Clinical and Serologic Parallels to APS-I in Patients with Thymomas and Autoantigen Transcripts in Their Tumors

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Clinical and Serologic Parallels to APS-I in Patients with Thymomas and Autoantigen Transcripts in Their Tumors

Anette S. B. Wolff,* Jaanika Kärner,† Jone F. Owe,‡§ Bergithe E. V. Oftedal,* Nils Erik Gilhus,*‡§ Martina M. Erichsen,§ Olle Kämpe,** Anthony Meager,# Pärt Peterson,† Kai Kisand,‡ Nick Willcox,** and Eystein S. Husebye*§

Patients with the autoimmune polyendocrine syndrome type I (APS-I), caused by mutations in the autoimmune regulator (AIRE) gene, and myasthenia gravis (MG) with thymoma, show intriguing but unexplained parallels. They include uncommon manifestations like autoimmune adrenal insufficiency (AI), hypoparathyroidism, and chronic mucocutaneous candidiasis plus autoantibodies neutralizing IL-17, IL-22, and type I IFNs. Thymopoiesis in the absence of AIRE is implicated in both syndromes. To test whether these parallels extend further, we screened 247 patients with MG, thymoma, or both for clinical features and organ-specific autoantibodies characteristic of APS-I patients, and we assayed 26 thymoma samples for transcripts for AIRE and 16 peripheral tissue-specific autoantigens (TSAgs) by quantitative PCR. We found APS-I–typical autoantibodies and clinical manifestations, including chronic mucocutaneous candidiasis, AI, and asplenia, respectively, in 49 of 121 (40%) and 10 of 121 (8%) thymoma patients, but clinical features seldom occurred together with the corresponding autoantibodies. Both were rare in other MG subgroups ($n = 126$). In 38 patients with APS-I, by contrast, we observed neither autoantibodies against muscle Ags nor any neuromuscular disorders. Whereas relative transcript levels for AIRE and 7 of 16 TSAgs showed the expected underexpression in thymomas, levels were increased for four of the five TSAgs most frequently targeted by these patients’ autoantibodies. Therefore, the clinical and serologic parallels to APS-I in patients with thymomas are not explained purely by deficient TSAg transcription in these aberrant APS-I deficient tumors. We therefore propose additional explanations for the unusual autoimmune biases they provoke. Thymoma patients should be monitored for potentially life-threatening APS-I manifestations such as AI and hypoparathyroidism. The Journal of Immunology, 2014, 193: 3880–3890.

Much is being learned about pathogenetic pathways from two human autoimmune syndromes and from the unexpected parallels between them. In autoimmune polyendocrine syndrome type I (APS-I), the autoimmunity against endocrine and ectodermal targets results from recessive mutations in the autoimmune regulator (AIRE) gene (1, 2). Expressed mainly in medullary thymic epithelial cells (mTECs), wild type AIRE is one factor that normally ensures that mTECs promiscuously express peripheral tissue-specific autoantigens (TSAgs) that then induce self-tolerance in thymocytes maturing nearby (3–5). According to current hypotheses, potentially autoreactive T cells escape this negative selection in AIRE-deficient thymus, emigrate, and cause autoimmune damage to target tissues (3, 4).

Starting in infants or young children, the typical diagnostic triad of APS-I comprises hypoparathyroidism, autoimmune adrenal insufficiency (AI), and chronic mucocutaneous candidiasis (CMC). Many patients develop other autoimmune manifestations, such as premature ovarian insufficiency (POI), vitiligo, alopecia, autoimmune hepatitis, keratitis, enamel dysplasia, and intestinal malabsorption (Table I) (4, 5). Phenotypes vary widely, even within families; some patients are first recognized in adulthood (4, 5).

Numerous autoantibodies recognize organ-specific autoantigens (6–10), and often correlate with the clinical manifestations in APS-I patients. For example, autoantibodies to NACHT leucine-rich-repeats protein 5 (NALP-5) correlate with hypoparathyroidism (6), 21-hydroxylase (21OH) with AI (10) and side-chain cleavage enzyme (SCC) with POI (9). Remarkably, at diagnosis almost 100% of these patients have autoantibodies neutralizing type I IFNs, especially IFN-α2, IFN-α8, and the related IFN-α (11–13). Moreover, autoantibodies against the Thy17 mediators IL-17A,
IL-17F, or IL-22, involved in mucous membrane defenses against *Candida albicans*, are prevalent (14, 15) and correlate with the CMC (14).

