Scientific Workshop

~Neuroinflammation~

Paris – January 18th, 2012

Report by

Christian Lobsiger

Foreword by

François Bourre, CNRS, France

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The Workshop was introduced by a word from the NEURON Coordinator Dr. Marlies Dorlöchter in order to explain the ERA-NET NEURON II Scheme and the scope of the Workshop.
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Development of a strategic Research Agenda of the NEURON II project,
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FOREWORD

The workshop in Paris on 18th January 2012 was entitled "Neuroinflammation" and was focused on different aspects of its implication in various diseases of the Central Nervous System (CNS) which has a serious impact on health but also at the economic and social level.

Pierre-Olivier Couraud described the biology of the Blood Brain Barrier (BBB) and highlighted the specific immune properties of the brain, which is not an immunologically inaccessible area as previously thought. In fact, based on our current knowledge, the brain is more a specialized immunological site with a very finely tuned regulation process that maintains homeostasis of the CNS. Deregulation or dysfunction of these processes is involved in various CNS diseases.

Angela Vincent focused her talk on autoimmune diseases of the CNS. Whereas some pathologies such as *myasthenia gravis* respond well to treatments including immunotherapy and plasma exchange to remove circulating antibodies, others are very difficult to treat due to our ignorance of the biological target of the auto-antibodies. An important point is that even when the target is known and an immunological treatment is efficient, it is sometimes necessary to treat patients for the rest of their lives without knowledge of the long-term consequences of such therapies.

Hugh Perry asked the question if the immune response observed in affected brain regions of patient with neurodegeneration pathology is neuroprotective or neurotoxic and how this neuroinflammation could contribute to the disease progression. At this time there is increasing evidence that systemic inflammation or infection leads to the acceleration of the disease by shifting the already primed, but still anti-inflammatory, microglial phenotype to a more deleterious pro-inflammatory state.

Alastair Compston pointed out that Multiple Sclerosis (MS) is more a "cascade of inflammatory events that culminates in the acute injury of axons and their myelin sheets" than a pure auto-inflammatory disease. A Genome Wide Association Study (GWAS) allowed the identification of 52 loci associated with MS. Most of them were located on genes linked to the immune system, with only very few genes related to classic neurodegeneration. The monoclonal antibody alemtuzumab that specifically targets mature lymphocytes leading to their rapid depletion and a consequent prolonged lymphopenia is now used in therapy with very good results. Nonetheless some patients were at risk for developing secondary autoimmunity with alemtuzumab treatment.

Martin Kerschensteiner described the neurobiology of MS with a particular emphasis on the loss of myelin sheaths and the death of oligodendrocytes, as well as decribing a significant axonal degeneration that ultimately leads to actual axonal loss. Several studies suggested that axonal damage most likely begins early in the disease process, supporting the idea that neuronal pathology is a crucial component of the MS disease pathophysiology. In conclusion axonal degeneration, although ultimately irreversible and linked to a detrimental loss of electrical activity, might have a specific window during which it is reversible and thus therapeutic intervention may be possible.

Markus Schwarz gave the last talk, focused on immunity and psychiatric disease. In particular, the imbalance of the glutamatergic system is suggested as a contributing factor in schizophrenia, and the tryptophan-serotonin-kynurenine pathway (TSKP) presents itself as an interesting candidate in this interface between immune and neurotransmission systems in schizophrenia. Indeed this pathway can be regulated by components of the immune system and components of the TSKP can reciprocally influence general immune function. This is illustrated by the fact that a classic 6-week anti-psychotic schizophrenia drug treatment induces changes in the plasma levels of the specific TSKP metabolite kynurenic acid, concomitantly with reduction of clinical symptoms.

Taking into account all these talks, it is clear that neuroinflammation is involved and is likely to be a significant factor in different CNS pathologies. Encouraging research that elucidates cellular and molecular pathways, the alphabet, word and language by which immune system and CNS cross-talk, is essential in order to find new targets and new therapies to combat these diseases.

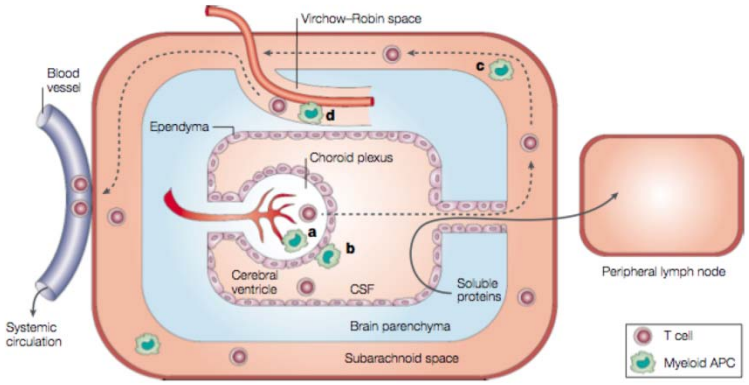
Speaker 1: Pr. Pierre-Olivier Couraud

Institut Cochin, Paris, France

TITLE: *"Introduction to neuro-immunity and brain inflammation".*

The classic text-book expression states that the central nervous system (CNS) is an immunologically "*privileged site*", with only very limited or even absent immune-responses. This was mainly based upon very early observations of a physical barrier, the *blood-brain-barrier (BBB)*, that became apparent when Paul Ehrlich and Edwin Goldman in 1900 tried to stain the CNS with peripherally injected dyes, later also due to the relative good survival of xenotransplants in brain tissue. There are a number of factors that indicate that a full-fledged immune-response within the brain would be very dangerous. One, the skull sets a physical limit to any major inflammation-associated brain swelling which would result in increased pressure and deleteriously reduced blood-supply. Two, damage to neurons would result not just in cell-loss but also in loss of the information content of these complex networks which can neither be regenerated nor rebuilt. Despite these dangers, it is now clear that the CNS can nevertheless mount a finely tuned immune response capable of fighting local infection and once this is activated, can result in beneficial or deleterious consequences during different neurodegenerative disorders.

Therefore, based upon our current knowledge, the CNS should now be characterized as an immunologically "*specialized site*". The brain is populated with *resident microglia* that constitute a powerful arm of the innate immune system which can phagocytose cellular debris, apoptotic cells and orchestrate via cytokines a local immune response when activated during injury or infection. However, the CNS lacks lymphatic vessels and contains no antigen-presenting dendritic cells (DCs) within the brain parenchyma, only resident microglia that have limited capacity for antigen presentation and are not known to leave the brain parenchyma. In addition, the BBB prevents large-scale cytokine and peripheral immune cell infiltration in contrast to what occurs in the periphery. Therefore, raising an adaptive immune response within the brain seems a challenging biological issue. In the periphery, the classic pathway of immune activation is characterized by antigen loaded DCs *(cellular route)* or soluble antigens *(soluble route)* that drain via the lymph system to lymph nodes where the actual activation of the adaptive immune response takes place. In the CNS, despite the lack of lymph vessels and no clear cellular route, it is now known that antigens can nevertheless exit. The brain is buffered against physical damage by floating in *cerebro-spinal-fluid (CSF)*, which is produced by the chorioid plexus lining the ventricles and which fills the *subarachnoid space* between the dura mater (at the skull) and the pia mater (at the brain parenchyma). There are principally two pathways by which antigens could exit the brain and arrive in the cervical lymph nodes. On one hand, via passage from the brain parenchyma through the brain-CSF-barrier into the CSF, as this barrier contains fenestrations ("windows") and on the other hand, via the so-called *perivascular spaces* (or Virchow-Robin-spaces) that are basically the entry-ports of blood-vessels into the brain-parenchyma, shortly before they get surrounded by the actual BBB. Both the CSF and the perivascular spaces are connected to the subarachnoid space where there are blood vessels and DCs that could take-up the brain-derived antigens. The physical connection from the subarachnoid space to blood circulation is located at the *arachnoidal villi* that extend directly into the venous sinuses of the cerebral hemispheres. There is also evidence of a direct connection between the CSF of the subarachnoid space around the *olfactory bulbs* above the nasal cavity and the draining lymphatics of the nasal submucosa. Once in the cervical lymph nodes, adaptive immune activation can take place, with either deleterious autoimmune



Blood-Brain-Barrier (BBB) and soluble antigen draining

consequences or beneficial actions to reduce local brain injury or infection.

