International Journal of Clinical Pharmacology and Therapeutics, Vol. 47 – No. 5/2009 (328-357)

ISSN 0946-1965

Whole genome analysis of the action of interferon-β

M.A. Kauffman[1], P. Yankilevich[1], P. Barrero[2], R. Bello[1], L. Marangunich[1], A. Vidal[1], M. Criscuolo[1], R.A. Diez[1] and A. Sterin Prync[1]

[1]Bio Sidus SA, Buenos Aires, Argentina, and [2]Laboratorio de Virología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina

Key words
interferon-β – multiple sclerosis – genomics – microarray – treatment

Abstract. Objectives: To characterize the IFNβ$_{1a}$-regulated gene expression on leukocytes of Multiple Sclerosis (MS) patients using microarrays with whole human genome representation. Methods: Genes differentially expressed by interferon-β were identified by a microarray in vitro study performed in leukocytes obtained from 5 MS relapsing-remitting patients. Results: Following the culture of peripheral blood mononuclear cells from MS relapsing-remitting patients for 24 hs with IFNβ$_{1a}$, the expression of 868 genes was modified: 545 increased (including CXCL11, CCL8, INDO, IFI27, CFB, CXCL10 and IFIT1) and 323 diminished (including RBP7, SEPT5, RNF8, ADORA2B and FOS). Conclusions: Since many of them were previously recognized as involved in MS pathogenesis, the IFNb$_{1a}$ mechanism of action could imply a compensatory regulation of systems deregulated in MS.

Introduction

Interferons were discovered in 1957 as the soluble substances mediating the phenomenon of viral growth interference in cell cultures [Isaacs and Lindenmann 1957]. Their antiproliferative and immunomodulatory actions were recognized later. Interferons have been studied extensively in multiple sclerosis (MS) for more than 20 years. As a consequence of the success of the drug in a few early pilot trials [Jacobs et al. 1987] and the confirmatory evidence of efficacy in large pivotal trials [PRISMS 1998, Sibley and Group 1993], recombinant interferons have been approved by the regulatory authorities of many countries for the treatment of relapsing-remitting MS.

The pleiotropic actions of interferon depend on an initial well-known intracellular cascade that includes the JAK/STAT/IRF complex [Takaoka and Yanai 2006] and the subsequent less-known transcriptional regulation of a large number of different genes [Rani and Ransohoff 2005]. The precise mechanism underlying the therapeutic effects of interferon-β (IFNβ) on MS remains to be fully elucidated. Furthermore, no single biochemical or genetic marker has been unequivocally linked to clinical or magnetic resonance imaging responses in individual patients.

DNA microarray technology is a novel technology that allows the simultaneous investigation of the expression of the whole genome. It has already given new insights into the complex pathophysiology of MS and their treatment. However, previous observations utilizing microarrays with partial representation of the human genome have not clearly demonstrated the molecular basis of complex biological effects of IFNβ in MS [Comabella and Martin 2007].

Our aim was to characterize the in vitro genomic effects of IFNβ on lymphocytes of multiple sclerosis patients using whole genome microarray assay.

Materials and methods

Patients, nonadherent mononuclear cells isolation and IFNβ$_{1a}$ treatment and RNA amplification, labeling and microarray hybridization

In vitro whole genome expression profiling of the effect of two preparations of IFNβ$_{1a}$ was performed on nonadherent mononuclear cells from 5 relapsing-remitting multiple sclerosis patients (RRMS, 2 males and 3 females, aged from 15 – 54 years) using micro-

Received
June 24, 2008;
accepted
December 17, 2008

Correspondence to
M.A. Kauffman,
MSc
Constitución 4234
(1254)
Buenos Aires, Argentina
m.kauffman@
biosidus.com.ar

arrays analysis (CodeLinkTM Human Whole Genome, GE Healthcare, Piscataway, NJ, USA). Data presented in this work are a complementary analysis of our previous work, therefore, a complete description of the patients' disease characteristics, as well as the procedures used for mononuclear cells isolation, IFNβ_{1a} treatment, RNA amplification, labeling and microarray hybridization has been published elsewhere [Sterin-Prync et al. 2008].

Bioinformatics and statistical data analysis

Raw data were imported into R statistical programming language environment, version 2.3.01. Graphic and statistical analysis was performed using Codelink and Linear Models for Microarray Data (LIMMA, 2.6.2) [Wettenhall and Smyth 2004] packages included in Bioconductor Project [Dudoit et al. 2003]. The preprocessing of the data sets to filter spots flagged as "bad", background correction and between microarrays normalization was done with Codelink package.

Log_2-fold change and p value of differential expression for each gene were obtained performing the following comparison: IFNβ_{1a} effect = (blastoferon plus rebif) minus control minus basal, i.e., differential gene expression due to IFNβ_{1a} effect, irrespective of pharmaceutical preparation used, since there are not genes differentially expressed between blastoferon and rebif [Sterin-Prync et al. 2008].

Differentially expressed genes were identified using an empirical Bayes-moderated t-test and ranked in order of evidence for differential expression with Limma. The empirical Bayes procedure provides an effective framework for studying the relative changes in gene expression for a large number of genes. It uses a simple nonparametric mixture prior to model the population of affected and unaffected genes, thereby avoiding parametric assumptions about gene expression [Efron and Tibshirani 2002]. The p values associated with the t-test were adjusted for multiple testing by using the Benjamini and Hochberg method [1990]. Thus, genes with an adjusted p value lower than 0.05 were identified as differentially expressed (DEG).

Ontology classification of differentially expressed genes

In order to categorize systematically the group of DEG, the classifying Panther system [Thomas et al. 2003] (Applied Biosystems, Foster City, CA, USA) was used. This system uses the binomial statistics to compare a list of genes under evaluation to a list of reference of the complete genome (NCBI: Homo sapiens genes) and determine statistically the functional groups of genes over-represented in the list under evaluation. These functional groups take their name from the biological process that characterizes each gene in the Panther system. Due to the multiple comparisons, the program corrects the p value by the Bonferroni method.

Gene set enrichment analysis

Gene set enrichment analysis (GSEA), a computational method that helps to rapidly connect gene expression with biology, was performed as described [Subramanian et al. 2005]. Our analysis used GSEA software version 2.0.1 and C2 curated 1,688 functional gene sets from the Molecular Signature Database (MSigDB) [Subramanian et al. 2005]. This analysis was performed on the full set of IFNβ_{1a} samples. The cumulative distribution function was constructed by performing 2,000 random gene set membership assignments. Gene sets satisfying the default multiple hypothesis testing threshold (false discovery rate, FDR, q value < 0.25) and having nominal p values lower than 0.05 were identified. Gene sets were ranked, first by consistent association with phenotype, then by FDR q value. This same GSEA analysis was performed on the C3 motif gene sets from MSigDB [Xie et al. 2005] which includes 837 gene sets representing different microRNA and transcription factors target genes. Finally, we built four additional gene sets based on previous publications on the effects of IFNβ, MS and experimental models of MS [Comabella and Martin 2007] to be analyzed and compared.

Confirmation by Real-Time RT-PCR

The expression of MX2, OAS2, GBP1 genes were independently quantified by RT2

Table 1a. Largest up-regulated genes (fold-change 10 or more).

Gene	GenBank accession	Description	Fold change	Adjusted p value
CXCL11	NM_005409	Chemokine (C-X-C motif) ligand 11	72.82	0.0003
CCL8	NM_005623	Chemokine (C-C motif) ligand 8	55.01	0.0025
INDO	NM_002164	Indoleamine-pyrrole 2,3 dioxygenase	36.64	0.0034
CFB	NM_001710	Complement factor B	31.40	0.0019
	NM_138397		28.68	0.0010
IFI27	NM_005532	Interferon, alpha-inducible protein 27	28.46	0.0032
IFI27	T47364	Interferon, alpha-inducible protein 27	27.63	0.0027
CXCL10	NM_001565	Chemokine (C-X-C motif) ligand 10	26.44	0.0029
RSAD2	NM_080657	Radical S-adenosyl methionine domain containing 2	22.04	0.0007
	CA425825	Transcribed locus	20.55	0.0000
	CA309689	Homo sapiens, clone IMAGE:4042735, mRNA	20.46	0.0004
IFIT1	NM_001548	Interferon-induced protein with tetratricopeptide repeats 1	17.77	0.0072
DEFB1	NM_005218	Defensin, beta 1	17.38	0.0089
	AW059912	Transcribed locus, strongly similar to XP_292184.4 PREDICTED: similar to immune-responsive gene 1 [Homo sapiens]	16.85	0.0008
APOBEC3A	NM_145699	Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A	16.52	0.0046
P2RY6	NM_004154	Pyrimidinergic receptor P2Y, G-protein coupled, 6	16.46	0.0306
KITLG	AK055903	KIT ligand	15.36	0.0036
	AI217164	Transcribed locus	13.86	0.0206
ETV7	NM_016135	Ets variant gene 7 (TEL2 oncogene)	13.83	0.0003
BCL2L14	NM_030766	BCL2-like 14 (apoptosis facilitator)	13.57	0.0028
EPSTI1	NM_033255	Epithelial stromal interaction 1 (breast)	13.51	0.0027
	NM_058184		12.66	0.0115
ISG15	NM_005101	ISG15 ubiquitin-like modifier	12.00	0.0028
IL1F9	NM_019618	Interleukin 1 family, member 9	11.95	0.0237
SERPING1	NM_000062	Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)	11.84	0.0036
	BF526295		11.82	0.0221
TCN2	NM_000355	Transcobalamin II; macrocytic anemia	11.68	0.0478
IFI44L	NM_006820	Interferon-induced protein 44-like	11.60	0.0113
IFIT2	NM_001547	Interferon-induced protein with tetratricopeptide repeats 2	11.29	0.0055
LAMP3	NM_014398	Lysosomal-associated membrane protein 3	11.17	0.0028
TNFSF10	NM_003810	Tumor necrosis factor (ligand) superfamily, member 10	11.09	0.0007
CCL15	NM_032964	Chemokine (C-C motif) ligand 15	10.97	0.0055
CXCL13	NM_006419	Chemokine (C-X-C motif) ligand 13 (B-cell chemoattractant)	10.83	0.0037
ADAMDEC1	NM_014479	ADAM-like, decysin 1	10.35	0.0041
CCL19	NM_006274	Chemokine (C-C motif) ligand 19	10.13	0.0193

Table 1b. Largest down-regulated genes (fold-change 5 or more).

Gene	GenBank accession	Description	Fold change	Adjusted p value
LOC646533	H95091	Hypothetical protein LOC646533	12.68	0.0046
RBP7	NM_052960	Retinol binding protein 7, cellular	11.27	0.0171
	BF445387		10.14	0.0423
LOC338758	BC040326	Hypothetical protein LOC338758	9.62	0.0218
FBP1	NM_000507	Fructose-1,6-bisphosphatase 1	9.13	0.0029
SEPT5	NM_000407	Septin 5	8.27	0.0287
RNF8	NM_003958	Ring finger protein 8	7.66	0.0434
FBP1	NM_000507	Fructose-1,6-bisphosphatase 1	7.61	0.0028
ADORA2B	NM_000676	Adenosine A2b receptor	7.49	0.0063
FOS	NM_005252	V-fos FBJ murine osteosarcoma viral oncogene homolog	7.24	0.0434
C21orf67	AK127059	Chromosome 21 open reading frame 67	7.18	0.0004
CDA	NM_001785	Cytidine deaminase	6.87	0.0115
FCER2	NM_002002	Fc fragment of IgE, low affinity II, receptor for (CD23)	6.66	0.0028
CCL20	NM_004591	Chemokine (C-C motif) ligand 20	6.49	0.0201
AREG	NM_001657	Amphiregulin (schwannoma-derived growth factor)	6.48	0.0254
CMTM2	NM_144673	CKLF-like MARVEL transmembrane domain containing 2	6.23	0.0179
PRAM1	NM_032152	PML-RARA regulated adaptor molecule 1	6.14	0.0021
	AK127644	CDNA FLJ45742 fis, clone KIDNE2016327	5.94	0.0007
S100A8	NM_002964	S100 calcium binding protein A8 (calgranulin A)	5.84	0.0048
PCDH9	BC042366	Protocadherin 9	5.68	0.0040
ITGAM	NM_000632	Integrin, alpha M (complement component 3 receptor 3 subunit)	5.64	0.0197
S100A4	NM_002961	S100 calcium binding protein A4 (calcium protein, calvasculin, metastasin, murine placental homolog)	5.52	0.0104
GALNAC4S-6ST	BE552243	B cell RAG associated protein	5.39	0.0186
	BQ182533	Transcribed locus	5.39	0.0126
PPBP	NM_002704	Pro-platelet basic protein (chemokine (C-X-C motif) ligand 7)	5.09	0.0225
118 NRGN	NM_006176	Neurogranin (protein kinase C substrate, RC3)	5.01	0.0216

Real-Time® RT-PCR assays (SuperArray Bioscience Corporation, Frederick, MD, USA) using the SYBR® Green (Molecular Probes Inc, Eugene, OR, USA) detection method with high quality gene-specific PCR primer sets and master mixes for microarray data validation. Briefly, 1.5 μg of template was annealed with random hexamers before the reverse transcription step and the first strand cDNA reaction (1 μl) served as template for Real-Time® PCR Assay for each gene. The 3-step PCR cycling followed by melting curve program was assayed and the ΔΔCt method was used to calculate the relative values of gene expression.

