



# TREATMENT AND IMMUNE CONTROL OF MALARIA AND TB

Unit 5

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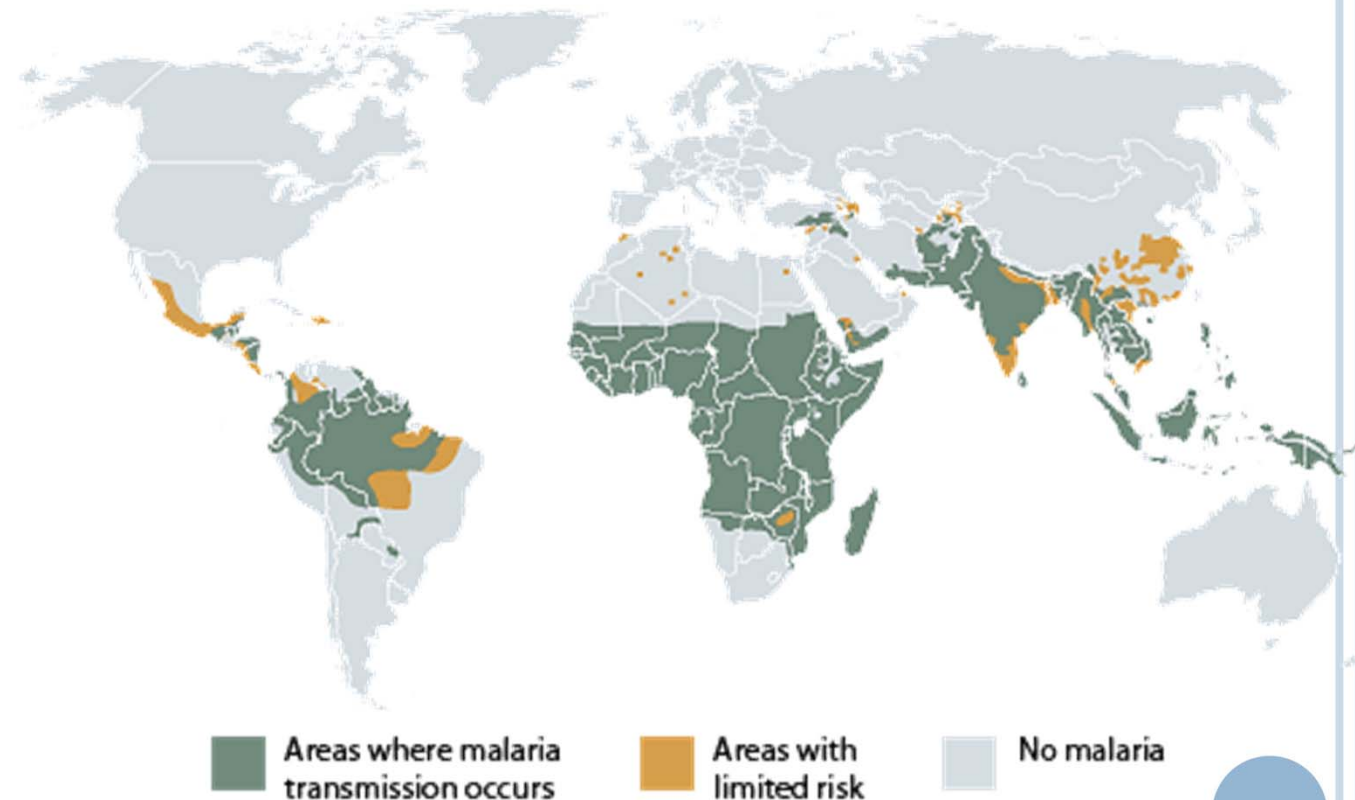
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# MALARIA PREVALENCE

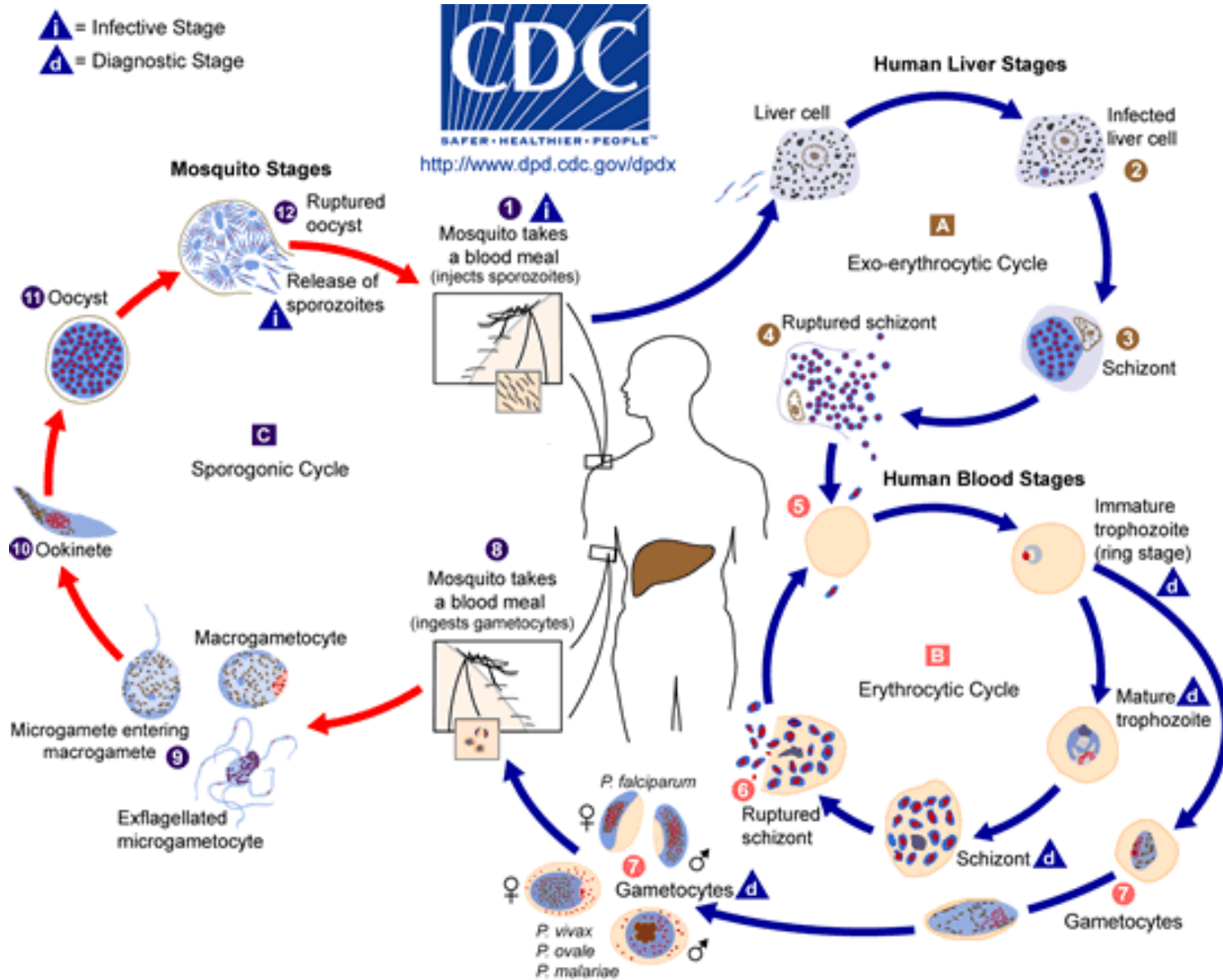
- ~350-500 million infections/year
- 1-2 million deaths/year, most of them among children under 5

Malaria-endemic countries, 2006



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

# MALARIA LIFE CYCLE



# *PLASMODIUM SPP.*

## ○ In humans:

- **Plasmodium falciparum:** causes the most severe form of malaria and can be fatal. Can cause chronic infections (up to 2–3 years), but does not form hypnozoites (dormant stages that persist in hepatocytes) and does not relapse.
- **Plasmodium vivax:** a major cause of clinical malaria, but is rarely fatal. Distribution is restricted by the absence of Duffy antigen (which determines entry into red blood cells) in African populations. This parasite forms hypnozoites and might relapse many years after apparent cure.
- **Plasmodium malariae:** infrequent cause of clinical malaria, especially in Africa. Untreated infections can persist as low-grade parasitaemia for several decades.
- **Plasmodium ovale:** infrequent cause of mild–moderate clinical malaria, but might be found in mixed infections with other species. Forms hypnozoites and might relapse.

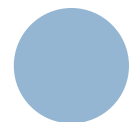
## ○ In mice:

- **Plasmodium chabaudi** (*P. chabaudi chabaudi* AS and *P. chabaudi adami*): used to study immune mechanisms and immunoregulation by cytokines, to identify susceptibility loci and to study the immune basis of pathology. *P. chabaudi chabaudi* AS causes non-lethal infection in resistant mouse strains and lethal infection in susceptible mouse strains. *P. chabaudi adami* causes a mild, non-lethal infection.
- **Plasmodium berghei** (*P. berghei* ANKA and *P. berghei* K173): widely used to study pathogenesis. *P. berghei* ANKA serves as a model of experimental cerebral malaria (ECM); there is genetic variation in the development of ECM between inbred mouse strains, which correlates with the production of pro-inflammatory cytokines.
- **Plasmodium yoelii** (*P. yoelii* 17XL, *P. yoelii* 17XNL and *P. yoelii* YM): used to study immune mechanisms and pathogenesis, including ECM, as recombinant merozoite surface protein 1 (MSP1) is available. *P. yoelii* 17XL is widely used to identify vaccine-induced immune responses.
- **Plasmodium vinckei:** *P. vinckei vinckei*, which causes a lethal infection, is used to study pathogenesis and for chemotherapy studies; *P. vinckei petteri*, which causes a non-lethal infection, is used to study immune mechanisms.

## ○ From Nature Reviews Immunology 4, 169-180 (March 2004)

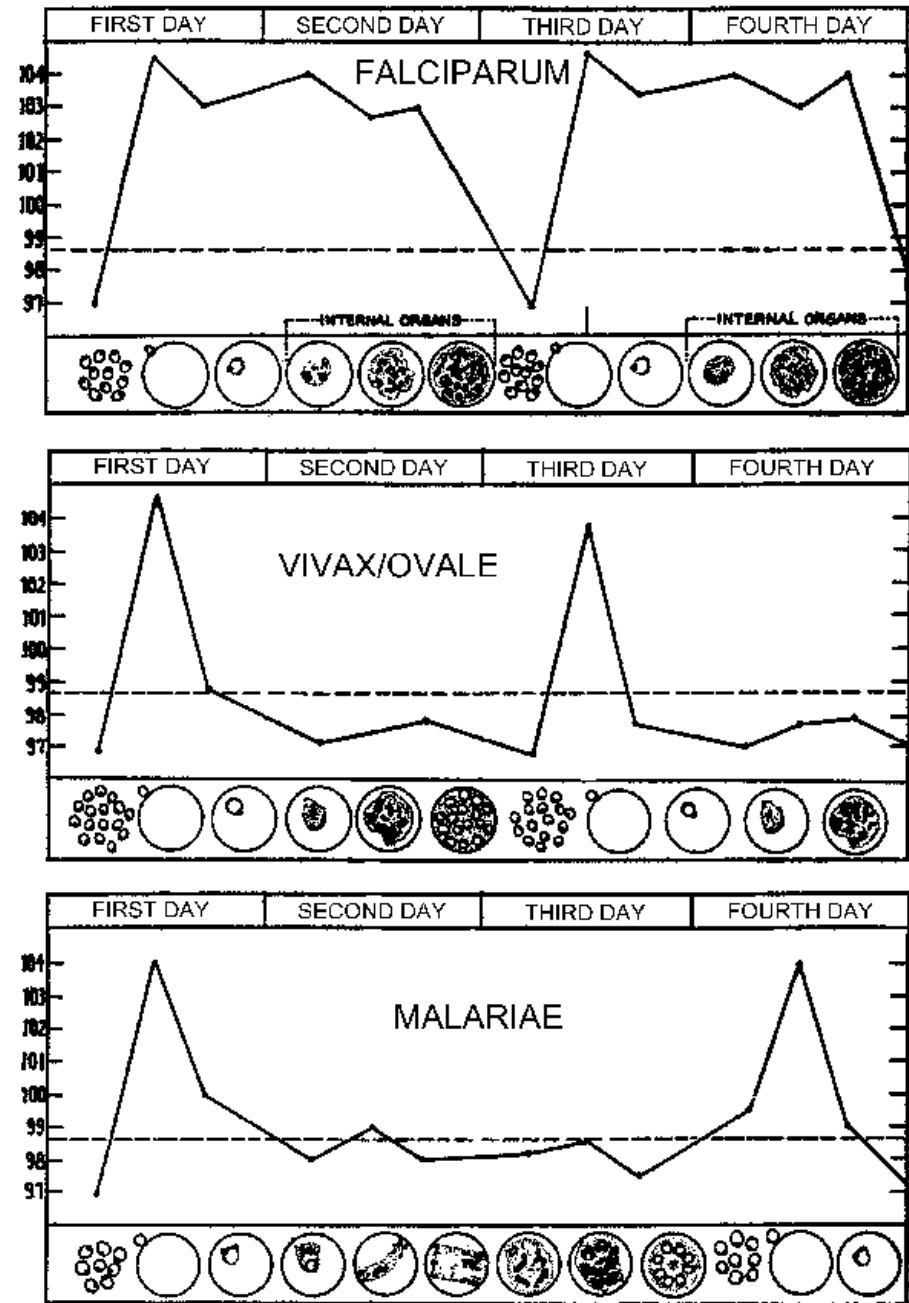
## *PLASMODIUM SPP.*: LIFE CYCLE DETERMINE DISEASE SEVERITY

| Exoerythrocytic schizogony and prepatent and incubation periods |                      |                 |                 |                    |
|---|----------------------|-----------------|-----------------|--------------------|
|   | <i>P. falciparum</i> | <i>P. vivax</i> | <i>P. ovale</i> | <i>P. malariae</i> |
| Prepatent period (days)   | 6-9                  | 8-12            | 10-14           | 15-18              |
| Incubation period (days)  | 7-14                 | 12-17           | 16-18           | 18-40              |
| Merozoite maturation (days)                                     | 5-7                  | 6-8             | 9               | 12-16              |
| Merozoites produced   | 40,000               | 10,000          | 15,000          | 2000               |



## *P. FALCIPARUM* ALSO CAUSES A MORE SEVERE DISEASE PRESENTATION

- Severe fevers are characteristic of any malaria, but the frequency and duration of relapse are the key features of falciparum and vivax malaria





## VARIATION OF SYMPTOMS AMONG PLASMODIUM SPECIES

- While falciparum is the only species associated with severe mortality, the others (particularly vivax) inflict a high degree of morbidity
- Some species can persist in the liver for years, while others persist in the blood stages as a low level infection

| Disease Severity and Duration                         |                    |              |                    |                   |
|---|--------------------|--------------|--------------------|-------------------|
|   | <b>vivax</b>       | <b>ovale</b> | <b>malariae</b>    | <b>falciparum</b> |
| Initial Paraoxysm Severity                            | moderate to severe | mild         | moderate to severe | severe            |
| Average Parasitemia (mm <sup>3</sup> )                | 20,000             | 9,000        | 6,000              | 50,000-500,000    |
| Maximum Parasitemia (mm <sup>3</sup> )                | 50,000             | 30,000       | 20,000             | 2,500,000         |
| Symptom Duration (untreated)                          | 3-8+ weeks         | 2-3 weeks    | 3-24 weeks         | 2-3 weeks         |
| Maximum Infection Duration (untreated)                | 5-8 years          | 12-20 months | 20-50+ years       | 6-17 months       |
| Anemia  | ++                 | +            | ++                 | ++++              |
| Complications   |                    |              | renal              | cerebral          |
| Modified from Markell and Voge's Medical Parasitology |                    |              |                    |                   |

# MALARIA IN MICE

- Early during infection, *P. berghei* strain ANKA is a good model of cerebral malaria (CM), and processes identified using this model have been subsequently validated in humans. Inbred mouse strains differ markedly in their susceptibility, showing the importance of host genetic variation in immunopathogenesis. Similarly, different strains of *P. berghei* (K173 versus ANKA) differ in some aspects of pathogenesis, indicating the influence of parasite genetic variation in induced pathology.
- Infection with *P. yoelii* strain 17XL (a lethal strain) induces CM that is associated with the sequestration of parasitized red blood cells, and it has been used together with *P. yoelii* strain 17XNL (a non-lethal strain) to study experimental vaccine-induced immune responses.
- *P. chabaudi chabaudi* strain AS causes a non-lethal infection in resistant mouse strains and a lethal infection in susceptible mouse strains. Lethality, however, results from haemolysis that is secondary to hyperparasitaemia, which might not be relevant to the human disease processes. This *Plasmodium* strain has been used to study experimental vaccines and immunological processes that control hyperparasitaemia. Infections with *P. chabaudi adami* are self-resolving, non-pathogenic and non-lethal.
- *P. vinckei vinckei* causes an aggressive, overwhelming hyperparasitaemia.

[Immunological processes in malaria pathogenesis](#)

Louis Schofield and Georges E. Grau

Nature Reviews Immunology 5, 722-735 (September 2005)

doi:10.1038/nri1686



# IMMUNE RESPONSES MEDIATE PLASMODIUM PATHOLOGY

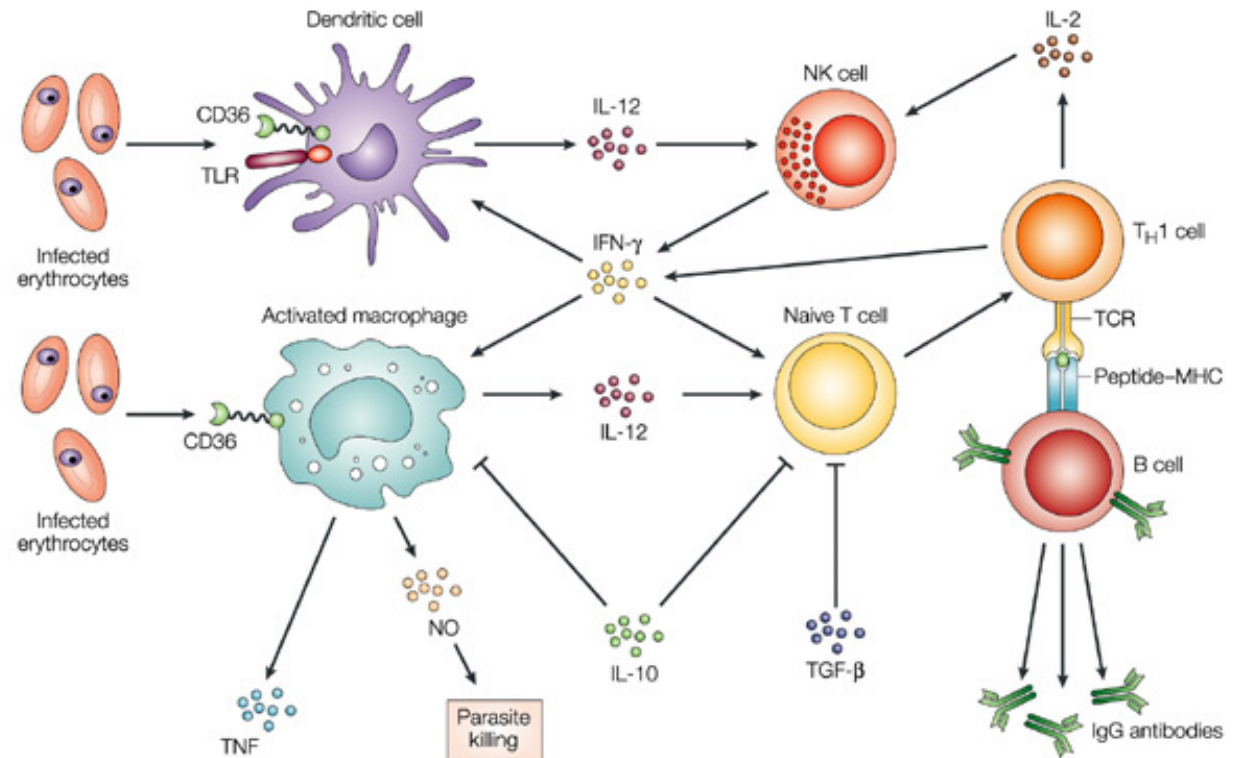
Table 2 | **Malaria products and their bioactivities**

| Parasite product                                 | Receptor and cell type   | Pathological and cellular effects  |
|--|--|--|
| <i>Plasmodium falciparum</i> 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 V $\alpha$ 14-V $\beta$ 8 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 <sub>H</sub> 1- or T <sub>H</sub> 2-cytokine production |
| Haemozoin  | TLR9 on DCs  | Contradictory reports: both T <sub>H</sub> 1- and T <sub>H</sub> 2-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- $\gamma$ production; regulates balance of T <sub>H</sub> 1 and T <sub>H</sub> 2 cytokines produced by NKT cells   |
| Isopentenyl pyrophosphate                        | $\gamma\delta$ TCRs  | Activates $\gamma\delta$ T cells; induces IFN- $\gamma$ production   |
| Protein antigens                                 | Diverse TCRs on CD4 <sup>+</sup> and CD8 <sup>+</sup> T cells  | Activates $\alpha\beta$ T cells; induces T <sub>H</sub> 1- or T <sub>H</sub> 2-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- $\gamma$ , interferon- $\gamma$ ; 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<sub>H</sub>, T helper; TLR, Toll-like receptor; TNF, tumour-necrosis factor; VCAM1, vascular cell-adhesion molecule 1.

