RUNX3 MEDIATED IMMUNE CELL DEVELOPMENT AND MATURATION

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ABSTRACT: The transcription factor RUNX3 is a prominent regulator of multiple hematopoietic cell lineages. Gene loss of function studies demonstrated the unique and essential roles of this master regulator in differentiated lymphoid and myeloid cells. As a complementary approach, RUNX3 was upregulated in various leukocyte subsets to probe the instructive role of this 'multi-lineage' specific transcription factor. In this report, we overview the immunomodulatory functions of RUNX3 within the hematopoietic compartment to get insight to the consequences of *Runx3* deletion or overexpression in committed immune cells. Genetic studies revealed the essential role of RUNX3 in Langerhans cell development, moreover, this transcription factor is necessary for the differentiation and maintenance of the cytotoxic CD8+ T cells, in addition, T helper, natural killer and B cells are also influenced by RUNX3. Importantly, the ectopic expression of *Runx3* enhances the immunogenicity of cytotoxic T cells and pluripotent stem cell derived dendritic cells suggesting that this protein can be applied in cell based immunotherapies.

KEY WORDS: RUNX3, cell differentiation, transcription factors, cytotoxic T cells, dendritic cells

ABBREVIATIONS: **ES-DC**, embryonic stem cell-derived DC; **DC**, dendritic cell; **HSC**, hematopoietic stem cell; **IEL**, intraepithelial lymphocyte; **Ig**, immunoglobulin; **LC**, Langerhans cell; **MHC**, major histocompatibility complex; **NK** cell; natural killer cell; **RUNX**, Runt related transcription factor; **TCR**, T cell receptor; **Tfh** cell, follicular helper T cell; **Th** cell, T helper cell; **TGF**, transforming growth factor; **Treg** cell, regulatory T cell

I. INTRODUCTION

Runt-related transcription factors (RUNXs) represent an ancient protein family, which was detected in many metazoan animals.[1] Three distinct mammalian RUNX proteins were identified, termed as RUNX1 (also known as AML1 or CBFα2), RUNX2 (AML3 or CBFα1) and RUNX3 (AML2 or CBFα3), each of them encoded by separate genes. RUNXs are orthologous to the Runt protein of Drosophila containing a conserved 'runt domain', which mediates DNA binding to a specific consensus sequence (TGt/cGGt/c) or its extended/altered versions of this core motif.[1-3] RUNX binding elements are frequently located within gene regulatory regions (promoters and enhancers) and are implicated in transcriptional activation or repression. For example, RUNX proteins contribute to gene activation by recruiting histone acetyltransferase containing coactivator complexes such as p300, CBP, MOZ and MORF.[4, 5] On the other hand, RUNXs often elicit suppressive effects on gene expression via binding of the TLE or the mSIN3 corepressors.[6-9] A specific peptide sequence (VWRPY) of the RUNX C-terminal part acts as a platform to recruit the TLE corepressor.[9] Moreover, this transcription factor family contributes to gene silencing by recruiting the SUV39H1 histone methyltransferase which catalyzes the formation of the repressive H3K9 histone methylation.[10, 11] Finally, histone deacetylase containing corepressor complexes also interact with the RUNX proteins and together they can elicit epigenetic silencing.[12]

In addition to the coregulators, RUNX proteins interact with the core binding factor-β (CBF-β), a non-DNA-binding RUNX partner, which increases the affinity and specificity of their DNA binding.[13, 14] Moreover, RUNX proteins could collaborate with additional transcription factors to orchestrate gene expression on composite regulatory elements (e.g. ETS-RUNX or AP1-RUNX).[15, 16] Thus, RUNX proteins have the potential to operate in numerous signaling events and lineage specific gene regulatory networks. Mammalian RUNX proteins have a distinct tissue-specific expression pattern, although overlapping expressions of the three

RUNXs have also been found.[17] For example, RUNX1 can be detected in hematopoietic stem cells (HSCs) and hemogenic endothelial cells of arteries and aorta, in addition, RUNX1 is expressed in numerous blood cell lineages including myeloid, B- and T-lymphoid cells.[17, 18] RUNX2 is present in chondrocytes, osteoblasts and mesenchymal tissues, but this protein can also be detected in several blood cell lineages including T cells.[19] RUNX3 is expressed in multiple hematopoietic lineages as well as in numerous tissues including cranial and dorsal root ganglia, thymus, chondrocytes, and the mesenchyme of epidermal appendages.[17] Despite the overlapping expression pattern, RUNXs have their own well-defined functions in the course of lineage specification in mammals. RUNX1 is required for definitive hematopoiesis and represents the most frequent target of chromosomal translocations in leukemias.[18, 20] RUNX2 has been identified as a master regulator of bone differentiation, and its haploinsufficiency is able to cause abnormal bone formation (cleidocranial dysplasia).[21, 22] *Runx3* null mice exhibit ataxia due to the loss of TrkC proprioceptive neurons in the dorsal root ganglia.[23, 24] Of note, RUNX transcription factors also exhibit many additional functions. For example, RUNX3 is required for the proper development of cytotoxic T-lymphocytes and Langerhans cells as we will discuss in this paper. To define the specific role of RUNX3, loss of function studies were carried out by using *Runx3*-deficient mice (including tissue-specific knock out animals) or engineered cell lines. As a complementary genetic approach, *Runx3* was overexpressed in various cell lines as well as in transgenic animals. In this article we first overview the specific roles of RUNX3 in the hematopoietic compartment, followed by tackling the role of RUNX3 in committed immune cells. We also aimed to highlight the immunoregulatory potential of this ‘multi-lineage’ specific transcription factor in both lymphoid and myeloid cells.

II. CONTRIBUTION OF RUNX3 TO STEADY STATE HEMATOPOIESIS

Before we start to discuss the specific functions of Runx3 in committed blood cell lineages, it is important to stress that this transcription factor also has a putative regulatory function during steady state hematopoiesis. HSCs have the potential to give rise to committed progenitors, which differentiate progressively to various blood cell types. Even though the origin of HSCs is still controversial, several transcription factors are able to regulate the development and the maintenance of this special stem cell type.[25, 26] A large number of studies demonstrated that RUNX1 exerts a prominent role on HSC specification and on the onset of definitive hematopoiesis during embryogenesis.[18, 27] However, RUNX3 plays only a marginal role at this stage, because it is barely expressed in the yolk-sack blood islands and in embryonic HSC precursor cells.[28] In addition to this finding, embryonic lethality was observed in *Runx1*, but is absent in *Runx3* null embryos due to impaired hematopoietic development.[18] However, in contrast to early embryonic blood cells, *Runx3* could readily be detected in fetal liver and also in adult HSCs and its expression pattern overlapped with *Runx1*.[28] In agreement with these results, Wang et al. described that the concurrent elimination of *Runx1* and *Runx3* elicited a stronger hematopoietic phenotype in adult animals as compared to the *Runx1* null mice. *Runx1*/*Runx3* double knock out mice exhibited a lethal phenotypes due to bone marrow failure or to myeloproliferative disorder.[29] Unexpectedly, the double KO cells showed mitomycin C hypersensitivity associated with some repair proteins defectively recruited to damaged DNA sites. This finding suggests that *Runx1*/*Runx3* composite gene deficiency leads to corrupted hematopoiesis due to the impaired DNA repair and genomic toxicity.[29] An additional study also supported the role of RUNX3 in blood cell development: elevated number of hematopoietic progenitors as well as enhanced myeloid cell proliferation was detected in *Runx3* (Mx1-Cre) single knockout mice leading to a myeloproliferative disorder in aged animals.[30] Finally, reduced number of blood cells was observed in runx3-depleted Zebrafish embryos, whereas overexpression of runx3 led to the

enhanced formation of runx1 expressing blood cell precursors.[31] In zebrafish, runx1 also acts as a key hematopoietic regulator, thus in this vertebrate model runx3 stimulates definitive hematopoiesis presumably indirectly via the activation of runx1.[32]

In summary, the prevailing view suggests that RUNX1 is a master regulator of hematopoiesis, but in some cases RUNX3 may also participate in the modulation of steady state blood cell development presumably in cooperation with RUNX1. Considering that RUNX3 exhibits numerous unique functions in differentiated immune cells, in the following sections the regulatory roles of RUNX3 in committed myeloid and lymphoid cells will be described.

