Immunopathology and Infectious Disease

Myasthenia Gravis Thymus

Complement Vulnerability of Epithelial and Myoid Cells, Complement Attack on Them, and Correlations with Autoantibody Status

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In early-onset myasthenia gravis, the thymus contains lymph node-type infiltrates with frequent acetylcholine receptor (AChR)-specific germinal centers. Our recent evidence/two-step hypothesis implicates hyperplastic medullary thymic epithelial cells (expressing isolated AChR subunits) in provoking infiltration and thymic myoid cells (with intact AChR) in germinal center formation. To test this, we screened for complement attack in a wide range of typical generalized myasthenia patients. Regardless of the exact serology, thymi with sizeable infiltrates unexpectedly showed patchy up-regulation of both C5a receptor and terminal complement regulator CD59 on hyperplastic epithelial cells. These latter also showed deposits of activated C3b complement component, which appeared even heavier on infiltrating B cells, macrophages, and especially follicular dendritic cells. Myoid cells appeared particularly vulnerable to complement; few expressed the early complement regulators CD55, CD46, or CR1, and none were detectably CD59*. Indeed, when exposed to infiltrates, and especially to germinal centers, myoid cells frequently labeled for C1q, C3b (25 to 48%), or even the terminal C9, with some showing obvious damage. This early/persistent complement attack on both epithelial and myoid cells strongly supports our hypothesis, especially implicating exposed myoid cells in germinal center formation/autoantibody diversification. Remarkably, the similar changes place many apparent AChR-seronegative patients in the same spectrum as the AChR-seropositive patients. (Am J Pathol 2007, 171:893–905; DOI: 10.2353/ajpath.2007.070240)

More than 80% of patients with typical generalized myasthenia gravis (MG) have IgG autoantibodies (IgG1 and IgG3) against the muscle acetylcholine receptor (AChR) in its native conformation (and are designated AChRab†).1,2 These antibodies cause receptor loss, and thus weakness, by accelerating AChR degradation1,3 and especially by activating complement.1,4 Another 5 to 10% of cases instead have (predominantly IgG4) autoantibodies against the muscle-specific kinase (MuSK).5–8 These MuSKab* patients’ MG tends to be...
more severe, more bulbar, and apparently harder to control with corticosteroids and azathioprine. It is extremely rare to find anti-AChR and anti-MuSK antibodies in the same patient. The remaining 10 to 15% of MG patients seem to have neither antibody in standard radioimmunoprecipitation tests and are usually termed seronegative (“SNMG”). Their MG nevertheless improves after plasma exchange, implying that they too have autoantibodies. Identifying their target(s) and developing an equally clear antibody test should save many delays in diagnosis.

In patients with early-onset anti-AChRAb+ MG (EOMG), the myasthenia often ameliorates after thymectomy, and characteristic thymic changes are found in >80% of cases. These include epithelial hyperplasia and extra-paracrine infiltration by lymph node-like tissue with T-cell areas and germinal centers (GCs). We have hypothesized that autosensitization is a two-step process: First, helper T cells are primed by unfolded AChR subunits that are expressed in medullary thymic epithelial cells (mTECs). Next, early antibodies against these subunits then attack rare muscle-like myoid cells nearby. These express intact AChR and have long been implicated in autoimmune response, leading to autoantibody diversification. Myoid cells are the only cells known to express whole AChR outside muscle, where lymphoid infiltrates are minimal in MG. By contrast, in the thymus, myoid cells colocalize significantly with these GCs, especially in cases of recent MG onset, which clearly incriminates them still further in pathogenesis. Attack on them and/or destruction by complement could explain their uneven distribution and/or their occasional rarity in EOMG. In MuSKAb+ MG, the thymus is typically normal-for-age, and such hyperplasia is rare, but some infiltrates are seen in 30% to 50% of SNMG cases.

