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Autologous M2-like macrophage applications in children with cerebral palsy

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Abstract

Following injury to the central nervous system (CNS), immune-mediated inflammation profoundly affects the ability of neural cells to survive and to regenerate. The role of inflammation, comprises mostly of macrophages, is controversial, since macrophages can both induce neuronal and glial toxicity and promote tissue repair. The opposite effects of macrophages may be conditioned by their functional heterogeneity. Thus, classical pro-inflammatory macrophages (M1) are tissue-destructive, while anti-inflammatory (M2) macrophages mediate tissue repair. In addition, M2 macrophages predominantly induce the Th2 response, which is particularly beneficial in CNS repair. Using growth factor deficiency conditions we have generated M2-like macrophages and evaluated the safety and clinical efficacy of endolumbar introduction of these cells in treatment of children with cerebral palsy (CP). Sixteen children from 2.0 to 8.0 years old with severe forms of CP were enrolled in this trial. Endolumbar administration of M2-like cells was accompanied by cytokine reactions in 10 (62.5%) persons. There was no evidence of local and systemic immediate hypersensitivity reactions, hematoma or infection complications related to cell transplantation. At 3 months after therapy the average Ashworth score decreased from 3.9 ± 0.2 to 3.1 ± 0.2 in the lower extremities ($p<0.01$). Gross Motor Function Measure (GMFM) test improved from 12.1 ± 9.0 to 60 ± 19 points ($p<0.01$). Three of six children experienced seizures arrest, and four children improved mental functions (improvement of speech and understanding). M2-like macrophage introduction was not accompanied by an increase of serum levels of interferon-gamma and interleukin-17, but resulted in significant enhancement of brain-derived neurotrophic factor (from 695 ± 60 to 1183 ± 153 pg/ml; pU=0.015) and a strong tendency to enlargement of vascular endothelial growth factor (from 190 ± 41 to 240 ± 40 pg/ml; pU=0.07). Our data indicate that transplantation of M2-like macrophages via lumbar puncture is safe and improves neurological status in children with CP. However, to better define the therapeutic effect of these cells in CP, randomized controlled prospective trials and long-term follow-up are required.

Keywords: M2-macrophages, cerebral palsy, cytokines, neurotrophic factors

Introduction

With damage to the nervous system, the activation of immune system and immune-mediated inflammatory reactions profoundly affect the ability of neurons to survive and to regenerate damaged axons. The role of inflammation, comprised mostly of macrophages, is controversial. Macrophages can cause neuronal and glial toxicity through

the proinflammatory cytokines, free radicals, eicosanoids and proteases [9,23]. However, recent studies have demonstrated that macrophages can enhance neuroprotection and promote long-distance axon growth, sprouting, and remyelination [20,33]. The positive effects of macrophages in CNS repair are considered to be mediated through the several mechanisms including phagocytosis and clearance of cell debris and inhibitory molecules [3,8]; limiting of glutamate-mediated excitotoxicity [31]; production of cytokines and growth factors with neuroprotective and regenerative activity [10,14,19,26]; attraction and activation of stem cells and neural precursors [21,38,1], and recruitment of T-cells capable of local production of neurotrophic factors and regulating glial cells [17,24]. The opposite effects of macrophages on CNS repair may be conditioned by the macrophage functional type. Thus, classical pro-inflammatory macrophages (M1) are tissue destructive, while anti-inflammatory (M2) macrophages mediate tissue repair [25,15,22,20]. In addition, M2 macrophages predominantly induce the Th2 response, which is particularly beneficial in the CNS repair [16]. Therefore, M2-like macrophages may be prospective candidates for cell therapy of brain injuries [7].

