Convegno monotematico SIF

# Neuroimmune Pharmacology: Challenging Paradigms Beyond Boundaries

*Integrating pharmacology, immunology, and neurosciences in a therapeutic perspective*

**Center of Research in Medical Pharmacology, University of Insubria**
**November 15th-16th, 2013**
Aula Magna, Via Dunant n. 2, Varese

# ABSTRACT BOOK

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Center of Research in Medical Pharmacology
University of Insubria

*15 august 2013*

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**Center of Research in Medical Pharmacology, University of Insubria**
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**Scientific Committee**
Sujit Basu (USA), Marco Cosentino (I), Salvatore Cuzzocrea (I), Monica Di Luca (I), Marco Ferrari (I), Howard E. Gendelman (USA), Franca Marino (I), Olimpia Meucci (USA)

**Topics**
AUTOIMMUNE DISEASE - NEURODEGENERATIVE DISEASE – CANCER - CARDIOVASCULAR DISEASE
NEUROIMMUNE PSYCHOPHARMACOLOGY - PHARMACOLOGY OF NEUROIMMUNE CROSS-TALK

## PRELIMINARY PROGRAM

**Friday, November 15th, 2013**

14:00-15:00 Welcome reception and registration

15:00-15:15 Opening addresses
**Howard E. Gendelman**
*Department of Pharmacology and Experimental Neuroscience, Nebraska Medical Center, Omaha, NE, USA,*
*Editor-in-Chief of the Journal of Neuroimmune Pharmacology*

15:15-16:00 Keynote Lecture
**Sujit Basu**
*Department of Pathology, The Ohio State University, Columbus, OH, USA*
**DOPAMINE-MEDIATED REGULATION OF ANGIOGENESIS IN TUMORS AND IN NORMAL WOUND TISSUES**

16:00-16:45 Coffee Break and Tour of Posters

16:45-19:00 Oral communications (selected from abstracts - 10+5 min/each)

**Saturday, November 16th, 2013**

9:00-10:30 Oral communications (selected from abstracts - 10+5 min/each)

10:30-11:15 Coffee Break and Tour of Posters

11:15-12:45 Oral communications (selected from abstracts - 10+5 min/each)

12:45-13:00 Closing remarks

**Organizing Committee**
Marco Cosentino, Franca Marino, Marco Ferrari

**Organizing Secretariat**
Mrs. Paola Gervasini, Center of Research in Medical Pharmacology, University of Insubria
Via Ottorino Rossi n. 9, 21100 Varese VA, Italy

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# ABSTRACTS

**# 01**

## Dopaminergic pathways in human CD8+CD28- T regulatory lymphocytes: novel targets for the pharmacological modulation of the anti-tumor immune response?

Ahmed Tanzeel (1), Rasini Emanuela (1), Legnaro Massimiliano (1), Bombelli Raffaella (1), Fenoglio Daniela (2), Filaci Gilberto (2), Marino Franca (1), Cosentino Marco (1)

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Regulatory T lymphocytes (Tregs) are specialized T cells crucial to the maintenance of immune homeostasis, contributing to immunologic self-tolerance and being critically involved in immunologic diseases, tumor immunity, and transplantation tolerance. Among Tregs, CD4+CD25+ T cells have received so far extensive attention, however several types of Tregs exist which have distinct phenotype and function. Recently, the presence and functional relevance of CD8+CD28- Tregs have been reported in human tumors, where they inhibit T cell proliferation and cytotoxicity, thus suggesting that these cells may have pathogenic relevance and implications for cancer immunotherapy [1].

Dopamine is a key neurotransmitters acting on five distinct dopaminergic receptors (DR) classified into D1-like (DRD1 and DRD5) and D2-like (DRD2, DRD3 and DRD4). Emerging evidence indicates that dopamine also exerts profound effects on the immune system. In particular, some of us reported that human CD4+CD25+ Tregs selectively express tyrosine hydroxylase (TH, the rate-limiting enzyme for the synthesis of catecholamines) and contain endogenous dopamine subserving an autocrine/paracrine inhibitory functional loop involving dopaminergic pathways and resulting in down-regulation of Treg function [2].

The aim of the present study was to investigate the existence and the funtional relevance of dopaminergic pathways in human CD8+CD28- Tregs. To this end, human CD8+CD28- and CD8+CD28+ T lymphocytes were isolated from peripheral blood mononuclear cells by means of immunomagnetic sorting. Expression of DR was assessed at the membrane level by flow cytometry and at the mRNA level by real-time PCR. Expression of TH and of the vesicular monoamine transporter 2 (VMAT2, which mediates the transport of dopamine in synaptic vesicles in neuronal terminals) was examined by immunocytochemistry as well as by real-time PCR. Catecholamines (dopamine, noradrenaine and adrenaline) were assayed by HPLC-ED.

Both CD8+CD28- and CD8+CD28+ T lymphocytes express all the DR types, both D1-like (DRD1 and DRD5) and D2-like (DRD2, DRD3 and DRD4), on the cell membrane to a non significantly different extent (frequency, range: 6.1-15.0%; MFI, range: 506.7-846.7). CD8+CD28- Tregs in comparison to CD8+CD28+ T lymphocytes however preferentially expressed mRNA for all DR (ratio CD8+CD28- vs CD8+CD28+: DRD1, 10.7, DRD5, 1.9, DRD2, 3.0, DRD3, 2.6, DRD4, 2.1, $P<0.05$ in all the cases) as well as for VMAT2 (ratio: 4.0, $P<0.05$), but not for TH (ratio: 1.0, $P = 0.99$). Intracellular expression of VMAT2 and TH was confirmed by immunocytochemistry. Compared to CD8+CD28+ T lymphocytes, CD8+CD28- Tregs also contained significantly higher amounts of catecholamines (ratio CD8+CD28- vs CD8+CD28+: dopamine, 14.9, noradrenaline, 17.7, adrenaline, 15.2, $P<0.05$ in all the cases).

Results thus suggest that human CD8+CD28- Tregs preferentially express DR and contain high amounts of dopamine (as well as of the other catecholamines noradrenaline and adrenaline), possibly due to their ability to store these transmitters in intracellular compartments by means of VMAT2. Functional assessment of the functional responses of CD8+CD28- Tregs after pharmacological manipulation of dopaminergic pathways is now strongly warranted, as positive results could provide the conceptual basis to exploit as immunomodulating and antitumor drugs the several dopaminergic agents already in clinical use for non-immune indications and with a favourable risk-benefit profile.

[1] Filaci G, Fenoglio D, Fravega M, Ansaldo G, Borgonovo G, Traverso P, Villaggio B, Ferrera A, Kunkl A, Rizzi M, Ferrera F, Balestra P, Ghio M, Contini P, Setti M, Olive D, Azzarone B, Carmignani G, Ravetti JL, Torre G, Indiveri F. CD8+ CD28- T regulatory lymphocytes inhibiting T cell proliferative and cytotoxic functions infiltrate human cancers. J. Immunol. 2007, 179: 4323-34.

[2] Cosentino M, Fietta AM, Ferrari M, Rasini E, Bombelli R, Carcano E, Saporiti F, Meloni F, Marino F, Lecchini S. Human CD4+CD25+ regulatory T cells selectively express tyrosine hydroxylase and contain endogenous catecholamines subserving an autocrine/paracrine inhibitory functional loop. Blood 2007, 109: 632-42.

*This study was supported in part by from the Italian Ministry of Instruction, University and Research–MIUR (PRIN 2008, project no. 2008WXF7KK_003 to F.M.)*

**# 02**

**Metabolic changes induced by interferon-α exposure in an *in vitro* model of human neurons**

Alboni Silvia (1), Schenetti Luisa (1), Brunello Nicoletta (1), Pariante Carmine M. (3), Righi Valeria (2)

*(1) University of Modena and Reggio Emilia, Department of Life Sciences, Modena, Italy; (2) University of Bologna, Department for Life Quality Studies, , Rimini, Italy; (3) Institute of Psychiatry, King's College London, UK*

The human neuroblastoma SH-SY5Y cell line is a third successive subclone of the SK-N-SH line, originally established from a bone marrow biopsy of a neuroblastoma patient. These cells possess many characteristics of neurons, and they represent one of the most-used models for studying cellular events and mechanisms involved in neurotoxicity and neurodegeneration or even in neuroprotection. We have been using these cells as a tools to evaluate the largely unexplored effects induced by interferon (IFN)-alpha (a clinically used type I IFN) exposure on neurons. Interferons are cytokines endowed with a pleiotropic spectrum of biological properties, including immunomodulation, antiviral and pro-inflammatory activity. Beside the periphery, and cells of the immune system, type I IFNs may have broad-ranging actions also in the brain, affecting neuronal differentiation, survival and synaptic plasticity. We recently demonstrated that exposure to IFN-α induces neurotoxic effects in a time e dose dependent manner in SH-SY5Y cells by impairing mitochondrial integrity and activity, recruitment of Bcl-2 family members, induction of oxidative stress (increases reactive oxygen species). Indeed following 72 hours exposure to this cytokine we found increased early apoptosis [1]. This prompted us to investigate the changes in the metabolic profile of live SH-SY5Y cells exposed to IFN-α (72 h) using 1H High Resolution-Magic Angle Spinning nuclear magnetic resonance (HR-MAS NMR) spectroscopy.. Firstly, this technique enabled us to characterize the metabolic signature of intact SH-SY5Y cells. Several metabolites, including amino acids, osmolites, phospholipids, organic acids, sugars and polyols have been identified. Moreover, we found that human neuronal cells exposed to IFN-α for 72 h had significantly increased concentration of lactate (Δ% 93.9), taurine (Δ% 117.7), myo-inositol (Δ% 123.2), scyllo-inositol (Δ% 117.4), glycerolphosphocholine (Δ% 135.2) and creatine (Δ% 159.9) compared with the vehicle-treated control cells. These data provide the demonstration that IFN-α exposure induces metabolic changes in human neuron-like cells. Moreover, these results may contribute to explain IFN-α-induced central side-effects often observed following IFN-α treatment for viral infection and malignancies.

[1] Alboni S, Gibellini L, Montanari C, Benatti C, Benatti S, Tascedda F, Brunello N, Cossarizza A, Pariante CM. N-acetyl-cysteine prevents toxic oxidative effects induced by IFN-α in human neurons. Int. J. Neuropsychopharmacol. 2013, 16: 1-17.

