ORIGINAL ARTICLE

The Initiating Immune Response of Acute Pancreatitis May be Mediated by the T-Helper 17 Pathway

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ABSTRACT

Objectives Acute pancreatitis is characterized by a systemic inflammatory response. We hypothesized that the fundamental inflammatory response observed during the initial stages of all acute pancreatitis is not a Th1 but rather a Th17 response. **Methods** Seven patients with mild AP presenting within three days of symptom onset were recruited. Peripheral blood was drawn for five consecutive days and plasma Th1/Th2/Th17 cytokine levels compared to eleven healthy controls. Plasma cytokine measurements were performed using Th1/Th2/Th17 Cytokine Bead Array assay and data quantified using FCAP Array software. **Results** IL-6 levels were significantly elevated in AP patients compared to controls; IL-10 levels were significantly elevated by day 3; IL17A levels increased on day 2 and significantly elevated at day 3 compared to controls declining to non-significant levels by day 4. IFNγ and TNFα levels were low at all time-points. **Conclusion** IL-17A and IL-10 (an anti-inflammatory cytokine implicated in suppressing Th17 cytokines secreted by macrophages and T cells) were elevated by day 3. In addition IL-6, which helps drive development of Th17 cells, was significantly elevated at all time-points. These preliminary results imply that the underlying AP induced systemic inflammation is polarized to a Th17 rather than a Th1 response.

INTRODUCTION

Acute pancreatitis (AP) is an inflammatory condition of the pancreas that initiates a systemic inflammatory response the severity of which either presents as self-limiting mild acute pancreatitis (MAP) or severe acute pancreatitis (SAP) characterized by organ failure. The initial immune mechanism instigating the development of inceptive acute pancreatitis remains unclear.

Pro-inflammatory cytokines responsible for the inflammatory processes associated with AP have been studied especially with regards to SAP. Increased plasma levels of IL-6, IL-8, macrophage migration inhibitory factor as well as the anti-inflammatory cytokine IL-10 have been demonstrated as potential predictive and prognostic markers of SAP [1, 2, 3]. Furthermore a significant depletion of CD4 T lymphocytes observed in patients with AP [4, 5] and down-regulation of HLA-DR (a marker of immune activation) on circulating monocytes have been associated with late mortality in patients with SAP [6, 7].

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To date published data has supported both Th1 [5] as well as Th17 pathways [8]. However most studies have heterogenous groups consisting primarily of SAP or mixed groups of SAP and MAP patients, different AP etiologies, mostly once off blood samples as opposed to regular samples and were designed to identify prognostic markers as opposed to potential immune mechanism. We hypothesize that all AP with systemic manifestation has an initial common SIRS pathway with common inflammatory mediators which may after a specific point develop into SAP or continue as MAP. An understanding of this fundamental inflammatory pathway is required to better understand and potentially prevent the progression of MAP to SAP, hence the need for this study.

MATERIALS AND METHODS

The study was approved by the University of the Witwatersrand Human Research Ethics Committee (M140885). Seven black African patients with mild alcohol-induced acute pancreatitis (MAP) diagnosed and classified according to the revised Atlanta Criteria [9] who presented within three days of onset of their abdominal pain with a history of a preceding alcohol binge to the pain were recruited into the study. Patients with medical co-morbidities, chronic medication use, previous hospital admissions for acute pancreatitis or potential immune modulating medication use within four weeks of their current admission were excluded from the study. Together with the routine clinical biochemistry tests EDTA peripheral blood was drawn at the same time each day for five consecutive days.

A control group with no current illness, medical co-morbidities or history of previous acute or chronic pancreatitis was recruited. A peripheral blood sample was drawn at one time-point.

Peripheral blood mononucleocytes were isolated using Ficoll–Paque density gradient. Time between sample collection and blood processing did not exceed four hours.

Plasma cytokine measurements were performed using Th1/Th2/Th17 Cytokine Bead Array (CBA) assay (BD Biosciences, La Jolla, Ca. USA) as per manufacturer instructions. Briefly, antibody coated beads are used to capture specific analytes such as cytokines present in patient plasma samples. Each bead has a specific fluorescence that is detected by a flow cytometer to allow for differentiation between various cytokines in a sample.

An LSRFortessa™ flow cytometer (BD Biosciences La Jolla, Ca. USA) was set up for acquisition using a template provided by the manufacturer and capture beads acquired as per manufacturer instructions. CBA data was quantified using Flow Cytometeric Analysis Program™ (FCAP Array™) software (BD Biosciences La Jolla, Ca. USA). Cytokines measured in a single assay were IL-2, IL-6, IL-10, IL-17A, IFNγ and TNFα. C-reactive protein and white cell counts were processed as usual by the hospital laboratory.

Statistical comparisons with a 95% confidence interval were performed using non parametric Mann-Whitney U tests. A p-value of <0.05 was considered significant.

RESULTS

Mean age of the MAP group was 39 (Range 31-55 years old) and the control group was 41 (Range 25-57 years old), basic laboratory results are listed in **Table 1**; liver function tests and calcium-magnesium-phosphate admission levels were normal for all seven patients. During their admission an abdominal ultrasound revealed no gallstones in all seven patients.

