The role of TNFα and lymphotoxin in demyelinating disease

Christopher Lock, Jorge Oksenberg, Lawrence Steinman

Multiple sclerosis (MS) is an inflammatory demyelinating disease of central nervous system (CNS) white matter.[1] The aetiology is unknown but the condition is probably the result of a misdirected immune response against myelin antigens. Pathologically there are multiple plaques or areas of white matter inflammation, demyelination, and glial scarring or sclerosis. In addition to myelin damage, axon loss may occur as there is a close relation between myelin and axon.[2] The inflammatory lesions are disseminated in time and space, and clinically the illness is characterised by relapsing episodes of neurological dysfunction.

In a commonly proposed sequence of events, autoreactive myelin specific CD4+ Th1 are activated in the periphery, probably by non-self antigens with a resemblance to myelin proteins.[3] T cells interact with adhesion molecules, such as selectins and integrins, on the capillary endothelium and then migrate into the brain parenchyma in response to chemotactic signals. Matrix metalloproteases (MMPs) are important in facilitating T cell penetration through the endothelial basement membrane. Microglia and astrocytes reactivate T cells locally in the CNS by presentation of myelin proteins bound to class II MHC molecules. T cells stimulate macrophage activity by release of proinflammatory cytokines such as IL2, IFNγ, tumour necrosis factor α (TNFα) and lymphotoxin (LT). Activated macrophages phagocytose myelin, and damage myelin by release of proteases, nitric oxide metabolites, reactive oxygen species, eicosanoids, complement components and TNFα. Autoantibodies directed against myelin basic protein (MBP) and myelin oligodendrocyte glycoprotein (MOG) are involved in myelin damage.[4 5]

Experimental allergic (or autoimmune) encephalomyelitis (EAE) can be induced in a variety of animal species, including non-human primates, by immunisation with spinal cord homogenates, myelin proteins or their peptide derivatives. A number of different myelin proteins can induce EAE, including proteolipid protein (PLP), MBP, MOG, myelin associated glycoprotein (MAG), and 2'3' cyclic nucleotide 3'-phosphdiesterase (CNP). Immunisation induces brain inflammation accompanied by varied signs of neurological disease. EAE can be adaptively transferred by myelin sensitised T cells. EAE and MS share common clinical, histological, immunological and genetic features, and EAE is widely considered to be a relevant model for the human disease. TNFα and LT are key elements in the pathogenesis of MS and EAE (reviewed by Steinman[6]).

TNFα and LT in EAE

Many studies indicate that TNFα activity is increased during active disease. TNFα and LT mRNA can both be detected in the CNS in acute EAE, and are made predominantly by microglia and infiltrating macrophages.[7] LT increases before the onset of clinical signs of EAE, while TNFα peaks at the height of clinical disease and during relapses.[7–9] TNFα given systemically worsens the severity and duration of EAE and can trigger relapses.[10 11] TNFα and LT are both directly toxic in culture to oligodendrocytes, cells that form the myelin sheath in the CNS.[12 13] TNFα given directly into the vitreous chamber in mice causes demyelination of the optic nerve.[14]

TNFα production by astrocytes can be induced in culture. There is higher production of TNFα in astrocytes from rodent strains which are susceptible to EAE.[15] The ability of MBP reactive T cell clones to transfer EAE correlates with their level of production of TNFα and LT.[16] Altered peptide ligands reduce the production of TNFα and Th1 cytokines, and can reverse EAE.[17 18]

Bacterial superantigens can induce relapsing attacks of paralysis in EAE, an effect blocked by antibody against TNFα.[19] TNFα delivered locally by a T cell clone carrying a TNFα encoding retrovirus construct exacerbates EAE.[20]

Reduction of TNFα activity by a number of different means abrogates disease. Treatment of mice with anti-TNFα antibody[21 22] or neutralisation of TNFα and LT activity with soluble p55 TNF receptor[23–25] blocks development of EAE. Rolipram, a selective phosphodiesterase type IV inhibitor that reduces production of TNFα and LT, reduces clinical signs of EAE.[26–28]

In summary, these studies lead to the conclusion that increased levels of TNFα activity exacerbate disease, while blockade of TNFα lessens disease in EAE models.

TNFα and LT transgenic and knockout mice

Transgenic experiments indicate that overexpression of TNFα can induce spontaneous disease. Overexpression of TNFα in the CNS of transgenic mice causes spontaneous inflammatory demyelination.[29–31] Oligodendrocyte apoptosis and myelin vacuolation is observed in these animals.[32] The effects of TNFα are prevented if the p55 TNF receptor is knocked out.[32] Overexpression of TNFα in the CNS,

Department of Neurology and Neurological Sciences, Stanford University, Beckman Center B002, Stanford, CA 94305, USA
C Lock
L Steinman

Department of Neurology, University of California at San Francisco, School of Medicine, San Francisco, USA
J Oksenberg

Correspondence to: Dr L Steinman.

even in mice lacking CD4, β-2 microglobulin, immunoglobulin µ chain, and RAG-1, is sufficient to induce demyelination.[33]

Gene knockout studies have examined the role of TNFα and LT in susceptibility to induced EAE. A complicating factor in several studies[34 35] is that disruption of these genes causes developmental defects, such as abnormal lymph nodes, altered splenic architecture, and abnormal immune function. In a more recent study of TNFα and LT knockout mice, immunodeficiency was corrected in LTα-/- mice by reconstitution with bone marrow cells.[36] Mice with TNFα knocked out, but with LTα present, had a delayed onset and shorter duration of disease. The absence of TNFα had the effect of impairing lymphocyte migration into the CNS. When LTα was knocked out, but TNFα was present, EAE developed normally. Similar results were seen in an earlier study of TNFα knockout mice.[37] The conclusion from these knockouts is that TNFα plays an important part in lymphocyte trafficking into the CNS.

Although TNFα is generally proinflammatory in EAE experiments, TNFα may be anti-inflammatory under some conditions. TNFα reduced EAE when given systemically using a recombinant vaccinia virus.[38] The effects of TNF and other cytokines vary depending on whether they are given systemically versus locally, timing of administration, and other factors. In another study of TNFα-/- knockout mice, MOG induced EAE was more severe in TNFα-/- mice than in littermate controls.[39] TNFα given systemically was protective and prevented development of EAE. Presumably the explanation is one of cytokine redundancy, with other molecules substituting functionally for TNFα in this situation. Similarly, Th1 cells are generally implicated in EAE, and Th1 clones reactive to MBP can transfer disease.[40] However in the study of Lafaille *et al*,[41] anti-MBP Th2 cells were derived from MBP specific TCR transgenic mice, and transferred into RAG-1 knockout mice. The transferred anti-MBP specific Th2 cells were able to cause EAE. IL4 was present in the lesions, but no TNFα or other Th1 cytokines were present.

