From T cell receptor repertoire to systems immunology: an integrative translational approach for biomarkers discovery

Encarnita Mariotti-Ferrandiz

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SCIENCES
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CANDIDATURE A

L'HABILITATION A DIRIGER DES RECHERCHES

Spécialité : IMMUNOLOGIE

Encarnita Mariotti-Ferrandiz

Maitre de conférences en Immunologie

Sorbonne Université

From T cell receptor repertoire to systems immunology: an integrative translational approach for biomarkers discovery

24 Octobre 2019

Jury : Dr. Anne Eugster
Dr. Nicolas Fazilleau
Dr. Sylvie Garcia
Dr. Frédérique Peronnet
Prof. Rodolphe Thiébaut
Dr. Gur Yaari

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UMRS959 (INSERM/SORBONNE UNIVERSITE)
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Paris – France

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This manuscript represents an additional step in my career as an academic scientific researcher, a step that I reached thanks to key mentors I met, worked with and, for several of them, still work with since my first footsteps in research. Prof. Pierre-André Cazenave and Prof. Adrien Six, for their initial support when I was a young MSc student and for offering me the initiatory journey in systems immunology at a time when such approach was not really supported. Dr. Shohei Hori, for trusting me when we met in Pasteur Institute before I completed my PhD and offering me a unique experience as a post-doc in his laboratory. And finally, Prof. David Klatzmann who welcomed me 7 years ago to join his lab. I am particularly grateful for his trust, support, and for sharing his ambition, vision, knowledge and sense of challenges.

Thank you also to all those persons who contributed from near or far to my scientific shaping, Sophie Dulauroy, Olivier Gorgette, Jacques Roland, Sylviane Pied, Petter Hoglund, Jelena Berglund-Petrovic, Valerie Soulard, Dominique Rueff-Juy, Anne-Marie Drapier, Aude Thiriot, Sidonia Fagarasan, Osami and Edith Kanagawa, Gwladys Fourcade, Wahiba Chaara, … and all the members of the different labs and environments I have been navigating through. A special thanks to Caroline Aheng, a key person in the i3 laboratory, without who my last year's achievements would have been way more difficult.

A second special thank also to Adrien Six, Bertrand Bellier, Stéphanie Graff-Dubois and all the teaching iTeam for welcoming me in their innovative and active team at Sorbonne Université and offering me the opportunity to build-in new courses and tracks for students. Your experience and enthusiasm have been, are and will be critical in my professional and personal development.

I want to thank also the students I have been in charge of, especially Pierre Barennes and Vanessa Mhanna. Thank you for your trust, your dedication and I wish you the best once we will be done!

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à Emi, Leia et Fred
à mes parents

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Abbreviation list

AAV-IL2	Adeno-associated virus expressing IL2
AID	Autoimmune and Inflammatory disease
AIRR	Adaptive Immune Receptor Repertoire
APC	Antigen presenting cell
BCR	B-cell receptor
CD4SP	CD4 single positive thymocyte
CD8SP	CD8 single positive thymocyte
CDR3	Complementary Determining Region 3
CM	Cerebral malaria
dLN	deep lymph node
DPCD3-	Double positive CD3 positive thymocyte
DPCD3+	Double positive CD3 negative thymocyte
eCRF	electronic case report form
Ig	Immunoglobulin
IL2	Interleukine 2
ILN	inguinal lymph node
IPEX	Immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrom
IQR	Interquartile range
LN	Lymph node
MHC	Major Histocompatibility Complex
MI	Myocardial infarction
m-PCR	multiplex PCR
NGS	next generation sequencing
NOD	Non-Obese Diabetic
nt-ES	nuclear-transferred embryonic stem cell
PbA	Plasmodium berghei ANKA
PCA	Principal component analysis
PLN	pancreatic lymph node
RA	Rheumatoid arthritis
RACE-PCR	Rapid amplification of cDNA-ends by PCR
RTE	recent thymic emigrant
RT-PCR	reverse transcription and PCR
SLE	Systemic lupus erythematous
sLN	superficial lymph node
T1D	Type-1 Diabetes
TCR	T-cell Receptor
Teff	Effector T cell
Tfh	Follicular helper T cell
Tfr	Follicular helper Regulatory T cell
TRAJ	TCR alpha J gene
TRAV	TCR alpha V gene
Treg	Regulatory T cell

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Curriculum Vitae

Encarnita MARIOTTI-FERRANDIZ

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Assistant-Professor, UFR927, Immunology department, Sorbonne Université

EDUCATION

2007	PhD in Immunology, Université P. et M. Curie (UPMC- Paris 6, France)
2003	Certificate of Advanced Immunology course, Pasteur Institute (France)
2003	Master of Science, Immunology, UPMC-Paris 6 (France)
2001	Bachelor of Science, Cell biology and physiology, UPMC-Paris 6 (France)

CAREER

Since 2014	**Assistant-Professor**, Sorbonne Université (SU), Paris (France) UMRS959, Immunologie - Immunopathologie - Immunothérapie, Pr. Klatzmann (SU, Paris)
2012	**Qualification as an Assistant-Professor applicant**
2012-2014	**Senior research scientist**, UPMC, Paris (France) UMRS959, Immunologie - Immunopathologie - Immunothérapie, Pr. Klatzmann (UPMC, Paris)
2008-2012	**Research scientist**, RCAI-Riken, Yokohama (Japon) Immune homeostasis, Dr. S. Hori
2003-2007	**PhD Student**, UPMC, Paris (France) Immunophysiopathologie infectieuse (IPPI) (Institut Pasteur, France), Pr. P-A. Cazenave and Pr. A. Six

AWARDS AND FELLOWSHIPS

2017	FOCIS 2017 Center of Excellence, Poster of Excellence
2008-2010	Post-doctoral fellowship, Japan Society for Promotion of Science (JSPS)
2006-2007	Doctoral fellowship, Fondation de France & CANAM, Institut Pasteur de Paris FOCIS 2006 Poster of Distinction
2003-2006	Doctoral fellowship (MNERT - French Ministry of research and education)
2002-2003	Study fellowship (UPMC)

RESEARCH TOPICS AND MAJOR ACHIEVEMENTS

Since 2012	**Coordination of human and mouse T cell receptor (TCR) repertoire studies, in health and disease** - Set-up and implementation of TCR repertoire analysis by next-generation sequencing: from molecular biology to computational analysis (*« Publication list »: 3 published articles n°T8, T11, T12; 1 review n° R2; 1 article in revision n°P1 and 2 article in preparation n°P3 & P5*). **-** Precise characterization of follicular regulatory T cells (Tfr) and their TCR repertoire in Mice (*« Publication list »: 3 published articles n°T9, T10, T13*)

- Analysis of the TCR repertoire diversity of regulatory T cells (Treg): Demonstration of major expansions within activated/memory Tregs compared to naive Tregs and T effectors (*« Publication list »: 1 published article n°T6 ; 1 article in preparation n°P2*)

Coordination of Systems Immunology activity for the study of autoimmune and autoinflammatory diseases (AID)

- Molecular biology methods and protocols benchmarking for transcriptome, microbiota and TCR analysis and their implementation in observational and interventional clinical trials (*« Publication list »: 1 published article n°T15; 1 article in preparation n°P4*)

- Coordination of clinical data integration activity for the follow-up of patients with autoimmune and inflammatory diseases (*« Publication list »: 1 published article » n°T14*)

- Coordination of multiparametric data analysis (*« Publication list »: 1 published article » n°P4*)

2008-2012 **Analysis of the repertoire of murine Tregs by high-throughput sequencing** *:*

- Characterization of transient Treg subpopulations (*« Publication list »:* 1 published article n°T4)

- Development and implementation of an experimental protocol by high-throughput sequencing of the TCR repertoire in mice (*« Publication list »: 1 article in preparation n°P6*)

- Development of a tool for TCR sequences annotation from high-throughput sequencing (clonotypeR), (*« Publication list »:* 1 published article *n°T7*).

2003-2007 **Kinetics of TCR repertoire modifications in pathological murine models**

- Demonstration of major and progressive alterations of the entire TCR repertoire during mouse cerebral malaria and identification of pathological TCR signatures (*« Publication list »:* 1 published article *n*°T1, T8)

- Highlighting early alterations of the TCR repertoire in young NOD mice before spontaneous autoimmune diabetes diagnosis ((*« Publication list »:* 1 published article *n*°T3*, collaboration: Dr Hoglund and Dr Petrovic , Karolinska Institute*)

GRANT APPLICATIONS

2019 **EIT-Health BP2020**

Co-coordinator with D. Klatzmann **-** TRIGAM : T-cell receptor Repertoire Insights : offering a full service of data Generation, Analysis & Modelling. Stage 1.

2018 **SC1-BHC-05-2018 (H2020) – iReceptor+ -** Coordinator Dr. G. Yaari (Bar-Ilan University, Israel)

Workpackage leader - MultiOMICS data integration for integrated analysis of the T and B lympocyte directory - funded until 2022

ERA-NET on Cardiovascular Diseases (JCT2018/H2020) - Coordinator Dr. P. Rainer (Medical University of Graz, Austria)

Co-principal investigator - Identification of TCR biomarkers associated with myocardial infarction - funded until 2021

WB-2018-10690 (Fondation de France) – Coordinator Dr. P. Ellul (Hôpital Debré, France)

Collaborateur - immunophenotyping of patients with autism spectrum disorders - Project selected for autition

2017 **2018 "New research collaboration" program** (Franco-Canadian Fund for Research) - Coordinator Prof. F. Breden (Simon Fraser University, Canada)

Partner – unfunded

University Research Institute 1st call (Ecole Universitaire de Recherche , ANR): ImSysT project

Co-coordinator with Prof. D. Klatzmann - unfunded

2015	RHU (ANR) 2nd call - iMAP project - Coordinator Pr. D. Klatzmann **Workpackage leader** - Integrating and analyzing MultiOMICS data from patients with Systemic Lupus Erythematosus under Low-dose IL-2 Therapy - Funded until 2021 RHU (TRIADE) 1st call: TRiADE - Coordinator Pr. D. Klatzmann **Collaborator** - Integration and analysis of multiOMICS data - pre-selected but not funded

FELLOWSHIP AND AWARDS

2018	Teaching and research premium Sorbonne Université (2018-2022)
2017	FOCIS Center of Excellence Travel Award
2008-2010	Japan Society for Promotion of Science (JSPS) post-doctoral fellowship
2007	Pasteur Institute PhD fellowship
2006	Fondation de France PhD fellowship
	FOCIS 2006 Poster of Distinction
2003-2006	French government doctoral fellowship (MNERT)
2002-2003	University of Paris Merit fellowship

SCIENTIFIC COMMUNICATIONS (Selection)

2020	Germany	Control and Decision Making in Immune Repertoire Dynamics (IMMREP 20) – **invited speaker**
	France	Qlife Spring school - Systems Immunology - **invited speaker**
2019	Israel	3rd International symposium on *Stochasticity and Control of Adaptive Immune Repertoire* – **invited speaker**
	Italy	4th Adaptive Immune-Receptor Repertoire (AIRR) meeting - **invited speaker**
2018	France	2nd International symposium on *Stochasticity and Control of Adaptive Immune Repertoire* - **invited speaker**
2017	USA	FOCIS 2017 – **poster**
	USA	3rd Adaptive Immune-Receptor Repertoire (AIRR) meeting - **invited speaker**
2016	USA	2nd Adaptive Immune-Receptor Repertoire (AIRR) meeting - **invited speaker**
2015	France	Systems Immunology and Genetics of the Infectious Diseases (SIGID) symposium - **invited speaker**
2014	France	Quantitative immunology workshop - **invited speaker**
2013	France	ImmunocomplexiT symposium - **invited speaker**
2012	Japan	22nd Kyoto T Cell Conference (KTCC) - **invited speaker**
2011	Japan	40th Japanese Society of Immunology meeting - **selected oral presentation**
2010	Japan	2nd Synthetic Immunology Symposium - **invited speaker**
	Japan	14th International Congress of Immunology (ICI) – **poster**
2009	Japan	19th KTCC – **poster**
2006	France	1st European Congress of Immunology (ECI) – **poster**
	USA	6th Federation Of Clinical International Societies (FOCIS) Congress – **poster**
2005	France	Institut Pasteur Immunology Department Meeting - **selected oral presentation**
2003	France	Annual Congress of French Society of Immunology (SFI) - **selected oral presentation**

RESEARCH EVALUATION ACTIVITY

2018	**Thesis committee** Cédric Schleiss (ED 414, Université de Strasbourg) **«** *Anomaly of lymphocyte response after BCR stimulation in chronic lymphocytic leukemia»;* Supervisors Pr. Philippe Georgel & Dr. Laurent Vallat, defence December 21st, Strasbourg.

	Thesis committee Nicolas Coatnoan, (ED 515, Sorbonne Université) **«** *Trypanosoma cruzi proline racemase, a diagnostic and monitoring marker for Chagas disease? »;* Supervisor Dr. P. Minoprio**,** defence December 14th 2018, Paris.
Since 2018	**Member of selection of temporary research assistant (ATER),** Sorbonne Université, Faculty of Science, Biology department
Since 2017	**PhD mid-term committee** as Sorbonne University representative and scientific expert (ED394 et ED515) - 3 PhD fellows
Since 2015	**Referee** pour PlosOne, Journal of Immunological Methods, Bioinformatics, Frontiers in Immunology (publons.com/a/1422670/)

SCIENTIFIC DISSEMINATION: CONFERENCES ORGANIZATION AND COMMUNITY WORKING GROUPS

2019	Israel	3rd symposium "Stochasticity and control of Adaptive Immune Repertoires" - **co-organization with Uri Hershberg, Michal O'Guil, Gur Yaari, David Klatzmann –** 100 expected participants, 31 speakers (november 2019)
2018	France	2nd symposium "Stochasticity and control of Adaptive Immune Repertoires" - **co-organization with Uri Hershberg, Michal O'Guil, Gur Yaari, David Klatzmann –** 128 participants, 29 orateurs (28th - 31st october 2018)
Since 2017	International	Adaptive Immune-Receptor Repertoire (AIRR) Biological Resources Working Group – **co-group leader with Maggie Bostick (TAKARABio, USA)**
Since 2016	International	Adaptive Immune-Receptor Repertoire (AIRR) Minimal Standards Working Group – **Active Member** (1 publication)
2016	France	Translational Data Analysis: A new engine for biomedical discovery meeting, June 2016, Paris, (France) – **co-organization with D. Chaussabel** (SIDRA Institute) - 20 participants
2015	France	New Technologies for Autoimmune/Inflammatory Disease Analysis (nTAïDA) workshops (4 series, DHU i2B funded) - **organizer** 40 to 70 participants per worshop, 4 speakers per workshop
2008-2012	Japan	Sciencescope (Association of french students and scientists in Japan) – **Treasurer, Secretary, organization of the Francophone Research Days (JFR) (JFR'09, JFR'10, JFR'11)**
Depuis 2004	France	Société Française d'Immunologie (SFI) – **member**

MANAGEMENT ACTIVITY AND RESEARCH TRAINING

2018-2019	Louisa Drouiche ; BSc3 **Analysis of the splenic TCR repertoire of NOD and B6 mice** (TRiPoD project, Vanessa Mhanna) Nicolas Huntzinger; MSc1 Bioinformatics, 3 months; **Development of a data curation tool for heterogeneous clinical data and their integration into tranSMART** (Transimmunom Projects, iReceptor +)
2017-2018	Grégoire Bohl, MSc1, Bioinformatics & Modelling, Sorbonne Université; 8 months, co-supervisor HP Pham (ILTOO-Pharma, Statistician); **Implementation of an interoperable web application for the exploration of TCR data** (Transimmunom, iMAP and TRiPoD projects) Nina Papaya, 3rd year of professional degree, ESTBB (Lyon); 6 months; **Validation of quality controls for the preparation of human and murine "TCR" DNA libraries** (Transimmunom, iMAP and TRiPoD projects) Louna Fruchard, BSc2 Biology, Sorbonne Université, 1 month; **Analysis of the human TCR thymic repertoire** (TRiPoD project, PhD Valentin Quiniou)

	Nicolas Huntzinger; BSc3 Biology, Sorbonne Université, 1 month; **Programming of interoperable R scripts for the exploitation of multi-platform TCR annotation files** (Transimmunom, iMAP, TriPoD projects) Anne-Sophie Wanegue, BSc3 Biology, Sorbonne Université, 1 month; **Analysis of the splenic TCR repertoire of aged NOD mice** (TRiPoD project, PhD Vanessa Mhanna) Nidhi Patel, MSc2, Interdisciplinary Research Center (CRI); 3 months. **Network-based gene expression in human autoimmune & inflammatory diseases** (Transimmunom project) Vanessa Mhanna, PhD student, recipient of the ministerial scholarship, Sorbonne University, ED Complexity of the Living Doctoral School (ED515); co-supervision with Adrien Six. **Mapping of the TCR repertoire in the mouse** (TRiPoD project) («Publications List», article n ° P2) Pierre Barennes, PhD student, Paris Diderot; co-supervisor with David Klatzmann. **Dynamics of the Treg repertoire during low-dose IL-2 treatment in SLE patients** (iMAP project) - 2nd year of PhD ("Publications List", article n° P1)
2016-2017	Vanessa Mhanna, MSc2, Université de Grenoble; 6 months; co-supervision with Adrien Six. **Analysis of the C57BL/6 mouse splenic TCR repertoire** (TRiPoD project) Mickael Mendes, MSc2, Sorbonne Universités - UPMC; 6 months; **Analysis of the NOD mouse splenic TCR repertoire** (TRiPoD project) Pierre Barennes, Research assistant; Optimization of the human TCR library preparation protocol (International Collaboration, iMAP Project) Cherazade Benbekhti, BSc3 Biology, Sorbonne Universités-UPMC, 2 months; **Adaptation of flow cytometry protocols** Marie-Charlotte Firobind, BSc3 Biology, Sorbonne Universités-UPMC, 2 months; **Analysis of the splenic TCR repertoire of aged NOD mice** (TRiPoD project, M2 Vanessa Mhanna)
2015-2016	Pierre Barennes, MSc2, Université de Limoges; 6 months ; Optimization of the human TCR bank preparation protocol (Transimmunom Projects, TriPoD iMAP)
2014-2015	Lea Sebag, Master 2, Université Paris Descartes; 6 months. **Implementation of a high-throughput sequencing platform for the analysis of the TCR repertoire** Wahiba Chaara, PhD student (directors: Adrien Six / Pierre-André Cazenave), Sorbonne University; **coordination, design and implementation of a workflow for the analysis of the TCR repertoire** ("Publications List", article n ° T12) Roberta Lorenzon, MD, PhD student (David Klatzmann/Patrice Cacoub), Sorbonne Université ; **Design and implementation of the Transimmunom eCRF** («Publications List» articles n ° T14, T15)
2009-2012	Technical staff (RIKEN, Japan) – Technical training on TCR Rep-Seq analysis
2004-2006	M.Sc. students from UPMC-Paris6 – TCR repertoire in mouse
2003-2005	Technical training of M.Sc and PhD students to T cell receptors diversity

TEACHING

2019	In charge of the MSc2 Systems Immunology (i2s) program within the Sorbonne Université Immunology curricula In charge of the BSc3 “Big data in health and immunology” course (starting September 2019)
Since 2014	Duties in basic immunology (BSc., MSc.), Systems immunology (MSc.), Immune repertoire in biomedical research (MSc.), Flow-cytometry training (MSc.) - Plenary lectures & training sessions
2013	Basic Immunology courses at the « Immune regulation : Autoimmunity and Immune Tolerance » doctoral degree (YTD9020), Tallin University (Estonia) Immune repertoire lectures at Immunotechnology Master degree, UPMC (France)
2004-2006	Immune repertoire lectures at Immunotechnology Master Degree, UPMC-Paris6

Publication list

Research articles

In preparation

P6 Encarnita Mariotti-Ferrandiz, Charles Plessy, Guanying Wang, Ruyji Iida, Osami Kanagawa, Teruhiko Wakayama, Ri-Ichiro Manabe, Shohei Hori. *High-throughput sequencing provides quantitative evidence for spatio-temporal shaping of Treg TCR repertoires*

P5 Valentin Quiniou, Pierre Barennes, Hang-Phuong Pham, Mikhail Shugay, Federica Martina, Vanessa Mhanna, Thomas Dupic, Thierry Mora, Aleksandra Walczak, Adrien Six, **Encarnita Mariotti-Ferrandiz**, David Klatzmann. *Human thymocytes selection favors T cell receptors for pathogens recognition by mimicry*

P4 Felipe Leal Valentim, Océane Konza, Roberta Lorenzon, Alexia, Nidhiben Patel, **Encarnita Mariotti-Ferrandiz**, Adrien Six, David Klatzmann. *Heterogeneity Aware Evaluation of Gene signatures on whole blood from type 1 diabetic patients*

