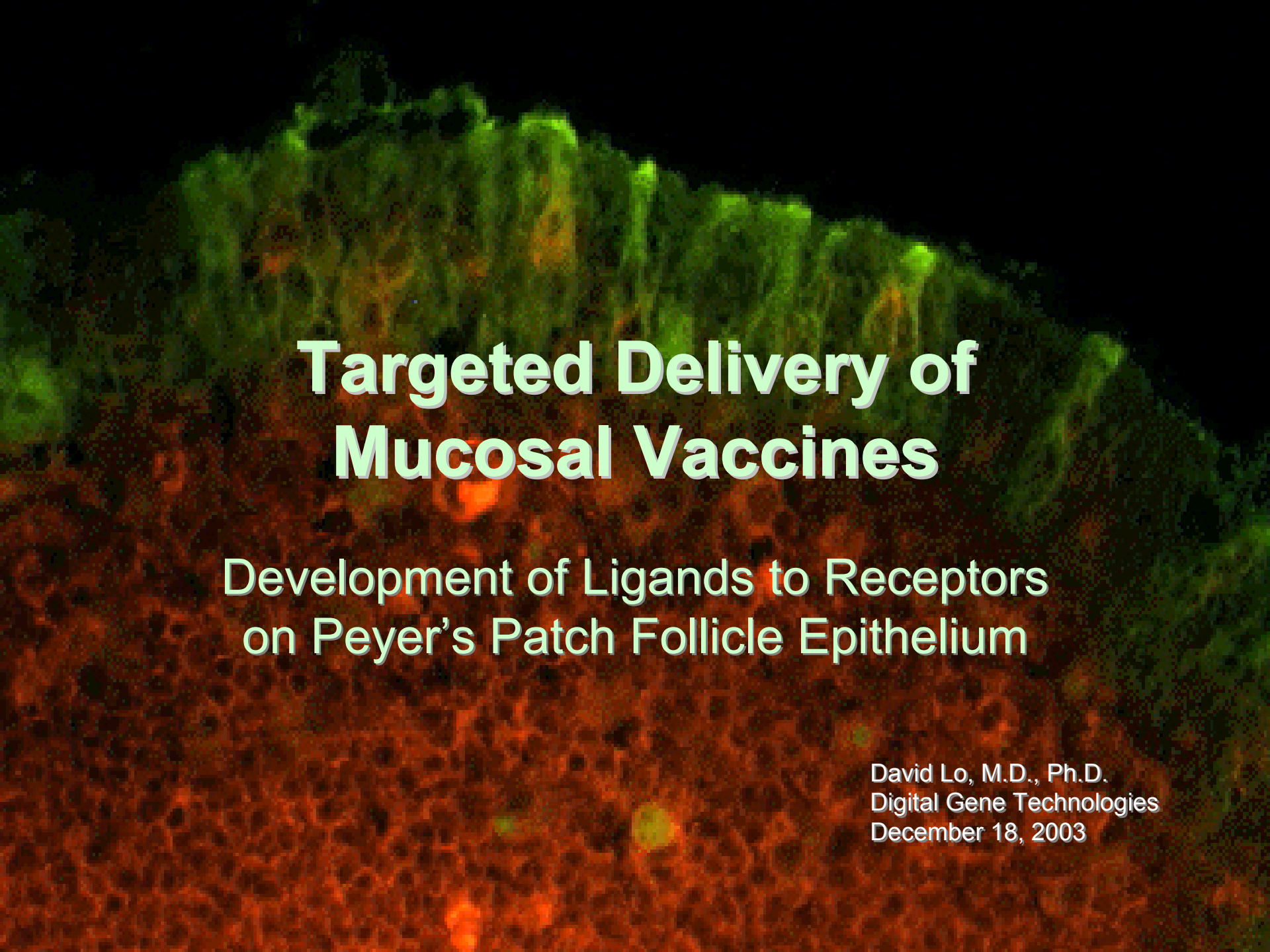

Targeted Delivery of Mucosal Vaccines

Development of Ligands to Receptors on Peyer's Patch Follicle Epithelium

David Lo, M.D., Ph.D.
Digital Gene Technologies
December 18, 2003

Ligands to Receptors on Peyer's Patch Follicle Epithelium

- Peyer's Patches are critical in protective mucosal immunity BUT ALSO in mucosal tolerance induction
- PP Follicle Associated Epithelium is central to this surveillance function; can we exploit the system for vaccine delivery?

- Q1: Can the FAE and M cells be molecularly defined?
- Q2: Does the FAE have functions helpful to mucosal immunity?
- Q3: How do we exploit the biology for vaccine delivery?

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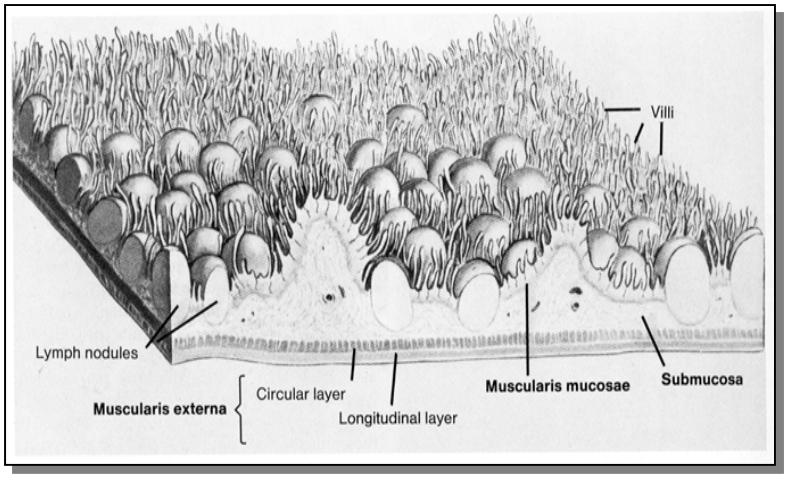
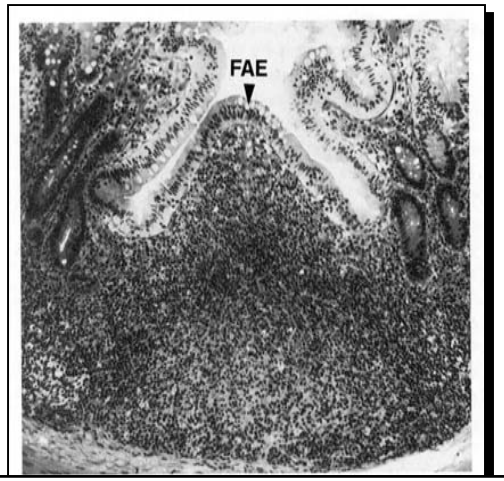
FAE/M Cell Biology

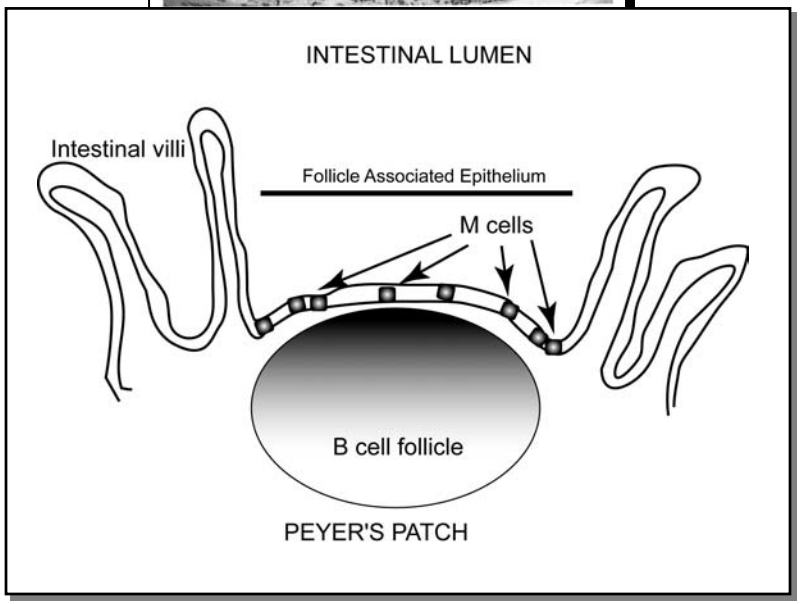
- In the small intestine, immune responses in Peyer's Patches may be activated against pathogens (viruses, bacteria), or tolerance may be induced to food antigens
- Sampling of antigens is through active transport of proteins across Peyer's Patch epithelium
- Hypothesis: FAE/M cell specific genes exist that can explain their function
  - M cell specific receptors can be exploited for development of vaccine/drug delivery systems

