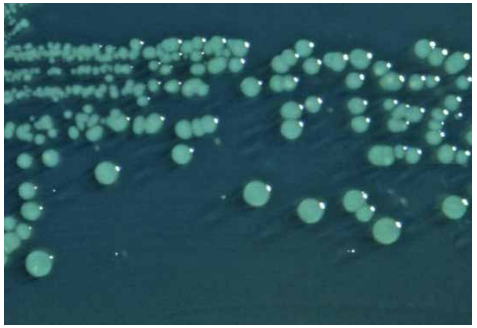
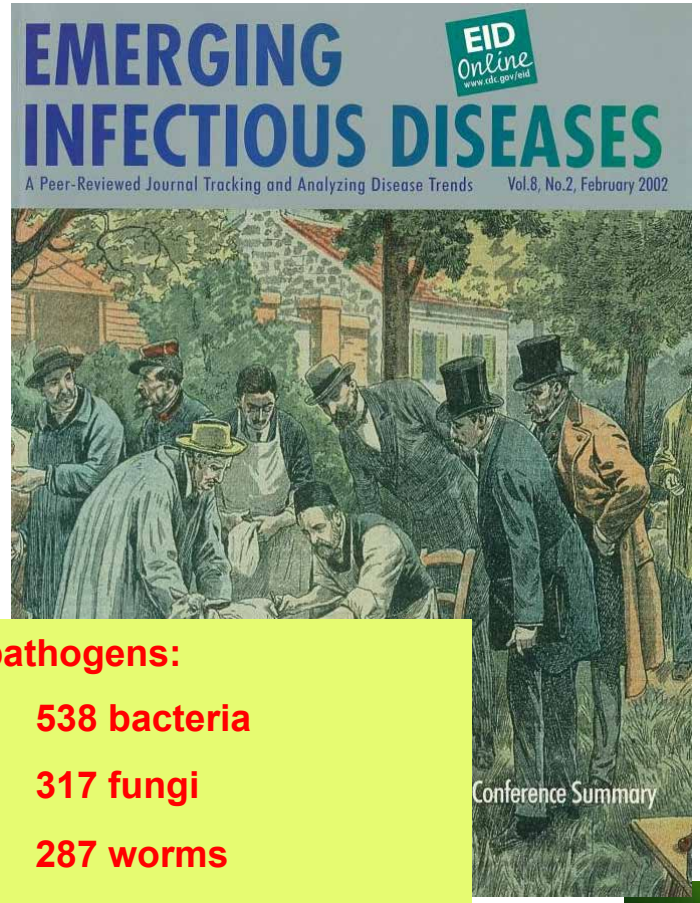
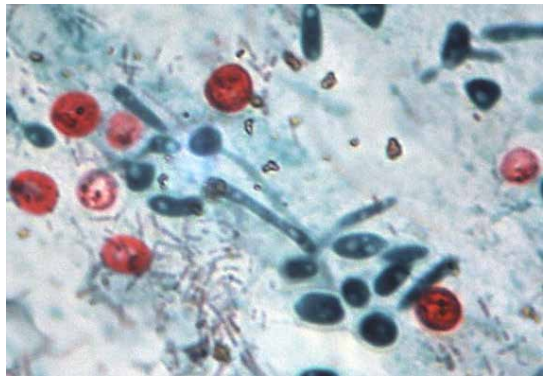
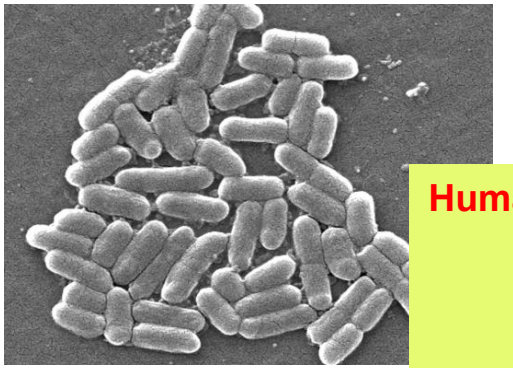
The Immunology of Infectious Diseases

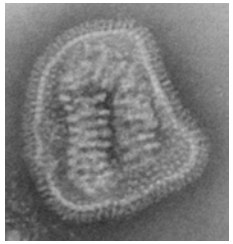
Rich Locksley, Nov 2018





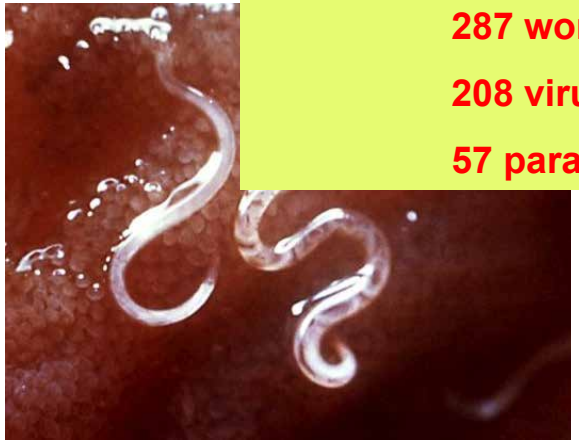






Human pathogens:

- **538 bacteria**
- **317 fungi**
- **287 worms**
- **208 viruses**
- **57 parasitic protozoa**



Infectious diseases: definitions

Colonization	**Persistence on skin or mucosal sites**
Infection	**Invasion and multiplication at sterile site**
Disease	**Pathology resulting from infection**

Infectious organisms

Commensals	**Normal flora; often symbiotic**
Pathogens	**Virulence genes - toxins, receptors, etc.**
Opportunists	**Immunodeficient or otherwise compromised host**

A remarkable engine…

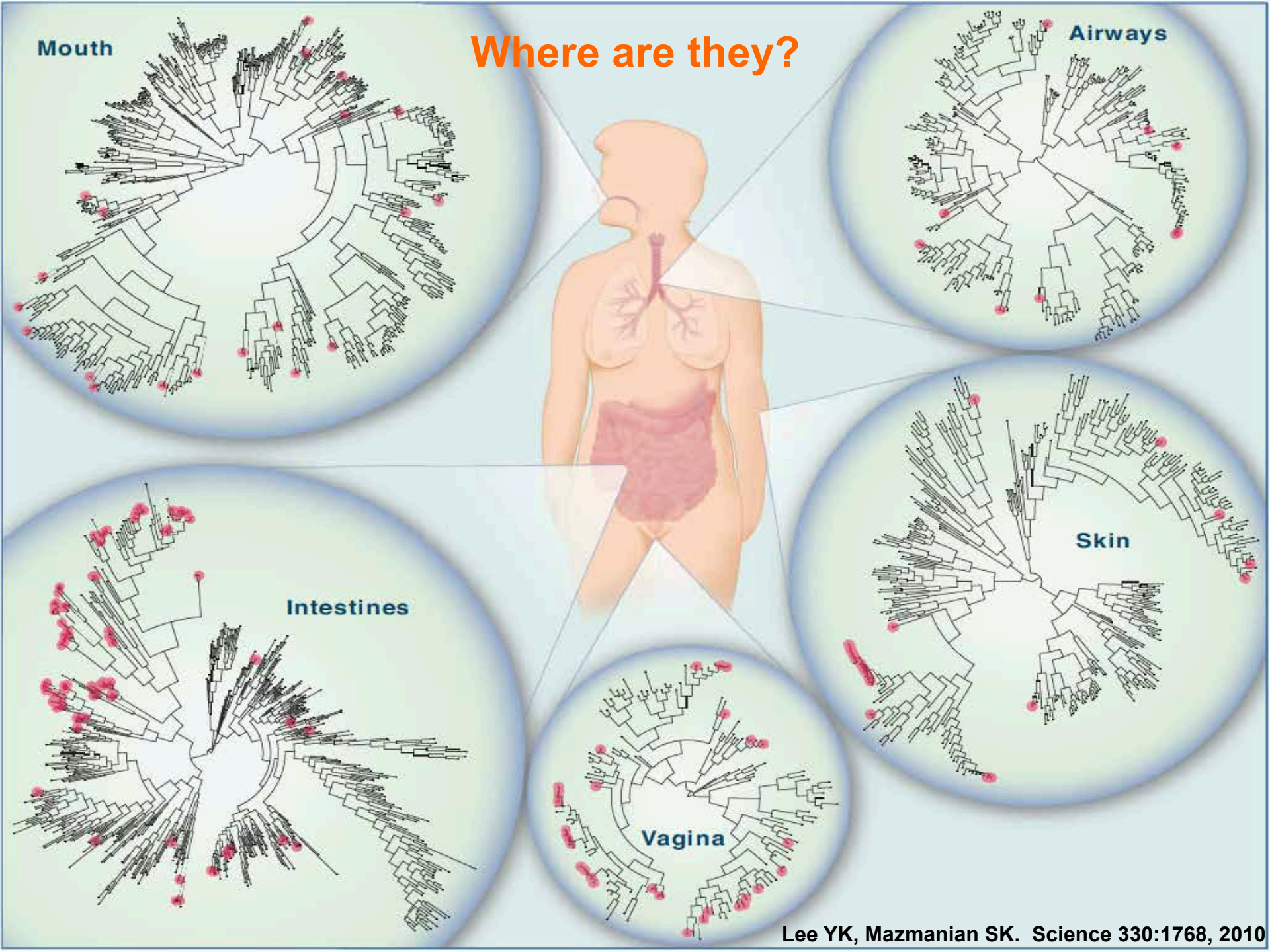
10 trillion human cells…

Plus 100 trillion bacterial cells…

For every HUMAN gene in your body, there are 360 microbial genes.

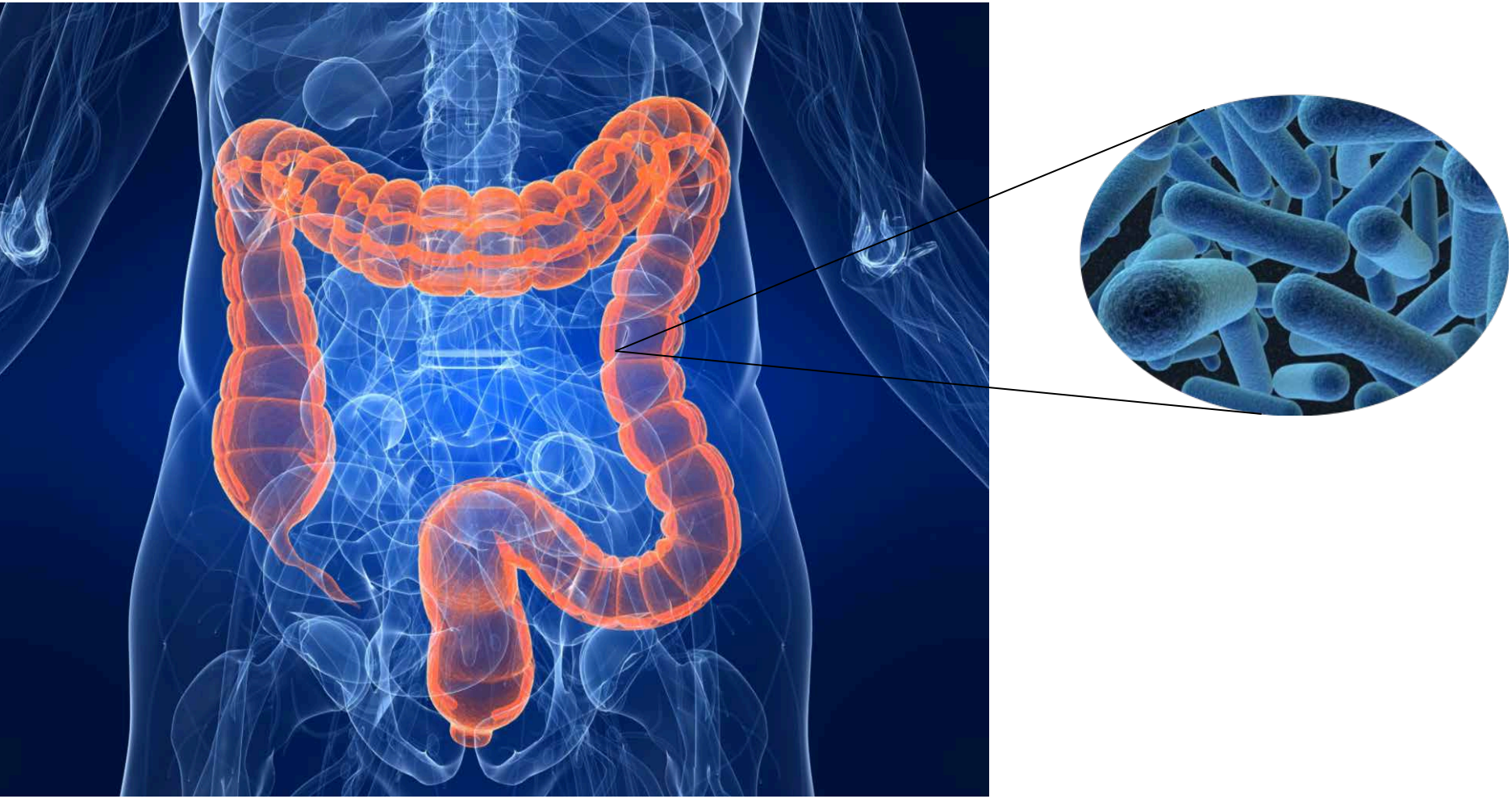
us

them



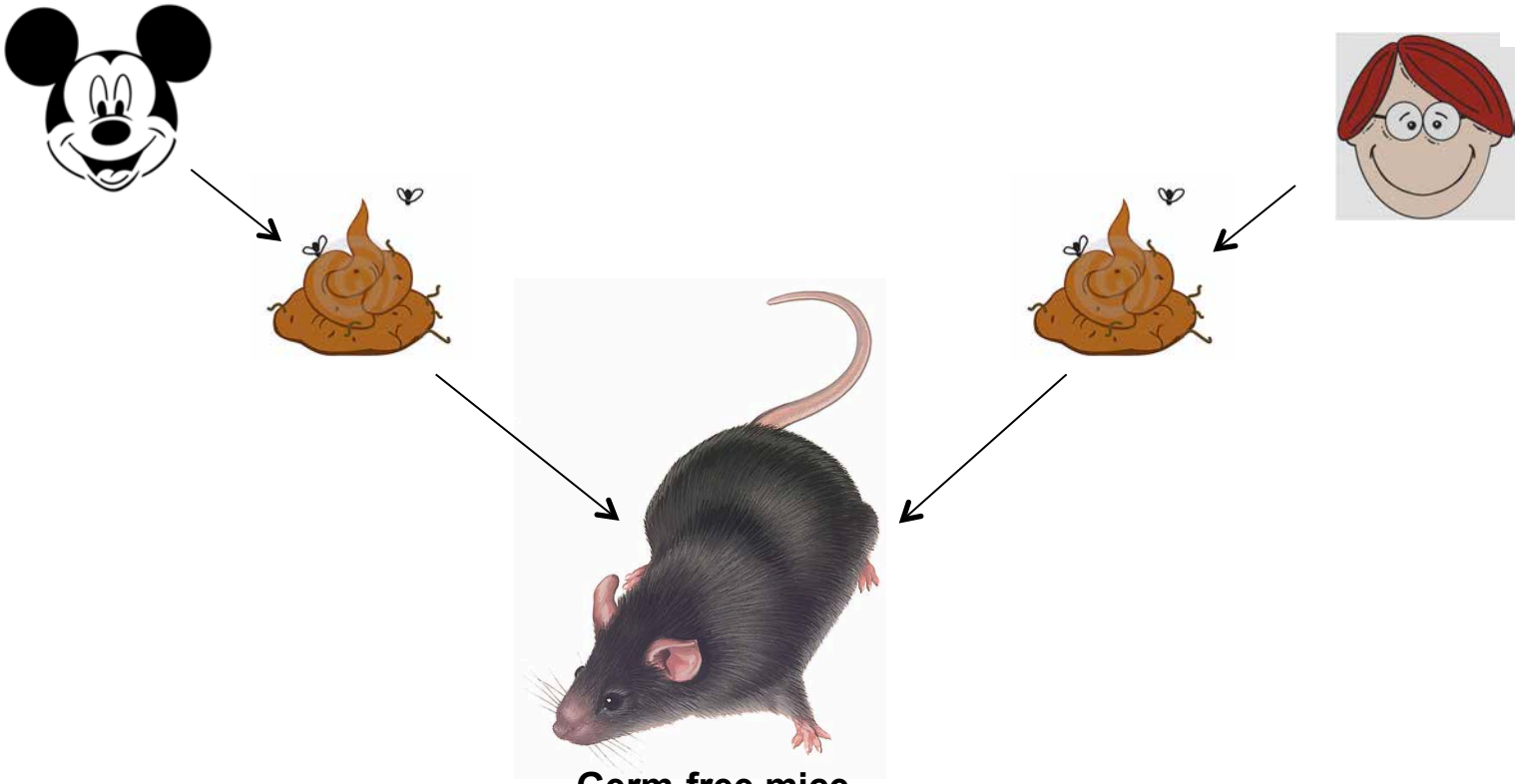
Lee YK, Mazmanian SK. Science 330:1768, 2010

Most resident bacteria reside in the bowel



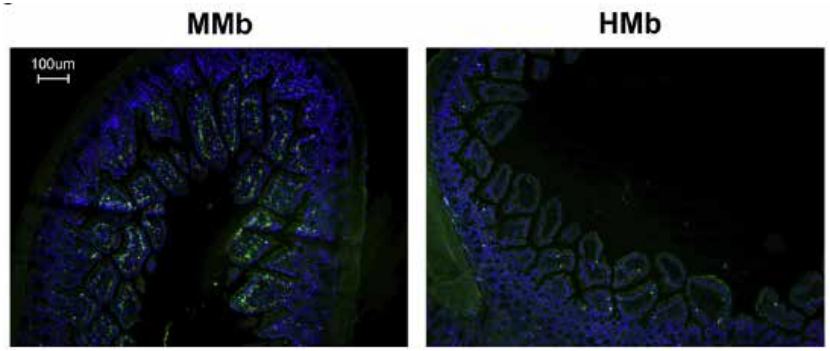
Role: Nutrient scavenging (complex polysaccharide diet); micronutrient synthesis; protection from ingested toxins in plants and other foods; competition for pathogenic organisms

Immune system development: attacking pathogens but not innocuous elements (self tissues, food, etc.)

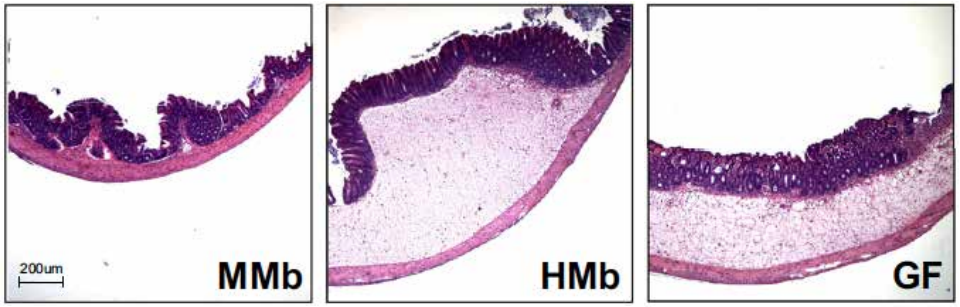


Germ-free mice

Gut immune cells



Protection from pathogens

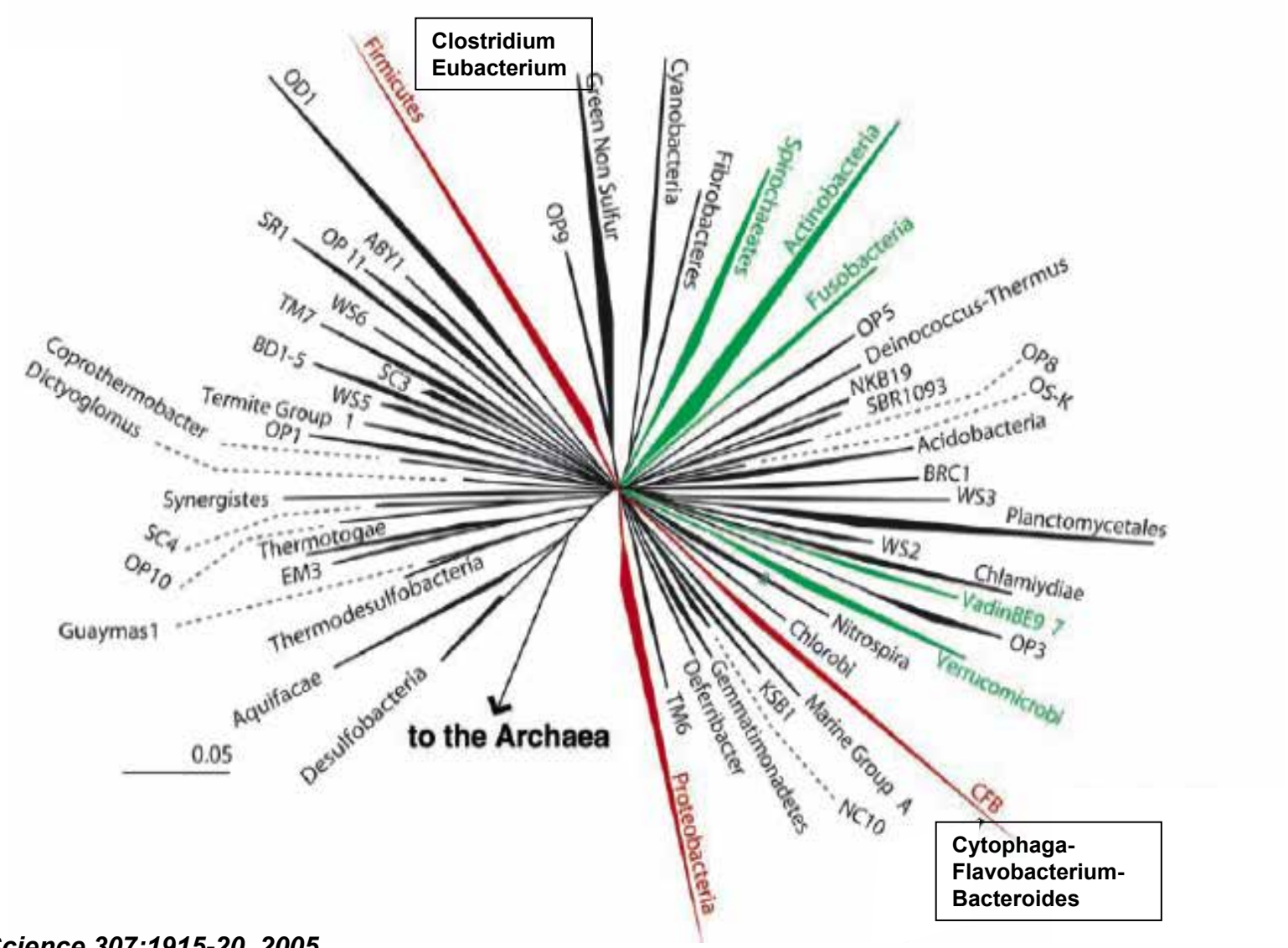


Chung H et al, Cell 149:1578-93, 2012

Biggest infectious disease risk?

