Role of complement activation in brain development

(Cindy)

The complement system consist of more than 25 proteins that are mostly synthesized in the liver as well as by cells that are involved in inflammation. This system is very critical and it is the most immediate response to mostly bacteria. The main functions of the system is to work together with antibodies to aid the process of eliminating bacteria. This can be achieved through a series of reactions that ultimately lead various activities such as facilitating opsonization, activating anaphylatoxin and killing a pathogen. The proteins in the complement system circulate in the blood stream as well as tissue fluids in an inactive state that does not attack the body. However when a pathogen such as bacteria presents in the body, the complement system activates. This activation occurs by a complement cascade where many complement proteins interact to activate each other. This cascade creates 3 different pathways of activation which are the Classical, Alternative and Lectin pathway.

The classical pathways is triggered by the initiating serum component C1 that binds with an antigen-antibody complex. C1 complex consist of three different subcomponents C1q, C1r and C1s. When C1q which is composed of 6 molecules binds with the constant region of either (2) IgG or 1 IgM, it activates the subcomponents C1r and C1s. C1s in its esterase form it's able to cleave onto different proteins in specific amino acid sequences, specifically C4 and C2. C4, C2 are cleaved into C4a/ C4b and C2a/C2b. Both C4a, and C2b remain in fluid phase because they are the smaller fragments. C4b and C2a bind on the surface of the cell which forms a C4b2a complex. This complex is called the classical pathway C3 convertase which cleaves C3. C3 is the most abundant complex of this system. The C3 convertase then proceeds to cleaving the C3 into the C3a and C3b complex. The remaining steps are shared by Lectin and the alternative pathway.

The alternative pathway differs from the Classical pathway in the manner that it does not need the presence of an antibody-antigen complex in order for it to be activated and it can be triggered by various agents. This specific pathway is activated by the binding of C3b to a protein or carbohydrate on the pathogen surface. The C3 protein circulates around blood stream and has the ability to spontaneously split into C3b and C3a. After the binding of C3b, this joins with the serum Factor B which forms C3bB. The presence of Factor D then cleaves to Factor B C3bB complex. Ba and Bb are fragments generated due to this action. Ba remains in the fluid while Bb goes on to attach to the remaining C3b. This form produces the complex C3bBb which is the C3 convertase of this pathway. C3bBb has the ability to activate more C3b molecules therefore enhancing or amplifying the pathway. This particular feature it's known as the amplification loop.

(Sandra)

The lectin pathway is intiated when the terminal polysaccharide residues of a pathogen such as a bacterium binds to MLB, which is structurally homologous to C1q of the classical pathway. TFicolin also has a similar structure to MLB and binds carbohydrates on microbial surfaces such as N-acetylglucosamine. MLB and ficolin are both found in circulation complexed with proteases known as mannose-associated serine proteases. After the complex is bound to the bacterium, one of the proteases, specifically MASP-2, cleaves C4 and C2 to form C4b2a on the bacterium's surface. C4b2a is also formed via the classical pathway. This is where the lectin and classical pathways converge in which C3 convertase cleaves the next component for both pathways.

The point that all three pathways converge at is the cleavage of the complement component C3. When C3 cleaves, it forms C3b and a small fragment called C3a. Once these components cleave, C3a is released in the fluid as an anaphylatoxin while C3b covalently bonds to the surface of the pathogen. This binding continues the complement activation cascade by initiating the alternative pathway. C3b is an important opsonin, which means that its deposition on the pathogen surface enhances pathogen uptake by phagocytic cells. This is one of the most important functions of complement in host defense. Regardless of the pathway, C3 convertase then binds and cleaves C5, which is the next component in the sequence. The cleavage of C5 produces C5a, and C5b. Similar to C3a, C5a is release in the fluid as the most potent anaphylatoxin. While C5b binds to the cell surface and forms the nucleus for the binding of the terminal complement components. The terminal components of the complement cascades include C5b, C6, C7 and C9. These components bind to one another and form a membrane attack complex know as MAC, that results in the lysis of the cell on which they deposit on. The formation of a MAC on the surface of the cell that reults in cell lysis is the terminal step of the three complement activation pathways

(Hannah & Katherine)

Weaker synapses are eliminated to create more efficient circuitry.

