Neuroinflammation in CNS disorders: 
*priming* a target for new therapies

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Organizer
Prof. Filippo Drago
Dear colleagues,

epidemiological data show that the disorders of the central nervous system (CNS) are some of the most prevalent, devastating and yet poorly treated illnesses. The development of new drugs for CNS disorders has the potential to provide patients with significant improvements in quality of life, and to reduce the future economic burden on health-care systems. Because the approval of CNS drugs with novel mechanisms of action has been rare in recent years, there is the need to ameliorate the R&D process in this field. Focusing on treatments that target disease pathophysiology will improve the chances of developing therapies that go beyond current symptomatic therapies. Indeed, the identification of new molecular targets involved in the pathogenesis of CNS disorders represents the essential step for the design of new and efficacious drugs able to modify the clinical course of these disorders.

In the last ten years a significant progress has been made in diverse areas of the neurosciences such as neurobiology, neuroimaging, neuroimmunology, social neuroscience and the ‘network approach’ to brain function. These advancements have suggested new pharmacological approaches for the treatment of CNS disorders which should be validated in the next years.

The present edition of the Summer School of Neuroscience, organized by the International PhD Program in Neuropharmacology, University of Catania, has witnessed in the last ten years the most important advances in this field with the contribute of more than 300 outstanding European neuroscientists. The first two editions of the International Summer School of Neuroscience, organized in 2003 and 2004, were initially focused on the role of neurotransmitters and their receptors in neuropsychiatric disorders, whereas the following editions were dedicated to drug discovery in neuroscience as well as to specific CNS disorders such as Alzheimer’s disease, major depression, Parkinson’s disease, schizophrenia, drug addiction and pain.

To celebrate the first 10 years of the Summer School of Neuroscience, the International PhD Program in Neuropharmacology, Department of Clinical and Molecular Biomedicine, Section of Pharmacology and Biochemistry, University of Catania organizes this year a Special Edition dedicated to “Neuroinflammation in CNS disorders: priming a target for new therapies”, where outstanding neuropharmacologists in Europe are invited as teachers to release a lecture.

Over the last two decades several studies have demonstrated that inflammation of the CNS (neuroinflammation) plays a central role in the pathophysiology of numerous CNS disorders. Originally viewed as an immune-privileged organ, the CNS is now recognized to have a constant interplay with innate and the adaptive immune systems, where resident microglia and infiltrating immune cells from the periphery have important roles. Common diseases of the CNS, such as stroke, multiple sclerosis, Alzheimer’s disease, Parkinson’s disease, pain and psychiatric disorders, elicit a neuroinflammatory response with the goal to limit the extent of the disease and to support repair and regeneration. However, various disease mechanisms leading to neuroinflammation contribute to the disease process itself. An extensive dataset describes neuroinflammation to have detrimental consequences, but results emerging largely over the past decade have indicated that aspects of the inflammatory response can also be beneficial for CNS outcomes. The goal of the 10th Edition of the Summer School of Neuroscience is to provide the local scientific community with the most relevant findings in the field of neuroinflammation and the new pharmacological approaches for the clinical management of CNS disorders.
Scientific Program

Saturday July 7th
Sunday July 8th
Monday July 9th
Tuesday July 10th
Wednesday July 11th
Thursday July 12th
Friday July 13th

10th Summer School of Neuroscience 2012
Saturday July 7th, 2012

13.45-14.15  Registration

14.15-14.30  Opening remarks
Filippo Drago (Italy)

14.30-15.30  Opening lecture
*Introducted by* Filippo Drago
Cytokines in CNS physiology and pathology
Robert Dantzer (USA)

The interaction between CNS and immune system (1)
*Introducted and moderated by* Nicoletta Brunello (Italy)

15.30-16.30  Inflammatory signalling pathways in CNS disorders
*Abstract*
Cinzia Dello Russo (Italy)

16.30-17.30  Nerve-Driven Immunity: Neurotransmitters and Neuropeptides in the Immune System
*Abstract*
Mia Levite (Israel)

17.30-18.00  Discussion

18.00-19.00  Immune proteins in synaptic and neuronal plasticity
*Abstract*
Raffaella Molteni (Italy)

19.00  Welcome reception
Sunday July 8\textsuperscript{th}, 2012

**Stress, inflammation and neuroimmune interactions**
*Introduced and moderated by Pierluigi Navarra (Italy)*

10.00-11.00  The stressed CNS: role of glucocorticoids  
Ron De Kloet (The Netherlands)

11.00-12.00  Role of GITRL/GITR system as a modulator of immune response and inflammation: therapeutic perspectives  
Carlo Riccardi (Italy)

12.00-12.30  Discussion

**The interaction between CNS and immune system (2)**
*Introduced and moderated by Mia Levite (Israel)*

16.00-17.00  Immune influence on adult neural stem cell regulation and function  
Maria Grazia Grilli (Italy)

17.00-18.00  Microglia and neuroprotection: from in vitro studies to therapeutic applications  
Barbara Monti (Italy)

18.00-18.30  Discussion

18.30-19.30  **Special Lecture**  
Immune activation in brain aging: too much or too little?  
Claudio Franceschi (Italy)
Fibromyalgia and depressive disorders
Introduced and moderated by Giovanna Maria Scoto (Italy)

10.00-11.00  Neuroendocrine immunology of fibromyalgia  
Riccardo Torta (Italy)

11.00-12.00  Fibromyalgia syndrome: from diagnosis to pharmacological treatment  
Piercarlo Sarzi Puttini (Italy)

12.00-12.30  Discussion

12.30-13.30  Neuroinflammation in the pathophysiology of depression: evidence from animal models  
Nicoletta Brunello (Italy)

Multiple Sclerosis
Introduced and moderated by Filippo Caraci (Italy)

16.00-16.45  The role of regulatory T cells in multiple sclerosis  
Roland Liblau (France)

16.45-17.30  The link between inflammation, synaptic transmission and neurodegeneration in multiple sclerosis  
Gianvito Martino (Italy)

17.30-18.15  Natural History of multiple sclerosis in the pediatric age: implications for outcome  
Martino Ruggieri (Italy)

18.15-18.30  Discussion

18.30-19.30  Special Lecture  
Treatment of multiple sclerosis: current concepts and future perspectives  
Bernard Hemmer (Germany)

19.30-19.40  Questionnaire
**Stroke**
*Introduced and moderated by Salvatore Salomone (Italy)*

10.00-11.00  The role of immune system in the pathogenesis of ischemic stroke: identification of new pharmacological targets  
*Abstract*  
Alberto Chiarugi (Italy)

11.00-12.00  Neuroinflammation and cerebrovascular disease in old age: a translational medicine perspective  
*Abstract*  
Mario Di Napoli (Italy)

12.00-12.30  Discussion

**Alzheimer’s disease**
*Introduced and moderated by Giancarlo Pepeu (Italy)*

16.00-17.00  Neuroinflammation: implications for the pathogenesis of AD  
*Abstract*  
Claudio Cuello (Canada)

17.00-18.00  Passive immunotherapy in AD  
*Abstract*  
Beka Solomon (Israel)

18.00-18.30  Discussion

18.30-19.30  Special Lecture  
Active immunization strategies in AD: where do we stand? where should we go?  
*Abstract*  
Cinzia A. Lemere (USA)
Neuroimmune interactions in neurodegenerative disorders
*Introduced and moderated by* Maria Angela Sortino (Italy)

10.00-11.00  Neuroinflammation in amyotrophic lateral sclerosis: new targets for the treatment  
Francesco Fornai (Italy)

11.00-12.00  mTOR as a multifunctional therapeutic target in HIV infection  
Ferdinando Nicoletti (Italy)

12.00-12.30  Discussion

Parkinson’s disease and other neurodegenerative disorders
*Introduced and moderated by* Claudio Cuello (Canada)

16.00-17.00  Modeling neuroinflammatory pathogenesis of PD  
Fabio Blandini (Italy)

17.00-18.00  Cytokines and immunity in PD  
Mario Zappia (Italy)

18.00-18.30  Discussion

18.30-19.30  Special lecture  
Neuroinflammation in PD: a target for neuroprotection?  
Stephane Hunot (France)

19.30-19.40  Questionnaire
**Migraine**
*Introduced and moderated by Stephan Hunot (France)*

- **10.00-11.00**  The role of neurogenic inflammation in the pathophysiology of migraine  
  [*Abstract*] Salvatore Salomone (Italy)

- **11.00-12.00**  Novel therapeutical targets in the treatment of migraine  
  [*Abstract*] Pierangelo Geppetti (Italy)

- **12.00-12.30**  Discussion

**Pain**
*Introduced and moderated by Pierangelo Geppetti (Italy)*

- **16.00-17.00**  Current challenges in glia-pain biology  
  [*Abstract*] Marzia Malcangio (UK)

- **17.00-18.00**  The role of neuroinflammation in neuropathic pain: mechanisms and therapeutic targets  
  [*Abstract*] Paola Sacerdote (Italy)

- **18.00-18.30**  Discussion

- **18.30-19.30**  Special lecture  
  Modulation of peripheral sensory neurons by the immune system: implications for pain therapy  
  [*Abstract*] Cristophe Stein (Germany)
Schizophrenia
*Introduced and moderated by Eugenio Aguglia (Italy)*

10.00-11.00  Role of perinatal inflammation in the pathogenesis of schizophrenia  
*Abstract*  
Joram Feldon (Switzerland)

11.00-12.00  Inflammatory processes in schizophrenia: a promising neuroimmunological target for the treatment of negative/cognitive symptoms  
*Abstract*  
Norbert Müller (Germany)

12.00-12.30  Discussion

12.30-13.30  Sponsored lecture by Janssen-Cilag  
Second-generation LAI antipsychotics in the management of schizophrenia  
*Abstract*  
Edoardo Spina (Italy)

Neuroinflammation in CNS disorders
*Introduced and moderated by Donatella Marazziti (Italy)*

16.00-17.00  Special lecture  
Neuroinflammation and docosanoid signaling in synaptic circuitry integrity: new mediators for neuroprotection and long term rescue  
*Abstract*  
Nicolas G. Bazan (USA)

17.00-17.30  Discussion

17.30-18.30  Special lecture  
Sickness behavior versus clinical depression: from inflammation to oxidative/nitrosative stress, autoimmune responses to neoepitopes, and neuroprogressive pathways  
*Abstract*  
Michael Maes (Belgium)

18.30-18.45  Closing remarks  
Filippo Drago (Italy)

18.45-18.55  Questionnaire
10th Summer School of Neuroscience 2012

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Cytokines in CNS physiology and pathology

Robert Dantzer
Anderson Cancer Center - Division of Internal Medicine
Department of Symptom Research, Houston Texas, USA

