TRANSCRIPTIONAL ANALYSIS OF TARGETS IN MULTIPLE SCLEROSIS

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Large-scale analyses of messenger RNA transcripts and autoantibody responses, taken from the actual sites of disease, provide us with an unprecedented view of the complexity of autoimmunity. Despite an appreciation of the large number of pathways and pathological processes that are involved in these diseases, a few practical targets and several new strategies have emerged from these studies. This review focuses on multiple sclerosis and on the approaches that are being used to identify new targets that might be manipulated to control this disease.

MICROGLIAL CELLS
Bone-marrow-derived macrophages that are resident in the central nervous sytem.

Our understanding of pathological processes in autoimmunity has been limited until recently by an inability to follow these complex processes on a large scale. For example, it has been impractical to measure the immune response to all the components of a complicated target organ. Although it has been possible to measure a few gene transcripts (messenger RNA) to see if these genes are differentially activated at the site of disease, it has not been practical to measure the mRNA levels of all the genes in the genome at the site of disease, until recently. The advent of technologies for the large-scale analysis of transcription[1,2] and of autoantibody responses[3] has enabled an unprecedented appreciation of the complexity of autoimmune disease. This review focuses on multiple sclerosis (MS), an autoimmune disease of the central nervous system (CNS) that culminates in neurodegeneration[4–6].

MS affects approximately one million people worldwide, with 350,000 cases in North America alone[3,4]. Women are affected two times more frequently than men[4–6]. The disease often begins in young adulthood with recurrent inflammatory attacks against the white matter of the brain, producing a myriad of neurological impairments, including blindness, loss of sensation, lack of coordination, bowel and bladder incontinence, and difficulty walking[5]. About one third of patients who start out with the relapsing–remitting form of MS progress to a more chronic form with more widespread disability.

The hallmark pathology in MS is the plaque — an area of myelin that has been denuded by inflammation and subsequent scarring by non-neural cells in the brain, including bone-marrow-derived MICROGLIA and brain-derived star-shaped astroglia. The cause of MS is enigmatic, although most investigators believe that the immune attack against white matter is paramount, with the resulting degeneration of axons and myelin being secondary to this inflammatory process[4,6] (FIG. 1). However, the possibility that the immune response is itself a reaction to some initiating neurodegenerative process must also be considered, analogous to the sequence of events that occur during wound healing. First there is the injury, then the process of repair; the repair process itself, in response to the wound, evokes a cascade of immunological activity. Therefore, by examining mRNA levels on a large scale from specimens taken both early and late in the disease process, it might be possible to assess whether neurodegeneration is actually the primary response and neuroinflammation of the white matter is a secondary response.

Large-scale approaches

Large-scale sequencing of mRNA from complementary DNA libraries derived from brain plaques of patients with MS[1], and gene microarray analysis of transcripts from MS lesions of various types[2], have shown both the complexity of the pathological response in MS and several targets that might be manipulated to control this disease. The general approach of using a broad-ranging analysis of the immune response to search for unusual patterns has so far led to some practical approaches for the containment of this disease. Several strategies have been used to analyse mRNA transcripts that are over or

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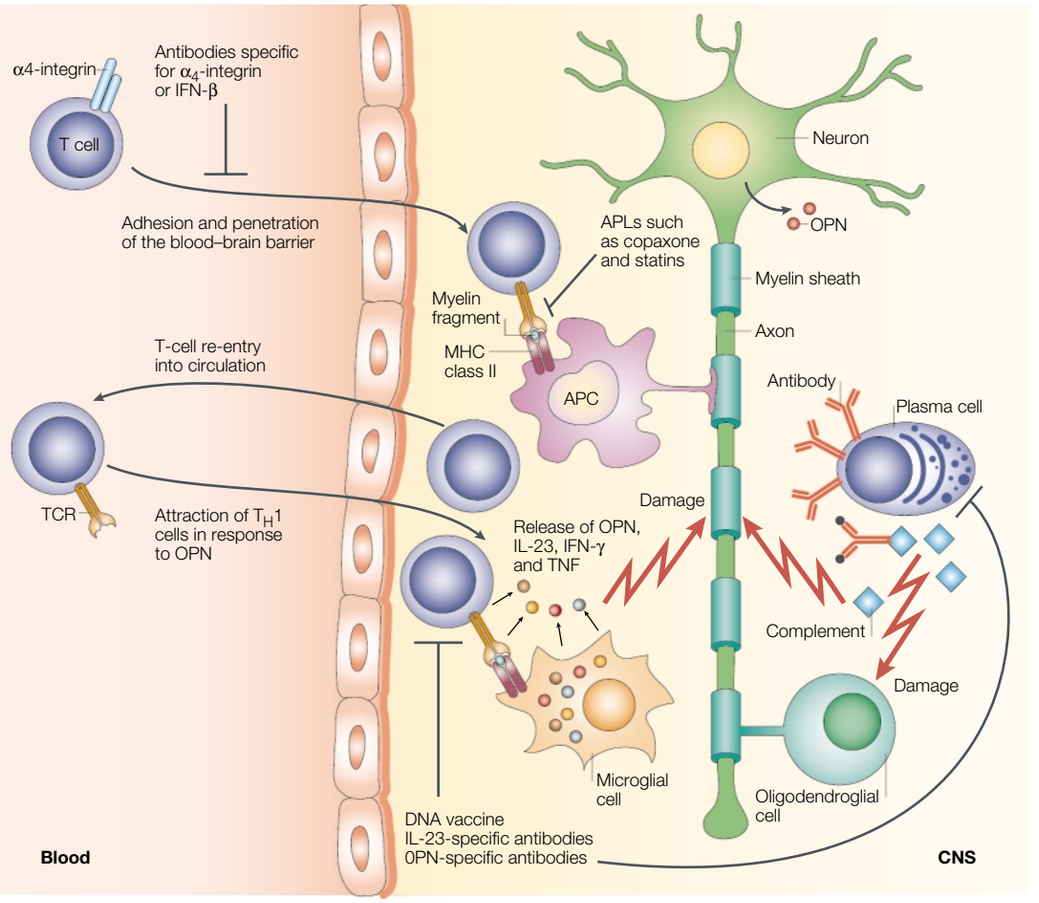

