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Neuroimmune Pharmacology as a Sub-discipline of Medical Neuroscience in the Medical School Curriculum

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Abstract

The emerging field of neuroimmune pharmacology (NIP) is the confluence of three distinct disciplines: neuroscience, immunology, and pharmacology (Gendelman and Ikezu 2008). NIP was born from the realization that inflammation within the central nervous system (CNS) plays a crucial role in many neurological pathologies and as such offers a rich array of novel pharmacological targets as potential therapeutics. As this field is likely to have a major impact in medical science, educating future physicians on this area will help increase awareness and may potentially inspire them to pursue careers in the field of NIP. However, a key challenge for medical educators, is how best to incorporate new material on emerging fields, such as NIP, into the medical school curriculum, specifically in the context of a medical neuroscience course. We propose the addition of two 50-min lectures plus an additional optional 2-h lab module to the standard first year medical neuroscience class curriculum. Lecture 1 will focus on how the CNS and the immune system inter-communicate with one another with emphasis on neuroanatomical features and chemical signal transduction between the two systems. Lecture 2 provides an introduction to inflammation in the CNS and provides a series of clinical correlates to describe how CNS inflammation contributes to the disease process. The lab module provides detailed visual examples of how CNS inflammation influences disease processes and provides two examples of how application of an immunomodulatory pharmacological agent can modify disease processes.

Keywords

Medical school curriculum; Education; Neuroimmune pharmacology; Medical students; Neuroscience

Introduction

The emerging field of neuroimmune pharmacology (NIP) is the confluence of three distinct disciplines: neuroscience, immunology, and pharmacology (Gendelman and Ikezu, 2008). NIP was born from the realization that inflammation within the central nervous system (CNS) plays a crucial role in many neurological pathologies, e.g., neurodegenerative disorders, viral/bacterial CNS infections, and autoimmune disorders of the CNS (Gendelman and Ikezu, 2008). In addition, many neuropsychiatric disorders have profound impacts on the immune system, “the mind-body connection”, e.g., drugs of abuse and various mood disorders (Gendelman and Ikezu, 2008). Lastly, a growing realization that pharmacological interventions which modulate the immune system’s affect within the CNS or CNS’ influence over the systemic immune system offers a hereto-untapped avenue of novel therapeutics as treatments of various pathologies.

As with all new emerging fields with profound health implications, such as NIP, it is challenging for medical school educators to determine how best to incorporate this new material into the medical school curriculum. NIP, being such a multi-disciplinary field presents its own challenges to medical school educators since neuroscience and immunology are traditionally first year classes, dealing with basic science topics of how the body ‘normally’ functions—“the Happy-path”. However, pharmacology is traditionally taught as a second year class with a goal of introducing the medical student to the many therapeutics applied in the treatment of different pathologies—“the Unhappy-path”.

The purpose of this article is to propose how best to incorporate NIP as a section taught to medical students within the first year medical neuroscience class. We propose two additional 50-min lectures and an optional laboratory module to the current medical neuroscience curriculum, as proposed in Table 1—lectures 48 and 49, hereto referred to as lecture 1 and lecture 2, respectively. Lecture 1’s focus is on how the CNS and the immune system communicate with one another along with a detailed anatomical view of the pharmacologically relevant structure of the CNS, the blood–brain barrier (BBB), emphasizing the challenges for both small molecules and cells to transit the BBB. Lecture two is an introduction to CNS inflammation aimed to dispel the old paradigm that the CNS is an “immuno-privileged” region of the body. Further, the second lecture primarily looks at glial cell activation during an inflammatory state and compares/contrasts inflammation in the CNS versus inflammation in the periphery. Lastly, an optional lab module is provided as a visual learning tool to demonstrate how neuroinflammation manifests and influences the various disease pathologies, as well as demonstrating the benefits of pharmacological intervention of the immune system.

Our intention is to provide pre-made syllabi that can be used “as-is” or modified by medical school educators/neuroscience course directors at their discretion or as new material becomes available. As such, the syllabi provided include key learning objectives and a mini-synopsis of either larger texts or a summary of the current literature to-date focusing on the key ‘must-know’ items that each medical student needs to master to fully comprehend NIP. In addition, the tone and style of the syllabi is written more in a condensed, note-taking form to facilitate learning of the material to students with little or no prior exposure of the material. This change in style is intentional.

Further, we realize that pharmacology is a subject typically introduced to medical students during their second year, as previously mentioned. We feel strongly that exposing first year medical students to the concepts of NIP and raising awareness that pharmacological intervention targeting the immune response in the CNS offers novel treatment modalities to many CNS pathologies. One has to keep in mind that there are no formal educational

standards dictating to medical schools either when to introduce the subject of pharmacology or the specific content of pharmacological material that medical students must master, as defined by the Liaison Committee on Medical Education the accreditation authority for all medical schools in the US (Candler et al., 2007). Further, recent studies have demonstrated better long-term retention in medical education when material is repeated with some interval between repetitions, e.g., first year and then repeated again in second year, versus a once-only 'bolus' dose of material to master (Kerfoot et al., 2007, Kerfoot, 2008). Overall, we feel a better approach is to introduce NIP concepts early during the first year as part of a the medical neuroscience class and assume further iterations of the material in the second year and first-hand exposure as medical students enter their neurology rotation(s) in years 3 and 4; thus, solidifying the concepts of NIP and its therapeutic benefits to new clinicians.

Syllabus—lecture 1: CNS—immune communication pathways

Learning objectives

1. Understanding of the BBB and the impact the BBB has on pharmacological targets within the CNS.
2. Know the neuroanatomical locations of the circumventricular organs (CVOs) and putative monitoring functions these may enable the CNS to perform.
3. Know key neuroendocrine and neuropeptide secretion patterns and what their impact is on the systemic immune system.
4. Know key immune-related cytokines and their role in the CNS as it relates to the behavioral responses fever, sickness, and Stress.
5. Understand the proposed mechanism by which peripheral cytokine release can translate into neuronal action potentials and which neuronal circuits are activated and how.
6. Know the role that key neuronal receptors, classically found on neurons, act as signaling molecules to modulate microglia activation and alter the immune response in the CNS.

Neuroanatomical structures relevant to NIP: BBB and CVOs

The CNS (brain and spinal cord) is perhaps the single most protected organ within the body, first being entirely encased within bone, the skull, or vertebral column. Further, the exterior of the CNS is encased within three membranes—the dura mater (outer), arachnoid membrane (middle), and the pia mater (inner) that separate the brain parenchyma from the exterior (Wilson et al., 2010). Lastly, the BBB, blood cerebral spinal fluid barrier, and the blood arachnoid barrier all separate the external blood compartment from the brain parenchyma and the specialized extracellular fluid bathing the brain, the colony-stimulating factor (CSF; Abbott et al., 2010, Pasha and Gupta, 2010). These barriers pose critical challenges for both the natural immune system and clinicians alike when combating disorders within this very limited and tightly regulated organ.

Blood–Brain Barrier—The phenomena of the BBB was initially observed over 100 years ago by a German immunologist, Paul Ehrlich, when he injected various dyes intravenously, intraarterially, or subcutaneously and showed a differential staining patterns between the brain, staining weakly or not at all, and other organs, staining strongly (Bechmann et al., 2007, Gendelman and Ikezu, 2008; Ch 4). To this day, the BBB still remains an enigma and is perhaps the most significant hurdle in finding viable therapeutic treatments for psychiatric and neurological disorders (Carvey et al., 2009). Our current understanding is that the BBB primarily contributes to the maintenance of the homeostatic micro-environment around the

neuropil, which then enables proper neuronal function, through precise regulation of transit across the brain capillary endothelial cells of ions, neurotransmitters, macromolecules, glucose and other various nutrients, and potentially neurotoxins (Abbott et al., 2010). Further, the BBB is not just passive physical barrier, but also an active barrier controlling the exchange of solutes between the blood compartment and the brain parenchyma through a combination of both efflux and influx transporter mechanisms (Abbott et al., 2010, Pasha and Gupta, 2010).

