

Manipulation of brain DNA synthesis is achieved by using a systemic immunological disease

(cerebellum development/graft-versus-host disease/autoradiography)

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ABSTRACT Previously, we showed that as early as postnatal day 11 an immunological disease, graft-versus-host disease, induced by grafting allogeneic lymph node cells into an immunoincompetent neonatal rat significantly decreases cerebellar histogenesis—i.e., DNA synthesis and the number of newly formed neurons. Here, we report that, subsequent to successful immunotherapy, there was a reversal of the deleterious graft-versus-host disease-induced alterations in DNA synthesis in individual cerebellar germinal cells. Immunotherapy involved treating the diseased rats on postnatal days 11, 12, and 13 with alloantisera specifically directed against the grafted lymph node cells injected on the day of birth. On postnatal day 14, diseased, serum-treated, and control littermates were injected with [³H]thymidine and, 15 min later, the cerebella were excised and autoradiographed. A 0.72-mm segment of the external granular layer in the cerebellar fissure between lobules VIB and C was searched for labeled cells. The control group had the greatest number of labeled cells, defined by the presence of six or more autoradiographic grains, (43 ± 4 , mean \pm SEM) and the greatest number of grains per cell (9.5 ± 0.2). Rats with the disease had few labeled cells (4 ± 2) and the number of grains per cell was low (6.6 ± 0.6); however, serum treatment increased both the number of labeled cells (26 ± 8) and the number of grains per cell (7.4 ± 0.2). These results show that without mononuclear infiltrates or inflammation in the cerebellum, a systemic immunological disease can dramatically decrease DNA synthesis per germinal cell and, moreover, that halting the disease by alloantisera therapy can reverse this effect. These findings emphasize the sensitive plastic nature of neuronal cell acquisition in the normally developing brain.

To determine the extent to which neural tissue can repair, by cellular proliferation, after a period of arrested growth or injury, manipulation of DNA synthesis is necessary—i.e., a relatively rapid (a few days) suppression and subsequent restoration of DNA synthesis paralleled by a decrease and subsequent increase in the formation of identifiable neuronal cell types. Graft-versus-host disease (GVHD), which can be arrested by treatment of host animals with alloantisera, provides such a manipulatory process (1). Because newborn rodents are not competent to recognize foreign tissues, immunocompetent allogeneic lymph node cells grafted on the day of birth (day 0) attack and destroy host lymphomyeloid cells, thus inducing the systemic immune syndrome, GVHD (2, 3). In the course of the disease, a decrease in cell proliferation in a number of vital organs occurs (4). Furthermore, an altered gait, suggestive of central nervous system involvement, is known to be one of the manifestations of GVHD. The cerebellum is indeed one of the organs adversely affected by neonatally induced GVHD, as indicated by a dramatic decrease in DNA synthesis, by postnatal day 11, an event that precedes the wasting process character-

izing the later stages of the disease (5). Here, we present autoradiographic evidence that the GVHD-induced decrease in DNA synthesis is due not only to fewer germinal cells synthesizing DNA but also to less DNA synthesis per individual cell. In addition, 3 days of therapy with alloantisera partially reversed these symptoms, resulting in greater numbers of DNA-synthesizing cells and more synthesis per cell.

METHODS

GVHD was induced in two-thirds of the Fischer rats in each litter by intravenous injection of 20×10^6 Dark Agouti lymph node cells on the day of birth (day 0). On postnatal day 11, when the GVHD-induced decrease in DNA synthesis had become apparent (5), treatment with alloantisera was begun. One-half of the diseased animals in each litter were given three daily 0.2-ml injections of Fischer serum (alloantisera) containing high titers of antibodies directed specifically against Dark Agouti histocompatibility antigens on the grafted lymphocytes. The remaining one-third of each litter served as controls. We previously determined that injection of either 40×10^6 syngeneic lymph node cells at birth (5) or 0.2 ml of alloantisera on postnatal days 11–13 had no detectable effect on the growth of control neonatal rats.

