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Autonomic Nerves in Experimental Allergic Neuritis in the Rat*,**

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Summary. After experimental allergic neuritis (EAN) was induced in 16 male Lewis rats with bovine peripheral myelin and adjuvants, peripheral nerves were examined morphologically at intervals of 12–21 days post inoculation (dpi). Signs of motor involvement were present in ten rats and were first elicited 12 dpi. They ranged from tail droop to complete lower limb paralysis. Autonomic nervous system (ANS) involvement was studied by contrasting morphological findings in the cervical sympathetic nerves (CSN), which are poorly myelinated and vagal nerves (VN) which contain numerous myelinated fibers in the endoneurium. Edema, perivenular infiltrates, and demyelination appeared in the VN of seven of nine neurologically affected rats, while the CSN showed edema and infiltrates in only one rat. ELISA assays were negative for anti-galactocerebroside antibody, and electron microscopy failed to show abnormalities of Schwann cells.

Key words: Galactocerebroside – Myelin – Schwann cells

Introduction

In recent years the specificity of immune phenomena occurring in peripheral nerve has received close attention, especially in experimental allergic neuritis (EAN). Earlier morphological studies of EAN [14, 31] showed that the myelin sheath is the principal target of infiltrating mononuclear cells which appear to displace Schwann cells during lysis and digestion of myelin. Except for minor reactive changes [9] the Schwann cell does not appear to be involved [14, 15, 19, 22, 31, 32]. In spite of this, the possibility of other antigens becoming targets of the immune process deserves consideration, particularly since the studies of Saida et al. [25] have shown that injection of anti-galactocerebroside antibodies into the endoneurium precipitates demyelination.

Galactocerebroside is a hapten, common to the myelin sheath and Schwann cells, which has been considered as a possible antigen in EAN [5, 6]. Electron microscopy of peripheral nerves within hours after intraneural injection of anti-galactocerebroside antibody has shown lysis of Schwann cells followed by demyelination [20, 24–26]. These observations in galactocerebroside neuritis prompted us to re-examine the status of Schwann cells during various phases of EAN induced by whole myelin.

Our experiment addressed the question of Schwann cell vs. myelin specificity in classical EAN by contrasting the histological appearance of cervical sympathetic nerves, which contain few myelinated fibers, with the densely myelinated vagus nerve. Autonomic involvement is common in severe cases of GBS [18] and has been demonstrated in EAN [28] so this approach was designed to ascertain whether the autonomic lesions were confined to the myelinated fiber population. We reasoned that if the principal immunologic target of allergic neuritis is the myelin sheath, one could expect significantly greater involvement in the vagus. However, involvement of both nerves would indicate that the Schwann cell is a subsidiary target. These morphological experiments were supplemented by immunologic assays of anti-galactocerebroside antibody activity using the ELISA assay.

Material and Methods

Male Lewis rats from the Charles River Labs., weighing 400–450 g, were used in this study. The rats were inoculated with an emulsion containing bovine peripheral myelin (which had been

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resuspended in normal saline, 33 mg/ml), emulsified with an equal volume of complete Freund's adjuvant containing purified Mycobacterium tuberculosis H37Ra (Difco) (5 mg/ml). Each rat was administered a total of 0.1 ml of inoculate intradermally (i.d.) into the footpad during ether anesthesia.

Animals were examined daily for weight loss and neurologic involvement, which was graded according to the following scale: 0 = normal, 1 = limp tail, 2 = weakness of hind limbs, 3 = severe weakness of hind limbs, and 4 = paralysis of hind limbs. Animals were anesthetized between day 12 and day 21, bled, and perfused with 2.5% phosphate-buffered glutaraldehyde. The cervical sympathetic chain and vagus nerve were removed, postfixed in osmium tetraoxide, dehydrated in alcohol, and embedded in Araldite. For light microscopy, 1-μm sections were stained with either paraphenylene diamine or methylene blue and fuchsin. About 40–50 sections of each nerve were examined. Ultrathin sections were cut from selected blocks for electron microscopy and stained with uranyl acetate and lead acetate.

In the serum of experimental animals IgG and IgM with galactocerebroside specificity were sought using an ELISA assay [16]. For this procedure 96-well PVC microtiter plates were saturated overnight with the antigen solution containing GC, lecithin, and cholesterol in ethanol at 4°C. They were then washed serially with 0.5% gelatin in phosphate-buffered saline (PBS) and 1% BSA in PBS. The sera was serially diluted from 1:20 to 1:640 with 1% BSA in PBS and incubated for 2 h at room temperature. The wells were rinsed as before, then incubated with peroxidase conjugated goat anti-rat IgM IgG for 2 h. The wells were then rinsed and subsequently developed with the peroxidase substrate containing hydrogen peroxide and ortho-phenylenediamine in PHS citrate buffer. The reaction was stopped with 2 M H_2SO_4. A blank of 1% BSA was utilized for each serially diluted serum. In addition, a positive control serum was utilized as well. The results were obtained with a micro ELIZA Reader.

