Brief Definitive Report

RECOGNITION OF HISTOCOMPATIBILITY DETERMINANTS CONTROLS REACTIVITY OF AUTO-SENSITIZED LYMPHOCYTES AGAINST NEURAL TISSUES

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Little attention has been paid to the genetic background of animals used as a source of antigenic material for sensitization in the investigation of allergic encephalomyelitis, and, with some exceptions (1–3), spinal cord tissue and basic myelin protein have been harvested from donors that were at least allogeneic, and often xenogeneic, with respect to the future recipient (4–6). The tacit assumption has been that variation between donor and recipient of sensitizing material would be unlikely to affect the disease process. As our experiments concerned the role of anti-idiotypic responses in the control of anti-self reactivity, it was mandatory that syngeneic central nervous system tissue be used for sensitization if the results were to be interpretable as valid anti-self responses. Cultured cerebellar tissues were used as a target for the encephalitogenic activity of lymphocytes from rats sensitized by injection of spinal cord tissue. The inbred strain of the donors of cultured cerebellum, lymphocytes, and spinal cord all exerted a decisive influence on the outcome of culture of nervous system cells with lymphocytes.

Materials and Methods

Rats of the inbred Lewis, PvG, and DA strains and (PvG × Lewis)F_1 hybrid rats were used in our study.

Sensitization against central nervous system tissues was with a 40% wt/vol saline suspension of spinal cord that was emulsified with an equal quantity of Freund's complete adjuvant. 0.05 ml of this emulsion was injected subcutaneously into each hind footpad, and 10^9 killed *Bordetella pertussis* organisms were injected into the dorsum of each hind paw. Thoracic duct cannulation used Gowans' modification (7) of the method of Bollman et al. (8), and cells were collected between 5 and 7 d after sensitization.

Culture of central nervous system tissue was performed as described by Bornstein and Murray (9), with cerebella removed within 48 h of birth. Several fragments, of ~1- to 2-mm diameter, were introduced into each Rose chamber (10), the lower coverslip of which had been coated with rat tail collagen. The chambers were filled with Hepes-buffered medium TC 199 that contained 50% heat-inactivated fetal calf serum and was supplemented with 50 μg of gentamicin, 100 U of penicillin, 3 mg of glucose, and 0.2 mg of L-glutamine/ml and then incubated at 37°C. Medium was changed after 24 and 72 h of incubation and then weekly. Lymphocytes were introduced into chambers after the 14th d by which time emigration from the explants had produced a confluent growth on the lower coverslip. Living cultures were examined by phase contrast and fixed preparations after defatting and staining with toluidine blue. Cultured cells resembled those described in cat cerebellum by Pomerat and Costero (11) (Fig. 1). The cultured cells were: (*a*) Large polyhedral epitheloid cells, presumably pial, at the periphery; and mesenchymal and fibroblastic cells, often binucleate, with well-defined nuclear membranes. (*b*) Oligodendroglia with globose form, indistinct nucleus, perinuclear cytoplasmic

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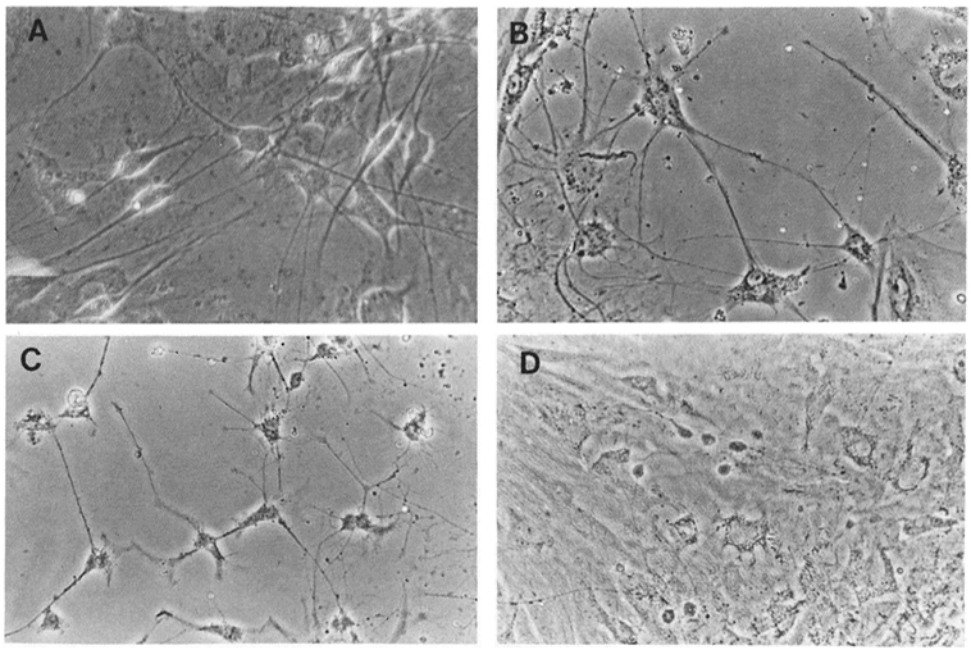

