Altered IgG₄ renal clearance in patients with inflammatory bowel diseases. Evidence for a subclinical impairment of protein charge renal selectivity

Giovanni Monteleone¹, Giuseppe Cristina², Tiziana Parrello¹, Susanna Morano³, Livia Biancone¹, Patrizia Pietravalle², Elisabetta Sagratella², Patrizia Doldo¹, Francesco Luzza¹, Umberto Di Mario¹ and Francesco Pallone¹

¹Dipartimento di Medicina Sperimentale e Clinica, Universita’ di Catanzaro and ²Cattedra di Endocrinologia, Universita’ di Roma ‘La Sapienza’, Roma, Italy

Abstract

Background. A loss of intestinal glycosaminoglycans (GAGs) has been shown in inflammatory bowel diseases (IBD). Since GAGs are involved in the regulation of renal protein filtration and GAGs disruption is associated with anionic proteinuria, we examined whether changes in the selectivity of renal protein filtration occur in IBD.

Methods. From 46 patients with IBD (17 with Crohn’s disease (CD), and 29 with ulcerative colitis (UC)) and 21 healthy subjects, urine and serum samples were obtained. Albumin, total IgG and IgG₄ clearances were measured using sensitive methods. Serum p-ANCA and TNF-α were tested.

Results. Median IgG₄ clearance was 0.041 ml/min/10⁻³ in patients with UC and 0.10 ml/min/10⁻³ in CD patients, both significantly higher than in controls (0.03 ml/min/10⁻³) (P<0.03). IgG₄ clearance was above the upper normal limit in 9/17 CD (53%) and in 10/29 UC (34.5%). Eighteen of 19 patients showing abnormal IgG₄ clearance were taking mesalazine. In patients on maintenance oral mesalazine, IgG₄ clearance was higher than that in patients off treatment (0.12 vs 0.03 ml/min/10⁻³, P=0.04). No clinical/laboratory sign of renal dysfunction was documented in patients with altered IgG₄ clearance and maintained on mesalazine treatment.

Conclusion. Renal protein charge permselectivity is impaired in 40% of patients with IBD with no overt proteinuria. Our data suggest that altered IgG₄ clearance may represent a subclinical marker of renal involvement in IBD.

Keywords: antineutrophil antibodies; Crohn’s disease; proteinuria; renal permselectivity; ulcerative colitis
in the renal protein charge or size selectivity are involved. Using sensitive methods, we provide evidence that renal charge selectivity is impaired in 40% patients with IBD without overt proteinuria.

**Subjects and methods**

**Patients**

Forty-six patients with IBD, 17 Crohn’s disease (CD) and 29 ulcerative colitis (UC), mean age 35 ± 9, diagnosed according the usual clinical, endoscopic and radiological criteria were enrolled. In the CD group, the primary site of involvement was ileal in nine, ileocolonic in four, and colonic in four patients. In the UC group, disease extent was distal in 10, left-sided in nine, and substantial in 10 patients. Disease was active in 20/29 UC patients by the Truelove criteria [8] while the Crohn’s Disease Activity Index (CDAI) [9] was >150 in 6/17 CD patients. Disease duration was 7.9 ± 5.5 years in CD and 5.56 ± 5.3 years in UC. Thirty-six patients (24 UC and 12 CD) were on medical treatment (22 mesalazine and 14 mesalazine plus steroids). No patient had been prescribed immunosuppressive or antibacterial drugs over the last 12 months.

Ten patients (five UC and five CD) were not receiving any medical treatment: four were newly diagnosed, while six patients in stable remission for more than 6 years had not received any drug over the last 4 years. No IBD patient had clinical and/or laboratory signs of metabolic, renal, cardiovascular, or neoplastic diseases. As control group, 21 healthy subjects without any evidence of IBD (mean age 34 ± 8 years) were considered.

**Blood and urine sampling**

Timed overnight urine collections and serum samples were obtained from all subjects and stored at −20°C until tested. For all urinary samples, proteinuria assays were performed within 2 weeks of collection.

**Measurement of proteinuria**

In all patients and controls the urinary albumin excretion rate (UAER) and the clearances of albumin, total IgG, and IgG4 were measured as previously reported [10–12]. All urinary tests were performed on three consecutive overnight collections and the mean was used as representative of the subject’s value. Protein concentrations were measured in serum and urinary samples using a solid-phase RIA for albumin and total IgG while an ELISA was used for IgG4. Protein clearances were defined as abnormal when values higher than mean ± 3 standard deviations (SD) of those found in 20 normal blood donors were detected.

