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Experimental Autoimmune Encephalomyelitis

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1. Introduction

Experimental autoimmune encephalomyelitis (EAE) is an animal model of multiple sclerosis (MS). MS is also named encephalomyelitis disseminata (ED). EAE has been also called experimental allergic encephalomyelitis, but the word `allergic` is more and more replaced by the word `autoimmune`. This has to do with the deeper understanding of the disease biology that has been gathered over the last years. MS is a chronic autoimmune disease of the central nervous system (CNS) that leads to inflammation, demyelination and axonal loss. The CNS lesions cause neurological deficits.

In the beginning the disease course of MS is in most cases relapsing-remitting but changes after several years into a secondary chronic progressive disease course (Table 1). More rarely, there are also primary progressive disease courses. The diagnosis of MS is often preceded by a single demyelinating event for example the appearance of Optic Neuritis (ON). There are indications that MS is caused by the activation of autoreactive CNS-specific T cells and possibly antibodies that lead to subsequent activation of additional immune cascades and lesion formation (Fig. 1). The relapsing phase of MS is thought to be predominately mediated by the adaptive phase of the immune response, while progressive forms are more driven by innate immune mechanisms (Bhat and Steinman, 2009; Weiner, 2009).

The EAE models that are used in the laboratory for assessment of immunological, neurobiological and therapeutic studies are all induced models or genetically modified models. There is no naturally occurring spontaneous EAE model that is accepted as a valuable laboratory model for MS. EAE can be induced in various animal strains and species. Most used species are mice and rats. The reason for this is the size, the availability of inbred strains and the possibility for genetic modification as well as the immense number of tools to characterize rodents. In addition certain monkey species like marmosets are used for specific questions that cannot be easily assessed in rodents (Hart et al., 2011).

What has become clear over the last years is the fact that EAE is no perfect model for all aspects of MS. Rather various different models represent facets of MS. Some models are more suited for immunological analyses and this can be further divided into adaptive and innate immunity related aspects as well as cellular aspects, like the analysis of influences of T and B cells on disease precipitation and maintenance. There are models that are more suited for analysis of certain neurobiological aspects of the disease, like axonal and neuronal pathology and detailed lesion characterization.

Course	Characteristics	Estimated Prevalence
Relapsing MS (RR-MS, rSP-MS, PR-MS)		
RR-MS (Relapsing-remitting MS)	• Presence of relapses and remissions • Disability due to residual symptoms	22 %
rSP-MS (SP-MS with superimposed relapses)	• Presence of relapses • Progressive form, steadily increasing disability	3.5 %
PR-MS (Progressive relapsing MS)	• Presence of relapses • Progressive from onset, steadily increasing disability	1.5 %
Progressive MS without relapses (nrSP-MS, PP-MS)		
nrSP-MS (SP-MS without superimposed relapses)	• No relapses • Progressive form, steadily increasing disability	60 %
PP-MS (Primary progressive MS)	• No relapses • Progressive from onset, steadily increasing disability	13 %

Table 1. Different disease courses of MS. Relapsing disease courses of MS are mainly driven by the adaptive immune response (T and B cells) while progressive disease is thought to be predominantly mediated by innate immunity. In most cases, relapsing disease changes over the course of MS to secondary progressive disease. As outlined in Table 10, specific EAE models can be used to mimic the aspects of different types and variants (Devic´s, ADEM) of MS.

MS variants can be modeled in rodents. For example Acute Disseminated Encephalomyelitis (ADEM), an acute inflammatory reactive disease of the CNS, and Neuromyelitis Optica (Devic`s disease) can be modeled in certain rodent species as outlined further below. The researcher who wants to use EAE as a model should be aware of all the various types of EAE and should be careful in selecting the correct species, strain and immunization protocol, since depending on these factors, the outcome and interpretability of the research will differ. The selection of the specific model might differ strongly between an immunologist who wants to assess basic principles of organ specific autoimmunity in contrast to the MS researcher who wants to analyze specific disease aspects or a new therapeutic approach in preclinical pharmacology studies.

