
بسم الله الرحمن الرحیم

Thymic development of NKT cells

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Outlines:

- Positive and negative selection of NKT cells
- Stages of NKT cell development
- Key signaling molecules
- transcription factors
- Human NKT cell development

Introduction

- NKT cells recognize lipid-based antigens, presented by the β 2M-associated MHC class-I-like molecule CD1d, and are separated into two broad classes: type 1 and type 2 NKT cells.
- Type 1 NKT cells recognize the prototypic NKT cell lipid antigen, α–galactosylceramide (α-GalCer) and express a CD1d-restricted semi-invariant αβ TCR comprising an invariant α–chain (Vα 14–Jα18 in mice, Vα 24–Jα18 in humans) coupled to a limited array of β-chains (Vβ 8, Vβ 7 and Vβ 2 in mice, Vβ 11 in humans).
- Type 1 NKT cells are relatively abundant in mice, representing between 1% and 3% of T cells in most tissues, and up to 50% of T cells in the liver. Human type 1 NKT cells are less frequent, representing fewer than 1% of T cells in the blood and liver.
- Humans appear to have greater numbers of type 2 NKT cells, which express diverse TCRs that confer broader lipid antigen specificities, but these cells are difficult to identify, and little is known about how they develop.

Positive and negative selection of NKT cells

- NKT cell development is thymus-dependent and takes place after birth:
  - Athymic nude mice and neonatally thymectomized mice do not develop NKT cells.

- CD1d-restricted NKT cells and conventional MHC-restricted T cells appear to be subject to the same principles of thymus selection, a process driven by the recognition of endogenous antigen(s).

Positive and negative selection of NKT cells

- Positive selection:
  - NKT cells require recognition of endogenous lipid antigens presented by CD1d in order to undergo positive selection.
  - CD1d expression on CD4+CD8+ double- positive cortical thymocytes is essential for NKT cell development.
- mutations of the CD1d intracellular domain
- deficient for adaptor protein complex 3 (AP3)

→ surface expression of CD1d alone is not sufficient

Positive and negative selection of NKT cells

- Candidate glycolipids identified as a selecting ligand for NKT cells:
  - isoglobotrihexosylceramide (iGb3)
  - plasmalogen lysophatidylethanolamine (pLPE) and lysophosphatidic acid (eLPA) appear to be important for NKT cell development.
  - It was recently revealed that mouse thymocytes express trace amounts of α-GalCer and α-GluCer. Although it remains to be determined whether mammalian-derived α-linked glycolipids are involved in NKT cell development.

