Clin. exp. Immunol. (1971) **9**, 329–337.

ANTIBODIES TO SKELETAL MUSCLE DEMONSTRATED BY IMMUNOFLUORESCENCE IN EXPERIMENTAL AUTOALLERGIC MYOSITIS

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(*Received* 7 *April* 1971)

SUMMARY

Immunization of inbred guinea-pigs with xenogeneic skeletal muscle can induce the development of a focal segmental myositis associated with the presence of a serum antibody which binds to the cross-striations of isogeneic or xenogeneic skeletal muscle as shown by indirect immunofluorescence. This antibody also produces striational staining of chick myoid cells. The association of striation-binding antibody with some forms of human and animal myositis is emphasized. It is concluded, however, that myositis is unlikely to be directly due to humoral antibody.

INTRODUCTION

Although experimental autoallergic myositis (EAM) is readily produced by the administration of skeletal muscle in Freund's complete adjuvant (FCA) (Dawkins, 1965; Kakulas, 1966; Takayanagi, 1967), the disease remains to be characterized serologically. It has been amply demonstrated by immunofluorescence that immunization with muscle or its derivatives can lead to the production of antibody which specifically binds to the cross-striations of skeletal muscle (see Pepe, 1968), and since this type of antibody could be involved in the pathogenesis of EAM, it was decided to test the sera of affected animals. The presence of a striation-binding antibody in myasthenia gravis and in *Mastomys* polymyositis (Strauss *et al.*, 1968), and the histopathological similarities between EAM and these diseases, suggested that such an antibody might be important.

MATERIALS AND METHODS

Experimental autoallergic myositis

Details of immunization have appeared previously (Dawkins, 1965). In brief, young adult strain thirteen guinea-pigs received repeated intramuscular injections of a crude homogenate of rabbit skeletal muscle emulsified in Freund's complete adjuvant. Injections were

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placed into the posterior cervical musculature. The intervals between injections varied from 2 to 4 weeks and animals were killed 2–12 weeks after the first injection. At post-mortem, blocks of quadriceps muscle, kidney and thymus were taken for histological examination.

The results obtained by immunization with rabbit muscle were compared with those obtained by substituting homogenates of rabbit thymus and kidney, chick muscle and kidney, and isogeneic strain thirteen guinea-pig muscle and kidney.

Immunofluorescence

Sera were collected before immunization and when the guinea-pigs were killed, and were tested immediately or stored at −20°C. Some animals were bled by cardiac puncture every 2–4 weeks.

Indirect immunofluorescence was used (Holborow & Johnson, 1967). The tissue substrate generally consisted of isogeneic guinea-pig skeletal muscle but isogeneic thymus, oesophagus and kidney; allogeneic muscle; rabbit muscle, oesophagus, heart and thymus and chick muscle and thymus were also used. Fresh tissue was embedded in OCT medium (Tissue-Tek, Ames) snap frozen in isopentane in liquid nitrogen and then stored at −70°C. Sections 6 μ thick were cut at −20°C. After air-drying they were covered with antiserum diluted 1/10–1/640. After 20 min, sections were washed with buffered saline pH(7·2) and conjugate (rabbit antiguinea-pig IgG FITC (Nordic)) was applied at a dilution of 1/8 for 30 min. After further washing they were mounted in buffered glycerine and viewed in a Leitz Ortholux using a Wratten 18B primary filter and a K430 secondary filter.

Absorption

Aliquots of the homogenates used for immunization were repeatedly washed in phosphate buffered saline by centrifugation at 800 *g* for 5 min. The resulting residue was then resuspended in an equal volume of antiserum. After incubation at 37°C for 1 hr and 4°C for 18 hr the supernatant was centrifuged at 3000 *g* for 30 min. After recentrifugation the absorbed antiserum was tested at dilutions of 1/5–1/80.

Blocking studies

After treatment with antirabbit muscle serum but before addition of conjugate, representative muscle sections were incubated with unconjugated antiguinea-pig IgG for 1 hr.

RESULTS

Results are summarized in Table 1.

