Cell Therapy of Cerebral Palsy

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The paper presents the results of a controlled study of cell therapy in 30 patients with severe forms of cerebral palsy. A cell suspension from immature nervous and hemopoietic tissues was injected into the subarachnoidal space of a recipient through a spinal puncture. Immune sensitization to donor antigens (detected by suppression of lymphocyte migration) was noted in few patients. In none patients laboratory and clinical signs of tissue-destructive autoimmune reactions were observed. One year after treatment activity of the major psychomotor functions in treated patients considerably surpassed the normal. No delayed complications of cell therapy were noted. These findings suggest that cell therapy is an effective, safe, and immunologically justified method of therapy for patients with cerebral palsy.

Key Words: *cell therapy; cerebral palsy*

Cerebral palsy (CP) is a severe disabling disease caused by brain injury during its active development. The main symptoms of this condition are inability to retain normal posture and perform targeted locomotor acts, psychic, speech, vision, and hearing disorders. Children with severe CP are disables requiring permanent care [1,7].

The efforts of clinical specialists are directed towards correction of various clinical symptoms of multifactorial disontogeny of CNS developing in CP [1,7]. Standard approaches cannot solve the main problem of CP associated with reparative recovery of the damaged brain. Compensatory capacities of CP patients are limited by the severity of brain damage and initially low reparative activity of the nervous tissue.

It was shown that transplanted low-differentiated (stem) cells can considerably enhance reparative capacities of the nervous tissue and improve the function of damaged brain [5,6,8]. Clinical studies also confirmed the efficiency of application of cell technologies in the therapy of severe brain injuries characterized by different etiopathogenesis and clinical manifestations [3-5]. Despite convincing substantiation and evident prospects of cell therapy in neurological disorders, only few attempts to apply cell technologies in the treatment of CP were made [5]. Therefore, it seems interesting to evaluate the role of cell therapy in rehabilitation of children with severe CP, which are usually resistant to standard treatment and require great social expenditures.

MATERIALS AND METHODS

The protocol of clinical studies was approved by the Scientific Council and Ethic Committee of the Institute of Clinical Immunology, Siberian Division of the Russian Academy of Medical Sciences. Informed consent was obtained from close relatives of each participant.

Tissues were isolated from human fetuses 16-20-week gestation (spontaneous or prostaglandin-induced abortions). Cell suspension was prepared by gentle squeezing of the tissue specimens [10]. The cells were cryopreserved routinely in fetal calf serum with 10% DMSO and stored in liquid nitrogen vapors. On the day of transplantation the cell suspension was defrosted at 37°C and cell viability was evaluated routinely by erythrosin staining.

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The dose of cells for one transplantation depended on patient age and body weight (mean dose 1×10^8 cells). The ratio of nervous cells to hemopoietic liver cells in the transplanted suspension was 10:1. The cells were injected into the subarachnoidal space of the recipient through a spinal puncture.

Cell reactivity to antigens was evaluated by inhibition of cell migration *in vitro* by using the method of evaluation of delayed-type hypersensitivity reaction in our modification *in vitro* [2]. Nucleated peripheral blood cells (2×10^5) were transferred into round-bottom wells of a 96-well plate (BDSL) and cultured for 18 h in RPMI 1640 supplemented with 10% autologous plasma, 10 mM HEPES, 4 mM L-glutamine, 5×10^{-5} M mercaptoethanol, and antibiotics in the presence of 10% lysate of donor cells (2×10^7/ml) or myelin (1μg/ml) at 37°C and 5% CO_2. Nonadherent cells were removed by washout with warm medium (37°C) and the number of adherent cells was determined by accumulation of formazan as described earlier [9,11]. All reagents were from Sigma.

The titers of serum antigens to myelin, DNA, cardiolipin, thyroglobulin, and thyrocyte microsomal fraction were measured using Vektor-Best kits and reagents according to manufacturer's instructions.

