Neutrophil-reactive autoantibodies have long been described in patients with a variety of disease states with immune neutropenia. In those early studies, the detection of autoantibodies was achieved by an indirect immunofluorescence (IIF) technique (17) with control neutrophils as the substrate (19). Subsequently, the receptors for the immunoglobulin G (IgG) Fc domain (Fc gamma receptors [FcγRs]), and occasionally their allotypic determinants, were shown to belong to the antigenic targets of these antibodies (4, 16). More recently, antibodies against different classes of FcγR, expressed or not on resting neutrophils, were found in sera from patients with autoimmune diseases, including primary Sjögren’s syndrome (pSS) and rheumatoid arthritis, irrespective of neutropenia. These anti-FcγR autoantibodies appear to be a rather heterogeneous population reactive with one or several classes of FcγRs. We have developed direct methods for the detection of such autoantibodies by enzyme-linked immunosorbent assays (ELISAs) that use each type of FcγR separately as the substrate. Given the description of sera positive by the FcγRII ELISA and negative by IIF, these assays might complement very usefully the IIF screening test with neutrophils and identify all types of anti-FcγR antibodies so that distinct autoantibodies would be characterized.

**MATERIALS AND METHODS**

**Sera.** Sera were collected from 66 patients with pSS, with all patients fulfilling the European preliminary criteria for the disease (20). Normal controls consisted of 34 sex- and age-matched healthy subjects. Three patients with idiopathic neutropenia were also tested and served as positive controls in the IIF screening test.

**Immunoelectrophoretic assay.** The ELISA used to measure immune complexes (ICs) has been described previously (1). Briefly, ICs were allowed to bind to Clq-coated plates, and IgG-containing ICs were detected by incubating the wells with horseradish peroxidase (HRP)-conjugated F(ab')2 anti-human IgG (Dakopatts, Copenhagen, Denmark).

**Screening by flow cytometry.** Sera were screened for neutrophil-reactive autoantibody by the IIF technique. Normal neutrophils were isolated from blood samples drawn into EDTA-containing tubes prepared in the laboratory. Cell suspensions containing more than 90% neutrophils were obtained by dextran T500 sedimentation (Pharmacia, Uppsala, Sweden); this was followed by Ficol- Hypaque (Eurobio, Paris, France) density gradient centrifugation and hypotonic lysis of residual erythrocytes. The cell viability exceeded 98%, as determined by staining with ethidium bromide (Sigma Chemical Co., St. Louis, Mo.).

Since some neutrophil antibodies have been shown to recognize allotypic specificities on FcγRIIB, neutrophil donors had previously been phenotyped for the NA1/NA2 and NB1+/NB1− systems (5). This preliminary phenotyping was performed by using specific polyclonal antiserum of the First International Granulocyte Serology Workshop to identify NA1+/NA2−/NB1−, NA1+/NA2+/NB1+−, and NA1+/NA2+/NB1− individuals (12). We thus identified heterogeneous NA1+/NA2+/NB1+ or NA1+/NA2−/NB1− and homozygous NA1+/NA1+ or NA2+/NA2−/NB1− individuals (J. Cartron, Kremlin-Bicêtre Hospital, Paris, France) for the ensuing IIF assays.

Two procedures were used to obviate irrelevant binding of circulating IgG to the cells. Firstly, circulating ICs were removed from the sera by precipitation with 2% polyethylene glycol (PEG; Merck, Darmstadt, Germany). PEG was dissolved in a buffer containing 0.1 M boric acid, 0.025 M disodium tetraborate, and 0.075 M NaCl (pH 8). The tubes were agitated, left on ice for 1 h, and centrifuged. In addition, the neutrophils used in the IIF assay were fixed to prevent binding of IgG-containing ICs or IgG aggregates to FcγR via their Fc domains. The fixation was carried out by a 5-min incubation at room temperature (RT) with 1% paraformaldehyde (PFA) in cold phosphate-buffered saline (PBS). In order to evaluate the effect of PFA treatment, fixed and unfixed neutrophils were incubated with heat-aggregated IgG for 1 h at 37°C. After three washes in PBS supplemented with 2% bovine serum albumin (BSA) (PBS-BSA), bound IgG was revealed as described for the autoantibodies in the IIF assay (see below). Aggregated IgG was prepared by heating Cohn fraction II (Sigma) at a concentration of 20 mg/ml in PBS for 1 h at 63°C.