FAE/M Cell Biology





- Peyer's Patches in intestine
  - Dependent on lymphocytes and Lymphotoxin-beta
  - Organized B follicles
  - Interfollicular T/DC zone



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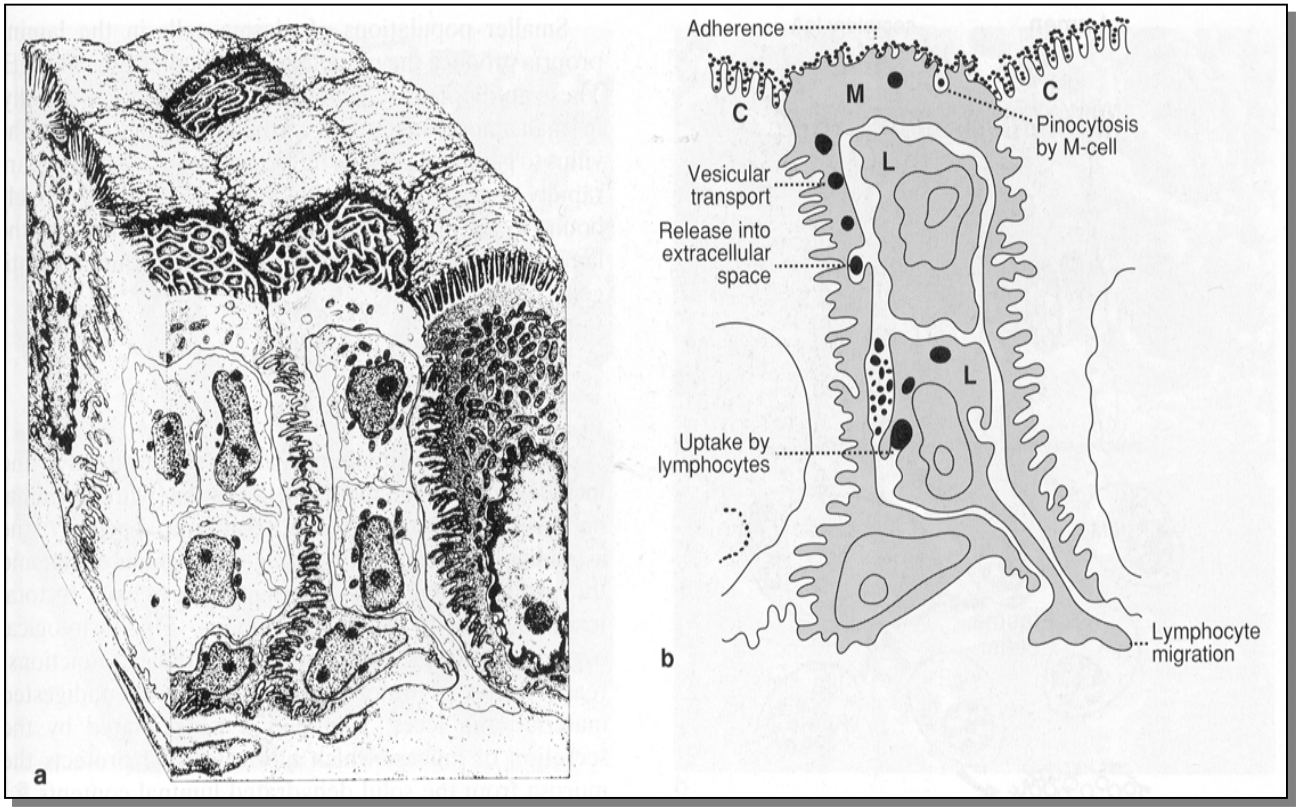
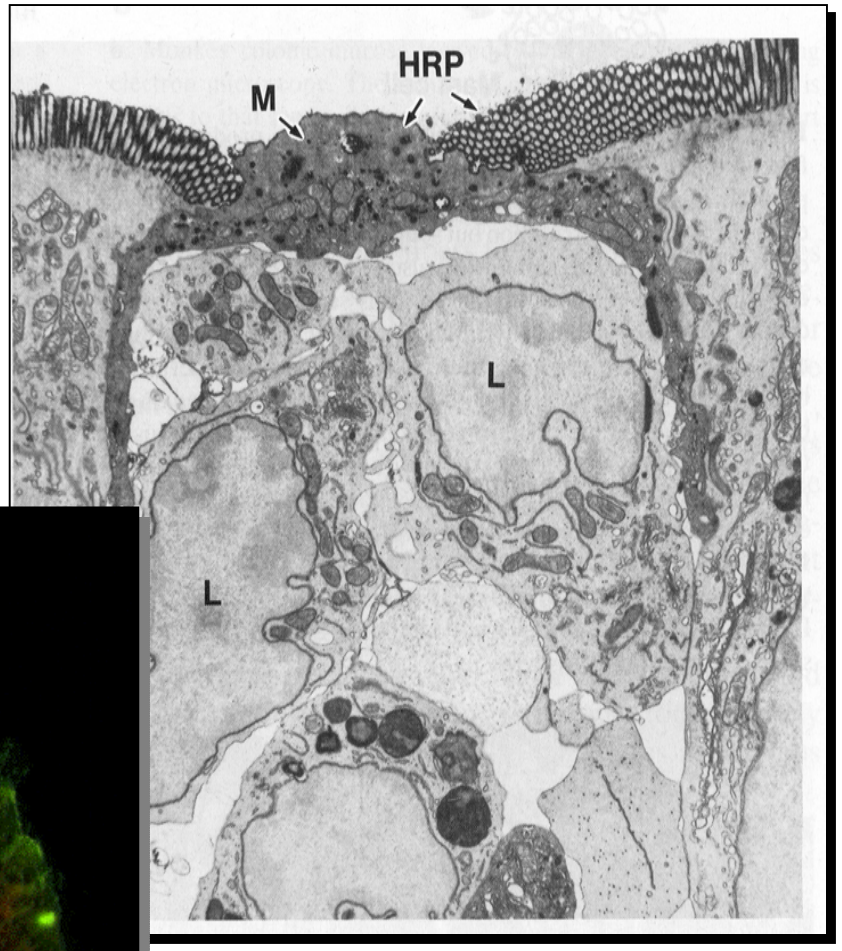
FAE/M Cell Biology

