Lymphocyte stimulation with muscle homogenate in polymyositis and other muscle-wasting disorders

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Polymyositis is a non-hereditary disorder chiefly affecting the musculature of the limb girdles, neck, and pharynx (Walton and Adams, 1959; Barwick and Walton, 1963); it has features suggesting an immunological pathogenesis—notably the histology, the association with collagen disorders and malignancy, and the responsiveness to steroids and immunosuppressive agents (Walton and Adams, 1959; Barwick and Walton, 1963; Rose and Walton, 1966; Logan, Bandera, Mikkelsen, and Duff, 1966; Winkelman, Mulder, Lambert, Howard, and Diessner, 1968; Malaviya, Many, and Schwartz, 1968). Direct evidence is scanty and conflicting as to whether the immunological processes involved are of the direct humoral or the delayed hypersensitivity type. Tests of skin sensitivity in cases of dermatomyositis associated with malignancy and the passive transfer of this sensitivity by sera (Grace and Dao, 1959; Curtis, Heckaman, and Wheeler, 1961) suggest a humoral mechanism, while the frequency of circulating antibodies is not higher in cases of polymyositis than in control cases (Caspary, Gubbay, and Stern, 1964). A delayed hypersensitivity mechanism is implied for human polymyositis if the myositis induced by injections of muscle and adjuvant (Dawkins, 1965)—and possibly mediated by lymphocytes (Kakulas, 1966a; Kakulas, 1966b)—is taken as an experimental analogue of the disease. On the other hand, polymyositis has occurred in association with Hodgkin's disease (Deep, Fraumeni, Tashima, and McDivitt, 1964), in which there is an early depression of delayed hypersensitivity (Aisenberg, 1966) and impaired lymphocyte transformation with phytohaemagglutinin (Hersh and Oppenheim, 1965).

'Specific' antigens, many of which cause delayed hypersensitivity reactions, may transform from 2 to 40% of lymphocytes from previously sensitized

subjects (Oppenheim, 1968), and this is a means of detecting lymphocyte sensitization to a particular antigen. In this paper we report the results of lymphocyte stimulation with homogenized muscle antigen in patients with polymyositis, with other muscle-wasting disorders, and unaffected controls.

MATERIAL AND METHODS

Eleven patients with polymyositis, 11 with other muscle-wasting disorders, and 10 unaffected controls were studied. The cases of polymyositis were acceptable on clinical, electromyographic and/or histological grounds. The details of the patients with polymyositis are summarized in Table I. They were grouped according to the classification of Walton and Adams (1959). The muscle-wasting group consisted of nine patients with muscular dystrophy, one with adult glycogen storage disease affecting muscle, and one patient with the Kugelberg-Welander syndrome. Functional grading of disability was based on that used by Rose and Walton (1966), modified from Swinyard, Deaver, and Greenspan (1957). This ranges from grade 1—normality to grade 6—inability to walk without aid or assistance. From clinical and investigatory evidence an estimate was made of the degree of activity in each patient with polymyositis, ranging from 0 (inactive) to +++ (very active). Each patient receiving steroids at the time of study had been on therapy almost from the commencement of the disease; in one patient, K.H., the dose had recently been increased.

Peripheral blood was defibrinated with glass beads, and lymphocytes separated by the method of Coulson and Chalmers (1967). They were cultured in tightly stoppered conical tubes containing 1 to 1.5×10^6 lymphocytes, 10% normal AB serum, and TC 199. Sterile human erector spinae muscle—obtained at operation—was homogenized as a 20% W/V preparation and stored at -40°C . Serial dilutions of antigen were added to cultures set up in triplicate at each concentration. Homogenates of liver and kidney were prepared in a similar manner and used as control antigens.

TABLE I
POLYMYOSITIS—DETAILS OF PATIENTS

Type	Patient	Sex	Age	Duration of disease (yr)	Activity of disease	Functional grading	Serum PCK i.u. <i>N</i> < 60 i.u.	Daily steroid dosage (prednisone mg)
I Polymyositis	D.H.	F	54	1.2	0	2	31.6	5
	K.H.	M	9	2.5	0	3	—	5
	D.K.	F	9	3.5	+	3	26.6	10
	M.S.	F	51	11.5	+	4	30	0
II Polymyositis with some evidence of collagen disorder	D.B.	F	32	1.5	++	4	393	0
	K.B.	F	56	0.5	+++	5	2,550	40
	I.S.	F	38	0.2	++	3	38.3	0
III Definite collagen disorder	D.C.	F	65	1.7	+++	6	10	10u. ACTH
	E.C.	F	48	8.0	++	5	65	10
	V.N.	F	57	3.6	+++	6	51.6	0
	F.W.	F	59	c. 3.0	+++	5	186	0

Lymphocytes were cultured for six days and labelled with (³H) thymidine (specific activity 2.5 µC/m-mole, Radiochemical Centre, Amersham) for a four hour pulse. Cells were washed and prepared for liquid scintillation counting (Hughes, Caspary, and Field, 1968). Results were expressed as an index of response which is the maximum uptake in stimulated cultures divided by the basal unstimulated uptake.

RESULTS

The results obtained in the three groups are expressed in Fig. 1. There is a significantly higher index of response in polymyositis compared with unaffected controls ($P < 0.001$) and with other muscle wasting disorders ($P < 0.001$) using Cochran's modification of the standard *t* test. No significant difference was found between other muscle-wasting disorders and controls. Two patients with muscular dystrophy showed probable stimulation with muscle homogenate; three with polymyositis showed no response, but two of these were clinically inactive. The patient, K.B., who was severely affected and who had a very high creatine phosphokinase level, showed the highest index of response. The degree of stimulation in all except one patient (E.C.) correlated reasonably with the activity of the disease.

DISCUSSION

In contrast to the negative findings recorded in myasthenia gravis (Housley and Oppenheim, 1967), the response to muscle homogenate in the group of patients with polymyositis appears to indicate that lymphocyte sensitization to some component in whole muscle may occur. The nature of the antigen

FIG. 1. Response of lymphocytes to muscle homogenate. Polymyositis v. normals: $P < 0.001$; polymyositis v. muscle wasting: $P < 0.001$; normals v. muscle wasting: not significant.

is of course unknown, and the demonstration of sensitization to muscle may be a secondary phenomenon. Two patients with muscular dystrophy

showed a response to the antigen and more careful selection of active cases of muscular dystrophy might show a greater number of responses in this 'control' group. Failure to respond to muscle antigen in three cases of polymyositis could be explained on the grounds of inactivity of the disease in two instances. In the third case the disease was active; as this was one of the more recently studied, the possibility of deterioration in the antigen has to be considered (Housley and Oppenheim, 1967). Furthermore, polymyositis may comprise a group of disorders and, if sensitization to a muscle antigen plays a fundamental role in the pathogenesis of the disease, the same antigen may not be responsible for sensitization in each case. Further work needs to be done on the immunological aspects of polymyositis.

SUMMARY

Lymphocyte stimulation with human muscle homogenate was studied in 11 patients with polymyositis, 11 with other muscle-wasting disorders, and 10 unaffected controls. Patients with polymyositis showed a significantly increased index of response compared with the other two groups.

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