EXPERIMENTAL ALLERGIC ENCEPHALOMYELITIS

Identification of the myelin component responsible for the appearance of brain esterified cholesterol

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1. Introduction

Animals with experimental allergic encephalomyelitis (EAE), an autoimmune demyelinating disease induced by injection of whole central nervous system (CNS) homogenate, show preferential changes of the glycosphingolipid (cerebroside and sulphatide) content and increased amounts of esterified cholesterol in the CNS [1,2]. By studying the CNS lipid composition of animals injected with purified constituents of myelin we were able to identify the CNS components which separately induced some of the above alterations. Thus, the encephalitogenic basic protein of myelin induced the classical neurological symptoms of EAE [3] together with alterations in the content of CNS sulphatides but no change in the content of cerebrosides or esterified cholesterol [2,4]. Changes in the level of cerebrosides were only induced when animals were sensitized with mixtures containing CNS lipids [2,4]. Also, since lipid alterations were induced in animals injected with non-encephalitogenic preparations a dissociation between paralysis and lipid changes was established [4]. However, none of the purified CNS components so far injected were able to induce the appearance of increased amounts of esterified cholesterol which, apart from occurring in EAE, is also a characteristic alteration of several human demyelinating diseases [5]. We will present here evidence indicating that the purified apoprotein of Folch's proteolipid can induce alterations in the level of esterified cholesterol. In addition, a hypothesis that explains the different lipid alterations on the basis of disturbed molecular interactions in the myelin membrane is proposed.

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2. Materials and methods

Rats 50–60 day old were used. Freund's complete adjuvant was prepared with lanolin in paraffin oil (10%, w/v) containing 2 mg/ml of heat-killed *Mycobacterium tuberculosis* (BCG Döerr, Bs. As., Argentina). Crude myelin was prepared from white matter of beef brain as in [6] and lyophilized. Purified apoprotein of Folch's proteolipid was obtained from bovine brain white matter as in [7] and rendered soluble in water [8]. Preparation of myelin basic protein from bovine spinal cord, CNS lipids, and quantitative determinations have been described [2]. The identification of the cholesterol moiety of the sterol esters by gas–liquid chromatography (GLC) was done essentially as reported [1]. Details of the injection procedures are given under table 1. All animals were killed by decapitation when neurological signs of EAE were present in animals injected with encephalitogenic preparations (10–13 days after injections).

3. Results and discussion

Animals sensitized with apoprotein of Folch's proteolipid which showed no paralytic symptoms had an increased amount of esterified cholesterol in brain (fig.1); after saponification, the sterol portion of the ester was further identified as cholesterol by both thin-layer chromatography (TLC) and GLC. The injection of CNS white matter homogenates or a myelin preparation, both of which contain the Folch's proteolipid as a constituent, also induced a similar increase. The amount of sterol esters in normal adult rat brain is below 0.1% of the free cholesterol fraction [9] and the increase observed in animals sensitized with the apoprotein of Folch's proteolipid amounted approximately to thirty times that quantity, as estimated by GLC by comparison with a known amount of standard. Besides, the injection of the purified apoprotein induced a significant alteration in the level of brain total cholesterol and sulphatides (table 1). We do not have a clear explanation at present of the cholesterol decrease induced by the Folch's apoprotein, which was not observed in the spinal cord. The metabolism of cholesterol in the CNS is not thoroughly known, however, lithocholic acid has been found in the brain of EAE animals and it has been proposed that cholesterol degradation could have been the source of this metabolite which is rapidly expelled from the brain [10]. As found in guinea pigs [2], neither purified myelin basic protein nor a mixture of it with CNS lipids induced the appearance of detectable amounts of esterified cholesterol and the changes for sulphatides were similar to those reported [2,4]. Our data have been obtained analyzing the whole brain; nevertheless, the changes described will be interpreted on the basis of a modified myelin membrane structure since both, the altered lipids and the purified components injected are major constituents of myelin [3,8,11].