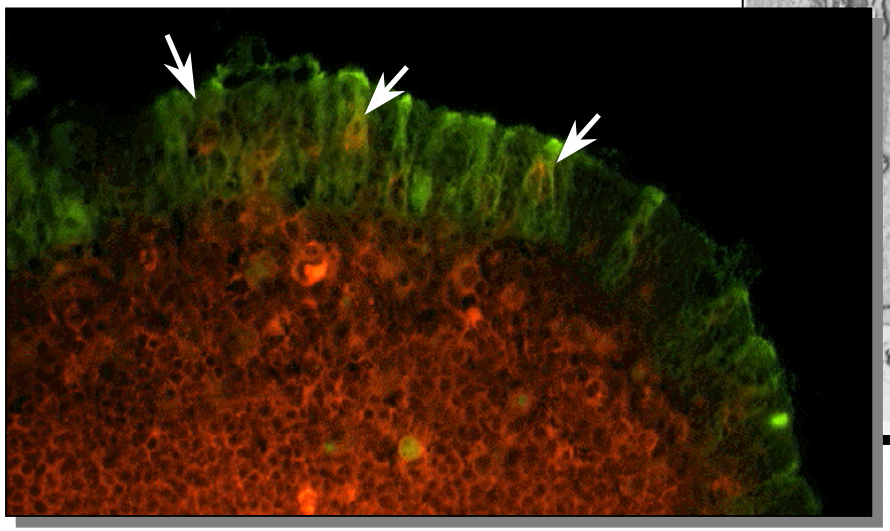
- Clues to FAE specialization:
  - Induction of differentiated phenotype dependent on interaction with lymphoid cells
  - Distinct morphology of M cells



FAE/M Cell Biology

- M cells develop in contact with B lymphocytes (arrows)
- Lectin UEA-1 (green) identifies mouse M cells





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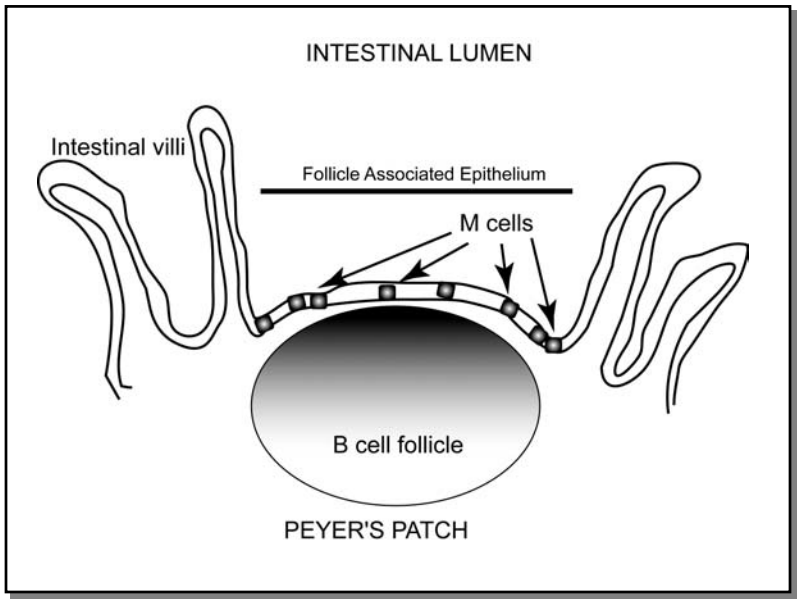
FAE/M Cell Biology

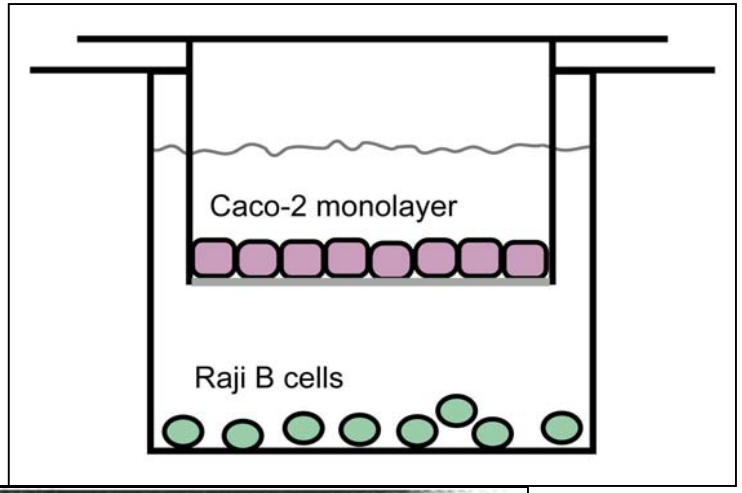
- Peyer's Patches in intestinal mucosa:
- TOGA® Gene Expression Profiling Studies to Find Candidate Receptors:
  - Human cell culture, Caco-2 co-culture with Raji B cells
    - Induces M cell phenotype
  - Peyer's Patch tissue from mouse and macaque, microdissection of FAE

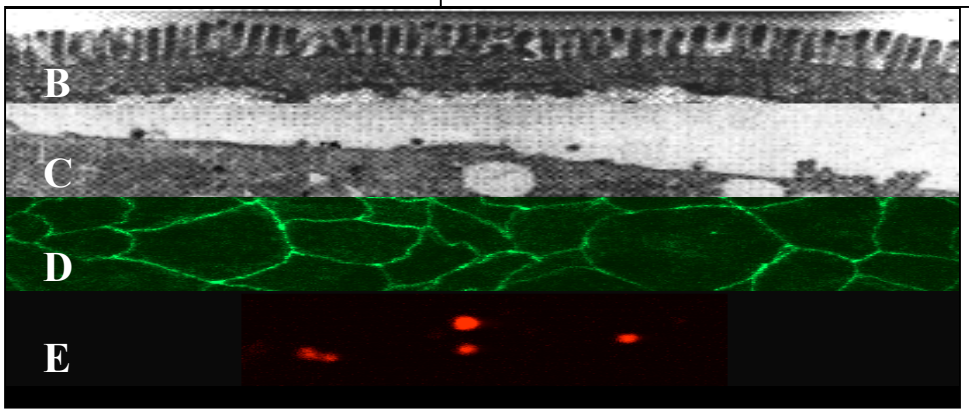


Caco-2/Raji B Co-culture

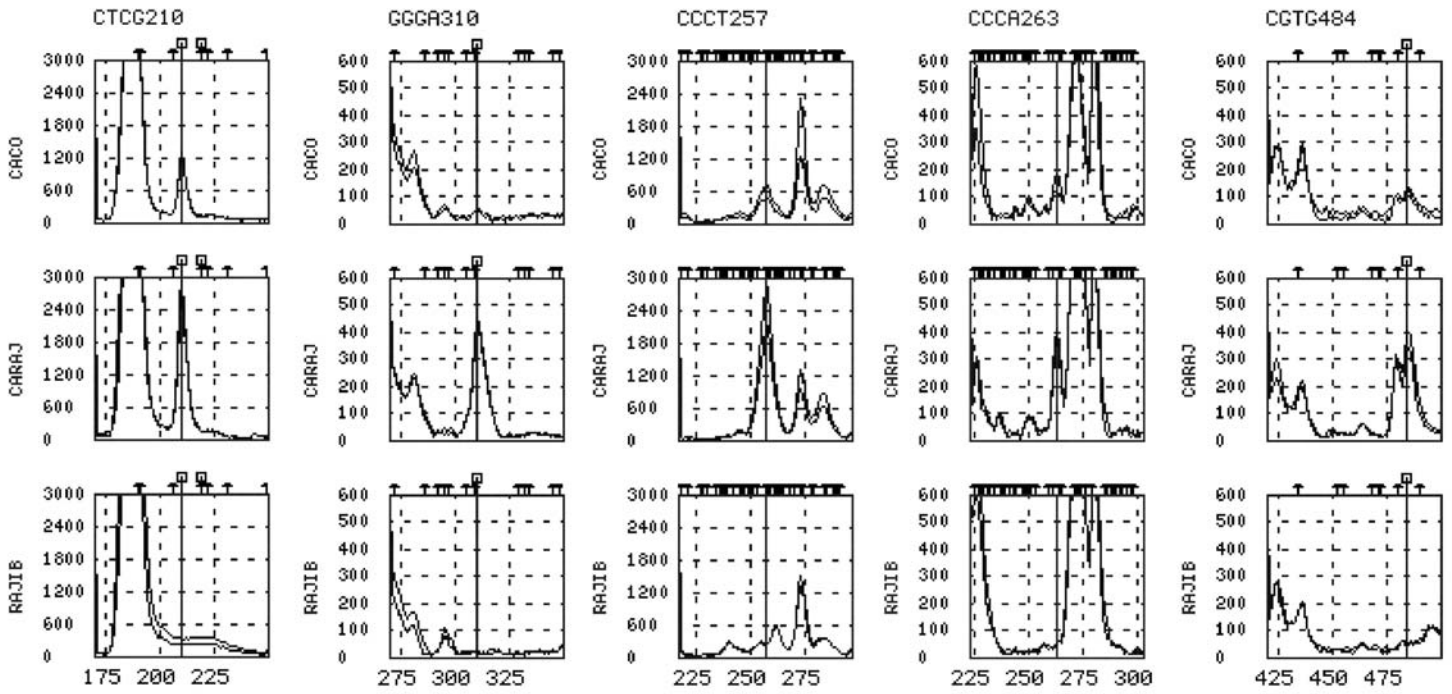
- Soluble factors provided by Raji B cells
- Loss of brush border
- Microparticle transcytosis

