Complex Pathogens, Helminths and Immunology

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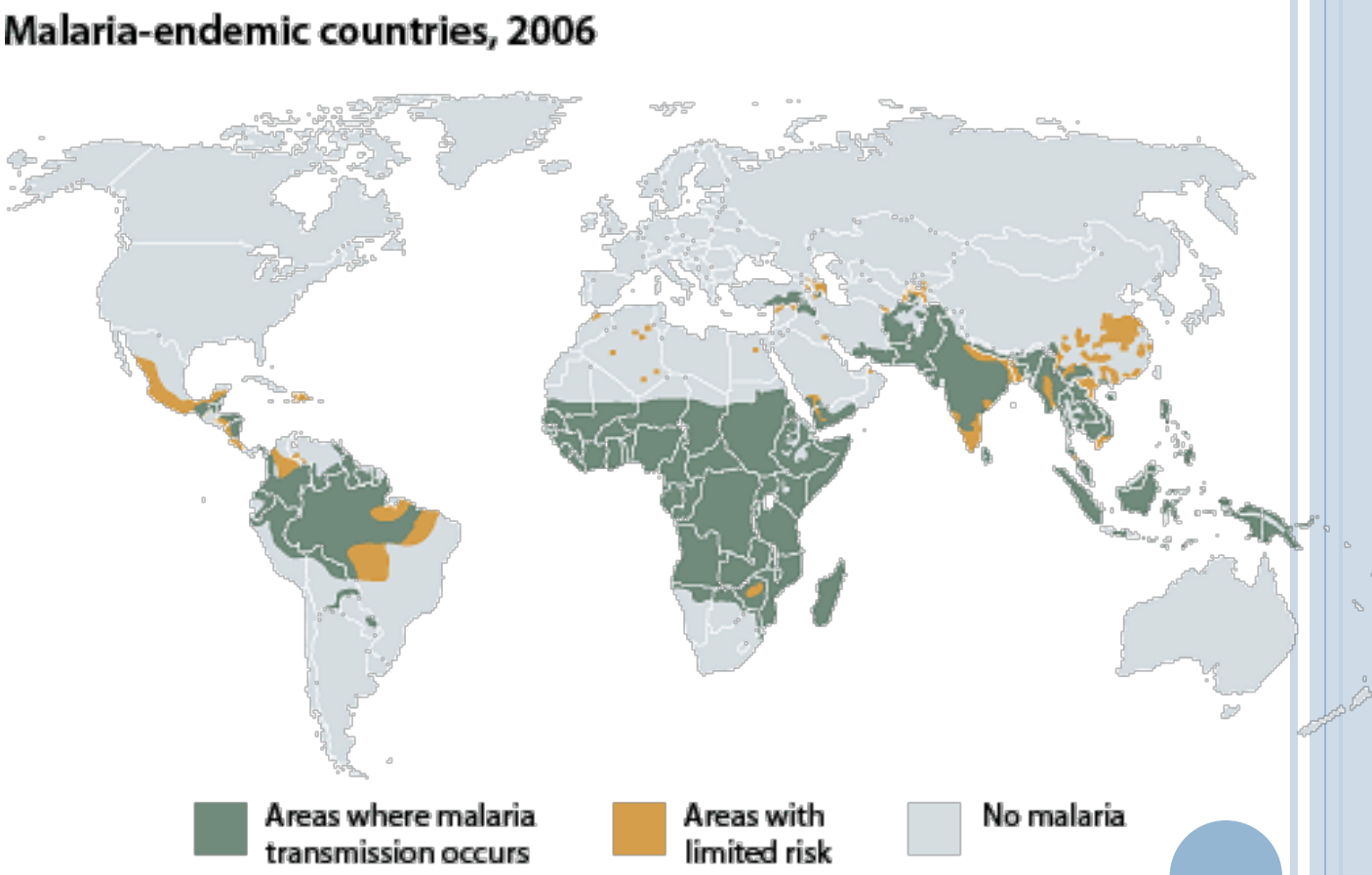
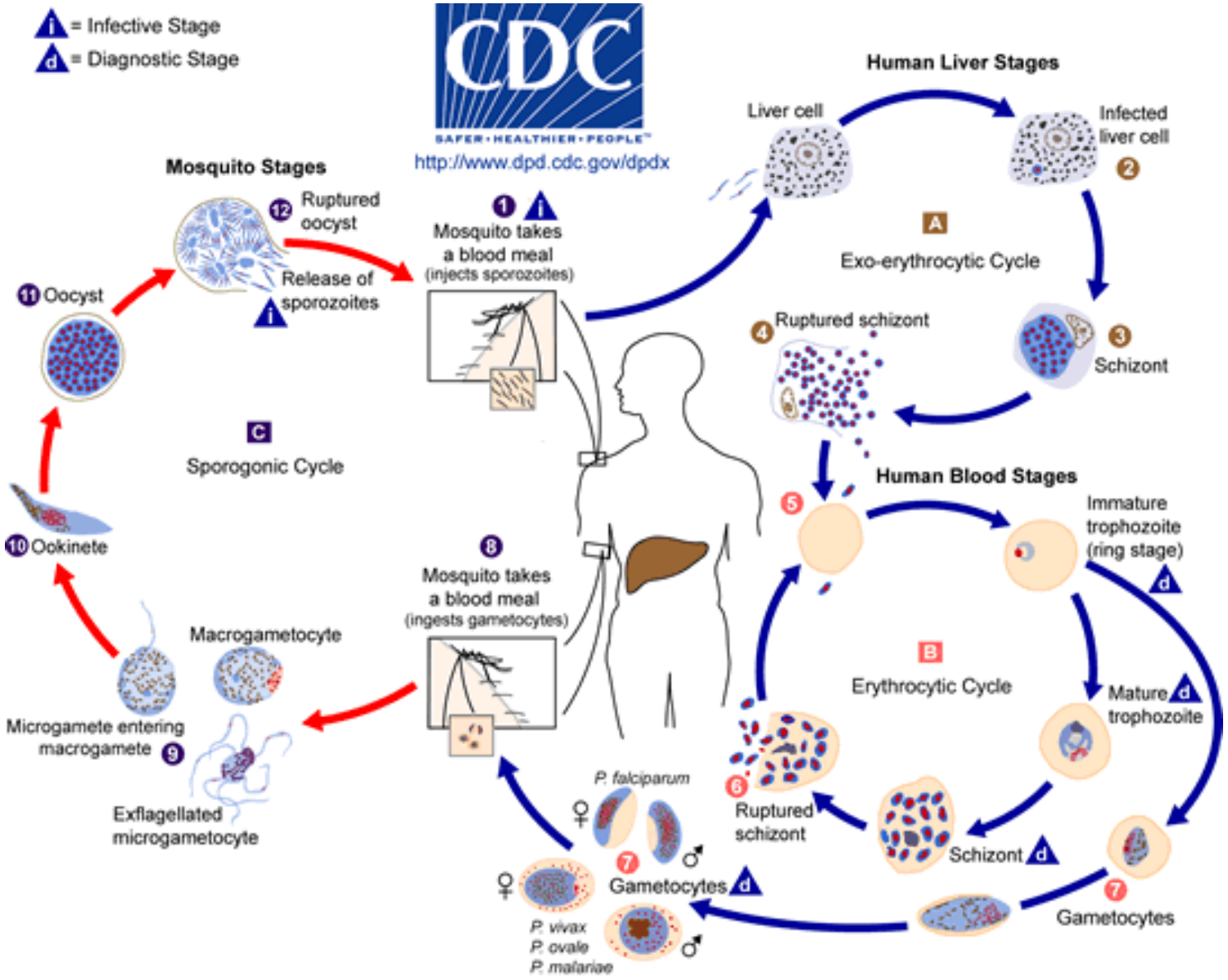
Malaria Prevalence

- ~350-500 million infections/year
- 1-2 million deaths/year, most of them among children under 5



Source: International travel and health 2008 page. World Health Organization website. Available at: www.who.int/ith/en/. Accessed July 31, 2008.

Malaria life cycle



Immune Responses mediate plasmodium pathology

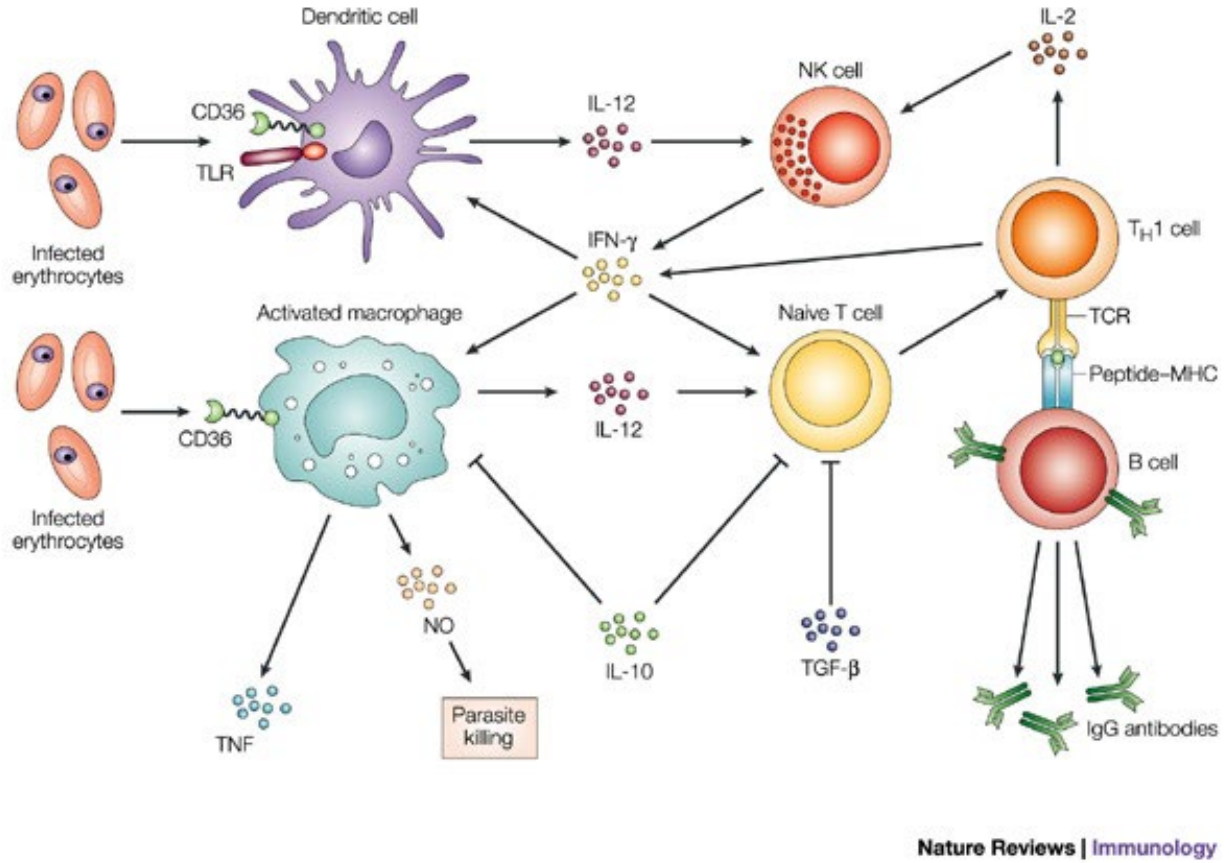
Table 2 | **Malaria products and their bioactivities**

Parasite product	Receptor and cell type	Pathological and cellular effects
Plasmodium falciparum EMP1-family members	ICAM1, VCAM1, CD36, thrombospondin, E-selectin, chondroitin sulphate A, hyaluronic acid and CD31 on endothelial cells and trophoblast cells; CD36 on DCs	Binding directs parasite to the brain, placenta and possibly other target organs; CD36 engagement proposed to suppress DC and macrophage activation
GPI	TLR2, TLR4 and/or possibly C-type lectins on several cell types, including DCs, macrophages, endothelial cells and adipocytes; CD1d and Vα14–Vβ8 TCR on NKT cells	Induces widespread expression of genes encoding pro-inflammatory proteins (including TNF, IL-1, IL-6, IL-12, iNOS, ICAM1, VCAM1); activates NKT cells; induces T_H1- or T_H2-cytokine production
Haemozoin	TLR9 on DCs	Contradictory reports: both T_H1- and T_H2-cell activities; induces and inhibits DCs; suppresses macrophages; induces IL-10 production; broadly immunosuppressive
Unknown ligands	NKC-encoded receptors on NK and NKT cells	Activates NK cells; induces IFN-γ production; regulates balance of T_H1 and T_H2 cytokines produced by NKT cells
Isopentenyl pyrophosphate	γδ TCRs	Activates γδ T cells; induces IFN-γ production
Protein antigens	Diverse TCRs on $CD4^+$ and $CD8^+$ T cells	Activates αβ T cells; induces T_H1- or T_H2-cytokine production
Unknown sugar(s)	MBL in plasma	Possible binding provides protection; low levels of MBL are associated with disease

DC, dendritic cell; EMP1, erythrocyte membrane protein 1; E-selectin, endothelial-cell selectin; GPI, glycosylphosphatidylinositol; ICAM1, intercellular adhesion molecule 1; IFN-γ, interferon-γ; IL, interleukin; iNOS, inducible nitric-oxide synthase; MBL, mannose-binding lectin; NK, natural killer; NKC, natural killer complex; NKT, natural killer T; TCR, T-cell receptor; T_H, T helper; TLR, Toll-like receptor; TNF, tumour-necrosis factor; VCAM1, vascular cell-adhesion molecule 1.

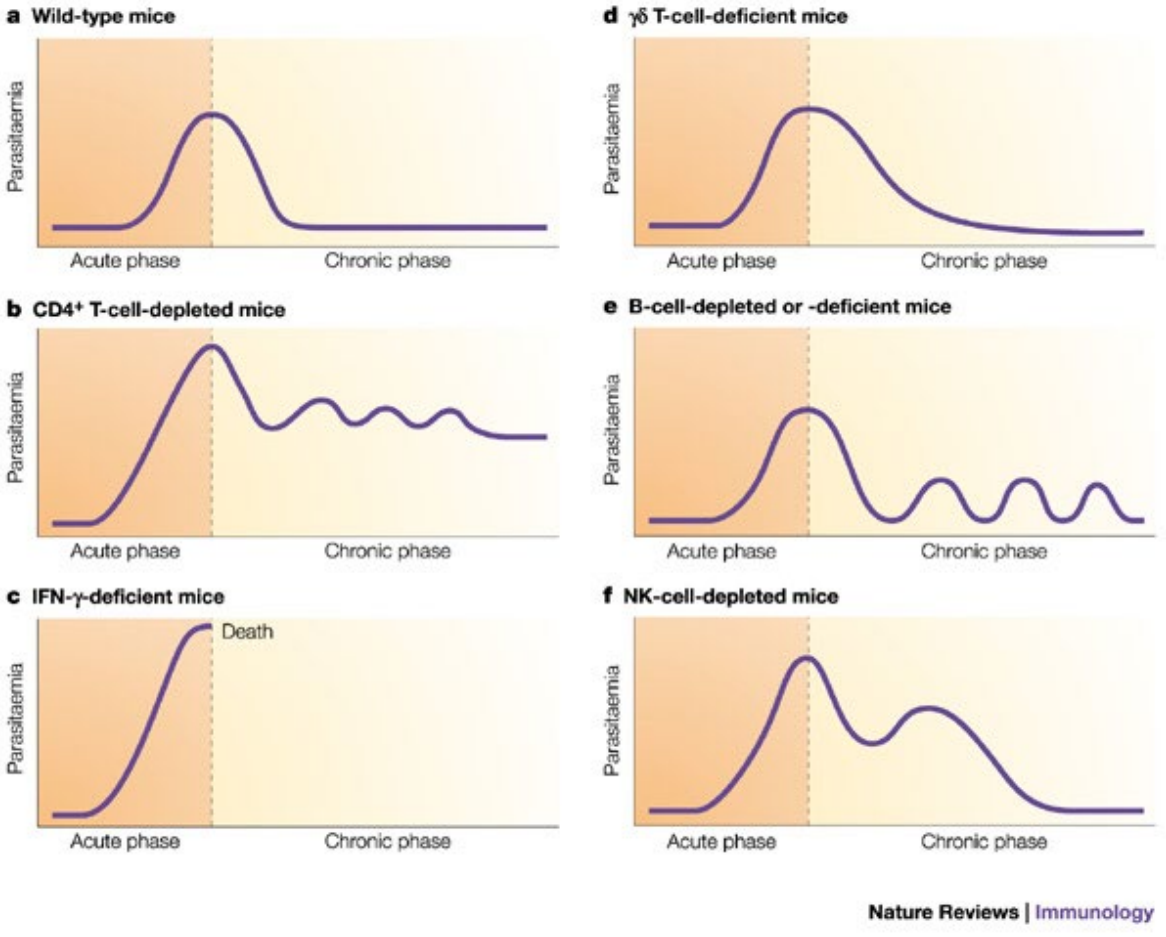
Typical "Th1-Type" immunity contributes to malaria protection and pathology