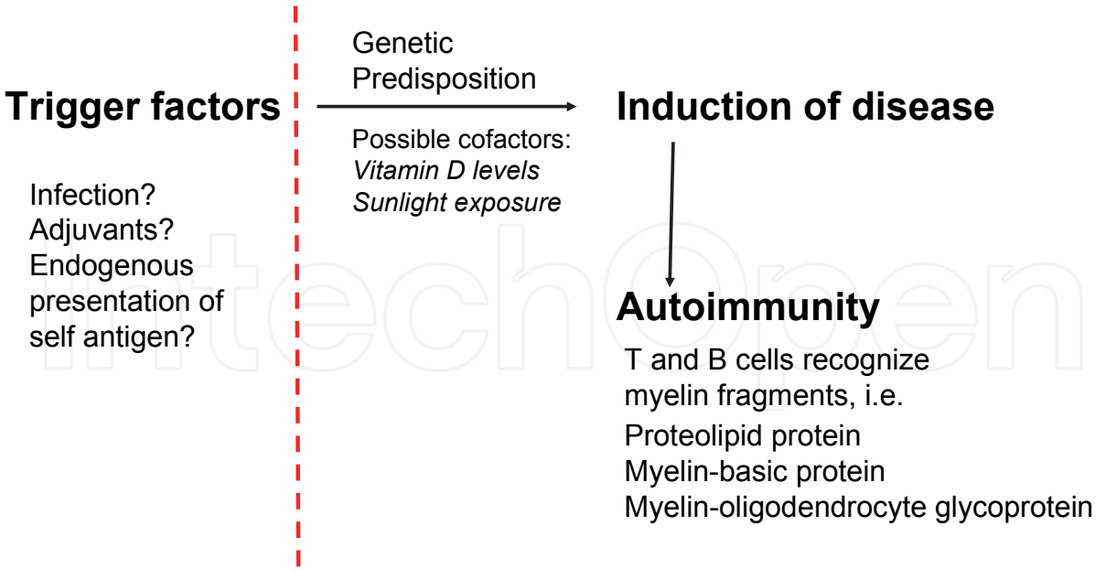

Fig. 1. Possible cascade for induction of MS. So far it is not clear what induces MS. Trigger factors are possibly infections, adjuvants or the endogenous presentation of self antigens (Fissolo et al., 2009). On the ground of a genetic predisposition with the possible cofactors vitamin D and sunlight exposure the disease is induced and autoimmunity is maintained by aberrant immune cascades that will finally lead to myelin/oligodendrocyte pathology as well as axonal and neuronal loss.

2. EAE models

Nowadays widely used EAE models in rats are the monophasic EAE in LEW rats that is induced with Myelin Basic Protein (MBP) or MBP peptides and Complete Freund´s Adjuvant (CFA) (Table 2). CFA consists of the mineral oil Incomplete Freund´s Adjuvant (IFA) mixed with heat killed extract from Mycobacterium Tuberculosis (MT). As prototype chronic EAE model in rats, EAE induced in DA rats with whole myelin extract in IFA or the extracellular domain of Myelin Oligodendrocyte Glycoprotein (MOG) in CFA or IFA have high value for studies regarding MS disease biology and therapeutic interventions. Whole myelin extract for this model is typically obtained from autologous spinal cord of DA rats. Interestingly DA rats develop EAE with the myelin extract/MOG with IFA alone.

In mice the most used model is the chronic model in C57BL/6 mice induced with the extracellular domain of MOG or MOG peptide 35-55 and CFA as well as Pertussis Toxin (PT) as adjuvant (Table 2). Other valuable models are the chronic relapsing EAE model induced with PLP or PLP peptide 139-151 in SJL mice with CFA and PT as ajduvants, the chronic relapsing EAE model induced in Biozzi mice with the extracellular domain of MOG or MOG peptide 92-106 in CFA and PT, the chronic progressive EAE induced in autoimmunity prone NOD mice with MOG peptide 35-55 and the monophasic EAE induced with MBP peptide Ac1-11 (or MBP peptide Ac1-9) with CFA and PT in PL/J mice.

There are relapsing progressive EAE models that can be induced with Theiler`s Murine Encephalitis Virus (TMEV) (Table 2). Interestingly in the TMEV model, antigen spreading to the classical myelin antigens can be observed during the disease course. Genetically

