



Poster Session 1: Neuroimmunology and Neuroinflammation

PI - 1-1

The influence of purine metabolism on excitotoxic neuronal injury

*J. Kleine¹, F. Ebrahimi¹, T. Hohmann¹, C. Ghabban¹, U. Grabiec¹, F. Dehghani¹

¹Martin Luther University Halle-Wittenberg, Department of Anatomy and Cell Biology, Halle (Saale), Germany

Objective: Stroke is one of the leading causes for disability and mortality in adults. Current therapies harbor many limitations and therefore are not suitable for all patients. The immunosuppressant mycophenolate mofetil (MMF) has the capacity to inhibit microglial and astrocytic activation and to reduce the extent of cell death after neuronal injury.

This study was designed to analyze the therapeutic windows of MMF and the temporal dynamics of cellular responses and signaling cascades.

Methods: Using N-methyl-D-aspartate (NMDA)-lesioned organotypic hippocampal slice cultures (OHSCs) treated with 100 µg/mL mycophenolate mofetil (MMF) within specific time frames, we determined the number of propidium iodide (PI) positive degenerating neurons and isolectin B4 positive microglial cells. The role of microglia and astrocytes in MMF – mediated effects was investigated after depletion with the bisphosphonate clodronate in NMDA lesioned OHSC. Furthermore, the involvement of further targets of MMF was identified in the cell culture.

Results: MMF treatment after excitotoxic damage significantly reduced both microglial and astroglial proliferation rates without affecting apoptosis. A crucial time-frame of significant neuroprotection was identified between 12 and 36 hours after injury.

Conclusion: Our data indicates that MMF significantly reduces the extent of neuronal cell death in a specific crucial time frame after injury. Therefore, long term immunosuppression seems unnecessary. Currently, the mechanism of MMF action in glial cells is still unexplored; however, it seems to be an interesting target to understand the interactions in proliferation between microglia and astrocytes.

PI - 1-3

Under hypoosmolar conditions substance P initiates and perpetuates waves of self-regenerating cortical spreading depolarization (CSD) in rat in vivo

*F. Richter¹, A. Eitner¹, J. Leuchtweis¹, R. Bauer², A. Ebersberger¹, A. Lehmenkühler³, H. G. Schaible¹

¹University Hospital Jena - Friedrich Schiller University, Institute of Physiology I / Neurophysiology, Jena, Germany

²University Hospital Jena - Friedrich Schiller University, Institute of Molecular Cell Biology, Jena, Germany

³Pain Institute and Center for Medical Education, Düsseldorf, Germany

Questions: The tachykinin substance P (SP) increases neuronal membrane excitability and is physiologically involved in homeostatic control. After stroke or brain trauma, however, SP participates in the development of brain edema and probably contributes to secondary damages. We asked whether SP is able to induce cortical spreading depolarization (CSD) and may induce primary brain damage.

Methods: We recorded in spontaneously breathing anesthetized adult rats (sodium thiopentone, 100 mg/kg, i.p.) CSD in cerebral cortex with two pairs of glass micropipettes (distance 5-6 mm) at depths of 400 and 1200 µm in two separated areas of the brain that were divided by a ring made from dental acrylic. Using microelectrodes CSD were recorded in a non-treatment and in the treatment area. Either SP dissolved in aqua at 10⁻⁵; 10⁻⁶; 10⁻⁷; 10⁻⁸ mol/L; or SP dissolved in artificial cerebrospinal fluid (ACSF) at 10⁻⁵ mol/L or aqua alone were



administered into the local cortical treatment area. To test for specificity, the treatment area was pretreated in some experiments with 250 nM of the NK-1 receptor antagonist (NK-1RA) L703.606 and SP was applied afterwards. Heart rate and systemic arterial blood pressure were continuously recorded. In a subgroup of rats also regional cerebral blood flow (rCBF) was measured. Plasma extravasation in cortical grey matter was assessed with Evans Blue.

Results: Only SP dissolved in aqua but not SP dissolved in ACSF induced series of self-regenerating CSD indicating that hypoosmolarity is a cofactor. Significant more self-recurring CSD were seen after 10^{-5} and 10^{-6} mol/L SP in aqua than after 10^{-7} and 10^{-8} mol/L SP in aqua. Aqua alone elicited as few CSD as the lowest concentration of SP (10^{-8} mol/L). The electrocorticogram confirmed that CSD induced by SP did not differ from those elicited by KCl and were real spreading depressions of electrocorticographic activity. Changes in rCBF induced by CSD had the same duration after SP and after KCl, but were smaller in amplitude after SP. A pretreatment with the NK-1RA prevented the initiation of CSD by SP. Plasma extravasation was observed after SP dissolved in aqua, but not after pretreatment with the NK-1RA or after topical application of aqua alone.

Conclusions: Topical application of SP dissolved in aqua to the cerebral cortex causes repetitive self-regenerating CSD waves in a dose-dependent manner. Higher doses of SP evoked more CSD waves overall and longer series of CSD waves than lower doses of SP. An additional triggering by a lowered osmolarity was necessary, since SP dissolved in ACSF at physiological osmolarities did not ignite CSD. The elicitation of CSD waves by SP is specifically linked to the functional NK-1R as it is the plasma extravasation in the superficial cortical layers in the cortical area that was superfused with SP. Thus, under hypoosmolar conditions SP is a candidate to elicit CSD and may contribute to secondary brain damage.

PI - 1-4

Gene Expression Levels in Acute Large Vessel Stroke in Humans

*S. Martha¹, L. Collier¹, S. Davis¹, A. Alhajeri¹, S. Grupke¹, J. Fraser¹, K. Pennypacker¹

¹University of Kentucky, Nursing, Lexington, United States

Introduction: In the setting of mechanical thrombectomy for emergent large vessel occlusion (ELVO), we have for the first time collected and evaluated blood immediately distal and proximal from the removed intracranial thrombus. These samples provide a unique resource in evaluating acute gene expression changes at the time of ischemic stroke. The purpose of this study was to evaluate gene expression changes occurring within the thrombus and across the occlusion in the intravascular space in acute ischemic stroke patients.

