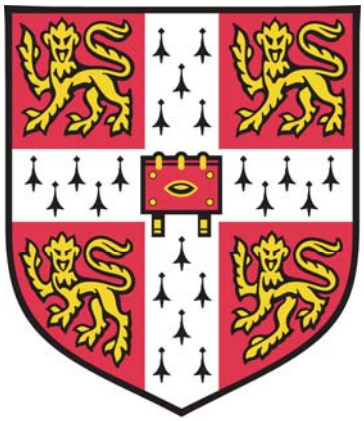
Characterisation of the transcriptional response to cytokine induced polarisation in human $CD4^+$ T cells and monocyte derived macrophages



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This dissertation is submitted for the degree of Master in Philosophy in Biological Science

August, 2017

Abstract

The immune system is composed of plastic cell populations which are capable of acquiring specialized functions upon cytokine stimulation. This phenomenon, known as cytokine induced cell polarisation, generates different effector states within cell types, and has been associated to autoimmune inflammation. In this study, we characterised the transcriptional response of different cell types to polarisation with cytokines linked to autoimmunity.

First, we isolated human naive $CD4^+$ T cells and monocytes from peripheral blood of two healthy individuals. Next, we polarised the cells with different cytokine combinations linked to autoimmunity and performed low coverage RNA-seq of 26 different conditions: ten $CD4^+$ T cell samples activated with anti CD3/CD28 antibodies and polarised with cytokines (Th0, Th1, Th2, Th17, iTreg, IL-10, IL-21, IL-27, IFN-β, and TNF-α) at two different time points, and six monocyte polarisations (M0, M1, M2, IL-23, IL-26, and TNF-α). In order to study differences between stimulation in the presence or absence of cytokines, we performed differential gene expression analysis. Furthermore, for selected conditions we also characterised the whole proteome. Together, these data were used to identify genes and pathways involved in cytokine induced cell polarisation.

This study generated a valuable resource for future investigation of gene expression in immune cells. Furthermore, our results confirmed that the response to type I interferons is reduced upon Th2 cell differentiation, and revealed that downregulation of genes in the phosphatidylinositol-3-kinase (PI3K) pathway may be involved in the differentiation of Th17 and iTreg cells. Finally, using a gene co-expression network analysis, we also identified transcription factors which coordinate the response to cytokine induced polarisation in $CD4^+$ T cells and macrophages such as IRF8, IRF9, and Sox4.

Declaration of originality

I hereby declare that this dissertation is the result of my own work and includes nothing which is the outcome of work done in collaboration except where specifically indicated in the text and in the initial collaboration note. It is not substantially the same as any that I have submitted for, or is being concurrently submitted for a degree or other qualification at the University of Cambridge or any other University or similar institution. I further state that no part of my dissertation has already been or is being concurrently submitted for any such degree, diploma or other qualification. Finally, I hereby declare that my dissertation does not exceed the word limit prescribed in the Special Regulations of the MPhil examination for which I am a candidate (This thesis consists of 19,393 words, exclusive of abstract, tables, references and appendix).

Date: _____

Signature: _____

Collaboration note

The work described in this dissertation was carried out by myself and members of the Immune Genomics Group at The Wellcome Trust Sanger Institute, Cambridge, and is part of a study designed by Gosia Trynka and Blagoje Soskic. I performed the cell isolation, and stimulation experiments in collaboration with Blagoje Soskic and Marta Baldrighi. These experiments included cell culture, flow cytometry, RNA isolation and quantification. Blagoje Soskic performed the Immunophenotyping of monocyte derived macrophages. In addition, library preparation and RNA-sequencing were carried out by the Sequencing facility at The Sanger Institute, while protein isolation, mass spectrometry, and peptide quantification analysis were performed by Theodoros Romeliotis and the Proteomics facility at The Sanger Institute, in collaboration with Marta Baldrighi. Fluorescence activated cell sorting was carried out by the cytometry facility at The Sanger Institute. I performed all the RNA-sequencing data analysis, including read mapping, differential gene expression, functional annotation, and gene co-expression network analysis. This project is done in collaboration with the public-private initiative for drug discovery Open Targets, and consequently the pharmaceutical firms GSK and Biogen were also involved in the study design.

Acknowledgments

“I am grateful to intelligent people. That doesn’t mean educated. That doesn’t mean intellectual. I mean really intelligent”, said American poet Maya Angelou. I am grateful to all the intelligent people I found myself surrounded with during this year. Firstly, I want to thank my supervisor Dr Gosia Trynka for accepting in her lab a student with virtually no experience in the field and who travelled 8600 kilometres with a suitcase full of dreams. Thank you for your incisive feedback and for providing an environment where I can wake up every day to do world leading science. Furthermore, I want to thank people at The Sanger Institute for introducing me to completely new ways of approaching biology and tackling issues which were unsolvable only a few years ago. I am particularly grateful to Blagoje Soskic, who has been my immunology mentor and who introduced me to the red queen paradox, the promiscuity of T cells, the danger model and all sorts of fascinating ideas. I also want to thank all members of the Immune Genomics group who were always willing to help me when I was lost, Dr Daniel Gaffney and Dr Matthias Zilbauer for providing feedback on this project, and everyone listed in the collaboration note for making this work possible. Moreover, I am also grateful to Rosana Pelayo and her laboratory in Mexico City for letting me do cell culture for the first time during my undergraduate studies. Lastly, I am grateful to all the professors who made me fall in love with science: Jacqueline Oliva who first taught me immunology, Carlos Prado who showed me mathematics can describe anything, Juan Bravo who let me design and experiment for the first time, and Eric Lander who taught me molecular biology via his online videos without ever meeting me.

I am most grateful to my family for their unconditional support. To my sister Tábata Cano, who discussed science with me over dinner for years before I even started University, and who eventually took me to Cambridge. I cannot thank my mum and my dad enough. They believed in me even at moments when I couldn’t believe in myself. Thank you for making me who I am today: you are Maya Angelou’s really intelligent people. I am also grateful to my extended family, specially to my grandmother.

Last, I thank the Gates Cambridge trust and community not only for funding my studies (twice), but also for caring for each and every student they have and for building a unique space where we can find a home. Special thanks go to all the people who helped me endure the long Cambridge winter and the emotional challenges of this degree. Particularly to Alex Kong for showing me what kindness is about, and to Noor Shahzad and Francesca Brown, who fed me books and lines of poetry when I was about to starve.

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