The BBB precisely regulates the cellular and non-cellular components that are allowed to enter the brain both with respect to physiological nutrients and pathological immune components. The BBB basically consists of pericytes that partially surround the endothelium of the blood vessels and of astrocytic endfeet that completely surround the pericytes and the endothelium. In addition, endothelial and parenchymal basal membranes are localized between endothelial cells and pericytes and between pericytes and astrocytic endfeet and it is within these basal membranes that perivascular macrophages and DCs are localized. Importantly, continuous intercellular tight junctions between the endothelial cells provide the necessary tightness of the vessels, composed mainly of claudins, occludin and junctional adhesion molecules (JAMs). Indeed, knocking-out claudin-5 in mice allows peripherally injected dyes to penetrate the brain, partially breaking down the BBB. In addition to the tight junctions that block simple diffusion of molecules from the blood into the brain, polarized expression of specific transporters within the apical (facing the vessel lumen) and basolatera*l* (facing the brain) sides of the blood vessel endothelial cells are an essential component of a functional BBB. Three types of transporters exist*: i)* receptor mediated transporters (e.g., IGF1-R, importing IGF1 from the blood), *ii)* carrier-mediated transporters (GLUT1), importing glucose from the blood) and *iii)* active efflux transporters (e.g., ABC-transporters, including multi-drug resistance proteins ABCB1/MDR-1). With respect to GLUT1, polarized expression means a higher transporter expression on the basolateral than on the apical side, therefore pumping glucose preferentially from the blood *into* the brain. On the other hand, MDR-1 is preferentially localized to the apical side, pumping potentially toxic molecules (and unfortunately also some therapeutic drugs) from the brain/endothelial cells back into the blood. Astrocytes also show polarization with respect to the BBB, for example, the water-transporter aquaporin-4 is specifically expressed on the astrocytic endfeet facing the blood vessels. The so-called_neurovascular unit describes the entire structure of the blood vessel, pericytes and the associated BBB. The pericytes can regulate, in response to neural activity, the diameter of the tiny blood capillaries branching out from the large vessels and thus help control local neural blood-supply. Interestingly, in the periphery, capillary-associated pericytes are much less common than in the brain and in mice partially deficient for pericytes (PDGF-B retention motif KO) display a leakier BBB.

The development of the BBB in mice starts at E12.5 with neural progenitors, by activation of the Wnt-signaling pathway, acting on endothelial cells and inducing both tight junction protein expression (e.g., claudin-3) and subsequently, polarized transporter expression (e.g., GLUT1). Pericytes are then associating with the forming vessels and lastly at birth (P1) astrocytic endfeet start to surround the structure. Comparable to the Wnt-pathway, the sonic-hedgehog pathway (probably of astrocytic origin) is important for the formation and integrity of BBB tightness both during development and adulthood by up-regulating tight-junction proteins and down-regulating the expression of proinflammatory molecules (including adhesion molecules) from CNS blood endothelial cells. Taking advantage of this developmental insight, BBB structures can be partially modeled *in vitro* by combining in transwell co-culture assays brain endothelial primary cells with glial cells and pericytes.

The migration of leukocytes into the brain is possible although highly regulated by a tight BBB. Due to tight-junctions, leukocytes cannot enter the brain by the classic paracellular ("in-between") migration but instead by a process called *trans-endothelial* ("through") migration. It is now believed that in the brain no actual "rolling" of leukocytes along the endothelial wall takes place, but rather there is a direct arrest by leukocyte $\alpha 4\beta 1$-integrin and vessel adhesion molecule (VCAM-1) interaction. It has to be assumed that a local need for leukocyte infiltration exists, due to brain damage or infection, which then leads to a local activation of the vessel membrane for targeted infiltration. Thus, local vessel chemokine expression or presentation induces activation of the leukocyte trans-migration process (via the corresponding leukocyte chemokine receptors) with further help of the LFA1-1/ICAM-1 integrin/adhesion couple. This transcellular migration process has been captured by the electronmicrographic visualization of outward (luminal) bound "transmigratory cups" taking up a leukocyte. After passing the endothelial cell, the leukocyte still has to cross both laminin-containing basal membranes and can actually get "stuck" between them, specific laminins being more permissive than others, laminin-$\alpha 5$ inhibiting transmigration while laminin-$\alpha 4$ promoting it for example. Under conditions like multiple sclerosis (MS) or its mouse model experimental autoimmune encephalopathy (EAE) pathological infiltration of leukocytes into the brain takes place. Recent studies have shown that the chemokine couple CCL20/CCR6 is instrumental in disease susceptibilty in the EAE model. CCR6 is expressed by Th17 T-cells that are essential for EAE

pathogenesis. Interestingly, CCR6-KO mice were resistant to EAE and it has been shown that CCL20 is constitutively expressed by ependymal cells of the choroid plexus, allowing for entry of the Th17 cells into the CSF. This leads to a broad cytokine/chemokine production within the linked subarachnoidal space containing the blood vessels. After this first CCR6-dependent wave of T-cell infiltration, a second much broader but CCR6-independent wave leads to the infiltration of different leukocytes across the activated parenchymal vessels. Some of the main therapeutic strategies in MS are to limit immune interactions at the BBB. This includes reducing pathological leukocyte infiltration by *i)* blocking specific integrin molecules on leukocytes (e.g., natalizumab, a monoclonal Ab against the $\alpha 4\beta 1$-integrin VLA4), or by interfering with cell adhesion molecules (VCAM-1, ICAM) on endothelial cell walls (e.g., effects of interferon-β) or *ii)* by directly removing a specifically deleterious T-cell (e.g., alemtuzumab, a monoclonal Ab against the CD52 T-cell epitope) or B-cell (e.g., rituximab, a monoclonal Ab against the CD20 B-cell epitope) population. Under conditions of pathological brain infection, including bacterial meningitis, a comparable trans-migratory mechanism of infiltration takes place. Meningococci bacteria (Neisseria meningitidis) can induce changes in the brain endothelial cells to form the necessary luminally-directed trans-migratory cups. Interestingly, it seems that bacteria are able to hijack normal trans-migratory signaling pathways and suppress leukocyte transmigration across endothelial cells in order to support their own transmigration.

In conclusion, the CNS is an immunologically "privileged" site. However, despite the lack of dedicated lymphatic draining vessels and the BBB, the brain is nevertheless able to raise a controlled and limited immune response. Controlling the integrity of the BBB and the trans-migratory processes of peripheral leukocyte infiltration is an important therapeutic pathway to limit pathological BBB breaching during autoimmune conditions like MS.