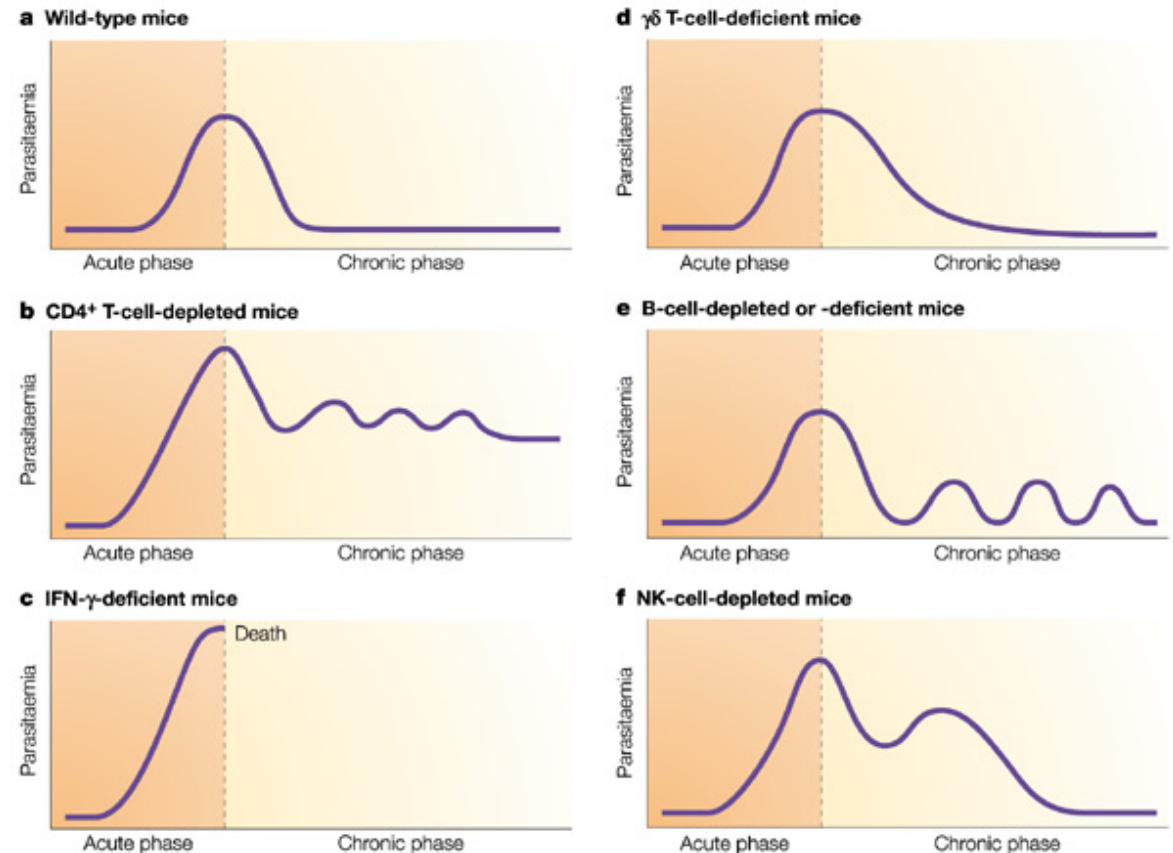
# TYPICAL “Th1-TYPE” IMMUNITY CONTRIBUTES TO MALARIA PROTECTION AND PATHOLOGY

- 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<sup>+</sup> 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<sup>+</sup> 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





# FATAL DISEASE IN MALARIA INFECTION

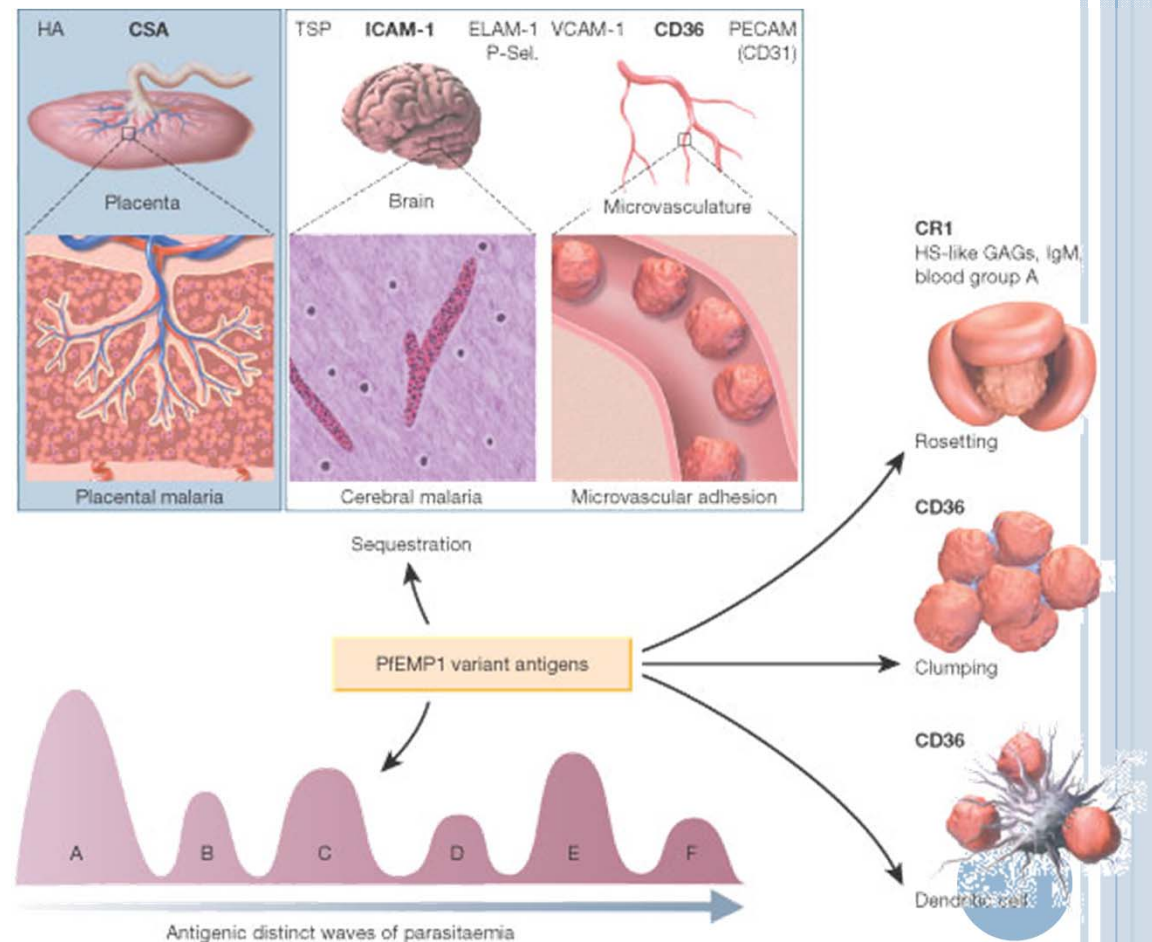
Table 1 | **Severe and fatal disease syndromes in malaria**

| 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 <sup>+</sup> , CD8 <sup>+</sup> and $\gamma\delta$ T-cell involvement; IFN- $\gamma$ production; neurological metabolic derangements; possibly hypoxia |
| Placental malaria  | Placental insufficiency, low birth weight, premature delivery, loss of fetus                  | <i>Plasmodium falciparum</i> 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 <sup>+</sup> T cells, $T_H1/T_H2$ cytokine balance (TNF and IFN- $\gamma$ 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- $\gamma$ , interferon- $\gamma$ ; IL-10, interleukin-10; RBC, red blood cell;  $T_H$ , T helper; TNF, tumour-necrosis factor.

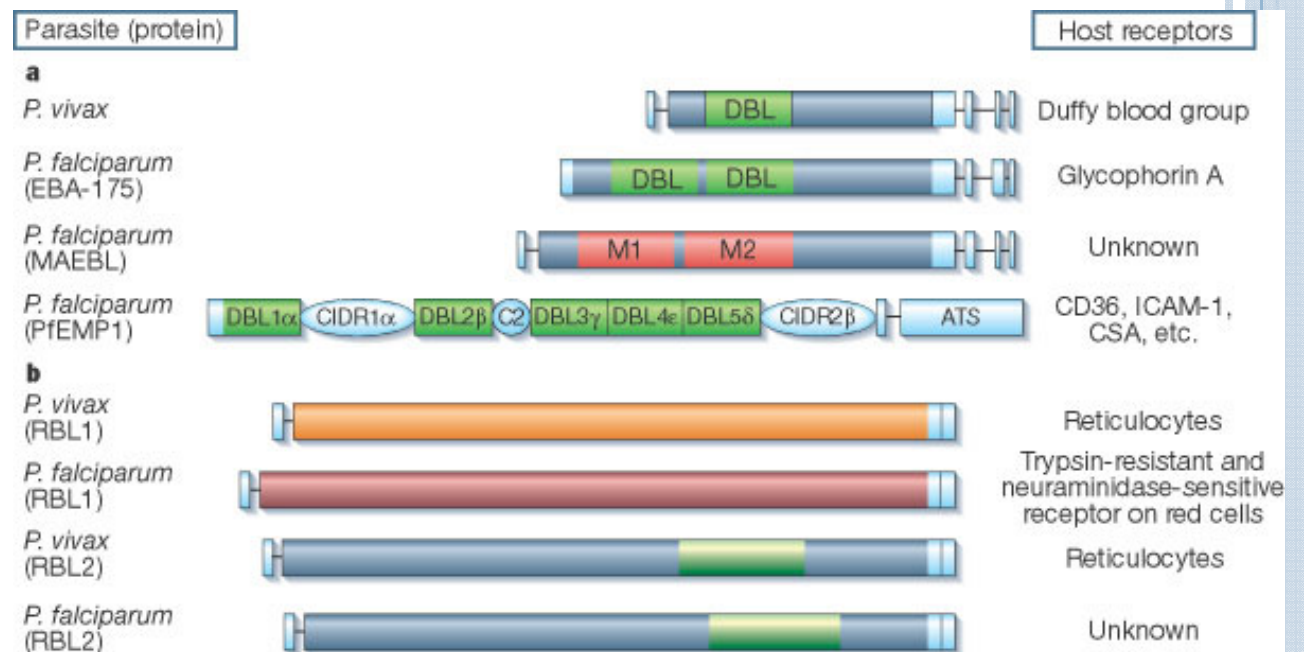
# ANTIGENIC VARIATION AND SEQUESTRATION: PATHOLOGICAL IMPLICATIONS

- PfEMP1 variation leads to distinct waves of parasitemia
- This receptor also causes RBC clumping and sequestration to avoid clearance and detection in the spleen
- These effects can be particularly deleterious in the placenta and the brain



# DIFFERENT MALARIA SPECIES UTILIZE DIVERSE HOST RECEPTORS

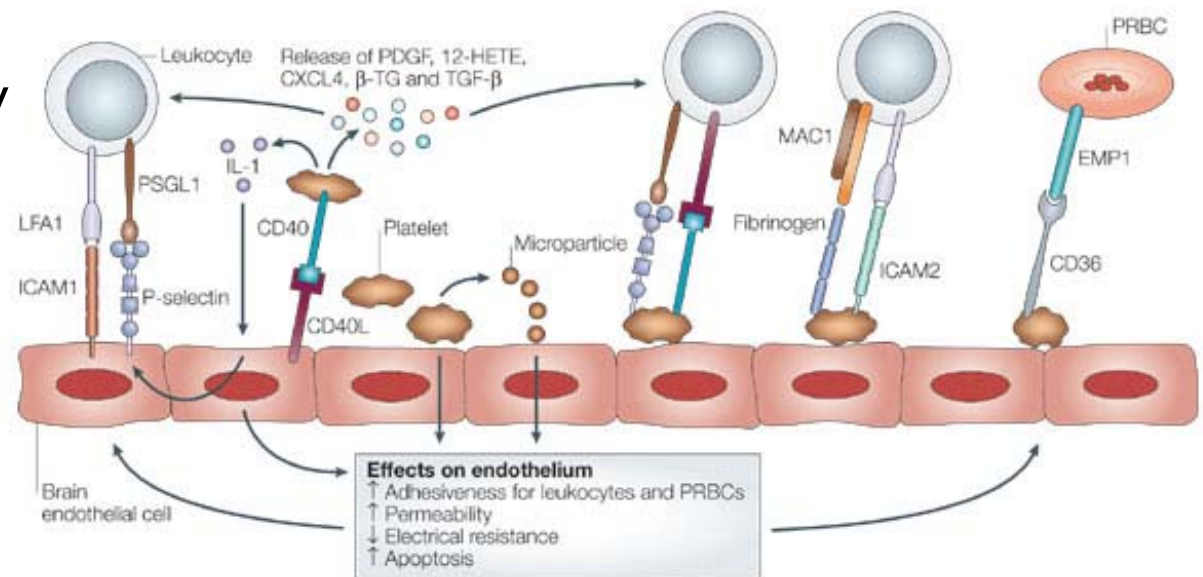
- The strength of malaria as an evolutionary pressure can be observed in West Africa, where mutations to eliminate expression of the Duffy blood group protect individuals from *P. vivax*





# PLATELETS CAN PLAY IMPORTANT IMMUNOLOGICAL ROLES

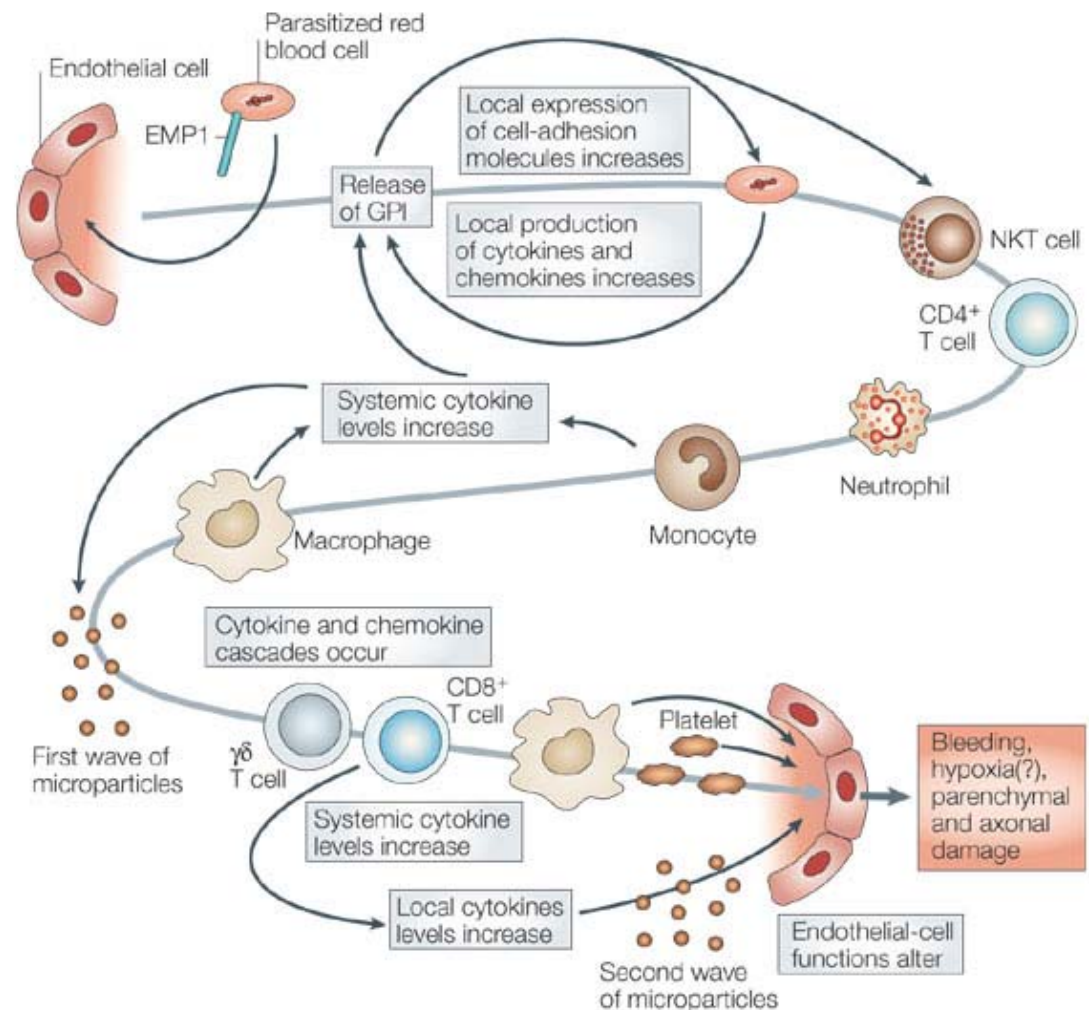
- Platelets are activated by TNF and can secrete other pro-inflammatory mediators such as IL-1, leading to an increased local inflammation and providing additional receptors for the recruitment of parasitized blood cells



