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Central Nervous System Immunopathology

1. Principles of Central Nervous System Immunity
2. Autoimmune epilepsies
3. Immnunotherapeutics in Pediatric Autoimmune Central Nervous System Disease

Research into how the CNS and the immune system communicate has accelerated in the past 20 years leading to a better understanding of pathways controlling the immune activation and neuroinflammation that have guided the approval of new disease-modifying therapies to treat CNS immunopathology. The principles of CNS immunity will provide us with an introduction into the basic principles underlying immune responses within the CNS, then we will go through the immune system in pediatric seizures and epilepsies and lastly the immunotherapeutics.

A Gregory P. Owens. Neuroprimer: Principles of Central Nervous System Immunity.Seminars in Pediatric Neurology, Volume 24, Issue 3, 145-151 (2017)

Despite longstanding perceptions, robust innate and adaptive immune responses occur within the central nervous system (CNS) in response to infection and tissue damage. Herein, principles underlying immune surveillance and the initiation of inflammatory innate and adaptive immune responses within the CNS will be discussed.

CNS immune privilege

Historically the notion that the brain was an immune privileged organ was founded on experiments that showed survival or growth of tumours transplanted into the brain and not peripheral sites. Subsequent studies informed the idea that once immune cells where primed peripherally via the afferent arm (immune activation within the lymph nodes) that they were able to migrate and enter into the brain parenchyma, but that the brain itself was incapable of eliciting an immune response.

CNS immune surveillance:

There are different cell types that survey the meninges and brain parenchyma for signs of infection and tissue damage.

Meninges and CSF:

1. T cells: predominant immune cell type and account for more than 80% of cells in normal CSF. They enter the CSF via transmigration across the choroid plexus.
2. B cells: typically rare, account < 1% of CSF lymphocytes
3. Macrophages (MHC class II): line the perivascular spaces, are within the subarachnoid space and choroid plexus

4. Dendritic cells: are in the CSF and choroid plexus in very small numbers

Brain parenchyma:

1. Microglial cells: are the primary innate immune cells within the brain parenchyma and function as good antigen presenting cells to T cells in models of infection.

CNS has pattern recognition pattern receptors which are found on the cell surfaces and within the cytoplasms and these are able to detect the danger patterns, once activated there is a subsequent cascade of cytokine signalling events that would lead to recruitment of peripheral inflammatory cells and adaptive immune cells into the CNS further amplifying the inflammatory response.

Once a problem is detected, brain specific antigens (neural antigens) are delivered to the deep cervical lymphoid tissue via the CSF where T cell priming occurs and this is made possible through these 2 pathways:

1. Meningeal lymphatic system: structures within the dura, along the cerebral venous sinuses that resemble the lymphatic vessels drain from the subarachnoid space into the deep cervical lymph nodes
2. Glymphatic system: free mixing of CSF with brain interstitial fluid along the Virchow- Robin spaces allow transfer of soluble CNS antigens from CSF and the interstitial brain fluid into the nasal mucosa where the afferent lymphatic vessels drain into the deep cervical lymph nodes

In the deep cervical lymph nodes the adaptive T and B cell immune responses are activated followed by migration of leukocytes into the CNS.

CSF T cell response, B cell ontogeny and migration into the CNS:

The primed T cells encounter the resident myeloid antigen presenting cells and gets reactivated, once reactivated, they secrete cytokines with invade the brain parenchyma to execute their function.

With infections and autoimmune diseases there is production of immunoglobulins and CSF oligoclonal bands with recruitment and expansion of B cells within the CNS. Just like the T cells, the antigen stimulation in the lymph nodes with the help of T helper cells lead to germinal centre reaction with clonal expansion, B cell switching, somatic mutation and release of long lived memory B cells and antibody secreting plasma blasts back into the circulation. It is postulated that the activated memory cells cross into the CNS, gets restimulated by resident target antigens and then differentiate into antibody secreting plasma blasts. Alternatively peripherally generated migratory plasma blasts could enter the CNS directly.

