A Striational Muscle Antigen and Myasthenia Gravis-Associated Thymomas Share an Acetylcholine-Receptor Epitope

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The coincidence of autoantibodies against the acetylcholine receptor (AChR) and muscle striational antigens (SA) is a characteristic finding in thymoma-associated myasthenia gravis (MG), but their origins are still unresolved. Some common muscle antigens that were shown to be targets of anti-SA autoantibodies in thymoma-associated MG have also been detected in normal or neoplastic thymic epithelial cells, suggesting that the release of (eventually altered) antigens from the thymic tumors could elicit SA autoimmunity. In contrast to this model, we report here that titin, which is a recently reported target of SA autoimmunity, is not expressed in thymomas. In addition, we show that skeletal muscle type-II fibers exhibit a striational immunoreactivity with monoclonal antibody mAb155, which was previously identified to label a very immunogenic cytoplasmic epitope of the AChR and neoplastic epithelial cells of MG-associated thymomas. We conclude from these findings that titin autoimmunity in thymoma-associated MG is either due to a molecular mimicry mechanism involving tumor antigens (other than titin) or is a secondary phenomenon following release of titin from muscle. Based on the common immunoreactivity of the AChR, a striational antigen and thymoma, we suggest as the pathogenetic mechanism of thymoma-associated MG a “circulus vitiosus” in which SA autoimmunity could help maintain the AChR autoimmunity that is primarily elicited by the thymomas.

KEYWORDS: Thymoma, thymus, myasthenia gravis, acetylcholine receptor, titin, autoimmunity.

INTRODUCTION

Myasthenia gravis (MG) is characterized by an abnormal fatiguability of muscle produced in most patients by autoantibodies against the nicotinic acetylcholine receptor (AChR). These autoantibodies are thought to impair neuromuscular transmission by reducing the number of muscle endplate AChR or blocking their function (Drachman et al., 1980; Burges et al., 1990).

In addition to anti-AChR autoimmunity, 70% of MG patients exhibit thymitis with or without lymphofollicular hyperplasia (Müller-Hermelink et al., 1986; Kirchner et al., 1986) and 10% have a thymoma (Kirchner and Müller-Hermelink, 1989). As an important diagnostic feature, only the latter group of MG patients almost invariably has high titers of heterogeneous autoantibodies against striational muscle antigens (SA) in addition to anti-AChR autoantibodies (Peers et al., 1977; Aarli et al., 1990; Connor et al., 1990; Ohta et al., 1990). In thymoma patients without MG anti-SA autoantibodies occur in only 24% (Williams and Lennon, 1987).

How SA autoimmunity is elicited in thymomas is not known. However, the finding that myosin and some less well-defined muscle proteins are anti-SA-autoantibody targets that also occur in
normal or neoplastic thymic epithelial cells (Gilhus et al., 1984; Williams and Lennon, 1987; Dardenne et al., 1987) has led to the hypothesis that thymomas might be the sites of SA autosen-
sitization. Because an antigenic relationship between striational antigens and the AChR was
not known, it is obscure whether autoimmune reactions against SA and AChR are interrelated.

Recently, titin was identified as a major target of anti-SA autoantibodies in MG (Aarli et al.,
1990). In the present paper, we report that titin is not expressed in thymomas, suggesting that
this kind of SA autoimmunity is either not due to intratumorous autosensitization or occurs by
molecular mimicry mechanisms, that is, by titin-related antigenic determinants in thymoma pro-
teins that may cross-react with titin—"specific" T cells, which could induce an antititin autoanti-
body response when released to the periphery. In addition, an immunoreactivity is demonstrated
that is shared by the AChR alpha subunit and a striational antigen different from titin. Because
the respective epitope—corresponding to AChR alpha371–378—is the only one so far detected in
MG-associated thymomas, we suggest that AChR autoimmunity and part of SA autoimmunity in
MG-associated thymomas could be interrelated.

RESULTS

Titin Is Expressed in Thymic Myoid Cells but Not in Thymomas

Frozen sections of two normal thymuses were probed with antititin mAb to three different titin
epitopes. As shown in Fig. 1, immunoreactivity with all three mAbs was seen in a few round or
spindle-shaped cells inside the medulla of normal thymuses close to Hassal's corpuscles. Both
the localization and the morphology of these cells are typical of thymic myoid cells (Kirchner et al.,
1988). This was confirmed by double immunolabeling of thymic myoid cells by both antide-
smin or anti-AChR mAb and by antititin mAb T12 (Figs. 2a and 2b). There was no expression of titin
in keratin-positive nonneoplastic thymic epithelial cells (Fig. 2c).

