Innate immune memory process in *Biomphalaria glabrata* snails: a comparative multi-omic approach to decipher the function and role-played by the hemocyte immune cells

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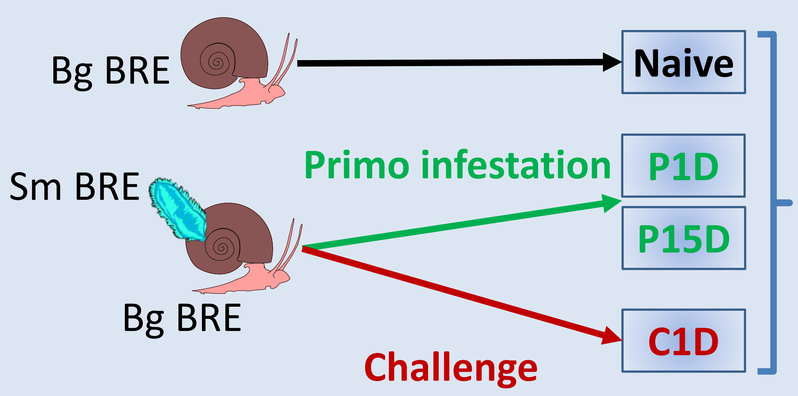
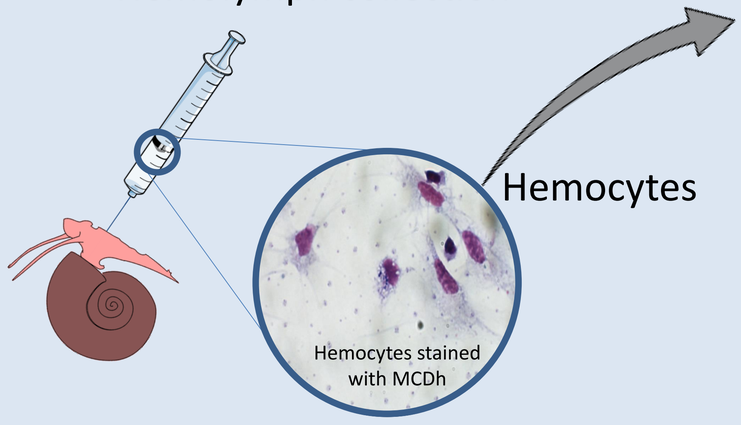
First molecular characterization of *B. glabrata* hemocyte populations at a single cell level

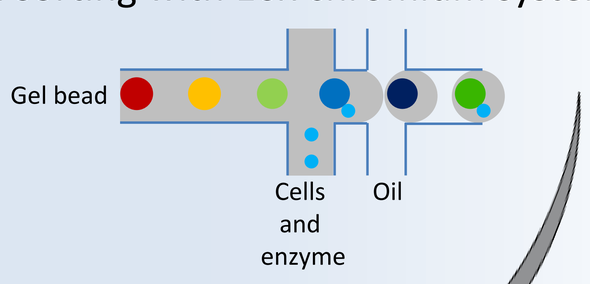
In the early 2000's, several studies have highlighted the ability of invertebrates immune system to be primed, protecting the individual when meets the same pathogen twice during its life span, this process is called « innate immune memory » (IIM). This IIM has recently been demonstrated for *B. glabrata* snails against *S. mansoni* infestation and it is characterized by a shift from a cellular response to a humoral response, certainly carried by the snail innate immune cells, the hemocytes.

Objectives : Identify hemocyte sub-populations of *B. glabrata* involved in IIM response towards *S. mansoni* parasite based on different transcriptomic profiles using Single-Cell RNA sequencing technology

scRNA seq protocole





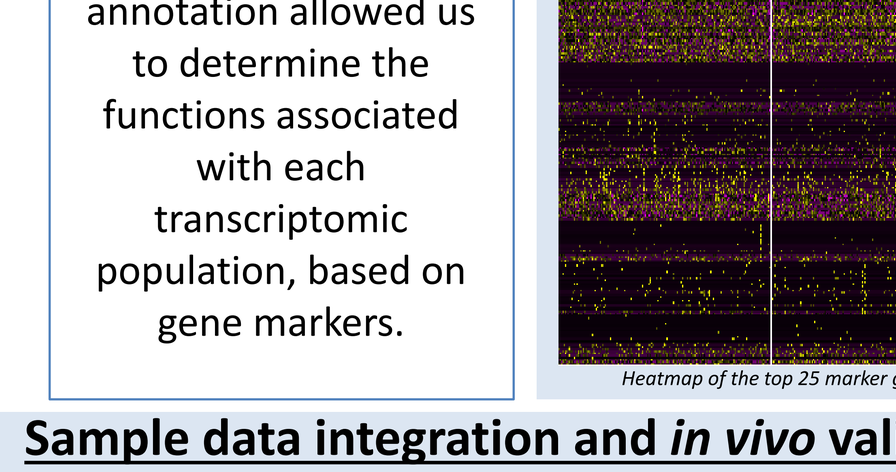


RNA sequencing using Novaseq technology from the MGX platform

Hemocyte samples recovery for naives, primo infested and challenged snails.