Cytokines are intercellular communication molecules that activate or stimulate the proliferation of target cells. They have been first identified in the immune system but have since been characterized together with their receptors in other organs including the central nervous system. This presentation will be restricted to proinflammatory cytokines such as interleukin-1beta and tumor necrosis factor-alpha that are mainly produced by activated monocytes and T-cells. At the periphery these cytokines coordinate the cellular and molecular events that ultimately result in the mounting of an inflammatory response. They are expressed constitutively in the central nervous system and they function as modulators of neurotransmission and learning and memory. However, they are also expressed in an inducible manner in response to peripheral or local immune stimulation and they serve to coordinate the central component of the acute phase response. Alterations in the CNS cytokine compartment play a pivotal role in the development of chronic pain, neuro-pathies, severe fatigue, mood disorders, and cognitive dysfunction that develop in physically ill patients. Some of the pathophysiological mechanisms that are involved in these disorders have been elucidated, making cytokine signaling pathways a viable target for the development of new therapies. Examples will be given in the field of pain and depression.
Inflammatory signalling pathways in CNS disorders

Cinzia Dello Russo
Institute of Pharmacology
Catholic University Medical School
Rome, Italy

The central nervous system (CNS) represents an immune privileged organ, in which immune and inflammatory responses are under a tight regulatory control, in order to prevent detrimental neuronal damage (Galea E et al., 2003). Microglial cells, the macrophages resident within the CNS, are the main immune cells in the brain and play an important role in initiating and sustaining inflammatory responses. These highly specialized cells are influenced by the local microenvironment and have a number of distinctive features, such as a ‘ramified’ morphology and downregulated phenotype. However, they express a wide variety of receptors of the innate immune system (sensor receptors) and undergo rapid activation in response to danger. Once activated, these cells can release inflammatory mediators, including cytokines, chemokines, neuritrophic factors and possibly neurotoxic factors, like nitric oxide (NO). Neuroinflammation, a chronic inflammatory process largely characterized by activated microglial cells has a pathophysiological role in virtually all CNS disorders (Griffin WS, 2006). Although the inducers of inflammation may be generated in a disease-specific manner, there is a robust evidence for a convergence in the mechanisms of sensing (i.e. Toll-like receptors), transduction (activation of transcription factors, like NFκB), and amplification (release of pro-inflammatory cytokines) of inflammatory processes leading to the production of neurotoxic mediators (Glass CK et al., 2010).

Recent experimental evidence from our group and others support the notion that the mammalian target of rapamycin (mTOR) is involved in microglial pro-inflammatory activation, thus making this kinase a possible target for therapeutic intervention to reduce brain inflammatory responses. In this regard, we have shown that mTOR mediates cytokine dependent cell activation and proliferation in primary cultures of rat cortical microglia (Dello Russo C et al., 2009). Moreover, we found that mTOR activation participates in the processes involved in the upregulation of the inducible form of NO synthase (NOS2) in astrocytes (Lisi L et al., 2011). Together these results suggest that inhibition of mTOR kinase activity in glial cells can result in antiinflammatory actions. Thus, mTOR inhibitors, like rapamycin and its analogs, may exert beneficial effects in inflammatory CNS disorders, like multiple sclerosis (MS). In this regard, rapamycin has been shown to prevent the induction and the progression of the relapsing-remitting experimental autoimmune encephalomyelitis (RR-EAE), a widely used animal model to study RR-MS pathology. This beneficial effect has been associated to suppression of effector T cell function and simultaneous increase of the percentage of T regulatory (Treg) cells (Esposito et al., 2010). In addition, RR-EAE rats treated with rapamycin exhibited a milder inflammatory infiltration of the spinal cord with smaller areas of demyelination and increased...
number of splenic Tregs in comparison to control animals (Donia et al., 2009). In a recent study, we have tested the effects of rapamycin in a chronic model of EAE (Lisi L et al., 2012a). Active immunization with myelin oligodendrocyte glycoprotein (MOG) or MOG35–55 peptide in C57BL6 mice yields a chronic monophasic disease, characterized by sustained central inflammation, demyelination and axonal damage (Iglesias et al., 2001). In our experience, animals usually develop a long-lasting disease, showing clinical symptoms 5-7 days after the MOG35–55 booster injection and reaching the peak of disease at 10-14 days. The disease tends to remain stable or progress over the time; in fact animals barely recover unless effectively treated (Feinstein DL et al., 2002; Murphy P et al., 2002). Therefore, this model appears to be a better experimental paradigm to study chronic forms of MS. Rapamycin ameliorates clinical and histological signs of chronic EAE when administered to already ill mice, at the peak of disease (therapeutic approach). Moreover, the drug significantly reduced the hyperalgesia, detected at the clinical onset of disease (Lisi L et al., 2012a). These findings may have important clinical implications for the therapy of MS, in particular its chronic forms.

Neuroinflammation plays also a prominent role in the physiopathology of HIV-1 related neurological disorders. Despite the success of the highly active antiretroviral therapy in suppressing plasma viral load as well as improving CD4+ cell counts, neurological disorders, including headaches, mild cognitive impairments, peripheral neuropathies, HIV-associated dementia, and HIV encephalitis, continue to affect approximately 50% of HIV-infected patients (for a recent review, Grovit-Ferbas and Harris-White 2010). HIV enters the CNS early during acute infection; it does not productively infect neurons but resides primarily in microglial cells and perivascular macrophages. Once infected these cells become activated thus promoting inflammation within the brain. Moreover, HIV infected microglia and macrophages continuously release HIV proteins that can increase the inflammatory response and lead to neuronal damage. A new class of antiretroviral drugs includes small molecular weight antagonists of the CCR5 co-receptor, among which maraviroc is the first approved drug for the treatment of HIV-experienced patients, namely patients with detectable HIV RNA load, multi-resistant to other antiretroviral drugs and infected by R5-tropic virus. We have tested the hypothesis that maraviroc, by blocking a chemokine receptor, may affect microglial activation during HIV-1 infection. In fact, maraviroc displayed opposite effects on microglial activation, depending on whether or not interferon γ (IFNγ) was used to stimulate microglial cells in vitro. Maraviroc down-regulated pro-inflammatory gene expression elicited by the viral protein gp120, whereas increased microglial activation when cells were stimulated with gp120 and IFNγ (Lisi L et al., 2012b). Taken together these data suggest that maraviroc can have the potential to exacerbate microglial activation in vivo, thus increasing in the long term the incidence of neurological complications in HIV-infected patients.
‘Nerve-Driven Immunity’ is a novel term (coined by the author of this abstract) and refers to the direct and potent effects that neurotransmitters and neuropeptides have in immune system. This topic is covered in a novel international book entitled: “Nerve-Driven Immunity: Neurotransmitters and Neuropeptides in the Immune System” (Publisher: Springer; Editor: Mia Levite), and was also discussed in an International meeting with a similar name held recently in Vienna on 9–10 March 2012.

Each book chapter, alike each talk in the meeting dealt with the unrecognized yet very important receptors and effects in the immune system of a different neurotransmitter or neuropeptide. The neurotransmitters and neuropeptides that exert potent effects on immune cells include the following: dopamine, adrenaline, noradrenaline, acetylcholine, glutamate, GABA, serotonin, somatostatin, neuropeptide Y (NPY), vasoactive intestinal polypeptide (VIP), calcitonin gene-related peptide (CGRP), opioids and cannabinoids, and most probably also others.

The four general lines take-home messages of book and meeting are as follows:

1) **Neurotransmitter’s receptors in the immune system**: Specific receptors for many neurotransmitters and neuropeptides receptors are expressed in most, if not all, types of immune cells. Yet, different immune cells have a different composition and level of neurotransmitter receptors. Also, the expression of the neurotransmitter receptors in immune cells is very dynamic and sensitive to the activation state of the cells. Resting na?ve immune cells differ markedly from activated immune cells in regards to the neurotransmitter receptors they express.

2) **Neurotransmitter’s effects in the immune system**: Neurotransmitters and neuropeptides by themselves trigger or rather inhibit many key immune functions. The same is true for various neurotransmitter analogs. The neurotransmitter-induced immune effects depend on the context and many factors among them: the neurotransmitter’s, the neurotransmitter’s receptor subtype being activated, the activation state of the immune cell, the simultaneous presence or absence of other stimuli that affect the immune cells, and several other factors.

3) **Neurotransmitter’s production in the immune system**: most of the neurotransmitters and neuropeptides are produced in immune cells, and under certain conditions they can be released to the extracellular milieu and affect other cells.
4) Involvement of neurotransmitters in immune diseases: Most of the neurotransmitters and neuropeptides are involved in various diseases of the immune system, among them autoimmune diseases, immunodeficiency diseases, inflammatory diseases and immune cancers: Leukemia and Lymphoma. All the above have wide implications on health and various diseases, and on the essential dialogue between the nervous system and the immune system. In my talk I will first discuss ‘Nerve-Driven Immunity’ in general, along the above-mentioned points, and then focus on dopamine receptors and effects in human T cells.
It is known that a large number of proteins that were first discovered in the immune system have been detected in healthy, uninfected nervous system, thus suggesting the possibility that they may have neuronal additional functions. Indeed, accumulating data demonstrates that different “immune proteins” not simply function in an immune setting, but are also crucial for normal brain development, neuronal differentiation, synaptic plasticity and even behavior. Furthermore, several studies reveal interesting parallels between cellular signaling mechanisms in the immune and nervous systems that may provide unexpected insights into the development, function, and diseases of both systems. These findings lead to strong clinical implications pointing to new directions for research and implying that “immune proteins” could be involved in the etiology and expression of several SNC disorders. Accordingly, this presentation aims to review and discuss several examples of novel synaptic and neuronal functions for immune molecules in the brain, and vice versa.
In response to stress, the brain activates several neuropeptide-secreting systems. This eventually leads to the release of adrenal corticosteroid hormones, which subsequently feed back on specific limbic brain circuits to modulate information processing. To exert these actions the hormone binds to two distinct types of receptors that coordinate fast membrane events with regulation of transcription. By targeting multiple genes, the two corticosteroid receptor types function in a binary fashion, serving as a master switch in the control of neuronal and network responses that underlie emotional arousal, cognitive processes and behavioural adaptation. In individuals predisposed either genetically or epigenetically by previous experiences, imbalance in this binary control mechanism can introduce a bias towards stress-related brain disease after adverse experiences. New candidate susceptibility pathways are now being identified that may serve as biomarker to predict a phenotype vulnerable to disease or as potential target to promote resilience still present in the diseased brain.

