SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Investigation of proinflammatory gene-and protein expression within the superficial spinal dorsal horn upon CFA induced chronic inflammation

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The Examination takes place at UD, Department of Anesthesiology and Intensive Care, training room, at 10.00, 2023.01.18.

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The PhD Defense takes takes place at UD, Department of Emergency Care and Oxyology, lecture room, at 12.00, 2023.01.18.

1. INTRODUCTION

1.1 Chronic inflammation

Chronic inflammation places an extra burden on the physical and mental well-being, and considerably impairs the quality of life. The reduction or loss of work ability and the increased use of health care affect the entire society. Based on the present categories, the nature of chronic pain may be nociceptive, neuropathic, or nociplastic. Prior to nociceptive pain (somatic or visceral) mechanical, chemical and thermal stimuli activate high threshold receptors, which convey signals into the central nervous system. Conversely, the main cause of neuropathic pain is either the injury of the signaling system, or in the wider context the impair of the somatosensory system. During nociplastic pain the signal processing is undoubtedly altered, however its mechanism remains unknown without tissue damage. The proinflammatory cytokines, which exert their effects on the entire extent of neuraxis, are crucial mediators of nociceptive and neuropathic pain. The inflammasomal system also contributes to the production of certain amount of cytokines.

1.2 Neuron-glia interaction, the tripartite synapse

Non-excitable glial cells (which were formerly deemed by researchers as passive providers of neuronal homeostasis) play a vital role in the establishment and maintenance of pain. Glial cells can be activated with numerous nociceptive stimuli, during which their number, morphology and function are markedly altered. The elements of the signal pathways are mitogen-activated protein kinases (MAP kinases), extracellular regulated kinases (ERK), p38 kinases, c-jun N-terminal kinase (JNK), which may regulate the synthesis of several mediators including proinflammatory cytokines.

Beyond the traditional pre-and postsynaptic members, neuron-glia interactions also play an important role in central sensitisation, hence glial cells are able to process synaptic information and contribute to neuronal plasticity. Their operations may perpetuate the excitatory tone by producing proinflammatory cytokines via positive feedback regulation. In addition, the reuptake of glutamate may be reduced in astrocytes resulting in a long-term excitatory synaptic tone.

1.3 Significance of cytokines

The term cytokine is a collecting name, which involves lymphokines, monokines, and chemokines as well. Based on the distance of their effects on cells, autocrine, paracrin or endocrine mechanisms are distinguished. The cytokine effects are oftentimes divergent and redundant. Their production occurs with a cascade-like mechanism, in which cytokine stimulated target cells secrete new cytokines. Cytokine secretion can be induced even in the nervous system, including spinal cord and dorsal root ganglion, their presence is necessary for cell growth and tissue regeneration, furthermore they are involved in immune responses against infections and tissue injuries.

One of the main groups, the proinflammatory cytokines attract immune cells to the site of injury or infection at early stage of the immune response. These proteins may induce robust receptor-ligand interactions, fewer than ten receptor activation may be enough to evoke physiological response. The reaction formed can be extremely dangerous not entirely because of the pathogenes, but the potential damage of immune system by the so-called cytokine storm. Cytokine storm can be induced mainly by pathogenic viruses and bacteria, as they shift the balance from normal inflammatory response towards potentially damaging response of the host, which has a severe aftermath.

1.4 The IL-1β and its receptor the IL-1R1

The increased production of the interleukin-1β (IL-1β) as the member of the proinflammatory cytokines indicates pathological processes. Peripheral injection of IL-1β provokes marked mechanical and thermal hyperalgesia potentiating the effects of other proinflammatory mediators.

The administration of IL-1R1 antagonist (IL-1ra, Anakinra), which acts on the interleukin-1 receptor-1 (IL-1R1), significantly decreases the mechanical hyperalgesia following intraplantar injection of complete Freund adjuvant (CFA). IL-1β is present not only in peripheral tissues, but brain and spinal cord. Its intracerebroventricular injection evokes thermal hyperalgesia, intrathecal application leads to mechanical allodynia and hyperalgesia. IL-1β directly enhances the neuronal excitability via modulation of transient receptor potential channel (subfamily V, TRPV1), voltage-gated

sodium channels, gamma-amino-butyric acid (GABA) and N-metil-D-aspartate (NMDA) receptors. The ionotropic NMDA receptors have a highlighted role in the activity dependent synaptic plasticity and chronic pain.

The colocalisation of NMDA receptor NMDA receptor 1 (NR1) subunit and the IL-1R1 can be detected in neurons. In injury induced central sensitisation proinflammatory cytokines including IL-1β secreted by activated spinal glial cells contribute to the maintenance of chronic pain. The cytokine was investigated in several neuropathic pain models, increased protein expression was observed in dorsal root ganglion as well as in spinal cord.

The IL-1R1 acts as the receptor of IL-1β, IL-1α and IL-1ra ligands. An other protein the IL-1R3 (formerly known as IL-1R accessory protein) functions as a coreceptor, with the ligand binding unit and its ligands (IL-1α, IL-1β) it forms a trimeric signal complex, which iniates signal cascade. Binding of IL-1ra prevents the interaction between the receptor and coreceptor, therefore the signal transduction can not commence. Key elements of the trimeric complex are the TIR domain dimerisation of IL-1R1 és az IL-1R3, which recruit the myeloid differentiation primary response 88 (MYD88), the Toll-interaktive protein (TOLLIP), and the IL-1 receptor associated kinase 4 (IRAK4) adaptor proteins. The connection of MYD88 iniates the phosphorylation of IRAK4, IRAK2 and IRAK1, which result in the recruitment and oligomerisation of tumor necrosis factor associated factor 6 (TRAF6). The TRAF6 together with IRAK1 and IRAK2 proteins associate with TGF-β activated kinase 1 (TAK1) enzyme and its binding proteins (TAK binding protein 2 and 3, TAB2 and TAB3). Thereafter, TRAF6 undergoes ubiquitination, and the TAK1 protein will be phosphorylated. Following phosphorilation the signaling divides into two main pathways.

At one of the pathways TAK1 activates nuclear factor kappa β (NFκB) beta subunit inhibitor (IKKb), which subsequently phosphorylates NFκB inhibitor (IkB), resulting in the degradation of this protein, hence (NFκB) freely translocates into the cell nucleus to iniate transcription of target genes.

