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Multiple Sclerosis-linked and Interferon-beta-regulated Gene Expression in Plasmacytoid Dendritic Cells

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Abstract

The cause of multiple sclerosis (MS) is not known and the mechanism of interferon-beta, a disease-modifying treatment, is not well-understood. We studied gene expression in plasmacytoid dendritic cells (pDCs), antigen-presenting cells implicated in MS pathogenesis. PDCs were separated from healthy donors and MS patients at two time points: before and after initiation of treatment with interferon-beta. Expression of selected MS-linked and interferon-beta-regulated genes was validated with single assays. We have identified 60 genes which were abnormally expressed in MS patients and were corrected after treatment. These genes could be studied as potential MS biomarkers and possible therapeutic targets in MS.

Keywords

Multiple sclerosis; Plasmacytoid dendritic cells; Gene expression; Interferon-beta; Biomarkers

1. INTRODUCTION

Multiple Sclerosis (MS) is an immune-mediated chronic inflammatory and neurodegenerative disease of the central nervous system. The etiology of MS is unknown. Multiple genetic and environmental factors have been implicated in the disease pathogenesis. Global gene expression profiling analysis in relatively homogenous cell populations, such as tumor cells, has been proven a powerful tool to identify abnormally expressed genes linked to disease pathogenesis (Chen et al., 2003, Ring and Ross, 2002). Gene expression studies done in patients with MS have used mostly a heterogeneous population of peripheral blood mononuclear cells (PBMC) (Gandhi et al., 2010, Bomprezzi et al., 2003, Mandel et al., 2004, Satoh et al., 2005) or postmortem autopsy samples comprised of several cell subsets with different morphology, gene expression profile, and function. As a result, the gene expression changes in key cell subsets involved in immunopathogenesis of MS, e.g., dendritic cells or B cells, could be easily masked/diluted by changes in other more abundant PBMC subsets.

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DNA viruses such as EBV and herpes zoster virus have been implicated in MS pathogenesis (Haahr and Hollsberg, 2006, Serafini et al., 2007, Kang et al., 2011). Plasmacytoid dendritic cells (pDCs) are involved in early recognition of viral pathogens, express the highest level of Toll-like receptor 9 (TLR9) compared to other PBMC subsets, and strongly contribute to innate and adaptive immune responses. TLR9 recognizes unmethylated CpG dinucleotides in bacterial and viral DNA sequences. Upon activation, pDCs secrete multiple cytokines such as interferons and promote T cell responses (Rissoan et al., 1999, Cella et al., 2000, Kuwana et al., 2001, Gilliet and Liu, 2002). The function of pDCs is impaired in a number of human disorders including HIV infection, Systemic Lupus Erythematosus, and cancer; conditions associated with abnormal immune responses to self-antigens and pathogens (Chehimi et al., 2002, Feldman et al., 2001, Farkas et al., 2001, Vallin et al., 1999, Zou et al., 2001, Munn et al., 2004). pDCs have been found in the brain and cerebrospinal fluid (CSF) of MS patients (Lande et al., 2008, Longhini et al.) and the concentration of pDCs is increased in the CSF of MS patients during an MS exacerbation (Longhini et al., 2011). One of the factors responsible for pDCs recruitment to the CSF is Fms-like tyrosine kinase 3 ligand, which is constitutively expressed in brain (Curtin et al., 2006) and is involved in the pathogenesis of experimental autoimmune encephalomyelitis, an animal model of MS (Greter et al., 2005, Whartenby et al., 2005).

IFN-beta 1a and 1b are FDA-approved disease modifying treatments (DMT) for MS. IFN-beta has multiple anti-viral and immunomodulatory mechanisms, including modulation of cell proliferation and apoptosis (Noronha et al., 1993, Sharief et al., 2001), expression of MHC class II and costimulatory molecules, and cytokine secretion (Arnason et al., 1996, Huang et al., 2001, Liu et al., 2001, McRae et al., 1998, Noronha et al., 1993). IFN-beta impairs maturation of pDCs (Lande et al., 2008) and affects TLR9 processing and TLR9-mediated production of cytokines and chemokines (Aung et al., 2010, Balashov et al., 2010).

