Inhibition of Experimental Allergic Encephalomyelitis in Rats Severely Depleted of T Cells

Abstract. *Lewis rats depleted of thymus-derived cells (B rats) failed to develop either experimental allergic encephalomyelitis or antibody against myelin basic protein. Lewis B rats reconstituted with 690 × 10^6 thymocytes developed experimental allergic encephalomyelitis and levels of antibody against myelin basic protein comparable to those of controls. The Lewis B rat model should be useful in the analysis of the role of thymus-derived cell populations and antibody in the induction of experimental allergic encephalomyelitis.*

Experimental allergic encephalomyelitis (EAE) is induced in several species by inoculation of central nervous tissue or by encephalitogenic basic protein of central myelin with or without adjuvants (*1*). Several studies implicate the thymus-derived lymphocyte (T cell) in the induction of the disease. Thymectomy of newborn rats and chickens has been shown to reduce the incidence of EAE by 50 to 80 percent in immunized animals (*2–4*), although in neither case was it demonstrated by appropriate reconstitution that the relevant lesion was the deficiency of thymus-derived cells. Experimental allergic encephalomyelitis can be induced in agammaglobulinemic chickens (*3*). The roles played by antibody and effector cells, presumably but not conclusively T cells, in the pathogenesis of the tissue damage (demyelination) in EAE have not yet been defined. One approach to elucidating mechanisms that relate effector cells, antibody, and target organ (central nervous system myelin) is the use of animal models with known genetic disposition toward EAE and controlled immunocompetent cell populations. These animals could be used to examine both the induction and expression of the disease.

We now report that rats of the inbred Lewis strain, normally highly susceptible to the induction of EAE by myelin basic protein or spinal cord emulsions (*5–8*), can be rendered completely resistant to the disease by experimental procedures that cause a drastic and specific reduction in the number of T cells (*10, 11*).

Lewis rats were purchased from Microbiological Associates, Walkersville, Maryland, and from Simonson Laboratories, Gilroy, California. Two series of thymus-deprived (B) rats were prepared (*10, 11*) as follows. Male and female rats were thymectomized when 5 weeks old, and 4 weeks later they were given 900 or 1000 roentgens (whole body) of gamma rays from a ^{137}Cs source, delivered at 109 roentgens per minute. On the day of irradiation, the rats were injected via the lateral tail vein with 10^7 viable bone marrow cells from a Lewis donor that had been thymectomized at 5 weeks of age and subjected to chronic lymphocyte drainage via an indwelling thoracic duct catheter for the 7 days immediately preceding their use as marrow donors. In one series of such recipient animals, the in vitro phytohemagglutinin response of peripheral blood lymphocytes was assayed 2 weeks after marrow reconstitution and compared with the phytohemagglutinin response of blood lymphocytes from two normal Lewis rats. The phytohemagglutinin responses of peripheral blood lymphocytes were measured by [^{3}H]thymidine incorporation (*12*). This response provides a sensitive indicator of T cell depletion in lymphocyte populations (*13, 14*). The results (Table 1) show the extreme T cell depletion caused in B rats by the procedures outlined above. In the second series of B rats, some animals were also reconstituted with an intravenous injection of 690 × 10^6 Lewis thymocytes obtained from 6-week-old donors and given on the day of immunization with encephalitogen. Guinea pig spinal cords (Pel-Freeze Biologicals, Inc., Rogers, Arkansas) were kept frozen at − 60°C until use. Basic protein was prepared and purified from spinal cords (*15*) with minor modifications (*8*). Purity of preparations was ascertained by acrylamide disc electrophoresis at neutrality with 0.2 percent sodium dodecyl sulfate or by electrophoresis in 5 percent acrylamide gels in 8*M* urea and 1*M* acetic acid with 0.2 percent sodium dodecyl sulfate (*8, 9*). All immunizations were given bilaterally to the hind foot pads in complete Freund's adjuvant (CFA) (Difco) containing 5 mg (dry weight) of *Mycobacterium butyricum* per 10 ml.

Table 1. Phytohemagglutinin response; dpm, disintegrations per minute.