Cytokine profiles are depicted in **Figure 1**. Bloods were drawn from patients at the same time (between 7am and 8am) on a daily basis. IL-17A levels were varied and widely scattered between MAP patients. Overall, plasma IL17A levels showed an increase on day 2 and by day 3 were significantly increased compared to healthy controls but declined to non-significant levels by day 4. IL-6 levels varied between patients, fluctuated on a daily basis, however were significantly elevated compared to the control group.

IFNγ and TNFα levels were low in both groups (at the lower end of detectable limits of the kit used in the study) and remained stable over time in the MAP group.

DISCUSSION

The aim of this study was to investigate the early cytokine profile of patients diagnosed with alcohol induced MAP in an attempt to identify the initial immune mechanism that is most likely common to all grades of acute pancreatitis. Neither IFNγ or TNFα cytokines were elevated when compared to healthy individuals suggesting that the inflammatory response is not a T helper cell 1 induced pathway. IL-17A plasma levels reached significant levels by day 3, and there were significant concurrent IL-10 increases with IL17-A. All patients demonstrated elevated IL-6 levels. These results indicate that the early systemic inflammatory response of MAP may be a Th17 mediated event.

Cytokines are secreted in a pattern that broadly divides T helper cells (Th) into three subgroups when an antigen is presented to a naïve T helper cell: Th1 cells that produce pro-inflammatory cytokines including IFNγ, TNF-β and IL-2; Th2 cells which include anti-inflammatory IL-10 responses and pro-inflammatory IL-4, IL-5 and IL-6 cytokines; pro-inflammatory Th17 cells characterized by IL-17A and IL-17F secretion and play an important role in mediating autoimmunity [10, 11]. The differentiation of IL-17A producing CD4 T cells requires distinct transcription factors which in turn are upregulated by signals from transforming growth factor and IL-6 [12].

IL-10 is an anti-inflammatory cytokine implicated in suppressing Th17 cytokines secreted by macrophages and T cells [13]. IL-10 may either be increased through an independent simultaneous Th2 pathway in an attempt to restore the immune system to a state of homeostasis, or IL-10 is being secreted in a Th17 dependent manner through increased levels of IL-17.

IL-2 may act as either a Th1 or a Th2 cytokine. In a Th1 response IL-2 is secreted by activated T cells and mediates proliferation of activated T cells; in a Th2 response it is necessary for survival of antigen specific T cells and development of immune memory. Co-incident with IL-17A and IL-10 plasma levels, IL-2 plasma levels were significantly elevated by day 3 **(Figure 1)** which may be the result of immune memory cell activation that occurs during an inflammatory process.

Table 1. Laboratory blood values for early acute pancreatitis.

	Admission Serum Amylase (U/L)		White cell count (x10^9/L)		C-reactive protein (mg/L)	
	Median	Range	Median	Range	Median	Range
Day 1			9.07	4.39-14.21	61.8	5-178.1
Day 2			11.27	4.37-13.8	94.7	5-267.1
Day 3	946	544-2135	9.72	4.53-14.75	88	11-310
Day 4			6.59	5.13-18.22	73.7	25.5-309.8
Day 5			9.34	4.61-14.29	47	15-247.3