Figure 1 | **The inflammatory phase of multiple sclerosis.** T cells, B cells and antigen-presenting cells (APCs), including macrophages, enter the central nervous system (CNS), where they secrete certain chemicals known as cytokines that damage the oligodendroglial cells. These cells manufacture the myelin that insulates the neuronal axon. The injured myelin cannot conduct electrical impulses normally, just as a tear in the insulation of a wire leads to a short circuit. Lymphocytes diapedese into the CNS through use of a surface receptor known as α4-integrin. This step is impeded by antibodies specific for α4-integrin or by interferon-β (IFN-β). Once the blood–brain barrier is breached, other inflammatory cells accumulate in the white matter. Inside the brain, T cells and accompanying macrophages and microglial cells release osteopontin (OPN), interleukin-23 (IL-23), IFN-γ and tumour-necrosis factor (TNF), all of which damage the myelin sheath. Also, the presence of OPN might lead to the attraction of T helper 1 (T_H1) cells. T-cell activation can be blocked by altered peptide ligands (APLs), such as copaxone, or by statins. Concomitantly, B cells (plasma cells) produce myelin-specific antibodies, which interact with the terminal complex in the complement cascade to produce membrane-attack complexes that further damage oligodendroglial cells. DNA vaccination can be used to tolerize T- and B-cell responses to myelin.

under expressed in tissue from patients with MS, enabling transcripts that are unique to MS plaques or that are differentially expressed in acute compared with chronic MS material to be identified. As shown in FIG. 2, we have used two parallel approaches. The first strategy involved large-scale sequencing of mRNA transcripts from cDNA libraries prepared from brain tissue. The second approach used OLIGONUCLEOTIDE MICROARRAYS[1,2,7]. These strategies in no way diminish the importance of other 'genomic' approaches that are being undertaken to understand the regulation of the immune response. Recently, several groups have embarked on the analysis of SINGLE-NUCLEOTIDE POLYMORPHISMS (SNPs) across the entire genome for evidence of linkage to susceptibility to MS. Discussions of the various approaches for the statistical analysis and hierarchical clustering of data are beyond the scope of this review. The reader is referred to our own papers, and reviews of them[1,2,7], and to the discussions by Schadt and colleagues[8] on statistical analysis, and by Chu and colleagues[9] on hierarchical clustering.

High-throughput sequencing of cRNA from EXPRESSED SEQUENCE TAGS (ESTs), using non-normalized cDNA brain libraries generated from brain lesions of patients with MS and from control individuals, has indicated the most prominent transcripts that are found in the brains of patients with MS. Using this protocol, the mRNA populations that are present in the brain specimens are accurately represented, enabling the quantitative estimation of transcripts and comparisons between specimens. We have sequenced more than 11,000 clones from these libraries from patients with MS and from controls[1], and have concentrated our analysis on mRNA species that are present in MS libraries, but absent in control libraries. This analysis yielded 423 genes, including 26 novel genes.

OLIGONUCLEOTIDE MICROARRAYS
Short chains of nucleotides are chemically coupled to a solid surface. There, they hybridize with complementary DNA sequences.

SINGLE-NUCLEOTIDE POLYMORPHISMS
(SNPs). Single base-pair changes that are inherited as Mendelian traits and might associate with a trait such as susceptibility to disease.

EXPRESSED SEQUENCE TAG
(EST). A single-pass, short read of complementary DNA that is generated from a transcribed region of the genome.

Of those, 54 genes showed a mean increase in their level of expression of 2.5-fold or greater in libraries derived from patients with MS compared with controls. Transcripts encoding αB-crystallin, an inducible heat-shock protein that is localized in the myelin sheath and targeted by T cells in MS, were the most abundant transcripts found to be unique to MS plaques. The next five most abundant transcripts included those that encode prostaglandin D synthase (PGDS), prostatic binding protein, ribosomal protein L17 and osteopontin (OPN; also known as ETA1). The potential role of OPN in the progression of MS is discussed in detail later[1].