Species	Strain	Induction	Disease type	Reference
Rat	LEW	MBP or MBP 68-88 peptide in CFA	Acute monophasic	(Mannie et al., 1985; McFarlin et al., 1975)
Rat	DA	Spinal cord in IFA or MOG 1-125 in CFA or IFA	Relapsing/progressive	(Lorentzen et al., 1995; Weissert et al., 1998b)
Mouse	PL/J	MBP, MBP Ac1-11 (or Ac1-9) in CFA + PT	Acute monophasic	(Zamvil et al., 1986)
Mouse	SJL	PLP protein or PLP 139-151 peptide in CFA + PT	Chronic relapsing	(Kuchroo et al., 1991)
Mouse	C57BL/6	MOG protein or MOG 35-55 peptide in CFA + PT	Progressive or acute	(Mendel et al., 1995; Oliver et al., 2003)
Mouse	Biozzi	MOG protein or MOG 92-106 peptide in CFA + PT	Chronic relapsing	(Baker et al., 1990)
Mouse	NOD	MOG 35-55 peptide in CFA + PT	Chronic progressive	(Maron et al., 1999)
Mouse	SJL	Theiler's murine encephalitis virus	Relapsing progressive	(Olson et al., 2001)
Mouse	B10.PL-H2^{u}	TCR^{MBP} transgenic	Spontaneous acute	(Goverman et al., 1993)
Mouse	C57BL/6	TCR^{MOG} transgenic	Optic neuritis	(Bettelli et al., 2003)
Mouse	C57BL/6	TCR^{MOG}XIgH^{MOG} transgenic	Spontaneous Optico-spinal disease	(Bettelli et al., 2006; Krishnamoorthy et al., 2006)

Table 2. Presently most used EAE models for laboratory research. The list only provides a part of all available EAE models. CFA = Complete Freund`s Adjuvant; IFA = Incomplete Freund`s Adjuvant; PT = Pertussis Toxin.

modified models include the spontaneous EAE in C57BL/6 mice that have a T Cell Receptor (TCR) specific for MBP peptide 1-9. Another genetically modified C57BL/6 mouse with a MOG specific TCR and a MOG specific B Cell Receptor (BCR) or surface Immunoglobulin (sIg) develop spontaneous EAE and can be very useful for assessment of specific scientific questions.

There are rodent EAE models that can be induced by passive transfer of T cells. These models are not subject of this chapter and are therefore not outlined in detail. T cell transfer models have been very suitable for immunological investigations mainly in regard to the dissection of the role of T cells in organ-specific autoimmunity (Krishnamoorthy and Wekerle, 2009).

3. Establishment of Myelin-Oligodendrocyte-Glycoprotein (MOG)- induced EAE in rats

The rat is a good species to perform EAE studies due to its larger size as compared to mice, the availability of many well characterized inbred strains, its strong standing for pharmacological studies and its great value for behavioral outreads. In addition novel ways for genetic modification are increasingly becoming available. The rat Major Histocompatibility Complex (MHC) is called RT1. The classical MHC I molecule is called RT1.A, the MHC II molecules are named RT1.B and RT1.D that are equivalent to HLA-DQ (RT1.B) and HLA-DR (RT1.D) (Fig. 2, Table 3).

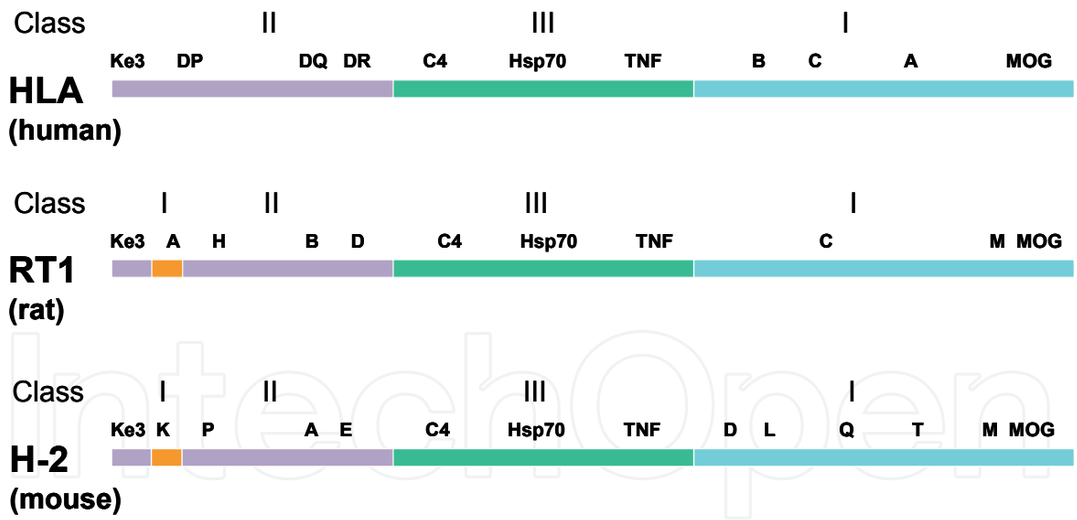

Fig. 2. Organization of MHC in different species (human [HLA], rat [RT1], mice [H2]). There are considerable differences in the organization between HLA, H2 and RT1 (Günther and Walter, 2001).