Synapses in the brain are remodeled and eliminated throughout life as part of a process known as synapse plasticity, which helps contribute to the effectiveness of the CNS circuit. To complete this circuit in the developing brain, neurons are wired together through a process known as synaptic pruning. Synaptic pruning helps elect the most efficient synapses and eliminate weaker ones. In postnatal development an excess of synaptic connections are formed during a period of synaptic proliferation. This is when the brain has its highest synaptic density. These synapses are then pruned according to efficacy, with over half of the synapses being lost by puberty (Blakemore et al., 2006; Chechik et al., 1999; Paolicelli et al., 2011). How synapses are chosen is currently unknown, but it has been proposed that synapse elimination is a result of competition between neighboring synapses. The punishment model suggests that strong synapses, or effective synapses, eliminate weaker synapses through a series of signals. The first signal being a local, protective signal and the second being a long range elimination signal (Veerhuis et al., 2011).

Immune molecules play a role in the facilitation of synapse pruning and plasticity. In the immune system, complement proteins opsonize pathogens, thus tagging them for engulfment by phagocytic cells. Tight junctions between adjacent cells in the blood brain barrier form a semipermeable membrane, constituting the brain as an immunoprivileged cite. In the brain, C proteins are shown to facilitate removal of misfolded proteins, damaged cells, and apoptotic neurons. The majority of C proteins are synthesized by the liver and cannot cross the blood brain barrier (Veerhuis et al., 2011). The C proteins in the brain are synthesized locally by resident glial cells, astrocytes and microglia. Emerging studies suggest that these glial cells, including microglia and astrocytes, are not limited to immunologic function but also play a role in synapse formation and pruning in the developing brain. (Stephan et al., 2012). This has been investigated principally in the retinogeniculate system of the mouse. The retinogeniculate system proves to be an excellent model to study synapse elimination in the CNS. During development, axons from retinal ganglion cells migrate to eye specific domains and synaptic pruning occurs. C proteins are highly upregulated during eye specific segregation, suggesting they play a role in this process (Stevens et al., 2007). C1q, the initiator protein in the complement cascade, is highly upregulated in mouse retinal ganglion cells. This expression in the retinal neurons is limited to the period of eye specific segregation and synaptic pruning. Retinal ganglion cells in C1q and C3 knockout mice show a failure to migrate to appropriate eye specific regions (Stephan et al., 2011). C1q and C3 knockout mice also show increased levels of epileptic activity and have more structural synapses than control mice, and have defects in CNS connectivity (Veerhuis et al., 2011; Stephan et al., 2011). Active microglia and C3 proteins have been seen to differentiate and migrate in response to the presence of C proteins in the developing hippocampus, cerebellum, and olfactory region and neuronal cells (Stephan et al., 2012; Veerhuis et al., 2011).

The complement systems tags weak synapses for engulfment. One of the key components to the complement system is the opsonization of pathogens. In the classical pathway, antigen-antibody complexes are the activators. However, C1 has been shown to activate in the developing brain without the presence of a pathogen (Stevens et al., 2007). C1q binds its globular head to lipids, surface proteins, or other opsonins including IgM and IgG. C1q's ability to bind a vast range of molecules allows it to bind CNS molecules to facilitate synapse refinement. Once C3 is deposited on the cell surface, the pathogenic cells is opsonized and in kind is targeted for phagocytosis (Sunshine and Coico, 2015; Stephan et al., 2012). This process is proposed for how C proteins regulate synapse formation and elimination. Through electron microscopy and high resolution imaging, microglia were seen engulfing synaptic inputs of retinal ganglion cells. Genetic or pharmacologic manipulation of microglial phagocytosis results in failures of eye specific segregation and synaptic pruning (Stephan et al., 2012). Studies suggest that C1q spontaneously activates the complement system in the brain and C3b is used to tag weaker synapses for elimination, possibly through the punishment model (Stevens et al., 2007). Crosstalk between neurons and microglia may allow microglia to choose which synapses to opsonize. This crosstalk is found to be facilitated by CX3CL1 and its conjugate

receptor, CX3CR1 expressed only on CNS microglia. Studies show mice deficient in CX3CR1 fail to recognize synapses, resulting in abnormal amounts of immature synapses (Zabel et al., 2013).