III. RUNX3 MODULATED DENDRITIC CELL ACTIVATION AND LANGERHANS CELL DEVELOPMENT

Runx3 is expressed in numerous myeloid cell types including dendritic cells (DCs), which can be classified as conventional and plasmacytoid DCs. In addition to this canonic function, DC like cells can also be generated *ex vivo* from monocytes (moDCs), bone marrow cells (BM-DCs) or pluripotent embryonic stem cells (ES-DCs). *Runx3* exerts an instructive role on the conventional CD4+ DC activation, while *Runx3*-deficient splenic DCs exhibit impaired major histocompatibility complex class II (MHCII) expression and T cell priming activity. Preferentially the Esam++ splenic DCs were underrepresented in *Runx3*-deleted animals suggesting that RUNX3 positively regulates the development and the immunogenicity of this particular DC subtype.[33] Consistent with this view, we observed that ectopic expression of *Runx3* was able to enhance the maturation potential of mouse ES cell-derived DCs (ES-DCs). RUNX3-instructed ES-DCs exhibited elevated expression levels of MHCII/CD80 and CD86, and these *ex vivo* differentiated cells displayed enhanced migratory capacity and superior T cell priming activity.[34] Remarkably, in human moDCs *RUNX3* overexpression led to the elevated expression of CD11a/CD18 and CD49d and might contribute to the elevated migratory, antigen presenting and T-cell stimulatory capacities of these cells.[35] In contrast to

these positive effects, TGF-β instructed, *Runx3*-null BM-DCs displayed accelerated maturation and enhanced potency to stimulate T-cells suggesting that RUNX3 could interfere with the TGF-β dependent DC maturation.[36] In line with this result, the chemokine receptor CCR7 was upregulated in *Runx3*-null DCs, which led to increased accumulation of activated DCs in the lung draining lymph nodes, and this contributed to the enhanced allergic airway inflammation.[37] Collectively, these findings indicate that DC maturation can either be accelerated or inhibited depending on the DC subtypes as well as the environmental cues (Fig. 1). Further kinetic and genomic analyses are needed to define the exact molecular functions acting RUNX3 in the course of DC activation.

The TGF-β pathway has previously been shown to get modulated by RUNX3, and this signaling pathway is also critical for the maintenance of the Langerhans cell (LC) compartment.[38-40] LCs belong to mononuclear phagocytic cells localized to the epidermis and are classified as macrophages originated from yolk sac-derived myeloid precursors and/or fetal monocytes.[41] However, unlike classical macrophages but similar to DCs, LCs are capable to migrate to lymph nodes and present antigens to T cells. Interestingly, Fainaru and his colleagues observed that LCs were missing completely from the epidermis of *Runx3*-null mice.[36] An additional study confirmed this finding and observed that the forced expression of *Runx3* rescues the differentiation of bone marrow-derived LCs even in the absence of Pu.1.[42] These results strongly suggest that RUNX3 is a critical regulator of the LC development, even though most of the mechanistic data were extracted from bone marrow derived *ex vivo* differentiated LC-like cells. In future experiments it would be interesting to probe other effects of *Runx3* by using *bona fide* LC precursors.

IV. RUNX3-DRIVEN CYTOTOXIC T CELL DEVELOPMENT

In mammals CD8+ T lymphocytes represent the key cytotoxic cell phenotype of the adaptive immune system. These effector T cells are derived from bi-potential CD4/CD8 double positive precursors, which undergo positive selection through T cell receptor (TCR) interactions with the MHC proteins in the thymus. Thereafter, the post-selected precursor T cells become diverted to CD4+ (helper) or CD8+ (cytotoxic) single positive T-cells. Those T cells, which receive signals from MHCII-restricted TCRs differentiate to CD4+, whereas T cells activated by MHCI-restricted TCRs differentiate to CD8+ T cells. Regardless of the specificity of the TCR, the selection of T cell fate can be compromised in the absence of some lineage specific transcription factors. In this context genetic studies demonstrated the central role of RUNX3 in the lineage commitment of CD8+ effector T cells. While the *Runx3*-null animals exhibited reduced number of CD8+ cytotoxic T cells and the recovered CD8+ cells possessed impaired proliferative and cytotoxic potential.[43, 44] RUNX3 dependent lineage selection is mediated partially by the suppression of CD4+ T cell fate together with RUNX3 acting as a direct transcriptional repressor on the CD4 gene via the binding to a specific silencer element.[44] Consistent with this finding, a subset of the *Runx3*-null CD8+ positive T cells exhibited retained CD4 expression.[43] To examine the molecular mechanism of the RUNX3 dependent CD4 regulation, it was found that the suppression of this gene was depended on the VWRPY motif of the RUNX3 protein.[45] The VWRPY penta-peptide can act as a docking module for the TLE corepressor complex, and this interaction is essential for the RUNX3-dependent CD4 silencing. In addition to CD4 suppression, Th-POK, the master regulator of CD4+ T cell development, is also repressed by RUNX3.[46] Strikingly, *Runx3* is suppressed by Th-POK indicating that these two key T-cell regulators antagonize with each other mutually.[47, 48] These results suggest that post selected T cells, which express *Runx3*, repress the CD4+ T cell specific pathway and remain committed to the CD8+ cytotoxic T cell program. In agreement with this, RUNX3 is readily detected in CD8+ but not in CD4+ T

cells. However, it is still poorly defined which signaling pathways initiate the *Runx3* expression upon the commitment of the cytotoxic T cells. The prevailing view is consistent with the kinetic signaling model, which suggests that TCR signaling of CD4/CD8 double positive thymocytes terminates CD8 expression and thus allows CD8-dependent TCR signaling to cease. Termination of TCR signaling allows the releases of intra-thymic cytokines such as IL-7, while induces *Runx3* expression, which specifies the CD8+ lineage fate.[49] In addition to the aforementioned RUNX3 regulated genes (*CD4* and *Th-POK*), many other putative RUNX3 target genes could also be operative in CD8+ T cells. A genome-scale gene expression analysis revealed that several hundred genes exhibited altered expression in both the *Runx3*-null resting and the cytokine activated T cells.[16] Moreover, many follicular helper T cell (Tfh) affiliated genes (*Tcf7*, *Bcl6*, *Maf*, *Il6ra*, *Cxcr5* and *Il21*) were turned on in *Runx3*-deficient CD8+ T cells suggesting that RUNX3 can mediate a general suppressive activity on the Tfh developmental program in committed cytotoxic T cells.[50] This study revealed that Runx3 can establish a repressive trimethylated H3K27 chromatin mark in the regulatory regions of the Tfh specific genes. This Runx3 dependent epigenetic silencing contributes for the suppression of the Tfh program in cytotoxic T cells. In summary, loss of function genetic analyses demonstrated the critical role of RUNX3 during the selection of CD8+ T cell lineage integrity (Fig. 2). This set of data altogether marked that RUNX3 is able to exert broad suppressive effects on the T-helper and Tfh specific transcriptional programs in developing T cells.