**# 03**

## Interferon alpha exposure increases the expression of the enzymes belonging to the kynurenine pathway in an in vitro model of human neurons: SH-SY5Y cells

Alboni Silvia (1), Benatti Cristina (1), Montanari Claudia (1), Benatti Stefania (1), Iarini Martina (1), Tascedda Fabio (1), Cannazza Giuseppe (1), Pariante Carmine M. (2), Brunello Nicoletta (1)

*(1) University of Modena and Reggio Emilia, Department of Life Sciences, Modena, Italy; (2) Institute of Psychiatry, King's College London, UK*

The past two decades have witnessed a burgeoning area of pre-clinical and clinical research linking psychiatric illnesses – particularly major depression (MD) – to activation of the inflammatory immune system. One of the stronger evidence supporting a causal role for inflammation in leading MD comes from reports indicating that depressive symptoms frequently develop in patients undergoing immunotherapy with cytokines, such as interferon (IFN)-α, for the treatment of malignancies or chronic viral infection. Although IFN-alpha-induced effects on the brain made IFN-α a model to study the influence of pro-inflammatory cytokines in the CNS and behavior, the molecular mechanisms underlying these effects are far from being fully understood.
It has been proposed that IFN-α may contribute to the etiology of MD by inducing indolamine 2,3-dioxygenase (IDO) expression and thus unbalancing the tryptophan/kynurenine metabolism toward the production of neurotoxic metabolites and\or reducing serotonin (5-HT) availability. IDO catalyzes the initial rate-limiting step in tryptophan degradation along the kynurenine pathway (KP). Kynurenine, the initial product of tryptophan degradation, is further catalysed into neurotoxic end-products through steps catalyzed by kynurenine 3-monooxygenase (KMO) and kynureninase (Kynu). However, Kynurenine can also be catabolised by kynurenine aminotransferase (KAT), into kynurenic acid, a potentially neuroptotective agent. A role for a disturbance in the equilibrium between neurotoxic/ neuroprotective KP endproducts producing an alteration in the neuroprotective–neurodegenerative balance in the brain of patients with MD, has been proposed in the neurodegeneration hypothesis of depression.
Studies recently performed in our laboratory have demonstrated that IFN-α induces toxic effects in an in vitro model of human neurons (human SH-SY5Y neuroblastoma cells) [1] thus our aim was to investigate the effects of IFN-α on KP in these cells.
Our studies show that IFN-α exposure increased the expression of all the kynurenergic enzymes investigated (IDO, KMO, Kynu and KAT). More particularly IFN-α strongly induced the expression of IDO mRNA (more than 900 –fold) in SH-SY5Y cells. Similar effects on kynurenergic enzyme expression were also observed when SH-SY5Y cells where differentiated with all-trans retinoic acid (in presence of neurotrophic support and in serum deprived conditions). We also found that INF-α decreased 5-HT levels whereas increased the kynurenine levels in the medium of both differentiated as well as not differentiated SH-SY5Y cells.
Our findings provide further information on the molecular pathways involved in IFN-α-induced effects in the brain and add a piece to the puzzle of what and how this cytokine may contribute to the pathogenesis of MD.

[1] Alboni S et al. N-acetyl-cysteine prevents toxic oxidative effects induced by IFN-α in human neurons. Int J Neuropsychopharmacol. 2013; 16:1-17.

**# 04**

## Increased neuroplasticity and microglia activation in the hippocampus in a mice model of successful antidepressant treatment

Brambilla Valentina (1,2), Calcaterra Lorenza (1,2), D'Adamo Patrizia (3,4), Martino Gianvito (1), Muzio Luca (1), Benedetti Francesco (2)

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Background - It is still unclear which biological changes are needed to recover from a major depressive episode. The search of biomarkers of antidepressant effects is a priority to improve current treatment options and to facilitate more rapid, successful treatment. New approaches focused on intracellular signalling pathways involved in the regulation of synaptic plasticity, such as the Wnt signalling cascade, and on pro-inflammatory markers. Sleep Deprivation (SD) provides a model antidepressant treatment successfully exploited to reverse-translate findings from in vivo human research to model organisms, with the aim of better understanding the biological underpinnings of the rapid antidepressant response observed in humans to this treatment.
Method - We studied the effects of repeated SD (for 12 or 24 hrs), administered with an automated system, either alone or combined with exercise (slow spinning wheel, SSW), in 136 C57BL/6J male mice (age: 3.5/4-month, weight: 28±1 g) divided in three groups (SD, SD+SSW, control). Forced Swimming Test (FST) was used to detect antidepressant-like effects. Unbiased evaluation of the transcriptional responses in the hippocampus were obtained by Illumina Bead Chip Array system followed by probing an independent confirmatory sample with real time PCR. Moreover, Wnt signalling activity was evaluated by assessing β-galactosidase activity in 12 Wnt reporter (BAT-gal) mice in the C57BL/6 background. Spine densities in granular neurons of the dentate gyrus (DG) were assayed by standard Golgi staining. Activation of Microglial/Macrophages cells was evaluated by immunufluorescence of Iba1. Rate of cell proliferation was estimated with a single pulse of the S-phase tracer IdU.
Results - Both SD and SD+SSW significantly decreased the floating time at the FST, confirming antidepressant-like effects of treatment. Analysis of hippocampal gene expression showed increased levels of the immediate early gene Arc/Arg3.1 in both SD and SD+SSW treated mice; and increased Microglia/Macrophages genes Iba-1 and chemokine receptors Cx3cR1 and CxcR4 in SD+SSW mice only. No effects were detected on Wnt reporter activity in BAT-gal mice, but significantly increased expression levels of the canonical Wnt signaling gene Wnt7a were observed after SD+SSW, which also increased the number of dendritic spines in CA4 neurons of the DG. Finally, SD+SSW up-regulated both the number of Iba1+ cells and rates of cell proliferation in the subgranular region of the DG.
Discussion - Antidepressant-like effects of SD and exercise were paralleled by up-regulation of Wnt 7a and of the innate immune system of the brain, and by an increase of the number of neuronal spines suggesting increased synaptogenesis in the DG of the hippocampus. Wnt 7a is a member of the Wnt-Canonical pathway that is functionally modulated by GSK-3β and activated by lithium, the mainstay for the treatment of mood disorders. Our present findings suggest that its increased expression could be a novel biomarker of rapid antidepressant effects. Observational studies associated microglia activation and disrupted circadian production of pro-inflammatory cytokines with depression severity, but clinical trials gave inconsistent results with antidepressants even increasing cytokines. Our data show that behavioral antidepressant-like effects are not hampered by microglia activation, thus questioning the simplistic view that activated microglia causes depression.

**# 05**
**NPY stimulates U87 human glioma cell proliferation**

Businaro Rita, Borghini Giovanni, Corsi Mariangela, Azzara Gabriella, Cannizzaro Cristina, Rosa Paolo, Pacini Luca, Calogero Antonella

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NPY is a 36-amino-acid peptide and is one of the most abundant peptide expressed in the central nervous system (CNS) of all mammals, including humans. It produces its biological effects through four G-protein-coupled receptors (Y1, Y2, Y4, Y5); it is an important neurotransmitter that regulates mood, endocrine function, blood pressure, nociception, appetite, body weight regulation, and cognition. NPY increases chemotaxis, inducing a preferential mobilization of activated monocytes and regulates the release of cytokines and oxidative reagents. On the other hand, cytokines such IL-1beta, modulate NPY production in human astrocytes. NPY has been involved in the regulation of several processes related to stress. It is expressed in numerous regions of the brain, including the hypothalamus, amygdala, and hippocampus and it has been shown to be downregulated in the amygdala during acute stress, but upregulated after repeated stress exposure. The finding that NPY (both peptide and transcript) is expressed close to the CNS, overlapping with the zone in which incoming axons are sorted before they enter the CNS is suggestive of a functional role related to growth, pathfinding and/or trophism. A bulk of studies strongly suggests that stress favors the onset and the progression of tumors. Aim of the present study was to evaluate the putative proliferative role of NPY on human glioma cell lines. NPY treatment of U-87 human glioma cell line promoted a concentration-dependent increase in proliferation, reaching the maximum effect at 10-9 M. We hypothesized that this activity may be dependent on Egr-1 down-regulation, since emerging evidence indicates that Egr-1 has significant tumor suppressor properties. Real time PCR results demonstrate that NPY is able to down-regulate Egr-1 after 1 h NPY treatment of U87 glioblastoma human cell line. These data agree with previous observations in mouse hypothalamic cell line [1], suggesting an NPY-driven suppression of Egr-1 promoter. Experiments are currently in progress in order to understand the biological role of this observation in glioblastoma tumors.

[1] Cyr NE, Toorie AM, Steger JS, Sochat MM, Hyner S, Perello M, Stuart R, Nillni EA. Mechanisms by which the orexigen NPY regulates anorexigenic α-MSH and TRH. Am. J. Physiol. Endocrinol. Metab. 2013, 304: E640-50

**# 06**

**Absence of TLR4 reduces neuroinflammation in an experimental in vivo model of Parkinson's disease.**

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The innate immune response in the brain is initiated by pathogen-associated molecular patterns (PAMPS) or danger-associated molecular patterns (DAMPS) produced in response to central nervous system (CNS) infection or injury. These molecules activate members of the Toll-like receptor (TLR) family, of which TLR4 is the receptor for bacterial lipopolysaccharide (LPS). Recent data also indicate that TLR4 is up-regulated by MPTP treatment in a mouse model of Parkinson Disease (PD). Parkinson's disease (PD) is a neurodegenerative disease characterized by the degeneration of dopaminergic nigrostriatal neurons, progressive loss of substantia nigra and inflammation. In this study we want to investigate the role of TLR4 in neurodegenerative disorders like PD using Neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) in vivo model using TLR4 knockout (KO) mice (C57BL/10ScNJ); MPTP animal model is a useful model for the study of neurodegeneration in PD because it produces clinical, biochemical and neuropathological changes similar to those observed in human PD. All mice received intraperitoneal injections of MPTP-HCl for 7 days, after this time point, mesencephalon was removed: behavioural and survival testing and biochemical analysis were performed. When compared to WT mice, TLR4 KO mice had lower mortality and better outcomes in behavioural tests (evaluated by force swim test, elevated plus maze test and catalepsy test). Mice that lacked TLR4 had minor expression of MPTP-induced GFAP, IL-1β, iNOS, and COX-2 mediators implicated in neuroinflammation. The translocation of NF-κB was also lower in mesencephalon from TLR4 KO mice. Our results clearly demonstrated that absence of TLR4 reduces the development of neuroinflammation associated with PD. Therefore, TLR4 could be considered as a possible therapeutic target in a neurodegenerative disorders like PD.

