Multiple sclerosis

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Multiple sclerosis is a complex genetic disease associated with inflammation in the CNS white matter thought to be mediated by autoreactive T cells. Clonal expansion of B cells, their antibody products, and T cells, hallmarks of inflammation in the CNS, are found in MS. This review discusses new methods to define the molecular pathology of human disease with high-throughput examination of germline DNA haplotypes, RNA expression, and protein structures that will allow the generation of a new series of hypotheses that can be tested to develop better understanding of and therapies for this disease.

Historical perspective

A French neurologist at the Salpetrière in Paris, Jean Martin Charcot, first described multiple sclerosis (MS) in 1868, noting the accumulation of inflammatory cells in a perivascular distribution within the brain and spinal cord white matter of patients with intermittent episodes of neurologic dysfunction (1–3). This led to the term *sclérose en plaques disseminées*, or multiple sclerosis. The more recent observation in 1948 by Elvin Kabat of increases in oligoclonal immunoglobulin in the cerebrospinal fluid of patients with MS provided further evidence of an inflammatory nature to the disease (4, 5). In the past half-century, several large population–based MS twin studies demonstrated a strong genetic basis to this clinical-pathologic entity (6–13). Lastly, the demonstration of an autoimmune, at times demyelinating, disease in mammals with immunization of CNS myelin (experimental autoimmune encephalomyelitis, or EAE), first made by Thomas Rivers at the Rockefeller Institute in 1933 with the repeated injection of rabbit brain and spinal cord into primates (14), has led to the generally accepted hypothesis that MS is secondary to an autoimmune response to self-antigens in a genetically susceptible host (see box, *What we know about MS*). It should be pointed out that although the inflammation found in the CNS of patients with MS is thought to represent an autoimmune response, this is based on negative experiments where investigators have not been able to consistently isolate a microbial agent from the tissue of diseased patients. Nevertheless, primary viral infections in the CNS may induce an autoimmune response (15), and the recurring lesson from the EAE model is that the minimal requirement for inducing inflammatory, autoimmune CNS demyelinating disease is the activation of myelin-reactive T cells in the peripheral immune system (16, 17).

Advances in immunology have provided clinicians with powerful tools to better understand the underlying causes of MS, leading to new therapeutic advances. The future calls for extending the original observations of Charcot and Kabat by defining the molecular pathology of MS at the level of DNA haplotype structure, CNS and peripheral mRNA and protein expression, leading to the generation of a new series of disease-related hypotheses.

Pathology

Gross examination of brain tissue of individuals with MS reveals multiple sharply demarcated plaques in the CNS white matter with a predilection to the optic nerves and white matter tracts of the periventricular regions, brain stem, and spinal cord. As was recognized early on and so elegantly investigated in more recent studies, substantial axonal injury with axonal transections is abundant throughout active MS lesions (18).

The inflammatory cell profile of active lesions is characterized by perivascular infiltration of oligoclonal T cells (19) consisting of CD4+/CD8 α/β (20, 21) and γ/δ (22) T cells and monocytes with occasional B cells and infrequent plasma cells (23). Lymphocytes may be found in normal-appearing white matter beyond the margin of active demyelination (24). Macrophages are most prominent in the center of the plaques and are seen to contain myelin debris, while oligodendrocyte counts are reduced. In chronic-active lesions, the inflammatory cell infiltrate is less prominent and may be largely restricted to the rim of the plaque, suggesting the presence of ongoing inflammatory activity along the lesion edge. Recently four pathologic categories of the disease were defined on the basis of myelin protein loss, the geography and extension of plaques, the patterns of oligodendrocyte destruction, and the immunopathological evidence of complement activation. Two patterns (I and II) showed close similarities to T cell–mediated or T cell plus antibody–mediated autoimmune encephalomyelitis, respectively. The other patterns (III and IV) were highly suggestive of a vasculopathy or primary oligodendrocyte dystrophy, reminiscent of virus- or toxin-induced demyelination rather than autoimmunity (25). It was of interest that the pattern of pathology tended to be the same in multiple lesions from any single individual with MS.

