

Modifications of the T cell repertoire during experimental cerebral malaria

M2 Immunotechnologie

Encarnita Mariotti-Ferrandiz, Doctorante

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1-Introduction (1)

Malaria:

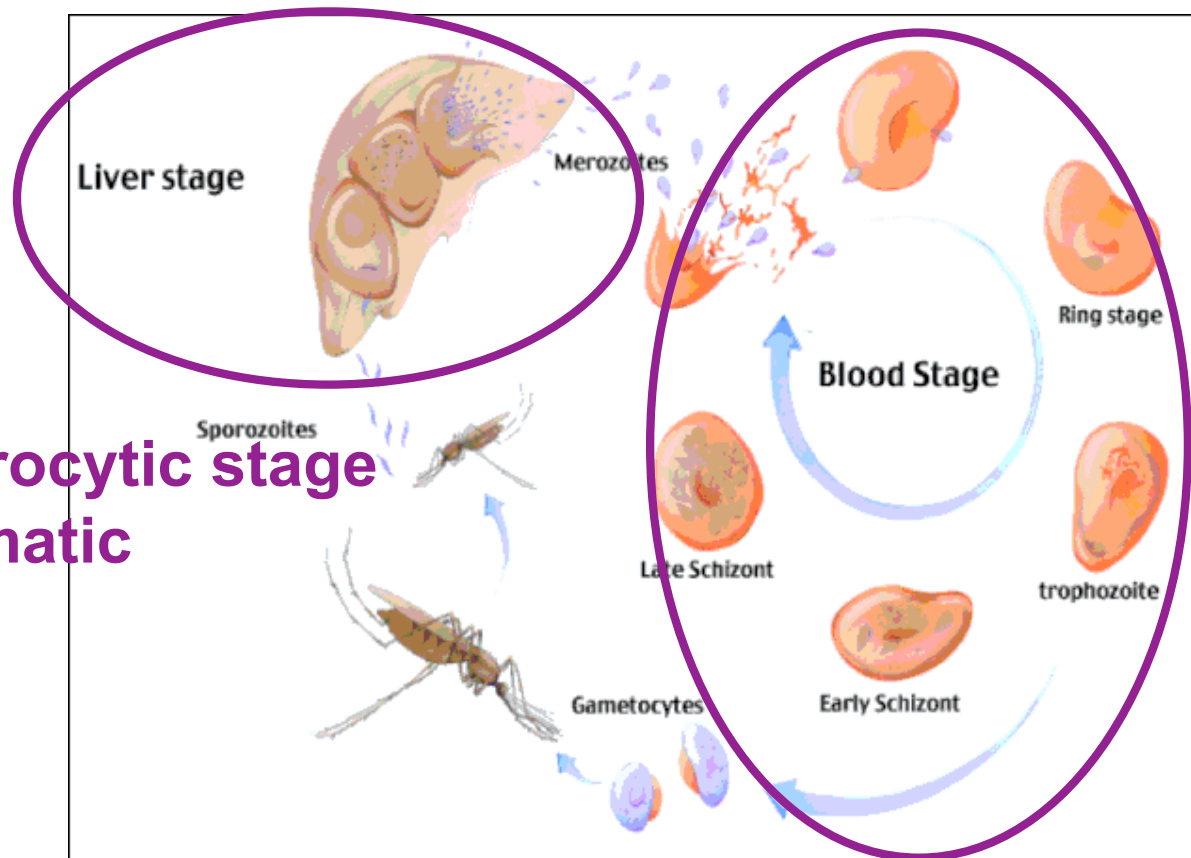
- *Malaria came from italian word mal'aria = mauvais air*
- Transmission of **Plasmodium** protozoa (*Alphonse Laveran, 1880*) by female *Anopheles* mosquitoes (*Ronald Ross, 1897*)
- Four species are involved in human malaria diseases : **P.vivax**, **P.ovale**, **P.malariae** and **P.falciparum**
- *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%)

1- Introduction (2)

- Complex parasite life cycle : 2 hosts
- x mosquito
- x mammals

**Erythrocytic stage
Symptomatic**

**Pre-erythrocytic stage
asymptomatic**



1- Introduction (3)

- *P. falciparum* infection → severe complications
 - x Severe anemia
 - x Acute respiratory deficiencies
 - x **Cerebral Malaria** => **30% *P. falciparum* related death**
(children < 5y. Old ; pregnant women)

- 2 hypotheses regarding the mechanism leading to CM:
 - x **PRBC** increase the vascular permeability by binding to endothelial molecules (ICAM-1, etc) (Blue Evans infiltration)

 - x **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:

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

x **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:

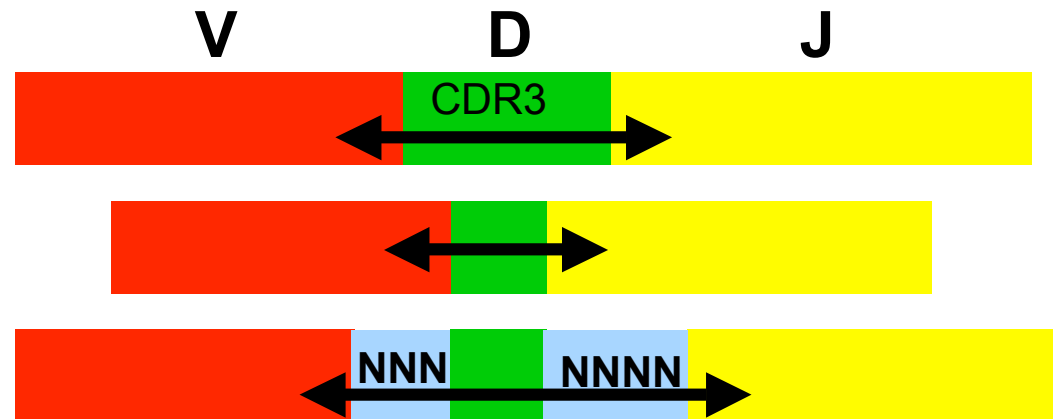
- Infection of B10.D2 mice with ***P. berghei ANKA*** (clone 1.49 L)
- Some physiopathological similarities with *P. falciparum* infection: “coma”, fever, ischemia, cytokines?
- Cerebral Malaria developing mice (CM⁺) die after 7 to 14 days p.i

2-T Cell Receptor (2)