Antibiotics - depletes commensals

Enrichment of the human intestinal microflora from the environment



Major

Minor

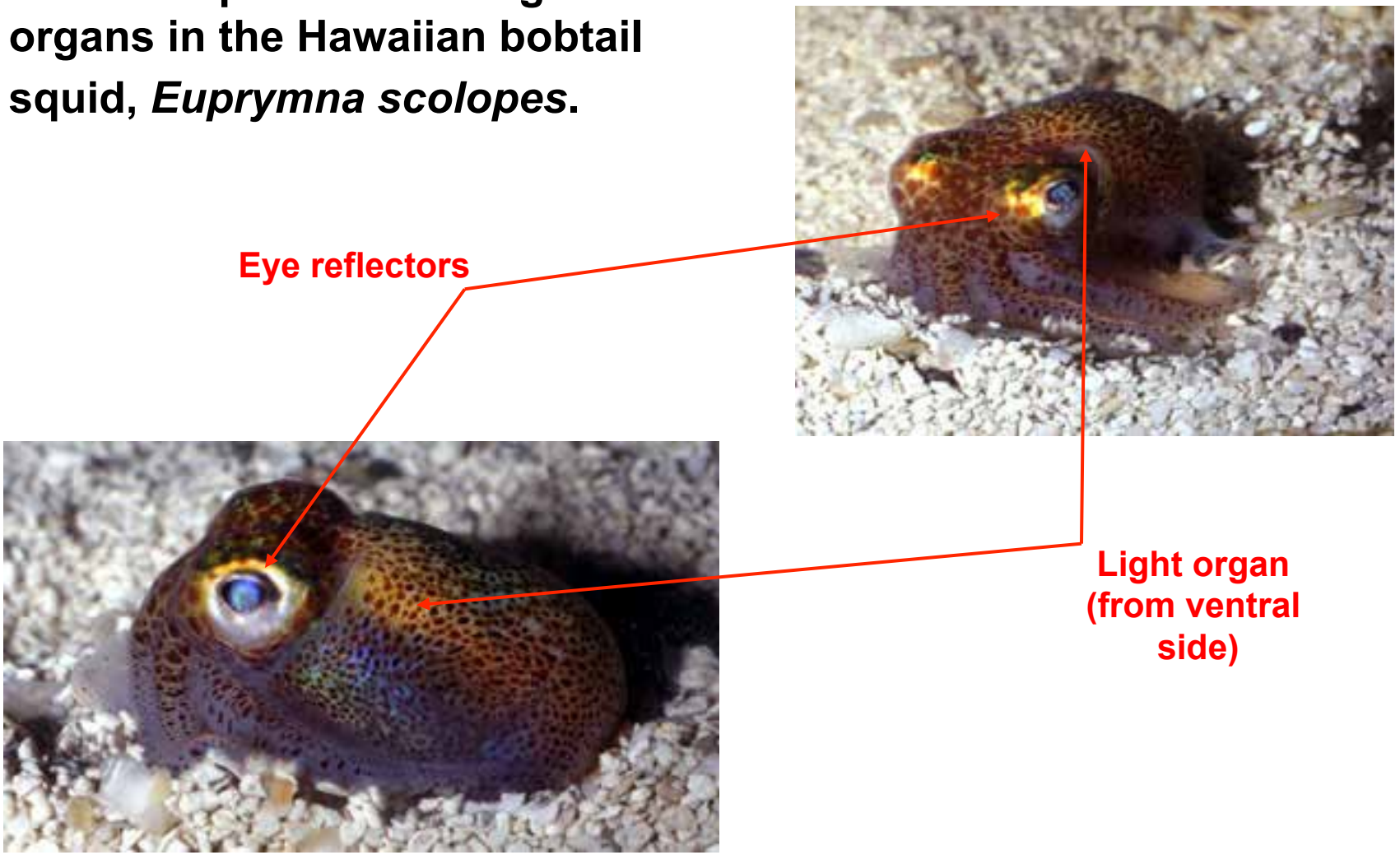
Absent

Backhed et al., Science 307:1915-20, 2005

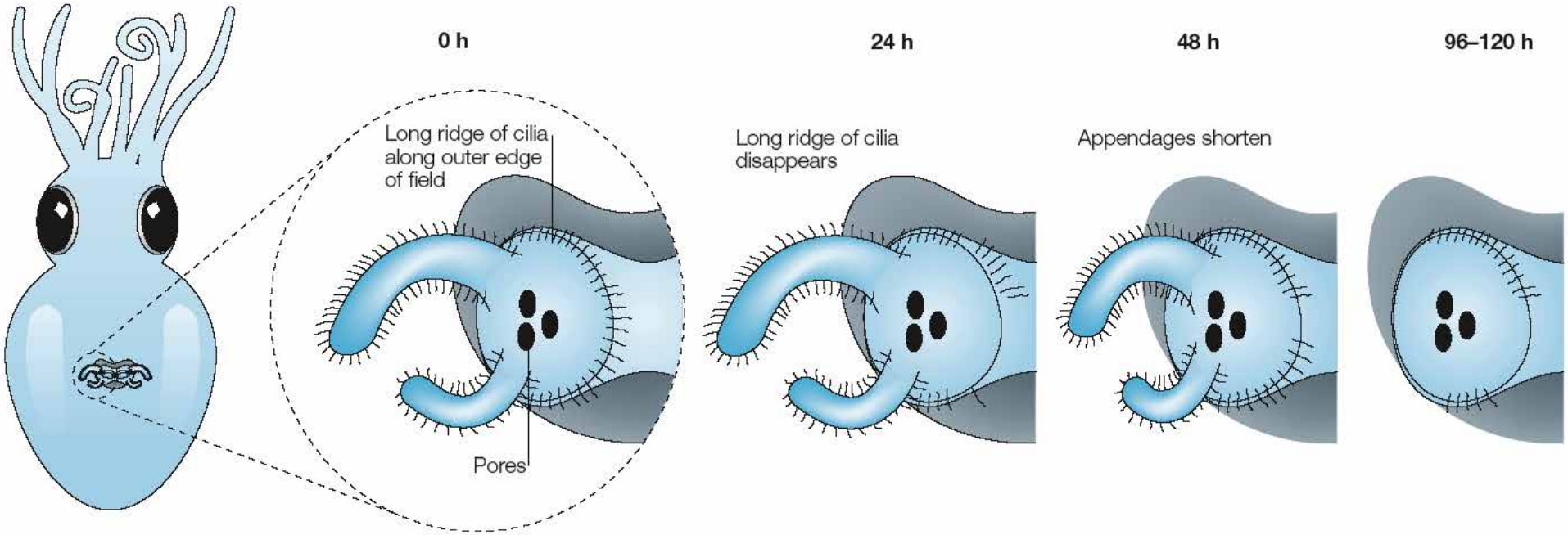
How do we do it?

Underwater clues…

Colonization by *Vibrio fischeri* expressing luciferase required for development of the light organs in the Hawaiian bobtail squid, *Euprymna scolopes*.



Light organ morphogenesis in response to *V. fischeri*



Sea water colonization by microbes

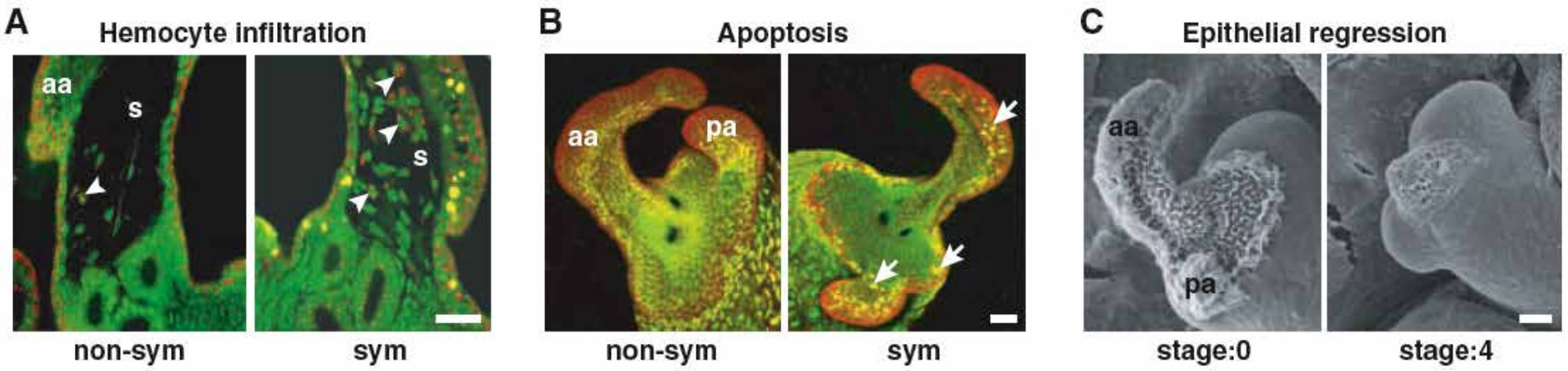
2 hr

Colonization by *V. fischeri*

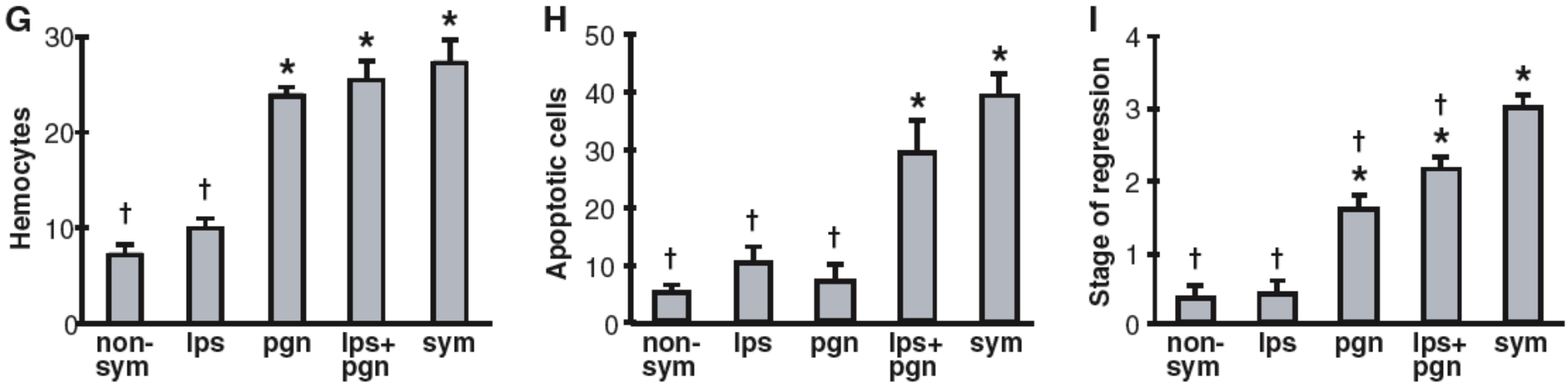
~10^6 total bacteria/ml salt water

~100 *V. fischeri* /ml salt water

Stages of light organ morphogenesis



…induced by PG and LPS from symbiont Vibrios

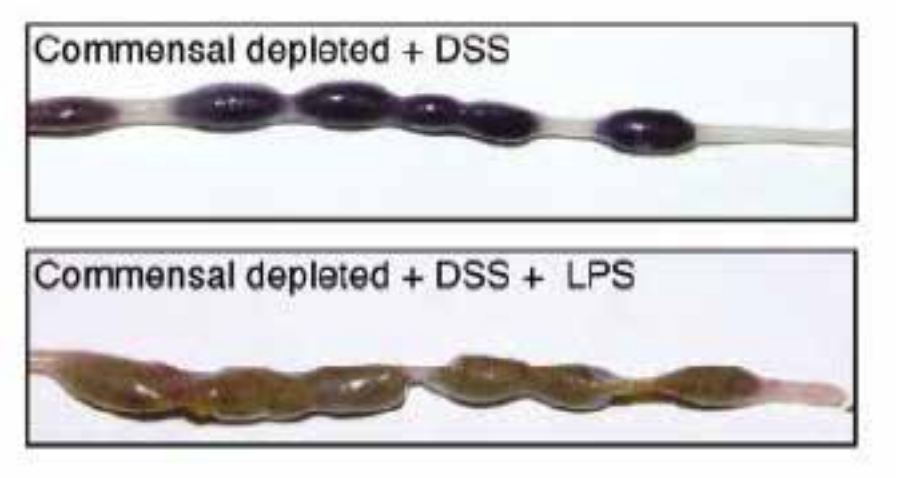


Koropatnick et al., Microbial factor-mediated development in a host-bacterial mutualism. Science 2004, 306:1186-8.

Mice unable to sense intestinal bacteria through TLRs cannot heal intestinal injury

Rakoff-Nahoum et al., Recognition of commensal microflora by Toll-like receptors is required for intestinal homestasis. Cell 118:229-41, 2004.

Intestinal bacteria can be replaced by a defined TLR ligand to mediate intestinal repair



Rakoff-Nahoum et al., Recognition of commensal microflora by Toll-like receptors is required for intestinal homestasis. Cell 118:229-41, 2004.

Microbial associated molecular patterns (MAMPs)

Genetically encoded sensors: Pattern Recognition Receptors (PRRs)

1. **Distinct types in distinct cellular compartments**

Toll-like receptors (TLRs)(10)	**cell surface, endosomes**
C-type lectin receptors (CLRs)(~15)	**cell surface, endosomes**
RNA/DNA sensors (NRs)(~15)	**cytosol, endosomes**
Nucleotide-binding-like leucine rich receptors (NLRs)(~20)	**cytosol**

2. **Sensors are in epithelial cells, tissue cells and hematopoietic cells**

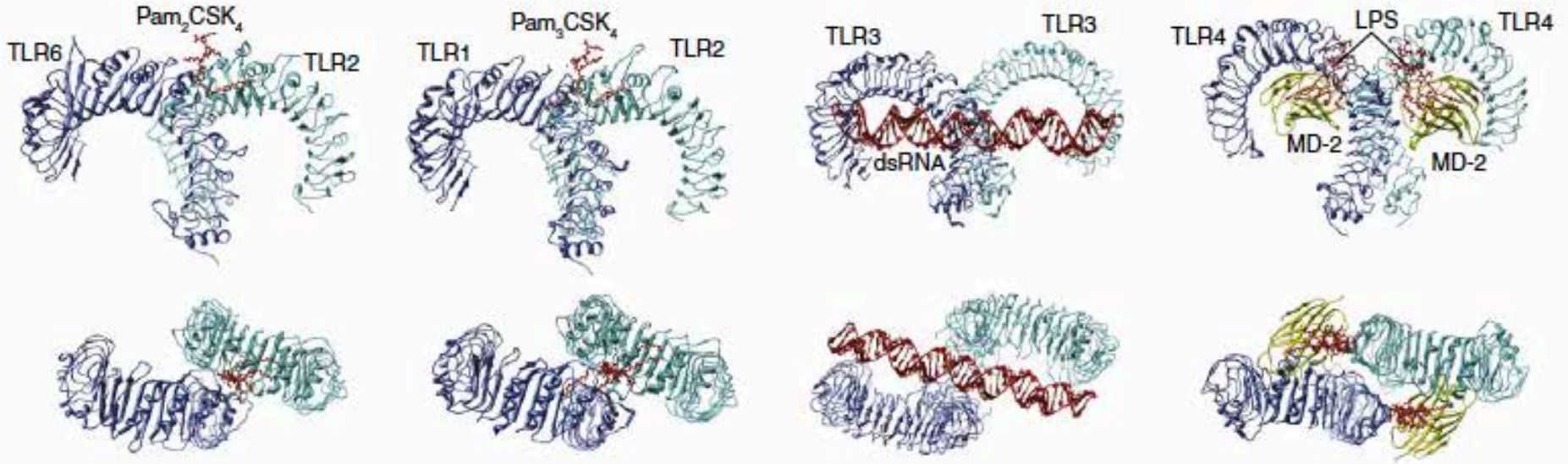
3. **TLRs, CLRs, NLRs, NRs set the immunologic 'tone' by inducing release of molecular signals for other cells: cytokines and chemokines**

TLRs encode diverse MAMP recognition elements

A

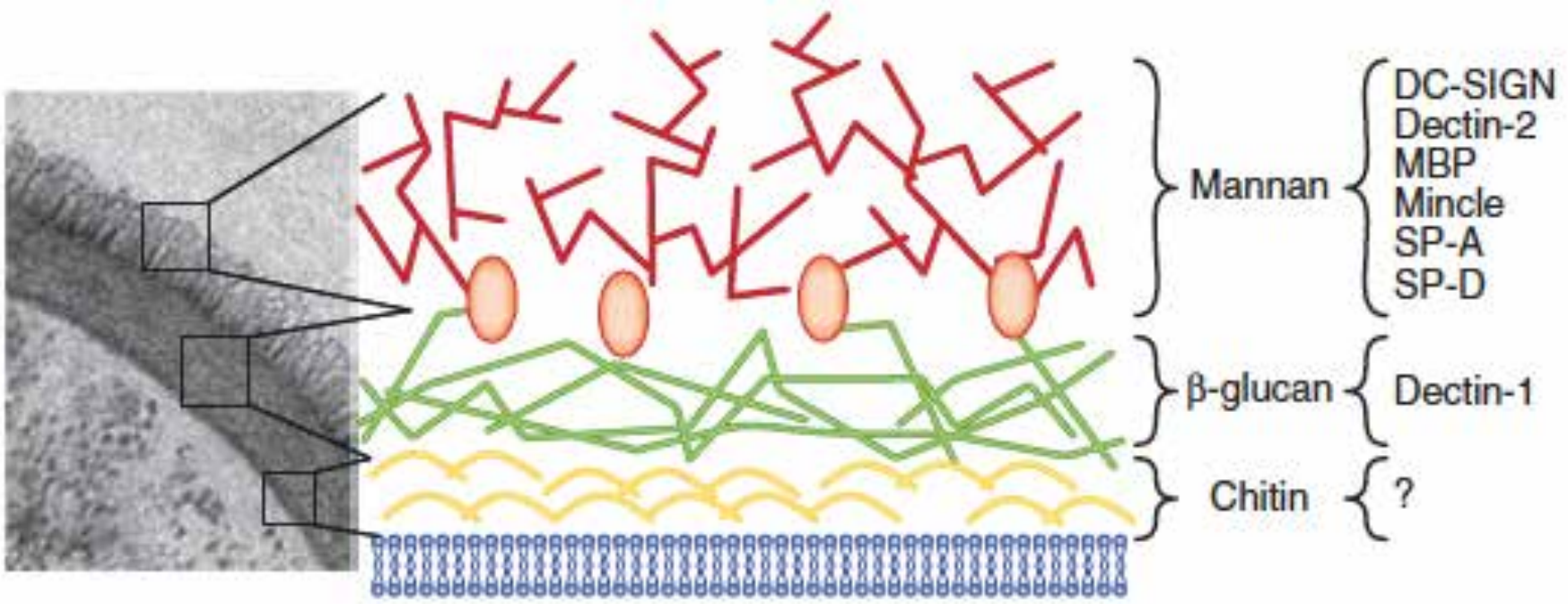
Receptor	TLR2/1 or 2/6	TLR3	TLR4	TLR5	TLR7	TLR9
Ligands	Lipopeptides	Poly I:C, dsRNA	LPS	Flagellin	ssRNA, resiquimod, imiquimod, loxoribine	Unmethylated DNA, CpG-DNA
Source	Gram-positive bacteria, fungi	Viruses	Gram-negative bacteria	Bacterial flagellum	Viruses	Bacteria
Examples	Pam_2CSK_4	dsRNA	LPS	Flagellin	ssRNA, Resiquimod, Imiquimod, Loxoribine	CpG-DNA

B



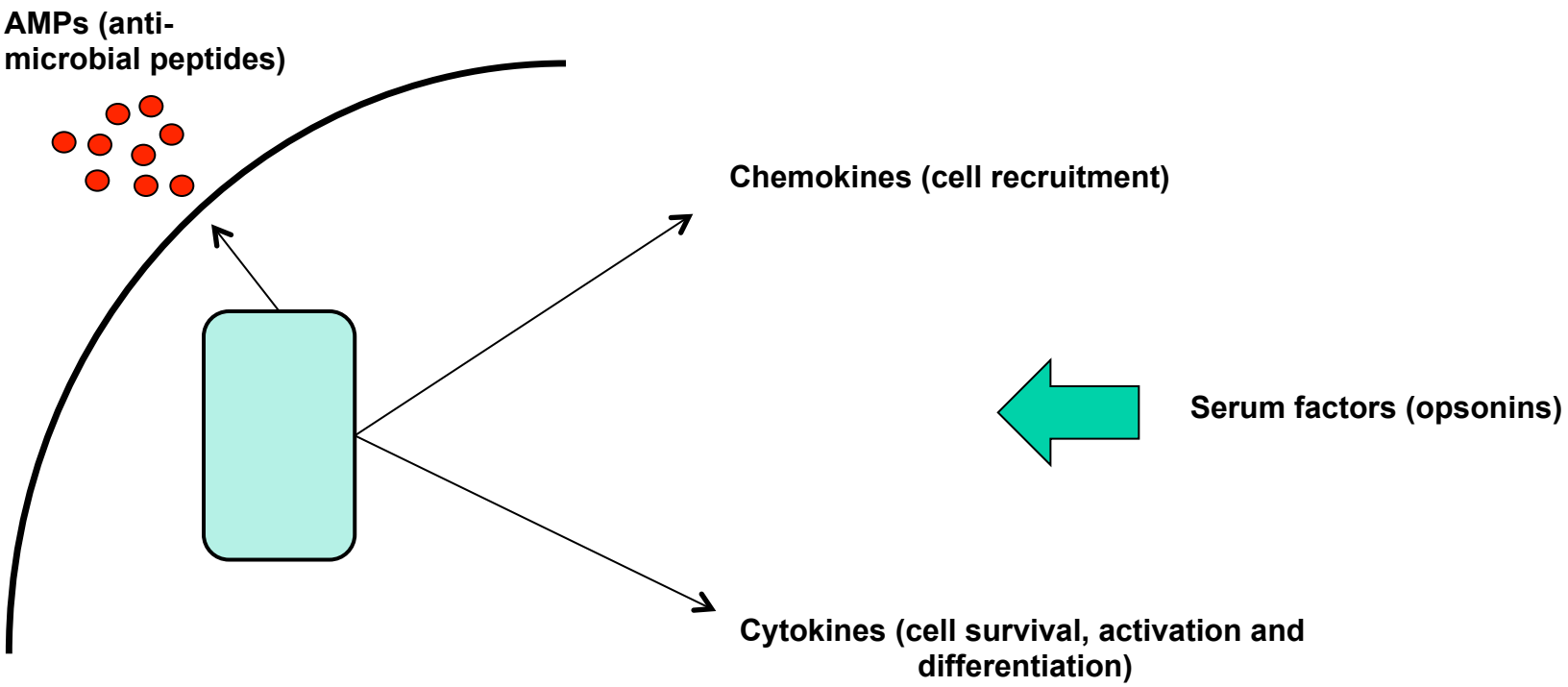
Moresco et al, Current Biology 21:R488, 2011