Natural history

MS, like other presumed autoimmune diseases, is more common in females and often first manifests clinical symptoms during young adulthood. At its onset, MS can be clinically categorized as either relapsing-remitting MS (RRMS, observed in 85–90% of patients) or primary progressive MS (PPMS). Relapses or "attacks" typically present subacutely, with symptoms developing over hours to several days, persisting for several days or weeks, and then gradually dissipating. The attacks are likely caused by the traffic of activated, myelin-reactive T cells into the CNS, causing acute inflammation with associated edema. The ability of high dose steroids to so quickly abrogate MS symptoms suggests that the acute edema and its subsequent resolution underlie the clinical relapse and remission, respectively. Studies in acute disseminated encephalomyelitis

(ADEM) in humans (26) and EAE in rodents (27) suggest that immunologically, acute attacks are self-limited by regulatory T cells.

The outcome in patients with RRMS is variable; untreated, approximately 50% of all MS patients require the use of a walking aid within ten years after clinical onset (28), although the consequences on prognosis of newer treatment regiments are not as yet clear. Increased attack frequency and poor recovery from attacks in the first years of clinical disease predict a more rapid deterioration. Multiple MRI lesions, particularly those that gadolinium enhance on the first MRI scan, also predict a more severe subsequent course. Early on in the disease, there are frequent gadolinium-enhanced MRI lesions, consistent with acute fluxes of activated, autoreactive T cells into the CNS causing a breakdown of the blood-brain barrier which may be associated with clinical events. However, with time, the extent of recovery from attacks is often decreased, and baseline neurological disability accrues. Ultimately, approximately 40% of relapsing-remitting patients stop having attacks and develop what may be a progressive neurodegenerative secondary disorder related to the chronic CNS inflammation, known as secondary progressive multiple sclerosis (SPMS) (29). The evolution to this secondary progressive form of the disease is associated with significantly fewer gadolinium-enhanced lesions and a decrease in brain parenchymal volume (30, 31). Similarly, while earlier RRMS is sensitive to immunosuppression (32), as times goes on, responsiveness to immunotherapy decreases and may in fact disappear in later forms of SPMS. Thus, rather than conceiving MS as first a relapsing-remitting and then a secondary progressive disease, it could be hypothesized that MS is a continuum where there are acute inflammatory events early on with secondary induction of a neurodegenerative process refractory to immunologic intervention. This hypothesis awaits experimental verification where early immunotherapy prevents the onset of secondary progressive disease. Such critical investigations require new models of investigation using natural history studies that can be performed over decades.

The primary progressive form of MS is characterized from the onset by the absence of acute attacks and instead involves a gradual clinical decline. Clinically, this form of the disease is associated with a lack of response to any form of immunotherapy (32). This leads to the notion that PPMS may in fact be a very different disease as compared to RRMS. A recent commentary points out the similarities between PPMS and human T lymphotropic virus type I–associated myelopathy (15), where there is a progressive decline in neurologic function from the disease onset.

Diagnosis

In the absence of a specific immune-based assay, the diagnosis of MS continues to be predicated on the clinical history and neurological exam; that is, finding multiple lesions in time and space in the CNS. The use of MRI has had a major impact on allowing the early and more precise diagnosis of the disease (33). In a recent prospective study, patients experiencing their first episode suggestive of CNS demyelination and having MRI evidence of at least three typical lesions were followed for an average of 42 months. Within that period, a significant proportion of patients developed an additional relapse, thus qualifying for the diagnosis of clinically definite MS. If there were no MRI lesions, the probability of developing MS was substantially less. More than half of those developing MS experienced the additional relapse within one year of their first episode (34). Thus, it seems reasonable to label the first attack of what appears to be MS as "singular sclerosis" and to explain to patients that there is a high likelihood of developing MS. This indicates to the patient that you have an understanding of the underlying problem but that the prognosis is not as yet clear, allowing patients who never have another attack to be saved from carrying a diagnosis of MS.