Seeking more direct evidence to implicate thymic myoid and/or epithelial cells in the response, we have now looked for signs of complement attack on them and for expression of the complement-regulatory proteins CD46, CD55, and CD59. The ability to label these markers in routine paraffin sections has enabled us to study a large series of these uncommon cases collected over ~25 years. Our findings further implicate myoid cells and mTECs in the pathogenesis not only of EOMG but also of SNMG.

**Materials and Methods**

**Clinical Material**

With informed consent and ethical committee approval, we studied thymic tissue from the same 11 adult age-matched controls (mostly undergoing thyroid or parathyroid surgery in Würzburg) and the same 67 patients with generalized MG as in Leite and colleagues (detailed in Supplemental Table at http://ajp.amjpathol.org). Their MG was diagnosed by clinical and electromyographic criteria in several centers; these patients comprised 23 with AChRAb+ MG (=EOMG), 14 with MuSKAb+ MG, and 30 with SNMG (clearly seronegative for both antibodies). We also included another eight generalized MG cases with previously borderline antibodies that now proved low-positive (0.5 to 2 nmol/L) with the higher AChR concentrations currently available (and negative against MuSK); these cases are designated AChRAb+ here.

**Thymic Sections and Antibodies**

Thymic sections (5 μm) from routine formalin-fixed, paraffin-embedded blocks were mounted on 3-amino-propyl-triethoxy-silane-coated slides. The sections were dewaxed and rehydrated by graded ethanol solutions and then either microwaved in Target Retrieval Solution (DakoCytomation, Glostrup, Denmark) for 10 minutes at 900 W (for most antibodies) or pretreated with protease type XXIV [0.0125% solution (w/v) in phosphate-buffered saline (PBS; Sigma, Gillingham, Dorset, UK)] at 37°C for 30 minutes for the antibodies asterisked in Table 1, which lists all of the antibodies used.28–32

**Immunohistochemistry**

Microwaved sections were incubated at 20°C in a peroxidase-blocking reagent for 10 minutes (DakoCytomation) and for 30 minutes with monoclonal antibodies to human CD3, CD1a, or cytokeratin at optimized dilutions in PBS. After two washes in PBS, binding was detected with the peroxidase-based Envision method, before washing and counterstaining with hematoxylin, washing in tap water, and mounting.

**Double-Immunofluorescence Labeling**

Pretreated sections were incubated for 30 minutes at 20°C with a mixture of two primary antibody dilutions. After two 5-minute washes in PBS, the sections were then incubated with isotype-specific secondary antibodies conjugated to Alexa Fluor 488 or Alexa Fluor 568 (Molecular Probes, Leiden, The Netherlands) at 1:200, for 45 minutes at 20°C. After two further washes, slides were mounted, and nuclei counterstained with 4,6-diamidino-2-phenylindole in fluorescence mounting medium (DakoCytomation).

We used paraffin sections of tonsils or biopsies from rejecting or IgA-nephropathic kidneys as positive controls. Negative controls included either irrelevant primary antibodies matched for species/isotype or none at all. The slides were stored at 4°C for 24 hours and then analyzed on a Zeiss fluorescence microscope (Welwyn Garden City, UK). All of the images were captured via a cooled digital camera, using MacProbe V3.4 software (Applied Imaging, Newcastle-upon-Tyne, UK).
Immunofluorescence Analysis

All slides were coded and analyzed systematically by a single blinded observer (M.I.L.). Entire sections labeled for CD3, CD1a, or cytokeratin (CK) were used to measure areas of total thymic tissue and its compartments; GCs were counted throughout entire anti-CD20/CD21/CD35-labeled sections to calculate their frequencies/mm² of thymic tissue.

Myoid cells were counted throughout two entire sections from each case (one double-stained for desmin/CD20 and the other for desmin/cytokeratin); counts were averaged when we calculated the percentage of exposed myoid cells, ie, those in direct contact with, or wholly within, any infiltrates (see Figure 6). We assessed their disposition in different compartments in all of the sections stained for desmin/cytokeratin. Optimal staining for desmin (in myoid cells) required different retrieval conditions from C3b and C9 (but not from CD59). Double labeling therefore demanded compromises; these were made at the expense of desmin, which normally stains strongly. We might thus have overlooked some small/weakly desmin cells.