Furthermore, all serum samples were extensively absorbed with a pellet of established human endothelial cells expressing major histocompatibility complex class I and class II antigens but devoid of FcγR, as described in every detail elsewhere (13).

Finally, the IIF assay was performed with suspensions of neutrophils adjusted to 5 × 10⁶/ml in PBS-BSA. One hundred microliters of cell suspension was incubated with 50 μl of serum diluted 1/10 in PBS. After 30 min at 37°C, the cells were washed three times and were incubated with a previously absorbed fluorescein isothiocyanate (FITC)-conjugated F(ab')2 anti-human immunoglobulin (Tago, Burlingame, Calif.) for 30 min at RT. This reagent had been incubated with a pellet of neutrophils, cleared by centrifugation, and tested by direct immunofluorescence to ensure that it did not bind to neutrophils. The cells were then washed another three times and were immediately analyzed by flow cytometry. An Elite flow cytometer (Coultronics, Hialeah, Fla.) equipped with a 488-nm argon laser was used to analyze 10,000 neutrophils per test. Positive cells were determined by defining a threshold with reference to an irrelevant anti-CD3 FITC-conjugated monoclonal antibody (MAb) and to the fluorescent second layer reagent applied without test serum (see Fig. 1A1 and A2). Control sera were used to set the normal threshold (see below).

**Preparation of pure FcγRs.** FcγRI and FcγRII were isolated from the monocytic cell line U937 (14) as described by Kulczycki (7) and Suzuki (18) with minor modification. The U937 cell line was purchased from the American Type Culture Collection (Rockville, Md.) and was maintained in culture in RPMI 1640 (Eu-
robi) supplemented with 10% heat-inactivated fetal calf serum (LabSystem, Sergy-Pontoise, France), 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 200 mmol of glutamine per liter (Biomerieux, Marcyl’Etoile, France) at 37°C with 5% CO2. To increase the density of FCγRI on their membranes, U937 cells were treated with 250 IU of gamma interferon (Genzyme, Cambridge, Mass.) per ml for 18 h.

To extract the FCγRI, U937 cells were solubilized. A pellet of 10^9 cells was washed three times in cold PBS. The resulting cells suspension was chilled in an ice-water bath and lysed with 0.5% Nonidet P-40 (Sigma) in the presence of protease inhibitors (10 mM iodoacetamide [Merck-Clevenot, Nogent-Sur-Marne, France] and 0.3 IU of aprotinin per ml, 1 μM pepstatin, and 2 mM phenylmethylsulfonyl fluoride [the last three compounds were from Sigma]). After 1 h, the lysates were clarified by centrifugation at 30,000 g for 40 min at 4°C. The U937 lysates were then sequentially applied to a Sepharose 4B column (Sigma) and a Sepharose-BSA column to remove nonspecific debris. The flowthrough was collected, and FCγRI and FCγRII were affinity purified on an IgG-Sepharose column. FCγRI was then separated from FCγRII by using an anti-FCγRII column (anti-CD32 MAb, clone 2E1, kindly donated by Michel Hirn, Immunotech, Marseille, France).

The F(ab’)2 anti-CD32 fragments were recovered following a 2-h pepsin digestion by passing the fraction over a protein G column and then a G-100 column (Pharmacia). The purities of the F(ab’)2 fragments were checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and their binding abilities were ascertained by testing the reagent with neutrophils in the flow cytometer with FITC-conjugated F(ab’)2 anti-mouse IgG (Tago) as a second layer. The anti-CD32 column was made by standard procedures, and the mixture of FCγRI and FCγRII was run through the column three times. FCγRI was collected in the effluent, and FCγRII eluted with 0.5 N CH₃COOH and 0.05% Nonidet P-40 in Tris (pH 8.6). Both the FCγRI and the FCγRII preparations were homogeneous, as judged by SDS-PAGE, in which proteins were silver stained and confirmed by Western blotting (immunoblotting) with anti-CD32 (Immunotech) and anti-CD64 (Seropec, Oxford, United Kingdom) MAbs to develop the blots. Purified FCγRI and FCγRII were quantitated by the Micro-BCA protein assay (Pierce, Rockford, Ill.).