ADEM is a monophasic demyelinating illness that can present with clinical, imaging, and laboratory manifestations indistinguishable from an acute MS attack (35). However, typical ADEM is seen in pediatric populations and has more of an explosive course associated with alterations in mental status, and a post-viral or post-vaccination history is often elicited. This disease is associated with significant responses to myelin proteins, indicated both by T cell and antibody measurements (26, 36). It is usually not difficult to distinguish this disease entity from "singular sclerosis," and the laboratory measurement of circulating high affinity antimyelin basic protein antibodies in ADEM but not MS may aid in the diagnosis. It should be noted that there are many reports demonstrating low affinity antimyelin autoantibodies by ELISA in sera and CSF of patients with MS, though their role either in the disease's pathogenesis or in predicting outcome is still not well defined. However, high affinity antimyelin basic protein or myelin oligodendrocyte antibodies appear to be more difficult to detect in the serum or CSF of patients with MS while antimyelin oligodendrocyte antibodies can be found in MS CNS plaque tissue (37).

MRI is the optimal imaging modality for MS. From a diagnostic standpoint, it is important to keep in mind that the typical appearance of multiple lesions on MRIs is not specific for MS. In the appropriate clinical setting, however, this appearance provides an important ancillary diagnostic tool that may establish the multifocality of CNS involvement. MRI has also been used to assess MS disease activity, disease burden, and the dynamic evolution in these parameters over time (38). MRI is 4–10 times more sensitive than the clinical evaluation in capturing CNS lesions (39), and serial studies have unequivocally demonstrated that clinically apparent changes reflect

| What we know about MS	
Inflammation in CNS white matter	Different pathological subtypes. Early $\gamma\delta$ T cell infiltration. Later CD4 and CD8 T cells with loss of myelin and axons.
Increase in IgG in CSF	CSF Ig is oligoclonal. B and T cells in CNS and CSF are also oligoclonal.
Complex genetic disease	λs of 20–40. High degree of inheritability. So far, only MHC region on chromosome 6 clearly associated with MS.
Antibody autoreactivity	Variable results among different studies. Agreement on presence of autoantibodies in MS plaque tissue. No high affinity autoantibodies in serum.
T cell autoreactivity	Autoreactivity to self-myelin antigens in patients with MS and normal subjects. In patients with MS, modest increase in frequency of myelin-reactive, activated T cells.
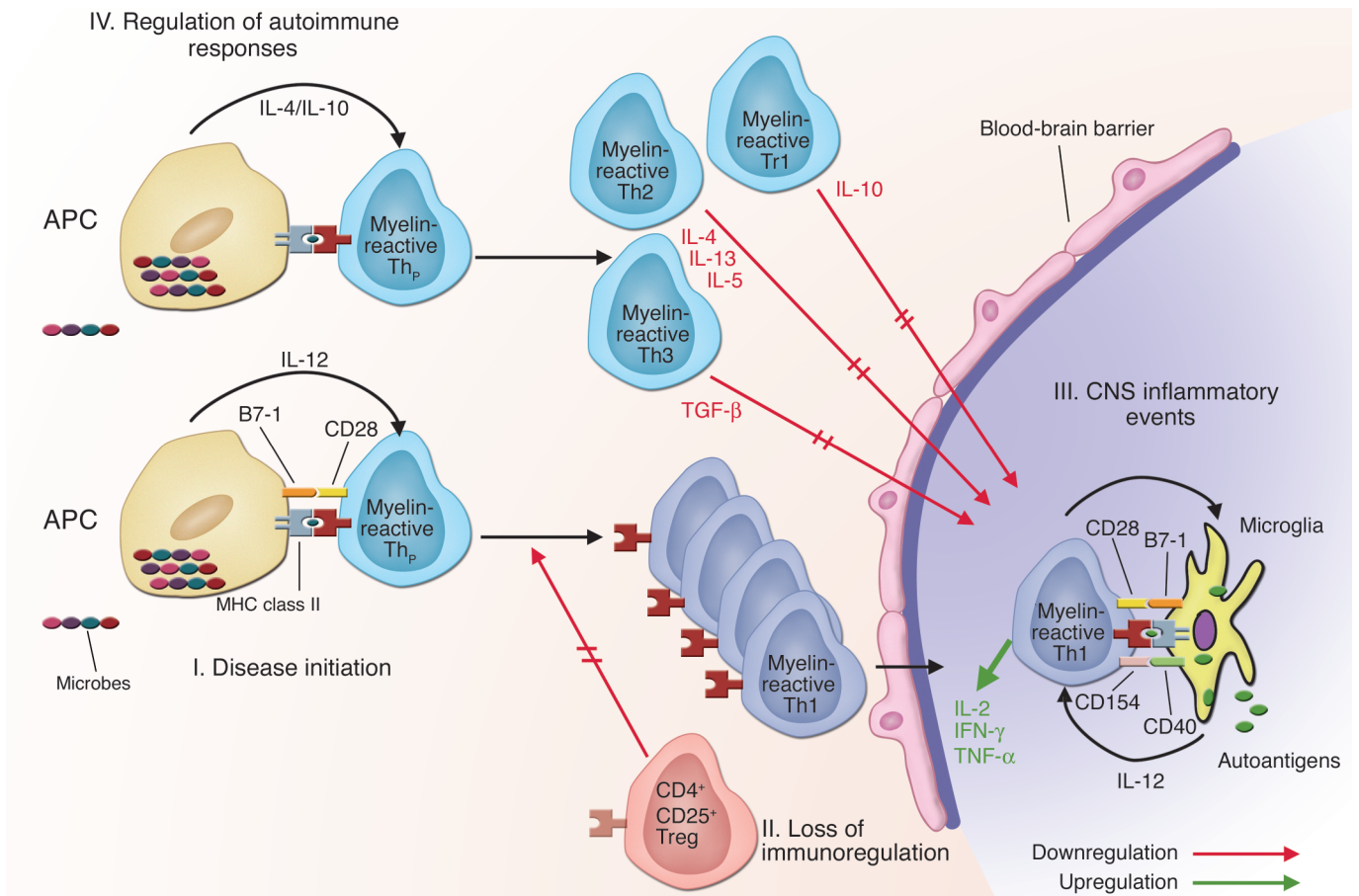
Figure 1