Results

Differential interferon-induced gene expression

Out of the 53,877 oligonucleotide sequences on the array, 868 were identified as differentially expressed after the interferon treatment, among which 60% were upregulated. Table 1 shows the genes with the largest responses, showing only those with a positive fold-change of 10 or more and a negative fold-change of 5 or more (Tables 1a,b, respectively).

Table 2. Gene sets enriched in interferon-β-treated samples. GSEA analysis.

N°	Name	Size	FDR q-val
1	IFNA_HCMV_6HRS_UP	41	0,00
2	SANA_IFNG_ENDOTHELIAL_UP	57	0,00
3	IFNA_UV-CMV_COMMON_HCMV_6HRS_UP	22	0,00
4	TAKEDA_NUP8_HOXA9_3D_UP	160	0,00
5	RADAEVA_IFNA_UP	40	0,00
6	SANA_TNFA_ENDOTHELIAL_UP	64	0,00
7	TAKEDA_NUP8_HOXA9_8D_UP	127	0,00
8	DER_IFNA_UP	53	0,00
9	BRCA_BRCA1_POS	85	0,00
10	IFNALPHA_NL_UP	22	0,00
11	ROTH_HTERT_UP	13	0,00
12	WIELAND_HEPATITIS_B_INDUCED	80	0,00
13	DER_IFNB_UP	78	0,00
14	LINDSTEDT_DEND_UP	39	0,00
15	IFN_ANY_UP	71	0,00
16	BENNETT_SLE_UP	19	0,00
17	TAKEDA_NUP8_HOXA9_10D_UP	148	0,00
18	CMV_8HRS_UP	27	0,00
19	CMV_ALL_UP	74	0,00
20	CMV-UV_HCMV_6HRS_UP	103	0,00
21	GRANDVAUX_IFN_NOT_IRF3_UP	13	0,00
22	IFN_BETA_UP	56	0,00
23	IFN_ALPHA_UP	33	0,00
24	CMV_HCMV_TIMECOURSE_12HRS_UP	19	0,00
25	IFNALPHA_HCC_UP	24	0,00
26	GRANDVAUX_IRF3_UP	12	0,00
27	UV-CMV_UNIQUE_HCMV_6HRS_UP	87	0,00
28	NF90_UP	22	0,01
29	APPEL_IMATINIB_UP	30	0,01
30	RIBAVIRIN_RSV_UP	18	0,01
31	LEE_MYC_E2F1_UP	54	0,01
32	CMV_24HRS_UP	55	0,02
33	DER_IFNG_UP	52	0,02
34	LINDSTEDT_DEND_8H_VS_48H_DN	58	0,02
35	IFNALPHA_NL_HCC_UP	14	0,02
36	ZHAN_MM_CD138_HP_VS_REST	26	0,03
37	REOVIRUS_HEK293_UP	200	0,03
38	DAC_FIBRO_UP	16	0,03
39	JISON_SICKLE_CELL	26	0,04
40	CMV_HCMV_6HRS_UP	19	0,04
41	TAKEDA_NUP8_HOXA9_16D_UP	128	0,06

Ontology analysis of differentially expressed genes

To categorize systematically DEG into functional groups, we applied Panther ontology classification system. The list of differentially upregulated genes showed an overrepresentation of several ontology/functional groups that included biological processes involved in innate and acquired immunity, apoptosis, signal transduction, cell signaling and nucleotide metabolism, as well as molecular functions involved in protein and nucleotides metabolism and the protein ubiquitin ligase pathway. Some of these biological processes were also overrepresented among the downregulated genes as well as inflammation mediated by chemokine and cytokine signaling pathway, immunoglobulin receptor family member, and molecular functions involved in calcium and calmodulin signaling pathways.

Pathway-level analysis of differentially expressed genes

In order to understand the pathways associated with interferon response in lymphocytes, we performed a pathway-level analysis. This is based on the premise that subtle coordinated changes in gene expression levels across the whole network of genes within a certain pathway can indeed have major effects on the pathway. To find such coordinated shifts of gene expression values, we applied the GSEA pathway-level algorithms that are based on the usage of the whole array data set. The application of this bioinformatic tool revealed the enrichment of 136 different functional gene sets. The most represented upregulated gene sets include genes mostly involved in apoptosis, acquired and innate immunity, interferon-α and β action under different conditions, dendritic cell activation and endothelial cell activation. Supplementary Table 2 shows the gene sets significantly enriched at FDR < 25% in the interferon-treated samples. To further illustrate this approach, Supplementary Tables 3 and 4 show list of genes contained by two of those enrichment gene sets: “IFNA HCMV 6hs UP”, and “Lindsted Dendritic up”, with the fold change obtained for each gene in our assay. As shown, both lists include some of the genes

Table 2. Part II.

N°	Name	Size	FDR q-val
42	KIM_TH_CELLS_UP	42	0,07
43	IFN_GAMMA_UP	34	0,08
44	RUTELLA_HEPATGFSNDCS_UP	131	0,07
45	TGFBETA_C3_UP	12	0,07
46	MOREAUX_TACI_HI_VS_LOW_DN	132	0,08
47	XU_ATRA_PLUSNSC_UP	13	0,09
48	LINDSTEDT_DEND_8H_VS_48H_UP	56	0,09
49	CMV_UV-CMV_COMMON_HCMV_6HRS_UP	17	0,09
50	IFN_ALL_UP	16	0,09
51	BECKER_TAMOXIFEN_RESISTANT_UP	33	0,09
52	MOREAUX_TACI_HI_IN_PPC_UP	58	0,11
53	GREENBAUM_E2A_DN	14	0,11
54	RUTELLA_HEMATOGFSNDCS_DIFF	533	0,12
55	CMV_HCMV_TIMECOURSE_ALL_UP	387	0,12

with the largest-fold changes. On the other hand, Supplementary Table 5 shows the groups significantly enriched at FDR < 25% in the noninterferon-treated samples, and Supplementary Tables 6 and 7 present the list of genes contained by two of those enrichment gene sets "Ferrando T cell differentiation pathway", and Lindsted Dendritic Down" with the fold change obtained for each gene in our assay. These sets include some of the genes with the largest negative fold changes.

Transcription factor-level analysis of differentially expressed genes

We applied the GSEA transcription factor-level algorithms to identify the transcription factors potentially mediating the regulation of the different genes by interferon. We only found the enrichment of sets that are formed by genes with known binding sites for STAT1, STAT2, IRF1, IRF7, IRF2 and IRF10. Some of these transcription factors are well known interferon responsive genes.

Multiple sclerosis-relevant genes analysis

We also performed a multiple sclerosis-relevant genes analysis. This analysis was based on gene sets built from the review by Comabella and Martin [2007] and reveals that 3 out of 4 groups are enriched at FDR < 25% in the interferon-treated samples. These groups were "differentially expressed genes identified in at least two independent microarray studies evaluating the in vitro and/or ex vivo effects of IFN-β in MS", "differentially expressed genes identified in at least three independent microarray EAE studies", and "differentially expressed genes identified in at least two independent microarrays studies using peripheral blood cells in MS". On the other hand, "Multiple Sclerosis genes in at least 2 independent microarray studies using brain tissue" was not enriched (data not shown). Supplementary Tables 8, 9 and 10 present the genes included in each of those groups with the fold change obtained in our study. An almost perfect correlation (κ 0.9) was found between interferon-regulated genes as reported in previous studies and ours. It is worth mentioning that, Supplementary Tables 9 and 10, which include genes consistently deregulated in MS, show that in our assay interferon produced an opposite change in the expression of almost 40% of those genes (bold type in Tables).

Discussion

We have explored the effect of IFNβ_{1a} in leukocytes of relapsing-remitting MS patients by means of a whole genome microarray assay. Under these conditions, an important number of genes were upregulated in response to the treatment with IFNβ_{1a}, whereas a markedly smaller number were down. Other studies that used similar methodologies, employing a partial genome representation, showed changes proportionally comparable to those described in this study [Satoh et al. 2006, Weinstock-Guttman et al. 2003].

Interferon-β is a pleiotropic molecule modestly effective in the treatment of MS [Sibley and Group 1993]. In this report, about 40% of the genes relevantly modified in MS are changed in the opposite sense by IFNβ suggesting a compensatory action. Among them, we found a marked down-regulation of IL1A and IL1B, the integrins ITGAM and ITGB2 the growth factor PBEF1, CSF1R and fibronectin. At diagnosis, several IFN-re-

Table 3. Genes included in IFNA HCMV 6HRS UP gene set with corresponding fold changes obtained in our experiment.

Probe	Rank in gene list	Core enrichment	Fold change	GenBank accession
KIF5C	1284	No	–1,32	NM_004522
RGL1	3614	No	6,52	NM_015149
TLR3	6026	No	–1,09	NM_003265
RABGAP1L	6204	No	1,82	BX107100
MAP2	6305	No	–1,35	AI123529
STX11	8595	No	–1,48	BF431882
C1ORF38	9028	No	1,02	NM_004848
SAMHD1	9546	No	1,15	NM_015474
PSCD1	12141	No	1,31	NM_017456
MYD88	14167	No	1,45	NM_002468
MELK	14416	No	1,24	NM_014791
GBP2	14511	No	1,86	NM_004120
PSMB9	15432	Yes	2,43	NM_002800
UBE2L6	15602	Yes	2,93	NM_198183
TRIM14	15676	Yes	1,58	NM_033221
BAZ1A	15860	Yes	2,27	NM_013448
WARS	15914	Yes	2,19	NM_004184
IRF7	16017	Yes	3,93	NM_004031
ECGF1	16019	Yes	2,77	NM_001953
TREX1	16061	Yes	3,5	NM_016381
NMI	16081	Yes	3,29	NM_004688
GMPR	16082	Yes	2,52	M24470
STAT1	16099	Yes	4,19	NM_007315
ISG20	16127	Yes	4,67	NM_002201
PLSCR1	16128	Yes	4,58	NM_021105
IFIT5	16194	Yes	2,65	NM_012420
IL15	16205	Yes	3,56	NM_000585
PML	16267	Yes	3,05	BQ000334
MX2	16294	Yes	5,05	NM_002463
TRIM22	16316	Yes	–1,21	AK122668
TRIM21	16329	Yes	3,63	NM_003141
IFIT3	16361	Yes	8,09	NM_001549
MX1	16392	Yes	9,12	NM_002462
IFI44L	16406	Yes	11,6	NM_006820
IL15RA	16438	Yes	7,14	NM_002189
GCH1	16444	Yes	5,67	NM_000161
TNFSF10	16449	Yes	11,09	NM_003810
CCL8	16465	Yes	55,01	NM_005623
RSAD2	16467	Yes	22,04	NM_080657
INDO	16470	Yes	36,64	NM_002164
IFI27	16471	Yes	28,46	NM_005532

Footnotes for supplementary Tables 3, 4, 6 and 7:

In order to understand the pathways associated with interferon response in lymphocytes, we performed a pathway-level analysis. This is based on the premise that subtle coordinated changes in gene expression levels across the whole network of genes within a certain pathway can indeed have major effects on the pathway. To find such coordinated shifts of gene expression values, we applied the GSEA pathway-level algorithms that are based on the usage of the whole array data set. This table shows the genes included in one of the functional sets differentially expressed in our experiment.

sponsive genes have been found modified in peripheral blood of MS patients [van Baarsen et al. 2006], so, genes relevant for IFN therapeutic effect probably include more than those defined as modified in a compensatory-manner by IFN in this study.