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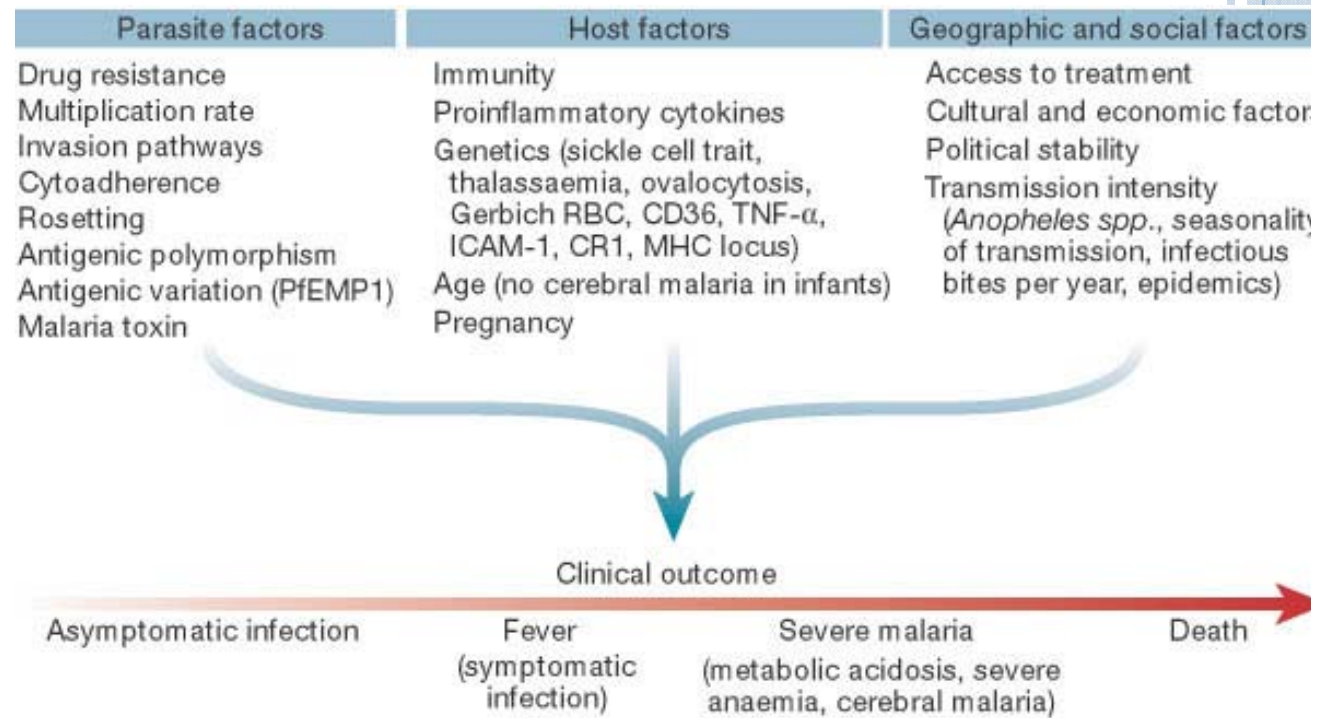
# PATHOLOGY OF CEREBRAL MALARIA

- A cascade of immunological stimulation leads to the accumulation of effector cell types and systemic hypercytokinemia, permeabilizing endothelial layers and contributing to blockages



# HOST/PARASITE/TREATMENT COMBINE TO DETERMINE OUTCOME FROM DISEASE

- Important to consider that only 1/200-500 infections is lethal
- The most important considerations are likely the parasite (only falciparum is commonly lethal) and immunity



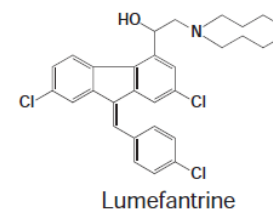
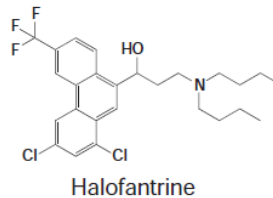
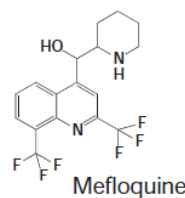
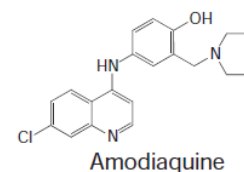
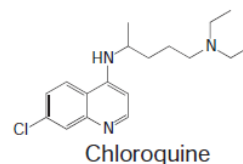
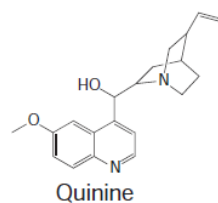
# ANTI-MALARIAL DRUGS

Box 1

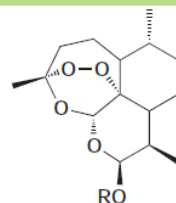
## Overview of antimalarial drugs

| Drug  | Main limitations  |
|---|---|
| Chloroquine<br>Quinine<br>Amodiaquine<br>Mefloquine<br>Halofantrine | Resistance<br>Compliance/safety/resistance<br>Safety/resistance<br>(Safety)/resistance/(cost)<br>Safety/resistance/cost |
| Artemisinins<br>(artemether, arteether, artesunate)                 | Compliance/(safety)/(GMP)/<br>(cost)  |
| Sulphadoxine–<br>pyrimethamine                                      | Resistance  |
| Atovaquone–<br>proguanil  | Resistance potential/cost   |
| Lumefantrine–<br>artemether   | (Compliance)/resistance<br>potential/(cost)   |

### Quinoline and related antimalarials

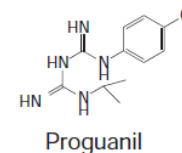
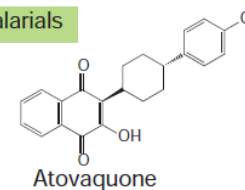
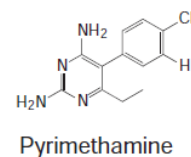


### Artemisinin antimalarials



R=H Dihydroartemisinin  
R=Me Artemether  
R=Et Arteether  
R=CO(CH<sub>2</sub>)<sub>2</sub>CO<sub>2</sub>H Artesunate

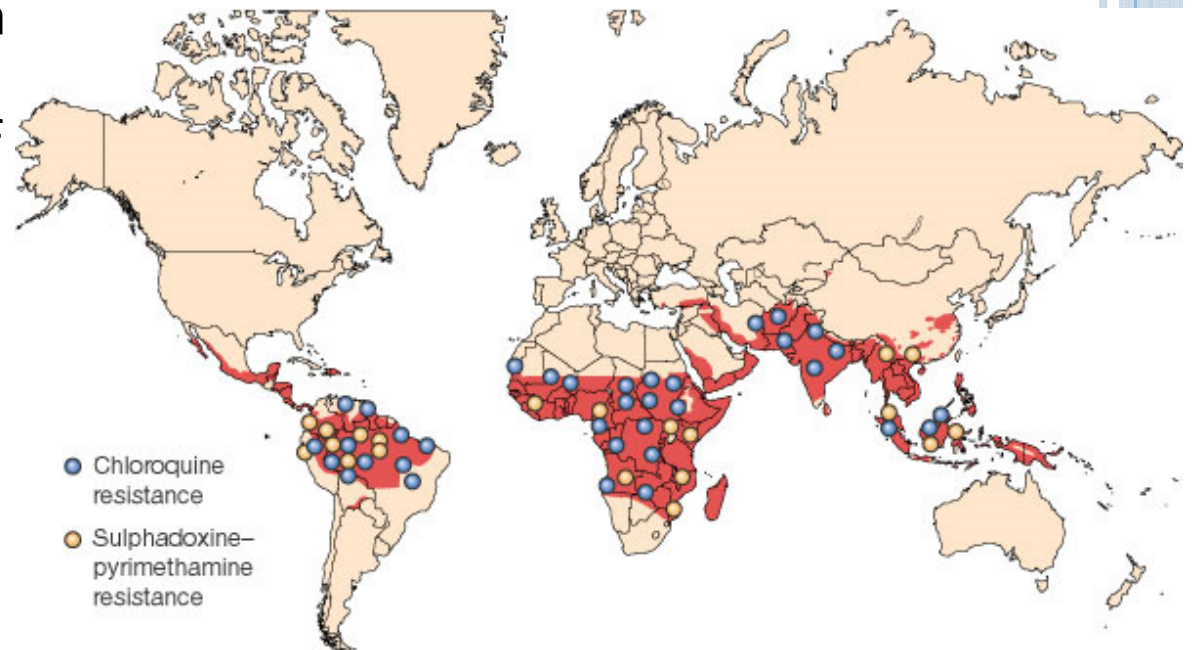
### Other antimalarials



Drugs that are currently in use as antimalarials have many attributes, but also possess certain liabilities that might be improved by further drug discovery and development. Liabilities placed in brackets refer to issues that are less serious for the drugs in question than those liabilities not placed in brackets. The structures of the main antimalarial drugs are provided for reference.

# RESISTANCE LIMITS DRUG EFFICACY

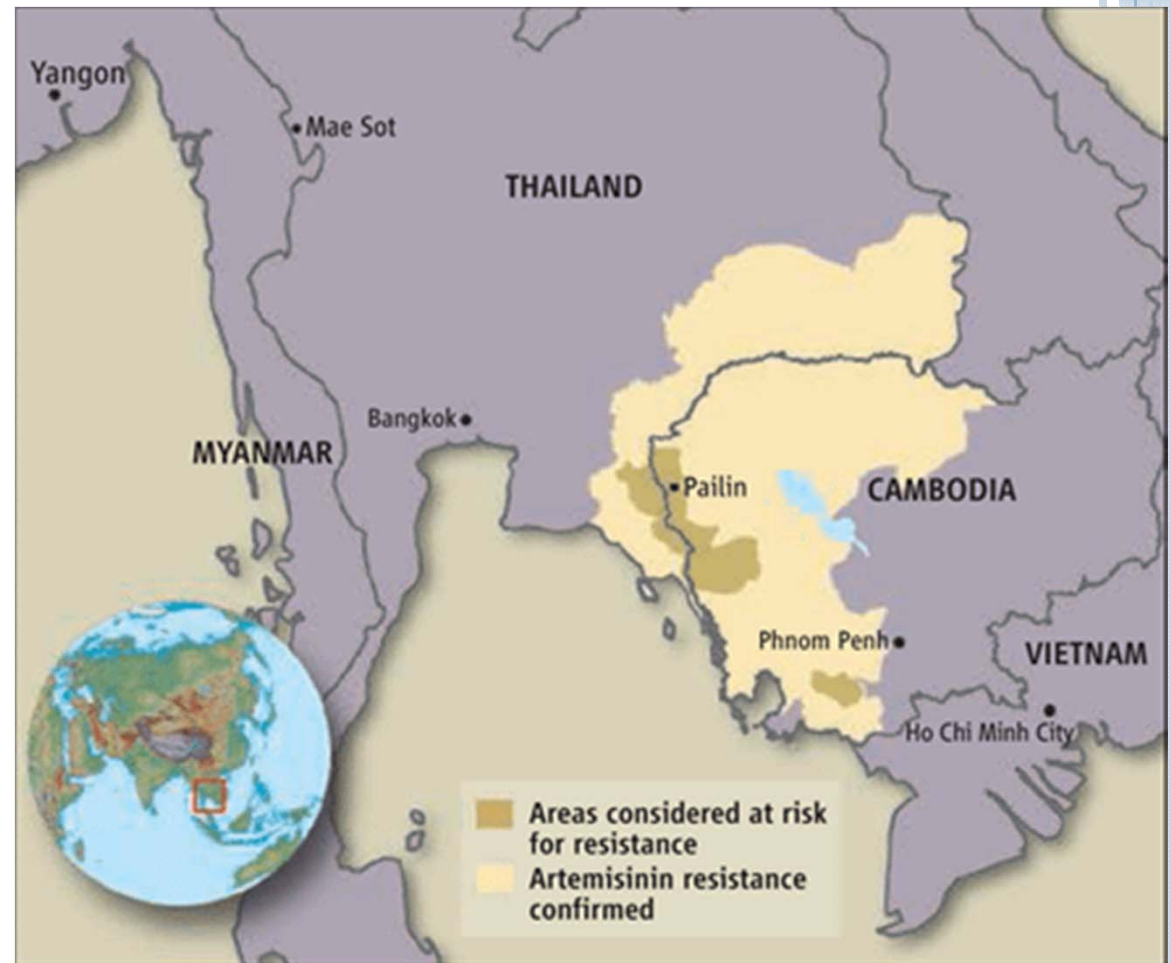
- Each new drug introduction has seen a rapid development and spread of resistance worldwide
- Most drug targets are enzymatic allowing parasite mutation to overcome drug sensitivity
- Chloroquine targeted a chemical process—resistance developed more slowly, but is now widespread





# EMERGING RESISTANCE TO ARTEMISININ

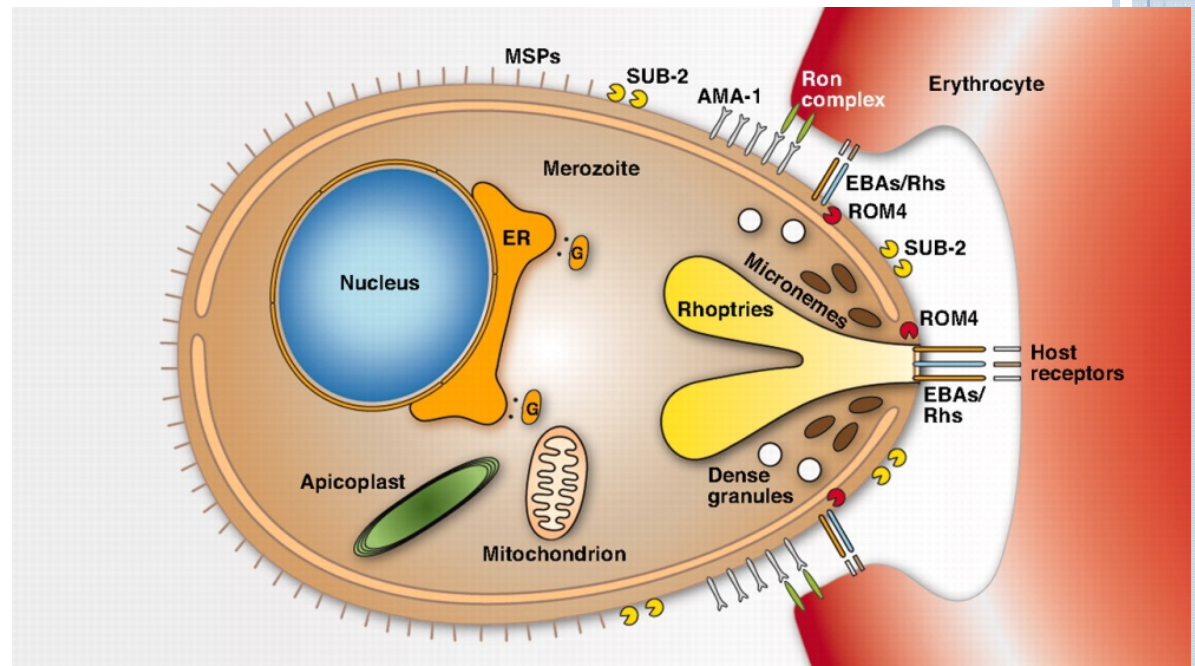
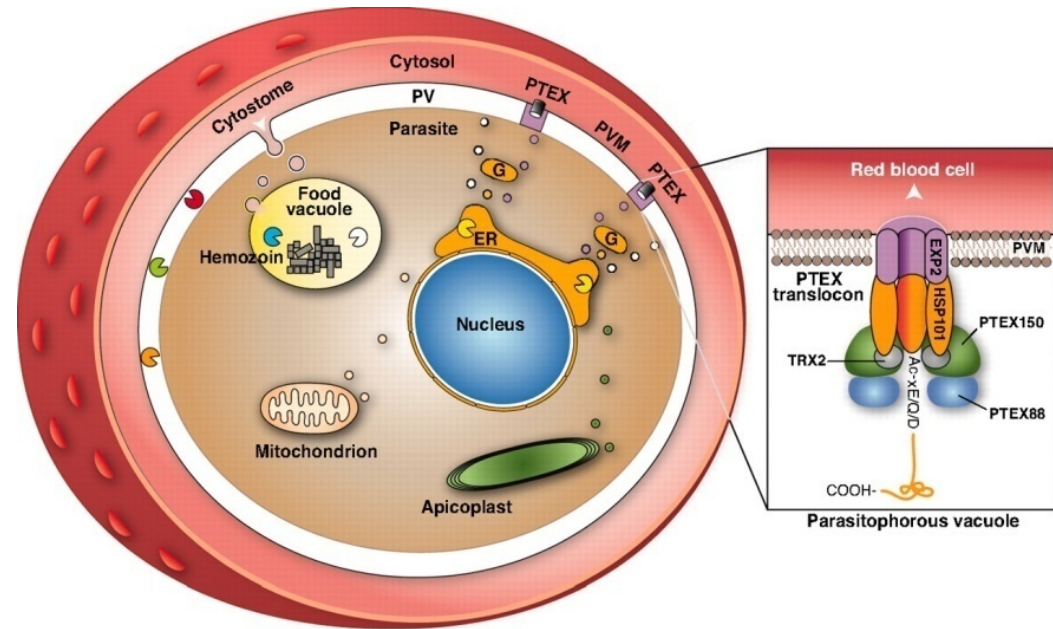
- No total resistance noted, but increased clearance times developing
- Monotherapies may be one cause of increased resistance (now banned in Cambodia)
- Mass treatment approach in heavily endemic areas also considered





# NEW AND OLD TARGETS OF THE MEROZOITE STAGE

- Molecular characterization of the merozoite itself and the process of RBC invasion may provide novel targets that will hopefully have minimal side effects due to their unique structure and function



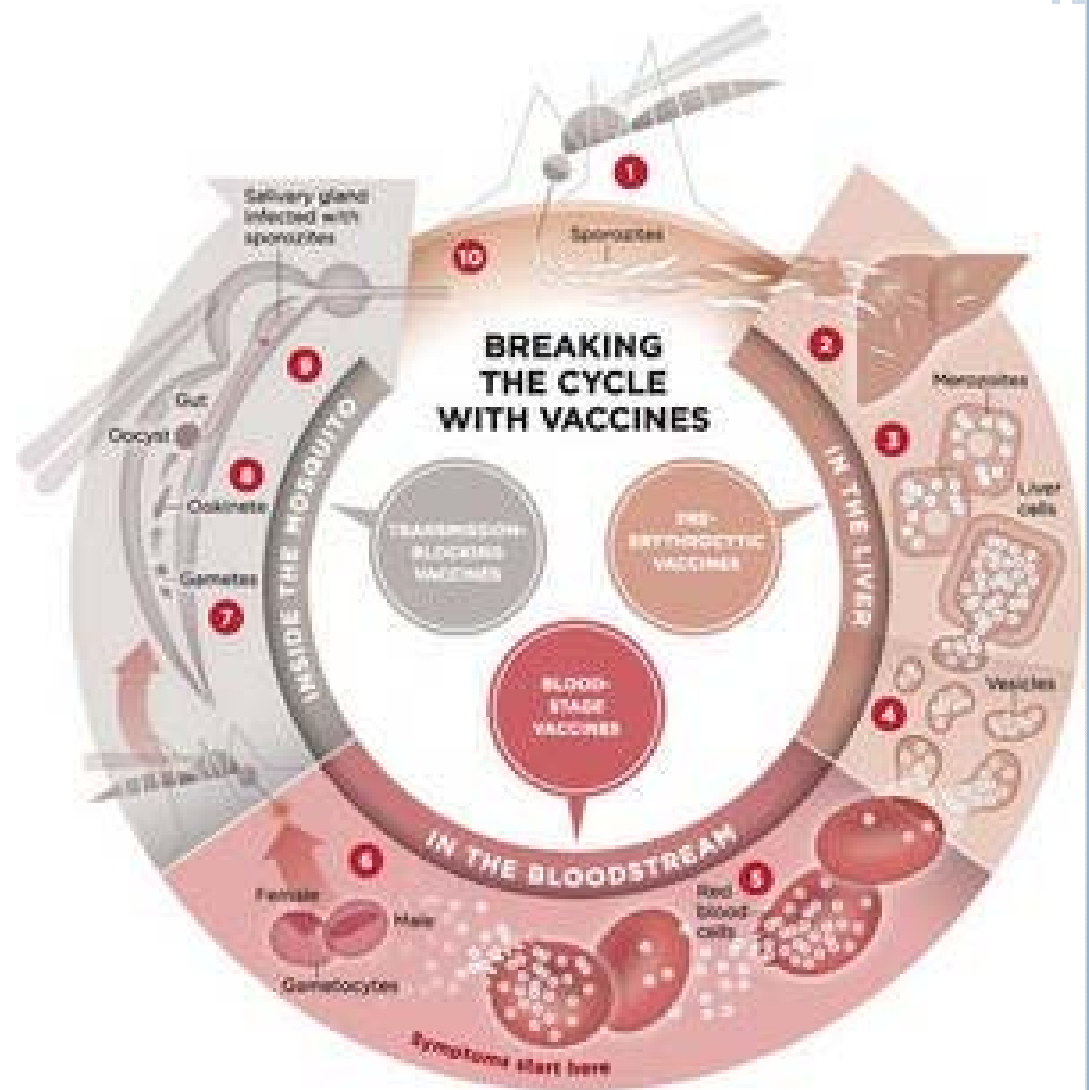
## VECTOR CONTROL

- 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)