Cerebral palsy (CP) is defined as a chronic non-progressive motor impairment syndrome due to a problem in the developing brain [28]. CP is manifested with spastic paralysis often in combination with epileptic seizures and/or mental impairment, caused by damage to the frontal cortical (mainly motor) area of the developing brain, mostly during pregnancy [29]. Sigmund Freud was the first who hypothesized that cerebral palsy may be closely associated with natal development. However, brain development continues during the first two years of life, so CP can result from brain injury occurring during the prenatal, perinatal, or postnatal periods. CP affects at least 2 in 1000 children, leading to more than 1 million chronic patients under the age of 21 [36]. Since this disease does not substantially reduce the lifespan, cerebral palsy is an important social and economic problem.

No evidence exists that the brain damage can be reversed; however, maturational and adaptive processes may change the clinical picture of the child over time. Treatment for cerebral palsy, therefore, has the goal not to cure or to achieve healthy subject states but to increase functionality, improve capabilities, and sustain health in terms of locomotion, cognitive functions, social interaction, and independence. Most children with cerebral palsy require lifelong medical and physical care, including physical, occupational, and speech/swallowing therapy.

At the moment there is no cure for CP, so currently available treatments for patients suffering from CP are only supportive, not curative [27]. This forces the search for new therapeutic approaches in the field of brain pathology. In this context cell transplantation has become a promising therapeutic option for CP treatment, and macrophages are considered to be prospective candidates for cell therapy.

Using growth factor deficiency conditions we have generated M2-like macrophages that had low antigen-presenting and proinflammatory activity and possessed considerable regenerative potential (in particular, produced high amounts of IGF-1 and VEGF) [7]. We hypothesized such macrophages may be useful in CNS repair, so evaluated the safety and therapeutic efficacy of endolumbar introduction of autological macrophages in treatment of children with cerebral palsy.

Patients and methods

Study design

This was a phase I/II non-randomized open-labeled clinical study of chronic children who had severe cerebral palsy and received transplantation of autologous M2-like macrophage. Treatment of CP children using autologous M2-like macrophages transplantation and all studies were performed in accordance with study protocols after obtaining written informed consent from patients' parents. The clinical trial protocols and consent form were approved by the Institutional Academic Board and Institutional Review Board (Local Ethics Committee). The purpose of this study was to assess the safety and therapeutic efficacy of M2-like macrophages for treatment of CP patients. The families of all eligible patients were properly informed about the nature of the study.

Patients and selection criteria

Sixteen severely brain-injured, cerebral palsy children (n=16, 10 boys and 6 girls) were examined and subjected to cell transplantation therapy. Cerebral palsy was diagnosed in these children at 12 months. The age of the patients varied from 2 to 8 years (median 4.5 years). The time from diagnosis of CP to cell therapy ranged from 1 to 7 years. According to the developed protocol, generated macrophages were injected via lumbar puncture. All patients were followed up for the following 9 months after cell therapy. The inclusion criteria were: 1) age $\geq$ 12 months and $\leq$ 8 years; 2) diagnosis: spastic cerebral palsy with quadriplegia; 3) performance status: Gross Motor Function Classification System at level IV–V; and 4) parental consent. The exclusion criteria were: 1) autism and autistic spectrum disorders without motor disability; 2) progressive neurologic disease; 3) HIV or uncontrolled bacterial, fungal, or viral infections; 4) impaired renal or liver function (as determined by serum creatinine >1.5mg/dL and/or total bilirubin >1.3mg/dL); 5) genetic disease or phenotypic evidence of a genetic disease on physical examination; 6) requires ventilatory support; 7) unable to obtain parental consent.

Macrophage generation and assessment

Human peripheral blood mononuclear cells (PBMCs) were obtained through density gradient centrifugation (Ficoll-Paque, Sigma-Aldrich, Germany) of heparinized whole blood samples. For monocyte separation PBMCs were plated at 3–5 x10^6/ml in tissue culture dishes (TPP, Switzerland) in RPMI-1640 (Sigma-Aldrich, Germany) with 5% FCS (Biolot, Russia) for 18 h and then washed to remove non-adherent residual lymphocytes. The percentage of CD14-positive cells was demonstrated by flow cytometry to be greater than 90–93% of the total cells recovered. The generation of macrophages from plastic-adherent cells was performed according previously developed protocol

[7]. In brief, adherent cells were cultured in RPMI-1640 with supplements at 37°C with 5% CO2. To receive M2-like macrophages we used recombinant human GM-CSF (rhGM-CSF, 50 ng/ml, R&D Systems, USA) and serum deprivation conditions (low percent of autologous plasma). In 7 days the macrophages were harvested by using EDTA in Hanks' balanced salt solution, washed and counted. Then the generated M2-like macrophages were resuspended in 2 ml sodium chloride 0.9 % and infused into the spinal cord fluid of the patient.

