Chapter 4

Gene Expression Pattern Characterises Development of Multiple Sclerosis

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1. Introduction

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). MS remains the most frequent cause of neurological disability, with the exception of trauma, for young adults. Investigations on twins show higher concordance rates of MS in monozygotic compared to dizygotic twins. In addition, familial susceptibility studies show that around 15% of MS patients have an affected relative. Familial risk for MS is thus very high compared to the lifetime prevalence in the general population of approximately 0.2%. Genome wide screens for MS have provided potential data for finding specific chromosomal loci involved in MS susceptibility. A series of whole genome screens for linkage to MS have been undertaken and resulted in the discovery of significant chromosomal susceptibility loci in the genome. These data have triggered a lot of interest in the regions found associated with MS and interestingly there are a number of genes that may plausibly be involved in the aetiology and pathophysiology of MS. These candidate genes have been implicated in a variety of approaches but usually involve immunological and/or genetic studies. One of the most consistent findings has been an association of specific major histocompatibility molecules which genes are located in the chromosome 6p21. However, other significant Non HLA regions pinpoint the involvement of several candidate genes that are currently under investigation at the sequencing and proteomic levels. 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 (EAE) and post mortem MS brain tissues. The genetic hallmarks of MS genetics, found to date, will be discussed in this chapter and particular conclusion on gene pathways and interactions proposed to possibly unravel the unknown aetiology of MS. Discussions on the effect of some MS medication and their effect in both cellular and molecular levels will be discussed.

2. MS genetics and overview

MS is a complex disease affecting the central CNS showing demyelinating nervous events due to an active autoimmune activity. Several patches of white matter degeneration are observed and are the results of multifocal entry of inflammatory immune cells in the CNS. These lesions scattered in the CNS vary in diameter and are most prominent within the periventricular myelin but can be present in various other parts of the CNS (Lumsden, 1970). The sclerotic appearance follows a classification where plaques are categorised as acute or chronic active, chronic silent plaques and importantly poor correlation is observed with the clinical classification of the disease. Clinical pathology is characterised by varying severity with MS being variable in onset and progression. These include Relapsing Remitting MS (RR-MS), Secondary Progressive MS (SP-MS) and Primary Progressive MS (PP-MS). While the lesions and symptoms are disseminated in time and space, the clinical classification of MS is mainly based on the occurrence of attacks, recovery states, and neurological deficits (Lumsden, 1970). Several concordance rate studies undertaken in twins showed a higher MS concordance in monozygotic, compared to dizygotic twins (Sadovnick et al., 1993) showing a clear involvement of genetics in MS. In addition, fifteen precents of MS patients have an affected relative that strengthen the genetic of MS. Interestingly, MS is more prevalent in women and accounts for more than two thirds of all MS sufferers (Weinshenker et al.,1994). Current and exponential knowledge in MS genetics enhances the clinical diagnosis for MS sufferers with the findings of genetic susceptibility loci and molecular markers. Some approaches have been and are currently investigated to generate new avenues to better diagnose MS, comprehend its pathophysiological cascades and importantly identify possible curative methods. Molecular genetic is one the scientific research area of choice to potentially unravel the yet unknown idiopathic disease. MS research investigations have concentrat-

ed efforts on gene expression profiling, determining DNA blue print, and comprehending the epigenetic in MS. All these efforts have provided an exciting opening of incoming transcriptomic, interactomic, epigenetic and proteomic discoveries all in relation to MS. Gene expression microarrays, Genome wide association studies, DNA methylation and miRNA profiling, copy number variation in MS that start to unravel specific loci in the genome, expression signatures and modulators of MS patho-physiology. Pro-inflammatory and anti-inflammatory take place in the pathophysiology of MS and include several cells of the immune system including the very important T regulatory T cells.

