Early defects in human T-cell development severely affect distribution and maturation of thymic stromal cells: possible implications for the pathophysiology of Omenn syndrome

Pietro Luigi Poliani, Fabio Facchetti, Maria Ravanini, Andrew Richard Gennery, Anna Villa, Chaim M. Roifman, and Luigi D. Notarangelo

Thymocytes and thymic epithelial cell (TEC) cross-talk is crucial to preserve thymic architecture and function, including maturation of TECs and dendritic cells, and induction of mechanisms of central tolerance. We have analyzed thymic maturation and organization in 9 infants with various genetic defects leading to complete or partial block in T-cell development. Profound abnormalities of TEC differentiation (with lack of AIRE expression) and severe reduction of thymic dendritic cells were identified in patients with T-negative severe combined immunodeficiency, reticular dysgenesis, and Omenn syndrome. The latter also showed virtual absence of thymic Foxp3+ T cells. In contrast, an IL2RG-R222C hypomorphic mutation permissive for T-cell development allowed for TEC maturation, AIRE expression, and Foxp3+ T cells. Our data provide evidence that severe defects of thymopoiesis impinge on TEC homeostasis and may affect deletional and nondeletional mechanisms of central tolerance, thus favoring immune dysreactive manifestations, as in Omenn syndrome.

Methods

Patients and thymic biopsies

Clinical, immunologic, and molecular features of the patients studied are listed in Table 1. Thymic biopsies from patients P3, P4, P6, P7, and P9 were obtained before hematopoietic cell transplantation in 5 patients, in agreement with protocols approved by the Institutional Review Board, Hospital for Sick Children (Toronto, ON) with informed consent obtained in accordance with the Declaration of Helsinki. Thymic samples from patients P1, P2, P5, and P8 were retrieved during postmortem examination. Anonymous normal thymi were obtained from infants with no immunologic abnormalities who underwent elective surgery for cardiovascular disease.
Table 1. Clinical, immunologic, and genetic features of the patients

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age at biopsy, years</th>
<th>ALCL, cells/μL</th>
<th>CD8, cells/μL</th>
<th>CD19, cells/μL</th>
<th>CD16, cells/μL</th>
<th>CD56, cells/μL</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>10</td>
<td>400</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
<td>Pancytopenia, IUGR, AK2 mutation</td>
</tr>
<tr>
<td>P2</td>
<td>7.5</td>
<td>729</td>
<td>114</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>PCP</td>
</tr>
<tr>
<td>P3</td>
<td>2</td>
<td>860</td>
<td>35</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>Pneumocystis pneumonia, FTT, RAG2 mutation</td>
</tr>
<tr>
<td>P4</td>
<td>10</td>
<td>2500</td>
<td>1600</td>
<td>1000</td>
<td>475</td>
<td>475</td>
<td>PCP, IL2RG mutation</td>
</tr>
<tr>
<td>P5</td>
<td>2</td>
<td>323</td>
<td>45</td>
<td>6</td>
<td>39</td>
<td>26</td>
<td>Disseminated candidiasis, ADA-SCID</td>
</tr>
<tr>
<td>P6</td>
<td>2</td>
<td>1090</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Positive family history, CD303/BDCA2</td>
</tr>
<tr>
<td>P7</td>
<td>3</td>
<td>900</td>
<td>0</td>
<td>9</td>
<td>1</td>
<td>2</td>
<td>Positive family history, T-B-SCID</td>
</tr>
<tr>
<td>P8</td>
<td>3</td>
<td>8600</td>
<td>3698</td>
<td>946</td>
<td>1462</td>
<td>NA</td>
<td>ED, CD, FTT, myocarditis, IP, RAG2 mutation</td>
</tr>
<tr>
<td>P9</td>
<td>6.5</td>
<td>4591</td>
<td>2670</td>
<td>1669</td>
<td>125</td>
<td>924</td>
<td>ED, FTT, CD, bronchiolitis, lymphadenopathy, hepatosplenomegaly</td>
</tr>
</tbody>
</table>

NA indicates not available; UGR, unilateral growth retardation; PCP, Pneumocystis pneumonia; FTT, failure to thrive; ED, erythroderma; CD, chronic diarrhea; IP, interstitial pneumonia; RD, reticular dysgenesis; SCID, severe, combined immunodeficiency; ADA, adenosine deaminase; OS, Omenn syndrome; and 6-6T, ribonuclease mitochondrial RNA-processing mutation.

*Postmortem examination.
†Died of disseminated adenovirus infection after T-cell-depleted haploidentical hematopoietic cell transplantation.
‡Diagnosis of ADA deficiency was established based on lack of ADA activity in red blood cells.