In every staining combination for complement regulators, components, or receptors, the overall distribution of the labeling was noted (diffuse versus patchy, blood vessels versus parenchyma), and each main feature was graded in each entire section according to its extent and intensity (−, no staining; +/−, very weak or very rare; +, weak or sporadic; ++, moderate or frequent; +++, strong or extensive; ++++, very strong staining throughout the section). We recorded labeling on myoid cells according to the thymic compartments in which they were found. Most staining combinations were studied systematically in every thymus, but CD46, CD55, and C1q only in two representative samples from each subgroup (including one MuSKAb⁺ thymus with infiltrates and one without).

Statistical Analysis

We used the Kruskal-Wallis one-way analysis of variance test followed by Dunn’s post test (for heterogeneity), linear regression, and χ² with Yates’ correction.

Results

Overview of Distinct Thymic Compartments

In all of the MG thymi, the cortex was essentially normal, as expected, apart from some evidence of atrophy/fatty replacement. This was more evident after steroid treatment in some cases (Supplemental Table at http://ajp.amjpathol.org), which thus enriched the medulla and infiltrates, but had very few other obvious effects. Nearly all MG samples also included areas of relatively normal medullary parenchyma (nMed) with abundant CD4 and CD8 T cells, dendritic cells, macrophages, and thymic epithelial cells (TECs), as well as numerous CD20⁺ B cells mainly around the Hassall’s corpuscles.

In many of the MG thymi, other parenchymal areas were compressed into characteristic medullary epithelial bands (MEBs) by expanding perivascular infiltrates, which were negative for cytokeratin. These infiltrates consisted primarily of lymph node-type T-cell areas, including many antigen-presenting cells, high endothelial...
Figure 1. Distribution of complement receptors C3aR, C5aR, and CR1 (receptor for C3b and C4b) (all in red) in epithelial areas and/or infiltrates in thymi from non-MG controls (A and B), AChRAb+ (C–E), or SNMG (F) MG patients. A and B: In control thymi, occasional mTECs are weakly C5aR, as in some areas in MG thymi, but labeling for C3aR is almost absent, even in blood vessels (arrowheads) (female donor 29 years of age). C–F: In MG, both C3aR and C5aR are expressed strongly in most samples with infiltrates (INF) ± GCs. C5aR is mainly on epithelial cells (cytokeratin in C; green), whether in the relatively normal medulla (nMed) or MEBs (C, D, and F), whereas most C3aR labeling is seen in the infiltrates and particularly in the GC (D–F), and in blood vessels (white arrowheads) (D and E). MG donors all female: C, 17 years of age; D, 20 years of age; E, 29 years of age; and F, 38 years of age (only F was taking steroids). Original magnifications, ×200.
venules, and some B cells, as well as GCs varying in number and size. Infiltrates are quantitated (in the same samples) in Leite and colleagues.\(^\text{25}\) In brief, they were rare/small in the controls, although they were age-matched adults. However, they were seen in nearly all of the AChRAb\(^+\) MG thymi (including the AChRAb\(^+\)) and also in >50% of the SNMG samples but in only 3 of the 14 with MuSKAb\(^+\) MG.\(^\text{25}\) The distribution of myoid cells is detailed in the final section. The changes in MG are described below in order of increasing abnormality.

Expression of Complement Receptors \([C5\alpha R, C3\alpha R, and CR1 (=CD35)]\)

Numerous cell types (T, B, and dendritic cells and macrophages) normally express receptors for activated complement components. In both MG and normal thymi, some TECs stained for C5\alpha R, mainly in subcapsular and medullary areas (especially around the Hassall’s corpuscles) rather than in the cortex (Figure 1A). Surprisingly, in the MG samples with infiltrates, we saw increased staining on many of the mTECs in the MEBs as well as in the nMed, but it was minimal in the infiltrates (Figure 1C; summarized in Table 2).