**# 07**

## Intracarotid infusion of mesenchymal stem cells in an animal model of Parkinson's disease

Cerri Silvia (1), Greco Rosaria (2), Mangione Antonina Stefania (2), Ghezzi Cristina (1), Levandis Giovanna (1), Avanzini Maria Antonietta (3), Maccario Rita (3), Armentero Marie-Therese (1), Blandini Fabio (1)

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra. Current PD therapies are purely symptomatic and do not modify disease progression. Advances in stem cell field have provided attractive options for PD treatment. Particular attention have been focused on mesenchymal stem cells (MSCs), which show potent neurotrophic, immune-regulatory and anti-inflammatory properties. We have previously demonstrated MSC neuroprotective potential following intrastriatal transplantation in a rodent PD model (Blandini et al., 2010). However, intra-cerebral injection represents a quite invasive method to be applied in patients. Alternative routes of administrations should be therefore explored. To this aim, we developed and validated a protocol for intra-arterial infusion of autologous rat MSCs.
Bone marrow-derived MSCs were co-labelled with Far Red and Near-Infrared fluorochromes and infused into Wistar rats through the internal carotid, at different time intervals after 6-hydroxydopamine intrastriatal injection. Hyperosmolar solution of mannitol was used to transiently permeabilize the blood-brain barrier. At designed times after cell infusion, brain and peripheral organs were excised and underwent Near-Infrared imaging to evaluate whole-body MSC distribution. Microscopic analysis of organ sections was then executed, to assess cell localization.
MSC clusters were found in brain, spleen, liver and lungs. Surgery and cell distribution to peripheral organs did not affect long-term animal survival and health, confirming safety and tolerability of the intra-arterial infusion.
Evaluations of MSC neuroprotective and neuroreparative effects are currently ongoing. Successful achievement of this work will set the ground for clinical trials employing intra-arterial MSC transplantation in PD patients.

**# 08**

## Adrenergic receptor agonists and inverse agonists: role in the HT-29 human colon cancer cell line

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There has been growing evidence suggesting that stress increases the incidence and promotes the development of several types of cancers. Colon cancer is one of the most common causes of cancer mortality worldwide. Adrenaline (AD) and noradrenaline (NA), released by the sympatoadrenomedullary system, are chronically elevated after chronic exposure to stress, and have been implicated in tumor cell proliferation, adhesion, migration and invasion. Both hormones exert their effects through interaction with alpha- and beta-adrenergic receptors (AR). In colon cancer cells, beta-adrenergic activation has been implicated in carcinogenesis and tumor progression. Based on this connection, beta-AR blockers have been studied as possible additions to cancer treatment.

This study aimed to investigate the effect of adrenergic ligands upon proliferation of human colon adenocarcinoma cell line HT-29. Cells were incubated during 24 h in the absence (control) or presence of the AR-agonists, AD (1, 10μM) and isoprenaline (ISO) (1, 10 μM). We next looked at the effect of beta-blockers [propranolol, carvedilol, atenolol and ICI 118,551 (0-100μM)], on cellular proliferation to determine whether inverse agonist activity would be observed.

Cell proliferation was assessed by 5' bromodeoxyuridine (BrdU) incorporation and MTT assays. Chronic treatment with the AR agonists induced a significant increase of HT-29 cell proliferation. AD, respectively at 1μM and 10 μM, significantly increased cell proliferation by 241% ($p<0.001$; n=9) and 185% (p=0.009; n=9). ISO enhanced by 179% (p=0.010; n=9) and 190% (p=0.008; n=9), respectively at 1 and 10 μM, comparing to controls. The IC50 values were calculated as 65.4 μM (33.7 to 126.9 μM) for propranolol (n=10-12), 52.9 μM (21.7 to 128.7 μM) for atenolol (n=8-12), 8.9 μM (6.5 to 12.0 μM) for ICI 118, 551 (n=12) and 8.0 μM (6.0 to 10.6 μM) for carvedilol (n= 12) from the dose-response curve of beta-blockers after 24 hours of exposure. In our study, carvedilol was the most potent antiproliferative agent. Further studies are needed to elucidate which of the beta-blockers currently in clinical use are the most effective in affecting proliferation, as well as in reverting catecholamine-induced proliferation, in colon cancer cells. This relative safe category of drugs could thus have new therapeutic implications for the prevention and treatment of colon cancer.

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**# 09**

## Monomethylfumarateinduces a switch of activated microglia from a pro-inflammatory to a neuroprotective phenotype

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BG-12,a fumaric acid ester with immunomodulatory and neuroprotective potential, is under investigation as treatment for multiple sclerosis. We hypothesize that its beneficial effects are mediated, at least in part, through its action on microglia. This possibility was investigated in vitro using activated microglial cell line and primary cell cultures in the absence or presence of its bioactive metabolite, monomethylfumarate (MMF).MMF inhibited microglia activation by lipopolysaccharide (LPS), significantly reducing the production and expression of pro-inflammatory molecules, TNFα, IL1β, and iNOS, as well as inhibiting the expression of PU.1, a transcription factor that is considered a marker of activation phenotype of microglia. MMF also inhibited the proliferation of LPS-activated primary microglia. Conversely, MMF significantly increased the expression of receptors typical of "steady-state microglia", NURR1, CD200R, and CX3CR1. MMF induced functional changes in activated microglia increasing calcium concentration and phagocytosis, itself associated with an increased expression ofTREM2, through which signaling in microglia facilitates debris clearance, creating a pro-regenerative environment. These findings suggest that upon exposure to MMF, microglia revert from a pro-inflammatory to a neuroprotective phenotype.
We have postulated that the effects of MMF are mediated, at least in part, through binding to the nicotinic acid receptor HCA2, for which it is an agonist. We have now shown that HCA2 is expressed on microglia and will present results of experiments designed to ascertain if signaling through HCA2 pathway is involved in the mode of action of MMF in microglia, and how this leads to downregulation of the inflammatory phenotype.

**# 10**

## Levodopa-induced leukopenia in Parkinson's disease: a case report

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Background: After more than 40 years of clinical use, levodopa (LD) remains the gold standard regarding symptomatic efficacy in the drug treatment of Parkinson's disease (PD). Compared with other available dopaminergic therapies, dopamine replacement with LD is associated with the greatest improvement in motor function, as assessed by reduced scores in the Unified Parkinson's Disease Rating Scale [UPDRS]). In addition, responsiveness to LD (required to exceed 25%–30% reduction in the motor part of the UPDRS) is a diagnostic criterion for PD. In clinical practice, LD slows the progression of disability as assessed by the Hoehn and Yahr staging system, and is associated with a reduction in mortality. Importantly, LD is one of the best tolerated drugs to treat PD, particularly in the elderly population. However, accumulated evidence shows that the therapeutic efficacy of LDOPA is gradually lost over time, and abnormal involuntary movements, dyskinesias, gradually emerge as a prominent side effect of the previously beneficial doses of the drug.
Case report: we report the case of a male patient of 82 years of age, without previous history of neurological diseases, who came to our attention in January 2009 for progressive resting tremor, started about 5 years ago. At admission, his neurological examination was positive for right upper limb resting tremor, micrographia, diffuse hypokinesia, decreased physiological syncinesis in left arm and gait difficulties with tendency to festination. DATscan showed bilateral qualitative and quantitative nigrostriatal dopaminergic impairment. At the clinical follow-up in June 2010 he presented with reduced motor autonomies and was thus started on Levodopa–Carbidopa. At the beginning of therapy, the patient showed reduction of hypokinesia and rigidity but also a significant reduction in leukocytes count (2,470 x $mm^3$), particularly neutrophils (0,710 x $mm^3$), confirmed by a second control three weeks later. After these findings, Levodopa-Carbidopa therapy was stopped with normalization of blood studies. On April 2011, the patient began treatment with Levodopa-Benserazide to control motor symptoms. For the second time the blood tests underscored an important neutropenia (2,40 x $mm^3$), that forced the suspension of therapy followed by normalization of leukocyte count. To date, the patient is taking dopamine agonists with benefit and no evidence of hematological or other adverse effects. By means of rt-PCR we showed that, in comparison to circulating neutrophils from healthy subjects, cells from our patient underexprressed mRNA for D2-like dopamine receptors (DR) and overexpressed mRNA for D1-like (D5) DR as well as for tyrosine hydroxylase, the first and rate-limiting enzyme in the synthesis of dopamine.
Discussion and conclusions: Our case shows an adverse reaction to LD that to our knowledge has not been previously reported. Normalization after LD dechallenge as well as relapse after rechallenge with LD in association with a different inhibitor of L-Dopa decarboxylase supports a strong causal relationship between LD and leukopenia. Additional studies are warranted to assess whether DR and/or TH mRNA expression in circulating neutrophils may be a marker of vulnerability to LD-induced leukopenia.

**# 11**

## The potent inhibitor of poly(ADP-ribose) polymerase-1, 5-aminoisoquinolinone (5-AIQ), promotes autophagy and reduces neural tissue damage and locomotor impairment after spinal cord injury in mice

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Poly (ADP-ribose) polymerase (PARP)-1 is a DNA-binding protein that is primarily activated by nicks in the DNA molecule. It regulates the activity of various enzymes - including itself- that are involved in the control of DNA metabolism. Upon binding to DNA breaks, activated PARP cleaves NAD+ into nicotinamide and ADP-ribose and polymerizes the latter on nuclear acceptor proteins including histones, transcription factors and PARP itself. Poly(ADP-ribosylation) contributes to DNA repair and to the maintenance of genomic stability. Evidence indicate that PARP plays an important role in cerebral ischemia/reperfusion, stroke and neurotrauma. Overactivation of PARP consumes NAD+ and ATP culminating in cell dysfunction and necrosis. PARP has also been shown to associate with and regulate the function of several transcription factors. PARP is involved in the up-regulation of numerous pro-inflammatory genes that play a pathogenetic role in the later stage of neurotrauma. The aim of this study was to investigate the effects of a water-soluble and potent PARP-1 inhibitor, 5-aminoisoquinolinone (5-AIQ), on secondary inflammatory damage associated with an experimental model of spinal cord injury (SCI). SCI was induced in mice through a spinal cord compression by the application of vascular clips (force of 24 g) to the dura via a four-level T5 to T8 laminectomy, and 5-AIQ (3 mg/kg, intraperitoneally, 1 and 6 hours after SCI) was injected into mice and once daily thereafter for 10 days.
Our results demonstrated that the administration of 5-AIQ significantly decreased the phosphorylation of the p70S6K protein and led to higher expression levels of LC3 and Beclin 1 in the injured spinal cord. In addition, neuronal loss and cell death in the injured spinal cord were significantly reduced in the 5-AIQ-treated mice compared to the vehicle-treated mice. Furthermore, the 5-AIQ-treated mice showed significantly higher locomotor function in Basso Mouse Scale (BMS) scores than did the vehicle-treated mice. These results indicate that 5-AIQ promoted autophagy, and reduced neural tissue damage and locomotor impairment after SCI.

