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Induction of experimental allergic encephalomyelitis in Lewis rats with purified synthetic peptides: Delineation of antigenic determinants for encephalitogenicity, *in vitro* activation of cellular transfer, and proliferation of lymphocytes

(autoimmunity/myelin basic protein/encephalitogenic determinants/T lymphocytes/adoptive transfer)

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ABSTRACT **Four highly purified synthetic peptides encompassing segments of the 68–86 region [for the numbering system used, see Eylar, E. H., Brostoff, S., Hashim, G., Caccam, J. & Burnett, P. (1971) *J. Biol. Chem.* 246, 5770–5784] of myelin basic protein (MBP), a region known to induce experimental allergic encephalomyelitis (EAE) in Lewis rats, were used to define and compare structure–function relationships between the primary structure of the 68–86 sequence and the three following biological activities: induction of EAE in Lewis rats, stimulation of T lymphocytes *in vitro* as measured by augmented cellular transfer of EAE to syngeneic recipients, and lymphocyte proliferation, as measured by [^{3}]thymidine incorporation. Guinea pig (GP) MBP was approximately 60 or 1500 times more active than the GP68–84 (Y G S L P Q K *S* Q R *S* Q D E N; single-letter amino acid abbreviations) or the modified bovine (MB) 68–84 (Y G S L P Q K *A* Q R *P* Q D E N) peptides for induction of EAE, respectively. Furthermore, lymphocytes primed with either GPMBP, GP68–84, or MB68–84 crossreacted *in vitro* with either GPMBP, GP68–84, or MB68–84 for activation of lymphocyte transfer activity. In contrast, lymphocytes primed with either GP68–84 or MB68–84 exhibited antigen-specific proliferation *in vitro* exclusively in response to either GP or MB sequences, respectively. Neither GP75–84 (S Q R S Q D E N) nor GP75–86 (S Q R S Q D E N P V) induced EAE, activated lymphocytes for EAE transfer, or stimulated lymphocyte proliferation under conditions and doses tested. We conclude that (*i*) structurally distinct determinants, reflecting existence of functionally independent classes of antigen receptors, specify encephalitogenic and proliferative responses of primed lymphocytes and (*ii*) determinants for EAE induction, cellular transfer of EAE, and lymphocyte proliferation include amino acid residues in the 68–74 (Y G S L P Q K) sequence of GPMBP.**

Experimental allergic encephalomyelitis (EAE) is a cell-mediated autoimmune response directed against autologous central nervous system myelin. Disease manifestations include clinical neurologic signs and perivascular infiltration of inflammatory mononuclear cells within the central nervous system (1, 2). Sensitization with myelin basic protein (MBP), a major protein constituent of central nervous system compact myelin, emulsified in complete Freund's adjuvant, will produce the full clinical and histological picture of EAE in a wide variety of animal species. In Lewis rats, sensitization with MBP in complete Freund's adjuvant induces the generation of lymphocytes in peripheral lymphoid organs with the capacity to adoptively transfer EAE into syngeneic Lewis recipients (3, 4). When lymphocytes sensitized to MBP/complete Freund's adjuvant are cultured in medium containing MBP for 3–4 days, they exhibit an ≈100-fold increase in EAE transfer activity, as evidenced by transfer of the paralytic signs of EAE with as few as 10^7 activated lymphocytes, and a vigorous antigen-specific proliferative response, as evidenced by [^{3}H]thymidine incorporation (5–8).

The encephalitogenic determinants responsible for EAE induction are species-specific. That is, different sequences of amino acid residues located at unique positions within the MBP molecule are critical for the induction of EAE in each mammalian species. For example, the 65–74 sequence or the 114–122 sequence of MBP selectively induce EAE in rabbits and guinea pigs, respectively (9). Thus, the capacity of MBP in complete Freund's adjuvant to induce an encephalitogenic immune response against autologous central nervous system myelin in a given mammalian species appears to be dictated by latent, species-specific immune response genes which presumably encode antigen-receptor molecules recognizing specific MBP sequences.