The changes in the lipid content induced by the purified apoprotein of Folch's proteolipid give further

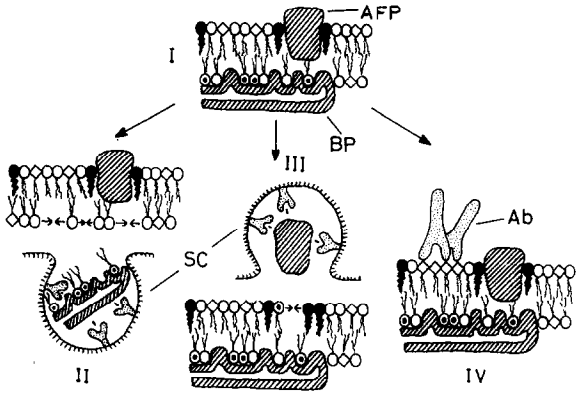

Fig.2. Possible mechanisms leading to selective lipid alterations in EAE. (I) Simplified structure of part of a normal myelin membrane [13]. The myelin basic protein (BP) and apoprotein of Folch's proteolipid (AFP) are shown interacting preferentially with sulphatides (○) and with cholesterol (●), respectively. (II) Modified membrane after interaction with a sensitized cell (SC) removing BP with associated sulphatides. (III) Only AFP is being removed by the SC leaving cholesterol available for alteration. (IV) Interactions of cerebrosides (◇) with other membrane components have been disturbed by a humoral antibody (Ab).

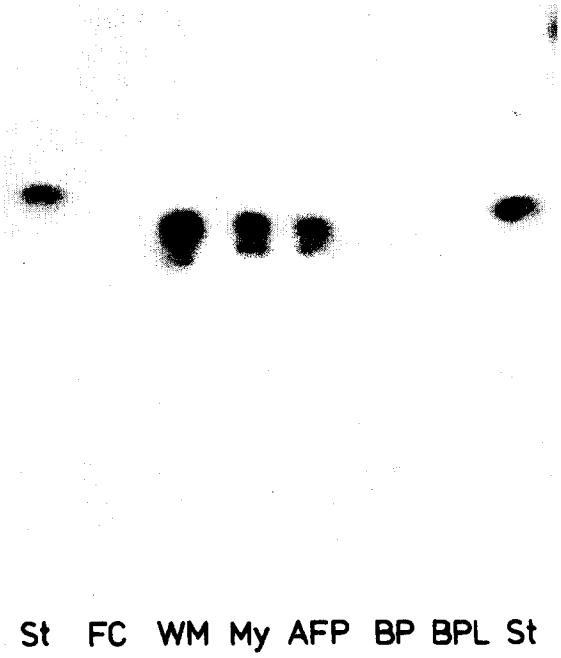

Fig.1. TLC of brain cholesterol esters. Esterified cholesterol was purified from a lower phase aliquot of Folch's partition, corresponding to 0.1 g tissue as in [23]. After running with heptane–ethyl ether (24 : 1) spots were visualized with Liebermann-Bouchard reagent. St, standard of cholesterol oleate. FC, WM, My, AFP, BP or BPL represent materials from animals of the different groups defined in table 1. A similar pattern was found for spinal cord.

Table 1
Brain lipid content of rats sensitized to different CNS components

Animals injected with			Sulphatides (mg/g w.w.)	Total cholesterol (mg/g w.w.)	Total phospholipids (μmol lipid P/g w.w.)
Freund's complete adjuvant	(FC)	(8)	4.26 ± 0.24	22.9 ± 0.9	70.3 ± 2.2
	Percentage differences with respect to animals FC				
White matter	(WM)	(7)	−43.9[a]	7.4	10.7
Myelin	(My)	(6)	−36.3[b]	1.3	− 3.3
Apoprotein of Folch's proteolipid	(AFP)	(5)	−37.8[a]	−18.8[c]	− 3.3
Myelin basic protein	(BP)	(6)	−29.8[b]	3.9	1.7
Myelin basic protein plus lipids	(BPL)	(5)	−39.9[a]	11.8	4.1

