Suppression of Experimental Allergic Neuritis with P_2 Protein of PNS Myelin

J. R. McDermott and A. B. Keith

Medical Research Council Demyelinating Diseases Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE

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Experimental allergic neuritis was induced in the guinea pig with bovine PNS myelin. Treatment with P_2 protein at the onset of the disease markedly reduced the severity of the clinical signs compared with untreated controls and animals treated with CNS myelin basic protein. The mortality was reduced from 94% in the combined control group to 50% in the treated group. Saline was a better vehicle for administration of the suppressive inoculation than incomplete Freund's adjuvant.

Introduction

Experimental allergic neuritis (EAN) is an autoimmune demyelinating diesease of the peripheral nervous system (PNS) which can be induced in a variety of species by sensitization with PNS tissue [1] or myelin [2] in Freund's complete adjuvant (FCA). It is recognized as the experimental counterpart of human idiopathic polyneuritis [3] (Guillain-Barré syndrome). In experimental allergic encephalomyelitis, the analogous CNS disease, the basic protein of the myelin sheath (MBP) is the causative antigen [4]. PNS myelin also contains MBP (referred to as P_1 protein in the PNS) although its encephalitogenic activity is only normally expressed when it is isolated from the myelin [5]. The two major PNS myelin-specific proteins are P_0, a glycoprotein of m.w. 28,000, and P_2, a basic protein of m.w. 13,000 [5]. Both P_0 and P_2 have been implicated in the pathology of EAN by the finding of delayed-type hypersensitivity to these two proteins (but not to MBP) in affected guinea pigs [6]. Mononuclear cells which bind P_2 protein have also been found in the lymph nodes of rabbits with EAN [7]. Recently, EAN has been induced in rats with isolated P_2 protein [8].

EAE can be suppressed by repeated injections of MBP in the period following the first development of clinical signs of the disease [9]. By analogy, therefore, it should be possible to suppress EAN with the neuritogenic component of PNS myelin. Here we show that injection of P_2 protein at first clinical signs significantly alters the course of EAN in the guinea pig.

Reprint requests to Dr. J. R. McDermott, MRC Demyelinating Diseases Unit, Newcastle General Hospital, Westgate Road, Newcastle upon Tyne NE4 6BE, U.K.

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Materials and Methods

PNS myelin was isolated from bovine intradural roots [10]. P_2 was isolated from 50 mg of an acid extract (0.1*N* HCl) of delipidated (ether:ethanol, 3:2) bovine intradural root myelin by chromatography on G75 Sephadex (2.5 × 69 cm) in 0.1 *M* acetic acid. The P_2 peak at 230–278 ml was collected and rechromatographed under the same conditions. The purity of the freeze dried product was assessed by polyacrylamide gel electrophoresis [10]. MBP was isolated from bovine brain myelin [11]. EAN was induced by injection of bovine intradural root myelin (1 mg) in an emulsion of saline (0.1 ml) and FCA (0.1 ml; containing 0.5 mg H37 Ra) into the hind feet of Hartley guinea pigs (300–400 g). Treatment was started when there was a loss in weight accompanied by the first signs of mild paraparesis (11–16 days post sensitisation).

A solution or suspension of P_2 (5 mg), MBP (5 mg) or PNS myelin (50 mg) in saline (5 ml) was emulsified with incomplete Freund's adjuvant (5 ml). 0.1 ml of the emulsion was injected over the nuchal region, and on the ten following days.

Clinical signs were scored on the following scale: 1. weight loss, mild paraparesis; 2. paraparesis (with characteristic "waddling" gait); 3. as 2 with respiratory distress and/or quadriparesis; 4. severe paraparesis/quadriparesis and respiratory distress with up to 45% loss in weight; 5. death or killed when moribund. Animals were killed when moribund or at 6–10 weeks by exsanguination under ether anaesthesia. Sciatic nerves and spinal cord with spinal roots and dorsal root ganglia (L7 and S1) were removed and fixed in 10% formalin for routine H and E histology.

