SUPPRESSION OF ALLERGIC ENCEPHALOMYELITIS IN RATS BY MEANS OF ANTIBRAIN SERUM*

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There is suggestive evidence that certain diseases of man, *e.g.* disseminated encephalomyelitis and thyroiditis, may result from immune responses directed against his own (autologous) tissues. This "autoallergic" concept of disease derives most support from several interrelated, well established observations in experimental animals.

Certain tissues, *e.g.* brain, thyroid, and testis, have long been known to possess antigenic activity and, when injected into experimental animals, elicit circulating antibodies which react specifically with the corresponding tissue *in vitro* (1–7). It is also known that animals injected with these tissues emulsified in Freund's adjuvant may develop striking tissue damage restricted to the organ in question; *viz.*, allergic encephalomyelitis, thyroiditis, and aspermatogenesis, respectively (reviewed by Paterson, reference 8). Each of these diseases may be induced by injection of an animal's own (autologous) tissues (9–11). The antibodies produced by a sensitized animal react specifically *in vitro* with his own brain, thyroid, or testes (10–12).

In a recent review (8) of allergic encephalomyelitis (AE) and other analogous experimental "autoallergic" diseases, it was stressed that the existing data did not yet justify the selection of one type of immune response over another as the responsible mechanism for these diseases. Events during the past 4 years have not required modification of this view. There is ample experimental evidence that the complement-fixing (CF) antibrain antibodies, called forth by brain sensitization, and directed against ethanol-soluble brain antigens do not cause AE (8). The strongest support for this conclusion is the observation that the CF antibrain antibodies may be elicited by non-mammalian (frog, turtle, and snake) nervous tissue which lack the capacity to induce AE (13). The unsuccessful attempts by several workers to transfer AE passively with antibrain immune serum (reviewed by Chase, reference 14) have been cited often as convincing evidence that circulating antibody plays no role in AE (see reviews by Waksman, references 15–17). As discussed elsewhere (18), other

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interpretations are possible. For example, passively administered antibrain antibody may not cross the blood-brain barrier in sufficient amounts to cause AE in intact normal animals. It is also possible that the unsuccessful attempts to transfer AE using immune serum reflect current lack of information about key factors necessary for such transfer.

In the last few years, much has been written about the importance of delayed-type hypersensitivity to brain in the pathogenesis of AE (see reviews by Waksman, references 15–17). According to this view, AE is believed to result from an interaction between nervous tissue antigen and "sensitized cells" immunologically committed to brain and spinal cord antigen(s) but not producing conventional serum antibody. The evidence for this view is indirect and not always consistent. For example, rabbits with AE or destined to develop AE may exhibit cutaneous reactivity resembling delayed-type reactions to brain extracts (19). Such reactions, however, also occur in other rabbits which do not develop the disease (19). In the rat, which regularly develops AE, convincing cutaneous reactivity of this type has not been demonstrated in spite of serious attempts to do so in our laboratory (20). It should be stressed that no direct evidence exists that delayed-type hypersensitivity either does or does not play an important role in this disease. The transfers of AE in rats (21, 22) by means of living lymph node cells derived from spinal cord–sensitized donors did not clarify this issue. While these studies did provide direct evidence that the disease has an immune mechanism and that lymph node cells produce the immune factor, they have not defined its nature. For as stated previously (18, 21, 22) and worthy of reemphasis here, it is impossible to determine whether the lesions of AE in recipient animals result from minute quantities of conventional serum antibody produced and released by the donor lymph node cells after their transfer. Or, alternatively, whether the lesions are due to a direct interaction between antigen in the brain of recipients and donor lymph node cells engaged in a delayed-type hypersensitivity response.

There have been clues which suggest a protective role for the CF antibrain antibodies in AE. In 1950, Thomas, Paterson, and Smithwick (23) noted that AE occurred more often and was more severe in dogs without CF antibrain antibody. It was suggested then that "... the antibody might represent a protective reaction." Subsequently it was found that rabbits sensitized to brain or spinal cord in adjuvant *via* the subcutaneous route almost invariably produced high titer CF antibody but rarely developed AE (13). In contrast, guinea pigs sensitized by any route, developed rapidly fatal AE and CF antibody could rarely be detected (24). More recent observations revealed that rats although severely paralyzed 2 or 3 weeks after sensitization, almost always recovered and appeared clinically well 6 to 9 weeks after sensitization (see below and reference 25). CF antibrain antibody was observed commonly in rats during recovery from the disease. Finally, rats which had recovered at 6 weeks, at a time when CF antibody was present, upon reinjection of nervous tissue plus adjuvant usually failed to exhibit again any clinical signs of AE or have pathological evidence of the disease (25). The preliminary finding of Florey, summarized by Vulpé (26), that guinea pig antibrain serum may interfere with active induction of AE in this species of animals, further supported the concept that CF antibrain antibody might have a protective function. For the above reasons, it seemed probable that passively administered immune rat serum containing CF antibrain antibody might suppress induction of AE in rats actively sensitized to nervous tissue.

The purpose of this paper is to present data which show that AE is suppressed in rats sensitized to nervous tissue and treated with rat antibrain serum. Evidence is presented consistent with the view that the suppressive or protective effect of antibrain serum is due to CF antibrain antibody.