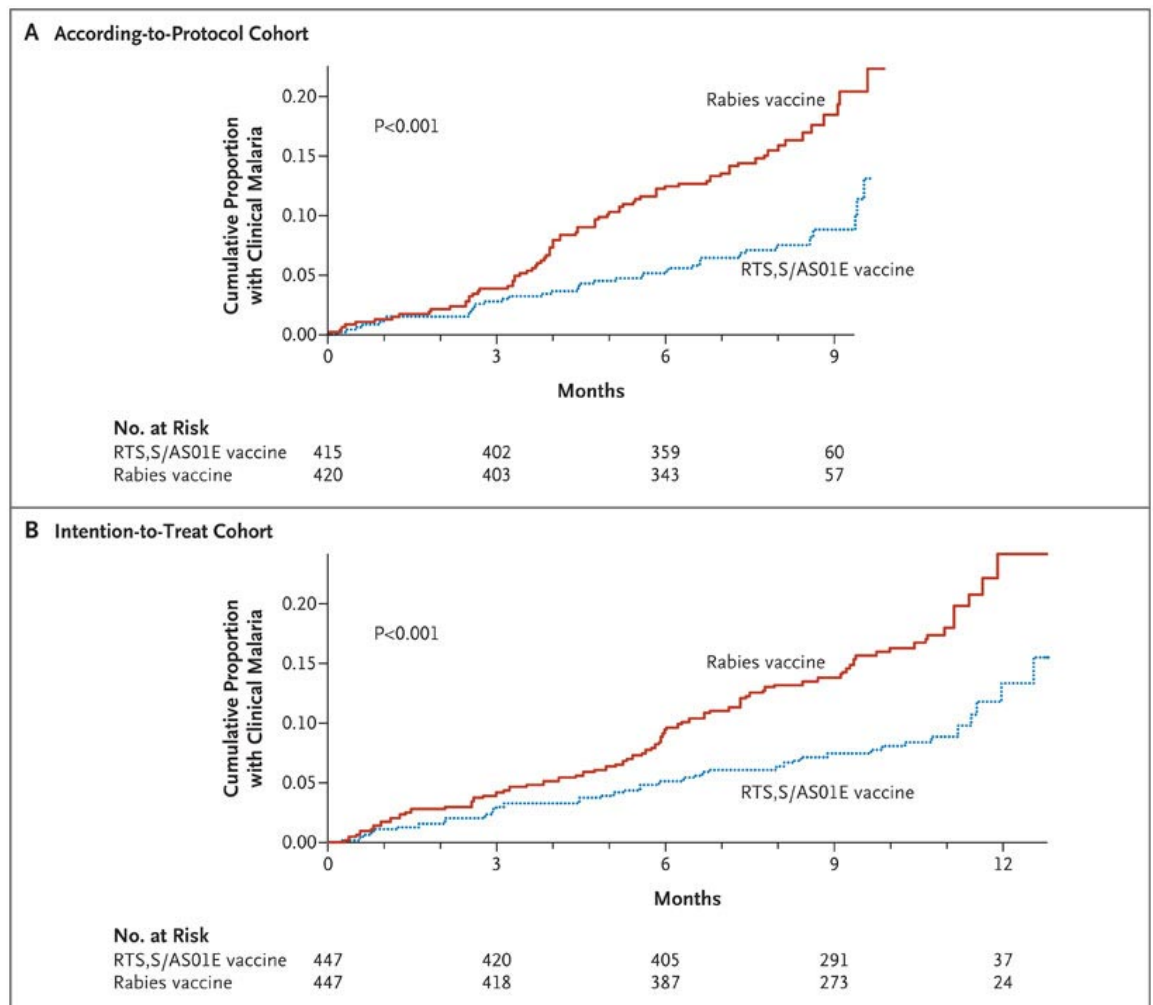
# MALARIA VACCINE TARGETS

- Three types of vaccines have been proposed, with variations in each group:
  - Pre-erythrocytic 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 circumsporozoite 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

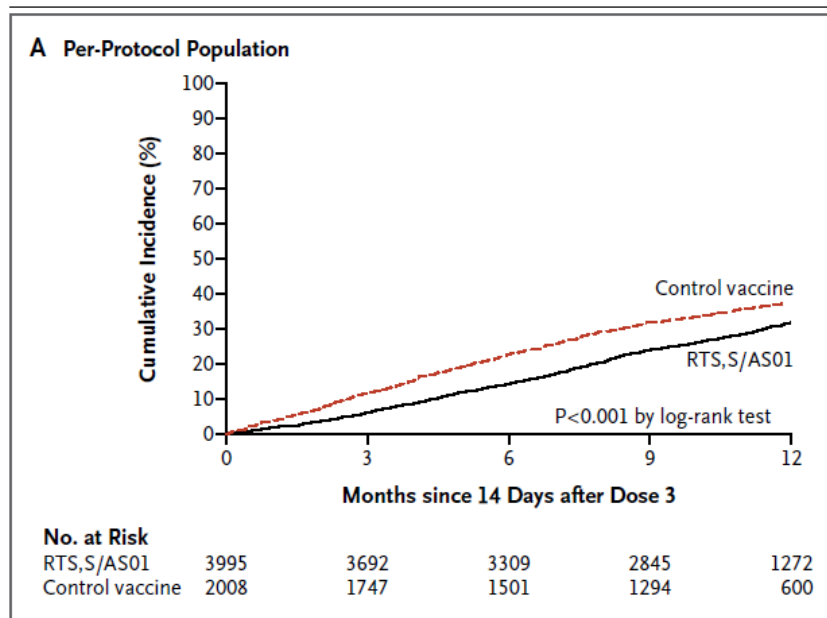
The NEW ENGLAND JOURNAL of MEDICINE

### 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

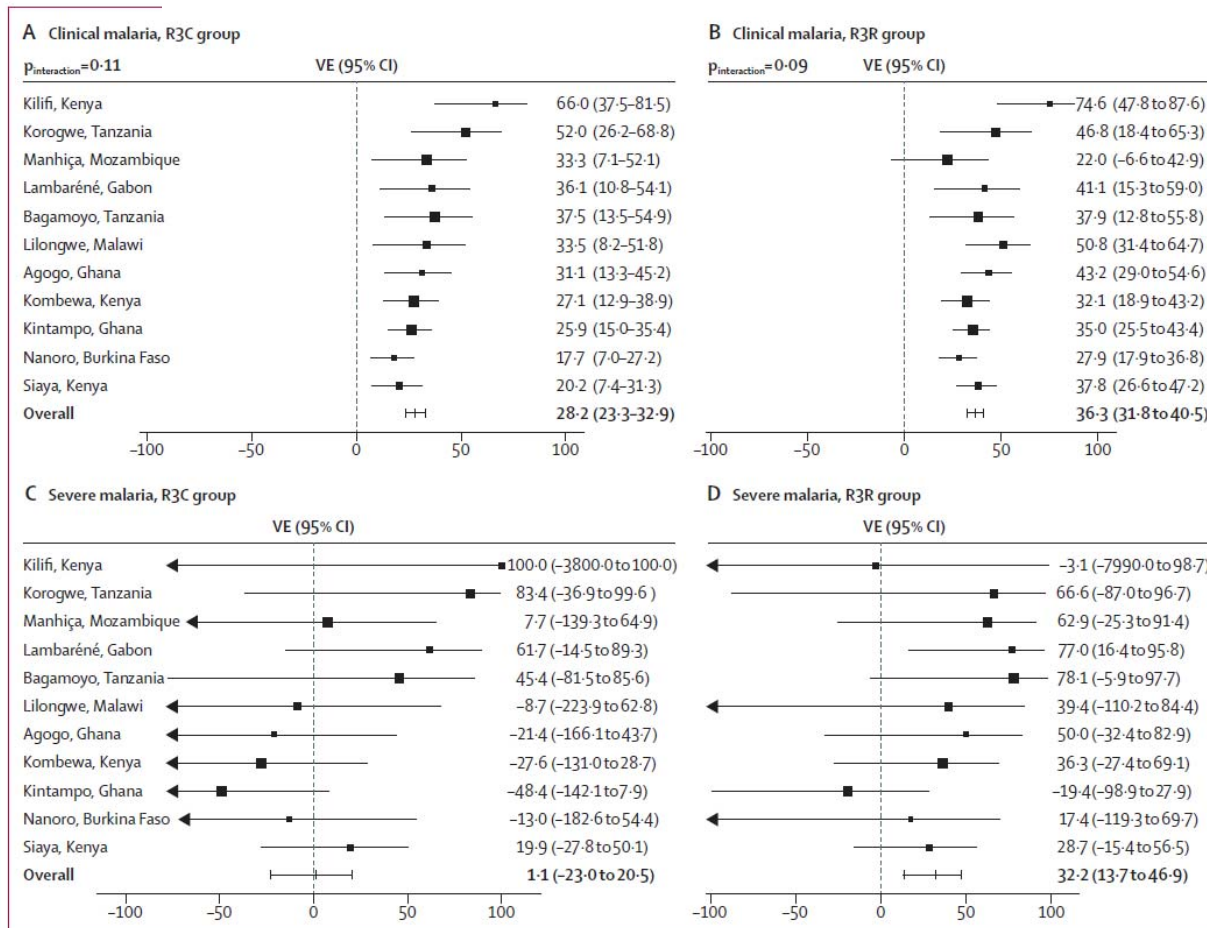


# Efficacy and safety of RTS,S/AS01 malaria vaccine with or without a booster dose in infants and children in Africa: final results of a phase 3, individually randomised, controlled trial



RTS,S Clinical Trials Partnership\*

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



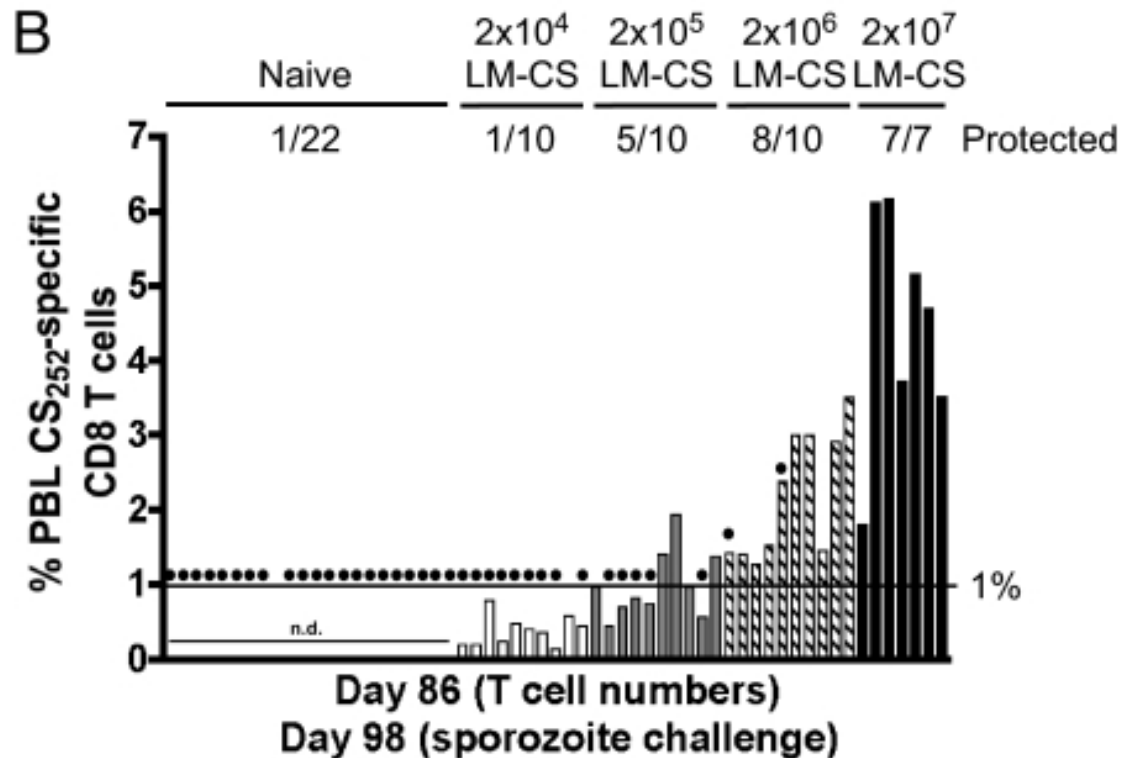
**Figure 3: Vaccine efficacy against clinical and severe malaria by study site in the 5-17 months age category**

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.  $p_{\text{interaction}}$  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.



# CAN CELLULAR RESPONSES WORK AGAINST INFECTED LIVER CELLS?

- Prime-boost-boost approach to drive extraordinarily high numbers of antigen-specific CD8 T cells (1% of all T cells needed)
- “Threshold” effects demonstrating effective surveillance of liver stage infection by CD8 T cells



**C**

| %CS <sub>252</sub> <sup>-</sup> specific PBL | Challenged | Protected | % Protected |
|--|------------|-----------|-------------|
| Day 98-422                                   |            |           |             |
| ≥1%  | 58         | 56        | 97%         |
| <1%  | 16         | 1         | 6%          |

Proc Natl Acad Sci U S A. 2008 September 16; 105(37): 14017–14022.

## 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

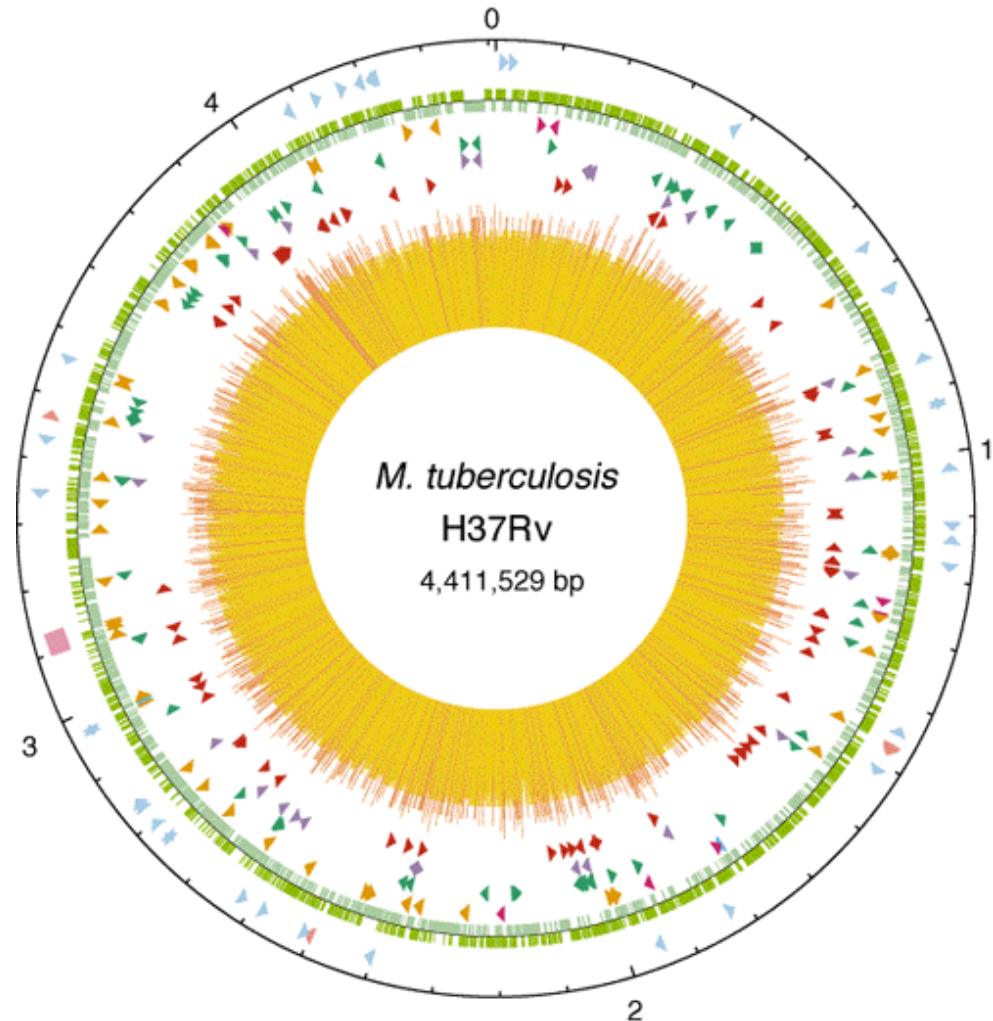




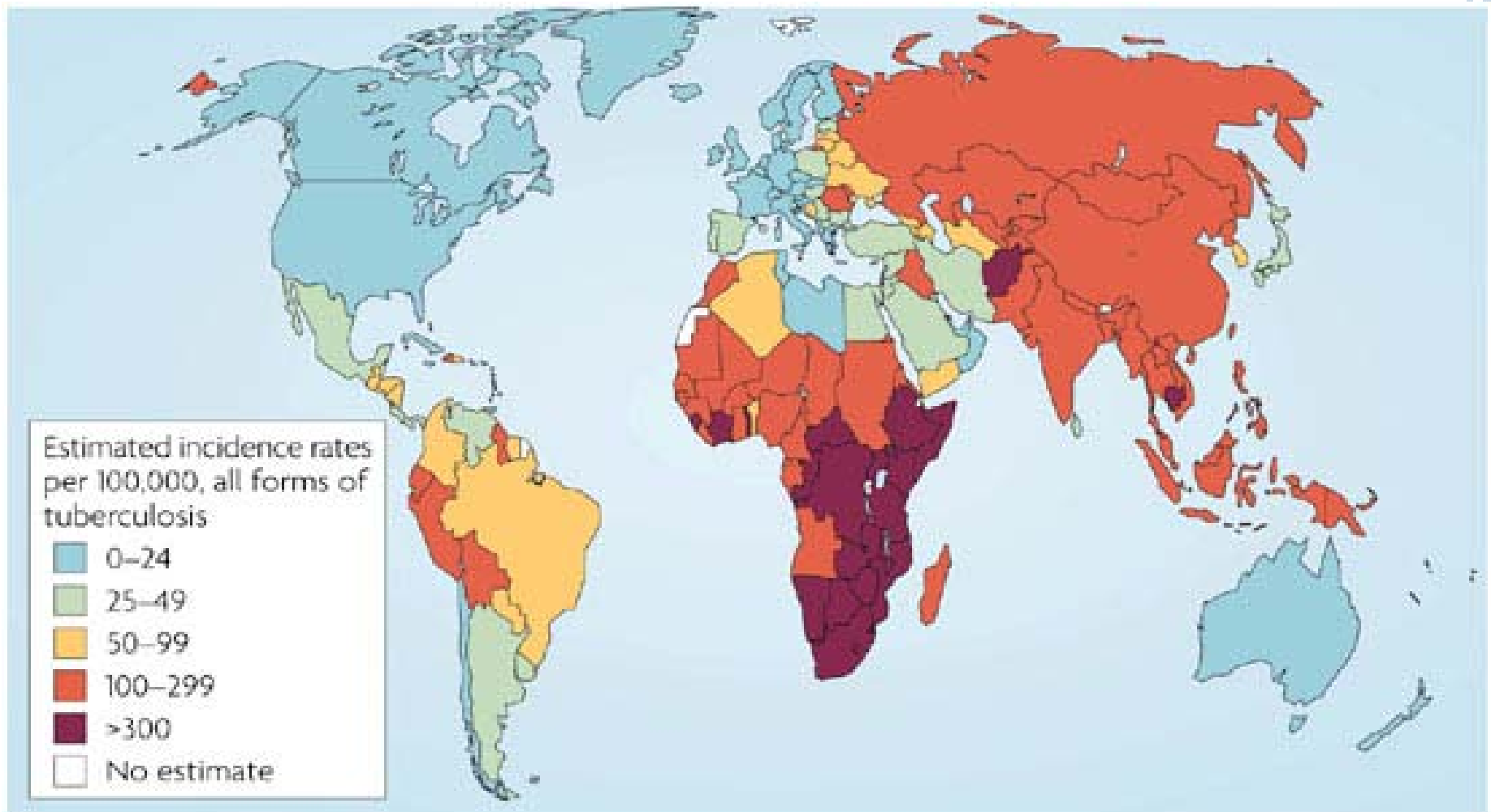
# IMMUNE CONTROL OF *MYCOBACTERIUM* *TUBERCULOSIS*