Lewis rats	No.	Response (dpm)*	
		Mean	Range
Normal	2	77,250	67,500 to 87,000
B rats†	24	225	60 to 748

* [^{3}H]Thymidine (0.25 μc) was added to each 1.0 ml of culture containing 10^6 peripheral blood leukocytes for the last 16 hours before harvest after 72 hours of cultures. † Lewis B rats used in the first experiment were assayed 14 days after irradiation and marrow reconstitution.

In the first series of rats, individuals received encephalitogen, either as 500 μg of guinea pig myelin basic protein (GPBP) in 0.2 ml of 0.15*M* NaCl emulsified with 0.2 ml of CFA, or as 110 mg of guinea pig spinal cord (GPSC) emulsified with 0.35 ml of CFA. In the second series of rats, individuals received either 100 μg of GPBP, or 110 mg (wet weight) of GPSC, both with CFA. In the first series, ten normal Lewis rats of both sexes, and in the second series, eight normal Lewis rats of both sexes were immunized similarly to provide a positive control for both immunogens. Antibody against GPBP was estimated only in serums from rats immunized with GPBP by immune coprecipitation with rabbit antiserum to rat immunoglobulin G (IgG) as the precipitant (*8, 16*). The GPBP was iodinated with carrier-free $Na^{125}I$ (New England Nuclear) by a modification of the Hunter-Greenwood method with chloramine-T as oxidant (*8, 17*). The iodinated GPBP was encephalitogenic at doses of 50 to 100 μg. The central nervous system was examined histologically according to standard methods (*6, 8, 18*).

The first experiment showed the effect of extreme T cell deprivation on the induction of EAE (Table 2, series 1). A total of 24 Lewis B rats was immunized with encephalitogen 17 days after marrow reconstitution, 12 receiving GPBP, and 12 receiving GPSC. Of the 24 immunized B rats, 16 survived to 14 days after immunization. Autopsy and histologic study in six of the eight immunized B rats dying before day 14 did not reveal EAE or any other lesions in the central nervous system; one animal had pneumonitis. In the other two rats that died before assay, autolysis was too far advanced when the rats were discovered to allow a histological examination. Some mortality is associated with the procedure for preparing B rats; this is usually accentuated by further experimental interference (unpublished observations). In addition, ten normal Lewis rats were also immunized with

Table 2. Protection from EAE in Lewis B rats. In series 1, half of each group received 110 mg of GPSC and half received 500 μg of GPBP; in series 2, half of each group received 110 mg of GPSC and half received 100 μg of GPBP. The intensity of histological EAE was measured on a modified scale of Alvord and Kies (*6, 18*). The antigen-binding capacity (ABC) of serum was determined by the method of Lisak *et al.* (*8, 16*).

Immunized Lewis rats	No. immunized	No. surviving to assay	Day of killing	Histologic EAE (No.)	Intensity of EAE	Serum ABC (%)
			Series 1			
Normal	10	10	14, 35	10	++ to +++	45 to 95
B rats	24	16*	14, 21	0	0	1.0 to 3.6
			Series 2			
Normal	8	8	16, 25	8	++ to +++	58 to 63
B rats	18	17†	16, 25	1	0(16) ± (1)	9 to 12; 20 for one animal
B rats reconstituted with thymocytes	18	18	16, 25	17	0(1) ++ to +++(17)	30 to 73

* Of eight rats dying before assay, six were examined histologically postmortem and found free of EAE. † One B rat immunized with GPSC died 4 days after immunization; necropsy showed focal hemorrhages in the white matter of the spinal cord.

the same encephalitogen, five with GPBP and five with GPSC. At 14 and 21 days, a group of B rats and six normal rats (of a group of ten) were killed, and their entire central nervous systems were prepared for histological diagnosis of EAE; blood serums of animals injected with GPBP were assayed for antibody to basic protein. Four immunized normal rats were also examined by the same assays 35 days after immunization.

