Brief Definitive Report

PASSIVE TRANSFER OF EXPERIMENTAL AUTOIMMUNE MYASTHENIA BY LYMPH NODE CELLS IN INBRED GUINEA PIGS

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Experimental autoimmune myasthenia gravis (EAM) is an autoimmune disease of the neuromuscular junction, recently induced in animals by injection of purified acetylcholine receptor (AChR) extracted from electric fish, in complete Freund's adjuvant (CFA). Rabbits (1–3) and monkeys (4) usually display acute muscle weaknesses and die of severe difficulties in breathing. Guinea pigs and rats (5) usually develop a mild disease, often transient, manifested clinically by hypoactivity, sinking of head, and weight loss. The experimental muscle disease may temporarily be reversed by the administration of anticholinesterase agents, and a decremental response of muscle activity to repetitive nerve stimulation can be demonstrated in electromyographic examination. Thus, EAM serves as an experimental model for the human autoimmune disease myasthenia gravis (MG) in which the same clinical, pharmacological, and electrophysiological phenomena are observed as a result of neuromuscular junction block probably due to autoimmunization toward the postsynaptic AChR.

In this report we demonstrate the passive transfer of EAM in strain 13 guinea pigs. This was achieved by transfer of lymph node cells from guinea pigs immunized with purified AChR from *Torpedo californica* to recipient animals.

Materials and Methods

Acetylcholine Receptor Antigen. Purified AChR from the electrogenic tissue of *T. californica* was obtained by solubilization of membrane fragments with 1% Triton X-100, followed by affinity chromatography on a *Naja naja siamensis* neurotoxin-Sepharose resin, as described by Aharonov et al. (6), with slight modifications, for isolation of AChR from the electric eel . The specific activity of the purified AChR was 8,000–10,000 pmol toxin bound per mg of protein.

Animals. Histocompatible strain 13 female guinea pigs weighing 300–400 g were used in all experiments.

Transfer Procedure. Donor animals were injected into foot pads with 100 μg of the purified AChR in CFA (Difco Laboratories, Detroit, Mich.). 8–10 days after challenge, donor animals were anaesthized and exsanguinated by cardiac puncture. Lymph nodes were removed and placed in minimal essential medium for suspension (Microbiological Associates, Jerusalem), finely minced, and filtered through nylon mesh. The cell suspension was centrifuged for 5 min at 200 *g* in the cold and the packed cells were resuspended in the above medium and washed twice. Portions were removed for cell counting and viability was determined by trypan blue exclusion. Approximately 2×10^8 cells with 70–80% viability were obtained from each donor. 5×10^8 viable cells were then injected

into each recipient [2.5×10^8 intravenously (i.v.) and 2.5×10^8 intraperitoneally (i.p.)]; this represents a donor:recipient ratio of 4:1.

Skin Test. Intradermal injection of 15 μg AChR in 0.15 ml of the AChR purification buffer (0.01 M Tris-Cl, pH 7.5 containing 0.1 M NaCl, 1 mM EDTA and 0.1% Triton X-100) were given to actively challenged animals 10 days after immunization, to cell recipients 5 days after cell transfer, and to normal control animals. The appearance of erythema at the site of injection was observed 24 h later, and the extent of delayed hypersensitivity was quantitated by measuring the diameter of the skin reaction. Skin reactions with a diameter larger than 5 mm were considered positive.

Humoral Response. Quantitative microcomplement fixation reactions were carried out, as described by Levine (7) in donor animals 8–10 days after challenge.

Pharmacological and Neurophysiological Studies. The anticholinesterase, tensilon (edrophonium hydrochloride, 0.1 mg), was injected i.v. to sick animals. Electromyographic examinations (EMG) were performed on actively challenged animals 12–18 days after challenge and in recipients 8–12 days after cell transfer, using an HP Electromyograph. The stimulating electrodes were inserted subcutaneously at the buttock, above the sciatic nerve, about 0.5 cm apart. A tetanic train of rectangular pulses, of 150 V amplitude and 100 μsec duration, at a frequency of 40 Hz, was administered for about 20 sec. The EMG was recorded by coaxial electrode which was inserted deep into the calf muscle.

Results

Passive transfer of EAM was accomplished, in three experiments, with lymph node cells obtained from 56 donors sensitized with AChR and 14 recipient guinea pigs. In a typical experiment, clinical signs of EAM were observed in 6 out of 10 actively challenged animals 12–22 days after injection of AChR. Such signs were observed in 8 out of 14 recipients 7–14 days after cell transfer. The disease was