In general, C3\alpha R showed an inverse distribution to that of C5\alpha R (Figure 1, D and F; Table 2). It was seen in only two of the controls, where it was largely confined to blood vessels (Figure 1B, arrowheads). In most MG samples, it was seen also on occasional patches of mTECs (Figure 1D). Although many extraparenchymal T and B cells were C3\alpha R\(^+\) too, it was strongest/most abundant on the follicular dendritic cells in the GC (Figure 1, D–F) and also in blood vessels (Figure 1, D and E). The receptor for C3b, CR1 (CD35), was essentially confined to GCs (Figure 1E) and was not detected in the controls.

### Table 2. Summary of the Range and Intensity of Labeling in the Main Thymic Compartments in MG Samples with Infiltrates

<table>
<thead>
<tr>
<th>Complement receptors:</th>
<th>Complement regulators:</th>
<th>Activated complement components:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C5\alpha R</td>
<td>C3\alpha R</td>
</tr>
<tr>
<td>Cortex</td>
<td>(−)</td>
<td>(+)</td>
</tr>
<tr>
<td>MG (Con)</td>
<td>(+)</td>
<td>(−)</td>
</tr>
<tr>
<td>nMed</td>
<td>(+)</td>
<td>(+)</td>
</tr>
<tr>
<td>MG (Con)</td>
<td>(++/+ +/−)</td>
<td>(+/−)</td>
</tr>
<tr>
<td>MEBs</td>
<td>(+/−)</td>
<td>(+)</td>
</tr>
<tr>
<td>MG Infiltrates</td>
<td>(++/−)</td>
<td>(+/−)</td>
</tr>
<tr>
<td>MG</td>
<td>(+/−)</td>
<td>(+/−)</td>
</tr>
<tr>
<td>Myoid cells</td>
<td>(+/−)</td>
<td>(+/−)</td>
</tr>
<tr>
<td>Blood vessels</td>
<td>(+/−)</td>
<td>(+++/+ −−)</td>
</tr>
<tr>
<td>MG (Con)</td>
<td>(+)</td>
<td>(+)</td>
</tr>
</tbody>
</table>

Maximal levels for controls (Con) are shown in parentheses where applicable. Each staining was graded in each entire section from − to +++ according to its extent and intensity: (−), weak or very weak; (+), moderate or frequent; (+/+), strong or extensive; and ++++, very strong staining throughout the section. The controls had virtually no MEBs or infiltrates and MG samples without infiltrates (eg, in MuSKAb\(^+\) MG) were broadly similar. Labeling in septa and surrounding connective tissue was similar to that in blood vessels, but generally weaker, especially for C3\alpha R and CD59. Distributions were very similar for CD46 and CD55. nMed, relatively normal medullary areas; MEBs, medullary epithelial bands.

In the various MG subgroups, the above changes occurred in any thymus with sizeable infiltrates (summarized in Table 2), correlating with their frequency and extent. Therefore, they were somewhat more prominent in the AChRAb\(^+\) than the AChRAb\(^−\) and apparently SNMG samples but were rare in MuSKAb\(^+\) thymi. The C3\alpha R and C5\alpha R expression implies the potential to respond to any available activated complement component, subject to complement regulation.

Expression of Complement Regulators \([CR1 (=CD35), CD46, CD55, and CD59]\)

On binding their targets, antibodies may initiate the classical complement cascade by activating C1, leading to formation of the C3 convertase (C4b2a) and activation (conversion) of C3, so generating both active C3b fragments and the C5 convertase (C4b2a3b). This leads to cleavage of C5 and assembly of pore-forming membrane-attack complexes (MAC; C5b-C9) in target cell membranes.\(^\text{35}\)

The major cell-bound complement regulators are CD55 (DAF), which accelerates decay of the C3 convertase, CD46, which enhances enzymatic degradation of C3b and C4b, and CD59, which blocks assembly of C9 into the MAC.\(^\text{34}\) CR1 (CD35) can also regulate the C3 convertase but is primarily a receptor for activated C3. We screened all thymi for CD59 and CR1 and two representative samples from each patient subgroup for CD46 and CD55. We detected CR1 in all GCs (Figure 1E), but not elsewhere.

