ideal (~ 50 percent), it is a necessary step in isolating and quantifying extracellular metabolites from this marine dinoflagellate (*25*). Without concentration, iron(III)-specific chelators from marine eukaryotes may remain undetected, whereas these compounds from marine cyanobacteria can be measured (*7*). Whether or not marine eukaryotic phytoplankton produce less siderophore than do marine cyanobacteria is unclear. Although cyanobacteria have a higher specific requirement for iron (especially when fixing nitrogen) (*26*) than do marine eukaryotic phytoplankton, quantitative comparisons between the two groups cannot be made until further information is available on the production, excretion, and cellular uptake of siderophores.

Prorocentrin is the first siderophore to be isolated from a eukaryotic marine phytoplankter. Its discovery suggests that the iron uptake mechanism of dinoflagellates may closely parallel that of prokaryotic siderophore-producing organisms (*8, 18*). The production of a strong trace metal chelator by a red tide organism has many important ecological implications. Blue-green algae can effectively eliminate competing algal species in freshwater lakes by sequestering all the available iron as a siderophore complex (*5*), and there is some indication that exogenous siderophores can both positively and negatively affect the growth of marine phytoplankton (*6*). Strong trace metal chelators can also reduce the toxicity of cupric ions to sensitive marine phytoplankton species (*27*). It will be interesting to test whether this dinoflagellate siderophore is readily available to prokaryotic organisms.

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15 June 1982; revised 12 October 1982

Multiple Sclerosis: Distribution of T Cell Subsets Within Active Chronic Lesions

Abstract. *The distribution of T cells and T cell subsets was examined within the human central nervous system in active lesions from seven patients with chronic multiple sclerosis. The monoclonal antibodies anti-T11, anti-T4, and anti-T8 were used to detect total (whole) T cells, helper T cells, and suppressor-cytotoxic T cells, respectively, and a monoclonal antibody against human Ia was used for macrophages and B cells. Lesion progression was associated with large numbers of $T4^+$ cells at the lesion margin and these extended great distances into the adjacent normal-appearing white matter. $T8^+$ cells were most commonly concentrated around the lesion margin and displayed a preferential perivascular distribution. Within the lesion center, only a few T cells were found. Ia^+ macrophages were most numerous within the centers of active lesions and were always present in the adjacent normal white matter. The monoclonal antibodies to T cells did not cross-react with glial cells including oligodendrocytes. These results indicate that $T4^+$ cells are actively involved in lesion extension and Ia^+ cells, in demyelination.*

Evidence from several sources has implicated cell-mediated immunity in multiple sclerosis (MS), the paradigm of the human demyelinating diseases. For example, levels of circulating T cell subsets fluctuate according to disease activity (*1*), lymphocyte studies support sensitization against nervous tissue (*2*), and the lesion pathology is similar to that occurring during T cell–dependent autoimmune demyelination (*3*). However, with the exception of a few studies on humoral factors and plasma cells in MS (*4*), little is known about immunologic events at the level of the target organ, the central nervous system (CNS). Lacking in MS (and, indeed, in any CNS condition) are studies in situ on T cells and their subsets, since these cells have never been identified within human nervous tissue. The present study has demonstrated T cell involvement in the brains of patients with MS by means of monoclonal antibodies against whole T cells, T cell subsets, and Ia-positive cells (macrophages and B cells). In CNS tissue containing active chronic MS lesions, that is, chronically demyelinated, gliotic lesions displaying abundant inflammation at the margins, we found distinct variations in T cell distribution in the CNS, suggesting that certain T cell subsets predominate at different stages of lesion development.