Materials and Methods

General Plan.—The protocol of the experiments designed to evaluate the role of antibrain serum in suppression of AE is presented in schematic form in Fig. 1. A large number of pro-

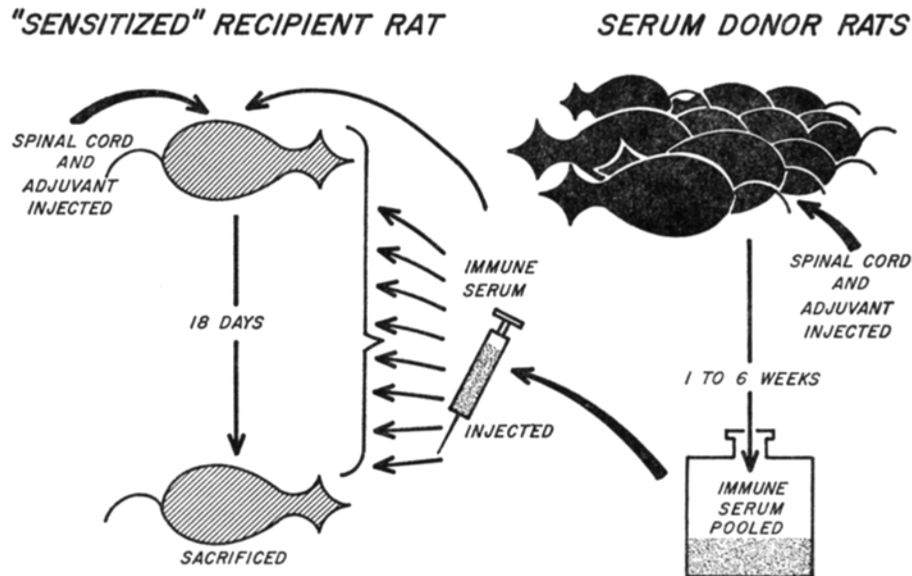

FIG. 1. Design of experiments for evaluating effect of antibrain serum on induction of AE.

spective serum donor rats (60 to 70 animals in most of the experiments) were sensitized with guinea pig spinal cord emulsified in adjuvant. 1 to 6 weeks later, the donor rats were bled, their sera collected and pooled. Then, prospective serum recipients were sensitized to spinal cord plus adjuvant in the same manner as the serum donors. In most experiments, each recipient was injected intravenously with a pool of serum 4 to 5 hours prior to sensitization. Thereafter, injections of the serum were given on alternate days through the 18th day after sensitization, during which time the rats were examined daily for clinical signs of AE. On the 19th day, the actively sensitized and serum-treated rats were sacrificed and their brains and spinal cords examined for microscopic lesions of AE.

Animals.—Albino Wistar rats,[1] of either sex, weighing 125 to 300 gm were employed. Hartley guinea pigs,[2] of either sex, weighing 500 to 800 gm were used as a source of spinal cord

[1] Wistar rats purchased from Hemlock Hollow Farms, Wayne, New Jersey or Carworth Farms, New City, New York.

[2] Hartley guinea pigs obtained from Hemlock Hollow Farms or Miss Lucy V. Via, Free Union, Virginia.

antigen. Animals were housed in wire cages and fed a standard diet of commercial food pellets (supplemented with "greens" in the case of guinea pigs) and tap water *ad lib.*

Tissue-Adjuvant Inocula and Sensitization of Animals.—Guinea pig spinal cord was removed and stripped of meninges aseptically and stored at −17°C for 1 to 7 days before used. The guinea pig kidneys had been aseptically removed and stored at −17 to −24°C for 1 year prior to use. Thawed portions of tissues were homogenized in a Ten Broeck grinder with 0.25 per cent phenol in distilled water to give a 33 per cent tissue (wet weight) homogenate and emulsified in complete adjuvant as previously described (13, 21, 24). Each rat was injected intracutaneously with 0.1 ml of tissue-adjuvant inoculum in each of 6 sites over the back and 0.1 ml in the ventral neck.

The Serum Pools and Their Use.—Serum-donor rats were bled aseptically by cardiac puncture under ether anesthesia. The blood was allowed to clot in sterile siliconized glass tubes[3] for 1 to 2 hours at room temperature followed by 4 to 8 hours at 4°C. The sera were collected by centrifugation at 4°C and pooled. In several experiments, serum pools were deliberately prepared to include only serum of rats which had exhibited clinical signs of AE before bleeding. Other pools were prepared from sera of rats which had remained clinically well. These serum pools were designated by the letters "A" (prior clinical signs of AE) or "B" (no prior signs), respectively. Once so designated, an "A" or "B" serum pool was used for all serum treatment of a given group of sensitized recipients. All serum pools were stored at 4°C in sterile rubber-stoppered glass vials. From 3 to 14 days elapsed between preparation of the pool and initial injection of recipients. The serum pool from rats sensitized to guinea pig kidney was prepared by pooling individual rat sera which had been stored at −17 to −25°C for 14 months. One serum pool, derived from rats sensitized to guinea pig spinal cord plus adjuvant 1 to 6 weeks prior to bleeding, was treated with 2-mercaptoethanol in a final concentration of 0.1 M as previously described (27), a procedure known to destroy CF antibrain antibody. Two serum pools, checked for bacterial contamination at the time they were prepared and after their use some 4 weeks later gave no bacterial growth on sheep blood.

Rats were usually kept in an incubator at 37°C for 2 to 10 minutes prior to intravenous injection of serum to induce peripheral venous dilatation and facilitate injection. Each animal was etherized lightly and gently hand-held by an assistant during injection of serum into either a leg vein (exposed by prior application of a depilatory) or lateral vein of the tail. Injection of volumes of 1.0 to 2.0 ml of serum required only 2 to 3 minutes and gave no untoward clinical signs.

Assay of Rat Serum for Complement-Fixing (CF) Antibrain Antibodies.—A 50 per cent hemolytic end-point technique was employed.[4] This technique as well as the details concerning preparation of ethanol extracts of rat brain for use as antigens has been described in previous reports from this laboratory (21, 24). If more than six 50 per cent hemolytic units of complement ($C'H_{50}$), of exactly 100 $C'H_{50}$ available, were fixed specifically by serum in presence of brain antigen, that serum was considered to contain antibrain antibody. Justification for this interpretation, based on prior experience with appropriate control studies, has been described in detail (21, 24).

