Immunome Research
ISSN: 1745-7580

Kozela et al., Immunome Res 2016, 12:1
DOI: 10.4172/1745-7580.1000108

Research Article Open Access

Insights into Gene Expression of Activated Pathogenic Autoimmune T Cells - Studies in Experimental Multiple Sclerosis-like Model

Ewa Kozela[1,2]*, Ana Juknat[1,2], Fuying Gao[3], Giovanni Coppola[3], Nathali Kaushansky[2] and Zvi Vogel[1,2]

[1]*The Dr Miriam and Sheldon G. Adelson Center for the Biology of Addictive Diseases, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 6997801, Israel*
[2]*Neurobiology Department, Weizmann Institute of Science, Rehovot 76100, Israel*
[3]*Departments of Psychiatry and Neurology, Semel Institute for Neuroscience and Human Behavior, David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA*

Abstract

Multiple Sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), are autoimmune diseases driven by pathogenic memory T cells. Using myelin oligodendrocyte glycoprotein (MOG) 35-55-specific encephalitogenic T cells (T_{MOG}) isolated from MOG35-55-immunized EAE mice we describe here their gene expression profile following antigen specific activation. A vast number of pro-inflammatory genes including cytokines, chemokines and growth factors (e.g., Csf2, Il3, Ccl1, Ccl3) as well as signaling pathways (e.g., iNOS, MAPK, JAK/STAT, NFκβ) were dramatically upregulated following MOG35-55 stimulation of T_{MOG} cells. A number of Th17-related pathways were induced confirming potent Th17-like activation of T_{MOG}. Interestingly, genes known for their anti-inflammatory role (Sit1, Hsd11b1, Pias3, Pparg, Lgmn, Klk3, Tnfaip8l2) were down-regulated in response to MOG35-55 suggesting that silencing of intrinsic suppressory mechanisms may underlie the hyperactivation of memory T cells. MOG35-55 activation led to lower transcription of pro-apoptotic/autophagic genes (Ddit4, Bbc3, Dapk2, Wbp1) and to enhanced level of anti-apoptotic transcripts (Bcl2l1). Transcripts related to toll-like receptors and MyD88-signaling were induced, revealing the involvement of innate immunity pathways in T cell driven autoimmunity. This study reveals the transcriptional events that lead to enhanced cytotoxicity, proliferation and resistance to apoptosis of activated autoimmune T cells. We suggest that encephalitogenic T cells may serve as a reliable *in vitro* model for screening for possible therapeutics against T cell driven autoimmune diseases.

Keywords: Encephalitogenic T cells; Th17; MOG; Multiple sclerosis; Autoimmune diseases; Gene expression

Abbreviations: APC: Antigen Presenting Cells; CNS: Central Nervous System; EAE: Experimental Autoimmune Encephalomyelitis; IL: Interleukin; IPA: Ingenuity Pathway Analysis; MOG: Myelin Oligodendrocyte Glycoprotein; MS: Multiple Sclerosis; RORγt: Retinoic acid Receptor-Related Orphan Receptor γt; STAT3: Signal Transducer and Activator of Transcription 3; T_{MOG}: MOG35-55-Specific $CD4^+$ T cell Line

Introduction

Multiple Sclerosis (MS) is an autoimmune demyelinating disease of the central nervous system (CNS) and the leading cause of paralysis in young adults [1]. The cause of MS is not well defined but it is acknowledged that pro-inflammatory, self-myelin-reactive clones of $CD4^+$ T cells play a critical role in MS pathology and progression [2,3]. These myelin-specific T cells are primed by antigen presenting cells (APC) to target the subject own myelin. Indeed, an immunization with myelin peptides or myelin containing particles induces T cell-driven MS-like pathology in animals, defined as experimental autoimmune encephalomyelitis (EAE), a common model of MS-like condition [4,5]. Memory T cells, despite their low quantity, exhibit enhanced proliferation, high cytotoxic effector functions, prolonged lifespan and re-activation in response to self-antigens. These distinct features are controlled by unique pattern of signaling pathways and reprogrammed gene expression [6]. It was observed that autoreactive memory T cells secrete interleukin(IL)-17 cytokine via retinoic acid receptor-related orphan receptor γt (RORγt)/signal transducer and activator of transcription 3 (STAT3)-dependent manner and were therefore defined as Th17 phenotype [7-9]. Adoptive transfer of such autoreactive encephalitogenic Th17 cells to healthy animals results in rapid and severe EAE defined as passive EAE [4,5]. Indeed, T lymphocytes of the Th17 phenotype were found to be present in MS patients as well as in several other T cell driven autoimmune conditions in humans [10].

Antigen re-activation of quiescent, circulating memory T cells may contribute to MS relapses [11]. Targeting these activated memory T cells appears to be an efficient strategy in MS treatment [12]. However, this approach is still challenging due to the difficulty in distinguishing autoreactive T cells from their non-pathogenic counterparts. Thus, further mapping of T cell specific pathways and co-regulated genes are of high importance.

In our current study, we analyzed the changes in gene expression following antigen-specific re-activation of autoreactive T cells. For this purpose, we used a mouse memory myelin oligodendrocyte glycoprotein (MOG)35-55-specific $CD4^+$ T cell line (T_{MOG}) isolated from MOG35-55-immunized EAE C57BL/6 mice [13-15]. Previously, we showed that these T_{MOG} cells produce and secrete IL-17 cytokine in response to MOG35-55 stimulation [16]. IL-17 production was accompanied by increased STAT3 phosphorylation [17] confirming self-antigen-induced Th17-like activity of these T_{MOG} cells. Here we performed whole gene expression analysis of the activated T_{MOG} and

***Corresponding author:** Ewa Kozela, The Dr Miriam and Sheldon G. Adelson Center for the Biology of Addictive Diseases, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 6997801, Israel, Tel: +972 584543248; E-mail: kozelae@post.tau.ac.il or ewa.kozela@weizmann.ac.il

Received February 18, 2016; **Accepted** March 23, 2016; **Published** March 28, 2016

Citation: Kozela E, Juknat A, Gao F, Coppola G, Kaushansky N, et al. (2016) Insights into Gene Expression of Activated Pathogenic Autoimmune T Cells - Studies in Experimental Multiple Sclerosis-like Model. Immunome Res 12: 108. doi: 10.4172/17457580.1000108

identified the main pathways and gene networks that are driving their acute activation and effector functions. Mapping these pathways and affected genes could lead to better understanding of the processes taking place in autoimmune diseases and eventually to better treatment.

Materials and Methods

Reagents

Lyophilized MOG35-55 peptide [MEVGWYRSPFSRVVH-LYRNGK] purchased from GenScript (Piscataway, NJ, USA) was re-constituted in sterile PBS and the stock solution stored in aliquots at -20°C. The working MOG35-55 solutions were prepared fresh just before the experiments. Fetal calf serum (FCS) and other tissue culture reagents were obtained from Biological Industries (Kibbutz Beit HaEmek, Israel).

T_{MOG} stimulation and CD4+ microbead purification from APC/T_{MOG} co-cultures

The MOG35-55-specific T_{MOG} cell line was isolated and maintained as described earlier [14-16]. T_{MOG} were stimulated with MOG35-55 in the presence of mouse spleen-derived APC as described before [16,17] with few modifications. Freshly isolated APC (50×10^6 cells/10 cm plate) were pre-adhered for 2 h at 37°C, washed with PBS containing Ca^{++} and Mg^{++} to remove floating cells and then co-cultured with 2.5×10^6 T_{MOG} cells. The APC/T_{MOG} co-cultures were stimulated with 5 μg/ml of MOG35-55. After 8 h of incubation, the media containing mostly T_{MOG} cells (but not the adherent APC cells) were carefully collected and the T_{MOG} cells further purified using anti-CD4 (L3T4) magnetic beads (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) as described earlier [17]. As shown by us before, $CD4^+$ column purification allows the separation of the T_{MOG} cells from any residual APC cells while preserving the antigen specificity and antigen-induced Th17-function of the T_{MOG} cells [16]. mRNA isolated from these purified T_{MOG} cells was subjected to global mRNA expression analysis using Illumina microarray as described below.

The MOG35-55 incubation time of 8 h was chosen based on previous time- and dose-response experiments, including MOG35-55-induced T_{MOG} proliferation, IL-17 cytokine production and release as well as intracellular pathways' activation [16-18].

RNA extraction and microarray transcript analysis

Purified T_{MOG} cells were lysed and subjected to RNA extraction (5Prime, Darmstadt, Germany) as described earlier [17,19]. For comparative microarray analysis, 200 ng samples of total RNA were amplified, labelled and hybridized onto Illumina MouseRef-8 v 2.0 Expression Bead-Chip, querying the expression of 25,600 RefSeq-curated gene targets and 796 random sequences used for the assessment of background noise. Arrays were processed and scanned with Illumina BeadStation platform according to the manufacturer's protocol. Raw data were log2 transformed and normalized using quantile normalization.

Statistical microarray analysis and gene expression analysis of the raw data were performed at the Informatics Center for Neurogenetics and Neurogenomics core at UCLA using R scripts (www.r-project.com) and Bioconductor packages (http://www.bioconductor.org; [20]) as described before [21].

Pathway and global functional analyses were performed using QIAGEN's Ingenuity Pathway Analysis (IPA®, QIAGEN Redwood City CA, USA www.qiagen.com/ingenuity). A data set containing gene identifiers and corresponding expression values was uploaded into the application to identify the biological functions as well as the pathways, gene networks and upstream regulators from the IPA library that were most significantly affected by the treatment with MOG35-55 as compared to control, purified, not stimulated T_{MOG} cells. Genes that met the p-value cutoff of 0.05 (using Fisher's exact test) were used to build the network plots using the IPA tool.

qPCR analysis

Selected gene products found by microarray analysis to be affected by MOG35-55 stimulation (as compared to unstimulated, purified T_{MOG}) were validated by qPCR as described [17,19] using β2-microglobulin (β2MG) gene product for normalization. Supplementary Table S1 presents the list of specific primers used. The qPCR runnings were repeated 3-4 times using mRNA preparations from independent experiments and analyzed statistically using t test.

Study approval

Mice used in this study were maintained according to the guidelines of the Institutional Animal Care and Use Committee (IACUC). Animal experiments were approved by the Weizmann Institute of Science and the Tel Aviv University IACUCs.

