CELLULAR EVENTS IN THE INDUCTION OF EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS IN RATS*

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Experimental allergic encephalomyelitis (EAE)[1] has been widely studied as an animal model for determining the factors that participate in the pathogenesis of demyelinating diseases. The evidence indicates that these disorders have an immunological basis, in that injection of either nervous system tissue or protein and peptides isolated from this tissue, emulsified in complete Freund's adjuvant (CFA), induces EAE and immunoreactivity in laboratory animals. The main pathological feature of this disease is the acute vasculitis associated with perivascular myelin damage that results in the clinical signs of EAE (1–3).

The antigen responsible for eliciting EAE exists within nerve tissue and may serve as an autoantigen causing an autoimmune response in the sensitized host. The capacity of an animal to react against its own cells may involve any of several possible cellular events: (*a*) an absence of a tolerant state to sequestered antigens, (*b*) bypass of the tolerant state in a particular cell type, or (*c*) a modulation of a normal network of regulatory mechanisms. Recently, attempts have been made to gain some insight into the cellular parameters of certain autoimmune phenomena through determination of antigen-specific unresponsiveness at the level of the thymus (T)- and bone marrow (B)-derived cells. It has been shown that thyroiditis and production of autoantibodies to thyroglobulin in the mouse result from the stimulation of competent B cells by nonspecific activation of T cells, thus bypassing the tolerant T cells (4). The presence of B cells in the mouse (5) and peripheral blood lymphocytes in the human (6) that specifically bind syngeneic and homologous thyroglobulin, respectively, support the existence of competent B cells for thyroglobulin. The inability of mice to respond to syngeneic thyroglobulin and the absence of antigen-binding lymphocytes (ABL) in the thymus of normal mice would be compatible with the hypothesis that unresponsiveness is maintained by the T

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[1] *Abbreviations used in this paper:* ABC, antigen-binding capacity; ABL, antigen-binding lymphocytes; BGG, bovine gamma globulin; BN, Brown Norway; BP, basic protein; BRBC, burro erythrocytes; BSS, balanced salt solution; CFA, complete Freund's adjuvant; EAE, experimental allergic encephalomyelitis; FCS, fetal calf serum; IFA, incomplete Freund's adjuvant; LNC, lymph node cells; PFC, plaque-forming cells; Tx, thymectomy.

cell (5). These observations offer some insight into the possible mechanisms involved in the induction of autoimmunity in at least one model. It is therefore of importance to investigate the mechanism(s) of antigen recognition whereby EAE is produced since the cellular parameters of this disease appear to differ from those of experimental autoimmune thyroiditis.

Another important aspect of EAE is the ability to modify the disease by the treatment of animals with nonencephalitogenic antigens. If the central nervous system (CNS) antigen is injected in a nonencephalitogenic form, i.e. either alone or incorporated in incomplete Freund's adjuvant (IFA), the disease produced as the result of a previous injection of the encephalitogen in CFA is suppressed. Several mechanisms have been implicated as being responsible for this effect, i.e. the existence of blocking antibody (7), immunologic tolerance (8), or the induction of suppressor T cells (9).

This paper is concerned with the immune status of T and B cells to basic protein of myelin and the role of these cells in induction of EAE in rats. Furthermore, data are presented which deal with the nature of suppression of EAE by nonencephalitogenic forms of basic protein.

Materials and Methods

Animals. Female Lewis and Brown Norway (BN) rats (Microbiological Associates, Bethesda, Md.) were used throughout the experiments. The animals were 4-wk old at thymectomy (Tx) and more than 6-wk old when immunized. Animals were maintained on Purina Laboratory Chow (Ralston Purina Co., St. Louis, Mo.) and water ad libitum.

Myelin Basic Protein (BP). Rat myelin BP was prepared by the method of Martenson et al. (10). Analysis by polyacrylamide electrophoresis demonstrated only two bands associated with the 16,000 and 12,000 molecular species previously described for myelin BP of the rat. Further analysis of ^{125}I-labeled preparations by polyacrylamide gel electrophoresis demonstrated that only 5% of the ^{125}I content was not associated with the major bands of BP. Thus, it appears that the purity of these preparations was 95% or greater.

Sensitization of Rats with Syngeneic BP. Lewis rats were sensitized by inoculations into the rear foot pads of a mixture of 100 μg BP in CFA containing 100 μg *Mycobacterium tuberculosis* H37Ra (Difco Laboratories, Detroit, Mich.). Control animals received inoculations of normal saline emulsified in CFA.