Results (Table 1)

Nine of the 14 experimental rats developed neurologic abnormalities. Average time of onset was 12.8 dpi, and the extent of involvement ranged from limp tail to complete hind limb paralysis. All affected animals underwent weight loss (Table 1). Histological abnormalities were observed only in neurologically affected rats. The vagus was involved in seven such rats; five unilaterally in five and bilaterally in two. Involvement consisted of edema, perivenular lymphocytic infiltrates, lipid-laden macrophages, demyelinated axons, and possible remyelination (Figs. 1–4).. Electron microscopy did not reveal any abnormalities of Schwann cells. The perivenular infiltrates were composed of plasma cells, lymphocytes, mononuclear cells, and some occasional polymorphonuclear cells.

Although specimens of cervical sympathetic nerve from ten animals with histological evidence of EAN were sampled, only one nerve showed signs of inflammation. By light microscopy, subperineurial edema was present in a single block, and occasional polymorphs, macrophages, and plasma cells were seen by electron microscopy (Fig. 5). There was no evidence of demyelination, and all Schwann cells were intact. The remaining blocks of CSN from this rat appeared normal.

In comparing the vagus and cervical sympathetic nerves, allowance must be made for the much greater size of the vagus nerve. The average cross-sectional area of the vagus was 5.56 times greater than the CSN in these rats. Nevertheless, when the appropriate morphometric correction is made, the area of vagal damage remained 1.62 times greater than that of the single CSN lesion. Furthermore, single comparison of affected areas understates the severity of the changes in these two nerves. Qualitatively, the disease in the

Table 1. Results

	dpi	Clinical		Histology			
		Weight loss %	Neuro signs	Vagus	Cervical sympathetic nerve	Anti-GC serum[a]	
						IgG	IgM
Experiment	12	3	–	–	–	–	0
	12	3	–	–	–	–	0
	13	3	–	–	–	0	0
	13	17.3	2	+	+	0	0
	13	−1	–	–	–	0	0
	14	17	4	–	–	0	–
	14	17	3	+	–	0	0
	15	17	4	–	–	0	0
	17	28	4	+	–	–	–
	20	17.2	3	+	–	–	0
	20	34	4	+	–	–	–
	20	22	3	+	–	–	0
	21	−12.8	–	–	–	–	0
	21	18.5	2	+	–	–	–
Control	–	−5	–	–	–	0	0
	–	−8	–	–	–	0	0

