

Restoring Epithelial Integrity With Plant-Based Therapeutics

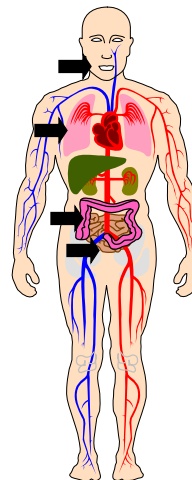
Tiffany W. Glavan
 Dr. Satya Dandekar
 Department of Medical Microbiology and Immunology
 CREATE-IGERT symposium
 September 16th 2008



Mucosal Surfaces of the Body

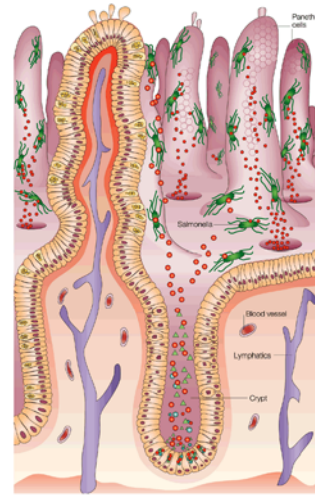
Site of host-microbe interactions

- Mucosal epithelial surfaces are found in the nasopharynx, lungs, gastrointestinal tract, and urogenital tract
- Important interface between the outer and inner environment
 - Mucosal surfaces are constantly exposed to foreign environmental antigens
 - Provide the site of transmission for the majority of human pathogens



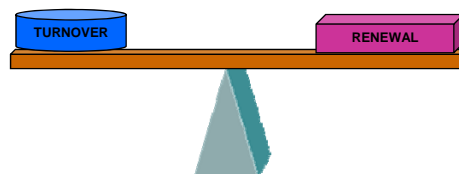
Gut-associated Lymphoid Tissue (GALT)

- GALT comprises the largest lymphoid organ in the body
- Protects the body against pathogenic invasion at mucosal surfaces in the small and large intestines
- Cells of the immune system constantly sampling luminal contents and making the “decision” of whether or not to mount an immune response
- Mucosal barrier function integral to human health



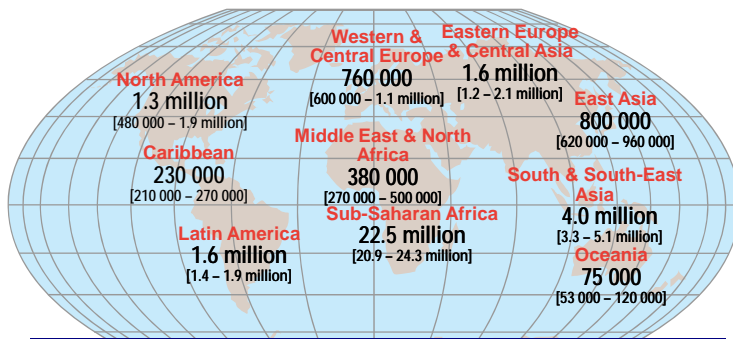
Epithelial Renewal is an Important Balancing Act

- Epithelial cells are continually differentiating and migrating upward, rapidly replenishing the epithelial layer
- Cell turnover in the epithelial layer is important both in the absorption of nutrients and in the containment of foreign antigens and commensal microorganisms



HIV poses a global challenge

Adults and children estimated to be living with HIV, 2007
 Total: 33.2 (30.6 – 36.1) million



