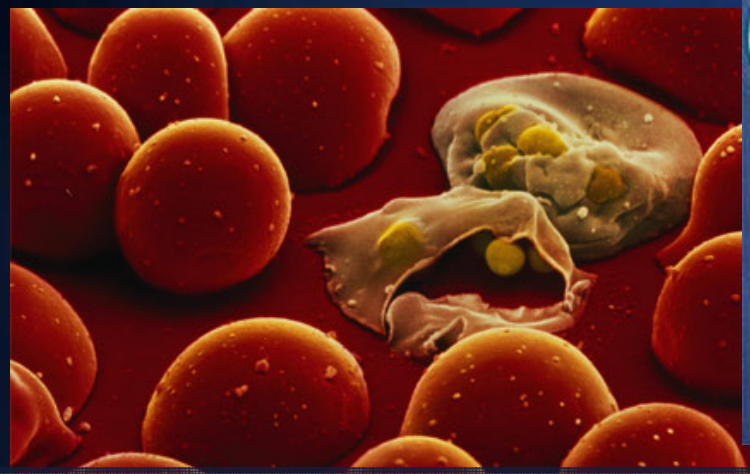
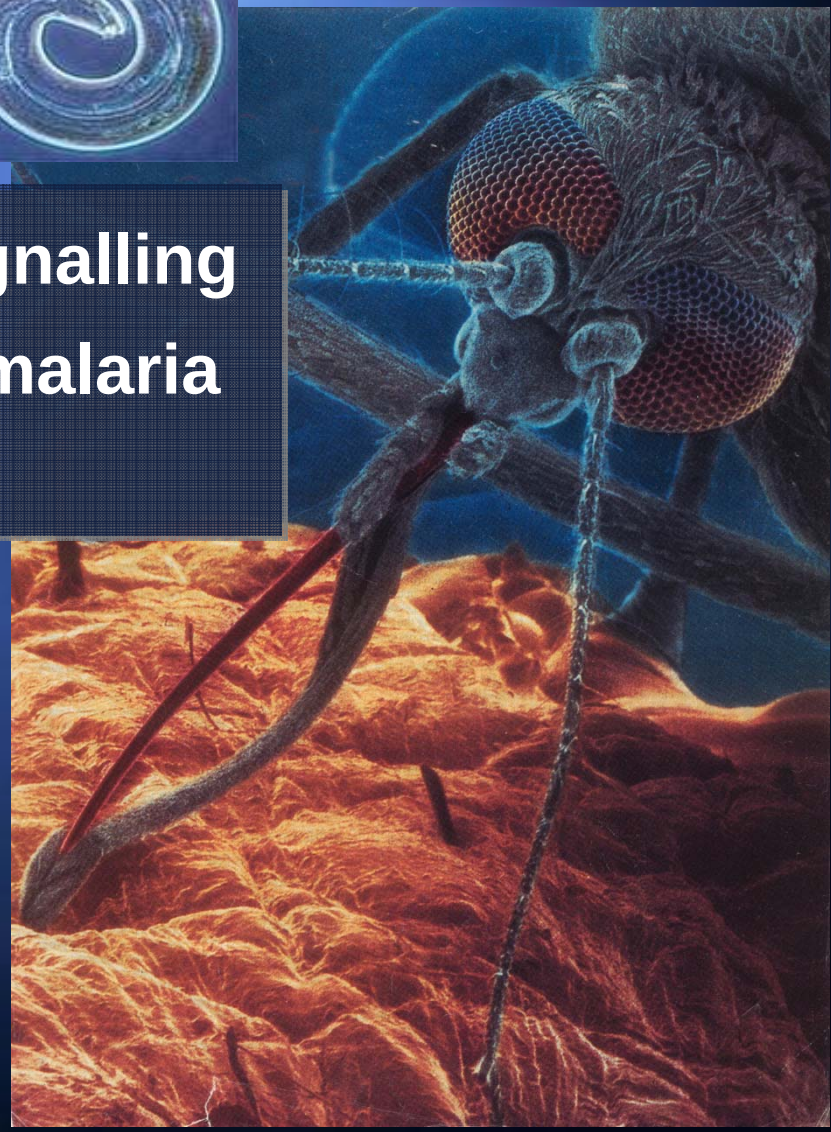


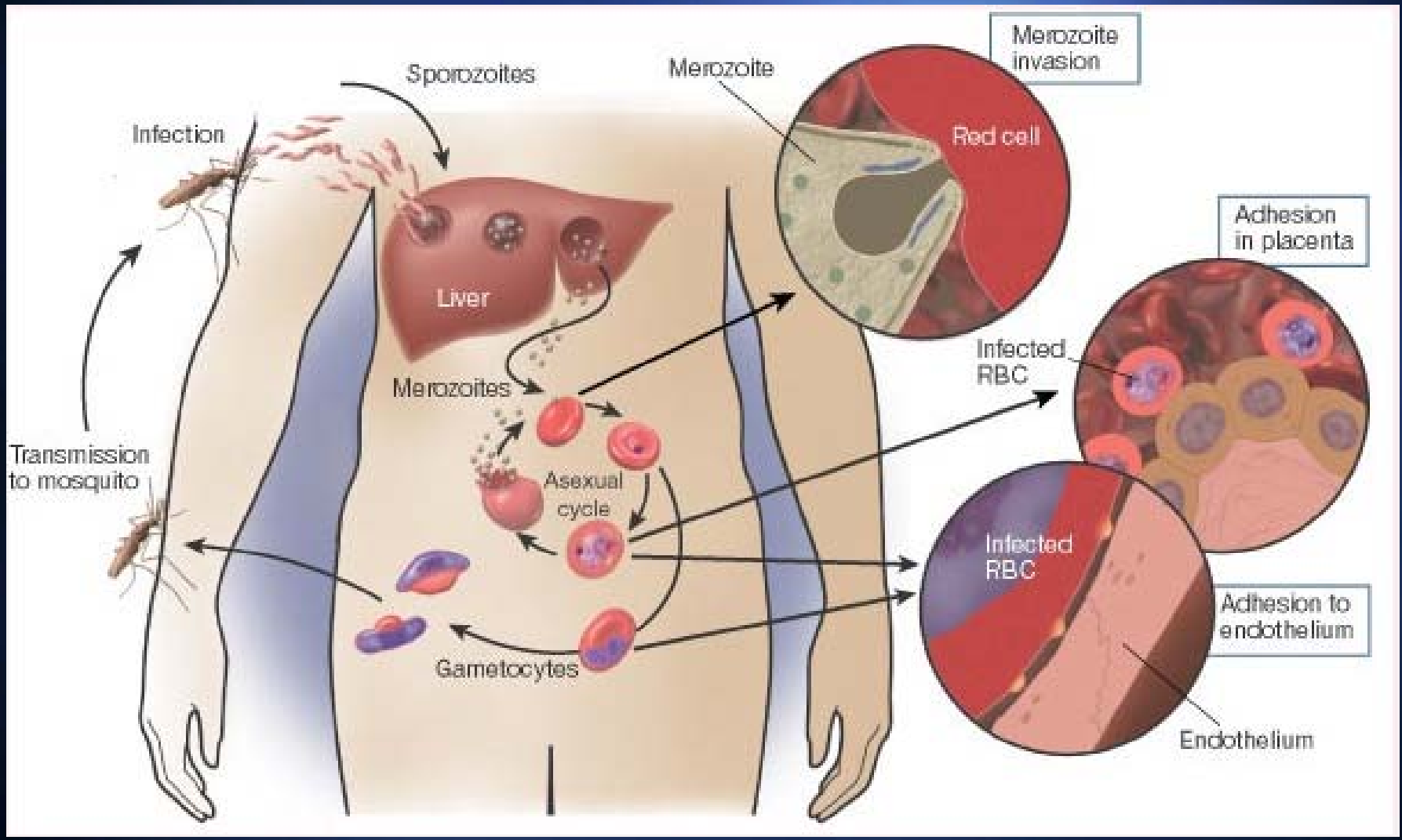


Inhibition of histamine-mediated signalling confers protection against severe malaria in murine models of disease

Brad S. Schneider, Ph.D.