3. Immune tolerance disturbance in MS

Along with this immunosuppressive function, an important immune tolerance is known to take place. Immuno-tolerance characteristics, that directly have effects on pro-inflammatory cells, do rely on particular cells called T regulatory cells or Tregs (Kuniyasu et al., 2000). Tregs can be T4 lymphocytes or T8 lymphocytes, these cells are mostly immune-modulator actors particularly in inflamed regions. Such modulatory action is mediated interestingly by contact inhibition towards non Treg cells such as subsets of T4 and T8 lymphocytic cells. Specific markers are responsible are expressed to differentiate these subsets with regulatory or non-regulatory effective T-cell functionality (Teffs). Tregs CD25 markers denote CD4+ CD25+ and CD8+CD25+ cells as well as FoxP3+ marker, a repressor activator of activated T-cells found in CD4+CD25+FoxP3+ Treg cells or Cd8+ CD25+ CDFoxP3+ Treg cells. Other Treg marker can be encountered and include CXCR3+, a molecule present in CD8+ CXCR3+ Treg cells. The Treg functional role in restoring tolerance can be developed through different mechanisms. Tolerance could be undertaken by contact interaction such as Fas- Fas ligand interaction dictating an apoptotic faith to the Teff cells (Watanabe et al., 2002). In addition, Tregs installs tolerance on Teff cells by inhibiting Teff cytokine synthesis and subsequently Teff cytolytic activation is halted as well as Teff proliferation reduced (Duthoit et al., 2005). Teff cells can be either CD8+ or CD4+ cells with CD4+ classified as TH1 and TH2 types with both differing in action as pro-inflammatory and anti-inflammatory actions respectively. Briefly, the Th1 activation pathway is mediated by interferon γ on binding to interferon γ surface receptor on T cells with subsequent intracellular cascade activation. Such cascade leads to the activation of the transcription factor T-bet that ultimately binds DNA responsive elements of genes within the nucleus. The main responsive elements controlled and activated by T-bet are the interferon γ and IL-12 receptor β2 chain genes. Upon activation, IL-12 receptor expression becomes widely available at higher amounts and the proteins co-locate in the cellular T-cell membrane surface. This receptor on the membrane becomes available for activation in the presence of local IL-12 cytokine. IL-12 receptor activation and expression induces an intracellular Stat 4 dependent cascade that enhances further the expression of the T-bet transcription factor. Relation between Tregs and pro-inflammatory TH1 and CD8+ T-cells demonstrate an interesting phenomenon that is built around the competition for Interleukin 2 binding. In sites of inflammation, binding of IL-2 by Tregs diminishes the availability of IL-2 to Teffs and therefore would limit their growth, function and even at early stage turning Teffs to become anergic towards antigens. Briefly, T-cells

are activated through the physical contact of antigens with their T-cell receptor. The antigen is presented by antigen presenting cells under the restriction of MHC class molecules. Such binding activates p56lck tyrosine kinase with activation of downstream phosphorylation of proteins and activation of phospholipase C. Such phospholipase generates turns phosphatidyl inositol diphosphate into two compounds; the diacyl glycerol and inositol tri-phosphate, IP3. The endoplasmic reticulum IP3 receptor is therefore activated by IP3 and enables the release of calcium in the cytosol. Such Ca2++ induces a membrane activation of the cell membrane calcium release activated calcium channel named CRAC to subsequently increase highly the intracellular pool Ca2++ concentration. High levels of Ca2++ activate caclineurin, a phosphatase that dephosphorylates the transcriptional factor NFAT (nuclear factor of activated T-cells). Such dephosphorylated form of NFAT can translocate consequently into the nucleus to reach and bind responsive elements of Il-2, AP1 and NFKB genes. Beside the roles of Tregs as pro-apoptotic inducers of cytolytic Teff cells and inhibitors of Teff cells expansion, Tregs are also capable to modulate inflammation and modulate the pattern observed in inflammation sites. Inflammation modulation of Tregs is mediated by their capacities to synthesize and secrete both anti-inflammatory TGFβ and interleukin 10 molecules. In addition, Teffs in presence of TGFβ expresses an additional pool of Interleukin 10 which maintains a positive feedback as Il-10 action dictates Teffs to respond with much higher affinity to TGFβ. Interestingly, expression of the transcription factor Foxp3 in Tregs is also subject to TGFβ action (Pyzic et al., 2007. In multiple sclerosis, both Foxp3 and TGFβ have been found to be down regulated in expression (Huan et al., 2005 and Mirshafley et al., 2009)

