UNIVERSITY OF ALCALÁ

Medicine Department

Unit I+D Associated to CNB-CSIC

Laboratory of Oncology and Immune System Diseases

ANALYSIS OF IMMUNE SYSTEM COMMITMENT IN ACUTE PANCREATITIS

Mª Esther San Antonio Sánchez

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SUMMARY

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Background: acute pancreatitis (AP) is an acute inflammatory process of the pancreas, with variable involvement of peripancreatic tissues and remote organ systems depend on severity of disease. A range of morphological findings exists, from interstitial oedema in mild disease (APM) to confluent areas of necrosis and haemorrhage in severe disease (APS). Cause inflammatory character, the involvement of immune system in the pathogenesis of AP constitutes an important study area. The pancreatic autodigestion cause by inappropriate activation of the proteolytic enzyme trypsin provokes an inflammatory cascade mediated by cytokines, complement factors and migration of activated leukocytes (macrophage, lymphocyte and neutrophil). Previous studies on experimental models and patients with AP reveal a strong leukocytosis and marked lymphopenia, higher in APS than APM, in peripheral blood. Also, an increase of inflammatory cytokines, some of these are implicated in disease progression (TNFα, IL6, IL8), chemokines, adhesion molecules (CD11b, CD62L), and decrease of HLA-DR expression in macrophage (another predictive marker) have been found. Recently, the involvement of apoptosis in pancreatic injury and lymphopenia has been proposed. Several prognostic scoring systems, with clinical, immunological, laboratory, and radiological criteria, have been developed to prediction of the severity of an attack at the time of admission, but APACHE II and Ranson Criteria, Balthazar Score, and C-reactive protein are more used. The **aim** of this study is to investigate the functional state of peripheral mononuclear cells from mild and severe acute pancreatitis patients, and to determinate their clinical significance.

Material and methods: heparinised blood samples from twenty nine APM and seventeen APS patients according to inclusion criteria based Atlanta classification were obtained at the time of admission, after 48h and 5 days. Sixty blood samples from healthy controls with age and sex matched were obtained too. The specific objectives of study are to evaluate in different subsets of peripheral lymphocytes and monocytes from these patients: a) the expression of surface antigens associated to cell activation, costimulation, tissular migration by lymphocytes (T, B and NK cells), b) the intracellular production of inflammatory cytokines and apoptosis by T lymphocytes; c) the expression of activation and tissular migration molecules, intracellular production of pro-inflammatory cytokines, phagocytosis and nitric oxide production by monocytes. Antigen surface expression and intracellular cytokine production were determined by immunostaning with monoclonal antibodies; apoptosis, phagocytosis and nitric oxide production levels were measured by fluorescent products; four colours flow cytometry quantitative were used.

Results: Significant differences in the number of cells and distribution of lymphocyte subsets, including lymphopenia, decrease of ratio CD4/CD8 and increment of effector/memory

lymphocytes, were observed between patients and healthy controls at the time of admission. The expression levels of activation markers (CD25, CD69, CD71, HLA-DR, CD56, CD95), costimulatory molecules (CD152, CD154) and adhesion molecules ($CD11a^{high}$, CD11b) in T cells were significantly higher in both patients groups, APM and APS, than healthy controls. Also, an increment of chemokine receptors (CkRs) expression (CCR2, CCR5, CCR6, CXCR3, CXCR4) were found in all lymphocyte subsets from these patients. Because characteristic lymphopenia of patients with AP, the absolute number of cells was lower in most analyzed subsets compared to controls except, an augment of CD4+CD95+ and CD8+CD95+ lymphocytes and no differences in number of lymphocytes that express CkRs. These immunological alterations were usually normalized with inflammatory episode remission. The functional studies about intracellular cytokine production and apoptosis in T cells showed a greater cytokines production (IL2, INFγ, TNFα) and spontaneous apoptosis in patients respect to healthy controls, with no significant changes during clinical course. IL4, IL6 and IL10 expression levels and spontaneous apoptosis were higher and lower respectively than APS. When we analyzed monocytary population, an increase of activation markers (CD80, CD40, CD62L) and CkRs, and decrease of intracellular cytokine production (IL1β, IL6, TNFα), phagocytosis and nitric oxide (NO) production were found in patients respect to healthy controls, and values were significantly lower in patients with APS compared to APM except CkRs and CD62L expression. Also, according previous studies, a strongly decreased HLA-DR expression were observed in patients with APS respect to patients with APM and healthy controls.

Conclusions: Our results have demonstrated that there is an intense alteration of immune system in patients with acute pancreatitis since early phases of disease. This anomalous immune response affects to principal cellular compartments, including T and B lymphocytes and monocytes, and is manifested by a prevalence of activated subsets with increment of adhesion and recirculation molecules, a predominant proinflammatory and Th2 pattern with associated decrease anti-inflammatory ability, and a striking impaired cytokine production, phagocytosis and oxidative capacity in monocytes. On the other hand, there are clear differences in system immune behaviour related to severity of disease in patients with AP. Despite the mechanisms which trigger these alterations are not clearly defined, an anomalous activation of immune system have been demonstrated and, therefore, we could reasonably suggest its involvement in the development of systemic and local tissular injury associated to AP.