Interleukin 33 is differentially expressed in the thymus by developmental stage and cell type.

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The thymus is the site of positive and negative selection (1-5). Comparing transcriptome data from cortical and medullary epithelial cells of the murine thymus (cTEC and mTEC) at 1, 3, and 6 months of age using a published dataset (6) revealed that the IL-1 family cytokine Interleukin 33 (IL-33) (7) was among the genes whose expression varied most significantly through mouse development in mTEC expressing low levels of the class II major histocompatibility complex MHC-II. At 6 months, the IL-33 was among the genes whose expression was most different between cell types. The alarmin IL-33 is differentially expressed between cells of the epithelium of the thymus and its expression is developmentally-patterned in the medulla of the thymus.

Keywords: thymus, medullary thymic epithelial cell, mTEC, cortical thymic epithelial cell, cTEC, interleukin 33, IL-33

Introduction

The thymus is the site of positive and selection, the processes by which only T-cells that are able to interact with self MHC are chosen and by which developing T-cells that can recognize self antigen are deleted (1-5). Positive selection occurs in the epithelial cells of the cortex (cTEC) (5, 8, 9) while negative selection occurs in the epithelial cells of the medulla (mTEC) (4, 5, 10), which can be distinguished based on expression levels of MHC-II as mTEC^{lo} and mTEC^{hi} (11). In this study, we compared that transcriptomes of cTEC, mTEC^{lo}, and mTEC^{hi} at 1 month, 3 months, and 6 months of murine life to understand the most significant transcriptional differences between these cell types during mammalian development, using a published dataset (6). We found that the IL-1 family cytokine IL-33 was among the genes whose expression was most different between mTEC^{lo}, mTEC^{hi} and cTEC at 6 months. IL-33 expression was also among the top 1% of genes with respect to change in expression through mammalian development in mTEC^{lo}. Interleukin 33 (IL-33) drives type 2 T-helper responses (11) and is has the ability to activate basophils, mast cells, and eosinophils (13, 14, 15). It is released into the extracellular space by endothelial cells following cellular insult or damage and is thought to function, like HMGB1, as an alarmin, and function as an innate immune indicator of danger (16). IL-33 may serve some immunologically important function in the cortex and medulla of the thymus during the course of mammalian development.

Methods

GSE56928 (6) was utilized for this differential gene expression analysis, performed using GEO2R. This public dataset was generated by Ki et al. using microarray (Affymetrix Mouse 430 2.0 array) of cTEC, mTEC^{hi} and mTEC^{lo}, $n=2$ for each developmental time point (1 month, 3 month, and 6 months) (6). The Benjamini and Hochberg method of p-value adjustment was utilized for p -value calculation by GEO2R, and auto-detect option was used for log-transformation applied to global expression data. The NCBI generated category of platform annotation was used. For statistical test to determine the significance of difference between

average RNA expression values across each developmental time point for both mTEC^{hi} and mTEC^{lo}, an ordinary one-way ANOVA with multiple comparisons (Tukey's multiple comparisons test) was utilized (PRISM 8.1.2) (227).

Results

We performed systems-level analysis of transcription in the thymus to understand the most significant differences in the transcriptional behavior of mTEC and cTEC in mammals. We used a published dataset for this analysis (6).

IL-33 was identified as among the genes most significantly different in mTEC across mammalian development (Table 1). Thus, we first compared the expression of IL-33 in each cell type across each time point (e.g., mTEC^{lo} at 1, 3 and 6 months), to understand in an unbiased fashion how IL-33 expression is patterned in the thymus.

mTEC^{lo} express IL-33 in a stage-specific fashion with a peak at 3 months of life

We identified differential stage-specific expression of IL-33 specifically in mTEC expressing low levels of MHC-II (mTEC^{lo}) (Table 1 and Figure 1). When performing a three-way transcriptome analysis of mTEC^{lo} at 1 month, 3 months and 6 months, IL-33 was ranked 112 out of 45101 with respect to differential expression globally (Table 1) ($p=1.04E-05$). IL-33 was expressed at significantly higher levels at 3 months than at 1 month (Figure 1, $p=0.0306$), and at significantly lower levels at 6 months than at 3 months (Figure 1, $p=0.0074$). The difference in expression of IL-33 between 1 and 6 months was not significant (Figure 1). Thus, IL-33 appears to peak in expression at 3 months of murine life in the mTEC^{lo} cells of the thymus.