Working hypothesis as to the cause of MS. (I) In a genetically susceptible host, common microbes both activate the APCs through toll receptors and contain protein sequences cross-reactive with self myelin antigens. This leads to what can be defined as the minimal requirement for inducing an autoimmune, inflammatory CNS disease in mammals. (II) Underlying immunoregulatory defects, such as decreases of regulatory T cells in the circulation of patients with MS, allow the further pathologic activation of autoreactive T cells (96). (III) Activated myelin-reactive T cells migrate into the CNS and recognize antigen presented by microglia, local APCs. Th1 cytokines are secreted and an inflammatory cascade is initiated. (IV) Regulation of autoimmune responses. Naturally occurring mechanisms may exist to regulate autoimmune responses including the induction of autoreactive Th2 (IL-4, IL-5, IL-13), Th3 (TGF-β), or Tr1 (IL-10) cytokine–secreting T cells that migrate to the CNS and downregulate (red arrow) inflammatory Th1 autoreactive T cells (green arrow). Therapies may attempt to induce Th2 (Copaxone, altered peptide ligands), Th3 (mucosal antigen), or Tr1 (β-IFNs, steroids). Th$_P$, precursor T cell.

only a minor component of disease activity. Lesions in the cerebrum are much more likely to be clinically silent, as compared to lesions in the brainstem or spinal cord.

Therapy

Therapies for MS have emerged over the last two decades with the demonstration of efficacy of three classes of immunomodulating therapies that impact the course of early MS: immunosuppressive drugs such as mitoxantrone and cyclophosphamide; β-IFNs; and an MHC-binding protein that engages the T cell receptor (TCR), glatiramer acetate (GA). The underlying pathology of MS as an inflammatory CNS disease was instrumental in leading to the drug treatments presently used. While these drug therapies were not prospectively designed based on a detailed understanding of the disease's pathophysiology, examination of these drug's mechanisms of action has provided insight into the etiology of MS. Newer therapies in clinical trials are based on a more rational understanding of the disease, and these will be discussed in more detail.

