SOCIÉTÉ DE PATHOLOGIE EXOTIQUE

Avec le parainnage de :

PARASITES ET IMMUNITE

Journée Scientifique de la SPE

dédiée à Pierre Ambroise-Thomas

Amphithéâtre Jacob, Institut Pasteur
25 - 28 rue du Dr Roux, 75015 Paris

Mardi 9 mai 2017

Le parasite face à l'immunité : P. Buffet (Paris)

Retour vers le début

Le parasite : Comment échapper à l'immunité ?
L'orateur : comment échapper à l'immunologie ?

Malaria Immunity in Man and Mosquito: Insights into Unsolved Mysteries of a Deadly Infectious Disease*

Annu. Rev. Immunol. 2014. 32:157–87

Peter D. Crompton,[1] Jacqueline Moebius,[1]
Silvia Portugal,[1] Michael Waisberg,[1] Geoffrey Hart,[1]
Lindsey S. Garver,[2] Louis H. Miller,[2]
Carolina Barillas-Mury,[2] and Susan K. Pierce[1]

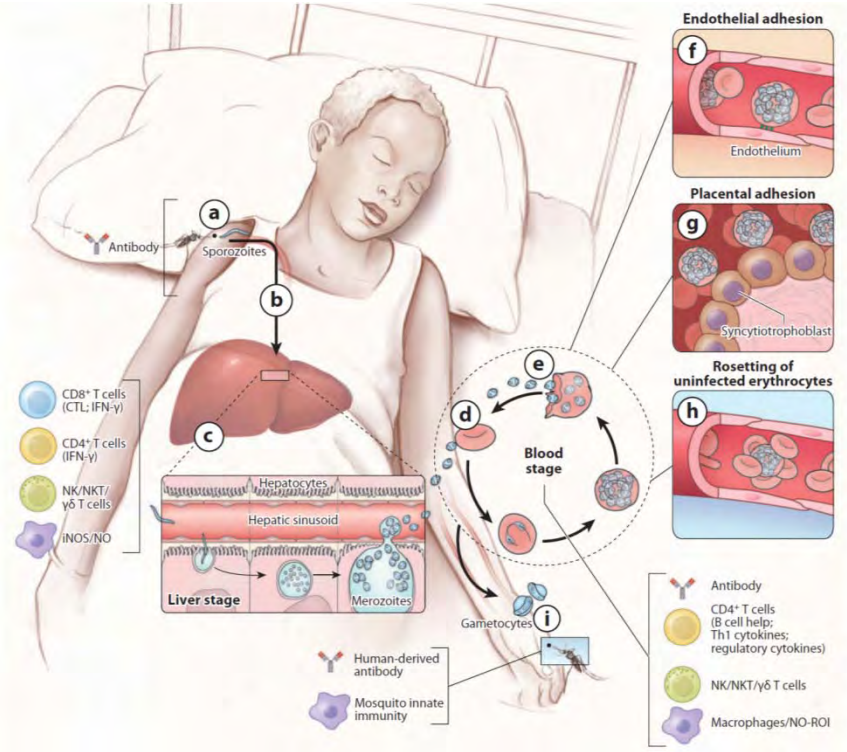
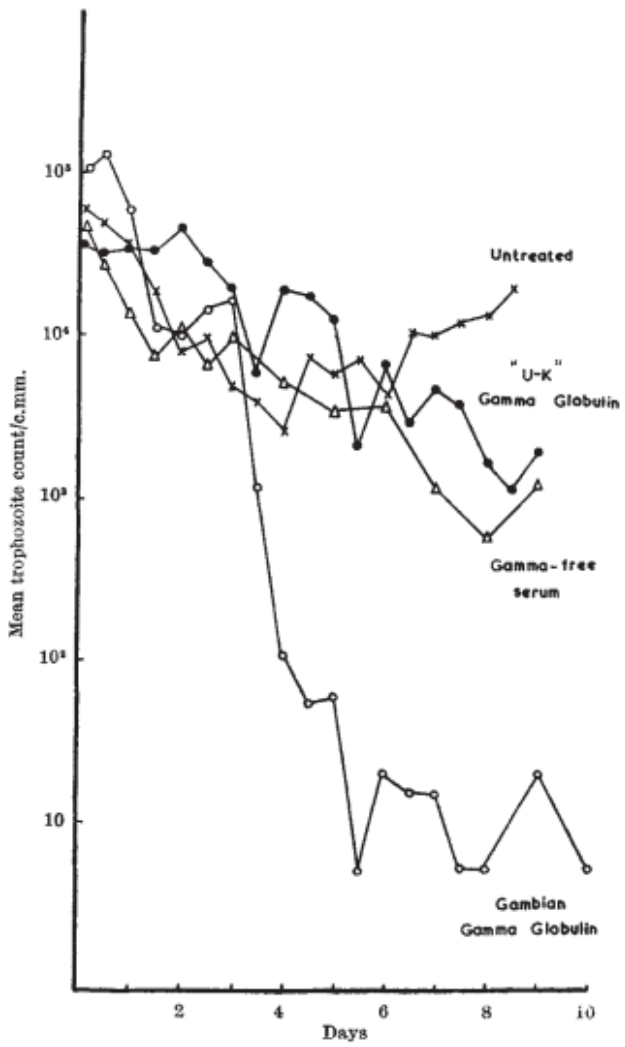


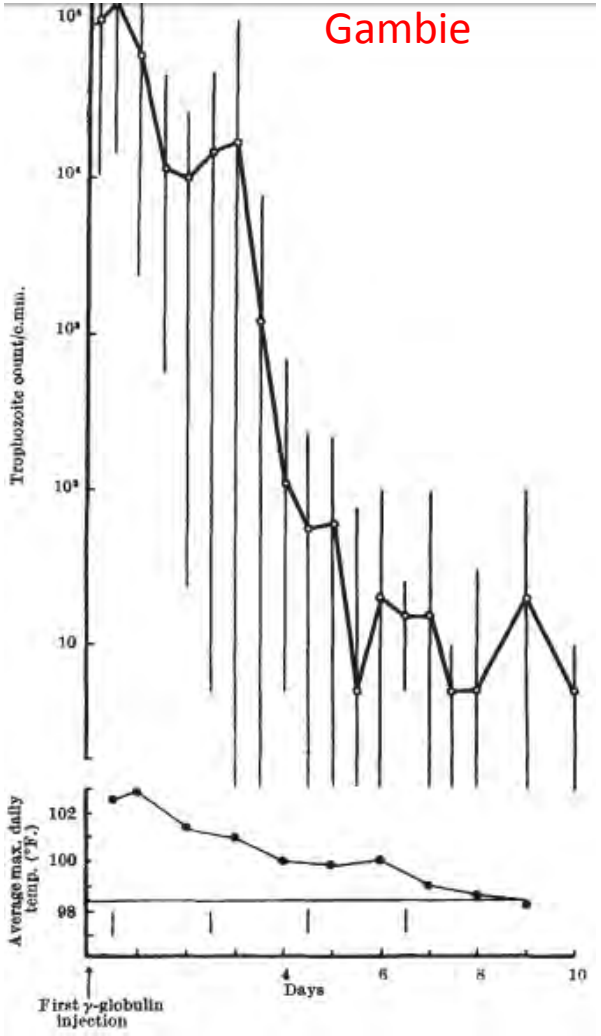
Figure 1

The *Plasmodium* life cycle in humans includes the asymptomatic liver stage; the blood stage, which causes disease; and the sexual gametocyte blood stage, which infects mosquitoes that transmit the parasite. Infection begins when a female *Anopheles* mosquito injects saliva that contains sporozoites into the skin and blood as it takes a blood meal (*a*). At this point, the infection is clinically silent, and there is no evidence for naturally acquired immunity. However, experimental infection with attenuated sporozoites has been shown to induce sterilizing immunity, and in this case the only known immune effectors that can reduce or block sporozoites in the skin are antibodies. In mouse models, sporozoites enter draining lymph nodes from the skin, where they are presented by dendritic cells and prime $CD8^+$ T cells. The highly motile sporozoites migrate to the liver, transverse Kupffer cells, and invade a small number of hepatocytes (*b*). In humans, the infection continues to be clinically silent at the liver stage, and sterilizing immunity is not naturally acquired. However, in humans and in mice, immunization with attenuated sporozoites induces sterilizing immunity that appears to rely on adaptive $CD8^+$ and $CD4^+$ T cells; on the innate production of inducible nitric oxide synthase (iNOS) and nitric oxide (NO); and on natural killer (NK) cells, NKT cells, and γδ T cells. Each sporozoite-infected hepatocyte gives rise to tens of thousands of asexual parasites called merozoites (*c*). Approximately one week after hepatocyte invasion, merozoites exit the liver into the bloodstream and begin a 48-h cycle (*d*) of red blood cell (RBC) invasion, replication, RBC rupture, and merozoite release (*e*). Clinical symptoms of malaria occur only during the blood stage and can begin as early as three days after the release of merozoites from the liver. Inside RBCs, the parasite dramatically remodels the RBC, a process that involves exporting variant surface antigens (VSAs) such as *P. falciparum* membrane protein 1s (PfEMP1s) to the RBC surface. VSAs act as receptors for a variety of endothelial cell ligands and mediate binding of infected RBCs (iRBCs) to the microvascular endothelium of various organs (*f*), allowing parasites to avoid splenic clearance. However, the sequestration of iRBCs in the microvasculature promotes the inflammation and circulatory obstruction associated with clinical syndromes of severe malaria, including cerebral malaria with iRBC sequestration in the brain and pregnancy-associated malaria with iRBCs in the placenta (*g*). VSA-mediated rosetting of iRBCs to uninfected RBCs may also contribute to disease (*h*). Coincident with the rupture of iRBCs and the release of merozoites and various parasite products are inflammation and the clinical symptoms of malaria. Both adaptive and innate immune responses are readily detected. The key immune effector at this stage is antibody. $CD4^+$ cytokine-producing T cells also play a role as do NK, NKT, and γδ T cells and macrophages through the production of NO and iNOS. A small number of blood-stage parasites differentiate into sexual gametocytes, which are taken up by mosquitos in blood meals (*i*). In the mosquito, the gametes fuse, ultimately forming sporozoites that enter the mosquito salivary gland to complete the life cycle (see **Figure 2**). In the mosquito, innate immune mechanisms serve to control parasite development. Immunization of the vertebrate host with proteins expressed by the parasite in the mosquito host results in the production of antibodies that are taken up by the mosquito with the blood meal, block parasite development, and consequently block transmission.

Importance de l'immunité humorale dans le paludisme

Efficacité du transfert passif chez l'être humain N°1





Cohen S et al. Nature 1961

Antibodies that Protect Humans against *Plasmodium falciparum* Blood Stages Do Not on their Own Inhibit Parasite Growth and Invasion In Vitro, but Act in Cooperation with Monocytes

By Hasnaa Bouharoun-Tayoun,* Phanorsi Attanath,‡
Arunee Sabchareon,‡ Tan Chongsuphajaisiddhi,‡ and Pierre Druilhe*

*From the *Laboratoire de Parasitologie Bio-Médicale, Institut Pasteur, 75015 Paris, France; and the ‡Faculty of Tropical Medicine, Mahidol University, 10400 Bangkok, Thailand*

