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# Blood Lymphocytes in Patients with Chronic Pancreatitis- Distributional and Function

**Ludmila CN, Ferguson AJ, Rådström W and Suganya D**

Division of Clinical Chemistry, Lund University, University Hospital Malmo, Malmo, Sweden

***Corresponding Author:** Suganya D, Division of Clinical Chemistry, Lund University, University Hospital Malmo, Malmo, Sweden

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## Abstract

**Objective:** To investigate whether the chronic inflammatory process in patients with chronic pancreatitis affects their immune function.

**Summary Background Data:** Chronic pancreatitis is a chronic inflammatory disease of the exocrine pancreas. In approximately 30% of patients, an inflammatory mass of the pancreatic head is found, representing an indication for surgery.

**Methods:** This study comprised 28 patients with chronic pancreatitis. Sixteen patients were also reevaluated 1 year after resection of the pancreatic head for chronic pancreatitis.

**Results:** Compared with an age- and gender-matched control group, the number of CD3+ cells was significantly increased in patients with chronic pancreatitis, with an increase of both CD3+CD4+ and CD3+CD8+ cells. The number of natural killer cells or B lymphocytes did not differ between the patients and the control group. After stimulation with phytohemagglutinin or anti-CD3 antibodies, the blastogenic response was significantly attenuated in the patients with chronic pancreatitis. One year after resection of the pancreatic head for chronic pancreatitis, the distribution and the blastogenic response to phytohemagglutinin and anti-CD3 antibodies had returned to normal compared with preoperative values.

**Conclusion:** The chronic inflammatory process in chronic pancreatitis markedly affects the distribution and function of peripheral immunocompetent blood cells, and elimination of the chronic inflammatory focus by pancreatic head resection restores the suppressed immune function in these patients.

## INTRODUCTION

Chronic pancreatitis is characterized by a painful, progressive destruction of the pancreatic gland, with increasing loss of exocrine and endocrine function. Approximately 30% of patients with chronic pancreatitis develop pancreatic head enlargement; this can lead to complications such as obstruction of the bile duct, pancreatic duct, and duodenum or compression of the portal vein or mesenteric artery. These complications represent an indication for surgical treatment. 1–3 Besides removal of the local complication, the resection of the enlarged pancreatic head offers several other advantages, because the inflamed pancreatic head is suspected to be the element that supports chronic pancreatitis. 4 However, recent studies have provided evidence that patients with chronic pancreatitis have up to a 10-fold increased risk for pancreatic carcinoma. 5

In recent studies, several groups have investigated the infiltrating lymphocytes in tissue specimens from patients with chronic pancreatitis. 6–9 All investigators found an increased number of infiltrating lymphocytes, and most groups support the hypothesis of involvement of cell-mediated cytotoxicity in the pathogenesis of chronic pancreatitis, as shown by increased frequencies of perforin-expressing cells 10 or CD8+CD103+ T cells 6 in chronic pancreatitis. In contrast to this hypothesis of cell-mediated toxicity, Emmrich et al 8 found a more common inflammatory type of infiltrating lymphocytes in chronic pancreatitis tissue specimens.

With regard to alterations of the systemic immune function, few data are available. In previous studies we have investigated serum levels of im-

munologic parameters such as neopterin, sIL-2Rα, sCD44s, and sCD44v6 in chronic pancreatitis. Interestingly, we found reduced serum levels of sCD44v6, whereas serum levels of sIL-2Rα and neopterin were not altered in patients with chronic pancreatitis. 11

The aim of our study was to investigate the distribution and proliferative capacity of phytohemagglutinin (PHA), concavalin A (ConA), pokeweed mitogen (PWM), and anti-CD3 antibodies of peripheral blood lymphocytes in patients with chronic pancreatitis compared with a gender- and age-matched group of healthy volunteers. We also compared these data in 16 patients with chronic pancreatitis before and 1 year after resection of the pancreatic head to evaluate whether resection of the inflamed pancreatic head leads to a return to normal of the distribution and function of peripheral blood lymphocytes.

