
What did we learn over the past
2 days?

Genetics is cool

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- But you have to achieve impossible p values

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- You have to replicate your findings in many, many, many centers

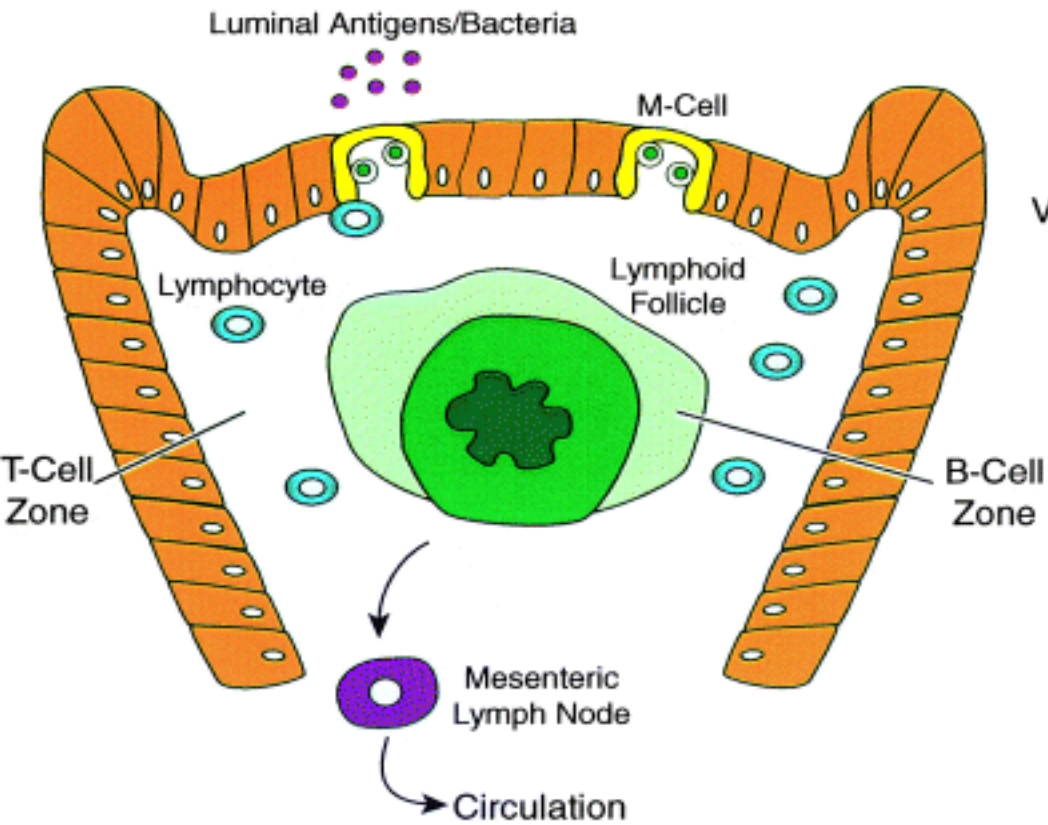
Genetics is cool

- But you have to achieve impossible p values
- You have to replicate your findings in many, many, many centers
- And you still need the biologists and physiologists to provide disease relevance