Physical and psychoemotional state of patients was scored using a 100-point scale characterizing 10 main psychomotor functions usually evaluated in neurological practice [1,7]. Seven parameters characterized motor functions (head holding, toy holding, turning from the abdomen to the back, crawling, sitting, standing, and walking), while other 3 parameters characterize the psychoemotional sphere (watching the toy, speech perception, and speech function). The absence, partial and complete performance of each function was scored as 0, 5, and 10. The performance was incomplete, if the patient makes attempts, but cannot attain the result. The scale is simple and convenient, it allows to exclude descriptive methods and to minimize subjectivism in the evaluation of the neurological state. This scale can be used for children of different ages and with different manifestations of the disease.

Incomplete functions do not extent functional capacities of the patient. Therefore during evaluation of self-service capacities, the absence of function and its incomplete performance was scored as 0. Similarly to evaluation of the main psychomotor function, unassisted performance was scored as 10.

The main indications for cell therapy were severity and unfavorable prognosis of the disease, and inefficiency of standard therapy for 1-2 years. Contraindications to cell therapy were chronic and acute infectious processes and severe concomitant diseases.

Treatment group included 30 children (19 boys and 11 girls) aging 1.5-7 years. The majority of these children (26, 87%) had double hemiplegia, the least curable form of CP. They had profound motor disorders due to increased muscular tone (spasm-rigidity), psychoemotional disturbances (oligophrenia, imbecillity, or idiocy), and speech disorders. Two patients (7%) had spastic diplegia and two had atonic-astatic form of CP. Magnetic resonance tomography revealed dilation of subarachnoidal spaces and atrophic hydrocephalus in 20 patients (67%). One patient (3%) had internal hydrocephalus (he underwent shunting procedure). In one patient severe inherited pathology of the brain was found. Six patients (20%) had intracranial hypertension. The overwhelming majority of patients had pronounced changes in EEG in the form of diffuse and local pathological rhythm disturbances.

The control group included 30 children receiving standard complex therapy (including drug treatment). This group was formed according to the paired principle: for each patient of the treatment group we selected a control patient with similar clinical parameters. Due to this approach the groups were well matched by clinical and prognostic parameters and time of observation (Table 1).

The results were analyzed by the Student and Mann—Whitney test.

RESULTS

A total of 73 transplantations were carried out in 30 patients (15 and 14 patients received 2 and 3 procedures, respectively, and one patient had one transplantation). Premedication including hormones, diuretics, and antiinflammatory drugs was aimed at prevention of early complications manifesting in meningism, vomiting, headache, and fever. These symptoms were effectively corrected in most cases and the patients stay in the hospital for not longer than 5-7 days.

Blood and liquor tests were performed before and one month after transplantation. Cell transplantation had practically no effect on blood cells and biochemical parameters and on liquor cytosis and protein content (data not shown). Thus, transplantation did not induce potentially dangerous toxic and inflammatory encephalitic reactions.

Cell reactivity to donor antigens was tested before and after transplantation in 20 children (12 boys and 8 girls) aging 2-12 years (mean age 5 years). Only 3 patients (15%) demonstrated considerably increased cell reactivity to donor antigens (Table 2). Subarachnoidal cell transplantation had practically no effect on the content of autoantibodies to myelin, DNA, cardiolipin, thyroglobulin, and thyrocyte microsomal fraction (Table 2).

TABLE 1. Characteristics of Patients

Parameters	Main group	Control group
Total number of patients	30	30
Boys	19	18
Girls	11	12
Mean age	3.1	3.2
Double hemiplegia	26	26
Spastic diplegia	2	2
Atonia-astatia	2	2
Mean score of functional activity ($M\pm m$)	14±2	14±3

The main psychomotor functions were evaluated before and one year after treatment. Before the therapy 24 of 30 patients in the treatment group had no coordinated muscular activity allowing them to hold the head. The absence or incomplete performance of this function was observed in 11 and 13 patients, respectively. After transplantation 21 (87%) of 24 patients can hold the head. In the control group, of 28 patients initially not holding the head (0 and 5 points in 6 and 22 patients, respectively) only six (21%) performed this function after one year.