The other signaling pathway recruits MAPK-kinase (MKK) proteins, whose substrates are the MAPK, JNK and ERK enzymes. These enzymes act on c-Jun, c-Fos, c-Myc and activating transcription factor (ATF2) promoting cell proliferation.

1.5 The secretion of IL-1β, the basics of the inflammasomal system

Some proinflammatory members of the interleukin-1 family reach active form and visibility for their specific receptor via enzymatic cleavage. The active cytokine is produced by a intracellular multienzyme complex termed inflammasome.

Inflammasomes are composed of three major structural domains: cytosolic NLRP, prokaspase-1 enzyme, and the interacting adaptor proteins. NLRP proteins contain leucin rich repeats (LRR) on their carboxyterminal, the center is formed by a conserved NACHT domain (abbreviation derives from: neuronal apoptosis inhibitor protein-*NAIP*, MHCII. transcriptional activator-*CIITA*, incompatible locus protein from *Podospora anserina* species, *HET-E* and telomerase-associated protein-*TP1),* which controls the nucleotid binding and the protein oligomerisation, the aminoterminal possesses a variable pyrin (PYD) domain, which is distinct in every NLRP sensor.

During canonical activation the inactive domains of NLR proteins homotypically connect with each other (triggering), thereafter a protein complex is assembled with the contribution of the apoptosis associated speck-like protein (ASC) containing a PYD and caspase activation and recruitment domain (CARD) and the procaspase-1. The cleavage of the autocatalytically activated caspase-1 on pro-IL-1β (31 kDa) prepares the extracellular secretion of the active IL-1β (17 kDa) form. The genetical basis of the inflammasomal system is the NFkB transcription factor, which is responsible for the transcription of several proinflammatory cytokines, including the *de nevo* synthesis of pro-IL-1β protein.

The formation of the IL-1β may take place via noncanonical route as well, which is the action of the caspase-11 enzyme (in humans caspase 4/5). The lipopolisacharide (LPS) was the first molecule, which was suspected as the direct activator of the noncanonical way. Upon internalisation LPS is recognised by caspase-11, the assembled LPS-caspase 11 complexes undergo oligomerisation by CARD domains, followed by autoproteolytic activation. The enzymatic cleavage involves another protein, such as gasdermin (GSDMD), which divides into an N-terminal pore-forming domain and a C-terminal autoinhibitory domain. The pore-forming domain as it associates with the cell membrane, controls the secretion of numerous cytoplasmic molecule, including IL-1β.

1.6 Inflammasomal systems of the central nervous system

The intracellular NLRP proteins of the inflammasomal system, which are reported to play a cardinal role in the central nervous system include NLRP1 in neurons, NLRP2 in astrocytes, and NLRP3 in microglia cells.

1.6.1 NLRP1 inflammasome

The neuronal NLRP1 inflammasome may influence the activation of the caspase-1 enzyme in a bidirectional manner. During the direct route, the CARD domain, during the indirect one, the PYD domain regulates their interaction. Its unique feature is to have a CARD domain on the carboxiterminal, furthermore it contains autoproteolytically active function to find (FIIND) domain, which generates two polipeptides connected with non-covalent bonds. The FIIND domain is essential for the function of the NLRP1, whose gene encodes only one human protein, however in mice it encodes numerous paralog enzymes.

We had a better insight into the operation of NLRP1, when it turned out, that caspase-1 can be also activated by the lethal toxine of the *Bacillus anthracis* (LeTx). The bicomponent toxine contains lethal factor protease (LF), and channel-forming protective antigen (PA), which enables toxine transport into the cells. Although LeTx cleavage on aminoterminal destabilises NLRP1 structure with ubiquitination and proteosome mediated degradation, the protein will be still activated. The autoprocessing of FIIND domain does not allow the operation of the protease enzyme on the peptidechain, the bioactive fragments, which are adjacent to the carboxyterminal interact with caspase-1. This phenomenon is termed proteosomal degradation induced inflammasomal activation or „functional degradation”

Involvement of NLRP1 complex in cognitive disorders and neurodegenerative diseases is thoroughly proved.

1.6.2 NLRP2 inflammasome

The NLRP2 inflammasome is also termed as NALP2, NBS1, PAN1, PYPAF2. In parallel with the remaining NLR receptors, NLRP2 may cooperate with ASC protein to activate caspase-1 enzyme, but it can inhibit the NFkB signaling pathway. One of its allele variants prevents the inhibition, hence its role in chronic inflammatory diseases such as Muckle-Wells syndrome is confirmed.

NLRP2 elicits high amount of IL-1β production. Pannexin channel blocker probenecid and purinergic P2 receptor X7 (P2X7) receptor inhibitor Brilliant Blue G (BBG) significantly reduce the ATP induced NLRP2 activation. Similar conclusions can be drawn from silencing experiments with small interfering RNAs (siRNAs).

1.6.3 NLRP3 inflammasome

NLRP3 receptor is the most studied inflammasomal multiprotein complex. The assembled elements involve the NLRP3, ASC, and the caspase-1, which connect to each other upon ligand binding, but inflammasomal activation may also occur via caspase-11 mediated noncanonical way.

Microglial activation of NLRP3 complex was investigated in some degenerative diseases. Accumulation of fibrillary α-synuclein in dopaminergic neurons may induce NLRP3 activation within monocytes and microglial cells derived from peripheral blood. These cells release IL-1β, IL-18 and other neurotoxic products, which damage nearby cells resulting in Parkinson disease. According to recent literature NLRP3 contributes to accumulation of amyloid-β protein (Aβ) plaques in Alzheimer disease and frontopolar dementia.

2. AIMS

Since, our knowledge is still scanty related to chronic pain, we aimed to investigate different nociceptive relay stations (dorsal root ganglion, spinal dorsal horn, cingulate gyrus) in a systematic manner. In this present work we discuss the findings of experiments performed within the spinal dorsal horn, which is the first relay station of the nociceptive processing.

As an experimental model, complete Freund adjuvant (CFA) induced inflammatory (recently termed as nociceptive) pain model was used, which was also studied in our laboratory earlier. Our initial experiments focused on the expression of 45 genes in samples taken from the spinal dorsal horns of control and CFA treated animals. Taqman Low Density Array (TLDA) revealed significant increase in the fold change of four genes in CFA model, tremendous increase of expression was observed exclusively in case of IL-1R1, which acts a ligand binding unit of the receptor.

