Impaired thymic expression of tissue-restricted antigens licenses the de novo generation of autoreactive CD4\(^+\) T cells in acute GVHD

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Key Points

- Loss of thymic ectopic self-antigen expression during murine acute GVHD is responsible for the de novo generation of autoreactive T cells.
- Functional impairment of the thymus medulla mechanistically links acute GVHD to posttransplantation autoimmunity.

Introduction

Acute graft-versus-host disease (aGVHD) and chronic graft-versus-host disease (cGVHD) remain primary complications of allogeneic hematopoietic stem cell transplantation (alloHSCT). 1, 2 Acute graft-versus-host disease is initiated by alloreactive donor T cells, which target a restricted set of tissues including the thymus. 3, 4 Human aGVHD predisposes to cGVHD with autoimmune manifestations that are integral components of the disease. 5, 6 It remains uncertain how autoimmunity is mechanistically linked to alloimmunity, but the thymus may play a role in this process. 1, 4, 7, 8

In the thymus, self-tolerance of the nascent T-cell receptor repertoire is attained through negative selection. 9 Essential for clonal deletion is the exposure of developing T cells to self-antigens, including those with highly restricted tissue expression. Thymic ectopic expression of tissue-restricted peripheral self-antigens (TRA) is a distinct property of mature medullary thymic epithelial cells (mTEC\(^{\text{high}}\)) expressing the transcription factor autoimmune regulator (Aire). 10 Importantly, intimate associations exist between perturbations in TRA expression (independent of cause), and the susceptibility to autoimmunity in both animals and humans. 10, 11, 12

We and others have demonstrated that mTEC\(^{\text{high}}\) are targets of donor T-cell alloimmunity during aGVHD, 3, 7, 13 and that thymic aGVHD interferes with the capacity of Aire\(^+\) mTEC\(^{\text{high}}\) to sustain TRA diversity. 14 Mechanistic links between altered thymic TRA expression and hence deviations in the TRA repertoire, the thymic production of autoreactive T-cells, and ultimately their peripheral appearance during aGVHD have not yet been established. Here we provide direct evidence in transgenic mice that de novo production of TRA-specific T-cells during acute GVHD is a direct consequence of impaired thymic ectopic OVA expression in mTEC\(^{\text{high}}\) cells. Our data, therefore, indicate that a functional compromise of the medullary mTEC\(^{\text{high}}\) compartment may link alloimmunity to the development of autoimmunity during chronic GVHD. (Blood. 2015;125(17):2720-2723)

Study design

Female C57BL/6 (H-2\(^b\)), Balb/c (H-2\(^d\)), CBy.PL(B6)-Thy1\(^+\)/ScrJ (Balb/c-Thy1.1,H-2\(^b\)), B6.Cg-Tg(TcraTcrb)425Cbn/J (OT-II;H-2\(^b\)), and C57BL/6-Tg (Ins2-TFRC/OVA)296Wehi/WehiJ (rat insulin promoter [RIP]-membrane-bound form of ovalbumin [mOVA];H-2\(^b\)) were purchased from the Jackson Laboratory and were kept in accordance with institutional regulations. RIP-mOVA mice express a membrane-bound form of OVA (mOVA) under control of the RIP. 15 These mice express mOVA in the pancreas, but also in the thymus specifically in mTEC. 16 We bred Rag2-deficient OT-II mice, producing transgenic V\(\alpha2\)V\(\beta5\) T-cell receptor (TCR) specific for OVA\(\text{323-339}\) with B6.SJL-PtprcaPep3b/BoyJ (B6.CD45.1;H-2\(^b\)) for OVA

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Results and discussion

We reported before that aGVHD causes a quantitative decline in the Aire⁺ mTEC<sup>high</sup> pool and consequently a less diverse TRA repertoire, thus impairing the molecular platform for central tolerance induction.<sup>14</sup> It remained uncertain, however, whether such mechanism sufficed for the escape of TRA-specific TCR from thymic deletion. Because the precise antigen specificities of auto-reactive effector T cells in cGVHD remain unidentified,<sup>17</sup> we used mOVA as a surrogate self-antigen and tested whether loss of mOVA expression affected central deletion of OVA-specific T cells during aGVHD. We chose the OT-II→RIP-mOVA system because (1) thymic mOVA expression is restricted to mTEC<sup>16</sup>; (2) TCR selection against mOVA recapitulates physiological tolerance induction to TRA in the thymus medulla<sup>16</sup>,<sup>18</sup>–<sup>21</sup>; and (3) a reduction of
mOVA mRNA in mTEC by <30% suffices for RIP-mOVA thymi to fail to delete OT-II cells.22

We studied aGVHD in lethally irradiated RIP-mOVA recipients of fully MHC-mismatched Balb/c donors (designated [d→RIP-mOVA]); Figures 1A and supplemental Figure 1). Consistent with previous data that reduction in mTEC compartment size is a universal manifestation of thymic aGVHD,14 total mTEClow, and mTEChigh, cells were diminished in numbers to ≤10⁶ cells/mouse at 4 weeks after alloHSCT (Figure 1B). In addition, the presence of thymic aGVHD in [d→RIP-mOVA] mice (supplemental Figure 1) reduced global OVA mRNA levels in total residual mTEChigh cell pools isolated after transplantation (Figure 1C). Our data also consistently demonstrated a reduction in the expression of both Aire mRNA and protein as a consequence of aGVHD-mediated TEC injury (Figure 1D). Because Aire regulates OVA expression19 and because the Aire⁺mTEChigh subset is reduced in numbers during aGVHD,14 our data argues that loss of Aire⁺mTEChigh was responsible for the deficiency in thymic OVA during aGVHD.