- A true M cell phenotype?





Candidate Selection



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Genes Regulated in Caco-2 Co-culture

Gene (accession no.)	Caco	CaRaj	RajiB	Fold regulation by TOGA, qPCR
Up-regulated:				
Transcription factor (AK000232)	44	2165	51	49.2, 119.3
Jagged-1 (AW369026)	43	328	46	7.6, 1.8
c-Maf (AV648578)	134	1273	49	9.5, 14.7
DEC-1 (AB004066)	169	972	78	5.8, 3.7
RAB-13 (W46375)	73	345	23	4.7, 9.3
Glutaredoxin (AW128930)	135	890	270	6.6, 3.6
GPx-4 (X71973)	92	277	55	3.0, 4.4
ULK1 (AF045458)	119	333	61	2.8, 2.5
CDC2-related kinase (Q14004)	56	149	53	2.7, n.t.
Down-regulated:				
Ubiquitin B (BC000379)	1492	665	979	0.45*
Mitochondrial gene (E27671)	3360	138	3918	0.04*
3-pgdh (AF006043)	1091	268	997	0.25*
farnesyl diphos synthase (BC010004)	2066	831	2168	0.40*
transketolase (BC008615)	1123	416	1155	0.37*

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Candidate Receptor Genes

Gene	Fold-regulation by TOGA, qPCR	Tissue Expression
Biliary glycoprotein A	3.9, 1.8	FAE=villi
Mu protocadherin	10.6, 19.5	FAE=villi
Tetraspan TM4SF5	2.6, 1.0	FAE>=villi
LDL-R	5.0, 1.1	FAE>villi
Apolipoprotein B	2.3, 3.5	FAE>villi
Tetraspan TM4SF3	8.9, 4.8	FAE>>villi, crypts
C. perfringens enterotoxin R	4.2, 1.8	FAE, M cells, villi
MMP15	2.7, 0.7	FAE
Laminin beta 3	3.0, 1.9	FAE

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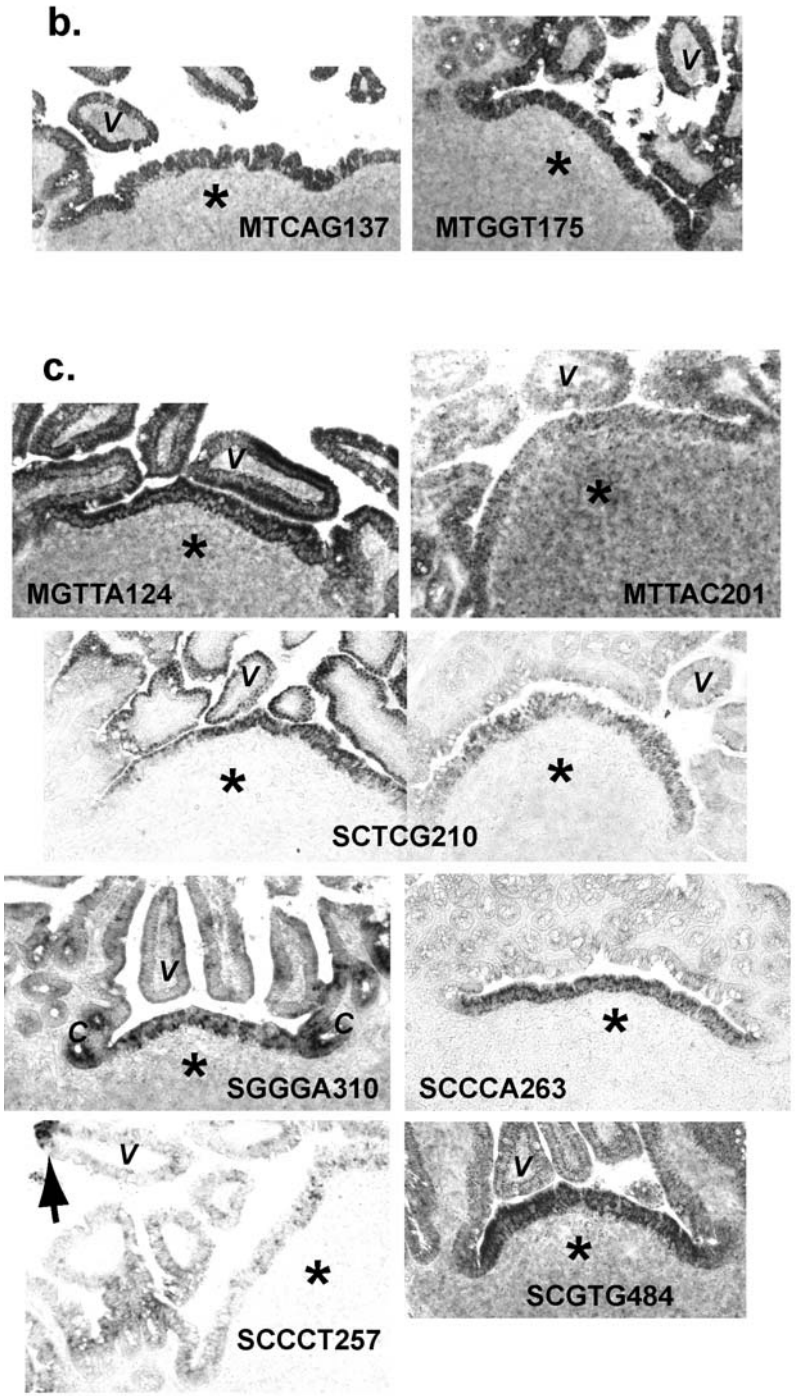
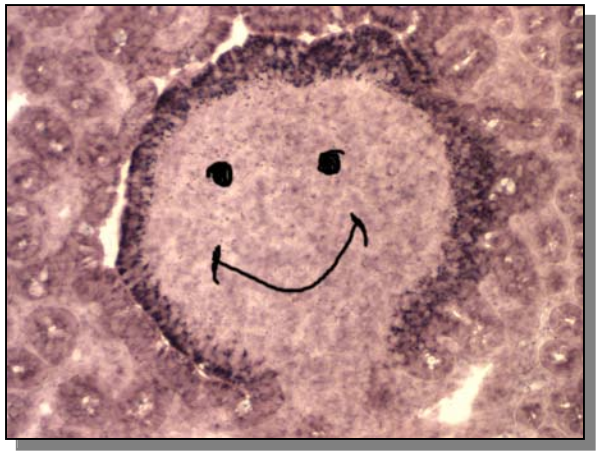


The Proof Is in the Patches



- Many epithelial specific patterns, not all restricted to FAE
- Some restricted to FAE, or subset of FAE