CLRs can be PRRs for fungal MAMPs



Hardison & Brown, Nat Immunol 13:817-22, 2012

PRR activation alerts other cells



Myeloid cells in inflammation (Innate Immunity)

Tissue cells

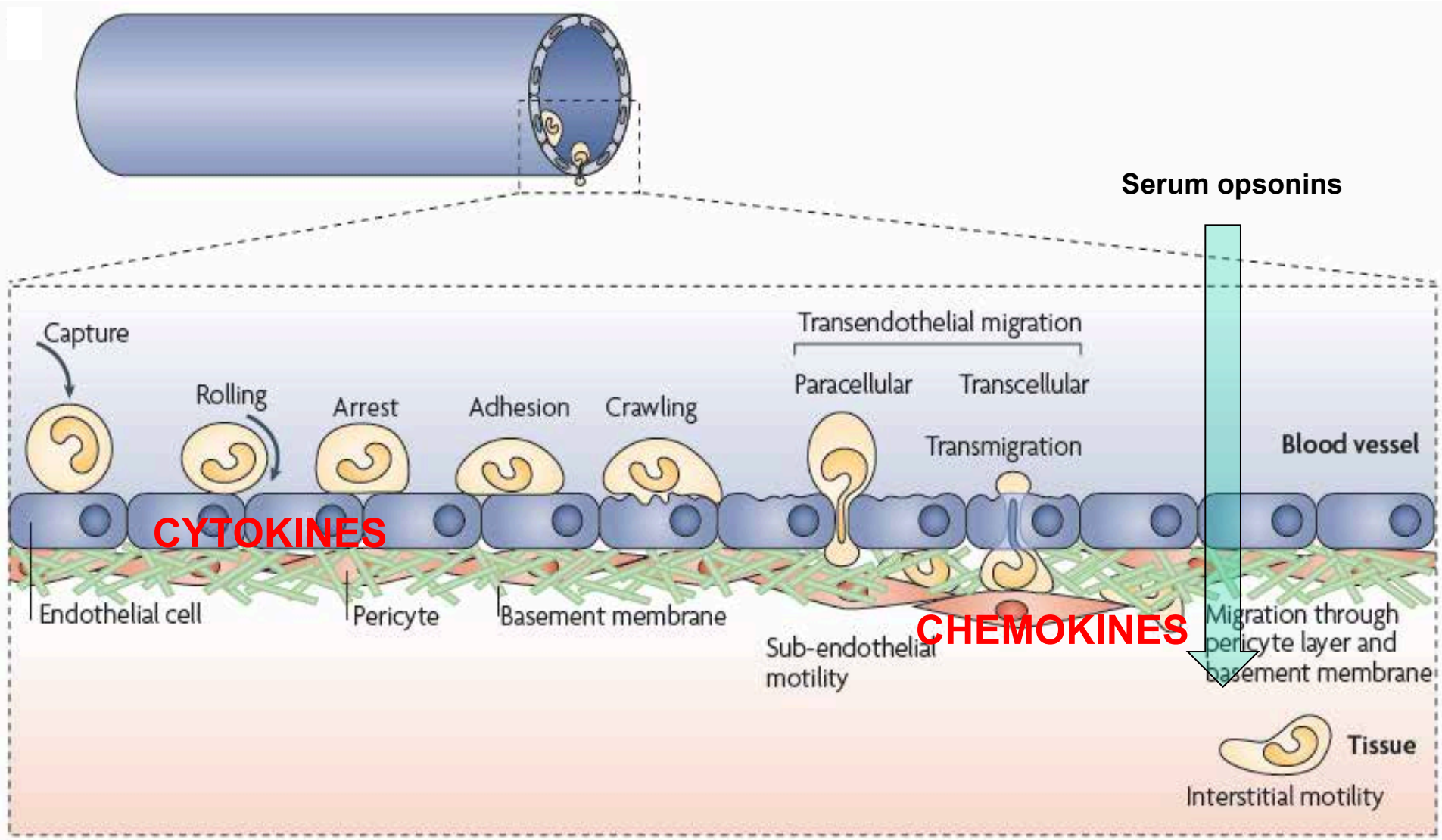
Macrophages: Phagocytose and kill (opsonized) microbes; release cytokines and chemokines; mediate tissue repair and homeostasis; *sentinel cells in all tissues*

Mast cells: Mediate immediate reactions to toxins by increasing blood vessel permeability; near vessels in skin and mucosa

Dendritic cells: Alert the adaptive immune system – T and B cells – by migrating from tissues to lymph nodes; *sentinel cells in all tissues*

Recruited (blood) cells

PMNs (neutrophils): take up and kill (opsonized) microbes (granule enzymes; toxic O_2 and N_2 radicals; NETosis to limit spread; die



Nourshargh et al, Nature Rev Molec Cell Biol 11:366, 2010

Acute phase response mediated by IL-6 cytokine family

Brain - Fever (prostaglandin EP3 receptor)

Liver - Acute Phase Proteins (soluble recognition factors)

Fat - Leptin (energy, wound repair)

Bone Marrow -

Leukocyte precursors

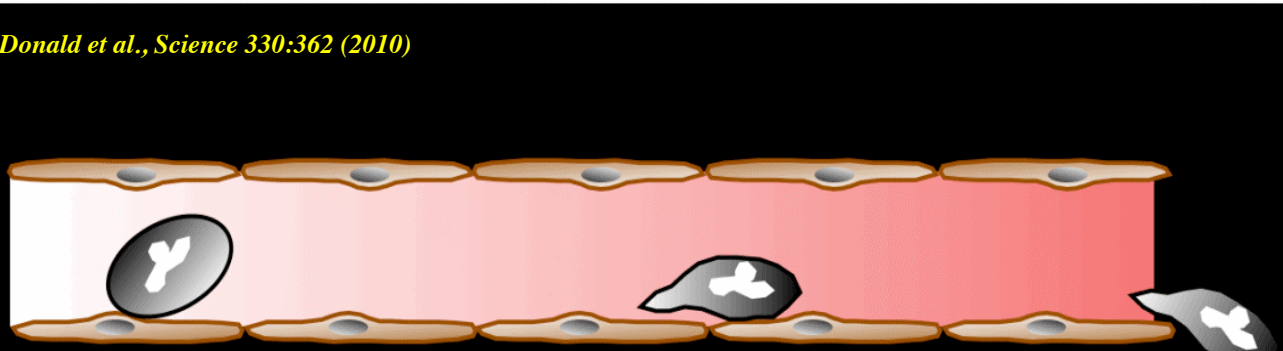
Pro-Inflammatory Danger Signal

Intravascular Chemoattractant Gradient

Focusing the Inflammatory Response

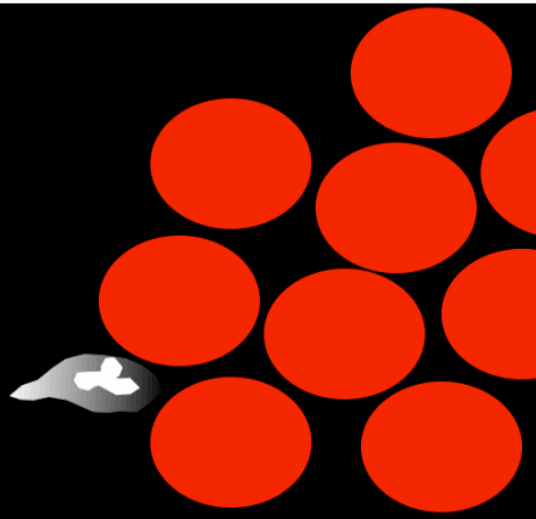
Adhesion → Chemotaxis → Necrotaxis

B McDonald et al., Science 330:362 (2010)



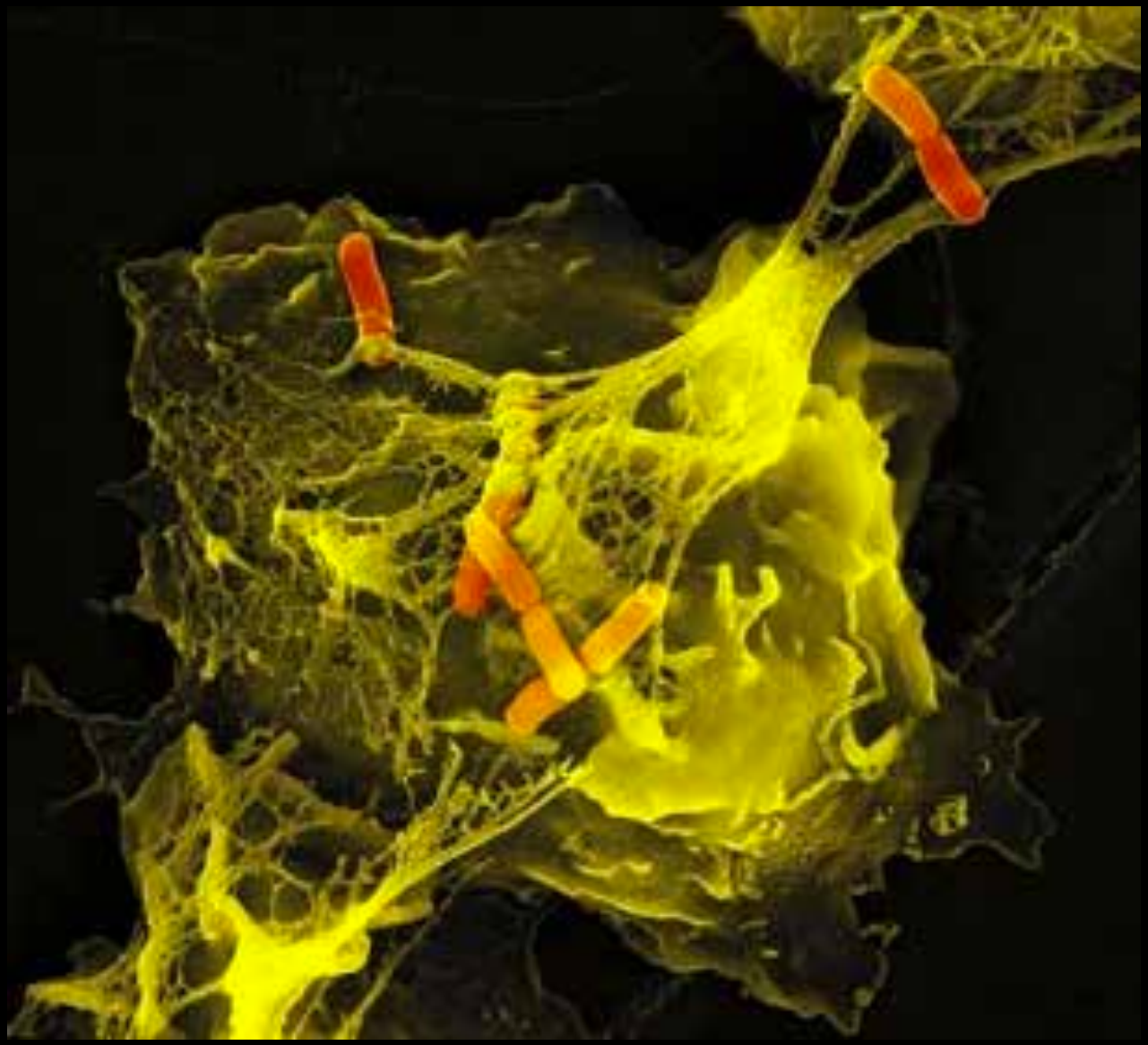
Extracellular ATP
↓
$P2X_7R$
↓
Nlrp3 Inflammasome
↓ IL-1β
IL-1R/MyD88 Signaling
↓
Mac1-ICAM1 dependent neutrophil adhesion

MyD88-dependent signals (non-bone marrow derived cell)
↓
Intravascular MIP-2/KC gradient
↓
CXCR2 Signaling
↓
Intravascular chemotaxis (Mac1 dependent crawling)

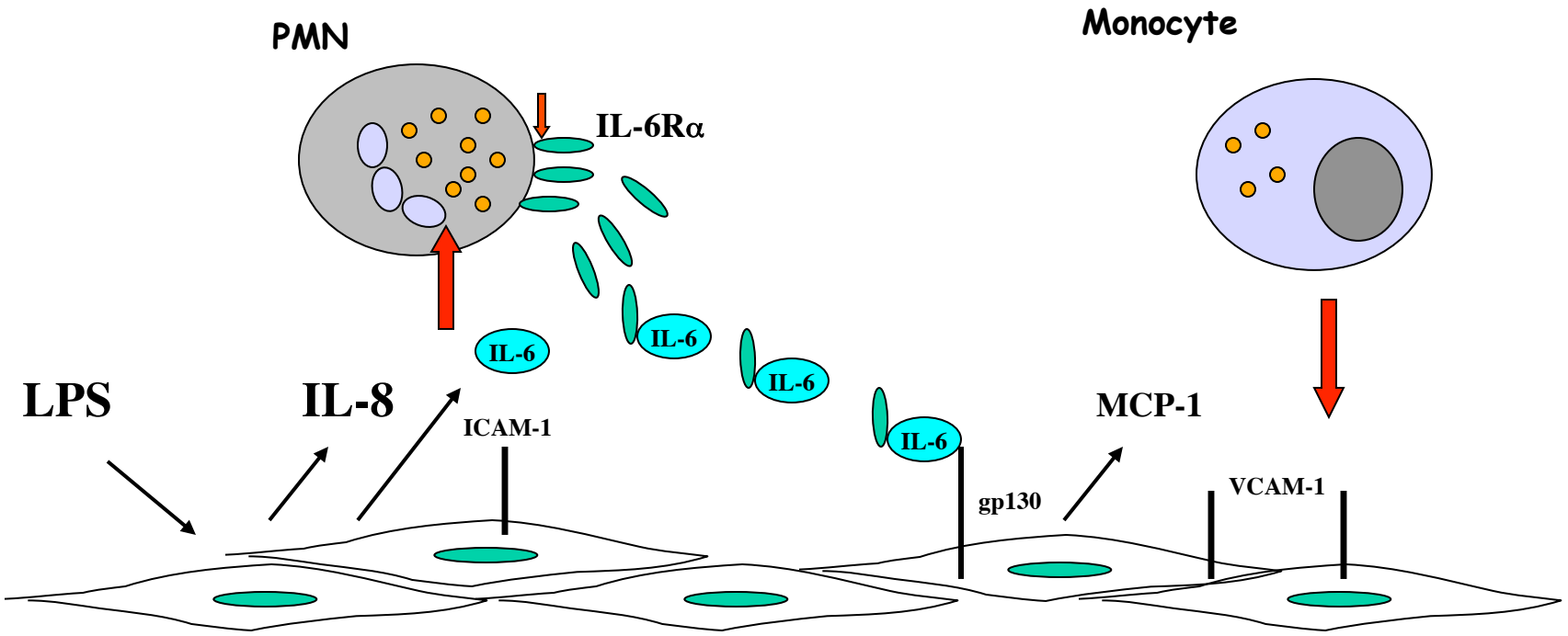


Formyl peptide necrotaxis signal
↓
formyl peptide receptor-dependent crawling into lesion
↓
Precise localization within focus of necrosis

NETosis: DNA



PMN to monocyte transition during inflammation



Short-lived

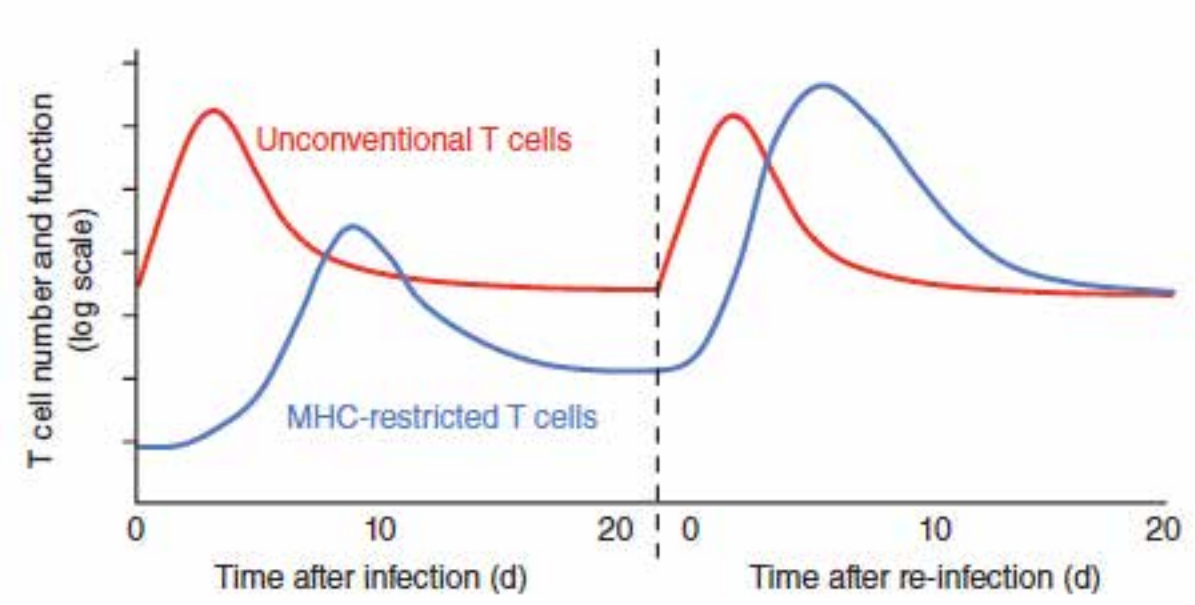
Toxic

Long-lived

Tissue Remodeling

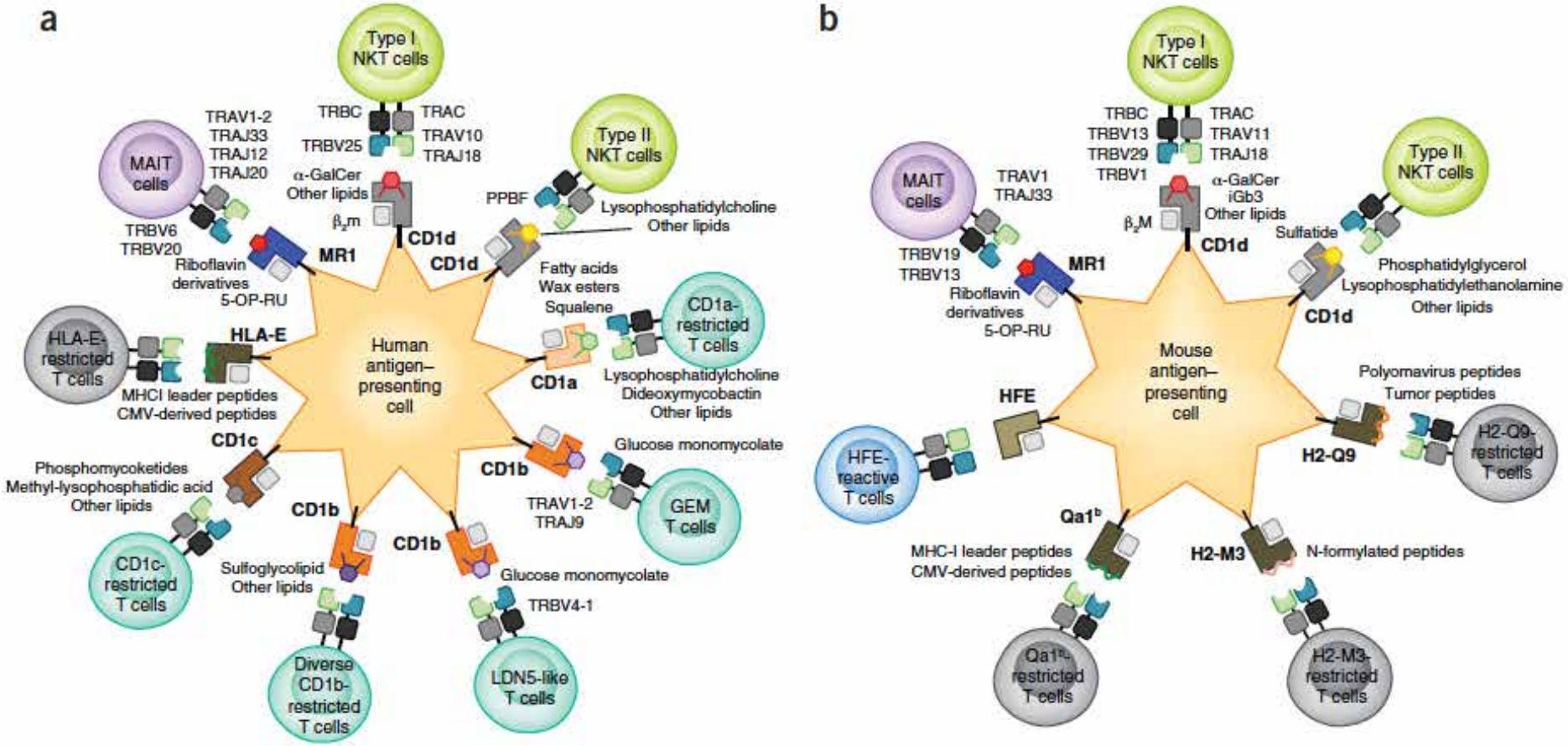
Macrophage maturation

'Invariant' lymphocytes



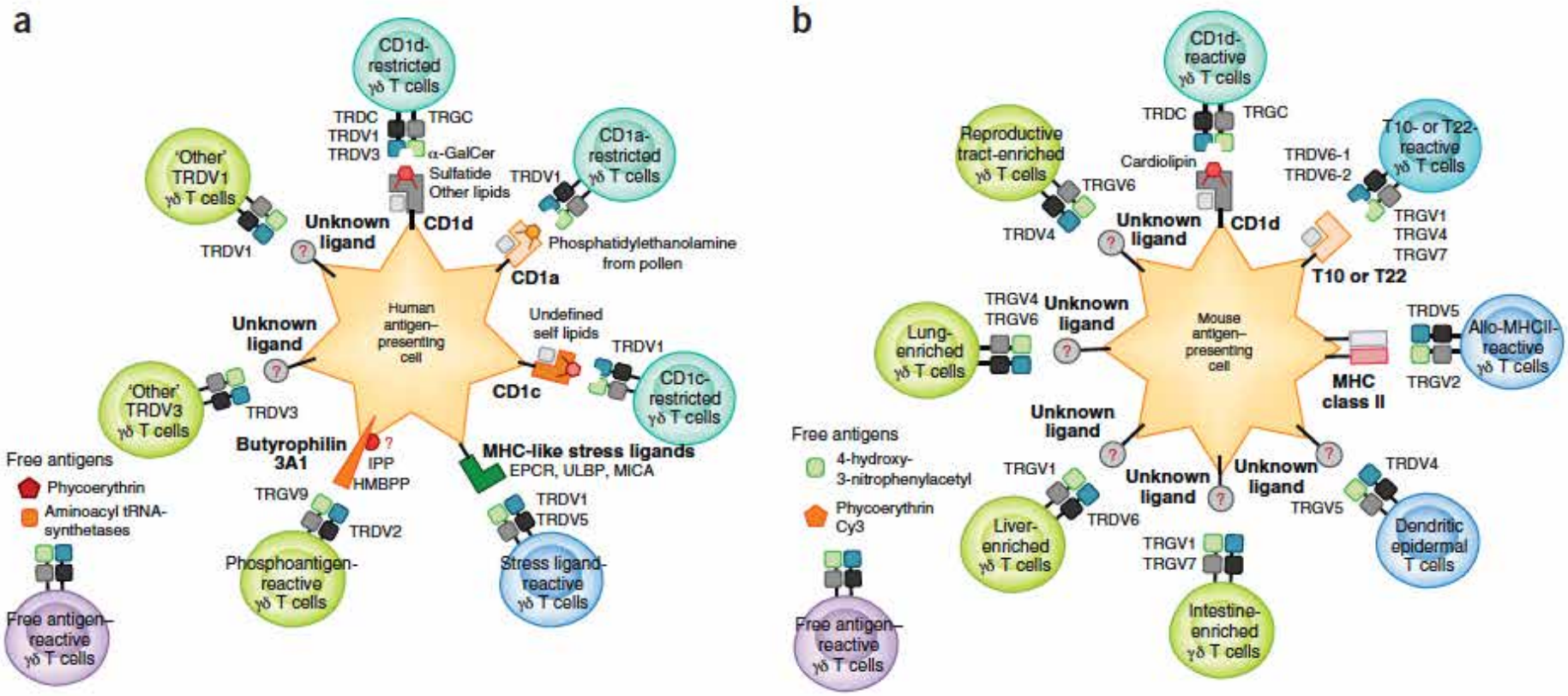
Godfrey DI, et al, The burgeoning family of unconventional T cells. Nat Immunol 11:1114, 2015.