For evaluation of the phenotype, cell suspensions were incubated for 20 min at 4°C with FITC- or PE-conjugated antibodies specific for human CD14, CD86, and HLA-DR or isotype controls (all from BD Biosciences, USA). Then cells were washed with PBS/ 0.1% sodium azide/ 0.1% bovine serum albumin, and analyzed with a FACSCalibur using CellQuest software (BD Biosciences).

The antigen-presenting/allostimulatory activity of the macrophages was determined by measuring macrophage capacity to induce T -cell proliferation in the mixed lymphocyte culture (MLC). Briefly, $1x10^4$ macrophages were plated in complete RPMI-1640 with $1x10^5$ allogeneic responder PBMCs for 5 days. Cell proliferation was measured by [^{3}H]thymidine incorporation (1 μCi/ well for 18 h). The stimulatory capacity of macrophages in MLC was expressed by the stimulation index (SI) = cpm in MLC (PBMCs+macrophages) / cpm in control culture (PBMCs alone). Th1, Th17 and Th2 - stimulatory activity was assessed by measuring of IFN-γ, IL-17 and IL-4/IL-10 in supernatants of MLC induced by macrophages. The stimulation index (SI) was calculated as ratio of cytokine level in MLC to spontaneous cytokine production in PBMCs alone.

The CP children's serum samples obtained before and after macrophage introduction were collected and frozen at –80°C until the measurement. The concentration of secreted cytokines and growth factors was determined using ELISA following the instructions of manufacturers: IFN-γ, IL-17, IL-4 (all from Protein Contour, St-Petersburg, Russia), brain-derived neurotrophic factor (BDNF; R&D Systems, USA) and vascular endothelial growth factor (VEGF; Invitrogen Corp., USA).

Measurement of safety and efficacy

All patients were evaluated according to a protocol by 3 independent experts including a neurologist, a neurosurgeon and one child's parents. Study evaluations consisted of thorough physical and neurological examinations and evaluations of adverse effects. The time from macrophage introduction to response was 3 months. The primary measure of efficacy was the improvement of motor functions of CP patients. To assess patients' motor abilities we used the Gross Motor Function Measure (an 88-item GMFM test), a criterion-referenced observational measure that was developed and validated to assess children with cerebral palsy [32]. The GMFM test includes evaluation of 88 items divided into 5 sections: 1, lying and rolling; 2, sitting; 3, crawling and kneeling; 4, standing; and 5, walking, running, and jumping. It evaluates the skills of the child in the individual items by using a 4-point scale on a quantitative basis. A secondary objective was to determine the effects on spasticity and muscle strength as well as mental faculties in these children. The five-point Ashworth scale was used for evaluating the degree of spasticity, and the six-point Medical Research Council Weakness Scale (MRC) served for muscle strength estimation.

In addition, we monitored the immediate reactions possibly related to the endolumbar introduction of autologous M2-like macrophages, which included allergic reactions (tachycardia, fever, skin eruption, leukocytosis) and local complications (hematoma or local infection).

Statistical analysis

The data were expressed as means ± SE. Statistica 6.0 software for Windows, StatSoft Inc. USA was used for analysis of data. Mean differences between groups were compared by a sign test. Additionally, the Mann–Whitney U test was used to compare nonparametric values.