There are intriguing parallels and differences in the autoimmune associations with thymic epithelial neoplasms. Overall, myasthenia gravis (MG) occurs in ≥30% of all patients with thymomas, especially of the thymopoietic types B2 and B3, plus characteristic autoantibodies against the acetylcholine receptor (AChR), titin, and other muscle Ags (16–19). Other autoimmune features in thymoma patients (with or without MG) are also sharply focused, especially on hemopoietic targets (in ~5%), causing various bone marrow aplasias (20–23) and possibly hypogammaglobulinemia. At diagnosis, ~70% have autoantibodies neutralizing type I IFNs (24, 25), similar to those in APS-1, and again with an IgG4 bias (26). Occasional thymoma patients have been reported with autoimmune endocrine (27–29) or ectodermal (30) manifestations, or even CMC plus autoantibodies neutralizing IL-17 and IL-22 deficiencies in TH17 and TH22 cells (14), similar to those observed in APS-1 (4, 5, 14). Whereas autoantibodies against AChRs are clearly pathogenic in MG, the organ-specific Abs in APS-I are useful diagnostic markers, and typically directed against intracellular TSAgs, as are those against titin and ryanoide receptor in many late-onset MG (LOMG; onset after age 45) and most thymoma patients (16–19).

The most obvious link between these disparate syndromes is that, in nearly all thymomas, the neoplastic thymic epithelial cells (TECs) fail to express AIRE detectably, implying reduced expression or active autoimmunization against certain common targets in aberrant thymic tissue (33). To distinguish between these hypotheses, we assessed whether the clinical and serologic similarities between APS-I and thymoma patients extend to APS-I–typical organ-specific autoantibodies, and whether these correlate with underexpression of AIRE and TSAg transcripts in their thymomas. These tumors must hold clues to autoimmunizing mechanisms, which are otherwise difficult to study in humans.

Materials and Methods

Patients

All samples were taken with informed consent and ethics committee approval in each referral center (Bergen, London, Oxford, and Tartu). The demographics of the patients and controls are shown in Supplemental Table I. Over 200 of the 247 patients with MG, thymomas, or both were seen (and many followed for long periods) by a single U.K. neurology team (34); all clinical information was taken from hospital and autopsy records. Among the 121 patients with thymomas (with or without MG), 10 (8%) had a total of 15 APS-I–typical manifestations, including AI in non-MG patient P2 (Tables I, II). Several of these disorders occurred together, even including asplenia or nail dystrophy (in patients P1 or P3). Both are unusual in autoimmune patients such as those who had no genomic AIRE mutations (asterisked in Table II and Supplemental Table III), as is the CMC (plus IL-22 autoantibodies) that severely afflicted P8-10. Many of the APS-I–typical manifestations appeared long after the thymomas, for example, in 6 of 29 U.K. cases with intervals > 5 y (21%) versus only 5 of 70 (7%) of patients with shorter intervals (p = 0.051).

Of the 121 MG patients without thymomas at diagnosis, only one with LOMG (P11) showed clear APS-I–typical features, whereas another (P12) had pernicious anemia, thyroid disease, and a strong family history of thyroid autoimmunity (Table II). Although this study is retrospective, all patients were assessed similarly and thoroughly by the same experts in each referral center. Thus, the EOMG and LOMG groups are ideally matched...
typing was available for 71 MG thymomas; we found thymocytes/TECs varied greatly). World Health Organization MG status or thymoma histology (where the proportions of
in the thymoma patients showed no significant correlations with
where these drugs are used only rarely (35).The autoantibodies
suppressive drug doses, although similar variations occur in APS-I
antibodies showed minor variations at different time points (Supple-
(20 of 65 [31%]), although not quite significantly (p = 0.061).

In total, 49 of 121 thymoma patients (40%) had ≥1 APS-I–
related organ-specific autoantibodies, and they tended to occur together with each other (Table IV), though often not with the corresponding clinical manifestations (Table II). For example, the one patient with AI (P2) tested negative against 17OH or 21OH, but positive against GAD-65. She was one of the seven thymoma patients without MG, of whom two also had GAD65 autoanti-
odies, and one gave low signals versus 17OH and 21OH. Notably,
APS-I–type autoantibodies were found in more patients with disease durations >5 y (13 of 25 [52%]) than with more recent onset (20 of 65 [31%]), although not quite significantly (p = 0.061).