Le CDR3 concentre la majorité de la diversité

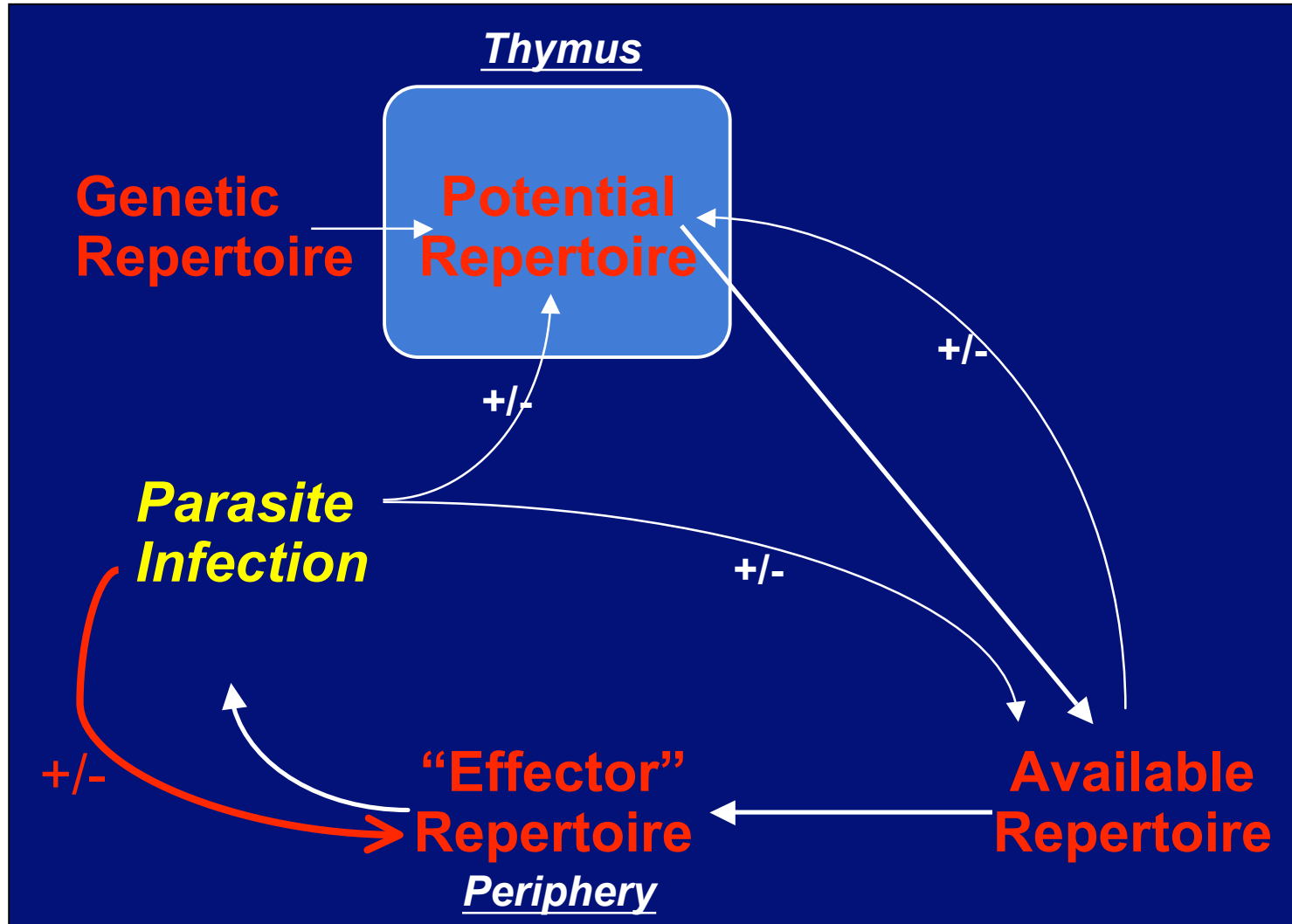
Diversité du TcR

- 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 due to **a higher perturbation**



- Description of the global T cell 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/ISEA peaks strategy



Serial **TcR BVBC PCR**
PCR product labelling

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

ISEApeaks Strategy

Data Extraction
(Immunoscope)

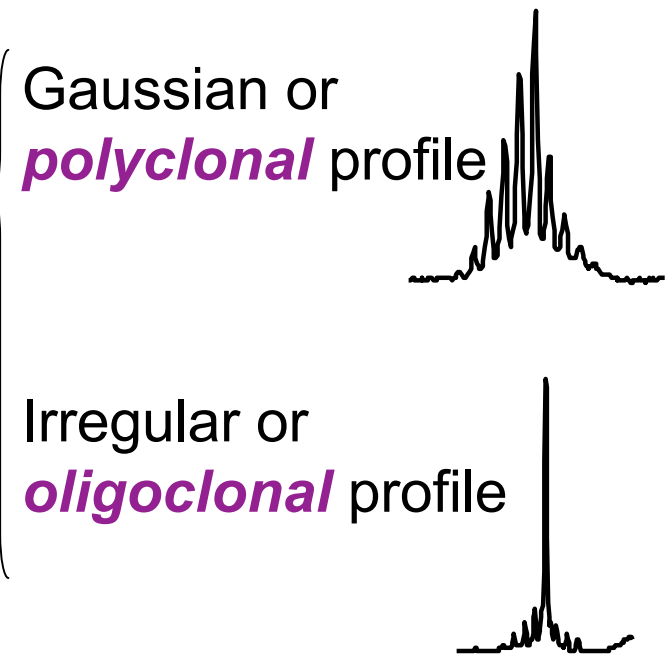
Peak Database

Estimation of perturbation:

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

Statistical analysis

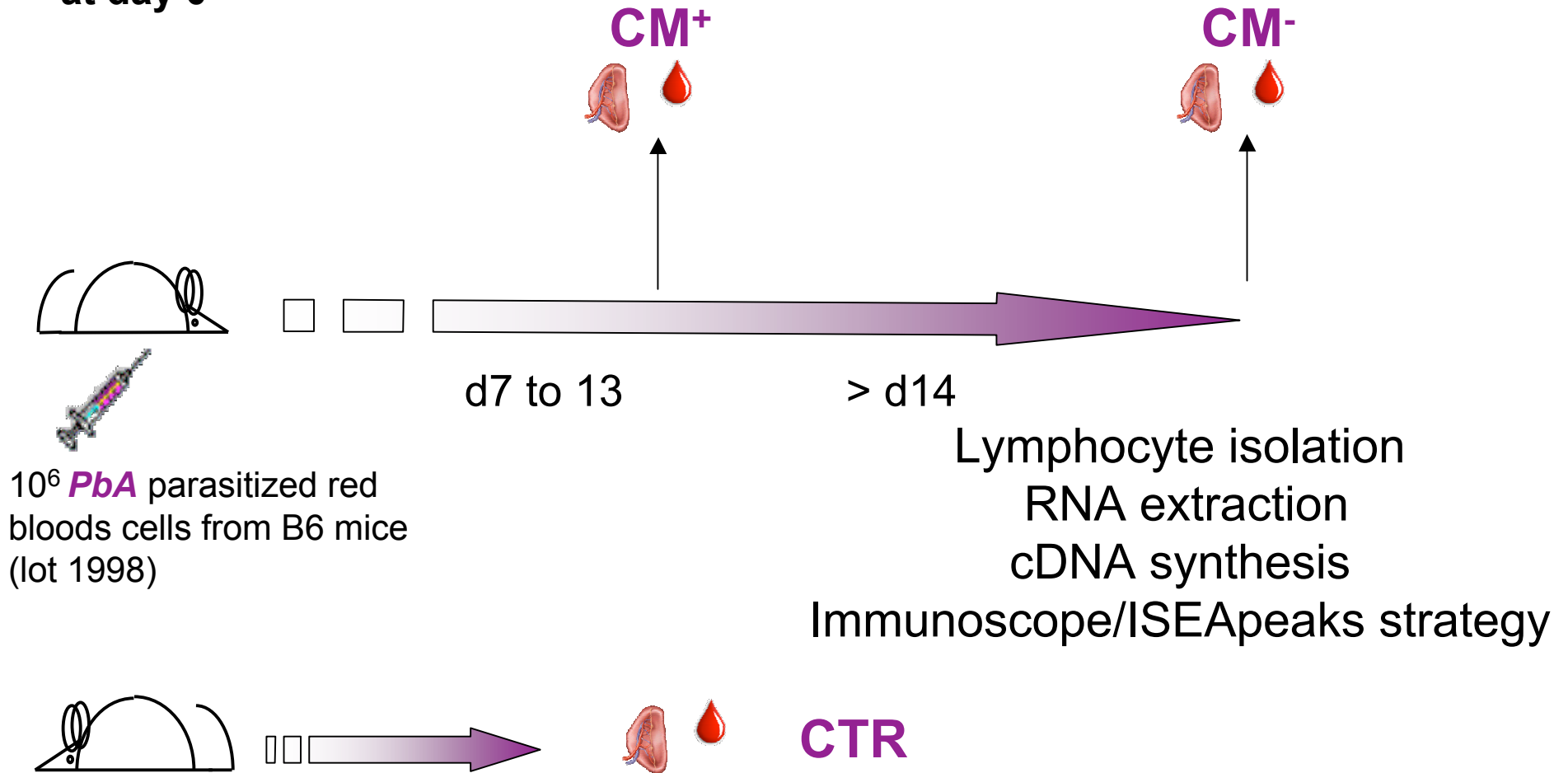
(Kruskal-Wallis, Mann-Whitney)



6- Experimental design (1)