In silico analysis using Cell ranger and Seurat

Hemocyte transcriptomic populations



T-disctributed stochastic neighbor embedding (tSNE) projection of 10 naive hemocyte clusters

Results in number :

Genome mapping rate : 90,1%

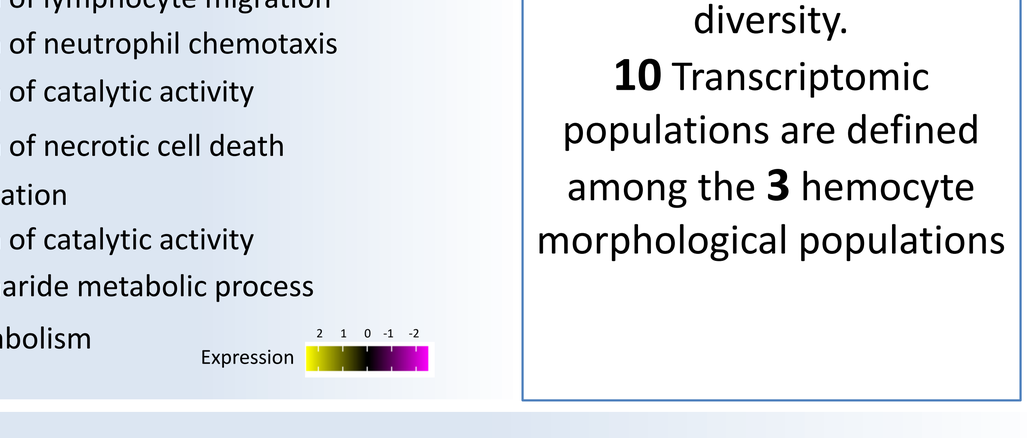
Recovered cells : 2 073

Unique genes detected : 16 926

Median genes per cell : 1 885

Number of reads : 185 257 268

Hemocyte morphological populations

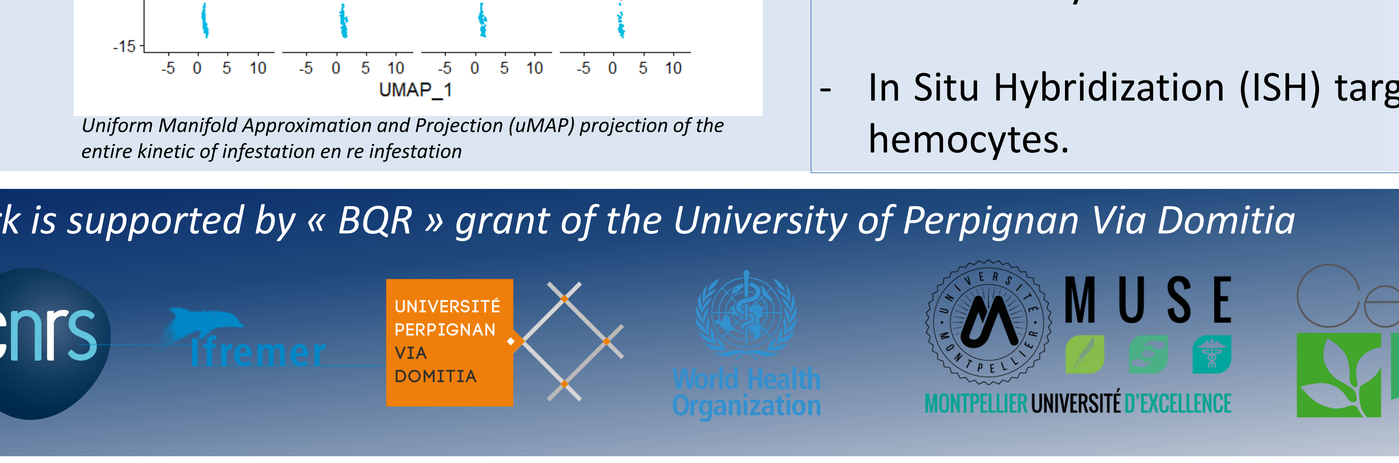


Granulocytes Hyalinocytes Blast like cells

Light microscopy imaging of the three hemocyte populations from B. glabrata stained with MCDh

Enrichment analysis using Gene Ontology annotation allowed us to determine the functions associated with each transcriptomic population, based on gene markers.

Transcriptomic populations and gene markers

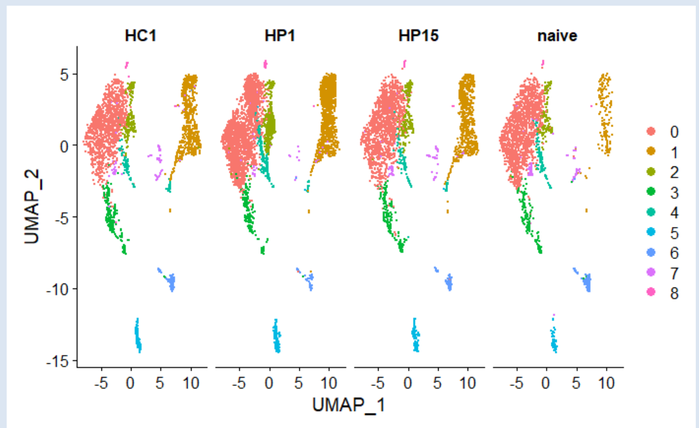


Heatmap of the top 25 marker genes by transcriptomic cluster and gene ontology enrichment

Use of scRNA seq allows us to demonstrate a greater hemocyte population diversity.
10 Transcriptomic populations are defined among the **3** hemocyte morphological populations

Sample data integration and *in vivo* validation

Integration of all data may allow us to identify the transcriptomic populations able to respond to the infection and thus support IIM.



Uniform Manifold Approximation and Projection (uMAP) projection of the entire kinetic of infestation en re infestation

Two technics will be used to make the link between hemocyte transcriptional and morphological populations :

- Flow cytometry technique : sorting hemocytes based on morphological characteristics and test each population using qPCR for expression of gene markers previously identified by scRNA.
- In Situ Hybridization (ISH) targeting scRNA gene markers on hemocytes.

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