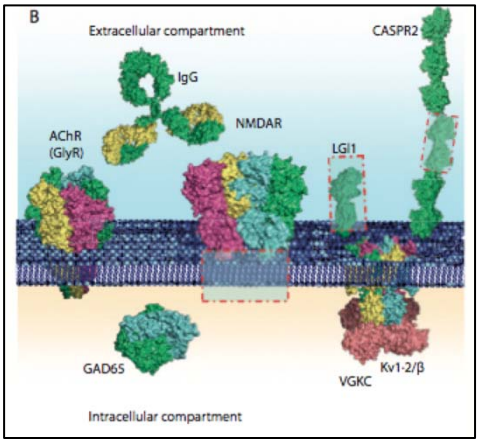
Speaker 2: Pr. Angela Vincent

Oxford University, England

TITLE: *"Auto-Immune Diseases of the Nervous System".*

The classic example of *Myasthenia gravis (MG)* presents an autoimmune disease in which auto-antibodies against the postsynaptic acetylcholine receptor (AChR) lead to a neuromuscular disorder characterized by muscle weakness and fatiguability. Comparable, in the Lambert-Eaton myasthenic syndrome, auto-antibodies are present against a plasma-membrane voltage-gated calcium channel at the neuromuscular junction. Treatments including immunosuppression lead to a rapid patient improvement after plasma exchange which removes circulating antibodies. Although these diseases are usually not progressive disorders (and symptoms can decrease), patients often need to be treated for the rest of their lives. On the other hand, when auto-antibodies are present in the central nervous system (CNS), usually this either suggests different infection-based encephalopathies, paraneoplasic neurological syndromes (PNS) or multiple sclerosis (MS). In the case of PNS, onconeural antibodies, which are produced by an immune response against certain peripheral tumors, can lead, after breaking immune tolerance, to neurological disorders by attacking CNS neuronal structures. Interestingly, auto-antibodies of these disorders are directed (in contrast to MG) to intra-cellular neuronal proteins and the attack against neurons is mediated directly by T-cells. Unfortunately, these syndromes often do not respond well to immunotherapies, but stabilization can only be achieved by directly treating the underlying tumor. In order to better study these PNS, an European network has been founded, the PNS Euronet, that has recruited during 2001-2009 a total of 979 patients from 20 different countries in order to establish a comprehensive clinical, serological and tumor database of the different PNS forms. Although auto-antibodies are present in most cases, MS is not thought to be a purely auto-antibody mediated disease. On the other hand, *Neuromyelitis optica (NMO),* (basically an ancient and more focal version of MS) is now considered to be a purely auto-antibody mediated disease. The specific epitope was identified as the water channel aquaporin-4 (Aqp4) localized on the astrocytic endfeet lining the endothelial cells of the blood-brain barrier (BBB). Both auto-antibodies and complement deposition are present with actual loss of the Aqp4 protein at sites of lesion. This epitope is different from classic MS auto-antigens despite similarities between the two conditions. NMO affects mostly females and is like MS, a relapsing-remitting disease with increasing disabilities, including common wheelchair-dependency within 5 years after onset.

Beside these three classic groups of disorders linked to CNS-directed auto-antibodies (MS, PNS and infection-based encephalopathies) a fourth new group of auto-antibody-diseases has been discovered during the last 10 years. In contrast to the classic PNS that are characterized by auto-antibodies against intra-cellular neuronal targets (and thus mediated rather by T-cells), auto-antibodies linked to these new disorders are targeted to neuronal cell-surface proteins. Patient symptoms are varied and can include amnesia, seizures and psychiatric features and can further develop to encephalopathy, movement disturbances and loss of consciousness. These symptoms are thought to be mediated directly by the action of the auto-antibodies against their neuronal targets and thus in contrast to PNS, these disorders respond quite well to immunosuppressive therapy and plasma exchange. Although the group of discovered auto-antibodies fitting to this group is constantly growing, the two best studied examples are syndromes linked to either sets of antibodies targeting voltage-gated potassium channels (VGKC) and antibodies targeting NMDA receptors (NMDAR).



Auto-antibodies against cell-membrane CNS epitopes
Vincent et al. The Lancet Neurology 2011 ; 10 :759-72

Disorders linked to VGKC-antibodies are less associated with tumors than classic PNS and can present themselves as either limbic encephalitis (characterized by psychiatric features, amnesia and facial dystonic seizures) or so-called *Morvan's syndrome* (characterized by peripheral nerve hyper-excitability and muscle twitching). Interestingly, in the UK, patients with high VGKC-antibodies were more often males and onset was after 40 years of age, likewise serum antibody levels were higher than CSF. Important questions include to determine if peripheral antibodies cross the BBB or are produced directly in the CNS and how this set of VGKC- antibodies can produce such symptomatic diversity (from peripheral muscle twitching to central amnesia). Although VGKC complexes are ubiquitously expressed in the CNS there is increased targeting of the hippocampus. Unexpectedly, it was just recently discovered that these so-called anti-VGKC antibodies were actually directed at region-specifically expressed VGKC associated proteins, rather than the potassium channel itself. Mainly two associated proteins are actually the target of VGKC-Abs, LGI1 and Caspr2. Interestingly, LGI1 is strongly expressed in the hippocampus and mutations are linked to epilepsy, while Caspr2 is associated with juxtaparanodes at the node of Ranvier along both peripheral and central nerves. Thus, the differential expression of the VGKC-associated proteins, targeted by the antibodies, can partially explain the distinct symptomatic phenotypes with respect to anti-LGI and anti-Caspr2 VGKC-associated auto-antibodies. Clinically, to detect these auto-antibodies in the patient's serum, an efficient method consists of cell-based immuno-assays, in which fluorescently tagged target proteins are expressed in cell-layers and binding auto-antibodies detected by fluorescent secondary antibodies.

Disorders linked to NMDAR-antibodies are characterized by encephalitis, seizures and mutism. There are described in young females with associated ovarian teratoma and the symptoms respond well to immunotherapy and tumor-treatment. The antibody targets preferentially the NR1/N2b NMDAR subunits of the hippocampus and leads to actual cross-linking and internalisation. However, no complement activation could be detected in postmortem samples and likewise no actual neuronal loss. Why specifically the hippocampus is targeted is not clear, but it might be linked to leakiness of the BBB. Likewise, it is not clear if intrathecal antibody production is essential for the syndrome. In an impressive therapeutic approach with a 12 year old girl that developed complete mutism, the symptoms were fully reversed after plasma exchange. In general, patients with NMDAR-antibodies develop the syndrome rather acutely over the length of 40 days, but one can distinguish two phases with an initial, rather cognitive, followed by a second physically disabling phase, including motoric defects, mutism and even lack of consciousness and of other autonomic functions. So far, only the two main groups of anti-VGKC (Lgi1/Caspr2) and anti-NMDAR auto-antibodies are regularly detected. However, an emerging set of other auto-antibodies based disorders against neuronal cell-surface proteins are continuously identified, with auto-antigens including the AMPA receptor, GABAb receptor and glycine receptors (GlyR). In the case of GlyR auto-antibodies, patients present with so-called "stiff-person syndrome", characterized by encephalomyelitis with rigidity, for which auditory or tactile stimuli trigger whole-body jerks. Plasma-exchange and other immunothera-

pies (during an almost 4-year treatment phase) were able to completely reverse this condition and get patients back to normal capabilities.

In conclusion, for all these different subgroups of neuronal cell-surface auto-antibody syndromes, immunotherapies are quite efficient but vary in the application. For some patient groups no continued immunosuppression is needed for recovery, while others need constant treatment with little knowledge about the long-term consequences of such treatment. In other groups relapses are possible after/during treatments or even spontaneous improvement is possible. These extreme examples demonstrate the gravity of these syndromes and the importance to push research to discover targets of these auto-antibodies mediated neurological syndromes. So far, only VGKC(LGI2/Caspr2)-antibody and AMPA receptor antibody detection kits are worldwide available. Likewise, active and passive immunisation models in mice need to be developed for studying mechanistic questions.