dpi, days post inoculation

[a] A positive control with hyperimmune rabbit anti-GC serum was obtained

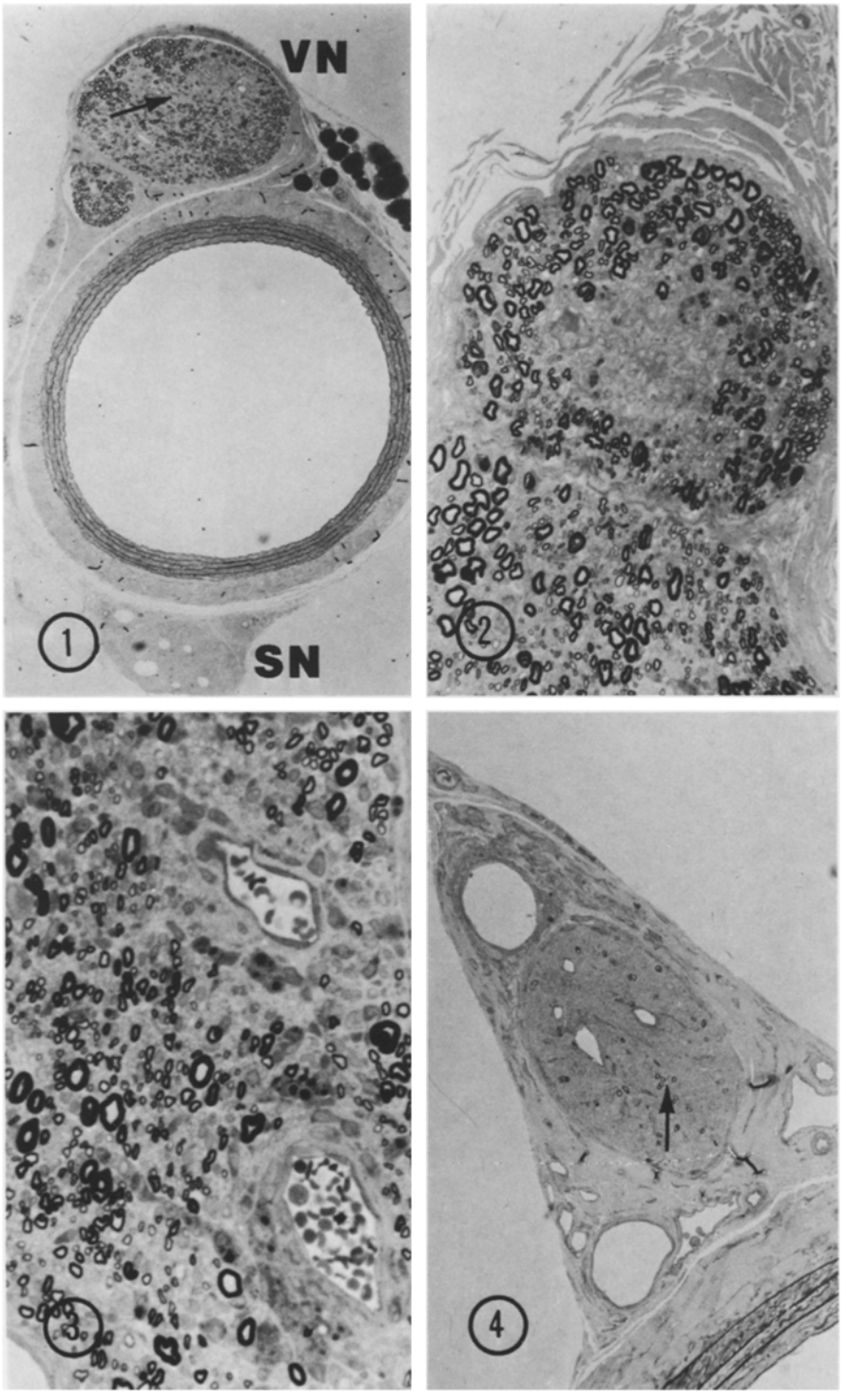

Fig. 1. EAN rat. Transverse section of the common carotid artery flanked by the vagus nerve (*VN*) and the cervical sympathetic (*SN*). Extensive demyelination (*arrow*) is present in the vagus. × 50

Fig. 2. Demyelination and cellular infiltrates in the vagus nerve in an EAN rat. × 200

Fig. 3. Perivenular infiltrates and demyelination in the vagus nerve. × 1,125

Fig. 4. Cervical sympathetic nerve is seen adjacent to the common carotid artery (*bottom left*). A few myelinated fibers (*arrow*), but no inflammation is seen in the nerve. × 200

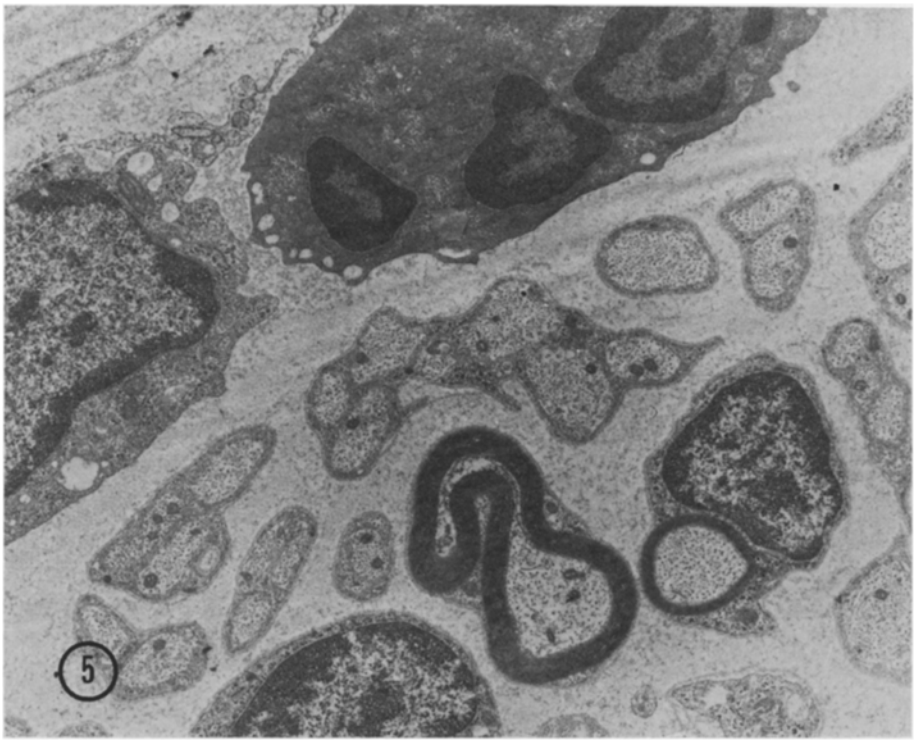

Fig. 5. Electron micrograph showing subperineurial inflammatory cells in a cervical sympathetic nerve. A macrophage (*left*) and a polymorphonuclear cell (*upper*) were identified but no demylination was seen. EAN rat

vagus nerve was much more severe histologically than the CSN.