Evaluation of EAE. Animals were examined daily for clinical symptoms of EAE which included evidence of weakness, paralysis, difficulty in righting, fecal impactation, loss of tail tonicity, or ruffled fur. Rats were killed 14 days after inoculations. Sections of CNS tissue were stained with hematoxylin and eosin and examined microscopically for lesions of EAE.

Detection of Plaque-Forming Cells (PFC). The plaquing procedure was a modification (11) of the method used by Jerne and Nordin (12) to assay direct PFC. Guinea pig serum served as the source of complement and rabbit anti-rat immunoglobulin serum diluted 1:50 was added in order to detect indirect PFC as described previously (13). BP was conjugated to sheep erythrocytes (SRBC) via a specific antibody to SRBC by means of carbodiimide reagent (14). In brief, 200 mg of a freshly prepared aqueous solution of 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide hydrochloride (Story Chemical Co., Muskegon, Mich.) was added to the mixture of 20 mg of BP and 10 mg of IgG anti-SRBC in 1 ml of 0.15 M NaCl. The mixture was gently agitated at room temperature for 30 min. The conjugated material was then dialyzed against 0.15 M NaCl for 24 h and the hemagglutinating activity of the conjugated material was measured against SRBC. The BP-IgG conjugate was reacted with SRBC at a dilution of the BP-anti-SRBC IgG slightly higher than its hemagglutinating titer. This allowed the specific antibody (conjugated to BP) to coat the erythrocytes without causing hemagglutination.

Iodination of BP. Myelin BP was iodinated as described by McConahey and Dixon (15). For the autoradiographic and antigen-suicide studies the sp act ranged from 40 to 75 and 200 to 400 μCi/μg protein, respectively. For quantitative determination of serum antibody to BP by the

antigen-binding capacity (ABC) assay, the BP was labeled to a sp act of approximately 0.1–0.3 μCi/μg protein.

Nitrogen Determinations. Protein nitrogen determinations were performed by a modification of the micro-Kjeldahl technique using the Technicon autoanalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) (16).

Autoradiography of ABL. The autoradiographic method employed was similar to that described by Clagett and Weigle (5). In brief, thymuses and spleens were removed from the rats after exsanguination and single cell suspensions were made from each organ in minimal essential medium (MEM) (Grand Island Biological Co., Grand Island, N. Y.) supplemented with 10% fetal calf serum (FCS) and 0.1% sodium azide. 200 μl of medium containing 20×10^6 nucleated cells was added to 100 ng of ^{125}I-BP and the cell suspensions were held at 4°C for 30 min. To remove unbound ^{125}I-BP from the cell suspension, each cell preparation was layered over 7 ml of FCS and centrifuged at 200 *g* for 10 min. This step was repeated three times. Cell smears were made on precleaned slides, air-dried and fixed, and the slides were immersed in NTB-2 (Eastman Kodak Co., Rochester, N. Y.). Exposure times varied from 7 to 14 days. The slides were developed and stained with Giemsa, and in each preparation at least 10^5 cells were scanned for the presence of grains.

In blocking experiments with unlabeled BP, the cell suspensions were mixed first with a 100- to 1,000-fold excess of BP diluted in MEM and incubated at 4°C for 30 min. The cell suspensions were then incubated with ^{125}I-BP for 30 min at 4°C. In blocking experiments with antisera, the cell suspensions (200 μl) were incubated with an equal volume of appropriately diluted antisera for 2 h at 4°C before the addition of ^{125}I-BP.

Antigenic Suicide of Normal Bone Marrow and Thymus Cells. Syngeneic rat BP was iodinated with ^{125}I at a specific activity of 200–400 μCi/μg protein as described previously (15). Normal thymuses and bone marrow were collected from 6- to 8-wk-old Lewis rats. The cells were suspended in MEM with 10% FCS at 5×10^7 cells/ml and were incubated separately with ^{125}I-BP at a ratio of approximately 3 μg/10^8 cells (5). The cell suspensions were held at 4°C for 5 h. Then, the cells (5 ml) were layered over 20 ml FCS and centrifuged at 200 *g* for 15 min in the cold, and the process was repeated twice. After the final wash, the treated cells together with untreated lymphocytes from the other cooperating cell line were suspended in MEM and injected intravenously into lethally (900 R) irradiated, Tx recipients. A control group in which cold BP was used instead of iodinated antigen was included. 4–6 days after reconstitution, the rats were injected with BP-CFA as previously described. Anti-thymocyte serum was prepared and titrated as described previously (17).