Speaker 3: Pr. Victor Hugh Perry

Southampton University, England

TITLE: *"Systemic Inflammatory Influences on Neurodegeneration"*

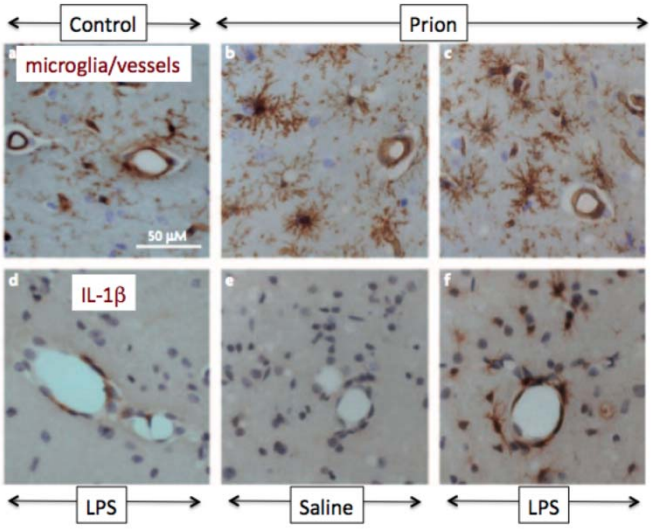
When our body is fighting an infection, our brain is setting us in a very distinct mode that is best described as *"sickness behavior".* We are feeling lethargic, apathetic, anhedonic and even show reduced social interactions, and can't concentrate. Combined with more metabolic effects, like increased body-temperature (fever), sleepiness and weight-loss, this sickness behavior is far from a simple annoyance, but a precisely regulated mechanism to produce optimal conditions for our immune system to successfully eliminate the pathogen. Importantly, early studies have shown that cytokines (e.g. IL-1β and TNFα) produced peripherally during systemic inflammation are able to induce the generation of this sickness behavior within the brain. Patients and rodents alike, injected peripherally with IL-1β, TNFα or LPS (lipopolysaccharides, simulating bacterial infections) develop flu-like symptoms, thus suggesting a signaling pathway from a peripheral infection into the central nervous system (CNS). The simplest concept would be that the peripheral cytokines gain access to the brain and communicate to the CNS resident microglia, which in turn would signal to neurons to induce the described sickness linked behavioral changes. Under normal conditions, brain microglia are in a non-activated surveillance state, thus, peripheral inflammation would simply alert them to induce the necessary neural adaptions.

However, what would happen if such peripheral inflammation arose when the CNS is itself already in a pathological state, as would be the case in patients with neurodegenerative disorders? Despite the brain's immune-privileged state, disorders like Alzheimer's (AD), Parkinson's, ALS (amyotrophic lateral sclerosis) and prion-diseases clearly show evidence of strong innate immune responses in the affected brain regions, including microglial activation. A central question in the field of neurodegeneration asks whether this neuroinflammation is a neuroprotective or a neurotoxic response and how it contributes to the different states of pathology progression. It has long been known from the periphery that acute systemic infections can cause flare-ups of underlying asthma or rheumatoid arthritis conditions. A tempting hypothesis thus would propose that common systemic infections and inflammations could produce a detrimental acceleration and worsening of an ongoing neurodegenerative process in affected patients. Hints for this can be seen in multiple sclerosis patients where certain peripheral infections can cause acute relapses. With respect to classic non-autoimmune neurodegenerative conditions like AD, it could even be hypothesized that a potential rather benign local immune response in the affected brain could be turned into a deleterious disease propagating neurotoxic response.

The idea behind this concept states that microglial cells under neurodegenerative conditions enter into a *"primed status"* that would allow them to act in a potentially more aggressive fashion in the CNS upon additional peripheral inflammatory stimuli. It has even be shown that due to normal aging, microglia in the brain become more activated (or "primed") than in young individuals (both human postmortem and animal tissues). Correlative hints come from epidemiological studies that state that individuals consuming long-term non-steroidal anti-inflammatory drugs (NSAIDs) show a modestly decreased risk to develop conditions like AD or PD. In order to experimentally study if peripheral infection and inflammation could aggravate neurodegenerative conditions by shifting the brain innate immune response to a more neuro-

toxic phenotype, a mouse model of prion-disease was used. Stereotaxic injection of a scrapie-infected brain homogenate (ME7) into the hippocampus of C57BL/6J mice induces a slow neurodegenerative process over almost 5-6 months paralleled by a neuroinflammatory response. Microglial activation is present long before actual symptoms appear and neural death is induced, thus presenting a chronic inflammation. In general, microglia can be present in different resting or activation states. The classic resting state is characterized by highly branched phenotype and low expression of classic activation and antigen-presenting markers. This surveillance state is most likely induced by several cell-cell molecular interactions, including CD200, CD22 and CX3CL1 on the neuronal side and corresponding receptors (CD200R, CD45 and CX3CR1) on microglial cells. Microglial activation results both in morphologic change leading to a more compact star-like shape and in a change of molecular markers and secreted cytokine-sets that can adopt any state between the two extremes of a fully pro-inflammatory (potentially neurotoxic) *M1* and a fully anti-inflammatory (potentially neurotrophic) M2 phenotype. Interestingly, microglial activation in the prion-mouse model, despite showing a clear change in morphology, results rather in a M2-phenotype than the expected M1-phenotype (as seen in an AD brain). In addition, despite slow degeneration of synapses, activated microglia seem not to be associated but a neuro-autophagozytic process could be involved. Thus, so far, this activation of the brian's innate immune system in the prion-mouse model, seems rather to be beneficial. However, are these microglia already "primed" and would a 2nd hit switch them to a pro-inflammatory M2-phenotype?

A set of experiments assessed this by injecting peripherally LPS into mice 18 weeks after prion-inoculation (a timepoint without obvious symptoms or neural loss, but with microglial activation). In control prion-mice that were simply challenged with peripheral saline injections, there was microglial activation in the brain, although the classic M1 pro-inflammatory marker, IL-1β, was absent from both glia and endothelial cells, confirming the M2-directed microglial activation, dominated by TGFβ production. When control non-prion-mice were injected with LPS, there was a clear IL-1β induction on brain endothelial cells. However, surrounding microglia did not induce this marker. Surprisingly, LPS injected prion-mice showed strong induction of IL-1β on both endothelial cells and microglia. Importantly, there was an increased sickness behavior, prion-model associated symptom onset was accelerated and final neural loss increased. In addition to increased IL-1β expression, there was also increased expression of other pro-inflammatory markers detectable, including TNFα, IL-6 and microglial iNOS. Therefore, although prion-inoculation clearly activated microglia, this activation stayed at an M2 phenotype, and only by additional peripheral LPS stimuli, these primed microglia were shifted to M1-activation state, which was not achieved by either treatment alone. Interestingly, TNFα mRNA levels can be elevated in just prion-inoculated mice, indicating an M1-shift. However, no protein elevation was detectable, suggesting that such primed microglia have an elevated ability to respond to rapid TNFα protein production when stimulated in addition. It is known that anti-inflammatory cytokines like IL-10 can block translation of primed TNFα mRNA levels.



Peripheral Inflammation affects CNS pathology

This synergistic inflammatory exacerbation of a CNS neurodegenerative condition by a peripheral inflammatory challenge is also present in other models. Age-related local microglial activation in mice can be increasingly shifted towards M1 by peripheral LPS injections. Likewise, in transgenic mouse models of AD and ALS, peripheral LPS injections increase the local neuroinflammation and accelerate neurodegenerative pathology. It can be assumed that the signaling pathway resulting from peripheral LPS stimulation reaches the CNS via perivascular macrophages that then transfer the signal to local brain microglia. However, how does peripheral inflammation lead to this "switching" of neurodegeneratively primed, but relatively benign microglial phenotype to an aggressive disease accelerating phenotype? Recent studies in the prion-mouse model suggest that this might mainly be due to an LPS-induced increased expression

of different microglial receptors that play key roles in macrophage activation, including M1-shifting pro-inflammatory FcγR III/IV (but not anti-inflammatory M2-shifting FcγR II). In addition to these receptor changes, increased levels of IgGs were detected in brain parenchyma of LPS-stimulated prion-model mice as compared to unstimulated ones, providing a link between prion-mediated antibody response and increased microglial sensitivity. In this respect it is of interest to mention that it has been observed that mice kept under recommended clean laboratory conditions (SPF) were more resistant to this kind of 2-hit neurodegenerative/peripheral-inflammation models than mice kept in more dirty conventional facilities, supporting the idea that increased systemic inflammation influences neurodegenerative conditions.

