Gene Expression Studies in Multiple Sclerosis

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Abstract: Multiple sclerosis (MS) is a serious neurological disorder affecting young Caucasian individuals, usually with an age of onset at 18 to 40 years old. Females account for approximately 60% of MS cases and the manifestation and course of the disease is highly variable from patient to patient. The disorder is characterised by the development of plaques within the central nervous system (CNS). Many gene expression studies have been undertaken to look at the specific patterns of gene transcript levels in MS. Human tissues and experimental mice were used in these gene-profiling studies and a very valuable and interesting set of data has resulted from these various expression studies. In general, genes showing variable expression include mainly immunological and inflammatory genes, stress and antioxidant genes, as well as metabolic and central nervous system markers. Of particular interest are a number of genes localised to susceptible loci previously shown to be in linkage with MS. However due to the clinical complexity of the disease, the heterogeneity of the tissues used in expression studies, as well as the variable DNA chips/membranes used for the gene profiling, it is difficult to interpret the available information. Although this information is essential for the understanding of the pathogenesis of MS, it is difficult to decipher and define the gene pathways involved in the disorder. Experiments in gene expression profiling in MS have been numerous and lists of candidates are now available for analysis. Researchers have investigated gene expression in peripheral mononuclear white blood cells (PBMCs), in MS animal models Experimental Allergic Encephalomyelitis (EAE) and *post mortem* MS brain tissues. This review will focus on the results of these studies.

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1- INTRODUCTION

MS is a demyelinating disease with an active immune component. Myelin, composed of a lipid bilayer and proteins, forms the extended membrane of oligodendrocytes and insulates neurons to provide rapid conduction of the action potential along axons. The CNS in MS is affected with patches of myelin degeneration produced by multifocal inflammatory events. These MS white matter lesions vary in diameter from less than one centimeter to several centimeters and are most prominent in the periventricular white matter. Other regions affected include the optic nerve and chiasm, pons, the cerebellar peduncles, medulla oblongata, the spinal cord and also in the periphery of cerebral gyri [40]. Histologically, MS lesions are classified as acute or chronic (active/silent), with no relation to the clinical classification of the disease.

MS is variable in onset and progression. Females account for approximately 60% of MS cases [70] with the incidence of MS in Northern Europe, Canada, and the Northern United States being approximately 1 new case per year per 10,000 persons (20-50 years). Twin studies show higher concordance rates of MS in monozygotic, compared to dizygotic twins [54], and 15% of MS patients have an affected relative. Diagnosis of MS can only be confirmed using high technology aids, such as computerized tomography, magnetic resonance imaging or analyses involving the detection of immu-

noglobulin oligoclonal bands in the cerebrospinal fluid of MS patients. Lesions and symptoms are disseminated in time and space and MS classification is therefore based on the occurrence of attacks, recovery states, and neurological deficits [40]. Three main types are encountered: (i) Relapsing-Remitting MS (RRMS); (ii) Secondary-Progressive MS (SPMS); and (iii) Primary-Progressive MS (PPMS).

Some molecular genetic methods that can and have been used to investigate MS include: *i) Comparative Expression Microarray Analysis:* Fluorescent or radio-labeled microarray technology provides a powerful tool in understanding biological systems. Using this technology the relative activity of genes and gene pathways in two different samples can be compared. In fluorescence microarray, total RNA or mRNA is extracted from two tissues, and alternately labelled with one of either two fluorescent dyes: commonly Cy3 and Cy5. The two 'probes' are then hybridised together, and unmatched probe specifically bound to a slide containing DNA encoding many thousands of known genes. The relative fluorescent intensity of each signal is then analysed, and used to determine the relative fold difference in gene expression between test and control tissues. Statistical T-tests are commonly used to determine whether differences in gene expression observed following scanning is significant. Since differential incorporation chemistries are associated with Cy3 and Cy5, it is usual to swap the dyes and repeat the initial experiments to enable optimization of the protocol and to validate results. To date, this emerging technology has been applied to examine gene expression patterning in a variety of common biological systems, identifying pathways in cancers of the skin [53], and breast [48], as well as aiding to understand aspects of development and embryogenesis.