**# 12**

## Fingolimod significantly modulates peripheral effector and regulatory T cells in MS patients

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Multiple sclerosis (MS) is a chronic, inflammatory disease of the central nervous system (CNS), driven by autoreactive lymphocytes and inflammatory T cells, possibly specific for myelin antigens, are still considered to play a pivotal role in MS pathogenesis. In particular, both Th1 and Th17 cells seem contribute to MS development. Several evidences support also the involvement of IL-17-producing CD8+ T cells subset, named Tc17, among cells infiltrating MS tissues. Moreover, proinflammatory $CD161^{high}CD8+$ T cells was recently observed in the peripheral blood of MS subjects and detected in CNS immune infiltrates. On the contrary, the abnormal activation of inflammatory cells might associate with an impairment of regulatory cells, that has been described in MS patients.

Fingolimod (FTY720), a new sphingosine 1-phosphate (S1P) receptor modulator, is approved for the treatment of MS based on results from phase II and III clinical trials in patients with relapsing remitting MS. Its phosphorylated form causes internalization and degradation of cell membrane-expressed S1P receptor 1, one of the five known S1P receptors, which is critical for T and B lymphocyte egress from secondary lymphoid organs and thymus. As consequence, lymphocytes retention in the lymph nodes is favored based on the prevalence of the signaling through CCR7, a receptor expressed by memory T cell. In fact, fingolimod has been shown to interfer differently on naïve and memory T cell subsets.

In this study we evaluated the impact of fingolimod on the inflammatory T cells of relapsing-remitting MS patients, analyzing CCR6 and CD161-positive CD4+ and CD8+ T cell subsets relevant for MS pathogenesis; as well as CD4+CD25+T reg cells, focusing on their expression of immunosuppressive ectonucleotidase CD39, involved in the hydrolysis of ATP and correlated to Treg activity.

Fingolimod treatment decreased frequencies mainly of CCR6+ and CD161+ T cells producing IL-17 and IFNγ or IFNγ alone in the CD4+ and in the CD8+ compartments when compared to baseline. In particular, fingolimod significantly reduced levels of $CD8+CD161^{high}CCR6+$ T cells producing IFNγ alone or with IL-17. These results confirm that fingolimod affect significantly classical CD4+ Th17 cells but also IFNγ producing non classical Th1/Th17 cells, which are dynamically generated from classical Th17 cells under inflammatory condition. In addition, these findings further support a selective effect of fingolimod on different T cell populations in MS individuals, probably based on its ability to modulate S1PR1 on lymphocytes and therefore controlling their egress from lymphoid organs .

To concern Treg cells, fingolimod treatment resulted in a statistically significant increment of circulating Treg cells in MS patients to levels similar to those observed in healthy controls. We confirmed also a reduced CD39 expression on $CD4+CD25^{high}CD127^{low}$ T cells in MS patients at baseline compared to controls, but CD39 levels were increased on Tregs one month after fingolimod was started, whereas frequencies of CD39 expressing CD8+ were not changed between the two time points.

Taken together these data suggest a double beneficial role of fingolimod in negatively modulating inflammatory T cell circulation and/or activation and restoring Treg homeostasis.

**# 13**

## Polymorphisms of dopaminergic receptor genes and peripheral immunity in Parkinson's disease

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Parkinson's disease (PD) is a neurodegenerative disorder which stems mainly from a massive loss of dopaminergic innervation in the striatum, as a result of chronic neuroinflammation involving activated microglia and astrocytes. Evidence obtained in animals and in humans however strongly suggest a key contribution to the pathogenesis of PD also by the peripheral immune system. In particular, T lymphocytes have been identified close to dopaminergic neurons in brains of both PD patients and animals with experimental neurodegeneration, and in peripheral blood of PD patients specific alterations of T cell subsets have been described which may be related to the clinical progression of the disease [1].
Involvement of the immune system in PD deserves attention since dopamine, besides being one of the main neurotransmitters, is also a key molecule bridging the nervous and immune systems. Immune cells express dopaminergic receptors (DR) and produce dopamine, which therefore can act as an autocrine/paracrine transmitter between immune cells as well as between these cells and neurons and glia [2]. Available evidence strongly supports investigation of dopaminergic mechanisms modulating the immune system as potential targets for novel neuroprotective strategies in neurodegeneration and in particular in PD.
As a part of a study devised to characterize the relevance of dopaminergic pathways in PD in the modulation of peripheral immunity (and in particular of CD4+ T cells), we investigated the possible relationship between functional polymorphysms of DR genes and the immunological profile in peripheral blood of PD patients.
Preliminary results are so far available for 25 patients (age [mean±SD]: 70.4±7.9 years, 6 females/19 males, UPDRS 17.9±6.8, H/Y [median/range]: 2/1-3), who were genotyped for the following single nucleotide polymorphisms (SNPs) in DR genes: DRD1, A-48G; DRD2, G2137A, C957T and C845G; DRD3, G-712C, A-205G and G25A. Genotyping was performed by Real Time PCR on an Applied Biosystems GeneAmp 9700 PCR System using a predesigned genotyping assay (Applied Biosystems, CA, USA). Correlation was sought between each SNP and number of total leukocytes, as well as of individual leukocyte populations (neutrophil, eosinophil and basophil polymorphonuclear leukocytes, lymphocytes and monocytes).
A significant correlation was found between the G-712C SNP in the DRD3 gene and the number of lymphocytes: 3 subjects carrying the -712G/C heterozygous genotype had significantly less circulating lymphocytes in comparison to 22 subjects with the -712 G/G homozygous genotype (1.8±0.5x109 cells/l vs 1.0±0.1x109 cells/l, P=0.037).
We plan now to assess the possible correlation of the various SNPs in DR genes with the number and frequency of specific lymphocyte subsets as well as to examine their relationship with DR responsiveness in immune cells. Furthermore, we will investigate additional SNPs in the remaining DR genes DRD4 and DRD5 as well as in other genes which encode for key proteins of the dopaminergic system, such as catechol-O-methyltransferase, monoamine oxidase type B, vesicular monoamine transporter 2 and the dopamine transporter. Results will eventually provide additional evidence for a functional link between dopaminergic pathways and the immune response by use of a novel and unprecedented pharmacogenetic approach.

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**# 14**

## Stem cells as advanced therapy of neuropathic pain

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Neuropathic pain can occur secondarily to injury of the central or peripheral nervous system. A pathological interaction between the neuron and non neural cells in the nerve and spinal cord is crucial for neuropathic pain. Stem cells appeared as the ideal tool to obtain a physiological repair and to restore normal conditions in the damaged tissues. We successfully treated peripheral nerve lesion-derived neuropathic pain (CCI in mouse), with iv administration of murine Neural Stem Cells (NSC). In parallel to the anti-allodynic and anti-hyperalgesic effect, we observed a reduction of pro-inflammatory cytokines. Behavioural and biochemical effects were evident by day 3 after NSC, while nerve repair initiated 4 weeks later. NSC reached the lesion shortly after administration and disappeared in the following days although the behavioural and biochemical effects were maintained. Since we wanted to repair or modulate a damage of the nervous system, the use of NSC should give a plus in neuropathic pain. However mesenchimal stem cells (MSC) are easier to be obtained and have strong anti-inflammatory properties. We replicated NSC effects using human MSC derived from adipose tissue (hASC). hASCs reversed hyperalgesia and allodynia starting 24 h after injection. The levels of IL-1β and IL-6, enhanced in CCI mice, were restored, while hASCs induced an increase in IL-10 level. In conclusion peripheral administration of stem cells of different origin and species therapeutically reversed neuropathic pain in the CCI mouse model. A bidirectional interaction between stem cells and lesioned nerve might be at the basis of the positive effects.

**# 15**
**Neuroactive steroids and multiple sclerosis**

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Neuroinflammation is a coordinated process aimed to cope with dangerous stimuli to the central nervous system (CNS), in the attempt to restore tissue integrity. When the fine-tuned regulation of this process is lost, chronic neuroinflammation become detrimental, as reported in several neurodegenerative diseases (Glass et al., Cell. 2010, 140,918-34; Tansey MG, Neurobiol Disease. 2010, 37,491-92).
Neuroactive steroids are cholesterol-derived molecules that can exert their action on CNS components (Melcangi et al., Cell Mol Life Sci. 2008, 65,777-797). They derived from peripheral steroidogenic glands or can be produced and metabolized directly in the CNS, by neurons and glial cells (Garcia-Segura and Melcangi, Glia. 2006, 54,485-98). These molecules, like progesterone (PROG), testosterone (T) and their derivatives, are physiological modulators in many processes, and exert anti-inflammatory and protective actions in pathological situations, as observed in humans and in animal models of many neurodegenerative diseases (Giatti et al., J Mol Endocrinol, 2012, 49:R125-34). Multiple sclerosis (MS) is an inflammatory and neurodegenerative pathology with unknown etiology, where the role of sex steroids in incidence and progression has been extensively described (Tomassini and Pozzilli, J Neurol Sci. 2009, 286:35-9; Greer and McCombe, J Neuroimmunol. 2011, 234:7-18). Recently, steroid-based therapies have been proposed in two different clinical trials, with promising results (Sicotte et al., Arch Neurol. 2007, 64:683-688; Vukusic et al., J Neurol Sci. 2009, 286:114-8). Therefore, we are interested in the protective effects of neuroactive steroids in an animal model of MS, the rodens experimental autoimmune encephalomyelitis (EAE). Indeed, male Dark Agouti (DA) rats were induced with EAE in order to develop a relapsing-remitting progression of this chronic pathology; subsequently they were treated with neuroactive steroids. As we recently reported, chronic PROG administration was able to ameliorate the progression of the disease and to improve neuroinflammatory and functional parameters impaired in EAE animals. Liquid chromatography tandem mass spectrometry analysis on spinal cord samples revealed that levels of dihydroprogesterone (DHP), a PROG metabolite, were increased after PROG administration, suggesting that the effects we observed were probably due to the conversion of PROG into DHP (Giatti et al., J Neuroendocrinol. 2012, 24:851-61).
Recently, we also evaluated the administration of dihydrotestosterone (DHT) to male EAE DA rats. DHT is a potent ligand for androgen receptor, and, unlike T, could not be metabolize into estradiol, a well-known protective compound in EAE. Here we present our preliminary data after treatment with DHT at 14 day (acute inflammatory phase) and 45 day (chronic phase) post induction. The results so far obtained show that DHT administration was able to promote neurological deficits observed in EAE animals, and to ameliorate inflammation, probably acting on microglia and astrocytes, resident immune cells of the CNS. Altogether these observations suggest neuroactive steroids as promising protective agents in MS.

