Cell-specific transcriptional modulation induced by fingolimod treatment in Relapsing Remitting Multiple Sclerosis patients

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BACKGROUND AND AIMS

Fingolimod (FTY), a second-line drug approved for aggressive form of Relapsing Remitting Multiple Sclerosis (RRMS), is a Sphingosine 1 Phosphate (S1P) analogue, that binds to S1P Receptors thus preventing lymphocytes egress outside lymph nodes and inducing peripheral blood lymphopenia. However its effect on a molecular level has not yet been fully outlined.

The present study aimed at investigating transcriptional changes induced by the treatment in specific immune cell subtypes.

PATIENTS AND METHODS

24 RRMS patients that started FTY were sampled at baseline and after 6 months of continuous treatment (Table 1). Patients previously treated with interferon, immunosuppressants, or natalizumab were excluded due to the known effect on gene expression. No patients received IV steroids in the month before enrollment.

In order to take into account the changes in peripheral blood cell composition, CD3+ T cells, CD20+ B cells and CD 14+ monocytes were purified from PBMC through sorting with the MACS MicroBeads system (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) with positive selection according to manufacturer's instructions. RNA sequencing on each cell subtype was performed using the Illumina TruSeq-Stranded mRNA preparation kit and the NextSeq500 platform (Illumina, SanDiego, CA). DESeq2 R package was used to identify differentially expressed genes (DEGs), defined as genes with a fold change (FC)>2 or <0.5 after treatment and a false discovery rate (FDR) < 5%. A pathway enrichment analysis was performed on these DEGs using the WebGestalt online tool, based on the KEGG database.

Sex (F/M)	16/8	**ARR pre FTY**	1.15
Mean Age (±SD)	36 ±9	**Median EDSS (range)**	2.5 (1.0-5.0)
Age at onset (±SD)	27.9 ±8.4	**Therapy**	17 No tp 7 GA
Disease Duration (±SD)	7.6 ±7.5		

Table 1: Clinical and demographic characteristic of included patients. No tp: no therapy; GA: glatiramer acetate.

RESULTS

A marked transcriptional modulation was found in both T and B lymphocyte populations. Specifically, 243 genes were upregulated and 181 were downregulated in T cells while 298 and 70 genes were up- and downregulated respectivey in B cells. On the contrary, in monocytes we only found 36 genes that were downregulated according to the above definition, and no upregulated genes.

The top 10 modulated genes for each cell type are reported in Table 2.

T Cells		
Gene	**P-value**	**FC**
SYT11	3,03E-67	2,25
APMAP	5,48E-60	2,37
ABI3	8,28E-58	2,78
CCL4	1,35E-56	4,37
PRF1	3,92E-55	4,12
TNFSF14	5,64E-52	2,89
S1PR5	4,37E-51	3,93
CD300A	4,52E-51	3,10
KLRG1	2,58E-50	3,02
FASLG	2,58E-50	4,75

B Cells		
Gene	**P-value**	**FC**
PASK	8,58E-58	0,38
CCR7	2,43E-41	0,21
FCGR3A	2,91E-37	4,81
FAM102A	4,67E-36	0,42
CX3CR1	3,18E-35	5,87
B4GALT5	1,95E-34	2,15
COL18A1	2,41E-34	0,32
CDC42EP3	2,03E-33	2,10
MDS2	3,18E-32	0,23
CTBP2	9,72E-32	3,78

Monocytes		
Gene	**P-value**	**FC**
LEF1	5,61E-40	0,22
TNFRSF25	4,26E-24	0,32
SLC38A1	3,54E-23	0,37
CARMIL2	2,44E-21	0,35
TCF7	2,44E-21	0,32
SPTBN1	2,44E-21	0,50
PLCG1	5,39E-21	0,41
FAM102A	5,04E-20	0,40
RHOH	5,56E-20	0,37
ETS1	1,9E-19	0,42

Table 2: Top 10 modulated genes in T cells, B cells and Monocytes..

Most of FTY-responsive genes had immune-related function, such as CCL4 and TNFSF14 in T lymphocytes, CCR7, FCG3A and CX3CR1 in B lymphocytes and TNFRSF25 in Monocytes.

We also noticed a good overlap between genes that were up- and downregulated in B e T cells, while Monocytes appear to have a quite separate trascriptional signature (Fig. 1).