## METHODS

### Patients

Between March 1997 and December 1998, 28 patients with the diagnosis of chronic pancreatitis were enrolled into the prospective study. In all patients included in this study, the time between the last exacerbation of chronic pancreatitis and the immunologic analyses was more than 3 months, to avoid having an acute inflammatory process influence the results. The main indication for pancreatic head resection was chronic pain (20/21) and obstruction of the common bile duct (12/21). In patients with compression of the common bile duct, endoscopic stenting had been performed before surgery. Twenty-one patients underwent resection of the pancreatic head as a result of chronic pancreatitis. In 19 patients, a duodenum-preserving pancreatic head resection was performed; in 2 patients, a pylorus-preserving duodenopancreatectomy was performed. In one patient resection of the pancreatic tail was performed. In six patients no resection was performed (one cystojejunostomy, one ligamentum arcuatum resection, four biopsies). In all patients, the diagnosis was confirmed by histologic examination. The mean age was 48 years (range 32–67 years). Twenty-five patients were men and three were women. Twenty of the 28 patients had a history of chronic alcohol abuse (defined as alcohol consumption > 50 mL/day). In all patients, 15 mL heparinized blood plus 4 mL ethylene diamine tetraacetic acid (EDTA) blood was drawn before surgery and immediately processed for the immune status examinations. In 16 patients the immune status was again evaluated 1 year after pancreatic head resection. The control group of healthy volunteers comprised 20 patients (mean age 46.8 years [range 28–71]); 4 subjects were women and 16 were men.

### Peripheral Blood Lymphocytes

To identify peripheral blood lymphocytes in whole blood, we used a four-color immunofluorescence method as described previously. 12 The following directly labeled mouse-antihuman antibodies were used: anti-CD3-FITC, anti-CD8-PE, anti-CD4-APC, anti-CD45-Percp, anti-CD56-PE, and anti-CD19-APC (all Dianova, Hamburg, Germany).

### Proliferative Capacity of Peripheral Blood Lymphocytes

We determined the proliferative capacity of peripheral blood lymphocytes after stimulation with PHA, ConA, PWM, and anti-CD3 antibodies by using a commercially available kit (Blastest, YLEM, Rome, Italy). Immediately after puncture, 250 µL heparinized whole blood was incubated in 3 mL culture medium and incubated in a 5% CO2 atmosphere at 37°C in a glass vial. Every blood sample consisted of five probes, containing PHA, ConA, PWM, anti-CD3 stimulation, or control. After a 72-hour incubation period, nuclei were isolated and stained with propidium iodide according to the manufacturer's instructions. The nuclei were analyzed on a FACScan (Becton Dickinson, Heidelberg, Germany) using the Cell Quest program. The corrected stimulation index was calculated by subtracting the S/G2 phase (%) in control culture from the S/G2 phase (%) in stimulated culture.

### Transforming Growth Factor Levels in Peripheral Blood

Levels of transforming growth factor beta 1 (TGF-β1) were determined in EDTA plasma from the patients and the control subjects using a commercially available enzyme-linked immunosorbent assay according to the manufacturer's instructions (R&D Systems Inc., Minneapolis, MN).

### Statistical Analysis

All statistics were calculated using the Medcalc software (Mariakerke, Germany) package Significance was set at $P < .05$. Significances were calculated using the Student t test.

