Minireview

Large-scale gene-expression studies and the challenge of multiple sclerosis

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Abstract

In multiple sclerosis, a complex neurodegenerative disorder, a combination of genetic and environmental factors results in inflammation and myelin damage. Recent transcription-profiling studies have found distinct gene-expression patterns in diseased tissue; such large-scale studies at different stages of the disease are contributing to understanding multiple sclerosis and developing effective therapy.

Multiple sclerosis (MS), a chronic disease of the central nervous system (CNS), is characterized by a relapsing-remitting or progressive clinical course and by the pathological triad of inflammation, demyelination, and gliosis (scarring). MS is one of the most common causes of neurological disability arising in early to middle adulthood and thus is a major health problem, particularly in Western societies. A large body of evidence supports the idea that MS is a complex disorder resulting from an interaction between an inherent genetic susceptibility and undefined environmental exposures. This interaction unleashes a cascade of molecular events that culminate in a relapsing inflammatory, and ultimately progressive, neurodegenerative process [1,2].

The histopathological hallmark of MS is the demyelinating plaque, usually a sharply circumscribed lesion in the CNS white matter that evolves dynamically [3]. In the early (acute) stage, lymphocytes (predominantly T cells) pass through the walls of blood vessels and infiltrate white matter, apparently orchestrating the demyelinating process. In some inflammatory lesions, a distinctive pattern of myelin damage can be seen and appears to be associated with the deposition of myelin-specific autoantibodies. As lesions evolve over time, inflammation lessens and astrocytes (star-shaped glial cells that respond to injury) proliferate extensively, resulting in a gliotic scar. Oligodendrocytes, the myelin-producing glial cells, are either destroyed as the inflammatory and gliotic process evolves or may proliferate and mediate partial remyelination. Some MS plaques appear to gradually enlarge by concentric outward growth.

Recent ultrastructural studies of MS lesions suggest that fundamentally different underlying pathologies may be present in different patients. Heterogeneity has been identified both in terms of the fate (that is, death or survival) of oligodendrocytes and by the presence or absence of deposition of antibody and complement. Rare cases may even have a primary oligodendrogliopathy without prior inflammation.

In most MS cases, tissue damage and neurological impairment reflect the downstream outcome of a coordinated series of events that includes peripheral lymphocyte activation, disruption of the blood-brain barrier, infiltration of inflammatory cells into the brain parenchyma, further stimulation of myelin-reactive T cells in the nervous system, autoantibody deposition, demyelination, and axonal loss. Cytokines, adhesion molecules, growth factors, and other molecules such as free radicals, proteases and vasoactive amines are thought to induce and regulate numerous critical disease-associated cellular functions (Figure 1). The