We postulated that aGVHD interfered with negative selection of the OVA TCR because (1) Aire⁻/⁻ RIP-mOVA mice cannot efficiently delete OT-II T-cells19 and (2) total thymic mOVA expression levels correlate with deletion efficacy of OVA-reactive TCR.16,18,19,21,22 To test our hypothesis, transgenic recipients with or without aGVHD were reirradiated and transplanted with syngeneic OT-II TCDBM (designated as OT-II→[d→RIP-mOVA]; Figure 1A). Thymic OT-II CD4⁺ T-cell development was monitored by assessment of CD45.1⁺ cells. The frequencies of CD45.1⁺ OT-II cells among total thymic CD4SP cells are shown as mean ± SD. The figure represents combined data from 3 independent experiments. *P < .05, Kruskall-Wallis test with Dunn’s multiple comparison test. Top right: Flow cytometric analysis of CD4SP thymocytes (live gate defined by 4,6-diamidino-2-phenylindole cells). The frequencies of CD45.1⁺ OT-II cells among total thymic CD4SP cells are shown as mean ± SD. Lower panels: Emergence of OT-II cells in the periphery. The frequencies of OT-II cells (CD45.1⁺ CD4SP), among total CD4⁺ T-cells in the spleens and lymph nodes are shown as mean ± SD. The figure represents combined data from 3 independent experiments with ≥6 mice analyzed per group. *P < .05, Kruskall-Wallis test with Dunn’s multiple comparison test. (B) Intracellular Foxp3 expression was analyzed in splenic CD4⁺ T cells isolated from OT-II→[d→RIP-mOVA] mice with or without aGVHD at 4 weeks after the second syngeneic HSCT. Flow cytometry plots depict surface CD45.1 and intracellular Foxp3 expression. (C) Quadrants [a], [b], [c], and [d] were further analyzed for surface expression of folate receptor 4 (FR4) and CD73. Data are representative of at least 2 independent experiments with ≥6 mice analyzed per group. (D) Cultures of carboxyfluorescein diacetate succinimidyl ester (CFSE)-labeled CD4⁺ T-cells isolated from spleens and lymph nodes of transplanted mice were used to detect ex vivo the proliferative response to OVA323-339 peptide presented by syngeneic APC (see supplemental Methods). Histograms of CFSE fluorescence in CD4⁺ responder cells are shown (log fluorescence intensity and cell numbers). Data are representative for ≥6 mice analyzed per group. The data substantiate that peripheral OT-II cells are responsive to their cognate antigen and therefore do not enter into an anergic state.
mTEC<sup>high</sup> resulted in an unopposed escape of “forbidden” OVA-specific V<sup>e</sup>2<sup>V</sup>B<sup>5</sup>CD<sup>4</sup> T-cell clones (Barnden et al.23; supplemental Figure 2) within the host thymus. OT-II cells were also present in the lymph nodes and spleens of transgenic mice with aGVHD (Figure 2A, bottom). Because mature OT-II T-cells were not passively transferred from donor grafts (supplemental Figure 2), formation of the peripheral OT-II pool was thymus-dependent.

In transgenic recipients with aGVHD, the fraction of C57BL/6 (CD45.1<sup>+</sup>) donor bone marrow–derived Foxp3<sup>+</sup> regulatory T-cells (T<sub>reg</sub>) among total splenic CD4<sup>+</sup> cells were reduced in frequency from a normal average of 10% to an average <1% (Figure 2B, upper left quadrants [a]). Among Foxp3<sup>+</sup>CD45.1<sup>+</sup> cells, some were FR<sup>4<sup>+</sup></sup>CD73<sup>high</sup>, documenting their anergic phenotype<sup>24</sup> (Figure 2C, far left panels [a]). In contrast, emerging OT-II (CD45.1<sup>+</sup>) cells were exclusively Foxp3<sup>+</sup> conventional T-cells whose FR<sup>4</sup>CD73 phenotype suggested that they were nonanergic<sup>25</sup> (Figure 2C, panels [c]). Indeed, CD45.1<sup>+</sup>CD4<sup>+</sup> (OT-II) cells, but not CD45.1<sup>+</sup>CD4<sup>+</sup> (non-OT-II) cells, isolated from aGVHD mice vigorously responded to OVA peptide in culture (Figure 2D).

Taken together, we provide direct evidence in transgenic mice using OVA as model TRA that intrathymic de novo production of TRA-specific CD4<sup>+</sup> T-cells during aGVHD is triggered by impaired ectopic TRA expression. These OVA-reactive T cells are exported into a periphery that is characterized by T<sub>reg</sub> deficiency. We advocate that functional compromise of the mTEC compartment may provide a pathogenic link between alloimmunity and the development of autoimmunity.<sup>26</sup> The identification of the specificities of autoreactive effector T cells in cGVHD will allow to test whether such a mechanism operates not only for a surrogate TRA, but is universal for thymic ectopic expression of those TRA that are present in tissues known to be targets of cGVHD.

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Authorship

Contribution: S.D. and M.H.H. designed and performed the study; M.V. performed the study; W.K. and G.A.H. shared senior authorship; W.K. and G.A.H. designed the work; and S.D. and W.K. wrote the paper.

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