- In infected mice, antigen is presented in the spleen where Th1 cells regulate innate and adaptive immune responses, including stimulating anti-parasite antibody and effector mechanisms such as ROI and RNI



IMMUNE MECHANISMS TO CONTROL MALARIA

- Antibodies block invasion of sporozoites into liver cells
- Interferon- (IFN-) and $CD8^+$ T cells inhibit parasite development in hepatocytes
- Antibodies block invasion of merozoites into erythrocytes
- Antibodies prevent sequestration of infected erythrocytes by preventing binding to adhesion molecules on the vascular endothelium
- IFN- and $CD4^+$ T cells activate macrophages to phagocytose intra-erythrocytic parasites and free merozoites
- Antibodies neutralize parasite glycosylphosphatidylinositol and inhibit induction of the inflammatory cytokine cascade
- Antibodies mediate complement-dependent lysis of extracellular gametes, and prevent fertilization of gametes and the development of zygotes



Mary M. Stevenson & Eleanor M. Riley
Nature Reviews Immunology 4, 169-180 (March 2004)

Fatal Disease in malaria infection

Table 1 | **Severe and fatal disease syndromes in malaria**

Syndrome	Clinical features	Possible sequence or mechanism of disease
Cerebral malaria	Sustained impaired consciousness, coma, long-term neurological sequelae	Cerebral parasite sequestration; bioactive GPI; pro-inflammatory cytokine cascade; endothelial-cell activation; natural killer T-cell activation; T_H1/T_H2-cell balance; chemokine production; monocyte, macrophage and neutrophil recruitment; platelet and fibrinogen deposition; $CD4^+$, $CD8^+$ and γδ T-cell involvement; IFN-γ production; neurological metabolic derangements; possibly hypoxia
Placental malaria	Placental insufficiency, low birth weight, premature delivery, loss of fetus	*Plasmodium falciparum* EMP1-mediated binding to placental endothelium and syncytiotrophoblast through chondroitin sulphate A and hyaluronic acid; cytokine production; chemokine-mediated recruitment and infiltration of monocytes; intravascular macrophage differentiation
Severe malarial anaemia	Pallor, lethargy, haemoglobin level of 4–6 g per 10 ml	Erythropoietic suppression by toxins and cytokines; increased RBC destruction, owing to parasitization, RBC alterations, complement and immune complex or antigen deposition, erythrophagocytosis, splenic hyperphagism, $CD4^+$ T cells, T_H1/T_H2 cytokine balance (TNF and IFN-γ versus IL-10)
Metabolic acidosis	Respiratory distress, deep breathing (Kussmaul breathing), hypovolaemia	Molecular mechanisms unknown. Possibly widespread parasite sequestration; bioactive toxins; increased vascular permeability; reduced tissue perfusion; anaemia; pulmonary airway obstruction; hypoxia; increased host glycolysis; repressed gluconeogenesis. Some overlap with shock-like syndrome
Shock-like syndrome (systemic inflammatory-response-like syndrome)	Shock, haemodynamic changes, impaired organ perfusion, disseminated intravascular coagulation	Bioactive toxins; T_H1 cytokines; acute-phase reactants

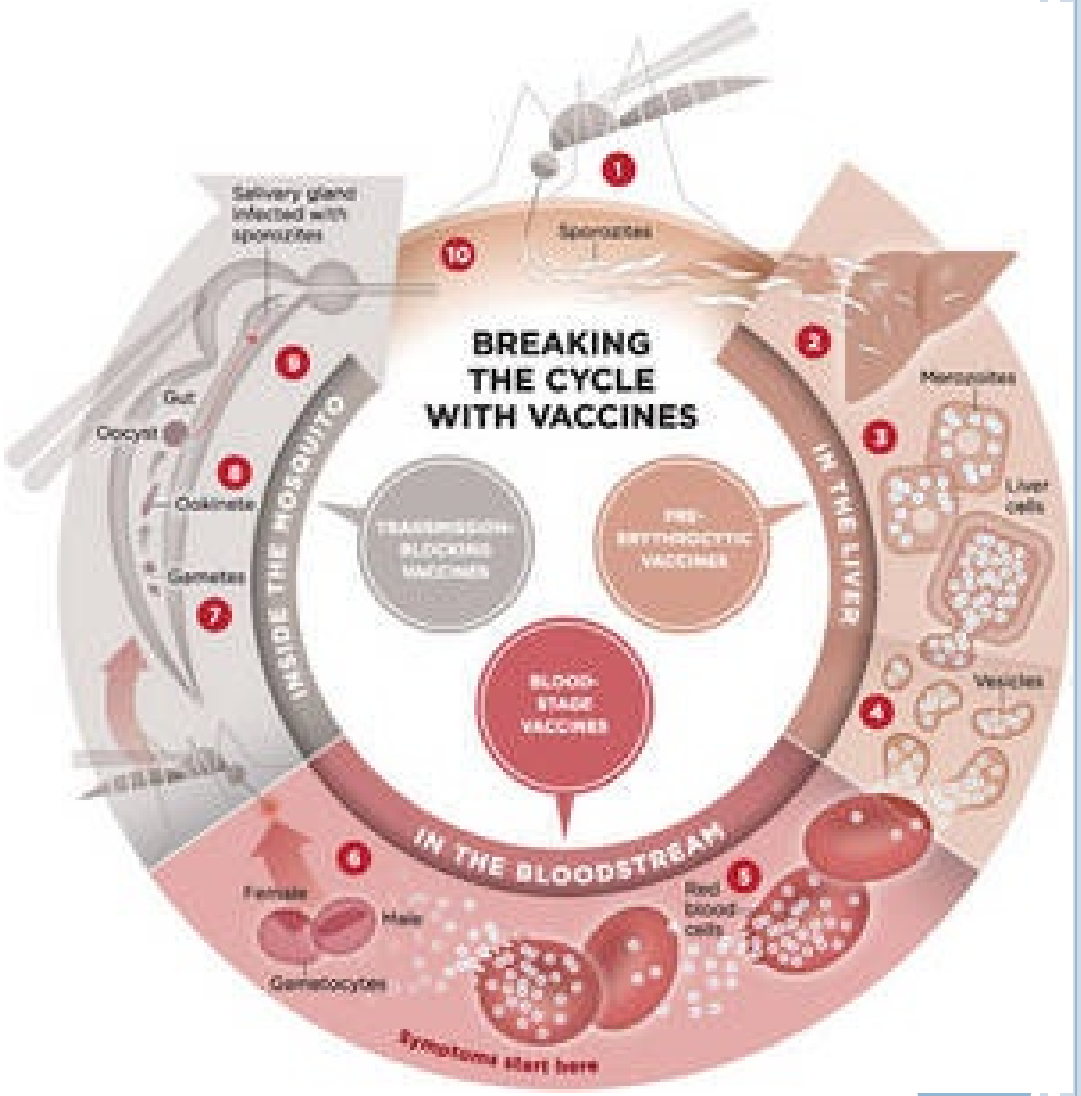
EMP1, erythrocyte membrane protein 1; GPI, glycosylphosphatidylinositol; IFN-γ, interferon-γ; IL-10, interleukin-10; RBC, red blood cell; T_H, T helper; TNF, tumour-necrosis factor.

Vector Control

- Vector control efforts range from basic bed nets, to spraying insecticides externally and on house walls, to more sophisticated "vector engineering" efforts to produce malaria-resistant mosquitoes, among many others
- Math modeling of infectious spread has led to some hypotheses about which of these methods are the most effective (bed nets, house wall spraying) and which are unlikely to be effective (releasing resistant mosquitoes)

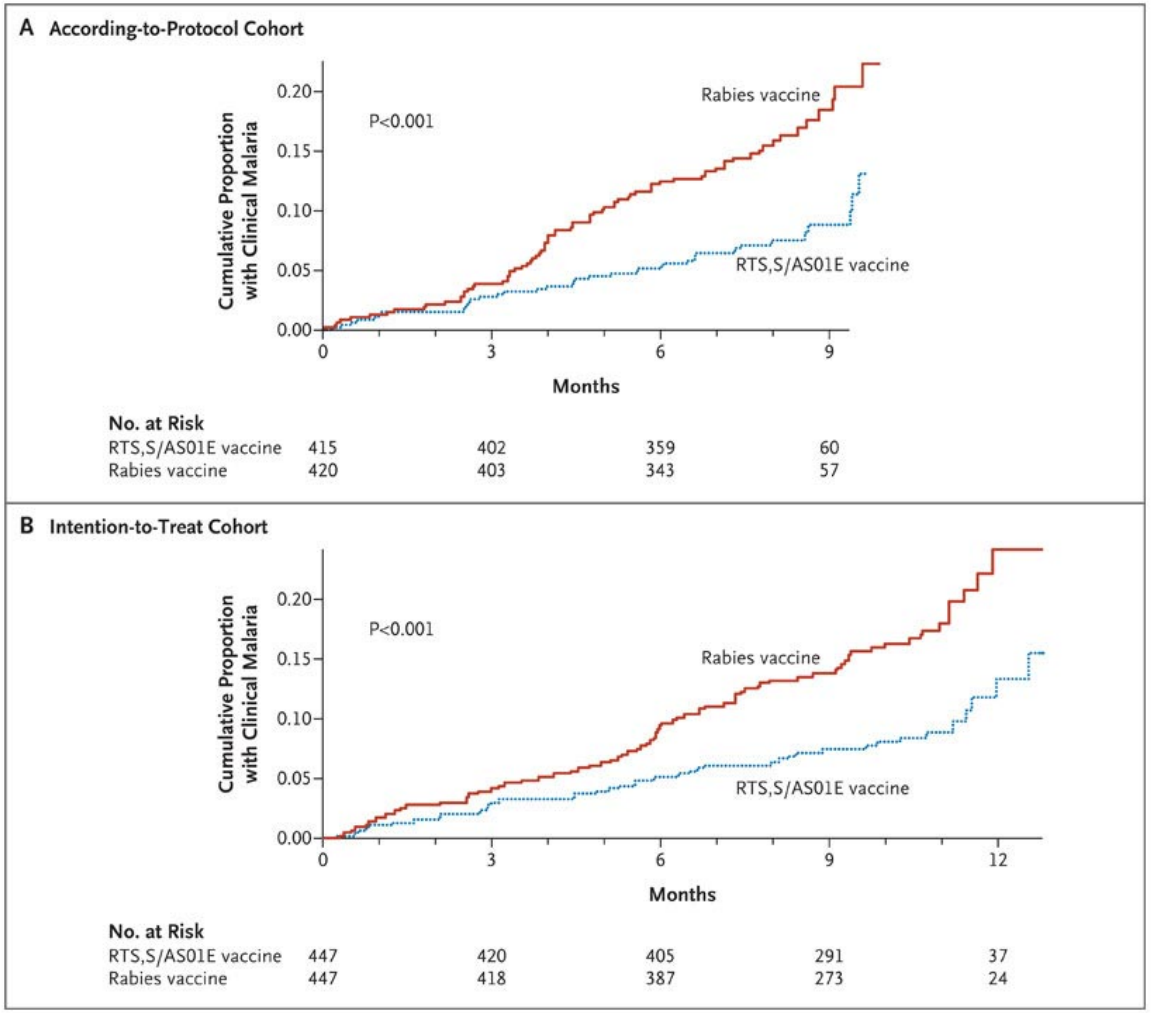
Malaria Vaccine Targets

- Three types of vaccines have been proposed, with variations in each group:
  - Pre-erthyrocytic vaccines: the only truly "sterilizing" protection, but hard to generate enough antibody immunity (to prevent any infection) or CD8 immunity (to clear every single infected liver cell)
  - Blood-stage vaccine: designed to enhance clearance of infected red blood cells, therapeutic but not sterilizing
  - Gametocyte vaccines: Potentially strong antigen candidates and immune complexes can be carried to the mosquito—"altruistic vaccine"



RTS,S Vaccine Approved for Stage III in Infants

- The vaccine candidate farthest along is RTS,S, pre-erythrocytic vaccine against the circumsporozite protein
- Mechanism is presumed to be antibody, but cellular responses have been shown
- Vaccine is adjuvanted and protein is linked to hepatitis B antigen



RTS,S SHOWED MODEST EFFICACY IN INFANTS

- ~30% efficacy shown in latest trial
- Generally viewed as disappointing, but still moving forward (previous trial had ~61% efficacy, but was much smaller and in a different transmission area)

N ENGL J MED 367;24 NEJM.ORG DECEMBER 13, 2012

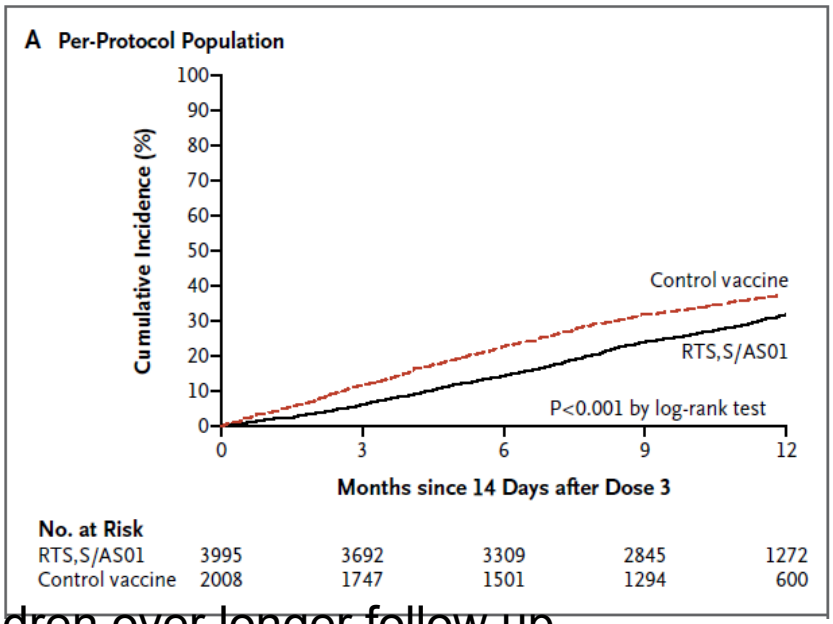
The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

A Phase 3 Trial of RTS,S/AS01 Malaria Vaccine in African Infants

The RTS,S Clinical Trials Partnership

ABSTRACT



Late 2013 results—47% efficacy in children over longer follow up

2019—target rollout in 3 sub-Saharan countries for childhood vaccination

Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial

RTS,S Clinical Trials Partnership*

www.thelancet.com Published online April 24, 2015 http://dx.doi.org/10.1016/S0140-6736(15)60721-8

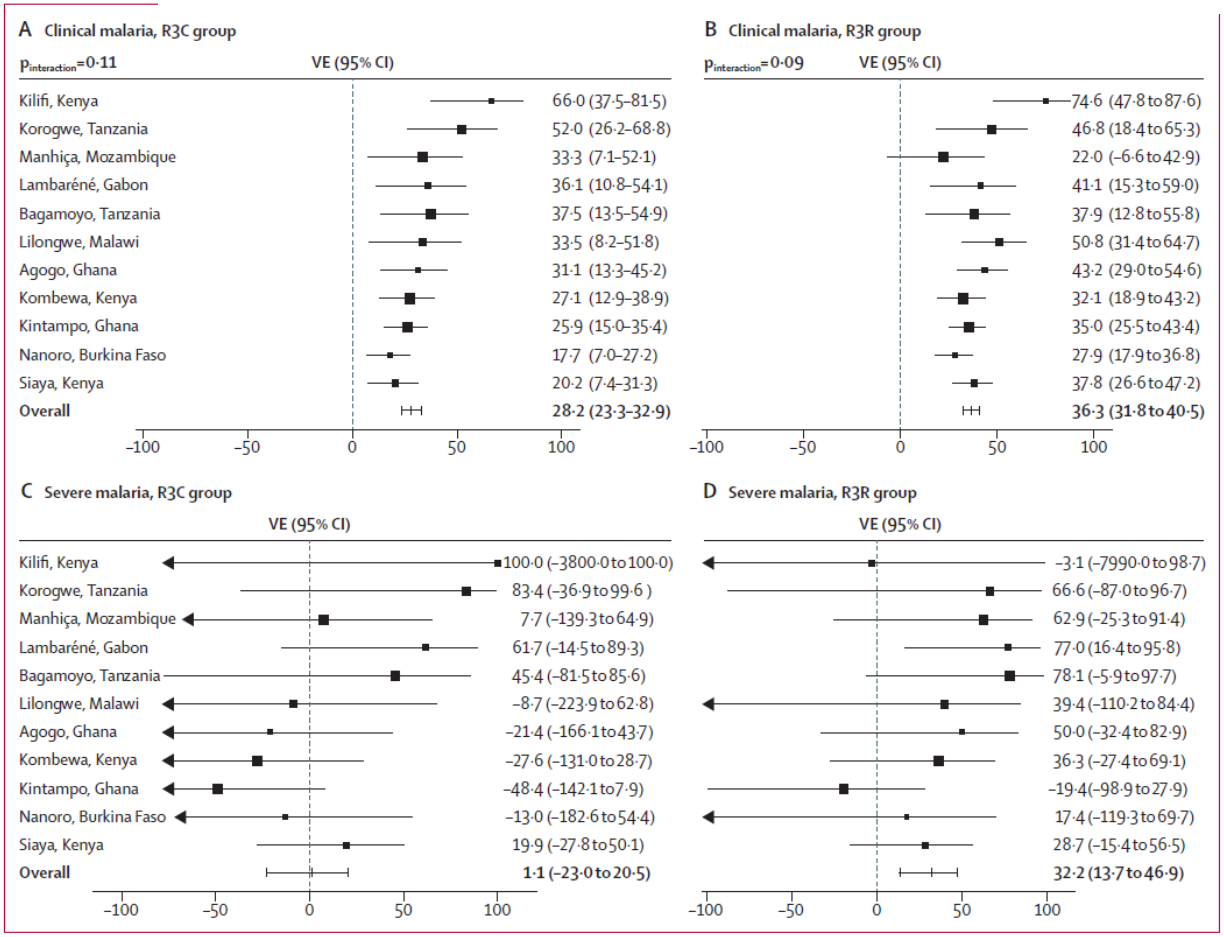


Figure 3: **Vaccine efficacy against clinical and severe malaria by study site in the 5–17 months age category**

VE against all episodes of clinical malaria (primary case definition) in (A) the R3C group and (B) the R3R group from month 0 to study end; and VE against severe malaria (primary case definition) in (C) the R3C group and (D) the R3R group from month 0 to study end. Study sites are ordered from lowest (Kilifi) to highest (Siaya) incidence of clinical malaria (secondary case definition) measured in control infants 6–12 weeks of age at enrolment during 12 months of follow-up. $p_{interaction}$ not calculated for (C) or (D). Analyses were by modified intention to treat. Bars are 95% CIs. The size of each square is proportional to the number of participants enrolled at each study site. The following numbers of children aged 5–17 months were enrolled by site for all three groups (R3R, R3C, and C3C) together: 600 in Kilifi, 912 in Korogwe, 1002 in Manhiça, 704 in Lambaréné, 903 in Bagamoyo, 800 in Lilongwe, 600 in Agogo, 1000 in Kombewa, 1002 in Kintampo, 600 in Nanoro, and 799 in Siaya. R3C=RTS,S/AS01 primary schedule without booster. C3C=control group. R3R=RTS,S/AS01 primary schedule with booster. VE=vaccine efficacy.

