Spontaneous Ocular and Neurologic Deficits in Transgenic Mouse Models of Multiple Sclerosis and Noninvasive Investigative Modalities: A Review

Archana A. Gupta,¹ Di Ding,^{1,2} Richard K. Lee,^{1,3,4} Robert B. Levy,⁵
and Sanjoy K. Bhattacharya^{1,2,4}

Multiple sclerosis (MS) is an autoimmune, inflammatory, neurodegenerative, demyelinating disease of the central nervous system, predominantly involving myelinated neurons of the brain, spinal cord, and optic nerve. Optic neuritis is frequently associated with MS and often precedes other neurologic deficits associated with MS. A large number of patients experience visual defects and have abnormalities concomitant with neurologic abnormalities. Transgenic mice manifesting spontaneous neurologic and ocular disease are unique models that have revolutionized the study of MS. Spontaneous experimental autoimmune encephalomyelitis (sEAE) presents with spontaneous onset of demyelination, without the need of an injectable immunogen. This review highlights the various models of sEAE, their disease characteristics, and applicability for future research. The study of optic neuropathy and neurologic manifestations of demyelination in sEAE will expand our understanding of the pathophysiological mechanisms underlying MS. Early and precise diagnosis of MS with different noninvasive methods has opened new avenues in managing symptoms, reducing morbidity, and limiting disease burden. This review discusses the spectrum of available noninvasive techniques, such as electrophysiological and behavioral assessment, optical coherence tomography, scanning laser polarimetry, confocal scanning laser ophthalmoscopy, pupillometry, magnetic resonance imaging, positron emission tomography, gait, and cardiovascular monitoring, and their clinical relevance. (*Invest Ophthalmol Vis Sci.* 2012;53:712–724) DOI:10.1167/iov.11-8351

Multiple sclerosis (MS) is an autoimmune, inflammatory, demyelinating, neurodegenerative disease of the central nervous system, predominantly involving myelinated neurons of the brain, spinal cord, and optic nerve.¹ MS can manifest with features of visual loss, extraocular muscle motility disorders, paresthesias, loss of sensation, weakness, dysarthria, spasticity, ataxia, and bladder dysfunction.² Ocular manifestations often develop early in the disease course. Vision loss, double vision, and painful ocular movements are observed in a large

subset of patients.^{3,4} MS has a multifactorial etiology,⁵ frequently multiphasic⁶ and multifocal.⁷ MS has heterogeneous clinical manifestations and is often disabling through deficits of sensory, motor, autonomic, and neurocognitive function. Approximately 400,000 people have been diagnosed with MS in the United States alone, and 2.5 million have been diagnosed worldwide.⁸ Factors such as age, sex, and seasonal variation can affect the presentation of MS.^{9–14} Other risk factors that can influence the development or progression of MS include viruses (such as Epstein-Barr virus), vitamin D, smoking, diet, obesity, pregnancy, hormones, and stress.^{11,12,15,16}

Based on the intensity and frequency of the symptoms and the rate of progression, demyelinating disorders can be categorized into five distinctive types: monophasic, relapsing-remitting, primary progressive, secondary progressive, and progressive relapsing. Relapsing-remitting MS is the most common form of the disease (80%)² and predominantly affects young and middle-aged persons.⁹ Relapsing-remitting type may lead to secondary progressive MS in 10% to 20% of patients. Primary progressive and progressive relapsing MS are the other recognized but rare forms of MS.

Rodents that exhibit MS signs and symptoms are termed experimental autoimmune encephalomyelitis (EAE) models. Transgenic mice with spontaneous neurologic manifestations eliminate the necessity of immunogen injection. This reduces the variability secondary to treatments and has provided new insights about disease pathogenesis. Noninvasive techniques used for studying these murine models highlight structural and molecular changes within the nervous system.

The first part of this review will summarize the available spontaneous models of EAE with respect to their genetics, phenotype, and avenue for future visual and ophthalmologic research. The second part of the review will focus on noninvasive investigative modalities for the evaluation of visual defects in MS in mice and will be followed by a brief overview of general methods for the evaluation of neurologic deficits.

EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

EAE, a parallel of MS in rodents (mice, rats), is important for studying the underlying pathophysiology of MS and for evaluating novel therapeutic and reparative approaches for treating MS. Animal models of MS also facilitate studying the role of environmental factors in disease initiation and progression.^{17,18} Current mouse models of EAE can be broadly categorized into two major classes: induced models in which EAE is initiated by active immunization with a myelin-like myelin basic protein (MBP), myelin oligodendrocyte glycoprotein (MOG), and myelin proteolipid protein (PLP) or by adoptive transfer by injecting myelin antigen-specific T cells; and spontaneous transgenic models in which genetic manipulation of immune cell molecules or antigens facilitates the

From the ¹Bascom Palmer Eye Institute, the Departments of ²Biochemistry and Molecular Biology, ³Cell Biology and Anatomy, and ⁵Microbiology and Immunology, and the ⁴Neuroscience Program, University of Miami Miller School of Medicine, Miami, Florida.

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Corresponding author: Sanjoy K. Bhattacharya, Bascom Palmer Eye Institute, University of Miami Miller School of Medicine, 1638 NW 10th Avenue, University of Miami, Miami, FL 33136; sbhattacharya@med.miami.edu.

development of spontaneous EAE (sEAE) in transgenic mice. In addition, toxin-induced EAE (cuprizone and lysophospholipid) and intracerebral virus inoculation-induced EAE are other available mouse models of MS.^{19–22} Notably, sEAE models provide the opportunity to investigate the intrinsic signals and pathways leading to MS without the need for external manipulation. However, it is pertinent to note that the spontaneous evolution of disease in transgenic mouse models inherently makes it difficult to precisely predict the temporal evolution and development of MS in each animal. EAE may occur at different times in different mice within the same line, rendering investigational drug trials challenging to run and difficult to interpret. In this context, a potential advantage of induced EAE models is that they may offer a better approach to address certain questions because the onset is controlled and the disease course is more predictable.

EAE is characterized by a prodromal phase, which is followed by atonicity in the tail, hind limb weakness, and paralysis of the forelimbs (classic EAE) (Table 1). In 30% to 60% of patients with MS, optic neuritis (ON) is also a common initial sign.^{3,4} Characterization of ON and associated visual loss can be performed with invasive and noninvasive testing methodologies. Interestingly, several, but not all, transgenic EAE models have been known to manifest ON symptoms.