Institut Pasteur

Plasmodium life cycle



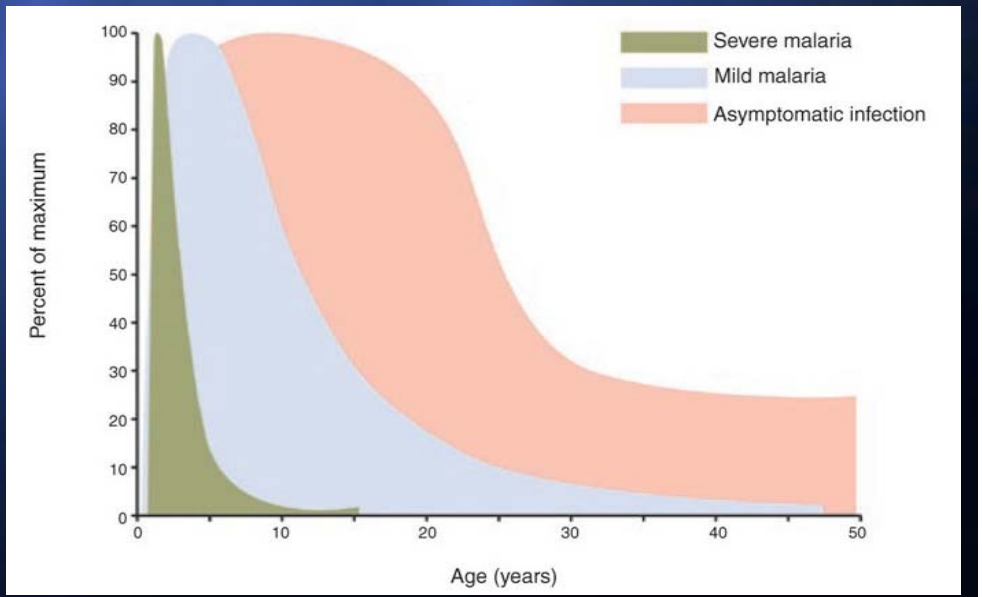
Miller L *et al.* Nature 415, 673-679

Malaria: disease progression

Parasite factors	Host factors	Geographic and social factors
Drug resistance	Immunity	Access to treatment
Multiplication rate	Proinflammatory cytokines	Cultural and economic factors
Invasion pathways	Genetics (sickle cell trait, thalassaemia, ovalocytosis, Gerbich RBC, CD36, TNF-α, ICAM-1, CR1, MHC locus)	Political stability
Cytoadherence	Age (no cerebral malaria in infants)	Transmission intensity (*Anopheles spp.*, seasonality of transmission, infectious bites per year, epidemics)
Rosetting	Pregnancy	
Antigenic polymorphism		
Antigenic variation (PfEMP1)		
Malaria toxin		

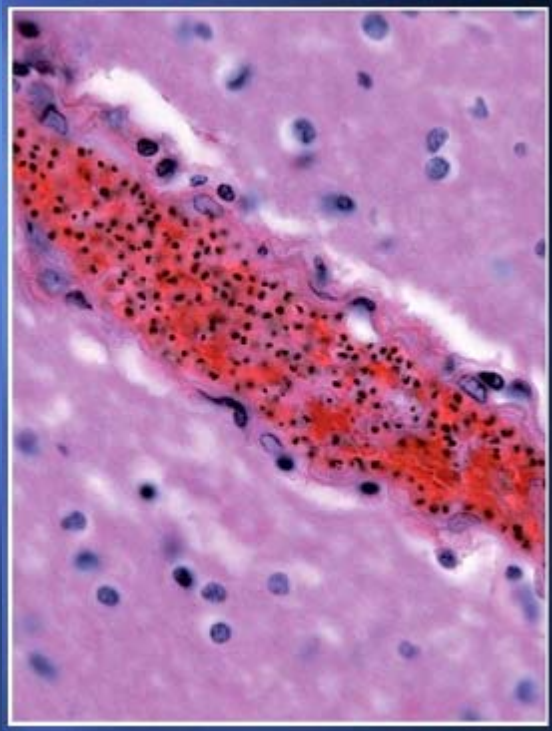
Clinical outcome

Asymptomatic infection	Fever (symptomatic infection)	Severe malaria (metabolic acidosis, severe anaemia, cerebral malaria)	Death



Cerebral Malaria

- Previously cerebral malaria was thought to result primarily from blockage of cerebral vessels by packed RBCs.
  - Leading to ischemia and hemorrhages.
- Human data show a proportion of cerebral malaria cases without RBC sequestration.
- This suggests that there are different mechanisms of pathogenesis leading to cerebral complications including:
  - Influx of activated cells ($CD8^+$ T cells)
  - Production of inflammatory mediators (IFNγ)
  - This may occur with or without evidence of sequestration.



The pathogenic mechanism is likely a more complex combination of events

Inflammation and Malaria

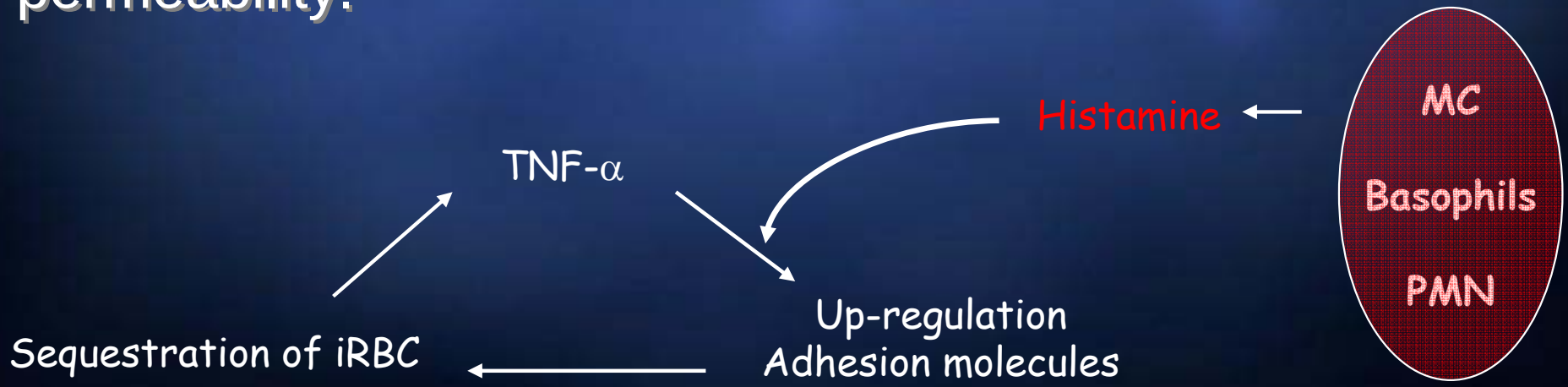
- Previous human studies suggest a role for eosinophils, basophils, and mast cells in the pathogenesis of malaria.
- Increased levels of histamine in the serum is associated with severity of *P. falciparum* disease (Srichaikul *et al.* 1976).
- Elevated IgE, which via binding with basophils and mast cells liberates histamine, is also associated with disease severity (Perlmann *et al.* 1999).
- Inflammatory cytokines (IFNγ and TNFα) are poor prognostic indicators for cerebral malaria.
- TCTP - a *Plasmodium*-derived homologue of human histomine-releasing factor (HRF).
  - Shown to cause histamine release from basophils and IL-8 release from eosinophils.

Study Aim

- To directly assess the relevance of histamine signaling in malaria pathogenesis and its association with disease severity.

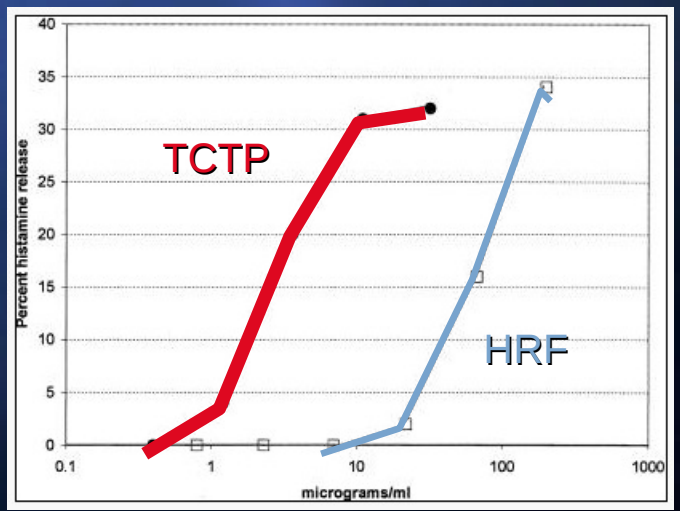
Rationale: Histamine

- Has pro-inflammatory and immunosuppressive activity
  - Reduces neutrophil chemotaxis.
  - Inhibits T-lymphocyte proliferation
  - Alters immune cell migration, activation, and maturation.
- **In DCs** histamine promotes an increase in cAMP and IL-10, while inhibiting IL-12.
- **In vascular endothelium cells** histamine induces P-selectin, cell adhesion, nitric oxide, and enhanced permeability.