# *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



## MTB IMPACT

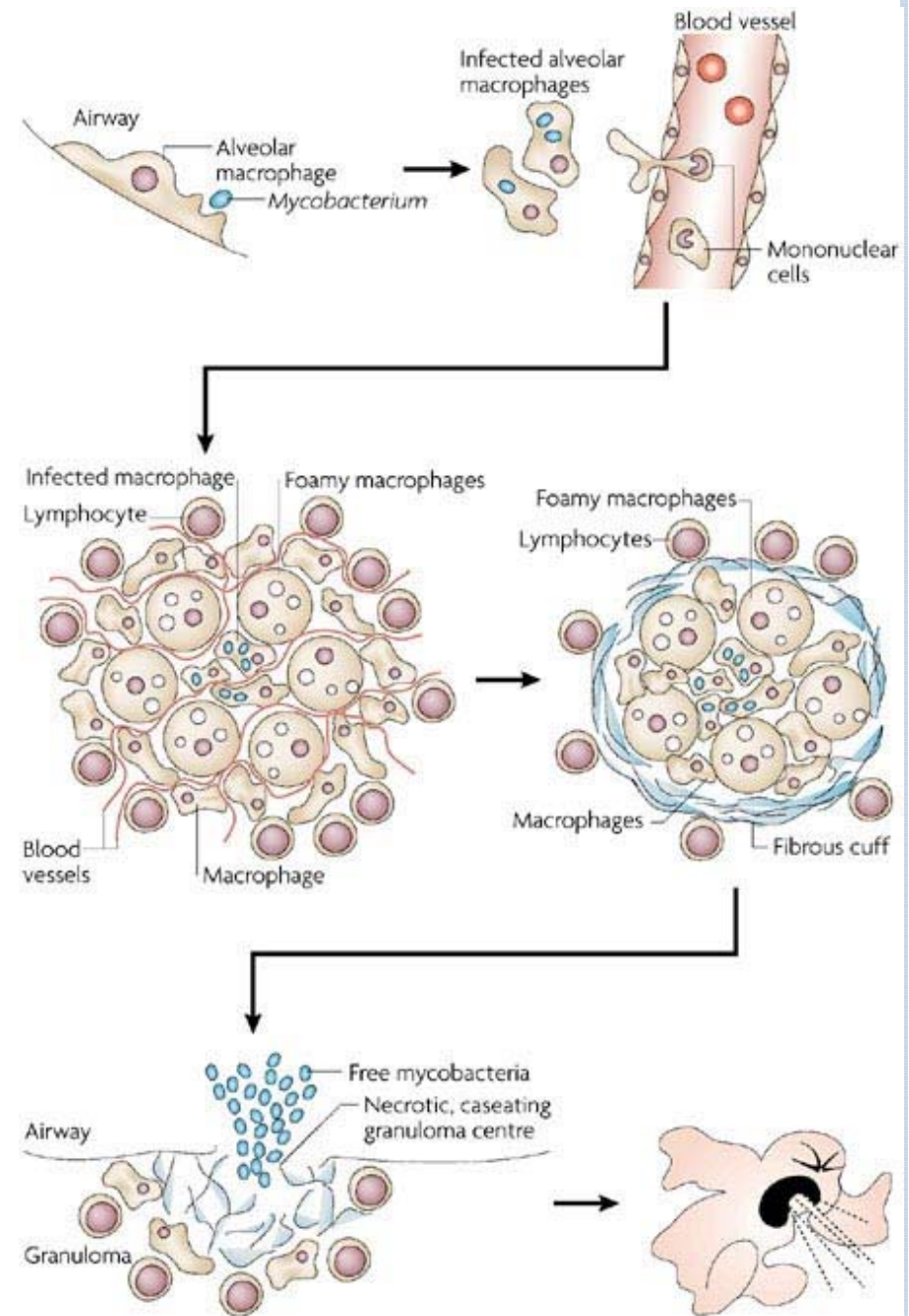
- 2.2 million deaths/year
- Burden of diseases in DALY (disability-adjusted life years)
- Total Disability Adjusted Life Years: 45 million (3.1%).
- 2 billion individuals infected with M. tuberculosis
  - 10% risk of developing disease following infection
  - Untreated, disease mortality is 50%
- 8 million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



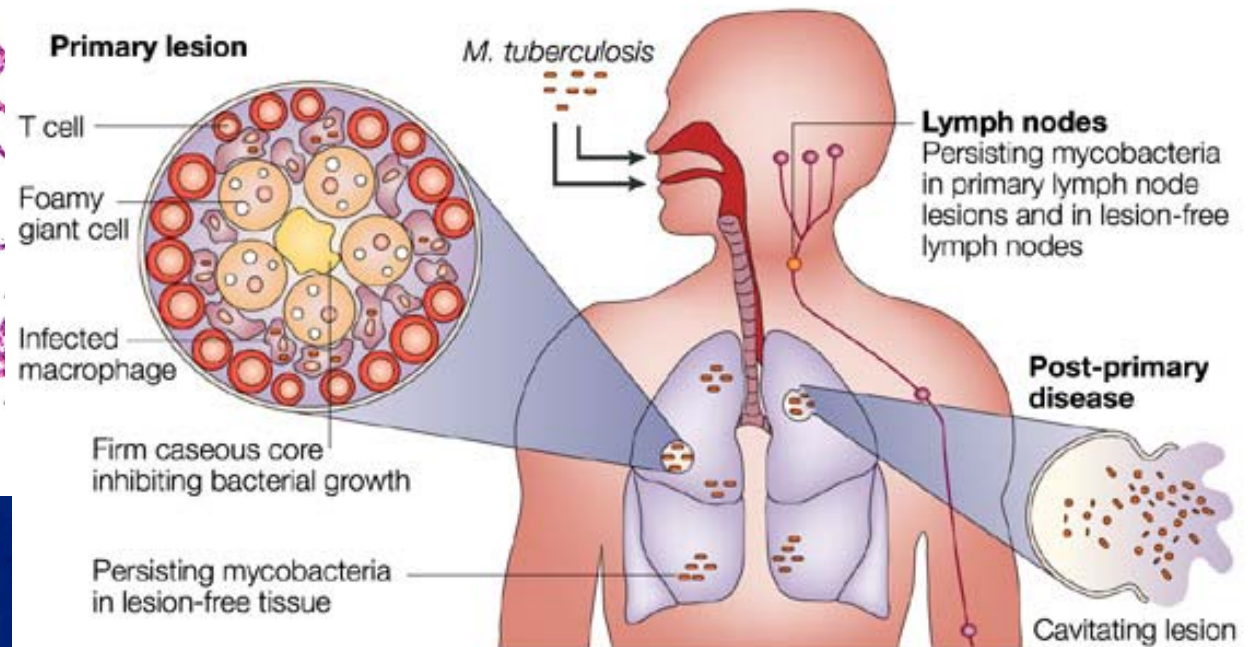
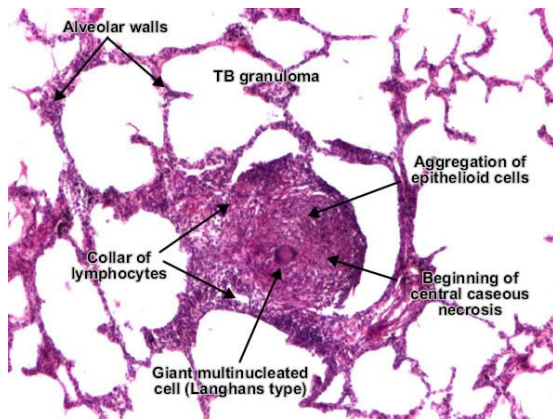


# MTB LIFE CYCLE

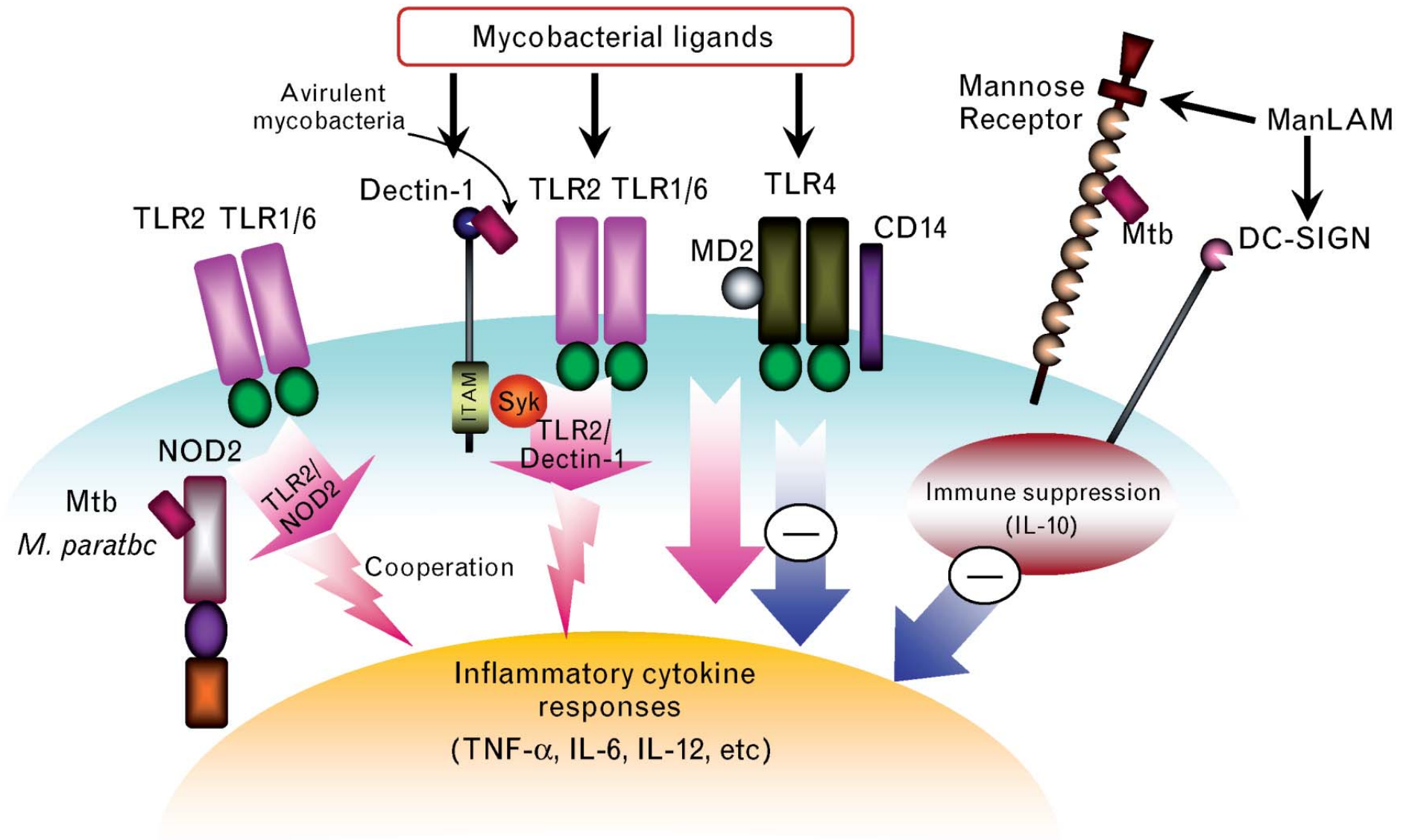
- MTb replicates in and accumulates in macrophages, mostly in the lung (though other tissue sites are possible)
- The accumulation of infected macrophages, surrounded by other leukocytes forms a unique structure called the granuloma, the characteristic feature of MTb-associated lung damage



# MTB LIFE CYCLE PART 2



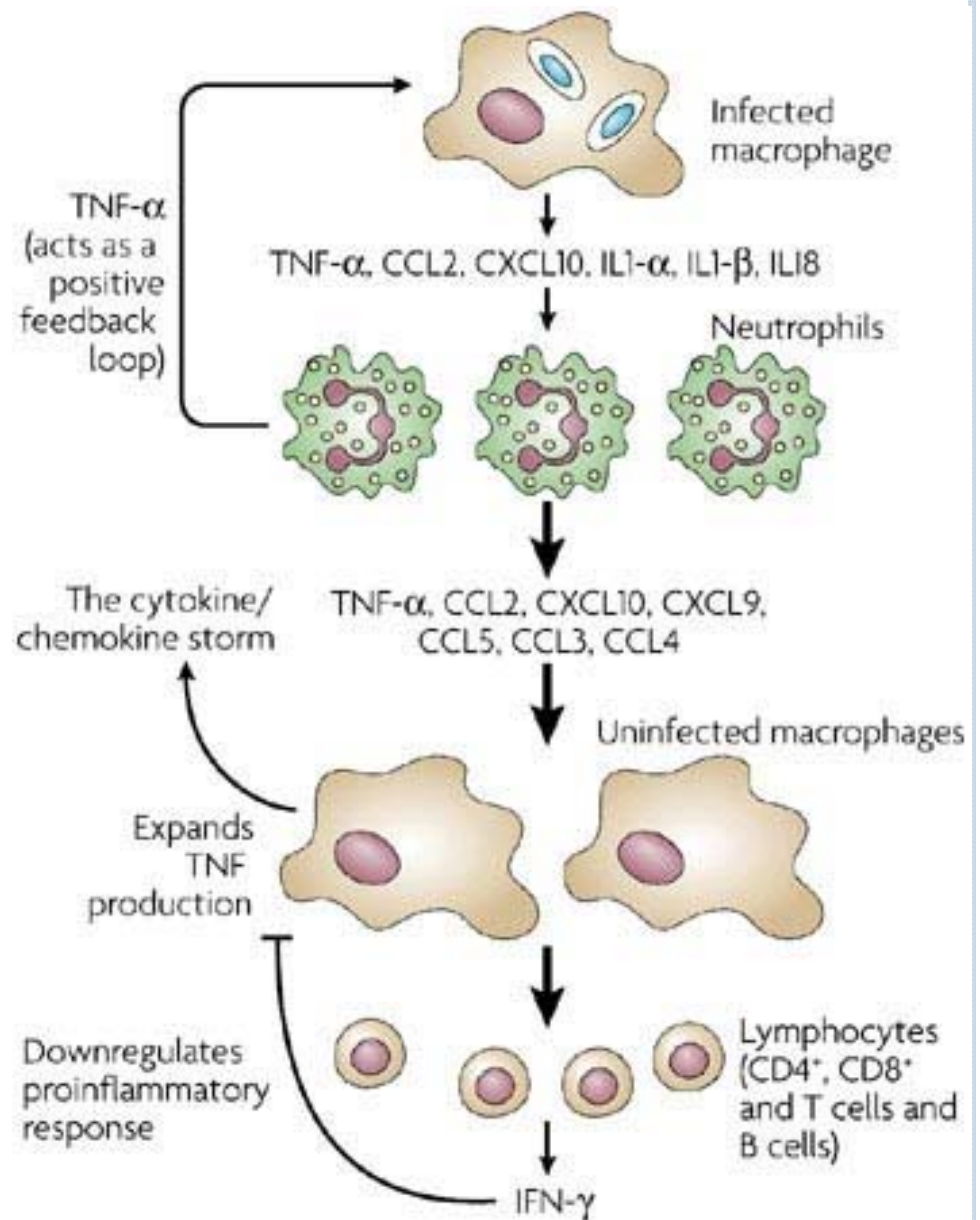
# INNATE RECOGNITION OF MTB





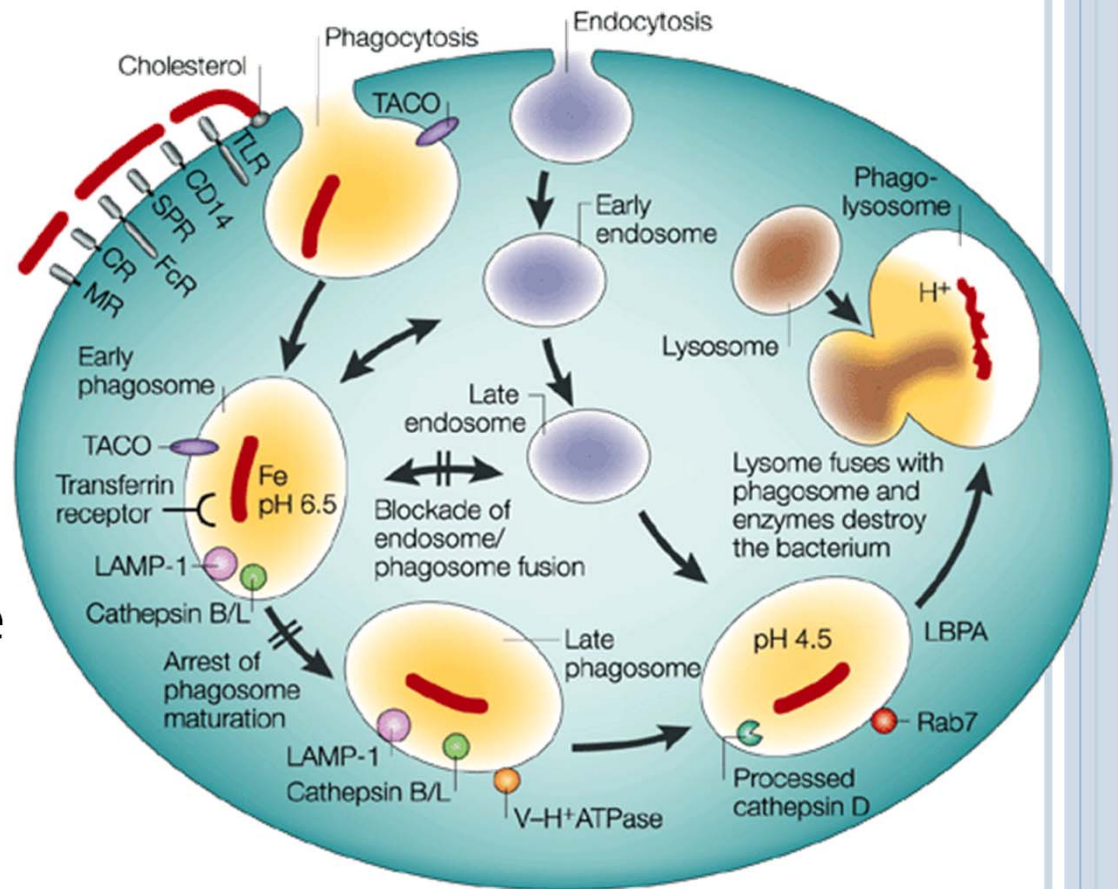
# ACTIVATION OF CYTOKINE STORM

- 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

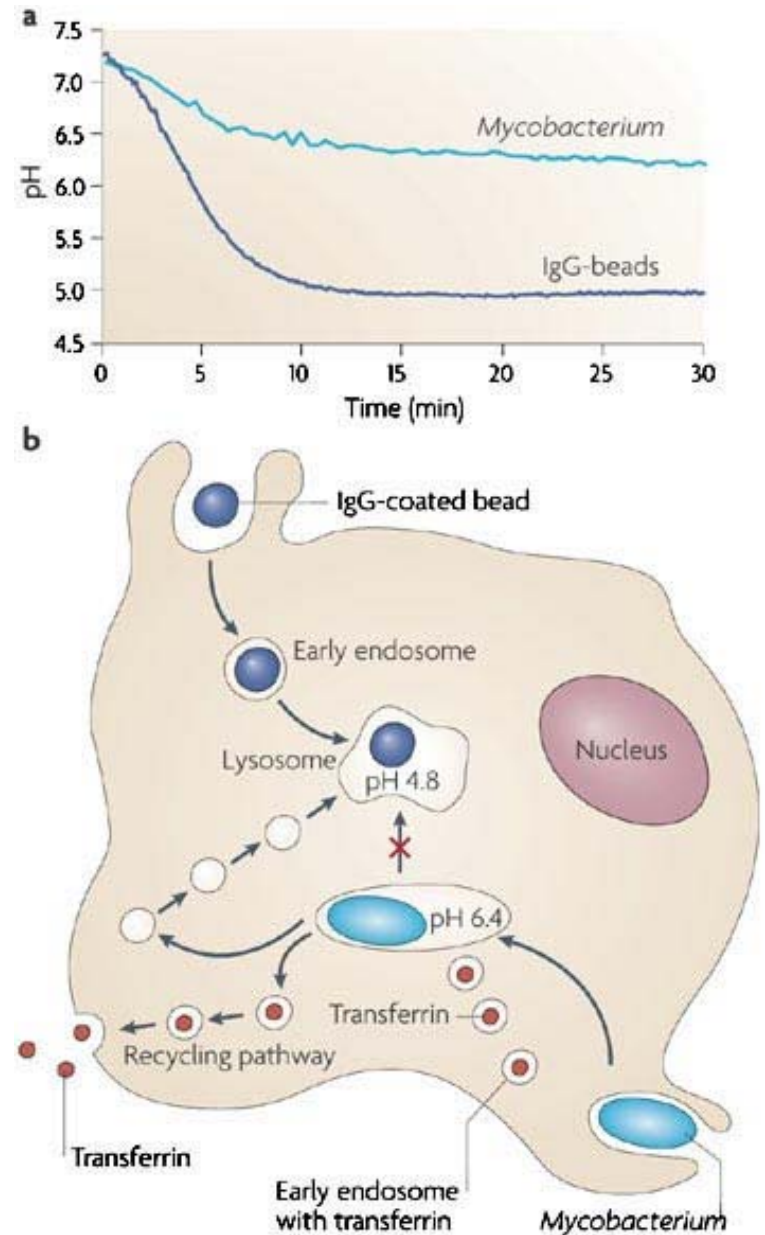
- After uptake by scavenger receptors, MTb arrests the maturation and fusion of the phagosome with the endosome
- Highly activated macrophages (IFN- $\gamma$  stimulation) can complete maturation and destroy the bacteria—otherwise, the bacteria remain latent or can grow





