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10. Five normal and three colchicine-treated (50 μg of colchicine in 25 μl of 0.9 percent NaCl injected intraventricularly 48 hours before death) male Sprague-Dawley rats (200 to 250 g body weight) were anesthetized with pentobarbital and each animal was perfused via the ascending aorta with 250 ml of ice cold 4 percent freshly depolymerized paraformaldehyde in a 0.1*M* sodium phosphate buffer at *p*H 7.4. Brains, pituitaries, and cervical spinal cords were removed and further fixed for 2 hours. They were then placed in a 5 percent solution of sucrose in phosphate-buffered saline (PBS) overnight at 4°C. The tissue was frozen in liquid dichlorodifluoromethane and 14-μm-thick sections were cut in a cryostat. Sections were mounted on gelatin-coated slides and incubated at 4°C overnight with primary antiserum diluted 1:400 with PBS containing 0.3 percent Triton X-100 and 2.5 percent bovine serum albumin. The sections were then washed and incubated for 2 hours at room temperature with fluorescein-conjugated goat or sheep antiserum to rabbit immunoglobulin G (Cappel Laboratories) diluted from 1:10 to 1:40 in Triton X-100 buffer without albumin. After being rinsed the sections were covered with a 1:1 mixture of glycerol and PBS and examined with a Leitz Orthoplan light-microscope equipped with an epifluorescence attachment. Anatomical distribution of immunoreactive fibers and cell bodies was correlated with coronal sections from the stereotaxic atlases of J. F. R. Konig and R. A. Klippel [*The Rat Brain: A Stereotaxic Atlas of the Forebrain and Lower Parts of the Brain Stem* (Williams & Wilkins, Baltimore, 1963)] and of J. L. Pellegrino, A. S. Pellegrino, A. J. Cushman [*A Stereotaxic Atlas of the Rat Brain* (Plenum, New York, 1979)].
11. The antibodies to Met-enkephalin were raised in a rabbit by injecting Met-enkephalin linked by glutaraldehyde to bovine serum albumin as described [R. J. Miller, K. J. Chang, B. Cooper, P. Cuatrecasas, *J. Biol. Chem.* **253**, 531 (1978)]. The antibodies to YGGFMRF were raised by the same method except that bovine thyroglobulin was used as a carrier. Specific immunofluorescence of rat brain sections produced by the Met-enkephalin antiserum was fully blocked by first incubating the antiserum with a 10-μ*M* concentration of Met-enkephalin. YGGFMRF also blocked the immunostaining but only at a 100-fold higher concentration. The immunofluorescence produced by the antiserum to YGGFMRF was blocked by 10-μ*M* YGGFMRF and also by Met-enkephalin, but only at a ten-fold higher concentration.
12. The commercially available synthetic peptides were obtained from Peninsula Laboratories or from Bachem. The YMRF-NH_2, FMRFY, and MRF-NH_2 were synthesized by J. K. Chang at Peninsula Laboratories. The YGGFMRF-NH_2 and RF-NH_2 were a gift from D. Price, Tallahassee, Fla.
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19. Two micrograms of YMRF-NH_2 were labeled with 1 mCi of ^{125}I by the chloramine-T method of W. M. Hunter and F. C. Greenwood [*Nature (London)* **194**, 495 (1962)]. Radioiodinated peptide was purified on a Sephadex G-10 column in 50 percent acetic acid, and radioimmunoassays with antiserum R3-1 at a final dilution of 1:12,000 were performed according to a procedure described by E. Weber *et al.* (*21*). The 50 percent inhibition concentration of the radioimmunoassay was 500 p*M*. Interassay variability was < 11 percent.
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24. Address correspondence and reprint requests to E.W. We thank D. Price, Tallahassee, for the YGGFMRF-NH_2 and RF-NH_2, J. K. Chang, Peninsula Laboratories, for synthesizing fragments and analogs of FMRF-NH_2, and N. Ling, La Jolla, for providing synthetic γ_1-MSH. We also thank I. Inman for technical assistance and S. Poage for preparing the manuscript. Supported by NIMH grant MH 23861, NIDA grant DA 01207, and ONR award SRO 001.

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Autoimmune Encephalomyelitis: Simultaneous Identification of T and B Cells in the Target Organ

Abstract. *Monoclonal antibodies to guinea pig T cells and antibodies to guinea pig immunoglobulin G were used in immunofluorescence studies to identify T and B cells in central nervous system tissue from guinea pigs with acute autoimmune encephalomyelitis. T cells appeared before B cells and were distributed within the white matter parenchyma, while B cells remained in perivascular spaces.*

Although it is recognized that experimental allergic encephalomyelitis (EAE) is a T cell–mediated autoimmune demyelinating condition (*1*), T cells have never been specifically identified in the target organ, the central nervous system (CNS). In the present study, fluorescein labeling of monoclonal antibodies to guinea pig T cells and rhodamine-labeled immunoglobulin G (IgG) were used to identify T and B cells in the CNS of guinea pigs with EAE. By means of this technique we found that T and B cells display different distribution patterns in the CNS. We also observed that, with increasing severity of the disease, there are correlated variations in the distribution of T and B cells, IgG, and the third component of complement (C3).

