Article

Application of autologous peripheral blood mononuclear cells into the area of spinal cord injury in a subacute period: a pilot study in pigs

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Simple Summary: Spinal cord injury is one of relevant medical and social issues causing severe disability. The possibility to overcome consequences of spinal cord injury is related to using cell therapy. Peripheral blood is an available source of adult stem cells for clinical use. In our study we have evaluated a therapeutic potential of peripheral blood mononuclear cells in a model of pig spinal cord injury. In subacute period (6 weeks after injury), peripheral blood mononuclear cells enclosed in fibrin glue were applied into the dorsal area of injured spinal cord. This pilot study demonstrated that the tissue structure could be improved and the conduction along spinal axons could be partially recovered in pigs with above cell therapy.

Abstract: Peripheral blood is an available source of adult stem cells for both fundamental research and clinical use. Peripheral blood mononuclear cells (PBMCs) contain a number of different multipotent populations of progenitor cells and possess a potential to differentiate into various types of cells under appropriate conditions. We have evaluated a regenerative potential of PBMCs embedded into fibrin matrix (FM) in a model of pig spinal cord injury. The distribution of transplanted PBMCs in the injured spinal cord was previously evaluated, which demonstrated the ability of these cells to penetrate into deep layers of the spinal cord and to concentrate mainly in the grey matter. The pilot study conducted demonstrated that the tissue structure could be improved and the conduction along posterior columns of the spinal cord could be partially recovered in animals in the setting of FM+PBMCs application. The multiplex analysis of blood serum and cerebrospinal fluid (CSF) showed the cytokine imbalance to occur without significantly shifting towards pro-inflammatory or anti-inflammatory cytokine cascades in the setting therapy provided.

Keywords: peripheral blood mononuclear cells; spinal cord injury; pigs; fibrin matrix.

1. Introduction

Spinal cord injury (SCI) is one of relevant medical and social issues causing severe disability. Most post-traumatic tissue degeneration is caused by a multiple secondary injury, which involves several closely related processes such as a blood-brain barrier dysfunction, local inflammation, neuronal death, demyelination and impaired neural pathways [1]. SCI can lead to severe motor, sensory and autonomic dysfunction. At present, there is no effective method to restore an injured spinal cord [2].

The possibility to overcome consequences of SCI is related to using cell therapy. For this purpose, neural stem cells, oligodendrocyte progenitors, mesenchymal cells, Schwann cells, olfactory epithelial cells, embryonic and induced pluripotent stem cells are used [2–4]. Nevertheless, stem and progenitor cells, which can be easily and invasively isolated from an adult body for subsequent autotransplantation to a recipient are most promising for clinical use. Peripheral blood is therefore the most convenient source where patients' stem and progenitor cells can be isolated, frozen and stored for subsequent use. For this purpose, peripheral blood mononuclear cells (PBMCs) are studied, as they can differentiate into alternative lineages *in vitro* and *in vivo* depending upon culture conditions or the site of transplantation [3]. PBMCs are found to contain a multitude of progenitor cells populations including hematopoietic stem cells, endothelial progenitor cells, mesenchymal stem cells (MSCs), populations of circulating fibrocytes, suggesting that PBMCs may possess the potential to differentiate into a number of mature functional cell types in specific microenvironments [3]. Several populations of progenitor cells isolated from PBMCs can also differentiate into neurons and glia [5–7]. Kim et al. (2006) reported that MSCs, derived from PBMCs, can be successfully induced in order to form neurospheres in the presence of epidermal growth factor and basic fibroblast growth factor and to differentiate into nerve cells *in vitro*, indicating a potential source of neuronal precursors for the treatment of central nervous system disorders [8]. Kijima et al. (2009) demonstrated that human $CD133^+$ PBMCs, locally transplanted into an injured peripheral nerve of rats can enhance vasculogenesis (blood vessel formation), providing the microenvironment required for axonal regeneration. Moreover, mRNAs of human GAPDH was identified in regenerated nervous tissue in eight weeks after PBMCs transplantation [9]. Results of these studies strongly suggest that PBMCs possess a significant potential for creating a microenvironment promoting regeneration of neurons or "a biological bridge" facilitating recovery.

Scaffolds are used as a vehicle to deliver stem cells into a site of injury; they are also used to bridge the gap of the lesion as contact guidance for axonal growth [10]. Both natural and synthetic biomaterials increase stem cell survival *in vivo*, by providing the cells with a controlled microenvironment, where cell growth and differentiation are facilitated [10–12]. The findings of Itosaka et al. (2009) suggest that fibrin matrix may be one of the promising candidates for a potential, minimal invasive scaffold for an injured spinal cord and this strategy of tissue engineering could be a hopeful option in regenerative therapy for patients with SCI [13].

We have studied a therapeutic potential of the application of peripheral blood mononuclear cells embedded in fibrin matrix in a subacute period of SCI in pigs based on the injured spinal cord structural and functional recovery criteria.

2. Materials and Methods

Isolation and adenoviral transduction of PBMCs

The study was approved by the Kazan Federal University Animal Care and Use Committee (Permit Number 2, 5 May 2015). Peripheral venous blood taken from healthy 4-month-old female pot-bellied pigs was separated by centrifugation in ficoll gradient density.

