AMAZING PAPERS IN NEUROSCIENCE
Primary Literature for Teaching Neuroimmunology – An Instructor's Resource

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Supplementing textbooks with primary literature in teaching neuroscience is a growing practice associated with several positive outcomes, such as increased content knowledge, research and data skills, and critical thinking. This pedagogical approach, however, still needs further development to make it accessible to instructors and valuable to students. This article describes a series of published articles we used in an undergraduate neuroimmunology course. Articles were selected to supplement the teaching of significant principles in the neuroimmunology of disease in neuro-infections, autoimmune diseases, and neurodegenerative diseases. Specifically, articles on multiple sclerosis, experimental autoimmune encephalitis, Herpes Simplex Virus 1, SIV/HIV infections, Alzheimer's, and Parkinson's diseases are described, and the pedagogical value of each is enunciated. These sources could be incorporated into a range of undergraduate and graduate courses to introduce several topics and principles of neuroimmunology.

Key words: neuroimmunology; primary literature; neuro-infection; autoimmune; neurodegenerative

INTRODUCTION

Neuroimmunology is a relatively new discipline in which scientists study immune and nervous system interactions in health and disease states. It is a multidisciplinary field involving the application of neurobiology, immunology, pathology, virology, and psychiatry as it relates to the central nervous system. Neuroimmunologists focus on neuroinflammatory disorders such as multiple sclerosis, viral infections of the central nervous system (CNS), and several other disorders where the immune response may be involved, including neurodegenerative diseases and aging. The discipline is not without challenges or earlier misconceptions, such as the idea that the brain was considered immune-privileged and that the blood-brain barrier prevented the crosstalk of the immune system and the brain (Nutma et al., 2019). Recent evidence shows that the nervous and immune systems can communicate through several cell types, molecules, and mechanisms (Nutma et al., 2019).

As new reports that address the significant questions of neuroimmunology continue to be released, teachers may find it challenging to identify critical readings on topics that are distant from their areas of expertise. Here, we review articles that address specific learning objectives and may be useful for teaching neuroimmunology. We have organized these articles by topics that address major subject area questions. The first article focuses on the role of antibodies in immune-mediated neuro-immunological disorders exemplified by Multiple Sclerosis (MS). The following two articles address the contribution of infiltrating immune cells that originate from the periphery in CNS disease development. We turn next to articles on viral infections and animal models of neuroimmunological disorders. The final two articles attempt to answer whether specific approaches, such as gene or cell depletion, help reduce CNS disorders. We hope these summaries will be a valuable guide for instructors wishing to include primary literature in their courses.

ROLE OF ANTIBODIES IN IMMUNE-MEDIATED NEUROIMMUNOLOGICAL DISORDERS

Topics: Autoimmune disorders, cause of MS, role of B cells and antibodies in MS.
Reference: Cepok *et al*. (2005).
Description: Many neuroimmunology classes will start by describing Multiple Sclerosis (MS), a disease that affects the nervous system. The precise cause of MS, however, remains unknown, and the article by Cepok et al. (2005) set out to investigate how the immune system might be related. They discovered that immune cells made antibodies against two Epstein-Barr Virus (EBV) proteins. They found that the disease was most likely due to viral proteins resembling the brain's protective myelin, causing the immune system to attack. In addition, cells in MS patients had a stronger reaction to the EBV proteins than controls, suggesting EBV's essential role in MS pathogenesis (Cepok et al., 2005).
Value: MS is a significant neuroimmunological disease characterized partly by the occurrence of multiple bands of antibodies known as oligoclonal IgG. This paper asks if the persistent production of antibodies in MS is specific to disease-relevant antigens, a subject that motivates students to discuss the causes of MS, which remains enigmatic. Discussing such difficult-to-understand areas of neuroimmunology provides room for creative and critical thinking in many ways. One area for student discussion could focus on the autoimmunity hypothesis - a prominent hypothesis for the cause of MS. This paper is valuable since the specificity of the B cell response to EBV proteins is described. Additional reading of other more recent epidemiological studies, which show that a large percentage of MS patients are positive for EBV and have a history of mononucleosis caused by EBV (Abrahamyan et al., 2020),

can enrich the discussion. From a technical perspective, this paper introduces the students to protein expression arrays, western blotting, IgG-binding epitope discovery techniques, and the use of the flow cytometer to analyze immune cell responses. These techniques are critical not only for neuroimmunology but also for providing a practical basis for understanding neurobiology.