Results

Effects of MOG35-55 on gene expression

Stimulation of T_{MOG} cells with MOG35-55 led to significant increases in a number of pro-inflammatory transcripts. Altogether, 247 gene transcripts were upregulated following specific activation of T_{MOG} by ≥ 2 fold and within this cohort 47 transcripts were upregulated by ≥ 4 fold ($p<0.005$). The highest MOG35-55-upregulated gene products (≥ 4 fold increase) are presented in Table 1 and include various proinflammatory cytokines (e.g. Csf2, Il3, Spp1, Tnf, Ifng, Lta), chemokines (e.g. Ccl1, Ccl3, Ccl4, Xcl1, Cxcl10, Cxcl9) and adhesion molecules (Amica1, Sema7a). MOG35-55 stimulation also resulted in increased mRNA levels of T cell transcription factors (Egr2, Egr1, Ier3, Atf3, Irf4, Axud1), signaling elements (e.g. Rgs16, Ndrg1, Tagap, Nr4a3), including those forming interferon signaling pathway (e.g. Ifit3, Oas2, Oasl2), TNF family signaling elements (Tnfsf11, Tnfsf14, Tnfrsf9) and members of the ubiquitination pathway (Usp18, G1p2). The remaining 200 transcripts that were upregulated by ≥2 fold (but less than 4 fold) are presented in Supplementary Table S2 and include Il12a cytokine, co-activating membrane molecules and receptors (e.g., Cd69, Il1r2, Tnfrsf4, Ccrl2, Il2ra, Tlr7, Cd40), signaling elements (e.g., Cdk5r1, Rgs1, Gbp2, Map3k8, Nfkbiz, Gbp4, Ifit2, Map2k3) and transcription factors (e.g. Irf7, Nr4a2, Socs3, Stat5a, Tbx21, Irf1, Junb). This large change in transcriptional effects by exposure to MOG35-55 demonstrates the powerful activation of MOG35-55-exposure on T_{MOG} cells and is in correlation with our previous observations on the Th17 function of these activated T_{MOG} cells cells [16,18]. In addition to the gene products that were upregulated, MOG35-55 stimulation led to downregulation of a large number of genes. Table 2 shows the list of genes whose expression was significantly suppressed (by 60% or more, $p<0.005$) in response to MOG35-55 stimulation of T_{MOG}. Among the most down-regulated transcripts we found various subunits of IL-17 receptors (e.g., Il17re, variants 2 and 1 and Il17rc). This may suggest protective mechanism acquired by T_{MOG} against their own cytotoxic activity induced via IL-17. Moreover, the transcription of several pro-apoptotic/pro-autophagic genes was decreased following MOG35-55 stimulation including of Ddit4, Bbc3, Dapk2, and Wbp1.

Accession	Symbol	Definition	Fold change
CYTOKINES and CHEMOKINES			
NM_011329.1	Ccl1	chemokine (C-C motif) ligand 1	91.8
NM_009969.2	Csf2	colony Stimulating Factor 2 (Granulocyte-Macrophage)	77.0
NM_011337.1	Ccl3	chemokine (C-C motif) ligand 3	54.1
NM_013652.1	Ccl4	chemokine (C-C motif) ligand 4	40.2
NM_008510.1	Xcl1	chemokine (C motif) ligand 1	30.0
NM_010556.2	Il3	interleukin-3	25.4
NM_009263.1	Spp1	secreted phosphoprotein 1	19.0
NM_013693.1	Tnf	tumor necrosis factor	15.3
NM_008337.1	Ifng	interferon gamma	10.8
NM_010735.1	Lta	lymphotoxin A	9.4
NM_021274.1	Cxcl10	chemokine (C-X-C motif) ligand 10	9.0
NM_008599	Cxcl9	chemokine (C-X-C motif) ligand 9	7.4
ADHESION MOLECULES			
NM_001005421.2	Amica1	adhesion molecule, interacts with CXADR antigen 1	5.0
NM_011352.2	Sema7a	sema domain, Ig domain and GPI membrane anchor, (semaphorin) 7A	4.6
TRANSCRIPTION FACTORS			
NM_010118.1	Egr2	early growth response 2	20.1
NM_007913.2	Egr1	early growth response 2	10.7
NM_133662.1	Ier3	immediate early response 3	5.5
NM_007498.2	Atf3	activating transcription factor 3	4.7
NM_013674.1	Irf4	interferon regulatory factor 4	4.5
NM_153287.2	Axud1	AXIN1 up-regulated 1	4.4
SIGNALING ELEMENTS			
NM_011267.1	Rgs16	regulator of G-protein signaling 16	16.0
NM_011451	Sphk1	sphingosine kinase 1	12.3
NM_011613.2	Tnfsf11	tumor necrosis factor (Ligand) superfamily, member 11	11.8
NM_145227.1	Oas2	2'-5' oligoadenylate synthetase 2	9.3
NM_025427.1	1190002H23Rik	regulator of cell cycle (Rgcc, Rgc32)	9.0
NM_019418.1	Tnfsf14	tumor necrosis factor (ligand) superfamily, member 14	8.5
NM_148933.1	Slco4a1	solute carrier organic anion transporter family, member 4a1	7.4
NM_013807.1	Plk3	polo-like kinase 3	7.1
NM_010501.1	Ifit3	interferon-induced protein with tetratricopeptide repeats 3	6.2
NM_010884.1	Ndrg1	N-myc downstream regulated gene 1	6.2
NM_001033399.1	Gfod1	glucose-fructose oxidoreductase domain containing 1	6.2
NM_153564	Gbp5	guanylate Binding Protein 5	6.1
NM_013606	Mx2	MX Dynamin-Like GTPase 2	6.0
NM_011854.1	Oasl2	2'-5' oligoadenylate synthetase-like 2	5.9
XM_138237.2	BC049975	serine (or cysteine) peptidase inhibitor, clade A, member 3F (Serpina3)	5.5
NM_011909.1	Usp18	ubiquitin specific peptidase 18	5.3
NM_152804.1	Plk2	polo-like kinase 2 (Drosophila)	4.9
NM_021384.2	Rsad2	radical S-adenosyl methionine domain containing 2	4.9
NM_011612.2	Tnfrsf9	tumor necrosis factor receptor superfamily, member 9	4.8
NM_008681	Ndrl	N-myc downstream regulated-like	4.7
NM_145968.1	Tagap	T-cell activation Rho GTPase-activating protein	4.7
NM_007532.2	Bcat1	branched chain aminotransferase 1, cytosolic, transcript v.2	4.4
NM_015783.1	G1p2	ISG15 ubiquitin-like modifier	4.1
NM_015743.1	Nr4a3	nuclear receptor subfamily 4, group A, member 3	4.1
NM_172142.1	AY078069	cDNA sequence AY078069	4.1
NM_008102.2	Gch1	GTP cyclohydrolase 1	4.1
NM_020557.3	Tyki	cytidine monophosphate (UMP-CMP) kinase 2, mitochondrial (Cmpk2)	4.0

Table 1: List of genes which expression was highly upregulated (≥4 fold, p <0.005) following MOG35-55-stimulation of T_{MOG}.

Accession	Symbol	Definition	Decrease
RECEPTORS and MEMBRANE PROTEINS			
NM_001034029.1	Il17re	interleukin 17 receptor E (Il17re), transcript variant 2	-80%
NM_145826.2	Il17re	interleukin 17 receptor E, transcript variant 1	-70%
NM_134159.2	Il17rc	interleukin 17 receptor C	-70%
NM_010654.1	Klrd1	killer cell lectin-like receptor, subfamily D, member 1	-70%
NM_025326.2	Tmem176a	transmembrane protein 176A	-70%
NM_177155.2	A530090P03Rik	killer cell lectin-like receptor family I member 2	-70%
NM_153590.2	Klre1	killer cell lectin-like receptor family E member 1	-60%
NM_023056.2	1810009M01Rik	transmembrane protein 176B	-60%
NM_030712.1	Cxcr6	chemokine (C-X-C motif) receptor 6	-60%
NM_177836.2	AW046396	disintegrin and metallopeptidase domain 23 (adam23)	-60%
NM_053110.2	Gpnmb	glycoprotein (transmembrane) nmb	-60%
NM_027995.1	Mpra	progestin and adipoQ receptor family member VII	-60%
NM_010819.1	Clecsf8	C-type lectin receptor CLECSF8 (Clec4d)	-60%
NM_010185.2	Fcer1g	Fc fragment of IgE, high affinity I, receptor for γ polypeptide	-60%
APOPTOSIS REGULATORS			
NM_029083.1	Ddit4	DNA-damage-inducible transcript 4	-70%
NM_031874.3	Rab3d	RAB3D, member RAS oncogene family	-60%
NM_007537.1	Bcl2l2	Bcl2-like 2	-60%
NM_133234.1	Bbc3	Bcl-2 binding component 3	-60%
NM_010019.2	Dapk2	death-associated kinase 2	-60%
NM_016757.1	Wbp1	WW domain binding protein 1	-60%
ANTI-INFLAMMATORY MOLECULES			
NM_019436.1	Sit1	suppression inducing transmembrane adaptor 1	-70%
NM_008288.1	Hsd11b1	hydroxysteroid 11-beta dehydrogenase 1	-60%
SIGNALING ELEMENTS			
NM_001040088.1	Sytl2	synaptotagmin-like 2 (Sytl2), transcript variant 5	-80%
NM_175514	D430039N05Rik	family with sequence similarity 171, member B (Fam171b)	-70%
NM_023049.1	Asb2	ankyrin repeat and SOCS box-containing protein 2	-70%
NM_146167.2	Ian3	GTPase, IMAP family member 7	-70%
NM_207668.1	Acpp	acid phosphatase, prostate	-70%
NM_133198.1	Pygl	liver glycogen phosphorylase	-70%
NM_029631.2	Abhd14b	abhydrolase domain containing 14b	-70%
NM_027307	Golph2	golgi membrane protein 1, transcript variant 1	-70%
NM_001035122.2	Golph2	golgi membrane protein 1, transcript variant 2	-60%
NM_175002.1	BC025076	membrane magnesium transporter 2 (Mmgt2)	-60%
NM_172574.1	Pqlc3	PQ loop repeat containing	-60%
NM_001013384.1	Podnl1	podocan-like 1	-60%
NM_009180.3	St6galnac2	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1, 3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 2	-60%
NM_009985.2	Ctsw	cathepsin W	-60%
NM_153119.2	AI840980	pleckstrin homology domain containing, family Q member 1	-60%
NM_178162.2	Hrbl	HIV-1 Rev binding protein-like, transcript variant 2	-60%
NM_016772.1	Ech1	enoyl coenzyme A hydratase 1, peroxisomal	-60%
NM_053214.1	Myo1f	myosin IF (Myo1f)	-60%
NM_177617.2	D330014H01Rik	dynein, axonemal, heavy chain 2 (Dnah2)	-60%
NM_010492.2	Ica1	islet cell autoantigen 1	-60%
NM_025329.1	0610012D17Rik	Tctex1 domain containing 2 (Tctex1d2)	-60%
OXIDATION REGULATORS			
NM_153783.2	Paox	polyamine oxidase (exo-N4-amino)	-70%
NM_153420	C130099A20Rik	2-phosphoxylose phosphatase 1 (Pxylp1)	-60%
NM_008160.1	Gpx1	glutathione peroxidase 1	-60%
NM_053108.2	Glrx	glutaredoxin	-60%
NM_180962.1	Cyhr1	cysteine and histidine rich 1, transcript variant 2	-60%
NM_029100.1	Sepn1	selenoprotein N, 1	-60%
NM_153783.2	Paox	polyamine oxidase (exo-N4-amino)	-60%
TRANSCRIPTION FACTORS			
NM_013646.1	Rora	RAR-related orphan receptor alpha	-60%

Table 2: List of genes whose expression was down-regulated following MOG35-55 stimulation of T_{MOG} cells, (by 80% to 60%), $p<0.005$.