The encephalitogenic region of MBP for the Lewis rat has been described and structure–function relationships have been explored by use of MBP fragments prepared by enzymatic cleavage of MBP and by use of synthetic peptides. Chou *et al.* (10) found that the full EAE-inducing capacity of guinea pig (GP) MBP resided in an enzymatic fragment containing the 19 COOH-terminal amino acids (-G S L P Q K S Q R S Q D E N P V V H F) of a highly encephalitogenic cleavage fragment comprising residues 44–89.‖ Further enzymatic reduction of this 69–89 sequence at either the COOH-terminus, to give peptide 69–85 (G S L P Q K S Q R S Q D E N P), or the NH_2 terminus, to give peptide 73–89 (Q K S Q R S Q D E N P V V H F), resulted in an order-of-magnitude decrease in activity of both encephalitogenic peptides (11). Cleavage of the 69–89 peptide at the arginine^{79}-serine^{80} bond (G S L P Q K S Q R/S Q D E N P V V H F) abolished EAE-inducing activity (10). From these observations, Chou *et al.* (11) concluded that the 72–86

Abbreviations: EAE, experimental allergic encephalomyelitis; $EAED_{50}$, dose inducing EAE in 50% of sensitized rats; GP, guinea pig; MB, modified bovine; MBP, myelin basic protein; SI, stimulation index. Single-letter amino acid abbreviations are used.

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‖ See the legend to Table 1 for an explanation of the residue numbering.

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sequence of GPMBP (P Q K S Q R S Q D E N P V) comprises the shortest possible sequence possessing the full encephalitogenic activity of MBP.

From synthetic peptides prepared by the Merrifield solid-phase method on *tert*-butyloxycarbonylglycyl resins, Hashim (12) demonstrated that the 69–84 region was a major EAE-inducing region of MBP in the Lewis rat. Furthermore, all peptides with the GP sequence (P Q K S Q R S Q D E N G, Q K S Q R S Q D E N G, or S Q R S Q D E N G) or the modified bovine (MB) sequence (G S L P Q K A Q R P Q D E N G, P Q K A Q R P Q D E N G, or A Q R P Q D E N G) were reported to possess encephalitogenic potency comparable to that of GPMBP (12–14). Thus, in contrast to the work reported with enzymatic fragments, deletions at the NH_2 terminus up to but not including serine-75 (GP) or alanine-75 (MB) did not materially alter the encephalitogenic activity of the two synthetic nonapeptides. Hashim and co-workers (13, 14) concluded that the NH_2-terminal G S L P Q K sequence was not necessary for EAE induction and that the complete determinant responsible for full EAE-inducing activity in Lewis rats resided in the 75–84 sequence of GPMBP (S Q R S Q D E N).

Definition of the structure of the encephalitogenic determinant permitted characterization of the cellular and humoral immune responses restricted to minimal-length encephalitogenic peptides. McFarlin *et al.* (15) and Kibler *et al.* (16) demonstrated that the GP45–89 and GP69–89 fragments were substantially more active than the corresponding 45–89 and 69–89 fragments of rat MBP with respect to EAE induction, *in vitro* lymphocyte proliferation, and induction of antibody formation. A single substitution of serine (GP) for threonine (rat) appeared to account for the greater biological activities of guinea pig versus rat fragments. These investigators (15, 16) postulated that the GP sequence might contain a helper T-cell determinant that served to amplify a separate population of lymphocytes effecting EAE activity. Investigations in guinea pigs conducted by Spitler and co-workers (17, 18) showed that GPMBP and the GP114–122 encephalitogenic peptide shared EAE-inducing activity but contained separate determinants for cell-mediated responses associated with development of disease. These findings were consonant with the concept of two determinants acting synergistically for induction of EAE.