## RESULTS

No differences were observed between patients and control subjects in terms of leukocyte count and red blood cell count. However, the number of lymphocytes per microliter was significantly increased in the patients (Fig. 1, $P < .05$). This increased number of peripheral blood lymphocytes was mainly due to an increased number of CD3+ cells ($P < .005$). Further analyses showed that CD3+CD4+ and CD3+CD8+ lymphocytes were both elevated in the peripheral blood of patients ($P < .01$ and $P < .05$). The number of B cells (CD19+) or natural killer cells (CD16+) did not differ between patients and control

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subjects. When peripheral blood was stimulated with PWM or ConA, no differences were observed between patients and control subjects. However, when peripheral blood lymphocytes were stimulated by either PHA or anti-CD3, the corrected stimulation index differed significantly between patients and control subjects. After stimulation with PHA, the corrected stimulation index was 19.9 ± 1.54% in patients and 25.3 ± 1.2% in control subjects (P < .02). After stimulation with anti-CD3, these differences were even more pronounced: the corrected stimulation index was 7.99 ± 1.1% in patients and 16.1 ± 1.6% in control subjects (P < .0001). This points to a possible immune dysfunction, especially in CD3+ lymphocytes, in patients with chronic pancreatitis (Fig. 2). Determination of TGF-β1 plasma levels revealed significantly increased levels in patients versus control subjects (5.9 ± 0.21 ng/mL vs. 4.2 ± 0.3, P < .005).

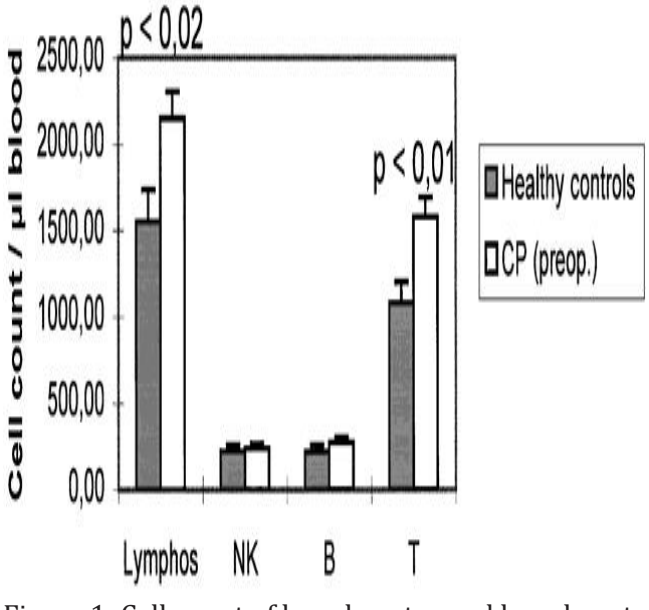


Figure 1: Cell count of lymphocytes and lymphocyte subsets in patients with chronic pancreatitis (CP) and healthy control subjects. In patients, the total number of lymphocytes (lymphos) was significantly greater than in control subjects. This increase was mainly due to an increased number of CD3+ T cells (T). The number of natural killer cells (NK) or B lymphocytes (B) did not differ between patients and control subjects.

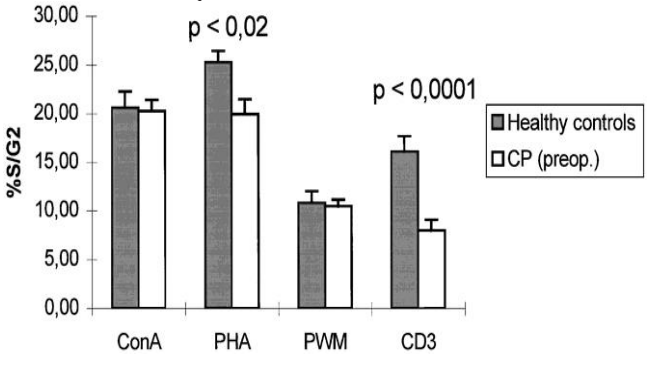


Figure 2. Blastogenic response of peripheral blood lymphocytes after stimulation with concavalin A (ConA), phytohemagglutinin (PHA), pokeweed mitogen (PWM), and anti-CD3. In patients with chronic pancreatitis (CP), the blastogenic response was significantly reduced after stimulation with PHA or anti-CD3 antibodies compared with healthy control subjects.