After noting stage-specific expression of IL-33 in mTEC^{lo} at 3 months, we used this transcriptome data to assess the expression at 1 month, at 3 months, and at 6 months by comparing each cell type in one-on-one comparisons (cTEC vs mTEC^{lo} at 1 month), and comparing the three cell types together at each time point (e.g., mTEC^{lo} vs mTEC^{hi} vs cTEC at 1 month).

At 6 months, IL-33 expression distinguishes the epithelium of the medulla from the epithelium of the cortex

When comparing the transcriptomes of all three cell types at 6 months (Table 1), IL-33 was identified as the 13th most differentially expressed gene in the transcriptome (Table 1; $p=2.27E-08$). When comparing the cTEC transcriptome against either mTEC transcriptome individually at 6 months (mTEC^{lo} vs. cTEC and mTEC^{hi} vs. cTEC), IL-33 was ranked 2nd and 150th with respect to global differential expression (Table 1; mTEC^{lo} vs. cTEC at 6 months: $p=2.14E-08$; mTEC^{hi} vs. cTEC at 6 months: $1.13E-06$). IL-33 was not differentially expressed between mTEC^{lo} and mTEC^{hi} at 6 months (Table 1; rank 2481). IL-33 was expressed at significantly higher levels in cTEC than in mTEC^{hi} as well as in mTEC^{lo} (Figure 2; mTEC^{hi} vs. cTEC $p<0.0001$; mTEC^{lo} vs. cTEC: $p<0.001$) at six months. Whether comparing the transcriptomes of all three cell types at 6 months, or the cTEC transcriptome versus either mTEC transcriptome individually (mTEC^{lo} vs. cTEC and mTEC^{hi} vs. cTEC), IL-33 was among the genes whose expression was most significantly different between cTEC and mTEC, suggesting that IL-33 expression uniquely marks the thymic cortex from the medulla at that developmental stage.

Discussion

We studied total transcription in the murine thymus by comparing the transcriptomes of cortical epithelial cells and medullary epithelial cells expressing either high or low levels of MHC-II, at 1 month, 3 months and 6 months to understand the major transcriptional differences across mammalian development and between these three cell types, using a published dataset (6). We found that IL-33 was differentially expressed in mTEC^{lo} in a stage-specific fashion and that at 6 months, IL-33 expression was among the genes whose expression was most different between mTEC^{lo}, mTEC^{hi} and cTEC.

IL-33, like IL-1 and IL-18, is first produced in precursor form, and is subject to proteolytic cleavage by caspase-1 (16). However, unlike IL-1, the full-length precursor form of IL33 is the active form. Its receptor consists of the ST2 receptor along with the IL-1 receptor

accessory protein (17). IL-33 activates eosinophils, mast cells and basophils and promotes type 2 T-helper responses (13, 14, 15, 11)). The expression of IL-33 is associated with multiple medical conditions (18, 19). The epithelial and airway smooth muscle cells of patients with bronchial asthma express higher levels of IL-33 than non-asthmatic patients (20, 21). One study demonstrated that IL-33-induced innate lymphoid cells contribute to atopic dermatitis (22). IL-33 has been shown to reduce both macrophage foam cell formation and the risk of developing atherosclerosis (23, 24). IL-33 controls adaptive immunity to nematodes (25). Regulatory T-cells (Treg) in the intestine express the IL-33 receptor ST2 and IL-33 promotes adaption of Treg to pro-inflammatory contexts by promoting accumulation of Treg in inflamed tissues and enhancing transformation growth factor beta (TGF-beta) signaling to enforce Treg differentiation through TGF-beta (26). IL-33 can display nuclear localization, associates with heterochromatin, and possesses a homeodomain-like helix-turn-helix motif found in molecules with transcriptional regulation properties (27).

