THYMIC B LYMPHOCYTE CLONES FROM PATIENTS WITH MYASTHENIA GRAVIS SECRETE MONOCLONAL STRIATIONAL AUTOANTIBODIES REACTING WITH MYOSIN, α ACTININ, OR ACTIN

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Myasthenia gravis (MG)¹ is an autoimmune disease of skeletal muscle that is characterized by prominent thymic pathology (reviewed in 1). Lymphoid follicles in the medulla are increased in number, often with germinal centers, and B lymphocytes (2) and DR⁺ cells are numerous (3). The thymus in MG is a site for the production of antibodies to nicotinic acetylcholine receptors (AChR) (4), the serologic hallmark of acquired MG and the cause of impaired neuromuscular transmission. Thymoma, a slowly growing epithelial tumor that rarely metastasizes, occurs in ~15% of patients with MG, and more impressively, it has been estimated that 35% of patients with thymoma either have MG or will develop MG (5). The basis of this striking association between thymoma and MG is not known.

In 1960, Strauss et al. (6) reported that sera of patients with MG contained antibodies that reacted with cross-striations of skeletal muscle. Although these striational autoantibodies (StrAb) were the first organ-specific autoantibodies found in MG, the pathogenic significance of StrAb, if any, is unknown. They are very infrequent in patients <20 yr old but are found in 55% of MG patients ≥60 yr old (7). StrAb are frequently found with thymoma, occurring in 80–90% of patients with MG and thymoma, and in 24% of patients with thymoma in the absence of clinical signs of MG (8–10). They are rarely found in other conditions. In MG, autoantibodies to AChR are found in higher titer in association with StrAb than in their absence (7).

The stimulus for production of StrAb is unknown. Their association with thymoma and the findings that these antibodies bind to thymic epithelial cells (8, 11) and thymic myoid cells (12, 13), in addition to skeletal muscle, suggest that the thymus has a central role in the induction of StrAb. Identification of the antigens to which StrAb bind in muscle and thymus should provide insight into the nature of the thymus’ association with these antibodies.

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¹ Abbreviations used in this paper: AChR, acetylcholine receptor; EBNA, Epstein-Barr nuclear antigen; MG, myasthenia gravis; StrAb, striational antibodies.
This paper describes the secretion of StrAb by lymphocytes cultured from thymuses of MG patients, and the establishment of B cell clones secreting monoclonal StrAb. B cells from thymus, thymoma, and peripheral blood were transformed fortuitously by EBV during attempts to hybridize lymphocytes from MG patients with the human myeloma, GK5. The monoclonal StrAb recognized myosin, α-actinin, and actin in both thymus and muscle. Based on these and other findings, we have developed a hypothesis concerning the stimulus for production of StrAb in association with MG and thymoma.

Materials and Methods

**Patients.** The diagnosis of MG in all patients participating in this study was supported by electromyographic examination and by the presence of AChR antibodies in serum (17). The 72 patients from whom thymic lymphocytes were cultured had a median age of 34 yr (range, 19 mo to 74 yr); 54 were female and 18 were male; 18 had thymoma. All had generalized muscle weakness and 23 were being treated with prednisone at the time of surgery. A clinical synopsis of each patient from whom B cell clones were established is shown in Table I.

**Thymic Cell Cultures.** Thymus tissues obtained at therapeutic thymectomy were dissociated mechanically, and 10⁶ cells/ml were cultured for 3–14 d at 37°C in a humidified atmosphere of 5% CO₂ and 95% air in medium (RPMI 1640) containing 10% FCS, PWM at 1/120 dilution (Grand Island Biological Co., Grand Island, NY), L-glutamine (0.06 mg/ml), 2-ME (10⁻⁴ M), streptomycin sulfate (0.2 mg/ml), and penicillin G (20 U/ml).

