Inflammatory bowel disease & liver disease

• Primary sclerosing cholangitis or autoimmune hepatitis develop in
  – 3-10% of patients with IBD
  – 70% of patients with PSC suffer from IBD
  – Broome et al. Dis Col Rec 1995; Loftus Gut 2005
Extraintestinal Manifestations of inflammatory bowel disease

**Eye**
- Uveitis
- Iritis
- Episcleritis

**Skin**
- Erythema Nodosum
- Pyoderma Gangrenosum

**Joints**
- Type 1 and 2 Arthropathy
  - Orchard, Wordsworth and Jewell
- Ankylosing spondylitis

**Liver**
- Primary sclerosing cholangitis
- Autoimmune hepatitis

Broome U et al. Gut 1996
Chapman, R.W. Gut 1991
Diseases associated with active bowel disease

- Pyoderma gangrenosum
- Erythema nodosum
- Episcleritis
Liver disease runs a course that is independent of bowel disease

- PSC can develop after colectomy for UC
- UC can develop after liver transplant for PSC
What is the Link?

ENTEROHEPATIC RECIRCULATION OF LYMPHOCYTES

- Lymphocytes primed in the gut recirculate through the liver

- Memory cells are long-lived and will continue to recirculate for many years

- The chance of responding subsequently to modified-self or foreign antigens in the liver is increased
Thymocytes → Thymus → Naïve T cells - CD45RA⁺, CCR7⁺, LFA-1⁻

Lymph node → Activated T cells - CD45RO⁺, CCR7⁻

Target Organ – Liver or Gut

DC

DC – Dendritic Cells

Antigens

• Antigen recognition
• Lymphocyte activation
• Tissue specific homing molecules

Long-lived Memory cells
CD45RA⁺ LFA-1⁺
TISSUE SPECIFIC HOMING RECEPTORS

DC

TISSUE SPECIFIC HOMING RECEPTORS
SIGNAL 1
Tethering
Selectins

SIGNAL 2
Trigger
Chemokine

SIGNAL 3
Arrest
Integrins

SIGNAL 4
Transmigration
Integrins
JAMs
Chemokine

Migration and
retention in tissue
Integrins
Chemokine
Naïve T cell homes to lymph node

<table>
<thead>
<tr>
<th>Chemokine receptor</th>
<th>Chemokine ligands</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR7</td>
<td>CCL21</td>
<td>HEV</td>
</tr>
<tr>
<td>CCR7</td>
<td>CCL19</td>
<td>Stroma of LN</td>
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</tbody>
</table>

Lymphocyte

L-selectin

αLβ2 (LFA-1)

PNAd

ICAM-1

CCL21 attached to GAGs

High Endothelial Venule
Skin Homing memory T cells

<table>
<thead>
<tr>
<th>Chemokine ligands</th>
<th>Comment</th>
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</thead>
<tbody>
<tr>
<td>CCR4</td>
<td>CCL17</td>
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<tr>
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<td>Skin vessels</td>
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</tbody>
</table>

Lymphocyte

- CCR4
- CLA
- αLβ2
- ICAM-1

Endothelial cell

- CCL17 attached to GAGs
MApCAM-1 A GUT ADDRESSIN

Briskin Am J Path 1997

GUT
Colon & small bowel
Appendicitis
CD & UC
MLN
Lung, liver, kidney, heart

LP/submucosal endothelium
HEV endothelium
LP/submucosal endothelium
HEV endothelium
Negative
MAdCAM-1 A GUT ADDRESSIN

Berlin et al Cell 1993

GUT
Colon & small bowel LP/submucosal endothelium
Appendicitis HEV endothelium
CD & UC LP/submucosal endothelium
MLN HEV endothelium
Lung, liver, kidney, heart Negative
**Gut Homing T cell**

*Svensson et al J Clin Invest 2003*  
*Kunkel et al J Exp Med 2000*

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<thead>
<tr>
<th>Chemokine receptor</th>
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</tr>
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<tr>
<td>CCR9</td>
<td>CCL25</td>
<td>Small bowel</td>
</tr>
</tbody>
</table>

CCL25 confined to thymus and small bowel  
Recruits CCR9+ T cells & IgA secreting B cells  
L-selectin

Peripheral node addressin

Lymph node homing Naïve T cells

L-selectin

Small bowel homing lymphocytes

Skin homing lymphocytes
What drives the unique homing phenotype of mucosal T cells?

- *Gut dendritic cells induce expression of α4β7 on T cells*  
  Stagg et al Eur J Immunol 2003

- *Selective generation of gut tropic T cells in gut-associated lymphoid tissue*  

- *Selective imprinting of gut-homing T cells by Peyer’s patch dendritic cells*  
  J. Rodrigo Mora et al Nature 2003

Peyer’s Patch DCs imprint gut homing on responding T cells whereas DCs from spleen or PLN cannot
Novel role for retinoic acid in imprinting gut-homing specificity on T cells

Retinoic acid imprints gut-homing specificity on T cells
Immunity Oct 2004

• Retinoic acid, enhances expression of $\alpha4\beta7$ and CCR9 on T cells and imprints them with gut tropism.