It should be mentioned that the observations described herein are made on a small sample of heterogeneous patients, for which, however, adequate statistical methods were employed. Observations on a much larger homogeneous population may be worthwhile. The rather prolonged 24-h incubation with interferon was selected intentionally to measure major changes in genetic expression, although it might have failed to detect transient early or yet later changes. Since IFNβ was present throughout the period of culture, it is highly probable that most genes regulated by this cytokine would show persistent changes after 24 hs of IFNβ exposure [Weinstock-Guttman et al. 2003], an exposure time frequently used in the evaluation of IFNβ. In addition, the method used for lymphocyte enrichment is the one commonly employed, but does not necessarily allow complete purification or selection of the final cell population. Taking into consideration these possible limitations, we were able to identify about 95% of the changes reported for IFNβ-regulated genes in similar studies [Comabella and Martin 2007]. The analysis of pathway levels shows that the most conspicuous gene sets regulated by IFNβ were formed by well-known IFNβ-responsive genes. Indeed, many of the genes differentially expressed possess binding sites for IFNβ-responsive transcription factors.

Cytokines regulate all phases of the immune response acting in paracrine and auto-

Table 3. Part II.

Description
Kinesin family member 5C
Ral guanine nucleotide dissociation stimulator-like 1
Toll-like receptor 3
RAB GTPase activating protein 1-like
Microtubule-associated protein 2
Syntaxin 11
Chromosome 1 open reading frame 38
SAM domain and HD domain 1
Pleckstrin homology, Sec7 and coiled-coil domains 1(cytohesin 1)
Myeloid differentiation primary response gene (88)
Maternal embryonic leucine zipper kinase
Guanylate binding protein 2, interferon-inducible
Proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)
Ubiquitin-conjugating enzyme E2L 6
Tripartite motif-containing 14
Bromodomain adjacent to zinc finger domain, 1A
Tryptophanyl-tRNA synthetase
Interferon regulatory factor 7
Endothelial cell growth factor 1 (platelet-derived)
Three prime repair exonuclease 1
N-myc (and STAT) interactor
Guanosine monophosphate reductase
Signal transducer and activator of transcription 1, 91kDa
Interferon stimulated exonuclease gene 20kDa
Phospholipid scramblase 1
Interferon-induced protein with tetratricopeptide repeats 5
Interleukin 15
Promyelocytic leukemia
Myxovirus (influenza virus) resistance 2 (mouse)
Tripartite motif-containing 22
Tripartite motif-containing 21
Interferon-induced protein with tetratricopeptide repeats 3
Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)
Interferon-induced protein 44-like
Interleukin 15 receptor, alpha
GTP cyclohydrolase 1 (dopa-responsive dystonia)
Tumor necrosis factor (ligand) superfamily, member 10
Chemokine (C-C motif) ligand 8
Radical S-adenosyl methionine domain containing 2
Indoleamine-pyrrole 2,3 dioxygenase
Interferon, alpha-inducible protein 27

crine networks of great complexity [Charo and Ransohoff 2006]. Homeostasis is maintained when pro- and antiinflammatory signals are balanced. A loss of this delicate balance is present in MS [Krumbholz et al. 2006]. Interestingly, several of the observed changes in cytokine genes expression appear to be compensatory, as was previously reported as being deregulated in microarray studies of MS [Comabella and Martin 2007]. For example, the production of IL-23 is increased in dendritic cells of MS patients [Vaknin-Dembinsky et al. 2006] and the blocking of this cytokine has been effective in animal models of MS [Chen et al. 2006]. In our study, in vitro treatment with IFNβ_{1a} reduced 3-fold the expression of this cytokine. When expressed in astrocytes, the chemokine CCL20 regulates the migration of immature dendritic cells into the CNS [Ambrosini et al. 2005], moreover, in MS patients' PBMC, high levels of CCL20 have been found [Furlan et al. 2005]. In our assay, a 6-fold downregulation of CCL20 was observed, suggesting a possible mechanism of therapeutic action of IFNβ_{1a}.

Complement activation and particularly terminal complex assembly plays an important role in MS pathogenesis [Rus et al. 2005]; sublytic levels of this complex could prevent apoptosis of oligodendrocytes [Cudrici et al. 2006]. IFNβ_{1a} increased the expression of CD59, which is a relevant regulator of the complement system by blocking its assembly [Mead et al. 2004], this finding also could be of importance in the action of IFNβ_{1a}. During periods of disease remission, MS patients express increased levels of IL-10 [Perrella et al. 2006], an interleukin that induces hyporesponsiveness of T cells through upregulation of LILRB2 [Beinhauer et al. 2004]. Our observation of a 5-fold increase in the expression of LILRB2 could illustrate another immunomodulatory mechanism of IFNβ_{1a}. Further studies should evaluate whether a transient early expression of IL-10 was not detected at 24 h.

The enrichment of dendritic cells gene set between both up- and down-regulated genes is meaningful because of the role of these cells in the regulation of immune response. Lopez et al. [2006] proposed that IFNβ promotes a Th2 environment in MS patients by both increasing the survival of plasmacytoid

Table 4. Genes included in Lindsted dendritic-up gene set with corresponding fold changes obtained in our experiment.

Probe	Rank in gene list	Core enrich-ment	Fold change	GenBank accession
GADD45A	1327	No	–1,79	NM_001924
KLF5	1345	No	–1,44	CB306177
TRAF1	1577	No	2,87	NM_005658
LAMB3	1770	No	–1,34	NM_000228
TNFAIP3	3228	No	2,28	NM_024873
NFKBIA	4726	No	–1,51	NM_020529
HIVEP1	7168	No	–2	BF982800
CXCR4	7268	No	–1,09	NM_003467
SIAH2	9483	No	1,04	NM_005067
PNRC1	9621	No	1,07	NM_006813
USP12	9860	No	–1,36	AI218190
BTG1	9984	No	–3,32	BC009050
NFKB1	11767	No	1,65	BX091379
LYN	11886	No	1,85	NM_002350
NR4A3	12148	No	2,83	NM_006981
REL	12362	No		
CFLAR	12716	No	2,42	NM_003879
MARCKSL1	12793	No	1,15	NM_023009
PSCDBP	12872	No	1,32	NM_004288
PLA2G6	13708	No	–1,87	AI472855
STK4	14077	Yes	1,61	NM_006282
ADM	14363	Yes	1,6	NM_001124
IL7R	14616	Yes	2,02	NM_002185
PSME2	14676	Yes	2,14	NM_002818
BIRC3	14862	Yes	1,76	NM_001165
RELB	14902	Yes	1,66	NM_006509
TNFRSF9	15075	Yes	2,92	AK125490
TNFAIP2	15409	Yes	1,75	NM_006291
PALM2-AKAP2	15736	Yes	2,78	NM_053016
CSF2RA	15871	Yes	2,48	NM_006140
CD83	15951	Yes	2,75	NM_004233
EED	16029	Yes	2,61	NM_003797
OPTN	16106	Yes	2,57	D54580
TAP1	16266	Yes	4,12	NM_000593
MARCKS	16320	Yes	1,91	NM_002356
LAMP3	16428	Yes	11,17	NM_014398
IL15RA	16438	Yes	7,14	NM_002189
IL2RA	16442	Yes	4,77	NM_000417
CD80	16473	Yes	7,42	NM_005191

dendritic cells (DC2) and decreasing the number of circulating myeloid dendritic cells (DC1) [Lopez et al. 2006]. Our results show the upregulation of the DC2 marker TLR7 [Liu et al. 2001] (4-fold increase) and the downregulation of the DC1 marker CD_{1a} (3-fold decrease). Moreover, dendritic cells are key players in T cell activation mechanisms [Lee and Iwasaki 2007]. T cell activation is a tightly regulated event, involving complex receptor-ligand interactions, ultimately leading to downstream signaling events. Optimal activation of naVve T cells requires at least two signals, antigen recognition and co-stimulation. Signals provided through CD28-CD80/CD86 interactions are essential for initial naVve T cell activation leading to increased IL-2 production and IL-2Ra (CD25) expression [Howard et al. 2005]. T cell activation generally incorporates a self-limiting mechanism, such as inhibitory co-stimulators to regulate T cell tolerance and attenuate the immune response. The expanding set of inhibitory co-stimulators currently includes CD152 (CTLA-4), PD-1, and BTLA [Bandyopadhyay et al. 2007, Schreiner et al. 2004]. The outcome of an immune response likely depends on the balance between these positive and negative signals. We found remarkable effects of $IFN\beta_{1a}$ on some co-stimulatory genes. CD80 was markedly up-regulated by $IFN\beta_{1a}$ (7.4-fold increase) and since a Th2 environment is favored by CD80 signaling, this could be another important mechanism of action of IFNβ in MS. Furthermore, we found the positive regulation of two of the three negative co-stimulators, such as CTLA-4 and PD-1. Since autoimmunity results from an imbalance in the regulatory mechanisms involved in the control of autoreactive T clones, IFNβ could be effective in modulating this process. Nevertheless, a recent study that investigated the in vivo short-term gene induction in response to a single dose of $IFN\beta_{1b}$ have not found a clear shift to a Th2 environment. However, some of the genes, more extensively regulated in this study, had a similar behavior in our experiment, such as IFIT3, IFIT2, OAS1, CCL8 and CXCL10 [Reder et al. 2008].

We found INDO, the gene coding for indoleamine-pyrrole 2,3 dioxygenase (IDO) remarkably up-regulated with a 31-fold change

Table 4. Part II.

Description
Growth arrest and DNA-damage-inducible, alpha
Kruppel-like factor 5 (intestinal)
TNF receptor-associated factor 1
Laminin, beta 3
TNFAIP3 interacting protein 3
Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
Human immunodeficiency virus type I enhancer binding protein 1
Chemokine (C-X-C motif) receptor 4
Seven in absentia homolog 2 (Drosophila)
Proline-rich nuclear receptor coactivator 1
Ubiquitin specific peptidase 12
B-cell translocation gene 1, anti-proliferative
Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)
V-yes-1 Yamaguchi sarcoma viral related oncogene homolog
Nuclear receptor subfamily 4, group A, member 3
CASP8 and FADD-like apoptosis regulator
MARCKS-like 1
Pleckstrin homology, Sec7 and coiled-coil domains, binding protein
Phospholipase A2, group VI (cytosolic, calcium-independent)
Serine/threonine kinase 4
Adrenomedullin
Interleukin 7 receptor
Proteasome (prosome, macropain) activator subunit 2 (PA28 beta)
Baculoviral IAP repeat-containing 3
V-rel reticuloendotheliosis viral oncogene homolog B, nuclear factor of kappa light polypeptide gene enhancer in B-cells 3 (avian)
Tumor necrosis factor receptor superfamily, member 9
Tumor necrosis factor, alpha-induced protein 2
PALM2-AKAP2 protein
Colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)
CD83 molecule
Embryonic ectoderm development
Optineurin
Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)
Myristoylated alanine-rich protein kinase C substrate
Lysosomal-associated membrane protein 3
Interleukin 15 receptor, alpha
Interleukin 2 receptor, alpha
CD80 molecule

in response to IFNβ treatment. IDO is a key enzyme, acting as the first step in a metabolic pathway that degrades tryptophan. Sakurai et al. [2002] have shown that IDO, by both tryptophan depletion and metabolites, contributes to the remission phase of experimental autoimmune encephalitis and decreases its severity [Sakurai et al. 2002]. Moreover, therapeutic targeting of IDO tolerance induction has been suggested [Penberthy 2007, Platten et al. 2005]. Furthermore, during pregnancy, IDO is also increased and contributes to fetus tolerance [Zhu et al. 2007]. Interestingly, the relapse rate of MS decreases during pregnancy, mainly in the third trimester [Vukusic et al. 2004]. Thus, the up-regulation of INDO may contribute to the effect of IFNβ.