P3 Laura Maria Florez, Gwladys Fourcade, Pierre Barennes*, Karina Gan-Fernandez*, Felipe Leal-Valentim*, Julien Lion , Federica Martina, Djamel Nehar-Belaid, Tristan Courau, Ariadna Gonzalez-Tort, Maria-Grazia Ruocco, Serena Zegninia, Mauro Giacca, Nuala Mooney, **Encarnita Mariotti-Ferrandiz**, David Klatzmann. *Resident effector memory Tregs of the uterus regulate trophic functions during mouse early pregnancy*

P2 Vanessa Mhanna, Gwladys Fourcade, Hang P. Pham, Pierre Barennes, Faustine Brimaud, Guillaume Churlaud, Bruno Gouritin, Valentin Quiniou, Paul-Gydeon G. Ritvo, Adrien Six†, **Encarnita Mariotti-Ferrandiz**†, David Klatzmann†. *Regulatory T Cells Have an Exhausted TR Repertoire In NOD Mice That Is Restored After IL2 Administration*

P1 Pierre Barennes, Valentin Quiniou, Mikhail Shugay, Evgeniy S. Egorov, Alexey N. Davydov, Dmitriy M. Chudakov, Imran Uddin, Mazlina Ismail, Theres Oakes, Benny Chain , Anne Eugster, Darko Sam, Amy Ransier, Daniel C. Douek, David Klatzmann, **Encarnita Mariotti-Ferrandiz.** *Systematic study of T-cell receptor repertoire profiling reveals large methodological biases: lessons from a multi-center study. In revision*

2018

T15- Lorenzon R*, **Mariotti-Ferrandiz E***, Aheng C, Ribet C, Toumi F, Pitoiset F, Chaara W, Derian N, Johanet C, Drakos I, Harris S, Amselem S, Berenbaum F, Benveniste O, Bodaghi B, Cacoub P, Grateau G, Amouyal C, Hartemann A, Saadoun D, Sellam J, Seksik P, Sokol H, Salem JE, Vicaut E, Six A, Rosenzwajg M, Bernard C, Klatzmann D. Clinical and multi-omics cross-phenotyping of patients with autoimmune and autoinflammatory diseases: the observational TRANSIMMUNOM protocol. **BMJOpen**. PMID: 30166293 *(* co-authors)*

T14- Lorenzon R, Drakos I, Ribet C, Harris S, Cordoba M, Tran O, Dasque E, Cacoub P, Hartemann A, Bodaghi B, Saadoun D, Berenbaum F, Grateau G, Ronco P, Benveniste O, Mariampillai K, Sellam J, Seksik P, Rosenzwajg M, Six A, Bernard C, Aheng C, Vicaut E, Klatzmann D, **Mariotti-Ferrandiz E**. Clinical data specification and coding for cross-analyses with omics data in autoimmune disease trials. bioRxiv 360719; doi: https://doi.org/10.1101/360719

T13- Ritvo PG, Saadawi A, Barennes P, Quiniou V, Chaara W, El Soufi K, Bonnet B, Six A, Shugay M, **Mariotti-Ferrandiz E**, Klatzmann D. High-resolution repertoire analysis reveals a major bystander activation of Tfh and Tfr cells. **PNAS**. PMID: 30158170

T12- Chaara W, Gonzalez-Tort A, Florez L, Klatzmann D, **Mariotti-Ferrandiz E*** and Six A* RepSeq data representativeness and robustness assessment by Shannon entropy, **Frontiers in Immunology** (*co-last authors) PMID: 29868003

2017

T11- Rubelt F*, Busse C.E*, Chan Bukhari S.A*, Bürckert J-P, **Mariotti-Ferrandiz E.**, Cowell L.G, Watson C.T, Marthandan N, Faison W.J, Hershberg U, Laserson U, Corrie B.D , Davis M.M, Peters B, Lefranc M-P, Scott J.K, Breden F, The AIRR Community, Luning Prak E.T^ and Kleinstein SH^.

Adaptive Immune Receptor Repertoire (AIRR) Community Recommendations for Sharing Immune Repertoire Sequencing Data. **Nature Immunology**, PMID: 29144493 *(* co-authors)*

T10- Ritvo P.G, Churlaud G., Quiniou V., Florez L., Brimaud F., Fourcade G., **Mariotti-Ferrandiz E**., Klatzmann D. Bona fide Tfr cells lack IL-2Ra but express antagonist IL-1R2 and IL-1Ra and suppress the IL-1-dependent activation of Tfh cells. **Science Immunology**. PMID: 28887367

T9- Maceiras AR, Almeida SCP, **Mariotti-Ferrandiz E**, Chaara W, Six A, Klatzmann D, Faro J, Graca L. Different TCR-specificity requirements in T follicular helper and T follicular regulatory cells from the same germinal centers. **Nature Communication.** PMID: 28802258

2016 **T8**- **Mariotti-Ferrandiz E**, Pham HP, Gorgette O, Dulauroy S, Klatzmann D, Cazenave P-A, Pied S et Six A. A TCRb repertoire signature can predict experimental cerebral malaria. **PlosOne.** PMID: 26844551

2015 **T7**- Plessy C, **Mariotti-Ferrandiz E**, Manabe R, Hori S. (2015) ClonotypeR – high throughput analysis of antigen receptor sequences. **bioRxiv**, DOI: doi.org/10.1101/028696

2014 **T6**- Bergot A-S, Chaara W, Ruggiero E, **Mariotti-Ferrandiz E**, Dulauroy S, Schmidt M, von Kalle C, Six A & Klatzmann D. TCR sequences and tissue distribution discriminate the subsets of naïve and activated/memory Tregs in mice. **Eur. J. Immunol**. PMID: 25726757

2010 **T5**- Montaudouin C., Boucontet L., Mailhé-Lembézat M-P, **Mariotti-Ferrandiz M.E.**, Six A. Pereira P., Freitas A.A, Garcia, S. Endogeneous TCR expression in RAG-deficient TCR transgenic mice. **PlosOne**. PMID: 20454452

2009 **T4**- Komatsu, N, **Mariotti-Ferrandiz M.E.**, Wang, Y, Malissen, B, Waldmann H and Hori, S. Heterogeneity of natural FoxP3+ Tcells: A commited regulatory T cell lineage and an uncommitted minor population retaining plasticity. **PNAS**. PMID: 19174509

2008 **T3**- Petrovic-Berglund J.*, **Mariotti-Ferrandiz M.E***, Rosmaraki E., Hall H., Cazenave P-A., Six A. and Höglund P.- TCR repertoire dynamics in the pancreatic lymph nodes of non-obese diabetic mice at the time of disease initiation. **Molecular Immunology**. PMID: 18471883 *co-author

T2- Guillot-Delost, M, Cherai, M, Hamel, Y, Rozenzwajg, M, Baillou, C, Simonin, G, Leclercq, V, **Mariotti-Ferrandiz M.E**, Six, A, Cheraud, P, Bon-Durand, V, Cohen, J, Klatzmann, D, Lemoine, FM. Clinical-grade preparation of human natural regulatory T-cells encoding the thymidine kinase suicide gene as a safety gene. **J. Gene Med**. PMID: 19514009

2004 **T1**- Collette A, Bagot S, **Ferrandiz M.E**, Cazenave P-A, Pied S et Six A. A profound alteration of blood TCRB repertoire allows prediction of cerebral malaria. **Journal of Immunology**. PMID: 15383590

Reviews

2018 **R3**- Ellul P, **Mariotti-Ferrandiz E,** Leboyer M, Klatzmann D. Regulatory T Cells As Supporters of Psychoimmune Resilience: Toward Immunotherapy of Major Depressive Disorder. **Frontiers in Neurology.** PMID: 29615964

2013 **R2**- Six A, **Mariotti-Ferrandiz M.E**, Chaara W, Magadan S, Pham H-P, Lefranc M-P, Mora T, Thomas-Vaslin V, Walczak AM and Boudinot P. (2013) - The Past, Present, and Future of Immune Repertoire Biology – The Rise of Next-Generation Repertoire Analysis. **Frontiers in Immunology**. PMID: 24348479

2008 **R1**- Boudinot P., **Mariotti-Ferrandiz M. E.**, Du Pasquier L., Benmansour A., Cazenave P. A., and Six A.. New perspectives for large-scale repertoire analysis of immune receptors. Mol.Immunol. PMID: 18279958

Book chapter

2009 B1- **Mariotti-Ferrandiz M.E**. and Adrien Six – Pain, Inflammation and autoimmunity, Chapter: Concept of autoimmunity during pain processes – Sorel M, Arnette edition, ISBN: 9782718411972

Introduction

One of the fundamental feature of the adaptive immune system is the extraordinary diversity of lymphocyte antigen receptors, immunoglobulins (Ig) and T cell receptors (TCR). This diversity endows the immune system with a potential to recognize, in an unanticipated manner, the diverse antigenic universe of “non-self” including pathogens and autologous tumours. The generation of the Ig and TCR repertoire depends on a somatic recombination machinery which randomly assembles a set of functional Ig or TCR genes from a pool of discontinuous gene segments, called variable (V), diversity (D) and joining (J) segments [1,2]. During the recombination process, which occur in central lymphoid organs (CLO, bone marrow and thymus for B and T cells respectively), nucleotides are added and removed randomly between V, D and J segments and lead to a junctional region, variable in length and sequence. This region, called the CDR3, is the signature of the rearrangement and contributes greatly to the high diversity of the TCR (Figure 1).

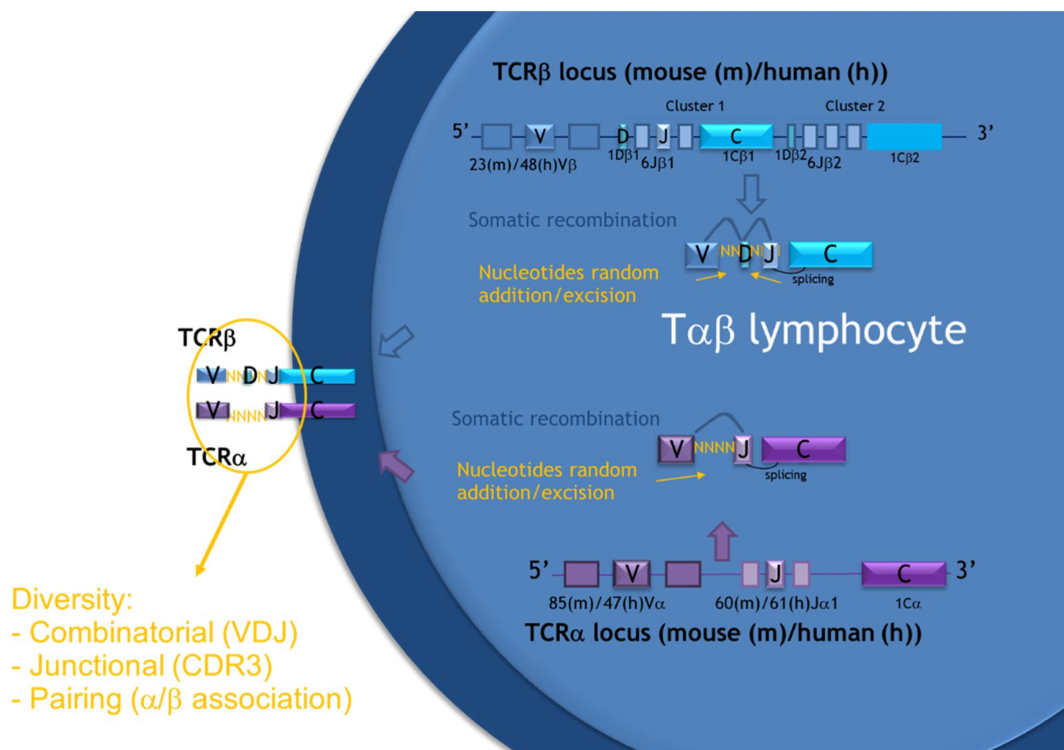

*Figure 1 : **Somatic recombination of the TCRα and TCRβ locus in thymocytes***

TCR Repertoire diversity generation and selection

After several processes of selection of the potential repertoire generated in the central lymphoid organs, the resulting collection of peripheral lymphocytes, each expressing a given BCR or TCR characterized by a particular combination of V, D and J segments and a singular CDR3 length, constitutes the available T cell repertoire (Figure 2). Such diversity will increase the chance to recognize foreign molecules and preclude from self-antigen pathogenic reactivity.

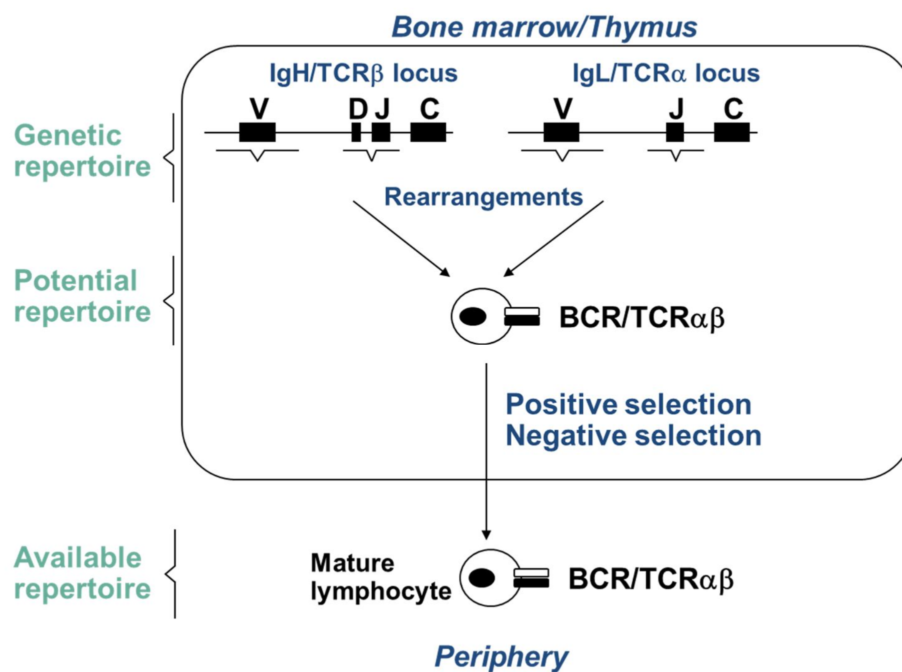

Figure 2 : ***Repertoire level according to the differentiation stage of T and B cells***

For αβ TCR, the potential diversity thus generated has been calculated to be as large as 10^{19} or even up to 10^{61} as very recently predicted by statistical modelling [3,4]. Obviously, only a small fraction of the potential repertoire can actually be used, because of the limitation in the number of cells a human and a mouse can harbour (up to ~10^{12} and ~10^{8} αβ T cells, respectively), in the body. Furthermore, the actual repertoire is shaped by various selection events that depend on TCR interactions with self- as well as non-self-peptides presented by the major histocompatibility complex (MHC) molecules in the thymus and periphery. However, the actual TCR repertoire

remains complex enough to have precluded attempts to comprehensively and quantitatively measure it for decades.

TCR repertoire diversity and specificity

The diversity of lymphocyte repertoire appears essential for host defence against “non-self” organisms. Decades of studies highlighted the presence and need for T cells harbouring specific TCR of various peptides derived from pathogens. This has been the ground for the concept of adaptive immune memory, the process by which an individual once exposed to an array of antigens expressed by a pathogen will be able to efficiently respond after a second exposure to the same pathogen [5]. This concept has been crucial in the improvement of health care by the introduction of vaccination as a prevention therapy to control infectious disease spreading and protect individual at the population level.

However, the adaptive immune system play also a major role in the maintenance of immune homeostasis and immunological tolerance to the “self” organism. Autoimmune diseases (ADs) in general have been associated with an alteration of the T cell repertoire, as it has been shown in Type I Diabetes (T1D) mouse model [6–9]. This holds true also in human, with most advanced results in T1D [10–13], Systemic Lupus Erythematosus [14–18] and Rheumatoid Arthritis [16,19–24]. Thus, I truly believe that the description of the T-cell population diversity would help to understand the complex mechanisms underlining the immune response, in particular in ADs.

TCR repertoire and function

Besides the antigen specificity, T cell population is extraordinarily functionally diverse as well. Since the identification of T4 and T8 in the early 80s [25], several subsets, more or less stable, have been characterized based on their location, activation markers, functional properties through cytokines/chemokines production, activation/inhibition surface markers, immune regulation [26,27]. Still the link between the function and the antigen-specificity is poorly understood, notably in regards with helper/effector T cells (Teffs) and regulatory T cells (Tregs)

(i) differentiation, (ii) selection and (iii) their balance, two major cell subsets with opposite function in the regulation of the immune response (Figure 3).

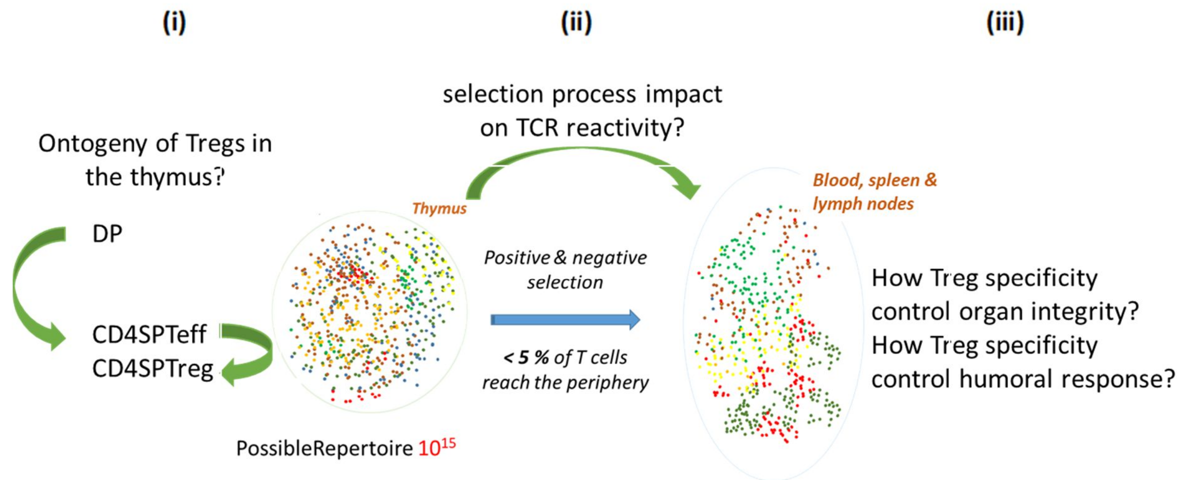

Figure 3: ***Schematic view of the link between TCR specificity and differentiation, selection and balance of Tregs and Teffs***

TCR repertoire unresolved questions

Considering all the characteristics of T cells, some fundamental questions have remained unresolved in immunology for more than half a century since the proposition of the clonal selection theory by Macfarlane Burnet [28]. From a generation and selection processes' perspectives: how much is the diversity of the actual repertoire (i.e., how many different lymphocyte clones are there) and how large is the clonal size [=number of cells] of each clone (i.e., how abundant is each clone) in an individual at a given time point? How does the actual repertoire change in time during development, or under steady state, or upon external and internal perturbations? How the actual repertoire is shaped through various selection events? From a functional perspectives: How does a skewed immune repertoire lead to the development of pathological autoimmunity? Conversely, is there a minimal repertoire required to efficiently respond to pathogens? How come that at a population level humans are able to, most generally, establish an efficient immune response against pathogens expressing non-self-antigens for which T cells are not positively selected still maintaining the integrity of their organs? Since the beginning of my career, I have been working to address part of these unresolved questions regarding T cell repertoire diversity using systems biology approaches.

TCR repertoire and systems immunology

Systems immunology is an apparent emerging field of research. Formally termed in 2006 by Ron Germain and Christoph Benoist [29], systems immunology can be naively defined as a systems biology approach applied to the immune system. Yet, the definition of systems biology is probably as diverse as the number of systems biologists as mentioned by Rainer Breitling [30], so it is for systems immunology. If I had to define my vision of systems immunology I would say that it is a global approach aiming at characterizing the immune system (whatever the level of the system considered) through the integration of the diversity of individual components forming that system. Retrospectively, systems immunology (as well as systems biology) is probably the original approach of research, since antiquity when complex systems where observed as a whole and not as the sum of their parts. This phrase, "the whole is greater than the sum of the parts", sometimes attributed to Aristotle and sometimes just as an interpretation of what Aristotle and others tried to express, became viral and somehow the foundation of modern systems biology.

After decades of reductionist approach, critical for the dissection of cellular and molecular mechanisms involved in the immune responses, technological progresses in molecular and cellular biology as well as in computational science now offers the possibility to integrate the parts of a complex system to rebuild the whole and eventually identify how the whole system behave under perturbations [31–35]. Such approach now provide opportunities to better characterize a system (un-exhaustively) at steady-state [36–40], in vaccinology [41–48], in pregnancy [49–51] and in various pathological conditions, such as autoimmune diseases [52–61], infection [62–68], cancer [51,69–73], … Most of the studies initially focused on methods for which global approaches were introduced and validated more than 15 years ago, such as transcriptomics, genomics, proteomics and cell phenotype…. Adaptive Immune receptor repertoire (AIRR) methodological progresses in the last ten years offered a renewal of the field and is now part of the system immunology approached [3,31,33,34,74,75].