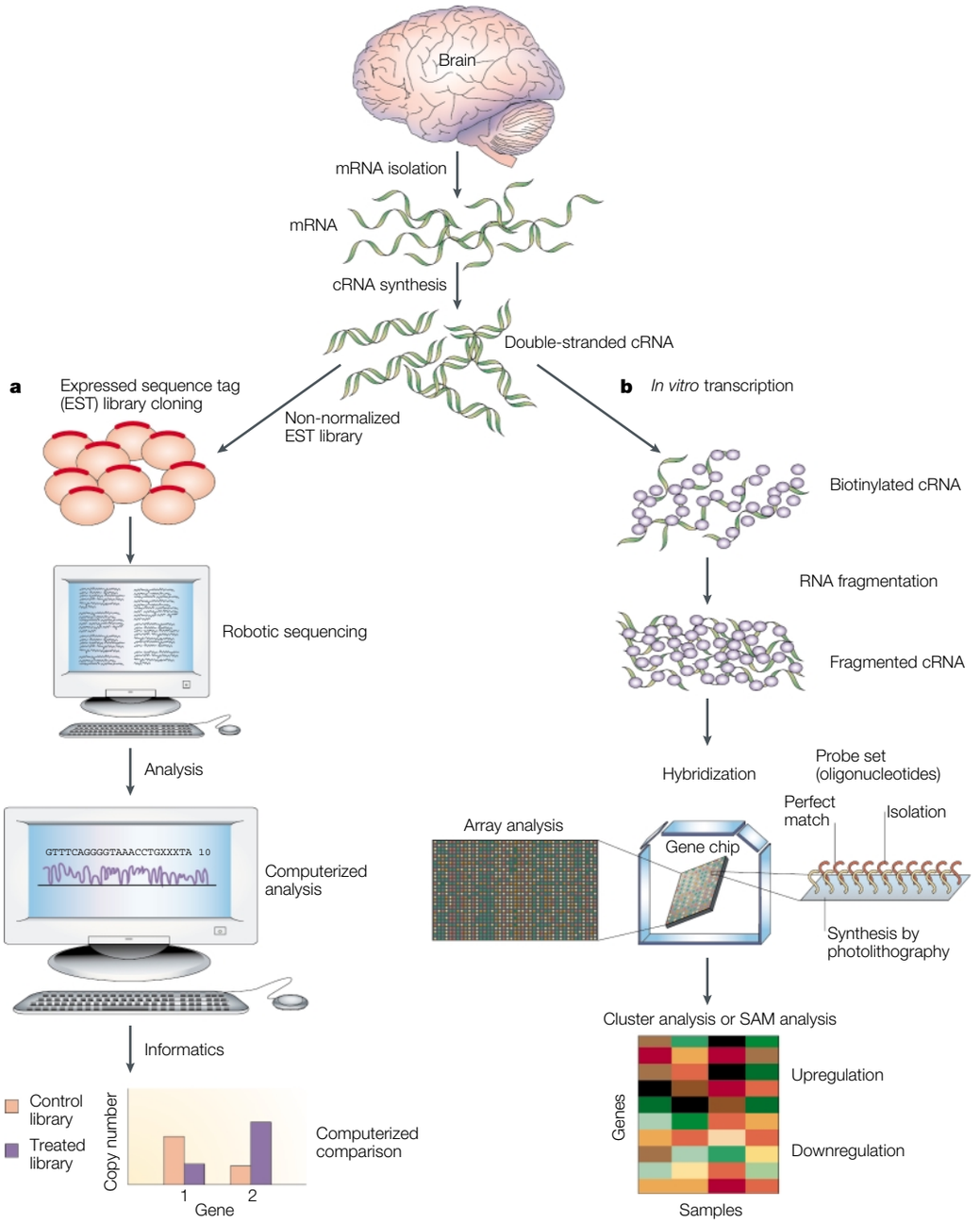

Figure 2 | **Large-scale analysis of gene transcription from MS lesions. a** | Robotic sequencing of expressed sequence tags (ESTs) produced by reverse transcription of messenger RNA from multiple sclerosis (MS) lesions. **b** | Strategy for the analysis of complementary RNA tagged with biotin on oligonucleotide microarrays. These microarrays either contain oligonucleotides that are printed on the surface by a process similar to photolithography, where certain chemical groups are protected whereas others are printed, or are made by printing complementary DNA on a glass surface. The output from these microarrays is analysed by statistical methods that allow groupings to become apparent. Cluster analysis or statistical analysis of microarrays (SAM) looks at correlations between different variables. This is similar to the methods used by pollsters, who might correlate variables such as voting preference and socioeconomic status. Statistical methods for handling data in hierarchical clusters are reviewed in REFS 1,2,8,9.

Few studies have looked at transcriptional profiles in MS lesions. We compared our results with those of Biddison and colleagues[10], who used cDNA microarrays to profile MS lesions. They studied two MS lesions from one brain and identified 29 genes that had an increased level of expression in acute MS plaques compared with control brain. All of these 29 genes were represented on the HuGeneFL chip that we used in our study[2], with the exception of 2-chimerin, which was replaced by chimerin. We found an increased level of expression of eight of these 29 genes in at least two of the four MS samples[2]. Another recent study by Selmaj and colleagues[11] directly compared different regions of MS lesions with different activity from the same individuals. A comparison of raw data sets between the study by Selmaj and colleagues[11] and the other previously reported analyses[1,2,10] has not been carried out yet. The studies by Chabas and colleagues[1], Lock and colleagues[2], and Whitney and colleagues[12] looked for similarities between MS lesions and lesions from the animal model EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE)[1,2,12]. Other studies[13,14] have analysed transcriptional profiles of EAE lesions. The investigation by Ibrahim and colleagues[13] looked at acute EAE induced by injection of myelin oligodendrocyte glycoprotein (MOG) in complete Freund's adjuvant (CFA) plus *Bordetella pertussis* toxin, which is an additional immune adjuvant. The CFA and *B. pertussis* toxin might have contributed to the alterations in gene transcription that were observed. Furthermore, only two time points on day 16 and day 22 after immunization, at the onset and peak of acute clinical disease, were examined. It would be useful in the future to study not only a model of acute EAE, but also one of the several other varieties of this disease[15]. It is also desirable to compare such results from different models of EAE with those obtained from studies of MS tissue, similar to the attempts that have been made by groups from Stanford and the National Institutes of Health[1,2,12].

The pros and cons. It is possible to look at the transcription level of all genes in the genome at the site of disease using microarrays or robotic sequencing. This allows an unprecedented view of gene activity. However, the alternative splicing of genes might be missed or mRNA transcripts that are unproductive might be detected. Therefore, it is crucial to support transcriptional analysis with identification of the translated protein at the site of disease, preferably using immunohistochemistry. Then, of course, it is necessary to understand the biology of the gene of interest, to understand the role that it might have in the pathogenesis of disease. This is a major challenge in terms of time and financial resources, and given the large amount of data resulting from large-scale transcriptional analysis, it is difficult to mount an in-depth effort to understand the biological role of more than a few interesting genes at any one time. Nevertheless, we describe here some unexpected pathways involved in autoimmune disease, the discovery of which has resulted from such studies.

EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS (EAE). Refers to a set of related animal models of multiple sclerosis. Typically, disease is induced by the injection of components of myelin, which leads to demyelination in the central nervous system.

Osteopontin: a role in MS progression

As mentioned earlier, molecular mining of two sequenced libraries from brain plaques of patients with MS and their comparison with a normal brain library (matched for size and tissue type, and constructed using an identical protocol) showed that transcripts encoding OPN are frequently detected in and exclusive to the mRNA population of MS plaques. Given the known pro-inflammatory role of OPN, we investigated the potential role of this protein in MS progression. First, we examined the cellular expression pattern of this protein in human MS plaques and in control tissue by immunohistochemistry. In active MS plaques, OPN was found to be expressed on microvascular endothelial cells and macrophages, and in the white matter adjacent to plaques. Reactive astrocytes and microglial cells also expressed OPN[1].

