Allergic Encephalomyelitis: Inhibition of Cellular Passive Transfer by X-Radiation* (34024)

SEYMOUR LEVINE, JOHN PRINEAS AND LABE C. SCHEINBERG

Pathology Department, New York Medical College, Center for Chronic Disease, Bird S. Coler Hospital, Welfare Island, New York, N. Y. 10017; and the Saul R. Korey Department of Neurology, Albert Einstein College of Medicine, New York, N. Y. 10461

Radiation has been reported to enhance or to suppress antibody formation and delayed hypersensitivity (1) including the active induction of experimental allergic encephalomyelitis (EAE) (2). The varying effects on EAE are probably related to the species studied and to the dose, timing, and manner of administration of antigen, adjuvant, and radiation. Radiation before the evocative inoculation or during the incubation period may affect various processes involved in active immunization. Of equal interest are the effects of radiation on fully immunized lymphoid cells and on their interaction with the target organ. These aspects can be studied with the aid of passive transfer of lymph node cells from unirradiated, actively immunized donors into radiated, immunologically naive, isohistogenic recipients. In the present work, late effects of radiation and recovery from early effects have been avoided by use of a rapid technique (3) in which recipients were killed 1 day after passive transfer of EAE cells and 1 or 2 days after radiation. Under these conditions, inhibition of EAE has been produced by radiation of recipients before as well as after they received encephalitogenic cells.

Methods. Donors of lymphoid cells were female or male Lewis rats, 150–250 g, immunized with 0.05 ml of a water-in-oil emulsion of 40% guinea pig spinal cord homogenate in an equal volume of Freund's complete adjuvant. The emulsion was injected intradermally into a right hind foot pad, and 0.1 ml of pertussis vaccine concentrate (20 billion organisms) was injected into the dorsum of the same foot for additional adjuvant effect. Seven days later, when the donors began to develop signs of EAE, the right popliteal, lumbar, sacral, renal, inguinal, and axillary lymph nodes were removed, dissociated into a cell suspension and injected intravenously into the recipients (1.5 to 3.0 $\times$ 10^8 cells each) (3).

Recipients were immunologically naive, isohistogenic, male 150–240 g Lewis rats. Two–4 days before cell transfer, a closed thermal brain injury was induced in the recipients by application of a preheated soldering iron to the intact skull (3). This precedure reduced the threshold for EAE, so that unirradiated animals killed 24 hr after injection of EAE cells invariably had numerous perivascular mononuclear inflammatory infiltrates adjacent to the zone of cerebral coagulation necrosis. Previous studies revealed that no such perivascular lesions developed after injection of saline, Lewis rat lymphoid cells immunized against nonneural antigens, heat-killed Lewis EAE cells, or living EAE cells from donor rats that had major histocompatibility differences from the Lewis recipients (3, 4). The term "passive" is applied to the cell transfer because these data and the short survival period suggest that the recipient does not participate in the active immune response to the specific neural antigen; "passive" is *not* meant to imply total lack of reactivity (see "Discussion").

Radiation of recipients was done on the day of transfer, the day before, or the day after, as specified below. Radiation was delivered by a Picker Vanguard X-ray unit operating at 280 kV and 20 mA through additional filtration of 1.94 mm of copper equivalent. The source-to-target distance was 43–48 cm. The dose rate was measured at the target site using a Victoreen thimble chamber each

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time the machine was used and ranged from 95 to 105 R/min. Scatter irradiation to the head in those experiments in which this portion of the animal lay outside the irradiated field was measured by lithium fluoride dosimetry and amounted to 1.5–2.5 R/min. Unanesthetized animals were immobilized for radiation in glass tubes. The tail was passed through a central hole in a rubber stopper at one end of the tube and it was fixed to the side of the tube with adhesive tape. From the other end of the tube, cotton swabs were inserted and packed around the animal's trunk and head. Sham irradiated control animals were treated similarly and for a corresponding period each time the experimental groups were irradiated.

Bilateral adrenalectomy was performed 2 or 4 days before transfer through a mid-dorsal incision; rats were maintained on saline thereafter. All recipients were killed 24 hr after cell transfer. Forebrain, thymus, spleen, and vertebrae were fixed in Bouin's solution. Sections were stained with hematoxylin and eosin. Scoring of EAE lesions was done on randomized slides without knowledge of the experimental group.

Results. Five recipients were given 250 R of X-radiation to the trunk (head shielded) 2 hr and again 20 hr after the cell transfer. None developed perivascular infiltrates, whereas five unirradiated and five sham irradiated controls had many EAE lesions. However, radiation *after* cell transfer in this experiment undoubtedly entailed direct effects on the transfused cells as well as on the host's lymphoid system. In the next experiment, two groups of 3 recipients were given 1000 R to the trunk either 2 hr before or 2 hr after cell transfer. Equally good inhibition of EAE was obtained in both groups.