Muscle changes and muscle binding antibody

Guinea-pigs repeatedly immunized with rabbit muscle in Freund's complete adjuvant (FCA) usually developed a focal segmental myositis (Fig. 1). The histopathological picture was similar to that previously described (Dawkins, 1965). Significant changes in the thymus were not found; there were inflammatory changes in and around the thymus of most animals injected with FCA irrespective of the type of tissue homogenate received. Ten of twelve animals immunized with rabbit muscle developed antibody capable of binding to the striations of isogeneic guinea-pig muscle (Figs 2–4). The pattern of staining was consistent throughout. Phase contrast microscopy suggested that the localization of antibody was

TABLE 1. Incidence of myositis and antimuscle, antimyoid cell and antithymocyte antibodies

Antigen	Animal	Injections	Myositis	Immunofluorescence*		
				Muscle	Myoid cell	Thymocyte
Rabbit muscle	15	4	−	++	N.E.	N.E.
	18	3	−	++	N.E.	N.E.
	19	4	N.E.	++	N.E.	N.E.
	25	1	−	+	+	−
	26	4	+	++	++	−
	27	4	+	++	++	−
	28	3	+	+	++	−
	29	4	+	++	++	−
	52	2	+	−	−	−
	53	2	+	−	+	−
	75	1	−	+	+	−
	88	1	N.E.	+	+	−
Rabbit thymus	30	3	−	−	−	+
	31	4	−	−	−	+
	32	4	−	−	−	+
	33	2	−	−	−	+
	34	4	−	−	−	+
	91	1	N.E.	−	N.E.	N.E.
Rabbit kidney	20	3	−	−	−	−
	21	3	−	−	−	−
	22	1	−	−	−	−
	23	1	−	−	−	−
	92	1	−	−	−	−
	93	1	−	−	−	−
Isogeneic muscle	40	4	−	−	−	−
	41	4	−	−	−	−
	42	3	N.E.	−	N.E.	N.E.
	43	1	−	−	N.E.	N.E.
Isogeneic kidney	44	4	−	−	N.E.	N.E.
	45	4	−	−	−	−
	46	2	−	−	N.E.	N.E.
	47	4	+	−	−	−
Chick muscle	48	1	−	−	−	(−)
	49	3	N.E.	+	+	(−)
	50	4	−	+	+	(−)
	51	3	−	−	+	(−)
	54	2	−	+	+	(−)
	55	2	+	+	+	(−)
	72	1	−	−	−	(−)
	76	1	+	+	N.E.	N.E.
	94	1	N.E.	+	+	(−)

Results given are those obtained with serum collected at the time of post-mortem and tested on isogeniec guinea-pig muscle or chicken thymus.

* ++, Strong staining, i.e. positive at dilutions of 1/40–1/80; +, definite staining, i.e. positive at dilutions of 1/10–1/20; −, equivocal or negative staining at dilution of 1/10; (−) no cell specific staining but increased background. N.E., not examined.

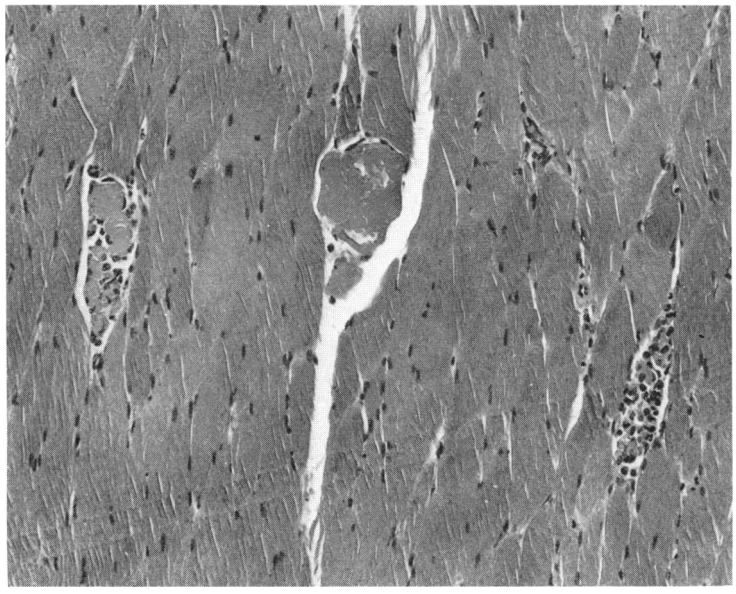

FIG. 1. The typical focal myositis following immunization of an adult guinea-pig with rabbit muscle in FCA. Three lesions in different stages of development are shown. H & E.

