Adhesion Molecules
And Lymphocyte Homing
Sharon S. Evans, Ph.D.
Dept. Immunology, RPCI
Feb. 9 & 14th, 2017
<table>
<thead>
<tr>
<th>Year Range</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1982 – 1986</td>
<td>Studies proposing to study cell adhesion IRRELEVANT, “…lacking overall functional relevance…” (NIH)</td>
</tr>
<tr>
<td>1986 – 1992</td>
<td>Scramble to identify cell adhesion molecules/ligands</td>
</tr>
<tr>
<td>14 $\alpha$, 8 $\beta$ chains of integrins identified $\rightarrow$ 24 integrins</td>
<td></td>
</tr>
<tr>
<td>L, E, and P-selectin structure determined</td>
<td></td>
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<tr>
<td>1990s-present</td>
<td>Identification of 50 chemokines and 19 chemokine receptors</td>
</tr>
<tr>
<td>1990 – present</td>
<td>Adhesion molecules, chemokines and signal transduction</td>
</tr>
<tr>
<td>$\beta$2, $\beta$1 integrins</td>
<td></td>
</tr>
<tr>
<td>L-selectin</td>
<td></td>
</tr>
<tr>
<td>homeostatic &amp; inducible chemokines</td>
<td></td>
</tr>
</tbody>
</table>
**Lecture 1:**

Review class slides


**Lecture 2:**

Objectives

- Be able to read and understand field of adhesion and leukocyte trafficking.

- Working knowledge of experimental approaches used to dissect role of adhesion molecules/chemokines in leukocyte trafficking.

- Understand structure/function relationship of trafficking molecules that direct leukocyte migration under homeostatic versus inflammatory conditions.
Outline

- General introduction to lymphocyte trafficking – homeostatic recirculation vs active recruitment.

- Homeostatic trafficking of lymphocytes across specialized high endothelial venules (HEVs)
  - Structure/function of adhesion molecules involved in initial tethering/rolling (L-selectin/PNAd)
  - Structure/function of chemokine/chemokine receptors that mediate activation (CCR7/CCL21)
  - Structure/function of adhesion molecules required for firm arrest/transendothelial migration (LFA-1/ICAM-1 & α4β7 integrin/MAdCAM-1)

- Leukocyte recruitment in acute and chronic inflammation.
Cellular Mediators of the Adaptive and Passive Immune Response

- Thymus
- Spleen
- Lymph nodes
- Gut-associated lymphoid tissue
- Bone marrow
- T cell
- Macrophage
- Antibodies
- B cell

- Cellular Mediators of the Adaptive and Passive Immune Response
Pathways of Lymphocyte Trafficking

Peripheral Blood Lymphocytes

- Peyer's Patches
- Peripheral Lymph Nodes
- Skin
- Spleen
- Tumor Sites
- Pregnant Uterus
- Autoimmune Diseases
- Infection Inflammation Injury
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- Leukocyte recruitment in acute and chronic inflammation.
Lymphocyte Extravasation Occurs Primarily Across Post-Capillary High Endothelial Venules

Post-Capillary Venules

High Endothelial Venules (HEV)
Pathways of Lymphocyte Recirculation
Tenants of T Cell-Based Immune Response

1. Generate pathogen-specific effector T cells

2. Deliver T cells to peripheral sites
   - Infected, injured tissue

Peripheral Site
- Skin
- Gut
- Lung
- Genital
- Urinary

Pathogen (virus, bacteria, parasites)

Entry from peripheral tissues:
- Dendritic cells
- Lymphocytes (activated)
- Antigen
- Soluble factors

Afferent lymphatics

Draining Lymph Node

HEV

Efferent lymphatics

von Andrian and Mempel
Nature Reviews / Immunology 2003
Tenants of T Cell-Based Immune Response

1. Generate pathogen-specific effector T cells

2. Deliver T cells to peripheral sites
   - Infected, injured tissue

Peripheral Site
- Skin
- Gut
- Lung

Pathogen *(virus, bacteria, parasites)*

Draining Lymph Node

Afferent lymphatics

Major site of naïve lymphocyte entry (~1-10 in $10^6$ T cells recognize a specific Ag)

3x$10^6$ cells recruited/day

Efferent lymphatics

von Andrian and Mempel
Nature Reviews / Immunology 2003
Tenants of T Cell-Based Immune Response

