Low-molecular-weight enzyme inhibitors suppress the development of experimental allergic encephalomyelitis[1]

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Summary. We tested the activity of low-molecular-weight enzyme inhibitors with immunomodifying actions on the suppression of experimental allergic encephalomyelitis (EAE). Of the agents tested the inhibitors of alkaline phosphatase, aminopeptidase B and esterase gave significant protection against the clinical expression of EAE in guinea pigs.
Key words. Enzyme inhibitors; experimental allergic encephalomyelitis; cell surface enzymes.

Of the low-molecular-weight enzyme inhibitors discovered in our institute, those acting against the enzymes located on cellular surfaces, such as aminopeptidases, esterase and alkaline phosphatase proved to have immunomodifying effects[2]. One of the alkaline phosphatase inhibitors, levamisole, is known to have favorable effects in patients with autoimmune diseases in spite of its immunopotentiating action[3]. Thus it seemed worthwhile to test the effects of immunomodifiers on the development of experimental allergic encephalomyelitis (EAE), which is known to be a useful model for studies of cell-mediated autoallergic immune diseases. The inhibitors tested in the present study are those with immunomodifying actions[2,4,5].

Materials and methods. Outbred male Hartley guinea pigs, weighing 300–350 g, were used throughout this study. Myelin basic protein (MBP) was prepared from the white matter of bovine brain by the modification of Eylar's method described previously[6]. The product caused 100% incidence of EAE in guinea pigs at a dose of 10 μg in Freund's complete adjuvant (FCA). In this study 30 μg of MBP were used for the immunization of each guinea pig. Briefly, aqueous solutions of MBP

Figure 1. Microscopical findings showing the normal structure of hindlimb muscle in a control guinea pig (× 90).

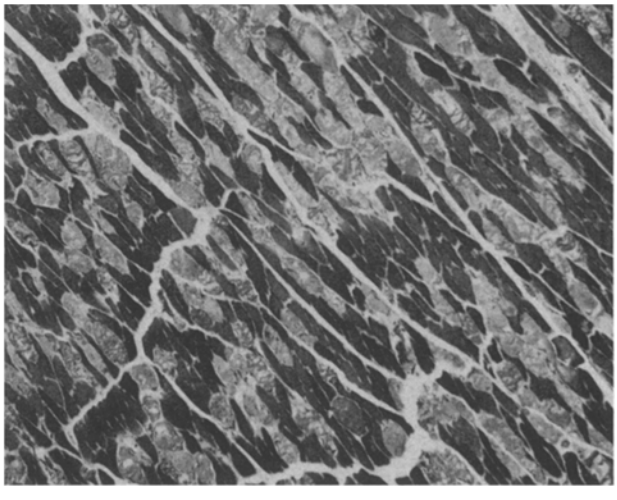

Figure 2. Severe structural damages in hindlimb muscle of a guinea pig afflicted with EAE (× 90).

The effects of various inhibitors on survival of the animals injected with the EAE antigen

Inhibitors	Dose (μg/guinea pig)	No. of animals	Average day of onset	Mean day of death	Survivors (35 days after immunization)	Statistical significance
None	–	10	11.5	13.3	0/10	
PBS	–	10	14.0	16.3	0/10	
Arphamenine B	500	4	13.0	14.8	0/4	NS
	50	5	13.6	> 19.8	1/5	NS
	10	5	14.5	> 27.0	3/5	$p < 0.02$
Amastatin	500	5	15.0	> 22.0	1/5	NS
	50	5	15.0	> 24.2	2/5	NS ($p < 0.1$)
Forphenicine	500	5	16.0	> 31.4	4/5	$p < 0.01$
	50	5	16.0	> 26.6	2/5	NS ($p < 0.1$)
Forphenicinol	500	5	15.3	> 24.6	2/5	NS ($p < 0.1$)
	50	5	15.4	> 22.4	1/5	NS
Esterastin	1000	5	17.0	> 23.6	1/5	NS
	100	5	14.8	> 28.0	3/5	$p < 0.02$
Ebelactone A	1000	5	13.4	> 25.2	2/5	NS ($p < 0.1$)
	100	5	14.9	> 23.6	1/5	NS

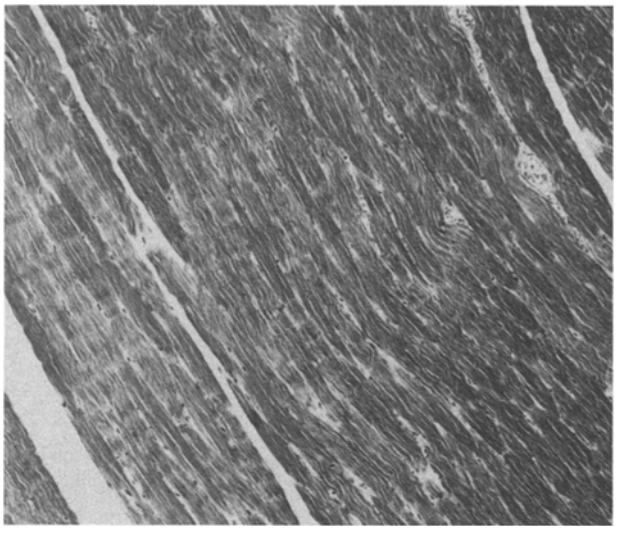

Figure 3. Microscopical findings of hindlimb muscle in a forphenicine-treated guinea pig. Structura damage is suppressed, but some irregularities of the lining of muscular fibers are seen to remain when compared with the control specimen (× 90).