J. Exp. Med. © The Rockefeller University Press • 0022-1007/90/12/1633/09 $2.00
Volume 172 December 1990 1633–1641

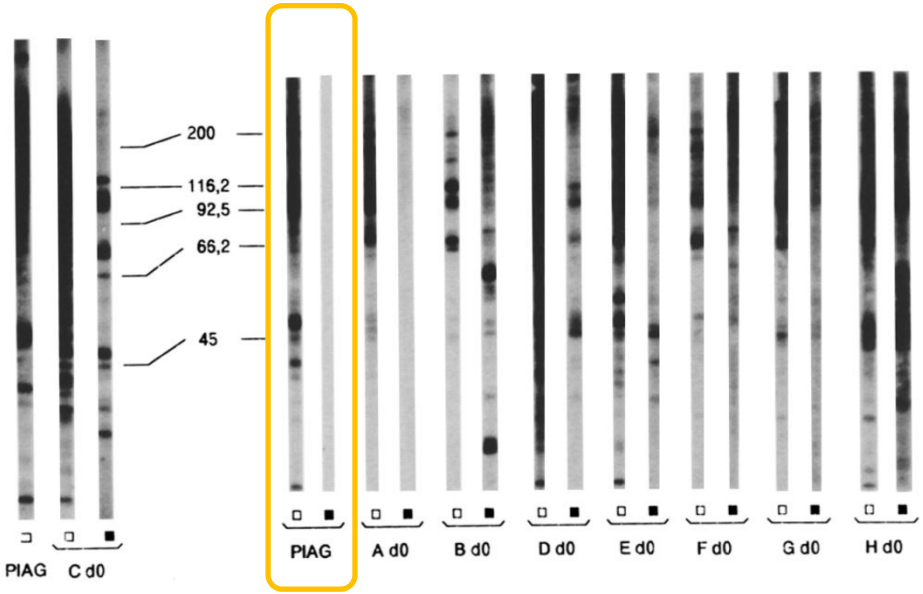
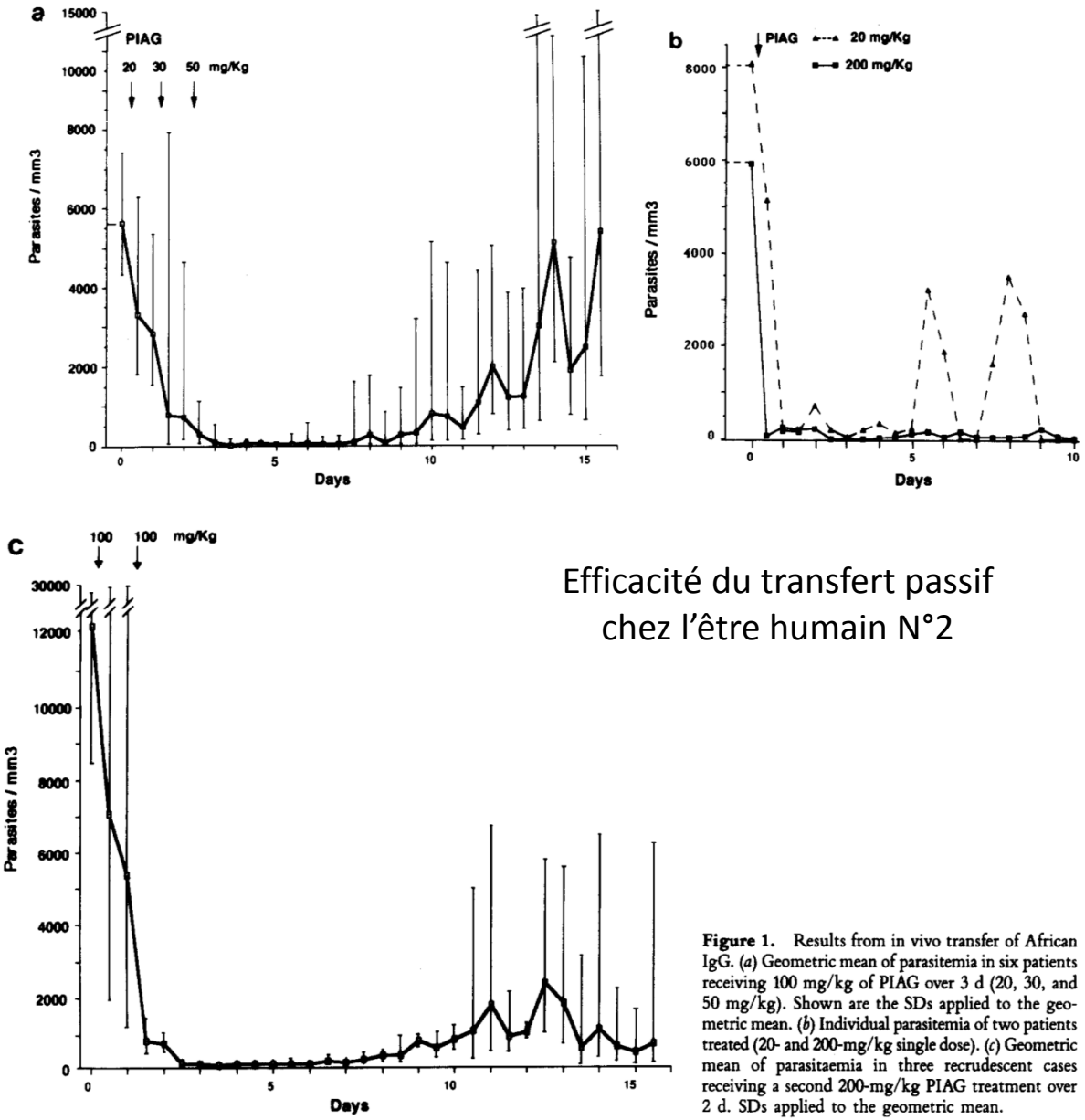


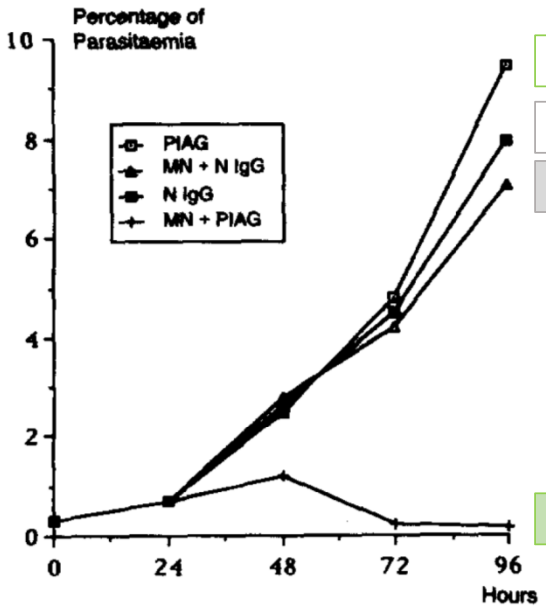
Figure 2. Comparative analysis of the antibody content in the African pool (PIAG) and in each of the receivers before Ig transfer (sera A–H on day 0) by Western blots performed on extract of parasites from patient C, revealed with ^{125}I-labeled anti-human IgG (□) or IgM (■).



Efficacité du transfert passif chez l'être humain N°2

Figure 1. Results from in vivo transfer of African IgG. (*a*) Geometric mean of parasitemia in six patients receiving 100 mg/kg of PIAG over 3 d (20, 30, and 50 mg/kg). Shown are the SDs applied to the geometric mean. (*b*) Individual parasitemia of two patients treated (20- and 200-mg/kg single dose). (*c*) Geometric mean of parasitaemia in three recrudescent cases receiving a second 200-mg/kg PIAG treatment over 2 d. SDs applied to the geometric mean.

Croissance parasitaire *in vitro* en présence d'anticorps seuls ou en présence d'anticorps et de monocytes



IgG hyperimmunes seules

IgG contrôles seules

IgG contrôles + monocytes

IgG hyperimmunes + monocytes

Figure 3. Antibody-dependent cellular inhibition assay (ADCI). 96-h cultures were performed using *P. falciparum* isolate from patient C with daily medium change, either in the presence (△, +) or absence (⊡, ■) of monocytes from healthy donors, using control IgG (△, ■) or the African IgG used in the in vivo transfer experiment (⊡, +) at a 2.5-mg/ml concentration in RPMI + 10% human serum medium.

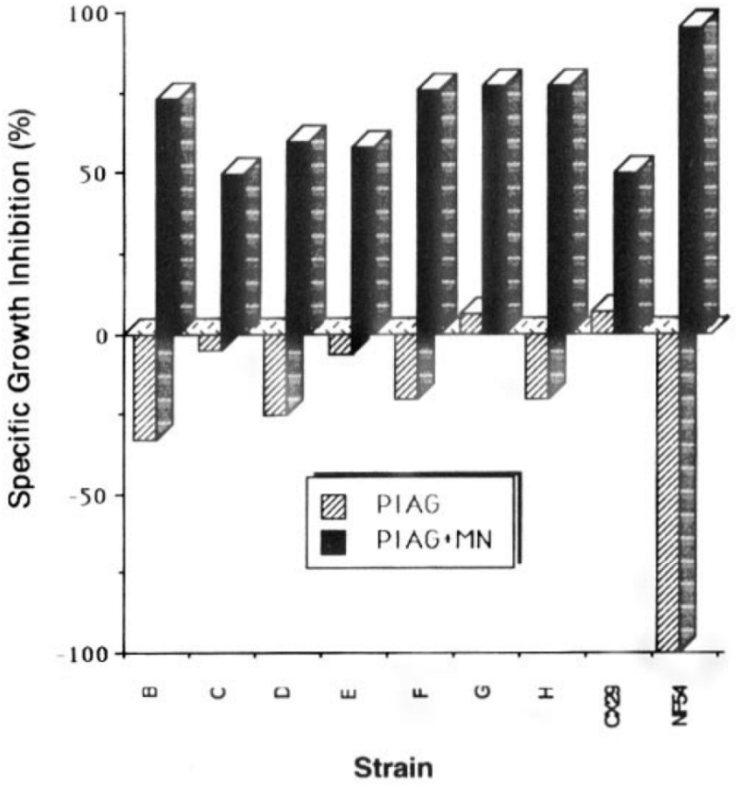


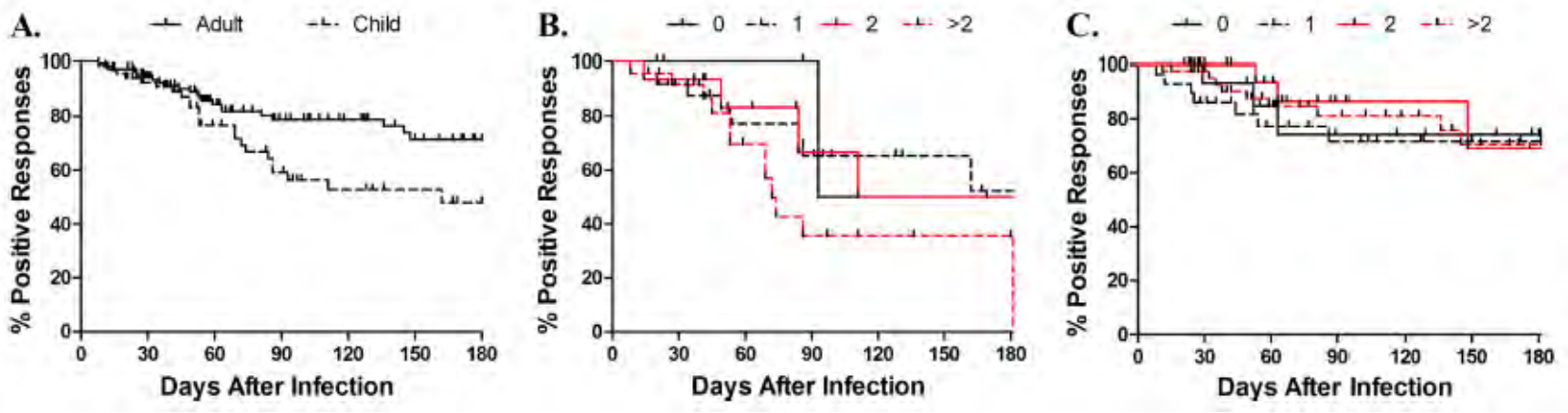
Figure 4. Summary of the 48-h ADCI assays performed with nine *P. falciparum* isolates and the African IgG (PIAG). Results are expressed as the specific growth inhibition in percent, which takes into account the possible inhibitory or facilitation effect of either cells or antibodies alone. Results obtained by ADCI appear as black bars (monocytes + antibodies). Controls in the presence of antibodies alone appear as hatched bars (the negative SGI represents an increase of *P. falciparum* growth as compared with control IgG). (*B–H*) Thai strains isolated from patients B to H before PIAG transfer. (*CX29*) The strain isolated from the Thai patient C on day 29 after transfer upon recrudescence of parasitemia. (*NF54*) An African strain used as control in each experiment.

Les grands type d'antigènes dans l'optique vaccinale

Antigène non variant non immunogène	Antigène non variant et immunogène	Antigène immunogène mais divers ou variant
Mauvaise cible vaccinale	Cible vaccinale parfaite	Mauvaise cible vaccinale
Sauf si on peut le rendre immunogène par altération et/ou adjuvantation	Si elle existait *Plasmodium falciparum* ne donnerait pas d'infections répétées ou chroniques	Sauf si on identifie les variants majeurs en fréquence ou en impact
Modèle « anatoxine	Et cela fait longtemps que nous aurions un vaccin	Modèle « Prévenar »
Approche PfRH5		Approche VarCSA2

