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In vitro neutrophil activation by ANCA from patients with ANCA associated vasculitis; degranulation and oxidative burst

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Introduction.– Some patients with ANCA associated vasculitis (AAV) have very high ANCA levels, despite clinical remission. Other patients with AAV relapse without any impressive rise in ANCA levels, and some patients are even ANCA negative. An ANCA test today will tell us the patients’ levels of anti-PR3 ANCA or anti-MPO ANCA, without further specification. Are some ANCA more pathogenic then others? Is there a pathogenic epitope? Or can the explanation be found in the neutrophils? There are some previous studies in the field, where ANCA from different patients have been tested on neutrophils from healthy donors. In this study we also explore ANCA stimulation of neutrophils from patients, measuring degranulation and oxidative burst.

Methods.– Purified IgG fractions were prepared using a protein G column. Peripheral blood was drawn in heparinized tubes and neutrophils were isolated by density centrifugation. The neutrophils were primed with TNF alpha and cytochalasin B. Oxidative burst was analyzed by means of DHR. Degranulation was evaluated by ELISA measurements of albumin, MMP-9, lactoferrin and myeloperoxidase. PMA, FMLP and a monoclonal ANCA were used as positive controls.

Results.– Neutrophils from five healthy controls and three patients have been investigated. IgG fractions from 40 ANCA positive patients and seven healthy controls have been tested. Only six of the ANCA fractions generated oxidative burst, and five of them also caused significant degranulation with mobilization of azurophil granules. The other IgG fractions from patients did not differ compared to IgG fractions from healthy controls, which also showed potential of neutrophil activation, although less pronounced. The results were dependent on the neutrophil donor. Overall the neutrophils from patients were less activated than the healthy controls, although the numbers are so far very small.

Discussion.– The impact on neutrophils of ANCA stimulation seems to be dependent not only of the ANCA, but also of the neutrophils. A larger number of patient neutrophil donors is needed in order to draw any conclusions, but the tendency is that the patients’ neutrophils are less prone to be activated – perhaps related to exhaustion from chronic inflammation. In this ongoing study, we aim to investigate this further by increasing the neutrophil donors numbers and by characterization of the IgG fractions.

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Introduction.– Proteinase 3 (PR3) is a serine-proteinase contained in azurophilic granules, secretory vesicle granules and is also present at the plasma membrane. We have previously shown that PR3 membrane expression was a risk factor in ANCA-associated vasculitis. We hypothesized that the proinflammatory activity of PR3 could be due to its ability to inactivate anti-inflammatory mechanisms, among them, annexin-A1 an endogenous mediator of resolution of inflammation. Annexin-A1 (AnxA1) acts as a “brake” for PMN adhesion to the microvascular wall preventing further cell transmigration to the inflammatory site and stimulates clearance of apoptotic cells. We previously showed that AnxA1 was clipped by PR3, limiting its biological functions.

Methods.– AnxA1 expression was evaluated on isolated neutrophils by facs analysis and by Western blotting on membranes. Anti-AnxA1 autoantibodies were detected by ELISA in the sera of patients with GPA using recombinant human AnxA1.

Results.– Whilst AnxA1 expression—as measured by flow cytometry—was not different between controls and GPA patients under the basal state or after TNF-alpha activation, it was increased after apoptosis. In the PMN membrane fraction from GPA patients, AnxA1 cleavage has been detected, under TNF-induced activation. When compared with the cleavage occurring in healthy control PMN, more AnxA1 was cleavage was evident alongside with a faster kinetics. Concentrations of anti-AnxA1 autoantibodies measured by ELISA were significantly raised in sera from GPA patients compared to normal controls.

Conclusion.– Collectively, our data suggest that AnxA1-dependent anti-inflammatory mechanisms could be dysregulated in GPA either by excessive cleavage by PR3 or by the presence of anti-AnxA1 Abs that could inhibit the anti-inflammatory effect of AnxA1. Restoring the anti-inflammatory potential of AnxA1 may be a future therapeutic option in GPA.

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Secondary vasculitis

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Thrombotic vasculopathy with neovascularisation associated with probably levamisole-contaminated cocaine: Report of 2 cases

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Introduction.– A substantial number of cases of vasculitis associated with the use of levamisole-adulterated cocaine have been reported. However, we present the first two known cases of vasculitis associated with the use of probably levamisole-adulterated cocaine in our country, severely affected by increasing drug traffic and consumption, with a peculiar histopathologic finding.

Methods.– Description of the clinical and histological findings in two cases.

Results.– Case 1: 31-year-old male who presented with bilateral ear lobe purpuric lesions. A biopsy showed thrombotic vasculopathy and intravascular papillary endothelial hyperplasia. After prednisone, his lesions resolved completely. Case 2: 38-year-old male with recurrent fever, myalgias and arthralgias plus cutaneous lesions after cocaine use. After the last inhalation he presented retiform purpura, stellate atrophic plaques and ulcers on cheeks, earlobes and legs. Biopsy showed vessel neoformation, fibrosis and intraluminal thrombi on small vessels, without vessel wall inflammation (figure 1). The patient asked for voluntary discharge. After hospital release, he died in another centre...