**# 16**

## Role of Toll like receptor 4 signaling pathway in the secondary damage induced by experimental spinal cord and brain injury

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The Toll like receptors (TLRs), as key mediators of innate immunity, responding to diverse microbial products, and injury-induced endogenous ligands. The activation signal diverges, following either of two inflammatory cascades, the myeloid differentiation primary response 88 (MyD88) pathway to nuclear factor kappa-B (NF-κB) activation or the toll receptor-containing adaptor inducing interferon IFN-β (TRIF) pathway, also called the MyD88-independent pathway. Although they have important functions in the innate immune system, emerging evidences also indicate their role in brain and spinal cord injury. We have therefore investigated the role played by TLR4 signaling pathway in the development of mechanisms of secondary inflammatory process in traumatic brain and spinal cord injury (TBI and SCI) in mice that lack a functional TLR4 signaling pathway. Controlled cortical impact injury and spinal cord injury were performed on TLR4 knockout (KO) mice (C57BL/10ScNJ) and wild-type (WT) mice. Brains and spinal cords were collected at 24 h after trauma. For TBI experiments, when compared to WT mice, TLR4 KO mice had lower infarct volumes and better outcomes in neurological and behavioral tests. Mice that lacked TLR4 had minor expression of TBI-induced glial fibrillary acidic protein (GFAP), Chymase, Tryptase, interleukin (IL-1b), inducible nitric oxide synthase (iNOS), Poly (ADP-ribose) polymerase (PARP) and Nitrotyrosine mediators implicated in brain damage. The translocation of expression of p-JNK, IkB-a and NF-kB pathway were also lower in brains from TLR4 KO mice. When compared to WT mice, resulted in significant augmentation of all the above described parameters. In addition, apoptosis levels in TLR4 KO mice had minor expression of Bax while on the contrary with Bcl-2. Therefore, MyD88- dependent pathway is also correlated with the development of spinal cord secondary injury via inflammatory reaction, consequently, for SCI experiments, we predicted that recovery would be improved in mice deficient in TLR4 signaling. Instead, we found that deficiencies in TLR4 signaling impaired functional recovery, neutrophil infiltration and caused excessive microglia and astroglial activation, increased production of inflammatory cytokines, iNOS, PARP, nitrotyrosine expression and apoptosis level. Accordingly, we showed that the absence of TLR4 also reduced the TRIF-mediated pathway by decreasing the expression of phosphorylated interferon regulatory transcription factor (pIRF-3) and consequently, reducing the release of IFN-β that can counteract the inflammation caused by MyD88- dependent pathway activation. In conclusion, how and when TLR4 signaling recruits MyD88 versus TRIF is the subject of much research and however, the exact role of these pathways in the pathophysiology of TBI and SCI remain unclear, but common TLR4 ligands appear to utilize the same pathway or both pathways consistently. Based on these data, we conclude that TLR4 signaling is important in regulating the pathophysiological sequel of SCI and TBI.

**# 17**

## Changes in CD4+ T lymphocyte subsets and expression of dopaminergic receptors in peripheral blood of patients with Parkinson's disease

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Parkinson's disease (PD) is a chronic neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons in the midbrain. Pathogenetic mechanisms include mitochondrial dysfunction, oxidative stress, environmental toxins leading to neuroinflammation and degeneration. Several lines of evidence however increasingly point to the peripheral immune system as a key player in PD. In particular, recent evidence indicates that peripheral CD4+ T lymphocytes infiltrate the brain and contribute to glial activation and neuroinflammation. The present study was thus devised to investigate peripheral immunity in PD patients with particular regard to CD4+ T lymphocytes and their membrane expression of dopaminergic receptors (DR), also in view of the role of dopamine as an autocrine/paracrine transmitter between immune cells as well as between these cells and neurons and glia.

We enrolled so far 35 patients with PD (age [mean±SD]: 64.9±6.2 years, 9 females/26 males, UPDRS [median/range]: 19/5-31, Hoehn and Yahr scale (median/range): 2/1-3) and 24 healthy subjects (age 68.6±6.5 years, 16 females/8 males). Blood samples were obtained from each subject to determine the frequency of the following CD4+ T cell subsets by means of flow cytometry, according to their expression of CD45RA and CCR7: T central memory (Tcm, CD3+CD4+CD45RA-CCR7+), T naive (CD3+CD4+CD45RA+CCR7+), and T effector memory (Tem, CD3+CD4+CD45RA-CCR7-). Immunophenotyping of DR on CD4+ T cells was performed by a 5-color flow cytometric analysis in whole blood with preliminary staining of the cells for DR by an indirect labelling procedure (primary antibody (Ab) + secondary Ab, PE).

Results show that PD patients have significantly less total lymphocytes (mean±SEM: 28.2±1.3% of total leukocytes in controls vs. 21.9±1.0% in patients; $P<0.01$). Within lymphocytes, PD patients have reduced CD3+ T cells (72.3±1.7% of total lymphocytes in controls vs. 69.7±1.4% in PD patients; $P<0.05$), as well as of CD4+ T cells (66.6±1.5% of CD3+ T cells in controls vs. 56.2±0.9% in PD patients, $P<0.0001$). PD patients had also increased Tem (24.8±1.9% of CD4+ T cells in controls, vs. 29.3±1.6% in patients; $P<0.01$), decreased Tcm (28.7±1.9% in controls vs. 26.4±1.5% in PD patients; $P<0.05$) and decreased T naive cells (43.4±2.7% in controls vs. 37.2±2.5% in PD patients; $P<0.01$).

Preliminary analysis of DR expression on CD3+CD4+ T cells showed decreased percentage of cells expressing several types of DR, namely: (controls vs. PD patients) DRD1, 11.6±1.4% vs. 9.1±1.9% ($P<0.05$); DRD2, 3.1±0.5% vs. 3.8±0.7% ($P>0.05$); DRD3, 6.6±1.0% vs. 5.4±0.7% ($P>0.05$); DRD4, 8.1±1.6% vs. 5.0±0.9% ($P<0.05$); DRD5, 13.1±1.2 vs. 8.6±1.3 ($P<0.01$).

The present results document significant changes in peripheral immunity of PD patients, suggestive of a chronic activation of the adaptive immune system. Decreased DR expression on CD4+ T lymphocytes deserves consideration in view of the role of dopaminergic agents in the treatment of PD, and warrant in-depth investigation to assess the influence of antiparkinson drugs on peripheral immunity and its possible consequences for the therapeutic response as well for disease progression. Better knowledge of the contribution of peripheral immune activation to the pathogenesis of PD will allow establishing its relevance as potential therapeutic target, possibly through DR expressed on lymphocytes.

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**# 18**

## Expression of dopaminergic receptors on human CD4+ T cell subsets: a five-colour flow cytometric analysis in whole blood

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Dopamine (DA) is one of the most important neurotransmitters in the central nervous system and exerts its action through five different dopamine receptors (DR), classified into D1-like (DRD1 and DRD5) and D2-like (DRD2, DRD3, and DRD4) on the basis of pharmacological and biochemical differences. The expression of DR is well characterized in nervous system, but little work has been done to examine their occurrence in other cells and tissues, especially in immune system cells, despite growing evidence concerning the immunomodulatory role of DA. Growing amount of evidence indeed indicates that DA exerts direct effects on the immune system which are relevant in health and disease. Exploitation of the immune effects of DA for therapeutic purposes requires however better understanding of the pattern of expression of DR on immune cells.
In the present study we examined the expression of DR on circulating CD4+ T lymphocytes by means of a novel flow cytometric assay in whole blood. Samples of venous blood were obtained from healthy donors (F/M: 17/8; age [mean±SD]: 55.9±15.7 years). Phenotyping of DR on CD4+ T cells was performed by a 5-color flow cytometric analysis in whole blood by use of a two-step protocol: during the first step, cells are stained for DR by an indirect labelling procedure (primary antibody (Ab) + secondary Ab, conjugated with PE); during the second step, cells were incubated with a cocktail of anti-human CD3, CD4, CD45RA and CCR7 Ab conjugated with fluorochromes to identify the subsets of interest of CD4+ cells. Expression of CD45RA and CCR7 allowed identifying the following subsets: T central memory (Tcm, CD3+CD4+CD45RA-CCR7+), T naive (CD3+CD4+CD45RA+CCR7+), and T effector memory (Tem, CD3+CD4+CD45RA-CCR7-).
CD4+ T cells were (mean±SEM) 66.3±2.4% of total CD3+ T cells. Among CD4+ T cells, Tcm were 28.7±1.9%, Tem 24.8±1.6% and T naive 43.4±2.7%. All five DR were expressed on CD4+ T cells. The D2-like DRD2, DRD3 and DRD4 were expressed by 3.1±0.5%, 6.6±1.1% and 8.1±1.6%,, while the D1-like DRD1 and DRD5 were expressed in slightly higher range, 11.7±1.4% and 13.2±1.2%, respectively ($P<0.05$ and $P<0.0001$ vs DRD2, DRD3 and DRD4). The same DR expression pattern was observed in CD4+ T cell subsets, although statistical significance of the differences between D2-like and D1-like DR was reached only in the subset of T naive cells ($P<0.05$ for DRD1 and $P<0.0001$ for DRD5).
In conclusion, by developing a flow cytometric assay which allows the straightforward analysis of expression of DR on human CD4+ T cell subsets, we have found that human CD4+ T lymphocytes express both D1-like and D2-like DR with a subset-specific pattern. Studies are now warranted to establish the functional role of DR receptors on the different CD4+ T cell subsets. Clarifying the role that DR on immune cells will allow to better understand the role of DA in the neuroimmune network in health and disease and to exploit the immunomodulatory potential of the several dopaminergic agents already in clinical use for non-immune indications and with a usually favourable risk-benefit profile.