On postnatal day 14, all animals in two litters were injected with $10 \mu\text{Ci}$ of [³H]thymidine ($1 \text{ Ci} = 3.7 \times 10^{10}$ becquerels) and decapitated 15 min later. A 15-min interval was chosen because of excessive grain density per cell at longer survival times. The cerebellar vermis and part of the brainstem were excised and fixed for 24 hr in Bouin's solution, washed in 70% ethanol for 1 wk, embedded in methacrylate (Sorvall), and sectioned at $3 \mu\text{m}$. Histological slides were coated with Kodak NTB-2 emulsion, exposed in a light-tight box for 4 wk at 4°C, developed with Kodak D-19, and stained with Mayer's hematoxylin/eosin. Labeled germinal cells were counted along a 0.72-mm length of the matrix zone of the cerebellar external granular layer of lobules VIB and C. A cell was considered to be labeled if it had six or more silver grains above it. The total number of grains per labeled cell was used as an indicator of DNA synthetic activity in that cell. As a measure of total cerebellar DNA synthesis in similarly labeled cerebella from other affected 14-day-old animals, uptake of [³H]thymidine into the DNA fraction from the acid-soluble fraction during a 15-min interval was assessed (Table 1).

RESULTS

Total cerebellar DNA synthesis (cpm in the DNA fraction/cpm in the acid-soluble fraction ratio) 15 min after injection of [³H]thymidine was significantly less in diseased animals than in littermate controls or alloantisera-treated animals (Table

Abbreviation: GVHD, graft-versus-host disease.

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Table 1. Analysis of DNA synthesis by autoradiography

Group*	Cells having ≥6 grains, no.	Grains in cells having ≥6		DNA synthesis
		No.	Range	
E	4 ± 2 ^{††}	6.6 ± 0.1 ^{††}	6–9	0.07 ± 0.01 ^{††}
I	26 ± 8 [†]	7.4 ± 0.2 [†]	6–15	0.13 ± 0.01 [†]
C	43 ± 4	9.5 ± 0.2	6–17	0.18 ± 0.01

Grains were counted in autoradiographs of midsagittal sections of cerebella excised 15 min after injection of 10 μ Ci of [³H]thymidine. Cells having 6 or more grains were counted in a 0.72-mm length of matrix in the external granular layer of four littermates from each group. The cpm in the DNA fraction/cpm in the acid-soluble fraction ratio represents DNA synthesis 15 min after injection of 10 μ Ci of [³H]thymidine. Appropriate values are expressed as mean ± SEM.

* E, experimental Fischer neonates were injected intravenously with 20×10^6 Dark Agouti lymph node cells on the day of birth; I, Fischer neonates were injected with Dark Agouti cells on the day of birth and then subjected to immunotherapy by intraperitoneal injection of 0.2 ml of Fischer anti-Dark Agouti hyperimmune serum on postnatal days 11, 12, and 13; C, untreated littermates (controls).

[†] Different from C ($P < 0.05$).

^{††} Different from I ($P < 0.05$).

1). Since the cpm in the acid-soluble fraction was similar in all groups, the availability of [³H]thymidine was not the limiting factor in the ability of diseased animals to incorporate precursor into DNA. Analysis of autoradiographs showed two causes of this decrease in total cerebellar DNA synthesis. First, there were fewer germinal cells synthesizing DNA (Fig. 1 and Table 1) in affected animals than in control animals and, second, there was less DNA synthesis in individual cells (Table 1).

Total cerebellar DNA synthesis in alloantiserum-treated animals was almost twice that of affected littermates, being 75% of control values (Table 1). Furthermore, the numbers of labeled cells and of grains per labeled cell were much greater in alloantiserum-treated neonates than in diseased littermates. Although the number of labeled cells and the number of grains per cell in cerebella from alloantiserum-treated animals was less than control levels ($P < 0.05$), the arrest of GVHD by alloantiserum treatment resulted in the resumption of DNA synthesis as assessed both biochemically in [³H]thymidine uptake studies and autoradiographically in silver-grain labeled-cell counting experiments.

DISCUSSION

Induction and subsequent termination of neonatal GVHD provides a model for manipulating proliferation of individual brain germinal cells repressing and then derepressing the rate of DNA synthesis. As there is no lymphocytic infiltration or evidence of inflammation in the cerebellum and DNA synthesis is rapidly restored by therapy, the as yet unrecognized agent(s) responsible for turning off DNA synthesis is probably a soluble factor(s).