Studies in EAE models. The role of OPN in inflammatory demyelinating disease was next examined using two models of EAE in mice — one relapsing–remitting model, and one model that is initially relapsing but is followed by a progressive phase, often culminating in death. The relapsing–remitting model of EAE was first used to compare the cellular expression of OPN at different stages of the disease. Disease was induced in SJL mice by immunization with a peptide of proteolipid protein (PLP139–151) in CFA, and the animals were scored daily for signs of disease. Histopathological analyses showed that OPN was expressed broadly in microglia during both relapse and remission from disease, and this expression was focused near perivascular inflammatory lesions. In addition to the expression of OPN by glial cells, expression by neurons was detectable during acute disease and relapse, but not during remission. These results indicated that the level of expression of OPN in lesions correlates with the severity of disease.

The potential role of OPN in demyelinating disease was next tested using OPN-deficient mice. EAE was induced using MOG35–55 in CFA in OPN-deficient mice and wild-type controls. EAE was observed in all OPN-deficient and -sufficient mice after immunization with MOG35–55. However, the severity of disease was reduced in all animals in the OPN-deficient group, and these mice were totally protected from EAE-related death. So, OPN significantly influenced the course of progressive EAE induced by MOG35–55 (REF. 1).

The rate of relapses and remissions was tested. During the first 26 days, OPN-deficient mice had a distinct evolution of EAE, with a much higher percentage of mice undergoing remissions compared with the control mice. Although the clinical courses in the two groups were different, there were similar numbers and appearances of inflammatory foci in the CNS. Therefore, although OPN might not influence the extent of the inflammatory response, this protein might influence whether or not the course of disease is progressive, or whether relapses and remissions develop.

To examine whether different immune responses were involved in OPN-deficient and OPN-sufficient animals, we tested the profile of cytokine expression in

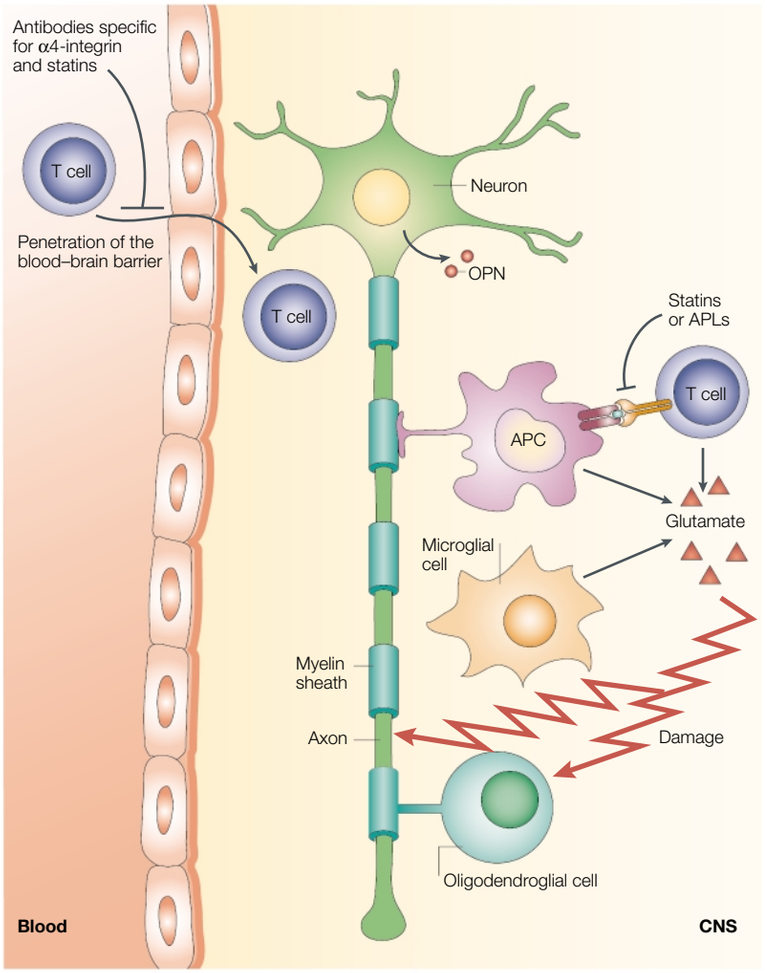

Figure 3 | **The neurodegenerative phase of multiple sclerosis.** T cells and antigen-presenting cells (APCs) such as macrophages produce glutamate, a toxic substance, which injures oligodendroglial cells and underlying axons. The activation of T cells can be blocked by various approaches, including statins and altered peptide ligands (APLs). Statins also inhibit the secretion of metalloproteases, and it has been suggested that they might, therefore, also act to block T cells from entering the central nervous system (CNS). Antibodies specific for α4-integrin also block the movement of T cells into the brain. OPN, osteopontin.

these mice. T cells from OPN-deficient mice, compared with OPN-sufficient T cells, had a reduced proliferative response to MOG35–55. In addition, the production of interleukin-10 (IL-10) was increased in T cells reactive to MOG35–55 in OPN-deficient mice that had developed EAE, compared with T cells from OPN-sufficient mice. At the same time, the production of interferon-γ (IFN-γ) and IL-12 was reduced in cultures of spleen cells from OPN-deficient mice stimulated with MOG. IFN-γ and IL-12 are important pro-inflammatory cytokines in MS[4,6]; the finding that there is reduced production of these cytokines in OPN-deficient mice is consistent with the hypothesis that OPN might have a crucial role in the modulation of T helper 1 (T_H1) immune responses in MS and EAE. Sustained expression of IL-10 might, therefore, be an important factor in the reversal of relapsing MS, and its absence might allow the development of secondary progressive MS.

