CHANGES IN PERIPHERAL BLOOD LYMPHOCYTE POPULATIONS IN PATIENTS WITH ACUTE PANCREATITIS

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ABSTRACT
We tested peripheral blood total lymphocyte and lymphocyte subsets in 32 consecutive acute pancreatitis patients (18 women and 14 men; age 33-80 years, median 63 years) studied at admission, as well as, at 48h and on day 5 of hospitalization, using a flow cytometric analysis. Seventeen healthy subjects comparable for sex and age were studied as controls. On admission the percentage of total lymphocyte, CD3+ T cells and CD4+ T cells was significantly decreased in patients with acute pancreatitis as compared to controls but was significantly increased at 48h and on day 5 with respect to those on admission. Conversely, the percentage of CD8+ T cells in patients was significantly decreased compared to controls at all points of investigation. The percentage of CD56+ NK cells was increased in patients on admission, but significantly decreased on day 5 compared to those on admission and to controls, as well. The percentage of activated CD3+ CD25+ T cells, CD4+CD25+ T cells and CD8+CD25+ T cells was significantly increased compared to controls at all points of investigation. We conclude that cell-mediated immunity was compromised in the early stage of acute pancreatitis, along with significant activation of T lymphocytes, especially T helpers cells.

Key words: Acute pancreatitis, Flow cytometry, Lymphocytes, T cells

INTRODUCTION
Acute pancreatitis (AP) is an acute inflammatory disease of the pancreas, with variable involvement of peripancreatic tissues and remote organ systems depend on severity of disease. The clinical presentation of AP varies from a relatively mild, self-limiting disorder to severe disease with development of multiple organ failure. (1-5).

Excessive inflammatory reaction is considered to contribute to the development of organ failure as a leading cause of complications in early AP. (6, 7). It is characterized by systemic release of pro-inflammatory cytokines (8). The systemic inflammation is accompanied by development of an anti-inflammatory reaction that results in high serum levels of anti-inflammatory cytokines (9) followed by immunosuppression with infectious complications in the late phase of AP.

Cause inflammatory character, the involvement of immune system in the pathogenesis of AP constitutes an important study area. Lymphocytes play a central role in the modulation of the inflammatory reactions in different disease, including AP (10). Several studies have reported a reduction of the peripheral lymphocyte count as well as CD3+, CD4+, CD8+ CD20+ and CD3+DR+ lymphocytes in acute pancreatitis (11- 24). T cell activation has been demonstrated in AP by expression of activation markers CD25 (20) and CD69 (18). Ueda et al. 2006 (21) have demonstrated that the CD4+ T helper cell type 1/type2 (Th1/Th2) balance tends to Th1 suppression in experimental severe AP and suggest that immunosuppression may occur from the early phase in patients with AP. Moreover,
some markers of immunosuppression have been proposed to predict subsequent infection in AP (25).

The data available so far are not sufficient to determine the role of different lymphocyte populations in AP.

The aim of this study is to clarify changes in lymphocyte subsets in the development of early AP.

**MATERIALS AND METHODS**

**Patients and controls**

This study was approved by the Ethical Commission of Medical Faculty, Trakia University and informed written consent was obtained from each patient.

EDTA blood samples from thirty-two patients (18 women and 14 man; age 33-80 years, median 63 years) with acute pancreatitis were obtained at admission (P0), at 48h (P1) and on day 5 (P2) of hospitalization. The control group comprised of 17 healthy volunteers (10 women and 7 man; age 29-75 years, median 60 years) without the history of recent inflammatory disease.

**Flow Cytometry Analysis of Lymphocyte Subsets**

Peripheral blood EDTA samples (100 μL) were incubated with 10 μL relevant anti-human antibodies to lymphocyte cell surface antigens, including CD3, CD4, CD8, CD19, CD25 and CD56 (Beckman Coulter) and treated on TQ-Prep Workstation & IMMUNOPREP Reagent System (Beckman Coulter) according to the manufacturer’s instructions. Four-color flow cytometric analysis was performed using Flow Cytometer FC500 Cytomics, Beckman Coulter.

**Data analysis**

The results were expressed as mean ± SEM. Statistical analysis was performed using the nonparametric Mann-Whitney’s U test. P values less than 0.05 were considered statistically significant. Data were analyzed using Statistica Ver.6 software.

**RESULTS**

The data analysis of lymphocytes and main lymphocyte subsets (CD19+B cells, CD56+ NK cells, CD3+ T cells, CD4+ T cells and CD8+ T cells) is shown on **Figure 1**.

**Figure 1.** Peripheral blood lymphocytes in patients with AP. P0 – patients at admission, P1 – patients at 48h, P2 – patients on day 5 of hospitalization. Results are expressed as mean ± SEM. *P<0.05 vs controls; **P<0.01 vs controls; ^P<0.05 P0 vs P1; ^^P<0.01 P0 vs P1; ^P<0.05 P1 vs P2.
Lymphocytes
The percentage of total lymphocytes was significantly lower in patients at all points of investigation compared to controls (P<0.01). Trend of gradual increase was marked by significant rising on the P2 compared with that on P0 (P<0.01), without reaching control value until the fifth day of investigation (P0).

