Models of Multiple Sclerosis

Multiple sclerosis (MS) is a major disabling disease of the central nervous system (CNS), which has been described for over two hundred years, yet it is still enigmatic and inadequately controlled.[1] As the CNS cannot easily be sampled, to gain ideas about disease mechanisms, a number of models have been developed. These include: myelin mutants, chemically-induced lesions, viral and autoimmune models, all of which show some evidence of demyelination, a pathological hallmark of MS.[2] Myelin mutants, such as the taiep rat, *Shiverer* (myelin basic protein (MBP) mutant), *Rumpshaker* and *Jimpy* (proteolipid protein (PLP) mutants) mice, as well as gene knockout animals such the myelin associated glycoprotein (MAG) knockout show dysmyelination, altered neurotransmission and in some instances clinical disease, and have been used to study myelination. The delivery of oligodendrocyte-selective toxins such as cuprizone, which causes focal demyelination notably to the cerebellar peduncle, or direct injection of ethidium bromide or lysolecithin into the CNS produces demyelination. These are usually effectively repaired once macrophages clear the myelin debris and glial precursor cells repopulate the lesion and remyelinate. These models have largely been used to study mechanisms of de/remyelination, notably after transplantation of myelinating glial cells and are currently seldom used as pre-clinical drug screening tools for MS.

Viral Models of MS

A number of viruses, including Semliki Forest Virus and Theiler's Murine Encephalomyelitis Virus, have been found to induce disease by neurotrophic infection of the CNS, specifically oligodendrocytes. Whilst some viral strains may be cytopathic to the oligodendrocyte, in many instances virally-infected cells are attacked by T cell and humoral responses, leading to demyelinating disease.[2,3] TMEV has notably been used to demonstrate mechanisms by which autoimmunity may develop following a viral infection. This paradigm is consistent with the aetiology of MS, where viral molecular mimicry and determinant spread, where damage from infection may stimulate subsequent autoimmunity, may contribute to the generation of an autoaggressive immune response.[3]

Autoimmune Models of MS

Experimental allergic encephalomyelitis (EAE) has received the most attention as a model of MS and is routinely used in testing therapeutic strategies for MS (Figure 1). This disease exhibits many clinical and histological features of MS and is caused by the induction of autoimmunity to antigens that are either naturally (typically myelin antigens) or artificially (such as implanted mycobacteria or ovalbumin that, following peripheral sensitisation to these antigens, allows local targeted lesions to be developed) expressed in the CNS.[2-4] Following sensitisation to myelin antigens animals develop disease, typified by limb paralysis. This is associated with blood:brain barrier dysfunction, mononuclear cell infiltration into the CNS and conduction block resulting in impaired neurotransmission. This can occur in the absence of demyelination and highlights a misconception by many that clinical EAE is due to demyelination. In some models disease is also associated with significant axonal loss, which is the underlying cause of persistent disability.[2] EAE is polygenic and susceptibility and the clinical course can vary depending on the immunising antigen (such as MBP and PLP) and the strain/species of animal being investigated.[2,4] For example, ABH and SJL mice develop relapsing EAE to disease induced by whole myelin, whereas C57BL/6 mice are resistant.[2] However, the discovery that MOG, a minor myelin protein, can induce chronic paralytic EAE in the C57BL/6 mice has allowed the numerous gene-knockout mice bred on that background to be used to investigate EAE.[2] Therefore EAE is not a single model, but a number of models that have varying degrees of similarity to MS.[2] As such, a similar clinical phenotype may be achieved via different routes of genetic control[2] and likewise suggests that there is likely to be some heterogeneity in the pathways leading to disease in MS.