Following resolution of CNS infections, intrathecal IgG synthesis and oligoclonal bands eventually dissipate. Importantly our understanding of these immune mechanisms has led to a wide array of new disease-modifying therapies with the purpose of reducing CNS inflammation. Drugs and monoclonal antibodies that target egress from secondary lymphoid follicles, migration of T-lymphocytes across the BBB and specific immune cell subsets are now being used to treat CNS inflammatory diseases. CNS immune processes are also regulated by a wide array of cytokines, chemokines and immune receptors which are also becoming the targets for potential disease intervention.

B) AUTOIMMUNE EPILEPSIES:

1. **Yeshokumar, Anusha K. et al. Autoimmune Epilepsies. Seminars in Pediatric Neurology, Volume 24, Issue 3,161-167(2017)**

2. **Korff CM and Dale RC. The Immune System in Pediatric Seizures and Epilepsies. Pediatrics.2017;140(3):e20163534**

Autoimmune epilepsies are clinical syndromes wherein the immune system is suspected to be involved in the pathogenesis of seizures or as a mechanism for neuronal injury following seizures. These articles discuss the current understanding of the diagnostic considerations, clinical features, proposed pathophysiologic mechanisms and commonly considered treatments of the autoimmune epilepsy. Consideration of autoimmune aetiology early in the clinical course is important to ensure timely initiation of immunotherapy as conventional antiepileptic drugs are typically unable to control these seizures.

<u>Mechanisms linking immune activation with seizures:</u>

1. An initial injury that occurs either in the CNS or in the periphery with subsequent activation of the immune system in one or both compartments (systemic or neuroinflammatory). Various events that have been identified to play such a role include peripheral infections, autoimmune diseases, CNS vascular diseases (thrombosis, emboli and haemorrhage), vasculitis, neurotrauma, metabolic disorders, CNS infections, seizures and status epilepticus
2. Release of mediators by lymphocytes in the periphery or activated glial or neuronal cells cause blood brain barrier breakdown, adhesion and penetration of activated peripheral lymphocytes, immunoglobulins and albumin into the brain with increasing extracellular potassium concentration as well as functional changes in neurons, glial cells and astrocytes.
3. Neuronal function changes occur which increase seizure susceptibility. Examples of these functional changes include:
   -increased expression of IL-1R1 (the target and mediator of the biological response to IL-1β)
   -activation of intracellular kinase families (eg. inducing phosphorylation of a subunit of glutamatergic NMDA-R)
   -inhibition of the glutamate reuptake
   -increased glutamate release in the extracellular space by astrocytes
   -promotion of synaptic reorganization
   -dysfunction of ion channels

<u>When should one suspect an immune etiology</u>? The clinical presentation

-is typically of an explosive onset in a previously normal child with seizures, encephalopathy, cognitive deterioration and other focal neurological deficits.

-Immune function analyses should be specifically considered in children if **3 of these 5 criteria** are present:

1. Seizures: high frequency from onset with early refractoriness to classic antiepileptic drugs
2. Acute or subacute onset of signs of diffuse CNS involvement

3. Signs and symptoms consistent with specific auto-antibodies.
4. Personal or family of autoimmune disease
5. Identifiable epilepsy syndrome or other structural and or metabolic causes are excluded by history

Diagnostic evaluation may provide evidence of an inflammatory process with increased protein and lymphocytic pleocytosis in CSF, abnormal signal on MRI and an EEG with diffuse or focal slowing and epileptiform discharges.

Initial workup:

1. Brain MRI (T_2W, FLAIR)
2. Blood auto antibodies panel
3. EEG
4. CSF:- cell count
   -protein level
   -oligoclonal distribution
   -auto-antibody panel

Specific workup to be considered:

1. Extended blood analyses for systemic autoimmune diseases
2. Tumor search

The autoimmune encephalitis syndrome are increasingly being defined by their associated autoantibody biomarkers. Clinical syndromes summarised in these articles are:

Syndrome	Pathogenic mechanism
Anti-NMDA receptor encephalitis	Anti-NMDA receptor antibodies, possibly T-cell mediated cytotoxicity
Limbic encephalitis	Antibodies against NMDA receptor, VGKC, GAD65, Ma2 or Hu neuronal antigens
Refractory/ intractable seizures	Antibodies against $GABA_A$ receptor and other neuronal antigens
Rasmussen encephalitis	T-cell mediated cytotoxicity
Febrile infection-related epilepsy syndrome	?Neuroinflammation, ?Viral infection

The spectrum of autoantibodies in autoimmune epilepsy is still unfolding, there is therefore a likely additional autoantibodies on the currently known autoantibodies. There are still patients with immune mediated epilepsies who are antibodies negative, therefore a negative result does not eliminate the possibility of an autoimmune etiology.