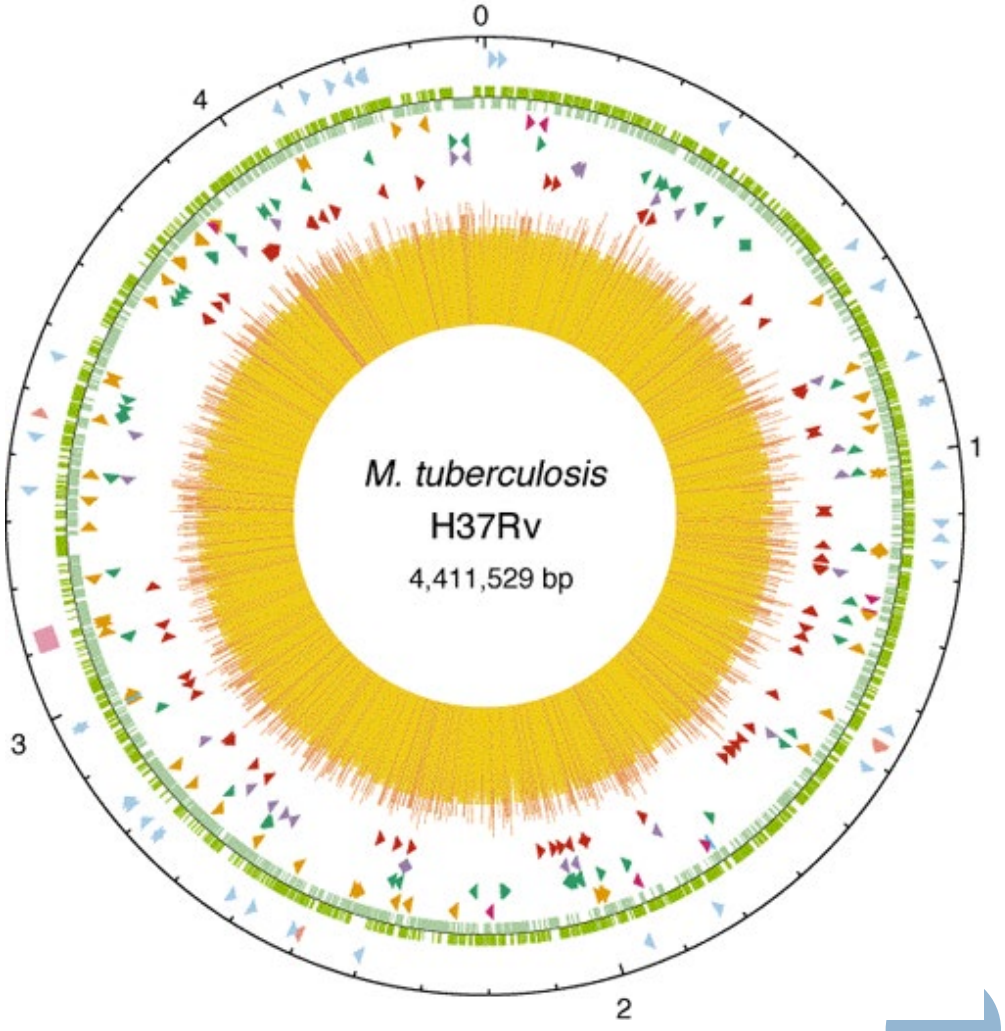
Math Modeling and Malaria

- Transmission models have contributed substantially to the understanding of malaria control
- Within host modeling is crucial to determine the potential efficacy of the three types of vaccine candidates
- The "threshold" effects of malaria infection (immunity is helpful in endemic regions, but requires frequent low grade re-infection) are particularly suited to a quantitative approach

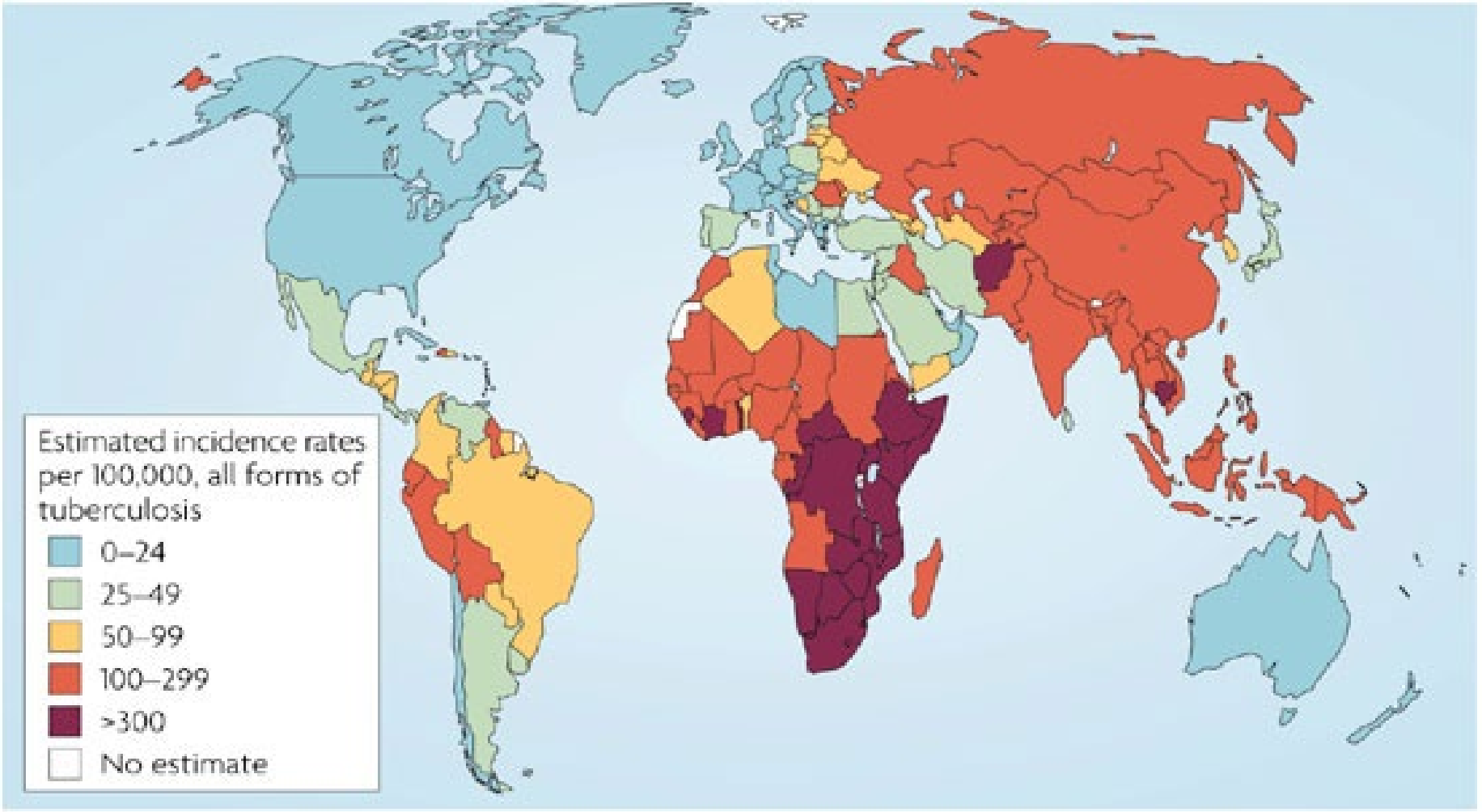
Immune Control of *Mycobacterium tuberculosis*

Mycobacterium Tuberculosis (Mtb)

- Acid-fast, rod-shaped bacillus
- Unique wax-rich cell wall composed of long chain fatty acids and glycolipids
- 250 genes dedicated to fatty-acid metabolism
- Slow, 20 hour replication time



MTB INFECTION WORLDWIDE



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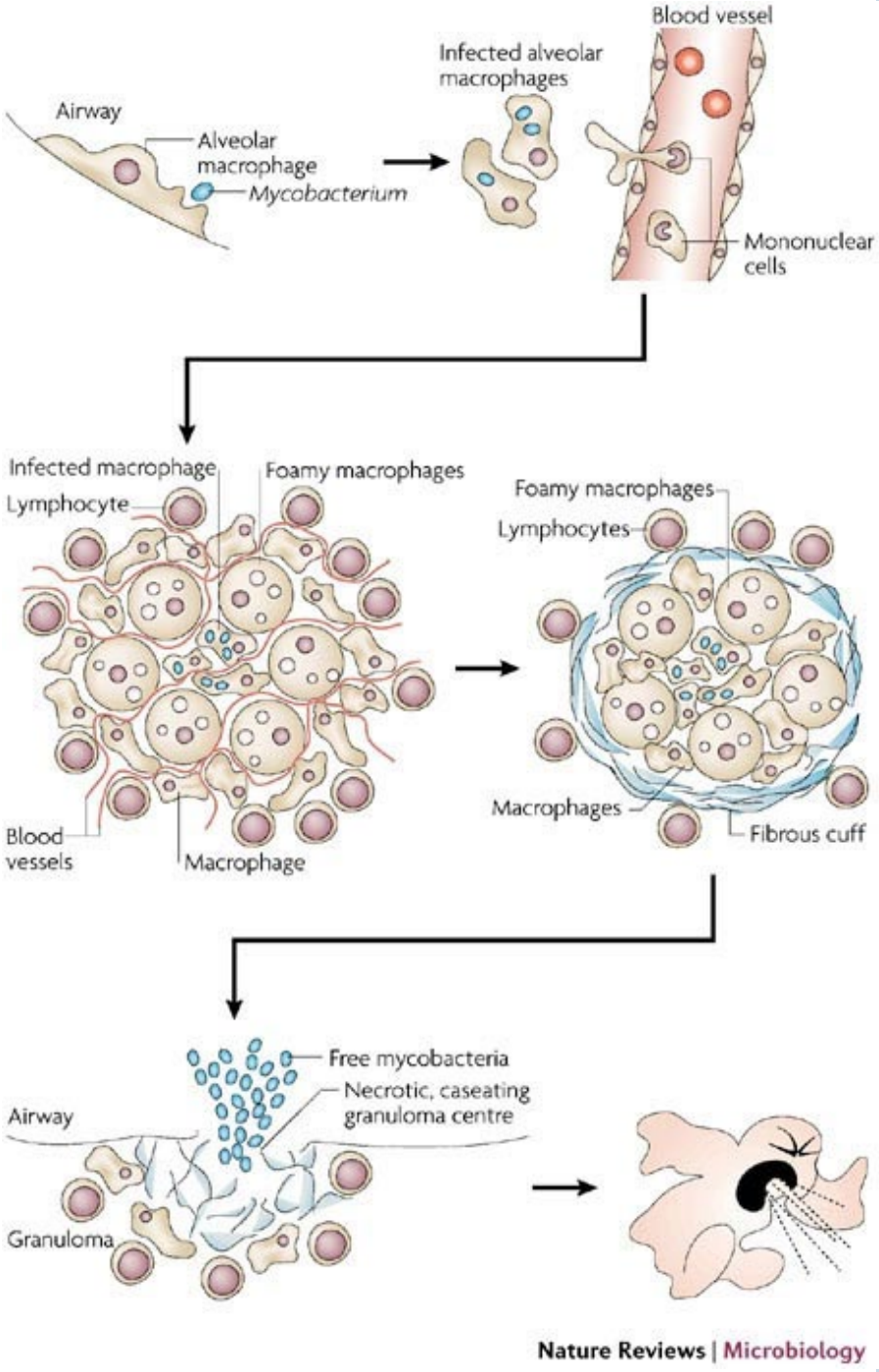
MTB IMPACT

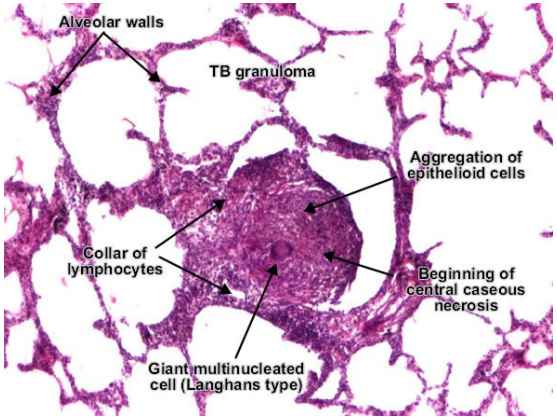
- 2.2 million deaths/year
- Burden of diseases in DALY (disability-adjusted life years)
- Total Disability Adjusted Life Years: 45 million (3.1%).
- 2 billion individuals infected with M. tuberculosis
  - 10% risk of developing disease following infection
  - Untreated, disease mortality is 50%
- 8 million new tuberculosis cases per year (1 new case every 4 seconds)
- 10–15 individuals infected annually by a single untreated patient

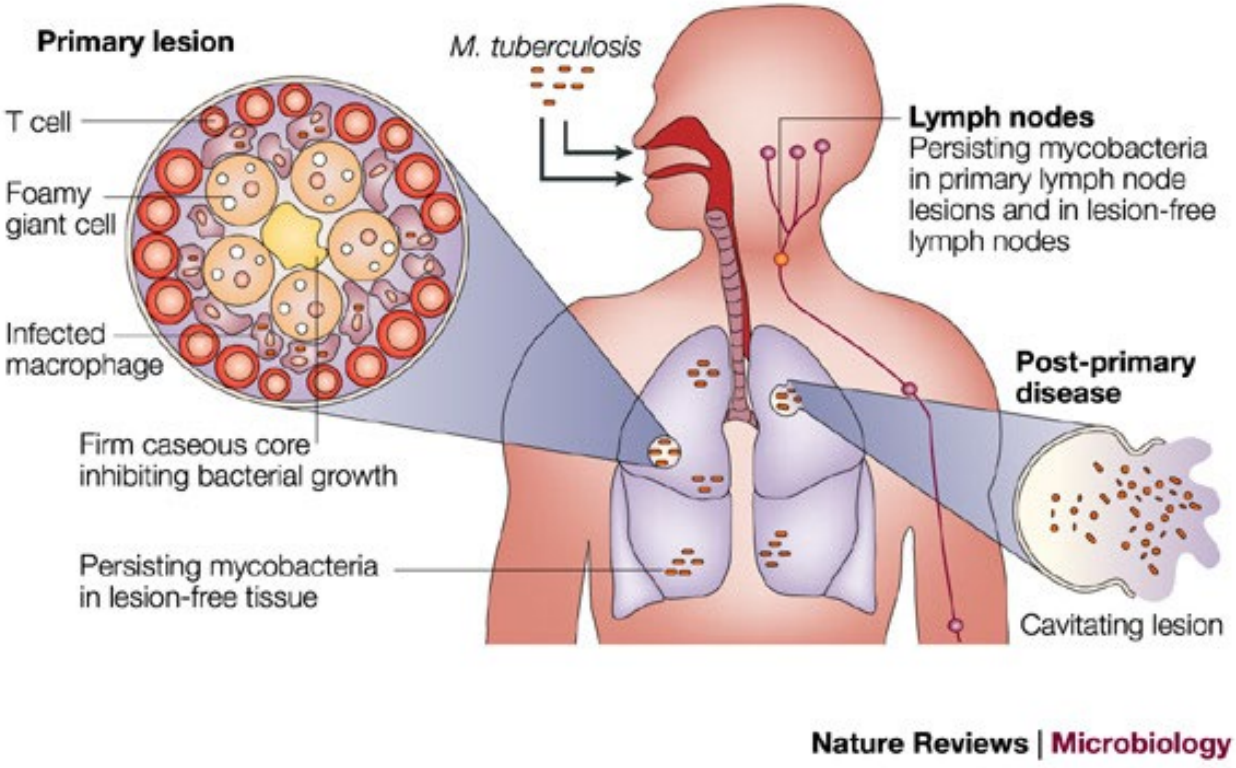
MTB LIFE CYCLE

- MTb replicates in and accumulates in macrophages, mostly in the lung (though other tissue sites are possible)
- The accumulation of infected macrophages, surrounded by other leukocytes forms a unique structure called the granuloma, the characteristic feature of MTb-associated lung damage