Because of the high incidence of antititin auto-
antibodies typical of MG/thymoma patients
(Aarli et al., 1990), we looked for an aberrant
expression of titin in thymoma because the de-
ovo expression of other proteins has previously
been reported as a typical feature of neoplastic
thymic epithelium (Willcox et al., 1987; Marx et
al., 1990, 1991). However, with respect to the

FIGURE 1. Immunoreactivity of a few round or spindle-shaped cells inside the human thymic medulla with antititin
monoclonal antibodies T4 (a), T12 (b), and T32 (c). Morphology and localization are typical of thymic myoid cells (cf. Fig. 2). No
immunoreactivity is encountered in epithelial cells. Immunoperoxidase technique on frozen sections (×160).
absence of titin, the thymoma epithelium resembles its normal counterpart (Figs. 1 and 3a). The lack of titin expression in thymomas was independent of tumor type and independent of aberrant expression of the AChR epitope alpha371–378 in the thymoma epithelial cells (Fig. 3b).

A Striational Antigen, MG-Associated Thymomas, and the AChR Share a Common Immunoreactivity

Investigations of skeletal muscle by anti-AChR mAbs showed a striational pattern only with mAb155 (Fig. 4a) that is known to be directed against the AChR alpha-subunit epitope alpha371–378 (Tzartos et al., 1986) and against MG-associated thymomas (Fig. 3b and Kirchner et al., 1988). A cross-reaction of mAb155 with muscle was reported previously (Kirchner et al., 1988) but the striational staining pattern was not appreciated then. No striational pattern was seen with mAb195 (Fig. 4b) directed against alpha67–76, the main immunogenic region (MIR) of the AChR (Tzartos et al., 1988). Obviously, the intensity of the striational staining by the mAb155 is unevenly distributed among different muscle fibers of the quadriceps (Fig. 4a) in contrast to the even distribution of antititin staining (Fig. 4c). As shown in Fig. 5, mAb155 immunoreactivity is mainly present in type-II fibers.

DISCUSSION

Many steps in the pathogenesis of AChR autoimmunity in MG are now well understood. In particular, a pathogenetic link between autoimmunity and thymic pathology has been suggested by characterizing AChR epitopes (Kao and Drachman, 1977; Wekerle et al., 1978; Schluep et al., 1986; Kirchner et al., 1988a, 1988b; Marx et al., 1991) and AChR-specific autoagressive B and T cells in both MG thymus and thymoma (Fuji et al., 1984; Vincent et al., 1987; Hohlfeld et al., 1984; Melms et al., 1989; Sommer et al., 1990). In contrast, the events triggering the autosensitization process and the role of concomitant autoimmunity to striational muscle antigens (SA) in thymoma-associated MG have not yet been defined (Aarli et al., 1990; Hohlfeld, 1990).

However, four recent findings favor the
exclude this hypothesis, but offer other pathogenetic possibilities as well. The absence of detectable titin in MG thymomas (Fig. 3) could imply that an epitope shared by some other thymoma protein is responsible for antititin autoimmunity. The latter could instead be a delayed consequence of titin release from muscle damaged by anti-AChR autoantibodies, but that would not explain why MG patients without thymoma seldom make a similar response. Alternatively, titin released from myoid cells (Figs. 1 and 2) in the residual thymus close to the tumor could be the autosensitizing molecule, but the same objection would apply here, too. In any case, these possibilities are all different from the model of tumor-triggered striational autoimmunity due to proteins shared by thymomas and muscle (Williams and Lennon, 1986; Dardenne et al., 1987). It is tempting to invoke some common cryptic epitope that initiates both autoimmune reactions, in view of the high and almost identical frequency of antititin and anti-AChR autoantibodies in thymoma-associated MG on the one hand (Newson-Davis et al., 1987; Aarli et al., 1990), and the absence of detectable molecules of either antigen in MG-associated thymoma on the other (Fig. 3 and Kirchner et al., 1988a; Marx et al., 1989; Geuder et al., 1989). Whatever proteins or epitopes may be involved, we assume that cross-reacting T cells are either stimulated (Kirchner et al., 1988; Marx et al., 1989) or erroneously tolerised (Marx et al., 1991) within the thymomas and induce an autoantibody response after export to the periphery.

Pathogenetic connections between the three hallmarks of paraneoplastic MG—autoantibodies against the AChR and striational antigens, and the occurrence of thymomas—have yet to be proven. Here we show that a special AChR epitope that is expressed in most MG-associated thymomas (corresponding to the AChR alpha-subunit sequence alpha371-378; Kirchner et al., 1988a; Geuder et al., 1989) is also present in a striational antigen (Fig. 4a). So far, the nature of the antigen bearing this AChR epitope is unresolved, but it is clearly different from titin (Fig. 3a versus 3b, and Fig. 4a versus 4c). The preferred expression in one muscle fibre type (Fig. 5) suggests that it is either an isoform of a protein of the contractile apparatus or a protein related to the metabolic function of a particular muscle fibre type (Dubowitz, 1985). Further investigations
FIGURE 4. Immunoreactivity of a striational muscle antigen in about 50% of quadriceps muscle fibers with anti-AChR mAb155 directed against the AChR epitope alpha371-378 (a). No reactivity of muscle with anti-AChR mAb195 directed against the AChR "main immunogenic region" (Tzartos et al., 1983) (b). In contrast to the striational immunoreactivity of mAb155, the immunoreactivity with antititin mAb T12 is encountered in 100% of muscle fibers (c), demonstrating the nonidentity of titin and the AChR-epitope-bearing striational antigen in human quadriceps muscle (all sections #5361). Frozen sections, immunoperoxidase (×400).