BBB physical architecture: The physical BBB is created by CNS capillary endothelial cells that form tight junctions, which prevent most substances in the blood from passively diffusing into the brain parenchyma through normal starling forces (Bechmann et al., 2007, Carvey et al., 2009, Abbott et al., 2010, Pasha and Gupta, 2010). Capillary endothelial cells contain a zonula occludens, which effectively block substances as low as 3.5 Å in radius, i.e., nearly any ion (Abbott et al., 2010). At the molecular level, the tight junctions have been experimentally determined to comprise primarily of Claudin 3 and 5 and Occludin, and are linked to the intracellular cytoskeleton of the endothelial cells, thus forming a contiguous barrier along the capillary (Abbott et al., 2010). The next layer is a basal lamina that is elaborated by the endothelial cells, which forms the innermost layer of the perivascular space and is often embedded with pericytes connecting to the endothelial cells through a peg and socket junction (Abbott et al., 2010). A second outer, relative to the capillary vessel, basal lamina completes the perivascular space, which is elaborated by astrocyte end-foot process. The astrocyte end-foot processes cover the entire surface of all blood capillaries in the CNS and begins what is referred to as the glia limitans (Abbott et al., 2010). Each of these physical barriers must be penetrated before any substance reaches the neuropil or brain parenchyma.

Solute and small molecular therapeutic transit across the BBB: In general, *passive diffusion* across the BBB is limited to only substances that are small (<500 Da) and either lipid soluble/hydrophobic or non-polar. The vast majority of CNS-targeted pharmacological agents in clinical use fit this description and passively cross the BBB as their method of entry into the CNS. However, most solutes, and pharmacological agents, are not lipid soluble and normally do not transit the BBB unless special transport mechanisms are present. Brain endothelial cells are equipped with an array of both influx or efflux transporters to supply the CNS with key nutrients or remove undesired solutes, e.g., glucose and neurotoxins, respectively. Further, transporters can be polarized to either the luminal (capillary side), abluminal (brain side) or both sides to specifically pump solutes into the cell, out of the cell, or across the cell (Abbott et al., 2010). Specifically, this enables the selectivity in the regulation of what actually can cross the BBB, in a similar fashion as seen in the proximal convoluted tubule of the kidney and solute re-absorption employed during urine production (Abbott et al., 2010).

A key challenge for drug development and successful delivery of a therapeutic agent targeted to the CNS is to overcome these various pumps, which provide a 'second line of defense' against undesired entry into CNS (Pasha and Gupta, 2010). The most significant pumps expressed in brain endothelial cells belong to the adenosine triphosphate (ATP)-binding cassette (ABC) transporter family, such as the multi-drug resistance protein, P-glycoprotein, and the breast cancer protein (Abbott et al., 2010, Pasha and Gupta, 2010). All ABC transporters act as efflux pumps, which actively work by pumping metabolites, neurotoxins, even lipid-soluble solutes from the CNS and endothelial cells into the capillary lumen, thus acting in a neuroprotective and detoxifying role (Abbott et al., 2010, Pasha and Gupta, 2010). A core strategy for increasing the delivery of drugs to the CNS is by combining the drug with an inhibitor of the ABC efflux pumps to curtail their activity thereby allowing more of the drug to ultimately enter the CNS (Pasha and Gupta, 2010).

Large macromolecules, such as antibodies and cytokines, can potentially transit the BBB not through transporters, but by receptor-mediated transcytosis in endocytic vesicles on the luminal side, which ultimately exocytose on the abluminal side (Abbott et al., 2010, Pasha and Gupta, 2010). A second form of transcytosis that does not depend on receptor–ligand interaction is adsorptive-mediated transcytosis, which is charge dependant, requiring excess positive charge on the solute to initiate the endocytic vesicle (Abbott et al., 2010, Pasha and Gupta, 2010). Either method requires that endocytic vesicles avoid fusion with endolysosomes, which would result in the degradation of the contents (Abbott et al., 2010, Pasha and Gupta, 2010). Currently, the significance of this a mechanism under nominal conditions is unclear, but electron microscopy studies currently suggest that under neuroinflammatory conditions increase the number of endocytic vesicles observed transiting brain endothelial cells (Abbott et al., 2010).

Cellular transit across the BBB: As we will see, a number of pathogens can infect the CNS either acutely or latently and potentially result in disease. Therefore, there is a real need for immune surveillance within the CNS, just as in the periphery, which requires a method to transit immune cells across the BBB and into the CNS itself under non-inflammatory conditions (Wilson et al., 2010). In addition, this feature needs to be responsive to the immune system upon detection of a pathogen that enables recruitment of the immune system to fight off and defend against said pathogen (Abbott et al., 2010, Wilson et al., 2010).

Just as in the periphery, migrating leukocytes undergo diapedesis across the endothelial cells to enter the perivascular space due to a combination of adhesion molecules and integrins. Under non-inflammatory states, brain endothelial cells express constitutively P-selectin, vascular cell adhesion molecule 1 (VCAM1), and intercellular adhesion molecule (ICAM1; Wilson et al., 2010). VCAM1's ligand, very late antigen-4 (VLA-4) a.k.a. $\alpha 4\beta 1$ integrin, expressed on the leukocytes underwent clinical trials as a therapeutic target by the monoclonal antibody—natalizumab in the treatment of multiple sclerosis, a demyelinating autoimmune CNS disorder (Wilson et al., 2010). In addition, lymphocyte function-associated-1 (LFA-1), the ligand for ICAM1, was recently targeted in the treatment of psoriasis by another monoclonal antibody, efalizumab (Wilson et al., 2010). Both monoclonal antibody therapeutics apparently prevented leukocyte entry into the CNS and resulted in a small percentage of patients developing progressive multifocal leukoencephalopathy associated with the reactivation of latent JC polyomavirus, highlighting the need for immunosurveillance in the CNS (Wilson et al., 2010).

Once across the brain endothelial cell, the leukocyte enters the perivascular space. It is thought that matrix metalloproteinases facilitate the degradation of the basal lamina of both the brain endothelial and the outer basal lamina of the glial limitans and other loose connective tissue elements. Traditionally, chemokines have been linked to leukocyte migration in both the periphery and the CNS, but recently CXCR3, a chemokine receptor found on activated T and NK cells, and high concentration of its ligands, CXCL9, 10, and 11, have been implicated in the retention of these cells within the perivascular space (Muller et al., 2007, Wilson et al., 2010). Recently, using the experimental autoimmune encephalitis (EAE) model for multiple sclerosis, Savarin et al. (2010) demonstrated that efficient T-cell transit of the glial limitans is dependent on activated and recruited monocytes, by showing that inhibition of recruited monocytes increased retention and in the perivascular space and delayed penetration of T-cells across the glial limitans boundary.

Circumventricular organs—Humans have been provided five core senses to experience the external world, mediated through the CNS. However, very little is understood how the mind senses the internal body, one core aspect of the “mind-body connection”. CVOs are

emerging as the critical link that enable the CNS to sense the internal body's milieu by sampling circulating blood plasma, as well as the ability for the CNS to directly secrete neuropeptides and neurohormones directly into systemic circulation (Duvernoy and Risold, 2007, Rodriguez et al., 2010). CVOs are specialized organs within the brain that have two common features: all are located along the third or fourth ventricle wall and all have special fenestrated (leakier) capillary walls than normal BBB capillaries (Duvernoy and Risold, 2007). Currently, there are six identified locations in the brain with CVOs each with a putative function based on neuroanatomical location, summarized in Table 2 and showed in Fig. 1b.