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**Table I: Clinical Information about Patients from Whom B Cell Lines Were Established**

| Patient | Sex | Age at thymectomy (yr) | Thymic pathology | Muscle weakness Duration (mo) | Severity* | Serum antibodies Striational (reciprocal titer) | ACh receptor titer nM |
|---------|-----|------------------------|------------------|-----------------------------|-----------|-----------------------------------------------|---------------------|
| I       | M   | 56                     | Thymoma          | 30                          | 3         | 60                                            | 7.4                 |
| II      | M   | 22                     | Hyperplasia      | 2                           | 3         | 120                                           | 11                  |
| III     | M   | 62                     | Normal           | 16                          | 4         | Negative                                      | 80                  |
| IV      | F   | 25                     | Hyperplasia      | 36                          | 3         | Negative                                      | 38                  |
| V       | F   | 23                     |Hyperplasia      | 16                          | 4         | Negative                                      | 19                  |
| VI      | F   | 70                     | Unknown          | 16                          | 6         | 3,840                                         | 361                 |
| VII     | F   | 33                     | Normal           | 18                          | 3         | Negative                                      | 2.6                 |

* All were generalized: 3, mild; 4, moderately severe with mild bulbar signs; 6, chronic severe, respirator-dependent (18).
‡ Assayed by indirect immunofluorescence using rat skeletal muscle as substrate (7).
§ Assayed by immunoprecipitation of solubilized human muscle AChR complexed with ¹²⁵I-α-bungarotoxin (7); normal, ≤0.05 nM.
Serum supplements were heated at 56°C for 30 min. Supernatants were assayed for StrAb by an ELISA using human skeletal muscle protein antigens extracted in 0.6 M KCl, as described below.

Establishment of Lymphocyte Clones. Thymus tissues of patients II-V were dissociated mechanically and peripheral blood lymphocytes of patients V-VII were isolated by centrifugation on Isopaque Ficoll of density 1.077 (19). Cells were cultured for 3 d as described above, with addition of human AB serum (5%), except for the thymoma cells (patient I), which were used immediately after dissociation with collagenase (0.8%), trypsin (0.25%), and DNase (0.02 mg/ml) in PBS. All cells were washed in medium and mixed 2:1 with the human myeloma cell line, GK5, in 43% PEG 1500 (M.A. Bioproducts, Walkersville, MD) for 2 min at 37°C. After slow dilution with medium, the cells were pelleted by centrifugation at 400 g at 22°C, and were resuspended at 10^6 cells/ml in RPMI 1640 containing FCS (15%), aminopterin (10^-6 M), thymidine (2 x 10^-5 M), deoxycytidine (2 x 10^-5 M), hypoxanthine (10^-4 M), and antibiotics, and they were then cultured for 1 mo in 96-well flat-bottomed plates. Wells with proliferating cells were screened for StrAb by ELISA. Positive colonies were cloned at least four times by limiting dilution and were grown in RPMI 1640 FCS (15%).

In an attempt to produce mAbs in ascites fluid, 5 x 10^7 cells of each antibody-producing line were injected intraperitoneally into groups of six male athymic nude mice (three BALB/C and three C57/BALB/C) 3 d after intraperitoneal injection of 0.5 ml sterile Pristane (Aldrich Chemical Co., Milwaukee, WI).

Antigens. Purified myosin, actin, and α-actinin from bovine and chicken skeletal muscle and chicken gizzard were purchased from Sigma Chemical Co. (St. Louis, MO). Cytoskeletal proteins were extracted from prefusion fetal human myoblasts (i.e., cultured for 5 d) in 1% Triton X-100 (20), and cytoskeletal proteins of adult human erythrocytes were prepared by the method of Bennett and Stenbuck (21). Thymus and skeletal muscle were obtained from a young female Lewis rat, weighing ~200 g, which had been anesthetized with Pentobarbital and subjected to whole-body perfusion with PBS. Proteins were extracted from rat tissues or frozen human limb muscle (amputated because of osteosarcoma) by homogenizing for 30 s (setting 6, Polytron homogenizer; Brinkman Instruments Co., Westbury, NY) in NaHCO_3 (1 M, pH 7.5). After 30 min at 22°C, the homogenate was centrifuged (2,800 g, 10 min) and the resulting pellet was resuspended in KCl (0.6 M), Na_2HPO_4 (0.01 M), and Na_2HPO_4 (0.01 M) and was incubated for 30 min at 22°C. The supernatant of a second centrifugation (7,800 g, 15 min) was dialyzed for 24 h against NaH_2PO_4 (0.01 M) and Na_2HPO_4 (0.01 M) (pH 7.5 at 4°C). Protease inhibitors included in all steps were: Pepstatin A (1 μg/ml), PMSF (0.01 mM) (Sigma Chemical Co.), Aprotinin (1 KIU/ml) (Boehringer Mannheim Biochemicals, Inc., Indianapolis, IN), EGTA (1 mM), and EDTA (1 mM).