4. Which gene expression microarrays started to enlighten gene expression in MS

4.1. Major post mortem brain tissue microarrays undertaken in MS

In 1999, the work of Whitney (Whitney et al., 1999) described the analysis of MS acute lesions from a single female MS patient with PP-MS. Patient's plaques and white matter were compared for gene expression and results showed 62 differentially expressed genes. The genes with increased expression in acute plaques included leukotriene A-4 hydroxylase, TNF α receptor, the auto-antigen 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 several candidates. Two years later, Withney (Whitney et al., 2001) undertook microarray experiments using 2 MS patients. One patient's 16 chronic inactive (silent) plaques and the second patient's acute and chronic active plaque were used in the investigation. Gene expression analysis compared the levels of mRNA plaques and compared with control normal white matter RNA. Several gene candidates were found dysregulated in expression in these human tissues and validated in animal MS models. It included thrombin receptor, proteinase activated receptor 3 (PAR3) which is a gene previously found up-regulated in macrophages while in presence of granulocyte-macrophage colony-stimulating factor (GM-CSF) (Colognato et al., 2003). Jun-D and the putative ligand

for the IL-1 receptor- related molecule (T1/ST2) were also found to be overexpressed in their animal studies. Interestingly, the arachidonate 5-lipoxygenase gene (5-LO) was found up-regulated in expression in MS. 5-LO is a gene coding for a key enzyme in the leucotriene pathway and responsible for the conversion of arachidonic acid to leukotriene A4 (LTA4). Interestingly their previous microarray study using human MS brain tissue (Whitney et al., 1999) showed an over-expression of leukotriene A4 hydrolase (LTA4H). The gene LTA4H is responsible for the conversion of LTA4 to LTB4. LTBA4 that acts on leukotriene B4 receptor 1 (BLTR), is a potent chemotaxic factor for neutrophils and induces leucocyte adhesion to endothelial cells (Yokomizo et al., 1997). These findings show clearly the importance of the leukotriene cascade in MS pathology. Genes involved in both the chemoattraction events and genes involved in the formation of the LTB4 chemoattractor molecule such as LTB4 omega hydroxylase or Cytochrome P450 family 4 subfamily F polypeptide 3 (LTBAH or CYP4F3) have also previously been studied. LTBA4H is a gene encoding two possible isoforms, CYP4F3A and CYP4F3B that aim at catabolising the effect of LTB4 action (Shak et al., 1984). Interestingly a study in 2009 (Parkinson et al., 2009) has shown that LTA4h is a marker in inflammatory perivascular cuffs and actively demyelinating plaques in relapsing-remitting and progressive human MS.

4.2. Discussion on key hallmark: Arachidonic pathway in MS

The over expression of prostaglandin D synthase interrogates once again about the important role that may play arachidonic acid related metabolites in MS neuroinflammation. Whitney et al. (Whitney et al., 1999 and 2001), showed the enzymatic involvement of the 5-lipoxygenase and leukotriene A4 hydrolase gene in the production of leucotriene pro-inflammatory molecules in MS disorder. In addition, Chabas (Chabas et al., 2001) 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 prostaglandin D synthase, the enzyme that Chabas et al. found in high amounts in MS cDNA libraries (Chabas et al., 2001). The prostaglandins and leucotrienes are both pro-inflammatory molecules and might play a significant role in MS pathology. Ligand of the peroxisome proliferator activated receptor (PPAR gamma). PPAR gamma acts as an anti-inflammatory element and inhibits the pro-inflammatory IL12 cytokine. IL12p40 production correlates with disease activity and is found increased in expression in SP-MS individuals (Balashove et al, 1999; Soldan et al, 2004). However, research demonstrated that IL23 rather than IL12 plays a higher role in brain autoimmune inflammation (Cua et al., 2003). PPAR gamma was found with higher gene expression levels in EAE mice treated with Lovastatin drugs (Paintlia et al., 2004). Further evidence was implicating the cycloxygenase enzymatic pathway in which Lovastatin treated EAE mice showed reduced expression of the COX2 enzyme. Taken together, this suggests 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 synthase (PFS), PGF2, was reported to be involved in acute demyelination of peripheral nerves (Hu et al., 2003).

The first MS gene expression study was investigated in 1997 by Becker (Becker et al., 1997). To undertake such investigating, a normalized cDNA library from CNS lesions of a PP-MS sufferer was studied. 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, Chabas's study (Chabas et al., 2001) 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 Tajouri et al., 2003, over expression of HSP 70 within chronic active plaques was found. The inducible form of HSP 70 has been shown to promote myelin autoantigen presentation in APCs (Mycko et al., 2003). Of note, HSP 70 was though found to be down-regulated in other studies (Bomprezzi et al., 2003 and Lock et al., 2002).

Additionally, in Chabas (Chabas et al., 2001) 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. However, a comment made on Chabas's work has been raised (Blom et al., 2003) with the publication of an independent study using a knockout mouse for the osteopontin gene (OPN-/- 129/C57/BL10 with q haplotype: B10.Q usually susceptible to EAE). In Blom's study 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 knockout 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 chemo-attractant factor localised on chromosome 4q21.