In this study, we analyzed gene expression in pDCs of healthy controls, untreated and IFN-beta treated MS patients. We were able to identify 60 genes which were abnormally expressed in untreated MS patients and were corrected after treatment with IFN-beta. Expression of selected genes was further validated by RT-qPCR. We discuss the function and potential involvement of these particular genes in MS pathogenesis and response to IFN-beta.

2. MATERIAL AND METHODS

2.1. Patients and Controls

We studied cells from 18–50 year old healthy donors (HD), patients with relapsing-remitting MS (RRMS) diagnosed according to the 2010 McDonald criteria (Polman et al., 2011). The study was approved by the Institutional Review Board IRB of UMDNJ-Robert Wood Johnson Medical School. Each healthy subject had one blood draw. Each MS patient had two blood draws: before and three months after treatment with IFN-beta. Exclusion criteria included: any immunomodulatory treatment in the 3 months prior to the first blood draw, e.g., corticosteroids, mitoxantrone, glatiramer acetate, IFN-beta, cyclophosphamide; presence of other disorders that may be associated with abnormal immune response (e.g., HIV, lupus, lymphoma) or demyelinating disease (e.g., Lyme, B12 deficiency); current or a pregnancy planned for anytime over the three months following the initial evaluation; baseline Expanded Disability Status Scale score greater than 5 (Kurtzke, 1983); clinical exacerbation/IV steroid treatment between blood draws; a clinical MS exacerbation less than 1 month prior to 1st blood draw to exclude the effect of MS exacerbation on pDC function; history of treatment with IFN-beta prior to first blood draw to reduce the possibility of IFN-beta neutralizing antibody occurrence and medical conditions that preclude IFN-beta

treatment, e.g., severe depression, or abnormal lab tests. The patients and healthy subjects are described in Table 1.

2.2. Preparation of PBMC and enrichment of pDCs

PBMC were separated from blood samples by Ficoll density gradient (MediaTech Inc). Separation of pDCs was done by immunomagnetic sorting using BDCA-4 cell isolation kit (Cat # 130 090 532, Miltenyi Biotec) according to the manufacturer's protocol. Separated pDCs were immediately resuspended in RNA cell protect reagent solution (Qiagen) to protect RNA from degradation and were stored at -80°C .

2.3. Microarrays experiment and data analysis

Total RNA was extracted from homogenized samples using Trizol reagent (Cat# 15596-026, Invitrogen, USA) and further purified with Qiagen RNAeasy columns with DNase treatment (Cat# 74106, Qiagen, Valencia, CA). RNA quality was assessed by capillary electrophoresis using the Agilent Bioanalyzer 2100 and spectrophotometric analysis prior to cDNA synthesis. Fifty nanograms of total RNA from each sample was amplified to cDNA, fragmented and biotinylated using the Nugen kits WT Ovation Pico (Cat# 3300-60, NuGen, San Carlos, CA) and FL Ovation Biotin (Cat# 4200-60, NuGen, San Carlos, CA). The labeled samples were hybridized to Affymetrix GeneChip® Human Genome U133 Plus 2.0 Arrays according to manufacturer's recommendations for hybridization, washing and scanning.

Gene expression data analysis was done by GeneSpring software (Agilent Technologies) to get the relative fold change of expression between different groups of subjects. An unpaired t-test was applied to select genes with significant difference in expression between healthy donors and untreated MS patients. A paired t-test was applied to select genes with significant difference in expression in MS patients before and after IFN-beta treatment. To select differentially expressed/regulated genes, the cut-off criteria consisted of a P value < 0.05 and fold change > 1.5 . Further statistical analysis was done with Chi-square test and Fisher's exact test using the GraphPad Prism program (GraphPad Software La Jolla, CA). We used Panther (Thomas et al., 2006) for gene ontology analysis (classification of genes according to biological and molecular function). In addition, we used the KEGG pathway database (Kanehisa et al.) to uncover the pathways for each gene (Table 2).