Blood samples were collected in vacuum test tubes (Apexlab, Russia) supplemented with an ethylenediaminetetraacetic acid (EDTA) solution. All procedures were performed in the biosafety class 2 cell culture laboratory. Primarily, 5 ml of whole blood were mixed with an equal volume of DPBS (PanEco, Russia) in a sterile 15 ml tube. The diluted blood sample was accurately applied to a ficoll solution (ficoll density - 1.077 g/cm^3, PanEco), then centrifuged at 1900 rpm for 20 min without a break.

After centrifugation a fraction of white blood cells was transferred into a new 15 ml tube. The cells were washed with DPBS twice (5 min at 1400 rpm). Red blood cells were lyzed in a lysis solution (155mM NH4Cl, 10mM KHCO3, 0.1mM EDTA, pH 7.3) for 5 min. The cells were washed with DPBS and resuspended in a RPMI-1640 media (PanEco) supplemented with 10% fetal bovine serum (HyClone, USA), 2 mM L-glutamine and 1% mixed penicillin-streptomycin (PanEco).

Then they were seeded on 10 cm cultural dishes at the amount of $8x10^6$ cells per plate. Isolated PBMCs were genetically modified with Ad5-EGFP with MOI of 10 and incubated in a humidified chamber containing 5% CO2 at 37°C. Green fluorescence was measured in 24 hours after transduction using an AxyObserver Z.1 microscope (Carl Zeiss, Germany).

Spinal cord injury and experimental groups

Animals were exposed to surgical manipulations after intubation anesthesia, appropriate pre-operation preparation and adequate analgesia/pain control. Premedication consisted of intramuscular injection of xylazine (0.6 mg/kg, Bimeda, Ireland) and ketamine (5 mg/kg, Hospira, USA). After propofol induction (IV, 2-6 mg/kg, Fresenius Kabi, Austria) endotracheal intubation was conducted using isoflurane (1.3%, Laboratorios Karizoo, Spain) throughout the intervention.

After laminectomy anesthetized pigs were subjected to a dosed contusion at the spinal T 10 level with a metal impactor weighing 50 g dropped from a height of 20 cm, followed by compression with the same weight for 10 min. Then muscles of the back were sutured layer by layer. A urinary catheter (10 Fr, Jorgensen Laboratories Inc., Loveland, CO) was inserted in 3-5 days after surgery. Cefazolin (25 mg/kg, Sintez, Russia) and ketoprofen (1 mg/kg, AVZ, Russia) were given as intramuscular injections. The pigs were housed separately within the first 48 h, then in pairs.

In 6 weeks after injury, PBMCs enclosed in fibrin glue (Tissucol, Baxter) (the experimental group - FM + PBMCs, n = 5) were applied to the damaged area (after removing synechiae and making several longitudinal incisions in the dura mater: 2-3 incisions with a length of 2-3 mm at a distance of 2-3 mm from each other). Animals of the control group were applied with cell-free FM under similar conditions (the control group –FM, n = 5). In addition, two pigs were used to evaluate the distribution of LV-EGFP-transduced PBMCs in the area of SCI at day 14 after application. After the wound was sutured layer by layer, cefazoline (25 mg/kg, Pharmasyntez, Russia) and ketoprofen (1 mg/kg, Moscow Endocrine Plant, Russia) were injected (1 mg/kg) as intramuscularly for 5 days.

A sub-acute period was chosen for cell transplantation as autologous transplantation of PBMCs is technically feasible in clinical practice in case of indications for re-surgery when autologous PBMCs derived from a patient can be applied.

Motor function testing with PTIBS

To evaluate the effectiveness of motor function recovery the PTIBS (Porcine Thoracic Injury Behavioral Scale) was used [14]. The PTIBS is a 10 point scale that describes various stages of hindlimb function. Score 1 represents no active hindlimb movement, rump and knees are on the ground, with Score 10 describing normal ambulation with normal balance. Locomotor recovery in the study groups was video-recorded as previously described [15]. Motor function assessments were scored simultaneously by two observers who were blinded to the study groups.

Electrophysiological Studies

Electrophysiological tests were performed for intact and experimental pigs 2 and 11 weeks after SCI as previously described [15]. The animals neuromotor function was assessed by stimulating electromyography. M- and H-waves from the gastrocnemius muscle were recorded in response to stimulation of the sciatic nerve. Monopolar needle electrodes were used for both recording and reference. An active electrode was inserted into the middle of the muscle belly, with the reference one implanted within a region of the tendomuscular junction at the Achilles tendon. Electrical stimulation of the sciatic nerve was carried out with square-wave single stimuli lasting for 0.2 ms. For stimulation monopolar needle electrodes were inserted subcutaneously within an area where the sciatic nerve exits from the pelvis.

To evaluate pyramidal tracts transcranial electrical stimulation (TES) was used. Motor evoked potentials (MEPs) were registered from the gastrocnemius muscle using the same technique as that for M-response. Transcranial stimulation was performed by needle electrodes inserted under the scalp up to the contact with the skull bone. Cathode was placed in the middle approximately 0.5 cm

caudally from the interorbital line. Anode was placed in the middle near the occipital bone. 0.04 ms stimuli with the intensity ranged from 20 to 400 V were used.