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ii) *Q-PCR Analysis*: Representative amplification of individual mRNA molecules can be achieved by reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Conventional RT-PCR is however, not suitable for quantitation due to the non-linear (exponential) nature of PCR product amplification. Several methods have been developed to overcome this deficiency. These include competition based assays [27] and real time-fluorescent detection of PCR product [25]. Real time-quantitative RT-PCR (or Q-PCR) has the advantage of being able to allow researchers to conveniently determine the PCR cycle at which specific product is amplified, in a linear way [75]. This value is referred to as the cycle threshold (CT) number, where a single CT difference represents a two-fold difference in the amount of specific target [35]. Modern real time PCR detection systems allow for the examination of many different gene products over a broad range of target expression. This type of data can then be used to produce standard curves, derived from known amounts of specific PCR product, which have the same primer binding sites as that of the specific cDNA target. Standard curve analysis then allows investigators to determine the amount of specific target in an unknown sample (copy number). The values derived can then be used to determine the relative abundance of a particular transcript with respect to others within the same tissue. This methodology is referred to as absolute Q-PCR analysis.

2- MICROARRAY RESULTS USING MS BRAIN TISSUE

MS is a complex autoimmune disorder of the CNS with both genetic and environmental contributing factors. Clinical symptoms are broadly characterised by initial onset, and progressive debilitating neurological impairment associated with the presence of MS plaques in the CNS. In 1999, Whitney *et al.* [72], described the analysis of MS acute lesions from a single female MS patient with PP-MS. Such plaques were compared with the white matter of the same patient and results showed 62 differentially expressed genes. The genes with increased expression in acute plaques included leucotriene A-4 hydroxylase, TNF α receptor, the autoantigen annexin XI, interferon regulatory factor 2 (IRF-2), activin Type II receptor (ACVR2), protein kinase C type β -1 (PRKCB1), myelin transcription factor-1 (MYT1) and many others. In 2001, the same team [73] undertook microarray experiments using 2 patients. The first patient had a total of 16 chronic inactive (silent) plaques and the second had an acute and a chronic active plaque. The authors compared the expression of the genes coming from these plaques to a pool of normal white matter gathered from controls. The authors found a set of differentially expressed genes in their MS tissues and confirmed their results using EAE mouse differential expression studies. Different candidates were found up-regulated in expression within EAE mice and human tissue. One of these genes consisted of the thrombin receptor gene or proteinase activated receptor 3 (PAR3). This gene was previously found up-regulated in macrophages previously stimulated with granulocyte-macrophage colony-stimulating factor (GM-CSF) [13]. The putative ligand for the IL-1 receptor-related molecule (T1/ST2) and Jun-D were also over-expressed in comparison to control mice. An interesting candidate, the arachidonate 5-lipoxygenase (5-LO), was found

up-regulated in expression in MS. This gene codes for a key enzyme in the leucotriene formation and is responsible for the passage of arachidonic acid to leucotriene A4 (LTA4). Interestingly the same team in their previous microarray study undertaken in human MS brain tissue [72], found an over-expression of a second gene, the leucotriene A4 hydroxylase (LTA4H). LTA4H is actually responsible for the passage of LTA4 to LTB4. LTB4, acting on leucotriene B4 receptor 1 (BLTR), is a potent chemotactic factor for neutrophils and induce leucocyte adhesion to endothelial cells [76]. These findings show the potential importance of the leucotrienes in MS pathology. To validate this hypothesis involving the chemoattraction pathway and the genes involved in the formation of the LTB4 chemoattractor molecule, one could focus their attention on the LTB4 omega hydroxylase or Cytochrome P450 family 4 subfamily F polypeptide 3 (LTBAH or CYP4F3). LTBA4H is a gene encoding two possible isoforms, CYP4F3A and CYP4F3B that aim at catabolising the effect of LTB4 action [59].

In 1997 Becker *et al.* [6], actually undertook the first MS gene expression studies investigating a normalized cDNA library from CNS lesions of a PP-MS sufferer. These results and other microarray expression studies are outlined in Table 1. The most important finding was a set of 16 genes all involved in autoimmunity. Three of these genes coded for proteins previously implicated in MS and include MBP, PLP and α - β crystallin. Of note, seven of these 16 genes are autoantigens associated with systemic lupus erythematosus (SLE) and two are associated with insulin dependent diabetes mellitus (IDDM). In 2001, a study [11] was performed involving a high throughput sequencing of expressed sequence tags. The authors used non-normalised cDNA brain libraries from MS brain lesions and normal control brains. They identified 330 gene transcripts common for all libraries with several of these involved in inflammatory response. Genes that were found highly expressed included Prostaglandin D synthase (PTGDS), prostatic binding protein (PBP), ribosomal protein L17 (RPL17), osteopontin (SPP1), heat shock protein 70 (HSP70), myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP). In our research, we found as well an over expression of HSP 70 within chronic active plaques. The inducible form of HSP 70 has been shown to promote myelin autoantigen presentation in APCs [44]. Of note, HSP 70 was found to be down-regulated in other studies [9, 37]. The over expression of PTGDS interrogates once again about the important role that may play arachidonic acid related metabolites in MS neuroinflammation. Whitney *et al.* [72-73], showed the enzymatic involvement of the 5-LO and leucotriene A4 hydrolase gene in the production of leucotriene proinflammatory molecules in MS disorder. In addition, Chabas *et al.* [11], showed that the second enzymatic pathway that metabolises acid arachidonic might also be playing a significant role in MS pathology. The cyclo-oxygenase pathway, with prostaglandin-endoperoxide synthase 1 and 2 (COX 1 and COX2), transforms arachidonic acid (AA) into prostaglandins (PGG2 series and PGH2 series). PGH2 is turned into PPD2 by PTGDS, the enzyme that Chabas *et al.* found in high amounts in MS cDNA libraries [11]. The prostaglandins and leucotrienes are both proinflammatory molecules and might play a significant role in MS pathology. Of interest, PGJ2 a molecule derived