Cinétique de la réponse Ac anti $MSP1_{19}$

Pérou



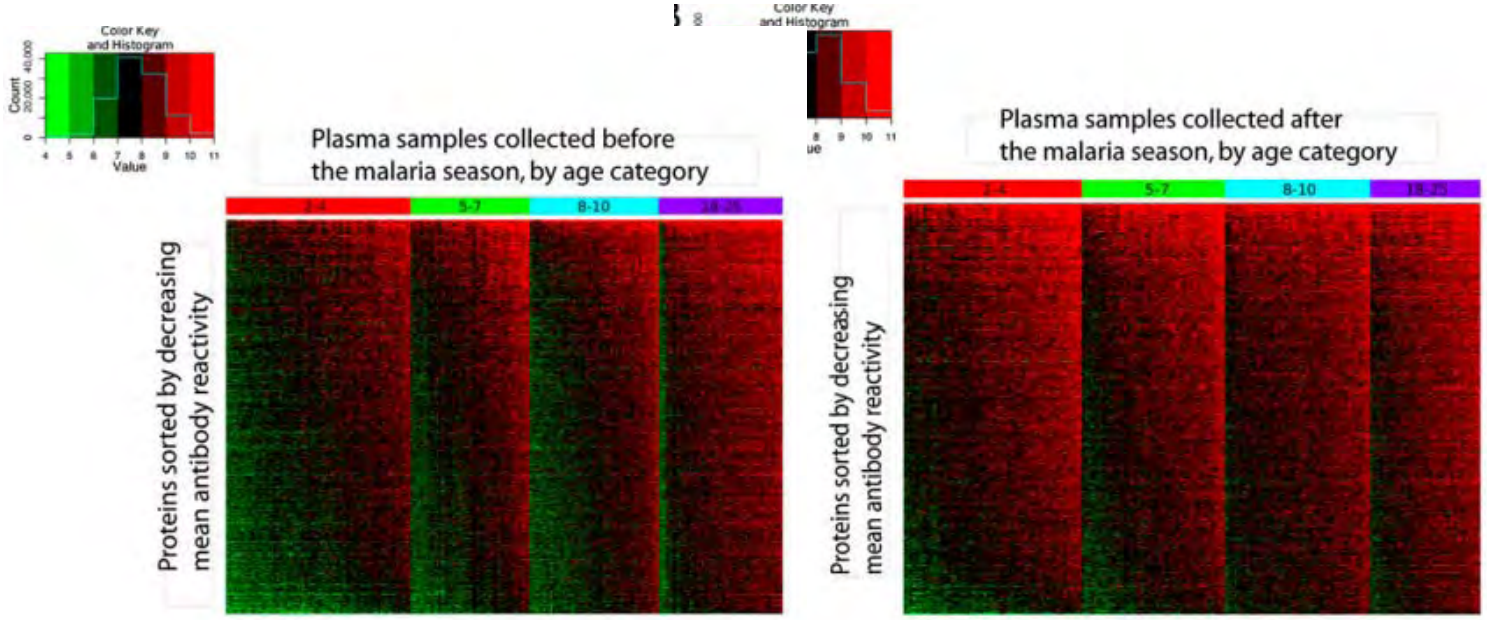
Enfants

Adultes

Clark et al. IAI 2012

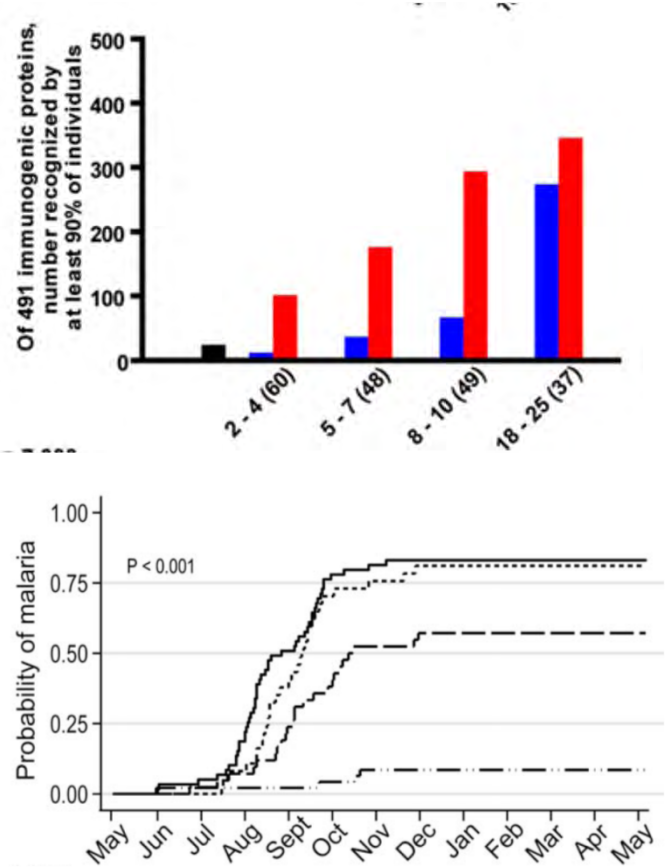
Ac anti *Pf:* acquisition graduelle & diversification

Mali



Pré saison de transmission

Post saison de transmission



Crompton P *et al.* PNAS 2010 Portugal Malaria J 2012

En résumé

L’immunité protectrice anti *P. falciparum* est essentiellement humorale

Tous les Ac anti P. falciparum ne sont pas protecteurs

La demi vie des IgG anti P. falciparum est variable selon Ag mais globalement brève

L’abondance et la diversité des réponses Ac anti P. falciparum s’acquièrent graduellement

ARTICLES

nature genetics

Genome-wide and fine-resolution association analysis of malaria in West Africa

Muminatou Jallow[1,34], Yik Ying Teo[2,3,34], Kerrin S Small[2,3,34], Kirk A Rockett[2,3], Panos Deloukas[3], Taane G Clark[2,3], Katja Kivinen[3], Kalifa A Bojang[1], David J Conway[1], Margaret Pinder[1], Giorgio Sirugo[1], Fatou Sisay-Joof[1], Stanley Usen[1], Sarah Auburn[2,3], Suzannah J Bumpstead[3], Susana Campino[2,3], Alison Coffey[3], Andrew Dunham[3], Andrew E Fry[2], Angela Green[2], Rhian Gwilliam[3], Sarah E Hunt[3], Michael Inouye[3], Anna E Jeffreys[2], Alieu Mendy[2], Aarno Palotie[3], Simon Potter[3], Jiannis Ragoussis[2], Jane Rogers[3], Kate Rowlands[2], Elilan Somaskantharajah[3], Pamela Whittaker[3], Claire Widden[3], Peter Donnelly[2,4], Bryan Howie[4], Jonathan Marchini[2,4], Andrew Morris[2], Miguel SanJoaquin[2,5], Eric Akum Achidi[6], Tsiri Agbenyega[7], Angela Allen[8,9], Olukemi Amodu[10], Patrick Corran[11], Abdoulaye Djimde[12], Amagana Dolo[12], Ogobara K Doumbo[12], Chris Drakeley[13,14], Sarah Dunstan[15], Jennifer Evans[7,16], Jeremy Farrar[15], Deepika Fernando[17], Tran Tinh Hien[15], Rolf D Horstmann[16], Muntaser Ibrahim[18], Nadira Karunaweera[17], Gilbert Kokwaro[19], Kwadwo A Koram[20], Martha Lemnge[21], Julie Makani[22], Kevin Marsh[19], Pascal Michon[8], David Modiano[23], Malcolm E Molyneux[5], Ivo Mueller[8], Michael Parker[24], Norbert Peshu[19], Christopher V Plowe[25,26], Odile Puijalon[27], John Reeder[8], Hugh Reyburn[13,14], Eleanor M Riley[13,14], Anavaj Sakuntabhai[27], Pratap Singhasivanon[28], Sodiomon Sirima[29], Adama Tall[30], Terrie E Taylor[25,31], Mahamadou Thera[12], Marita Troye-Blomberg[32], Thomas N Williams[19], Michael Wilson[20] & Dominic P Kwiatkowski[2,3], Wellcome Trust Case Control Consortium[33] &

Mutation
HbS

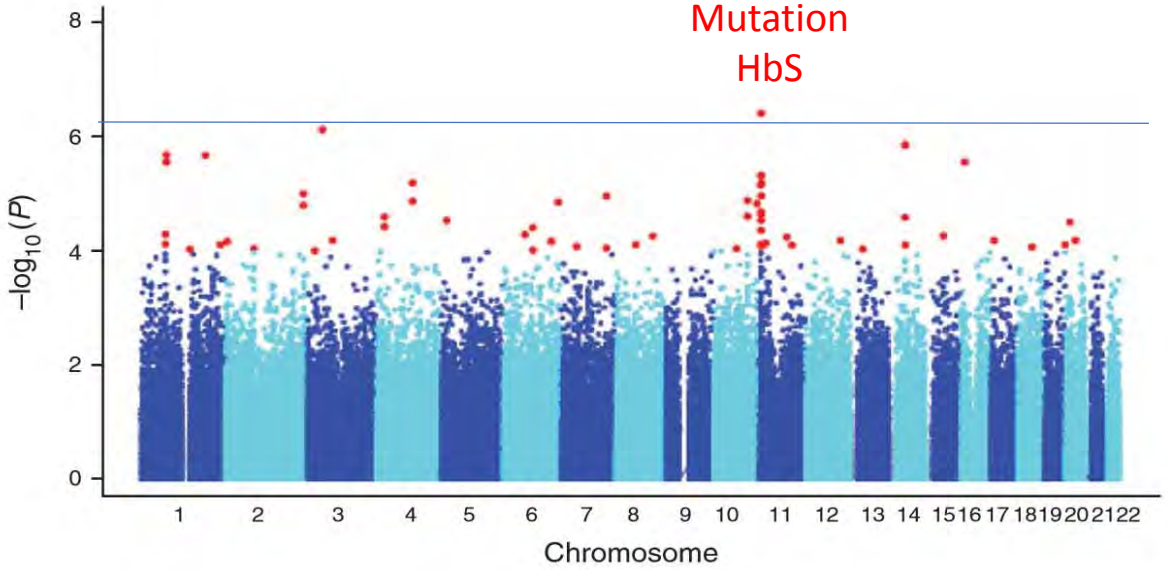


Figure 4 Genome-wide signals of association with severe malaria. Plot of the $-\log_{10} P$ values for the trend test correcting for the first three principal components from EIGENSTRAT. Each point represents a SNP from the 402,814 remaining after quality control filters were applied. Different bands of blue are used to differentiate SNPs on consecutive autosomal chromosomes. SNPs with P values less than 10^{-4} are represented by red points.

Dans l'état actuel des études « génôme entier »
Les associations les plus fortes concernent

1. les mutations de l'hémoglobine (HbS)
2. Le groupe sanguin O
3. Un transporteur de la membrane érythrocytaire
4. Une molécule impliquée dans la perméabilité endothéliale

In addition, numerous immune system genes show association with resistance or susceptibility to malaria, including those encoding MHC molecules, FcγRIIB, components of both the type I and type II IFN responses, IL-12, and NO synthase (NOS) (15). However, few of these associations have been tested in more than one endemic setting, and when tested results have varied.