In control thymi, labeling for CD46 (Figure 2A) and CD55 (not shown) was minimal and primarily restricted to blood vessels in the medulla and septa (Table 2). By contrast, CD59 was more widespread, being found, in addition, on scattered macrophages and dendritic...
cells, as well as on some subcapsular and medullary TECs (especially around Hassall’s corpuscles; Figure 2D), like C5aR, but more variable in extent and intensity.

In MG, CD46 and CD55 were again patchily up-regulated in the nMed, and especially in the MEBs (Figure 2B, C, and E-I). Cytokeratin (CK, green). A: In controls, CD46 (A) and CD55 (not shown) expression is minimal, in MG, both are much stronger in the MEBs than in the nMed in both AChRAb+ (B and F) and SNMG (C) MG. They are also seen on blood vessels, some mTECs, and other cells (eg, macrophages), and especially in the GC, CD59 sometimes shows a linear distribution at MEB borders (F, bottom left) like that of laminin. D: In controls, CD59 is expressed by numerous medullary TECs and some septal macrophages. In MG, CD59 labeling is variable: it is extensive in nMed (I) and in many MEBs, infiltrates, and GC in both AChRAb+ (E) and SNMG (H) MG, but not universally, even where there are nearby infiltrates (G). White arrowheads mark blood vessels. (Donors all female: A, 17 years of age; B, 16 years of age; C, 40 years of age; D, 21 years of age; E, 20 years of age; F, 16 years of age; G, 20 years of age; H, 38 years of age; and I, 53 years of age; *taking steroids). Original magnifications, ×200.

Table 2), although not in every infiltrated area (Figure 2G). With rare exceptions, all these findings were broadly similar in the infiltrated areas in all of the MG subgroups, although CD55 was less evident in an unusual MuSKAb+ sample with infiltrates (not shown).

Deposition of C1q, C3b, and C9 Components

In control thymi, labeling for C1q and C3b was largely restricted to the Hassall’s corpuscles, blood vessel endothelium, and connective tissue and was otherwise minimal in both cortex and medulla (Table 2, Figure 3C). In MG, by contrast, we saw clear signs of activation of the classical complement pathway; surprisingly, most sam-

Figure 2. Distribution of complement regulators CD46, CD55, and CD59 (all in red) in epithelial areas and infiltrates in control (A and D) and MG thymi (B, C, and E-I). Cytokeratin (CK, green). A: In controls, CD46 (A) and CD55 (not shown) expression is minimal, in MG, both are much stronger in the MEBs than in the nMed in both AChRAb+ (B and F) and SNMG (C) MG. They are also seen on blood vessels, some mTECs, and other cells (eg, macrophages), and especially in the GC, CD59 sometimes shows a linear distribution at MEB borders (F, bottom left) like that of laminin. D: In controls, CD59 is expressed by numerous medullary TECs and some septal macrophages. In MG, CD59 labeling is variable: it is extensive in nMed (I) and in many MEBs, infiltrates, and GC in both AChRAb+ (E) and SNMG (H) MG, but not universally, even where there are nearby infiltrates (G). White arrowheads mark blood vessels. (Donors all female: A, 17 years of age; B, 16 years of age; C, 40 years of age; D, 21 years of age; E, 20 years of age; F, 16 years of age; G, 20 years of age; H, 38 years of age; and I, 53 years of age; *taking steroids). Original magnifications, ×200.
Figure 3. Labeling for C1q and C3b complement fragments (both in red) in epithelial areas and infiltrates in MG and control thymi. Cytokeratin (CK, green). A and B: In MG, there is extensive patchy labeling for C1q in mTECs and other cells in MEBs and in infiltrates and GC in AChRAb+/H11001 (A) or SNMG (B) samples. C: In controls, C3b labeling is seen in Hassall’s corpuscles (HC), fat, and connective tissue septa and blood vessels. In MG, whether AChRAb+ or SNMG, it is most evident in MEBs (focally in D and F), in the adjacent infiltrates (D and E), and in their GC (F). The nMed areas are negative for C3b in most MG thymi (E and F). (Donors all female: A, 16 years of age; B, 38 years of age; C, 24 years of age; D, 24 years of age; E, 43 years of age; and F, 28 years of age; *on steroids). Original magnifications, ×200.
samples with infiltrates showed substantial labeling for C1q on patches of densely packed mTECs in samples with infiltrates (Figure 3, A and B). Many such mTECs also labeled strongly for C3b (Figure 3, D–F), which sometimes showed a linear laminin-like pattern (Figure 3D). However, other apparently similar areas in the same sections were negative for either C1q or C3b (Figure 3D), as was the nMed (Figure 3, E and F; except for C3b in three AChRAb+ cases). Within the infiltrates, labeling for C1q, and especially for C3b, was strongest/most consistent in the GCs (on follicular dendritic cells and B cells as expected35; Figure 3, A and F; see also Figure 5C) but was also evident on some B lymphocytes and macrophages (Figure 3, D and E).

