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Modeling the intrathymic pathogenesis of myasthenia gravis

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ABSTRACT

Myasthenia gravis is (MG) a prototypic autoimmune disease; the immune effector mechanisms and autoantigenic target have been delineated. However, the events that lead to the abrogation of self-tolerance to neuromuscular acetylcholine receptors (nAChRs) remain a mystery. The thymus gland has long been considered to hold the key to solving this mystery, although the nature of its involvement remains to be elucidated. The nAChR was one of the first self-proteins associated with a defined autoimmune disease that was found to be expressed on thymic stromal populations. The studies described herein represent our efforts to determine how this "promiscuous" autoantigen expression may be involved in the immunopathogenesis of MG. We review our work, characterizing the expression of the nAChR alpha subunit in the thymus, and advance a hypothesis and experimental model, which explore how intrathymic expression of this autoantigen may contribute to the immunopathogenesis of this autoimmune disease.

1. Introduction

My relationship with Bob Lisak goes back to 1974 when I was an Allergy/Immunology fellow at Penn and continued when I joined the Penn faculty in the Division of Allergy and Immunology in 1978. Bob was a spectacular mentor, who along with his good friend and colleague Burt Zweiman, was instrumental in getting my academic career off to a successful start. Just as importantly, he was a terrific role model, a veritable triple threat, always incredibly enthusiastic about his science, teaching, and patient care. Intense – yes – but able to kick back and enjoy other important life pursuits including, music, art, sports, a good joke, good food, wine, and single malt. I salute Bob for his long, distinguished career as a consummate academician and neuroimmunologist.

Bob is singularly responsible for my interest in myasthenia gravis (MG) and it is this topic which I will address in this monograph. Specifically I will focus on studies carried out in my laboratory that address the role of the thymus in the pathogenesis of MG. In particular, I focus on how perturbations of the thymus may lead to a breach in self-tolerance and the induction of anti-AChR antibodies.

2. Evidence supporting a primary role of the thymus in the pathogenesis of MG

MG is an autoimmune disease characterized by weakness of striated muscles. It is caused by IgG antibodies directed at neuromuscular acetylcholine receptors (nAChRs) (reviewed in [1]). These autoantibodies impair neuromuscular transmission by causing a reduction in the number of these receptors. MG is a prototypic autoimmune disease; the immune effector mechanisms and autoantigenic target have been delineated (reviewed in [1]). However, the events that lead to the abrogation of self-tolerance to nAChRs remain a mystery. The thymus gland has long been considered to hold the key to solving this mystery, although the nature of its involvement remains to be elucidated. Interest in a primary role for the thymus has been fueled by pathologic, clinical, and immunologic lines of evidence (summarized in Table 1).

Attention was originally focused on a potential pathogenic role of the thymus following the recognition of striking pathologic changes in this organ at the time of autopsy. A pattern of germinal center hyperplasia is observed in the thymus of 70% of patients with early onset of MG (prior to age 40 years) (reviewed in [2,3]). Another 10% of MG patients' thymi display thymomas, i.e., tumors of epithelial cell origin (reviewed in [2]). The architecture of the hyperplastic thymi is generally preserved with well demarcated cortical and medullary regions. However the medulla is crowded by numerous germinal centers (GCs), which display the architectural features and cellular constituents of GCs seen in the secondary follicles of peripheral lymph nodes from healthy subjects. The GCs extend into thymic medullary perivascular spaces. By contrast, the thymic architecture is severely altered in patients with thymoma. Normal appearing thymocytes are admixed with neoplastic epithelial cells with the loss of a distinct corticomedullary demarcation. Thymoma lymphocytes show the immunophenotypic properties of normal immature cortical thymocytes.

Clinical evidence incriminating the thymus comes from empiric trials of thymectomy in MG patients [4,5]. Although no controlled study has ever been carried out, the accumulated experience indicates that thymectomy, particularly in young patients with germinal

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Table 1
Evidence for role of thymus in pathogenesis of MG.

- Pathological
  - GC hyperplasia and thymoma
- Clinical
  - Apparent benefit of thymectomy
- Immunological
  - nAChRα reactive thymic CD4+ T and B cells
  - Decreased function and number of thymic regulatory T cells
  - Increased expression of CXCL13 and CCL21 MG hyperplastic thymi
  - Increased thymic epithelial cell expression of IL-1 and IL-6
  - Increased MG thymic expression of BAFF and APRIL
- Structural
  - nAChR subunits expressed on myoid cells and thymic epithelia cells

center hyperplasia is associated with an excellent outcome, measured as either remission or an improvement in clinical symptoms. The mechanisms responsible for the beneficial effect of thymectomy remain to be elucidated. Although anti-AChR antibody titers gradually fall post thymectomy, it is not clear that the fall in titer is linked causally to improvement. No obvious effects on immunoregulatory mechanisms have been demonstrated. Thymectomy is also the recommended treatment for patients of all ages suspected of having thymoma.

From an immunologic standpoint the MG thymus has unique features that could reflect pathogenic involvement and help explain some of the histologic abnormalities. B cells and immunoglobulin-secreting cells are rare inhabitants of normal thymi but they are found in increased numbers in cell suspensions of MG thymus, particularly hyperplastic thymus relative to control thymus (obtained from subjects undergoing elective cardiothoracic surgery) [6–9]. This finding suggests that such B cells have undergone antecedent in vivo activation.