However, spinal expression of IL-1R1 was earlier verified, there were no data about the distribution of the receptor and its ligand within the spinal dorsal horn. Therefore in the next step of experiments following TLDA method we tried to precisely quantify the spinal distribution of IL-1R1 and its ligand in control and CFA injected animals.

We were curious whether the deletion of IL-1R1 gene caused any difference in the nociceptive response. To address this question electronic von Frey and Hargreaves behavioural tests were performed with wild-type C57BL6 and IL-1R1 KO mice.

Hence, in the spinal dorsal horn tissue extracts we demonstrated the cleaved form of IL-1β protein (active, 17 kDa) with immunoblot on post-injection day 3, we aimed to study that inflammasome type, from which there are no available data in CFA model in literature, and may be involved in the secretion of the active form of IL-1β.

3. MATERIALS AND METHODS

3.1 Experimental animals, animal model of chronic inflammation

Experimental work was performed on male *Wistar-Kyoto* rats (Gödöllő, Hungary, n=78), wild-type male C57/BL6 mice (n=18), and genetically modified (created by Labow and his colleagues) male IL-1R1 KO mice (from Jackson Laboratories) (n=18) (B6.129S7-Il1r1 tm1/mx,/J stock #:003245). Animals were kept under standard conditions of light and dark. Food and water were provided *ad libitum*. In our CFA model 1:1 mixture of 100 µl (mice) or 150 µl (rat) CFA (water, paraffinoil, thermally inactivated Mycobacterium suspension) and saline were injected into the right hindpaw of animals according to earlier works. Some groups of the wild-type C57/BL6 and IL-1R1 KO mice (n=6-6) received pseudotreatment (saline solution) into the right hindpaw. Control rats and mice were not treated.

3.2 TaqMan Low Density Array method (TLDA)

By means of TLDA method we aimed to investigate the relative gene expression changes within the spinal dorsal horn. Following laminectomy, spinal lumbar segments (L4-L5) were removed from control (n=9), and CFA treated rats (n=9) on post-injection day 3 (n=9). The ipsilateral spinal dorsal horns of CFA treated animals were separated from the remaining tissues for further processing. Following tissue removal, RNA was isolated, RNA quality was determined by Agilent microelectrophoresis and Nanodrop photometry. We generated cDNA library, whose 100 ng amount was loaded into the ports of TLDA microfluid cards provided by the manufacturer. The parallel analysis of 48 genes were performed via 8 ports. The obtained data were retrieved from three parallel experiments. The individual values of gene expression were determined with $2^{-\Delta\Delta Ct}$ method of Livak and his colleagues.

3.3 Mechanical and thermal behavioural tests

During experiments the nociceptive responsiveness of rats was tested by only mechanical behavioural test (*Dynamic plantar aesthesiometer, Ugo Basile*), mice were examined with both mechanical and thermal behavioural tests (*Plantar Test Instrument-Hargreaves Apparatus, Ugo Basile*). The mechanical and/or thermal nociceptive paw withdrawal threshold (MWT and TWL) values were measured daily from both paws before and after injections of CFA and saline. In some mice (n=36) and rat experimental groups (n=18), exclusively mechanical and/or thermal allodynia tests were performed without further investigations, these animals were examined for 11 days upon CFA injection. In case of those rats, which were used in further experiments (n=60), the animals were examined for 3 day after CFA treatment (at the lowest mechanical nociceptive threshold value).

In mechanical behavioural test animals were placed upon a network platform and covered with a Perspex enclosure that rendered the animals unrestrained for the duration of the measurement. The hind paw of the animal was positioned above a metal von Frey-type filament with a tip diameter of about 0.5 mm. The filament exerted an increasing pressure to the plantar, surface until the animal removed its paw. At this point, the measurement was terminated and the actual force at which paw withdrawal occurred was recorded.

In thermal allodynia animals were also placed upon a similar plastic platform, which was mentioned previously. The hind paw of the animal was positioned above an infrared light source which was focused onto the plantar surface. The illumination which exerted a heat stimulus to the plantar surface was applied until the animal removed its paw.

3.4 Western blot

Detection of target proteins, and their putative expression change upon CFA injections was carried out immunoblot. We collected lumbar spinal dorsal horn similarly to the TLDA method from control (n=6) an CFA treated (n=6) experimental animals. Tissue extracts were first sonicated in lysisbuffer supplemented with protease inhibitors, then cell debries were removed with centrifugation.

Protein concentration of the samples was determined with compatible BCA assay, they were dissolved in reducing buffer, then run on 10% sodium-dodecyl sulphate (SDS) polyacrylamide gel and electroforetically transferred on polyvinylidene difluoride (PVDF) membrane, which was blocked with Tris-buffered saline (TTBS) supplemented with 10% calf serum albumin, then samples were incubated with one of the primary antibodies as follows: goat anti-IL-1R1 receptor antibody (1:500, RnD Systems), rabbit anti-IL-1β antibody (1:500, Peprotech), rabbit anti-NLRP1 antibody (1:1000, Abcam), rabbit anti-NLRP2 antibody (1:500, Abcam), rabbit anti-NLRP3 antibody (1:500, Abcam), and internal control (mouse anti-β-tubulin, 1:2000, Sigma).

Following numerous washing steps in TTBS, blots were incubated with either rabbit anti-goat, goat anti-rabbit or rabbit anti-mouse IgG secondary antibodies conjugated with horseradish peroxidase (1:1000 DakoCytomation). Protein bands were visualised with 3,3'-Diaminobenzidine (DAB) chromogen reaction. Quantification of immunoblots was conducted with Image J software, values of optical densities were normalised to β-tubulin.

3.5 Enzyme-linked immunosorbent assay (ELISA) method

Rat IL-1β Quantikine ELISA kit was used to determine the IL-1β protein concentration from spinal dorsal horn tissue homogenisates of control (n=3) and CFA treated (n=3/day) animals at post-injection day 1-4.

Tissue samples were mechanically homogenised in icecold RIPA buffer supplemented with protease inhibitors, then following 20 minute shake and centrifugation the insoluble tissue debries were removed. 50µl volume of the supernatant was used in triplicates to detect IL-1β amount of the homogenisates. For the subsequent steps we followed instructions of the manufacturer.