Blood and spleen repertoires

B10.D2
at day 0



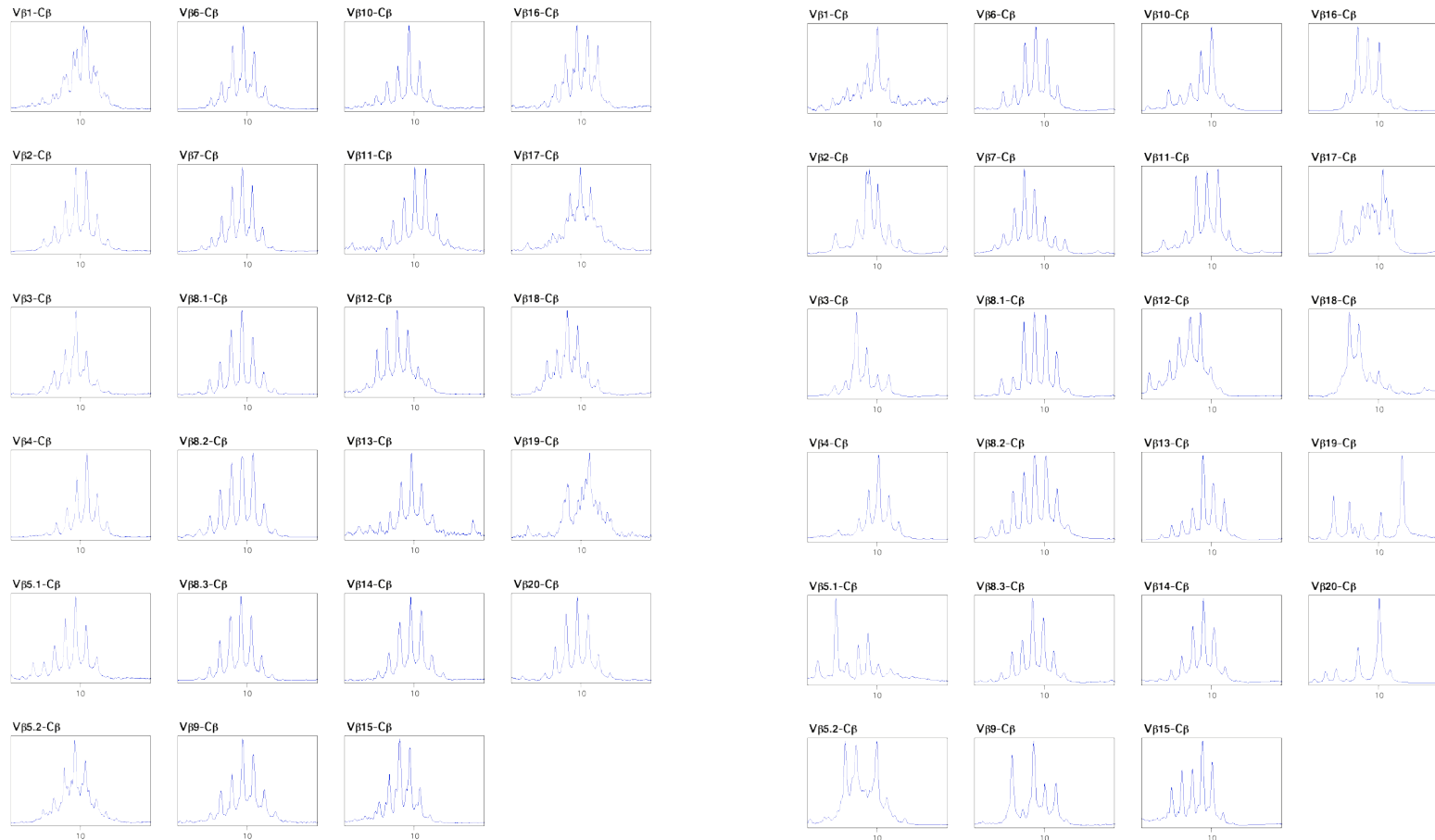
10^6 *PbA* parasitized red
bloods cells from B6 mice
(lot 1998)

Lymphocyte isolation
RNA extraction
cDNA synthesis
Immunoscope/ISEApeaks strategy

CTR

6-Results (1)

Répertoires des PBL B10.D2



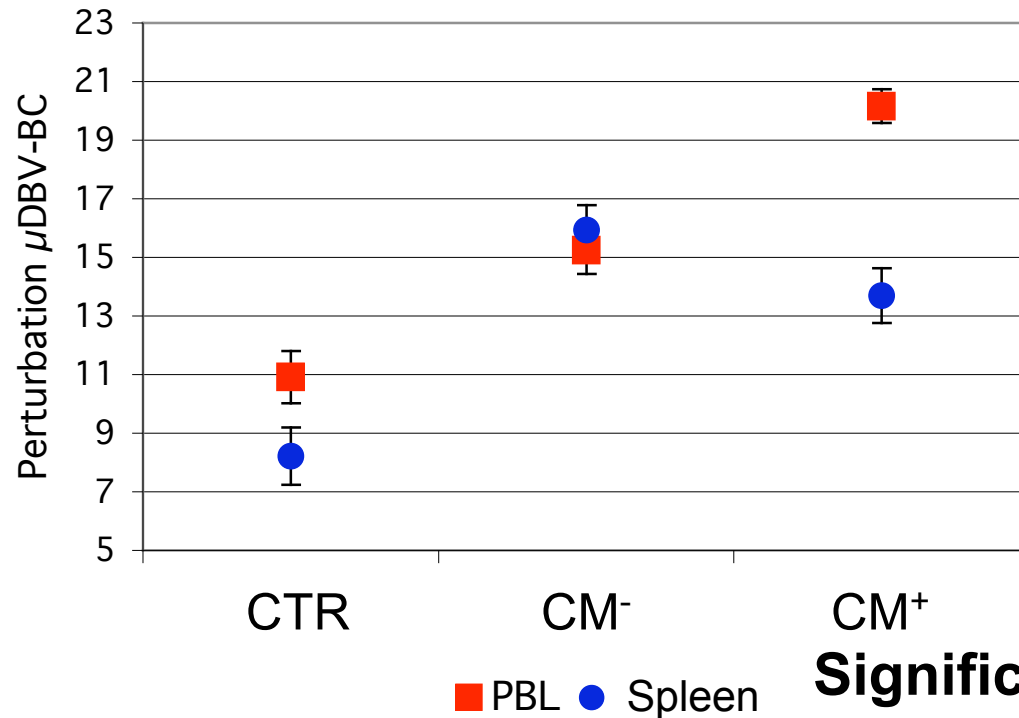
contrôle

NP+

6- Results (2)