FIG. 1. Cerebellar cultures (phase contrast). (A) Neuronal cells in 52-d culture. × 375. (B) Neuronal cells in 13-d culture. × 400. (C) Astrocytes in 6-d culture. × 200. (D) Oligodendroglia in 12-d culture. × 200.

halo, and two or three narrow processes. (*c*) Astrocytes, with cell body and long ramifying processus. (*d*) Neurons that survived well and had clear nuclei without well-defined nucleolus and granular cytoplasm; and processes, if visible, were long and branching. Cultures remained in excellent condition for at least 2 mo.

Results

Lymphocytes from Unsensitized Donors Coexist with Cerebellar Cultures. After the addition of 10^7 normal syngeneic or allogeneic thoracic duct lymphocytes, both cerebellar and lymphoid cells remained healthy for 2 mo. Table I (A) summarizes strain combinations of the cerebellum and lymphocytes that were examined. Mixtures of equal numbers (5×10^6) of lymphocytes from mutually allogeneic strains persisted indefinitely on cerebellar cells (Table I [B]). The survival of lymphocytes that were cocultured with cerebellar cells was vastly superior to that of thoracic duct cells alone.

Syngeneic Lymphocytes Sensitized Against Syngeneic Spinal Cord Damage Cerebellar Cells. Lymphocytes from syngeneic donors sensitized against syngeneic spinal cord invariably damaged cultured cerebellar cells severely. The strains of rats used are summarized in Table I (C). The first difference from cultures with unsensitized lymphocytes was observed on the 3rd d and consisted of the dense aggregation of lymphocytes over cerebellar cells (Fig. 2 A). It was not clear if lymphocytes clustered selectively over any specific cell but, when overlying lymphocytes did not obscure morphology, damaged cerebellar cells were apparent by 4 d. Many central nervous system cells were dead by the 8th d (Fig. 2 B and C).

Lymphocytes Sensitized Against Syngeneic Cord Do Not Damage Allogeneic Cerebellar Cells. Sensitized cells from the same lymph collections used above were added in

TABLE I

Addition of Lymphocytes to Cultured Cerebellar Tissue

	Strain of origin			Number of lymphocyte donors‡	Result§
	Sensitized lymphocyte donor*	Sensitizing spinal cord donor	Cerebellar culture donor		
A	Normal, nonsensitized rats				
	PvG	None given	PvG	2	N
	PvG	None given	Lewis	2	N
	PvG	None given	(PvG × Lewis)F_1	2	N
	Lewis	None given	Lewis	3	N
	Lewis	None given	PvG	3	N
	(PvG × Lewis)F_1	None given	(PvG × Lewis)F_1	4	N
	(PvG × Lewis)F_1	None given	Lewis	4	N
	(PvG × Lewis)F_1	None given	PvG	4	N
B	Normal, nonsensitized rats				
	PvG + Lewis	None given	PvG	2	N
	PvG + Lewis	None given	Lewis	2	N
C	Lewis	Lewis	Lewis	3	A
	PvG	PvG	PvG	2	A
	(PvG × Lewis)F_1	(PvG × Lewis)F_1	(PvG × Lewis)F_1	>8	A
D	Lewis	Lewis	PvG	3	N
	PvG	PvG	Lewis	2	N
	(PvG × Lewis)F_1	(PvG × Lewis)F_1	DA	6	N
E	(PvG × Lewis)F_1	(PvG × Lewis)F_1	Lewis	6	A
	(PvG × Lewis)F_1	(PvG × Lewis)F_1	PvG	6	A
F	Lewis	PvG	Lewis	4	A
	Lewis	PvG	PvG	3	A
G	Lewis	PvG	DA	3	N
	PvG	Lewis	DA	3	N

