

An Animal Replacement Alternative for the Investigation of Cerebro-Vascular Diseases

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Discipline of Pathology



The University of Sydney
Sydney Medical School



**Marie Bashir
Institute**

Sydney Institute for Emerging Diseases & Biosecurity

Overview

- experimental approaches
- main disease studied
- our co-culture model system
- other clinical applications

- “Le fait qu’on se soucie des animaux aujourd’hui est un signe que l’humanité progresse”
- “The fact that we care about animals nowadays is a sign that mankind is progressing”



Boris CYRULNIK

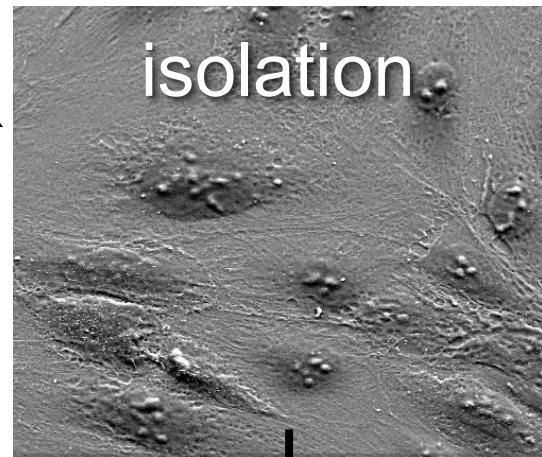
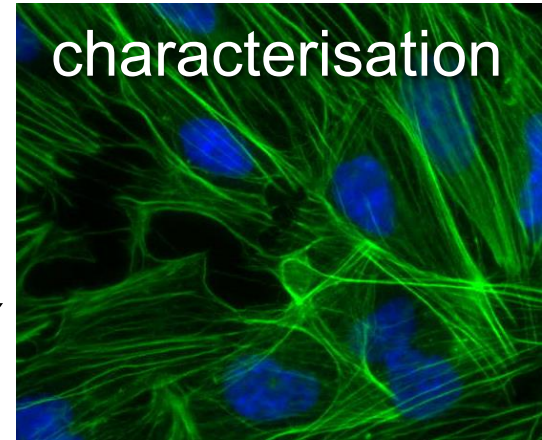
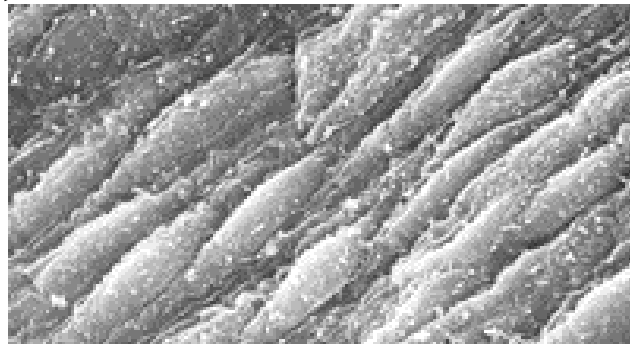
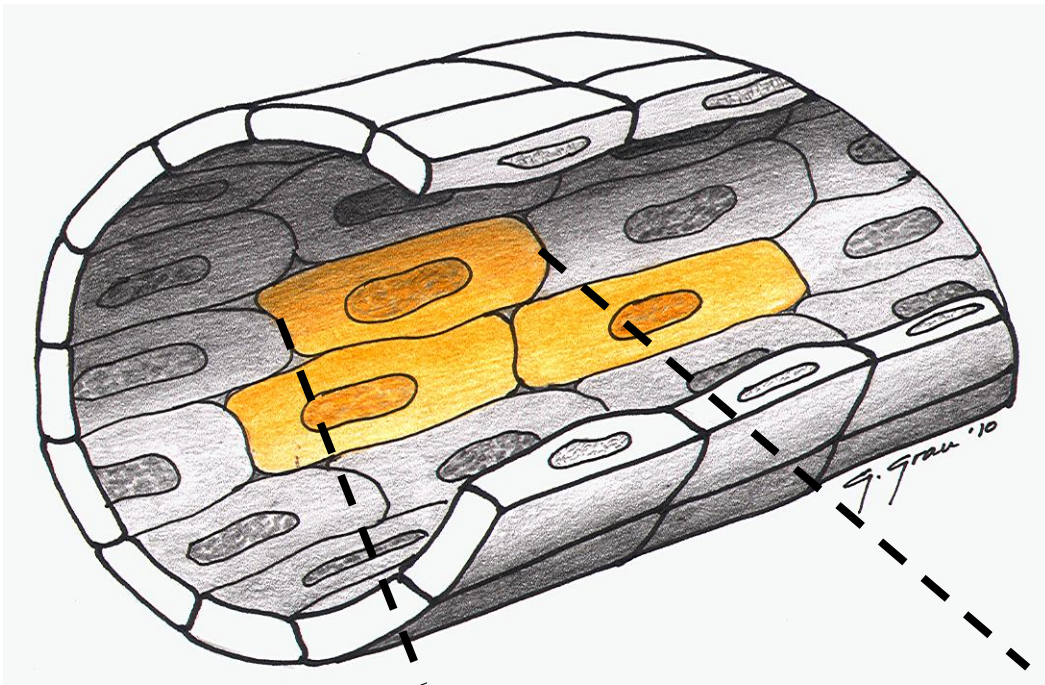
“All models are wrong, but some are useful”

George E.P. Box
(1919-2013)

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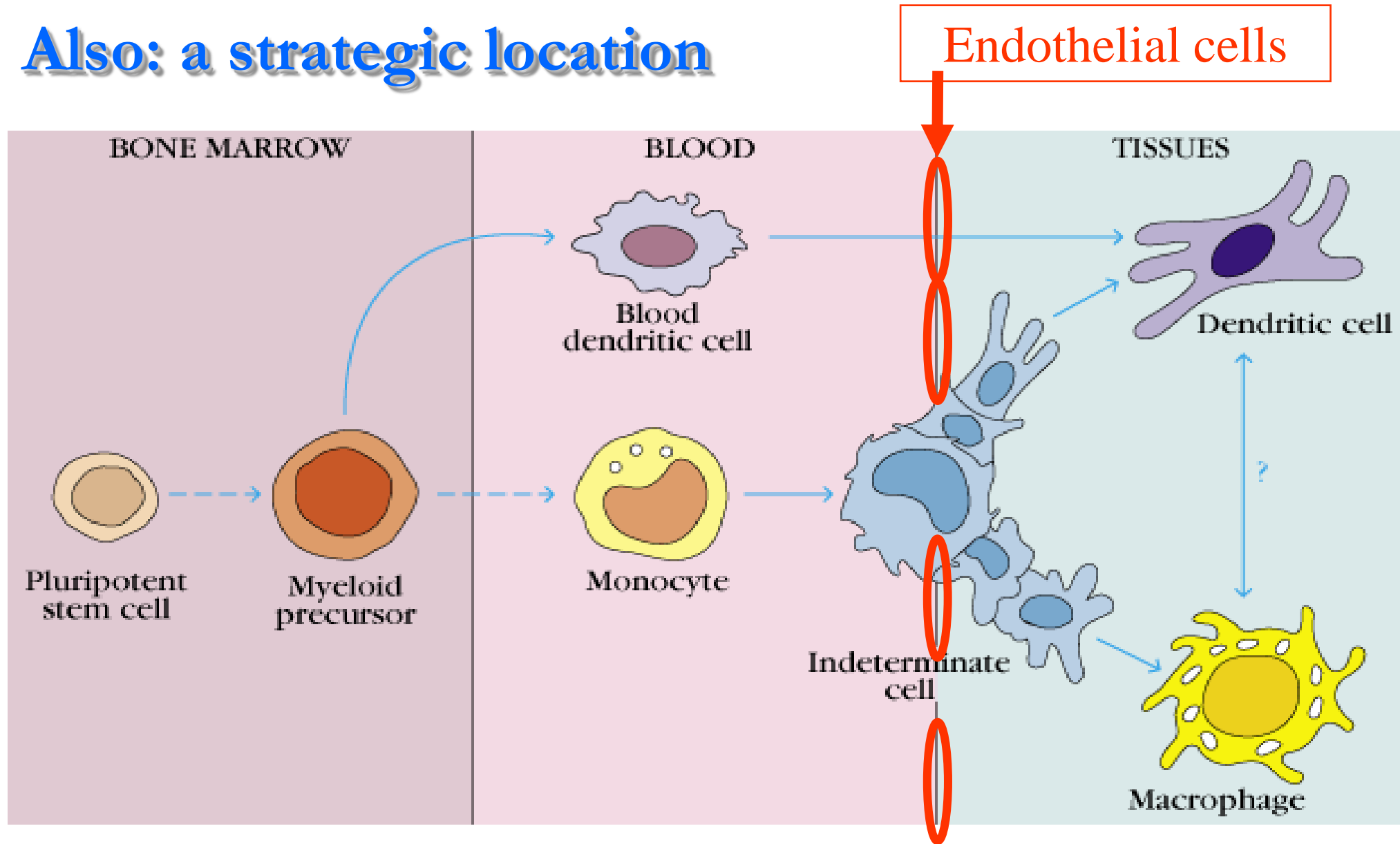
Endothelial cells (EC)



culture



Also: a strategic location



Approches expérimentales *in vivo*

observation : *immunohistopathologie*

intervention :

- perfusion de cytokines*
- modulation de la réponse immune*
- blocage de molécules d'adhérence (mAbs, souris KO)*
- déplétion en leucocytes ou plaquettes*

mécanismes des lésions



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Cerebral Malaria (CM)

Major life-threatening complication:
a diffuse encephalopathy due to untreated infection
with *Plasmodium falciparum*