3.6 Immunohistochemistry

3.6.1 Tissue preparation

Immunohistochemical procedures were carried out on control (n=7) and CFA treated rats (n=8) on post-injection day 3. Animals were deeply anesthetised by injection of sodium-pentobarbital, then transcardial perfusion was performed

with saline and 0.1 M phosphate buffer containing 4% paraformaldehide. After removal of L4-L5 lumbar spinal cord segments from the animals, samples were postfixed for 4 hours, then they were placed into 0.1 M phosphate buffer supplemented with 10% and 20% sacharose overnight. Following antigen retrieval with liquid nitrogen and embedding the samples into agar, 50 μm thick sections were cut with Vibratome. These sections were used at the subsequent steps of the reactions.

3.6.2 Immunoperoxidase reactions

Laminar expression and distribution of target proteins were studied in spinal dorsal horn of free-floating sections. Following endogenous peroxidase inhibition sections were gently shaken in 0,01 M Tris-PBS (TPBS) supplemented with 10 % normal goat or rabbit serum (NGS or NRS) for 50 min, then upon washing with 1% TPBS based NGS or NRS for 15 min, they were incubated with one of the primary antibodies used also in Western blot experiments for 3 days. Upon washing steps in 1% NRS or NGS, sections were placed into 0,01 M TPBS solution supplemented with 1% biotinylated rabbit anti-goat (1:200,Vector Labs) or biotinylated goat anti-rabbit secondary IgG antibody (1:200,Vector Labs) for 5 hours. After washing sections were incubated with avidin-biotinylated horseradish peroxidase complex (ABC 1:100, Vector Labs) overnight. Immunoreaction was visualised later with DAB reagent. Following washing steps in TRIS and PB solution, the sections were dehydrated in ascending series of alcohol. After xylol treatment the sections were fixed on glass slide by DPX medium (Sigma). Image capturing was conducted by Olympus CX-31 epifluorescent microscope.

3.6.3 Double immunofluorescent labelings

In case of the IL-1R1 colocalisation analysis the primary antibodies applied in 0.1 M PBS solution containing 1% NDS were as follows: goat anti-IL-1R1 antibody (1:500) and one of the antibodies listed here: guinea pig anti-CGRP (1:2000, Peninsula Labs), biotynilated IB4 (1:2000, Sigma), guinea pig anti-VGLUT2 (1:2000, Chemicon), mouse anti-VGAT (1:2000, Synaptic Systems), mouse anti-glial fibrillary acidic protein (GFAP, 1:1000, Chemicon), mouse anti-CD11b (1:500, Bachem), rabbit anti-KCC2 (1:2000, Upstate

Biotechnology), mouse anti-postsynaptic density protein 95 (PSD95, 1:100, Frontier), or mouse anti-gephyrin (1:100, Synaptic Systems) antibodies. The reactions were incubated for 3 days at 4 ℃.

After washing steps in 0.1 M PBS supplemented with 1% NDS, the sections were incubated with donkey anti-goat IgG secondary antibody conjugated with Alexa 555 (1:1000, Molecular Probes) and one of the antibodies for 2 hours listed here: Alexa Fluor 488 conjugated donkey anti-guinea pig IgG (1:1000, Molecular Probes), Alexa Fluor 488 conjugated streptavidin (1:1000, Molecular Probes), Alexa Fluor 488 conjugated donkey anti-mouse IgG (1:1000, Molecular Probes) or Alexa Fluor 488 conjugated donkey anti-rabbit IgG (1:1000, Molecular Probes).

The expression of IL-1β and the inflammasomal sensor NLRP1, NLRP2 and NLRP3 proteins were studied in glial cells by using rabbit anti-IL-1β (1:500), rabbit anti-NLRP1 (1:100), rabbit anti-NLRP2 (1:500), and rabbit anti-NLRP3 (1:500) antibodies and one of the antibodies as follows: mouse anti-GFAP (1:1000), or guinea pig anti-ionised calcium binding adaptor molecule (Iba1, 1:2000, Synaptic Systems). Primary antibodies were used with the same method as described earlier. Following incubation with secondary antibodies (Alexa Fluor 488 conjugated goat anti-rabbit IgG 1:1000, Thermo Fisher Scientific, Alexa Fluor 555 conjugated goat anti-mouse IgG, 1:1000, Thermo Fisher Scientific, or Alexa Fluor 555 conjugated goat anti-guinea pig IgG, 1:1000, Thermo Fisher Scientific) and washing steps (1% NGS serum, then PBS) sections were collected on glass slides and covered with Vectashield medium (Vector Labs).

3.6.4 Quantitative analysis of confocal microscopy

Single 1-μm-thick optical sections were scanned with an Olympus FV1000 confocal microscope. A 10 × 10 standard square grid with an edge-length of 4 μm was put onto confocal images obtained from superficial spinal dorsal horn. The colocalization of IL-1R1 with neuronal and glial markers was quantitatively analysed in the double-stained sections. IL-1R1-immunostained spots over the edges of the standard grid were counted in laminae I and II. The selected IL-1R1-immunostained spots were then examined to determine whether they were located within the confines of areas immunoreactive for the

axonal (CGRP, IB4-binding, VGLUT2, VGAT), glial (GFAP, CD11b), and postsynaptic membrane (PSD95, gephyrin) markers. In case of sections double-stained for IL-1R1 and KCC2, the selected IL-1R1-immunostained spots were checked to define whether they were aligned along KCC2-immunostained membranes (considered as somatodendritic membrane expression of IL-1R1) or located within areas surrounded by KCC2-immunostained membranes (considered as cytoplasmic expression of IL-1R1 at the somatodendritic compartment of neurons).

In case of IL-1β the colocalisation values were determined in a similar way with glial markers (GFAP, Iba1). Changes of IL-1β expression as well as volume of GFAP positive astrocytes were also studied by using IMARIS software (Bitplane). Distance of IL-1β immunoreactive spots from GFAP positive astrocyte profiles was calculated with distance matrix. We investigated the overlapping of NLRP1, NLRP2, NLRP3 inflammasomal sensors with GFAP, and data were completed with distance matrix. Data obtained from control and CFA treated animals were analysed on three random sections of each animal within Rexed lamina I and II.