'Invariant' lymphocytes



Godfrey DI, et al, The burgeoning family of unconventional T cells. Nat Immunol 11:1114, 2015.

'Invariant' γδ T cells



Godfrey DI, et al, The burgeoning family of unconventional T cells. Nat Immunol 11:1114, 2015.

Nonpolymorphic MHC molecules

Molecular chaperones for distinct cellular compartments

Tissue-specific distribution

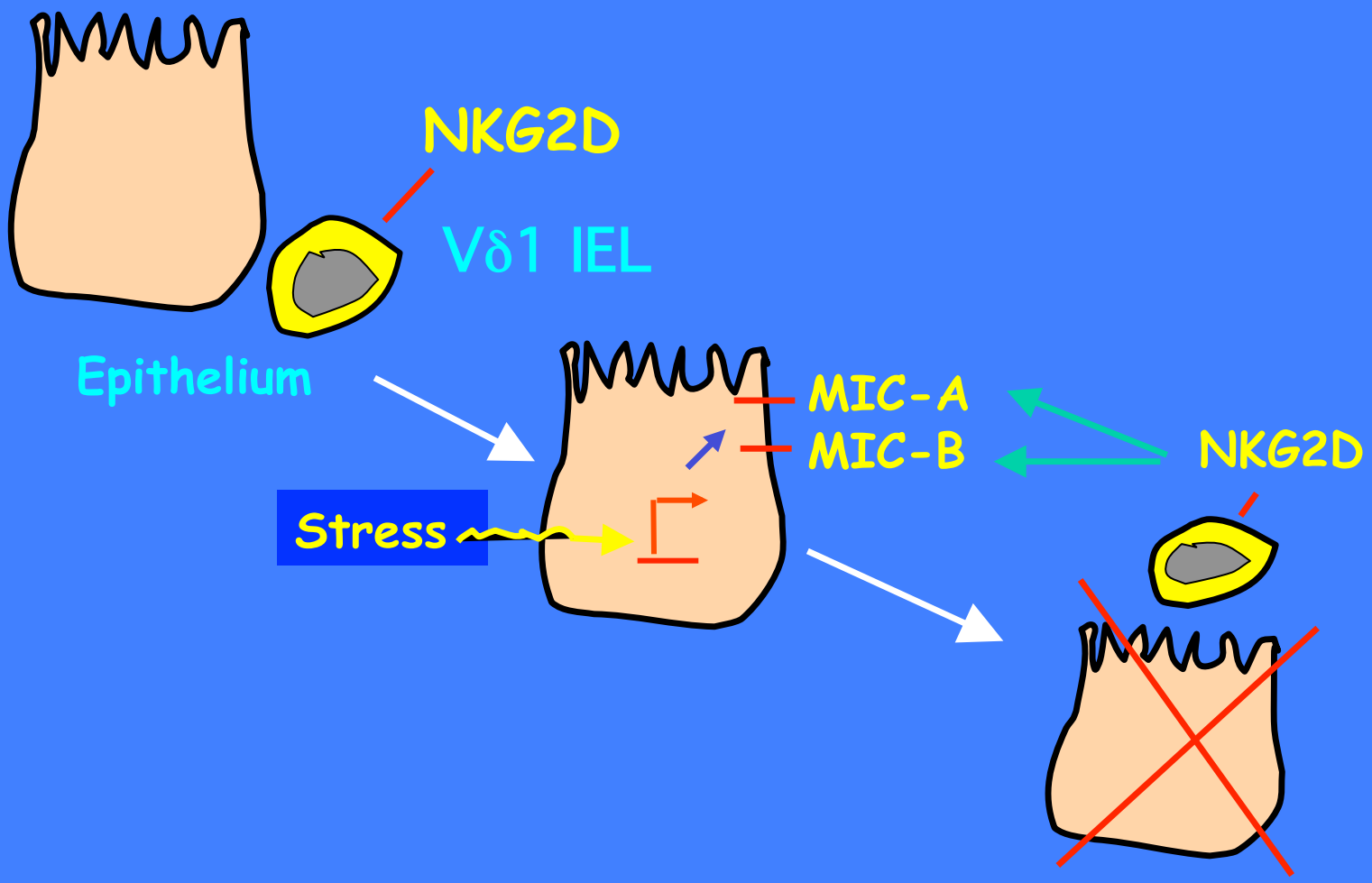
Can be stress- or cytokine-induced

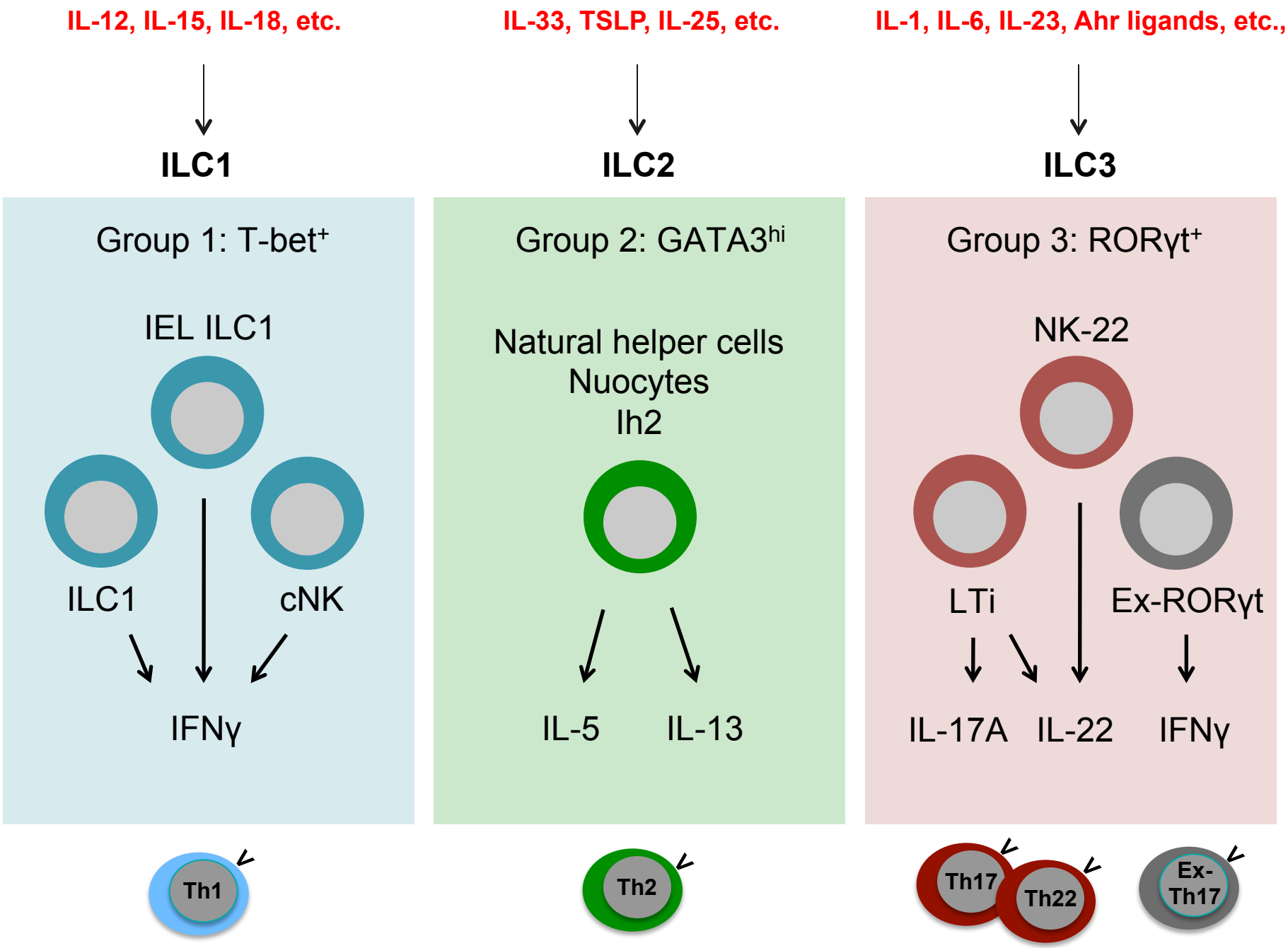
Many interact with invariant lymphocyte populations

Non-peptide or self-peptide-based recognition

MIC-A, MIC-B, RAE-1

Stress-induced cytotoxicity




IL-12, IL-15, IL-18, etc.
IL-33, TSLP, IL-25, etc.
IL-1, IL-6, IL-23, Ahr ligands, etc.,
ILC1
ILC2
ILC3
Group 1: T-bet+
Group 2: GATA3hi
Group 3: RORγt+
IEL ILC1
Natural helper cells
Nuocytes
Ih2
NK-22
ILC1
cNK
LTi
Ex-RORγt
IFNγ
IL-5
IL-13
IL-17A
IL-22
IFNγ
Th1
Th2
Th17
Th22
Ex-Th17

Activation of immunity

Inflammatory cytokines/chemokines

-acute phase response

-phagocyte recruitment/activation

Nonpolymorphic MHC and/or ligand expression

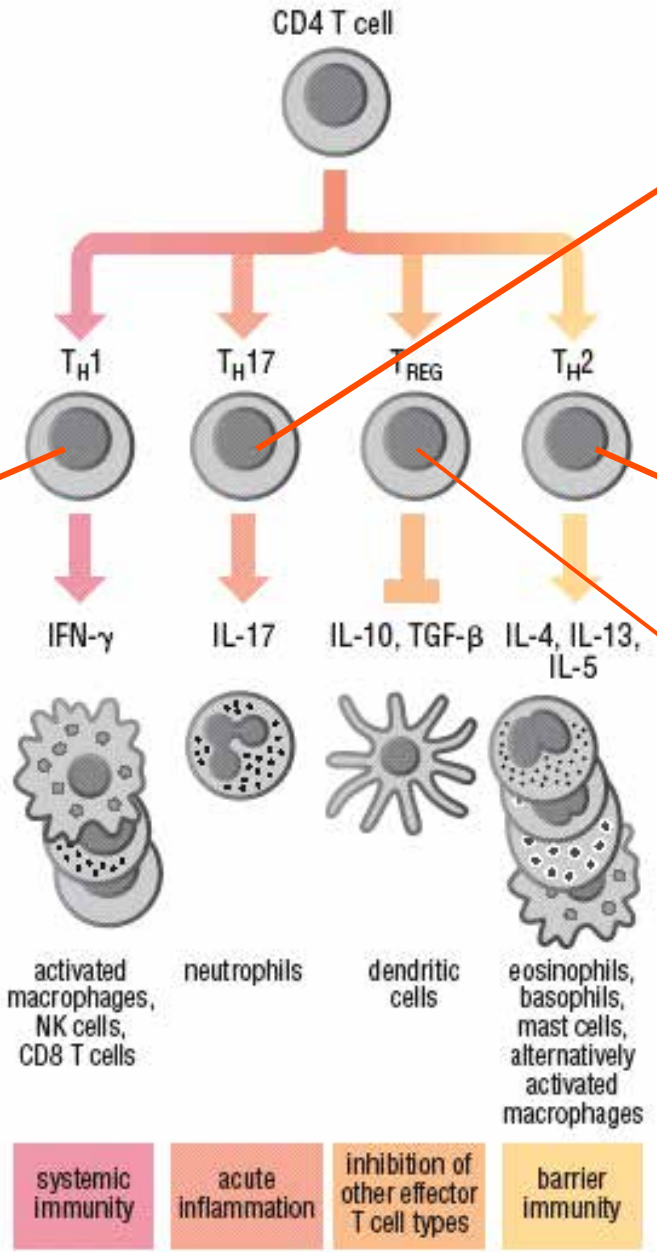
Activation of ‘innate’ and ‘invariant’ lymphoid cells

Migration and maturation of dendritic cells

Activation of adaptive T and B cells

All in setting of normal commensal flora

Immunity is 'modular'



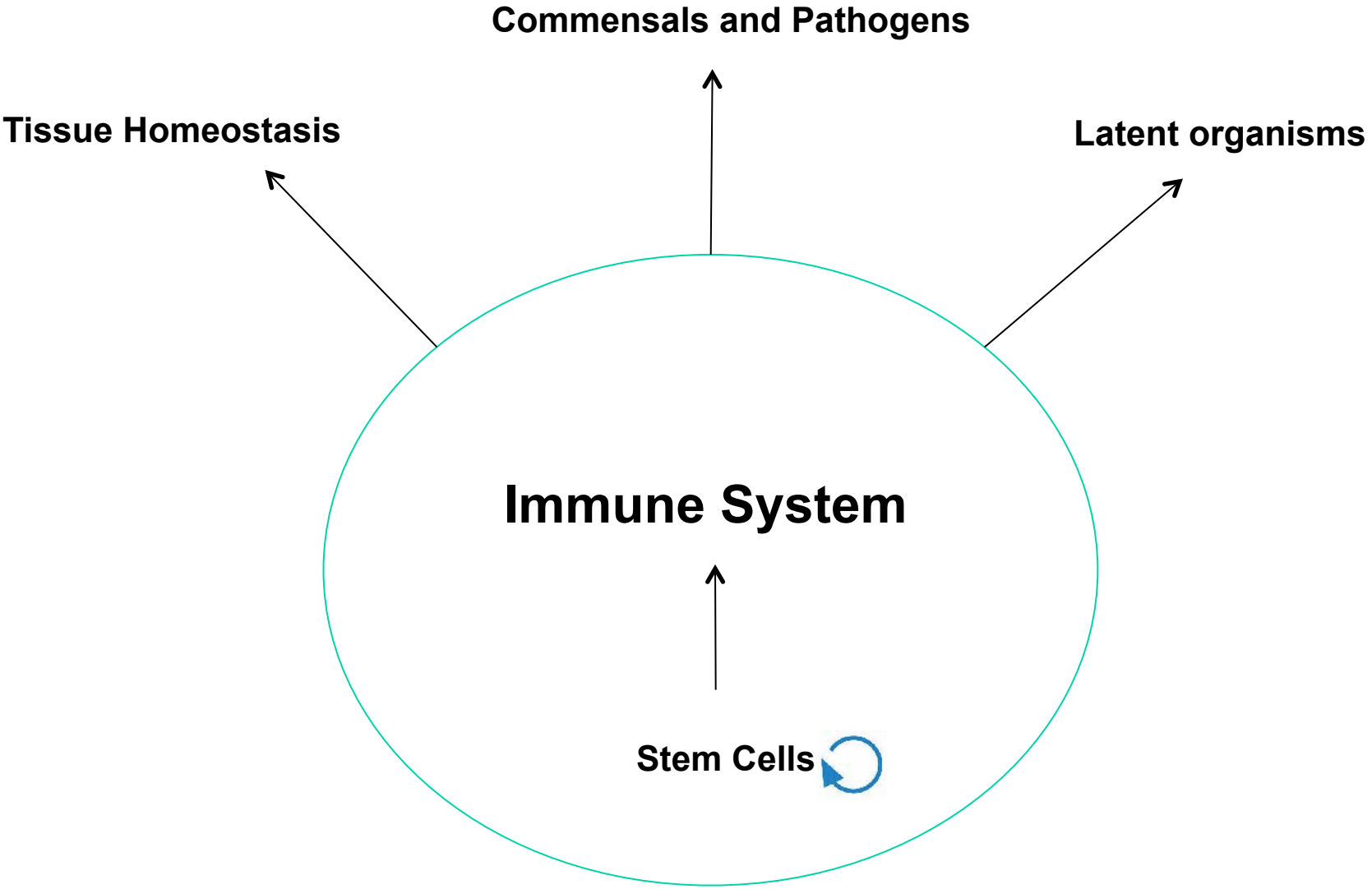
Extracellular bacteria and fungi

Facultative and obligate intracellular organisms

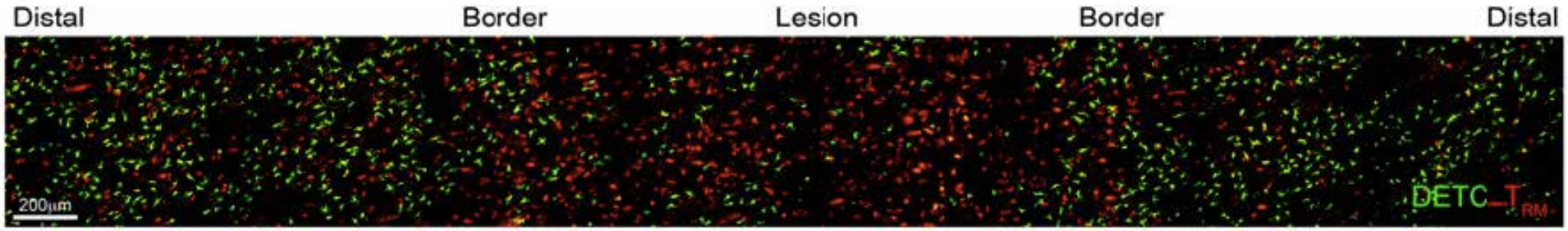
Helminths and biting insects

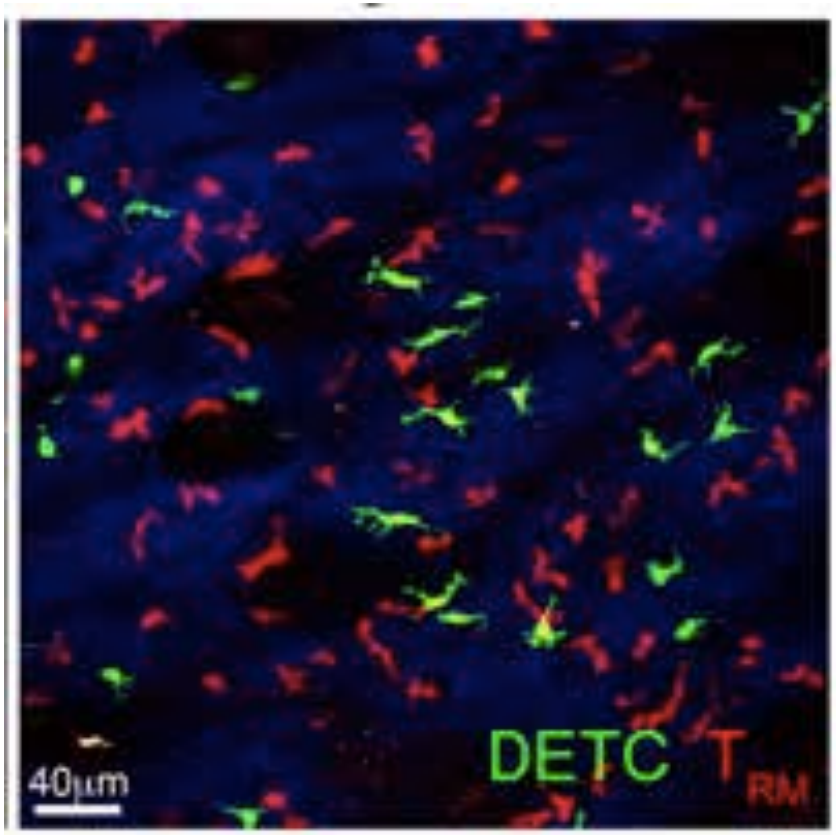
Sustain homeostasis in chronic infections

Immunity is ‘constant’



Age creates ‘memory’





Zaid et al, PNAS 111:5307, 2014

SUMMARY AND THOUGHTS

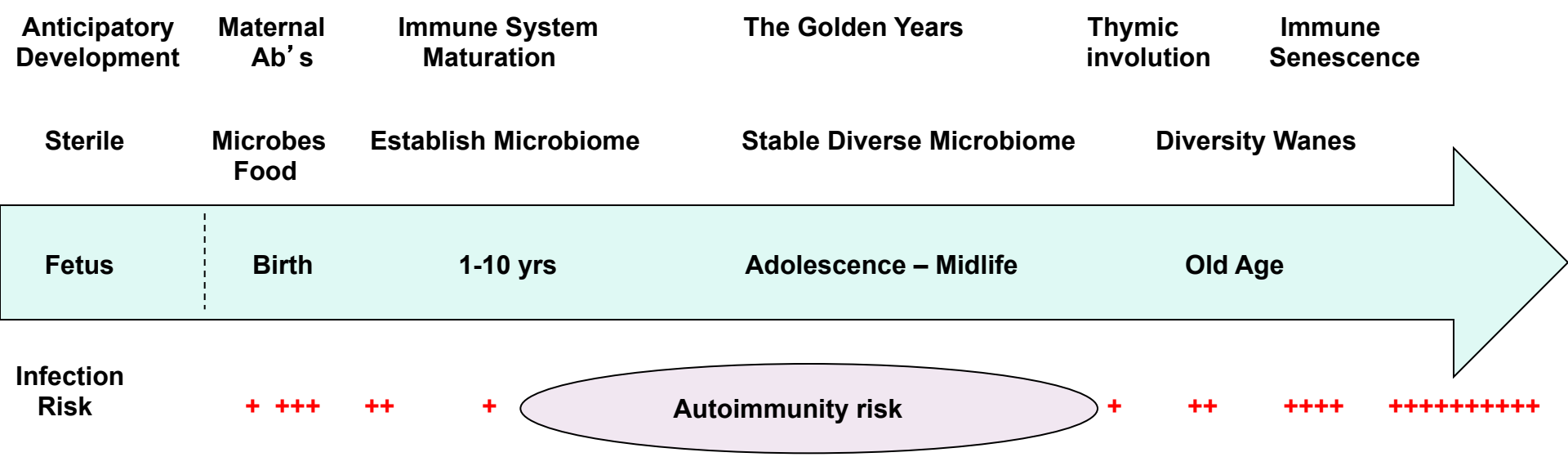
Immune system develops in anticipatory way and matures in presence of microbes/food (developmental window).

