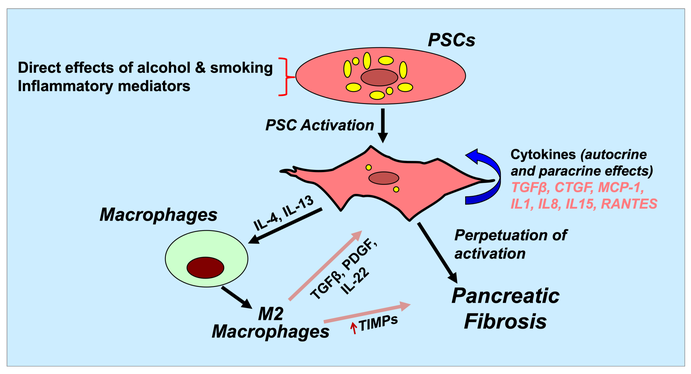
Pancreatic Stellate Cells and Macrophage Interactions Mediated by IL-4 Promote Alcoholic Chronic Pancreatitis Progression

P Rajan[1,2], Z Xu[1,2], B Lee[3], C Perera[1,2], S. M. Zahid Hosen[1,2], Tanzila Khan[1,2], T Cohen-Hyams[2], M Killingsworth[2], S Husain[3], R Pirola[1,2], J Wilson[1,2], S Pandol[4] and Apte M[1,2].

[1]Pancreatic Research Group, South West Sydney Clinical Campuses, School of Clinical Medicine, Faculty of Medicine & Health, UNSW Sydney, Australia; [2]Ingham Institute for Applied Medical Research, Sydney, Australia; [3]Stanford University, Stanford, USA and [4]Cedars-Sinai Medical Center, Los Angeles, USA.

Introduction

- Chronic pancreatitis is associated with significant morbidity. Novel therapeutic approaches are urgently needed.
- Pancreatic stellate cells (PSCs) produce the fibrosis of chronic pancreatitis.
- Inflammatory cells, particularly macrophages play an important role in pancreatitis.
- PSCs produce IL-4 and IL-13, which can promote differentiation of macrophages into a profibrogenic (M2) phenotype. In turn, macrophages produce TGF-β, PDGF and IL-22 to activate PSCs, forming a feed-forward loop (Figure).



- Cigarette smoke is reported to accelerate progression of alcoholic chronic pancreatitis as evidenced by early calcification and fibrosis.
- However, the mechanisms of alcohol ± smoking-induced progression of alcoholic chronic pancreatitis have not been fully elucidated.

Hypothesis

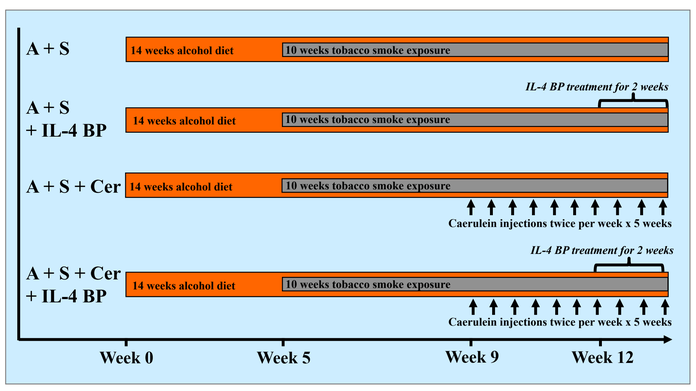
Interactions between PSCs and infiltrating macrophages mediated by PSC-secreted IL-4, potentiate pancreatic necroinflammation and fibrosis.

