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Retinoic acid signalling in thymocytes regulates T cell development

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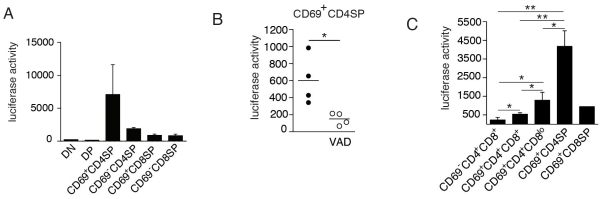
Introduction

- The Vitamin A derivative retinoic acid (RA) works as a ligand for a family of nuclear RA receptors (RARα, RARβ and RARγ) which form heterodimers with retinoid X receptors (RXR). These complexes function as ligand-activated transcription factors, recognizing specific RA responsive elements in the regulatory regions of target genes.
- RA has been reported to play a direct role in regulating multiple aspects of peripheral T cell responses[1], but whether endogenous RA signalling occurs in developing thymocytes and the potential impact of such signals in regulating T cell development remains unclear.
- To address this question, we have crossed dnRARα mice, that carry a dominant-negative (dn) RARα transgene behind a floxed transcriptional STOP cassette, with CD4Cre mice. In the resulting dnRARα-CD4Cre$^+$ mice, CD4-promotor driven Cre-expression removes the STOP cassette and leads to constitutive expression of the dnRARα. This blocks RA signalling in developing thymocytes from the DN3/4 stage onwards and thus allows us to study the role of RA in T cell development.

Single positive (SP) cells preferentially receive retinoic acid signals in the thymus



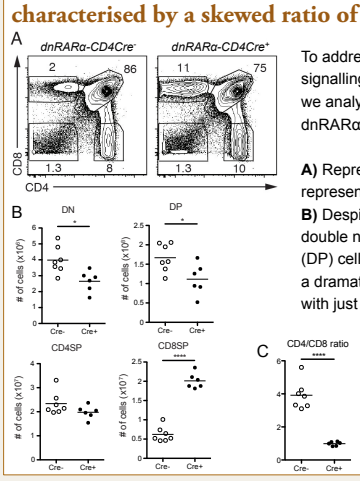
To investigate whether developing thymocyte subsets receive RA signals *in vivo*, we used DR5-luciferase reporter mouse mice, which carry a RA-inducible luciferase transgene, and assessed luciferase activity in sorted thymocyte subsets.

A) While luciferase was barely detected in DN and DP thymocytes, it was readily measured in CD4SP and CD8SP cells. CD4SP cells displayed a notably enhanced RAR response compared to CD8SP cells, mainly due to higher activity in recently generated CD69$^+$ CD4SP cells.

B) The luciferase signal in CD69$^+$ CD4SP cells was confirmed to be retinoid-dependent, as luciferase activity was reduced when mice were raised under Vitamin A-deficient conditions (VAD).

C) Evaluation of RA signalling at stages directly linked to positive selection and lineage specification events revealed 2-fold and 5-fold upregulation of luciferase activity in positively selected CD69$^+$CD4$^+$CD8$^+$ and CD4$^+$CD8^{lo} cells, compared to pre-selection DP (CD69$^-$CD4$^+$CD8$^+$).

dnRAR α -CD4Cre mice display perturbed thymopoeisis characterised by a skewed ratio of CD4/CD8 SP cells



To address the biological significance of RA signalling in CD69$^+$ DP and SP thymocytes we analysed T cell development in 8w old dnRARα-CD4Cre mice.

A) Representative FACS profiles. Numbers represent percentage of cells in gate.

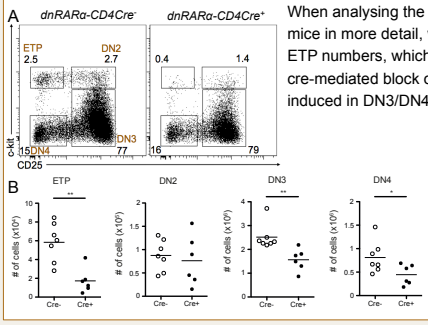
B) Despite a marked reduction in early double negative (DN) and double positive (DP) cells, dnRARα-CD4Cre$^+$ mice displayed a dramatic increase in CD8SP cell numbers, with just a minor impact on CD4SP cells.

C) The increase in CD8SP numbers resulted in a 4-fold reduction in the CD4SP:CD8SP ratio of dnRARα-CD4Cre$^+$ mice.