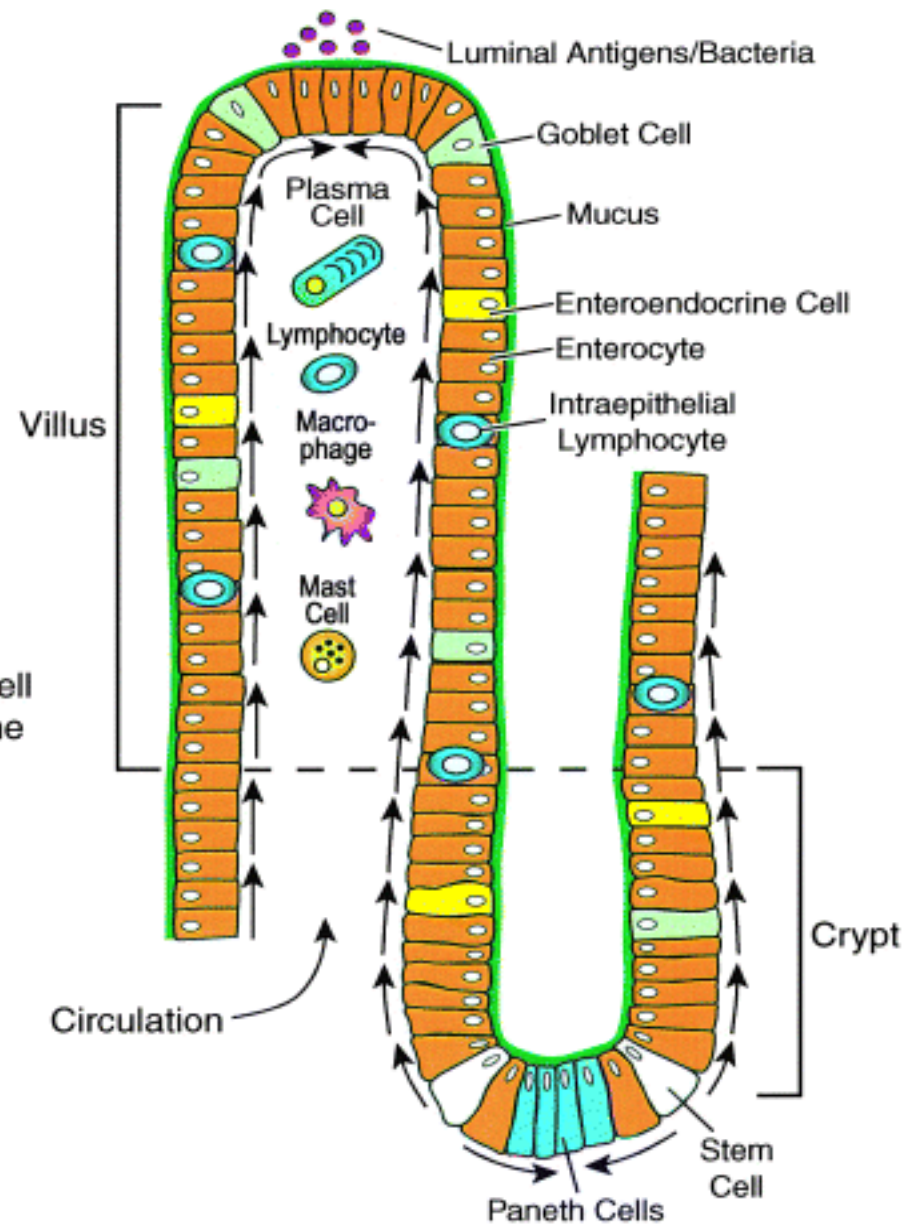
Genetics is cool

- NOD2 appears to be involved in Peyer's patch development
- In the knock out
 - Increases in permeability
 - Increased bacterial translocation
 - Increased cell number in PPs
 - Increased numbers of ILFs
 - Increased IFN γ , IL4, TNF in PP
- ? Role in protection against enteric organisms (Y pestis) and genetic selection

INDUCTIVE SITE (Peyer's Patch)



EFFECTOR SITE (Lamina Propria)



Genetics is cool

- NOD2 mutations are associated with a higher incidence of GVHD
- NOD2 mutations are associated with a poorer prognosis in SB transplant
 - The effect on α defensins (as well as HBD5) is indirect

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 - The effect on α defensins (as well as HBD5) is indirect -
 - Stange is wrong

Genetics is cool

- Adiponectin mutations/polymorphisms in CD patients
 - need greater numbers - Not ready for prime time
- Occludin, claudin 1, claudin 4, JAM 1 mutations/polymorphisms in CD patients
 - need greater numbers - Not ready for prime time

Genetics is cool (ultimate lesson)

- Wait for the geneticists to do their thing - then do genetic profiling to follow a phenotype or physiological observation

**Is there really a single pathogen
responsible for the development of IBD
- CD vs UC?**

Is there really a single pathogen responsible for the development of IBD - CD vs UC?