Somatosensory evoked potentials (SEPs) were used to evaluate posterior columns of the spinal cord. To register them monopolar needle electrodes were subcutaneously inserted. To register potentials from the lumbar level an active electrode was inserted over the upper lumbar vertebrae, the reference electrode inserted over the middle thoracic vertebrae. For registration from the scalp an active electrode was inserted in the middle approximately 0.5 cm caudally from the interorbital line, the reference electrode inserted in the middle near the occipital bone. Electrical stimulation of the tail was performed by round electrodes with stimulus duration/dupigion of 0.2 ms. Stimulus intensity was chosen by tail movements (the smallest stimulus evoking tail movements was used).

Histological methods

In 4 months after treatment the animals were anesthetized and perfused with a 4% paraformaldehyde solution (4°C). An 8 cm fragment of the spinal cord was taken from the spinal column and fixed in a 4% paraformaldehyde solution for 2 days. Then the sample was transferred into 30% sucrose. Cryostat cross-sections of the spinal cord over 1 cm from the injury epicenter rostrally and cadually were stained with azur-eosin. Stained sections were embedded into vitrogel and studied under the APERIO CS2 scanner (Leica). The Aperio imagescope software was used to measure the tissue area.

Cytokine Assay

The multiplex analysis was performed using the MILLIPLEX MAP Porcine Cytokine/Chemokine (magnetic) kit #PCYTMG-23K-13PX (Millipore) as previously described [15]. Each experiment was done in triplets. The kit permits a simultaneous multiplex analysis of 13 pig cytokines/chemokines/interleukins in a 25-µL aliquot of porcine cerebrospinal fluid (CSF) and blood serum obtaining before SCI and on post-injury days 7 and 14.

Statistical Analysis

To process the results obtained the Origin 7 Pro software was used. Data are presented as mean ± standard deviation (SD). A one-way analysis of variance (ANOVA) with a Tukey's test were used for multiple comparisons between all study groups. All analyses were performed in a blinded manner with respect to the study groups. The significance level lower than 0.05 ($P<0.05$) was accepted for all statistical data.

3. Results

Distribution of transplanted PBMCs in injured spinal cord

In 14 days after application Ad5-EGFP-transduced PBMCs, specific fluorescence was detected at least 8 mm rostrally and cadually from the epicenter of injury. EGFP-labeled PBMCs with intense fluorescence clustered in pots-traumatic cavities and around them in the epicenter of injury (Fig.1A). At a distance from the site of SCI, $EGFP^+$-cells were primarily identified in the grey matter and in a small number in ventral and lateral funiculi of the white matter (Fig.1B, C). We also detected EGFP-labeled PBMCs in the area of epidural fibrosis, where more PBMCs were found above the caudal part of the spinal cord, with fewer PBMCs found in the synechias above its rostral part (Fig.1D-G). At the same time there was a positive correlation cadually –the more the distance from the epicenter of injury, the greater number of $EGFP^+$-cell were found in the synechias. Rostrally there was a negative correlation –the farther from the site of SCI, the fewer $EGFP^+$-cells in the synechias.