Histological Studies.—Brains and spinal cords were fixed in 10 per cent formaldehyde and then cut into blocks, paraffin-embedded, cut at 5 to 10 microns, and stained with hematoxylin-eosin. A minimum of 7, in most cases 14, different sections of brain-spinal cord of each animal were examined microscopically for lesions of AE. The brain sections included trans-

[3] Siliconizing done with desicote, Beckman Instruments, Inc., Fullerton, California. All glassware in this study was processed through sulfuric acid–dichromate and repeatedly rinsed in tap water and then distilled water before being sterilized for use.

[4] Complement, sheep red cells, and anti-sheep cell hemolysin obtained from Certified Blood Donor Service, New York.

verse cuts through (*a*) the cerebrum at level of the thalamus, (*b*) the mesencephalon including the 3rd ventricle, and (*c*) the cerebellum-pons in the region of the 4th ventricle. The spinal cord sections included at least 3 longitudinal cuts through the cervico-thoracic segments and 1 cut through the lumbar region.

Criteria for Clinical Aspects of AE and Grading of Histological Lesions of AE.—Rats were observed daily and checked for neurological signs noting, in particular, the gait, strength of hindlegs, and righting reflexes. Any animal exhibiting gross ataxia on 2 or more consecutive days and/or paralysis of one or both hindlegs for 1 or more days was recorded as showing clinical signs of AE.

The 7 (or more) stained sections of brain and spinal cord from each rat were examined microscopically by each author for lesions of AE. It was usually easy to determine whether an animal did or did not have characteristic lesions, positive or negative, (24). Since it soon was apparent that minimal disease (as judged by relatively few lesions) had occurred in many of the rats treated with serum, the following arbitrary and reproducible grading system was used. Even if only 1 discrete focal area of definite vasculitis was found, the animal was considered positive. If up to 6 discrete, focal areas of vascular-perivascular inflammation characteristic of AE were found in the 7 to 14 sections examined, the animal was classified as having "few lesions." If more than 6 areas were demonstrable, the animal was classified as having "many lesions." In most instances, those animals classified as having "many lesions" had disseminated, intense lesions within the brain and/or spinal cord.

EXPERIMENTAL

Time-Course of AE in Rats.—For purposes of reference, experience with induction of AE in albino rats during the past 3 years is set out in Table I. The data provide a basis for predicting the occurrence of AE in rats in our laboratory at different times after sensitization. The observations have direct bearing on the serum-suppression data to be presented below. As can be seen (Table I), rats given a single injection of guinea pig spinal cord plus adjuvant remain clinically well through the 8th day after sensitization. Demonstrable histological evidence of AE can be found by the 7th or 8th day in a few rats. Clinical signs and lesions of AE reach peak incidence between the 15th to 18th day after sensitization. At this time, approximately half of the sensitized animals exhibit clinical signs of AE. Virtually all sensitized rats have demonstrable lesions of AE. During the 3rd week after sensitization rats uniformly begin to improve clinically with gradual waning of neurological signs. Lesions of AE are now less frequent. It is of considerable interest that by the 29th to 39th day after sensitization full clinical recovery has usually ensued; and only an occasional animal exhibits persistence of clinical signs. An invariable accompaniment of this clinical recovery is the marked decrease in proportion of rats with lesions of AE during this time. Around the 42nd to 44th day an occasional rat exhibits recurrence of clinical signs or may, for the first time, exhibit neurological signs. This apparent resurgence of clinical signs at this time is accompanied by an increase in proportion of rats with lesions of AE. By the 63rd day after sensitization rats are clinically well and lesions have virtually disappeared. Thus, in the rat sensitized in this fashion, AE is a

TABLE I

Time-Course of Allergic Encephalomyelitis (AE) in 433 Control Rats Sensitized to a Standard Nervous Tissue and Adjuvant Emulsion during the Period 1959 to 1962

Time between sensitization and sacrifice	No. of experiments	No. of rats sensitized*	Clinical-pathological status of rats on day sacrificed		Proportion of rats with AE on day sacrificed
			No. rats with clinical signs of AE‡	No. rats with lesions of AE	
days					
6	3	18	0	0	0/18§ (0%)
7	4	42	0	4	4/42 (10%)
8	1	24	0	2	2/24 (8%)
12	1	5	1	2	2/5 (40%)
14	2	9	0	6	6/9 (67%)
15	1	4	2	4	4/4 (100%)
16	1	5	5	5	5/5 (100%)
17	3	22	12	21	21/22 (95%)
18	3	17	6	17	17/17 (100%)
19	10	72	30	68	68/72 (95%)
20	5	20	5	16	16/20 (80%)
21	3	19	9	14	14/19 (74%)
27	3	13	4	8	8/13 (62%)
29	2	16	1	10	10/16 (62%)
39	1	32	0	2	2/32 (6%)
42	2	56	3‖	6	6/56 (11%)
44	1	26	1‖	4	4/26 (15%)
63	1	33	1‖	1	1/33 (3%)

* All rats sensitized to standard guinea pig spinal cord–adjuvant emulsion *via* the intra cutaneous route as described in Materials and Methods.

‡ Except for 3 rats, all animals in this table and Tables II to V with clinical signs were subsequently found to have lesions of AE.

§ Numerator, No. of rats with lesions of AE with or without associated clinical neurological signs; denominator, No. of rats sensitized and sacrificed on postsensitization day indicated.