Table 1. Results of Microarray Experiments on Human MS Brain Samples

Gene Expression in MS	MS Patients	Control	Type of Array or cDNA Libraries	Candidate Gene Validation Technique
Becker <i>et al.</i> , [6]	PP-MS:3 CA with NAWM	2 control libraries	Normalised cDNA library	None
Whitney <i>et al.</i> , [72]	1 PP-MS: 2 A	1 NAWM©	RCA(³³ PdCTP) ~ 5000 genes	IHC
Whitney <i>et al.</i> , [73]	1 PP-MS:16 CS 1RR-MS:1 A +1 CA	3 NWMΘ	RCA (³³ PdCTP) 2798 genes	EAE (SJL/J <i>via</i> MBP87-99 and C57Bl/6 MOG 35-55) + IHC
Lock <i>et al.</i> , [37]	CP-MS: 1A + 1CA SP-MS:1CA + 1CS SP-MS:1CA + 1CS CP-MS4:1CS	2: NWM + WB	OFA ~5000 genes	EAE (C57BL/6 <i>via</i> MOG 35-55)
Mycko <i>et al.</i> , [44-45]	4 SP-MS: 2CA + 2CS	©	RCA (³² PdATP) 588 genes	Real time PCR
Chabas <i>et al.</i> , [11]	3 MS	1 control library	Non normalised cDNA libraries ~ 4000 clones/library	EAE (SJL/J <i>via</i> PLP 139-151; 129/C57Bl/6 <i>via</i> MOG 35-55)
Tajouri <i>et al.</i> , [63]	5 MS	5 NWM	~5000 genes	Real time PCR
Lindberg <i>et al.</i> , [34]	6 SP-MS (acute lesions and NAWM)	12 controls Θ	12633 genes	Real time PCR

© Tissue obtained from the same MS sufferer; CP-MS: Chronic progressive MS; EAE: Experimental allergic encephalomyelitis; IHC: Immunohistochemistry; NAWM: Normal apparent white matter; NWM: Normal white matter (None MS patient); OFA: Oligonucleotide fluorescence based arrays; RCA: Radioactive cDNA based arrays; WB: Whole brain; Θ Pooled tissues.

from PGD2, is a natural ligand of the peroxisome proliferator activated receptor (PPAR γ). PPAR γ acts as an anti-inflammatory element and inhibit the pro-inflammatory IL12 cytokine. PPAR γ was found with higher gene expression levels in EAE mice treated with Lovastatin drugs [46]. Further implicating the cyclooxygenase enzymatic pathway can be found in Paintlia *et al.* study [46] in which Lovastatin treated EAE mice showed reduced expression of the COX2 enzyme. Taken together, this implicates that the transformation of PGD2 into PGJ2 might play a potential role in MS. Enzymatically, PGD2 can be either transformed into PGJ2 or PGF2. Of note, the product of prostaglandin F synthase (PFS), PGF2, was reported to be involved in acute demyelination of peripheral nerves [21]. Additionally, in Chabas *et al.*, [11] decreased transcription levels were observed for synaptobrevin (VAMP3), amyloid beta precursor protein-binding, family B, member 1 (APBB1), LDL-receptor related protein (LRP1), glycogen synthase kinase 3 alpha (GSK3A), brain specific sodium-dependent inorganic phosphate co-transporter or solute carrier family 17 (SLC17A7). Chabas's team placed their attention on the increase of osteopontin transcripts in MS. A closer analysis of this candidate was performed on EAE mice. Interestingly, a knock out mouse for osteopontin showed in their study a decrease in EAE severity when compared to control mice [11]. However, Blom *et al.*, [8] published a comment on Chabas's work [11] after their studies using a knockout mouse for the osteopontin gene (OPN-/- 129/C57/BL10 with q haplotype: B10.Q usually susceptible to EAE). The gene OPN was solely and completely inactivated with the use of fully backcrossed mice. EAE mice were induced by injections of recombinant rat MOG myelin proteins emulsified in complete Freund

