Letter to the Editor

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Lack of Evidence of Antineutrophil Cytoplasmic Antibodies Involvement in the Acute Phase of Thrombotic Thrombocytopenic Purpura

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Dear Sir,

A consistent amount of experimental data suggest the hypothesis that neutrophils participate in the pathophysiology of the hemolytic-uremic syndrome (HUS) [1–3]. As a matter of fact, an increased adherence of neutrophils to the endothelium has been demonstrated in HUS [1], together with an augmented release of neutrophil-derived proteinases, such as elastase [3].

Antineutrophil cytoplasmic antibodies (ANCA) have been demonstrated to be able to induce neutrophil degranulation and produce oxygen radicals in vitro [4], when primed by cytokines, such as TNF-α or IL-1, or products from infectious agents, as occurs in Wegener's granulomatosis, following a respiratory tract infection [5]. Therefore, Rollino et al. [6] who recently found the serum of one HUS patient (out of 4 tested) positive for both antiproteinase 3 (PR3-Abs, or C-ANCA) and antimyeloperoxidase (MPO-Abs, or P-ANCA) antibodies, postulated the participation of ANCA, together with anti-elastase antibodies, in the pathophysiology of HUS, at least in certain patients. Thrombotic thrombocytopenic purpura (TTP) is a rare hematological disorder, which shares a lot of striking similarities with HUS, especially in adults. Extremely debated is still the hypothesis that these two diseases are simply the qualitative different expression of a unique physiopathological substrate. Even if the microthrombi found in the microcirculation of TTP patients usually lacks polymorphonuclear cells, consisting only of platelets and hyaline, PAS-positive substances, we decided to look for the eventual presence of ANCA in sera of acute-phase TTP patients. It is in fact theoretically possible that neutrophils could initiate, via ANCA-induced degranulation and free radicals production, the endothelial damage that ultimately leads to the clinical pattern of acute TTP, without leaving behind them clear evidence of their involvement.

Twenty-nine adult TTP patients were studied during the acute phase of the disease; TTP had been diagnosed in the presence of the following signs: thrombocytopenia ($<100 \times 10^9$/l), microangiopathic anemia (demonstrated by the presence of schizocytes in peripheral blood smears), no disseminated intravascular coagulation, no anti-erythrocyte and antiplatelet antibodies, high LDH levels, and signs of neurological involvement. The diagnosis of HUS was ruled out since all our patients showed only slight signs of renal involvement; as a matter of fact, none of our patients showed serum BUN and creatinine exceeding 60 and 1.3 mg%, respectively, the only signs of kidney compromise being the presence of microhematuria (in all patients) and cylindruria (in 6 of them) at urinalysis. Furthermore, in 4 of these patients, stool examination led us to exclude the presence of enterotoxins producing *Erchericchia coli* strains.

ANCA were searched for by an indirect immunofluorescence technique according to the method of Wiik [7]. Granulocyte isolation was performed from heparinized whole blood sample by 6% dextran sedimentation and a Ficoll-Hypaque density gradient, as described elsewhere [8]. Isolated granulocytes were repeatedly washed in PBS, pH 7.4, containing 1% human serum albumin, cytocentrifuged on defatted glass slides, and then fixed in absolute ethanol for 5 min at 4°C. Granulocyte substrate was incubated with patient serum diluted 1:20 in PBS for 20 min in a humidity chamber, then washed three times in PBS and incubated with FITC-conjugated rabbit Ig specific for human IgG (Dakopatts, Glostrup, Denmark) diluted 1:50 in PBS. After three additional washings, the slides were covered by a 2:1 glycerol-PBS mixture and a cover glass and read in an epi-illumination Leitz microscope.

Different to the observation of Rollino et al. [6] in 1 HUS patient, a completely negative ANCA assay was found in all TTP patients tested.

ANCA can be found in the circulation of several patients affected by systemic necrotizing vasculitis, such as Wegener's granulomatosis, microscopic polyarteritis nodosa, leukocytoclastic angiitis, necrotizing and crescentic glomerulonephritis, Churg-Strauss syndrome, classic polyarteritis nodosa and Tolosa-Hunt syndrome, and have also been recently proposed as possible initiators of the vascular damage which can be found in HUS [6]. The absence of ANCA in the sera of our 29 acute-phase TTP patients,

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seems to exclude the involvement of these autoantibodies in the pathogenesis of TTP. Moreover, even in HUS, we are far from having a clear demonstration of ANCA's participation in the etiopathogenesis of this disease; the concomitant C-ANCA and P-ANCA positivity reported by Rollino et al. [6] in 1 patient could be a casual association, since patients with vasculitis virtually never have both types of antibodies [9, 10] as would be expected if the autoantibody formation was a secondary phenomenon [10]. However, the role of other autoantibodies in the etiopathogenesis of TTP deserves further investigation; as a matter of fact, the complex relationship existing between anticardiolipin (aCL-Abs), TTP and systemic lupus erythematosus (SLE), which often shares a number of similarities with TTP and is sometimes associated with it, is currently under scrutiny by our group.

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