The production of recombinant human FCγRIIIb has been fully described elsewhere (4a). Briefly, a CD16-encoding full-length complementary DNA-containing plasmid was used as the template, and two oligonucleotides (Xo123, 5’-GAATTCGGATCCTTAGTACCAGGTGGAGAGAATGATGA-3’; and Xo124, 5’-AAGCTTGAATTCATATGCGGACTGAAGATCTCCAAAGG-3’) were prepared to amplify the segment corresponding to the extracellular part of the molecule. A BamHI fragment was inserted into the vector M13 mp18 at the EcoRI site. One of the recombinant clones that expressed large amounts of recombinant FCγRIIIb as inclusion bodies was selected to prepare a 608-bp fragment, and the fragment was inserted into the vector pET3a at the NdeI and BamHI sites and transfected into Escherichia coli C600. Following growth in culture, bacterial cells were digested with lysozyme and DNA was lysed with 50 U of benzonase per ml. The homogenate was centrifuged so that the pellet represented CD16-enriched inclusion bodies. These were dissolved, and the solubilized material was filtered through an 8-μm-pore-size Millipore membrane and a 0.2-μm-pore-size filter. SDS-PAGE showed a silver-stained major band with a molecular mass of approximately 27 kDa. Automated NH₂-terminalsequencing established the presence of the nine expected amino acid residues.

Anti-FCγR autoantibody analysis by ELISAs. Three ELISAs were developed by using purified FCγRI, purified FCγRII, and recombinant FCγRIIIb, respec-

FIG. 1. Profiles of control and test sera in the IIF assay with normal neutrophils as the substrate. Fluorescence was analyzed on a logarithmic scale. (A) As negative controls, cells were directly incubated with an FITC-conjugated nonspecific MAb (A1) or an FITC-conjugated anti-human Ig (A2); the serum from a patient with idiopathic neutropenia was used as a positive control (A3). (B) Binding of heat-aggregated IgG on unfixed cells (B1) was prevented by parafomaldehyde treatment of the cells (B2), but the control described above for panel A3 remained positive with fixed cells (B3). (C) Representative profiles of a normal serum sample (C1), a weakly positive serum sample from a patient with pSS (C2), and a strongly positive serum sample from a patient with pSS (C3). Sera were tested on fixed cells, and the percentage of stained cells was calculated according to the threshold defined for panel A1.
tively, as capture agents. To prevent binding of IgG through its Fc portion in the IgG autoantibody test, FcRs were denatured by heating them at 95°C for 5 min and treating them with β-mercaptoethanol, which was subsequently removed by dialysis. Successful denaturation was evaluated by incubating serial dilutions of monomeric or aggregated human IgG instead of sera in the three ELISAs.

Appropriate conditions for coating the FcRs were defined in preliminary experiments in which increasing amounts of each FcR were diluted in either citrate or carbonate-bicarbonate buffer. In the subsequent anti-FcγRIII autoantibody test, microtiter plates (Dynatech, Marne-la-Coquette, France) were coated with 100 μl of FcγRIII diluted to 1 μg/ml in citrate buffer (pH 3.5) for an overnight incubation at 4°C. In the anti-FcγRI and anti-FcγRII autoantibody tests, microtiter plates (Nunc, Roskilde, Denmark) were coated with 100 μl of either antigen diluted to 5 μg/ml in carbonate-bicarbonate buffer (pH 9.6) for 2 h at 37°C and overnight at 4°C. The results were obtained by subtracting the background optical density (OD) from that of the test serum.

Rheumatoid factor (RF) was depleted from all test sera by using a column of IgG-Sepharose 4B. Bound RF was eluted from the column with acetate, and the background optical density (OD) from that of the test serum.

Validation of the anti-FcγR ELISAs. The conditions of the three ELISAs were optimized by testing three concentrations of the coated antigen in carbonate-bicarbonate and citrate buffers. Standard curves were drawn with increasing amounts of MAb anti-FcγRI, anti-FcγRII, and anti-FcγRIII to define the optimum conditions. Figure 2 shows the curves obtained in the anti-FcγRII assay.

It was essential to avoid binding of circulating IgG to the coated FcγR via the Fc portion. A treatment that combined heating and β-mercaptoethanol incubation was therefore applied to the FcγRs. Serial dilutions of human IgG prepared from Cohn fraction II were tested for their ability to recognize treated and untreated FcγRs. Human IgG had previously been heat aggregated in the FcγRII- and FcγRIII-specific assays. We observed that this pretreatment of the FcγRs efficiently

mean proportion of positive cells was 5.5% ± 6.5%. This led us to set the normal threshold at 20% stained cells, i.e., the mean plus two standard deviations. Test sera, diluted 1/10, were thus considered positive when they stained at least 20% of the cells. Positive sera were then serially diluted, and the autoantibody titer was defined as the highest dilution giving at least this percentage of stained cells. Representative profiles of sera from a healthy control (Fig. 1C1) and two patients (Fig. 2C2 and C3) are also shown.

