Imunidade ao *Plamodium*: mais questões que respostas

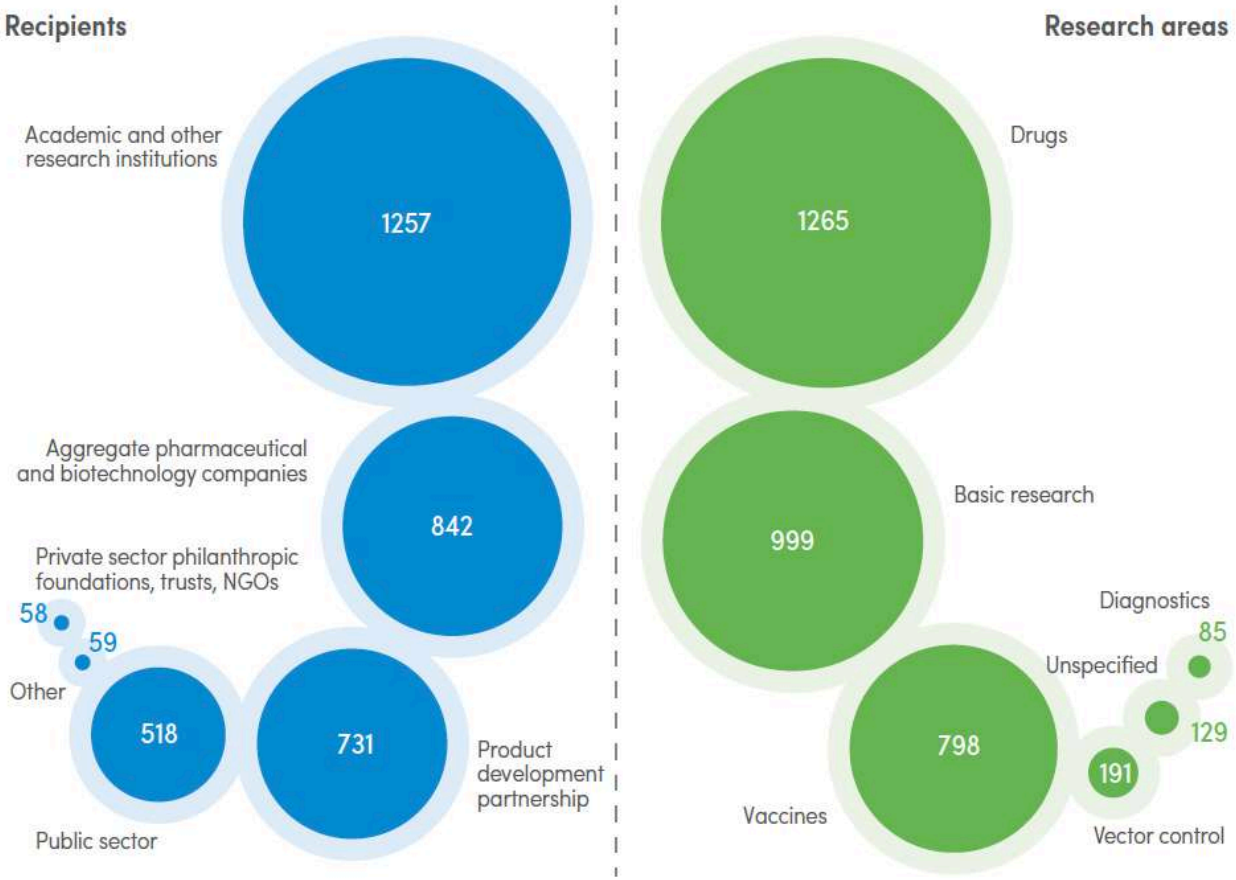
Claudio Marinho
Laboratório de Imunoparasitologia Experimental
Departamento de Parasitologia / ICB-USP

marinho@usp.br

Imunidade ao plasmódio: mais questões que respostas

1) Intodução
2) Mecanismos de defesa
3) Mecanismos de escape
4) Mecanismos de patogênese

Em 2016, cerca de 2,7 bilhões de dólares foram investidos para o controle e a eliminação da malária no mundo, tanto por ações governamentais de países endêmicos de malária, quanto por parceiros internacionais.



WORLD HEALTH ORGANIZATION, 2017

Imunidade ao plasmódio: mais questões que respostas

1) Introdução
2) **Mecanismos de defesa**
3) Mecanismos de escape
4) Mecanismos de patogênese

Immunobiology of Malaria

Adaptive immune responses
(adults in endemic areas)

Clinically silent infections

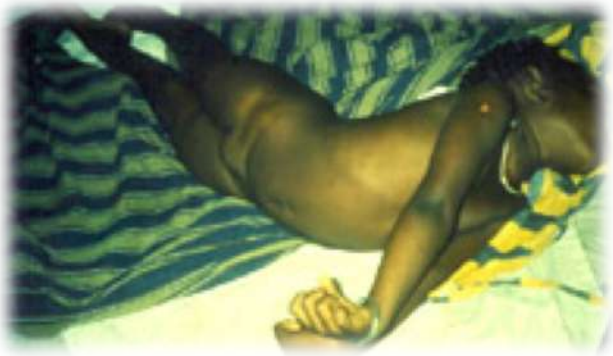
Clinically overt infections

Non-immune individuals
(children in endemic areas, non-immune adults)

Determinants of malaria syndromes and disease severity

Parasitised organ(s)

Local and systemic action of parasite toxins

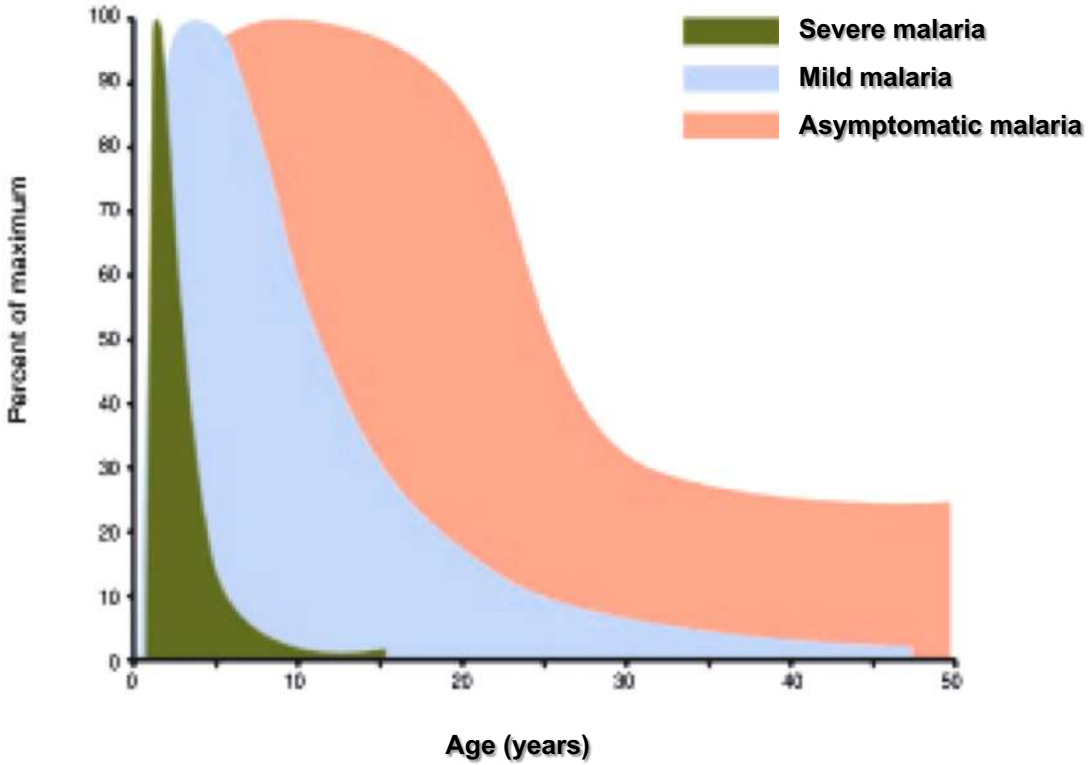


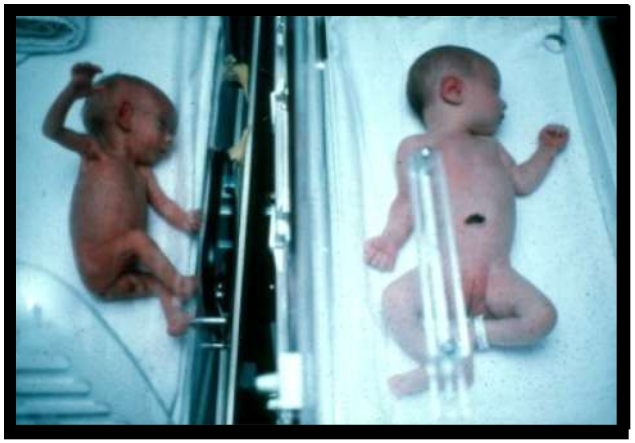
Action of pro-inflammatory and counter-regulatory cytokines

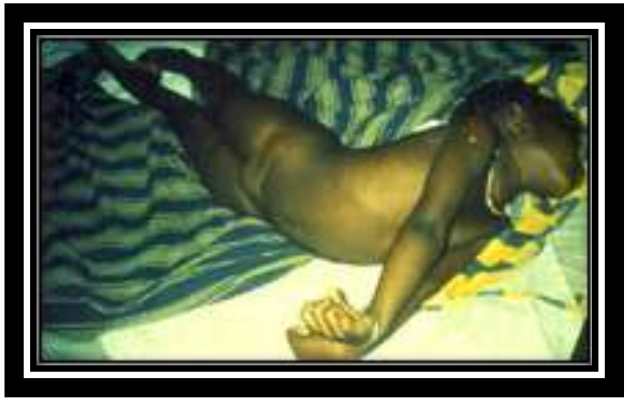
Recruitment and activation of inflammatory cells

Appropriate regulation of immune responses is key to disease outcome

Population indices of immunity in an endemic area of *P. falciparum* transmission







Marsh et al. Nature Immunology, 2008

THE JOURNAL OF BIOLOGICAL CHEMISTRY

Vol. 280, No. 9, Issue of March 4, pp. 8606–8616, 2005
Printed in U.S.A.

Induction of Proinflammatory Responses in Macrophages by the Glycosylphosphatidylinositols of *Plasmodium falciparum*

CELL SIGNALING RECEPTORS, GLYCOSYLPHOSPHATIDYLINOSITOL (GPI) STRUCTURAL REQUIREMENT, AND REGULATION OF GPI ACTIVITY*

Received for publication, December 2, 2004, and in revised form, December 17, 2004
Published, JBC Papers in Press, December 28, 2004, DOI 10.1074/jbc.M413541200

Gowdahalli Krishnegowda‡§, Adeline M. Hajjar§¶, Jianzhong Zhu‡§, Erika J. Douglass¶, Satoshi Uematsu, Shizuo Akira**, Amina S. Woods‡‡, and D. Channe Gowda‡§§**

*From the ‡Department of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, Hershey, Pennsylvania 17033-0850, ¶Department of Immunology, University of Washington, Seattle, Washington 98195, **Department of Host Defense, Research Institutes for Microbial Diseases, Osaka University, Osaka 565-0871, Japan, and ‡‡NIDA, National Institutes of Health, Baltimore, Maryland 21224*

PNAS

SEE COMMENTARY

Malaria hemozoin is immunologically inert but radically enhances innate responses by presenting malaria DNA to Toll-like receptor 9

Peggy Parroche*, Fanny N. Lauw*, Nadege Goutagny*, Eicke Latz*, Brian G. Monks*, Alberto Visintin*, Kristen A. Halmen*, Marc Lamphier†, Martin Olivier‡, Daniella C. Bartholomeu‡, Ricardo T. Gazzinelli*§¶, and Douglas T. Golenbock*‖

Division of Infectious Diseases and Immunology, University of Massachusetts Medical School, Worcester, MA 01605; †Centro de Pesquisas Rene Rachou, FIOCRUZ, 1715 Belo Horizonte, Brazil; ‡Department of Biochemistry and Immunology and Department of Parasitology, Biological Sciences Institute, Federal University of Minas Gerais, 6627 Belo Horizonte, Brazil; §Department of Microbiology/Immunology, McGill University, Montreal, Quebec, Canada H3A 2T8; and ¶Eisai Research Institute, 4 Corporate Drive, Andover, MA 01810

Experimental Parasitology 128 (2011) 205–211

Contents lists available at ScienceDirect

Experimental Parasitology

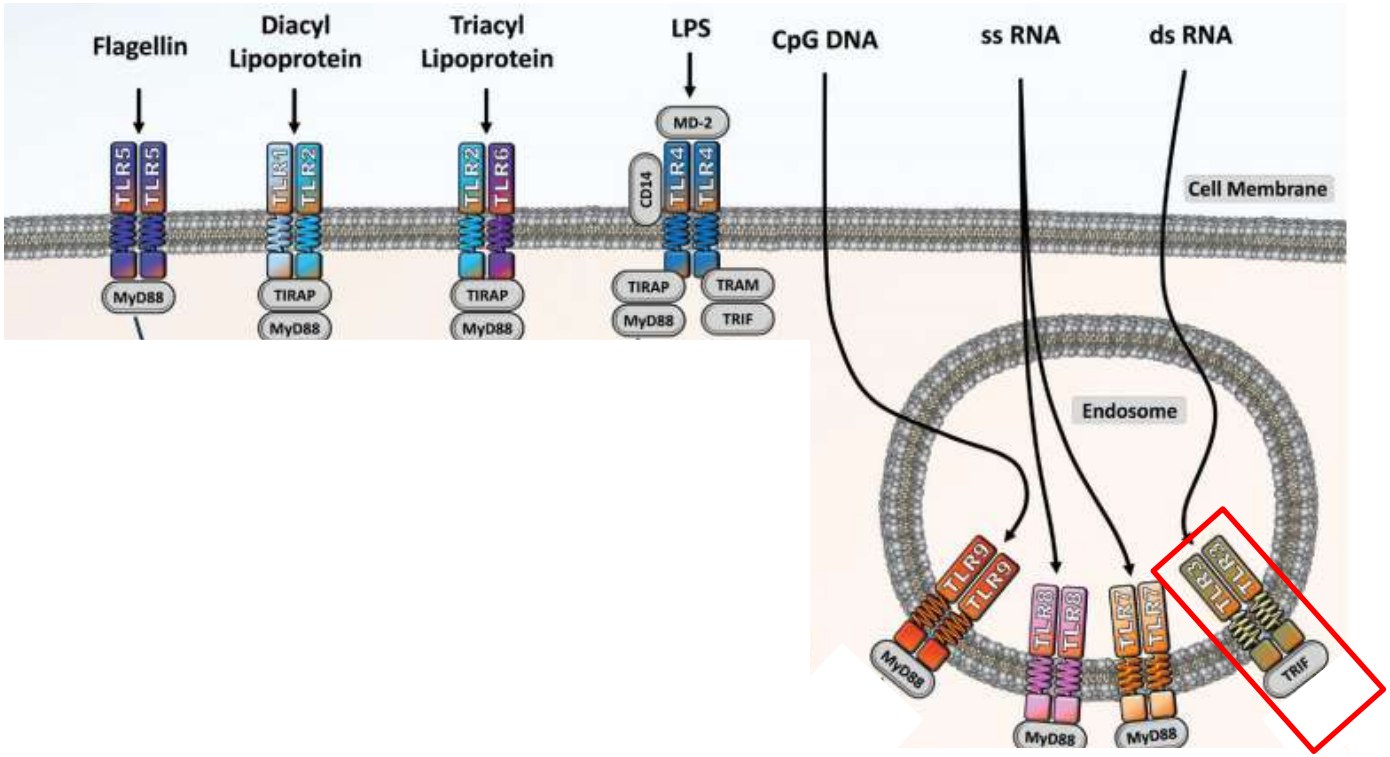
journal homepage: www.elsevier.com/locate/yexpr

Proinflammatory responses by glycosylphosphatidylinositols (GPIs) of *Plasmodium falciparum* are mainly mediated through the recognition of TLR2/TLR1

Jianzhong Zhu *, Gowdahalli Krishnegowda, Guangfu Li, D. Channe Gowda

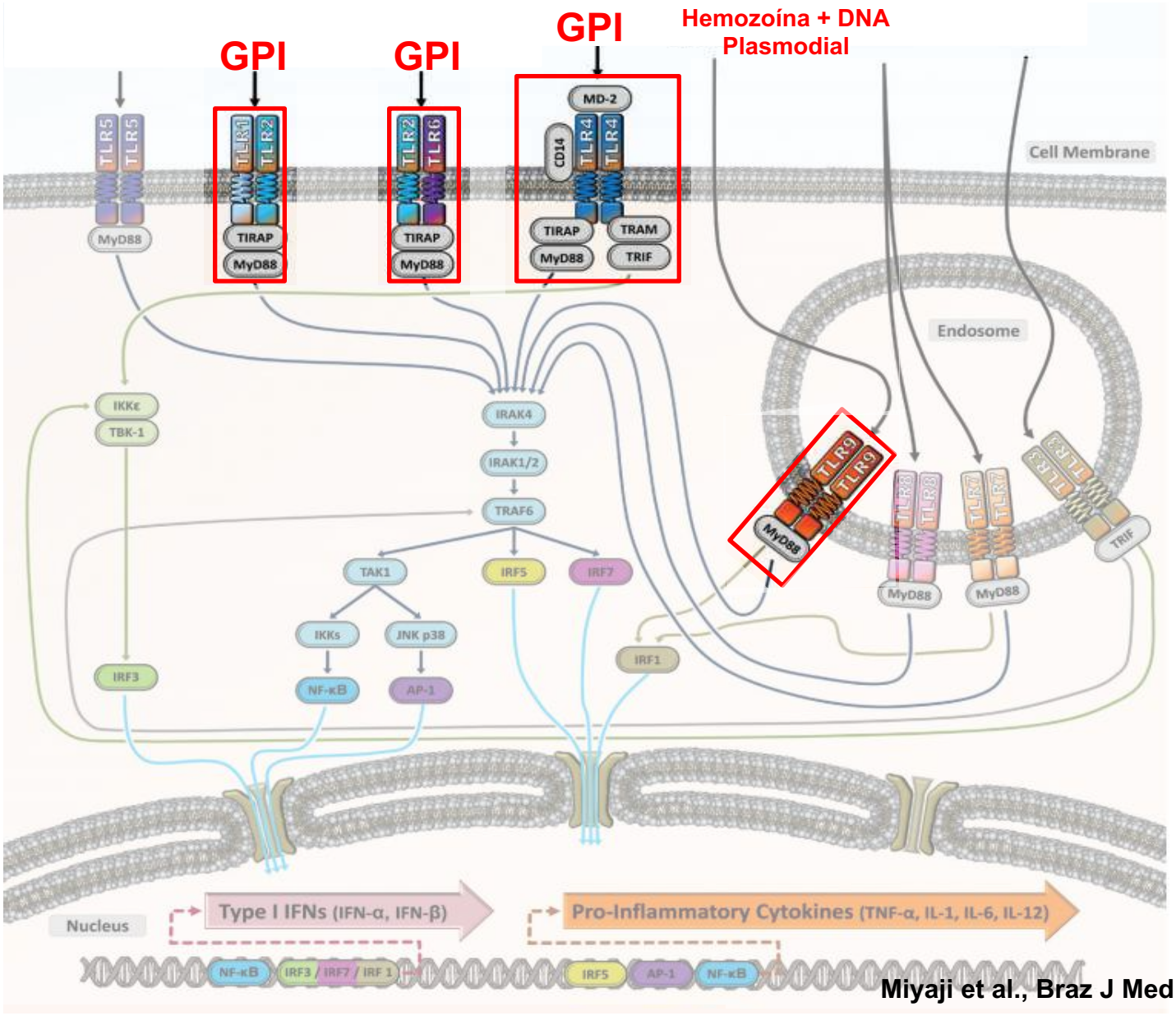
Department of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, Hershey, PA 17033, USA