Immune system to CNS interfaces

It has been a mere 15 years since the initial paper linking inflammation to behavior changes, specifically the cytokine-induced sickness behavior (Dantzer and Kelley, 2007, Gendelman and Ikezu, 2008; Ch 15 and 34). Since that time it has become clearer that peripheral inflammation induces more traditional psychiatric behavior changes where the mind and body work together to promote survival and recovery during infection (Dantzer and Kelley, 2007). Behavioral changes have been demonstrated upon either the detection of circulating pathogens that can illicit an innate immune response (e.g., endotoxin or double-stranded RNA) or circulating pro-inflammatory cytokines with interleukin-1beta (IL-1 β) being the most potent trigger (Cunningham et al., 2007, Dantzer and Kelley, 2007). The brain responds by initiating the sickness and febrile response, hypothalamic–pituitary–adrenal (HPA) axis, and most recently demonstrated to induce depression (Dantzer and Kelley, 2007).

The behavior changes of the sickness response manifest itself as malaise, fatigue, sleepiness, anorexia, apathy, and irritability (Dantzer and Kelley, 2007). The febrile response increases the core body temperature, a putative means of promoting immune cell proliferation and inhibiting bacterial or viral proliferation (Dantzer and Kelley, 2007). Activation of the HPA axis leads to the release of corticotrophic-releasing factor released from the paraventricular and supraoptic nuclei (PVN/SON), which in turn triggers the release of adrenocorticotrophic hormone (ACTH) from the anterior hypophysis (Sternberg, 2006, Dantzer and Kelley, 2007). Systemic ACTH acts on the adrenal glands to trigger the release of cortisol, which then feeds back on both the PVN/SON and anterior hypophysis as a feedback inhibition mechanism (Sternberg, 2006). However, more importantly, cortisol also suppresses the immune system (Sternberg, 2006).

Peripheral cytokine to neuronal modulation signal transduction—A general molecular mechanism appears to underlay all these behavioral changes observed during a peripheral inflammatory response. Regardless of the type of stimulus, either a pathogenic stimulus or cytokine (IL-1 β) secretion, can lead to eventual interaction with brain endothelial cells and perivascular microglia cells upon entering systemic circulation and can trigger the following cascade (Rivest, 2010):

1. Receptor–ligand binding on the brain endothelial cells and activated microglia triggers nuclear factor kappa B (NF- κ B) and mitogen-activation protein kinase pathways. Interestingly, this occurs strongest where the BBB is weakest, which is at regions where CVOs are present.
2. Upregulation of cyclooxygenase 2 (COX2) and microsomal prostaglandin E synthase (mPGES) and cytosolic phospholipase A2 (cPLA2- α) transcription. Note: this is primary site of action for the anti-pyrogenic activity of non-steroidal anti inflammatory class of drugs as non-specific COX inhibitors.

3. Within seconds, prostaglandin E2 (PGE2) synthesis occurs via release of arachidonic acid by cPLA2- α and subsequent conversion to PGE2 in a two-step conversion via COX2 and then mPGES activity.
4. PGE2 then diffuses freely across the glial limitans barrier and into the brain parenchyma. PGE2 release appears to be a general feature throughout the BBB upon IL-1 β detection and is therefore non-specific. However, PGE2's half-life is only 30 s, so diffusion is severely localized.
5. PGE2 binds to a prostanoid receptor, which are G-coupled receptors with four different possible versions. It is unclear which receptor is most important, but the specificity of prostanoid receptors determines the specificity of which neuronal-glial circuits are activated. Regions that show binding include the thalamic, PVN/SON, locus coeruleus, parabrachial nucleus, bed nucleus of the stria terminalis, and the central nucleus of the amygdala.

CNS to immune system interfaces

Microglia, the brain's resident immune cell, are ubiquitous throughout the brain (Pocock and Kettenmann, 2007, Gendelman and Ikezu, 2008; Ch 12.5). For over 20 years, it has been known that macrophages and T-cells possess receptors for classical neurotransmitters; and in the last 10 years, first glutamate and then gamma-aminobutyric acid (GABA) receptors were demonstrated on microglia (Farber et al., 2005, Pocock and Kettenmann, 2007). As we have just looked at the ability of the CNS to interpret cytokines and other immune stimuli, it only makes sense that immune cells, particularly microglia, have the ability to receive and interpret traditional neuronal signaling and thus providing contextual data about their surrounding environment.

A key challenge in this signal transduction mechanism is that microglia fails to form synapses, at least in the traditional neuron context of a synapse (Pocock and Kettenmann, 2007). This was overcome by the realization that neurotransmitters were not just limited to the synapse that some escape and form a concentration gradient around the synapse, extrasynaptic expression of neurotransmitter receptors are involved in excitotoxicity (Pocock and Kettenmann, 2007). This led researchers to hypothesize that glial cells, like microglia, receive signals from neurons not via traditional "wiring transmission" (synaptic connection), but by "volume transmission", based on the surrounding extra-synaptic concentration of a particular neurotransmitter (Pocock and Kettenmann, 2007). This section will focus on how microglia respond to various neurotransmitters and their effect on neuroinflammation, with a goal to demonstrate that the immune system can be modulated/regulated by the CNS.

Dopaminergic and adrenergic receptors—Microglia respond to both dopamine and norepinephrine (Pocock and Kettenmann, 2007). In addition, application of beta-adrenergic agonists mirror and agonists inhibit the effects of norepinephrine. Microglia express D1, D2, D4, D5 dopamine receptors and a full complement of α 1, α 2, β 1, and β 2 adrenergic receptors (Farber et al., 2005). Overall, both dopamine and norepinephrine have anti-inflammatory effects, but they have differential effects (Farber et al., 2005, Pocock and Kettenmann, 2007). Specifically, both dopamine and norepinephrine have been demonstrated to reduce levels of the cytokine IL-12p40, but only norepinephrine reduces the combination of TNF- α , IL-6, production of nitric oxide and modulated microglial proliferation (Farber et al., 2005, Pocock and Kettenmann, 2007). This provides a mechanistic way to understand how the selective serotonin reuptake inhibitors, such as fluoxetine used in the treatment of depression, can act as an anti-inflammatory since they also cross react with the norepinephrine reuptake receptor (Abdel-Salam et al., 2003).

Purinergic receptors—ATP is a neurotransmitter, but can be found in higher concentration during local brain injury, presumably from traumatic, necrotic cell death, and can induce rapid microglia activation (Pocock and Kettenmann, 2007). Microglia express a number of purinergic receptors that mainly respond to ATP, but also other purines as well, e.g., UDP, have key roles too (Pocock and Kettenmann, 2007). The effects on microglia of ATP are wide and generally aimed at pro-inflammatory activation of chemotaxis, increased cytokine release, and phagocytosis (Pocock and Kettenmann, 2007). Specifically, chemotaxis on resting microglia is controlled by the P2Y₁₂ receptor and its activation triggers the rapid attraction of microglial processes upon detection of ATP directed along the chemical gradient of ATP (Pocock and Kettenmann, 2007). UDP interaction with P2Y₆ receptor triggers phagocytosis, more than likely aimed at detection of necrotic cells requiring removal (Pocock and Kettenmann, 2007). Lastly, the P2X₇ receptor has been implicated in the increase release of TNF- α , IL-1 β , and superoxide production via depletion of intracellular K⁺ (Pocock and Kettenmann, 2007).

Neurotransmitter: glutamate—Glutamate is the major excitatory neurotransmitter of the CNS, possessing two major classes of receptors: ionotropic (fast) and metabotropic (slow; Gendelman and Ikezu, 2008). Glutamate can not only stimulate microglia through release volume transmission from the surrounding excitatory synapses, but is also actively secreted by the microglia upon stimulation from LPS or amyloid- β peptides (A β ; Pocock and Kettenmann, 2007). Microglia express both the ionotropic and metabotropic glutamate receptors each providing unique functionality (Pocock and Kettenmann, 2007).