adjuvant. The results from Blom *et al.* showed no decrease in severity of these EAE OPN-/- mice and such data were in direct contradiction with Chabas's findings. Blom hypothesized that the knock out mouse model used in Chabas's work could have knock-out OPN-linked polymorphic genes and explain the decrease in EAE severity. The genes closely linked to OPN that have potential inflammatory functions were cited and accounted for 14 genes. This would include the IFN-gamma-inducible protein 10 (IP-10 or CXCL10) a chemoattractant factor localised on chromosome 4q21. CXCL10 is a chemokine that preferentially attracts Th1 lymphocytes through its receptor CXCR3, expressed at high levels on these cells [38]. IP-10 is induced in a variety of cells in response to the Th1 cytokine IFN-gamma [41]. IP-10 expression is most often associated with Th1-type inflammatory diseases, where it is thought to play an important role in the recruitment of Th1 lymphocytes into tissues. Of note, Tajouri's work [63] showed that CXCL10 was over-expressed in chronic active plaques by a fold increase of 2.5 whereas this increase was more prominent in acute plaques in secondary progressive MS brains. Relapses in MS often are preceded by increased TH1 cytokine levels and decreased levels of TH2 cytokines. Remissions, on the other hand, exhibit a rise in the anti-inflammatory TH2 cytokines [74, 77]. CXCL10 levels are related to clinical relapses in EAE [15, 10] and the source of production of CXCL10 is from astrocytes in EAE mice [65]. Immunoreactivity to CXCL10 was shown in demyelinating plaques [22] Also, this protein is found in higher levels within the CSF of MS patients compared to healthy controls [16] and such levels of expression correlate with the count of leucocytes in the CSF [61]. Anti- CXCL10 reduces disease activity in common

EAE [15]. In viral model of MS (chronic demyelinating phase of mouse hepatitis virus infection of the CNS), mice showed a decrease severity of their pathology [36]. CXCL10 acts on a receptor, the CXC-chemokines Receptor 3 (CXCR3) that is localised genetically on chromosome X (Xq13). The gene for CXCR3 was localised on human chromosome Xq13 which is in clear contrast to all other chemokine receptor genes, suggesting unique function(s) for this receptor and its ligands that may lie beyond their established role in T cell-dependent immunity [38]. CXCR3 is found over-expressed in macrophages, T cells and reactive astrocytes in MS plaques [60]. Perivascular cuffs in *post mortem* MS lesions showed CXCR3+ cells presence correlating with an increase of interferon gamma production (Balashov *et al.*, 1999). In 2002, Sorensen *et al.* showed a continuous accumulation of CXCR3 +cells in lesion formation of MS patients [61]. Targeting the CXCR3 receptor *via* antagonists could alter T-cell diapedesis through the CNS in MS [51]. Hong *et al.*, [20] demonstrated that treatment with Glatiramer acetate was significantly reducing the expression of CXCR3.

Tajouri *et al.*, [63] used RNA from MS chronic active and MS acute lesions. RNA was extracted, and compared with patient matched normal white matter by fluorescent cDNA microarray hybridisation analysis. This resulted in the identification of 139 genes that were differentially regulated in MS plaque tissue compared to normal tissue. Of these, 69 genes showed a common pattern of expression in the chronic active and acute plaque tissues investigated; while 70 transcripts were uniquely differentially expressed (≥ 1.5 -fold) in either acute or chronic active tissues. These results included known markers of MS such as the myelin basic protein (MBP) and glutathione S-transferase (GST) M1, nerve growth factors, such as nerve injury-induced protein 1 (NINJ1), X-ray and excision DNA repair factors (XRCC9 & ERCC5) and X-linked genes such as the ribosomal protein, RPS4X. Several genes were involved in inflammation including a number of leucocyte markers that are present in MS plaques. As an example, the gene granulins has been found to be slightly up-regulated compared to normal controls. Granulin is a novel class of growth regulators expressed by leucocytes [4]. This gene is normally not expressed in normal brains but in brain glial tumour cells [33] and located at 17q21.32, a region of suggestive linkage in MS pathology [17]. In addition complement molecules or acute phase proteins such as Complement component 1, q subcomponent, beta polypeptide (C1QB) were found to be up-regulated in expression in the most inflammatory forms of plaque types, the acute plaques. The expression of C1QB may originally come from blood vessel endothelial cells and could act detrimentally on the CNS with this complement inflammatory molecule [29]. Interestingly, this inflammatory gene is involved in sporadic amyotrophic lateral sclerosis neurodegeneration in which high levels of gene expression are found in *post mortem* tissues [18]. In parallel, anti-inflammatory proteins such as endothelial protein C receptor (PROCR) were found, in our study, to be dramatically down-expressed in acute inflammatory plaques but this effect was less pronounced in chronic active plaques.