‖ Of the 3 rats with clinical signs when sacrificed on 42nd day, the ataxia observed in 2 had persisted since the 11th and 12th day after sensitization whereas the ataxia noted in the 3rd rat made its initial appearance on day sacrificed. The 1 rat with signs when sacrificed on the 44th day had been paralyzed previously, had recovered and appeared clinically well on the 17th day after sensitization and remained well until 39th day when severe ataxia reappeared. The ataxia in the 1 rat sacrificed on the 63rd day had persisted since it initially appeared on the 14th day after sensitization. Routine sections of brain–spinal cord from this rat as well as the 2 rats with persistence of clinical signs up to 42nd day had no microscopic lesions of AE.

transitory disease. Almost all animals are afflicted by the 3rd week after sensitization, most show full recovery by the 5th to 6th week, and virtually no trace of disease exists by the 9th week.

This pattern of AE in the rat was an important clue which prompted the search for a protective factor in antibrain rat serum.

Suppression of AE in Rats Treated with Antibrain Serum.—A serum-suppression experiment is shown in detail in Table II. Twelve rats were sensitized to spinal cord plus adjuvant; each received 2.0 ml of pooled antibrain rat serum on alternate days. All 12 animals remained clinically well through the 19th day at which time they were sacrificed. In only 6 animals could AE lesions be found. Notable is the fact that 6 of the 12 serum-treated animals had no evidence of AE whatsoever. In contrast, 18 control rats sensitized with the same inoculum but not treated with serum exhibited the expected incidence

TABLE II

Suppression of Allergic Encephalomyelitis (AE) *in Rats Sensitized to Nervous Tissue Plus Adjuvant and Treated With Antibrain Rat Serum* (*Experiment R-8-60*)

Serum treatment*	No. of rats sensitized	No. with clinical signs on days after sensitization			No. with lesions‡		Proportion rats *without* evidence of AE§
		1–7	8–14	15–19	Many lesions	Few lesions	
2.0 ml pooled serum IV on day sensitized and alternate days thereafter through 18th post-sensitization day	12	0	0	0	2	4	6/12‖ (50%)
No serum	18	0	4	8	13	4	1/18 (6%)

* Serum pool used for treatment derived from sera collected from rats 6 weeks after sensitization to spinal cord plus adjuvant as described in Materials and Methods.

‡ All rats sacrificed on 19th day after sensitization = 1 day after last injection of immune serum. Lesions of AE recorded as few or many in number as described in Materials and Methods.

§ In this table and in Tables III to V, final results expressed in terms of rats failing to develop AE.

‖ Numerator, No. of rats remaining clinically well and having no demonstrable lesions of AE; denominator, No. of rats sensitized.

and picture of AE. Four rats developed paralysis during the 2nd week. During the 3rd week, 1 of these 4 had recovered but 5 additional rats had developed neurological signs. Seventeen of the 18 rats were found to have characteristic AE lesions. In most of these 18 control animals the lesions were numerous, intense, and readily found in the brain and spinal cord. Only 1 of the 18 control rats failed to develop evidence of AE.

Suppression of AE; Combined Results with Different Antibrain Serum Pools.—The results of several experiments, employing 8 different serum pools are presented in Table III. The results of treatment of small groups of rats with pooled serum derived from either previously paralyzed serum donors (*e.g.* pools 3-A, 4-A, and 5-A) or donors clinically well before bleeding (pools 3-B, 4-B, or 5-B) gave comparable results and for convenience have been combined

in tabulating the data in the upper part of Table III. The control groups (not serum-treated) for the serum-treated groups of rats are listed in the bottom of Table III in corresponding sequence.

Of 48 animals sensitized and treated with serum, only 3 developed clinical

TABLE III

Suppression of Allergic Encephalomyelitis (AE) in Sensitized Rats Treated with Pooled Antibrain Rat Serum

Serum treatment*					No. of rats sensitized to cord plus adjuvant	No. with clinical signs of AE‡	No. with lesions of AE§		Proportion rats *without* evidence of AE
Serum pool No.	Donors bled at:	Volume serum injected	Injection schedule postsensitization	Route of injections			Many lesions	Few lesions	
	wks.	*ml*	*days*						
8	6	2.0	Alternate	i.v.	12	0	2	4	6/12‖
5-A, B	3	2.0	Alternate	i.v.	9	1	4	1	4/9
3-A, B	3	1.0	Alternate	i.v.	6	0	0	5	1/6
4-A, B	6	2.0	Even	i.v.	9	1	7	0	2/9
		1.0	Odd	i.v.					
VIII	6	1.5	Alternate	i.p.	12	1	2	7	3/12
Total					48	3/48	15/48	17/48	16/48 (33%)
No serum (control groups of rats for those groups listed above)					18	9	13	4	1/18
					5	2	3	2	0/5
					5	2	5	0	0/5
					4	2	3	1	0/4
					12	8	9	3	0/12
Total					44	23/44	33/44	10/44	1/44 (2%)

* Serum pools prepared from sera of rats collected 3 or 6 weeks after sensitization to spinal cord–adjuvant and used for treatment as indicated.

‡ The 3 rats sensitized and serum-treated exhibited initial neurological signs 10, 14, and 16 days after sensitization, respectively. Clinical signs in control rats first noted 12 to 17 days after sensitization.

§ Rats sacrificed on 19th postsensitization day except those animals treated with serum pools 3-A, B (and corresponding control rats) which were sacrificed on 21st day. Lesions of AE recorded as few or many in number as described in Materials and Methods.

‖ Numerator, No. of rats failing to develop AE; denominator, No. of rats sensitized.

signs of AE. The remaining 45 rats remained clinically well. Although 32 rats were found to have lesions, in the majority of these animals these lesions were few in number and tended to be inconspicuous in character. Sixteen (33 per cent) failed to develop either clinical or histological evidence of AE. In contrast, similarly sensitized control rats (given no serum) regularly developed

AE. The majority of these animals had severe disseminated disease. Only 1 of the 44 (2 per cent) failed to develop AE.