In the serial samples available from these patients, the auto-
antibodies showed minor variations at different time points (Sup-
mental Fig. 1); some of them might reflect changes in immunosuppressive drug doses, although similar variations occur in APS-I
where these drugs are used only rarely (35).The autoantibodies
in the thymoma patients showed no significant correlations with MG status or thymoma histology (where the proportions of thymocytes/TECs varied greatly). World Health Organization typing was available for 71 MG thymomas; we found ≥1 APS-I–
like autoantibody in 2 of the 12 (17%) patients with types A or AB
versus 20 of the 56 (36%) with types B2 and B3 (p = 0.20). Type
B1 is the only subtype reported to express AIRE (in ∼50%) (32).
Among four such patients, P5 had high-index autoantibodies
against 21OH and 17OH (Table II), but unfortunately we did not have access to his thymoma.

In the LOMG, EOMG, and ocular MG subgroups, by contrast, none of these autoantibodies were more common than in the controls, even in P11, who had several APS-I–like manifestations (Table II). Because the above clinical and serologic associations thus appear to be with the thymomas rather than the MG, and although we had only seven non-MG thymoma patients, we group all the thymoma patients together from this point forward.

MG-specific autoantibodies in APS-I patients

None of the APS-I patients had detectable autoantibodies against either AChR (0 of 38 versus 114 of 114 of the MG/thymoma patients; p < 0.001) or titin (p < 0.001; not shown), nor did any of them have any obvious MG-like neurologic features.

Transcript levels for AIRE and TSAgs in thymomas and adjacent thymic remnants

To test the hypothesis that these autoantibodies correlate with decreased AIRE and TSAg expression, we assayed AIRE and 16 TSAg transcripts in the available thymoma, remnant or control thymus blocks. To adjust for content of any TEC subtype (36, 37), which varies greatly between thymomas (17, 34), we normalized the qPCR by the keratin-8 (KRT8) which varies greatly between thymomas (17, 34), we normalized the qPCR by the keratin-8 (KRT8) signals; these correlated strongly, but inversely, with the thymocyte content estimated when the tumors were first processed (34) (not shown). They were broadly consistent in the duplicate blocks available from five thymomas and one remnant, which have each been averaged.

Relative AIRE transcript levels were low in almost all thymoma subtypes, but there were large individual differences (Fig. 2A). Values were high in one of the two available type B1 thymomas, in line with previous reports (31, 32). As expected, AIRE expression was much lower in the thymomas than in the adjacent autologous thymic remnants in all five available pairs (one was non-MG; Fig. 2B).

Data from these paired thymomas or thymic remnants also illustrate the variability between different patients and different TSAgs (Table V). TDRD6 and H+/K+ adenosine triphosphatase (ATPase) transcript values were lower in most of the thymomas than in the remnants, as expected. In contrast, the steroidogenic enzyme transcripts mostly showed similar or even higher values in the thymomas.

Table I. APS-I–like manifestations given as number (%) in the different patient groups

<table>
<thead>
<tr>
<th>Manifestation</th>
<th>APS-I (n = 38)</th>
<th>Thymoma with MG (n = 114)</th>
<th>No MG (n = 7)</th>
<th>LOMG (n = 63)</th>
<th>EOMG (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any APS-I–like manifestation</td>
<td>38 (100)</td>
<td>9 (8)</td>
<td>1 (14)</td>
<td>2 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Hypoparathyroidism</td>
<td>26 (68)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Addison disease</td>
<td>25 (66)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gonadal failure</td>
<td>8 (21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoimmune thyroiditis</td>
<td>5 (13)</td>
<td>2 (2)</td>
<td>2 (3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I diabetes</td>
<td>3 (8)</td>
<td>1 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adiopcia</td>
<td>12 (32)</td>
<td>5 (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitiligo</td>
<td>6 (16)</td>
<td>2 (2)</td>
<td>1 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nail pitting</td>
<td>5 (13)</td>
<td>1 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keratitis</td>
<td>3 (8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Enamel dysplasia</td>
<td>12 (32)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malabsorption</td>
<td>8 (21)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoimmune hepatitis</td>
<td>3 (8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pernicious anemia</td>
<td>2 (5)</td>
<td>2 (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dry eyes or mouth</td>
<td>1 (3)</td>
<td></td>
<td>1 (2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>1 (3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic mucocutaneous candidiasis</td>
<td>28 (74)</td>
<td>3 (3)</td>
<td>1 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypogammaglobulinemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pure red cell aplasia</td>
<td>2 (2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asplenia</td>
<td>2 (5)</td>
<td>1 (1)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

