Intra-abdominal Adipose Tissue as a Major Source of IL-6 in Experimental Colitis

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Adipose tissue – What is “fat”?

- Heterogenous tissue composed of mature adipocytes, preadipocytes, endothelial cells, macrophages, leukocytes, and fibroblasts

- In addition to energy storage, adipose tissue is now known to have important endocrine role in metabolism, immunity, and inflammation.

- Over 50 known adipokines including inflammatory cytokines (TNF-α, IL-6, MCP-1), growth factors (IGF-1, VEGF), and adipocyte-specific “adipormones”
Adipose tissue and Inflammation

- Obesity is now recognized as a chronic inflammatory condition
  - Macrophage infiltration of adipose tissue
  - Increased circulating CRP, IL-6, and adipokines
  - Increased fecal calprotectin levels

- Obesity-associated diseases are increasingly attributed to inflammation
  - Atherosclerosis
  - Prevalence of colorectal cancer
  - Severity of acute pancreatitis

- Visceral fat correlates with disease states more strongly than total body fat
Adipose tissue and IBD

• Hypertrophic mesenteric adipose tissue and fat-wrapping are hallmarks of Crohn’s disease
  – higher levels of TNF-a, IL-6, MCP-1, leptin, resistin, and adiponectin than healthy subjects
• Obesity associated with higher year-by-year disease activity in Crohn’s
Obesity and IBD

Prevalence and Epidemiology of Overweight and Obesity in Children with Inflammatory Bowel Disease

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- Prevalence of obese/overweight in pediatric IBD population is 23.6% (20% in CD, 30.1% in UC)
Questions

• Does induction of colitis result in increased expression of inflammatory cytokines from adipose tissue in an animal model of IBD?

• Does intra-abdominal fat respond differently than subcutaneous fat?

• Do adipose-derived cytokines contribute to severity of colitis?
Methods

• Model
  – 2% dextran sulfate sodium in drinking water for 5 days
  – C57BL

• Mice sacrificed at Day 0, 3, 7, 14, and 21

• Outcomes
  – Representative animal for histology
  – Plasma cytokines by multiplex
  – Tissue mRNA levels by qRT-PCR
Results – Severity peaks at Day 14

Fig 1: After treatment with DSS, mice experienced significant decreases in body weight (a). Increases in Plasma IL-6 levels (b) and histologic evidence of colonic inflammation (c) were evident by Day 7 and peaked at Day 14.
Cytokine Expression in Colon

**Fig 2.** Colon mRNA levels of IL-6, TNF-α, and IL-1β were significantly increased by DSS treatment as early as Day 3. IL-6 levels were increased by 230 fold at Day 7 (a) making this the most prominent cytokine induced in colonic tissue.
IL-6 is induced in intra-abdominal adipose tissue

Fig 4. (a,b) IL-6 mRNA levels in Mesenteric (8.6 fold) and Epididymal (3.8 fold) Fat at Day 7 were significantly increased from baseline (c) No significant increase in Subcutaneous Fat IL-6 expression occurred with treatment. *P<0.05
**IL-6 levels at Day 7**

![Graph showing IL-6 mRNA levels](image)

**Fig 5.** Day 7 mRNA levels of IL-6 are significantly increased above control in colon, mesenteric fat, and epididymal fat. Adipose tissue levels at Day 7 are significantly higher than kidney or liver.
Preliminary Conclusions

• During acute experimental colitis, adipose tissue is a major source of IL-6 production.

• IL-6 is significantly induced from intra-abdominal, but not subcutaneous adipose tissue

• Peak in adipose-derived mRNA precedes peak plasma levels, suggesting a contribution by adipose tissue to circulating levels

• Epididymal fat pad changes suggest tissue-specific response rather than mere local lymphoid reaction to tissue injury
Further Questions/Plans

• Does adipose-derived IL-6 contribute to the severity of colitis?
  • Mice fed a high-fat diet have been shown to have more severe response to DSS
  • Preliminary data suggesting improved survival in SIRS with removal of epididymal fat

• Compare severity of colitis between caloric-restricted and high-fat diet mice, with and without removal of epididymal fat pad.
The Use of High Resolution Colonoscopy for Development of a Novel Orthotopic Murine Model of Colorectal Cancer
Animal models

- Needed to study mechanisms of pathogenesis but also to assess potential therapies
- Cannot recapitulate all aspects of human disease
- Advantages and limitations depending on outcome of interest
Murine Models of Colorectal Cancer

• Sporadic Models
  – Genetically-engineered (\textit{Apc} mutant, MutS/MutL, \textit{Muc2-/-})
  – Chemically-induced (AOM/DSS)

• Transplant Models
  – Heterotopic Xenografts
  – Metastasis Assays (IV or intra-splenic)
  – Surgical Orthotopic Implantation
Purpose

• Establish an orthotopic model of colorectal cancer via intramural cell injection using high-resolution colonoscopy

• Theoretical advantages
  – Orthotopic location
  – Minimally-invasive
  – Serial evaluation
Materials

• Equipment
  – Mini-endoscope
    • Karl-Storz Coloview ®
    • 1.9mm scope, sheath, biopsy forceps
  – Standard laparoscopic set-up
    • Donation from Stryker ®
  – Custom injection needle and Microliter syringe
    • Hamilton® Company
    • 6” 30G needle
Methods

• Cell suspension prepared in phosphate-buffered saline

• Mice anesthetized with ketamine-xylazine

• Scope inserted to mid-descending colon

• 25 mL cell-suspension injected into submucosa

• Weekly surveillance with colonoscopy
Procedure
Procedure
Methods
Video
Results

• Procedure tolerated well
  – 1 death due to perforation

• Tumors first visible at 14 days

• Histology confirmed tumor establishment in submucosa

• Tumor establishment rate of 83% (25/30 injections)
Complication
Conclusions

- Intramural injection in descending colon is possible

- Tumor growth can be reliably monitored on serial endoscopy

- Successful tumor establishment with multiple cell lines in immunocompetent and immunodeficient mice
Conclusions

• Disadvantages/Shortcomings
  – Technically challenging
  – Growth duration limited by obstruction
  – Inconsistency precludes comparative studies

• Applications
  – Cancer cell interactions with microenvironment
  – Tissue-specific gene knockout with Cre-recombinase