Modifications of the T cell repertoire during experimental cerebral malaria

M2 Immunotechnologie

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04.12.2006

1-Introduction (1)

Malaria:

- *Malaria came from italian word mal'aria = mauvais air*

- Transmission of ***Plasmodium*** protozoa *(Alphonse Laveran, 1880)* by female *Anopheles* mosquitoes *(Ronald Ross, 1897)*
- Four species are involved in human malaria diseases : ***P.vivax***, ***P.ovale***, ***P.malariae*** and ***P.falciparum***

- *Ancestral disease: India, Vs b JC*
- *Eradicated from Europe and US in 50's*

- But still present in Africa, Asia, Central and South America
- 300 to 600 million people infected per year

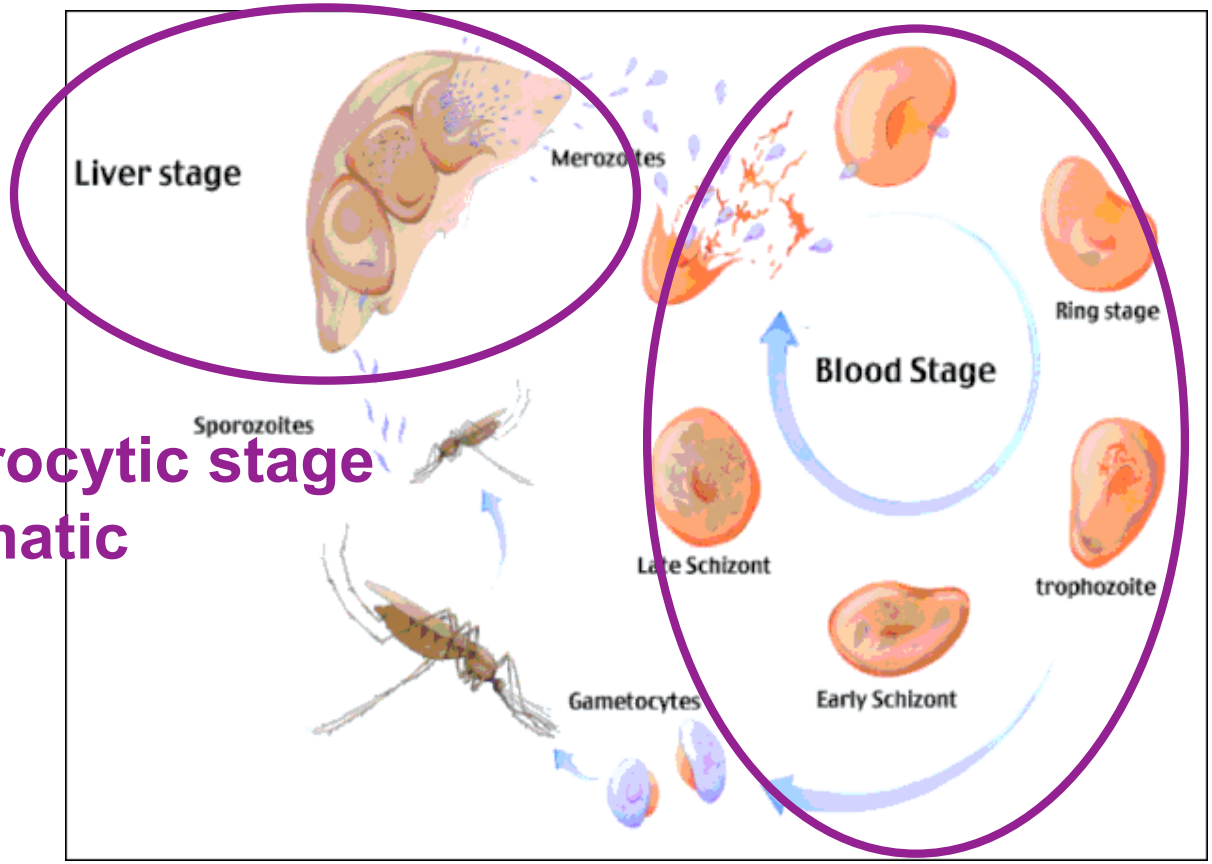
- *P.falciparum induces **severe** and **lethal** disease*
- >1 million people die, mostly in Sub-saharian Africa (90%)

The Africa Malaria Report - WHO 2005

1- Introduction (2)

- Complex parasite life cycle : 2 hosts
  - ✗ mosquito
  - ✗ mammalians

Erythrocytic stage
Symptomatic



Pre-erythrocytic stage
asymptomatic

1- Introduction (3)

- *P. falciparum* infection → severe complications

✗ Severe anemia

✗ Acute respiratory deficiencies

✗ **Cerebral Malaria => 30% *P. falciparum* related death**

(children < 5y. Old ; pregnant women)

- 2 hypotheses regarding the mechanism leading to CM:

✗ **PRBC** increase the vascular permeability by binding to endothelial molecules (ICAM-1, etc) (Blue Evans inflitration)

✗ **Humoral and cellular immune** responses lead to brain inflammation (proinflammatory cytokines, autoantibodies, cerebral T cells infiltration)

1- Introduction (4)

T lymphocytes and cerebral malaria (CM):

- In mice:

✗ CD4 and CD8 T cells **contribute to neuropathology** (KO, Ab depl.)

✗ **$CD8^+$ T$\alpha\beta$** observed in cerebral microvascular endothelium (Belnoue et al. 2002; Bagot et al., 2003)

- In humans:

T cells number in the peripheral blood decreases in CM+ children compared to non-CM children (Hviid, L et al, Infection and Immunity, 1997, 65: 4090-93)

Experimental Cerebral Malaria in mouse:

- Infection of B10.D2 mice with ***P. berghei ANKA*** (clone 1.49 L)
- Some physiopathological similarities with *P. falciparum* infection: "coma", fever, ischemia, cytokines?
- Cerebral Malaria developing mice (CM^+) die after 7 to 14 days p.i

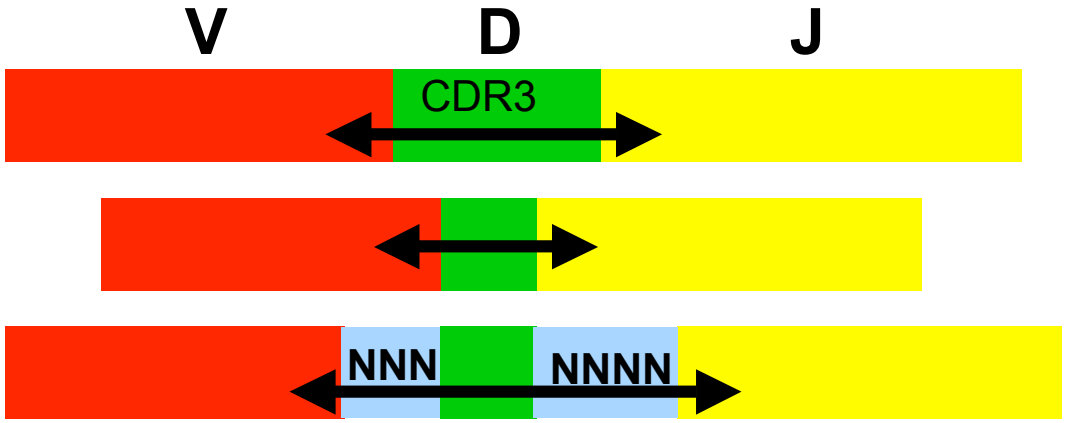
2-T Cell Receptor (2)