Quantitation of Antibody to Rat BP. Antibody to BP was determined by the sodium sulphate method described by Day and Wexler (18). Results are reported as micrograms of BP bound per milliliter of serum. Background values obtained in the sera of unimmunized rats were subtracted but were usually lower than 1–2 μg of BP/ml of serum.

Suppression with Soluble BP. Rats were sensitized with 100 μg of BP-CFA. Suppression treatment was started 3 days later with 800 μg of BP by intradermal (i.d.) injection. The same treatment was repeated 6, 9, and 12 days after the sensitizing injections.

Suppression with BP in IFA. 3 days after rats were injected with 100 μg of BP-CFA, they were injected with 100 μg BP in IFA for suppression of EAE as previously described (9). A total of eight i.d. injections were given over a 2 wk period.

Adoptive Transfer Experiments to Study the Role of Suppressor Cells. For adoptive transfer experiments, donor rats were either treated with the soluble BP or BP-IFA as described above. Spleens and lymph nodes collected from both normal rats and rats injected with BP were excised separately into balanced salt solution (BSS). The single cell suspensions were centrifuged at 200 *g* for 10 min and washed three times with BSS. Viabilities were determined by trypan blue dye exclusion. Cells were combined according to the particular protocol and suspended in 1 ml of BSS. The cells were adoptively transferred into Tx, irradiated (900 R) rats by intravenous injection via the lateral tail vein. Challenges with BP-CFA were performed on the day of the transfer or 4–6 days later. Rats were examined daily for symptoms of EAE as previously described and sacrificed 14 days after challenge.

Statistical Analysis. The significancy in the number of ABL found in the nonimmunized control rats and the ABL observed after different experimental treatments was determined by the Poisson distribution (19).

TABLE I
Frequency of ABL to BP in Spleen and Thymus of Normal Lewis and BN Rats

Amount of ^{125}I BP	Source of normal lymphoid cells	Rat strain	ABL/10^5 cells
ng			
100	Thymus	Lewis	8
100	Thymus	BN	0
100	Spleen	Lewis	17
100	Spleen	BN	11

Results

Autoradiographic Detection of ^{125}I-BP-Binding Lymphocytes in Unimmunized Rats. The frequency of thymus and spleen cells from unprimed Lewis rats that bound ^{125}I-labeled syngeneic BP is shown in Table I. When thymus cells were incubated with ^{125}I-BP, 8/10^5 cells were positive, whereas there were 17/10^5 ABL in the spleen cells. On the other hand, thymus cells from BN rats did not bind ^{125}I-BP. However, a normal complement of ABL was detected in their spleen cells. This finding, together with the inability of the anti-thymus serum to inhibit the ABL in the spleen, indicates the absence of ABL in T cells of BN rats.

Antigen-Suicide of Normal Thymus and Bone Marrow Cells by ^{125}I-Labeled Syngeneic BP. The results obtained by determining ABL indicated the presence of immunocompetent T and very likely B cells in the Lewis strain of rats. Therefore, it was of interest to determine if such cell populations could be rendered unresponsive by "suicide" with syngeneic ^{125}I-BP of high specific activity. The data in Table II clearly show the following: (*a*) Normal thymus cells in the presence of B cells treated with heavily iodinated ^{125}I-BP were able, when transferred to Tx irradiated rats, to support the sensitization of the adoptively transferred animals and the development of EAE. These animals, however, were unable to produce PFC to BP. Despite the inability to form PFC to BP, they mounted a humoral immune response to an unrelated antigen, namely, burro erythrocytes (BRBC); (*b*) When thymus cells were suicided and transferred together with normal bone marrow cells to Lewis rats, the animals could not be sensitized when injected with BP-CFA. This inability to respond was manifested by an absence of both EAE and PFC to BP. However, the animals formed PFC to a thymus-dependent antigen, i.e. BRBC, showing the specificity of the cells killed by the radioiodinated antigen; (*c*) Normal thymus cells transferred together with thymus cells which were previously suicided and normal bone marrow cells, however, restored the capacity of rats to become sensitized with BP-CFA, resulting in both EAE and PFC to BP; (*d*) The treatment of both cell populations by radioiodinated antigen prevented the induction of EAE and the formation of PFC to BP, but not to the BRBC; (*e*) The transfer of thymus cells which were previously treated with cold BP and normal bone marrow cells to rats resulted when sensitized with BP-CFA, in the development of EAE and PFC to BP; (*f*) Rats reconstituted with bone marrow alone,