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

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

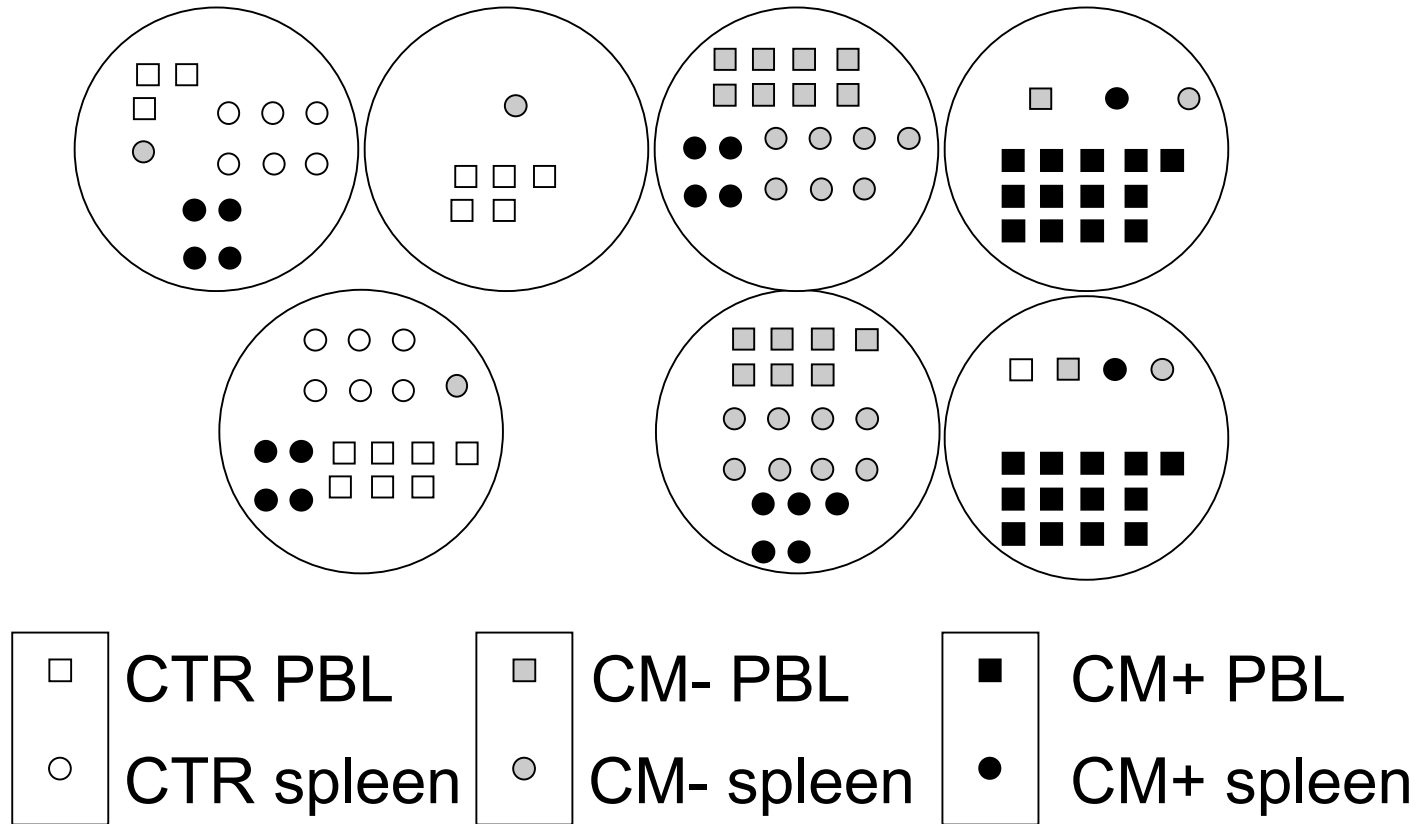


Significant higher perturbation of the PBL repertoire in CM⁺ mice compared to:

- Control PBL and Spleen
- CM⁺ Spleen

6- Results (3)

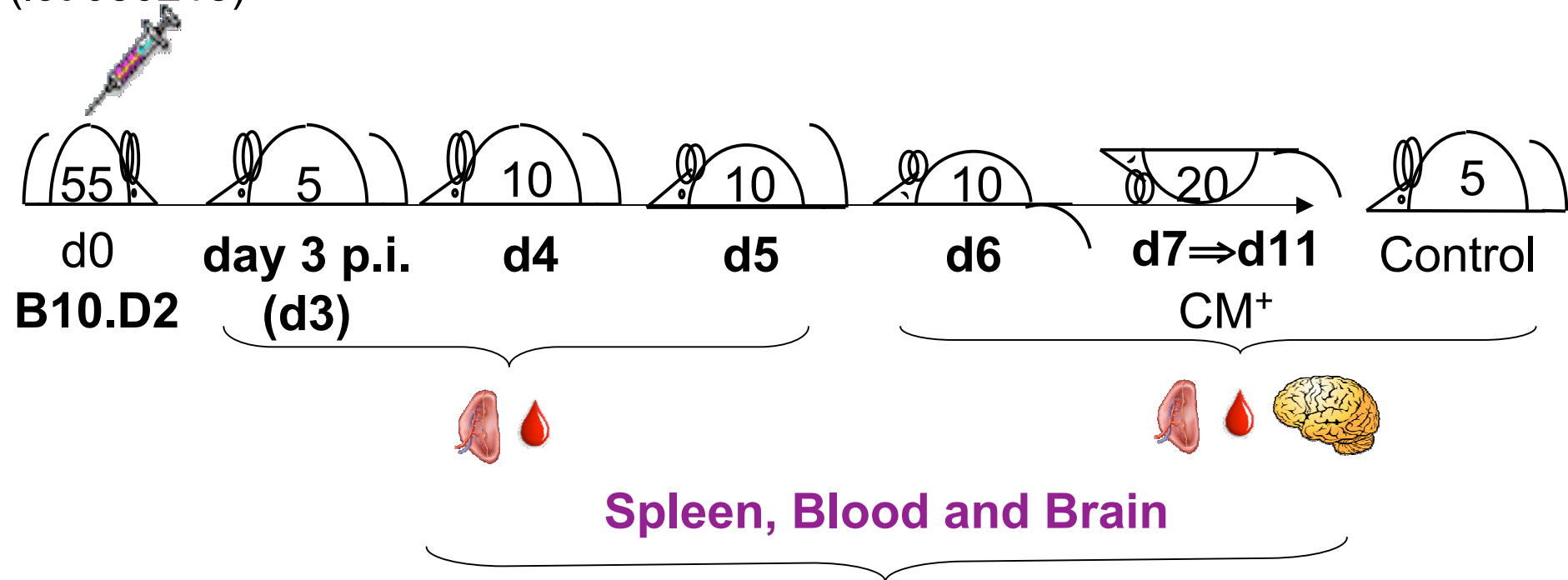
Clustering



7. Experimental design (2)

Kinetic of the infection

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

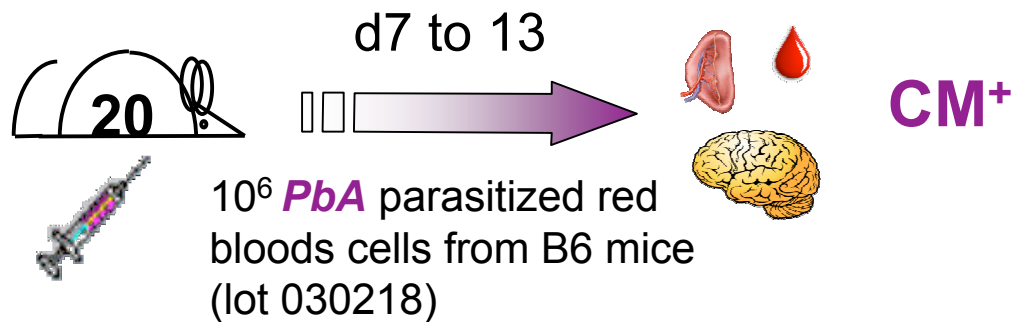


Lymphocyte isolation
RNA extraction
cDNA synthesis
Immunoscope/ISEApeaks strategy

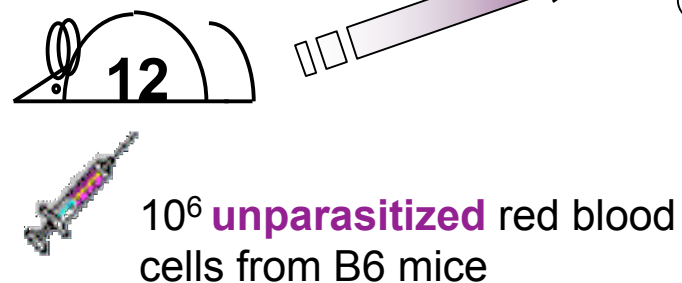
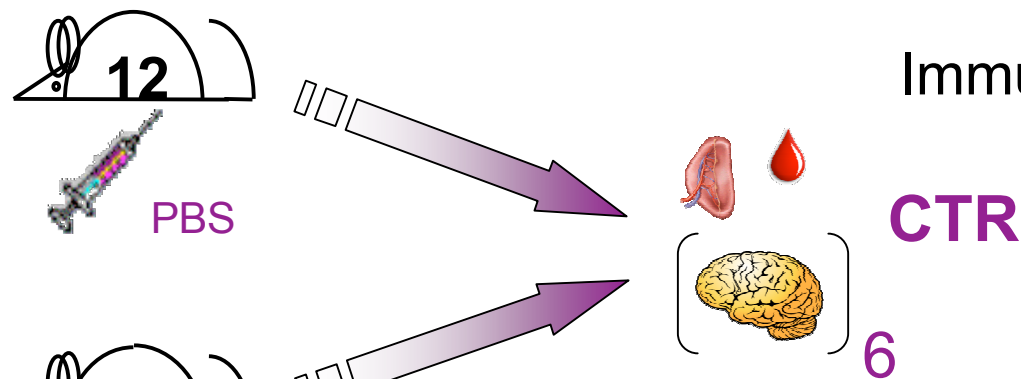
7. Experimental design (3)