Disease mechanisms

Immunopathophysiology of MS. It is often asked whether EAE, briefly discussed above, is really MS. Our laboratory investigates the pathophysiology of MS by directly studying patients with the disease and does not directly investigate mouse models. Some of our colleagues will even note in good humor that MS is a superb model of EAE! (V. Kuchroo and S. Miller, personal communication.) The truth is, we are and we will always be appropriately limited with human experimentation. Experimental models, be they Newtonian physics or rodent models of autoimmunity, are just that — models. They do not represent truth (try to investigate the velocity of a sub-atomic particle approaching the speed of light using Newtonian physics), and are only as useful as the question one asks of the model. For example, it was shown almost a decade ago that the α4β7 integrin, VLA-4, was critical for T cell traffic into the CNS of mice with EAE (40). This resulted in a highly successful phase II trial of anti–VLA-4 in patients with RRMS (41), which is now in phase III investigations. This is an excellent exam-

ple of how the EAE model, if used to ask the correct question, might be highly useful in developing therapies for MS.

A second critical lesson from the EAE model is that of epitope spreading, first observed by Eli Sercarz (42). With the injection of a single myelin protein epitope into mice with subsequent development of EAE, it was observed that T cells became activated against other epitopes of the same protein; this was followed by T cell activation in response to other myelin proteins that become capable of adoptively transferring the disease to naive mice. The epitope spreading requires costimulation with B7/CD28, suggesting that with tissue damage in the CNS an adjuvant is created in the CNS with the expression of high amounts of B7.1 costimulatory molecules associated with antigen release (43). Moreover, we have recently observed that a transgenic mouse expressing DR2 (DRB1*1501) and a TCR (Ob1A12) cloned from the blood of a patient with MS recognizing an immunodominant myelin basic protein peptide p85–99 (MBP p85–99) spontaneously developed EAE with epitope spreading to a number of antigens implicated in MS including α-β crystalline, and proteolipid apoprotein (D. Altman, V. Kuchroo, and D. Hafler, unpublished observations). As we have observed high expression of B7.1 costimulatory molecules in the CNS white matter of patients with MS (44), and as most patients exhibit T cell reactivity to a number of myelin antigens (45), it is likely that by the time a patient develops clinical MS there has been epitope spreading with reactivity to multiple myelin epitopes. However, the presence of clonally expanded T cells in the CSF and brain tissue of patients with the disease raises the issue that there may be clonal reactivity to just a few myelin antigens. Single cell cloning of T cells from the inflamed CNS tissue screened against combinatorial and protein libraries may allow new insights into the pathophysiology of MS.

Data from a number of laboratories combined with experimental data from the EAE model where myelin antigen is injected with adjuvant into mammals indicate that there are autoreactive T cells recognizing myelin antigens in the circulation of mammals. It appears that the activation of these T cells is the critical event in inducing autoimmune disease (Figure 1). We and others first demonstrated over a decade ago that T cell clones isolated from the blood of patients with MS frequently exhibit exquisite specificity for the immunodominant p85–99 epitope of myelin basic protein (45–47). However, while the TCR appears to be highly specific in recognizing this peptide, altering the peptide ligand can change the TCR conformation to yield a higher degree of T cell cross-reactivity (48). Using combinatorial chemistry, even a greater degree of cross-reactivity could be demonstrated, and a number of viral epitopes were identified that could trigger autoreactive T cell clones in a manner that would not be predicted by simple algorithms (49). Indeed, one MBP-reactive T cell clone recognized an epitope of an entirely different self-protein, the myelin oligodendrocyte glycoprotein. Hence, a significant degree of functional degeneracy exists in the recognition of antigens by T cells. This is consistent with the hypothesis that MS is triggered by autoreactive T cells activated by microbial antigens cross-reactive with myelin (50). The high frequency of activated, myelin-reactive T cells in the circulation and CSF of patients with MS (51, 52) is consistent with the hypothesis that the disease is initiated by a microbial infection.