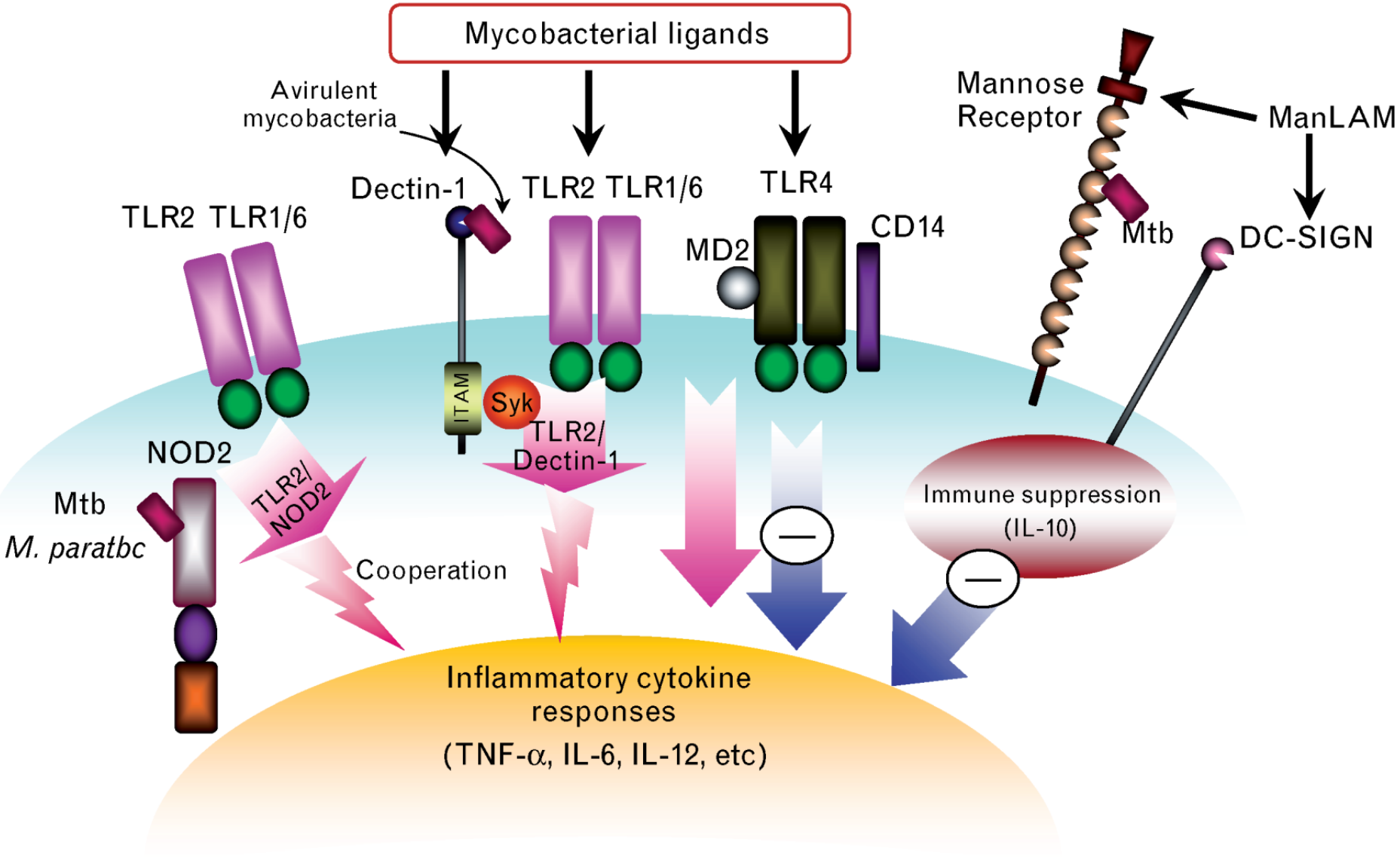


MTB LIFE CYCLE PART 2



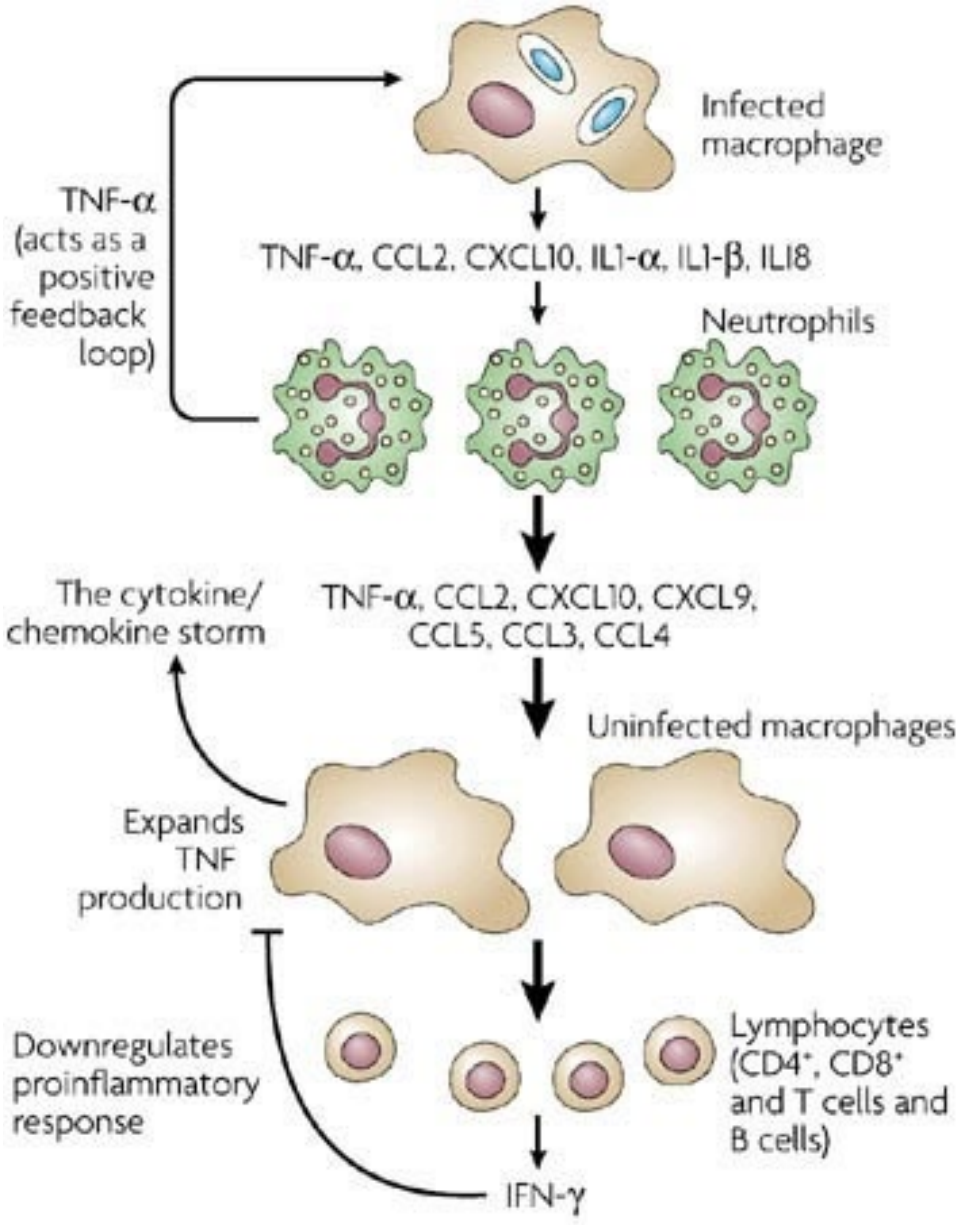


Innate Recognition of Mtb



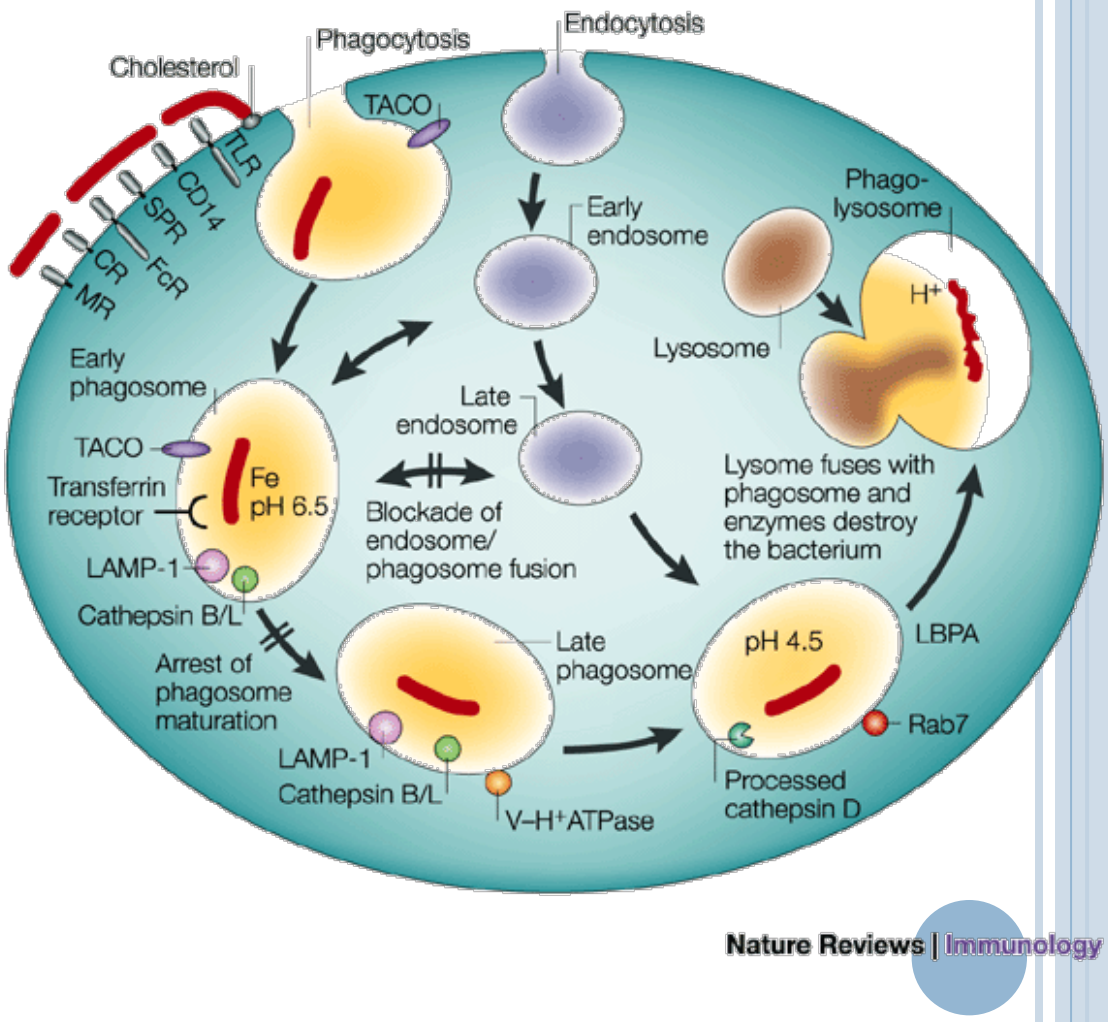
Activation of Cytokine Storm

- Macrophages do respond to the infection, even if they fail to clear
- Recruitment of other monocyte/macrophages/inflammatory cells to the lesion, promoting granuloma formation and enhancement of cytokine signaling
- Eventually recruits adaptive response which acts through "traditional" cell-mediated clearance and regulation of macrophage effector function



