GENETICS

Can Neurografts from Mice with Chromosome 16 Trisomy Serve as a Model of Alzheimer Disease?

E. A. Lushchekina, T. Schtal', R. Shlibs, A. Goldammer, M. Beck, and V. Bigl'

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Basal forebrain regions were transplanted from mouse embryos with chromosome 16 trisomy into the lateral brain ventricle of healthy adult mice. After 1-12 months, the grafts contained immunoreactive choline- and GABAergic neurons and astroglial cells. Quantitative analysis revealed no neurodegenerative changes in these grafts compared to diploid ones. Pronounced infiltration with T and B cells and activation of micro- and astroglia were found in the grafts with chromosome 16 trisomy and in control specimen.

Key Words: *Alzheimer disease; neurotransplantation; immunocytochemistry*

Mice with chromosome 16 trisomy (TS16) are used as a model of Down syndrome, because mouse TS16 corresponds to human TS21 responsible for this disease. Detailed genetic analysis of mouse chromosome 16 revealed the presence of segments similar to those in human chromosome 21 responsible for Alzheimer disease (AD) [15]. This is why many Down patients develop AD symptoms [5] and both diseases are associated with cholinergic deficit [11]. Thus, TS16 mice are now widely used as a model of both human diseases [9,10,15]. However, TS16 mice die before or immediatelly after birth due to multiple noncerebral pathologies, which makes impossible long-term study of their brain. On the other hand, long-lasting changes in the brain underlie the development of AD. The lifetime of this tissue can be prolonged by transplantion brain regions from TS16 embryos to the brain of healthy mice [3,4].

The purpose of the present study was to reveal AD-specific degenerative changes in basal forebrain grafts from TS16 embryos.

Institute of Higher Nervous Activity and Neurophysiology, Russian Academy of Sciences, Moscow; P. Flechsig Institute of Brain Research, Leipzig

MATERIALS AND METHODS

TS16 embryos were obtained by crossing double heterozygous males with Robertson translocation (Rb (11.16)8 Lub; Rb(16.17)2M) and normal completely acrocentric females. Glycoprotein Thy 1.2 (Thy 1 allele) was present in the genotype of parent males and females. Basal forebrain regions including theseptum and diagonal band nuclei were dissected from TS16 and control embryos and transplanted in the brain of adult male Rfy mice (Thy 1.1). Presence of TS16 was confirmed by karyotypic analysis of the liver tissue [13].

One, 2, 3, 9 and 12 months after transplantation, the recipients (5 animals in each group) were killed under ether narcosis and perfused intracardially with 50 ml physiological saline and 100 ml 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). The brain was dehydrated in 30% sucrose and frosen at -20°C. Brain sections (25 μ) were treated with 0.6% hydrogen peroxide in Tris buffer (pH 7.4) for inhibition of endogenous peroxidase, washed in Tris buffer and placed in Tris buffer containing 5% normal goat serum and 2% BSA. The sections were incubated with the following primary monoclonal anti-

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bodies: rat antibodies to Thy 1.2 (1:100, Pharmigen), glial fibrillary acid protein (GFAP, 1:4000, Dako), panneuronal antiserum PGP-9.5 (1:1000, Chemicon), dioxygenated mouse antibodies to parvalbumin (PA-235 clone, 1:1000, Sigma).

Primary antibodies were visualized by incubation with the corresponding biotinylated goat antibodies (1:200-1:500, Dianova) in Tris buffer with 2% BSA. Then the sections were washed and treated with biotin-streptavidin-peroxidase complex (ABC complex: 12.5 μg streptavidin and 2.5 μg biotinylated horseradish peroxidase in 1 ml Tris-BSA). Peroxidase-labeled antibodies to digoxigenin and nickel-diaminobenzidine were used as chromogens. After reactions for Thy 1.2 and GFAP the sections were counterstained with cresyl violet or methylene blue. T and B lymphocytes were revealed by incubation with polyclonal antibodies to CD3 (1:1000, Dako) and monoclonal rat (clone RA3-6B2) antibodies to Ly 5/B220 (1:1000, Cedar Lane), respectively. Before reaction with primary antibodies the sections were treated with proteinase K (2.5 μg/ml) and antibodies to CD3 or citrate buffer (0.1 M sodium citrate, pH 5) and antibodies to CD45. Microglia was identified immunocytochemically with rat monoclonal F 4/80 antibodies (50 μg/ml, Secotec) on proteinase K pretreated sections and histochemically with biotinylated lectin from *Lycopersicon esculentum* (20 μg/ml, Vector).Quantitative studies were performed with a Sony camcorder combined with a computer and light microscope (Zeiss). KS software was used. Neuron diameter was determined by image analysis at a 40-fold magnification. Statistical analysis was carried out by Wilcoxon test using SPSS 7.5 software.