In this manuscript, I will introduce briefly my achievements during the past years and my current research to finish with my perspectives for the next 5 years.

T cell receptor diversity inception (2004-2012)

During my early career, I had the chance to join two major laboratories in the field of T cell biology, including TCR repertoire diversity and regulatory T cells differentiation and characterization:

- From my MSc degree internship, I joined Prof. Adrien Six team in Prof. Pierre-André Cazenave laboratory at Pasteur Institute (Paris, France) to study the TCR repertoire alteration during the course of *Plasmodium berghei* ANKA infection in mouse.
- Right after my PhD, I joined Dr. Hori's laboratory at Riken Institute (Yokohama, Japan) to work on Treg specificity and repertoire in mouse physiological condition.

Identification of a blood TCRβ signature predicting Cerebral Malaria

Background

During my MSc degree and my PhD internships, the later supported by a French Ministery of Education and Research Doctoral fellowship (MNERT), I worked on the characterization of the T lymphocytes diversity in different mouse models. My main research project, supervised by Prof. Adrien Six, aimed at characterizing brain T lymphocyte diversity in a mouse model of cerebral malaria (CM), one of the severe complications of Plasmodium falciparum infection, in part associated with an uncontrolled immune response. In particular, Tαβ lymphocytes play an important role as shown by the protection of Tαβ cell-deficient mice infected by the appropriate parasite, *Plasmodium berghei ANKA* (PbA) [76,77]. Moreover, CD8+ Tαβ cells reach the brain of CM-developing mice (CM+ mice) [78–80]. The complex composition of micro-organisms in general including antigenic, superantigenic and mitogenic activities, alter the diversity, leading to polyclonal proliferations and/or oligoclonal expansions [81,82]. Such modifications can lead to inappropriate responses that, in turn, scramble or divert the protective appropriate response. We hypothesized that during Plasmodium infection, the diversity of Tαβ repertoire is highly modified. At that time, we made used of Immunoscope technique [83] (also known as CDR3 spectratyping [84]).

Method: From qualitative to quantitative TCR profiling by Immunoscope

Immunoscope technique developed at the Pasteur Institute in early 90's [83] generates CDR3 length profiles, at that time mostly analysed qualitatively. Although such approach was promising and efficient for the study of pathological conditions associated with major expansions of clones, it reveals lack of quantitative reliability [85]. To overcome such issue, Adrien Six and his former PhD student developed a software able to quantitatively manage the information output from Immunoscope, namely ISEApeaks [86]. The principle was simple: rearrangements were labelled during amplification by PCR and separated at the molecular level using sequencing methods. The labelling allowed to identify and measure the intensity of each rearrangement. The separation based on the size, the output corresponded to the CDR3 length of each rearrangement. The data from the CDR3 length profiles obtained from the sequencer could therefore be retrieved and gathered in a peak database. CDR3 spectratypes could then be quantitatively analysed by applying different measures such as perturbation indexes, translating the modifications between samples but also reflecting the emergence of recurrent oligoclonal expansions. Associated to multivariate statistical methods implemented in ISEApeaks, this strategy allowed a rigorous analysis of the repertoire and can be seen as the grounds of TCR database integration.

Results

In a first study, we showed a perturbation of blood TCRß repertoire in CM+ mice, compared to healthy mice and also to infected mice without cerebral symptoms, partly due to recurrently expanded T cell clones [87]. Moreover, the cerebral damages associated to the infiltration of CD8+ T lymphocytes in the brain of CM+ mice suggested that these modifications were directly associated with the outcome of CM. In order to evidence the dynamics of this alteration, we performed a kinetic analysis of blood, spleen and brain TCRß repertoires, from day 3 post-infection (p i) until the death following the development of neurological signs of PbA-infected mice. The repertoire of total blood, spleen and brain lymphocytes was analysed by comparison between infected and non-infected mice. We observed a global alteration of the repertoire of infected mice from day 5 in the spleen and day 6 in the blood. The perturbation increases for all Vß in the blood and the spleen. Interestingly, perturbation of most of the Vß is peculiar to each compartment analysed suggesting a compartmentalization of the diversity during the infection.

Strikingly, the comparison of brain repertoires between healthy and CM+ mice revealed a perturbation limited to 5 Vß out of 23, and recurrent oligloclonal expansions among 2 Vß families. These 5 Vß are perturbed in the spleen and the blood, following a similar kinetic. These results confirmed our previous observations of a huge perturbation of the repertoire associated to the development of cerebral malaria. Moreover, they indicate a possible targeted alteration of the brain TCRß diversity, suggesting an oriented recognition, of parasitic or self-antigens, in the brain. We therefore applied a strategy for TCR signature detection based on an approach developed by Adrien Six team for transcriptome analysis [88]. We could identify a TCRβ signature in the blood discriminating with > 90% accuracy the development of CM. In addition, this blood signature was also found in the spleen and more importantly in the brain, strongly suggesting a pathogenic role of T cells bearing those TCR in the development of CM in mice [89].

Conclusion

With this work we brought a dynamic dimension to the repertoire description, showing for the first time (1) the evolution of TCRß diversity during the course of the infection, (2) the description of the global brain TCRß repertoire in mouse and (3) the biomarker potential of TCR signatures. This work was awarded in 2006 by the "FOCIS poster of distinction".

TCRβ repertoire is modified at early stage of T1D onset in mouse

In parallel, I was involved in a collaborative study of the Tαβ cell repertoire in young NOD mice. Initiation of the T cell response that cause autoimmune diabetes in NOD mice occurs in the pancreatic lymph nodes (PLN) around two weeks of age. To identify T cells that were activated early in response to beta cell antigens at this site, we investigated the TCRβ diversity in the pancreatic lymph nodes (PLN) in comparison with inguinal lymph nodes (ILN) as controls, of 10, 14, 18 and 22¬ day-old NOD females. An analysis of all Vβ chains by Immunoscope and ISEApeaks (see methods) revealed intensive perturbations of the repertoire through time in the PLN as well as in the ILN, suggesting that the overall TCR repertoire is subject to major changes between 10 and 22 days of age. In contrast, when a selected set of Vβ chains was analysed at the Vβ-Jβ level, several unique TCR perturbations and oligoclonal expansions were observed in the PLN compared to the ILN. Different oligoclonal expansions were found at different time points,

suggesting that the initiation of diabetes in NOD mice may involve several T cell specificities that are activated in a sequential manner [90].

Altogether, 5 research articles, one review and one book chapter were published out of my PhD work (**PUBLICATION ACHIEVEMENTS A**).

PUBLICATION ACHIEVEMENTS A : PHD
Main research projects:
Mariotti-Ferrandiz E, et al PlosOne. PMID: 26844551 (T8)
Petrovic-Berglund J.*, Mariotti-Ferrandiz M.E*, et al. Molecular Immunology. PMID: 18471883 (T3)
Collette A, et al.. Journal of Immunology. PMID: 15383590 (T1)
Collaborative research projects
Montaudouin C et al. PlosOne. PMID: 20454452 (T5)
Guillot-Delost et al. J. Gene Med. PMID: 19514009 (T2)
Reviews & book chapter:
Boudinot P et al. Molecular Immunology. PMID: 18279958 (R1)
Mariotti-Ferrandiz M.E. and Adrien Six. Arnette edition, ISBN: 9782718411972 (B1)

TCR repertoire and specificity of regulatory T cells

Soon after my PhD defence, I joined Dr Hori's laboratory at Riken Institute in Japan with the aim to link TCR repertoire diversity with T cell subset functions. This post-doctoral internship was supported by a Japan Society for Promotion of Science (JSPS) post-doctoral fellowship that I obtained soon after joining Dr Hori's laboratory.

Seminal work from Shimon Sakaguchi [91–93], further confirmed by others [94], established that the normal αβ T cell repertoire consists of not only "conventional/effector" T (Teff) cells that exhibit ridding or aggressive functions but also regulatory T (Treg) cells that display protective or suppressive functions over aggressive immune activities to ensure immunological tolerance to "self" and innocuous environmental antigens including commensal bacteria [95,96]. This notion has been firmly established by the findings by Hori's lab and others that the development of functional Treg cells is controlled by the transcription factor Foxp3 and that their defective generation underlies the catastrophic autoimmune, inflammatory and allergic disease that develops in Foxp3-mutant mice and humans suffering from the IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked) syndrome [97–101]. Still, Treg deficiency or insufficiency was not clearly addressed at that time. Two main hypothesis were reasonably

stated: The first hypothesis would suggest that AIDs are due to a quantitative defect of Tregs explained by a decrease number of cells leading to an imbalance between the Treg and the Teff compartment. This has been supported by the description of an IL-2 insufficiency in T1D patients (as well as the NOD mouse model) for instance. The second hypothesis would rather explain AIDs development due to a limited/altered Treg cell repertoire diversity that could lead to the development of various autoimmune or allergic diseases by creating "holes" in the antigenic universe recognized by Treg cells. For my post-doctoral internship, I proposed to tackle the second hypothesis by first characterizing Treg TCR repertoire diversity in physiology. In addition, Treg specificity was (and is still) unknown. I therefore established a mouse model to try to resolve it.

Treg TCR specificity

Background

Studies on TCR specificity often relied on transgenic mice expressing only one single TCRβ chain and characterized the diversity of their TCRα repertoire, in order to make the analysis and data interpretation straightforward by eliminating the diversity of TCRβ chains and the diversity caused by pairing of TCRα and β chains. It remained unclear, however, whether the picture obtained from such transgenic systems can be generalized to the fully diverse T cell repertoire in normal, non-transgenic individuals. In addition, since the TCRα repertoire and the consequent antigenic recognition by the resulting αβ TCR have been shown to be somehow skewed in such TCRβ "fixed" mice compared to normal non-transgenic mice, it is possible that different pictures may be obtained from different transgenic systems.

My first project aimed at establishing a more physiological mouse model to address the TCR repertoire and antigen specificity of Treg cells. In collaboration with Dr. Teruhiko Wakayama (Riken Institute), the lab established Treg cell-derived nuclear-transferred ES (nt-ES) cell lines. Briefly, they sorted Treg cells based on CD25Hi expression by FACS sorting, retrieve the respective nucleus and transferred them onto ES denucleated cell line using an established protocol [102]. The efficiency of such nuclear transfer with Treg cell derived nuclei was very low, leading to only 6 established nt-ES cell line. Each of those were characterized for their TCRαβ by Sanger

sequencing. By germ-line transmission, they obtained one functional TCRβ gene from one of the nt-ES lines, called 1D2β. Although they failed to obtain the functional 1D2 TCRα gene by germ-line transmission, we decided to take advantage of the 1D2β allele and utilized this unique Treg-derived TCRβ fixed mouse model for characterization of TCR repertoire and specificity of Treg cells.

Results

First, I determined whether the original 1D2 nt-ES cells are indeed derived from T_{reg} cells by "retrospective" sequencing. We introduced the $Foxp3^{hCD2}$ reporter allele that was generated in the by Herman Waldmann lab in UK [103], as a more reliable reporter-gene of Foxp3 expression [104], and one TCRCα-null allele to prevent expression of two TCRα chains on the surface [105] into the 1D2β homozygous mice, sorted $Foxp3^{hCD2+}$ Treg and $Foxp3^{hCD2-}$ Tconv cells, and performed conventional sequencing of the original Vα44/Jα44 rearrangements. As a result, we found that the 1D2α clonotype was expressed preferentially in Treg cells, although it was also expressed in Tconv cells albeit at a lower frequency, indicating that this 1D2 αβ TCR was indeed derived from Treg cells. Second, to reconstitute the original 1D2αβ TCR *in vivo* and to address its specificity, I cloned both 1D2α and β cDNA into a retroviral vector, infected $RAG^{-/-}$ BM cells with the retrovirus, and transferred the 1D2αβ-transduced BM cells into irradiated host mice, using a published protocol for retrogenic mice generation [106,107]. We found that both $Foxp3^{+}$ and $Foxp3^{-}$ T cells expressing the 1D2αβ TCR did develop in such retrogenic mice, further confirming that the 1D2 αβ TCR can instruct developing T cells to differentiate into Treg cells. Importantly, such differentiation could be achieved only in BM chimeras reconstituted with 1D2αβ-transduced BM and wild type BM cells, establishing Hsieh's observations that Treg differentiation is highly dependent on intraclonal competition [108].

Conclusion

Those results also supported previous observations by others [109–112] that Tregs and Teffs may differentiate independently of their TCR and therefore may share part of their diversity. Indeed, as Foxp3+ cells have been found to function in a wide range of immunological contexts, their specificity has been questioned, and especially the overlap with conventional T cells

(Foxp3⁻). While some groups showed distinct Foxp3+ and Foxp3− repertoires and revealed self-reactivity among Foxp3⁺ TCRs [110,113,114] others conclude to a high overlap between both populations arguing that Foxp3⁺ have a non-self-reactive repertoire [95,112]. Therefore, from this project we moved to the characterization of TCR repertoire diversity of the 1D2β mouse.

Treg TCR repertoire diversity

Background

At that time, and since the discovery of the TCR loci [1], several attempts have been made to estimate the repertoire diversity of various T cell subsets including Treg cells in humans and in mice [112–118] by using conventional sequencing approaches, i.e., RT-PCR amplification of rearranged TCRα or β transcripts followed by cloning and sequencing of individual clones by the Sanger method. However, the information obtained from such studies has been limited - although informative - and sometimes contradictory, primarily because of technical limitations of the conventional approach (labour- and cost-intensive, inherently “low-throughput”). As a result, one could only infer the diversity of the whole repertoire from such small samples based on some statistical assumptions on the underlying clonal size distributions [119]. To further develop our ongoing research activity, I decided to establish a novel strategy for comprehensive and quantitative measurement of the TCR repertoire diversity of Treg and Tconv cells by taking advantage of the next-generation sequencers that have been fully functional at the Riken Institute. To this end, I established a collaboration with Drs. Plessy (Bioinformatician) and Manabe (Head of the sequencing facility at Riken Institute) and obtained a Riken President Fund for feasibility studies in 2009.

Method

As for the next-generation sequencing platform, we have used the 454 FLX Titanium from Roche, which could generate up to $1.6x10^6$ individual reads per run with a read length of up to 400-bp, a length sufficient to determine the nucleotide sequence of the CDR3 and the Vα2 and Jα gene segment usage.

We used the 1D2β mice harbouring the $Foxp3^{hCD2}$ reporter allele and one TCRCα-null allele [120] and sorted peripheral CD4⁺ T cells into Foxp3⁺ Treg and Foxp3⁻ Tconv cells by flow cytometry

based on the hCD2 reporter expression from the thymus of 5 weeks old mice and peripheral secondary lymphoid organs from 5 and 8 weeks old mice.

Because there are numerous Vα gene segments, we have restricted our analyses to cells expressing the TRAV14 (Vα2) family (which is one of the most major family). I designed a specific primer that covers all the 6 TRAV14 family members and a reverse primer specific for the constant region so that we can amplify TRAV14 transcripts.

Results

This collaboration allowed me to access to the state-of-the-art sequencers and to develop in tight collaboration with a bioinformatician a tool, clonotypeR, for TCR data obtained from next generation sequencing (NGS), unavailable at that time [121]. clonotypeR does include functions for automatic TCR sequences analysis as well as statistical modelling of repertoire diversity, by computing diversity indexes (richness, Morisita-Horn, Species Abundance Distribution).

Our quantitative comparisons of V and J segments frequency, CDR3 length and clonotype distribution between samples, showed that Foxp3+ and Foxp3− T cells can be discriminated by these different parameters from the TCRα in a fixed TCRβ background, in line with the works of Hsieh et al [113] and Pacholczyk et al. [112]. In our study, as we sequenced millions of CDR3, we could show that the differences between Foxp3+ and Foxp3− repertoires are quantitative rather than qualitative, and that a frequent CDR3 in one population will tend to be rare but not completely absent from the other population. This challenges the concept of private clonotype in such high-resolution studies. Strikingly, at the level of TRAV14 and TRAJ segments, we found differential usage between Foxp3+ and Foxp3− in 1D2β. Similar observations were also reported earlier with a different fixed β chain [113], although no statistical comparisons could be achieved. Given that the Vα and Jα nucleotides contributing to the CDR3 region are the same in each TRAV14 segments respectively, those results suggest a contribution of the conserved regions from the V segments to T cells function. Structure analysis of self-reactive human and murine TCRs showed that TCRs specific for self-antigens display an unconventional structure of V regions compared to non-self-reactive TCR [122]. In addition, this is a crucial point for fundamental analysis of TCR repertoire since most of studies focused the immune diversity analysis to the

CDR3 region, arguing that peptide/MHC recognition is limited to CDR3 region. However, if we consider the contribution of TRAV segments as well as TRAJ, this may revise upwards the extent of the TCR diversity.

As in previous studies, we found a certain overlap between regulatory T cells and conventional T cells, in periphery and in the thymus. To try to distinguish thymic versus peripheral shaping of the repertoire, we analysed also recent thymic-emigrants by using Rag1-GFP model, as it is known that RAG-expression, required for the rearrangement, is abolished prior to thymus exit. The reporter gene GFP allowing to track those cells which recently expressed RAG, presumably recently emigrating from the thymus.

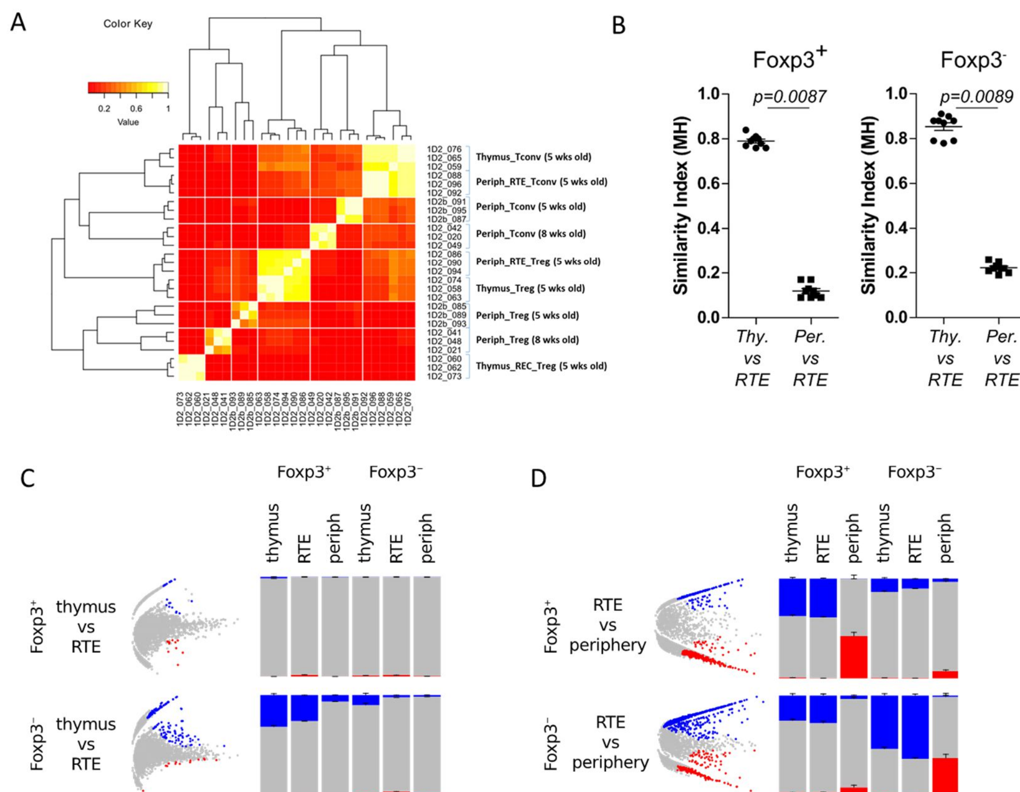

*Figure 4 : **Recent thymic emigrants reveal TCR repertoire maturation dynamics***

(A) Hierarchical clustering of the 1D2b libraries calculated based on the Morisita-Horn similarity. (B) Morisita-Horn index calculated between thymus and RTE repertoires (left) or peripheral and RTE repertoires (right) using 6000 sequences sub-sampled libraries from Foxp3+ (red) and Foxp3− (black) samples. In graphs, the bar represent the median and statistical significant tested with t-test. (C) Differential representation of clonotypes in thymus and RTE Foxp3+ or Foxp3− cells. The dot plots represent each clonotype, with on the vertical axis the fold change between the compared populations (up, thymus; down, RTE), and on the horizontal axis the average expression level.

Statistically significant fold changes are indicated by a red dot. With each statistical comparison defining three groups (up, no difference, down), the stacked bar charts represent the contribution of these three groups to the thymic, RTE and peripheral Foxp3+ or Foxp3− populations. (D) Same as C, except that the comparison is RTE vs. periphery.