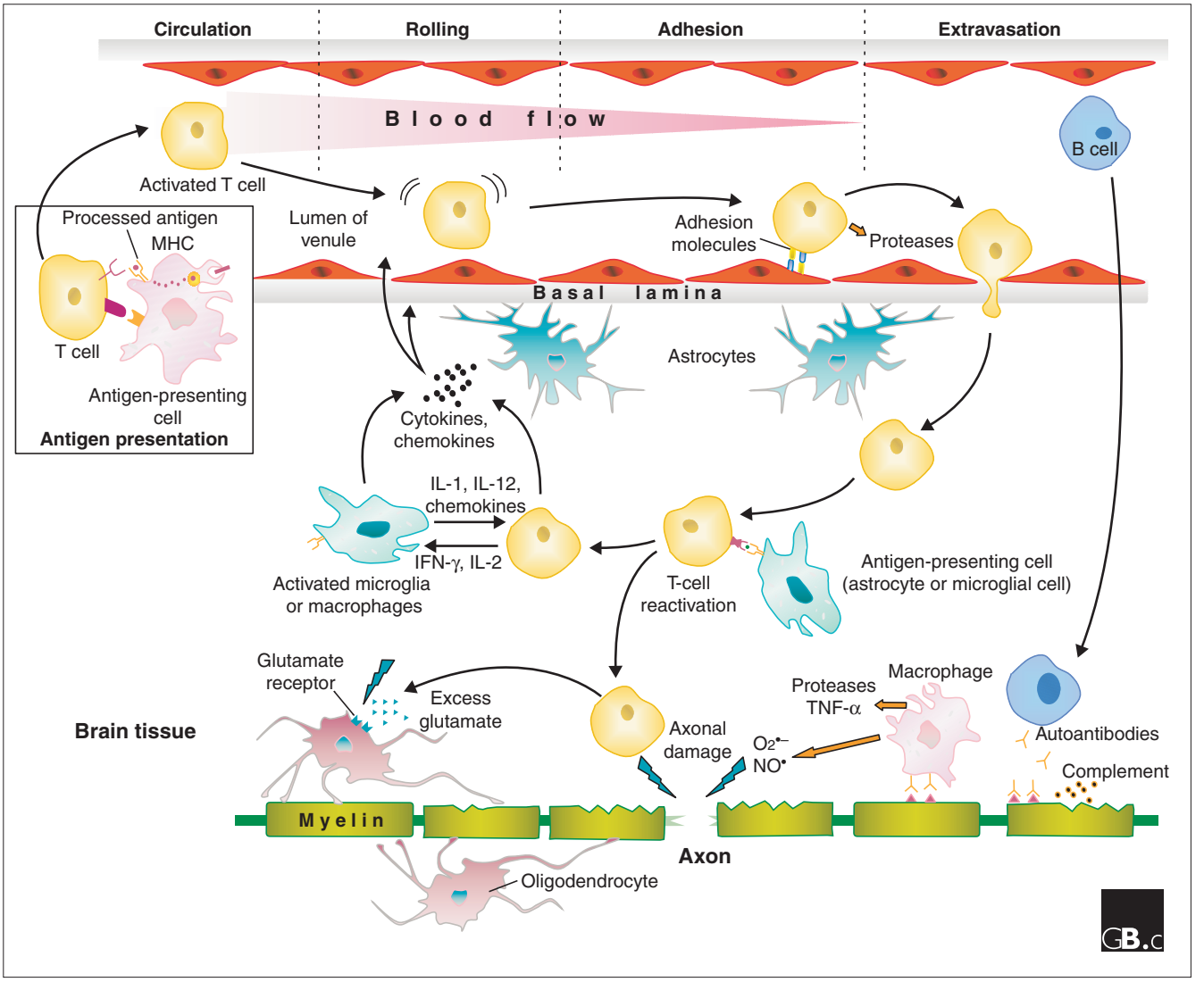

Figure 1
A model of MS pathogenesis. T cells become activated in the periphery by processed peptides expressed by antigen-presenting cells in the context of MHC molecules (see inset). In MS, these peptides are thought to mimic the molecular shape of some CNS antigens. Activated T cells undergo transcriptional changes resulting in the expression of adhesion molecules and proteolytic enzymes that favor their adhesion to the basal lamina of the capillary vessels of the blood-brain barrier. The T cells then pass out of the blood vessels (extravasation) to the brain parenchyma where they are reactivated by astrocytes or microglial cells now presenting CNS antigens. This second activation step triggers a new wave of inflammation in which numerous cytokines, chemokines, and other molecules such as NO, glutamate, and free radicals are produced. This process is maintained by positive feedback loops acting on effector cells, and eventually results in damage to myelin, oligodendrocytes, and neurons.

comprehensive analysis of these cellular transcriptional programs - the transcriptome - both in the CNS and the periphery may identify one or several distinct molecular fingerprints and may contribute to a more accurate model of MS pathogenesis.

The Human Genome Project has facilitated large-scale high-throughput analysis of differential gene expression, allowing progress in functional interpretation of genomic information [4-7]. Of these methods, DNA microarrays and quantitative real-time PCR have been the most utilized. Whichever type of microarray is used for hybridization with a labeled cRNA or cDNA population from the sample(s) of interest, sophisticated detection systems can then read and quantitate the amount of each specific mRNA present in the original sample(s) (see Figure 2). A structured classification of genes or experiments according to temporal and/or topographic transcriptional patterns can thus be obtained, most commonly by using hierarchical clustering [8]. The differential expression of a manageable number of targets can be validated by sensitive real-time PCR, and these can be further assessed using *in vivo* models.

