Deregulated platelet-activating factor levels and acetylhydrolase activity in patients with idiopathic IgA nephropathy

Yves Denizot, Corinne Aupetit, Franck Bridoux¹, Jean-Claude Alphonse², Michel Cogne and Jean Claude Aldigier

EP CNRS 118, Faculté de Médecine, 2 rue Dr Marcland, Limoges and Service de Néphrologie, CHU Dupuytren, 2 avenue Luther King, Limoges, ¹Service de Néphrologie, CHU La Milétrie, BP 577, Poitiers and ²Service de Néphrologie, CHU Gabriel Montpied, 63000 Clermont-Ferrand, France

Abstract

Background. Platelet-activating factor (PAF) is a phospholipid mediator with potent inflammatory activities. PAF stimulates IgA synthesis by B cells while IgA aggregates enhance PAF production by neutrophils and mesangial cells. These results led us to investigate blood PAF levels and plasma acetylhydrolase (AHA, the PAF catabolic enzyme) activity in patients with idiopathic IgA nephropathy (IgAN).

Methods. PAF and AHA levels were investigated using the platelet aggregation assay and degradation of ³H-labelled PAF, respectively. The genotype of AHA with regard to the G994/T mutation in exon 9 was assessed by an allele-specific polymerase chain reaction.

Results. Blood PAF levels were significantly (P = 0.003, Mann–Whitney U-test) elevated in IgAN patients (50.6 ± 6.8 pg/ml, n = 33) compared with healthy controls (18 ± 5 pg/ml, n = 18). In contrast, plasma AHA levels were significantly (P = 0.0001, Mann–Whitney U-test) reduced in patients with IgAN (61 ± 2 nmol/ml/min, n = 51) compared with healthy controls (78 ± 4 nmol/ml/min, n = 53). G994→T transversion in exon 9 of AHA was not found in any of the IgAN patients.

Conclusion. Elevated circulating levels of PAF in IgAN patients might result from an insufficient AHA probably related to environmental factors rather than genetic ones. The mechanism and the precise role of the PAF/AHA deregulation in IgAN patients remain to be clarified.

Keywords: acetylhydrolase activity; Berger’s disease; IgA nephropathy; platelet-activating factor

Introduction

Berger’s disease is a frequent idiopathic IgA nephropathy (IgAN) that occurs in the absence of any systemic disorder. It is characterized by predominant deposits of IgA in the kidney mesangium [1]. End-stage renal disease requiring dialysis or renal transplantation develops in many patients with IgAN. The pathophysiology of IgAN has not been elucidated. Immunological mechanisms seem to be involved, with a significant elevation of serum IgA1 and the formation of immune circulating complex ultimately leading to pathogenic deposits in the kidney [1].

Platelet-activating factor (PAF), a phospholipid molecule with numerous potent inflammatory activities, is implicated in several inflammatory ailments in humans [2]. Regulation of PAF concentration is of importance since elevated levels of PAF could result in pathological effects [2]. Blood PAF concentrations are regulated by acetylhydrolase activity (AHA) found in plasma [3]. The AHA gene is located on chromosome 6 and comprises 12 exons. Deficiency of PAF and AHA in IgAN. Modifications of PAF and AHA levels are reported in blood and urine from patients with renal involvement in diabetes mellitus or systemic lupus erythematosus [6,7]. PAF stimulates IgA synthesis by human B cells [8]. In turn, IgA aggregates enhance PAF production by human polymorphonuclear neutrophils [9] and human mesangial cells [10].

All these results prompted us to investigate blood PAF levels and plasma AHA in patients with idiopathic IgAN. We have also assessed patient genotypes with regard to the G994→T mutation in exon 9 of the PAF AHA gene.

Subjects and methods

Subjects

Blood samples were obtained from patients with idiopathic IgAN (originating from the Limoges, Poitiers and Clermont-Ferrand areas of France) and healthy individuals with their
informed consent and the agreement of the local ethics committee. Blood PAF levels were investigated in 33 patients (mean age 44 years, 24 men, nine women, mean creatinine clearance 88 ml/min) without haemodialysis, kidney transplantation or renal insufficiency and 18 healthy controls (mean age 47 years, seven men, 11 women). Plasma AHA was assessed in 51 IgAN patients (mean age 40 years, 44 men, seven women) and 53 healthy controls (mean age 36 years, 23 men, 30 women). Among patients, 17 exhibited renal failure (creatinine clearance > 80 ml/min) at the time they entered this study. Genotyping of the G994→T mutation of the AHA gene was investigated in 34 patients with AHA < 40 nmol/ml/min, i.e. less than half of the mean AHA in healthy controls.