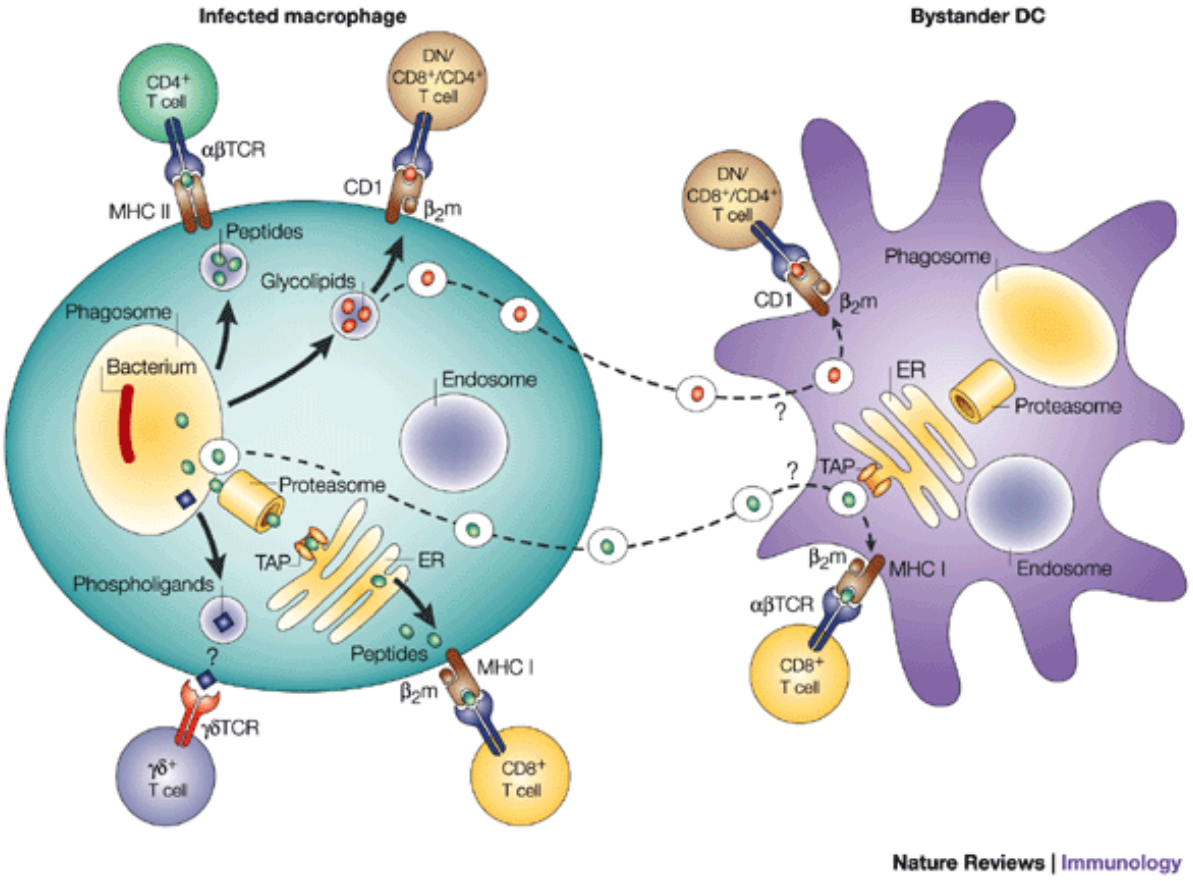
Endosomal/Lysosomal Dysregulation

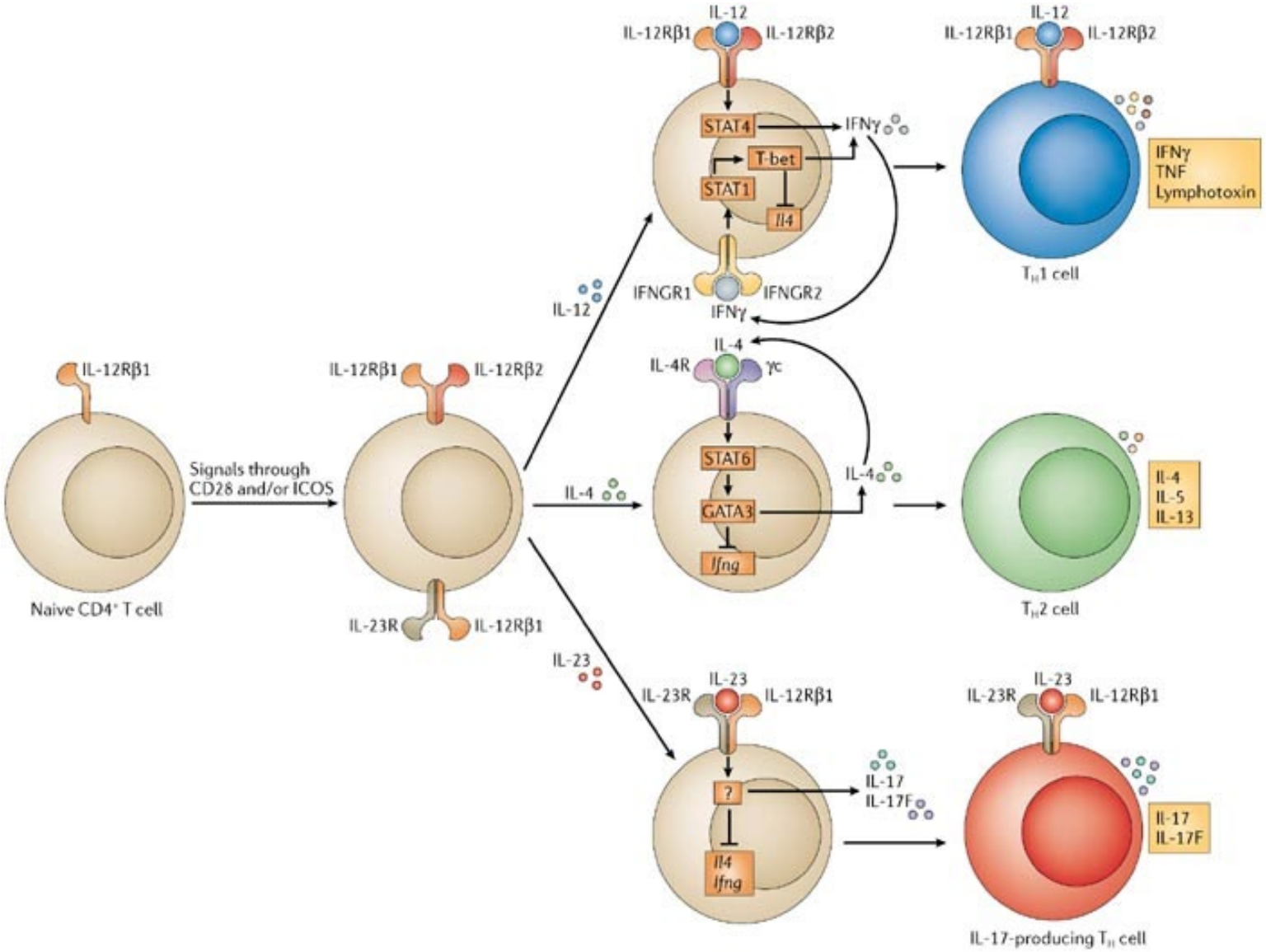
- After uptake by scavenger receptors, MTb arrests the maturation and fusion of the phagosome with the endosome
- Highly activated macrophages (IFN-g stimulation) can complete maturation and destroy the bacteria—otherwise, the bacteria remain latent or can grow



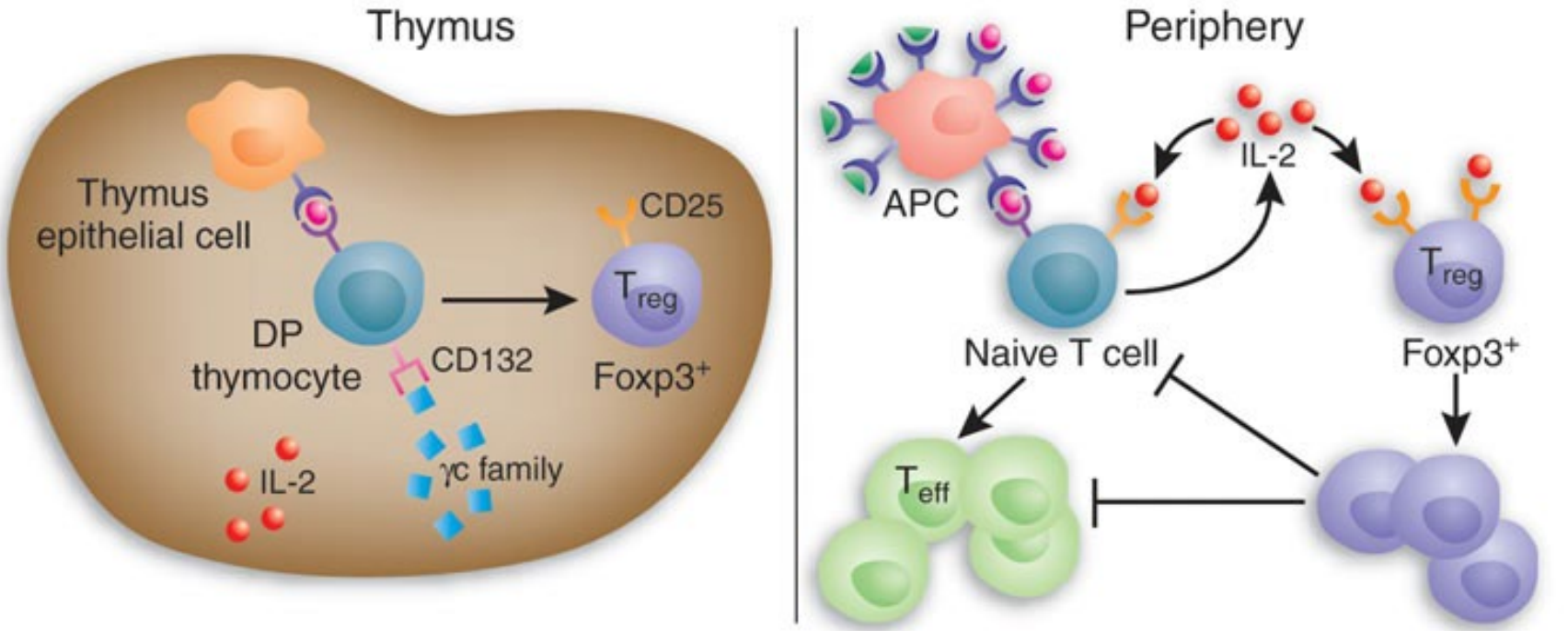
Initiation of the Adaptive Response

- The cytokine storm initaited by the innate response determines the character of the ensuing adaptive response
- Non-classical T cells (gamma-delta, CD1 restricted) play an important role in MTb control, but are not conserved between humans and mice, making their study difficult (one reason why guinea pigs are often used in MTb studies)
- Both CD_4 and CD8 functions (cytokine regulation and direct cell clearance) are associated with protection from disease



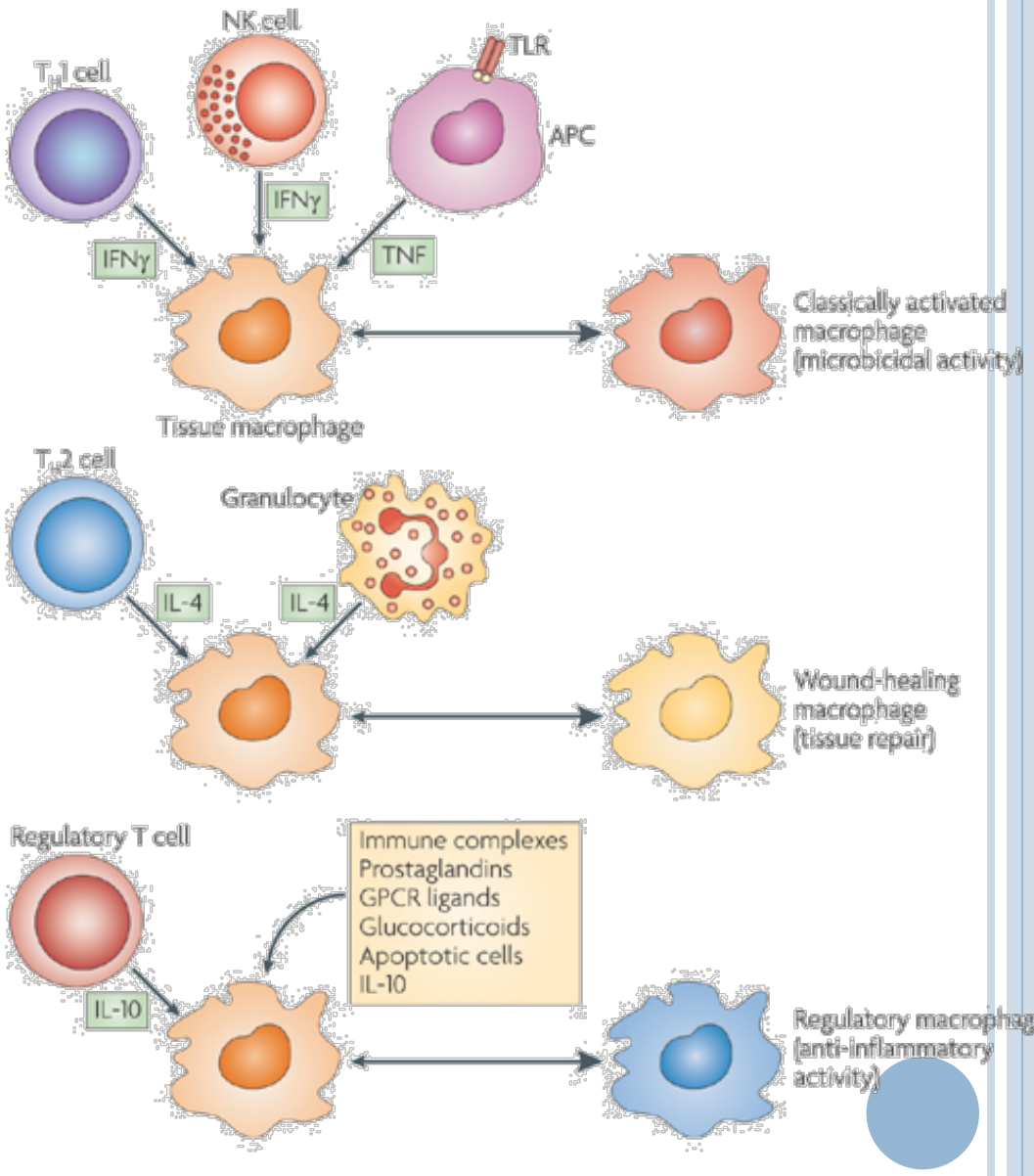


Generation of Antigen-Specific Regulatory T cells



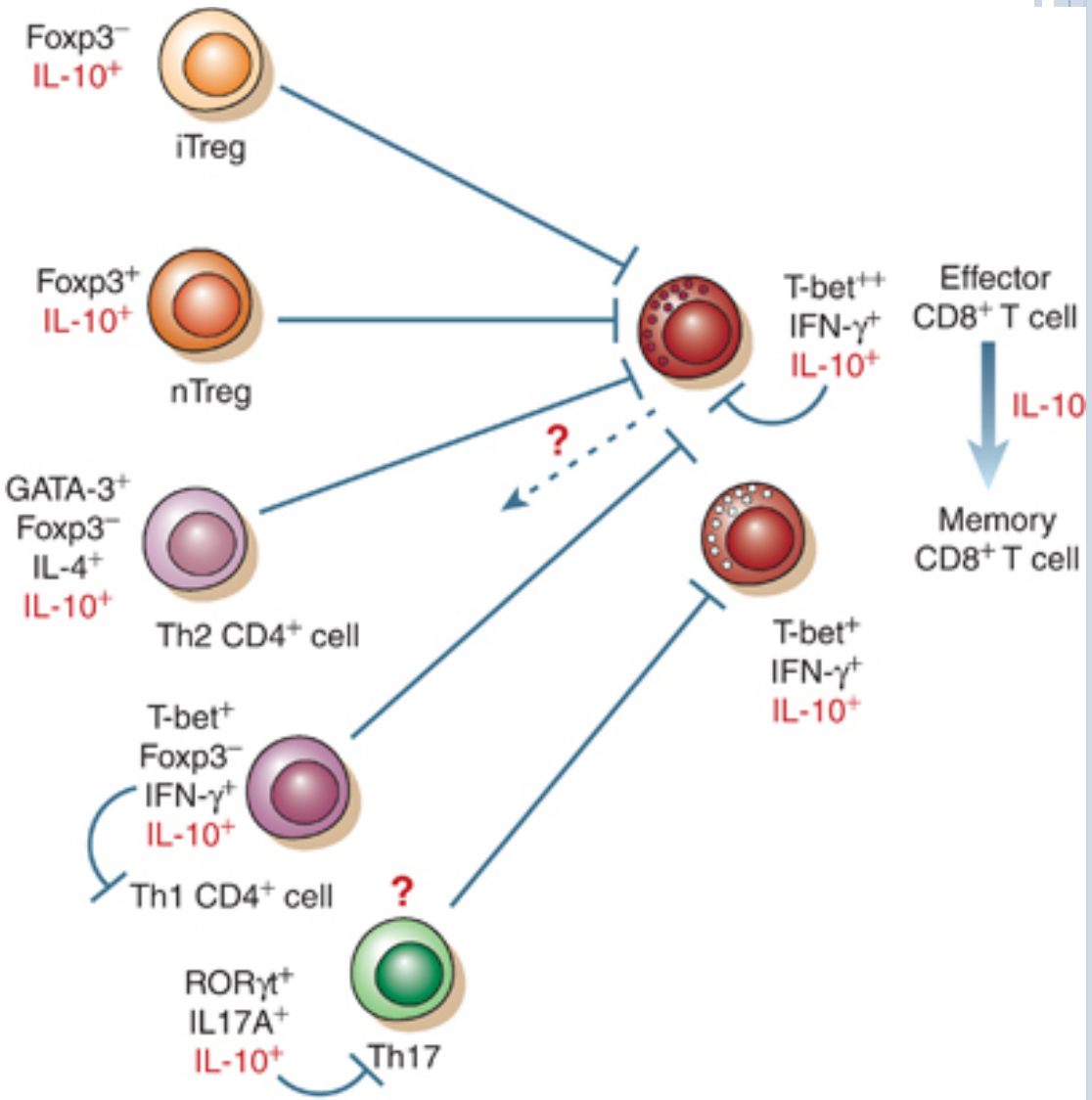
T cell regulation of Macrophage effector Function

- The balance of regulatory vs. effector signals (and the various types of those signals) determine the activation mileu of the granuloma and the infected macrophage
- Immune-associated pathology is also a risk, so some regulatory balance is required to maintain the lung physiology while achieving clearance or control

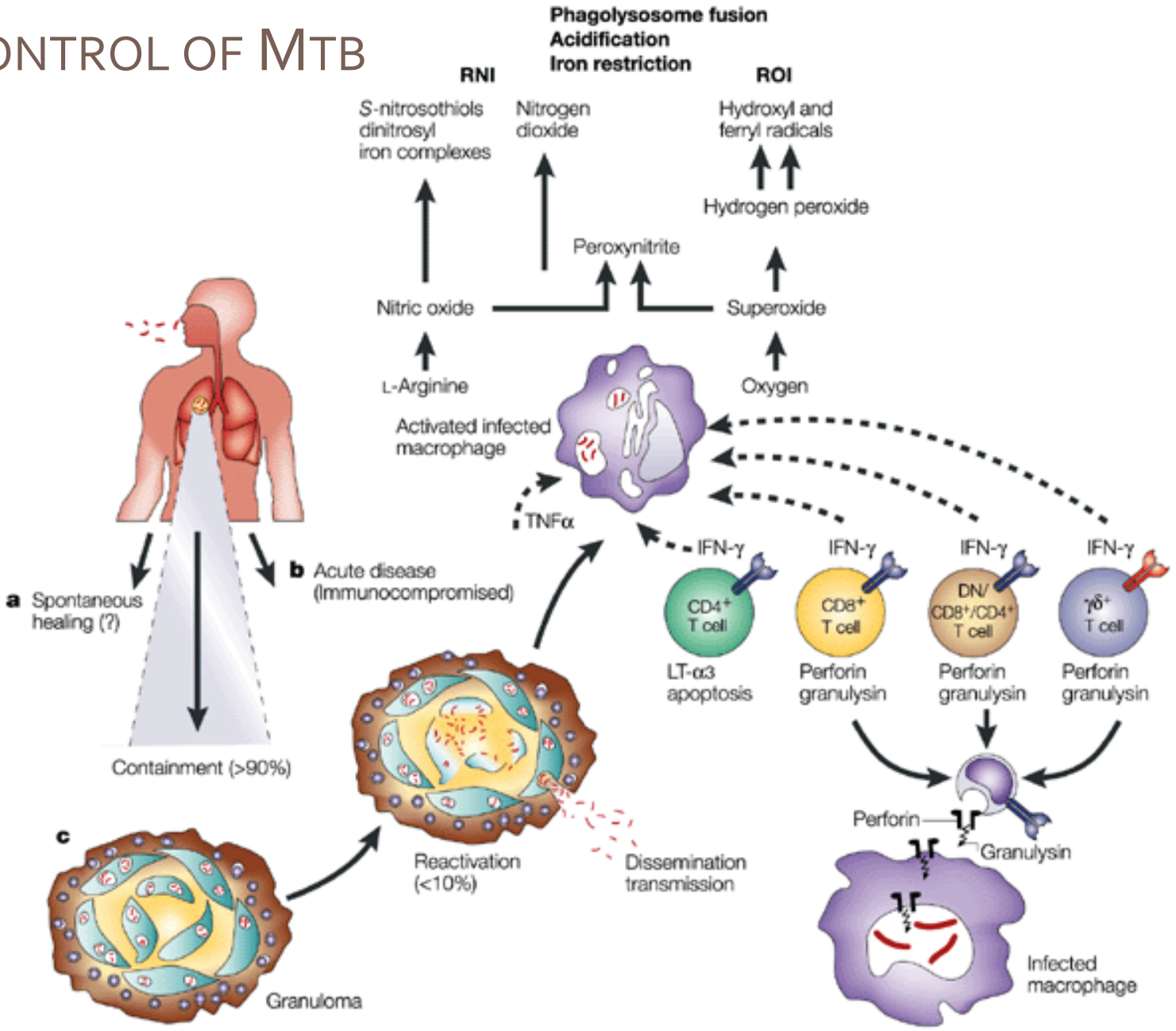


IL-10 REGULATION OF LUNG PATHOLOGY

- IL-10 has been shown in multiple infections to be a key regulatory of pathology
- In influenza, IL-10 produced by multiple cell types is required for survival in certain models of infection
- The pleiotropic effects of this cytokine are still poorly understood at a mechanistic level