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**# 19**

**Dopaminergic system in peripheral blood mononuclear cells: what role in inflammatory central obesity?**

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A state of chronic immune activation is a major feature of central obesity (CO) and monocytes are hallmarks of inflammatory processes and of an higher risk for cardiovascular disease [1] Dopamine (DA) is synthesized by different immune cells and modulates their function through DA receptors (DRD1-5) [2], but whether peripheral dopaminergic system is involved in the low grade inflammation linked to CO is unknown. The migration of peripheral blood mononuclear cells (PBMCs) towards sites of inflammation such as the adipose tissue is crucial to the development of sustained tissue inflammation. We aimed to study dopamine receptors and tyrosine hydroxylase (TH), the rate-limiting step in DA synthesis, expression in PBMCs and its relation with inflammatory and metabolic parameters in blood donors.
To investigate dopaminergic pathways in CO, we measured the expression of DRD1-5 and TH by semiquantitative real-time PCR in PBMCs obtained from 15 healthy donors, 8 with CO and 7 without CO, according to the International Diabetes Federation criteria by using waist circumference (≥80 cm for women and ≥94 cm for men). The ratio (R) was calculated between DRD and TH mRNA expression between individuals with and without CO. A ratio <0.5 was considered under expression and >2.0 over expression. Plasma lipid profile and leptin levels were determined. Blood monocyte subpopulations (pro-inflammatory CD14+CD16+ and classical CD14+CD16-) were also investigated by flow cytometry using CD14, CD16, CD11b, and CD36 markers. CD16+ pro-inflammatory monocytes immunophenotypically present a lower complexity and lower expression of CD14, CD11b and CD36 in comparison with classical monocytes. Centrally obese individuals showed higher plasmatic levels of total cholesterol (TC) ($p=0.05$), VLDL cholesterol ($p=0.022$), triacylglycerol ($p=0.022$) and leptin ($p=0.022$) in comparison with subjects without CO. While DRD1 was undetected in PBMCs, no differences were found in both DRD3 and DDR4 expression between groups. In CO, DRD2 ($R=0.11$; $p<0.001$), DRD5 ($R=0.07$; $p=0.001$) and TH ($R=0.38$; $p=0.015$) were under expressed, in comparison with non-CO. The number of pro-inflammatory monocytes was similar in both groups with or without CO, but in CO their immunophenotypic characteristics showed a higher size ($p=0.031$) and lower CD11b expression ($p=0.011$). DRD2 expression was positively correlated with both TH ($\rho=0.786$; $p=0.021$) and CD11b ($\rho=0.928$; $p=0.008$). Plasmatic levels of VLDL and triacylglycerol correlated negatively with the expression of CD11b in pro-inflammatory monocytes, whereas both TC and LDL levels negatively correlated with the complexity and CD14 expression in these cells. These preliminary results suggest that circulating peripheral mononuclear cells from centrally obese individuals show a distinct dopaminergic pattern associated not only with a higher inflammatory phenotype, but also with a more atherogenic lipid profile in plasma. These findings seem very challenging to further investigate the role of dopaminergic pathways in inflammatory obesity.

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**# 20**

## Neuroscience and Psychoneuroimmunology: a more complex vision of patient in chemotherapy

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The evidences from many field of the research has showed a more complex vision of the human organism than in the past. In this perspective, Psychoneuroimmunology and Neuroscience have highlighted the link between emotions, cognitions, immunity and diseases. In the literature, the weight of stress-related psychosocial factors on the onset and the course of the cancer is more than in the past. In fact, studies from many field of research has showed that an integrated psychological and medical approach together increase the efficacy of the treatments. By this point of view, dysfunctional metacognitive beliefs can play an important role for depression and anxiety of the patients in chemotherapy.
The aim of this study was to examine the the role of metacognitions to predict anxiety and depression levels of patients in chemotherapy.
A clinical group of 175 patients in chemotherapy of the Medical Oncology Unit of the Department of Human Pathology of Policlinico Universitario of Messina were consecutively selected and underwent the following tests: Hospital Anxiety and Depression (HADS) [1]; Metacognitions Questionnaire 30 (MCQ-30) [2].
Inclusion criteria were a chemotherapy at the time of this study and the exclusion criteria were the presence of any psychiatric disorder included in Axis I and/or II of the DSM-IV-TR.
The final sample of patients consisted of 141 females and 34 males (mean age in years = 58.21; DS = 11.66). Forty-six percent of patients had a breast cancer diagnosis, thirty percent of patients had a bowel cancer diagnosis and nine percent had a lung cancer diagnosis.
The main hypotheses were test using two sets of hierarchical multiple regression analyses for each criterion or dependent variable (DV). In the first analysis, anxiety was the DV. In the second analysis, depression was the DV. The independent variables were the dysfunctional metacognitive beliefs and demographic aspects for both hierarchical multiple regression analyses.
The results of the first analysis showed that negative beliefs about worry concerning uncontrollability and danger, and positive beliefs about worry predicted anxiety as DV. Those dysfunctional metacognitive beliefs explained 57 percent of variance in anxiety score ($R2 = .568$; ANOVA: $F = 76.501$; $P \leq .000$).
The results of the second analysis showed that negative beliefs about worry concerning uncontrollability and danger, beliefs about the need to control thoughts and the age as demographic variable predicted depression as DV. Those variables explained 40 percent of variance in depression score ($R2 = .387$; ANOVA: $F = 35.342$; $P \leq .000$).
The findings of this study have a number of clinical and theoretical implications regarding the bond between metacognitive process, emotional suffer, anxiety and depression in a more complex way. In fact, these results allow us to confirm the link between Neuroscience and Psychoneuroimmunology also in the field of cancer research. On the other hand, there are specific relations between dysfunctional metacognitive beliefs and anxiety and depression for patients in chemotherapy.

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**# 21**

## Distinct effects of antidepressant treatment on anhedonia and inflammation in stressed rats

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Major depressive disorders (MDD) originate from the interaction between vulnerability genes and adverse environmental factors, such as stress. Depression is characterized by dysfunctions in multiple systems, including neurotransmitters, hormones as well as, neurotrophic and neuroplastic molecules. In recent year the role for immune/inflammatory systems has re-gained attention based on a number of observations, including elevated blood levels of the pro-inflammatory cytokines found in depressed patients and the high comorbidity existing between depression and inflammation-related diseases.
On these bases, the purpose of our study was to investigate the potential relationship between inflammation and anhedonia, a classical feature of depression. To this aim, adult male rats were exposed to a chronic mild stress (CMS) paradigm for 8 weeks and the cerebral mRNA levels of pro-inflammatory cytokines and microglial markers were evaluated in parallel with the anhedonic phenotype. Moreover, a group of animals (sham or CMS) were chronically treated with the antidepressant imipramine (10 mg/kg/day starting from week 2), in order to evaluate the ability of the antidepressant treatment to restore behavioral defects and interfere with the inflammatory alterations.
We found that the expression of IL-1β, IL-6, and TNF-α were significantly increased in the hippocampus of CMS rats. These changes were accompanied by a gradual decrease of sucrose consumption over the 8-week period, taken as an index of anhedonia. Moreover, while chronic imipramine treatment was able to normalize the anhedonic phenotype caused by CMS, it did not normalize the increased expression of inflammatory markers.
Our findings suggest that exposure to CMS is associated with a significant elevation of inflammatory markers, which do not appear to contribute to the 'anhedonic' phenotype observed in stressed animals. Under a translational perspective, the inability of chronic antidepressant treatment to normalize cytokines levels may be relevant for residual symptoms and could lead to enhanced risk of relapse and to heightened vulnerability to other pathologic conditions.

**# 22**

## Impact of Major and Minor Depression on the ischemic heart disease outcome over one year from the onset

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Objective: Lifetime prevalence of depression is about 20% and up 25% in women's population; in western society, ischemic heart disease is the first cause of morbidity and mortality and, according to WHO, soon it will became the first cause of death all over the world. Furthermore, acute coronary syndromes (ACS) are themselves risk factors for depression and depression is supposed to be a negative prognostic factor on cardiac outcome. The aim of the present study is to evaluate if, in the year after their first episode of acute coronary syndrome, patients with no history of mood disorders, develop depressive symptoms and if the onset of depression influences the cardiac outcome.
Methods: 304 consecutive patients at their first ACS and without a history of depression were recruited at the Coronary Intensive Care Unit. Patients were evaluated for depression and anxiety with Hospital Anxiety and Depression Scale (HADS) and Primary Care Evaluation of Mental Disorder (PRIME-MD) both at baseline and a 1,2, 4, 6, 9 and 12 months follow up. At baseline were collected clinical and socio-demographical variables.
Results: we found that 39 patients (12.8%) developed depression whereof 25 (8.2%) with Minor Depression (md) and 14 (4.6%) with Major Depression (MD), whether 265 patients (87.2%) were non depressed. In this sample 21.4% of MD and 16% of md patients had a second acute coronary event compared to 7.9% of non depressed patients. These findings suggest that depression influences negatively the cardiac outcome, confirming his role as a risk factor for new cardiac events (O.R = 2.89 ; I.C. 0.98 – 8.54 p = 0.056).
Conclusion: our study confirms literature data according to which there's a double link between depression and acute coronary syndromes. According our results depression represents a risk factor for new cardiac events in patients with acute coronary syndromes. These finding on such a narrow population (no history of depression and at their first acute coronary syndrome), underline how these two diseases are inter-connected, sharing a common pathogenesis and neuro-endocrine modification on which further studies are needed.

**# 23**

## Neuroprotective effect of palmitoylethanolamide and luteolin on secondary inflammatory process associated with CNS pathologies

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It has recently been demonstrated that palmitoylethanolamide (PEA), an endogenous lipid amide belonging to the N-acylethanolamine family, exerts neuroprotection in central nervous system (CNS) pathologies. Since oxidative stress is considered to play an important role in neuroinflammatory disorders, in the present work we studied a new composite, a formulation including PEA and the antioxidant compound luteolin (Lut), subjected to an ultramicronization process, co-ultraPEALut. We investigated the effect of co-ultraPEALut (in the respective fixed doses of 10:1 in mass) in a CNS pathology such as in spinal cord injury (SCI), through an ex vivo organotypic spinal cord culture model and an in vivo model of SCI, and in the traumatic brain injury (TBI). For the organotypic cultures, spinal cords were prepared from mice at postnatal day 6 and were cut into transverse slices of 400 μm thickness to generate the lumbar organotypic slice cultures. After 7 days of culturing, the slices were mechanically injured onto the center of the slice and the co-ultraPEALut was applied at different concentration. For the in vivo study, SCI was induced in mice through spinal cord compression by the application of vascular clips (force of 24 g) and co-ultraPEALut (1 mg/kg ip) was administered at 1 and 6 hours after SCI. For the TBI model a cortical contusion was produced on the exposed cortex of the mice, using a controlled impactor device to produce a brain injury. One hours after TBI the mice were treated with PEA (3mg/kg body weight) + Lut (10mg/kg body weight) as combination therapy. In all of the CNS trauma models that we used, the mice were sacrified after 24 hours and the parameters involved in the inflammation process were evaluated. In the present study we demonstrated that the treatment with co-ultraPEALut resulted in a significant improvement of motor and cognitive recovery after SCI as well as after TBI markedly reducing lesion volumes. Moreover, our results revealed the ability of co-ultraPEALut to reduce brain trauma through modulation of NF-κB activation. In addition, treatment with co-ultraPEALut significantly enhanced the expression of the neuroprotective neurotrophins (BDNF, and GDNF) compared to vehicle. Thus, our data demonstrated that PEA and Luteolin can exert neuroprotective effects and the combination of both by co-ultramicronization, could improve their ability to counteract the neurodegeneration and neuroinflammation possibly due to its antioxidant properties of Luteolin.

