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Graft-versus-host disease impairs cerebellar growth

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The mechanisms of mental retardation are of critical interest to both basic developmental neurobiologists and those in the clinical community concerned with perinatal development. The possibility of a deleterious immunological interaction between mother and fetus has long been recognised as a potential developmental hazard¹. However, there have been no reports of specific conditions which involve immunological injury to the developing brain. Graft-versus-host disease (GVHD) is a model disease that can be induced naturally or experimentally by grafting mature, immunocompetent lymphoid cells into an immature, immunoincompetent host. The failure of the host, for example a newborn rat pup, to recognise the graft as non-self leads to an attack by the grafted cells on host lymphomyeloid tissues, resulting in many symptoms, including wasting, anaemia, alterations of lymphoid organ weights, hyperexcitability and an ataxic-like gait². The last two features suggest possible brain dysfunctions and have motivated our investigation of immunologically induced perinatal brain injury. The rat cerebellum was used here as a model system for the study of brain alterations during GVHD because of its well known cytoarchitecture³. In addition, its postnatal development renders it sensitive to many forms of perinatal stress^{4,5}. In this study, we demonstrate that significant alterations in the cerebellar cellular content and capacity for DNA synthesis occur in rats suffering from neonatally induced GVHD.

GVHD was induced by injecting 40×10^6 parental strain lymph-node cells (PSLNC) on the day of birth (day 0) into the F₁ hybrid offspring of two inbred strains of rats (Fischer and DA). Newborn F₁ hybrid animals were used because they are genetically tolerant of grafts from either parent and, thus, especially susceptible to immunological attack by parental lymphocytes. Non-injected littermates or littermates injected with harmless, syngeneic F₁ hybrid LNC were used as controls. At various ages, the pups were killed and alterations in cerebellar development assessed. First, cell acquisition was estimated by measuring total cerebellar DNA content. Second, the uptake of ³H-thymidine (³H-TdR) into the DNA tissue fraction from the acid-soluble tissue fraction was assessed 1 h after an intraperitoneal injection of 2 µCi per g body weight. General growth of the cerebellum was measured by determining organ weight changes and total organ RNA and protein content.

The time course of GVHD effects on cerebellar maturation is characterised by a rapid appearance of changes. Thus, at the ninth postnatal day, injected animals were relatively normal by the parameters measured here. However, at postnatal day 11 cerebellar development was noticeably altered.

As seen in Table 1, cerebellar weights were affected in animals injected with PSLNC. Between days 9 and 11 there was only a 19% increase in cerebellar weight in animals with GVHD, compared with a 26% increase in controls. Subsequent to this initial change in cerebellar weight gain, cerebella from GVHD animals remained significantly smaller than those of non-injected littermates. In addition to our assessment of GVHD effects on cerebellar weights we also weighed each intact animal, its spleen and thymus, and found weight alterations similar to those previously described in animals with neonatal GVHD⁶.

In animals injected with PSLNC, both cell proliferation and RNA and protein contents were decreased compared to either non-injected controls or controls injected with syngeneic cells. This effect was proportional to changes in cerebellar weight (Table 1).

At each day including and after day 11, there was significantly less DNA, RNA and protein, indicating a marked decrease in accretion of cell number and cellular content (Table 1). There was actually less DNA at day 14 than day 13 in cerebella from GVHD animals, possibly reflecting a degree of cell death in addition to the slowed cell proliferation. Compared to controls, the uptake of ^3H -TdR into the DNA fraction from the acid-soluble fraction was less in GVHD animals at each day measured except day 9. Labelling of the acid-soluble fraction was not significantly different in GVHD compared to controls, but there was a great difference ($P < 0.001$) in the amount of label in the DNA fraction, suggesting that although cerebellar cells in GVHD animals can take up ^3H -TdR, the mechanism for incorporating the label into DNA is altered. It is especially noteworthy that these actions on brain growth occur at a time before body wasting is evident. Animals which received syngeneic cells, that is, lymphocytes not reactive against the host, were similar to non-injected controls with respect to either cellular acquisition or cellular content.