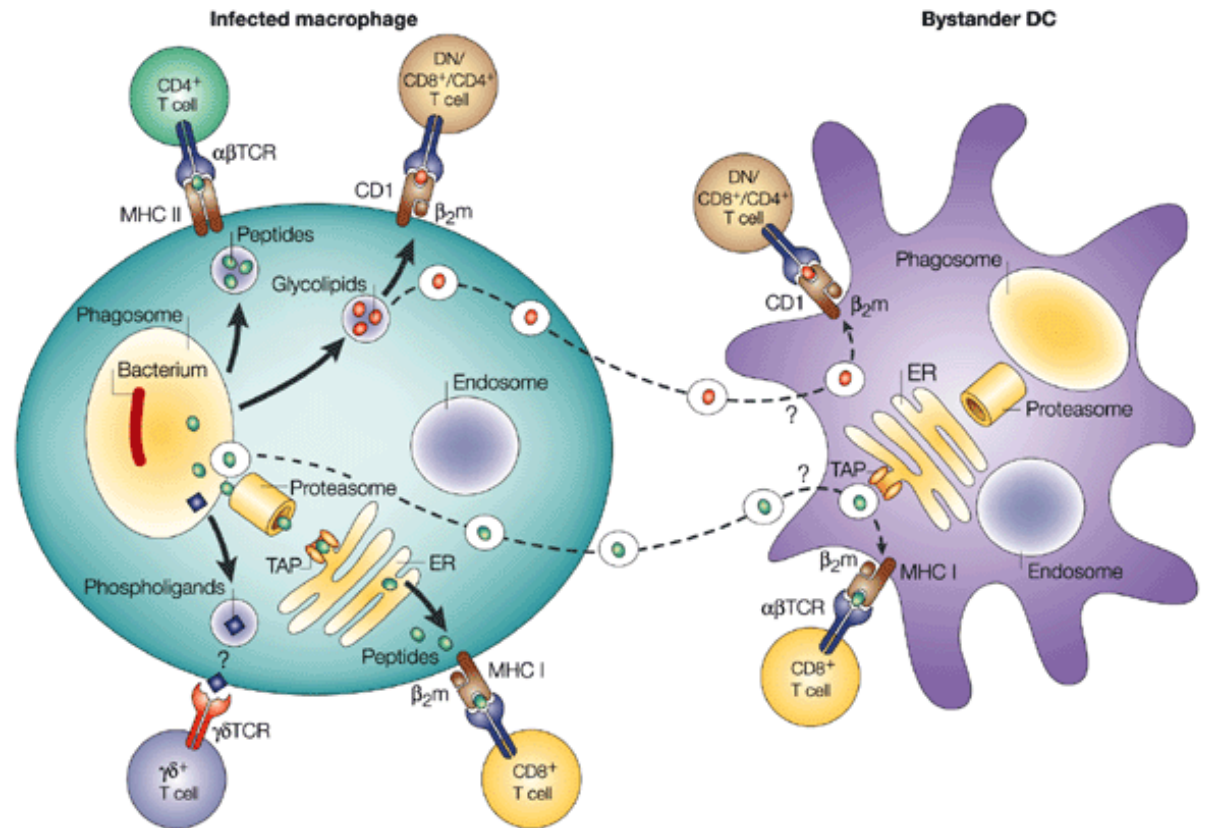
## PREVENTION OF ACIDIFICATION LEADS TO PERSISTENCE

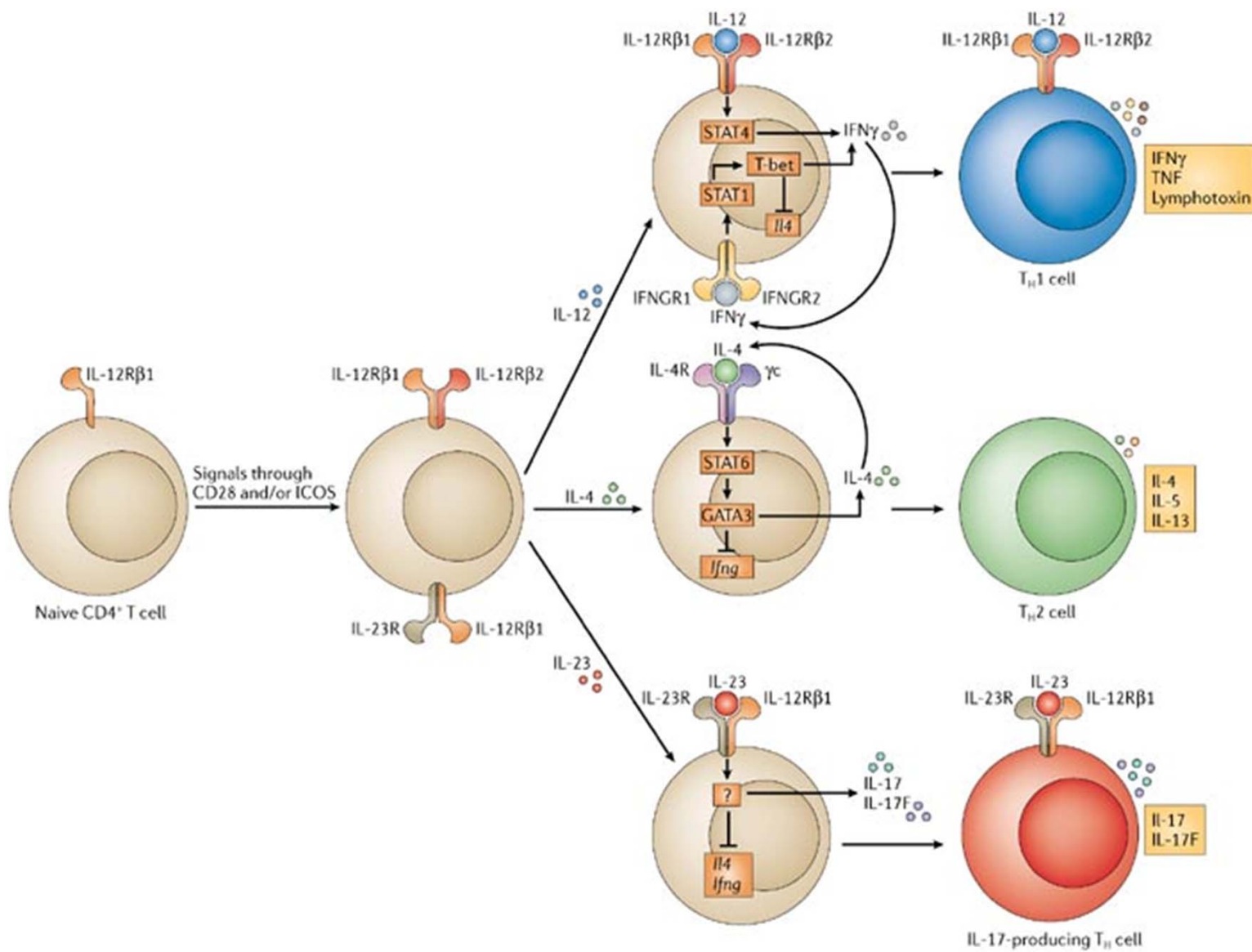
- MTb uses active processes to prevent acidification
- Inert latex beads complete the cycle and are rapidly “acidified” within five minutes of uptake
- Persistence also requires important metabolic adaptations (low oxygen environment) that allow continued bacterial growth and survival



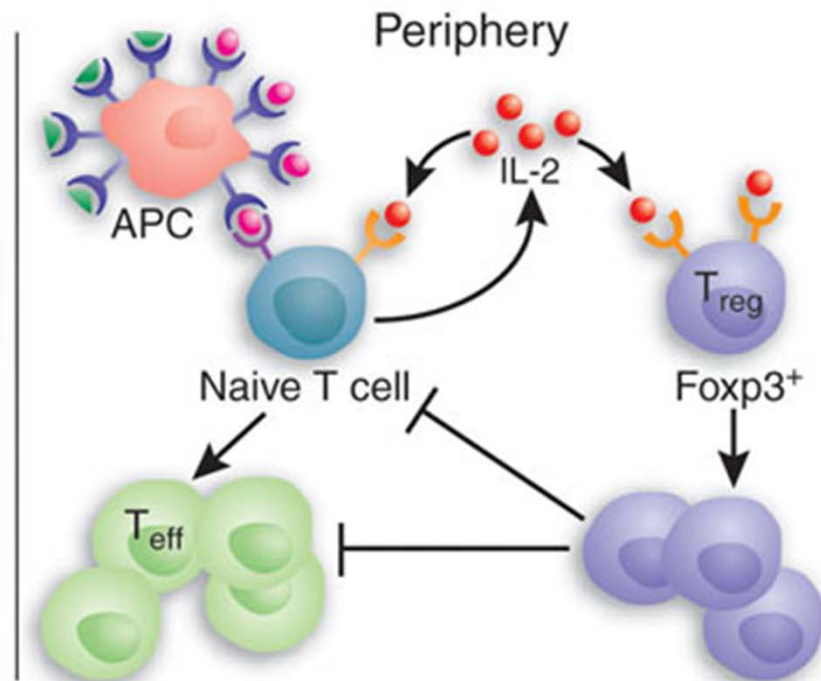
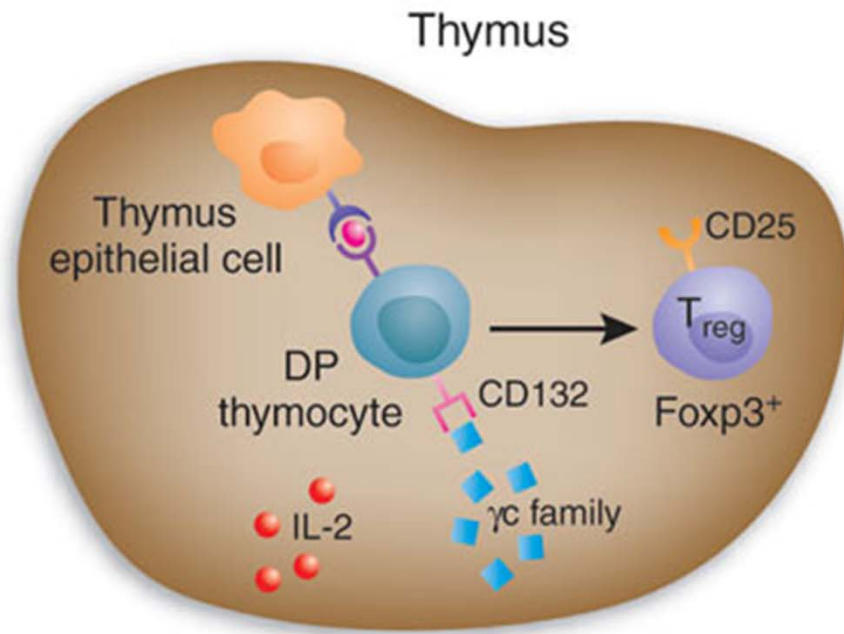
# INITIATION OF THE ADAPTIVE RESPONSE

- The cytokine storm initiated 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 CD4 and CD8 functions (cytokine regulation and direct cell clearance) are associated with protection from disease





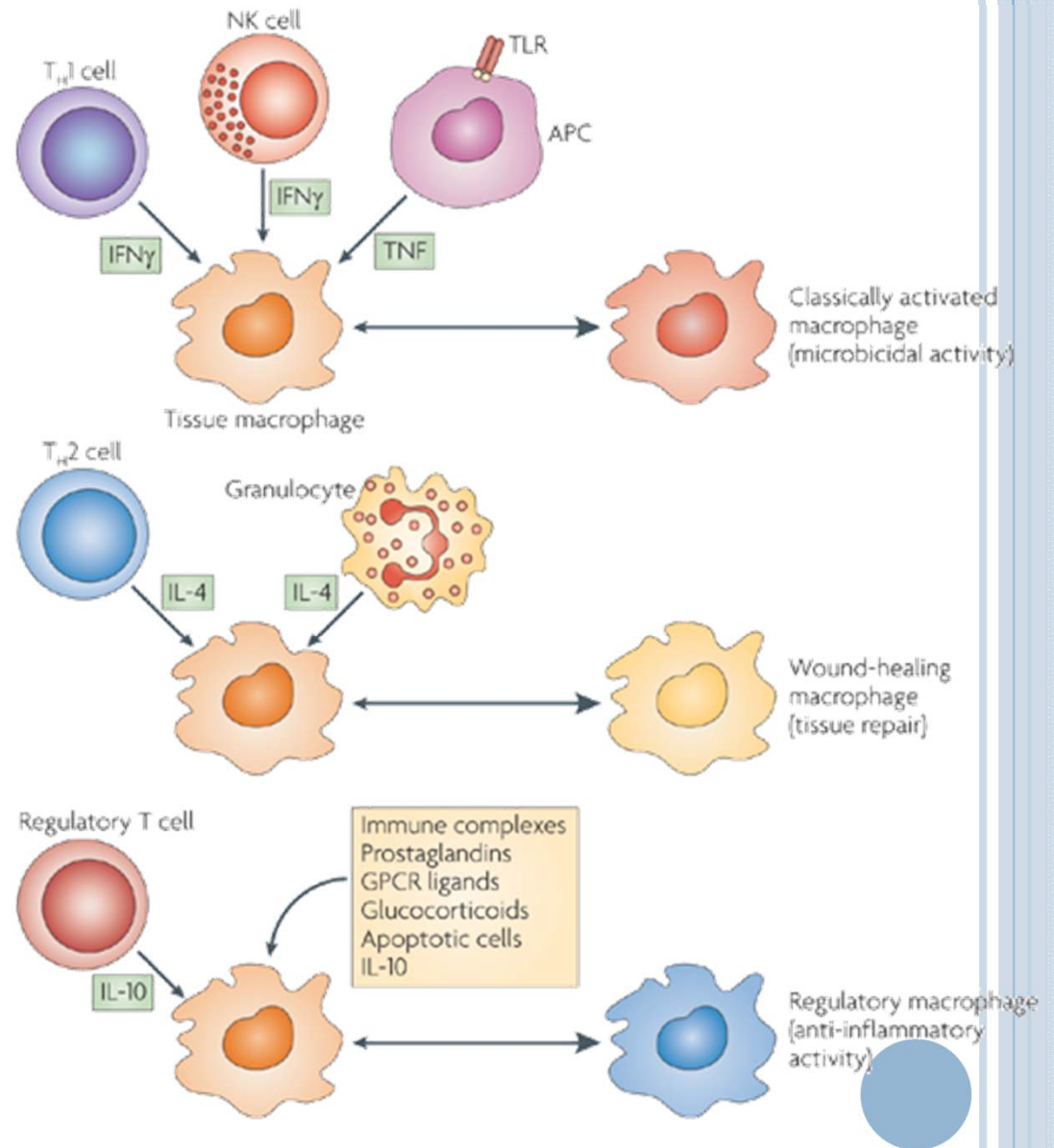
# GENERATION OF ANTIGEN-SPECIFIC REGULATORY T CELLS





# T CELL REGULATION OF MACROPHAGE EFFECTOR FUNCTION

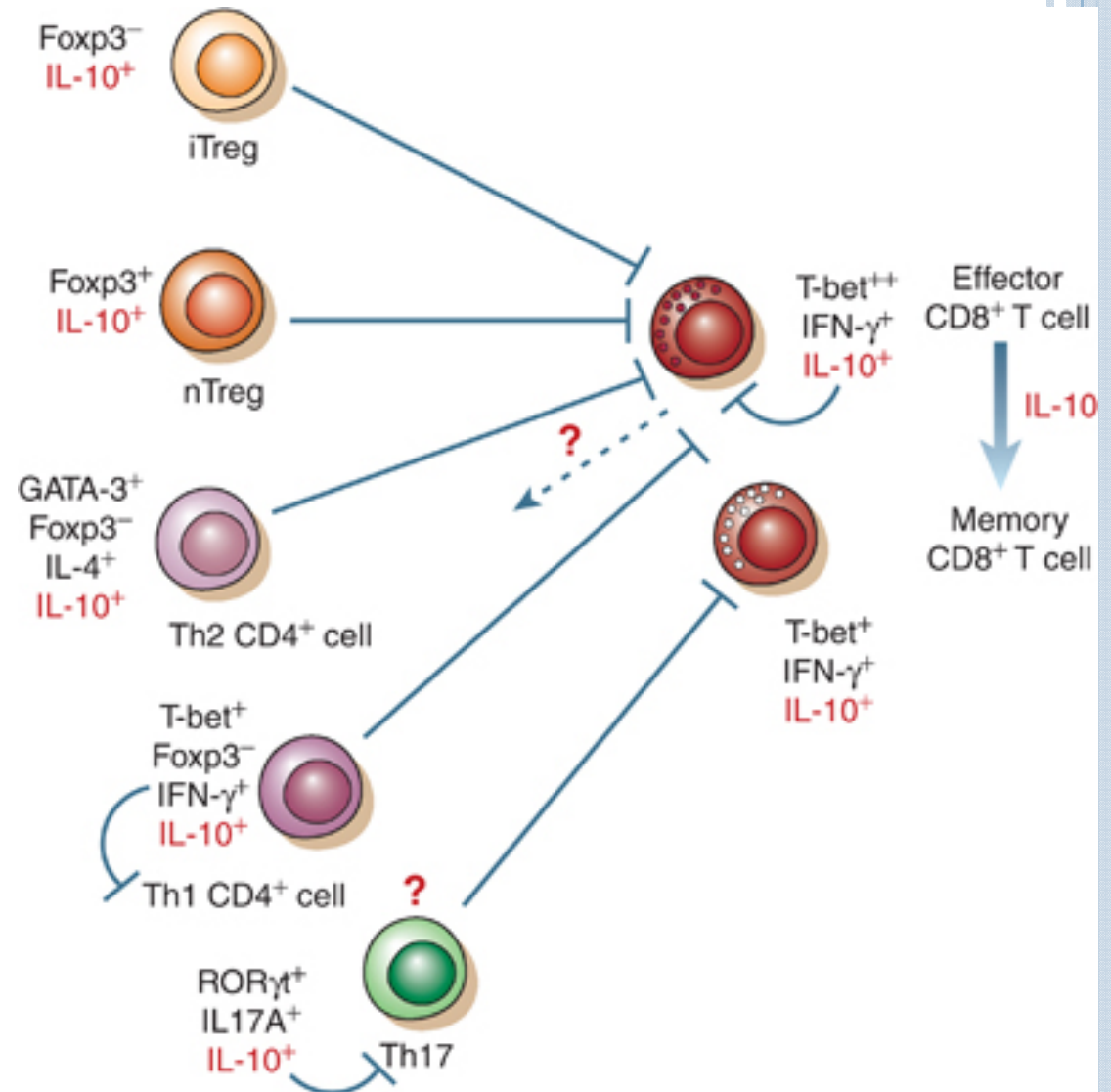
- The balance of regulatory vs. effector signals (and the various types of those signals) determine the activation milieu of the granuloma and the infected macrophage
- Immune-associated pathology is also a risk, so some regulatory balance is required to maintain the lung physiology while achieving clearance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