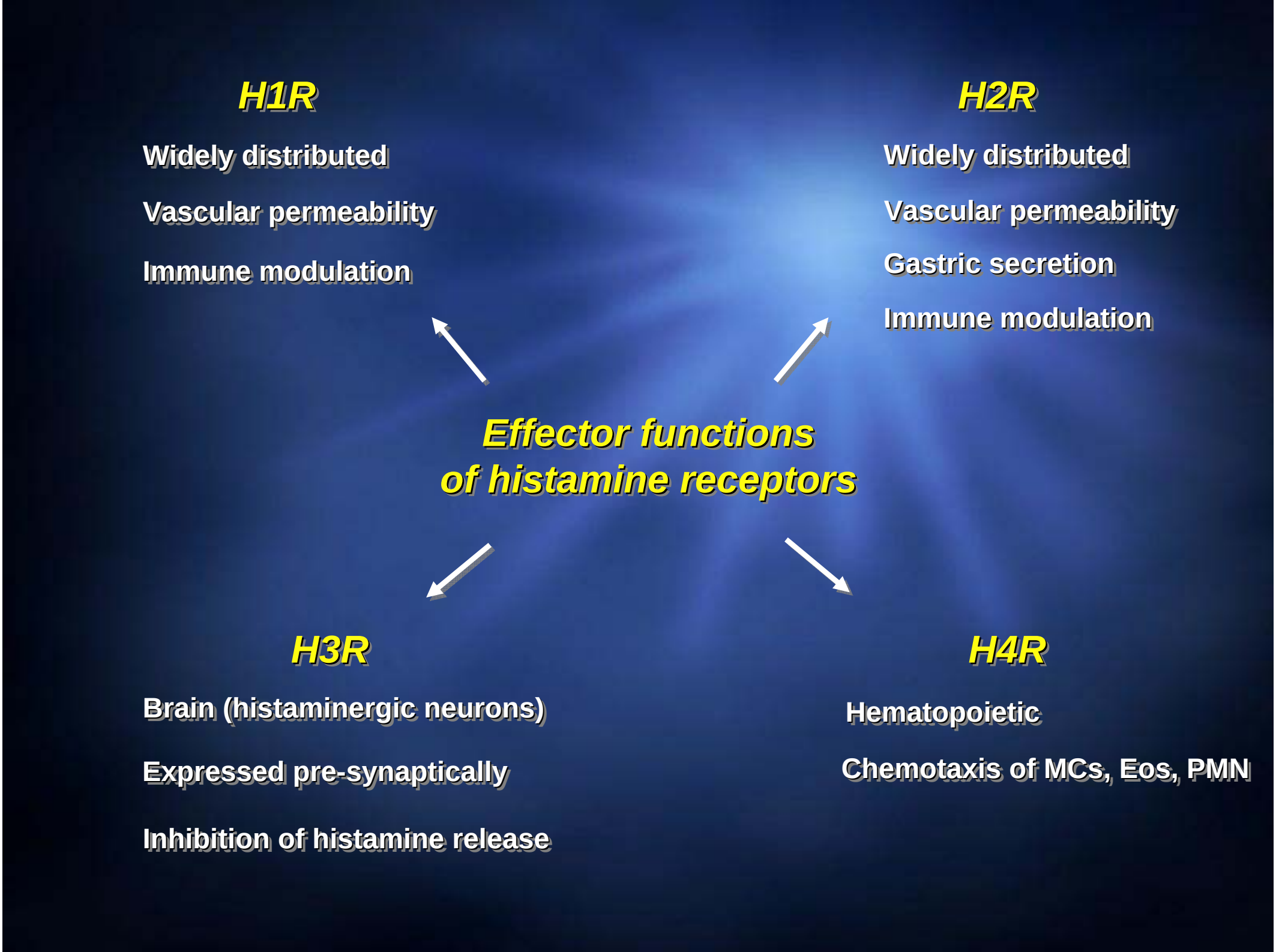


Rationale: Histamine

- Histamine can increase expression of thrombomodulin which is implicated in parasitized RBC sequestration.
- *Plasmodium* TCTP (transitionally controlled tumor protein)
  - Found in the plasma of *P. falciparum*-infected patients
  - Calcium binding (Gnanasekar et al. 2002)
  - Heat stress adaptation (Mak et al. 2001)
  - Antioxidant activity (Gnanasekar et al. 2007)
  - Histamine release