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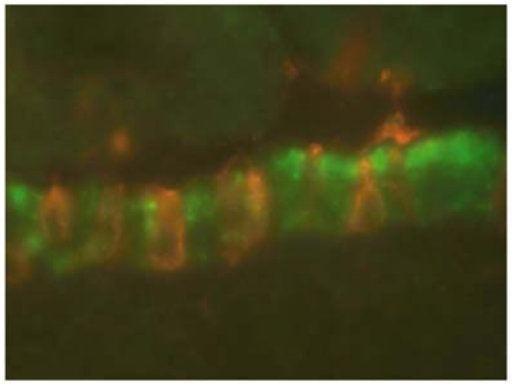
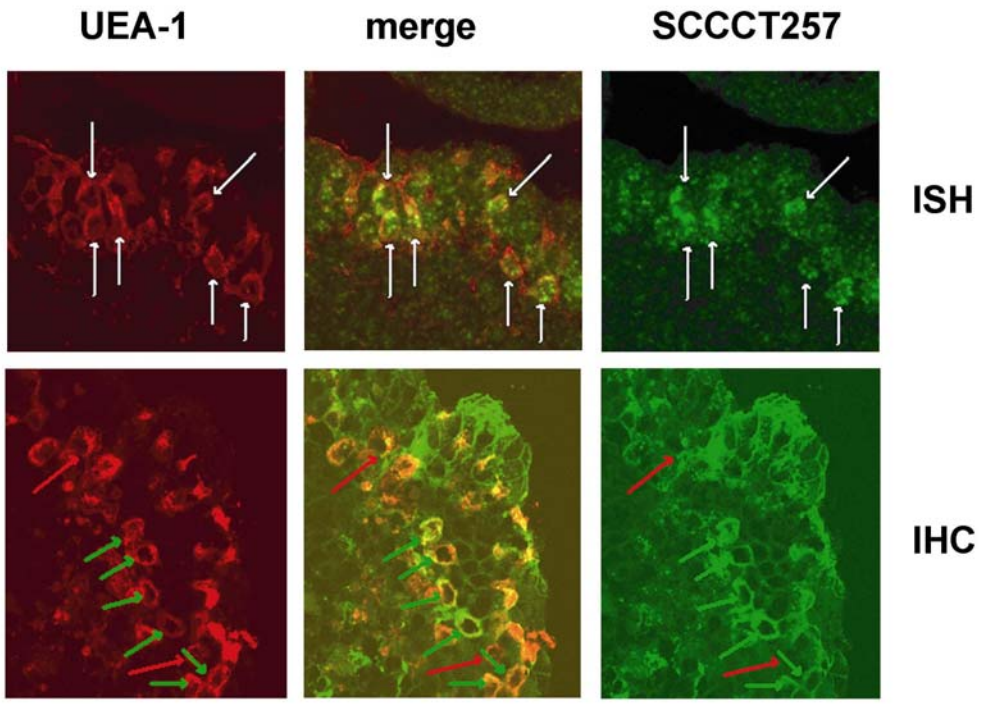
Co-localization With M Cell Marker UEA-1

a. SCGTG484



b. SCCCT257



- SCGTG484 (laminin beta 3) showed FAE specific distribution, but not on M cells
- SCCCT257 (CPE-R) showed epithelial tight junction distribution, but also higher expression and cytoplasmic distribution in M cells

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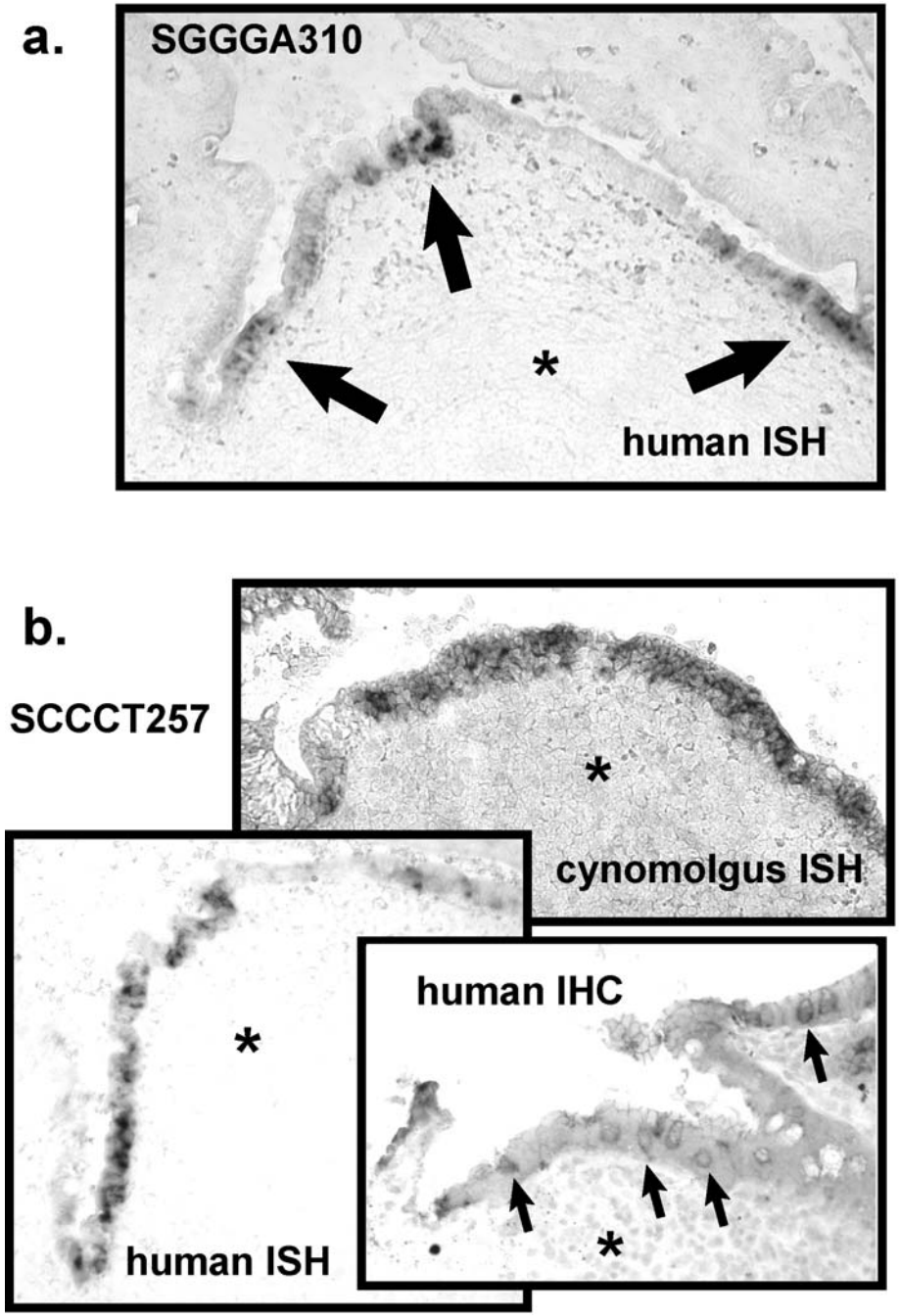

Expression in Monkey and Human Peyer's Patch

- TM4SF3 expressed in FAE subset
- CPE-R again shows cytoplasmic distribution in subset of FAE