Despite these exceptions, the same generalisations hold true from experiments with TNFα transgenic and knockout mice. TNF expression in transgenic mice is sufficient to cause spontaneous inflammatory demyelination, while inactivation of the TNFα gene impairs lymphocyte migration into the CNS.

TNFα and LT in MS
As in EAE, TNFα and LT protein and mRNA can be demonstrated in MS plaques.[42 43] TNFα positive cells include lymphocytes, macrophages, endothelial cells, astrocytes, and microglia.[44 45]

TNFα is present in the CSF of subjects with MS,[46–48] and the level of TNFα correlates with severity and progression of disease.[49] TNFα increases the permeability of CNS endothelial cells.[50] TNFα levels in CSF are higher in MS subjects with active disease and correlate with blood-brain barrier damage.[51] CSF mononuclear cells from MS subjects show increased TNFα mRNA levels, compared with blood mononuclear cells, or compared with cells from the CSF of control subjects.[52 53] PBLs maintained in culture from MS subjects produce more TNFα and LT than controls.[54] Disease exacerbation is correlated with higher levels of TNFα and LT mRNA in PBLs.[55–58] TNFα production after mitogen stimulation of PBLs from MS subjects is increased before exacerbations.[59]

As well as being toxic to oligodendrocytes, TNFα, and to a lesser extent LT, is mitogenic for astrocytes in culture, and may contribute to reactive gliosis found in MS.[60 61]

Like EAE, these data suggest that higher levels of TNFα correlate with increased disease activity in MS.

TNF genes and susceptibility to MS
The genes encoding TNFα and LT are embedded within the major histocompatibility complex (MHC) in the human chromosomal segment 6p21, about 250 kb centromeric to the HLA-B gene and 355 kb telomeric to C2. Multiple polymorphisms have been identified in or near the TNF genes. However case-control population based studies have failed to detect significant genetic association with MS susceptibility or progression. Results indicating distortions of the expected allelic frequencies could be attributed to the effect of linkage disequilibrium with HLA-DR2.[62]

Current treatments for MS: effects on TNFα
Several currently used treatments for MS have effects on TNFα activity. Methylprednisolone intravenously, or oral prednisone, is commonly used in the treatment of MS exacerbations. The effects of corticosteroids include a decrease in TNFα activity by inhibiting transcription.[63] There is a reduction of MMP-9 activity (gelatinase B), and increased levels of TIMP-3.[64]

Two forms of IFNß are currently used for the treatment of MS, IFNß1b (Betaseron) and IFNß1a (Avonex, Rebif). IFNß has several mechanisms of action (reviewed by Yong *et al*[65]). Pre-treatment of T cells with IFNß decreases VLA-4 integrin expression and reduces subsequent TNFα production by microglia.[66] IFNß may favour the production of anti-inflammatory cytokines,[67–71] and decrease TNFα production.[72 73] IFNß decreases TNFα receptor levels on T cells.[74] IFNß decreases MMP-2 and MMP-9 activity and reduces T cell migration into the CNS.[75 76]

Copolymer-1 (Copaxone), a random copolymer of alanine, glutamic acid, tyrosine, and lysine, is the most recent immunomodulatory drug approved for the treatment of MS. Recent studies suggest that copolymer-1 acts as an altered peptide ligand, causing a shift from a Th1 to a Th2/Th3 response, with a decrease in TNFα mRNA levels.[77]
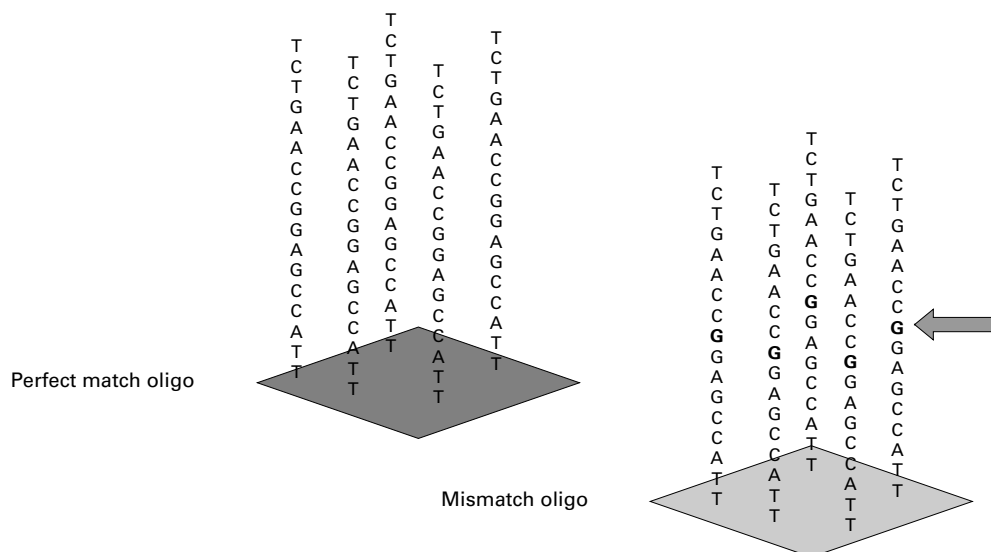
Figure 1   Chip design. A perfect match (PM) and an adjacent mismatch (MM) 25-mer DNA oligonucleotide probe pair is illustrated. The MM oligonucleotide has a single change at position 13.

Therapeutic blockade of TNFα activity in MS

A study of two rapidly progressive MS subjects treated with the anti-TNFα antibody cA2 (Remicade, Centocor) was reported by Van Osten *et al* in 1996.[78] The subjects were given two infusions of 10 mg/kg of antibody at intervals of two weeks. There was no reported clinical worsening of disease. However, an increase in the number of gadolinium enhancing lesions on magnetic resonance imaging, a rise in CSF IgG index, and an increase in the number of lymphocytes in the CSF was observed after each infusion. The magnetic resonance imaging and CSF findings were felt to indicate intrathecal immune activation. VCAM-1 was detectable in CSF post-infusion, while levels of VCAM-1 and ICAM-1 were lower in serum. TNFα production by stimulated WBCs was lower after treatment. cA2 antibody could not be detected in CSF, and was probably not able to cross the blood-brain barrier. Unfortunately, there were no monthly pre-treatment scans to estimate ongoing disease activity in this small study.