Methods: We developed the Blood and Clot Thrombectomy Registry and Collaboration (BACTRAC) protocol: an IRB-approved tissue banking strategy for ELVO (clinicaltrials.gov NCT03153683). We evaluated relative concentrations of gene expression in 84 inflammatory molecules in thrombi removed from adults who received thrombectomy for ischemic stroke, in static blood distal to thrombus, and in peripheral circulation.

Results: We analyzed the first 39 subjects (age = 67 ± 13.2 , 16 males) in the BACTRAC registry. Results from microarray analyses demonstrate that 21 genes (CCL1, CCL11, CCL13, CCL17, CCL26, CCL8, CSF3, CX3CL1, CXCL1, CXCL9, IFNA2, IL13, IL17C, IL17F, IL1A, IL27, IL3, IL33, LTA, TNFSF11, and TNFSF13) had at least 25 mean fold change in the distal blood compared to the peripheral blood. Fourteen genes (AIMP1, CCL11, CCL13, CCL15, CCL16, CCL23, CCR2, CCR4, CCR8, CD40LG, CXCL10, IL10RA, IL15, and TNFSF13B) had at least 10 mean fold change in the thrombus compared to the peripheral blood. Overall, these genes are associated with chemo-attraction and cell proliferation of monocytes, neutrophils, and T cells.

Conclusion: These findings provide a novel insight into the initial pathology of large vessel stroke in humans, particularly in regard to identifying acute gene expression changes that occur during stroke.



PI - 1-5

Assessment of a potential therapeutic strategy for neuroinflammation involving moderate hypothermia and cyclosporine A in an ischemia/reperfusion murine OHSC model

*S. Wowro¹, G. Tong¹, N. Ebermann¹, F. Berger¹, K. Schmitt¹

¹German Heart Center Berlin, Department of Congenital Heart Disease – Pediatric Cardiology, Berlin, Germany

Question: Ischemic stroke is a leading cause of death and disability worldwide that is exacerbated by limited treatment options (e.g. tPA), which is applicable only for a selective cohort. Therapeutic hypothermia (TH) has been established as a neuroprotective treatment for various acute brain injuries, but its efficacy in the treatment of ischemic stroke is still uncertain. Cyclosporine A (CsA), a calcineurin inhibitor and immunosuppressor, has also been shown in experimental as well as clinical studies to potentially be neuroprotective. However, Phase II studies with CsA for ischemic stroke showed no benefits. Therefore, we investigated the effects of TH alone and in combination with CsA on the neuroinflammatory response in a simulated ischemia/reperfusion injury model using murine organotypic hippocampal slice cultures (OHSCs).

Methods: Murine OHSCs isolated from P1-3 C57BL/6N mice were exposed to simulated ischemia (1h oxygen-glucose deprivation, OGD; 0.2% O₂) followed by 24h of simulated reperfusion (complete supplemented medium; 21% O₂) at 37 or 33.5 °C. CsA treatment group was preconditioned with 10 μM CsA for 1h before experimental start, and remained during OGD and reperfusion. Cytotoxicity was determined by glutamate and lactate dehydrogenase (LDH) releases and inflammatory cytokines and chemokine gene expressions were measured by RT-qPCR.

Result: OHSC exposed to 1h OGD resulted in necrotic cell death as indicated by a significant increase in glutamate release. Treatment with 10 μM CsA augmented the neurotoxic effect of OGD as observed in even higher levels of glutamate and LDH releases, but showed no neurotoxic effect in the non-injured normoxic group. 1h OGD followed by 24h reperfusion (OGD/R) resulted in an inflammatory response that was unexpectedly worsened by TH, as indicated by significant increases in IL-6 and MCP-1 expressions. Interestingly, IL-1β expression was significantly suppressed by cooling. Non-cooled OGD/R injured OHSC treated with CsA resulted in similar increases in IL-6, and significant higher TNF-α and MCP-1 expressions. Interestingly, the combined treatment of TH and CsA on the OGD/R injured OHSCs had an additive inflammatory effect, as observed in the highest levels of IL-1β, IL-6, and MCP-1 expressions.

Conclusion: Our results showed that moderate TH initiated after OGD has variable effects on inflammatory cytokines and chemokine expressions in a murine OHSC model. Cooling protectively attenuated IL-1β expression, but also increased IL-6 expression. Treatment with CsA also induced an inflammatory response that was even greater when applied in combination with TH. We did not observe any putative neuroprotective effects of CsA in our OGD/R-induced injury hippocampal slice culture model. In fact, the neuroinflammatory response was greatest when CsA was administered in combination with moderate hypothermia.

PI - 1-6

Opposite effects of neuroprotective cannabinoids, Palmitoylethanolamide and 2-Arachidonoylglycerol on function and morphology of microglia

*U. Grabiec¹, M. Pelzer¹, J. Kleine¹, T. Hohmann¹, F. Dehghani¹

¹MLU-Faculty of Medicine, Department of Anatomy and Cell Biology, Halle, Germany

Question: Various studies performed observed increased cannabinoid concentrations after stroke in in vitro and in vivo experiments showed that cannabinoids exert a neuroprotective effect. Furthermore, it is known that inflammation and neuronal damage are coupled and microglia cells are the main immunological effector cells within the brain. Treatment with cannabinoids, like N-arachidonoyl dopamine, 2-Arachidonoylglycerol (2-AG), Palmitoylethanolamide (PEA), led to a decrease in the number of damaged neurons after excitotoxicity lesion in organotypic hippocampal slice cultures (OHSC) and to alterations in the activation of microglial cells. While 2-



AG and PEA are shown to exert neuroprotective and anti-inflammatory properties, possible interactions (entourage effect) between those cannabinoids were not examined yet.