2.4. RT-qPCR

Validation of THBS1, IL6ST, FCGR2A, and HSPA1A gene expression was done by RT-qPCR. The primer and probe assays (Hs00962908_m1 for THBS1, HS00174360_m1 for IL6ST, Hs00234969_m1 for FCGR2A and Hs00359163_s1 for HSPA1A) for PCR were purchased from Applied Biosystems and the assay conditions were selected according to the manufacturer's instructions. The RT-qPCR reactions were run on the iCycler iQ Real-Time detection System (Bio-Rad Laboratories, Hercules, CA) according to the manufacturer's protocol. All RT-qPCR measurements were done in three replicates and data analysis was performed using the formula $2^{-\Delta\text{Ct}}$ and normalized against HPRT1, the house-keeping gene. The statistical analysis was done with unpaired (healthy subjects vs. MS patients) and paired (the same MS patients tested before and after IFN-beta treatment) *t* test using the GraphPad Prism program (GraphPad Software, La Jolla, CA).

3. RESULTS

3.1. Identification of IFN-beta-regulated genes potentially involved in MS pathogenesis

First, we compared gene expression in pDCs separated from untreated MS patients and healthy donors. Based on our experimental data and criteria of gene selection (P value $<$

0.05 and fold change >1.5) described in Materials and Methods, we identified 1,213 genes differentially expressed in pDCs of untreated MS patients which we refer to as “disease-linked” genes. Assuming that the number of identified human genes is 22,333 (Pertea and Salzberg, 2010), the proportion of “disease-linked” genes abnormally expressed in pDCs in MS was 1,213 out of 22,333 or 5.4 %.

In the second part of the study, MS patients were tested before and after treatment. The expression of 283 genes in pDCs was found to be affected by IFN-beta treatment. Those genes are further called “IFN-beta-regulated”. If one assumes that “IFN-beta-regulated” genes are not linked to MS pathogenesis, it would be expected that only 5.4% of those genes would be “disease-linked”, similar to the proportion of “disease-linked” genes among human genes (see above). However, based on the results presented in Figure 1, a significantly higher proportion (fourfold) of “IFN-beta-regulated” genes (64 out of 283 genes or 22.6%) overlapped with the set of 1213 “disease-linked” genes, $p < 0.0001$ based on Chi-square test and Fisher’s exact test. This strongly suggests that the identified subset of 64 genes which is further called “disease-linked and IFN-beta-regulated” is involved in MS pathogenesis and not just a random occurrence.

The subset of 64 “disease-linked and IFN-beta-regulated” genes was further separated into the following groups based on the pattern of gene expression change depicted in Figure 2: The first groups (group A) consisted of 37 genes whose expression was significantly increased in untreated MS patients (as compared to healthy subjects) and were “corrected” or had significant reverse (decreased) change in their expression after IFN-beta treatment (Supplementary Table 1). The second groups (group B) consisted of 23 genes with significantly decreased expression in untreated MS patients and were “corrected” or had significant reverse (increased) change in their expression after IFN-beta treatment (Supplementary Table 2).

The third groups (group C) consisted of four genes which had increased expression in untreated MS patients and with further increased expression after IFN-beta treatment.

In summary, 60 out of the 64 genes (94%) were “corrected” after the treatment with IFN-beta, which is significantly higher than the randomly expected 50% chance of “correction”, $p < 0.0001$ based on Chi-square test and Fisher’s exact test.

3.2. Gene Ontology and Pathway Analysis

As the major function of pDCs is antiviral-surveillance and regulation of immune system, we decided to focus on the aberrant expression of genes involved in immune system process. We could identify 10 genes which are involved in immune system process using Panther, the gene ontology analysis program. Among these ten genes, THBS1, HDGFRP3, IL6ST, GADD45A, FCGR2A, and CHEK1 are from the gene set A (Figure. 2) and HSPA1A, OAS2, BTN3A3, and TNS1 belong to the gene set B (Figure. 2). The molecular functions of each gene are described in the Table 2. We focused on these ten genes to discover the pathways using KEGG database (Table. 3). This analysis could not identify pathways for three genes (BTN3A3, TNS1, and HDGFRP3). Among other seven genes, gene FCGR2A is associated with SLE, gene HSPA1A is involved in antigen-processing, genes THBS1 and IL6ST are associated with cytokine-cytokine receptor induced signaling, and genes GADD45A, THBS1, and CHEK1 are associated with p53 signaling pathway.