4. RESULTS

4.1 Results of TLDA relative gene expression

Putative gene expression involved in nociceptive signaling and chronic pain including IL-1R1 was examined rat superficial spinal dorsal horn upon CFA injection. We intended to elucidate whether significant gene expression changes were detectable at the mRNA level of the receptor. In addition, we wanted to reveal the possible spinal expression of 43 other selected genes upon CFA injection. In comparison with control, in CFA treated spinal dorsal horn IL-1R1 expression was significantly increased (6.02-fold, p=0.0002).

From the remaining genes of interest (excitatory and inhibitory neurotransmitters, cytokines, protein-kinases, ionchannels) the expression of NAPE-PLD (involved in endocannabinoid synthesis) gene was moderately increased (1.11-fold, p=0.0016), the expression of Faah gene (involved in endocannabinoid hydrolysis) (0.78-fold, p=0.0368) and excitatory ionotropic glutamate AMPA receptor subunit 2 (Gria2) gene were also reduced compared to control (0.77-fold, p=0.0447).

4.2 Results of mechanical behavioural test in rats

Before the earlier described methods, the validation of nociceptive responsiveness was conducted with mechanical behavioural test in male Wistar rats. Mechanical withdrawal threshold (MWT) values of control and left contralateral hindpaw of CFA treated animals were comparable throughout the experimental period. Upon CFA injection significant decrease of MWT values was measured (p=0.001) from the right ipsilateral hindpaw of CFA treated animals compared to control. The lowest average values were registrated on post-injection day 3 (11.46±1.01 g).

4.3 Results of mechanical and thermal behavioural tests in mice

Due to the importance of IL-1R1-IL-1β signalisation mechanical and thermal threshold of wild type C57BL6 and IL-1R1 KO male mice were also registrated

upon CFA injection (day 0) until day 11. Certain groups of animals received saline as a pseudotreatment. MWT values of control wild-type and IL-1R1 KO animals were quite comparable. In wild-type mice MWT values between 4.8-5.0 g were registrated, with an average value of 4.81±0.29 g, in IL-1R1 KO animals range between 3.0-5.0g was measured with an average value of 3.94± 0.51 g. Prior to CFA injection the discrepancy between the registrated basal values of wild-type and IL-1R1 mice certained to be highly significant. ($p=0.0000002$). Injection of physiological saline into the right ipsilateral hindpaw evoked detectable difference neither in wild-type nor in KO animals. However, CFA injection into the right hindpaw induced marked decrease in MTT values of wild-type mice. Reduction of MTT values reached its maximum on post-injection day 4 (1.68±0.06 g). In IL-1R1 KO mice CFA treatment evoked only a moderate decrease in MWT data, which lasted approximately one week. Reduction of MWT values was significantly registrated on post-injection day 1-7 ($p=0.00004–0.006$), however significant MWT differences were observed between wild-type and KO animals on days 1-9 ($p=0.000004–0.01$).

Results of CFA injection induced thermal allodynia were parallel with the mechanical allodynia in both wild-type and IL-1R1 KO animals. In wild-type mice thermal withdrawal latency (TWL) values were registrated in the range of 4.9-10.2 s with an average value of 7.47±1.22 s, in IL-1R1 KO animals these values were measured between 6.3-10.0 s with an average value of 8.15 ± 0.85s during the duration of the experiment. Injection of saline did not induce robust difference in TWL values in agreement with mechanical behavioural test. However, CFA injection evoked a significant reduction in TWL values measured from right hindpaw of wild-type mice. The maximum of the reduction was observed on day 2 and 3 upon CFA injection (1.74±0.01 s and 1.72±0.08 respectively). In TWL data of KO mice only moderate decrease was detected, which lasted only 1-7 days upon CFA treatment. Statistically significant decrease was permanently observed during the experiment ($p= 0.0005–0.029$). Significant TWL differences between wild-type and KO animals were detectable on day 1-9 ($p=0.000003–0.021$).

4.4 Results of IL-1R1 protein expression

4.4.1 Controls

As included in the immunohistochemical protocol recombinant rat derived protein (IL-1R1-(Sino Biological Inc Peking) was applied to test the specificity of the anti-IL-1R1 primary antibody. The recombinant peptid and the appropiate antibody were equivalently mixed and used as primary serum on free-floating sections to induce antibody depletion. Mixture was stored at 4 °C-for 18 hours, then centrifuged. Thereafter we followed the steps of the immunoperoxidase reaction, specific immunoreaction was not identifiable. As a different control we also performed an antibody specificity test described earlier in the immunoblot chapter. Immunoreaction revealed a band with a 80 kD molecular weight, which corresponds to the molecular weight of the receptor according to literature.

4.4.2 Investigation of IL-1R1 protein expression with immunoblot

Gene expression changes of IL-1R1 obtained from TLDA method were validated at a protein level with Western blot from spinal dorsal horn samples of control (day 0) and CFA treated rats (day 3). The densitometric analysis demonstrated almost 1.5 fold significant increase at the protein level of IL-1R1 in CFA treated animals compared to control ($p=0.029$).

4.4.3 Investigation of IL-1R1 protein expression with immunohistochemistry

4.4.3.1 Results of IL-1R1 protein expression with immunoperoxidase method

The distribution and expression of IL-1R1 protein were studied by immunoperoxidase method in control and CFA treated circumstances. Spot-like immunoreaction was detected in Rexed lamina I and II of the spinal dorsal horn of rats, even immunoreactive band was identified at the inner part of Rexed lamina II, which was markedly increased upon CFA treatment compared to control.

4.4.3.2 Results of double immunofluorescent labeling

4.4.3.2.1 IL-1R1 expression on axonterminals

In order to study the axonal expression of the receptor, we performed colocalisation analysis between IL-1R1 and peptidergic primary afferents (CGRP), non-peptidergic primary afferents (IB4), axonterminals of excitatory (VGLUT2) and inhibitory interneurons (VGAT). According to double immunofluorescent labelings in control sections complete segregation was observed between IL-1R1 and these markers. The axonal distribution of IL-1R1 immunoreactive puncta is as follows: 0.1±0.01% on marker CGRP, 0.3± 0.10% on marker IB4, 1.2± 0.46% on marker VGLUT2, 0.1±0.04% on marker VGAT. CFA injection did not considerably change the values taken from control.

