Inhalation studies are used in the pharmaceutical industry, the introduction of the proposed 3D primary cell culture system in such companies has a large potential for 3R. A successful introduction of the proposed model in industry may reduce a substantial number of painful animal experiments, replace animal experiments by in vitro testing and refine in vitro model systems used today to study particle-lung interactions.

**Background and aim**

Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) affecting about one out of 700 young adults in Switzerland. Histological data and results from animal experiments indicate that T lymphocytes, B cells, macrophages and antibodies contribute to the formation of immune-mediated lesions in the brain and spinal cord of MS patients. The presence of clonally expanded immunoglobulins in the cerebrospinal fluid (CSF) of MS patients is one of the most prominent hallmarks of the disease. However, neither the specificity of these immunoglobulins nor the triggers causing demyelination and axonal damage are known (Sospedra and Martin, 2004).

A large proportion of current MS research is currently performed in a model system called experimental autoimmune encephalomyelitis (EAE). For the induction of EAE, rodents or nonhuman primates are actively immunised with myelin antigens, thus inducing an inflammation of the CNS. This inflammation leads to a progressive paralysis of the animals before they eventually are sacrificed. While CNS inflammation can also be caused by adoptive transfer of MOG-reactive T cells alone, in rats and nonhuman primates antibodies are additionally required for the induction of demyelination. Likewise, deposits of immunoglobulin and complement can be detected in MS lesions, supporting the role of immunoglobulins in MS pathogenesis.

**References**


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**Fig. 1**: Cerebellar slices were incubated in the presence of complement with a mouse IgG1-Isotype control (left panels) or with an anti-MOG antibody (right panel). Slices were fixed and stained for myelin basic protein.
Although EAE has provided many valuable insights into the pathology of MS, it only partially reflects MS and it is difficult to discern the role of different arms of the immune system in respect to the induction of CNS damage. Using organotypic CNS slice cultures, we have therefore established an experimental model system in which the complex architecture of the CNS is maintained and which allows the detailed investigation of interactions between the CNS and components of the immune system, as well as mechanisms of CNS damage and repair.

**Method and Results**

Our lab currently focuses on the characterisation of the role of antibodies in MS. We have established methods to amplify immunoglobulin genes from single expanded CSF plasma cells and to express corresponding heavy and light chain immunoglobulin genes as full human recombinant monoclonal antibodies in a eukaryotic expression system. Organotypic slice cultures provide an excellent tool to study the pathophysiological relevance of these antibodies. In order to establish antibody-mediated tissue damage, we used an antibody specific for MOG (myelin oligodendrocyte glycoprotein) in the presence of complement. As shown in Figure 1, the anti-MOG antibody was capable to cause demyelination in organotypic slice cultures (right panel). Complement alone did not alter the integrity of the myelin sheath (left panel). Currently, we are evaluating the effect of the antibodies derived from clonally expanded plasma cells of MS patients mentioned above. To this end, we not only rely on morphological changes, but also on the conductance of electrical stimuli in slice cultures of the hippocampus. In Figure 2, a hippocampal slice is shown on top of multigrid electrodes.

Neurons in the dentate gyrus are stimulated and a response is measured in the CA1 region of the hippocampus. Figure 3 shows such a response pattern of hippocampal neurons.

**Conclusions and relevance for 3R**

*Reduce:* Using slice cultures of hippocampus and cerebellum, several experimental conditions can be tested with tissue obtained from a single mouse. In contrast, for the induction of EAE, several mice have to be included per experimental group. *Refine:* Induction of EAE leads to severe disability and suffering of mice. Organotypic slice cultures are performed after euthanasia, which substantially refines the experimental procedures. Bystander reactions are less likely to happen in an *in vitro* setup than *in vivo*. Additionally, organotypic slice cultures reflect the three-dimensional *in vivo* structure more precisely than conventional cell cultures.

*Replace:* Organotypic slice cultures present an alternative tool to study the interactions between the CNS and specific components of the immune system. Therefore, many questions which are now investigated in the animal model of MS can alternatively be studied using this *in vitro* system, thus replacing many *in vivo* experiments.

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