Identification of functional networks in activated T_{MOG}

MOG35-55 significantly affected genes were uploaded onto the IPA analysis program to identify the main gene networks associated with diseases, functional subsets, pathways and upstream regulators. Figure 1 shows the IPA-identified functional categories that were the most significantly ($p<0.005$) enriched following MOG35-55 stimulation. Supplementary Figure S1 complements this list with functional categories falling into lower significance values (between $p>0.005$

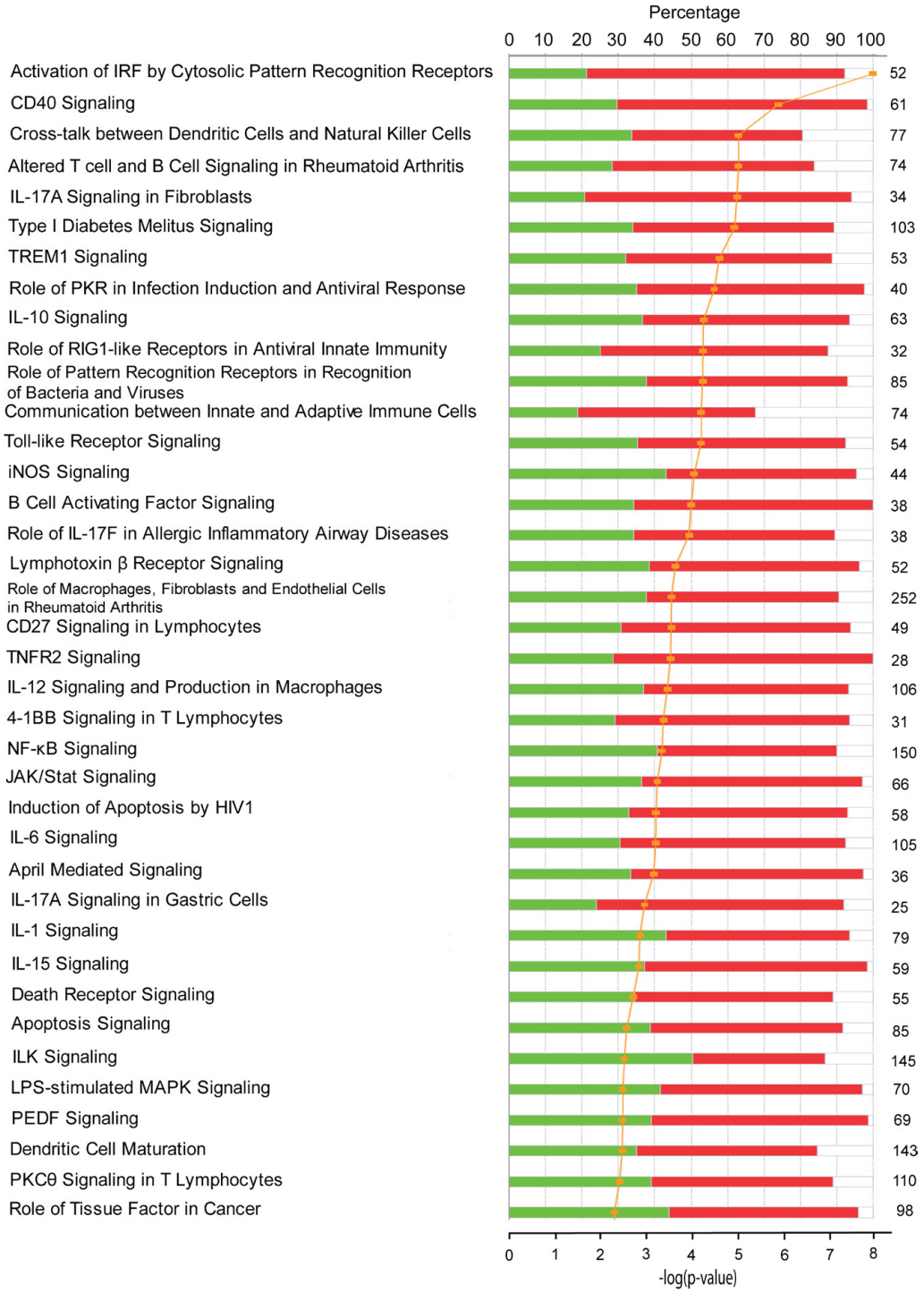

Figure 1: IPA analysis identified functional categories that were significantly ($p<0.005$) enriched following MOG35-55 stimulation of T_{MOG}. The total number of MOG35-55-affected genes within each of the listed categories is indicated on the right side of each bar. Green color represents the percentage of down-regulated genes, the red color the percentage of up-regulated genes and the non-colored area represents genes that were either not affected or affected insignificantly following MOG35-55-activation. The percentage scale is located at the top of the diagram. The orange line indicates the significance level according to the scale given at the bottom of the graph.

and p<0.05). The IPA analysis presented in Figures 1 and S1 links the MOG35-55 affected genes to several known autoimmune pathologies such as rheumatoid arthritis and type I diabetes (p<0.005, Figure 1) as well as systemic lupus erythematosus and multiple sclerosis (p<0.05, Figure S1) suggesting prevailing autoimmune type of inflammatory activation. Furthermore, among the IPA-identified categories we find recurring annotations related to Th17 activity. These annotations include "IL-17A Signaling in Fibroblasts", "Role of IL17F in Allergic Inflammatory Airway Diseases" and "IL-17A Signaling in Gastric Cells" (at p<0.005) as well as "IL-17 Signaling", "Role of IL-17A in Arthritis", "Differential Regulation of Cytokine Production in Macrophages and T Helper cells by IL-17A and IL-17F" and "IL-17A Signaling in Airway Cells" (at p>0.005-p<0.05) suggesting a dominant Th17 transcriptional mode of the activated T_{MOG} cells. In parallel, several pathways related to other cytokines were also recognized as significantly enriched in activated T_{MOG}. These include: IL-1, IL-6, IL-8, IL-10, IL-12, IL-15 and their cognate receptors such as Lymphotoxin β Receptor, TNFR2, (p<0.005) followed by IL-2, IL-9 and IGF1 signaling (p<0.05). IPA analysis highlighted the role of iNOS, MAPK, JAK/STAT, PKCθ, ILK and NFκβ signaling, pathways that are known to be downstream of 4-1BB (Tnfrsf9), CD28, April (Tnfsf13), CD27, CD40, TREM1 and RIG1-like receptors, in MOG35-55 stimulated T cells (p<0.005).

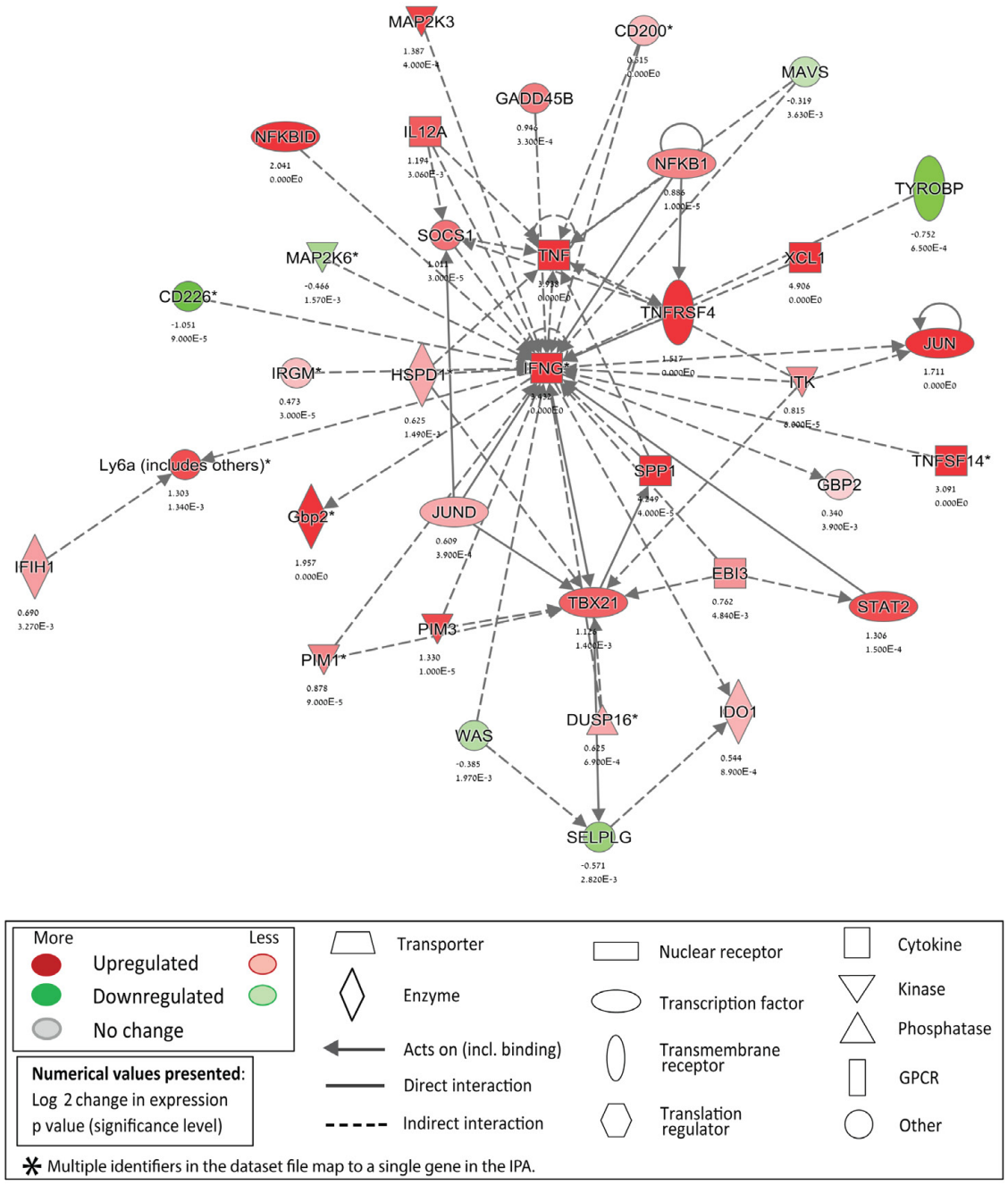

Figure 2: IFNγ-dependent gene network identified by IPA as the most significantly affected (up-regulated) following MOG35-55 stimulation of T_{MOG} cells.

Several pathways related to antigen presentation were recognized by IPA to be enriched following MOG35-55, emphasizing the role of dendritic cells (the most competent APC) and B cells (the major type of APC in the periphery) in the process of T_{MOG} stimulation. This includes "Cross Talk Between Dendritic and Natural Killer Cells", "Altered T Cell and B Cell Signaling in Rheumatoid Arthritis", "B Cell Activating Factor Signaling" and "Dendritic Cell Maturation". Interestingly, the IPA identified a substantial number of pathways that relate to a crosstalk between innate and adaptive immunity as being involved in MOG35-55 stimulation. These include "Activation of IRF by Cytosolic Pattern Recognition Receptors", "Role of PKR in Interferon Induction and Antiviral Response", "Role of RIG1-like Receptors in Antiviral Innate Immunity", "Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses", "Toll-like receptor signaling" and "LPS Stimulated MAPK Signaling".