• Vitamin A deficiency causes a reduction in $\alpha4\beta7$ memory T cells in lymphoid organs and intestinal lamina propria.
Vitamin A deficiency is associated with gut infections and chronic diarrhoea
Retinoic acid imprints gut-homing specificity on T cells

Iwata M et al Immunity Oct 2004

Retinal \[\rightarrow\] Retinol \[\rightarrow\] Retinoic acid

ADH

Retinal dehydrogenase
Retinoic acid imprints gut-homing specificity on T cells
*Iwata M et al Immunity Oct 2004*

ADH

Retinal → Retinol → Retinoic acid

Retinal dehydrogenase

Selectively expressed in gut DCs
Retinoic acid imprints gut-homing specificity on T cells

Iwata M et al Immunity Oct 2004

Retinal → Retinal dehydrogenase → Retinol → Retinoic acid

Selectively expressed in gut DCs

RAR
Retinoic acid receptors
Retinoic acid imprints gut-homing specificity on T cells
Iwata M et al Immunity Oct 2004

Retinal → Retinol → Retinoic acid

Selectively expressed in gut DCs

RA-responsive enhancer sequence

3p21.3

β7 Chromosome 15

Retinal dehydrogenase

ADH

RAR Retinoic acid receptors

CCR9
DCs from human mesenteric LN imprint CCR9 on responding lymphocytes

Activation of CD3+ PBL at 7 days

Generation of Gut-Homing IgA-Secreting B Cells by Intestinal Dendritic Cells

Mora, Eksteen et al *Science* Nov 18 2006

MLN

MAdCAM-1

PORTAL LN

MAdCAM-1 in PSC

VAP-1

MADCAM-1

α4β7

CCR9
CCL25 confined to thymus & small bowel

- Critical for recruiting CCR9+ T cells and IgA secreting B cells to the small bowel

CCR9 and a4b7 are selectively expressed on mucosal lymphocytes
  - Papadakis Gastro 2001
CCL25 upregulated on endothelium only in PSC and autoimmune hepatitis

In PSC 20% of liver lymphocytes express CCR9

![Graph showing mean % CCR9+ T-cells for different groups.]

- PSC LIL n=8
- PSC PBL n=8
- PBC LIL n=6
- NL PBL n=6
- NL LIL n=6
- CD PBL n=3
- CD LPL n=3
In PSC 20% of liver lymphocytes express CCR9
In PSC 20% of liver lymphocytes express CCR9

These are primed T cells
Gut homing lymphocytes

Endothelial cell

PSC Liver infiltrating lymphocytes

VCAM-1

MAdCAM-1

CCL25

CCR9

α4β1

α4β7

VAP-1R

VAP-1
Where are gut-homing T cells in PSC imprinted?
In the liver or the gut?
Neo lymphogenesis is seen in portal-associated lymphoid follicles in PSC

CCL21 on HEV

CCL19 on lymphatics and stromal cells

Grant et al. Am J Path 2002
Can PSC liver-derived DCs imprint CCR9 and α4β7 on responding T cells?

Isolation of DCs from PSC liver tissue
Goddard et al Am J Pathol 2004

Fresh liver pieces incubated over night at 37°C in RPMI 10% FCS

Immunomagnetic selection to deplete CD3 and enrich CD11c

DCs matured with LPS and used at 1:5 ratio to activate naïve allogeneic T cells +/- 10nM ATRA

CCR9 and α4β7 expression measured day 0,3,7,14
Can Liver Dendritic Cells induce Gut Homing Molecules?

DCs from human liver CANNOT imprint CCR9 on responding lymphocytes.
VCAM-1 MAdCAM-1

α4β1

β7

CCR9

VAP-1R

Endothelial cell

Gut homing lymphocytes

PSC Liver infiltrating lymphocytes

DCs from human liver CANNOT imprint CCR9 on responding lymphocytes

CCR9+ T cells in the liver are mucosal T cells imprinted by gut DC
What retains T cells in the liver?