Crosstalk between immune molecules mediate opsonization. Other molecules of the immune system are posited to mediate synaptic pruning and refinement as well, including neuronal pentraxins and MHC class 1 molecules. Pentraxins of the immune system are involved in opsonization of dead cells and have homology with neuronal pentraxins. Therefore, neuronal pentraxins may mediate microglial opsonization and phagocytosis. MHC-1 proteins has also been shown to colocalize with synaptic proteins. Again, mice deficient in neuronal pentraxins and MHC-1 molecules show lowered levels of eye specific segregation (Stephan et al., 2012).

(Rachel)

The complement system in the brain may have implications in Alzheimer's. Alzheimer's disease is classified as a degenerative brain disease, and the most common form of dementia. Alzheimer's disease is largely characterized by the deposition of B-amyloid peptide (B-AP) by the central nervous system (CNS); however, additional studies have shown that the brain affected by alzheimer's have immunohistochemically detected classical markers of immune-mediated damage and are primarily associated with B-AP structures such as senile plaque. Amyloid fibrils are an essential part of the neuropil saline plaques (P. Eikelenboom, M. Rozemuller). They consist of clusters of A4 proteins which are believed to be neuronal cell receptors derived from glycoproteins. Amyloid (also called saline) plaques are a build-up of the amyloid proteins that accumulates outside the central nervous system, and when these amyloid proteins divide improperly they form a neurotoxin called B-AP. B-AP contains C1q and C3 fragments involved in complement activation and not C1s and C3a concluding that complement components are not passively bound to the the amyloid plaque, but it is evident that they are the result of the activation of the complement component of the innate immune system (Joseph Rogers). It is known that the first step of the classical pathway is the binding of C1q and later activation of C1r and C1s, but it has been seen that B-AP can activate the complement cascade without the aid of an immunoglobulin. Studies show that B-AP actively binds to the first component of the C1 complex, the C1q. In vitro experiments have confirmed that B-AP can directly activate the complement system without the mediation of an immunoglobulin, and is shown by the formation of the membrane attack complex (MAC) which is a strong indicator that the complement system has been fully activated. The complement- activated membrane attack that occurs is normal in the removal of damaged cells, but in Alzheimer's patients, the complement-mediated attack occurs adjacent to the B-AP ducts, and thus destroying the surrounding cells which are healthy. In a healthy body, the regulatory factor C1 inhibitor prevents the binding of C1q to non-immunoglobulins, and thus prevent from the complement cascade from occurring. Although many of these theories have been proven by in-vitro experimentation, we have only touched the surface in understanding the role of

complement activation in Alzheimer's. Future studies will better our understanding of the relations of serine proteases (C1r, C1s C2..) interactions in complement activation, and in hope to eventually be able to prevent and revert the degenerative result of this process. The complement system has shown to have an integral role in neuron death in Alzheimer's disease, and future medication, and treatment hopes to prevent the activation of the complement system by amyloid plaques by inhibiting the interactions of complement proteins and B-AP to help reduce the loss of neurons.

Excessive pruning from an upregulation in C proteins shows indications of Autism. Autism is defined as a neurodevelopmental disorder that results in impaired social interaction and communication, and is often coupled with epilepsy and mental retardation. Enzyme- linked immunosorbent assays, cytokine protein arrays, and immunochemistry have all been used to study the immune-mediated mechanisms that are involved in the cause of this neurodevelopmental disorder. The complement activation process that removes infectious organisms in the blood has been thought to cause cellular apoptosis (cellular killing) in the brain. There are 3 kinds of complement pathways that can occur, but only the classical pathway is present in the brain. The classical pathway is initiated with the binding of the C1q molecule to IgG oe IgM immune complexes. This results with the formation of C3 convertase. C1q has been shown to be upregulated in patients with autism in compared with patients without, and thus has a direct effect in the phagocytosis of more cellular targets than in individuals without (Lawrence Fourgeaud). Some patients with an increased amount of C1q have shown to accumulate most of their excess C1q in the epithelial line of their gastrointestinal tract, and were colocalized with IgG molecules. This result explains the apoptosis of the epithelial cells in the lining of these organs, and helps explain how the increased levels of C1q in the brain have a detrimental effect on neurons, and thus the development of the brain (John T morgan, Gursharan Chana). Studies have also shown that C1q knockout mice have a depleted number of phagocytic cells which leads to the conclusion that with the knowledge that neurons are notably more sensitive to the complement system an increased level of C1q especially in the brain results in the increased level of apoptotic cells. In the brain, the only cells that express C3 receptors are called microglial cells (B A Corbett). An increased number of microglial cells have been seen in many neurodevelopmental disorders like Autism, and is thought to be directly related to the pathological changes due to connectivity in these disorders. This idea has only been developed this year, and requires an extensive amount of more research, but the facts are clear that patients with neurodevelopmental disorders have an increased amount of C1q and microglial cells in their body and brain, and in turn have an increased percentage of cells dying via apoptosis. The correlation is clear, but the mechanisms, and the causes are still being studied. One idea is clear though, the complement system is one of the most vital portions of innate immunity, but when even the smallest problems arise, it can be one of the most detrimental effects on your body.