- Subtractive hybridization vs viral chip - Is there really a signal?
- Antimicrobial antibodies reflect defect in tolerance rather than specific infection - selectivity may reflect colonization vs. immunodominance
- MAP should be dead - no evidence for an immune response against this organism in IBD patients
 - Immune response is hallmark of mycobacterial diseases

We have found the cure for colitis

- In mice

We have found the cure for colitis

- Inhibit cholinergic nicotinic receptor
- ATN-161 (anti- $\alpha3V\beta3$ peptide involved in angiogenesis)
- ? Adiponectin
- ? LMP20 (TNF inhibitor) - no effect in chronic DSS model - effects only seen at low dose in acute DSS
- Galectin 1 homodimer - decreases IFN γ and increases IL10 in anti-CD3 stimulated PBMC

We have found the cure for colitis

- Necatur - Hookworm-
 - increases in IL5 and eosinophils in humans
 - Increasing evidence suggests the epidemiologic associations are telling us that the modification of the hygiene hypothesis may have some merit
 - Assess the plausibility of the cold chain hypothesis
 - Need to define mechanism of activation of peripheral immune system by systemic trafficking
 - Need to define antigens in helminths more rigorously
 - Need to show clear cause/effect
 - Effect of glycans may be the key in atopy, regulatory cell activation and anti-cancer immunity

We have found the cure for colitis

- MCH antagonists - decreased TNBS colitis in MCHR-/- mice
- HSP 60 and HSP 70 - conserved across species and bacteria - stress responses, anti-inflammatory. Why is adding so little through the upper GI tract so effective in TNBS model when you have kgs of HSPs in the colon?
- TLR cell permeable peptide targeting the juxtamembrane highly charged PIP2 binding sequences -
 - Protects from septic shock - if given before the insult
 - effective in TNBS but TLR2 not 4 peptide

We have found the cure for colitis

- Photodynamic therapy - induction of apoptosis in activated CD4+ T cells in the inflamed mucosa -
 - dose effect
 - Maintenance of response
- IL22 - TH17 driven, increased in CD>>UC tissues.
 - Activates STAT3 but not ERK1/2 in innate cells preventing activation of pro-inflammatory pathways (ERK1/2 mediated)
 - Decreased inflammation in TcR α -/- with increased MUC1, MUC3 and MUC13.
 - Atsushi Mizoguchi makes neat slides

We have found the cure for colitis

- Autologous vs allogeneic BMT for immune mediated inflammatory disorders
 - Data from many centers suggests that it works for some but not all such diseases
 - Suggest that this approach alters the immune repertoire - this can and should be studied in detail
 - In Chicago the data are promising but the key question remains as to whether this is related to the induction regimen as opposed to the transplant itself
 - The Hawkey study will address this
 - Is it worth the potential increase in mortality
 - Most of us believe that CD is not a fatal disease

So why do all of these agents work so effectively?

- Are there common pathways?
 - Is the interplay of cell types in the mucosal environment so critical that if you correct one you correct them all?
 - But then why is it that if we have a defect in one pathway the default is to mucosal inflammation (basically all of the mouse models)?

So why do all of these agents
work so effectively?
(ultimate lesson)

Mice are not men

The enteric nervous system is important in intestinal inflammation

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- This was for John Bienenstock's sake - I really don't believe it

The enteric nervous system is important in intestinal inflammation

- Glial cell ablation leads to small bowel inflammation (T Savidge - J Exp Med)
- SST is anti-inflammatory - effective in the treatment of IL10^{-/-} model
- Enteric ganglia are depleted in active IBD (CD and UC)
- Vagotomy increases severity of DSS induced colitis but not (indirectly inferred) in CD4⁺ CD62L⁺ -->SCID transfer model
- Probiotics do not work through vagal innervation