Acute EAE was induced in adult strain 13 guinea pigs by inoculation with an emulsion containing bovine white matter or isogeneic spinal cord tissue in complete Freund's adjuvant (*2*). A severe form of acute EAE was also induced in juvenile strain-13 guinea pigs similarly inoculated (*3*). Control animals consisted of uninoculated age-matched guinea pigs. Animals sensitized for EAE developed neurologic signs (paraparesis and incontinence) 12 to 24 days after inoculation. The animals were anesthetized and perfused with phosphate-buffered saline (PBS) and their CNS (brain and spinal cord) was removed. Slices were embedded in optimal cooling temperature medium and frozen in a bath of acetone and dry ice for cryostat sectioning. The sections were cut 10 μm thick and fixed for 20 minutes in ethanol at 4°C. Staining for T cells was achieved with mouse monoclonal antibodies to guinea pig T cells (*4*). This antibody (5CC2) was raised against peripheral lymphocytes from guinea pigs and gave immunofluorescent staining of circulating T cells and mature thymocytes (it also gave weak staining of B cells when used at a dilution of $1:10^4$ or lower). Tissue was exposed to 5CC2 at a dilution of $1:10^6$ in PBS and then incubated with fluorescein isothiocyanate–conjugated $F(ab')_2$ fragments of rabbit antibodies to mouse immunoglobulin at a dilution of 1:50 in PBS. For simultaneous demonstration of B cells, other IgG-containing cells (macrophages), and IgG deposits, we used rhodamine-conjugated $F(ab')_2$ fragments of rabbit antibodies to guinea pig IgG (specific for heavy and light chains) at a dilution of

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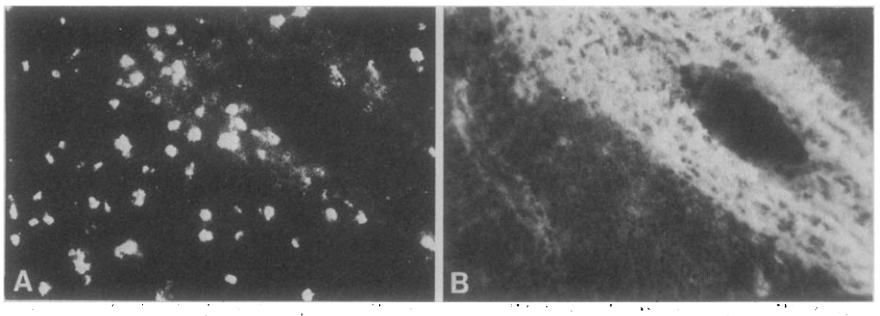

Fig. 1. Frozen section of brain tissue from a guinea pig with acute EAE. Tissue is double-stained with fluorescein and rhodamine conjugates to show T and B cells, respectively. (A) T cells diffusely distributed in the parenchyma; only an occasional T cell (probably *en passage*) is within the perivascular cuff (upper right). Fluorescein filter (×500). (B) Same field as in (A), but stained for B cells. Note the dense packing of immunoglobulin-positive cells (B cells and some macrophages) in the perivascular space. Rhodamine filter (×500).

1:50 in PBS. Deposits of guinea pig C3 were identified by a rhodamine-conjugated IgG fraction of rabbit antibodies to guinea pig C3.

In the CNS of adult guinea pigs with acute EAE, T cells were predominantly located in the white matter parenchyma, while B cells were largely restricted to perivascular and meningeal infiltrates (Fig. 1, A and B). Double labeling of the same cell by both fluorescein and rhodamine was not seen in the CNS. In the spinal cord, inflammation was most severe in the lumbar area and decreased in intensity toward the cervical region. Sections from multiple levels of the same spinal cord revealed that although lesion topography differed greatly from level to level, T and B cells maintained a parenchymal and perivascular distribution, respectively. An occasional T cell was found in the spinal cord gray matter around the central canal and at the junction between the gray and white matter. In the brain, T cells predominated in the subependymal area of the lateral ventricles and in the white matter of the brainstem, whereas B cells and macrophages were present in the meninges and in some perivascular infiltrates. Deposits of IgG occasionally were found in the brain and spinal cord, usually in the anterior root exit zone and anterior fissure of the spinal cord. The distribution of C3 was similar to that of IgG. Control guinea pig CNS tissue did not stain for T and B cells or for IgG and C3.

