Understanding the immune system in Chronic Immune-Mediated inflammatory diseases

Our immune system is a complex network of cells and organs that together defend the body against harmful foreign invaders such as bacteria and viruses. Inflammation is a normal, controlled part of these defences but sometimes this control breaks down to cause immune-mediated inflammatory diseases such as spondyloarthritis (SpA), psoriasis, Crohn's disease, and rheumatoid arthritis.

These diseases affect around 7 percent of the population worldwide and often strike at a young age causing lifelong illness. This represents a considerable burden for the affected individuals and society.

White blood cells called T helper (Th) lymphocytes play a critical role in such inflammatory processes. Dr Elisabetta Bianchi, a senior staff scientist at the Pasteur Institute in Paris, France is using Qlucore's Omics Explorer to study a subset of these Th lymphocytes, called Th17 cells, which are involved in chronic immune-mediated inflammatory diseases.

The ability of the immune system to mount efficient responses depends on the differentiation of naive CD4+ T cells (lymphocyte cells that have never been activated or seen an antigen) into functionally distinct T helper (Th) subsets, each characterised by the secretion of specific 'cytokine signatures'. Cytokines are messenger molecules that send chemical instructions to the rest of the immune system, causing it to ramp up its response against an antigen. Different Th subsets make different cytokines including interleukins, interferons, and tumor necrosis factors (TNF).

It was thought there were only two Th subsets: Th1 cells that produce interferon gamma for defense against intracellular pathogens, and Th2 cells, which produce interleukin IL-4, IL-5, IL-13 involved in the protection against parasitic infections. Th17 cells were discovered in 2006. They secrete IL-17, IL-21, IL-22, and IL-26 in humans, and contribute to immune responses against extracellular bacteria and fungi.

Dr Bianchi's Immunoregulation group has a particular interest in the role of Th17 cells in Ankylosing Spondylitis (AS), a type of Spondyloarthritis that affects the spinal joints and causes severe chronic pain and functional impairment.

First step for overviewing data

Qlucore Omics Explorer is an important tool for the group's research, according to Dr Bianchi. Her team has been using it for nearly a decade mainly for analysing gene expression data. "We use Qlucore as a first step to get an overview of the data, and to identify deeper questions we should be asking, " she says. "I typically start with a PCA [principal component analysis], as a first quality analysis of the samples and to start identifying patterns in the data. We then perform statistical analysis and visualize the data using a heatmap."

"Before we had the Qlucore tool, the group was analyzing data on a smaller scale. We would focus on a few genes and we could analyze them using Excel and Graphpad. Now that we carry out large scale analyses that generate more data, Qlucore is vital."

The Institute has a dedicated bioinformatics department too but like many such departments, it is overloaded with work. "We consult them only after we have performed an initial analysis, and have formulated a specific question that requires a complex statistical approach," says Dr Bianchi.

Recently, the group has used Omics Explorer to understand how the transcription factor RORγt (encoded by the RORC gene) modulates Th17 polarization and function. In a paper published in *Nature Communications* Dr Bianchi and her team have defined several regulatory elements at the human RORC locus in thymocytes and peripheral CD4+ T lymphocytes, with CRISPR/Cas9-guided deletion of these genomic segments supporting their role in RORγt expression.

"If we stimulate T lymphocytes with different cytokines that induce the differentiation of Th17 cells, we know that the cells will express the Transcription Factor RORγT but we didn't know exactly what are the pathways and the transcription factors that induce RORγT in humans," she says.

As well as analysing the expression of RORγT, the team analysed a panel of 450 immune related genes using Nanostring technology, and used Qlucore Omics Explorer to look at the results.

"We found that by deleting the regulatory regions that we thought might have enhancer activity for RORγT expression, RORγT levels were strongly reduced, " explains Dr Bianchi.

"We ended up with cells that looked like Th1 cells that make interferon gamma. It was a surprising result but one that confirms observations by other scientists that Th1 and Th17 lineages seem closely related. "

Dr Bianchi hypothesises that the Th1 phenotype may be the "default" pathway of T helper cell differentiation; it is much easier to get cells to make interferon gamma than to make Il-17.

