Immunological and Clinical Aspects of Cell Therapy in the Treatment of Aftereffects of Craniocerebral Injury

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Cell suspension consisting of cells from immature nervous and hemopoietic tissues was transplanted subarachnoidally to patients with craniocerebral injury aftereffects. In some patients cell therapy led to immune sensitization to donor antigens, detected by the leukocyte migration inhibition test. No signs of tissue-destructive autoimmune reactions were detected in patients receiving cell therapy. Follow-up of 56 patients showed that cell therapy was associated with significant improvement of the neurological status. No serious complications of this treatment modality were observed. Presumably, cell therapy is a safe method which can be used in the treatment of craniocerebral injury aftereffects.

Key Words: *cell therapy; immunity; craniocerebral injury*

Craniocerebral injury (CCI) is the main cause of severe disability in young patients. The treatment of CCI aftereffects is difficult because of low reparative potential of the nervous tissue. Drug therapy including neuroprotectors, antioxidants, hormones, and drugs improving cerebral circulation is not always effective in this patient population [7]. Today great attention is paid to potentialities of cell therapy in the treatment of severe neurological disorders. The CNS is a privileged organ from immunological viewpoint. It means that donor allogenic cells transplanted into the CNS are largely protected from immune reactions directed against them. It was shown that cells derived from immature nervous tissue produced a totality of mediators stimulating the growth and myelination of nerve fibers. Moreover, they can be involved in the formation of new routes for nerve pulses conduction [5]. Realization of the functional potential of these cells can lead to an appreciable reduction of neurological deficiency caused by brain injury [2]. However, the attitude of physicians to the use of cell technologies in neurosurgical practice remains watchful. This attitude is largely due to the lack of information on various aspects of clinical use of cell technologies. This paper presents clinical and immunological results of transplantation treatment of patients with manifest consequences of CCI.

MATERIALS AND METHODS

Clinical trials were carried out in accordance with the protocol approved by Academic Council and Ethic Committee of Institute of Clinical Immunology. Each patient gave informed consent to participation in the study.

Tissues were derived from human fetuses (16-22 week gestation) after spontaneous or prostaglandin-induced abortions. Cell suspension was prepared as described previously [10]. The resultant cells were cryopreserved by the standard method in fetal calf serum with 10% dimethylsulfoxide and

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stored in liquid nitrogen. Cell suspension was defrosted on the day of transplantation at 37°C. Cell viability was evaluated by the standard method (Trypan Blue staining).

Cell reactivity to antigens was evaluated by cell migration inhibition in the presence of cell lysates by means of delayed hypersensitivity test in our *in vitro* modification [1]. Peripheral blood nuclear cells were placed (2×10^5) into round-bottom wells of a 96-well plate (BDSL) and cultured for 18 h in RPMI 1640 with 10% autologous plasma, 10 mM Hepes, 4 mM L-glutamine, 5×10^{-5} M mercaptoethanol, and antibiotics in the presence of 10% donor cell (2×10^7/ml) lysate or myelin (1 μg/ml) at 37°C and 5% CO_2. Nonadherent cells were removed by washout with warm (37°C) medium and the number of adherent cells was evaluated as described previously by accumulation of formazan, formed from 3-(4,5-dimethylthiasol-2-yl)-2,5-diphenyltetrazoleum bromide (MTT) [9,11]. All reagents from Sigma.

Serum antibodies to myelin, DNA, cardiolipin, thyroglobulin, and thyrocyte microsomal fraction were evaluated using Vector-Best reagents and commercial kits according to the instructions.

Delayer results of cell therapy were studied in 56 patients (19 women and 37 men) aged 18-73 years with severe CCI aftereffects (psycho-organic syndrome). Time after injury did not exceed 2 years. All patients developed cystic adherent arachnoiditis with signs of hemispheric degeneration and hydrocephalus. Twenty-three patients had subarachnoidal and 11 intracerebral cysts, porencephalic in 5 cases. Intracerebral hypertension was combined with disorders in the intellectual mnestic functions with focal organic symptoms. Ten patients suffered from generalized convulsive syndrome difficult to arrest.