Interestingly, RUNX3 also activates the cytotoxic machinery in CD8+ T cells, and some of these positive effects were identified and characterized by gain of function studies. As an example, overexpression of RUNX3 positively regulated the CD103 expression in T cells.[51] In addition, the number of CD8+ T cells was elevated in *Runx3* transgenic thymus demonstrating, that ectopic expression of this protein forced thymocytes to select the CD8+

developmental pathway.[52] Moreover, RUNX3 can directly stimulate this gene via the regulation of the stage specific enhancers of CD8.[53] To carry on this study Sato and his colleagues have engineered a *Runx3* transgenic, as well as a dominant negative Runt domain expressing mouse strain. As expected, the presence of a dominant negative RUNX resulted in less CD8+ T cells from the CD4+CD8low post-selected cells, while the expression of the CD8 marker remained high in the committed T cells. Activation of naive CD8+ T cells, together with various peripheral antigens promoted the differentiation of these cells into cytolytic effector killer T cells. To characterize this process further, *Runx3* was re-expressed retrovirally in CD8+ T cells derived from *Runx3*-null mice, and as expected, RUNX3 restore the expression of several cytotoxic T cell specific genes (*Eomes*, *perforin*, *granzyme B*, and *IFNg*).[54] Moreover, this study revealed that Runx3 can bind to the putative regulatory elements of genes encoding perforin, granzyme B, and IFN-γ suggesting that these T cell specific genes are regulated by RUNX3 in effector T cells directly. Finally, overexpression of *Runx3* was found to accelerate CD69+ CD103+ tissue resident memory T cell differentiation *in vivo* indicating that CD8+ memory T cell development is also modulated by this transcription factor.[55] In conclusion, several gain of function studies confirmed that the RUNX3 mediated transcriptional program is necessary for the maintenance of effector and memory functions of cytotoxic T cells (Fig. 2).

V. RUNX3 DEPENDENT TH1 POLARIZATION AND ENHANCED SUPPRESSIVE T CELL DEVELOPMENT

Despite the general suppressive role of RUNX3 on CD4+ T cell development, curiously, this transcription factor is also involved in T helper cell polarization. Upon encountering and capturing antigen, naive CD4+ T helper (Th) cells become polarized to effector cell subsets, which exhibit distinct cytokine profiles. Non-polarized CD4+ T cells have the potential to differentiate into Th1, Th2, Th9, Th17 or follicular helper T cells in the periphery.

Furthermore, CD4+ T lymphocytes can give rise to immunosuppressive regulatory T (Treg) cells. The Th1 cells release IFN-γ and activate macrophages as well as CD8+ T cells to induce immunity against intracellular pathogens. Whereas, Th2 cells secrete IL-4, IL-5, IL-13 and stimulate humoral responses against extracellular pathogens concomitant with isotype switching to immunoglobulin E (IgE).[56] An early study demonstrated that ectopic expression of *Runx1* negatively regulates Th2 polarization of splenic CD4+ T cells by suppressing *Gata3* expression while maintaining Th1 idendity.[57] In contrast to these data, later it was suggested that *Runx3* represents the principal RUNX component in Th1 cells, because this gene was upregulated during Th1 polarization, while *Runx1* remained barely expressed in Th1 primed cells.[58] Consistent with this, impaired IFN-γ production was detected in *Runx3*-null Th1 cells, whereas ectopically expressed *Runx3* markedly potentiated T-bet dependent IFN-γ production. Curiously, RUNX3 also interfered with the Th2 expression of IL-4 via binding to the IL4 silencer (DNase I hypersensitivity site IV in the mouse IL4 locus).[58] Furthermore, in the absence of *Runx3* IL-4 was reactivated in non-polarized or Th1 skewed T helper cells, which provoked asthma-like symptoms due to accelerated Th2 responses.[59] Several additional studies confirmed the instructive role of RUNX3 during Th1 polarization. For example, overexpression of *Runx3* in CD4+ T-cells led to polarization to the Th1 phenotype even under conditions that promote Th2 differentiation via suppression of GATA3 transcriptional activity.[60, 61] Opposing to these findings, the forced expression of *Gata3* redirect CD4+ T cell development into IL-4 producing Th2 type cells via repressing the capacity of RUNX3 to induce *Ifng* expression. Finally, the transcription factor TWIST1 has the potential to regulate Th1 polarization negatively via interfering with RUNX3, while the forced expression of RUNX3 could revert the suppressive effect of TWIST1 exerted on IFN-γ production.[62] In conclusion, RUNX3 acts as a positive regulator of murine Th1 polarization via the transcriptional stimulation of *Ifng* expression and the suppression of IL-4 production (Fig. 3).

Interestingly, *RUNX3* resides in the human chromosome 1p36.1, a genomic region containing putative susceptibility genes for asthma, hence *RUNX3* dysfunction might provoke accelerated Th2 responses in these patients.[63]

In addition to Th1/Th2 polarization, RUNX3 also exhibits diverse roles on Th17 development. Committed Th17 cells produce IL-17 and IL-22 cytokines to protect mucosal surfaces against extracellular pathogens; moreover, these cells are implicated in the development of various autoimmune diseases.[64] Forced expression of *Runx1* or *Runx3* in differentiated Th17 cells were found to enhance the development of IL-17A and IFN-γ producing Th17 cells, conversely, *Runx1* or *Runx3* deficiency impaired the formation of IFN-γ producing cells.[65] These findings imply that both RUNX1 and RUNX3 have the potential to reprogram Th17 cells into pathogenic IL17/IFN-γ releasing cells, which are implicated in various autoimmune diseases. Contrary to these results, upregulation of *Runx3* suppress Th17 polarization, whereas *Runx3*-deleted CD4+ T cells conferred enhanced Th17 differentiation capacity suggesting that *Runx3*-instructed CD4+ T cells are highly tolerogenic and thus are able to deal with gut specific antigens without provoking inflammation.[66] In agreement with the putative tolerogenic effects of this factor, several lines of evidence suggest that RUNX proteins facilitate the conversion of CD4+ T cells into immunosuppressive Treg cells. The RUNX1-Cbfβ heterodimer is a putative regulator of Treg cell functions because *Cbfb*-deleted Treg cells exhibit impaired T cell suppressive capacity; in addition, *RUNX1* silenced human Treg cells expressed less *FOXP3*.[67, 68] Despite of these findings, later studies also suggested the central role of RUNX3 in Treg cell generation. For example, ectopically expressed *Runx3* more consistently regulated the *Foxp3* expression in Treg cells compared with *Runx1*.[69] In addition, *RUNX3* and *FOXP3* were highly expressed in circulating human CD4+ CD25++ Treg cells.[70]

The possibility that RUNX3 promotes the differentiation of additional suppressive cell types, i.e. the intraepithelial lymphocytes (IELs) was also emerged. Retinoic acid and TGF-β could elicit the differentiation of CD8αα IEL cells in CD4+ T cells in a RUNX3-dependent manner.[71] Furthermore, T-bet and RUNX3 together repressed CD4+ T helper cell functions and elicited the IEL program including the expression of CD8αα.[72] These observations altogether suggest that the intestinal environment provides cues for RUNX3 dependent Treg and/or IEL cell differentiation.

VI. RUNX3 INSTRUCTED NK CELL PROLIFERATION AND MATURATION

Natural killer (NK) cells provide cytotoxic innate cell defense against virus-infected as well as transformed cells. Similarly to CD8+ cytotoxic T cells, RUNX3 is highly expressed in mouse and human NK cells and is able to modulate the activity of this blood cell lineage.[73-75] It was documented that overexpression of a dominant negative RUNX protein reduced NK cell numbers in *ex vivo* differentiated cells. Furthermore, semimature ($CD43^{high}Mac\text{-}1^{high}$) splenic NK cells were obtained from the dominant negative *Runx* gene carrying transgenic mice.[73] A loss of function study confirmed the regulatory role of *Runx3* on NK cell activation. Lower percentage of mature NK cells was detected in *Runx3*-null spleen, moreover, defective IL-15-dependent NK cell proliferation and expansion was observed in the *Runx3*-deficient animals, preferentially in the uterus.[75] Despite of these alterations, the remaining *Runx3*-deficient NK cells still exhibited normal cytotoxic activity. In agreement with these findings, forced expression of *Runx3* was sufficient to preserve NK cell responses upon induction of brain ischemia suggesting that the sustained expression of *Runx3* contributed to the maintenance of NK cell function *in vivo*.[76]

RUNX3 can directly modulates the transcription of *Ncr1* (also known as *NKp46*) an NK cell affiliated gene via binding to promoter specific RUNX response elements.[77] This study also described that a dominant negative form of RUNX was able to repress *Ncr1* expression,

whereas, overexpression of *RUNX3* positively regulated the NKp46 level in a murine NK cell line (KY-2). To further characterize the genomic impact of RUNX3 on NK cells a genome-wide gene expression analysis was employed. This study revealed that 891 genes exhibited altered expression in *Runx3*-deleted splenic NK cells.[75] More importantly, 73% of the altered genes could regulate this factor directly, since RUNX3 occupancy was detected in the regulatory regions of these genes. However, further studies are needed to characterize the contribution of these putative RUNX3 regulated genes on NK cell development and activity. Moreover, it will be important to probe those common targets, which are modulated by RUNX3 similarly to cytotoxic CD8+ T cells as compared to NK cells.[16] In summary, NK cell maturation and proliferation can be modified with RUNX3 (Fig. 4), however the genomic targets of these factors is still poorly characterized in NK cells.