See Table II and Supplemental Table III for more details on the patients.
<table>
<thead>
<tr>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>P 1 (F, 40)</td>
</tr>
<tr>
<td>P 2 (F)</td>
</tr>
<tr>
<td>P 3 (M, 46)</td>
</tr>
<tr>
<td>P 4 (F, 41)</td>
</tr>
<tr>
<td>P 5 (M, 28)</td>
</tr>
<tr>
<td>P 6 (F, 50)</td>
</tr>
<tr>
<td>P 7 (F, 64)</td>
</tr>
<tr>
<td>P 8 (F, 46)</td>
</tr>
<tr>
<td>P 9 (M, 27)</td>
</tr>
<tr>
<td>P 10 (F, 35)</td>
</tr>
<tr>
<td>P 11 (M, 74)</td>
</tr>
<tr>
<td>P 12 (F, 53)</td>
</tr>
</tbody>
</table>

**Thymoma**
- Patient (Sex, MG Onset Age [y])
- Thymoma:
  - WHO Classification
  - Clinical Features (Age in Years at Onset or Death)
- APS-I–type Autoantibodies:
  - Duration (y)
  - Autoantibodies:
    - 21OH
    - 17OH
    - NALP5
    - SCC
    - GAD65
    - TPH-1
    - AADC
    - IFN-α
    - IL-12
    - ANA

**Other Autoantibodies**
- IL-12
- ANA

**Notes**
- *Delay between first signs of thymoma and of APS-I–type features.*
- *Two ages indicate both onset and death.*
- *No genomic AIRE-mutations.*
- *Common APS-I manifestations are marked in bold.*
- *Noted at autopsy.*
- *Lost to follow-up at 74 years of age.*

**Autoantibody Data**
- Autoantibody positive but index < 200; ++, autoantibody index ≥ 200; AI, adrenal insufficiency; Alo, alopecia; ANA, anti-nuclear Abs; As, asplenia; CMC, chronic mucocutaneous candidiasis; F, female; hypo-γ, hypogamma-globulinemia; IFNs, autoantibodies to type 1 IFNs; M, male; Nail dysr, nail dystrophy; NMT, neuromyotonia; PA, pernicious anemia; pulm fibr, pulmonary fibrosis; SLE, systemic lupus erythematosus; SPS, stiff-man syndrome; T, thyroid autoimmunity; TID, type 1 diabetes mellitus; Th17, autoantibodies to IL-17A, IL-17F, or IL-22; TLP, tongue lichen planus; Urt, urticaria; V, vitiligo.
The 26 thymomas (including two non-MGs) showed the most striking variability in TSAg transcripts, even when AIRE expression was low (black circles in Fig. 3). Values for AADC, H+/K+ ATPase and AChR-α clearly followed the AIRE expression pattern: highest in control thymi, intermediate in remnants, and low in thymomas (Fig. 3). We also noted significantly lower values for

Table III. Prevalences (%) of APS-I–type organ-specific autoantibodies in the different cohorts

<table>
<thead>
<tr>
<th>Autoantibody</th>
<th>APS-I</th>
<th>Thymoma ± MG</th>
<th>LOMG</th>
<th>EOMG</th>
<th>Healthy Controls</th>
<th>AI/APS-II</th>
</tr>
</thead>
<tbody>
<tr>
<td>21OH</td>
<td>55**</td>
<td>13*</td>
<td>1.6</td>
<td>3.6</td>
<td>3.5</td>
<td>86</td>
</tr>
<tr>
<td>17OH</td>
<td>24**</td>
<td>8.8*</td>
<td>0</td>
<td>1.8</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>SCC</td>
<td>40**</td>
<td>11**</td>
<td>3.2*</td>
<td>7.3*</td>
<td>0</td>
<td>6.5</td>
</tr>
<tr>
<td>AADC</td>
<td>41**</td>
<td>6.2</td>
<td>0</td>
<td>3.6</td>
<td>4.4</td>
<td>2.2</td>
</tr>
<tr>
<td>NALP-5</td>
<td>35**</td>
<td>3.5</td>
<td>1.8</td>
<td>1.8</td>
<td>1.6</td>
<td>NA</td>
</tr>
<tr>
<td>TPH-1</td>
<td>28**</td>
<td>14**</td>
<td>0</td>
<td>1.8</td>
<td>0</td>
<td>NA</td>
</tr>
<tr>
<td>GAD65</td>
<td>29**</td>
<td>15*</td>
<td>4.8</td>
<td>1.8</td>
<td>3.4</td>
<td>21</td>
</tr>
</tbody>
</table>