Main gene networks affected by MOG35-55 stimulation

The genes significantly affected by MOG35-55 stimulation were used by IPA analysis to build gene networks that are involved in MOG35-55 activation of encephalitogenic T cells (see Figures 2-9 and supplementary Figures S2-S5). These IPA-constructed networks include the genes that were significantly affected ($p<0.05$) by MOG35-

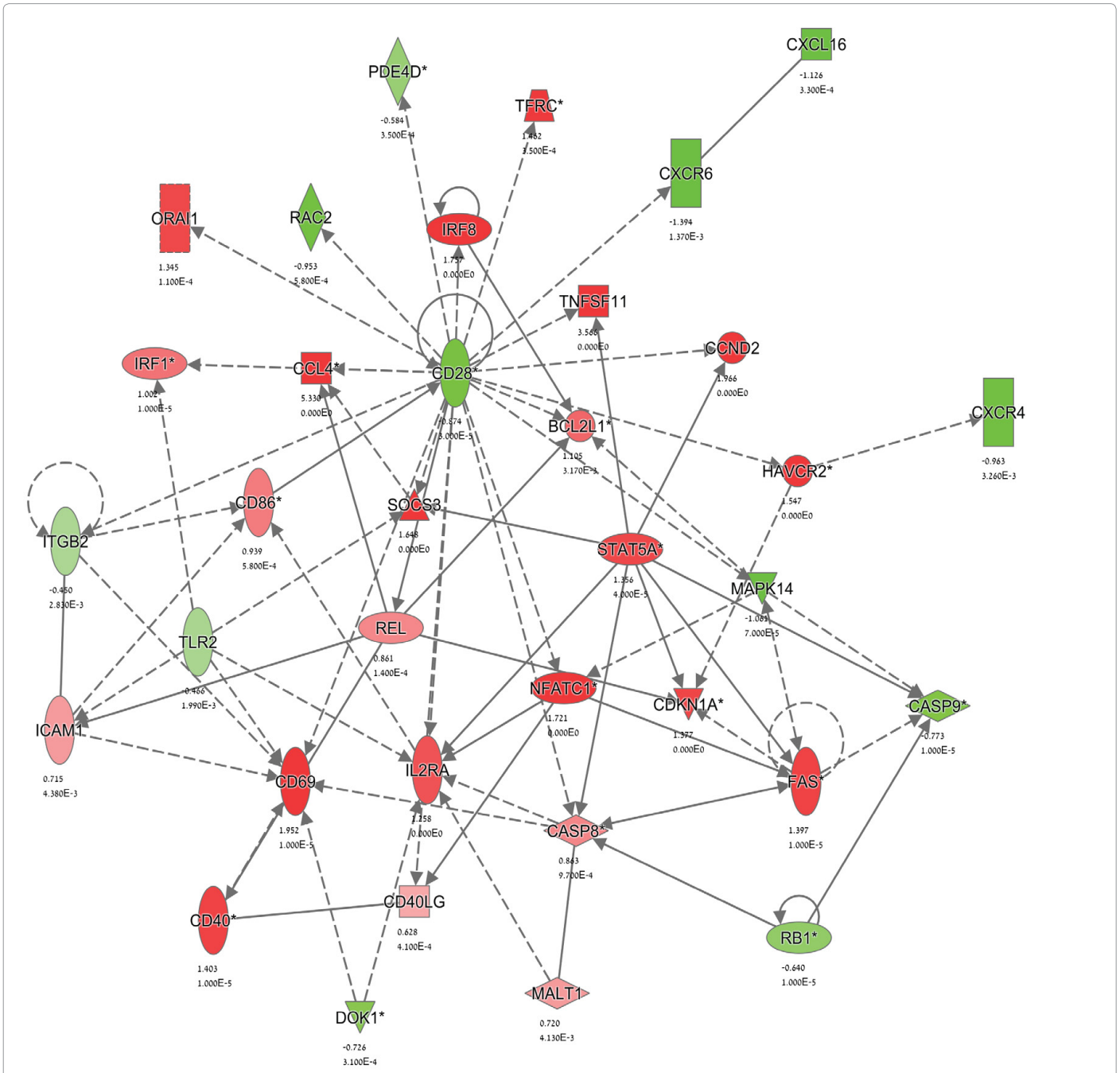

Figure 3: IPA-identified gene network showing involvement of CD28 and IL2RA-related gene expression following MOG35-55-stimulation of T_{MOG} cells.

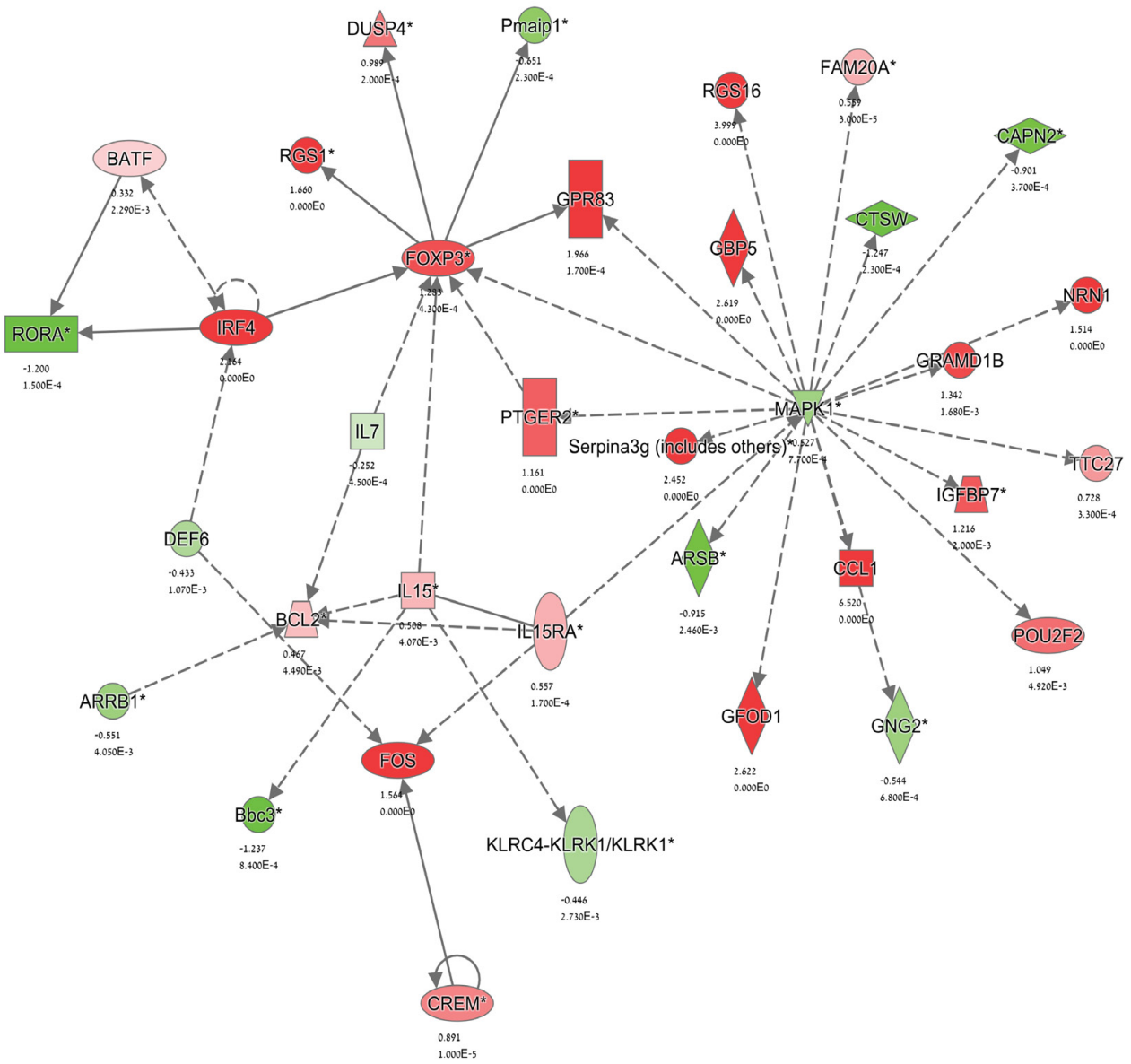

Figure 4: IPA-identified gene network showing involvement of MAPK, FoxP3 and Fos signaling in MOG35-55-activated T_{MOG} cells

55 stimulation even if they did not meet our previous criteria of 4 fold or 2 fold upregulation or 80-60% downregulation. The network identified by IPA as the most significantly contributing to MOG35-55-induced proinflammatory effects is composed of IFNγ and a vast number of IFNγ-related genes (Figure 2). Most of the genes within this group were identified as highly upregulated including a major IFNγ transcription factor (Tbx21), elements of IFN signaling pathway(s) (Stat2, Socs1, Jun, Jund, Mapk2k3, Dusp16), cytokines (Il12a), chemokines (Xcl1) and T cell activating proteins (e.g. Ly6a). The expression of Tnf and TNF signaling elements were identified as co-related to the IFNγ pathway activity.

Subsequent interactomes reveal the involvement of CD28 signaling (Figure 3), MAPK, Fos and FoxP3 (Figure 4), IL-6, CSF2 and TNFα (Figure 5), STAT5, Rel, Myc (Figure 6), IL-4 (Figure 7), IL-2 (Figure 8) and MOG35-55 affected anti-inflammatory pathways including IL-4, CTLA4 and CBFβ-related genes (Figure 9). Moreover, the supplementary Figures S2- S5 illustrate pathway interactions including IFNAR2 and SATB1 signaling (Figure S2), link between TNFα, CCL13 and TLR4 (Figure S3), between IFNγ, IL-2 and TLR3 signaling pathways (Figure S4) and between STAT4, TNFα and IFNγ (Figure S5). It is worth noting that the genes comprising the interactomes presented in Figure S3, and Figure S4 (TLRs and TLR-dependent Myd88 pathway) support a role of the innate immunity processes in T cell driven autoimmunity.

Several genes attributed by IPA into these interactomes include transcripts of genes known to exert anti-inflammatory functions that were found to be downregulated following MOG35-55 activation. These genes include: signaling threshold-regulating transmembrane adapter 1 (Sit1, Table 2), 11β-hydroxysteroid dehydrogenase type 1 (Hsd11b1, Table 2, Figure 7), Pias3 (Figure 8) and peroxisome proliferator-activated receptor gamma (Pparg,