Results and Discussion

The disease was uniformly fatal in untreated contros after a 6–16 day progressive deterioration (Table). Perivascular infiltration of mononuclear cells

Table. Suppression of EAN in guinea pigs challenged with bovine PNS myelin.

Therapy [a]	Mean day of onset	No. improved after 5 days therapy	Clinical Score		Mean day of death
			End of therapy	End of experiment	
a) None	13.1	–	3,3,3,4,4,5,5,5	5,5,5,5,5,5,5,5	25
b) Saline/IFA	14.6	0/5	2,3,4,5,5	0,5,5,5,5	27
c) MBP/IFA	14.5	1/4	2,3,4,5	5,5,5,5	29
d) P_2/IFA	14.0	4/5	0,0,0,2,2	1,2,5,5,5	29
e) P_2/IFA [c]	14.8	3/5	0,0,1,2,5	0,0,5,5,5	36
f) P_2/saline [c, b]	14.7	5/6	0,0,0,0,1,5	0,0,0,0,5,5	28
g) PNS myelin/IFA	13.8	3/5	0,1,1,1,2	2,3,5,5,5	32

[a] Commencing at onset of clinical signs and given over eleven days.
[b] P_2 (5 mg) dissolved in saline (10 ml) without IFA.
[c] 0.5 ml given on day 1.

and demyelination were seen on histological examination of the sciatic nerve and spinal roots (L 7, S 1). There was little or no overlap of lesions into the CNS.

None of the animals receiving saline and only one receiving MBP showed any clinical improvement over the first five days of treatment. The overall course of disease in these two groups was no different from the untreated control group. In contrast, a definite clinical improvement occurred in the majority of the animals treated with P_2 or PNS myelin. At the end of the treatment period, all four groups (d, e, f, and g) had a significantly ($p \leqq 0.01$; Kolmogorov-Smirnov test) lower clinical score than the control group (a). After therapy was withdrawn, several animals suffered a relapse and followed the normal progressive course to death. However, the percentage mortality in the combined P_2-treated animals (50%) was lower than in the combined (a,b,c) control group (94%). The most effective suppression was obtained with P_2 in saline, possibly because here the protein is more readily accessible to the circulating immune system than it is in myelin or in an oily emulsion. Morphological studies are being carried out to assess the degree of residual damage and remyelination in the PNS of the recovered animals.

The mechanism of the suppressive effect of MBP on EAE is thought to involve the interaction of lymphocytes with the injected MBP. Thus it was shown that the passive transfer of EAE with lymph node cells could be blocked by treating the donor animal with MBP [12]. It is not known whether this is simply a "defusing" of MBP-sensitized lymphocytes or whether a more complex process is involved, for example the induction of antigen-specific suppressor T cells [13].

The suppressive effect of P_2 on EAN implies that this protein is involved in the pathogenesis of EAN, even though EAN cannot be induced in guinea pigs with P_2 in FCA (McDermott, Keith, Madrid unpublished). This interpretation must be made with caution however since successful suppression of EAE has also been achieved with the non-encephalitogenic co-polymer, Cop-1 [14]. It is possible that more than one PNS myelin component is involved in the induction of EAN. More likely though, the isolation of P_2 from its lipid environment in myelin changes its antigenic properties and lessens its neuritogenicity. There is also evidence that isolated P_2 protein regains its neuritogenicity when recombined with gangliosides from peripheral nerve prior to injection [15]. However, the fact that P_2 will suppress EAN indicates that the isolated protein is still capable of interacting with lymphocytes sensitized to myelin-bound P_2.

This work may have relevance to the treatment of Guillain-Barré syndrome, since sensitivity of lymphocytes to P_2 has been demonstrated in patients with this disease [16]. The fatal form of EAN used in this study is perhaps not the best model for assessment of potential therapy since the human disease has a mortality of around 10–20%. By using lower doses of challenging antigen a more appropriate model could be produced. From the present study, P_2 in saline would seem to offer the best prospects for further therapeutic experiments.

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