Le CDR3 concentre la majorité de la diversité

Diversité du TcR

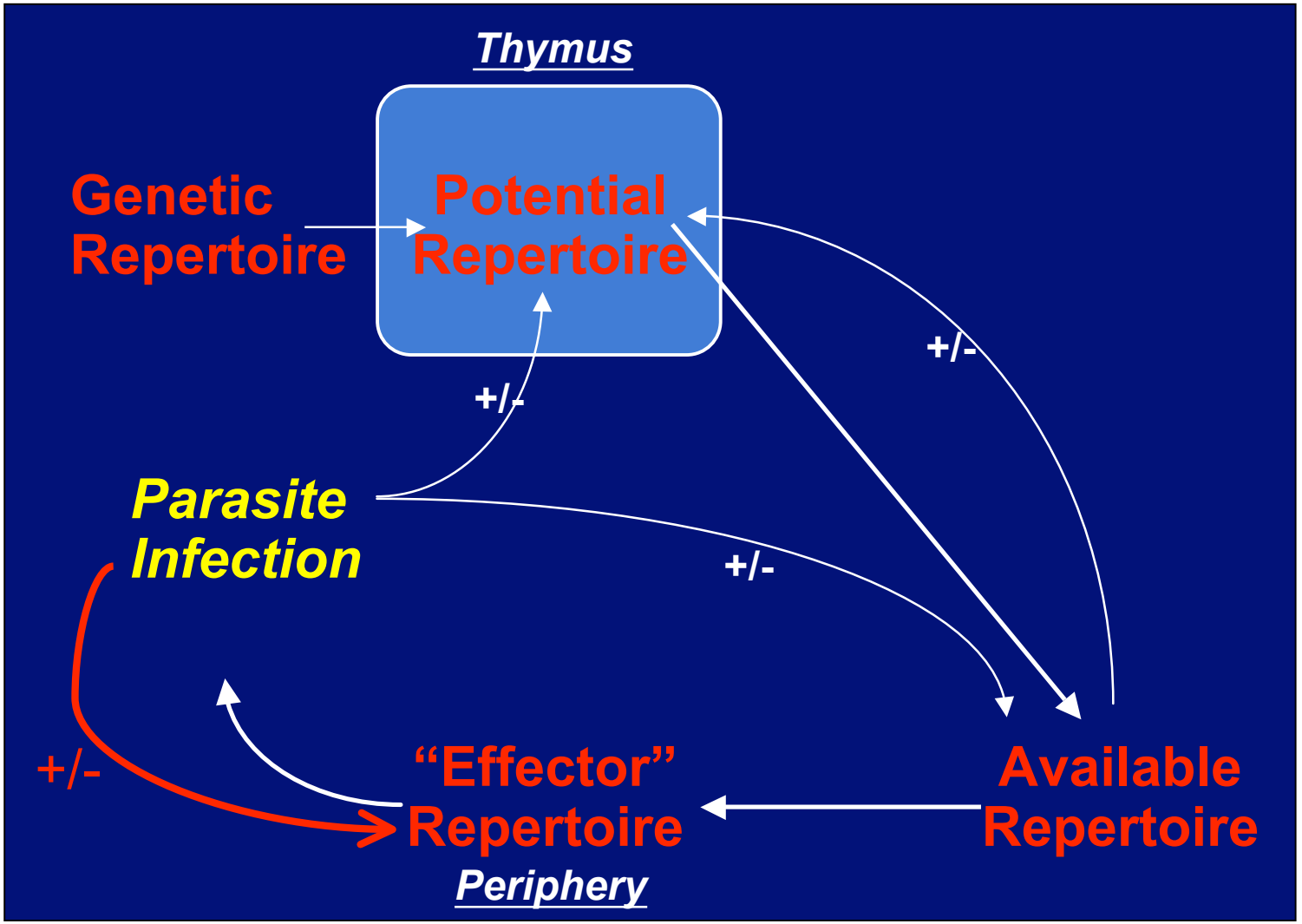
- Diversité combinatoire = combinaison des segments V(D)J
- Diversité d'appariement = TCRa/TCRb, TCRg/TCRd
- Diversité jonctionnelle = addition aléatoire de nucléotide au niveau de la jonction V(D)J

→ la région CDR3 est ainsi variable en séquence et en taille : *signature du réarrangement*



Variable en taille

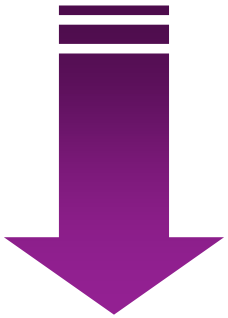
3-Immune repertoires



4- Hypotheses and Objectives

• *PbA* expresses a high diverse antigen repertoire => infection leads to **massive** peripheral lymphocytes repertoire modifications

• Infiltration of T cells in the brain => Cerebral malaria is associated with and might be to due to **a higher perturbation**



→ Description of the global Tab repertoire perturbation during the course of infection, **before and during neuropathology**

→ Characterization of the nature of this perturbation in different organs: **spleen**, **blood** and **brain**

5- Immunoscope/ISEApeaks strategy

Serial **TcR BVBC PCR**
PCR product labelling

Electrophoresis **in acrylamide gel**
(automated sequencer)

Data Extraction
(**Immunoscope**)

ISEApeaks Strategy

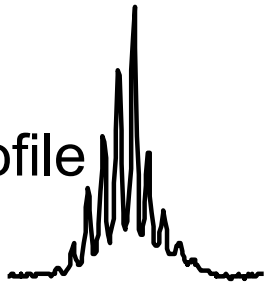
Peak Database

Estimation of perturbation:

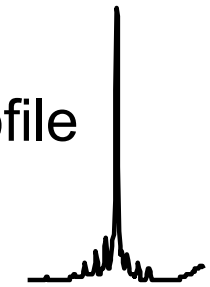
- Global Perturbation: **Gorochov** index
- Recurrent oligoclonality: **Oligoscore** index

Statistical analysis
(Kruskal-Wallis, Mann-Whitney)

Gaussian or
polyclonal profile



Irregular or
oligoclonal profile



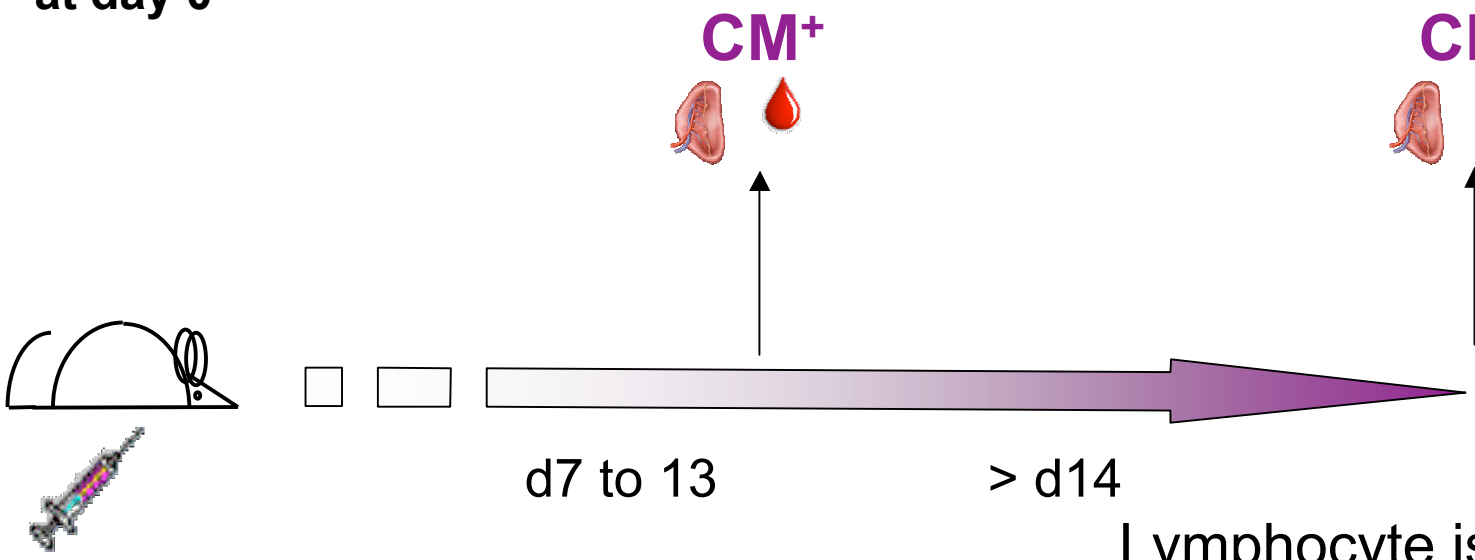
Collette et Six, 2000, *Bioinformatics*

Pannetier et coll., 1995 *Trends Immunol*

6- Experimental design (1)

