Pathogens and commensals: War and Peace at mucosal surface

Nothing in biology makes sense except in the light of evolution
Theodosius Dobzhansky

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Co-evolution has created an « immunological conundrum » that forces to conjugate **tolerance** to commensal bacteria and quick and efficient **recognition** and **elimination** of bacterial pathogens.

**Symbionts**

**Pathogens**

Recognition network: **PRRs**: TLRs, NLRs, Rig1, MDA5…

Amplification loop: TREM, HMGB1, Gal3, Sepsis, Septic shock

10^{12}/ml in colon

Rupture of homeostasis = IBD, obesity, diabetes, cancer?

Adaptive immunity

Pathogens recognition, capture, completion of eradication process, protection

Regulation

Loss of control

Sansonetti & Di Santo, 2007, Immunity
Sansonetti & Medzhitov, 2009, Cell
Sansonetti, 2010, Mucosal Immunology
How does one identify a harmful pathogen hidden amidst a herd of harmless symbionts?
Bacterial life at mucosal surfaces
« Seating on a volcano »

**Symbionts/commensals:** survive at distance or in particular niches, escape (regulate) innate defenses

**Pathogens:** engage epithelium and subvert immune defenses
- Blocking of danger signaling
- Post-translational modification of key immune signaling molecules

- O2, NO, ROS
- Antimicrobial peptides
- Lysozyme, proteases, lectins, phospholipases
- Transmigrating phagocytes
- sIgA

Cationic antimicrobial peptide hBD3
Sperandio et al.

Kim et al., PNAS, 2005
Arbibe et al., Nature Immunol., 2007
Sperandio et al., J. Exp. Med., 2008
Marteyn et al., Nature, 2010
Konradt et al., Cell Host Microbe, 2011
Puhar et al., Immunity, 2013
Peace: symbiotic bacteria maintain gut homeostatic mechanisms

**SYMBIONTS**

- Absence (limitation) of virulence factors
- PAMPs less agonist?
- Sequestration, weak activity of TLRs
- Life in biofilms on mucus surface (at distance of epithelium)
- Controled diffusion and sampling of PAMPs and prokaryotic signalisation molecules
War: pathogenic bacteria disrupt / by-pass gut homeostatic mechanisms

**PATHOGENS**

1 – Mucinases
   Eradication of microbiota (niche occupancy)
   Adhesins / Invasins
   Secretory systems / effectors
   Hemolysins
   Massive engagement of PRRs
   Pathogenic properties sensed as exogenous danger signals

2 - Release of endogenous danger signals (DAMPs / small molecules) before initiation of proinflammatory transcriptional reprogramming.

3 – Subversion / dampening of innate (inflammatory) and adaptive immune responses
Pathogenesis of *Shigella* infection: central role of Type Three Secretory Apparatus (T3SA)

- Development of inflammation
- Rupture of epithelial barrier
- Facilitation of invasion
- Stimulation of epithelial bactericidal capacities

- Pyroptosis = proinflammatory apoptosis
- Activation of caspase-1, Release of IL-1β and IL-18

- CCL-20

- Basolateral macropinocytosis (TTSS)

- Motility, cell to cell spread (TTSS)

- Vacuole lysis (TTSS)

- Escape to autophagy

- «facilitated translocation»

- Defensins and other bactericidal molecules

- Follicle-associated epithelium

- PNN

- Nod1

- NF-κB

- JNK

- Pro-inflammatory genes

- IL-8, other cytokines, chemokines

- MΦ

- M cell

- T lympho

- B lympho
Shigella - danger signaling 1: cytosolic sensing of PGN by Nod molecules

Inflammatory programing based on sensing abnormal localisation of bacterial cell wall fragments and transcription of innate immunity genes

Girardin et coll., EMBO Reports, 2001
Girardin et coll., Science, 2003
**Shigella**
- **danger signaling 2**: pyroptosis inflammasome

![Diagram](image-url)

- **Flagellin**
  - *L. pneumophila*
- **Flagellin**
  - *S. typhimurium*
  - *P. aeruginosa*
- **S. flexneri**
- **Pro-caspase-1**
- **Caspase**
- **Macrophages**
- **TTSS translocator**
- **Pannexin-1**
- **NLRP3 inflammasome**
- **Pro-caspase-1**
- **Caspase-1**
- **Apoptosis**
- **Inflammation**