Conclusions

In summary, the RNA of non-adherent cells of MS patients stimulated in vitro by IFN β_{1a} was analyzed in whole genome microarray. We have expanded the list of IFNβ-responsive genes in leukocytes of MS patients, providing the largest catalogue available. Noteworthy changes in gene expression, due to IFNβ treatment described herein, appear to be compensatory to many previously described abnormalities found in MS patients, reinforcing the hypothesis that IFNβ counter-regulates the perturbed immune response of these patients. A previously unrecognized role is suggested for the regulation of dendritic cells by IFNβ as well as for INDO induction and the regulation of co-stimulatory signals. Further studies are required to test these hypotheses.

Acknowledgments

The authors wish to thank the patients for their collaboration, Mariana Papouchado, PhD, and Mrs. Virginia Tavernese for skillful technical assistance, and Mrs. Miriam Denegri for assistance in the English version of the text. PRB is a member of the Research Career of CONICET (Argentina National Research Council).

This study has been sponsored by Bio Sidus S.A.

References

Ambrosini E, Remoli ME, Giacomini E, Rosicarelli B, Serafini B, Lande R, Aloisi F, Coccia EM. Astrocytes produce dendritic cell-attracting chemokines in vitro and in multiple sclerosis lesions. J Neuropathol Exp Neurol. 2005; *64:* 706-715.

Bandyopadhyay G, De A, Laudanski K, Li F, Lentz C, Bankey P, Miller-Graziano C. Negative signaling contributes to T cell anergy in trauma patients. Crit Care Med. 2007; *35:* 794-801.

Beinhauer BG, McBride JM, Graf P, Pursch E, Bongers M, Rogy M, Korthauer U, de Vries JE, Aversa G, Jung T. Interleukin 10 regulates cell surface and soluble LIR-2 (CD85d) expression on dendritic cells resulting in T cell hyporesponsiveness in vitro. Eur J Immunol. 2004; *34:* 74-80.

Charo IF, Ransohoff RM. The many roles of chemokines and chemokine receptors in inflammation. N Engl J Med. 2006; *354:* 610-621.

Chen Y, Langrish CL, McKenzie B, Joyce-Shaikh B, Stumhofer JS, McClanahan T, Blumenschein W, Churakovsa T, Low J, Presta L, Hunter CA, Kastelein RA, Cua DJ. Anti-IL-23 therapy inhibits multiple inflammatory pathways and ameliorates autoimmune encephalomyelitis. J Clin Invest. 2006; *116:* 1317-1326.

Comabella M, Martin R. Genomics in multiple sclerosis-Current state and future directions. J Neuroimmunol. 2007; *187:* 1-8.

Cudrici C, Niculescu F, Jensen T, Zafranskaia E, Fosbrink M, Rus V, Shin ML, Rus H. C5b-9 terminal complex protects oligodendrocytes from apoptotic cell death by inhibiting caspase-8 processing and up-regulating FLIP. J Immunol. 2006; *176:* 3173-3180.

Dudoit S, Gentleman RC, Quackenbush J. Open source software for the analysis of microarray data. Biotechniques. 2003; *(Suppl.):* 45-51.

Efron B, Tibshirani R. Empirical bayes methods and false discovery rates for microarrays. Genet Epidemiol. 2002; *23:* 70-86.

Furlan R, Rovaris M, Martinelli Boneschi F, Khademi M, Bergami A, Gironi M, Deleidi M, Agosta F, Franciotta D, Scarpini E, Uccelli A, Zaffaroni M, Kurne A, Comi G, Olsson T, Filippi M, Martino G. Immunological patterns identifying disease course and evolution in multiple sclerosis patients. J Neuroimmunol. 2005; *165:* 192-200.

Hochberg Y, Benjamini Y. More powerful procedures for multiple significance testing. Stat Med. 1990; *9:* 811-818.

Howard LM, Kohm AP, Castaneda CL, Miller SD. Therapeutic blockade of TCR signal transduction and co-stimulation in autoimmune disease. Curr Drug Targets Inflamm Allergy. 2005; *4:* 205-216.

Isaacs A, Lindenmann J. Virus interference. I. The interferon. Proc R Soc Lond B Biol Sci. 1957; *147:* 258-267.

Jacobs L, Salazar AM, Herndon R, Reese PA, Freeman A, Jozefowicz R, Cuetter A, Husain F, Smith WA, Ekes R, et al. Intrathecally administered natural human fibroblast interferon reduces exacerbations of multiple sclerosis. Results of a multicenter, double-blind study. Arch Neurol. 1987; *44:* 589-595.

Krumbholz M, Theil D, Cepok S, Hemmer B, Kivisakk P, Ransohoff RM, Hofbauer M, Farina C, Derfuss T, Hartle C, Newcombe J, Hohlfeld R, Meinl E. Chemokines in multiple sclerosis: CXCL12 and CXCL13 up-regulation is differentially linked to CNS immune cell recruitment. Brain. 2006; *129:* 200-211.

Lee HK, Iwasaki A. Innate control of adaptive immunity: dendritic cells and beyond. Semin Immunol. 2007; *19:* 48-55.

Liu YJ, Kanzler H, Soumelis V, Gilliet M. Dendritic cell lineage, plasticity and cross-regulation. Nat Immunol. 2001; *2:* 585-589.

Lopez C, Comabella M, Al-zayat H, Tintore M, Montalban X. Altered maturation of circulating dendritic cells in primary progressive MS patients. J Neuroimmunol. 2006; *175:* 183-191.

Mead RJ, Neal JW, Griffiths MR, Linington C, Botto M, Lassmann H, Morgan BP. Deficiency of the complement regulator CD59a enhances disease severity, demyelination and axonal injury in murine acute experimental allergic encephalomyelitis. Lab Invest. 2004; *84:* 21-28.

Penberthy WT. Pharmacological targeting of IDO-mediated tolerance for treating autoimmune disease. Curr Drug Metab. 2007; *8:* 245-266.

Perrella O, Sbreglia C, Perrella M, Spetrini G, Gorga F, Pezzella M, Perrella A, Atripaldi L, Carrieri P. Interleukin-10 and tumor necrosis factor-α model of immunomodulation in multiple sclerosis. Neurol Res. 2006; *28:* 193-195.

Platten M, Ho PP, Youssef S, Fontoura P, Garren H, Hur EM, Gupta R, Lee LY, Kidd BA, Robinson WH, Sobel RA, Selley ML, Steinman L. Treatment of autoimmune neuroinflammation with a synthetic tryptophan metabolite. Science. 2005; *310:* 850-855.

PRISMS SG. Randomised double-blind placebo-controlled study of interferon-β_{1a} in relapsing/remitting multiple sclerosis. PRISMS (Prevention of Relapses and Disability by Interferon-β_{1a} Subcutaneously in Multiple Sclerosis) Study Group. Lancet. 1998; *352:* 1498-1504.

Rani MR, Ransohoff RM. Alternative and accessory pathways in the regulation of IFN-β-mediated gene expression. J Interferon Cytokine Res. 2005; *25:* 788-798.

Reder A, Velichko S, Yamaguchi K, Hemancioglu K, Ku K, Beekman J, Wagner T, Perez H, Salomon H, Croze E. IFN-β_{1b} induces transient and variable gene expression in relapsing-remitting multiple sclerosis patients independent of neutralizing antibodies or changes in IFN receptor RNA expression. J Interferon Cytokine Res. 2008; *28:* 317-331.

Rus H, Cudrici C, Niculescu F. C5b-9 complement complex in autoimmune demyelination and multiple sclerosis: dual role in neuroinflammation and neuroprotection. Ann Med. 2005; *37:* 97-104.

Sakurai K, Zou JP, Tschetter JR, Ward JM, Shearer GM. Effect of indoleamine 2,3-dioxygenase on induction of experimental autoimmune encephalomyelitis. J Neuroimmunol. 2002; *129:* 186-196.

Satoh J, Nanri Y, Tabunoki H, Yamamura T. Microarray analysis identifies a set of CXCR3 and CCR2 ligand chemokines as early IFNβ-responsive genes in peripheral blood lymphocytes in vitro: an implication for IFNβ-related adverse effects in multiple sclerosis. BMC Neurol. 2006; *6:* 18.

Schreiner B, Mitsdoerffer M, Kieseier BC, Chen L, Hartung HP, Weller M, Wiendl H. Interferon-β enhances monocyte and dendritic cell expression of B7-H1 (PD-L1), a strong inhibitor of autologous T cell

activation: relevance for the immune-modulatory effect in multiple sclerosis. J Neuroimmunol. 2004; *155:* 172-182.

Sibley W, Group IMSS. Interferon-β_{1b} is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomized, double-blind, placebo-controlled trial. The IFNB Multiple Sclerosis Study Group. Neurology. 1993; *43:* 655-661.

Sterin-Prync AE, Yankilevich P, Barrero PR, Bello R, Marangunich L, Vidal A, Criscuolo M, Benasayag L, Famulari AL, Dominguez RO, Kauffman MA, Diez RA. Two recombinant human interferon-β_{1a} pharmaceutical preparations produce a similar transcriptional response determined using whole genome microarray analysis. Int J Clin Pharmacol Ther. 2008; *46:* 64-71.

Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA. 2005; *102:* 15545-15550.

Takaoka A, Yanai H. Interferon signalling network in innate defence. Cell Microbiol. 2006; *8:* 907-922.

Thomas PD, Campbell MJ, Kejariwal A, Mi H, Karlak B, Daverman R, Diemer K, Muruganujan A, Narechania A. PANTHER: a library of protein families and subfamilies indexed by function. Genome Res. 2003; *13:* 2129-2141.

Vaknin-Dembinsky A, Balashov K, Weiner HL. IL-23 is increased in dendritic cells in multiple sclerosis and down-regulation of IL-23 by antisense oligos increases dendritic cell IL-10 production. J Immunol. 2006; *176:* 7768-7774.

van Baarsen LG, van der Pouw Kraan TC, Kragt JJ, Baggen JM, Rustenburg F, Hooper T, Meilof JF, Fero MJ, Dijkstra CD, Polman CH, Verweij CL. A subtype of multiple sclerosis defined by an activated immune defense program. Genes Immun. 2006; *7:* 522-531.

Vukusic S, Hutchinson M, Hours M, Moreau T, Cortinovis--Tourniaire P, Adeleine P, Confavreux C. The Pregnancy In Multiple Sclerosis G: Pregnancy and multiple sclerosis (the PRIMS study): clinical predictors of post-partum relapse. Brain. 2004; *127:* 1353-1360.

Weinstock-Guttman B, Badgett D, Patrick K, Hartrich L, Santos R, Hall D, Baier M, Feichter J, Ramanathan M. Genomic effects of IFN-β in multiple sclerosis patients. J Immunol. 2003; *171:* 2694-2702.

Wettenhall JM, Smyth GK. limmaGUI: a graphical user interface for linear modeling of microarray data. Bioinformatics. 2004; *20:* 3705-3706.

Xie X, Lu J, Kulbokas EJ, Golub TR, Mootha V, Lindblad-Toh K, Lander ES, Kellis M. Systematic discovery of regulatory motifs in human promoters and 3'UTRs by comparison of several mammals. Nature. 2005; *434:* 338-345.

Zhu WH, Lu CZ, Huang YM, Link H, Xiao BG. A putative mechanism on remission of multiple sclerosis during pregnancy: estrogen-induced indoleamine 2,3-dioxygenase by dendritic cells. Mult Scler. 2007; *13:* 33-40.

Table 5. Gene sets enriched in control samples. GSEA analysis.