Disorientation

Coma

Delirium

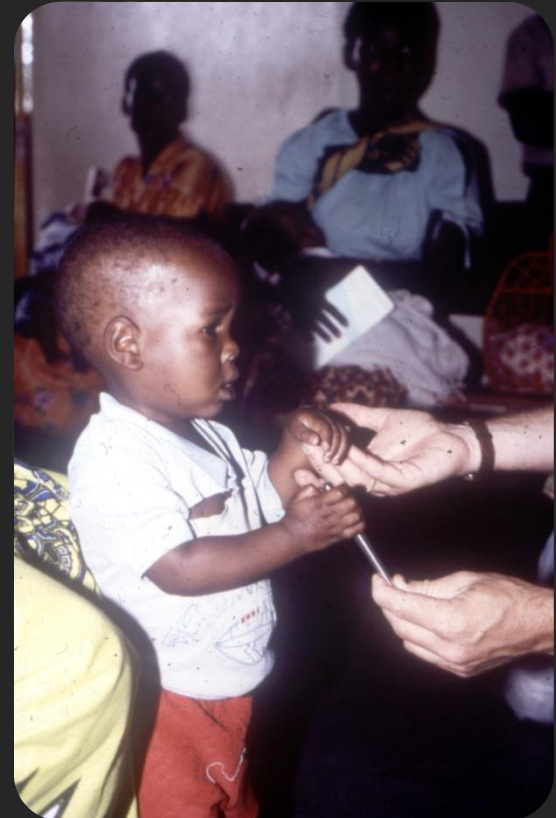
Seizures

Severe
metabolic acidosis



Multisystem dysfunction

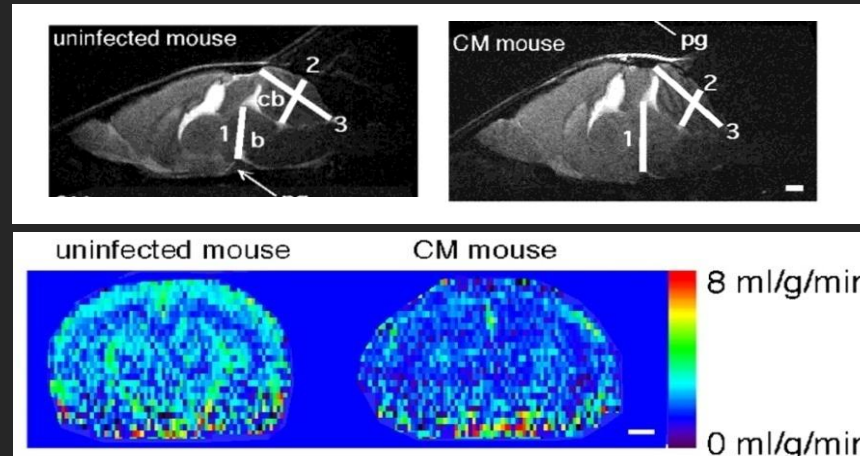
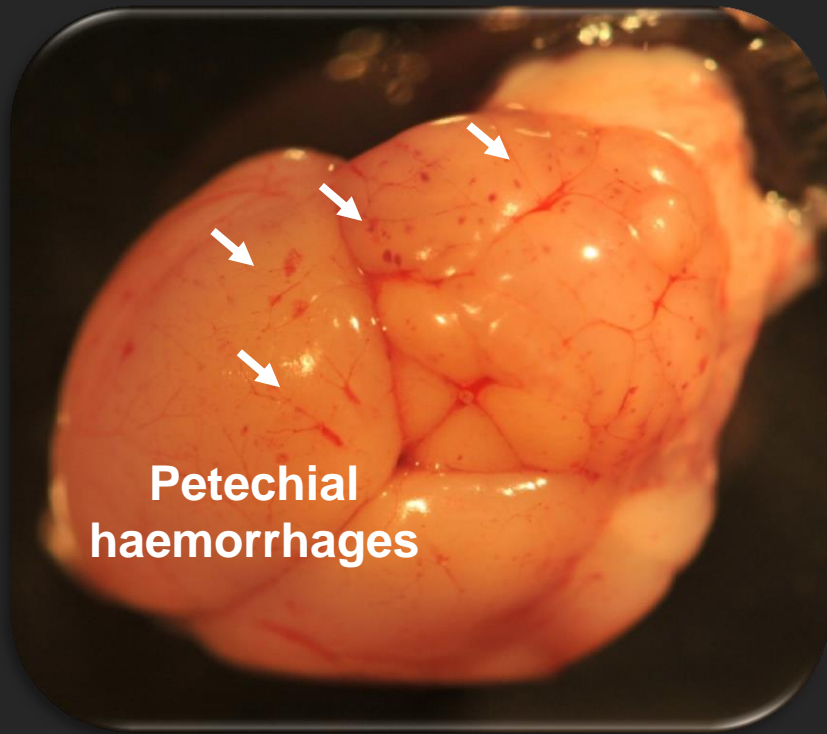
up to 30% mortality rate



Neurological sequelae

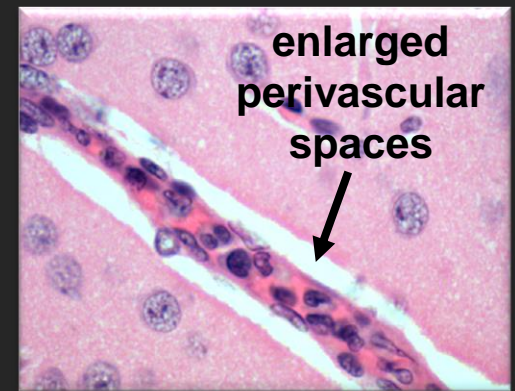
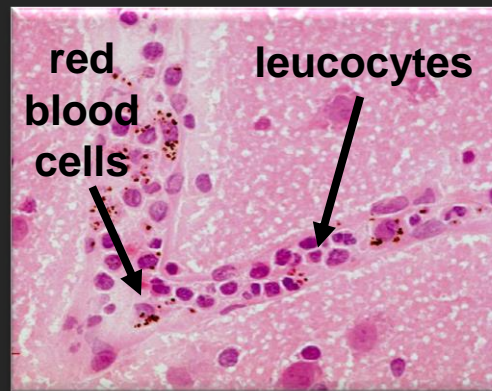
Experimental cerebral malaria

↑↑↑ pro-inflammatory cytokines
(TNF, IFN- γ , LT)



brain
oedema

engorgement of capillaries +
enlargement of perivascular spaces



Cerebral malaria (CM) pathogenesis: remains incompletely understood

Current approaches for the study of CM



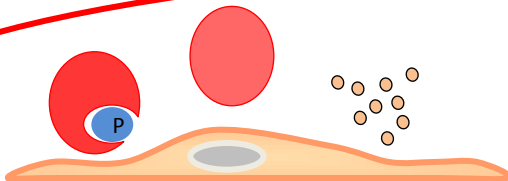
Clinical studies in endemic areas



Ex vivo – post-mortem histopathology on human brain tissue



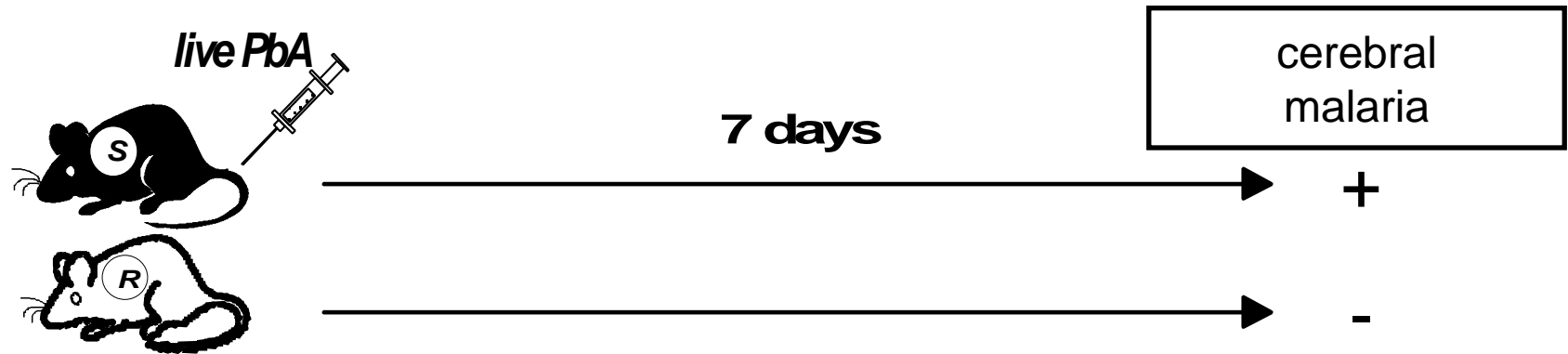
In vivo – animal models



In vitro - modelling of CM lesion

I

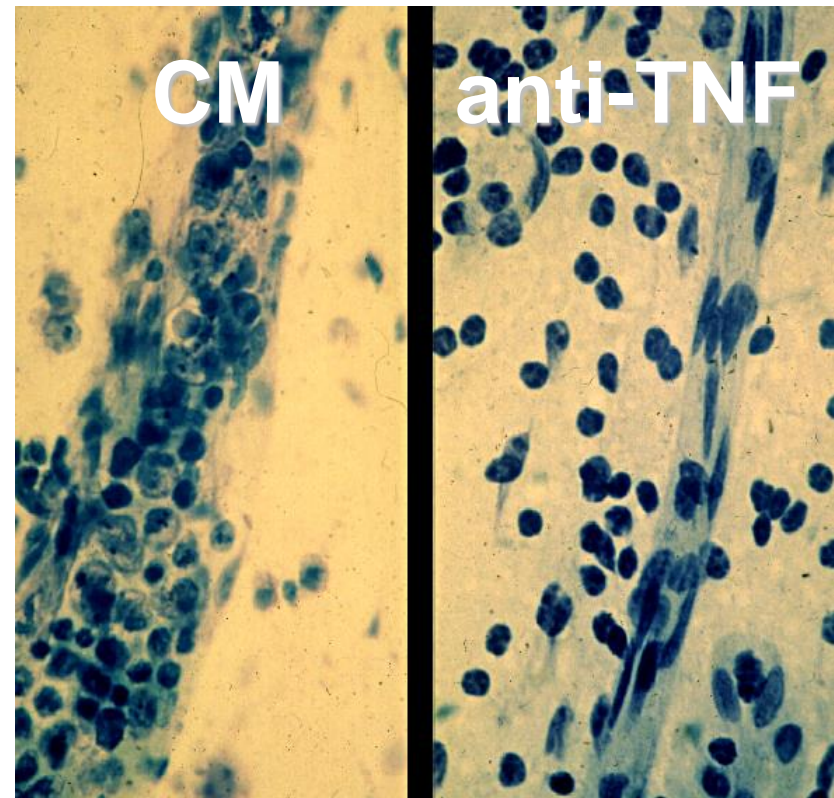
CM is a strictly T-cell dependent pathology



II

TNF is an essential mediator in CM

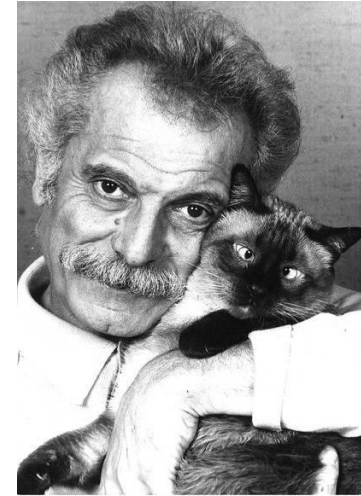
- high serum levels during CM
- its neutralisation prevents CM
 - antiserum
 - mAb
 - pXF
- induces CM in resistant mice
- absence of CM in
 - transgenics for sTNFR
 - TNF knock-outs



“Sans technique, le genie n’est rien qu’une sale manie”

“Without technique, genius is nothing more than a lousy habit”

- **RESPECT**
- **CARE**
- **MINIMUM BURDEN**
- **...**



GD et Kiko (Photo Schabus)

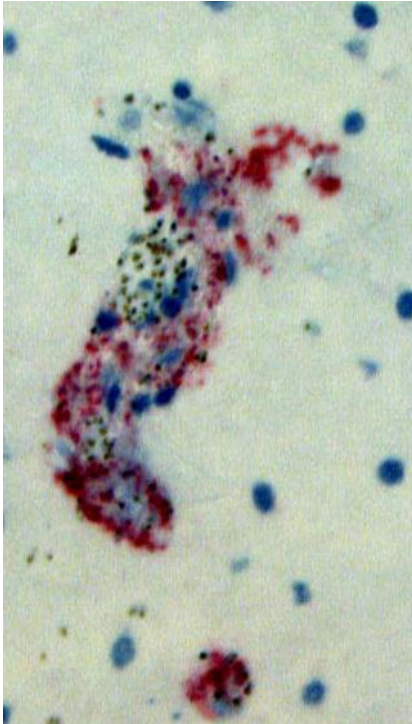
Georges BRASSENS

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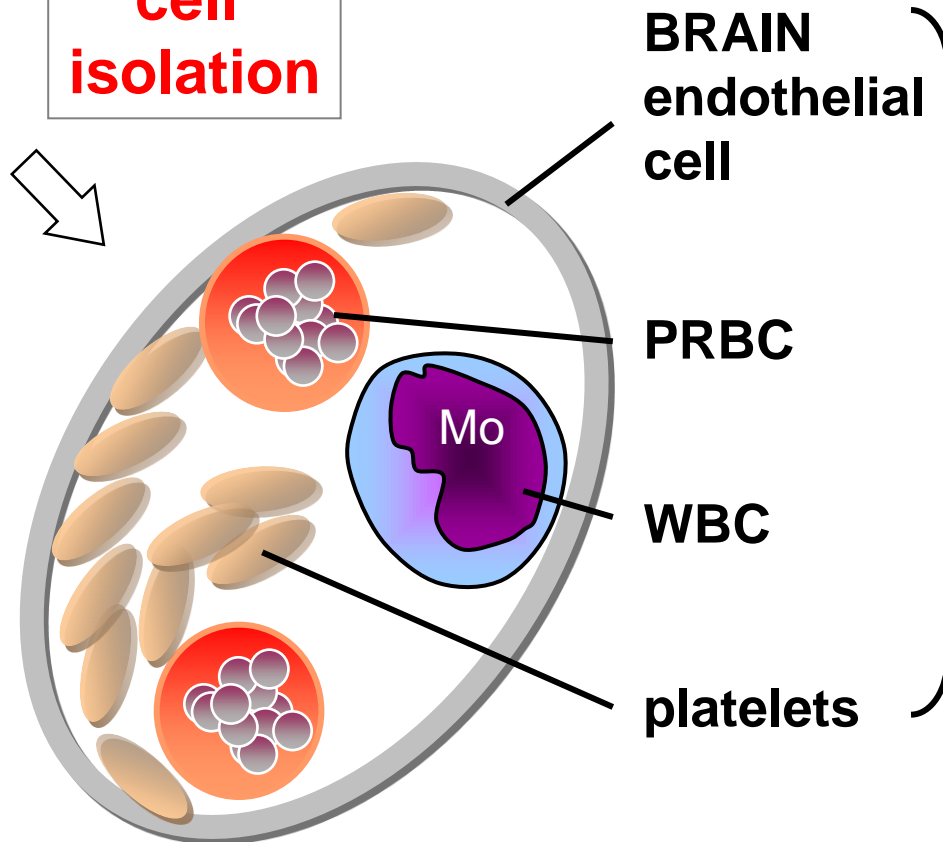
1