MODELS OF SPONTANEOUS EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

The selection of a suitable animal model of MS will help in identifying underlying disease mechanisms and testing new treatment modalities. sEAE animal models of MS share similarities with human MS disease. Genetic alterations induced by insertion of a specific transgene predispose an animal to develop MS. There are a number of transgenic mouse models for MS disease. Various transgenic mouse models that present with spontaneous development of ocular and neurologic manifestations have been reviewed here and are summarized in Table 2. Such transgenic mouse models typically have an altered expression of molecules involved in immune cell signaling, which ultimately leads to EAE as depicted in Figure 1 and summarized in Table 3. Both T cells and B cells have been shown to play a role in EAE pathogenesis.^{23,24} Transgenic mice can express CD4⁺ and CD8⁺ T cells specific for epitopes of myelin protein components.^{25–27} Transgenic mice can also be considered in relation to the specific component of myelin that is targeted by the immune cells PLP, MBP, and MOG.^{17,28,29}

Some transgenic mice develop ON and other ocular manifestations, whereas others do not. MOG is a major protein component of myelin in the optic nerve and spinal cord. The predilection of mice expressing MOG-specific T cells for the development of ON can be explained by the physiologically higher concentration of MOG in the optic nerve compared

with the concentration in the spinal cord. Bettelli et al.²⁹ generated 2D2 (also called TCR^{MOG}) transgenic mice expressing MOG-specific T cells. Isolated spontaneous ON (sON) was demonstrated in 35% of 2D2 transgenic mice, when observed for a period of 1 year. 2D2 mice also developed eyelid swelling and tearing. In some, these changes evolved into partial or complete atrophy of the eye. The incidence of ON on histologic evaluation was found to be 49%. Adjacent oculomotor nerves that have myelin derived from the peripheral nervous system did not show any signs of inflammation. 2D2 mice are open to evaluation of ON and visual defects by other modalities, including noninvasive methods, which has not been performed as yet. Only 4% of 2D2 mice developed sEAE. Bettelli et al.²⁹ suggested that the manifestations of the CNS-specific autoimmune disease vary according to the target autoantigen involved. In another experiment with 2D2 mice, Bettelli et al.²⁴ characterized the development of opticospinal encephalomyelitis or Devic disease. The 2D2 mouse was crossed with MOG-specific immunoglobulin (Ig) heavy-chain knock-in mice (IgH^{MOG}). In 2D2 × IgH^{MOG} double-transgenic mice, one-third of B cells were found to be specific to MOG.²⁴ A high incidence (59%) of spontaneous Devic disease was observed in these double-transgenic mice, in contrast to 2D2 transgenic mice (6%). The increased incidence of sEAE in 2D2 × IgH^{MOG} mice indicates the efficient MOG presentation to transgenic T cells by B cells.²⁴ In the presence of transgenic T cells, B cells undergo class switching to produce massive amounts of IgG1. In addition, B cells enhance active proliferation and activation of transgenic T cells. This active cooperation between T and B cells leads to the development of severe and spontaneous autoimmune Devic-like disease.²⁴ Krishnamoorthy et al.³⁰ also reported a similarly high incidence (50%) of spontaneous Devic disease in 2D2 × IgH^{MOG} double-transgenic mice. MOG^{−/−} and MOG deficient IgH^{MOG} mice with B cells (not T cells) specific for MOG remained healthy. The incidence of disease in MOG-deficient 2D2 mice (B-cell specific) was similar to that of its wild-type counterparts (15%–20%). Disease was observed in <15% of 2D2 × IgH^{MOG} × MOG^{−/−} mice. Thus, the incidence appears to be higher in MOG-sufficient mice. However, the clinical manifestations of sEAE were found to be similar in both MOG-deficient and MOG-sufficient mice.³⁰

Mastronardi et al.²¹ and Johnson et al.³¹ described ND4 transgenic mice manifesting sEAE. ND4 mice have 70 copies of the DM20 isoform generated by alternative RNA splicing from the PLP gene.²¹ ND4 mice are clinically healthy until approximately 3 months of age; thereafter, they spontaneously manifest intrinsic symptoms of MS, which culminate in death by 8 to 10 months of age.^{21,31,32} DM20 is a major PLP isoform in young mice that becomes a minor component in adults. High DM20 protein levels in adult mice result in an abnormal myelin assembly with a high propensity for disruption. Thus, the persistence of immature myelin into adult life induces demyelination in ND4 mice.³¹ Enriquez-Algeciras et al.³² used various noninvasive modalities for characterization of sEAE in ND4 mice and found associated functional visual deficit and optic nerve defects that are manifested before the onset of clinical neurologic deficits in ND4 mice.

The HLA-A3–2D1-TCR double-transgenic mice described by Friese et al.³³ revealed evidence of ON on histopathologic examination. Early and late ON (after induction by injecting antigen) in the absence of any motor deficits was demonstrated histopathologically in a small group of mice immunized with PLP fragment 45–53. sON was not observed in this model, suggesting differences between different models with respect to ON and inherent heterogeneity in MS disease. They studied the predisposition to disease by HLA-A*0301 in mice transgenic for the 2D1 human T-cell receptor (TCR) (2D1-TCR), which is specific for the PLP 45–53 epitope presented by HLA-A3. PLP

TABLE 1. Expanded Disability Status Scale or Clinical Scoring

Score	Sign
0	No disease
0.5	Distal limp tail
1	Limp tail
2	Mild paraparesis, ataxia
3	Moderate paraparesis; the mouse trips from time to time
3.5	One hind limb is paralyzed, the other moves
4	Complete hind limb paralysis
4.5	Complete hind limb paralysis and incontinence
5	Moribund; difficulty breathing; unable to eat or drink

TABLE 2. Transgenic Mouse Models Manifesting sEAE

Serial Number	Transgenic Model	Transgene	Background Strain (mouse)	Onset (days)	Lifespan/ Duration of Observation (days)	Incidence of sEAE (%)	Incidence of sON (%)	Other Ocular Features	Neurologic Features	Noninvasive Methods	References
1	ND4	DM-20 (All cells)	CD-1	90	240 to 300/-	—	+	—	Tremor, unsteady gait, seizure	PERG, FERG, OCT, MRI	21,31,32
2	2D2 × IgH ^{MOG} /OSE/Devic	MOG 35-55-specific TCR (CD4 ⁺ T cell)	C57BL/6	49-70	-/-	—	sOSE: 50	—	Paralytic and spastic EAE	—	30
3	2D2 × IgH ^{MOG} × MOG ^{-/-}	MOG 35-55 specific TCR (CD4 ⁺ T cell)	C57BL/6	49-70	-/-	—	sOSE: 15	—	Paralytic and spastic EAE studied with cage grip test	—	30
4	2D2 × MOG ^{-/-} × Rag2 ^{-/-}	MOG 35-55 specific TCR (CD4 ⁺ T cell)	C57BL/6	49-70	-/-	—	33	—	Paralytic and spastic EAE studied with cage grip test	—	30
5	2D2 × MOG ^(cre/cre)	MOG 35-55-specific TCR (CD4 ⁺ T cell)	C57BL/6	49-70	-/-	—	15	—	Paralytic and spastic EAE studied with cage grip test	—	30
6	2D2/TCR ^{MOG}	MOG 35-55 specific TCR (CD4 ⁺ T cell)	C57BL/6	73.5 ± 7.7	-90	—	sOSE: 6	—	Decreased tail tone; hind limb and forelimb weakness/partial/complete paralysis	—	24
7	2D2 ^{MOG} × IgH ^{MOG}	MOG 35-55-specific TCR (CD4 ⁺ T cell)	C57BL/6	44.1 ± 2.8	-90	4	sOSE: 69	—	Decreased tail tone; hind limb and forelimb weakness/partial/complete paralysis	—	24
8	2D2/TCR ^{MOG}	MOG 35-55 specific TCR (CD4 ⁺ T cell)	C57BL/6	75-150	—	4	35 (clinically), 49 (IH)	Tearing, eyelid swelling, partial/complete eye atrophy	Limp tail, hind limb paralysis	—	29
9	DR2/TCR	HLA-DR2; MBP peptide complex specific TCR (CD4 ⁺ T cell)	DBA/2 × C57BL/6	6-12	—	4	ON detected on H	—	Hind leg paralysis	—	35
10	DR2/TCR × Rag2 ^{-/-}	HLA-DR2; MBP peptide complex-specific TCR (CD4 ⁺ T cell)	DBA/2 × C57BL/6	48-102	—	100	ON detected on H	—	Hind leg paralysis	—	35
11	ODC-OVA/OT1	OVA-specific TCR (CD8 ⁺ T cell)	C57BL/6	12 to 19	-/-	90	ON detected on H	—	Ascending paralysis of extremities, uncoordinated limb movements, tremor	—	27
12	5B6	PLP139-151-specific TCR (CD4 ⁺ T cell)	SJL CD90.1	40-100	-160	80	—	—	Uneven gait, impaired righting reflex, total hind limb or forelimb paralysis	—	22
13	5B6	PLP139-151-specific TCR (CD4 ⁺ T cell)	B10.S/SJL	—	-44	B10.S: 4, SJL: 40	—	—	Limp tail, impaired righting reflex, waddled gait, hind limb paralysis	—	38
14	5B6	PLP139-151-specific TCR (CD4 ⁺ T cell)	SJL/J (H-2s)	—	-360	40	—	—	4E3 developed more severe EAE than 5B6 clone in SPF/VAF conditions	—	28
15	4E3	PLP139-151-specific TCR (CD4 ⁺ T cell)	SJL/J (H-2s)	—	-360	60-83	—	—	Uneven gait, impaired righting reflex, total hind limb or forelimb paralysis	—	28
16	HLA-A3-2D1-TCR	PLP45-53-specific TCR (CD8 ⁺ T cell)	Humanized Tg (HLA-A3)	—	-100	4	Induced ON on H	—	Paralytic EAE	—	33
17	αβ	MBP1-11-specific TCR (CD4 ⁺ T cell)	B10.PL	35-150	-360	14-44	—	—	Limp tail, uneven gait, hind limb or forelimb paralysis	—	17
18	Vα2.3/Nβ8.2	MBP (NAcl-11) specific TCR (CD4 ⁺ T cell)	B10.PL	13.63 ± 4.17	-360	80	—	—	Limp tail, waddling gait, ataxia, partial or total hind limb paralysis	—	25