Recent findings suggest that the above concept of the complementary MR- and GR-mediated actions of corticosterone operating in binary fashion on brain and behavior can be expanded to coordination with peripheral systems. This coordination of mind, brain and body can occur through centrally driven hormonal cascades and sympathetic outflow as well as by a direct action exerted by the naturally occurring glucocorticoid on the neuro-immune system. This is exemplified by the emerging evidence for an MR-mediated pro-inflammatory action and immune activation as opposed to the well known anti-inflammatory action and immune suppression via GR. Supported by the Royal Netherlands Academy of Science, EU-lifespan (www.lifespannetwork.nl), Eurostress and TI-Pharma.

Glucocorticoid-Induced TNFR-Related (gitr) is a gene coding for a member of the TNF receptor super-family (TNFRSF). It is expressed in several cells and tissues, including T and Natural Killer (NK) cells, and is activated by its ligand, GITRL, mainly expressed on Antigen Presenting Cells (APCs) and endothelial cells. GITR activation by its ligand (GITRL) influences the activity of effector and regulatory T cells thus participating in the development of immune response against tumours and infectious agents, as well as in autoimmune and inflammatory diseases. In particular, we and others have demonstrated that GITR plays a pivotal role in murine experimental colitis, acute and chronic inflammation of the lung, collagen-induced arthritis, splanchnic artery occlusion (SAO) shock, thyroiditis, acute pancreatitis and multiple organ dysfunction syndrome (MODS). GITR is crucial also in the inflammatory response within CNS as demonstrated in the experimental autoimmune encephalomyelitis and spinal cord injury models. These effects are due to several concurrent mechanisms including: co-activation of effector T cells, inhibition of regulatory T (Treg) cells, NK cell co-activation, activation of macrophages, modulation of DC function and regulation of the extravasation process. Notably, treating animals with GITR-Fc fusion protein ameliorates autoimmune/inflammatory diseases while GITR triggering, by treatment with anti-GITR mAb, is effective in treating viral, bacterial and parasitic infections as well as in boosting immune response against tumours. Indeed, GITR modulation has been indicated as one of the top 25 most promising research areas by the American National Cancer Institute and a clinical trial testing the efficacy of an anti-GITR mAb in melanoma patients has been started. Moreover, we have recently identified a CD4+CD25lowGITR+ cells in the blood of human healthy donors. This population posses regulatory activity and is expanded in patients affected by autoimmune diseases. The expression of GITR in cells different from those of immune system opens the possibility of using GITR modulators in diseases unrelated to immune system but also indicates there are possible risks deriving from GITRL/GITR system pharmacological modulation. Moreover, structural and functional studies clearly demonstrate differences between GITRL/GITR systems of mice and humans, suggesting that results from mice should be applied to humans cautiously. In conclusion, GITR triggering and inhibition could be useful in treating tumours, infectious diseases as well as autoimmune and inflammatory diseases.
Immune influence on adult neural stem cell regulation and function

Maria Grazia Grilli
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Neurogenesis, the formation of new neurons from neural stem/progenitor cells (NSC, NPC), occurs in the hippocampal dentate gyrus and in the subventricular zone of mammals throughout life. Although the exact function of adult neurogenesis is currently uncertain, recent studies suggest that at least in the hippocampus the newly formed neuronal population plays important roles in hippocampal-dependent cognitive abilities, including memory. Interestingly, the process of adult neurogenesis is greatly influenced by the interaction between cells of the adaptive immune system and CNS-resident immune cells. During this presentation special attention will be given to the current knowledge on the cross-talk existing between NSC/NPCs and CNS-resident microglia as well as CNS-infiltrating immune cells from the circulation. According to the current knowledge, the regulation of such immune-cell activity appears crucial since too little immune activity (as for example in immune deficiency syndromes) or too much immune activity (as in severe inflammatory diseases) can lead to impaired hippocampal neurogenesis, which could then result in impaired hippocampal-dependent cognitive abilities.

The interaction of immune system with adult neural stem/progenitor cells will be discussed in view of its potential influence on aging-associated decline in neurogenesis and on deregulated neurogenesis in neuropsychiatric disorders, including major depression and neurodegenerative diseases. Finally the therapeutic implications of such interaction will be extensively discussed.
Microglia are the main immune cells in the brain, playing a role in both physiological and pathological conditions. Microglial involvement in neurodegenerative diseases is well-established, being microglial activation and neuroinflammation common features of these neuropathologies. Microglial activation has been considered harmful for neurons, but inflammatory state is not only associated with neurotoxic consequences, but also with neuroprotective effects, such as phagocytosis of dead neurons and clearance of debris. This brought to the idea of protective autoimmunity in the brain and to devise immunomodulatory therapies, aimed to specifically increase neuroprotective aspects of microglia. During the last years, several data supported the intrinsic neuroprotective function of microglia through the release of neuroprotective molecules. These data led to change the traditional view of microglia in neurodegenerative diseases: from the idea that these cells play an detrimental role for neurons due to a gain of their inflammatory function, to the proposal of a loss of microglial neuroprotective function as a causing factor in neuropathologies. This “microglial dysfunction hypothesis” points at the importance of understanding the mechanisms of microglial-mediated neuroprotection to develop new therapies for neurodegenerative diseases. In vitro models are very important to clarify the basic mechanisms of microglial-mediated neuroprotection, mainly for the identification of potentially-effective neuroprotective molecules, and to design new approaches in a gene therapy set-up. Microglia could act as both a target and a vehicle for CNS gene delivery of neuroprotective factors, endogenously produced by microglia in physiological conditions, thus strengthening the microglial neuroprotective phenotype, even in a pathological situation.
Immune activation in brain aging: too much or too little?

Claudio Franceschi  
Department of Experimental Pathology  
University of Bologna, Italy

Aging is a complex phenomenon characterised by reduced fitness and increased risk of morbidity and death. Most of the more prominent age-related diseases including neurodegeneration share an inflammatory pathogenesis and the same holds for many autoimmune diseases of the CNS, such as Multiple Sclerosis. Data will be presented regarding the emerging concept that a chronic inflammatory status characterises old age (inflamm-aging) therefore favouring the onset of these diseases. Moreover, a summary of the last results obtained by high-throughput techniques (“omics”) in the study of aging and longevity will be presented, showing the complexity of the phenomenon and the need for an integrated approach of Systems Biology for the analysis and interpretation of these high-dimensionality data.
Fibromyalgia syndrome (FMS) is a common chronic condition, that affects at least 2% of the adult population. Chronic widespread pain is the defining feature of FM, but patients may also exhibit a range of other symptoms, including sleep disturbance, fatigue, irritable bowel syndrome, headaches, and mood disorders.

The pathophysiology of fibromyalgia (FM) is not completely understood: interactions among external stressors, behavioural constructs, neuro-transmitters, hormones, immune, and sympathetic nervous systems alterations appear to be involved (Stisi et al., 2008). Dysregulation of the HPA axis can partially explain some symptoms of FM, including fatigue, depression and sleep disturbance. This dysregulation might be directly related to pain perception, as demonstrated by a significant association between levels of CRH in CSF and pain levels (Lyon et al., 2011). Studies in patients with FM uniformly observed subtle alterations in hypothalamic pituitary adrenal functioning and structural and functional changes in the brain (Geenen and Bijlsma, 2010), that are related to a sympathetic hyperactivity (Stisi et al., 2008), but also to an autonomic nervous system hyporeactive responsiveness to stressors. Recent data highlight the putative role of cytokines in the pathogenesis of FM (Muzammil and Cooper, 2011; Maes et al., 2012), but not all studies agree (Menzies et al., 2011; Uçeyler et al., 2011). The autonomic nervous system is implicated in the maintenance of the physiological homeostasis: dysautonomia in FMS could at least contribute to several others symptoms such as pain, fatigue and morning stiffness (Kingsley, 2012). Relentless sympathetic hyperactivity may explain sleep disorders (Lerma et al., 2011), anxiety, pseudo-Raynaud’s phenomenon, sicca symptoms, and intestinal irritability. In contrast several data demonstrate an attenuated activity of both the sympathetic (adrenal medulla component) and the parasympathetic branch (Kadetoff and Kosek, 2010; Riva et al., 2012). Favorable neuroendocrine changes should be expected after successful pharmacological or non-pharmacological interventions that target pain and associated symptoms.

References
-Kadetoff D, Kosek E. Evidence of reduced sympatho-adrenal and hypothalamic-pituitary activity during static muscular work in patients
Fibromyalgia syndrome: from diagnosis to pharmacological treatment

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Introduction
Musculoskeletal pain conditions are universally prevalent among all age and gender groups. They constitute a diverse group of disorders with regard to pathophysiology but are linked anatomically and by their association with pain and impaired physical function. They encompass a spectrum of conditions, including inflammatory diseases such as rheumatoid arthritis or gout; age-related conditions such as osteoporosis and osteoarthritis; common conditions of unclear aetiology such as back pain and fibromyalgia; and those related to activity or injuries such as occupational musculoskeletal disorders, sports injuries or the consequences of falls and major trauma. Among them, Fibromyalgia (FM) represents the intersection of a considerably abnormal and reduced pain threshold with a series of clinical distress variables including pain, fatigue, sleep disturbance, anxiety, and depression, among others.

Diagnosis
The construct of FM derived from an article by Smythe and Mofsky entitled ‘Two contributions to the understanding of the “fibrositis” syndrome’ published in 1977. The authors identified the characteristics of the syndrome, then called ‘fibrositis’, and proposed criteria based on what they saw as its key features: non-refreshing sleep and tender points. Tender points were defined as pre-specified points on the body that, in persons with the syndrome, were particularly sensitive to pressure. The presence of ‘widespread aching for longer than three months’ and ‘disturbed sleep with morning fatigue and stiffness’ was also a requirement in these criteria. Decreased pain threshold was measured by a count of tender points.

By the late 1980s there were many different formal and ad-hoc criteria sets. There was no clear agreement on which tender point sites should be examined or how they should be examined, nor how many sites had to be tender for a positive examination. Similarly, the format and content of symptom questions was unknown. Both in the clinic and in research settings, the reliability and validity of the available criteria was not known.

The 1990 ACR classification criteria
Based on comparing patients with similar but non-fibromyalgia pain complaints, the ACR-committee found that the presence of widespread pain (WSP) combined with at least 11 of 18 tender points best separated patients with fibromyalgia and controls, even though...
some combinations of symptoms (e.g., fatigue, cognitive problems) were not evaluated. This occurred because the authors did not recognize the importance of these symptoms at the time of the study. The authors suggested that the presence of 11 of 18 tender points and the simultaneous presence of WSP for at least 3 months should be the classification criteria for FM.