**Microalbuminuria assay**

Microalbuminuria was measured in all patients by an immunoturbidimetric method using a commercially available assay (MicroAlbs, Ames, Bucks, UK). The method was based on the reaction of albumin with a specific antibody. Precipitating immunocomplexes were formed in the presence of polyethylene glycol, producing turbidity. The turbidity was photometrically measured at 340 nm wavelength.

**p-ANCA**

ANCA were detected as previously reported [13]. Briefly, a monolayer of 200,000 neutrophils, isolated from peripheral blood of healthy subjects, was air dried in each of 96 wells in a microtitre plate, fixed in 95% ethanol, air dried again, and blocked with 0.25% bovine serum albumin in phosphate-buffered saline, pH 7.38. Sera were tested at a 1:100 dilution and bound antibody was detected using alkaline phosphatase conjugated anti-human IgG (Sigma, MO, USA). The test was considered positive for antipolymorphonuclear cells when optical density (OD) readings were above the mean + 2 SD of negative control samples (OD values of negative control sera were 0.048 ± 0.020). All samples positive by ELISA were examined by indirect immunofluorescence as previously indicated [13]. Only samples showing a characteristic perinuclear pattern (p-ANCA) were considered as positive. Sera of 20 unaffected blood donors were used as negative controls.

**TNF-α assay**

TNF-α was measured in all serum samples using a commercially available ELISA assay (Medgenix Diagnostics SA, Fleurus, Belgium). Results were expressed as pg/ml and the lowest detectable concentration was 15 pg/ml.

**Statistical analysis**

The non-parametric two-tailed Wilcoxon’s rank sum test and Student’s t-test were used as appropriate for the statistical analysis of the data. The relation between protein clearances and clinical variables was also assessed by logistic regression analysis. Prevalences of altered protein clearances in study groups were analysed by χ²-test and by Fisher’s exact test when n < 5. Relationship between serum TNF-α concentrations and IgG4 clearances was performed by Pearson’s test.

**Results**

In all but one IBD patient the albumin excretion rate was within the normal range (value < 20 µg/min). Median albumin excretion rate was 3.4 µg/min (range 0.34–19.5) in UC, 3.8 µg/min (range 0.35–33.7) in CD and 3.9 µg/min (range 1.02–12.07) in controls (P = 0.6 and 0.9 respectively). Albumin clearance was above the normal range (values > 0.3 ml/min/10⁻³) in 3/46 (6.5%) IBD patients and the median values did not differ between UC, CD, and controls (Table 1). Microalbuminuria was detected by the immunoturbidimetric assay in one CD patient showing an abnormal albumin excretion rate.

As shown in Table 1, median IgG clearance in IBD patients did not differ from that in controls. IgG clearance was above the normal range (values > 0.4 ml/min/10⁻³) in two CD patients, both with a long-standing disease and on chronic continuous steroid plus mesalazine treatment.

In 19 IBD patients (41.3%), 10 UC and nine CD, IgG4 clearance was above the upper normal limit (values > 0.09 ml/min/10⁻³). In all 19 patients the IgG4/IgG ratio was also increased. In UC and CD patients, median IgG4 clearance (0.041 and 0.10 respectively) was significantly higher than that in controls (0.03 ml/min/10⁻³) (P < 0.03) (Table 1). In
Table 1. Albumin, IgG, and IgG₄ clearances and IgG₄/IgG ratio in Crohn’s disease, ulcerative colitis, and controls

<table>
<thead>
<tr>
<th></th>
<th>Crohn’s disease</th>
<th>Ulcerative colitis</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>Interquartile range</td>
<td>Median</td>
</tr>
<tr>
<td>Albumin</td>
<td>0.09</td>
<td>0.06–0.131</td>
<td>0.04</td>
</tr>
<tr>
<td>IgG</td>
<td>0.152</td>
<td>0.053–0.245</td>
<td>0.05</td>
</tr>
<tr>
<td>IgG₄</td>
<td>0.1</td>
<td>0.02–0.5</td>
<td>0.0415</td>
</tr>
<tr>
<td>IgG₄/IgG</td>
<td>1.7</td>
<td>0.03–6</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Numbers indicate ml/min/10⁻³.

Of 10 patients (five CD and five UC) with altered IgG₄ clearance and keeping on continuous mesalazine treatment, routine urinalysis was also performed and the levels of urinary urea, electrolytes, protein and creatinine were estimated 2 years after assaying protein clearances. No alteration in renal laboratory parameters was observed in these 10 patients. Moreover, no clinical sign of renal dysfunction was documented in patients with altered IgG₄ clearance.

Discussion

In this study we report that 40% patients with IBD have abnormal renal IgG₄ clearance. In no patient with normal IgG₄ clearance were there alterations in the clearance of IgG₁ (i.e. cationic immunoglobulins), suggesting that a charge selectivity impairment was responsible for the observed change in protein clear-
 rowCount}