(continues)

TABLE 2 (continued). Transgenic Mouse Models Manifesting SEAE

Serial Number	Transgenic Model	Transgene	Background Strain (mouse)	Onset (days)	Lifespan/ Duration of Observation (days)	Incidence of sEAE (%)	Incidence of sON (%)	Other Ocular Features	Neurologic Features	Noninvasive Methods	References
19	Vα4/Vβ8.2	MBP (NAc1-11) specific TCR (CD4 ⁺ T cell)	B10.PL	32.33 ± 8.08	—/360	30	—	—	Limp tail, waddling gait, ataxia, partial or total hind limb paralysis	—	25
20	T/R [−] & T/α [−] β [−]	MBP (Ac1-11) specific TCR (CD4 ⁺ T cell)	B10/PL	35	—/28-100	100	—	—	sEAE: acute phase for 1 week, chronic progressive phase for weeks to months. Weight loss, limp tail, front or hind leg weakness/partial/complete paralysis	—	40
21	T/R [−] & T/R ⁺	MBP (Ac1-11) specific TCR (CD4 ⁺ T cell)	B10/PL	91	≥90/—	T/R [−] : 100, T/R ⁺ : 11 to 14	—	—	Weakness or paralysis of hind limb and fore limb	—	39
22	Line 7	HLA-DRA1*0101 and DRB1*1501 and anti-MBP 85-99/DR15-specific TCR (CD4 ⁺ T cell)	H2Aβ ^{−/−}	120-150	—/280	60-100	—	—	Limp tail, impaired righting reflex, waddling gait, partial/total hind limb or forelimb paralysis	—	23
23	I-Aβ ^{−/−} B7.2	B7.2 (microglial cell)	C57BL/6	59 ± 10	—/250	100	—	—	Hind limb claspings when lifted by tail, uncoordinated hind limb splaying and weakness with difficulty to right when overturned, weak tail movements and poor proprioception when walking on cage bars	—	26
24	I-Aβ ^{+/+} B7.2	B7.2 (microglial cell)	C57BL/6	133 ± 26	—/250	100	—	—	Hind limb claspings when lifted by tail, uncoordinated hind limb splaying and weakness with difficulty to right when overturned, weak tail movements, and poor proprioception when walking on cage bars	—	26
25	CD4 ^{−/−} B7.2	B7.2 (microglial cell)	C57BL/6	66 ± 20	—/250	100	—	—	Hind limb claspings when lifted by tail, uncoordinated hind limb splaying and weakness with difficulty to right when overturned, weak tail movements, and poor proprioception when walking on cage bars	—	26

sOSE, spontaneous optico-spinal encephalomyelitis/spontaneous Devic's like disease; PLP, proteolipid protein; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; +, present; −, not studied/specified in the referenced article.

FIGURE 1. Schematic overview of immune cell responses in transgenic models of EAE. The peptide epitopes presented by APCs induced the activation of CD8 through MHC class I (A) and of CD4 through MHC class II (B). TCR transgenic T cells leading to the induction of EAE. The 2D2 model (C) includes T- and B-cell transgenic antigen receptor populations. The individual T-cell receptor transgenic mouse models differ with respect to type of T-cell and effector molecules involved (see Table 3). APC, antigen-presenting cell; SR, scavenger receptor; TCR, T-cell receptor; BCR, B-cell receptor.

constitutes 50% of the CNS myelin protein. 2D1-TCR was derived from CD8⁺ T cells cloned from an individual with typical relapsing-remitting MS, which kills PLP-expressing HLA-A*0301-positive cells and thus recognizes a naturally processed epitope. Although major histocompatibility complex (MHC) class II alleles and CD4⁺ T cells are most commonly implicated in EAE, MHC class I and CD8⁺ T cells have also been demonstrated to be involved.³⁴ Friese et al.³³ suggested MHC class I-restricted CD8⁺ T-cell involvement in initiating the first subtle immune insult, but disease progression required additional contributions from MHC class II-restricted CD4⁺ T cells. The HLA-A3-2D1-TCR strain is a double-transgenic mouse expressing two human MHC class I alleles. The sEAE has been found in 4% of these double-transgenic mice. HLA-A*0301 in these mouse models has been shown to predispose to EAE, whereas HLA-A*0201 has a protective role.³³

Madsen et al.³⁵ described DR2/TCR, a double-transgenic mouse line expressing DRA*0101/DRB1*1501 and TCR. Optic and vestibular nerve involvement have been demonstrated in 4% of DR2/TCR double-transgenic mice using histopathology and immunohistochemistry, which increases up to 100% when DR2/TCR double-transgenic mice were backcrossed to RAG2 (recombination activating gene 2)-deficient (Rag2^{-/-}) mice.³⁵ This indicates that T cells specific for the HLA-DR2 (MHC complex II)-bound MBP peptide are sufficient and necessary for the development of EAE. The prevention of endogenous TCR rearrangement mediated by RAG2 and a resultant increased frequency of MBP-specific T cells, together with the decreased frequency of regulatory T cells, appears to be responsible for the high incidence of sEAE in DR2/TCR transgenic Rag2^{-/-} mice.³⁵