OPN might have pleiotropic functions in the pathogenesis of demyelinating disease (FIG. 1). The production of OPN by glial cells might attract T_H1 cells, and its expression by glial and ependymal cells might allow inflammatory T cells to penetrate the brain. Finally, our data indicate that neurons might also secrete this pro-inflammatory molecule and participate in the autoimmune process. Potentially, neuronal secretion of OPN could modulate inflammation and demyelination, and influence the clinical severity of the disease. Consistent with this idea, a role for neurons in the pathogenesis of MS and EAE has been described recently[4,6].

CD44 is a known ligand of OPN, mediating a decrease in the production of IL-10 (REF. 16). OPN-deficient mice produce increased levels of IL-10 during the course of EAE. We showed recently that CD44-specific antibodies prevented EAE[17], indicating that the pro-inflammatory effect of OPN in MS and EAE might be mediated by CD44. The binding of OPN to its integrin fibronectin receptor $\alpha_V\beta_3$ through the arginine–glycine–aspartate tripeptide motif might also perpetuate T_H1-cell-mediated inflammation[1,16,17]. In active MS lesions, the α_V subunit of this receptor is overexpressed by macrophages and endothelial cells, and the β_3 subunit is expressed on the luminal surface of endothelial cells. Through its tripeptide-binding motif, OPN inhibits the function of inducible nitric-oxide synthetase (iNOS), which is known to participate in autoimmune demyelination[1]. So, in conclusion, OPN is situated at several checkpoints that would allow diverse activities in the course of autoimmune-mediated demyelination.

Recently, Cantor's group[18] has described concordant results in another model of EAE. They studied a model of relapsing EAE induced by PLP172–183 in C57BL/6×129 OPN-deficient mice that had been backcrossed to C57BL/6 mice for six generations. Wild-type OPN-sufficient mice on the C57BL/6×129 background, which were littermates of the OPN-deficient mice, were used as controls. The incidence of clinical disease and the mean day of disease onset were similar in OPN-deficient C57BL/6×129 mice compared with controls. OPN-deficient C57BL/6×129 mice had lower maximum clinical disease scores and recovered faster without spontaneous relapses in this model. Decreased levels of inflammatory infiltration and demyelination were seen in this model in the OPN-deficient C57BL/6×129 mice. Therefore, these results were even more marked than those seen by our group in the MOG35–55-induced model of EAE[1]. In the study by Cantor's group[18], $CD4^+$ T cells reactive to PLP172–183 produced less IFN-γ and tumour-necrosis factor (TNF) after restimulation with the myelin peptide. Furthermore, OPN-deficient C57BL/6×129 mice produced increased levels of IL-10 after co-stimulation with CD3-specific antibody, compared with wild-type littermates, indicating that a shift to a T_H2-cell phenotype had occurred. Taken together, our results[1] and those of Cantor's group[18] indicate that OPN is a potent modulator of autoimmune demyelinating disease. The role of OPN in the pathogenesis of MS is

described in FIG. 1. Note the inclusion of IL-23, a recently discovered cytokine that contains the p40 subunit of IL-12 (REF. 19). The potential interactions between IL-23 and OPN are the subject of intense investigation. It will be important to know where IL-23, IL-12 and OPN fit into the hierarchy of regulation of T_H1-cell responses in the brain in human autoimmune disease.