**Immunostaining
(Malawian patient)**



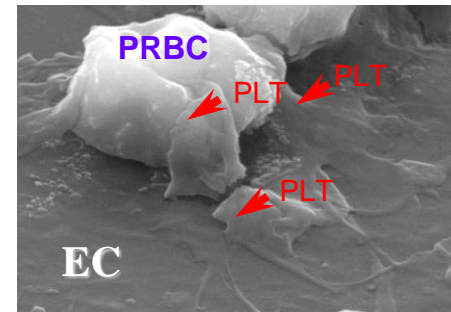
2

**cell
isolation**

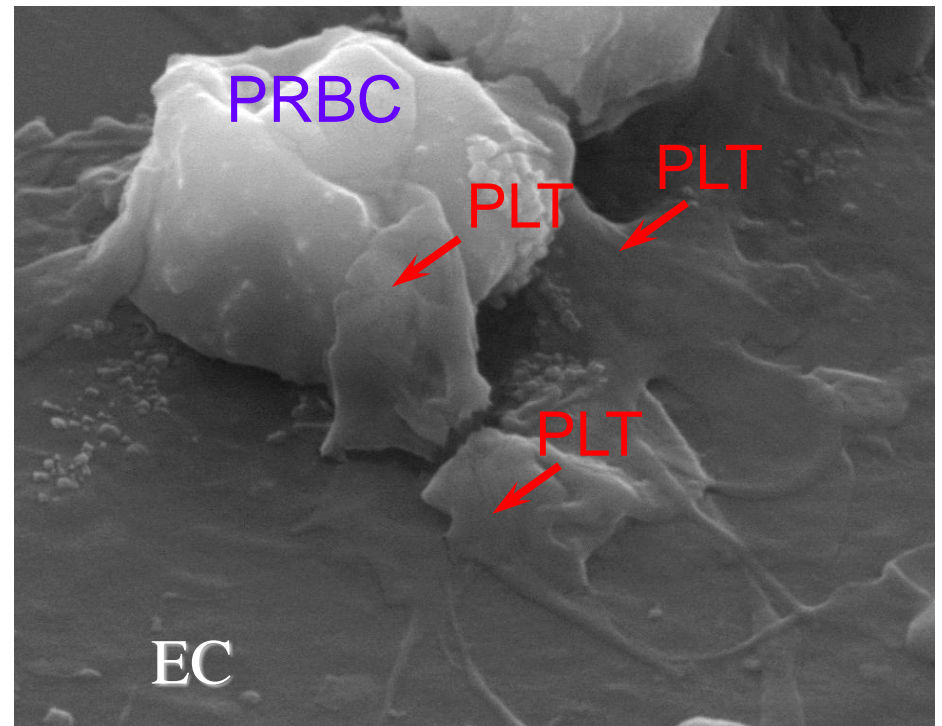
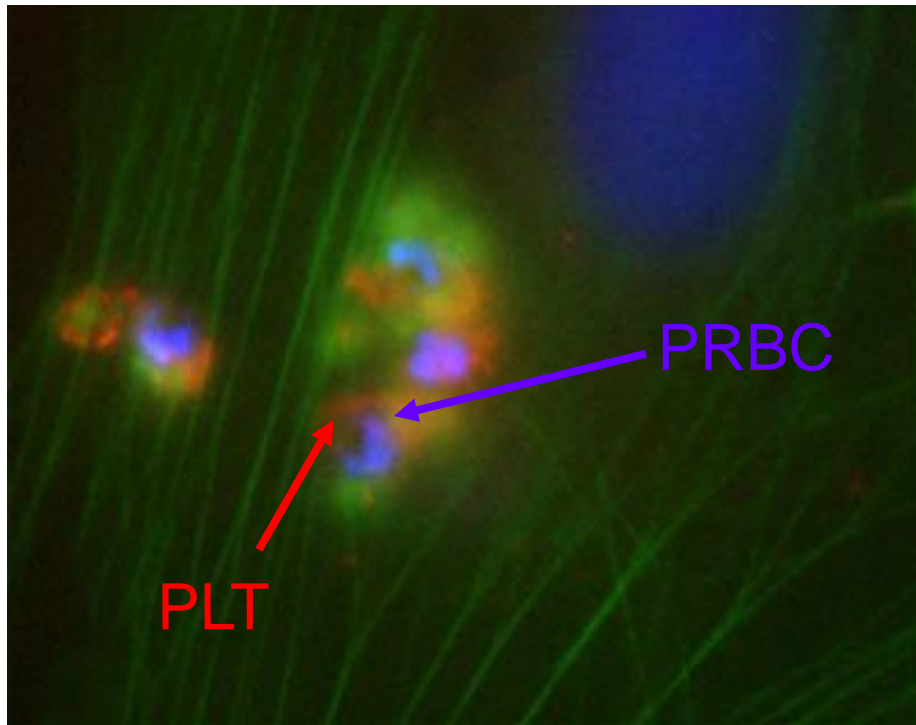


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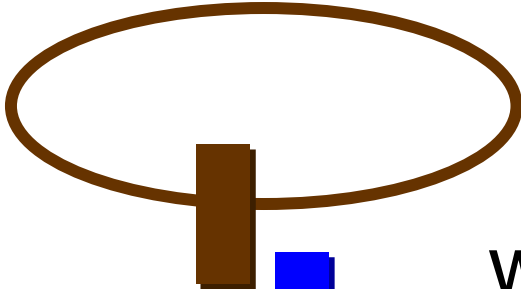
co-cultures



In vitro evidence for a role of platelets in PRBC-EC bridging



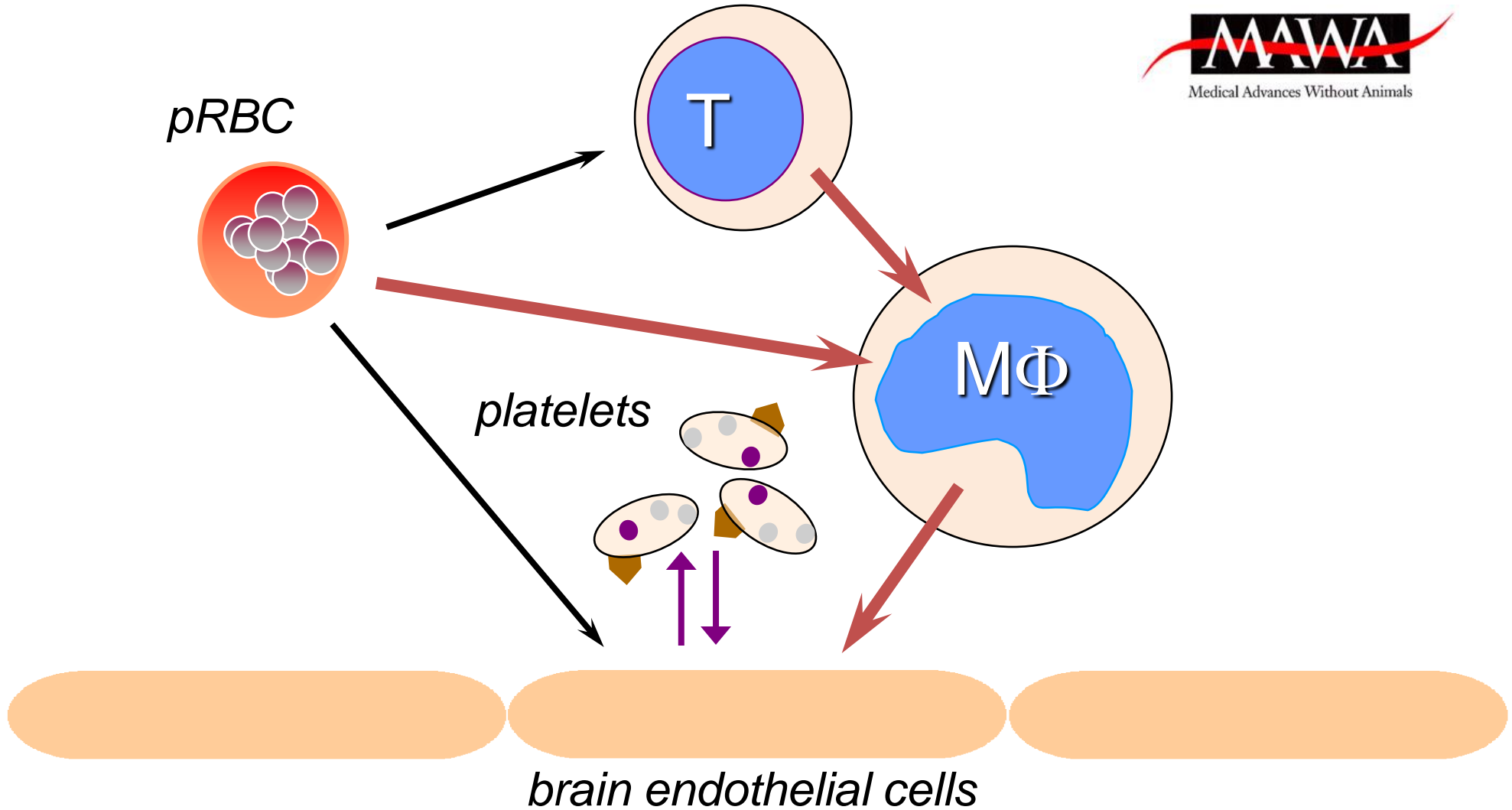
platelet



which molecules ?