1. Generate pathogen-specific effector T cells

2. Deliver T cells to peripheral sites
   - Infected, injured tissue

Peripheral Site
- Skin
- Gut
- Lung

Pathogen *(virus, bacteria, parasites)*

Lymphocyte migration through LN parenchyma is active process

Afferent lymphatics

Draining Lymph Node

HEV

Efferent lymphatics

von Andrian and Mempel
*Nature Reviews / Immunology 2003*
Tenants of T Cell-Based Immune Response

1. Generate pathogen-specific effector T cells

2. Deliver T cells to peripheral sites
   - Infected, injured tissue

**Peripheral Site**
- Skin
- Gut
- Lung

**Pathogen** (virus, bacteria, parasites)

**Afferent lymphatics**

**Draining Lymph Node**

**HEV**

**EXIT: S1P/S1P₁**

**Efferent lymphatics**

**Exit from peripheral LN:**
- Naïve lymphocytes
- Activated lymphocytes

*von Andrian and Mempel
Nature Reviews / Immunology 2003*
Architecture and Vascular Organization of Lymph Node (H & E)

T cell-rich paracortex
Morphological Comparison of Squamous/Flat vs. Cuboidal/High Endothelial Venules (HEV)
Leukocyte – HEV Interactions

- Thome (1889) – described HEV structure
- Schumacher (1899) – noted presence of lymphocytes in walls of HEV
- Direction of lymphocyte migration (from blood → tissue or from tissue → to blood) not known until 1964 (Gowans and Knight)
- Gowans & Knight injected radiolabeled lymphocytes into mouse (i.v.). 1 hour later, recovered radioactivity in tissues – supporting blood → tissue route of migration.

Gowans and Knight, 1964
Dynamic Regulation of Leukocyte-Endothelial Adhesion

Free flowing
~ 500 μm/sec

Tethering & Rolling
< 25 μm/sec
(1-3 sec)

Firm Sticking
0 μm/sec
(10 min)

Lymphocyte
HEV

Transmigration
(10-15 min)

HOMING

Adapted from Immunol. Rev. 2002
In vivo Imaging of Lymphocyte – HEV Interactions in PLN by Intravital Microscopy (IVM)

Femoral Artery
(injection of Calcein-labeled lymphocytes)

Inguinal LN

von Andrian Cur Opin Immunol 2004
Chen et al Cancer Immunol Immunother 2006
Chen et al Nature Immunol 2006
Lymphocyte – Endothelial Interactions Observed Under IVM in Inguinal LN

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- Leukocyte recruitment in acute and chronic inflammation.
Classes of Cell Adhesion Molecules

- **mucins**
- **selectins**
- **Ig-CAMs**

**Tethering/rolling**

**Firm arrest**
Dynamic Regulation of Leukocyte-Endothelial Adhesion

1. Tethering/Rolling
   - L-selectin/PNAd
   - $\alpha_4\beta_7$/MAdCAM-1

2. Activation
   - CCL21/CCR7

3. Sticking
   - LFA-1/ICAM1

4. Transmigration

Adapted from Immunol. Rev. 2002
Protein Motifs of L-Selectin PLN Homing Receptor

Seed 1989
Tedder 1989
Rosen 1989
Immunogold Labeling of L-Selectin on Lymphocyte Microvilli

Endothelial Cell

Leukocyte

P-selectin; CD62P (GMP-140/PADGEM)

E-selectin; CD62E (ELAM-1)

L-selectin; CD62L (MEL-14/LAM-1/DREG-56)

Selectin Family

Endothelial E-selectin, lectin and EGF repeats

Ligand-binding loop

Lectin Module

EGF Module
Lymphocytes Traffic Across PNAd\(^+\) (MECA-79 mAb reactive) HEV in Peripheral Lymph Nodes

Remove PLN

Stain with anti-PNAd mAb

\((\text{MECA-79 mAb, green})\)

5 min.