Acetylhydrolase assay

Plasma samples obtained after blood centrifugation (600 g, 10 min) were stored at −80°C until AHA assay. AHA was measured by the degradation of [3H]PAF as previously reported [11]. Briefly, 105 d.p.m. of [3H]acetyl-PAF (10 Ci/mmol), 0.1 mM PAF, HEPES buffer (pH 7.8) in a final volume of 450 μl and 50 μl of diluted plasma (1:50 dilution in HEPES buffer) were incubated for 20 min at 37°C. The reaction was stopped with 100 μl of bovine serum albumin (10%) and 400 μl of trichloroacetic acid (20%). Samples were centrifuged (1500 g, 15 min) and supernatants counted in a liquid scintillation counter. Results are expressed as nanomoles of degraded PAF per ml of plasma (nmol/ml/min), as means of duplicate determinations. The variation between duplicates was < 6%.

Genotyping of the G994→T mutation of the AHA gene

DNA was prepared from isolated leukocytes. The genotype of the AHA gene with regard to the G994→T mutation was determined by an allele-specific polymerase chain reaction (PCR) as previously described [3]. Both rounds of PCR were performed under the following conditions: first, one cycle at 94°C for 5 min; secondly, five cycles at 94°C (60 s), 56°C (60 s) and 72°C (60 s); thirdly 25 cycles at 94°C (60 s), 52°C (60 s) and 72°C (60 s); and fourthly one cycle at 72°C (5 min). The sense and antisense primers for the entire exon 9 were 5′-CTATAATTTATATCATGCTT-3′ and 5′-TTTACTATTCTCTTGCTTTAC-3′, respectively. The sense and antisense primers for the partial exon 9 containing the normal sequence were 5′-CTATAATTTATATCATGCTT-3′ and 5′-TCACTAGAGGTCTGAATAAC-3′, respectively. The sense and antisense primers for the partial exon 9 containing the mutation were 5′-CTATAATTTATATCATGCTT-3′ and 5′-TCACTAGAGGTCTGAATAAC-3′, respectively. The sizes of the products formed were 160 bp for the entire exon 9 and 108 bp for the partial exon 9 containing the normal sequence or the mutation.

Platelet-activating factor assay

A 2 ml aliquot of fresh blood samples was mixed immediately with 4 vol of ethanol (80% final). Lipids were ethanol-extracted and purified on thin-layer chromatography (TLC) plates [Silica gel 60 (20 × 20 cm, 0.5 mm), Merck] as previously reported [11]. Plates were developed in a mixture of chloroform/methanol/water (70:35:5, v/v/v) with [3H]PAF as marker. Areas of samples on TLC plates with Rf values corresponding to the PAF standard were extracted, suspended in 60% ethanol and assayed for PAF activity by platelet aggregation assay [11]. Briefly, aspirin-treated washed rabbit platelets were stirred in 300 μl of Tyrode buffer containing 0.25% gelatin, 1 mM creatine phosphate and 10 U/ml creatine phosphokinase (pH 7.4). The aggregating activity of the samples was measured using a calibration curve obtained with 2.5–20 pg of synthetic PAF (Novabiochem, Switzerland). The lipid compound extracted from blood was characterized further on the basis of its aggregating activity in the presence of 0.1 mM CV 3988 (Takeda Chemical Industries, Japan) and BN 52021 (Tebu, France), two specific PAF receptor antagonists, and its retention time during TLC.

Statistical analysis

Differences between groups were assessed by Mann–Whitney U-test. A P < 0.05 was considered as significant.

Results

We first investigated the levels of PAF in blood of patients with idiopathic IgAN. As shown in Figure 1A, we found a 2.7-fold increase of PAF in the blood of these patients compared with healthy controls (50.7 ± 6.8 pg/ml vs 18 ± 5 pg/ml, P = 0.003, Mann–Whitney U-test). The PAF recovered from blood had biological and physicochemical characteristics identical to those of authentic PAF. Firstly, it induced the aggregation of washed rabbit platelets that were refractory to arachidonic acid and ADP-mediated pathways. Secondly, the platelet-aggregating activity was totally inhibited by 0.02 mM CV 3988 and BN 52021, two PAF receptor antagonists; and thirdly, on TLC, the aggregating activity exhibited a retention time similar to that of synthetic PAF (data not shown).