Each of the 8 serum pools exerted a consistent and marked suppressive influence on development of AE. This fact is best appreciated by the rarity of clinical signs, the scanty nature of lesions, and more important, the relatively high proportion of rats without either clinical or histological evidence of AE in contrast to the situation in the control animals (bottom half of Table III, also see Table I). It may be seen, however, that the different serum pools varied in their capacity to suppress AE. This did not appear to be clearly related to time serum donors were bled, *i.e.* 3 or 6 weeks postsensitization, the volume of serum injected, or the route of injection. For example, using pool 8, disease was completely suppressed in half of actively sensitized recipients, each of which received 2.0 ml on alternate days. Using pools 4-A and 4-B, also collected from donors at 6 weeks, less suppression of disease was noted in spite of the fact that each rat received one and a half times as much serum.

One additional experiment (not shown in Table III) should be mentioned. Two groups of 8 rats each were sensitized to spinal cord–adjuvant. One group was treated with antibrain serum (collected from donors 3 to 6 weeks after sensitization) in the amount of 2.0 ml per rat just prior to sensitization of serum recipients and again 48 hours after sensitization, respectively. Another group was not treated with serum. Both groups developed severe AE. At least 6 animals in each group showed paralysis and virtually all had severe lesions. This lack of suppression in rats receiving only 2 injections of antibrain serum suggests that suppression of the disease shown in Table III may require serum treatment for an appreciable period (even as long as 18 days) after exposure to nervous tissue–adjuvant emulsion.

It seemed important to exclude the remote possibility that the occurrence of disease in the serum-treated rats was in part the *result* of injecting antibrain serum. To this end, an antibrain serum pool, comparable in every respect to those previously used (Table III) was injected intravenously into a group of 8 *normal* rats. Each rat received 2.0 ml of serum on alternate days over an 18 day observation period. All remained clinically well. One animal found dead on the 18th day and the remaining 7 rats sacrificed on the 19th day were found to have no demonstrable lesions of AE. The results of this experiment are in agreement with prior unsuccessful attempts to transfer AE passively with immune serum carried out in this laboratory (28) and by other workers (14). This observation supports the view that suppression of AE by passively administered immune serum represents the equivalent of transfer of protection against the disease.

Control Serum Pool Experiments.—Additional experiments, in which similarly sensitized rats were treated with appropriate control serum pools are shown in Table IV. These experiments were usually carried out at the same

time as those shown in Table III but are presented separately here in this fashion for convenience. Pools of serum prepared from rats sensitized to kidney plus adjuvant or adjuvant alone or pools of normal rat serum exerted no appreciable suppression of the disease. Over 50 per cent of the 57 sensitized rats treated with control serum developed clinical signs and the majority of

TABLE IV

Lack of Suppression of Allergic Encephalomyelitis (AE) in Sensitized Rats Treated with Various Types of Control Rat Sera

Type of serum treatment (i.v. route)				No. of rats sensitized to cord and adjuvant and treated with serum	No. with clinical signs of AE*	No. with Lesions of AE‡		Proportion of rats *without* evidence of AE
Status of serum donors	Donors bled at:	Volume serum injected	Injection schedule postsensitization			Many	Few	
	wks.	*ml*	*days*					
Sensitized to kidney plus adjuvant	3	1.0	Alternate	5	4	4	1	0/5§
Sensitized to adjuvant only	3	2.0	Alternate	7	3	4	3	0/7
Sensitized to adjuvant only	6	2.0	Alternate	15	6	11	1	3/15
Normal rats	—	2.0	Alternate	9	7	7	0	2/9
Normal rats	—	2.0 1.0	Even Odd	10	5	6	4	0/10
Normal rats	—	2.0 1.0	Even Odd	11	5	4	4	3/11
Total				57	30/57	36/57	13/57	8/57 (14%)

* Initial appearance of clinical signs on 11th to 17th postsensitization day.

‡ All rats sacrificed 19 days after sensitization (1 day after last injection of serum) except those rats treated with antikidney-adjuvant serum which were sacrificed 21 days after sensitization. Lesions of AE recorded as few or many in number as described in Materials and Methods.

§ Numerator, No. of rats failing to develop AE; denominator, No. of rats sensitized.

the animals were found to have many lesions. Only 8 of the 57 rats were free of AE. It should be noted that the time of collection of serum from the control donors, the volume of serum pools injected into recipients, and the schedules of serum injection were comparable in all respects to the conditions of the antibrain serum pools which gave suppression (Table III). The data (Table III and IV) strongly suggest that suppression of AE by antibrain serum administered passively is due to a factor directed against nervous tissue, most likely antibrain antibody. The data in control animals exclude the possibility

that the serum suppression of AE (Table III) is due to such non-specific factors as ether anesthesia, handling of animals, and trauma associated with the frequent injections; *e.g.*, non-specific "stress."

Is the Protective Effect of Antibrain Serum Due to Complement-Fixing Antibody?—The antibrain serum pools were prepared from donors 3 or 6 weeks after sensitization, at a time known from previous work (25) when close to 80 per cent of sensitized rats have detectable complement-fixing (CF) antibrain antibodies. It seemed conceivable that the suppressive effect the antibrain serum pools exerted on the disease might be a function of CF antibrain antibody, known to be present in the pools.