Before the therapy none of the patients in the treatment group held the toy in the hand (0 and 5 points in 27 and 3 patients, respectively), while after treatment this function appeared in 27 patients. Of 30 control children unable to hold the toy in the hand (0 and 5 points in 22 and 8 patients, respectively) improvement was noted in only 4 patients.

Of 29 children of the treatment group unable to turn from the abdomen to the back (0 and 5 points in 28 and 1 patients, respectively) this function appeared in 20 patients, while none of 30 controls (0 and 5 points in 27 and 3 patients, respectively) attained this result.

Before the treatment 29 children in the main group (0 and 5 points in 24 and 5 patients, respectively) and 30 children in the control group (0 and 5 points in 24 and 6 patients, respectively) could not sit without support. After cell therapy this function appeared in 17 patients, while in the control group only 5 patients were able to retain sitting posture.

Initially, the ability to stay was absent in 29 children in the main group (0 and 5 points in 25 and 4 patients, respectively) and 30 children in the control group (0 and 5 points in 22 and 8 patients, respectively). After treatment this function appeared in 10 patients receiving cell therapy and in none of the controls.

Before treatment walking was absent in 29 patients of the main group (0 and 5 points in 28 and 1 patients, respectively) and 30 patients of the control group (0 and 5 points in 29 and 1 patients, respectively). After treatment this function appeared in 6 children receiving cell therapy, and in none of the controls.

Initially, 27 children in the main group (0 and 5 points in 20 and 7 patients, respectively) and 28 controls (0 and 5 points in 19 and 9 patients, respectively) could not focus on the toy. After treatment 23 children receiving cell therapy could watch the toy, while in the control group this function appeared in only 3 patients.

Before treatment 23 children in the main group (0 and 5 points in 20 and 3 patients, respectively) and 26 children in the control group (0 and 5 points in 15 and 11 patients, respectively) did not understand speech addressed to them. After therapy this function appeared in 18 patients receiving cell therapy and in 8 controls.

Initially, the speech was absent in all children of both groups. After therapy speech appeared in 12 patients receiving cell transplantation and in 1 patient in the control group.

In general, the level of the main psychomotor functions in the main group 2-fold surpassed that in the control (Fig. 1, *a*). Functional improvement in a patient of the control group usually did not attain the level allowing unaided realization of this function. At the same time, functional improvement in children of the main group extended their functional capacities and considerably increased self-service level (Fig. 1, *b*). All intergroup differences are reliable ($p<0.001$).

TABLE 2. Antigen-Specific Immunoreactivity of Patients after Subarachnoidal Cell Transplantation

Antigen	Number of patients	Type of immunoreactivity	Number of patients with positive reaction (%)
Donor alloantigens	20	Migration test	3 (15)
Myelin	12	Autoantibodies	0 (0)
Cardiolipin	17	Autoantibodies	0 (0)
DNA	7	Autoantibodies	0 (0)
Thyroglobulin	17	Autoantibodies	0 (0)
Thyrocyte microsomal fraction	17	Autoantibodies	0 (0)