DO INFILTRATING IMMUNE CELLS FROM THE PERIPHERY CONTRIBUTE TO CNS DISEASE PATHOGENESIS?

Topics: Blood Brain Barrier, neurodegeneration, Experimental Autoimmune Encephalitis (EAE), Parkinson's disease.

References: Ajami et al. (2011); Harms et al. (2018).

Description: An essential question in neuroimmunology has been the distinct roles played by CNS resident microglia and infiltrating myeloid cells such as monocytes in the development of disorders including MS or Parkinson's disease (PD). If microglia are already in the brain, it remains to be understood why peripheral cells such as monocytes need to enter the brain and whether they play a different role. To address this question, we chose two articles: the first by Ajami et al. (2011) features a mouse model of experimental autoimmune encephalitis, and the second by Harms et al. (2018), a model of Parkinson's disease.

Ajami et al. (2011) used a combination of a surgical procedure, known as parabiosis, a technique that creates blood chimeras between two animals, and removal of myeloid cells. They found a correlation between monocyte entry into the brain and progression to the paralytic stage of EAE (Ajami et al., 2011). Inhibiting the receptors necessary for monocyte recruitment resulted in reduced EAE progression. Furthermore, infiltrating monocytes were transient and did not contribute to the resident microglial pool in the long term (Ajami et al., 2011). This article proved that monocytes are distinct from the resident microglia and have different roles in neuroinflammation and disease progression (Ajami et al., 2011).

The second article in this series asked whether monocyte entry is essential for neuroinflammation and degeneration in a mouse model of Parkinson's disease (Harms et al., 2018). When a protein linked to PD, called alpha-synuclein, was expressed in mice, it was found that monocytes from the periphery entered the brain and caused inflammation in PD-relevant brain areas (Harms et al., 2018). Furthermore, genetic deletion of an entry receptor expressed on monocytes prevented monocyte entry and reduced microglial activation and neuronal degeneration.

Value: The article by Ajami et al. (2011) offers a dual advantage in illustrating the role of infiltrating monocytes in the CNS and modeling MS using the EAE mouse model. Firstly, the article provides a unique introduction to the presence of peripheral cells in the CNS. Indeed, the authors show that the brain resident microglia are distinct from monocytes and are maintained through self-renewal and not by recruitment of outside cells. This discovery was an essential advancement to our knowledge of the cellular developmental process. They also show that the incoming monocytes have a different role in disease progression. Discussion about monocytes in the CNS clarifies some challenging ideas in the field, including the distinct cellular roles of monocytes and microglia in disease initiation and progression, which may be difficult to grasp through usual lecture materials. Second, modeling MS has several problems since it is uniquely a human disease with an unknown origin. The two major types of models for MS are based on autoimmunity or viral infection in rodents. Students can learn and discuss the characteristics of the models and compare them to MS in humans. Experimental details for parabiosis and chimerism are challenging for undergraduates to understand but not impossible. Images for monocytic infiltration into the CNS using GFP-positive cells at different levels of disease severity are convincing and an exciting demonstration of the beauty of combining two powerful techniques. With this material, students get early exposure to advanced techniques and ways of thinking they otherwise would not have exposure to at this stage in their career.

The second article by Harms et al. (2018) demonstrates that peripheral monocyte entry into the CNS plays a role in neurodegeneration in a model of Parkinson's Disease. Students could review the role of the blood-brain barrier in health, how it is compromised in disease states, how monocytes enter tissues, and the functions of chemokines and their receptors. Delving into these areas helps students grasp the complexity of the relationship between the brain and the immune system in the context of neurodegenerative diseases. From a technical perspective, students can get exposure to the techniques for designing and delivering viral constructs into the brain, immunohistochemistry, and flow cytometry. To sum up, MS and PD are significant diseases, and these two articles' similar research objectives are a simple way for instructors to illustrate complex topics in well-known neuroimmunological diseases.