First of all, by measuring the Morisita-Horn similarity distance, we found that RTE repertoire are highly similar with that of thymic cells, for both Treg and Teff (Figure 4 A&B). Interestingly, Foxp3- clonotypes enriched in the thymus compared to RTE were found to be abundant in thymic and RTE Foxp3+ population, suggesting that these clonotypes were auto-reactive, either expressed by both thymic Foxp3^{+} and Foxp3^{-} and then deleted from the Foxp3- subset, or expressed by Foxp3^{-} and redirected toward the Foxp3^{+} stage upon thymic maturation (Figure 4 C&D). This result was apparently in contradiction with previous results showing that TCR engagement is required to upregulate the IL2Rα chain (CD25) expression and the corresponding signalling pathways which in turn led to the transcription of Foxp3 [110,123] arguing for the existence of Tregs precursors among the CD4SP cells CD25^{+}. However, since we separated the cells based on the expression or the absence of expression of Foxp3, our Foxp3− samples may contain precursors of Tregs. It is reasonable to think that, Foxp3− clonotypes found in the Foxp3^{+} compartment may correspond or at least be enriched in Foxp3+ precursors which did not up-regulate CD25 yet. If so, they must be found preferentially in the peripheral Foxp3^{+} compartment. To cope with this point, we carefully analysed the peripheral distribution of thymic clonotypes. Interestingly, while 16% and 20% of exclusive-thymic Foxp3^{+} and exclusive-thymic Foxp3− clonotypes respectively are found in the periphery, 60% of clonotypes shared between thymic Foxp3^{+} and Foxp3^{-} are selected to the periphery and 78% are found in peripheral Foxp3^{+} compartment (among which half are also shared with Foxp3^{-} cells) supporting indirectly the two step model. However, the direct evidence requires the comparison of Foxp3^{-} CD25^{-} cells [124].

Conclusion

During those 5 years of post-doc in Japan, I expanded my skills in cellular and molecular immunology and developed new skills in bioinformatics in particular for TCR repertoire tools and strategies development. With the pioneer works of TCR repertoire studies by NGS, I could show that (i) TCR repertoire diversity from thymus and periphery discriminate Tregs from Teffs, (ii) total Treg repertoire is highly diverse and (iii) that the clonal size distribution of Treg and Teffs reflects

central and peripheral selection events. This in turn suggests that, by tracking changes of the clonal size distributions during T cell development or during immune responses, we will be able to delineate how each selection event impinges on the shaping of the actual Treg and Tconv cell repertoires.

Altogether, 2 research articles were published and one is (still) in preparation out of my first post-doc work (**PUBLICATION ACHIEVEMENTS B**).

PUBLICATION ACHIEVEMENTS B : POST-DOC 1
Main research projects:
Mariotti-Ferrandiz E et al. *High-throughput sequencing provides quantitative evidence for spatio-temporal shaping of Treg TCR repertoires* (P6)
Plessy C, Mariotti-Ferrandiz E et al. bioRxiv, DOI: doi.org/10.1101/028696 (T7)
Komatsu, N, Mariotti-Ferrandiz M.E. et al. PNAS. PMID: 19174509 (T4)

Towards a systems immunology approach for biomarker discovery (since 2012)

In 2012, I joined Prof David Klatzmann laboratory as a post-doctoral fellow to work on two recently granted projects obtained by David Klatzmann:

- A European Research Council Advanced Grant project aiming at "Deciphering the regulatory T cell repertoire: towards biomarkers and biotherapies for autoimmune diseases" (TRiPoD project).
- A Laboratory of Excellence project funded by the local National Agency for Research (Agence Nationale pour la Recherche, ANR) aiming at revisiting the nosography of autoimmune and inflammatory diseases through a systems immunology approach (Transimmunom project).

In those two project, there was a major focus on Treg biology as well as on their TCR repertoire composition and specificity, in human and/or mouse physiology and pathology.

I therefore had two major missions to fulfil the expectations on those projects.

Implementation and coordination of the Treg TCR repertoire studies in mouse and human physiological and pathological conditions

Development and implementation of TCR repertoire diversity analysis approaches by NGS

Background

Systematic sequencing of “all” Ig/TCR transcripts expressed in a lymphocyte population of interest can be achieved by NGS technologies which become available after the seminal work of Weinstein et al. in 2009 [125]. NGS allows to produce several tens of thousands of sequences in a short amount of time. A benefit of this approach is clearly that several angles of analysis can be looked at such as clonotypes frequency, Ig/TCR V-C or V-J usage, CDR3 diversity, CDR3 sequence analysis, V gene allele identification… However, while several studies at that time, including mine at Riken, have highlighted the feasibility of using deep sequencing for the analysis of Ig/TCR repertoire diversity [126], now called AIRRSeq (for Adaptive Immune Receptor Repertoire Sequencing) [127,128], the ability to process the complexity of the information provided by such amounts of data remained limited and specific software developments for automatic annotation of TCR sequences and statistical modelling of repertoire diversity were needed, as we summarized in a Frontiers in Immunology review [129].

In addition, besides the computational needs, the molecular approaches used and proposed by different laboratories and/or companies, such as Adaptive Biotechnologies and iRepertoire in the US, were at their early birth and there was no consensus nor grounded experience, as it was a newly born field. Such absence of consensus was raised by the AIRR community, launched in 2015 by Felix Breden and Jamie Scott and is currently tackled by different working groups, including two I belong to.

In tight collaboration with Prof. Adrien Six Integrative Immunology team (i2) in the laboratory, I have been involved in the development and implementation of (i) TCR repertoire modelling strategy and tools and (ii) setup and validation of a robust experimental design for TCR library preparation and sequencing.

Results

Bringing my experience and developments from my first post-doctoral internship, I have been working on the establishment of a workflow to systematically compute various metrics and analysis strategies, allowing to model the TCR repertoire diversity, composition and variation in human and mouse samples [130–132].

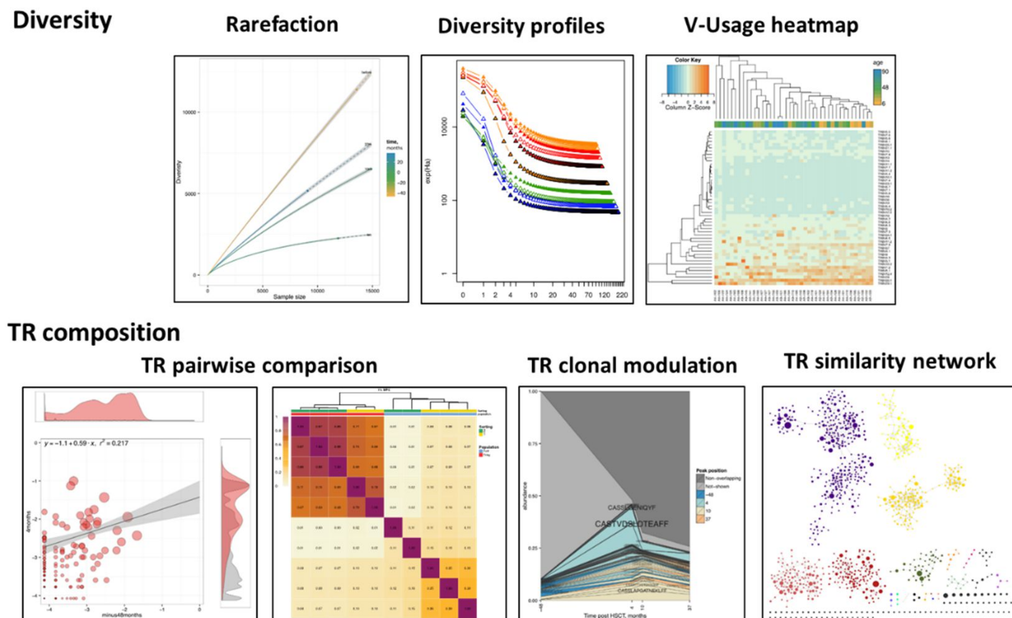

*Figure 5 : **TCR repertoire exploration modelling***

Starting with clonotypeR, I worked with Wahiba Chaara, a former PhD student of Adrien Six, and we setup a full R package, which is continuously improved with new data and questions arising from our studies, in collaboration with two PhD students I co-supervise, Pierre Barennes and Vanessa Mhanna (Figure 5). This package includes (i) several diversity measures borrowed from the ecology field, such as rarefaction curves, diversity indices (Renyi Profiling, Gini Index, …) or from information theory field (Shannon diversity) as well as V and J gene usage modelling based on their count and frequencies (Figure 5, top & [130,132–134]) and (ii) several visualization and quantification tools for TCR composition comparison, such as differential TCR clonotype expression [124], similarity matrix [134–137] as well as clone tracking (Figure 5, bottom). Finally, deep sequencing repertoire analysis calls for advanced statistical analysis and graphical representations, such as multivariate analysis (e.g. hierarchical clustering, principal component

analysis, multidimensional scaling…) and probabilistic or network modelling of sequence distributions [4,138–141]. Through a long lasting collaboration with Thierry Mora and Alexandra Walczak's team (Ecole Normale Supérieure de Paris) as well as with Mikhail Shugay (Skoltech Faculty, Moscow), we improved, adapted and extended our workflow with innovative and state-of-the art approaches developed in the field.

Most of those measures have been incorporated in a web-application, named DiversiTR, available internally and developed under my supervision with Dr. Hang Phuong Pham, a statistician and collaborator as well as Gregoire Bolt, a computer science MSc student that I supervised during his MSc internship (Figure 6).

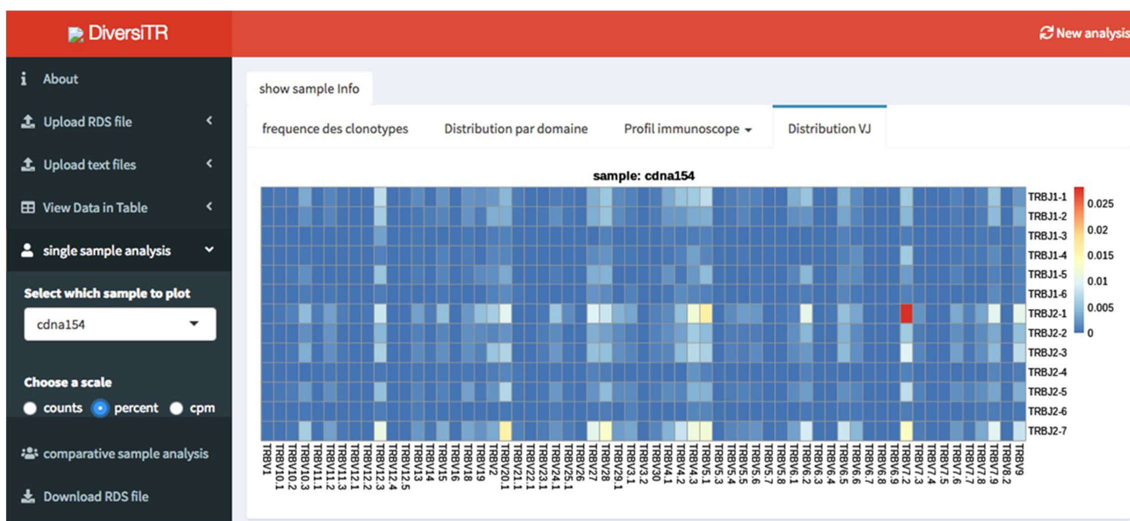

*Figure 6 : **Screen shot of DiversiTR, a web-app for TCR repertoire analysis***

Finally, because we are generating millions of TCR data, we needed a framework to store but most importantly to query our tremendous database. We therefore worked on the specification of the TCR database with Adrien Six and Wahiba Chaara as well as with Hadrien Oubert (a former Bioinformatics MSc student supervised by Adrien Six). The idea was to have a clear knowledge on what we would need to store for efficient and scientifically meaningful queries. We come up with a schema and a finalized version of an in house database. However, lacking the human resources to maintain and scale-up the database in the laboratory, we joint the forces with the iReceptor team from a Simon Fraser University (Vancouver, Canada) who developed the

iReceptor gateway for AIRR-seq data integration and query [142] and was willing to expand its tool to other laboratories. I have been supervising, with the help of the IT manager of the laboratory and the iReceptor team, the setting up of an iReceptor repository in the laboratory, now functional and waiting for data loading.

In addition to the tool development, in a co-supervised work with Adrien Six, we revealed that over sequencing small samples may lead to artificial diversity that can eventually be overcome by using Shannon entropy as a normalization method [132] as it is well known to identify what is called the true diversity [143]. These results led us to realize the necessity in establishing a robust method for TCR repertoire analysis by NGS.

Conclusions

In tight collaboration with statisticians, computer scientists and bioinformaticians, I implemented a framework for advanced still user friendly TCR repertoire modelling that can now be applied for (i) diversity exploration and (ii) TCR signature identification in mice and humans.

Portraying TCR repertoire diversity of Treg subsets in mouse

Background

Treg have been shown as previously introduced as an heterogeneous population, based either on their differentiation origin (thymus vs. periphery [144] but also on their activation status. Indeed, the i3 laboratory showed that in the periphery, activated/memory Tregs (amTregs), characterized as CD4+Foxp3+CD44hiCD62L, are enriched in self-antigen specific Tregs, in comparison with their naïve counterpart (nTregs, CD4+Foxp3+CD44hiCD62L) [145–147] and that they are enriched in deep lymph nodes (dLN) draining deep organs [130]. In addition, a first TCR repertoire study, focusing on only one TCRVβ by NGS in physiological mouse background (C57BL/6), revealed that (i) amTregs and nTregs were characterized by more expansions, compared with Teffs, (ii) expansions of Treg subsets are observed in deep LN compared with their superficial LN counterparts and (iii) amTregs and Teffs display a higher overlap than nTregs and Teffs as well as nTregs and amTregs. Overall, the results suggested an instructive model for mice Treg selection in the thymus as well as a self-antigen specificity of amTregs enriched in deep LN, in line with other studies performed in TCRb transgenic mice [148,149]. This first study prompted

us to further characterize Tregs subset repertoires in different tissue LN as well as in the thymus in mice but also in humans, where indeed seminal studies suggested a global Treg repertoire as diverse as Teff cells [117].

Experimental design

To answer that question, I coordinated the experimental design for Treg subset sampling in mouse by FACS sorting as well as the TCR repertoire analysis.

In human, a similar approach has been implemented by Valentin Quiniou (PhD Student) under the supervision of David Klatzmann to obtain LN, thymus, spleen and blood from organ donors enrolled in an approved protocol by the local ethical committee at APHP. I am not directly involved in the supervision of this study, so I will not further detail it [150].

In mice, we selected two mouse background: (i) the commonly used C57BL/6 mouse background, as a reference model for physiological Treg differentiation and function and (ii) the Non-Obese-Diabetic (NOD) mouse background as an autoimmune disease model where Treg function is impaired notably due to a defect in IL2 [151–153] and specific tissue (the pancreas) is attacked by effector CD4 and CD8 cells [154]. In both backgrounds, a Green Fluorescent Protein (GFP) reporter gene for Foxp3 expression was added by mouse mating in order to unambiguously identify Foxp3^{+} Tregs. amTregs, nTregs, Teffs (CD4+Foxp3-) and CD8+ T cells were sorted from 6 LN (3 deep LN and 3 superficial LN) as well as from the spleen. In addition, thymic subsets where sorted in order to reproduce the major steps of thymocytes differentiation, including Tregs: double positive (DP) CD3- thymocytes, as representative of cells that did not undergo TCRβ selection, DPCD3+ as representative of TCRβ selected cells, and CD4 Single Positive (CD4SP) Foxp3-, CD4SPFoxp3+ and CD8SP as thymocytes positively selected and committed to their respective peripheral subsets (CD4 Teffs, CD4 Tregs and CD8 Teffs). Importantly, those later subsets may include cells that did not undergo negative selection at the time of sampling. This protocol has been applied to young (8-10 weeks old), old (20-26 weeks old) and very old (60 weeks old) mice, female and male separately. Since some Treg subset where very rare in some LN (especially renal and para-aortic), we decided to pool the cells from 6 to 8 age, sex and genetic background matched animals. This protocol was validated by the local ethical committee. All the

sorted cells were systematically stored in lysis buffer at -80°C. Since the beginning of the study in 2013, we collected more than 4000 samples across more than 100 experiments.

Results

During sample collection, we started the analysis of the TCR repertoire of the different T cell subset sorted from the spleen. I supervised two master students, among which one continued as a PhD student in the lab under my co-supervision together with Adrien Six, and granted with a 3 years doctoral fellowship from the French Ministry of Education. A manuscript focusing on splenic CD4 subsets spleen is currently in preparation [155] where we show that amTregs in NOD mice are more diverse than in C57BL/6 probably as a results of interleukine-2 (IL2) production impairment in NOD mouse.

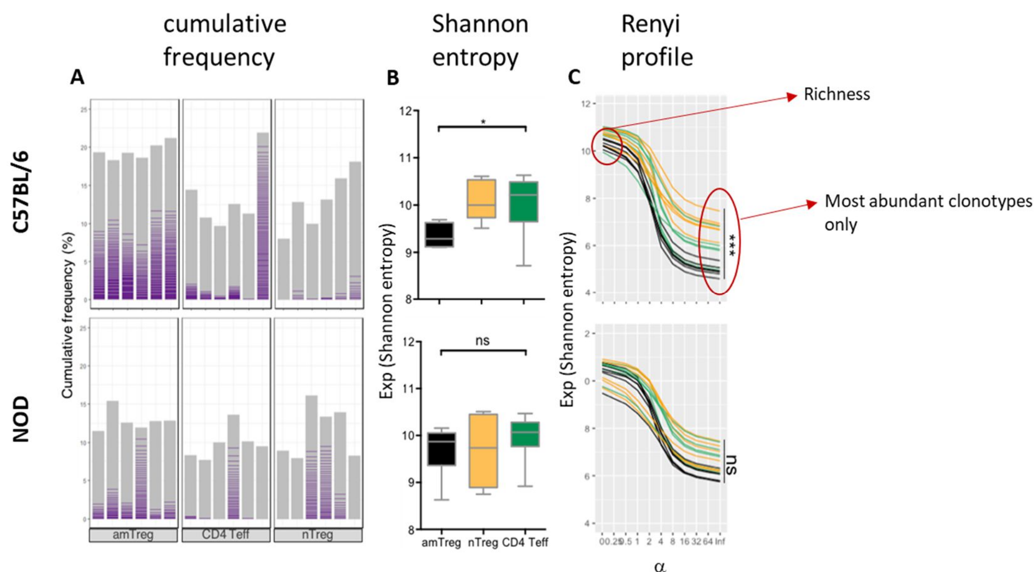

Figure 7 : ***B6 but not NOD amTregs show lower diversity and more expansions than nTregs and Teffs.***
(A) Cumulative frequency of the 1% most predominant clonotypes in each metamouse within the three cell populations, with each purple line representing a unique clonotype (grey lines indicate rare clonotypes) in B6 (upper plot) and in NOD (lower plot) backgrounds. (C) Shannon entropy index was calculated for amTregs (in black), nTregs (in yellow) and Teffs (in green). Statistical analyses were performed using the two-way ANOVA test to compare the different cell subsets (: p<0.05). (D) Renyi profiles display the diversity as a function of the parameter α for amTregs, nTregs and Teffs in B6 (upper plot) and NOD (lower plot). An analysis of similarities (ANOSIM) revealed a statistical difference between the groups in B6 (***: p<0.001) but not in NOD (ns: p≥0.05).*

We therefore applied the same strategy of cell sorting in NOD mice treated with AAV-IL2, a vector restoring IL2 production in mice [156]. This time, cells were sorted from individual mice to

appreciate inter-individual variation. Our results show that IL-2 treatment induces amTregs repertoire expansions (as well as nTregs to a less extend) but not Teffs. In addition, we inferred the specificity of the most expanded clonotypes in each subset and condition (C57BL/6, NOD, NOD+IL2) by comparing them with T1D associated TCR published elsewhere. We found that the expanded clonotypes in amTregs and Teffs (but not in nTregs) were enriched in T1D specific clonotypes in NOD treated by IL2, as well as in C57BL/6 but not in NOD non treated mice.