Staining for C9/MAC was generally weak and was not seen on either TECs or even C3b+ follicular dendritic cells (not shown), implying a bias toward earlier stages of complement activation. Thus, C9/MAC showed an inverse distribution to that of CD59 in reactive GC. Overall, as in our previous studies, these thymic GCs show labeling essentially identical to that in reactive tonsillar GC for all of the markers we have now applied for the first time in MG.

Again, all such labeling was rare in the relatively normal thymus but was broadly similar in any MG sample with infiltrates, regardless of the subgroup (as summarized in Tables 2 and 3), although it did vary in degree. Moreover, C3b deposition on mTECs was equally prevalent in thymi from the most recent onset and the longer-standing AChRAb+ and apparently SNMG patients (Supplemental Figure A at http://ajp.amjpathol.org).

### Involvement of Myoid Cells

In general, myoid cells (desmin+) were haphazardly distributed in the nMed, often near the Hassall’s corpuscles, and were sometimes clustered. In any thymus with infiltrates, other very typical locations were at the edges of MEBs, ie, where they interfaced with infiltrates (Figures 4B and 5, A, B, and D), especially where there were GCs nearby, or even wholly within the infiltrates (Figures 4A and 5C); together, these are designated exposed myoid cells.

### Table 3.

Numbers of Samples Showing C3b Deposition in the MEBs, in the Infiltrates, and on Myoid Cells Exposed to the Infiltrates in Each Subgroup, and Estimated Percentages of the Myoid Cells Positive for C3b in Each Compartment

<table>
<thead>
<tr>
<th></th>
<th>C3b in MEB</th>
<th>C3b in infiltrates</th>
<th>C3b on exposed myoid cells</th>
<th>% of the myoid cells in nMed that labeled for C3b</th>
<th>% of the exposed myoid cells that labeled for C3b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls (n = 11)</td>
<td>2 (18%)</td>
<td>NA</td>
<td>NA</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>AChRAb+ (n = 23)</td>
<td>20 (87%)</td>
<td>17 (74%)</td>
<td>18 (78%)</td>
<td>3.8% (0.7 to 5.6)</td>
<td>38% (8 to 48)</td>
</tr>
<tr>
<td>AChRAbH (n = 8)</td>
<td>4 (50%)</td>
<td>4 (50%)</td>
<td>4 (50%)</td>
<td>3.3% (1.9 to 5.1)</td>
<td>25% (14 to 27)</td>
</tr>
<tr>
<td>SNMG (n = 30)</td>
<td>20 (67%)</td>
<td>15 (50%)</td>
<td>19 (63%)</td>
<td>2.7% (0.4 to 3.7)</td>
<td>24% (4 to 33)</td>
</tr>
<tr>
<td>MuSKAb+ (n = 14)</td>
<td>3 (21%)</td>
<td>2 (14%)</td>
<td>2 (14%)</td>
<td>1.2%</td>
<td>14% (11.17)</td>
</tr>
</tbody>
</table>

*Occasional areas of mTEC adjacent to small perivascular expansions. NA, not applicable.