Sets immunologic 'health' by establishing basal tone for tolerance versus immunoprotection. Weak PRR stimulation favors tolerance, strong PRR stimulation favors immunity and generation of memory (protection).

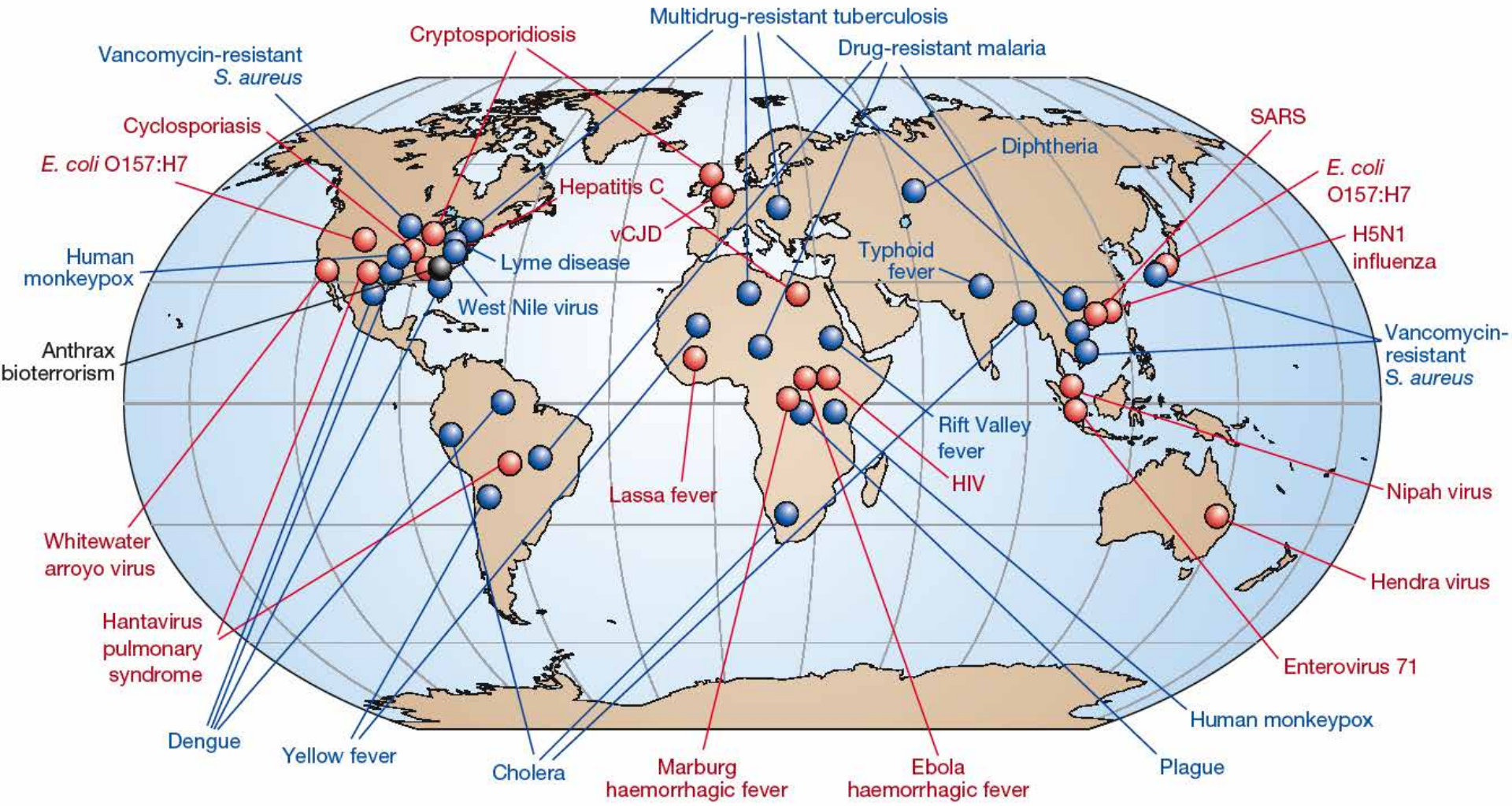
Innate immunity sets the 'tone'. Adaptive immunity sets the 'flavor' – matched to the kind of stimulus – and generates memory.

Works very well – infections tend to happen in very young (before immune system matures, very old when immune system ages, and a little bit in pregnancy).

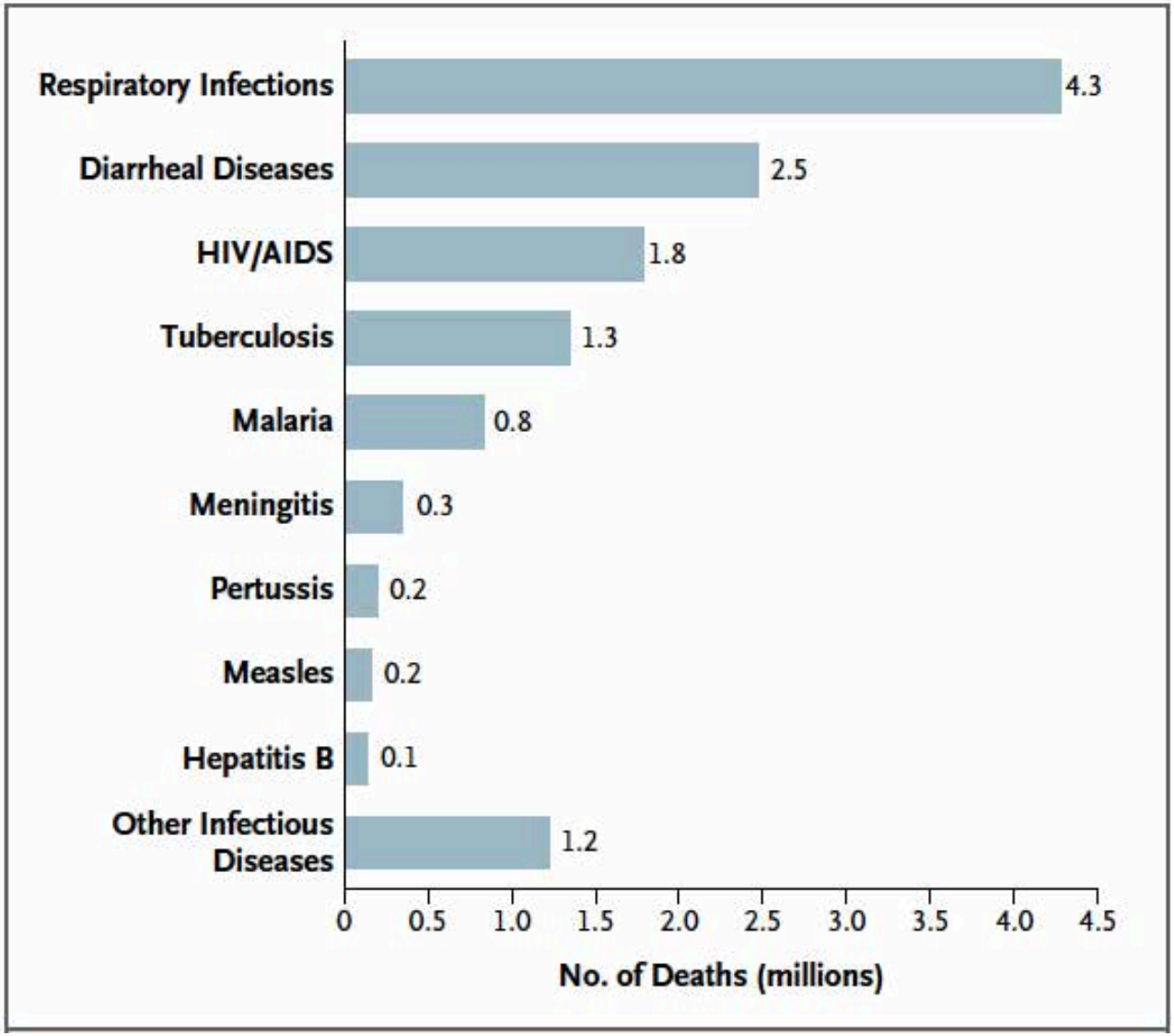
Certain diseases of Westernized civilizations may be tied to improved standard of living, antibiotics and subsequent development of a dysregulated immune system.



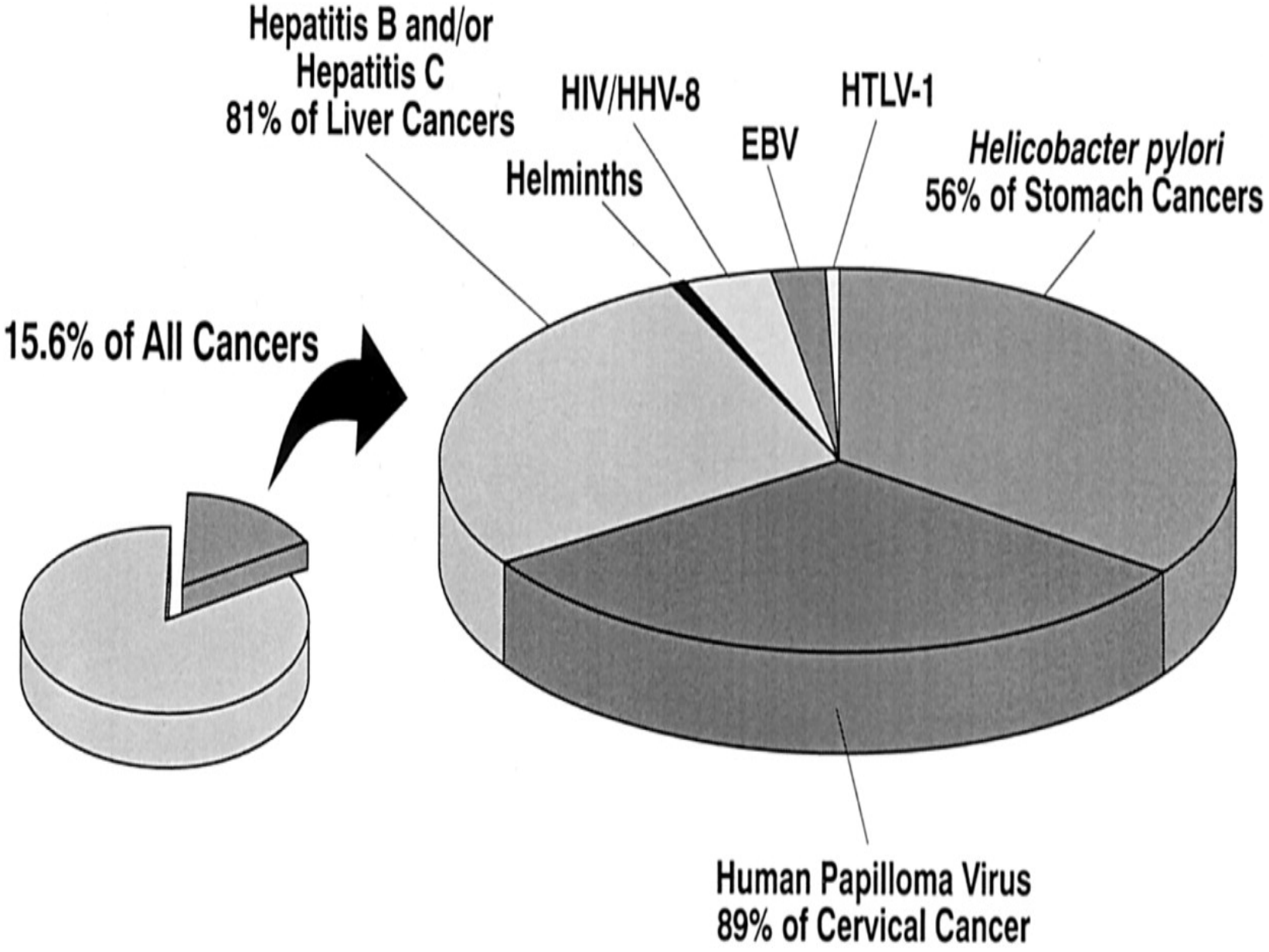
Emerging and re-emerging infectious diseases



Morens DM et al., Nature 2004, 430:242-9.



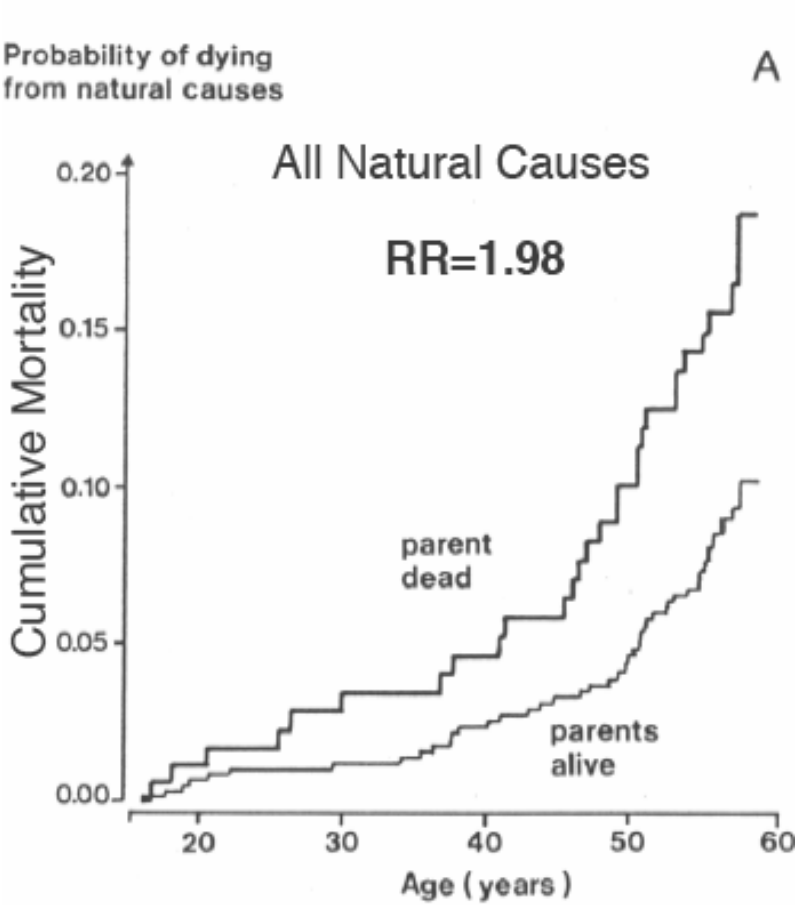
15/58.8 million deaths worldwide (25.5%), not counting secondary causes like rheumatic heart diseases, liver cancer, cervical cancer, etc. // AS Fauci, DM Morens. The perpetual challenge of infectious diseases. NEJM 366:454-61, 2012.

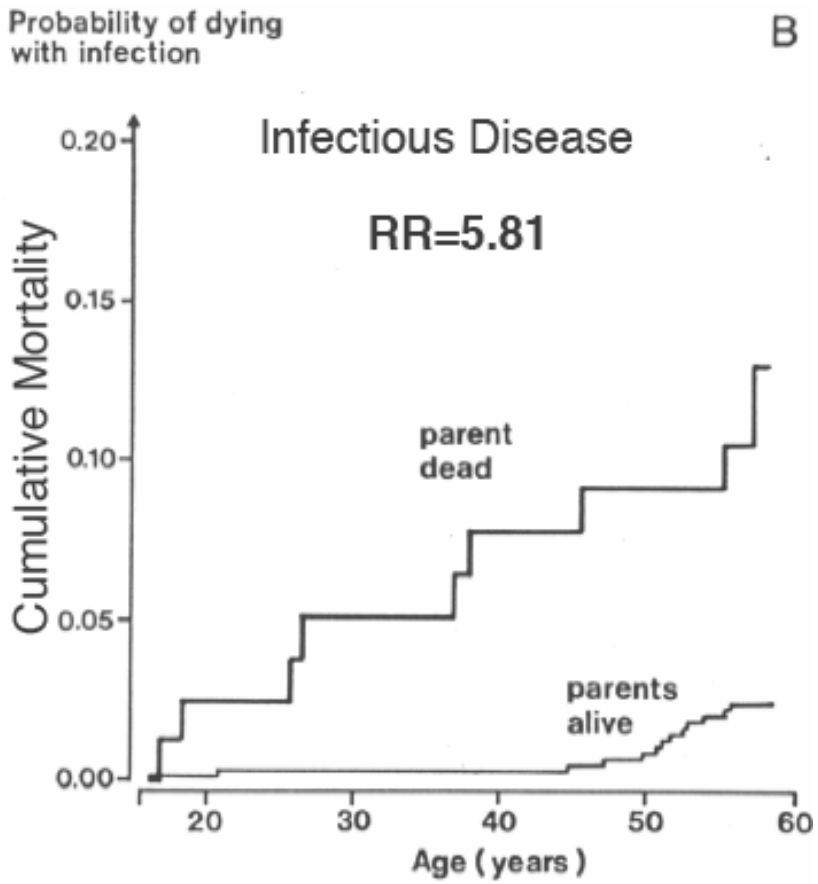

Hepatitis B and/or
Hepatitis C
81% of Liver Cancers
HIV/HHV-8
HTLV-1
EBV
Helminths
Helicobacter pylori
56% of Stomach Cancers
15.6% of All Cancers
Human Papilloma Virus
89% of Cervical Cancer

Heritability of premature death among 960 adoptees

TI Sorenson et al., Genetic and environmental influences on premature death in adult adoptees. N Engl J Med 318:727-32, 1988

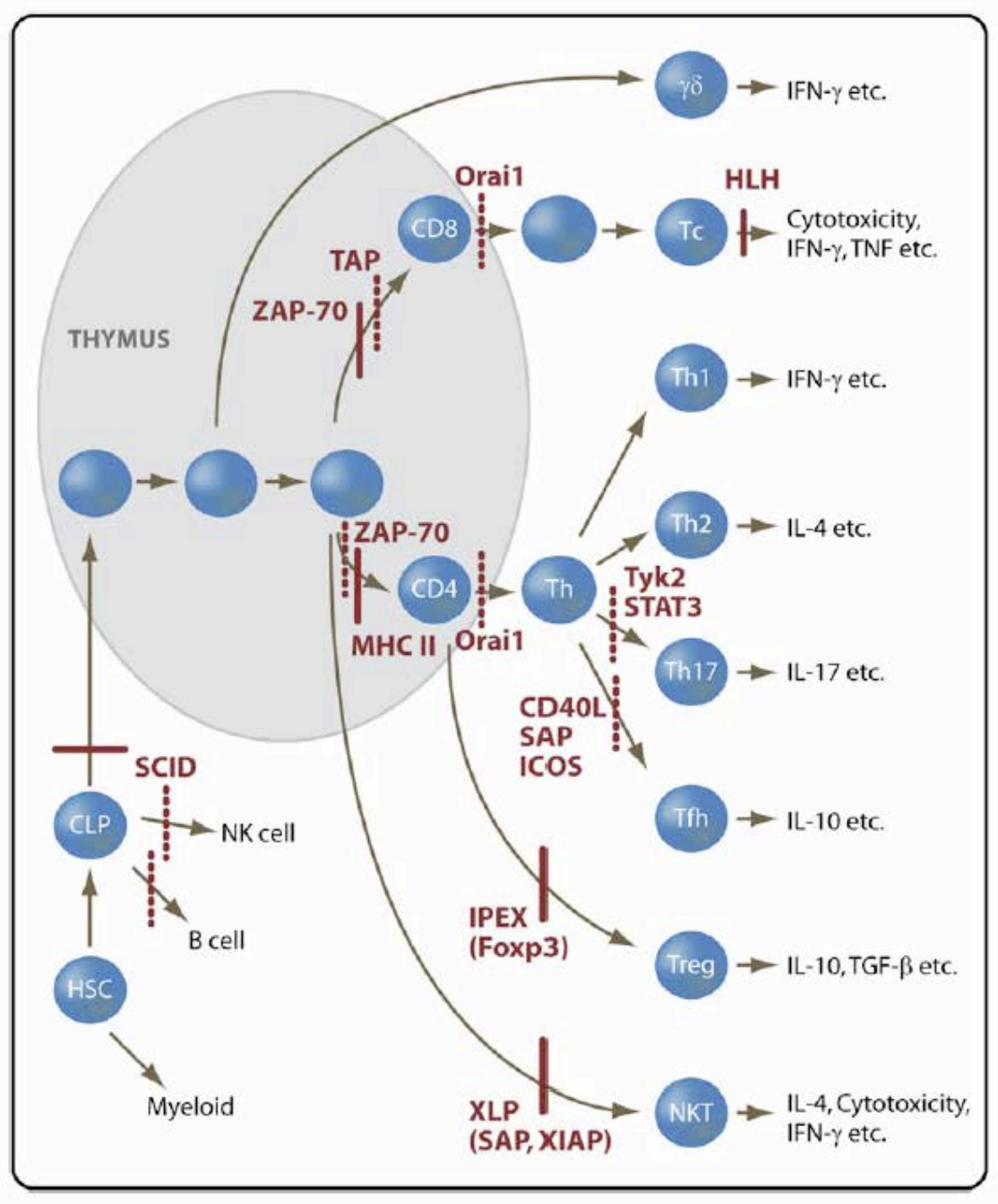
Implies risk of infectious diseases largely heritable - we need to understand the genes involved





Severe combined immunodeficiency

- *Requires isolation and antibiotics*
- *Ultimately requires bone marrow transplantation*
- *Amenable to gene therapy*