RESULTS

Reactivity of peripheral blood cells of 34 patients to donor cell lysates and to myelin was studied in the migration test 1 month after cell transplantation. Cells were tested only from patients who did not react to the above antigens before transplantation. Enhanced cell reaction (>15% cell migration inhibition index) to cell lysates and myelin was detected in 8 (23%) and 5 (15%) patients, respectively (Table 1).

In order to evaluate the effects of transplanted cells on humoral autoimmune reactions, levels of antimyelin autoantibodies, and autoantibodies routinely determined in clinical practice, the tests were carried out before and 1 month after transplantation. Eight (42%) of 19 patients had initially elevated levels of antibodies to myelin before cell transplantation. Presumably, this was due to CCI. Only 1 of 19 patients the titer of antimyelin antibodies markedly increases (2-fold) during the posttransplantation period (Table 2).

Initially elevated (compared to normal) levels of antibodies to DNA, cardiolipin, thyroglobulin, and thyrocyte microsomal fraction were detected in 1 (3%), 7 (19%), 3 (8%), and 4 (11%) of 37 (100%) patients, respectively. In none of examined patients the levels of test autoantibodies increased in response to cell transplantation (Table 2), but remained at the basal level in all cases.

Sixteen patients received single subarachnoidal cell transplantation, 32 received two, and 8 patients received 3 transplantations. Clinical effect of different intensity presenting as regression of common cerebral symptoms, decreased extrapyramidal tone, and improvement of the mental emotional sphere was noted in all patients. Epileptic attacks ceased in 4 patients, due to which anticonvulsants could be discontinued 3-4 months after therapy. In other patients with epileptic syndrome the dose of anticonvulsants was reduced 2-3-fold for 1.5 years. One year after transplantation magnetic resonance tomography showed regression of degenerative hydrocephalus and EEG showed normalization of biorhythm. All patients felt stronger, more energetic, with better working capacity and moods. No serious side effects of cell therapy were detected. Due to treatment all patients later appreciably extended the range of rehabilitation measures.

TABLE 1. Patients' Cell Reactivity to Donor Antigens and Myelin

Antigen	Number of patients	Number of reactive patients (%)
Donor alloantigens	34	8 (23)
Myelin	34	5 (15)

TABLE 2. Humoral Autoreactivity of Patients after Cell Therapy

Autoantigen	Number of patients	Number of reactive patients (%)
Myelin	19	1 (5)
DNA	37	0 (0)
Cardiolipin	37	0 (0)
Thyroglobulin	37	0 (0)
Thyrocyte microsomal fraction	30	0 (0)

Addition of fetal liver cells (belonging mainly to hemopoietic differentiation lines) to the transplant was based on the data indicating that hemopoietic tissues contain stem cells differentiating into cells forming nervous tissue [4]. It is noteworthy that immature hemopoietic tissue contains endotheliocyte precursor cells providing neovascularization of ischemic tissues [8].

Immunological privileges of the CNS are not absolute. High reactivity to donor antigens detected in the migration test (Table 1) was induced in some patients. However, the proliferative response of T-lymphocytes to donor antigens in these patients as a rule did not surpass the basal level (data not presented). We explain these results by low percentage of sensitized lymphocytes in the total T-cell population.

Migration test showed increased reactivity to myelin in some patients. Similarly as with transplantation antigens, this reactivity was not detected in the proliferative test and as a rule was not associated with the development of humoral immune response. Low autoimmune reactivity did not seem to lead to the development of tissue destruction. However, we observed no induction of total systems pathological autoimmune reactions in treated patients (Table 2). It is known that immunocompetent cells penetrating into the focus of brain injury can become the main source of the neurotrophic factor and of other mediators maintaining cell survival and stimulating axonal growth [3,6]. Hence, the development of autoimmune reactions to brain antigens can play a positive role in reparation of damaged CNS.

No serious complications of cell therapy were recorded during the entire period of observation. Some patients had fever (up to 38.5°C) and meningisms for 48 h after cell transplantation. These symptoms were arrested by appropriate drug therapy. Cytosis of the liquor in recipients persisted for 7 days after transplantation because of high content of live and dead mononuclear cells.

The group of patients was extremely heterogeneous by the manifestations of CCI consequences and duration of period of observation. We therefore failed to form a retrospective control group for objective and statistical processing of the results. However, we consider our first experience gained in the treatment of CCI aftereffects encouraging and plan to carry out clinical studies in this direction on a wider scale.

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