Os Toll Like Receptors (TLR) são uma família de receptores que reconhecem padrões moleculares (PRR)



Miyaji et al., Braz J Med Biol Res, 2011

Os Toll Like Receptors (TLR) são uma família de receptores que reconhecem padrões moleculares (PRR)



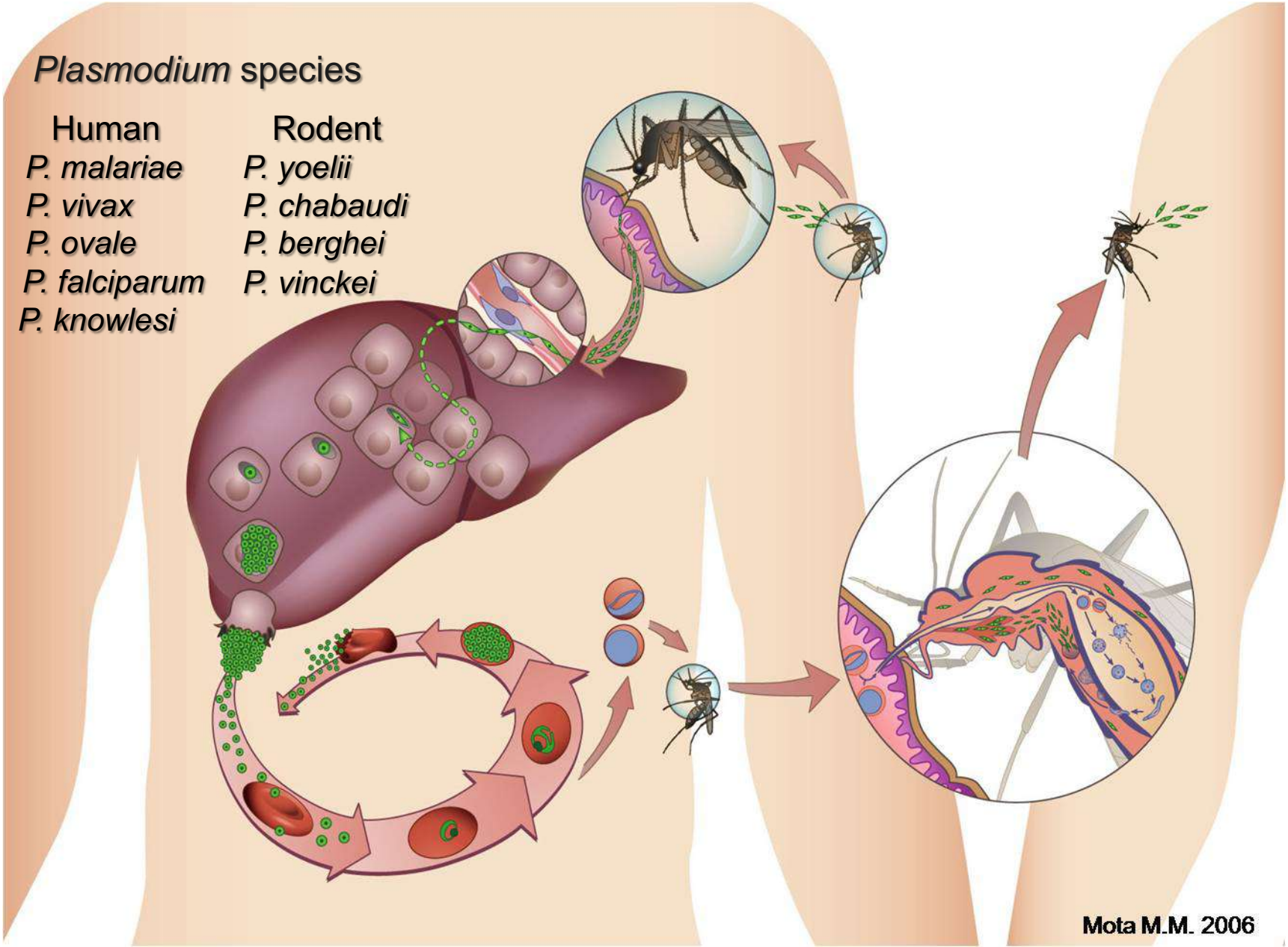
Miyaji et al., Braz J Med Biol Res, 2011

Malaria products and their bioactivities

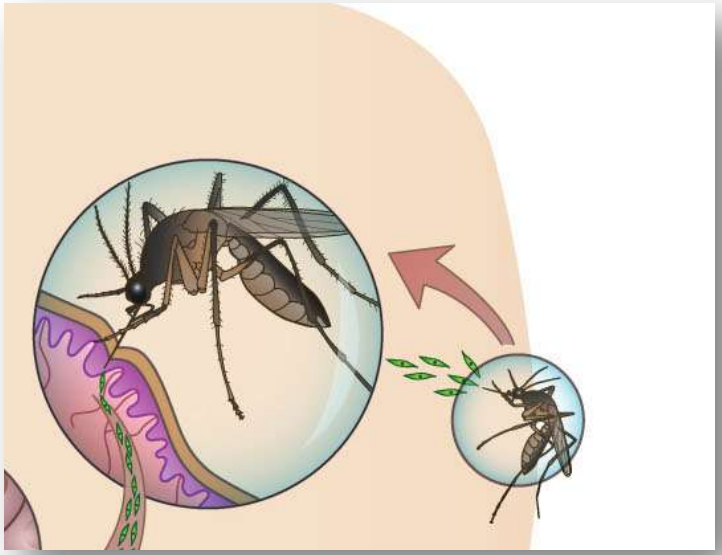
Parasite product	Receptor and cell type	Pathological and cellular effects
***Plasmodium falciparum* EMP1-family members**	**Endothelial and trophoblast cells :** ICAM1, VCAM1, CD36, thrombospondin, E-selectin, CSA, HA and CD31 **DCs:** CD36	Binding directs parasite to the **brain, placenta** and possibly other target organs; CD36 engagement proposed to suppress DC and macrophage activation
GPI (glycosylphosphatidylinositol)	**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 TH1- or TH2-cytokine production
Haemozoin	TLR9 on DCs	**Contradictory reports:** both TH1- and TH2-cell activities; induces and inhibits DCs; suppresses macrophages; induces IL-10 production; broadly immunosuppressive

Adaptado de: Schofield *et al.*, 2005, NATURE.

Plasmodium life cycle



Que mecanismos efetores do sistema imune podem atuar nos esporozoítos durante a primo-infecção?



- Fagocitose direta por neutrófilos e macrófagos (se houver reconhecimento)

- Opsonização ou lise pelo complemento (via alternativa, lectinas ou clássica se houver anticorpo IgM de baixa afinidade ou IgG adquiridos da mãe)

- Neutralização ou inibição da penetração (se houver anticorpos)

Opsoninas:

C3b e C4b na superfície de microrganismos se encaixam no receptor do C (CR1) em células fagocitárias e promovem fagocitose.

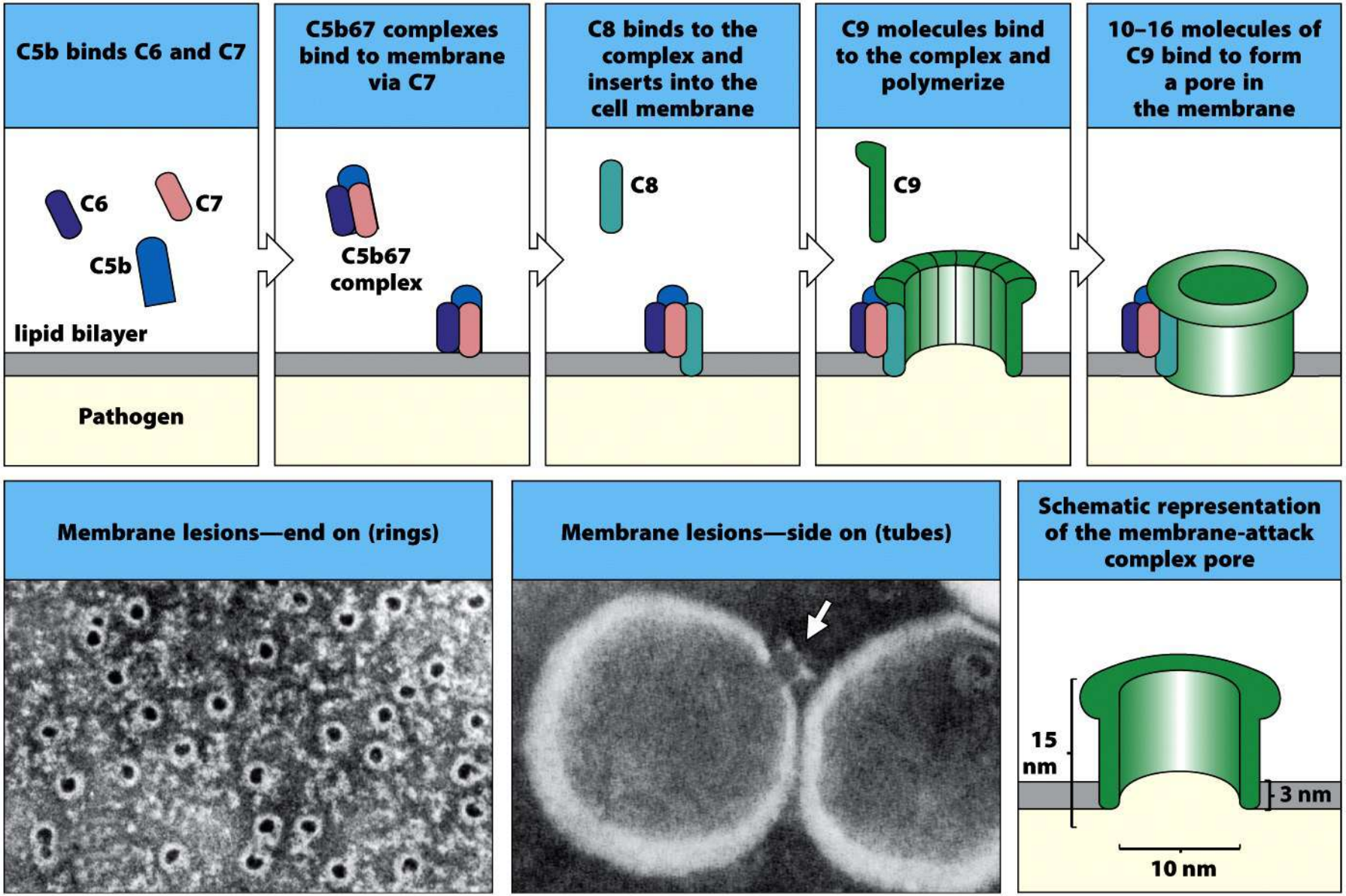
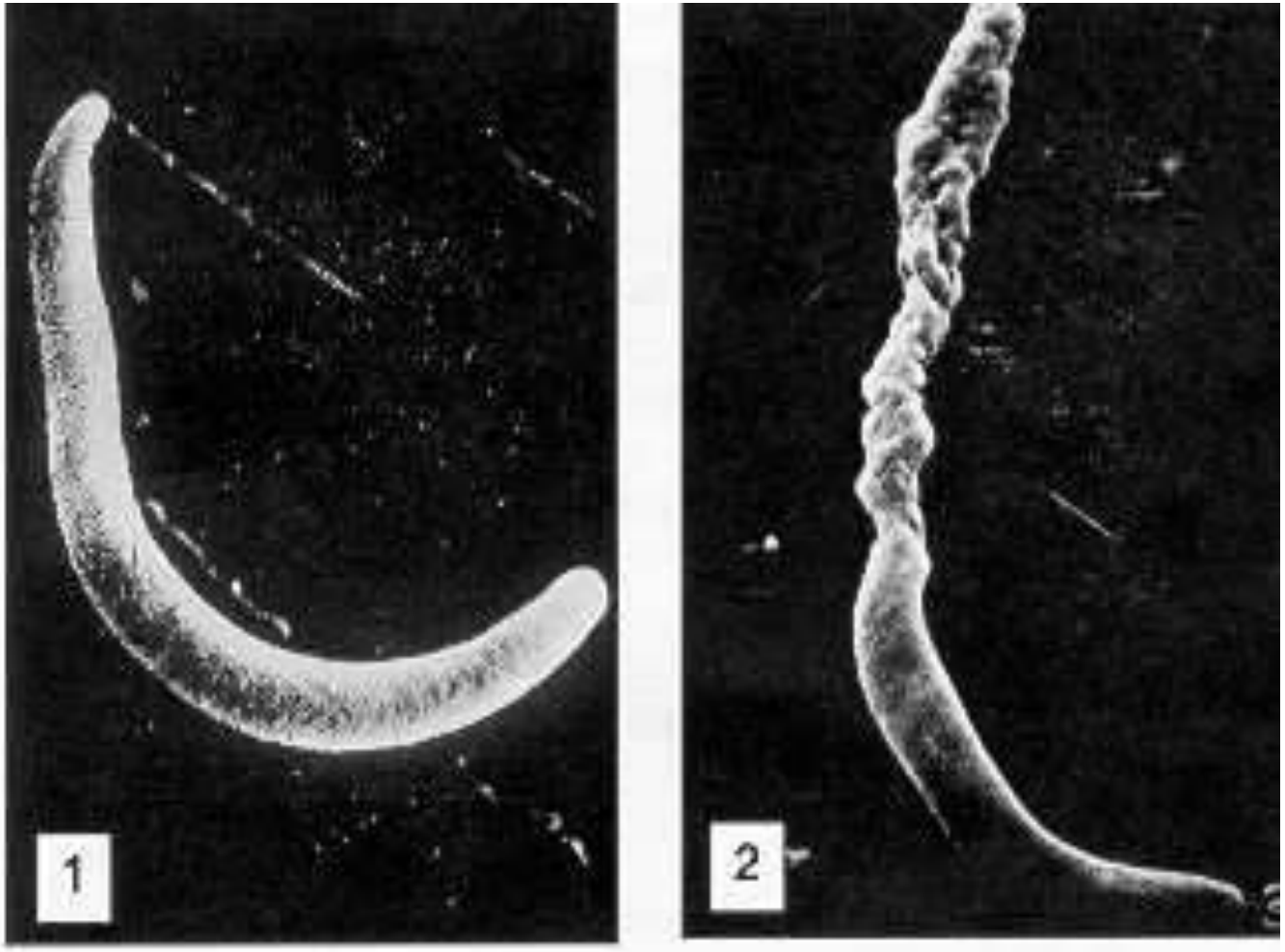
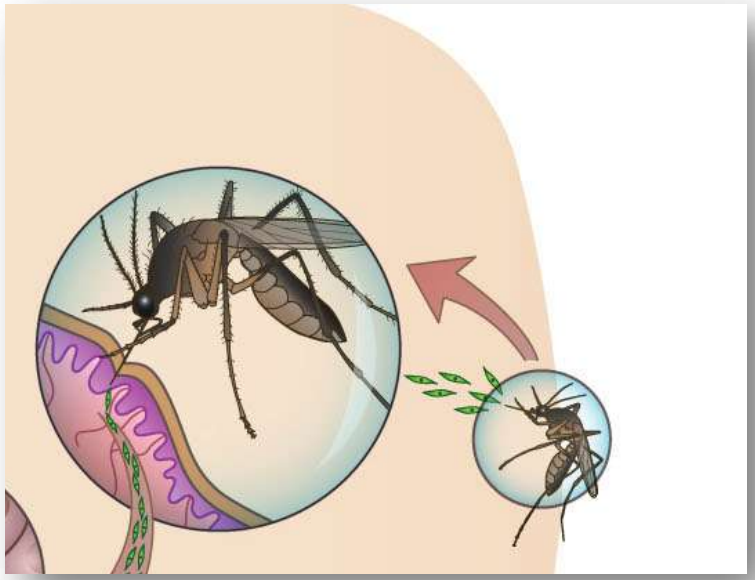


Figure 2-41 Immunobiology, 7ed. (© Garland Science 2008)

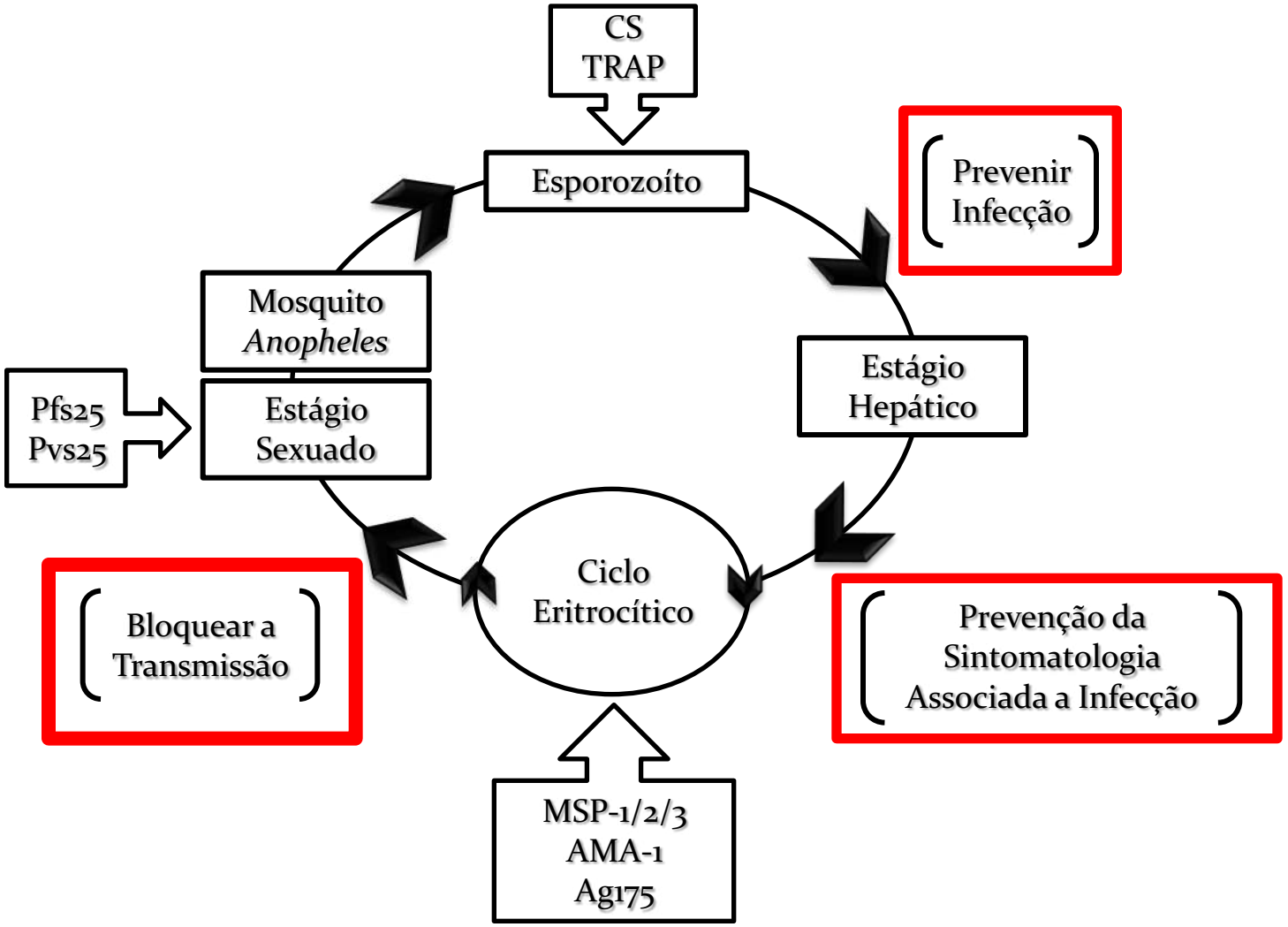

1
2

Que mecanismos efetores do sistema imune podem atuar nos esporozoítos durante a re-infecção?