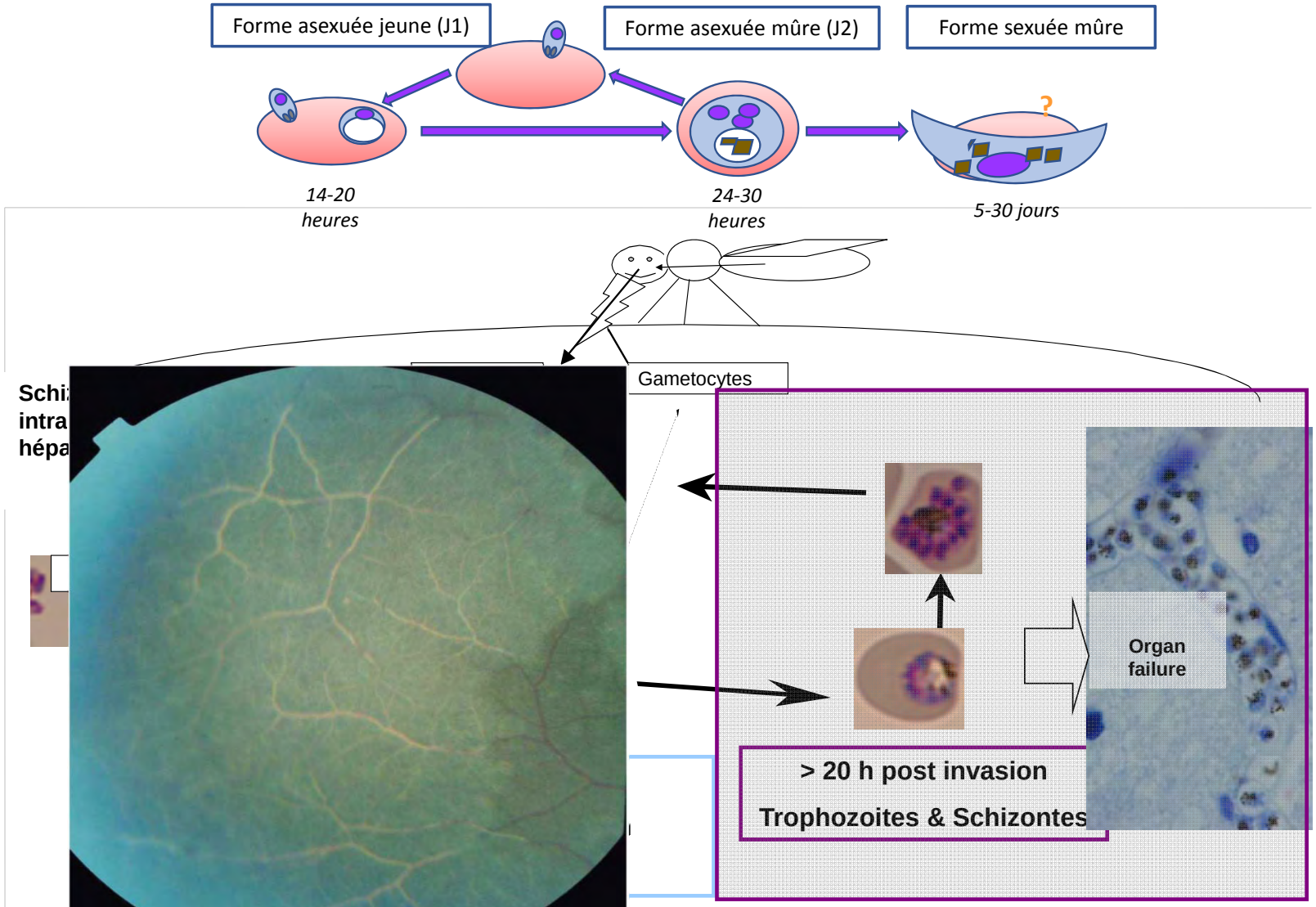
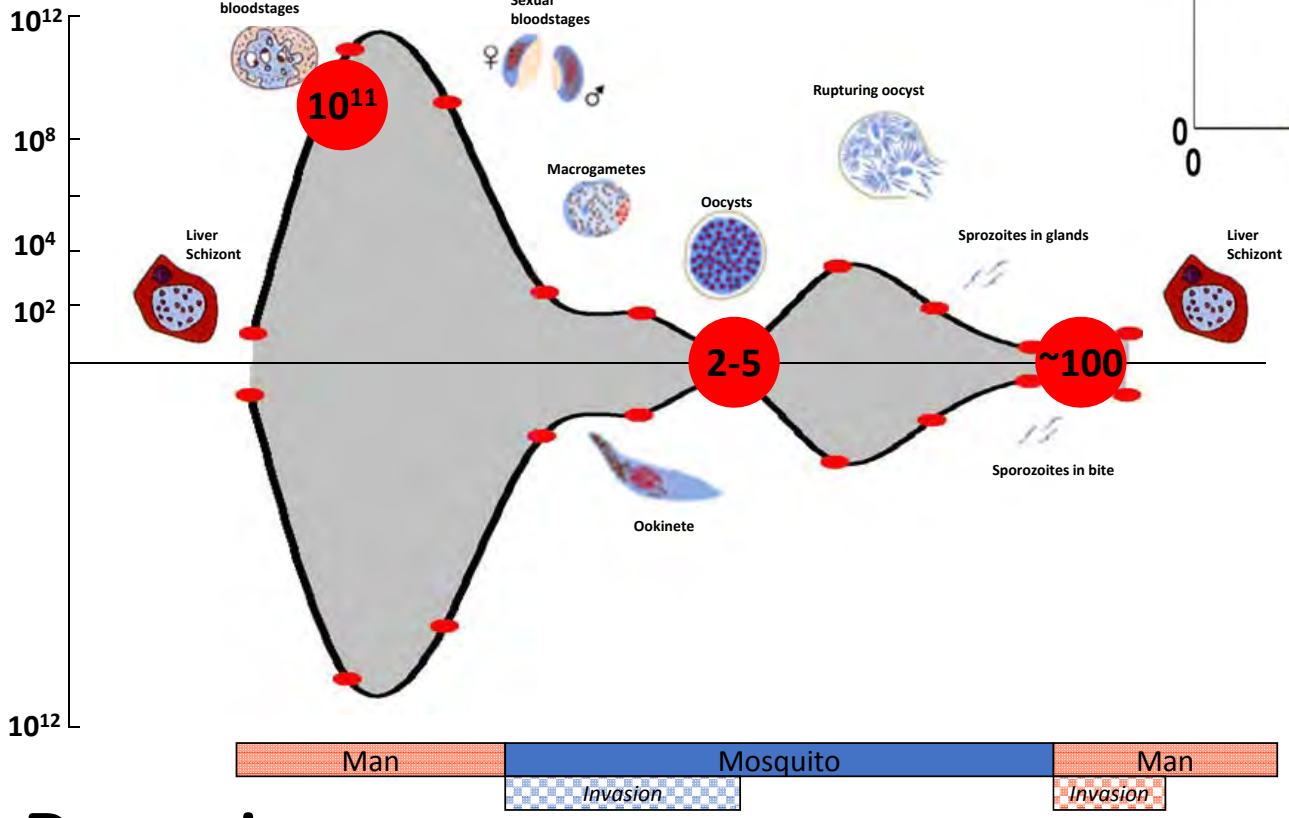
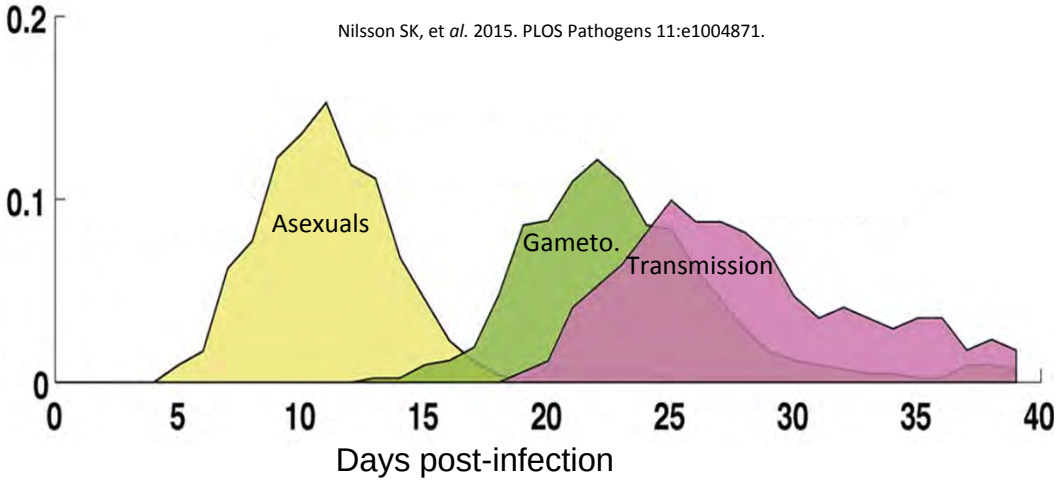


FIGURE 3. White retinal vessels in an area of confluent peripheral retinal whitening.





Nilsson SK, et *al.* 2015. PLOS Pathogens 11:e1004871.

Parasite: Developmental Bottlenecks

Alavi et al 2003

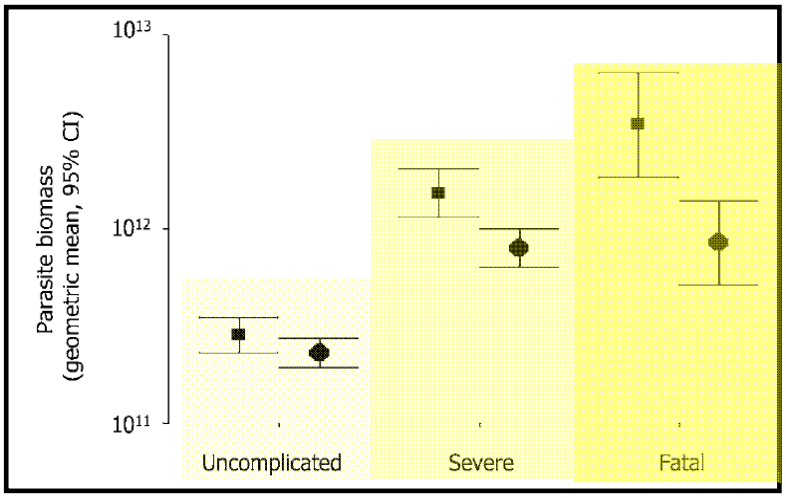
SIDELIGHTS ON MALARIA IN MAN OBTAINED BY SUBINOCULATION EXPERIMENTS.

BY

Brigadier N. HAMILTON FAIRLEY, C.B.E., F.R.S., *et al.**
(*From the Land Headquarters. Medical Research Unit (A.I.F.), Cairns, Australia*).

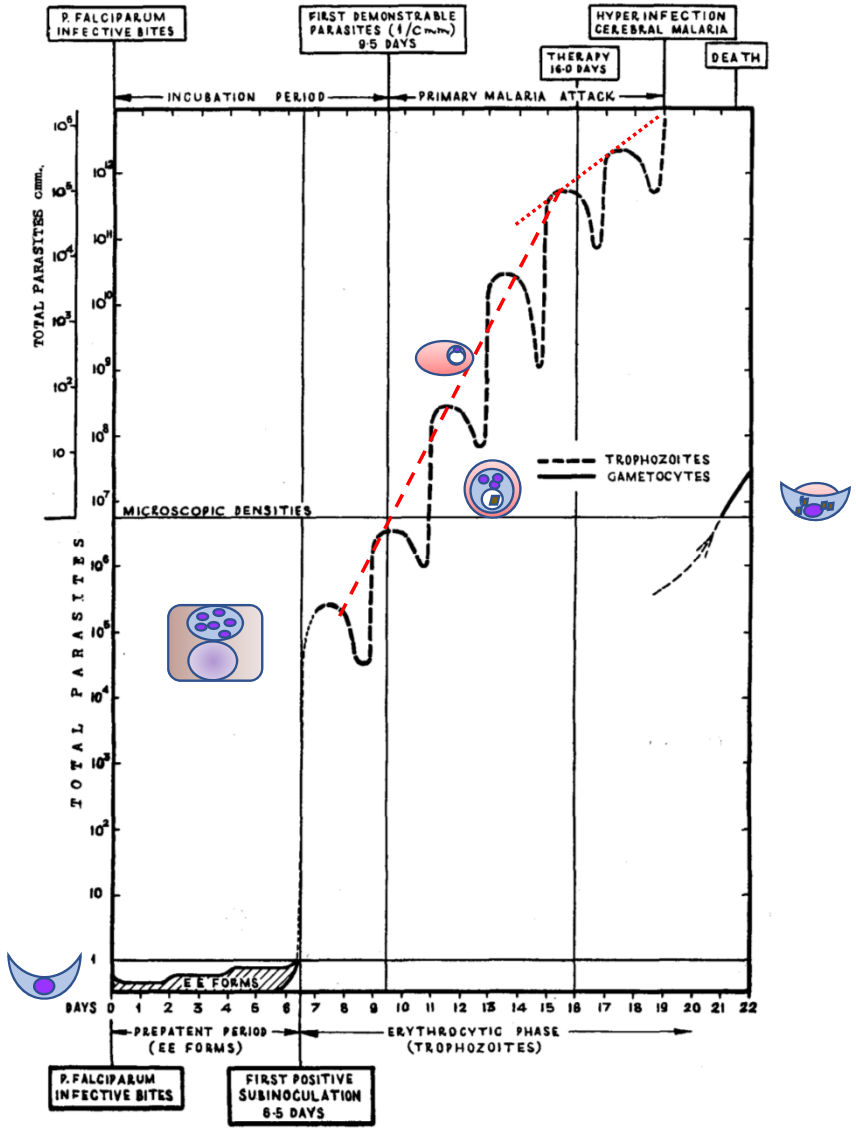
Note that pre-erythrocytic or hypothetical e.e. forms are considered not to persist after 6 days being completely transformed into erythrocytic parasites (micromerozoites). The erythrocytic parasites steadily increase from submicroscopic densities to hyper-infection; the fall in the parasitic curve during each cycle is due to the larger pigmented erythrocytic parasites being withdrawn from the circulating blood.

Fairley NH. Sidelights on malaria in man obtained by subinoculation experiments. Trans R Soc Trop Med Hyg. 1947;40:621–76.



Dondorp et al. PLoS Medicine 2005;2(8):e204

CHART 7. THE NATURAL HISTORY OF *P. falciparum* IN MAN FROM SPOROZOITE INOCULATION TO THERAPY FOR PRIMARY ATTACK.



Transmission

ELSEVIER

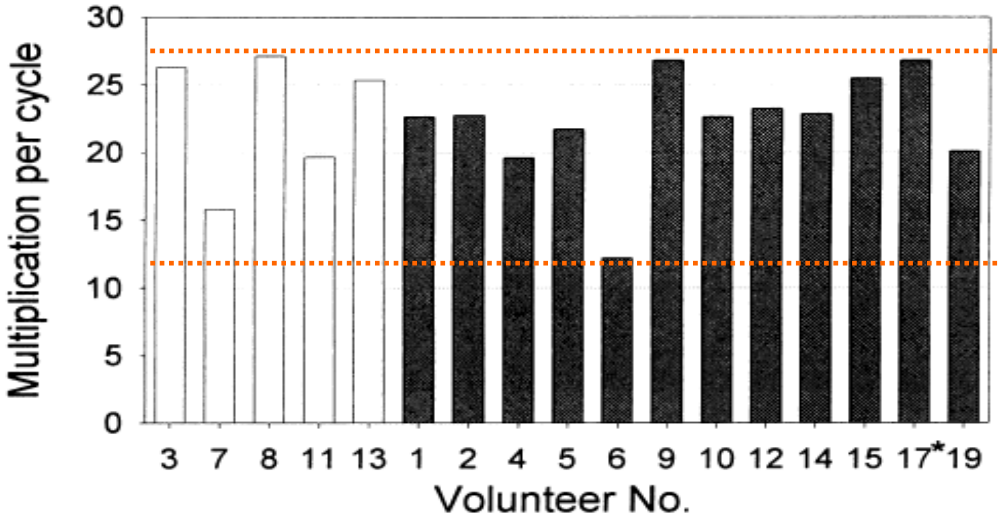
Vaccine 18 (2000) 1925–1931

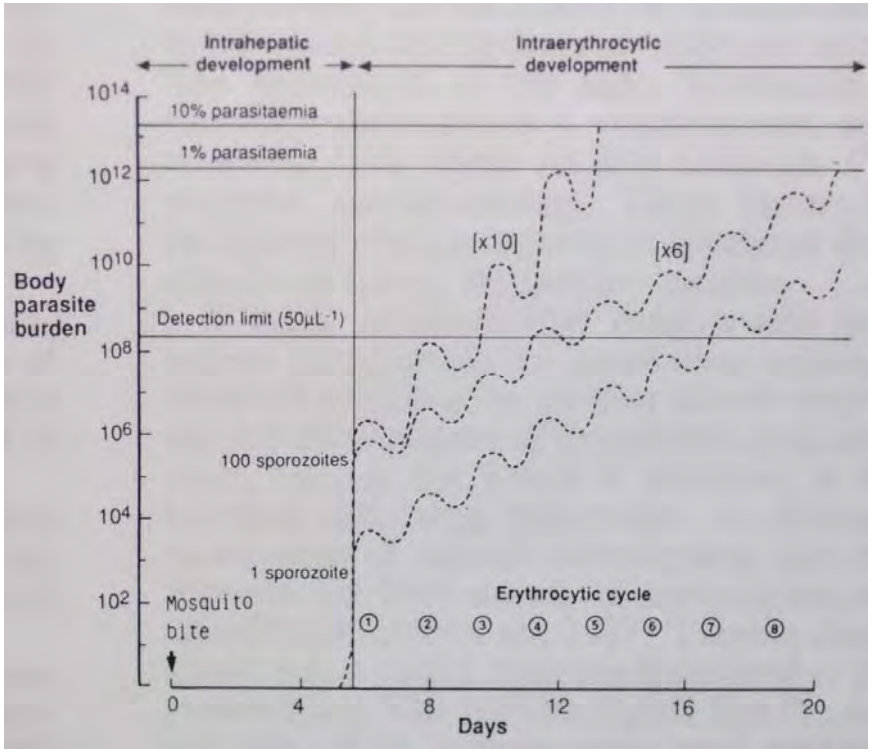
Vaccine

www.elsevier.com/locate/vaccine

Effect of vaccination with 3 recombinant asexual-stage malaria antigens on initial growth rates of *Plasmodium falciparum* in non-immune volunteers