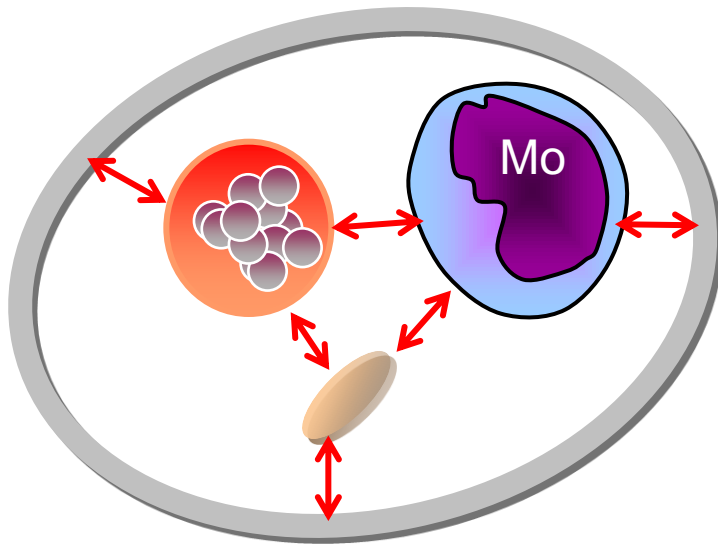
brain endothelial cell

Tri-partite, quadri-partite cultures

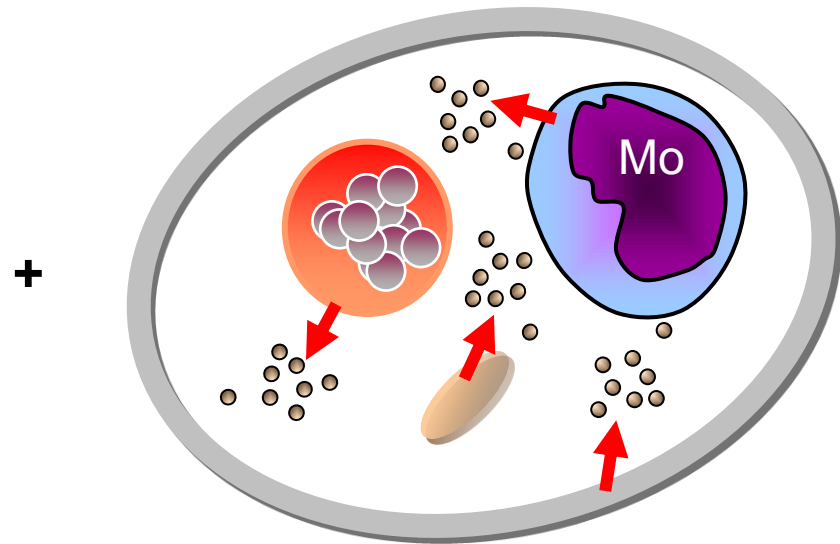


Modelling cerebral malaria *in vitro*: 2 levels of complexity

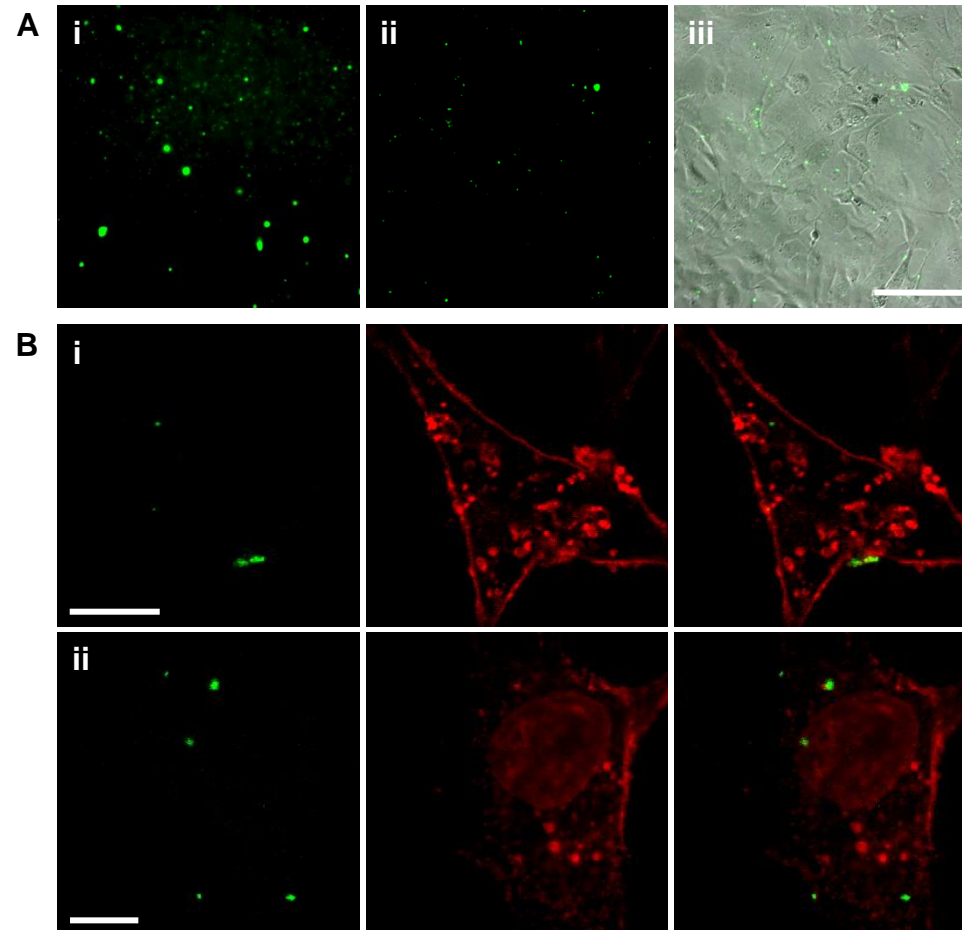
cell-cell interactions



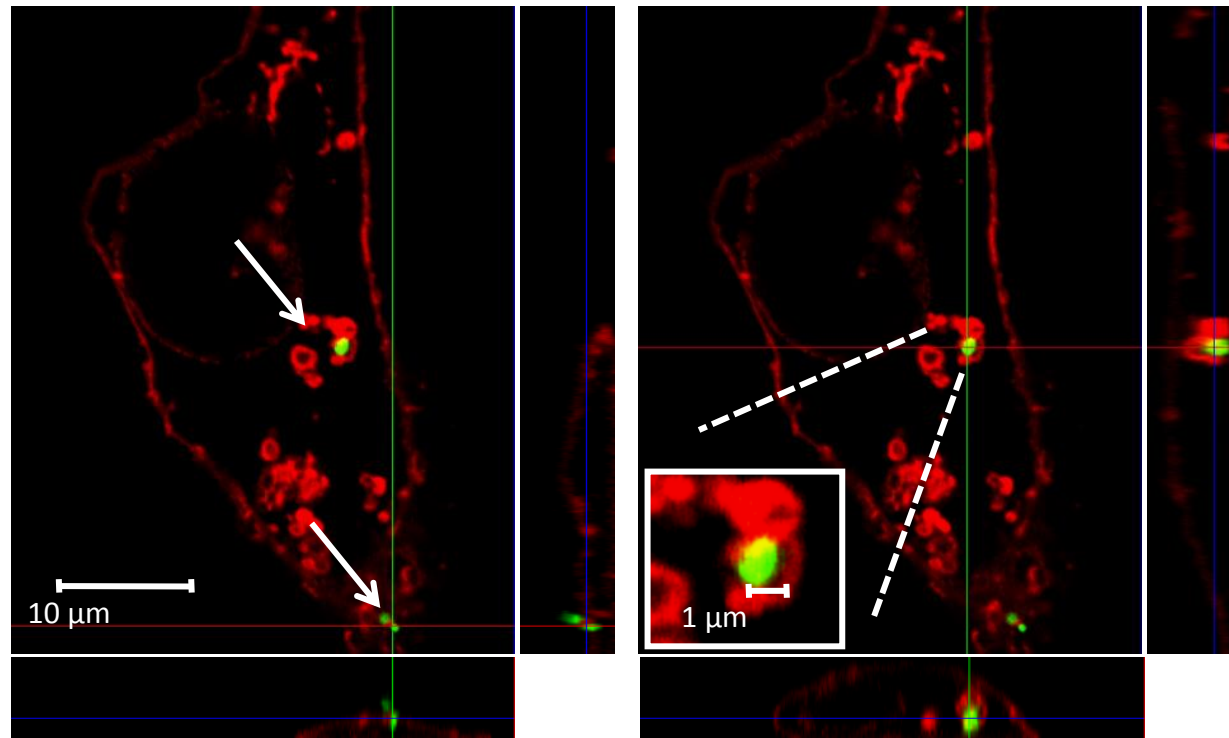
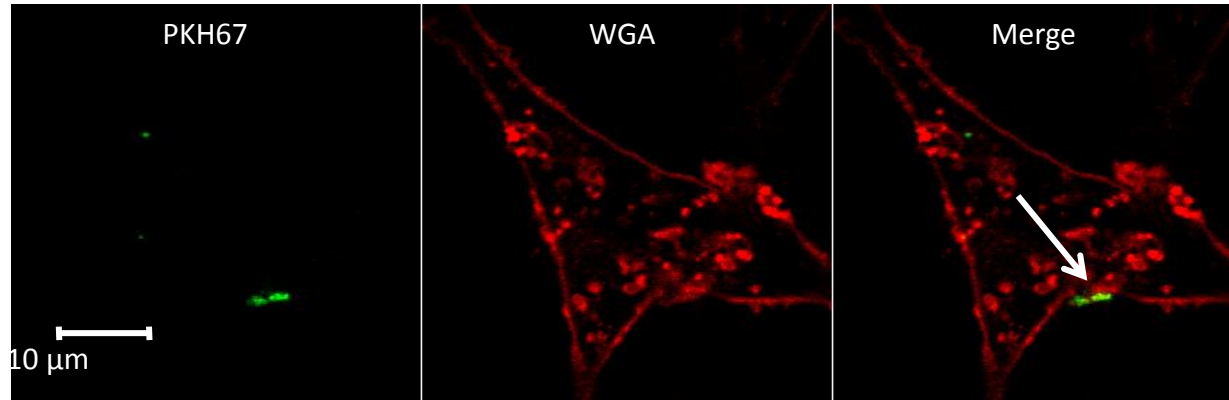
cell-derived microparticles



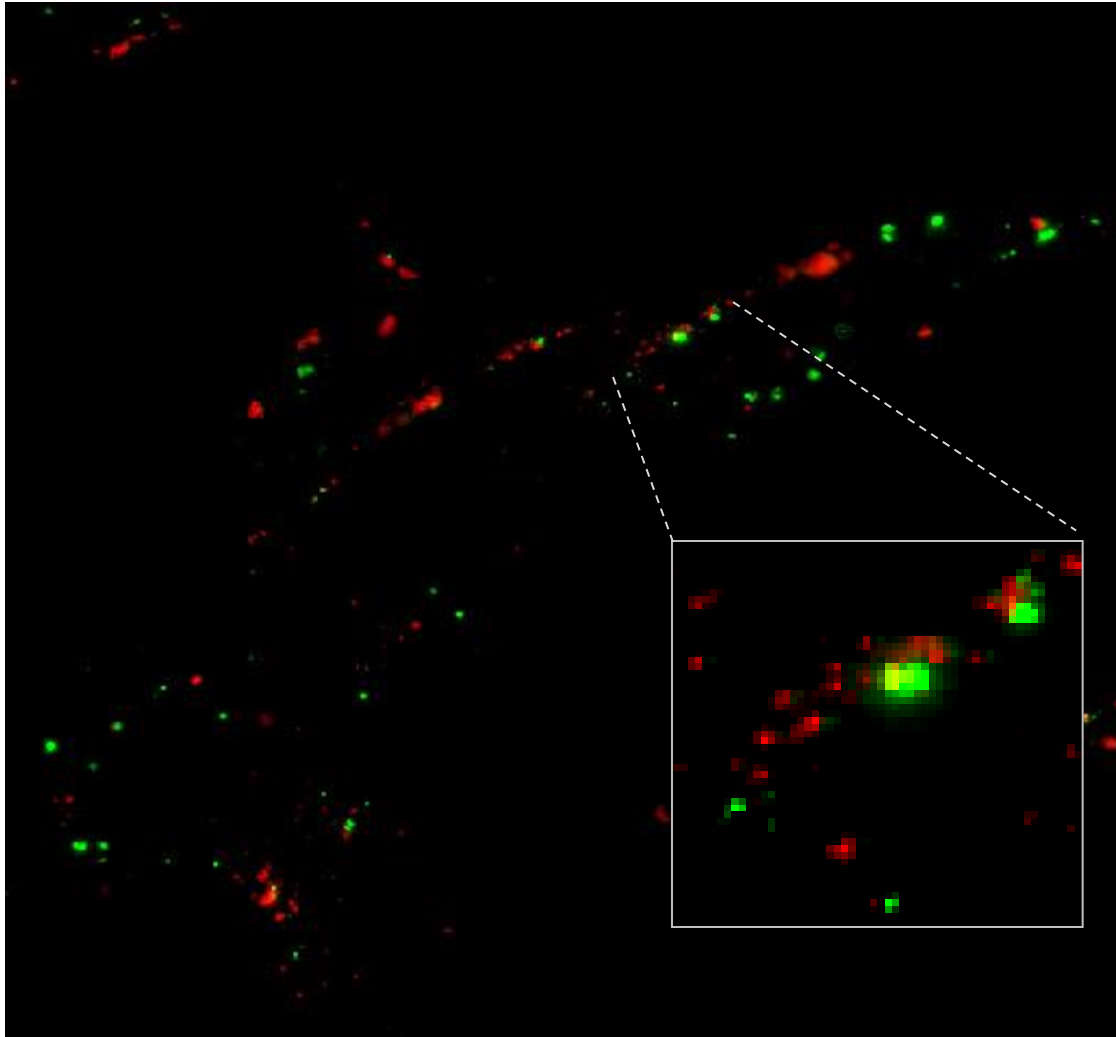
Platelet MP (PMP) bind to and are internalised in brain EC