- neutralização ou inibição da penetração (principalmente IgG de alta afinidade)
- fagocitose ou citotoxicidade por macrófagos e neutrófilos (opsonização por C3b ou IgG)
- Aumento da fagocitose pelo IFN-γ produzido pelos linfócitos Th1
- lise pela via clássica

Estratégias e Alvos Vacinais



Adaptado de *Ballou* et al

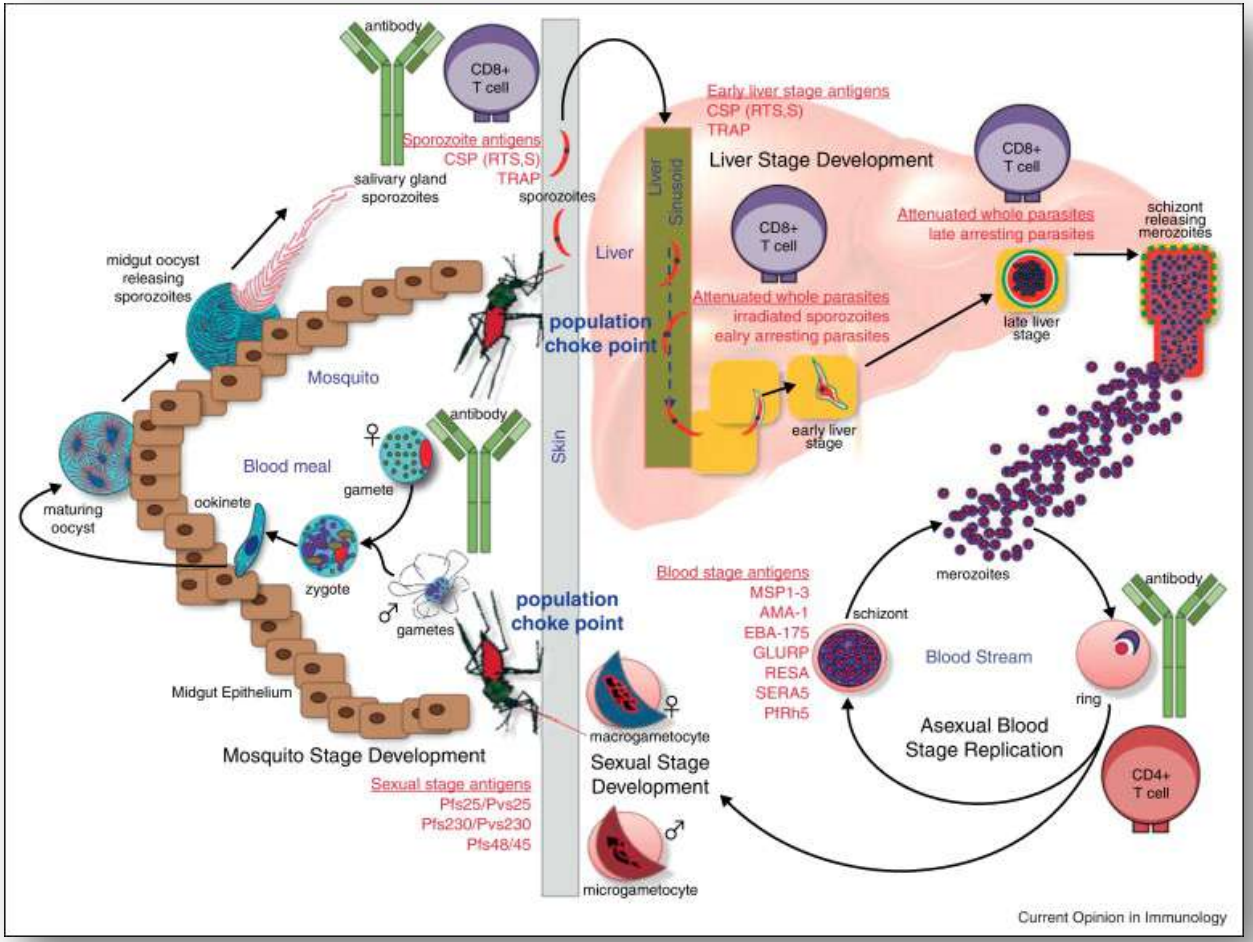
Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Immunology

Malaria vaccine development: persistent challenges

Ashley M Vaughan[1] and Stefan HI Kappe[1,2]



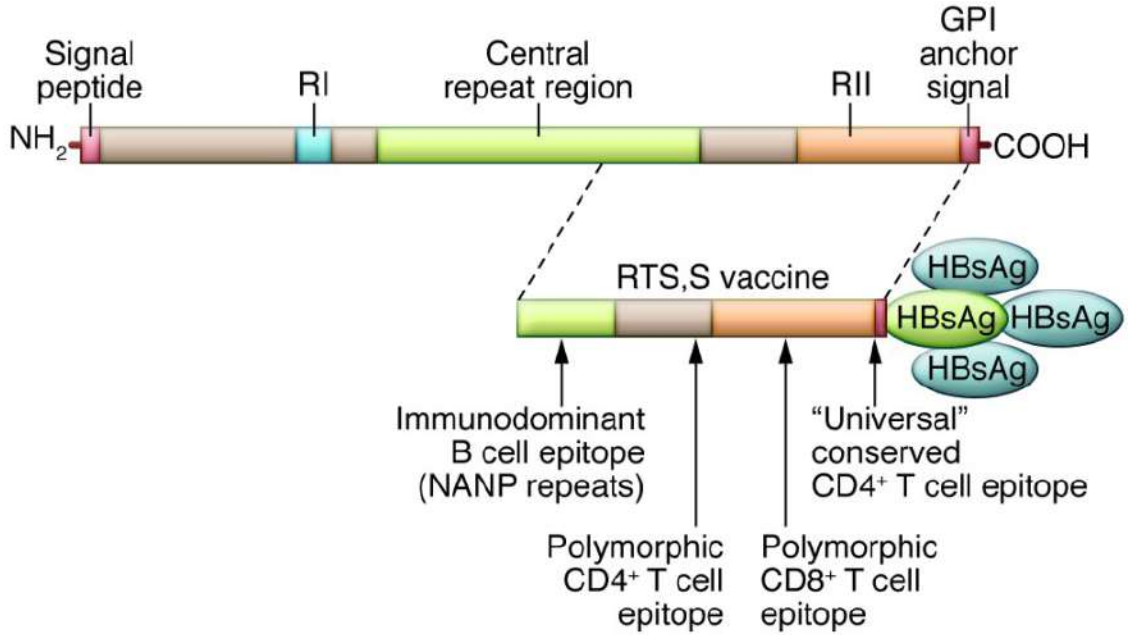
RESULTS OF RTS,S MALARIA VACCINE TRIALS

- Many trials have shown that RTS,S malaria vaccine is safe and stimulates the immune system
- RTS,S efficacy trial in Mozambique – enrolled 2022 children aged 1-4 years
- 50% protection against severe malaria and severe anemia
- Malaria causes 1 million childhood deaths per year in Africa
- If this vaccine could reduce by half the cases of severe disease, we would be saving thousands of lives

▪GSK , Walter Reed Army Institute the PATH Malaria Vaccine Initiative (MVI)—with grant money from the Bill & Melinda Gates Foundation.

✓CSP (proteína circunsporozoíta)

The **CSP protein** is responsible for parasite binding to **hepatocytes' highly sulphated heparan sulphate proteoglycans (HSPGs),** blocking the CSP protein through high avidity antibodies prevents parasite binding to HSPGs and therefore prevents through an as yet uncharacterised mechanism the parasites' gaining entry into hepatocytes



The **RTS,S** is a fusion protein of the **CSP and a hepatitis B surface antigen protein** (HBsAg) expressed in fungi, HBsAg is a highly immunogenic component used for this purpose (12).

The RTS,S/A01E formulation reached 59.1% vaccine efficacy over 17 months of phase III trials after all three doses were administered.

J Clin Invest. 2010; 120(12):4168–4178

Nature. 1967 Oct 14;216(5111):160-2.

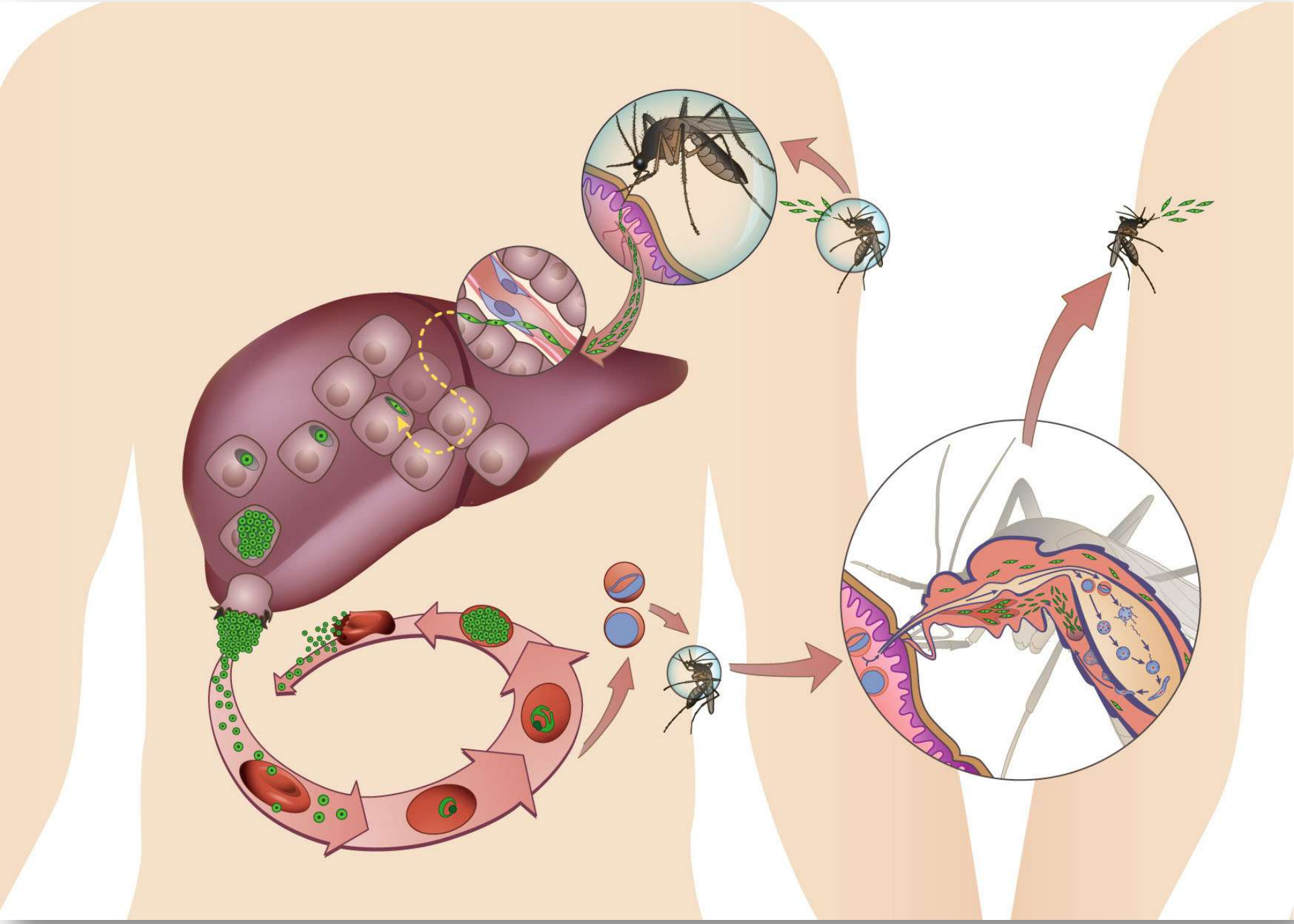
Protective immunity produced by the injection of x-irradiated sporozoites of plasmodium berghei.

Nussenzweig RS, Vanderberg J, Most H, Orton C.

Protective Immunity produced by the Injection of X-irradiated Sporozoites of *Plasmodium berghei*

STUDIES with avian malaria have shown that killed sporozoites as well as sporozoites inactivated with ultraviolet light can produce a partial immunity after injection into birds[1,2]. On the other hand, attempts to use the erythrocytic stages of the parasite as the source of antigen have met with only limited success with avian[3], rodent[4] and monkey malaria[5,6]. Previous attempts to use killed sporozoites of the rodent malarial parasite, *Plasmodium berghei*, to immunize rodents have been unsuccessful. We therefore sought to determine whether protective immunity to this parasite could be achieved by partial inactivation of the injected sporozoites as opposed to injection of dead parasites. X-irradiation was chosen as the inactivating agent, because of the partial immunity

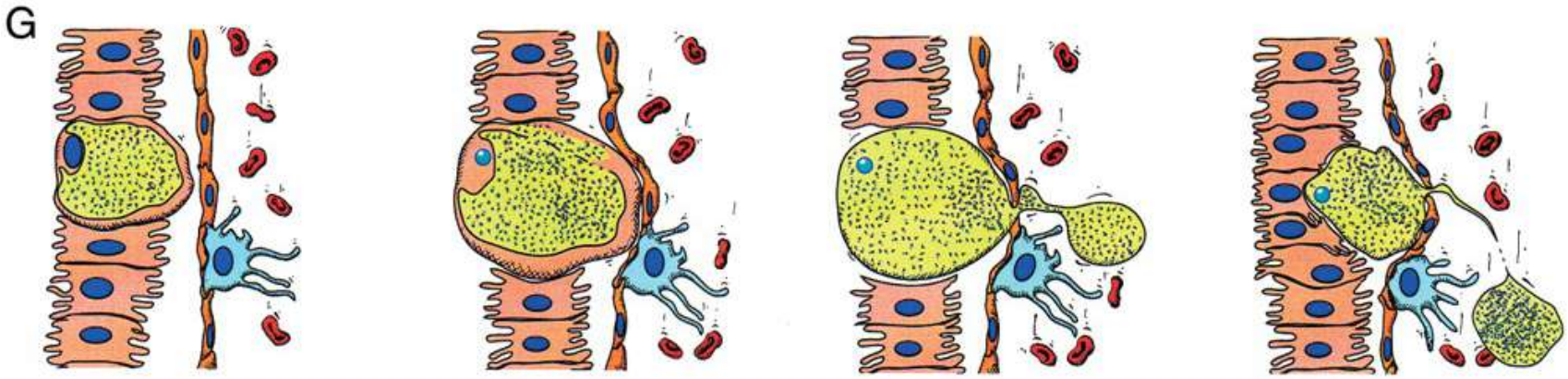
"O trabalho de Ruth e Victor Nussenzweig fundamentou a concepção e o desenvolvimento da vacina", afirma Joe Cohen, da farmacêutica GSK Bio, autor do artigo no *The Lancet* que descreve os resultados na África. O casal Ruth e Victor vive nos EUA desde 1964 e trabalham na New York University (NYU).



