ORIGINAL ARTICLES

Induction of Experimental Allergic Encephalomyelitis in C57/BL6 Mice: An Animal Model for Multiple Sclerosis

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ABSTRACT

Basic research on the autoimmune disease multiple sclerosis has been performed mainly on its animal model namely experimental allergic encephalomyelitis. There are many different approaches established to get this model. Despite the existence of many references in literature in this regard, we have been faced with many difficulties generating the model suitable for studying different therapies. After a long time of challenging to get a reliable and replicable method, we came up with the following major points: First, the key element for getting a maximum number of sick animals at a defined time is to consider the most appropriate animal body weight (19-20 gr). Even though the age of immunized animals (6-8 week old) is highlighted in literature, we found out that body weight is of a greater importance. Secondly, because the only available susceptible mice strain in Iran is C57/BL6, the choice of peptide for immunization would be myelin oligodendrocyte glycoprotein (35-55 sequence of this peptide 200 μg/animal). Finally, pertussis toxin which is a costly reagent plays a key role in stimulating the immune response. Altogether, we recommend that considering the above mentioned tricks and tracks, one would definitely be able to generate a chronic progressive type of model, for basic research on therapies of multiple sclerosis.

Keywords: Experimental allergic encephalomyelitis; C57/BL6 mice; Multiple sclerosis; Myelin oligodendrocyte glycoprotein

INTRODUCTION

Multiple sclerosis (MS) is an inflammatory and demyelinating disease of the central nervous system with no applicable treatment so far. Experimental allergic encephalitis (EAE) animal models have been extensively used to investigate potential therapeutics for MS. The clinical course of the disease typically consists of four different types: acute fatal EAE, chronic progressive EAE, chronic relapsing EAE and chronic EAE with delayed onset.[1]

In acute fatal EAE, there is an abrupt weight loss, weakness of hind limbs, altered gait of animals, rapidly progressing to paralysis of the involved extremities, incontinence and impaired respiration which lead to animals death shortly after the immunization. In chronic progressive EAE, the disease develops slowly but progressively within two weeks. In chronic relapsing EAE, the animals suffer from acute disease with variable intensities. The signs would either be mild consisting of weak hind limbs, altered gait and incontinence or severe paraplegia of hind legs. After a complete recovery which lasts for a month or so, relapses occur. In chronic EAE with delayed onset, there is a weight loss and general weakness two weeks after the immunization with neurological symptoms starting in one or two months.[1] Among the different types mentioned, chronic progressive EAE would be the best to get a uniform group in terms of number and

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time of incidence while having enough time for studying different therapies.

Adoptive transfer of encephalitogenic T cells and active immunization with myelin antigen are known as passive and active ways of inducing EAE, respectively.[2,3] In active immunization, susceptible animals of different species can be immunized by varieties of neuroantigens. Different types of neuroantigens such as myelin basic protein (MBP), proteolipoprotein (PLP) or myelin oligodendrocyte glycoprotein (MOG) are the most commonly used encephalitogenic peptides.

Among different mice species suggested to be used as a model, C57/BL6 is the only one available in Iran which can be purchased from the Pasteur Institute of Iran. Therefore the choice of peptide with encephalitogenic potency in this species would be MOG.[4] According to Devaux and colleagues,[5] different sequences of the MOG peptide could have different effects. For example while sequence of 91-110 is effective in the therapy of EAE, that of 35-55 is effective in induction of the disease.

By simultaneously applying 35-55 sequence of MOG and Pertussis toxin in both male and female C57/BL6 mice with the animal body weight of 19-20 gr, we have successfully been able to generate the model repetitively in this study, analysing both clinically and histologically.

MATERIALS AND METHODS

Peptide

A P35-55 sequence of MOG peptide: M-E-V-G-W-Y-R-S-P-F-S-R-V-V-H-L-Y-R-N-G-K[5] was synthesized in our center (the national research center of genetic engineering and biotechnology) by Dr. H. Mostafavi who has currently moved to Tabriz University. The peptide was tested and found to be as equally effective as commercially available MOG (Sigma).

Induction of EAE

Male and female C57/BL6 mice were purchased from Pasteur institute of Iran with body weight ranging from under 18 gr, between 19-20 gr, and over 20 gr. After a week of acclimatization, they were divided into three groups of the above mentioned body weight range (n= 7 in each group). Mice were immunized with 200 μg of MOG peptide (the 35-55 sequence) emulsified in complete Freund's adjuvant supplemented with 4 mg/ml killed mycobacterium tuberculosis. A volume of 0.1 ml of the mixture was injected at the base of the tail for each mouse. Pertussis toxin (500 ng/mouse) was injected immediately and 48 hours later. Clinical assessment was performed on the basis of the following scales: 0- normal, 1- limp tail or mild hind limb weakness, 2- limp tail and moderate hind limb weakness, 4- limp tail and severe hind limb weakness or moderate ataxia, 5- paraplegia with no more than moderate forelimb weakness, 6- limp tail and paraplegia with severe forelimb weakness or severe ataxia followed by death.