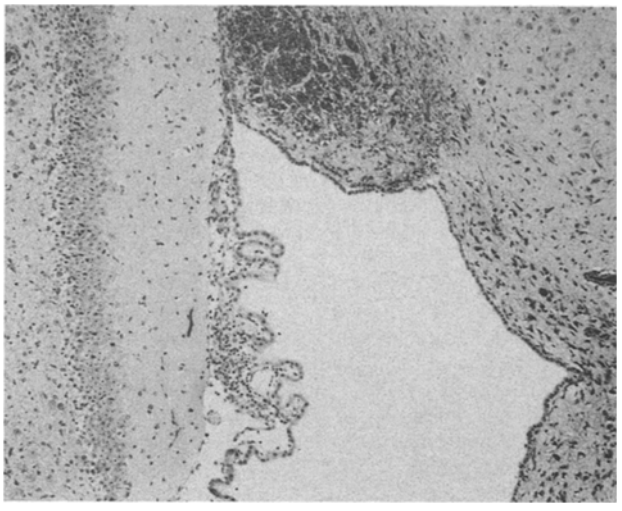

Figure 5. The tissues around the cerebral ventricle of an EAE animal *not* treated by enzyme inhibitors. The dark areas representing the tissue degeneration include many demyelinated nerves (× 75).

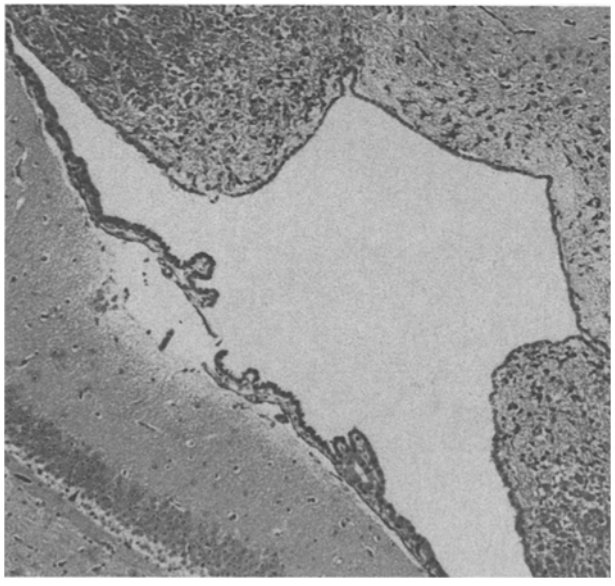

Figure 4. Cerebral tissue around the cerebral ventricle of a control animal (× 75).

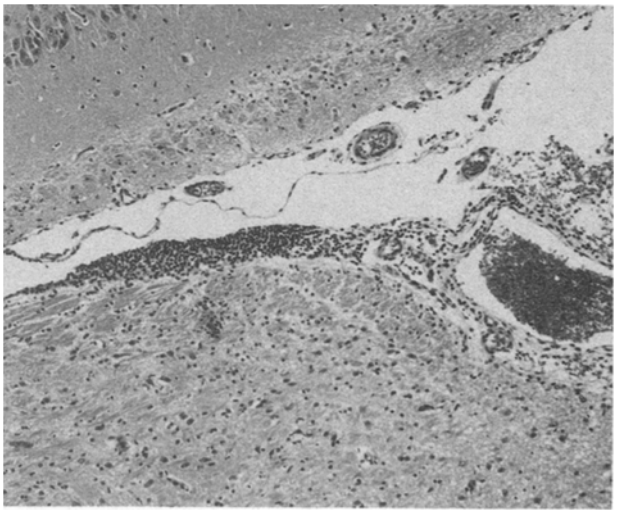

Figure 6. Tissues around the cerebral ventricle in the animal treated with forphenicine. Although mononuclear cells have infiltrated in the meningeal area, the destructive changes in the nervous tissues were very slight (× 75).

and of mycobacteria (0.25 ml each) were emulsified in a syringe with 0.1 ml of Arlacel A and 0.4 ml of Bayol F. Aliquots of 0.1 ml were injected into the foot pads of both hind legs of guinea pigs. Heat-killed *Mycobacterium tuberculosis*, virulent Aoyama B strain, suspended in Arlacel A was used for the preparation of FCA so that each 0.1 ml of MBP-FCA emulsion contained 100 µg of mycobacteria. Starting on the day of immunization, enzyme inhibitors were injected i.p. once daily[7]. Preliminary studies confirmed that the inhibitors chosen have no significant toxicity for the animals within the dose range adopted in the present study.