Therapeutic approaches:

As a general rule, situations in which neuronal antibodies are found on the target surface antigens have a much higher therapeutic response rate than those in which antigens are intracellular. Immunotherapy include high dose intravenous steroids, intravenous immunoglobulin, plasma exchange and rituximab.

Important questions that remain open include the understanding of the precise timing and sequence of elements of the immune response to seizures, the detection of reliable diagnostic biomarkers of CNS inflammation in children with epilepsies, the identification of specific clinical, radiological and electrophysiological features that may aloe early suspicion of immune epilepsy, and the development of optimal therapeutic strategies and molecules targeted against various inflammatory mediators described above.

C) Nosadini, Margherita et al. Immunotherapeutics in Pediatric Autoimmune Central Nervous System Disease: Agents and Mechanisms. Seminars in Pediatric Neurology,Volume 24,Issue 3,214-228(2017)

A comprehensive knowledge of the range of available immune therapies and deeper understanding of their action should benefit therapeutic decision making. Immune mediated brain conditions are regarded as a treatable group of conditions and should be high on the differential list of any child with a new presentation or change in neurological function. Clinically they can be separated into autoimmune encephalitis, autoimmune demyelination syndromes and autoimmune neuropsychiatric syndromes. It is important to always consider that there are multiple immune processes happening in individual patients, hence one needs to be flexible in therapeutic decision making until there are improved molecular techniques to define the immune system in individual patients.

First line agents:

Immune therapy	Mechanism of action and effects
Glucocorticoids Methylprednisolone 30mg/kg/day iv for 3 to 5 days followed by oral prednisone taper over weeks to months Or monthly pulses (3 days) of intravenous methylprednisone Or 3 to 4 weekly (3 days) of oral dexamethasone	Have both a genomic and non-genomic mode of action Decrease in T-cell activity by targeting dendritic cell, downregulating the expression of MHC Class II and of other co-stimulatory molecules and proinflammatory cytokines and interfering with T-cell receptor signalling Promote the expression of anti-inflammatory cytokines
Intravenous Immunoglobulins High dose of 1 to 2g/kg over 5 days	Blockade of cell-cell interactions that are mediated by cell surface receptors Neutralization of cytokines, complements, immune cell receptors and pathogenic circulating autoantibodies Inhibits receptor expression on the innate effector cells and B cells Increased autoantibodies clearance

Therapeutic apheresis: **Therapeutic plasma exchange and immunoadsorption** 5 to 7 plasma exchanges over 10 to 14 days **NB:** refer to table 2 in the immunotherapeutics article for neurological indications for therapeutic apheresis	Elimination of autoantibodies and immune complexes Possible role on the removal of cytokines, soluble cytokine receptors and soluble adhesion molecules.

Second line agents:

Immune therapy	Mechanism of action and effects
Rituximab	B lymphocyte destruction mediated by binding to cell surface CD20 located on the B lymphocytes through 3 main mechanisms 1. Antibody-dependant cellular cytotoxicity 2. Complement-dependant cytotoxicity 3. Transient dose dependent decrease T-cell inactivation: decreased inflammatory cytokine production, proliferation capacity and expression of T cell activation markers
Cyclophosphamide	Irreversible alkylation of DNA bases with formation of DNA crosslinks resulting in impaired essential DNA replication and transcription leasing to cell apoptosis