The mucosal immune response may hold the key to understanding HIV pathogenesis

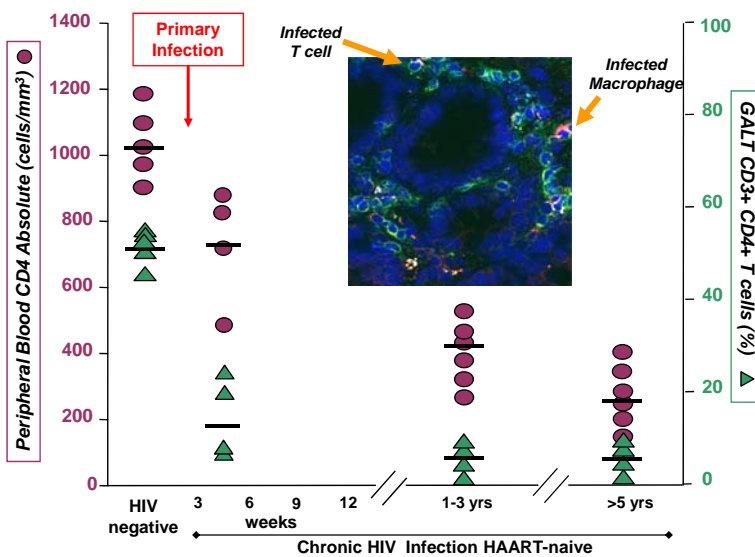


World Health Organization

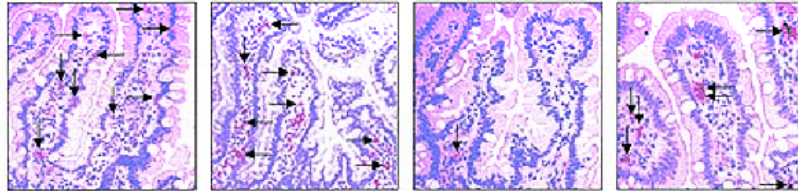


UNAIDS
 The Joint United Nations Programme on HIV/AIDS

HIV guts the gut's immune response rapidly and severely



HIV therapy fails to fully restore CD4+ T-cells in the gut



HIV
negative

HIV infected
no therapy

HIV infected
5 years therapy

Although CD4+ T-cell levels are restored in the peripheral blood compartment following HAART, restoration in the gut mucosa is limited and viral reservoirs persist here

Research Focus of the Lab

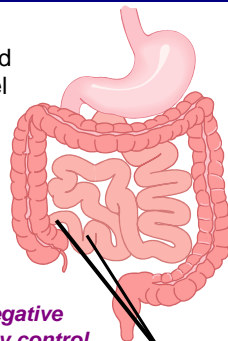
- Determine pathogenic mechanisms of HIV associated mucosal damage
- Develop novel experimental approaches to enhance and restore mucosal repair and regeneration and to achieve protection against HIV disease

We are interested in answering the questions:

- How does the loss of gut CD4⁺ T-cells impact the early innate immune response to other microbes?
- Does the loss in CD4⁺ T-cells from the gut contribute to chronic inflammation at a local and systemic level?
- How do viral reservoirs in the gut change and evolve over time?

How does a pre-existing SIV infection alter the immune response to *Salmonella*?