B lymphocytes
The percentage of B lymphocytes was higher in patients at all points of the study compared to controls, but the differences were only significant in comparison of P0 vs. controls (P<0.01). Gradually decrease was observed without reaching the control value by the fifth day (P2).

NK cells
The percentage of NK cells was higher without significance on P0 compared to controls. Gradually reduction without significance was observed, reaching a lower value in patients at P2 compared to controls.

T cells
Significant decrease was observed in patients at P0 compared to controls (P<0.05). Significant elevation with reaching the value of controls was observed in patients at P1 vs.P0 (P<0.05) and patients at P2 vs.P0 (P<0.05).

CD4+ T cells
There was no significant differences in the percentage of CD4+ T cells when compared patients at all points of investigation to controls. But significant elevation was observed in patients at P1 vs. P0 (P<0.05) and patients at P2 vs.P0 (P<0.05).

CD8+ T cells
The percentage of CD8+ T cells was significantly lower in patients at all points of investigation compared to controls (P0 vs controls P<0.05; P1 vs controls P< 0.01; P2 vs. controls P<0.05)

CD25+ T cells, CD25+CD4+ T cells and CD25+CD8+ T cells
The data analysis of activated populations of CD25+ T cells, CD25+CD4+ T cells and CD25+CD8+ T cells is shown on Figure 2.

Figure 2. Activated T lymphocyte populations in patients with AP. P0 – patients at admission, P1 – patients at 48h, P2 – patients on day 5 of hospitalization. Results are expressed as mean ± SEM. *P<0.05 vs controls; **P<0.01 vs controls; ^P<0.05 P0 vs P1; #P<0.05 P1 vs P2.
All three cell populations were significantly elevated in patients at all points of investigation compared to controls (P<0.01). There was significant decrease of CD25+ T cells and CD25+CD8+ T cells when compared patients at P0 vs. P1 (P<0.05), and significant decrease when compared patients at P1 vs. P2 (P<0.05), as well.

**DISCUSSION**

In this study, we analyzed the changes in peripheral blood lymphocyte subsets with reference to different time points of acute pancreatitis without taking into account the severity of the disease. The design of this study aimed to trace the dynamics of changes in lymphocyte populations characterized acute pancreatitis and their possible role in the development of pro-inflammatory and anti-inflammatory immune response.

Our results confirm the significant decrease in peripheral blood lymphocytes observed by other authors (11-24). Despite a tendency to increase lymphocytes remained significantly reduced throughout the follow-up period. Similar dynamics showed T lymphocytes which were reduced in patients at admission, but at 48h almost reached the control values. A significant reduction of T lymphocytes in early pancreatitis was observed in other study (14, 16,15,20,24), but the data on B lymphocytes and NK cells are relatively small and inconsistent. Pietruczuk M. et al found significant depletion of B lymphocyte subset at days 2 in patients with mild and severe pancreatitis (20). Depletion of the B lymphocytes in early AP was found by Pezzili R. et al. (19), but significant increase of B lymphocytes in infected AP patients was observed by Snen et al (23). We found a relative increase in B lymphocytes and NK cells early in the disease, with a tendency to decrease more pronounced for NK cells, while B lymphocytes remained persistently elevated. A comparison with the results of other authors is difficult due to different methodological formulations, for example the separation of AP patients into mild and severe pancreatitis, a study of absolute lymphocyte count, different time periods of pancreatitis.

Among T lymphocytes we observed decrease in both CD4+ T cells and CD8+ T cells in AP patients at admission. Significant increase at 48h and on 5th day was observed for CD4+ T cells while CD8+ T cells remain significantly lower throughout the follow-up period. It created a temporary imbalance with the apparent prevalence of CD4+ over CD8+ cells. Significant depletion of peripheral blood CD4+ and CD8+ subpopulations of T lymphocytes in the course of AP has previously been reported (13-15, 20-24). In contrast to our results Pietruczuk M. et al (20) have observed significant depletion of the CD4+ T cells, while CD8+ T cells were in the normal range and temporary imbalance in the cell ratio with the prevalence of CD8+ cells. The above mentioned differences in methodological formulations may be the cause of these discrepancies. Furthermore CD4+ T cells and CD8+ T cells are heterogeneous populations and different subsets of these cells may respond differently in the course of AP.

Study of the activation status of CD3+ T cells, CD4+ T cells, CD8+ T cells defined by expression of the activation marker CD25 showed significant and sustained increase of the three populations tested. Significant activation of T lymphocytes defined by CD25 expression (20) and activation marker CD69 has been observed in previous study (18,19).

In conclusion, we found a significant depletion of peripheral blood lymphocytes in patients with AP. Imbalance of the main lymphocyte populations with decrease of T lymphocytes and CD8+ T cells was observed in the earliest stages of the development of AP, followed by a rapid increase of T lymphocytes due to an increase of CD4+ T helper cells. Significant activation of the T cells and the immune system was the feature of the earliest stages of AP.

**REFERENCES**