Results

As shown in Table 1, the majority of children were diagnosed with severe spastic cerebral palsy, which can be considered the hallmark of our investigation. We did not have even a single child with a mild form of CP (when the use of both hands and/or gait is clumsy) and only one patient was diagnosed with a moderate degree of disease (characterized by the ability to use the affected hand in bimanual activities and/or impaired gait) [18]. It is evident from Table 1 that the examined children formed quite a homogenous group presumably with spastic forms of cerebral palsy (spastic quadriplegia was revealed in 14/16 children). The GMFM-88 score at entry was 12.1 ± 9.0. The majority of CP children had the fifth level of movement abnormalities, wher the child did not hold ,his head and back, all motor functions were limited, and these movement defects were not compensated by additional means.

Evaluating the degree of spasticity based on the Ashworth scale evidenced a considerable (4–5 point) increase in muscle tone in 14 out of 16 (87.5%) of CP children with an average Ashworth score of 3.9 ± 0.2. In two children even passive movements were hampered, and spasticity achieved 5 points. The MRC weakness score reflecting muscle strength in forearms was 1.8 ± 0.15, which indicated a marked reduction in muscle strength in all children at baseline. More than one third of CP children (6/16) had epileptic seizures. Mental faculties were impaired in virtually all patients. In fact, 14 out of 16 children had no capacity to speak, and 9 out of 16 did not understand addressed speech.

Each of sixteen trial patients received one grafting of autologous macrophages generated from their own peripheral blood according to our protocol (see Patients and methods). The motor and mental faculties of these CP children were evaluated at 3 months after the cell transplantation by 3 independent experts including a neurologist, a neurosurgeon and one child's parents. It is of great importance that their opinions practically coincided and the difference in their assessments was minimal.

First, we proved the principle possibility of generating M2-like macrophages in children with CP. Mean cell yield of macrophages was 77.1 ± 13.4x10^3 from 1x106 peripheral blood mononuclear cells. On average 0.8 ± 0.15 x 10^6/kg M2-like macrophages (0.18–2.58 x 10^6/kg) were used for the introduction (Table 2). The viability of the obtained cells in all cases was more than 90%.

Number	16
Gender (Boy/Girl)	10/6
Age (median), years	4.5 (2-8)
CP forms: - spastic - dystonic - atonia-ataxia - mixed	 12 (75%) 1 (6.2%) 2 (12.5%) 1 (6.2%)
Motor dysfunctions (according to Gross Motor Function Classification System): - level IV - level V	 2 (12.5%) 14 (87.5%)
Degree of spasticity (based on Ashworth scale): - level I–III - level IV - level V	 12.5% 75 % 12.5%
Muscle strength (points, MRC): - 1 point - 2 points - 3 points	 37.5% 50% 12.5%
Epileptic seizures	6 (37.5%)
Mental deficiency - non-understanding of addressed speech - non-speaking	 9 (56%) 14 (88%)
Cell Ther Transplant. 2011;3:e.000092.01. doi:10.3205/ctt-2011-en-000092-table1	

Table 1. Patients' characteristics

Macrophage characteristic	
Macrophage yield (from 1x10^6 MNCs)	77.1 ± 13.4x10^3
Number of macrophages x10^6/kg (M ± m, median)	0.8 ± 0.15 (0.6)
Cell viability	
Immunophenotype (% positive cells, min-max): HLA-DR CD14 CD86	 22–87 47–90 11–20
The ability to induce allo-T-cell response in MLC (stimulation index, SI)	0.36 – 1.46
Cell therapy safety	
Febrile temperature ± 1–2 fold vomiting in the first two days	10 (62.5%)
Subfebrile temperature without vomiting	1 (6.25%)
Local inflammatory or allergic reactions	0
Meningism	0
Local or systemic infections	0
Exacerbation of comorbidity - Atopic dermatitis	 1 (6.25%)
Cell Ther Transplant. 2011;3:e.000092.01. doi:10.3205/ctt-2011-en-000092-table2	