Brain repertoire

B10.D2
at day 0



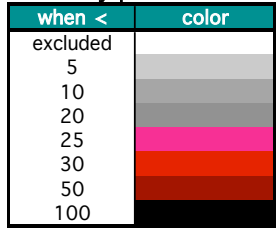
Lymphocyte isolation
RNA extraction
cDNA synthesis
Immunoscope/ISEApeaks strategy



7. Results (4)

Global TCR BV perturbation

DrawArray parameters



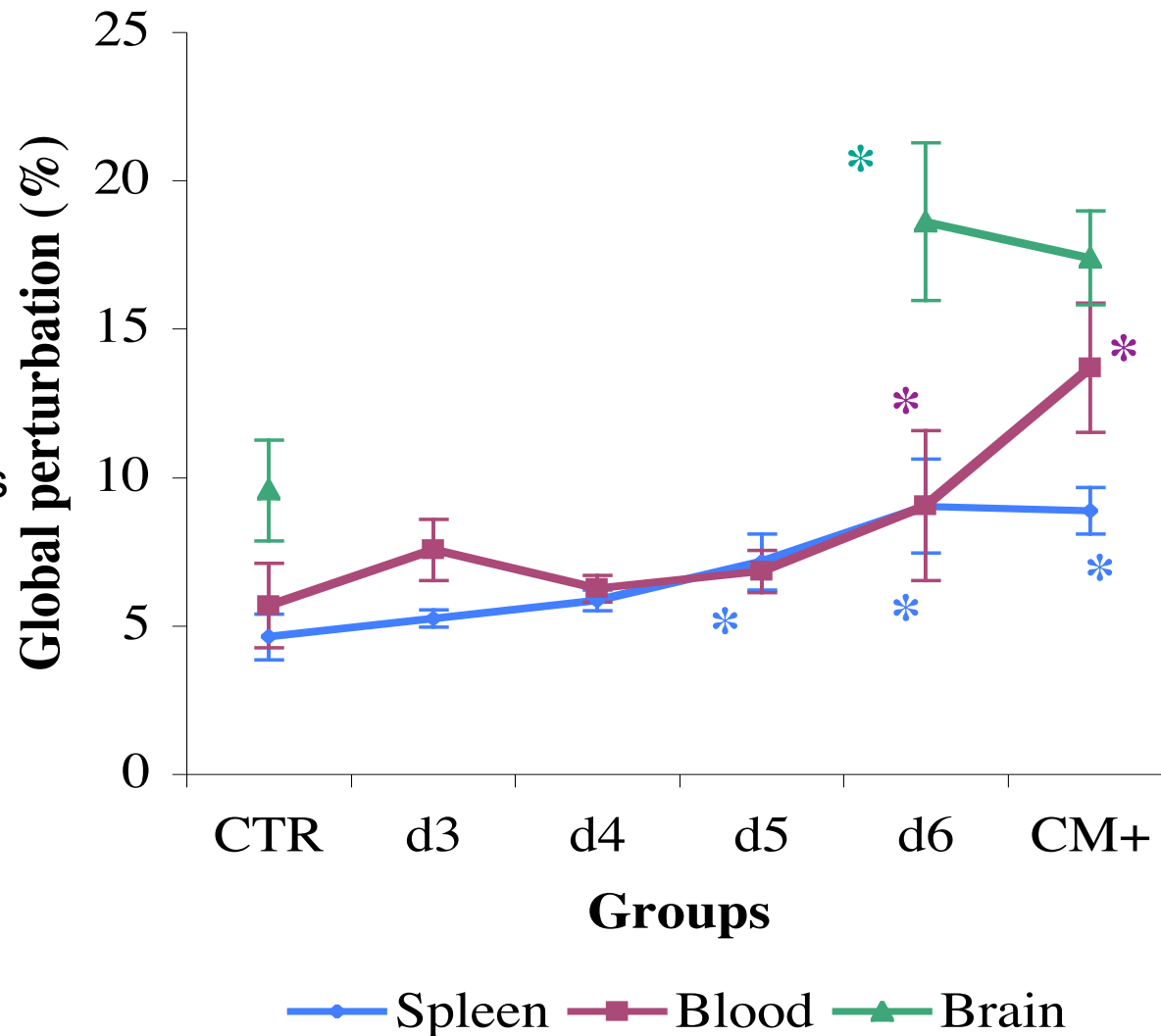
	TCRVO1	TCRVO2	TCRVO3	TCRVO4	TCRVO5.1	TCRVO5.2	TCRVO6	TCRVO7	TCRVO8.1	TCRVO8.2	TCRVO9	TCRVO10	TCRVO11	TCRVO12	TCRVO13	TCRVO14	TCRVO15	TCRVO16	TCRVO17	TCRVO18	TCRVO19
TCR01	2.85	4.96	3.73	excluded	11.94	6.31	4.13	3.14	3.29	2.21	4.25	9.12	2.72	10.92	10.83	15.63	7.12	4.06	9.39	4.27	6.10
TCR02	2.85	7.60	1.27	excluded	10.72	6.89	4.02	2.83	2.77	2.77	4.85	excluded	2.77	2.77	2.77	15.63	7.12	4.06	9.39	4.27	6.10
TCR03	2.85	excluded	4.93	excluded	12.33	6.31	4.13	3.14	3.29	2.21	4.25	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR04	2.85	4.24	4.24	4.24	10.72	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR05	2.85	1.74	3.22	3.09	11.73	7.10	4.33	3.00	4.36	5.66	3.80	6.13	5.47	11.87	6.52	15.14	8.45	3.42	3.99	5.10	10.56
TCR06	2.85	3.46	3.46	3.46	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR07	3.28	33.85	5.00	excluded	5.75	4.30	4.87	3.19	4.46	3.99	3.19	3.38	3.47	19.71	11.85	12.55	3.40	3.11	4.48	10.20	20.21
TCR08	3.28	3.28	3.28	3.28	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR09	5.05	4.48	4.24	2.92	8.79	4.18	4.18	2.38	3.82	1.74	4.41	excluded	5.88	11.20	2.39	18.12	2.30	3.47	2.59	excluded	excluded
TCR10	5.05	4.44	4.44	4.44	6.67	4.09	4.09	3.47	4.07	3.47	4.07	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR11	3.01	3.10	4.40	3.26	10.80	3.67	3.07	4.00	3.66	3.79	2.08	11.27	3.40	21.02	7.79	18.12	2.30	3.47	2.59	excluded	excluded
TCR12	3.01	3.01	3.01	3.01	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR13	3.67	4.14	excluded	4.23	7.11	4.00	3.28	6.19	4.40	2.80	6.65	excluded	2.26	14.63	8.26	16.83	5.60	8.79	2.95	5.01	4.21
TCR14	3.67	4.14	4.14	4.14	10.72	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR15	4.24	6.26	3.70	3.34	6.49	10.53	3.44	4.28	3.98	4.40	2.80	excluded	2.65	5.63	5.31	12.50	10.09	3.48	3.44	3.41	4.28
TCR16	4.24	6.26	4.24	4.24	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR17	5.67	3.67	4.93	excluded	9.38	3.83	3.83	2.16	6.37	4.30	6.30	excluded	3.41	11.81	7.73	18.01	3.50	excluded	4.02	7.95	18.45
TCR18	5.67	3.67	3.67	3.67	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR19	7.10	6.87	5.92	6.00	14.74	4.91	5.80	5.35	3.43	5.27	4.33	excluded	7.15	18.39	5.49	13.46	6.78	3.77	4.40	10.31	19.74
TCR20	7.10	6.87	6.87	6.87	17.86	4.91	5.80	5.35	3.43	5.27	4.33	excluded	7.15	18.39	5.49	13.46	6.78	3.77	4.40	10.31	19.74
TCR21	9.81	3.61	3.61	3.61	11.90	2.41	4.81	2.97	4.01	2.97	2.97	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR22	9.81	3.61	3.61	3.61	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR23	3.32	24.38	2.71	3.42	10.80	5.76	8.84	3.10	3.10	5.58	2.22	14.60	4.96	excluded	10.24	15.92	3.03	5.75	5.30	4.85	11.43
TCR24	3.32	3.32	3.32	3.32	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR25	5.71	7.59	4.95	8.25	2.91	4.22	6.46	2.57	3.32	6.76	4.70	excluded	10.52	12.68	10.38	12.26	6.07	5.73	5.59	7.95	5.11
TCR26	5.71	7.59	7.59	7.59	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR27	6.09	6.00	4.22	7.24	22.22	5.46	4.26	2.09	4.41	4.43	4.43	excluded	5.03	11.46	6.41	14.13	6.08	2.73	2.19	9.07	13.65
TCR28	6.09	6.00	6.00	6.00	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR29	7.25	6.66	3.56	6.00	11.73	7.10	4.33	3.00	3.00	3.00	3.00	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR30	7.25	6.66	6.66	6.66	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR31	6.15	8.64	10.82	3.73	9.44	7.14	excluded	2.05	3.41	3.82	2.04	21.74	4.35	16.95	10.32	20.40	7.14	5.49	10.59	13.58	6.81
TCR32	6.15	8.64	8.64	8.64	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR33	7.14	7.24	5.80	3.87	29.13	8.26	6.83	5.88	3.20	4.05	3.18	32.93	2.79	18.07	11.81	16.67	5.87	3.40	5.82	7.33	12.49
TCR34	7.14	7.24	7.24	7.24	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR35	4.35	7.16	8.84	3.89	excluded	4.00	7.20	4.95	4.75	6.05	4.46	4.73	12.55	12.78	32.64	4.80	4.38	5.86	16.88	8.56	16.88
TCR36	4.35	7.16	7.16	7.16	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR37	4.74	17.65	20.31	13.53	23.00	23.50	6.79	6.73	18.26	14.32	5.05	6.76	7.80	10.98	9.50	18.36	excluded	13.20	16.09	17.76	23.05
TCR38	4.74	17.65	17.65	17.65	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR39	11.10	14.05	15.54	7.94	32.71	8.40	8.27	2.40	3.68	7.02	7.02	excluded	11.96	18.57	excluded	20.12	3.64	5.78	9.36	14.76	16.81
TCR40	11.10	14.05	14.05	14.05	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR41	15.52	5.91	11.04	8.48	24.64	12.63	11.90	10.40	6.11	9.61	18.68	7.55	10.90	11.69	15.47	10.20	11.32	10.26	20.09	18.77	20.59
TCR42	15.52	5.91	5.91	5.91	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR43	20.65	10.39	20.46	8.28	21.13	11.08	12.89	5.39	8.89	7.69	5.69	excluded	10.88	16.99	7.67	13.63	6.39	9.45	7.06	15.76	10.57
TCR44	20.65	10.39	10.39	10.39	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR45	20.65	10.39	20.65	20.65	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR46	20.65	10.39	20.65	20.65	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR47	20.65	10.39	20.65	20.65	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR48	20.65	10.39	20.65	20.65	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR49	20.65	10.39	20.65	20.65	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR50	20.65	10.39	20.65	20.65	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR51	20.65	10.39	20.65	20.65	13.89	6.89	4.02	2.83	2.77	2.77	4.85	excluded	6.30	14.67	9.08	15.63	7.12	4.06	9.39	4.27	6.10
TCR52	20.65	10.39	20.65	20.65	13.89	6.89	4.02	2.83	2.77	2.77</											