**American College of Rheumatology 2010 preliminary diagnostic criteria**
The 2010 criteria addressed a number of problems with the 1990 criteria. They eliminated the TPE, a physical examination item, substituting the widespread pain index (WPI), a 0–19 count of the number of body regions reported as painful by the patient. In addition, the 2010 criteria assessed on a 0–3 severity scale a series of symptoms that were characteristic of fibromyalgia: fatigue, non-refreshed sleep, cognitive problems, and the extent of somatic symptom reporting. The items were combined into a 0–12 Symptom Severity (SS) scale. Finally, the Widespread Pain Index (WPI) and SS-scales could be combined into a 0–31 fibromyalgianess scale, a second measure of polysymptomatic distress or FM severity.

Instructions, a criteria worksheet, and a patient pain location report are available on-line as an aid to ACR2010 assessments (www.arthritis-research.org/research/fibromyalgia-criteria).

**Pharmacological treatment**
Once a diagnosis of FM is made, patients are usually started on pharmacological treatment. Boomershine and Crofford suggest that the three drugs currently approved by the American Food and Drugs Administration (FDA) should now be used as ‘anchor drugs’ and, although still important, could later be complemented by older approaches. Unfortunately, no direct comparative studies have yet been published, and there is still no consensus as to where to start.

FM patients experience amplified ascending sensory input on which drugs such as anticonvulsants may act, and defects in the diffuse noxious inhibitory control system (DNIC), which could be treated using serotonin-norepinephrine re-uptake inhibitors (SNRIs). This may be an over-simplification, but it does show that there are at least two complementary symptom control strategies.

Pregabalin binds to the α2δ (alpha2delta) subunit of the voltage-dependent calcium channel in the central nervous system; it has analgesic, anxiolytic-like and anticonvulsant activity in animal models. Alpha2-delta is an auxiliary protein associated with voltage-gated calcium channels, and the potent binding of pregabalin at the α2δ (alpha2delta) site reduces calcium influx at nerve terminals and thus reduces the release of a number of neurochemicals, including glutamate, noradrenaline and substance P, which may explain the analgesic, anticonvulsant and anxiolytic-like activity of pregabalin in animal models. It has also been suggested that reducing neurotransmitter release from neurons in the spinal cord and brain may be clinically beneficial for FM patients.

Pregabalin is approved for the treatment of FM and neuropathic pain and, in some countries, also for the management of generalised anxiety disorder and as an adjuvant medication for seizures. The dose indicated for the treatment of FM is 300-450 mg/day divided into two administration, although many clinicians start with smaller nightly doses, as it seems to have a specific beneficial effect on sleep. Anxiety is also very common in FM patients and, as patients with concomitant sleep disorders and anxiety almost always experience...
initial insomnia, pregabalin is a rational choice. The dose can subsequently be increased to the recommended dose, but escalation may be limited by side effects such as weight gain, edema and dizziness that is generally self-limiting over time. Although it is not approved by the FDA, the use of gabapentin has also been studied in FM patients, and daily doses ranging from 1200 mg to 2400 mg have been found to have some effect. Although the gabapentin trials involved far fewer patients than the pregabalin trials, the effect sizes seemed to be similar, and so gabapentin can be considered an option especially when pregabalin is not available. The other two FDA-approved medications for the treatment of FM are duloxetine and milnacipran. Duloxetine is also FDA-approved for depression, generalised anxiety disorder and painful diabetic neuropathy, and milnacipran is also approved for the management of major depression in Europe and Japan. Both are SNRI antidepressants, and it has been hypothesised that pain, anxiety, chronic stress and depression have common pathogenetic backgrounds (neurotransmitters and immune responses), and depression can be considered a systemic disease related to unbalanced neurotransmission also to other neurotrophic, neurosteroidal, central nervous system (CNS) hormonal modifications, and widespread autonomic, immunological and metabolic somatic changes. According to this hypothesis, antidepressants restore neurotransmitter levels and modulate receptor expression in the hypothalamus, which normalises hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis. Autonomic system alterations, such as sympathetic overactivity, are encountered in both depression and fibromyalgia. Finally, pro-inflammatory cytokines inside the CNS play a role in the pathophysiology of mood disorders and pain, and their modulation by means of chronic antidepressant administration contributes to improving both.

The trials of duloxetine have shown that 70% of its effect on pain is due to its analgesic rather than its antidepressant action, although it is still a good choice for patients with FM, depression and anxiety. The FM dose is 60 mg once a day, but it is usually started at a daily dose of 30 mg. Duloxetine seems to have a neutral effect on sleep.

The recommended milnacipran doses range from 50 mg twice a day to 100 mg twice a day. It has been found that it significantly improves fatigue and fibro-fog (especially at the 200 mg dose), possibly because of its greater adrenergic effect.

**Analgesic treatment**

Tramadol has been found to be beneficial in FM patients. It is an atypical pain reliever that has a different action on the CNS (the re-uptake of serotonin and norepinephrine) from that of other narcotics. Its most common side effects are drowsiness, dizziness, constipation and nausea, and it should not be given in combination with tricyclic antidepressants (TCAs). Alone or in combination with acetaminophen, it is commonly prescribed at a dose of 200-300 mg/day to relieve FM-related pain. Its potential for drug abuse is fortunately negligible, but there is a theoretical risk of seizures and serotoninergic syndrome when it is combined with selective serotonin re-uptake inhibitors (SSRIs), SNRIs, monoamine oxidase inhibitors (MAOIs) and tryptans, although only a few cases have been described. There is no scientific evidence that NSAIDs alone are effective in FM patients, although they may be useful for analgesia when combined with TCAs. However, the results obtained when NSAIDs are combined with benzodiazepines have been inconsistent. The CNS mechanisms of FM (central sensitisation and disinhibition, and a dysfunctional HPA axis) may explain the relatively reduced efficacy of NSAIDs and opioids, particularly as the latter are more effective for peripheral pain. However, NSAIDs can be helpful in reducing pain.
flares induced by excessive physical activity, tendinitis or bursitis, although they should only be used on an as needed basis in order to avoid side effects. COX2 inhibitors have much fewer side effects, but are less effective against pain.

One recent study has found that transdermal buprenorphine, a strong opioid, has beneficial effects on severe widespread pain (VAS >6/10), but it is less effective on the other symptoms typical of FM.

A subset of FM patients do not respond to opioids, but other patients who may have overlapping conditions such as diabetes, chronic myofascial pain, temporomandibular joint disorder, arthritis, degenerative disc disease and so on, may receive a significant benefit. The doses of immediate-release opioids should be increased slowly until the pain is reduced, and then the patients should be switched controlled-release opioids as most patients with chronic, non-malignant pain can be managed with <200-300 mg/day of morphine (or equivalent).

Opioids may be helpful in treating FM-related pain, but they may also induce tolerance and become habit forming, and are also associated with adverse effects such as constipation, sedation and nausea. Physicians should obtain a careful medical and psychological profile of the patient before prescribing opioids.

Treating fatigue, sleep and mood disorders

Fatigue and sleep disturbances are major complaints among FM patients. Appropriate treatment of sleep disturbances and physical rehabilitation are the best means of managing fatigue in the long term. The medications commonly used in narcolepsy have been used to treat fatigue in FM patients. Modafinil has been approved by the FDA for the treatment of excessive somnolence associated with narcolepsy, shift workers and sleep apnea, and can be useful if fatigue prevents patients from starting physical rehabilitation. The initial dose is 50 mg in the morning, and can be increased to 400 mg daily, although modafinil and other compounds carry a risk of abuse and drug interactions.

Small doses of the amitriptyline or cyclobenzaprine represent the first approach to the treatment of delayed sleep onset in FM patients, but they are only effective in about 30% of cases and most patients cannot tolerate higher doses because of their sedative and cholinergic side effects [44-46]. Although it has not been formally tested, trazodone is a usually well-tolerated sedating antidepressant. Hypnotic drugs as zolpidem, zopiclone and eszopiclone can also be used, and act mainly through the BZ1 receptor. Benzodiazepines should be used cautiously as they may disrupt sleep architecture.

Sodium oxybate is the sodium salt of gamma-hydroxybutyrate (GHB), an endogenous short chain fatty acid, used for the oral administration of exogenous GHB. The supraphysiological concentrations induced by exogenous administration probably lead to qualitatively different neuronal activity from that of endogenous GHB. GHB may play a neuromodulating/neurotransmitting role, and sodium oxybate is approved by the FDA for the treatment of cataplexy and excessive daytime sleepiness. It has recently also been shown to improve pain and fatigue in phase III FM trials.

Thirty percent of FM patient suffer from depression at the time of diagnosis, and 50-60% sometime during their lifetime. There is also evidence that anxiety can be as common as depression in FM patients, and post-traumatic stress disorder (PTSD) is more prevalent in
FM patients than in the general population. The central monoaminergic neurotransmission abnormalities observed in depression may play a role in FM pathophysiology because dysfunctioning 5-HT- and NE-mediated descending pain-inhibitory pathways are important mechanisms in FM-related pain. Antidepressants that increase 5-HT- and NE-mediated neurotransmission are frequently used to treat FM and other chronic pain conditions, particularly neuropathic pain. Inhibiting both 5-HT and NE re-uptake transporters using TCAs or SNRIs seems to be more effective in treating pain and FM than inhibiting either transporter alone with selective SSRIs or noradrenergic re-uptake inhibitors (NARIs) [52,53], but the efficacy of TCAs is counterbalanced by their side effects. SSRIs such as citalopram [54], escitalopram, paroxetine and sertraline are not effective against pain but, like fluoxetine, could be used to treat associated depression.

**Combination therapy**
In general, about half of all treated patients with medication seem to experience a 30% reduction of symptoms, suggesting that many patients with fibromyalgia will require additional therapies. The number of randomized controlled trials of exercise or behavioral interventions in the fibromyalgia literature has increased dramatically in the past decade. Progressive walking, simple strength training movements, stretching activities, aerobic exercise improve functional status, and self-efficacy in women with fibromyalgia actively being treated with medication. Thus, other forms of treatment, including exercise, cognitive behavioural therapies and self-management strategies, may be necessary to achieve satisfactory treatment outcomes. Surprisingly no controlled randomized data emerge from the literature which explains how much improvement it’s possible to get from combining a pharmacological treatment with a structured rehabilitation or psychological program.

**Conclusions**
It is clear that, as in the case of other disorders, the most efficacious treatment of FM needs to combine the main elements of pharmacotherapy, exercise, physical therapy and CBT. A number of medical treatments have been used to treat the various symptoms of FM (pain, sleep disturbances, anxiety and depression) with the final aim of improving the patients’ quality of life. Psychological and physical therapy may sometimes be more effective than pharmacological treatment. A number of studies have also evaluated the effect of moderately intense exercise, the level that is most suitable for usually deconditioned and unfit FM patients.