Science
AAAS

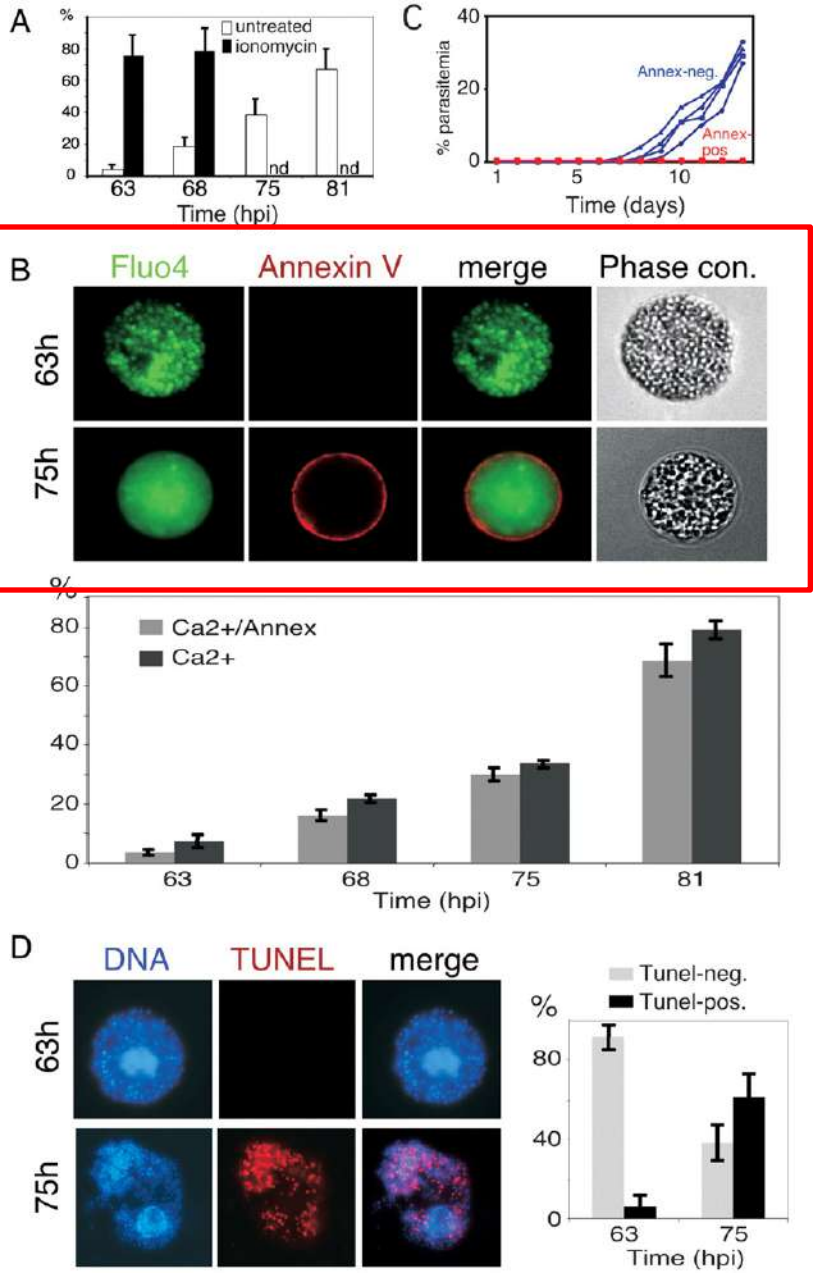
Manipulation of Host Hepatocytes by the Malaria Parasite for Delivery into Liver Sinusoids

Angelika Sturm,[1*] Rogerio Amino,[2,3*] Claudia van de Sand,[1] Tommy Regen,[1] Silke Retzlaff,[1] Annika Rennenberg,[1] Andreas Krueger,[1] Jörg-Matthias Pollok,[4] Robert Menard,[2] Volker T. Heussler[1]†



"The merosomes discovery"

Fig. 4. Merosomes do not expose PS at their surface for several hours



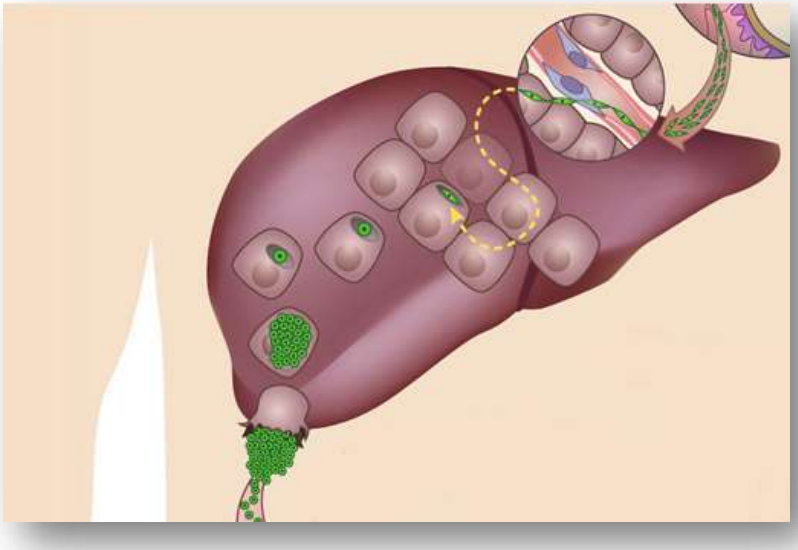
(**A**) Detached cells were isolated at different time points and stained with Annexin-V—fluorescein isothiocyanate and propidium iodide.

(B) Merozoites accumulate Ca^{2+} and inhibit the PS switch in the host cell membrane.

(**C**) Detached, Annexin-V–stained cells at 63 or 75 hpi were isolated with a micropipette and transferred to 24-well plates; Annexin-V–positive or Annexin-V–negative floating cells were separated and subsequently injected into mice. Mouse parasitemia were determined daily for 2 weeks [four mice infected with Annexin-V–positive cells (red symbols); four mice infected with Annexin-V–negative cells (blue symbols)].

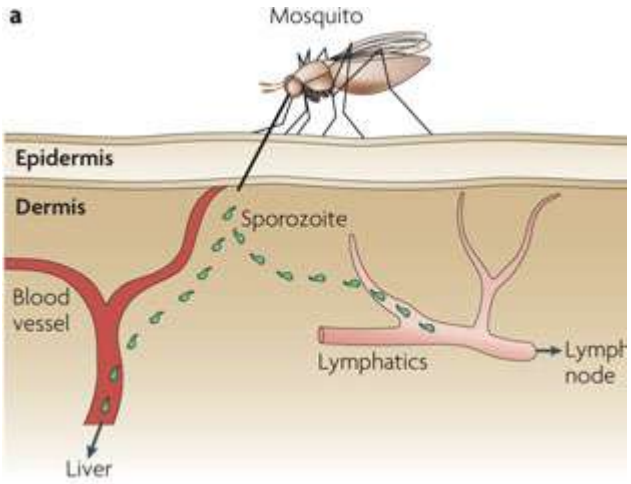
(**D**) TUNEL (terminal deoxynucleotidyl transferase–mediated dUTP nick-end labeling) staining of floating cells at 63 and 75 hpi. Red, TUNEL stain; blue, Hoechst 33258 stain.

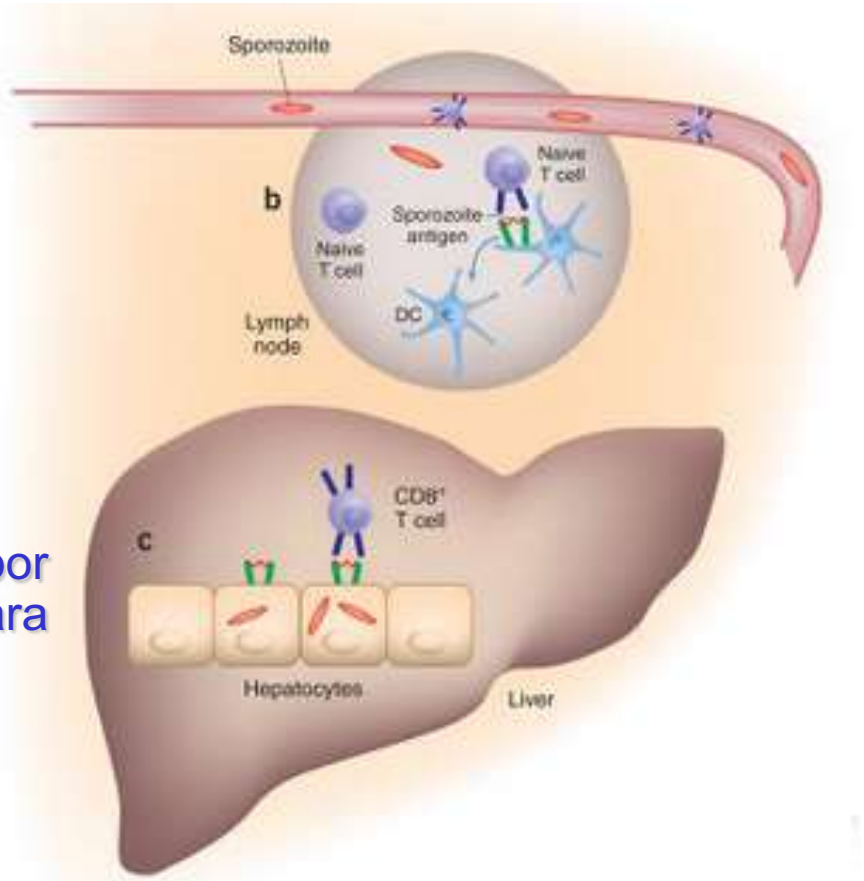
Que mecanismos efetores do sistema imune podem atuar nas formas intra-hepáticas durante a primo-infecção?



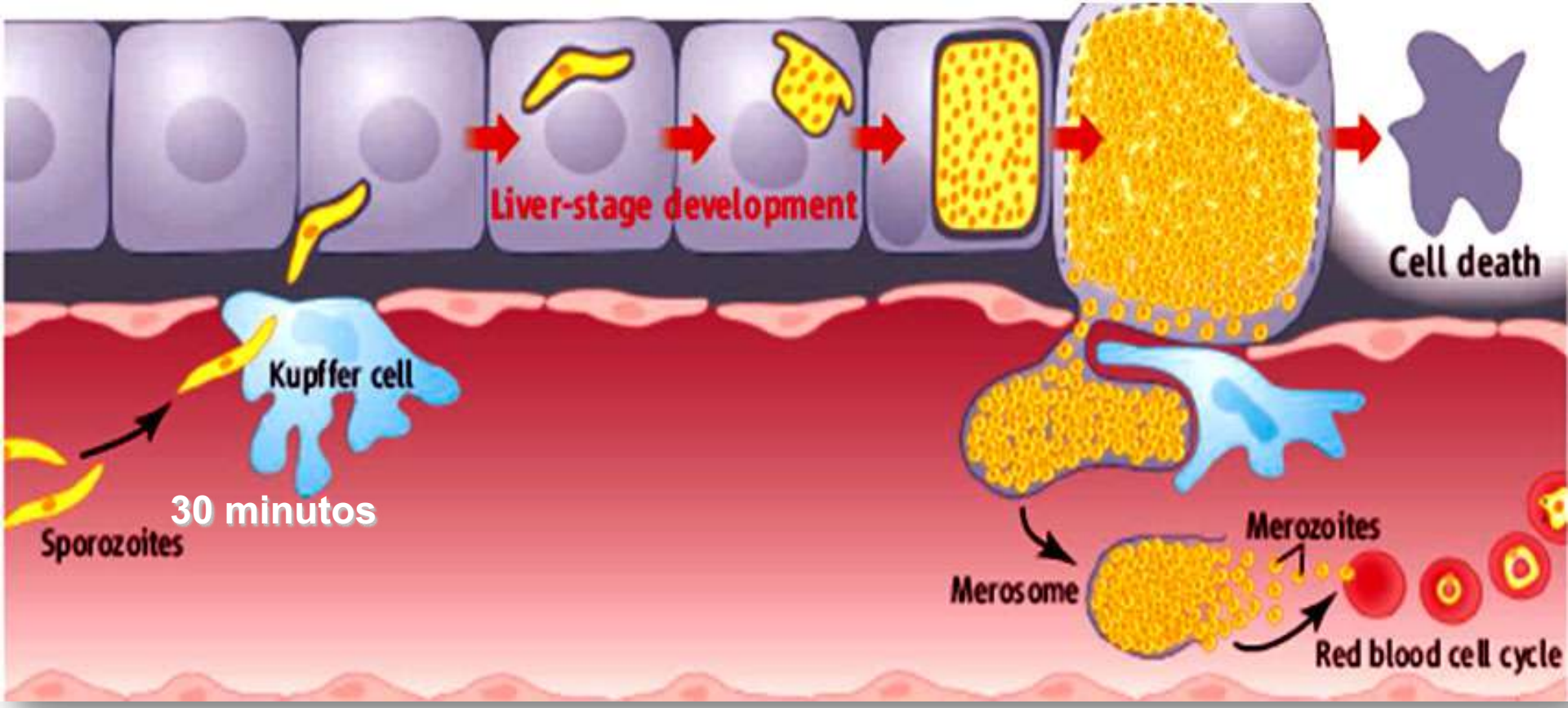
- citotoxicidade por células NK
  (se houver reconhecimento)

Que mecanismos efetores do sistema imune podem atuar nas formas intra-hepáticas durante a re-infecção?



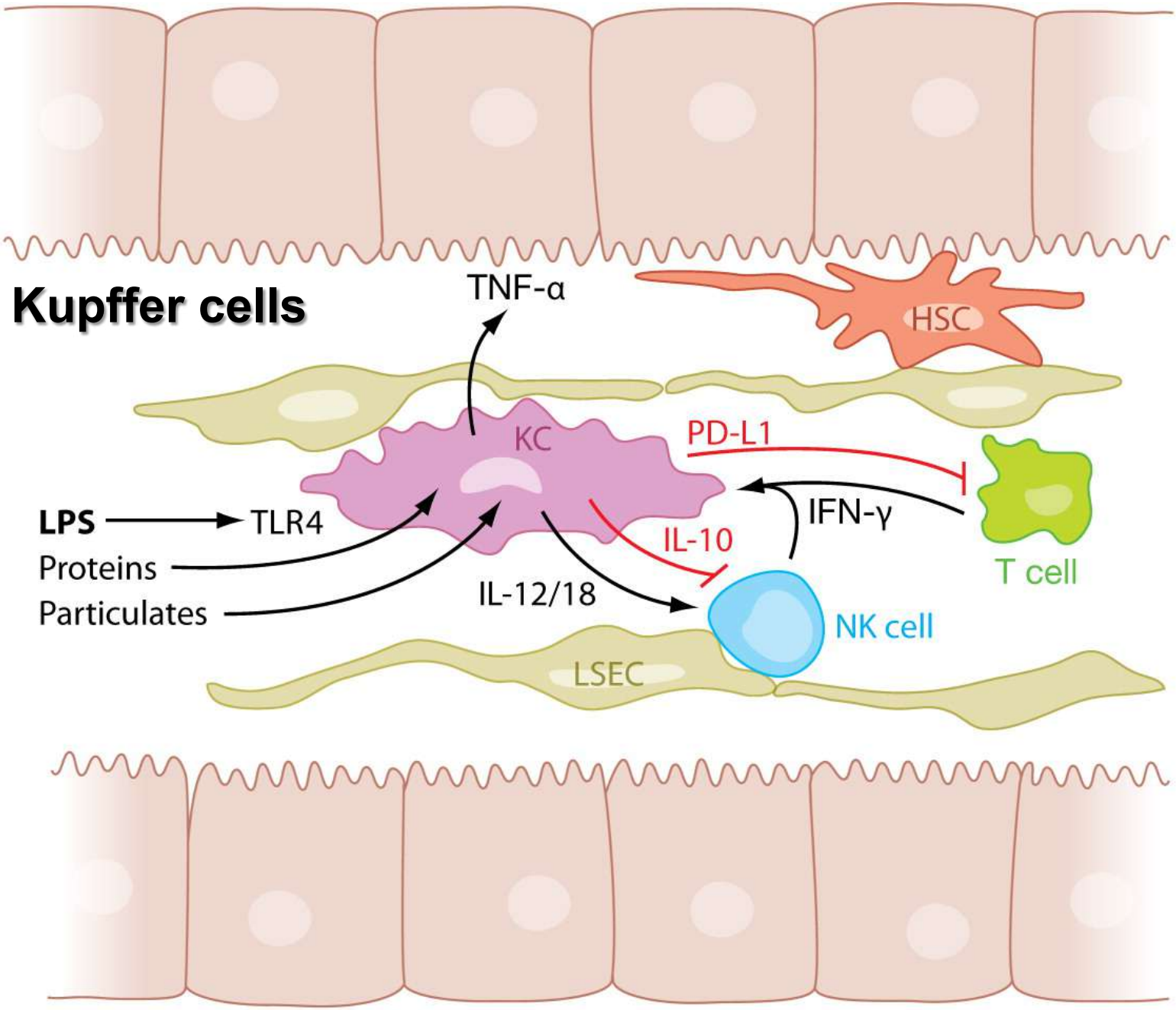


- destruição dos hepatócitos infectados por linfócitos Tc1 ($CD8^+$) específicos para peptídeos do plasmódio

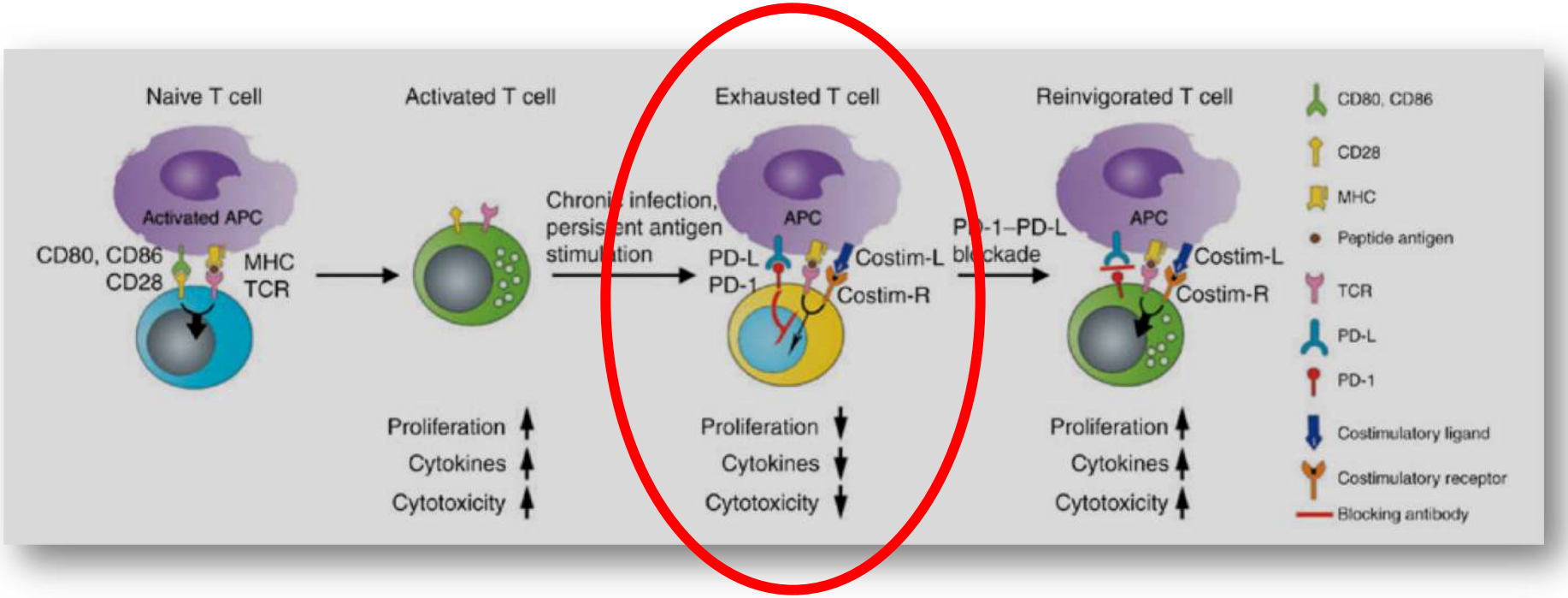


Duração da esquizogonia pré-eritrocítica			
P. vivax	*P. malariae*	*P. falciparum*	*P. ovale*
8 dias	12-16	6	9