3.3. Validation of microarrays result by RT-qPCR

We used RT-qPCR to validate abnormal expression of four selected genes (THBS1, HSPA1A, FCGR2A, and IL6ST) from the above list of 10 genes involved with the immune system. The results of RT-qPCR for THBS1 and HSPA1A were consistent with the results

of microarrays (Fig. 3). THBS1 expression (Mean \pm SEM) was significantly increased in untreated MS patients (n=8) as compared to HD (n=8) (0.1454 ± 0.0427 vs. 0.03216 ± 0.0153 respectively, $p = 0.0259$). After IFN-beta treatment, mean THBS1 expression was significantly decreased (0.0938 ± 0.0292 , $p=0.0198$ as compared to the same group of patients before treatment). HSPA1A expression was significantly decreased in untreated MS patients as compared to HD (2.853 ± 0.3129 vs. 5.815 ± 0.9422 , respectively, $p = 0.0099$). After IFN-beta treatment, HSPA1A expression was significantly increased (5.22 ± 0.9611 , $p = 0.0174$ as compared to the same group of patients before treatment). The microarray results for IL6ST could not be validated by RT-qPCR (data not shown). For FCGR2A, the difference in RT-qPCR gene expression between healthy donors (n=8) and untreated MS patients (n=8) was consistent with the microarrays data. The FCGR2A expression in healthy donors and untreated MS patients were 0.3031 ± 0.0518 and 0.5475 ± 0.0978 , respectively (unpaired t test p value = 0.0444. However, RT-qPCR did not confirm FCGR2A microarray data in treated MS patients (data not shown).

4. DISCUSSION

We have identified 60 genes which were abnormally expressed in MS patients and corrected after treatment with IFN-beta. Based on the Panther analysis, ten of those genes are likely to be involved in immune system processes. In an attempt to evaluate the contributions of the aberrant expression of these genes in the pathogenesis of MS, we discuss their potential relevance in the context of pDC biology such as antigen presentation, cytokine secretion, and regulation of T cell responses.

We found that FCGR2A, also known as CD32, was overexpressed in pDCs of untreated MS patients. It has been shown that FCGR2A internalizes and delivers DNA-containing immune complexes to interact with TLR9 which is required for activation of pDCs to secrete IFN- α and other pro-inflammatory cytokines/chemokines (Means et al., 2005). It can be hypothesized that pDCs in MS patients are hypersensitive to nucleic acid-immune complexes which can lead to abnormal immune responses.

Three other genes (HSPA1A, BTN3A3, and GADD45A), which can influence T cell responses, had abnormal expression in pDCs of MS patients. HSPA1A is a heat shock protein (HSP). HSPs are stress response proteins well known as molecular chaperones for mediating protein folding and preventing protein aggregation which are critical steps for proteins to function properly. HSPs have been implicated in T cell immune response regulation. It has been suggested that self-HSPs contribute to the generation of regulatory T cells. Tanaka et al demonstrated that immunization with the conserved HSP70 peptide prevents the development of adjuvant arthritis (Tanaka et al., 1999). HSP60 expression was decreased in children with autoimmune idiopathic thrombocytopenia purpura (Xiao et al., 2004). Surprisingly, the possible role of HSPA1A in the pathogenesis of MS has not been studied even though decreased expression of HSPA1A was discovered in many gene expression studies of MS patients (Gandhi et al., 2010, Bompreszi et al., 2003, Mandel et al., 2004, Satoh et al., 2005, Satoh et al., 2006). One can hypothesize that decreased expression of HSPA1A in pDCs of MS patients could affect the ability of pDCs to induce regulatory T cells. HSPA1A or factors which are responsible for its inhibition could be potential specific therapeutic targets to treat MS.

It is well known that B7 co-stimulatory family ligands can stimulate or inhibit T cell responses. Butyrophilin family (BTN1, BTN2, and BTN3) and butyrophilin-like family (BTNL2, BTNL3, BTNL8, and BTNL9) of proteins have structural homology to the B7 family ligands. Our microarray gene expression data revealed decreased expression of BTN3A3 in untreated MS patients. BTN3A3 as well as other members of the family such as

BTN1A1, BTN2A2, BTNL1, and BTNL2 are negative co-stimulatory regulators of T cell responses (Arnett et al., 2007, Nguyen et al., 2006, Yamazaki et al., Smith et al., Yamashiro et al.). We hypothesize that there is impaired inhibition of T cell response due to the decreased expression of BTN3A3 in MS patients which can be restored by IFN-beta treatment.

