



2010 Research Grant Program Winning Abstract

The Role of Dendritic Cell Precursors in Experimental Autoimmune Encephalomyelitis

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Experimental autoimmune encephalomyelitis (EAE) is an inflammatory disease of the central nervous system (CNS) in mice that models the human disease, multiple sclerosis (MS). CD4 T cells are critical mediators of disease, since they are required for EAE. Additionally, encephalitogenic IL-17- and IFN- γ -producing CD4 T cells can transfer disease passively. In standard models of EAE, disease is induced by subcutaneous immunization with myelin components (e.g. myelin oligodendrocyte glycoprotein (MOG)) emulsified in adjuvant. MHC class II (MHCII)-dependent antigen presentation by antigen presenting cells (APCs) is critical for generating pathogenic CD4 T cells in EAE. We have previously shown that dendritic cells (DCs) are capable of mediating all APC functions during EAE. Further, an accumulation of DCs within the brain and spinal cord occurs during disease, suggesting that there is active recruitment of DCs during neuro-inflammation. We hypothesize that DCs participating in neuro-inflammation arise from bone marrow-derived DC precursors (pre-DCs). Recent work has characterized the developmental pathway for conventional (i.e. CD11b+ or CD8 α +) DCs (e.g. Liu et al, *Science*, 324(5925):392-7, 2009). Using flow cytometry, the identification of these cells requires at least six colors. We have successfully employed the BD™ LSR II in the flow cytometry core at Washington University in St. Louis to identify pre-DCs within the blood and in lymphoid organs of mice.

We aim to characterize the contribution of pre-DCs to the pathogenesis of EAE in two ways. First, we will define the temporal frequency of pre-DCs during the course of EAE development. We anticipate that the number of pre-DCs will rise during disease development if they contribute as a source of DCs in the CNS during EAE. Pre-DCs will be identified by six color flow cytometry (acquired on a BD LSR II) in the bone marrow, blood and spleen at multiple timepoints after immunization. This is a novel approach, since currently there is no information on the modulation of steady-state DC development during inflammatory responses. Second, we plan to isolate pre-DCs from the blood, bone marrow and spleen of naïve mice and transfer them to mice with EAE. Isolation of pre-DCs from congenic animals will be performed using the BD FACSAria™ II in the flow cytometry core at Washington University in St. Louis. We hypothesize that enlarging the pre-DC pool will result in greater disease after immunization with MOG. Alternatively, pre-DCs may not affect disease severity or even reduce clinical disease. We will evaluate mice with EAE that receive pre-DCs from wild-type or MHCII-/- mice to determine the requirement for antigen presentation in any effect elicited by transferred pre-DCs. Following transfer, we will attempt to recover mature DCs from the CNS of mice with EAE. We plan to separate the transferred DC population from host DCs using the BD magnetic separation technique (e.g. using congenic marker CD45.1-PE, followed by BD IMag™ antibodies and separation using a BD IMagnet). Overall, these experiments will provide insight into the regulation of DC homeostasis during inflammation. Furthermore, this study will clarify the contribution of pre-DCs to antigen presentation during EAE, potentially opening a line of research into targeted therapeutics



for patients with MS.

A BD Biosciences Research Grant would be a distinctive way of providing support for this research project. First, this research is being undertaken in a new laboratory that has just started at Washington University in St. Louis. As such, limited resources exist for de novo projects such as this one. Further, the techniques utilized for the proposed research - including flow cytometry, FACS sorting and cell separation - are all linked to BD products and supplies. Finally, this is a unique pathway of investigation within the neuro-immunology community. Research focusing on immune cell lineage commitment as it pertains to a neurologic disease is challenging due to the complexities of multiple disciplines. Financial support from BD for this work would be an endorsement of the cross-disciplinary collaboration between developmental biologists, neuroscientists, immunologists and clinicians required for success of this project.

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