Número de merozoítas por esquizonte tecidual			
P. vivax	*P. malariae*	*P. falciparum*	*P. ovale*
10.000	2.000	40.000	15.000


Kupffer cells
TNF-α
HSC
KC
PD-L1
LPS
TLR4
Proteins
Particulates
IL-10
IFN-γ
T cell
IL-12/18
NK cell
LSEC

Interação PD1 (Programmed Death 1) & PDL1/PDL2



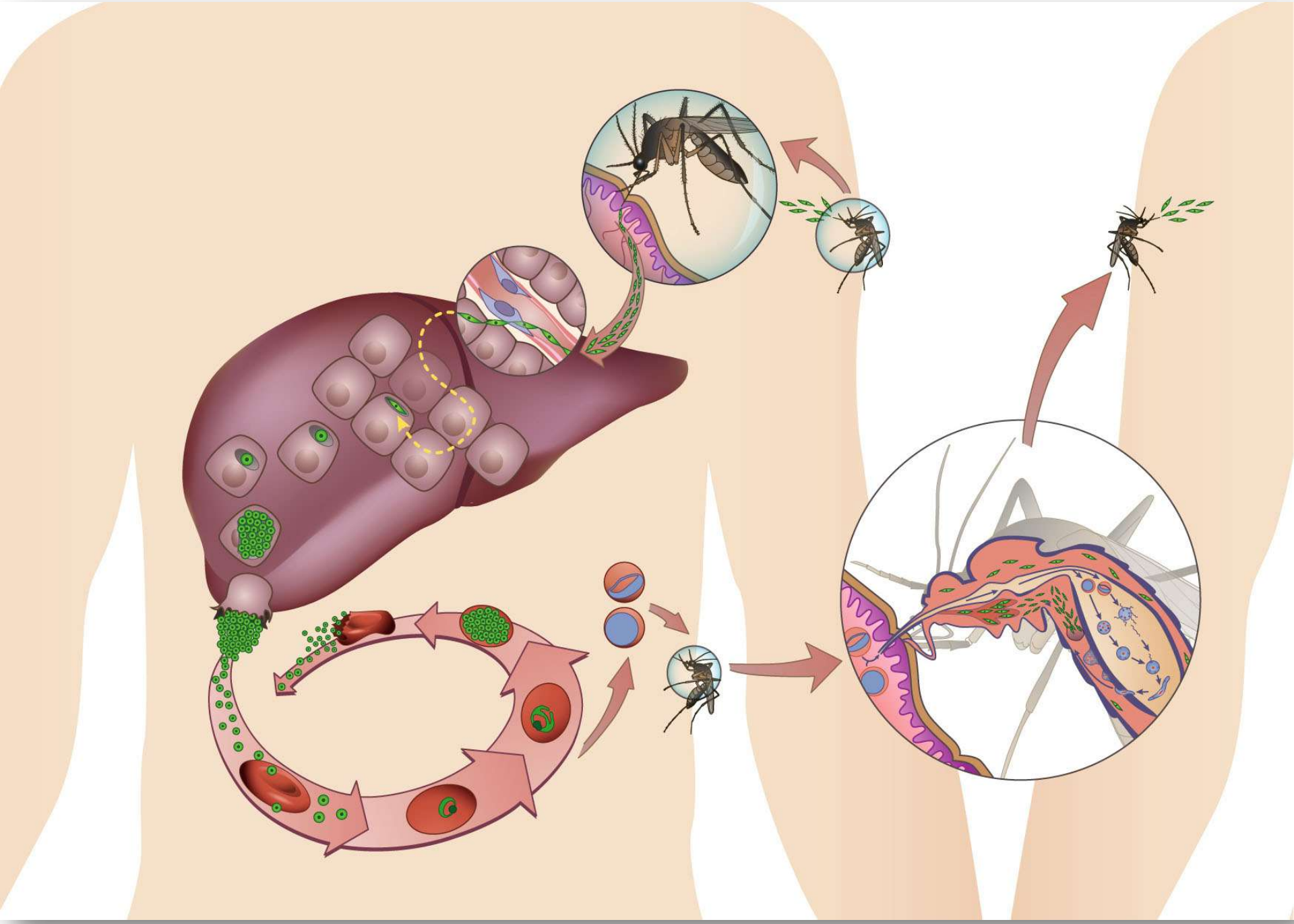
A interação PD-1 com PD-L1 determina:

-<u>Inibição da proliferação e secreção de citocinas pelas células T</u>

-O sinal via PD-1 pode inibir níveis baixos de coestimulação via CD28.

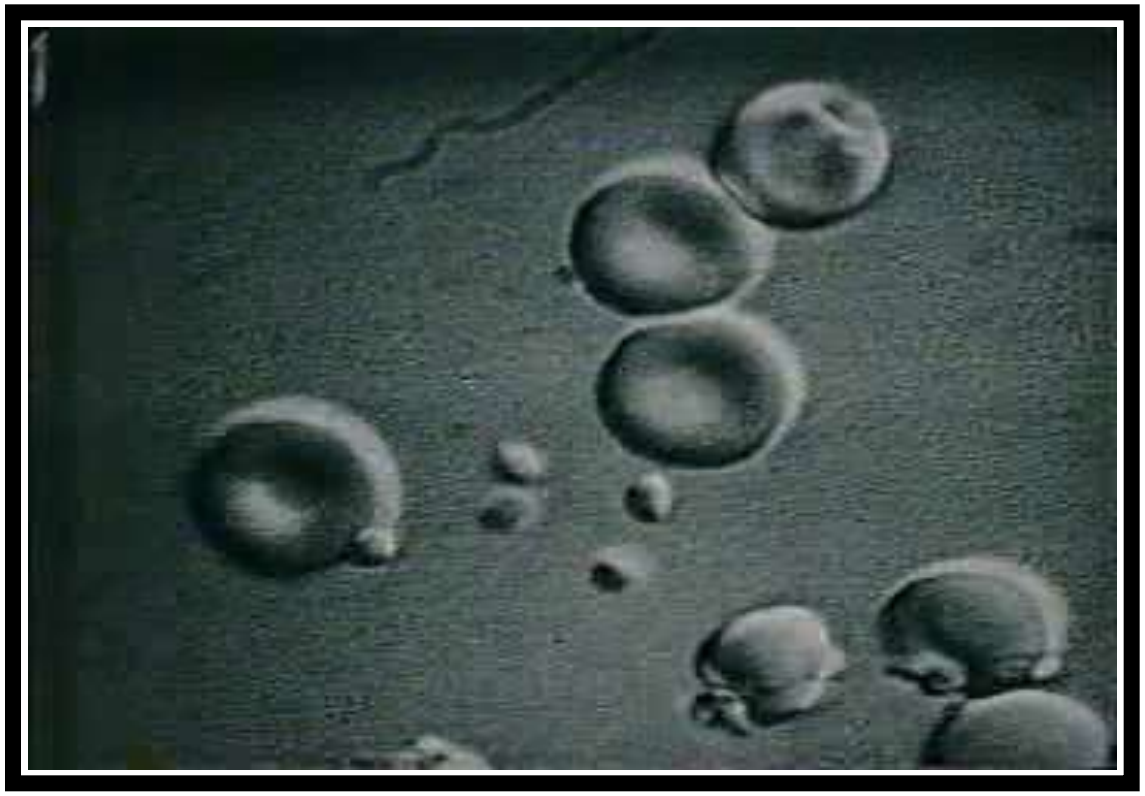
Coestimulação negativa

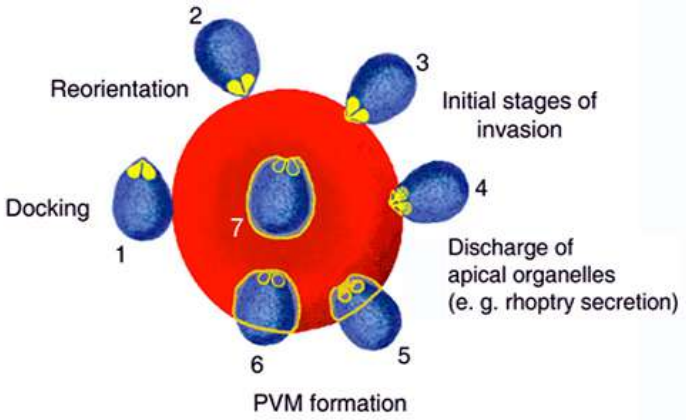
Nature Immunology **8**, 239 - 245 (2007)



- A invasão da hemácia é facilitada pela <u>secreção de enzimas na róptria</u>

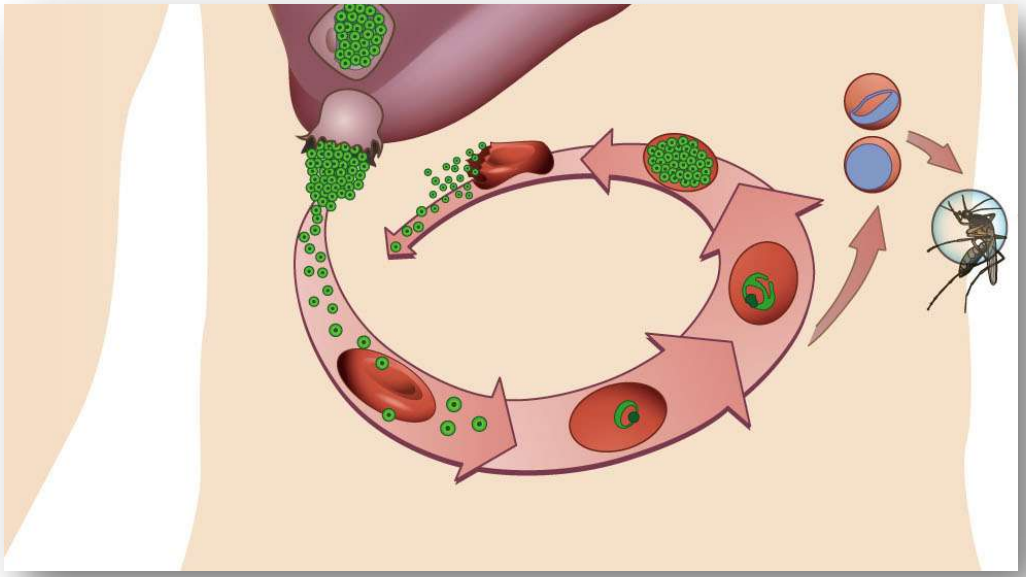
- A interação firme/invasão depende de proteínas da superfície do merozoíta e da hemácia:
    - *P. vivax*: Duffy binding protein 1 e 2 (parasita) e fator Duffy (RBC)
    - *P. falciparum*: EBA-175 (parasita) e glicoforinas (RBC)





Fonte: NHI

Que mecanismos efetores do sistema imune podem atuar nos merozoítos no início da infecção?

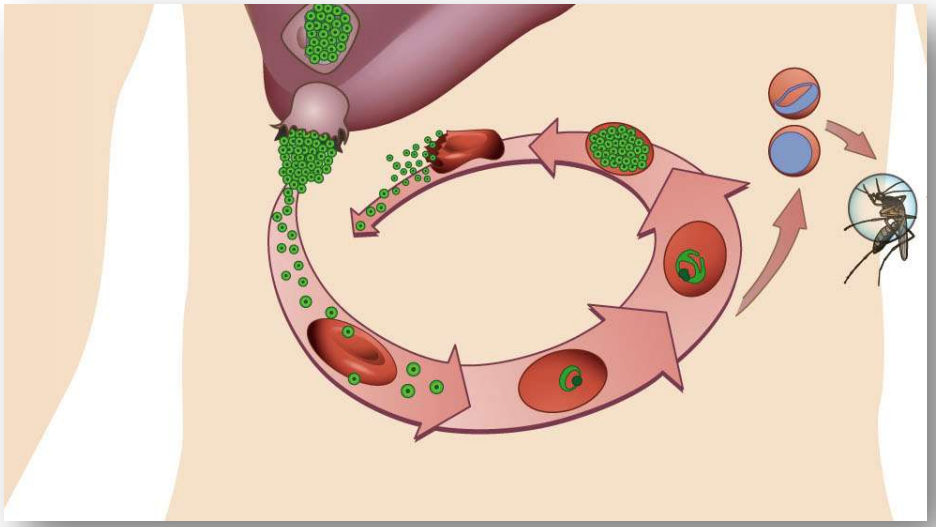


- fagocitose ou citotoxicidade direta por neutrófilos e macrófagos (se houver reconhecimento)

- opsonização ou lise pelo complemento (via alternativa, ou clássica se houver anticorpo IgM de baixa afinidade ou IgG adquiridos da mãe)

- neutralização (se houver anticorpo)

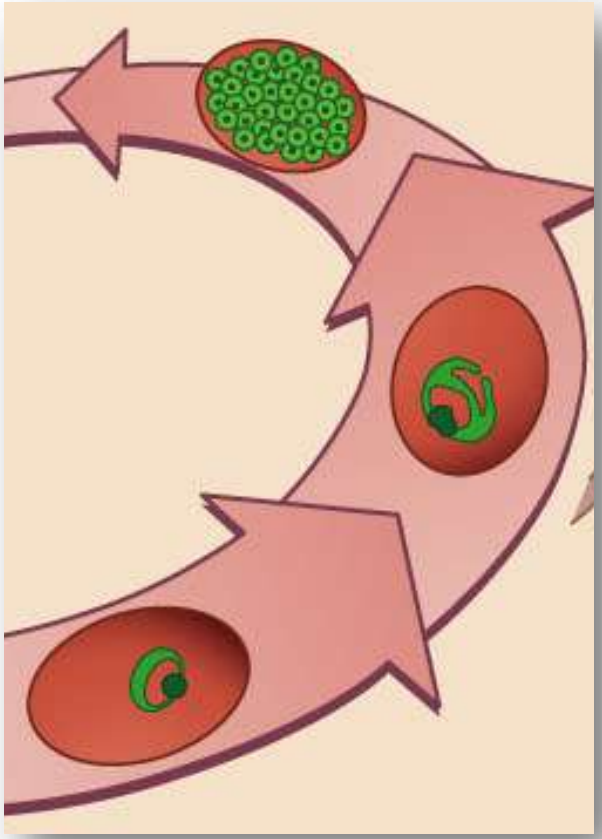
Que mecanismos efetores do sistema imune podem atuar nos merozoítos na infecção tardia?



- neutralização ou inibição da penetração (principalmente IgG de alta afinidade)
- fagocitose ou citotoxicidade por macrófagos e neutrófilos (opsonização por C3b ou IgG)
- Aumento da fagocitose pelo IFN-γ produzido pelos linfócitos Th1
- lise pela via clássica

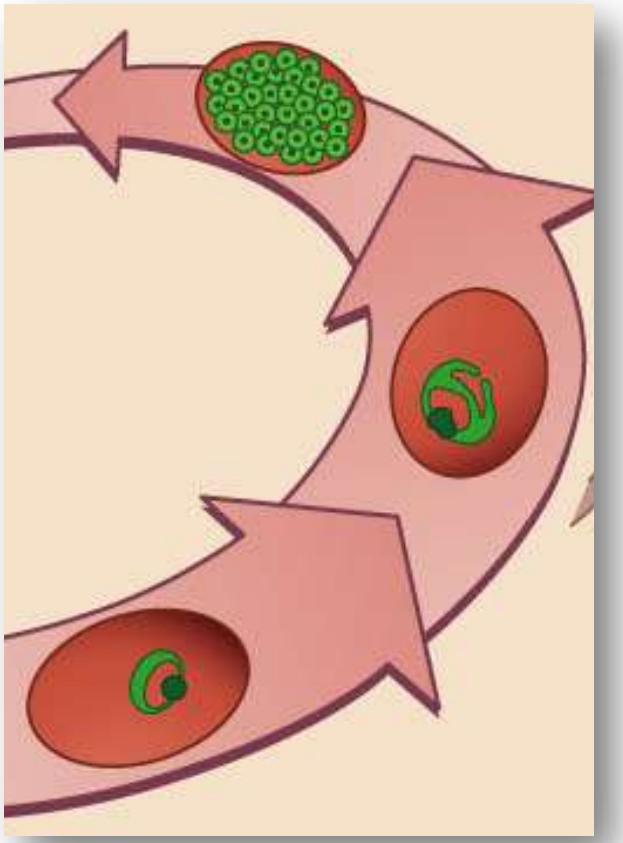
Que mecanismos efetores do sistema imune podem atuar nas formas intra-eritrocíticas no início da infecção?

- nenhum

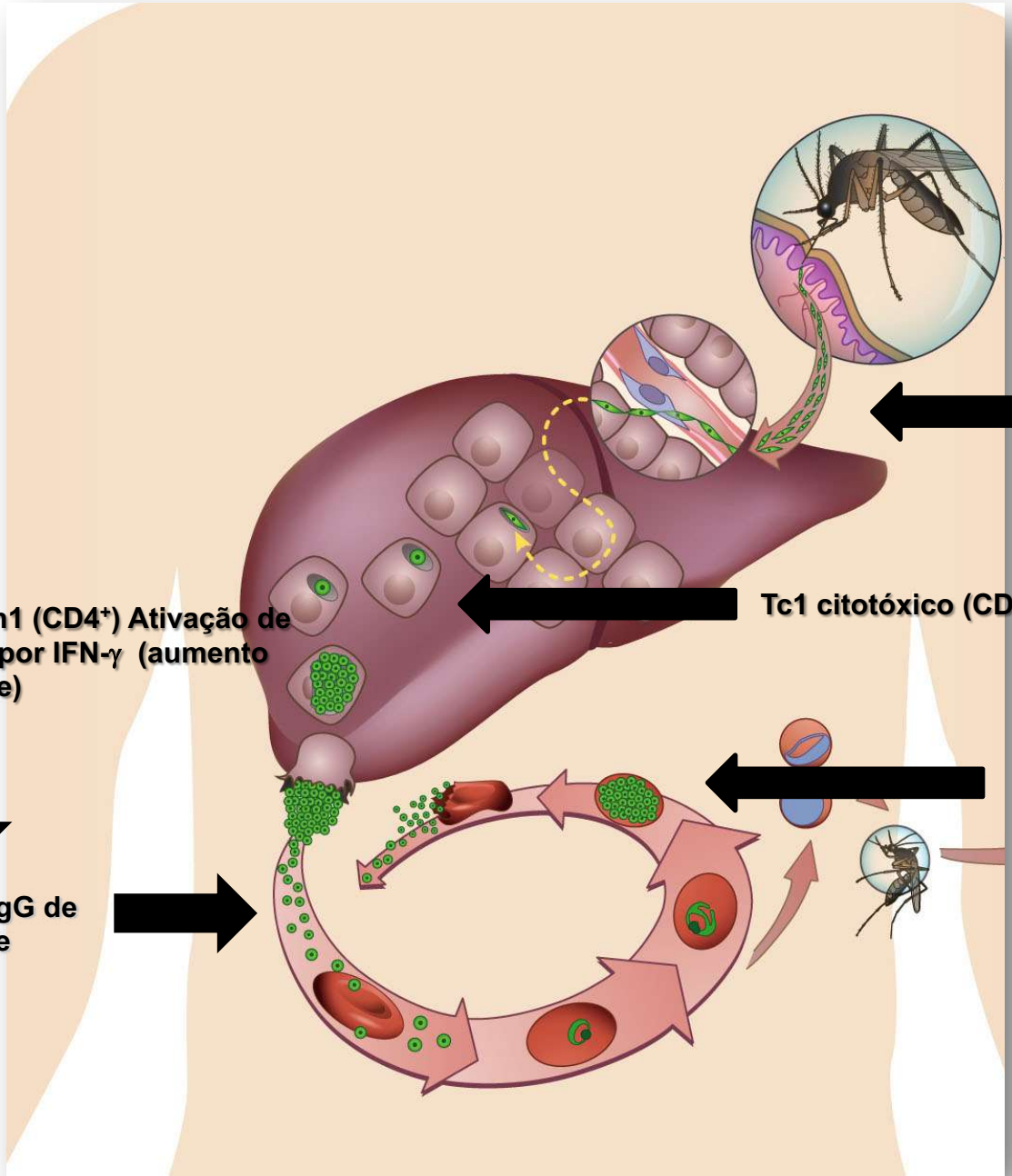


Que mecanismos efetores do sistema imune podem atuar nas formas intra-eritrocíticas na infecção tardia?