Methods: Excitotoxic lesioned OHSC were treated with PEA, 2-AG, the combinations of both and the number of damaged neurons was evaluated. To investigate the role of microglia on neuroprotection and the entourage effect, primary microglial cell cultures were treated with Lipopolysaccharide (LPS) and 2-AG, PEA and their combinations. Thereafter, NO production, ramification index, PPAR- α distribution and proliferation of microglial cells were measured.

Results: We found that the co-application of 2-AG and PEA did not increase the neuroprotective effect in the presence or in absence of microglia. PEA and 2-AG had contrary effects on ramification index and on NO production. No significant changes were observed in the proliferation rate of microglia.

Conclusions: Even though the two neuroprotective cannabinoids 2-AG and PEA have different targets, no entourage effect was observed. 2-AG and PEA exert contrary effects on morphology and function of microglia.

PI - 1-7

LINGO-1 directed siRNA nanoparticles promote central remyelination in ethidium bromide induced demyelination in rats

*O. Saed¹, A. E. Hassan¹

¹Faculty of medicine, Alexandria university, Medical physiology, Alexandria, Egypt

Questions: LINGO-1 inhibition showed promising results in enhancing remyelination. The impact of intranasal administration of LINGO-1 directed small interference RNA (siRNA) loaded chitosan nanoparticles on demyelination and remyelination processes has been investigated using the toxic ethidium bromide model of demyelination.

Methods: Sixty adult male Wistar rats were randomly assigned to one of 6 groups (n = 10 each) and subjected to stereotaxic intrapontine injection of ethidium bromide (EB) (n=40) or saline (n=20). EB injected rats were either remained untreated or received intranasal LINGO-1 directed siRNA-chitosan nanoparticles from day 1 to day 7 (demyelination group) or from day 7 to day 21 (remyelination group) after injection. All rats were subjected to behavioural testing and after scarification biochemical essays and histological examination of pontine tissues were done. (write name of some of estimated parameter) (results). Multiple variables were evaluated by one-way ANOVA test, followed by Post Hoc test (tukey). Results for beam balance and foot fault tests were analyzed by Kruskal-Wallis test followed by Mann Whitney U test for comparison.

Results: Evident histological features of demyelination were observed in pontine tissue and higher levels of caspase-3 activity were detected compared to control rats. With LINGO-1 directed siRNA- chitosan nanoparticles treatment, animals performed better than controls. Remyelination treated group showed better motor performance than demyelination group. MBP was significantly high when treatment was administrated for 2 weeks starting from 7th day compared to demyelination treated group or control (7.06 ± 1.10 versus 4.90 ± 0.32 and 1.09 ± 0.09 , $p = 0.000$).

Conclusion: The present study concluded that LINGO-1 directed siRNA loaded chitosan nanoparticles can improve neurological, neurochemical disturbance and enhance remyelination in EB-induced demyelination in a rat model. Intranasal way of administration of LINGO-1 directed siRNA nanoparticles appears to be an effective tool for drug delivery. It is worth mentioning that the current study revealed that the treatment regimen may exert great effect on the outcome. Improvement of biochemical parameters, motor performance and histopathological finding were significantly better when the treatment strategy is applied for 2 weeks in remyelination phase when compared to its usage as neuroprotective agent in demyelination phase.



PI - 1-8

Endothelial $\alpha 6 \beta 4$ integrin protects against experimental autoimmune encephalomyelitis by maintaining vascular integrity and tight junction protein expression

S. Halder¹, R. Kant¹, *R. Milner¹

¹The Scripps Research Institute, Molecular Medicine, La Jolla, United States

Background: Extracellular matrix (ECM) proteins play important functions regulating vascular formation and function. Laminin is a major component of the vascular basal lamina and transgenic mice deficient in astrocyte or pericyte laminin show defective blood-brain barrier (BBB) integrity, indicating an important instructive role for laminin in cerebral blood vessels. As we previously showed that in the normal brain, endothelial expression of the laminin receptor $\alpha 6 \beta 4$ integrin is predominantly restricted to arterioles, but induced on all vessels during neuroinflammation, the goal of this study was to define the role of this integrin in the maintenance of BBB integrity.

Methods: $\alpha 6 \beta 4$ integrin expression was analyzed using dual-immunofluorescence of brain sections taken from the experimental autoimmune encephalomyelitis (EAE) mouse model of multiple sclerosis. To investigate the role of endothelial $\alpha 6 \beta 4$ integrin, transgenic mice lacking endothelial expression of the $\beta 4$ integrin ($\beta 4$ -EC-KO) and wild-type (WT) littermates were subject to EAE, and clinical score and several neuropathological parameters examined by immunofluorescence. In vitro, $\beta 4$ integrin null brain endothelial cells (BECs) were examined for expression of tight junction proteins using immunocytochemistry and flow cytometry.

Results: EAE progression was associated with marked upregulation of cerebrovascular $\beta 4$ integrin expression, with the vast majority of blood vessels expressing $\beta 4$ integrin at the acute stage of EAE (day 21). While $\beta 4$ -EC-KO mice showed the same time of EAE onset as WT littermates, they developed significantly worse clinical disease over time, resulting in elevated clinical score at the peak of disease and maintained elevated thereafter. In keeping with this, the brains of $\beta 4$ -EC-KO mice showed enhanced leukocyte infiltration and BBB breakdown as well as increased loss of the endothelial tight junction proteins claudin-5 and ZO-1. Under pro-inflammatory conditions, $\beta 4$ KO BECs in vitro also showed increased loss of claudin-5 and ZO-1 expression.

Conclusions: Our findings support the concept that $\alpha 6 \beta 4$ integrin upregulation is an inducible protective mechanism that stabilizes the BBB under neuroinflammatory conditions.

PI - 1-9

Influence LPS-induced inflammation to disruption blood-brain barrier in mice

*K. Astakhova¹

¹Lomonosov MSU, Moscow, Russian Federation

The inflammation accompanies chronic and acute processes such as neurodegenerative diseases and ischemic injury in the organism. Proinflammatory cytokines can not only increase the tissue permeability but also recruit cells, inducing secondary reactions and developing inflammation. The mechanism of spreading inflammation and influence inflammatory response on distant organs is not clear. In this research, we investigated lipopolysaccharide (LPS, E.coli O111:B4) – induced inflammation and disruption blood-brain barrier on BALB/c mice.