The GADD45 genes (GADD45A, GADD45B, and GADD45gamma) participate not only in cell cycle and apoptosis processes, they have been also implicated in regulating of Th1 immune responses (Lu et al., 2004, Lu et al., 2001, Jirmanova et al., 2007). More specifically, the expression of GADD45A in dendritic cells plays the critical role in induction of Th1 responses (Jirmanova et al., 2007). Thus, increased expression of GADD45A in pDCs of MS patients could contribute to increased Th1 polarization. IFN-beta treatment inhibits GADD45A expression which may lead to decreased Th1 responses. Further experiments are required to confirm the suggestion.

THBS1 is a distinct matricellular protein which has diverse functions in different biological processes. The expression of several extracellular matrix (ECM) proteins including THBS1 was increased in MS brain lesions (Mohan et al.). The authors could not identify the cellular source of those upregulated ECMs, but they suggested that increased TGF-beta expression in MS lesions (Baranzini et al., 2000) is responsible for the increased expression of ECM proteins which in turn stimulate immune cells to produce pro-inflammatory chemokines. It is well known that THBS1 activates latent TGF-beta1 (Schultz-Cherry and Murphy-Ullrich, 1993, Schultz-Cherry et al., 1994b, Schultz-Cherry et al., 1994a). Taken together, increased expression of THBS1 in pDCs of MS patients could activate latent TGF-beta1 resulting in increased expression of ECM proteins and increased pro-inflammatory cytokines/chemokines secretion. The role of THBS1 has been described in other peripheral inflammatory diseases. Increased level of THBS1 and TGF-beta were found in plasma of rheumatoid arthritis (RA) patients (Rico et al., 2008). THBS1 has been suggested as a new therapeutic target in RA (Rico et al.). Therefore, it is conceivable that the aberrant expression of THBS1 in pDCs of MS patients could contribute to abnormal immune responses.

2',5'-oligoadenylate synthetase (OAS) family genes (OAS1, OAS2, OAS3) are interferon response genes which are well known for their antiviral function by activation of RNase L (Zhou et al., 1993, Hovanessian, 1991). Three distinct forms of OAS genes (OAS1, OAS2, and OAS3) exist in different cellular components and have different enzyme activities (Marie et al., 1997, Marie et al., 1990). It is possible that each protein has other specific functions in addition to antiviral properties. For example, OAS1 and OAS3, but not OAS2, prevent the replication of Dengue virus (a flavivirus). In our microarray data, only OAS2 expression was deficient in untreated MS patients. All OAS family genes were up-regulated by IFN beta treatment.

Cell migration is one of the critical factors for pDCs to serve as antigen-presenting cells. First, pDCs need to travel to seek and capture self/non-self antigens. Then, pDCs migrate to lymph nodes to prime T cells in order to induce tolerance or immune responses. Tensin regulates cell migration (Chen et al., 2002). Decreased expression of Tensin1 (TNS1) has been associated with different types of human cancers (Rhodes et al., 2004, Chen et al., 2000). Decreased expression of TNS1 in pDCs of untreated MS patients could affect their migratory pattern and the ability to regulate immune responses.

IL6ST (gp130) is the signal transducer of IL6, LIF, CT-1, OSM, CNTF, IL-11, and IL27 (Taga and Kishimoto, 1997) and it is a critical component of a wide variety of biological responses such as inflammation and cell proliferation through JAK-STAT or MAPK or

PI3K/AKT pathway. MS patients have increased gp130 expression in serum and decreased gp130 expression in CSF (Padberg et al., 1999). In addition, increased expression of gp130 was discovered in brain tissue of both MS patients and animal models of MS (Tseveleki et al., Dutta et al., 2007). Our microarray data showed overexpression of gp130 in pDCs. However, the difference between MS patients and healthy controls was not significant based on RT-qPCR. CHEK1 (check point kinase 1) is involved in multiple pathways and interacts with many proteins. For instance, CHEK1 can manipulate TLR and TGF-beta signaling pathways by interacting with RelA (Rocha et al., 2005) and SMAD4 (Barrios-Rodiles et al., 2005), respectively. Through the interaction with SMURF1 and SMURF2 (Barrios-Rodiles et al., 2005), HDGFRP3 regulates TGF-beta signaling. Taken together, TGF-beta signaling in pDCs of MS patients could be affected by overexpression of CHEK1 and HDGFRP3.