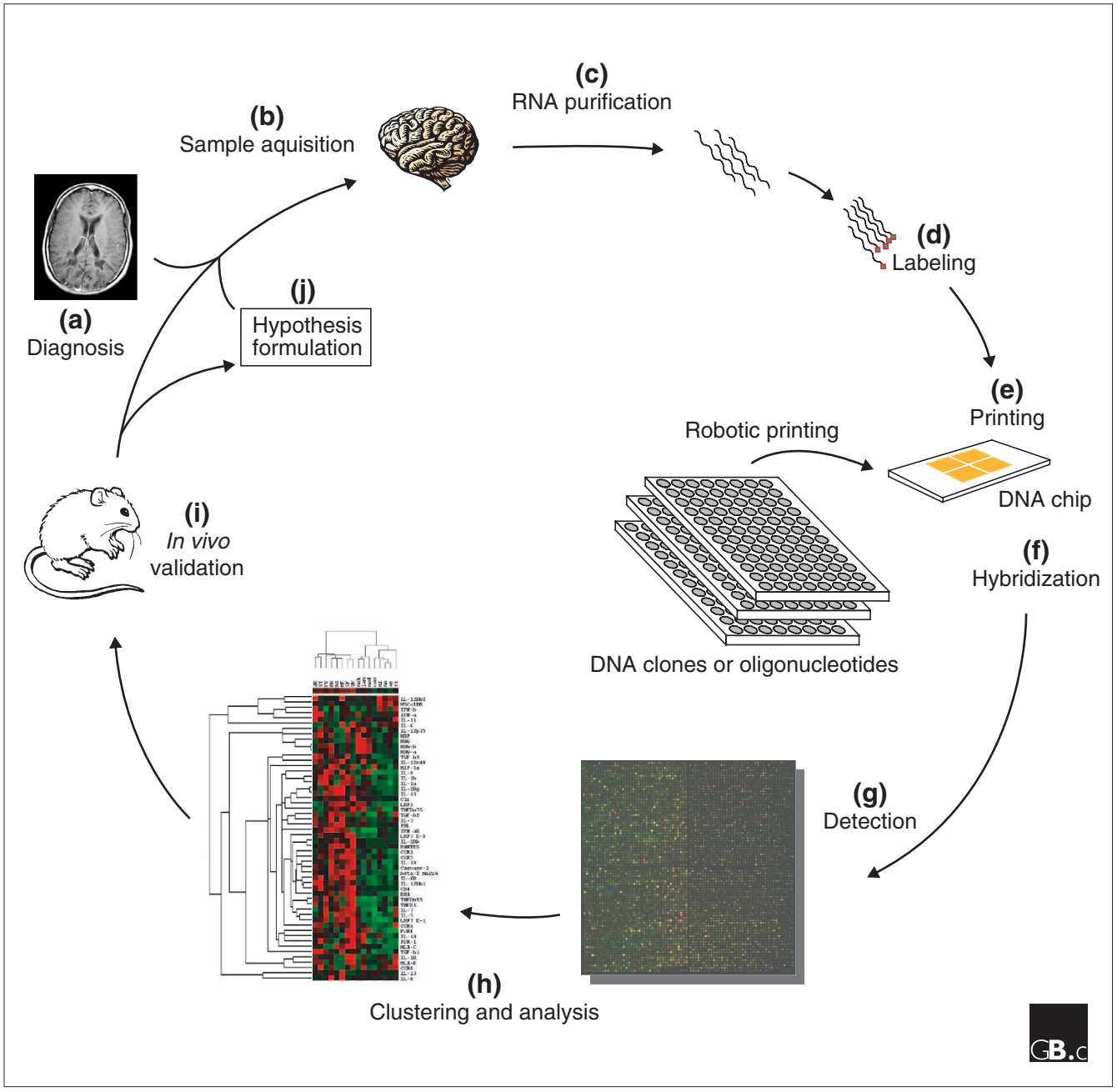

Figure 2
Functional genomics of MS. **(a)** Accurate diagnosis of patients based on clinical evidence, particularly magnetic resonance imaging, and the patients' historical data, is pivotal for the validity of genomic analysis. **(b)** Typically, post-mortem samples with short autolysis times are used, although biopsy specimens are sometimes available. Immunohistological analysis and laser capture microdissection at this stage can add substantially to subsequent data interpretation. **(c)** High-quality RNA is then obtained from the tissue and subjected to quantitation and integrity analysis. **(d)** Depending on the type of array to be used, the RNA sample is converted into fluorophore-labeled cDNA (spotted cDNA arrays) or cRNA (oligonucleotide-based arrays). **(e)** A nucleic-acid-containing solid support - the DNA chip or microarray - is obtained either by spotting cDNA clones or by *in situ* synthesis of oligonucleotides onto a glass surface. **(f)** The labeled sample is then laid on top of the array and hybridized for several hours. In the case of spotted cDNA arrays, an equal amount of two differently labeled samples (usually one is a control) is mixed prior to the hybridization step. **(g)** A confocal laser microscope can be used to scan and measure the fluorescence emitted by the hybridized probes. The intensity of the signal is directly related to the amount of mRNA originally present in that sample. In spotted cDNA arrays, the ratio of the two fluorophores is measured and the relative intensity of each probe is then calculated for each cDNA-containing spot. **(h)** Different classification algorithms can be used to organize the expression of all genes analyzed in a particular experiment. In this way, genes with correlated patterns of expression are clustered together and so can be readily identified. **(i)** On the basis of the expression results, a particular gene or group of genes can be selected for validation *in vivo*. At this stage, animal models can be used to assess the effect of a genetic deletion or overexpression affecting the gene(s) of interest. **(j)** At the end of this process, a hypothesis can be generated that is consistent with the results obtained. New rounds of experimentation are usually required to refine a particular hypothesis.

Large-scale gene-expression studies in MS