All experiments were performed on 2,5-3 month-old male mice of BALB/c. The weight of animals was 25-30 g. Different doses of LPS (1.6, 3.2, 4.8, 6.4 mg/kg) were injected animals of four experimental groups, respectively. Control group received a physiological solution. Leucocyte level and different types of cells were determined at 4, 24 and 72 hours after LPS-induced inflammation by hematology analyzer and light microscopy. Tissue permeability was estimated by Evans Blue at 24, 72 and 96 hours after the injection of LPS 3.2 mg/kg. BBB-permeability was investigated in following structures of a brain: hemispheres, cerebellum and brain stem. All data were analyzed by GraphPad Prism 5.1, two-way ANOVA test.



The administration of LPS increased a permeability of all biological barriers. It depends not only on the dose of LPS but also on the exposure time. The most pronounced inflammatory response was obtained at 24 hours after LPS-injection in the dose of 3.2 mg/kg. At this time point, total number and the ratio of leucocytes types were statistically significant changed relatively to control mice. We identified appearance immature neutrophils in peripheral blood. This fact can be associated with active infiltration mature white blood cells to tissues with inflammation. Moreover, proinflammatory cytokines migration to the neurons and glia and the following of neuroinflammation were can induced by the increase of tissue and BBB permeability.

Accordingly, the stabilization of tissue barriers through reduction damaged vascular endothelium may be another target of complex neuroinflammation therapy.

This work was supported partly by a grant (Mol_A, 18-34-00977) from the Pirogov Russian National Research Medical University in 2018.

P I - 1-10

Adaptive immune cell activation in acute pediatric traumatic brain injury

*S. Ortega¹, V. O. Torres¹, F. Tian², J. Windsor¹, K. Poinatte¹, J. Tweed³, C. Greenwell³, D. Miles⁴, L. Raman⁴, A. Stowe¹

¹UT Southwestern Medical Center, Neurology and Neurotherapeutics, Dallas, United States

²UT Arlington, Arlington, United States

³Children's Health, Dallas, United States

⁴UT Southwestern Medical Center, Pediatrics, Dallas, United States

Question: Traumatic brain injury (TBI) arises in two phases: the first is caused by mechanical injury to the brain resulting in tissue shearing, bleeding, and cerebral ischemia, and a secondary phase mediated by excitotoxicity, edema, delayed axonal injury, and neuroinflammation. In fact, chronic inflammation following TBI predisposes individuals to other neurological diseases that are particularly relevant for pediatric TBI (pTBI). Clinical studies investigating neuroinflammation are limited and rarely focus on adaptive immune responses in pTBI patients.

Methods: Pediatric TBI patients (1-18 yrs.) and controls (0-18 yrs., no CNS injury, n=10) were recruited according to approved IRB protocol. Blood and cerebral spinal fluid were collected at days 1, 3 (both n=14) and day 5 (n=13) following TBI and placement of an external ventricular drain. Peripheral blood immune cells were enumerated using fluorescent antibodies and flow cytometry. Statistical analysis used Kruskal-Wallis non-parametric one-way ANOVA for comparison between pTBI and controls, while Friedman non-parametric one-way ANOVA was used for between day comparisons. Neuronal autoreactivity was evaluated by labeling peripheral blood mononuclear cells with carboxyfluorescein succinimidyl ester (CFSE) and culturing with neuronal and control antigens. Response to antigen was determined by dilution of CFSE (proliferation) and expression of CD25 (activation).

Results: pTBI patients exhibited a higher quantity of leukocytes (CD45⁺) relative to controls (p=0.01) on day 1 post-TBI, indicating an immediate and robust immune modulation due to CNS injury, that decreased at days 3 and 5. Major subsets of adaptive immune cells (CD3, CD19, CD4, and CD8) did not exhibit altered cellularity relative to controls but activated adaptive T cells (CD4⁺CD161⁺ and CD8⁺CD161⁺) were substantially higher at day 1 (p=0.01 and p=0.03, respectively). Autoreactivity analysis of pTBI patients (n=3) revealed a CD4 T cell response to various CNS-derived antigens (e.g. MAP2, PLP, β -amyloid) which is absent in controls (n=3). Interestingly, the CD8 T-cell analysis revealed CNS-specific autoimmune responses absent in pTBI patients which were present in controls.

Conclusions: The observable differences within the CD45⁺, CD4⁺CD161⁺ and CD8⁺CD161⁺ cell populations showed alterations in cellularity that coincided with CNS injury. This may suggest alterations in cellular tracking as CNS injury evolves. Detection of CNS-specific CD4 T-cells and loss of CNS-specific CD8 T-cells suggest specific activation of adaptive immune cells which may have reciprocal roles in brain injury and/or repair. Based on these initial observations, we hypothesize that the adaptive immune system is activated upon TBI and can potentially



target CNS resident cells in young patients. Thus, these data warrant further investigation into the role of the adaptive immune system after brain injury and its potential role in immunotherapeutic targeting.

P II - 1-11

Impact of NKG2D signaling on T cell and NK cell function in cerebral ischemia

*C. David¹, T. Ruck², S. Menci¹, F. Langhauser¹, S. Meuth², C. Kleinschnitz¹

¹University Hospital Essen, Department of Neurology, Essen, Germany

²University Hospital Münster, Department of Neurology, Münster, Germany

Questions: Stroke is the second leading cause of death worldwide. However, therapeutic strategies are currently limited to pharmacological and mechanical recanalization therapy and past translational research efforts did not proceed from preclinical stage to clinical practice. Therefore, only a deeper understanding of the pathophysiology will help to identify new treatment targets. Post-stroke inflammation has been recognized for many years and T cells as well as NK (natural killer) cells have been implicated as drivers of the inflammatory responses. NK immunoreceptors expressed on both NK and T cells might be involved in stroke development. NKG2D is a member of the c-type lectin NK immunoreceptors and previous studies demonstrated an important role of NKG2D signaling in autoimmune disorders such as multiple sclerosis as well as in cardiac and renal ischemia reperfusion injury. In this study, we aimed to elucidate a potential contribution of NKG2D signaling to stroke pathogenesis.