TRITC-labeled cells

MECA-79 / Lymphocyte

MECA-79\(^+\) HEV

Lymphocyte

Chen et al., Nature Immunol. 2006
Lymphocytes Traffic Across PNAd+ (MECA-79 mAb reactive) HEV in Peripheral Lymph Nodes

Remove PLN Stain with anti-PNAd mAb (MECA-79 mAb, green)

TRITC-labeled cells

1 h

MECA-79 / Lymphocyte

MECA-79+ HEV

Lymphocyte

50 µm

Chen et al., Nature Immunol. 2006
PNAd is the Major L-Selectin Ligand in Peripheral Lymph Node HEV

Mucin Stalk ~ 30 nm

Posttranslational Modifications of Endothelial L-Selectin Ligands

- O-linked carbohydrate (glycosyl transferases substitute CHO on serines and threonines)
- Sulfated saccharide (GlcNAc-6-O-sulfotransferase, 6-O-ST). This enzyme restrictively expressed in HEV. 6-O-SO$_4$ is the recognition epitope of MECA-79 mAb. HEC-GlcNAc6ST-deficient mice (Rosen).
- Fucose (Fucosyl transferase (FucT-IV, FucT-VII))
- Sialylated polysaccharides (Sialyl Lewis X [sLex]; sialyltransferase 3 Gal IV; ST3GalIV))

*Peripheral Lymph Node Addressin [PNAd]*
HEC-GlcNAc6 sulfotransferase (HEC-GlcNAc6ST)

Requirement for sulfation during tethering/rolling shown in HEC-GlcNAc6ST-knock-out mice; Rosen & Fukuda’s group Nature Immunol 2005

6-sulfo sialyl Lewis X

Sialyl Transferase 3 Gal IV (ST3GalIV)

Fucosyl Transferase VII (FUT IV/VII)

PNAd

*Sialic acid
*Galactose
*GlcNAc
*GalNAc
*S
*SO₃

SO₃

*HEC-GlcNAc6 sulfotransferase (HEC-GlcNAc6ST)

MECA-79 epitope


*Requirement for sulfation during tethering/rolling shown in HEC-GlcNAc6ST-knock-out mice; Rosen & Fukuda’s group Nature Immunol 2005
L-Selectin – PNAd Interactions in Peripheral Lymph Node HEV

Glycocalyx - 10 nm

LN HEV

PNAd

SO$_3^-$

SO$_3^-$

SO$_3^-$

SO$_3^-$

SO$_3^-$

Ca$^{++}$

L-selectin

Lymphocyte

Lectin
Analysis of Lymphocyte Homing In Vivo

Inject i.v.:
- $3 \times 10^7$ PKH-26-labeled Wild-type Cells
- $3 \times 10^7$ FITC-labeled L-selectin$^{-/-}$ (knock-out) Cells or with L-selectin blocking mAb (DREG-56 or Mel-14)

Collect:
Sacrifice mouse one hour after injection of mixed cell populations

Analyze:
Determine ratio of FITC-/PKH26-labeled cells in tissues
Homing Capabilities of L-Selectin-Deficient Lymphocytes

Relative Homing

1 hour

Spleen | PP | MLN | PLN

Wildtype | L-selectin-/-

Quantifying Lymphocyte – Endothelial Interactions in PLN

Measure the velocity of rolling cells (order IV)

Count the numbers of rolling, sticking and non-interacting cells in certain time period
Quantifying Lymphocyte – Endothelial Interactions in PLN

Blood flow velocity at level IV venules: 500 µm/sec

Chen et al., Nature Immunol. 2006
Quantifying Lymphocyte – Endothelial Interactions in PLN

Rolling Fraction = Rolling cells / Total cells

Sticking Efficiency = Sticking cells / Total cells

L-selectin Ab

LOV

HOV (HEV)

Venular order

I  II  III  IV  V

Rolling fraction (%)

80  60  40  20  0

Sticking efficiency (%)

80  60  40  20  0
Lymphocyte Extravasation in LN HEV

Evans SS, Repasky EA, Fisher DT
Nature Reviews Immunol 2015
Outline

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- Leukocyte recruitment in acute and chronic inflammation.
Chemokines

C

CC

CXC

CXXXC

CCL (ligand)
- CCL21 (SLC)

CCR (chemokine receptor)
- CCR7

CXCL (ligand)
- CXCL12 (SDF-1)

CXCR (chemokine receptor)
- CXCR4
Structure of CC Chemokines

3-dimensional structure at N-terminus required for binding to chemokine receptor

Basic (positively charged) amino acids in C-terminus required for binding to glycosaminoglycans (heparin sulfate) that make up glycocalyx on endothelial cells

α-helix

Anti-parallel β-pleated sheet
FIG. 4. Human leukocyte receptors for chemokines. Human leukocytes express multiple seven-transmembrane receptors that interact with one or more of the chemokines. (Adapted from drawings and data provided by Drs. Charles Mackay, Barrett Rollins, and Andrew Luster.)
Chemokines Stimulate Chemotaxis and Migration