Some juvenile strain 13 guinea pigs, ostensibly inoculated for chronic relapsing EAE (*5*), unexpectedly developed signs of severe acute EAE after 2 to 3 weeks. This form of the disease, which frequently proved fatal (*3*), was comparable to that reported elsewhere (*6*). In these severely afflicted animals, perivascular cuffing was more extensive throughout the spinal cord and brain. Infiltration by T and B cells was widespread in these regions; T cells were uniformly distributed throughout gray and white matter and B cells and macrophages were located in the numerous perivascular cuffs, particularly at the gray-white matter border and, on occasion, in the CNS parenchyma. Binding of IgG was more pronounced and extensive around the dense infiltrates, and some diffuse binding was noted in the subpial white matter. The distribution of C3 was similar to that of IgG.

Previous studies of acute EAE (*7*) and "hyperacute" EAE (*8*) showed similar distribution of B cells, IgG, and C3 by methods comparable to those employed here. However, T cells had not been identified in the CNS in EAE, and they still have not been convincingly demonstrated in sections from target organs in other inflammatory diseases. The present study is innovative in that it combined a monoclonal antibody to T cells with several other technologies to give a montage of immunological events in the CNS of animals with two different forms of EAE.

During the study it was found that 5CC2 weakly stains B cells. Therefore, a second monoclonal antibody to guinea pig T cells (8BE6) was tested (*4*). This antibody is highly specific for all peripheral T cells, does not stain B cells (as tested by two-color immunofluorescence on a fluorescence-activated cell sorter), and is believed to be equivalent to monoclonal antibodies to human T cells (OKT-3) (Orthodone, Raritan, New Jersey) (*9*). Using 8BE6 at a dilution of $1:10^4$, we obtained results that were identical to those obtained with 5CC2 at $1:10^6$.

Studies of the order of events in the CNS in EAE indicate that T cells appear in the parenchyma 5 to 6 days after inoculation—before the appearance of clinical signs. Infiltrates containing B cells and macrophages are seen on days 10 to 11, a period coinciding with the onset of clinical signs (*10*). These findings contrast with the suggestion by researchers experimenting with other autoimmune diseases, such as Sjögren's syndrome, that B cells are the first lymphocytes to appear in the target organs (*11*). The functions of these invading lymphocytes are not precisely known, but it is unlikely that more than a minute percentage are specifically sensitized (*12*).

The staining patterns in EAE imply a difference in the mobility of T and B cells in the target organ. It is known from in vitro (*13*) and in vivo (*14*) studies that T cells are more mobile than B cells. The extent to which local factors influence this differential mobility is not known, but it has been suggested that the lower *p*H in inflammatory lesions (*13*) inhibits B cell movement. It is likely that in EAE and multiple sclerosis (MS), the human disease for which EAE serves as a model, other factors are operative since in both conditions chronic, silent lesions contain many B cells (plasma cells) perivascularly in the absence of active disease (*15*); plasma cells in MS and chronic EAE are rare in the CNS parenchyma (*16*).

The sequence of lymphocyte events in MS remains to be elucidated. With the availability of monoclonal antibodies to human T cells (*9*), this problem may now be solved. Such information would be useful not only in MS research but also in studies of other inflammatory diseases.

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Slow-Wave Sleep: A Recovery Period After Exercise

Abstract. *Sleep recordings were carried out on athletes on four successive nights after completing a 92-kilometer road race. Significant increases in total sleep time and slow-wave sleep were found after this metabolic stress. The results show a definite exercise effect on sleep and support sleep-restoration hypotheses.*

Recent research has lent credence to the hypothesis that sleep and in particular slow-wave sleep (SWS) (*1*), is a recovery period for daily metabolism (*2, 3*). Evidence in support of this theory includes the synchrony of growth hormone release with SWS in humans (*4*), the suggestion that optimum conditions for anabolism prevail during sleep (*5*), and studies showing SWS duration to be proportional to preceding wakefulness (*6*). Although many other studies (*7*) have yielded supportive evidence for the theory, the prediction that daytime exercise would increase SWS has produced equivocal results (*8*). Possible reasons for these conflicting findings include the variable fitness of the subjects tested (*9*), the time during the day at which the exercise is performed (*10*), and the absolute amount of exercise (*11*). The absolute amount of exercise is relevant since it is the increase in energy expenditure during exercise over and above basal metabolism that would be expected to influence the amount of SWS. To evaluate the theory that SWS is a recovery process and to resolve the question of the effect of exercise on sleep, an experiment was carried out in which the sleep patterns of six subjects were studied after a 92-km marathon. We thought that this extreme event would highlight the effect of a large increase in energy expenditure on sleep.