NEURO-INFECTIONS AND ANIMAL MODELS OF NEUROIMMUNOLOGICAL DISORDERS

Topics: Neuro-infection, neurovirology, substance use disorders, Human Immunodeficiency Virus (HIV), Herpes Simplex Virus-1 (HSV-1).

References: Fox et al. (2022); Katzilieris-Petras et al. (2022).

Description: Some pathogens, such as HIV and HSV-1, can enter the CNS and cause infections. Different locations within the nervous system, such as brain meninges, spinal cord, or peripheral nerves, could be targets of infections. Furthermore, significant interactions between pathogens and substance abuse can increase the adverse effects of these agents. Fox et al. (2022) exemplify the dual impact of substance use disorders and comorbid infection with HIV/SIV on the brain. By use of a Macaque model of Simian Immunodeficiency Virus (SIV) infection, anti-retroviral therapy, and morphine injection, they showed that morphine suppresses the peripheral immune response but alters the brain myeloid gene expression into a neurodegenerative phenotype that enhances the development of SIV (Fox et al., 2022). Among other outcomes, microglia from macaques in the SIV and morphine groups had elevated expression of

the cytokine known as osteopontin, which can change the state of viral reservoirs in the CNS (Fox et al., 2022).

Katzilieris-Petras et al. (2022) demonstrate that Herpes Simplex Virus can enter the brain and cause Herpes Simplex Encephalitis (HSE). Following entry of HSV-1, brain resident microglia and infiltrating monocytes have been implicated in sensing HSV-1 and induction of an antiviral response and virus control. The authors found that when microglia were removed in a mouse model of HSE, the brain produced fewer cytokines and chemokines, such as Interferon type 1 (IFN), at early time points after virus entry into the CNS (Katzilieris-Petras et al., 2022). Consequently, viral levels increased, and disease symptoms worsened in microglia-depleted mice. In vitro experiments with primary cells validated that microglia were essential for IFN RNA induction and control of HSV-1 replication (Katzilieris-Petras et al., 2022). This data shows a critical role for microglia in activating an early antiviral response against HSV-1, thus preventing neuronal damage.

Value: Fox et al. (2022) highlight the interactive effects of substance use and SIV in the CNS, a subject where increased knowledge is vital because neurocognitive impairments associated with this combination continue to be observed despite access to and the presence of anti-retroviral medications. A significant population in the US suffers from opioid use disorder, and in addition, opioids are used for pain management, making opioid use a risk factor for HIV transmission. To study the effects of opioids and SIV in specific cell types, the authors used advanced techniques such as single-cell and single-nucleus RNA sequencing (scRNA-seq and snRNA-seq). These new techniques offer an intriguing method for students to see firsthand the nuances of RNA patterns in individual cells that will pique their interest and spark conversations. Furthermore, in this study, SIV-infected macaques were used as a non-human primate model for HIV. This non-human primate model is excellent for studying the interactive effects of drug abuse and SIV in the brain and will undoubtedly generate exciting discussions about sample sizes needed to create meaningful data, the associated ethical considerations, and the role of institutional animal use committees (IACUCs) in ensuring ethical research practices.

In the second article in this series, Katzilieris-Petras et al. (2022) elegantly demonstrated the role of microglia in controlling HSV-1 infection in the brain. Students can review core concepts on how some neurotropic viruses can enter the brain by hijacking the natural ways these cells transport proteins to and from the CNS. Students could dive into neurotropic viruses' life cycles, exploring how lytic and latent phases contribute to neuroimmunological disease. The discussion could also attempt to understand whether peripheral immune cells are recruited into the CNS and whether they can control HSV-1 infection, thus promoting a fascinating investigation into a current enigma. By analyzing easy-to-follow figures, students can clearly understand the process of microglia depletion. They will also be able to track viral titers, observe the development of HSV-1 disease-like symptoms, and induction of an antiviral response in a mouse model. For the many undergraduate students who don't get much laboratory experience, exposure to such research data from primary literature is invaluable and will set them up for future success.

DO SPECIFIC APPROACHES SUCH AS GENE OR CELL DEPLETION HELP REDUCE THE EFFECTS OF CNS DISORDERS?

Topics: Therapy in neuroimmunological diseases, translational neuroimmunology, monoclonal antibodies, gene depletion.

References: El Khoury et al. (2007); Hauser et al. (2008).