Na et al.²⁷ demonstrated ON using histopathology in ODC-OVA transgenic mice. These mice were generated using ovalbumin (OVA) cDNA under the control of an oligodendrocyte-specific MBP promoter. The ODC-OVA mouse was crossed with OT-I and OT-II transgenic mice to generate ODC-OVA/OT-I and ODC-OVA/OT-II double-transgenic mice. In ODC-OVA transgenic mice, OVA is sequestered in the cytosol of the oligodendrocytes and thus inaccessible to OT-II/CD4⁺ T

cells expressing OVA-specific MHC class II-restricted receptors. OVA has been observed to be accessible in these mice to naive CD8⁺ T cells expressing MHC class I-restricted (OT-I) T-cell receptors, which results in antigen release in the periphery of these mice within the first 10 days of life.²⁷ Thus, naive CD8⁺ T cells that can gain access into an immune-privileged organ, such as the CNS, can initiate autoimmunity through an IFN- γ -mediated amplification loop without the antigen in question being spontaneously released for presentation by professional antigen-presenting cells resulting in sEAE in 90% of ODC-OVA mice on a C57BL/6 background.²⁷ In summary, the foregoing section presented a review of a number of sEAE models that present ON and visual defects, which has been characterized with invasive or noninvasive methods. In human MS disease, the measurement of visual acuity alone often provides the misleading inference that the visual defects are transient, but rigorous studies with a number of noninvasive methods clearly demonstrate the nontransient nature of functional loss.^{36,37} As in human MS, the ON and visual defects in mouse MS are irreversible.³²

In contrast to the mouse models described, a number of other transgenic sEAE models have been developed in which visual defects or associated ocular pathology has not been investigated. We will review a brief account of antigens and cellular players in these sEAE models and summarize their sEAE incidence. It is evident from the foregoing discussion that the onset and severity of ON and visual defects differ in MS in different model systems, and their evaluation and characterization are poised to provide greater insight into the different aspects of MS. The lack of investigation of ON and visual defects in the transgenic models described next renders them a rich resource for future investigation using both invasive and noninvasive methods. Characterization of visual defects in these models will further enhance our understanding of ON related to MS.

Some sEAE models have been developed using TCR transgenes. We will discuss the transgenic mouse models that express immune cells with subsequent sEAE development. Waldner et al.²⁸ isolated genomic rearranged TCR genes from the

TABLE 3. Alteration in Innate and Adaptive Immune System Cells and Molecules in Transgenic Mouse Models of sEAE

Myelin Epitope Reactive T Cell	Peptide/Antigen	Amino Acid Sequence	MHC on APCs	Cell/Molecule Alteration	Transgenic Mouse Model	Reference
CD4 ⁺	PLP	139-151	MHC class II	TLR 4 and TLR 9 associated break in tolerance	5B6	38
				Increased expression of IFN- γ and IL-17, decreased IL-10 and regulatory T cells	5B6	22
				Increased expression of IFN- γ and IL-17	5B6	28
				Increased expression of IFN- γ and IL-17	4E3	28
				Overexpression of DM-20 (a product of alternative RNA splicing from PLP gene) in mature myelin	ND4	21,31,32
	MBP	1-11		Associated environmental factor (viral infection) implicated	$\alpha\beta$	17
				Increased expression of CD69 and CD44, decreased CD45RB and increased expression of T _H 1 cells	V α 2.3/V β 8.2	25
				Increased expression of T _H 2 cells (IL-4 and TGF- β)	V α 4/V β 8.2	25
				CD28 costimulation and triggering of B7 ligands by endogenous danger signal	T/R ⁻ & T/ α ⁻ β ⁻	40
				Absence of protective nontransgenic lymphocyte	T/R ⁻	39
				T-cell proliferation and increased expression of IFN- γ and IL-2	Line 7	23
				HLA-DR2 can mediate sEAE by presenting antigen to T cell	DR2/TCR	35
				Enhanced T-cell response through B-cell antigen presentation	2D2 $\times$ IgH ^{MOG}	24
				Modulating the immune response in a dose-dependent manner, dependent on target autoantigen	2D2/TCR ^{MOG}	29
CD8 ⁺	Neoself antigen OVA	—	MHC class I	CD8-mediated antigen release from oligodendrocytes required for OVA-presentation in draining lymph nodes	ODC/OVA/OT1	27
	PLP presented by HLA-A3	45-53		CD8 ⁺ -mediated induction of EAE and CD4 ⁺ mediated progression. HLA-A*0201 has a protective role	HLA-A3-2D1-TCR	33
Unknown	Constitutive expression of B7.2 on microglia	—		Constitutive costimulation through B7 and severely deficient in CD4 ⁺ T cell	I-A β ^{-/-} B7.2	26
				CD4 ⁺ gene-depleted B7.2	CD4 ^{-/-} B7.2	26
				Constitutive costimulation through B7	I-A β ^{+/+} B7.2	26

PLP, proteolipid protein; MOG, myelin oligodendrocyte protein; MBP, myelin basic protein; HLA, human leucocyte antigen; TLR, toll-like receptor; IFN, interferon; IL, interleukin; TCR, T cell receptor.

PLP-139-151-specific T-cell clones 5B6 (V α 4 and V β 6) and 4E3 (V α 11 and V β 16) to generate 5B6 and 4E3 TCR transgenic mice on the SJL background (Table 2). Each T-cell clone was MHC II (I-As) restricted.²⁸ The mice were housed under specific pathogen-free (SPF) and virus antibody-free conditions and were observed for 1 year. Interestingly, they observed sEAE in each backcross generation of 5B6 and 4E3 transgenic mice but not in nontransgenic mice. The incidence of sEAE was 40% to 67% in 5B6 mice and 60% to 83% in 4E3 mice, respectively. Female gender bias was observed only in one of the lines of 4E3 mice.²⁸ In another study by Waldner et al.,³⁸ 5B6 transgenic mice were developed on the B10.S backgrounds. In contrast to those on the SJL background, a lower incidence (4%) of sEAE was observed in 5B6 mice on the B10.S

background.³⁸ A diminished endogenous activation state and T-cell-activating capacity of antigen-presenting cells of transgenic mice on the B10.S background may explain this variation in autoimmune disease induction.³⁸ In 5B6 transgenic mice, Zhang et al.²² illustrated cervical lymph nodes, but not the spleen, to be the initial peripheral activation site. They found CD4⁺ cells from cervical lymph nodes in these mice to be hyperactivated. They deduced the role of heterogeneous populations of regulatory T cells in regulating the onset of sEAE. The study findings indicated that the induction of peripheral tolerance could be exploited to prevent or treat sEAE.²²

We now present the discussion of mice expressing MBP-specific TCR. Gorman et al.¹⁷ generated a TCR mouse model with CD4⁺ cells specific for MBP 1-11. The $\alpha\beta$ transgenic

mice have functionally rearranged TCR genes cloned from a B10.PL background. The development of sEAE was observed in 14% to 44% of mice maintained under non-SPF conditions. In contrast, none of the mice housed in SPF conditions developed sEAE in this study, suggesting an environmental or infectious etiology.¹⁷

Song et al.²⁵ compared V α 2.3/V β 8.2 and V α 4/V β 8.2 transgenic mice for the development of sEAE. V α 2.3/V β 8.2 and V α 4/V β 8.2 transgenic mice, developed on a B10.PL background, overexpressed TCR specific for the MBP epitope NAc1-11. They found that approximately 15% of V α 2.3/V β 8.2 mice housed under SPF conditions developed sEAE compared with 80% of mice housed under non-SPF conditions. In contrast, only 11% and 10% of V α 4/V β 8.2 mice developed sEAE in SPF and non-SPF facilities, respectively.²⁵