IL-33 is an important cytokine and we found here that its expression changes more across mammalian development by epithelial cells of the thymus than 99% of the rest of the genome. IL-33 differentially marks the cortex of the thymus from the medulla at 6 months of life, and IL-33 peaks in expression at 3 months of life in the medullary epithelial cells of the thymus that express low levels of MHC-II. IL-33 has generally been understood to function at barrier sites (28, 29), and only loss-of-function experiments specifically ablating IL-33 in the mTEC and cTEC of the thymus will be able to adequately evaluate a role for IL-33 signaling in positive and negative selection or related processes of the thymus.

References

1. Blackman, M., Kappler, J. and Marrack, P., 1990. The role of the T cell receptor in positive and negative selection of developing T cells. *Science*, 248(4961), pp.1335-1341.
2. Surh, C.D. and Sprent, J., 1994. T-cell apoptosis detected in situ during positive and negative selection in the thymus. *Nature*, 372(6501), pp.100-103.
3. Ashton-Rickardt, P.G., Bandeira, A., Delaney, J.R., Van Kaer, L., Pircher, H.P., Zinkernagel, R.M. and Tonegawa, S., 1994. Evidence for a differential avidity model of T cell selection in the thymus. *Cell*, 76(4), pp.651-663.
4. Murphy, D.B., Lo, D., Rath, S., Brinster, R.L., Flavell, R.A., Slanetz, A. and Janeway, C.A., 1989. A novel MHC class II epitope expressed in thymic medulla but not cortex. *Nature*, 338(6218), pp.765-768.
5. Laufer, T.M., DeKoning, J., Markowitz, J.S., Lo, D. and Glimcher, L.H., 1996. Unopposed positive selection and autoreactivity in mice expressing class II MHC only on thymic cortex. *Nature*, 383(6595), pp.81-85.
6. Ki, S., Park, D., Selden, H.J., Seita, J., Chung, H., Kim, J., Iyer, V.R. and Ehrlich, L.I., 2014. Global transcriptional profiling reveals distinct functions of thymic stromal subsets and age-related changes during thymic involution. *Cell reports*, 9(1), pp.402-415.
7. Arend, W.P., Palmer, G. and Gabay, C., 2008. IL-1, IL-18, and IL-33 families of cytokines. *Immunological reviews*, 223(1), pp.20-38.
8. Bill, J. and Palmer, E., 1989. Positive selection of CD4⁺ T cells mediated by MHC class II-bearing stromal cell in the thymic cortex. *Nature*, 341(6243), pp.649-651.
9. Cosgrove, D., Chan, S.H., Waltzinger, C., Benoist, C. and Mathis, D., 1992. The thymic compartment responsible for positive selection of CD4⁺ T cells. *International immunology*, 4(6), pp.707-710.
10. Liston, A., Lesage, S., Wilson, J., Peltonen, L. and Goodnow, C.C., 2003. Aire regulates negative selection of organ-specific T cells. *Nature immunology*, 4(4), pp.350-354.
11. Yang, S.J., Ahn, S., Park, C.S., Holmes, K.L., Westrup, J., Chang, C.H. and Kim, M.G., 2006. The quantitative assessment of MHC II on thymic epithelium: implications in cortical thymocyte development. *International immunology*, 18(5), pp.729-739.
12. Schmitz, J., Owyang, A., Oldham, E., Song, Y., Murphy, E., McClanahan, T.K., Zurawski, G., Moshrefi, M., Qin, J., Li, X. and Gorman, D.M., 2005. IL-33, an interleukin-1-like

cytokine that signals via the IL-1 receptor-related protein ST2 and induces T helper type 2-associated cytokines. *Immunity*, 23(5), pp.479-490.