**References**
Hauser W, Wolfe F. Diagnosis and diagnostic tests for fibromyalgia (syndrome). Reumatismo 2012, in press.
Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Katz RS, Mease P et al. The American College of Rheumatology preliminary diagno-
Major depression is a common and sometimes fatal disorder that is a leading cause of disability worldwide. Available antidepressant medications, which largely target monoamine pathways, are effective; however, more than 30% of depressed patients fail to achieve remission despite multiple treatment trials. Thus, there is a pressing need to identify novel pathophysiologic pathways relevant to depression that 1) reveal neurobiological targets for the development of new medications and 2) elucidate related biomarkers for the identification and monitoring of potentially responsive patients. One promising development in this regard is the emergence of inflammation as a common mechanism of disease. Indeed, numerous studies have demonstrated a clear relationship between inflammation and the development of cardiovascular disease, diabetes, and cancer. Mounting data indicate that inflammation may also play a role in neuropsychiatric diseases, including major depression. There is a rich animal literature demonstrating that administration of cytokines or cytokine inducers can profoundly affect the metabolism of serotonin, norepinephrine, and dopamine. Moreover, drugs (serotonin and norepinephrine reuptake inhibitors) and gene polymorphisms (serotonin transporter gene) that affect monoamine metabolism have been shown to influence the development of cytokine-induced depressive-like behavior in laboratory animals and humans. Regarding the mechanisms involved, much attention has been focused on the enzyme, indoleamine 2,3 dioxygenase (IDO). Through stimulation of multiple inflammatory signaling pathways, including signal transducer and activator of transcription 1a (STAT1a), interferon regulatory factor (IRF)-1, NF-kB, and p38 mitogen activated protein kinase (MAPK), cytokines can activate IDO. Indoleamine 2,3 dioxygenase, in turn, breaks down tryptophan (TRP), the primary amino acid precursor of serotonin, into kynurenine (KYN). The breakdown of TRP is believed to contribute to reduced serotonin availability. Supportive of the role of IDO in cytokine-induced depression, decreased TRP and increased KYN in the peripheral blood have been associated with the development of depression in patients administered IFN-alpha. Moreover, blockade of IDO has been shown to inhibit the development of LPS-induced depressive-like behaviour in mice. Of note, cytokine-induced IDO activation and the generation of KYN appear to have important effects on neurotransmitters and mood independent of effects on serotonin. For example, administration of KYN alone has been shown to induce depressive-like behavior in mice.

Given the accelerating development of biomarkers and treatments focused on the inflammatory response, there is tremendous promise that these advances, in addition to their relevance to general medicine, may have unique applications in psychiatry.
New concepts in fundamental immunology, in particular the identification of a lineage of CD4 T cells endowed with regulatory properties (Tregs) have revolutionized the understanding of immune-mediated diseases both in experimental models and in humans. The impact of the Tregs, characterized by the expression of CD25 and the Foxp3 transcription factor, on the development and remission from CNS inflammation, and their therapeutic potential, is being aggressively studied in preclinical animal models. Recent data indicate that the CD4+ CD25+ Foxp3+ Tregs act both at the level of secondary lymphoid organs and in the inflamed CNS during experimental autoimmune encephalomyelitis. They contribute to the natural protection against autoimmunity and participate in the spontaneous remission of disease. Their role in multiple sclerosis (MS) is still unclear, but convergent data indicate that circulating CD4+CD25+ T cells from MS patients exhibit defective regulatory properties. Several disease-modifying therapies act on Treg cells and their beneficial effects on MS could, in part, result from this mode of action. A better understanding of the induction of Treg cells, of their mechanisms of action, and of approaches to manipulate them in vivo may offer new therapeutic opportunities for MS patients.
Inflammation and degeneration are the usual pathological processes occurring within the central nervous system (CNS). They are only apparently distinct process because as soon as the pathological process becomes chronic they have the tendency to become strictly interrelated. As such, primary neurodegeneration triggers a secondary inflammatory reaction while primary inflammatory reactions lead to neurodegenerative phenomena. Several molecular and cellular events sustaining intrinsic brain repair mechanisms (the brain repair system) occurring within the CNS as a consequence of chronic inflammatory and/or degenerative processes have been described so far. They can be divided into three distinct – although strictly interrelated – categories: inflammation-driven processes, CNS plasticity and neuro(glio)genesis. By one hand, humoral and cellular inflammatory components shift sense (function) over time from a tissue-damaging mode to a mode promoting tissue repair (e.g. neurotrophic support from inflammatory cells). By the other hand, the recruitment of alternative “non-damaged” functioning neuronal pathways (cortical maps) – occurring mainly via axonal branching and synaptogenesis – takes place as a consequence of brain damage. Whether or not (and to what extent) the recapitulation of precise developmental pathways underlies the whole phenomenon of brain plasticity is still matter of investigation. Finally, endogenous neural stem/precursor cells (NPCs) – the self-renewing and multipotent cells of the CNS capable of driving neurogenesis and gliogenesis in adult life – may adapt targeted migration into damaged areas and promote repair via several mechanisms of action (e.g. neuro and gliogenesis, immunomodulation, neuroprotection). It is still a matter of investigation whether (or not) equally robust brain repair/protection can occur following the recruitment within the CNS of trans-differentiating stem cells of a different embryonic origin (e.g. developmental plasticity vs. cell fusion). In multiple sclerosis (MS), a CNS-confined disorder characterized by primary inflammation, cellular degeneration leading to irreversible neurological deficits is predominantly viewed as the consequence of an uncontrolled – but still undiscovered – pathogenic alien. However, there is accumulating evidence suggesting that the failure of the brain repair system may also be considered as one of the contributing factors leading to irreversible neurodegeneration.
Natural History of multiple sclerosis in the pediatric age: implications for outcome

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10th Summer School of Neuroscience

International PhD Program in Neuropharmacology
Treatment of multiple sclerosis: current concepts and future perspectives

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Although the cause of multiple sclerosis (MS) has remained obscure, many findings support an autoimmune pathogenesis on the background of a complex interaction between multiple genes and environmental factors. Accordingly, targeting the immune system has been a rational approach for the treatment of MS. The development of disease-modifying immunomodulatory drugs with partial efficacy, coupled with advances in understanding the pathophysiology and pathology of MS, has provided momentum to explore more specific and hopefully more effective immune-based therapeutic strategies. Future treatments will likely need both to target inflammation and to focus on promotion of neuroprotection and repair. I will discuss current treatment strategies and the most promising therapeutic approaches for MS currently in the pipeline.
The role of immune system in the pathogenesis of ischemic stroke: identification of new pharmacological targets

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Despite its health burden, stroke treatment remains an unmet need. Energy impairment and excitotoxicity are early events contributing to ischemic neuronal death, whereas activation of brain inflammatory cells causes delayed ischemic injury. Players contributing to the harmful inflammatory responses accompanying ischemic stroke have been deeply investigated, and their molecular nature is in part understood. Among these, receptors of the innate immune system (such as Toll-like receptors (TLRs) and scavenger receptors), as well as specific lymphocytes subpopulations have now emerged as key contributors of the inflammatory response prompted by an ischemic challenge to the brain. In this presentation, current knowledge on molecular mechanisms triggering inflammatory cell recruitment and activation within the ischemic brain will be discussed, as well as the therapeutic potential of strategies targeting the post-ischemic inflammatory response.
Neuroinflammation and cerebrovascular disease in old age: a translational medicine perspective

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Stroke is among the most common diseases of advanced age becoming a steadily increasing financial healthcare problem in the industrialized world with the increasing longevity and aging of the population. The incidence of cerebrovascular disease is highest in the elderly population, representing one of the commonest causes of disability and mortality worldwide. Over the past decades, a tremendous amount of research has been undertaken into developing effective therapeutic strategies for the treatment of acute stroke. Unfortunately, many neuroprotective agents that have shown successful results in treating animal models of acute stroke have failed to translate into clinical treatments, because ischemic changes in the commonly used young animal stroke models do not reflect the molecular changes associated with the aged brain. Only tissue-plasminogen activator (t-PA) is currently licensed for use in the treatment of acute ischemic stroke. The pathophysiological mechanisms of brain response to cerebral ischemia in old age are currently poorly understood. However, during the last years promising findings suggest that systemic inflammation and neuroinflammation are central features in cerebrovascular disease in the elderly. Inflammation in the central nervous system (CNS) or in the periphery may be a risk factor for the initial development of cerebral ischemia. Peripheral infection and inflammatory processes are likely to be important in this respect. CNS inflammation is important in the pathophysiologic processes occurring in ischemic stroke, subarachnoid hemorrhage and following hemorrhagic stroke. Stroke elicits an inflammatory response in the injured brain that is accompanied by a marked local inflammatory reaction that is initiated by ischemia or hematoma-induced expression of cytokines, adhesion molecules, and other inflammatory mediators, including prostanoids, extracellular proteases, reactive oxygen species and nitric oxide, leading to the accumulation of inflammatory cells, such as leukocytes and microglia. Many of these compounds are known to promote and sustain inflammatory responses at local and systemic level, producing a neuroinflammatory response and a systemic acute phase response. The inflammatory reaction, which has a rapid onset and continues after the stroke, is thought to acutely contribute to the evolution of tissue injury and repair. The acute phase inflammatory response after stroke is a reflection of an unspecific Systemic Inflammatory Response Syndrome (SIRS). Classic acute-phase reactants and body temperature are also modified in stroke, and may be useful in the prediction of events, outcome, and as therapeutic targets. The pentraxin C-reactive protein (CRP) is found as a stronger and independent predictor of stroke risk and of prognosis in stroke patients when compared to other prothrombotic and inflammatory markers. Its synthesis is markedly upregulated in stroke. Since it, as known to activate the complement cascade in an antibody-independent fashion and chronic activation, can cause destruction of host tissue, this pentraxin may be important initiators of an autodestructive process influencing short-term and
long-term prognosis of stroke patients. Better understanding of the role of the post ischemic-induced inflammatory response and its potential for modulation might have profound implications for patient treatment. Preclinical studies suggest that interventions that are aimed at attenuating such inflammation reduce the progression of brain damage that occurs during the late stages of cerebral ischemia. In particular, strategies that block the activity of inflammation-related enzymes reduce ischemic damage with an extended therapeutic window. Although at the moment, clinical trials using anti-inflammatory strategies did not show benefit in patients with ischemic stroke, there is a strong rationale for continuing to explore the efficacy of anti-inflammatory therapies in the treatment of the late stages of cerebral ischemia. With increasing use of reperfusion therapies for the treatment of acute ischemic stroke, inflammatory pathways and oxidative stress remain attractive therapeutic targets for the development of adjuvant neuroprotective agents. It is plausible that in the near future, additional strategies using neuroprotective drug cocktails that target inflammation could offer exciting new promise in the therapeutic approach to ischemic stroke.
Neuroinflammation: implications for the pathogenesis of AD

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Classical histopathological studies in postmortem brain samples from Alzheimer’s disease (AD) sufferers have demonstrated the existence of a significant inflammatory process. This inflammatory process is characterized by the recruitment of activated microglia around amyloid plaques. Such a process can be replicated in most transgenic animal models of the Alzheimer’s pathology. In addition, there is strong epidemiological evidence that long term treatment with anti-inflammatory reagents diminish significantly the incidence of Alzheimer’s disease. This body of information has led to a number of prospective clinical trials which, unfortunately, had an unsuccessful outcome.