Table 2. Macrophage characteristic and cell therapy safety

Moreover, evaluation of these M2-like cells to stimulate various T–helper cells revealed that M2-like cells similar to M1 macrophages induce T lymphocytes to produce IL-4 and presumably IL-10, but in contrast to M1 did not induce secretion of Th1 (IFN-γ) and Th17 (IL-17) cytokines. In M1-stimulated cultures, a pronounced stimulation of IFN-γ and IL-17 was observed: 344 ± 104 pg/ml (SI 32.6 ± 11.1) and 402 ± 167 pg/ml (SI 80 ± 33), respectively. In marked contrast, M2-like macrophages did not induce nor IFN-γ (32 ± 21 pg/ml; SI 3.2 ± 2.1), or IL-17 (11 ± 4 pg/ml; SI 2.2 ± 0.76). At the same time both M1 and M2-like macrophages stimulated an equal production of Th2 cytokines (IL-4 and IL-10) in MLC: 115 ± 39 and 78 ± 38 pg/ml for IL-10 as well as 109 ± 24 and 97 ± 15 pg/ml for IL-4.

Endolumbar administration of M2-like cells was accompanied by fever and 1–2 fold vomiting in 10 out of 16 children (63%). These cell-therapy-related reactions never lasted longer than one or two days and were easily abrogated by the use of Dexasone and Cerucal. We did not observe any local or systemic immediate hypersensitivity reactions, local hematoma, or infection complications due to cell transplantation. However, one child demonstrated the exacerbation of atopic dermatitis.

As shown in Table 3, three months after cell therapy a significant decrease in spasticity was revealed. In the lower extremities of the CP children Ashworth scores decreased from 3.9 ± 0.2 to 3.1 ± 0.2 ($p<0.01$) while the muscle strength in the forearms was enhanced from 1.8 ± 0.15 to 2.9 ± 0.22 ($p<0.05$). The Gross Motor Function Measure test improved significantly from 12.1 ± 9.0 to 60 ± 19 points ($p <0.01$).

Apparent clinical improvements were noted in 11 out of sixteen cell-grafted CP children (68.8%; responding group). With the cell-based therapy, two-thirds of CP children (11/16) initially unable to retain their head in the vertical position in-

dependently became able to consistently execute this function. Among 15 children who initially failed to sit, after cell therapy 9 could sit without assistance. The majority of CP children involved in our investigation had no capacity to crawl. Only one boy could crawl on his abdomen, and two from 16 children could crawl/move on their backs by pushing their feet. With M2-therapy, eight children (43%) became able to crawl/move on their backs. Three of six children with seizure syndrome experienced seizures arrest, which persisted after the discontinuation of anticonvulsants.

Cell therapy significantly influenced mental functions. We observed a decrease in aggression (63%) and improvement of contact with outsiders (69%). Four children improved mental functions; they became able to understand or understand better the addressed speech (2/9) and showed the appearance of a meaning-bearing speech (2/14).

It should be noted that improvement of motor functions and mental abilities once registered at 3 months following cell administration persisted without reversion during the whole period of observation (until one year in some children).

Comparing children responding and not responding to cell therapy, we found some trends. First, the CP children with improved motor and/or mental functions were younger (4.6 ± 0.6 vs 6.4 ± 0.8 years; pU >0.05). Second, they received higher number of input cells (0.74 ± 0.15 vs 0.59 ± 0.17 x 106/kg; pU >0.05). And, finally, the development of cytokine reactions in children who responded to cell therapy was observed twice as often (in 82% vs. 40%) as in the non-responsived group.

Analysis of some cytokines and growth factor levels in the serum of CP children showed that the macrophage introduction was not accompanied by an increase of IFN- γ, IL-17, and IL-4. At the same time, such a therapy resulted in significant enhancement of brain-derived neurotrophic factor (BDNF; from 695 ± 60 to 1183 ± 153 pg/ml; pU =0.015) and strong tendency to an increase of vascular endothelial growth factor (VEGF; from 190 ± 41 to 240 ± 40 pg/ml; pU =0.07). It is of great importance that these changes were the most pronounced in the responder group.