As the gene set B included a number of Type I IFN responsive genes (USP18, IFIH1, XAF1), one could hypothesize that pDCs, one of the main cell sources of IFN-alpha, are impaired in patients with MS. However, neither the frequency of IFN-alpha producing pDCs nor the total amount of IFN-alpha produced upon stimulation with TLR9 agonists were decreased in patients with MS based on our studies (Aung et al., 2010, Balashov et al., 2010). The gene expression levels of IFN-alpha were not significantly changed in pDCs of MS patients based on our microarray results (data not shown). The question whether Type I IFN receptor-mediated signaling pathways are impaired in pDCs of patients with MS remains open. In summary, our studies identified novel genes involved in immunomodulatory mechanisms and linked to IFN-beta therapy in MS. These genes may be potential therapeutic targets for MS. Further studies are needed to validate the pathophysiologic involvement of these genes in MS and to elucidate their exact role in MS pathogenesis and treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

MS	multiple sclerosis
RRMS	relapsing-remitting MS
IFN-beta	interferon-beta
pDCs	plasmacytoid dendritic cells
DMT	disease-modifying treatment
PBMC	peripheral blood mononuclear cells
TLR9	Toll-like receptor 9
CSF	cerebrospinal fluid
RT-qPCR	real-time quantitative polymerase chain reaction

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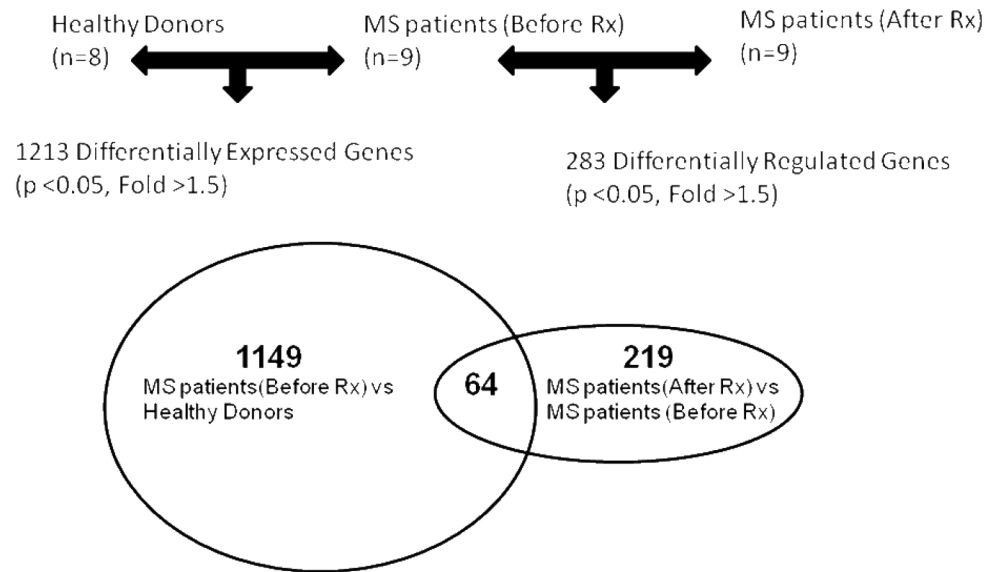


Figure 1. Pharmacogenomic analysis of plasmacytoid dendritic cells (pDCs)
pDCs from 8 healthy donors and 9 MS patients before and after IFN-beta treatment (Before Rx and After Rx) were separated and gene expression analysis was examined by microarrays. The numbers of differentially expressed/regulated genes between subject groups (based on the criteria of p < 0.05 and fold change > 1.5) are shown.