References

[1]Brown, CC & Noelle, R *(Eur J Immunol*, 2015); [2]Matloubian, M. *et al.* (*Nature*, 2004)

Early thymic progenitors (ETP) are reduced in dnRAR α -CD4Cre mice

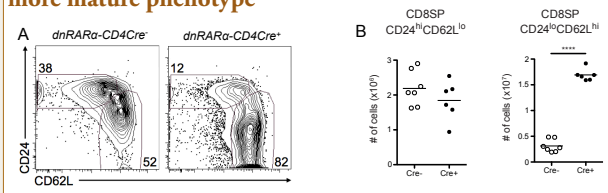


When analysing the DN subsets of dnRARα-CD4Cre$^+$ mice in more detail, we found a marked reduction in ETP numbers, which was particularly striking as the cre-mediated block of RA signalling is thought to be induced in DN3/DN4 subsets and later.

A) Representative DN subset plots (pregated on CD45$^+$Lin$^-$ CD8$^-$CD4$^-$ TCRβ$^-$)

B) DN subsets were defined as follows: ETP (c-kit$^+$CD44$^+$CD25$^-$) DN2 (c-kit$^+$CD44$^+$CD25$^+$) DN3 (c-kit$^-$CD44^{lo}CD25$^+$) DN4 (c-kit$^-$CD44$^-$CD25$^-$)

CD8SP thymocytes from dnRAR α -CD4Cre mice exhibit a more mature phenotype

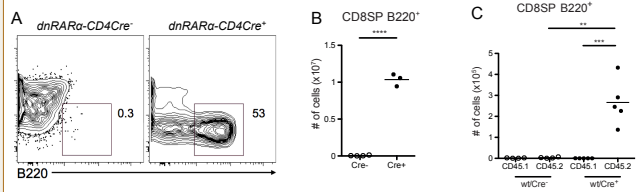


The accumulating CD8SP thymocytes in dnRARα-CD4Cre$^+$ mice were mainly CD24^{lo}CD62L^{hi} CD69$^-$, suggesting a mature phenotype. A similar phenotype has been reported for egress-incompetent SP cells in S1P1-knockout mice[2], indicating that the increased numbers of mature CD8SP in dnRARα-CD4Cre mice are partly due to prolonged thymic retention and/or defects in thymic egress.

A) Representative FACS profiles (pregated on CD45$^+$Lin$^-$CD4$^-$ CD8$^+$TCRβ$^+$).

B) Total numbers of immature CD24^{hi}CD62L^{lo} and mature CD24^{lo}CD62L^{hi} CD8SP subsets.

Aberrant expression of B220 on CD8SP thymocytes in dnRAR α -CD4Cre mice



Unexpectedly, approximately 50% of CD8SP thymocytes in dnRARα-CD4Cre$^+$ mice expressed the B cell marker B220. In mixed bone marrow chimeras with Cre$^+$ (CD45.2) and WT (CD45.1) bone marrow, Cre$^+$ BM derived CD8 SP thymocytes expressed B220, thus demonstrating that the aberrant expression of B220 was T cell intrinsic.

A) Representative FACS profiles (pregated on CD45$^+$Lin$^-$CD4$^-$ CD8$^+$TCRβ$^+$ CD19$^-$).

B) Total number of B220 expressing CD8SP cells in Cre$^-$ and Cre$^+$ mice.

C) Mixed bone marrow chimeras were set up with bone marrow input from WT (CD45.1$^+$) and Cre$^-$ or Cre$^+$ (CD45.2$^+$) mice at a 1:1 ratio into lethally irradiated B6 (CD45.1$^+$CD45.2$^+$) mice. B220$^+$ CD8SP numbers were assessed 8w after reconstitution.

Conclusion

- The induction of RAR transcriptional activity in positively-selecting DP thymocytes and post-selection CD4SP and CD8SP suggests a potential role for RA in lineage specification events.
- Increased ETP numbers in dnRARα-CD4Cre$^+$ mice indicate that impaired RAR signals in DP and SP thymocytes indirectly affect thymocyte precursor entry and/or survival, as RA signalling is supposed to be blocked only from the DN3/4 stage onwards.
- A skewed CD4SP:CD8SP ratio and phenotypical alterations of the accumulating CD8SP subset in dnRARα-CD4Cre$^+$ mice suggest that RA signalling is required for functional SP thymocyte homeostasis and maturation, and potentially thymic egress.