Finally it is worth mentioning that RUNX3 regulates the development of two additional innate lymphoid cell types. Deletion of *Runx3* from NKp46-expressing cells, results in innate lymphoid cell type 1 (ILC1) and cell type 3 (ILC3) deficiency. It seems that RUNX3 can maintain the survival of ILC1 cells, and this factor also governs the differentiation of ILC3 via regulation of RORγ expression.[78]

VII. RUNX3 DEPENDENT B CELL PROLIFERATION AND IMMUNGLOBULIN-A CLASS SWITCH

Accumulating evidence indicates that the Epstein-Barr virus (EBV) provoked B cell proliferation is influenced by RUNX3. In the course of EBV infection, the viral transcription factor EBNA2 activates *RUNX3* expression through a specific DNA element within a super-enhancer.[79, 80] In addition to this observation, virus induced RUNX3 can downregulate the expression of RUNX1 via the P1 promoter specific response element in B lymphoid cells.[81] Of note, the impaired RUNX1 expression is correlated with the enhanced B cell proliferation.

The authors of these reports suggested that RUNX3 positively regulates the EBV-mediated B cell expansion and immortalization (Fig. 4) probably via the repression of RUNX1 expression.

In addition to cell proliferation, RUNX3 can also regulate the immunoglobulin A (IgA) class switch recombination in B cells. IgA antibodies are preferentially produced by gut associated B cells, protect against mucosal pathogens and modulate the size and composition of the intestinal microbiome. B cells can undergo IgA class switch recombination upon CD40-CD40L ligation promoted by TGF-β signaling.[82] Remarkably, RUNX3 is part of the TGF-β dependent complex, and binds to the promoter of the mouse germline Ig α gene thus stimulating the transcription of Ig α in splenic B and I.29μ cells.[83] It is worth mentioning that the transcription of germline Ig α is a pre-requisite for the IgA class switch recombination.[84] Additional studies also confirmed that TGF-β activated SMAD2, 3 and RUNX3 can cooperate at the promoter of the germline Ig α gene and together they stimulate Ig α transcription.[85-87] In accord with these results, impaired IgA class switch recombination was detected in *Runx3*-deleted splenic B cells in vitro.[36] Unexpectedly, *Runx3*-null mice were found to produced more IgA *in vivo* suggesting that other RUNX proteins might compensate for *Runx3*-deficiency upon IgA class switch recombination[36]. In agreement with this, impaired IgA production was detected in *Runx2*/*Runx3* double-deficient animals, and the retinoic acid- or the TGF-β-dependent inductions of germline Ig α transcription were also blocked in the absence of *Runx2*/*Runx3*. Moreover, re-expression of either *Runx2* or *Runx3* retained the IgA producing capacity of these B cells suggesting that both RUNX2 and RUNX3 modulate the TGF-β-dependent IgA class switch recombination in murine B cells.[88]

VIII. RUNX-DRIVEN LINEAGE CONVERSIONS AND IMMUNE CELL MATURATION

In this report we presented several examples for functional alterations in immune cells due to the overexpression or deletion of RUNX3. These cell type specific genetic modifications helped us to define the steady state and the pathological roles of this important regulatory protein. Of note, nowadays overexpression of transcription factors has become a feasible and robust tool to manipulate cell fate choices especially after the discovery that exogenously added factors could reprogram differentiated cells into induced pluripotent stem (iPS) cells.[89] This seminal discovery has altered fundamental ideas on the stability of cellular identity, stimulating novel cell reprogramming strategies including direct lineage conversions.[90, 91] In this context, fibroblasts could successfully be transdifferentiated to cardiomyocytes or neurons with a cocktail of cardiac or neuronal specific transcription factors, respectively.[92, 93] Moreover, fibroblasts were reconverted to hematopoietic cells with transcription factor mediated lineage reporgramming.[94-97] In addition, pluripotent stem cell-derived blood cell differentiation was improved with ectopically expressed transcription factors.[34, 98-103]

To modify cellular identity, the core gene regulatory networks need proper reactivation with lineage determining (pioneer) transcription factors.[104] In case of neuronal reprogramming Ascl1 acts as a lineage determining transcription factor, while engineering fibroblast derived liver cells Foxa1-3 were considered as pioneer transcription factors.[105, 106] Importantly, lineage determining transcription factors by themselves are usually not sufficient to generate functional target cells, additional maturation promoting transcription factors are required to stimulate the formation of fully mature cells.[90] Interestingly, RUNX1 is an active component of several transcription factor cocktails, which have previously been used to engineer hematopoietic cells derived from fibroblasts, endothelial cells or pluripotent stem cells.[94, 101, 107, 108] Until now, the hierarchy of the selected transcription factors has not been determined in these blood cell conversion studies. However, it is well known that RUNX1 plays a pivotal role in transforming the hemogenic endothelium to hematopoietic cells, suggesting that

RUNX1 acts as a blood lineage determining transcription factor.[18] In contrast to RUNX1, our analysis suggested that RUNX3 can be classified as a maturation promoting factor in the course of ES-DC development. During the *ex vivo* differentiation process, *PU.1* and *Irf8* were identified as highly expressed genes in ES-DCs, and these two pioneer transcription factors might be able to drive the development of these cells.[34] Even though *ex vivo* differentiated DCs had a limited T cell activating potential, the enforced expression of RUNX3 still could facilitate strong DC dependent immunogenicity suggesting that RUNX3 acts as a *bona fide* maturation factor in this pluripotent stem cell derived DC differentiation model. Of note, RUNX3 also stimulates the differentiation and the cytotoxic activity of CD8+ T and NK cells. Thus it seems feasible, that the forced expression of RUNX3 could facilitate the maturation of the cytolytic effector cells. Importantly, fully mature immune cells could be harnessed for cell-based immunotherapies.

Immunotherapy represents a powerful weapon against many cancer types. For example, ex vivo generated autologous DCs, were pulsed with tumor antigens and injected back into cancer patients to boost antitumor adaptive immune response. Some of these DC-based vaccines have been shown promising clinical outcomes.[109] In addition, immune checkpoint inhibitor antibodies were applied to facilitate T-cell response by antagonizing co-inhibitory signals (for example, CTLA-4 or PD1/PD-L1). Strikingly, durable responses were observed in increasing numbers of tumors upon the application of these checkpoint blockers.[110, 111] Finally, adoptive cell therapies were employed using tumor infiltrating lymphocytes or the patient's peripheral T cells were engineered to target cancer specific antigens, via physiological T cell receptors or chimeric antigen receptors (CARs).[112] Importantly, recent clinical trials with CD19 targeting CAR T cells has shown a remarkable, 90% remission in patients with acute B lymphoblastic leukemia, underscoring the great potential of this cell-based therapeutic strategy.[113] However, despite the high rate of response observed with these

novel methods, unfortunately, a few patients failed to response to these treatments and many responders relapsed after a longer period of time suggesting that these protocols must be further optimized.[114] Genetically encoded tools like ectopically expressed transcription factors may allow cell engineers to boost the survival and the immunogenicity of the ex vivo manufactured immune cells.

RUNX3 has a critical regulatory role in cytotoxic T cells, raising the possibility that this particular transcription factor could be harnessed for cellular therapy. In agreement with this, overexpression of RUNX3 in adoptively transferred murine T cells enhanced the abundance of tumor infiltrating lymphocytes and impacted tumor growth negatively suggesting, that RUNX3 activation or overexpression can be used to ameliorate the anticancer response.[55] In addition, our results indicated that RUNX3-instructed ES-DCs exhibited a superior T cell activation capacity which may be beneficial to provoke antitumor response.[34] In the future, these murine data must be extended with human studies to prove or disprove the therapeutic potential of the RUNX3-primed antigen presenting or cytotoxic T cells.