Lock *et al.*, [37], investigated the differences in gene expression between acute and chronic silent plaques from 4

MS individuals and found 1080 genes with a fold change of >2 in at least 2 out of 4 MS samples. Genes expressed in 4/4 MS samples were classified according to the type of lesion studied. Over-expressed genes included T- B and macrophage cell related genes, growth and endocrine factors, granulocyte and mast cell related genes as well as neurogenic and remyelinating factors. As an example, interleukin 17 (IL-17), transforming growth factor 3 (TGF- β 3), adrenocorticotrophic hormone receptor (ACTHR), tryptase-III and immunoglobulin E receptor β chain (Ig $\epsilon\beta$) and matrix metalloproteinase 19 (MMP-19) were up-regulated in expression only in chronic silent plaques. In acute plaques, melanocortin-4 receptor (MC4R), signal transducer and activator of transcription 5B (STAT5B), insulin like growth factor 1 or somatomedin C (IGF1), granulocyte colony stimulating hormone (G-CSF) and interferon, alpha-inducible protein (G1P2) transcripts were over represented. Of note, G-CSF was also found over-expressed in the acute phase of EAE animals [10]. Of interest as well, some pregnancy related genes were differentially expressed such as an increased of expression of pregnancy-specific β 1 glycoprotein (PSG3) in acute plaques, a decreased expression for PSG11 in chronic silent. In Tajouri *et al.*, [63] experiments, a dramatic increase of PSG3 occurs in acute plaques and interestingly this gene is genetically localised on 19q13.2, a promising MS linked susceptibility locus [47]. Of note, PSG molecules are actually co-expressed in the late stage of placenta formation with gut-enriched Kruppel-like zinc finger protein gene (GKLF4) [7]. Of interest, GKLF4 is found prominently decreased in expression with interferon β therapy [62], a treatment of high efficacy in treating relapsing remitting MS (RR-MS) affecting mostly women.

Mycko *et al.*, [44] established arrays to compare MS chronic active plaques and chronic inactive plaques. They investigated as well the differential gene expression in between the centre and the margin of such plaques. This resulted in the identification of very interesting features such as an increased level of expression of adenosine A1 receptor (ADORA1) in the marginal zone of the chronic active plaques. Studies on EAE animals depleted of the ADORA1 gene showed an increased severity of the disease course [66]. Consequently, ADORA1 may be involved in reducing the ongoing worsening effect of inflammation in MS lesions. The purine nucleoside adenosine inhibits IL-12 and this effect results in the increase of the Th2 type IL 10 mediator [19]. Additionally in Mycko *et al.* [44], an up regulation of expression was observed for the myelin transcription factor (MyT1) in the margins of chronic active lesions. Such MyT1 factor, precluding of ongoing attempts of remyelination in MS plaques, was previously identified as over-expressed in acute plaques in Whitney *et al.*, [72]. DNA repair related genes such as the X-ray repair complementing defective repair in Chinese hamster cells 9 (XRCC9) were also found up-regulated in the margins of chronic active and silent plaques. In our array data of this current thesis, XRCC9 gene was down regulated in MS acute and chronic active plaques.

Lindberg *et al.*, [34], used oligonucleotide DNA chips that included a total of 12 633 probes. He investigated the gene expression of MS lesions and NAWM (surrounding these lesions) that were extracted from SP-MS brain patients. Common immune responsive and neural homeostatic related

genes were altered in expression. As an example, the neural development factor Ephrin receptor (EPBR), the cytoskeletal genes tubulin A and B and the pro-inflammatory interleukin 6 receptor were all increased in expression. The gene lysosome-associated membrane protein 2 (LAMP2), a neuro-lysosomal protector was down-expressed as well as synaptojanin 2b (SYNJ2), a gene involved in vesicle recycling.