Methods: Wildtype (WT) mice underwent transient middle cerebral artery occlusion (tMCAO). NK cell infiltration was characterized in the brain 1, 3 and 7 days after stroke by histological analyses and flow cytometry. Further fluorescence stainings allowed the identification of NK cells in human brain tissue. Moreover, WT mice were prophylactically or therapeutically treated with an anti-NKG2D blocking antibody or the respective isotype after tMCAO. Infarct volume and animal survival as well as the extent of immune cell infiltration and NKG2D ligand MULT-1 expression in the brain were measured by flow cytometry and qPCR 24h or 72h after stroke surgery. Furthermore, we investigated the impact of different immune cell subsets in an animal model lacking mature T and B cells (*Rag*^{-/-}) by determining absolute numbers of lymph node cells relative to WT mice.

Results: Preliminary data showed NK cell infiltration in both murine and human brain after stroke, demonstrating a role of NK cells in post-stroke inflammation. Prophylactic and therapeutic blockade of NK cell signaling via NKG2D significantly reduced infarct volumes compared to isotype controls 24h after tMCAO. The effects were even more pronounced 72h after stroke and were paralleled by improved survival. Moreover, expression of MULT-1 was highly increased in the cortices ipsilateral to the stroke indicating facilitated NKG2D-NKG2D ligand interactions. Interestingly, MULT-1 expression and the number of brain infiltrating immune cells were significantly reduced after pharmacological depletion of NK signaling. Furthermore, we found reduced absolute peripheral immune cell numbers in *Rag1*^{-/-} mice due to the lack of functional $\alpha\beta$, $\gamma\delta$ - and NK-T as well as B cells.

Conclusion: Our findings provide evidence for an important role of the NKG2D signaling pathway in early stroke development and might hint towards a new therapeutic target in stroke therapy. Preliminary data strengthen the relevance of NK cells in human disease.

P II - 1-12

Effect of spinal cord root compression on pain sensitivity in white rats

*A. Semina¹

¹Moscow State University, Moscow, Russian Federation

According to epidemiological studies, up to 64% of the human population suffer from various types of pain. The chronic pain syndrome creates greatest danger and problem, whereas it continues for a long period of time even



after the cause is eliminated. Neuropathic pain is a pain that occurs because of direct damage or disease of the somatosensory system. Chronic neuropathic pain accompanies a number of diseases (diabetes, infectious diseases, rheumatoid arthritis, etc.) and are potentiated by inflammatory processes and violation of the blood-brain barrier. Radicular (compression) syndrome - one of the most common neurological syndromes - which are due to a compression of the nerve roots of the spinal cord. It is characterized by acute pain that dramatically reduces the quality of life and can lead to disability of patients.

The aim of this work was to study the state of pain sensitivity in the post-compression period in rats with radicular syndrome.

Experiments were conducted on Wistar rats weighing 250-300 g. The experimental group included rats with the hemilaminectomy of 6-7 cervical vertebrae and the compression of a right sensitive C7 nerve root of the spinal cord. Animals of the control group was subjected hemilaminectomy; without of a nerve root compression. The pain sensitivity was measured by the compression of the front paw with the help of algometer UgoBasil on day 1, 2, 4 and 7 after surgery.

The study found that the 15-minute compression of the nerve root in rats led to the significant increase the pain sensitivity (by 10.8%) at 24 hours of the compression. These data point to the development of hyperalgesia only on the ipsilateral limb. The high pain sensitivity was maintained at a reliable level in relation to the baseline (before surgery) up to 48 hours. Pain sensitivity was restored to the initial values on the 7th day after the compression of nerve root. Pain sensitivity of the contralateral limb had no significant differences in relation to the initial level throughout the study.

Thus, 15-minute compression of the nerve root of the spinal nerve leads to the development of hyperalgesia during the next 48 hours, with the restoration of normal pain sensitivity on the 7th day. Further study of the mechanism of pain sensitivity changes at the damage of the nerve roots will reveal new ways of relief of pain at this type of neurological disease.

P II - 1-13

Study of the effect of co-application of the *E. Coli* lipopolisaccharide and toxic doses of glutamate to the cultured neurons

A. Surin¹, Z. Bakaeva^{2,3}, *N. Lizunova⁴, V. Pinelis², I. Krasilnikova², L. Gorbacheva^{4,5}, D. Boyarkin², I. Tarzhanov³

¹Institute of General Pathology and Pathophysiology, Moscow, Russian Federation

²FSAI "National Medical Research Center of Children's Health" Russian Ministry of Health, Laboratory of neurobiology, Moscow, Russian Federation

³Peoples' Friendship University of Russia, Institute of Medicine, Moscow, Russian Federation

⁴M.V. Lomonosov Moscow State University, Faculty of biology, Moscow, Russian Federation

⁵N. I. Pirogov Russian National Research Medical University, Moscow, Russian Federation

Questions: The effects of bacterial endotoxin (*E. Coli* lipopolysaccharide, LPS) and LPS in combination with overstimulation of glutamate receptors on intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$), mitochondrial potential (MP) and cell survival in mixed primary neuroglial cultures from the cerebellum and the cerebral cortex of the rat (9-12 DIV) were studied.

Methods: Primary cultures of cortical or granular neurons were prepared from the cortex 1-2 day old or from the cerebellum of 6-7-day old Wistar rats. Fluorescence microscopy measurements were performed employing inverted microscope. For single cell $[Ca^{2+}]_i$ and MP detection, neurons were loaded with Fura-FF and Rh123, respectively. The survival of cultured neurons subjected to Glu and LPS was studied using vital fluorescent dyes and MTT-test.