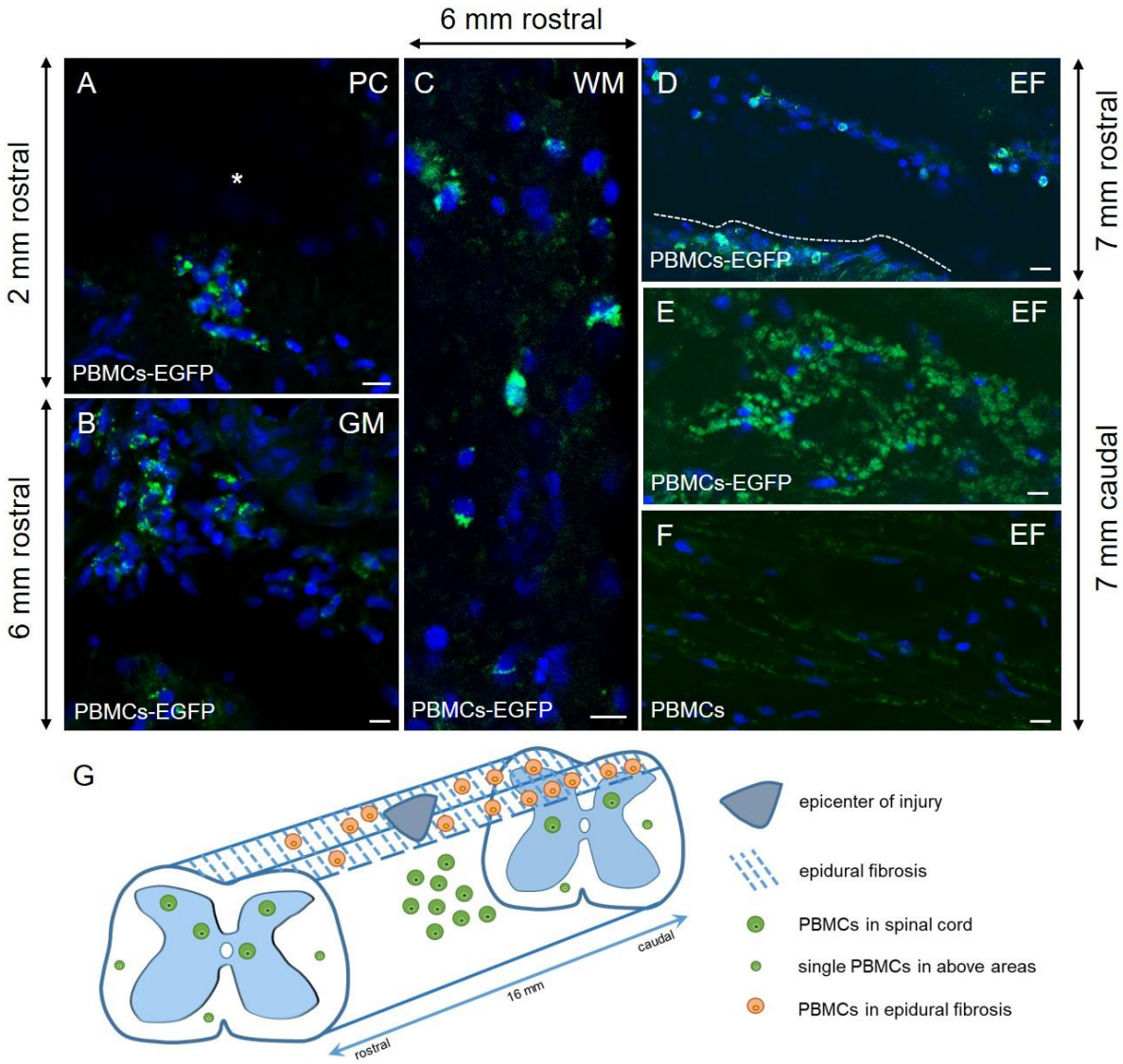

Figure 1. Distribution and survival of PBMCs-EGFP in the area of SCI in pigs. On day 14 Ad5-EGFP-labeled (green) PBMCs were located mainly in post-traumatic cavities (PC, asterisk) and around them at the epicenter of trauma (A). At a distance from the site of SCI, EGFP-PBMCs were found mainly in the grey matter (B, GM) and in small amounts in the white matter (C, WM). The EGFP-PBMCs distribution can be seen in the area of epidural fibrosis (EF); fewer $EGFP^+$-cells were found above the rostral part of the spinal cord (D, dashed line indicates the dorsal surface of the spinal cord), and more in the synechias above its caudal part (E). The area of epidural fibrosis in pig applied with Ad5-EGFP-unlabeled PBMCs is shown in F. Nuclei are stained with DAPI (blue). Scale bars: 10 μm. Schematic presentation of the EGFP-PBMCs distribution in the area of epidural fibrosis and spinal cord tissue according to the distance from the site of injury (G).

Histological evaluation of an injured spinal cord after PBMCs transplantation

There were significant differences in the area of intact tissue and a total area of abnormal cavities between the study groups (Fig. 2A-C). The application of FM+PBMCs improved the morphometry after cell therapy as compared to the group treated with FM only: the area of intact tissue was more at a distance of 2 mm rostrally and 3 mm cadually from the epicenter of injury ($p<0.05$), a total area of abnormal cavities was less at a distance of 3-4 mm cadually from the epicenter of injury ($p<0.05$). Nevertheless, the morphometry improved in distal areas (6-10 mm rostrally and cadually) from the injury epicenter in the control group treated with FM only, while the tissue integrity did not

significantly improve and achieved the plateau in the experimental group, with a total area of abnormal cavities significantly varying.