3- MS MICROARRAY RESULTS USING MS PERIPHERAL BLOOD MONONUCLEAR CELLS

Peripheral blood cells (PBMC) from MS individuals have been used to extract mRNA and to investigate gene expression levels were investigated by microarray experiments. Bompreszi *et al.*, [9] used a set of PBMC from fresh blood obtained from 14 MS patients and 7 controls but also frozen blood from 3 MS patients and 2 controls. A second set of cells was investigated and obtained from frozen blood of 10 MS patients and 10 controls. All of these patients were chosen under the condition of non-previous therapy. The differential gene expression from this study revealed 303 differentially expressed candidate genes. Among these, the platelet activating factor acetyl hydrolase (PAFAH1B1), a gene involved in brain development and chemoattraction during inflammation and allergy, was found with an increased transcript expression in MS peripheral blood cells when compared to controls. Tumour necrosis factor receptor (TNFR or CD27) is found also highly regulated in these MS cells. This gene is a costimulator for T cell activation and is crucial for immune response development. The T cell receptor (TCR) gene was also found increased in expression as well as the zeta chain associated protein kinase (ZAP70). TCR is essential for T cell mediated immune response and has been implicated in MS susceptibility [5]. ZAP70 is directly implicated in TCR induced T cell activation [12]. Other candidates such as zinc protein 128 (ZNF128) and transcription factor 7 (TCF7) play a role in T cells and both were found at higher expression levels in MS blood cells. Cytokines are numerous and act on cytokine receptors during inflammation. The interleukin 7 receptor (IL7 R) is up-regulated in Bompreszi *et al.* [1], as well as the myelin and lymphocyte protein (MAL). This receptor plays roles in B cells and T cells activation and particularly is involved in $\gamma\delta$ T cells. $\gamma\delta$ T cells are present in MS lesions and their inhibition decrease the severity of EAE mice and induced the reduction of proinflammatory cytokines and iNOS expression [49]. The main down-regulated genes under-expressed were tissue inhibitor of metalloproteinase 1 (TIMP1), plasminogen activator inhibitor 1 (SERPINE 1), the histone coding genes, and the heat shock protein 70 (HSP70), an autoantigen implicated in the ubiquitin proteasome pathway for the degradation of cytokines.

A second study by Ramanathan *et al.*, [50] investigated RR-MS patients within their clinical remission to investigate around 15 thousand genes. The results have shown common differential gene expression implicated in TCR activation such as the cAMP responsive element modulator and lymphocyte specific protein tyrosine kinase (LCK), both found at a high level of expression. Interleukin receptor gene was also found up-regulated in MS blood compared to controls. Detoxification genes were increased in expression such as

haemoglobin scavenger receptor (M130 or CD163 antigen), as well as high expression levels of autoantigens such as autoantigen PM-SCL. Interestingly, a high level of gene transcripts was found for the melanocyte specific transporter protein gene (P protein) a gene involved in the oculocutaneous albinism disorder [32].

Other studies on PBMCs were undertaken but differential expression studies have focused on MS patients treated with particular therapeutics and comparison of their response was made against non treated controls. Interferon β therapy (Betaferon and Avonex drugs) in MS is effective due to its immunosuppression activity and was investigated in a few studies. The action of interferon beta is thought to play a role in decreasing the MHC class II molecules on the surface of glial cells (thus diminishing their capacity as antigen presenting cells) [55]. Also, interferon β is thought to decrease the disruption of the blood brain barrier [77] and to shift a proinflammatory Th1 mediated immunity to Th2 immunity [26]. Koike *et al.*, [30] performed microarray experiments on T cells using 13 MS patients, before and after interferon β therapy. Data showed 21 differentially expressed genes after treatment with beta interferon and nine of these genes possess interferon responsive elements. Of particular interest, this study upon interferon beta treatment showed the down regulation of gene expression of tumour necrosis factor alpha induced protein 6 (TNFAIP6 or TSG-6). TSG-6 is a gene previously found implicated with murine experimental arthritis, another form of autoimmune disease [3]. An interesting conclusion held by the author is the exclusion of the hypothesis that interferon β treatment in MS actually shifts immunity from a Th1 to Th2 shift. This is in concordance with the work of Wandinger *et al.*, [68] and Sturzebecker *et al.*, [62]. Sturzebecker *et al.*, [62] investigated the gene expression profile of PBMCs ex vivo and *in vitro* from 10 RR-MS patients with interferon therapy. The authors noted altered gene expression for interferon related genes such as an up-regulation of STAT1. Interestingly, they found the down regulation of IL 8 gene, a known chemoattractant for neutrophils, but as well a down-regulation of a fair number of proliferative effectors. This anti-proliferative effect was evident especially *via* the down regulation of gene expression of FBJ murine osteosarcoma viral [v-fos] oncogene homolog (cFos), protooncogene cJun (c-Jun) and FMS-related tyrosine kinase 3 (Flt-3). The gut-enriched Kruppel-like zinc finger protein (GKLF4) was found prominently decreased in expression with interferon β therapy. This gene is thought to play a role in pregnancy specific glycoproteins (PSG) gene expression control since both GKLF4 and PSG molecules are co-expressed in the late stage of placenta formation [7]. Of interest, studies on Pregnancy in Multiple Sclerosis (PRIMS) show that the third trimester of pregnancy is the subject of a marked reduction in relapse rate [67]. Surprisingly, Sturzebecker reports an up-regulation of pro-inflammatory chemokines such as interferon-gamma-inducible protein 10 (IP-10 or CXCL10), monocyte chemoattractant protein 1 (MCP1 or SCYA2 or CCL2) and karyopherin beta-2 (Mip1). Previous gene profiling studies by the same research team by Wandinger *et al.*, [68], has shown that proinflammatory factors such as interleukin 12 receptor β 2 (IL12R β 2) chain as well as chemokine, CC motif, receptor 5 (CCR5) were also up-regulated in expression in MS peripheral blood cells after