Laffaile et al.³⁹ generated anti-MBP T/R⁻ and T/R⁺ transgenic mice by injection of α and β TCR constructs derived from an encephalomyelitogenic T-cell clone into the pronuclei of fertilized C57BL6 eggs. The H-2^u haplotype was introduced into the transgenic background by crosses with either PL/J or B10.PL mice. RAG^{-/-} deficient mice were originally generated in the 129/Sv genetic background.³⁹ The incidence of sEAE was found to be very different in T/R⁻ and T/R⁺ mice, despite their having the same number of encephalitogenic T cells and being housed in same SPF facility. sEAE developed in only 14% of T/R⁺ mice at 12 months; in contrast, it developed in 100% of T/R⁻ mice. This implies that sEAE can be mediated by anti-MBP CD4⁺ T cells in the absence of any other lymphocytes. Nontransgenic lymphocytes have been shown to have a protective role. In situ activation of CD4⁺ anti-MBP cells in the CNS may trigger sEAE.³⁹

Furtado et al.⁴⁰ achieved monoclonality of the $\alpha\beta$ T-cell repertoire by crossing MBP-specific TCR transgenic mice with either RAG^{-/-} mice (generating T/R⁻ mice) or TCR $\alpha^{-/-}$ TCR $\beta^{-/-}$ double knockout mice (generating T/ $\alpha^{-/-}\beta^{-/-}$ mice). In most mice, sEAE was observed after 35 days, with 30% manifesting symptoms by 42 days, 50% by 50 days, 80% by 70 days, and 100% after 100 days under SPF conditions.⁴⁰ Notably, sEAE occurs with high frequency in germ-free mice, thereby challenging the microbial theory of disease induction. Peripheral activation of T cells in CNS draining lymph nodes is an early occurrence in sEAE. This may facilitate the swift migration of T cells into the CNS in response to IFN- γ . Lymphadenectomy in healthy pre-EAE transgenic mice has been demonstrated to delay the onset and to diminish the severity of EAE.⁴⁰

Certain epitopes of human neuroproteins have been known to trigger EAE. EAE-associated demyelination and neuronal degeneration correlate well with intermolecular and intramolecular spread of T-cell responses to HLA-DR15-restricted epitopes of MBP, MOG, and β -crystalline.²³ Ellmerich et al.²³ generated mice (termed line 7) that are MBP 85-99-specific TCR mice. Almost 60% of line 7 mice were noted to develop sEAE by 6 months. sEAE was significantly exacerbated on crossing line 7 mice with RAG-deficient mice (100%).²³

Zehntner et al.²⁶ and Brisebois et al.⁴¹ generated a transgenic mice that overexpresses the mouse B7-2 protein in the lymphoid cells. Seven of 8 initial founders carrying 8 to 20 copies of transgene were successfully crossed with C57BL/6 mice to establish lines (7, 16, 23, 26, 27, 31, and 33).⁴² B7.2 transgenic (lines 31 and 33) mice constitutively express the T-cell costimulatory ligand B7.2/CD86 on microglial cells. They spontaneously develop neurologic manifestations and demyelinating lesions in the spinal cord. T-cell activation in the CNS by B7.2-expressing microglia apparently is a prerequisite for disease development. Earlier onset of sEAE is observed in B7.2 transgenic mice deficient in CD4⁺ T cells.²⁶ Interestingly, mice deficient in IFN- γ have been shown to be resistant to EAE.²⁶ Because IFN- γ is involved in microglial activation, it is pro-

posed that autoreactive CD8⁺ T cells in the CNS may play a key role in the pathogenesis of MS in mice. The I-A $\beta^{+/+}$ B7.2 mice strain contains both CD8⁺ and CD4⁺ T cells, and 100% of these mice develop sEAE.²⁶ The I-A $\beta^{-/-}$ strain of C57BL B7.2 transgenic mice that predominantly express CD8⁺ T cells also develop sEAE with 100% incidence. A high preponderance of CD8⁺ T cells was observed in the neurologic lesions of these mice, correlating with the symptomatic state and suggesting a significant role of this T-cell subset in MS development.^{26,41} In the foregoing section, we describe a number of transgenic models of sEAE that differ with respect to onset, severity, and environmental effects on of the disease, together with differences in antigenic molecules and cellular players in which the ON and visual defects have not been investigated. In the ensuing sections, we will describe noninvasive methods that enable the characterization of visual defects and optic nerve in living animals and the methods that enable assessment of neurologic deficits.

NONINVASIVE IMAGING METHODS

Early diagnosis combined with prompt treatment of MS can delay disease progression and relapse⁴³ and the associated loss of axons.⁴⁴ A number of noninvasive methods are now available for the assessment of various aspects of MS and EAE, and their numbers are also expanding. Serial measurements with these noninvasive methods facilitate the monitoring of disease evolution, the effect of modulation of various factors, the efficacy of interventions, and the assessment of ocular structures and visual function in MS.³² Use of noninvasive methods also reduces the number of animals needed for the study. We will provide an overview of the noninvasive modalities for the assessment of visual function and optic nerve health. This will be followed by a summary of the noninvasive investigative modalities for general neurologic deficits, including simultaneous assessment of ocular pathology associated with EAE and MS.

NONINVASIVE MODALITIES FOR VISUAL AND OPTIC NERVE HEALTH ASSESSMENT

We summarize here the noninvasive modalities frequently used for monitoring ocular disease in mice with EAE.

Electrophysiological Assessment

Pattern Electrophoretogram and Flash Electrophoretogram. Pattern electrophoretogram (PERG) provides an objective assessment of retinal function and a direct functional assessment of retinal ganglion cell activity.⁴⁵ Light-adapted flash electrophoretogram (FERG) is an index of outer retinal function. The combination of PERG and FERG is commonly used to detect specific dysfunctions in vision.³⁷ PERG response has two main components, a positive waveform at 50 ms (P50) and a negative waveform at 95 ms (N95).⁴⁵ The P50 component is abnormal in macular dysfunction, with simultaneous reduction in N95 waveforms. N95 originates from retinal ganglion cells and is abnormal in optic nerve disease. Unlike visually evoked potentials (VEPs), which are altered in both macular and optic nerve dysfunction, PERG more specifically assesses optic nerve dysfunction. PERG measurements show discernible changes in visual function before the onset of ophthalmic symptoms. PERG analysis detects a marked decrease in amplitude with advancing age and severity of disease in ND4 mice. A discernible decrease in amplitude of PERG at 3 months and a marked decrease at 8 months have been reported, without any concomitant alterations in FERG.³² PERG measurement detects visual dysfunction and is more sensitive to early decline com-

pared with disability score. Interestingly, PERG detects changes even before MRI can pick up demyelination.^{32,37}