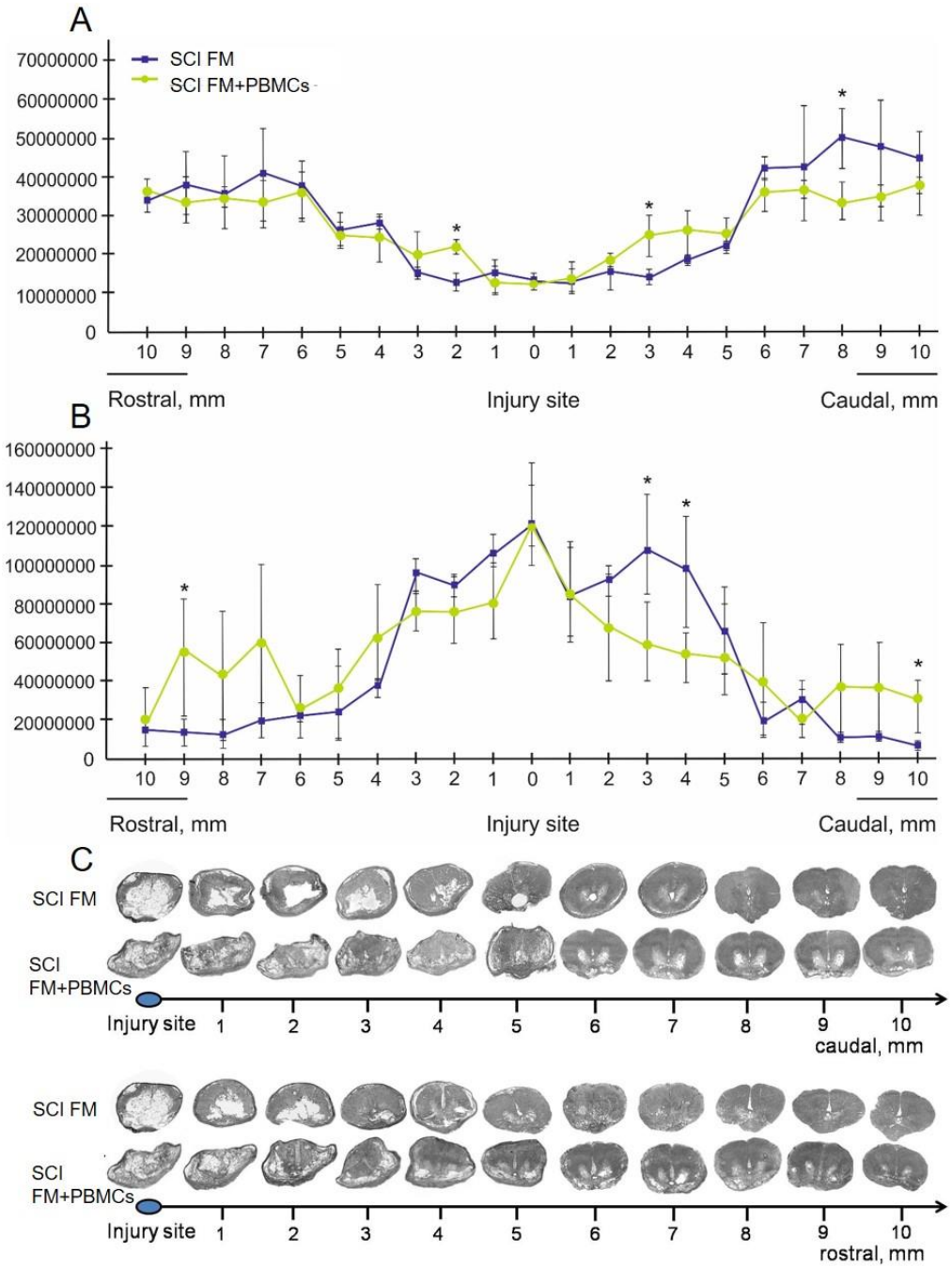

Figure 2. Spinal cord morphometry in experimental pigs. An area of the spared tissue (A) and a total area of abnormal cavities (B) 10 mm rostrally and caudally from the injury epicenter in 22 weeks after FM (blue line) or FM+PBMCs (yellow line) application. * $p < 0.05$, one-way ANOVA followed by a Tukey's post hoc test. (C) Cross-sections of the injured spinal cord at a distance of 10 mm rostrally and caudally from the injury epicenter in 22 weeks after SCI in experimental pigs. Azur-eosin staining.

Changes in behavioral and electrophysiology results

During 6 weeks after SCI, the pigs had a PTIBS score of 1.64±0.63; active hindlimb movements were either lacking or insignificant (Fig. 3A). After the application of FM only or FM+PBMCs, the animals had a similar rise of PTIBS scores within 4 weeks. In 10 weeks, there was no positive progress in pigs of the control group (FM), with an average score being 3.17±0.13. At the same time in the

experimental group (FM+PBMCs) motor activity scores slightly increased for up to 12 weeks of the experiment when subsequently reaching the plateau and with an average score of 3.74±0.08. However, differences in PTIBS scores between FM only and FM+PBMCs groups were not statistically significant throughout the study.

Electrophysiological parameters were recorded prior to injury and in 6 and 22 weeks after SCI. No significant differences in M-wave amplitude and latency were observed before and after the injury. TES recorded no MEPs and SEPs in all the animals in 6 weeks after SCI (Fig. 3B-D). These changes suggested the lack of conduction along both lateral and posterior columns of the spinal cord, that indicated the injury adequacy.

After application of FM or FM+PBMCs there were no differences in the conduction recovery along lateral columns: in 22 weeks the only animal of the control group treated with FM application had MEPs on one side, with MEPs recorded from both limbs in only 1 pig applied with FM+PBMCs. It should be noted that in animals with the conduction recovery the latency of MEPs was lower in that applied with FM+PBMCs. This may suggest a positive effect of PBMCs on restoration of conduction along spinal axons.

The main differences were related to the state of posterior columns of the spinal cord in the groups (Fig. 3E, F). In 22 weeks no SEPs from the cortex were recorded in the group of animals applied with FM only and the only pig had a lumbar peak. At the same time point, both lumbar and cortical peaks were recorded in 2 animals of the experimental group (treated with FM + PBMCs), with one animal having a cortical peak without a lumbar one. Thus, the conduction along posterior columns of the spinal cord partially recovered in 3 out of 5 pigs applied with FM+PBMCs.