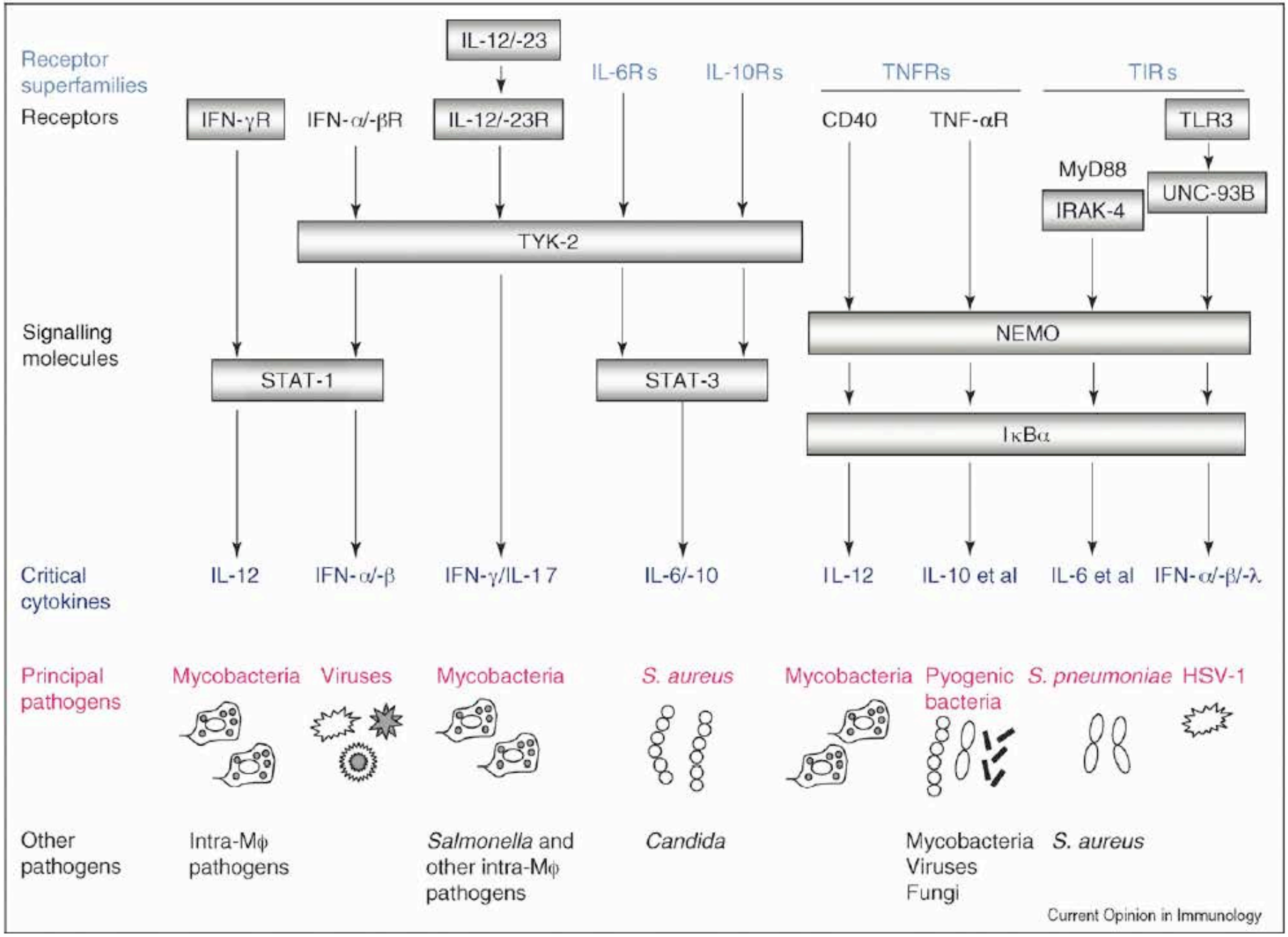


Primary Adaptive Immunodeficiencies

Proximal defects: multiple lymphocyte lineages; broad spectrum, life-threatening infections; bone marrow reconstitution or gene therapy required

Distal defects: narrower spectrum of infections; can be life threatening (XLP, HLH, IPEX), chronic (Stat3, Tyk2) or unexpectedly mild (TAP, CD8)

Primary Innate Immunodeficiencies

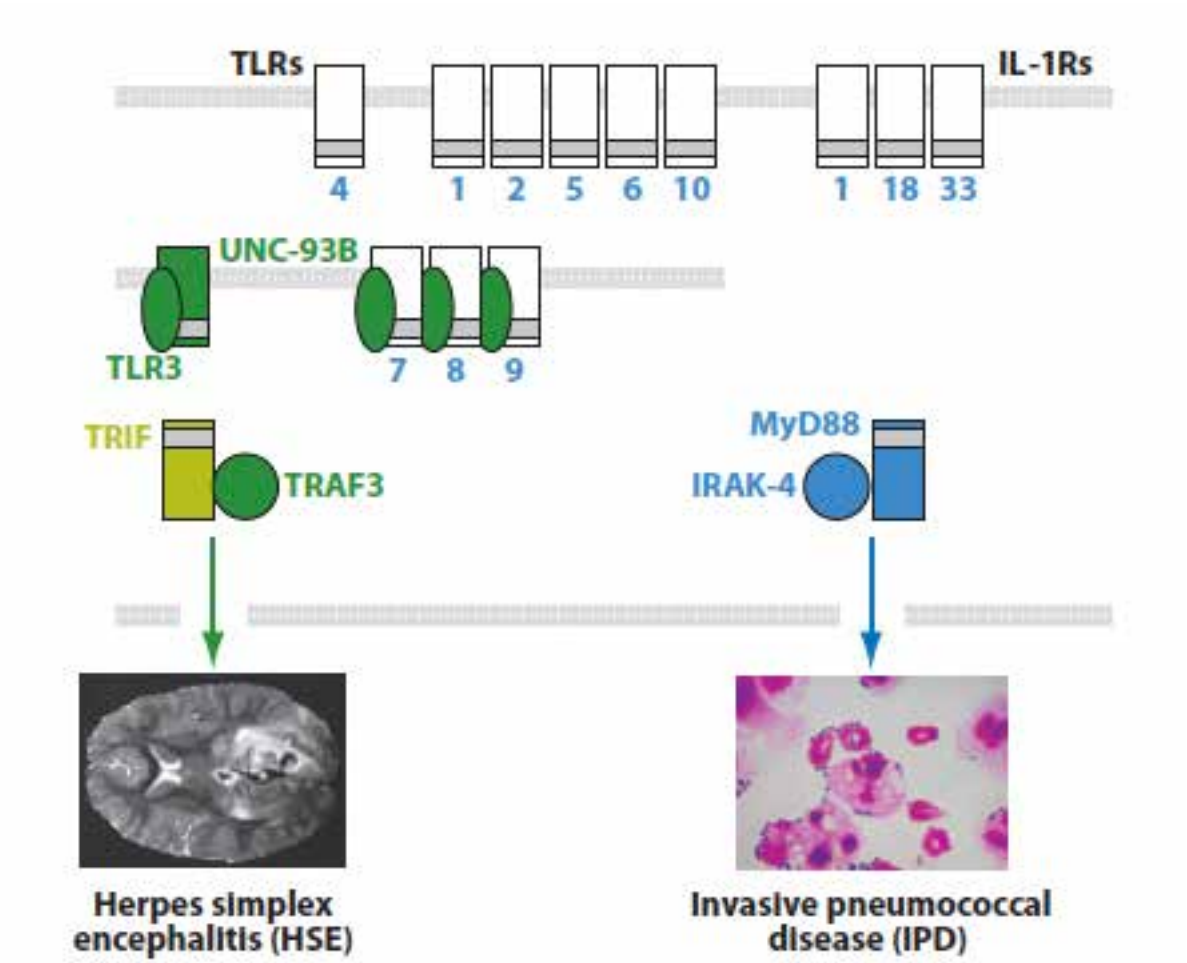


'Immunodeficiencies' can result from immune gene and non-immune gene defects

Genetic mutation	Common Pathogens
DARC	Plasmodium vivax
HgbS	Plasmodium falciparum
Fut2	Norovirus
SAP, XIAP	EBV
EVER1, 2	HPV
Terminal complement components	Neisseria
IFNγ/IL-12	Mycobacteria, Salmonella
TLR4, IRAK, MyD88	Encapsulated bacteria
Unc93B, TLR3	HSV encephalitis
CCR5	HIV
CXCR4	HPV
IL-17 pathway	Candida albicans

Why is the immune deficiency so 'narrow'?

Pathogen-sensing is necessary for 'colonization'



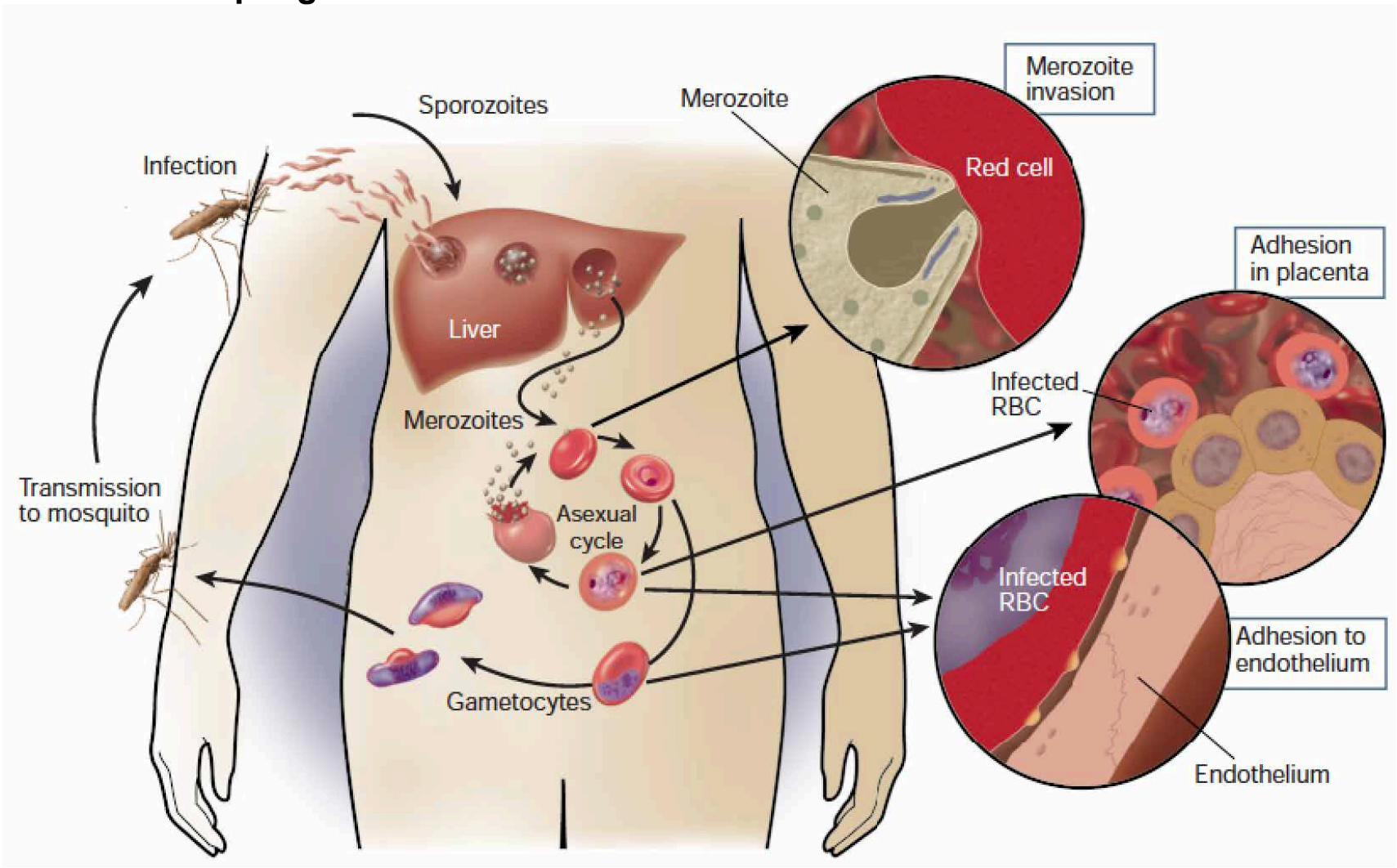
Casanova J-L, et al. Human TLRs and IL-1Rs in host defense: natural insights from evolutionary, epidemiological and clinical genetics. Annu Rev Immunol 29:447-91, 2011.

The Big 3

Organism	Type	Infected /Deaths	Reservoir	Asympt. Infection	Sequestration (latency)	Antigenic variation	Sterile immunity
HIV	Retrovirus	34 million /30 million (total)	Human	Yes	Yes (CD4 cells)	Yes	No
TB	Mycobacteria	2 billion /2.5 million (annual)	Human	Yes	Yes (macrophages)	Yes	No
Malaria	Protozoan	350-500 million /1.5 million (annual)	Human (mosquito)	Yes	Yes (red cells)	Yes	No

MALARIA:
(5300 genes)

Highly adapted mosquito-borne protozoan; derived from gorilla ancestor; infects RBCs (no MHC); dangerous forms adhere to blood vessels in the microvasculature of peripheral organs to escape removal by spleen macrophages



Miller LH, et al. Nature 415:673, 2002.

Malaria immunity

Genetic selection for abnormal hemoglobins that impair parasite maturation (sickle hemoglobin, others)

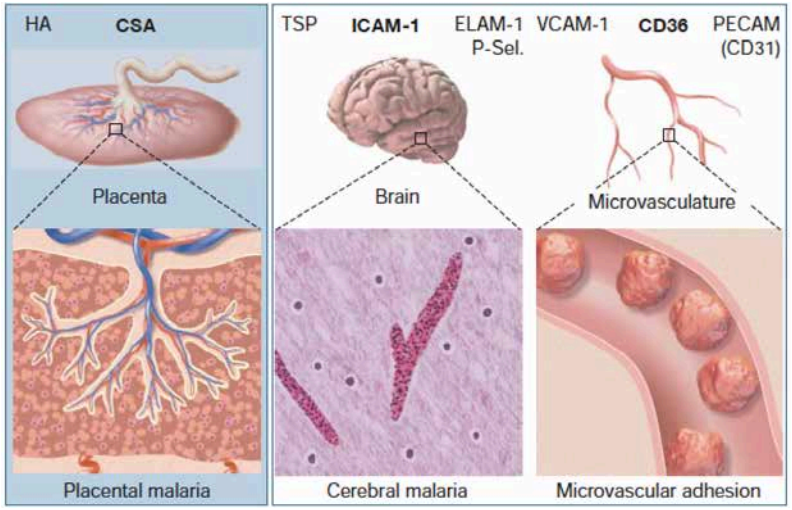
CD4-dependent antibodies that block attachment of infected RBC's

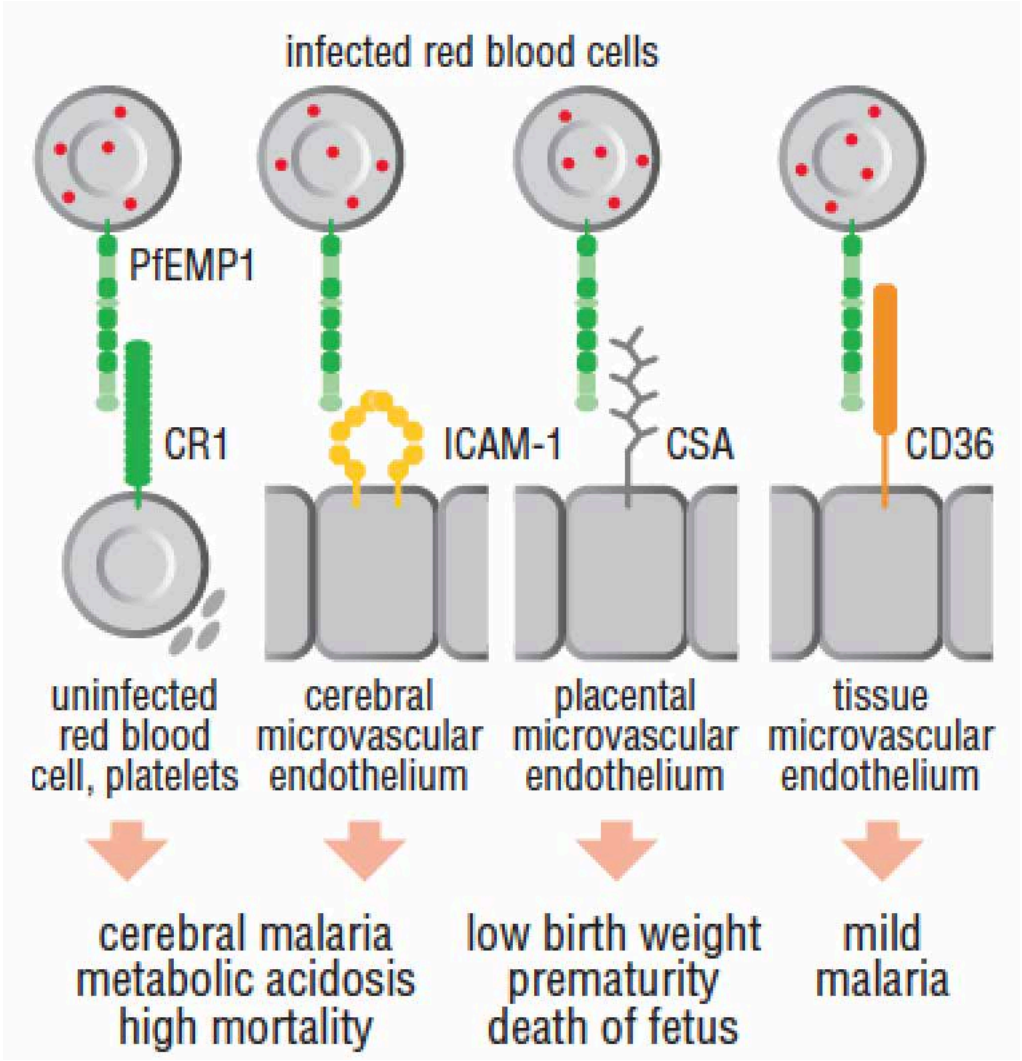
CD8 T cells that kill infected liver cells

Develops slowly over many years

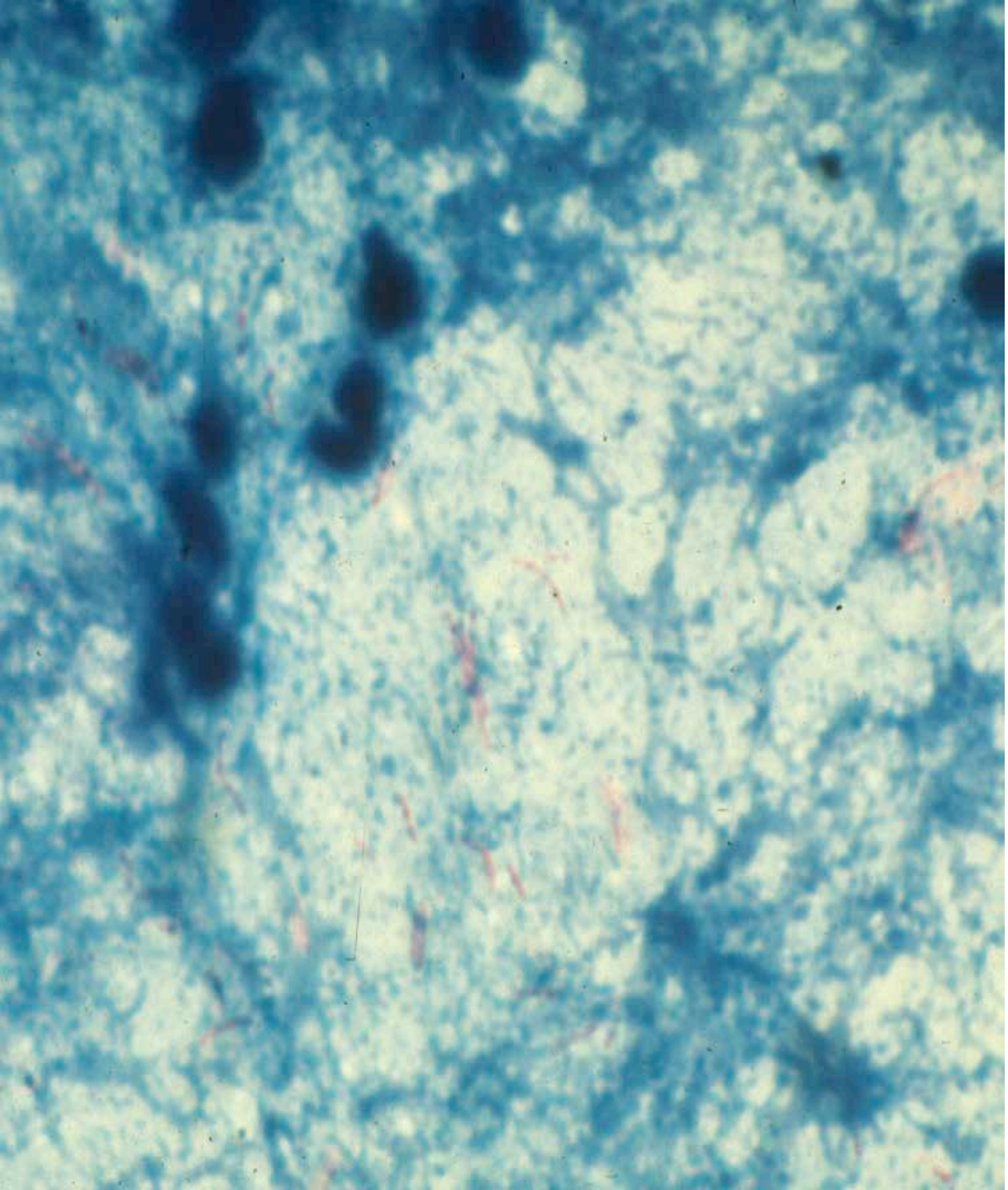
Asymptomatic parasitemia common with age (reservoir)

Highly variant gene family (>100 of the 5300 parasite genes) mediates attachment of infected red cells to host microvascular endothelial cells





DeFranco, Locksley, Robertson. Immunity, New Sciences Press, 2007.