Visual Evoked Potential. An evoked response is an electrical potential recorded from the nervous system after presentation of a visual stimulus. Pattern VEP (PVEP) is an evoked response to a reversing checkerboard stimulus. PVEP is represented by a waveform with a small negative component at 80 ms (N80), followed by a major positive component at around 100 ms (P100).⁴⁶ Most P100 is generated from the central portion of the visual field (30°), with the lower part of the field contributing a greater percentage than the upper part.⁴⁷ VEPs can identify demyelination in the absence of clinical features. VEP changes persist even after clinical recovery from ON. In MS, VEP demonstrates optic nerve conduction delay (increased latency) with a less marked change in amplitude. A delay in the latency of VEP can be attributed to myelin abnormality, whereas a decrease in intensity occurs secondary to axonal damage.⁴⁸ VEP is a sensitive modality for objective assessment of functional impairment of the visual pathway and is used as a diagnostic tool for the identification of early MS onset.⁴⁹ VEP appears to be more specific in identifying early changes pertaining to demyelination than MRI.⁵⁰ In shiverer mutant mice (nontransgenic mice), which have a severe myelin deficiency, VEP latency was longer than in normal wild-type mice. Compared with wild-type mice, VEP latency was found to be 30% in mice homozygous for MBP^{shi} with little or no CNS myelin and 7% in mice heterozygous for MBP^{shi} with apparently normal myelin.⁵¹

The multifocal VEP (mfVEP) has the potential for earlier recognition of patients (an initial MRI scan is suggestive but not diagnostic for MS), enabling precise monitoring.⁵² With further advances in technology, mfVEP should be capable of measuring potentials from many regions in the visual field simultaneously.⁵³

Optical Coherence Tomography. Optical coherence tomography (OCT), a type of optical imaging, was initially developed to image the transparent tissue of the eye, but with the use of infrared rays it can also be applied to image nontransparent tissues. In a highly scattered tissue, the back-scattered light from a specific depth is selected interferometrically. OCT allows two- and three-dimensional imaging using backscattering as endogenous contrast.⁵⁴ OCT measures retinal nerve fiber layer (RNFL) thickness, which correlates with ganglion cell number. Peripapillary RNFL contains mostly axons, and the macula is largely composed of retinal ganglion cells (RGCs). Unmyelinated axons in RNFL are ideal to characterize neurodegeneration, neuroprotection, and potentially even neuronal repair. RNFL thinning has been demonstrated in asymptomatic MS.⁵⁵ OCT imaging has been used in mouse models, as in ND4 mice.³²

Confocal Scanning Laser Ophthalmoscopy. Confocal scanning laser ophthalmoscopy (CSLO) is specifically designed to analyze the optic nerve head and to provide an indirect evaluation of RNFL thickness. CSLO constructs a three-dimensional map of the retinal tissue around the optic nerve head, permitting derivation of planimetric and volumetric parameters in reference to the optic nerve head and the RNFL.⁵⁶ CSLO enables a noninvasive, multimodal microscopy of the fundus in mouse and in vivo retinal imaging.⁵⁷

Using a mouse model with labeled RGCs, CSLO visualized RGCs undergoing apoptosis in vivo.⁵⁸ CSLO provides information complementary both to the fundus camera and to OCT. CSLO can capture rapid images at low light levels and allows unique insight into inflammatory and degenerative CNS processes in the eye at cellular levels. Recruitment of leukocytes into tissue from the blood circulation is an important pathophysiological phenomenon in MS. Precise tracking of different types of leukocytes, such as CD4⁺ and CD8⁺ T cells, mono-

cytes, and neutrophils by CSLO, could help distinguish different phases of immune responses during MS-related ON.^{59,60}

Scanning Laser Polarimetry. Scanning laser polarimetry (SLP) measures the retardation of the polarized rays from RGCs.⁶¹ The extent of retardation correlates with RNFL thickness. The SLP phase shift is due to the arrangement and density of RNFL, microtubules, and other directional elements and tissue thickness. SLP provides an objective and quantitative measure of RNFL loss.⁶¹ A significant correlation has been found in SLP parameters in patients with ON, suggesting that ON causes morphologic changes in RNFL. Recurrent ON aggravates RNFL damage and causes diffuse peripapillary RNFL damage.⁶² SLP has been suggested to be a useful adjunctive modality in patients with demyelinating ON.⁶³ SLP can potentially be used as a noninvasive investigative modality to characterize RNFL in mouse models of EAE.

Pupillometry. Pupillary reflex metrics is a measurement of baseline pupillary diameter during the direct and consensual pupillary reflex. Pupillometry can potentially be used to characterize demyelinating and neurodegenerative lesions and has a future potential for use in MS patients. It has been evaluated in mice but remains to be used in mouse models of EAE. It can facilitate the monitoring of neuroprotective and restorative treatments in murine models of EAE.⁶⁴ A pupillometry device tailored for rodents can be an inexpensive, simple tool to monitor disease progression in mice.⁶⁴

Virtual Optokinetic System. The virtual optokinetic system (VOS) consists of a virtual cylinder with vertical sine wave gratings that can be projected in three-dimensional coordinate space on a quadrangle of computer monitors in a testing area.⁶⁵ Neuro-ocular plasticity in an adult mouse has been characterized with VOS.⁶⁵ In a study, maximal spatial frequency was evaluated in response to monocular deprivation (MD). The presence of experience-dependent plasticity in an adult mouse visual system characterized by modulation of the normal function of subcortical visual pathways by the visual cortex has been demonstrated.⁶⁵ VOS responses with or without MD are modulated in MS. VOS has great potential for facilitating understanding of the visual pathway and cortical plasticity in the response to treatment and recovery.⁶⁵ VOS has recently been applied in mice with EAE to access visual function.⁶⁶

Noninvasive Investigative Modalities for General Neurologic Deficits and Visual Health

We present an account of general methods for the evaluation of bona fide neurologic deficits associated with MS. Several of these methods also enable evaluation of visual health and optic nerve structure.

Gait Evaluation. Gait can be monitored using several methods, including a complex wheel assay (DigiGait; Mouse Specifics, Inc., Quincy, MA) and the cage grip test.^{26,67,68} Complex wheel assay characterizes bilateral sensorimotor coordination. Mice are housed in a cage equipped with an optical sensor to quantify the number of revolutions of wheel per unit time interval. The mice are first trained on a training wheel before introduction to the "complex wheel," which has 22 missing rungs. Total distance run, $v_{\max}$ among daily runs, number of runs, and maximum run interval can be determined.⁶⁷

The complex wheel assay (DigiGait; Mouse Specifics, Inc.) monitors gait changes in EAE. It assists in imaging the ventral side of the mouse as it walks on a motorized transparent treadmill belt. A high-speed camera captures the dynamics and quantifies the stance and swing components of stride without the need of paw inking. It then calculates stride length, duration, frequency, and numerous other spatial and temporal gait indices for each limb.^{68,69} In the cage grip test, the mouse is

gently lifted by its tail and held inverted in close vicinity to a cage. The animal is allowed to grip the grid of the cage with its front limbs and is then monitored for paralysis, spasticity, and altered proprioception.

Clinical Scoring or Expanded Disability Status Scale.

The Expanded Disability Status Scale (EDSS) grades the severity of disease based on neurologic features. In most mouse models, EAE begins with the paralysis of the tail and hind legs and progressively ascends to the forelimbs (referred to as classic EAE). The disease may be associated with ataxia, incontinence, and seizures. The scoring system is principally formulated based on neurologic motor deficits called EDSS to quantify neurologic abnormalities. A score is assigned to each abnormality (Table 1). The scoring system may vary among different studies.^{32,70}

Cardiovascular Parameter Monitoring. MS is a paralytic inflammatory autoimmune disease that may alter the autonomic regulation of physiological states, including heart rate and blood pressure. Alterations in cardiovascular parameters in sEAE can be monitored with telemetry (invasively)⁷¹ or a cardiac function instrument (noninvasively) (ECGenie; Mouse Specifics, Inc.).