Histology

Mice were perfused with 4%paraformaledehyde (PFA), the spinal vertebrae were taken and postfixed in bouin's fixative for 2-3 days to be decalcified. Using a fine forceps, vertebra was removed and spinal cord soaked in ethanol 70% until the day of embedding. For paraffin embedding, tissues were first dehydrated in 90%, 96%, 100% (2 changes) each for 1 hour and then cleared by incubations in ethanol/toluene (2:1), ethanol/toluene (1:1), toluene 100% (2 changes) each for 30-45 minutes. Finally the embedding was started by toluene/paraffin (1:1) for half an hour and continued for four changes of paraffin baths, each for 15 minutes. Blocks were allowed to be cooled and sectioned by using a rotary microtome at 7 μm. Sections were stained by luxol fast blue (myelin stain) and cresyl violet (nucleus stain) and mounted by depex.

RESULTS

Clinical Assessment of EAE Induction

The results in figure 1 show that in 19-20 gr body weight animals the increase in clinical scores (bottom panel) starts early after the immunization (day second) and corresponds well with the decline in body weight (top panel); at the time the mean score increases from 0 to 3, the mean body weight decreases from 19.63 gr to 14.33 gr. Figure 2 shows an EAE mouse with a score of 3, paralyzed hind limb and tail. In other groups however, either there was a delayed increase in clinical score; chronic EAE with delayed onset (day 12 in group over 20 gr) or there was a significant weakness of the animal (in group under 18 gr) which resulted in an early animal death; acute fatal EAE (day 6 after the immunization). Altogether a body weight range

between 19-20 gr was found to be ideal to get the chronic progressive type of EAE which can easily be repeated.

Histological Assessment of EAE Induction

Histological analysis of spinal cords from EAE induced animals showed that there was a significant neuron death and demyelination (Figure 3b) in comparison with normal intact animals (Figure 3a). This result indicates that there is a consistency between histological and clinical assessments.

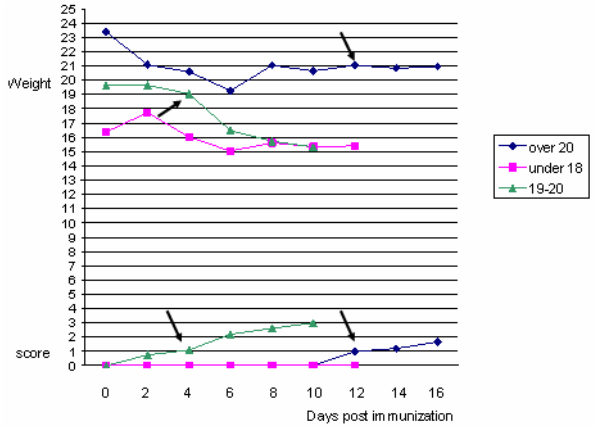

Figure 1. Summary of disease pattern changes observed in EAE induced animals with different body weights. In 19-20gr body weight animals the increase in clinical scores (bottom panel) starts early after the immunization (2 dpi; day second post immunization) and corresponds well with the decline in body weight (top panel). In group over 20gr however, there is a delayed increase in clinical score as well as a delayed onset (12 dpi). In group under 18gr, there is a significant weakness of the animal which results in an early animal death (day 6 dpi).

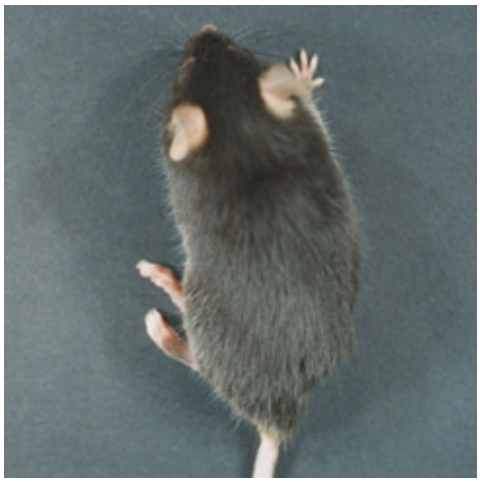

Figure 2. Photograph of EAE induced BL6/C57 mouse with paralyzed hind limbs and tail (score of 4).