Elevated levels of PAF can result from lower PAF catabolism and, thus, we assessed plasma PAF AHA levels in patients with idiopathic IgAN. As shown in Figure 1B, plasma AHA levels were markedly (P = 0.0001, Mann–Whitney U-test) reduced in patients with idiopathic IgAN (61 ± 2 nmol/ml/min, n = 51) compared with healthy controls (78 ± 4 nmol/ml/min, n = 53). No difference could be documented (P = 0.13, Mann–Whitney U-test) between patients with (66 ± 5 nmol/ml/min, n = 17) and without (58 ± 2 nmol/ml/min, n = 34) renal failure. By using an allele-specific PCR, we then investigated the putative presence of the G994→T transversion in exon 9 of AHA that is responsible for the loss of its enzymatic activity. As reported in Figure 2, patients with IgAN exhibiting a plasma AHA reduced by 50% (i.e. 40 nmol/ml/min) were homozygous for the normal allele. Only one representative sample is shown but all 34 IgAN patients had the same pattern (data not shown).

Discussion

Some studies have highlighted elevated blood PAF levels in patients with several kidney diseases [6,7].
PAF is known to stimulate the release of various cytokines [such as interleukin (IL)-1, IL-6 and tumour necrosis factor α] involved in IgAN [2]. Elevated levels of PAF can result from lower PAF catabolism [2]. We have focused our attention on this hypothesis particularly since plasma PAF AHA level is affected in a wide range of pathologies with a component of inflammation [18]. We observed reduced plasma PAF AHA levels in patients with idiopathic IgAN compared with healthy controls suggesting that the decreased plasma AHA levels in patients with IgAN may partially explain the elevated circulating blood PAF levels. It is of interest that the plasma AHA levels are similar in patients with and without renal failure, indicating that the fall of the plasma AHA does not result merely from kidney failure. However, despite the fact that elevated blood PAF levels fit well with the decreased AHA levels, we cannot exclude that these elevated levels of PAF might be linked to an elevated PAF production through IgA Fc alpha receptors (FcαR) from neutrophils rather than to a reduced PAF catabolism. Indeed, neutrophils are a major source of PAF [19], IgA induces PAF release from neutrophils [9], and occupation of FcαR is increased on neutrophils from patients with IgAN [20]. Clearly this hypothesis deserves further investigation.

A reduced plasma AHA has been associated with a G994→T mutation in exon 9 of the AHA gene in the Japanese population [3,4,11,18]. This mutation is reported to influence the degree of proteinuria and the extent of mesangial proliferation in Japanese childhood IgAN [21]. While this mutation is not detected in a healthy Caucasian population, no result is available concerning patients with IgAN. The results of the present study indicate that this deficiency, assessed by an allele-specific PCR, is not found in Caucasian
patients with idiopathic IgAN. One hypothesis to explain the fall of plasma AHA during IgAN might be decreased AHA production by the diseased kidney. However, data have been reported that anephric patients have normal serum AHA levels [22] and that most of the AHA in human plasma originated from haemopoietic lineage cells [23]. Another hypothesis for the low AHA levels in IgAN patients might be the potential role of free oxygen radicals. Indeed, free radicals play a role in IgAN [24]. They are also known to inactivate AHA which might represent one mechanism by which oxygen radicals enhance and/or prolong the pro-inflammatory effect of PAF [25]. Clearly, further experiments are needed to clarify this hypothesis.

In conclusion, blood PAF levels are elevated in patients with idiopathic IgAN; this increase is associated with markedly reduced plasma AHA levels. The mechanism and the precise role of the PAF/AHA deregulation in these patients remain to be clarified. However, in view of the beneficial effects of PAF blockade in several experimental models of acute renal failure [26], PAF receptor antagonists might have clinical applications for IgAN patients. Made possible by molecular cloning of AHA [27], replacement of this circulating anti-inflammatory enzyme might represent an interesting potential therapy for patients with IgAN.

References


Received for publication: 20.6.99
Accepted in revised form: 12.4.00