Transwell Migration Assays

Chemokine (CCL21)

5 μm pore
Chemokines Stimulate Chemotaxis and Migration

Transwell Migration Assays

Chemokine (CCL21)

5 μm pore
Evidence that CCL21 is a Tissue Specific Homing Chemokine

- Tissue specific localization of CCL21 in HEV
**CCL21 (SLC) is Expressed by Lymph Node HEV**

<table>
<thead>
<tr>
<th>In Situ Analysis (mRNA)</th>
<th>IHC Analysis (protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCL/CXCL13</td>
<td>CCL21/SLC</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>WT(^+/-)</th>
<th>plt/plt</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNAd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCL21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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BCL/CXCL13 – binds CXCR5 (involved in B cell migration)
SLC/CCL21 – binds CCR7 (mediates B & T cell homing across HEV)

**Science 286:2098, 1999**

**J. Exp. Med. 191:61, 2000**
In vivo Imaging of CCL21 Localization on PLN HEV by Intravital Microscopy (IVM)

Femoral Artery
Injection of FITC-labeled anti-CCL21 Ab

Inguinal LN

CCL21 is Expressed by Lymph Node HEV in Wild Type But Not *plt/plt* Mice

- HEV express CCL21 [SLC] mRNA and protein
- HEV of *plt/plt* mice lack CC21 [SLC] and CCL19 [ELC/MIP3β]); can’t support firm adhesion or homing
- Reconstitution of CCL21 in HEV of *plt/plt* mice restores firm adhesion
- T cells from CCR7^-/- suppressed in ability to enter LN, PP
- Chemokine desensitization of CCR7 (using high concentrations of CCL21) blocks arrest of rolling cells

Evidence that CCL21 and CCR7 receptor critical for T lymphocyte extravasation across HEV

Chemokine (CCL21)

Signaling

- Proliferation
- Differentiation
- Migration
- Adhesion to HEV, EC, or ECM

Chemokine Receptor (CCR7)

$G_{\alpha i}$ $G_\gamma$ $G_\beta$

PTX

$PI3K$ $ERK$

$Ca^{++}$

Gene Transcription

Cytoskeletal Reorganization

Proliferation Differentiation
CCL21 Is Required for Lymphocyte Trafficking Across HEV

Short-Term Homing Assay (PLN)

Chen et al., Nature Immunology, 2006
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- Leukocyte recruitment in acute and chronic inflammation.
Lymphocyte Extravasation in LN HEV

Evans SS, Repasky EA, Fisher DT
Nature Reviews Immunol 2015
LAD – Leukocyte Adhesion Deficiency

LAD patient leukocytes do not express LFA-1 (leukocyte-function associated antigen-1) – cannot make β2 integrin chain
Integrin Family
LFA-1 is Localized on Planar Membranes of Lymphocytes
Ig Superfamily
LFA-1 is Required for Lymphocyte Homing Across HEV of Lymphoid Organs

Quantify red-labeled cells in organs

Chen et al., Nature Immunol. 2006
LFA-1 Binds to Domain 1 (D1) of ICAM-1 Dimers

Y Yang, Molecular Cell 14:269-276, April 23, 2004
CCL21 and ICAM-1 Engagement are both Required for Maximal LFA-1 Affinity

Alon et al., Nature Immunol. 2005
Dynamic Regulation of Leukocyte-Endothelial Adhesion

**HEV**

1. **Tethering/Rolling**
   - L-selectin/PNAd
   - α4β7/MAdCAM-1

2. **Activation**
   - CCL21/CCR7

3. **Sticking**
   - LFA-1/ICAM-1/2

4. **Transmigration**
   - LFA-1/ICAM-1/2

Adapted from Immunol. Rev. 2002
ICAM-1-Enriched Microvilli-Like Projections Form Cup-Like Structure that Guides Transendothelial Migration

Shaw et al., J. Exp. Med. 2004
Yang et al., Blood 2005
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- Leukocyte recruitment in acute and chronic inflammation.
Lymphocyte Recirculation and Recruitment

**Recruitment**
- Trafficking to extralymphoid sites (HEV-like):
  - Low basal level
  - Active recruitment induced by infection, injury, acute & chronic inflammation