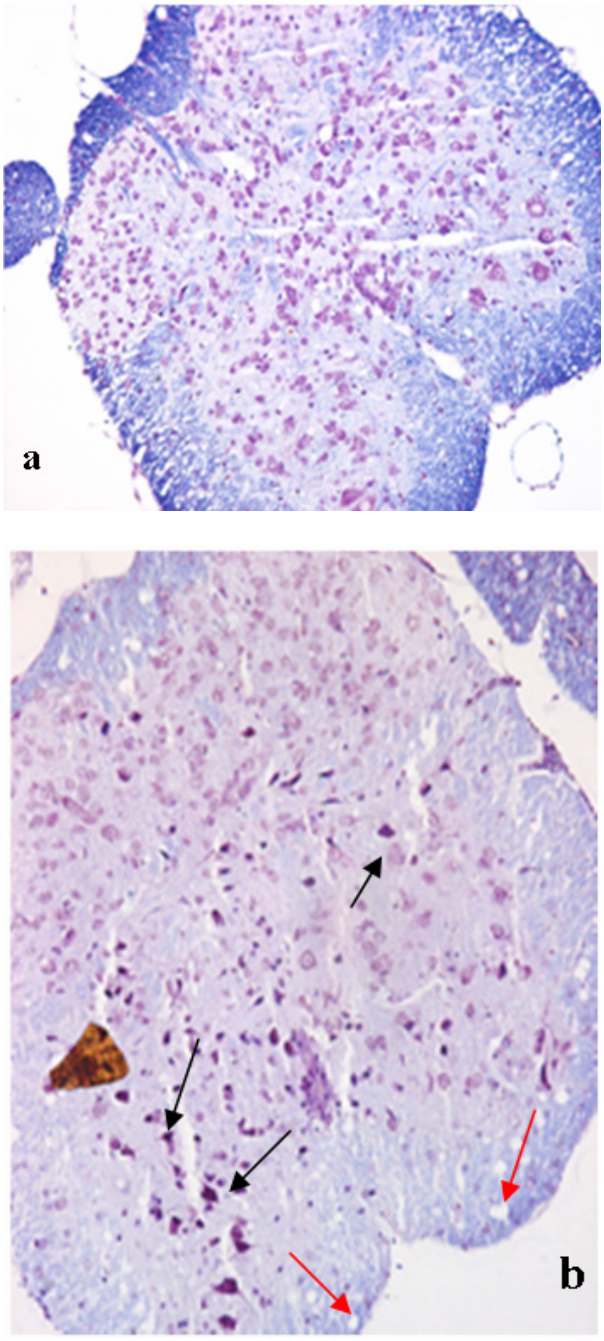

Figure 3. Photomicrograph of cross sections from spinal cords in (a) intact and (b) EAE mice. Black arrows point at extensively stained piknotic neurons (dead neurons) that are ubiquitously found in both dorsal and ventral horns of the spinal cord. Red arrows point at vacuolated myelin in EAE induced animals. Note the difference in myelin stained intensities between the intact and EAE spinal cords. Sections were stained with luxol fast blue (a myelin stain) and cresyl violet (nucleus stain).

DISCUSSION

Due to the importance of the animal model of EAE in understanding mechanisms involved in multiple sclerosis, many different approaches have been performed to get the model. In 1970's and 1980's, EAE was readily generated with whole homogenate of spinal cord or brain white matter in guinea pigs, rats, rabbits and monkeys,[6-9] but with many difficulties in mice.[10] Developing sophisticated methods, researchers finally came up with the advantage of using peptides instead of whole brain and spinal cord homogenates.

Major myelin proteins, myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) have been considered to be potential autoantigens to induce EAE in rodents.[5,11-13] Moreover, Sobel and colleagues[14] showed that there was variability in responses to certain sequences of different encephalitogenic peptides. Depending on the methods used, the period of the disease varied from 14-21 days, during which the induction of the disease should be verified by histological examination of central nervous system. Oligodendrocytes, producers of myelin in central nervous system (CNS), are the main targets in this disease and myelin destruction can therefore be easily detected by routine histological techniques.

With limited and available research resources and a long term trial of different methods, we came up with some important tricks and tracks through which a model could be generated and tested thereafter. These clues provide the least frustrating and the most feasible and reproducible method in generating the model. The only available susceptible mouse strain is C57/BL6 and therefore the most effective encephalitogenic peptide would be sequence 35-55 of MOG.[5]

Four different clinical courses of the disease have been classified: acute fatal EAE, chronic progressive, chronic relapsing and chronic with delayed onset. It seems that animals suffer from hyperacute EAE die during the first few days after the immunization,[1] therefore they may not be a good model for investigating the effects of therapies. To our experience, 6-8 week old mice with an average body weight of under 18gr die soon after the immunization, probably suffering from hyperacute EAE, therefore they are not an appropriate age to be used. On the other hand, using 16-18 week old mice with the animal body weight greater than 20 gr, we do get a response but a delayed and prolonged response. The fact that different types of EAE can be generated depending on age has also been shown by Lassmann and Wisniewski[1] in guinea pigs. One possible explanation for the differences in clinical course and pathology at different ages would be that chronic disease is much more frequent in sensitized young animals. Therefore a small variation in the time of sensitization may have a dramatic effect on clinical expression of the disease.

One other important issue is the use of pertussis toxin, a costly reagent that plays a key role in the initiation of autoimmune disease by modulating the interaction between the innate and adaptive immune systems in response to self antigens.[15] Double immunization, boosting on day 8, with the same dose of MOG and pertussis toxin can also be considered if noticeable clinical symptoms were not observed.

In conclusion, key elements in generating a progressive EAE are: using a susceptible strain to a known peptide, an appropriate animal body weight, injection of pertussis toxin at the time of immunization and double immunization. Following this schedule, one would surely be able to get a reproducible response.

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