Some examples of applying the cell transplantation therapy for cerebral palsy are described below.

The 2-year-old boy N. was evaluated for developmental delay at the age of 2 years. He was born as a result of premature delivery at 28 weeks, weighing 1200 g. The boy was from the second pregnancy (the first childbirth) with danger of fetus wastage. He stayed in the Intensive Care Unit for 16 days and in the Department of Newborn Pathology for 1 month.

On admission all motor and mental functions were profoundly defective. The patient demonstrated global developmental delay. The boy was incapable of turning from abdomen to back, holding a toy in his hand, sitting, standing, and walking. He kept his head in an upright position with great difficulty. He was incapable of tracking a toy with his eyes, speaking, and understanding addressed speech. Epileptic seizures occurred up to 6 times a day. The GMFM-88 score was 0, degree of spasticity according to the Ashworth scale was 4, and muscle strength in the forearms was reduced, at only 2 points.

The treatment included endolumbar administration of generated autologous M2-like macrophages (total dose $3.1x10^6$; 0.22x $x10^6$/kg). Cell introduction was accompanied by subfebrile fever and one episode of vomiting. Three months later the patient could turn from abdomen to back, hold a toy in his hands, sit, stand with support and retain his head in a vertical position. His epileptic seizures have completely stopped. The GMFM-88 score increased up to 164 and muscle strength in the forearms enhanced up to 4, while spasticity decreased to 2 points.

Parameters	Before cell therapy	After cell therapy
Degree of spasticity (Ashworth scale)	3.9 ± 0.2	3.1 ± 0.2**
Muscle strength in the forearms (MRC)	1.8 ± 0.15	2.9 ± 0.2*
Motor dysfunctions (GMFM-88)	12.1 ± 9.0	60 ± 19**
Note: * - p<0.05 and ** - p<0.01; sign test was used to determine the significance		
Cell Ther Transplant. 2011;3:e.000092.01. doi:10.3205/ctt-2011-en-000092-table3		

Table 3. Neurological improvement at three months after macrophage introduction

Cytokine/growth factor (pg/ml)	Before cell therapy	After cell therapy
	M ± m	
BDNF	695 ± 60	1183 ± 153 *
VEGF	190 ± 41	240 ± 40
IFN-γ	1.0 ± 0.6	1.0 ± 0.4
IL-17	<OOR	1.0 ±1.0
IL-4	14 ± 4.1	10.0 ± 2.7
Note: * - p<0.05 and ** - p<0.01; sign test was used to determine the significance		
Cell Ther Transplant. 2011;3:e.000092.01. doi:10.3205/ctt-2011-en-000092-table3		

Table 4. Cytokine and growth factor levels in the serum

At 6 months after therapy the boy could understand addressed speech. The child gained weight. He is without anticonvulsant therapy for 1 year.

The 5-year-old boy G. with cerebral palsy was from the fourth pregnancy with danger of fetus wastage. The childbirth was the second, premature at 32 weeks, as a result of Cesarean section. The newborn child had a weight of 2025 g. Prematurity II. He stayed in the Department of Newborn Pathology for 1 month. At one year, following a detailed assessment, the child was assigned a diagnosis of moderately severe spastic quadriplegia cerebral palsy.

On admission the patient could crawl, sit, stand with assistance, understood addressed speech and spoke. At the same time the boy was unable to walk without assistance and could not retain his head in a vertical position. Intelligence was unaffected. The GMFM-88 score was 158, degree of spasticity according to the Ashworth scale was 4, and muscle strength in the forearms was reduced, at 2 points. The treatment included endolumbar administration of generated autologous M2-like macrophages ($5.2x10^6$; $0.31x\ x10^6$/kg). Three months later the patient could walk without support and retain his head in a vertical position. The boy could make attempts to run with wide-set legs and walk up the stairs without support. The GMFM-88 score increased to 217 and muscle strength in the forearms was enhanced to 4, while spasticity decreased to 2 points.