4.3. Discussion on key hallmark: Chemokine IP-10 in MS

CXCL10 or IP-10 is a chemokine that preferentially attracts Th1 cells through its receptor CXCR3, expressed at high levels on these cells (Loetscher et al., 1996). IP-10 is induced in a variety of cells in response to the Th1 cytokine IFN-gamma (Luster et al., 1985). IP-10 expres-

sion 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 (Tajouri et al., 2003) 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 (Wingerchuk, et al., 1997 and Young et al., 1998). CXCL10 levels are related to clinical relapses in EAE (Fife et al., 2001 and Camody et al., 2002) and the source of production of CXCL10 is from astrocytes in EAE mice (Tani et al., 1996). Immuno-reactivity to CXCL10 was shown in demyelinating plaques (Huang et al., 2000). Also, this protein is found in higher levels within the CSF of MS patients compared to healthy controls (Franciotta et al., 2001) and such levels of expression correlate with the count of leucocytes in the CSF (Sorensen et al., 2002). Anti- CXCL10 reduces disease activity in common EAE (Fife et al., 2001). In viral model of MS (chronic demyelinating phase of mouse hepatitis virus infection of the CNS), mice showed a decrease severity of their pathology (Liu et al., 1997). 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 (Loetscheret al., 1996). CXCR3 is found over-expressed in macrophages, T cells and reactive astrocytes in MS plaques (Simpson et al., 2000). Perivascular cuffs in post mortem MS lesions showed CXCR3+ cells presence correlating with an increase of interferon gamma production (Balashov et al., 1999). Additional findings of elevated chemokine receptors CXCR3 has been reported in peripheral blood of progressive forms of MS (Vacknin- Dembinski et al, 2006). In 2002, Sorensen et al showed a continuous accumulation of CXCR3 +cells in lesion formation of MS patients (Sorensen et al., 2002). Targeting the CXCR3 receptor via antagonists could alter T-cell diapedesis through the CNS in MS (Ransohoff et al., 2000). Hong's study (Hong et al., 2004) demonstrated that treatment with Glatiramer acetate was significantly reducing the expression of CXCR3. In Tajouri's study (Tajouri et al., 2003), the author 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 granulin has been found to be slightly up-regulated compared to normal controls. Granulin is a novel class of growth regulators expressed by leucocytes (Bateman et al., 1993). This gene is normally not

expressed in normal brains but in brain glial tumour cells (Liau et al., 2000) and located at 17q21.32, a region of suggestive linkage in MS pathology (GAMES and the Transatlantic Multiple Sclerosis Genetics Cooperative., 2003). 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 (Klegeris et al., 2000). Interestingly, this inflammatory gene is involved in sporadic amyotrophic lateral sclerosis neuro-degeneration in which high levels of gene expression are found in post mortem tissues (Grewal et al., 1999). In parallel, anti-inflammatory proteins such as endothelial protein C receptor (PROCR) were found, in Tajouri et al., 2003 to be dramatically down-expressed in acute inflammatory plaques but this effect was less pronounced in chronic active plaques.

Lock's study (Lock et al., 2002) 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), adrenocorticotropic hormone receptor (ACTHR), tryptase-III and immunoglobulin E receptor 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 (Camody et al., 2002). Of interest as well, some pregnancy related genes were differentially expressed such as an increased of expression of pregnancy-specific β1 glycoprotein (PSG3) in acute plaques, a decreased expression for PSG11 in chronic silent. In Tajouri study (Tajouri et al., 2003), the author experimented 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 (Pericak-Vance et al., 2004). 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) (Blanchon et al., 2001). Of interest, GKLF4 is found prominently decreased in expression with interferon β therapy (Sturzebecher et al., 2003), a treatment of high efficacy in treating relapsing remitting MS (RR-MS) affecting mostly women.

The author Mycko (Mycko et al., 2003) 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 de-

pleted 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's study (Mycko et al., 2003), 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's study (Whitney et al., 1999). 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.

The author Lindberg (Lindberg et al., 2004) used oligonucleotide DNA chips that included a total of 12 633 probes. Lindberg 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.