Blood and spleen repertoires

B10.D2
at day 0



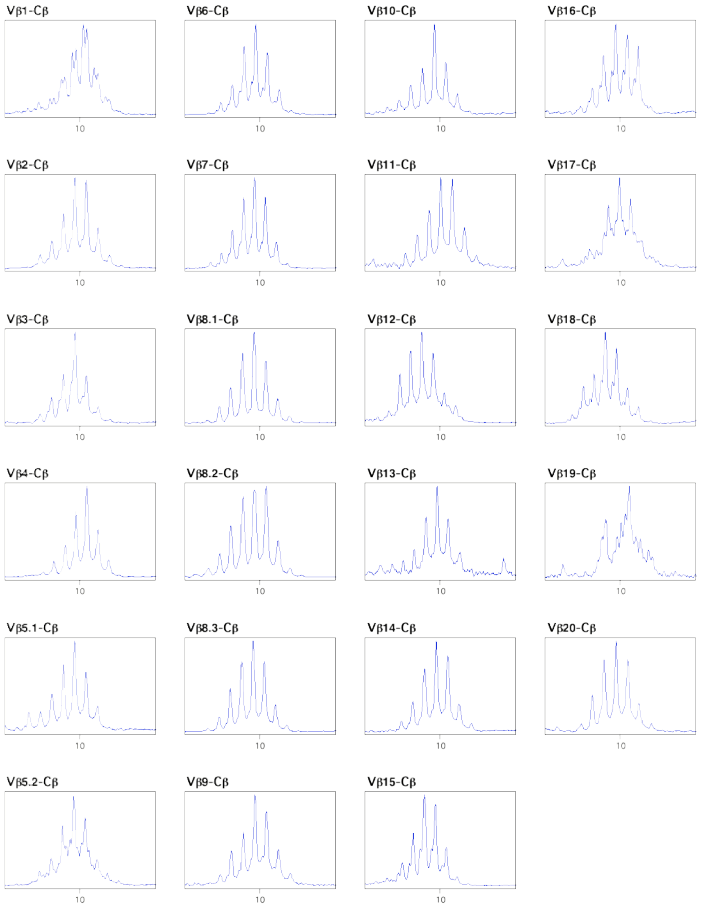
10^6 ***PbA*** parasitized red bloods cells from B6 mice (lot 1998)

Lymphocyte isolation
RNA extraction
cDNA synthesis
Immunoscope/ISEApeaks strategy

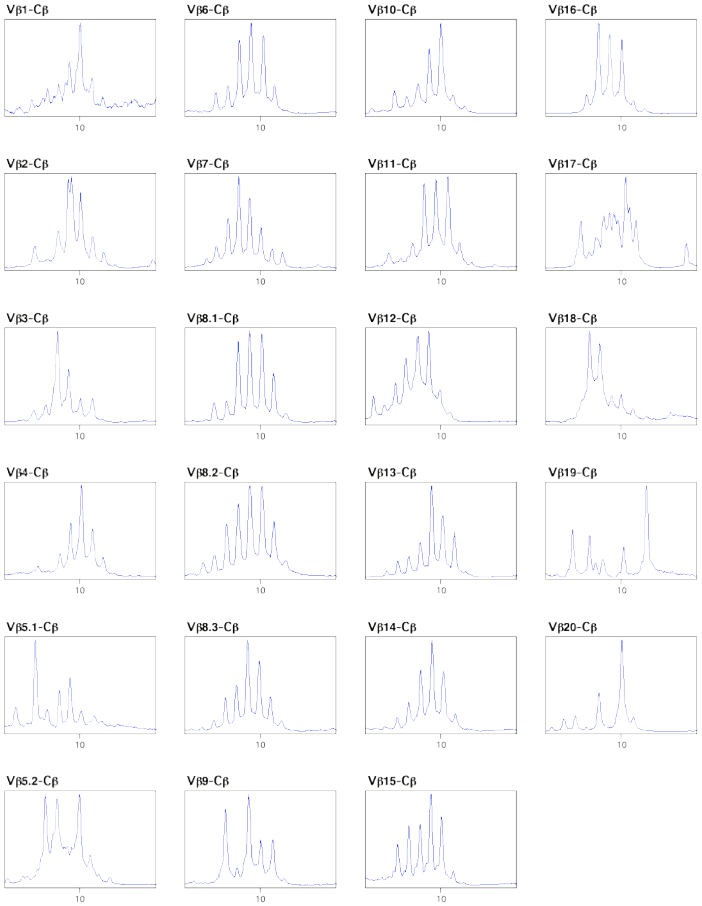
CTR

6-Results (1)

Répertoires des PBL B10.D2



contrôle

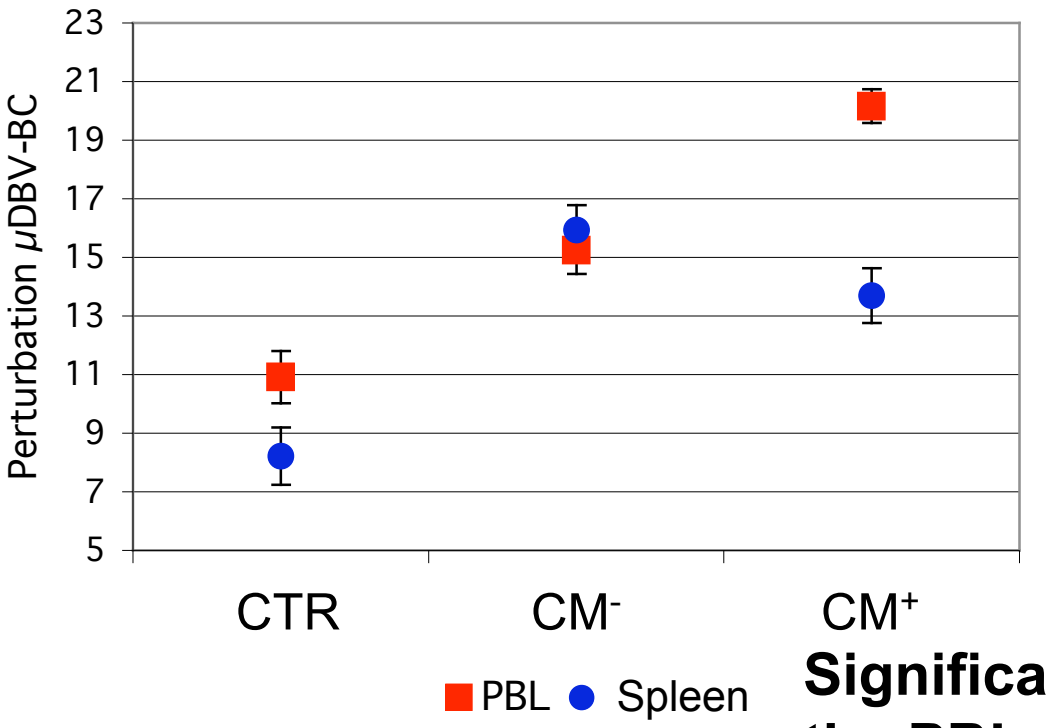


NP+

6- Results (2)