N°	Name	Size	FDR q-val
1	ZHAN_MULTIPLE_MYELOMA_VS_NORMAL_DN	34	0,00
2	FERRANDO_T_CELL_DIFFERENTIATION_PATHWAY	15	0,04
3	FERRANDO_TAL1_NEIGHBORS	12	0,07
4	LINDSTEDT_DEND_DN	55	0,06
5	IDX_TSA_UP_CLUSTER1	22	0,05
6	WNT_TARGETS	21	0,05
7	CROONQUIST_IL6_STROMA_UP	37	0,06
8	MARTINELLI_IFNS_DIFF	18	0,05
9	NAB_LUNG_UP	24	0,08
10	TAKEDA_NUP8_HOXA9_8D_DN	165	0,09
11	IGF_VS_PDGF_DN	35	0,11
12	UVB_NHEK1_C2	21	0,12
13	DIAB_NEPH_UP	53	0,13
14	FEEDERPATHWAY	8	0,15
15	KIM_TH_CELLS_DN	15	0,14
16	ROS_MOUSE_AORTA_DN	64	0,14
17	ZUCCHI_EPITHELIAL_DN	42	0,14
18	HALMOS_CEBP_UP	45	0,17
19	1_2_DICHLOROETHANE_DEGRADATION	7	0,16
20	ASCORBATE_AND_ALDARATE_METABOLISM	7	0,15
21	HALMOS_CEBP_DN	35	0,16
22	TGFBETA_C1_UP	15	0,18
23	PREADIP_VS_FIBRO_UP	10	0,23
24	GALINDO_ACT_UP	68	0,23
25	AD12_48HRS_DN	11	0,24
26	CELL_ADHESION	160	0,24
27	EXTRINSICPATHWAY	13	0,23
28	ADIP_DIFF_CLUSTER2	36	0,22
29	GNATENKO_PLATELET_UP	33	0,23
30	VERHAAK_AML_NPM1_MUT_VS_WT_UP	148	0,22
31	GNATENKO_PLATELET	33	0,22
32	MUSCLE_MYOSIN	12	0,21
33	MYOD_NIH3T3_UP	69	0,21
34	CDMACPATHWAY	15	0,22
35	STRIATED_MUSCLE_CONTRACTION	33	0,24

Table 6. Genes included in Ferrando t cell differentiation pathway gene set with corresponding fold changes obtained in our experiment.

Probe	Rank in gene list	Core enrichment	Fold change
CD1A	2	Yes	-2,98
ITGB2	115	Yes	-4,11
TMSL8	225	Yes	-1,66
CD1B	305	Yes	-2,63
CD1D	491	Yes	-2,61
CRIP1	1228	Yes	-1,87
CD34	2031	Yes	-2,23
FCGR3B	2059	Yes	
CD1E	2595	Yes	-1,38
PTPRF	2979	Yes	-1,35
CD1C	3127	Yes	-1,34
DNTT	5561	No	-1,46
MME	6458	No	-1,94
CD6	9719	No	1,04
SELL	12971	No	1,33

Table 6. Part II.

GenBank Accession	Description
NM_001763	CD1a molecule
NM_000211	Integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
NM_021992	Thymosin-like 8
NM_001764	CD1b molecule
NM_001766	CD1d molecule
NM_001311	Cysteine-rich protein 1 (intestinal)
M81104	CD34 molecule
NM_030893	CD1e molecule
BG988050	Protein tyrosine phosphatase, receptor type, F
AW024273	CD1c molecule
BX348545	Deoxynucleotidyltransferase, terminal
NM_007289	Membrane metallo-endopeptidase (neutral endopeptidase, enkephalinase)
NM_006725	CD6 molecule
NM_000655	Selectin L (lymphocyte adhesion molecule 1)

Table 7. Genes included in Lindstedt dendritic down gene set with corresponding fold changes obtained in our experiment.

Probe	Rank in gene list	Core enrichment	Fold change	GenBank accession
CLEC10A	12	Yes	–4,76	NM_006344
FCER2	14	Yes	–6,66	NM_002002
ITGAM	30	Yes	–5,64	NM_000632
LST1	112	Yes	–4,33	NM_007161
TGB2	115	Yes	–4,11	NM_000211
FCGRT	299	Yes	–3,21	NM_004107
F13A1	654	Yes	–1,68	NM_000129
CDH2	814	Yes	–1,42	NM_001792
ASAHL	857	Yes	–1,94	NM_014435
PTPRE	948	Yes	–1,58	NM_130435
CCNH	1058	Yes	–1,78	AK094534
TGFBI	1164	Yes	–1,9	NM_000358
TPM1	1267	Yes	1,58	D29134
DAB2	1342	Yes	1,67	BQ013870
PECAM1	1719	Yes	–1,96	BG570355
PRSS3	1926	Yes	–1,42	AA453364
IFNGR1	1945	Yes	–2,2	CA771718
ACTN1	2256	Yes	–1,52	NM_001102
SLC6A2	2390	Yes	–1,58	H04623
QSCN6	2439	Yes	–1,43	AI004674
NPR1	3122	No	–1,35	NM_000906
ALOX15	3272	No	1,45	NM_001141
HLA-DMB	3516	No	–1,34	NM_002118
AQP3	3543	No	–1,31	NM_004925
MAN2B1	3705	No	–1,29	NM_000528
NCF2	3710	No	–1,32	NM_000433
ARHGDIB	3770	No	–1,48	NM_001175
SLC1A5	4853	No	–1,18	NM_005628
MKNK1	4988	No	–1,3	AI525788
EGR2	5136	No	–1,19	NM_000399
CDH1	5336	No	1,89	NM_004360
CCL22	5339	No	–1,22	NM_002990
DOCK1	5605	No	1,66	AI379570
PTGER3	6605	No	–1,1	BG212233
ITGAV	6619	No	–1,32	AK021459
RAB31	6747	No	–1,39	BG746044
PPAP2B	6875	No	2,56	NM_003713
CTNND1	7848	No	1,35	BG539024
TOP1	8279	No	1,66	NM_003286
ANXA1	8743	No	–1,69	NM_004306
ITGA5	8974	No	–1,08	AI809671

Table 7. Part II

Probe	Rank in gene list	Core enrichment	Fold change	GenBank accession
IGSF6	10123	No	1,1	NM_005849
RCBTB2	10235	No	1,34	AK125170
TNFRSF13C	10488	No	1,06	NM_052945
CD99	11197	No	1,26	R94508
NCBP2	11258	No	1,58	NM_007362
HLA-DRB1	11821	No	–1,42	L76566
CLEC2B	12217	No	1,39	NM_005127
ADCY7	13436	No	–1,24	NM_001114
ATP6V1A	13510	No	1,61	BQ438490
LAPTM4A	13612	No	1,52	NM_014713
ACP5	14099	No	1,56	NM_001611
DNMT1	14826	No	1,52	NM_001379
MAF	15619	No	2,01	AF055376
CCR1	15698	No	2,17	NM_001295

Table 7. Part III.

Description
C-type lectin domain family 10, member A
Fc fragment of IgE, low affinity II, receptor for (CD23)
Integrin, alpha M (complement component 3 receptor 3 subunit)
Leukocyte specific transcript 1
Integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
Fc fragment of IgG, receptor, transporter, alpha
Coagulation factor XIII, A1 polypeptide
Cadherin 2, type 1, N-cadherin (neuronal)
N-acylsphingosine amidohydrolase (acid ceramidase)-like
Protein tyrosine phosphatase, receptor type, E
Cyclin H
Transforming growth factor, beta-induced, 68kDa
Tropomyosin 1 (alpha)
Disabled homolog 2, mitogen-responsive phosphoprotein (Drosophila)
Platelet/endothelial cell adhesion molecule (CD31 antigen)
Protease, serine, 3 (mesotrypsin)
Interferon gamma receptor 1
Actinin, alpha 1
Solute carrier family 6 (neurotransmitter transporter, noradrenalin), member 2
Quiescin Q6-like 1
Natriuretic peptide receptor A/guanylate cyclase A (atrionatriuretic peptide receptor A)
Arachidonate 15-lipoxygenase, type B
Major histocompatibility complex, class II, DM beta
Aquaporin 3 (Gill blood group)
Mannosidase, alpha, class 2B, member 1

Table 7. Part IV.

Description
Neutrophil cytosolic factor 2 (65kDa, chronic granulomatous disease, autosomal 2)
Rho GDP dissociation inhibitor (GDI) beta
Solute carrier family 1 (neutral amino acid transporter), member 5
MAP kinase interacting serine/threonine kinase 1
Early growth response 2 (Krox-20 homolog, Drosophila)
Cadherin 1, type 1, E-cadherin (epithelial)
Chemokine (C-C motif) ligand 22
Dedicator of cytokinesis 1
Prostaglandin E receptor 3 (subtype EP3)
Integrin, alpha V (vitronectin receptor, alpha polypeptide, antigen CD51)
RAB31, member RAS oncogene family
Phosphatidic acid phosphatase type 2B
Catenin (cadherin-associated protein), delta 1
Topoisomerase (DNA) I
Annexin A13
Integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
Immunoglobulin superfamily, member 6
Regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 2
Tumor necrosis factor receptor superfamily, member 13C
CD99 molecule-like 2
Nuclear cap binding protein subunit 2, 20kDa
Major histocompatibility complex, class II, DR beta 1
C-type lectin domain family 2, member B
Adenylate cyclase 7
ATPase, H+ transporting, lysosomal 70kDa, V1 subunit A
Lysosomal-associated protein transmembrane 4 alpha
Acid phosphatase 5, tartrate resistant
DNA (cytosine-5-)-methyltransferase 1
V-maf musculoaponeurotic fibrosarcoma oncogene homolog (avian)
Chemokine (C-C motif) receptor 1

Table 8. Comparison between differentially expressed genes identified in at least two independent microarray studies evaluating the effect of IFNβ in multiple sclerosis along the fold change found for these genes in our assay conditions.

Probe	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in PBC of MS patients treated with IFN β	GenBank accession	Description
CASP1	1,44	↑	NM_033292	Caspase 1, apoptosis-related cysteine peptidase (interleukin 1, beta, convertase)
CASP3	3,28	↑	NM_032991	Caspase 3, apoptosis-related cysteine peptidase
CCL4	1,08	↑	AK024994	Chemokine (C-C motif) ligand 4
CCR5	6,1	↑	NM_000579	Chemokine (C-C motif) receptor 5
CD40	3,41	↑	NM_001250	CD40 molecule, TNF receptor superfamily member 5
CD69	–1,58	↑↓	BX108920	CD69 molecule
CSF2RB	1,44	↑↓	NM_000395	Colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)
CXCL10	26,44	↑	NM_001565	Chemokine (C-X-C motif) ligand 10
CXCR4	–1,09	↑↓	NM_003467	Chemokine (C-X-C motif) receptor 4
EIF2AK2	5,04	↑↓	NM_002759	Eukaryotic translation initiation factor 2-alpha kinase 2
FAS	2,5	↑↓	NM_000043	Fas (TNF receptor superfamily, member 6)
FOS	–7,24	↓	NM_005252	V-fos FBJ murine osteosarcoma viral oncogene homolog
5964 G1P2	12	↑	NM_005101	ISG15 ubiquitin-like modifier
G1P3	2,85	↑	NM_022873	Interferon, alpha-inducible protein 6
GBP1	7,44	↑	NM_002053	Guanylate binding protein 1, interferon-inducible, 67kDa
GBP2	1,86	↑	NM_004120	Guanylate binding protein 2, interferon-inducible
HLA-A	2,3	↑	BM715946	Major histocompatibility complex, class I, A
HLA-G	2,28	↑	NM_002127	HLA-G histocompatibility antigen, class I, G
ICAM1	–1,26	↑	NM_000201	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor
IFI30	1,6	↑	NM_006332	Interferon, gamma-inducible protein 30
IFIT1	17,77	↑	NM_001548	Interferon-induced protein with tetratricopeptide repeats 1
IFITM1	3,08	↑	NM_003641	Interferon induced transmembrane protein 1 (9-27)
IFITM2	2,06	↑	NM_006435	Interferon induced transmembrane protein 2 (1-8D)
IFITM3	5,81	↑	NM_021034	Interferon induced transmembrane protein 3 (1-8U)
IL15RA	7,14	↑	NM_002189	Interleukin 15 receptor, alpha
IL8	–3,55	↓	NM_000634	Interleukin 8 receptor, alpha
IRF7	3,93	↑	NM_004031	Interferon regulatory factor 7
ITGAL	–1,46	↓	T28925	Integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
JUN	–1,51	↓	NM_002228	Jun oncogene
MMP9	–1,41	↓	NM_004994	Matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)
MX1	9,12	↑	NM_002462	Myxovirus (influenza virus) resistance 1, interferon-inducible protein p78 (mouse)

↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated.