For our work using immunopharmacological and protein-binding techniques to define molecular interactions of EAE-inducing MBP determinants and their respective receptors on Lewis rat lymphoid cells, securing and testing highly purified synthetic peptides of minimal length containing these determinants was a crucial first step. To this end, we obtained four synthetic peptides consisting of sequences analogous to the reported minimal length encephalitogenic determinant (GP75–84) (13) or with the sequence extensions at either the COOH terminus (GP75–86) or the NH_2 terminus (GP68–84 or MB68–84) reported to increase the potency of encephalitogenic immune responses in Lewis rats (11). We tested each peptide for its capacity to induce EAE and its ability to stimulate the two EAE-associated lymphocyte responses paralleling the encephalitogenic immune response: (*i*) *in vitro* activation of lymphoid cells expressing EAE-transfer activity, corresponding to *in vivo* triggering of encephalitogenic effector lymphocytes, and (*ii*) *in vitro* proliferation of lymphocytes, corresponding to *in vivo* expansion of the EAE-inducing lymphocyte population. Our observations suggest that (*a*) determinants specifying the encephalitogenic activity of lymphocytes are structurally distinct from determinants engendering *in vitro* lymphocyte proliferation and (*b*) determinants for EAE induction, *in vitro* activation of cells transferring EAE, and *in vitro* proliferation of lymphocytes include amino acid residues in the 68–74 sequence of GPMBP (Y G S L P Q K).

MATERIALS AND METHODS

Animals. Adult male Lewis rats (8–12 weeks old, 200–225 g) were purchased from M. A. Bioproducts, Walkersville, MD. Rats were housed 6–7 per cage with pine shavings for bedding and were given free access to food and water.

Antigens. GPMBP was isolated from spinal cord by the method of Swanborg *et al.* (19). A high degree of homogeneity of each MBP preparation was confirmed by polyacrylamide gel electrophoresis. Lysozyme (Sigma) was used as a control antigen because its molecular weight and isoelectric point are similar to those of MBP.

The designation, primary structure, and other pertinent features of the four synthetic peptides used in this investigation, together with the corresponding structure of the relevant region of GPMBP under study, are listed in Table 1. Different systems exist for numbering amino acid positions in MBP to variably accommodate specific additions or deletions in MBPs of diverse vertebrate origin. We used one (20) of the two (20, 21) widely used numbering systems based on sequences reported for the bovine MBP molecule.

The four peptides were custom-prepared by the Merrifield solid-phase method and were purified, via preparative HPLC by Peninsula Laboratories (Belmont, CA), to at least 98% homogeneity as ascertained by TLC, electrophoresis, amino acid analysis, and HPLC. The homogeneity of the peptides was verified in our laboratory via analytical HPLC using a Waters Associates HPLC system; the relative elution volume for each of the peptides is reported in terms of the capacity factor k_n (22). The capacity factor is a measure of the column retention volume for eluted compound n, with $k_n = (V_n - V_o)/V_o$, where V_n is the retention volume from the time of injection to the peak maximum and V_o is the void volume or

Table 1. Designation, primary structure, molecular weight, and analytical HPLC data of synthetic peptides

MBP or peptide	Amino acid sequence	Commercial lot no.	Molecular weight	HPLC	
				% solvent B	k_n
	6 77 8				
	8 69 6				
GPMBP	– Y G S L P Q K S Q R S Q D E N P V –		18,500		
GP68–84	Y G S L P Q K S Q R S Q D E N	005757	1,737	17	4.5
MB68–84	Y G S L P Q K A Q R P Q D E N	004952	1,731	12	5.0
GP75–86	S Q R S Q D E N P V	005709	1,159	9	2.0
GP75–84	S Q R S Q D E N	005063	963	0	0.6

The amino acid residue numbering system is that originally published by Eylar *et al.* (20) for native bovine MBP, containing glycine and histidine at positions 77 and 78, respectively. Molecular weights are that published for MBP (1, 20) and those calculated on the basis of amino acid sequences for the synthetic peptides. The HPLC data for each peptide were obtained using isocratic elution with the indicated percentage of solvent B [CH_3CN/0.5% H_3PO_4 (3:2, vol/vol)] in water. The capacity factor, k_n, is described in *Materials and Methods*.

volume from the time of injection to the unretained solvent peak (22).