Samples of CNS tissue were obtained at autopsy from seven patients with documented MS for 3 to 13 years. For control purposes, CNS tissue was taken from one case each of systemic lupus erythematosus (SLE), leukemia, and carcinoma of the lung. Seventy blocks of MS tissue containing grossly visible le-

sions sampled from paraventricular and subcortical white matter were embedded in Optimal Cooling Temperature Compound (Lab-Tek Products), frozen in a bath of acetone and dry ice, and cut into 10-μm sections on a cryostat. The sections were stained for routine histology, and 18 blocks were found to contain active chronic lesions. These were selected for the present study. To locate T cells and Ia^+ cells, we used a four-step peroxidase-antiperoxidase (PAP) technique (*5*) with some modifications. Sections were fixed for 8 minutes in acetone and then incubated consecutively with 3 percent H_2O_2 for 10 minutes and tris-saline containing 3 percent normal swine serum for 20 minutes. The T cells were then labeled with monoclonal antibodies as follows: total (whole or pan) T cells with anti-T11, helper (inducer) T cells with anti-T4, and suppressor-cytotoxic T cells with anti-T8. Ia^+ (antigen presenting) cells were localized by a monoclonal antibody to Ia (New England Nuclear). The monoclonal antibodies to the T cells were diluted $1:10^4$ and the antibody to Ia was diluted 1:50 in tris-saline containing 5 percent normal swine serum and applied for 90 minutes. The sections were then incubated with an immunoglobulin G (IgG) fraction of rabbit antiserum to mouse Ig, previously absorbed against human serum, for 50 minutes at a dilution of 1:20. Afterward, the sections were treated with swine antiserum to rabbit Ig (1:40) for 50 minutes and then with a purified complex consisting of peroxidase and rabbit antibody to peroxidase (rabbit PAP) (1:50) for 30 minutes.

Before being applied to CNS tissue, the monoclonal antibodies were tested with the above technique on sections of human lymph node tissue, and each was found to display the same staining patterns as described previously (*5*). Initial experiments on the control human CNS tissue revealed a lack of binding to nervous tissue elements. Staining of T cells was generally absent in control tissue except for the SLE specimens which contained isolated positively staining T cells throughout the gray and white matter parenchyma. Routine histology of active chronic MS lesions demonstrated well-demarcated edges and a gradient of inflammatory activity involving dense perivascular and parenchymal infiltrates (Fig. 1A). Myelin, axons, and lipid stains confirmed the presence of ongoing demyelination.

In more active (that is, intensely infiltrated) chronic MS plaques stained immunocytochemically with anti-T11, heavy infiltrates of T cells were present at the lesion edge (Fig. 1B) and, surprisingly, these penetrated deeply into the parenchyma of the adjacent white matter which appeared normal by routine histology. However, relatively small numbers of total T cells were detectable in the center of such lesions. Helper-inducer T cells ($T4^+$) occurred in similar numbers at the lesion edge (Fig. 1C) from which they also extended deeply into the adjacent white matter. Like total T cells, helper T cells were found less frequently within the lesion where their numbers decreased progressively toward the center, a region made up mainly of astrocytic scar tissue and a few macrophages. In contrast, suppressor-cytotoxic T cells ($T8^+$) were localized mainly at the lesion edge and in a narrow zone of adjacent, normal-appearing white matter; they were rare at a distance from the lesion edge. In the lesion center proper, only small numbers of $T8^+$ cells were present. In general, $T8^+$ cells showed some predilection for perivascular areas (Fig. 1D), more so than $T4^+$ cells. In active lesions large Ia^+ cells (probably all of which were macrophages by virtue of their large size) were numerous and predominated throughout the lesion center but decreased slightly toward the lesion edge (Fig. 1E). Some Ia^+ cells could always be detected in normal white matter and were sometimes seen in close contact with lymphocytes; a few of the small Ia^+ cells may have been activated T cells. Neural elements did not stain with the antibody to Ia.

In less active, chronic MS lesions displaying only a few inflammatory cells, T cell involvement was not as widespread as in more active plaques. Ia^+ cells were less frequent in the lesion center and accumulated at the lesion edge from which they penetrated deeply in large numbers into adjacent normal white matter. In long-established lesions, perivascular cuffs contained more macrophages than T cells, whereas more recent areas displayed larger numbers of T cells in perivascular infiltrates.