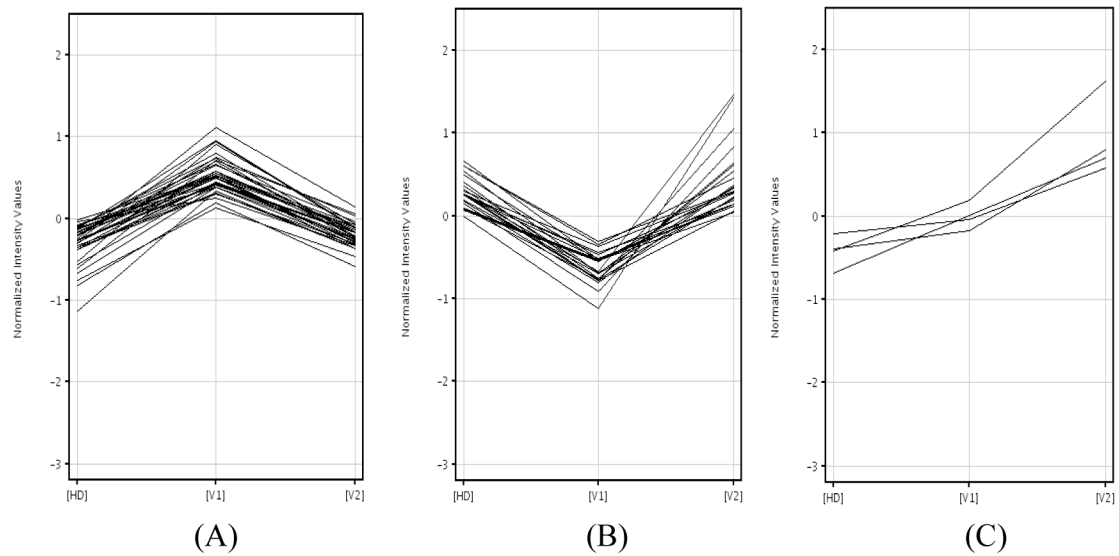


Figure 2. Expression pattern of “disease-linked and IFN-beta-regulated” genes

The 64 “disease-linked and IFN-beta-regulated” genes were separated into several groups based on change in their expression pattern in the 8 healthy donors (HD), 9 MS patients before treatment (V1), and the same 9 MS patients after treatment with IFN-beta (V2). Thirty-seven genes were up-regulated in untreated MS patients and down-regulated (corrected) by IFN-beta treatment (A). Twenty-seven genes were down-regulated in untreated MS patients and up-regulated (corrected) by IFN-beta treatment (B). Four genes were up-regulated in untreated MS patients and further up-regulated by IFN-beta treatment (C). The Y-axis is the normalized intensity values and each line represents the average normalized expression of a single gene.

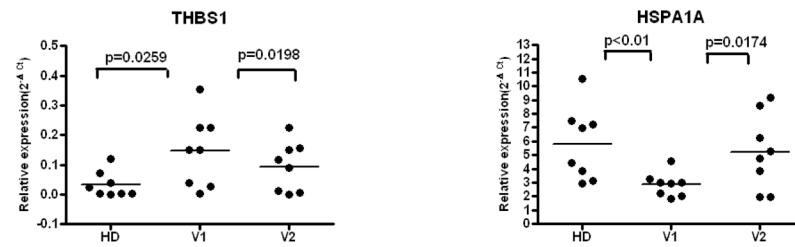


Figure 3. Validation of Microarrays Gene Expression results by RT-qPCR

Expression of THBS1 and HSPA1A relative to HPRT1 (house-keeping gene) was measured by RT-qPCR in healthy donors (HD) (n=8) and MS patients before (V1) and after IFN-beta treatment (V2) (n=8). The primers and RT-qPCR conditions are described in the method section. Fold difference in gene expression was calculated using the formula $2^{-\Delta C_t}$.

Table 1

Patients and healthy subjects enrolled in the study.

Subjects:	Type of MS (Number)	Age (Mean \pm SEM)	Female/Male ratio	Disease Modifying Drugs (number of patients)
HD	N/A (8)	34.78 \pm 3.196	6/2	None
MS: No Rx *	RR MS (9)	35 \pm 3.193	7/2	None
MS: IFN- β *	RR MS (9)	35 \pm 3.193	7/2	IFN beta-1b, subcutaneous (5) IFN-beta-1a, intramuscular (4)

* The same MS patients were tested at two time points: before treatment and 3 months after treatment with IFN-beta.