EAE induced with whole myelin extracts or MBP in LEW ($RT1^l$) rats has been used over many years (Table 2). This model is monophasic and was the prototype EAE model in the past. Much understanding regarding MS pathogenesis, immunology and neurobiology has been obtained over the years. The model is very useful to study basic immunological principles regarding T cell migration to the CNS and T cell related immunity of MS. This

model is often used for pharmacological investigations in EAE. Beside with whole myelin extracts the model can be induced with MBP protein (various isoforms) or peptides from the main encephalitogenic regions MBP 89-101 and MBP 68-88. Interestingly MBP peptide 89-101 binds to RT1.D^{l} (HLA-DR-like), while MBP 68-88 binds to RT1.B^{l} (HLA-DQ-like) (de Graaf et al., 1999; Weissert et al., 1998a). There are species dependent effects of MBP on disease development. We could demonstrate that the MBP peptide 63-88 derived from Guinea Pig (GP) as compared to the MBP peptide 63-88 derived from rat is much more encephalitogenic. Immunization with MBP_{GP}63-88 results in the agglomeration of T cells in the CNS that express up to 30% the TCRBV chain 8.2 (TCRBV8S2), while after immunization with MBP_{RAT}63-88 there is not such a strong overusage of such T cells in the CNS (Weissert et al., 1998a). Based on this fact it can also be understood that therapeutic manipulation in the context of immunization with MBP_{GP}63-88 is more demanding as compared to MBP_{RAT}63-88. We equally demonstrated that MBP derived peptides of different species can act as superagonists, agonists or antagonists depending on the expressed MHC II haplotype (de Graaf et al., 2005).

Strain*		***Class I***	***Class II***		***Class III***	***Class I***
	Haplotyp	RT1.A	RT1.B	RT1.D		RT1.C
DA	av1	a	a	a	av1	av1
COP	av1	a	a	a	av1	av1
ACI	av1	a	a	a	av1	av1
PVG-RT1^{a} (DA)	av1	a	a	a	av1	av1
LEW.1AV1 (DA)	av1	a	a	a	av1	av1
LEW	l	l	l	l	l	l
LEW.1N (BN)	n	n	n	n	n	n
LEW.1A (AVN)	a	a	a	a	a	a
LEW.1W (WP)	u	u	u	u	u	u
LEW.1AR1	r2	a	u	u	u	u
LEW.1AR2	r3	a	a	a	u	u
LEW.1WR1	r4	u	u	u	a	a
LEW.1WR2	r6	u	a	a	a	a
BN	n	n	n	n	n	n

Table 3. RT1 haplotypes of inbred rat strains (Weissert et al., 1998b). RT1.B is the rat equivalent to HLA-DQ and RT1.D to HLA-DR. *Donor strain in brackets

It was demonstrated that as compared to LEW rats, DA rats develop in a much higher incidence chronic disease after immunization with autologous whole spinal cord

homogenates (Lorentzen et al., 1995). In DA rats immunization of autologous whole spinal cord in IFA is sufficient to induce this type of chronic disease. Interestingly it was found that DA rats develop antibodies against the MOG extracellular domain MOG 1-125. Based on this fact we were interested to assess the encephalitogenic potential of MOG 1-125 in DA rats and other inbred rat strains. We found that immunization with MOG 1-125 in CFA resulted in relapsing-remitting disease (Weissert et al., 1998b). Also the immunization of MOG 1-125 in IFA causes to this type of disease. Interestingly for the first time it was observed in rodents that the rats developed ON in addition to classical EAE symptoms (Storch et al., 1998). In addition, specific immunization protocols allowed the selective induction of ON. ON is a typical aspect of MS. The CNS lesions in this model have much greater similarity to MS as compared to the lesions in LEW rats immunized with MBP or MBP peptides. Widespread demyelination was present and the analysis of lesions resulted in the important insight that beside T cells also antibodies contribute to lesion formation in this model. In contrast to MBP, MOG is expressed on the exterior part of the myelin sheath and the Ig-like domain can be recognized by antibodies. Binding of antibodies to MOG can activate a number of immune mechanisms like the activation of macrophages and complement deposition that can result in increased tissue damage as compared to purely T cell mediated pathology.

Based on the findings in DA rats and immunization with MOG we were wondering about the encephalitogenic potential in rat strains with different MHC haplotypes. This question appeared important to us, since it has been demonstrated for a long time that the MHC II region has a strong genetic influence on MS (Sawcer et al., 2011). Therefore we used a large number of inbred congenic LEW rat strains that express a wide variety of MHC haplotypes. In addition we used various inbred rat strains (Table 3) (Weissert et al., 1998b).

