Reduced Numbers of Mature Medullary Thymic Epithelial Cells in SKG Mice

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Abstract

Attenuated T cell receptor (TCR) signalling contributes to the susceptibility for autoimmunity as shown via mutants of PTPN22 and Zap70 genes. We here set out to investigate the effect of an attenuated TCR signal on the composition of the thymic epithelial cell (TEC) compartment. To that extent, we combined flow cytometry and histology and compared the TEC subpopulations of Zap70 wild type with SKG mutant mice. We found an increased cortical TEC compartment in SKG thymi at the expense of reduced numbers of mature medullary TECs and a 4.8-fold reduced medulla area. We also found reduced proportions of CD69\(^+\) activated thymocytes among double-negative, double-positive and CD4\(^-\)CD8\(^+\) single-positive stages, reduced absolute numbers of single-positive thymocytes, diminished expression of Lta and Ltb by CD4\(^-\)CD8\(^+\) single-positive thymocytes and a diminished expression of Ccl19, a target gene of the lymphotixin-b-receptor. While the reduced thymocyte numbers together with the attenuated TCR signal explain the diminished expression of lymphotoxins, the latter is required for an AIRE-independent expression of tissue-restricted antigens as well as attracting positively selected thymocytes to the medulla. Our results describe altered TEC compartments in SKG mice that are likely to support the development of autoimmunity.

Introduction

The aetiology of autoimmunity is based on the interaction between genetic and environmental factors. For human autoimmune diseases, the major histocompatibility complex (MHC) confers the main genetic risk to individuals [1]. However, over the last years, evidence has accumulated that genes involved in T cell receptor (TCR) signalling can also contribute to the susceptibility. For instance, variations in the PTPN22 gene that encodes the protein tyrosine phosphatase Lyp have been associated with rheumatoid arthritis (RA), systemic lupus erythematosus, type I diabetes and Graves disease [2]. Another molecule involved in TCR signalling and susceptibility for autoimmunity is the zeta-chain-associated protein kinase 70 (ZAP70).

Murine mutant strains of Zap70 provided important insights into the role of the TCR signal strength in shaping the TCR repertoire and leading to T cell-driven autoimmunity. As of yet, three distinct mutations of Zap70 have been described and analysed for their effects on thymic T cell selection and subsequent immunological consequences in the periphery. Siggs and colleagues analysed two variants of the Zap70 gene. On the one hand, the mutant variant mildly decreased TCR signalling which was tolerated on the systemic level. On the other, the wireless variant led to a strongly diminished TCR signalling and an abolished positive selection with subsequent immunodeficiency [3]. In addition, Tanaka and colleagues investigated the skg variant and made use of Zap70\(^{+/+}\), Zap70\(^{skg/+}\), Zap70\(^{skg/skg}\), Zap70\(^{skg/+}\) and Zap70\(^{−/−}\) mutant strains that are also characterized by a graded attenuation of TCR signalling. They proposed a ‘selection shift’ model whereby the extent of the TCR signal attenuation controls the selection of autoimmune TCR clones and subsequent autoimmunity. According to this model, T cell clones selected under mildly diminished signalling conditions led to predominantly organ-restricted autoimmunity while a strongly attenuated TCR signalling led to the selection of T cell clones that mediated severe systemic autoimmunity [2, 4].

Interestingly, in all these models, the counterparts of the thymocytes during thymic selection – the thymic epithelial cells (TEC) – have been neglected. However, the first selection process during T cell development is mediated by cortical thymic epithelial cells that present a specific set of antigens in an MHC-restricted fashion [5]. Thymocytes recognizing self-MHC and binding ubiquitous self-antigens with low affinity are positively selected.
These thymocytes then migrate into the thymic medulla and are subjected to negative selection mediated by medullary TECs and dendritic cells [6, 7]. Importantly, medullary TECs express genes that outside the thymus are tissue restricted, a process called promiscuous gene expression [8]. Within the thymus, dendritic cells and medullary TECs cooperate to eliminate autoreactive thymocytes with high affinity to self-proteins [9]. In contrast, thymocytes with lower affinities for self-antigens are allowed to escape into the periphery for immune surveillance. And finally, thymocytes with medium affinity to self-antigens are driven to differentiate into regulatory T cells that will mediate suppression [10].