Gregor Lawrence[a,*], Qin Cheng[a,1], Carol Reed[a,2], Darrin Taylor[a], Anthony Stowers[a,3], Nicole Cloonan[a], Christine Rzepczyk[a], Anne Smillie[a], Karen Anderson[a], David Pombo[a], Anthony Allworth[b], Damon Eisen[a,b], Robin Anders[c], Allan Saul[a]





White N, Chapman D, Watt G. The effects of mutltiplication and synchronicity on the vascular distribution of parasites in falciparum malaria. Trans R Soc Trop Med Hyg 1992;86(6):590

Infection à *Plasmodium falciparum* chez un sujet adulte naïf

Taux de croissance de plus de 10 par cycle (48h) de la charge parasitaire corporelle totale

Log-linéaire sans inflexion pendant les 8 premiers jours (interprétable comme absence d'impact de l'immunité adaptative)

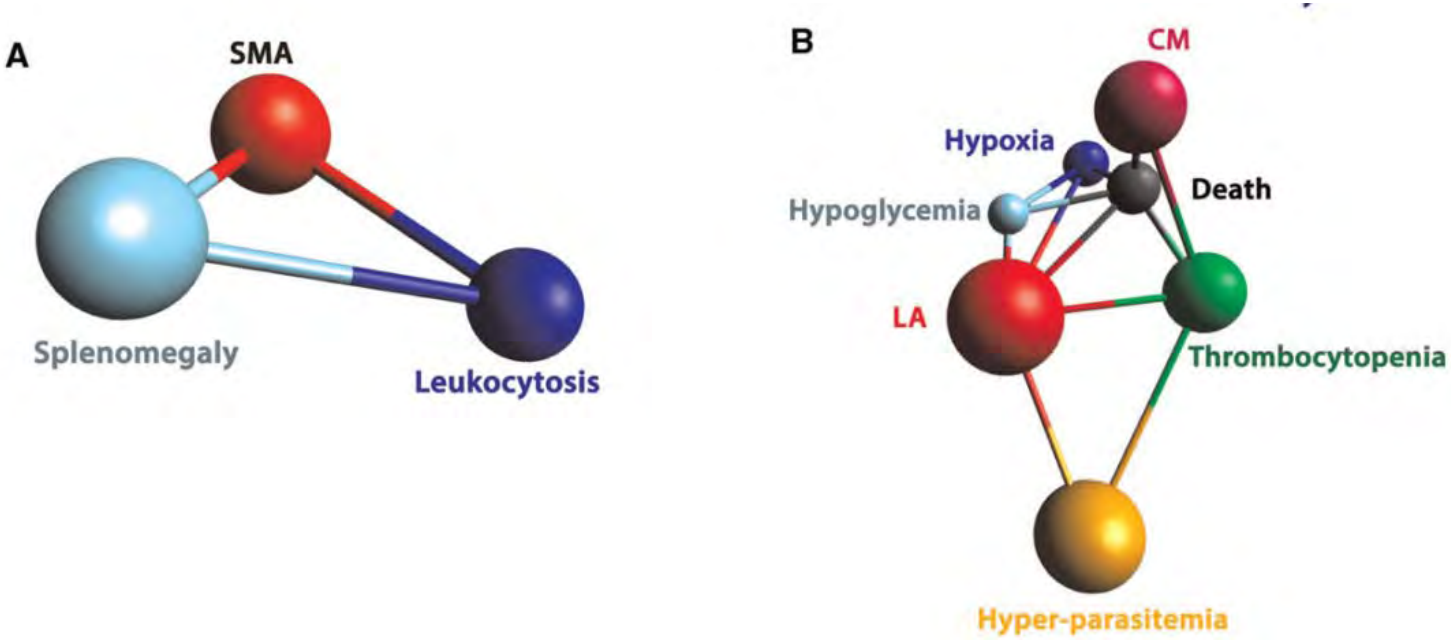
Variations interindividuelles de ce taux d'un facteur 2-3

Am. J. Trop. Med. Hyg., 88(4), 2013, pp. 747–756
doi:10.4269/ajtmh.12-0668

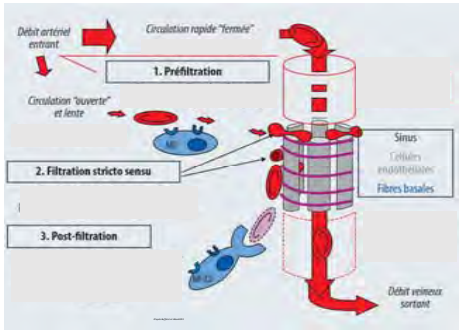
Inter-Relationships of Cardinal Features and Outcomes of Symptomatic Pediatric *Plasmodium falciparum* Malaria in 1,933 Children in Kampala, Uganda

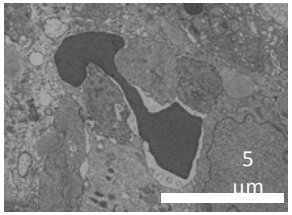
Christine M. Cserti-Gazdewich, Aggrey Dhabangi, Charles Musoke, Isaac Ssewanyana, Henry Ddungu, Deborah Nakiboneka-Ssenabulya, Nicolette Nabukeera-Barungi, Arthur Mpimbaza, and Walter H. Dzik*

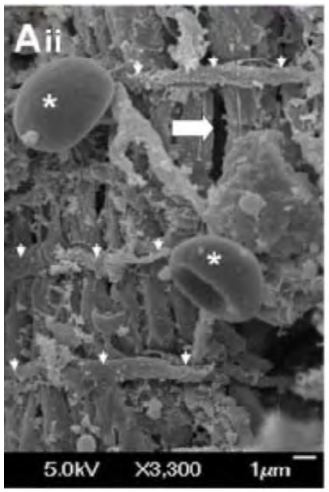
University Health Network, University of Toronto, Toronto, Canada; Mulago Hospital, Makerere University College of Health Sciences, Kampala, Uganda; Joint Clinical Research Center, Kampala, Uganda; Uganda Cancer Institute and the African Palliative Care Association, Kampala, Uganda; Massachusetts General Hospital, Harvard University, Boston, Massachusetts



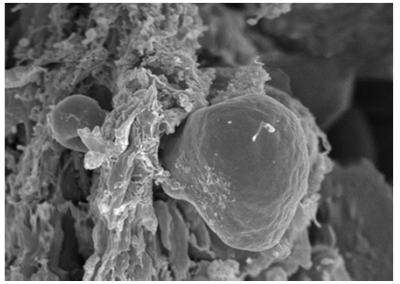
Exploration du globule rouge mobile (1)



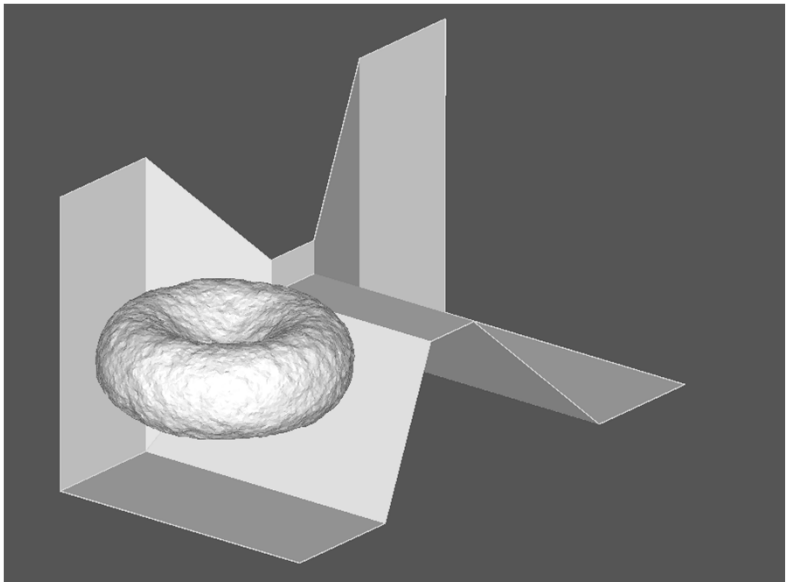




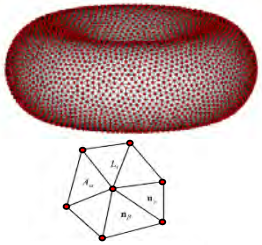
Modélisation *in silico* de la deformation d'un globule rouge franchissant une fente inter-endothéliale splénique



Dimension de la fente: Hauteur =1.2 microns, largeur = 4 microns
Durée médiane de franchissement 1/10ème de seconde (rat)



Spectrin-Level Model



The total Helmholtz free energy of the system:

The bending free energy

The in-plane energy:

Membrane viscosity:

D.E. Discher et al. **Biophys.J** (1998), J. Li et al., **Biophys.J**, 88 (2005), Pivkin et al. **PRL** (2008), Peng, Li, Pivkin et al **PNAS** (2013)

Lien avec Equipe N°2 ?

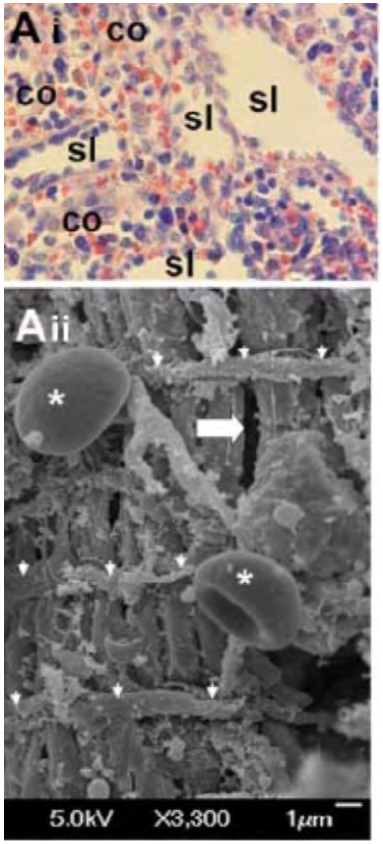
PNAS

Biomechanics of red blood cells in human spleen and consequences for physiology and disease *2016*

