CHAPTER 4
8.1 Focus of the Present Study

In order to establish a reliable animal model in Wistar rats, two different myelin antigens were used to induce active experimental autoimmune encephalomyelitis (EAE) with similarity to human M.S of remitting relapsing type (RRM.S). The present study was aimed at:

- Study of the disease course of myelin oligodendrocyte glycoprotein (MOG) induced EAE in Wistar rats.
- Study of the disease course of myelin basic protein (MBP) induced EAE in Wistar rats.
- Selection of the antigen (model) based on the inclusion and exclusion criteria for the further studies.

8.2 Experimentation

- Two myelin antigens, myelin basic protein and myelin oligodendrocyte protein were (MOG and MBP) were prepared by dissolving them in 0.98% saline and were finally emulsified with equal volume of Freud's complete adjuvant (CFA).
- Immunization with the antigens was carried out by subcutaneous injections on the anesthetized rats.
- The neurological deficits that occurred in rats following induction of EAE in both types of models (MBP injected and MOG injected) were checked for a period up to 31 days post immunization.
- Body weights of the all the animals were recorded to compare their weights, before the immunization, 20 days post immunization and after the experiment i.e. 31 days post immunization.
- Based on the analysis of the patterns of the neurological deficits and criteria described in experimental procedures (Table 3.1), a reliable model system with similarity to human RR type M.S was selected for further experimentation.
4.3 Introduction

Human multiple sclerosis in complex disease with variations in its pathological course. More than 85% of the M.S patients experience this disease in a remitting relapsing form termed as RRMS (remitting relapsing type multiple sclerosis).

Several types of animal models, collectively called as “experimental autoimmune encephalomyelitis” (EAE) of demyelinating disease have been described that represent human M.S. These models have been classified into active EAE; passive transferred EAE and co-transferred EAE. However, active EAE resembles human M.S in its induction and effector phases (Denic et al., 2011; Serres et al., 2009) while others lack the induction phase because encephalitogenic cells are used in lieu of the myelin antigens, thus lacking the basic mechanism of human demyelinating disease.

Autoimmunity to myelin antigens has been described as the most plausible mechanism of the inflammatory type demyelination in human as well as the experimental disease (Zozulya et al., 2010). EAE has been described in a number of laboratory animals including rats, mice and guinea pigs etc. However different kinds of antigens elicit varied kinds of responses (Derfuss et al., 2010) in a particular species and same antigen can behave distinct in different species making it difficult to ascertain the course followed by a particular antigen in a specific strain of animals (Baker et al., 2011). It is has been seen that a particular antigen can elicit remitting relapsing type EAE in rats while other may develop a monophasic disease.

A large variety of myelin antigens have been established to elicit autoimmunity in rats (Derfuss et al., 2010). These include myelin oligodendrocyte glycoprotein (MOG), myelin associated glycoprotein (MAG), oligodendrocyte basic protein (OBP), proteolipid protein (PLP) and myelin basic protein (MBP) and many others not listed here. The mechanisms regulating the onset of the autoimmune response with respect to a particular antigen are also different. Some of the antigens induce strong T-lymphocyte response that may be accompanied with a subtle auto-antibody response. Others basically induce auto-antibody response associated with T-lymphocyte activation (Bornstein and Appel, 1961). Therefore, it becomes clear that target organ specific autoimmunity is a mixed response of T-lymphocytes.
and auto-antibodies. The type of response a particular antigen can develop has been ascribed to the spatial arrangement of the antigen in the cellular membrane. It is now an established fact that the two mostly used myelin antigens viz. MOG and MBP reside on the extracellular (Breithaupt et al., 2003) and intracellular (Reynolds et al., 1989) aspects of the cells respectively.

We tested these two myelin antigens (MOG and MBP) in the same rat strain viz. Wistar rats (Rattus Norvegicus) to understand which of the two antigens can develop EAE much similar to the RR type human M.S with well defined phases and allows a stable course with minimal mortality which is a prerequisite for an ideal model for extensive studies.

4.4 Results

4.4.1 Active immunization with MOG

4.4.1.1 Neurological deficits followed a RR type pattern after MOG immunization.

Rats after receiving 100-200μL of inoculum containing 50μg of MOG emulsified with complete Freud’s adjuvant (CFA) started showing signs of EAE; 8-9 days post immunization (p.i) with evident motor impairment in about 18/20 (90%) animals. However, definite signs of EAE were visible only days 11-13, p.i. The disease at this stage was characterized by limb weakness, limping and need for support because rats preferred to stay along the walls of the cage. But as the course progressed, animals reached at the peak of the disease on the day 15 p.i (mean score 3.5) with hind limb paralysis and no mortality. This stage passed with the tapering of the symptoms and minimal sign were present 18-20 days p.i (Fig.4.1 A & B) with mean score of about 1.6 and were considered as the end of the first acute phase of EAE in Wistar rats. The acute phase was succeeded by a phase of remission extending from 20-28 days p.i) which involved restoration of motor function and other signs to a remarkable extent. As the disease progressed from this stage, the signs of relapse could be seen after 28 days (p.i). In the first relapse, the signs of EAE reached a mean score of about 2.5.

4.4.1.2 Weight changes after MOG immunizations.

The body weight of the rats was checked before, after the first acute phase of the disease and at the first relapse. Paralleling the disease course, weight of the EAE rats did not grow as
compared to adjuvant injected animals. All the animals at the time of induction, weighed 150± 5gm. The mean weight of the EAE (MOG) rats at the first acute phase was 136gm and after the first relapse the mean weight was only 154gm. It is evident that EAE lead to a significant weight loss in the rats.