**# 24**

## Pilot pharmacological experimental study in obese obsessive-compulsive personality traits patients treated with sertraline after plastic surgery

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Correlations between psychiatric disorders and overweight/obesity are reported in the literature. However, temperament/personality traits have been less frequently studied even though the correlation with Axis-1 diseases is well defined. Seventy overweight/obese patients (age 18-60 years, BMI 25-34.9 at recruitment) referring to the outpatient service for obesity-related lipodystrophism were enrolled. Psychiatric disorders, temperament traits, and body image perception were evaluated and compared with a control group (N = 33) from the general population sharing clinical/demographic features. Patients had higher scores in lifetime depression, with moderate/mild concern with body shape. Regarding personality traits, tests revealed higher scores on subscale RD4 (dependence/independence) in patients, whereas controls scored higher on the "openness to experience" NEO Five Factory Inventory sub-scale. Obese patients had a higher prevalence of obsessive characteristics.The affective sphere is an important feature in obese patients, as are obsessive traits, since a distorted body shape perception, temperament and personality traits appear to be involved in leading patients to seek surgical advice. These characteristics appear really relevant not only for obesity pathology diagnosis but also for medical follow-up such as compliance in control visits, diets and rehabilitation programs. After this pilot-study, we have hypothesed the possibility to pharmacologically treat these patients (sertraline at maximum dosage (150-200 mg/die) in the clinical manifestation of their personality assessment in order to optimize their clinical management.The possibility to detect these patients could be an important instrument to avoid early post- treatment relapse and to give patients the possibility to refer also to a psychiatric care not only before but also after surgery.

**# 25**

## Weight gain in atypical antipsychotics treatment and PKC-β

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Atypical antipsychotics (APD) are currently used in clinical practice for a variety of mental disorders but weight gain is a common side effect, resulting frequently in obesity. The mechanisms underlying pharmacologically induced weight gain are still controversial.
We tested in vitro the effects of different APDs on adipogenic events in cultured human pre-adipocytes and in rat muscle-derived stem cells (MDSCs), aiming to identify a common intracellular event contributable to these drugs. Culture behavior was evaluated in terms of cell proliferation, lipid accumulation, gene expression and morphological features. Results indicate that APDs influence adipogenic events through changes in the differentiation and proliferation of pre-adipocytes and MDSCs that are brought on by protein kinase C-β (PKC-β) activation. These data identify a signaling route that could be a potential target of pharmacological approaches for preventing the weight gain associated with APD treatment.
Our further step is now to demonstrate in vivo that PKC-β null and wild-type mice treated with PKC-β inhibitors are protected from APD-induced weight gain and yet retain their ability to counteract anxiety (otherwise, what is the benefit of preventing weight gain if APDs are also inhibited from performing their main function?), as well as defining the detailed mechanism by which APDs are activating PKC-β is needed.

**# 26**

## Rapamycin modulates lipopolysaccharide-induced neuroinflammatory response and absence seizures in in a genetic rat model of absence epilepsy

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The mammalian target of rapamycin (mTOR) signaling pathway has been recently indicated as a suitable drug target for the prevention of epileptogenesis; however, the exact mechanism underlying this antiepileptogenic effect still remains unclear (Russo et al., 2012. Mol Neurobiol. 46(3):662-81). The mTOR pathway is known for its involvement in the control of the immune system (as well as other important physiological functions) (Powell et al., 2012. Annu Rev Immunol. 30: 39-68). Since neuroinflammation is now recognized as a major contributor to epileptogenesis and associated changes in neuronal excitability (Devinsky et al., 2013. Trends Neurosci. 36: 174-84), we wished to examine whether the beneficial neuroprotective effects of mTOR modulation could involve a suppression of the neuroinflammatory process in epileptic brain. We therefore examined the effects of rapamycin, an mTOR inhibitor, on the production of pro-inflammatory cytokines in a genetic (WAG/Rij) rat model of absence epilepsy. We have previously shown that rapamycin inhibits the aggravation of absence seizures produced by intracerebral administration of the bacterial endotoxin lipopolysaccharide (LPS) in WAG/Rij rats (Russo et al., 2013. Neuropharmacology. 69:25-36). We now provide evidence that this effect is correlated with the ability of rapamycin to dampen and delay the increase in neuroinflammatory cytokines (IL-1β and TNF-α) produced by LPS, most likely through inhibition of the activation of nuclear factor-κB (NF-κB), a transcription factor known to be involved in the regulation of neuroinflammatory processes. Our results suggest that such a mechanism could contribute to the antiseizure and antiepileptogenic effects of rapamycin in this absence epilepsy model and further highlight the potential therapeutic usefulness of mTOR inhibition in the management of human epilepsy disorders.

**# 27**

## ω-3 and ω-9 fatty acids control adrenomedullary catecholamine content and secretion

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The prevalence of obesity is dramatically increasing worldwide, and is associated with an increased risk of cardiovascular (CV) disease and metabolic disorders. The optimal dietary strategy for the prevention of chronic diseases remains a challenging and highly relevant preventive health issue. Adrenaline (AD) and noradrenaline (NA), the main mediators of the sympathoadrenomedullary system, play crucial roles in the regulation of metabolic and CV homeostasis. AD and NA seem to behave very differently in metabolic syndrome: whereas NA levels positively correlate with obesity and CV risk, AD shows an inverse association with CV mortality.
Fatty acids (FA) are not only important sources of energy but they also play key roles in regulating a range of physiological responses, and dysfunction of their metabolism can impair energy homeostasis. The typical western diet contains insufficient omega-3 FA, which could be the cause of many chronic diseases including CV disease, since omega-3 FA are usually associated with cardioprotective benefits. In contrast, consumption of trans FA, even at low levels of intake, significantly increases the risk of coronary events. Although FA can have marked effects on neurosecretion, very limited information is available concerning the effect of these molecules on catecholamines (CA) handling by adrenal chromaffin cells.
This work aimed to investigate the effect of chronic exposure to both omega-3 [eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)] and omega-9 [oleic acid (OA) and, its trans isomer, elaidic acid (EA)] FA on CA cellular content and release (quantified by high-performance liquid chromatography), using bovine adrenal chromaffin cells. The effect of these molecules on tyrosine hydroxilase (TH), the rate limiting step in CA synthesis, and phenylethanolamine N-methyltransferase (PNMT), the enzyme that converts NA to AD, expression was also evaluated by quantitative PCR.
Within the omega-9, while EA selectively impaired AD cellular content to about 51% of control, OA was devoid of effect. EA also inhibited the release of both CA (by about 28% and 51%, respectively for AD and NA) and was able to selectively decrease KCl-induced AD release to about 79%. Chronic exposure to EPA and DHA did not significantly modify both CA cellular content and KCl-evoked release. On the other hand, these FA led to a significant reduction of NA basal release (to about 72% and 62% of control, respectively with EPA and DHA ), but had no effect over AD. EPA was the only FA capable to significantly change TH expression, decreasing its mRNA levels ($p = 0.022$). EA, EPA and DHA significantly decreased the expression of PNMT, being DHA the most effective in this reduction.
Our results suggest that FA under study affected CA production, cellular content and release through different pathways. Further studies are required to identify and characterize the intracellular responses involved in the actions of these dietary FA over CA responses. That knowledge, together with data obtained from “in vivo” studies, might help to sustain future nutritional recommendations in order to improve health.

**# 28**
**Sympathoadrenergic modulation of human neutrophils**

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Background: Catecholamines (CA) are mediators of the central nervous system and of the sympathetic branch of the autonomic nervous system (ANS) that is involved in the genesis of the stress response [1]. The chronic stress have a negative effect on the cardiovascular system [2]. Several studies suggest that polymorphonuclear cell (PMN) play an important role on cardiovascular disease (CVD), in particular atherosclerosis development [3] and it has been known that PMN express adrenergic receptor (AR; 1). Therefore we aimed to investigated catecholaminergic pathways in human PMNs and whether CA influence PMN functions. In particular, we assessed the ability of Adrenaline (A), Noradrenaline (NA) and of the β-AR agonist isoprenaline (ISO) to modulate migration, reactive oxygen species (ROS) generation, interleukin (IL)-8 production, β2-integrin expression and their ability to modulate the morphogenesis process and the adhesion of PMN to HUVEC monolayer's, steps involved in vascular damage and atherosclerotic plaque development.
Methods: PMNs were obtained from venous blood of healthy subjects and tyrosine hydroxylase (TH) and AR expression was assayed by real-time PCR. ROS were detected by spectrofluorimeter assay and β2-integrin expression and cell adhesion, by flow cytometry. Migration was evaluated by Boyden chamber and CA assayed by HPLC. IL-8 production was measured by ELISA and morphogenesis with fluorescence microscopy evaluation.
Results: PMN expressed detectable levels of mRNA for TH as well as for several subtypes of α- and β-AR. Stimulation with fMLP 0,1 μM significantly increased α1A, α2A, β1, β2 and β3-ARs, but not TH.
A did not affect spontaneous migration but concentration-dependently (10 nM–1 μM) significantly reduced fMLP-induced chemotaxis. Incubation with the α1-AR antagonist prazosin (PRA), the α2-AR antagonist yohimbine (YOH) or the β-AR antagonist propranolol (PRO), all at 10 μM, did not affect spontaneous migration or fMLP-induced chemotaxis, while PRO but not YOH or PRA reverted the inhibitory effect of A on fMLP-induced chemotaxis.
Spontaneous ROS production was unaffected by A (0.01 μM–1 μM) and NA (1 μM) , while ISO (1 μM) induces a significant reduction. Pretreatment with A, NA and ISO significantly reduced fMLP-induced ROS production. YOH, but not PRA or PRO, significantly increased spontaneous ROS production, while fMLP-induced ROS generation was unaffected by PRA and YOH, but was significantly reverted by PRO.
A was unable to affect either resting or fMLP-stimulated IL-8 production. A, NA, or ISO did not modify resting β2-integrin expression (measured as CD11b and CD18 expression) but reverted fMLP-induced expression. Preliminary data shown that if PMN were treated with lipopolysaccharide (LPS), they adhered to the endothelium and their adherence appears to be reduced by the coincubation with A (1 μM).
Conclusions: CA are able to modulate several PMN functional responses involved in the inflammatory component of the atherosclerotic process. PMNs could represent a target for adrenergic agents aimed at decreasing inflammation and vascular damage in CVD.