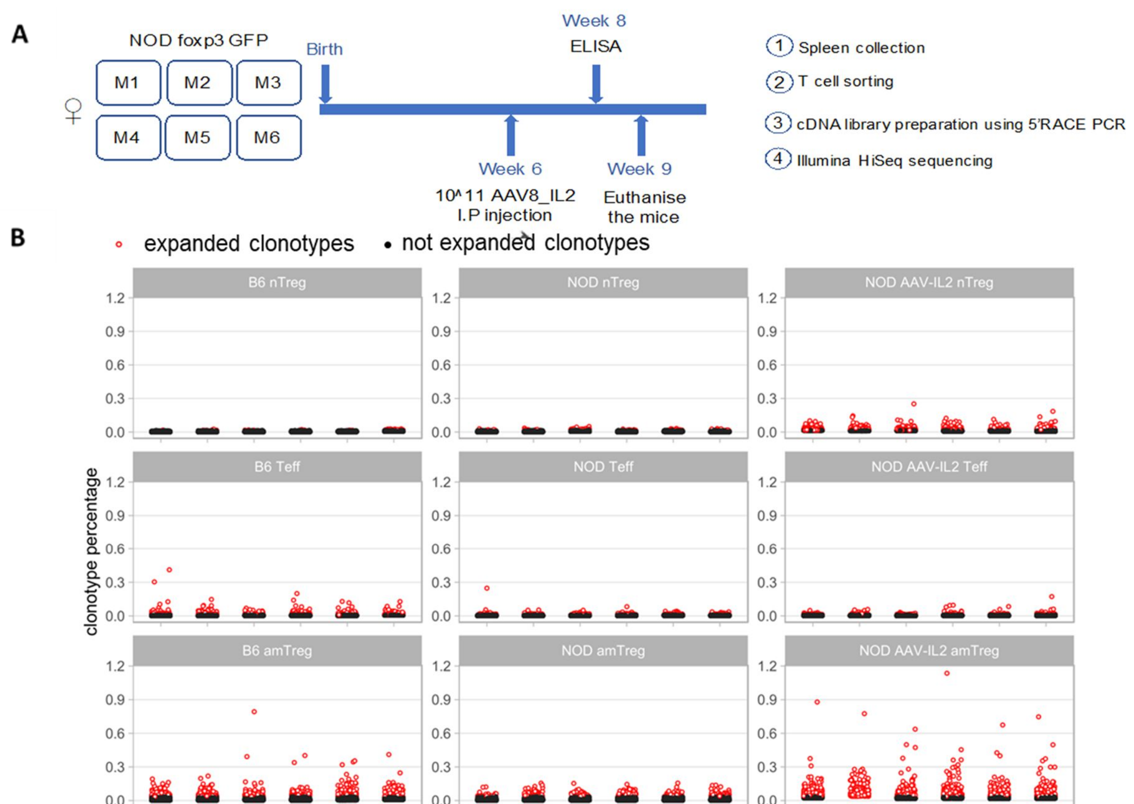

*Figure 8 : **amTregs and nTregs are are expanded in NOD upon low-dose IL-2 treatment**.*

*(A) Experimental design. (B) The abundance (in %) of the expanded clonotypes are plotted for each sample (n=6) within the three populations and mouse models. Red circles represent sequences that lie 3*IQR or more above the third quartile calculated across all 18 samples within each population.*

Altogether, those results suggest (i) that pancreas self-antigen TCRs are naturally arising in mice and enriched in amTregs, (ii) that amTregs are exhausted in NOD background due to impaired IL2 production leading to a poor control of autoreactive Teffs that escape negative selection in the thymus. Further characterization of the overlap between amTregs and Teffs will be required. In addition, since non treated mice repertoires were generated from cells pooled from several mice while NOD treated with IL2 repertoires were analysed from individual mice, we cannot exclude

that the differences, especially in terms of specificity are due to private response of individual mice that might be diluted in NOD pooled repertoires.

In two collaborative studies within the laboratory and with the team of Luis Graca (Instituto Gulbenkian, Portugal), I have been involved in the precise characterization of regulatory follicular T cells (Tfr) and their TCR repertoire in NOD and C57BL/6 mice [133,134,157].

Conclusions

This study has been setting up the ground for our TCR repertoire modelling strategy and already provide insights into fundamental questions regarding T cell differentiation and TCR repertoire diversity importance to better understand pathological conditions.

Coordination of Systems Immunology Activity for the Study of AIDs

Background

Autoimmune and inflammatory diseases (AIDs) have been shown to form a continuum of diseases from pure autoimmune to pure inflammatory diseases [158] mainly based on genetic studies. It is also known that most of the patient diagnosed for one AID, may progressively develop other AIDs. The most representative situation is that of T1D patients, who may develop by time renal and cardiac autoimmune disorders. Since (i) the immune system play a major role in such diseases, (ii) most of the treatments associated with each of the AIDs are not fully beneficial for all the patients and (iii) treatment for one disease may be detrimental in case of a secondary AID occur. The Transimmunom project aims at revisiting the nosography of AIDs through a systems immunology approach consisting in the cross-analysis of multi-parametric data, including blood transcriptome, serum cytokines and auto-antibodies, sorted blood Treg and Teffs TCR repertoire as well as gut microbiome and clinical data.

In the framework of this project, I participated to the overall experimental design of sample collection, benchmarking of data integration tools and molecular biology methods [159] and led the clinical data integration in a harmonized database [160] as well as the validation of molecular biology approaches for TCR repertoire sequencing by NGS [161].

AID patient clinical data integration

In tight collaboration with Roberta Lorenzon, the medical doctor of the Transimmunom trial, we established a workflow (Figure 9).

Figure 9 : ***eCRF design and implementation workflow.***
The figure represents the 3-steps workflow adopted for the eCRF design and implementation: (1) Protocol design, (2) eCRF design and (3) eCRF validation. In each box are listed the actions, its aim and the person in charge of it. Colour code: Blue: clinical team, purple: biological team, green: computer scientist, and orange: the trial monitor team and brown the Core management team (see methods for description).

Results

To tackle the challenge, we assembled a clinical expert consortium (CEC) to select relevant clinic-biological features to be collected for all patients and a cohort management team comprising biologists, clinicians and computer scientists to design an electronic case report form (eCRF). After more than a year and a half (100h of meetings), we (i) choose as an eCRF platform an open-source CFR-part 11 compliant electronic data capture system, OpenClinica, (ii) selected with the CEC 865 clinical and biological parameters covering all the diseases and to be (for more than 80%) collected for all the patient and healthy volunteers recruited in the trial, (iii) coded the parameters using CDISC standards into 5835 coded values and (iv) designed 28 structured eCRFs

by categorizing the 865 parameters. Examples of coding are check boxes for clinical investigation, numerical values with units, disease scores as a result of an automated calculations, and coding of possible treatment formulas, doses and dosage regimens per disease. Technical adjustment have been implemented to allow data entry and extraction of this amount of data, rarely achieved in classical eCRFs designs.

Conclusions

This task was one of my first mission when I joined the i3 laboratory. Although it can appear far from my initial background, I have been proposed this task based on the experienced I acquired in interdisciplinary projects since the beginning of my PhD. On top of this, I learned a lot regarding translational research and clinical trials, which offered me an additional knowledge to undertake new multidisciplinary studies, including translational and clinical trial research programs. This work will be valorised through upcoming collaboration within the new Medical & University Department of Immunology and Biotherapies at Pitié-Salpêtrière as well as the Hu-Precimed consortium, a project structuring the Precision Medicine sector in France to which I am participating, together with several members of the Transimmunom project, with regards with clinical data integration.

Establishment of a robust experimental method for TCR library preparation and sequencing

Background

Since the advance of NGS, analysis of the TCR repertoire is increasingly being used to understand disease pathogenesis and lymphocyte dynamics in health as well as in various pathological contexts such as autoimmune diseases, infections or cancers [75,162–167]. Indeed, with the high resolution provided by NGS should contribute to (1) deciphering the specificity or the breadth of a given adaptive immune response under pathological conditions, (2) identifying TCRs as biomarkers of diseases or clinical response to a treatment and (3) stratifying patients according to their TCR, therefore fostering new personalized therapeutic development.

However, because of the broad immunological situations where TCR-Seq can apply with the aim either at characterizing subtle modifications of the repertoire [10,11,166–174] or at identifying

and tracking major clonal lymphomas [175–177], the requirement in terms of specificity and sensitivity of the method is different. Technically speaking, there are a number of caveats for which there is a lack of consensus in the field. Indeed, each step from sample preparation to analysis is critical and may have a profound impact on the actual data generated and subsequent interpretation of the results [178]. Still, there is no precise answer on the impact of methodology on TCR diversity measures, such as gene usage, rearrangement insertion and deletion, clonotype distribution, and quantification of rare versus abundant sequences. Two major approaches are used: 5'RACE-PCR (exclusively on RNA) and multiplex-PCR (either on RNA or DNA). To solve those issue and identify a robust and reproducible method that we could use in our different ongoing projects, I launched a broad comparative study and succeeded to mobilize key leaders in the field.

Results

We compared 8 different methods, including three from commercial entities, using as starting material a standardized set of input samples (Figure 10). Our aims were for each method to evaluate (i) their robustness in identifying and quantifying clonotypes of different sizes and (ii) their sensitivity to the input material quantity. Using the same analysis strategy on all the data generated, we observed differences between methods for (i) intra- and inter-method reproducibility–and (ii) the accuracy of TCR determination. Notably, our study revealed that starting from RNA, RACE-PCR based methods performed better than multiplex-PCR. While DNA based methods provided high quality data, the absence of commercialized methods for TCRα repertoire limits the interest of such approaches. This study highlighted the advantages and limitations of different TCR-seq methods, which may be used to guide approaches to the study of human diseases in which the TR plays a fundamental role. This work has been handle by Pierre Barennes, PhD student under my co-supervision and the manuscript is now under revision [161].

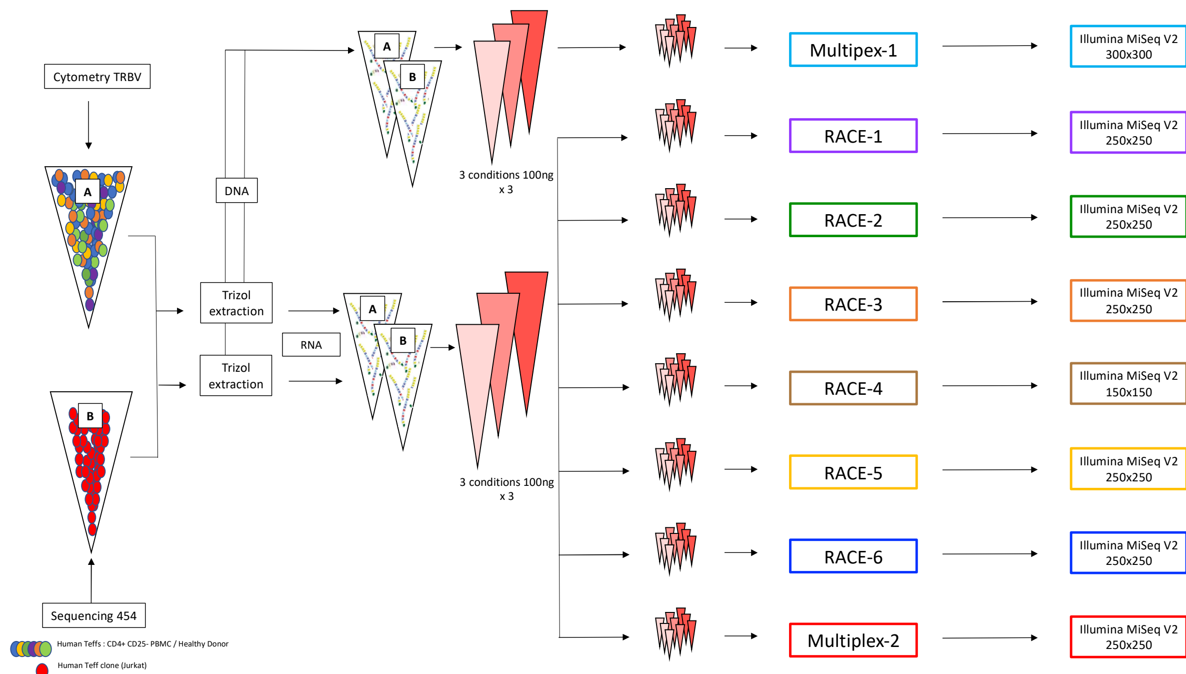

Figure 10 : ***TCR library method comparison - Experimental design.***
DNA and RNA of FACS-sorted effector T cells (CD4+CD25-CD127+) from one donor and cultured Jurkat leukemic T-cells were extracted. Jurkat RNA has been then added to effector T cells RNA at the following ratios: 1:1000, 1:100, 1:10 to a final quantity of 100ng. Same dilutions have been performed on DNA samples with DNA from Jurkat cells. Triplicate of each ratio condition were processed by 7 TR library preparation methods for RNA and by one TR library preparation for DNA. All the libraries were sequenced on Illumina MiSeq as indicated in the figure panel.

In addition to the molecular method validation, in collaboration with the LIGAN EquipEx to fine-tune the sequencing method, with the team of Michelle Rosenzwajg to set an efficient and handy method for Treg vs. Teff cell separation from blood samples and recently with a French biotech-company (Primadiag), commercializing automatization systems for NGS library preparation, together with Pierre Barennes we established a complete workflow for TCR library preparation and sequencing from patient blood samples.

General Conclusions

During my second post-doc and my starting position as an assistant professor in i3 lab, I (i) develop a framework for TCR repertoire modelling in mouse and humans including wet and dry lab developments, (ii) characterize several Treg cell subset TCR peculiarities and (iii) set-up a systems immunology approach for AIDs study.

Altogether, 8 research articles were published and 2 reviews were published out of my first post-doc work (**Publication achievements C**).

Publication achievements C : Post-doc 2 and assistant professor
Main research projects:
As co-author:
Lorenzon R*, **Mariotti-Ferrandiz E*** et al. **BMJOpen**. PMID: 30166293 (T15)
Bergot A-S et al. **Eur. J. Immunol**. PMID: 25726757 (T6)
Ritvo P.G et al. **Science Immunology**. PMID: 28887367 (T10)
As (co-)Principal Investigator:
Lorenzon R et al. bioRxiv 360719; doi: https://doi.org/10.1101/360719 (T14)
Chaara W et al. **Frontiers in Immunology** PMID: 29868003 (T12)
Ritvo PG et al. **PNAS**. PMID: 30158170 (T13)
Collaborative research projects
Maceiras AR et al. **Nature Communication.** PMID: 28802258 (T9)
Rubelt F* et al. **Nature Immunology**, PMID: 29144493 (T11)
Reviews:
Ellul P et al. **Frontiers in Neurology**. PMID: 29615964 (R3)
Six A et al. **Frontiers in Immunology**. PMID: 24348479 (R2)

Perspectives (2019-2023)

In the next four years, I will continue my research work on (i) the analysis of the TCR repertoire of T lymphocytes in physiological and pathological conditions with the integration of single-cell approaches and (ii) the integration of multi-OMICS data for the implementation of systems immunology approaches in translational research.

The TCR, a potential biomarker of the physiopathological state in humans

Main research areas

(i) The "biomarker" potential of the TCR repertoire in the context of AIDs with the aim of characterizing the nature of the modulations of the Treg and Teff peripheral TCR repertoire in two situations:

(a) according to the pathology and to identify the presence of pathology-dependent TCR signatures in the circulating repertoire.

My work will be based on the TCR Treg / Teff repertoire data currently produced as part of the Transimmunom [159] project, including 400 patients and 100 healthy volunteers. From Treg and Teff sorted from peripheral blood, the TCR repertoire data will be obtained by high-throughput sequencing according to the protocol validated under my direction at the laboratory [161]. I will coordinate the implementation of the statistical modelling of the data using the tools available in the laboratory [130,132–134]. Our preliminary results comparing blood Treg and Teff repertoires from healthy donors and T1D patients and using probabilistic model revealed disease and cell-subset signatures (Figure 11).

[Transimmunom Project, ANR-11-LABX-0069, funded until 2020; 1 doctoral student under my co-supervision with Prof. David Klatzmann (currently 2nd year of PhD)].

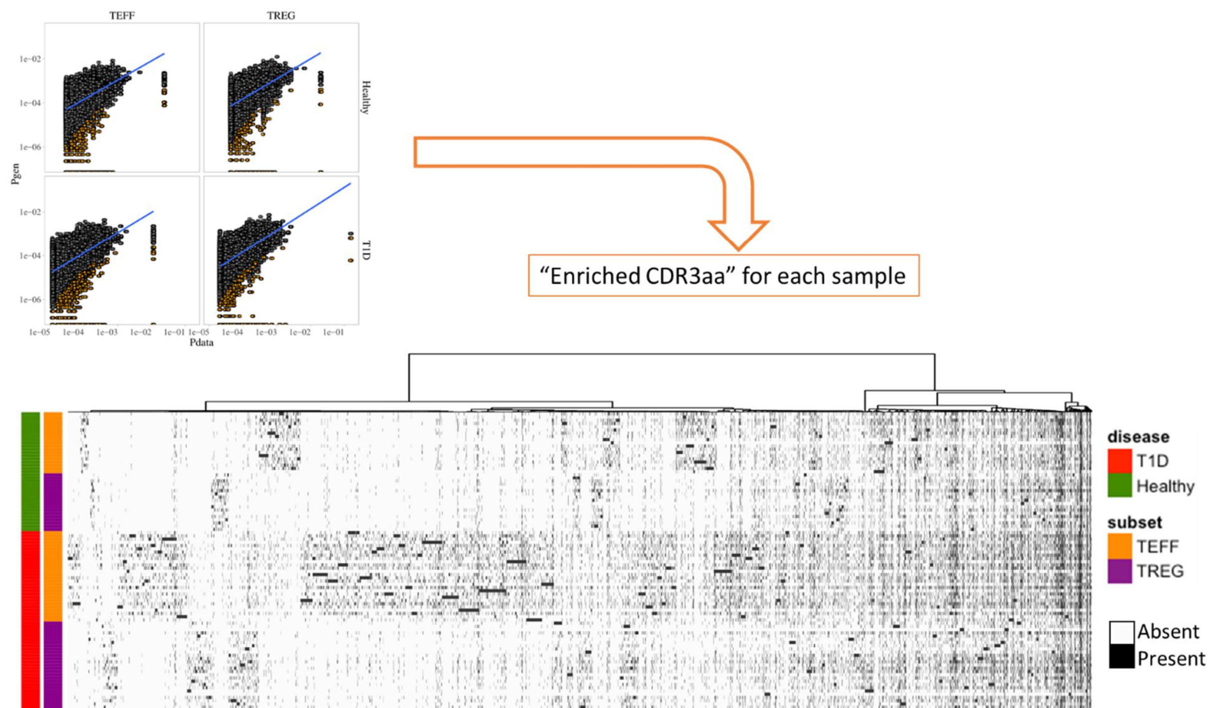

*Figure 11 : **Probability distribution reveals clinical condition and cell-subset CDR3aa signatures***
For each dataset, the probability of each unique CDR3 (Pdata) has been evaluated in regards with the probability of generation (Pgen) based on Mora et al. model. All the CDR3s with a Pdata significantly higher than the theoretical Pgen is highlighted in orange (p. adjusted <0,05) and considered as enriched CDR3s in the dataset (Top). All the enriched CDR3s identified in each sample have been then clustered using hierarchical clustering (below).

(b) in response to immunomodulatory therapy and to identify its predictive value by comparing the repertoire before and after treatment of responder vs. non-responder patients.

This project will be based on the interventional clinical trial LUPIL-2 (NCT02955615) for which I coordinate the activity "multi-omics integration" (see below) and the analysis of the TCR repertoire. Recent work have shown the clinical potential of ILT-101 immunotherapy (IL-2 low dose) in patients with different AIDs, including SLE [56,57,151,179]. This activation can lead to modifications of the Treg and Teff repertoire, as we found in mice, which can serve as a "predictive biomarker of response to treatment". The TCR repertoire data will be produced according to the same experimental design as in the Transimmunom project using longitudinal samples (n = 5) obtained from 50 placebo patients and 50 patients treated with ILT-101.

In both contexts, I will coordinate the implementation of statistical data modelling using the tools available in the laboratory as described earlier.

[IMAP project (ANR-16-RHUS-0001), financed until 2021; 1 doctoral student under my co-supervision with Prof. David Klatzmann (currently 2nd year of PhD)]

ii) Characterization of TCR of Treg and Teff in myocardial infarction (MI) by a single-cell approach. In October 2018, I obtained an ERA-NET funding (for young researchers) with the aim of characterizing the TCR repertoire of Treg and Teff in an experimental MI model using a single-cell approach. MI is accompanied by T-cell infiltration in the myocardium, which in turns is implicated in the remodelling necessary for a good post-infarction prognosis [180] with a role for Treg in myocardial repair [181]. By isolating T cells from tissue and mediastinal lymph nodes of mice triggering mechanically induced myocardial infarction, it will then be possible to identify the diversity of the cellular infiltrate. Combining repertoire analysis with single-cell transcriptome, my objectives will be to coordinate the statistical modelling of single-cell data in order to (a) characterize the diversity of the repertoire of cells infiltrating the post-infarction heart and (b) identify the TCRs associated with the healing function of Treg vs. the inflammatory function of Teff. The samples will be obtained in collaboration with the University of Wurzburg (Gustavo Campos-Ramos, project partner). In parallel, samples from peripheral blood and tissues biobanked under a cohort of patients established 10 years ago at the Wurzburg Hospital will be processed for TCR repertoire by us and the third partner (and project coordinator), Peter Rainer. I will coordinate the analysis of those data. Together with mouse data, we expect to identify markers of MI healing.

[AIR-MI project (ERA-NET on Cardiovascular Diseases (JCT2018 / H2020), 1 PhD student (recruitment in progress) under my supervision, funded until 2022].