TABLE II

Antigen Suicide of Thymus and Bone Marrow Cells by ^{125}I-Labeled Syngeneic BP

Cells transferred		Recipients sensitized with	EAE*		PFC to:	
B cells	T cells		Clinically	Histologically	BP‡	BRBC§
250 × 10^6 Normal bone marrow	200 × 10^6 Normal thymocytes	BP-CFA	10/10	10/10	20	8,742
250 × 10^6 Bone marrow and ^{125}I BP	200 × 10^6 Normal thymocytes	BP-CFA	10/10	10/10	0	11,196
250 × 10^6 Normal bone marrow	200 × 10^6 Thymocytes and ^{125}I BP	BP-CFA	0/5	0/5	0	17,763
250 × 10^6 Normal bone marrow	200 × 10^6 Thymocytes and ^{125}I BP + 200 × 10^6 normal thymocytes	BP-CFA	10/10	10/10	28	16,240
250 × 10^6 Bone marrow and ^{125}I BP	200 × 10^6 Thymocytes and ^{125}I BP	BP-CFA	0/10	0/10	0	14,706
250 × 10^6 Normal bone marrow	200 × 10^6 Thymocytes and cold BP	BP-CFA	9/10	10/10	31	15,322
250 × 10^6 Bone marrow and ^{125}I BP	None	BP-CFA	0/10	0/10	0	75
250 × 10^6 Normal bone marrow	None	BP-CFA	0/4	0/4	0	0
250 × 10^6 Bone marrow and ^{125}I BP	200 × 10^6 Normal thymocytes	CFA	0/5	0/5	0	16,962
250 × 10^6 Bone marrow and ^{125}I BP	200 × 10^6 Normal thymocytes	CFA	0/5	0/5	0	112‖

* Positive/total.

‡ PFC/10^6 popliteal lymph node cells.

§ PFC/spleen.

‖ Background to BRBC. Rats were not immunized with BRBC.

either treated or not treated with the radioiodinated antigen, were not sensitized by BP-CFA and did not form PFC to a thymus-dependent antigen, i.e., BRBC. In control groups transferred with both normal T and B cells, sensitization was represented by the appearance of both EAE and PFC to BP. Results from control rats given CFA alone eliminated the possibility that CFA caused the sensitization.

Suppression of EAE. In the two experiments shown in Table III, Lewis rats treated with BP without adjuvant and subsequently sensitized with BP-CFA were unable to develop EAE. However, they did form similar levels of antibodies to BP as the nonsuppressed group in which EAE was manifest. On the other hand, control rats receiving only the BP (without CFA) developed neither EAE nor antibodies to BP.

Similar results were obtained when animals were injected with BP in IFA after sensitization with BP-CFA (Table IV) in that none of the treated animals that received a prior injection of BP-CFA developed EAE. In this case, however, antibodies were produced in all groups including the group which received BP in IFA.

Determination of Suppressor Cells in the Spleens and Lymph Nodes of Lewis Rats Protected Against EAE by Immunosuppressive Treatment. Attempts were made to determine how treatment with the soluble BP in rats sensitized with BP-CFA caused suppression. Adoptive transfers were performed to detect suppressor T cells in the spleen cells as well as in the lymph node cells (LNC) of rats pretreated with either BP alone or BP in IFA, as indicated before, and

TABLE III
*Experimental Suppression of EAE in Lewis Rats by Intradermal Injection of Soluble BP from Spinal Cord**

Exp. no.	Group no.	Injected with	Sensitized with‡	EAE§		Serum ABC‖
				Clinically	Histologically	
1	1	BP	–	0/5	0/5	0.4
	2	BP	BP-CFA	0/5	1/5	4.2
	3	–	BP-CFA	4/4	4/4	5.8
2	1	BP	–	0/5	0/5	0
	2	BP	BP-CFA	0/5	2/5	3.9
	3	–	BP-CFA	3/5	5/5	4.0

* Rats received four i.d. injections of soluble BP (800 μg each) every 3 days.
‡ Rats were sensitized with 100 μg of BP in CFA. Group 2 received the initial dose of soluble BP 3 days after sensitization.
§ Positive/total.
‖ Values reported represent micrograms of BP bound per milliliter of serum. Determinations were done 14 days after sensitization.