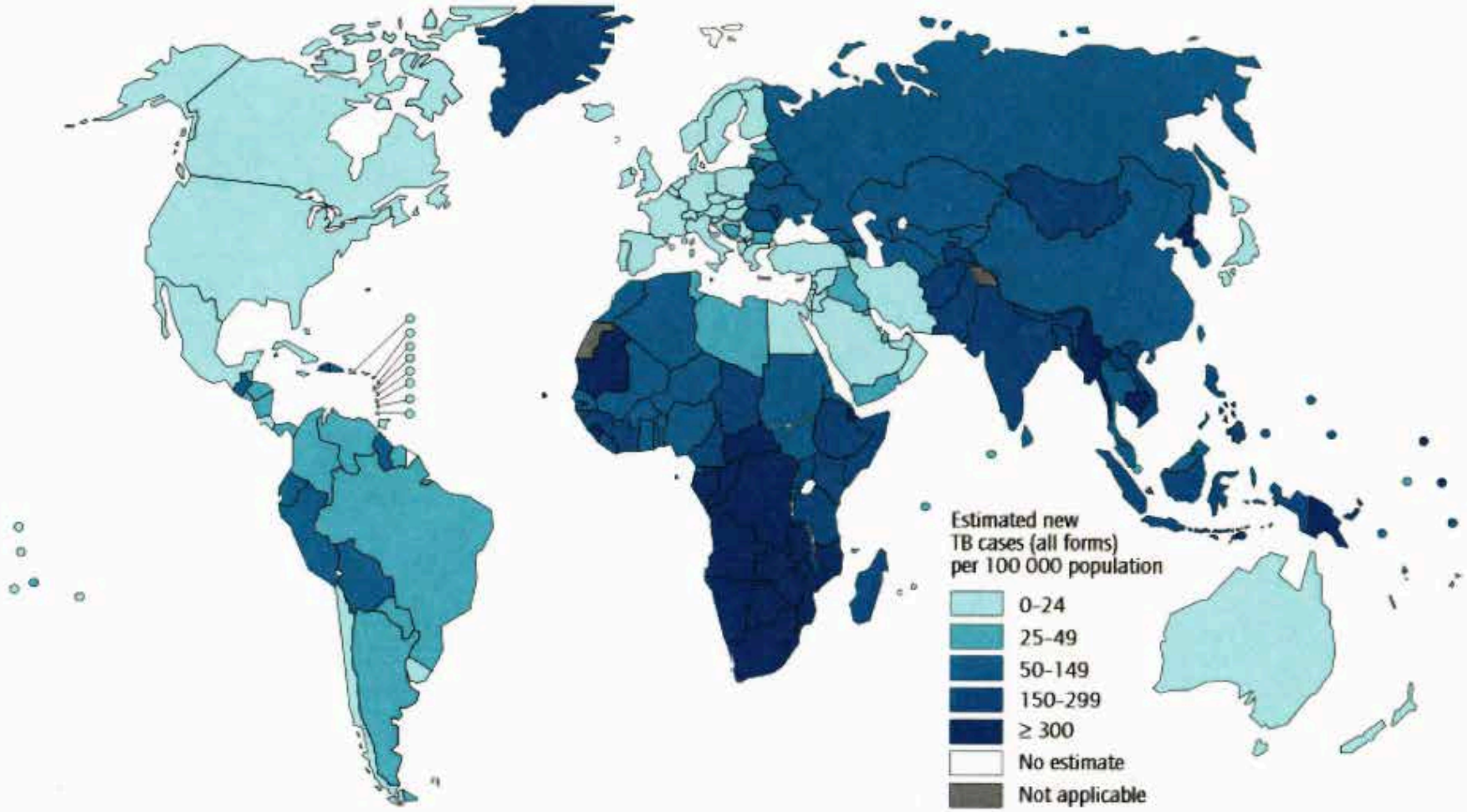


M. Tuberculosis

(3959 genes)

‘Acid-fast’ mycobacteria

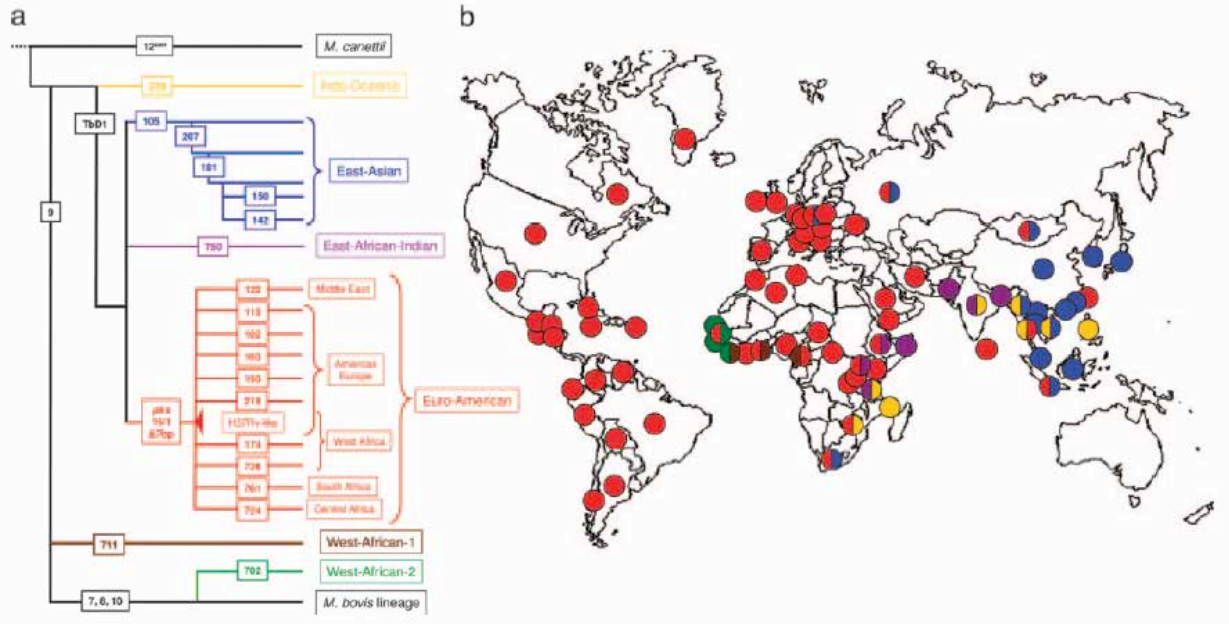
Global map of TB incidence 2011

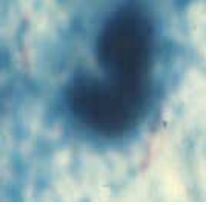


- 2 billion people infected with *M. tuberculosis*
- 8 million new cases each year
- 1.5-2 million deaths each year
- ~5700 deaths per day

SOURCE: WHO Global Tuberculosis Report 2012

M. tuberculosis Genome - Clonotypic Lineages





Tuberculosis Pathogenesis

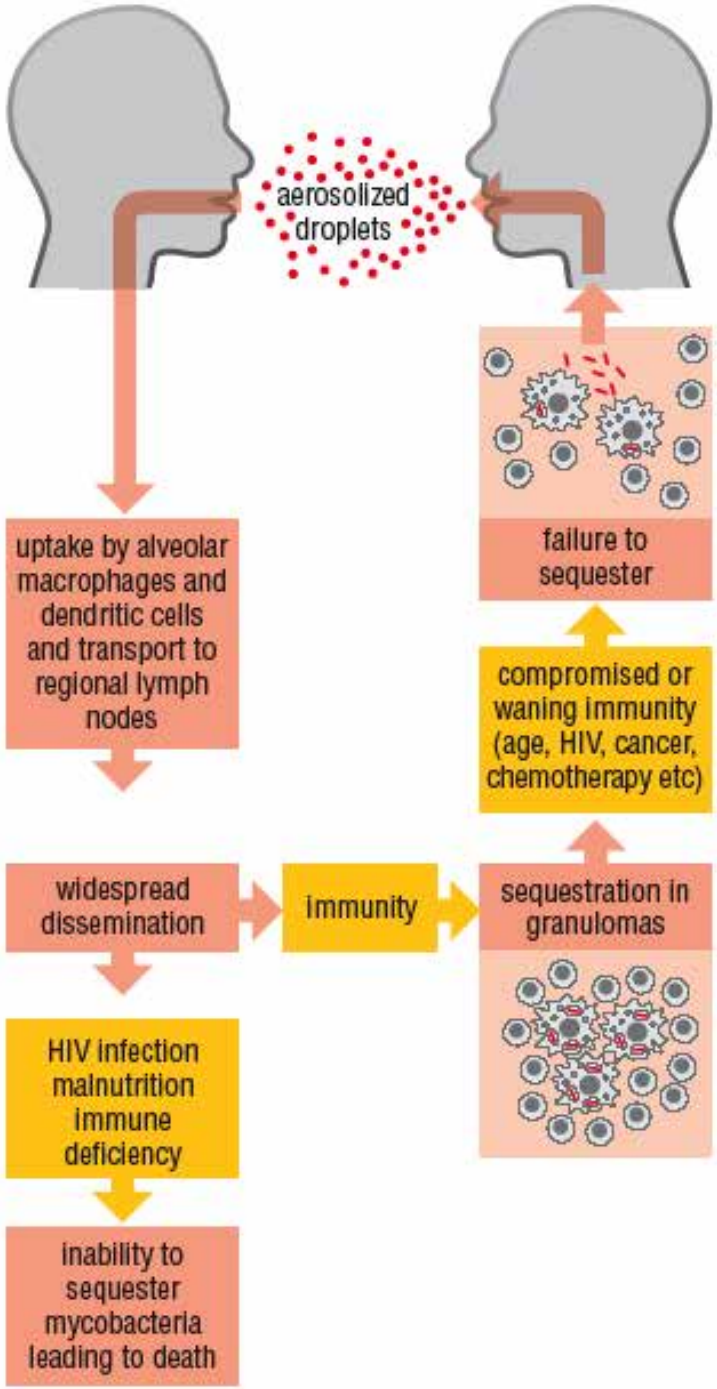
Inhalation of infected droplets (human-to human transmission)

Facultative intracellular pathogen of macrophages

Spread to regional lymph nodes with bacteremia and diffuse metastatic foci

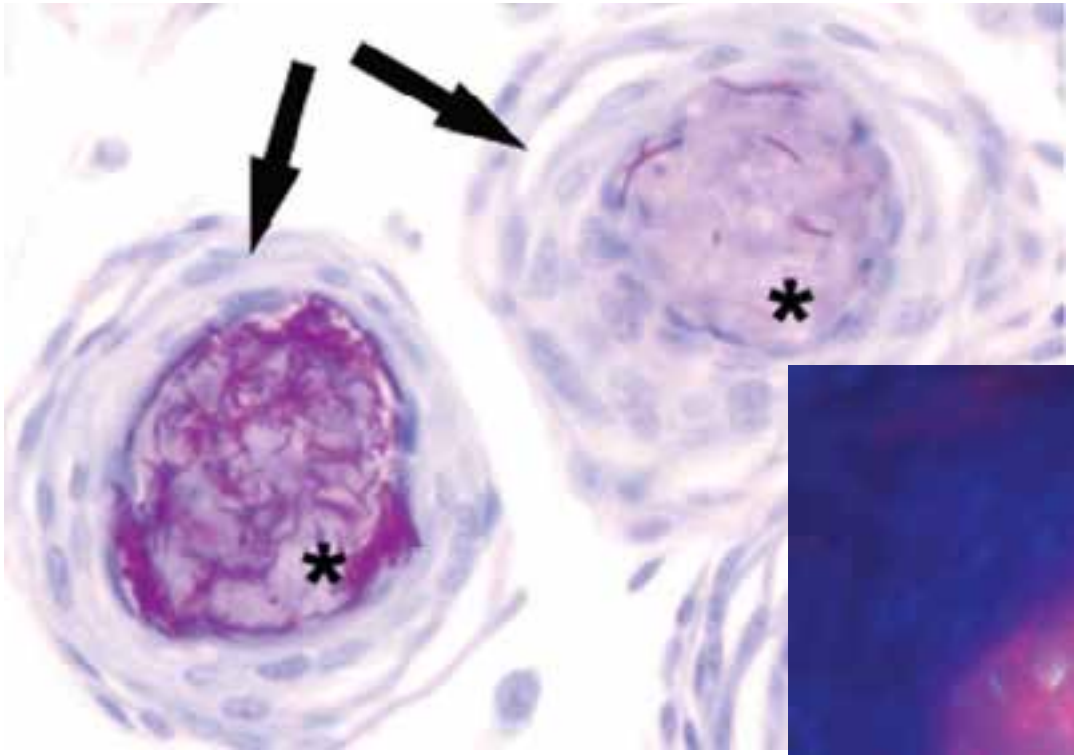
Control by Th1 cell immunity and macrophage activation

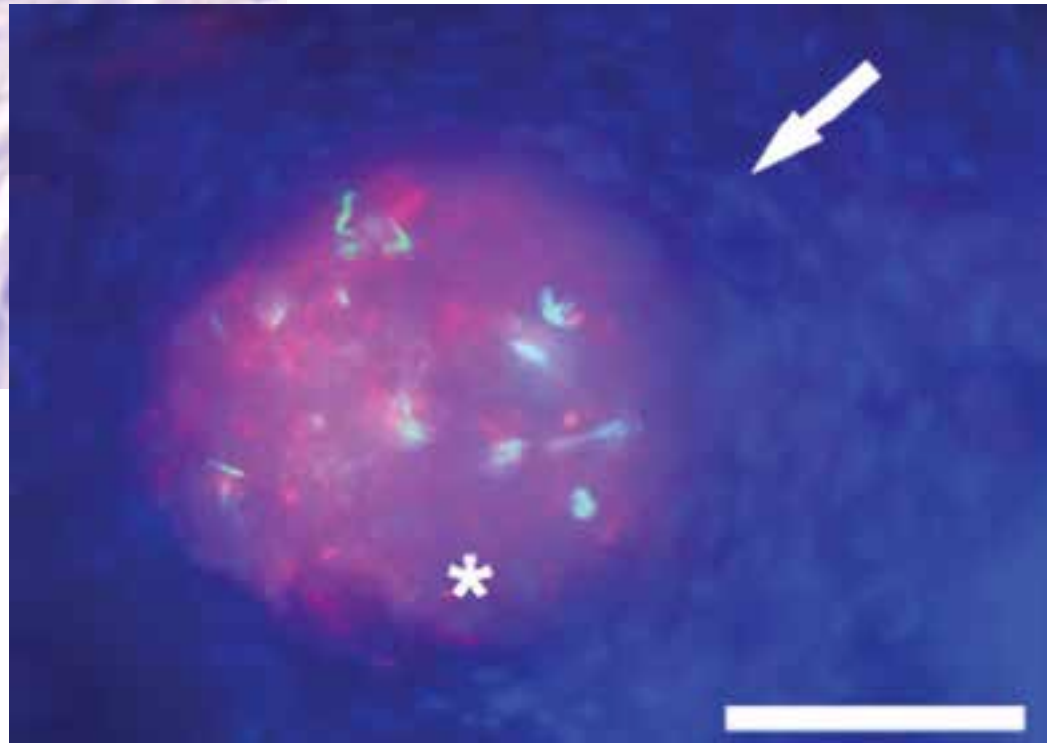
Latent for years in caseating granulomas in aerobic sites with poor lymphatic drainage (lung apices, kidneys, vertebral bodies, meninges)



Maintenance of TB in humans - sequestration in granulomas by Th1-mediated immunity

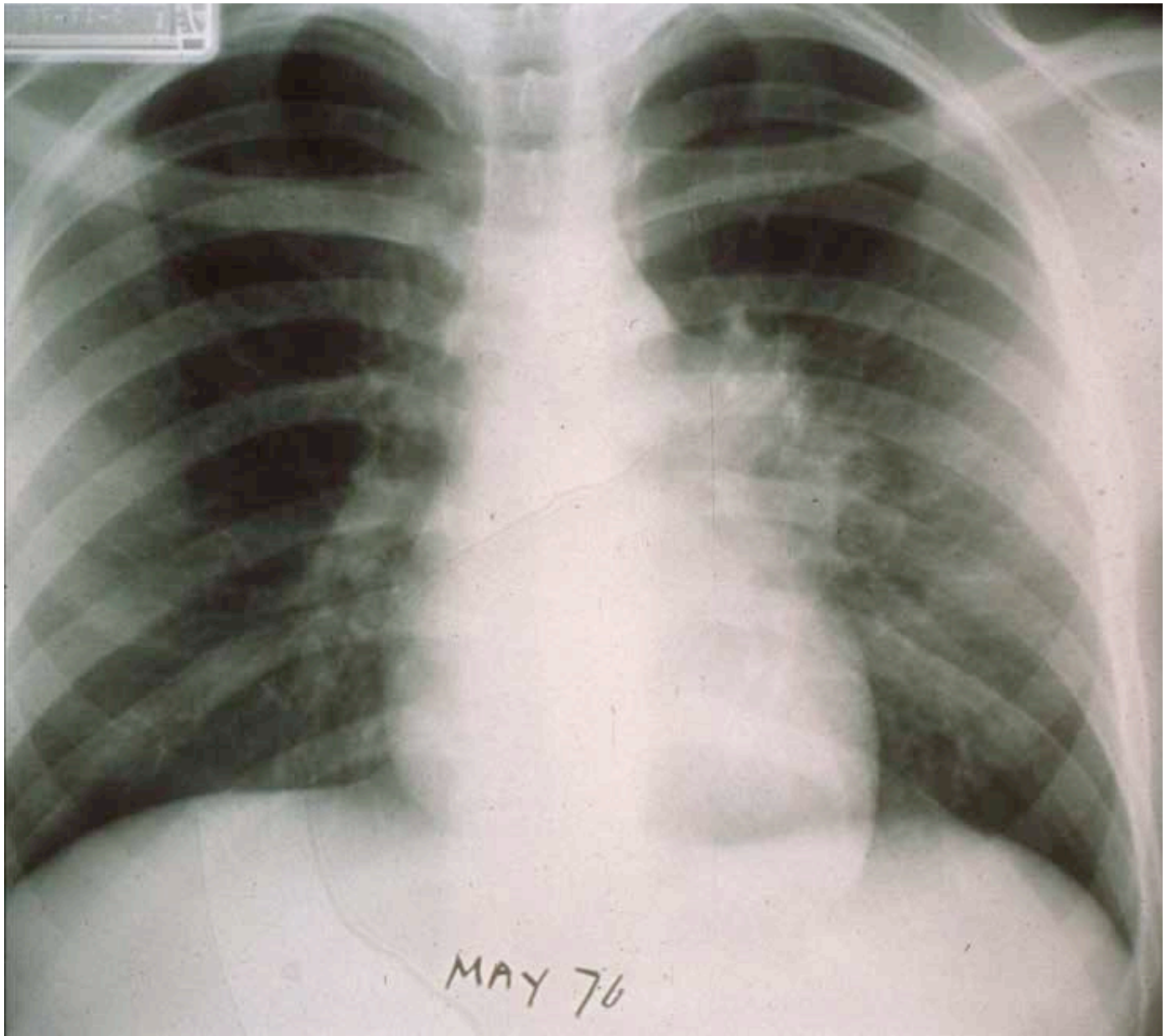
M. tuberculosis homes to and persists in caseous granulomas



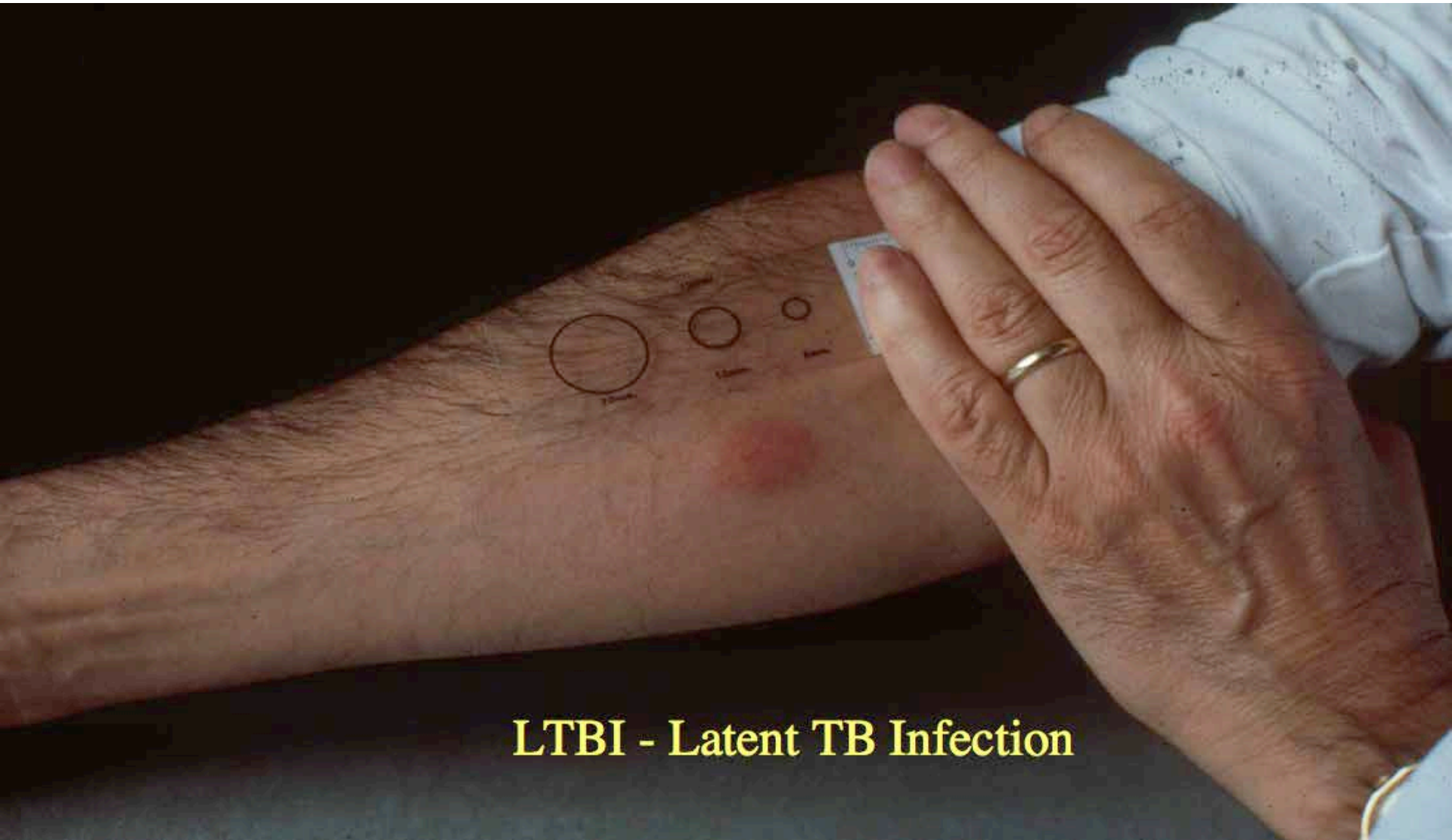


Cosma CL et al, Nat Immunol 5:828, 2004

Primary Tuberculosis



Tuberculin Skin Test (DTH)



