Lymphocyte Stimulation by Acetylcholine Receptor in Polymyositis

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Summary. Lymphocytes of twenty-seven patients with polymyositis were incubated in vitro with cholinergic receptor rich membranes obtained from the electric organs of Torpedo Marmorata. Lymphocytes of polymyositis patients were slightly stimulated; positive responses were present mainly in patients affected from more than a year. Sensitization against the nicotinic cholinergic receptor may explain the occurrence of the myasthenic syndrome with polymyositis.

Key words: Polymyositis – Myasthenia gravis – Acetylcholine receptor – Lymphocyte stimulation.

Zusammenfassung. Lymphocyten von 27 Patienten mit Polymyositis wurden in vitro zusammen mit Membranen des elektrischen Organes von Torpedo Marmorata, die reich an Anteilen von Acetylcholinrezeptoren waren, inkubi-

iert. Die Lymphocyten von Polymyositispatienten erwiesen sich als leicht stimuliert. Positive Ergebnisse waren vor allem in jenen Fällen festzustellen, bei welchen die Patienten mehr als 1 Jahr schon erkrankt waren. Die Über-

empfindlichkeit gegenüber den Acetylcholinrezeptoren mag für das Vor-

handensein myasthenischer Syndrome in Fällen von Polymyositis verantwort-

lich sein.

Introduction

An experimental disease similar to human myasthenia gravis can be induced in animals by immunization with the nicotinic cholinergic receptor (AChR) obtained from the electric organs of fish (Patrick et al., 1973; Sugiyama et al., 1973; Tarrab-Hazdai, 1975; Heilbronn et al., 1975; Lindstrom et al., 1976). However the

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muscles of the animals affected are heavily infiltrated by macrophages and lymphocytes, and phagocytosis and some fiber atrophy occur, lesions not frequently observed in the human disease (Clementi et al., 1976; Schwartz et al., 1976). It has been proposed that this experimental disease can be considered an allergic polymyositis because of the histological similarities to human polymyositis (Schwartz et al., 1976). On the other hand, some electromyographic features of polymyositis indicate a certain degree of denervation and fatigue, and mixed forms, in which polymyositic and myasthenic symptoms overlap, have also been described (Namba et al., 1971; Oosterhuis et al., 1973; De Reuck et al., 1976). Sensitization of lymphocytes to cholinergic receptor has recently been reported in myasthenic patients (Abramsky et al., 1975; Conti-Tronconi et al., 1977). In view of these experimental results we investigated sensitization to cholinergic receptor in polymyositis patients.

Patients and Methods

Lymphocytes from 25 healthy subjects and from 27 patients with polymyositis were studied. The patients were 3 to 62 years old. Four also had systemic lupus erythematosus, one had rheumatoid arthritis and one scleroderma. The muscular involvement was demonstrated by electromyographic findings and by the usual clinical abnormalities. The duration of the illness ranged from 2 months to 5 years. Twenty-one patients were receiving steroid treatment and one was also treated with azathioprine. Lymphocytes, isolated from peripheral blood according to Böyum (1968), were cultured according to the micromethod of Hartzman (Hartzman et al., 1971) with minor modifications (Conti-Tronconi et al., 1977). For each subject, the culture was carried out for 4 days at 37°C in the presence of a standard human AB serum (I.S.M. Belfanti, Milano, Italy). Lymphocytes were tested with phytohemoagglutinin (PHA 10 μl/ml, Wellcome, Beckenham, England) or with our antigen preparation (Ag, 50 or 100 μg/ml). The degree of lymphocytic stimulation induced by PHA or by Ag was expressed as the stimulation index (SI), that is the ratio between the ³H-thymidine incorporation obtained in the presence of a mitogen (PHA or our Ag), and the basal incorporation in the absence of any stimulation. Our antigen preparation consisted of a postsynaptic membrane suspension isolated from Torpedo Marmorata electric organs by sucrose density gradient ultracentrifugation (Clementi et al., 1976). The postsynaptic fraction was rich in AChR (about 35% w/w of proteins, as demonstrated by binding with ¹²⁵I-alphabungarotoxin). It was almost devoid of AChE activity. These membranes were pelleted and resuspended in RPMI 1640 (Eurobio, Paris, France). We previously showed that a) stimulation by antigen is dose dependent, the maximal stimulation being obtained in the range of concentrations used in our experiments; b) AChR is the only membrane component responsible for the stimulation of lymphocytes from myasthenic patients (Conti-Tronconi et al., 1977). Hence, in these cases of polymyositis, we infer that the AChR is responsible for lymphocytic stimulation.

Results

Normal lymphocytes were never stimulated by our antigen; the mean SI obtained was 0.94 (Table 2). The antigen had a mild inhibitory action on the normal lymphocytes, so that the SI was often less than 1 (Conti-Tronconi et al., 1977). When individually considered, the normal SI never exceeded 1.5, which we took as the upper normal limit (Fig. 1).