When comparing the results of the IIF screening test before and after the removal of circulating ICs by PEG precipitation of 66 serum samples from pSS patients, 13 serum samples were positive before, and 10 of these remained positive thereafter (Table 1). Three serum samples appeared to be positive in the IIF test, and indeed, they displayed high levels of IgG-containing ICs, as established by the specific ELISA (ODs = 0.710, 0.632, and 0.438, respectively). However, another three IgG-IC-containing serum samples were negative in the original IIF assay (ODs = 0.602, 0.376, and 0.311, respectively).

To address the question of whether the NA1/NA2 and NB1+/NB1− phenotype of the neutrophil donor may influence the assay, 10 serum samples from pSS patients (found to be positive with heterozygous NA1+NA2+/NB1+ and following PEG precipitation) were tested again with heterozygous NA1+NA2+/NB1− and homozygous NA1+NB1+ and NA2+NA2+ neutrophils. As shown in Tables 1 and 2, the results of the IIF assay remained unchanged with NA2− or NB1− cells, whereas five serum samples did not react with NA1− cells. Our conclusion is that it is unnecessary to consider the NB1+/ NB1− phenotype of the neutrophil donor, while the use of NA homozygous neutrophils as the substrate in the IIF screening would miss some autoantibody-positive serum samples. Finally, 10 of 66 serum samples from pSS patients and no serum samples from healthy subjects were considered to be positive. Consequently, the IIF screening for neutrophil-reactive antibody had to be performed by using fixed cells from NA heterozygous donors with sera from which ICs had been removed.

**RESULTS**

**Optimization of the IIF screening test.** The IIF screening validation process began with the definition of positive sera. The negative and positive controls were analyzed by using heterozygous NA1+NA2+/NB1+ unfixed neutrophils as the substrate, to define the cutoff proportion of positive cells on the logarithmic fluorescence scale. According to this threshold, no stained cells were detected by the FITC-conjugated anti-CD3 MAb (Fig. 1A1) or the reagent in the second layer (Fig. 1A2), while up to 90% stained cells were obtained with the three positive controls (Fig. 1A3). Whereas nonspecific staining of unfixed neutrophils by aggregated IgG was efficiently prevented by the PFA treatment (Fig. 1B1 and B2), these positive controls showed comparable profiles when PFA-fixed neutrophils were used (Fig. 1B3).

Following the definition of these conditions, 34 serum samples from healthy subjects were tested as described earlier. The

### TABLE 1. Remaining percentages of stained cells following the precipitation of ICs from the serum of 13 patients with pSS

<table>
<thead>
<tr>
<th>Serum sample no.</th>
<th>% stained cells in the IIF assay</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before IC precipitation</td>
<td>After IC precipitation</td>
</tr>
<tr>
<td>1</td>
<td>92.9</td>
<td>92.3</td>
</tr>
<tr>
<td>2</td>
<td>49.7</td>
<td>30.4</td>
</tr>
<tr>
<td>3</td>
<td>44.6</td>
<td>32.5</td>
</tr>
<tr>
<td>4</td>
<td>92.6</td>
<td>77.0</td>
</tr>
<tr>
<td>5</td>
<td>47.5</td>
<td>55.7</td>
</tr>
<tr>
<td>6</td>
<td>63.0</td>
<td>31.0</td>
</tr>
<tr>
<td>7</td>
<td>43.7</td>
<td>38.3</td>
</tr>
<tr>
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<td>76.5</td>
</tr>
<tr>
<td>9</td>
<td>60.0</td>
<td>55.3</td>
</tr>
<tr>
<td>10</td>
<td>49.0</td>
<td>47.2</td>
</tr>
<tr>
<td>11</td>
<td>32.5</td>
<td>6.8</td>
</tr>
<tr>
<td>12</td>
<td>29.0</td>
<td>1.3</td>
</tr>
<tr>
<td>13</td>
<td>41.2</td>
<td>1.9</td>
</tr>
</tbody>
</table>