The present results are in agreement

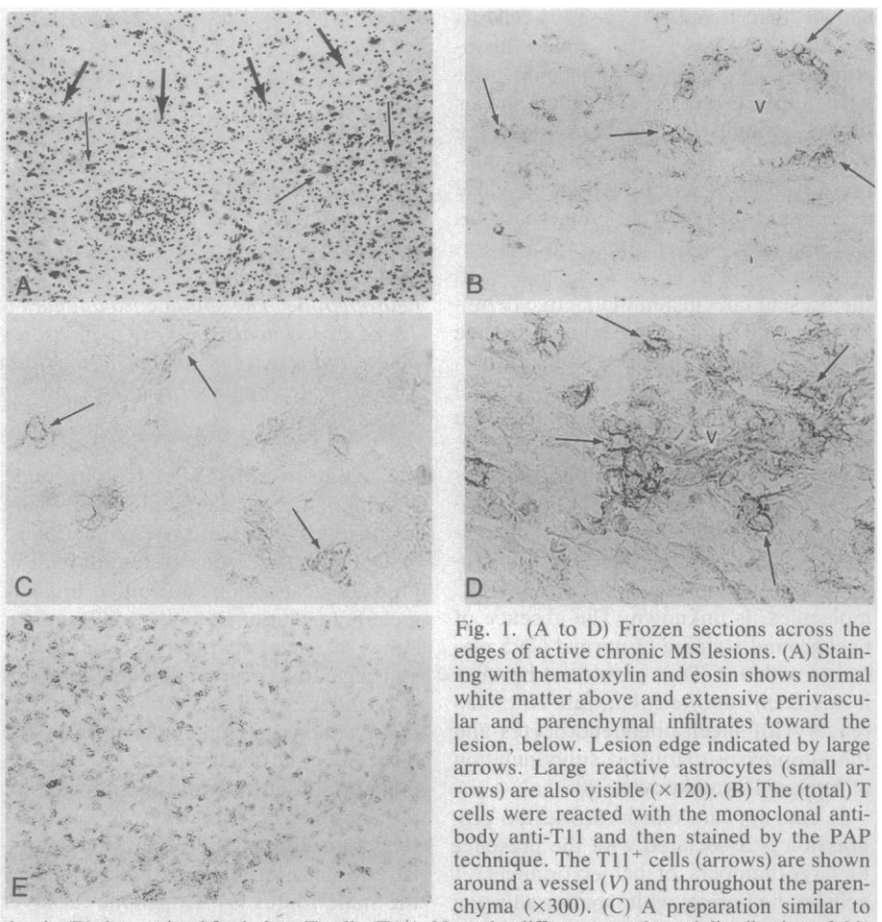

Fig. 1. (A to D) Frozen sections across the edges of active chronic MS lesions. (A) Staining with hematoxylin and eosin shows normal white matter above and extensive perivascular and parenchymal infiltrates toward the lesion, below. Lesion edge indicated by large arrows. Large reactive astrocytes (small arrows) are also visible (×120). (B) The (total) T cells were reacted with the monoclonal antibody anti-T11 and then stained by the PAP technique. The $T11^+$ cells (arrows) are shown around a vessel (*V*) and throughout the parenchyma (×300). (C) A preparation similar to that in (B), but stained for helper T cells ($T4^+$). Note the diffuse parenchymal distribution of this T cell subset (arrows) (×480). (D) The edge of an active lesion stained with anti-T8 (suppressor-cytotoxic T cells) showing the predominant perivascular distributon of this T cell subset (arrows). A vessel (*V*) is shown in the center (×480). (E) A section across the center of a lesion showing that the Ia^+ cells decrease in density from the lesion center (left) toward normal white matter (right) (×120).

with previous morphologic observations that lymphocytes occur less frequently in the center of MS lesions than in the adjacent normal white matter (*6*). However, more precise identification of these small mononuclear cells as T cells or T cell subsets has not previously been possible in the CNS in this or any other condition. With the aid of monoclonal antibodies, we have characterized further these infiltrating cells and have demonstrated that T cells, in particular $T11^+$ and $T4^+$ cells, are numerous at the lesion margin and in the normal-appearing white matter surrounding the active chronic MS plaque but occur only in small numbers within the lesion center.