Results. As can be seen in the table, all of the 20 untreated guinea pigs and those treated only with phosphate buffered saline (PBS) died within 3 weeks. However, of the 64 animals treated with one of the inhibitors, 23 (35.9%) survived. A statistically significant difference was found ($p < 0.02$) between the two groups. The most effective inhibitor used was forphenicine[8]. At a dose of 500 µg/day, four of the five animals treated ($p < 0.01$) were saved. However, this agent was less effective at a dose of 50 µg/day, saving only two out of the five animals ($p < 0.1$). Also, arphamenine B[9], at a dose of 10 µg/day and esterastin[10] at a dose of 100 µg/day each saved three out of the five animals ($p < 0.02$). Amastatin[11], at a dose of 50 µg/day, ebelactone A[12] at 1000 µg/day, and forphenicinol[13] at 500 µg/day each saved two out of the five animals treated, but their effects were not statistically significant ($p < 0.1$).

Pathological findings also confirmed the efficacy of the inhibitors. Figures 1–3 demonstrate the findings in hindlimb muscle of the control animals, the untreated diseased animals and the diseased animals treated with forphenicine at a dose of 500 µg/day. It is evident that the muscular structures were severely damaged by the disease processes and that the development of

the pathological changes was suppressed by the effects of forphenicine. Also, the findings in cerebral tissues were compatible with these results (figs 4–6). Namely, the demyelination of the central nerves occurred in the diseased animals but not in the forphenicine-treated ones, although meningeal infiltration of mononuclear cells persisted in some of the latter animals. The findings in the animals saved by the other inhibitors were similar to these.

Discussion. Previously, Brosnan et al. reported that inhibitors of plasminogen activators and other neutral proteinases gave significant protection against the clinical expression of EAE in Lewis rats[14]. The present study, using other inhibitors, clearly demonstrated their suppressive effects on pathological changes in the central nervous tissues and muscles. Our previous studies suggested that this disease model presents unexpectedly extensive changes involving multiple organs[7]. Accordingly, the beneficial effects of these inhibitors may have a broader significance, encompassing even systemic autoimmune diseases. The effects of the inhibitors against alkaline phosphatase, aminopeptidase B and esterase were remarkable.

Judging from our observations, as well as those of others, it seems difficult to relate the pathogenic mechanisms of this disease to any specific enzymes. It seems more reasonable to explain the effects of these inhibitors by their actions on immune-responsive cells. In this respect, it is noteworthy that 500 μg of forphenicine saved 80% of the experimental animals. This agent resembles levamisole[15] in that it is an inhibitor of alkaline phosphatase. Also, both of these substances are known to be immunopotentiators rather than immunosuppressors. The reason why the immunopotentiators favorably affect the autoallergic state is not known yet. If autoimmunity were a manifestation of immunodeficiency it would not be surprising if drugs stimulating immune responsiveness produced improvement in autoimmune diseases[3]. These paradoxical phenomena concerning the so-called immunomodifiers should be reanalyzed in the light of an improved understanding of the antigens, the cellular interactions and the suppressor cell system[16]. Another question arising from this work is that of the optimal dosage of each enzyme inhibitor. Since it is possible that some of the immunomodifiers have dual effects depending on the doses administered, a more detailed study should be done to decide their optimum dosage precisely. Such a study, using the most promising agent, is being planned now.

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Chemotactic and random movement of cord-blood granulocytes

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Summary. Chemotactic responsiveness and random movement of cord-blood granulocytes were studied with a modified Boyden's method. Cord-blood granulocytes were less active chemotactically than granulocytes from healthy children and adults, whereas the random filter movement of the cells from all three sources was about the same.

In cord sera, concentrations of cell directed chemotaxis inhibitors were equal to those in sera from other age groups. Compared with the situation in healthy children and adults, the generation of chemotactic factors in cord-blood sera was impaired. This impairment was not related to an increased activity of chemotactic factor inactivators.

Measurement of the cyclic nucleotide levels in granulocytes from cord-blood and from children belonging to various age groups revealed that the cord granulocytes have significantly lower concentrations of cAMP and cGMP, which could have been responsible for the decreased chemotactic responsiveness.

Key words. Granulocytes, cord-blood; random movement; chemotactic responsiveness; chemotaxis inhibitors; cyclic nucleotide levels.

Introduction. Rapid migration of neutrophils into tissues invaded by bacteria is essential for host defense. An association between depressed neutrophil chemotaxis and susceptibility to infections in patients with recurrent bacterial infection has been established[1–5]. Chemotactic disorders can be divided into cellular and humoral defects, and can be caused by specific