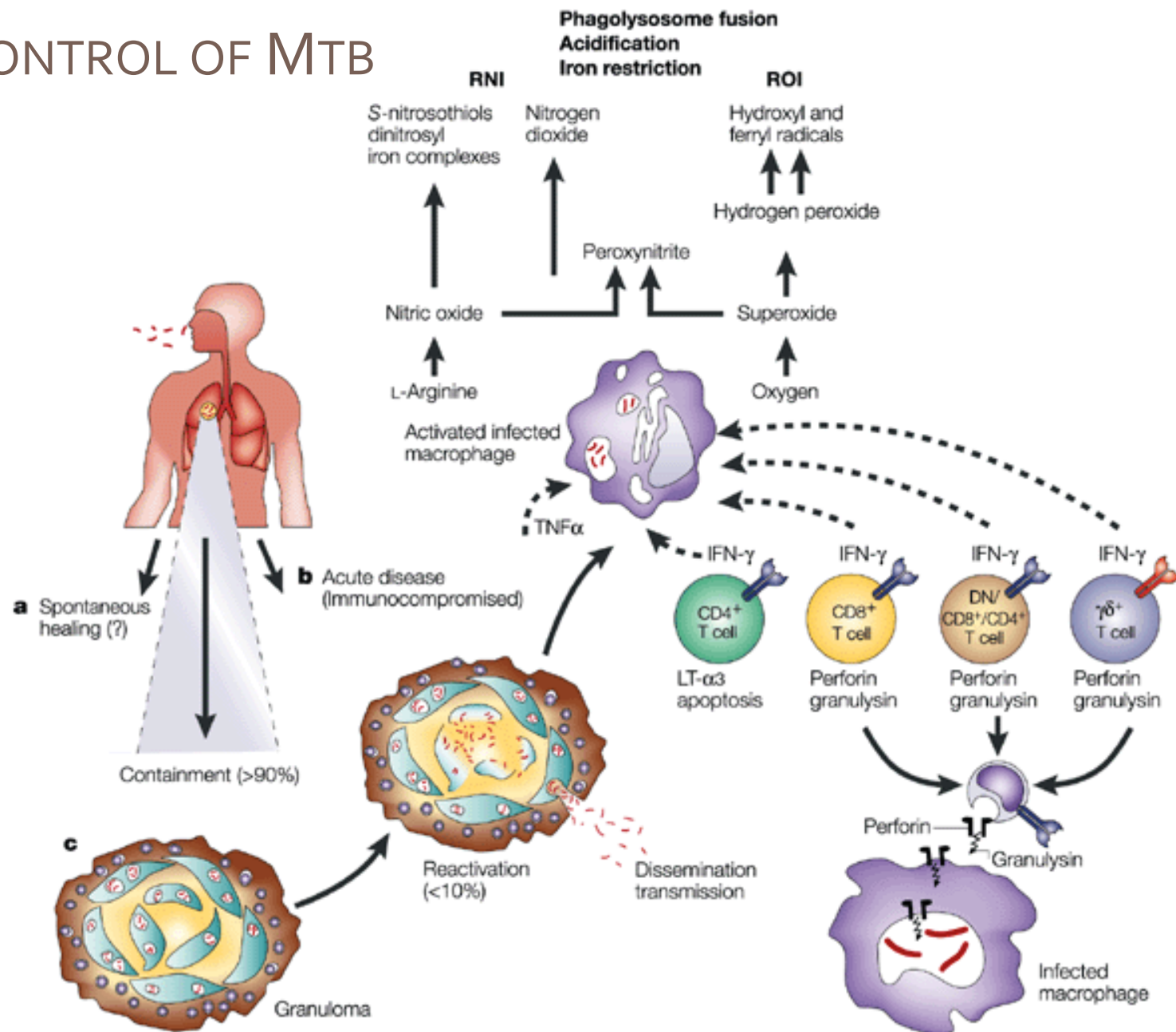


# IL-10 REGULATION OF LUNG PATHOLOGY

- 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

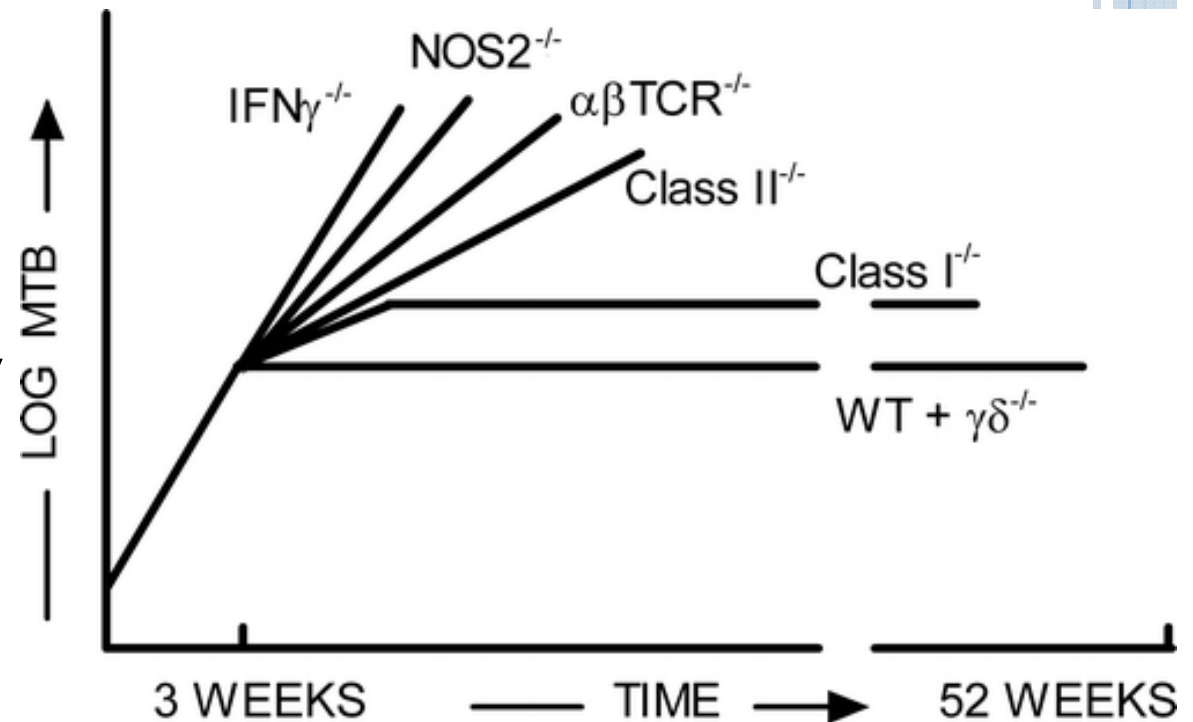


# CONTROL OF MTB

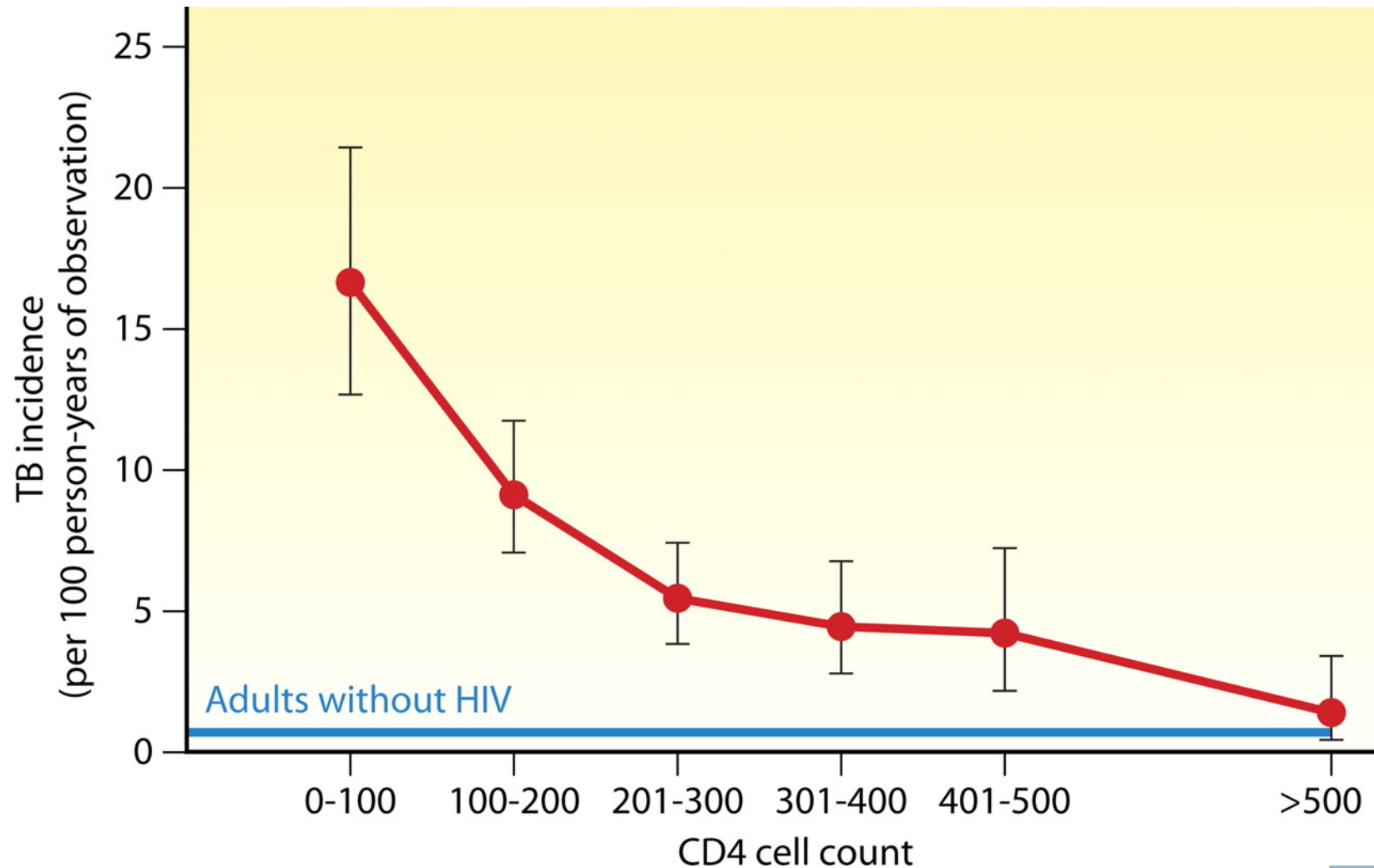


# SUMMARY OF CONTROL MECHANISMS

- Phagolysosomal destruction is the most important mechanism for removing bacteria
- IFN $\gamma$  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



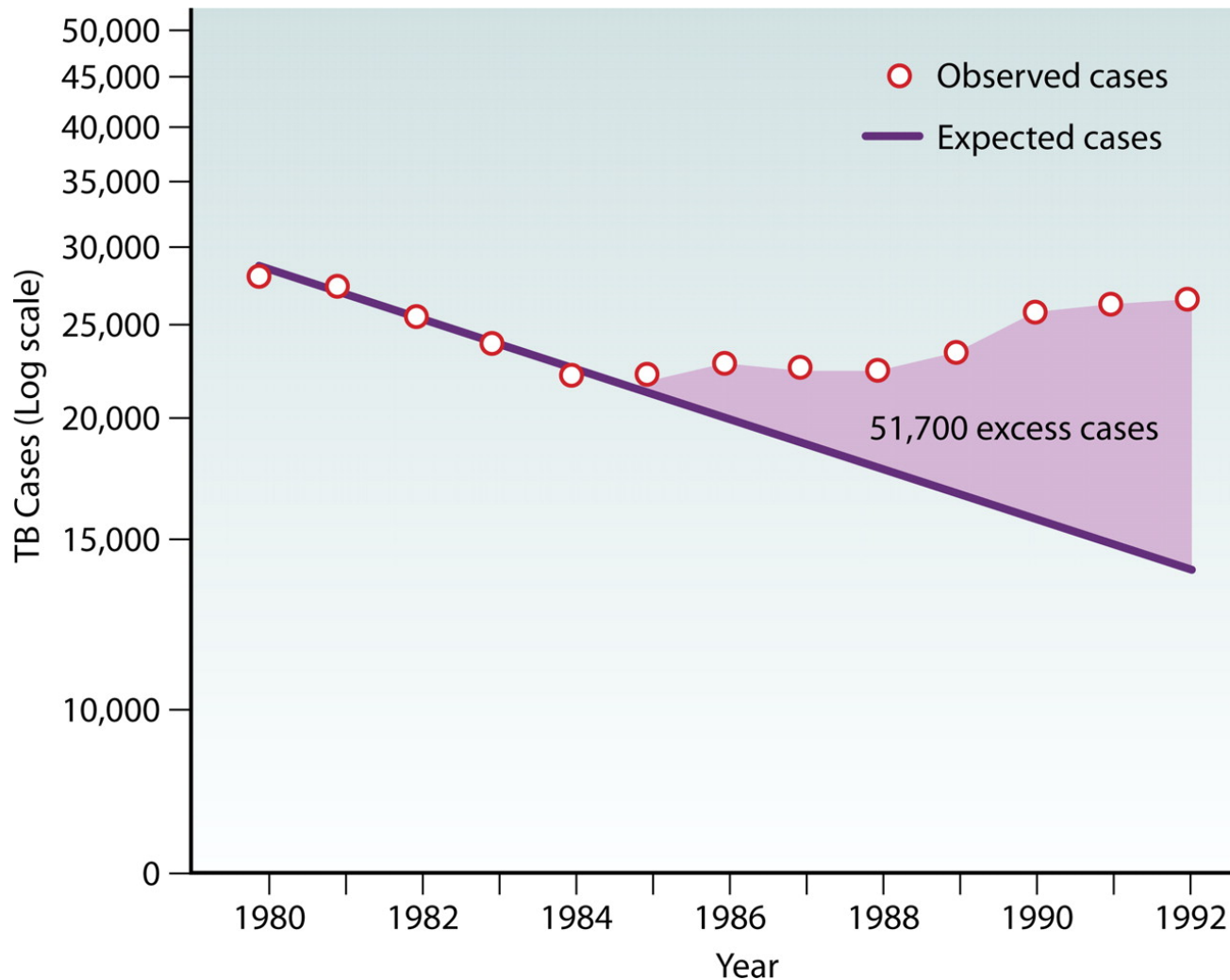
**TB incidence rates decrease with recovery of CD4 cell counts during antiretroviral therapy.**



Candice K. Kwan, and Joel D. Ernst Clin. Microbiol. Rev.  
2011;24:351-376

Clinical Microbiology Reviews

## Estimated excess TB cases attributed to the worsening HIV epidemic in the United States from 1985 to 1992.



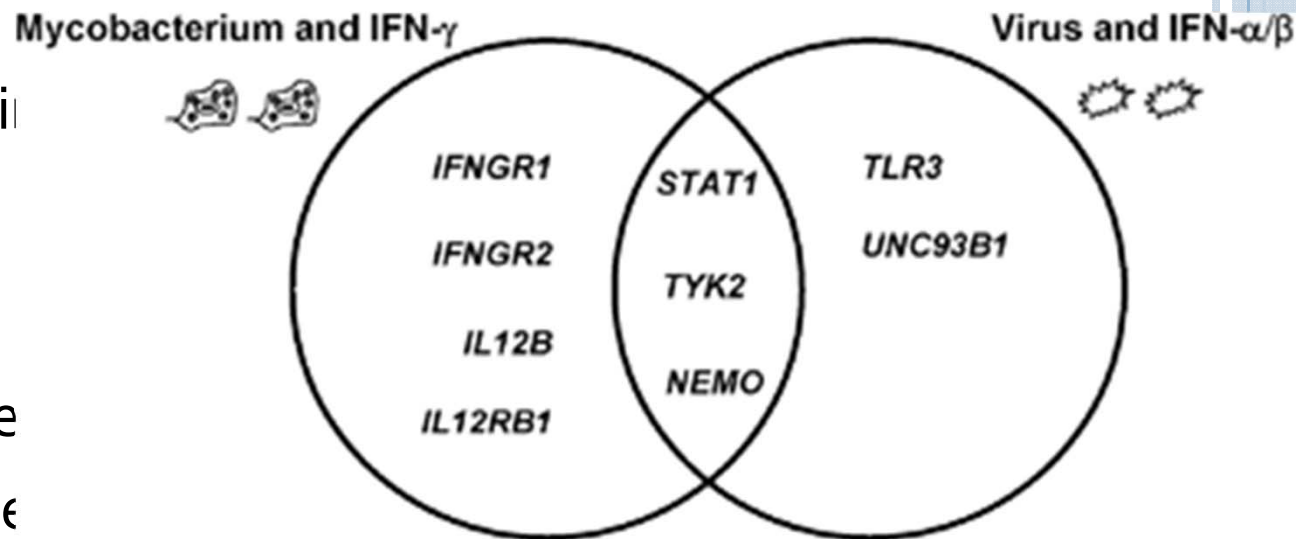
Candice K. Kwan, and Joel D. Ernst Clin. Microbiol. Rev.  
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Clinical Microbiology Reviews



# HUMAN GENETIC DEFICIENCIES

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



# WHAT CAUSES REACTIVATION?

- In developing countries, post-primary disease rate peaks in young adult age groups (common feature of several types of infections—endocrine influence?)
- Immunosuppression of innate or adaptive immunity results in reactivation (HIV, TNF blockade)
- Immune senescence (aging)