Assessment of Encephalitogenic Activity. *Active sensitization.* Synthetic peptides or MBP were dissolved in phosphate-buffered saline and emulsified in an equal volume of complete Freund's adjuvant by use of a Sorval omnimixer (Model 17105, Dupont Instruments, Sorvall Division, Newtown, CT). Each Lewis rat received 0.1 ml of an emulsion consisting of a specified dose of MBP or peptide in complete Freund's adjuvant containing 200 μg of *Mycobacterium tuberculosis* H37Rv, Jamaican strain. Sensitizing inoculum (50 μl) was injected intradermally into each hindleg footpad. The dose of MBP or peptide that induced clinical signs of EAE in 50% of the sensitized population ($EAED_{50}$) was calculated according to the principles of the Reed and Muench method (23).

Clinical and histopathological measurements of EAE. Rats were monitored daily for clinical signs of EAE according to the following scale: lack of tonicity in the distal half of the tail, 0.25; lack of tonicity in the entire tail, 0.5; ataxia, 1.0; hindleg paresis, 2.0; and hindleg paralysis, 3.0. Sections of brain and spinal cord were examined microscopically for EAE lesions and the lesions were graded (with a maximal value of 3.0) as described by Carbone *et al.* (24).

***In Vitro* Culture and Adoptive Transfer of Lymphocytes.** Nine to eighteen days after sensitization, the popliteal, inguinal, and periaortic lymph nodes or the spleens were trimmed of fat and processed to a single-cell suspension by teasing through a stainless steel wire mesh screen. The cells were washed three times in Hanks' balanced salts solution (GIBCO) and resuspended in complete RPMI 1640 medium (Northwestern University, Chicago, IL) containing 5% fetal bovine serum (GIBCO), 2 mM glutamine (Sigma), streptomycin (100 μg/ml) (GIBCO), penicillin (100 units/ml) (GIBCO), and 2-mercaptoethanol (Eastman-Kodak). Cells were adjusted to a density of 5×10^6 per ml and incubated 84 hr at 37.5°C with 5% CO_2 and the designated antigen in 75-cm^2 culture flasks (Corning). Each culture flask contained 40 ml of medium and was placed on its side (horizontal orientation) to maximize surface area during incubation. After incubation, cells were washed three times in Hanks' balanced salts solution and counted with determination of viability (trypan blue exclusion test). Selected numbers of *in vitro*-activated cells in 0.7 ml of Hanks' balanced salts solution were injected into the lateral tail vein of recipient Lewis rats.

Lymphocyte Proliferative Assay. Lymph node cells and splenocytes, processed and resuspended in complete RPMI medium as described above, were dispensed into flat-bottomed microtiter plates (Nunc), with each well containing 5×10^5 cells and appropriate concentrations of antigens in a volume of 167 μl. After 72 hr of incubation at 37.5°C, with 5% CO_2, 1 μCi of [^{3}H]thymidine (2 Ci/mmol, Amersham, IL; 1 Ci = 37 GBq) was added to each well and incubation was continued for 24 hr. Cultures were harvested on fiberglass filters and the incorporation of [^{3}H]thymidine was measured by liquid scintillation counting.

RESULTS

Encephalitogenic Activities of Peptides. Data obtained from more than six experiments in which various doses of GPMBP and the four synthetic peptides were tested for encephalitogenic activity are summarized in Table 2. The $EAED_{50}$ of GPMBP was 0.02 nmol. Peptides GP68–84 and MB68–84 also induced EAE in a dose-dependent manner. Based on $EAED_{50}$, GP68–84 ($EAED_{50}$ 1.2 nmol) was ≈25 times more active than MB68–84 ($EAED_{50}$ 30 nmol). Neither GP75-84 (tested at 1, 10, and 100 μg doses) nor GP75-86 (tested at 5 and 50 μg doses) induced clinical or histopathological changes of EAE. The rank order of encephalitogenic activities for GPMBP and the two EAE-active peptides would appear to be: GPMBP > GP68–84 > MB68–84.