Spontaneous CNS autoimmunity

Rodent EAE studies have demonstrated that disease develops once sufficient T cells escape the control mechanisms that keep autoimmunity in check. Furthermore, by transgenically introducing myelin (MBP, PLP or MOG)-specific T cell receptors (TCR) into all T cells, then even the slightest trigger can lead to spontaneous CNS disease.[2,4] These animals have proved to be important tools in understanding autoimmunity and both $CD4^+$ and $CD8^+$ TCR transgenic models of EAE have been generated,[4] thus accommodating thoughts that there may be a $CD8^+$ T cell bias in some MS lesions.[5] More recently, transgenic mice expressing MS-associated major histocompatibility class II haplotypes and human derived myelin (MBP)-specific TCR with or without human CD4 have been shown to spontaneously develop EAE.[7] These humanised models have been suggested to be significant improvements over standard models.[7] However, such animals are usually produced on the C57BL/6 mouse background, because of the availability of embryonic stem cells required for transgenesis, and this strain typically develops EAE that rapidly shows a chronic paralytic course, due to the nerve loss that this strain quickly accumulates.[2,4] As such, it is more difficult to manipulate EAE compared to other strains.[2] Furthermore, the incidence and phenotype can be so variable in such humanised-TCR mouse models[7] that they do not offer advantage over existing standard models for purposes of routine drug screening, unless there is an a priori reason for testing agents that are specific for these human components. Nevertheless, humanising models, such that they can accept MS-patient derived cells may lead to new tools for the future.[7]

Is EAE a misleading tool for MS research?

MS appears to be a uniquely human condition and no other animal spontaneously develops a disease identical to MS. Furthermore, it must be recognised that immunisation of mammals, including humans, with CNS proteins does not induce MS, but acute disseminated encephalomyelitis. This was recognised over a century ago when rabies vaccine containing residual CNS material was injected into humans or more recently when encephalomyelitis developed following amyloid beta protein vaccination in Alzheimer's disease.[8] As such, EAE will always be an imperfect model, but nevertheless it has shaped the therapeutic approaches applied to MS for decades.[5,6,9,10] However, because of the many failures to clinically translate experimental findings in EAE into MS, opinions have been voiced that animal models are of limited value in the search for treatments in MS.[5,9] This opinion is not entertained by all,[6,10] but in addition to arguments made by critics of EAE, such as differences in the cellular and cytokines responses between some EAE models and MS,[5,9] the failure of EAE studies to detect viable treatments[5,9] may also relate to how the results of the studies are interpreted by the scientific and clinical fraternity.