Up to now, knowledge about the impact of high and low TCR signal strength on the TEC compartment is scarce. However, a cross-talk between thymocytes and TECs has been described to be essential for mTEC maturation [11–13]. We therefore postulated that the reduced TCR signal strength in SKG mice will be reflected in the medullary TEC compartment.

Materials and methods

Ethics statement. All animal experiments were performed in accordance with the guidelines of the local animal use and care committee. All organ removals were reported to the responsible authority ‘Landesamt für Landwirtschaft, Lebensmittelsicherheit und Fischerei Mecklenburg-Vorpommern’ in line with §7(2) German animal protection law. Animal housing was carried out by professional animal keepers, and all efforts were made to minimize suffering.

Mice. Balb/c (Charles River, Sulzfeld, Germany), C57BL/6J (Charles River), NMRI (Charles River), DBA/1J (Charles River), MRL/MpJ (Jackson Laboratory, Bar Harbor, ME, USA) and SKG (kind gift from Ulf Hamann, DRFZ in Berlin, Germany; the skg genotype was verified by sequencing in our laboratory) mice were maintained in a specific pathogen free unit on a 12 h light / 12 h dark cycle with 21 ± 2 °C, the humidity was 60 ± 10%, and the room air change is 20-fold. Mice were housed using a stocking density of 3–5 mice per cage. Mice were given water and ssniff R/M-H diet (ssniff Spezialdiäten GmbH, Soest, Germany) ad libitum.

Histology. Thymi from SKG and Balb/c mice were excised and fixed in 4% paraformaldehyde for 5 days. Paraformaldehyde was removed under floating tap water for 30 min, tissues were paraffin-embedded, and 5 μm thin sections were prepared. Sections were deparaffinized and rehydrated prior to staining with haematoxylin/eosin (H&E). Stained sections were digitalized using an AxiosCam MRc5 on an Axio Imager.M2 microscope with an EC Plan-Neofluar 20 × /0.50 M27 objective (all from Carl Zeiss Jena GmbH, Jena, Germany). Regions were quantified using ImageJ (v. 1.51n).

Immunofluorescence. Thymi from SKG and Balb/c mice were excised, embedded in TissueTek (SakuraFinetek Europe B.V., Alphen aan den Rijn, Netherlands), mounted on steel blocks and immediately frozen in liquid nitrogen.

Cryosections of 6 μm were performed with a Carl Zeiss Cryotome (Carl Zeiss Jena GmbH, Jena, Germany) and transferred to glass slides. Sections were fixed in –20°C precooled acetone (Merck Millipore, Darmstadt, Germany) for 10 min and were then allowed to dry for 20 min at RT. Slides were washed in 1 × TBS for 5 min each, and thymi were encircled with a wax pen (Thermo Fisher Scientific, Waltham, MA, USA). Blocking was performed for 1 h in a humidified chamber at RT with 1 × blocking buffer (1 × TBS containing 2.5% skim milk (USBiologicals, Salem, MA, USA) and 2.5% FCS (Biochrom, Berlin, Germany). Given dilutions of primary reagents were administered after removing blocking buffer and slides were incubated overnight in a humified chamber at 4 °C. For the detection of mTEC areas biotinylated UEA-1 (Vector Laboratories, Burlingame, CA, USA; dilution 1:100) was used, detection of terminally differentiated mTECs was performed using rabbit-anti-mouse-involucrin (BioLegend, San Diego, CA, USA; dilution 1:1000).