RESULTS

Grafts from embryos with TS16 and control diploids transplanted into the left ventricle of recipients showed good survival for 1 year and could be easily identified on brain sections incubated with Thy 1.2 (Fig. 1, *a*). They were intimately integrated in the ventricle wall, intensively vascularized (Fig. 1, *a, b*), and contained usual number of neurons and astroglial cells positively stained with panneuronal PGP-9.5 antiserum and GFAP, respectively (Fig. 1, *b, c*). TS16 and control grafts contained choline- (choline acetyltrasferase-reactive) and GABAergic (parvalbumin-positive) neurons (Fig. 1, *d*). Light microscopy revealed no significant differences between TS16 and diploid tissues at all terms of the study (1, 3, 6, 9 and 12 month after transplantation). Quantitative analysis revealed no statistically significant differences in the mean diameter of cholinergic neurons in TS16 (12.0-13.6 μ) and diploid (12.0-14.2 μ) grafts. The same was true for the mean diameter of GABAergic (12.1-14.2 μ in TC16 and control grafts) and PGP-9.5-reactive (10-11.9 μ) neurons. However, moderate but significant decrease in the mean diameter of cholinergic (from 13.6 to 12 μ) and GABAergic (from 14.0 to 12.1 μ) neurons was observed in TS16 and control grafts from month 1 to month 12 of the experiment. Besides, the mean diameter of cholin- (12.0-14.2 μ) and GABAergic (12.1-14.0 μ) neurons was significantly higher than that of other neurons visualized with panneuronal marker PGP-9.5 (10.0-11.9 μ). Histochemical immune reaction revealed activated microglia and reactive astrocytes and lymphocytes in the grafts. Starting from month 1 posttransplantation, astrogliosis, pronounced infiltration with T (Fig. 2, *a*) and B (Fig. 2, *b*) lymphocytes, and marked activation of microglia (Fig. 2, *c*) were found in TS16 grafts. T lymphocytes were evenly distributed in the grafts, while B lymphocytes were less abundant and accumulated mainly along the graft-recipient border and perivascularly. Similar inflammatory reaction was noted in the control diploid grafts.

There are contradictory data on the development of AD-specific pathology in neurografts from TS16 mice. AD-specific changes were observed in hippocampal grafts as soon as 6 months after transplantation [13], while other authors revealed no immunoreactive AD-specific signs in basal forebrain, hippocampus, and dorsal root ganglia grafts even 14 months after transplantation [6,11,14]. These contradictions are still unexplained. The present study confirms that TS16 grafts exhibit no AD-typical changes and cannot be used as an adequate model of this disease. Why transplanted cells develop no genetically determined pathology? Numerous reports describe neurodegeneration of trisomic cells in culture. In cultures containing trisomic neurons and glia, choline acetyltransferrase activity and the number of cholinergic neurons decrease, while in the presence of diploid glial cells cholinergic neurons from TS16 mice show normal expression of cholinergic markers [12]. These data indicate the key role of astroglial cells in the impairment of cholinergic function in TS16 mice. It was shown that nerve growth factor of glial origin prevents TS16-induced degeneration of cholinergic neurons [7]. Cholinergic neurons from TS16 animals show normal survival in the recipient hippocampus, while denervation of the hippocampus causes degeneration of transplanted neurons due to their deprivation of neurotrophic factors [7]. Thus, TS16-induced neurodegeneration depends on trophic influence of glial cells. This was confirmed by electron microscopy revealing no pathological changes in TS16 neurons from 15-day-old embryos [11]. Hence, mental retardation in Down patients and intellectual and memory dysfunctions during AD depend on functional and biochemical impairment rather than structural changes in neurons [11]. Thus, neu-