- nenhum (a não ser que antígenos do parasita se fixem na superfície das hemácias)



Que mecanismos efetores do sistema imune que efetivamente atuam na malária?



Anticorpos IgG de alta afinidade

Tc1 citotóxico ($CD8^+$)

Linfócitos Th1 ($CD4^+$) Ativação de macrófagos por IFN-γ (aumento da fagocitose)

IgG específica contra antígenos do parasita expressos na membrana do eritrócito

Anticorpos IgG de alta afinidade

Imunidade ao plasmódio: mais questões que respostas

1) Introdução
2) Mecanismos de defesa
3) **Mecanismos de escape**
4) Mecanismos de patogênese

Estratégias de escape

Estratégias modular a resposta imunológica do hospedeiro

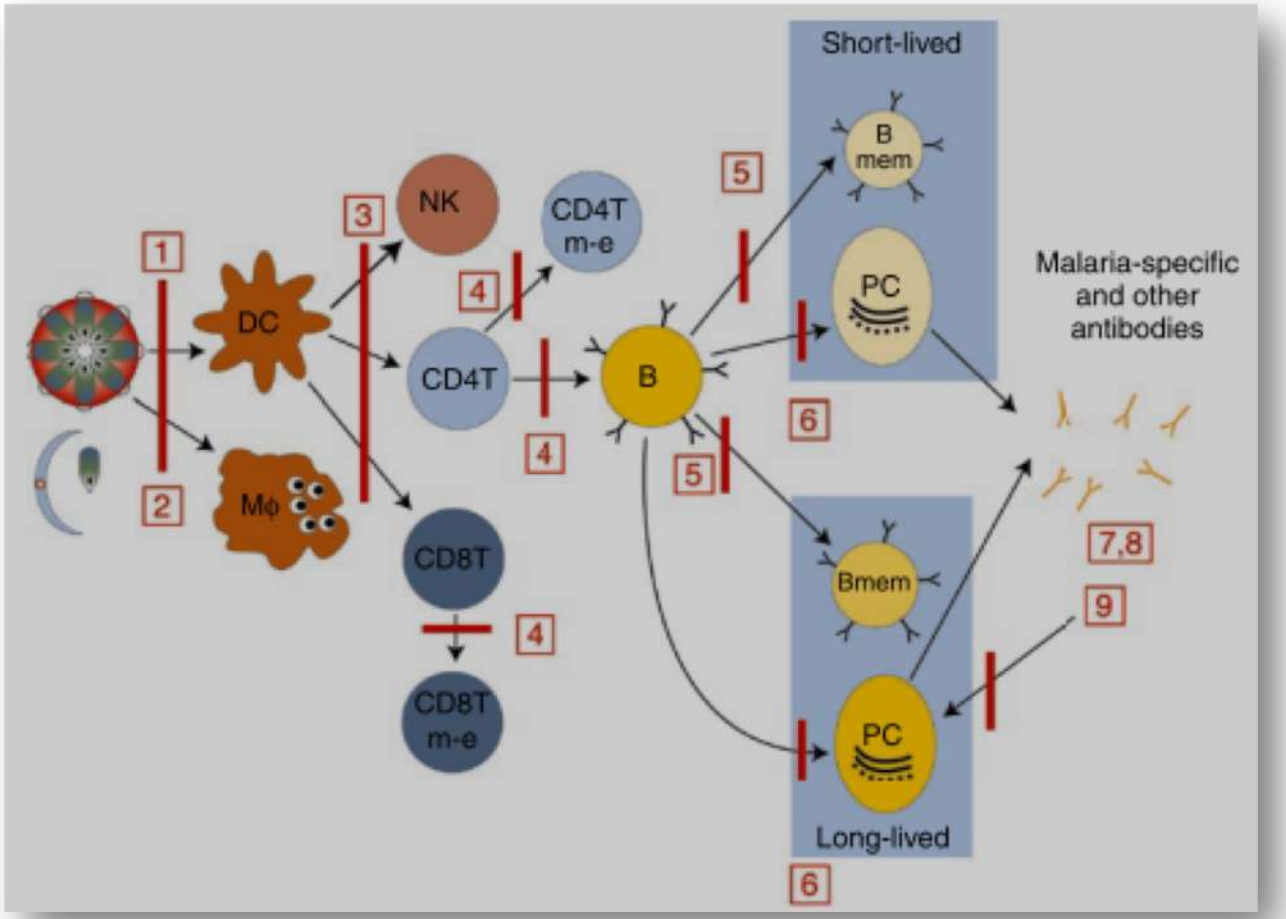
- Crescimento intracelular em células com pouco ou sem apresentação em MHC classe 1

- Não deixar a hemácia infectada passar pelo baço: citoaderência (apenas *P. falciparum*) e variação antigênica

- Interferência imunológica: anergia, inibição da resposta celular contra estágios hepáticos

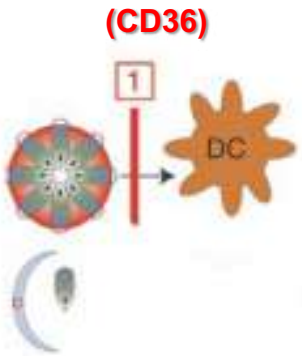
Estratégias de escape

Estratégias modular a resposta imunológica do hospedeiro

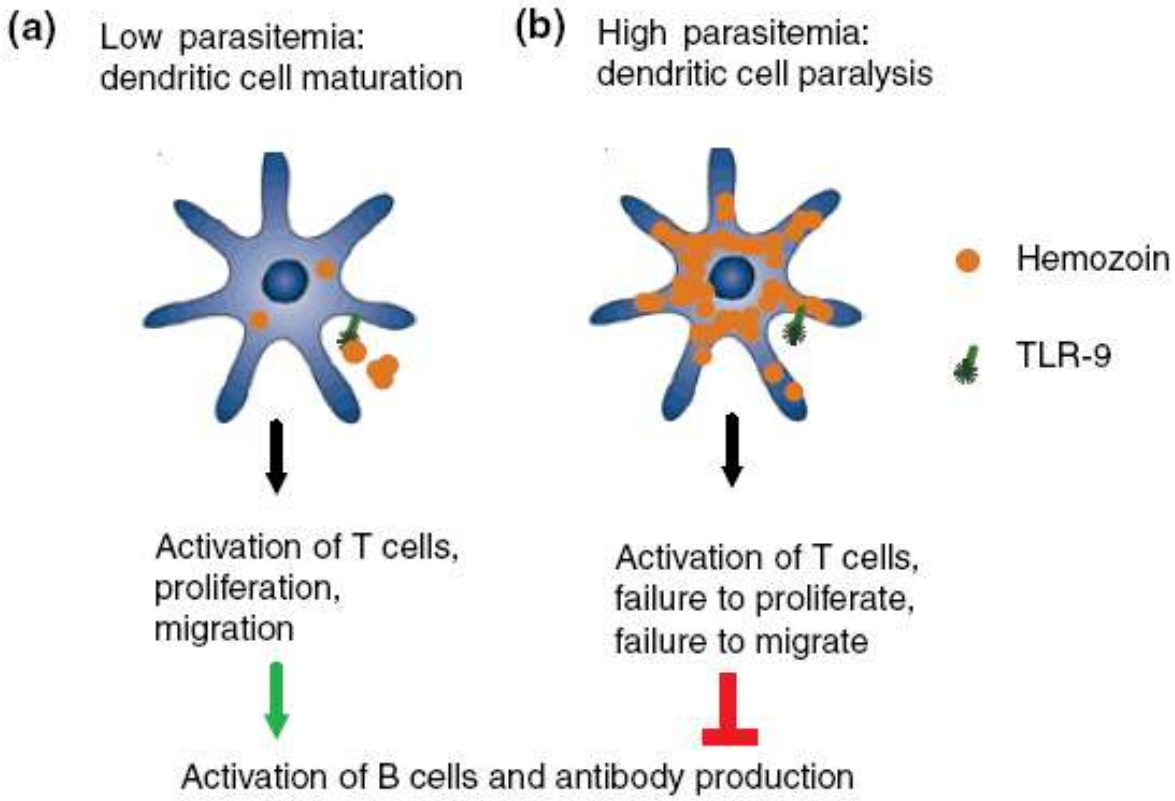


Marsh et al. Nature Immunology, 2008

Estratégias de escape



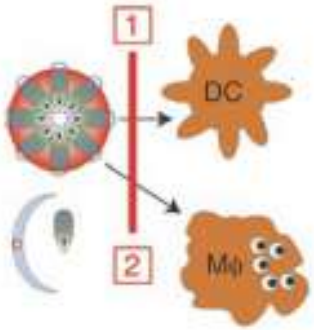
1. Interação entre iRBC e células Dendríticas pode inibir a maturação das células Dendriticas



Urban&Todryk. *Journal of Biology, 2006.*

Estratégias de escape

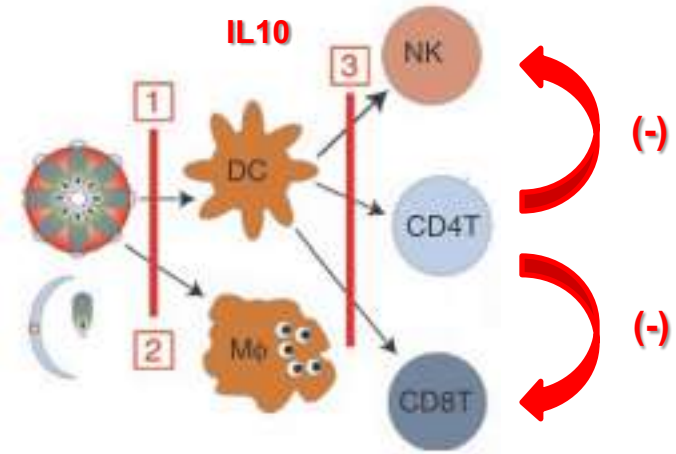
2. Hemozoína pode inibir a ativação de macrófagos



