

Team : IALTA Under the supervision of Pr Gilles Foucras UMR INRAE ENVT 1225 – IHAP



REIDSOCS project: Description of lactating mammary gland immune cells using single-cell RNA-Seq approach.



Problematic of REIDSOCS project: milk production

Mammary gland:





Milk : Positive trade balance of 3.5 billions €

Milk quality decreases → 300 000 somatic cells (CCS)/mL

EC 2004/853 standard, April 29th 2004 → 400 000 cells/mL





→ Mastitis: mammary gland infections

Problematic of REIDSOCS project: ewe genetic mutation







SNP SOCS-2 (suppressor of cytokine signaling 2)

Rupp Et.al 2015

SOCS-2: negative regulator of multiple signaling pathways:



Non functionnal SOCS-2 protein

Bullock AN, [...], Knapp S, PNAS 2006

Aims of the REIDSOCS project

- Determine the immune cells composition of lactating mammary gland using unprior approach: scRNA-Seq
- Compare the immune cells composition between WT and SOCS-2 R96C KI mice using flow cytometry and scRNA-Seq
- Identify the immune function alteration during Staphylococcus aureus infection associated with SOCS-2 R96C phenotype

SOCS2^{R96C} mice







SOCS-2 invalidation: phenotype confirmation (gigantism)

Mammary gland immune cells transcriptome by scRNA-Seq









Multiplexing using CD45 TotalSeq antibody (biolegend)



FACS sort of CD45+ viable cells



scRNA-SEQ libraries (10X genomics) + NGS Illumina sequencing

6

Systemic injection of CD45 antibody



Lactating mammary gland (without LN) cells extraction



Cellular composition

Gene expression levels



Data analysis

1) FACS sort



Thanks to Alexia Zakaroff-Girard and Elodie Riant

2) Librairies preparation

We inject 18000 cells > almost 6000 sequencing cells (catch rate of 33%) in 2 injection wells.





Thanks to Frederic Martins

3) Analysis pipeline of scRNASEQ data analysis



https://hoohm.github.io/CITE-seq-Count/ https://satijalab.org/seurat/ https://cole-trapnell-lab.github.io/monocle3/ Lane A Cellranger results

Lane B Cellranger results





17,407 Post-Normalization Mean Reads per Cell 16,744 Nedian Genes per Cell 1,360

Estimated Number of Cells

Cells	
Estimated Number of Cells	17,407
Fraction Reads in Cells	88.6%
Pre-Normalization Mean Reads per Cell	16,989
Post-Normalization Mean Reads per Cell	16,744
Median Genes per Cell	1,360
Median UMI Counts per Cell	4,232

Sequencing	
Pre-Normalization Number of Reads	295,740,486
Post-Normalization Number of Reads	291,469,620

Aggregation	
SOCS1 Fraction of Reads Kept	97.1%
SOCS2 Fraction of Reads Kept	100.0%
SOCS1 Pre-Normalization Total Reads per Cell	17,238
SOCS2 Pre-Normalization Total Reads per Cell	16,747
SOCS1 Pre-Normalization Confidently Mapped Barcoded Reads per Cell	10,135
SOCS2 Pre-Normalization Confidently Mapped Barcoded Reads per Cell	9,843

Sar	nple
Name	SOCS
Description	
Transcriptome	mm10
Chemistry	Single Cell 3' v3
Cell Ranger Version	3.0.2

Demultiplexing with hashtag oligos and doublet exclusion under Seurat



Ridge plots indicated the enrichment for selected HTOs



UMAP representation of the cell clusters (n=19) of all WT and KI mices



CD45 expression level in the cell clusters - All mices





2913 cells 560 cells 597 cells 449 cells 3624 cells 523 cells

UMAP representation of CD45 positive cells – Without WT2 (LN)



Clusters cell type automatic attribution: clustifyrdata



https://github.com/rnabioco/clustifyrdata

UMAP representation of CD45 positive cells – Without WT2 (LN)



Specific macrophages supopulations in lactating mammary gland?



Monocle analysis on F4/80 CD11c macrophages supopulations



WT vs KI immune cell composition difference? – scRNA-Seq and HTO



Macrophages and neutrophils altered in KI phenotype?

4) Immunophenotyping of SOCS2^{R96C} mice by flow cytometry

Phenotying analysis strategy by flow cytometry (Miltenyibiotec MACSQ10)



Lactating MG (d7) of females WT (n = 10) vs KI (n = 10)





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scRNA-Seq

WT

KI





Conclusions



eol

Prospects on REIDSOCS project



Identification of SOCS-2 function during Staphylococcus aureus infection in mice lactating mammary gland

> Lactating females (7-10 days)



Determine the transcriptome of both immune and non immune lactating mammary gland cells during bacterial infection (Staphylococcus aureus) with spatial information

Collaboration with Spatial Transcriptomics Pilot Facility (Annelie Mollbrink), Sweden

THANK YOU FOR YOUR ATTENTION













Centre Régional d'Exploration Fonctionn et Ressources Expérimentales

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Genotoul Bioinfo

Genotoul

GeT





Poids des 2 glandes mammaires N°4



N = 5 WT / 8 KI

CD45 positive cells – Without WT2 (LN)