Afterwards, the slides were washed in 1 × TBS for 5 min each and incubated for 1 h in a humidified chamber at RT with the given dilutions of secondary reagents: NeutraVidin Alexa488 (Molecular Probes Inc., Eugene, OR, USA; dilution 1:500) and goat-anti-rabbit Alexa 546 (Molecular Probes Inc., OR, USA; dilution 1:500). The slides were washed two times in 1 × TBS, and the nuclei were counter-stained for 15 min at RT with DAPI (Molecular Probes Inc., Eugene, OR, USA; 1:1000). Finally, the slides were washed two times with 1 × TBS, four times each under floating tap water and 5 min with distilled water. After carefully removing the excess of water, the sections were embedded in mounting medium (DiaSorin Inc., Stillwater, MN, USA) and the coverslips were encircled with nail polish to protect them from dehydration. Fluorescence imaging was performed with Confocal Microscope LSM780 (Carl Zeiss Jena GmbH, Jena, Germany) and Zen2011 Software.

Enrichment of TEC. Thymic cells were isolated from 4- to 5-week-old male mice [14]. Thymi from five mice were carefully excised and adjacent connective tissue was removed. Thymi were cut into small pieces and digested in 500 μl RPMI containing 0.02% collagenase B (Roche, Basel, Switzerland), 2 U/ml dispase (BD Bioscience, Heidelberg, Germany) and 100 U/ml DNase (Roche) per thymus. Thymic pieces were incubated at 37° C, and cells were dissociated by careful pipetting using glass pipettes with decreasing opening diameter. Single cell suspension was centrifuged, resuspended in 4 ml dense Percoll (density: 1.115 g/ml, Sigma Aldrich, Germany) and transferred into a 15 ml tube. Cell suspension was overlain with 2 ml less dense Percoll (density: 1.065 g/ml) and...
2 ml D-PBS (Gibco, Life Technologies, Germany). Density gradient was established by centrifugation at 1450 g at 4 °C for 30 min. TEC enriched in the second interphase was used for subsequent flow cytometry analysis.

Flow cytometry analysis and cell sorting. TEC enriched and TEC depleted cell populations were stained for surface markers in ice-cold PBS pH 7.4, 0.5% bovine serum albumin, 0.1% sodium azide. The following antibodies were used for TEC staining and sorting: Ly-51:FITC (clone: 6C3, BD Bioscience, Germany), CD80:PE (clone: 16-10A1, Biolegend, Germany), CD45:PerCP (clone: 30-F11, Biolegend), EpCAM:Alexa647 (clone: G8.8, Biolegend). The cell numbers for the analysed TEC populations were calculated by adding up the respective cell numbers contained in the TEC enriched and TEC depleted cell fractions after the Percoll density gradient divided by the number of thymi used to isolate the cells. TEC data were obtained in two independent experimental procedures with different settings during acquisition of the data. Therefore, data were normalized for these different experimental settings.

Thymocytes were flushed from minced thymi and the following antibodies were used for the activation panel and for sorting; CD4:FITC (clone: GK1.5, Biolegend), CD8: PE/Cy7 (clone: 53-6.7, Biolegend) and CD69:BV510 (clone: H1.2F3, Biolegend). Cells were sorted and data were acquired on a FACS Aria II machine (BD, Germany) and analysed by FACS Diva Software (BD, Germany).