* Thoracic duct lymphocytes were collected from normal rats, or those sensitized with central nervous system tissue, 5–7 d previously.

‡ At least three replicate cultures were established with the lymphocytes from each individual donor.

§ All of the replicates in each set of cultures followed a similar course. (A) Activity was observed. Aggregation of lymphocytes over cultured central nervous system cells was seen after 3 d, and damage to underlying cells followed in 1–5 d, as described in the text and illustrated in Fig. 2. (N) Lymphocyte activity was not observed. Lymphocytes and cerebellar cells remained in morphologically excellent condition, as illustrated in Fig. 3.

similar numbers to cerebellar cultures from unrelated rats. Combinations of lymphocytes and cerebellar cells are summarized in Table I (D) as is the invariable lack of damage to the cultures. Cerebellar cells invariably survived with sensitized allogeneic lymphocytes for indefinite periods (Fig. 3).

Sensitized Semiallogeneic Lymphocytes Damage Cerebellar Cells. Absolute identity between spinal cord, lymphocyte, and cerebellum is not essential for damage to the latter cells. Lymphocytes from F_1 hybrid rats sensitized with syngeneic cord regularly damaged cerebellar cells of either parental strain (Table I [E]).

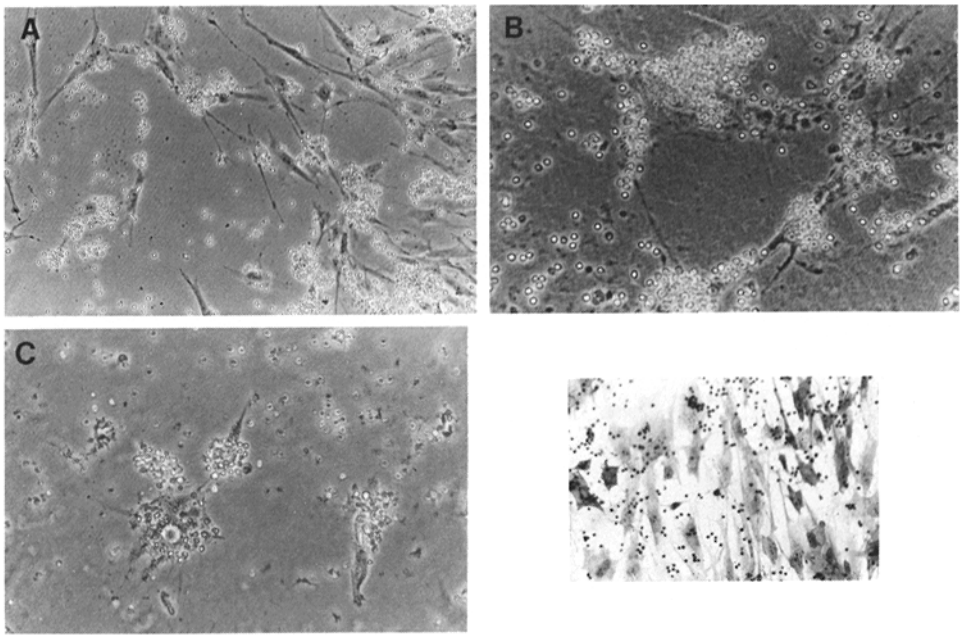

FIG. 2. Cerebellar cultures that contain syngeneic lymphocytes sensitized against syngeneic cord (phase contrast). (A) Lymphocytes over healthy cerebellar cells 3 d after addition. × 100. (B) Lymphocytes still clustered with some deterioration in underlying cerebellar cells after 5 d. × 200. (C) Marked degenerative changes in cerebellar cells after 6 d. × 200.