7. Results (5)

Global TcR BV perturbation



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



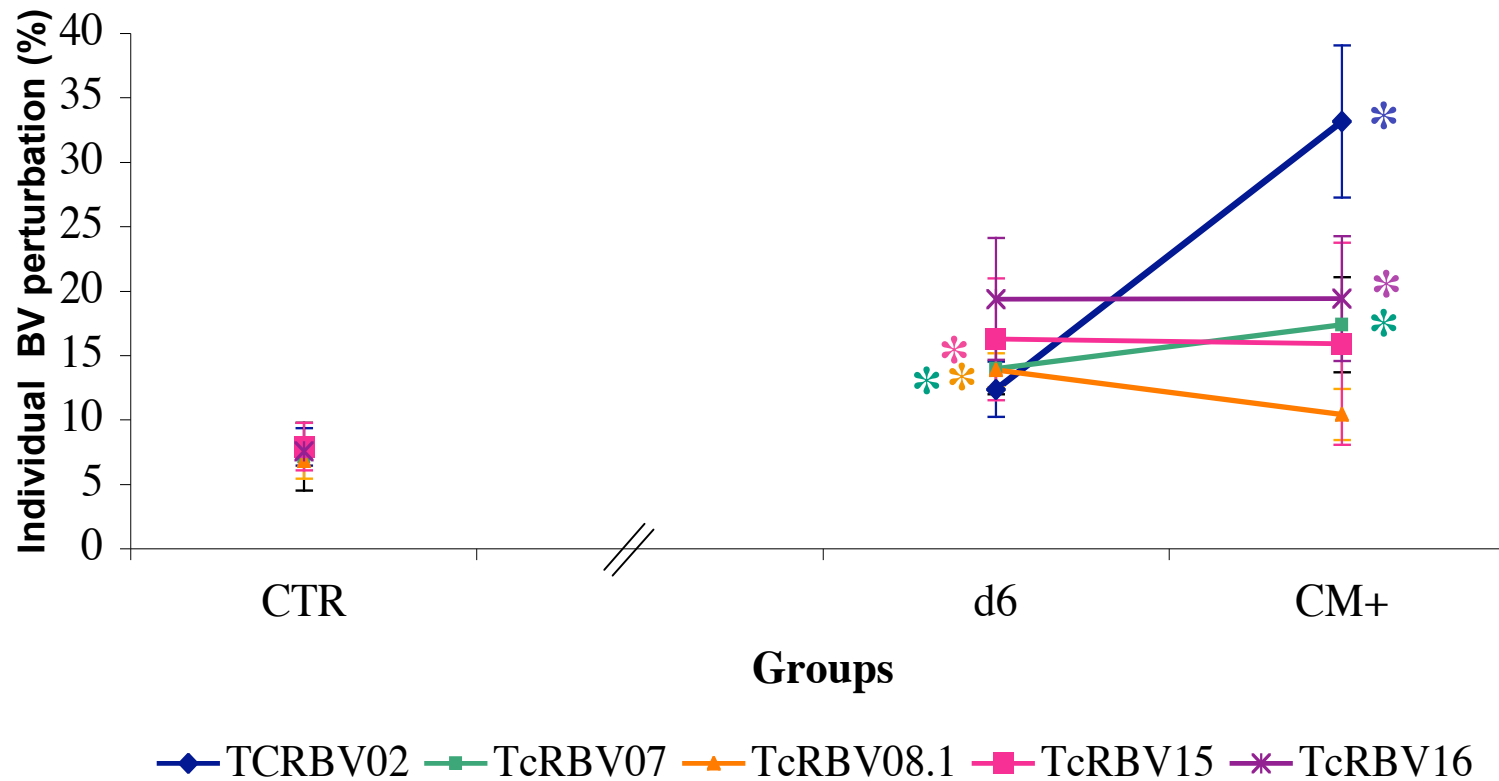
Progressive increases of the perturbation

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 TcR β 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