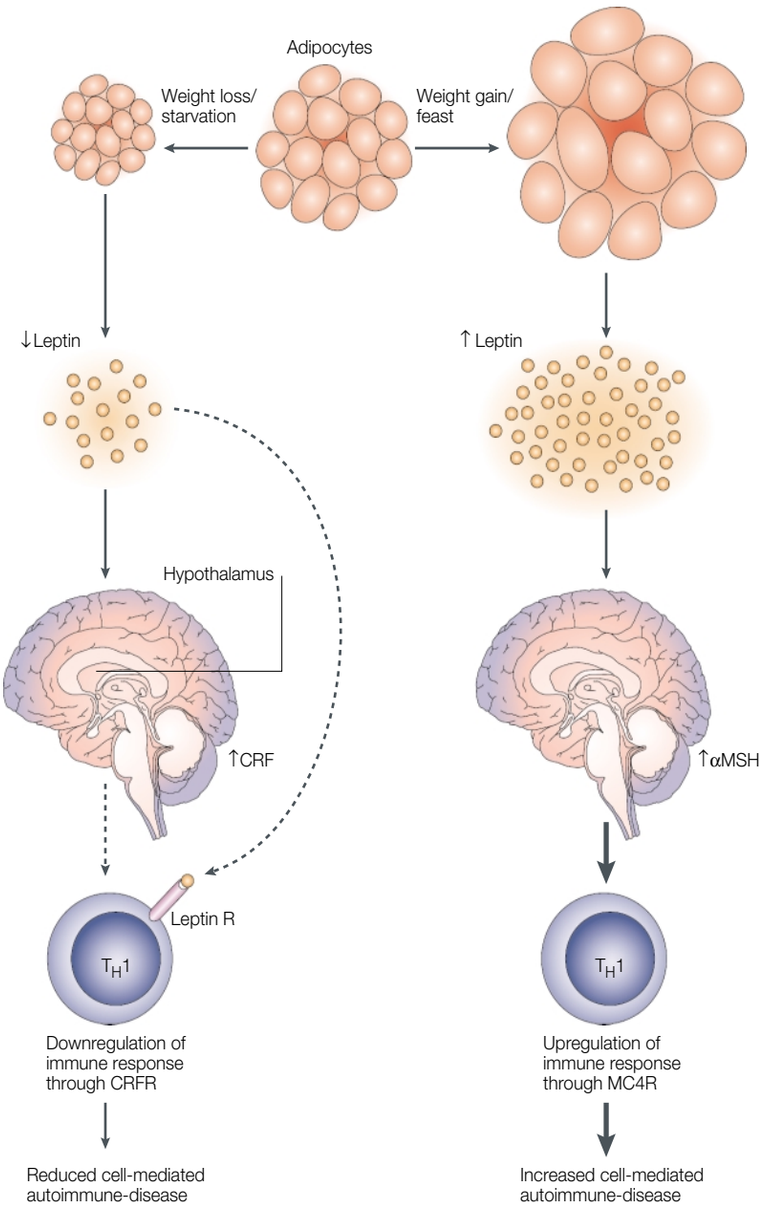

Figure 4 | **The intricate interplay between leptin, corticotropin-releasing factor and the melanocortins in the regulation of T_H1 immunity.** Leptin, corticotropin-releasing factor (CRF) and the melanocortins modulate T helper 1 (T_H1)-cell autoimmunity. Decreases in the level of body fat or starvation decrease leptin levels and increase CRF levels. Increased levels of CRF downregulate T_H1-cell-mediated autoimmunity, as do decreased levels of leptin. Other molecules of the α-melanocyte-stimulating hormone (αMSH) family modulate T_H1-cell-mediated autoimmunity through their melanocortin-4 receptor (MC4R). Leptin R, leptin receptor; CRFR, corticotropin-releasing factor receptor. Reproduced, with permission from *The Journal of Clinical Investigation*, from REF. 39.

OPN polymorphisms and disease. Further studies have been undertaken looking at OPN polymorphisms and disease course in patients with MS. In 821 patients with MS that were analysed, a trend for association with disease course was detected in patients carrying at least one 1284A allele of the *OPN* gene, indicating that this polymorphism has an effect on disease course. Patients with this genotype were less likely to have a mild disease course and were at increased risk for a secondary-progressive clinical type of disease[20]. In patients with MS in Japan, polymorphisms in OPN were shown to be crucial for determining susceptibility to progressive or relapsing MS[21]. Levels of OPN were increased in the spinal fluid of patients with MS during relapses[22]. In addition, OPN mRNA levels were increased in patients with Huntington's disease, and levels of OPN mRNA and protein were increased in brain tissue from a transgenic mouse model of Huntington's disease after successful treatment with an inhibitor of a key enzyme, transglutaminase, that is involved in the pathogenesis of this neurodegenerative disease[23]. Levels of OPN mRNA and protein were also increased in an experimental model of epilepsy[24]. So, the role of OPN in a wide variety of neuroinflammatory and neurodegenerative conditions continues to be an area worthy of intense interest. Contradictory results published by Blom and colleagues[25] might be explained at several levels[26].

The results described by Blom and Holmdahl[25] might be due to differences between the EAE model that they studied and the models used by our group[1] and by Cantor's group[18]. Holmdahl's group induced EAE using recombinant rat MOG1–125, whereas we used a peptide fragment of MOG, MOG35–55, which is the immunodominant epitope in C57BL/6 mice. Cross and colleagues[27,28] have shown that the form of inducing antigen (protein compared with peptide) has a role in the pathogenesis of EAE[27], such that B-cell-deficient mice do not develop EAE when immunized with full-length MOG, although they are susceptible to MOG35–55-induced disease. So, there are likely to be different mechanisms at work in EAE induced using recombinant proteins compared with that induced using peptide fragments of the same protein. Furthermore, the recombinant MOG1–125 of rat origin used by Holmdahl and colleagues has many differences compared with the mouse sequence. The importance of OPN in EAE is further shown by the fact that DNA vaccination to OPN ameliorated EAE, by inducing OPN-specific antibodies[26]. Finally, to implicate linked genes definitively as the basis for the differences seen between the data of Holmdahl and colleagues[25] and those of our group[1] and of Cantor's group[18], EAE induced in earlier backcrosses of their[25] OPN-deficient strain would have to be examined. Until such differences are explicitly analysed, it is difficult to draw any definitive conclusions. It is not at all unusual for divergent results to arise from studies of autoimmunity in knockout mice, especially when different animal models are examined[26,29].

Table 1 | **Validation of the role of neuroendocrine genes found in MS lesions**

Gene product	Biological function	Indication in MS*
Leptin	Involved in modulation of body weight and adiposity; regulates T_H1 cells	Upregulated in acute plaques
Melanocortin-4 receptor	Involved in modulation of body weight and adiposity; regulates T_H1 cells	Upregulated in acute plaques
ACTH receptor	Involved in regulation of stress responses	Upregulated in chronic plaques
Pregnancy glycoprotein 13	N.D.	Upregulated in chronic plaques
Pregnancy glycoprotein B1	N.D.	Upregulated in chronic plaques

ACTH, adrenocorticotropic hormone; MS, multiple sclerosis; N.D., not determined; T_H1, T helper 1. *Based on data from REF. 2.

Lipid and cholesterol metabolism

Transcriptional profiling of MS tissue has indicated many changes in genes that are involved in lipid and cholesterol metabolism[2]. Expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase) was downregulated in brain tissue from MS patients, as were the expression levels of other genes involved in crucial pathways of lipid metabolism, such as those encoding stearoyl CoA desaturase (SCD), acetoacetyl-CoA thiolase, propionyl-CoA carboxylase and enoyl-CoA hydratase. Initially, we have focused our attention on the potential role of HMG-CoA reductase, because of its pleiotropic effects on the immune system, including reduction in the expression of inducible MHC class II molecules and the ability to block leukocyte function-associated antigen 1 (LFA1) and its interactions with intercellular adhesion molecule 1 (ICAM)[30–32]. The class of drugs known as statins reduce cholesterol synthesis by inhibiting HMG-CoA reductase. We showed more than twenty years ago that inhibition of expression of MHC class II molecules could reverse autoimmune disease in several animal models, including EAE, experimental autoimmune myasthenia gravis and experimental autoimmune thyroiditis[30,33–35]. Recently, promising results in pre-clinical studies have ignited interest in the potential application of the cholesterol-lowering HMG-CoA reductase inhibitors (statins) for the therapy of MS[36–38].