To test whether the distribution or function of peripheral immunocytes in patients with chronic pancreatitis is dependent on risk factors or comorbidity (exocrine or endocrine insufficiency), the patients were analyzed with regard to gender, age, duration of symptoms, diabetes, exocrine insufficiency, nutrition status (determined by body mass index), and levels of the acute phase parameter C-reactive protein. Gender, duration of symptoms, serum protein level, and serum C-reactive protein level had no influence on the distribution or function of peripheral blood lymphocytes. With regard to nutritional status, patients with a body mass index of less than 20 (6/28 patients) had an increased number of CD3+CD4+ peripheral blood lymphocytes (mean 1,399.5 ± 127/µL) compared with patients with a body mass index of more than 20 (22/28 patients; mean 947.5 ± 79/µL;P < .02). The other evaluated parameters did not differ. With regard to endocrine and exocrine function, patients with endocrine insufficiency (n = 15) had a significantly higher number of peripheral CD56+ cells (299 ± 56/µL) compared with patients without endocrine insufficiency (n = 13, 192 ± 17/µL;P < .05) (Table 1). Further, the T4/T8 ratio was significantly lower in patients with diabetes and chronic pancreatitis versus patients with chronic pancreatitis but without endocrine insufficiency (diabetes, 1.87 ± 0.2; no diabetes, 2.58 ± 0.2;P < .05). This difference was mainly due to an increased number of CD3+CD8+ cells in patients with diabetes, although this did not reach statistical significance (diabetes, 584 ± 69/µL; no diabetes, 468 ± 56/µL;P = .16). Patients with exocrine insufficiency, as determined by a pathologic result on the pancreatolauryl test (n = 20), had significantly greater amounts of peripheral blood CD19+ B lymphocytes (337 ± 38/µL) compared with the B-lymphocyte counts of patients with chronic pancreatitis but without exocrine insufficiency (n = 8; 179 ± 35/µL, P < .01). The other parameters were not dependent on endocrine or exocrine insufficiency. The proliferative capacity after mitogenic stimulation with ConA, PHA, PWM, or anti-CD3 was not dependent on gender, age, duration of symptoms, diabetes, exocrine insufficiency, nutritional status, and C-reactive protein levels.

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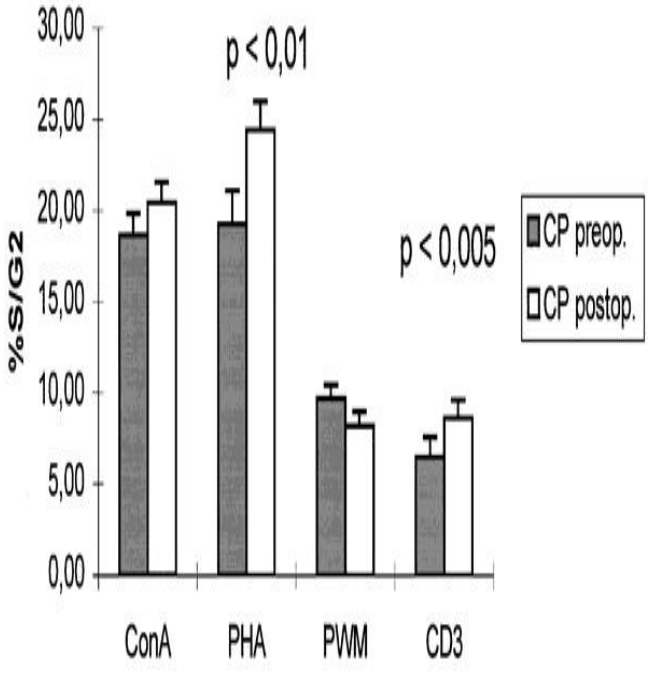


Figure 3. Blastogenic response of peripheral blood lymphocytes following stimulation with with concavalin A (ConA), phytohemagglutinin (PHA), pokeweed mitogen (PWM), or anti-CD3 in patients with chronic pancreatitis (CP) before and 1 year after pancreatic head resection. After pancreatic head resection, the blastogenic response to PHA and anti-CD3 increased significantly.