Compartmentalisation of PMP in brain EC



PMP bind to and transfer platelet antigens on brain EC surface



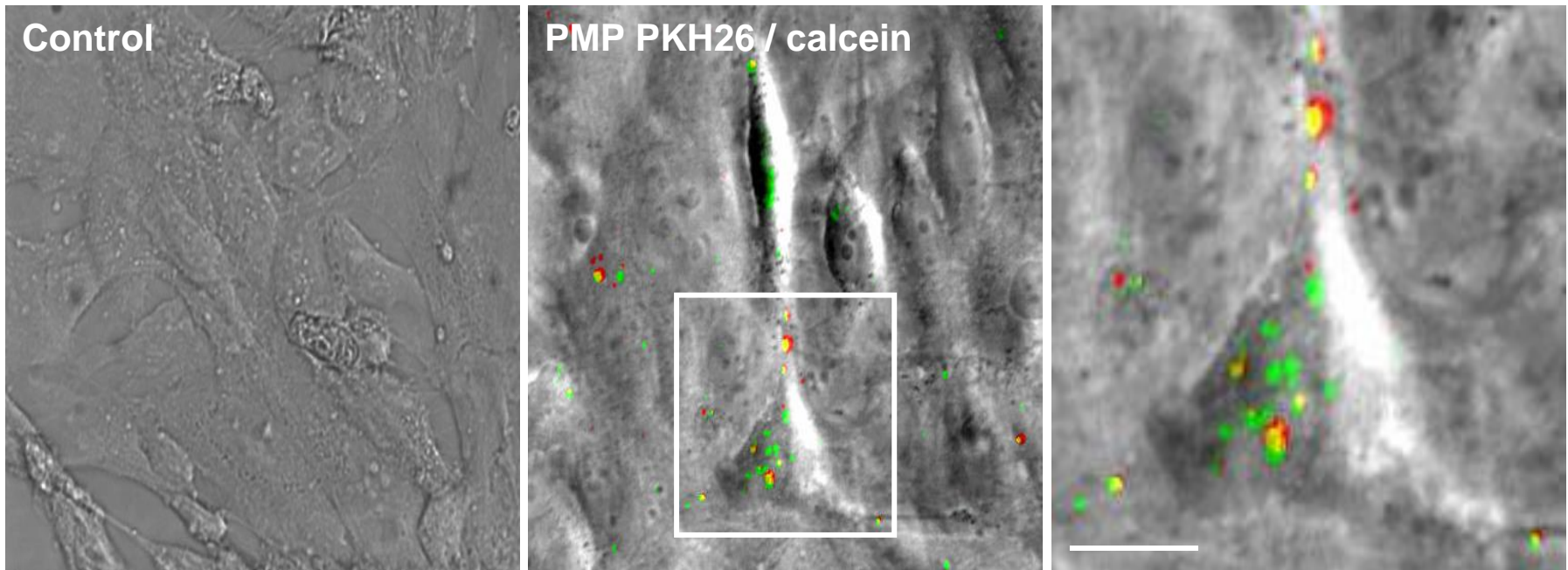
PMP membrane PKH67

CD36 / GPIV



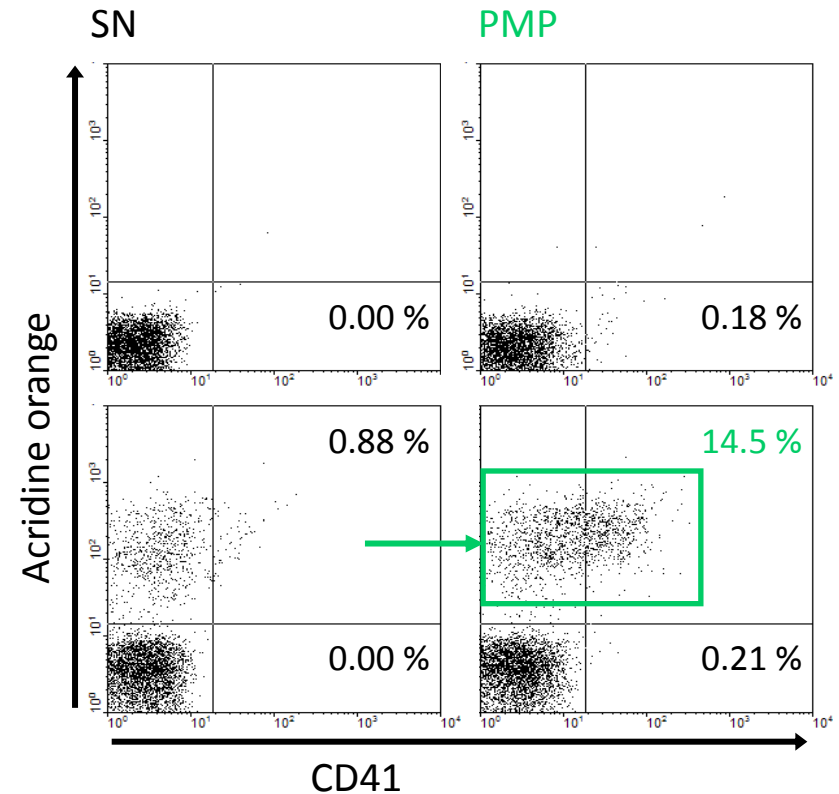
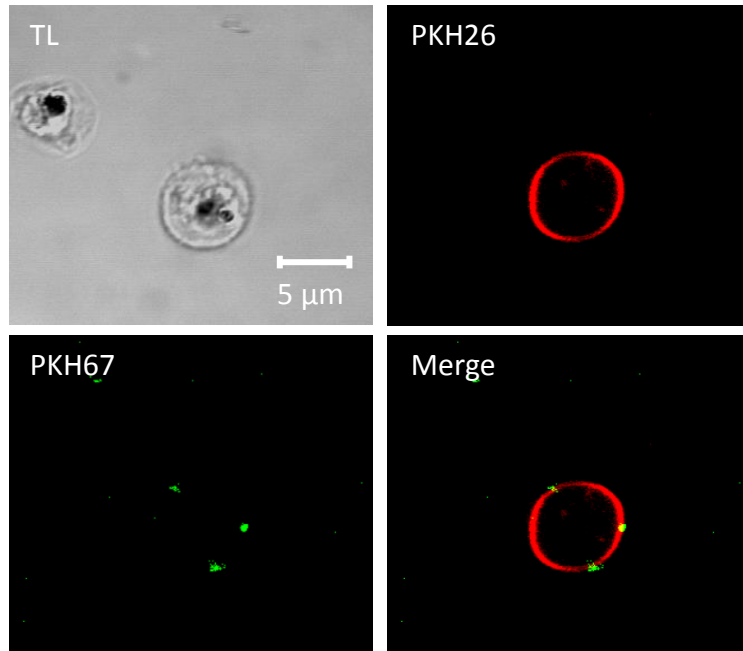
New surface phenotype
for brain EC

PMP membrane and content have a different fate after contact with EC membrane

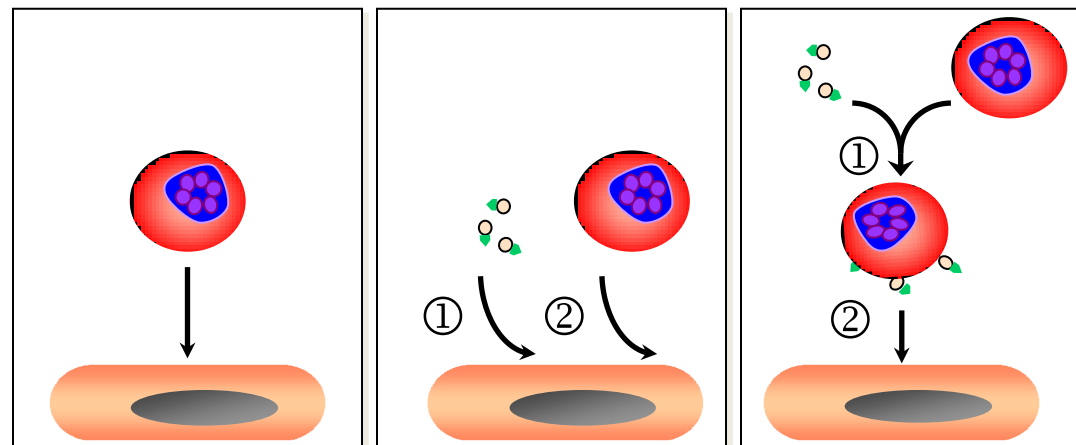
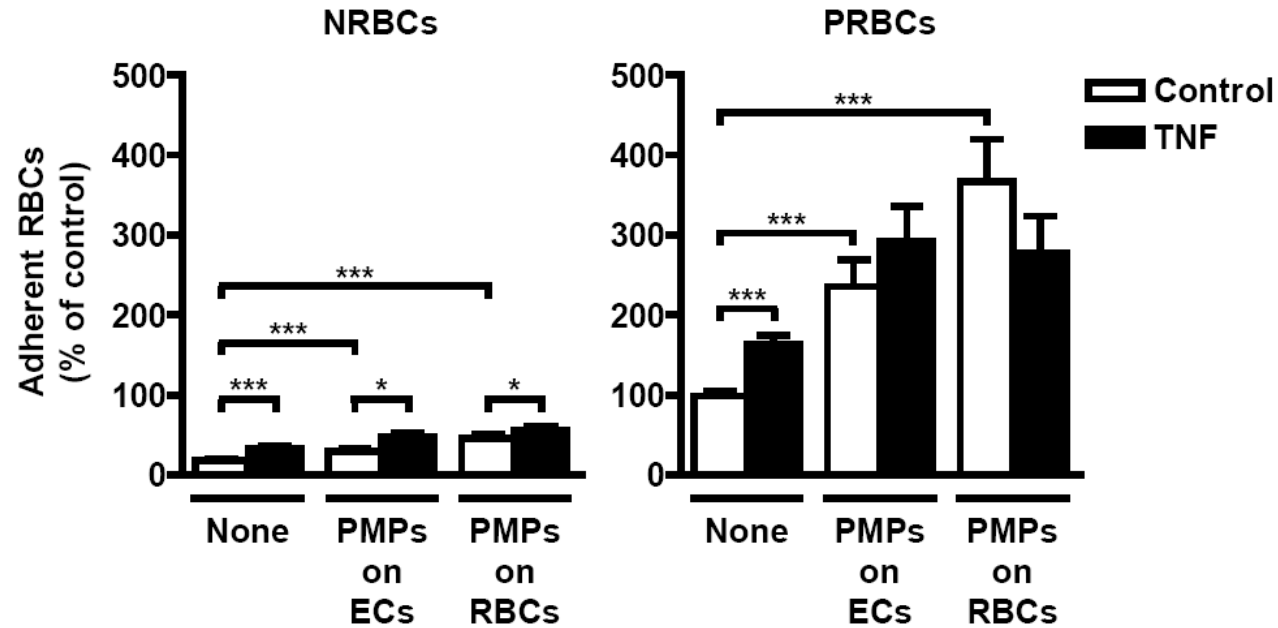


