Importance of the Non-responder in Deciphering Animal Behavior of Experimental Autoimmune Encephalomyelitis

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Animal Models of Multiple Sclerosis

To bring new drugs and therapies to the clinic, preclinical studies are mandated to establish the efficacy and possible mechanism of action. For the best results, animal models that closely mimic the target disease are required. Prior to initiating large-scale multi-centered clinical trials, the Food and Drug Administration in the United States often dictates that the efficacy and safety of new drugs need to be demonstrated in two animal models, and not just two different strains of mice. This set of rules has not always been followed when testing drugs for autoimmune diseases. Given that many of these diseases, including fibromyalgia, multiple sclerosis, arthritis, and even autism fall into spectrums of disorders, determining an appropriate animal model is complicated. With regard to multiple sclerosis, the primary animal model is experimental autoimmune encephalomyelitis (EAE) [1,2]. In mouse or rat models of EAE, there are several ways to induce the disorder including viral infection, genetic or adoptive transfer of activated T cells, or chemical/antibody producing systemic injections [1-5]. These models have been described by numerous investigators with each pointing out the strengths and weaknesses [2,6,7]. However, less attention is paid to the major form of MS which is a relapsing-remitting form, and few animal models of EAE provide a reliable and consistent profile of this disease. Viral infection and adoptive transfer result in a progressive form of EAE – most likely mimicking chronic progressive MS. However, chronic progressive MS only affects 15% of the population, leaving a majority of the people with relapsing-remitting MS at onset without a reasonable animal model.

Of the current models, induction of chronic progressive EAE by immunization with myelin oligodendrocytic glycoprotein (MOG<sub>35-55</sub>) is the most popular as reflected by publication frequency [8]. A relapse-remitting form of EAE can be induced by immunization with proteolipid protein (PLP<sub>139-151</sub>) [9]. Despite the lack of similarity in etiology, the pro-inflammatory components of both EAE and MS are similar, as the levels of IFN-γ, IL-1β, and TNF-α are upregulated. In response to this external insult, both disorders are characterized by CNS demyelination and neurodegeneration. Initially the peripheral immunization stimulates reactive T cells to proliferate and begin secreting cytokines [10]. The autoreactive T cells migrate to the CNS, proliferate again and become activated to secrete more cytokines leading to an inflammatory process. This CNS inflammation is subsequently related to eventual neuronal death, axonal damage and neurodegeneration. The hallmark of EAE progression is the deterioration of locomotor behavior. It is apparent that this cascade of events is individualized even within a genetically homogeneous population of mice. Not only are induction rates variable, but the clinical manifestations within the “inflicted” group often differ dramatically. While individualized differences may be acceptable for the study of precision or personalized medicine, variations in response within an animal model defeat the purpose of using animals that are in most cases genetically bred to be relatively similar. The experimental supposition is that all animals will respond to the stimulus, in this case viral challenge or antigen in a correspondingly similar manner. However, with EAE and other autoimmune disorders, the frequency of variation in response is exceptionally high. Both responders and non-responders to the onset of disease as well as to therapeutic treatment are important players in the interpretation of the experiments. The failure to respond is not always a negative.

Preclinical Data Interpretation

Some of the most difficult data to interpret is the subjective call on clinical behavior in animals. Given that mice and rats cannot respond to verbal commands/questions, the interpretation and anthropomorphizing of gestures to mean anxiety, pain, or depression are clearly subjective and prone to a wide range of variation. One must assume that the classification of clinical signs of MS in mice with EAE is open to interpretation. Some researchers describe behaviors that range from normal to paralysis and fit the observations into a scale of 0-5 [11]. Other researchers, including ourselves, have attempted to provide more clarity by expanding the scale to 10 [12,13]. In our studies we have examined movement and posture for each limb of the animal and develop a final summative score. Our justification is in part based on the fact that EAE presents in a caudal to cranial manner and hindlimbs develop limppness and wobbliness several days before the front limbs. The summation allows for each limb to be given a weighted consideration in the overall clinical score. Even with careful observation, some mice do not appear to be “EAE-like”, much less demonstrating responsiveness to a pharmacotherapy.

However, the clinical scores in studies on treatments are often the hallmark for determining whether a drug is a likely candidate for further clinical studies. It is understandable that most drugs that are successful in preclinical studies are failures in the clinic. This aspect of behavioral interpretation is accepted as being subjective. However, a more comprehensive approach to interpreting EAE animal studies is the consideration of “non-responders” and “responders”. The concept of some individuals responding positively or negatively to a treatment is common in medical literature on humans [14-18]. Drug therapies are almost “assumed” to be effecte for some, but not all, subjects. In a recent search of the PubMed website, more than 27,400 entries had “responder” as a keyword; of these 95% were related to humans, and approximately 900 entries were related to mice/rat/rabbits. If the search was restricted to the use of “responder” in the title, less than 2% were related to animal models [8].

The opinion among most researchers is that the use of genetically related rats or mice should result in “all or none” reactions. However, more animal models are using outbred mice or rats, and therefore the...
normal variation in response is normal. Secondly, researchers tend to not publish the number of animals injected with a virus or antigen that did not develop the disease. In most cases, the point of the study is to examine a variable within a disease – not to report to the efficiency for generating the disorder. This is particularly true for diseases such as EAE that are induced by antigen injections or viruses, type 1 diabetes induced by streptozotocin injections, and even mouse models of cancer that are chemically induced.

However, it is in fact, the non-responders to therapy that are the most interesting and perhaps provide new clues to the pathophysiology of the disease. For example, if 20 mice are injected with the same MOG_{35} antigen and all other conditions (volume, time, age) are consistent, why is it that 3 or 4 mice do not develop disease? Would not their immunological response be of interest?

Moreover, in terms of therapeutic treatments, again, most drug makers and researchers are accepting of the fact that everyone in a cohort will not be “cured” by the new pharmaceutical. And yet, in basic research, few papers will report on “non-responders” to their therapeutic treatments. This discrepancy is particularly relevant to immunomodulating therapies as their mechanisms and pathways of action are not well defined. Autoimmune diseases in mice or rats most likely are not consistent manifestations of the same disorder. It would seem likely that each animal reacted to the antigen or viral challenge differently and thus would mount a slightly different response, allowing for variation in their overall clinical behavior, and interpretation of their “response” to therapy. These biases in non-responders are rarely published or discussed, but we believe they are perhaps the most important elements of the research paradigm – why did an animal not respond to the treatment when 90% of his cohort was ‘cured’, for example? Did the research fail to look at a contributing pathway? Did the research fail to consider biofeedback mechanisms that would increase or decrease specific factors/hormones/cytokines?

In conclusion, authors are encouraged to present all data in basic science experiments and to begin to interpret possible reasons for the non-responders. Inclusion of their behavior may provide insight into future studies that would support development of personalized biotherapeutics.

References