TABLE V

Relationship between Complement-Fixing (CF) Antibrain Antibody Content of Serum Pools and Effectiveness in Suppressing Allergic Encephalomyelitis (AE) in Rats

Serum pool No.*	$C'H_{50}$ fixed by serum pool with brain antigen‡	No. of rats sensitized and serum-treated	No. with clinical signs of AE	No. with lesions of AE	Proportion of rats *without* evidence of AE
5-B	61	4	0	2	2/4§
5-A	35	5	1	3	2/5
4-B	39	6	1	4	2/6
4-A	36	3	0	3	0/3
3-B	25	3	0	2	1/3
3-A	56	3	0	3	0/3
VIII	34	12	1	9	3/12
Total		36	3/36	26/36‖	10/36 (28%)
Control pools	4, 4, 3, 1, 0	50	27	42	8/50
VII	8	11	6	11	0/11
6-2M	5	5	2	4	1/5
Total		66	35/66	57/66‖	9/66 (14%)

* Serum pools, except VII and 6-2M, same as those recorded in Tables II to IV. Pool VII derived from sera of rats collected 7 days after sensitization to spinal cord–adjuvant. Pool 6-2M prepared from sera collected from rats 1 to 6 weeks after spinal cord–adjuvant sensitization and found to fix 55 $C'H_{50}$ *before* treatment with 2-mercaptoethanol (2M).

‡ No. of 50 per cent hemolytic units of complement ($C'H_{50}$), of exactly 100 $C'H_{50}$ available, fixed by 0.25 ml of serum in presence of rat brain antigen diluted usually 1 to 100 or 1 to 250 in physiological saline.

§ Numerator, No. of rats failing to develop AE; denominator, No. of rats sensitized.

‖ Of the 26 rats with lesions of AE, 13 had many lesions and 13 had few lesions; of the 57 rats with lesions, 44 had many, severe, disseminated lesions and 13 had few lesions (see Materials and Methods).

The relationship between the quantity of CF antibrain antibody in the serum

pools (expressed in terms of $C'H_{50}$ fixed specifically with brain antigen *in vitro*) and the suppressive effect the serum pools exerted on AE is shown in the upper portion of Table V. Similar data are set out in the bottom portion of Table V with respect to 5 control serum pools (those already shown in Table IV) and 1 antibrain serum pool prepared from sera collected from rats

TABLE VI

Levels of Passively Administered Complement-Fixing (CF) Antibrain Antibody in Rats Sensitized to Nervous Tissue and Treated with Antibrain Serum Pools

Serum pool		Rat No. (sensitized and serum-treated)	Volume serum pool injected‡	$C'H_{50}$ fixed by rat serum on 4th postsensitization day	Occurrence (+) or absence (0) of AE§
Pool No.	$C'H_{50}$ fixed with brain antigen*				
			ml		
3-A	56	E 551	2.0	17	+
		E 553	2.0	6	+
		E 554	2.0	4	+
3-B	25	E 555	2.0	1	+
		E 556	2.0	10	0
		E 557	2.0	0	+
5-A	35	E 750	1.0	9	0
		E 751	1.0	12	+
		E 752	1.0	5	+
		E 753	1.0	3	+
		E 755	1.0	0	0
5-B	61	E 746	1.0	8	+
		E 747	1.0	11	0
		E 748	1.0	5	+
		E 749	1.0	12	0

* No. of 50 per cent hemolytic units of complement ($C'H_{50}$), of exactly 100 $C'H_{50}$ available, specifically fixed by 0.25 ml of serum in presence of rat brain antigen.

‡ Serum injected just prior to sensitization of rats and on alternate days thereafter.

§ Presence or absence of microscopic lesions of allergic encephalomyelitis (AE) in nervous tissue of rats on 19th or 21st postsensitization day.

7 days after sensitization. These serum pools in agreement with prior work contained little, if any, CF antibody. In addition, data are also presented concerning the suppressive activity of an antibrain serum pool collected from donors 1 to 6 weeks after nervous tissue sensitization and shown to contain CF antibody. This pool (6-2M) before used was treated with 2-mercaptoethanol in order to destroy the CF antibody. In earlier work we had observed (27) that the CF antibrain antibody produced by rats belongs to the high molecular

weight (19S) class of gamma globulins which are characteristically destroyed by treatment with 2-mercaptoethanol.

The data (Table V) indicate clearly that all serum pools containing CF antibrain antibody exerted a suppressive effect on AE. Those pools lacking CF antibody had little, if any, suppressive influence on the disease. The most telling experiment is that employing the 2-mercaptoethanol-treated antibrain serum pool. This pool before 2-mercaptoethanol treatment fixed 55 $C'H_{50}$ with rat brain antigen and was comparable in this and all other respects to the antibrain serum pools which did exert a suppressive effect (upper portion, Table V). This serum pool, following treatment with 2-mercaptoethanol, had neither demonstrable CF antibrain antibodies nor suppressive activity.

It is clear that no direct relationship exists between the level of CF antibrain antibody in a given serum pool and the suppressive activity of that pool (see Table V). It seemed likely that one explanation for this observation might be variation in the level of CF antibrain antibody passively achieved in individual recipient rats. Direct support for this interpretation is provided by the data presented in Table VI.

Fifteen sensitized and serum-treated rats were bled from the retroorbital venus plexus on the 4th postsensitization day, just prior to the third injection of antibrain serum. The individual serum samples were assayed for CF antibrain antibodies. Since it is known that rats actively sensitized to nervous tissue plus adjuvant rarely produce CF antibrain antibody before the 5th to 7th postsensitization day (25), any CF antibrain antibody present in the sera of these rats on the 4th postsensitization day represents passively administered antibody. Seven (Nos. 551, 556, 750, 751, 746, 747, and 749) of the 15 recipient rats had small amounts of CF antibrain antibody. The sera of these 7 rats fixed from 8 to 17 hemolytic units of complement ($C'H_{50}$) specifically with brain antigen. The remaining 8 rats had little, if any, CF antibrain antibody (less than 7 $C'H_{50}$-fixed). It is of interest that of the 7 rats with CF antibrain antibody on the 4th postsensitization day, 4 were completely protected from AE. In contrast, only 1 of the other 8 rats in which appreciable antibody was not demonstrable, failed to develop evidence of the disease. These observations suggest that a key factor in suppression of AE with antibrain antibody is the level of CF antibody passively achieved.