TABLE IV
*Experimental Suppression of EAE in Lewis Rats by Injection of BP in IFA**

Group no.	Injected with	Sensitized with‡	EAE§		Serum ABC‖
			Clinically	Histologically	
1	BP-IFA	–	0/5	0/5	2.4
2	BP-IFA	BP-CFA	0/5	0/5	3.6
3	–	BP-CFA	5/5	5/5	4.7

* Rats received eight subcutaneous injections of BP (100 μg each) in IFA in a 2-wk period.
‡ Rats were sensitized with 100 μg of BP in CFA. Group 2 received the initial dose of BP-IFA 3 days after sensitization.
§ Positive/total.
‖ Values reported represent micrograms of BP bound per milliliter of serum. Determinations were done 14 days after sensitization.

combined with either LNC or spleen cells of untreated rats as outlined in Table V. No protection was afforded by the spleen cell population of suppressed rats, when mixed with normal LNC. This group and the group receiving only normal spleen and LNC behaved similarly in the capacity to develop EAE and to form antibodies to BP after sensitization with BP-CFA. The same result was observed when LNC from suppressed rats were combined with spleen cells obtained from unimmunized rats.

The results of adoptive transfers of cells obtained from animals immunized with BP-IFA were similar. Again, no suppressor activity could be demonstrated in either the spleen cell or LNC populations.

Determination of ABL in Thymus and Spleen Cells of Rats Protected Against EAE by Immunosuppressive Treatment. Cell suspensions from Lewis rats treated with BP alone and then challenged with BP-CFA were used for ABL

TABLE V

Effect of Inoculating Lymphoid Cells from Immunosuppressed Rats Together with Lymphoid Cells from Unimmunized Animals on the Induction of EAE

Group no.	Suppressive treatment	Source of cells*		Sensitized with‡	EAE§		Serum ABC‖
		Spleen	Lymph node		Clinically	Histologically	
1	None	Normal	Normal	BP-CFA	9/10	10/10	4.4
2	BP	Suppressed	Normal	BP-CFA	7/9	9/9	3.9
3	BP	Normal	Suppressed	BP-CFA	5/5	5/5	4.5
4	BP-IFA	Suppressed	Normal	BP-CFA	4/5	5/5	5.0
5	BP-IFA	Normal	Suppressed	BP-CFA	6/6	6/6	5.0
6	None	Normal	Normal	CFA	0/5	0/5	0

* Single cell suspensions from either spleen cells or LNC from Lewis rats protected against EAE by injection of encephalitogenic BP from rat spinal cord, were mixed with either spleen or LNC from normal rats and transferred into previously Tx, irradiated (900 R) rats.

‡ Animals were sensitized with BP emulsified in CFA.

§ Positive/total.

‖ ABC determinations were done at sacrifice 14 days after sensitization with BP-CFA. Values represent micrograms of BP bound per milliliter of serum.

TABLE VI

Effect of Suppression of EAE on ABL in Lewis Rats

Rats injected with*	ABL per 10^5 cells	
	Spleen	Thymus
BP-CFA	125	16
BP-CFA and BP‡	55	4
BP§	11	0

* Organs for study of ABL were taken from the experimental animals previously shown in Table V.

‡ The reduction in the number of ABL is significant when compared with the group treated with BP-CFA alone; i.e., spleen: $P < 0.01$; thymus: $P < 0.01$.

§ The reduction in the number of ABL in the thymus is significant when compared with the untreated control group (Table I), $P < 0.01$.

studies. For this purpose, rats were sacrificed 14 days after challenge with BP-CFA, and ABL were determined in the thymuses and spleens as described. Table VI shows that injection of rats with soluble BP diminished to a significant level the number of ABL present after a subsequent challenge with BP-CFA. The decrease was greater in the thymus cell population, although the number of ABL also decreased in the spleen cells. On the other hand, the group that received the BP, but was not challenged afterward, showed no thymus cells binding BP ($P < 0.01$) and a diminished number of ABL in the spleen cells, when compared to those from unimmunized rats ($17/10^5$ cells). Experiments in which ABL were blocked with cold BP showed no differences when compared to the ABL found in unprimed animals.