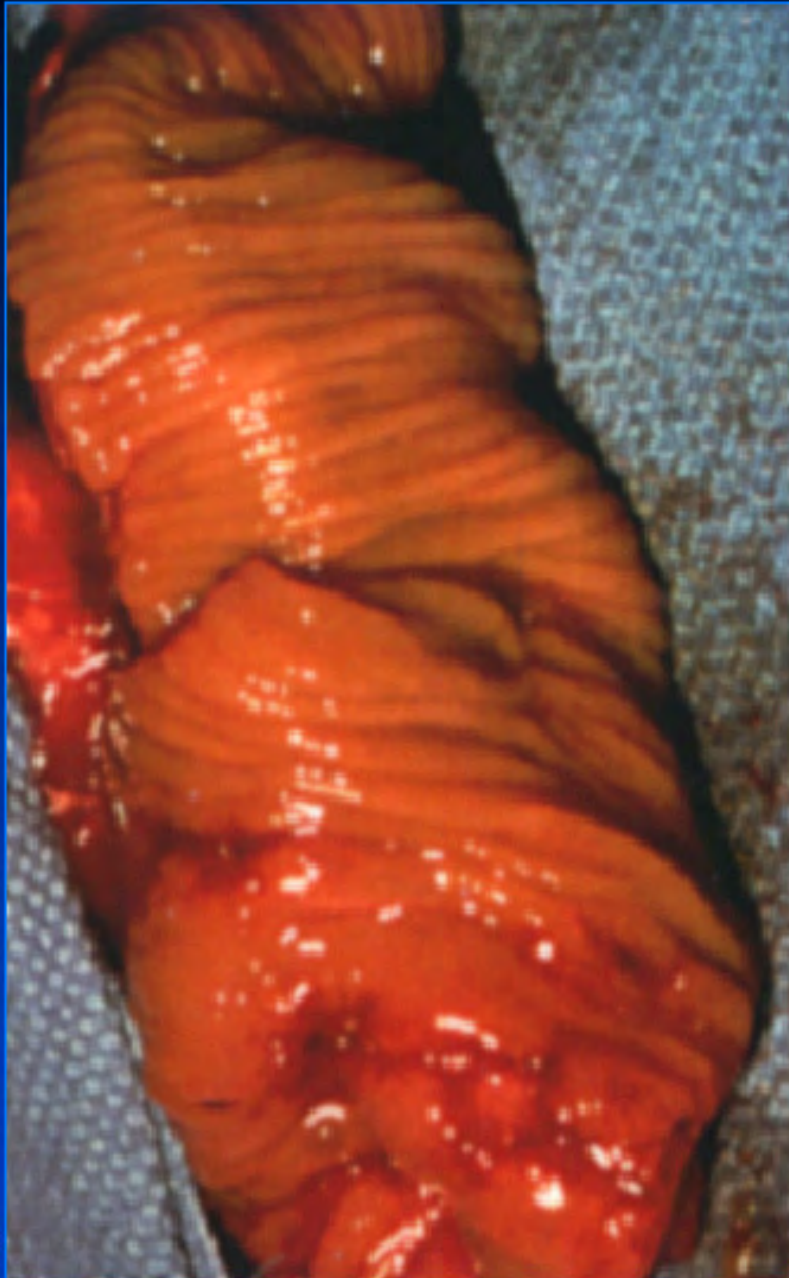
The enteric nervous system is important in intestinal inflammation

- Melanin concentrating hormone (MCH) makes you fat, is increased in active colitis, is produced by enteric nerves and its blockade ameliorates TNBS colitis (without an effect on weight)
- Increases in neuropeptides are associated with post-inflammatory pain in diverticulitis