Collaborative research projects

Mapping of the diversity of the TCR repertoire of lymphocyte subpopulations T in humans and mice [*TriPoD project, 1 doctoral student under my co-supervision with Adrien Six funded until October 2020*] "; TCR repertoire in NSC breast cancer patients (Isabelle Cremer, Centre de Recherche des Cordeliers, Paris, France).

Systems Immunology: a Holistic Approach to the Study of Autoimmune and Inflammatory Diseases (AIDs)

The complexity of cellular interactions and regulation during immune responses requires comprehensive approaches aimed at not only studying the individual components involved, but also their complex interactions [29,31,182]. Systems biology approaches are increasingly applied to the study of immune diseases. The i3 laboratory therefore introduced a systems immunology approach in 2008 by studying the immune system [159] as tissue and combining cytomic, genetic, transcriptomic and proteomic studies [51,58,61,88,132,134,157,160,183,184]. These approaches are at the heart of the Transimmunom and iMAP projects.

Main research area

Towards integrating adaptive Immune receptor repertoire (AIRR) data together with clinical and multi-OMICS data. After the coordination of the technological benchmarking for the production of high quality OMICS data, in particular the transcriptome and the TCR repertoire (Transimmunom and iMAP), as well as the design of the Transimmunom project's clinical database [160], I will now focus on the integration of multiomics data with the AIRR-sequencing databases as part of the Project iReceptor+ project (SC1-BHC-05-2018 (H2020) for which I obtained funding in 2018.

The analysis of the TCR and BCR repertoire covers many clinical conditions, however the current available databases, such as iReceptor [142], VDJserver [185], VDJdb [186] and Mc-PAS [187] are non-harmonized and do not take into account all the parameters of the immune system and clinical data that are needed to better interpret the link between adaptive Immune receptor repertoires (AIRR) and the physiopathological condition studied. iReceptor+ project aims to strengthen the value of public data and their exploitation for basic and translational research towards clinics. The first goal of the project is to build, based on iReceptor framework, a federated and harmonized AIRR database. The second is to link modelling tools with this database to foster data analysis. The third aim, which I am responsible for, is to build a framework for the integration of multi-OMICS and clinical data together with AIRR data from iReceptor. To this end, I will take advantage of the implementation within Transimmunom project of an

integration tool to combine clinical and multi-OMICS data on a patient-oriented database, using tranSMART platform [188–190], and coordinate the design the of framework to make tranSMART interoperable with iReceptor federated repository. This work will consist mainly in (i) establishing the AIRR variables to be integrated in tranSMART for multi-OMICS cross-analysis, (ii) establishing a prototype for minimal standard systems immunology data and (iii) designing a tranSMART-API for multi-OMICS including AIRR data.

[*Project iReceptor + (SC1-BHC-05-2018 (H2020), 1 research engineer recruited in June 2019, 1 doctoral fellow (recruitments in progress); funded until 2022*].

Collaborative research projects

Integration and analysis of multi-OMICS data from patients with AIDs (Transimmunom Project [Transimmunom Project, ANR-11-LABX-0069] and SLE patients under ILT-101 treatment [iMAP Project (ANR -16-RHUS-0001)]; Role of regulatory T lymphocytes in a model of autism induced by "maternal Immune Activation" (Hôpital Debré, Paris, France); Participation in the structuring of a precision medicine sector, Hu-PreciMED; Immune related adverse events in cancer patients under Immune check-point inhibitors treatment: TCR as a biomarker of IrAEs outcome, Departement Medical Universitaire, Pitie-Salpêtrière Hospital (consortium establishment and fund raising on going).

Conclusions

With time, I acquired an expertise on the TCR repertoire field, conceptually, scientifically and technically as well as in high-throughput data analysis. In the last years, I further expanded my interest towards systems immunology with the aim to integrate function, cell-cell interaction and T cell repertoire information together. My future research will aim at bridging the gap between TCR repertoire diversity and immune cell function towards translational systems immunology in tight collaboration with clinicians, bioinformaticians and community networks within the framework of the four main projects I am involved in (Figure 12).

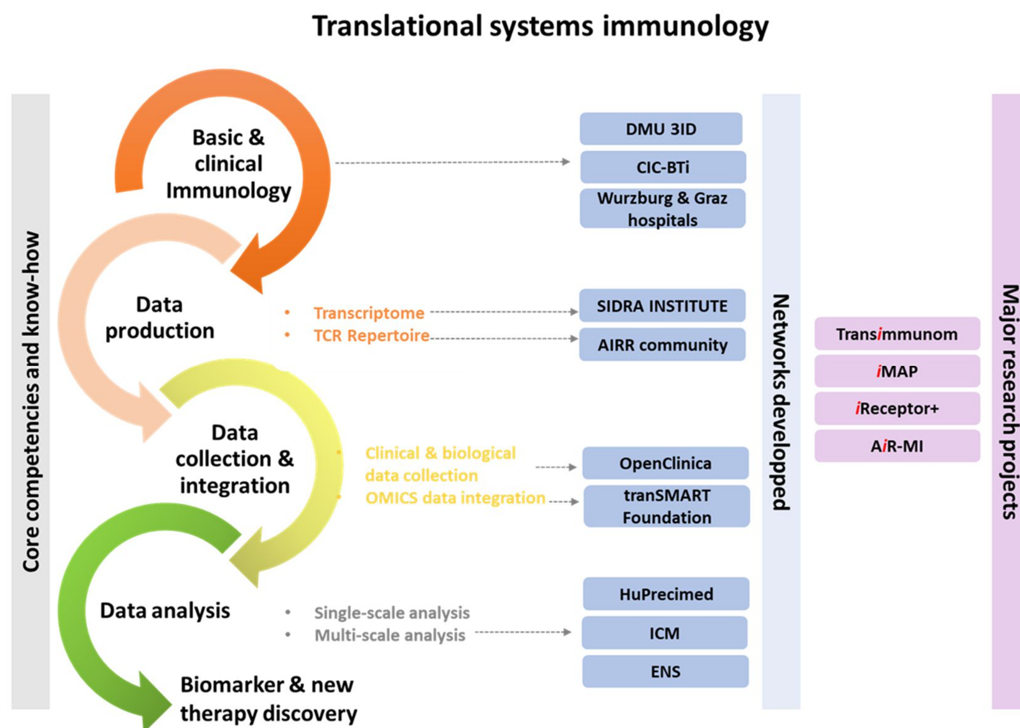

*Figure 12 : **Schematic representative of my research perspectives***

As an assistant professor recruited in Sorbonne Université (ex-UPMC), I have been involved since the beginning and now with the leadership of a master degree in Systems Immunology (starting next September 2019; https://bit.ly/2GDJfHf), in disseminating and training young generation of Immunologists to this "Renaissance" of the systems immunology field.

References

1. Chien YH, Gascoigne NR, Kavaler J, Lee NE, Davis MM. Somatic recombination in a murine T-cell receptor gene. Nature. 1984;309: 322–326.

2. Tonegawa S. Somatic generation of antibody diversity. Nature. 1983;302: 575–581. doi:10.1038/302575a0

3. Bradley P, Thomas PG. Using T Cell Receptor Repertoires to Understand the Principles of Adaptive Immune Recognition. Annu Rev Immunol. 2019;37: 547–570. doi:10.1146/annurev-immunol-042718-041757

4. Mora T, Walczak A. Quantifying lymphocyte receptor diversity. ArXiv Prepr ArXiv160400487. 2016; Available: https://arxiv.org/abs/1604.00487

5. Esser MT, Marchese RD, Kierstead LS, Tussey LG, Wang F, Chirmule N, et al. Memory T cells and vaccines. Vaccine. 2003;21: 419–430. doi:10.1016/S0264-410X(02)00407-3

6. Baker FJ, Lee M, Chien Y, Davis MM. Restricted islet-cell reactive T cell repertoire of early pancreatic islet infiltrates in NOD mice. Proc Natl Acad Sci. 2002;99: 9374–9379.

7. Ferreira C, Palmer D, Blake K, Garden OA, Dyson J. Reduced Regulatory T Cell Diversity in NOD Mice Is Linked to Early Events in the Thymus. J Immunol. 2014;192: 4145–4152. doi:10.4049/jimmunol.1301600

8. Marrero I, Hamm DE, Davies JD. High-Throughput Sequencing of Islet-Infiltrating Memory CD4+ T Cells Reveals a Similar Pattern of TCR VÎ² Usage in Prediabetic and Diabetic NOD Mice. von Herrath MG, editor. PLoS ONE. 2013;8: e76546. doi:10.1371/journal.pone.0076546

9. Marrero I, Aguilera C, Hamm DE, Quinn A, Kumar V. High-throughput sequencing reveals restricted TCR Vβ usage and public TCRβ clonotypes among pancreatic lymph node memory CD4+ T cells and their involvement in autoimmune diabetes. Mol Immunol. 2016;74: 82–95. doi:10.1016/j.molimm.2016.04.013

10. Eugster A, Lindner A, Catani M, Heninger A-K, Dahl A, Klemroth S, et al. High Diversity in the TCR Repertoire of GAD65 Autoantigen-Specific Human CD4 $^{+}$ T Cells. J Immunol. 2015;194: 2531–2538. doi:10.4049/jimmunol.1403031

11. Gomez-Tourino I, Kamra Y, Baptista R, Lorenc A, Peakman M. T cell receptor β-chains display abnormal shortening and repertoire sharing in type 1 diabetes. Nat Commun. 2017;8: 1792. doi:10.1038/s41467-017-01925-2

12. Jacobsen LM, Posgai A, Seay HR, Haller MJ, Brusko TM. T Cell Receptor Profiling in Type 1 Diabetes. Curr Diab Rep. 2017;17. doi:10.1007/s11892-017-0946-4

13. Seay HR, Yusko E, Rothweiler SJ, Zhang L, Posgai AL, Campbell-Thompson M, et al. Tissue distribution and clonal diversity of the T and B cell repertoire in type 1 diabetes. JCI Insight. 2016;1. doi:10.1172/jci.insight.88242

14. Costa N, Pires AE, Gabriel AM, Goulart LF, Pereira C, Leal B, et al. Broadened T-cell Repertoire Diversity in ivIg-treated SLE Patients is Also Related to the Individual Status of Regulatory T-cells. J Clin Immunol. 2013;33: 349–360. doi:10.1007/s10875-012-9816-7

15. Kolowos W, Herrmann M, Ponner BB, Voll R, Kern P, Frank C, et al. Detection of restricted junctional diversity of peripheral T cells in SLE patients by spectratyping. Lupus. 1997;6: 701–707.

16. Liu X, Zhang W, Zhao M, Fu L, Liu L, Wu J, et al. T cell receptor β repertoires as novel diagnostic markers for systemic lupus erythematosus and rheumatoid arthritis. Ann Rheum Dis. 2019; annrheumdis-2019-215442. doi:10.1136/annrheumdis-2019-215442

17. Sui W, Hou X, Zou G, Che W, Yang M, Zheng C, et al. Composition and variation analysis of the TCR β-chain CDR3 repertoire in systemic lupus erythematosus using high-throughput sequencing. Mol Immunol. 2015;67: 455–464. doi:10.1016/j.molimm.2015.07.012

18. Thapa DR, Tonikian R, Sun C, Liu M, Dearth A, Petri M, et al. Longitudinal analysis of peripheral blood T cell receptor diversity in patients with systemic lupus erythematosus by next-generation sequencing. Arthritis Res Ther. 2015;17. doi:10.1186/s13075-015-0655-9

19. Di Sante G, Tolusso B, Fedele AL, Gremese E, Alivernini S, Nicolò C, et al. Collagen Specific T-Cell Repertoire and HLA-DR Alleles: Biomarkers of Active Refractory Rheumatoid Arthritis. EBioMedicine. 2015;2: 2037–2045. doi:10.1016/j.ebiom.2015.11.019

20. Kim K-J, Kim M, Adamopoulos IE, Tagkopoulos I. Compendium of synovial signatures identifies pathologic characteristics for predicting treatment response in rheumatoid arthritis patients. Clin Immunol Orlando Fla. 2019;202: 1–10. doi:10.1016/j.clim.2019.03.002

21. Lissina A, McLaren JE, Ilander M, Andersson EI, Lewis CS, Clement M, et al. Divergent roles for antigenic drive in the aetiology of primary versus dasatinib-associated CD8+ TCR-Vβ+ expansions. Sci Rep. 2018;8: 2534. doi:10.1038/s41598-017-18062-x

22. Musters A, Klarenbeek PL, Doorenspleet ME, Balzaretti G, Esveldt REE, van Schaik BDC, et al. In Rheumatoid Arthritis, Synovitis at Different Inflammatory Sites Is Dominated by Shared but Patient-Specific T Cell Clones. J Immunol. 2018;201: 417–422. doi:10.4049/jimmunol.1800421

23. Rossetti M, Spreafico R, Consolaro A, Leong JY, Chua C, Massa M, et al. TCR repertoire sequencing identifies synovial Treg cell clonotypes in the bloodstream during active inflammation in human arthritis. Ann Rheum Dis. 2017;76: 435–441. doi:10.1136/annrheumdis-2015-208992

24. Schneider-Hohendorf T, Görlich D, Savola P, Kelkka T, Mustjoki S, Gross CC, et al. Sex bias in MHC I-associated shaping of the adaptive immune system. Proc Natl Acad Sci. 2018;115: 2168–2173. doi:10.1073/pnas.1716146115

25. Engleman EG, Benike CJ, Glickman E, Evans RL. Antibodies to membrane structures that distinguish suppressor/cytotoxic and helper T lymphocyte subpopulations block the mixed leukocyte reaction in man. J Exp Med. 1981;154: 193–198. doi:10.1084/jem.154.1.193

26. DuPage M, Bluestone JA. Harnessing the plasticity of CD4(+) T cells to treat immune-mediated disease. Nat Rev Immunol. 2016;16: 149–163. doi:10.1038/nri.2015.18

27. Brummelman J, Pilipow K, Lugli E. Chapter Two - The Single-Cell Phenotypic Identity of Human CD8+ and CD4+ T Cells. In: Galluzzi L, Rudqvist N-P, editors. International Review of Cell and Molecular Biology. Academic Press; 2018. pp. 63–124. doi:10.1016/bs.ircmb.2018.05.007

28. Burnet FM. Clonal selection theory of acquired immunity. Cambridge: University Press; 1959.

29. Benoist C, Germain RN, Mathis D. A plaidoyer for 'systems immunology.' Immunol Rev. 2006;210: 229–234.

30. Breitling R. What is systems biology? Front Physiol. 2010;1: 9. doi:10.3389/fphys.2010.00009

31. Burel JG, Apte SH, Doolan DL. Systems Approaches towards Molecular Profiling of Human Immunity. Trends Immunol. 2016;37: 53–67. doi:10.1016/j.it.2015.11.006

32. Chaussabel D, Baldwin N. Democratizing systems immunology with modular transcriptional repertoire analyses. Nat Rev Immunol. 2014;14: 271–280. doi:10.1038/nri3642

33. Davis MM, Tato CM, Furman D. Systems immunology: just getting started. Nat Immunol. 2017;18: 725–732. doi:10.1038/ni.3768

34. Or-Guil M, Boyd SD. Systems immunology of human humoral immunity. Curr Opin Syst Biol. 2018;12: 70–77. doi:10.1016/j.coisb.2018.11.002

35. Shay T, Kang J. Immunological Genome Project and systems immunology. Trends Immunol. 2013;34: 602–609. doi:10.1016/j.it.2013.03.004

36. Piasecka B, Duffy D, Urrutia A, Quach H, Patin E, Posseme C, et al. Distinctive roles of age, sex, and genetics in shaping transcriptional variation of human immune responses to microbial challenges. Proc Natl Acad Sci. 2018;115: E488–E497. doi:10.1073/pnas.1714765115

37. Thomas-Vaslin V, Altes HK, De Boer R, Klatzmann D. Comprehensive assessment and mathematical modeling of T cell population dynamics and homeostasis. J Immunol. 2008;180: 2240–2250.

38. Thomas-Vaslin V, Six A, Ganascia J-G, Bersini H. Dynamical and Mechanistic Reconstructive Approaches of T Lymphocyte Dynamics: Using Visual Modeling Languages to Bridge the Gap between Immunologists, Theoreticians, and Programmers. Front Immunol. 2013;4. doi:10.3389/fimmu.2013.00300

39. Urrutia A, Duffy D, Rouilly V, Posseme C, Djebali R, Illanes G, et al. Standardized Whole-Blood Transcriptional Profiling Enables the Deconvolution of Complex Induced Immune Responses. Cell Rep. 2016;16: 2777–2791. doi:10.1016/j.celrep.2016.08.011

40. Vaeth M, Wang Y-H, Eckstein M, Yang J, Silverman GJ, Lacruz RS, et al. Tissue resident and follicular Treg cell differentiation is regulated by CRAC channels. Nat Commun. 2019;10: 1183. doi:10.1038/s41467-019-08959-8

41. Blazewicz J, Borowski M, Chaara W, Kedziora P, Klatzmann D, Lukasiak P, et al. GeVaDSs - decision support system for novel Genetic Vaccine development process. BMC Bioinformatics. 2012;13: 91. doi:10.1186/1471-2105-13-91

42. Dérian N, Bellier B, Pham HP, Tsitoura E, Kazazi D, Huret C, et al. Early Transcriptome Signatures from Immunized Mouse Dendritic Cells Predict Late Vaccine-Induced T-Cell Responses. Altan-Bonnet G, editor. PLOS Comput Biol. 2016;12: e1004801. doi:10.1371/journal.pcbi.1004801

43. Fourati S, Ribeiro SP, Blasco Tavares Pereira Lopes F, Talla A, Lefebvre F, Cameron M, et al. Integrated systems approach defines the antiviral pathways conferring protection by the RV144 HIV vaccine. Nat Commun. 2019;10: 863. doi:10.1038/s41467-019-08854-2

44. Gonçalves E, Bonduelle O, Soria A, Loulergue P, Rousseau A, Cachanado M, et al. Innate gene signature distinguishes humoral versus cytotoxic responses to influenza vaccination. J Clin Invest. 2019;130. doi:10.1172/JCI125372

45. Matthijs AMF, Auray G, Jakob V, García-Nicolás O, Braun RO, Keller I, et al. Systems Immunology Characterization of Novel Vaccine Formulations for Mycoplasma hyopneumoniae Bacterins. Front Immunol. 2019;10: 1087. doi:10.3389/fimmu.2019.01087

46. McKay PF, Cizmeci D, Aldon Y, Maertzdorf J, Weiner J, Kaufmann SH, et al. Identification of potential biomarkers of vaccine inflammation in mice. eLife. 2019;8. doi:10.7554/eLife.46149

47. Six A, Bellier B, Thomas-Vaslin V, Klatzmann D. Systems biology in vaccine design: Systems biology in vaccine design. Microb Biotechnol. 2012;5: 295–304. doi:10.1111/j.1751-7915.2011.00321.x

48. Thiébaut R, Hejblum BP, Hocini H, Bonnabau H, Skinner J, Montes M, et al. Gene Expression Signatures Associated With Immune and Virological Responses to Therapeutic Vaccination With Dendritic Cells in HIV-Infected Individuals. Front Immunol. 2019;10: 874. doi:10.3389/fimmu.2019.00874

49. Aghaeepour N, Ganio EA, Mcilwain D, Tsai AS, Tingle M, Gassen SV, et al. An immune clock of human pregnancy. Sci Immunol. 2017;2: eaan2946. doi:10.1126/sciimmunol.aan2946

50. Blazkova J, Gupta S, Liu Y, Gaudilliere B, Ganio EA, Bolen CR, et al. Multicenter Systems Analysis of Human Blood Reveals Immature Neutrophils in Males and During Pregnancy. J Immunol. 2017;198: 2479–2488. doi:10.4049/jimmunol.1601855

51. Nehar-Belaid D, Courau T, Derian N, Florez L, Ruocco MG, Klatzmann D. Regulatory T Cells Orchestrate Similar Immune Evasion of Fetuses and Tumors in Mice. J Immunol. 2016;196: 678–690. doi:10.4049/jimmunol.1501834

52. Banchereau R, Hong S, Cantarel B, Baldwin N, Baisch J, Edens M, et al. Personalized Immunomonitoring Uncovers Molecular Networks that Stratify Lupus Patients. Cell. 2016;165: 551–565. doi:10.1016/j.cell.2016.03.008

53. Chaussabel D, Quinn C, Shen J, Patel P, Glaser C, Baldwin N, et al. A Modular Analysis Framework for Blood Genomics Studies: Application to Systemic Lupus Erythematosus. Immunity. 2008;29: 150–164. doi:10.1016/j.immuni.2008.05.012

54. Jourde-Chiche N, Burtey S, Bardin N, Whalen E, Gondouin B, Presnell S, et al. Modular Transcriptional Neutrophil Signature As Predictive of Nephritis and of Its Severity in SLE Patients. Arthritis Rheumatol. 2014;66: S711–S712.