Control of Mtb



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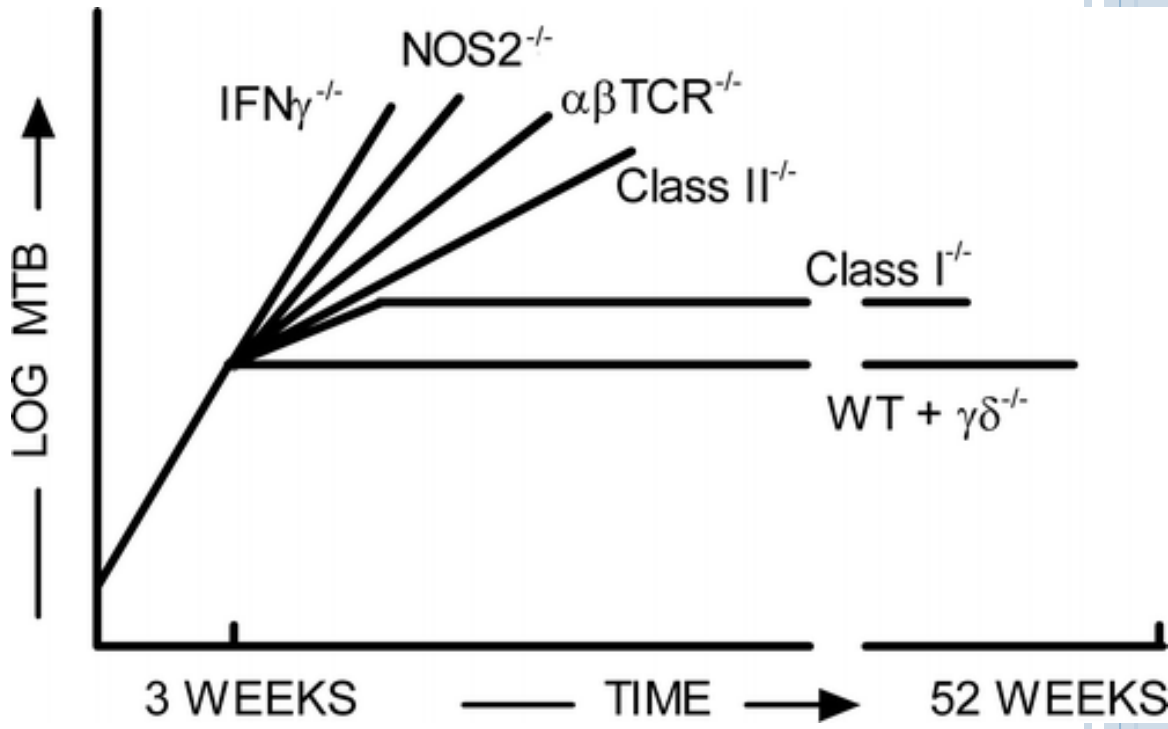
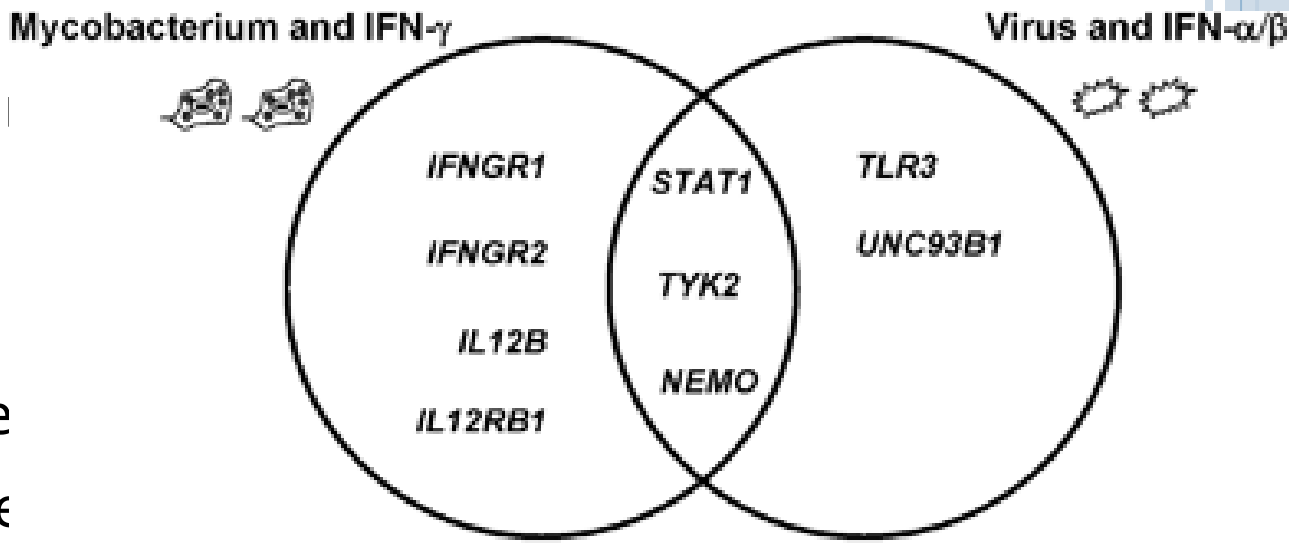
Summary of Control mechanisms

- Phagolysosomal destruction is the most important mechanism for removing bacteria
- IFNg stimulates the maturation of the phagolysosome, overcoming the inhibitory signals used by MTb
- The most effective form of this killing involves ROI and RNI
- Adaptive immunity is important for regulating the cytokine environment and, to a smaller extent, for cytolytic killing



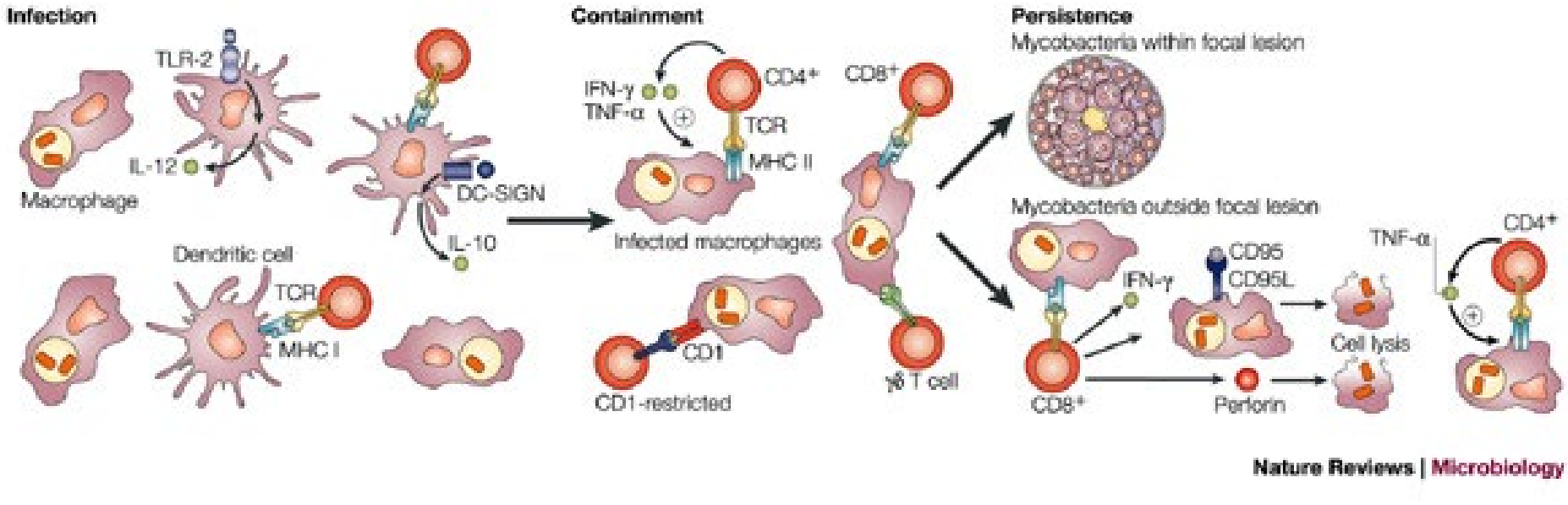
Human Genetic Deficiencies

- The primary phenotype of individuals with genetics deficiencies i IFN-g signaling or activation is susceptibility to Mycobacterial disease
- In contrast, deficiencie in Type I IFNs result in viral susceptibilities



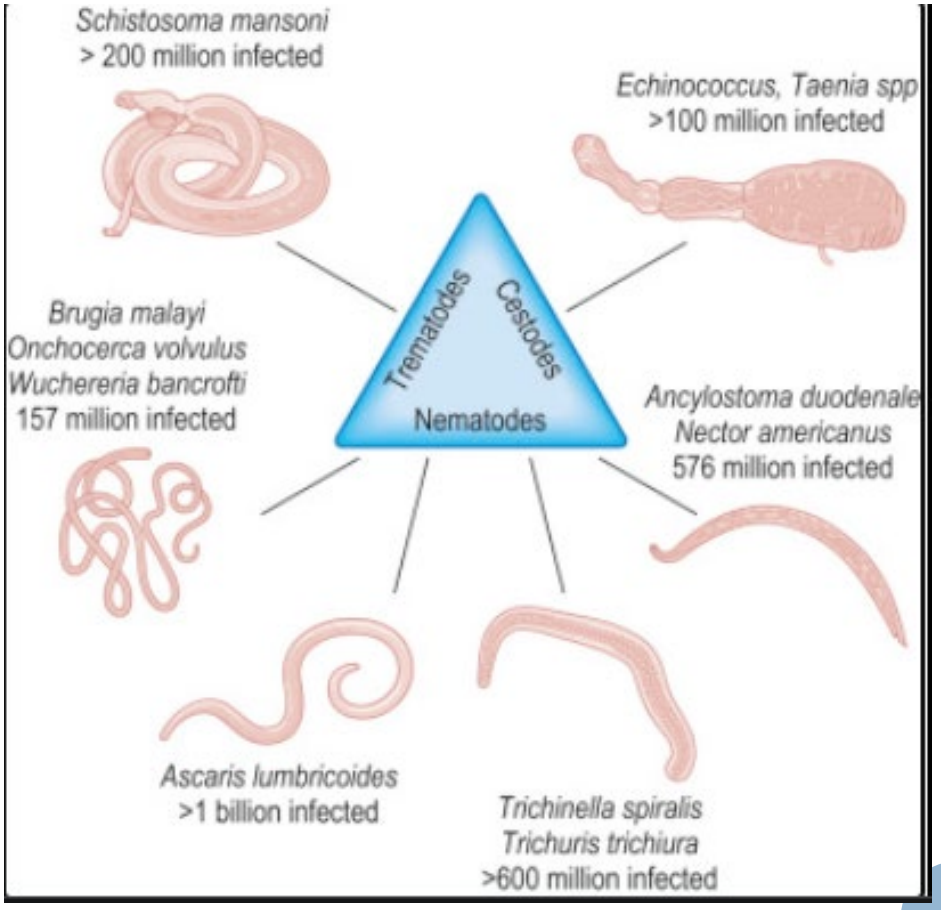
Summary and Perspectives

- MTb is never completely cleared following initial infection
- The primary effector mechanisms are macrophage bactericidal functions, but their success is determined by the cytokine and cellular regulatory environment
- Small subtle shifts over time or dramatic short-term changes lead to reactivation and disease