Table 2

Molecular functions of genes involved in immune system process.

Gene Symbol	Gene Title	Molecular Function
HSPA1A (Set A)	Heat shock 70kDa protein 1A	DNA/RNA helicase activity hydrolase activity RNA splicing activity RNA binding
OAS2 (Set A)	2'-5'-oligoadenylate synthetase 2	nucleic acid binding nucleotidyltransferase activity
BTN3A3 (Set A)	Butyrophilin, subfamily 3, member A3	ubiquitin-protein ligase activity
TNS1 (Set A)	Tensin 1	hydrolase activity phosphatase activity protein binding structural molecule activity
THBS1 (Set B)	thrombospondin 1	receptor binding enzyme regulator activity
HDGFRP3 (Set B)	Hepatoma-derived growth factor, related protein 3	DNA binding receptor binding transcription factor activity
IL6ST (Set B)	Interleukin 6 signal transducer (gp130, oncostatin M receptor)	cytokine receptor activity receptor binding
GADD45A (Set B)	Growth arrest and DNA-damage- inducible, alpha	protein binding
FCGR2A (Set B)	Fc fragment of IgG, low affinity IIa, receptor (CD32)	receptor activity
CHEK1 (Set B)	CHK1 checkpoint homolog (S. pombe)	kinase activity

Table 3

Pathway analysis of genes involved in immune system process.

Gene Symbol	Gene Title	KEGG pathway (category)	Pathway
HSPA1A	heat shock 70kDa protein 1A	Genetic Information Processing; Transcription	Spliceosome
		Genetic Information Processing; Folding, Sorting and Degradation	Protein processing in endoplasmic reticulum
		Environmental Information Processing; Signal Transduction	MAPK signaling pathway
		Cellular Processes; Transport and Catabolism	Endocytosis
		Immune System	Antigen processing and presentation
		Human Diseases; Neurodegenerative diseases	Prion diseases
		Human Diseases; Infectious Diseases	Measles, Influenza A, Toxoplasmosis
OAS2	2'-5'-oligoadenylate synthetase 2	Human Diseases; Infectious Diseases	Hepatitis C, Measles, Influenza A, Herpes simplex infection
THBS1	thrombospondin 1	Environmental Information Processing; Signal Transduction	TGF-beta signaling pathway
		Environmental Information Processing; Signaling Molecules and Interaction	ECM-receptor interaction
		Cellular Processes; Transport and Catabolism	Phagosome
		Cellular Processes; Cell Growth and Death	p53 signaling pathway
		Cellular Processes; Cell Communication	Focal adhesion
		Human Diseases; Cancers;	Bladder cancer
		Human Diseases; Infectious Diseases	Malaria
IL6ST	interleukin 6 signal transducer (gp130, oncostatin M receptor)	Environmental Information Processing; Signal Transduction	Jak-STAT signaling pathway
		Environmental Information Processing; Signaling Molecules and Interaction	Cytokine-cytokine receptor interaction
GADD45A	growth arrest and DNA-damage-inducible, alpha	Environmental Information Processing; Signal Transduction	MAPK signaling pathway
		Cellular Processes; Cell Growth and Death	Cell cycle, p53 signaling pathway
FCGR2A	Fc fragment of IgG, low affinity IIa, receptor (CD32)	Cellular Processes; Transport and Catabolism	Phagosome
		Immune System	Fc gamma R-mediated phagocytosis
		Development	Osteoclast differentiation
		Human Diseases; Immune System Diseases	Systemic lupus erythematosus
		Human Diseases; Infectious Diseases	Staphylococcus aureus infection, Tuberculosis, Leishmaniasis

Gene Symbol	Gene Title	KEGG pathway (category)	Pathway
CHEK1	CHK1 checkpoint homolog (S. pombe)	Cellular Processes; Cell Growth and Death	Cell cycle, p53 signaling pathway
		Human Diseases; Infectious Diseases	HTLV-I infection