Membrane : PKH26
Content: calcein-AM

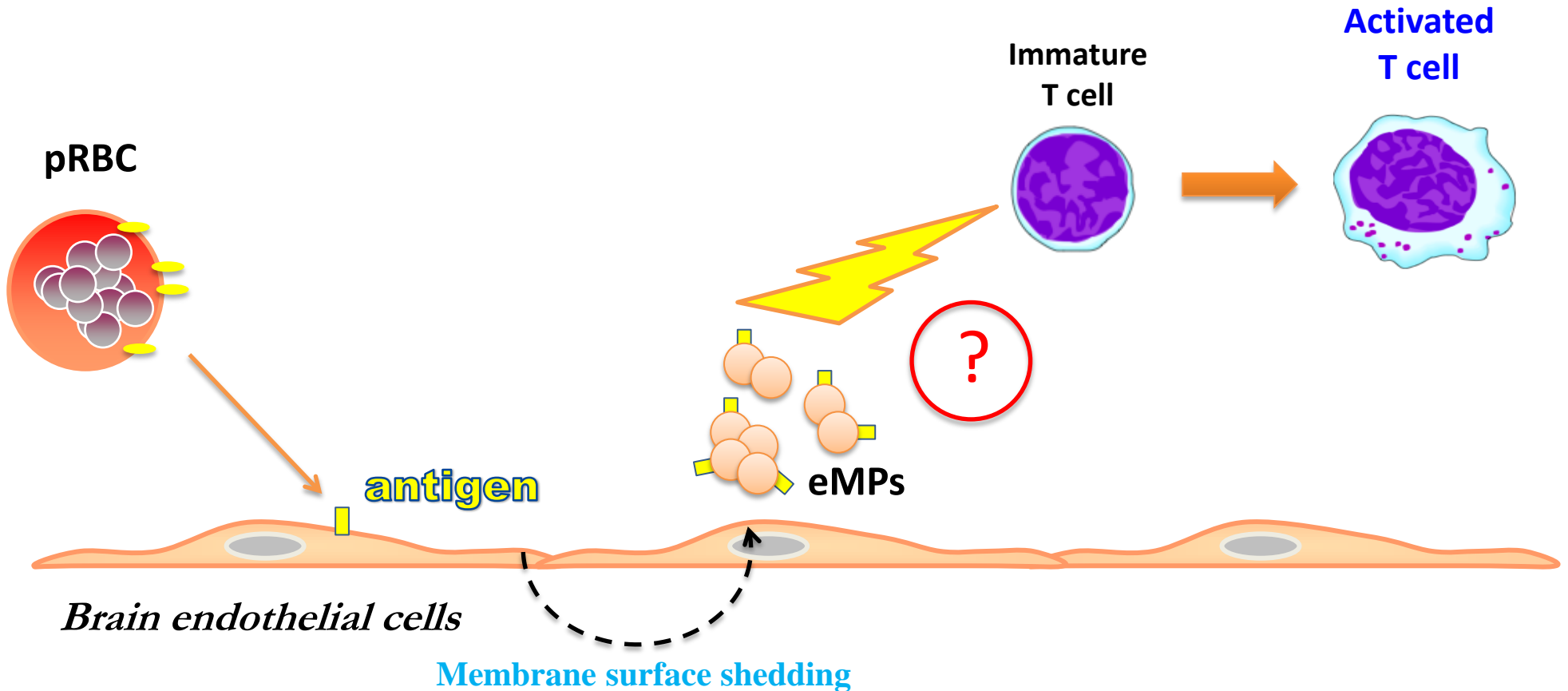
PMP bind and transfer platelet antigens to PRBC



PMP enhance PRBC cytoadherence on brain EC



Are endothelial MPs immunomodulatory?



Overview

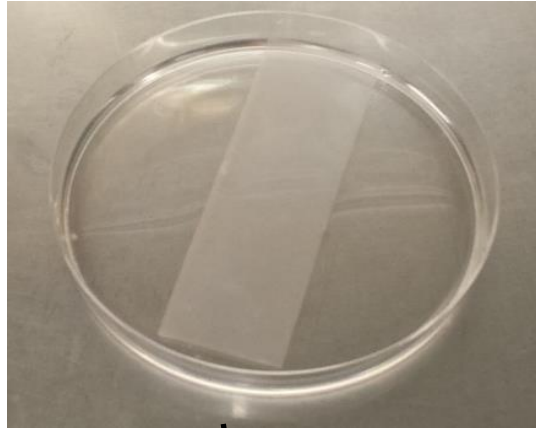
- experimental approaches
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Novel applications of our brain endothelium co-culture model

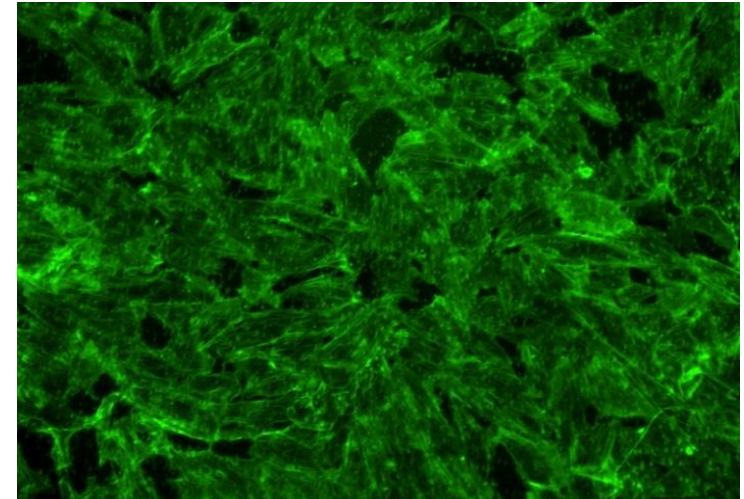
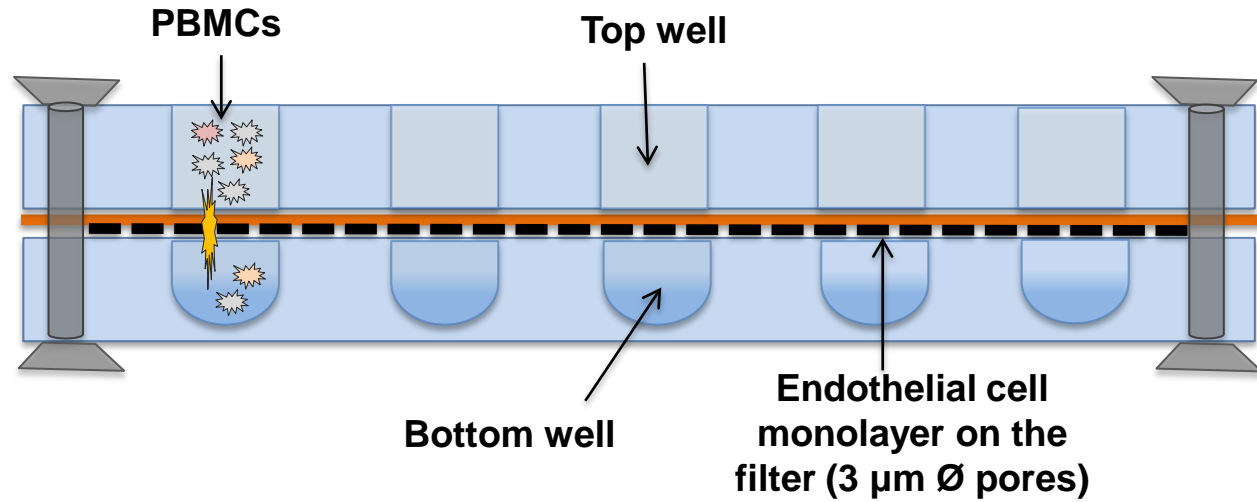
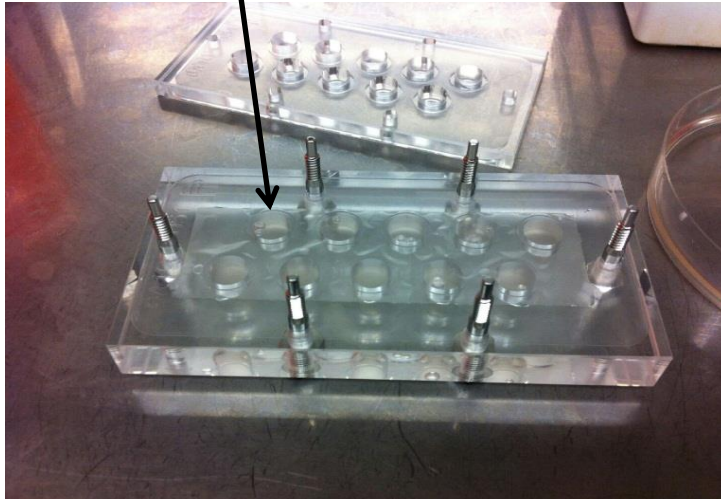
- Multiple sclerosis (*coll. Prof. S. Hawke*)
- Septic shock
- Cryptococcal meningo-encephalitis (*coll. Prof. T. Sorrell*)
- Viral meningitides (*coll. Prof. N. King*)

Multiple Sclerosis

Trans-endothelial migration (TEM) *in vitro* model

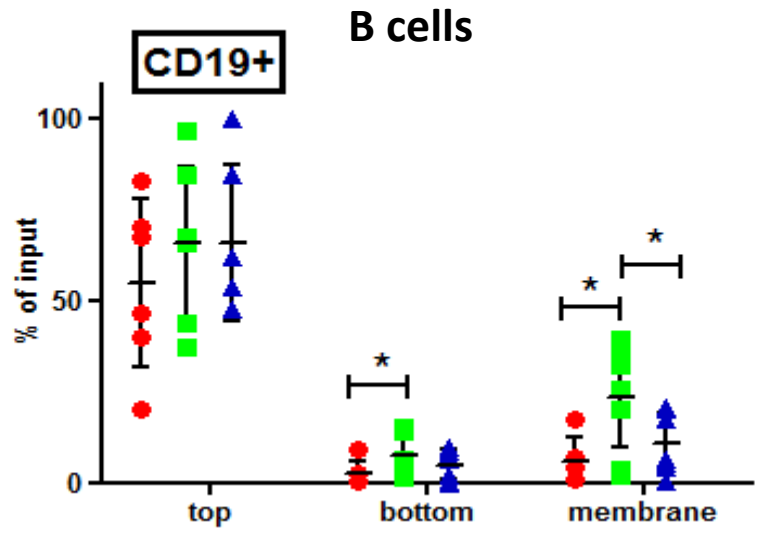
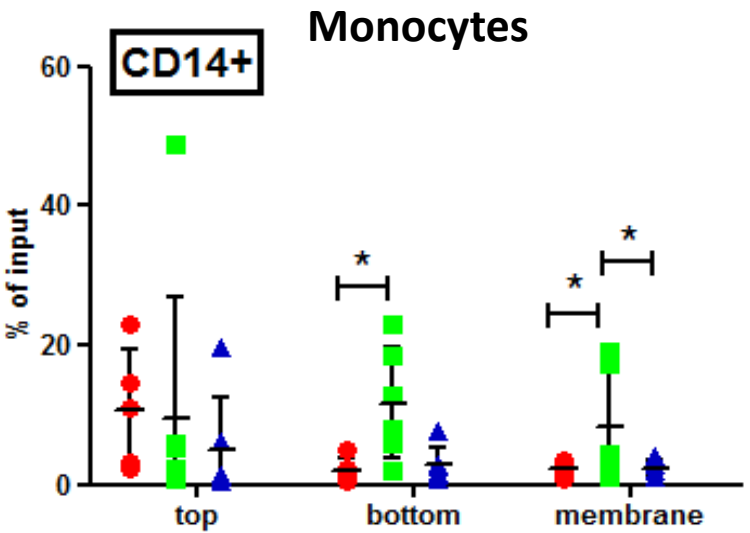
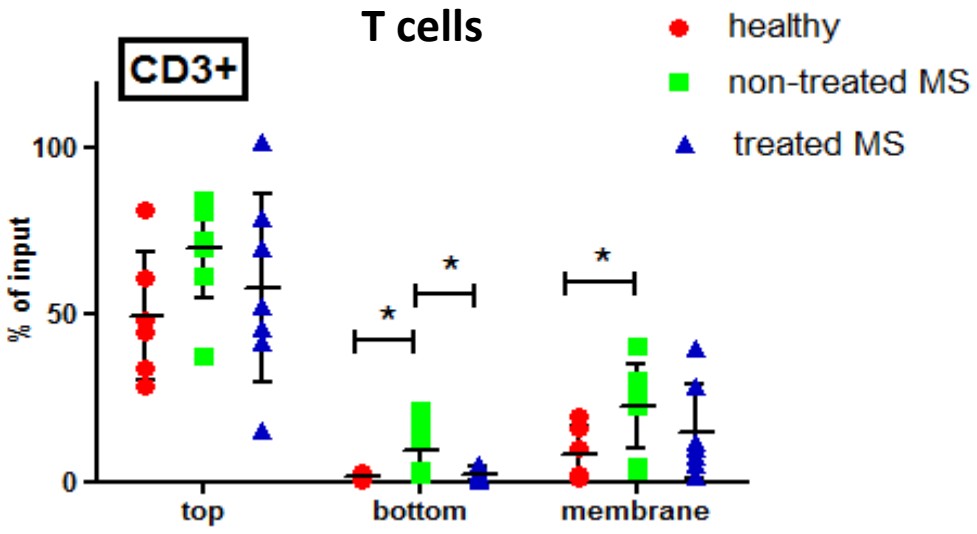
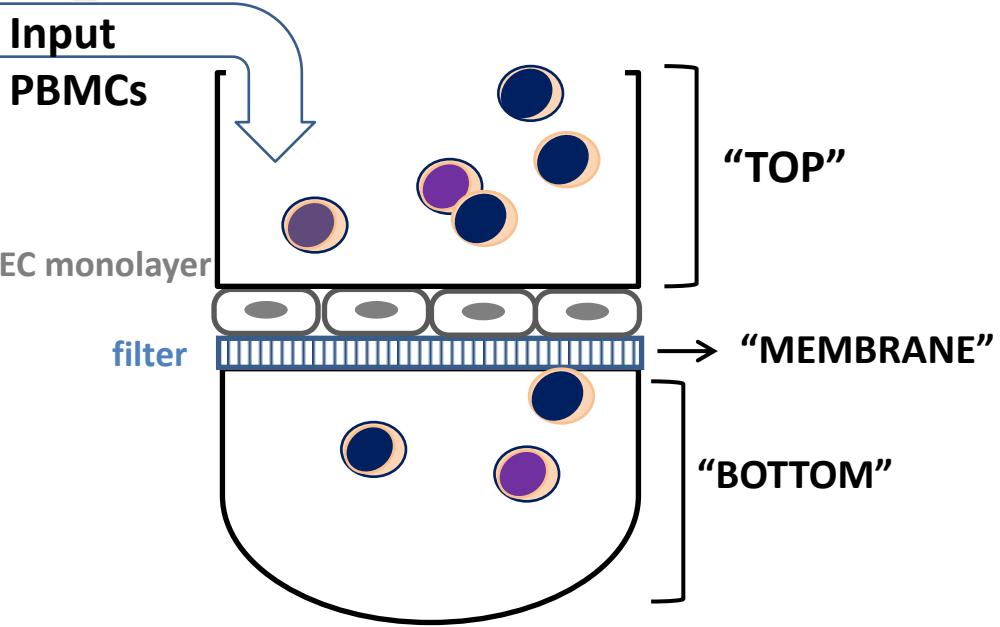