Aims

1) To assess the effect of IL-4 receptor blocking peptide (IL-4 BP) on the progression of alcoholic pancreatitis induced in mice by alcohol feeding, cigarette smoke exposure and caerulein
2) To determine whether PSC – macrophage interactions mediate the fibro-inflammatory response in vitro

Methods

1) In vivo: Mouse alcoholic chronic pancreatitis model with smoke exposure



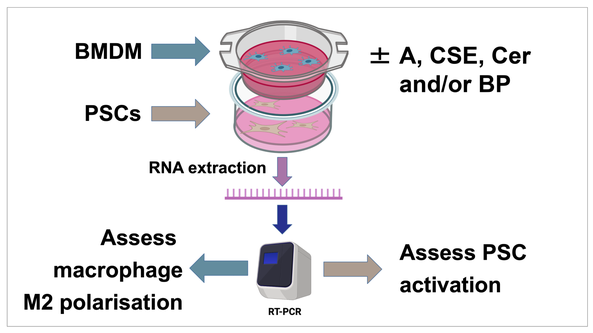
Male C57BL/6 mice

- fed Lieber-DeCarli alcohol diet for 14 weeks
- exposed to cigarette smoke equivalent to heavy smoking (25 cigarettes/day) from week 5 to 14

Methods (continued)

- caerulein (Cer), an analogue of pancreatic enzyme secretagogue cholecystokinin, at 50 µg/kg or PBS (6 IP injections at hourly intervals per day, twice weekly) in week 9 to 14 to induce pancreatitis
- IL-4 receptor blocking peptide (BP) at 2.5 mg/kg (IP daily) in week 13 to 14
- pancreas examined histologically for injury, fibrosis and macrophage infiltration

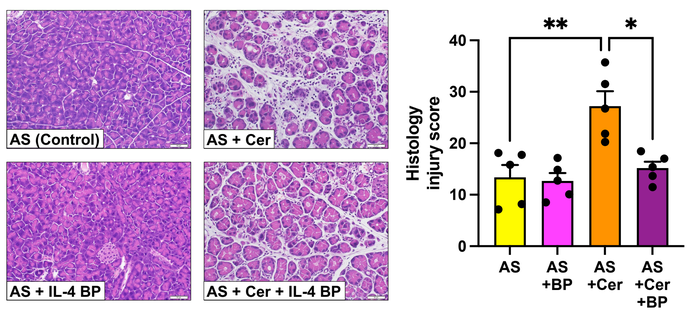
2) In vitro: Mouse PSCs cocultured with mouse bone marrow-derived macrophages (BMDM) in the presence or absence of 50 mM alcohol (A), 40 ng/ml cigarette smoke extract (CSE), 100 nM Caerulein (Cer) and/or 1 µM IL-4 receptor blocking peptide (BP) to assess macrophage differentiation at 24 hours and PSC activation at 48 hours



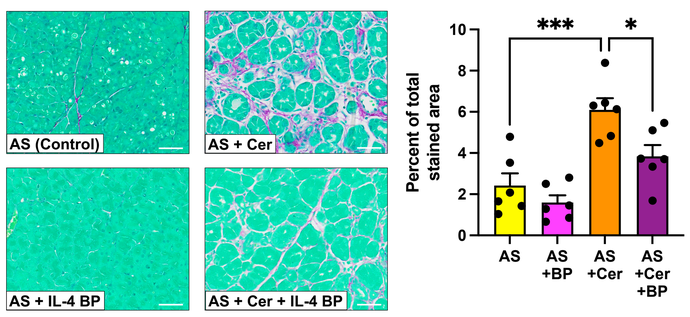
Results

1) In vivo:

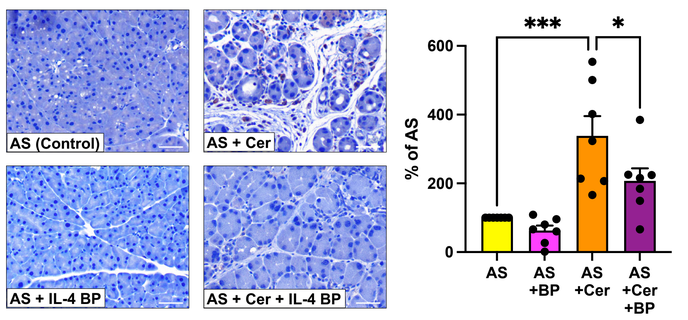
Pancreatic Injury



Pancreatic Fibrosis



Macrophage Infiltration

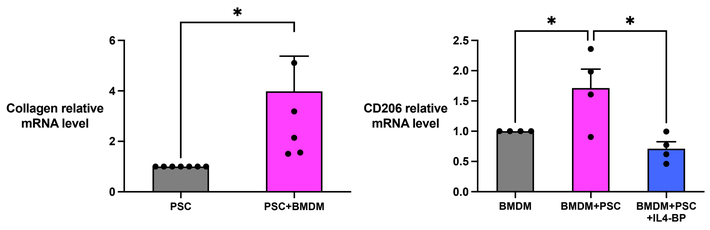


*Compared to mice in AS and AS + IL-4 BP groups, AS + Cer group mice exhibited significantly increased i) pancreatic injury (assessed by oedema, necrosis, vacuolisation, inflammation and haemorrhage); ii) fibrosis (assessed by Sirius Red staining); and iii) M2 macrophage infiltration (assessed by CD206 staining). These effects were blocked in AS + Cer mice treated with IL-4 receptor blocking peptide (BP). *p<0.05, **p<0.01, ***p<0.001, n = 5 – 7 mice per group.*

Results (continued)

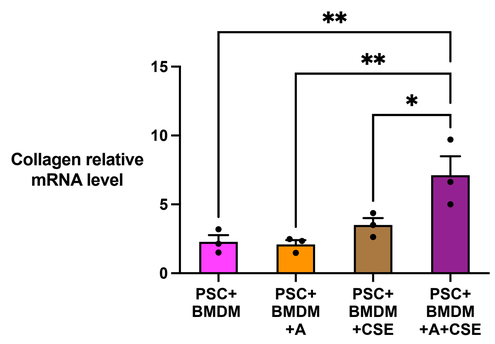
2) In vitro:

PSC-Macrophage Coculture
Collagen in PSCs; CD206 in BMDM



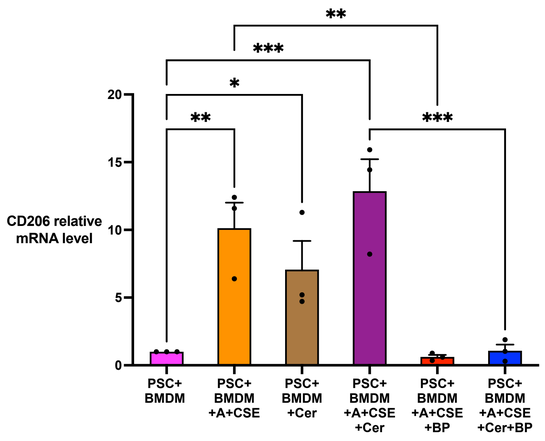
*In the presence of BMDM, PSCs were activated as evidenced by significantly increased collagen mRNA expression. Vice versa, CD206 mRNA expression in BMDM was significantly increased, indicating a change to the profibrogenic phenotype M2. This effect was blocked by IL-4 receptor blocking peptide. *p<0.05, n = 4 or 6 different cell preparations.*

Effect of Alcohol and CSE on Collagen Expression in PSCs Cocultured with BMDM



*In the presence of alcohol (A) + cigarette smoke extract (CSE), the macrophage-induced collagen mRNA expression in PSCs was significantly increased. *p<0.05, **p<0.01, n = 3.*

Effect of Alcohol, CSE, Caerulein with/without IL-4 BP on CD206 Expression in BMDM Cocultured with PSCs



*CD206 mRNA expression in macrophages (BMDM) was significantly increased in the presence of alcohol (A) + cigarette smoke extract (CSE) and/or caerulein (Cer). This effect was abolished by IL-4 receptor blocking peptide. *p<0.05, **p<0.01, ***p<0.005, n = 3 different cell preparations.*

Conclusions & Implication

Caerulein challenge significantly increased pancreatic injury, collagen deposition and M2 macrophage infiltration in alcohol-fed, smoke exposed mice; these inductions were inhibited by IL-4 receptor BP treatment.

The coculture of PSCs and macrophages significantly increased the activation of PSCs and the polarisation of macrophages to an M2 phenotype. These effects were further significantly increased in the presence of alcohol + smoke compounds and/or caerulein, but were abolished by IL-4 receptor blocking peptide.

IL-4 may represent a novel therapeutic target to inhibit PSC-macrophage interactions thereby reducing the fibro-inflammatory reaction in alcoholic pancreatitis and retarding disease progression.