5. Major peripheral blood mononuclear cell gene expression microarrays undertaken in MS

Peripheral blood cells (PBMC) from MS individuals have been used to extract mRNA and to investigate gene expression levels by microarray experiments. Bomprezzi et al., 2003 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 chemo-attraction 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 co-stimulator 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 (Beall et al., 1993). ZAP70 is directly implicated in TCR induced T cell activation (Chan et al., 1992). 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 in Bomprezzi's study (Bomprezzi et al., 2003) 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 γδ T cells. γδ T cells are present in MS lesions and their inhibition decrease the severity of EAE mice and induced the reduction of pro-inflammatory cytokines and iNOS expression (Rajan et al,. 1996). 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 auto-antigen implicated in the ubiquitin proteasome pathway for the degradation of cytokines.

A second study (Ramanathan et al., 2001) 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 CD 163 antigen), as well as high expression levels of auto-antigens such as auto-antigen 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 (Lee et al., 1994).

6. Treatment regimen and consequences in gene expression of MS patients

6.1. Common treatment available in MS

The existence of spontaneous remissions makes treatment difficult to evaluate. Several accepted regimes exist with indication being dependent upon the stage of the disease. Acute stages are treatable with oral prednisone, or dexamethasone until manifestation remit. Interferon β in high doses given every other day (subcutaneously) may reduce the frequency of neurological exacerbations in patients with RR-MS. The processes of demyelination and relapse are currently being treated with the drugs Avonex (interferon β-1a), Betaferon (interferon beta-1β), Copaxone (Glatiramer acetate, Co-polymer-1 or COP-1), Rebif (interferon β-1a), and Novantrone (chemotherapeutic agent) in the United States and United Kingdom. Of particular interest is the drug Copaxone. This drug is an oligopeptide (L-glutamic acid, L-alanine, L-tyrosine and L-lysine) leading to a diminution of exacerbation rates in RR-MS (Johnson et al., 1998) inhibiting the migration of lymphocytes (Prat et al., 1999) and inhibition of T cell activation (Miller et al., 1998). Copaxone was shown as well to act on T cells by increasing their secretion of neurotrophic factors such as the brain derived neurotrophic factor (BDNF) (Chen et al., 2003) with relevance to new research investigations for therapeutics (Ziemssen et al., 2003). Recent advances in the discovery of new treatments are encouraging. The classical immuno-modulator, β-interferon decreases the level of inflammation and has

been shown to decrease the blood brain barrier monocyte infiltration within the CNS of MS animal models (Floris et al., 2002). However, patients on β-interferon therapy tend to produce neutralising antibodies against the drug, reducing overall beneficial effects of this type of therapy (Bertolotto et al., 2003). Furthermore, discontinuation of β-interferon treatment further increases antibody production and as a result leads to further reduction of interferon efficiency (Reske et al., 2004). Steroids are usually used in an attempt to reduce neutralising antibody production (Bagnato et al., 2003), however benefits of interferon therapy are now debatable following a recent study that showed no effect of interferon β in PP-MS (Leary et al., 2003). New therapeutic attempts are under investigation with the example of the use of pluripotent cells. Haematopoietic stem cell implantation in humans offers new hopes to patients with MS (Burt et al., 2003) and studies using neural precursor cells in EAE rat models published promising results with a decrease of disease severity and reduced CNS inflammation (Ben-Hur et al., 2003). Additionally, some other studies have also been carried out with the use of statins (3-hydroxy-3-methylglutaryl conenzyme A reductase inhibitors). As an example, results of a recent study showed a 43 % decrease in the mean number of MRI lesions using Simvastatin (Zocor drug) in 30 patients investigated with relapsing remitting MS (Vollmer et al., 2004). Patients with RR-MS currently follow the ABC therapy (Avonex, Betaseron and Copaxone) to minimise the neuro-inflammation course of the disease but presently, there is no curative therapy for MS. New drug discoveries show preliminary promising data and researchers are attempting to find new strategies to cure MS. With new trials under way and increasing research undertaken in MS, sufferers may hope for a normal life with newly developed drugs.

6.2. Major peripheral blood mononuclear cell gene expression microarrays undertaken in therapeutic treated MS patients

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) (Satoh et al., 1995. Also, interferon β is thought to decrease the disruption of the blood brain barrier (Young et al., 1998) and to shift a pro-inflammatory Th1 mediated immunity to Th2 immunity (Karp et al., 2000).