Discussion

The results presented above showed that T cells were the only cells required for the induction of EAE after immunization with BP-CFA, albeit both T and B cells contained specific receptors capable of recognizing syngeneic BP. ABL to BP have been previously reported in the spleens of guinea pigs immunized with BP-CFA (20). Conversely, no significant ABL have been found in rats sensitized with BP-CFA (20). The low numbers of ABL to BP found in thymuses and spleens of normal rats in the present study could explain the previous failures of others to detect them (20). In addition, the experimental conditions used in the present studies, i.e. higher specific activity of the ^{125}I-BP, amount of ^{125}I-BP added to the reaction, and time of exposure, also favored the detection of ABL to BP in this model.

The relationship between ABL detected in vitro and their role in initiating the induction of EAE in vivo was established with antigen-suicide experiments (21–23) involving elimination of specific T- and B-cell functions by treatment with heavily iodinated (^{125}I) BP. Thus, no EAE was observed when T cells were suicided with high specific activity ^{125}I-BP and transferred into rats with immunocompetent B cells (B rats) which were then challenged with the BP in CFA, indicating that pretreatment with labeled BP eliminated specific T cells and thus abolished cell-mediated immunity to this neuroantigen. In addition, the treatment of T cells with the ^{125}I-labeled antigen specifically inactivated the T-cell collaborative effect with the B cell for the formation of antibody to BP, but not for the nonspecific anti-BRBC response. Furthermore, supplementing the antigen-suicided thymus cell inoculum with normal thymus cells effected a reversal of this inactivation, i.e., the appearance of clinical EAE and antibody formation to BP. In contrast to the inhibition of EAE by specific suicide of T cells, EAE and PFC to BP developed in response to BP-CFA injection in Tx, irradiated rats that were reconstituted with thymocytes previously treated with cold BP and normal bone marrow cells. In addition, EAE was observed after BP-CFA challenge in Tx, irradiated rats that were transferred with normal T cells, and bone marrow which had previously been suicided in vitro with ^{125}I-BP of high specific activity. The absence of antibody formation to BP in this group strongly indicated that suicide of specific antibody precursor cells did not alter the induction of EAE by the neuroantigen. In this regard, there have been numerous reports concerning the possible role of antibody in EAE. In agreement with the above observation, attempts to induce EAE by injection of sera obtained from sensitized animals have been unsuccessful (24–26). In addition, the disease may be induced regularly in bursectomized chickens (27). Furthermore, it has been reported that sera from animals with EAE have a protective effect on the development of EAE after injection of BP-CFA (28). The antibody may protect by interfering either with the sensitization of lymphocytes by the CNS antigen or with the activity of ABL or, less likely, by reacting with target tissue in the brain and thereby protecting against sensitized lymphocytes or their products. It is also possible that antibodies may participate in the progression of the disease initiated by sensitized cells. The present results indicate that whether antibodies protect from or enhance EAE, they are not a necessary factor for the initiation and induction of the disease.

It has been postulated that some autoantigens that circulate in low concentrations maintain unresponsiveness in specific populations of T but not in B cells (29–30). For example, thyroglobulin has been shown to circulate in sufficient concentrations to maintain unresponsiveness in T cells but not in B cells (31). Induction of tolerance to heterologous antigen in T cells requires extremely small amounts of tolerogen as compared to the relatively large amounts required for B-cell tolerance (29, 31). The demonstration by Clagett and Weigle (5) that specific unresponsiveness to autologous thyroglobulin exists in the T cells while the B cells remain responsive confirms this dichotomy with self-antigens. Therefore, B cells can recognize both cross-reacting and self-antigens on heterologous thyroglobulin, whereas the T-cell population is capable of recognizing only the unrelated determinants on these cross-reacting antigens. This is an important consideration in EAE since most work has been done with allogeneic or xenogeneic rather than syngeneic BPs. The available data, for the most part, indicate that EAE is produced primarily, if not solely, by a cell-mediated response to BP, strongly implicating T cells (32–35). In contrast to the immune status of T and B cells to thyroglobulin, apparently there is no unresponsiveness in the T cells to BP which prevents the natural induction of EAE. In fact, the data with both ABL and the suicide experiments can only be explained if both T and B cells specific for BP are immunocompetent.