mRNA expression analysis. Sorted cells were centrifuged, lysed with RLT+ buffer (RNasy Plus Mini Kit) and stored therein at −80°C. RNA isolation was performed using the RNasy Plus Mini Kit (QIAGEN, Germany) according to manufacturer’s protocols. RNA quality was checked on a Bioanalyzer System (Agilent Technologies). Isolated RNA was then transcribed into cDNA using the High Capacity RNA 6000 Pico Kit (Agilent Technologies, CA, USA) in a Bioanalyzer System (Agilent Technologies). cDNA was synthesised with the SuperScript III reverse transcriptase kit (Life Technologies, Germany) using a specific primer set for mTEC. The following were then transcribed into cDNA using the High Capacity RNA 6000 Pico Kit (Agilent Technologies, CA, USA) in a Bioanalyzer System (Agilent Technologies). Isolated RNA was then transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Life Technologies GmbH, Germany) according to the manufacturer’s instructions. Quantification of the gene expression for Tnfsf11 (Rankl, Mm00441906_m1), Cd40lg (Mm00441911_m1), Ltb (Lymphotixin β, Mm00434774_g1), Lta (Lymphotixin α, Mm00440228_g1), Fgf7 (Fibroblast growth factor 7, Mm00 433291_m1), Aire (Autoimmune Regulator, Mm00 477461_m1), Ccl19 (Chenokine Ligand 19, Mm008399 67_g1), Ccl21 (Chenokine Ligand 21, Mm03646971_gH), Fzr2 (Fzr Family Zinc Finger 2, Mm01320619_m1), Rebp18 (Regulated Endocrine Specific Protein 18, Mm00 485697_m1), Krt10 (Keratin 10, Mm03009921_m1) and In2 (Insulin2, Mm00731595_gH) was performed by TaqMan Gene Expression Assays according to manufacturer’s instructions. Gapdh was used as a reference gene and analysed by TaqMan Rodent GAPDH Control Reagents (Life Technologies). Real-time PCR data were acquired on an Applied Biosystems 7900HT Fast Real-Time PCR System (Life Technologies). Statistics. Statistical analyses were performed in R (version 3.3.1).

Results

SKG harbour reduced numbers of mature medullary TECs

We here investigated the cellular composition and size of the murine TEC compartment comparing SKG mice harbouring a mutant Zap70 gene and Balb/c as the corresponding background strain. We also analysed additional mouse strains with a wild type Zap70 gene including models for induced and spontaneous autoimmunity, respectively. To that extent, we performed flow cytometry and calculated both, absolute numbers and percentages of cortical as well as immature and mature medullary TECs in the thymus (Fig. 1A). TECs were identified as EpCAMCD45+ cells. Among them, cortical TECs are identified by a high expression of Ly51 whereas medullary TECs express none or low levels of Ly51. Medullary mTECs are further differentiated into a mature (CD80high) and an immature stage (CD80low).

For the cortical TECs, we found a significant increase in numbers as well as percentages in SKG mice compared to Balb/c (Fig. 1B). Consequently, there were significantly decreased percentages alongside a trend towards lower numbers of medullary TECs in SKG mice. The reduction in the numbers of medullary TECs was limited to the mature TEC compartment as shown by a 75% decrease in mature medullary TECs and constant numbers of immature medullary TECs. These data resulted in a significant increase in the percentages of immature medullary TECs and a marked decrease in the percentages of mature medullary TECs among medullary TECs in SKG mice. Of note, the percentages (Figure S1) and numbers (Figure S2) of mature medullary TECs in SKG mice are also reduced compared to the mouse strains C57BL/6J, DBA/1J, MRL and NRMI.

We further investigated the thymic morphology by histology and performed H&E stainings of thymic thin sections (Fig. 1C). In line with the reduced numbers of mature medullary TECs, the proportion of the medulla in SKG mice was significantly reduced in comparison with Balb/c and appears as sparsely scattered patches. Thus, the reduction seen in histology is even more prominent than the reduction in cell numbers of medullary TECs.

We here considered CD80low medullary TECs as immature. However, terminally differentiated medullary TECs also downregulate CD80. To exclude the possibility that the increased percentages of CD80low medullary TECs result from an increase in terminally differentiated medullary TECs, we performed immunofluorescence on thymic thin sections and stained with involucrin, a specific marker for terminally differentiated medullary TECs. As

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expected, we did not see an increase in involucrin+ cells in SKG compared to Balb/c mice (Figure S3).

In summary, our data show a relative and absolute increase in cortical TECs alongside a decrease in mature medullary TECs in autoimmune-prone SKG mice with a mutant Zap70 gene.

SKG thymocytes show a reduced capacity to drive maturation of medullary TECs

Next, we investigated the mechanism that may lead to a delay in the differentiation of medullary TECs from the immature to the mature stage. It has been shown
previously that positively selected thymocytes provide signals which drive medullary TEC maturation [15]. Therefore, we analysed the thymocyte compartment of SKG and Balb/c mice in more detail.