This distribution of T cells and Ia^+ cells in the CNS of patients with MS suggests several possibilities of relevance to the pathogenesis of this disease. In addition to supporting the concept of MS being a T cell–mediated condition, the presence of dense infiltrates of $T4^+$ cells in nearby normal white matter might herald an early pathogenetic event. These cells could function to recruit macrophages or mediate cytotoxicity themselves. In this regard, it has been shown that a subset of $T4^+$ cells is cytotoxic for class II MHC (major histocompatibility complex) molecules (*7*). Also, the presence of Ia^+ macrophages in the normal white matter might be indicative of antigen presentation, supported perhaps by the observed close association between Ia^+ macrophages and lymphocytes. Furthermore, ultrastructural studies are in agreement with the present conclusion that active demyelination in MS depends on the presence of macrophages (*6*). The apparent restriction of $T8^+$ (suppressor-cytotoxic) cells to the edge of the lesion and to perivascular areas might reflect a difference in the mobility of helper and suppressor T cells. Whether these cells trigger suppression of autoreactivity or mediate another function, such as conventional cytotoxicity against class I MHC molecules, is unknown. The absence of background staining of normal CNS elements with anti-T8 is of interest because it has been reported that anti-T8 cross-reacts with sheep oligodendrocytes and the suppressor-cytotoxic subpopulation of human T lymphocytes (*8*).

It thus appears that in MS lesion progression is associated with the presence of large numbers of helper (inducer) T cells in the normal white matter adjacent to an existing lesion, whereas suppressor-cytotoxic T cells are limited to the lesion margin. However, demyelination seems to depend on the presence of Ia^+ macrophages. These observations might prove of relevance to future considerations on the pathogenesis of multiple sclerosis.

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* On leave of absence from the Neurologic Institute, Vienna, Austria.

12 October 1982

Evidence for the Neuropeptide Cholecystokinin as an Antagonist of Opiate Analgesia

Abstract. *The endogenous neuropeptide cholecystokinin, when administered systemically or perispinally, potently antagonizes opiate analgesia produced by foot shock and morphine. Nonopiate foot-shock analgesia is not reduced by this neuropeptide. The spinal cord appears to be a critical site of cholecystokinin action. These experiments suggest a physiological role for cholecystokinin as a specific opiate antagonist in analgesia-mediating systems. A similar mode of action may explain other behavioral effects of cholecystokinin, such as suppression of food intake.*

Cholecystokinin (CCK), a polypeptide existing in a variety of amino acid chain lengths, has been isolated from peripheral and central nervous system tissue (*1*). Physiological actions attributed to CCK include regulation of satiation (*2*), modulation of catecholaminergic activity (*3*), and regulation of hypothalamic peptides (*4*). Under certain conditions several of these actions are opposite those of the opiates. Food intake decreases after administration of CCK intraperitoneally or intracerebroventricularly (*2*), whereas food consumption increases after administration of morphine or β-endorphin by the same routes (*5*). Dopamine turnover is decreased by CCK (*3*) and increased by morphine in specific brain regions (*6*). Stress-induced feeding is mediated by endogenous opiates (*7*) and blocked by CCK (*8*). Also, genetically obese mice have elevated levels of β-endorphin in the brain (*9*) but reduced levels of CCK (*10*). We therefore hypothesized that CCK antagonizes endogenous opiates.

Since the endogenous opiates are best known for their analgesic properties, we tested our hypothesis by determining whether the sulfated octapeptide of CCK (CCK-8) blocks opiate-mediated analgesia produced by certain environmental stimuli. Watkins *et al.* (*11*) showed that analgesia can be produced by brief shock of the front or rear paws. Only shock of the front paws was found to produce opiate-mediated analgesia. They also demonstrated that opiate-mediated analgesia can be evoked by a classical conditioning paradigm in which brief foot shock is used as the unconditioned stimulus (*12*). We therefore used these paradigms to determine whether CCK-8 an-