Adoptive Transfer of Sensitized Lymphocytes Cultured with Either MBP or Peptides. Data obtained from more than eight experiments concerning *in vitro* activation of sensitized lymph node cells are summarized in Table 3. Lymph node cells that were sensitized to GPMBP, GP68–84, or MB68–84 crossreacted *in vitro* with either GPMBP, GP68–84, or MB68–84 as evidenced by occurrence of EAE in recipients injected with the activated cells. Other data, not shown in Table 3, established two additional points. First, cultured splenocytes (usually obtained from the same donors as the lymph node cells) exhibited crossreactivity similar to that observed with lymph node cells with respect to *in vitro* activation; i.e., splenocytes of rats primed with either GP68–84 or MB68–84 were activated with either one of the two peptides or MBP. Second, either lymph node cells or

Table 2. Comparative encephalitogenic activities of GPMBP and synthetic peptides

Sensitizing antigen	Dose		Occurrence of EAE		$EAED_{50}$, nmol
	μg	nmol	Signs (score)	Lesions (score)	
GPMBP	3.0	0.16	4/4 (3.0)	4/4 (3.0)	0.02
	0.5	0.03	4/4 (1.5)	3/4 (1.5)	
	0.1	0.005	1/4 (0.5)	0/4	
GP68–84	25	14.5	3/3 (3.0)		1.2
	5	2.9	9/13 (1.9)	2/2 (2.0)	
	1	0.6	4/10 (0.6)	1/4 (1.0)	
	0.2	0.12	0/3	0/3	
MB68–84	50	29	4/8 (0.44)	6/8 (0.88)	30
	5	2.9	0/7	0/3	
GP75–86	50	43	0/4	0/4	
	5	4.3	0/4	0/4	
GP75–84	100	104	0/7	0/7	
	1	1.0	0/2	0/2	

MBP or peptides were solubilized in phosphate-buffered saline and emulsified in an equal volume of complete Freund's adjuvant. Each Lewis rat received a total of 0.10 ml of the emulsion, containing indicated dose of antigen plus 200 μg of *M. tuberculosis*, via injection of each hindleg footpad. In all cases, rats were sacrificed for histological assessment 20–28 days after sensitization, or sooner when moribund due to EAE. Occurrence rates of EAE signs and lesions are expressed as ratios, with average score of clinical signs and lesions (see *Materials and Methods*) for each group of animals shown in parentheses. Precipitous deaths of three rats sensitized to the highest dose of GP68–84 precluded securing tissue for histological examination.

Table 3. Adoptive transfer of EAE with donor lymph node cells (LNC) after *in vitro* activation with MBP or peptide

Primary sensitization of donors	*In vitro* activation of donor LNC	LNC transferred per recipient, no. $\times 10^{-6}$	Occurrence of clinical signs of EAE in recipients (clinical score)
GPMBP (50 μg)	GPMBP	60–85	4/4 (3.0)
		10–55	9/9 (3.0)
		4	2/2 (0.6)
	GP68–84	40	1/1 (3.0)
		25	3/3 (2.2)
	MB68–84	100	6/6 (1.6)
	GP75–84	85–170	0/6
	Lysozyme	180	0/2
GP68–84 (25–50 μg)	GPMBP	10	1/1 (3.0)
	GP68–84	40	4/4 (2.5)
	MB68–84	40–80	3/3 (2.0)
MB68–84 (25–100 μg)	GPMBP	50	2/2 (3.0)
		10	1/1 (2.0)
	GP68–84	50	1/1 (1.0)
	MB68–84	100	2/2 (3.0)
		25	1/1 (0.5)
		5–20	0/4

Donor Lewis rats were sensitized with MBP or peptide emulsified in complete Freund's adjuvant. Draining LNC were obtained from donors 9–18 days later and cultured with various concentrations of MBP or peptide (1–4 μM, except GP75–84, which was tested at 2, 6, and 20 μM) for ≈84 hr. Surviving lymphocytes were extensively washed, counted, and injected into the lateral tail vein of each recipient Lewis rat. Recipients were monitored daily for clinical signs of EAE (see *Materials and Methods*).

splenocytes primed with either GP68–84 or MB68–84 could be activated with a preparation of syngeneic rat MBP.