**Rapid inflammatory programming based on release of a presynthesized pool of pro-inflammatory cytokines (IL-1β, IL-18)**

**References**
- Zychlinsky et al., 1992, Nature
- Zychlinsky et al., 1994, J Clin Invest
- Hilbi H et al., 1998, J Biol Chem
**Shigella - danger signal 3**: early epithelial cell release of ATP across connexin-based hemichannels (Danger-associated molecular patterns)

Rapid inflammatory programming based on release of intracellular ATP (DAMPs)
= Inflammasome activation
= Th17 lymphocytes differentiation

Tran Van Nhieu et al., 2003, Nat Cell Biol
Puhar et al., 2013, Immunity
Expression, regulation, subversive function of T3SA effectors

- before secretion
- after TTSS activation (target cell recognition)

**VirB**
- *ipaA*, *ipaB*, *ipaC*, *ipaD*,
- *ipgB1*, *ipgD*, *icsB*,
- *ospC2/3/4*, *ospD1*, *ospD2*

**MxiE**
- *ospB*
- *ospF*
- *ospC1*
- *virA*
- *ospD3*, *ospE1/2*,
- *ospG*,
- *ipaH1/2*, *ipaH4*,
- *ipaH7*, *ipaH9.8*

**INVASION**
- IpaB, IpaC, IpaA,
- IpgB1, VirA, IpgD

**INHIBITION OF SECRETION**
- IpaB: (Mounier et al., 2012. Cell Host & Microbe)

**OTHER PHENOTYPES**
- IcsB: inhibition or autophagy (Ogawa et al., 2005, Science)
- VirA: inhibition of microtubules, facilitates actin-based motility (Yoshida et al., 2006, Science)

**MODULATION OF INNATE RESPONSES**
- **OspG**: kinase, binds/blocks ubiquitin transfer protein E2, protects I-kB from degradation. Anti-inflammatory +++ (Kim et al., 2005, PNAS).
- **OspF**: dephosphorylation of Erk1/2, epigenetic regulation of pro-inflammatory genes - i.e. IL-8. Regulates transmigration of PMNs through epithelium (Arbibe et al., 2007, Nat.Immunol.). Phosphothreonine lyase (Li et al., 2007, Science).
- **IpaHs**: (5 + 5 chromosomal copies): New family of Ubiquitin ligases (E3) (Rohde et al., 2007, Cell Host & Microbes) IpaH9.8 targets NEMO (Ashida et al., 2010, Nat.Cell Biol.)
Shigella T3SA effector IpgD dampens ATP-mediated danger signaling

Histopathology, HES
Rabbit ileal loop
8 h infection

IpgD

IpgD

HEMICANAL (Connexins)

PURINERGIC RECEPTOR

Pi(4,5)P2

Pi(5)P

IpgD

PI(4,5)P2

PI(5)P

ATP release in luminal fluid, rabbit ileal loop. 4h infection

Niebuhr et al., EMBO J. 2002
Pendaries et al., EMBO J 2006

Puhar et al., 2013, Immunity
Immunosuppressive environment induced by *Shigella* (innate)

**Suppression of humoral defense mechanisms**
- AMPs

**Suppression of cellular defense mechanisms**
- ATP

**Cellular factors**
- Follicle-associated epithelium
- «facilitated translocation»
- M cell
- MΦ
- Lympho B
- ATP
- PNN
- DC

**Molecular factors**
- TTSS
- Osp(s)
- Pyroptosis
- IcsA
- lyse vacuole (TTSS)
- motilité/passage cellule-cellule (TTSS)
- cellules épithéliales

**Pathological outcomes**
- Suppression of danger signaling
- Suppression of humoral defense mechanisms
- Suppression of cellular defense mechanisms

**Immunosuppressive mechanisms**
- Activation of caspase-1
- Pyroptosis = pro-inflammatory apoptosis
- Release of IL-1β and IL-18

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- Kim et al. 2005. PNAS
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