First, we quantified the gross developmental stages of thymocytes in SKG and Balb/c mice (Fig. 2). To that extent, we differentiated CD4°CD8° double-negative (DN), CD4°CD8° double-positive (DP), CD4°CD8° (SP4) and CD4°CD8° single-positive (SP8) thymocytes, respectively. As shown before, we could confirm reduced percentages and numbers of SP4 and SP8 thymocytes in SKG mice compared to Balb/c [4]. Moreover, we here revealed significantly reduced proportions of CD69+-activated thymocytes among DN, DP and SP8 stages (Fig. 2).

Second, we sorted DN, DP, SP4 and SP8 thymocytes and analysed their expression of molecules known to be crucial for medullary TEC maturation (Fig. 3A). We found a significant reduction in the expression of Lta, Ltb and Tnfsf11 in DN and a significantly lower expression of Lta and Ltb in SP8 thymocytes in SKG compared to Balb/c mice.

Third, we sorted medullary TECs from SKG and Balb/c thymi and analysed the expression of selected Ltb receptor target genes (Fig. 3B). We found a significantly lower expression of Ccl19 in mature mTECs from SKG mice. However, no difference could be detected for Ccl21 and Fstf2. Furthermore, we determined the expression of Fstf2-dependent (Resp18 and Krt10) and Aire-dependent (Ins2) TRAs and found no difference in either expression. The same holds true for the expression of Aire. However, all genes were significantly differentially expressed between mature medullary TECs and immature medullary TECs independent of the mouse strain.

In summary, the activation of thymocytes as well as their expression of signalling molecules for medullary TEC maturation is reduced in SKG mice.

Discussion

We here demonstrate a marked decrease in the mature medullary TEC compartment in SKG mice in comparison with BALB/c and four additional mouse strains carrying the Zap70 wt gene. This decrease in SKG mice resembles an intermediate phenotype between Zap70 wt and knockout mice, the latter showing a complete lack of medullary structures as well as severe systemic autoimmunity. In contrast, SKG mice show a more restricted autoimmunity resulting in arthritis and some systemic manifestations such as reduced bone density, rheumatoid nodules and inflammation of the lungs [4, 16–18].

We aimed to investigate what caused the reduction in mature medullary TECs in SKG mice. Previous studies already suggested an intricate cooperation between thymocytes and TEC compartments by showing that the development of mature medullary TECs is crucially dependent on the expression of signalling molecules expressed by positively selected thymocytes [11, 12].

The present manuscript describes reduced numbers of both, SP and CD69+-activated DP thymocytes. These reduced numbers together with the attenuated TCR signal strength in SKG mutant mice are predicted to result in a net reduction in signalling molecules. Indeed, we here found a reduced expression of the signalling molecules Lta and Ltb in SP8 thymocytes. Of note, a reduction in lymphotoxin β is expected to lead to two different effects, the first one being a reduction in the AIRE-independent expression of several TRAs in murine medullary TECs, including collagen type II [19–22]. The second effect implies a reduction in lymphotoxin β-regulated chemokines that have been described to attract positively selected thymocytes to the medulla [20]. Our results thus combine quantitative and qualitative findings that provide an explanation for both, the lack of positively selected thymocytes in the medulla of SKG mice and autoimmunity resulting from inaccurate selection [21, 23].