IX. CONCLUSIONS

In this review we have analyzed the immunoregulatory functions of RUNX3 in various lymphocyte and dendritic cell subsets. Genetic perturbation of *Runx3* helped us to understand the diverse roles of this transcriptional regulator in both lymphoid and myeloid cells. In many cases RUNX3 exhibits overlapping functions with RUNX1 but RUNX3 also exerts several unique and essential roles in committed immune cells. To understand the diverse molecular functions of this important factor, numerous RUNX3 target genes and pathways have already been identified in various immune cell types and transgenic models but the RUNX3 dependent genome-wide transcriptional and epigenetic program is just beginning to be elucidated. Additional ChIP-seq analyses of RUNX3 binding and histone modifications would be needed for the identification and characterization of novel immune cell-specific RUNX

genomic regulatory elements. Moreover, these gene expression analyses must be expanded with single cell genomic approaches to further elucidate the cell type specific roles of this master gene. In addition, increased number of human data would be needed to uncover the possible pathological roles of RUNX3 in immune cell disorders. Finally, RUNX3 can facilitate the terminal differentiation and/or the maturation of cytotoxic T lymphocytes and some DC subsets. Based on this, the forced expression or the reactivation of RUNX3 might be applied for developing immunotherapeutic approaches to provoke relevant immune cell responses able to be used in clinical settings.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Hughes S, Woollard A. RUNX in Invertebrates. Adv Exp Med Biol. 2017;962:3-18. PubMed PMID: 28299647.
2. Tahirov TH, Inoue-Bungo T, Morii H, Fujikawa A, Sasaki M, Kimura K, Shiina M, Sato K, Kumasaka T, Yamamoto M, Ishii S, Ogata K. Structural analyses of DNA recognition by the AML1/Runx-1 Runt domain and its allosteric control by CBFbeta. Cell. 2001 Mar 09;104(5):755-67. PubMed PMID: 11257229.
3. Bowers SR, Calero-Nieto FJ, Valeaux S, Fernandez-Fuentes N, Cockerill PN. Runx1 binds as a dimeric complex to overlapping Runx1 sites within a palindromic element in the human GM-CSF enhancer. Nucleic Acids Res. 2010 Oct;38(18):6124-34. PubMed PMID: 20483917. PMCID: PMC2952845.
4. Yang XJ. The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. Nucleic Acids Res. 2004;32(3):959-76. PubMed PMID: 14960713. PMCID: PMC384351.

5. Kitabayashi I, Yokoyama A, Shimizu K, Ohki M. Interaction and functional cooperation of the leukemia-associated factors AML1 and p300 in myeloid cell differentiation. The EMBO journal. 1998 Jun 1;17(11):2994-3004. PubMed PMID: 9606182. PMCID: PMC1170639.
6. Yarmus M, Woolf E, Bernstein Y, Fainaru O, Negreanu V, Levanon D, Groner Y. Groucho/transducin-like Enhancer-of-split (TLE)-dependent and -independent transcriptional regulation by Runx3. Proc Natl Acad Sci U S A. 2006 May 09;103(19):7384-9. PubMed PMID: 16651517. PMCID: PMC1464349.
7. Imai Y, Kurokawa M, Yamaguchi Y, Izutsu K, Nitta E, Mitani K, Satake M, Noda T, Ito Y, Hirai H. The corepressor mSin3A regulates phosphorylation-induced activation, intranuclear location, and stability of AML1. Mol Cell Biol. 2004 Feb;24(3):1033-43. PubMed PMID: 14729951. PMCID: PMC321417.
8. Lutterbach B, Westendorf JJ, Linggi B, Isaac S, Seto E, Hiebert SW. A mechanism of repression by acute myeloid leukemia-1, the target of multiple chromosomal translocations in acute leukemia. J Biol Chem. 2000 Jan 7;275(1):651-6. PubMed PMID: 10617663.
9. Javed A, Guo B, Hiebert S, Choi JY, Green J, Zhao SC, Osborne MA, Stifani S, Stein JL, Lian JB, van Wijnen AJ, Stein GS. Groucho/TLE/R-esp proteins associate with the nuclear matrix and repress RUNX (CBF(alpha)/AML/PEBP2(alpha)) dependent activation of tissue-specific gene transcription. J Cell Sci. 2000 Jun;113 (Pt 12):2221-31. PubMed PMID: 10825294.
10. Reed-Inderbitzin E, Moreno-Miralles I, Vanden-Eynden SK, Xie J, Lutterbach B, Durst-Goodwin KL, Luce KS, Irvin BJ, Cleary ML, Brandt SJ, Hiebert SW. RUNX1 associates with histone deacetylases and SUV39H1 to repress transcription. Oncogene. 2006 Sep 21;25(42):5777-86. PubMed PMID: 16652147.
11. Chakraborty S, Sinha KK, Senyuk V, Nucifora G. SUV39H1 interacts with AML1 and abrogates AML1 transactivity. AML1 is methylated in vivo. Oncogene. 2003 Aug 14;22(34):5229-37. PubMed PMID: 12917624.
12. Durst KL, Hiebert SW. Role of RUNX family members in transcriptional repression and gene silencing. Oncogene. 2004 May 24;23(24):4220-4. PubMed PMID: 15156176.
13. Kagoshima H, Nimmo R, Saad N, Tanaka J, Miwa Y, Mitani S, Kohara Y, Woollard A. The C. elegans CBFbeta homologue BRO-1 interacts with the Runx factor, RNT-1, to promote stem cell proliferation and self-renewal. Development. 2007 Nov;134(21):3905-15. PubMed PMID: 17933794.
14. Golling G, Li L, Pepling M, Stebbins M, Gergen JP. Drosophila homologs of the proto-oncogene product PEBP2/CBF beta regulate the DNA-binding properties of Runt. Mol Cell Biol. 1996 Mar;16(3):932-42. PubMed PMID: 8622696. PMCID: PMC231075.
15. Wotton D, Ghysdael J, Wang S, Speck NA, Owen MJ. Cooperative binding of Ets-1 and core binding factor to DNA. Mol Cell Biol. 1994 Jan;14(1):840-50. PubMed PMID: 8264651. PMCID: PMC358432.
16. Lotem J, Levanon D, Negreanu V, Leshkowitz D, Friedlander G, Groner Y. Runx3-mediated transcriptional program in cytotoxic lymphocytes. PLoS One. 2013;8(11):e80467. PubMed PMID: 24236182. PMCID: PMC3827420.
17. Levanon D, Groner Y. Structure and regulated expression of mammalian RUNX genes. Oncogene. 2004 May 24;23(24):4211-9. PubMed PMID: 15156175.
18. de Bruijn M, Dzierzak E. Runx transcription factors in the development and function of the definitive hematopoietic system. Blood. 2017 Apr 13;129(15):2061-9. PubMed PMID: 28179276.
19. Vaillant F, Blyth K, Andrew L, Neil JC, Cameron ER. Enforced expression of Runx2 perturbs T cell development at a stage coincident with beta-selection. J Immunol. 2002 Sep 15;169(6):2866-74. PubMed PMID: 12218099.
20. Sood R, Kamikubo Y, Liu P. Role of RUNX1 in hematological malignancies. Blood. 2017 Apr 13;129(15):2070-82. PubMed PMID: 28179279. PMCID: PMC5391618.
21. Komori T. Regulation of osteoblast differentiation by transcription factors. J Cell Biochem. 2006 Dec 01;99(5):1233-9. PubMed PMID: 16795049.
22. Otto F, Thornell AP, Crompton T, Denzel A, Gilmour KC, Rosewell IR, Stamp GW, Beddington RS, Mundlos S, Olsen BR, Selby PB, Owen MJ. Cbfa1, a candidate gene for cleidocranial dysplasia