Microglia expression of ionotropic glutamate receptors is mainly AMPA (D,L- β -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) ionotropic, with fewer kainate receptors, and no identified NMDA (N-methyl-D-aspartate) receptors are found on microglia (Pocock and Kettenmann, 2007). Ionotropic glutamate receptors have a differential role on microglia. AMPA receptor activation acts in an anti-inflammatory role, reduced TNF- α levels, whereas kainate receptors act in a pro-inflammatory role by increasing microglial activation and TNF- α levels (Pocock and Kettenmann, 2007).

Metabotropic glutamate receptors (mGluRs) have either a neuroprotective or neurotoxic effect depending on specific subtype expressed on the microglia. Neurotoxic effects are mediated through Group II mGluRs triggering the release of TNF- α and Fas ligand, which in turn initiates neuronal apoptosis (Pocock and Kettenmann, 2007). Activation of the group II mGluRs has been speculated to occur in an autocrine fashion from the glutamate released from the microglia itself, as mentioned above (Pocock and Kettenmann, 2007).

Neuroprotective effects are mediated through group III mGluRs and the neuroprotective effect can be modulated by the application of specific group III mGluR agonists (Pocock and Kettenmann, 2007). Affinity differences for glutamate between the two groups, with group II having greater affinity than group III, favors a neurotoxic phenotype (Pocock and Kettenmann, 2007).

Neurotransmitter: GABA—GABA is the major inhibitory neurotransmitter of the CNS (Gendelman and Ikezu, 2008). All three main GABA_B receptors are present on microglia and upregulated upon activation (Pocock and Kettenmann, 2007). As in neuronal cells, GABA_B receptors activate a K⁺ current, which has been demonstrated to reduce cytokine release upon LPS stimulation, thereby acting in as a potential anti-inflammatory pathway (Pocock and Kettenmann, 2007, Gendelman and Ikezu, 2008).

Syllabus—lecture 2: intro to CNS inflammation and clinical correlates

The following syllabus is proposed for day 2, which covers an introduction to CNS inflammation and relates CNS inflammation to key neurological disorders.

Learning objectives

1. Understand the concept of immune-privileged vs. immune-suppressed as it relates to CNS inflammatory response.
2. Understand the stages of the inflammatory reaction in the CNS and the role that microglia, astrocytes, T-cells, and neurons play.
3. Understand and be able to differentiate the different states of microglia and the functional role of each state.
4. Understand key differences in the inflammatory reaction within the CNS versus an inflammatory reaction within the periphery.
 - a. Higher set point/threshold
 - b. Microglia proliferation versus neutrophil recruitment
5. Know the role that inflammation and microglia play in following clinical correlates:
 - a. Neurodegenerative disorders
 - b. Stroke
 - c. NeuroAIDs
 - d. Multiple sclerosis
 - e. Depression

CNS inflammation

One of the most widely studied environmentally cued physiological and pathological conditions known to mammalian systems is that of inflammation. Today, inflammation is thought of more as an adaptive response, primarily to either infection or tissue injury, with the overall goal of restoring a homeostatic balance to the affected region (Medzhitov, 2008). While acute inflammation is a physiological process and in general is beneficial, e.g., removal of a pathogen and tissue regeneration, if dysregulation occurs can be equally detrimental to the host resulting in conditions like sepsis, chronic inflammatory states (e.g., rheumatoid arthritis), or various autoimmune disorders (e.g., multiple sclerosis; Medzhitov, 2008). Inflammation, whether in the peripheral or the CNS, is governed by the inflammatory process comprising of the following four steps, a.k.a “The four R’s of the immune response”: recognition, recruitment, removal, and resolution/repair (Gendelman and Ikezu, 2008; Ch 2, 8.4.4, 9, 16.4, 45).

Summary of the immune response in the periphery—Typically, in the peripheral the general acute inflammatory response is initiated by detection of some external pathogen (e.g., bacterial or viral entity) or tissue injury (e.g., hypoxic stress) by resident cells of the innate immune system, e.g., macrophages or mast cells, that results in local production of cytokines, chemokines, and many other bioactive compounds that trigger the inflammatory response to many cell types in the surrounding vicinity (Medzhitov, 2008). Cytokines send messages to activate local post-capillary endothelial cells, which in turn become more leaky and allow for the extravasation of neutrophils (Medzhitov, 2008). Chemokines direct

neutrophils to the site of the infection or injury, and once there, the neutrophils attempt to kill the detected pathogen or affected injured cells via release of reactive oxygen and reactive nitrogen species from their granules (Medzhitov, 2008). A round of monocyte recruitment follows the neutrophil recruitment to the affected regions that are phagocytic to clear damage or infected cells (Medzhitov, 2008). Once region is cleared of the infection or damage acute inflammation response then transitions to a repair and regenerative response mediated by different biochemical signals, e.g., transforming growth factor- β (TGF- β ; Medzhitov, 2008). TGF- β recruits monocytes which work to remove dead cells, by phagocytosis, and initiate tissue remodeling and/or scar formation if the tissue cannot be regenerated (Medzhitov, 2008).

CNS Under active immuno-suppression at rest—Initially it was originally thought that the CNS was an ‘immune-privileged’ region of the body (Gendelman and Ikezu, 2008, Wilson et al., 2010). A concept put forth due to the observation of the BBB restriction of cytokines and leukocyte entry (as described in “Neurodegenerative Diseases and Inflammation” section), lack of lymphatic drainage, and low levels of constitutive major histocompatibility complex (MHC)-class I and II expression (Gendelman and Ikezu, 2008, Wilson et al., 2010). However, a more realistic view of the CNS is that of an actively ‘immune-suppressed’ region by multiple mechanisms, that raise the threshold to initiate an immune response higher than elsewhere in the body (Ouchi et al., 2009). For example, neuronal expression of CD200 on their surface interacting with CD200 receptor on microglia (Gendelman and Ikezu, 2008), electrical stimulation from neurons that is detectable by microglia, and astrocyte secretion of TGF- β all function to suppress activation of an immune response in the CNS from insult and maintain microglia in a resting state, see below (Ouchi et al., 2009). Overall, this constitutes the first way the immune response in the CNS is different than in the periphery, i.e., microglia are actively suppressed leading to a higher threshold of insulting agent before microglia respond.

Microglia role in CNS inflammation—Microglia has long been described as the resident immune cell of the CNS, with key roles in homeostasis maintenance, host defense, and repair (Gendelman and Ikezu, 2008, Lynch, 2009). Microglia comprise 5–10% of the cells in the CNS and are found in higher concentration in gray matter versus white matter of the brain (Lynch, 2009). Interestingly, unlike resident macrophages in peripheral organs that originate from recruited monocytes, microglia is thought to originate from early embryonic macrophage-like mesodermal precursor cells prior to the closure of the BBB (Lynch, 2009, Streit and Xue, 2009). In the adult, there is “overwhelming evidence” that microglia coordinate and orchestrate the inflammatory response in the CNS, through a series of phenotypically observable state changes (Gendelman and Ikezu, 2008, Lynch, 2009, Streit and Xue, 2009).

Initially, prior to some disruption of the homeostatic balance, microglia’s role is one of surveillance/recognition of stimuli and scavengers of debris that can potentially perturb the steady-state balance to a given tissue (Gendelman and Ikezu, 2008, Medzhitov, 2008). In microglia, this surveillance state is referred to as “resting (monitoring) microglia” and has a characteristic phenotype of small soma and highly ramified processes, i.e., very long and arborized cytoplasmic projections, illustrated in Fig. 2 (Town et al., 2005). Microglia cells themselves are non-motile in this state; hence, the term resting, but their cytoplasmic processes are highly motile sampling repetitively a particular territory with little overlap (Town et al., 2005, Lynch, 2009, Streit and Xue, 2009). Further, microglia possess an array of sensors (see “CNS to immune system interfaces” section) that sample the local micro-environment and surrounding cellular elements ranging from the ability to detect pathogens and to assess neuronal functionality (Lynch, 2009).