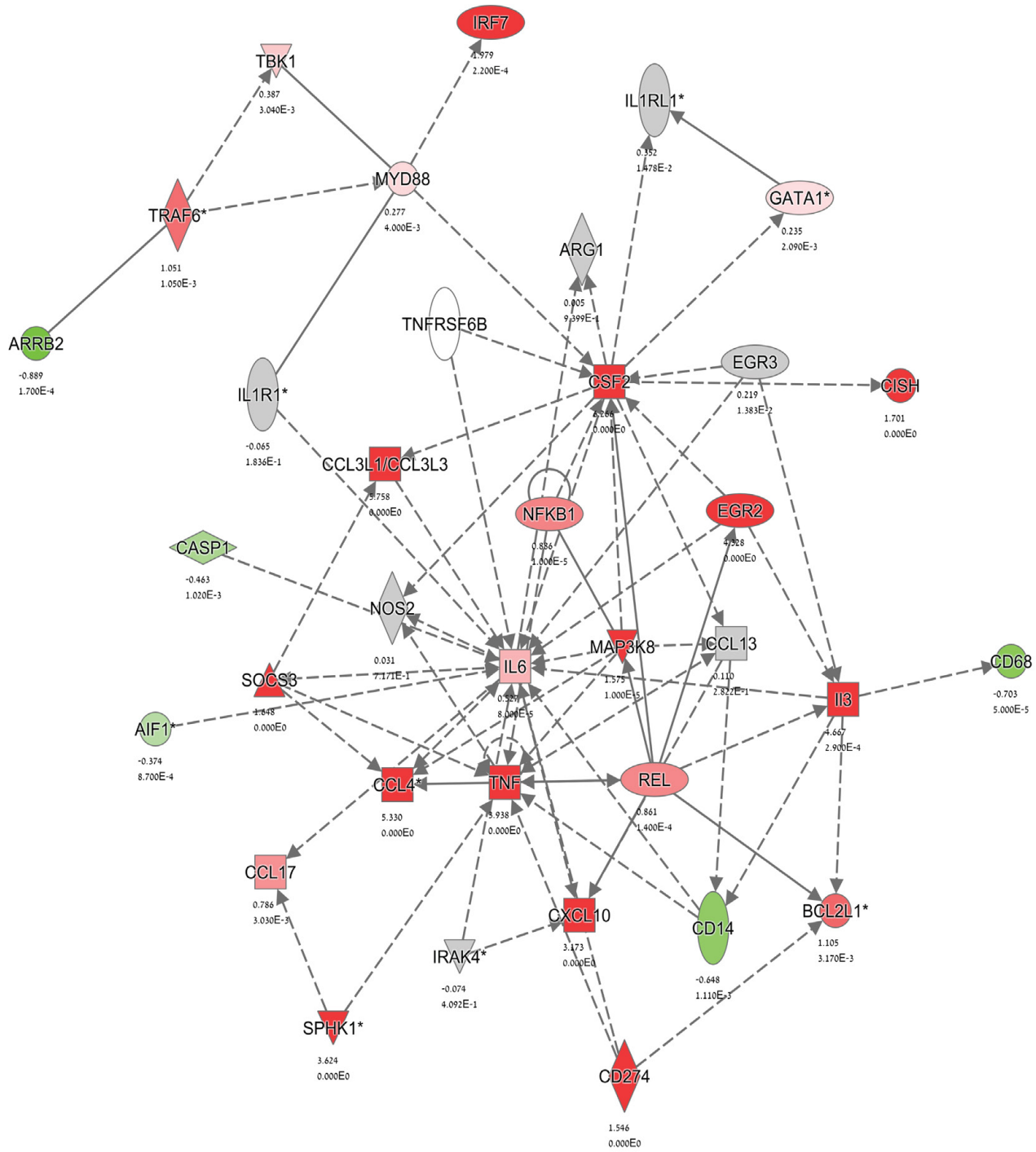

Figure 5: IPA-identified gene network showing TNF, IL-6 and GMCSF (Csf2) cytokine mRNA regulation and mutual interactions of the cytokine-related genes following MOG35-55 stimulation of T_{MOG} cells.

Figure S3). Figure 9 shows other anti-inflammatory genes that were suppressed following MOG35-55 activation, i.e. legumain (Lgmn), kallikrein 3 (Klk3) and tumor necrosis factor (TNF)-alpha-induced protein 8-like 2 (Tnfaip8l2) and links these effects via CBFβ, a known transcriptional co-regulator of FoxP3 to IL-4-dependent genes (Figure 7) and to CTLA4, both known for their potent regulatory role in inflammatory processes, including autoimmunity. These findings suggest that intrinsic regulatory mechanisms may be suppressed in activated memory T cells to facilitate efficient activation. On the other hand, several other gene products known to exert anti-inflammatory effects in Th17 cells like Stat5a (Figure 3), Foxp3 (Figure 4) and Egr2 (Figure 5) were found to be upregulated,

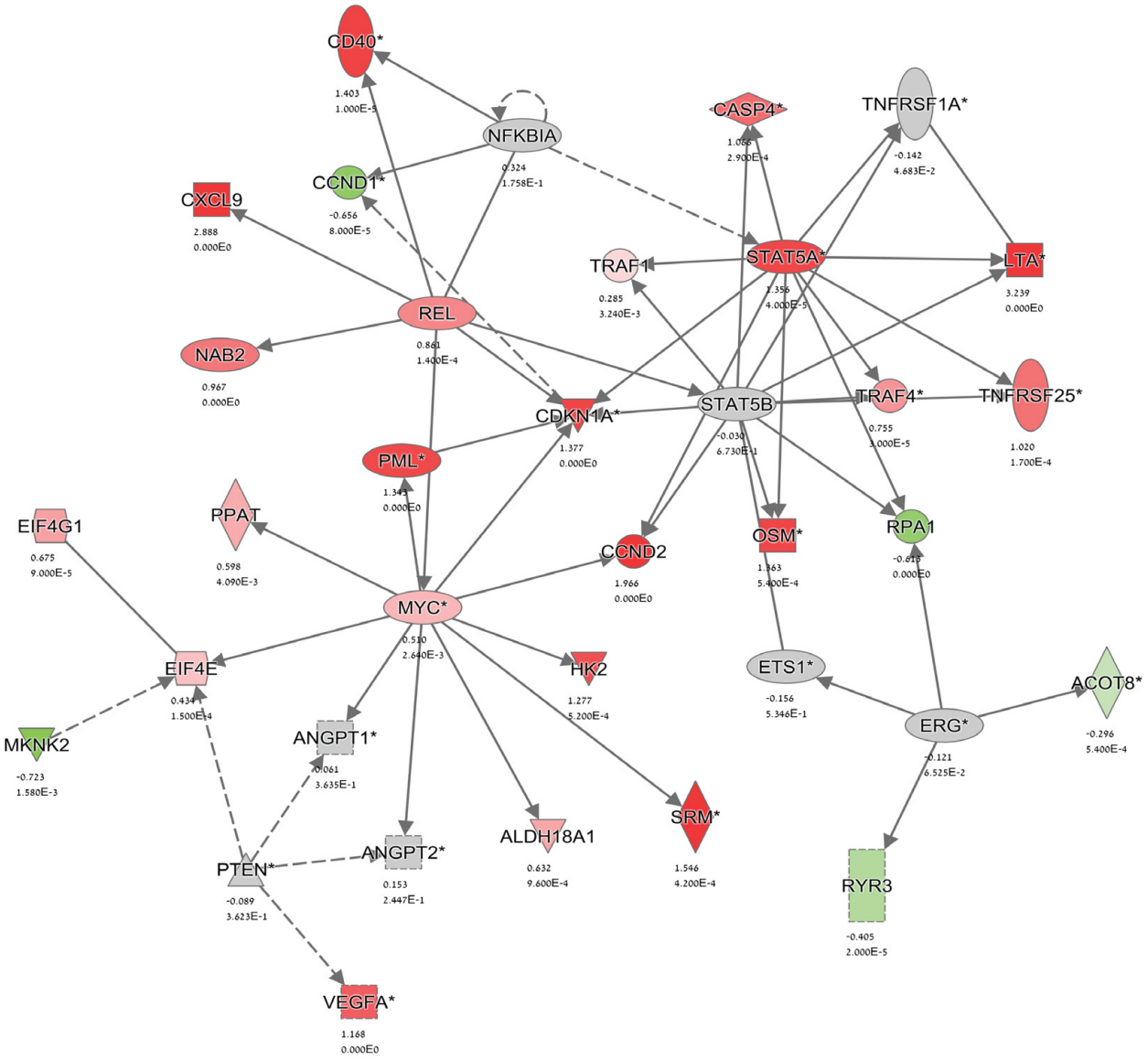

Figure 6: IPA-identified gene network showing the involvement of STAT5, REL and MYC signaling in MOG35-55-stimulated T_{MOG} cells.

suggesting that feedback homeostatic processes may be initiated in parallel with T cell activation.

Validation by qPCR

Several genes that were identified by microarray analysis as differentially regulated were subjected to validation by qPCR using β2-microglobulin as a reference gene. Table 3 shows examples of qPCR analysis of selected gene products in stimulated T_{MOG} cells. MOG35-55 induced an increase in the mRNA level of the cytokines Il12α (by 4.3 fold) and Xcl1 (62.7 fold), of the transcription factors Irf4 (by 3.4 fold) and Stat5 (3.3 fold) and of signaling molecule Mx1 (by 3 fold) signaling molecule. These observations are in agreement with the mRNA expression observed using the gene profiling method.

Symbol	Gene name	MOG35-55 [fold change]
Il12a	Interleukin 12	4.4 ± 1.0*
Xcl1	Chemokine (C motif) ligand 1	62.7 ± 11.8**
Irf4	Interferon regulatory factor 4	3.4 ± 0.7*
Stat5	Signal transducer and activator of transcription 5A	3.3 ± 0.6*
Mx1	MX dynamin-like GTPase 1	3.0 ± 0.3**

Table 3: qPCR validation of several transcript levels in MOG35-55-stimulated T_{MOG} cells. The data are expressed as fold change in MOG35-55-stimulated cells vs control, not stimulated cells (equal to 1) and analyzed with t test, *p<0.05, **p<0.01 vs control.

Discussion

We show here for the first time the transcript profiling of encephalitogenic T cells following activation by exposure to their antigen. The present report identifies the main pathways and gene networking mediating the MOG35-55-induced proinflammatory functions of memory T_{MOG} cells as well as the interactions between them.

MOG is a CNS specific myelin glycoprotein that is expressed

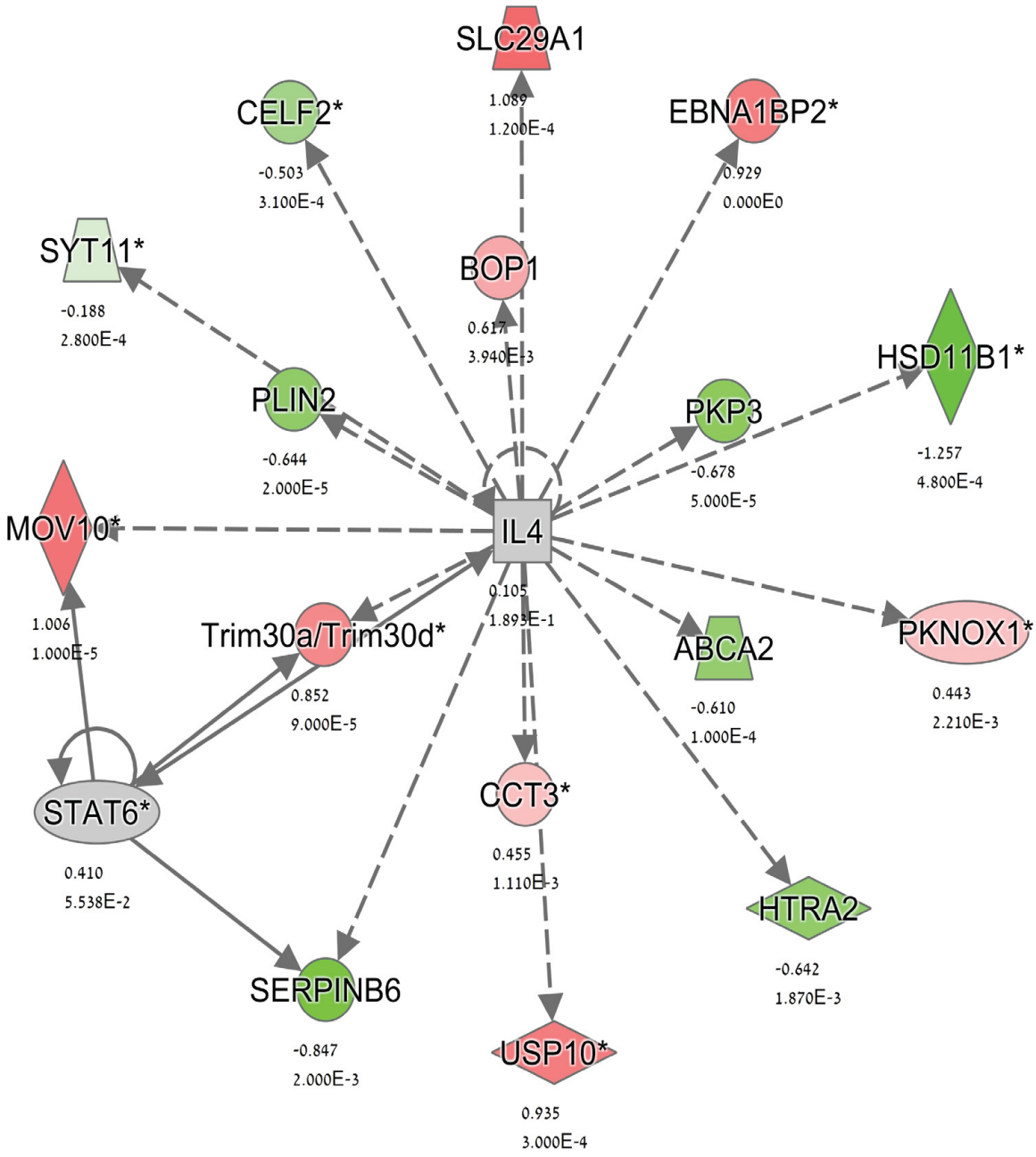

Figure 7: IPA-generated gene network showing IL-4 as a main regulator and IL-4 dependent gene expression in MOG35-55-activated T_{MOG}.