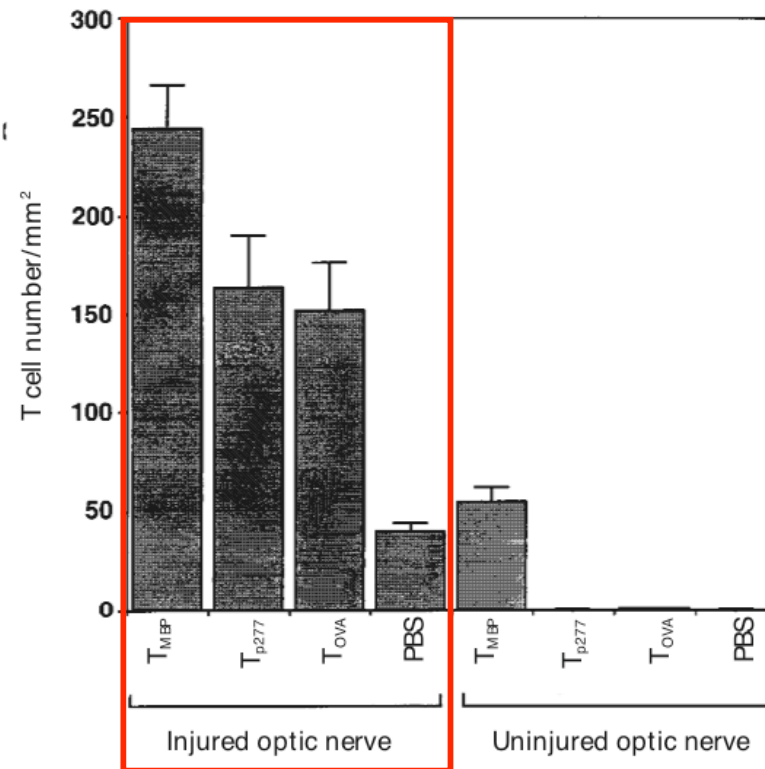


Figure 1

=> 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

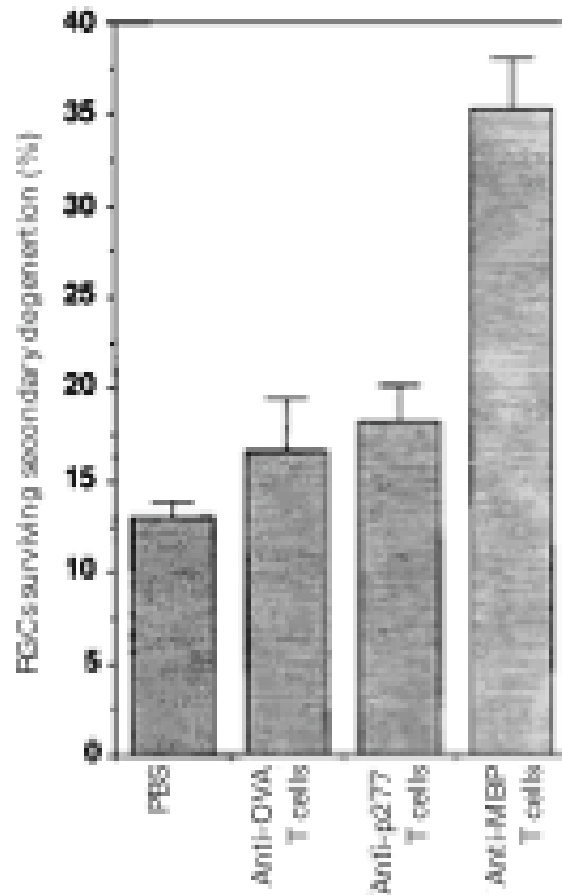
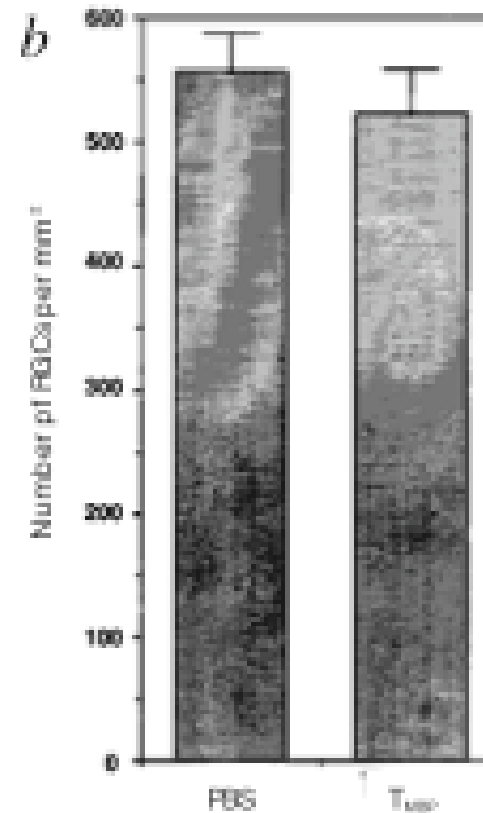


Figure 4b



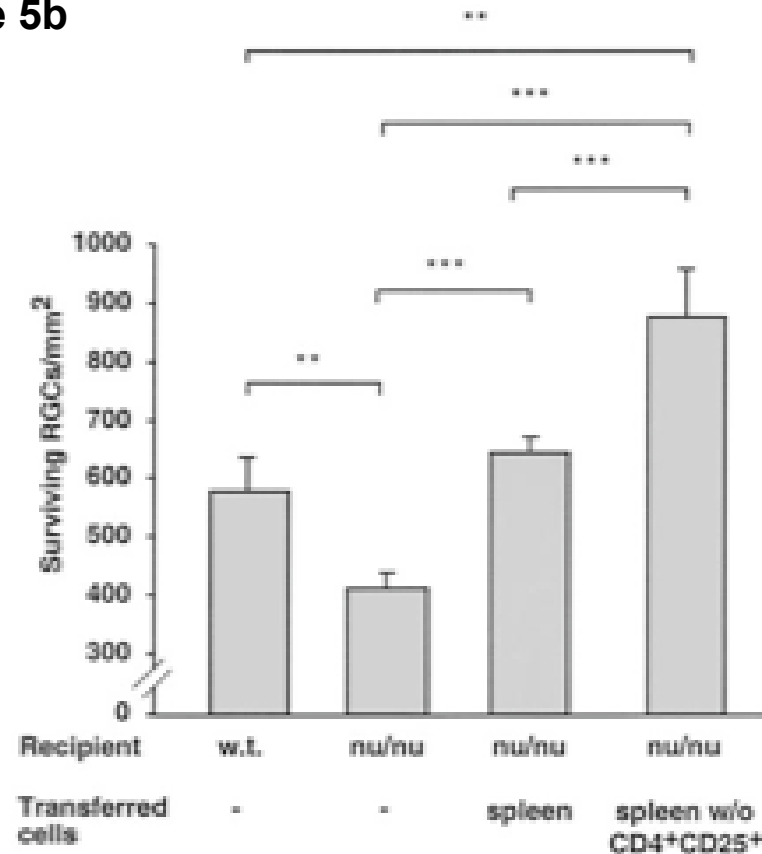
Les cellules T rencontrant leur antigène (donc auto-réactives) facilitent la réparation de la lésion (Figure 2), mais ne sont pas agressives en contexte physiologique (Figure 4b).