Global TcR BVBC perturbation in B10.D2 mice infected by *PbA*

Collette, A et al., 2004, *JI*

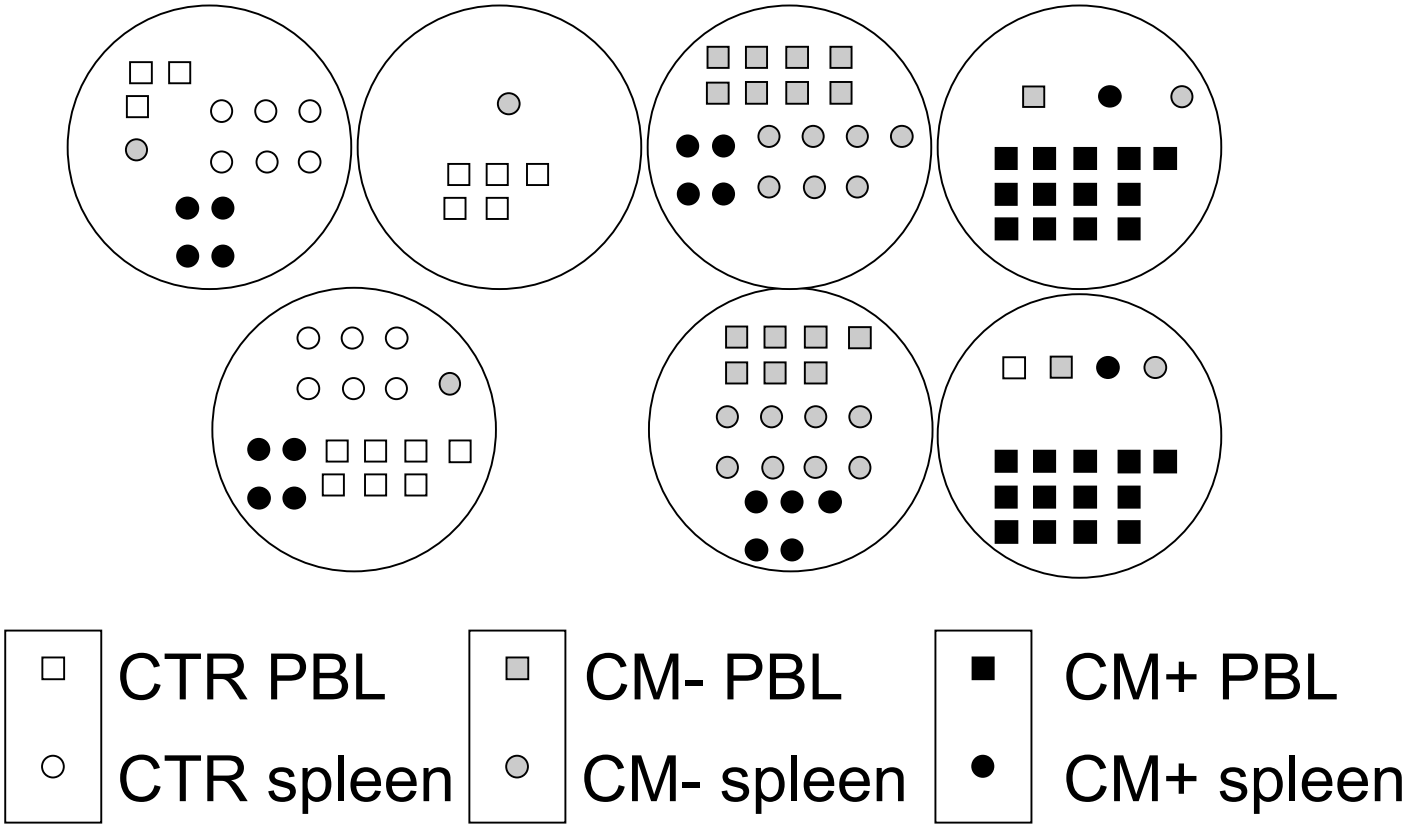


Significant higher perturbation of the PBL repertoire in CM+ mice compared to:

- Control PBL and Spleen
- CM+ Spleen

6- Results (3)

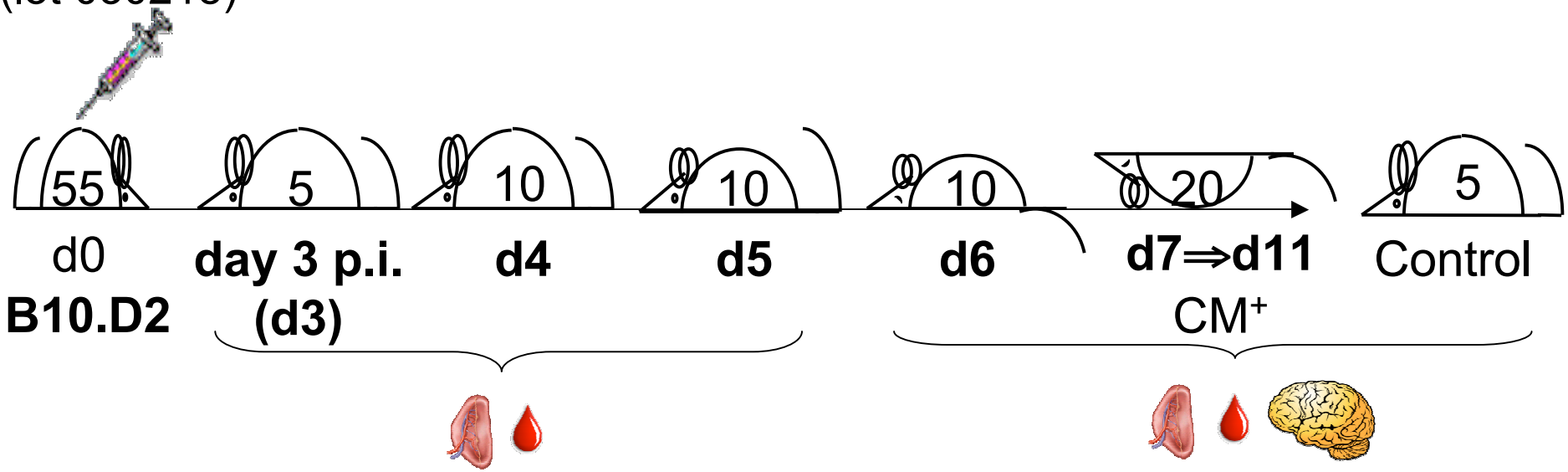
Clustering



7. Experimental design (2)

Kinetic of the infection

10^6 *PbA* parasitized red blood cells from B6 mice (lot 030218)



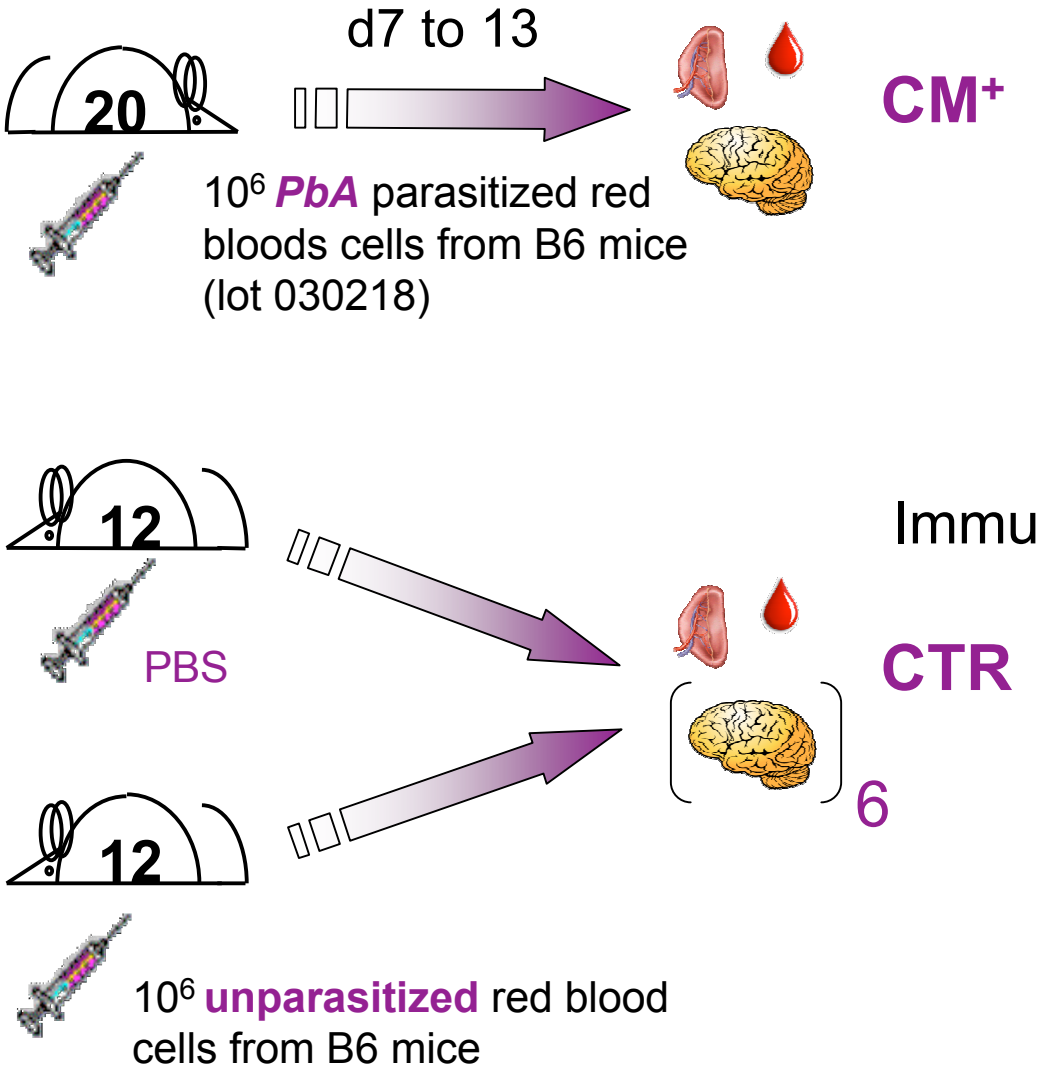
Spleen, Blood and Brain

Lymphocyte isolation
RNA extraction
cDNA synthesis
Immunoscope/ISEApeaks strategy