Taken to the human disease, especially AD, it has been long known by caring personnel that patients with systemic infections, even rather common ones, show accelerated disease progression and worsening of their cognitive condition. Interestingly, in clinical studies, systemic infections and increased TNFα and IL-1β levels have been linked to an increasing rate of cognitive decline. This is further supported by a variety of AD-risk factors (including smoking, obesity, diabetes and age) that all are linked to increased systemic inflammatory markers. The idea of using activated microglia to therapeutically remove the potential neurotoxic plaques in AD has been assessed in both mouse models and actual clinical trials. Likewise, it is essential to note that there are crucial differences between microglial responses in mice and human. Microglial concentration is higher in the white matter in humans, while higher in the grey matter in mice. Likewise, there are differences with respect to superoxide and NO production between activated human and mouse microglia. Despite the fact that immunization strategies against Aβ peptides were successful in reducing the plaque load, especially in the patients, they were not able to change the actual cognitive decline. This underlines the need for further studies to define which components of the neurodegenerative and neuroinflammatory pathology are actually deleterious, beneficial or neutral for the underlying disease.

In conclusion, there is increasing evidence that systemic inflammation or infections can influence an already present neurodegenerative condition and lead to its acceleration and worsening by shifting the already primed, but still anti-inflammatory, microglial phenotype to a more deleterious pro-inflammatory state.

Speaker 4: Pr. Alastair Compston

Cambridge University, England

TITLE: *"Genes and Immunity in Multiple Sclerosis"*

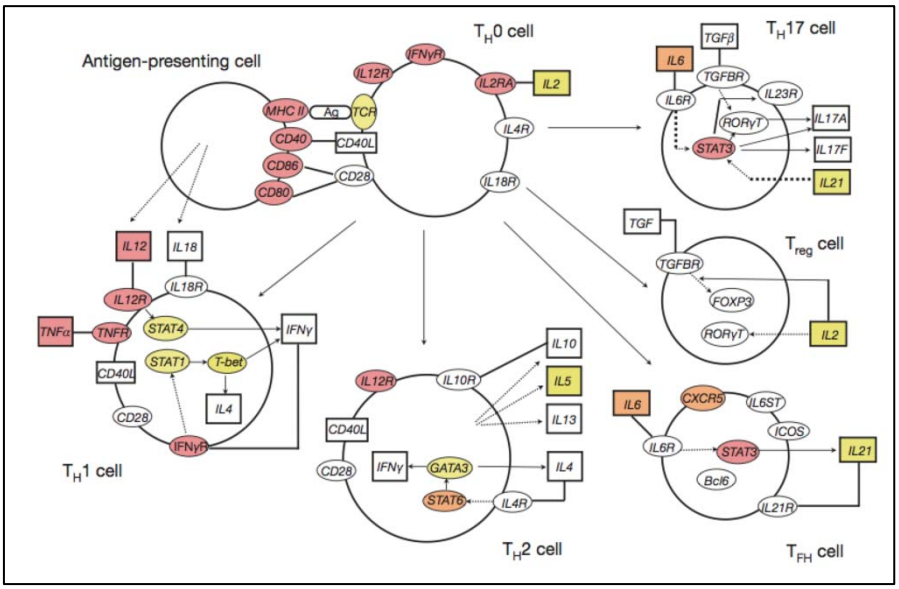
Multiple sclerosis (MS) is the most common neurological disorder affecting young adults and is more prevalent in women than men. MS is most often called an auto-immune disease, but an accurate description would be a "*cascade of inflammatory events that culminates in the acute injury of axons and their myelin sheets*". It is important to realize that the symptoms of this disease are both linked to the demyelination due to the immune-attack but also to the physical interruption of the underlying axonal tracts. It is principally the axons of the central nervous system (CNS) that are affected while nerves in the PNS are mostly spared. The pathological hallmarks of MS are the loss of myelin in diverse white matter structures of the brain and spinal cord with associated scarring and inflammation of the affected regions. Both *innate and adaptive (auto-)immune responses* are present with activation of resident microglia, astrocytes and infiltration of peripheral lymphocytes (together with auto-antibodies) and macrophages. Immune attack of the myelin results in death of the oligodendrocytes with only very limited remyelination from resident oligodendrocyte progenitors. Neuroimaging of MS patients (MRIs) can clearly detect such regions of myelin loss in the brain and spinal cord as well as pathological auto-antibody immunoglobulin bands in the CSF.

The neurological symptoms of MS are very diverse and accumulate over different disease stages. Initial symptoms include general weakness, diminished dexterity, gait instability, ataxia and visual impairments. Onset may be abrupt or insidious and as disease worsens, bladder dysfunction, fatigue and heat sensitivity occur. Additional symptoms could be linked to discharges originating along demyelinated axons and include neck flexion evoked electric shock-like pain, hemifacial pains and brief tonic spasms. Advanced stages often are linked to the appearance of cognitive deficits and depression is present in at

least half of all MS patients. Progression of MS can vary considerably, but the underlying pathological mechanism could be described in three stages: i) functional impairment of myelinated nerve fibers due to the deleterious effects of direct immune attack, although the actual axons are still preserved, ii) loss of myelin and partial damages to the underlying naked axons (as a consequence of immune mediators), paralleled by attempts of remyelination and adaptive plasticity, iii) ongoing myelin loss and lack of sufficient remyelination, ultimately leads to an irreversible axonal degeneration and physical loss of axons, probably due to chronic lack of neurotrophic support from the lost oligodendrocytes. Therefore, MS consists of two phases, a neuroinflammatory and a neurodegenerative phase, although it is debated if the latter is purely a consequence of the former or not itself a primary factor. Overall, symptom progression of MS can be grouped in the two extremes of a relapsing-remitting MS, with unpredictable attacks and phases of partial or even complete remission and a primary progressive MS, with steadily accumulating worsening of the symptoms. However, mixtures of these two classes exist and a relapsing-remitting MS can become with time a progressive MS. The well-known remission phases are likely due to partially successful attempts of remyelination, avoiding irreversible damages to the axons. Current treatment of MS is based upon immune-modulating drugs like interferon-beta and glatiramer acetate (copaxone), strong immune-suppressive corticosteroids and monoclonal antibodies directly targeting the deleterious lymphocytes (natalizumab). The idea behind these drugs is to reduce recurrence of acute relapses and thus slow worsening of symptoms. But despite certain successes, the underlying progressive nature of MS and resulting irreversible neurodegeneration cannot be stopped. This insight already suggests that pure anti-inflammatory therapy-approaches will not be sufficient and regenerative as well as neuroprotective strategies are additionally necessary.

An estimated 2.5 million people worldwide are affected with MS. However, the prevalence depends heavily on the geographic region and ethnicity, and varies considerably, but can reach levels of 1/1500 (in average) to up to 1/500. There is an increased prevalence of MS when traveling north and south of the equator, with low levels in equatorial lands and exceptionally high levels in Canada and Scotland. A northern European origin of the disease has been proposed and an increased risk in both females and white populations, especially those of northern European ancestry. In addition, a tempting idea suggests that MS "evolved" (influenced by cultural and genetic population change) from a related but much more restricted demyelinating disorder, *Neuromyelitis optica*. Historically, Jean-Martin Charcot in 1865 recognised MS as a distinct entity and made basically a clinical description that partially already existed before, finally coherent. Although the causes for MS are unknown, there are clear indications for geographical and ethnic influences, strongly suggesting that MS is a classic mixture of genetic and environmental factors. There are even indications, with only rare clinical descriptions in the past, that MS might be a rather "new" disease and showing increased incidence during the 20th century.