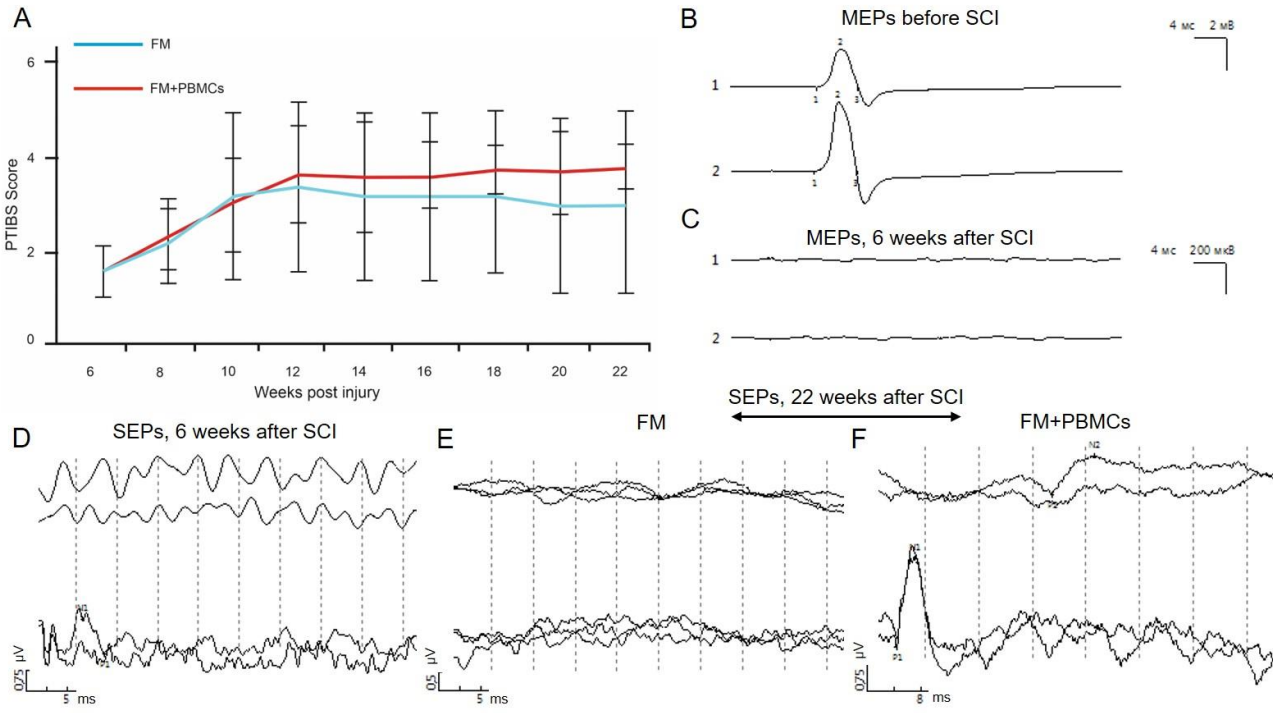

Figure 3. Behavioral testing and electrophysiology results in pigs. Locomotor PTIBS scores of FM (blue line) and FM+PBMCs (red line) groups (A). No significant differences were found between the study groups throughout the study. Electrophysiology results show MEPs before SCI (B) and MEPs/SEPs 6 weeks after SCI (C, D). In 22 weeks no cortical or lumbar SEPs were recorded in 4 pigs of the group applied with FM (E). Both lumbar and cortical peaks (F) were recorded in 2 animals of the group with FM + PBMCs application.

Cytokine Profile

Blood serum and CSF cytokine/chemokine profiles were evaluated on days 7 and 14 after application of FM or FM+PBMCs (Fig. 4). On day 7 after the beginning of treatment a CSF IL-18 level

increased and that of IL-1Ra decreased significantly in animals of the experimental group (FM+PBMCs) as compared to similar parameters in the control group (FM) (Table 1). In 2 weeks after application these differences alleviated and CSF cytokine/chemokine concentrations did not significantly differ in animals of the study groups.