Immunofluorescence Assays. Clonal cell lines were tested for Epstein-Barr nuclear antigen (EBNA) by a complement-fixation assay (22). Monoclonal and serum antibodies were tested for reactivity with nuclei, mitochondria, smooth muscle, and gastric mucosal cells by using mouse stomach and kidney substrates (Quantafluor fluorescent autoantibody test; Kallestad Laboratories, Inc., Austin, TX). Sections of rat, human and frog skeletal muscle, frog thymus (4 μm), or stretched myofibrils prepared from glycercinated rat muscle (14) were used to assay StrAb in patients' sera (1:40) or in undiluted growth media from cultures of the B cell clones by using fluoresceinated goat anti-human IgG, IgM, or IgA (Southern Biotechnology Assoc., Inc., Birmingham, AL), diluted 1:100. In a dual fluorescence study of a mitochondrial-reactive IgM mAb, cultured human myoblast cells were fixed with acetone and exposed first to the IgM mAb then to serum from a patient with primary biliary cirrhosis (IgG antibodies) at 1:640 dilution. After washing, fluorescein-labeled goat anti-human IgM and rhodamine-labeled anti-human IgG (Southern Biotechnology Assoc., Inc.) were added together, each at a dilution of 1:100. All samples were examined with a Carl Zeiss, Inc. (Thornwood, NY) fluorescence microscope using epi-illumination at 440 nm (fluorescein) or 510 nm (rhodamine).

Immunoblotting. Extracted muscle, thymus, and cytoskeletal proteins and standards of known molecular weights (Bio-Rad Laboratories, Richmond, CA) were incubated for 30
TABLE II
Frequency of StrAb in Sera and in Growth Media of Cultured Thymic Cells from 72 Patients with MG

| StrAb produced by thymic cells | Serological evidence of StrAb | Total |
|-------------------------------|------------------------------|-------|
|                               | Positive | Negative |       |
| Positive                      | 13       | 9        | 22    |
| Negative                      | 9        | 41       | 50    |
| Total                         | 22       | 50       | 72    |

18 patients had thymoma and 6 of those were being treated with corticosteroids. StrAb were detected in sera of 14 of the 18 with thymoma, and in the thymic growth media of 8. Five of the 10 thymoma patients whose growth media were negative for StrAb, and 2 of the 4 whose sera were negative, were receiving corticosteroids. Media were assayed by ELISA using human skeletal muscle protein antigens, and sera were assayed by indirect immunofluorescence on sections of rat skeletal muscle.

Results

Antibodies reactive with skeletal muscle were detected in supernatant media of thymic cells cultured from 59% of MG patients in whom StrAb were detectable in serum and in 18% of patients without StrAb detectable in serum (Table II). These values were significantly different (p <0.005) in χ² analysis.

Proliferating B cell lines were obtained from thymus, thymoma, or peripheral blood of all patients whose lymphocytes we attempted to fuse with the GK5 myeloma (Table III). However, antibodies reactive with skeletal muscle were secreted only by cells from patients whose sera contained StrAb. Clones secreting mAbs SA-1A and SA-1B were derived from patient I. The clone secreting mAb SA-2 was from patient II. These clones have secreted StrAb for over 12 mo in continuous culture without any change in antigenic specificities (determined by immunoblot analyses and immunofluorescence assays), and periodic recloning has produced subclones only with the same antigenic specificity as the parental clone. Use of isotype-specific anti-Ig in indirect immunofluorescence assays and in immunoblots revealed that the three mAbs were of the IgM class.

The three clones were normal and diploid by chromosomal analysis, and they
were EBNA+*. The GK5 myeloma, also EBNA+, had a 7;8 balanced reciprocal translocation; the formal karyotype was 46,XY,t(7;8)(p22;q22). Therefore, there was no cytogenetic evidence that the antibody-secreting clones were hybridomas derived from somatic fusion of GK5 and patients' B lymphocytes. Because athymic nude mice injected with the clones did not develop ascites, unfiltered growth media from cultured clones were used as sources of mAbs.