[a] Significant at $P < 0.001$
[b] Significant at $P < 0.01$
[c] Significant at $P < 0.02$

P was calculated by Student's t test for non-correlated samples

Results from animals FC are mean values ± SEM, the number of animals in each group is given in brackets. Rats were injected intradermally in each hind foot with: animals FC, 0.25 ml Freund's complete adjuvant emulsified with 10% water. Animals WM, same as FC but including 35 mg homogenized bovine white matter. Animals My, AFP and BP, same as FC but including 4.2 mg lyophilized bovine myelin, 0.2 mg apoprotein Folch's proteolipid or 0.2 mg myelin basic protein, respectively. Animals BPL, same as BP plus 0.16 mg of sulphatides, 0.7 mg of cerebrosides, 0.85 mg of cholesterol and 2.5 μmoles of dipalmitoylphosphatidylcholine. The preparations were sonicated for 3 min at 20 kHz in a Branson sonifier before injection. Similar results to those in brain were obtained in spinal cord except that total cholesterol in animals AFP was not significantly altered

support to a previous interpretation [4] in which we suggested that the alterations of brain lipids in EAE may be the consequence of independent and specific disruptive responses directed towards some membrane constituents, mediated by immunological agents. These facts can probably be better understood at present in view of the recent advances in the knowledge of the dynamic structure of membranes [12], of the particular interactions occurring between lipids and proteins of myelin [13] and of the modifications of cell surface interactions by immunological agents [14].

The alterations of the lipid content in our animals after the injection of purified myelin constituents appear to be closely related to the behaviour of myelin components in model membranes. Thus, the significant interactions between sulphatides and myelin basic protein or the apoprotein of Folch's proteolipid described in lipid monolayers [15] may correlate to the changes we observed in the CNS sulphatide content of animals injected with those purified proteins. Similarly, the increased levels of esterified cholesterol and the altered CNS cholesterol content induced by the apoprotein of Folch's proteolipid can be related to the preferential interactions between this protein and cholesterol in monolayers [15]. It is possible that cellular or humoral immunological agents originated in response to protein [16] and lipid haptens [17] that are myelin constituents, known to occur in EAE animals, may modify the normal interactions between the membrane components. Surface immunoglobulins of limphoid cells [14] as well as membrane components of myelin [18] can undergo topographical rearrangements

involving patching phenomena which seem to be a general mechanism leading to selective removal of certain membrane molecules [14].

Figure 2 represents an interpretation of the results described in EAE. Alternative mechanisms that would result in selective lipid alterations are shown. One possibility is the concomitant removal of the target (i.e., the basic protein) and associated component by a sensitized cell (fig.2,II). However, the loosening of normal lipid–protein associations brought about by removal of protein molecules (i.e., the apoprotein of Folch's proteolipid) by the perturbing immunological agent might also lead to lipid alterations (fig.2,III). In the case of brain cerebroside alterations induced by injection of CNS lipids [4] the disturbing molecule could be an antibody directed against this lipid hapten [19] (fig.2,IV). The lipid molecules thus loosened from specific interactions could be made available to enzymatic activities which, because of a changed environment in the membrane, might be less restrained in the damaged tissue. Mechanisms of the type proposed would lead to quantitative alterations in the lipid content without requiring an independent activation of several enzymes in response to each of the different components injected. In this connection it is to be noted that the activity of arylsulphatase A, an enzyme involved in the metabolism of sulphatides, was not increased in the brain of animals with EAE in a magnitude that could be correlated to the changes in the content of this lipid [20]. Esterified cholesterol and glycosphingolipid changes similar to those described in EAE occur in a variety of human demyelinating diseases [5,21]. Since cellular or humoral immunological agents against myelin components occur in both EAE [16,17] and multiple sclerosis [22], a mechanism of the type discussed above might also be applied to some of the human disorders.

Acknowledgements

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