Novel therapeutics

Peptides bound to MHC as therapeutic options. It was recognized almost a decade ago that the strength of signal delivered through the TCR determines which cytokines are secreted by the T cell (53). The cell apparently measures affinity in part by timing the engagement between the TCR and the peptide/MHC complex. With longer engagement, a qualitatively different TCR complex has time to form, and the extent of ζ chain phosphorylation increases correspondingly (54). Altered peptide ligands (APLs), which bind with low affinity to the TCR, weaken this signal. The ability of APLs to change the cytokine program of a T cell from a Th1 to a Th2 response was exploited first by Kuchroo and coworkers as a therapy for autoimmune disease (55). Using the EAE model of MS, these authors showed that APLs can activate IL-4 secretion by both encephalitogenic T cells and naive T cell clones that cross-react with self-antigens.

Injection of APLs is of clear therapeutic value in treating different models of EAE (56, 57), and autoreactive human T cell clones can also be induced to secrete the anti-inflammatory cytokines IL-4 and TGF-β after TCR engagement by APLs (58, 59). However, it was noted that while APLs can induce Th2 cytokine secretion of MBP-reactive T cells isolated from the peripheral blood T cell of patients with MS, they can also induce a heteroclitic response in some patients, activating these MBP-reactive T cells against the patient's own tissues (60). These data provide a strong rationale for the therapeutic use of APLs in patients with autoimmune disease. However, they also raise the issue that in some instances, highly degenerate TCRs can recognize APLs as self-antigens.

A recently published phase II clinical trial testing an altered MBP p85–99 peptide confirms both of these conclusions. At the higher peptide dosage tested, two of seven MS patients developed remarkably high frequencies of myelin basic protein–reactive T cells, and these responses were likely associated with significant increases in MRI-detectable lesions (61) and perhaps even disease exacerbations. In contrast, patients treated with lower doses of the APL showed no such disease flare-ups and may have indeed exhibited some degree of immune deviation towards increases in IL-4 secretion of MBP-reactive T cells (61, 62). Thus, APLs represent a classic double-edged sword. In our outbred population, given the high degree of degeneracy in the immune system, it is unclear whether it is possible to find APLs of self-peptides that pose no risk of cross-reactivity with self.

An alternative approach to the use of a single APL is the administration of peptide mixtures that contain many different antigen specificities. Random copolymers that contain amino acids commonly used as MHC anchors and TCR contact residues have been proposed as possible "universal APLs." GA (Copaxone) is a random sequence polypeptide consisting of four amino acids (alanine (A), lysine (K), glutamate (E), and tyrosine (Y) at a final molar ratio of A:K:E:Y of 4.5:3.6:1.5:1) with an average length of 40–100 amino acids (63). Directly labeled GA binds efficiently to different murine H-2 I-A molecules, as well as to their human counterparts, the MHC class II DR molecules, but does not bind MHC class II DQ or MHC class I molecules in vitro (64). In phase III clinical trials, GA subcutaneously administered to patients with RRMS decreases the rate of exacerbations and prevents the appearance of new lesions detectable by MRI (65, 66). This represents perhaps the first successful use of an agent that ameliorates autoimmune disease by altering signals through the TCR.

A "universal antigen" containing multiple epitopes would be expected to induce proliferation of naive T cells isolated from the circulation, due to its expected high degree of cross-reactivity with other peptide antigens. Indeed, GA induces strong MHC class II DR-restricted proliferative responses in T cells isolated from MS patients or from healthy controls (64). In most patients, daily injection with GA causes a striking loss of responsiveness to this random polypeptide antigen, accompanied by greater secretion of IL-5 and IL-13 by CD4+ T cells, indicating a shift toward a Th2 response (67–70). In addition, the surviving GA-reactive T cells exhibit a high degree of

degeneracy, as measured by their ability to cross-react with a large variety of peptides represented in a combinatorial library (68).