In contrast with currently approved treatments for MS, which are administered parenterally, statins are given orally and are well tolerated. Atorvastatin (Lipitor®), which is given orally and is currently the most potent statin for cholesterol reduction, can prevent or reverse chronic and relapsing EAE[36]. *In vivo* treatment with atorvastatin induced the secretion of anti-inflammatory T_H2 cytokines by T cells and suppressed the secretion of T_H1 cytokines by T cells. *In vitro*, atorvastatin promoted T_H0 cells to differentiate into T_H2 cells, which could adoptively transfer protection against MS to recipient mice. Atorvastatin also suppressed the expression of MHC class II molecules by microglia and, when tested *in vitro*, it inhibited IFN-γ-inducible expression of co-stimulatory and MHC class II molecules by antigen-presenting cells (APCs). So, atorvastatin has immunomodulatory effects on both APCs and T cells. These findings indicate that statins could be useful in the early inflammatory phase of MS (FIG. 1), as well as in the neurodegenerative phase of MS (FIG. 3). Statins also inhibit the secretion of metalloproteases, which indicates that they might prevent T cells from entering the CNS. Lovastatin, which partially suppressed acute EAE in rats, was shown to inhibit the production of iNOS and TNF — pro-inflammatory molecules that are neurotoxic — indicating that statins might be beneficial in the chronic phase of MS[38]. At the moment, there is one open-label trial testing simvastatin in relapsing–remitting MS being carried out, and other trials using atorvastatin are being planned. Because of their anti-inflammatory and potential neuroprotective effects, statins are also being evaluated for potential efficacy for the treatment of other CNS disorders, including Alzheimer's disease[36].

Neuroendocrine mediators in autoimmunity

Large-scale analysis of gene transcripts in MS lesions showed that levels of the neuroendocrine mediators leptin, melanocortin-4 receptor and adrenocorticotropic hormone receptor (ACTHR) are increased at the site of inflammation in the brain[1,2].There are indications that other neuroendocrine mediators, such as the melanocortins[2] and corticotrophin-releasing factor (CRF)[2,39–42], have important roles in diseases such as MS and rheumatoid arthritis that are thought to be autoimmune in nature. Leptin is thought to produce its profound effects on appetite and body weight by altering the balance between the anorectic (appetite suppressing) neuropeptides α-melanocyte-stimulating hormone (α-MSH) and CRF, and the orexigenic (appetite enhancing) neuropeptides Agouti-related protein (AGRP) and neuropeptide Y (NPY). Shifts in the balance between these neuropeptides also contribute to the cytokine-mediated loss of appetite and body weight, and malaise that is induced by lipopolysaccharide[39]. One important mechanism of this regulation occurs at the level of the pro-opiomelanocortin (POMC)-expressing neurons in the arcuate nucleus of the hypothalamus, which are activated by leptin to release α-MSH[39]. It seems that this interplay between neuropeptides in relation to hypothalamic function might be reproduced at sites of autoimmune pathology (FIG. 4). All three of these neuropeptides — leptin, melanocortin 4 and ACTH — have pleiotropic roles in neuroendocrine and immune physiology.

Table 2 | **Validation of the role of allergy-associated genes found in MS lesions**

Gene product	Biological function	Indication in MS*
Prostaglandin D synthase	Involved in allergic inflammation	Upregulated in plaques
High-affinity IgE receptor β-chain	Involved in allergic inflammation	Upregulated in chronic plaques
Histamine 1 receptor	Involved in allergy; recently found to be present on T_H1 cells	Upregulated in chronic plaques
Mmcp7 (tryptase)	Degranulation product	Elevated in acute plaques and CSF
Pafr	Role in mouse anaphylaxis; chemotactic for inflammatory cells	Elevated in CSF and plasma of patients with MS

CSF, cerebrospinal fluid; Mmcp7, mouse mast cell protease 7; MS, multiple sclerosis; Pafr, platelet-activating factor receptor; T_H1, T helper 1. *Based on data from REFS 1,2.

The microsomal enzyme SCD1 is required for biosynthesis of the monounsaturated fats palmitoleate and oleate from saturated fatty acids[39,43]. *Scd1* mRNA levels are highly elevated in the livers of *ob/ob* mice, which contain a mutation in the leptin receptor and develop obesity. Indeed, SCD1 probably has a decisive role in the metabolic effects of leptin. Interestingly, SCD1 was downregulated in brain tissue from patients with MS[2], and both mRNA levels and activity of this enzyme are repressed by leptin[39]. *ob/ob* mice with additional mutations in *Scd1* are markedly less obese than *ob/ob* controls[39]. The role of leptin in autoimmune brain disease, and in the immune system in general, might be mediated by downregulation of this enzyme (SCD1) involved in the biosynthesis of monounsaturated fats. Once again, we witness the remarkable choreography of molecules that are involved in the regulation of body weight and energy metabolism, and the parallel roles of these same molecules in the finely tuned immune response (FIG. 4 and TABLE 1).

Earlier work had shown that CRF, which is the main regulator of the stress response in the hypothalamus–pituitary–adrenal axis, or urocortin, which is a naturally occurring paralogue of CRF, acting directly on T cells in adrenalectomized mice ameliorated EAE. Antagonists of CRF blocked these effects[42]. So, CRF, similar to leptin, is produced by the brain and might act directly on the immune system. Expression of CRF itself can be regulated by cytokines, adding another layer of complexity and a further target for intervention. Another neuropeptide, ACTH, which is an important mediator of the stress response produced in the pituitary gland, has been used for more than 40 years to treat patients with MS. ACTHR is expressed in MS lesions[2].

These results involving neuroendocrine mediators of autoimmunity imply that stress might be beneficial in autoimmunity and that brief interludes of starvation might help to reverse disease. In fighting microbial infections, perhaps 'stress' is detrimental and 'eating' is recommended, but in the case of autoimmunity, the opposite appears to hold[39].