No IgG or IgM anti-galactocerebroside activity was detected by the ELISA assay in our rats. A positive (1:320) control was obtained using hyperimmune rabbit anti-galactocerebroside serum.

Discussion

Autonomic nervous system involvement was prominent with inflammatory changes encountered in seven of nine affected rats. The significantly greater inflammation of the densely myelinated vagus compared with the sparsely myelinated CSN reinforces the view that the disease process of EAN has a proclivity for myelin. Furthermore, electron microscopy confirmed that the Schwann cell is not involved in either the vagus nerve or CSN. In only one rat did the CSN contain edema and occasional inflammatory cells.

These findings of myelin rather than Schwann cell involvement are in accord with previous observations in both EAN and Guillain Barre-syndrome (GBS) [2–4, 7–11, 13, 14, 20–22, 31, 32], which are consistent with a cell-mediated immune response against the myelin sheaths of peripheral nerves. In this experiment, a cell-mediated mechanism would explain the delayed onset 9–10 dpi, lymphocytic perivenular infiltrates in the infiltrate, and lack of anti-galactocerebroside activity in the serum.

A role for antibody in the pathogenesis of EAN has also been sought. Immunoglobulin as well as cells have been demonstrated in endoneurial edema fluid during early EAN in the rat [19]. However, it is not known at present whether specific antibodies are involved in demyelination in rat EAN. Direct intraneural injection of antisera to several myelin antigens has been carried out in non-sensitized rats [10, 24, 25, 27]. Demyelination can be elicited by injecting antibodies to surface antigens, such as galactocerebroside [25] and P_0 [10], while antibodies to myelin basic protein (MBP) and P_2 which are internally located within the myelin sheath fail to induce it. There is no doubt that cell-mediated immune responses to MBP and P_2 can be evoked with subsequent demyelination in EAE [13] and in EAN, respectively [9, 10]. Galactocerebroside (GC) is of particular interest because it is a glycolipid hapten common to Schwann cells and the myelin sheath [17] and antibody to GC has been demonstrated in experiments involving rabbits. Galactocerebroside neuritis in rabbits differs in many respects from ordinary EAN in the rat. Firstly, its time course is more protracted [24–27] and, secondly, the mechanism of demyelination involves a complement-dependent attack on Schwann cells. A similar attack on Schwann cells with demonstrable lysis of their cytoplasm can be induced by injection of a humoral factor obtained from rabbits sensitized against GC [25]. In contrast to ordinary

EAN, in which cell-mediated immunity gives rise to neuritis 9–10 dpi [9], direct injection of anti-galactocerebroside antibody causes edema and Schwann cell injury as little as 20 min after endoneurial injection [10, 20]. No lymphocytic perivenular infiltrates are observed [20, 25]. Rabbits are more apt to produce GC antibodies than rats. Normal rabbit sera are known to contain antibodies to glycolipids [23], and we have found that rabbits immunized with Freund's complete adjuvant alone produce complement-fixing antibodies to galC, sometimes in high titer [20]. Antibody to GC in Lewis rats with EAN was not detected in either the present study nor in previous experiments [7, 8, 10], nor has anti-GC been detected in Guillain-Barré syndrome [17].

Apart from these observations, ultrastructural studies provide further basis for distinction between ordinary EAN in the various experimental animals in which it has been induced. Uniform separation of myelin lamellae, also known as biphasic myelinopathy [21], is found in chickens with either EAN- or Marek's disease-induced neuritis [15] and occasionally occurs in rabbits with ordinary EAN [1], but has not been reported in the rat. This distinctive change which involved altered periodicity of myelin lamellae [12] has been suggested to signify antibody priming of myelin before lysis by macrophages [15]. Electron microscopy has also been reported to show macrophage activity within the neurilemma of unmyelinated fibers in rabbit EAN [1]. Damage to Schwann cells would not be unexpected in this model in view of the proclivity for anti-GC antibody production by these animals. Differences in the morphology of EAN are not confined to the experimental models mentioned above. A fourth species, the guinea pig, is subject to severe axonal degeneration in the course of ordinary EAN [28–30]. As many as 30% of teased nerve fibers showed axonal degeneration, while 42% underwent segmental demyelination in this model [28]. In summary, the pathology of EAN is quite diverse.

To date the mechanism of GBS is not understood, yet EAN remains the best model as judged by morphological criteria. By specifically addressing the relative involvement in the cervical sympathetic and vagus nerves, the effect on myelin can be examined separately; thus, the significantly lesser involvement of the cervical sympathetic confirms the myelin specificity of EAN.

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