Table 8. Part II.

Probe	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in PBC of MS patients treated with IFN β	GenBank accession	Description
MX2	5,05	↑	NM_002463	Myxovirus (influenza virus) resistance 2 (mouse)
OAS1	7,47	↑	NM_016816	2',5'-oligoadenylate synthetase 1, 40/46kDa
PECAM1	–1,96	↓	BG570355	Platelet/endothelial cell adhesion molecule (CD31 antigen)
PSMB9	2,43	↑	NM_002800	Proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)
SP110	4,4	↑	NM_004509	SP110 nuclear body protein
STAT1	4,19	↑	NM_007315	Signal transducer and activator of transcription 1, 91kDa
TNFAIP6	3,34	↑	NM_007115	Tumor necrosis factor, alpha-induced protein 6
TNFSF10	11,09	↑	NM_003810	Tumor necrosis factor (ligand) superfamily, member 10

↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated.

Table 9. Comparison between differentially expressed genes identified in at least two independent microarray studies that have made use of peripheral blood cells of MS patients reported in the literature along the fold change found for this genes in our assay conditions.

Probe	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in PBC of MS patients	GenBank accession	Description
ATF3	3,06	↑↓	NM_004024	Activating transcription factor 3
ATM	–1,41	↑↓	BX095231	Ataxia telangiectasia mutated (includes complementation groups A, C and D)
BCL2	1,34	↑↓	BF513340	B-cell CLL/lymphoma 2
BRCA1	1,17	↑↓	NM_007305	Breast cancer 1, early onset
BTG1	–3,32	↓	BC009050	B-cell translocation gene 1, anti-proliferative
CASP10	**1,85**	↓	NM_001230	Caspase 10, apoptosis-related cysteine peptidase
CASP8	1,27	↑↓	BG258036	Caspase 8, apoptosis-related cysteine peptidase
CCL2	2,75	↑↓	NM_002982	Chemokine (C-C motif) ligand 2
CCL20	–6,49	↑↓	NM_004591	Chemokine (C-C motif) ligand 20
CCL3	–1,46	↑↓	NM_002983	Chemokine (C-C motif) ligand 3
CCL8	54,47	↑↓	NM_005623	Chemokine (C-C motif) ligand 8
CCR5	6,1	↑↓	NM_000579	Chemokine (C-C motif) receptor 5
CD24	–1,12	↑↓	NM_013230	CD24 molecule
CD83	**2,75**	↓	NM_004233	CD83 molecule

↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison with the description with the PBC of MS patients microarrays.

Table 9. Part II.

Probe	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in PBC of MS patients	GenBank accession	Description
CDC25B		↑↓		
CDK2	1,31	↑↓	NM_001798	Cyclin-dependent kinase 2
CR2	–1,46	↑↓	AW968617	Complement component (3d/Epstein Barr virus) receptor 2
CTSB	3,57	↑↓	NM_001908	Cathepsin B
DAXX	1,18	↑↓	NM_001350	Death-associated protein 6
DDIT3	–1,23	↓	S62138	DNA-damage-inducible transcript 3
DGKA	**–1,49**	↑	NM_001345	Diacylglycerol kinase, alpha 80kDa
DNAJB1	**–1,5**	↑	NM_006145	DnaJ (Hsp40) homolog, subfamily B, member 1
E2F5	**1,43**	↓	NM_001951	E2F transcription factor 5, p130-binding
GNA13	1,6	↑↓	BQ710423	Guanine nucleotide binding protein (G protein), alpha 13
GZMB	1,04	↑	NM_004131	Granzyme B (granzyme 2, cytotoxic T-lymphocyte-associated serine esterase 1)
HBEGF	–3,27	↑↓	NM_001945	Heparin-binding EGF-like growth factor
HSPA1A	**2,11**	↓	NM_005345	Heat shock 70kDa protein 1A
HSPA1L	–1,16	↓	NM_005527	Heat shock 70kDa protein 1-like
IFI16	3,95	↑↓	NM_005531	Interferon, gamma-inducible protein 16
IL16	–1,1	↑↓	BF974121	Interleukin 16 (lymphocyte chemoattractant factor)
IL1B	–7,19	↓	NM_000576	Interleukin 1, beta
IL1R1	**1,03**	↓	BX108587	Interleukin 1 receptor, type I
IL1R2	–1,08	↑	NM_004633	Interleukin 1 receptor, type II
IL1RN	1,99	↑↓	NM_000577	Interleukin 1 receptor antagonist
IL6R	–1,05	↑↓	AV735883	Interleukin 6 receptor
IL7R	2,02	↑	NM_002185	Interleukin 7 receptor
ITGA4	**–1,2**	↑	NM_000885	Integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)
ITGA6	**–1,57**	↑	BQ898221	Integrin, alpha 6
ITGAL	**–1,46**	↑	T28925	Integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)
ITGB2	–4,11	↑↓	NM_000211	Integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
JUN	–1,51	↓	NM_002228	Jun oncogene
LTB	–1,1	↑	NM_009588	Lymphotoxin beta (TNF superfamily, member 3)
MAPK1	1,84	↓	AL157438	Mitogen-activated protein kinase 1
MAPK14	–1,04	↑↓	NM_001315	Mitogen-activated protein kinase 14
MAPK6	–1,51	↑↓	AI610053	Mitogen-activated protein kinase 6
MX2	5,05	↑	NM_002463	Myxovirus (influenza virus) resistance 2 (mouse)

↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison with the description with the PBC of MS patients microarrays.

Table 9. Part III.

Probe	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in PBC of MS patients	GenBank accession	Description
MYC	1,37	↑↓	NM_002467	V-myc myelocytomatosis viral oncogene homolog (avian)
NFATC3	–1,31	↑↓	AI673006	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3
NFKB1	**1,65**	↓	BX091379	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (p105)
NFKBIA	–1,51	↑↓	NM_020529	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
NFRKB	–1,24	↑↓	BF446107	Nuclear factor related to kappaB binding protein
NR3C1	1,45	↑↓	U25029	Nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor)
NR4A1	–1,47	↓	BX094883	Nuclear receptor subfamily 4, group A, member 1
NR4A2	–3,01	↑↓	CD723630	Nuclear receptor subfamily 4, group A, member 2
NR4A3	**2,83**	↓	NM_006981	Nuclear receptor subfamily 4, group A, member 3
PKP4	**–1,79**	↑	AA605142	Plakophilin 4
PLAUR	–2,81	↓	NM_002659	Plasminogen activator, urokinase receptor
PMS1	–1,04	↑↓	NM_000534	PMS1 postmeiotic segregation increased 1 (S. cerevisiae)
POLE2	–1,39	↑↓	AI762202	Polymerase (DNA directed), epsilon 2 (p59 subunit)
PTGS2	**–5,66**	↑	NM_000963	Prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)
PTPRC	1,4	↑↓	NM_002838	Protein tyrosine phosphatase, receptor type, C
SCYE1	**–1,04**	↑	NM_004757	Small inducible cytokine subfamily E, member 1 (endothelial monocyte-activating)
SKIL	**3,71**	↓	NM_005414	SKI-like oncogene
SLC35A1	1,21	↑↓	NM_006416	Solute carrier family 35 (CMP-sialic acid transporter), member A1
SMAD7	–1,07	↑↓	NM_005904	SMAD family member 7
SRPK1	1,07	↑	BX103900	SFRS protein kinase 1
STAT1	4,19	↑↓	NM_007315	Signal transducer and activator of transcription 1, 91kDa
TGFB1	–1,36	↓	NM_000660	Transforming growth factor, beta 1 (Camurati-Engelmann disease)
TGFBR2	–1,08	↑↓	NM_003242	Transforming growth factor, beta receptor II (70/80kDa)
TIMP1	–1,42	↓	NM_003254	TIMP metallopeptidase inhibitor 1
TNF	–1,05	↓	NM_000594	Tumor necrosis factor (TNF superfamily, member 2)
TNFAIP3	2,28	↑↓	NM_024873	TNFAIP3 interacting protein 3
TNFAIP6	**3,34**	↓	NM_007115	Tumor necrosis factor, alpha-induced protein 6
TNFSF10	11,09	↑↓	NM_003810	Tumor necrosis factor (ligand) superfamily, member 10
TNFSF9	–1,08	↑↓	NM_003811	Tumor necrosis factor (ligand) superfamily, member 9
TOPBP1	1,04	↑↓	BM310955	Topoisomerase (DNA) II binding protein 1

↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison to the description with the PBC of MS patients microarrays.

Table 9. Part IV.

Probe	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in PBC of MS patients	GenBank accession	Description
TPR	1,62	↑↓	AI332534	Translocated promoter region (to activated MET oncogene)
TRA	1,13	↑↓	X73617	T cell receptor alpha locus
TRAF2	**2,16**	↓	BX117601	TRAF2 and NCK interacting kinase
TRAF5	1,39	↑	NM_004619	TNF receptor-associated factor 5
TRAP1	**1,12**	↓	AI673006	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 3
VAV2	–1,02	↑↓	CA427171	Vav 2 oncogene
VCAM1	–1,19	↓	NM_001078	Vascular cell adhesion molecule 1

↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison with the description with the PBC of MS patients microarrays.

Table 10. Multiple sclerosis genes identified in at least three independent microarray studies performed in EAE along the fold change found for these genes in our assay conditions.

Mouse probe	Homo sapiens homolog	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in EAE microarray studies	GenBank accession	Description
Adfp	ADFP	**–1,65**	↑	NM_001122	Adipose differentiation-related protein
Anxa1	ANXA1	1,69	↑	NM_004306	Annexin A13
Anxa2	ANXA2	1,92	↑	BG569830	Annexin A2
Anxa3	ANXA3	**–1,12**	↑	NM_005139	Annexin A3
Apoc2	APOC2	**–1,74**	↑	NM_000483	Apolipoprotein C-II
Arg1	ARG1	**–1,21**	↑	NM_000045	Arginase, liver
Arhgdib	ARHGDIB	**–1,48**	↑	NM_001175	Rho GDP dissociation inhibitor (GDI) beta
Arpc1b	ARPC1B	**–1,22**	↑	AI829701	Actin related protein 2/3 complex, subunit 1B, 41kDa
Atp1a1	ATP1A1	2,71	↑	BC069003	ATPase, Na+/K+ transporting, alpha 1 polypeptide
Atp2a2	ATP2A2	–1,25	↑↓	BI491798	ATPase, Ca++ transporting, cardiac muscle, slow twitch 2
B2m	B2M	1,74	↑↓	AI565931	Beta-2-microglobulin
Bcan	BCAN	**–1,08**	↑	NM_021948	Brevican

*Not homo sapiens homolog gene was found, #Gene not found in the list of probe of the gene array. Letter in *italics* = the name of the homo sapiens homolog probe is different of mouse. ↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison with the description with the PBC of MS patients microarrays.

Table 10. Part II.