FIGURE 5. Immunoreactivity of anti-AChR mAb155 with a striational antigen is confined to type-II fibers in the quadriceps muscle. Serial cross sections labeled with mAb155 (a; immunoperoxidase) and reacted for ATPase activity at pH 9.4 (b). Immunoreactivity with mAb155 coincides with a high ATPase activity (dark color) typical of type-II fibers (Dubowitz, 1985) (×400).

have to prove whether the striational antigen immunolabeled by mAb155 (Fig. 4a) is identical to the thymoma proteins bearing the AChR epitope detected by mAb 155 (Marx et al., 1989,
TABLE 1
Clinical and Pathological Findings in MG Patients with Thymic Epithelial Tumors and in Controls

| Case No. | Sex | Age (y) | Histologic diagnosis | AChR Epitope |
|----------|-----|---------|---------------------|--------------|
| 19964    | F   | 34      | Mixed thymoma       | -            |
| 29117    | F   | 64      | Mixed thymoma       | -            |
| 15977    | F   | 64      | Cortical thymoma    | +            |
| 6942     | M   | 50      | Cortical thymoma    | +            |
| 22401    | F   | 63      | Well-differentiated thymic carcinoma | + |
| 27920    | M   | 45      | Well-differentiated thymic carcinoma | + |
| Thy2     | F   | 2       | Normal thymus       | +b           |
| Thy40    | M   | 40      | Normal thymus       | +b           |
| S361     | M   | 2       | Quadriceps muscle   | +c           |
| S64      | F   | 81      | Quadriceps muscle   | +c           |

\(^{a}\)Immunoreactivity with anti-AChR mAb155 (Tzartos et al., 1986).

\(^{b}\)Immunoreactivity of mAb155 with thymic myoid cells and some medullary epithelial cells (Kirchner and Müller-Hermelink, 1989).

\(^{c}\)Striational pattern in type-II fibers.

1990), and whether either molecule is in fact a target of autoimmune B or T cells in vivo. If the latter question could be positively answered, our finding suggests a pathogenetic model in which striational autoimmunity might help to maintain AChR autoimmunity. Such a model of a “circulus vitiosus” would explain the clinical experience that removal of the thymoma does not ameliorate the MG.

MATERIALS AND METHODS

Patients and Tissues

Six thymomas from MG patients, two normal thymuses (obtained during thoracic surgery), and normal quadriceps muscle (obtained from autopsies within 6 hr after death) were investigated using cryostat sections from snap frozen tissue. Tumors were classified according to Kirchner and Müller-Hermelink (1990). Clinical data of patients are summarized in Table 1.

The diagnosis of generalized myasthenia gravis was based on clinical findings, including an abnormal muscle fatiguability, electrophysiological investigations and anti-AChR serum titers that were above 1 nmol/L.

Immunohistochemistry

The monoclonal antibodies (mAb) used in this study are described in Table 2. The immunohistochemical three-stage immunoperoxidase technique and the double-labeling procedure combining the immunoperoxidase with an alkaline phosphatase technique (as the first and second step, respectively) were as described previously, including the same control experiments (Kirchner et al., 1988).

Muscle Fiber-Type Determination

To investigate whether skeletal muscle immunoreactivity with anti-AChR mAb155 was confined to either type-I or -II muscle fibers serial frozen sections were processed for routine enzyme histochemical determination of ATPase activity at both pH 4.6 and pH 9.4 (Dubowitz, 1985).

TABLE 2
Antibodies Used in This Study

| Antibody  | Concentration or dilution applied | Antigen labeled | Source                        |
|-----------|----------------------------------|-----------------|-------------------------------|
| 35betaH11 | 1:100                            | Keratin No. 8/18| Gown and Vogel, 1982         |
| Desmin    | 1:1000                           | Desmin          | Laboserv Giessen, Germany     |
| T4        | 1:50                             | Nonrepetitive titin epitope inside the I band | Fürst et al., 1988 |
| T12       | 1:10                             | Nonrepetitive titin epitope close to the Z line | Fürst et al., 1988 |
| T32       | 1:5                              | Repetitive titin epitope within the A band | Fürst et al., 1989 |
| mAb155    | 1 µg/mL                          | AChR-epitope alpha371-378 | Tzartos et al., 1986 |
| mAb195    | 1 µg/mL                          | AChR-epitope alpha67-76 (MIR\(^{a}\)) | Tzartos et al., 1988 |

\(^{a}\)MIR=main immunogenic region (Tzartos et al., 1988).
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