4.4.3.2.2 IL-1R1 expression in neuronal cytoplasm and somatodendritic membrane

Intracellular chloride concentration is mainly regulated by the expression of KCC2 transporter protein. The protein is exclusively expressed on neurons, the receptor is integrated into the somatodendritic membrane, where it controls chloride mediated postsynaptic hyperpolarisation. IL-1R1 is not localised on axonterminals. When investigating the colocalisation between IL-1R1 and KCC2, the immunoreactive puncta of the receptorset were found either on the KCC2 positive somatodendritic surface (membrane) or within the cytoplasmic area surrounded by the marker (cytoplasm). Quantification of control sections with double immunofluorescent labelings showed that 19.9±2.27% of the receptorset was identified inside the KCC2 positive membrane, concurrently 49.9±2.56% of the receptorset was found to be cytoplasmic.

In CFA treated animals the ratio of IL-1R1 immunoreactive spots of the somatodendritic membrane compartment significantly enhanced ($p=0.002$) from 19.9±2.27% to 35.1±1.5 %, however cytoplasmic ratio was decreased to 44.39±0.56%.

4.4.3.2.3 IL-1R1 expression in the membrane of excitatory and inhibitory postsynaptic membrane

Putative overlapping of the receptor with excitatory and inhibitory postsynaptic markers was also investigated. According to earlier data, PSD95 is used as the marker of the excitatory glutamatergic neurons and gephyrin is the marker of the inhibitory GABAergic and glycinergic postsynaptic density.

IL-1R1 immunoreactivity was mainly observed in the excitatory postsynaptic density. 9.04±0.43% of the receptorset was localised on the PSD95 positive marker, which was nearly half of the immunoreactive spots on the KCC2 positive somatodendritic surface. The colocalisation between IL-1R1 and gephyrin certained to be significantly smaller, 1.2±1.1% of the receptor was found in the inhibitory postsynaptic density.

In CFA injected animals the distribution of the receptor was neglectably changed compared to control. 9.93±0.4% of the IL-1R1 colocalised with PSD95 marker, and 1.43±1.14% of the receptor colocalised with gephyrin.

4.4.3.2.4 IL-1R1 expression in astrocytes and microglial cells

In order to analyse the receptor expression on astrocyte and microglial profiles, we performed colocalisation analysis between IL-1R1 and GFAP positive astrocytes and CD11b positive microglial cells. In control animals 9.3±0.65% of the receptor were expressed on astrocytes, negligible fraction of the receptor 1.2±0.30% was expressed by microglial cells. After CFA injection receptorset expressed by GFAP profiles was negligibly reduced to 9.23±0.41%, however on microglial cells it increased to 2.5±0.35%.

4.5 Results of IL-1β and NLRP protein expression

4.5.1 Control

Similarly to IL-1R1 the anti-IL-1β antibody specificity was also tested by the earlier described antibody depletion protocol (Peprotech, catalog number: 400-01B), no specific immunoreaction was observed. Antigen peptides were not available against inflammasomal sensor proteins, therefore we omitted primary antibody, only secondary antibodies were used, specific immunreaction was also not detected in the superficial spinal dorsal horn. In order to support our

findings with NLRP2 marker, we carried out positive immunoperoxidase reaction (by using primary antibody) and negative immunoperoxidase reaction (by omitting primary antibody) on hematoxylin stained lung section.

4.5.2 Examination of IL-1β quantity with ELISA and immunoblot

The specificity of anti-IL-1β antibody was also confirmed with immunoblot. The weak immunoreactive band at 31 kDa showed molecular weight of pro-IL-1β from control spinal dorsal horn tissue extracts (day 0).

On post-injection day 3, at the peak of mechanical allodynia, both pro-IL-1β, and its active, caspase-1 cleaved 17 kDa form were identified in high amount in CFA treated spinal extracts.

We did not find any relevant data related to time-dependent changes of IL-1β protein in inflammatory pain, therefore we continued our experiment with ELISA method. The basal level of the cytokine measured from L4-L5 spinal dorsal horn tissue extracts was 124±20.05 pg/ml concentration, which was significantly enhanced ($p=0.040$) to 255±37.4 pg/ml on post-injection day 3, which was in agreement with results of the mechanical test. On day 4 concentration of IL-1β was markedly reduced to 174±12.03 pg/ml.

4.5.3 Examination of IL-1β expression with immunohistochemical methods

The results of immunoblot and ELISA were confirmed by immunoperoxidase reaction. Similarly to IL-1R1 spot-like immunoreaction of IL-1β was detected in superficial Rexed laminae, where the immunoreactivity certained to be more robust on the ipsilateral side of CFA treated animals. During quantification significant increase ($p=0.0000064$) of IL-1β immunoreactive puncta were calculated in CFA treated samples (day 3) in comparison with control, IL-1β production robustly increased with 78±7.48 %.

When analysed the colocalisation of IL-1β with glial cells we observed that 3.31±1.48% of microglial profiles expressed IL-1β, upon CFA injection the colocalisation increased negligibly to 4.03±1.57%. In case of astrocytes 15.15±3.38% of GFAP positive profiles produced the cytokine, however in inflammation this ratio was significantly increased ($p=0.002$) to 39.3±5.24%.

4.5.4 Examination of IL-1β expression with IMARIS

At the peak of the CFA induced chronic inflammatory pain the IL-1β production of astrocytes was significantly increased. For the investigation of morphofunctional differences and IL-1 production between astrocytes of control and CFA treated animals IMARIS software analysis was conducted. In CFA treated sections, the absolute number of IL-1β immunoreactive spots (IL-1, n=1337±229.2) and the colocalisation with GFAP marker are also significantly increased (COLOC, n=76±15.35) (p=0.009032 and p=0.022896 respectively), compared to control (IL-1, n=272.33±174 and COLOC, n=23.33±9.76). Furthermore, the volume of GFAP profiles taken from CFA treated animals was markedly, but not significantly (p=0.194406, 5620 µm3±1623) increased in comparison with control (3630 µm3±1020). If the colocalisation was normalised to the total number of the IL-1β immunoreactive puncta (COLOC/IL-1), then ratio of the colocalisation proved to be significantly higher in control (12.21%±2.95) (p=0.0366) compared with inflammatory conditions (5.99%±1.05).