Mouse probe	Homo sapiens homolog	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in EAE microarray studies	GenBank accession	Description
Bcl2a1b	*BCL2A1**	**–1,7**	↑	NM_004049	BCL2-related protein A1
Bst1	BST1	**–3,55**	↑	NM_004334	Bone marrow stromal cell antigen 1
Btg1	BTG1	3,32	↑	BC009050	B-cell translocation gene 1, anti-proliferative
C1qa	C1QA	**–1,19**	↑	NM_015991	Complement component 1, q subcomponent, A chain
C1qb	C1QB	1,54	↑	NM_000491	Complement component 1, q subcomponent, B chain
C1qc	C1QC	2,05	↑	NM_172369	Complement component 1, q subcomponent, C chain
C3	C3	2,89	↑	NM_000064	Complement component 3
C4	*C4BPA*	**–1,81**	↑	NM_000715	Complement component 4 binding protein, alpha
Cacna1g	CACNA1G	–1,5	↓	NM_198383	Calcium channel, voltage-dependent, alpha 1G subunit
Capg	CAPG	1,3	↑	BI825089	Capping protein (actin filament), gelsolin-like
Casp4	CASP4	3,31	↑	AL833655	Caspase 4, apoptosis-related cysteine peptidase
Ccl19	CCL19	10,13	↑	NM_006274	Chemokine (C-C motif) ligand 19
Ccl2	CCL2	2,75	↑	NM_002982	Chemokine (C-C motif) ligand 2
Ccl3	CCL3	**–1,43**	↑	NM_002983	Chemokine (C-C motif) ligand 3
Ccl5	CCL5	1,62	↑	NM_002985	Chemokine (C-C motif) ligand 5
Ccl6	CCL6	**–1,48**	↑	NM_002993	Chemokine (C-X-C motif) ligand 6 (granulocyte chemotactic protein 2)
Ccl9	CCL9	4,1	↑	NM_002416	Chemokine (C-X-C motif) ligand 9
Ccl12	CCL12	1,63	↑	CN479391	Chemokine (C-X-C motif) ligand 12 (stromal cell-derived factor 1)
Ccr1	CCR1	2,17	↑	NM_001295	Chemokine (C-C motif) receptor 1
Ccr2	CCR2	1	↑	NM_000648	Chemokine (C-C motif) receptor 2
Ccr5	CCR5	6,1	↑	NM_000579	Chemokine (C-C motif) receptor 5
Cd14	CD14	**–4,19**	↑	NM_000591	CD14 molecule
Cd3d	CD3D	**–1,77**	↑	AK098100	CD3d molecule, delta (CD3-TCR complex)
Cd3g	CD3G	**–1,11**	↑	NM_000073	CD3g molecule, gamma (CD3-TCR complex)
Cd14	CD14	**–4,19**	↑	NM_000591	CD14 molecule
Cd44	CD44	1,66	↑	BM764748	CD44 molecule (Indian blood group)
Cd48	CD48	1,34	↑	NM_001778	CD48 molecule
Cd52	CD52	**–1,88**	↑	NM_001803	CD52 molecule
Cd53	CD53	1,88	↑	AK124172	CD53 molecule
Cd68	CD68	2,19	↑	NM_001251	CD68 molecule

*Not homo sapiens homolog gene was found, #Gene not found in the list of probe of the gene array. Letter in *italics* = the name of the homo sapiens homolog probe is different of mouse. ↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison with the description with the PBC of MS patients microarrays.

Table 10. Part III.

Mouse probe	Homo sapiens homolog	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in EAE microarray studies	GenBank accession	Description
Cd72	CD72	1,31	↑	NM_001782	CD72 molecule
Cd74	CD74	**–1,59**	↑	AI820756	CD74 molecule, major histocompatibility complex, class II invariant chain
Cd9	CD9	**–2,1**	↑	BX093669	CD9 molecule
Cebpa	CEBPA	**–1,89**	↑	NM_004364	CCAAT/enhancer binding protein (C/EBP), alpha
Cebpb	CEBPB	**–1,26**	↑	NM_005194	CCAAT/enhancer binding protein (C/EBP), beta
Cebpd	CEBPD	**–1,26**	↑	NM_005195	CCAAT/enhancer binding protein (C/EBP), delta
Chi3l1	CHI3L1	**–1,54**	↑	NM_001276	Chitinase 3-like 1 (cartilage glycoprotein-39)
Chi3l3*			↑		
Chl1	CHL1	1,45	↑	R49177	Cell adhesion molecule with homology to L1CAM (close homolog of L1)
Col3a1	COL3A1	**–1,44**	↑	NM_000090	Collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)
Coro1a	CORO1A	**–1,37**	↑	NM_007074	Coronin, actin binding protein, 1A
Cp	CP	**–1,96**	↑	NM_000096	Ceruloplasmin (ferroxidase)
Crip1	CRIP1	**–1,87**	↑	NM_001311	Cysteine-rich protein 1 (intestinal)
Csf1r	CSF1R	**–3,34**	↑	NM_005211	Colony stimulating factor 1 receptor, formerly McDonough feline sarcoma viral (v-fms) oncogene homolog
Csf2ra	CSF2RA	2,48	↑	NM_006140	Colony stimulating factor 2 receptor, alpha, low-affinity (granulocyte-macrophage)
Csf2rb2	*CSF2RB*	1,44	↑	NM_000395	Colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)
Ctla2a*			↑		
Ctsc	CTSC	1,52	↑	NM_001814	Cathepsin C
Ctsd	CTSD	2,35	↑	NM_001909	Cathepsin D
Ctsh	CTSH	1,56	↑	BX098957	Cathepsin H
Ctss	CTSS	**–1,65**	↑	NM_004079	Cathepsin S
Ctsz	CTSZ	1,94	↑↓	NM_001336	Cathepsin Z
Cxcl9	CXCL9	4,1	↑	NM_002416	Chemokine (C-X-C motif) ligand 9
Cxcl10	CXCL10	26,44	↑	NM_001565	Chemokine (C-X-C motif) ligand 10
Cyba	CYBA	1,18	↑	NM_000101	Cytochrome b-245, alpha polypeptide
Dab2	DAB2	1,67	↑	BQ013870	Disabled homolog 2, mitogen-responsive phosphoprotein (Drosophila)
Dcn	DCN	1,48	↑	NM_133504	Decorin
Laptm5	LAPTM5	**–2,09**	↑	AW236220	Lysosomal associated multispanning membrane protein 5

*Not homo sapiens homolog gene was found, #Gene not found in the list of probe of the gene array. Letter in *italics* = the name of the homo sapiens homolog probe is different of mouse. ↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison with the description with the PBC of MS patients microarrays.

Table 10. Part IV.

Mouse probe	Homo sapiens homolog	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in EAE microarray studies	GenBank accession	Description
F10#	F10		↑		
Fabp7#	FABP7		↑		
Fcgr1	FCGR1	**–1,85**	↑	NM_000566	Fc fragment of IgG, high affinity Ia, receptor (CD64)
Fcgr2b	FCGR2B	–3,56	↑	U90938	Fc fragment of IgG, low affinity IIb, receptor (CD32)
Fcgr3	*FCGR3A*	**–1,94**	↑	NM_000570	Fc fragment of IgG, low affinity IIIa, receptor (CD16a)
Fn1	FN1	–3,47	↑	BQ020273	Fibronectin 1
Fgl2	FGL2	3,22	↑	NM_006682	Fibrinogen-like 2
Ftl1*			↑		
Fxyd5	FXYD5	**–1,49**	↑	BX648809	FXYD domain containing ion transport regulator 5
G1p2	G1P2	12	↑↓	NM_005101	ISG15 ubiquitin-like modifier
Gbp2	GBP2	1,86	↑	NM_004120	Guanylate binding protein 2, interferon-inducible
Gbp4	GBP4	1,53	↑	AI280680	Guanylate binding protein 4
Gfap	GFAP	1,81	↑	AI978562	Glial fibrillary acidic protein
Gnai2	GNAI2	1,28	↑	NM_002070	Guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2
Gp49a	CD300A	–1,45	↑	NM_007261	CD300a molecule
Gpx3	GPX3	**–1,63**	↑	NM_002084	Glutathione peroxidase 3 (plasma)
Grn	GRN	1,55	↑	BQ923463	Granulin
H2-aa	*HLA-DQA1*	1,88	↑	NM_002122	Major histocompatibility complex, class II, DQ alpha 1
H2-ab1	*HLA-DQB2*	1,09	↑	NM_182549	Major histocompatibility complex, class II, DQ beta 2
H2-b1	*HLA-DRA*	**–1,23**	↑	M60333	Major histocompatibility complex, class II, DR alpha
H2-d1	*HLA-A*	2,3	↑	BM715946	Major histocompatibility complex, class I, A
H2-d4*			↑		
H2-dma#	*H2-DMA*		↑		
H2-dmb1	*HLA-DMB*	**–1,34**	↑	NM_002118	Major histocompatibility complex, class II, DM beta
H2-ea	*HLA-DRA*	**–1,23**	↑	M60333	Major histocompatibility complex, class II, DR alpha
H2-eb1	*HLA-DRB1*	**–1,42**	↑	L76566	Major histocompatibility complex, class II, DR beta 1
H2-k1	HLA-G	2,28	↑	NM_002127	HLA-G histocompatibility antigen, class I, G
H2-l	HLA-A	2,3	↑	BM715946	Major histocompatibility complex, class I, A
H2-q2	HLA-F	1,85	↑	NM_018950	Major histocompatibility complex, class I, F
H2-q8	HRH2	**–1,44**		NM_022304	Histamine receptor H2
H2-t10*					
H2-t17*					
H2-t23	*HLA-E*	3,04		NM_005516	Major histocompatibility complex, class I, E

*Not homo sapiens homolog gene was found, #Gene not found in the list of probe of the gene array. Letter in *italics* = the name of the homo sapiens homolog probe is different of mouse. ↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison with the description with the PBC of MS patients microarrays.

Table 10. Part V.

Mouse probe	Homo sapiens homolog	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in EAE microarray studies	GenBank accession	Description
Hck	HCK	**–1,21**	↑	BF901596	Hemopoietic cell kinase
Hcls1	HCLS1	1,63	↑	NM_022460	HCLS1 binding protein 3
Hp	HP	**–2,15**	↑	NM_005143	Haptoglobin
Hpgd	HPGD	**–1,54**	↑	NM_000860	Hydroxyprostaglandin dehydrogenase 15-(NAD)
Icam1	ICAM1	**–1,26**	↑	NM_000201	Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor
Ifi30	IFI30	1,6	↑	NM_006332	Interferon, gamma-inducible protein 30
Ifi47*			↑		
Ifi203*			↑		
Ifi204	*IFI16*	3,95	↑	NM_005531	Interferon, gamma-inducible protein 16
Ifit1	IFIT1	17,77	↑↓	NM_001548	Interferon-induced protein with tetratricopeptide repeats 1
Ifit2	IFIT2	11,29	↑	NM_001547	Interferon-induced protein with tetratricopeptide repeats 2
Ifit3	IFIT3	8,09	↑	NM_001549	Interferon-induced protein with tetratricopeptide repeats 3
Ifit3	*IFITM3*	5,63	↑	NM_021034	Interferon induced transmembrane protein 3 (1-8U)
Igfbp2	IGFBP2	2,07	↑	NM_004031	Interferon regulatory factor 7
Igtp*			↑		
Lig1*			↑		
Lig2*			↑		
Il10rb	IL10RB	1,76	↑	AK123722	Interleukin 10 receptor, beta
Il18bp#	IL18BP		↑		
Il1a	IL1A	**–4,98**	↑	X02851	Interleukin 1, alpha
Il1b	IL1B	**–7,19**	↑	NM_000576	Interleukin 1, beta
Il1r2	IL1R2	–1,08	↑↓	NM_004633	Interleukin 1 receptor, type II
Il1rn	IL1RN	1,99	↑	NM_000577	Interleukin 1 receptor antagonist
Il2rg	IL2RG	1,56	↑	NM_000206	Interleukin 2 receptor, gamma (severe combined immunodeficiency)
Il4ra	**IL4R**	**–1,67**	↑	AW449273	Interleukin 4 receptor
Il6	IL6	–2,41	↑↓	NM_000600	Interleukin 6 (interferon, beta 2)
Inpp5d	INPP5D	**–1,1**	↑	AI806783	Inositol polyphosphate-5-phosphatase, 145kDa
Iqgap1	IQGAP1	**–2,73**	↑	AA477099	IQ motif containing GTPase activating protein 1
Irf1	IRF1	1,59	↑	NM_002198	Interferon regulatory factor 1
Irf5	IRF5	2,08	↑	NM_032643	Interferon regulatory factor 5

*Not homo sapiens homolog gene was found, #Gene not found in the list of probe of the gene array. Letter in *italics* = the name of the homo sapiens homolog probe is different of mouse. ↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison with the description with the PBC of MS patients microarrays.