FIG. 3. (Inset) Cerebellar cells after 42 d with allogeneic lymphocytes sensitized against cord of the same strain. Both cell components are well preserved. Toluidine blue staining was used. × 250.

Lymphocytes from Specifically Sensitized Allogeneic Donors Damage Cerebellar Cells. Lymphocytes from rats sensitized against allogeneic cord attacked cerebellar cells syngeneic with both cord and lymphocyte donors (Table I [F]). In contrast, lymphocytes sensitized against allogeneic cord failed to react against cerebellar cells from a third, unrelated strain (Table I [G]).

Discussion

Thoracic duct lymphocytes collected between 5 and 7 d after sensitization against spinal cord regularly damaged cerebellar cultures only if spinal cord lymphocytes and cerebellar cultures were syngeneic or semiallogeneic. The difficulty in relating these in vitro observations to the development of clinical encephalomyelitis stems from the difficulty apparent in published reports of transferring disease with cells from sensitized donors to normal rats. The unreliability with which transfer can be accomplished in this manner (12) has led to the use of indirect criteria for detection of lymphocyte reactivity. The relevance of perivascular cell accumulation in sensitized lymphocyte recipients is dubious in view of the poor temporal correlation between this feature and clinical disease in actively sensitized animals. Similarly, extrapolation from events in cultured tissue to the intact rat, should be cautious. Either or both of perivascular infiltration and antineuroantigen activity may only be epiphenomena that are not on the causative pathway to clinical disease. If many processes are involved in the development of clinical disease, any assay used to follow lymphocyte reactivity may represent only one of these.

The relationship of the mechanisms responsible for damage to cultured cerebellar cells to the host of cytotoxicity phenomena catalogued in the literature is as obscure as is the relationship of the latter to any processes of occurrence in intact animals. There are strong similarities between the current observations and "H-2 restriction" of cytotoxicity of lymphocytes against antigenically modified target cells (13, 14). The behavior of lymphocytes on cultured neural tissue differs from most descriptions of H-2 restriction in the absence of requirement for antigenic modification of the target cells. The present phenomenon is not readily explicable by the "modified self" hypothesis (15). Recent indications that sensitization against syngeneic spinal cord does not require its antigenic modulation during emulsification support this inference.

It is not clear whether restriction of the activity of lymphocytes from syngeneically sensitized rats was imposed at the time of sensitization or became apparent only at the effector stage. This may depend upon whether self recognition requires active stimulation of lymphocytes confronted with syngeneic cells, or merely failure to identify foreign determinants. The ability of lymphocytes from allogeneically sensitized rats to attack both allogeneic and syngeneic neural cultures might indicate that exposure to both organ-specific and self determinants is unnecessary for sensitization against self tissues. Sensitization against allogeneic cord may be more complex. Because central nervous system cells express significant quantities of histocompatibility determinants (16), it is possible that reactivity against allogeneic cerebellar cultures depends upon two subpopulations of lymphocytes–one sensitized against neural and histocompatibility determinants, respectively. The technique of removal of the subpopulations of specifically reactive lymphocytes by passage through a semiallogeneic host is being applied to this problem.

Ultimately, it may become a semantic question as to whether reactions between lymphocytes and syngeneic cerebellar cells are immunological or otherwise. However, at an admittedly simplistic level, it would not be surprising if cells derived from a single donor were to be at an advantage in mutual interaction by comparison with cells from unrelated donors.

Summary

Thoracic duct lymphocytes from rats sensitized against syngeneic spinal cord rapidly produce damage in cultures of syngeneic cerebellar cells but coexist indefinitely with allogeneic cultures. Lymphocytes from donors that have been sensitized against allogeneic spinal cord attack cultures of syngeneic and specific allogeneic cerebellum but not cells from rats of a third, unrelated strain.

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