Ileal Ligated Loop Model



SIV-negative healthy control animals

SIV-infected animals

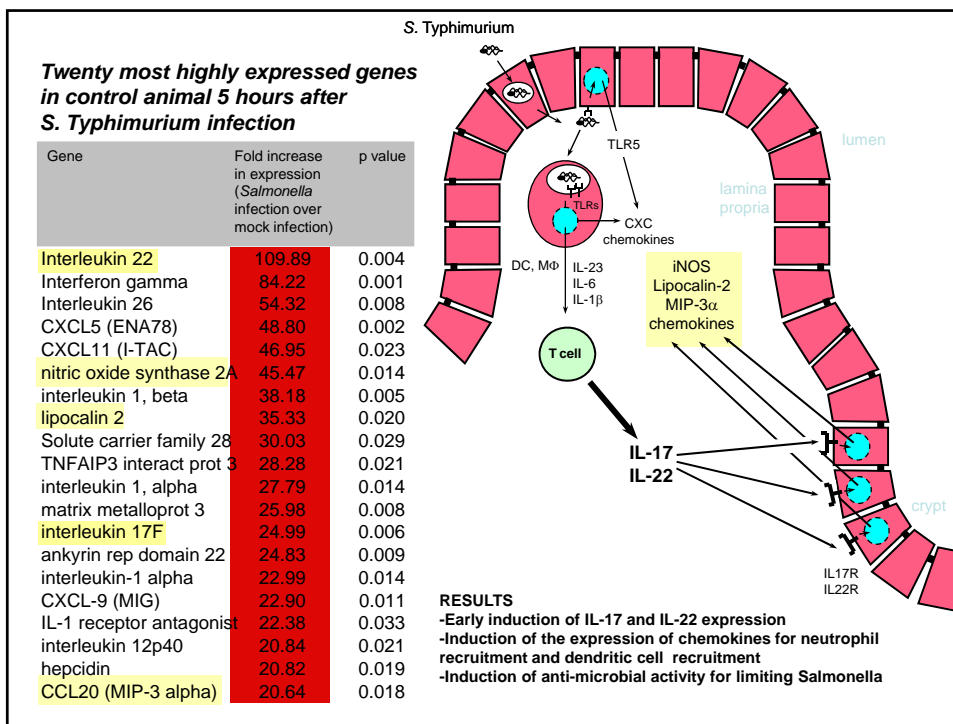


Inject with *S. Typhimurium* or with sterile medium

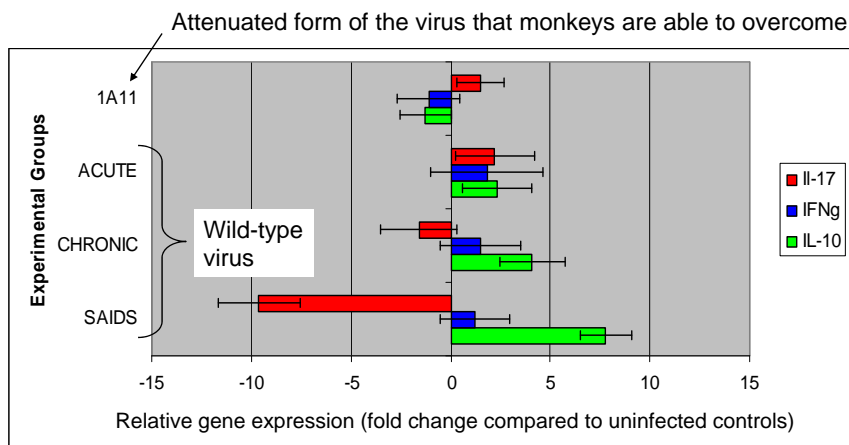


Collect loops at 5 hours after *Salmonella* challenge to investigate host response

Set up a co-infection study in the rhesus macaque model of AIDS to identify defects in mucosal immune response to *Salmonella typhimurium*

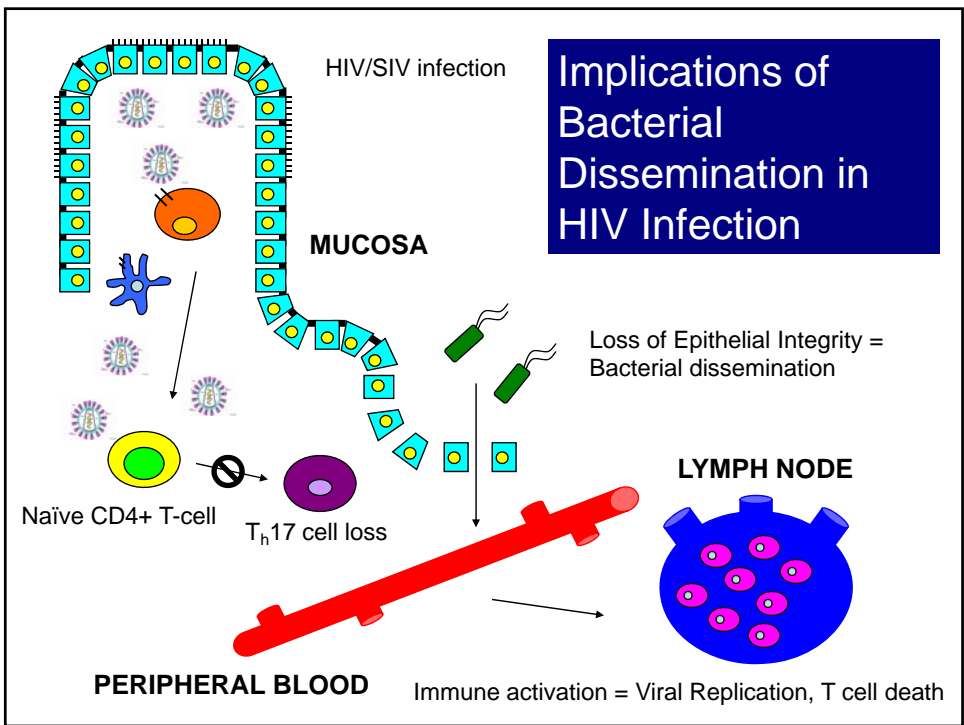
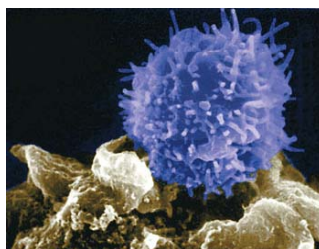