We observed that certain rat strains developed relapsing-remitting or chronic progressive disease, others hyperacute progressive disease, some slow progressive disease with predominance of cortical pathology and others were protected (Table 4). Also the selection of the adjuvant had additional effects. While LEW.1N ($RT1^n$) immunized with MOG 1-125 in CFA developed hyperacute progressive disease that often lead to death due to pontine lesions, immunization with MOG 1-125 in IFA resulted in chronic progressive disease with axonal pathology that has great similarity to MS (Kornek et al., 2000).

We also observed that LEW ($RT1^l$) rats immunized with MOG 1-125 did not develop EAE (Weissert et al., 1998b). This is in contrast to immunization protocols with MBP or MBP peptides which lead to EAE as outlined above (Weissert et al., 1998a). Based on the fact that LEW.1AV1 rats that carry the $RT1^{av1}$ haplotype derived from the DA rat develop EAE after immunization with MOG 1-125, we concluded that the MHC haplotype is operating in the context of the myelin antigen used for immunization (Weissert et al., 1998b). This was further supported by the observation that LEW.1N ($RT1^n$) rats and BN ($RT1^n$) rats do not develop disease after immunization with MBP, but strong disease after immunization with MOG 1-125. This finding appears of some importance since it might explain why MHC haplotypes might differ as susceptibility loci in different parts of the world: depending on the environmental challenges that differ in different regions of the world, the subsequent induction of immunity against certain myelin components might differ. For example in Western Europe and North America the main susceptibility HLA allele is HLA-DR2b, while in Sardinia it is HLA-DR4 (Marrosu et al., 1997).

We described rat strains that carried MHC haplotypes that allow disease development after immunization with MOG 1-125 but which were protected by non-MHC genomes. We defined that ACI rats as well as MHC congenic PVG-RT1^{av1} that both carry the RT1^{av1} haplotpye were protected from EAE (Weissert et al., 1998b). Subsequent large genetic screens allowed dissection of some of the susceptibility genes (Swanberg et al., 2005).

Species	Strain	Induction	Disease type	Reference
Rat	LEW.1A	MOG1-125 in CFA	Chronic progressive	(Weissert et al., 1998b)
Rat	LEW.1AV1	MOG1-125 in CFA	Relapsing-remitting	(Weissert et al., 1998b)
Rat	LEW.1AV1	MOG1-125 in IFA	Relapsing-remitting	(Kornek et al., 2000)
Rat	LEW.1N	MOG1-125 in CFA	Hyperacute progressive	(Weissert et al., 1998b)
Rat	LEW.1N	MOG1-125 in IFA	Chronic progressive	(Kornek et al., 2000)
Rat	LEW.1W	MOG1-125 in CFA	Slow progressive	(Weissert et al., 1998b)
Rat	LEW.1AR1	MOG1-125 in CFA	Slow progressive, cortical pathology	(Storch et al., 2006; Weissert et al., 1998b)
Rat	DA	MOG1-125 in CFA	Relapsing-remitting, optic neuritis	(Storch et al., 1998; Weissert et al., 1998b)
Rat	BN	MOG1-125 in CFA	Neuromyelitis optica	(Meyer et al., 2001; Weissert et al., 1998b)

Table 4. EAE models induced with MOG 1-125 in rats. Different disease courses and types of CNS pathology can be induced that are dependent on the MHC haplotype.

We found one LEW rat strain with a specific MHC haplotype that predominantly develops cortical lesions, LEW.1AR1 (RT1^{r2}) rats (Storch et al., 2006; Weissert et al., 1998b). Recently cortical lesions have been acknowledged as a primary cause of disability in MS. Cortical lesions can be also induced in LEW rats with a subencephalitogenic immunization with myelin components and subsequent local intrathecal application of Tumor Necrosis Factor alpha (TNFα) (Merkler et al., 2006). Marmosets immunized with MOG 1-125 can develop cortical lesions as well (Pomeroy et al., 2005). By now it has also been observed that certain mouse strains can develop cortical pathology (Mangiardi et al., 2011). In our observation LEW.1AR1 rats immunized with MOG 1-125 in CFA represent the most suitable and reproducible model for cortical pathology of MS and possible therapeutic manipulation.

