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5th World Congress on

Hepatitis & Liver Diseases

2nd International Conference on Pancreatic Cancer & Liver Diseases

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August 10-12, 2017 London, UK

Neutrophil apoptosis ex vivo in hepatosplenic patients with neutropenia pre-and post splenectomy

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Background: The pathophysiology of neutropenia seen in patients with schistosomiasis or hepatitis C infection that complicates the course of liver disease is poorly understood.

Aim & Methods: We evaluated the neutrophil apoptosis before and after splenectomy to clarify the role of apoptosis and splenomegaly in the occurrence of neutropenia. Neutrophils were isolated from 23 hepato-splenic patients with neutropenia, 8 hepatosplenic patients with normal neutrophil counts, 7 patients who were post splenectomy and a further ten normal control subjects. These were cultured for 24 hours and the time course of neutrophil apoptosis was assessed by determination of Annexin V and propidium iodide binding by flow cytometry. Fas and Bcl2 expression were determined on fresh neutrophils using flow cytometry. Levels of tumor necrosis factor alpha, interleukin 3 and gamma interferon were evaluated using an immunosorbent assay.

Results: Neutrophil apoptosis was minimal in the fresh neutrophils, however, cultured neutrophils exhibited significantly greater apoptosis in neutropenic patients when compared to non-neutropenic patients (P=0.01 at 4 hours and P<0.05 at 24 hours) and control group (P<0.01 at 4 hours and 24 hours). After splenectomy, the percentage of neutrophil apoptosis declined to the normal control levels (P>0.05). Fas and Bcl2 expression on neutrophil were significantly higher in the neutropenic group as compared to normal controls (P<0.05, P=0.01 respectively). Serum TNF alpha, IL-3, and IFN gamma levels were not significantly different in all studied groups.

Conclusion: Neutrophils from neutropenic hepatosplenic patients exhibit markedly accelerated apoptosis, which is normalized after splenectomy. Thus increased neutrophil apoptosis may in part be responsible for the occurrence of neutropenia.

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