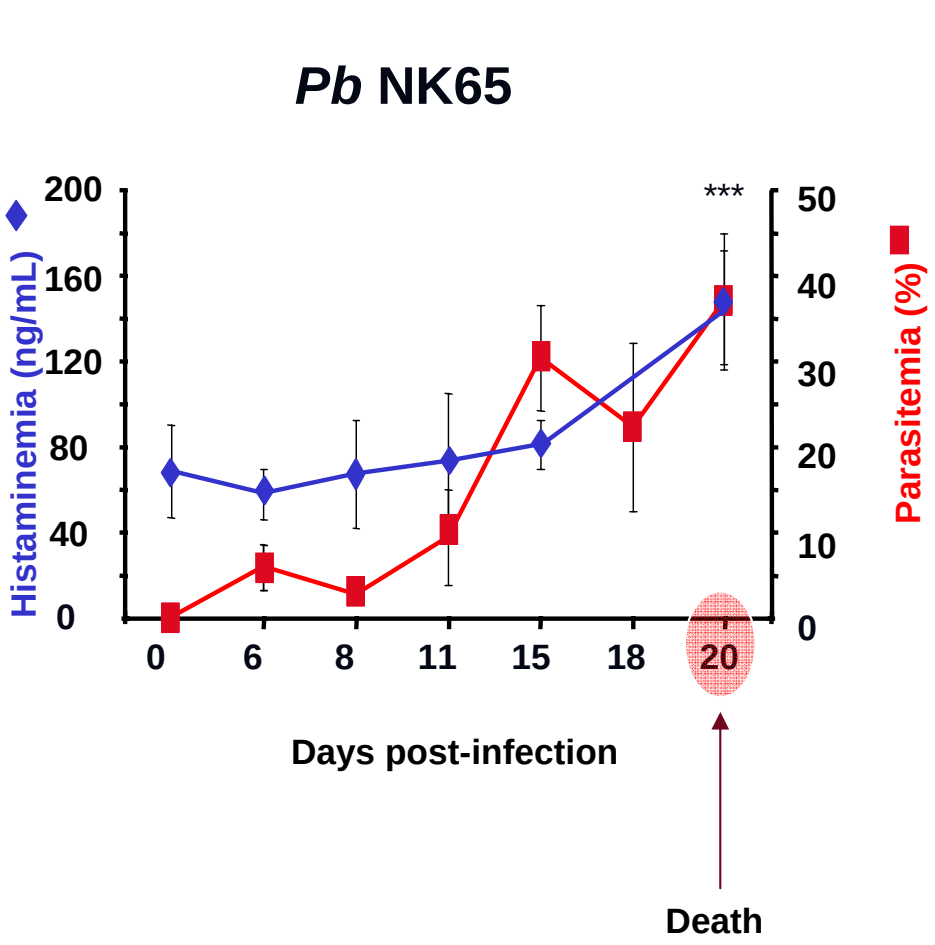
Mac Donald *et al*. 2001

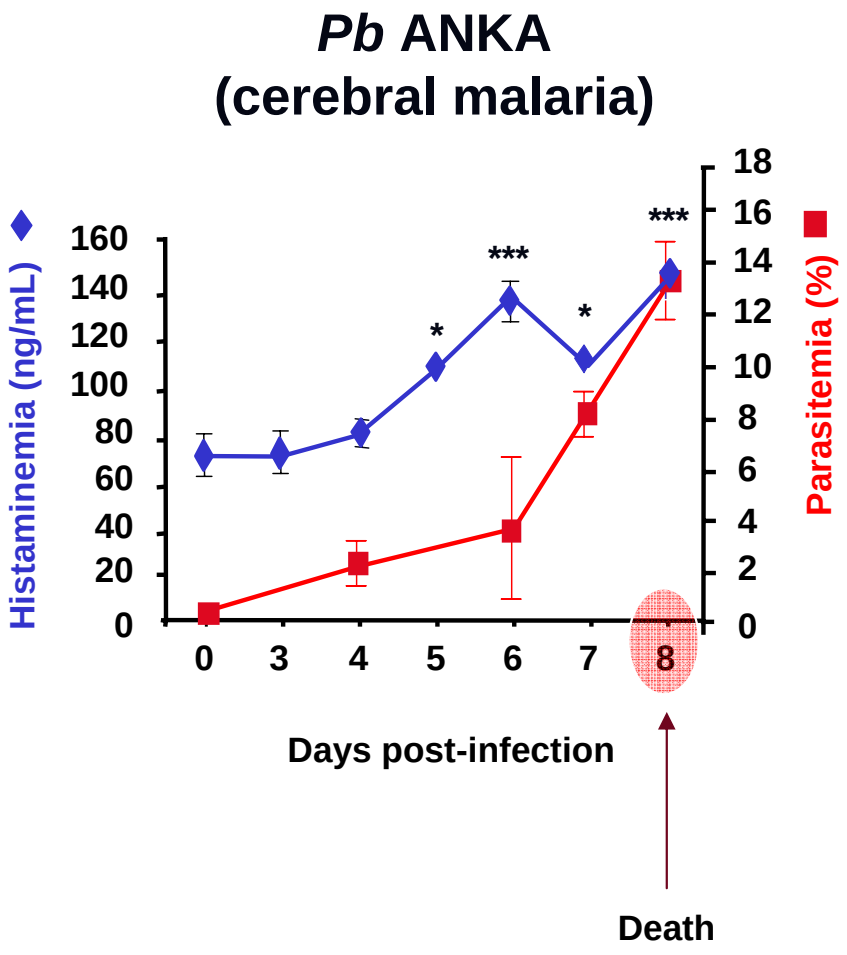

H1R
Widely distributed
Vascular permeability
Immune modulation
H2R
Widely distributed
Vascular permeability
Gastric secretion
Immune modulation
Effector functions
of histamine receptors
H3R
Brain (histaminergic neurons)
Expressed pre-synaptically
Inhibition of histamine release
H4R
Hematopoietic
Chemotaxis of MCs, Eos, PMN

Experimental Design

- Genetically
  - Histadine decarboxylase-deficient ($HDC^{-/-}$) mice
  - Histamine receptor knock-out (H1R/H2R) mice
- Pharmacologically
  - Antihistamines
- *Plasmodium berghei* strains
  - NK65: induces anemia
  - ANKA: induces cerebral malaria
- Routes of infection
  - Infected mosquitoes (*Anopheles stephensii*)
  - Infected RBCs
- Phenotypes
  - Parasitemia
  - Immune response
  - survival

Histamine levels post-infection

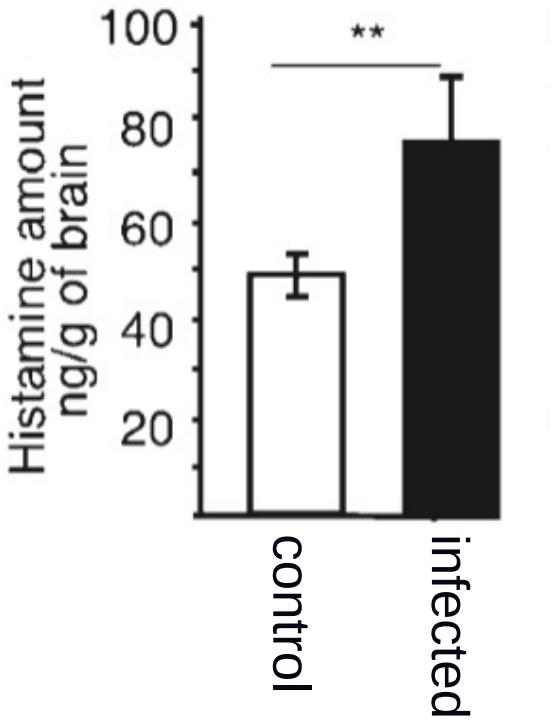




Beghdadi et al. J Exp Med, 2008

Histamine levels post-infection

Histamine level in the brain day 7 p.i.

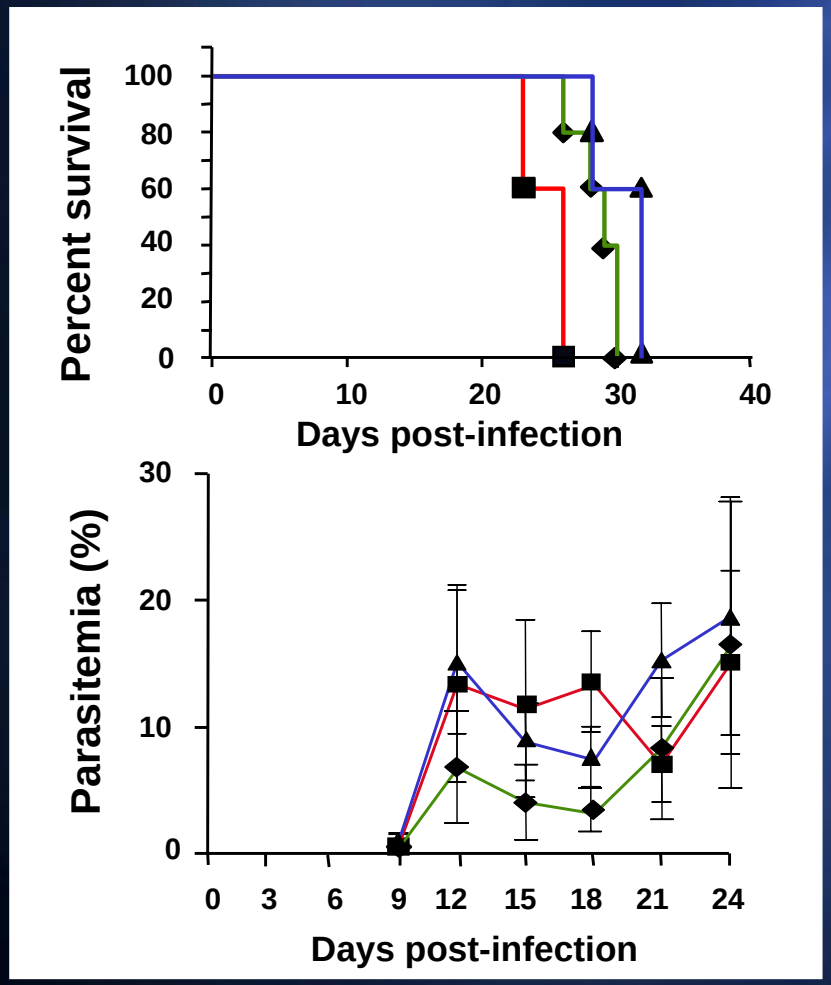


Beghdadi et al. J Exp Med, 2008

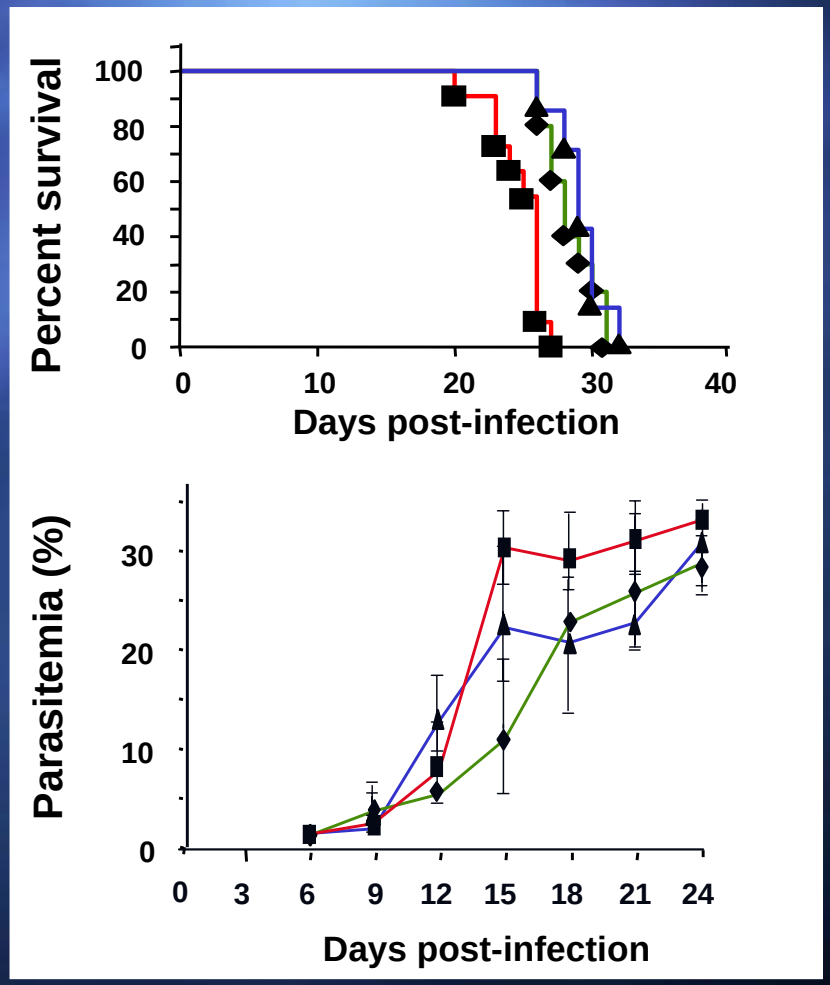
Role of H1R and H2R in *P. berghei* infection