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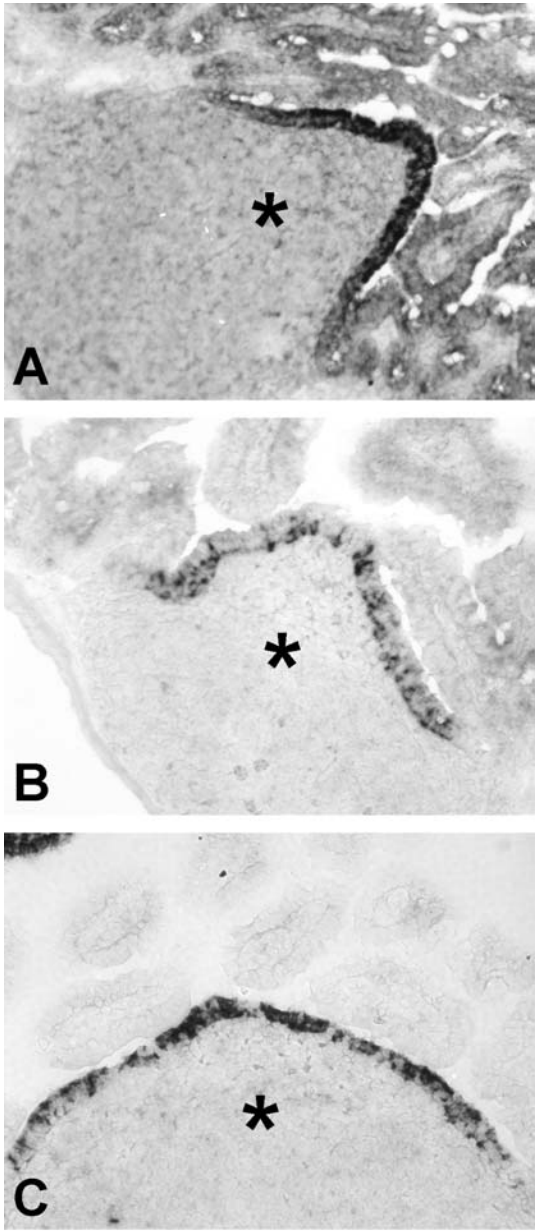
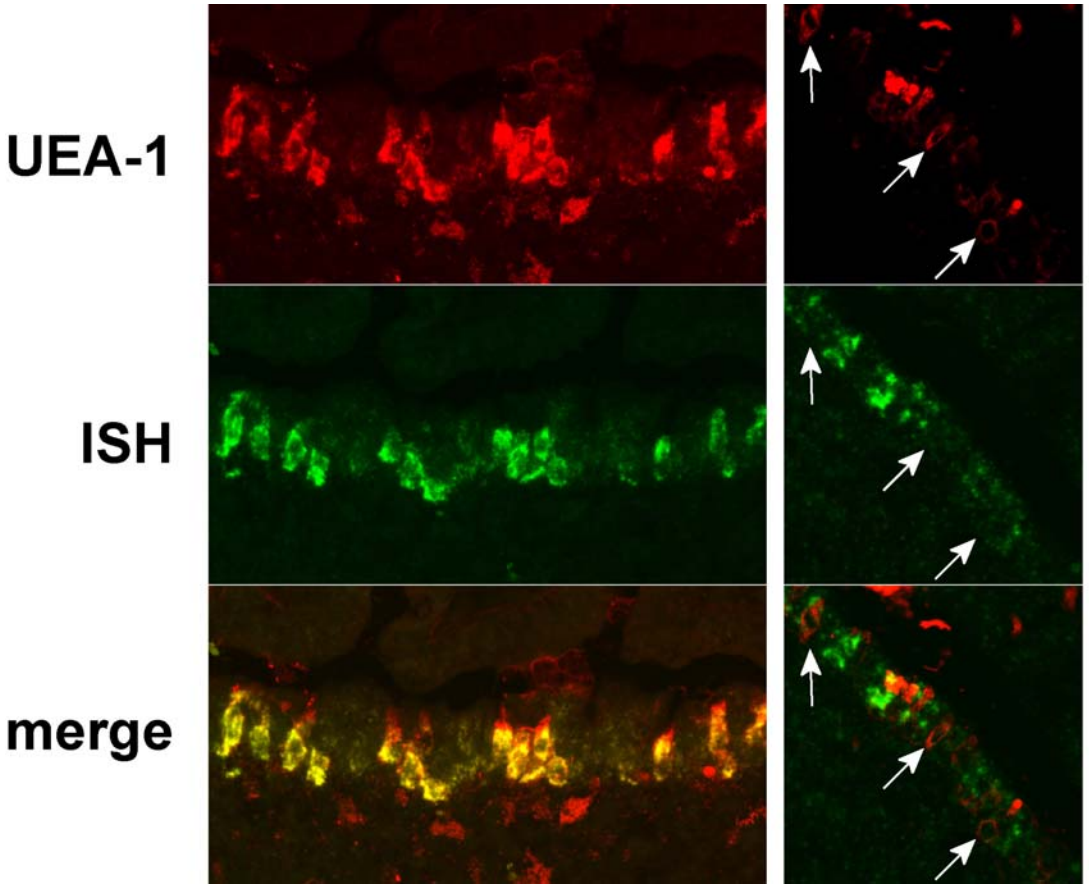
Gene Stories I: Cell Culture Modeling of Complex Tissue

- FAE specific genes identified, consistent with specialized epithelial development and function
  - Transcription factors
  - Laminin beta 3, MMP15, tetraspanins, protocadherin, RAB-13
- Genes identified showing FAE subset expression
  - CPE-R suggests M cell specific pattern
- Conservation of expression in mouse, human



More genes from study of dissected FAE:

- PGRP genes show expression in distinct FAE subsets
- PGRP-S is M cell specific, PGRP-L is not



Nasal Associated Lymphoid Tissue (NALT)

- Different mucosal immune system sites express similar sets of epithelial receptors

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Gene Stories II: Antigen and Adjuvant Receptors

- PGRP gene distribution in FAE suggests epithelial cell functional specialization

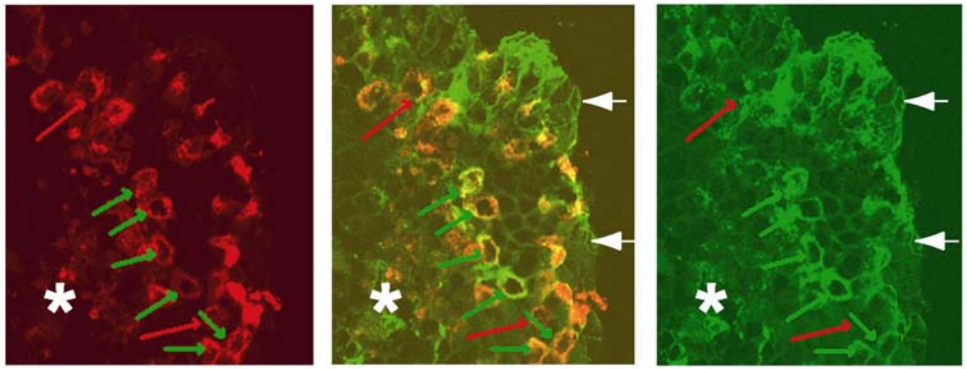
- Dual functions of PGRPs in FAE and M cells
  - Peptidoglycan receptors are “Pattern Recognition Receptors” (PRR) which trigger innate immunity
  - PRR triggering is the basis of vaccine adjuvants
  - Thus, receptors may therefore target both antigen delivery and adjuvant signaling