**TABLE 1** Mendelian immune disorders predisposing the patient to mycobacterial disease<sup>a</sup>

| Condition | Clinical form and molecular basis        | <i>Mycobacterium</i> species                | References                          |
|-----------|--|---|-------------------------------------|
| SCID      | T(−)B(−)NK(−):                           | BCG   | (51–57)                             |
|           | Reticular dysgenesis, ADA deficiency     | <i>M. avium</i> , <i>M. marinum</i>         | (59, 60)                            |
|           | T(−)B(−)NK(+):                           | <i>M. tuberculosis</i>                      | (131)                               |
|           | RAG-1/RAG-2, Artemis defects             |   |                                     |
|           | T(−)B(+)NK(−):<br>γ-chain, JAK-3 defects |   |                                     |
| HIES      | T(−)B(+)NK(+):<br>IL-7Rα, CD45 defects   |   |                                     |
|           | Not identified                           | BCG   | (61)                                |
|           |  | <i>M. intracellulare</i>                    | (62)                                |
| CGD       | XR-CGD:                                  | BCG   | (56, 64–69)                         |
|           | gp91-NADPH oxidase                       | <i>M. avium</i> , <i>M. flavescens</i> ,    | (70–73, 133)                        |
|           | AR-CGD:                                  | <i>M. fortuitum</i> , <i>Mycobacterium</i>  |                                     |
|           | p22, p47, p67-NADPH oxidase              | spp.<br><i>M. tuberculosis</i>              | (133, 134)                          |
| EDA-ID    | XR-EDA-ID:                               | <i>M. avium</i> , <i>M. kansasii</i> ,      | (74–79)                             |
|           | NEMO defect                              | <i>Mycobacterium</i> spp.                   |                                     |
|           | XR-OL-EDA-ID:                            | <i>M. chelonae</i>                          |                                     |
|           | NEMO defect                              | <i>M. tuberculosis</i>                      | (76, 135)                           |
| HIGM      | Cleft lip/palate-EDA-ID:                 |   |                                     |
|           | Not identified                           |   |                                     |
| MSMD      | XR-HIGM:                                 | <i>M. tuberculosis</i> , <i>M. bovis</i>    | (132)                               |
|           | CD154                                    |   |                                     |
| MSMD      | Response to IFNγ abolished:              | BCG   | (44, 79, 90, 95, 97, 99a, 99b, 101) |
|           | c-AR-IFN-γR1 deficiency                  | <i>M. avium</i> , <i>M. kansasii</i> ,      | (43, 44, 90, 94, 96–102, 99b)       |
|           | c-AR-IFN-γR2 deficiency                  | <i>M. szulgai</i> , <i>M. chelonae</i> ,    |                                     |
|           |  | <i>M. fortuitum</i> , <i>M. abscessus</i> , |                                     |
|           |  | <i>M. smegmatis</i> , <i>M. peregrinus</i>  |                                     |
|           | Impaired response to IFNγ:               | BCG   | (86, 87, 106, 107, 109a, 109b)      |
|           | p-AR-IFN-γR1 deficiency                  | <i>M. avium</i> , <i>M. kansasii</i> ,      | (86, 87, 90, 107, 109, 109a, 109c)  |
|           | p-AD-IFN-γR1 deficiency                  | <i>M. chelonae</i> , <i>M. abscessus</i> ,  |                                     |
|           | p-AR-IFN-γR2 deficiency                  | <i>M. goodii</i> , <i>M. asiaticum</i>      |                                     |
|           | p-AD-STAT1 deficiency                    | <i>M. tuberculosis</i>                      | (106)                               |
| MSMD      | Impaired IFNγ production:                | BCG   | (110–113, 115)                      |
|           | c-AR-IL-12Rβ1 deficiency                 | <i>M. avium</i> , <i>M. chelonae</i>        | (112–114, 116)                      |
|           | c-AR-IL-12p40 deficiency                 | <i>M. tuberculosis</i>                      | (115)                               |

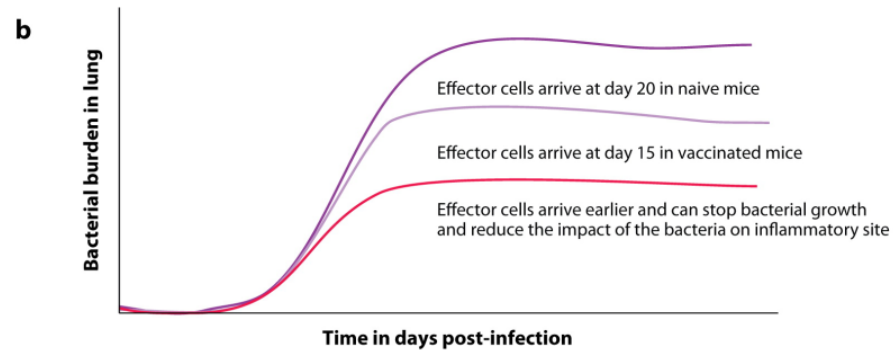
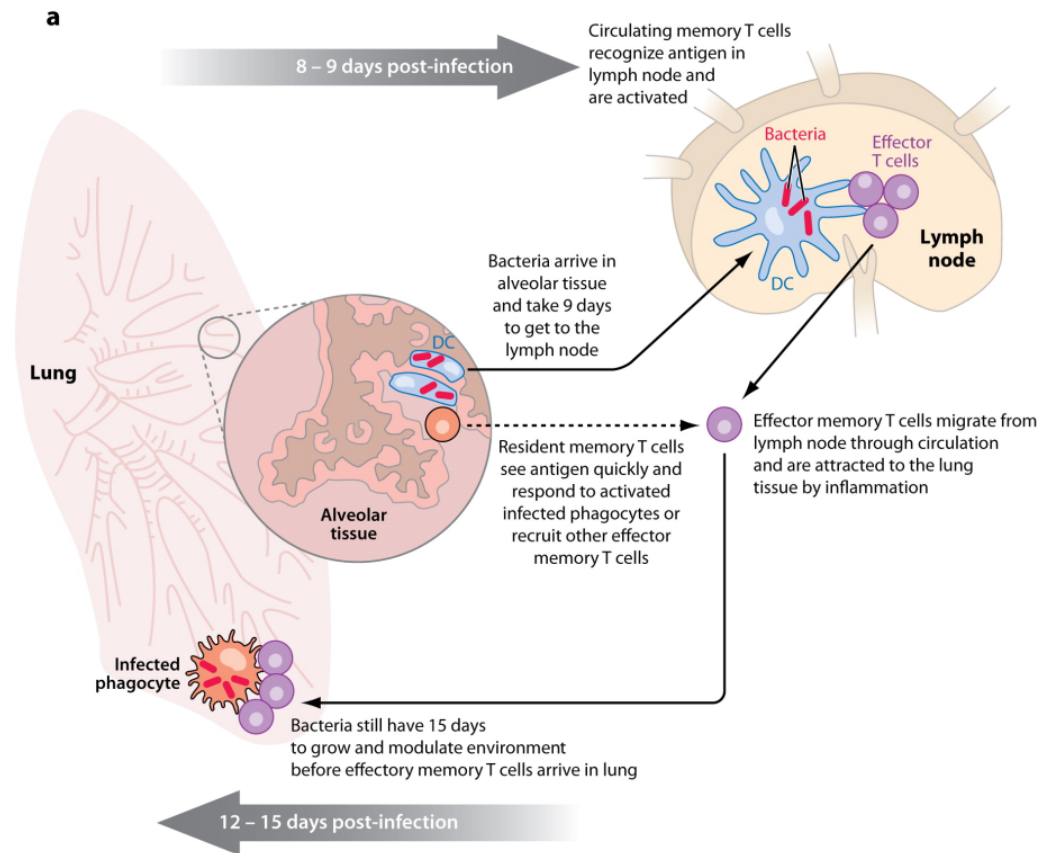
<sup>a</sup>The conditions are indicated, followed by their clinical forms and molecular defect. The *Mycobacterium* species isolated from patients suffering from the various conditions are indicated in three groups [BCG, EM (second line), and *M. tuberculosis* and related species (third line)]. References corresponding to each of the three groups of mycobacteria, for each condition or clinical form considered, are also indicated. Abbreviations: SCID, severe combined immunodeficiency; CGD, chronic granulomatous disease; HIES, hyper-IgE syndrome; EDA-ID, anhidrotic ectodermal dysplasia with immunodeficiency; OL-EDA-ID, anhidrotic ectodermal dysplasia with immunodeficiency, lymphedema, and osteopetrosis; HIGM, hyper-IgM syndrome; MSMD, Mendelian susceptibility to mycobacterial disease; AR, autosomal recessive; XR, X-linked recessive; c-AR, complete autosomal recessive; p-AR, partial autosomal recessive; p-AD: partial autosomal dominant; BCG: Bacille Calmette-Guérin.

Table 1 | **Tuberculosis vaccine candidates**

| Vaccine candidate   | Potential advantage                          | Potential disadvantage  | Examples  | Reference                      |
|---|--|---|---|--------------------------------|
| <b>Subunit vaccine</b>  |  |   |   |                                |
| Antigen in adjuvant   | Mild side effects only                       | Restricted number of T-cell clones, primarily CD4 <sup>+</sup> T cells, immunogenicity depends on adjuvant type | Culture filtrate (ill-defined antigen mixture)<br>Defined antigen: ESAT-6<br>Mtb 8.4<br>Ag85<br>Fusion protein: Ag85-ESAT-6                               | 112<br>113<br>114<br>112<br>98 |
| Naked DNA   | CD4 plus CD8 T cells                         | Restricted number of T-cell clones, conventional T cells, safety concerns                                       | Hsp60<br>Ag85<br>Mtb 8.4<br>Therapeutic vaccination   | 90<br>91<br>114<br>74          |
| Recombinant carrier expressing antigen                              | CD4 and/or CD8 T cells                       | Restricted number of T-cell clones, safety concerns   | r- <i>Vaccinia</i> expressing Ag85<br>r- <i>Salmonella</i> expressing Ag85  | 108<br>115                     |
| <b>Viable mycobacterial vaccine</b>                                 |  |   |   |                                |
| <i>Mycobacterium tuberculosis</i> deletion mutant                   | CD4 plus CD8 T cells, unconventional T cells | Safety concerns, immunosuppressive  | Erp- <i>M. tuberculosis</i><br>Acr- <i>M. tuberculosis</i><br>Icl- <i>M. tuberculosis</i><br>PcaA- <i>M. tuberculosis</i><br>Pdim- <i>M. tuberculosis</i> | 116<br>117<br>26<br>118<br>119 |
| Auxotrophic mutant  | Improved safety (BCG)                        | Reduced immunogenicity, safety concerns ( <i>M. tuberculosis</i> )  | Met, Leu, Iiv-BCG<br>Met, Pro, Trp <i>M. tuberculosis</i>   | 120<br>121                     |
| rBCG expressing cytolysin   | CD4 plus CD8 T cells, unconventional T cells | Devoid of TB-specific antigens, safety concerns   | rBCG-listeriolysin  | 100                            |
| rBCG expressing cytokine  | Improved immunogenicity                      | Primarily CD4 T cells, devoid of TB-specific antigens, safety concerns  | rBCG-IL-2, -IFN- $\gamma$   | 102                            |
| rBCG overexpressing antigen   | Improved immunogenicity                      | Primarily CD4 T cells, safety concerns  | rBCG-Ag85   | 122                            |
| <b>Combination vaccine</b>  |  |   |   |                                |
| rBCG coexpressing immunomodulator plus antigen                      | Improved immunogenicity, protective antigens | Safety concerns   | Not done  |                                |
| r <i>M. tuberculosis</i> deletion mutant expressing immunomodulator | Improved immunogenicity                      | Safety concerns   | Not done  |                                |
| Prime boost   | Improved immunogenicity                      | Safety concerns   | BCG→protein (Ag85)<br>Naked DNA→protein (Ag85)<br>Naked DNA→ <i>Vaccinia</i> (Ag85)<br>Naked DNA→BCG  | 106<br>107<br>108<br>109       |

Information adapted from REF. 1. Acr, a cognate of the  $\alpha$ -crystallin family of low-molecular-mass heat-shock proteins (Hsps); Ag85, antigen 85; Erp, a secreted protein of *M. tuberculosis*; BCG, Bacille Calmette-Guérin; ESAT-6, early secreted antigenic target 6 kDa protein; Icl, isocitrate lyase; IFN- $\gamma$ , interferon- $\gamma$ ; IL-2, interleukin-2; Iiv, either isoleucine, leucine or valine; PcaA, mycolic acid cyclopropane synthase; Pdim, phthiocerol dimycocerosate; rBCG, recombinant BCG; TB, tuberculosis.

# RATE OF ADAPTIVE RESPONSE CORRELATES WITH CONTROL



# WHAT CONTROLS REACTIVATION?

- Diagnosis and assessment of TB still relies on chest radiography
- Rapid sequencing approaches might provide a platform for greater diagnostic discernment with less invasive techniques
- These studies also generate hypotheses that can lead to better understanding and prediction of reactivation

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nature

## LETTERS

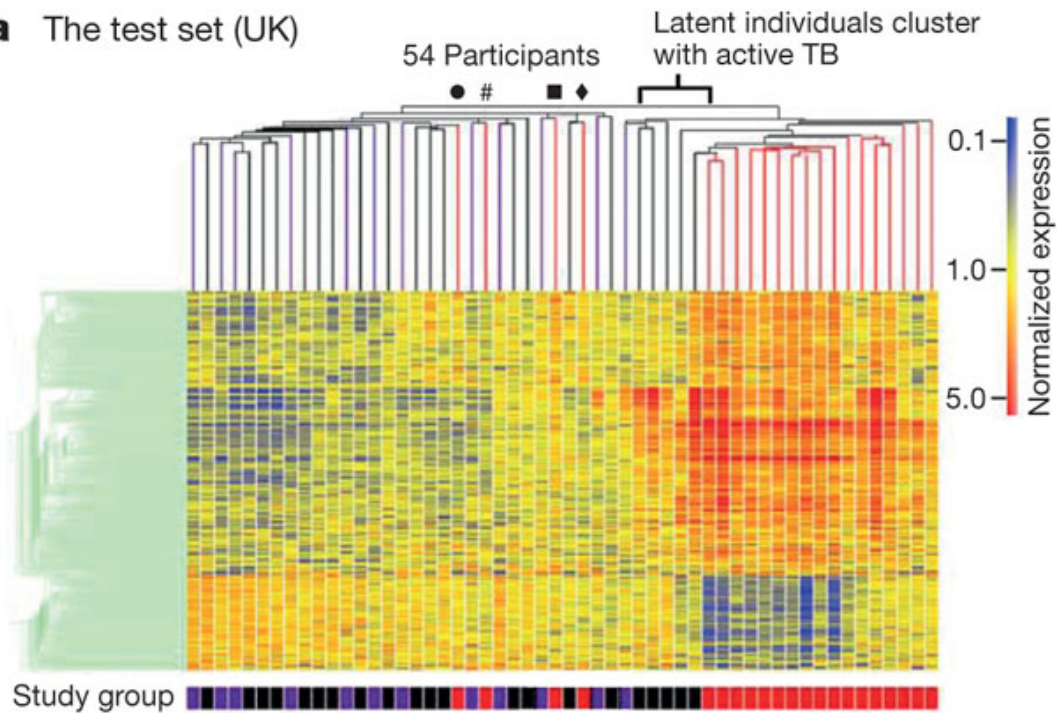
### **An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis**

Matthew P. R. Berry<sup>1</sup>, Christine M. Graham<sup>1\*</sup>, Finlay W. McNab<sup>1\*</sup>, Zhaohui Xu<sup>6</sup>, Susannah A. A. Bloch<sup>3</sup>, Tolu Oni<sup>4,5</sup>, Katalin A. Wilkinson<sup>2,4</sup>, Romain Banchereau<sup>9</sup>, Jason Skinner<sup>6</sup>, Robert J. Wilkinson<sup>2,4,5</sup>, Charles Quinn<sup>6</sup>, Derek Blankenship<sup>7</sup>, Ranju Dhawan<sup>8</sup>, John J. Cush<sup>6</sup>, Asuncion Mejias<sup>10</sup>, Octavio Ramilo<sup>10</sup>, Onn M. Kon<sup>3</sup>, Virginia Pascual<sup>6</sup>, Jacques Banchereau<sup>6</sup>, Damien Chaussabel<sup>6</sup> & Anne O'Garra<sup>1</sup>

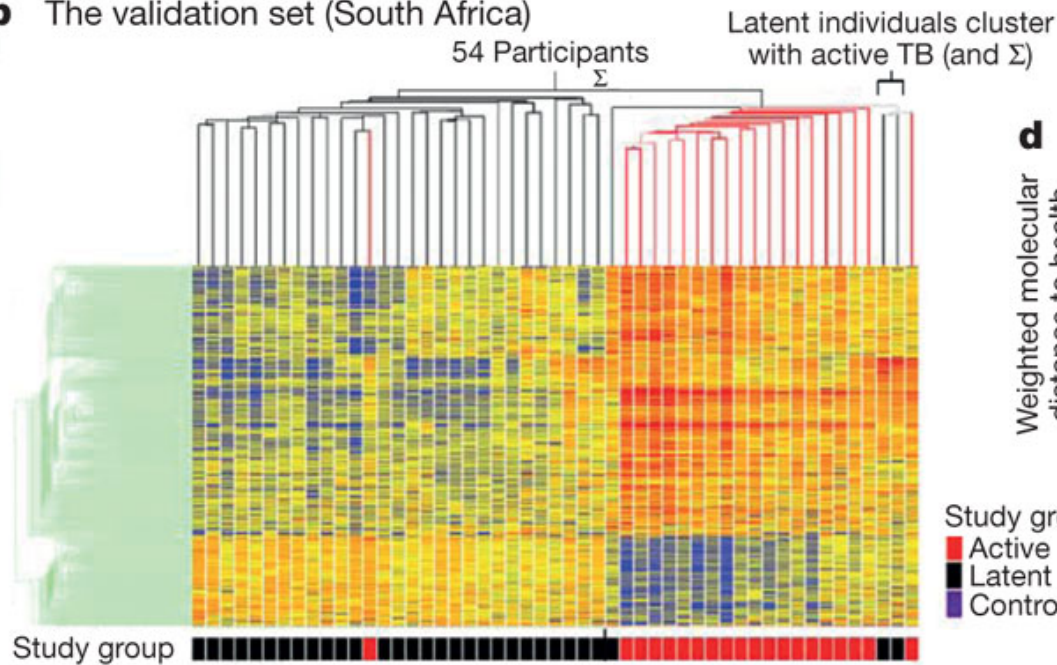




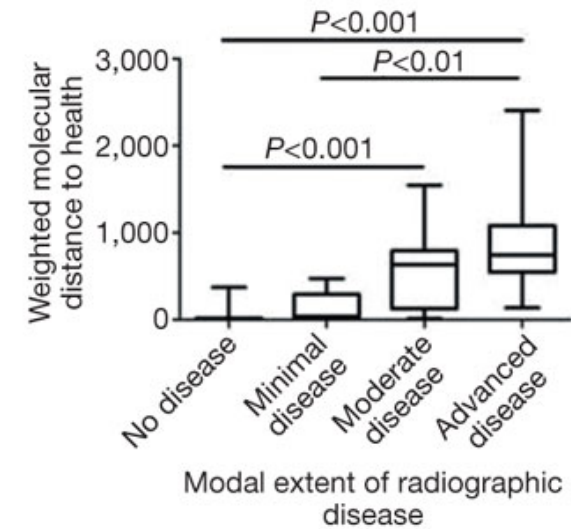
**a** The test set (UK)



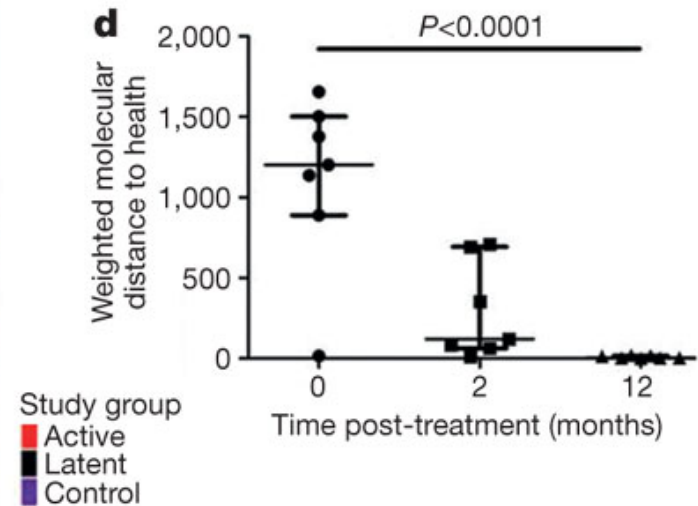
**b** The validation set (South Africa)



**c**



**d**



# 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