syndrome, is essential for osteoblast differentiation and bone development. Cell. 1997 May 30;89(5):765-71. PubMed PMID: 9182764.
23. Levanon D, Bettoun D, Harris-Cerruti C, Woolf E, Negreanu V, Eilam R, Bernstein Y, Goldenberg D, Xiao C, Fliegauf M, Kremer E, Otto F, Brenner O, Lev-Tov A, Groner Y. The Runx3 transcription factor regulates development and survival of TrkC dorsal root ganglia neurons. The EMBO journal. 2002 Jul 01;21(13):3454-63. PubMed PMID: 12093746. PMCID: PMC125397.
24. Inoue K, Ozaki S, Shiga T, Ito K, Masuda T, Okado N, Iseda T, Kawaguchi S, Ogawa M, Bae SC, Yamashita N, Itohara S, Kudo N, Ito Y. Runx3 controls the axonal projection of proprioceptive dorsal root ganglion neurons. Nat Neurosci. 2002 Oct;5(10):946-54. PubMed PMID: 12352981.
25. Gottgens B. Regulatory network control of blood stem cells. Blood. 2015 Apr 23;125(17):2614-20. PubMed PMID: 25762179.
26. Dzierzak E, Speck NA. Of lineage and legacy: the development of mammalian hematopoietic stem cells. Nat Immunol. 2008 Feb;9(2):129-36. PubMed PMID: 18204427. PMCID: PMC2696344.
27. Tober J, Maijenburg MW, Speck NA. Taking the Leap: Runx1 in the Formation of Blood from Endothelium. Curr Top Dev Biol. 2016;118:113-62. PubMed PMID: 27137656.
28. Landry JR, Kinston S, Knezevic K, de Bruijn MF, Wilson N, Nottingham WT, Peitz M, Edenhofer F, Pimanda JE, Ottersbach K, Gottgens B. Runx genes are direct targets of Scl/Tal1 in the yolk sac and fetal liver. Blood. 2008 Mar 15;111(6):3005-14. PubMed PMID: 18184866.
29. Wang CQ, Krishnan V, Tay LS, Chin DW, Koh CP, Chooi JY, Nah GS, Du L, Jacob B, Yamashita N, Lai SK, Tan TZ, Mori S, Tanuichi I, Tergaonkar V, Ito Y, Osato M. Disruption of Runx1 and Runx3 leads to bone marrow failure and leukemia predisposition due to transcriptional and DNA repair defects. Cell Rep. 2014 Aug 07;8(3):767-82. PubMed PMID: 25066130.
30. Wang CQ, Motoda L, Satake M, Ito Y, Taniuchi I, Tergaonkar V, Osato M. Runx3 deficiency results in myeloproliferative disorder in aged mice. Blood. 2013 Jul 25;122(4):562-6. PubMed PMID: 23741011.
31. Kalev-Zylinska ML, Horsfield JA, Flores MV, Postlethwait JH, Chau JY, Cattin PM, Vitas MR, Crosier PS, Crosier KE. Runx3 is required for hematopoietic development in zebrafish. Dev Dyn. 2003 Nov;228(3):323-36. PubMed PMID: 14579373.
32. Kissa K, Herbomel P. Blood stem cells emerge from aortic endothelium by a novel type of cell transition. Nature. 2010 Mar 04;464(7285):112-5. PubMed PMID: 20154732.
33. Dicken J, Mildner A, Leshkowitz D, Touw IP, Hantisteanu S, Jung S, Groner Y. Transcriptional reprogramming of CD11b+Esam(hi) dendritic cell identity and function by loss of Runx3. PLoS One. 2013;8(10):e77490. PubMed PMID: 24204843. PMCID: 3817345.
34. Takacs E, Boto P, Simo E, Csuth TI, Toth BM, Raveh-Amit H, Pap A, Kovacs EG, Kobolak J, Benko S, Dinnyes A, Szatmari I. Immunogenic Dendritic Cell Generation from Pluripotent Stem Cells by Ectopic Expression of Runx3. J Immunol. 2017 Jan 01;198(1):239-48. PubMed PMID: 27852743.
35. Dominguez-Soto A, Relloso M, Vega MA, Corbi AL, Puig-Kroger A. RUNX3 regulates the activity of the CD11a and CD49d integrin gene promoters. Immunobiology. 2005;210(2-4):133-9. PubMed PMID: 16164020.
36. Fainaru O, Woolf E, Lotem J, Yarmus M, Brenner O, Goldenberg D, Negreanu V, Bernstein Y, Levanon D, Jung S, Groner Y. Runx3 regulates mouse TGF-beta-mediated dendritic cell function and its absence results in airway inflammation. The EMBO journal. 2004 Feb 25;23(4):969-79. PubMed PMID: 14765120. PMCID: 380997.
37. Fainaru O, Shseyov D, Hantisteanu S, Groner Y. Accelerated chemokine receptor 7-mediated dendritic cell migration in Runx3 knockout mice and the spontaneous development of asthma-like disease. Proc Natl Acad Sci U S A. 2005 Jul 26;102(30):10598-603. PubMed PMID: 16027362. PMCID: PMC1180803.
38. Li QL, Ito K, Sakakura C, Fukamachi H, Inoue K, Chi XZ, Lee KY, Nomura S, Lee CW, Han SB, Kim HM, Kim WJ, Yamamoto H, Yamashita N, Yano T, Ikeda T, Itohara S, Inazawa J, Abe T, Hagiwara A, Yamagishi H, Ooe A, Kaneda A, Sugimura T, Ushijima T, Bae SC, Ito Y. Causal relationship between the loss of RUNX3 expression and gastric cancer. Cell. 2002 Apr 05;109(1):113-24. PubMed PMID: 11955451.