Studies with twins and sibling pairs clearly suggest a genetic influence (but not a classic Mendelian trait) on disease susceptibility, with an up to 30% chance of an identical twin for developing MS if the other twin does so (as compared to only 2-5% for fraternal pairs). In 1996 several studies have identified, using candidate gene approaches, a genetic linkage of MS to specific alleles of the major histo-compatibility complex (MHC; Chr6p21), MHC association with MS being already proposed in 1972. So-called *GWAS, genome wide association study*, are powerful tools to identify genetic risk factors, but require inherently large number of controls and patients. This technique basically compares hundreds of thousands of genotypes, represented by SNPs (single nucleotide polymorphisms identifying a specific allelic variant) between individuals. A first low-powered GWAS was conducted in 2007 by the "Multiple Sclerosis Genetics Consortium" that genotyped candidate SNPs in a total of 1540 family trios with MS, 2322 individual MS cases and 5418 controls (mostly from the U.K. and the U.S.). Despite the numbers, with respect to GWAS, this sample size is relatively small. However, the results clearly confirmed the strong association of the MHC locus with MS and refined it to multiple SNPs in the HLA-DRA locus. In addition, two new loci, one linked to IL2Rα (interleukin-2 receptor α) and one to IL7Rα (interleukin-7 receptor α), were discovered as heritable risk factors for MS. In total, an additional 23 loci (although less significant than the main three ones) were identified to have increased association with MS. In 2011 the same MS consortium (together with the Wellcome Trust Case Control Consortium, for control individuals) used a much higher powered GWAS with individuals from 12 European countries, Australia, New Zealand and the U.S. The total amount of MS patients analyzed was 9.772 (that resulted from a significantly larger number of affected individuals after applying stringent quality rules) compared to 17.376 controls and a full set of 500K SNPs were genotyped. Despite the statistical nightmare that country-specific controls pose on the final analysis, this unique GWAS was able to confirm 23 of the 26 previously suggested associations (including the major HLA-DRA, IL2Rα and IL7Rα) and uncovered at least 29 novel susceptibility loci (although, no evidence for genetic association with clinical course or severity of disease was detected). Strongest hits were found, as expected, in the MHC locus, but now with even higher resolution in HLA-A/B/C (for MHC-I) and with a major hit in HLA-DRB1/DQB1 (for MHC-II).



Most GWAS identified genes associated with MS are immune-related
Sawcer et al. Nature 2011 10;476(7359):214-9

Very interestingly, of all the 52 loci identified as associated with MS, the resulting candidate genes linked to these loci were mostly immune-system related genes, with only very few genes related to classic neurodegeneration. This represents compelling evidence that the critical disease mechanism of MS primarily involves immune dysregulation (although it does not decrease the crucial importance of neuroprotective strategies against irreversible axonal loss in MS). Interestingly, there were also links to previously reported vitamin D related environmental risk factors. When further analyzing the identified linked genes, it became obvious that most of them were directly or indirectly implicated in T-cell differentiation and/or maturation (with a preference for the T-helper and antigen-presenting cell population) and with many of them also known to be linked to other auto-immune diseases. Some of the major hits and its associated genes, included pathways that are already in therapeutic application against MS, especially by using targeting through humanized monoclonal antibodies as is the case against VCAM1 ('natalizumab'), IL2Rα ('daclizumab'), CD52 ('alemtuzumab') and CD86 ('abetacept', fusion protein, not mAB). Targeting

of these molecules principally suppresses the lymphocytes that express them and thus reduce the adaptive immune response in MS.

One of the most promising newer MS drugs, beside classic IFNβ treatment (in use since 1971), is the monoclonal antibody alemtuzumab that targets specifically mature lymphocytes leading to their rapid depletion and a consequent prolonged lymphopenia. Although there is recovery of the lymphocyte population, it is slow and variable with $CD4^+$ T-cells remaining partially depleted up to 5 years after a single pulse of treatment. The drug was originally used in 1991 with (rather advanced) secondary progressive MS patients and showed partial success in suppressing relapses, but was not efficient in reducing overall progression of disability. Interestingly, when the same drug was used with early relapsing-remitting MS (different trials in early 2000) it showed not just efficiency against relapses but reduced the accumulation of disability, suggesting actual slowing of the underlying progressive disease. Importantly, alemtuzumab was significantly more efficient (up to 70%) than classic IFNβ treatment. These observations indicate that MS might have two distinct inflammatory phases: an early phase that can be targeted and if successfully modulated can slow the appearance of irreversible axonal damage (and increasing disabilities) and a late phase that could be targeted, but even if successfully modulated, will not anymore be able to reverse (or stop) the already ongoing terminal axonal injury (post-inflammatory neurodegeneration). The therapeutic mechanism of alemtuzumab is most likely linked both to a partial T-cell depletion, but also to a surprising modulation of the recovering T-cell population that seems to adopt a more anti-inflammatory phenotype. However, in contrast to these promising findings, at least 30% of MS patients treated with alemzumab developed a deleterious secondary thyroid autoimmune disease. Subsequent analysis actually indicated that patients with high serum levels of IL-21 were exceptionally at risk to develop secondary autoimmunity with alemtuzumab treatment, therefore hinting at an opportunity to use IL-21 as biomarker to target alemtuzumab more efficiently to the appropriate MS-patient groups.

In conclusion, the future of MS-drug development has to find a better compromise between the high safety but low efficacy of classic interferone beta treatment and the high efficiency but high risk of alemtuzumab.

Speaker 5: Pr. Martin Kerschensteiner

Ludwig-Maximilian University, Munich, Germany.

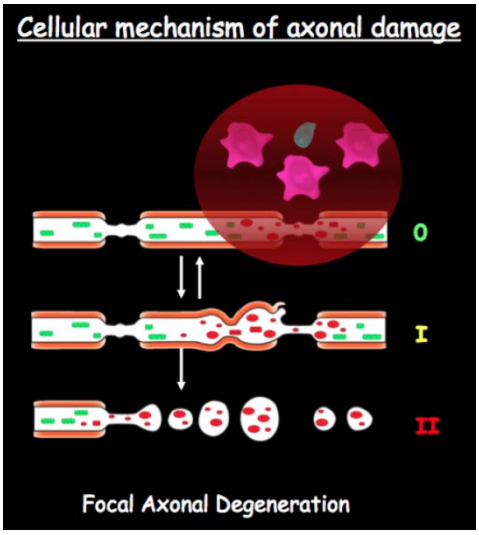
TITLE: *"Neurobiology of Multiple Sclerosis".*

Multiple sclerosis (MS) affects around 2.5 million persons worldwide and typically begins in early adulthood between 20-30 years of age. At least 50% of patients need physical help to walk 20 years after disease onset. In addition, life expectancy is often reduced by 10-15 years. MS is classically described as an auto-immune disease which leads to demyelination in the brain and spinal cord. The inflammation consists of infiltrating lymphocytes and macrophages as well as activation of resident microglia and astrocytes. The disease can progress anywhere between the two extremes of a relapsing-remitting MS or a primary progressive MS. The pathology can be described with *both a loss of the myelin sheath* and death of the oligodendrocytes, but also with a *significant axonal degeneration* that ultimately leads to actual axonal loss. Accumulating evidence supports the idea that axonal damage seems to correlate better with actual disease stage and permanent clinical deficits than classic inflammatory markers. Several studies suggested that axonal damage must already start early in the disease and can result, in extensive axonal loss (from 30 to up to 100%), supporting the idea that neuronal pathology is a crucial component of MS disease.