## DISCUSSION

Chronic pancreatitis is a disease that primarily affects the exocrine pancreas. In the course of chronic pancreatitis, a continuous destruction of pancreatic acinar and ductal cells occurs, and these cells are replaced by fibrous tissue. An overproduction of extracellular matrix molecules may further compromise the remaining parenchyma, leading to an ongoing process of tissue destruction that finally ends in exocrine and sometimes endocrine insufficiency. So far, several hypotheses for the development of chronic pancreatitis have been postulated, but its exact pathomechanisms remain unclear. Recent studies have provided evidence for an overproduction of several growth factors, such as epidermal growth factor (EGF) or members of the TGF-β family. 13–15 In particular, the overproduction of TGF-β in chronic pancreatitis seems to be an important factor for the excessive production of extracellular matrix molecules by inducing the connective tissue growth factor, which leads to the production of collagen, fibronectin, and proteoglycan. 13,16,17 However, TGF-β is also known to be a potent immunosuppressive agent in vitro and has been shown to inhibit immune function in chronic inflammatory lesions in vivo. 18 Especially in viral or bacterial chronic inflammatory processes such as hepatitis or streptococcal infections, TGF-β is supposed to be one of factors responsible for the immune dysfunction in the local inflammation site and the imbalance of systemic immune cells. 18,19 Corresponding to these results, we found a direct inverse correlation between TGF-β1 plasma levels and a reduced blastogenic response of peripheral T lymphocytes after stimulation with anti-CD3 antibodies.

We found an increased number of peripheral CD3+ cells, with an increase in both CD3+CD4+ and CD3+CD8+ cells. However, stimulation of the peripheral blood lymphocytes with anti-CD3 antibodies resulted in a decreased corrected stimulation index compared with that of a gender- and age-matched control group; this points to an immune imbalance in the peripheral blood lymphocytes in patients with chronic pancreatitis. Also, when peripheral blood was stimulated with PHA, the corrected simulation index in patients with chronic pancreatitis was markedly decreased compared with that in the control subjects. These observations could offer new answers to the question of whether patients with chronic pancreatitis have an increased risk for pancreatic cancer. Recently, special entities of chronic pancreatitis such as hereditary pancreatitis 20 or the tropical form of chronic pancreatitis 9 have been linked with a highly increased risk for pancreatic cancer. Even in alcoholic pancreatitis, the risk for pancreatic cancer seems to be increased. 5 However, these patients also seem to have an overall increased cancer risk, 21 which could be due to risk factors such as alcohol and smoking. In our patients with chronic pancreatitis, neither alcohol consumption nor smoking had a negative influence on the distribution of peripheral blood cells. The corrected stimulation index after stimulation with PHA, PWM, or anti-CD3 antibodies even tended to be greater in patients with a history of chronic alcohol abuse than in patients without, suggesting that the immune dysfunction and probably the reduced immunosurveillance in patients with chronic pancreatitis are not related to alcohol consumption. Nicotine abuse had no influence on the distribution or function in patients with chronic pancreatitis.

To confirm our hypothesis that chronic inflammatory processes are the reason for the reduced proliferative capacity of peripheral blood lymphocytes, we investigated the distribution and proliferative capacity of peripheral blood lymphocytes from 16 patients with chronic pancreatitis 1 year after pancreatic head resection. We found a significant restoration of the corrected stimulation index compared with the preoperative values; the distribution of peripheral blood lymphocytes had also returned to normal, supporting the idea that the local-

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In conclusion, we showed for the first time that the chronic inflammatory process in chronic pancreatitis markedly affects the distribution and function of peripheral immunocompetent blood cells and that elimination of the chronic inflammatory focus by pancreatic head resection restores the suppressed immune function in these patients.

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