The regenerative propensity of cerebellar germinal cells has previously been demonstrated by recovery of growth following x-irradiation (6), drug (7) and virus treatment (8), and malnutrition (9), all procedures that damage or slow the stem cell population growth. However, the superiority of GVHD as a manipulator of brain cell proliferation derives from the absence of serious drawbacks associated with other procedures, such as the four listed above. For example, the manifestations of manipulation by GVHD are dramatic but without the cell death that accompanies x-irradiation or drug or virus treatment and also are in contrast to small changes in cerebellar growth parameters accompanied by great decreases in body weight in malnutrition.

A clinically relevant aspect of these findings relates to the fact that young children, less than age 2, have suffered from GVHD

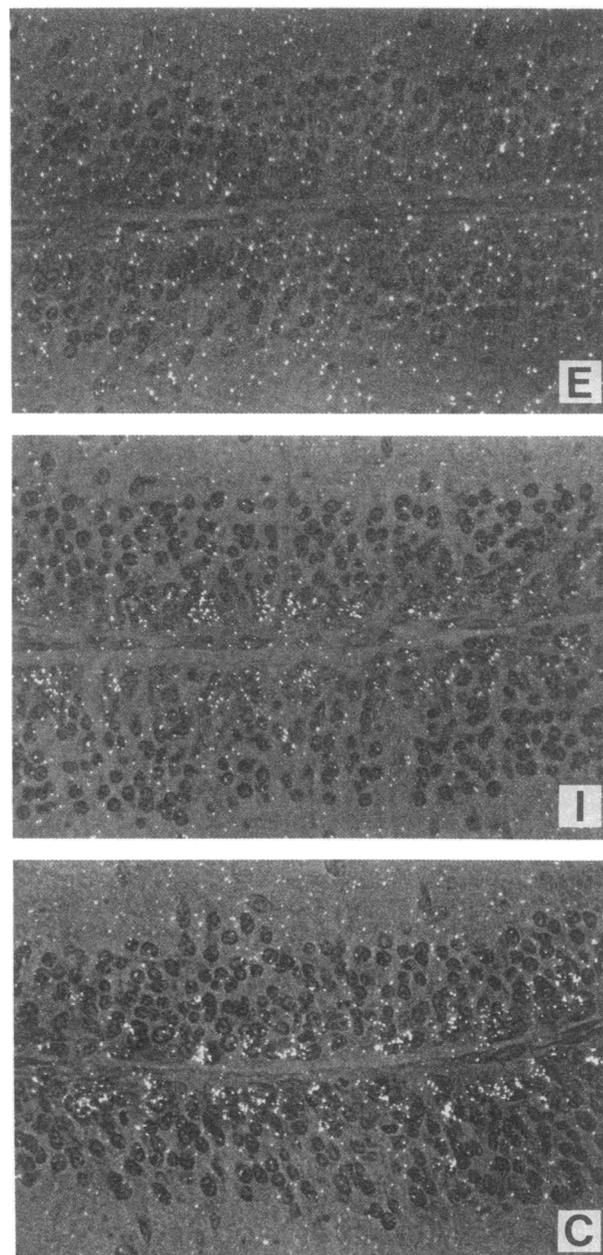


FIG. 1. Doubly exposed photomicrograph obtained by using bright-field and dark-field illumination of the cerebellar external granular layer at 1,000 diameters demonstrates the paucity of labeled cells in tissues from 14-day-old rats injected with allogeneic lymph node cells (E) compared with the number of labeled cells in diseased animals treated for three days with alloantiserum (I) and untreated littermate controls (C).

as a result of therapeutic bone marrow transplantation during the time of normal cerebellar maturation (10–13). Such episodes of GVHD may subject infants and children to alterations in brain growth similar to those we have demonstrated in the developing rat cerebellum. Furthermore, the restoration of DNA-synthesizing ability in brain germinal cells might be generalized to other developing tissues in individuals suffering from GVHD. Also, our data suggest that timely intervention in other perinatal maladies may similarly normalize DNA synthesis as well as organ development.

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