In view of these results, four experiments were done with whole body radiation 20–24 hr *before* cell transfer. These experiments (Table I) revealed that passive transfer of EAE was inhibited completely by 1000 or 700 R, partly by 400 R, and not at all by 100 R directed to the recipient. The complete inhibitory effect of 1000 or 700 R was manifest in rats whose adrenals had been removed beforehand, although adrenalectomy *per se* has an enhancing effect on passive transfer of EAE (3, 5). The partial inhibitory effect of 400 R was only slightly reversed by prior adrenalectomy. Although radiation causes nonspecific stress (6) and stress inhibits passive transfer of EAE (7), it is clear that the inhibitory effects of radiation were largely independent of adrenal-mediated stress reactions.

An attempt to reverse the effect of 700 R radiation by transfusion of normal marrow, spleen, or thymus cells was not successful. Twenty-four normal isohistogenic rats provided spleens, thymuses, and marrow from long bones for 6 irradiated recipients. The washed cell suspensions that contained 1.3×10^9, 2.1×10^9 and 0.97×10^9 nucleated cells/dose, respectively, were given intravenously 1 day after radiation and 1 hr before transfer of EAE lymph node cells. However, the inhibitory effect of radiation was not overcome by these cell supplements (Table I).

The inhibition of passive transfer of EAE by whole-body radiation of recipients was not due to a local effect on the thermal injury of the brain inflicted 1–3 days earlier, because the same result was obtained when the head was shielded from the X-ray tube (Table I). Furthermore, direct effects of radiation *pretreatment* on the brain (8) as well as indirect (humoral) effects of radiation on brain vessels (9) might be expected to increase permeability and enhance rather than inhibit EAE.

Sections of bone marrow, spleen, and thymus from radiated animals revealed necrosis and depletion of hemopoietic and lymphoid cells. The severity of damage was proportional to the dose of radiation. The injury was not reversed by adrenalectomy or by supplements of normal marrow, spleen, or thymus cells. Spleen and thymus weights were decreased by all doses of radiation, even 100 R which was not inhibitory for EAE (Table I).

Discussion. Inhibition of passive transfer by radiation of recipients after they had received EAE cells was expected. Of greater interest was the inhibition by radiation of

TABLE I. Passive Transfer of EAE to Radiated Recipients in Four Experiments.

Recipient treatment[a] (R)	Number with EAE score[b]			(% of control)[c]	
	0 or ±	1+	2+	Thymus	Spleen
1000	4			17	36
+ adrenex	3			24	49
(head shielded)	4			20	37
700	4			17	36
700	8			11	32
+ adrenex	4			12	38
400	2	2		14	40
+ adrenex		3	1	19	46
100			4	45	68
+ adrenex		1	3	60	70
700	4	2		24	42
+ marrow cells	5	1		24	46
+ spleen cells	6			26	53
+ thymus cells	6			21	59
Sham radiation[d]			20	100	100

[a] Each rat received passive transfer of EAE cells derived from one actively immunized donor. Radiation (whole body, except as specified) was done 1 day before, bilateral adrenalectomy was done 2 or 4 days before, and cell supplements were injected iv 1 hr before transfer of EAE cells.

[b] Scores based on number and intensity of EAE lesions at periphery of zone of thermal coagulation necrosis in brain; scale 0 to 2. Rats were killed 24 hr after cell transfer; slides randomized and read "blind."

[c] To facilitate comparisons, organ weights are given as percentage of average thymus or spleen weight of control, sham radiated group of the same experiment.

[d] Combined control groups of 4–8 rats from each of the four experiments.

recipients 2 or 24 hr *before* cell transfer. This result is in accord with the inhibition by prior radiation of passive transfer of delayed hypersensitivity to tuberculin or rabbit gamma globulin, reported by Cummings *et al.* (10) and Coe *et al.* (11). The inhibition in our experiments depended on the dose of radiation. It was not a direct effect on the brain and it was largely independent of the adrenal gland. It was associated with massive destruction of the host's lymphoid system and bone marrow. These results suggest that a host component is essential for success of passive transfer of EAE. It has been demonstrated that the majority of infiltrating inflammatory cells in other delayed hypersensitivity reactions originate from the host's bone marrow [reviewed in (12)]. Only a minority are derived from the injected donor cells. If this is true also of EAE, it may be that the specific encephalitogenic donor cells, unsupported by nonspecifically reacting host cells, were too few in number to be identified in the brain. The absence of EAE lesions is also compatible with the hypothesis that encephalitogenic cells produce a mediator which attracts nonspecific host inflammatory cells into the brain of normal recipients, but cannot do so in hosts depleted by radiation.