Koike et al., 2003 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 (Bardos et al., 2001). 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 (Wandinger et al., 2001 and Sturzebecher et al., 2003). Sturzebecher investigated the gene expression profile of PBMCs ex vivo and in vitro from 10 RRMS 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 chemo-attractant 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), proto-oncogene 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 (Blanchon et al., 2001). 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 (Vukusic et al., 2004). Surprisingly, Sturzebecker reports an up-regulation of pro-inflammatory chemokines such as interferon-gamma-inducible protein 10 (IP-10 or CXCL10), monocyte chemo-attractant 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., 2001, has shown that pro-inflammatory factors such as interleukin 12 receptor β2 (IL12Rβ2) chain as well as chemokine, CC motif, receptor 5 (CCR5) were also up-regulated in expression in MS peripheral blood cells after interferon beta treatment. IL12 Rβ2 is also found by Hong et al. (Hong et al., 2004), 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 (Wang et al., 2000), 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 (MIP1α and MIP1β). 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 Camody et al., 2002). CCR5 is found increased in expression on T cells in peripheral blood with this receptor only found up regulated in Progressive forms of MS and not in relapsing-remitting MS (Vaknin-Dembinsky et al, 2007) Interestingly, CCR5 is significantly down-expressed in MS with Glatiramer acetate drug treatment (Hong et al., 2004) 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 (Paintlia et al., 2004) and seems to be a key factor in remission in EAE mice (Camody et al., 2002). Also, the up-regulation of some pro-inflammatory markers after interferon β therapy has been noted.

An interesting study by Der et al., 1998 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 β. Consequently, MxA and MxB over-expression with interferon β are in favour and support the findings of Wandinger et al [68]. Significant increase of expression of MxA was also found in MS peripheral blood cells after interferon β therapy [20]. However, in Wandinger et al., 2001 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 (with interferon β treatment), the gene LMP2 is inversely modulated compared to Avonex non treated MS patients [(Igelsias et al., 2004). 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 (Der et al., 1998) 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., 2001 reports 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., 2004, investigated PBMC from 18 MS patients treated with interferon β-1a and a group of 12 MS patients 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 pro-inflammatory molecules were indeed down-expressed with this drug. Iglesisas et al., 2004 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 (Murga et al., 2001). 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., 2004 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 thymopoeitin (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 (Ren et al., 2002). 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 (Ren et al., 2002) 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., 2006 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 Satoh (Satoh et al., 2006b) 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 (Satoh et al., 2006b) again that pro-inflammatory chemokines are up-regulated following interferon β therapy. Such pro-inflammatory chemokines include CCR2 (monocytic) and CXCR3 (thymocytic).

7. Conclusions

Large power Microarray studies for gene expression in MS are undertaken with subcellular isolated immune cells and post-mortem brain tissues. This area of research is still revealing altered expression of candidate genes. A series of gene expression were studied to assess specific patterns of gene expression in MS patients and animal models. Data demonstrated a strong immunological and inflammatory gene involvement along with a number of stress and antioxidant related genes, metabolic and CNS markers. Particular interest is drawn on genes that are genetically located at MS susceptible loci, loci that were previously revealed by linkage and genome wide screen studies. Present research tends to replicate similar high throughput gene expression investigation but importantly the present investigations are using starting tissue material that presents both with higher quality & integrity. In addition, the research in MS gene expression is undertaken using homogenous cellular material where researchers carefully purify and isolate subpopulation of cells to undertake the gene expression profiling in post mortem brains and blood cells. Promising results are ahead of us; results that need to be replicated and emanating from large power studies with, in this case, the potential and probability to find

new gene candidates and their pathways. Genome wide association studies are enhancing the findings but MS remains still unknown at this stage with a complex pathological mechanism that needs to be unravelled. Combination of GWAS data and Microarrays is with no doubt narrowing the list of key genes that are possibly part of the aetiology of MS and represents therefore an interesting and exciting time for MS research. Clustering analysis should aid in providing a means to classify candidates into global functional groups in the disease course. The large amount of data arising from all these studies is daunting but enhanced statistical analysis and storage of data is promising and would be fast rewarding 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 pro-inflammatory 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. Of particular interest are a number of specific genes genetically localised at susceptible loci found to be in linkage with MS (largely reported in genome wide screen studies). However due to clinical complexity of the disease, the heterogeneity of the tissues used as well as the DNA chips/membranes used for the gene profiling, one is faced with a phenomenal load of differentially expressed genes. Although this information is essential for the understanding of the pathogenesis of MS, one must now depict and comprehend the gene pathways and interactome involved in the MS disorder.

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