mAb SA-1A discretely immunostained myofibrillar I bands (Fig. 1A). It did not react with nuclei, smooth muscle, mitochondria, or gastric mucosal cells. In agreement with its staining of I bands, mAb SA-1A bound to actin in immunoblots (Fig. 2) and ELISAs (Fig. 3A). It recognized actins from human adult muscle and erythrocytes, prefusion fetal myoblasts, and chicken gizzard (Figs. 2 and 3A). It also bound to α actinin from chicken gizzard and human muscle sources (Figs. 2 and 3A). mAb SA-1A did not stain myofibril Z lines, despite the demonstration of α actinin in that location by a rabbit antiseraum against α actinin (Fig. 1B).

mAb SA-1B also immunostained myofibrillar I bands (Fig. 1C). It reacted only with actin in ELISAs (Fig. 3B) and immunoblots (Fig. 4). mAb SA-1B additionally immunostained structures with the morphology and spatial distribution of mitochondria in a variety of cell types (Fig. 5). In sections of skeletal muscle (Fig. 5B), the staining pattern was similar to that seen with sera from occasional patients with MG and thymoma (Fig. 5D). With the exception of the striations, staining with mAb SA-1B matched the pattern obtained with control antimitochondrial antibodies in serum of a patient with primary biliary cirrhosis (Fig. 5C). In sections of other tissues mAb SA-1B bound only to mitochondria-rich structures, e.g., proximal tubules in kidney. Other structures and tissues were unstained, notably nuclei, smooth muscle, and gastric mucosal cells.

mAb SA-2 immunostained only myofibrillar A bands in muscle (Fig. 1D), and with the exception of thymus, it did not stain other structures or tissues, notably nuclei, smooth muscle, mitochondria, or gastric mucosal cells. Consistent with the A band localization in muscle, SA-2 reacted exclusively with myosin in ELISAs (Fig. 3C) and in immunoblot analyses (Fig. 6).
All the mAbs bound to proteins extracted from rat thymus that corresponded in electrophoretic mobilities to their respective contractile protein antigens in skeletal muscle (Figs. 6 and 7). mAb SA-2 selectively stained medullary epithelial
FIGURE 2. mAb SA-1A bound to actin (42,000) and α actinin (102,000) components of contractile proteins that were electrophoresed and electroblotted to nitrocellulose paper. Lanes A–E, Coomassie Blue staining; lanes F–J, SA-1A immunostaining. Lanes A and F, human skeletal muscle proteins; B and G, purified chicken gizzard actin; C and H, adult human erythrocyte proteins; D and I, human prefusion myoblast proteins; E and J, purified chicken gizzard α actinin. Numbers indicate M, × 10⁻³.

cells in sections of frog thymus (Fig. 8), but no specific staining was seen using SA-1A or SA-1B.

Discussion

This study has shown that thymus and thymoma tissues of patients with MG harbor B lymphocytes with the potential to secrete StrAb, which are a serologic marker of both MG and thymoma. StrAb were secreted by freshly isolated thymic cells cultured with PWM and by B cell clones derived from thymus, thymoma, and peripheral blood.

We did not anticipate the fortuitous transformation of B lymphocytes by EBV in attempts to fuse them with the human myeloma GK5. Presumably the manipulations with PEG either enhanced the transfer of EBV from GK5 (which is EBNA⁺) or activated endogenous EBV in the patients' lymphocytes. The latter possibility is suggested by a report (23) that diploid EBV-transformed human lymphocyte lines were generated in attempts to hybridize blood lymphocytes of cancer patients to a myeloma line from mouse, a species that is not susceptible to EBV infection.

The fact that lymphocytes committed to producing IgM are most susceptible to EBV transformation (24) may explain why all the mAbs we obtained were IgM, while StrAb in sera of patients with spontaneously acquired MG and thymoma generally are of IgG class. It is noteworthy that StrAb of IgM class appeared in sera of rheumatoid arthritis patients after treatment with D-penicillamine (25).
Reactivity with cytoskeletal proteins has been reported (26, 27) as a frequent specificity among human mAbs derived from subjects with a variety of clinical disorders. However, several lines of evidence suggest that the antigenic specificities of the monoclonal StrAb that we obtained were representative of the autoantibodies in sera of patients with MG and thymoma. First, although proliferating lymphocyte colonies were obtained from thymus or peripheral blood of all MG patients studied, B cell lines secreting StrAb were obtained only from patients who had StrAb in their sera. Second, the specificities of the mAbs for myosin, α actinin, and actin were exhibited by serum antibodies in the two patients from whom the B cell clones were established (28).