Pb NK65

Infected mosquito bites



Infected RBC

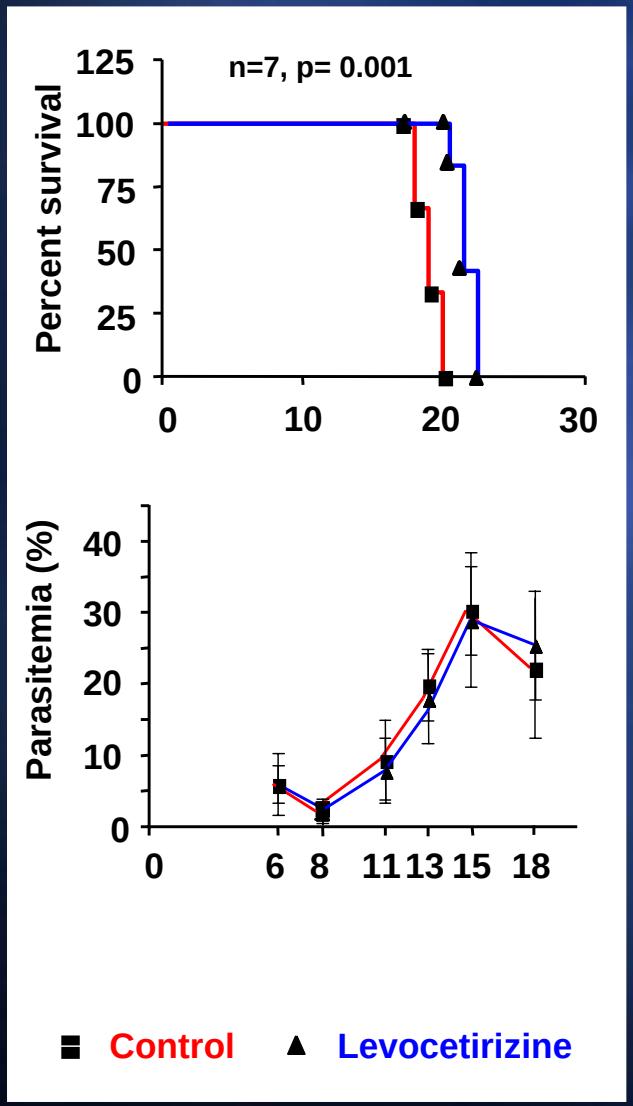


■ B6 ◆ H1R$^{-/-}$ ▲ H2R$^{-/-}$

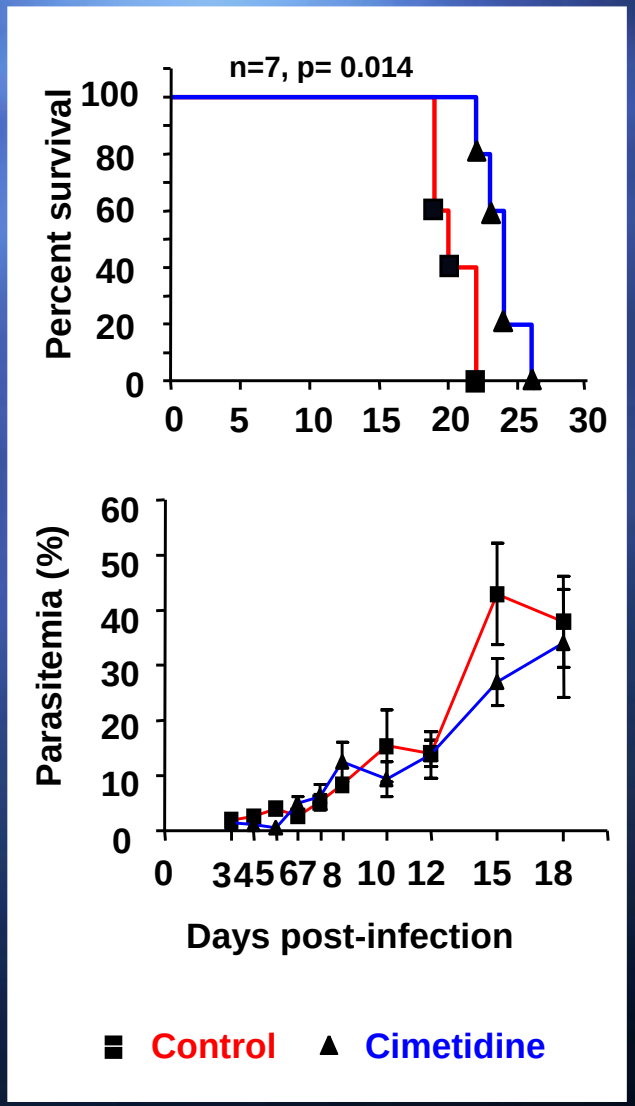
Beghdadi et al. J Exp Med, 2008

Prolonged survival following treatment with histamine inhibitors

H1 antagonist

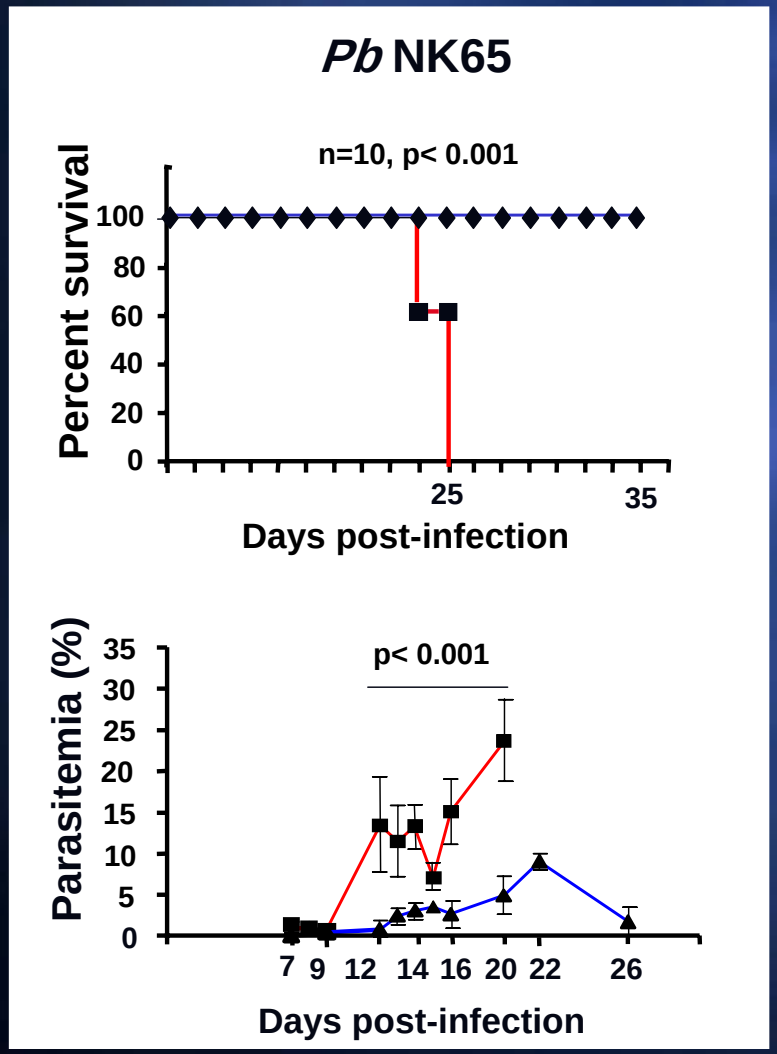


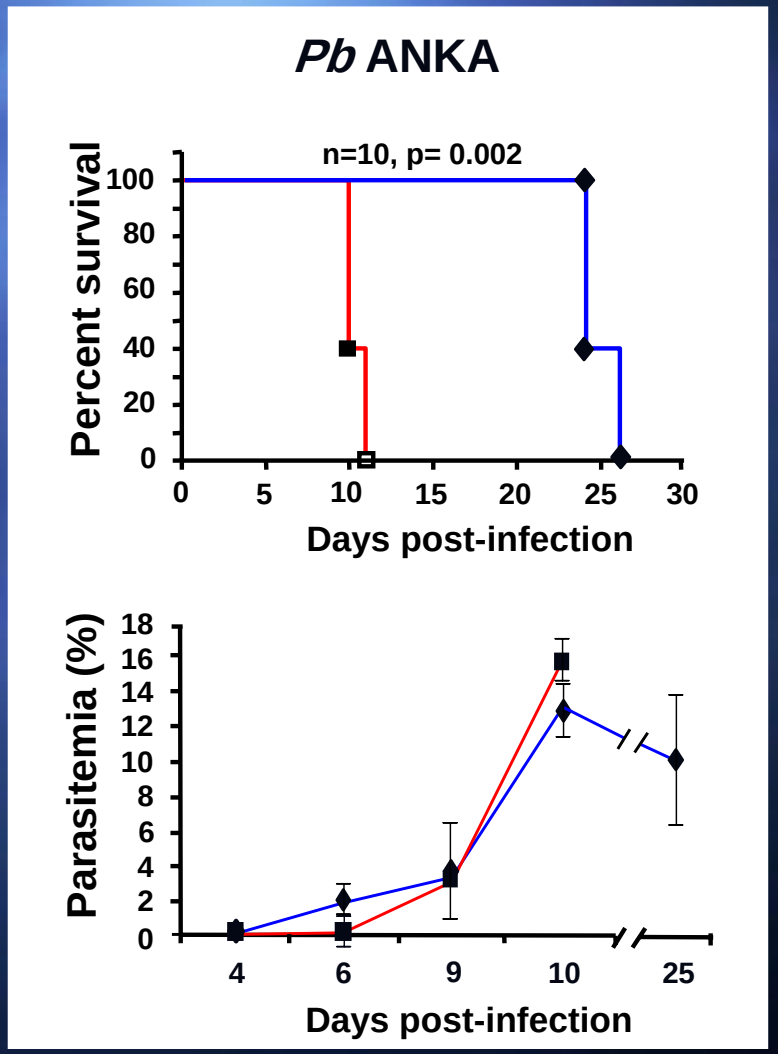
H2 antagonist