preferentially on the outermost surface of the myelin sheath. Although being a minor component of CNS myelin, MOG is highly immunogenic, and its targeting by encephalitogenic memory T cells induces severe EAE in various rodents and primates [22-24]. Autoimmunity to MOG was recognized as one of the primary demyelinating triggers in MS (For review see [25]). These observations substantiate the use of T_{MOG} memory cells as a tool to identify the molecular mechanisms mediating memory T cell pathological activity and especially the processes that can lead to the demyelination. In the present study, we applied gene array analysis to profile the changes in mRNA expression following MOG35-55 activation of memory T_{MOG} as well as to identify the main mechanisms driving the T cells' unique properties including Th17 function, antigen-specificity and cytotoxicity.

In recent years, few large-scale genetic approaches have been used to study the signature gene networks controlling autoimmune processes and Th17 functions. In many cases, this research was carried out on purified, naïve spleen-derived T cells differentiated *in vitro* (e.g., using IL-6+TGFβ or IL-6+TGFβ+IL-23 cytokines) into IL-17 releasing T helper cells [26,27]. Due to the absence of APC cells in these *in vitro* systems, the newly differentiated Th17 cells lack antigen specificity. Consequently, antigen-activated pathways and correlated gene networks in such T cells are usually not identified by the subsequent bioinformatics analyzes. In other cases, antigen specific molecular

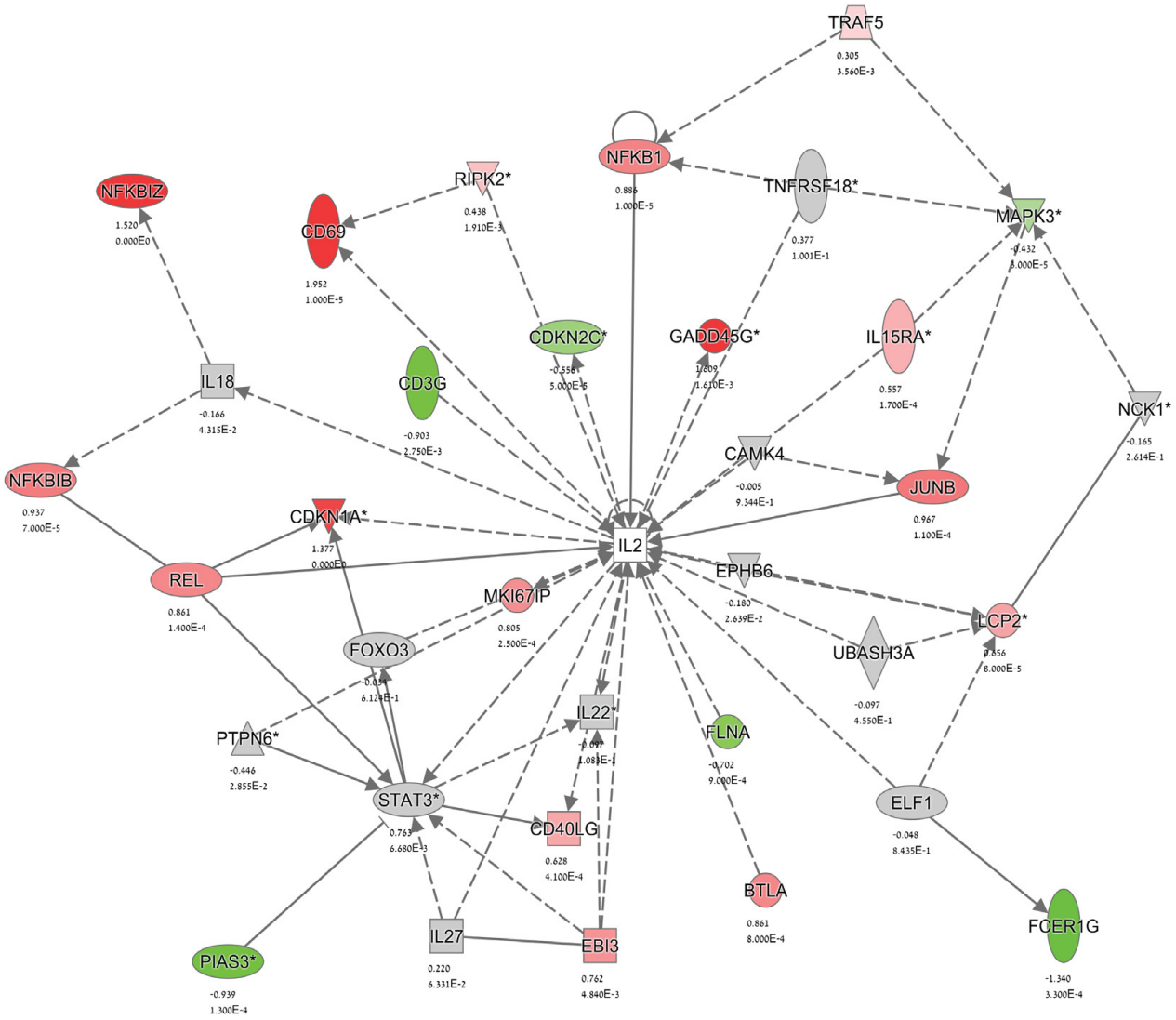

Figure 8: IPA-generated gene network identified IL-2 dependent gene expression to be highly involved in MOG35-55 activation of T_{MOG}.

patterns, including gene expression, were addressed using tissues isolated from EAE mice [28]. However, in these later cases the presence of other cell types in addition to the memory T cells in the whole tissue homogenates could have influenced the accuracy of the results obtained regarding the gene expression in the T cells of interest. We overcame above obstacles by utilizing pure antigen specific T cells which were incubated with the antigen in the presence of APC but were carefully purified afterwards before subjecting them to the mRNA preparation and gene transcription analysis.

We observed that 8 h of stimulation with MOG35-55 led to a dramatic pro-inflammatory activation of T_{MOG} cells manifested by increased transcription of cytokines, chemokines, growth factors and elements of pro-inflammatory signaling cascades. This result corresponds with the known profile of memory T cells defined by highly coordinated expression of array of cytokines, chemokines and transcription factors [29]. IPA analysis identified IFNγ, TNFα, IL-1, IL-2, IL-6, IL-12, IL-10, IL-15 signaling pathways as involved in MOG35-55 activation of T_{MOG}. This led to the identification of MAPK, NFkB, JAK/STAT and Jun pathways as the leading co-activated intracellular cascades in T_{MOG} activation in agreement with previous reports (for review see [30]).

Several "IL-17 signaling" pathways were recognized to be enriched in activated T_{MOG}, clearly indicating powerful Th17-like activation of T_{MOG}. Indeed, we previously showed that MOG35-55 stimulation leads to increased IL-17 mRNA production and to the release of IL-17 protein from T_{MOG} co-cultured with APC [16]. It is known that the generation of Th17 is mainly dependent on IL-6 and TGFβ activation and that Th17 encephalitogenicity is determined by co-expressed IL-1 and IL-23 [31]. Moreover, GM-CSF and IL-3 were recently shown to drive the activation of autoreactive T cells [32,33]. Indeed, all these cytokines, the relevant transcription factors and intracellular cascades (Tbx21, Stat4, Irf4) were recognized by our analysis to be highly activated following MOG35-55 stimulation. Annotations related to efficient antigen presentation and processing, including pathways activated in the T cells

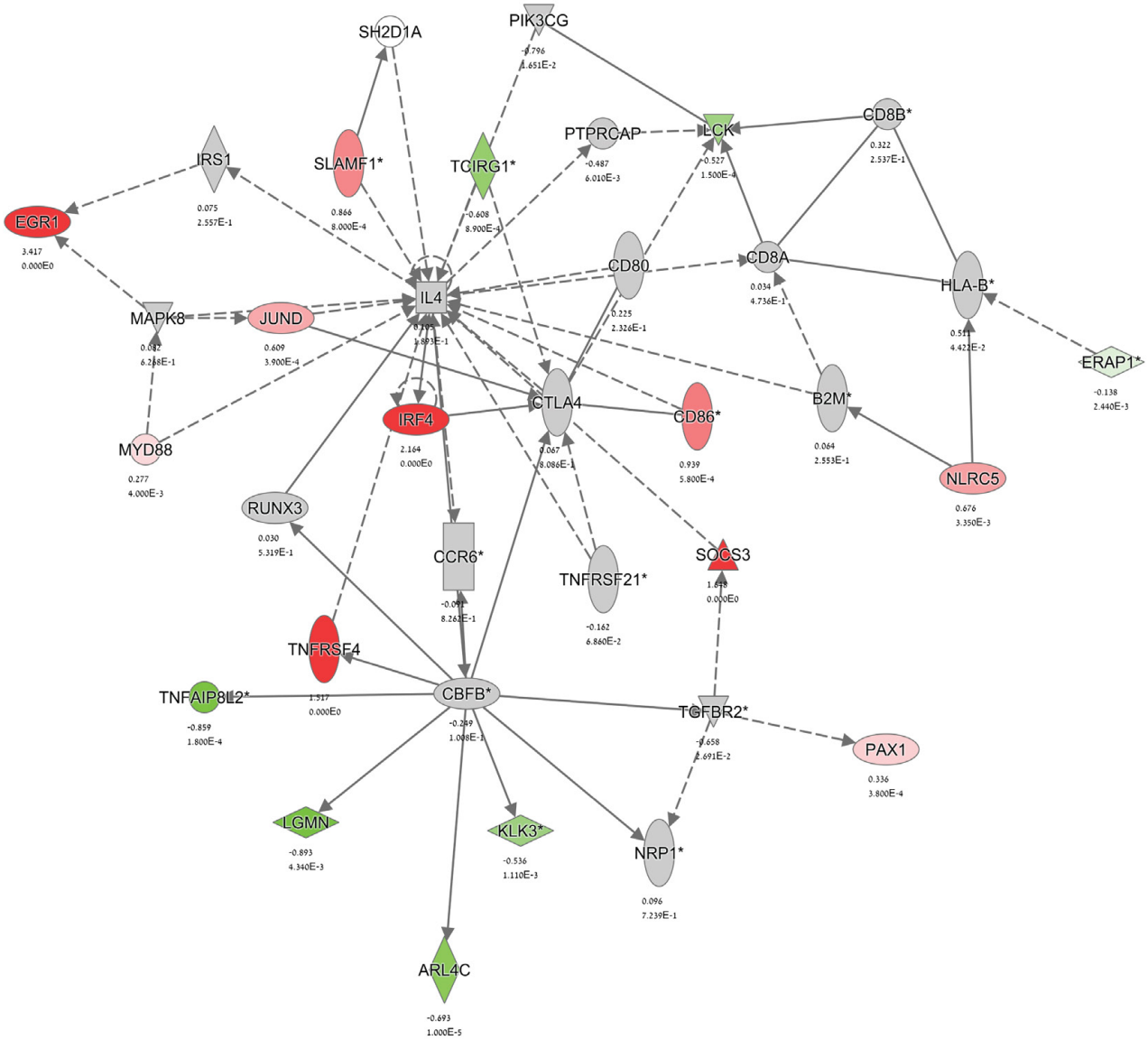

Figure 9: IPA-generated gene network showing MOG35-55 effect on anti-inflammatory pathways and genes.