The importance of the antigen receptor on T cells as a mechanism of antigen recognition and the participation of these cells in EAE could explain the fact that BN rats, which according to the present studies do not contain T-cell receptors for BP, have been shown by several investigators to resist sensitization with BP-CFA (36–39). Furthermore, Pitts et al. (40) obtained antibodies but not EAE after immunizing BN rats with BP-CFA and pertussis vaccine, an adjuvant known to stimulate B-cell proliferation (41). The latter results further support the presence of unresponsiveness at the T-cell but not B-cell level in the BN rat. However, EAE could be induced in these rats when more complex neural antigens were used (36), which might bypass the specificity of tolerant T cells. The latter model may resemble the immune status of T and B cells in thyroiditis, i.e., unresponsiveness at the T-cell level can be overcome by giving a heterologous antigen (42).

Another mechanism postulated in the control of autoimmune reactions is that of regulation by suppressor T cells. Our results do not support a role of suppressor T cells in inhibiting the induction of EAE by injection of nonencephalitogenic BP. It was reported that thymocytes from bovine gamma globulin (BGG) inoculated rats could, upon adoptive transfer to normal rats, specifically suppress both cell-mediated and antibody responses to BGG (43). Recently a population of suppressor cells, present in the lymph nodes and stimulated by treatment of rats with BP in IFA, was thought to exert control over the development of EAE in rats (9). In the present studies, adoptive transfer experiments provided no evidence for the presence of suppressor cells in either the spleens or lymph nodes of rats protected against EAE by treatment with BP either alone or incorporated in IFA. In this respect, the present results differ from those previously reported (9). These differences could be explained in that in the above experiments syngeneic antigen was used rather than the allogeneic antigen

used by others (9), and that the techniques differed. Suppressor activity has been shown to be lost with age in New Zealand mice (44, 45), and this loss was associated with the spontaneous induction of autoimmune disease. Although the present data suggest that suppressor activity is not involved in the abrogation of EAE afforded by nonencephalitogenic BP, these observations cannot be interpreted to mean that suppressor T cells play no role in regulation of autoimmune phenomena similar to that observed in New Zealand mice.

Although circulating antibody has been postulated to be responsible for the inhibition of the cellular immune response which presumably mediates EAE (46), such antibody does not appear to be involved in the inhibition of EAE by injection of BP without adjuvant or BP in IFA as shown in the present paper. First, rats injected with BP-IFA failed to develop EAE when also challenged with BP-CFA, but developed circulating antibodies to BP at the same level as rats injected only with the BP-CFA. Similarly, rats protected by injection of BP without adjuvant develop a normal level of circulating antibody when injected with BP-CFA. In this latter experiment, since the BP-CFA was given before (3 days) the suppressing injections of either BP-IFA or BP without adjuvant, the production of antibody in the suppressed group is not unexpected. Thus, effector T cells could well be suppressed although B cells have differentiated to the stage where T-cell help is no longer required for antibody synthesis. This possibility is compatible with the presence of a decreased number of ABL to BP in the suppressed group despite a normal level of circulating antibody. The failure to develop EAE but not antibody production in the suppressed rats is apparently the result of inhibition of an effector T-cell population after the precursor B cells have been committed and no further T help is required.

Summary

Although both the T and B cells of the Lewis rat have immunoglobulin receptors for basic protein (BP) of myelin, and both cell types are required for antibody production to BP, the present results demonstrate that the T cells are the only cells required for the induction of experimental allergic encephalomyelitis (EAE). Both EAE and anti-BP were readily induced in thymectomized, irradiated Lewis rats reconstituted with normal thymus and bone marrow cells and challenged with BP in complete Freund's adjuvant. If the thymus cells were first treated with BP heavily labeled with ^{125}I so as to eliminate (suicide) specific T cells, the recipients neither develop EAE nor produce antibody to BP. On the other hand, if the thymus cells were untreated and the specific B cells of bone marrow were eliminated by treatment with ^{125}I-BP, EAE was not inhibited, although no antibody was produced. These results strongly suggest that the T cell is responsible for the induction of EAE although both the T and B cells are competent to respond to BP.

Evidence was presented which suggests that neither suppressor T cells nor circulating antibody are involved in the inhibition of EAE by injection of Lewis rats with nonencephalitogenic preparations of BP. The immune status of T and B cells of the Lewis rat to BP was compared with the immune status of these cells in other species to thyroglobulin, where only the B cells appear to be competent. In this context, Brown Norway rats, which are resistant to the

induction of EAE, also appear to lack T cells reactive to BP, although competent B cells are present.

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