Mice deficient in histamine ($HDC^{-/-}$) are resistant to lethal infection by two strains of *P. berghei*

infectious mosquito bites



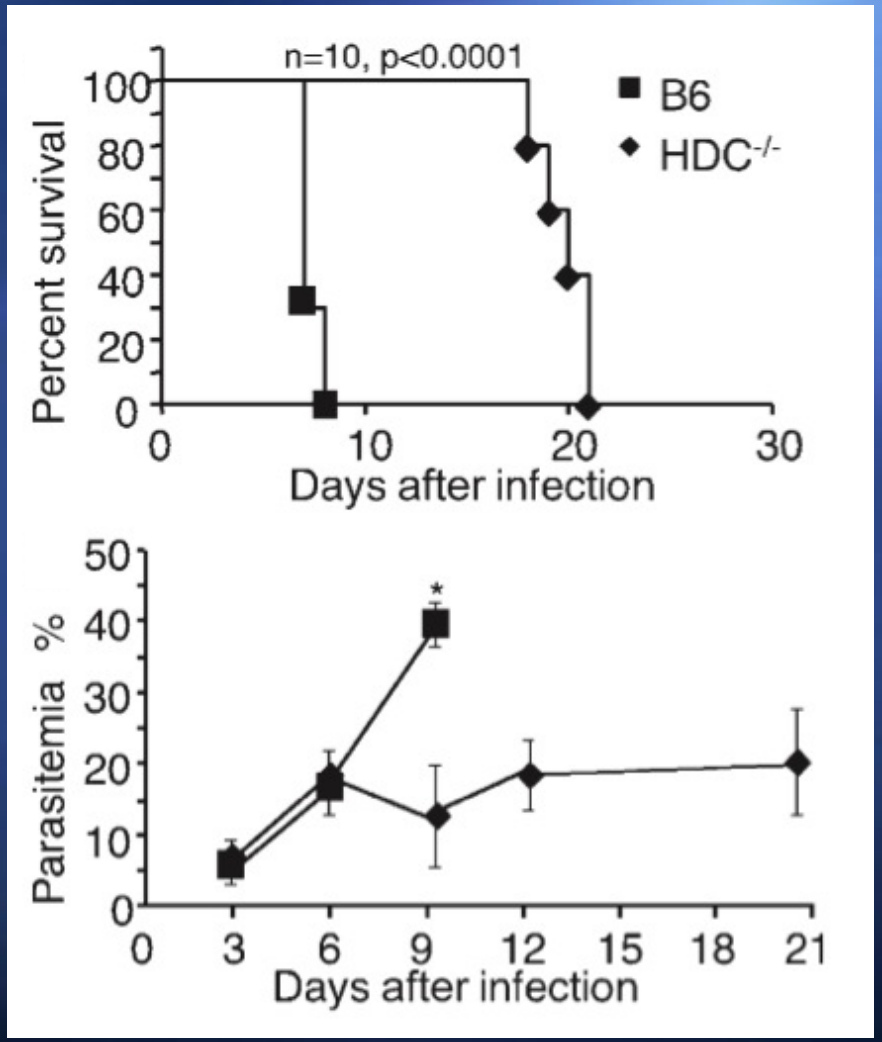


■ B6 ◆ $HDC^{-/-}$

Beghdadi et al. J Exp Med, 2008

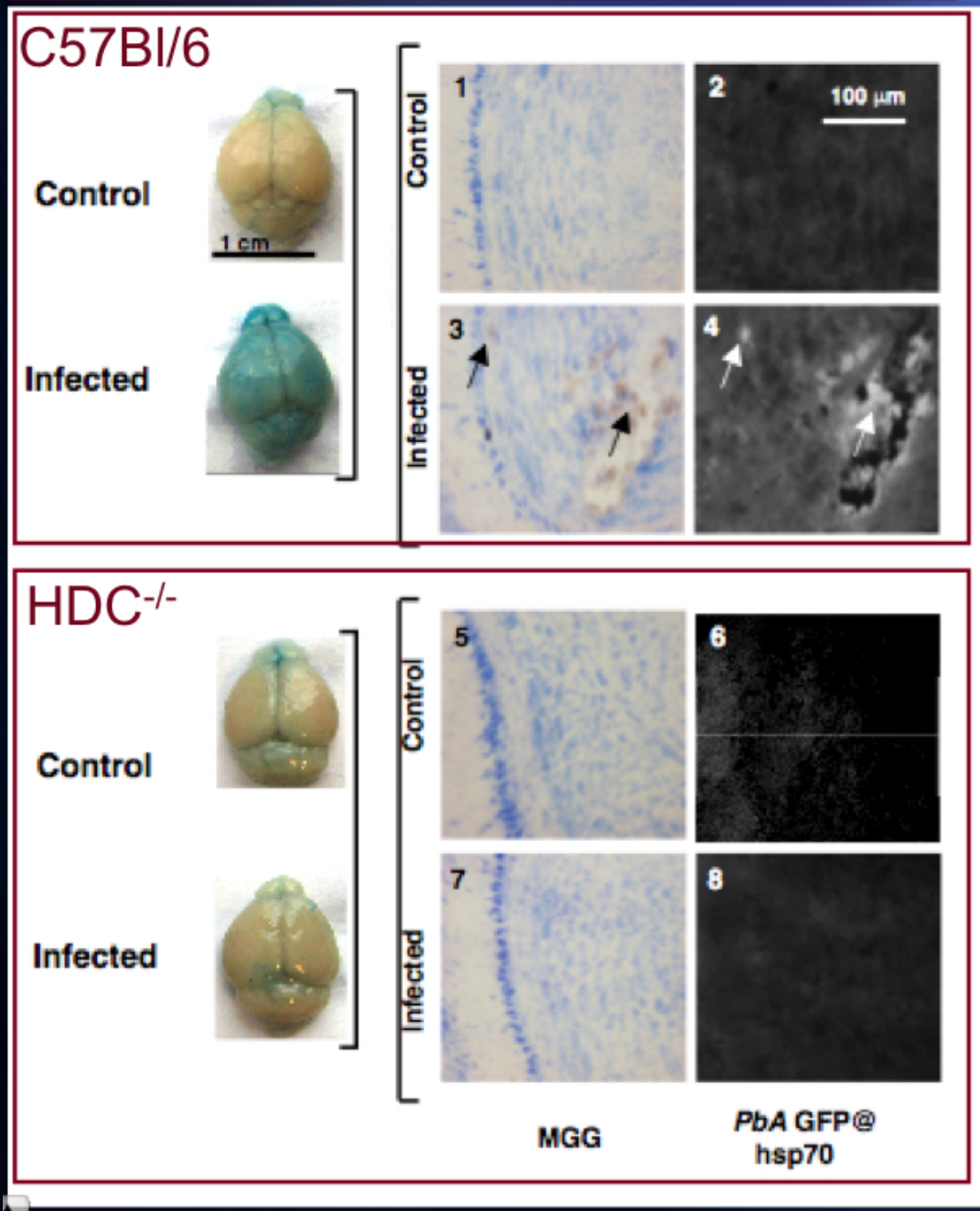
Mice deficient in histamine ($HDC^{-/-}$) are resistant to lethal infection by two strains of *P. berghei*