<u>Maintenance therapy:</u>

Immune therapy	Mechanism of action and effects
Mycophenolate mofetil (prodrug of mycophenolic acid which acts as an inhibitor of inosine monophosphate dehydrogenase)	Inhibition of inosine monophosphate dehydrogenase, a rate limiting enzyme in the synthesis of guanosine nucleotides Antiproliferative effects on T and B lymphocytes, monocytes, fibroblasts, endothelial, mesangial and vascular smooth cells Induction of apoptosis in lymphocytes and monocytes Induction of necrosis in lymphocytes Reduction of cytokine production by T lymphocytes, dendritic cells and endothelial cells Reduction of immunoglobulin production by B lymphocytes Reduction of chemotaxis to inflammation sites in monocytes and lymphocytes Inhibition of cell-cell interaction and endothelial adhesion in monocytes, dendritic cells, neutrophils and fibroblasts
Azathioprine (synthetic purine analog)	Interference with T and B cell proliferation by halting DNA replication and blocking the pathway for purine synthesis Cytostatic and immunosuppressive action
Methotrexate (antimetabolite, folic acid	Inhibition of purine and pyrimidine synthesis Promotion of adenosine release with adenosine-mediated

antagonist)	suppression of inflammation Diminishes stimulated neutrophil adhesion and leukocyte accumulation in inflammatory exudates Reduction of antigen-dependent T-cell proliferation Inhibition of T cell activation and suppression of intercellular adhesion molecule expression by T cells Selective downregulation of B cells
Tacrolimus (macrolide calcineurin inhibitor)	Inhibition of the production of inerleukin-2
Bortezomib (protease inhibitor)	Inhibition of proinflammatory cytokine cascades Reduction of autoantibody production by reducing plasma cell production
Oral agents that modulate cell signalling **Ruxolitinib and Tofacitinib** (agents targeting key enzyme JAK)	Reduction of cytokine production

<u>Future utility of Monoclonal antibodies:</u>

With improved understanding of pathophysiology and biomarkers, some of these agents could be used in the future in paediatric autoimmune CNS diseases.

1. Monoclonal antibodies that target lymphocytes
   -**Ocrelizumab:** humanized monoclonal anti-CD20 antibody like Rituximab, under consideration for use in MS
   -**Ofatumumab**: human monoclonal anti-CD20 antibody, used in MS
   -**Alemtuzumab**: targets CD52 (expressed on the cell surface of lymphocytes and at lower levels on monocytes, macrophages, eosinophils and natural killer cells, binds to CD52 on the cell surface causing long lasting depletion of lymphocytes and monocytes mediated by antibody-dependent cell mediated cytotoxicity, complement-dependent cytotoxicity and apoptosis, used in MS

2. Monoclonal antibodies that target complement: cause antibody-mediated complement cytotoxicity
   -**Eculizumab:** monoclonal antibody that targets the cleavage of C5 protein, prevents the release of C5a and activation of the terminal complement pathway
   -Indication: NMO spectrum disorders

3. Monoclonal antibodies that target lymphocyte adhesion across vessel walls
   -**Natalizumab**: targets α-4 intergrin, an essential adhesion molecule expressed on the surface of lymphocytes which is involved in lymphocyte trafficking into the CNS, inhibits the lymphocyte migration across the BBB by blocking α4 intergrin, used in MS

4. Monoclonal antibodies that target cytokines and cytokine receptors

-**Anakinra:** IL-1 receptor antagonist, reduces proinflammatory cytokines
-**Tocilizumab**: monoclonal antibody that targets IL-6 receptor inhibiting the binding of IL6 to its receptor
-**Daclizumab**: monoclonal antibody that targets IL-2 receptor α-chain
-**TNF-α inhibitors**: infliximab, adalimumab and etanercept

Clinical implications: what does the future hold?

Knowledge and deeper understanding of immune therapies and their modes of action should help clinicians in therapeutic decision making. Clinicians need to carefully consider and sometimes apply a "risk versus benefit" approach when it comes to choice of immune therapies especially in the setting where patients have seronegative immune CNS syndromes. A plethora of new treatments are currently being investigated and represent an exciting future in the field of pediatric neuroimmunology, some of the examples are: macrolides, nonsteroidal anti-inflammatory drugs, other immunomodulatory agents used in rheumatology like hydroxychloroquine, sulfasalazine, pyschotropic drugs like serotonin reuptake inhibitors and neuroleptics, extracellular vesicles, parasitic helminths, ketogenic diet, naturopathic agents like curcumin and the role of the gut-immune-brain-interaction.