In a next step we were interested to define the encephalitogenic stretches within the extracellular domain of MOG 1-125 (Tables 5, 6). In order to do this we used 18 amino acid

long overlapping peptides of the MOG 1-125 rat sequence (Weissert et al., 2001). We performed this study in many different inbred and inbred MHC congenic rat strains. Interestingly we found that only MOG 91-108 in CFA induced disease in rat strains with the $RT1^{av1}$ or $RT1^{n}$ haplotype. Rat strains with the $RT1^{av1}$ haplotype are DA rats and LEW.1AV1 rats and rats with the $RT1^{n}$ haplotype LEW.1N and BN rats. We found that MOG 91-108 bound well to purified $RT1.B^{a}$ molecules and to $RT1.D^{n}$ molecules. Immunization with MOG 91-108 induced a T and B cell response. In LEW.1N rats this T cell response was difficult to measure and we concluded early that other factors than classical Th1 mediated cytokines might be operative contributing to disease precipitation (Weissert et al., 2001).

In most instances the immunodominant peptides did not correspond to the peptides that were capable of inducing EAE. We concluded that the immune response to the immunodominant peptides might be also a signature of a regulatory T cell response (we called it `modulatory).

RT1	RT1.A	RT1.B/D	RT1.C	Strain	Disease inducing peptides	Peptides that raise an immuno-dominant T cell response
l	l	l	l	LEW	none	MOG 37-54 and MOG 43-60
u	u	u	u	LEW.1W	none	None
r4	u	u	a	LEW.1WR1		
r2	a	u	u	LEW.1AR1		
a	a	a	a	LEW.1A	MOG 91-108, MOG 96-104	MOG 73-90, MOG 91-108
r3	a	a	u	LEW.1AR2		
r6	u	a	a	LEW.1WR2		
av1	a	a	av1	LEW.1AV1		
av1	a	a	av1	DA		
av1	a	a	av1	COP		
av1	a	a	av1	PVT-$RT1^{av1}$	none	none
av1	a	a	av1	ACI		
n	n	n	n	LEW.1N	MOG 91-108, MOG 98-106	MOG 19-36
n	n	n	n	BN		

Table 5. MOG peptides that induce disease and immune responses. Most MOG stretches that induce strong immune responses in rats do not induce EAE (Weissert et al., 2001).

Species	Strain	Induction	Disease type	Reference
Rat	DA	MOG91-108 in CFA	Monophasic or chronic	(Weissert et al., 2001)
Rat	LEW.1AV1	MOG91-108 in CFA	Monophasic or chronic	(Weissert et al., 2001)
Rat	LEW.1N	MOG91-108 in CFA	Monophasic or chronic	(Weissert et al., 2001)
Rat	LEW.1AV1	MOG96-104 in CFA	Monophasic or chronic	(de Graaf et al., 2008)
Rat	LEW.1N	MOG98-106 in CFA	Monophasic or chronic	(de Graaf et al., 2008)

Table 6. EAE models induced with MOG peptides. Based on detailed immunological analysis the region MOG 91-108 was defined as the encepathalitogenic region in different rat strains. Further dissection allowed the narrowing of the disease inducing MOG stretches to nine amino acid long peptides.

We observed that complement depletion does lead to protection from disease, underscoring the influence of antibodies to MOG. Crystallographic studies have indicated that the region MOG 91-108 is accessible to antibodies (Breithaupt et al., 2008). In addition, we demonstrated by spectroscopic TCR analysis that depending on the expressed RT1 haplotype, the predominance of certain TCRBV chains was the same in rat strains with different non-MHC genomes underscoring the strong influence of the MHC II haplotype on TCRBV usage in the MOG model (de Graaf et al., 2008).

For the rat EAE models, we measured the binding strength of the encephalitogenic peptides by comptetitive binding assays (Table 7). We could show that the peptides that induce EAE are binding well to the MHC II molecules that present the peptides to T cells (de Graaf et al., 2008; de Graaf et al., 1999; Weissert et al., 2001; Weissert et al., 1998a). We dissected the binding qualities to purified RT1.B and RT1.D molecules and the T and B cell response to MOG 91-108 in LEW.1N and LEW.1AV1 rats. We found that the peptides that bound strongest, induced EAE. This were the peptides MOG 96-104 in LEW.1AV1 rats binding to $RT1.B^a$ and MOG 98-106 in LEW.1N rats binding to $RT1.D^n$. With increasing shortening of the peptides, the evolving disease was partly reduced, indicating that possibly there was a reduction in the activated encephalitogenic T cell repertoire (de Graaf et al., 2008).

That peptides which induce EAE bind strongly to the restricting MHC II molecule is in agreement with measured binding strengths in humanized mouse models, but contrasts findings in the PL/J mouse in which the encephalitogenic peptide MBP Ac1-9 binds only very weakly to the $I\text{-}A^u$ MHC II molecule. While in the first case the persistence of antigen might lead to breaking of tolerance, in the latter case, the escape from tolerance might be of primary importance in disease establishment.