A soluble dimeric p55 TNF receptor-immunoglobulin fusion protein (sTNFR-IgG p55; lenercept, Roche) was tested in a double blind placebo controlled study of 168 mainly relapsing-remitting MS subjects.[78a] Subjects were treated with 10, 50, or 100 mg of lenercept intravenously every four weeks, for a period of up to 48 weeks. Clinical evaluations and MRI were obtained every four weeks. The exacerbation rate was increased by 2%, 68%, and 50%, over the placebo rate, in subjects treated with 10, 50 and 100 mg of lenercept respectively (p = 0.007). There was a dose dependent decrease in the time to first exacerbation in treated groups (p = 0.006). There
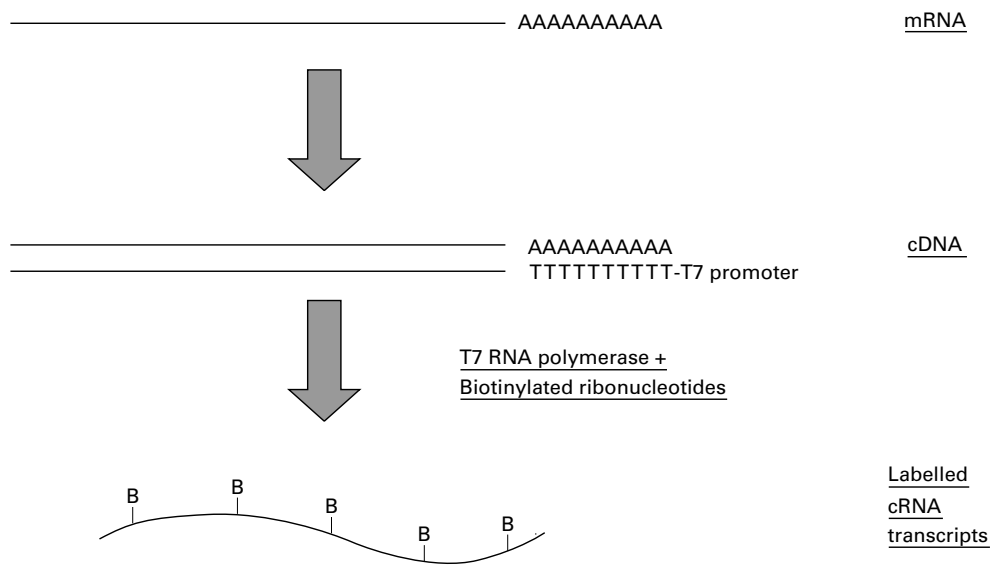

Figure 2   Sample preparation. A primer containing oligo-dT (T24) and a T7 RNA polymerase binding site is used for first strand cDNA synthesis. Double stranded cDNA is used for an in vitro transcription reaction, to make cRNA labelled with biotin-UTP and biotin-CTP.

Figure 3 Genechip image. The figure shows a scanned image of a HuGeneFL chip.

occurrences of rheumatoid factor or ANA were more common in the lenercept treated group. Despite clinical worsening, there were no significant changes in MRI measurements between groups. One explanation may be the timing of scans in relation to drug dosing. MRI scans were obtained four weeks after the preceding dose, and changes may therefore have been missed. However, it is also recognised that MRI changes have poor pathological specificity in MS, and poor correlation with clinical measures of disability. Newly active MRI lesions reflect changes in blood-brain barrier permeability, which may perhaps be separate from CNS inflammation.

Pentoxyfilline, a phosphodiesterase inhibitor, reduces TNFα production and prevents EAE[79 80] and has been studied in MS. TNFα production is reduced in vitro,[81] but there is no apparent effect on disease.[82 83] Perfenidone, an experimental drug that prevents gliosis and blocks TNFα synthesis, is currently being tested in MS.

To date the results of TNFα blockade in MS have been disappointing, and seem to make disease worse, or at best have no effect. These results are unexpected and in contrast with animal models, where TNFα blockade is effective in ameliorating disease. The reasons for this difference are not clear at the present time.

Metalloproteinase inhibitors

The MMPs are a group of zinc containing proteolytic enzymes involved in the degradation and remodelling of the extracellular matrix.[84] The family consists of approximately 18 members subdivided into collagenases, gelatinases, stromelysin, and membrane-type MMPs. T cells express predominantly MMP-2 (gelatinase A) and-9 (gelatinase B), while macro-
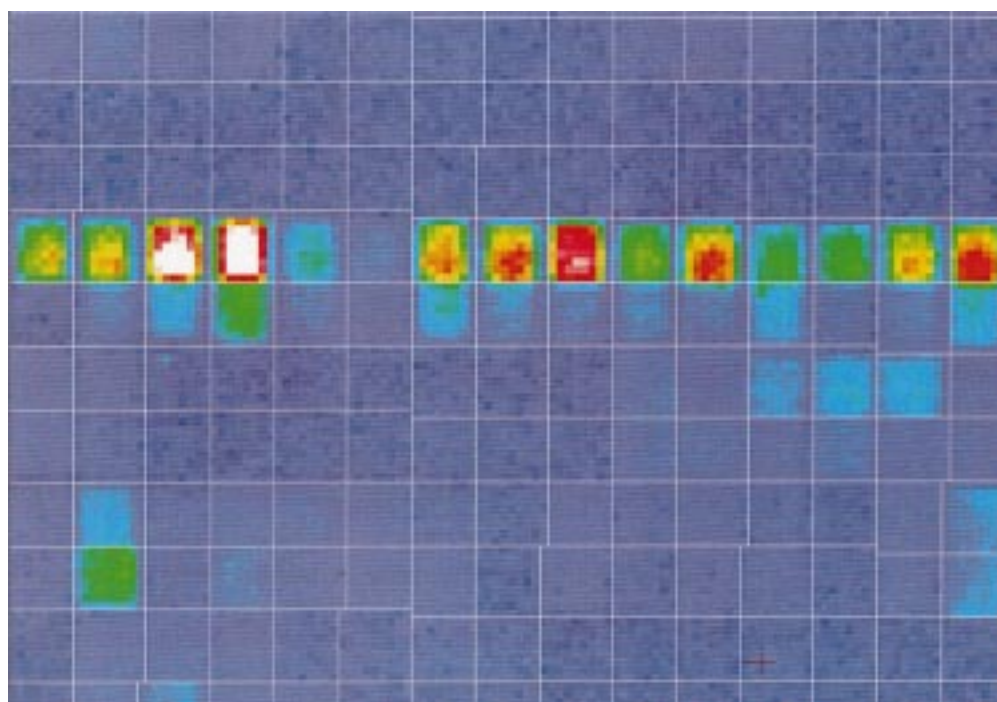
was a tendency for the duration and severity of exacerbations to be increased with lenercept, although this was not statistically significant. Side effects included headache, hot flushes, nausea, dyspnoea, and abdominal pain. Antibodies to lenercept were detected in most of the treated subjects. Trough serum concentrations of lenercept were detected in only a third of patients, and not in those subjects with high anti-lenercept antibody concentrations. New