similar to that described by Strauss & Kemp (1967) for myasthenic sera; namely, binding at the I bands and sometimes the H zones. The antibody was found 2 weeks after a single injection, but maximal titres (up to 1/80) were obtained only after repeated injections. A similar antibody could be demonstrated in six of nine guinea-pigs which received injections of chick muscle in FCA, but titres did not exceed 1/20. With sera from all other groups

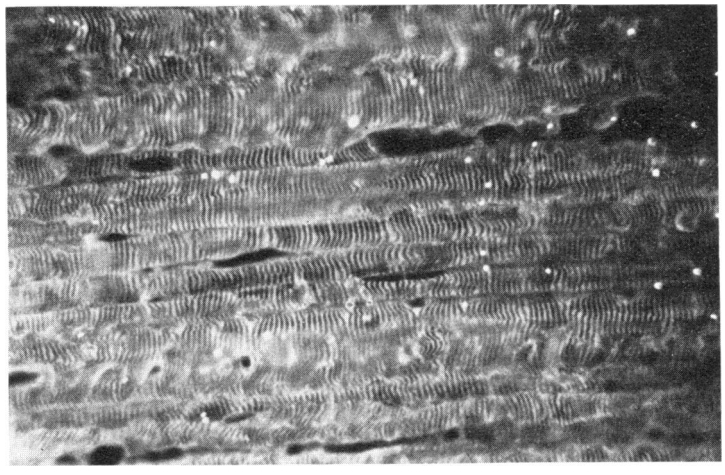

FIG. 2. Indirect immunofluorescence: Guinea-pig antirabbit muscle serum on a frozen section of guinea-pig muscle. Staining of alternate cross-striations.

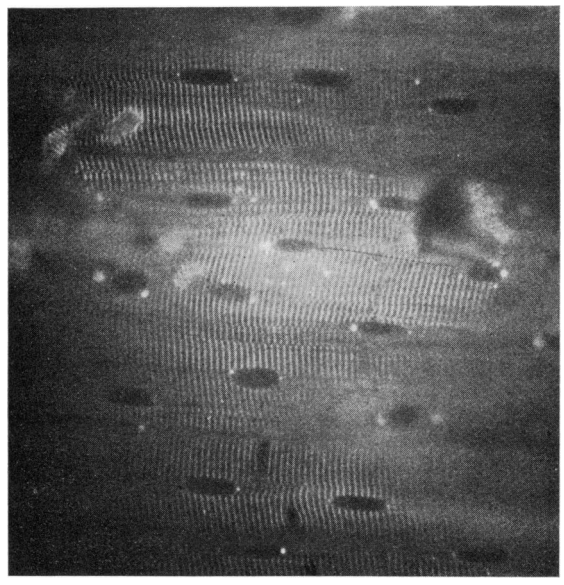

FIG. 3. Same as Fig. 2 demonstrating the absence of nuclear staining.

attempts to demonstrate striational staining were consistently negative. It was, however, possible to demonstrate some non-tissue-specific sarcolemmal or intercellular binding in most animals which received xenogeneic kidney or muscle. In addition occasional sera showed varying degrees of the 'zebra-staining' described by Feltkamp (1965) (Fig. 5).

Other tissue substrates

The striation-binding antibody could be demonstrated on allogeneic guinea-pig, rabbit and chick skeletal muscle. In sections of isogeneic guinea-pig or rabbit oesophagus, the

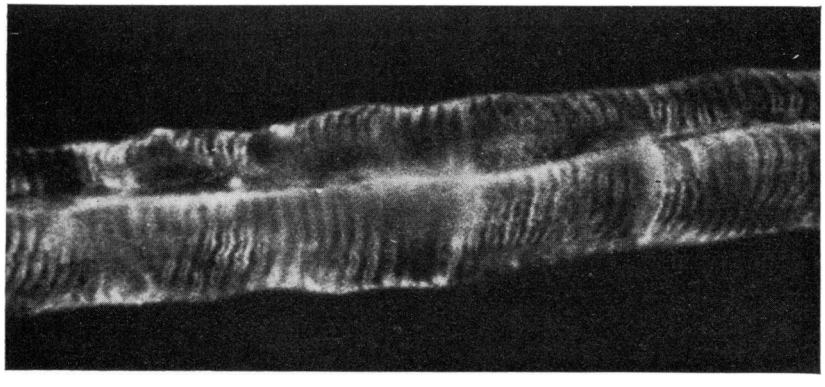

FIG. 4. Same as Fig. 2 demonstrating that stained striations consist of two closely approximated bands separated by tan unstained band.