Haplotype (Strain)	Encephalitogen	Restriction	IC_{50} (μM)	Reference
$RT1^{l}$ (LEW)	MBP_{gp}72-85	B^{l}	2,5	(Weissert et al., 1998a)
	MBP 87-99	D^{l}	0,02	(de Graaf et al., 1999)
$RT1^{av1}$ (DA, LEW.1AV1)	MBP 87-99	B^{a}	0,06	(de Graaf et al., 1999)
	MOG 91-108, MOG 96-104	B^{a}	9,5	(de Graaf et al., 2008; Weissert et al., 2001)
$RT1^{n}$ (BN, LEW.1N)	MOG 91-108, MOG 98-106	D^{n}	0,03	(de Graaf et al., 2008; Weissert et al., 2001)
$H\text{-}2^{s}$ (SJL/J)	MBP 81-100	$I\text{-}A^{s}$	0,36	(Wall et al., 1992)
	PLP 100-119	$I\text{-}A^{s}$	1,24	(Greer et al., 1996)
	PLP 139-151	$I\text{-}A^{s}$	0,04	(Greer et al., 1996)
	PLP 178-191	$I\text{-}A^{s}$	0,74	(Greer et al., 1996)
$H\text{-}2^{u}$ (PL/J)	MBP Ac1-9	$I\text{-}A^{u}$	> 100	(Fairchild et al., 1993; Liu et al., 1995)
HLA-DR2 transgenic mice	MBP 84-102	DRB1*1501	0,004	(Madsen et al., 1999; Wucherpfennig et al., 1994)
HLA-DR4 transgenic mice	MOG 91-108	DRB1*0401	0,3	(Forsthuber et al., 2001)

Table 7. Binding of EAE inducing peptides to purified MHC molecules. Binding strengths were assessed with competitive binding assays and affinity purified MHC II molecules.

In summary we made a major step in establishing more suitable models to study MS disease biology and therapeutic interventions. In addition we were able to obtain a large quantity of insight into the immune regulation in the context of genetic factors in a complex autoimmune disease. We demonstrated the major influence of MHC II haplotypes on disease regulation, but for the first time also of MHC I. Recently also for MS influences of MHC I loci on susceptibility could be confirmed (Sawcer et al., 2011).

4. Ways of EAE induction with either MBP, PLP or MOG and choice of the relevant animal model/strain

Beside the selection of the right species and strain, the selection of the model antigen is of great importance for the outcome of the EAE studies. The sequences of the most used

stretches of myelin proteins for disease induction used in different species are listed in Table 8. The peptides should have a high degree of purity and it is advisable to prepare large batches that can be used over long term for experimentation. In the case of EAE induction with recombinant antigens, also larger scale preparation and usage of identical batches over longer time is recommendable, since there is the danger of batch to batch variation that can dramatically affect the outcome of experimentation.

In addition to species, strain and antigen, the adjuvant is of major importance for the success of the EAE induction and the outcome of the experimentation (Table 9). While in the rat models, the usage of IFA or CFA is sufficient, nearly all mouse models require the addition of PT in the immunization protocol and booster immunizations. The preparation of the antigen/adjuvant mixture requires much care and the presence of a homogenate emulsion is needed for successful immunizations.

In the past immunizations have often been performed in the foot pads of the rodents. Due to obvious ethical reasons, this procedure is not any more applied. In addition this procedure results in the swelling of the footpads affecting gait. This type of gait disturbance can blur EAE symptoms with the consequence of wrongly reported EAE scores. Nowadays, in rats the immunization is done as a single injection in the base of the tail, while in mice multiple injections in the flanks are used for the procedure. It is advisable to establish the best suited immunization protocol in the laboratory with care, after the selection of the model based on scientific rationales has been performed.