7. Experimental design (3)

Brain repertoire

B10.D2
at day 0



Lymphocyte isolation
RNA extraction
cDNA synthesis
Immunoscope/ISEApeaks strategy

7. Results (4)

Global TcR BV perturbation

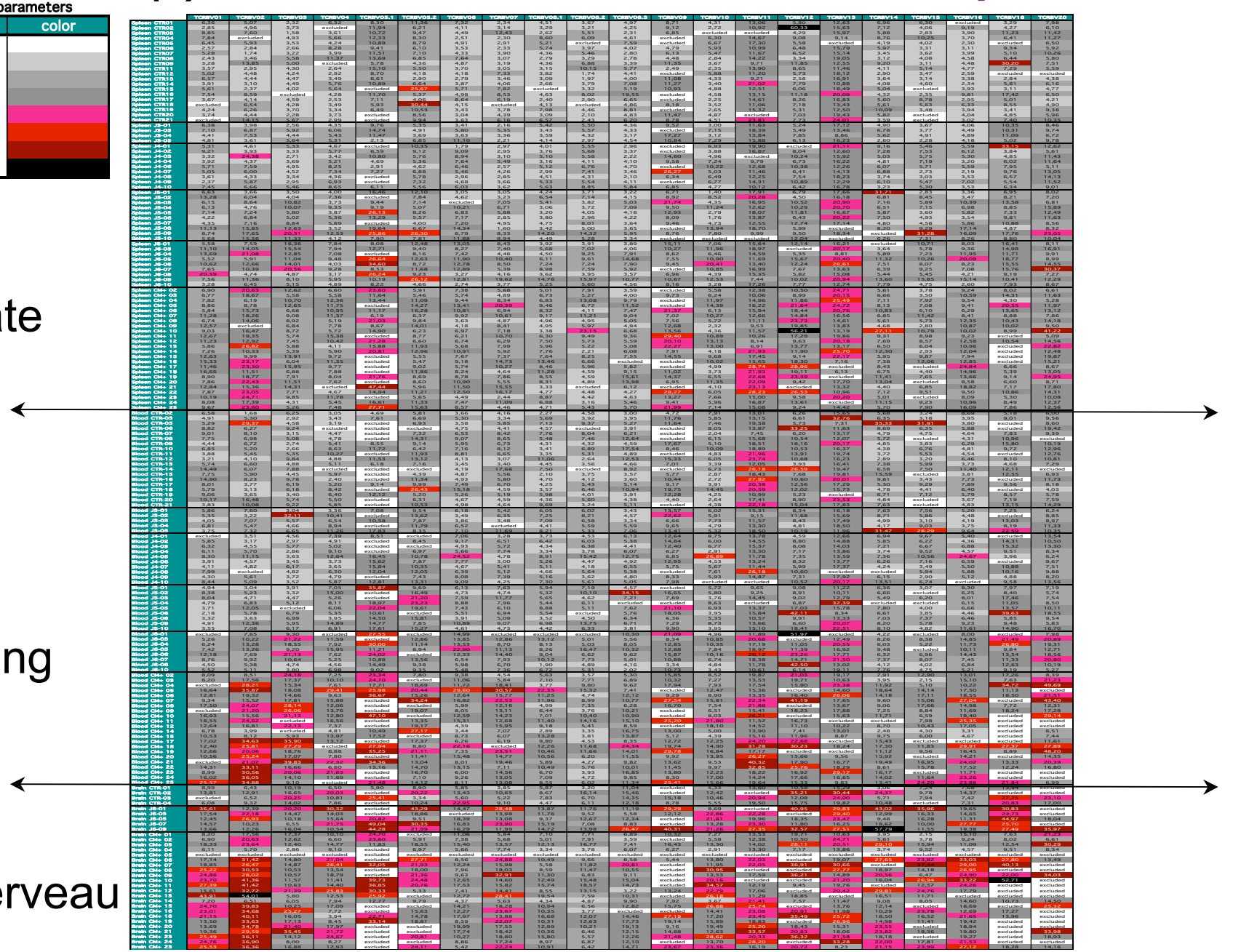
DrawArray parameters

when <	color
excluded	
5	
10	
20	
25	
30	
50	
100	

Rate

Sang

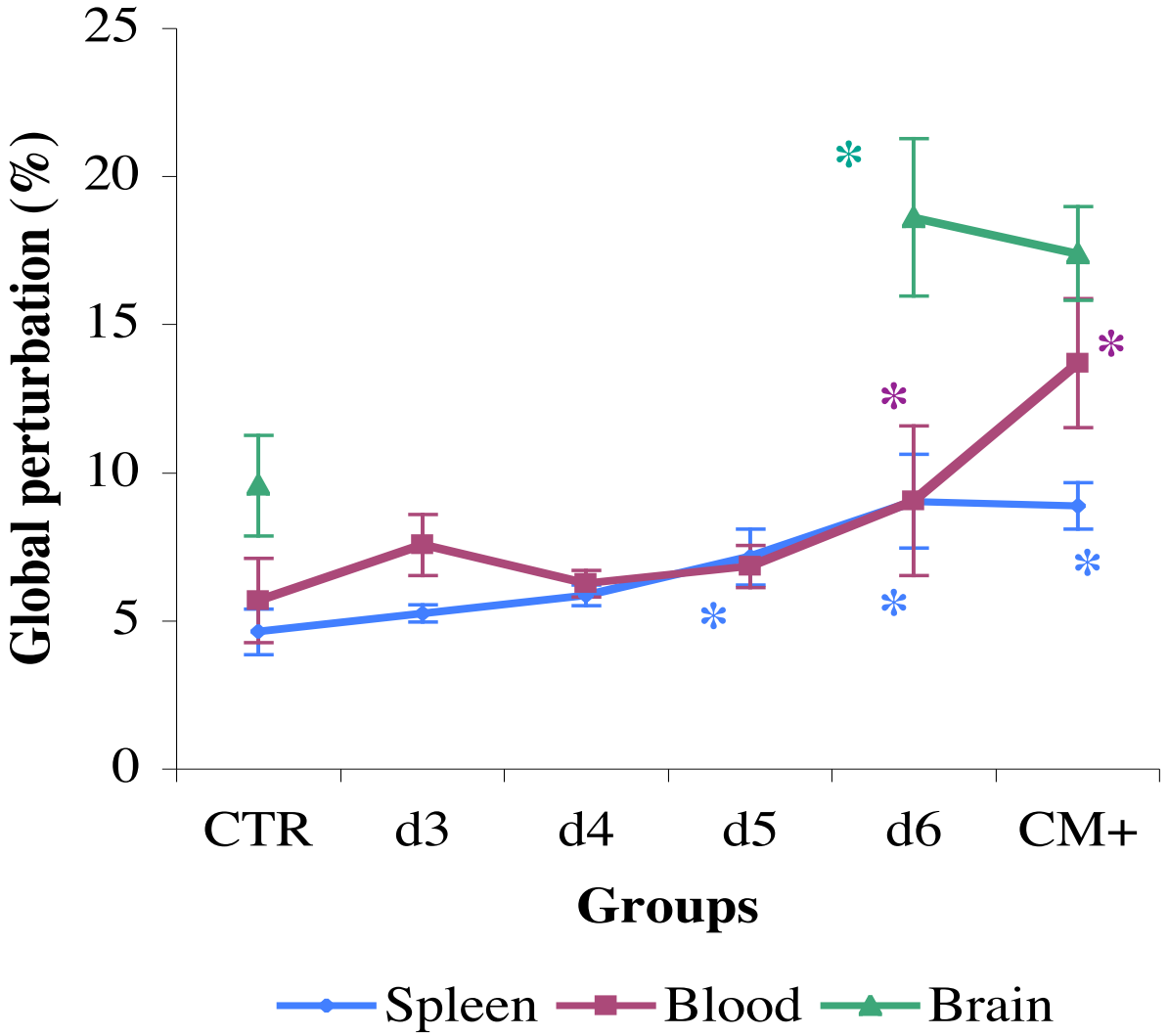
Cerveau



7. Results (5)

Global TcR BV perturbation

- 99 mice
- CTR Brain = pools
- non parametric tests
- comparison to CTR



Progressive increases of the perturbation

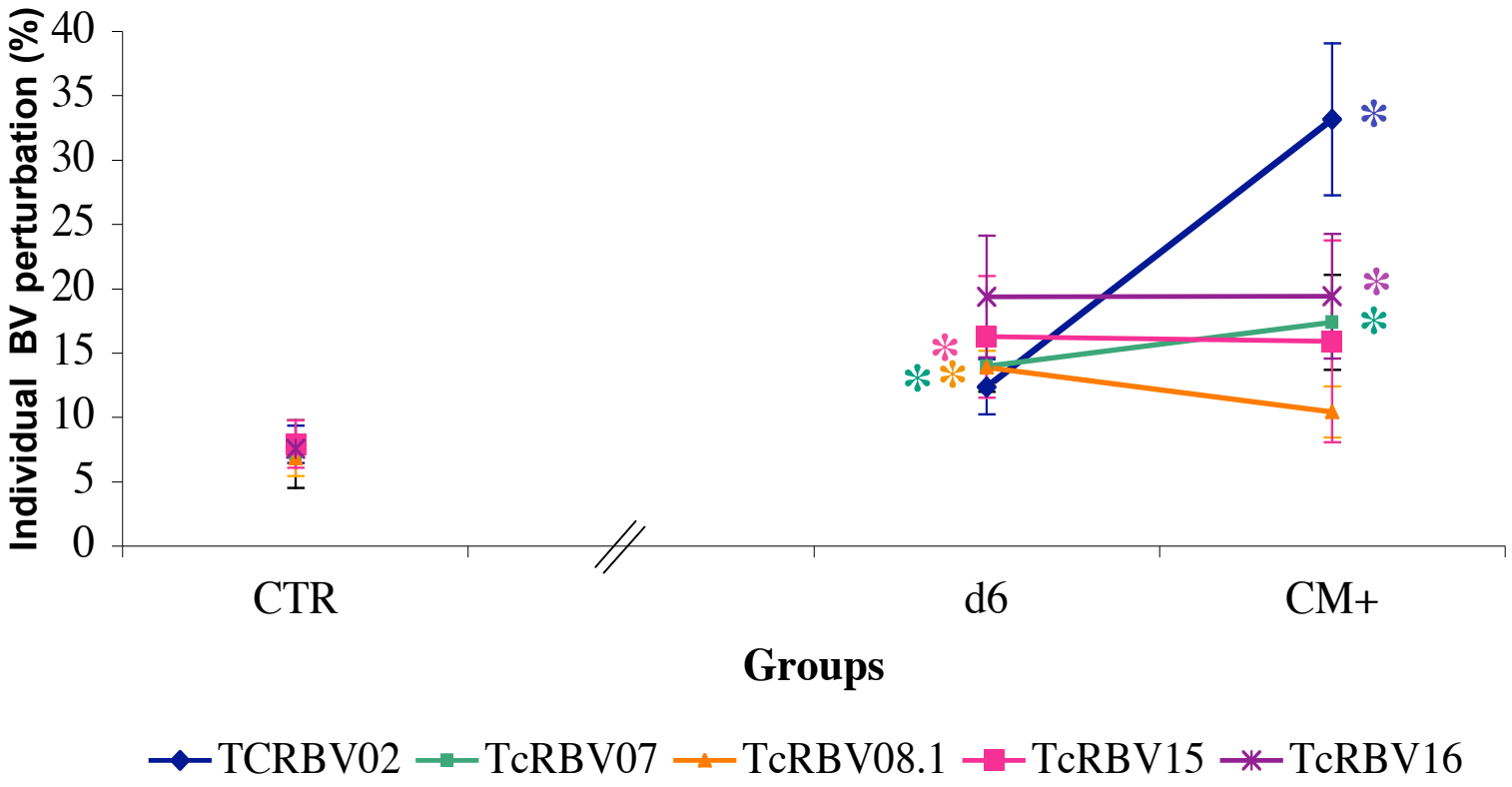
Significant perturbation from day 5 in spleen, and day 6 in blood and brain

7. Results (6)

Individual TcR BV perturbation

- **Most of the BV** are perturbed in **Spleen** and **Blood**

- Only **5 BV** are perturbed in the **Brain**



8. Summary

✓ Experimental cerebral malaria in B10.D2 mice is associated with a **significant perturbation of the TcRß repertoire** in spleen, blood and brain

✓ This perturbation is observed **during the course of the infection:**

- from day 5 in spleen
- from day 6 in blood and brain

✓ Individual TcR BV perturbation:

- **most of BV** are perturbed in **spleen** (d.3-4 p.i) and **blood** (d.6 p.i.)