Histological preparations showed the regular arrangement of the layers of the cerebellum to be undisturbed in 14-d-old animals with the classic symptoms of GVHD. In addition, there was no mononuclear cellular infiltration of the cerebellum, which argues against a direct, cell-mediated attack by invading lymphocytes against cerebellar cells.

The demonstrated effects of GVHD on maturing rat cerebellum may be highly significant with respect to the development of the human cerebellum, which acquires 80% of its total neuronal cell population, that is, granule cells, during the first two postnatal years⁷. Therapeutic measures, including exchange transfusion of the immunologically immature fetus *in utero*⁸, routine transfusion of infants suffering from congenital immunological deficiency syndrome⁹, and even of lymphocytes from the maternal to the infant circulatory system¹⁰ can all place infants at risk due to the induction of a GVHD syndrome. In addition, bone marrow transplantation in children with haematological disorders can result in GVHD, and affected infants often survive mild or moderate episodes of the disease¹¹. Human infants do, in fact, receive foreign lymphocytes in these instances at a time when cerebellar cells are still proliferating, but there has been no assessment of potential CNS damage due to induction of graft-versus-host disease.

The adverse actions described here of GVHD on cell proliferation in the developing rat cerebellum indicate that the CNS is a previously unrecognised site of damage during this syndrome. The mechanism by which GVHD causes damage to cerebellar growth is still obscure. No lymphocytic invasion of the cerebellar tissue was observed in affected animals, which suggests that a blood-borne factor may be involved. Alterations in cerebellar synthetic capacity could be the result of several different agents during the course of the disease; for example, hormones like cortisone¹² or factors from activated lymphocytes such as proliferation inhibitory factor.¹³ It is conceivable that the cerebellum could be affected as an innocent bystander in a similar way to other tissues such as the skin¹⁴ and intestine¹⁵ of animals with GVHD. Our preliminary histological studies have suggested a depletion of cells in the external germinal layer, an effect quite different from that found in malnutrition¹⁶ and some hormonal alterations¹⁷. The analysis of the process by which graft-versus-host disease selectively slows growth within the proliferative cell population in the cerebellum remains a challenge to the understanding of basic growth processes in neural development, and is under investigation in our laboratory.

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Table 1

Effect of graft-versus-host disease on several cerebellar growth parameters

Day killed	Injected cells	Cerebellar weight (mg)	Cerebellar contents (mg)			
			DNA	RNA	Protein	DNAF/ASF
GVHD-producing LNC injected						
9	None	63.3±1.7	0.45 ±0.02	0.37±0.01	4.4±0.1	0.43±0.03
	FLNC	61.1±1.2	0.48±0.01	0.38±0.01	4.4±0.1	0.46±0.04
11	None	85.4±1.2	0.65±0.02	0.44±0.01	6.7±0.3	0.81±0.04
	FLNC	75.3±1.4 *	0.54±0.02 *	0.39±0.01 *	6.1±0.3 *	0.53±0.02 *
13	None	110.9±1.7	0.89±0.03	0.54±0.01	7.2±0.1	0.54±0.02
	FLNC	102.6±1.8 *	0.77±0.01 *	0.47±0.02 *	6.4±0.2 *	0.35±0.03 *
14	None	119.0±2.1	0.95±0.03	0.55±0.02	9.0±0.2	0.76±0.05
	FLNC	82.2±4.2 *	0.73±0.07 *	0.43±0.04 *	7.2±0.4 *	0.34±0.03 *
Non-GVHD-producing LNC injected						
14	None	121.4±4.8	1.04±0.06	0.60±0.02	8.6±0.6	0.62±0.03
	F ₁ hybrid LNC	124.4±4.4	1.05±0.04	0.58±0.02	8.6±0.4	0.58±0.04

Animals injected with either 40×10^6 Fischer lymph node cells (FLNC) or F₁ hybrid LNC were compared with non-injected littermates. DNAF/ASF is the ratio of c.p.m. per g in the DNA fraction to c.p.m. per g in the acid-soluble fraction 1 h after an i.p. injection of ^3H -TdR (2 μCi per g body weight). Results are expressed as mean±s.e.m.

* Significantly less than non-injected controls (None), $P < 0.05$.