All subjects (age, 18 to 26 years; mean age, 21.7 years) slept for two nonconsecutive nights (with two intervening nights) in the sleep laboratory 2 weeks before the marathon. The first of these was to allow for the "first-night effect" (*12*) and was not recorded. The second of these was used as a prerace baseline level. Sleep patterns were recorded on the night of the marathon (night 1) and for the subsequent three nights (nights 2 to 4); they were recorded again 2 weeks after the marathon as a postrace control. Of the six recorded nights, the only day on which any specific exercise had been performed was that of the extended marathon (*13*). None of the subjects were taking medication, and they did not drink alcohol or coffee on the days of the study. Sleep recordings were made in the standard manner and were scored blind by two trained scorers according to standard criteria (*14*). All six subjects had previously completed several standard marathons over the preceding year, and three had in previous years completed this extended marathon. Five of the six subjects were tested for treadmill maximum aerobic power ($\dot{V}_{O_2\ max}$) and degree of fitness 3 weeks before the marathon. Lactic acid turn point (*15*) was over 70 percent of $\dot{V}_{O_2\ max}$ for all but one of these subjects, indicating a high state of fitness. The range of $\dot{V}_{O_2\ max}$ for these five subjects was 3.56 to 4.07 liters (55.8 ± 2.2 ml per kilogram of body weight per minute, mean ± standard error of the mean). The marathon started at 0600, and the subjects required between $8\frac{1}{2}$ and $10\frac{3}{4}$ hours to complete the 92 km (average speed, 10.7 to 8.6 km/hour). Body mass of the runners decreased (despite considerable fluid intake during the marathon) from 70.2 ± 2.6 to 68.1 ± 2.7 kg [intrasubject comparisons, t (5) = 4.20, $P < .01$], and rectal temperature increased from 37.6° ± 0.15° to 39.1° ± 0.16°C [t (5) = 13.33, $P < .001$].

Environmental wet- and dry-bulb temperature ranges during the race were 10.2° to 19.2°C and 10.4° to 26.2°C, respectively, and wind velocity ranged from 0.2 to 3.5 m/sec.

The analysis of the sleep records showed no significant differences between the 2-week pre- and 2-week post-marathon nights; therefore, the mean of these two recordings is used as the baseline sleep values. The baseline values were similar to those obtained in normative studies of males of similar age (*16*).

Total sleep time increased significantly over control times on each of the four nights after the marathon [F(4, 20) = 21.3, $P < .05$] (Fig. 1). Wakefulness was greatest on the night of the marathon, perhaps because of muscle and blister pains; this result could explain why the longest sleep period occurs on the second night after the marathon. Subjective sleep ratings for the seven laboratory nights showed that four of the six subjects reported having slept best on the night 2.

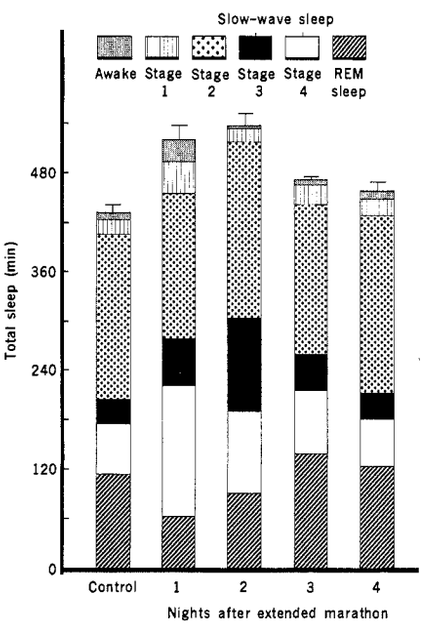

Fig. 1. Total sleep time (mean ± standard error of the mean) and mean number of minutes spent in each sleep stage on control nights, after the 92-km marathon, and on three subsequent nights.

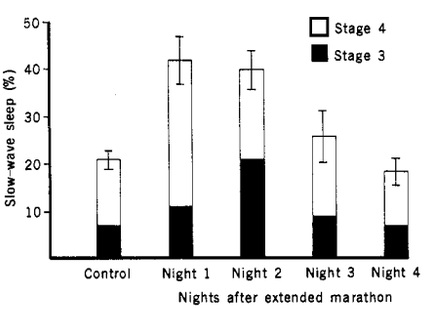

Fig. 2. Slow-wave sleep as a percentage of the total night's sleep on control nights, the night after the 92-km marathon, and on three subsequent nights.

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