Histologic and immunohistochemical procedures

Formalin-fixed, paraffin embedded thymic sections have been used for routine hematoxylin and eosin and single/double immunohistochemical staining using the following reagents: claudin-4 (Cl4d), Ulex Europaeus Agglutinin-1 (UEA-1) ligand, and Aire as markers of TEC maturation; S-100, CD208/DCLAMP, CD11c, and CD303/BDC2A2 as markers of DCs; CD163 to identify CD11c+ macrophages; and Foxp3 as a marker of nTreg. Double immunofluorescence staining for cytokertatin 5 (CK5) and cytokertatin 8 (CK8) have been used to evaluate TEC maturation and distribution. Detailed information is available in supplemental data (available on the Blood website; see the Supplemental Materials link at the top of the online article).

Slides were viewed with an Olympus Bx60 microscope using U Plan Apochromats 10×, 20×, and 40× lenses. Images were acquired using a DP70 model camera (Olympus) and were processed with CellF imaging software (Soft Imaging System GmbH) and Adobe photoshop version 7.0 (Adobe Systems). Aire+ cells, when present, have been counted on 10 different high power fields for each section and values expressed as number of cells/mm² plus or minus SD.

Results and discussion

As shown in Figure 1 and supplemental Figure 1, thymic sections from patients with null mutations in the AK2 gene (reticular dysgenesis, patient P1), IL2RG (patients P2 and P3), CD3D (patient P6), or RAG2 (patient P7) genes, or with complete lack of adenosine deaminase activity (patient P5), showed severe atrophy, loss of cortical and paracortical areas, marked decrease in thymus mass, and hypocellularity of the thymus (Figure 1). In contrast, biopsies from patient P4 (whose hypomorphic R222C mutation in the IL2RG gene was permissive for normal thymopoiesis) showed preserved thymic architecture.

Development of TEs is marked by differential expression of CK5 and CK8, with TEC progenitors being mostly CK5CK8+ and mature cTECs and mTECs CK8-CK5+ and CK8CK5+, respectively (Figure 1). Thymic biopsies from all patients, with the exception of patient P4, showed a diffuse epithelial network composed of CK5CK8+ cells, with predominance of CK8 expression. In contrast, normal compartmentalization of CK8CK5+ cTECs and CK8CK5+ mTECs was detected in patient P4 (Figure 1, supplemental Figure 1).

Maturation of mTECs is marked by expression of Cld4 and UEA-1 binding, with a subset of mature Cld4+UEA-1+ mTECs expressing Aire (Figure 1). With the exception of patient P4, thymic sections from all other patients in our series showed virtual absence of Cld4+UEA-1+ mTECs and lack of Aire expression, regardless of the genetic defect (Figure 1, supplemental Figure 1). In contrast, preserved UEA-1 binding (data not shown) and normal Cld4 and Aire expression (supplemental Figure 1) were observed in the thymus from patient P4, with no significant difference in the number of Aire+ mTECs compared with control thymus (392.2 ± 94.5 vs 414 ± 106.2 cells/mm²).