13. Cherry, W.B., Yoon, J., Bartemes, K.R., Iijima, K. and Kita, H., 2008. A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. *Journal of allergy and clinical immunology*, 121(6), pp.1484-1490.
14. Allakhverdi, Z., Smith, D.E., Comeau, M.R. and Delespesse, G., 2007. Cutting edge: The ST2 ligand IL-33 potently activates and drives maturation of human mast cells. *The Journal of Immunology*, 179(4), pp.2051-2054.
15. Suzukawa, M., Iikura, M., Koketsu, R., Nagase, H., Tamura, C., Komiya, A., Nakae, S., Matsushima, K., Ohta, K., Yamamoto, K. and Yamaguchi, M., 2008. An IL-1 cytokine member, IL-33, induces human basophil activation via its ST2 receptor. *The Journal of Immunology*, 181(9), pp.5981-5989.
16. Cayrol, C. and Girard, J.P., 2009. The IL-1-like cytokine IL-33 is inactivated after maturation by caspase-1. *Proceedings of the National Academy of Sciences*, 106(22), pp.9021-9026.
17. Chackerian, A.A., Oldham, E.R., Murphy, E.E., Schmitz, J., Pflanz, S. and Kastelein, R.A., 2007. IL-1 receptor accessory protein and ST2 comprise the IL-33 receptor complex. *The Journal of Immunology*, 179(4), pp.2551-2555.
18. Xu, D., Chan, W.L., Leung, B.P., Huang, F.P., Wheeler, R., Piedrafita, D., Robinson, J.H. and Liew, F.Y., 1998. Selective expression of a stable cell surface molecule on type 2 but not type 1 helper T cells. *The Journal of experimental medicine*, 187(5), pp.787-794.
19. Liew, F.Y., Pitman, N.I. and McInnes, I.B., 2010. Disease-associated functions of IL-33: the new kid in the IL-1 family. *Nature Reviews Immunology*, 10(2), pp.103-110.
20. Préfontaine, D., Nadigel, J., Chouiali, F., Audusseau, S., Semlali, A., Chakir, J., Martin, J.G. and Hamid, Q., 2010. Increased IL-33 expression by epithelial cells in bronchial asthma. *Journal of Allergy and Clinical Immunology*, 125(3), pp.752-754.
21. Préfontaine, D., Lajoie-Kadoch, S., Foley, S., Audusseau, S., Olivenstein, R., Halayko, A.J., Lemièrre, C., Martin, J.G. and Hamid, Q., 2009. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *The Journal of Immunology*, 183(8), pp.5094-5103.
22. Salimi, M., Barlow, J.L., Saunders, S.P., Xue, L., Gutowska-Owsiak, D., Wang, X., Huang, L.C., Johnson, D., Scanlon, S.T., McKenzie, A.N. and Fallon, P.G., 2013. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *Journal of Experimental Medicine*, 210(13), pp.2939-2950.

23. McLaren, J.E., Michael, D.R., Salter, R.C., Ashlin, T.G., Calder, C.J., Miller, A.M., Liew, F.Y. and Ramji, D.P., 2010. IL-33 reduces macrophage foam cell formation. *The Journal of Immunology*, 185(2), pp.1222-1229.
24. Miller, A.M., Xu, D., Asquith, D.L., Denby, L., Li, Y., Sattar, N., Baker, A.H., McInnes, I.B. and Liew, F.Y., 2008. IL-33 reduces the development of atherosclerosis. *The Journal of experimental medicine*, 205(2), pp.339-346.
25. Humphreys, N.E., Xu, D., Hepworth, M.R., Liew, F.Y. and Grencis, R.K., 2008. IL-33, a potent inducer of adaptive immunity to intestinal nematodes. *The Journal of Immunology*, 180(4), pp.2443-2449.
26. Schiering, C., Krausgruber, T., Chomka, A., Fröhlich, A., Adelmann, K., Wohlfert, E.A., Pott, J., Griseri, T., Bollrath, J., Hegazy, A.N. and Harrison, O.J., 2014. The alarmin IL-33 promotes regulatory T-cell function in the intestine. *Nature*, 513(7519), pp.564-568.
27. Carriere, V., Roussel, L., Ortega, N., Lacorre, D.A., Americh, L., Aguilar, L., Bouche, G. and Girard, J.P., 2007. IL-33, the IL-1-like cytokine ligand for ST2 receptor, is a chromatin-associated nuclear factor in vivo. *Proceedings of the National Academy of Sciences*, 104(1), pp.282-287.
28. Pichery, M., Mirey, E., Mercier, P., Lefrancais, E., Dujardin, A., Ortega, N. and Girard, J.P., 2012. Endogenous IL-33 is highly expressed in mouse epithelial barrier tissues, lymphoid organs, brain, embryos, and inflamed tissues: in situ analysis using a novel IL-33-LacZ gene trap reporter strain. *The Journal of Immunology*, 188(7), pp.3488-3495.
29. Martin, N.T. and Martin, M.U., 2016. Interleukin 33 is a guardian of barriers and a local alarmin. *Nature immunology*, 17(2), p.122.