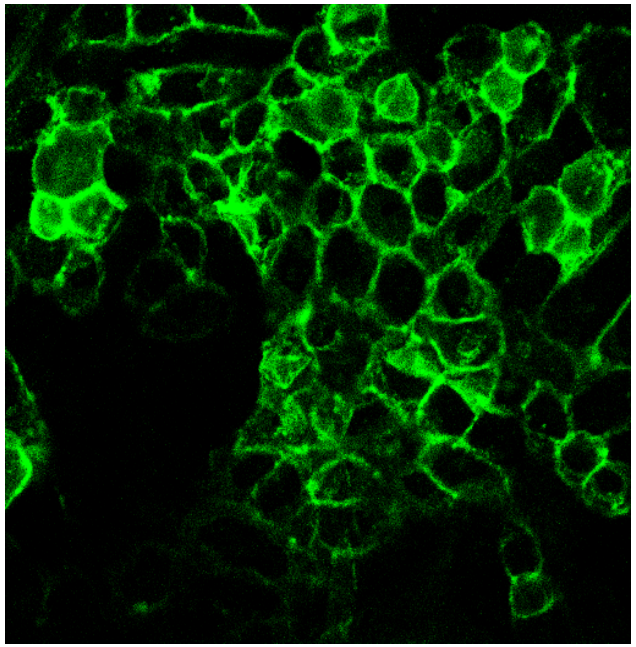
Cell Biology of FAE/M Cell Genes

- Are some of the candidate genes receptors for FAE/M cell transcytosis?
  - Cloned full length cDNA, stable transfectants
  - Select synthetic ligands (phage display)
  - Test for binding and transcytosis in vivo

- Are some of the candidate genes receptors for adjuvant signals?
  - Test ligands for adjuvant activity in the presence of an antigen challenge

CPE-R Subcellular Distribution





- Transfected cells and cells in vivo show different subcellular patterns
  - Enterocytes and transfected cells suggest tight junction distribution
  - M cells show cytoplasmic distribution

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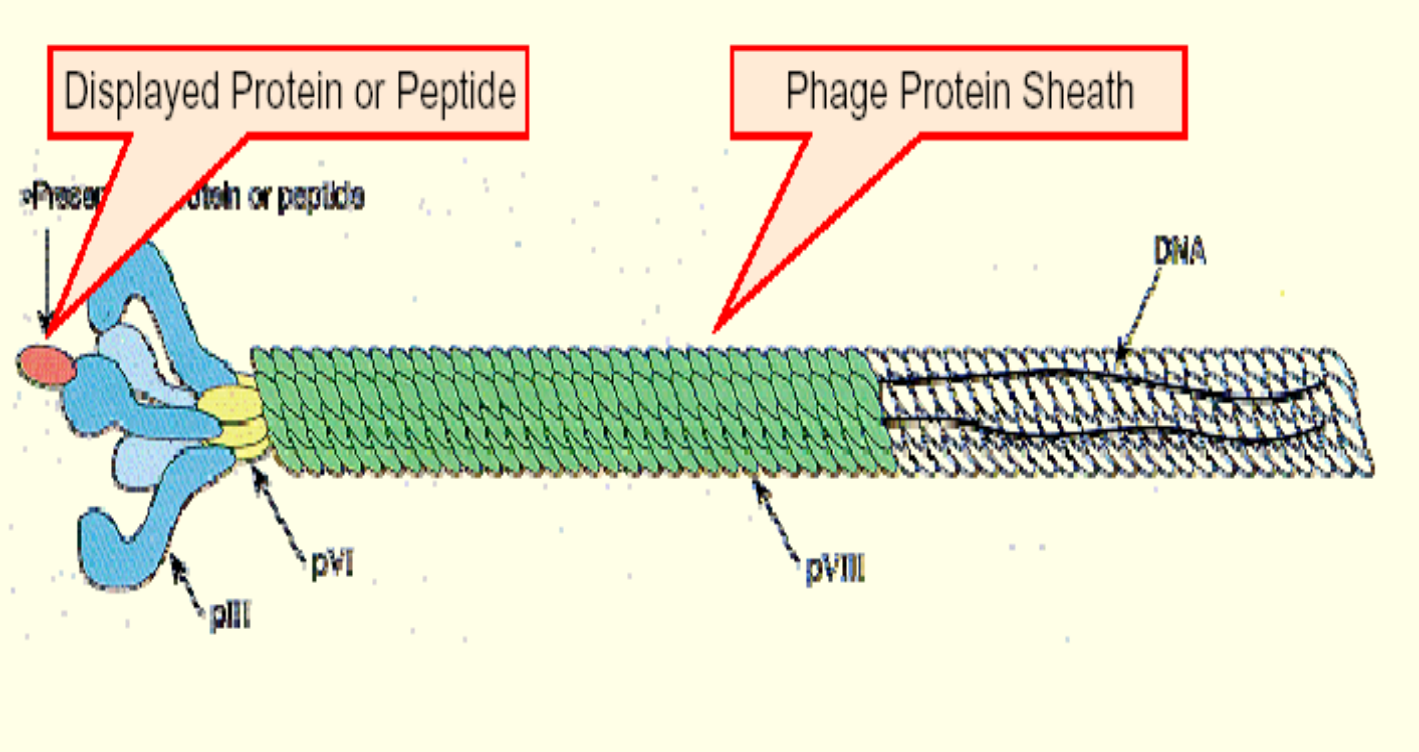
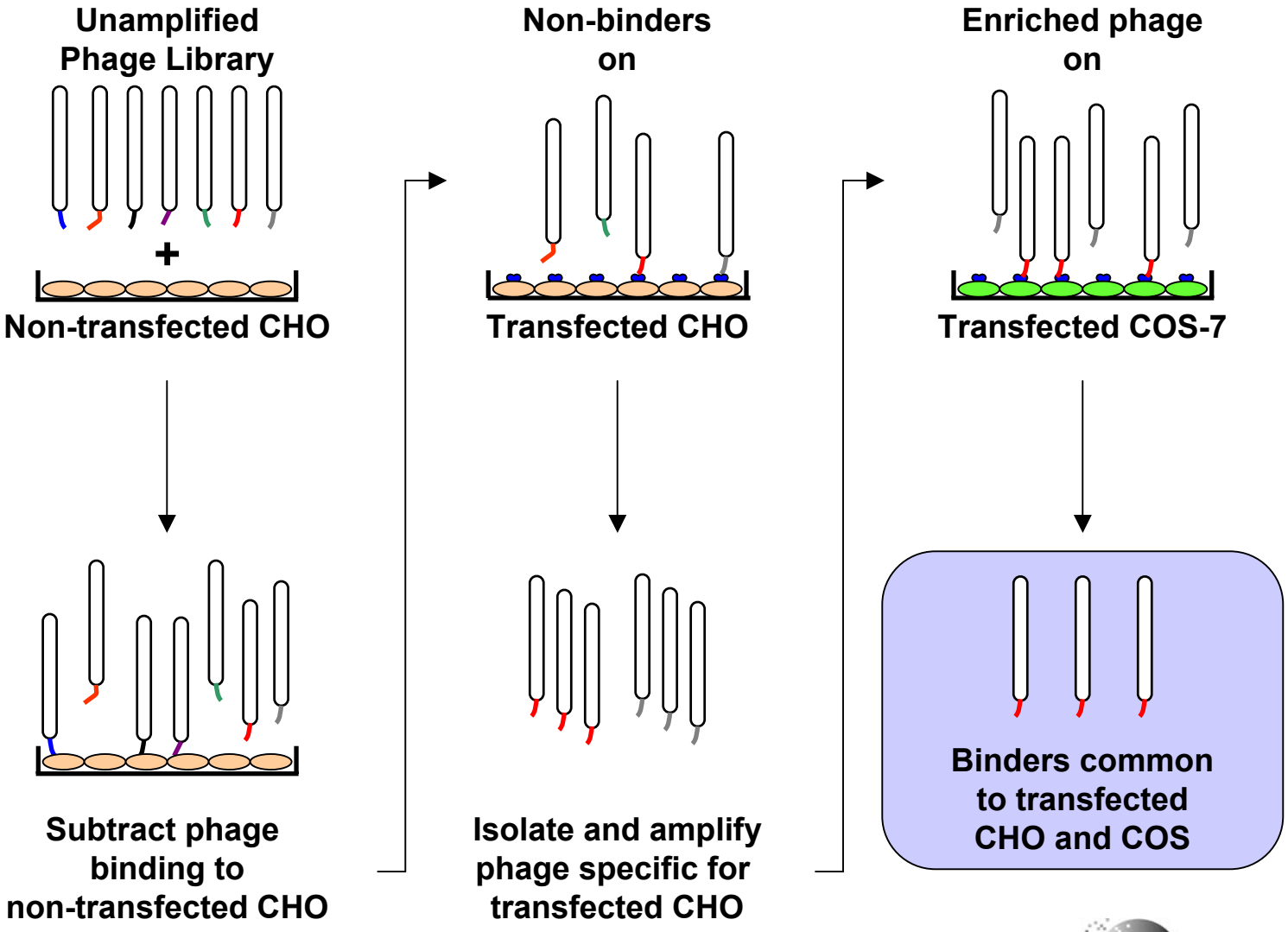
Phage Display Ligand Selection