Results: In cultured neurons from the cerebral cortex and cerebellum LPS (0.1, 1.0 and 10 $\mu\text{g/ml}$) did not affect the level of resting $[\text{Ca}^{2+}]_i$ and did not increase the proportion of neurons, in which glutamate (Glu, 33 and 100 μM , 10 μM Glu, 0 Mg^{2+}) induced delayed Ca^{2+} deregulation (DCD) and synchronous with DCD a strong decrease in MP. It was found that in cortical neurons the disturbance of Ca^{2+} homeostasis occurs at Glu concentrations lower than in granular neurons. 15 min pre-incubation with LPS did not affect the synchronicity of $[\text{Ca}^{2+}]_i$ changes and MP during the development of DCD, but the presence of LPS (1-10 $\mu\text{g/ml}$) slowed the recovery of $[\text{Ca}^{2+}]_i$ following Glu wash out only in cortical neurons. We have revealed that LPS (10 $\mu\text{g/ml}$) reduced by $10\pm 4\%$ the increase in oxygen consumption induced by Glu. The cause may be a disruption in the spatial structure of ion channels and transporters as a result of the amphiphilic LPS introduction into plasma membrane. Evaluation of the cell survival demonstrated that LPS and Glu individually reduced the survival of neurons in the primary culture from the cortex of the rat brain by $18\pm 5\%$ and $30\pm 4\%$, respectively. We did not observe synergism or additivity of toxic effects of LPS and Glu during their simultaneous application.

Conclusions: The results showed that under conditions of a complex system of neuroglial culture, the effects of LPS on Ca^{2+} homeostasis and cell death during excitotoxicity can be masked by the combination of different mechanisms of Ca^{2+} entry to and removal from the cytosol. Modulation by LPS of Glu-induced Ca^{2+} deregulation is, apparently, not dominant in the subsequent excitotoxic cell death.

The work was supported by grants of RFBR 16-04-00792, 17-00-00106, and grant of RSF 17-15-01487.

P II - 1-14

The influence of PAR1 agonists on the development of glutamate-induced toxicity

*I. Babkina^{1,2}, T. Molchanova², A. Surin³, L. Gorbacheva^{1,2}

¹Pirogov Russian National Research Medical University, Medicobiologic faculty, Moscow, Russian Federation

²Lomonosov Moscow State University, Faculty of Biology, Moscow, Russian Federation

³National medical research center for children's health, Laboratory of Neurobiology and the fundamentals of brain activity, Moscow, Russian Federation

Brain ischemia and neurodegenerative diseases accompany the key damage factor – glutamate(Glu)-induced toxicity, which leads to increasing of cytoplasm calcium and cell death. NMDA and AMPA receptors are involved in this type of toxicity and can be modulated by serine proteases of hemostasis.

The aim of this study was to investigate the protective actions of PAR1-agonists at Glu toxicity. Experiments were performed on cortical neurons from rat pups. The survival of cells was analyzed by MTT test. Excitotoxicity was modulated by the 1 hour-incubation of cells with Glu 100 μM . PAR1 were blocked by SCH79797. The cell calcium was loaded with FURA-2AM.

It was shown the activation of AMPA receptors causes pronounced cell death, in contrast to NMDA. At the same time, MK801 (antagonist of NMDA) decreased the Glu-induced cell death. At the first time we found that the new peptide(NPNDKYEPF amide, Pep9) the analogue of tethered ligand released at the hydrolysis of PAR1 by APC, prevented the neuronal death at Glu-toxicity, like APC. PAR1 was required for protective effect both Pep9 and APC.

We found that the development of a secondary calcium response typical for the Glu-application and mitochondrial depolarization were observed. To wash cells with a free-calcium solution did not cause the restoration of the basal calcium, it indicates a high sensitivity of cortical neurons to Glu and the development of a long-term secondary rise of calcium. The application of Thr(thrombin) 10 nM caused a transient rise in calcium, which subsequently returned to the basal level. At the same time, APC and Pep9 did not change calcium. It is possible that these PAR1-agonists lead to the initiation of various intracellular cascades.

The pretreatment cells with PAR1-agonists did not change the neuron number with calcium dysregulation at Glu. However, APC and Pep9 significantly increased the percentage of neurons that restored the basal level of calcium



after the Glu. Thr also increased the neuron number that restored the basal level of calcium, however, significant differences were not found.

NMDA led to a restoration of the calcium basal level in neurons, in contrast to the application of Glu. Thus, the irreversibility of Glu-induced calcium dysregulation is most likely due to AMPA or metabotropic receptors.

The estimation of intracellular calcium kinetics pointed to the absence of differences in the amplitude of the NMDA-induced calcium response in all groups of cells. At the incubation cultures with Pep9, the cell number restoring basal calcium was increase. At the same time, Thr enhanced the toxicity of NMDA and stabilized and lengthened the plateau phase. These data indicate the realization of "biased agonism" as the mechanism of Thr and Pep9 effects.

Thus, the protective effect of APC and Pep9 on cultured rat neurons at Glu excitotoxicity realized via stabilization of intracellular calcium and mediated by PAR1.

The work was supported by RFFR 16-04-01869,16-04-00792

P II - 1-15

Pentraxin-3 is expressed in different brain cells with a specific temporal pattern after traumatic brain injury.

*S. Ippati¹, M. Oggioni¹, D. De Blasio¹, S. Fumagalli¹, C. Perego¹, D. Mercurio¹, D. Minuta¹, M. G. De Simoni¹

¹Mario Negri Institute for Pharmacological Research, Neuroscience, Milano (MI), Italy

Questions: Pentraxin-3 is a pattern recognition molecule belonging to the family of long pentraxins involved in the humoral immunological response. Pentraxin-3 is known to interact with complement components and regulators including mannose-binding lectin and factor H. Pentraxin-3 is up-regulated following pro-inflammatory stimuli and may be involved in central nervous system diseases, however its role in traumatic brain injury is still unexplored. We investigated brain localization of pentraxin-3 over time in a mouse model of traumatic brain injury. We also evaluated pentraxin-3 interaction with brain cells and mannose-binding lectin deposition in the injured brain.