TLR9

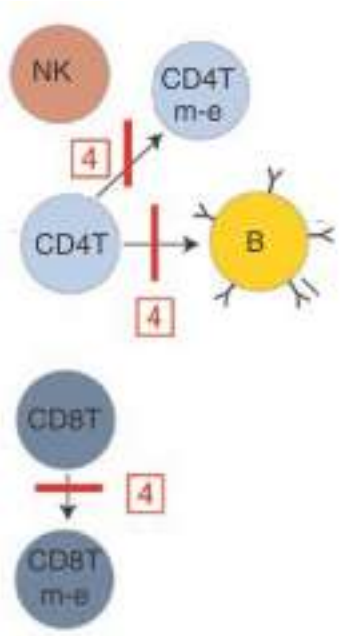
Estratégias de escape

3. IL10 produzida pelas DC e macrófagos inibe a ativação de LTCD4^{+}



Estratégias de escape

4. IL10 e TGF-β produzidos pelos LTCD4^{+} inibem a geração de células de memória (efetora e central)

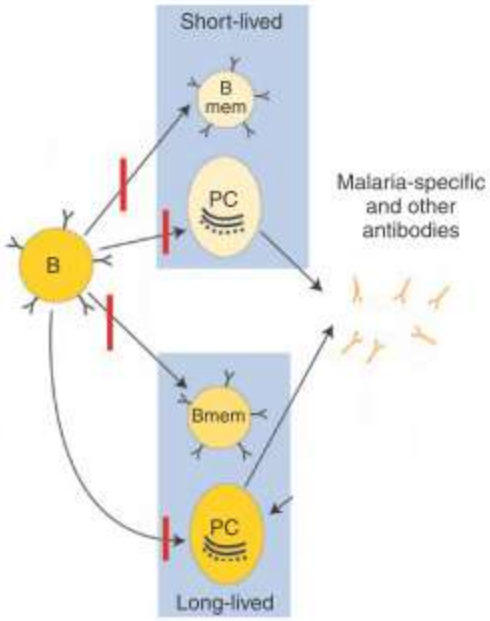


↑ **[Ag] na infecção aguda**

- Inapropriado "priming"
- Apoptose

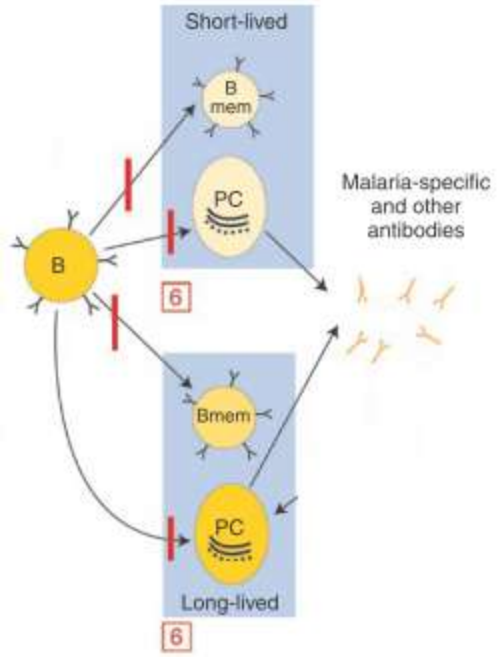
Estratégias de escape

5. iRBC induz apoptose e depleção de células B de memória



Estratégias de escape

6. iRBC induz respostas humorais para alvos polimorficos



- Hipergamaglobulemia
- Aumento do catabolismo de Ab
- Ativação policlonal

TH2

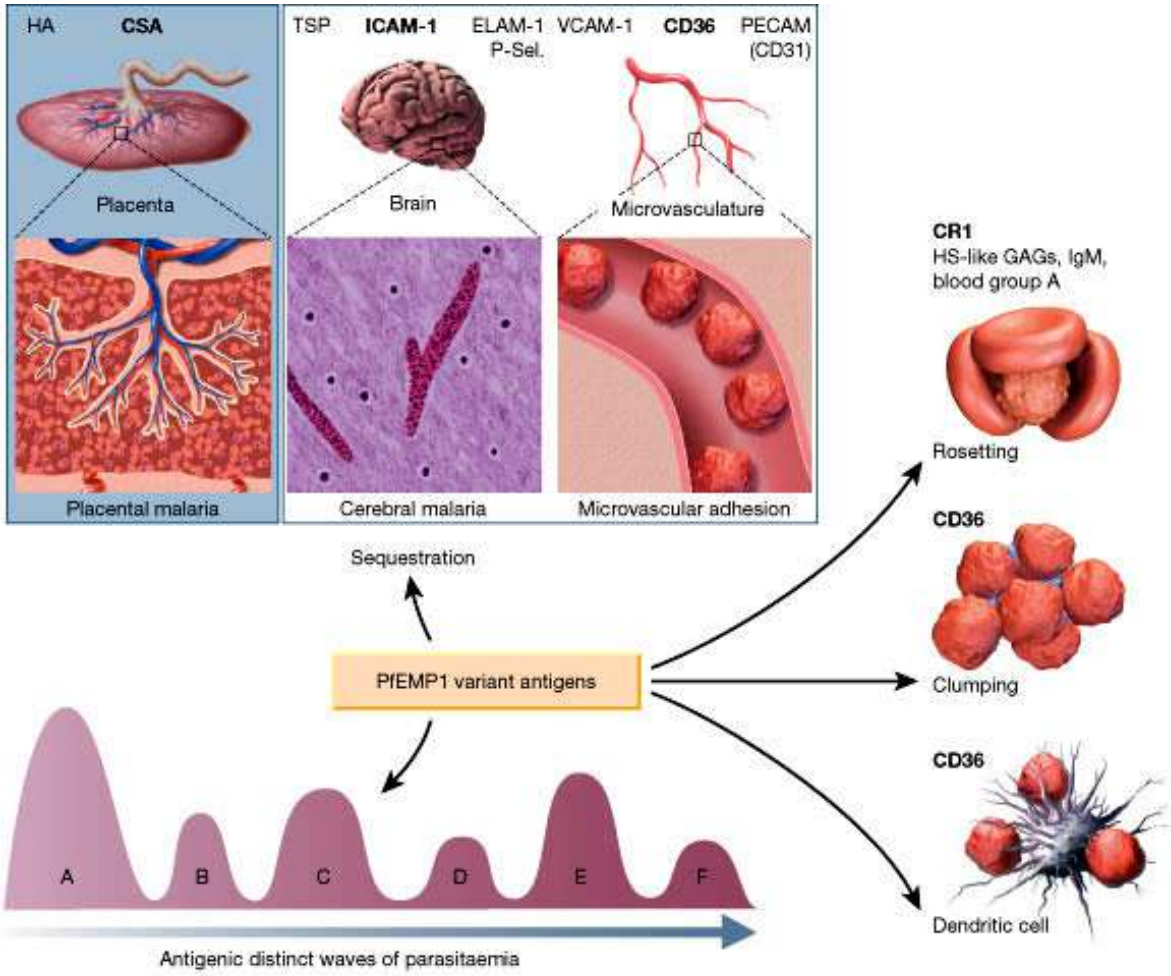
IgG1

TH1

IgG2a

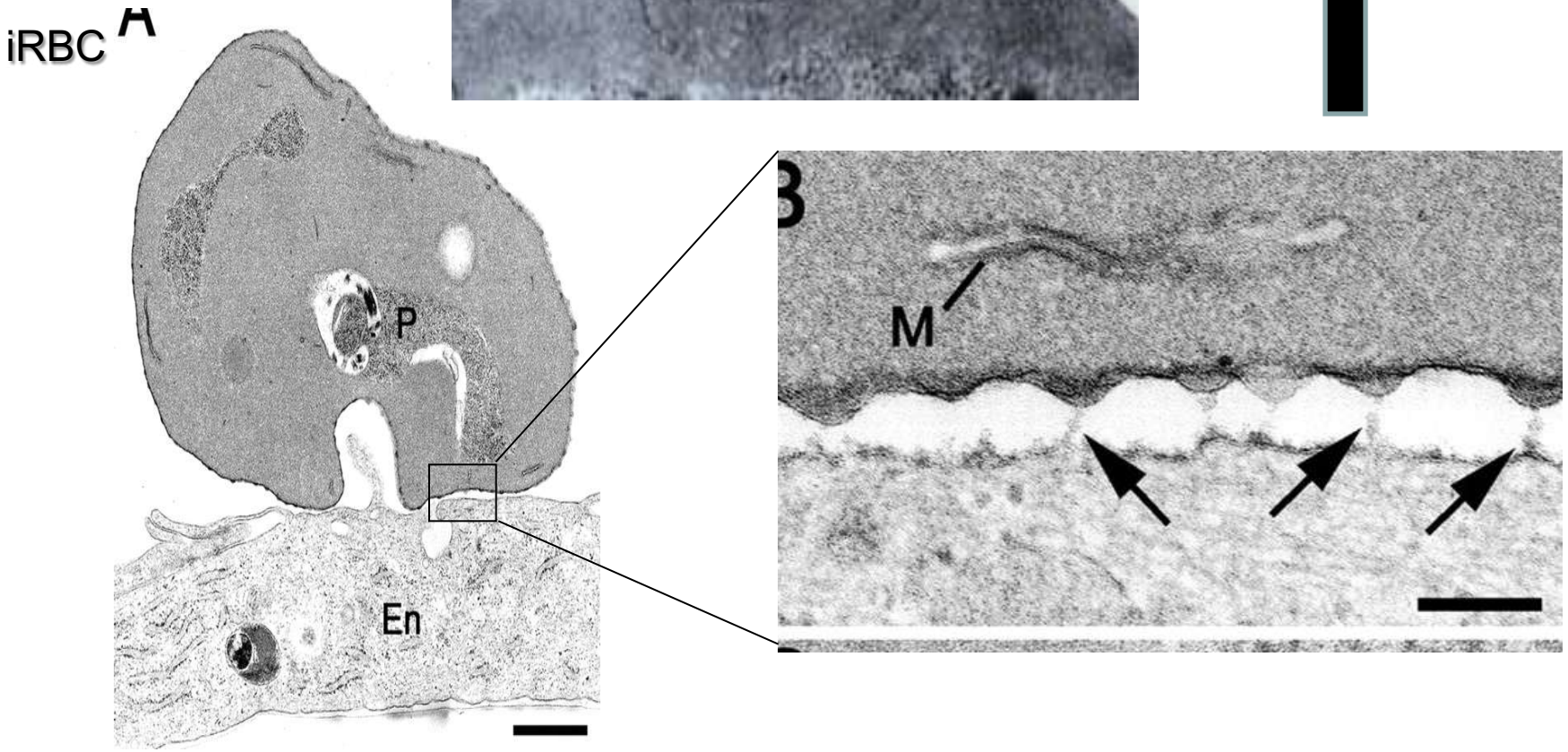
Estratégias de escape

7. Variação antigênica



Miller *et al.*, 2000

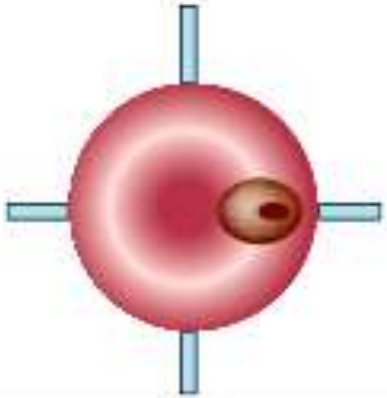
Hemácias infectadas com formas >20h após reinvasão tem protrusões (“knobs”)



Célula endotelial

(Horrocks et al. 2005)

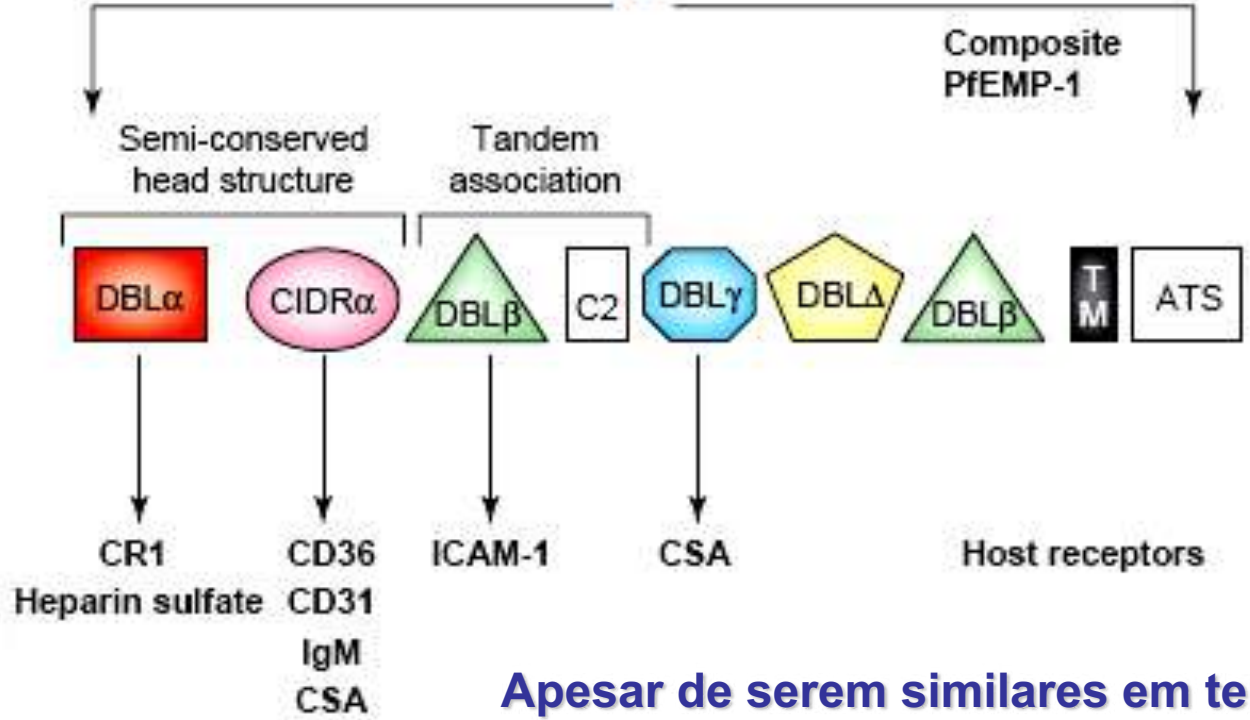
família *var gene* (±60 cópias)



pRBC

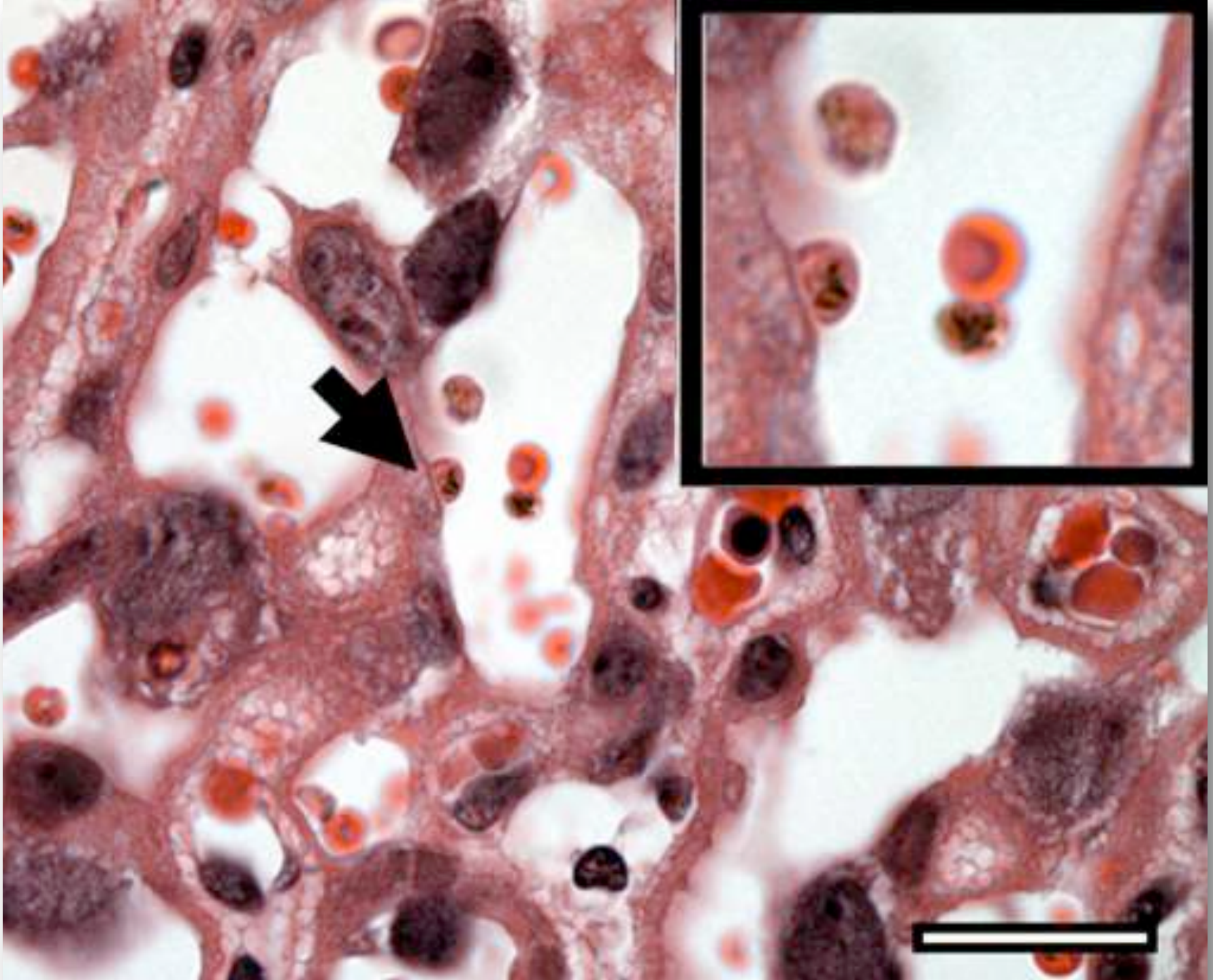
16 a 20 h após a invasão a proteína é inserida na membrana do eritrócito

Proteína PfEMP-1



Apesar de serem similares em termos de estrutura geral, cada PfEMP-1 é altamente polimórfica.

Mackintosh *et al.*, TRENDS in Parasitology, 2004.



Imunidade ao plasmódio: mais questões que respostas

1) Introdução
2) Mecanismos de defesa
3) Mecanismos de escape
4) **Mecanismos de patogênese**

Aspectos clínicos

Sintomas da infecção com *Plasmodium sp.* em pessoas não-imunes

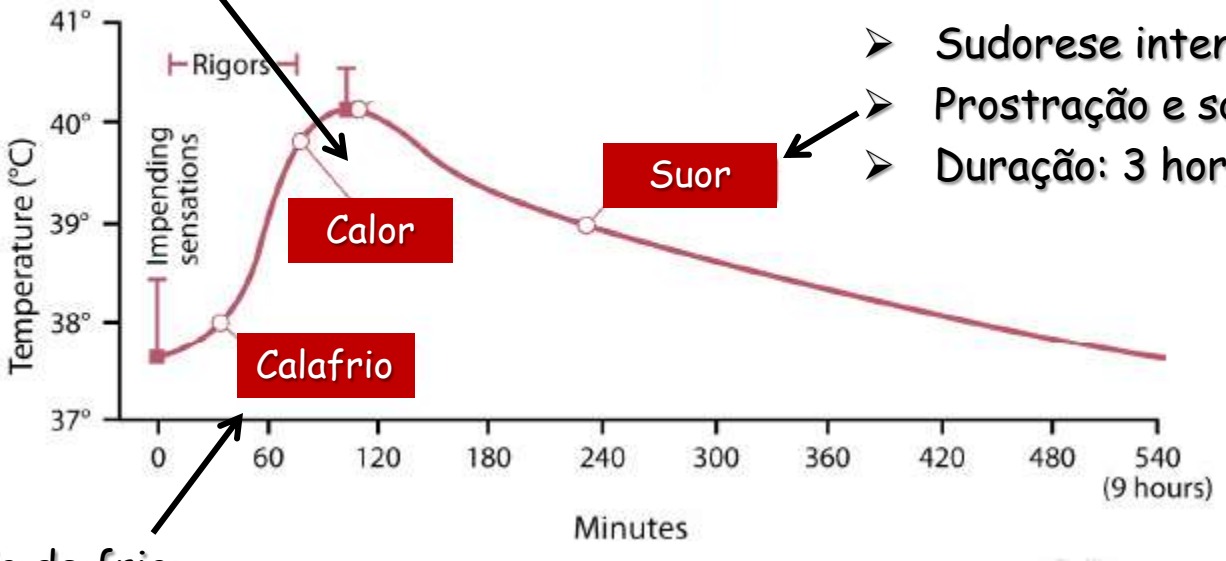
- febre
- cefaléia
- sudorese
- artralgia
- mialgia
- calafrios

Frequente: febre intermitente

as vezes: esplenomegalia, diarréia, vômitos e anemia

- Calor intenso
- Dor de cabeça
- Náusea e vômito
- Temperatura Alta (39-41°C)
- Duração: 2-8 horas

IL-1, IL-6 e TNF-α



- Sudorese intensa
- Prostração e sono
- Duração: 3 horas

- Forte sensação de frio
- Tremores incontroláveis
- Náusea e vômito
- Duração: 1-2 horas

P. vivax	*P. malariae*	*P. falciparum*	*P. ovale*
48 horas	72 horas	36-48 horas	48 horas
Terçã benigna	Quartã	Terçã maligna	Terçã leve

Fonte: CTLT

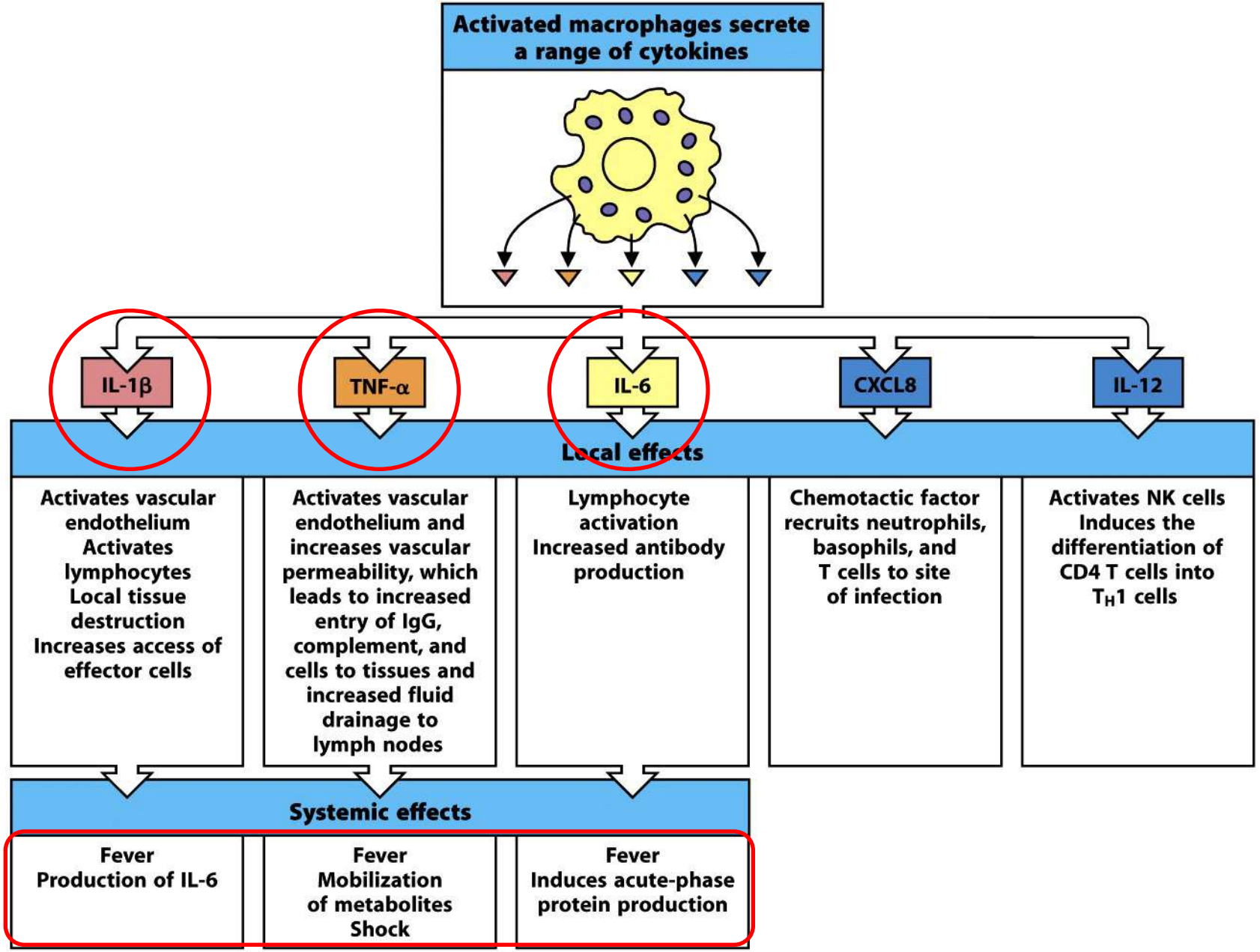
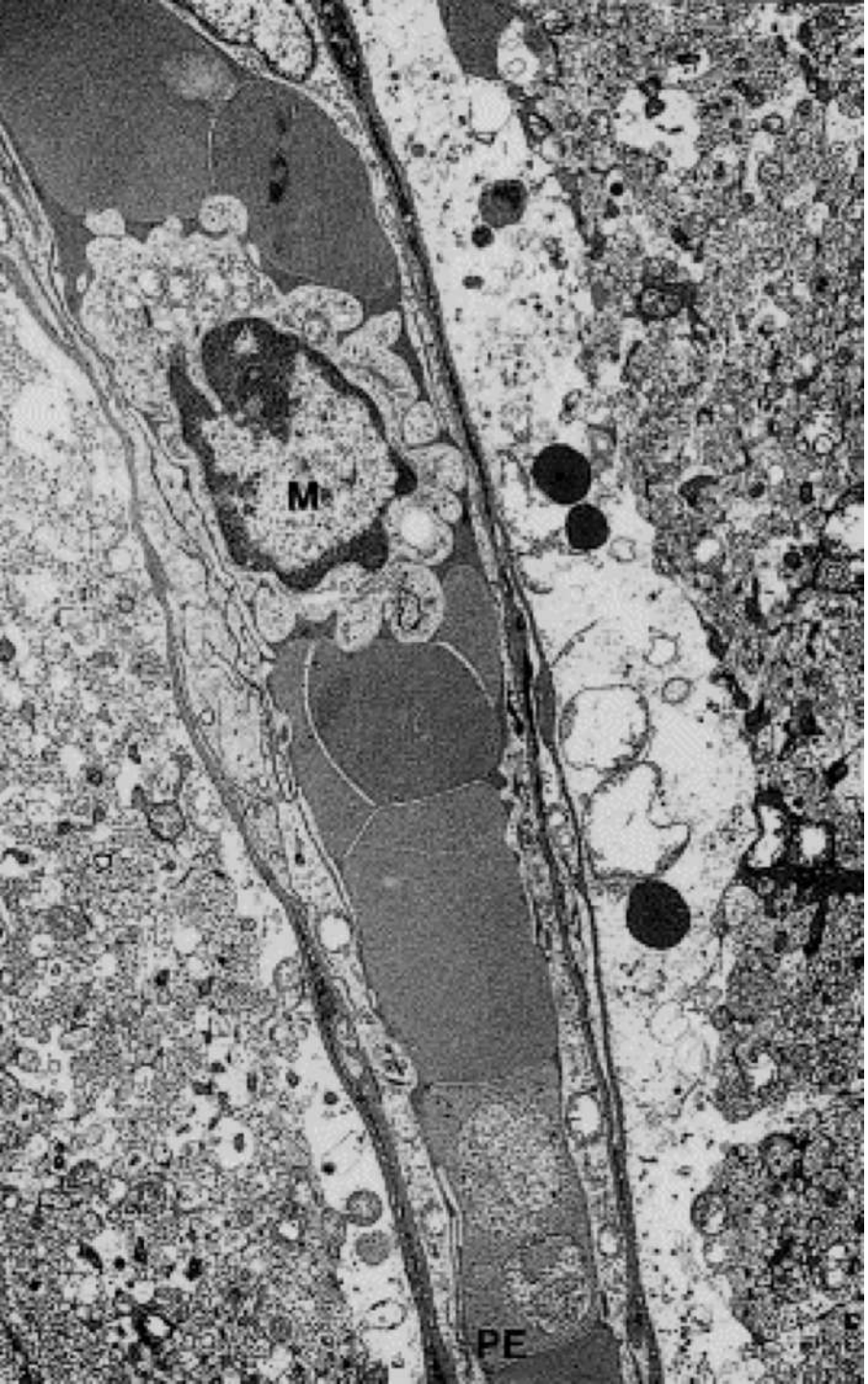