An early example of large-scale expression profiling of the MS lesion was provided by Becker *et al.* [9], who constructed and sequenced a normalized cDNA library from a brain sample obtained post-mortem from a patient with the primary progressive form of the disease. This study, albeit not statistically powerful, identified several inflammatory genes and known putative autoantigens that were present in the MS-derived libraries but absent from two normal control libraries. In a subsequent report by the same group [10], spotted cDNA microarrays were used to interrogate the expression levels of more than 5,000 genes in the same specimen. In this study the authors describe the differential expression of 62 genes, including those encoding the Duffy chemokine receptor, interferon regulatory factor-2, and tumor necrosis factor α. The absence of total or even partial replication between the screens is noteworthy. This observation highlights the large variability that is usually found in large-scale gene-expression profiling experiments when different experimental platforms are employed.

One of us (S.E.B.) recently reported the high-throughput sequencing of diseased brain-expressed transcripts using cDNA non-normalized libraries generated from MS lesions and control brain [11]. Over 11,000 clones were sequenced, and analysis focused on genes present in MS libraries but absent from the control library. The most abundant transcripts unique to MS plaques were those for α B-crystallin, an inducible heat-shock protein that is localized in the myelin sheath and targeted by T cells in MS [12]. The next five most abundant transcripts were those for prostaglandin D synthase, prostatic binding protein, ribosomal protein L17, and osteopontin (OPN), also called early T-cell activation gene-1, which has pleiotropic functions, including roles in tissue remodeling, cell survival and cellular immunity [13,14]. OPN was also found in lesions of experimental allergic encephalomyelitis (EAE), a murine model with similarities to MS. In addition, OPN expression in neurons was detectable during acute disease and relapse, but not during remission. OPN-deficient mice were resistant to progressive EAE and had frequent remissions. Responses mediated by type 1 T-helper cells involved in CNS autoimmunity may be regulated by OPN, making it a possible target for new therapies.

Ramanathan *et al.* [15] reported the analysis of brain samples from 15 relapsing-remitting MS patients and 15 controls by probing nylon cDNA arrays. Only 34 out of 4,000 genes interrogated showed statistically significant differential expression in MS samples when compared to controls. Surprisingly, only a very small fraction of these genes can be directly associated with current models of MS pathogenesis or with its downstream inflammatory effects. *Bona fide* regulatory changes in gene expression may have been obscured in this study by the low signal-to-noise ratio characteristic of molecular control processes.