Thus, in vivo administration of GA induces highly cross-reactive CD4^{+} T cells that are immune-deviated to secrete Th2 cytokines. We have proposed that GA-induced migration of highly cross-reactive Th2 (and perhaps Th3) cells to sites of inflammation allows their highly degenerate TCRs to contact self-antigens, which they recognize as weak agonists, much like APLs. These T cells then apparently secrete suppressive, Th2/Th3 cytokines, thus restricting local inflammation. Thus, knowledge of the strong genetic association for MHC in patients with MS has indirectly led to a number of therapeutic trials and new insights into the disease.

Cytokines and costimulatory signals. β-IFN has similarly had a major impact on the treatment of RRMS, though whether it can prevent the transition to SPMS is still not as yet known. The mechanism of action of β-IFN is also not as yet clear, and likely involves alterations of a number of different pathways including induction of IL-10 and inhibition of T cell traffic by blocking metalloproteinases (71). Clinical trials that block the common IL-12 and IL-23 p40 chain are about to begin, as are efforts to block costimulatory signals provided by B7-CD28 interactions with CTLA-4 Ig.

What remains unknown: the genetic basis of MS

In summary of over a century of research on MS, the scientific community has demonstrated that MS is a complex genetic inflammatory disease of the CNS white matter accompanied by T cell, B cell, and macrophage infiltration; the antigenic target of these immune cells is not certain, but are likely to be common myelin antigens shown to be encephalitogenic in the EAE model. To date, the MHC gene region is the only area of the human genome clearly associated with the disease, though the precise genes in that region responsible for MS are not as yet known. In the same way that Charcot and then others defined the key features of MS by simply examining brain pathology and observing inflammation, it is critical to redefine the *molecular pathology* of inflammatory human disease in terms of germline DNA sequence based on the haplotype map, transcription products by RNA microarrays (72), and translation products by tandem mass spectrometry. The combination of such approaches will likely generate a new series of hypotheses that can be examined by both animal and in vitro models of human disease. As MS is a complex genetic disease, understanding which combinations of genes provide the multitude of perhaps relatively minor risk factors which in the population as a whole provide protection from microbial disease but together, in unfortunate random combinations, result in human autoimmune disease is a central goal of present research efforts.

New approaches to understanding the genetic basis of MS

Approximately 15–20% of MS patients have a family history of MS, but large extended pedigrees are uncommon, with most MS families having no more than two or three affected individuals. Studies in twins (6–10, 12, 13) and conjugal pairs (73) indicate that much of this familial clustering is the result of shared genetic risk factors, while studies of migrants (74) and apparent epidemics (75) indicate a clear role for environmental factors. Detailed population-based studies of familial recurrence risk (76–78) have provided estimates for familial clustering with λs, the ratio of the risk of disease in the siblings of an affected individual compared with the general population equal to approximately 20–40 (79, 80). It has become clear that this represents a complex genetic disease with no clear mode of inheritance.

Genetic diseases may fundamentally be divided into two types. First are the "gene disruptions," where there is a gene mutation or deletion, which exhibits high penetrance, and where there is the emergence of a clear clinical phenotype. Sickle cell anemia and muscular dystrophy are two such examples with mutations of the hemoglobin and dystrophin genes, respectively. In these diseases, linkage studies, i.e., linking rather large segments of the human genome identified by so-called microsatellite markers among family members with the disease, followed by positional cloning of the disease gene, have been a powerful tool in human genetics. Such studies in families with multiple sib pairs with MS have been less successful. Specifically, to date, the only confirmed genetic feature to emerge from these efforts is the association and linkage of the disease with alleles and haplotypes from the MHC on chromosome 6p21 (81–86). In the mid 1990s, whole genome screens for linkage (87–89) were published. While these investigations have continued to accumulate whole genome linkage data and almost all of these screens have found more regions of potential linkage than would be expected by chance alone, no other clearly statistically significant region has emerged by linkage investigations.