Figure 4. Rarity of complement regulators on myoid cells. In both control (not shown) and MG thymi (A), myoid cells (MC) are uniformly CD59+ (red), even when exposed to infiltrates, but ~5% of the latter express detectable CD55 (red) (B, inset). (Donors both female: A, 20 years of age, B, 16 years of age). Desmin (De, green). Original magnifications: ×200, ×1000 (insets).
Figure 5. Labeling for C1q, C3b, or C9 (all in red) on exposed myoid cells (MC) in MG thymi. Desmin (De, green).

A AChRAb⁺

C1q/De

MEB

MC

INF

B ‘SNMG’

C1q/De

GC

MEB

C AChRAb⁺

C3b/De

GC

MEB

GC

D ‘SNMG’

C3b/De

INF

MEB

E AChRAb⁺

C9/De

MC

F ‘SNMG’

C9/De

MC

A and B: Some exposed myoid cells label for C1q in AChRAb⁺ (A) or SNMG (B) MG samples, in which many of them label for C3b (C and D, enlarged in insets) and some for C9 in AChRAb⁺ (E) or SNMG (F) samples. Note aggregation of desmin (B and E). (Donors all female: A, 16 years of age; B, 33 years of age; C, 20 years of age; D, 38 years of age; E, 35 years of age; and F, 33 years of age; *on steroids). Original magnifications: ×200 (C and D); ×1000 (A, B, E, F, and insets).
Complement Regulators and Receptors

We never observed significant staining in any of the control or MG samples for CD59 on any of the many thousands of myoid cells we examined (Figure 4A, Table 2). Likewise, they showed no labeling for CD46 or CD55 in the two controls tested, but these regulators were clearly detectable on ~10 and 5%, respectively, of the exposed myoid cells in the seven representative MG thymi from each subgroup with infiltrates (Figure 4B). Nearly all myoid cells must also be negative for CR1 because it was confined to the GC. In one control, they also appeared negative for C3aR and C5aR; however, in two AChRAb+ thymi, up to 5% of the exposed myoid cells were C3aR+ and up to 10% were C5aR+ (not shown). Hence, in general, myoid cells appear relatively deficient in complement regulators and therefore vulnerable to complement attack, for which we checked next.

Complement Components

In the control thymi, myoid cells very rarely showed any detectable C1q or C3b (Table 3). In MG, both were rare in thymi without infiltrates and uncommon in the nMed (Tables 2 and 3). In sharp contrast, ~10% of the exposed myoid cells showed clear C1q labeling (Figure 5, A and B), which implicates the classical pathway in activation. Moreover, in almost every MG thymus with infiltrates, C3b labeling was seen clearly and strongly on many of the exposed myoid cells (Figure 5, C and D), up to 48% of them in some samples (Table 3). At lower frequency, we also saw clear staining for C9/MAC on some of the exposed myoid cells (Figure 5, E and F). Although the C3b+ myoid cells showed no terminal dUTP nick-end labeling staining (for apoptosis; not shown), a few showed signs of damage (eg, desmin aggregation; Figure 5, B and E).

Exposed myoid cells were much more frequent in AChRAb+ than control thymi (Figure 6), and were also more common in most AChRAbο and many apparently SNMG thymi, whereas they were rare in the MuSKAb+ subgroup (Figure 6). Moreover, the frequencies of myoid cells under complement attack (by C3b) showed a parallel hierarchy, reaching up to 48% in the AChRAb+, 27% in the AChRAbο, and 33% in some SNMG samples (Table 3B) but only 17% in two of the three MuSKAb+ samples with occasional small infiltrates. Remarkably, this attack was already evident in the most recent-onset cases, in fact, regardless of MG duration at thymectomy (Supplemental Figure B at http://ajp.amjpathol.org), and also in some thymi with very modest infiltrates and few GC, ie, with signs of atrophy/burnout.