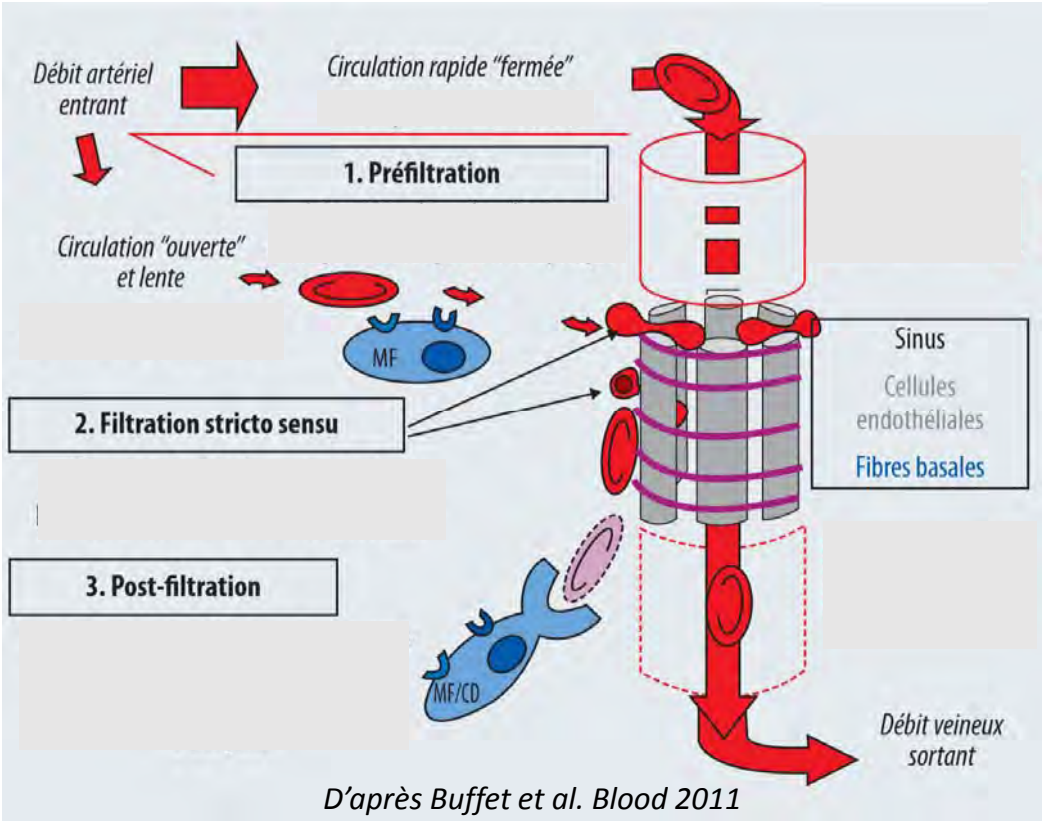
Igor V. Pivkin[a,b,1], Zhangli Peng[c,d,1], George E. Karniadakis[e], Pierre A. Buffet[f,g], Ming Dao[d,2], and Subra Suresh[h,i,j,2]

[a]Institute of Computational Science, Faculty of Informatics, University of Lugano, 6900 Lugano, Switzerland; [b]Swiss Institute of Bioinformatics, 1015 Lausanne, Switzerland; [c]Department of Aerospace and Mechanical Engineering, University of Notre Dame, Notre Dame, IN 46556; [d]Department of Materials Science and Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139; [e]Division of Applied Mathematics, Brown University, Providence, RI 02912; [f]Faculté de Médecine Université Paris Descartes, Institut National de la Transfusion Sanguine, 75015 Paris, France; [g]Laboratoire d'Excellence GR-Ex, F-75015 Paris, France; [h]Department of Biomedical Engineering, Carnegie Mellon University, Pittsburgh, PA 15213; [i]Computational Biology Department, Carnegie Mellon University, Pittsburgh, PA 15213; and [j]Department of Materials Science and Engineering, Carnegie Mellon University, Pittsburgh, PA 15213

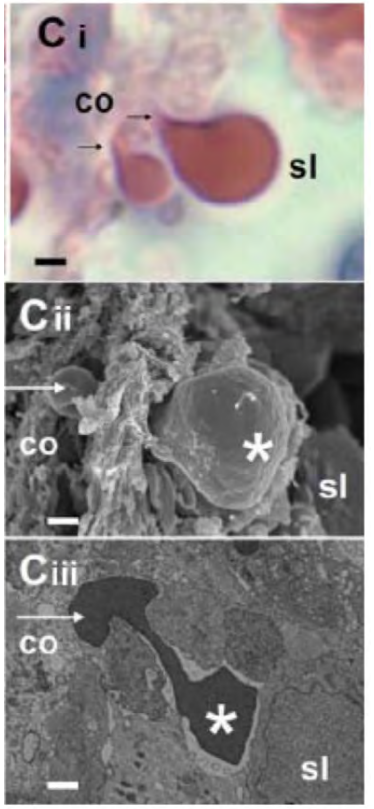
Equipe BioTIGR Concept central : Le splénon



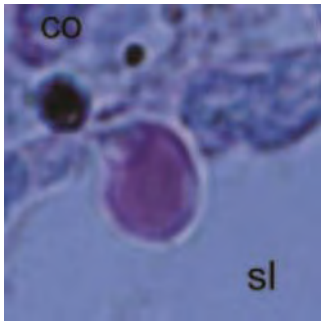
Interactions globule rouge – rate
Le splénon Unité fonctionnelle filtrante splénique



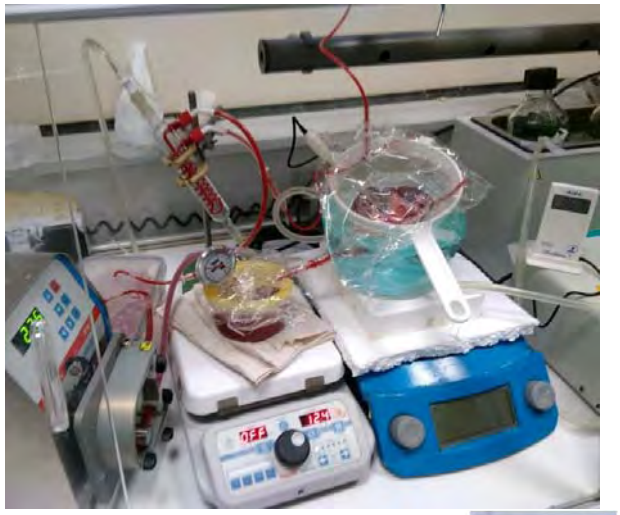
D'après Buffet et al. Blood 2011

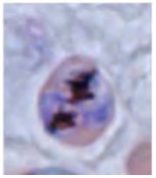


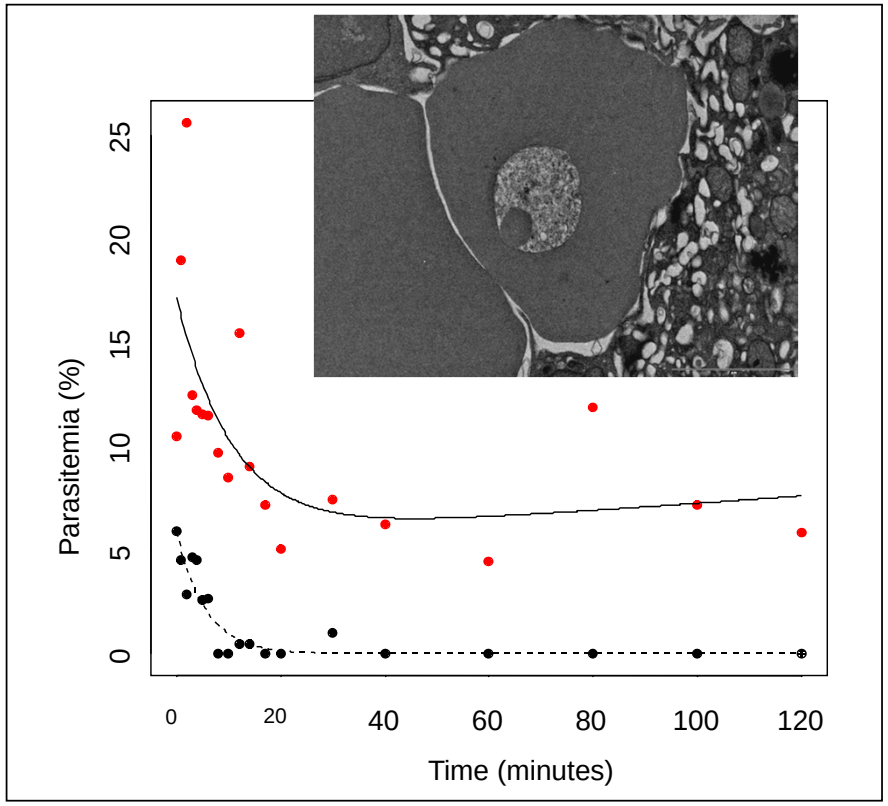
Deplaine et al. Blood 2011



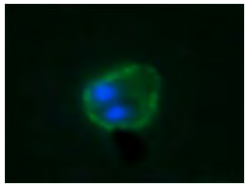
Isolated-perfused spleens retained rings
Mean clearance half-life = 7.5 min (8 experiments)



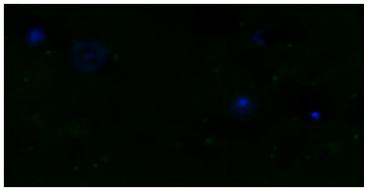




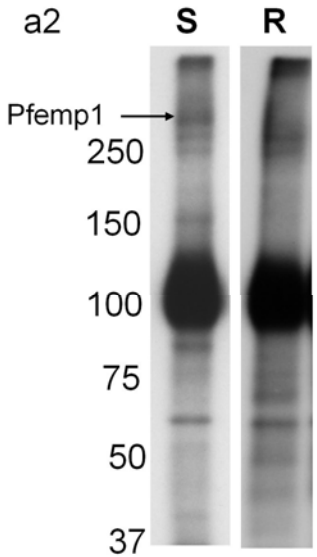
Schizonts



Rings



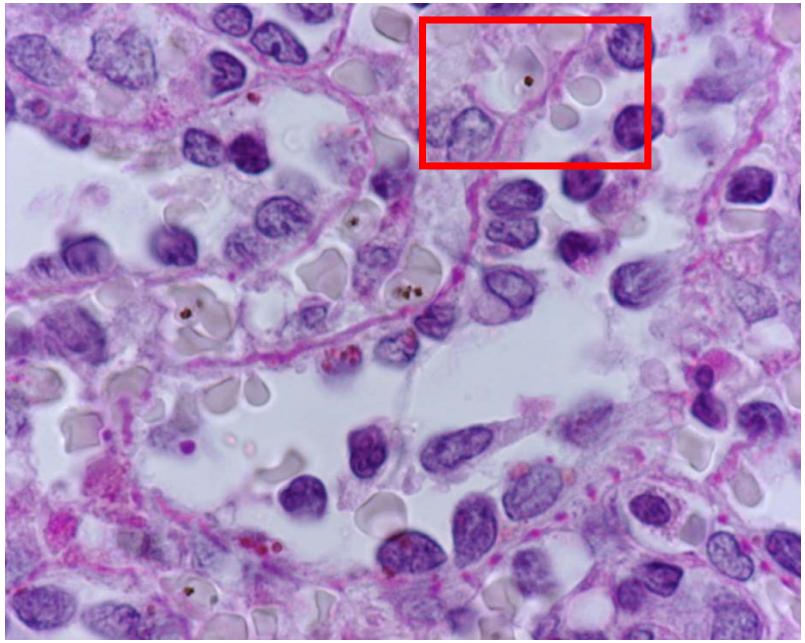
Negative surface IFA

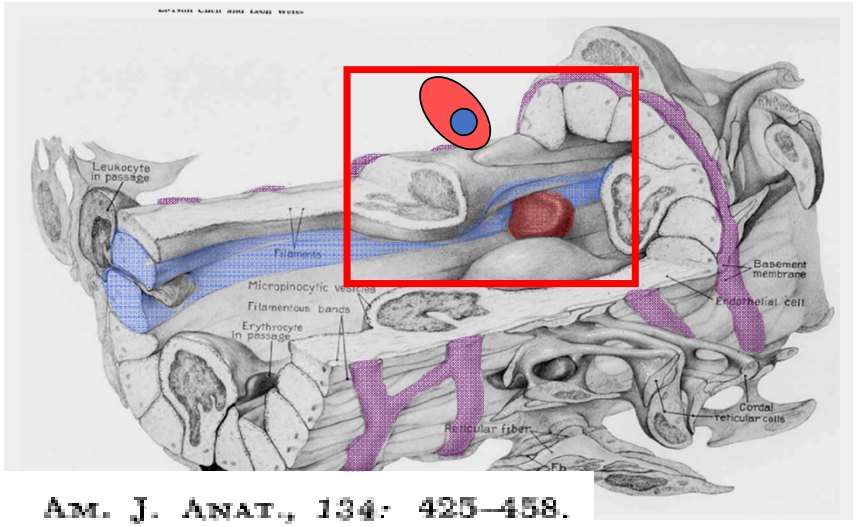


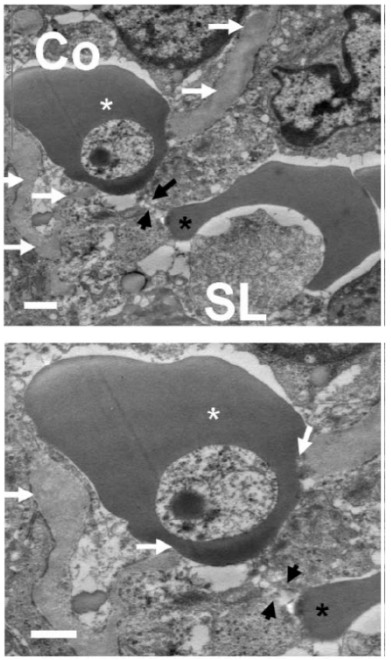
Safeukui et al. Blood 2008;112:2520-8

Topographie rétentionnelle et indice d'élongation des globules rouges parasités

Safeukui et al. Blood 2008;112:2520-8







Index d'élongation des GR parasités 0.47 at 30 Pa (ektacytometry / Lorca)