As a conclusion, one can say, that in chronic inflammatory pain the absolute number of IL-1β immunoreactive spots (IL-1) and the absolute value of colocalisation (COLOC) are significantly higher than that of control. Conversely, colocalisational ratio is higher if it is normalised to the total number of IL-1β (COLOC/IL-1). The volume of GFAP profiles was although increased, no significant changes were observed. We also measured the distance of IL-1β immunoreactive spots from astrocyte profiles by using IMARIS in control- and inflammatory conditions. The distance matrix did not show significant difference between the two groups, the majority of IL-1β puncta were found within the distance of 1 µm in both control and CFA treated animals.

4.5.5 Examination of NLRP markers with IMARIS software

IL-1β is produced by the previously mentioned inflammasomal system, which is relatively less studied within the superficial spinal dorsal horn. By means of immunoblot, the molecular weight of all the three inflammasomal proteins (NLRP1, NLRP2 and NLRP3) was detected in tissue extracts of spinal dorsal horn (NLRP1: 165 kDa, NLRP2 and NLRP3: 120 kDa).

From the three inflammasomal proteins, we detected robust increase only at NLRP2 protein with immunoblot in CFA treated animals (day 3) compared to control (day 0). According to our results the main source of IL-1β is astrocyte in CFA model. In the subsequent experiments we intended to reveal the putative inflammasomal system, which contributes to caspase-1 activation and consequently the production of IL-1β. Quantitative analysis showed that NLRP1 protein was entirely separated from the GFAP profiles in spinal dorsal horn of control and CFA treated rats. During evaluation of the colocalisation both in control and CFA injected animals upon double immunofluorescent labelings we found high amount of NLRP2 expression. 8.34±1.5% of NLRP2 positive immunoreactive puncta were colocalised with astrocytes in control sections, after CFA injection significant (p=0.000000148) more than 2-fold increase was experienced (20.59±1.6%). NLRP3 colocalisation with astrocyte was 5.36±1.21% in control, which neglectably increased to 6.21±1.29% during inflammation.

Precedingly, the immunoblot showed significant increase of NLRP2 protein in CFA treated samples. In order to support this finding, we calculated the absolute number of NLRP2 immunoreactive spots (control n= 20.66±1.32, CFA treated animals: n= 37.5±1.53), which also significantly increased (81.5± 7.65% upon CFA administration (p=0.00000011) compared to control.

4.5.6 Distance analysis of NLRP proteins

We also determined the distance matrix of NLRP proteins from astrocyte profiles by using IMARIS software, during which the distance of three NLRP markers was analysed from GFAP positive profiles. In case of NLRP1 and NLRP3, we did not find marked changes, however at NLRP2 sensor the immunoreactive puncta were significantly higher (p=0.0004) in number in CFA treated animals within the distance of 0-1µm.

5. DISCUSSION

Our experiments were based on the results of TLDA gene expression, therefore during the first part of our work we investigated the distribution of IL-1R1 in the spinal dorsal horn. To mimic chronic pain, CFA induced inflammatory (nociceptive) model was used. During the second part of our work we wanted to detect those cells, which may play a vital role in the secretion of the receptor ligand.

Animal models are important tools of the biomedical sciences, therefore numerous models are available to study the mechanisms of chronic pain. During our experiments intraplantar injection of Complete Freund Adjuvant (CFA) induced nociceptive model was applied. CFA model was an appropiate model for studying chronic inflammatory pain according to earlier descriptions (this definition was valid, when the first publication was written in 2017), in the latest IASP glossary (2020) our model was interpreted as nociceptive pain model.

5.1 Conclusions based on the results of IL-1R1 expression

In this present work, we studied the gene- and protein expression of IL-1R1 in superficial spinal dorsal horn of rodents in a detailed manner in control and CFA treated animals. Contribution of IL-1R1 to the mechanical and thermal allodynia as well as central sensitisation was confirmed by using IL-1R1 KO mice.

The findings of mechanical and thermal behavioural tests showed that, the effect of the subcutaneous intraplantar CFA injection on inflammation was also present in KO animals, but these mice demonstrated significantly reduced nociceptive responsiveness compared with wild type animals.

During TLDA experiment we were one of the first group, who detected more than 6-fold increase in IL-1R1 expression (6.02) upon CFA injection on the ipsilateral side of spinal dorsal horn. The proinflammatory IL-1β induced nociceptive responses with the contribution of IL-1R1, which was expressed by many tissues.

During our work, we were one of the first groups, who verified the necessity of IL-1R1 for the chronic inflammation and central sensitisation. Although the spinal expression of IL-1R1 was also studied earlier, first we characterised its precise cellular distribution in spinal dorsal horn by using immunoblot and immunoperoxidase techniques.

According to the results of double stainings, the majority of the receptorset was localised on neuronal somatodendritic compartment, and in a lesser extent on astrocyte cells. The activation of neuronal IL-1R1 was associated with excitatory glutamatergic transmission.

Kinases of IL-1β-IL-1R1 signalisation phosphorilate the GluN1 and/or GluN2B subunits, which leads to receptor activation. Spinal injection of IL-1β promotes the firing of C fibers, and elicits the postdischarge (wind-up) of wide-dynamic range neurons (WDR, termination of nociceptive and low-threshold mechanoreceptor afferents), which results in long-term potentiation (LTP) and central sensitisation.

From our results we can conclude that, IL-1R1 is associated with excitatory neurotransmission. The half of the receptorset integrated into the KCC2 positive somatodendritic membrane was colocalised with the excitatory postsynaptic marker PSD95. During CFA induced inflammation the existing somatodendritic population almost increased to its 2-fold number, but it was not correlated with PSD95, which meant that the receptor evoked effects were rather extrasynaptic. IL-1R1 was also detected in the inhibitory postsynaptic density. Although the receptor number was neglectable here, some authors suggested the presence of glycinergic synapses on GABAergic neurons, where IL-1β elicited disinhibition leading to a permanent excitatory tone.

IL-1R1 can be expressed by glial cells in spinal cord. Gruber-Schoffnegger and his colleagues showed that via glial IL-1R1 gliotransmitter secretion might have occured, which was necessary for AMPA and NMDA receptor activation. Our findings also supported IL-1R1 expression by astrocytes, however colocalisation with microglial cells was neglectable. Upon CFA injection we did not observe considerable changes in receptor number in either glial cell type.

As a final conclusion, we suppose that during CFA induced peripheral inflammation primary nociceptiv afferents fibers release ATP, which later evokes IL-1β secretion. IL-1β binds to rather neuronal, less frequently to glial IL-1R1, to either potentiate the NMDA receptor mediated synaptic currents or to act on glycinergic interneurons eliciting disinhibition.