Typically, the acute inflammatory response in the CNS is initiated when microglia detect an invading pathogen (e.g., bacterial or viral entity), tissue injury (e.g., hypoxic stress or trauma), or upon neuronal disconnection (e.g., peripheral nerve/spinal cord lesion) resulting in microglia activation (Town et al., 2005, Medzhitov, 2008, Ouchi et al., 2009). Microglial activation is the hallmark of neuroinflammatory response (Gendelman and Ikezu, 2008, Ouchi et al., 2009). Microglia, like peripheral macrophages, recognize potential non-self pathogens via a series of pathogen-associated molecular patterns receptors that detect various motifs specific to pathogens, e.g., toll-like receptor-4 (TLR-4) recognition of Gram-negative bacteria via LPS interaction (Town et al., 2005, Medzhitov, 2008). Activated microglia also have a characteristic morphology where ramified processes shorten and enlarge the soma, referred to as “amoeboid” phenotype, as illustrated in Fig. 2 (Town et al., 2005).

In addition, activated microglia upregulate a number of surface markers, cytokines, chemokines, and many other bioactive compounds that trigger the inflammatory response to many cell types in the surrounding vicinity (Town et al., 2005, Medzhitov, 2008). Upregulated cell surface markers cross activate the adaptive immune response, including major histocompatibility complex (I and II), co-stimulatory receptors B7.1 and B7.2, and CD40 or suppress activation CD200R and fractalkine R (Town et al., 2005, Lynch, 2009). Further, activated microglia significantly increases their phagocytic capability and secretes pro-inflammatory innate cytokines (TNF- α , IL-1 β , and IL-6; Town et al., 2005).

There is an emerging recognition that classifying microglia states as “resting” or “activated” is a gross oversimplification and the phenotypical alterations between the two may mask functional state differences (Lynch, 2009). As many as four functional states have been proposed to describe activating microglia: cytokine producing, surface marker expressing, phagocytic, and neurotrophin producing (promotes wound healing in the CNS; Lynch, 2009). Interestingly, these states loosely mirror peripheral macrophage states commonly described as M1, classically activated, and M2, alternatively activated, macrophages, which are pro-inflammatory/pro-recruitment or highly phagocytic, respectively (Ransohoff and Perry, 2009). A better understanding of these microglia states, how they apply to various neurological disorders, which states are neuroprotective versus neurotoxic, and most importantly how to trigger alterations in microglia states are all critical points that need to be addressed that will aid in the design of better therapeutical interventions to CNS inflammation (Lynch, 2009).

Recruitment of peripheral immune cells—Perhaps the most significant difference between CNS-driven inflammatory response and peripheral-driven inflammatory response is in the recruitment of neutrophils, within hours of insult, followed about a day later by monocyte/macrophage recruitment (Medzhitov, 2008). In a periphery inflammatory response, the released cytokines from resident macrophages activate local endothelial post-capillary cells, which in turn become more leaky and allow for the recruitment of neutrophils and macrophages to the location of the insult (Medzhitov, 2008). However, recruitment of neutrophils and macrophages is not as clear-cut and appears to be highly exclusive into the CNS, possibly not even occurring at all during the inflammatory reaction (Ferrari et al., 2004, Denes et al., 2007). Using an experimental stroke model, observers primarily detected a very robust microglia proliferative stage with little or no recruitment of exogenous macrophages despite a detectable disruption of the BBB (Denes et al., 2007). However, neutrophil infiltration was observed peaking at 48–72 h and increased proportionally relative to the duration of the occlusion in the same stroke model (Denes et al., 2007). Similarly, upon a viral infection resulting in vesicular stomatitis viral encephalitis, a rather robust neutrophil recruitment occurs centered around perivascular spaces within 24–36 h post-infection (Ireland and Reiss, 2006). Finally, under a simulated CNS chronic

inflammatory state induced by prolonged (>30 days) of IL-1 expression, neutrophil recruitment was not observed for 8 days, peaked at 14 post-IL-1 administration, and was associated with the breakdown of the BBB and demyelination (Ferrari et al., 2004). Interestingly, by day 30, there was no apparent neutrophil invasion and both reestablishment of BBB integrity and re-myelination occurred, in other words, a complete resolution of the inflammatory reaction despite persistent IL-1 expression (Ferrari et al., 2004).

Astrocyte activation in CNS inflammation—Astrocytes, the most abundant glial cell type in the brain, provide critical metabolic nutrients, comprise portion of the BBB (see “BBB Physical Architecture” section), and aid in the ionic and neurotransmitter homeostasis (Escartin and Bonvento, 2008, Gendelman and Ikezu, 2008). As with microglial activation, upon stimulation from cytokines (IL-1 β and/or TNF- α), astrocytes too are activated and undergo characteristic morphological and functional alterations (Escartin and Bonvento, 2008). Upon activation, astrocyte processes, stress fibers, soma hypertrophy, proliferation (leading to gliosis), and overexpression of characteristic intermediate filaments glial fibrillary acidic protein (GFAP), vimentin, and nestin (Escartin and Bonvento, 2008, Gendelman and Ikezu, 2008). However, the time course of astrocyte activation and function is complex and variable in nature depending on the actual stimuli (Escartin and Bonvento, 2008).

Experimental methods to inhibit/ablate astrocyte activation have demonstrated much more severe lesions to physical wounds and traumatic cortical injury, attributed to disruption of the BBB integrity resulting in more inflammatory cell infiltration and more neuronal cell death (Escartin and Bonvento, 2008). Therefore, proper astrocyte activation aids in maintaining BBB integrity and preventing peripheral immune cell infiltration (Escartin and Bonvento, 2008). In addition, through studies inducing astrocyte activation have demonstrated additional functions astrocytes provide during a CNS inflammation: enhanced glutamate homeostasis, antioxidant secretion, energy metabolism, and production of neurotrophic factors (Escartin and Bonvento, 2008). Specifically, an upregulation of glutamate excitatory amino acid transporters 1 and 2, which facilitates the removal of glutamate, and the secretion of the antioxidant glutathione both mechanisms enhance neuroprotection. Energy metabolism is altered, with more reliance on fatty acid β -oxidation and depletion of glycogen stores to maintain glucose supply to neuron and minimize reliance on glucose provided from systemic circulation, ideal in a time of stroke/ischemic condition (Escartin and Bonvento, 2008). Lastly, increased production of fibroblast growth factors 1 and 2 (FGF-1/2) and nerve growth factor, which generally function as neuroprotective can be potentially neurotoxic to damaged nerve cells (Escartin and Bonvento, 2008).

A hallmark of wound healing is scar formation, only if the original tissue cannot be remodeled, which is associated with the final resolution of the inflammatory process (Gendelman and Ikezu, 2008, Medzhitov, 2008). Astrocytes mediate CNS scar formation, termed gliosis, and is dependent on the nature of the insult (Escartin and Bonvento, 2008). A focused local disruption of the brain parenchyma leads to astrocytes extending their processes along a single plane demarcating the lesion from unaffected/healthy CNS parenchyma, termed anisomorphic gliosis and is irreversible (Escartin and Bonvento, 2008). Where lesions that are less focused, common in Alzheimer's disease, activated astrocytes are more evenly distributed and have randomly oriented processes, termed isomorphic gliosis, which is reversible (Escartin and Bonvento, 2008).