*aNumbers of sera assayed for the various autoantibodies varied slightly. We tested sera from ≥37 patients with APS-I, ≥113 patients with thymomas ± MG, ≥56 patients with LOMG, ≥55 patients with EOMG, and ≥57 controls. We tried to test the earliest bleeds from each patient, but many with thymomas were already taking immunosuppressive drugs for their MG.

*bData from 426 Norwegian AI/APS-II patients fully characterized by Erichsen et al. (1). APS II is defined as AI plus thyroid disease, type I diabetes, or both.

*p < 0.05, patient group versus control; **p < 0.01, patient group versus control.

NA, not analyzed.
Table IV. Percentages of patients without and from 1 to ≥3 APS-I–type organ-specific autoantibodies.

<table>
<thead>
<tr>
<th>No. of Autoantibodies</th>
<th>APS-I (n = 38)</th>
<th>With MG (n = 114)</th>
<th>No MG (n = 7)</th>
<th>LOM (n = 63)</th>
<th>EOMG (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8</td>
<td>57*</td>
<td>57</td>
<td>89*</td>
<td>82*</td>
</tr>
<tr>
<td>1</td>
<td>29</td>
<td>21</td>
<td>29</td>
<td>11</td>
<td>15</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>16</td>
<td>14</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>≥3</td>
<td>52</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*p < 0.0001, when comparing all thymoma patients (± MG) without thymoma (EOMG plus LOMG) using χ² test.

insulin, HDC, and TDRD6 in thymomas (p < 0.01; Kruskal–Wallis test) and for GAD65 when compared with the pooled control and remnant thymi (p < 0.01; Mann–Whitney U test; Fig. 3).

Surprisingly, even when AIRE values were low, we found higher TSAg transcript values per epithelial cell in many thymomas (numbers with z-scores > 3 are shown in brackets) than in any of the control thymi (Fig. 3) for: 21OH [10], 17OH [1], TG [3], TPO [5], TH [1], HDC [2], TDRD6 [2], insulinoma-associated tyrosine phosphatase–like protein (IA-2) [1], SOX9 [11], and TPH-1 [3] (Supplemental Table III). Thus, these TSAgs appear AIRE-independent, despite their frequent recognition by autoantibodies in thymoma and APS-I patients (Fig. 1, Table III). Transcript values showed no obvious correlations with MG status or thymoma histology.

Correlating APS-I–type features and autoantibodies with TSAg transcript values in thymomas

For manifestations that could be correlated with TSAg transcripts, there were only six informative patients (Supplemental Table III). In the two with alopecia (P6 and P7), TH transcript levels were similar or even higher than in control thymi (z-scores, 13.4 and 0.8). In three of the four patients with autoimmune thyroid disease (P1, P13, and P14), it presented later than their MG and their thymomas. Interestingly, two of them gave elevated transcript values for TPO (z-scores, 2.3 and 3.4), the target that correlates best with autoimmune thyroiditis, whereas values were low for both TPO and TG in P15, whose thyroiditis had presented several years earlier.

The autoantibodies likewise failed to show consistent negative correlations with TSAg transcript values. In 14 of 26 patients, we detected a total of 31 autoantibodies against informative targets, excluding AChR (Supplemental Table III). Transcript values gave positive z-scores in 14 of these 31 instances (for 21OH, TPO, and SOX9 they were >3 in five instances or more). Although AIRE values overlapped the controls in P19 and P20, together they had autoantibodies to five TSAgs, despite positive z-scores for three of these TSAgs (and AChR-α). Conversely, AIRE was low in P21, but she had adrenal autoantibodies despite z-scores >2.5 for 17OH and 21OH.

Overall, these findings provide no clear support for general TSAg underexpression in thymomas as the main cause of the associated autoimmunity.