Single cell suspensions prepared from MG thymi with germinal center hyperplasia secrete anti-AChR antibodies [10–12]. However, the thymic B-cells also secrete anti-influenza and anti-tetanus toxoid antibodies [10,11]. The latter are only detected when patients are booster immunized to tetanus toxoid 3–4 weeks prior to thymectomy. Thus, the B-cell repertoire in the MG thymus may reflect systemic as well as local immune events.

AChR-reactive CD4+ T cells have been detected in MG thymus (both thymoma and hyperplasia), but not in normal thymus [13,14]. These cells are enriched in the thymus relative to the blood of the same MG patient [14]. Migration of T cells reactive to foreign antigen into the thymus is known to occur [15–20]. Therefore, it is not clear whether AChR-reactive T cells in MG thymus are sensitized in the periphery with subsequent intrathymic localization or are sensitized in situ. The status of intrathymic T-regulatory cells is less clear with some reports finding decreased or normal numbers with perhaps decreased function [21–23].

Numbers of IL-1- and IL-6-producing epithelial cells are increased in hyperplastic MG thymus [24]. These cells were found largely in the perifollicular areas and connective tissue adjacent to the septae of disrupted cortex. Cells producing IL-2 were less prominent and confined largely to perifollicular areas. This distribution of cytokine production was not seen in normal thymus or in hyperplastic lymph nodes. MG thymus is also characterized by increased expression of B-cell activating factor of the tumor necrosis factor (BAFF) and A proliferation-inducing ligand (APRIL) relative to control thymus [25]. DNA microarray analysis has revealed overexpression of CXCL13 and CCL21 by epithelial cells in hyperplastic MG thymus [26,27]. These chemokines are involved in germinal center formation in the peripheral lymphoid system. It was suggested that the overexpression of CXCL13 and CCL21 served to attract B cells and activated T cells to the MG thymus.

3. Intrathymic expression of nAChR and its regulation

For over thirty years investigators have appreciated that nAChRs are expressed on/in cells resident in the thymus. This finding led to the hypothesis, first espoused by Werkele [28], that the thymus represents an important focus for initiating or perpetuating the autoimmune response in MG. This idea runs counter to current immunological dogma, which defines the pivotal role played by thymic self-proteins, particularly those expressed on epithelial cells, in the induction of self-tolerance [29].

nAChRs are expressed in two major forms (reviewed in [1]). The so-called mature or junctional form is expressed on innervated muscles and the immature or fetal form is expressed on non-innervated tissue. At the mature (innervated) myoneural junction, nAChRs are comprised of 4 subunits labeled α, β, δ, and ε. Two alpha subunits and one each of the other subunits are assembled, like the whalebone in a corset, to form an asymmetric hourglass channel spanning the membrane. Two alternatively spliced alpha subunit isoforms have been characterized, P3A− and P3A+. The larger P3A+ isoform, which includes an additional sequence of 25 amino acids between exons 3 and 4, is found only in humans and other primates. In fetal muscle, as in adult denervated muscle or nonjunctional membrane, a γ subunit replaces the ε subunit found on nAChRα at mature, innervated muscle endplates.

Expression of the receptor α chain has been reported on a wide variety of thymic cells including epithelial cells [30], thymocytes [31], and myoid cells [28,32,33]. Myoid cells were initially viewed as the principal AChR-expressing cells in thymus [28,33]. These cells, which phenotypically resemble skeletal muscle cells, are found in the medulla of both normal and MG thymi. We and others have been particularly interested in the intrathymic expression of the nAChR alpha subunit (nAChRα), since this subunit contains disease relevant B and T cell epitopes [34–37].

Using an RT-PCR, several groups, including our own, re-examined this question. We initially reported that mRNA for the nAChRα was expressed in normal mouse, normal human, and MG thymus (Table 2) [38,39]. In addition, we reported that nAChRα mRNA was expressed on transformed murine thymic cortical and medullary epithelial cell lines and thymic dendritic cell lines [38]. Moreover we provided evidence for the first time that mRNAs encoding both major isoforms of the human AChRα, i.e., P3A+ and P3A−, were expressed in normal and MG thymi and normal human thymic epithelial cells (TECs) [39,40]. We also showed that the nucleotide sequences of these isoforms were identical to their counterparts expressed at the myoneural junction and provided evidence for the expression of a third, albeit minor, nAChRα isoform. Subsequently, others reported that nAChRα protein as well as mRNA was expressed on human TECs [41].

The RT-PCR studies engendered considerable debate about the expression of other nAChR subunits on thymic cells and whether they are expressed as components of intact receptors. Some of the reported discrepancies may reflect differences in the ages of the thymus donors and differences in the design of the RT-PCRs. A distillation of these studies suggests that the nAChR β and ε mRNAs are expressed in most normal and MG thymus specimens with variable expression of δ and γ subunits [41,42]. Myoid cells appear to be the principal cell type expressing an intact nAChR, which has the characteristics of a fetal nAChR, namely it features a δ subunit rather than an

Table 2
Summary of Early nAChRα RT-PCR studies.

- nAChRα expression
  - Normal mouse, control human, MG thymus
  - Human thymic epithelial cell line
  - Mouse transformed thymic stromal cell lines
    - Dendritic cell lines
    - Cortical and medullary epithelial cell lines
  - Thymic nAChRα nucleotide sequences identical to counterparts at myoneural junction

Wheatley et al. J Immunol 1992;148;3105
Wheatley et al. Ann N Y Acad Sci 1993;681:74