Figure 4 Close view of genechip. Individual synthesis features or probe cells can be seen. Each 24 × 24 μm probe cell is imaged by the scanner as a series of 3 μm pixels. The software aligns a grid to the image after scanning. A row of brighter PM probes can be seen, above a row of corresponding MM probes.
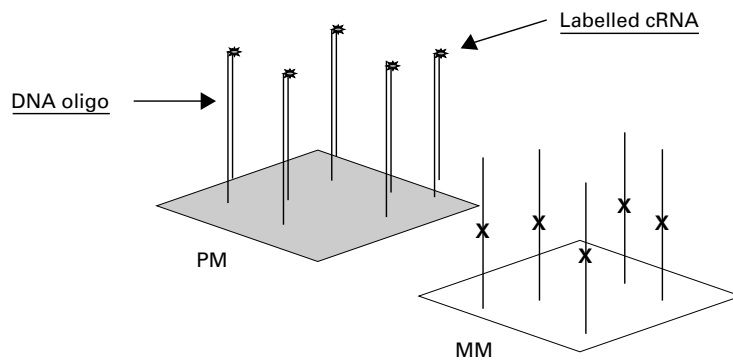
Figure 5   Hybridisation. Biotinylated cRNA, stained with streptavidin-phycoerythrin, binds preferentially to the PM oligonucleotide. Hybridisation is measured by calculation of PM-MM differences, and PM/MM ratios. The software makes a call of increased, decreased, or no change for each RNA, when comparing two chips.

phages secrete a broader range and larger amounts of MMPs,[85] including MMP-1, -2, -3, -7 (matrilysin), -9 and -12. Neurons, astrocytes, and oligodendrocytes also express MMPs.[86–89] The activity of MMPs is tightly regulated at the level of transcription, by proenzyme activation, and by the activity of tissue inhibitors of metalloproteinases (TIMPs). Gene transcription can be induced by growth factors, inflammatory cytokines, and via cell-ECM or cell-cell interactions. MMPs are initially produced as inactive pro-MMPs, containing a zinc atom bound to a cysteine residue in the catalytic domain. Activating factors disrupt the zinc-cysteine interaction and expose the catalytic site. After activation, MMPs are regulated by the formation of complexes with one of the four TIMPs. MMP activity is tightly controlled by these means, but excess MMP production and activation may be an important factor in autoimmune diseases, including MS.

Table 1   TNF related genes represented on HuGeneFL array

Accession	Entrez definition
HG4683-HT5108	Tumour necrosis factor receptor 2 associated protein Trap3
M16441	Lymphotoxin gene extracted from human tumour necrosis factor and lymphotoxin genes, complete CDs
M31165	Human tumour necrosis factor inducible (TSG-6) mRNA fragment, adhesion receptorCD44 putative CDS
M31166	Human tumour necrosis factor inducible (TSG-14) mRNA, complete CDs
U 12595	Human tumour necrosis factor type 1 receptor associated protein (TRAP1) mRNA, partial CDs
M32315	Human tumour necrosis factor receptor mRNA, complete CDs
M59465	Human tumour necrosis factor alpha inducible protein A20 mRNA, complete CDs
U77396	Human TNF alpha inducible responsive element mRNA, complete CDs
U86755	Human TNF alpha converting enzyme mRNA, complete CDs
U69611	Human TNF alpha converting enzyme mRNA, complete CDs
U78798	Human TNF receptor associated factor 6 (TRAF6) mRNA, complete CDs.
D78151	Human mRNA for 26S proteasome subunit p97, complete CDs
D38047	Human mRNA for 26S proteasome subunit p31, complete CDs
X02910	Human gene for tumour necrosis factor (TNF alpha)
V01512	Human cellular oncogene c-fos (complete sequence)
J04111	Human c-jun proto oncogene (JUN), complete CDs, clone hCJ-1
M58286	Homo sapiens tumour necrosis factor receptor mRNA, complete CDs
L41690	Homo sapiens TNF receptor-1 associated protein (TRADD) mRNA, 3' end of CDs
U69108	Homo sapiens TNF receptor associated factor 5 mRNA, partial CDs
Z22951	Homo sapiens gene encoding p65 subunit of transcription factor NF-kappaB
L04270	Homo sapiens (clone CD18) tumour necrosis factor receptor 2 related protein mRNA, complete CDs
Y10256	Homo sapiens mRNA for serine/threonine protein kinase, NIK

The HuGeneFL probe array is a high density, single chip array with over 6000 genes represented. Full length genes were selected from UniGene, GenBank, and TIGR databases by Affymetrix. TNFα, LT, TNF receptors, and a number of genes involved in TNFα signalling pathways are represented on the array.

MMPs are needed to facilitate T cell penetration through the basement lamina of the vascular endothelium into the CNS. Increased MMP expression can be demonstrated in the CNS of subjects with MS[90–95] and in animals with EAE.[96 97] MMP inhibitors block degradation of the extracellular matrix and reduce entry of T cells into the CNS. One mechanism of action of IFNß in MS is the inhibition of MMP-9 activity of T lymphocytes.[75 76]

TNFα is initially produced as a 26 kDa membrane anchored protein and is converted to the mature, secreted 17 kDa protein by TNFα converting enzyme (TACE). MMP inhibitors prevent the conversion of TNFα into an active form. In addition, MMPs contribute directly to the degradation of myelin proteins, and MMP inhibitors may block this activity.

MMP inhibitors can block induction of EAE.[98–102] A small trial in MS subjects of a combination of D-penicllamine and metacycline, which inhibit MMP-9 and t-PA, was reported. Ten patients with secondary progressive MS were treated over a period of one year. There was no improvement in Extended Disability Status Scale (EDSS) scores at one year, and there were problems with toxicity.[103] Several MMP inhibitors are currently being tested in clinical trials in MS, but there are no published data at the present time.

Genomics and microarray technology

Current estimates suggest that there are approximately 100 000 genes in the human genome, and about one half of these have been partially or completely sequenced. Determination of which of the 100 000 genes are expressed is a useful initial step in understanding a disease process.

Microarray technology allows a large scale readout of gene expression. Several different types of microarrays are available, based on cDNAs, PCR products, or oligonucleotides immobilised on a solid support. The Affymetrix Genechip is a high density oligonucleotide array synthesised directly onto glass slides by a combination of photolithography and light activated chemistry.[104 105] The expression arrays contain as many as 400 000 24×24 μm synthesis features. Each synthesis feature or probe cell contains approximately 10^7 copies of a specific 25-mer DNA oligonucleotide sequence. The synthesis features are organised in pairs, consisting of a perfect match (PM) oligonucleotide and a mismatch oligonucleotide (MM) immediately below (fig 1). The MM oligonucleotide is identical except for a single base change at the middle position, and is used as a control for hybridisation specificity. Each gene on the array is represented by a series of 20 probe pairs, which span the sequence.