Table 10. Part VI.

Mouse probe	Homo sapiens homolog	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in EAE microarray studies	GenBank accession	Description
Irf5	IRF5	2,08	↑	NM_032643	Interferon regulatory factor 5
Irf7	IRF7	3,93	↑	NM_004031	Interferon regulatory factor 7
Irf8	IRF8	1,22	↑	H53164	Interferon regulatory factor 8
Irg1	*LOC730249*	16,85	↑	AW059912	Similar to Immune-responsive protein 1
Irgm	IRGM	**–1,15**	↑	BI764111	Immunity-related GTPase family, M
Isgf3g	ISGF3G	2,93	↑	NM_006084	Interferon-stimulated transcription factor 3, gamma 48kDa
Itgam	ITGAM	**–5,64**	↑	NM_000632	Integrin, alpha M (complement component 3 receptor 3 subunit)
Itgb1	ITGB1	–3,96	↑↓	NM_032571	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
Itgb2	ITGB2	**–4,11**	↑	NM_000211	Integrin, beta 2 (complement component 3 receptor 3 and 4 subunit)
Jak3	JAK3	**–1,07**	↑	NM_000215	Janus kinase 3 (a protein tyrosine kinase, leukocyte)
Junb	JUNB	–1,36	↑↓	NM_002229	Jun B proto-oncogene
Laptm5	LAPTM5	**–2,09**	↑	AW236220	Lysosomal associated multispanning membrane protein 5
Lcn2	LCN2	**–1,51**	↑	NM_005564	Lipocalin 2 (oncogene 24p3)
Lcp1	LCP1	**–1,45**	↑	NM_002298	Lymphocyte cytosolic protein 1 (L-plastin)
Ldha	LDHA	1,31	↑	NM_005566	Lactate dehydrogenase A
Lgals1	LGALS1	**–1,55**	↑	NM_013268	Lectin, galactoside-binding, soluble, 13 (galectin 13)
Lgals3	LGALS3	**–1,03**	↑	NM_002306	Lectin, galactoside-binding, soluble, 3 (galectin 3)
Lgals3bp	LGALS3BP	3,05	↑	NM_005567	Lectin, galactoside-binding, soluble, 3 binding protein
Lgals9	LGALS9	2,75	↑	NM_009587	Lectin, galactoside-binding, soluble, 9 (galectin 9)
Lilrb3	LILRB3	4,75	↑↓	NM_006840	Leukocyte immunoglobulin-like receptor, subfamily B (with TM and ITIM domains), member 3
Lilrb4#	LILRB4		↑		
Lsp1	LSP1	**–1,06**	↑	NM_002339	Lymphocyte-specific protein 1
Lst1	LST1	**–4,33**	↑	NM_007161	Leukocyte specific transcript 1
Ly6a*			↑↓		
Ly86	LY86	2,22	↑	BF514291	Lymphocyte antigen 86
Mgst1	MGST1	2,52	↑	NM_145792	Microsomal glutathione S-transferase 1
Mki67	MKI67	1,04	↑	NM_002417	Antigen identified by monoclonal antibody Ki-67
Mmp12	MMP12	**–1,13**	↑	NM_002426	Matrix metallopeptidase 12 (macrophage elastase)
Mog	MOG	–1,28	↓	NM_206814	Myelin oligodendrocyte glycoprotein
Mpeg1#	MPEG1		↑		

*Not homo sapiens homolog gene was found, #Gene not found in the list of probe of the gene array. Letter in *italics* = the name of the homo sapiens homolog probe is different of mouse. ↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison with the description with the PBC of MS patients microarrays.

Table 10. Part VII.

Mouse probe	Homo sapiens homolog	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in EAE microarray studies	GenBank accession	Description
Mt2#	MT2A		↑		
Myc	MYC	**–1,3**	↑	NM_004688	N-myc (and STAT) interactor
Myo1f#	MYOIF		↑		
Ncf1	NCF1	1,32	↑	NM_000265	Neutrophil cytosolic factor 1, (chronic granulomatous disease, autosomal 1)
Ncf4	NCF4	**–1,12**	↑	BE931058	Neutrophil cytosolic factor 4, 40kDa
Nfatc1	NFATC1	**–1,25**	↑	BF476880	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1
Nfkbia	NFKBIA	**–1,51**	↑	NM_020529	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha
Nos2	*NOS2A*	**–1,24**	↑	T03803	Nitric oxide synthase 2A (inducible, hepatocytes)
Nupr1	NUPR1	8,59	↑	NM_012385	Nuclear protein 1
Pabpc1	PABPC1	**–2,16**	↑	AK098572	Poly(A) binding protein, cytoplasmic 1
Pbef1	PBEF1	**–4,26**	↑	BM451023	Pre-B-cell colony enhancing factor 1
Pfn1	PFN1	**–1,02**	↑	NM_005022	Profilin 1
Plp1	PLP1	–1,28	↓	NM_000533	Proteolipid protein 1 (Pelizaeus-Merzbacher disease, spastic paraplegia 2, uncomplicated)
Plp2	PLP2	**–2,53**	↑	NM_001642	Amyloid beta (A4) precursor-like protein 2
Ppp1r14b	PPP1R14B	**–3,96**	↑	NM_032571	Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)
Prg1	PRG1	–1,29	↑↓	AV650179	Proteoglycan 1, secretory granule
Psmb10	PSMB10	1,87	↑	NM_002801	Proteasome (prosome, macropain) subunit, beta type, 10
Psmb8	PSMB8	2,19	↑	NM_000064	Complement component 3
Psmb9	PSMB9	2,43	↑	NM_002800	Proteasome (prosome, macropain) subunit, beta type, 9 (large multifunctional peptidase 2)
Psme1	PSME1	1,43	↑	NM_006263	Proteasome (prosome, macropain) activator subunit 1 (PA28 alpha)
Ptpn1	PTPN1	**–2,15**	↑	AA015779	Protein tyrosine phosphatase, non-receptor type 13 (APO-1/CD95 (Fas)-associated phosphatase)
Ptpn6	PTPN6	1,17	↑	AW991455	Protein tyrosine phosphatase, non-receptor type 6
Ptprc	PTPRC	1,76	↑	BM720549	Protein tyrosine phosphatase, receptor type, C-associated protein
Rab3d	RAB3D	1,19	↑	NM_004283	RAB3D, member RAS oncogene family
rbm3	RBM3	**–1,09**	↑	NM_006743	RNA binding motif (RNP1, RRM) protein 3
Rras	RRAS	1,3	↑	NM_006270	Related RAS viral (r-ras) oncogene homolog
S100a11	S100A11	**–1,97**	↑	NM_003955	Suppressor of cytokine signaling 3
S100a4	S100A4	**–5,52**	↑	NM_002961	S100 calcium binding protein A4

*Not homo sapiens homolog gene was found, #Gene not found in the list of probe of the gene array. Letter in *italics* = the name of the homo sapiens homolog probe is different of mouse. ↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison with the description with the PBC of MS patients microarrays.

Table 10. Part VIII.

Mouse probe	Homo sapiens homolog	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in EAE microarray studies	GenBank accession	Description
S100a6	S100A6	**–1,81**	↑	NM_014624	S100 calcium binding protein A6
S100a8	S100A8	**–5,84**	↑	NM_002964	S100 calcium binding protein A8
S100a9	S100A9	–2,82	↑↓	NM_002965	S100 calcium binding protein A9
Saa3	*SAA2*	1,21	↑	NM_030754	Serum amyloid A2
Samhd1	SAMHD1	1,15	↑	NM_015474	SAM domain and HD domain 1
Sat1	SAT1	1,45	↑	NM_002970	Spermidine/spermine N1-acetyltransferase 1
Serpina3G*			↑		
Serpina3n	*SERPINA3*	1,22	↑	BG567659	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3
Serping1	SERPING1	11,41	↑	NM_000062	Serpin peptidase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)
Sfpi1	SFPI1	**–1,45**	↑	BC051714	Spleen focus forming virus (SFFV) proviral integration oncogene spi1
Sirpa	SIRPA	1,69	↑	NM_080792	Signal-regulatory protein alpha
Slc11a1	SLC11A1	**–12,68**	↑	H95091	Solute carrier family 11 (proton-coupled divalent metal ion transporters), member 1
Slfn2*			↑		
Socs3	SOCS3	1,08	↑	NM_003955	Suppressor of cytokine signaling 3
Spp1	SPP1	1,73	↑	NM_000582	Secreted phosphoprotein 1 (osteopontin, bone sialoprotein I, early T-lymphocyte activation 1)
Stat1	STAT1	4,19	↑	NM_007315	Signal transducer and activator of transcription 1, 91kDa
Stat6	STAT6	1,23	↑	NM_003153	Signal transducer and activator of transcription 6, interleukin-4 induced
Tagln2	TAGLN2	**–1,25**	↑	NM_003564	Transgelin 2
Tap1	TAP1	4,12	↑	NM_000593	Transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)
Tapbp	TAPBP	1,66	↑	NM_003190	TAP binding protein (tapasin)
Tcirg1	TCIRG1	1,07	↑	NM_006053	T-cell, immune regulator 1, ATPase, H+ transporting, lysosomal V0 subunit A3
Tcrb-j	*CENPJ*	**–1,02**	↑	NM_018451	Centromere protein J
Tgfb1	TGFB1	**–1,36**	↑	NM_000660	Transforming growth factor, beta 1 (Camurati-Engelmann disease)
Tgfbi	TGFBI	**–1,9**	↑	NM_000358	Transforming growth factor, beta-induced, 68kDa
Tgtp*			↑		
Tlr6#	TLR6		↑		
Tmsb4x	TMSB4X	1,42	↑	AV713423	Thymosin, beta 4, X-linked
Tnf	TNF	**–1,05**	↑	NM_000594	Tumor necrosis factor (TNF superfamily, member 2)

*Not homo sapiens homolog gene was found, #Gene not found in the list of probe of the gene array. Letter in *italics* = the name of the homo sapiens homolog probe is different of mouse. ↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison with the description with the PBC of MS patients microarrays.

Table 10. Part IX.

Mouse probe	Homo sapiens homolog	Fold change after in vitro treatment with IFNβ1 a in our assay	Gene expression described in EAE microarray studies	GenBank accession	Description
Tnfrsf1b	TNFRSF1B	1,15	↑	NM_001066	Tumor necrosis factor receptor superfamily, member 1B
Tyrobp	TYROBP	**–1,05**	↑	NM_003332	TYRO protein tyrosine kinase binding protein
ugt8a	*UGT8*	–1,24	↓	NM_003360	UDP glycosyltransferase 8 (UDP-galactose ceramide galactosyltransferase)
Vamp8	VAMP8	1,03	↑	NM_003761	Vesicle-associated membrane protein 8 (endobrevin)
Vasp	VASP	**–1,24**	↑	NM_003370	Vasodilator-stimulated phosphoprotein
Vcam1	VCAM1	**–1,19**	↑	NM_001078	Vascular cell adhesion molecule 1
Xdh	XDH	1,03	↑	NM_000379	Xanthine dehydrogenase
Ybx1	YBX1	1,39	↑	NM_004559	Y box binding protein 1
Zap70	ZAP70	**–1,26**	↑	NM_207519	Zeta-chain (TCR) associated protein kinase 70kDa

*Not homo sapiens homolog gene was found, #Gene not found in the list of probe of the gene array. Letter in *italics* = the name of the homo sapiens homolog probe is different of mouse. ↑ genes appear up-regulated by all the authors, ↓ genes appear down-regulated by all the authors, ↑↓ some authors found the gene up-regulated and others found it down-regulated, the minus before the fold change number means that the gene is down-regulated, the **bold** fold change number are the genes that change their expression orientation in our assay in comparison with the description with the PBC of MS patients microarrays.