### TABLE 2. Allospecificities recognized by 10 antineutrophil-reactive serum samples defined by the IIF test

<table>
<thead>
<tr>
<th>Target neutrophil</th>
<th>Result for the following serum sample no.:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>NA1+NA2+NB1+</td>
<td>+</td>
</tr>
<tr>
<td>NA1+NA2+/NB1−</td>
<td>+</td>
</tr>
<tr>
<td>NA1+NA1+NB1+</td>
<td>+</td>
</tr>
<tr>
<td>NA2+NA2+NB1+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Five serum samples did not react with NA1-negative cells (serum samples 1, 3 to 5, and 7).
prevented the nonspecific binding of IgG (Fig. 3) but did not affect the reactivity of the MAb specific for each FcγR except for the IgG anti-FcγRIII MAb (3G8), which showed a dramatically decreased OD when it was tested with denatured FcγRIII (data not shown). This prompted us to select the IgM anti-FcγRIII as a positive control in the corresponding ELISA.

The assays were then applied to 20 serum samples from healthy subjects. They were tested at a previously determined optimal dilution of 1/100. Their mean anti-FcγRI and anti-FcγRII activities were 0.050 ± 0.030 (mean ± 1 standard deviation) and 0.092 ± 0.047, respectively. Their IgM and IgG anti-FcγRIII activities were 0.096 ± 0.008 and 0.088 ± 0.008, respectively. Test sera were considered positive when they gave an OD higher than the mean for sera from healthy subjects plus 2 standard deviations. As defined, the anti-FcγRI and anti-FcγRII cutoff values were 0.110 and 0.186, respectively. For the anti-FcγRIII assay, the IgM and IgG cutoff values were 0.190 and 0.174, respectively. The reproducibility of the ELISA was established by calculating the interassay coefficients of variation, which were 7 and 6% for the IgM and IgG anti-FcγRIII tests, respectively, and 4 and 7% for the anti-FcγRI and the anti-FcγRII antibody assays, respectively.

Application to the patients’ sera. Sixty-six serum samples from pSS patients were screened for neutrophil-reactive antibody by the IIF technique and for anti-FcγRIII antibody in the ELISA. Ten serum samples were positive by the IIF technique, as shown above (with titers ranging from 1/80 to 1/1,280); 30 serum samples were found to contain at least one isotype of anti-FcγRIII antibody in the ELISA (24 IgM and 17 IgG). Five of the 10 IIF assay-positive serum samples were thus positive in the anti-FcγIIB ELISA. Interestingly, IgM and IgG autoantibodies were detected in four and three of these serum samples, respectively. Three distinct profiles of autoantibody-containing sera were observed: those positive in both assays and those positive in only one of them. Twenty serum samples were positive in the anti-FcγRIII-specific ELISA and negative in the IIF assay. Ten of these 20 serum samples were further tested in the FcγRI- and FcγRII-specific ELISAs. Autoantibodies from one serum sample recognized the three receptors (serum sample 1 in Table 3), those from six serum samples reacted with FcγRII in addition to FcγRIII (serum samples 2 through 7 in Table 3), and those from the remaining three serum samples (serum samples 8 through 10 in Table 3) proved to be specific for FcγRIII.

DISCUSSION

Our observations suggest that the “gold standard” IIF method for the detection of total anti-FcγR antibody activity must be associated with ELISAs with FcγRs as soluble antigens. Indeed, we found that anti-FcγR autoantibodies could be identified with a given form of FcγR but not with other forms. The variety of epitopes targeted by anti-FcγR autoantibodies is likely to account for this apparent discrepancy between the IIF assay and the ELISA. One may assume that those patients testing negative by the former assay but positive by the latter one have autoantibodies to the FcγRIIIb intracellular domain. Alternatively, changes to the receptor may occur after shedding upon activation in patients with connective tissue diseases, such as pSS (11) or rheumatoid arthritis (12). Prior to testing, all of the serum samples were extensively absorbed with human endothelial cells from clones established in our laboratory (13) and were shown to express major histocompatibility complex class I and class II antigens but were shown to be devoid of FcγR (as determined by using anti-CD16, anti-CD32, and anti-CD64 MAbs). Given that platelets and B lymphocytes express FcγRIIa and FcγRIIb, respectively (6), these cells would deplete sera of anti-FcγRIIIB antibodies cross-reactive with FcγRII, as established in pilot experiments.