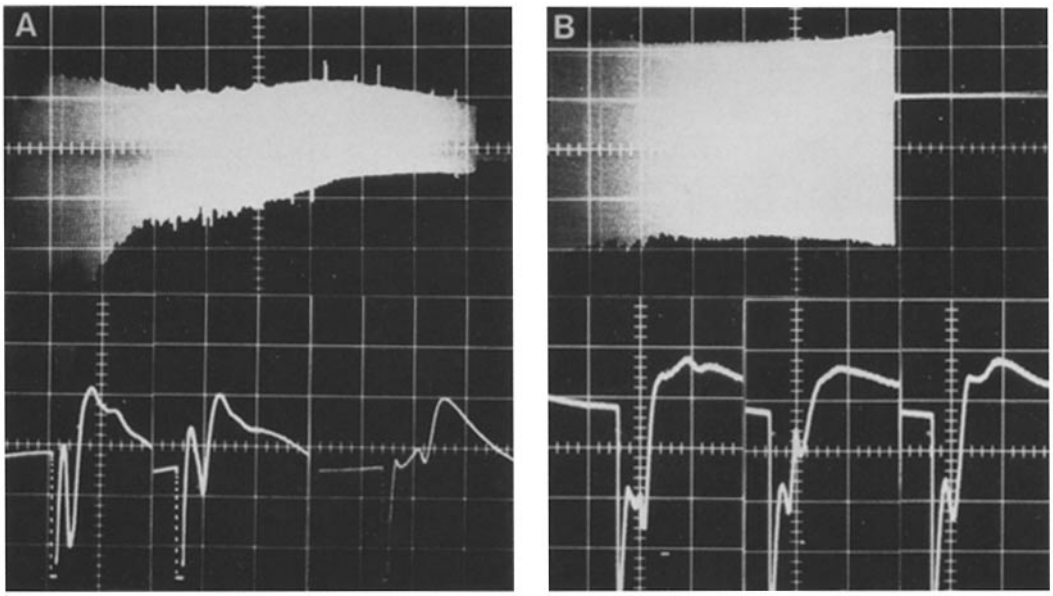

FIG 1. Evoked compound motor action potentials (ECMAP) in calf muscle of a recipient guinea pig with EAM (A) and of a normal guinea pig (B). The calibration in the upper traces is in both cases: horizontal, 2 sec/div; vertical, 2.5 mV/div. In the lower traces the calibration is: horizontal, 5 msec/div; vertical, 2.5 mV/div. The lower traces show single ECMAP evoked on early, intermediate and late stages of the repetitive stimulation shown in the upper traces.

characterized by mild generalized muscle weakness, sinking of the head, hypoactivity, anorexia, and weight loss. This syndrome was transient, usually lasting 3–4 days. Muscle weakness could be reversed for about 15 min immediately after i.v. administration of tensilon. Neurophysiological examinations revealed a decrease in amplitude during repetitive nerve stimulation in all AChR-injected animals, as well as in all recipients (Fig. 1 A). A normal response was observed in nonimmunized animals, and in animals injected with CFA alone (Fig. 1 B). All actively challenged animals and also all recipients exhibited delayed hypersensitivity as demonstrated by a positive skin test (above 5 mm diameter) with 15 μg of AChR. The average diameters of the skin reactions were 19.6 mm for actively challenged animals and 9.2 mm for the recipients. No response was revealed with a nonrelated control antigen (lysozyme). No circulating antibodies to AChR were detected by the microcomplement fixation assay in the sera of donor animals, when sacrificed 8–10 days after challenge.

Discussion

In the present study we have reported the passive transfer of EAM in strain 13 guinea pigs. The clinical picture of muscle weakness in about 60% of the animals as well as the characteristic improvement after anticholinesterase agent administration and the pathognomonic electromyographic decremental muscle response to repetitive nerve stimulation, were similar to the same phenomenon observed in parallel in actively challenged animals, and indicates that the recipients suffered from EAM. This experimental AChR-induced disease has the same neurological, pharmacological and electrophysiological manifestations as the human autoimmune disease MG, in which a specific cellular sensitization towards AChR (8–10), as well as antibodies in some cases[1], has been recently demonstrated by us. The induction of EAM in experimental animals by injecting AChR, and the immune response towards AChR in patients suffering from MG, provide evidence for the breakdown of tolerance to self-AChR as the cause of the disease. Both the immunopharmacological neuromuscular blockage and the thymic disorders which are characteristic of the disease may involve the AChR as autoantigen (11).

The results of the present study, as well as the demonstration of a prominent lymphocyte response to AChR in MG patients (9, 10), suggest that cellular immunity plays a significant role in the pathogenesis of myasthenia gravis. Such findings have also been reported for other autoimmune conditions which have been related to delayed hypersensitivity. For example, cellular immunity towards the myelin basic protein has been observed in acute postinfections disseminated encephalomyelitis (12), and passive transfer has been demonstrated in its experimental model, experimental allergic encephalomyelitis (13).

The mechanism by which suspensions of sensitized lymphocytes induce EAM in recipient animals is not clearly defined. Sensitized cells may act either directly on the neuromuscular junction, or indirectly, by releasing antibodies or nonim-

[1] Aharonov, A., O. Abramsky, R. Tarrab-Hazdai, and S. Fuchs. 1975. Humoral antibodies to acetylcholine receptor in patients with myasthenis gravis. *Lancet*. In press.

munoglobulin mediators. No conclusive information as to the role of cells vs. antibodies in the production of the motor end plate abnormality can be provided from the present study. The relative contribution of the cellular response and the humoral antibodies in both EAM induced in animals and in the human disease, MG, await further investigation.

Summary

Passive transfer of experimental autoimmune myasthenia (EAM) was performed with lymph node cells from donor guinea pigs immunized with purified acetylcholine receptor (AChR) from *Torpedo californica*. Recipient animals revealed the same clinical signs and electromyographic patterns as observed in actively challenged animals. These phenomena are parallel to the clinical manifestations of the human disease myasthenia gravis, in which cellular response to AChR was recently demonstrated.

The authors wish to thank Dr. Israel Silman for fruitful discussions, and Mrs. Dora Barchan for excellent technical assistance.

Received for publication 28 May 1975.

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