(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

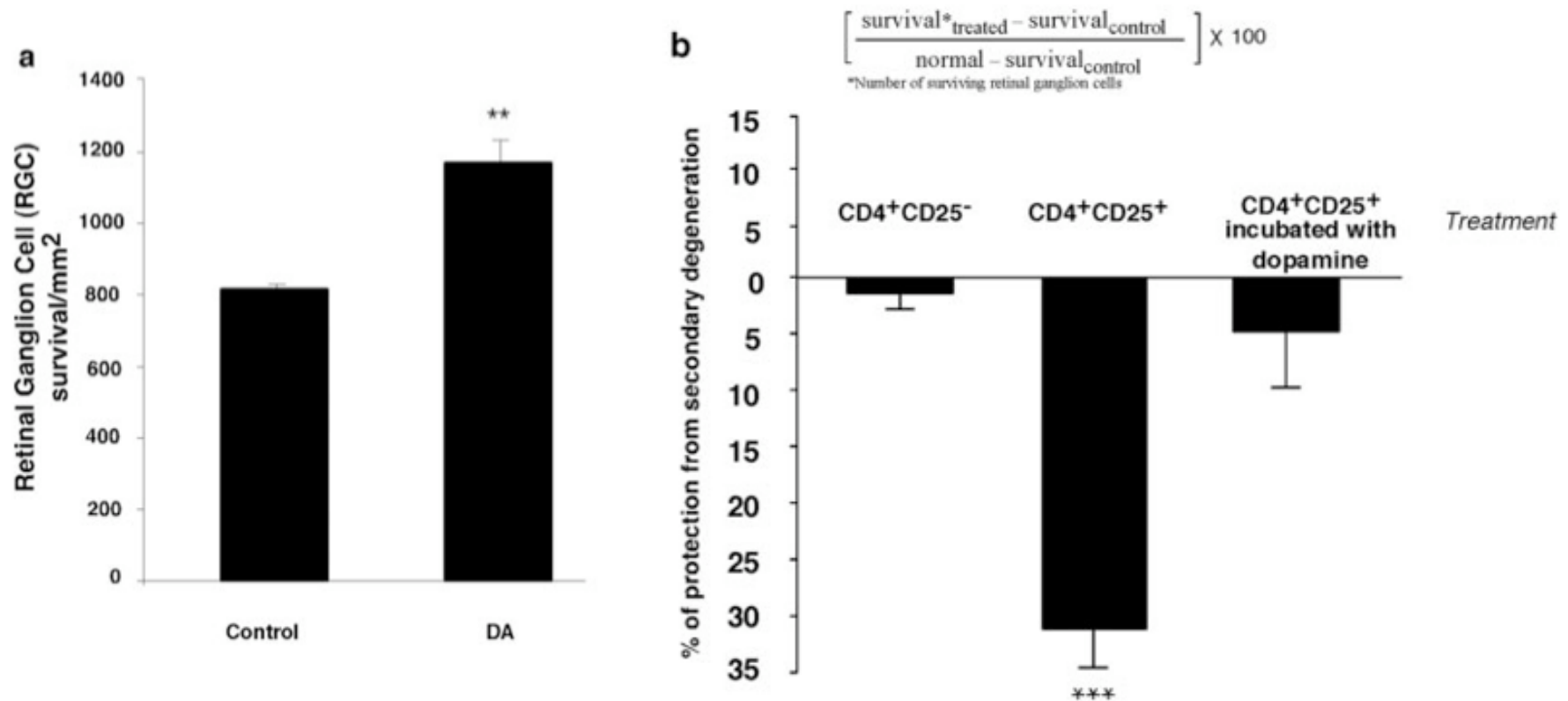


=> Régulation de l'auto-immunité naturelle par les cellules régulatrices

(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**

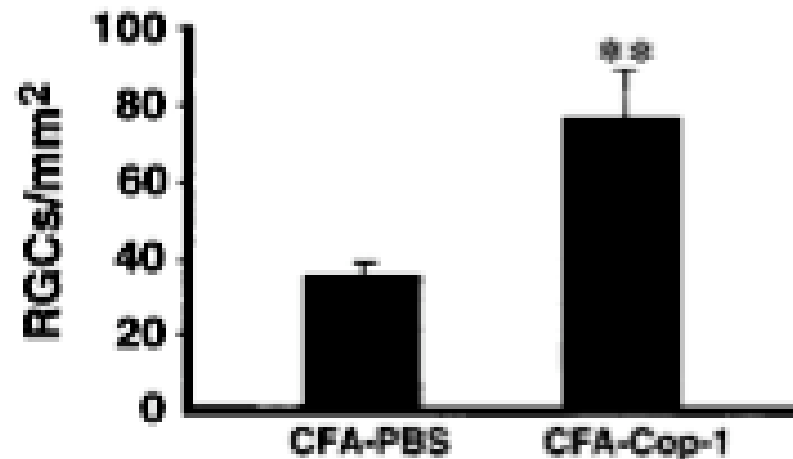
(Kipnis, J et coll, J. Neuroscience, 2004 vol.24, pp 6133–6143)

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 :



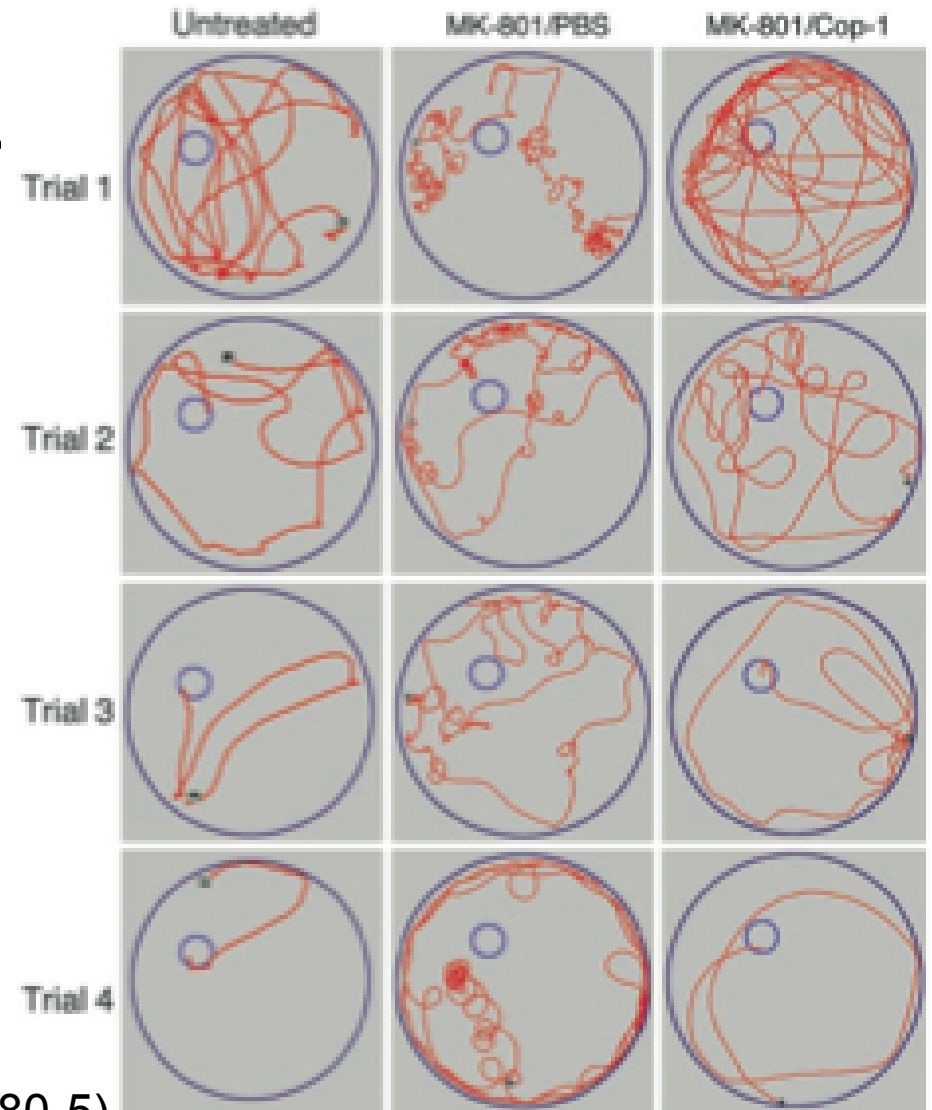
(Kipnis, J et coll, PNAS, 2000, vol. 97, pp.7446-7451)

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 envisager 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 Voegtli
- Christèle Sellier

***URA CNRS 1961, Institut Pasteur & Université
Pierre et Marie Curie (Paris 6)***