brazilian patients with SLE, with or without SLE nephritis and healthy controls

<table>
<thead>
<tr>
<th>Genotypes n (%)</th>
<th>Alleles</th>
<th>(P^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R/R</td>
<td>R131</td>
<td>0.58</td>
</tr>
<tr>
<td>H/R</td>
<td>H131</td>
<td>0.42</td>
</tr>
<tr>
<td>H/H</td>
<td></td>
<td>0.07</td>
</tr>
</tbody>
</table>

\(a^2\) test (vs healthy control).

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**Fig. 1.** Genotyping of the Fcγ receptor type IIa (FcγRIIa) by allele-specific primers using nested polymerase chain reaction (nPCR). (A) Strategy used consisted in a first genomic PCR (PCR\(_1\)) amplifying a DNA fragment between exon 4 and 5 containing the nucleotide A or G in position 494 (exon 4). A nested PCR (PCR\(_2\) and PCR\(_3\)) was then performed to amplify either the fragment containing 494A or 494G, which is responsible for the amino acid substitution at position 131 (arginine/histidine) in the second extracellular domain of FcγRIIa that underlines the FcγRIIa polymorphism. (B) Analysis of amplified DNA products of five SLE patients following PCR using the FcγRIIa-H131 specific primer (494A) or the FcγRIIa-R131 specific primer (494G) on agarose gel electrophoresis demonstrating the three possible allelic combinations (R/R131, H/H131 and H/R131, respectively).
Healthy R/R 131 genotype (FcγRIIa polymorphism and frequency of renal flares in patients with SLE nephritis

<table>
<thead>
<tr>
<th>Number of renal flares</th>
<th>Genotypes n (%)</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R/R</td>
<td>H/R</td>
</tr>
<tr>
<td>0–1</td>
<td>55</td>
<td>24 (44)</td>
</tr>
<tr>
<td>≥2</td>
<td>8</td>
<td>5 (62)</td>
</tr>
</tbody>
</table>

*Number of nephritis flares during 3 years of follow-up.  
Fisher’s exact test.

Table 3. Linkage between FcγRIIa polymorphism and severity of SLE nephritis

<table>
<thead>
<tr>
<th>n</th>
<th>Genotypes n (%)</th>
<th>Alleles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R/R</td>
<td>H/R</td>
</tr>
<tr>
<td>With renal failure</td>
<td>10</td>
<td>5 (50)</td>
</tr>
<tr>
<td>Healthy subjects</td>
<td>48</td>
<td>10 (21)</td>
</tr>
</tbody>
</table>

*Fisher’s exact test.

(70%) and a decreased frequency of H131 allele (30%), although not reaching statistical significance (P = 0.06) (Table 3).

Furthermore, analysis of FcγRIIa polymorphism in 14 SLE patients with class III and IV of WHO nephritis histological classification, SLE forms associated with inflammation, also pointed to a skewing toward the R/R 131 genotype (FcγRIIa R/R131 50%, H/R131 29% and H/H131 21%) compared with healthy controls, although not reaching statistical significance (P = 0.09).

Discussion

Our data provide evidence for a role of FcγRIIa polymorphism, as an inherited risk factor, in the pathogenesis of lupus nephritis in Brazilian patients.

The FcγRIIa genotype distribution in Brazilian healthy individuals of 21% R/R131 and 27% H/H131 is distinct from that observed in Japanese blood donors described to have >50% representation of H/H131 and <10% of R/R131 [12]. Conversely, the FcγRIIa genotype distribution described herein is similar to that observed in normal French Caucasians (18% with R/R131 and 31% with H/H131) [5] and Chinese (16% with R/R131 and 26% with H/H131) [13]. Discrepancy in genotype frequency of FcγRIIa associated with ethnic variation has been described for Japanese, Chinese and Caucasian [8]. In fact, FcγRIIa-H/H131 allotype had an increased frequency in the two former normal populations as compared with Caucasians [14].

The clearance of IC from the circulation of patients with SLE is dependent on expression and function of FcγR [2,3]. Impaired handling of IC by the mononuclear phagocyte system results in tissue deposition and is considered essential to development of inflammation such as that observed in SLE nephritis. Mechanisms of defective IC clearance in SLE involve quantitative and qualitative alterations of FcγR. Indeed, decreased FcγR expression associated with impaired function has been described in SLE [3,15,16]. In addition, FcγRIIa polymorphisms have been associated with non-optimal handling of IC in SLE [4].

The findings of the present study revealed a higher frequency of FcγRIIa-R131 genotype in Brazilian SLE patients, particularly significant for those with renal involvement when compared with ethnically matched healthy controls. This observation offers further support for an essential function of this receptor in lupus IC-mediated nephritis. The data are in agreement with previous studies of European [9], Afro-American [10] and Korean [8] SLE populations, although others have not observed a correlation between FcγRIIa polymorphism and renal involvement [6,7]. These variations point to the need of ethnically matched healthy controls from the same region of the world. In this regard, it is important to emphasize that this increased frequency of R131 genotype and alleles observed in Brazilian patients with SLE nephritis cannot be attributed to the over-representation of R131 allele (83%) reported among a population of Amazon Indians [17], as the Brazilian control populations studied here were ethnically matched.

Interestingly, the Arg-His polymorphism of FcγRIIa was shown to affect the clearance of IgG-IC in vivo favouring glomerular deposition of circulating IC in SLE patients, particularly of the IgG2 subclass [4]. Increased levels of circulating IC containing anti-nucleosome IgG2 are also found in SLE patients with renal relapses but not in SLE with extra-renal involvement, as well as in kidney biopsies [18]. Accordingly, we have observed that lupus patients presenting ≥2 renal flares in 3 years had a tendency for increased frequency of the R-131 allele (81%) when compared with patients with ≤1 renal flare (58%), although this failed to reach statistical significance. Reinforcing this finding, SLE patients with renal impairment also presented an over-representation of the R131 allele (70%) at the limit of statistically significant association when compared with normal control (47%) (P = 0.06). Nevertheless, although our data favour the role of FcγRIIa polymorphism in lupus nephritis, other types of IgG-containing IC, which are not dependent on FcγRIIa, such as IgG3 subclass, have been also found associated with renal deposits in lupus [19]. Association of FcγRIIa polymorphism with other FcR polymorphism (i.e.
FcγRIIA, which regulate IgG1 and IgG3 binding) and/or immune response alterations could provide an explanation for such findings [3].

Moreover, the apparent skewing towards the R/R 131 genotype in Brazilian SLE patients with class III and IV of WHO nephritis histological classification (50%) compared with normal controls (21%) (P = 0.064), further implicates the FcγRIIA in the pathogenesis of the proliferative forms of SLE nephritis. In this regard, a recent study has demonstrated that this low affinity FcγRIIA variant in SLE is in fact associated not only with proliferative GN but also with high chronicity indices and a more intense IgG2 glomerular deposition [20].

In conclusion, an abnormal distribution of the FcγRIIA allotypes, with an increased representation of R/R131 genotype, may contribute to the pathogenesis and/or the development of renal disease in Brazilian SLE patients.

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Conflict of interest statement. None declared.

References


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