Oligodendrocyte and neuronal cell role in CNS inflammation—Oligodendrocytes provide CNS neuronal axons myelin sheathing, insulation, with each supporting myelination for up to 50+ internode regions of multiple neurons. During inflammatory states, oligodendrocytes are particularly susceptible to damage from reactive oxygen and nitrogen

species (ROS/RNS) due to high lipid content of myelin and lipid peroxidation (Zeis and Schaeren-Wiemers, 2008). Recent data suggests that, like other glial cells, oligodendrocytes actively react to pro-inflammatory conditions around them, which in turn triggers the induction of protective mechanism designed to modulate the inflammatory response (Zeis and Schaeren-Wiemers, 2008). In response to IFN- γ , oligodendrocytes initially upregulate protective antioxidant producing enzymes and anti-inflammatory chemokines (CCL2, 3, and 5), but if over-whelmed by IFN- γ the oligodendrocyte induces apoptosis (Zeis and Schaeren-Wiemers, 2008). In addition, oligodendrocytes secrete TGF- β in response to a pro-inflammatory micro-environment, which is both immunosuppressive and potentially promotes remyelination (Zeis and Schaeren-Wiemers, 2008).

Clinical correlates

This section reviews the role the immune systems influences various disorders of the CNS and how pharmacological targeting of the immune system can be therapeutic to each condition.

Neurodegenerative diseases and inflammation—Collectively, the neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease are clinically disparate in etiology, but all share a common trait in their pathogenesis: Sustained activation of CNS inflammatory processes (Gendelman and Ikezu, 2008, Glass et al., 2010; Ch 39).

AD affects ~40% of people >80 years old in industrialized countries, with common symptoms including memory loss, progressive loss of cognition, and various behavioral disturbances (Gendelman and Ikezu, 2008, Glass et al., 2010). Two key pathologies are observed in AD are extracellular plaques composed of cleaved fragments of amyloid precursor protein (APP), A β 1-42 or A β 1-40, and intracellular neurofibrillary tangles composed of hyperphosphorylated microtubule binding tau protein (Gendelman and Ikezu, 2008, Glass et al., 2010).

Interesting, when looking microglia and astrocytes in AD brain, both cell types appear in their activated state, see "Microglia role in CNS inflammation" and "Astrocytes activation in CNS inflammation" sections, respectively, surrounding the A β plaques (Glass et al., 2010). Further, microglia are elaborating pro-inflammatory cytokines IL-1 β , TNF- α , IL-6, and have upregulated MHC-II expression, all markers of activated microglia, as well increased production of ROS/RNS and astrocyte activation (Glass et al., 2010). Activation of microglia by A β plaques is thought to occur through interactions with either TLR receptors and/or scavenger receptor for advanced glycosylation end-products receptors (Glass et al., 2010). It is thought that the proinflammatory factors and ROS/RNS are neurotoxic, particularly to the cholinergic neurons in the basal forebrain involved in short- to long-term memory conversion being the most sensitive, which in turn further activates microglia through the release of ATP and interactions with the P2X7 receptor, see "Purinergic receptors" section (Glass et al., 2010).

Confusion comes since there is also evidence to support microglia in a neuroprotective role in AD as well. One of the strongest genetic associations with AD is apolipoprotein E (apoE4), a protein involved in very low-density-lipoprotein clearance (Glass et al., 2010). Recent studies demonstrate apoE binding to its receptor microglia activation is attenuated and this affect is isoform specific, with apoE4 being the weakest stimulator of this pathway. In addition, over-production of microglia, through sustained stimulation by macrophage-colony stimulating factor (m-CSF) injections over a 4-month period increased both the number of microglia and clearance of A β plaque and conveyed neuroprotection, see "Alzheimer's disease and neuroinflammation" section for lab exercise (Glass et al., 2010).

PD is the second most common neurodegenerative disease and most common movement disorder in the US (Glass et al., 2010). PD is characterized by bradykinesia (slow to initiate movements), “pill-rolling” tremor, rigidity, and postural instability (Glass et al., 2010). Damage seems to focus in the dopaminergic neurons of the substantia nigra (SN) and is the hallmark pathology is the presence of Lewy bodies, aggregates of misfolded α -synuclein protein in neurons (Glass et al., 2010). The etiological cause of PD is unclear, but inflammatory process appears to play a role in the pathogenesis of PD (Glass et al., 2010). Activated microglia, ROS and increased levels of pro-inflammatory cytokines (TNF- α and IL-1 β) are observed in the SN of PD patients (Glass et al., 2010). Whether neuronal cell death leading to release of misfolded α -synuclein or extra cellular aggregation of misfolded α -synuclein play a role in initiating the immune response in PD is unclear (Glass et al., 2010). One thing is clear, once initiated, inflammation seems to exacerbate the damage through further production of ROS, which is already increased as a result of increased dopamine synthesis, making the remaining neurons highly susceptible to further cell death from oxidative stresses, see “Parkinson’s disease and neuroinflammation” section (Glass et al., 2010).

ALS, or Lou Gehrig’s disease, is a fatal neurodegenerative disease affecting both upper motor (motor cortex) and lower motor (anterior horn of spinal segments) neurons (Glass et al., 2010). ALS patients suffer from fasciculations, muscle wasting and weakness, increased spasticity and hyper-reflexia (Glass et al., 2010). Death is commonly due to progression of the disease to motor neurons controlling the diaphragm and hence affecting respiratory processes. Inflammation is prominent component of ALS and activated microglia and astrocytes are observed in all regions affected by ALS, see “ALS and neuroinflammation” section for lab exercise (Glass et al., 2010).

Stroke—Stroke is the third leading cause of death and most common cause of permanent disability worldwide (Gendelman and Ikezu, 2008, Jin et al., 2010; Ch 31). Two primary types of stroke, ischemic or hemorrhagic, are caused by either thrombosis or a ruptured arterial blood vessel in the brain, respectively, with the middle cerebral artery the most commonly affected vessel (Gendelman and Ikezu, 2008). CNS parenchyma downstream from the affected vessel loses its supply of oxygen and nutrients, and an ischemic condition is detected that elicits an inflammatory reaction (Gendelman and Ikezu, 2008, Jin et al., 2010).

The ensuing inflammatory response has been demonstrated to correlate to the severity of the infarct and subsequent neurological deficits (Jin et al., 2010). Microglia activation initiates the inflammatory response to stroke, occurring within minutes of the ischemic event (Jin et al., 2010). This triggers a period of microglia proliferation, peaking at 2–3 days post-event, and the elaboration of ROS, cytokines (IL-1 β , TNF- α , and IL-6), and chemokines (Denes et al., 2007, Jin et al., 2010). Neutrophil invasion is a hallmark of stroke, perhaps due to breakdown of BBB during the ischemic event, and the severity of damage in a stroke has been directly attributable to the number of invading neutrophils (Jin et al., 2010). Neutrophil invasion starts as early as 30-min after the ischemic event and peaks by the third day, with gradual reduction until complete clearance is achieved (Denes et al., 2007, Jin et al., 2010). Neutrophils are thought to mediate tissue damage by the excessive production of ROS/RNS, additional release of pro-inflammatory cytokines/chemokines (elastase and metalloproteinase-9), and increased expression of adhesion molecules (Denes et al., 2007, Jin et al., 2010). Microglia, on the other hand, are responsible for the clearance of infiltrating neutrophils, by induction of apoptosis and phagocytosis of neutrophils, and is now considered a key step in limiting tissue damage during an ischemic event in the CNS (Denes et al., 2007, Jin et al., 2010).

Therapeutic treatment of strokes have traditionally centered on clot dissolution, through the application of tissue plasminogen activator (Gendelman and Ikezu, 2008, Jin et al., 2010). However, an emerging idea in stroke treatments is to target anti-inflammatory therapy, mainly through the blockade of leukocyte invasion (Gendelman and Ikezu, 2008, Jin et al., 2010). Specifically, targeting of anti-adhesion molecules that facilitate leukocyte adherence to activated endothelial cells, e.g., CD11b/cd18, ICAM-1, and P-selectin (Jin et al., 2010). In vivo animal studies have all shown promising results with reductions in stroke volume, but this success has not translated well to humans (Jin et al., 2010).