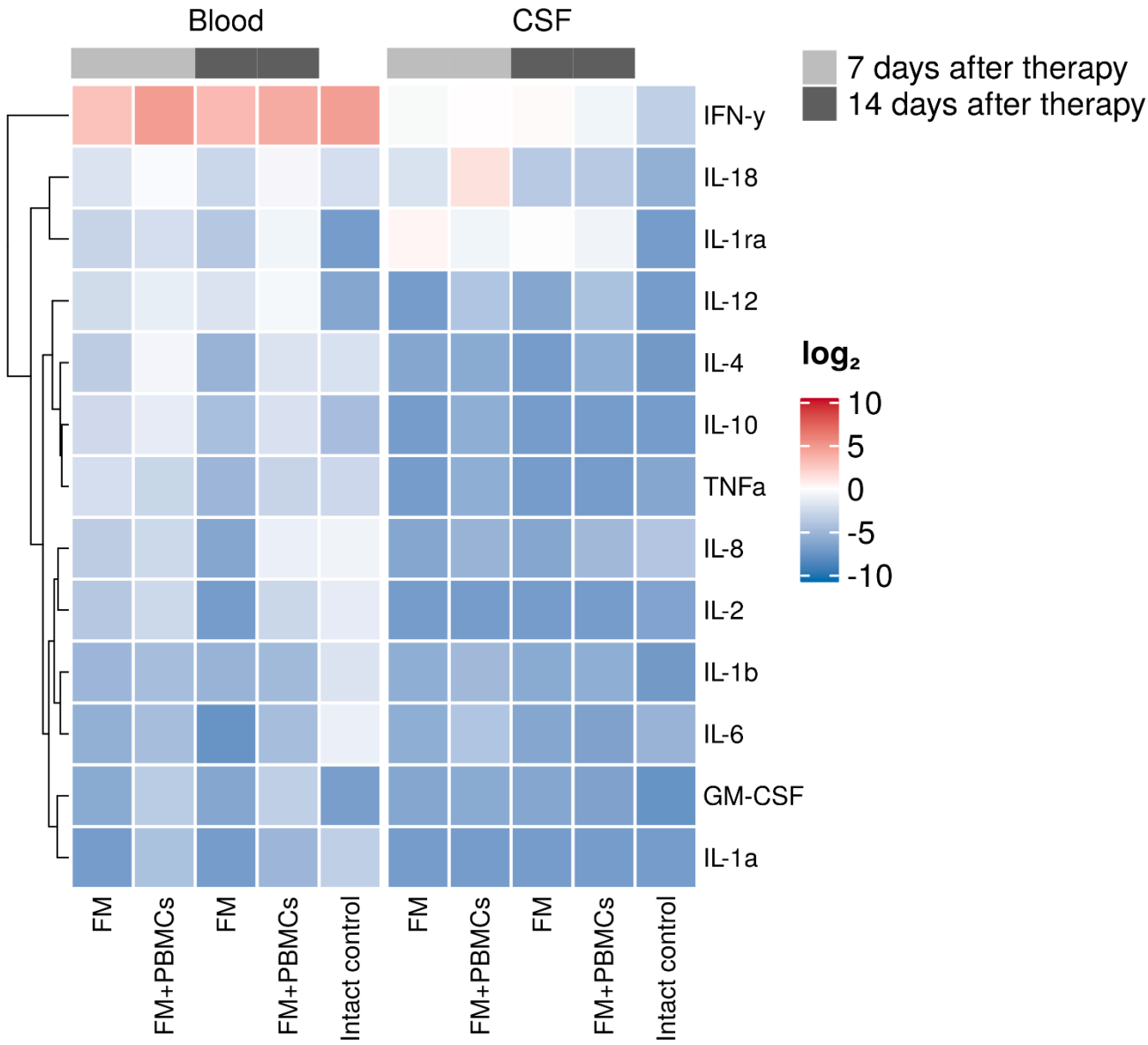

Figure 4. Heat maps showing cytokine/chemokine log10 concentrations, generated with the multiplex analysis of blood serum and cerebrospinal fluid before and after spinal cord injury in the study groups. Cytokine/chemokine profile on days 7 and 14 after FM or FM+PBMCs application. A dendrogram resulting from hierarchical clustering of cytokines is shown on the left.

On day 7 after application of FM+PBMCs a blood serum IFN-g level significantly increased as compared to the same parameter in the control group (FM). In 2 weeks after application IFN-g levels in both groups were similar; however, there were differences in IL-18 concentrations with higher levels in the FM+PBMCs group.

Table 1. Blood and cerebrospinal fluid cytokine/chemokine concentrations (ng/mL) on days 7 and 14 after FM or FM+PBMCs application. * $p<0.05$ when compared with similar analytes of intact samples, # $p<0.05$ when compared with similar analytes in this group in 14 days after therapy. Text in bold indicates significant difference ($p<0.05$) in this analyte between these groups in the corresponding

period after therapy. < - protein concentrations below the specified value could not be detected with the kit.

cytokine/ chemokine		Intact control	7 days after FM/ FM+PBMCs	14 days after FM/ FM+PBMCs
GM-CSF	blood	0.011±0.003	0.02±0.01/0.09±0.05*	0.01±0.005/0.11±0.09*
	CSF	0.006±0.003	0.01±0.006*/0.02±0.005*	0.016±0.005*/0.013±0.005*
IFN-g	blood	22.78±7.03	**7.16±6.34/25.54±4.46#**	8.99±5.35/15.81±2.61
	CSF	<0.12	0.79±0.47/1.05±0.17	1.12±0.05/0.63±0.35
IL-10	blood	0.05±0.02	0.19±0.15/0.44±0.47*	0.05±0.04/0.26±0.2*
	CSF	0.008±0.0007	<0.01/0.23±0.005*	<0.01/<0.01
IL-12	blood	0.01±0.007	0.21±0.16*/0.49±0.08*#	0.3±0.12*/0.72±0.14*
	CSF	0.009±0.0008	<0.01/0.07±0.01*	0.1±0.005*/0.6±0.3*
IL-18	blood	0.24±0.1	0.29±0.2/0.81±0.55*	**0.16±0.1/0.69±0.25***
	CSF	0.025±0.022	**0.29±0.3*/2.65±0.12***	0.09±0.01/0.09±0.01
IL-1a	blood	0.11±0.15	<0.01/0.06±0.03	0.01±0.005/0.03±0.01
	CSF	<0.008	<0.01/0.01±0	<0.01/<0.01
IL-1b	blood	0.29±0.32	0.03±0.007/0.04±0.02	0.03±0.015/0.04±0.005
	CSF	<0.009	0.02±0.005/0.04±0.005	0.02±0/0.02±0.005
IL-1Ra	blood	<0.01	0.15±0.09/0.22±0.05	0.08±0.03/0.62±1
	CSF	<0.01	**1.28±0.17#/0.62±0.02**	0.95±0.06/0.59±0.72
IL-2	blood	0.41±0.66	0.08±0.07/0.17±0.16	<0.01/0.16±0.05
	CSF	0.015±0.005	<0.01/<0.01	<0.01/<0.01
IL-4	blood	0.25±0.24	0.1±0.09/0.68±1.06	0.03±0.01/0.3±0.26
	CSF	<0.009	0.01±0.005/0.02±0	0.01±0/0.02±0.01
IL-6	blood	0.52±0.2	0.025±0.021*/0.047±0.042*	0.006±0.005*/0.05±0.06*
	CSF	0.03±0.012	0.02±0.005/0.07±0.1	0.016±0.005/0.01±0.005
IL-8	blood	0.6±0.42	0.1±0.02/0.18±0.08	0.01±0.005*/0.51±0.58*
	CSF	0.07±0.04	0.01±0.005/0.03±0.01	0.016±0.005/0.04±0.02
TNF-a	blood	0.17±0.19	0.2±0.03/0.15±0.06	0.03±0.02/0.15±0.06
	CSF	0.01±0.008	<0.01/0.023±0.005	<0.01/<0.01