Index d'élongation d'un GR normal = 0.6

La rate humaine retient des globules rouges dont la surface paraît normale

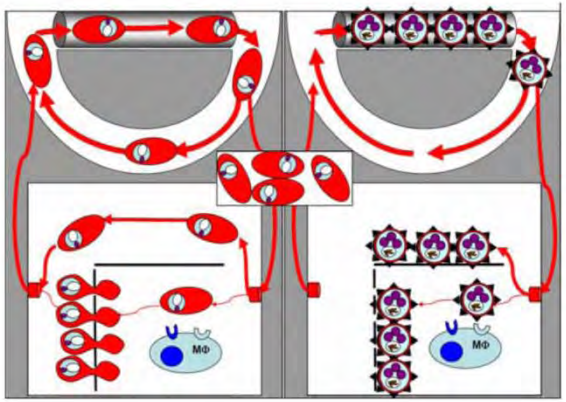
Rétention mécanique pure

Deplaine et al. Blood 2011
Safeukui et al. Blood 2012
Safeukui et al. PLoS1 2013
Roussel et al. Transfusion 2016

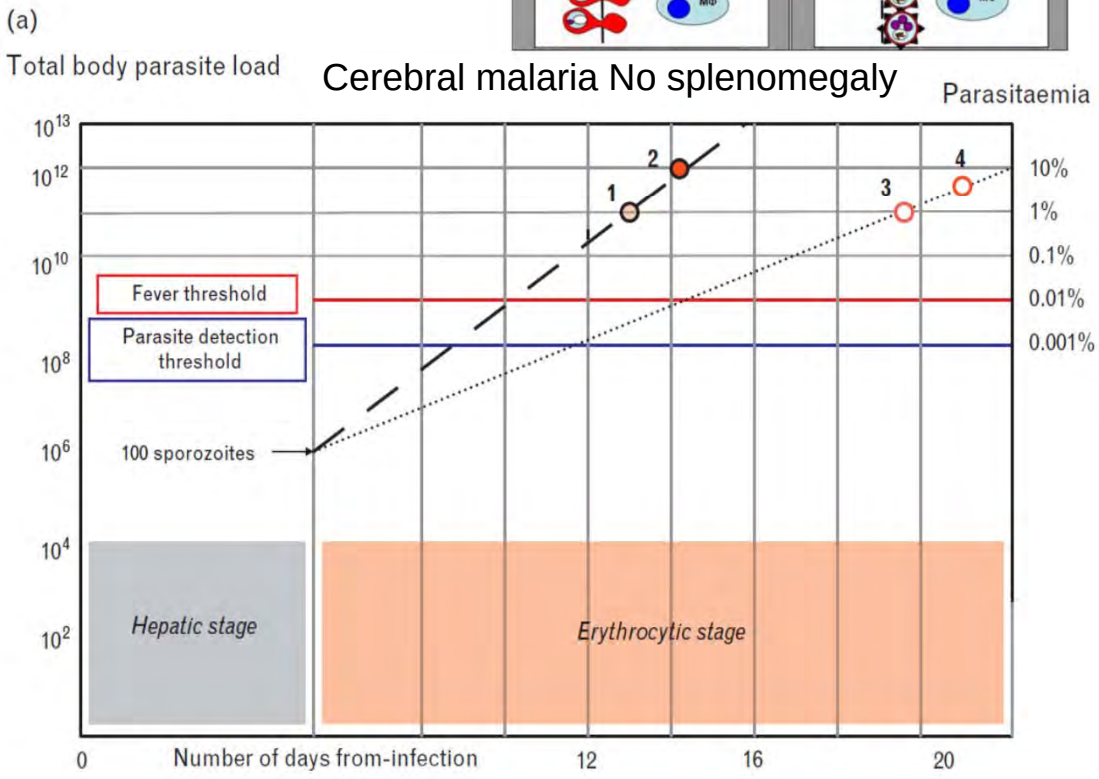
Retention of erythrocytes in the spleen: a double-edged process in human malaria

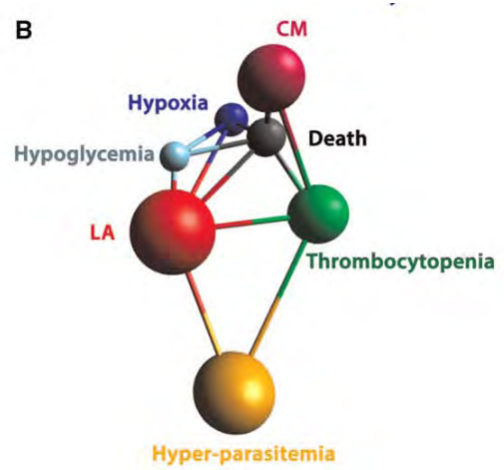
Pierre A. Buffet[a], Innocent Safeukui[b], Geneviève Milon[c], Odile Mercereau-Puijalon[b] and Peter H. David[b]

Current Opinion in Hematology 2009, 16:157–164

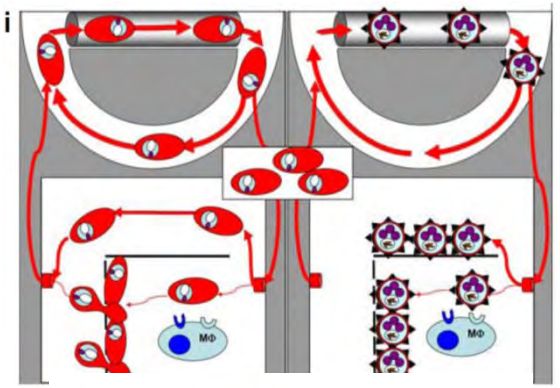


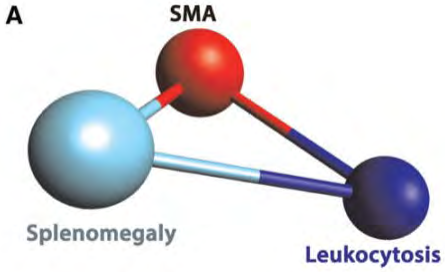
Cerebral malaria No splenomegaly

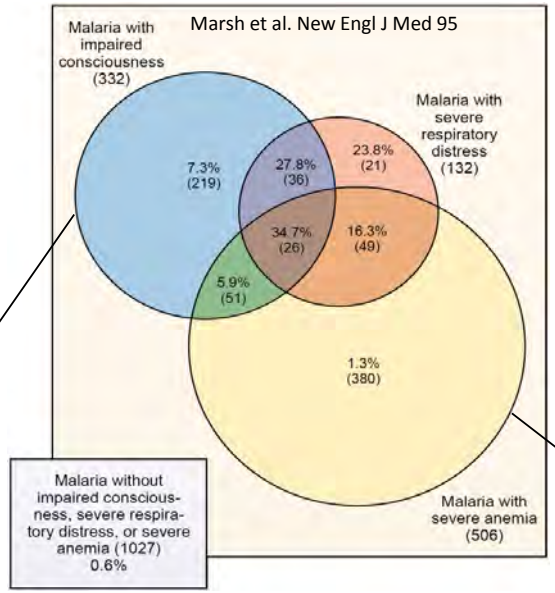




Severe anemia
Frequent splenomegaly







Cerebral malaria associated with

. rapid evolution (3-4 days of fever)

Bruneel 05, Giha 08

. high parasite loads

Giha 05, Dondorp 08

. no splenomegaly

Giha 08

Severe malarial anemia associated with

. relatively slow evolution (7 days of fever)

Giha 08, Sowunmi 09

. relatively low parasite loads

Giha 08, Sowunmi 09

. splenomegaly

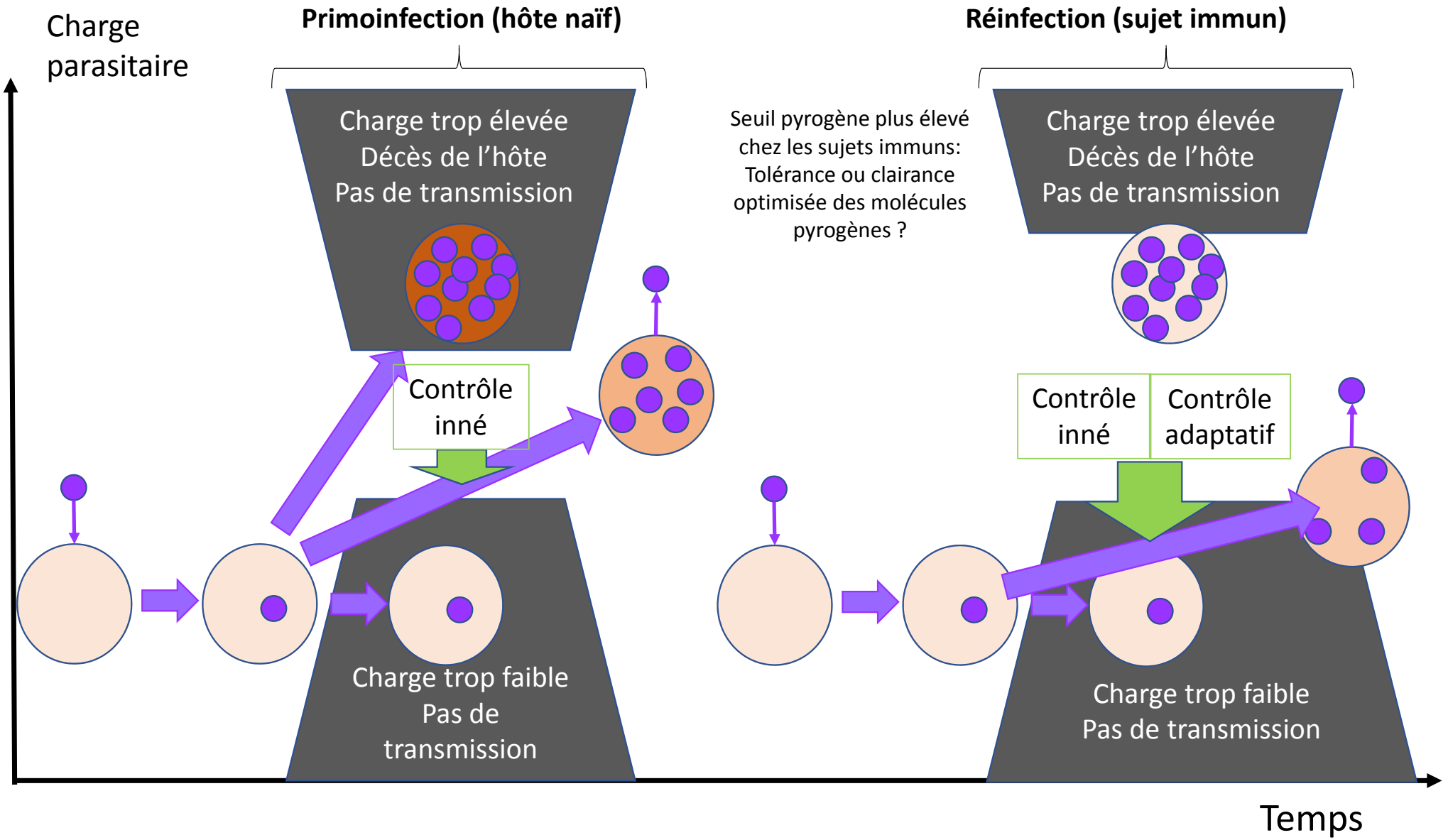
Looareesuwan 87, Price 01, Giha 08, Sowunmi 09