L litre; mg/L milligrams per litre; U/L units per litre

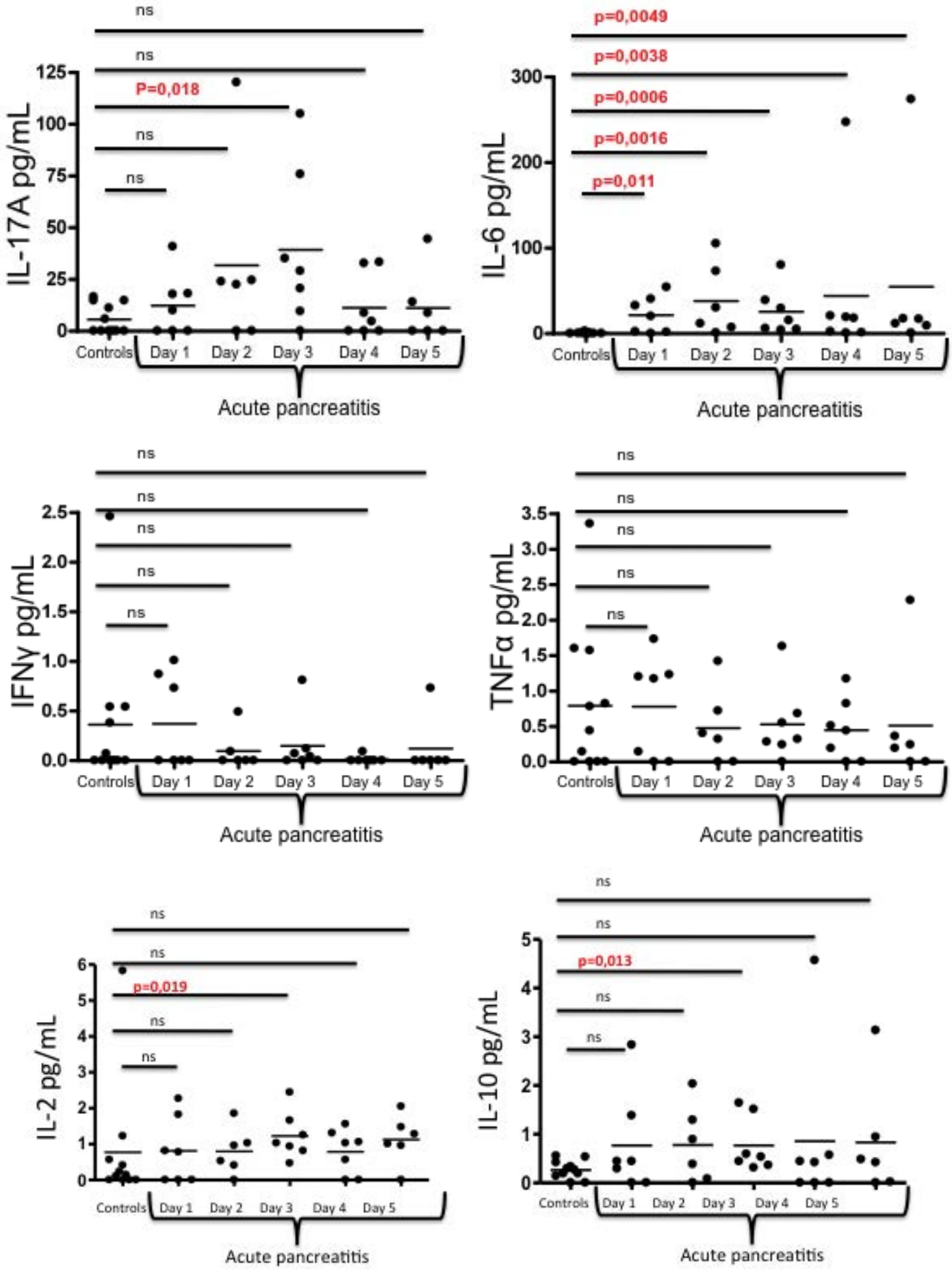

Figure 1. Scatterplots of Cytokine profiles, early acute pancreatitis versus controls.
IL Interleukin; IFN interferon; pg/mL picogram per milliliter; TNF tumor necrosis factor

IL-6 has both pro- and anti- inflammatory potential [14]. It is secreted by macrophages and T cells to either stimulate pro-inflammatory immune responses; alternatively IL-6 acts as an anti-inflammatory mediator by stimulating IL-10 secretion.

Unlike Th1 and Th2 immune responses which are driven by CD4 Th cells, Th17 inflammatory responses may be driven by various immune cells including γδ T cells, αβ T cells, natural killer cells, macrophages and Paneth cells [15]. The mechanisms regulating Th17 inflammatory responses are not well understood. It has been proposed that naive Th17 cells are generated in the presence of IL-6 and differentiated Th17 cells proliferate under the influence of IL-23 [16] in turn inducing Il23r mRNA expression. The resulting Th17 cells have been shown to have greater pathogenic potential. Furthermore, macrophage derived Th17 cytokine secretion is suppressed by IL-10 and IL-10 deficient macrophages stimulated with LPS produce high levels of IL-17 [17].

Lysosomal autophagic dysfunction has been shown to be a key point in the pathophysiology of AP [17], and direct interaction between autophagy proteins and innate immune signaling molecules such as pro-inflammatory cytokines has been demonstrated [18]. In addition the inhibition of autophagy promotes secretion of IL-23 by macrophages and dendritic cells, promoting IL-17 secretion by γδ T cells [19]. The effector cytokines of Th17 cells have been shown to mediate crosstalk between epithelial tissues and the immune system in various host defense actions, specifically infections and auto-immune diseases [20]; however, we have shown that this mediation may occur in the acute, non-infective systemic inflammatory response of early MAP.

This study is limited by a small number of participants, however the group is homogenous compromising one ethnicity, one grade and etiology of AP. In clinical practice it is rare that a patient with MAP presents within a day or two of their symptom onset hence to standardize sample collection we recruited them at Day 3 of symptom onset.

These results imply a Th17 immune response drives the initial phase of alcohol induced MAP. The cytokines that were elevated in this study are implicated in a Th17 driven pro-inflammatory immune response whereas cytokines associated with a Th1 driven pro-inflammatory response were not significantly elevated. We hypothesize the initial AP inflammatory response develops as naive Th17 cells are generated from macrophage derived IL-6. Potentially the development of SAP may occur as a result of Th17 responses with a greater pathogenic potential (IL-23 induced), possibly due to dysfunctional autophagy or the inability of monocytes to mount an adequate IL-10 anti-inflammatory response due to anergy.

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Conflict of Interest

All authors declare having no conflict of interests or financial disclosures.

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