11-027

TNF-α-ACCELERATED APOPTOSIS ABROGATES ANCA-MEDIATED NEUTROPHIL RESPIRATORY BURST BY A CASPASE-DEPENDENT MECHANISM

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Apoptosis is important for terminating inflammation. However, constitutive PMN apoptosis was also shown to upregulate membrane expression of autoantigens, including proteinase 3 (PR3) and myeloperoxidase (MPO). TNF-α is increased in patients with active ANCA vasculitis, primes PMN for an ANCA-induced respiratory burst, but also rapidly induces apoptosis. We investigated the effect of TNF-α-induced apoptosis on ANCA antigen expression and on ANCA-induced superoxide generation in human PMN. PMN were brought to apoptosis by 10 ng/ml of TNF-α or a combination of TNF-α and 2.5 μg/ml the protein synthesis inhibitor cycloheximide, or cycloheximide alone for 3 h. Apoptosis and ANCA antigen expression were assessed by FACS and microscopy. Superoxide was determined with the ferricytochrome C assay. TNF-α with cycloheximide for 3 h caused apoptosis in 87% PMN compared to 2% in untreated controls (n=18; $p<0.01$). Accelerated apoptosis was associated with an increase in ANCA-antigen expression for both proteinase 3 and myeloperoxidase ($p<0.05$). Nevertheless, apoptosis was paralleled by a decreased PR3 and MPO ANCA-induced respiratory burst ($p<0.05$). Blocking caspase-3 activity prevented apoptosis in TNF-α with cycloheximide-treated cells (83% to 2%) and prevented compromised respiratory burst in response to ANCA. Also, caspase-3 inhibition abrogated apoptosis-mediated ANCA antigen upregulation (PR3 141.6 ± 34.1 MFI to 33.9 ± 7.8; MPO 48.3 ± 12.9 MFI to 11.9 ± 3.2, n=6, $p<0.05$). We conclude that TNF-α-accelerated apoptosis is associated with increased ANCA antigen expression but with downregulated respiratory burst activity in response to ANCA. Specific inhibition of apoptosis by caspase-3 blockade prevented the increase in ANCA-antigen expression and preserved the capability of generating superoxide, thereby establishing a causative role for apoptosis. We suggest that TNF-α exhibits dual actions by both priming and terminating ANCA-mediated activation of human PMN.

12-071

MEMBRANE EXPRESSION OF NEUTROPHIL PROTEINASE 3 (PR3) IS ASSOCIATED WITH RELAPSE IN PR3-ANCA RELATED VASCULITIS

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Background: The highly specific presence in serum of autoantibodies directed against intracellular neutrophil proteins such as PR3 (PR3-ANCA) suggests a pathophysiological role of these autoantibodies in patients with necrotizing small-vessel vasculitis. A stable but interindividually highly variable membrane expression of PR3 has been found on resting neutrophils. We hypothesized that, in patients with PR3-ANCA related vasculitis, a higher expression of PR3 on neutrophil membrane would lead to more interaction with PR3-ANCA and could thereby influence the extent or course of the disease.

Methods: PR3 expression on unstimulated isolated neutrophils from patients with PR3-ANCA related vasculitis was determined by FACS analysis using the anti-PR3 murine mAb 12.8. Patients were divided according to the distribution of neutrophil membrane PR3 in 3 groups: low, bimodal, and high. Disease extent at diagnosis was scored with the Birmingham Vasculitis Activity Score (BVAS). Actuarial relapse-free survival was calculated from diagnosis to the first relapse and compared between groups with the log rank test.

Results: 89 patients (age 49 ± 16.6; 47 male/42 female) with PR3-ANCA related vasculitis followed at our department were included. At diagnosis, renal involvement was present in 52 (58%) and pulmonary involvement in 49 (55%) patients, BVAS was 23 ± 10.5. During follow-up (81 ± 67 months) 50 patients had one or more relapse. Age at diagnosis, organ involvement and BVAS at diagnosis were not different between patients with low (n=32), bimodal (n=26), and high (n=31) neutrophil membrane PR3 expression. However, median relapse-free survival was 104.5 months in patients with low PR3 expression as compared to 36.6 and 30.8 months in patients with bimodal and high PR3 expression, respectively ($p=0.023$). Clinical manifestations at first relapse of vasculitis were not different between these groups.

Conclusion: The level of individual PR3 expression on resting neutrophils is significantly associated with risk for relapse in patients with PR3-ANCA associated vasculitis, but not with disease extent or manifestations at diagnosis or relapse. These data support the hypothesis that interaction in vivo of ANCA with PR3 expressed on membranes of neutrophils plays a role in the pathophysiology of PR3-ANCA related vasculitis.

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