These findings (Table VI) underscore the difficulty in achieving uniformly high levels of CF antibrain antibody administered passively. The levels of CF antibrain antibody achieved passively in sensitized recipients did vary when this point was checked (Table VI) and can be assumed to have varied in other recipients receiving serum treatment. This fluctuation in antibody level seems adequate to account for the variability in degree of suppression of AE observed from one experiment to another, irrespective of the antibrain pool employed and its CF antibrain antibody content at the time it was assayed.

DISCUSSION

The clinical-pathological picture of AE in rats (Table I) has certain features which should be emphasized. Albino rats develop AE regularly following a single injection of a standard spinal cord homogenate–adjuvant emulsion. Approximately half of the sensitized animals exhibit readily detected clinical neurological signs by the 2nd to 3rd week after sensitization. At this time characteristic microscopic lesions of AE can be demonstrated in the brain and/or spinal cord of almost all sensitized rats. From this point on, the majority of rats exhibit steady improvement and tend to recover. By the 4th to 6th week after sensitization most of the animals appear clinically well once again although recurrence of neurological signs may be exhibited by an occasional rat at this time. In similar fashion, lesions of AE in this species of animal, *viz.* focal vasculitis with associated perivascular demyelination, are found with progressively decreasing frequency during this same postsensitization period. Around the 6th week a transitory resurgence of disease, lesions as well as signs, may occur. By the 9th week after sensitization virtually all rats are clinically well and microscopic lesions of AE are virtually non-existent. The transitory nature of AE in the rat, with spontaneous and full recovery from the disease being the rule, stands in contrast to the more progressive or even fulminating and lethal course of the disease in certain other susceptible species of animals; *e.g.*, the guinea pig (8). The fact that rats continue to produce CF antibrain antibody as they recover from acute manifestation of AE (25) led, in part, to the thesis that CF antibrain antibody might have a protective function.

The data (Tables II and III) indicate that pooled serum collected from rats 3 or 6 weeks after sensitization has the capacity to transfer passively protection against AE to actively sensitized rats. All antibrain serum pools tested to date have exerted a suppressive effect which although clear cut is variable in intensity. This observation agrees with the preliminary finding of Florey (summarized by Vulpé, reference 26), that immune sera collected from guinea pigs sensitized to nervous tissue plus adjuvant may confer protection against AE on actively sensitized guinea pigs. The use of appropriate control serum pools (Table IV) in the present study supports the view that the suppressive effect obtained with antibrain rat serum is due to antibrain antibody. All antibrain serum pools found to contain appreciable levels of CF antibrain antibody (Table V) exerted a suppressive effect. Control serum pools and antibrain serum pools lacking CF antibrain antibody (Table V) gave no suppressive effect. Noteworthy is the fact that a serum shown to have high titer CF antibrain antibody and treated with 2-mercaptoethanol, no longer has demonstrable CF activity and is devoid of any suppressive effect on AE.

While the data strongly suggest that the factor in antibrain serum responsible for suppression of AE is CF antibrain antibody, it cannot be concluded that this is indeed, the case. No direct correlation exists between the level of CF

antibrain antibody in a given serum pool and the suppressive effect that serum pool exerts. One explanation for this lack of correlation may be the difficulty in maintaining appreciable levels of passively administered CF antibrain antibody in recipient animals. Data supporting this point and set out in detail above (see text, discussion of Table VI) also reveal the importance of appreciable levels of passively administered CF antibody for suppression of AE in individual recipient rats. The *in vivo* absorption of passively administered antibody to the brain of actively sensitized recipients or to spinal cord in the inoculum used for sensitization as well as a short half-life, *e.g.* 5 to 6 days, of passively administered gamma globulin[5] in the rat (29) could well account for variation in CF antibrain antibody levels achieved passively in individual recipients and variation in degree of suppression of the disease.

Previous studies (reviewed by Paterson, reference 8) have shown no direct relationship between occurrence of CF antibrain antibody and occurrence of AE in individual animals. The fact that CF antibody may be elicited by sensitization with certain non-mammalian nervous tissues although these same nervous tissues do not cause AE indicates that CF antibody in all probability has no causal role in AE (13). These observations and the unsuccessful attempts by various workers to transfer AE to normal animals with antibrain serum (reviewed by Chase, reference 14) have been often cited (15–17) as evidence that the CF antibrain antibodies represent an irrelevant immune response to nervous tissue sensitization. And, by implication, that circulating antibrain antibody of any type has little to do with the pathogenesis of AE. The observations described in this paper deal directly with this issue. Passively administered antibrain serum consistently alters the expected response of rats to active sensitization to nervous tissue; *viz.*, AE is suppressed or prevented. This observation has one inescapable meaning. This meaning is that antibrain antibody does not represent an irrelevant immune response and must be given serious consideration as a factor in AE.

While the meaning of the observations concerning serum suppression of AE described in this paper is clear, the underlying mechanism(s) is not. It may be useful to outline three current lines of thinking, knowing that each may require modification in light of future work. These lines of thinking are presented in decreasing order of probability, based on data in hand and experience of others set out in the immunologic literature.

1. Passively Administered CF Antibrain Antibody Inhibits Active Production of the Immune Response Causing AE.—It has been shown that passively administered antibody may suppress or inhibit active production of antibody

[5] It may be important that the CF antibrain antibody produced by the rat is a heavy weight 19S gamma globulin (27). Its half-life may be shorter than that for, presumably, 7S rat gamma globulin (29), since half-life of homologous 19S antibody in the guinea pig and the rabbit may be of the order of only 1 to 2 days (J. Uhr, and M. S. Finkelstein, unpublished observations).

by an animal against the antigen in question. Uhr and Baumann (30) have reviewed key studies on this point and presented additional data in guinea pigs and rabbits to document it. The recent study of Neiders *et al.* (31) concerning suppression of active production of anti-sheep cell hemolysin in rats by repeated injections of the antibody in question is especially pertinent. It was shown that detectable levels of circulating hemolysin passively administered are necessary for inhibition of active production of hemolysin.