All 18 immunized normal Lewis rats showed severe histological EAE characterized by focal vascular lymphocytic and mononuclear infiltrates and by demyelination 14 days after immunization (Table 2, series 1 and 2). (Seven of these animals showed paresis of hind limbs 11 to 14 days after immunization.) Blood serums of all immunized control animals with GBPB also showed binding of 45 to 95 percent of the ^{125}I-labeled GBPB at optimum concentrations of antigens to antiserum (*8, 16*). None of the 16 Lewis B rats killed either at 14 or 21 days after immunization showed clinical or histological EAE. The antigen-binding capacity of the serums was within the normal range (Table 2, series 1). This experiment demonstrated that B rats were protected against the induction of EAE, and also against the induction of detectable antibody to the basic protein of myelin. It did not, however, permit the conclusion that the thymus deprivation of B rats specifically led to protection. Possibly other aspects of the procedure, such as whole-body irradiation, for producing B rats contributed to protection. Consequently, a second experiment was performed to rule out all such effects and to restrict the source of the protection specifically to the deprivation of T cells. In this experiment use was made of the observation that a single injection of normal thymocytes to B rats can restore cell-mediated immune reactions and thymus-dependent antibody formation (*11, 14*).

A second series of Lewis B rats was prepared and divided into four groups. Twenty-three days after irradiation and marrow reconstitution, two groups of nine B rats each received 690×10^6 normal Lewis thymocytes intravenously; two additional groups of nine B rats each received no further cells. On the same day, one group of thymocyte-reconstituted and one group of nonreconstituted B rats were immunized with 110 mg of GPSC; the other two groups received 100 μg of GPBP. Two groups of four normal Lewis rats similarly immunized provided a positive control.

Of the 36 B and thymus-reconstituted B rats, 35 survived until the time they were killed, either at 16 or 25 days after immunization; all eight of the normal Lewis rats survived (Table 2, series 2). As in the previous series, all normal Lewis rats showed histological EAE and their serums showed high antigen-binding capacity. Similarly, of 17 surviving immunized B rats not reconstituted with thymocytes, 16 showed no clinical or histological signs of EAE. The remaining B rat showed weak (±) histological EAE, consisting of only two perivascular cellular infiltrates in the lumbar segment of the spinal cord. However, of the 19 immunized B rats reconstituted with syngeneic thymocytes, 17 showed histological EAE. Four of these animals also showed clinical signs of paresis or paralysis of the hind legs. In the thymus-reconstituted animals, the cellular infiltrates were diffuse rather than focal in perivascular areas, and most cells were of the epithelioid type with large irregular vesicular nuclei and sparse cytoplasm. Only one individual of the thymocyte-reconstituted group immunized with GPBP and killed on day 25 failed to show any histological signs of EAE, although its blood serum showed 61 percent of antigen binding. Very low, but probably significant antigen-binding capacity was found in the serum of the nonreconstituted B rats, while the antigen-binding capacity of serum from the thymocyte-reconstituted B rats was in the same range as that of serums from normal immunized Lewis rats (Table 2).

A sufficient role for T cells in the induction and expression of EAE in experimental animals has been suggested by the demonstration that neonatally bursectomized and sublethally irradiated chickens are as susceptible to clinical and histological EAE as control animals (*3*). That immune T cells are also necessary for EAE is suggested by the findings that (i) EAE cannot be transferred to normal recipients with immune serum from affected donors and (ii) neonatally thymectomized and irradiated chickens or neonatally thymectomized rats are less susceptible to the induction of the disease than control animals (*2–4*), although nonspecific suppressive effects due to causes other than simple absence of T cells were not controlled by appropriate cellular reconstitution. Our experiments have demonstrated that adult thymectomized, irradiated marrow–reconstituted Lewis rats (B rats) are almost completely resistant to an encephalitogenic challenge, while simple neonatal thymectomy prevented induction of EAE in only 50 to 80 percent of animals (*2*); only one B rat out of 33 individuals showed EAE, and in this animal the disease was of minimal intensity (Table 2, series 2). This result suggests that T cells play a necessary role in the development of the disease. This suggestion is confirmed by the observation that reconstitution of B rats with normal thymocytes restored to essentially normal levels the ability of the B rat to react to the encephalitogenic challenge by displaying clinical and histolog-

ical EAE. Furthermore, the antibody response to determinants of the purified basic protein used as the assay antigen (and as encephalitogen in half of the control and T cell–depleted and thymus-reconstituted animals used) was almost, if not completely, thymus-dependent (Table 2, series 1). Injection of thymocytes restored the antibody responses of B rats to normal levels (Table 2, series 2).