![FIG. 3. Standard curves obtained with serial dilutions of heat-aggregated IgG incubated in microtiter plates coated with FcγRI (circles), FcγRII (triangles), or FcγRIII (squares). FcγR had been denatured (open symbols) or not denatured (closed symbols) prior to coating.](http://cvi.asm.org/)
The distinction of IgG bound to FcγR via the Fc fragment from autoantibodies attached to the cells through their Fab regions is a prerequisite for the detection of neutrophil-reactive autoantibodies. To inhibit binding of IgG to the cells, PFA treatment of neutrophils has already been used by several investigators, leading to divergent results (15, 19). As an additional precaution, ICs were therefore precipitated with PEG, which reduced the number of IIF assay-positive serum samples from 13 to 10. Three serum samples became negative. However, the IC ELISA data for these three serum samples indicated that the amount of aggregated IgG was far less than the amount of aggregated IgG which was used to test the neutrophil PFA fixation. It would thus have been interesting to know whether the ICs of these three patients had anti-FcγR reactivities. Assuming that FcγRs might be incorporated into the target of antineutrophil autoantibodies, it was necessary to validate this method in order to ensure whether it inhibits Fc binding of IgG without affecting the specific recognition of FcγRs by the corresponding autoantibodies. We confirmed this point in the present study, since the procedure reduced background fluorescence but had no effect on the antigenic epitopes of the FcγRIIib. Inasmuch as the NA1 allotypic determinant seems to be a target of some anti-FcγRIIb antibodies, we proposed that neutrophil donors heterozygous in the NA1/NA2 allotypic system be selected for the IIF screening of anti-FcγR antibodies. Five patients (patients 1, 3, 4, 5, and 7 in Table 2) showed apparent NA1 specificity. These are autoantibodies, not alloantibodies, because all of them were NA1 homozygotes.

We have developed two additional ELISAs in which FcγRI and FcγRII, purified from the surfaces of U937 cells, were used to coat the plates. Our purification method provides functional receptors or, at least, receptors that are still able to bind to aggregated human IgG. Such receptors may be potentially useful in affinity or function studies by using inhibition experiments. In return, we had to denature them before using them to coat the microtiter plates. We described in this report a very simple treatment sufficient to achieve this. It is advisable to apply it even in the IgM autoantibody assay, in which RF may interfere with the anti-FcγR activity. Indeed, anti-FcγR autoantibodies have been described in various systemic autoimmune diseases in which RF is commonly present. We must, however, acknowledge that by denaturing the FcγR antigens to prevent ligand binding, we limit ourselves to the detection of antibodies reactive with linear amino acid epitopes. Physiologically relevant three-dimensional complex epitopes may thus be missed. This conclusion is supported by our finding that the reactivity of MAB 3G8 was reduced after the denaturation of FcγRIIb. In addition, the expression of rhuFcyRIIib in E. coli prevents glycosylation and thus detection of antisera that involve glycosylated residues, a major feature of native FcγRIIib. These antibodies could possibly react with neutrophils in the IIF assay, because some serum samples bound to the neutrophil-extracted NA1+ receptor but did not bind to its deglycosylated form in the ELISA (8). However, neutrophil antibodies from other serum samples reacted with the nonglycosylated rhuFcyRIIib, as shown previously (10) by the ELISA and Western blotting (immunoblotting) methods.

Our third ELISA was based on the use of a whole human recombinant FcγRIIib. Recently, Boros et al. used truncated murine (2) or human (3) recombinant FcγRII and FcγRIII and described anti-FcγR activity in autoimmune patients. All of these methods for the detection of specific anti-FcγR antibodies could be used in parallel in order to further elucidate the implicated epitopes. Interestingly, the presence of such a reactivity is related to the clinical presentation of the disease (9), although neutropenia is not always due to autoantibodies.

Overall, three antibody profiles could be defined (8): 5 serum samples were positive in both assays, 5 serum samples were positive in the IIF test and negative in the ELISA, and 20 serum samples were positive in the ELISA and negative in the IIF test. Previous absorption experiments (8, 10) established that the IIF assay and ELISA were directed to the same antigen. Alternatively, steric hindrance may be incriminated. Given that 20 serum samples were positive in ELISA and negative in the IIF assay, another potential explanation relates to the fact that the sensitivity of the ELISA was too high, although five serum samples were negative by the IIF assay and positive by the ELISA.

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REFERENCES

11. Lamour, A., R. Le Corre, Y. L. Pennec, and P. Youinou. 1995. The presence of anti-Fc gamma receptor autoantibodies is related to the clinical presen-