Description: The article by El Khoury et al. (2007) found that mice lacking the CCR2 gene, which helps microglia cells to move, had fewer microglia around senile plaques. These plaques, consisting of amyloid beta, activated microglia, astrocytes, and degenerating neurons are a hallmark of Alzheimer's disease (El Khoury et al., 2007). Although microglia are often seen gathering on senile plaques in several AD models, their role remains to be fully explored. This article shows that Alzheimer's disease mice deficient in CCR2 accumulated amyloid beta earlier and died prematurely, suggesting that having microglia early on may help clear the senile plaques and protect against Alzheimer's early stages (El Khoury et al., 2007).

The article by Hauser et al. (2008) addresses the role of B lymphocytes in the pathogenesis of Multiple Sclerosis. Previous research suggested that abnormal immune activity in the brain involving the production of multiple bands of antibodies by B cells could contribute to MS (Siden, 1979; Cerrato et al., 1984; Owens et al., 2006; Franciotta et al., 2008). To test this, they conducted a 48-week-long phase-2 trial with 104 people with relapsing-remitting MS, using rituximab, a monoclonal antibody that selectively depletes $CD20^+$ B lymphocytes. The study found that individuals who received a dose of rituximab had reduced counts of inflammatory brain lesions and clinical relapses. This effect was sustained up to 48 weeks (Hauser et al., 2008).

Value: The article by El Khoury et al. (2007) demonstrates the protective role of microglia in Alzheimer's disease, which is mediated by these cells' ability to engulf amyloid beta. As students review neurodegenerative diseases, including AD, as presented in this article, they can explore how the different cell types may contribute to or mitigate disease progression. Furthermore, it has been shown that senile plaques composed of amyloid-beta and neurofibrillary tangles, which consist of Tau protein, are both observed in AD patients. The ongoing debate over whether amyloid beta or Tau is the primary driver of AD remains one of the most compelling questions in the field. Students can critically compare the evidence for amyloid versus Tau and methods to diagnose the disease, fostering students' creativity and the ability to develop innovative approaches to complex issues.

The second article in this series, Hauser et al. (2008), presents solid evidence for the role of B-cell depletion with rituximab in reducing inflammatory brain lesions and clinical relapses in MS patients. Traditionally, MS was thought to be driven by $CD4^+$ T cells. Thus, several MS therapies targeted these cells (Jacobs et al., 1996; Wolinsky et al., 2007). This paper sparks discussion that pushes us to rethink the predominant mechanisms responsible for MS. Additionally,

this paper is an invaluable example of a well-designed Phase-2 trial that demonstrates and stimulates discussion about randomized, double-blinded, and placebo-controlled studies. In addition, it provides a thinking point for the importance of institutional review boards, ethics committees, and written informed consent by research subjects.

AUDIENCE AND IMPLEMENTATION

These papers are suitable for advanced-level undergraduate or graduate-level neuroscience courses. I have used them in these classes and students have found them exciting and relevant to the lecture material. It's an enriching experience. At the heart of this teaching method, students are taught to read effectively. Those currently in the field always do this, so starting students early is beneficial. Developing scientific literacy by reading high-level and sometimes complex primary articles is a great thing. The more technically challenging papers are valuable for teaching core concepts in their respective areas, and students should not be expected to fully understand all the technical details. Some papers, however, may use one or two techniques that are well-explained and valuable for teaching both the core ideas and details of how they're done.

Implementation could vary and may include letting students read and present a paper to the class, assigning students to explain sections of the paper, or the instructor presenting a summarized version. As instructors, reading these texts gives us invaluable insights into neuroimmunology's current and evolving scope. By familiarizing ourselves with such important research, we can offer students a broader and more informed perspective. Asking students to answer brief questions about each paper and submitting responses before the class in which the paper is discussed helps prepare students for vibrant in-class discussions. It allows them to come in more prepared. These papers represent essential advances in our understanding of neuroimmunological complications, each a crucial step to solving complex disorders like Alzheimer's, Parkinson's, or even viral infections. For students, reading research out of class provides opportunities to engage deeply with the text, fostering critical thinking and textual analysis. It may even spark students' interest in specific career paths in neuroimmunology- a field where they may one day contribute their own research.

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