In the most recent report on the application of microarrays to MS, Lock *et al.* [16] described the analysis of four dissected brain specimens from chronic or secondary progressive MS patients and compared them with two specimens from non-inflammatory post-mortem brain tissue. The dissected lesions ranged from acute inflammatory to chronic silent, spanning a broad range of plaque activity. Lock *et al.* [16] found a set of 49 genes that showed increased expression and 39 genes with decreased expression in all four MS samples compared with the two control brain specimens. Among the overexpressed transcripts, HLA (MHC) class II and immunoglobulin genes were of particular interest, because they reflect an active immune response in the lesions. Also noteworthy was the higher expression of immunoglobulin genes in acute plaques than in chronic silent lesions. Similarly, granulocyte colony-stimulating factor (G-CSF) was upregulated for most of the active plaque types. This finding was validated *in vivo* using the EAE model: animals that previously received a subcutaneous dose of G-CSF developed a much milder disease than control animals, suggesting an active immunomodulatory mechanism upregulating the production of this trophic factor in EAE and MS lesions. Another gene differentially upregulated according to the type of lesion was the immunoglobulin Fc receptor γ subunit (FcγR), which showed a significantly higher expression in the chronic silent than the acute plaques. Transgenic mice with a reduced expression of the FcγR gene also showed a milder clinical course of EAE than did their normal littermates. Genes with decreased expression included those for several myelin components, such as proteolipid protein, myelin-associated glycoprotein, and myelin oligodendrocyte glycoprotein. This last finding may reflect not only the catabolic demyelinating process but also ineffective or absent myelin repair. Overall, this impressive study illustrates the validity of the functional genomics approach as a hypothesis generator.

The need for standards and crossvalidation

Studies such as those described here [9-11,15,16] can provide valuable information about the molecular mechanisms underlying plaque formation, but their interpretation is subject to a number of issues. Firstly, discrepancies observed between studies could be related to the use of different platforms for assessing the expression profiles, so rigorous crossvalidation is required before accurate comparisons can be performed. The different mechanisms of plaque formation present in different subsets of patients, or comparison of samples obtained at different time points in the evolution of the plaque, could lead to discrepancies.

A second issue in interpreting the recent studies is that in most large-scale gene-profiling studies only a small fraction of the expected candidate genes appear as differentially regulated. For example, genes involved in T-cell and proinflammatory functions would be expected to be abundantly expressed in an MS lesion. Controls - both normal and other

neurological disease controls - should be carefully selected in order to maximize the chance that disease-specific changes will be revealed. Similarly, differences in the effective concentration and stability of each mRNA species can be minimized by meticulous selection and systematic processing of fresh specimens.

A third issue is that the high costs involved mean that often a very few samples are analyzed, compromising the statistical significance of the experiments, and (equally important) only representing a momentary snapshot of the dynamic process they are meant to depict.

The importance of longitudinal analyses in dynamic disease states

Most gene-expression profiling reports on the MS plaque have involved single, descriptive experiments that capture only the physiological and molecular stages at which the sample under study was harvested. Given the dynamic nature of the inflammatory and degenerative processes that operate in MS, longitudinal studies using animal models may provide a context for the interpretation of snapshots derived from the limited human histopathological material available to investigators - material which by necessity reflects a sampling bias created by the clinical situations that resulted in availability of biopsy or autopsy tissue for examination.

In addition to different variants of MS, the clinical course in an individual person is characterized by abrupt or gradual perturbations in disease activity. The hallmark of dynamic disease states is the sudden, qualitative and quantitative change in the temporal pattern of physiological events that underlie the disease. Longitudinal monitoring of plaque activity or other biological compartments by expression analysis may allow the identification of temporal patterns and underlying cellular events that drive tissue damage in MS. In other dynamic diseases, recognition of these patterns already forms a basis for therapeutic decision-making [17].

In conclusion, large-scale gene-expression profiling methods have already provided an initial burst of information about MS to complement the histopathological and imaging data accumulated to date. The combined analysis of the genomic and transcriptional information, together with the modeling of genetic networks, may help to predict the responses of a particular biochemical pathway under a variety of different stimuli, simulated feedback loops, or other interactions. A useful conceptual model of the inflammatory events that drive demyelination and neurodegeneration in MS may emerge that can provide understanding of existing therapies as well as a rationale for novel treatment strategies.

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