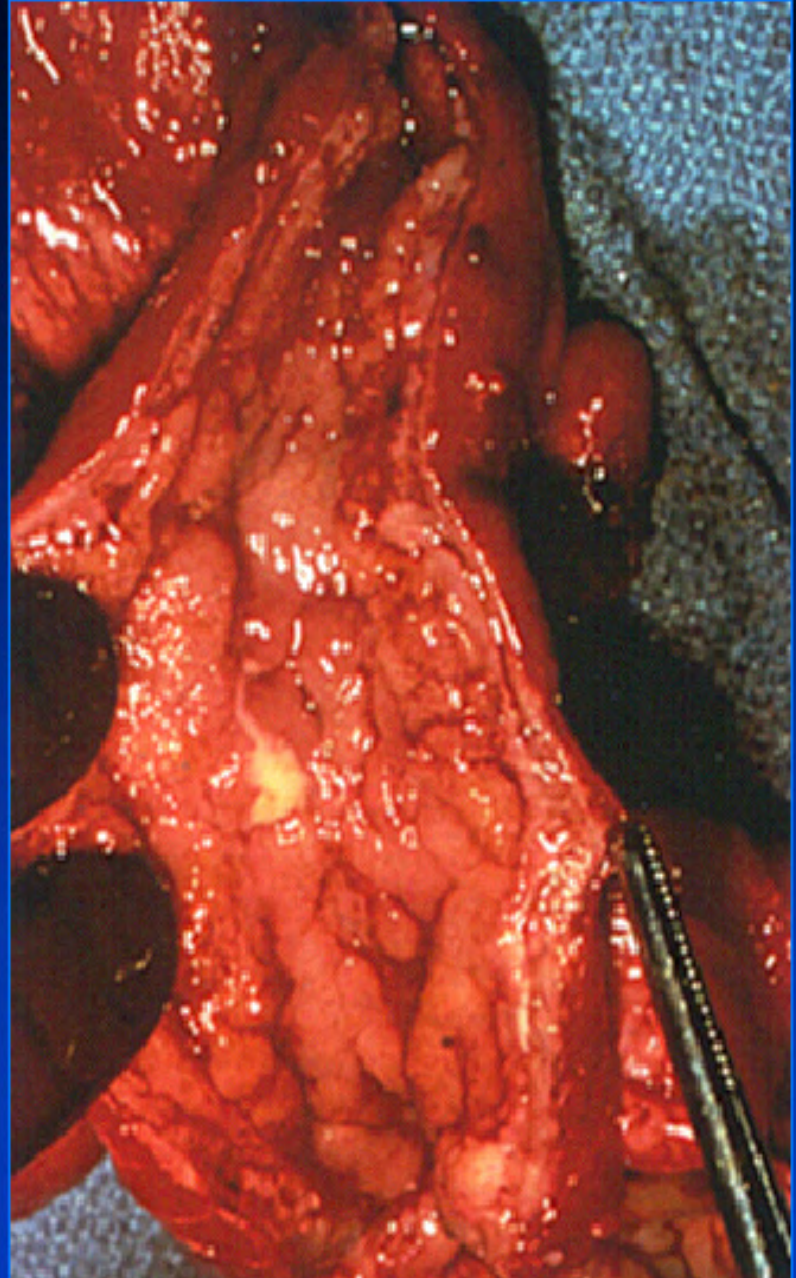
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- Vascular endothelium and lymphatics
 - Role in lymphoid organogenesis
 - Literature suggesting that IBD is an ischemic disease
 - Lymphocytes and APCs have to get into the bowel to do their dirty work
 - ATN 161 - induces decrease in vascular density associated with decreases in IL6 and IL12 in the tissue
- Adipocytes -
 - creeping fat. Seen in CD but not other inflammatory disorders - Why?
 - Adiponectin - PPAR γ induced, anti-proliferative and IL10 inducing
 - ? Role of leptin in immunoregulation



Normal colon



IBD - thick colon transmural disease



There are cell types that are ignored that shouldn't be

- Stromal cells -
 - They have to be there for a reason
 - source of TGF β in normal gut mucosa
- Regulatory cells that are not CD4+CD25+FoxP3+
 - HSP60/70 - MICA/B restricted Tregs

Regulatory cells

CD4+

CD8+

NK-T

T $\gamma\delta$

CD4-8-

B cells

DCs

Macrophages

Mouse

&

Human

Regulatory T cells

There are multiple types of Tregs -
all of them have been described in
conjunction with the gut