4. Discussion

The fundamentals of SCI cell therapy have specific features which should be taken into consideration for the regenerative potential of stem and progenitor cells to be most effectively employed. A spinal cord injury is generally an acute condition which requires urgent medical intervention. In this regard and taking the rapid development of inflammatory and immune processes into account, regenerative therapy with the use of autologous cells in a sub-acute period of the disorder is preferable [4], [16]. At the same time, there can be a delayed application of cells as part

of a scaffold in a repeated surgical intervention when a neurosurgeon has an access to a surface of the spinal cord. In this case peripheral blood is a most available and convenient source for a clinical use of autologous adult stem cells.

In our study PBMCs were for the first time tested in a model of SCI in large animals when evaluating functional and structural parameters as well as blood serum and CSF cytokine/chemokine profiles in a delayed period after therapy. In addition, the migration activity of PBMCs embedded in FM and applied on the area of SCI was evaluated. The findings indicate that PBMCs can penetrate into the spinal cord and concentrate primarily in the grey matter. However, it should be noted that a part of PBMCs have remained within epidural fibrosis by day 14 after application on the area of SCI. We suppose this might be due to a low PBMCs migration rate and a resulting enclosure of some cells in rapidly forming synechias. For example, in our previous study MSCs embedded in FM transplanted into the area of SCI in pigs had a higher migration rate into the spinal cord, with there being a less number of them in synechias [15]. It is worth noting that PBMCs were not detected in the spinal cord on day 7 after intravenous transplantation to rats with SCI [17]. When intraspinally transplanted PBSCs were detected 2 mm rostrally and cadually from the epicenter of SCI in mice, with their number gradually decreased according to the time course [18].

The behavior analysis with the PTIBS did not show significant differences in the motor function recovery in pigs of the control (FM) and experimental (FM + PBMCs) groups, despite the positive dynamics in the case of using the proposed cell therapy. The positive effect of PBMCs was evidenced by electrophysiological data confirming partial recovery of conduction in the posterior columns of the spinal cord. An intraperitoneal injection of PBMCs-secretome was previously shown in a rat SCI model to improve the locomotor activity [19]. Intraspinal transplantation of G-CSF-mobilized peripheral blood stem cells (PBSCs) also promoted functional recovery in a mouse model of SCI [18]. Even in a week after a single intravenous injection to rats with SCI human peripheral blood-derived $CD133^{+}$ cells provided significant functional recovery [20]. These results in rodents are encouraging; however, it seems to be impossible to compare them with our findings to the full extent as different populations of PBSCs and animal models are used.

That tissue structure can be improved in the setting of PBMCs transplantation to rodents with SCI has been previously shown; a decreased area of abnormal cavities and a less damage of the myelin sheath were noted [18], [20], [21]. In our study, the application of FM + PBMCs on the site of SCI improved tissue integrity and reduced an area of abnormal cavities nearby the epicenter of injury, without stimulating regenerative processes at a distance.

The CSF cytokine/chemokine profile showed an increased IL-18 level on day 7 after the application of FM+PBMCs. IL-18, being the main immunoregulatory cytokine, is important in protecting the body against infections and tumors [22]. In the same time point, a CSF IL-1Ra level increased in animals treated with cell therapy as compared to intact controls and was lower when compared with the SCI group and FM application. Thus, mechanisms preventing the activation of the intracellular inflammatory signaling cascade of IL-1 were noted to develop in both groups, though to a different extent.

The treatment of rat SCI with the PBMCs-secretome was previously found to increase CXCL-1 and IL-10 expression levels in plasma [19]. In our study, in SCI a serum IL-10 level increased in both study groups as compared to the intact control. However, on day 7 after the application of FM + PBMCs, there was an increased blood serum level of IFN-g known as a potent pro-inflammatory factor that triggers the activation of microglia and the subsequent release of neurotoxic factors [23]. In 2 weeks after application IFN-g levels were similar in both groups; however, there were differences in the IL-18 concentrations, with a higher value in the FM + PBMCs group. Thus, our results showed the cytokine imbalance to occur without significantly shifting towards pro-inflammatory or anti-inflammatory cytokine cascades in the setting therapy provided. The data obtained confirm our previous research [24], showing the complexity of inflammatory reactions and immunological response after SCI, as well as their widespread prevalence, which is confirmed by cytokine profile changes in blood serum. Further investigations of the complex cytokine imbalance that occurs after

SCI, including that associated with cell-based therapy, are required in order to determine whether it can be correlated with the severity of injury and a clinical prognosis.

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