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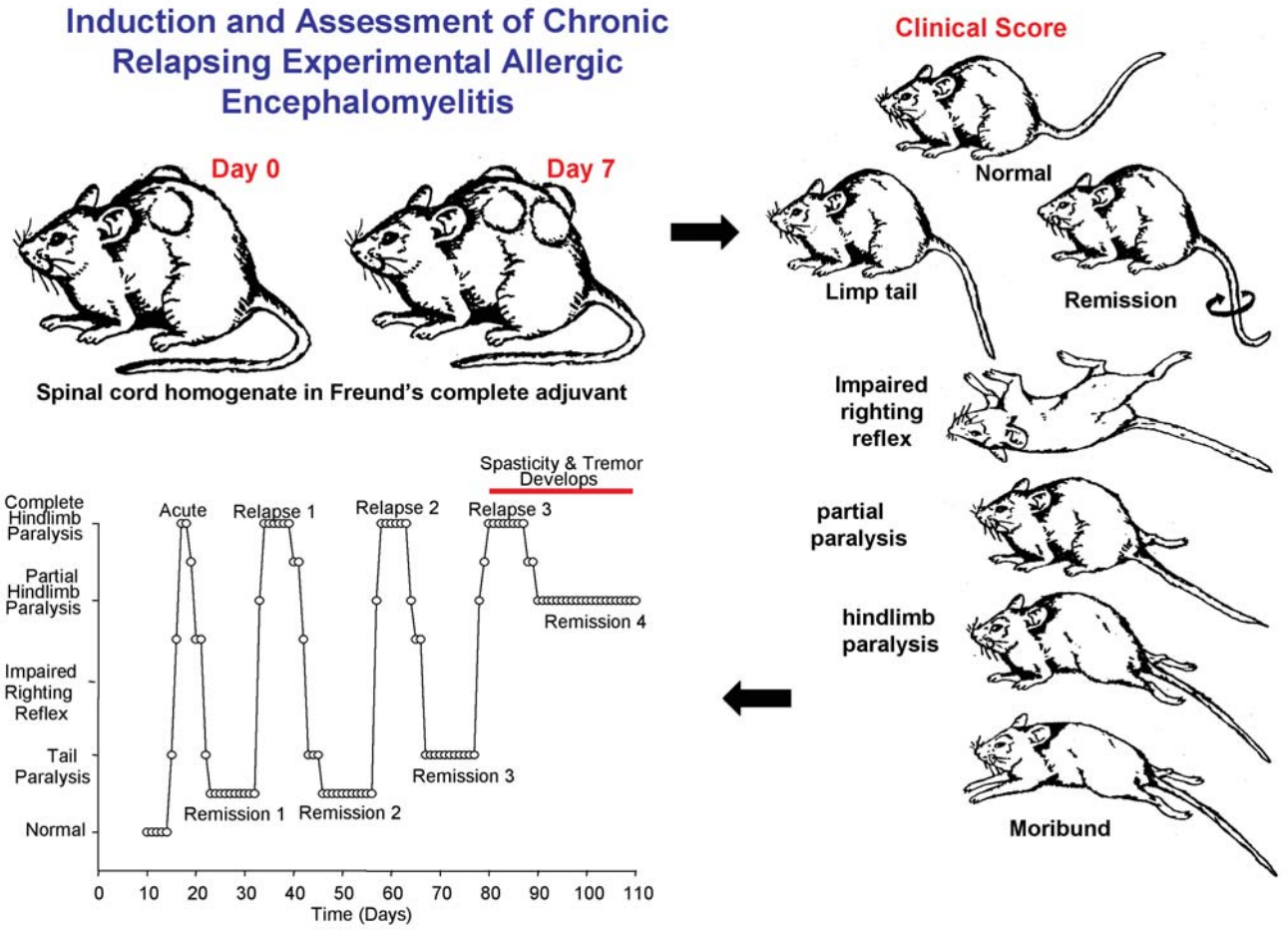

Figure 1: Induction and clinical course in an Experimental Allergic Encephalomyelitis Model.

EAE can be a leading tool for MS research

Disease in EAE is easy to detect and can relapse, unlike most other experimental autoimmune models, and is thus widely used. This is studied by many basic scientists who justify their research in terms of human disease, whilst they actually search for the unifying mechanism/theory for the development and control of autoimmunity. The EAEologist thus becomes preoccupied with the biology of a process and designs experiments as such, rather than concerning themselves with the biology of the disease, which by and large is complex. Therefore the experimental models and therapeutic agents may not have been used in a context where they are relevant to people with disease. It is relatively easy to stop an immune response from developing, but it is very much more difficult to switch off the immune response once it has been triggered.[11] This is because the immune response is designed to avoid the development of autoimmunity, but also to give us life-long protection from attack by infection, following previous activation. The vast majority of experiments in EAE are aimed at understanding the generation of the immune response. As such, prophylactic treatment, which probably has little relevance to treating the human condition, is the norm. Very few studies examine drug treatment once disease and demyelination is established in the CNS and the number of studies where treatment is initiated after only two attacks have occurred number very few. By contrast, many studies of immunological agents in MS have investigated their effect in late stage progressive disease, which may have thwarted them from showing any efficacy (see below). The successes of treatments of EAE, as a route to defining immune mechanisms, are published and lauded every month.[6] However, on closer inspection the results of some studies may show only a minor clinical improvement or a delay in disease course of just a few days. This is probably of marginal biological significance when viewed in terms of treating the heterogeneous human disease developing over years. Importantly, the doses of drugs given to animals may far exceed what one may be prepared to apply clinically to chronic disease in humans. Lower doses may be inactive but negative data seldom inspires editors to publish.