Samples are prepared for hybridisation to the array, first by isolation of polyA+ mRNA. Double stranded cDNA is then made, and the cDNA is used as a template to produce biotinylated cRNA (fig 2). The labelling procedure amplifies the mRNA population by about 150-fold. The cRNA is fragmented and hybridised to the array, forming hybrids

between the biotinylated cRNA and the DNA oligonucleotides on the chip. The array is washed, stained with streptavidin-phycoerythrin, and scanned with a confocal laser microscope. A scanned image of a genechip is shown in figure 3, and a close up view in figure 4.

The average fluorescence intensity is calculated for each probe cell. The presence or absence of a particular RNA is determined from the hybridisation pattern, using PM and MM differences and ratios (fig 5). The signal is proportional to the amount of bound, labelled cRNA. The relative concentrations of different RNAs in a population can be estimated from the signal intensity. A single sample is applied to each array, and the software compares arrays.

Using this system, we have analysed several rodent EAE models, and human MS samples (unpublished data), to look for differentially expressed genes. The aim is to identify novel targets for drug development. A number of TNF related genes are represented on the arrays (table 1). Many genes known to be involved in demyelination are differentially expressed, along with a number of less well characterised genes. The data are complex, but provide a more global view of gene expression. A difficulty with this type of data is that it does not give functional information regarding the identified genes. Functional experiments such as gene inactivation, cellular localisation, and other types of studies are needed. Cluster analysis may provide functional clues by grouping together genes whose expression is co-regulated.[106] The size of the data sets also presents a challenge.

Genomics has been likened to the biological equivalent of the chemical periodic table, but with 100 000 gene elements and an information space with more than two dimensions.[107] The microarray approach will hopefully provide a more comprehensive view of TNF related pathways involved in demyelinating and other diseases.

1 Steinman L. Multiple sclerosis: a coordinated immunological attack against myelin in the central nervous system. Cell 1996;85:299–302.

2 Trapp BD, Peterson J, Ransohoff RM, Rudick R, Mork S, Bo L, Axonal transection in the lesions of multiple sclerosis [see comments]. N Engl J Med 1998;338:278–85.

3 Hartung HP. Pathogenesis of inflammatory demyelination: implications for therapy. Curr Opin Neurol 1995;8:191–9.

4 Warren KG, Catz I, Steinman L. Fine specificity of the antibody response to myelin basic protein in the central nervous system in multiple sclerosis: the minimal B-cell epitope and a model of its features. Proc Natl Acad Sci USA 1995;92:11061–5.

5 Genain CP, Cannella B, Hauser SL, Raine CS. Identification of autoantibodies associated with myelin damage in multiple sclerosis [see comments]. Nat Med 1999;5:170–5.

6 Steinman L. Some misconceptions about understanding autoimmunity through experiments with knockouts. J Exp Med 1997;185:2039–41.

7 Renno T, Krakowski M, Piccirillo C, Lin JY, Owens T. TNF-alpha expression by resident microglia and infiltrating leukocytes in the central nervous system of mice with experimental allergic encephalomyelitis. Regulation by Th1 cytokines. J Immunol 1995;154:944–53.

8 Issazadeh S, Ljungdahl A, Hojeberg B, Mustafa M, Olsson T. Cytokine production in the central nervous system of Lewis rats with experimental autoimmune encephalomyelitis: dynamics of mRNA expression for interleukin-10, interleukin-12, cytolysin, tumor necrosis factor alpha and tumor necrosis factor beta. J Neuroimmunol 1995;61:205–12.

9 Begolka WS, Vanderlugt CL, Rabbe SM, Miller SD. Differential expression of inflammatory cytokines parallels progression of central nervous system pathology in two clinically distinct models of multiple sclerosis. J Immunol 1998;161:4437–46.

10 Kuroda Y, Shimamoto Y. Human tumor necrosis factor-alpha augments experimental allergic encephalomyelitis in rats. J Neuroimmunol 1991;34:159–64.

11 Crisi GM, Santambrogio L, Hochwald GM, Smith SR, Carlino JA, Thorbecke GJ. Staphylococcal enterotoxin B and tumor-necrosis factor-alpha-induced relapses of experimental allergic encephalomyelitis: protection by transforming growth factor-beta and interleukin-10. Eur J Immunol 1995;25:3035–40.

12 Selmaj KW, Raine CS. Tumor necrosis factor mediates myelin and oligodendrocyte damage in vitro. Ann Neurol 1988;23:339–46.

13 Selmaj K, Raine CS, Farooq M, Norton WT, Brosnan CF. Cytokine cytotoxicity against oligodendrocytes. Apoptosis induced by lymphotoxin. J Immunol 1991;147:1522–9.

14 Jenkins HG, Ikeda H. Tumour necrosis factor causes an increase in axonal transport of protein and demyelination in the mouse optic nerve. J Neurol Sci 1992;108:99–104.

15 Chung IY, Norris JG, Benveniste EN. Differential tumor necrosis factor alpha expression by astrocytes from experimental allergic encephalomyelitis-susceptible and -resistant rat strains. J Exp Med 1991;173:801–11.

16 Powell MB, Mitchell D, Lederman J, et al. Lymphotoxin and tumor necrosis factor-alpha production by myelin basic protein-specific T cell clones correlates with encephalitogenicity. Int Immunol 1990;2:539–44.

17 Karin N, Mitchell DJ, Brocke S, Ling N, Steinman L. Reversal of experimental autoimmune encephalomyelitis by a soluble peptide variant of a myelin basic protein epitope: T cell receptor antagonism and reduction ofinterferon gamma and tumor necrosis factor alpha production. J Exp Med 1994;180:2227–37.

18 Brocke S, Gijbels K, Allegretta M, et al. Treatment of experimental encephalomyelitis with a peptide analogue of myelin basic protein [published erratum appears in Nature 19989;392:630]. Nature 1996;379:343–6.

19 Brocke S, Gaur A, Piercy C, et al. Induction of relapsing paralysis in experimental autoimmune encephalomyelitis by bacterial superantigen. Nature 1993;365:642–4.

20 Dal Canto RA, Shaw MK, Nolan GP, Steinman L, Fathman CG. Local delivery of TNF by retrovirus-transduced T lymphocytes exacerbates experimental autoimmune encephalomyelitis. Clin Immunol 1999;90:10–14.

21 Selmaj K, Raine CS, Cross AH. Anti-tumor necrosis factor therapy abrogates autoimmune demyelination. Ann Neurol 1991;30:694–700.