The other types of genetic diseases are more complex; an alternative hypothesis emerging from the linkage studies is that MS, as a common disease, is caused by common allelic variants each with only subtle but important variations in function. Put another way, crude theoretical modeling of human population history suggested that variants which have a high population frequency as a whole, and are likely to be responsible for complex traits (the common disease–common variant hypothesis), will generally be very old and therefore accompanied by rather little linkage disequilibrium (90). Quantitatively, this may translate to dozens of gene regions each with risk factors of less than ×1.1–×1.4 but which in concert lead to major risk for disease development. It may be postulated that as populations emerged out of Africa 30,000 to 50,000 years ago, exposure to new microbes resulted in what are thought to be major population bottlenecks, with survival of individuals with allelic variants allowing for resistance to the novel infectious event. These combinations of different genes providing resistance to the population, when randomly coming together, result in a hyperresponsive immune system, with subsequent autoimmune diseases the price an individual may pay for protection of the general population. Organ specificity may have emerged because each infectious agent evolved with a population bottleneck would select for a single "MHC restricting" element and subsequent antigen specificity.

Identifying the common allelic variants that may underlie such common diseases requires a different approach from linkage studies. One method might be to actually sequence the whole genome among a group of 5,000 patients with MS as compared to an equal number of healthy controls. While this would be the most sensitive approach, as all variants would be identified, at this stage of technology it would be impossible to even consider. It could be argued that as there appear to be only about 10 million variant, single nucleotide polymorphisms (SNPs) in the population, we could just examine those in the patients with disease compared to control subjects. This would also be far beyond present technologies. The possible emerging solution is both elegant and simple, and is based on a recent observation that was in fact suggested by studies of the MHC region over a decade ago. The discovery is that genetic variants tend to occur together in what are called "haplotype blocks." That is, recent investigations (91) have shown that recombination is not uniformly distributed along chromosomes, as previously assumed, but rather is concentrated in hot spots that are on average some 20 to 40kb apart (haplotype blocks). It has also been shown that in Europeans and Americans of European descent there is very little haplotype diversity within

these genomic haplotype blocks (92). Again, this extensive linkage disequilibrium is most probably the consequence of a severe population bottleneck affecting Europeans some 30,000 to 50,000 years ago (93). The European population is thus ideal for screening for association of allelic variants with disease, since very few SNP markers from each of these linkage disequilibrium blocks will be required to screen the entire genome (94). It is expected that there will be approximately 100,000 such haplotype blocks. Assuming that three SNPs are required to interrogate fully the haplotype diversity associated with each block, the whole genome could be screened using approximately 300,000 SNPs (~10% of all SNPs). This approach has been used by Rioux, Daly, Lander, and coworkers to identify the *IBD5* locus in a previously identified linkage peak in patients with inflammatory bowel disease (95).

A whole genome association scan, while attractive, is only beginning to be feasible as the cost of genotyping continues to decrease. It is also possible that such an approach may fail because MS may be the result of more than the one genetic syndrome that it is generally believed to be or that hundreds or even thousands of genes, each representing only a fractional risk factor, are associated with the occurrence of MS. Epistatic effects of genes will also complicate the analysis. Nevertheless, large, properly powered experiments will definitively answer the question as to issues of disease heterogeneity and relative risk factors, and will prevent the wasting of resources on underpowered investigations that may provide no definitive answers.

The formation of international consortiums, which allow significant collections of patients, combined with high-throughput genotyping will be critical in performing whole genome scans based on the haplotype map. These collaborative efforts, although using many resources, will be necessary in providing a true road map for rational drug discovery. In this regard, the International MS Genetic Consortium was created two years ago by institutions around the globe including the University of Cambridge, the University of California at San Francisco, Duke University, Vanderbilt University, Harvard Medical School, the Massachusetts Institute of Technology, and the Brigham and Women's Hospital. These new partnerships in medical science requiring collaborations across scientific disciplines and medical institutions will challenge the fabric of funding, authorships, and scientific credit that have traditionally defined academic success. Finally, unlike "gene knockout diseases" which require gene therapy that has been difficult to achieve clinically, elucidation of specific pathways will likely require only minor modification of allelic gene functions. Studies in the EAE model have indicated that modification of only a few gene loci are required to eliminate disease risk. Thus, pharmacologic targeting of relatively few pathways (with proper safeguards for privacy) in populations screened for disease risk may be the ultimate treatment for both the inflammatory and degenerative components of MS.

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