Th17 defect found only when animals infected with wild-type virus



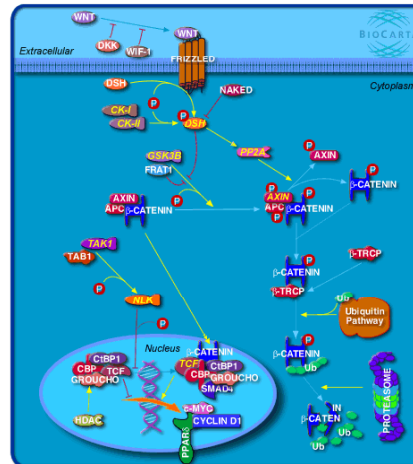
Th17 cells

- Th17 cells are a subset of CD4+ T cells that play a protective role in mucosal defense against extracellular bacterial or fungal pathogens
- Th17 cells produce IL-17, an important signaling molecule that orchestrates a neutrophil-mediated proinflammatory response at the intestinal mucosa
- Induce the synthesis of antimicrobial compounds
- Support intestinal barrier function by inducing tight junction formation



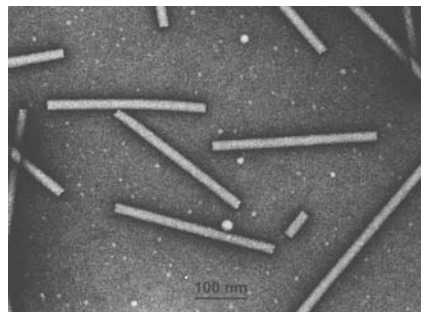
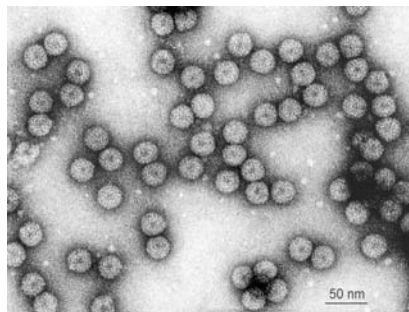
How can we restore the integrity of the mucosal epithelium?

- Epithelial Growth Factors
 - Mitogenic agents
 - Mediators of Wnt pathway
 - FGF-7
 - GLP2
 - R-spondin



Viral vector based plant expression systems

- Cucumber Mosaic Virus (CMViva)
- Tobacco Mosaic Virus (TRBO)



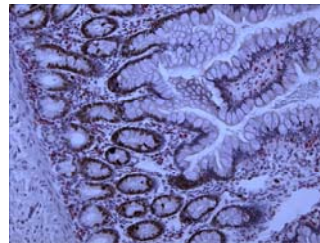
Advantages of Viral Vector Based Plant expression systems

- Speed
- Activity
- Safety
- Biocontainment
- Protein expression levels



The ultimate goal...

- Plan to evaluate efficacy of growth factors in restoring the epithelial integrity of the mucosal layer in SIV-infected macaques
- Qualitative and quantitative tools readily available for efficacy studies, including
 - Immunohistochemistry
 - Real-time PCR
 - Microarray analysis

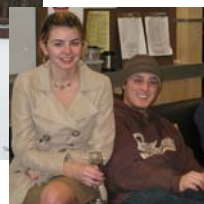


Acknowledgements

THANK-YOU!

The Dandekar Lab

Dr. Satya Dandekar
Monica Macal
Irina Grishina
Jeff Chan
Michael George
Sumathi Sankaran
Paula Lerner
William Hu



Our collaborators:

Dr. Karen McDonald, Chemical Engineering and Materials Science
Dr. Abhaya Dandekar, Pomology
Dr. Bryce Falk, Plant Pathology

**This work was made possible through funding by the National Science Foundation (DGE-0653984) via the Integrative Graduate Education and Research Traineeship (IGERT) program
National Institutes of Health grants**