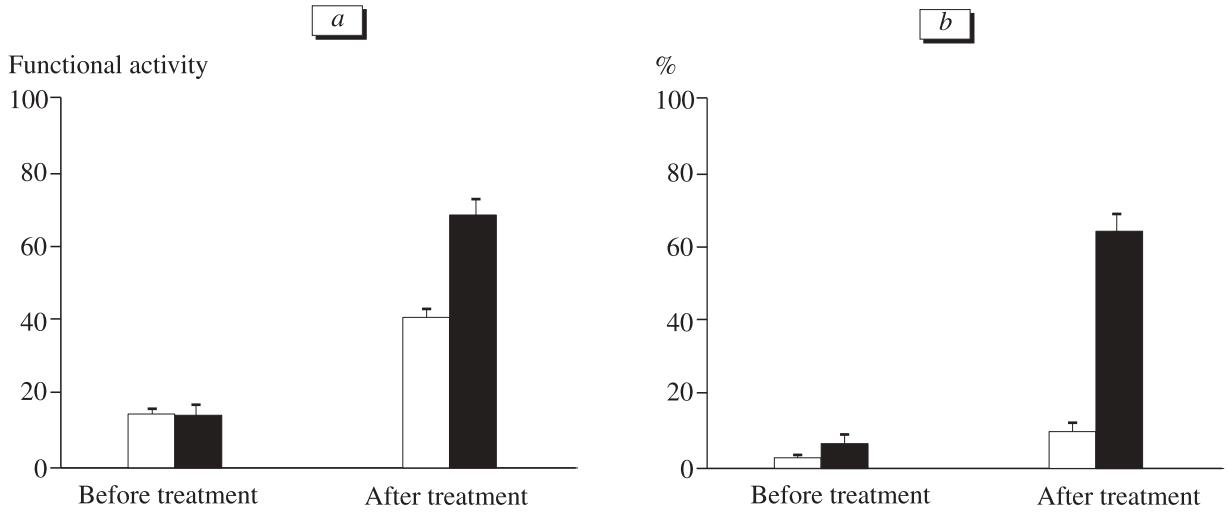

Fig. 1. Major psychomotor functions (*a*) and self-service capacity (*b*) in patients before and one year after therapy. Open bars: control; light bars: cell therapy.

Initially, convergent strabismus of the central genesis was observed in 18 patients. After cell transplantation correction of this condition was noted in 8 patients, while in others no improvement was achieved.

According to EEG data, epileptiform activity was found in 4 patients. After treatment, these episodes became less frequent and epileptiform activity in EEG disappeared in 3 patients.

The positive dynamics in the neurological status closely correlated with positive changes in the eye fundus. Initially, 25 of 30 patients in the main group had retinal hypertension angiopathy. After treatment normalization of the eye fundus was found in all patients with positive clinical dynamics, which attested to improved cerebral hemodynamics.

Increased reactivity to donor antigens (lymphocyte migration test) was observed in few patients receiving cell therapy. However, proliferative response of T cells to donor antigens in these patients did not surpass the initial level (data not shown). These findings can be explained by low percentage of sensitized lymphocytes in the total T cell population of the recipient.

Is was hypothesized that take of immunogenic allogenic cells can induce immune response directed not only towards donor antigens, but also against autoantigens with similar structure expressed on cells of different organs and tissues. However, our findings suggest that subarachnoidal transplantation of allogenic cells is not associated with the development of systemic autoimmune processes and, therefore, is a safe method of therapy.

Transplanted cells affect the brain of the recipient via a complex mechanism, but the leading role can be allocated to the production of neurotrophic factors and transmitters stimulating the formation of new blood vessels and improving cerebral hemodynamics. Neurotrophic factors can stimulate functional activity of nerve cells and brain structures (among them structures depressed due to the development of CP). Different transplanted cells can promote the growth and myelination of neuronal processes and restore damaged communications [5]. The fact that clinical improvement in the majority of patients was observed starting from the first few days of cell therapy indirectly confirms the important role of production of neurotrophic factors in the mechanisms of the therapeutic effects of donor cells on damaged brain. This does not exclude possible therapeutic role of the formation of new synaptic interactions between donor neural cells with recipient neurons and their participation in neural transmission. This possibility was demonstrated in many experimental studies [5,6,8]. However, it is unlikely, that small number of donor neurons migrating into the damaged foci and survived there can sharply improve brain functions due to the formation of new neural communications.

No one case of reversion of the clinical effect was noted over the entire observation period (more than 3 years for some patients). No delayed complications determined by functional activity of transplanted cells were observed.

Thus, cell therapy is an effective, safe, and immunologically justified method of treatment in CP. Our findings can provide the basis for planning wide-scale studies on the use of this method in children neurological practice.

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