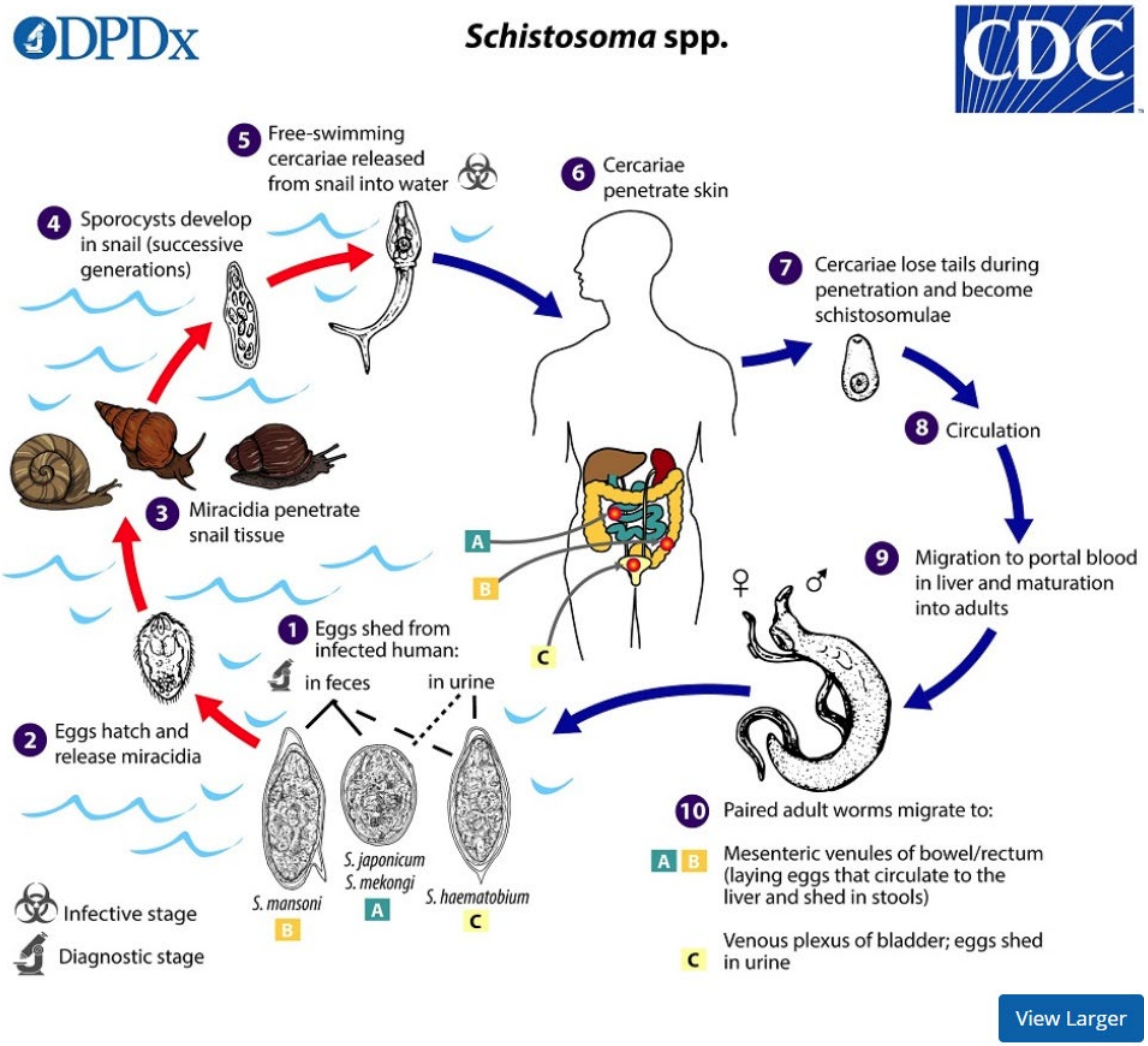


The Burden of Helminth Infections

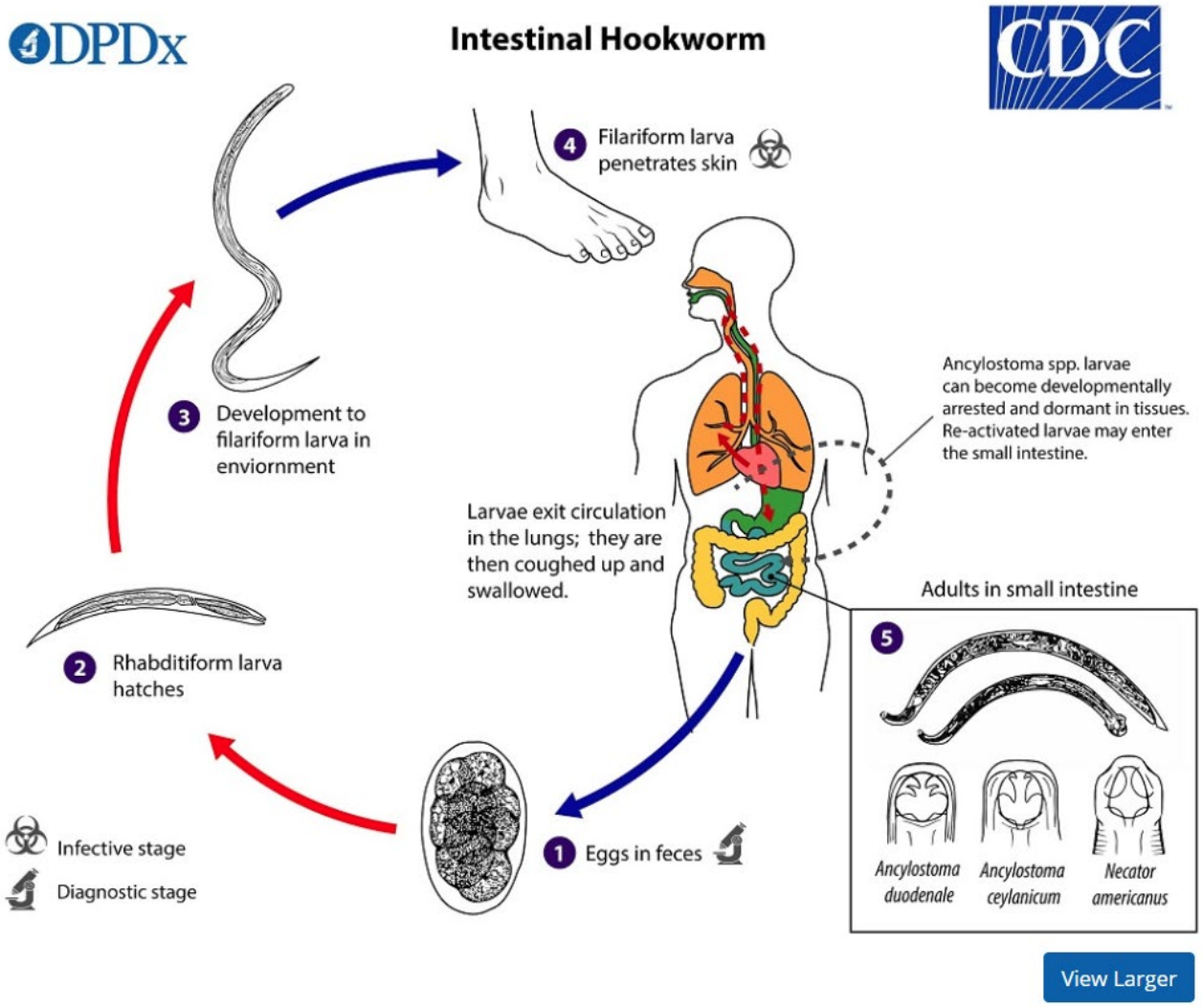
- After malaria, helminths represent the biggest DALY-impact of a parasitic infection in human populations
- Multiple life cycles drive distinct pathological outcomes



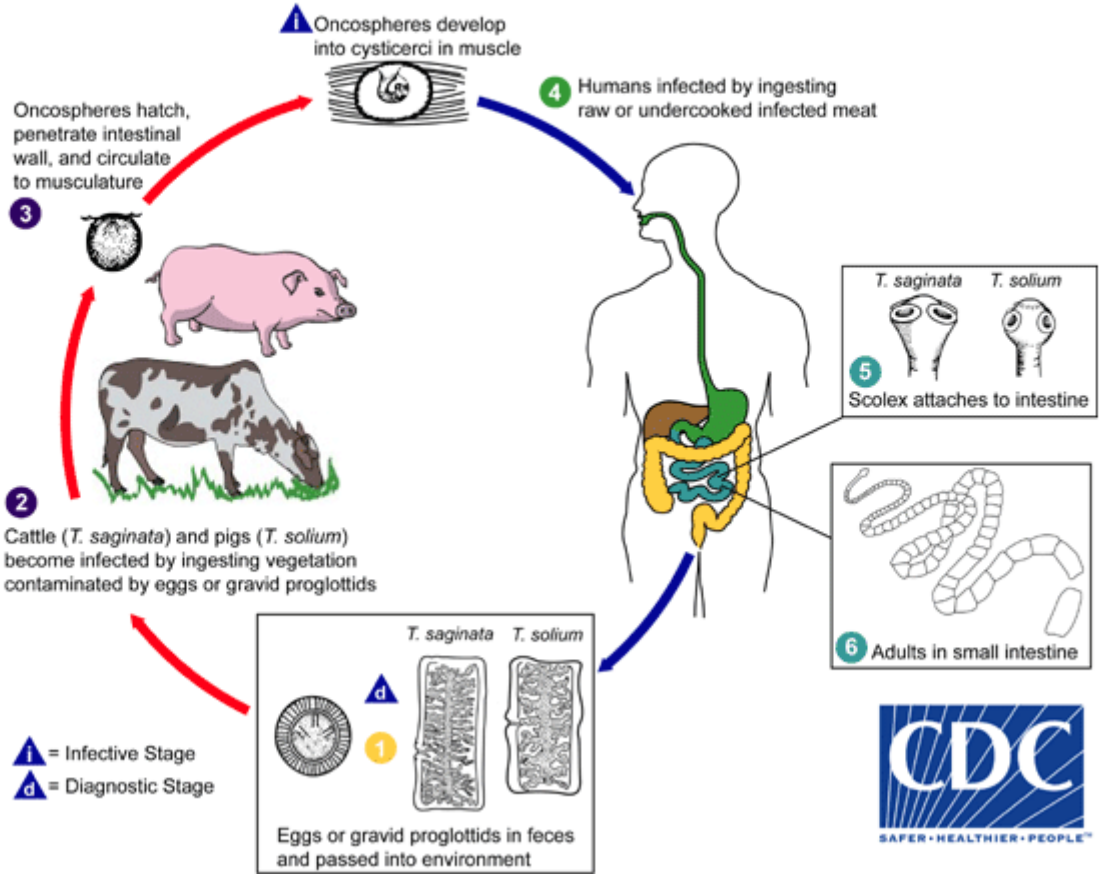
Schistosoma Life Cycle



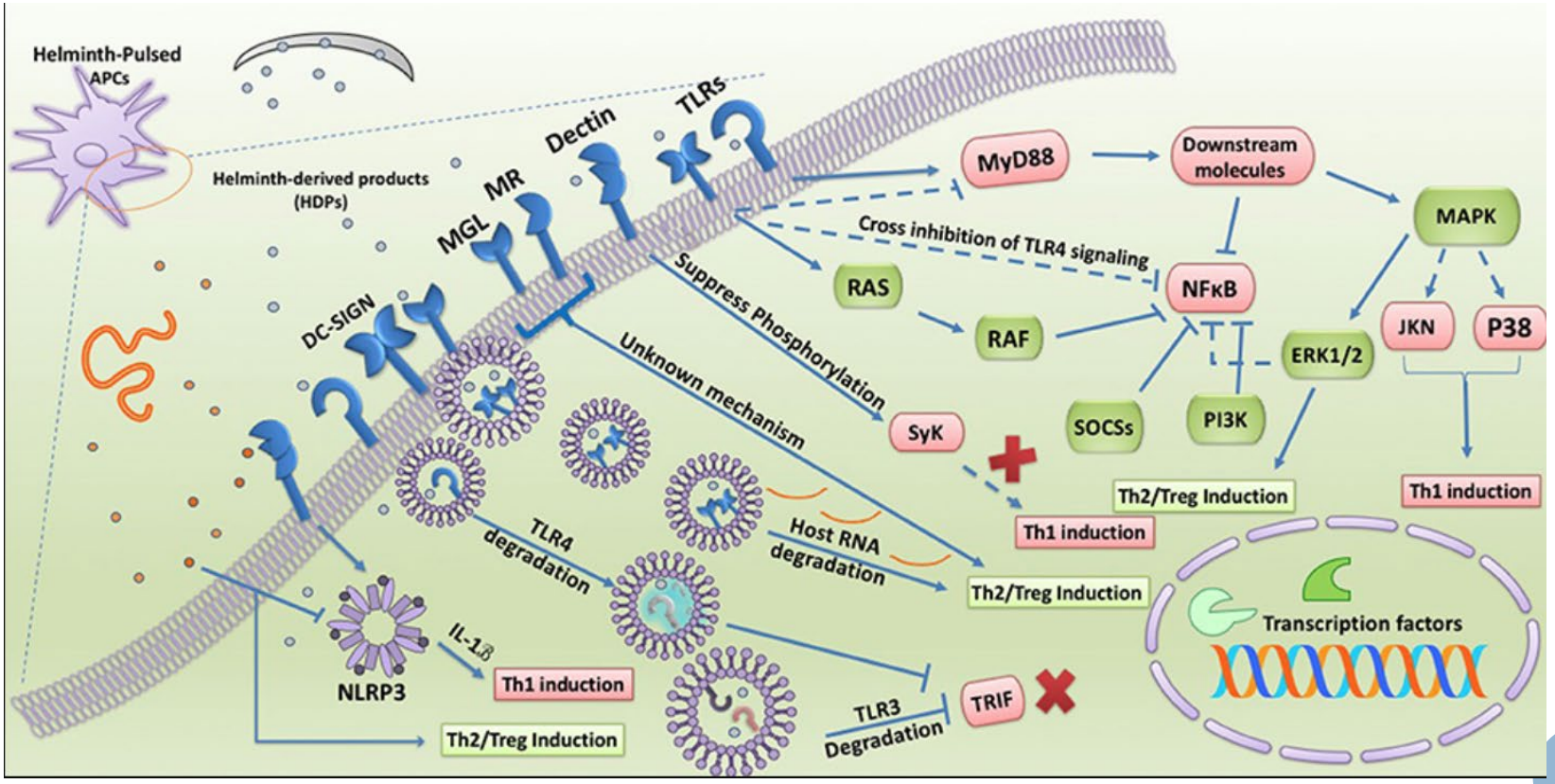
Hookworm Life Cycles

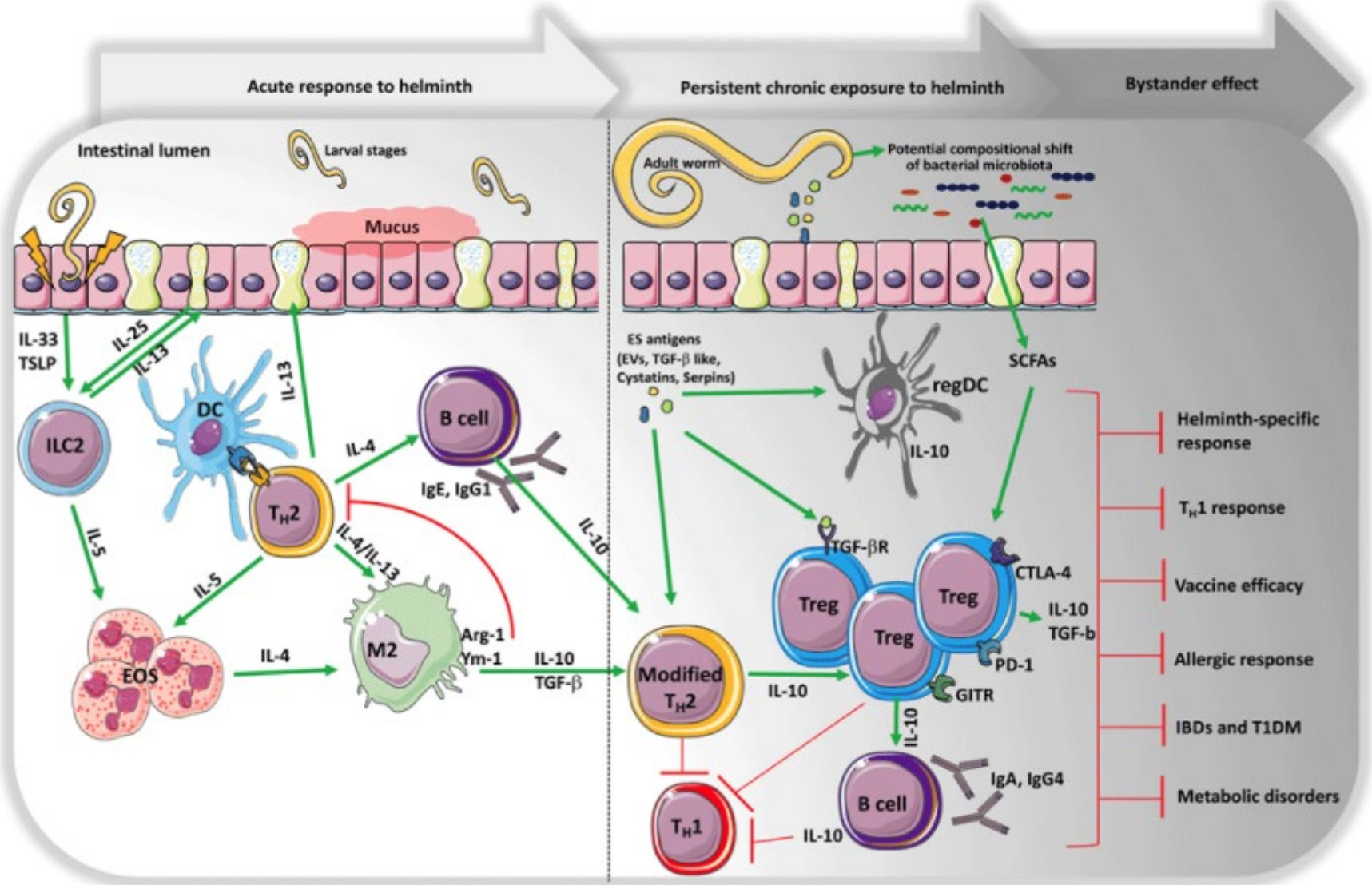


Taenia Life cycle

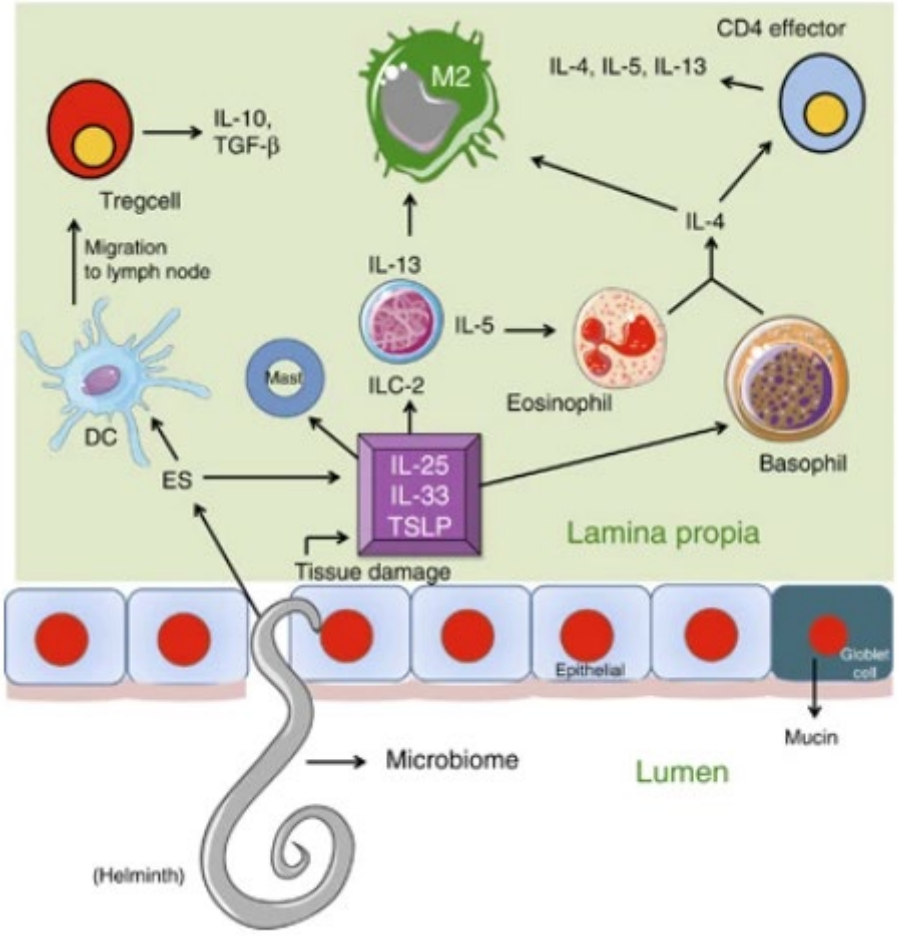


Innate signaling in response to helminth-derived products




Acute response to helminth
Persistent chronic exposure to helminth
Bystander effect
Intestinal lumen
Larval stages
Mucus
Adult worm
Potential compositional shift of bacterial microbiota
IL-33
TSLP
IL-25
IL-13
IL-13
DC
ILC2
T_H2
IL-4
B cell
IgE, IgG1
IL-5
IL-5
IL-4/IL-13
IL-10
EOS
IL-4
M2
Arg-1
Ym-1
IL-10
TGF-β
Modified T_H2
ES antigens
(EVs, TGF-β like,
Cystatins, Serpins)
regDC
IL-10
SCFAs
TGF-βR
Treg
Treg
Treg
CTLA-4
IL-10
TGF-b
PD-1
GITR
IL-10
IL-10
T_H1
IL-10
B cell
IgA, IgG4
Helminth-specific response
T_H1 response
Vaccine efficacy
Allergic response
IBDs and T1DM
Metabolic disorders