Discussion

The brain possesses a limited capacity for endogenous regeneration after various insults, including perinatal hypoxia/ischemia. Therefore, the treatment of cerebral palsy as neurologic sequelae of hypoxia/ischemia-induced damage demands regenerative strategies. Recent studies have demonstrated that macrophages can enhance neuroprotection and promote axon growth, sprouting and remyelination [11]. Moreover, promotion of neuroregeneration was shown to be mediated predominantly by M2 anti-inflammatory macrophages [33,20]. The present study provides the first evidence for the possible application of M2-like macrophages for the treatment of cerebral palsy.

The evaluation of macrophage capacity to activate various types of T-helper cells as the first step of the present study revealed that generated M2-like macrophages did not stimulate T cells to produce IFN-γ and IL-17. This can be considered as the basic difference between the two types of macrophages investigated in our study, since classical M1 macrophages did induce substantial Th1 and Th17 cytokine production. With regard to Th2-stimulatory activity, M2-like macrophages did not differ from the M1 analogue as both stimulate detectable levels of IL-4 and IL-10. The last data are of great importance since Th2 cells are known to support neuron survival better than Th1 cells and in contrast to Th1 significantly stimulate axonal outgrowth. Namely, Th2 cells stimulate glial cells to produce neurotrophic factors without inducing inflammation[16]. Therefore, Th2-stimulatory activity of M2-like macrophages constituted an additional reason for the application of these cells for CNS repair.

In our study, we have shown that M2-like macrophages may be successfully generated in children with CP, and macrophage yields and functions in these cases are compared with that of adult healthy individuals [7]. Moreover, we first demonstrated that the introduction of M2-like macrophages via lumbar puncture in children with CP was safe, well tolerated and did not induce serious adverse reactions. Fever following macrophage administration observed in more than half of the patients was simply stopped with medication. There was no evidence of local immediate hypersensitivity reactions, hematoma, or infection at the site of the cell injection, and any serious infection complications related to the cell transplantation. Aggravation of atopic dermatitis registered in one person suggested the capacity of generated macrophages to activate Th2 response in vivo. This fact calls for a careful examination of patients for allergic diseases and may be exclusion criteria for M2 macrophage application in children with severe and diffuse forms of atopic pathology. On the other hand, exacerbation of atopic dermatitis in only one out of 16 cases evidenced that endolumbar application of macrophages obviously did not induce systemic activation of the Th2 response. This suggestion is confirmed by the results of IL-4 measurements of the serum of treated children. Indeed, we did not observe any enhancement of IL-4 serum levels after the introduction of M2-like macrophages.

Despite our clinical trial enrolling children with severe impairment of motor functions (predominantly V level of Gross Motor Function Classification System), cell therapy was accompanied by significant decreases in spasticity, increased muscle strength and enhancement of GMFM scores. Along with the increase in motor functions, more than half of the children displayed a decrease in aggressiveness and an improvement in communication with strangers. Enhancement of mental functions was also confirmed by appearance of the capacity to understand in two children and to speak in another two participants. In addition, three of six persons experienced full arrest of their seizure syndrome. Current therapeutic strategies of CP management are aimed at preventing brain damage, but at present there are no effective means to repair the brain once damage has occurred. From this point of view, our data are of significant importance. Similar results have been described recently by Chen L. et al. after introduction of olfactory ensheathing cells (OECs). These authors showed that OECs derived from aborted fetal tissue and injected into the bilateral corona radiata in the frontal lobes resulted in a significant increase of GMFM-88 score and improvement of mental functions in CP, according to the Caregiver Questionnaire Scale score [6].