+PPD and Risk of Active TB

HIV-neg

CXR-neg

0.1% per yr

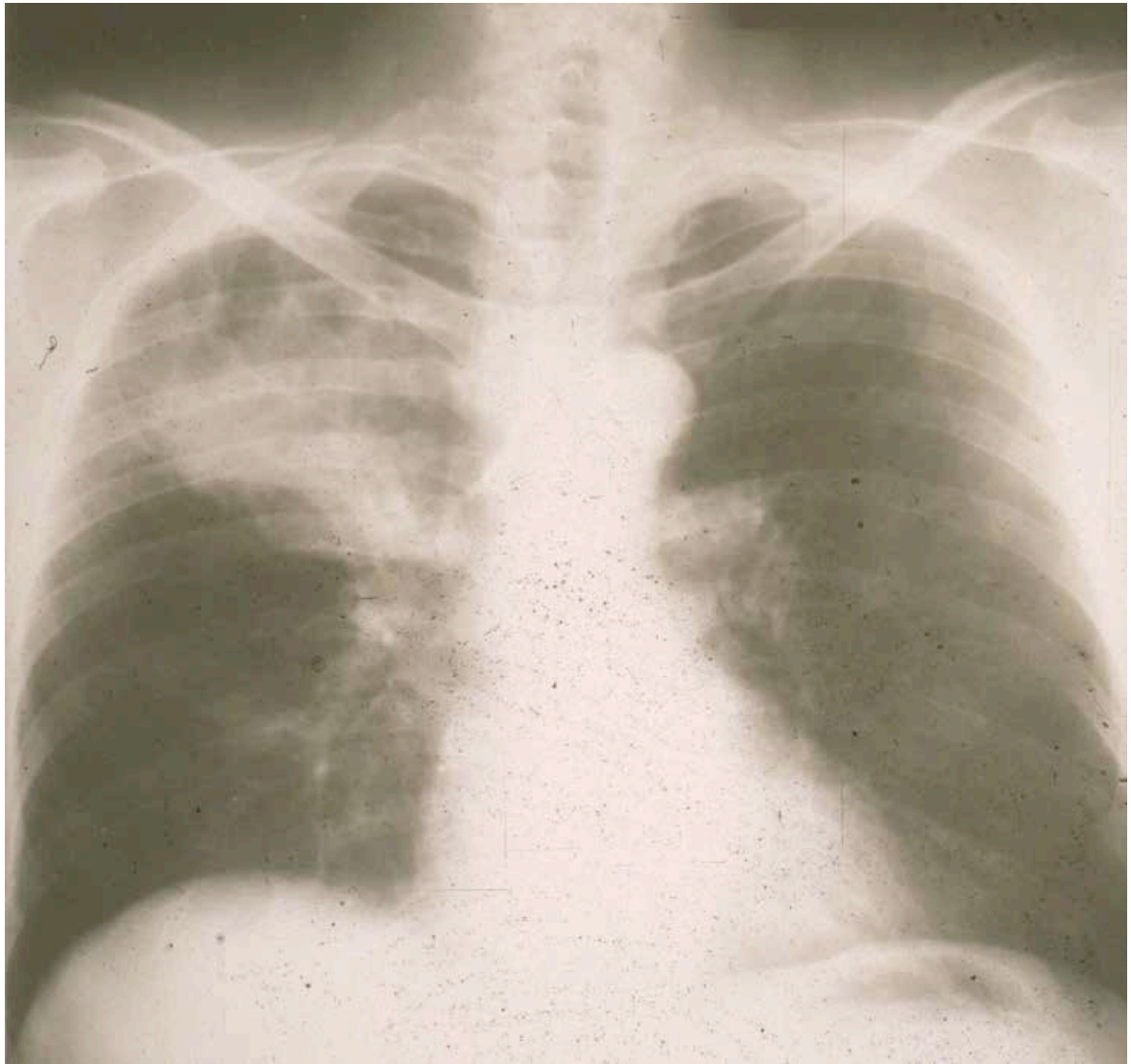
HIV-pos

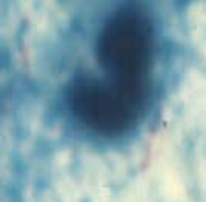
CXR-neg

7% per yr

Cavitary Latent Tuberculosis

Reactivation Tuberculosis





Immunity to Tuberculosis

Latency maintained by Th1 cell immunity

- **CD4>CD8; IL-12, IFN-γ, TNF**

Genetic risks (environmental mycobacteria, TB, salmonella)

- **IFN-γR, IL-12 p40, IL-12Rβ1, Stat1 mutations**

Acquired risks

- **HIV, anti-TNF, immunosuppression, IFN-γ autoantibodies (SE Asia)**

Candida albicans – scope of the problem

Aerobic eukaryote – 16 megabase haploid genome/~6600 genes

Commensal of human skin, mucosa

Opportunistic pathogen – most common fungal infection of humans; 4th most common bloodstream infection in US hospitals; >$1 billion in added healthcare costs

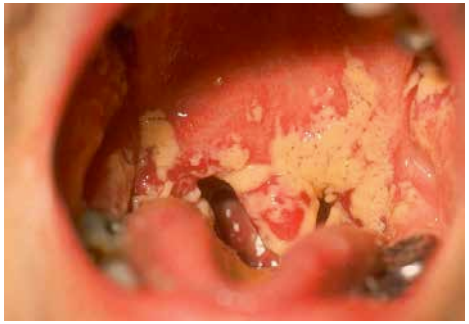
Predisposing conditions – antibiotics, barrier dysfunction (burns), neutrophil deficits, neutropenia, prosthetics/catheters, GI surgery, immunosuppression

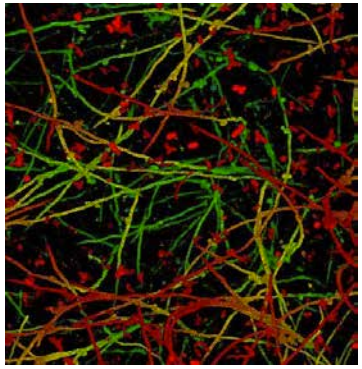
Related to capacity to form biofilms
Invades in setting of intense immunodeficiency (BM Tx)
Neither specific for Candida

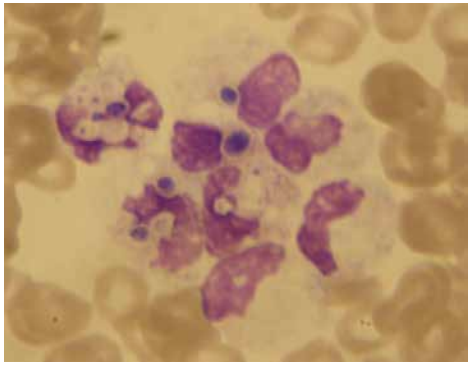
Genetic accidents – chronic mucocutaneous candidiasis

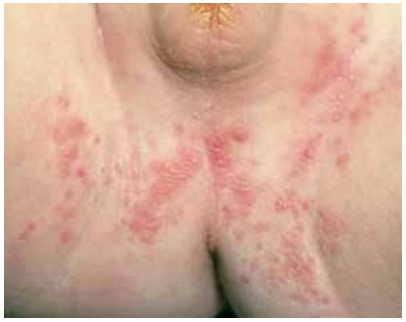
Peculiarly Candida-specific

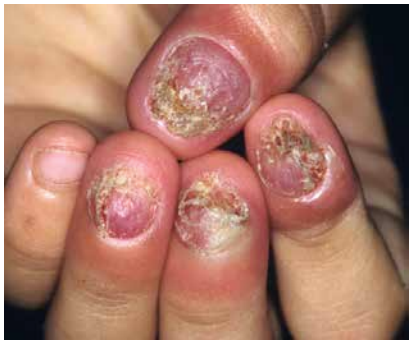
Spectrum of Candidiasis in humans











Cutaneous

Antibiotics
Barrier dysfunction
PMN deficits
(MBL deficiency)

Mucocutaneous

T cell deficiency (HIV)
APCED (AR Aire)

Invasive

Catheters/prostheses
GI perforation/surgery
Anti-TNF
Neutropenia
T cell deficiency
BM Tx

Systemic

Candida specificity: **+++**

APCED/APS-1

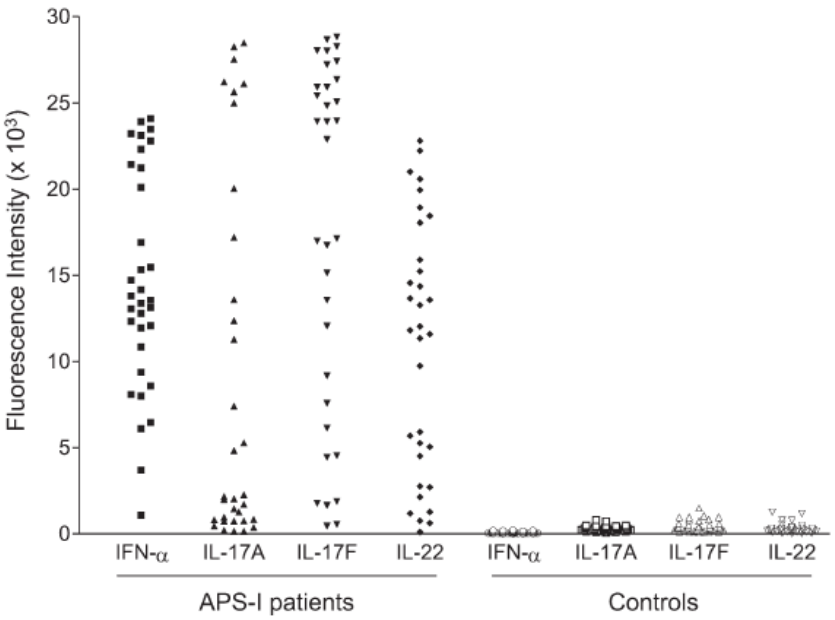
Rare autosomal recessive sydrome due to AIRE mutations. Autoimmune polyendocrinopathy with candidiasis and ectodermal dysplasia/autoimmune polyendocrine syndrome type 1. Early onset CMC (90%), hypoparathyroidism, adrenal failure common. High anti-type I IFN Ab's described in 2006, but no CMC in pts with deficits in type 1 IFN pathway (Stat1, NEMO, UNC-93B, TLR3). Pts with CMC have high-titer neutralizing Ab's to IL-7A, IL-7F, IL-22. Two patients with thymoma and CMC also had high titer anti-IL17 Abs.

Table I. APECED patients whose PBMCs were tested for cytokine responses

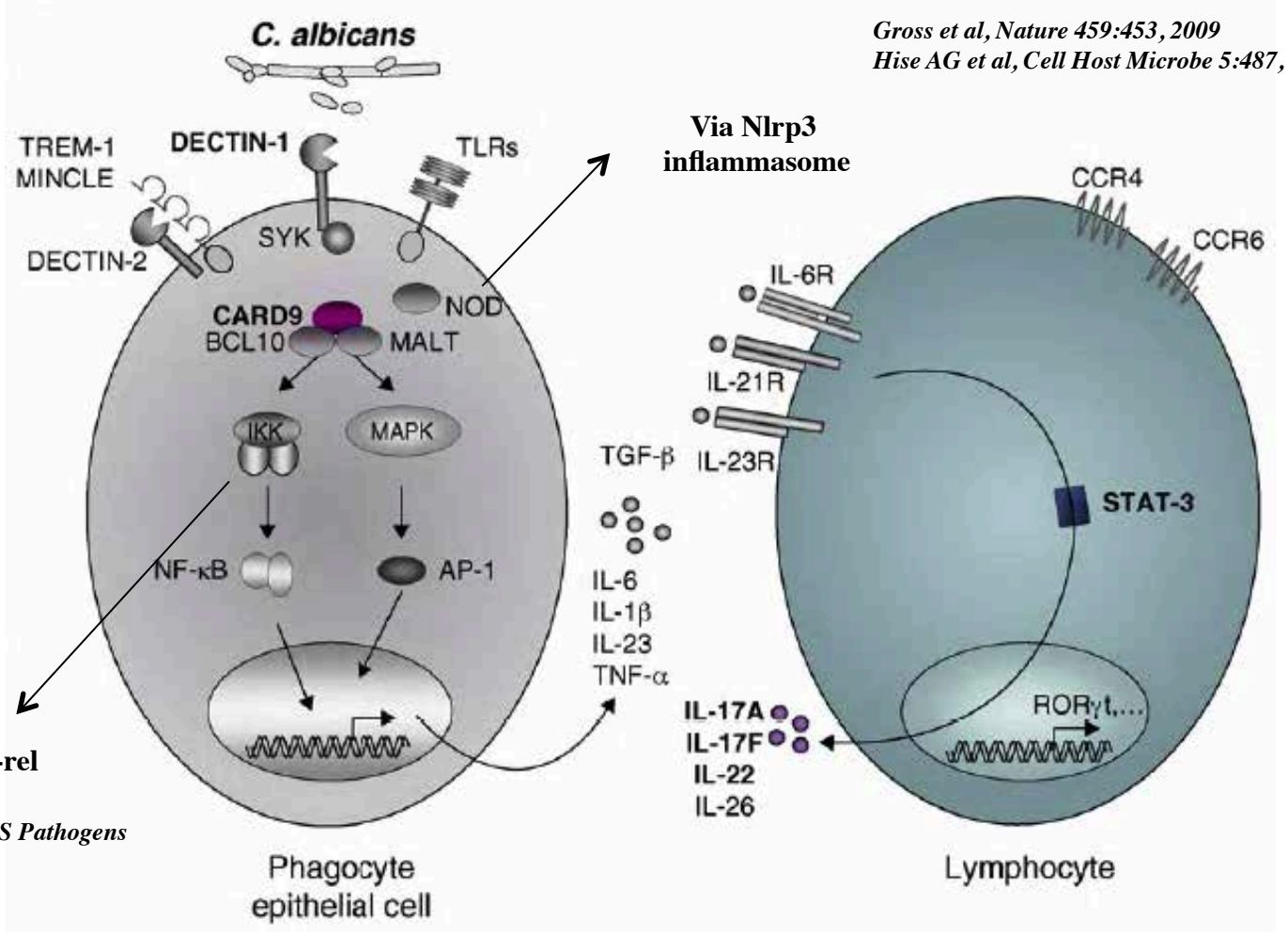
Patient	*AIRE* mutation	Age	CMC	FACS sample	Sample at 72 h	Neutralizing antibody titer against:				Binding antibodies against: [a]		
						IFN-ω	IL-17A	IL-17F	IL-22	IL-17A	IL-17F	IL-22
		yr										
A	c.[769C>T] + [769C>T][b]	29	Yes	No	No	20,000	<32	38	9,000	0.12	3.11	0.17
B[c]	c.[21_43dup23] + [21_43dup23]	10	Yes	Yes	Yes	>51,200	250,000	75	9,000	3.85	2.43	0.55
C	c.[769C>T] + [769C>T]	17	Yes	Yes	Yes	>51,200	<32	56	5,500	0.07	1.30	0.28
D	c.[1064-1068dupCCCGG] + [1064-1068dupCCCGG]	18	Yes	Yes	Yes	>51,200	<32	1,250	>525,000	0.83	3.64	1.81
E	c.[769C>T] + [769C>T]	18	Yes	No	No	>51,200	<32	350	160,000	0.70	3.52	1.01
F	c.[769C>T] + [769C>T]	23	Yes	No	Yes	20,000	15,000	1,300	95,000	3.90	3.90	0.79
G	c.[769C>T] + [769C>T]	60	Yes	Yes	Yes	12,800	<32	300	13,000	0.39	3.89	0.64
H	c.[769C>T] + [967_979del13]	20	Yes	No	Yes	100,000	<32	5,500	4,500	2.98	3.90	0.25
I	c.[967_979del13] + [1163_1164insA]	24	Yes	No	Yes	256,000	nd	9,200	3,300	0.09	3.90	0.56
J	c.[879 + 1G>A] + [879 + 1G>A]	48	No	Yes	No	30,000	<32	<32	<32	0.30	0.29	0.18
K[c]	c.[967_979del13] + [967_979del13]	21	No	Yes	Yes	>51,200	<32	<32	<32	0.17	0.40	0.24
L	c.[769C>T] + [c.274C>T]	55	No	No	Yes	12,000	<32	<32	<32	0.04	0.03	0.16
M	c.[967_979del13] + [c.274C>T]	29	No	Yes	Yes	200,000	<32	<32	930	0	0.03	0.21
N[d]	c. [682T>G] + [=]	40	No	Yes	Yes	9,000	<32	<32	<32	0.01	0.16	ND

Kisand K et al, J Exp Med 207:299, 2010

Ahlgren KM et al, Eur J Immunol 41:235, 2011



Activation of carbohydrate-recognition pathways leads to IL-1β, IL-23p19 and creates conditions favoring IL-17 production and Th17 differentiation *in vitro*



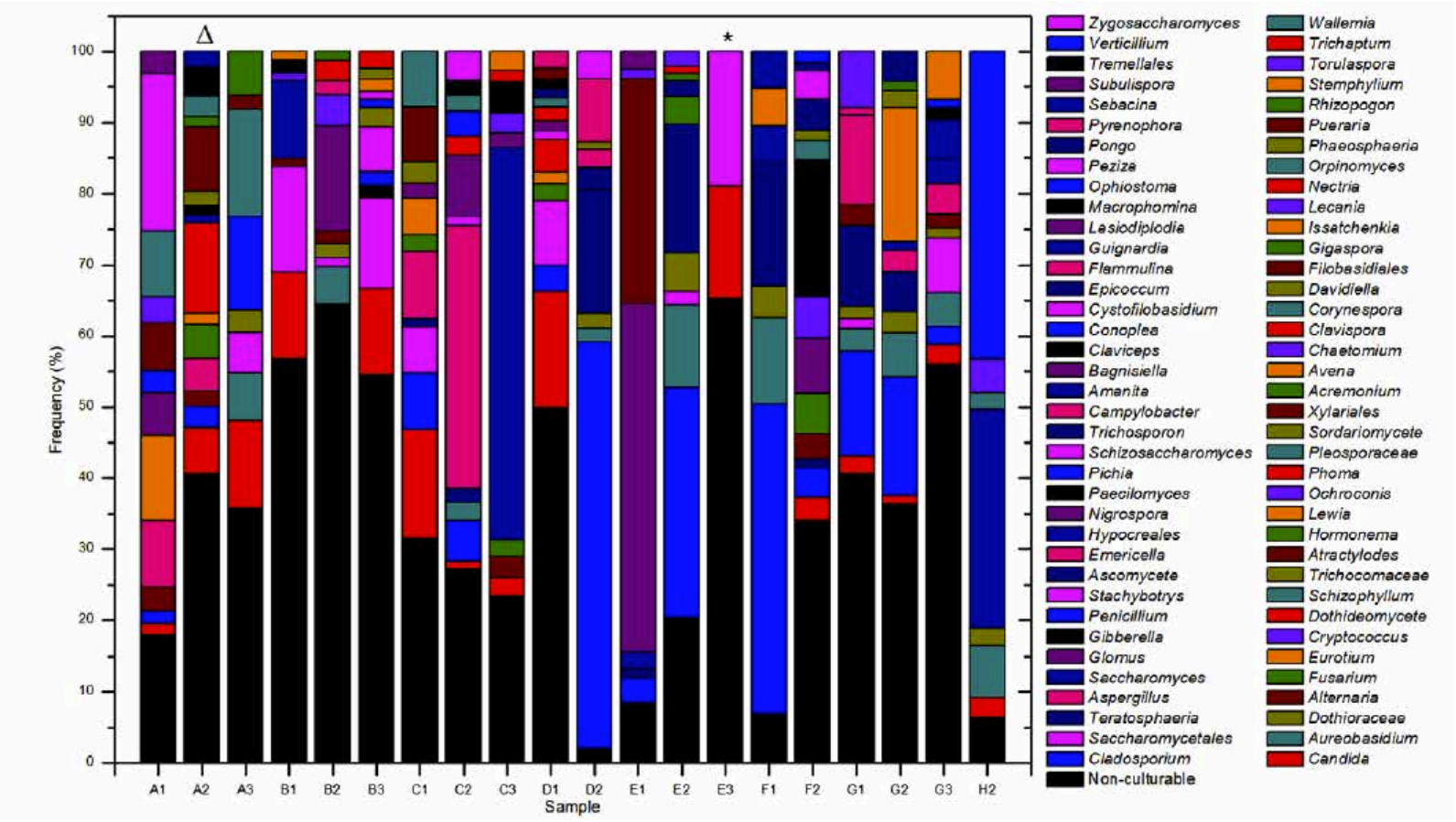
Gross et al, Nature 459:453, 2009
Hise AG et al, Cell Host Microbe 5:487, 2009

Gringhuis SI et al, PLOS Pathogens 7:e1001259, 2011

Puel A et al, Curr Opin Immunol 22:467, 2010

The human oral ‘Mycobiome’ is complex – why only Candida?

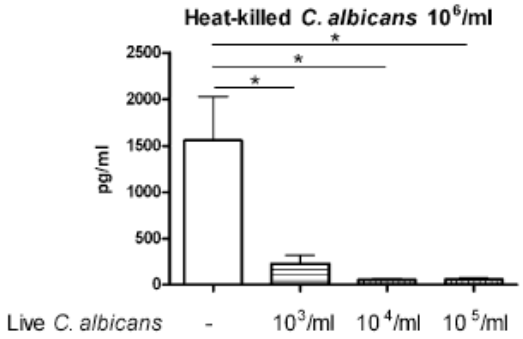
Ghannoum MA et al, PLOS Pathogens6:e1000713, 2010



Candida albicans Dampens Host Defense by Downregulating IL-17 Production

Shih-Chin Cheng, Frank van de Veerdonk, Sanne Smeekens, Leo A. B. Joosten, Jos W. M. van der Meer, Bart-Jan Kullberg, and Mihai G. Netea

IL-17 is one of the key cytokines that stimulate host defense during a *Candida* infection. Several studies have demonstrated the capacity of *Candida albicans* to induce a Th17 response. Surprisingly, experiments employing live *C. ablicans* demonstrated a specific downregulation of host IL-17 secretion in human blood mononuclear cells (PBMCs). By avoiding the direct contact of live *C. albicans* and PBMCs, we demonstrate that this inhibition effect is mediated by a soluble factor released by live *C. albicans*. However, this effect is due neither to the releasing of *C. albicans* pathogen-associated molecular patterns nor to the alteration of different Th cell subtypes. Rather, we found that live *C. albicans* shifts tryptophan metabolism by inhibiting IDO expression away from kynurenines and toward 5-hydroxytryptophan metabolites. In addition, we show that these latter 5-hydroxytryptophan metabolites inhibit IL-17 production. In conclusion, live *C. albicans* inhibits host Th17 responses by modulatory effects on tryptophan metabolism. *The Journal of Immunology*, 2010, 185: 2450–2457.



A different way to think about infectious diseases – frustrated commensalism