- only **5 BV** are perturbed in the **brain**
- **BV02** and **BV08.1** present the **same pattern** of perturbation in the **three compartments**

Compartmentalized TCR diversity during the infection

9. Next questions

Is the observed perturbation involved in neuropathology?

- Characterize the BVBJ repertoire of PBL, splenocytes and brain lymphocytes for the 5 BV perturbed in the brain

- Characterize the phenotype of brain T cells during pathology
- Determine the dependence between the three compartments

⇒ **Analysis using each group as reference for perturbation index calculation**

⇒ **Study of the relationship between the TCR diversity and the lymphocyte dynamic => B6 model** - *on going*

What is the naive repertoire in the brain of mice?

- few T cells in « naive » mice

- stochastic ?

=> The concept of protective autoimmunity

10- Le concept d'autoimmunité protectrice

Modèle d'étude: lésion du nerf optique de rat + cellules T anti-MBP

Objectif : Caractérisation du rôle de la réponse immune spécifique de la MBP dans la réparation nerveuse

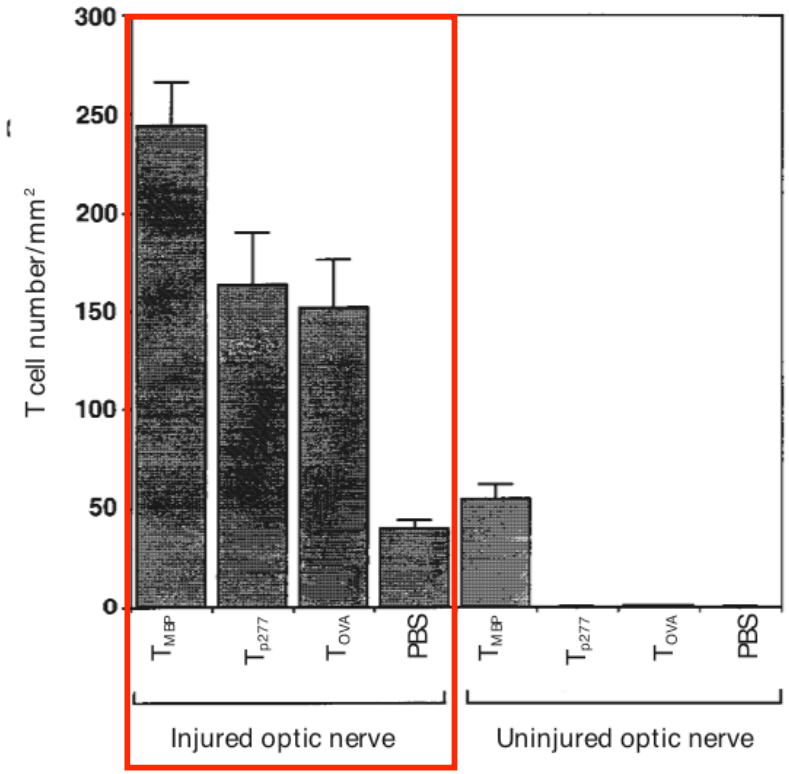


Figure 1

=> Accumulation de cellules T au site de lésion (spécificité quelconque)