The above conflicting data would suggest that a positive therapeutic outcome could only be achieved by arresting inflammatory process in preclinical stages of AD. It is now becoming increasingly clear that AD has a long “silent” or “latent” stage before MCI or AD can be diagnosed. Unfortunately, present methods cannot ascertain which patients will develop AD at such stages. Transgenic animal models instead allow the investigation of aspects of the “preclinical” AD pathology, including the participation of inflammatory processes. Thus, our laboratory is conducting studies in the McGill mice and the McGill rat transgenic models of the AD-like amyloid pathology. Both models develop the full amyloid AD-like pathology including cognitive impairments, the overt plaque-related inflammation and the occurrence of dystrophic neurites. These studies have permitted us to define a previously unnoticed “pre-plaque” inflammatory process which results in the production of elevated pro-inflammatory cytokines, enhanced synthesis of NO and the intermediate activation of microglia with its mobilization towards neurons in the hippocampus and the cerebral cortex burdened with Abeta amyloid peptides. In this early stage of the AD pathology there is an accumulation of Abeta oligomers preceding plaque formation. The application of minocycline (a tetracycline with known CNS anti-inflammatory properties) corrected the upregulation of inducible nitric oxide synthase and cyclooxygenase-2 observed in young transgenic placebo mice.

In addition, the down-regulation of inflammatory markers correlated with a reduction in amyloid precursor protein levels and amyloid precursor protein-related products. The studies suggested a minocycline effect on diminishing BACE 1 activity which was found to be up-regulated in transgenic placebo-treated animals. In addition some beneficial effects on cognitive outcomes could be detected in mice treated at the pre-plaque stage while, in contrast, a negative effect was found when the minocycline treatment was initiated at the post-amyloid plaque stages. These findings could explain the apparent contradictory clinical observations derived from the use of NSAIDS and they would indicate that an anti-inflammatory therapy should be considered as preventive intervention; only at very early stages of
the “silent” AD pathology; a staging for which we are still missing unequivocal biomarkers.

References


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The amyloid cascade hypothesis states that overproduction of amyloid-beta peptide (AβP) or failure to clear this peptide, leads to Alzheimer’s disease (AD) primarily through amyloid deposition, presumed to be involved in neurofibrillary tangles formation; these lesions are then associated with cell death which is reflected in memory impairment, the hallmarks of this dementia. Several labs have bred AD models of transgenic mice that produce human AβP and develop plaques and neuron damage as well as immunological aspects of the disease pathogenesis. The immune system appears to participate in AD pathogenesis. Animal models of the disease enabled to translate the immunological concept into treatment approaches of the disease. Active and passive immunotherapies are being pursued in order to clear brain amyloid plaques and associated side effects and thus open a new area for Alzheimer’s therapeutics.
Alzheimer’s disease (AD) is the most common form of dementia, affecting over 30 million people worldwide. Amyloid-beta protein (Abeta) has become a therapeutic target based on pathological, biochemical and genetic evidence supporting its role as a key component in the disease process. Active Abeta immunization has been effective in lowering cerebral Abeta levels and improving cognitive deficits in animal models of AD. In humans, dosing of an active Abeta vaccine in the AN1792 Phase II clinical trial was halted in 2002 when ~6% of the 300 immunized AD patients developed meningoencephalitis. Regional plaque clearance and lowering of CSF tau were reported however, only modest (or no) clinical improvements were observed in patients following immunization. Second-generation active Abeta vaccines designed to avoid the adverse events from the previous trial are under investigation. These include short peptide-conjugate vaccines, mimotope vaccines, DNA vaccines, and vaccines targeting RAGE/Abeta complexes. Currently, more than 900 AD patients are enrolled or being recruited worldwide for active Abeta vaccine Phase II clinical trials. In addition, active vaccines against pathological forms of tau protein, another hallmark protein in AD, are under development. On the basis of preclinical studies and the limited data from clinical trials, it appears that immunotherapy might be most effective in preventing or slowing the progression of AD when patients are immunized before or in the very earliest stages of disease. Biomarkers for AD and imaging technology have improved greatly over the past 10 years and, in the future, may be able to identify pre-symptomatic, at-risk individuals who could benefit from early Abeta and/or tau active immunization.
Amyotrophic lateral sclerosis is a rapidly progressive neurodegenerative disorder characterized by the loss of motor neurons in the spinal cord and brainstem accompanied by degeneration of corticospinal and corticonuclear neurons within the cerebral cortex. The loss of motor neurons leads to paralysis which progressively involves all the skeletal muscles.

A variety of mechanisms have been investigated in the pathogenesis of ALS. Excitotoxicity took a prominent role in the last decades. At the same time, a detrimental effect of glial cells surrounding motor neurons was postulated. Excitotoxicity and glial cells may be simultaneously involved given the metabolic relationship between astrocytes and motor neurons in regulating the availability of glutamic acid. Besides this, the role of glial cells appears to be more important than a mere buffer for an excess of extracellular glutamate. For instance, it is demonstrated that in the absence of glial cells pure motor neuron cultures carrying a mutation in the SOD1 gene which leads to ALS are not affected by the disease process while this occurs in the presence of glial cells. Specific glia-derived factors were demonstrated to be involved in such a detrimental cell-to-cell communication although the specific role of each molecule remains to be established. The existence of a toxic cell-to-cell communication led to the concept of ALS as a motor neuron disorder characterized by the occurrence of non-autonomous cell death in which the disease mechanism is not expressed at the level of isolated motor neurons. Such a concept was extended in recent years to cells other than motor neurons and glia. For instance Martin and co-workers (2007) found that synuclein positive interneurons degenerated more than motor neurons and further studies documented the involvement of multiple cell types from the anterior horn in the pathogenesis of ALS.

The present communication is aimed to disclose the role of abnormal cell to cell communication in sustaining and spreading the molecular mechanisms of cell death. This process is reminiscent of disease transmission in other neurodegenerative disorders featuring a prion-like cell to cell spreading. This is confirmed by prion domains typical of ALS-specific proteins. The role of cell membrane proteins belonging to the immune system as well as the expression of advanced glycation end products (AGE) will be analyzed along with different cell types and glia-derived cytokines.
mTOR as a multifunctional therapeutic target in HIV infection

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Patients undergoing long-term highly active antiretroviral therapy treatment are probably at a higher risk of various HIV-related complications. Hyperactivation of the mammalian target of rapamycin (mTOR) has been found to contribute to dysregulated apoptosis and autophagy which determine CD4+ T-cell loss, impaired function of innate immunity and development of neurocognitive disorders. Dysregulated mTOR activation has also been shown to play a key part in the development of nephropathy and in the pathogenesis of HIV-associated malignancies. These studies strongly support a multifunctional key role for mTOR in the pathogenesis of HIV-related disorders and suggest that specific mTOR inhibitors could represent a novel approach for the prevention and treatment of these pathologies.
Modeling neuroinflammatory pathogenesis of PD

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Although almost 50 years have passed since the dopaminergic defects was identified as the main neurochemical alteration of Parkinson's disease (PD), the cause of the disease remains unknown and the pathways of neurodegeneration still incompletely understood. A restricted number of biological mechanisms contribute to the process of cell death in the nigrostriatal pathway, including mitochondrial defects and enhanced formation of reactive oxygen species leading to oxidative damage, and abnormal protein aggregation. In addition to or, possibly, intermingled with these mechanisms of neuronal damage there is another crucial factor: neuroinflammation. Once believed to be an “immune privileged” system, impermeable to immune cells of the periphery, the central nervous system is, in fact, capable of dynamic immune and inflammatory responses, mainly mediated by the activation of microglia and astrocyte reactivity. Furthermore, it is now clear that communication between the brain and peripheral immune system, with passage of immune cells from the periphery to the cerebral parenchyma, is a rather common phenomenon. The inflammatory response associated with the onset and progression of cell loss in the dopaminergic nigrostriatal tract and, more in general, the role of immune mechanisms are increasingly recognized in the pathogenesis of PD. Neuroinflammatory changes have been repeatedly demonstrated in both neurotoxic and transgenic animal models of the disease, as well as in PD patients. Virtually all neurotoxins used to replicate the nigrostriatal degeneration that characterizes PD trigger signs of neuroinflammation at the sites of neurodegeneration. The vast majority of findings have been obtained in animals treated with 6-hydroxydopamine or MPTP. Further insights have been provided with the advent of PD transgenic models, particularly of those based on the expression of mutant α-synuclein – the key protein of PD pathogenesis - or overexpression of the wild type form of the protein. These latter models have provided crucial insights into the correlation between α-synuclein and the dichotomous response that microglia can activate, with the polarization toward a cytotoxic or cytoprotective phenotype (M1 or M2). The evolving concept of differential microglia polarization in response different neurotoxic insults, in particular, may set the ground for a fine tuning of the neuroinflammatory process that accompanies and sustains the evolution of the nigrostriatal neurodegeneration since the very early phases of PD, opening new perspectives for PD treatment.
Parkinson’s disease (PD) is a neurodegenerative disorder characterized by the progressive neuronal loss of the substantia nigra and other brain structures. Despite all recent advances derived from preclinical and clinical studies, the exact pathogenetic mechanisms underlying the selective dopaminergic neuronal death are still elusive. Recently, emerging evidence has pointed out the role of innate and adaptative immune response on pathology of PD. Persistent activation of microglia has been demonstrated in post-mortem brain of PD patients and in animal models, so that a close association between the activated microglia and the degeneration of dopaminergic cells has been postulated. Microglia could exert deleterious effects through both the release of pro-inflammatory cytokines, such as Tumoral Necrosis Factor α (TNF-α) and Interleukin-1β (IL-1β), and the increased production of reactive oxygen species such as NO, superoxide and peroxynitrite.

Additionally, the interaction between T lymphocytes and microglia may alter the functional profile of microglia toward a more pro-inflammatory state thus exacerbating neurodegenerative process. Infiltration of T cells subset in the nervous system seems also to influence PD progression of disease, while it is unclear whether alteration in peripheral blood T lymphocytes directly promote the development of PD or it is just a secondary response to the already exiting disease process. Better knowledge of the role of immune response and neuroinflammation in PD could pave the way to the discovery of future treatments of the disease.
Neuroinflammation in PD: a target for neuroprotection?