NeuroAIDS—NeuroAIDS is general term related to the neurocognitive deficits the result from HIV infection in the CNS (Gendelman and Ikezu, 2008, Gras and Kaul, 2010; Ch 41). HIV-associated dementia (HAD) is the most severe form of neuroAIDS, but more common today is mild neurocognitive deficits (Gendelman and Ikezu, 2008, Gras and Kaul, 2010). Before the discovery of highly active antiretroviral therapy (HAART), neuroAIDS was a very common complication of AIDS with prevalence as high as 66% of HIV-positive patients in the early 1990s, but after the application of HAART, HAD prevalence fell to only 1% in 2000 (Gendelman and Ikezu, 2008, Gras and Kaul, 2010). However, with HIV-positive patients now living much longer life span, due to more effective HAART combinations, neuroAIDS is once again on the rise (Gras and Kaul, 2010). One possible reason for this is that HAART pharmacological agents have variable CNS penetration so HAART is not as effective in the CNS versus the periphery (Gras and Kaul, 2010). In fact, recent studies demonstrate that microglia activation levels are comparable today to those seen in pre-HAART days, suggesting that once the HIV virus travels across the BBB, it is somewhat protected from HAART therapy (Gras and Kaul, 2010). Clinical signs of HIV encephalitis (HIVE), the acute form of neuroAIDS, include: microglia nodules, multinucleated giant cells, widespread reactive astrogliosis, and decrease in synaptic and dendritic density with neuronal cell loss (Gras and Kaul, 2010).

HIV's entry into the brain appears to occur early in the course of the infection via infected macrophages and/or lymphocytes without any BBB disruption (Gras and Kaul, 2010). HIV infection in the CNS persists primarily in perivascular macrophages and microglia, with more infected and activated microglia correlating to the severity of the NeuroAIDS, see "HIVE and neuroinflammation" section for lab exercise (Gras and Kaul, 2010). In addition, BBB alterations also figure prominent in HIVE with reduced expression of tight junctions leading to increased permeability and HIV+peripheral monocyte infiltration (Gras and Kaul, 2010). Taken together, this suggests that either microglia or the BBB are more suitable therapeutic targets than the CD4+ lymphocytes more commonly associated with HIV infection.

Multiple sclerosis—Multiple sclerosis (MS) is an inflammatory demyelination disease of the CNS, targeting the oligodendrocyte specifically (Gendelman and Ikezu, 2008, Zeis and Schaeren-Wiemers, 2008; Ch 40). Currently, the most accepted etiology of MS is that it is driven by the adaptive immune response, in the form of T-cell mediated (CD4⁺, CD8⁺, and γ/δ T cells) immunity against antigens found uniquely on oligodendrocytes (Gendelman and Ikezu, 2008, Zeis and Schaeren-Wiemers, 2008). In addition, secondary microglia activation exacerbates demyelination. Opsonized oligodendrocytes, with either antibody or complement, are commonly observed; see "MS and Neuroinflammation" section for lab exercise (Zeis and Schaeren-Wiemers, 2008). However, it is unclear what actually initiates the T-cell response against the oligodendrocytes. There are two competing hypotheses: (1) intrinsic oligodendrocyte damage and subsequent inflammatory response during which presentation of oligo specific proteins (e.g., myelin basic protein (MBP)) results a T-cell response against oligodendrocytes and subsequently drives the development of MS (Gendelman and Ikezu, 2008, Zeis and Schaeren-Wiemers, 2008). Alternatively, (2) a viral

pathogen with an antigen that has molecular mimicry to MBP that ultimately results in MS (Gendelman and Ikezu, 2008, Zeis and Schaeren-Wiemers, 2008).

Depression—A better appreciation for the inflammation either CNS or peripherally derived has recently been demonstrated to induce depression independent of the sickness response (Dantzer and Kelley, 2007, Frenois et al., 2007, Gendelman and Ikezu, 2008; Ch 35). A potential mechanism in the cytokine-induced depression is indoleamine-2,3-dioxygenase (IDO) enzyme, which is upregulated during inflammation (Dantzer and Kelley, 2007). IDO degrades the amino-acid tryptophan, the limiting factor in the synthesis of serotonin (Dantzer and Kelley, 2007, Frenois et al., 2007). Further, degradation of tryptophan results in the generation of neurotoxic metabolites e.g. quinolinic acid and 3-hydroxy-kynurenine that act as agonists on glutamatergic neurons (Dantzer and Kelley, 2007).

Neuroinflammation lab learning examples

This section is intended to visually demonstrate how neuroinflammation manifests and influences the various disease pathologies discussed above. Further, two examples are provided where modulation of inflammatory processes by pharmacological agents affect in a positive manner disease progression to illustrate the concept of NIP. Application of this, or similar material, can be employed in a group discussion setting along with a series of discussion questions, based on the clinical situation at hand, to walk and guide the student through examples to appreciate neuroinflammatory processes and realize it is a common underpinning of many neurological disorders. Further, to realize the therapeutic potential that modulating the immune system can play in the future treatment of many neurological disorders.

Alzheimer's disease and neuroinflammation

To illustrate characteristic microglial activation in AD, transgenic (Tg) (APP+PS1) mice were used that spontaneously develop A β plaque depositions. To illustrate activated microglia in AD, mouse samples from the hippocampus immunohistologically labeled with Iba1, a macrophage/microglia-specific calcium-binding protein that is expressed at any functional state (Suppl. Fig. 1a; Sanchez-Ramos et al., 2009). The instructor should point out and highlight the particular pattern of microglial staining in AD, clustering, surrounding, and invading the areas of amyloid deposits in the hippocampus.

As an example of a potential neuroimmune pharmacological therapeutic, application of granulocyte macrophage colony-stimulating factor (GM-CSF) subcutaneously in the periphery has implications for CNS inflammatory processes that potentially can affect the pathogenesis of AD. GM-CSF administration appears to act by increasing the overall microglial burden, even mildly in control NT mouse, but much more pronounced in the Tg mouse model of AD (Sanchez-Ramos et al., 2009). Again, note that the increased numbers in microglia are still centered around and in the amyloid plaque depositions.

To determine if the increased number of microglia had an impact on AD disease progression, immunostaining of A β plaques was measured, as shown in Suppl. Fig. 1b. Sections from both the hippocampus and entorhinal cortex in Tg mice from untreated versus treated with GM-CSF were stained (Sanchez-Ramos et al., 2009). Significantly less plaque was observed in either location for the GM-CSF treated versus the vehicle (Sanchez-Ramos et al., 2009). Overall, this suggests that bone marrow derived monocyte hematopoiesis is increased, providing more monocytes, with increased recruitment from the periphery for final differentiation to microglia. The more microglia present at the site of A β plaques, the

greater the ability to clear A β plaque and better performance on behavior tests (Sanchez-Ramos et al., 2009).

Parkinson's disease and neuroinflammation

This module is designed to demonstrate Neuroinflammation in PD by demonstrating microglial activation and the production of ROS using a standard drug induced, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), model for PD. The MPTP-treated mice were intraperitoneally injected hydroethidine (a marker for ROS), followed by immunofluorescence of substantia nigra pars compacta (SNpc) with Mac-1 (microglia marker, Suppl. Fig. S2; Wu et al., 2003). In the MPTP induced PD model, excessive amounts of microglia and ROS production primarily occurring in the SNpc (Wu et al., 2003). Further, a second example of a potential neuroimmune pharmacological therapeutic, the antibiotic minocycline is applied to block microglial activation and demonstrate reduced ROS production and microglia accumulation within the SNpc despite MPTP treatment (Wu et al., 2003).