(Moalem, G et coll, Nat. Med., 1999, vol5, pp 49-55)

10- Le concept d'autoimmunité protectrice

Figure 2

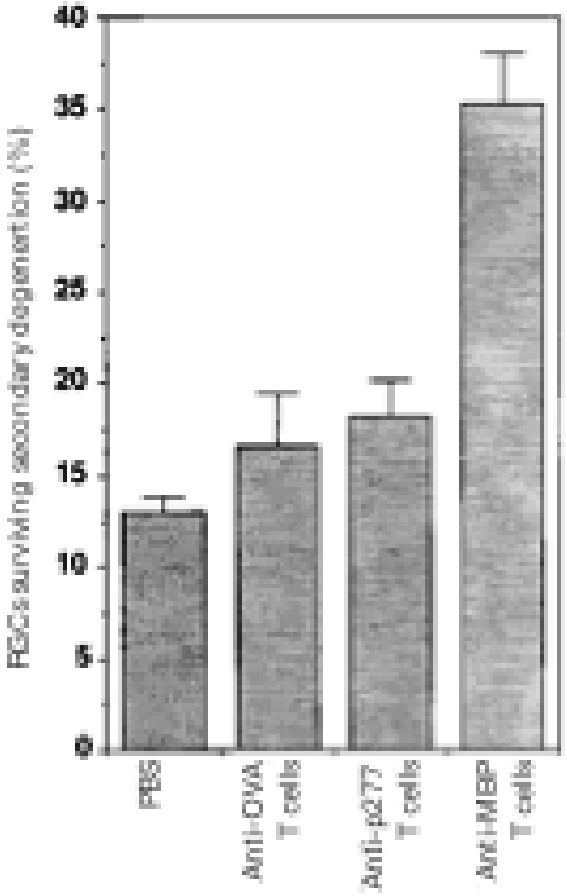
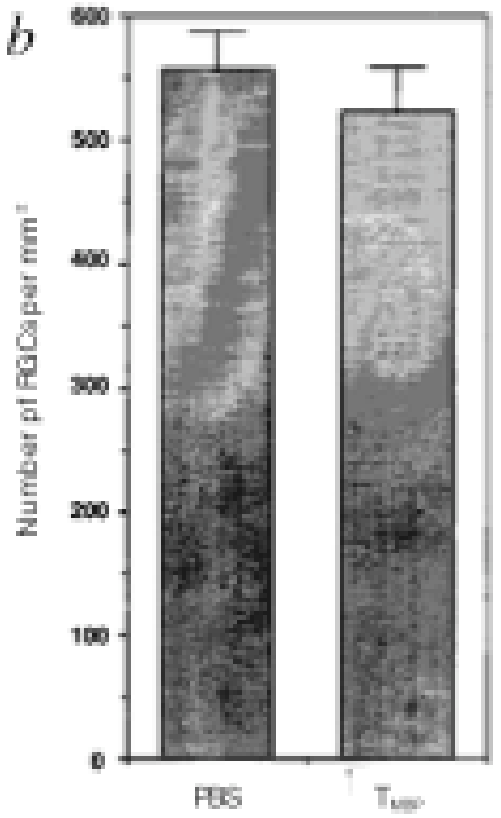


Figure 4b



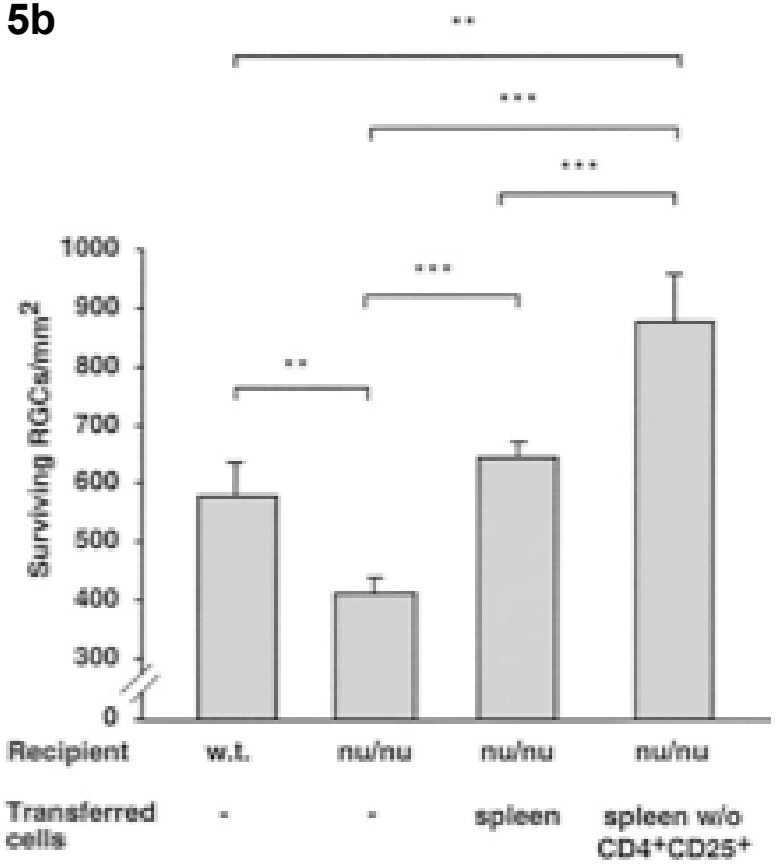
Les cellules T rencontrant leur antigène (donc auto-réactives) facilitent la réparation de la lésion (Figure 2), mais ne sont pas agressives en contexte physiologique (Figure 4b).

(Moalem, G et coll, Nat. Med., 1999, vol5, pp 49-55)