4.4.2 Active immunization with MBP.

4.4.2.1 Deficits followed a monophasic type pattern after MOG immunization

Rats were immunized with 100-200μL of inoculum containing 50μg of MBP emulsified with complete Freud’s adjuvant (CFA). Disease (EAE) developed approximately 5 days (p.i) in about 16/20 (80%) animals while rest showed no or haphazard signs of EAE. The actively induced population (80%) showed difference in the day of the onset of the disease. At average all the induced animals showed peak symptoms at 13 days, p.i with a severity score of 4.8 after which disease remitted until the 22nd day, p.i. There was considerable morbidity at this stage 4/16(25%). The phase after 22 days showed stable symptoms (Fig.4.1 C & D) which did not change for the rest of the course i.e. 31 days, p.i. MBP immunisation although was able to induce EAE in Wistar rats, showed haphazard pattern of disease course with severe symptoms and increased morbidity as compared to the MOG induced active EAE. In some rats, mild paw edema was noticed which could be due to the adjuvant and was considered as non specific immune response, but no such activity was noticed in MOG injected rats. The disease course was monophasic with no defined phases as some of the animals showed early remission of the signs and varying symptoms.

4.4.2.2 Weight changes following MBP immunizations.

Body weight of the EAE animals did not grow after MBP immunization when compared to adjuvant controls. At the start of the immunization rats weighed 150± 5 gm. MBP immunized rats showed a mean weight of 136gm at 20 days, p.i and 147gm at the end of the experiment. Therefore it can be concluded that MBP immunization leads to significant weight loss in Wistar rats.
4.4.3 Selection of animal model in Wistar rats.

It is evident from the above results that MOG immunization leads to a relapsing type EAE in Wistar rats while MBP immunization lead to a monophasic disease. Moreover, MOG induced EAE showed well defined phases which could be segregated into the first acute phase, first remission and the first relapse. In contrast, MBP EAE showed haphazard pattern of disease induction in only 80% of animals with significantly elevated morbidity (25%). Based on the inclusion and exclusion criteria described elsewhere, MOG induced EAE was selected as a reliable model to study human remitting relapsing multiple sclerosis despite producing feebler symptoms as compared to the MBP.

![Figure 4.1](image)

Figure 4.1- Disease course followed by the Wistar rats after immunization with MOG and MBP. A&B represent the scatter plots of the disease score induced by MOG. C&D represent the course of EAE induced by MBP.

4.5 Discussion

Myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein are the two myelin antigens, used extensively in the organ specific target autoimmunity in various laboratory animals to study aspects of human demyelinating disease (multiple sclerosis). Both of the
antigens have been proven to be successful in this respect. We tested these on Wistar rats in search of a reliable and reproducible model which could recapitulate human remitting relapsing type M.S.

Active immunization with MOG in Wistar rats elicited a remitting relapsing type disease with well defined phases. Since MOG domains are required for optimal demyelinating response, the disease can remit and relapse which can be explained by the remyelination of the demyelinating plaques in the remission phase while as MBP leads to a bout of monophasic acute transient neuroinflammation which eventually subsides. It is evident that MOG (35-55) leads to the generating of strong auto-antibody response (Ichikawa et al., 1996). MOG domains reside on the extracellular surface of the cellular membranes (Fig.4.2). This property makes it more accessible to the circulating antibodies. In the rats, the induction of clinical disease induced by MOG exhibits an absolute requirement for the MOG-specific antibodies (Stefferl et al., 1999). These findings are consistent with the recent studies which demonstrate the presence of anti-MOG antibodies in the serum and CSF of human M.S (Di Pauli et al., 2011; Klawiter et al., 2010). It is believed that anti-MOG antibodies may be responsible for the active demyelination in EAE but at the same time may not affect the course of neuroinflammation (Ohtani et al., 2011). Therefore the role of sensitized T-lymphocytes can’t be precluded. Myelin basic protein elicits strong T-lymphocyte responses (Kheradmand et al., 2009; Mao et al., 2007); since MBP domains reside on the intracellular side of the cells. This spatial arrangement of the epitope doesn’t allow the auto-antibodies to recognize their target since antigen–antibody interaction needs direct interaction. In this case inflammatory response is dominated by the sensitized T-lymphocytes which recognize their target cells by MHC molecules.
Therefore it may be suggested that auto-antibodies are the prerequisite for the active
demyelination (Waegemans, 2004, Van der Goes et al., 1999) while T-lymphocytes generate
stable inflammatory response (Codarri et al., 2010). This inference can be supported by the
finding that MBP rats showed a stable phase after initial disease in Wistar rats. This claim is
further supported by the fact that passively transferred EAE by sensitized encephalitogenic
T-lymphocytes induces a monophasic disease which subsides or leaves a residual deficit but
does not relapse (Ben Nun et al, 1981). Moreover, these differences are also governed by the
 genetic and environmental factors. These inferences point towards the role of sensitized T-
lymphocytes in the aetiology of the neuroinflammation in both MOG and MBP induced
EAE and highlight the role of auto-antibodies in the development of the chronic disease
which remits and relapses over a period of time.

As pointed earlier, the purpose of the study was to establish a reliable model in Wistar rats.
The fine specificity and mortality are equally important in the evaluating the candidacy of the
model. In our studies MBP immunization increased the mortality significant (25%) while no
mortality was recorded in case of the MOG immunizations. Moreover, no inflammation or
edema was noticed in the MOG immunized rats while a few rats in the MBP immunized rats
showed paw edema pointing towards the non specificity of the model. With all these
findings, it can be concluded that MOG induced EAE in Wistar rats is much alike human
RRMS and can be used as a reliable model for extensive studies.