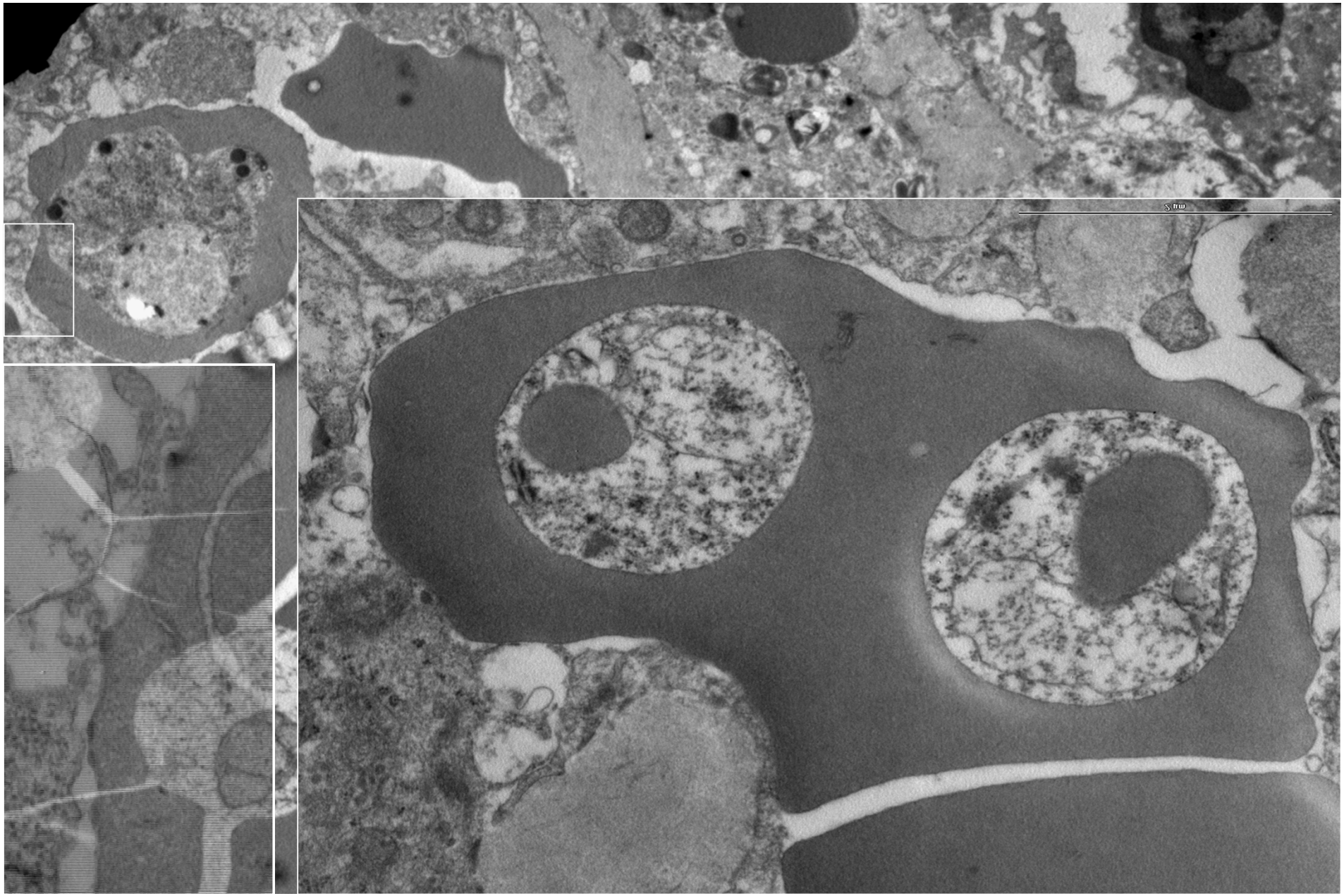
Hypothesis

Loose retention of rings and uninfected RBC in the spleen

Buffet et al. Curr Op Hematol 2009

Stringent retention of rings and uninfected RBC in the spleen


Charge parasitaire
Primoinfection (hôte naïf)
Réinfection (sujet immun)
Charge trop élevée
Décès de l'hôte
Pas de transmission
Seuil pyrogène plus élevé chez les sujets immuns: Tolérance ou clairance optimisée des molécules pyrogènes ?
Charge trop élevée
Décès de l'hôte
Pas de transmission
Contrôle inné
Contrôle inné
Contrôle adaptatif
Charge trop faible
Pas de transmission
Charge trop faible
Pas de transmission
Temps

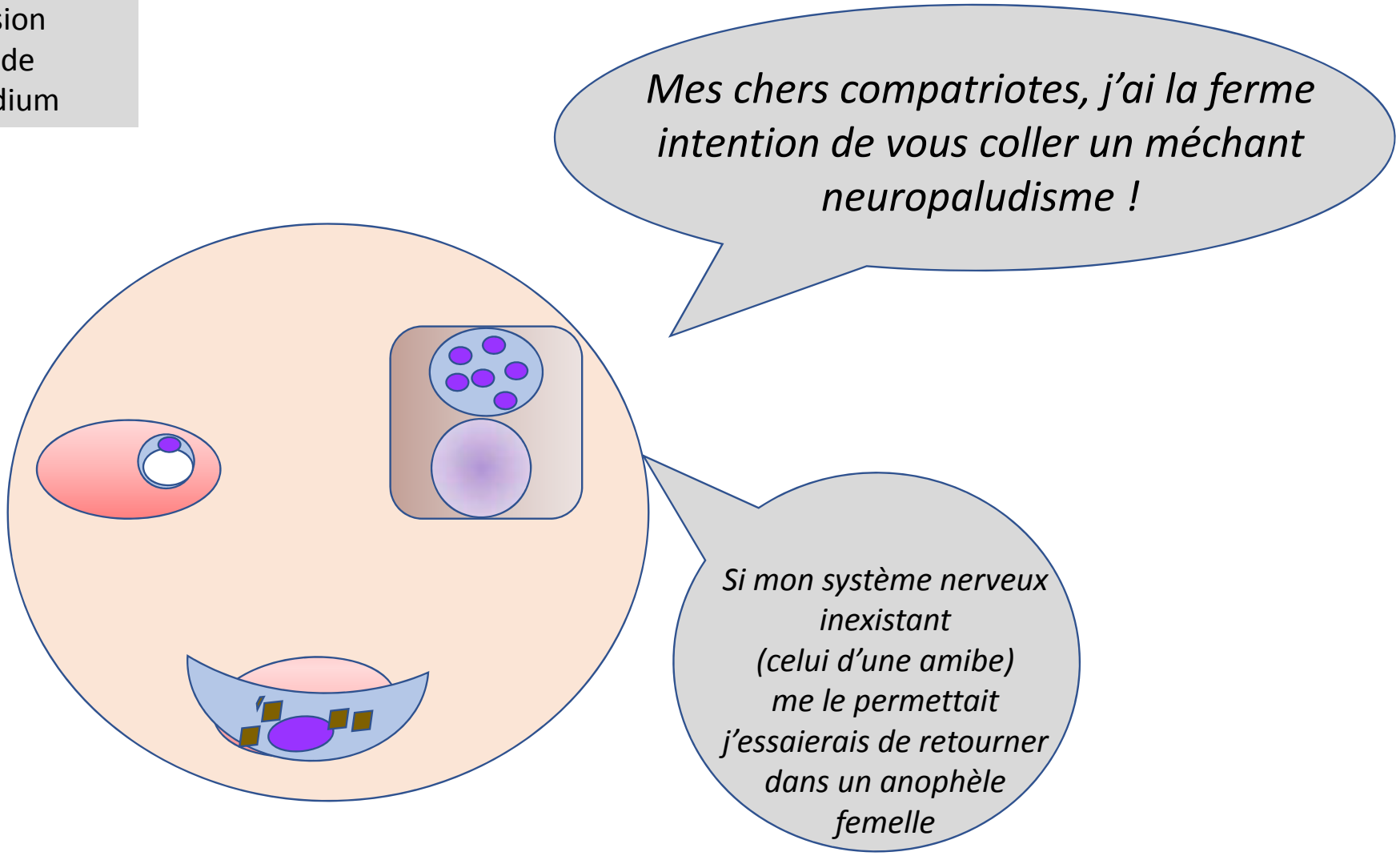



Mikado


le Sport à Villejuif-Léo Lagrange, l'Europe à Europe, le Cinéma à Bonne Nouvelle, Ils font le métro
à Montparnasse-Bienvenüe, la Création à Saint-Germain-des-Prés, l'Ecologie urbaine à Luxembourg
La Santé à Pasteur
j'ai la rate qui se dilate
Évoquer, sur un mode ludique, la santé au quotidien : du milieu du métro aux questions d'environnement en passant par les progrès de la recherche... A travers des sollicitations avant tout visuelles, chacun pourra explorer les enjeux médicaux d'aujourd'hui et de demain. A la découverte d'une nouvelle façon de prendre soin de soi, sous le signe du bien vivre et de la bonne humeur.

Comme **Pierre Ambroise-Thomas** qui y a consacré une partie importante de sa carrière, nous ne pouvons qu'être interpellés par les rapports qu'entretiennent les parasites avec leurs hôtes. Comment ces « étrangers », parfois de taille impressionnante et pas toujours dotés de très bonnes intentions, peuvent-ils survivre dans des organismes qui déploient pourtant, grâce au système immunitaire, des trésors d'ingéniosité pour se protéger de toutes les agressions ?

Profession
de foi de
Plasmodium



Quelle est la cible des anticorps protecteurs ?

Quel est le mécanisme de protection ?

Pourquoi leur acquisition est-elle si lente ?

Pourquoi persistent-ils assez peu si l’exposition cesse ?

Déficit mémoriel ou la troisième mamelle de la lenteur
(Qui oublie son passé peut être condamné à le revivre)

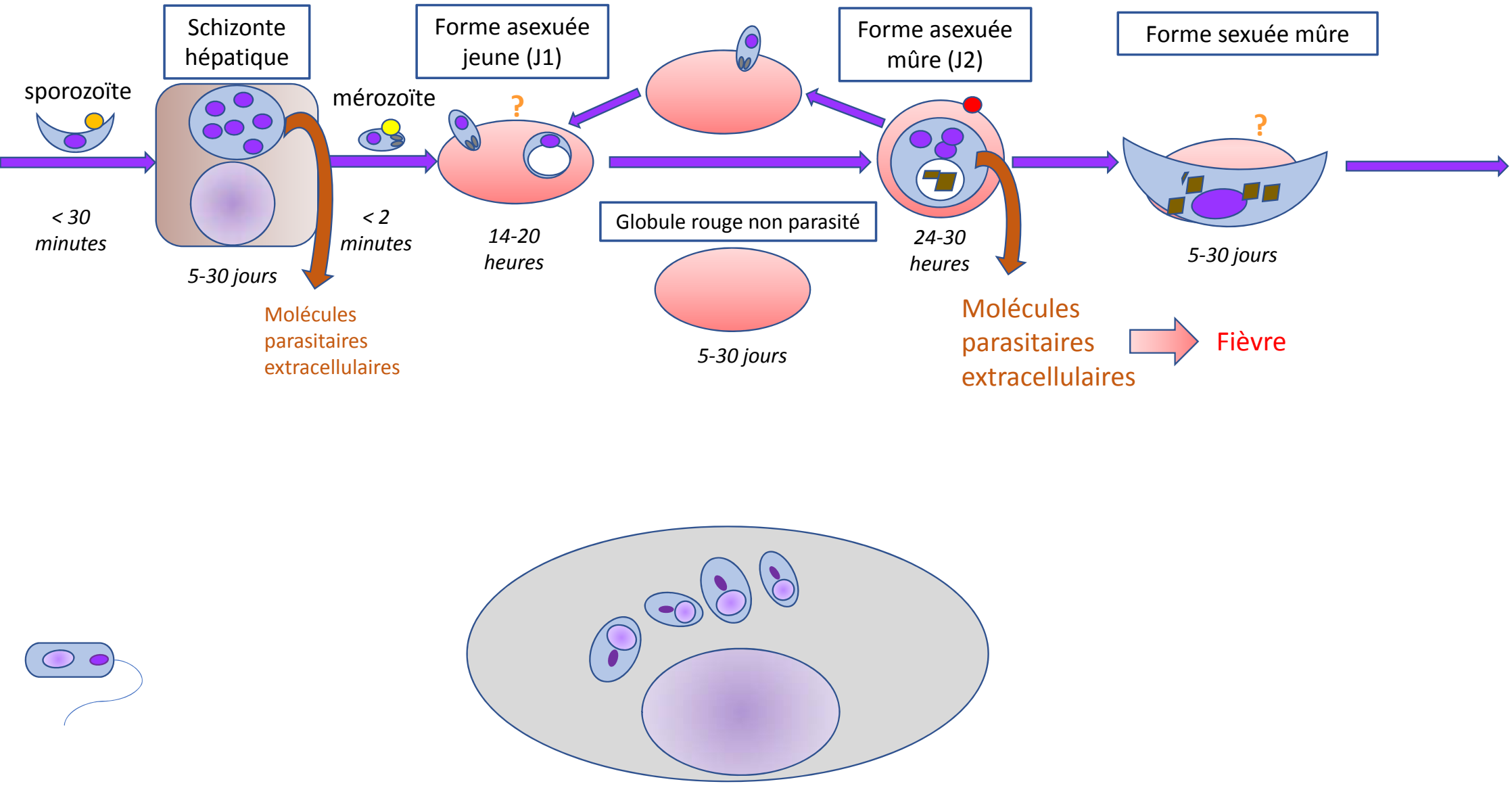
Décroissance rapide des anticorps anti-Pf chez les enfants africains

Apparition lente

Réapparition rapide des symptômes après réduction de la circulation des parasites

Apparition lente et inconstante des lymphocytes B mémoire anti-Pf (une fois acquis leur persistance est bonne)

Acquisition de lymphocytes B mémoire atypiques dont la fonction et l'impact sur l'infection restent à déterminer


Schizonte hépatique
Forme asexuée jeune (J1)
Forme asexuée mûre (J2)
Forme sexuée mûre
sporozoïte
mérozoïte
?
?
< 30 minutes
5-30 jours
< 2 minutes
14-20 heures
Globule rouge non parasité
24-30 heures
5-30 jours
5-30 jours
Molécules parasitaires extracellulaires
Molécules parasitaires extracellulaires
Fièvre

Accordingly, there is no convincing evidence for naturally acquired immunity capable of completely neutralizing the parasite at the skin or liver stages. Indeed, even after decades of repeated *P. falciparum* exposures, adults in malaria-endemic areas are at the same risk of becoming infected with parasites as young children (27). The cellular and molecular basis of this clinical silence and paucity of sterilizing immunity is only poorly understood. The relatively weak innate and adaptive immune responses to the skin and liver stages may reflect the low inoculum of parasites in the mosquitoes' saliva (10–100 sporozoites). The inherent immunoregulatory environment of skin (28) and liver (29) may also be exploited by the parasite to evade immune mechanisms. Normal