Consistent with early serological reports (29–31), the epitopes to which the mAbs bound were conserved across species, and one mAb (SA-2) was reactive with skeletal muscle myosin. mAb SA-2 bound to H chains of myosin from muscle and thymus. It also immunofluorescently stained structures containing myosin, skeletal muscle A bands and stress fibers of cultured nonmuscle cells.
FIGURE 4. mAb SA-1B bound to the actin (42,000) component of contractile proteins that were electrophoresed and electroblotted to nitrocellulose. Lanes A–D, Coomassie Blue staining; lanes E–H, SA-1B immunostaining. Lanes A and E, human skeletal muscle proteins; B and F, purified chicken gizzard actin; C and G, adult human erythrocyte proteins; D and H, human prefusion myoblast proteins. Numbers indicate M, $\times 10^{-3}$.

(28), indicating that it recognized an epitope found on cytoskeletal as well as myofibrillar forms of myosin. Additionally, it immunostained thymic medullary epithelial cells.

By using several different assays to analyze the monoclonal StrAb, we identified specificities of StrAb that were previously unrecognized. In immunofluorescence analyses on tissue sections or myofibrils, many epitopes may be inaccessible and thus apparently nonreactive, because contractile proteins in myofilaments, stress fibers, and related structures form multiprotein complexes. For example, neither mAbs SA-1A nor SA-1B immunofluorescently stained smooth muscle in sections of mouse stomach, but both reacted in immunoblots with $\gamma$ actin purified from smooth muscle.

Another unanticipated finding was that mAb SA-1A bound to both $\alpha$ actinin and actin. Antigenic relatedness between vertebrate actin and the actin-binding protein $\alpha$ actinin has not, to our knowledge, been shown previously. However, Maruta et al. (32) reported amino acid sequence homology and immunologic crossreactivity between actin and actin-binding proteins of Physarum polycephalum. Despite its reactivity with purified $\alpha$ actinin, SA-1A stained myofibril I bands, which contain actin, and not myofibril Z lines, which contain $\alpha$ actinin. If the SA-1A-reactive epitopes common to actin and $\alpha$ actinin are located at sites where actin and $\alpha$ actinin bind to each other, as depicted in Fig. 9, this anomaly would be explained, because antigenic sites in the myofibril Z line would be inaccessible to SA-1A due to the known saturation of $\alpha$ actinin with actin in that location (33).

Our observation that the actin-reactive mAb SA-1B bound to mitochondria
FIGURE 5. (A) Dual immunofluorescent staining of mitochondria in a human myoblast by the IgM mAb SA-1B (upper, fluorescein) and polyclonal IgG antimitochondrial antibodies (middle, rhodamine); corresponding phase contrast image (lower). Mitochondria in rat skeletal muscle immunofluorescently stained by mAb SA-1B (B), antimitochondrial antibodies from a patient with primary biliary cirrhosis (C), or serum from a 42-yr-old male MG patient with thymoma (D). Mitochondria-containing midpiece (arrowheads) in a human spermatozoan stained by mAb SA-1B (left); corresponding phase contrast image (right). Bars represent 5 μm.
FIGURE 6. Contractile proteins from perfused rat skeletal muscle (lane 1) and thymus (lane 2), and H chains of purified bovine myosin (lane 3) were electrophoresed in a 5% separating gel and silver stained (A) or immunostained with mAb SA-2 after blotting to nitrocellulose (B). In each preparation, mAb SA-2 reacted only with myosin H chains (200,000). Numbers at right, indicating $M_r \times 10^3$, were determined by staining coelectrophoresed proteins of known molecular weights with silver (A) or Coomassie Blue (B). Although proteins of $M_r < 100 \times 10^3$ are not shown, data reported elsewhere (28) showed that mAb SA-2 did not react with any proteins smaller than myosin H chains in a contractile protein preparation from skeletal muscle.