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**# 29**

## Adrenergic/dopaminergic pathways in circulating lymphocytes as early markers of CIS progressing to multiple sclerosis: the Clinically Isolated Syndrome and CAtecholamines in Lymphocytes-CISCALy Study

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Several lines of evidence indicate that dopaminergic receptor (DR)- and adrenoceptor (AR)-operated pathways are dysregulated in lymphocytes during multiple sclerosis (MS), and interferon-β restores their responsiveness, suggesting their relevance for the therapeutic response. Whether such dysregulation develops as a consequence of MS or precedes its onset is still debated. In the majority of cases, clinical onset of MS is represented by an acute or subacute episode that may be ascribed to a single lesion in the white-matter of the central nervous system. This presentation is known as a clinically isolated syndrome (CIS). By definition, patients with a CIS do not have MS, though they may be at a high risk to develop the disease and, since the earliest molecular events in MS are still unknown, research on CIS may provide new insights into early changes and pathogenetic mechanisms that might affect the initial course of MS. This study was therefore devised to investigate DR/AR-operated pathways in lymphocytes from subjects with CIS, to establish their possible early dysregulation and to assess their relationship with subsequent progression to MS.
We started a multicentric prospective study which enrolled CIS patients, studied at baseline and after 6 and 12 months. Circulating lymphocytes were isolated from venous blood and expression of mRNA for α- and β-AR subtypes, D1-like (DRD1, DRD5) and D2-like (DRD2, DRD3, DRD4) DR subtypes, and for tyrosine hydroxylase (TH, the rate-limiting enzyme for catecholamine synthesis) was evaluated in total lymphocytes and in purified CD4+CD25- T effector cells (Teff) and in CD4+CD25+ T regulatory cells (Treg). Production of catecholamines by lymphocytes, as well as their levels in urine, was assessed by means of HPLC-ED. Plasma ACTH and cortisol were measured by standard procedures. Age- and sex-matched healthy subjects (HS) were enrolled as control.
So far, results are available for 25 CIS patients (age [mean±SD]: 31.1±11.2 years, F/M: 14/11) and 15 HS (age: 32.7±10.6 years, F/M: 8/7). At baseline, in comparison to lymphocytes from controls, cells from CIS patients expressed significantly higher levels of TH, β1- and β3-AR mRNA, both at rest and after PHA (10 μg/mL for 48 h). After PHA, cells from CIS patients also expressed higher mRNA levels for DRD3. Overexpression of β1- and β3-AR mRNA was confirmed in Teff and Treg from CIS patients, which also expressed more α1B-AR mRNA. Teff from CIS patients also expressed more TH and DRD3 mRNA. So far, 16 CIS patients reached 12 months follow-up and 11 progressed to MS. In progressed subjects total lymphocytes at baseline expressed more PHA-induced α2B-AR mRNA and Teff expressed slightly increased DRD2 mRNA. Assay of catecholamines in cells and urine, as well as of plasma ACTH and cortisol, are ongoing.
Preliminary data suggest dysregulation of DR/AR-operated pathways in circulating lymphocytes in an early event occurring in CIS patients and that specific dysregulations may have some relationship with subsequent progression to MS. Completion of the study will allow to establish the relevance of these findings for the clinical development of MS.

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**# 30**

## Modifications of the intestinal dopaminergic tone in a rodent model of Parkinson's disease. Is the dorsal motor nucleus of the vagus nerve involved?

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Parkinson's disease (PD) is classically characterized by its motor symptoms. However, additional debilitating symptoms include non-motor manifestations, such as gastrointestinal (GI) dysfunctions, whose link with the central lesion is currently poorly understood.
In this study, we investigated dopaminergic neurotransmission and peristalsis in the GI tract of rats that received an injection of 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (a rodent model of PD-like neuronal degeneration). The relationship between central dopaminergic denervation and GI dysfunction was investigated studying the neurochemical changes in the dorsal motor nucleus of the vagus (DMV).
Evaluations were performed 4 weeks after surgery. Loss of dopaminergic terminals and neurons was evaluated by immunohistochemistry for the enzyme tyrosine hydroxylase. In the GI tract, peristalsis was evaluated on distal colon while HPLC determination of dopamine (DA) and gene dopamine D2 receptor (DRD2) expression were studied in isolated GI segments. Neuronal activity in the DMV was studied by analysis of FosB/Delta FosB expression.
Peristalsis showed no differences at basal recording between 6-OHDA and control groups. However, 6-OHDA lesioned animals completely lost the inhibitory response to DA-agonist rotigotine. A trend toward a reduced expression of DRD2 was observed in the colon of 6-OHDA lesioned rats, which was associated with moderate, nonsignificant increases in tissue levels of dopamine. 6-OHDA lesioned animals showed increased FosB/Delta FosB expression, compared to control animals, in DMV neurons.
Our results demonstrate that selective lesion of the nigrostriatal dopaminergic pathway triggers alterations of intestinal dopaminergic system that may be sustained by changes in DMV neuronal activity.

**# 31**

## Role of autophagy in the response of human cancer cell lines to chemotherapy under normoxic and hypoxic conditions

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Autophagy is a highly conserved and regulated, ATP-dependent, cellular process, occurring in eukaryotic cells, that has been assigned a key role in the regulation of cell survival under stress. Over the past decade, interest in autophagy has notably increased not only in antineoplastic, but also in neurodegenerative diseases research; despite some initial controversy as whether or not autophagy occurs in neurons, it is now evident that neurons undergo autophagy and that this process is activated following exposure to hypoxic and excitotoxic stimuli. The role of autophagy in cancer is also somewhat controversial and quite complex, as the process appears to be tumor suppressive during cancer development, but contributes to tumor cell survival during cancer progression. Recently, several studies have provided links between multiple hypoxic signaling events and the activation of autophagy as a survival strategy for hypoxic cells.

For example, the presence of hypoxic regions is a common feature of solid tumors, where they may serve as niches for the maintenance of tumor initiating cells and exert a selective pressure causing extensive adaptive changes at the cellular level. Such changes, largely dependent on activation of hypoxia-inducible factors (HIFs), lead to an increasingly aggressive tumor phenotype and to chemoresistance. Autophagy may contribute to both aspects, while at the same time playing a fundamental role in tumor escape following immunotherapy. In this regard, it is well known that HIF-1α stabilization activates signaling pathways leading to STAT3 accumulation and activation in tumor cells. Furthermore, activated STAT3 inhibits the cytotoxic potential of cytotoxic T-lymphocyte (CTL) in tumor cells, leading to resistance to immunotherapy.

The aim of this study was to assess the role of autophagy in the response of a human colon adenocarcinoma cell line, HCT116, to the standard chemotherapeutic drug 5-Fluorouracil (5-FU), and to the direct apoptosis activator, the BH3 mimetic compound Obatoclax (OBT), under different experimental conditions, namely, normoxia and chemical hypoxia, based on $CoCl_2$-induced stabilization of the inducible α subunit of HIF-1.

Our results confirm a decrease in the cytotoxic and pro-apoptotic effects of 5-FU under chemical hypoxia; interestingly, HIF-1α stabilization does not affect the response of HCT116 cells to OBT. At the same time, cytotoxicity tests, fluorescence microscopy and western blot analysis show that both compounds are able to induce a pro-autofagic response, that appears to be more pronounced under chemical hypoxia. Data obtained following combined treatment with 5-FU and OBT show a significant increase in pro-autophagic response of HCT116 cells, with respect to single drug treatments, in both experimental conditions. Inhibition of the autophagic process causes a significant reduction in survival fraction, in both experimental conditions, which suggests that autophagy plays a cytoprotective role in this experimental setup. It can be of interest to investigate if same cytoprotective effects can also occur or can be induced in the CNS.

These results support autophagy inhibition as a strategy to potentiate the antitumor effects of therapeutic combinations of conventional cytotoxic drugs and apoptosis-activating agents; further investigations in in vivo models are required to evaluate whether this strategy might also potentiate the effects of cancer immunotherapy.

**# 32**

## Asenapine: clinical effectiveness and incidence of collateral effects

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The Second-Generation Antipsychotics (SGA) in the treatment of schizophrenia and bipolar disorders have undoubtedly made significant improvements in quality of life for many patients, although they are associated with metabolic, endocrinological and electrocardiographic side effects. Asenapine, the last SGA, is characterized by affinity for dopamine receptors, serotonergic and adrenergic receptors.
The study enrolled patients observed for a period of three months. It consists of two parts: the first one is similar to the OSTER study proposed by Lundbeck, based on evaluations of clinical efficacy in patients with Bipolar Disorder type I which started an antipsychotic therapy and/or mood stabilizer; the second part is a spontaneous study focused specifically on Asenapine intended to record any side effects of the molecule among those most frequently related to antipsychotics. In first part of the study it was administered psychometric scales (YMRS, CGI-BD, MADRS, FAST) in five consecutive visits over three months. The second part is carried out in two stages (baseline and three months), recording the incidence of extrapyramidal side effects (with the scale DMOE), metabolic (changes in CBC, glycemia, total cholesterol, HDL, LDL, triglycerides, ALP, ALT, gamma GT, prolactin), electrocardiographic (lengthening of QTc) or changes in vital signs (weight, blood pressure and heart rate, BMI) in patients treated with Asenapine. The full study enlists patients referred to the Psychiatry Unit of the "Ospedale di Circolo – Fondazione Macchi" of Varese.
The first part has been implemented from May 2012 to April 2013. Our center has enrolled nine patients of mean age 51.4 years (± SD 17.9). There is a drop-out caused by poor patient compliance and it's to be underlined one adverse reaction: daytime sleepiness. The first data about the clinical response seen in the YMRS (remission are considered a score ≤12 at each visit and response ≥50% reduction in the scale score at each visit), there has been a marked decrease in mean scores from the time of enrollment in subsequent tests. In particular, starting from the third week (T3) therapy we achieved a response, compared to baseline score as the fifth week (T4) the remission of maniac symptoms. Unfortunately, given the small number of patients enrolled by our center, the data available so far do not allow us to process the results that have a validity statistically significant. This processing will only be possible by evaluating the data collected by all centers involved in the study and not yet at our disposal.
The second part, which began in early 2013 and is still ongoing, has so far enrolled 6 patients of mean age 44.83 years (± SD 20.73). The majority of patients treated with polypharmacy (SGA or FGA, mood stabilizers and BDZ) before the introduction of Asenapine, in most cases introduced with switch from SGA: 66.7% of the patients are men and mostly with low education.
Since the second part includes patients treated with asenapine regardless of the diagnosis, the disease of the patients enrolled so far are distributed as follows: 50% of bipolar disorder and 50% of psychotic disorders personality disorder. However, given the recent actual start of the trial, are not yet available to today results statistically analyzed with regard to the detection of side effects.

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