22 Ruddle NH, Bergman CM, McGrath KM, et al. An antibody to lymphotoxin and tumor necrosis factor prevents transfer of experimental allergic encephalomyelitis. J Exp Med 1990;172:1193–200.

23 Klinkert WE, Kojima K, Lesslauer W, Rinner W, Lassmann H, Wekerle H. TNF-alpha receptor fusion protein prevents experimental auto-immune encephalomyelitis and demyelination in Lewis rats: an overview. J Neuroimmunol 1997;72:163–8.

24 Selmaj K, Papierz W, Glabinski A, Kohno T. Prevention of chronic relapsing experimental autoimmune encephalomyelitis by soluble tumor necrosis factor receptor I. J Neuroimmunol 1995;56:135–41.

25 Baker D, Butler D, Scallon BJ, O'Neill JK, Turk JL, Feldmann M. Control of established experimental allergic encephalomyelitis by inhibition of tumor necrosis factor (TNF) activity within the central nervous system using monoclonal antibodies and TNF receptorimmunoglobulin fusion proteins. Eur J Immunol 1994;24:2040–8.

26 Genain CP, Roberts T, Davis RL, et al. Prevention of autoimmune demyelination in non-human primates by a cAMP- specific phosphodiesterase inhibitor. Proc Natl Acad Sci USA 1995;92:3601–5.

27 Sommer N, Loschmann PA, Northoff GH, et al. The antidepressant rolipram suppresses cytokine production and prevents autoimmune encephalomyelitis [see comments]. Nat Med 1995;1:244–8.

28 Sommer N, Martin R, McFarland HF, et al. Therapeutic potential of phosphodiesterase type 4 inhibition in chronic autoimmune demyelinating disease. J Neuroimmunol 1997;79:54–61.

29 Probert L, Akassoglou K, Pasparakis M, Kontogeorgos G, Kollias G. Spontaneous inflammatory demyelinating disease in transgenic mice showing central nervous system-specific expression of tumor necrosis factor alpha. Proc Natl Acad Sci USA 1995;92:11294–8.

30 Taupin V, Renno T, Bourbonniere L, Peterson AC, Rodriguez M, Owens T. Increased severity of experimental autoimmune encephalomyelitis, chronic macrophage/microglial reactivity, and demyelination in transgenic mice producing tumor necrosis factor-alpha in the central nervous system. Eur J Immunol 1997;27:905–13.

31 Akassoglou K, Probert L, Kontogeorgos G. Kollias G. Astrocyte-specific but not neuronspecific transmembrane TNF triggers inflammation and degeneration in the central nervous system of transgenic mice. J Immunol 1997;158:438–45.

32 Akassoglou K, Bauer J, Kassiotis G, *et al*. Oligodendrocyte apoptosis and primary demyelination induced by local TNF/p55TNF receptor signaling in the central nervous system of transgenic mice: models for multiple sclerosis with primary oligodendrogliopathy. Am J Pathol 1998;153: 801–13.

33 Kassiotis G, Bauer J, Akassoglou K, Lassmann H, Kollias G, Probert L. A tumor necrosis factor-induced model of human primary demyelinating diseases develops in immunodeficient mice. Eur J Immunol 1999;29:912–17.

34 Frei K, Eugster HP, Bopst M, Constantinescu CS, Lavi E, Fontana A. Tumor necrosis factor alpha and lymphotoxin alpha are not required. for induction of acute experimental autoimmune encephalomyelitis. J Exp Med 1997;185: 2177–82.

35 Suen WE, Bergman CM, Hjelmstrom P, Ruddle NH. A critical role for lymphotoxin in experimental allergic encephalomyelitis. J Exp Med 1997;186:1233–40.

36 Sean Riminton D, Korner H, Strickland DH, Lemckert FA, Pollard JD, Sedgwick JD. Challenging cytokine redundancy: inflammatory cell movement and clinical course of experimental autoimmune encephalomyelitis are normal in lymphotoxin-deficient, but not tumor necrosis factor-deficient, mice. J Exp Med 1998;187:1517–28.

37 Korner H, Riminton DS, Strickland DH, Lemckert FA, Pollard JD, Sedgwick JD. Critical points of tumor necrosis factor action in central nervous system autoimmune inflammation defined by gene targeting. J Exp Med 1997;186:1585–90.

38 Willenborg DO, Fordham SA, Cowden WB, Ramshaw IA. Cytokines and murine autoimmune encephalomyelitis: inhibition or enhancement of disease with antibodies to select cytokines, or by delivery of exogenous cytokines using a recombinant vaccinia virus system. Scand J Immunol 1995;41:31–41.

39 Liu J, Marino MW, Wong G, *et al*. TNF is a potent antiinflammatory cytokine in autoimmune-mediated demyelination. Nat Med 1998;4:78–83.

40 Baron JL, Madri JA, Ruddle NH, Hashim G, Janeway CA, Jr. Surface expression of alpha 4 integrin by CD4 T cells is required for their entry into brain parenchyma. J Exp Med 1993;177:57–68.

41 Lafaille JJ, Keere FV, Hsu AL, *et al*. Myelin basic proteinspecific T helper 2 (Th2) cells cause experimental autoimmune encephalomyelitis in immunodeficient hosts rather than protect them from the disease. J Exp Med 1997;186:307–12.

42 Raine CS, Bonetti B, Cannella B. Multiple sclerosis: expression of molecules of the tumor necrosis factor ligand and receptor families in relationship to the demyelinated plaque. Rev Neurol (Paris) 1998;154:577–85.

43 Woodroofe MN, Cuzner ML. Cytokine mRNA expression in inflammatory multiple sclerosis lesions: detection by non-radioactive in situ hybridization. Cytokine 1993;5: 583–8.

44 Selmaj K, Raine CS, Cannella B, Brosnan CF. Identification of lymphotoxin and tumor necrosis factor in multiple sclerosis lesions. J Clin Invest 1991;87:949–54.

45 Hofman FM, Hinton DR, Johnson K, Merrill JE. Tumor necrosis factor identified in multiple sclerosis brain. J Exp Med 1989;170:607–12.

46 Maimone D, Gregory S, Arnason BG, Reder AT. Cytokine levels in the cerebrospinal fluid and serum of patients with multiple sclerosis. J Neuroimmunol 1991;32:67–74.

47 Hauser SL, Doolittle TH, Lincoln R, Brown RH, Dinarello CA. Cytokine accumulations in CSF of multiple sclerosis patients: frequent detection of interleukin-1 and tumor necrosis factor but not interleukin-6. Neurology 1990;40: 1735–9.

48 Drulovic J, Mostarica-Stojkovic M, Levic Z, Stojsavljevic N, Pravica V, Mesaros S. Interleukin-12 and tumor necrosis factor-alpha levels in cerebrospinal fluid of multiple sclerosis patients. J Neurol Sci 1997;147:145–50.