FIGURE 7. Contractile proteins from perfused rat thymus (lane 1) or skeletal muscle (lane 2) were electrophoresed in a 10% separating gel and silver stained (A) or immunostained with mAb SA-1A (B) or mAb SA-1B (C) after blotting to nitrocellulose. In both preparations, mAb SA-1A reacted with a actinin (102,000) and actin (42,000) and mAb SA-1B reacted with actin (42,000). Numbers at right, indicating $M_r \times 10^3$, were determined by staining coelectrophoresed proteins of known molecular weights with silver (A) or Coomassie Blue (B and C). Distances between proteins in A do not correlate with those in B or C because of artefactual distortion in drying the gel in A.
suggests either that there is a similar epitope on mitochondria and all isoactins or that actin is closely associated with mitochondria (34). The latter proposal is more likely because it has been reported that affinity-purified polyclonal antibodies raised against γ actin react with mouse skeletal muscle mitochondria (35), and that human antimitochondrial antibodies bind to proteins with the molecular weight of actin (36, 37). We showed that mAb SA-1B could also react with actin
that is not associated with mitochondria, since it bound electroblotted actin from adult human erythrocytes, which do not contain mitochondria.

In the context of MG, a most intriguing reactivity of the monoclonal StrAb was their binding to muscle and nonmuscle isotypes of contractile proteins in both thymus and muscle. It was first reported ~25 yr ago (6) that StrAb immunostain nonlymphoid cells in the thymic medulla, but the reactive antigens have not previously been identified biochemically. Because AChR are expressed on medullary epithelial cells of the human thymus (38), it is noteworthy that the contractile proteins that are reactive with the monoclonal StrAb (myosin, α actinin, and actin) copurify or colocalize (39–43) with AChR of mammalian skeletal muscle. These cytoskeletal elements, presumed to integrate AChR in the plasma membrane (44, 45), could conceivably be released by the focal membrane lysis that follows activation of complement by anti-AChR antibodies (46–48). However, it is unlikely that the mere release of these proteins provides a sufficient immunogenic stimulus to induce StrAb because StrAb are rarely found (in the absence of thymoma) in young patients with MG, regardless of the degree of complement-mediated membrane damage.

The high prevalence of StrAb in patients with thymoma and in elderly MG patients could be explained if the neoplastic transformation of thymic epithelial cells to thymoma were a critical step in the induction of StrAb. Contractile proteins are altered biochemically in the process of neoplastic transformation (49). It is therefore conceivable that contractile proteins associated with AChR in thymic epithelial cells might be altered antigenically as an early event in the pretumorous evolution of thymoma. These proteins could be released extracellularly through a complement-mediated immune attack against AChR on thymic cells, or through incorporation of membrane proteins into the envelope of a budding putative thymomagenic virus (1, 50). The expression of new epitopes on contractile proteins associated with AChR and the release of those proteins from thymic epithelial cells undergoing neoplastic transformation may provide the immunogenic stimulus that elicits production of StrAb.

**Summary**

Striational autoantibodies (StrAb), which react with elements of skeletal muscle cross-striations, occur frequently in patients with thymoma associated with myas-
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thenia gravis (MG). Dissociated thymic lymphocytes from 22 of 72 MG patients secreted StrAb when cultured with PWM. A high yield of EBV-transformed B cell lines was established from thymus, thymoma, and peripheral blood of seven patients with MG, but clones secreting StrAb arose only from the three patients who had StrAb in their sera. The monoclonal StrAb bound to A bands or I bands in skeletal muscle of human, rat, and frog. One bound to mitochondria in addition to myofibrillar I bands. None bound to nuclei, smooth muscle, or gastric mucosal cells.

In immunoblot analyses and ELISAs the monoclonal StrAb bound to muscle and nonmuscle isotypes of myosin, α actinin, and/or actin. All bound to contractile proteins common to thymus and muscle, and one selectively immunostained epithelial cells of the thymic medulla. From these antigenic specificities we suggest that StrAb might arise as an immune response directed against the cytoskeletal anchoring proteins associated with nicotinic acetylcholine receptors in thymic epithelial cells undergoing neoplastic transformation to thymoma.

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Note added in proof: As an alternative explanation for the association of thymoma and StrAb, we suggest that protein products of a class of hybrid oncogenes exemplified by onc-D and v-fgr (truncated cytoskeletal proteins joined to a putative transmembrane receptor and/or tyrosine kinase) may be the immunogenic stimulus for StrAb. Thus, anti-StrAbs, and perhaps anti-AChR antibodies and MG also, could arise as the result of an intrathymic antitumor immune response to a novel oncogene product (51, 52).

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