TNF + IFN- γ
stimulation



Human brain microvascular endothelial cell line hCMEC/D3, on polycarbonate filters

Fingolimod reduces transmigration of PBMCs from MS patients across endothelium in a BBB *in vitro* model



Antibody panels for flow cytometric analysis of leucocyte (PBMC) subsets.

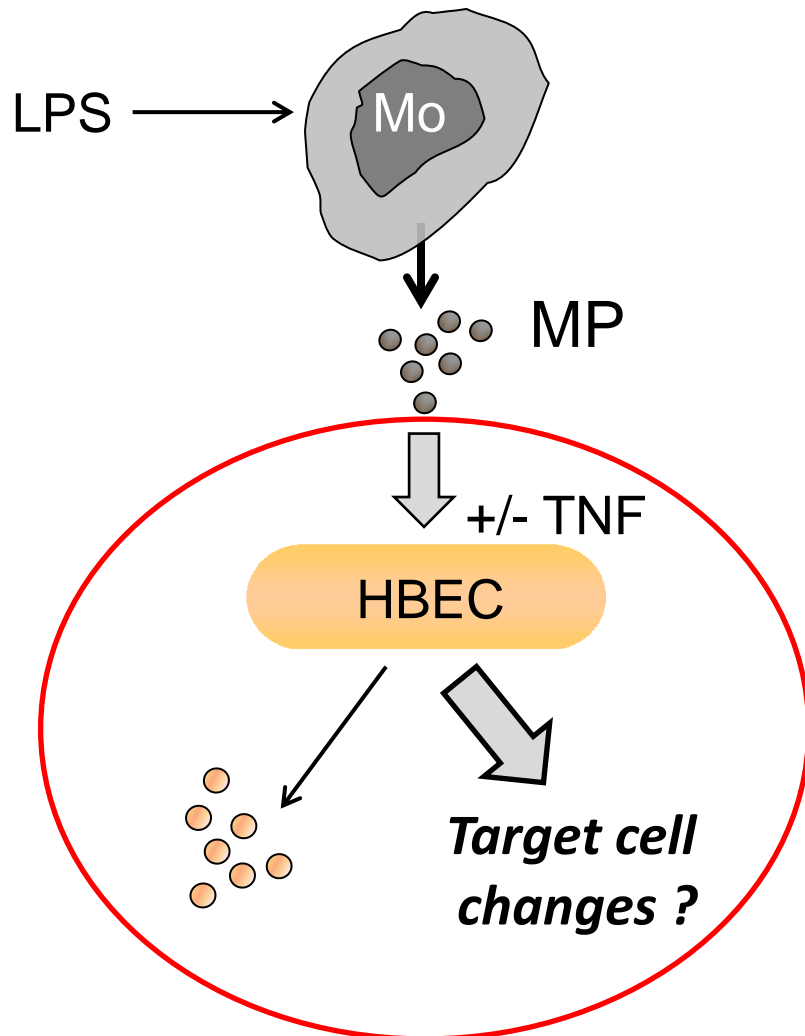
	405 Excitation		488 Excitation					633 Excitation		
	Pacific Blue	Krome Orange	FITC	PE	ECD	PC5.5	PC7	APC	APC-AF700	APC-AF750
B reg	CD4	CD3	CD19	CD86		CD80	CD274	CD62L	CD8	CD27
Activation	CD4	CD3	CD19	CD14		CD154	CD28	CD62L	CD8	CD27
Adhesion	CD4	CD3	CD11a	CD44 or CD40		CD56	CD25	CD62L	CD8	CD49d
NK, NKT and Th17	CD4	CD3	CD16	CD161		CD56	CD25	CD146	CD8	CD45RA

Conclusions / TEM in MS patients

- **PBMCs from non-treated MS patients adhere and migrate more efficiently**
- **Fingolimod**
 - **reduces TEM of T cells, B cells and monocytes towards the levels of healthy controls**
 - **might act on leucocytes, additionally to its effect on endothelial S1PR**

Septic Shock and the blood-brain barrier

microparticles in sepsis



- Do LPS-induced monocytic MP (mMP) functionally differ from MP released from resting cells?
- Do mMP display pro-inflammatory / procoagulant properties ?
- What are the effects of mMP on endothelium integrity?

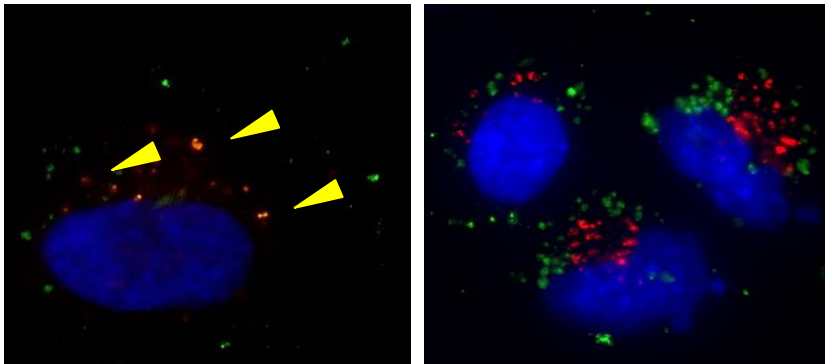


LPS-induced monocytic MP partially co-localise with endothelial lysosomes

Early endosomes

45 min

2 h

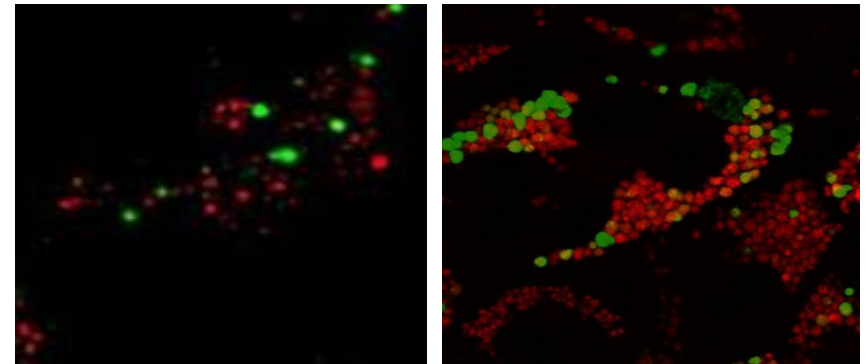


moMP: PKH67 / **EEA-1**

Lysosomes

45 min

2 h

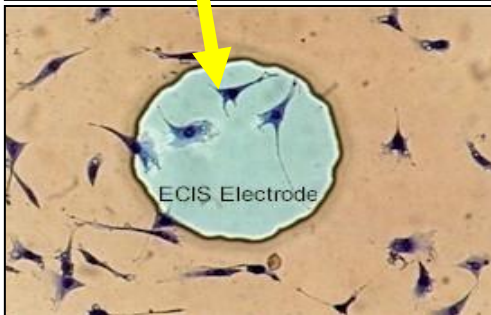
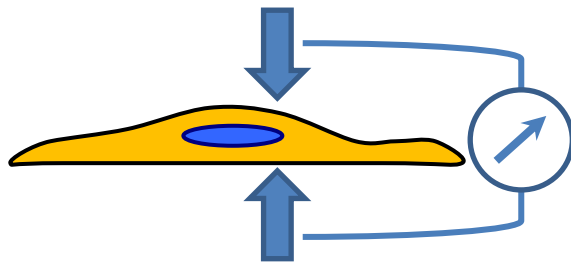