49 Sharief MK, Hentges R. Association between tumor necrosis factor-alpha and disease progression in patients with multiple sclerosis [see comments]. N Engl J Med 1991;325:467–72.

50 Duchini A, Govindarajan S, Santucci M, Zampi G, Hofman FM. Effects of tumor necrosis factor-alpha and interleukin-6 on fluid-phase permeability and ammonia diffusion in CNS-derived endothelial cells. J Investig Med 1996;44:474–82.

51 Sharief MK, Thompson EJ. In vivo relationship of tumor necrosis factor-alpha to bloodbrain barrier damage in patients with active multiple sclerosis. J Neuroimmunol 1992;38:27–33.

52 Monteyne P. Sindic CJ. Data on cytokine mRNA expression in CSF and peripheral blood mononuclear cells from MS patients as detected by PCR. Multiple Sclerosis 1998;4: 143–6.

53 Matusevicius D, Navikas V, Soderstrom M, *et al*. Multiple sclerosis: the proinflammatory cytokines lymphotoxin-alpha and tumour necrosis factor-alpha are upregulated in cerebrospinal fluid mononuclear cells. J Neuroimmunol 1996;66:115–23.

54 Mokhtarian F, Shi Y, Shirazian D, Morgante L, Miller A, Grob D. Defective production of anti-inflammatory cytokine, TGF-beta by T cell lines of patients with active multiple sclerosis. J Irnmunol 1994;152:6003–10.

55 Navikas V, He B, Link J, *et al*. Augmented expression of tumour necrosis factor-alpha and lymphotoxin in mononuclear cells in multiple sclerosis and optic neuritis. Brain 1996;119:213–23.

56 Correale J, Gilmore W, McMillan M, *et al*. Patterns of cytokine secretion by autoreactive proteolipid protein- specific T cell clones during the course of multiple sclerosis. J Immunol 1995;154:2959–68.

57 Rieckmann P, Albrecht M, Kitze B, *et al*. Tumor necrosis factor-alpha messenger RNA expression in patients with relapsing-remitting multiple sclerosis is associated with disease activity. Ann Neurol 1995;37:82–8.

58 Martino G, Consiglio A, Franciotta DM, *et al*. Tumor necrosis factor alpha and its receptors in relapsing-remitting multiple sclerosis. J Neurol Sci 1997;152:51–61.

59 Beck J, Rondot P, Catinot L, Falcoff E, Kirchner H, Wietzerbin J. Increased production of interferon gamma and tumor necrosis factor precedes clinical manifestation in multiple sclerosis: do cytokines trigger off exacerbations? Acta Neurol Scand 1988;78:318–23.

60 Selmaj KW, Farooq M, Norton WT, Raine CS, Brosnan CF. Proliferation of astrocytes in vitro in response to cytokines. A primary role for tumor necrosis factor. J Immunol 1990; 144:129–35.

61 Selmaj K, Shafit-Zagardo B, Aquino DA, *et al*. Tumor necrosis factor-induced proliferation of astrocytes from mature brain is associated with downregulation of glial fibrillary acidic protein mRNA. J Neurochem 1991;57: 823–30.

62 Oksenberg JR, Seboun E, Hauser SL. Genetics of demyelinating diseases. Brain Pathol 1996;6:289–302.

63 Andersson PB, Goodkin DE. Glucocorticosteroid therapy for multiple sclerosis: a critical review. J Neurol Sci 1998;160:16–25.

64 Rosenberg GA, Dencoff JE, Correa N Jr, Reiners M, Ford CC. Effect of steroids on CSF matrix metalloproteinases in multiple sclerosis: relation to blood-brain barrier injury. Neurology 1996;46:1626–32.

65 Yong VW, Chabot S, Stuve O, Williams G. Interferon beta in the treatment of multiple sclerosis: mechanisms of action. Neurology 1998;51:682–9.

66 Chabot S, Williams G, Yong VW. Microglial production of TNF-alpha is induced by activated T lymphocytes. Involvement of VLA-4 and inhibition by interferonbeta-lb. J Clin Invest 1997;100:604–12.

67 Noronha A, Toscas A, Jensen MA. Interferon beta decreases T cell activation and interferon gamma production in multiple sclerosis. J Neuroimmunol 1993;46:145–53.

68 Revel M, Chebath J, Mangelus M, Harroch S,Moviglia GA. Antagonism of interferon beta on interferon gamma: inhibition of signal transduction in vitro and reduction of serum levels in multiple sclerosis patients. Multiple Sclerosis 1995;1 (suppl 1):S5–11.

69 Rudick RA, Ransohoff RM, Peppler R, VanderBrug Medendorp S, Lehmann P, Alam J. Interferon beta induces interleukin-10 expression: relevance to multiple sclerosis. Ann Neurol 1996;40:618–27.

70 McRae BL, Picker LJ, van Seventer GA. Human recombinant interferon-beta influences T helper subset differentiation by regulating cytokine secretion pattern and expression of homing receptors. Eur J Immunol 1997;27:2650–6.

71 McRae BL, Semnani RT, Hayes MP, van Seventer GA. Type I IFNs inhibit human dendritic cell IL-12 production and Thl cell development. J Immunol 1998;160:4298–304.

72 Gayo A, Mozo L, Suarez A, Tunon A, Lahoz C, Gutierrez C. Interferon beta-lb treatment modulates TNFαlpha and IFNgamma spontaneous gene expression in MS [see comments]. Neurology 1999;52:1764–70.

73 Rep MH, Hintzen RQ, Polman CH, van Lier RA. Recombinant interferon-beta blocks proliferation but enhances interleukin-10 secretion by activated human T-cells. J Neuroimmunol 1996;67:111–18.

74 Bongioanni P, Mosti S, Moscato G, Lombardo F, Manildo C, Meucci G. Decreases in Tcell tumor necrosis factor alpha binding with interferon beta treatment in patients with multiple sclerosis. Arch Neurol 1999;56:71–8.

75 Stuve O, Dooley NP, Uhm JH, Antel JP, Francis GS, Williams G, *et al*. Interferon betalb decreases the migration of T lymphocytes in vitro: effects on matrix metalloproteinase-9. Ann Neurol 1996;40:853–63.

76 Leppert D, Waubant E, Burk MR, Oksenberg JR, Hauser SL. Interferon beta-1b inhibits gelatinase secretion and in vitro migration of human T cells: a possible mechanism for treatment efficacy in multiple sclerosis. Ann Neurol 1996; 40:846–52.

77 Miller A, Shapiro S, Gershtein R, *et al*. Treatment of multiple sclerosis with copolymer-1 (Copaxone): implicating mechanisms of Th1 to Th2/Th3 immune-deviation [In Process Citation]. J Neuroimmunol 1998;92:113–21.