**Recirculation**
- Efficient trafficking to lymphoid organs:
  - PLN (HEV)
  - Spleen (no HEV)
  - Peyer’s Patches (HEV)
Dynamic Regulation of Endothelial Adhesion at Sites of Infection/Injury/Inflammation

Role of cell adhesion receptors in inflammation. An inflammatory response to foreign antigens of bacteria begins with the attraction of leukocytes that release biologically active mediators (A). These cytokines or bacterial products stimulate the expression of adhesion molecules such as ICAM-1, E-selectin, P-selectin, and VCAM-1 on the surface of cuboidal endothelial cells (B). White blood cells then adhere to the activated endothelial cells, migrate through the endothelium and enter areas of infection or tissue damage (D). (*) Up-regulated adhesion receptors on the surfaces of cells; Endo, endothelium.

Local Cytokines Increase Lymphocyte Recruitment at Inflammatory Sites

Sites of Infection, Local Tissue Damage

Transcription/Expression of Chemokines & Vascular Adhesion Molecules
- ICAM-1
- VCAM-1
- E/P-selectin
- CXCL9/10
- CCL5
mRNA Expression in Human Umbilical Vein Endothelial Cells (HUVEC) Following TNF-α Treatment

VCAM-1 Terminal Ig domains

E-selectin Lectin, EGF Repeat

Hrs: 1 2 24 48 72 0

- 4.4
- 2.4
- 1.4

- 4.4
- 2.4
- 1.4
The secret killer

The surprising link between inflammation and heart attacks, cancer, Alzheimer's and other diseases

What you can do to fight it

Taming the flames within

The fires within

The surprising link between inflammation and heart attacks, cancer, Alzheimer's and other diseases

Read the story
Venules Lined by High Endothelium in Synovium of Rheumatoid Arthritis Patients

<table>
<thead>
<tr>
<th>Disease</th>
<th>Affected organ</th>
<th>Plump endothelium</th>
<th>CAMs on endothelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crohn’s disease</td>
<td>Gut</td>
<td>+</td>
<td>MAdCAM-1/PNAd/ICAM-1</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>Gut</td>
<td>+</td>
<td>MAdCAM-1/PNAd/ICAM-1</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>Pancreas</td>
<td>+</td>
<td>MAdCAM-1/PNAd/ICAM-1</td>
</tr>
<tr>
<td>Grave’s disease</td>
<td>Thyroid</td>
<td>+</td>
<td>PNAd/ICAM-1</td>
</tr>
<tr>
<td>Hashimoto’s thyroiditis</td>
<td>Thyroid</td>
<td>+</td>
<td>PNAd/ICAM-1</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Synovium</td>
<td>+</td>
<td>PNAd/ICAM-1</td>
</tr>
<tr>
<td>Cutaneous lesions</td>
<td>Skin</td>
<td>+</td>
<td>PNAd/ICAM-1</td>
</tr>
<tr>
<td>Sjogren’s syndrome</td>
<td>Lacrimal/salivary glands</td>
<td>+</td>
<td>PNAd/MAdCAM-1/ICAM-1</td>
</tr>
<tr>
<td>Cancer</td>
<td>Breast, melanoma, ovarian</td>
<td>+</td>
<td>PNAd</td>
</tr>
</tbody>
</table>

Mechanisms of Neutrophil Recruitment to Inflamed Tissues

PMN

CXCR2

LFA-1

ICAM-1

IL-8/MIP2 KC

E/P-Selectin

Mucin-like CAM

Endothelium

1

2

3&4
Intravital Microscopy
## Ischemia-Reperfusion Injury

<table>
<thead>
<tr>
<th>Control:</th>
<th>Cells move through venules at high velocity (~2400 μm/sec); few adherent cells.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemia:</td>
<td>Reduced blood flow; distinct cells visible.</td>
</tr>
<tr>
<td>Reperfusion:</td>
<td>Increased number of rolling (~40 μm/sec) and adherent cells; adhesion is L-selectin and P-selectin dependent.</td>
</tr>
</tbody>
</table>
Process of Metastasis of Malignant Cells Involves Similar Adhesive Mechanisms As Lymphocyte Trafficking

1. Initial Release
2. Initial Invasion
3. Invasion of Blood Vessel
4. Initial Arrest - Adhesion
5. Extravasation - Exit from Vessel
6. Growth of Metastatic Nodule

R. Hynes, Cell 68:303-322, 1992
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