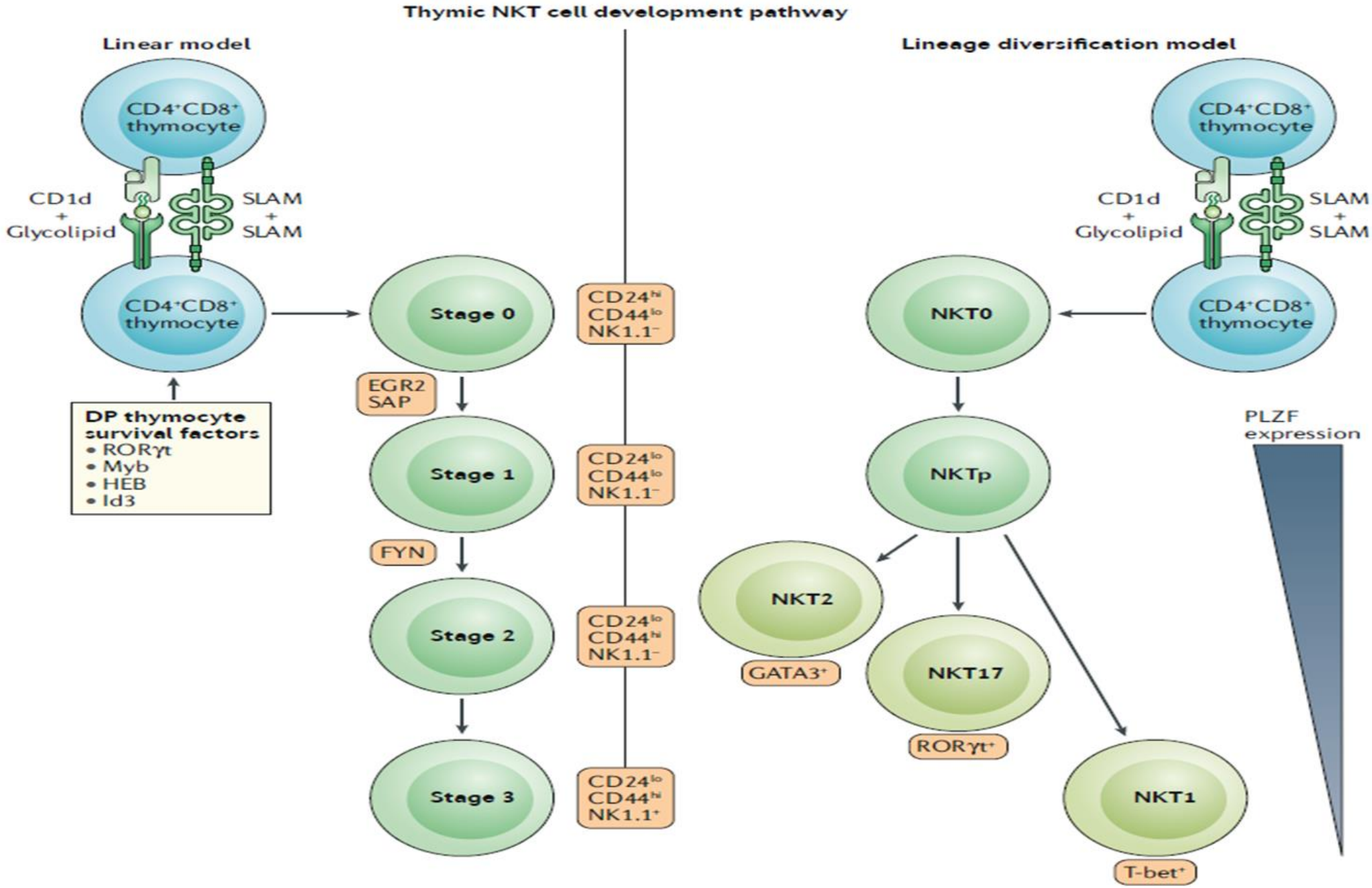
Positive and negative selection of NKT cells

- Negative selection:
  - either the injection of α-GalCer into neonatal mice or the addition of α-GalCer to fetal thymic organ cultures caused the deletion of developing NKT cells, which was mediated by $CD1d^+$ dendritic cells.
  - transgenic mice that overexpress CD1d, or that express a TCR β-chain with strong autoreactivity towards CD1d, also have fewer NKT cells, consistent with enhanced negative selection.

Stages of NKT cell development

- Most NKT cells branch off from conventional T cell development at the CD4+CD8+ DP stage.
- A small proportion of NKT cells may branch off earlier, from TCRβ^{+}CD4^{-}CD8^{-} DN thymocytes.

- Many NK1.1^{-} NKT cells become NK1.1^{+}, which support a precursor–progeny relationship between the NK1.1^{-} and NK1.1^{+} subsets.
- Reliance on NK1.1 expression as a defining measure of maturity is problematic, because not all mouse strains express it and because some NK1.1^{-} NKT cells are functionally mature.


Thymic NKT cell development pathway
Linear model
CD4+CD8+ thymocyte
CD1d + Glycolipid
SLAM + SLAM
CD4+CD8+ thymocyte
DP thymocyte survival factors
• RORγt
• Myb
• HEB
• Id3
Stage 0
CD24hi CD44lo NK1.1−
EGR2 SAP
Stage 1
CD24lo CD44lo NK1.1−
FYN
Stage 2
CD24lo CD44hi NK1.1−
Stage 3
CD24lo CD44hi NK1.1+
Lineage diversification model
CD4+CD8+ thymocyte
CD1d + Glycolipid
SLAM + SLAM
CD4+CD8+ thymocyte
NKT0
NKTp
NKT2
GATA3+
NKT17
RORγt+
NKT1
T-bet+
PLZF expression

Stages of NKT cell development

- Most NKT1 cells are $NK1.1^{+}CD44^{hi}$, and therefore resemble the aforementioned stage 3 NKT cells; however, NKT2 and NKT17 cells are both $NK1.1^{-}$, and on that basis could be mistakenly classified as immature stage 1 or stage 2 NKT cells.