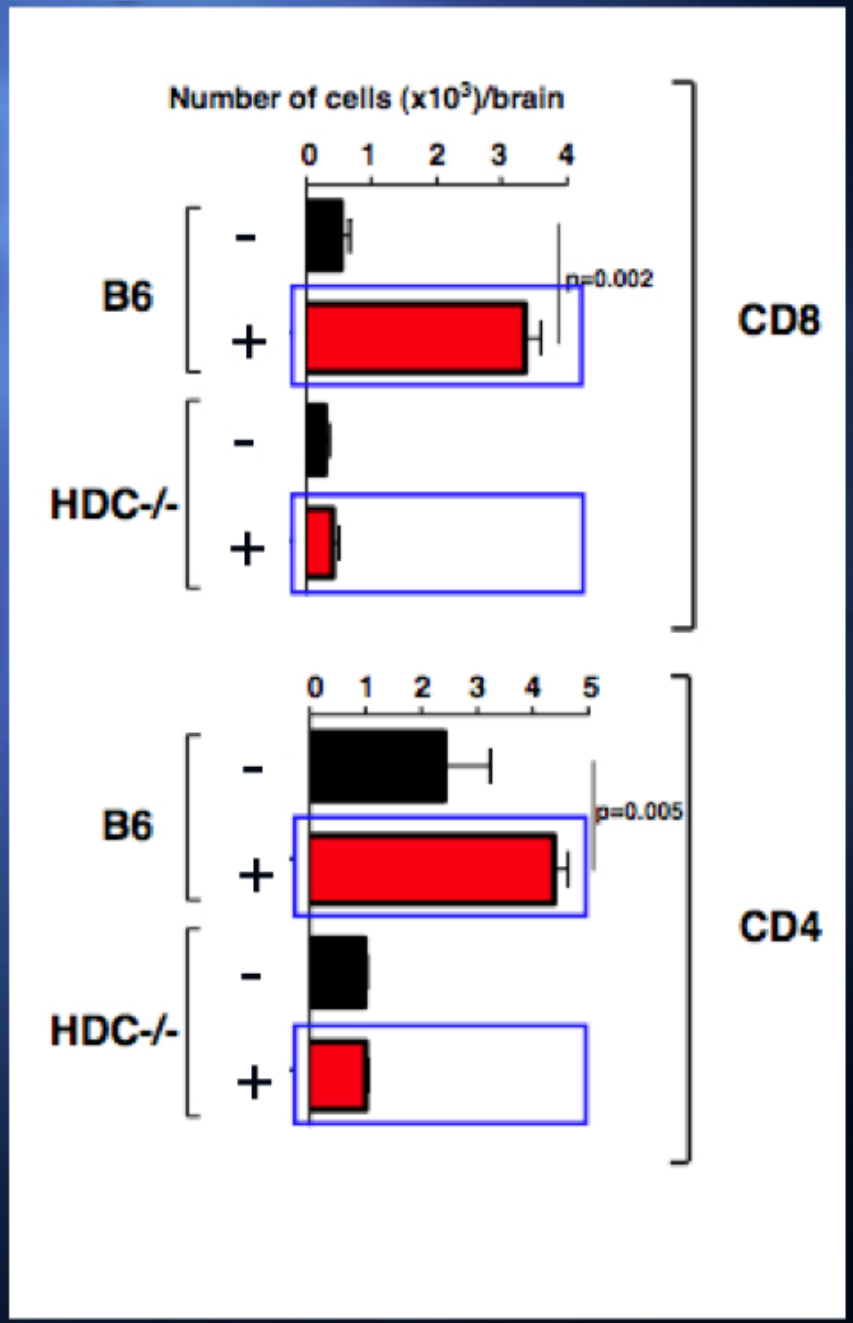
Pb ANKA infected RBCs



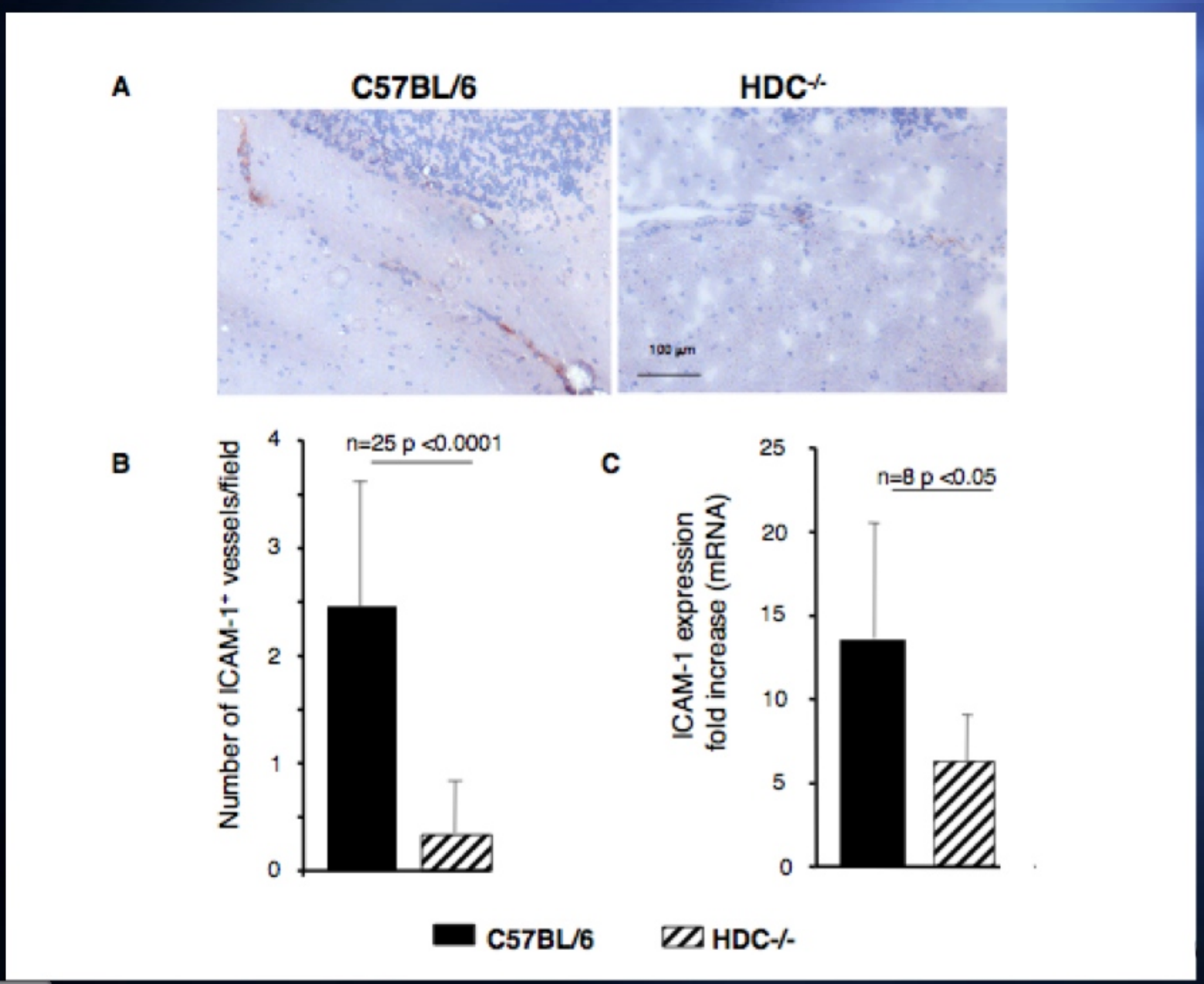
Beghdadi et al. J Exp Med, 2008

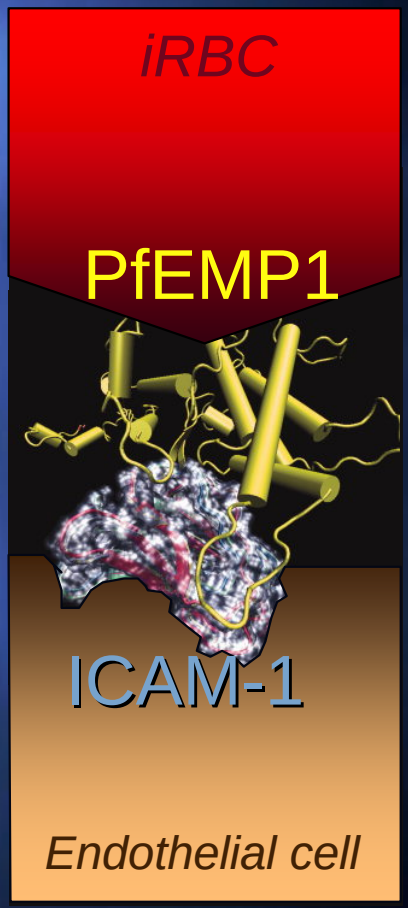
Preserved BBB integrity and lack of T cell sequestration in the brain of HDC$^{-/-}$ mice during infection