Discussion

This article reports three novel findings in the MG thymus. First, we found unexpected up-regulation of complement receptors and regulators on the hyperplastic medullary epithelial cells (mTECs), and evidence of early and persistent complement attack on them. These findings are consistent with the proposed roles of mTECs in auto-sensitizing AChR-specific helper T cells15–17 and in attracting the lymph node-type infiltrates.37 Second, the general lack of CD46, CD55, and especially CD59 on myoid cells indicates vulnerability to complement-mediated damage. Moreover, the deposition of C3b on many exposed myoid cells (often near GCs), and even of the terminal C5b-9 (MAC) complex on some, strongly supports their proposed role in provoking GC formation and thus in autoantibody diversification. Third, the changes in many patients with apparently seronegative (SNMG), and most with borderline AChRAb titers (AChRAbο), are very similar to those in EOMG (although somewhat milder). This argues strongly that these subgroups belong to the EOMG spectrum; it therefore also implicates AChR autoantibodies in both, which are evidently underestimated in standard assays with native AChR in dilute solution (M.I. Leite, S. Jacob, S. Viegas, J. Cossins, D. Beeson, B.P. Morgan, N. Willcox, and A. Vincent, in preparation). The rarity of similar thymic changes in MuSKAb+ MG again emphasizes the distinctness of this subgroup.

Involvement of mTECs

In our previous studies, the mTECs appeared hyperplastic because of their dense packing and more uniform staining for several integrins, especially αvβ5; to that we can now add increased expression of C5aR, CD46, and CD55 and CD59. As with other epithelial (and endothelial) cells,38–40 this up-regulation could be a result of attack by autoantibodies triggering the classical complement pathway, as indicated by the labeling for C1q, and especially for C3b. This, in turn, agrees very well with the previously reported autoantibodies against mTECs in EOMG,41 whereas our failure to detect factor B (not
shown) argues against any major concomitant role for the alternative pathway. Expression of peripheral tissue-specific autoantigens by mTECs is often focal, as with AChR subunits, which could explain the patchy labeling we observed for C5aR and complement regulators. Furthermore, sublytic MAC deposition can stimulate epithelial, endothelial, and Schwann cell proliferation, which might contribute to the mTECs’ hyperplastic appearance in MG. Their up-regulation of CXCL13 may be an important factor in provoking the nearby infiltrates in MG.

Involvement of Myoid Cells

In experimental MG in mice, cd55 or cd59 alone can each protect against motor endplate damage. However, unlike the mTECs, few myoid cells expressed CD46 or CD55 and none were CD59, even when there was marked local infiltration, implying general vulnerability to complement. Indeed, a remarkably high proportion of the exposed myoid cells in MG labeled for C1q, more for C3b, and a few even for the terminal membrane attack complex. That implies that GC, and adjacent infiltrates, develop in response to complement deposition on myoid cells. Their subsequent killing and/or proliferation might explain their variable numbers (Figure 6), or even virtual absence, in some EOMG samples; C9/MAC-exposed myoid cells in these patients’ thymi. The contrasting rarity of similar thymic changes in MuSKAb cases fits with the predominance of noncomplement-activating IgG4 anti-MuSK antibodies; the few patients with infiltrates might be the ones who also have some complement-activating IgG1 anti-MuSK.

There is one final notable correlate. The GCs were fewer and smaller in these low-affinity (SNMG) patients’ thymi, which might well imply more limited diversity of their autoantibodies than in typical AChRAbMG. Indeed, we are now able to detect anti-AChR antibodies, in ~50% of previously SNMG patients, by their binding to AChRs densely clustered on transfected human embryonic kidney cells. Interestingly, positivity in this new assay correlates with thymic infiltrates and C3b-exposed myoid cells in these patients’ thymi. The contrasting rarity of similar thymic changes in MuSKAb cases fits with the predominance of noncomplement-activating IgG4 anti-MuSK antibodies; the few patients with infiltrates might be the ones who also have some complement-activating IgG1 anti-MuSK.

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