Stages of NKT cell development

- It had been assumed that the NK1.1^{-} IL-4-producing NKT cells in the thymus were mostly stage 2 precursors of mature TH1-type cytokine producers.
- Now it appears they are mature thymic NKT2 cells that interact with thymic tuft cells and become the dominant source of IL-4 in the thymus.

Stages of NKT cell development

- The identification of thymic NKT2 cells was demonstrated when thymic hCD2+ NKT cells from BALB/c KN2 mice that express human CD2 as a surrogate marker for IL-4 expression did not give rise to T-bet^{hi} NKT1 cells after intrathymic injection,
  - which suggested that they were already mature.

Key signaling molecules

- SLAM–SAP–FYN pathway is crucial for the development of NKT cells in humans and mice.
- The importance of the SLAM–SAP–FYN pathway is evidenced by the absence of NKT cells in SAP- deficient mice and in patients with X- linked lymphoproliferative disease,
  - who have mutations in the gene encoding SAP
- SLAM molecules also promoted the development of NKT cells by reducing the TCR signal strength after positive selection. TCR signaling is still required for NKT cell development, because mice deficient for the TCR-proximal signaling kinase ZAP70 are devoid of $CD3^{+}NK1.1^{+}$ NKT cells.

transcription factors

- Mature subsets of NKT cells differentially express PLZF, a factor that is also critical for NKT cell development.
- PLZF interacts with many immunoregulatory genes that have critical roles in regulating the development and function of NKT cells.
  - PLZF expression is regulated by the transcription factors *EGR2* and *RUNX1.*

transcription factors

- PLZF binds genes that encode transcription factors that regulate cytokine production by NKT cells, such as *Gata3, Maf, Runx3* and *Rorc*.
  - ✓ *GATA3* is required for the expression of IFN-γ, IL-4 and IL-13 by peripheral NKT cells.
  - ✓ *Maf* was shown to be important for the expression of IL-4 and IL-17 by NKT cells.
  - ✓ *Rorc* encodes the transcription factor RORγ-t, which is absolutely essential for the expression of IL-17 by NKT17 cells.
  - ✓ *Runx3* binds to multiple regulatory elements of the *Ifng* gene and probably works in conjunction with T-bet to regulate IFNγ production.

Human NKT cell development

- The frequency of NKT cells in humans is more variable and much lower than in mice.
  - NKT cells making up ~1% of human liver mononuclear cells,
  - as compared to ~50% in mouse liver.
- immature NKT cells from human thymus resemble those from mice, which suggests that:
  - these cells undergo similar pathways of thymic development.
- human NKT cells express PLZF, suggesting a role for that protein in the development and function of NKT cells in both species

Human NKT cell development

- Some important differences have also been observed:
  - A higher proportion of human thymic NKT cells that lack CD161 (the human homologue of mouse NK1.1)
  - The presence of a CD8+ NKT cell subset in humans that is not found in mice.

summary

- NKT cells represent one of the best studied populations of unconventional T cells.
- Earlier studies that tracked NKT cell development using CD24, CD44 and NK1.1 are useful, but their findings may need to be re-evaluated in order to take into account previously unknown mature NKT cell subsets (for example, $NK1.1^{-}$) and the heterogeneity of gene expression within immature subsets.
- Studies characterizing the heterogeneity of subsets should enable researchers to better distinguish developing from mature NKT cell subsets without the need for transcription factor staining, and thus enable in vitro assays and transfer studies that will further delineate the NKT cell development pathway.

References

- Pellicci DG, Koay HF, Berzins SP. Thymic development of unconventional T cells: how NKT cells, MAIT cells and γδ T cells emerge. Nature Reviews Immunology. 2020 Dec;20(12):756-70.


Thank You
For Your Attention