Reduced expression of ICAM-1 in the cerebral microvasculature of HDC$^{-/-}$ mice

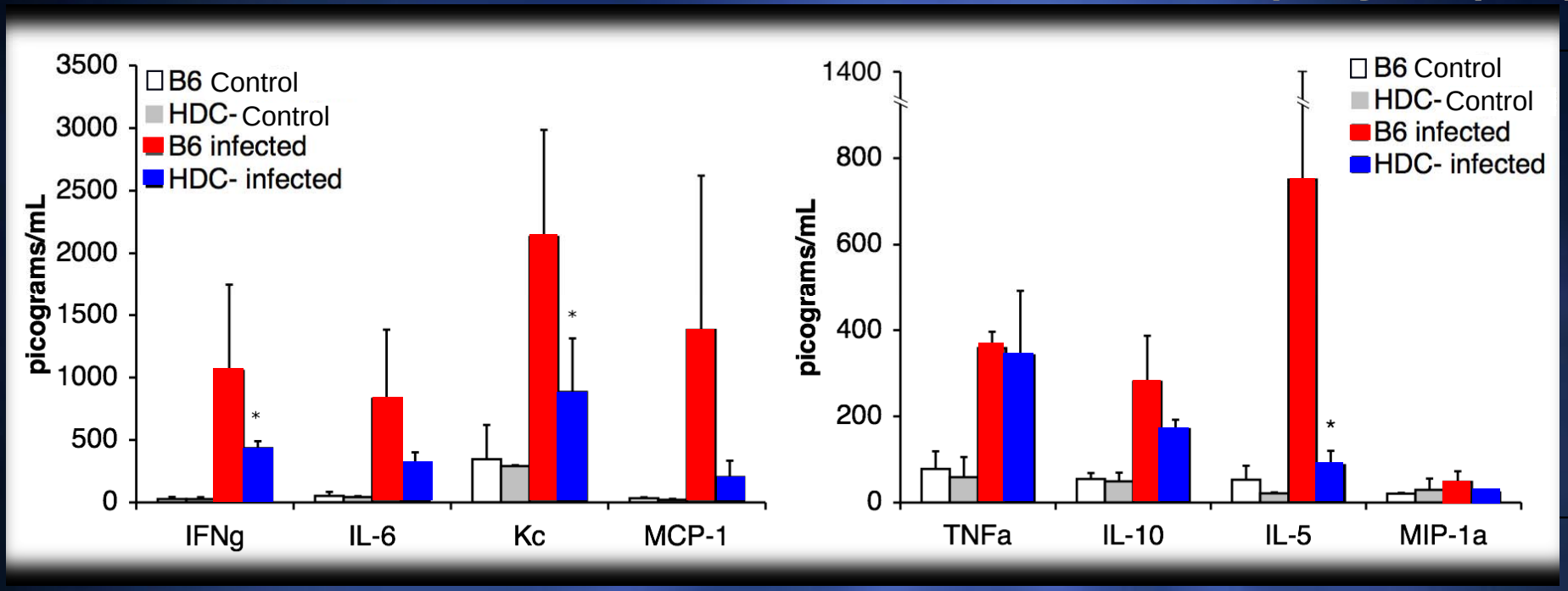




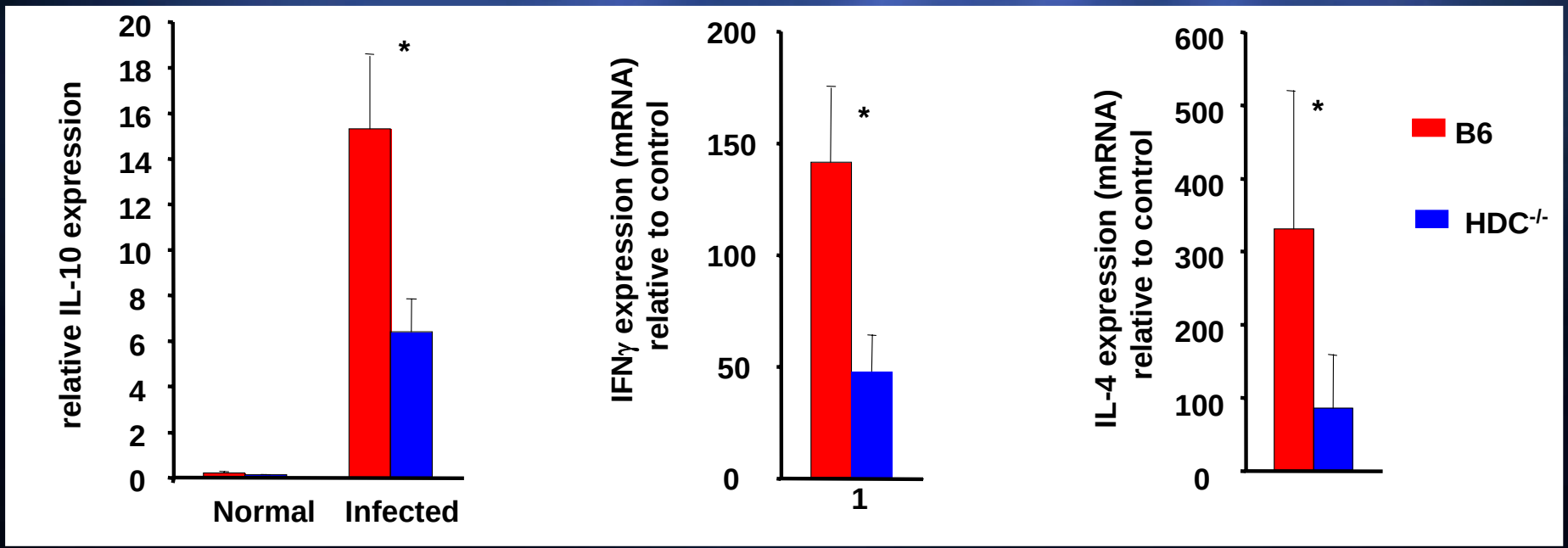
Adapted from Bertonati et al. Proteins. 2007

Differential production of cytokines and chemokines between infected C57Bl/6 and HDC$^{-/-}$ mice (day 6 p.i.)

PLASMA



BRAIN



Conclusions

- Histamine levels increase during malaria infection and is associated with disease severity.
- Mice genetically deficient in H1R and H2R have delayed mortality
- Mice treated with H1 and H2 antihistamines survive longer than untreated mice.
  - This suggests that histamine signaling via these receptors is deleterious to the host during *Plasmodium* infection.
  - However, blocking the action of these receptors has a limited effect in regards to survival time, suggesting that histamine mediates other factors involved in the pathogenesis.

Signaling through H3R and H4R is unlikely to account for the remainder as a nonselective H3/H4 inhibitor (Thioperamide) showed no protective effect.

Conclusions

- Mice lacking histamine were highly resistant to malaria disease when exposed to ***P. berghei*** **NK65-infected** mosquitoes.
  - ~100% survival
  - Only 30% developed blood-stage parasites.

This suggests that the absence of histamine results in:

- the generation of a more efficient effector response against the pre-erythrocytic or blood stages of the parasite.

- limits the immunopathology associated with the disease.

Conclusions

- Mice lacking histamine did not develop cerebral malaria following infection with ***P. berghei* ANKA**.
  - Absence of cerebral complications although mice do inevitably die of hyperparasitemia.
  - Pathogenic effects of histamine are likely to be during the blood stages of the parasite.

This suggests that histamine is involved in the progression to cerebral malaria.

The similar parasitemia between groups implies that the resistance of $HDC^{-/-}$ mice is not due to a decrease in infectivity.

Thus, histamine appears to be involved with immunopathology caused by damaging inflammation in the brain.

Conclusions

~~Histamine~~ =

1. Decreased BBB permeability
2. Decreased recruitment of $CD4^+$ and $CD8^+$ T cells
3. Decreased ICAM-1 expression
4. Decreased IL-10, IL-4, IL-5, and IFNγ

Thus, histamine appears to be involved with immunopathology caused by damaging inflammation in the brain.

Conclusions

- Our results suggest that antihistamines may have therapeutic value in the treatment of malaria.
  - Particularly in reducing the likelihood of adverse complications.
- Interestingly, some antihistamines have been shown to potentiate antimalarial drug activity (Nakornchai *et al*. 2006)
- Although malaria vaccine development remains the central goal, alternative chemotherapy-based approaches have the potential to be highly important and are readily available.

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