Th3

Tr1

CD4⁺CD25⁺ FoxP3⁺

CD8⁺

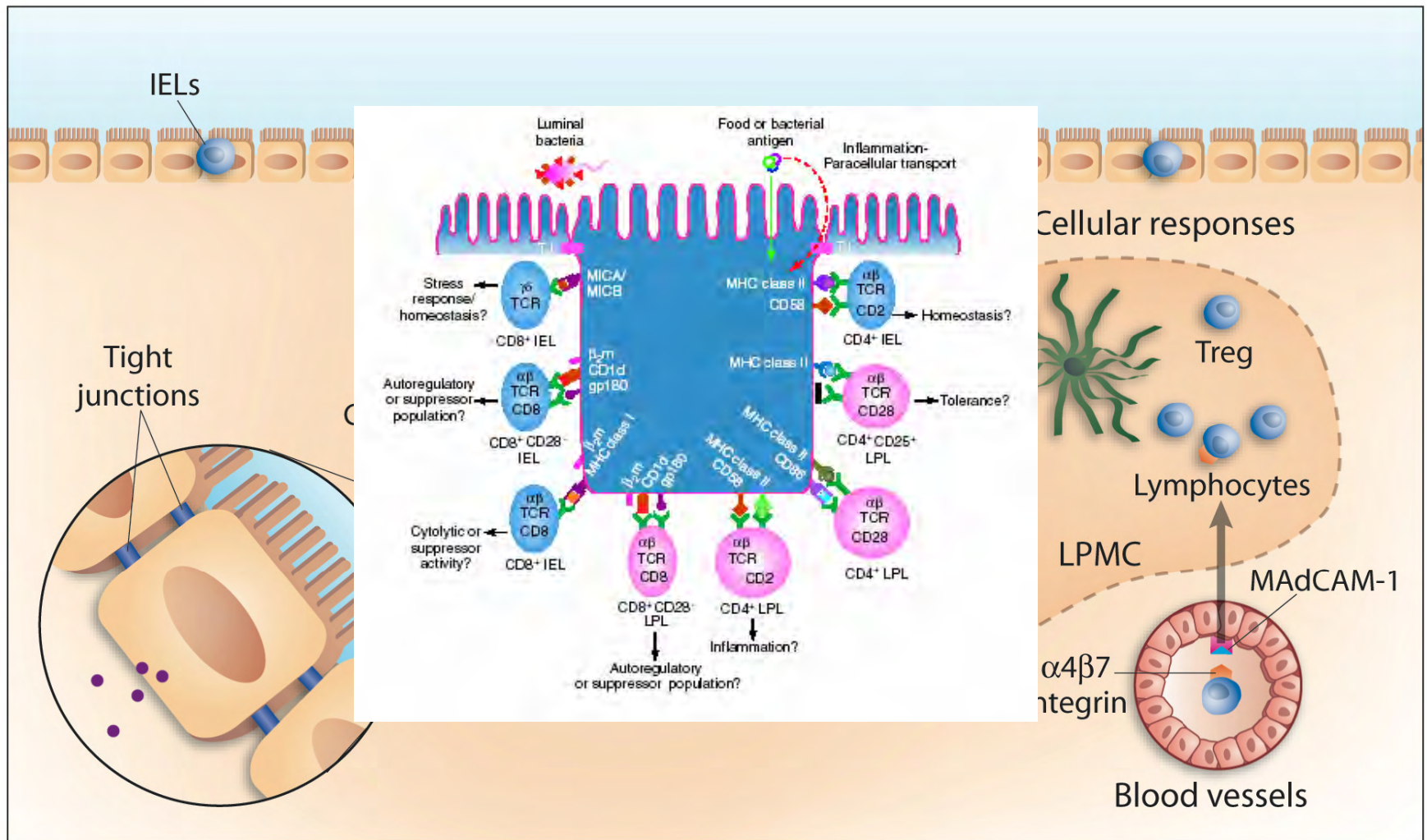
We need markers for disease specificity and activity

- CCFA RFA for biomarkers
- Genetic, serologic, radiologic and fecal
 - Development of designer chips (E coli, yeast, and autoantigen)
 - Refinement of available markers (A12 better than calprotectin)

Epithelial cells rule

- Involved in barrier function, innate and adaptive immunity in the GI tract. Must be the central cell involved in the development of IBD.
 - ? Role for mutations in the junctional complex proteins
 - Permeability defects in IBD. Are they real?
 - We need to develop better methods to characterize defects in permeability

Epithelial cells by multiple pathways (innate and adaptive) regulate mucosal immune responses



We love BMRP!!!