The noninvasive instrument (ECGenie; Mouse Specifics, Inc.) assesses cardiac function in conscious and ambulatory laboratory animals. It enables quick and precise electrocardiography in mice, notably without the need for anesthesia; it can be used to study the effect of MS on the cardiovascular system and can potentially be applied to mice.^{72,73}

Behavioral Assessment. Behavioral assessment can be performed using the resident intruder test of social interaction. Video recordings are used to quantify independent and interactive behaviors. Independent behaviors include sniffing the environment, rearing independently or alongside the cage, digging, freezing, and scratching. Interactive behavior assessment includes sniffing or lying next to other mice, running away from other mice, boxing or wrestling, mounting, pinning, and tail rattling. An increase in interactive behavior and a decrease in independent behavior may be observed with demyelination.⁶⁷

Magnetic Resonance Imaging. Inflammatory demyelinating plaque of white matter often precedes clinical manifestations in MS. Objective, noninvasive, and prompt detection of plaques with magnetic resonance imaging (MRI) is thus crucial in establishing a pre-symptomatic diagnosis, in initiating early preventive strategies, and in tracking the disease course. Active lesions can be characterized on T_1 - and T_2 -weighted images.

T_1 -weighted imaging is a fundamental type of MRI that provides good gray and white matter delineation.⁷⁴ T_2 -weighted imaging demonstrates similar hyperintensity for edema, infiltration, and demyelination, thereby precluding the precise identification of each of these pathologic alterations.

The combination of STIR-FSE (short tau inversion recovery-fast spin-echo), PDCSE (proton density conventional spin-echo), and T_1 -CSE + Gd-DTPA (gadolinium-diethylenetriamine pentaacetate) sequences can differentiate demyelinating lesions from those with pure inflammation.⁷⁵ Gadolinium-enhanced T_1 -weighted MRI aids in the diagnosis of acute inflammation. Early gadolinium leakage observed in EAE appears related to alterations of the blood brain barrier (BBB) and cellular infiltration rather than demyelination. Thus gadolinium-enhancing lesions are believed to herald acute but not chronic lesions.^{76,77}

Significant demyelination can be demonstrated with progressing age in various mouse models of EAE, including ND4, on T_2 rapid acquisition relaxation enhanced (RARE) MRI sequences (Fig. 2). Significant shrinkage of brain volume is also observed with advancing age.³² Notably, the size and number of lesions detected by MRI have been shown to only modestly

FIGURE 2. Sagittal T_2 RARE MRI brain images of 5-month-old control (A) and ND4 mice (B), as indicated. Arrows: areas undergoing structural changes and demyelination.

correlate with clinical features of MS.⁷⁸ Edema, inflammation, demyelination, and axonal damage cause change in tissue relaxation properties.⁷⁹

Diffusion of water molecules is facilitated along the nerve fiber tract (longitudinally) in the white matter but not perpendicularly to it (i.e., anisotropy). Contrast in diffusion-weighted imaging (DWI) is the result of an alteration in diffusivity and a loss of anisotropy caused by inflammation and demyelination.⁸⁰

Magnetic diffusion tensor imaging (DTI), a specific parametric MRI, can investigate actual vector properties of the nerve fiber tracts and provide detailed anatomic tractography. In vivo DTI is an exceptionally sensitive and specific measure of white matter injury, especially in the context of MS and EAE. Directional diffusivities of water molecules are altered by injury to white matter. DTI demonstrates that axonal injury leads to increased axial diffusivity and that myelin injury causes increased radial diffusivity. Respiratory motion causes error in tensor calculation, which can be nullified by immobilizing the head of the mouse in a holder or with respiratory gating control. Imaging slice thickness of 0.5 mm is the thinnest possible to date (usually 0.5–1 mm). Six white matter tracts were evaluated with DTI in a study by Sun et al.,¹¹ namely anterior commissure, corpus callosum, cerebral peduncle, external capsule, optic nerve, and optic tract. DTI identified the optic nerve and tract to be severely affected. There were approximately 19% and 18% decreases in axial diffusivity, suggesting axonal injury of optic nerve and tract, respectively, and 156% and 86% increases in radial diffusivity, suggesting myelin injury of optic nerve and tract respectively.¹¹

Axonal damage but not demyelination is suggested to be the prime etiology of long-term neurologic deficits in MS. In the ventrolateral white matter of mice, axial diffusivity has a negative correlation with disability score.⁸¹ Axial diffusivity was profoundly lower in mice with severe EAE than in those with moderate disease.⁸¹ Radial diffusivity and relative anisotropy are unable to differentiate moderate from severe EAE. More axonal damage leads to less axial diffusivity on DTI.⁸¹ Furthermore, greater magnitude of axonal damage was detected compared with myelin damage in the white matter in EAE mouse

models. Direct correlation has been demonstrated between axonal damage observed on DTI and on histologic examination with anti-amyloid precursor protein antibody.⁸²

Axial diffusivity and axonal damage on DTI also correlate well with quantitative staining for neurofilament (SMI31, a marker of axonal integrity), are decreased throughout white matter, and are not limited to areas of demyelination.⁸³

DTI aids in elucidating the relationship between directional diffusivities and ultrastructural properties of the optic nerve. Progressive axonal degeneration in ON resulted in a 23% reduction of parallel (axial) diffusivity with no alteration of perpendicular diffusivity. Axial diffusion changes had a strong correlation with total axolemmal cross-sectional area in a prechiasmal portion of optic nerve.⁸⁴

MRI uses the breakdown in BBB and subsequent leakage of conventional gadolinium to detect lesions. The contrast enhancement reflects BBB breakdown with leakage of paramagnetic particles, not active inflammation, and the two may not always match. MS may encompass subtle to significant BBB breakdown. Subtle breakdown may not be identified with conventional gadolinium. Bis-5-hydroxytryptamidediethylenetriamine-pentaacetate gadolinium [bis-5HT-DTPA (Gd)] is the myeloperoxidase (MPO)-activated paramagnetic sensor. MPO-targeted MRI facilitates earlier detection of smaller demyelinating lesions with increased precision.⁸⁵

Functional Magnetic Resonance Imaging. Functional magnetic resonance imaging (fMRI) is an advanced imaging technique that measures the hemodynamic response in brain and spinal cord. Defects in cortical plasticity in motor, cognitive, and visual systems have been found with fMRI,⁸⁶ facilitating studies of reorganization in the brain associated with the disease process. Blood oxygen level dependence (BOLD) acts as an MRI contrast. Hemoglobin is diamagnetic when oxygenated and paramagnetic when deoxygenated.⁸⁶ High BOLD intensities are noted with oxygenated hemoglobin. Neurons do not have internal reserves of glucose and oxygen, and increased neuronal activity requires increased supplies of the same.⁸⁷ As a result, surplus oxyhemoglobin is observed in local veins of the involved area of the brain. This causes a change in the local ratio of oxygenated and deoxygenated hemoglobin, which acts as a marker of BOLD for MRI. The BOLD signals are quantified using rapid volumetric acquisition of images with contrast weighted by T_1 or T_2 .⁸⁸