55. Ramilo O, Allman W, Chung W, Mejias A, Ardura M, Glaser C, et al. Gene expression patterns in blood leukocytes discriminate patients with acute infections. Blood. 2007;109: 2066–2077. doi:10.1182/blood-2006-02-002477

56. Rosenzwajg M, Churlaud G, Mallone R, Six A, Dérian N, Chaara W, et al. Low-dose interleukin-2 fosters a dose-dependent regulatory T cell tuned milieu in T1D patients. J Autoimmun. 2015;58: 48–58. doi:10.1016/j.jaut.2015.01.001

57. Rosenzwajg M, Lorenzon R, Cacoub P, Pham HP, Pitoiset F, El Soufi K, et al. Immunological and clinical effects of low-dose interleukin-2 across 11 autoimmune diseases in a single, open clinical trial. Ann Rheum Dis. 2019;78: 209–217. doi:10.1136/annrheumdis-2018-214229

58. Saadoun D, Rosenzwajg M, Joly F, Six A, Carrat F, Thibault V, et al. Regulatory T-cell responses to low-dose interleukin-2 in HCV-induced vasculitis. N Engl J Med. 2011;365: 2067–2077. doi:10.1056/NEJMoa1105143

59. Terrier B, Geri G, Chaara W, Allenbach Y, Rosenzwajg M, Costedoat-Chalumeau N, et al. IL-21 modulates Th1 and Th17 responses in giant cell arteritis. Arthritis Rheum. 2011; doi:10.1002/art.34327

60. Terrier B, Geri G, Chaara W, Allenbach Y, Rosenzwajg M, Costedoat-Chalumeau N, et al. Interleukin-21 modulates Th1 and Th17 responses in giant cell arteritis. Arthritis Rheum. 2012;64: 2001–2011. doi:10.1002/art.34327

61. Terrier B, Chaara W, Dufat L, Geri G, Rosenzwajg M, Musset L, et al. Serum biomarker signature identifies patients with B-cell non-Hodgkin lymphoma associated with cryoglobulinemia vasculitis in chronic HCV infection. Autoimmun Rev. 2014;13: 319–326. doi:10.1016/j.autrev.2013.11.001

62. Cardona P-J, Català M, Arch M, Arias L, Alonso S, Cardona P, et al. Can systems immunology lead tuberculosis eradication? Curr Opin Syst Biol. 2018;12: 53–60. doi:10.1016/j.coisb.2018.10.004

63. Kazmin D, Nakaya HI, Lee EK, Johnson MJ, Most R van der, Berg RA van den, et al. Systems analysis of protective immune responses to RTS,S malaria vaccination in humans. Proc Natl Acad Sci. 2017;114: 2425–2430. doi:10.1073/pnas.1621489114

64. Mikkaichi T, Yeaman MR, Hoffmann A, MRSA Systems Immunobiology Group. Identifying determinants of persistent MRSA bacteremia using mathematical modeling. PLoS Comput Biol. 2019;15: e1007087. doi:10.1371/journal.pcbi.1007087

65. Querec TD, Akondy RS, Lee EK, Cao W, Nakaya HI, Teuwen D, et al. Systems biology approach predicts immunogenicity of the yellow fever vaccine in humans. Nat Immunol. 2009;10: 116–125. doi:10.1038/ni.1688

66. Singhania A, Graham CM, Gabryšová L, Moreira-Teixeira L, Stavropoulos E, Pitt JM, et al. Transcriptional profiling unveils type I and II interferon networks in blood and tissues across diseases. Nat Commun. 2019;10: 2887. doi:10.1038/s41467-019-10601-6

67. Tran TM, Samal B, Kirkness E, Crompton PD. Systems immunology of human malaria. Trends Parasitol. 2012;28: 248–257. doi:10.1016/j.pt.2012.03.006

68. Vallejo AF, Read RC, Arevalo-Herrera M, Herrera S, Elliott T, Polak ME. Malaria systems immunology: Plasmodium vivax induces tolerance during primary infection through dysregulation of neutrophils and dendritic cells. J Infect. 2018;77: 440–447. doi:10.1016/j.jinf.2018.09.005

69. Patel AP, Tirosh I, Trombetta JJ, Shalek AK, Gillespie SM, Wakimoto H, et al. Single-cell RNA-seq highlights intratumoral heterogeneity in primary glioblastoma. Science. 2014;344: 1396–1401. doi:10.1126/science.1250220

70. Puram SV, Tirosh I, Parikh AS, Patel AP, Yizhak K, Gillespie S, et al. Single-Cell Transcriptomic Analysis of Primary and Metastatic Tumor Ecosystems in Head and Neck Cancer. Cell. 2017;171: 1611-1624.e24. doi:10.1016/j.cell.2017.10.044

71. Shalek AK, Satija R, Adiconis X, Gertner RS, Gaublomme JT, Raychowdhury R, et al. Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. Nature. 2013;498: 236–240. doi:10.1038/nature12172

72. Singer M, Wang C, Cong L, Marjanovic N, Kowalczyk M, Zhang H, et al. A Distinct Gene Module for Dysfunction Uncoupled from Activation in Tumor-Infiltrating T Cells. Cell. 2016;166: 1500-1511.e9. doi:10.1016/j.cell.2016.08.052

73. Tirosh I, Izar B, Prakadan SM, Wadsworth MH, Treacy D, Trombetta JJ, et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Science. 2016;352: 189–196. doi:10.1126/science.aad0501

74. Covacu R, Philip H, Jaronen M, Almeida J, Kenison JE, Darko S, et al. System-wide Analysis of the T Cell Response. Cell Rep. 2016;14: 2733–2744. doi:10.1016/j.celrep.2016.02.056

75. Davis MM. The αβ T Cell Repertoire Comes into Focus. Immunity. 2007;27: 179–180. doi:10.1016/j.immuni.2007.08.005

76. Grau GE, Piguet PF, Engers HD, Louis JA, Vassalli P, Lambert PH. L3T4+ T lymphocytes play a major role in the pathogenesis of murine cerebral malaria. J Immunol Baltim Md 1950. 1986;137: 2348–2354.

77. Sierro F, Grau GER. The Ins and Outs of Cerebral Malaria Pathogenesis: Immunopathology, Extracellular Vesicles, Immunometabolism, and Trained Immunity. Front Immunol. 2019;10. doi:10.3389/fimmu.2019.00830

78. Bagot S, Nogueira F, Collette A, do Rosario V, Lemonier F, Cazenave P-A, et al. Comparative Study of Brain CD8+ T Cells Induced by Sporozoites and Those Induced by Blood-Stage Plasmodium berghei ANKA Involved in the Development of Cerebral Malaria. Infect Immun. 2004;72: 2817–2826. doi:10.1128/IAI.72.5.2817-2826.2004

79. Belnoue E, Kayibanda M, Deschemin J-C, Viguier M, Mack M, Kuziel WA, et al. CCR5 deficiency decreases susceptibility to experimental cerebral malaria. Blood. 2003;101: 4253–4259. doi:10.1182/blood-2002-05-1493

80. Boubou MI, Collette A, Voegtlé D, Mazier D, Cazenave PA, Pied S. T cell response in malaria pathogenesis: selective increase in T cells carrying the TCR V(beta)8 during experimental cerebral malaria. Int Immunol. 1999;11: 1553–1562. doi:10.1093/intimm/11.9.1553

81. Pied S, Voegtle D, Marussig M, Rénia L, Miltgen F, Mazier D, et al. Evidence for superantigenic activity during murine malaria infection. Int Immunol. 1997;9: 17–25. doi:10.1093/intimm/9.1.17

82. Reina-San-Martin B, Cosson A, Minoprio P. Lymphocyte polyclonal activation: a pitfall for vaccine design against infectious agents. Parasitol Today Pers Ed. 2000;16: 62–67.

83. Pannetier C, Cochet M, Darche S, Casrouge A, Zoller M, Kourilsky P. The sizes of the CDR3 hypervariable regions of the murine T-cell receptor beta chains vary as a function of the recombined germ-line segments. Proc Natl Acad Sci U A. 1993;90: 4319–23.

84. Gorski J, Yassai M, Zhu X, Kissela B, Kissella B [corrected to Kissela B, Keever C, et al. Circulating T cell repertoire complexity in normal individuals and bone marrow recipients analyzed by CDR3 size spectratyping. Correlation with immune status. J Immunol Baltim Md 1950. 1994;152: 5109–5119.

85. Fozza C, Barraqueddu F, Corda G, Contini S, Virdis P, Dore F, et al. Study of the T-cell receptor repertoire by CDR3 spectratyping. J Immunol Methods. 2017;440: 1–11. doi:10.1016/j.jim.2016.11.001

86. Collette A, Cazenave P-A, Pied S, Six A. New methods and software tools for high throughput CDR3 spectratyping. Application to T lymphocyte repertoire modifications during experimental malaria. J Immunol Methods. 2003;278: 105–116. doi:10.1016/s0022-1759(03)00225-4

87. Collette A, Bagot S, Ferrandiz ME, Cazenave P-A, Six A, Pied S. A profound alteration of blood TCRB repertoire allows prediction of cerebral malaria. J Immunol Baltim Md 1950. 2004;173: 4568–4575. doi:10.4049/jimmunol.173.7.4568

88. Pham HP, Derian N, Chaara W, Bellier B, Klatzmann D, Six A. A novel strategy for molecular signature discovery based on independent component analysis. Int J Data Min Bioinform. 2014;9: 277–304.

89. Mariotti-Ferrandiz E, Pham H-P, Dulauroy S, Gorgette O, Klatzmann D, Cazenave P-A, et al. A TCRβ Repertoire Signature Can Predict Experimental Cerebral Malaria. Carvalho LJ de M, editor. PLOS ONE. 2016;11: e0147871. doi:10.1371/journal.pone.0147871

90. Petrovic Berglund J, Petrovc Berglund J, Mariotti-Ferrandiz E, Rosmaraki E, Hall H, Cazenave P-A, et al. TCR repertoire dynamics in the pancreatic lymph nodes of non-obese diabetic (NOD) mice at the time of disease initiation. Mol Immunol. 2008;45: 3059–3064. doi:10.1016/j.molimm.2008.03.009

91. Itoh M, Takahashi T, Sakaguchi N, Kuniyasu Y, Shimizu J, Otsuka F, et al. Thymus and autoimmunity: production of CD25+CD4+ naturally anergic and suppressive T cells as a key function of the thymus in maintaining immunologic self-tolerance. J Immunol Baltim Md 1950. 1999;162: 5317–5326.

92. Nishimura E, Sakihama T, Setoguchi R, Tanaka K, Sakaguchi S. Induction of antigen-specific immunologic tolerance by in vivo and in vitro antigen-specific expansion of naturally arising Foxp3+CD25+CD4+ regulatory T cells. Int Immunol. 2004;16: 1189–1201. doi:10.1093/intimm/dxh122

93. Sakaguchi S, Sakaguchi N, Asano M, Itoh M, Toda M. Immunologic self-tolerance maintained by activated T cells expressing IL-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. J Immunol. 1995;155: 1151–1164.

94. Shevach EM. Biological Functions of Regulatory T Cells. Advances in Immunology. Elsevier; 2011. pp. 137–176. Available: http://linkinghub.elsevier.com/retrieve/pii/B9780123878274000048

95. Cebula A, Seweryn M, Rempala GA, Pabla SS, McIndoe RA, Denning TL, et al. Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota. Nature. 2013;497: 258–262. doi:10.1038/nature12079

96. Russler-Germain EV, Rengarajan S, Hsieh C-S. Antigen-specific regulatory T-cell responses to intestinal microbiota. Mucosal Immunol. 2017;10: 1375–1386. doi:10.1038/mi.2017.65

97. Brunkow ME, Jeffery EW, Hjerrild KA, Paeper B, Clark LB, Yasayko S-A, et al. Disruption of a new forkhead/winged-helix protein, scurfin, results in the fatal lymphoproliferative disorder of the scurfy mouse. Nat Genet. 2001;27: 68–73.

98. Fontenot JD, Gavin MA, Rudensky AY. Foxp3 programs the development and function of CD4+CD25+ regulatory T cells. Nat Immunol. 2003;4: 330–336. doi:10.1038/ni904

99. Hori S, Sakaguchi S. Foxp3: a critical regulator of the development and function of regulatory T cells. Microbes Infect. 2004;6: 745–751. doi:10.1016/j.micinf.2004.02.020

100. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003;299: 1057–61.

101. Ramsdell F, Ziegler SF. FOXP3 and scurfy: how it all began. Nat Rev Immunol. 2014;14: 343–349. doi:10.1038/nri3650

102. Hikichi T, Kohda T, Wakayama S, Ishino F, Wakayama T. Nuclear Transfer Alters the DNA Methylation Status of Specific Genes in Fertilized and Parthenogenetically Activated Mouse Embryonic Stem Cells. Stem Cells. 2008;26: 783–788. doi:10.1634/stemcells.2007-0907

103. Kendal AR, Chen Y, Regateiro FS, Ma J, Adams E, Cobbold SP, et al. Sustained suppression by Foxp3+ regulatory T cells is vital for infectious transplantation tolerance. J Exp Med. 2011;208: 2043–2053. doi:10.1084/jem.20110767

104. Komatsu N, Mariotti-Ferrandiz ME, Wang Y, Malissen B, Waldmann H, Hori S. Heterogeneity of natural Foxp3+ T cells: a committed regulatory T-cell lineage and an uncommitted minor population retaining plasticity. Proc Natl Acad Sci U S A. 2009;106: 1903–1908. doi:10.1073/pnas.0811556106

105. Mombaerts P, Clarke AR, Rudnicki MA, Iacomini J, Itohara S, Lafaille JJ, et al. Mutations in T-cell antigen receptor genes alpha and beta block thymocyte development at different stages. Nature. 1992;360: 225–231. doi:10.1038/360225a0

106. Bettini ML, Bettini M, Vignali DAA. T-cell receptor retrogenic mice: a rapid, flexible alternative to T-cell receptor transgenic mice. Immunology. 2012;136: 265–272. doi:10.1111/j.1365-2567.2012.03574.x

107. Bettini ML, Bettini M, Nakayama M, Guy CS, Vignali DAA. Generation of T cell receptor-retrogenic mice: improved retroviral-mediated stem cell gene transfer. Nat Protoc. 2013;8: 1837–1840. doi:10.1038/nprot.2013.111

108. Bautista JL, Lio C-WJ, Lathrop SK, Forbush K, Liang Y, Luo J, et al. Intraclonal competition limits the fate determination of regulatory T cells in the thymus. Nat Immunol. 2009;10: 610–617. doi:10.1038/ni.1739

109. Hsieh CS, Lee HM, Lio CW. Selection of regulatory T cells in the thymus. Nat Rev Immunol. 2012;12: 157–67. doi:nri3155 [pii] 10.1038/nri3155

110. Lio C-WJ, Hsieh C-S. A Two-Step Process for Thymic Regulatory T Cell Development. Immunity. 2008;28: 100–111. doi:10.1016/j.immuni.2007.11.021

111. Pacholczyk R, Kern J. The T-cell receptor repertoire of regulatory T cells. Immunology. 2008;125: 450–8. doi:IMM2992 [pii] 10.1111/j.1365-2567.2008.02992.x

112. Pacholczyk R, Ignatowicz H, Kraj P, Ignatowicz L. Origin and T Cell Receptor Diversity of Foxp3+CD4+CD25+ T Cells. Immunity. 2006;25: 249–259. doi:10.1016/j.immuni.2006.05.016

113. Hsieh C-S, Liang Y, Tyznik AJ, Self SG, Liggitt D, Rudensky AY. Recognition of the peripheral self by naturally arising CD25+ CD4+ T cell receptors. Immunity. 2004;21: 267–277. doi:10.1016/j.immuni.2004.07.009

114. Wong J, Mathis D, Benoist C. TCR-based lineage tracing: no evidence for conversion of conventional into regulatory T cells in response to a natural self-antigen in pancreatic islets. J Exp Med. 2007;204: 2039–2045. doi:10.1084/jem.20070822

115. Arstila TP. A Direct Estimate of the Human T Cell Receptor Diversity. Science. 1999;286: 958–961. doi:10.1126/science.286.5441.958

116. Casrouge A, Beaudoing E, Dalle S, Pannetier C, Kanellopoulos J, Kourilsky P. Size Estimate of the TCR Repertoire of Naive Mouse Splenocytes. J Immunol. 2000;164: 5782–5787. doi:10.4049/jimmunol.164.11.5782

117. Fazilleau N, Bachelez H, Gougeon M-L, Viguier M. Cutting Edge: Size and Diversity of CD4+CD25high Foxp3+ Regulatory T Cell Repertoire in Humans: Evidence for Similarities and Partial Overlapping with CD4+CD25− T Cells. J Immunol. 2007;179: 3412–3416. doi:10.4049/jimmunol.179.6.3412

118. Hsieh C-S, Zheng Y, Liang Y, Fontenot JD, Rudensky AY. An intersection between the self-reactive regulatory and nonregulatory T cell receptor repertoires. Nat Immunol. 2006;7: 401–410. doi:10.1038/ni1318

119. Sepúlveda N, Boucontet L, Pereira P, Carneiro J. Stochastic Modeling of T cell receptor gene rearrangement. J Theor Biol. 2005;234: 153–165. doi:10.1016/j.jtbi.2004.11.018

120. Sant'Angelo DB, Lucas B, Waterbury PG, Cohen B, Brabb T, Goverman J, et al. A Molecular Map of T Cell Development. Immunity. 1998;9: 179–186. doi:10.1016/S1074-7613(00)80600-7

121. Plessy C, Mariotti-Ferrandiz E, Manabe R-I, Hori S. clonotypeR - high throughput analysis of T cell antigen receptor sequences. Biorxiv. 2015; doi:http://dx.doi.org/10.1101/028696

122. Sethi DK, Schubert DA, Anders A-K, Heroux A, Bonsor DA, Thomas CP, et al. A highly tilted binding mode by a self-reactive T cell receptor results in altered engagement of peptide and MHC. J Exp Med. 2011;208: 91–102. doi:10.1084/jem.20100725

123. Burchill MA, Yang J, Vang KB, Farrar MA. Interleukin-2 receptor signaling in regulatory T cell development and homeostasis. Immunol Lett. 2007;114: 1–8. doi:10.1016/j.imlet.2007.08.005

124. Mariotti-Ferrandiz E, Plessy C, Wang G, Iida R, Kanagawa O, Wakayama T, et al. High-throughput sequencing provides quantitative evidence for spatio-temporal shaping of Treg TCR repertoires. Prep.

125. Weinstein JA, Jiang N, White RA, Fisher DS, Quake SR. High-throughput sequencing of the zebrafish antibody repertoire. Science. 2009;324: 807–10. doi:324/5928/807 [pii] 10.1126/science.1170020

126. Benichou J, Ben-Hamo R, Louzoun Y, Efroni S. Rep-Seq: uncovering the immunological repertoire through next-generation sequencing: Rep-Seq: NGS for the immunological repertoire. Immunology. 2012;135: 183–191. doi:10.1111/j.1365-2567.2011.03527.x

127. Breden F, Luning Prak ET, Peters B, Rubelt F, Schramm CA, Busse CE, et al. Reproducibility and Reuse of Adaptive Immune Receptor Repertoire Data. Front Immunol. 2017;8: 1418. doi:10.3389/fimmu.2017.01418

128. Rubelt F, Busse CE, Bukhari SAC, Bürckert J-P, Mariotti-Ferrandiz E, Cowell LG, et al. Adaptive Immune Receptor Repertoire Community recommendations for sharing immune-repertoire sequencing data. In: Nature Immunology [Internet]. 16 Nov 2017 [cited 17 Nov 2017]. doi:10.1038/ni.3873

129. Six A, Mariotti-Ferrandiz ME, Chaara W, Magadan S, Pham H-P, Lefranc M-P, et al. The Past, Present, and Future of Immune Repertoire Biology – The Rise of Next-Generation Repertoire Analysis. Front Immunol. 2013;4. doi:10.3389/fimmu.2013.00413

130. Bergot A-S, Chaara W, Ruggiero E, Mariotti-Ferrandiz E, Dulauroy S, Schmidt M, et al. TCR sequences and tissue distribution discriminate the subsets of naïve and activated/memory Treg cells in mice. Eur J Immunol. 2015; n/a-n/a. doi:10.1002/eji.201445269

131. Chaara W. Caractérisation de la diversité du répertoire TCR par modélisation de données de séquençage haut-débit [Internet]. 2016. Available: http://www.theses.fr/2016PA066410/document

132. Chaara W, Gonzalez-Tort A, Florez L, Klatzmann D, Mariotti-Ferrandiz, Encarnita, Six, Adrien. RepSeq data representativeness and robustness assessment by Shannon entropy. Front Immunol. 2018;9. doi:10.3389/fimmu.2018.01038

133. Maceiras AR, Almeida SCP, Mariotti-Ferrandiz E, Chaara W, Jebbawi F, Six A, et al. T follicular helper and T follicular regulatory cells have different TCR specificity. Nat Commun. 2017;8: 15067. doi:10.1038/ncomms15067

134. Ritvo P-G, Saadawi A, Barennes P, Quiniou V, Chaara W, Soufi KE, et al. High-resolution repertoire analysis reveals a major bystander activation of Tfh and Tfr cells. Proc Natl Acad Sci. 2018;115: 9604–9609. doi:10.1073/pnas.1808594115

135. Greiff V, Bhat P, Cook SC, Menzel U, Kang W, Reddy ST. A bioinformatic framework for immune repertoire diversity profiling enables detection of immunological status. Genome Med. 2015;7. doi:10.1186/s13073-015-0169-8

136. Madi A, Shifrut E, Reich-Zeliger S, Gal H, Best K, Ndifon W, et al. T-cell receptor repertoires share a restricted set of public and abundant CDR3 sequences that are associated with self-related immunity. Genome Res. 2014;24: 1603–1612. doi:10.1101/gr.170753.113

137. Miho E, Yermanos A, Weber CR, Berger CT, Reddy ST, Greiff V. Computational Strategies for Dissecting the High-Dimensional Complexity of Adaptive Immune Repertoires. Front Immunol. 2018;9. doi:10.3389/fimmu.2018.00224

138. Ben-Hamo R, Efroni S. The whole-organism heavy chain B cell repertoire from Zebrafish self-organizes into distinct network features. BMC Syst Biol. 2011;5: 27.