The increase of the excitatory tone, and the reduction of inhibitory synapses promote central sensitisation.

5.2 Conclusions based on the results of IL-1β and inflammasomal proteins

In the second part of this present work the ligand of IL-1R1, as well as the inflammasomal protein expression were investigated. We were one of the first groups, who studied the time-dependent changes of the IL-1β cytokine, and validated with behavioural tests.

It is generally accepted, that at the early phase of chronic pain, first the spinal microglial cells are activated, but contradictory data are available about the dominance of glial cells in the late phase. In some models the microglial cells, in others the astrocytes cells predominate and produce proinflammatory cytokines. Our data showed the primary role of activated astrocytes in producing IL-1β upon CFA injection, which is in agreement with other authors.

IL-1β may exert its effects not only on neurons, but on glial IL-1R1 as well. IL-1R1 can influence not only neuronal excitability, but it promotes secretion of proinflammatory mediators. IMARIS method proved that these activated astrocytes were markedly different regarding their morphology and size from their counterparts taken from control animals. For the biological activity of the cytokine, the caspase-1 induced cleavage is also necessary from the inactive precursor proform, hence only the active, matured form can bind to its cognate receptor.

We have a contradictory picture about inflammasomal expression, data are mostly retrieved from neuropatic pain models. Although NLRP1 and NLRP3 proteins were earlier identified in astrocyte and microglial cells, however in our model even if we observed these proteins in spinal dorsal horn, no significant changes were detectable compared to control. NLRP2 expression was studied in a variety of diseases: skin lesions, chromosome damage, airway disorders, embryonic development of murine, but scanty data were available in central nervous system. Minkiewicz and his colleagues identified its functional expression in human astrocytes, but until date only one publication mentioned NLRP2 in chronic pain.

Their results were confirmed in spinal dorsal horn, where NLRP2 was significantly increased both in its absolute quantity and its expression in astrocyte cells. An important logical link was that NLRP2 inhibited NFκB in

macrophages, trophoblasts and glioblastoma cell lines to limit cytokine secretion.
It is possible, that although the protein induces cytokine production, it may inhibit the proinflammatory NFκB route after a certain period with self-regulation to prevent tissue damage.

As a final conclusion, we suppose that, IL-1 production within spinal dorsal horn is due to the NLRP2 activation in astrocyte cells. Following activation the cytokine binds to either its neuronal mainly extrasynaptic IL-1R1 to participate in excitatory neurotransmission and/ or disinhibition or binds to glial receptor to evoke production of proinflammatory cytokines via autocrine way.

6. SUMMARY

Nowadays there is a growing body of literature associated with chronic pain. The main reason behind this fact is the progressive health deterioration and substantial decline of life quality of patients, which may lead to severe depression. The potential loss of capability of work have a tremendous impact on society, hence the problems of this medical condition are to be resolved in the collective interest. Although our currently knowledge increasingly grows about the pathological mechanisms related to this sensory overload, the lack of deeper insights into the molecular pain processes and the scanty information being available about the interactions of neurons with glial cells still poses a challenge to research- and medical applications.

Regarding the statements above, our work here focused on the establishment of the CFA induced chronic inflammatory pain model to investigate the changes of gene- and protein expression within the spinal dorsal horn, especially the elements of the IL-1R1 signalisation. On one hand our findings can support the earlier results regarding the putative role of IL-1R1, on the other hand we provide here new data about its relatively less studied quantitative receptorial distribution. We were one of first laboratories, who reported a time-dependent correlation between the changes of the receptorial gene- and protein expression within the superficial Rexed laminae by means of TLDA array and other morphological methods following CFA injection.

Our results showed that, not only the IL-1β ligand, but its receptor is also significantly upregulated chiefly at extrasynaptic part of neurons to potentiate the inflammatory condition.

In accordance with the previous postulations, we can accept that, the cytokine release takes place from astrocytes at later phase of inflammation, whose morphology is markedly different in comparison with control cells.

We were the first to emphasize the importance of inflammasomal protein NLRP2 in spinal central sensitisation. Significant astrocytic upregulation of this protein can also contribute to the inflammatory amplification and the production of IL-1β and other proinflammatory agents.

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List of publications related to the dissertation

1. **Ducza, L.**, Szűcs, P., Hegedűs, K., Bakk, E., Gajtkó, A., Wéber, I., Holló, K.: NLRP2 Is Overexpressed in Spinal Astrocytes at the Peak of Mechanical Pain Sensitivity during Complete Freund Adjuvant-Induced Persistent Pain.
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2. Holló, K., **Ducza, L.**, Hegyi, Z., Dócs, K., Hegedűs, K., Bakk, E., Papp, I., Kis, G., Mészár, Z. M., Bardóczi, Z., Antal, M.: Interleukin-1 receptor type 1 is over-expressed in neurons but not in glial cells within the rat superficial spinal dorsal horn in complete Freund adjuvant induced inflammatory pain.
J. Neuroinflammation. 14 (1), 1-18, 2017.
DOI: http://dx.doi.org/10.1186/s12974-017-0902-x
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List of other publications

3. Mészár, Z., Kókai, É., Varga, R., **Ducza, L.**, Papp, T., Béres, M., Nagy, M., Szűcs, P., Varga, A.: CRISPR/Cas9-Based Mutagenesis of Histone H3.1 in Spinal Dynorphinergic Neurons Attenuates Thermal Sensitivity in Mice.
Int. J. Mol. Sci. 23 (6), 1-18, 2022.
DOI: http://dx.doi.org/https://doi.org/10.3390/ijms23063178
IF: 5.923 (2020)

4. Vágó, J., Kiss, K., Karanyicz, E., Takács, R. Á., Matta, C., **Ducza, L.**, Rauch, T. A., Zákány, R.: Analysis of Gene Expression Patterns of Epigenetic Enzymes Dnmt3a, Tet1 and Ogt in Murine Chondrogenic Models.
Cells. 10 (10), 1-20, 2021.
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5. Gajtkó, A., Bakk, E., Hegedűs, K., **Ducza, L.**, Holló, K.: IL-1[béta] Induced Cytokine Expression by Spinal Astrocytes Can Play a Role in the Maintenance of Chronic Inflammatory Pain.
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