It remains enigmatic, whether autoimmunity in SKG mice is caused or driven by the attenuated TCR signal strength, the diminished expression of lymphotoxins, the reduction in mature medullary TECs or by a combination of all above mechanisms. In favour of a role of the reduced numbers of mature medullary TECs, it was shown that a single medullary TEC expresses only a small group of 200–300 tissue-restricted antigens (TRA). In order for the whole spectrum of TRAs to be represented within the thymus, medullary TECs need to establish a mosaic expression pattern [24]. This mosaic expression pattern in turn requires a high mobility of the thymocytes so that they get contact to all possible TRAs and become selected. A reduced number of mature medullary TECs will simply increase the likelihood for autoreactive thymocytes to evade negative selection in the medulla. Indeed, a recent study could demonstrate that the specific depletion of medullary TECs led to an overt autoimmune hepatitis paralleled by autoantibodies against liver, lung, kidney and intestine as well as inflammatory infiltrations within lungs and kidneys [25]. Thus, a marked reduction in medullary TECs by itself seems to suffice to induce a restricted autoimmunity with systemic manifestations.

We here not only found a decrease in mature medullary TECs, but we also demonstrated an absolute and relative increase in cTECs in the SKG mice. Even though some knowledge exists about the factors driving medullary TEC maturation, the information on cortical TEC differentiation is rather poor. It has been shown that the presence of DN1-3 thymocytes drives cortical TEC maturation by as of...
yet unknown signals [26]. Another study suggested that thymocytes at the DP stage can induce cortical TEC maturation [27]. Indeed, we found an increase in the proportion of early CD69+ DP thymocytes in SKG mice without observing any quantitative changes in the DN compartment. Both, the increase in early DP thymocytes and an altered expression of signalling molecules in DN thymocytes may therefore be responsible for the increase in cortical TECs in SKG mice.

As of yet, it appears that autoimmunity in SKG mice may be induced by a combination of different parameters, among them the attenuated TCR signalling, a reduced medullary TEC compartment and a diminished \( Lta \) and \( Ltb \) expression. All of these should be considered when

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studying the effect of altered TCR signalling on autoimmunity.

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Author statement

RE was involved in the experimental design, in carrying out of experiments, in the interpretations of the data and in drafting the manuscript. BM was involved in the experimental design, in the interpretations of the data and in drafting the manuscript. AB, AJ and DK carried out the experiments and were involved in discussion and interpretation of the data. All the authors reviewed and finally
approved the manuscript and agreed to be accountable for all aspects of the work.

Declaration of commercial interest

The authors declare no competing financial interests.