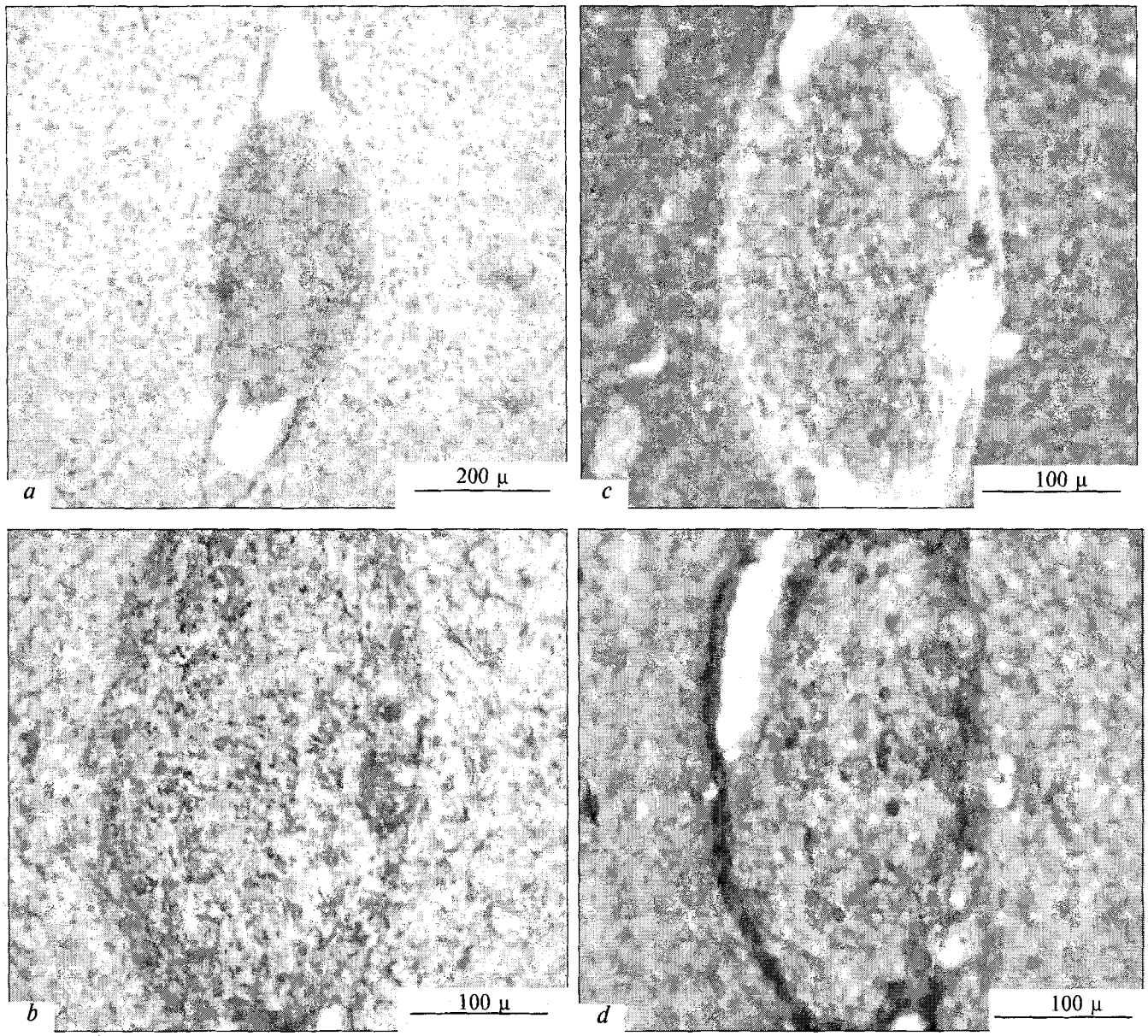

Fig. 1. TS16 grafts in recipient brain 3 months after transplantation: *a*) graft revealed by immunocytochemical reaction for Thy 1.2 looks like a dark structure, methylene blue counterstaining; *b*) astroglia visualized by immunocytochemical reaction for glial fibrillary acid protein, methylene blue counterstaining; *c*) neurons revealed with panneuronal PGP-9.5 antiserum; *d*) choline- and GABAergic cells revealed by double immunohistochemical reaction for choline acetyltransferase and parvalbumin; more and less intensely stained cells correspond tocholine and GABAergic cells, respectively. Note intense vascularization of the graft.

rotrophic factors play the key role in AD and other pathologies associated with cholinergic deficit. B and T lymphocyte infiltration and intensive vascularization of the graft observed in our study point to a significant metabolic integration between the graft and recipient brain ensuring sufficient supply of TS16 grafts with trophic factors from the recipient's brain, which prevents neurodegeneration. Since chronic inflammatory reaction does not induce neurodegeneration, it is possible that B and T lymphocytes together with glia contribute to trophic support of the transplanted tissue.