A major point remaining to understand is how these axonal damages and loss are produced and how the initiating deleterious immune-response and loss of oligodendrocytes contribute to it. *Does the immune-mediated injury to the axon produce only a local effect or does this effect spread along the axon? What are the dynamics of the reacting immune and glia cells, as well as the degenerating myelin producing oligodendrocytes with regard to the axonal degeneration and are there attempts (or windows) of regenerative efforts?* The best way to study such neuro-immune interactions would be to study them during the different disease stages and not just at the two extremes of pre-symptomatic and end-stage. To

achieve this goal, live in vivo imaging was applied in the classic Experimental Auto-immune Encephalomyelitis (EAE) mouse-model of MS. In this model injection of the myelin protein MOG, in combination with pertussis toxin to break down the blood-brain barrier, leads to an autoimmune mediated demyelination as well as strong axonal degeneration starting several days after inoculation. To visualize and distinguish the degenerating axon, the reacting microglia/macrophages as well as the infiltrating T-cells, different combinations of transgenic fluorescent reporter mice were used, marking each of the implicated players in distinct colors. To image the axonal degeneration induced in the EAE model, mice were anesthetized, intubated, fixed in an adapted stereotactic frame (to avoid that respiration-movements interfere with imaging) and finally a small "window" (laminectomy) was cut into the dorsal spinal cord in order to lower an objective into the tissue. Two-photon microscopy allowed to visualize the ongoing degenerative process, either during an extended imaging session of several hours, or repeatedly over several days, which required closing/re-opening of the laminectomy, and waking/re-anesthetizing the mice.

The classic view of MS predicts that axonal degeneration principally begins after the events of demyelination, when the axon becomes "naked" and thus susceptible to immune attack. However, what was observed in the EAE model using in vivo two-photon imaging, was that the axon already shows signs of degeneration, with the myelin sheath still intact. These first signs of axonal stress, after induction of EAE, were characterized by axonal "swellings", often besides nodes of Ranvier and with reacting macrophages/microglia present around the affected axonal fragment. In vivo imaging also suggested that macrophages/microglia were firstly associated with degenerating sites before the arrival of infiltrating T-cells. Interestingly, when repeated imaging of the same "focal axonal degeneration" (FAD) site was done, these pathological "swellings" seemed to be reversible during extended periods. Once these swellings persist, actual axon fragmentation follows, spreads slowly in both direction from the original site of FAD and leads to the formation of terminal "bulbs". Fine-structural analysis suggests that there are dysmorphic, swollen mitochondria present during the initial phase of focal axonal swelling. As the myelin sheath was often still intact during the swelling phase, the most obvious toxic molecule capable of crossing this barrier and damaging axonal mitochondria would be ROS (Reactive Oxygen Species) derived from the locally activated macrophages and microglia around the affected axon. However, such toxic molecules could also act directly at nodes of Ranvier, where the axon is locally less protected. In vivo imaging of ROS production confirmed the presence of these toxic molecules at FAD sites. Importantly, treatment of EAE mice with ROS scavengers was able to reduce and reverse early focal axonal degeneration (during the "swelling phase"), although it did not reduce the accumulation of immune cells at FAD sites. These observations from the EAE mouse model were also found in actual MS tissue, including axonal swellings with the myelin sheeth still present, as well as mitochondrial damage present within these sites of axonal degeneration. Beside ROS, a second toxic mechanism for axonal injury could be mediated by glutamate excitotoxicity. Indeed, it has been shown that there are functional glutamate receptors present along central nervous system axons. Support for such a toxic mechanism comes from observations that in EAE lesions local contacts of immune cells with axons were correlated with increased calcium levels in affected axons, a sign of potential excitotoxicity. Indeed, it was shown that these calcium fluctuations could be reversed by local application of glutamate receptor antagonists using in vitro systems. One of the challenges in studying axonal degeneration in MS and the search for neuroprotective compounds comes from the fact that many so-called neuroprotective compounds have also strong immune-modulatory effects. Simplified non-inflammatory axonal degeneration models are thus needed. Likewise, actual axonal fragmentation and loss are rather terminal disease signs in MS. In contrast, the process of axonal degeneration could last for extended periods and thus, functional deficits largely precede final structural loss. Therefore, methods to measure axonal dysfunction need to be developed to identify time windows for efficient therapeutic intervention.



MS is *classically seen as a white matter tract disease*. However, new results suggest that there is likewise *damage of myelin* (and oligodendrocytes) *in the grey matter*. This cortical pathology is already present in

38% of early stage MS patient tissue biopsies and is associated (like the white matter pathology) with infiltrating lymphocytes and macrophages. The importance of these observations comes from the fact that cortical pathology correlates well with the appearance of cognitive deficits in MS which develop in 50% of patients. Cortical pathology can be assessed with MRI and reveals both actual cortical (myelin) lesions but also reveals cortical atrophy and thinning, which appears focal and then becomes widespread. Cortical pathology dominates the pathological progress as MS develops and it is therefore of increasing clinical relevance to improve its detection, imaging and histopathological analysis. Likewise, modeling this cortical pathology of MS needs to be improved. A subclinical EAE mouse model was used in combination with delayed stereotactic injection of cytokines directly into the cortex. This model produces an auto-immune mediated demyelination outside of white matter tracts directly in the cortical grey matter. Using an adapted two-photon microscopy technique will allow to detect such cortical demyelination associated with (or preceding) neurodegeneration and pathological neuro-immune interactions, in vivo at a depth of several 100μm.

In conclusion, axonal degeneration, although ultimately irreversible and linked to detrimental loss of electrical activity, might have a specific window during which it is reversible and thus therapeutic intervention possible.

Speaker 6: Dr. Markus Schwarz

Ludwig-Maximilian University, Munich, Germany.

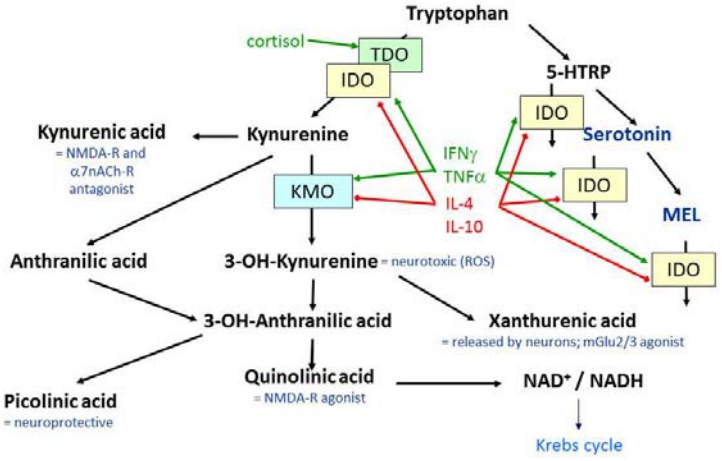
TITLE: *"Immunity and Psychiatric Disorders".*

There is a longstanding history of a potential association between immune-system dysfunction and psychiatric disorders, especially schizophrenia, although the field never came into the mainstream of research. Both direct viral infections as well as genetic associations have been proposed as risk factors for scizophrenia. For viruses, studies during the last decade have suggested links with CMV, HSV, Polio and Rubella infections. For the genetics, many polymorphisms in different cytokines were proposed and recently in 2009, GWAS studies have identified actual links to MHC-complex loci and increased risk of schizophrenia. Likewise, there is circumferential evidence that major depression is associated with a mild pro-inflammatory state. On the other hand, it has been known for a long time that the strong mood and autonomic changes, associates with general sickness behavior (lethargia, confusion and anorexia), are linked to the (direct/indirect) effects of peripheral cytokines (IL-1β, TNFα, IL-6) on specific central nervous system (CNS) functions. Comparable to that, immune-modulatory IFNα administration against chronic hepatitis has been associated with increased depressive behavior including risk of suicide. In addition, a significant proportion (40%) of patients with rheumatoid arthritis developed depression and anxiety disorders, psychiatric syndromes that are strongly reduced when patients are treated with anti-TNFα drug.