References

1 Gregersen PK, Silver J, Winchester RJ. The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum* 1987;30:1205–13.
2 Sakaguchi S, Benham H, Cope AP, Thomas R. T-cell receptor signaling and the pathogenesis of autoimmune arthritis: insights from mouse and man. *Immunol Cell Biol* 2012;90:277–87.
3 Siggs OM, Miosge LA, Yates AL. Graded attenuation of TCR signaling elicits distinct autoimmune diseases by altering thymic T cell selection and regulatory T cell function. *J Immunol* 2010;185:2290–305.
4 McCaughtry TM, Baldwin TA, Wilken MS, Hogquist KA. Clonal deletion of thymocytes can occur in the cortex with no involvement of the medulla. *J Exp Med* 2008;205:2575–84.
5 Anderson G, Takahama Y. Thymic epithelial cells: working class heroes for T cell development and repertoire selection. *Trends Immunol* 2012;33:256–63.
6 Klein L, Krywski B, Allen PM, Hogquist KA. Positive and negative selection of the T cell repertoire: what thymocytes see (and don’t see). *Nat Rev Immunol* 2014;14:377–91.
7 Krywski B, Derbinski J. Self-representation in the thymus: an extended view. *Nat Rev Immunol* 2004;4:688–98.
8 Hubert F-X, Kinkel SA, Davey GM et al. Antigen-driven autoreactive CD4+ T cells in the thymus. *J Immunol* 2009;182:13037–44.
9 Klein L, Kyewski B, Allen PM, Hogquist KA. Positive and negative selection of the T cell repertoire: what thymocytes see (and don’t see). *Nat Rev Immunol* 2014;14:377–91.
10 Modigliani Y, Coutinho A, Pereira P et al. Establishment of tissue-specific tolerance is driven by regulatory T cells selected by thymic epithelium. *Eur J Immunol* 1996;26:1807–15.
11 Akiyama T, Shimo Y, Yanai H et al. The tumor necrosis factor alpha receptor RANK and CD40 cooperatively establish the thymic medullary microenvironment and self-tolerance. *Immunity* 2008;29:423–37.
12 Irla M, Hugues S, Gill J et al. Autoantigen-specific interactions with CD4+ thymocytes control mature thymic epithelial cell cellularity. *Immunity* 2008;29:45–65.
13 Irla M, Guerri L, Guenot J et al. Antigen recognition by autoreactive CD4+ Thymocytes drives homeostasis of the thymic medulla. *PLoS ONE* 2012;7:e52591.
14 Derbinski J, Pinto S, Rösch S, Hessel K, Kyewski B. Promiscuous gene expression patterns in single medullary thymic epithelial cells argue for a stochastic mechanism. *PNAS* 2008;105:657–62.
15 Sun L, Luo H, Li H, Zhao Y. Thymic epithelial cell development and differentiation: cellular and molecular regulation. *Protein Cell* 2013;4:342–55.
16 Sakaguchi N, Takahashi T, Hata H et al. Altered thymic T-cell selection due to a mutation of the ZAP-70 gene causes autoimmune arthritis in mice. *Nature* 2003;426:454–60.
17 Negishi I, Motoyama N, Nakayama K et al. Essential role for ZAP-70 in both positive and negative selection of thymocytes. *Nature* 1995;376:435–8.
18 Keith RC, Powers JL, Redente EF et al. A novel model of rheumatoid arthritis-associated interstitial lung disease in SKG mice. *Exp Lung Res* 2012;38:55–66.
19 Chin RK, Zhu M, Christiansen PA et al. Lymphotoxin pathway-directed, autoimmune regulator-independent central tolerance to arthritogenic collagen. *J Immunol* 2006;177:290–7.
20 Seach N, Ueno T, Fletcher AL et al. The lymphotoxin pathway regulates aire-independent expression of ectopic genes and chemokines in thymic stromal cells. *J Immunol* 2008;180:5384–92.
21 Zhu M, Chin RK, Tumanov AV, Liu X, Fu Y-X. Lymphotoxin β receptor is required for the migration and selection of autoreactive T cells in thymic medulla. *J Immunol* 2007;179:8069–75.
22 Takaba H, Morishita Y, Tomonouji Y et al. Feef2 orchestrates a thymic program of self-antigen expression for immune tolerance. *Cell* 2015;163:975–87.
23 Kurobe H, Liu C, Ueno T et al. CCR7-dependent cortex-to-medulla migration of positively selected thymocytes is essential for establishing central tolerance. *Immunity* 2006;24:165–77.
24 Pinto S, Michel C, Schmidt-Glenewinkel H et al. Overlapping gene coexpression patterns in human medullary thymic epithelial cells generate self-antigen diversity. *PNAS* 2013;110:E3497–505.
25 Bonito AJ, Aloman C, Fiel MI et al. Medullary thymic epithelial cell depletion leads to autoimmune hepatitis. *J Clin Invest* 2013;123:3510–24.
26 Shabib S, Desanti GE, Jenkinson WE, Parnell SM, Jenkinson EJ, Anderson G. Checkpoints in the development of thymic cortical epithelial cells. *J Immunol* 2009;182:150–7.
27 Forni E, Ferrero I, Merck E et al. Cutting edge: thymic crosstalk regulates delta-like 4 expression on cortical epithelial cells. *J Immunol* 2008;181:8199–203.

Supporting Information

Additional Supporting Information may be found online in the supporting information tab for this article:

**Figure S1** Percentages of TEC populations in SKG, Balb/c, C57BL/6J, DBA/1J, MRL/MpJ and NMRI mice.

**Figure S2** Number of TEC populations in SKG, Balb/c, C57BL/6J, DBA/1J, MRL/MpJ and NMRI mice.

**Figure S3** Comparable numbers of involucrin-positive terminally differentiated mTECs in SKG and Balb/c mice.