Connections between allergy and autoimmunity

Previous work has shown that self-antigens can trigger allergic responses. In our recent work[44,45], we have brought to attention Paul Ehrlich's century-old scenario for autoimmunity, 'horror autotoxicus', in which the immune system attacks the body's own tissues[44]. Large-scale transcriptional sequencing of MS lesions has shown that there are a large number of allergy-related gene transcripts in MS lesions (TABLE 2). These transcripts include those encoding prostaglandin D_1, platelet-activating factor receptor (PTAFR), tryptase, Fcε receptor (FcεR) and eosinophilic cationic protein[2,45].

Since Rivers' description of EAE more than 60 years ago[46], our concepts of allergy and autoimmunity have been highly dichotomous[46–48]. However, this distinction has been increasingly blurred as drugs that are commonly used for the treatment of allergic diseases, such as antihistamines, have been shown to ameliorate EAE[46–48]. Furthermore, an association between sensitivity to histamine and susceptibility to EAE has been described[46–48]. *B. pertussis* toxin (PTX), which increases vasoactive-amine sensitization, is required as an adjuvant to induce EAE in those strains of mice that are not physiologically sensitive to histamine. *B. pertussis* histamine sensitization (*Bphs*) is the gene controlling PTX-induced vasoactive-amine sensitization, and susceptibility to EAE and other autoimmune diseases is linked to an allele of this gene[49]. Interestingly, Teuscher and colleagues[49] reported recently that *Bphs* encodes the histamine 1 receptor (H1R). Finally, the use of large-scale analysis of gene transcripts from MS lesions has identified several molecules that can also have important roles in the allergic response. Taken together, this evidence indicates that components of classical allergic responses can also markedly influence the pathogenesis of autoimmune disease in the EAE model.

Several molecules that can have important roles in allergic responses were shown to participate in EAE[1,2,46–49]. In MS lesions, we have shown that there is an increased level of transcription of the genes encoding H1R, PTAFR, tryptase, FcεRI and PGDS. Transcripts encoding tryptase, PTAFR and PGDS were present at an increased level in the CNS in EAE. Moreover, the expression of H1R was increased by T_H1 cells reactive to myelin, and immunohistochemical staining showed that H1R and H2R are present in inflammatory lesions. EAE was ameliorated in mice with disruptions of the γ-chain that is common to FcγRIII and FcεRI. Even if T_H1 cells are the main contributors to the pathogenesis of EAE and MS, molecules that are involved in allergic responses can potently modulate the disease also. In accordance with this conclusion, both H1R antagonists and PTAFR antagonists markedly blunted EAE[46–48].

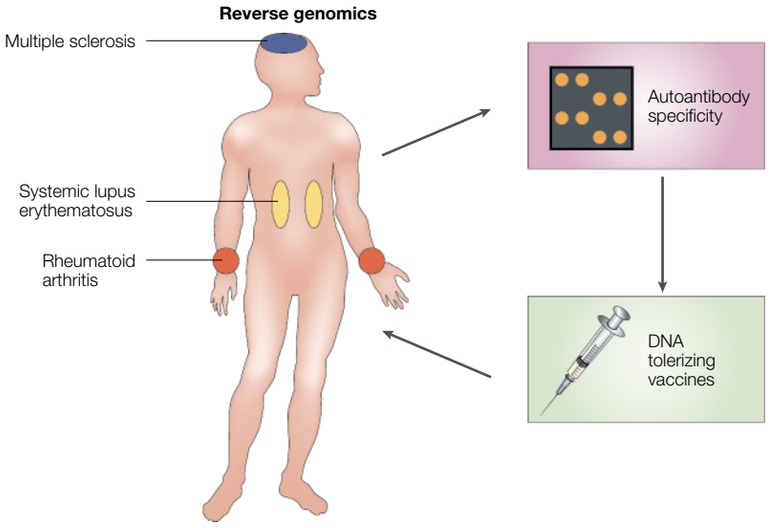

Figure 5 | **The 'reverse proteomic' approach.** Autoantibody microarrays[3] are constructed to identify the nature of the immune response. The specific autoantigens that have triggered immunity can then be targeted, and the immune response to them suppressed by encoding the autoantigens in a DNA plasmid. The plasmid contains specially engineered immunosuppressive DNA sequences[50,51,54].

A proteomic approach to autoimmune disease

Perhaps again with homage to Ehrlich's ideas[44], we can use DNA as a drug to manufacture a 'magic bullet' to reverse the course of autoimmunity. We have recently described a technology that allows the large-scale analysis of autoantibody responses[50,51]. We have adapted this technology to construct a myelin proteome, representing the main myelin proteins and their peptide epitopes. Using this microarray, we can analyse, on a large scale, the autoantibody response as it spreads to various components of the myelin sheath and targets the immune system for attack. Using an approach whereby DNA encoding these myelin components can tolerize or even deviate autoaggressive anti-myelin responses, we can now take the autoantibody data from the myelin microarray and construct a vaccine consisting of DNA encoding these myelin targets[52,53].

The use of DNA vaccines can even be applied on a wider scale. Using DNA constructs that actually target pathogenic cytokines, rather than promoting tolerance to self, we can reverse the effects of pathogenic molecules such as OPN by using a DNA vaccine to this pathogenic cytokine[26]. Such an approach can reverse ongoing paralytic disease. This approach has been used to target various pathogenic chemokines and cytokines[26,54–57]. So, DNA, when used as a drug, can rapidly and conveniently target molecules that have been discovered by the large-scale proteomic and transcriptional analysis of diseased tissues. DNA itself can be used as a 'magic bullet'. This 'reverse proteomic' approach, using DNA as a drug, is shown in FIG. 5.

Conclusions

Large-scale transcriptional profiling of lesions from the brains of patients with MS has determined a large number of new targets and new pathways for potential interventions. The course of inflammation has indicated molecules that most experts would have considered unlikely to have a role in autoimmune disease. OPN, neuroendocrine mediators, enzymes involved in the metabolism of cholesterol, and molecules associated with allergy all have important roles in the pathogenesis of MS. Large-scale approaches to understanding the immune response can be extended to proteomics, and these new technologies reveal further complexities of autoimmune inflammation.

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