by dendritic cells and B cells (both present in the APC population) were also recognized to be enriched (e.g., "Crosstalk between Dendritic and NK cells", "Altered T and B cell Signaling in Rheumatoid Arthritis", "B Cell Activating Factor Signaling", "Dendritic Cell Maturation"). These findings highlight the fact that MOG35-55-induced stimulation of T_{MOG} indeed comprises activation of both, (i) Th17 functional phenotype and (ii) antigen specific responses.

As described in Table 2 and the interactomes presented in Figures 8, 9 and S3, several transcripts of proteins with anti-inflammatory roles were down-regulated following MOG35-55 stimulation. These genes include Sit1, previously shown to negatively regulate TCR-mediated signaling in T-cells [34]; Hsd11b1, an enzyme participating in steroid biosynthetic pathways and shown to have restraining effects in MS-like demyelinations [35,36]. Pparg, well known for its anti-inflammatory role, including in MS-like pathologies [37]; Pias3, shown to serve as a potent suppressor of Th17 function [38]; Lgmn, a cysteine protease and a promoter of regulatory T cell function [39]; Klk3, exerting protective role in human systemic lupus erythematosus and lupus nephritis [40] and Tnfaip8l2 (TIPE2), a negative regulator of TLRs and T-cell receptor function, inhibiting JUN/AP1 and NFκB activation and promoting Fas-induced apoptosis [41]. Moreover, Tnfaip8l2, Lgmn and Klk3 gene products were shown by IPA to be directly regulated by CBFβ protein, a co-factor that (together with Runx1 or Runx3) binds to FoxP3 promoter and activates its regulatory function. Indeed, Cbfb-deleted regulatory T cells exhibit impaired suppressive function *in vitro* and *in vivo* with altered gene expression profiles including reduced expression of Tnfaip8l2, attenuated expression and activity of FoxP3 and high expression of IL-4, altogether resulting in autoimmunity [42,43]. These results imply that antigen–induced silencing of intrinsic inhibitory mechanisms may accompany memory T cell activation.

Interestingly, various IL-17 receptor transcripts (including Il17re, v1 and v2 and Il17rc) belong to the group of down-regulated genes following the stimulation with MOG35-55. IL-17RA and IL-17RC form a heteromeric complex in which IL-17RC is required for IL-17A

and IL-17F mediated signaling [44]. IL-17RC expression is low in haematopoetic tissues and is high in non-immune cells (e.g. prostate, liver, kidney, thyroid and joints). We hypothesize, that such down-regulation of IL-17R on T_{MOG} memory cells may protect these IL-17-releasing cells from their own cytotoxic activity induced by this cytokine, a mechanism that was suggested by Gaffen et al., [44]. We observed enhanced levels of the transcript of FoxP3, known to serve as a master regulator of suppressory transcription in autoimmune pathologies [45]. This is in agreement with a previous observation reporting that moderately increased FoxP3 activity transiently accompanies the activation of pathogenic T cells [46,47] while the classic regulatory T cell phenotypes are usually characterized by stable high levels of FoxP3 [48].

STAT5 was found by us to be slightly upregulated following MOG35-55 stimulation. STAT5 was recently described as a negative regulator of STAT3-dependent Th17 function [49]. Moreover, STAT5 seems to play diverse roles in T cell activation as it can also induce pro-proliferative IL-2 signals and is indispensable for the encephalitogenicity of autoreactive $CD4^+$ T cells via induction of pro-inflammatory GMCSF production [33]. Indeed, the levels of Csf2 mRNA (the gene transcript encoding GMCSF) were elevated in activated T_{MOG}, indicating that STAT5 may play a role in promoting the activation of T_{MOG}.

EGR2 is known as a main anergy regulator [50]. We found that Egr2 mRNA is up-regulated following MOG35-55 stimulation (see also [17]. This result suggests that feedback homeostatic processes may be initiated in parallel with T cell activation, tuning proper antigen activation of T_{MOG} as suggested by Miao et al. [51].

Interestingly, IPA identified certain cancer–related pathways to be enriched in activated TMOG. This may imply the induction of mechanisms regulating cell cycle and proliferation. Indeed, memory T cells respond to activating antigens by increased proliferation [13,52], a finding that was confirmed by us using T_{MOG} cells [15,18]. Accordingly, transcripts known for their pro-proliferative and anti-apoptotic roles in activated T cells e.g. regulator of cell cycle (Rgcc) and BCL2-Like 1 (Bcl2l1) [53] were upregulated and those with pro-apoptotic role were down-regulated in response to MOG35-55 stimulation, e.g. BCL2 binding component 3 (Bbc3). In agreement with this increase in anti-apoptotic gene products another study showed anti-apoptotic gene reprogramming (increase in anti-apoptotic Bcl2 activity) in quiescent MOG35-55 selected T cell line derived from MS patients long time after their last activation, thus suggesting that memory T cells possess reprogrammed gene function that contributes to their resiliency to apoptosis and prolonged survival [54].

Various cytokines involved in autoimmune processes, including TNFα, IFNγ and IL-4 found to be activated in our hands, play important roles in innate immunity as well, linking both types of immune responses. Our analysis revealed a significant role of innate immunity processes in the antigen activation of encephalitogenic T_{MOG} cells. This involved regulation of TLRs mRNAs (Tlr2, Tlr3, Tlr4, Tlr7) as well as activation of MyD88, a membrane-bound molecule initiating TLR-dependent signaling, mRNA. IPA analysis demonstrated substantial activation of IFNγ mRNA and of IFN-dependent pathways known to play important roles in innate immunity. Indeed, TLR-mediated activation of dendritic cells was shown to be critical for CD4+ and CD8+ T cells' effector functions [55]. Moreover, initiation of EAE requires functional MyD88-dependent signaling since MyD88-deficient mice are resistant to MOG-induced EAE [56,57]; for review see [58]). Indeed, Th17 cells are primarily activated in response to pathogen infections [59]). Our results indicate that these pathways are of high relevance not only at the initial phase of memory T cell differentiation but also during the re-activation of established antigen-specific T cell clones such as T_{MOG}. Thus, parallel targeting of both innate and adaptive processes may be of high relevance for restraining the pathogenicity of autoreactive T cells as suggested by Waldner et al. [58] and Mills et al. [60].

The present study reveals that the various MOG35-55-enriched pathways and gene networks are similar to the processes driving other T cell-dependent autoimmune pathologies including psoriasis, type 1 diabetes, systemic lupus erythematosus, rheumatoid arthritis and inflammatory bowel disease. Indeed, memory Th17 were shown to be involved in several autoimmune disorders including MS, psoriasis, rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus and asthma [59]. This similarity includes as well other cytokines, co-receptors, signaling pathways and transcriptional regulators accompanying the Th17 activity [59] and described in the present report. This strongly suggests that despite the high epitope-specificity of the T_{MOG} the mechanisms leading to autoimmune pathogenicity are shared by other types of autoreactive T cell lineages. Thus, using T_{MOG} cells *in vitro* should be useful for studying the mechanisms and pathways involved in other autoimmune diseases including those listed above. In addition, it should have significant predictive values in screening for therapeutic potentials of various materials not only for MS-like pathologies but for other T cell driven autoimmune diseases as well. We therefore suggest that the T_{MOG} system may serve as a reliable model for deciphering the pathways affected by potential therapeutics to diminish activity of autoimmune T cells.

Authors' contributions

EK and NK provided the know-how in T_{MOG} cell line culturing. AJ performed mRNA isolation. GC and FG performed the microarray data analysis. EK performed the biological experiments, carried out the IPA analysis and wrote the article. ZV critically reviewed the manuscript. All authors contributed to the discussions of the results and to reviewing and editing the manuscript.

Acknowledgments

The T_{MOG} cell line was kindly provided by the laboratory of Professor Avraham Ben-Nun from the Immunology Department at the Weizmann Institute of Science, Rehovot, Israel.

Funding

This work was supported by the Dr Miriam and Sheldon G. Adelson Medical Research Foundation. AJ is supported by the Israeli Ministry for Absorption in Science.

References

1. Sospedra M, Martin R (2005) Immunology of multiple sclerosis. Annu Rev Immunol 23: 683-747.
2. Burns J, Rosenzweig A, Zweiman B, Lisak RP (1983) Isolation of myelin basic protein-reactive T-cell lines from normal human blood. Cell Immunol 81: 435-440.
3. Van der AA, Hellings N, Bernard CC, Raus J, Stinissen P (2003) Functional properties of myelin oligodendrocyte glycoprotein-reactive T cells in multiple sclerosis patients and controls. J Neuroimmunol 137: 164-76.
4. Yura M, Takahashi I, Serada M, Koshio T, Nakagami K, et al. (2001) Role of MOG-stimulated Th1 type "light up" (GFP+) CD4+ T cells for the development of experimental autoimmune encephalomyelitis (EAE). J Autoimmun 17: 17-25.
5. Shevach EM (2011) Animal models for autoimmune and inflammatory diseases. In: Coligan JE, Kruisbeek AM, Margulies DH, Shevach EM, Strober W (eds) Current Protocols in Immunology. John Wiley & Sons, New York, pp. 15.0.1-15.0.5.
6. Podojil JR, Miller SD (2009) Molecular mechanisms of T-cell receptor and costimulatory molecule ligation/blockade in autoimmune disease therapy. Immunol Rev 229: 337-355.
7. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, et al. (2005)

Interleukin 17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type 1 and 2 lineages. Nat Immunol 6: 1123-1132.