Cell Based Subtractive Panning



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Consensus Ligand Sequences

- **M13 Phage DisplayTarget = CHO transfected with EDD1S_48 mouse + V5/poly-His**
-
-

```
48e2c3r2_s08 ~~TsflqaY~ ~~~~~~~~~~ ~~~
48e2c3r2_s12 ~~~~~idSYa AL~~~~~~~~ ~~~
48e2c3r2_s10 dMrTlld~~~ ~~~~~~~~~~ ~~~
48e2c3r2_s14 ~MqTvrnh~~ ~~~~~~~~~~ ~~~
48e2c3r2_s03 ~~TTinrSp~ ~~~~~~~~~~ ~~~
48e2c3r2_s16 ~vTTyvrf~~ ~~~~~~~~~~ ~~~
48e2c3r2_s02 ~~~~~msSdk Af~~~~~~~~ ~~~
48e2c3r2_s18 ~~~~~mtSqr sL~~~~~~~~ ~~~
48e2c3r2_s05 ~~~~~~~~~~ ~MsqsllP~~ ~~~
48e2c3r2_s17 ~~~~~~~~~~ ~LnisflP~~ ~~~
48e2c3r2_s19 ~~~~~~~~~~ ~~~mViiPpq ~~~
48e2c3r2_s07 ~~~~~~~~~~ ~~~~mltPWr h~~
48e2c3r2_s09 ~~~~~~~~~~ ~~~~~~APWa lar
48e2c3r2_s11 ~~~~~~~~~~ ~MTSIeAP~~ ~~~
48e2c3r2_s13 ~~~~~~~~~~ ~MTSIeAP~~ ~~~
48e2c3r2_s15 ~~~~~~~~~~ ~MTSIeAP~~ ~~~
48e2c3r2_s01 ~~~~~~~~~~ ~~~~~mAPsp Rm~
48e2c3r2_s20 ~~~~~~~~~~ ~~~~~mAPhf Rd~
Consensus    -MTT---SY- AMTSI-APW- R--
```

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Ligand-mediated Vaccine Delivery

- Q1: Can the FAE and M cells be molecularly defined?

- A1: Yes!
  - Specific genes define differentiation of FAE, distinguishing from enterocytes
  - FAE subsets can be further defined by expression of specific marker genes (e.g., PRGP-S versus PGRP-L)
  - M cell specific genes can be identified
  - Genes show conservation across species and across Mucosal-Associated Lymphoid Tissues

Ligand-mediated Vaccine Delivery

- Q2: Does the FAE have functions helpful to mucosal immunity?

- A2: Yes.
  - Genes specific to FAE (CPE-R, PGRPs) may provide specific antigen/particle binding and transport function
  - FAE specific Pattern Recognition Receptors (PRR) may play a role in adjuvant signaling

Ligand-mediated Vaccine Delivery

- Q3: How do we exploit the biology for vaccine delivery?

- A3: (Yes?)
  - Use synthetic ligands to provide targeted delivery of vaccine antigens
    - Doorstep versus Mailslot
  - Use Pattern Recognition Receptor (PRR) signaling to provide specific mucosal adjuvant activity

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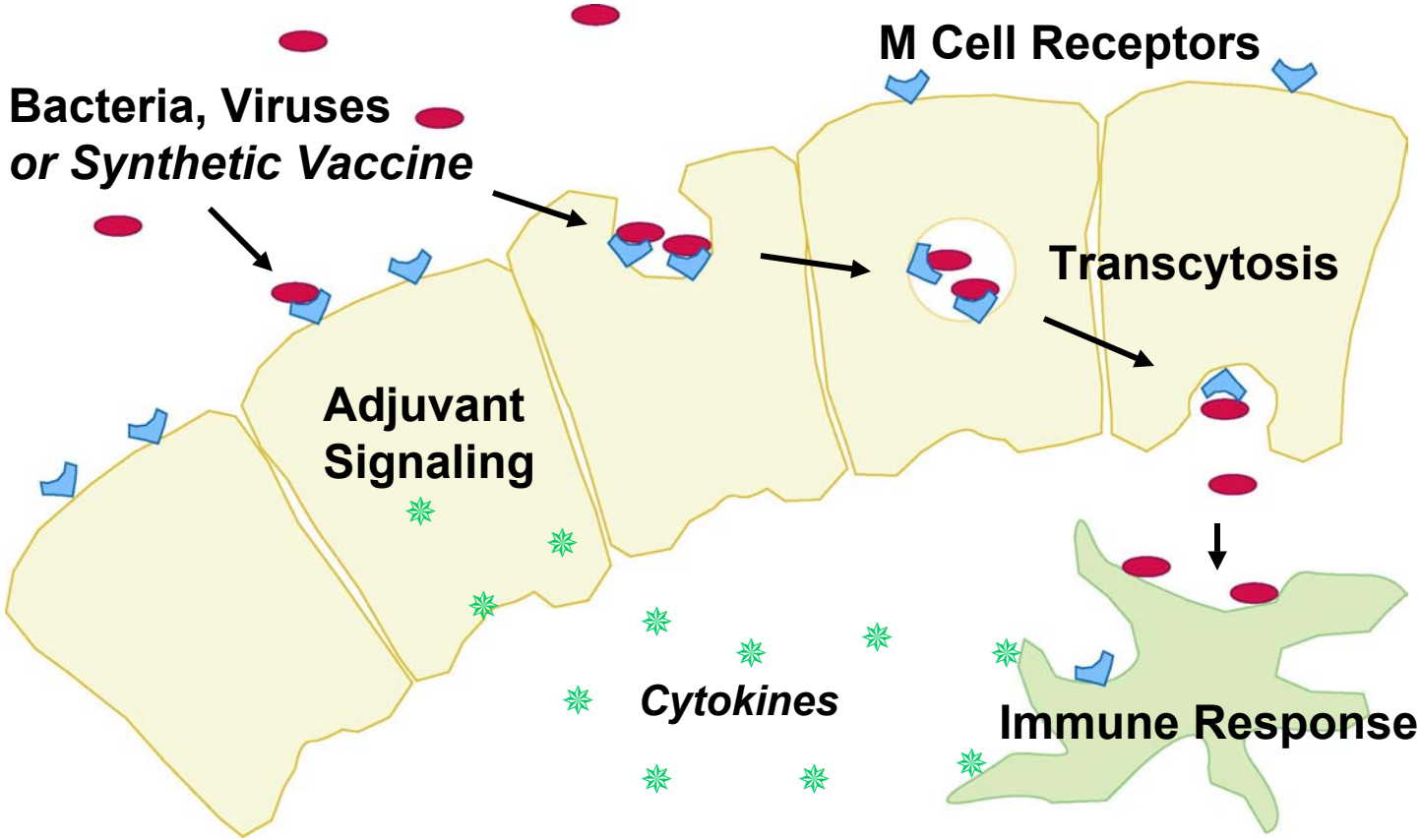
Exploiting Normal Biology in Vaccine Development



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