Methods: Male C57Bl/6 mice underwent sham surgery or controlled cortical impact brain injury (velocity: 5 m/s; depth: 1mm). Pentraxin-3 presence was assessed by immunofluorescence at 30 min, 24, 48 hours, 4 days, 1 week and 4 weeks after injury. Immunostaining for pentraxin-3 was quantified by segmentation of stained area using Fiji software. Pentraxin-3 co-localization or co-expression with astrocytes, oligodendrocytes precursors and neutrophils was assessed by confocal analysis. Pentraxin-3 relationship with the lectin pathway initiator mannose-binding lectin-C deposited on injured brain vessels was also investigated.

Results: Pentraxin-3 appeared in traumatized brains starting from 30 min after injury and progressively increased up to 1 week. At 4 weeks pentraxin-3 was still present in the injured area, although to a lower extent compared to 1 week. Co-localization studies in the injured area revealed that pentraxin-3 was expressed by oligodendrocyte precursors at every time points after trauma. Furthermore at 24h and 48h pentraxin-3 co-localized with neutrophil azurophilic granules, while at 4 days and at 1 week pentraxin-3 was mostly expressed by branched astrocytes. Lastly, at 4 days and 1 week after trauma co-presence of pentraxin-3 and mannose-binding lectin-C deposited on brain vessels was found in the injured area.

Conclusions: Pentraxin-3 is expressed in the perilesional cortical area early after traumatic brain injury and increases over time up to the sub-acute phase after injury. Pentraxin-3 is expressed by different brain cells, such as oligodendrocyte precursors, neutrophils and astrocytes, and interestingly in each of them with a specific temporal pattern. Also, Pentraxin-3 and mannose-binding lectin-C deposition on injured vessels appear to be closely related without co-localization. These results support the hypothesis that brain cell-produced pentraxin-3 may be implicated in the neuropathology of traumatic brain injury.



P II - 1-16

Weight-drop model of TBI results in more severe outcomes in animals with skull fracture

*G. Vikmane^{1,2}, E. Vavers^{1,3}, B. Zvejniece^{1,4}, E. Kupats³, L. Zvejniece¹, M. Dambrova^{1,3}

¹Latvian Institute of Organic Synthesis, Riga, Latvia

²Latvia University of Life Sciences and Technologies, Jelgava, Latvia

³Riga Stradins University, Riga, Latvia

⁴Latvia Pauls Stradins Clinical University Hospital, Riga, Latvia

Traumatic brain injury (TBI) is one of the leading causes of mortality and morbidity in people under the age of 45 years. According to the World Health Organization data, TBI will be the major cause of death in 2020. The weight-drop model is the most commonly used experimental method to advance the understanding of the pathophysiology of closed head injury. Previous results in the weight-drop model have provided evidence of more than 30% incidence of skull fractures. The aim of the present study was to evaluate and compare outcomes after a different severity of head injury with or without skull fracture in TBI model.

Swiss Webster (SW) male mice, weighing 28–40 g were included in this study. 2 mm and 5 mm diameter Teflon-tipped cones were used to induce TBI and an incidence of skull fracture and NSS was compared. The neurobehavioral status was assessed by the NSS 2 and 24 h after TBI. To measure skull thickness computed tomography scans were performed. Neuroinflammation related gene expression (interleukin (IL)-6, IL-1 β , tumor necrosis factor (TNF)- α and tissue inhibitor of metalloproteinases (TIMP)-1) was measured by quantitative RT-PCR analysis in the hippocampus and striatum 12 h, and 1, 3 and 14 days after TBI.

Parietal bone fractures occurred in 10% of animals using a 5 mm tip cone, while the 2 mm tip cone induced fractures in 33% of cases. 2 and 24 h after TBI the NSS was significantly more elevated in TBI than in sham-operated mice. There was a significant difference of NSS between animals with and without skull fractures 24 h after TBI. Skull thickness correlated with the age of the animals; moreover, a thicker parietal bone was associated with a decreased risk of fractures. 12 h after injury induced significant 4-, 20- and 125-fold increase in IL-6, TNF- α and TIMP-1 gene expression, respectively, in ipsilateral hippocampus. And a 6-fold increase in TNF- α gene expression was found in ipsilateral striatum in animals with the skull fracture. The significant 2-fold increase in TNF- α gene expression was observed in the ipsilateral striatum but not in the hippocampus of animals without skull fracture 12h after TBI.

TBI with skull fracture resulted in more severe neurobehavioural response and considerable increase of inflammatory process-related gene expression in brain tissue. After weight-drop TBI experiments, data obtained from animals with skull fractures must be analysed separately from those without skull fractures. To produce a homogenous type of injury and more reproducible NSS results, a cone with at least 5 mm tip diameter and animals with the same age should be used.

Acknowledgements This study was supported by the framework of EU-ERA-NET NEURON project CnsAflame.

P II - 1-17

Experimental stroke differentially affects discrete subpopulations of splenic macrophages

*L. McCulloch¹, S. Bui¹, A. Alfieri², A. Harris¹, B. McColl¹

¹UK Dementia Research Institute, University of Edinburgh, Centre for Discovery Brain Sciences, Edinburgh, United Kingdom

²University of Edinburgh, Centre for Discovery Brain Sciences, Edinburgh, United Kingdom

Introduction: Following stroke, there is a systemic deactivation of monocytes/ macrophages that may contribute to immunosuppression and the high incidence of bacterial infection experienced by stroke patients. The manipulation of macrophage subsets may be a useful therapeutic strategy to reduce infection and improve



outcome in patients. The spleen is the largest natural reservoir of immune cells, many of which are mobilised to the site of injury after stroke and is notable for the diversity of its functionally distinct macrophage subpopulations. We examined the effects of experimental stroke on splenic macrophages at a subpopulation specific level. Furthermore, as multiple stimuli are present in the spleen after experimental stroke, we would like to determine which of these factors are responsible for stroke-induced changes to macrophages that result in these functional deficits.

