Visualizing intestinal immune homeostasis: T cells patrol independent of a mucosal dendritic cell network

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Outline

• Background

• Mucosal Intravital Imaging Validation

• Description of Dendritic Cell Network

• Mucosal T cell Patrolling
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Immune homeostasis in gut

- $10^{14}$ Bacteria Reside in Normal Gut (approx. 2000 species)
  - Aids in fiber digestion
  - Produces some vitamin K and B

- Immune inflammation is controlled

- Protective immunity is preserved

- ~80% of total leukocytes are in the gut

- More effector lymphocytes in the gut than anywhere else (ex. Th17)

Enteric Flora

Quiescence and Protective Immunity

Inflammation Tumorigenesis

Host Genetics
Immune microanatomy of small intestine
Overall hypothesis: The study of the modulation of T cell patrolling will provide basic insights into regulation of T cell activation in the intestine.
Why IVM to understand gut immunobiology?

• Where do the important T cell-APC interactions occur?

• What is the physical nature of T cell-APC interactions in the intestine?
  - Stable versus scanning
  - Biochemistry of TCR-pMHC and signal strength
  - Does this change during inflammation (colitis or tumor rejection)

• How does a single T cell find antigen in volume ~50,000X its own size?
  - Volume of a T cell: \( \pi r^3 = 3.14 \times 5^3 \approx 400 \mu m^3 \)
  - Volume of a Villus: \( \pi r^2 h = 3.14 \times 25^2 \times 100 \approx 200,000 \mu m^3 \)
  - Volume of lamina propria in intestine: Vol. of villus x number of villi ~200,000 x 1,000 ~ 2e8\( \mu m^3 \)
Application of LSM intravital microscopy

• Intact microenvironment (blood, $O_2$, endocrine etc.)

• Conserved microanatomy

• Identify what cellular interactions and migratory behavior are important to maintain homeostasis and immunity

• Other unpredicted insights

• Many intravital immunologic studies have focused on secondary lymphoid organs, not effector sites like the gut
Challenges in applying fluorescence IVM

• Temperature
  - <35°C, cells stop migrating
  - >39°C, cells stop migrating

• Perfusion
  - Lack of blood flow, cells stop migrating and disrupted para/endocrine systems

• Photodamage and phototoxicity
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Intravital Microscopic Set-up

Surgical Exposure of Small Bowel

Imaging Platform

A. Oxygen
B. Temperature monitoring and modulation
C. Environmental chamber
Visualizing Gut Leukocytes via the Mucosal Surface

Olympus FV1000
Ti:Sapphire fs pulsed w/neg. chirp (MaiTai DeepSee HP)
4NDD
Inverted

Typically use 25X 1.05NA, hi transmission near IR

Typical acquisition parameter
~620 x 620 pixels
2 microsecond dwell
15 z @ <30sec intervals

Playback: 5fps
Localized tissue damage
Clearance of dextran from vasculature

10KDa Dextran – FITC
70KDa Dextran – TR

Analyze small bowel blood vessel fluorescence

Permeability

Clearance

Fluorescence Intensity

Outside : Inside

% Fluorescence

Time (min)
Validation Summary

• Limited tissue damage due to surgical procedure

• Tissue perfusion is intact

• Blood filtration from kidney is intact
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Small intestine villus DC activity and intact blood flow
Visualizing Gut Leukocytes

100 µm
Overlapping non-hematopoietic and DC networks

Lethal Irrad. → CX3CR1-EGFP Bone Marrow → Chimeric

Actin-CFP

Mucosal/epithelial interface 0µm

Lamina Propria 8µm

Lamina Propria 16µm

Lamina Propria 24µm

Dendritic cell
Non-hematopoietic cell
Blood vessel
DC network summary

- DCs form a continuous network in both small and large bowel
- Network spans from villi to crypt lamina propria
- Mucosal DCs express low amounts of gap junctions
- DC network overlaps a non-hematopoietic cell network
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  • Mucosal T cell Patrolling
Villus T cell migration
The role of DCs in homeostatic T cell patrolling
Role of dendritic cells in homeostatic T cell patrolling

Hypothesis: T cells do not require DCs for homeostatic patrolling
Homeostatic T cell patrolling
How is cell migration quantified?

- This diagram represents a cell whose migration that is observed over time.

- Each blue dot represents an observation.

- An observation is made every 30 seconds.

- Every cell in the imaging field is monitored and migration characteristics quantified.
Dendritic cells in T cell patrolling

Question 1: Do T cells *scan* or *stop-and-go* on dendritic cells during homeostasis?

**Scanning**

If T cell scan DCs, speed and arrest coefficient will remain unaffected in the absence of DCs.

**Stop-and-go**

If T cell stop-and-go on DCs, they will migrate faster and/or arrest less often in the absence of DCs.
Dendritic cells in T cell patrolling part I

T cell scan DCs as they migrate through tissue during homeostasis

Speed – mean rate of migration per cell

Arrest coefficient – percent of time a cell is migrating <2µm/min
Dendritic cells in T cell patrolling part II

MI – displacement / track length

CI – max. displacement / track length

DCs are required for directionality in homeostatic mucosal T cell patrolling

p=0.08

p=0.02
Summary and conclusion

• We established a system for studying mucosal biology
  - Tissue damage is limited to incision area

• Perfusion is intact and dextran clearance is consistent with intact kidney filtration

• Vasculature is permeable to low MW dextran

• Mucosal dendritic cells form a highly organized network

• Dendritic cell are sessile and actively probe during homeostasis, reminiscent of DCs in secondary lymphoid organs

• T cells patrol independent of mucosal DCs, scanning as they migrate through tissue

• T cell require mucosal DCs to provide directionality
Single- and MP- Excitation IVM

Quantification
- Cell migration
  - Chemotaxis
  - Confinement
  - Speed
  - Arrest
  - Randomness
- Perfusion
- Vascular permeability
- Cell viability

Application
- Homeostasis
- Infectious Disease
- Tumor formation/rejection

Organs
- Small bowel
- Viscera (skin)
- Parietal peritoneum
- Liver
- Footpad
- Spleen
- Inguinal Lymph Node

Labels
- Organic dye
- Soluble dye
- Antibody
- Genetic
- SHM
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