139. Dupic T, Marcou Q, Walczak AM, Mora T. Genesis of the αβ T-cell receptor. PLOS Comput Biol. 2019;15: e1006874. doi:10.1371/journal.pcbi.1006874

140. Mora T, Walczak AM, Bialek W, Callan CG. Maximum entropy models for antibody diversity. Proc Natl Acad Sci U S A. 2010;107: 5405–5410. doi:10.1073/pnas.1001705107

141. Murugan A, Mora T, Walczak AM, Callan CG. Statistical inference of the generation probability of T-cell receptors from sequence repertoires. Proc Natl Acad Sci. 2012;109: 16161–16166.

142. Corrie BD, Marthandan N, Zimonja B, Jaglale J, Zhou Y, Barr E, et al. iReceptor: A platform for querying and analyzing antibody/B-cell and T-cell receptor repertoire data across federated repositories. Immunol Rev. 2018;284: 24–41. doi:10.1111/imr.12666

143. Renyi A. On measures of information and entropy. Proc 4th Berkeley Symp Math Stat Probab. 1961; 547–561.

144. Abbas AK, Benoist C, Bluestone JA, Campbell DJ, Ghosh S, Hori S, et al. Regulatory T cells: recommendations to simplify the nomenclature. Nat Immunol. 2013;14: 307–308. doi:10.1038/ni.2554

145. Chen T, Darrasse-Jeze G, Bergot A-S, Courau T, Churlaud G, Valdivia K, et al. Self-Specific Memory Regulatory T Cells Protect Embryos at Implantation in Mice. J Immunol. 2013;191: 2273–2281. doi:10.4049/jimmunol.1202413

146. Darrasse-Jèze G, Bergot A-S, Durgeau A, Billiard F, Salomon BL, Cohen JL, et al. Tumor emergence is sensed by self-specific CD44hi memory Tregs that create a dominant tolerogenic environment for tumors in mice. J Clin Invest. 2009; doi:10.1172/JCI36628

147. Fisson S, Darrasse-Jeze G, Litvinova E, Septier F, Klatzmann D, Liblau R, et al. Continuous activation of autoreactive CD4+ CD25+ regulatory T cells in the steady state. J Exp Med. 2003;198: 737–46. doi:10.1084/jem.20030686

148. Föhse L, Suffner J, Suhre K, Wahl B, Lindner C, Lee C-W, et al. High TCR diversity ensures optimal function and homeostasis of Foxp3+ regulatory T cells. Eur J Immunol. 2011;41: 3101–3113. doi:10.1002/eji.201141986

149. Lathrop SK, Bloom SM, Rao SM, Nutsch K, Lio C-W, Santacruz N, et al. Peripheral education of the immune system by colonic commensal microbiota. Nature. 2011;478: 250–254. doi:10.1038/nature10434

150. Quiniou V, Barennes P, Pham H-P, Shugay M, Martina F, Mhanna V, et al. Human thymocytes selection favors T cell receptors for pathogens recognition by mimicry. Prep.

151. Klatzmann D, Abbas AK. The promise of low-dose interleukin-2 therapy for autoimmune and inflammatory diseases. Nat Rev Immunol. 2015;15: 283–294. doi:10.1038/nri3823

152. Long SA, Cerosaletti K, Bollyky PL, Tatum M, Shilling H, Zhang S, et al. Defects in IL-2R signaling contribute to diminished maintenance of FOXP3 expression in CD4(+)CD25(+) regulatory T-cells of type 1 diabetic subjects. Diabetes. 2010;59: 407–15. doi:db09-0694 [pii] 10.2337/db09-0694

153. Tang Q, Adams JY, Penaranda C, Melli K, Piaggio E, Sgouroudis E, et al. Central Role of Defective Interleukin-2 Production in the Triggering of Islet Autoimmune Destruction. Immunity. 2008;28: 687–697. doi:10.1016/j.immuni.2008.03.016

154. Verdaguer J, Schmidt D, Amrani A, Anderson B, Averill N, Santamaria P. Spontaneous autoimmune diabetes in monoclonal T cell nonobese diabetic mice. J Exp Med. 1997;186: 1663–1676. doi:10.1084/jem.186.10.1663

155. Mhanna V, Fourcade G, Pham H-P, Barennes P, Brimaud F, Churlaud G, et al. Regulatory T Cells Have an Exhausted TR Repertoire In NOD Mice That Is Restored After IL2 Administration. Prep.

156. Churlaud G, Jimenez V, Ruberte J, Amadoudji Zin M, Fourcade G, Gottrand G, et al. Sustained stimulation and expansion of Tregs by IL2 control autoimmunity without impairing immune responses to infection, vaccination and cancer. Clin Immunol. 2014;151: 114–126. doi:10.1016/j.clim.2014.02.003

157. Ritvo P-GG, Churlaud G, Quiniou V, Florez L, Brimaud F, Fourcade G, et al. Tfr cells lack IL-2Rα but express decoy IL-1R2 and IL-1Ra and suppress the IL-1–dependent activation of Tfh cells. Sci Immunol. 2017;2: eaan0368. doi:10.1126/sciimmunol.aan0368

158. McGonagle D, McDermott MF. A Proposed Classification of the Immunological Diseases. PLoS Med. 2006;3. doi:10.1371/journal.pmed.0030297

159. Lorenzon R, Mariotti-Ferrandiz E, Aheng C, Ribet C, Toumi F, Pitoiset F, et al. Clinical and multi-omics cross-phenotyping of patients with autoimmune and autoinflammatory diseases: the observational TRANSIMMUNOM protocol. BMJ Open. 2018;8: e021037. doi:10.1136/bmjopen-2017-021037

160. Lorenzon R, Drakos I, Ribet C, Harris S, Cordoba M, Tran O, et al. Clinical data specification and coding for cross-analyses with omics data in autoimmune disease trials. bioRxiv. 2018; doi:10.1101/360719

161. Barennes P, Quiniou V, Shugay M, Egorov ES, Davydov AN, Chudakov DM, et al. Systematic study of T-cell receptor repertoire profiling reveals large methodological biases: lessons from a multi-center study. Revis.

162. Attaf M, Huseby E, Sewell AK. αβ T cell receptors as predictors of health and disease. Cell Mol Immunol. 2015; doi:10.1038/cmi.2014.134

163. Boudinot P, Mariotti-Ferrandiz ME, Pasquier LD, Benmansour A, Cazenave P-A, Six A. New perspectives for large-scale repertoire analysis of immune receptors. Mol Immunol. 2008;45: 2437–2445. doi:10.1016/j.molimm.2007.12.018

164. Heather JM, Ismail M, Oakes T, Chain B. High-throughput sequencing of the T-cell receptor repertoire: pitfalls and opportunities. Brief Bioinform. 2017; doi:10.1093/bib/bbw138

165. Lindau P, Robins HS. Advances and applications of immune receptor sequencing in systems immunology. Curr Opin Syst Biol. 2017;1: 62–68. doi:10.1016/j.coisb.2016.12.009

166. Miles JJ, Douek DC, Price DA. Bias in the [alpha][beta] T-cell repertoire: implications for disease pathogenesis and vaccination. Immunol Cell Biol. 2011;89: 375.

167. Schrama D, Ritter C, Becker JC. T cell receptor repertoire usage in cancer as a surrogate marker for immune responses. Semin Immunopathol. 2017;39: 255–268. doi:10.1007/s00281-016-0614-9

168. Cui J-H, Jin Y, Lin K-R, Xiao P, Chen X, Pan Y, et al. Characterization of peripheral blood TCR repertoire in patients with ankylosing spondylitis by high-throughput sequencing. Hum Immunol. 2018;79: 485–490. doi:10.1016/j.humimm.2018.03.007

169. Cui J-H, Lin K-R, Yuan S-H, Jin Y-B, Chen X-P, Su X-K, et al. TCR Repertoire as a Novel Indicator for Immune Monitoring and Prognosis Assessment of Patients With Cervical Cancer. Front Immunol. 2018;9. doi:10.3389/fimmu.2018.02729

170. Dash P, Fiore-Gartland AJ, Hertz T, Wang GC, Sharma S, Souquette A, et al. Quantifiable predictive features define epitope-specific T cell receptor repertoires. Nature. 2017;547: 89–93. doi:10.1038/nature22383

171. Emerson RO, DeWitt WS, Vignali M, Gravley J, Hu JK, Osborne EJ, et al. Immunosequencing identifies signatures of cytomegalovirus exposure history and HLA-mediated effects on the T cell repertoire. Nat Genet. 2017;49: 659–665. doi:10.1038/ng.3822

172. Heather JM, Best K, Oakes T, Gray ER, Roe JK, Thomas N, et al. Dynamic Perturbations of the T-Cell Receptor Repertoire in Chronic HIV Infection and following Antiretroviral Therapy. Front Immunol. 2016;6. doi:10.3389/fimmu.2015.00644

173. Schober K, Buchholz VR, Busch DH. TCR repertoire evolution during maintenance of CMV-specific T-cell populations. Immunol Rev. 2018;283: 113–128. doi:10.1111/imr.12654

174. Ye B, Smerin D, Gao Q, Kang C, Xiong X. High-throughput sequencing of the immune repertoire in oncology: Applications for clinical diagnosis, monitoring, and immunotherapies. Cancer Lett. 2018;416: 42–56. doi:10.1016/j.canlet.2017.12.017

175. Brüggemann M, Kotrová M, Knecht H, Bartram J, Boudjogrha M, Bystry V, et al. Standardized next-generation sequencing of immunoglobulin and T-cell receptor gene recombinations for MRD marker identification in acute lymphoblastic leukaemia; a EuroClonality-NGS validation study. Leukemia. 2019; doi:10.1038/s41375-019-0496-7

176. Knecht H, Reigl T, Kotrová M, Appelt F, Stewart P, Bystry V, et al. Quality control and quantification in IG/TR next-generation sequencing marker identification: protocols and bioinformatic functionalities by EuroClonality-NGS. Leukemia. 2019; doi:10.1038/s41375-019-0499-4

177. Matos TR, de Rie MA, Teunissen MBM. Research Techniques Made Simple: High-Throughput Sequencing of the T-Cell Receptor. J Invest Dermatol. 2017;137: e131–e138. doi:10.1016/j.jid.2017.04.001

178. Greiff V, Miho E, Menzel U, Reddy ST. Bioinformatic and Statistical Analysis of Adaptive Immune Repertoires. Trends Immunol. 2015;36: 738–749. doi:10.1016/j.it.2015.09.006

179. Rosenzwajg M, Churlaud G, Hartemann A, Klatzmann D. Interleukin 2 in the pathogenesis and therapy of type 1 diabetes. Curr Diab Rep. 2014;14: 553. doi:10.1007/s11892-014-0553-6

180. Meng X, Yang J, Dong M, Zhang K, Tu E, Gao Q, et al. Regulatory T cells in cardiovascular diseases. Nat Rev Cardiol. 2016;13: 167–179. doi:10.1038/nrcardio.2015.169

181. Zacchigna S, Martinelli V, Moimas S, Colliva A, Anzini M, Nordio A, et al. Paracrine effect of regulatory T cells promotes cardiomyocyte proliferation during pregnancy and after myocardial infarction. Nat Commun. 2018;9. doi:10.1038/s41467-018-04908-z

182. Germain RN, Meier-Schellersheim M, Nita-Lazar A, Fraser IDC. Systems Biology in Immunology: A Computational Modeling Perspective. Annu Rev Immunol. 2011;29: 527–585. doi:10.1146/annurev-immunol-030409-101317

183. Allenbach Y, Chaara W, Rosenzwajg M, Six A, Prevel N, Mingozzi F, et al. Th1 response and systemic treg deficiency in inclusion body myositis. PLoS One. 2014;9: e88788. doi:10.1371/journal.pone.0088788

184. Pitoiset F, Cassard L, El Soufi K, Boselli L, Grivel J, Roux A, et al. Deep phenotyping of immune cell populations by optimized and standardized flow cytometry analyses: Deep Immunophenotyping for Clinical Studies. Cytometry A. 2018;93: 793–802. doi:10.1002/cyto.a.23570

185. Christley S, Scarborough W, Salinas E, Rounds WH, Toby IT, Fonner JM, et al. VDJServer: A Cloud-Based Analysis Portal and Data Commons for Immune Repertoire Sequences and Rearrangements. Front Immunol. 2018;9: 976. doi:10.3389/fimmu.2018.00976

186. Shugay M, Bagaev DV, Zvyagin IV, Vroomans RM, Crawford JC, Dolton G, et al. VDJdb: a curated database of T-cell receptor sequences with known antigen specificity. Nucleic Acids Res. 2018;46: D419–D427. doi:10.1093/nar/gkx760

187. Tickotsky N, Sagiv T, Prilusky J, Shifrut E, Friedman N. McPAS-TCR: a manually curated catalogue of pathology-associated T cell receptor sequences. Bioinforma Oxf Engl. 2017;33: 2924–2929. doi:10.1093/bioinformatics/btx286

188. Scheufele E, Aronzon D, Coopersmith R, McDuffie MT, Kapoor M, Uhrich CA, et al. tranSMART: An Open Source Knowledge Management and High Content Data Analytics Platform. AMIA Jt Summits Transl Sci Proc AMIA Summit Transl Sci. 2014;2014: 96–101.

189. Christoph J, Knell C, Bosserhoff A, Naschberger E, Stürzl M, Rübner M, et al. Usability and Suitability of the Omics-Integrating Analysis Platform tranSMART for Translational Research and Education. Appl Clin Inform. 2017;8: 1173–1183. doi:10.4338/ACI-2017-05-RA-0085

190. Christoph J, Knell C, Naschberger E, Stürzl M, Maier C, Prokosch H-U, et al. Two Years of tranSMART in a University Hospital for Translational Research and Education. Stud Health Technol Inform. 2017;236: 70–79.

Selected publications

Pierre Barennes, Valentin Quiniou, Mikhail Shugay, Evgeniy S. Egorov, Alexey N. Davydov, Dmitriy M. Chudakov, Imran Uddin, Mazlina Ismail, Theres Oakes, Benny Chain , Anne Eugster, Darko Sam, Amy Ransier, Daniel C. Douek, David Klatzmann, **Encarnita Mariotti-Ferrandiz.** *Systematic study of T-cell receptor repertoire profiling reveals large methodological biases: lessons from a multi-center study. In revision*

Lorenzon R, Drakos I, Ribet C, Harris S, Cordoba M, Tran O, Dasque E, Cacoub P, Hartemann A, Bodaghi B, Saadoun D, Berenbaum F, Grateau G, Ronco P, Benveniste O, Mariampillai K, Sellam J, Seksik P, Rosenzwajg M, Six A, Bernard C, Aheng C, Vicaut E, Klatzmann D, **Mariotti-Ferrandiz E**. Clinical data specification and coding for cross-analyses with omics data in autoimmune disease trials. bioRxiv 360719; doi: https://doi.org/10.1101/360719

Chaara W, Gonzalez-Tort A, Florez L, Klatzmann D, **Mariotti-Ferrandiz E*** and Six A* RepSeq data representativeness and robustness assessment by Shannon entropy, **Frontiers in Immunology** (*co-last authors) PMID: 29868003

Mariotti-Ferrandiz E, Pham HP, Gorgette O, Dulauroy S, Klatzmann D, Cazenave P-A, Pied S et Six A. A TCRb repertoire signature can predict experimental cerebral malaria. **PlosOne.** PMID: 26844551

Résumé substantiel (French)

Depuis le début de ma carrière dans la recherche académique, mes travaux ont porté sur l'une des caractéristiques fondamentales du système immunitaire adaptatif, à savoir **l'extraordinaire diversité des récepteurs de l'antigène, en particulier des lymphocytes T, aussi appelé TCR** (pour *T-cell Receptor*). En effet, cette diversité confère au système immunitaire **le potentiel de reconnaître l'univers antigénique du «non-soi»**, y compris les agents pathogènes et les tumeurs autologues, alors même que ces antigènes ne sont a priori pas présents dans l'organisme. Les lymphocytes T se différencient dans le thymus, où ils acquièrent l'expression en surface du TCR, hétérodimère constitué de deux chaines, au travers de mécanismes de réarrangements somatiques aléatoires entre plusieurs dizaines de gènes présents au niveau génomique sous forme germinale. Des étapes de sélection viennent façonner la diversité de cellules différenciées, évitant notamment l'export dans les sites périphériques de cellules inefficaces pour reconnaitre un antigène mais aussi et surtout éliminant les cellules, qui au hasard des réarrangements auraient pu acquérir un TCR capable de reconnaitre avec une très forte affinité des antigènes du soi, et donc contribuer à la mise en place d'une autoimmunité pathologique.

La diversité potentielle du répertoire a été à 10^{19} TCR différents (modélisée pour atteindre jusqu'à 10^{61} TCR différents très récemment !). De toute évidence, seule une petite fraction du répertoire potentiel peut réellement être utilisée, en raison du nombre limité de cellules qu'un individu peuvent contenir dans le corps (chez l'humain ~ 10^{12} et chez la souris ~ 10^{8} cellules T αβ). Une fois en périphérie, **l'ensemble des lymphocytes T sélectionnés dans le thymus va constituer le répertoire disponible pour le maintien de l'homéostasie de l'organisme**, impliquant à la fois des cellules capables de répondre à des infections par des agents pathogènes, à l'intrusion d'allergènes, à des transformations tumorales mais aussi à des cellules capables d'éviter des manifestations autoimmunes pathologiques en maintenant une tolérance pour le soi en périphérie. Lors de ces réponses immunitaires, le répertoire lymphocytaire T disponible va être engagé dans le contrôle de l'agent à circonscrire, conduisant potentiellement à une modulation,

plus ou moins marquée de la diversité du répertoire TCR. Caractériser ces modulations pourrait permettre de **(i) mieux comprendre les états pathologiques** (par exemple pour le développement de vaccins anti-infectieux ou de ciblage de cellules tumorales) et **(ii) d'identifier des marqueurs biologiques de l'état physiopathologique d'un individu** (par exemple pour une meilleure prise en charge thérapeutique).

Cette diversité de TCR ne peut être dissociée de la diversité des populations cellulaires T désormais identifiées sur la base de leurs fonctions respectives, inflammatoires vs.régulatrices, anti-pathogènes intracellulaires vs. anti-pathogènes extracellulaires, impliquées dans la régulation de la production d'anticorps…. **Aussi, l'étude du répertoire TCR n'a de sens que si l'on prend en compte cette dimension supplémentaire, et considérer le système immunitaire comme un tout et pas simplement la somme de ses sous-parties**. Cette approche, désormais appelée immunologie des systèmes ou immunologie intégrative, a constitué les fondations de l'ensemble des travaux de recherche que j'ai mené jusqu'à présent.

Ainsi, depuis mon doctorat en science, mes travaux portent sur **(i) la caractérisation des modulations du répertoire TCR en conditions pathologiques** (infection ou pathologie autoimmunes), **(ii) l'adaptation et l'amélioration des méthodes d'investigations de la diversité du répertoire TCR** par des outils de biologie moléculaire, **(iii) le développement de stratégies de modélisation statistique de cette diversité**. Ces travaux ont pris appuis sur une approche d'immunologie des systèmes, étayée au fil du temps et des développements technologiques. Mes perspectives pour les prochaines années visent à extraire l'information pertinente du répertoire TCR dans le contexte des maladies autoimmunes en particulier, en ciblant des sous-populations fonctionnellement caractérisées, afin d'en décrire la diversité mais aussi identifier des signatures de TCR associées aux situations pathologiques (en réponse ou non à un traitement). Mon intérêt se porte majoritairement sur l'équilibre entre les lymphocytes T régulateurs et les lymphocytes T effecteurs, critique pour le maintien de l'homéostasie du l'organisme.