39. Borkowski TA, Letterio JJ, Farr AG, Udey MC. A role for endogenous transforming growth factor beta 1 in Langerhans cell biology: the skin of transforming growth factor beta 1 null mice is devoid of epidermal Langerhans cells. J Exp Med. 1996 Dec 1;184(6):2417-22. PubMed PMID: 8976197. PMCID: PMC2196398.
40. Sere K, Felker P, Hieronymus T, Zenke M. TGFbeta1 microenvironment determines dendritic cell development. Oncoimmunology. 2013 Mar 1;2(3):e23083. PubMed PMID: 23687620. PMCID: PMC3655738.
41. Doebel T, Voisin B, Nagao K. Langerhans Cells - The Macrophage in Dendritic Cell Clothing. Trends Immunol. 2017 Nov;38(11):817-28. PubMed PMID: 28720426.
42. Chopin M, Seillet C, Chevrier S, Wu L, Wang H, Morse HC, 3rd, Belz GT, Nutt SL. Langerhans cells are generated by two distinct PU.1-dependent transcriptional networks. The Journal of experimental medicine. 2013 Dec 16;210(13):2967-80. PubMed PMID: 24249112. PMCID: 3865480.
43. Woolf E, Xiao C, Fainaru O, Lotem J, Rosen D, Negreanu V, Bernstein Y, Goldenberg D, Brenner O, Berke G, Levanon D, Groner Y. Runx3 and Runx1 are required for CD8 T cell development during thymopoiesis. Proc Natl Acad Sci U S A. 2003 Jun 24;100(13):7731-6. PubMed PMID: 12796513. PMCID: PMC164656.
44. Taniuchi I, Osato M, Egawa T, Sunshine MJ, Bae SC, Komori T, Ito Y, Littman DR. Differential requirements for Runx proteins in CD4 repression and epigenetic silencing during T lymphocyte development. Cell. 2002 Nov 27;111(5):621-33. PubMed PMID: 12464175.
45. Seo W, Tanaka H, Miyamoto C, Levanon D, Groner Y, Taniuchi I. Roles of VWRPY motif-mediated gene repression by Runx proteins during T-cell development. Immunol Cell Biol. 2012 Sep;90(8):827-30. PubMed PMID: 22370763.
46. Setoguchi R, Tachibana M, Naoe Y, Muroi S, Akiyama K, Tezuka C, Okuda T, Taniuchi I. Repression of the transcription factor Th-POK by Runx complexes in cytotoxic T cell development. Science. 2008 Feb 08;319(5864):822-5. PubMed PMID: 18258917.
47. Egawa T, Littman DR. ThPOK acts late in specification of the helper T cell lineage and suppresses Runx-mediated commitment to the cytotoxic T cell lineage. Nat Immunol. 2008 Oct;9(10):1131-9. PubMed PMID: 18776905. PMCID: PMC2666788.
48. Luckey MA, Kimura MY, Waickman AT, Feigenbaum L, Singer A, Park JH. The transcription factor ThPOK suppresses Runx3 and imposes CD4(+) lineage fate by inducing the SOCS suppressors of cytokine signaling. Nat Immunol. 2014 Jul;15(7):638-45. PubMed PMID: 24880459.
49. Kimura MY, Thomas J, Tai X, Guinter TI, Shinzawa M, Etzensperger R, Li Z, Love P, Nakayama T, Singer A. Timing and duration of MHC I positive selection signals are adjusted in the thymus to prevent lineage errors. Nat Immunol. 2016 Dec;17(12):1415-23. PubMed PMID: 27668801.
50. Shan Q, Zeng Z, Xing S, Li F, Hartwig SM, Gullicksrud JA, Kurup SP, Van Braeckel-Budimir N, Su Y, Martin MD, Varga SM, Taniuchi I, Harty JT, Peng W, Badovinac VP, Xue HH. The transcription factor Runx3 guards cytotoxic CD8+ effector T cells against deviation towards follicular helper T cell lineage. Nat Immunol. 2017 Jun 12. PubMed PMID: 28604718.
51. Grueter B, Petter M, Egawa T, Laule-Kilian K, Aldrian CJ, Wuerch A, Ludwig Y, Fukuyama H, Wardemann H, Waldschuetz R, Moroy T, Taniuchi I, Steimle V, Littman DR, Ehlers M. Runx3 regulates integrin alpha E/CD103 and CD4 expression during development of CD4-/CD8+ T cells. J Immunol. 2005 Aug 01;175(3):1694-705. PubMed PMID: 16034110.
52. Kohu K, Sato T, Ohno S, Hayashi K, Uchino R, Abe N, Nakazato M, Yoshida N, Kikuchi T, Iwakura Y, Inoue Y, Watanabe T, Habu S, Satake M. Overexpression of the Runx3 transcription factor increases the proportion of mature thymocytes of the CD8 single-positive lineage. J Immunol. 2005 Mar 01;174(5):2627-36. PubMed PMID: 15728469.
53. Sato T, Ohno S, Hayashi T, Sato C, Kohu K, Satake M, Habu S. Dual functions of Runx proteins for reactivating CD8 and silencing CD4 at the commitment process into CD8 thymocytes. Immunity. 2005 Mar;22(3):317-28. PubMed PMID: 15780989.
54. Cruz-Guilloty F, Pipkin ME, Djuretic IM, Levanon D, Lotem J, Lichtenheld MG, Groner Y, Rao A. Runx3 and T-box proteins cooperate to establish the transcriptional program of effector CTLs. J Exp Med. 2009 Jan 16;206(1):51-9. PubMed PMID: 19139168. PMCID: PMC2626671.

55. Milner JJ, Toma C, Yu B, Zhang K, Omilusik K, Phan AT, Wang D, Getzler AJ, Nguyen T, Crotty S, Wang W, Pipkin ME, Goldrath AW. Runx3 programs CD8(+) T cell residency in non-lymphoid tissues and tumours. Nature. 2017 Dec 14;552(7684):253-7. PubMed PMID: 29211713.
56. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol. 1986 Apr 01;136(7):2348-57. PubMed PMID: 2419430.
57. Komine O, Hayashi K, Natsume W, Watanabe T, Seki Y, Seki N, Yagi R, Sukzuki W, Tamauchi H, Hozumi K, Habu S, Kubo M, Satake M. The Runx1 transcription factor inhibits the differentiation of naive CD4+ T cells into the Th2 lineage by repressing GATA3 expression. J Exp Med. 2003 Jul 07;198(1):51-61. PubMed PMID: 12835475. PMCID: PMC2196077.
58. Djuretic IM, Levanon D, Negreanu V, Groner Y, Rao A, Ansel KM. Transcription factors T-bet and Runx3 cooperate to activate Ifng and silence Il4 in T helper type 1 cells. Nat Immunol. 2007 Feb;8(2):145-53. PubMed PMID: 17195845.
59. Naoe Y, Setoguchi R, Akiyama K, Muroi S, Kuroda M, Hatam F, Littman DR, Taniuchi I. Repression of interleukin-4 in T helper type 1 cells by Runx/Cbf beta binding to the Il4 silencer. J Exp Med. 2007 Aug 06;204(8):1749-55. PubMed PMID: 17646405. PMCID: PMC2118685.
60. Kohu K, Ohmori H, Wong WF, Onda D, Wakoh T, Kon S, Yamashita M, Nakayama T, Kubo M, Satake M. The Runx3 transcription factor augments Th1 and down-modulates Th2 phenotypes by interacting with and attenuating GATA3. J Immunol. 2009 Dec 15;183(12):7817-24. PubMed PMID: 19933870.
61. Yagi R, Junttila IS, Wei G, Urban JF, Jr., Zhao K, Paul WE, Zhu J. The transcription factor GATA3 actively represses RUNX3 protein-regulated production of interferon-gamma. Immunity. 2010 Apr 23;32(4):507-17. PubMed PMID: 20399120. PMCID: PMC2867662.
62. Pham D, Vincentz JW, Firulli AB, Kaplan MH. Twist1 regulates Ifng expression in Th1 cells by interfering with Runx3 function. J Immunol. 2012 Jul 15;189(2):832-40. PubMed PMID: 22685315. PMCID: PMC3392532.
63. Haagerup A, Bjerke T, Schiotz PO, Binderup HG, Dahl R, Kruse TA. Asthma and atopy - a total genome scan for susceptibility genes. Allergy. 2002 Aug;57(8):680-6. PubMed PMID: 12121185.
64. Khader SA, Gaffen SL, Kolls JK. Th17 cells at the crossroads of innate and adaptive immunity against infectious diseases at the mucosa. Mucosal Immunol. 2009 Sep;2(5):403-11. PubMed PMID: 19587639. PMCID: PMC2811522.
65. Wang Y, Godec J, Ben-Aissa K, Cui K, Zhao K, Pucsek AB, Lee YK, Weaver CT, Yagi R, Lazarevic V. The transcription factors T-bet and Runx are required for the ontogeny of pathogenic interferon-gamma-producing T helper 17 cells. Immunity. 2014 Mar 20;40(3):355-66. PubMed PMID: 24530058. PMCID: PMC3965587.
66. Reis BS, Rogoz A, Costa-Pinto FA, Taniuchi I, Mucida D. Mutual expression of the transcription factors Runx3 and ThPOK regulates intestinal CD4(+) T cell immunity. Nat Immunol. 2013 Mar;14(3):271-80. PubMed PMID: 23334789. PMCID: PMC3804366.
67. Rudra D, Egawa T, Chong MM, Treuting P, Littman DR, Rudensky AY. Runx-CBFbeta complexes control expression of the transcription factor Foxp3 in regulatory T cells. Nat Immunol. 2009 Nov;10(11):1170-7. PubMed PMID: 19767756. PMCID: PMC2764816.
68. Kitoh A, Ono M, Naoe Y, Ohkura N, Yamaguchi T, Yaguchi H, Kitabayashi I, Tsukada T, Nomura T, Miyachi Y, Taniuchi I, Sakaguchi S. Indispensable role of the Runx1-Cbfbeta transcription complex for in vivo-suppressive function of FoxP3+ regulatory T cells. Immunity. 2009 Oct 16;31(4):609-20. PubMed PMID: 19800266.
69. Bruno L, Mazzarella L, Hoogenkamp M, Hertweck A, Cobb BS, Sauer S, Hadjur S, Leleu M, Naoe Y, Telfer JC, Bonifer C, Taniuchi I, Fisher AG, Merkenschlager M. Runx proteins regulate Foxp3 expression. J Exp Med. 2009 Oct 26;206(11):2329-37. PubMed PMID: 19841090. PMCID: PMC2768863.
70. Klunker S, Chong MM, Mantel PY, Palomares O, Bassin C, Ziegler M, Ruckert B, Meiler F, Akdis M, Littman DR, Akdis CA. Transcription factors RUNX1 and RUNX3 in the induction and suppressive