moMP PKH67 / **LysoTracker**

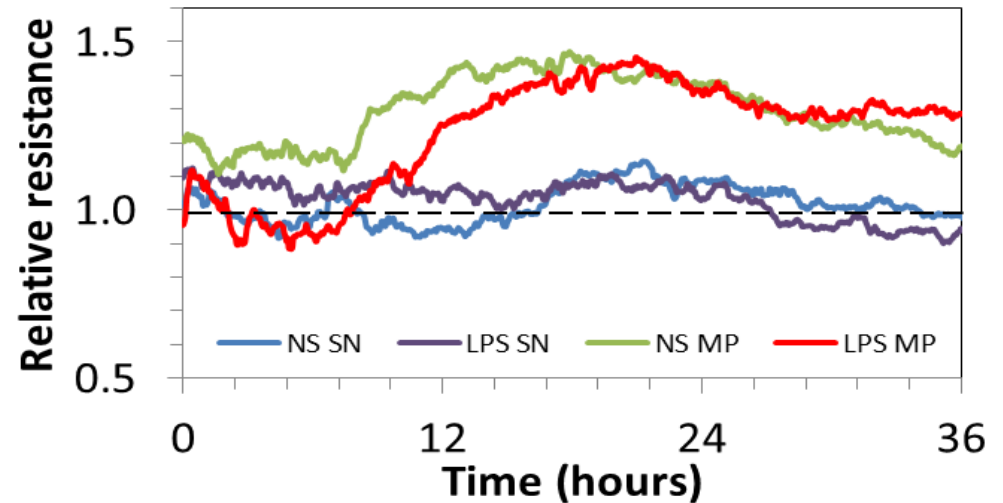


Beryl WEN

Effect of LPS-induced monocytic microparticles on endothelial integrity

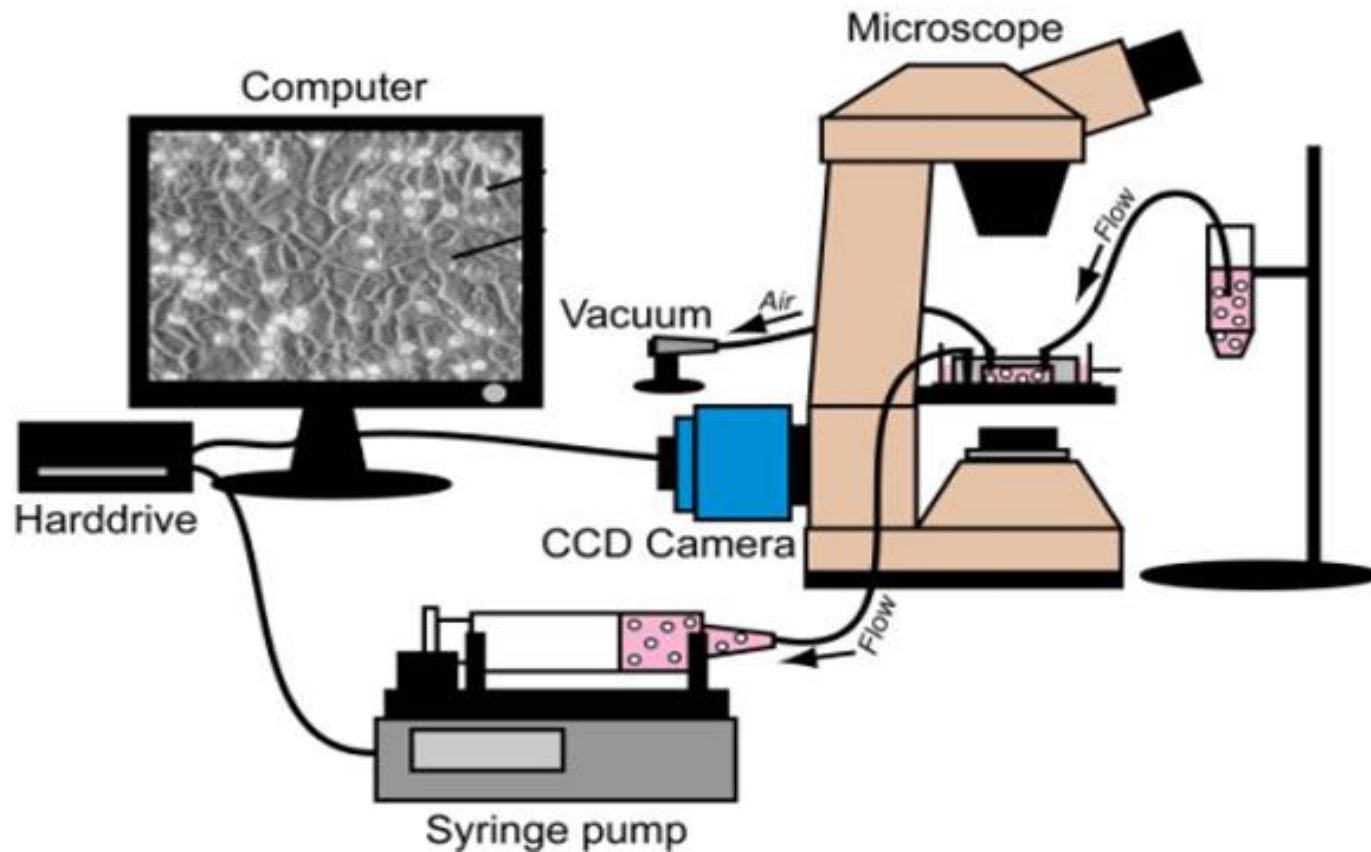


Resting EC

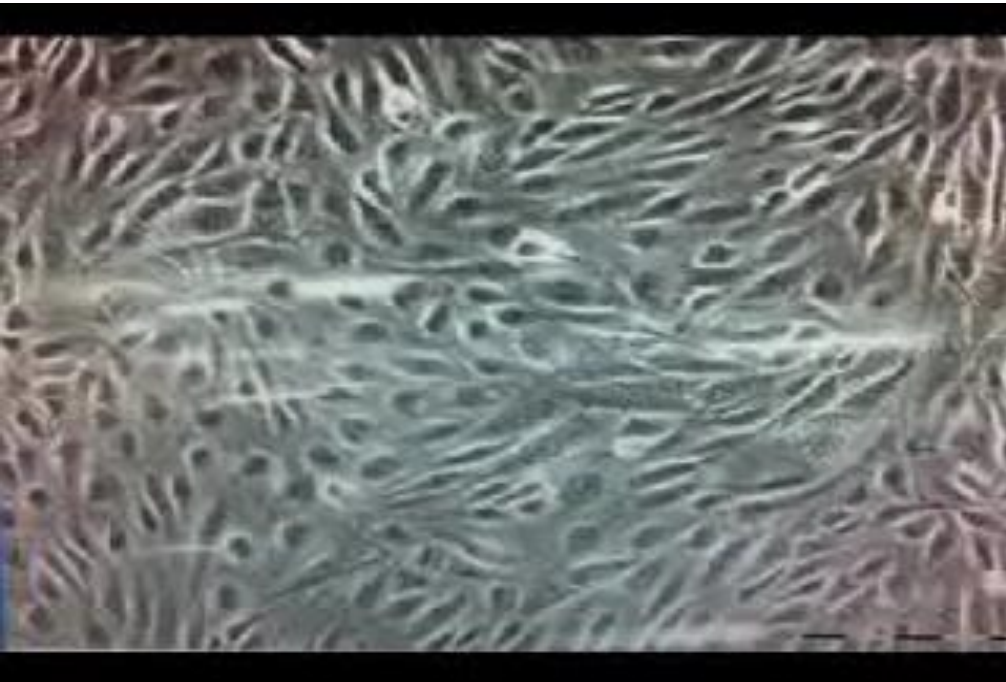


Cryptococcal Meningitis

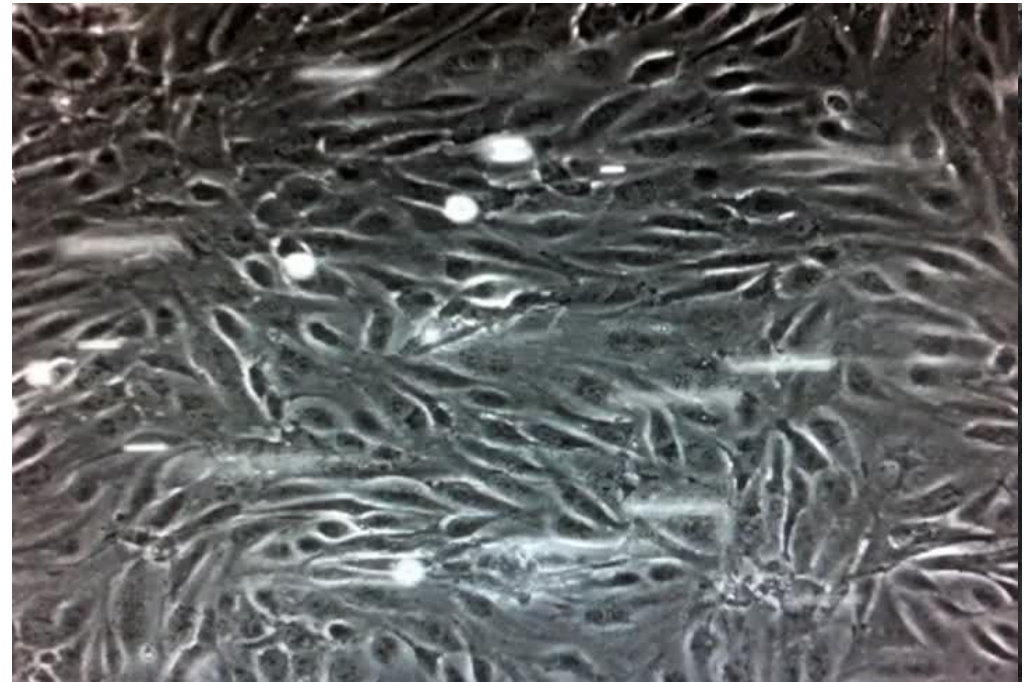
Flow chamber: to explore cells in movement



Effect of TNF on binding of phagocytosed cryptococci to brain endothelium

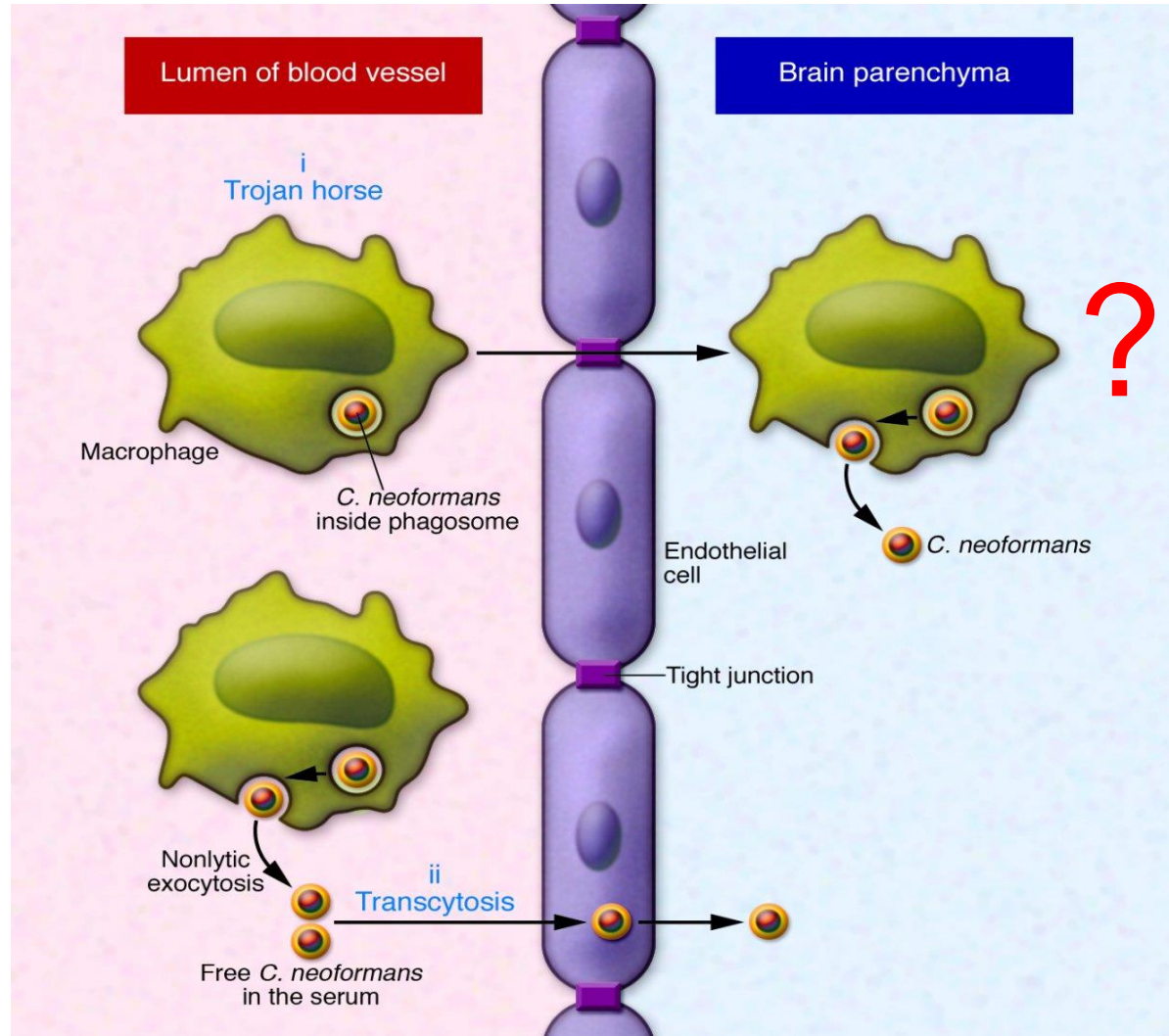


Resting endothelium



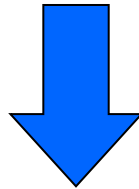
TNF-activated endothelium

Mechanisms of cryptococcal passage



Conclusion

Better knowledge



Better treatment(s)



The University of Sydney Australia



Vascular Immunology Unit

Valéry Combes
Fatima El-Assaad
Dorothee Faille
Sharissa Latham
Beryl Wen
Anna Zinger
Simon Hawke
Georges E. Grau

Molecular Immunopathology

Helen Ball

Andrew Mitchell

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Viral

Immunopathology

Zheng Ling

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Marie Bashir Inst.
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UNIVERSITY



University of Genève, Switzerland
Christine Chaponnier



Institut Cochin, Paris, France
Pierre-Olivier Couraud



Research posters on the wall include:
- "The human *Helicobacter pylori* strains contribute significantly to the diversity of the gastric microbiome through potent mucin-degrading activity"
- "Plasmodium falciparum... vascular endothelial cells involves transmembrane signaling and cytoskeletal reorganization"
- "SUMMARY: ..."