8. Kebir H, Kreymborg K, Ifergan I, Dodelet-Devillers A, Cayrol R, et al. (2007) Human TH17 lymphocytes promote blood-brain barrier disruption and central nervous system inflammation. Nat Med 13: 1173-1175.
9. Laurence A, Tato CM, Davidson TS, Kanno Y, Chen Z, et al. (2007) Interleukin-2 signaling via STAT5 constrains T helper 17 cell generation. Immunity 26: 371-381.
10. Rostami A, Ciric B (2013) Role of Th17 cells in the pathogenesis of CNS inflammatory demyelination. J Neurol Sci 333: 76-87.
11. Okuda Y, Okuda M, Apatoff BR, Posnett DN (2005) The activation of memory CD4(+) T cells and CD8(+) T cells in patients with multiple sclerosis. J Neurol Sci 235: 11-17.
12. Aharoni R (2014) Immunomodulation neuroprotection and remyelination - the fundamental therapeutic effects of glatiramer acetate: a critical review. J Autoimmun 54: 81-92.
13. Ben-Nun A, Wekerle H, Cohen IR (1981) The rapid isolation of clonable antigen-specific T lymphocyte lines capable of mediating autoimmune encephalomyelitis. Eur J Immunol 11: 195–199.
14. Ben-Nun A, Cohen IR (1982) Experimental autoimmune encephalomyelitis (EAE) mediated by T cell lines: process of selection of lines and characterization of the cells. J Immunol 129: 303-308.
15. Kaushansky N, Zhong MC, Kerlero de Rosbo N, Hoeftberger R, Lassmann H, et al. (2006) Epitope specificity of autoreactive T and B cells associated with experimental autoimmune encephalomyelitis and optic neuritis induced by oligodendrocyte-specific protein in SJL/J mice. J Immunol 177: 7364-7376.
16. Kozela E, Juknat A, Kaushansky N, Rimmerman N, Ben-Nun A, et al. (2013) Cannabinoids decrease the th17 inflammatory autoimmune phenotype. J Neuroimmune Pharmacol 8: 1265-1276.
17. Kozela E, Juknat A, Kaushansky N, Ben-Nun A, Coppola G, et al. (2015) Cannabidiol, a non-psychoactive cannabinoid, leads to EGR2-dependent anergy in activated encephalitogenic T cells. J Neuroinflammation 12: 52.
18. Kozela E, Lev N, Kaushansky N, Eilam R, Rimmerman N, et al. (2011) Cannabidiol inhibits pathogenic T cells, decreases spinal microglial activation and ameliorates multiple sclerosis-like disease in C57BL/6 mice. Br J Pharmacol 163: 1507-1519.
19. Juknat A, Pietr M, Kozela E, Rimmerman N, Levy R, et al. (2013) Microarray and pathway analysis reveal distinct mechanisms underlying cannabinoid-mediated modulation of LPS-induced activation of BV-2 microglial cells. PLoS One 8: e61462
20. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, et al. (2004) Bioconductor: open software development for computational biology and bioinformatics. Genome Biol 5: R80.
21. Coppola G (2011) Designing, performing, and interpreting a microarray-based gene expression study. Methods Mol Biol 793: 417-439.
22. Kerlero de Rosbo N, Milo R, Lees MB, Burger D, Bernard CC, et al. (1993) Reactivity to myelin antigens in multiple sclerosis. Peripheral blood lymphocytes respond predominantly to myelin oligodendrocyte glycoprotein. J Clin Invest 92: 2602-2608.
23. Adelmann M, Wood J, Benzel I, Fiori P, Lassmann H, at al. (1995) The N-terminal domain of the myelin oligodendrocyte glycoprotein (MOG) induces acute demyelinating experimental autoimmune encephalomyelitis in the Lewis rat. J Neuroimmunol 63: 17-27.
24. Genain CP, Hauser SL (1996) Allergic Encephalomyelitis in Common Marmosets: Pathogenesis of a Multiple Sclerosis-like Lesion Methods 10: 420-434.
25. Iglesias A, Bauer J, Litzenburger T, Schubart A, Linington C (2001) T- and B-cell responses to myelin oligodendrocyte glycoprotein in experimental autoimmune encephalomyelitis and multiple sclerosis. Glia 36: 220-234.
26. Yosef N, Shalek AK, Gaublomme JT, Jin H, Lee Y, et al. (2013) Dynamic regulatory network controlling TH17 cell differentiation. Nature 496: 461-468.
27. Gaublomme JT, Yosef N, Lee Y, Gertner RS, Yang LV, et al. (2015) Single-Cell Genomics Unveils Critical Regulators of Th17 Cell Pathogenicity. Cell 163: 1400-1412.
28. Whitney LW, Ludwin SK, McFarland HF, Biddison WE (2001) Microarray analysis of gene expression in multiple sclerosis and EAE identifies 5-lipoxygenase as a component of inflammatory lesions. J Neuroimmunol 121: 40-48.
29. Olsson T (1995) Critical influences of the cytokine orchestration on the outcome of myelin antigen-specific T-cell autoimmunity in experimental autoimmune encephalomyelitis and multiple sclerosis. Immunol Rev 144: 245-268.
30. Zhu S, Qian Y (2012) IL-17/IL-17 receptor system in autoimmune disease: mechanisms and therapeutic potential. Clin Sci (Lond) 122: 487-511.
31. El-Behi M, Ciric B, Dai H, Yan Y, Cullimore M, et al. (2011) The encephalitogenicity of T(H)17 cells is dependent on IL-1- and IL-23-induced production of the cytokine GM-CSF. Nat Immunol 12: 568-575.
32. Codarri L, Gyülvészi G, Tosevski V, Hesske L, Fontana A, et al. (2011) RORÎ³t drives production of the cytokine GM-CSF in helper T cells, which is essential for the effector phase of autoimmune neuroinflammation. Nat Immunol 12: 560-567.
33. Sheng W, Yang F, Zhou Y, Yang H, Low PY, et al. (2014) STAT5 programs a distinct subset of GM-CSF-producing T helper cells that is essential for autoimmune neuroinflammation. Cell Res 24: 1387-1402.
34. Marie-Cardine A, Kirchgessner H, Bruyns E, Shevchenko A, Mann M, et al. (1999) SHP2-interacting transmembrane adaptor protein (SIT), a novel disulfide-linked dimer regulating human T cell activation. J Exp Med 189: 1181-1194.
35. Heidbrink C, Häusler SF, Buttmann M, Ossadnik M, Strik HM, et al. (2010) Reduced cortisol levels in cerebrospinal fluid and differential distribution of 11beta-hydroxysteroid dehydrogenases in multiple sclerosis: implications for lesion pathogenesis. Brain Behav Immun 24: 975-984.
36. Pareek TK, Belkadi A, Kesavapany S, Zaremba A, Loh SL, et al. (2011) Triterpenoid modulation of IL-17 and Nrf-2 expression ameliorates neuroinflammation and promotes remyelination in autoimmune encephalomyelitis. Sci Rep 1: 201
37. Drew PD, Xu J, Racke MK (2008) PPAR-gamma: Therapeutic Potential for Multiple Sclerosis. PPAR Res 2008: 627463.
38. Bixler SL, Sandler NG, Douek DC, Mattapallil JJ (2013) Suppressed Th17 levels correlate with elevated PIAS3, SHP2, and SOCS3 expression in CD4 T cells during acute simian immunodeficiency virus infection. J Virol 87: 7093-7101.
39. Probst-Kepper M, Geffers R, Kröger A, Viegas N, Erck C, et al. (2009) GARP: a key receptor controlling FOXP3 in human regulatory T cells. J Cell Mol Med 13: 3343-3357.
40. Ponticelli C, Meroni PL (2009) Kallikreins and lupus nephritis. J Clin Invest 119: 768-771.
41. Lou Y, Zhang G, Geng M, Zhang W, Cui J, et al. (2014) TIPE2 negatively regulates inflammation by switching arginine metabolism from nitric oxide synthase to arginase. PLoS One 9: e96508.
42. Kitoh A, Ono M, Naoe Y, Ohkura N, Yamaguchi T, et al. (2009) Indispensable role of the Runx1-Cbfbeta transcription complex for in vivo-suppressive function of FoxP3+ regulatory T cells. Immunity 31: 609-620.
43. Rudra D, Egawa T, Chong MM, Treuting P, Littman DR, et al. (2009) Runx-CBFbeta complexes control expression of the transcription factor Foxp3 in regulatory T cells. Nat Immunol 10: 1170-1177.
44. Gaffen SL (2009) Structure and signalling in the IL-17 receptor family. Nat Rev Immunol 9: 556-567.
45. Kleinewietfeld M, Hafler DA (2013) The plasticity of human Treg and Th17 cells and its role in autoimmunity. Semin Immunol 25: 305-312.
46. Wang J, Ioan-Facsinay A, van der Voort EI, Huizinga TW, Toes RE (2007) Transient expression of FOXP3 in human activated nonregulatory CD4+ T cells. Eur J Immunol 37: 129-138.
47. Tran DQ, Ramsey H, Shevach EM (2007) Induction of FOXP3 expression in naive human CD4+FOXP3 T cells by T-cell receptor stimulation is transforming growth factor-beta dependent but does not confer a regulatory phenotype. Blood 110: 2983-2990.
48. Sakaguchi S, Ono M, Setoguchi R, Yagi H, Hori S, et al. (2006) Foxp3+ CD25+ CD4+ natural regulatory T cells in dominant self-tolerance and autoimmune disease. Immunol Rev 212: 8-27.
49. Yang XP, Ghoreschi K, Steward-Tharp SM, Rodriguez-Canales J, Zhu J, et

al. (2011) Opposing regulation of the locus encoding IL-17 through direct, reciprocal actions of STAT3 and STAT5. Nat Immunol 12: 247-254.

50. Harris JE, Bishop KD, Phillips NE, Mordes JP, Greiner DL, et al. (2004) Early growth response gene-2, a zinc-finger transcription factor, is required for full induction of clonal anergy in CD4+ T cells. J Immunol 173: 7331-7338.

51. Miao T, Raymond M, Bhullar P, Ghaffari E, Symonds AL, et al. (2013) Early growth response gene-2 controls IL-17 expression and Th17 differentiation by negatively regulating Batf. J Immunol 190: 58-65.

52. Pape KA, Khoruts A, Mondino A, Jenkins MK (1997) Inflammatory cytokines enhance the in vivo clonal expansion and differentiation of antigen-activated CD4+ T cells. J Immunol 159: 591-598.

53. Tegla CA, Cudrici CD, Nguyen V, Danoff J, Kruszewski AM, et al. (2015) RGC-32 is a novel regulator of the T-lymphocyte cell cycle. Exp Mol Pathol 98: 328-337.

54. Mandel M, Gurevich M, Lavie G, Cohen IR, Achiron A (2005) Unique gene expression patterns in human T-cell lines generated from multiple sclerosis patients by stimulation with a synthetic MOG peptide. Clin Dev Immunol 12: 203-209.

55. Walsh KP, Mills KH (2013) Dendritic cells and other innate determinants of T helper cell polarisation. Trends Immunol 34: 521-530.

56. Prinz M, Garbe F, Schmidt H, Mildner A, Gutcher I, et al. (2006) Innate immunity mediated by TLR9 modulates pathogenicity in an animal model of multiple sclerosis. J Clin Invest 116: 456-464.

57. Marta M, Andersson A, Isaksson M, Kämpe O, Lobell A (2008) Unexpected regulatory roles of TLR4 and TLR9 in experimental autoimmune encephalomyelitis. Eur J Immunol 38: 565-575.

58. Waldner H (2009) The role of innate immune responses in autoimmune disease development. Autoimmun Rev 8: 400-404.

59. Bedoya SK, Lam B, Lau K, Larkin J 3rd (2013) Th17 cells in immunity and autoimmunity. Clin Dev Immunol 2013: 986789.

60. Mills KH (2011) TLR-dependent T cell activation in autoimmunity. Nat Rev Immunol 11: 807-822.