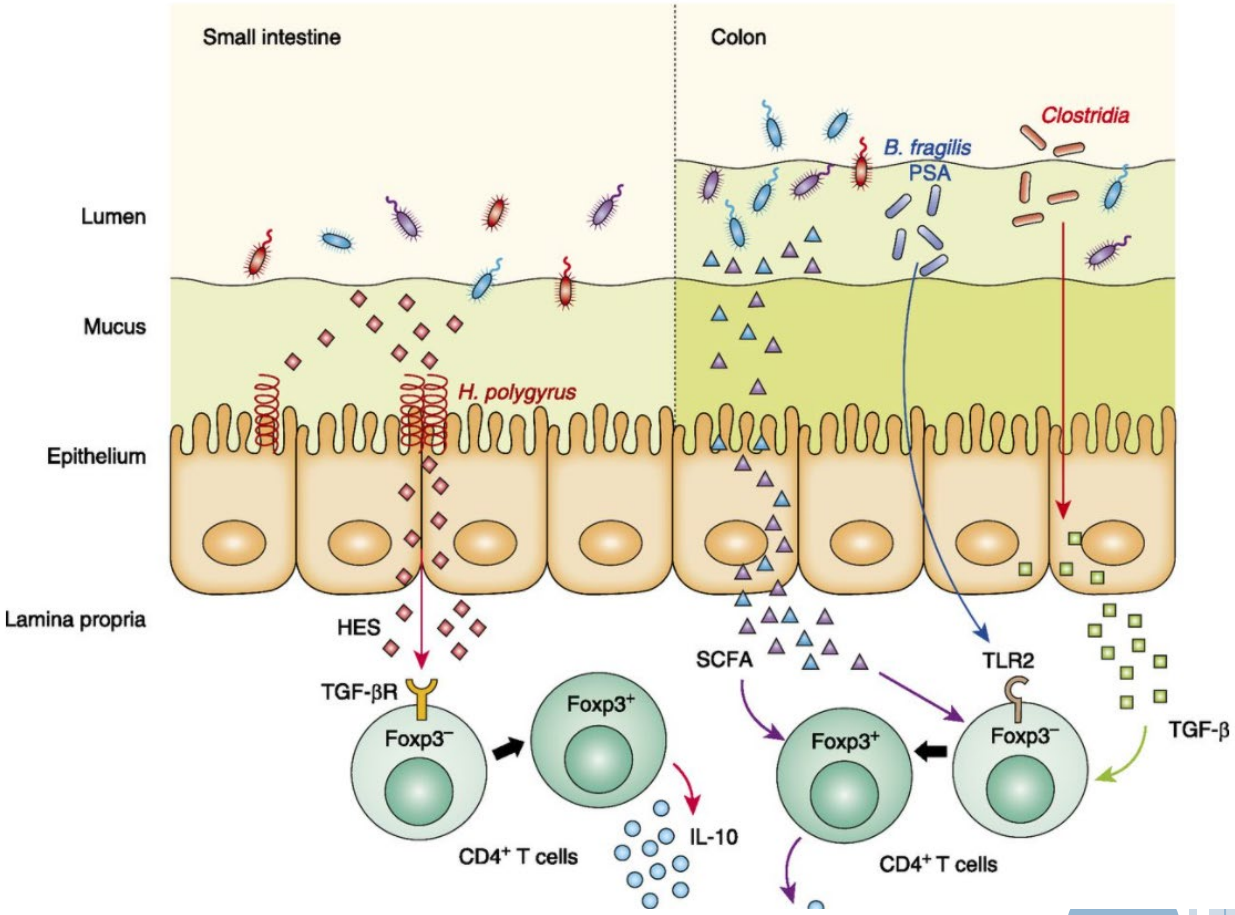
Local Tissue induction of regulatory responses



- *Mucosal Immunology* volume 7, pages753–762 (2014)

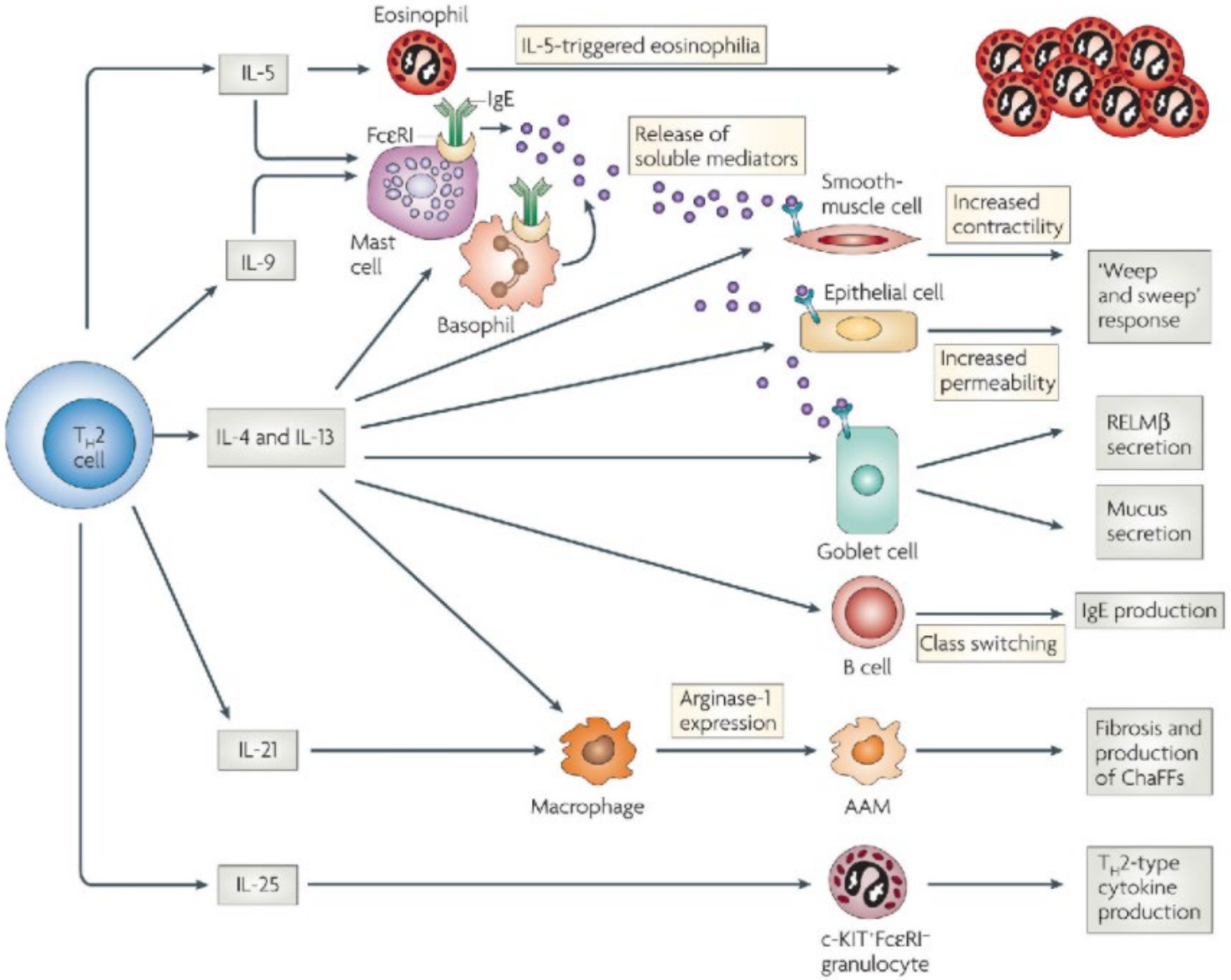
Helminth Interaction with the Microbiome

- Microbiome components and helminths likely co-evolved
- Similar effector and host manipulation pathways
- Immune tolerance vs. immune resistance



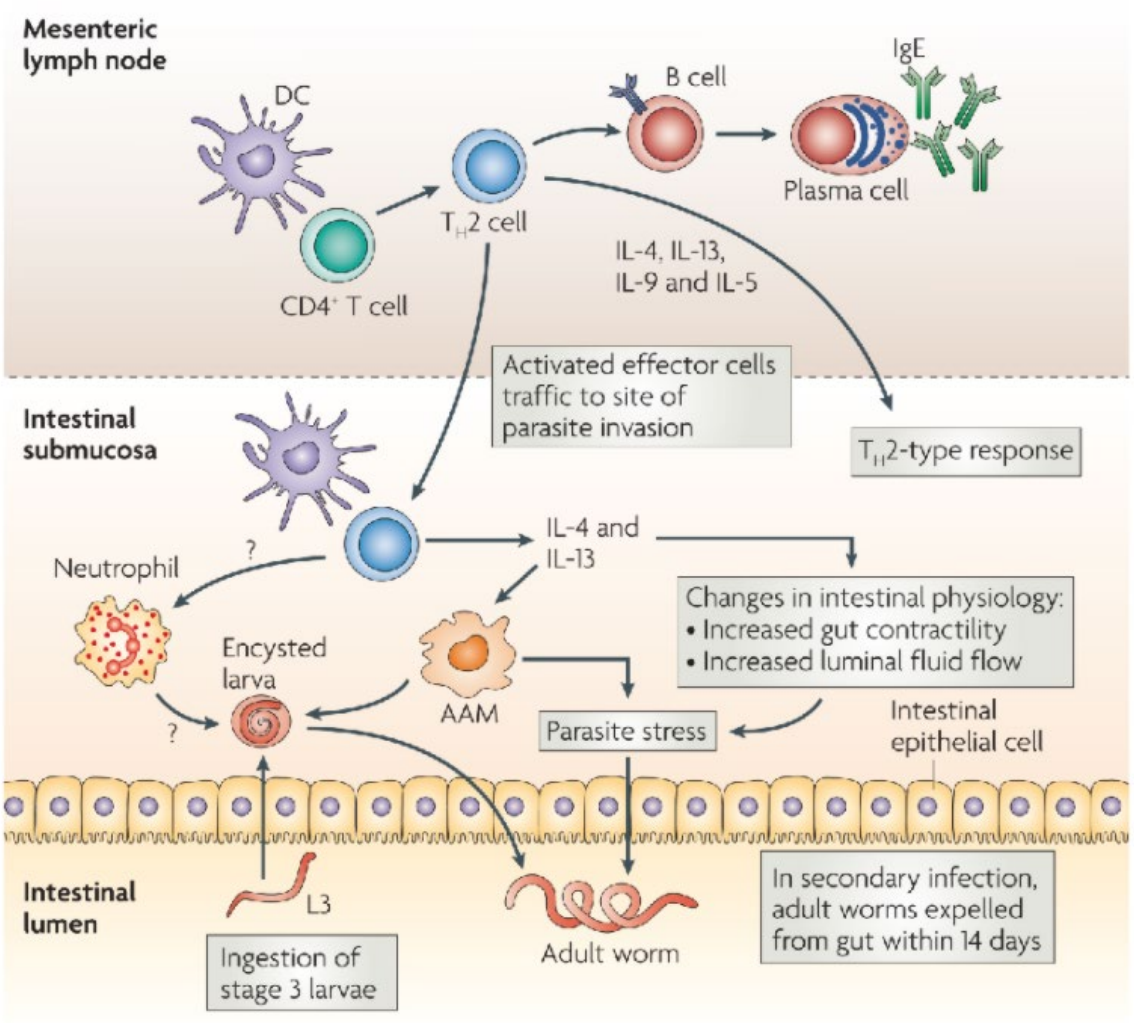
J Immunol November 1, 2015, 195 (9) 4059-4066; DOI: https://doi.org/10.4049/jimmunol.1501432

The Th2 response is the main effector of Helminth clearance

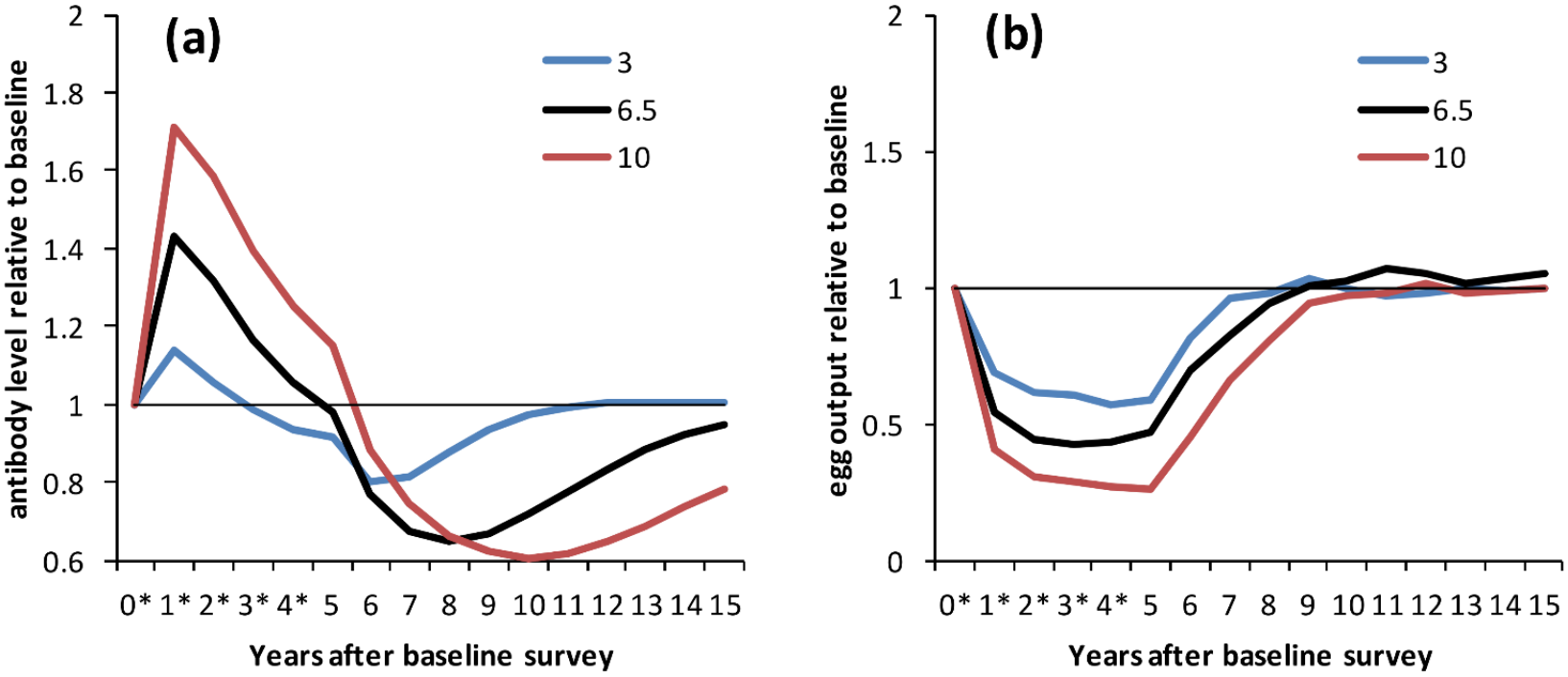


Effector Mechanisms for Helminth Damage and Clearance

- CD4 T cell orchestrate the response against helminths
- Th2 responses involve multiple effector mechanisms, not all fully understood
- Cell based an mechanical disruptions lead to worm death and explusion



Some Evidence for Protective Immunity



- IgE levels correlate with egg burdens and rates of re-infection
- https://doi.org/10.1371/journal.pntd.0003059

Conclusions/Discussion Points

- Parasitic helminths represent a major evolutionary burden on human development
- An entire arm of the immune response is dedicated to response to worm infections, but in many populations it fails to be engaged
- Evolutionary "assumption" may have led to increases of atopic conditions, such as allergy, resulting from lack of helminth targets