Red = HEA 125
Green = CD45
CCL28 and CCR10

- CCL28 expressed in mucosal epithelium
  - Pan et al J Immunol 2004
- CCR10 expressed by IgA secreting B cells
  - Kunkel JCI 2003

- CCL28 is expressed on inflamed bile ducts
Human cholangiocytes secrete CCL28 in response to LPS and IL-1

BEC in vitro culture x400

20% Liver infiltrating T cells express CCR10
VCAM-1 expression on bile ducts and cholangiocytes

Liver infiltrating lymphocytes bind to cholangiocytes via VCAM1 when activated by chemokines

Heydtmann et al. J Immunol 2005
Biliary epithelial cell (BEC) rescue of LIL from apoptosis

Activated Caspase 3 in PSC LIL cultured on plastic with IL-2 at 12Hrs
Biliary epithelial cell (BEC) rescue of LIL from apoptosis

Activated Caspase 3 in PSC LIL cultured on plastic with IL-2 at 12Hrs

VCAM-1 on bile ducts

VCAM-1 on TNFα treated cholangiocytes

Control

TNFα
Activated Caspase 3 in PSC LIL cultured on plastic with IL-2 at 12Hrs

Activated Caspase 3 in PSC LIL cultured on TNFα stimulated fixed BEC 12Hrs with IL-2

Biliary epithelial cell (BEC) rescue of LIL from apoptosis
CCR9 & CCL25 specific to PSC
PROMOTES RECRUITMENT

Eksteen et al J Immunol 2006

CCR10 & CCL28
Target cells to bile ducts

PSC Liver endothelium

PSC Biliary epithelial cell
IBD

Naïve T cell

MLN + PPs

IBD
Naïve T cell

MLN + PPs

VAP-1r

α4β7

CCR-9

CXCR3

Gut endothelium
MAdCAM VAP-1
CCL25, CXCR3

IBD
Naïve T cell

MLN + PPs

VAP-1r

α4β7

CCR-9

Memory T cell

Gut endothelium

MAdCAM

CCL25, CCL28

IBD
Naïve T cell

MLN + PPs

Gut endothelium

Liver endothelium

VAP-1r

α4β7

NO antigen

NO retention

release to lymphatics

RECIRCULATION

Memory T cell
Naïve T cell

MLN + PPs

Memory T cell

VAP-1r

α4β7

VAP-1

+antigen

activation

retention

GUT

INFLAMMATION
Naïve T cell

MLN + PPs

Memory T cell

Liver endothelium

MAdCAM-1, CCL25

Gut endothelium

MAdCAM, VAP-1

+antigen

activation

retention

INFLAMMATION

GUT

VAP-1r

α4β7

CCR9

CXCR3

α4β7

VAP-1r

MAdCAM-1, CCL25

CCL25, CCL28
CREDITS

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Chris Weston

Dept Pathology
Stefan Hubscher

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Ron Gladue and Nick Pullen (Pfizer)
Mike Briskin (Millenium)
Uli von Andrian and Rodrigo Mora (Harvard)
DCs from human liver imprint low levels of α4β7 on responding lymphocytes

* p<0.05; ** p<0.01
Human T cells activated in the presence of vitamin A upregulate CCR9

Eksteen et al 2007
The hepatic sinusoids are a unique, low-flow vascular bed.
Vascular adhesion protein 1 promotes lymphocyte recruitment to the liver

Constitutively expressed in the liver
Supports lymphocyte recruitment
McNab Gastro 1996; Lalor J Immunol 2002; Bonder et al Immunity 2005

Contact
Arrest
Extravasation

VAP-1 in normal human liver
CCL25 is over-expressed in PSC livers

*Eksteens et al J Exp Med 2004*

14 kDa -

<table>
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<tr>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Primary sclerosing cholangitis</td>
<td>Alc cirrhosis</td>
<td>Primary biliary cirrh</td>
<td>Other CLD</td>
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</tbody>
</table>

Fold increase in CCL25 mRNA

- PSC
- NL
- BAT
- ALD
- PBC
- SKIN

*P* < 0.05
CCR9 INDUCTION IS LOST FOLLOWING RESTIMULATION WITH HUMAN LIVER-DERIVED DCs
Chronic hepatitis

Cirrhosis

PSC
- Virus/infection
- Toxin
- Autoimmunity

Resolution

Bile duct fibrosis

CIRRHOSIS AND CHOLANGIOCARCINOMA
Chronic hepatitis

Resolution

Cirrhosis

PSC
- Virus/infection
- Toxin
- Autoimmunity

CIRRHOSIS AND CHOLANGIOCARCINOMA

Chronic hepatitis

Bile duct fibrosis

Resolution
Salmi M et al 1994
*Gastroenterology*

VAP-1 in IBD

Grant AJ et al 2001
*Hepatology*
L-selectin

Peripheral node addressin

**Lymph node homing Naïve T cells**

- CCR7
- αLβ2
- ICAM-1

**Small bowel homing lymphocytes**
*Kunkel 2000, Papadakis 2001; Svensson 2002.*

- CCR9
- α4β1
- VCAM-1
- MAdCAM-1
- VAP-1L

**Liver homing effector lymphocytes**

- CXCR3
- α4β7
- VAP-1L
- αLβ2
- VAP-1
- ICAM-1
DCs from human mesenteric LN but not those from human liver imprint CCR9 on responding lymphocytes.