The mechanisms underlying the clinical effects of M2-like macrophages in CP patients are not quite clear. We would like to point out some of them. As we have shown previously, M2-like macrophages are capable of spontaneous production of BDNF, IFG-1, EGF, bFGF, G-CSF, erythropoietin and VEGF, which possess neuroprotective activity and stimulate CNS regeneration [7]. Stimulation of CNS repair in ischemic brain damage may also be the result of increased angiogenesis and vasculogenesis [2,37]. In this connection an increase in serum levels of BDNF and VEGF following cell therapy indicate that clinical effects may be mediated by growth factors produced by macrophages or other paracrine-activated cells. Finally, recent

findings showed that monocytes/macrophages are able to differentiate into endothelium-like cells and function as precursors of endothelial cells [30] and thus participate in the repair of the vascular barrier after brain injury [12].

At the same time it should be emphasized that macrophage injection via lumbar puncture was not accompanied by an increase of serum IFN-γ or IL-17. Proinflammatory cytokines IFN-γ and IL-17 are known to have marked destructive effects on the nervous system [34,35]. From this point of view, the data that M2-like macrophages lack Th1- and Th17-stimulatory activity in vitro and in vivo are additional arguments evidencing the safety of their application. However, to better define the therapeutic effects of these cells in CP, randomized, controlled prospective trials and long-term follow-up are required.

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Применение аутологичных М2-подобных макрофагов у детей с церебральным параличом

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Резюме

Повреждение центральной нервной системы (ЦНС) сопровождается развитием иммуноопосредованных воспалительных реакций, которые оказывают существенное влияние на выживаемость и регенерацию нервных клеток. Роль воспаления, индуцированного главным образом макрофагами, противоречива, поскольку макрофаги могут проявлять как нейротоксическую активность в отношении нейронов и клеток глии, так и способствовать репарации нервной ткани. Оппозитные эффекты макрофагов могут быть обусловлены их функциональной гетерогенностью. Так классические провоспалительные макрофаги (М1) являются тканедеструктивными, а противовоспалительные макрофаги (М2) участвуют в репарации тканей. Кроме того, М2 макрофаги индуцируют преимущественно Th2 ответ, который в наибольшей степени способствует восстановлению ЦНС. Используя культуральные условия с дефицитом ростовых факторов, мы разработали метод получения М2-подобных макрофагов и исследовали безопасность и клиническую эффективность эндолюмбального введения этих клеток в лечении детей с церебральным параличом (ЦП).

В исследование были отобраны 9 детей в возрасте от 2 до 8 лет с тяжелыми формами ЦП. Эндолюмбальное введение М2-подобных макрофагов сопровождалось развитием цитокиновых реакций у 10 (62,5%) детей. Локальных или системных аллергических реакций немедленного типа, а также гематом или инфекционных осложнений, связанных с введением клеток, не отмечалось. Через три месяца после лечения уровень спастичности в нижних конечностях (балл по шкале Эшворта) снизился с 3,9 ± 0,2 до 3,1 ± 0,2 ($p<0,01$).

Уровень двигательной активности по шкале GMFM (Gross Motor Function Measure) возрос с 12,1 ± 9,0 до 60 ± 19 баллов (p<0,01). У трех из шести детей было отмечено полное прекращение судорожного синдрома, у четырех детей отмечено улучшение ментальных функций (способность понимать обращенную речь и говорить). Трансплантация М2-подобных макрофагов путем люмбальной пункции не сопровождалась возрастанием уровня интерлейкина-17 и интерферона-γ в сыворотке крови, однако приводила к достоверному увеличению нейроторофического фактора головного мозга (с 695 ± 60 до 1183 ± 153 пг/мл; pU=0,015) и выраженной тенденции к возрастанию концентрации фактора роста эндотелия сосудов (с 190 ± 41 до 240 ± 40 pg/ml; pU=0,07).

Полученные нами данные свидетельствуют о том, что эндолюмбальное ведение М2-подобных макрофагов является безопасным и улучшает неврологический статус у детей с ЦП. Однако для более полной оценки терапевтического потенциала этих клеток у детей с ЦП необходимы дальнейшие рандомизированные контролируемые проспективные исследования с оценкой отдаленных эффектов.

Ключевые слова: М2-макрофаги, церебральный паралич, цитокины, нейротрофические фактор

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