interferon β treatment. IL12R β 2 is also found by Hong *et al.* [20], to be significantly over-expressed with interferon β . Although, the inhibition of IL12R has been reported to be mediated by interleukin β induced interleukin 10 dependant activation pathway [69], such various findings show the eventual reason why some MS patients do fail to respond to interferon β treatment. The cytokine gene profiling results from Wandinger *et al.* [68] also rules out partially the hypothesis that interferon β therapy induces a Th1-Th2 shift in PBMC of MS patients. Such an idea is further supported by additional findings showing increased expression, after interferon β therapy, of other Th1 mediators such as Chemokine (C-C) receptor 5 (CCR5). CCR5 being the chemokine receptor for normal T-cell expressed and secreted (RANTES) and the two isoforms of the chemoattractor macrophage inflammatory protein 1 cited above (MIP1 α and MIP1 β). The gene CCR5 has already been found at high level of expression in acute phase of EAE animals and low in expression during the recovery phase of these animals [10]. Interestingly, CCR5 is significantly down-expressed in MS with Glatiramer acetate drug treatment [20] and such a treatment could compensate for the interferon β inability to decrease CCR5. Of note, CCR5 is also down-regulated in expression with Lovastatin drug treatment in EAE mice [46] and seems to be a key factor in remission in EAE mice [10].

Also, the up-regulation of some proinflammatory markers after interferon β therapy has been noted. An interesting study by Der *et al.* [14] performed oligonucleotide array experiments with untreated HT1080 cells and cells treated with interferon α - β or γ . The results attempted to identify levels of gene expression of interferon regulated and non-regulated genes. The interferon regulated genes such as interferon-induced protein P78 (MxA) (MxA is homolog to Myxovirus influenza resistance 1: MX1) and the interferon-inducible protein p78, second locus (MxB, homolog MX2) showed an up-regulation of gene expression following interferon β treatment but were not differentially expressed with interferon γ . Consequently, MxA and MxB over-expression with interferon β are in favour and support the findings of Wandinger *et al.* [68]. Significant increased of expression of MxA was also found in MS peripheral blood cells after interferon β therapy [20]. However, in Wandinger *et al.* [68], large multifunctional protease 2 (LMP2), with a role in antigen presentation and IL-15R α chain were found with high levels of transcripts after interferon β therapy. Additional microarray experiments examining interferon β -responsive transcripts in PBMC of MS patients, have shown that in Avonex-treated MS patients (interferon β treatment), the gene LMP2 is inversely modulated compared to Avonex non treated MS patients [24]. Such high levels of LMP2 in both studies may not be due to the interferon β therapy by itself but simply due to the increase of interferon γ concentration along with interferon β therapy. Der's [14] research has also shown that over representation of transcripts from LMP2 is dependent on interferon γ exclusively but not dependent on interferon β treatment. Interestingly, Wandinger *et al.*, [68] report that IFN- γ gene expression is actually increasing transiently after two months of interferon β therapy during the course of MS pathology.

Hong *et al.*, [20], investigated PBMC from 18 MS patients treated with interferon β -1a and a group of 12 MS pa-

tients treated with Glatiramer acetate. Interferon related genes were differentially expressed with interferon β but also Th1 type molecules were increased in expression. Additionally, Glatiramer acetate treatment shows that some of these proinflammatory molecules were indeed down-expressed with this drug.