Figure 1: mTEC^{lo} express IL-33 in a stage-specific fashion with a peak at 3 months of life

The expression of IL-33 is graphically represented by boxes from minimum to maximum value (n=2 for each cell type). A one-way ANOVA with multiple comparisons was performed to evaluate the significance of difference in IL-33 expression between each cell type and the results of this test, a p-value, is shown above each comparison.

Figure 2: At 6 months, IL-33 expression distinguishes the epithelium of the medulla from the epithelium of the cortex

The expression of IL-33 is graphically represented by boxes from minimum to maximum value (n=2 for each cell type). A one-way ANOVA with multiple comparisons was performed to evaluate the significance of difference in IL-33 expression between each cell type and the results of this test, a p-value, is shown above each comparison.

Table 1

Comparison	Rank	ID	p-value	t	B	Gene name
mTEC(hi) vs mTEC(lo) at 1 month	41671	1416200_at	8.6E-01	-1.82E-01	-7.164875	interleukin 33
mTEC(hi) vs cTEC at 1 month	1657	1416200_at	2.32E-05	-10.1550493	3.237716	interleukin 33
mTEC(lo) vs cTEC at 1 month	592	1416200_at	9.25E-05	-9.3846606	2.12143782	interleukin 33
mTEC(hi) vs cTEC at 3 months	413	1416200_at	2.23E-07	17.1025791	7.915417	interleukin 33
mTEC(hi) vs mTEC(lo) at 3 months	3223	1416200_at	2.71E-04	-6.6551352	0.563342	interleukin 33
mTEC(lo) vs cTEC at 3 months	1134	1416200_at	9.94E-04	5.1613269	-0.5035	interleukin 33
mTEC(hi) vs mTEC(lo) at 6 months	2481	1416200_at	4.76E-04	7.107052	0.27997	interleukin 33
mTEC(hi) vs cTEC at 6 months	150	1416200_at	1.13E-06	-1.89E+01	6.433875	interleukin 33
mTEC(lo) vs cTEC at 6 months	2	1416200_at	2.14E-08	-35.201719	9.04907	interleukin 33
	Rank	ID	p-value	F		Gene name
cTEC from 1 to 3 to 6 months	7492	1416200_at	3.16E-02	5.74		interleukin 33
mTEC(hi) from 1 to 3 to 6 months	31563	1416200_at	6.05E-01	5.37E-01		interleukin 33
mTEC(lo) from 1 to 3 to 6 months	112	1416200_at	1.04E-05	6.55E+01		interleukin 33
<u>1 month cTEC vs mTEChi vs mTEClo</u>	1312	1416200_at	9.64E-06	71.84983		interleukin 33
<u>3 months cTEC vs mTEChi vs mTEClo</u>	1181	1416200_at	2.96E-06	7.63E+01		interleukin 33
<u>6 months cTEC vs mTEChi vs mTEClo</u>	13	1416200_at	2.27E-08	4.83E+02		interleukin 33

Table 1: Differential expression of IL-33 in the thymus.

The transcriptomes being compared, the rank of differential expression out of 45101 total transcripts, Affymetrix probe ID, p-value with respect to differential expression out of 45101 transcripts, t, and gene name. For analyses involving three comparisons, the F statistic is provided rather than t and B (log odds of differential expression).