Figure 2-44 Immunobiology, 7ed. (© Garland Science 2008)



Sequestro

⬇

Obstrução da microvasculatura

⬇

Isquemia e Hipoxia

⬇

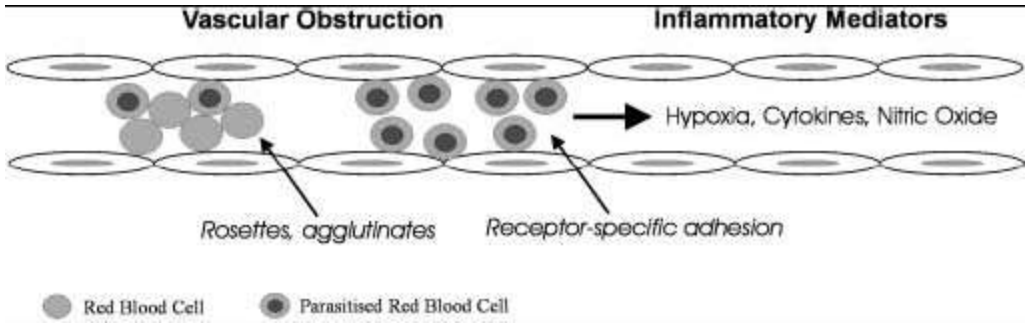
Patogênese

⬆

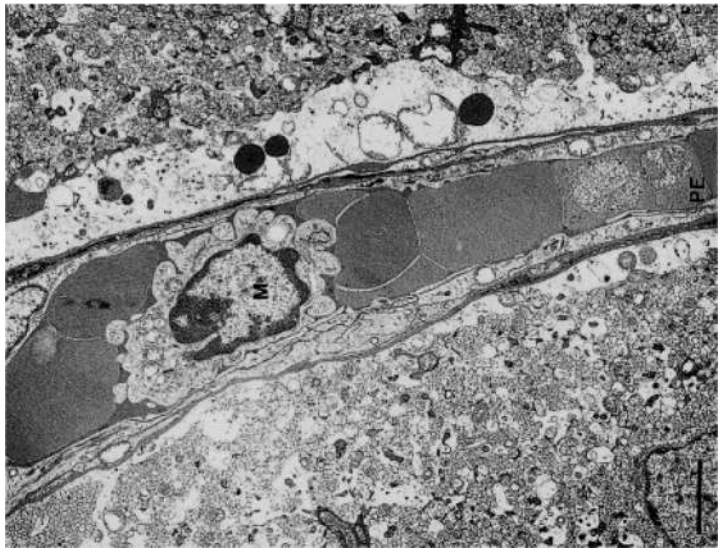
Citocinas vaso ativas

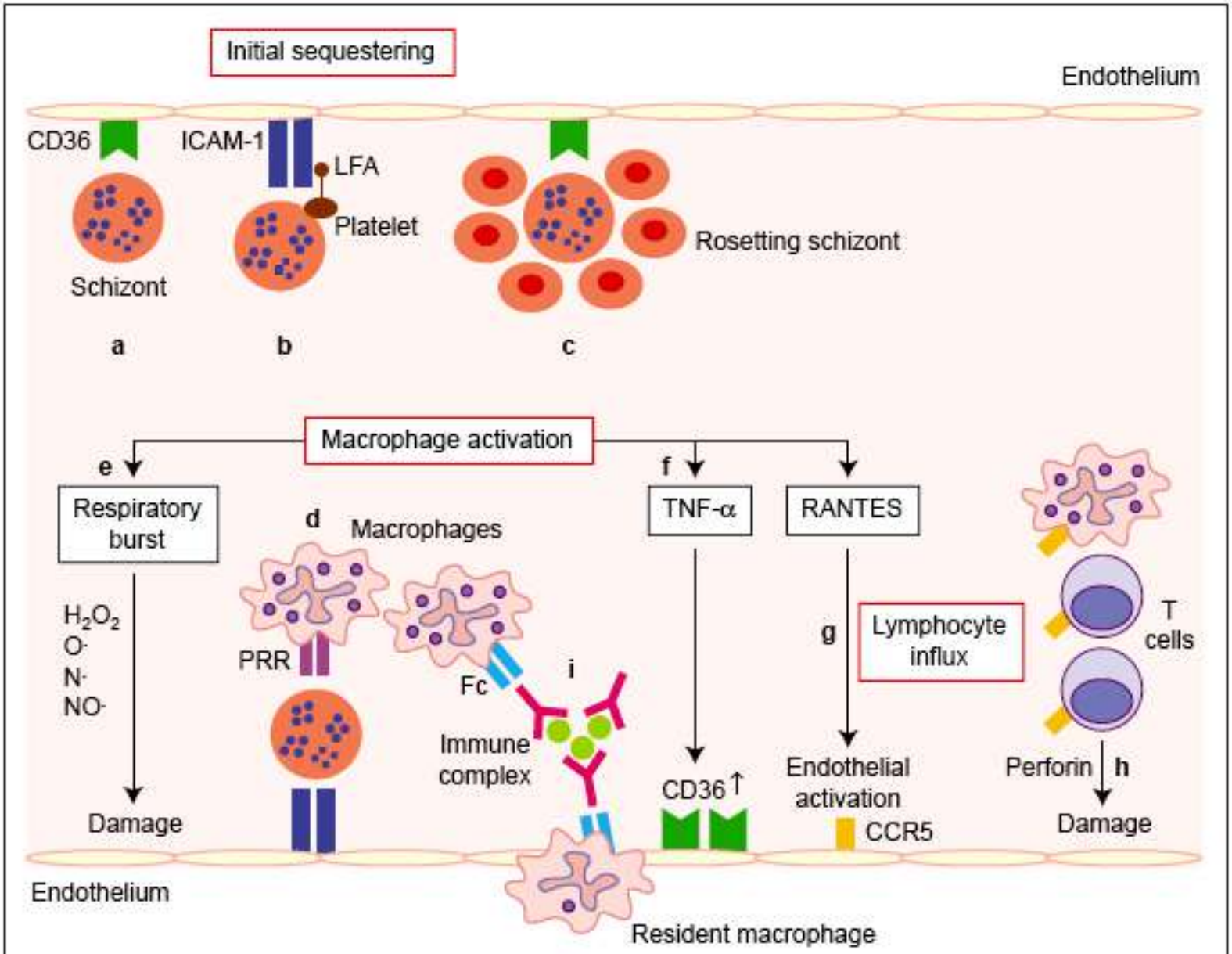
Severe and fatal disease syndromes in malaria

Cerebral malaria: Possible sequence or mechanism of disease



- Cerebral parasite sequestration
- Bioactive GPI
- Pro-inflammatory cytokine cascade
- Endothelial-cell activation
- NKT-cell activation
- TH1/TH2-cell balance
- Chemokine production
- Monocyte, macrophage and neutrophil recruitment
- Platelet and fibrinogen deposition
- CD4+, CD8+ and γδT-cell involvement
- IFN-γ production
- Neurological metabolic derangements and possibly hypoxia



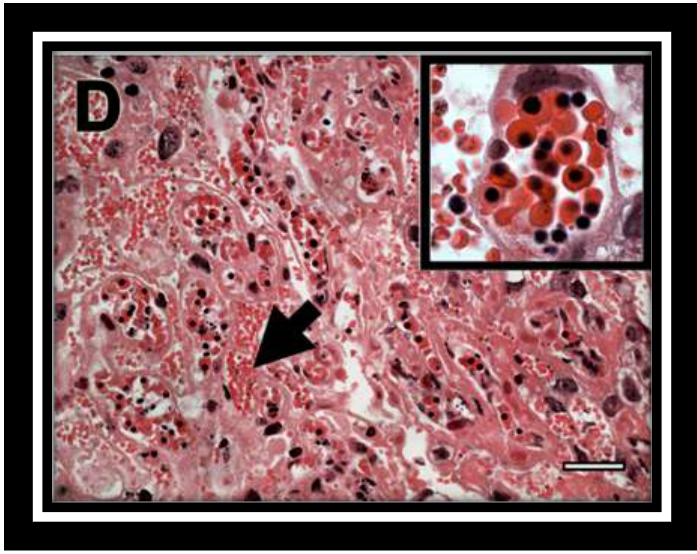


Possible pathways of immunopathological organ damage inside organ vessels

Severe and fatal disease syndromes in malaria

Severe malarial anaemia: Possible sequence or mechanism of disease

- Erythropoietic suppression by toxins and cytokines
- Increased RBC destruction owing to parasitization
- RBC alterations
- Complement and immune complex or antigen deposition
- Erythrophagocytosis and splenic hyperphagism
- CD4+T cells
- TH1/TH2 cytokine balance (TNF and IFN-g versus IL-10)



Severe and fatal disease syndromes in malaria

Metabolic acidosis: Possible sequence or mechanism of disease

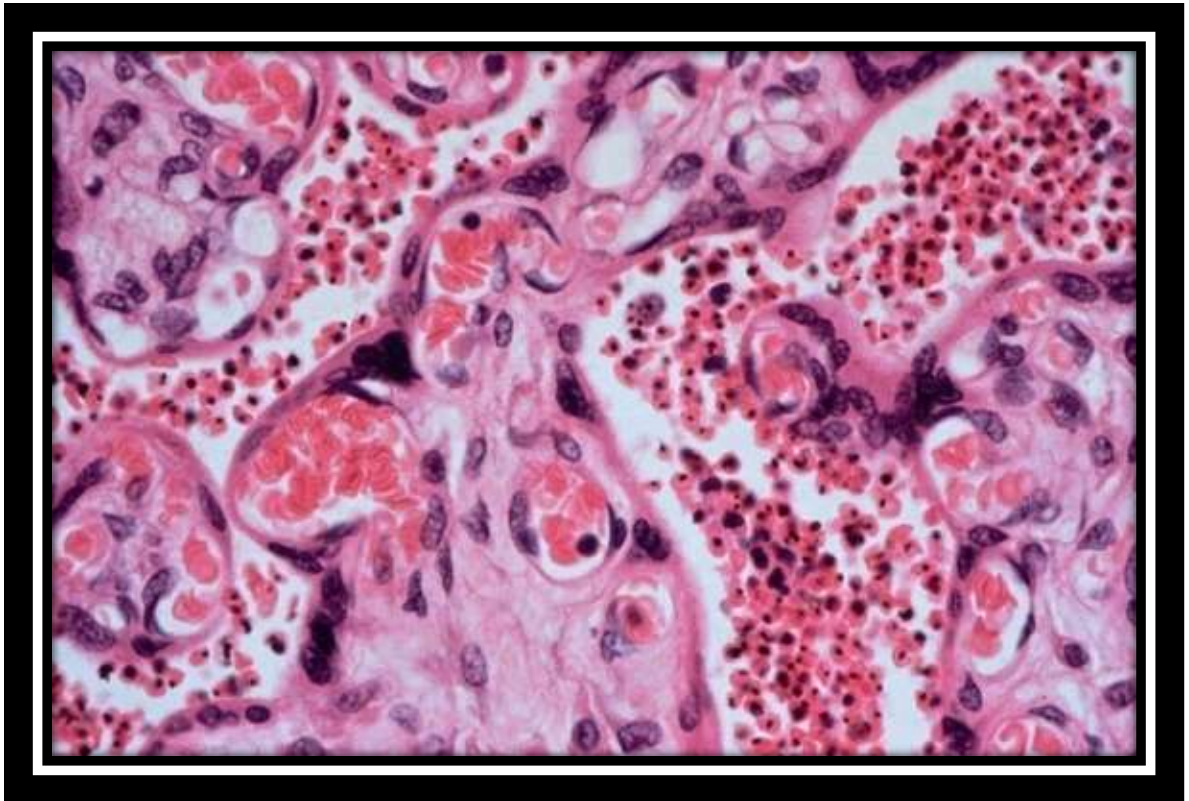
Molecular mechanisms unknown:

- Possibly widespread parasite sequestration
- bioactive toxins
- increased vascular permeability
- reduced tissue perfusion
- anaemia
- pulmonary airway obstruction
- hypoxia
- increased host glycolysis
- repressed gluconeogenesis

Severe and fatal disease syndromes in malaria

Placental malaria: Possible sequence or mechanism of disease

- PfEMP1-mediated binding to placental syncytiotrophoblast through CSA and HA
- Cytokine production
- Chemokine mediated recruitment and infiltration of monocytes
- Intravascular macrophage differentiation



Mother's Malaria Infection
(often asymptomatic)

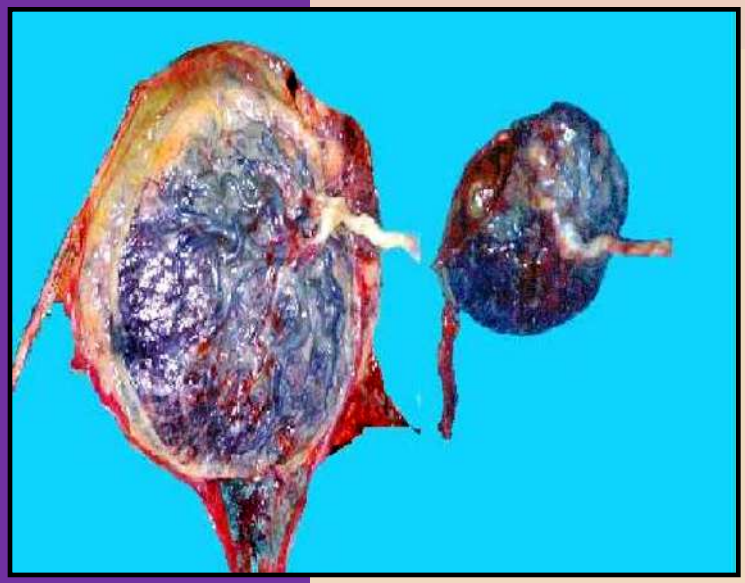
Anemia

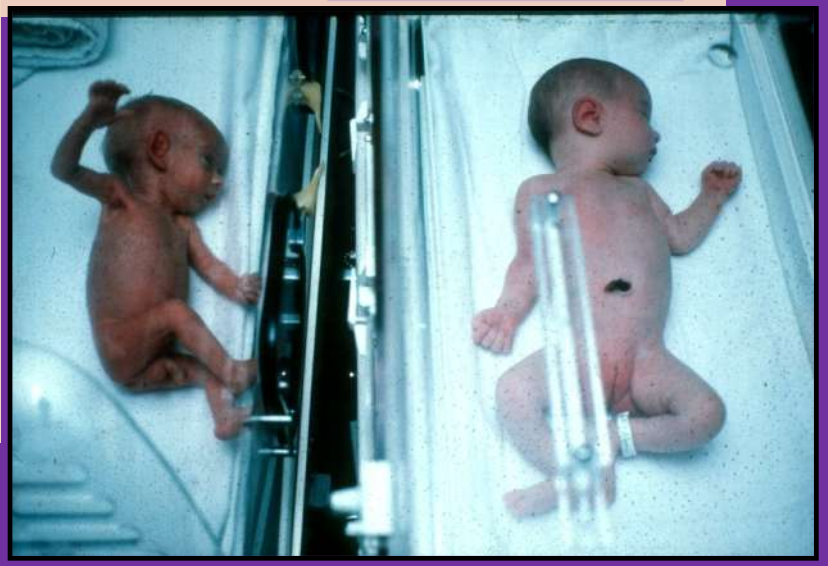
Placental Infection

Maternal Risk

Low Birthweight

Infant Risk





WHO, 2003

Fim