In both trisomic and control grafts, the mean size of choline- and GABAergic neurons decreases post-transplantation but significantly exceedes the size of other neurons. The decrease in the size of cholinergic neurons during aging was reported previously [8]. Thus, normal aging is reproduced in the grafts. Large choline- and GABAergic neurons can represent giant relay neurons visualized by Golgi method [1,2], while other neurons revealed in our study by panneuronal PGP-9.5 serum correspond to smaller interneurons described by the same authors. Interneurons to a lesser

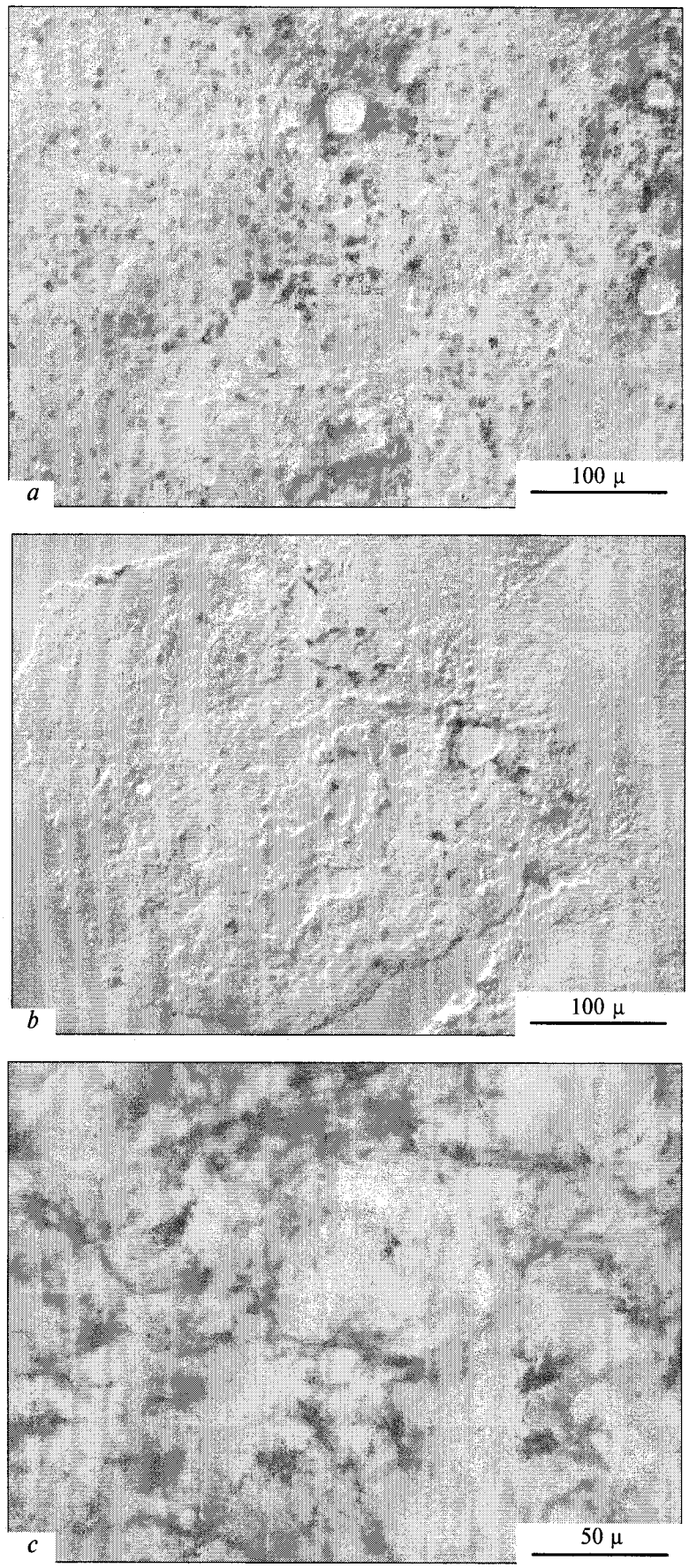

Fig. 2. Inflammatory reaction in TS16 grafts 3 months after transplantation: infiltration with T (*a*) and B (*b*) lymphocytes revealed by immunocytochemical reactions for CD3 and CD45, respectively; *c*) activated microglia on the border between the graft (upper left corner) and recipient's brain stained histochemically with *Lycopersicon esculentum* lectin.

extent depend on sensory input than relay neurons and, therefore, maintain their properties, in particular the size of soma, after transplantation.

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