Methods: Splenic macrophages were profiled by immunohistochemistry (IHC) and flow cytometry 1-7 d after 40 min transient occlusion of the middle cerebral artery using a transluminal filament. Microarray analysis of whole spleen RNA identified gene expression changes then validated by qPCR and IHC. Bone-marrow-derived macrophages were stimulated *in-vitro* with LPS and noradrenaline to determine if this recapitulated phenotypic changes seen *in-vivo* after experimental stroke

Results: Red pulp (RP) and marginal zone macrophages (MZM) showed increases in density and altered micro-anatomical location after experimental stroke. These changes were not due to increased recruitment of precursors from the bone marrow. Genes associated with phagocytosis and proteolytic processing were upregulated in the spleen after stroke with increased expression of the lysosome-associated protein, LAMP2, specifically in RP and MZM subsets. In contrast, MHC class II expression was reduced specifically in these populations. Furthermore, genes associated with macrophage ability to communicate with other immune cells, such as co-stimulatory molecules and inflammatory cytokine production, were also downregulated in the spleen. Stimulation of bone-marrow derived macrophages with LPS and noradrenaline *in-vitro* did not alter macrophage expression of MHC Class II but did result in reduced pro-inflammatory cytokine production. This suggests that macrophage phenotype characterised may be a cumulative effect of multiple stimuli present in the spleen after stroke.

Conclusions: These findings suggest that selective splenic macrophage functions could be impaired after stroke and the contribution of macrophages to stroke-associated pathology and infectious complications should be considered at a subset-specific level. Furthermore, multiple signalling pathways may contribute to the overall stroke-induced changes to macrophage phenotype. Further understanding of these mechanisms are required before optimal therapeutic manipulation of macrophages to improve stroke outcome can be achieved.

P II - 1-19

Spinal neural progenitor cells reduce inflammation after spinal cord injury via modulation of NFkB pathway

*K. Kárová¹, A. G. Amin², M. Jhanwar-Uniyal², P. Jendelova¹

¹Institute of Experimental Medicine, Academy of Sciences of the Czech Republic, Prague, Czech Republic

²New York Medical College, New York, Germany

Traumatic spinal cord injury (SCI) is marked by gliosis which is in part a result of local enhanced production of pro-inflammatory cytokines, resulting in exacerbated state of injury. Activation of nuclear factor- κ B (NF κ B) signaling pathway has been shown to be associated with inflammatory response induced by SCI. Here, we elucidate the pattern of activation of NF κ B in the pathology of SCI in rats, and investigate the effect of transplantation of spinal neural precursors (SPC-01) on its activity. In addition, levels of secretory TNF α and extent of glial scarring were estimated following SCI. Immunohistochemistry revealed that the activity of NF κ B pathway displays two peaks, at 3 and 28 days after SCI, with highest levels of p65 nuclear translocation observed at 28 days. Transplantation of SPC-01 cells resulted in reduction of secretory TNF α levels at 10 and 14 days after injury (3 and 7 days after transplantation), as determined by Multiplex analysis. In addition, SPC-01 cells transplantation significantly decreased p65 NF κ B nuclear levels at day 28 after injury, mainly in the gray matter along with strong attenuation of glial scarring. The results of this study demonstrated that SCI induced a distinct pattern of activation of NF κ B signaling pathway, which might play a crucial role not only in the inflammatory response that follows primary injury, but also in astrocyte reactivity. The therapeutic benefit of SPC-01 cell transplantation might be due to its effect on modulating TNF α /NF κ B signaling pathway and in turn inhibiting glial scar formation.



Supported by InterAction LTAUSA 17120, LOI 1309 and GACR304/12/G069.

P II - 1-20

Nerve Growth factor (NGF) and dehydroepiandrosterone (DHEA) attenuate microglia-mediated neuroinflammation

*G. Fodelianaki¹, F. Lansing¹, P. Bhattarai¹, M. Troullinaki¹, A. Neuwirth¹, C. Mund¹, S. Grossklaus¹, A. Gravanis², I. Charalampopoulos², C. Tsatsanis³, V. Alexaki¹

¹Institute for Clinical Chemistry and Laboratory Medicine, University Clinic Carl Gustav Carus, TU Dresden, Dresden, Germany

²Medical School, University of Crete, Department of Pharmacology, Heraklion, Greece

³Medical School, University of Crete, Department of Clinical Chemistry, Heraklion, Greece

Neuroinflammation is a pathophysiological hallmark of many neurodegenerative diseases. Microglia, the parenchymal immune cells of the central nervous system, play a critical role in neuroinflammation. In response to infection or damage they drive inflammation and tissue repair. However, aberration in microglial responses is a common feature of neurodegenerative diseases and critically contributes to disease progression. Thus, it is important to elucidate how microglia-mediated neuroinflammation is regulated by endogenous factors. Here, we explored the effect of Nerve Growth Factor (NGF), an abundant neurotrophin, on microglial inflammatory responses. NGF downregulated LPS-induced production of pro-inflammatory cytokines and NO in mouse microglial cells, acting through its high affinity receptor TrkA and acutely inhibited TLR4-mediated activation of the NF κ B and JNK pathways. Interestingly, NGF altered the LPS-mediated metabolic reprogramming in microglial cells reducing glycolysis. In accordance, the neurosteroid dehydroepiandrosterone (DHEA) exerted similar anti-inflammatory effects in microglia acting through TrkA. Studies using CX3CR1CreERTkAfl/fl mice subjected to acute LPS-induced neuroinflammation indicated that endogenous NGF activity may not be sufficient to influence LPS-mediated microglial inflammatory responses. However, systemic administration of DHEA attenuated LPS-induced microglial inflammation. Collectively, our data indicate that NGF and DHEA are potent regulators of microglial inflammatory responses, thereby providing the platform for potential future therapeutic interventions in neuroinflammatory pathologies.