78 van Oosten BW, Barkhof F, Truyen L, *et al*. Increased MRI activity and immune activation in two multiple sclerosis patients treated with the monoclonal anti-tumor necrosis factor antibody cA2. Neurology 1996;47:1531–4.

78a The Lenercept Multiple Sclerosis Study Group and the University of British Columbia MS/MRI Analysis Group. TNF neutralization in MS: results of a randomized, placebo-controlled multicenter study. Neurology 1999;53: 457–65.

79 Rott O, Cash E, Fleischer B. Phosphodiesterase inhibitor pentoxifylline, a selective suppressor of T helper type 1- but not type 2-associated lymphokine production, prevents induction of experimental autoimmune encephalomyelitis in Lewis rats. Eur J Immunol 1993;23:1745–51.

80 Nataf S, Louboutin JP, Chabannes D, Feve JR, Muller JY. Pentoxifylline inhibits experimental allergic encephalomyelitis. Acta Neurol Scand 1993;88:97–9.

81 Rieckmann P, Weber F, Gunther A, *et al*. Pentoxifylline, a phosphodiesterase inhibitor, induces immune deviation in

patients with multiple sclerosis. J Neuroimmunol 1996;64: 193–200.

82 Myers LW, Ellison GW, Merrill JE, *et al*. Pentoxifylline is not a promising treatment for multiple sclerosis in progression phase. Neurology 1998;51:1483–6.

83 van Oosten BW, Rep MH, van Lier RA, *et al*. A pilot study investigating the effects of orally administered pentoxifylline on selected immune variables in patients with multiple sclerosis. J Neuroimmunol 1996;66:49–55.

84 Yong VW, Krekoski CA, Forsyth PA, Bell R, Edwards DR. Matrix metalloproteinases and diseases of the CNS. Trends Neurosci 1998;21:75–80.

85 Goetzl EJ, Banda MJ, Leppert D. Matrix metalloproteinases in immunity. J Immunol 1996;156:1–4.

86 Gottschall PE, Deb S. Regulation of matrix metalloproteinase expressions in astrocytes, microglia and neurons. Neuroimmunomodulation 1996;3:69–75.

87 Apodaca G, Rutka JT, Bouhana K, *et al*. Expression of metalloproteinases and metalloproteinase inhibitors by fetal astrocytes and glioma cells. Cancer Res 1990;50:2322–9.

88 Colton CA, Keri JE, Chen WT, Monsky WL. Protease production by cultured microglia: substrate gel analysis and immobilized matrix degradation. J Neurosci Res 1993;35: 297—304.

89 Uhm JH, Dooley NP, Oh LY, Yong VW. Oligodendrocytes utilize a matrix metalloproteinase, MMP-9, to extend processes along an astrocyte extracellular matrix. Glia 1998;22:53–63.

90 Cossins JA, Clements JM, Ford J, *et al*. Enhanced expression of MMP-7 and MMP-9 in demyelinating multiple sclerosis lesions. Acta Neuropathol (Berl) 1997;94:590–8.

91 Cuzner ML, Davison AN, Rudge P. Proteolytic enzyme activity of blood leukocytes and cerebrospinal fluid in multiple sclerosis. Ann Neurol 1978;4:337–44.

92 Richards PT, Cuzner ML. Proteolytic activity in CSF. Adv Exp Med Biol 1978;100:521–7.

93 Maeda A, Sobel RA. Matrix metalloproteinases in the normal human central nervous system, microglial nodules, and multiple sclerosis lesions. J Neuropathol Exp Neurol 1996; 55:300–9.

94 Cuzner ML, Gveric D, Strand C, *et al*. The expression of tissue-type plasminogen activator, matrix metalloproteases and endogenous inhibitors in the central nervous system in multiple sclerosis: comparison of stages in lesion evolution. J Neuropathol Exp Neurol 1996;55:1194–204.

95 Leppert D, Ford J, Stabler G, *et al*. Matrix metalloproteinase-9 (gelatinase B) is selectively elevated in CSF during relapses and stable phases of multiple sclerosis [In Process Citation]. Brain 1998;121:2327–34.

96 Gijbels K, Masure S, Carton H, Opdenakker G. Gelatinase in the cerebrospinal fluid of patients with multiple sclerosis and other inflammatory neurological disorders. J Neuroimmunol 1992;41:29–34.

97 Gijbels K, Proost P, Masure S, Carton H, Billiau A, Opdenakker G. Gelatinase B is present in the cerebrospinal fluid during experimental autoimmune encephalomyelitis and cleaves myelin basic protein. J Neurosci Res 1993;36:432–40.

98 Gijbels K, Galardy RE, Steinman L. Reversal of experimental autoimmune encephalomyelitis with a hydroxamate inhibitor of matrix metalloproteases. J Clin Invest 1994;94: 2177–82.

99 Clements JM, Cossins JA, Wells GM, *et al*. Matrix metalloproteinase expression during experimental autoimmune encephalomyelitis and effects of a combined matrix metalloproteinase and tumour necrosis factor-alpha inhibitor. J Neuroimmunol 1997;74:85–94.

100 Hewson AK, Smith T, Leonard JP, Cuzner ML. Suppression of experimental allergic encephalomyelitis in the Lewis rat by the matrix metalloproteinase inhibitor Ro31–9790. Inflamm Res 1995;44:345–9.

101 Matyszak MK, Perry VH. Delayed-type hypersensitivity lesions in the central nervous system are prevented by inhibitors of matrix metalloproteinases. J Neuroimmunol 1996;69: 141–9.

102 Liedtke W, Cannella B, Mazzaccaro RJ, *et al*. Effective treatment of models of multiple sclerosis by matrix metalloproteinase inhibitors. Ann Neurol 1998;44:35–46.

103 Dubois B, DtHooghe MB, De Lepeleire K, Ketelaer P, Opdenakker G, Carton H. Toxicity in a double-blind, placebo-controlled pilot trial with D- penicillamine and metacycline in secondary progressive multiple sclerosis. Multiple Sclerosis 1998;4:74–8.

104 Wodicka L, Dong H, Mittmann M, Ho MH, Lockhart DJ. Genome-wide expression monitoring in Saccharomyces cerevisiae. Nat Biotechnol 1997;15:1359–67.

105 Lockhart DJ, Dong H, Byrne MC, *et al*. Expression monitoring by hybridization to high-density oligonucleotide arrays [see comments]. Nat Biotechnol 1996;14:1675–80.

106 Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genomewide expression patterns. Proc Natl Acad Sci USA 1998;95:14863–8.

107 Lander ES. Array of hope. Nat Genet 1999;21(suppl 1):3–4.