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In the field of neurodegenerative disorders, the concept of non-cell autonomous disease mechanisms suggests that neurodegeneration is not just mediated by damages within the affected neurons but is also influenced by interactions with neighboring glia and immune cells. This so-called neuroinflammatory response produces both neurotoxic and neurotrophic effects and it has been suggested that its imbalance could be at the core of the detrimental progressive nature of many neurodegenerative diseases including Parkinson's disease (PD), a neurological condition characterized by the progressive loss of dopamine-containing (DA) neurons in the substantia nigra (SN).

Mounting evidence from epidemiological, genetic, postmortem and animal studies suggest that innate neuroinflammatory processes associated with glial cell activation could be intricately linked not just to disease progression but also to selectivity of neurodegeneration. In addition to these well-recognized culprits, latest developments in the field now reveal that adaptive immune response to neurodegeneration may also play an important role in PD pathogenesis. In autopsy brain specimens from PD patients, increased number of infiltrated CD8 and CD4 T cells within the SN has been documented. Interestingly, similar T cell response can be recapitulated in preclinical models such as MPTP-intoxicated mouse, without massive blood-brain barrier disruption. This suggests that as for glial cell activation secondary to neuronal injury, T cell brain invasion is a highly regulated process. Importantly, it was noted that MPTP-induced DA cell loss is markedly attenuated in immunodeficient mice lacking mature T cells. This indicates that the adaptive immune system may represent a driving force for progressive neurodegeneration in PD. Although much remains to be learnt about the origin and development of such adaptive immune response, these preliminary observations open new exciting perspectives for immune-based therapeutics in PD.
Migraines were once thought to be initiated exclusively by problems with blood vessels, but the vascular changes of migraines are now considered to be secondary to brain dysfunction. Although cerebral vasodilation can trigger migraine attacks, blood vessel diameters return to normal more than an hour before the migraine headaches occur. When the constriction of blood vessels in the brain stops and the aura subsides, the blood vessels of the scalp dilate. The emerging evidence suggests that just as alterations in neuronal activity can lead to downstream effects on the cerebral blood vessel, so too can changes within endothelial cells or vascular smooth muscle lead to downstream alterations in neuronal activity. The phenomenon known as cortical spreading depression (CSD), which is associated with the aura of migraine, has been theorized as a possible cause of migraine. In CSD, neurological activity is initially activated, then depressed over an area of the cerebral cortex, which results in the release of inflammatory mediators, irritation of cranial nerve roots, most particularly the trigeminal nerve, which conveys the sensory information for the face and much of the head. Stimulation of the trigeminal ganglia causes the release of peptides within the trigemino-vascular system. The main peptides released by trigeminal axons projecting into the meninges are calcitonin gene related peptide (CGRP) and substance P. Parasympathetic efferents and trigeminal activation cause vasodilation, and plasma leakage within the dura Mater. Even though CGRP (and subsequent vasodilatation) does not reportedly excite or directly sensitize meningeal nociceptors, it can indicate migraine attacks. The local release from afferent neurons of inflammatory mediators including CGRP, substance P, nitric oxide, vasoactive intestinal polypeptide, 5-HT, Neurokinin A, is referred to as Neurogenic inflammation. Other mechanisms may enable levels of released brain and blood components to approximate and activate the trigeminovascular system during the aura. CSD increases proteases that degrade membrane proteins. During CSD, matrix metalloproteinases (MMP) degrade laminin, collagen type IV, a critical component of brain blood vessels. MMP-9 is activated within 15 minutes in blood vessels and lasts for many hours (12 hours). Current study of the treatment of migraine with CGRP blockers show promise. In early trials, the first oral nonpeptide CGRP antagonist, MK-0974 (Telcagepant), was shown effective in the treatment of migraine attacks, but elevated liver enzymes in two participants were found. Other therapies and other links in the neurogenic inflammatory pathway for interruption of disease are under study, including migraine therapies.
Novel therapeutical targets in the treatment of migraine

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The mechanism of migraine, a neurovascular disorder, which affects one fifth of the general population and involves patients from infancy through senescence is still elusive. However, novel and at least some of them promising targets are emerged in the last five years. In addition to activation of serotonin 5-HT1B/D, selective activation of 5-HT1F receptors seems to represent an effective mechanism for the acute treatment of migraine. The release of calcitonin gene-related peptide (CGRP) from terminals of trigeminal primary sensory neurons is now recognized as a major event of the underlying mechanisms of migraine. Thus, the molecular, neurochemical and anatomical pathways, whose activation results in the modulation of CGRP release, appear of relevance for migraine. Although some molecules showed some hepatotoxicity, additional clinical trials with new CGRP receptor antagonists are ongoing and they may provide a modality to treat migraine attacks alternative to NSAIDs and triptans. A variety of receptors and channels are expressed by trigeminal primary sensory neurons and regulate CGRP release. Among them, the transient receptor potential (TRP) family of cation channels is gaining increasing importance, principally because of the recent discovery of endogenous and exogenous TRP stimulants, which are known inducers of the migraine attack. However, pharmaceutical development of TRP antagonists is still in an early phase.
Current challenges in glia-pain biology

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Pain is an unpleasant sensory and emotional experience in response to an actual or potential threat to the integrity of the body and as such is a protective mechanism. When pain outlives its usefulness becomes chronic pain which results in plastic changes within the pain pathways. Acute pain perception, such as exposure to a hot surface, involves specialised sensory neurons, namely the nociceptors, which convey the noxious stimulus to dorsal horn neurons in the spinal cord which ascend to the thalamus on their way to the somatosensory cortex where the intensity and type of pain is perceived. A parallel pathway ascends from the dorsal horn to the limbic system particularly the amygdala and the hypothalamus where the emotional component is registered. Chronic neuropathic pain such as in diabetic neuropathy or rheumatoid arthritis can be objectively identified by increased sensitivity to painful stimuli (hyperalgesia) and painful response to non-noxious stimuli (allodynia). Several neuronal mechanisms underlie hyperalgesia and allodynia both peripherally and centrally including changes in the expression of peptides, receptors and ion channels in sensory nerves and the spinal cord where both increased neuronal excitability of dorsal horn neurons (central sensitization) and a reduced GABAergic inhibitory tone contribute to the hyperalgesic state. Recent evidence also points to a role for glial cells in the development and maintenance of neuropathic and inflammatory hyperalgesia/allodynia. Specifically, peripheral nerve and tissue damage can trigger microglial and/or astrocyte activation in the dorsal horn of the spinal cord. The signals that mediate spread of nociceptive signalling between neurons and glial cells in the dorsal horn are being actively investigated and include neuronal ATP which activates glial P2X4 and P2X7 receptors, microglial-derived BDNF which regulates the activity of GABA-A receptors and neuronal fractalkine which activates CX3CR1 receptors on microglia following proteolytic cleavage of the chemokine domain by cathepsin S. This microglial enzyme is released via a P2X7-mediated mechanism. Despite the lack of direct clinical evidence yet available to demonstrate the link between (micro)glia and pain in man, this lecture will argue that pursuing (micro)glial targets are a valid strategy for chronic pain therapy.
Neuropathic pain affects 1% of the population, it can occur secondarily to injury of the central nervous system, but most commonly peripheral nervous system. These injuries can be iatrogenic (amputation cholecystectomy etc), traumatic, due to tumors compressing peripheral nerves, drugs, metabolic (diabetes) or viral (Herpes Zoster, HIV) diseases, ischemia. Whatever the cause, neuropathic pain is unresponsive to classic analgesics and antidepressant or anticonvulsant drugs, Transcutaneous Electrical Nerve Stimulation and psychological/cognitive help are used. All these treatments relief a limited percentage of patients (30%, e.g. comparable to placebo), before pain inevitably reappears. The diversity of therapeutic approaches sharing an equal percentage of failure suggests that each of them targets only a few out of the multiple pathological changes observed during the development of the disease. In order to understand neuropathic pain, till now, most of the attention has been directed to the central anatomical and biochemical modifications that occur in the Central Nervous System (spinal cord, CNS) following peripheral nerves lesion. We and other Authors showed changes in several neurotransmitters and neuropeptides in the CNS but also that a pathological interaction between the neuron and non neural cells in the peripheral nerve and in the spinal cord is crucial for the development and maintenance of neuropathic pain. Following a peripheral lesion, activated Schwann cells, resident and infiltrating macrophages, activated glial cells in the DRG and glia and microglia in the spinal cord, secrete pro/anti-inflammatory cytokines in the peripheral and central nervous system and start a neuroinflammatory cascade of events that progressing toward the CNS (spinal cord and brain) leads to neuronal sensitization and the development and maintenance of neuropathic pain. We have described in different animal models of neuropathic pain, such as nerve injury and diabetes induced neuropathy, the temporal expression of the proinflammatory cytokines IL-1β and IL-6 and of the antinflammatory cytokine IL-10 along the pain pathways in the peripheral (sciatic nerve, DRG) and the central (spinal cord) NS. These cytokines appear to be activated/modulated in the nervous tissue in parallel with the occurrence of painful behaviour such as allodynia and hyperalgesia. We applied several therapeutic approaches in order to reduce painful symptoms, such as treatment with the non specific purinergic antagonist PPADs, the phytoestrogen genestein and a cell stem therapy with murine adult neural stem cells. All treatments were able to significantly ameliorate nociceptive hypersensitivity, and consistently re-established a correct balance between pro and antinflammatory mediators in the peripheral and central nervous system. The whole of these data suggest a pivotal role of immune system and inflammation in neuropathic pain and nerve degeneration. The modulation of inflammatory molecules is, in-fact, the common trait accomplished throughout different mechanisms by different drugs, converging in neuropathic pain modulation.
Modulation of peripheral sensory neurons by the immune system: implications for pain therapy

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The concept that the immune system can communicate with peripheral sensory neurons to modulate pain is mostly based on documented interactions between opioid ligands and receptors. Such findings may have broad implications for the development of safer pain medication. Innovative strategies take into account that analgesics should be particularly active in pathological states rather than producing a general suppression of the central nervous system, as with conventional morphine- or cannabinoid-like drugs. Inflammation of peripheral tissue leads to increased functionality of opioid receptors on peripheral sensory neurons and to local production of endogenous opioid peptides in immune cells. Endocannabinoids were also detected in leukocytes but their role in pain modulation is yet to be addressed. Future aims include the development of peripherally restricted opioid agonists, selective targeting of opioid-containing immune cells to sites of painful injury and the augmentation of peripheral ligand and receptor synthesis, e.g. by gene therapy. Similar approaches may be pursued for cannabinoids. The ultimate goal is to avoid detrimental side effects of currently available analgesics such as respiratory depression, cognitive impairment, addiction, gastrointestinal bleeding and thromboembolic complications.
Role of perinatal inflammation in the pathogenesis of schizophrenia