Global picture reveals new information

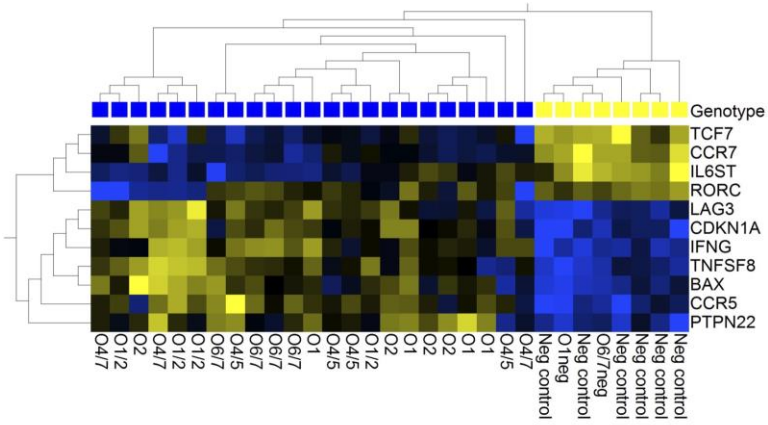

Figure: heatmap of multigroup comparison (ANOVA) of gene expression levels between control cells (Neg), and Th17 cells carrying genomic deletions at the RORC locus. Gene expression was measured by nCounter technology (Nanostring). 4 donors ($p = 0.002$, $q = 0.060$)

"With Omics Explorer it was easy to look at the differences in gene expression between cells with the regulatory regions that could differentiate towards Th17 phenotype, and cells with deleted regulatory regions, which could not express RORγT and couldn't differentiate to the Th17 phenotype, " says Dr Bianchi.

"It meant we could look at the full sample of 450 genes and identify which genes were differentially expressed, and we found an increase for genes expressed by Th1 type-cells."

She adds: "Without having a tool which lets you analyse easily the expression profile of 400 or so genes, you would just focus on the obvious gene candidates. By being able to look at a lot of data at one time, you see the global picture. Without that, we would never have seen this switching between phenotypes. We

would have focused on RORC2 and simply said, the gene is expressed less. We would not have seen that these were two different types of cell".

Larger data sets can still use the same tool

Long term, Dr Bianchi hopes that this work will help to guide the treatment of patients suffering from immune mediated diseases such as AS.

AS patients are often treated with a cytokine-blocking drug called anti-TNF. Blocking TNF can reduce inflammation and joint damage in two-thirds of patients. The rest don't respond. There is an alternative drug called Anti-IL-17 (that inhibits the IL-17A cytokine released by Th17) but again this only works for a subset of patients.

It is not clear why some patients respond and others don't. One of the studies that Dr Bianchi's group has been analyzing with the Qlucore tool compares immune responses in AS patients before and after treatment with anti-TNF.

"We are seeing a very strong effect on a whole panel of genes, and what's interesting is that IL-17 is not affected by anti-TNF treatment. Now we are keen to see what happens when we block IL-17," says Dr Bianchi.

She thinks it is possible that these two treatments might be complementary. So if anti-TNF doesn't work, then blocking IL-17 might work because it affects a different pathway.

For future research, the group is moving away from Nanostring technology to RNA sequencing. "We will be working with much larger datasets of thousands of gene expression profiles," explains Dr Bianchi. "It's a different order of magnitude of data but we can still use the same Qlucore tool in the same way to get a sense of what's going on, and which direction we need to go with our research. It is very powerful."

Reference

'NFAT primes the human RORC locus for RORγt expression in CD4+ T cells' by Hanane Yahia-Cherbal, Magda Rybczynska, Domenica Lovecchio, Tharshana Stephen, Chloé Lescale, Katarzyna Placek, Jérome Larghero, Lars Rogge & Elisabetta Bianchi; Nature Communications **10**, Article number: **4698 (2019)**