Description	P-value	<u>FDR q-value</u>	Cluster
immune response	2.87E-4	1	P2ry6_macro
antigen processing and presentation of pentide or polysaccharide antigen via MHC class II	5.09F-4	1	P2rv6 macro
antigen processing and presentation of peptide antigen via MHC class II	5.09E-4	7.79E-1	P2ry6_macro
antigen processing and presentation of peptide antigen	6 36E-4	7 3E-1	P2ry6_macro
antigen processing and presentation	6 36E-4	5 84E-1	P2ry6_macro
cytokine-mediated signaling nathway	6.81E-5	2.26E-1	Csn3 macro
signal transduction	1 82E-4	3.02E-1	Csn3 macro
granulocyte chemotaxis	2 3E-4	2 55E-1	Csn3 macro
	2.3E-4	1 91E-1	Csn3 macro
neutrophil migration	2.3E-4	1.51E 1	Csn3 macro
myeloid leukocyte migration	2.3E-4	1.33E 1 1.27E-1	Csn3 macro
cellular amide metabolic process	4 68F-6	8.00F-03	Socs3 macro
translation	1 14E-5	9 77E-3	Socs3 macro
amide biosynthetic process	1 14F-5	6 51E-3	Socs3 macro
neptide metabolic process	1 14E-5	4 88E-3	Socs3 macro
peptide inclusione process	1.14E-5	3.91F-3	Socs3 macro
negative regulation of developmental process	1.14E 9	6 5E-1	CD206 macro
cell differentiation	3 14E-4	6 15E-1	CD206_macro
positive regulation of chemotaxis	7.01E-4	9 15E-1	CD206_macro
regulation of anatomical structure morphogenesis	8.07E-4	7 9F-1	CD206_macro
negative regulation of cell proliferation	9.68F-4	7 58F-1	CD206_macro
	9.001 4	7.502 1	
cell cycle	2.83E-20	8.48E-17	Birc5_macro
cell cycle process	5.36E-20	8.02E-17	Birc5 macro
cell division	3.25E-16	3.24E-13	Birc5_macro
mitotic cell cycle process	1.54E-14	1.15E-11	Birc5_macro
regulation of cell cycle	2.52E-13	1.51E-10	Birc5_macro
regulation of cell cycle process	5.52E-10	2.76E-7	Birc5_macro
defense response	9.87E-11	6.52E-8	Mx1_macro
response to other organism	5 49E 10	1 91E 7	Mul maaro
defense response to virus	1.16E 0	3 20E 7	Mx1_macro
	5 70E 0	1.42E.6	Mx1_macro
defense regnonse to other organism	J.79E-9	2.45E.6	Mx1_macro
	1.3/E-0 2.32E 8	2.43E-0 1.62E-6	My1 macro
immune affector process	2.33E-0 3.22E 0	4.02E-0 5.81E-6	My1 magro
	3.23E-0	5.01E-0	My1 magro
immune response	1.62E 7	2.4JE-0	My1 magro
response to besterium	1.02E-7	2.4/E-J 0.1/E-2	My1 magro
cellular response to interform alpha	1.30E-3	9.14E-3 1 25E 2	My1 magro
regulation of innate immune regenese	1.10E-4	1.53E-2 1.50E-2	My1 macro
	1.44E-4	1.57E-2	IVIAT IIIaCIU

CD45 positive cells – Without WT2 (LN)

	WT1	WT3	KI1	KI2	КІЗ	wт	КІ
P2ry6_macro	28%	31%	20%	17%	27%	29%	21%
Csn3_macro	32%	7%	17%	21%	17%	20%	18%
Socs3_macro	25%	30%	12%	21%	21%	27%	18%
NK_cells	0%	9%	12%	5%	2%	5%	6%
Neutro	1%	5%	11%	7%	4%	3%	7%
CD206_macro_DC	2%	5%	5%	10%	4%	3%	6%
CD127_LT	4%	5%	6%	4%	1%	4%	4%
CD27_LT	0%	4%	7%	2%	1%	2%	3%
Birc5_macro	5%	0%	3%	3%	7%	3%	4%
B_cells	0%	0%	0%	7%	9%	0%	5%
Mx1_macro	2%	3%	2%	2%	2%	2%	2%
Cd3_Cd14_macro	0%	0%	4%	0%	0%	0%	1%
Tk1_macro	1%	1%	1%	1%	4%	1%	2%

Immunophenotyping of SOCS2^{R96C} mice by flow cytometry

Phenotying analysis strategy by flow cytometry (Miltenyi MACSQUANT10)

	Fluorochome	Target	Cells
V1	Pacific blue	Ly-6G	Neutrophils
V2	VioGreen	CD45	Immune cells
B1	FITC	Viobility Fixable Dye	Dead cells
B2	PE	Nkp46 (CD335)	NK cells
B3	PE-Vio615	CD19	LB
B4	PeCy7	CD11c	Myeloid cells (DC / macro)
R1	APC	F4/80	macrophages
R2	APC-Vio770	CD3	LT

Females WT (n = 10) vs KI (n = 10)

Lactating MG (day=7)



SOCS2^{R96C} mice: size alteration



NanoScan PET/CT, Mediso (Service Exploration Non-Invasive, CREFRE)