The T cell is instrumental to the development of EAE and thus most immunotherapies target the T cell in MS.[5] However, other factors besides T cell activity may contribute to pathology as already shown in EAE. B cell responses can shape the disease course once T cells have created the diseased environment, allowing entry of pathogenic/demyelinating or remyelination-inducing antibodies into the CNS.[12] In acute models, the first episode is often non-demyelinating and B cell responses may not have developed by the time this occurs. In contrast, marked demyelination occurs in EAE models, such as in strain 13 guinea pig and marmoset EAE, but this may take months to develop.[13,14] Likewise, macrophages/microglia have received relatively little attention as therapeutic targets in MS, yet they are probably instrumental in inflammatory pathogenic and repair mechanisms. Therefore some of the failures to translate findings from EAE into MS may not be simply the fault of the models, but the failure of the investigator to appreciate the very different scenarios in which the drugs were tested in animals and used in humans.

For many years drugs used in MS have had marginal efficacies on clinical course above placebo. Although it has to be accepted that a vast number of agents shown to ameliorate EAE have subsequently failed in the clinic,[5,9] the development of drugs such as Tysabri® have been critically dependent on biological studies in animals.[6] This and other drugs, such as CamPath®, can have a marked impact on the relapse rate but unfortunately this level of efficacy comes with the cost of increased adverse events. These can in some instances be fatal, such as the development of progressive multifocal leukoencephalopathy after treatment with Tysabri.[15] Whilst some people may berate the inability of animal studies to detect adverse events,[5,9] they in fact do so in the majority of cases for drugs that are destined to fail the Research and Development programme. Usually EAE studies aim to provide 'proof of concept' for therapeutic actions and are not designed to detect adverse events. Should risks of adverse events be in the range of 1:1000,[15] then it is unlikely that this would be detected as experimental group sizes used to test drug activity are considerably smaller. Furthermore, laboratory

animals are housed in environments free of animal pathogen, which removes the risk of infectious complications. However, when arresting the function of a significant portion of the immune system with potent immunomodulatory drugs, it is not surprising that development of infection and tumours become more probable.

EAE and MS are not just about autoimmunity

The concept that MS is just a problem of autoimmunity has been championed and directed by much EAE research, but immunotherapy has consistently failed in the clinic when progressive MS has been targeted.[1] Instead these studies suggest that while (auto)immunity may drive blood:brain barrier dysfunction and relapsing disease, this also appears to create a CNS microenvironment that is permissive to neurodegenerative processes that are no longer dependent on, or sensitive to inhibitors, of autoimmunity.[1] This has recently also been shown to be the case in long-established EAE,[11] which suggests that monotherapies solely targeting autoimmunity are insufficient to control EAE, let alone MS.[11] In addition, there is emerging evidence from studies employing chemical lesions or dysmyelinating genetic mutants that there is also 'slow burning' axonal loss in chronically demyelinated tissue. This indicates that the autoimmune paradigm is insufficient to describe both progressive EAE and MS and may go some way to explaining the clinical failures using anti-immunological therapies. Whilst aggressive immunotherapy early after diagnosis of MS may be desirable, once sufficient damage has accumulated, the additional use of neuroprotective agents will be needed to treat progressive MS. Whilst EAE may be able to detect agents that inhibit immunity, we have some way to go in determining which agents will inhibit (auto)immune-independent nerve loss and progression.

Conclusion

Experimental models of human disease, whether directly relevant to MS or not, help one to understand the underlying biology. Without these one would not have the base of knowledge to inform development of treatments towards the clinic. Importantly, they help provide the confidence to invest in the development of costly clinical trials that may ultimately lead to improvements in the lives of many people living with MS. It is important to realise that experimental models can only give sensible answers if we ask of them sensible and relevant questions!

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