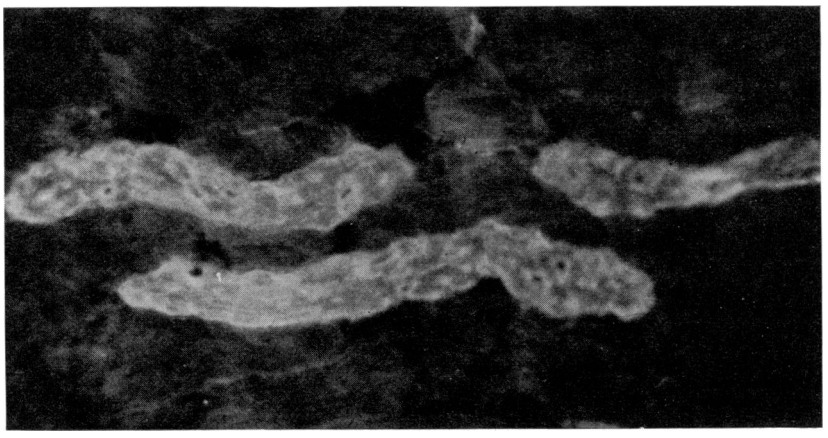

FIG. 5. Indirect immunofluorescence: Guinea-pig antirabbit thymus serum on guinea-pig muscle. 'Zebra' staining.

striated muscle fibres were stained but there was no cross-reactivity with smooth muscle. On rabbit heart there was some binding to cross striations, but this was weaker and more variable than with skeletal muscle.

When sections of chicken thymus were stained, cross-reaction between skeletal muscle and myoid cells was readily demonstrated; sera positive on skeletal muscle produced specific staining of myoid cells. With antirabbit muscle sera there was very selective staining of these cells and their striations were clearly seen (Figs 6 and 7). With antichick muscle on the other hand, there was considerably more associated background staining. Cross reactivity was also apparent when guinea-pig thymus was used, but myoid cells were smaller, non-striated and infrequent. Antisera raised against rabbit thymus produced specific staining of rabbit thymocytes but not of myoid cells. Sera which gave 'zebra-staining' were negative when tested on thymus.

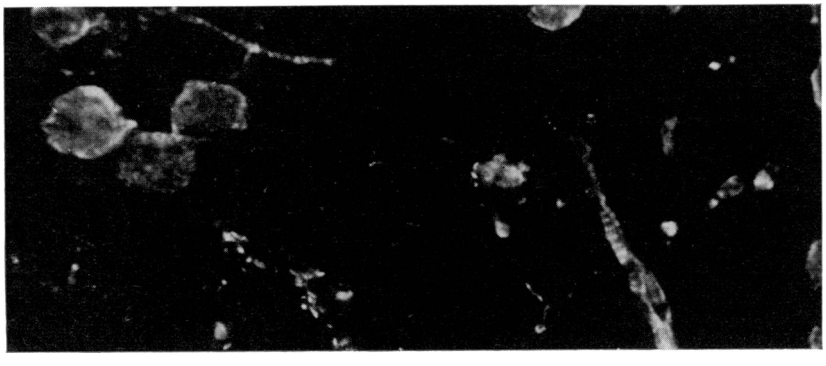

FIG. 6. Indirect immunofluorescence: Guinea-pig antirabbit muscle on young adult chicken thymus showing striational staining of the tails of myoid cells.

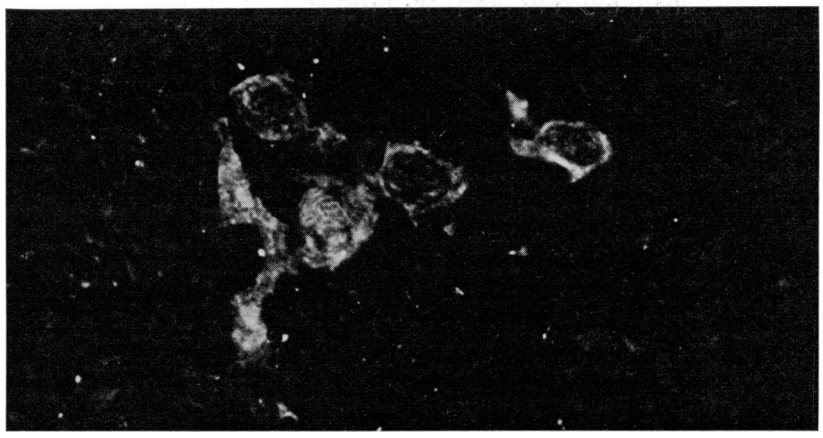

FIG. 7. Same as Fig. 6 showing circular striations of the bodies of myoid cells.