Myelin protein stretch	**Sequence**
MBP_{MOUSE} Ac1-9	Ac-ASQKRPSQR
PLP_{MOUSE} 139-151	HSLGKWLGHPDK
MOG_{MOUSE} 35-55	MEVGWYRSPFSRVVHLYRNGK
MBP_{RAT} 68-88	HYGSLPQKSQRTQDENPVVHF
MBP_{GP}68-88	HYGSLPQKSQRSQDENPVVHF
MBP_{RAT} 89-101	VHFFKNIVTPRTP
MOG_{RAT} 91-108	SDEGGYTCFFRDHSYQEE
MOG_{RAT} Ac96-104	Ac-YTCFFRDHS-NH2
MOG_{RAT}Ac98-106	Ac-CFFRDHSYQ-NH2

Table 8. Sequences of myelin peptides used for EAE induction in mice and rats

Species/ strain	Immunogen	Primary adjuvant	Secondary adjuvant	Injection site	Reference
Mouse					
PL/J	MBP Ac1-11	CFA	PT	Flanks	(Zamvil et al., 1986)
SJL	PLP 139-151	CFA	PT	Flanks	(Kuchroo et al., 1991)
Biozzi	MOG 1-125	CFA	PT	Flanks	(Baker et al., 1990)
C57Bl/6	MOG 35-55	CFA	PT	Flanks	(Oliver et al., 2003)
Rat					
LEW	MBP	CFA	None	Tail base	(Weissert et al., 2000)
LEW	MBP 68-88	CFA	None	Tail base	(Weissert et al., 1998b)
LEW	MBP 89-101	CFA	None	Tail base	(Weissert et al., 2000)
LEW	PLP	CFA	None	Tail base	(Zhao et al., 1994)
DA, LEW.1AV1, BN, LEW.1N, LEW1.AR1	MOG 1-125	CFA or IFA	None	Tail base	(Kornek et al., 2000; Storch et al., 2006; Weissert et al., 1998b)
DA, LEW.1AV1	MOG 91-108, MOG 96-104	CFA	None	Tail base	(de Graaf et al., 2008; Weissert et al., 2001)
LEW.1N or BN	MOG 91-108, MOG 98-106	CFA	None	Tail base	(de Graaf et al., 2008; Weissert et al., 2001)

Table 9. Immunization regimen for different EAE models and usage of adjuvants. CFA = Complete Freund`s Adjuvant; IFA = Incomplete Freund`s Adjuvant; PT = Pertussis Toxin.

5. Conclusions

It is well possible to induce different aspects of MS in rodent EAE models (Table 10). Some models are more suited for immunological analysis, while others better serve the neuroscience community. None of the models can model all aspects of MS. Based on the specific scientific question, the most suitable EAE model for a specific analysis should be selected based on the characteristics of the model. The selection of the best suited model will result in better results of the overall research project and will improve the interpretability of the results.

Course of MS	Type of EAE	Strain	Immunization
Relapsing forms of MS			
RR-MS	MOG-EAE	DA, LEW.1AV1	MOG 1-125
rSP-MS	MOG-EAE	DA	MOG 1-125
PR-MS	MOG-EAE	LEW.1N	MOG 1-125
Non-relapsing forms of MS			
nrSP-MS	MOG-EAE	LEW.1A	MOG 1-125
PP-MS	MOG-EAE	LEW.1W, LEW.1WR1	MOG 1-125
Specific pathologies or rare MS variants			
Predominance of cortical lesions	MOG-EAE	LEW.1AR1	MOG 1-125
Devic`s disease	MOG-EAE	BN	MOG 1-125
ADEM	MBP-EAE	LEW	MBP_{GP}68-88, MBP_{RAT}68-88

Table 10. Best suited rat model to investigate aspects of different MS types and MS variants

The MOG-EAE model in rats with the availability of various inbred and RT1 congenic strains provides a very good and well defined system for assessment of pertinent questions regarding MS disease biology and therapeutic interventions.

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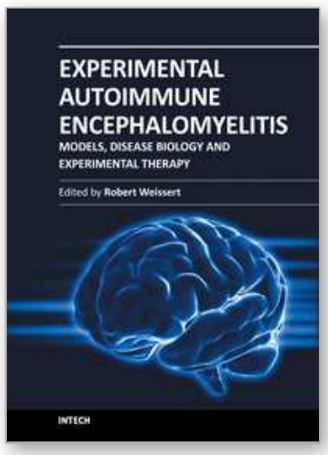

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Experimental Autoimmune Encephalomyelitis - Models, Disease Biology and Experimental Therapy is totally focused on the model of multiple sclerosis, experimental autoimmune encephalomyelitis (EAE). The book chapters give a very good and in depth overview about the currently existing and most used EAE models. In addition, chapters dealing with novel experimental therapeutic approaches demonstrate the usefulness of the EAE model for MS research. With an international perspective, this book features contributions from authors throughout the world, Australia, Germany, Japan, Spain, Taiwan, and USA. There is an impressive international Faculty that provides insight into current research themes. This further demonstrates the importance of EAE in research all over the world. The book will provide established researchers and students with novel insights and guidance for their research and will help to push the field forward.

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