Iglesias *et al.*, [24] undertook a study investigating Avonex treatment. The methodology consisted in comparing peripheral blood cells from 5 RR-MS, treated with the drug, to 5 RR-MS without Avonex free. A second comparison was made against healthy blood donors. A set of 6800 genes was screened in this microarray experiment and data were focused mainly on the E2F pathway, a pathway of high interest in autoimmunity [43]. This pathway is triggered by interleukin 2, a potent interleukin involved in maturation and activation of T cells. Briefly, IL2 acts on IL2 receptor leading to a phosphatidyl 3-kinase dependant intracellular cascade inducing subtypes of E2F proteins (E2F 1-3 are downstream activators; E2f 4-5 are repressors). E2F transcription factors bind to DNA and induce immune cell proliferation and S phase entry in the cell cycle. The listing of genes resulting from the microarray experiments in Iglesias *et al.*, [24] showed a common up-regulation of expression of histone genes in MS. Interestingly, the histone genes and Fas1, that are normally increased in MS pathology, and decreased in expression in the presence of the Avonex drug. Additionally, the gene GM-CSF receptor β chain (CSF2RB), E2F3 and histone H4/D (HIST1H4A), were increased in MS but were inversely modulated in PBMCs from Avonex-treated patients when compared to untreated MS patients. Of interest, the H4/D gene is localised at 6p21, a strong MS linked chromosomal locus. On the other hand the gene E2F2, found up-regulated in PBMC of MS patients, was not inversely modulated by the action of Avonex. Avonex appears to be inhibiting the E2F3 pathway and has a strong negative effect on the monocyte activation factor GM-CSF but no effect on the differentiation of thymocytes from precursor cells [absence of inverse modulation found for the gene thymopoietin (TMPO)]. The author also found the down-regulation of expression in MS PBMC of the gene O-6-methylguanine-DNA methyltransferase (MGMT), a gene involved in DNA repair. DNA repair mechanism may interact directly with the E2F pathway [52]. Of note, data from our array experiments showed two differentially genes expressed that relate to DNA repair mechanism, the base excision repair gene (UNG) and BRCA1-associated RING domain protein 1 (BARD1). These two genes are involved in the E2F pathway [52] and both were down-regulated with the UNG gene being down-regulated only in chronic active plaques and the BARD1 gene being down-expressed in both chronic active and acute plaques. Of note, BARD1 showed lower down expression in chronic active plaques than within acute plaques.

Satoh *et al.*, [57] established the gene expression pattern using T cells and non T cells of Japanese MS individuals. Their result showed a down regulation of genes involved in DNA repair but as well a very abundant number of apoptotic genes. Such genes included the down regulation in MS of BCL2, TRAIL and DAXX and E2F5. In addition, they confirmed the up regulation of genes associated with inflammation such as IL1 receptor type 2, CXCL2 and ICAM1. In

2006, the same author [58] demonstrated the influence of interferon β therapy in MS. A particular gene CXCL9 was suppressed in long term treatment of interferon β in RRMS patients. Besides the findings of the common genes known to be differentially expressed in MS such as CXCL10 expression, Satoh demonstrated [58] again that pro-inflammatory chemokines are up-regulated following interferon β therapy. Such pro-inflammatory chemokines include CCR2 (monocytic) and CXCR3 (thymocytic).

4- CONCLUSIONS

Microarray experiments for gene expression in MS have revealed hundreds of significantly altered expressed genes. Some of these genes have been further investigated and have provided increased understanding of the complex pathological mechanisms involved in MS. Many more genes need further analysis and represent an interesting and exciting future in MS research. This analysis needs both a biological and physiological context to define the gene pathways involved in the disorder. Clustering analysis should aid in providing a means to classify candidates into global functional groups.

The large amount of data arising from all these microarray studies is daunting and includes several gene profiling studies of MS brain tissue, MS PBMC and animal models of MS. To solve this puzzle, pharmacological studies in MS have been undertaken in humans and animals in order to pinpoint responsive genes known to have positive effects in MS. Both approaches should aid in unraveling factors responsible for triggering MS pathology and could allow means to find new therapeutics. However, the gene expression experiments in MS brains should be carried out in more accessible and other types of tissue to gain a better picture of MS. Pharmacologically, gene profiling analysis has indicated that some proinflammatory molecules are drug resistant to interferon β therapy and seem indeed to be repressed by Lovastatin drug. Intensive investigation of each candidate gene and implicated pathways is the next step in MS research and will require further research at the proteomic level and increased new pharmaceutical trials.

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