Staining for S-100, CD11c (Figure 1, supplemental Figure 1), and CD303/BDC2A2 (data not shown), covering the large majority...
Figure 1. Thymic compartmentalization and maturation of mTECs, DCs, and nTregs is abrogated in patients with severe defects in T-cell development but is preserved in a patient whose genetic defects are largely permissive for T-cell development. Detailed analysis of the thymic biopsy from a representative normal thymus (A) shows defined CMD (hematoxylin and eosin staining) with normal compartmentalization of CK8 \textsuperscript{+}/CK5 \textsuperscript{+} cTECs (CK8, red staining) and CK8 \textsuperscript{+}/CK5 \textsuperscript{+} mTECs (CK5, green staining). Mature mTECs express Cld4 and Aire (brown staining, top and bottom images, respectively). In contrast, mutations that abrogate T-cell development (reticular dysgenesis, B; γc-null SCIDX1, C; RAG2-null T, B, SCID, D) or that are only partially permissive for T-cell development (Omenn syndrome associated with RMRP mutations, E), are associated with profound atrophy and loss of CMD (hematoxylin and eosin staining), highlighted by the presence of a diffuse epithelial network mostly composed of CK5 and CK8 double-positive immature TECs (yellow staining). No expression of claudin-4 (Cld4) and Aire was detected in these samples. In contrast, as shown in panel F, the biopsy from the patient carrying a hypomorphic R222C mutation in the IL2RG gene, permissive for T-cell development, shows normal thymic architecture with CMD (hematoxylin and eosin staining), normal distribution of CK8 \textsuperscript{+}/CK5 \textsuperscript{+} cTECs (CK8, red staining) and CK8 \textsuperscript{+}/CK5 \textsuperscript{+} mTECs (CK5, green staining), and expression of Cld4 and Aire (brown staining, top and bottom images, respectively). In the normal thymus (A), CD11c \textsuperscript{+} (top image, red staining) and S-100 \textsuperscript{+} (bottom image, brown staining) DCs are distributed in the medullary areas. Combined CD11c and CD163 staining differentially marks CD11c \textsuperscript{+} DCs in the medullary region and CD163 \textsuperscript{+} macrophages, which are primarily distributed into the cortex, with only rare CD11c and CD163 double-positive cells. Conversely, severe depletion of DCs is present in the thymic biopsies of all patients whose genetic defects severely compromise T-cell development. (B-E) Absence of S-100 \textsuperscript{+} cells in all patients. In addition, CD11c \textsuperscript{+} cells, although present in good number, largely coexpress CD163, indicating a macrophage phenotype. Rare CD11c \textsuperscript{+}/CD163 \textsuperscript{+} DCs have been observed in the patient with Omenn syndrome (E). In the control thymus, Foxp3 \textsuperscript{+} nTreg clusters around mature activated CD208 \textsuperscript{+} DCs, as highlighted by double-staining procedures (A). In contrast, thymic biopsies from patients with genetic defects that are nonpermissive for T-cell development show absence of mature activated CD208 \textsuperscript{+} DCs and Foxp3 \textsuperscript{+} nTregs (B-D). The thymic biopsy from the Omenn syndrome patient (E) shows absence of CD208 \textsuperscript{+} DCs but focal expression of rare Foxp3 \textsuperscript{+} cells (E). In contrast, the thymus from the patient with hypomorphic R222C mutation in the IL2RG gene demonstrates normal distribution of both S-100 \textsuperscript{+} and CD11c \textsuperscript{+} DCs (F, CD11c \textsuperscript{+} top image, red staining; S-100 \textsuperscript{+} bottom image, brown staining) along with the evidence of Foxp3 \textsuperscript{+} nTreg interacting with mature activated CD208 \textsuperscript{+} DCs (F). Hematoxylin and eosin staining, original magnification ×10; immunofluorescence stainings, original magnification ×20; CK5 (green), CK8 (red), nuclei (blue), merge (yellow). Single immunohistochemical stainings, original magnification ×40; CD11c (blue) and CD163 (brown); Foxp3 (blue) and DCLAMP (CD208; brown).
of DCs populations showed virtual absence of thymic DCs in all patients with the exception of patient P4. Although a significant number of CD11c+ cells was detected in all patients, these cells largely coexpressed CD163 and, hence, represented macrophages (Figure 1, supplemental Figure 1). The presence of CD163+ and CD11c+CD163+ macrophages in patient P1 (Figure 1) is in keeping with the notion that the myeloid differentiation arrest in reticular dysgenesis affects the granulocytic but not the monocytic lineage. Overall, these data extend previous observations that the number of thymic DCs is markedly decreased in patients with X-linked SCID.20

Thymic DCs have been implied to mediate a critical role in the conversion of autoreactive T cells into nTregs.14 Staining of control thymi confirmed that Foxp3+ cells were distributed around mature/activated (CD208/DCLAMP+) DCs (Figure 1). No (patient P8) or very rare (patient P9) Foxp3+ cells were observed in thymic biopsies from patients with Omenn syndrome (Figure 1, supplemental Figure 1), despite the residual ability to generate T cells. In contrast, a normal number of Foxp3+ cells were present in patient P4, carrying the hypomorphic IL2RG R229C mutation that was permissive to T-cell development (Figure 1). To our knowledge, this is the first study in which thymic architecture and maturation of thymic stromal cells have been analyzed in detail in patients with a variety of defects that affect early stages in T-cell development. The lack of Aire expression and the severe depletion of thymic Foxp3+ cells may provide a unifying mechanism for the pathophysiology of Omenn syndrome in patients whose genetic defects severely reduce, but do not completely abrogate, T-cell development.

References


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Authorship

Contribution: P.L.P. performed the study and participated in critical interpretation of the findings and in writing the manuscript; F.F. participated in critical interpretation of the findings and in research design; M.R. performed some of the experiments; A.R.G. provided biologic specimens and participated in writing the manuscript; A.V. participated in writing and in critical interpretation of the findings; C.M.R. provided most of the thymic samples and participated in critical interpretation of the findings; and L.D.N. designed the study and participated in critical interpretation of the findings and in writing.

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