Our data strongly suggest that the suppressive influence of antibrain serum is a function of CF antibrain antibody, and that detectable levels of CF antibrain antibody passively administered may be important for suppression of AE (Table VI). CF antibrain antibody, although not causing AE itself, might suppress active production of another type of antibrain antibody. Such could occur if both antibodies are directed against basically dissimilar brain antigens which, however, share at least one antigenic grouping or determinant. For example, CF antibody might suppress active production of the cytotoxic antibody shown by Bornstein and Appel (32) to be present in sera of rabbits and guinea pigs sensitized to nervous tissue and developing AE. This cytotoxic antibody destroys myelin when added to tissue cultures of brain. In like manner, CF antibrain antibody may suppress delayed-type immune responses against brain. Little evidence exists, however, which can be used to support the view that induction of delayed-type hypersensitivity in fact may be suppressed by passively administered antibody.

2. *Passively Administered Antibrain Serum Inhibits Production of Antibody Responsible for AE.*—The factor in antibrain serum responsible for suppression of AE may not be CF antibody but another antibody, *e.g.* cytotoxic antibody, causing the disease. By providing this antibody passively, its active production may be suppressed. Viewed in this light, the transfer of protection against AE, rather than transfer of the disease itself, could be due to insufficient levels of antibody, *e.g.* cytotoxic antibody, being achieved, the quality of the antibody transferred or difficulty of passively administered antibody crossing the blood-brain barrier and reacting with antigen in the brain of the recipient. If this line of thinking is correct, transfer of protection against AE represents evidence strongly suggesting that it is serum antibody that causes AE. It should be mentioned that other work in our laboratory is consistent with this thesis. For example, AE is suppressed by whole body x-irradiation and suppression of the disease is associated with marked impairment in circulating antibrain antibody production (20). It is generally believed that killed mycobacteria are essential for rapid and regular induction of AE and that the importance of the mycobacteria lies in their capacity to induce immune responses of the delayed-type against nervous tissue antigen. This is not the case in the rat. AE may be induced in the rat equally well with or without killed mycobacteria

in the sensitizing inoculum (24, 33). In rats, omitting mycobacteria does not impair circulating CF antibrain antibody production and the lymph nodes draining sites of sensitization show conspicuous production of plasmacytes.

3. CF Antibrain Antibody May Act as "Blocking Antibody".—CF antibrain antibody when administered passively might combine with antigenic sites in the sensitized recipient's nervous tissue without consequent tissue damage. In this fashion, CF antibrain antibody might prevent antigenic sites from binding to or interacting with another immune factor causing AE, be this circulating antibody other than CF antibody, "sensitized" cells, or some as yet not recognized type of immune factor.

In its operational aspects, the suppression of AE in rats by passively administered antibrain serum is analogous to observations made by other workers in the field of tumor and transplantation immunology; *viz.*, tumor enhancement and prolongation of skin transplant survivals. This analogy can be readily appreciated if one considers autologous mammalian brain to represent potentially foreign tissue because it contains constituents which are antigenic for the individual concerned. In biological terms, the occurrence of AE may be considered to represent an attempt on the part of the animal sensitized to nervous tissue (by injection of brain or spinal cord) to inadvertently reject its own (autologous) nervous tissue. Thus, the development of AE is equivalent to the rejection of a tumor transplant or a skin graft. It is well known that passively administered immune serum, collected from appropriate donors, may interfere with or abolish the immune response required for rejection of tumor transplants (34, 35). Serum treatment may similarly interfere with the rejection of skin grafts (36, 37). It may be noteworthy that the mechanism responsible for tumor enhancement, and perhaps prolonged survival of skin grafts, by means of passively administered immune serum, most likely appears to be inhibition of active production of the immune factor in question (35).

Current studies are designed to answer, if possible, the general question as to whether passively administered antibrain serum suppresses AE by inhibiting active responses to antigenic stimulation or whether it exerts its suppressive effect by acting on the target organ; *i.e.*, the brain of the sensitized animal. The use of the AE transfer model (18, 21, 22) may permit one to distinguish between these two modes of action.

The work described in this paper is an outgrowth of continued studies of the relative role of serum factors, as well as cellular factors, in the pathogenesis of AE. It is hoped that the suppression of AE by antibrain serum described here may prove helpful in clarifying the role of CF antibrain antibody and other circulating antibrain antibodies in AE and help uncover clues that may lead to the eventual unraveling of the precise pathogenesis of AE.

SUMMARY

Rats regularly develop evidence of allergic encephalomyelitis (AE) 2 to 3 weeks following sensitization to nervous tissue plus adjuvant. Independent of the severity of AE which occurs, gradual recovery is the rule and by the 6th to 9th week after sensitization rats appear clinically well and microscopic lesions of AE have virtually disappeared.

Pooled serum collected from rats 3 or 6 weeks after sensitization contains complement-fixing (CF) antibrain antibodies. Such pooled serum exerts a striking suppressive influence on development of AE when passively administered to rats actively sensitized to nervous tissue. Serum pools which contain CF antibrain antibody suppress the disease. Serum pools lacking CF antibody do not suppress the disease. Serum containing CF antibrain antibody after treatment with 2-mercaptoethanol no longer fixes complement with brain antigen *in vitro* and no longer suppresses AE *in vivo*. The data suggest that transfer of protection against AE by passively administered antibrain rat serum is due to an antibrain antibody, possibly the CF antibodies. The meaning of these findings is discussed in terms of the role(s) of circulating antibrain antibody in the pathogenesis of AE.

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