[^{3}H]Thymidine Incorporation by Sensitized Lymphocytes Cultured with Either MBP or Peptides. Proliferation-response data [expressed as stimulation indices (SIs)] obtained from eight experiments using splenic lymphocytes of sensitized rats are shown in Table 4. Lymphocytes primed with GPMBP or GP68–84 exhibited significantly increased incorporation of [^{3}H]thymidine when cultured with GPMBP or GP68–84 (SI = 5–50); no appreciable increase was observed when these cells were cultured with MB68–84 (SI < 1.5). Conversely, lymphocytes sensitized to MB68–84 were stimulated to incorporate thymidine by culture with MB68–84 (SI = 5–15) but not with GPMBP or GP68–84 (SI < 2). The GP75–84 and GP75–86 peptides did not stimulate [^{3}H]thymidine incorporation in lymphocytes primed with either GPMBP, GP68–84, MB68–84, or GP75–84. Although not evident from the data presented in Table 4, it was observed that when cultured lymphocytes exhibited significant proliferative responses to MBP or peptide, the responses invariably were dose-dependent in nature. Two additional findings, not set out in Table 4, merit mention. First, syngeneic rat MBP stimulated proliferation of lymphocytes primed with GPMBP or GP68–84 but did not stimulate proliferation of lymphocytes primed with MB68–84. Second, sensitized lymph node cells exhibited a determinant-specific proliferation pattern identical to the one observed for the splenocyte population.

Table 4. *In vitro* thymidine incorporation of lymphocytes sensitized to MBP or peptide

Sensitizing antigen	SI of lymphocytes cultured with				
	GPMBP	GP68–84	MB68–84	GP75–84	GP75–86
GPMBP	28	10	1	1	1
GP68–84	38	11	1	1	1
MB68–84	1	1	13	1	1
GP75–84	1	ND*	1	1	1

Donors were sensitized with 25–100 μg of GPMBP, GP68–84, MB68–84, or GP75–84 in complete Freund's adjuvant. Spleens were removed from donors 9–18 days after sensitization and processed to a single-cell suspension. Splenocytes were aliquoted in 96-well plates (5×10^5 cells per 167 μl of medium in each well). After 72 hr of culture, 1 μCi of [^{3}H]thymidine was added to each well and incubation was continued for an additional 24 hr. The cells were harvested on fiberglass filters, and [^{3}H]thymidine incorporation was measured by liquid scintillation counting. The SI was defined as the ratio of the mean cpm of quadruplet wells with antigen to mean cpm of quadruplet wells without antigen. Standard deviations of the mean cpm were routinely <20%. Maximal values of antigen-stimulated [^{3}H]thymidine incorporation were usually obtained at the highest antigen concentration tested (10 μM) and were used for the calculation of the SI.

*ND, not determined.

DISCUSSION

The purpose of this investigation was to examine the structure–function relationships between the primary structure of the encephalitogenic region of MBP and three constituent immune responses representing EAE in Lewis rats. The three responses studied were induction of the disease in actively sensitized animals, *in vitro* activation of primed lymphoid cells expressing transfer of EAE in syngeneic recipients, and *in vitro* proliferative responses of sensitized lymphocytes. Comparison of the structure–function relationships for EAE induction with those of the two *in vitro* EAE-associated responses resulted in two conclusions: First, determinants stimulating expression of encephalitogenic activity in primed lymphocytes appear to be structurally distinct from determinants stimulating *in vitro* proliferation (compare Tables 3 and 4). Second, determinants for EAE induction and both *in vitro* immune responses include amino acid residues in the 68–74 (-Y G S L P Q K-) sequence of GPMBP (see Tables 2–4).