Discussion

This wide-ranging study unexpectedly shows APS-I–typical organ-specific autoantibodies in over 40% of thymoma patients, and APS-I–typical clinical manifestations in 8%, but often not in the same individuals. This study confirms and extends an earlier observation of “thymocopying” of APS-I development in a patient with a type B thymoma (27). Despite having no AIRE mutations, some thymoma patients developed major APS-I manifestations such as AI, CMC, or asplenia, but at much lower frequencies than in APS-I. However, APS-I patients showed no clinical or serologic signs of MG. Surprisingly, some of the autoantigens targeted in thymoma patients showed the expected underexpression in their AIRE-deficient tumors, but many did not, including several adrenocortical, gonadal, and neuroectodermal targets, some of which are considered APS-I–specific. Thus, our results highlight differences and similarities in the pathogenesis in these two syndromes. These results also question current hypotheses that a lack of AIRE-regulated TSAg expression in thymomas is the major cause of the unusually biased autoimmunity.

Clinical and serologic parallels implicating thymic aberrations in autoimmunization

The overlapping autoantibody reactivities were largely confined to patients with thymomas rather than LOMG or EOMG, even though thymoma and LOMG patients are well known to share several other autoantibodies, especially against internal muscle autoantigens (18), type I IFNs, or IL-12 (25). Thymoma patients without MG are difficult to collect; although their numbers in this study are too small to exclude differences in prevalences of...
APS-I–type autoantibodies or manifestations definitively, the present results—and the contrasts with LOMG—suggest that the thymoma alone, rather than the associated MG, is responsible for this serologic and clinical overlap. Thymic aberrations are further implicated by the type I IFN–, IL-17– and IL-22–neutralizing antibodies—not only by their prevalence in both APS-I and thymoma patients, but also by their rarity in numerous other autoimmune diseases, even where peripheral type I IFNs, dendritic cells, or Th17 or Th22 cells are involved in pathogenesis (14, 15, 25, 38). Conversely, most of the “standard autoantibodies” com-

**FIGURE 3.** Relative transcript signals for APS-I target autoantigens (normalized to keratin-8) and shown as fold change compared with one pediatric control sample. The bars for each group represent the medians. Gray squares and black circles indicate samples with AIRE expression >0.1 or <0.1, respectively. Black horizontal lines above indicate where the thymomas differed significantly from remnants or from control groups (one-way ANOVA [Kruskal–Wallis] with Dunn’s multiple comparison correction), and the red horizontal lines where they differed significantly from the combined remnants plus controls (Mann–Whitney U tests). **p < 0.01.
Monoclonal antibodies against thymic epithelial cells are commonly found in sporadic and systemic autoimmune diseases but are uncommon in thymoma patients (22, 32, 39, 40).

Thymic aberrations are also implicated in autoimmunization by our observations in patients with the 22q11.2 deletion (or Di George Syndrome) who have primary thymus aplasia. Approximately 15% have similar autoantibodies against SCC, 17OH, and 21OH, again with no signs of AI or POI (41, 42). Interestingly, MG has been reported in rare patients with hypomorphic RAG1 mutations who also have disorganized AIRE-deficient thymi (43), or IFN-α autoantibodies (44), which again implicates aberrant AIRE-deficient thymopoiesis in autoimmunization in these disparate syndromes.

Clinical and immunologic differences between APS-I and thymoma patients

It is widely accepted that the autoimmune endocrine tissue damage in APS-I is T cell–mediated (45). Whereas autoantibodies against AChR are directly pathogenic in MG, those against intracellular targets are valuable as diagnostic markers. In APS-I, the autoantibodies against steroidogenic enzymes correlate well with AI and POI, against TH with alopecia (46) and against TPH-1 with malabsorption (7); curiously, GAD65 autoantibodies correlate with malabsorption in APS-I instead of type 1 diabetes (7).

Although the APS-I–typical manifestations appear to be uncoupled from the autoantibodies in our thymoma patients, both were probably underestimated for several clinical reasons. There are often delays of many years between detection of autoantibodies and onset of the corresponding clinical feature in APS-I (35). Similarly, additional manifestations appeared ≥15 y after initial presentation in several thymoma patients (e.g., P1-P3, P8, and P9; Table II, Supplemental Table III), but they might have been missed in many others in whom prethymectomy follow-up was much shorter, as possibly in a previous study in which only 28 patients were tested for autoantibodies (32). Asplenia might have escaped notice because it requires imaging. Moreover, the immunosuppressive therapy often used for MG can cause repressed autoimmune T cell–mediated tissue destruction, and their glucocorticoid treatment might have compensated for unsuspected AI. There might also be genetic differences in responsiveness to certain TSAgs.