These examples suggest that a dysregulated immune function could contribute, could be associated with or even could cause psychiatric disorders, although molecular links between the two sides being still largely unknown. One possibility would be an interaction between immune effector molecules (cytokines) or immune cells directly with CNS neurotransmitter systems or their metabolism. Lack of serotonin has been strongly linked to depression while an imbalance in the glutamatergic system could be linked to schizophrenia. The tryptophan metabolism could be a major player in this interaction as it produces, on one hand, serotonin and on the other hand, it produces via the kynurenine pathway endogenous NMDA-receptor agonists (quinolinic acid) and antagonists (kynurenic acid). Several key enzymes of the tryptophan-serotonin-kynurenine pathway (TSKP) can be regulated by components of the immune system and reciprocally components of the TSKP can influence general immune function. Specific cytokines (especially IL-4/10, IFNγ, TNFα) are able to shift the degradation of tryptophan away from serotonin and towards kynurenine, thus leading to reduced serotonin levels (with potential effects on depressive states). Of interest are the clinical data that suggest an amelioration of major depression when antidepressants are used in combination with NSAIDs (Non Steroidal Anti-Inflammatory Drugs), e.g. COX-2 inhibitors like celecoxib. Even more surprising, high kynurenine vs tryptophan ratios were associated

with good responders in this clinical analysis, while low-KYN/TRP ratios were common in non-responders. This associates increased kynurenine with an inflammatory state in depression which is contributing to the psychiatric symptoms.

With respect to schizophrenia, apart from the suggested genetic and viral risk factors there is also evidence for progressive loss of brain volume, which is not directly linked to neurodegeneration but might represent a mild ongoing immune process or encephalitic state underlying. Although there is conflicting evidence from studies showing either increased pro-inflammatory states, increased adaptive anti-inflammatory Th2-shifts (T-cells) or pro-inflammatory innate monocyte responses in schizophrenic patients, deregulation of an immune response remains an interesting candidate for a pathophysiological contribution. Together with the aforementioned imbalance of the glutamatergic system as an additional player in schizophrenia, the tryptophan-serotonin-kynurenine pathway becomes an interesting candidate in this interface between immune and neurotransmission systems in schizophrenia. Specific pro-inflammatory mediators can shift tryptophan degradation towards the NMDA receptor agonistic quinolinic acid and thus produce potential neuronal damage (via excitotoxicity). On the other hand, the observed anti-inflammatory Th2 response could shift the tryptophan degradation towards the NMDA receptor antagonistic kynurenic acid and thus add to the psychotic and cognitive symptoms. Globally, the immune response could lead via modulating the TSKP to an imbalance of the schizophrenia associated glutamatergic neurotransmission. Interestingly, when transferred to the patients undergoing a classic 6-week anti-psychotic schizophrenia drug treatment, higher initial plasma levels of the specific TSKP metabolite kynurenic acid or increased kynurenic acid/kynurenine ratio after treatment were associated with reduction of clinical symptoms scores upon discharge. These results indicate that there is an imbalance in the kynurenine pathway in schizophrenia. The 6-week antipsychotic treatment may partially reverse the imbalance in the TSKP metabolism and that in turn induces clinical response. Interestingly, this correlation between TSKP metabolism and drug-treatment outcome could be linked to changes in the immune response: It has been shown that in schizophrenia patients after 3 months of classic anti-psychotic treatment, there is an increase in overall T-cells and a decrease in B-cells. Likewise, there is data suggesting that the anti-psychotic drugs can have direct effects on immune effectors and changing cytokine levels. Based upon that, several trials have been started to assess the effect (as already before in major depression) of combining classic anti-psychotic drugs with anti-inflammatory COX-2 inhibitors on schizophrenic patients. The difficulty with these studies was to determine at which stage of the disorder patients are still responsive for this anti-inflammatory treatment. As it turned out, schizophrenia patients who had developed the disease for not longer than 2 years were the best responders and did show a clear additional improvement when COX-2 inhibitors were used together with the anti-psychotic drug. Patients who had the disease for more than 10 years did not show any increased improvement when the COX-2 inhibitor was added.



The tryptophan-serotonin-kynurenine pathway

In conclusion, increasing evidence suggests that modulation of neuroinflammation in psychiatric disorders like depression and schizophrenia could have beneficial effects. This suggests that the two processes could be linked, both by inflammation being able to modulate underlying psychiatric conditions as well as anti-psychotic drugs having effects on both cognition and the immune response.

Annex I, Scientific Advisory Board

NEURON Scientific Advisory Board

1. **Prof. Celso Arango** (University of Madrid, Spain),
2. **Prof. Vania Broccoli** (San Raffaele Scientific Institute, Milan, Italy),
3. **Prof. Eero Castren** (University of Helsinki, Finland),
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9. **Dr. Ana-Maria Zagrean** (University of Medicine and Pharmacy, Bucharest, Romania)

Guests

1. **Dr. Sigrid Weiland**, European Commission
   sigrid.weiland@ec.europa.eu

Annex II, List of Participants

NEURON Meeting Participants

1. **Ignacio Baanante**, Instituto de Salud Carlos III, Spain
   ibaanante@isciii.es
2. **Dr. Julio Barbas,** MEC, Spain
   julio.barbas@micinn.es
3. **Prof. Bernard Bioulac**, CNRS, France
   bernard.bioulac@cnrs-dir.fr
4. **Prof. Alexis Brice**, INSERM, France
   brice@upmc.fr
5. **Dr. Francois Bourre,** CNRS, France
   francois.bourre@u-bordeaux2.fr
6. **Dr. Jenifer Clark,** ANR, France
   Jenifer.Clark@agencerecherche.fr
7. **Dr. Rafael de Andrés-Medina**, Instituto de Salud Carlos III, Spain
   rdam@isciii.es
8. **Priv. Doz. Dr. Marlies Dorlöchter**, DLR Projektträger des BMBF, Germany
   marlies.dorloechter@dlr.de
9. **Dr. Frank Glod,** Fonds National de la Recherche, Luxembourg
   frank.glod@fnr.lu
10. **Dr. Gaetano Guglielmi,** Ministry of Health, Italy
    g.guglielmi@sanita.it
11. **Dr. Anabela Isidro,** Fundação para a Ciência e Tecnologia, Portugal
    Anabela.Isidro@fct.pt
12. **Dr. Anne Jouvenceau,** Inserm, France
    anne.jouvenceau@inserm.fr
13. **Dr. Cinzia Kutschera,** Ministry of Health, Italy
    c.kutschera@sanita.it
14. **Dr. Benny Leshem,** Chief Scientist Office of the Israeli Ministry of Health, Israel
    benny.leshem@moh.health.gov.il
15. **Dr. Nava Levine**, Chief Scientist Office of the Israeli Ministry of Health, Israel
    nl@013.net
16. **Dr. Petra Lüers,** DLR Projektträger des BMBF, Germany
    Petra.Lueers@dlr.de
17. **Dr. Natalia Martin,** ANR, France
    natalia.martin@agencerecherche.fr
18. **Dr. Herbert Mayer,** FWF – der Wissenschaftsfonds, Austria
    herbert.mayer@fwf.ac.at
19. **Dr. Erkki Raulo,** Academy of Finland , Finland
    erkki.raulo@helsinki.fi
20. **Dr. Simonetta Taddei,** Ministry of Health, Italy
    s.taddei@sanita.it
21. **Dr. Mika Tirronen,** Academy of Finland , Finland
    mika.tirronen@aka.fi

22. **Dr. Elizabeth Theriault,** Institute of Neurosciences, Mental Health and Addiction, CIHR
    Elizabeth.Theriault@ubc.ca
23. **Dr. Katrin Valgeirsdottir,** The Icelandic Centre for Research
    katrin@rannis.is
24. **Malgorzata Zieminska**, NCBiR, Poland
    m.zieminska@ncbir.pl