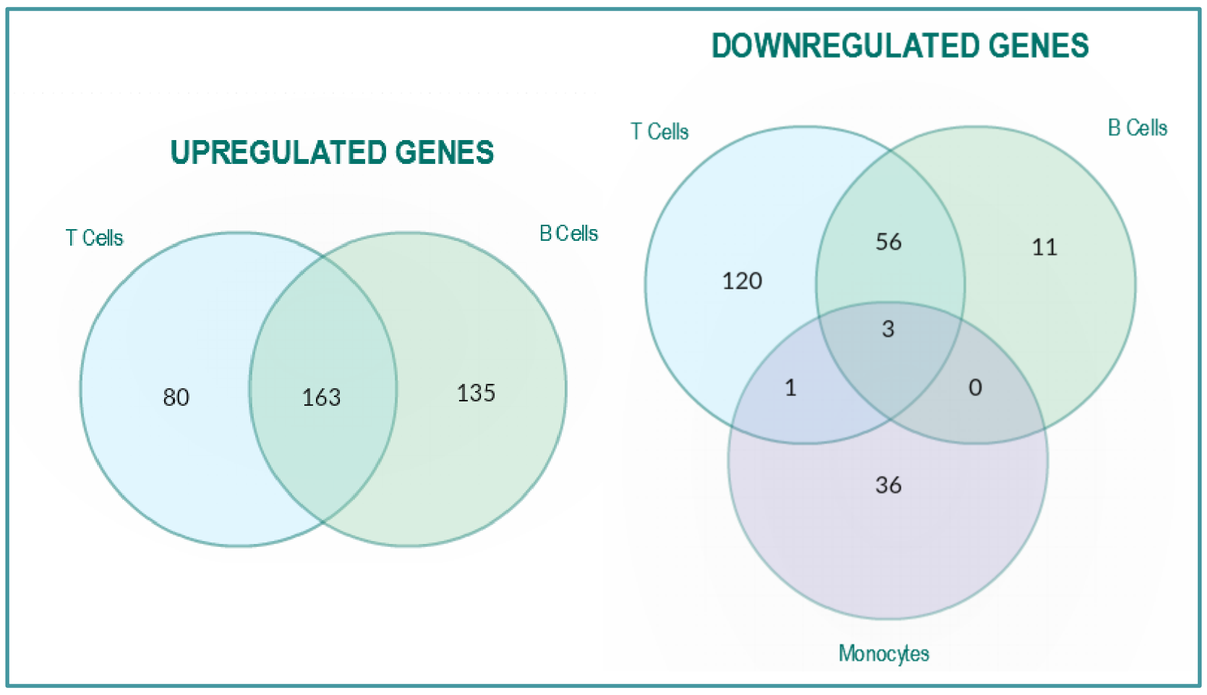

Fig 1: Venn diagrams showing the overlap between genes up- and downregulated in T. cells, B cells and Monocytes

Similarly, DEGs from T and B cells were enriched of genes involved in shared immune-related pathways, with 5 out of the first 10 selected pathways being common to both lymphocyte classes (Table 3 and 4).

T Cells				
Pathway	C	O	P-value	FDR
Cytokine-cytokine receptor interaction	82	24	2.31E-13	6.71E-11
Chemokine signaling pathway	105	17	9.51E-06	0.001384
Cell adhesion molecules (CAMs)	59	12	1.98E-05	0.001919
Natural killer cell mediated cytotoxicity	82	14	3.27E-05	0.002326
Neuroactive ligand-receptor interaction	36	9	4.00E-05	0.002326
Graft-versus-host disease	22	7	5.52E-05	0.002676
Osteoclast differentiation	87	14	6.48E-05	0.002692
Pathways in cancer	213	24	8.16E-05	0.002969
Hematopoietic cell lineage	41	9	0.00012	0.003885
Staphylococcus aureus infection	22	6	0.000493	0.014229

Table 3: Top 10 enriched pathways in T cells. C: n° of gene in the pathway; O: n° of DEGs observed in the pathway.

B Cells				
Pathway	C	O	P-value	FDR
Cytokine-cytokine receptor interaction	92	19	1.15E-08	3.37E-06
Neuroactive ligand-receptor interaction	39	12	5.99E-08	8.77E-06
Complement and coagulation cascades	22	8	2.55E-06	0.000198
Staphylococcus aureus infection	29	9	2.70E-06	0.000198
Malaria	26	8	1.07E-05	0.000626
Osteoclast differentiation	99	15	2.51E-05	0.001225
Natural killer cell mediated cytotoxicity	84	13	7.09E-05	0.002969
Chemokine signaling pathway	115	15	0.000149	0.005447
African trypanosomiasis	17	5	0.000675	0.021011
Antigen processing and presentation	56	9	0.000717	0.021011

Table 4: Top 10 enriched pathways in B cells. C: n° of gene in the pathway; O: n° of DEGs observed in the pathway.

The enrichment analysis in Monocytes also outlined pathways involved in immune-regulation but different from those in B and T cells (Table 5).

Monocytes				
Pathway	C	O	P-value	FDR
Primary immunodeficiency	22	5	1.71E-07	4.95E-05
T cell receptor signaling pathway	78	7	3.4E-07	4.95E-05
Hematopoietic cell lineage	53	5	1.63E-05	0.001586
Th1 and Th2 cell differentiation	64	5	4.14E-05	0.003015
NF-kappa B signaling pathway	74	5	8.39E-05	0.004883

Table 5: Pathways enriched in Monocytes. C: n° of gene in the pathway; O: n° of DEGs observed in the pathway.

CONCLUSIONS

FTY induces major transcriptional changes at the immune level, that appear to be shared between T and B lymphocytes, whereas the induced gene expression modulation in monocytes is quite different. Our data suggest that at least part of the immunomodulatory action of FTY could be regulated at the transcriptional level.

LITERATURE

Calabresi PA, Radue EW, Goodin D et al. Safety and efficacy of fingolimod in patients with relapsing-remitting multiple sclerosis (FREEDOMS II): a double-blind, randomised, placebo-controlled, phase 3 trial. Lancet Neurol. (2014) Jun;13(6); Jörg Friess, Michael Hecker, Luisa Roch t al. Fingolimod alters the transcriptome profile of circulating CD4+ cells in multiple sclerosis. Sci Rep (2017) Feb 3;7; Luisa Roch, Michael Hecker, Jörg Friess et al. High-Resolution Expression Profiling of Peripheral Blood CD8+ Cells in Patients with Multiple Sclerosis Displays Fingolimod-Induced Immune Cell Redistribution. Mol Neurobiol (2016) Sep 8

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