If there is some true uncoupling of autoimmunization of the B cell and pathogenic T cell responses in patients with thymomas, it might reflect several immunologic differences from APS-I. Normally, thymic AIRE plays its key roles when the T cell repertoire is being established in the fetus and infant. Indeed, its deletion in mice after postnatal day 3 does not cause autoimmunity (47), nor does thymectomy, even in children. Whereas APS-I patients have genomic AIRE mutations from conception, the AIRE-deficiency arises only in the tumors (31, 32) and decades later in life in thymoma patients. Second, APS-I patients also lack functional AIRE expression in spleen and lymph nodes, where it could play important roles in maintaining peripheral tolerance (48, 49). Those checkpoints presumably remain intact in patients with thymomas, and they could be sufficiently potent to restrain some of the potentially autoaggressive T cells exported by these tumors, again making the observed parallels seem the more remarkable.

Moreover, most thymomas show additional aberrations not to be expected in APS-I thymi, which are not available for study. These might also contribute to loss of tolerance, as they include absence of HLA class II on the neoplastic TECs and of muscle-like thymic myoid cells (17, 34). Interestingly, in both thymomas and Aire−/− mouse thymi, there are few (if any) Hassall’s corpuscles.

<table>
<thead>
<tr>
<th>Gene</th>
<th>P24 non-MG</th>
<th>P17</th>
<th>P25</th>
<th>P26</th>
<th>P15</th>
</tr>
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<tbody>
<tr>
<td>AIRE</td>
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<td>0.04</td>
<td>0.01</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>21OH</td>
<td>4.87</td>
<td>0.11</td>
<td>0.38</td>
<td>0.22</td>
<td>13.90</td>
</tr>
<tr>
<td>17OH</td>
<td>7.87</td>
<td>0.11</td>
<td>0.26</td>
<td>0.88</td>
<td>1.17</td>
</tr>
<tr>
<td>SCC</td>
<td>0.22</td>
<td>0.32</td>
<td>0.49</td>
<td>1.44</td>
<td>0.12</td>
</tr>
<tr>
<td>AADC</td>
<td>0.00</td>
<td>1.40</td>
<td>0.00</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>TPH-1</td>
<td>0.40</td>
<td>0.15</td>
<td>0.19</td>
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<tr>
<td>HDC</td>
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<td>0.01</td>
<td>0.04</td>
<td>0.37</td>
<td>0.65</td>
</tr>
<tr>
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<td>0.58</td>
<td>0.11</td>
<td>0.14</td>
<td>0.46</td>
<td></td>
</tr>
<tr>
<td>TPO</td>
<td>0.01</td>
<td>0.32</td>
<td>0.02</td>
<td>1.55</td>
<td>0.03</td>
</tr>
<tr>
<td>GAD65</td>
<td>0.01</td>
<td>0.06</td>
<td>0.00</td>
<td>38.30</td>
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<tr>
<td>INS</td>
<td>0.31</td>
<td>0.19</td>
<td>0.02</td>
<td>2.31</td>
<td>0.07</td>
</tr>
<tr>
<td>IA-2</td>
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<td>0.10</td>
<td>1.54</td>
<td>2.71</td>
<td>2.63</td>
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<tr>
<td>TDRD6</td>
<td>0.07</td>
<td>0.06</td>
<td>0.05</td>
<td>0.17</td>
<td>0.37</td>
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<td>H/K ATPase</td>
<td>0.01</td>
<td>0.05</td>
<td>0.01</td>
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<td>SOX9</td>
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</tr>
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<td></td>
<td>3.49</td>
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</tr>
</tbody>
</table>

Each number is the TSAg value in the patient’s thymoma block divided by the value in the autologous thymic remnant. Red indicates transcript values >4.00 (>4-fold higher in thymomas than in thymic remnants), green indicates transcript values <0.25 (<4-fold lower in thymomas than in thymic remnants), and yellow indicates intermediate transcript values (0.26–4.00).
around which AIRE+ TECs are normally frequent and Foxp3+ regulatory T cells (Tregs) are positively selected (17, 50–52). There are also changes in Tregs in both syndromes (51, 53–56).