Although the absence of a host cellular component is the most obvious explanation of our results, it is not the only one. It has been suggested that radiation may produce humoral substances that damage the transfused donor immunocompetent cells (13). We consider also the possibility that the generalized increase of permeability following radiation (14) may allow donor EAE cells to escape into and be lost in nonneural organs. Even without invoking permeability change, the entry of donor EAE cells into lymphoid

organs as part of their normal migration (15) may be followed by their mechanical entrapment in necrotic tissue detritus.

Reports of enhancement of passive transfer by prior radiation of prospective recipients are not necessarily at variance with our results. In homologous transfers, unlike our isohistogenic system, radiation of recipients may enhance immunologic activities of donor cells by preventing a homograft reaction directed against them (13). Furthermore, other passive transfer experiments have utilized immunologic systems that take several or many days to develop; during this interval there is considerable repair of radiation damage (16). Gell and Wolstencroft (17) and Coe *et al.* (11) reported that delayed hypersensitivity skin reactions in irradiated recipients of cell transfer were slight or absent from tests made on the day of transfer or the following day, but appeared and increased in succeeding days. These facts may explain the apparent beneficial effect of radiation on transfer of EAE in rabbits and rats (18). The brevity of our experimental design ensured that the donor cells suffered from the full brunt of host malfunction. The brevity of our experiments, however, may also be responsible for our failure to reverse the effects of radiation by infusing normal marrow or spleen cells. In radiated mice and guinea pigs, for example, bone marrow supplements did not begin to restore the depressed blood leukocyte level until 5 days after infusion (19). Similarly, in radiated and thymectomized rats, bone marrow cells had a greater restorative effect on passive transfer of tuberculin hypersensitivity when 7–10 days were allowed to elapse before injection of the immunized lymph node cells (12). Therefore, the failure of our marrow or spleen cell supplements does not rule out deficiency of host cells as the mechanism of radiation inhibition.

Summary. Experimental allergic encephalomyelitis (EAE) was produced by passive transfer of fully immunized lymph node cells into immunologically naive, isohistogenic recipients. Passive transfer of EAE was inhibited by X-radiation of recipients shortly after or 24 hr before the transfer. Inhibition depended on the dose of radiation. It did not require direct effects of radiation on the encephalitogenic lymph node cells or on the brain, or indirect effects through the adrenal. Inhibition was associated with massive destruction of the recipient's lymphoid and hemopoietic systems, and may be related to deficient capacity for mounting an inflammatory reaction.

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The Distribution of Rabbit Anti-Mouse Thymus Globulin After Injection into Mice* (34025)

CARLOS NAVA, H. O. O'KANE, AND R. G. SHORTER

Section of Tissue & Organ Transplantation, Mayo Clinic & Foundation, Rochester, Minnesota

The immunosuppressive action of heterologous antilymphoid sera (ALS) has been well established in the laboratory in various species of animal, and prolongation of survival of allografts of skin (1), kidney (2), and liver (3) and xenografts of tumor (4) has been achieved with their use. Such observations have led to the clinical trial of ALS as an adjunct to more conventional immunosuppressive therapy in the grafting of organs in man (5, 6). Although theories have been formulated as to the mechanism of the immunosuppressive action (7), no definitive explanation has been found. We considered that a study of the distribution of heterologous antilymphoid globulin *in vivo* after intravenous injection might be contributory in this regard and the distributions of radio labeled gamma globulin and IgG prepared from heterologous antithymus serum were investigated after their parenteral injection. Presented here is a report of the results of these experiments.

Materials and Methods. Rabbit anti-mouse thymus sera (RAMTS) were prepared by injecting intravenously each of three New Zealand rabbits (average weight 2 kg) with suspensions of 1×10^9 viable thymus cells obtained from neonatal mice. Each rabbit was injected twice at a 2-weekly interval and bled 1 week after the second injection. The sera were pooled and heated at 56° for 30 min. The initial titer of lymphocytotoxicity *in vitro* for murine lymphocytes was 1:4096 when tested by the method described by Terasaki and associates (8) and titrated to less than 10% kill. After repeated absorptions with murine red cells (1 vol erythrocytes: 4 vol serum) to remove *in vitro* hemagglutinating and hemolyzing activity, and a single absorption with a homogenate of murine kidney (1 vol kidney homogenate: 4 vol serum), the gamma globulin (ATG) was precipitated with 40% ammonium sulfate.

In preparation for labeling with radioactive iodine, one portion of the precipitate was reconstituted in sodium phosphate buffer, 0.05 *M*, pH 7.0, to the original volume of the serum and then dialyzed against the same buffer. At this stage the lymphocytotoxic titer of the globulin (ATG) was 1:4096 when tested *in vitro* against murine lymphocytes from the peripheral blood and titrated to less than 10% kill. After reconstitution in 0.1 *M* potassium phosphate buffer (pH 8.0) another portion of the precipitate was dialyzed against this same buffer prior to the separation of the IgG (ATIgG) by column chromatography using diethylaminoethanol (DEAE) cellulose (9). The immunoelectrophoretic pattern of this fraction showed the presence

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