Effects of absorption and blocking

Striation-binding antibody was readily removed by absorption with homogenates of rabbit and guinea-pig muscle. Chick muscle was also active although somewhat less effective. Rabbit thymus was inactive. Sarcolemmal staining was decreased by absorption with either muscle or kidney.

Treatment with unconjugated anti-IgG prior to application of the conjugate greatly decreased the intensity of striational staining obtained with antirabbit muscle serum.

DISCUSSION

These results confirm that immunization of guinea-pigs with xenogeneic skeletal muscle may lead to the appearance of at least one striation-binding antibody. Our data show that although this antibody reacted strongly with isogeneic tissue immunization with isogeneic antigen was ineffective. This therefore suggests that the tissue-specific antigen must be administered with foreign antigens before it evokes production of tissue-specific antibody. Examination of different tissue substrates and the effects of absorption indicate that there is a high degree of cross-reaction between the tissue specific muscle antigens of rabbit guinea-pig and chick. We have also demonstrated cross-reactivity between skeletal muscle and myoid cells of the thymus, as previously shown by other authors (Goldstein & Whittingham, 1967; Kalden *et al.*, 1969). In contrast, however, we have not found that immunization with muscle or thymus leads to a specific 'thymitis'. This difference could be attributed to the different route and schedule of immunization but different adjuvant and antigen preparations may be important.

The demonstration that animals with EAM have a striation-binding antibody is of particular interest because of the close association between myositis or lymphorrhages and a similar antibody in human myasthenia gravis (Oosterhuis *et al.*, 1968). The occurrence of myositis in some cases of myasthenia gravis has been emphasized (Russell, 1953; Osserman, 1958) and should be considered when animal models and theories of pathogenesis are proposed. The association of myositis and striation-binding antibody has also been noted in

Mastomys polymyositis (Strauss *et al.*, 1968; Stewart & Snell, 1968). Myositis and anti-muscle antibodies are not always associated however; in human polymyositis antimuscle antibodies appear to be unusual (Stern, Rose & Jacobs, 1967; Fessell & Raas, 1968; Dawkins & Eghtedari, 1970 (unpublished)).

Our data indicate that although guinea-pigs appear to require repeated immunization before myositis occurs in high incidence (Dawkins, 1965; Vetters *et al.*, 1969), the striation-binding antibody is often present after a single injection. Furthermore, the lack of a clear correlation between antibody titre and the presence of myositis (Table 1) suggests that the two phenomena may be two independent manifestations of immunization. In experiments using ^{51}Cr-labelled monolayers of chick muscle we have obtained no evidence that the striation-binding antibody is cytotoxic (Dawkins & Loewi, in preparation) although it does bind to muscle cells under appropriate conditions (Dawkins & Lamont, 1971). Thus we have no evidence to contradict previous suggestions that the pathogenesis of EAM is probably more dependent on cell mediated immunity than humoral antibody (Dawkins, 1965; Kakulas, 1966; Takayanagi, 1967).

ACKNOWLEDGMENTS

We are grateful to Dr J. N. McCormick, Dr J. R. Kalden, Dr T. E. W. Feltkamp and Dr Thea Feltkamp-Vroom for helpful suggestions and to Miss J. Snajdar for able technical assistance.

This work was supported by a grant from the Arthritis and Rheumatism Council of Great Britain.

NOTE ADDED IN PROOF

Furthur evidence that immunization with muscle can lead to severe destruction of muscle *in vivo* is provided by a recent report which demonstrated that serum creatine kinase is elevated in rats with EAM: G. Morgan, J. B. Peter & B. B. Newbould (1971) Experimental allergic myositis in rats. *Arthr. and Rheum.* (In press).

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