Magnetic Transfer Imaging. Magnetic transfer imaging (MTI) provides enhanced contrast imaging over MRI. MTI is sensitive to the exchange of magnetization between immobile protons bound to a macromolecular matrix and free water protons.^{89,90} It is difficult to decipher the temporal evolution of MS lesions with T_1 - and T_2 -weighted MRI; hence, some lesions may remain undetected. Magnetization transfer ratio precisely delineates normal-appearing white matter in MS patients from that of healthy persons. MTI is specific for demyelination and degeneration, which is confirmed on histopathology.^{91,92} However, MTI is a semiquantitative technique because it is dependent on the size of the macromolecules and the exchange among the mobile and bound proton pool, thus reducing its specificity for myelin characterization.⁹³ In combination with DWI MRI, MTI is additive and improves the overall specificity for white matter imaging.⁹⁴ Ultrafast MRI methods allow measurement of T_1 and T_2 relaxation times (relaxometry) in multiple brain slices. Ultrasmall superparamagnetic iron oxide (USPIO) particles are novel magnetic nanoparticles whose small size and surface characteristics are favorable for imaging. They aid in the detection of occult BBB alterations and in visualizing subtle macrophage infiltration in active inflammatory plaques. Thus, USPIO is a unique contrast medium with high sensitivity for neuroinflammatory processes.⁹⁵ The sensitivity of these nanoparticles to detect neu-

roinflammatory and demyelinating lesions when combined with MTI is significantly higher than that of DTPA.

Functional Molecular Imaging. Functional molecular imaging (FMI) is a novel imaging approach that provides accurate quantitative and qualitative assessments of MS. The image-guided functional interventions facilitate enhanced understanding of the pathogenesis of MS and are conducive for development and monitoring new treatment modalities. FMI can be performed with MRI and with optical and other imaging techniques.^{87,96} FMI has a great potential for future innovation and application in the field of MS and will open new horizons in the diagnosis and follow-up of MS management.^{86,97}

Nuclear Magnetic Resonance Spectroscopy. Nuclear magnetic resonance (NMR) spectroscopy investigates tissue bioenergetics. On excitation, complex chemical structures generate multiple signals at different frequencies that are deciphered into an NMR spectrum by Fourier transformation. The information from the NMR spectrum can be analyzed to determine the chemical structure of a sample noninvasively and nondestructively. NMR spectroscopy enables temporal monitoring of phenotypic changes in individual animals over a period without the need to euthanize them. NMR spectroscopy is similar to radioisotope imaging but does not involve radioisotopes. ³¹P-NMR of the brain has been used to analyze tissue phospholipids noninvasively and has the potential to be used for MS research.⁹⁸

Photon Emission Tomography. Photon emission tomography (PET) is a three-dimensional imaging modality used to examine metabolic processes in the body. The tracer for PET is a biologically active molecule, 2-[¹⁸F] fluoro-2-deoxy-D-glucose ([¹⁸F] FDG), an analog of glucose. [¹⁸F] FDG PET is an in vivo noninvasive modality efficient in detecting upregulated metabolism in the form of enhanced glycolysis, commonly observed with tumors. Increased glycolysis has also been observed in immune cell infiltrates.⁹⁹ [¹⁸F] FDG PET in conjunction with computed tomography (CT) enables serial quantification of increased glycolysis and inflammatory changes in MS. PET imaging of CNS changes correlates with disease onset. PET can also identify altered glycolysis in response to immunosuppressive therapy. Simultaneous acquisition of metabolic PET images with high-resolution CT (50–200 μ m) facilitates better localization of anatomic sites of increased glycolysis with the associated inflammation. Coregistration of PET and CT images in spontaneous models of EAE can aid in better understanding of the disease process.⁹⁶ Increased uptake of radiolabeled iNOS (inducible nitric oxide synthase) inhibitor in EAE mouse models implicates the potential of PET for quantifying MS in humans.¹⁰⁰

Activated glial cells express peripheral benzodiazepine receptors (PBRs)¹⁰¹ and are involved in MS immune responses. PET with [¹¹C]-PK11195 showed ligand uptake at PBRs that were present at sites of active lesions, corroborating MRI results. Increased PBR density as an index of the CNS inflammatory response can also be assessed with the radiolabeled ligand ¹²³I-CLINDE for PET.¹⁰² During disease progression, an increase in normal-appearing white matter uptake was observed, implicating active disease. Thus, with the help of biological markers, PET can identify cells (microglia) participating in the disease, something that is not possible with MRI.^{101,103} Similar results have been observed in mice manifesting EAE and in patients with MS.^{101,103} A synthesized fluorescent molecule, 1,4-bis (p-aminostyryl)-2-methoxy benzene (BMB), selectively binds to myelin, facilitating the identification and quantification of demyelinating lesions in EAE. Differential binding of BMB segregates remyelinated areas in shadow plaques arising from either demyelinated lesions or normal-appearing white matter. The ¹¹C-radiolabeled BMB can be used in both in vivo and ex vivo PET to image CNS myelin in mice. The ¹¹C-

radiolabeled BMB has been used ex vivo in the human brain and has potential for in vivo use in humans.⁵⁴

Optical Imaging. Light can be used to facilitate understanding of the function and structure of tissues. A variety of optical imaging methods in addition to OCT—near infrared spectroscopy (NIRS), infrared spectroscopy, fluorescence and bioluminescence imaging—are available.⁹⁷

Near Infrared Spectroscopy. NIRS, or laser optical tomography, is a spectroscopic method that uses the near infrared region of the electromagnetic spectrum (800–2500 nm). NIRS is a noninvasive method wherein two wavelengths are used to measure the difference in oxygen content of the hemoglobin in blood or tissues. NIRS can be considered a partial replacement for fMRI and has been safely used in infants because of the absence of radiation exposure and its portable nature.⁵⁵

Fluorescence Optical Imaging. Hemoglobin and cytochrome, indocyanine green dye, and NAD/NADH exhibit fluorescence properties. Each plays a crucial role in metabolic processes at the cellular level. Fluorescence optical imaging indirectly measures these processes. Although these fluorescent techniques have been restricted to ex vivo imaging, evaluation in small animals is possible noninvasively using minimally invasive endoscopic fiber optic systems.⁹⁷

Bioluminescence Imaging. Bioluminescence imaging (BLI) is a noninvasive in vivo molecular and cellular level optical imaging modality. BLI has high sensitivity and low background noise. EAE in transgenic mice expressing injury-responsive luciferase reporter in astrocytes (GFAP-luc) can be evaluated noninvasively with BLI.¹⁰⁴ BLI of the brain and spinal cord strongly correlates with the severity of clinical disease and the number of pathologic changes in EAE.¹⁰⁴ When performed early, BLI also predicts the severity of disease. BLI can potentially be used for monitoring neuroinflammation in EAE.^{105,106}

In summary, transgenic mouse models present with spontaneous ocular and neurologic disease and aid our knowledge of various aspects of MS pathogenesis and treatment. Transgenic mouse models facilitate understanding of the differences in antigenicity, rates of progression and disease severity, involvement of any environmental factors, and development of ON. Recovery from ON because of interventional strategies and comparison of MS kinetics with ON kinetics will also be important factors to study in these mouse models. Overall, these models will provide important insight into human MS. Advanced noninvasive investigative techniques facilitate the diagnosis and monitoring of disease processes. fMRI is a promising and novel modality with great scope for future application. Combinations of multiple diagnostic techniques have provided new insights into the structural and molecular changes occurring in the nervous system during MS progression.

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