In the study of EAE the Lewis B rat should thus be of value in the analysis of the role of different thymus-derived cell populations in restoring the inert recipient to susceptibility to the disease and in a further examination of the role of antibody in the disease process.

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Decline of DDT Residues in Migratory Songbirds

Abstract. *Analyses of ten species of migratory songbirds killed when the birds flew into television towers in Florida showed a progressive decline in the concentration of DDT and its metabolites (DDD and DDE) in their fat depots for the period 1964 to 1973. This decline is apparently correlated with the decreased usage of DDT in the United States during the same time.*

Introduced on a wide-scale basis since World War II, DDT (*1*) quickly became popular for the control of many agricultural pests and disease vectors. Because of its persistence in the environment, solubility in fat, dissemination through air and water, and widespread application, it is now found virtually all over the world in both terrestrial and aquatic ecosystems. By about 1958 there were indications that DDT and its metabolites might be associated with declines in avian populations at the top of food pyramids. Evidence was marshaled by Ratcliffe (*2*), Hickey and Anderson (*3*), and others that DDT accumulates in the fatty tissues of some raptorial and piscivorous birds at the top trophic levels. In birds such as the bald eagle (*Haliaeetus leucocephalus*), peregrine falcon (*Falco peregrinus*), and osprey (*Pandion haliaetus*) decreases in eggshell thickness and population declines from 1947 to 1967 have been correlated by some investigators with DDT usage and subsequent reproductive, metabolic effects on the birds (*2, 3*). A variety of laboratory experiments on other birds (Japanese quail, *Coturnix coturnix*; mallard, *Anas platyrhynchos*; black duck, *Anas rubripes*; American kestrel, *Falco sparverius*; and screech owl, *Otus asio*) subsequently revealed correlations between dietary DDE (*1*) or DDT and eggshell thinning and ensuing poor reproductive success. Hickey and Roelle (*4*) pessimistically reported ". . . the steady numerical decline in the 1950's of the breeding adults [peregrine falcons] in many regions. This decline continues [in 1965], and the end is nowhere in sight." Despite these effects since DDT came into widespread use, the sublethal effects of the chlorinated hydrocarbon pesticides on animals of lower trophic levels, namely, insectivorous and granivorous species, are virtually unknown.

For at least the last two decades hundreds of small birds have been killed as a result of striking tall television towers in northern Florida during nocturnal migratory flights between their breeding grounds in North America and wintering areas in the West Indies and Central and South America (*5*). Such migrants are conspicuously obese, especially in the autumn, when subcutaneous and abdominal fat depots comprise 30 percent or more of the body weight (*6*). These marked fat depots are valuable indicators of the pesticide burdens in the migrants (*7*). The analyses presented here are based upon autumnal samples of chiefly insectivorous birds collected the morning after their deaths at WCTV tower near Tallahassee and WJXT and WJKS towers at Jacksonville. All these south-bound birds (*8*) were classified as "very fat."

From each of five to ten adult males of the same species, fat was dissected from the interfurcular depot and pooled as a species-specific sample for analysis. An attempt was made to remove the same amount of fat from each bird; the pooled fat samples per species had a mean weight of 1.16 g (0.58 to 1.95 g). Subsequent lipids extracted from the fat samples averaged 0.76 g (0.14 to 1.56 g) for all the samples. Samples were then analyzed according to the technique described by Grocki and Johnston (*7*).

In the ten species (*8*) of small migrants totaling 319 individuals analyzed here (Fig. 1), no sample was devoid of DDT or its metabolites. As expected, *p,p'*-DDE was more abundant than either *p,p'*-DDT or *p,p'*-DDD (*1*), the mean ratio of DDE to DDT [in parts per million (ppm), lipid weight] in the 43 samples being 1/0.56. Declines to low concentrations of total DDT (DDT, DDD, and DDE) in 1973 are clearly evident from the regression lines calculated for the bird groups A and B.

One may obtain a further demonstration of the dramatic decline of the DDT burden over a 5-year span by comparing mean annual concentra-