From an autoreactive standpoint, any encephalitogenic determinant(s) present in any MBP species must crossreact with rat MBP in order to induce EAE or to stimulate cellular transfer of the disease in the Lewis rat. In our hands, the MBP and peptide preparations possessing encephalitogenic activity crossreacted with rat MBP in terms of *in vitro* activation of cells effecting transfer of the disease (see Table 3 and text related to Table 3). In contrast, the proliferative determinant

in the encephalitogenic MB68–84 peptide exhibited no detectable crossreactivity with GP68–84, GPMBP, or rat MBP in the lymphocyte thymidine-incorporation assay (see Table 4 and related text). Thus, either one or both of the amino acid differences between the GP68–84 and MB68–84 peptides [Y G S L P Q K (S/A) Q R (S/P) Q D E N] encoded the exquisite specificity of the proliferative determinant(s), whereas neither residue had any appreciable influence upon the specificity of the determinant(s) responsible for activation of cellular transfer. From such observations, we conclude that the *in vitro* activation of cells involves a determinant-specific receptor on lymphocytes that is directly implicated in mediating transfer of EAE, whereas the *in vitro* proliferative response reflects an equally determinant-specific but entirely separate set of antigen receptors. Our findings are consonant with work of others (13–16) that suggests that two functionally disparate determinants may need to act in concert for development of EAE. Our results reduce this concept to the level of specific amino acid sequences and residues within the rat-specific encephalitogenic region of GPMBP.

In several instances, we observed a clear-cut dissociation of *in vitro* activation and *in vitro* proliferative immune responses of primed lymphocytes. That is, lymph node cells or splenocytes sensitized to GP68–84 or MB68–84 manifested transfer of EAE after activation with MB68–84 or GP68–84, respectively (see Table 3), without detectable antigen-specific proliferation (see Table 4). These findings underscore the fact that *in vitro* cellular proliferation is not a necessary concomitance of *in vitro* T-lymphocyte activation resulting in transfer of EAE.

A second major observation reported here is that the 68–74 (-Y G S L P Q K-) sequence of rat MBP was essential for, or greatly augmented, autoreactivity to MBP. Our observation that the determinant(s) enabling proliferative responses include immunodominant amino acid residues at positions 75 or 80 (-X Q R X Q D E N-) along with other research indicating that determinant(s) stimulating encephalitogenic processes include asparagine-84 (- S Q R S Q D E N-) (25), indicates that structurally distinct proliferative and encephalitogenic determinants are located within the 75–84 sequence and that both require the 68–74 (-Y G S L P Q K-) sequence for expression of the respective activities. There are at least four interpretations for these findings. First, the -Y G S L P Q K- sequence may stabilize a conformation necessary for immune recognition of the -S Q R S Q D E N- sequence. Second, the proliferative and encephalitogenic determinants may include separate or possibly overlapping regions in the -Y G S L P Q K- sequence. Third, another independent determinant confined to this sequence may be required for the expression of both encephalitogenic and proliferative determinant activities. Fourth, the -Y G S L P Q K- sequence may facilitate the interaction of encephalitogenic peptides with antigen-presenting cells necessary for T-cell recognition.

In terms of encephalitogenic activity, the GP68–84 peptide was about 1/60th as active as GPMBP and, thus, does not represent the minimal sequence associated with full encephalitogenic activity. This finding is consistent with the work of Chou *et al.* (11) showing that cleavage of the 86–89 (-V V H F-) sequence from the 69–89 peptide fragment resulted in a 90% reduction of encephalitogenic activity. The GP75–86 peptide did not induce EAE at a dose of 50 μg per rat (Table 2). Additional testing of the GP75–86 peptide at very high doses will be necessary to determine whether the augmenting effect of the 85–86 (-P V-) sequence is dependent upon the presence of the 68–74 (-Y G S L P Q K-) sequence.

We have found that the GP75–84 (S Q R S Q D E N) peptide lacks encephalitogenic activity, even when tested in doses as high as 100 μg per rat (Table 2). In addition, we have found that the MB68–84 peptide has less encephalitogenic activity than might have been expected from the work reported by Hashim and his associates (12, 13). It is noteworthy that the synthetic peptides S53 and S49 (S Q R S Q D E N G and G S L P Q K A Q R P Q D E N G, respectively), reported by Hashim's group (12, 13) to possess EAE activity for Lewis rats, differ from peptides GP75–84 and MB68–84, respectively, by virtue of containing a COOH-terminal glycine. Whether the non-native COOH-terminal glycine confers either demonstrable or increased encephalitogenic activity, respectively, on GP75–84-glycine or MB68–84-glycine should be investigated.

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