(Hannah)

The complement system has a role in brain development. The complement system is an integral part of innate immunity and the removal of infectious pathogens in the body. Molecules of this system are activated by interaction with antigen-antibody complexes circulating in the body. C proteins then opsonize pathogens, tagging them for phagocytosis by macrophages, or microglia in the brain. C3b is the major opsonin found in the brain. Bacteria covered in these opsonins are targeted and destroyed by phagocytic cells of the immune system. This process has been posited as a process for synapse pruning and plasticity in the brain. Evidence of upregulated C proteins and protein localization supports this idea. Crosstalk between complement proteins and other molecules of the immune system may also help mediate these interactions. B-amyloid peptide in the central nervous system can activate the complement cascade causing destruction of healthy brain cells. Individuals with Alzheimer's have higher levels of C1q and C3, which is suggested to cause over pruning of healthy synapses. It is evident that complement components play a role in the developing brain, though the exact mechanisms are still being investigated.

Boulanger LM. 2009. Immune proteins in brain development and synaptic plasticity. *Neuron* 64:93–109

Corbett, B.A., Kantor, A.B., Schulman, H., Walker W.L., Lit, L., Ashwood, P., Rocke, D.M., Sharp, F.M.. (2007). A proteomic study of serum from children with autism showing differential expression of apolipoproteins and complement proteins. Molecular Psychiatry, 12 (3), pp. 292-306

Eikelenboom, P., Hack, C.E., Rozemuller, J.M., Stam, F.C. (1989). Complement activation in amyloid plaques in Alzheimer's dementia. Virchows Arch. Cell Pathol., 56, pp. 259-262

Fourgeaud, L., Boulanger, L.M. (2007). Synapse remodeling, compliments of the complement system. Cell, 131 (6), pp. 1034-1036

Morgan, J. T. *et al.* (2010). Microglial activation and increased microglial density observed in the dorsolateral prefrontal cortex in autism. *Biol. Psychiatry* 68, 368–376

Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Panzanelli P, Giustetto M, Alves Ferreira T, Guiducci E, Dumas L, Ragozzino D, Gross CT (2011) Synaptic pruning by microglia is necessary for normal brain development. Science 333:1456 –1458.

Piani, D., Spranger, M., Frei, K., Schaffner, A., Fontana, A. (1992). Macrophage-induced cytotoxicity of N-methyl-d-aspartate receptor positive neurons involves excitatory amino acids rather than reactive oxygen intermediates and cytokines. Eur J Immunol, 22, pp. 2429-2436

Rogers, J., Cooper, N.R., Webster, S., et al. (1992). Complement activation by beta-amyloid in Alzheimer disease. Proc Natl Acad Sci USA, 89, pp. 10016-10020

Stephan, A. H., Barres, B. A. & Stevens, B. The complement system: an unexpected role in synaptic pruning during development and disease. Annu. Rev. Neurosci. **35**, 369–389 (2012)

Stevens B, Allen NJ, Vazquez LE, Howell GR, Christopherson KS, et al.2007. The classical complement cascade mediates CNS synapse elimination. Cell 131: 1164–78

Uhlmann R. F., Larson E. B., Koepsell T. D., Rees T. S., Duckert L. G. (1991). Visual impairment and cognitive dysfunction in Alzheimer's disease. J Gen Intern Med; 6: 126–32

Veerhuis, R., Nielsen, H. M., & Tenner, A. J. (2011). Complement in the Brain. Molecular Immunology, 48(14), 1592–1603. Zabel MK, Kirsch WM. (2013). From development to dysfunction: Microglia and the complement cascade in CNS homeostasis. Ageing Res Rev, 12: 749–756.