ALS and neuroinflammation

To demonstrate that neuroinflammation is a component in ALS, cervical spinal cord hemisections from human samples of ALS patients and control patients were immunohistochemically stained for HLA-DR (MHC-II, a marker of activated microglia, Suppl. Fig. S3; Kawamata et al., 1992). Suppl. Fig. S3 a and c are control (non-disease) patients with little staining and b and d are ALS patients demonstrated significant numbers of microglia in the anterior and lateral funiculus as well as in the anterior horn (Kawamata et al., 1992); c and d are higher power magnification of the lateral funiculus.

MS and neuroinflammation

Perivascular inflammation, referred to as perivascular cuffing, is a region where infiltrating inflammatory cells (T-cells and macrophages) enter the CNS, which is thought to be an early indication of demyelination (Ge et al., 2005). Using an EAE model for MS, application of MRI using T2 weighting can be used to visualize perivascular cuffs and Hematoxylin and Eosin staining to confirm perivascular inflammatory infiltrates (Suppl. Fig. S4; Ge et al., 2005).

HIVE and neuroinflammation

To explore the initial phases of HIV entry into the CNS, which is thought to be mediated through macrophage transit from periphery to CNS, it is necessary to image macrophages originating from the bone marrow versus resident microglia (Liu et al., 2008). By application of a focal mouse model of HIVE, where severe combined immunodeficiency mice were intracranially injected with HIV⁺ human monocyte/macrophages and then transplanted with superparamagnetic *iron* oxide-labeled mouse bone marrow macrophages intravenously provided the ability to track the transit of monocytes in vivo (Liu et al., 2008). The results demonstrate iron staining with Prussian Blue of transplanted IV-injected monocytes in the brain parenchyma increased astrocyte activation by staining with GFAP, and increased neuron cell loss by staining with NeuN (Suppl. Fig. S5b, b; Liu et al., 2008). However, with little or no evidence for an invasion of peripheral macrophages, astrocyte activation, or neuronal loss in the control sham injection (Suppl. Fig. 5a, c, e; Liu et al., 2008).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Anatomy of the blood–brain barrier and circumventricular organs. **a** Anatomical representations of the blood–brain barrier (BBB), which should highlight the challenges in traversing from the blood to brain compartments by either small molecules or cells of the peripheral immune system, e.g., neutrophil, monocytes, or T-cells. The BBB is comprised of the following anatomical structures: The capillary lumen, brain endothelial cell with tight junctions, brain endothelial basement membrane, the perivascular space, glial (astrocyte) basement membrane, glial limitans (comprised of Astrocyte foot processes) and lastly the brain parenchyma. **b** Anatomical locations of the six circumventricular organs situated around the third and fourth ventricles. The neurohypophysis (NH) and median eminence, vascular organ of the lamina terminalis (OVLT), subfornical organ (SFO), subcommissure organ (SCO), pineal gland (PI), and the area postrema (AP)

Fig. 2.

Characteristic morphological changes between resting microglia and activated microglia (Stence et al., 2001). Microglial activation was induced by euthanization (hypoxic condition). **A** Resting microglia demonstrate small highly ramified morphology (Stence et al., 2001). **B** Upon activation microglia activate and their soma increase in size and their ramified morphology disappears (Stence et al., 2001). **C** Three examples of 3D rendering of isolated microglia cells to illustrate morphological changes (Stence et al., 2001)

Table 1

Proposed medical neuroscience syllabus with Neuroimmune pharmacology

Part I	Part II
Lecture 1—Course introduction	Lecture 29—Motor systems 1
Lecture 2—Intro to neurophysiology	Lecture 30—Motor systems 2
Lecture 3—Intro to neuroanatomy	Lecture 31—Motor systems 3
Lecture 4—Membrane potentials	Lecture 32—Motor systems 4
Lecture 5—Action potentials	Lecture 33—Motor systems 5
Lecture 6—Spinal cord anatomy 1	Lecture 34—Motor systems 6
Lecture 7—Spinal cord anatomy 2	Lecture 35—Cortex 1
Lecture 8—Brainstem anatomy 1	Lecture 36—Cortex 2
Lecture 9—Brainstem Anatomy 2	Lecture 37—Cortical EEG and epilepsy
Lecture 10—Brainstem anatomy 3	Lecture 38—Neural imaging
Lecture 11—Development of CNS	Lecture 39—Clinical correlate—basal ganglia
Lecture 12—Synaptic physiology 1	Lecture 40—Hypothalamus
Lecture 13—Synaptic physiology 2	Lecture 41—Limbic system 1
Lecture 14—Autonomic nervous system	Lecture 42—Limbic system 2
Lecture 15—Clinical correlation—brainstem and spinal cord	Lecture 43—Thalamic input to cortex
Lecture 16—Sensory receptors	Lecture 44—Clinical correlate—stroke
Lecture 17—Neurotransmitters 1	Lecture 45—Neuropsychology and autism
Lecture 18—Neurotransmitters 2	Lecture 46—Sleep, circadian rhythms, and EEG
Lecture 19—Somatosensory system	Lecture 47—Adult neurogenesis
Lecture 20—Pain	Lecture 48—CNS—immune communication pathways
Lecture 21—Auditory system 1	Lecture 49—Intro to CNS inflammation and clinical correlates
Lecture 22—Auditory system 2	Lecture 50—Whole brain section and forebrain pathways
Lecture 23—Visual system 1	Lecture 51—Neurobiological basis of cognitive disorders 1
Lecture 24—Visual system 2	Lecture 52—Neurobiological basis of cognitive disorders 2
Lecture 25—Visual system 3	Lecture 53—Intro to the neurological exam
Lecture 26—Taste and smell	
Lecture 27—Vestibular system 1	
Lecture 28—Vestibular system 2	

Adopted from 2008 Boston University School of Medicine Medical Neuroscience Class Schedule

Table 2

Circumventricular organs known functions (Duvernoy and Risold, 2007)

Name	Type	Location and Function
Neurohypophysis (posterior pituitary) Note: both regions have separate and distinct CVOs	Secretory	<i>Nervous lobe</i> : enables secretion of Vasopressin and Oxytocin from the megacellular paraventricular nucleus (PVN) and the supraoptic nucleus (SON) Median eminence: enables secretion of releasing factors (e.g. corticotropin-releasing factor) from the tuberohypophyseal that regulate secretion of adenohypophysis (anterior pituitary) hormones
Vascular organ of the lamina terminalis (OVL.T)	Sensory	The capillaries of lamina terminalis are in direct contact with nerves and nerve fibers that sample the following substances: 1 Angiotensin II—paraventricular and supraoptic nuclei, which enables <i>osmoregulation</i> through release of vasopressin. 2 Fibers of hypothalamic origin that release luteotropin hormone releasing hormone (LHRH) which may play a role in the reproductive cycle regulation through the release of Gonadotropins, follicle stimulating hormone (FSH) and Luteinizing hormone (LH)
Subfornical organ (SFO)	Sensory	Subfornical organ plays a role in osmoregulation by reacting to angiotensin ii, like the lamina terminalis, and its neurons project to the paraventricular and supraoptic nuclei inducing the sensation of thirst
Subcommissure organ (SCO)	Sensory	There is still much debate to the role of the subcommissure organ, but some data suggests a role in the regulation of CSF into the narrow aqueduct
Pineal gland (PI) (epiphysis cerebri)	Secretory	The Pineal gland secretes melatonin and serotonin to regulate diurnal cycles
Area postrema (AP)	Sensory	Capillaries are in direct contact with the nucleus of the solitary tract and the motor dorsal vagal nuclei. Functionally, the area postrema has known roles regulation of cardiovascular, respiratory and potentially the source of the emetic reflex, which controls the vomiting center in the reticular formation of the medulla