These clinical factors, plus the late onset of AIRE deficiency localized in aberrant thymoma microenvironments, probably contribute substantially to the apparent differences from APS-I.

**TSAg transcripts in thymomas**

Currently favored hypotheses assume that minimal or undetectable TSAg expression by the AIRE-deficient neoplastic TECs (31, 32) is the main cause of the associated autoimmunity. Although it is much easier to test TSAgs for Aire-dependence in *Aire−/−* mouse TECs (3, 57) than in humans, their AIRE-deficient thymomas seem a practical alternative. Although we recognize that the TSAg transcript values alone might not fully reflect protein expression levels, other options were not available to us.

We also recognize that other biologic factors might have affected the AIRE and TSAg signals we detected. For example, thymoma TECs apparently derive from progenitors with combined cortical and medullary markers (34); the normal counterparts of these progenitors are rare, and they have scarcely been studied. Because thymoma TECs appear clonal (58), AIRE and TSAg expression can vary between tumors, as they both do between individual mTECs (59, 60). They can vary within thymomas, too, although this was not obvious in the available duplicate samples. Expression might also change as thymomas evolve over time or react to changing blood supply or hormonal influences (34), which might explain the correlations we noted with thymoma durations. Some of these issues might be clarified by testing single TECs from thymomas and pediatric thymi.

Nevertheless, some TSAgs were clearly underexpressed in thymomas, notably AChR-α, H/K+ ATPase, AADC, insulin, GAD65, HDC, and TDRD6. These data partially fit with previous reports of AIRE-dependent, but variable, expression of insulin, AChR-α, IA-2, and H/K+ ATPase (32, 33, 60, 61).

Notably, AChR-α transcript levels were high in some thymoma patients, implying that underexpression is not the sole cause of their MG. That also seems unlikely because none of our APS-I patients had MG or detectable autoantibodies against AChR or titin. Other AChR subunits can be important targets, too (62), and so can pre-existing peripheral tolerance to any of them.

In striking contrast, transcript values for 21OH, TPO, and SOX9 were higher (with positive z-scores) in 40–65% of our thymomas than in the control thymi, even in tumors where AIRE transcript values were almost undetectable. Our data also suggest AIRE-independence of 17OH, SCC, TG, TH, and TPH-1. Intriguingly, these data include four of the five TSAgs most frequently recognized by autoantibodies in the thymoma group (Fig.1; *p* < 0.05 in Table III). Likewise, AIRE-independence has been reported for TG, TPO, and GAD67 in control TECs (60, 63), and for 17OH and 21OH in AIRE-negative thymomas (32). Moreover, TG, steroidogenic enzymes, and type I IFNs and Th17/Th22 cytokines are expressed by thymic cell types other than mTECs (14, 63, 64), and they should therefore be available for negative selection even in the absence of AIRE, again suggesting that additional mechanisms are operating.

**Toward a unifying hypothesis**

Among APS-I–typical TSAgs, 21OH, 17OH, SCC, and TPO stand out because of their apparent AIRE-independence and the significantly increased frequencies of autoantibodies against the first three in thymoma patients. Moreover, these autoantibodies—and others against IFNs, IL-17s, and IL-22—were among the first to appear in APS-I infants (65). By contrast, only autoantibodies against AADC and GAD65 clearly correspond to current thinking. Overall, our data question the generality of current hypotheses that the similar AIRE deficiency in APS-I and thymomas predisposes purely by impairing their expression of AIRE-dependent TSAgs/self-tolerance induction. We therefore propose that AIRE deficiency might also create dangerous, Treg-deficient microenvironments where available AIRE-independent autoantigens bias selection or actively autoimmune (33), as in paraneoplastic syndromes. To us and others (66), this proposal explains these unusual early dominant responses more neatly than current hypotheses, which apply better to those against other TSAgs like GAD65. Once again, key evidence has come from observations in patients.

Our findings also have implications for patient management. They argue for reconsideration of thymectomy in APS-I children, to halt the continuing supply of autoreactive T cells. Furthermore, in thymoma patients, subclinical adrenal insufficiency can mimic MG, with muscle weakness and fatigue (67), if not recognized, it could lead to an acute or even fatal Addisonian crisis.

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The authors have no financial conflicts of interest.

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