Disturbances directed at the maternal host during pregnancy can lead to direct physiological changes in the fetal environment and negatively affect the normal course of early brain development in the offspring. This can have long-lasting consequences for the development of postnatal brain dysfunctions, in which the primary cerebral insult or pathological process occurs during early brain development long before the illness is clinically expressed. One prominent example of such neuropathological outcome is schizophrenia: This disorder seems to be associated with aberrations in early neurodevelopmental processes caused by a combination of environmental and genetic factors, which predispose the organism to long-lasting neuropathology and psychopathology. A large body of human epidemiological data shows that maternal infection during pregnancy is one of the relevant environmental factors increasing the risk of this neurodevelopmental brain dysfunction in the offspring. Even though the precise neuroimmunological mechanisms involved still need to be delineated, one prevalent hypothesis suggests that infection-induced disruption of fetal neurodevelopmental processes may predispose the organism to long-lasting changes in subsequent brain and behavioral development, thereby facilitating the expression of postnatal brain dysfunctions relevant to schizophrenia. This hypothesis has been substantiated by numerous investigations in experimental rodent models demonstrating the emergence of altered fetal brain development and multiple long-term brain and behavioral abnormalities relevant to schizophrenia following prenatal exposure to infection and/or immune activation. The present talk will highlight the advances in modeling the epidemiological link between prenatal immune challenge and neurodevelopmental brain dysfunctions and will discuss the relevance of experimental findings to the prenatal infectious etiologies of human mental illness.
A persistent (chronic) infection as aetiological factor an inflammatory process in schizophrenia is discussed since many years. A prenatal immune challenge during the second trimenon of pregnancy seems to be crucial. Research points out that not one single pathogen but the immune response of the mother is related to the increased risk for schizophrenia. Several reports described increased serum IL-6 levels in schizophrenia. IL-6 is a product of activated monocytes and of the activation of the type-2 immune response. Moreover, several markers of the type-1 immune response are decreased in the majority of schizophrenic patients, while signs of activation of the type-2 immune response are described accordingly in schizophrenia. Mechanisms involved in the inflammatory process in schizophrenia will be outlined focussing on the role of microglia cells, the macrophages of the brain. Microglia activation in schizophrenia was shown by studies using positron emission tomography (PET).

Due to the imbalance of the immune system in schizophrenia which results in inflammation, antiinflammatory treatment would be expected to show advantageous therapeutic effects. Cyclo-oxygenase-2 inhibitors have been evaluated in schizophrenia. COX-2 inhibition reduces not only the levels of proinflammatory cytokines, COX-2 inhibition has also an impact to the glutamatergic neurotransmission and influences the tryptophan/kynurenine metabolism: all three components seem to be involved in the pathophysiology of schizophrenia. In the meantime, several studies with the COX-2 inhibitor celecoxib have been performed in schizophrenia. Short term studies (5 to 8 weeks) of the COX-2 inhibitor celecoxib show a therapeutic effect mainly in early stages of schizophrenia, the pertaining studies and interfering variables will be discussed. Moreover, also the mixed COX-1/COX-2 inhibitor aspirin might have beneficial effects in early stages of schizophrenia. In the meantime, a metaanalysis of studies on anti-inflammatory compounds showed therapeutic effects in schizophrenia. Further therapeutic strategies based on immune-modulatory effects will be discussed, too.
Second-generation LAI antipsychotics in the management of schizophrenia

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(...)

10th Summer School of Neuroscience
International PhD Program in Neuropharmacology
Neuroinflammation and docosanoid signaling in synaptic circuitry integrity: new mediators for neuroprotection and long term rescue

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The significance of the selective enrichment in omega-3 essential fatty acids (docosaheaxaenoyl –DHA- chains of membrane phospholipids, 22C and 6 double bonds) in the nervous system (e.g. synaptic membranes and dendrites) has remained, until recently, incompletely understood. While studying mechanisms of cell survival in neurodegenerations, we contributed to the discovery of a docosanoid synthesized from DHA by 15-lipoxygenase-1, which we dubbed neuroprotectin D1 (NPD1, 10R,17S-dihydroxy-docosa-4Z,7Z,11E,13E,15E,19Z hexaenoic acid). This mediator is a docosanoid because it is derived from a 22C precursor (DHA), unlike eicosanoids, which are derived from the 20 C arachidonic acid family of essential fatty acids not enriched in the nervous system. We found that NPD1 is promptly made in response to oxidative stress, seizures and brain ischemia-reperfusion, and in the presence of neurotrophins. NPD1 is neuroprotective in experimental brain damage, oxidative-stressed retinal pigment epithelial (RPE) cells, and in human brain cells exposed to amyloid-β peptide. Thus we envision NPD1 as a protective sentinel, one of the very first defenses activated when cell homeostasis is threatened by neurodegenerations. We provide here recent experimental examples that highlight the specificity and potency of NPD1, spanning beneficial bioactivity during initiation and early progression of neurodegenerations, epileptogenesis and stroke: 1) NPD1 increases during seizures in the hippocampus, and when we administered this docosanoid during pharmacologically–induced epileptogenesis it elicited a remarkable attenuation of pathological brain oscillations. This reflects attenuation of aberrant neuronal network activities that lead to spontaneous recurrent seizure. We used multi-microelectrode arrays in freely moving mice to record this data. Thus, docosanoid-mediated signaling rescues neuronal network disruptions; 2) In brain ischemia-reperfusion, DHA administered (i.v.) one hour after two hours of middle cerebral artery occlusion (MCAO) leads to penumbra protection with an extended window of protection (up to five hours) and with concomitant NPD1 synthesis. The availability of anti-apoptotic BCL-2 proteins is positively modulated by NPD1, whereas pro-apoptotic BCL-2 proteins are negatively regulated, as is the arrival of leukocytes due to neurovascular unit breakdown. Recently we identified a COX-2 mediated pathway in addition to the lipoxygenase-catalyzed route. The former gives rise to novel aspirin-triggered NPD1. This novel docosanoid is very potent in attenuating neuroinflammation and stroke mediated brain damage; 3) NPD1 is drastically reduced in CA1 areas from Alzheimer’s patients. Therefore we have explored the significance of NPD1 in cellular models that recapitulate part of the Alzheimer’s pathology. Human neurons and astrocytes challenged by amyloid-β or by overexpressing APPsw (double Swedish mutation) show that NPD1 downregulates amyloidogenic processing of amyloid-β precursor protein, switches off pro-inflammatory gene expression (TNF-α, COX-2 and B-94-TNF-α inducible pro-inflammatory element), and promotes neural...
cell survival. Moreover, anti-amyloidogenic processing by NPD1 targets α- and β-secretases and PPARγ receptor activation. The cell death cascade involves multiple checkpoints and signaling networks. NPD1 regulation targets upstream events of cell survival as well as neuroinflammatory signaling, in turn promoting homeostatic regulation of neural circuitry integrity.

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The first inkling that depression is an immuno-inflammatory disorder and that there are phenomenological similarities between clinical depression and sickness behavior and that both conditions may share a common pathway, i.e. inflammation, was published between 1990-1993 [1-3]. There are some phenomenological similarities between sickness behavior and clinical depression, e.g. behavioral inhibition, anorexia, weight loss, sleep and psychomotor disorders; physio-somatic symptoms (fatigue, hyperalgesia, malaise); anxiety; and mild cognitive impairment [3,4]. Nevertheless, clinical depression and sickness behavior are two completely different conditions [4]. Sickness behavior is an acute behavioral complex induced by acute infections and immune trauma and caused by pro-inflammatory cytokines. It is an adaptive response that enhances recovery by conserving energy to combat infection/inflammation and therefore is a behavioral part of a compensatory (anti)-inflammatory response system (CIRS), which limits an overzealous immuno-inflammatory response [4]. Clinical depression, on the other hand, is a lifelong disease with a tendency towards recurrent episodes, a chronic course, seasonal variation, (hypo)manic symptoms, sensitization of episodes, and progressive deterioration [4-7]. In clinical depression, and not sickness behavior, immuno-inflammatory response 2 sensitization, progressive damage by oxidative and nitrosative stress (O&NS) to fatty acids, proteins, DNA and mitochondria, and progressive autoimmune responses directed against self-epitopes (e.g. anchorage molecules and serotonin) are the substrate of a neuroprogressive process, whereby multiple depressive episodes cause neural tissue damage and consequent functional and cognitive sequelae [4-11]. Whereas sickness behavior is an acute CIRS response, clinical depression is accompanied by a CIRS response that tends to downregulate the primary immuno-inflammatory response [4]. Whereas acute infections/trauma typically elicit sickness behavior, less well defined trigger factors are associated with the onset of depression, i.e. psychosocial stressors and inflammatory disease. While traumatic life events quite likely cause an inflammatory state often leading to clinical depression, no association between psychosocial stressors and sickness behavior has been described. Clinical depression shows multiple “co-morbidities” with a large variety of a) brain disorders related to neurodegeneration, e.g. Alzheimer’s, Parkinson’s and Huntington’s disease, multiple sclerosis and stroke; b) medical disorders, such as cardiovascular disorder, chronic fatigue syndrome, chronic obstructive pulmonary disease, rheumatoid arthritis, psoriasis, systemic lupus erythematosus, inflammatory bowel disease, irritable bowel disease, leaky gut, diabetes type 1 and 2, obesity and the metabolic syndrome, and HIV infection; and c) conditions, such as hemodialysis, interferon-α-based immunotherapy and the postnatal period [12]. The common denominator of all these conditions is activation of (neo)inflammatory and O&NS pathways. The presence of concomitant depression is strongly associated with a lower quality of life and increased morbi-
dity and mortality in these medical diseases/conditions. All in all, while sickness behavior is an acute, beneficial CIRS response, clinical depression is a disabling, progressive disorder belonging to the spectrum of inflammatory-neurodegenerative diseases. It follows that rather than targeting only one immuno-inflammatory pathway, such as inflammation (e.g. cyclo-oxygenase-2 or the tryptophan catabolite (TRYCAT) pathway), a better strategy for depression treatment entails the multi-targeting of the relevant pathways, including a) inflammatory cytokines, such as IL-1, IL-6 and TNF; b) Th1 and Th17 responses; c) damage by O&NS and lowered antioxidant levels; d) autoimmune responses to oxidatively/nitrosatively modified neoantigenic determinants; e) damage to mitochondria and respiratory chain enzymes and adenosine triphosphate production; and f) neuroprogression [11]. A number of new drug candidates, which we will test in phase-2 placebo controlled trials, are discussed.

References.


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