10- Le concept d'autoimmunité protectrice

Modèle d'étude: lésion du nerf optique de souris + cellules T régulatrices

Figure 5b

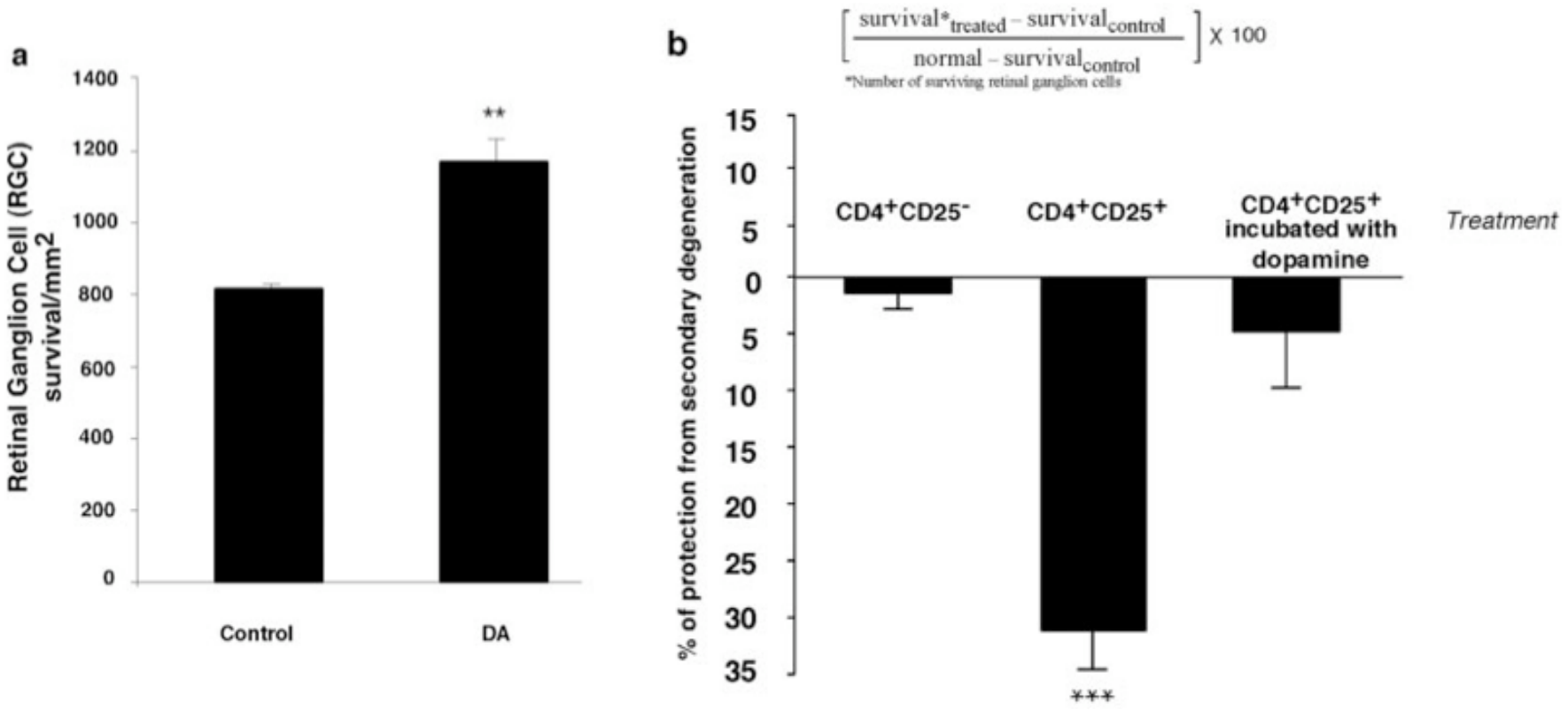


=> Régulation de l'auto-immunité naturelle par les cellules régulatrices

(Kipnis, J et coll, PNAS, 2002, vol.99, pp 15620-15625)

10- Le concept d'autoimmunité protectrice

Modèle d'étude: lésion du nerf optique de souris + cellules T régulatrices



=> Régulation de l'auto-immunité naturelle par les cellules régulatrices **dont la fonction est régulée par la dopamine**

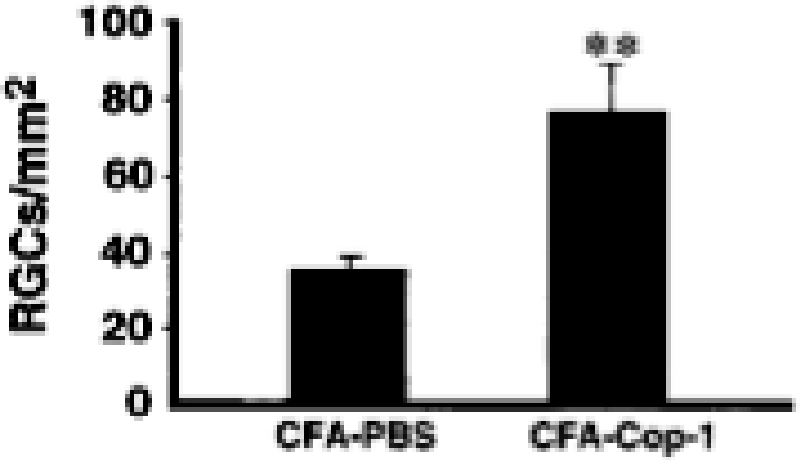
(Kipnis, J et coll, J. Neuroscience, 2004 vol.24, pp 6133–6143)

10- Le concept d'autoimmunité protectrice

=> Il existe donc une auto-immunité protectrice naturelle

Changement de perspective thérapeutique :
- Immunomodulation plutôt qu'immunosuppression (Cf. rôle négatif des Treg)

- Immunisation avec Cop1, peptide croisé avec MBP :



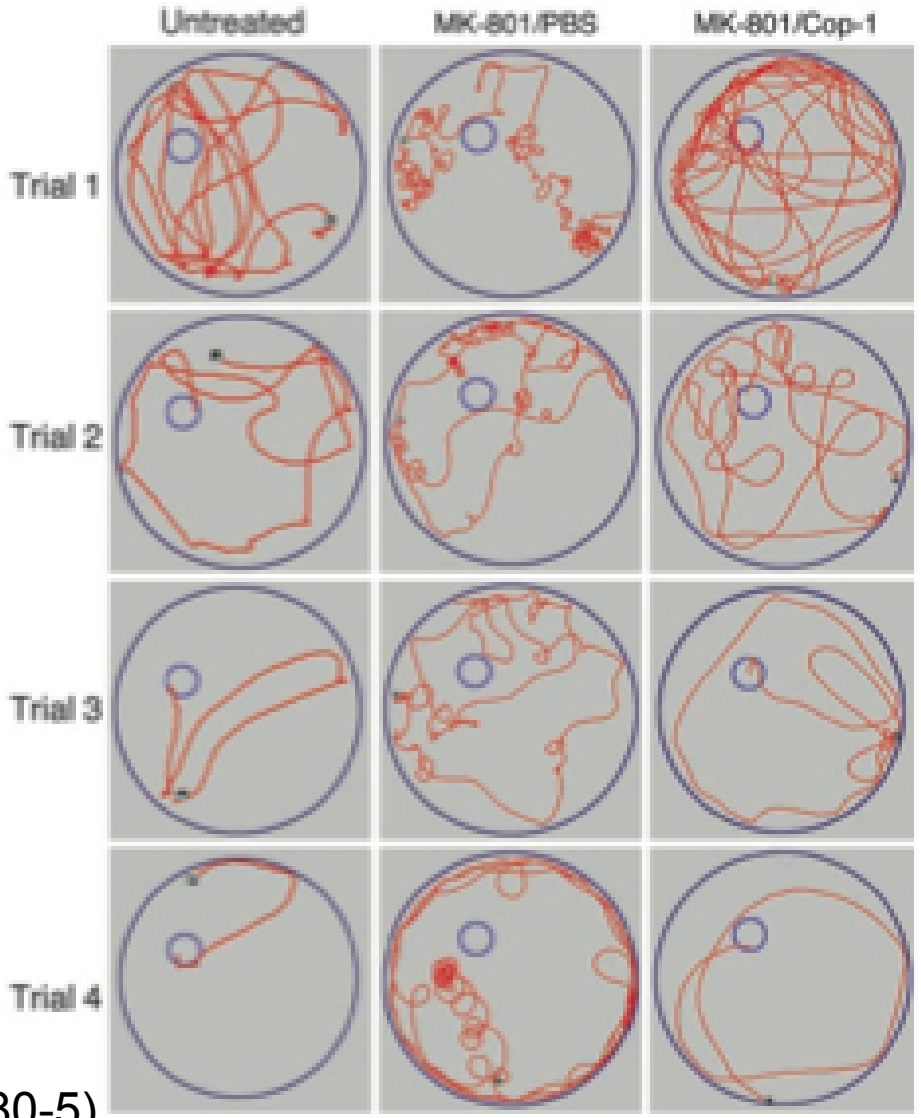
(Kipnis, J et coll, PNAS, 2000, vol. 97, pp.7446-7451)

10- Le concept d'autoimmunité protectrice

Immunisation avec Cop1

MK801 : antagoniste du récepteur NMDA
=> Symptômes psychotiques
(troubles du comportement)

=> Système immunitaire impliqué dans la régulation des atteintes du système nerveux central



(Kipnis, J et coll, PNAS, 2004, vol. 101, pp.8180-5)

11. Neuropaludisme et autoimmunité protectrice ?

1. **La perturbation observée est-elle impliquée dans la neuropathologie au cours de l'infection par *Plasmodium*?**
2. **Quel est le répertoire lymphocytaire T dans le cerveau chez les souris naïves?**

…

3. **Quel lien peut-on établir entre le concept d'autoimmunité protectrice et le neuropaludisme?**
4. **Peut-on induire/stimuler une réponse autoimmune protectrice chez les souris infectées par *PbA*?**
5. **Quelles perspectives peut-on envissager en terme d'immunointervention?**

Immunophysiopathologie infectieuse

Immune repertoire diversity

- Mélanie Bonnet
- Sophie Dulauroy
- Encarnita Ferrandiz
- Sami Ketari
- Ali Tebbi

- Adrien Six

****

- Pierre-André Cazenave
- Sylviane Pied

- Olivier Gorgette
- Jacques Roland
- Valérie Soulard
- Anne-Laurence Blanc
- Danielle Voegtlé
- Christèle Sellier

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