function of Foxp3+ inducible regulatory T cells. J Exp Med. 2009 Nov 23;206(12):2701-15. PubMed PMID: 19917773. PMCID: PMC2806624.
71. Nambu Y, Hayashi T, Jang KJ, Aoki K, Mano H, Nakano K, Osato M, Takahashi K, Itoh K, Teramukai S, Komori T, Fujita J, Ito Y, Shimizu A, Sugai M. In situ differentiation of CD8alphaalpha Tau cells from CD4 T cells in peripheral lymphoid tissues. Sci Rep. 2012;2:642. PubMed PMID: 22962636. PMCID: PMC3435563.
72. Reis BS, Hoytema van Konijnenburg DP, Grivennikov SI, Mucida D. Transcription factor T-bet regulates intraepithelial lymphocyte functional maturation. Immunity. 2014 Aug 21;41(2):244-56. PubMed PMID: 25148025. PMCID: PMC4287410.
73. Ohno S, Sato T, Kohu K, Takeda K, Okumura K, Satake M, Habu S. Runx proteins are involved in regulation of CD122, Ly49 family and IFN-gamma expression during NK cell differentiation. Int Immunol. 2008 Jan;20(1):71-9. PubMed PMID: 18003603.
74. Guo Y, Maillard I, Chakraborti S, Rothenberg EV, Speck NA. Core binding factors are necessary for natural killer cell development and cooperate with Notch signaling during T-cell specification. Blood. 2008 Aug 01;112(3):480-92. PubMed PMID: 18390836. PMCID: PMC2481540.
75. Levanon D, Negreanu V, Lotem J, Bone KR, Brenner O, Leshkowitz D, Groner Y. Transcription factor Runx3 regulates interleukin-15-dependent natural killer cell activation. Mol Cell Biol. 2014 Mar;34(6):1158-69. PubMed PMID: 24421391. PMCID: PMC3958033.
76. Liu Q, Jin WN, Liu Y, Shi K, Sun H, Zhang F, Zhang C, Gonzales RJ, Sheth KN, La Cava A, Shi FD. Brain Ischemia Suppresses Immunity in the Periphery and Brain via Different Neurogenic Innervations. Immunity. 2017 Mar 21;46(3):474-87. PubMed PMID: 28314594.
77. Lai CB, Mager DL. Role of runt-related transcription factor 3 (RUNX3) in transcription regulation of natural cytotoxicity receptor 1 (NCR1/NKp46), an activating natural killer (NK) cell receptor. J Biol Chem. 2012 Mar 02;287(10):7324-34. PubMed PMID: 22253448. PMCID: PMC3293567.
78. Ebihara T, Song C, Ryu SH, Plougastel-Douglas B, Yang L, Levanon D, Groner Y, Bern MD, Stappenbeck TS, Colonna M, Egawa T, Yokoyama WM. Runx3 specifies lineage commitment of innate lymphoid cells. Nat Immunol. 2015 Nov;16(11):1124-33. PubMed PMID: 26414766. PMCID: PMC4618046.
79. Gunnell A, Webb HM, Wood CD, McClellan MJ, Wichaidit B, Kempkes B, Jenner RG, Osborne C, Farrell PJ, West MJ. RUNX super-enhancer control through the Notch pathway by Epstein-Barr virus transcription factors regulates B cell growth. Nucleic Acids Res. 2016 Jun 02;44(10):4636-50. PubMed PMID: 26883634. PMCID: PMC4889917.
80. Spender LC, Cornish GH, Sullivan A, Farrell PJ. Expression of transcription factor AML-2 (RUNX3, CBF(alpha)-3) is induced by Epstein-Barr virus EBNA-2 and correlates with the B-cell activation phenotype. J Virol. 2002 May;76(10):4919-27. PubMed PMID: 11967309. PMCID: PMC136164.
81. Spender LC, Whiteman HJ, Karstegl CE, Farrell PJ. Transcriptional cross-regulation of RUNX1 by RUNX3 in human B cells. Oncogene. 2005 Mar 10;24(11):1873-81. PubMed PMID: 15688019.
82. Boyaka PN. Inducing Mucosal IgA: A Challenge for Vaccine Adjuvants and Delivery Systems. J Immunol. 2017 Jul 01;199(1):9-16. PubMed PMID: 28630108.
83. Shi MJ, Stavnezer J. CBF alpha3 (AML2) is induced by TGF-beta1 to bind and activate the mouse germline Ig alpha promoter. J Immunol. 1998 Dec 15;161(12):6751-60. PubMed PMID: 9862705.
84. Manis JP, Tian M, Alt FW. Mechanism and control of class-switch recombination. Trends Immunol. 2002 Jan;23(1):31-9. PubMed PMID: 11801452.
85. Zhang Y, Derynck R. Transcriptional regulation of the transforming growth factor-beta - inducible mouse germ line Ig alpha constant region gene by functional cooperation of Smad, CREB, and AML family members. J Biol Chem. 2000 Jun 02;275(22):16979-85. PubMed PMID: 10748032.
86. Pardali E, Xie XQ, Tsapogas P, Itoh S, Arvanitidis K, Heldin CH, ten Dijke P, Grundstrom T, Sideras P. Smad and AML proteins synergistically confer transforming growth factor beta1

responsiveness to human germ-line IgA genes. J Biol Chem. 2000 Feb 04;275(5):3552-60. PubMed PMID: 10652350.
87. Hanai J, Chen LF, Kanno T, Ohtani-Fujita N, Kim WY, Guo WH, Imamura T, Ishidou Y, Fukuchi M, Shi MJ, Stavnezer J, Kawabata M, Miyazono K, Ito Y. Interaction and functional cooperation of PEBP2/CBF with Smads. Synergistic induction of the immunoglobulin germline Calpha promoter. J Biol Chem. 1999 Oct 29;274(44):31577-82. PubMed PMID: 10531362.
88. Watanabe K, Sugai M, Nambu Y, Osato M, Hayashi T, Kawaguchi M, Komori T, Ito Y, Shimizu A. Requirement for Runx proteins in IgA class switching acting downstream of TGF-beta 1 and retinoic acid signaling. J Immunol. 2010 Mar 15;184(6):2785-92. PubMed PMID: 20142360.
89. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell. 2006 Aug 25;126(4):663-76. PubMed PMID: 16904174. Epub 2006/08/15. eng.
90. Xu J, Du Y, Deng H. Direct lineage reprogramming: strategies, mechanisms, and applications. Cell Stem Cell. 2015 Feb 5;16(2):119-34. PubMed PMID: 25658369.
91. Chambers SM, Studer L. Cell fate plug and play: direct reprogramming and induced pluripotency. Cell. 2011 Jun 10;145(6):827-30. PubMed PMID: 21663788.
92. Srivastava D, DeWitt N. In Vivo Cellular Reprogramming: The Next Generation. Cell. 2016 Sep 8;166(6):1386-96. PubMed PMID: 27610565.
93. Ang CE, Wernig M. Induced neuronal reprogramming. J Comp Neurol. 2014 Aug 15;522(12):2877-86. PubMed PMID: 24771471. PMCID: PMC4099045.
94. Batta K, Florkowska M, Kouskoff V, Lacaud G. Direct reprogramming of murine fibroblasts to hematopoietic progenitor cells. Cell Rep. 2014 Dec 11;9(5):1871-84. PubMed PMID: 25466247. PMCID: PMC4542300.
95. Pereira CF, Chang B, Qiu J, Niu X, Papatsenko D, Hendry CE, Clark NR, Nomura-Kitabayashi A, Kovacic JC, Ma'ayan A, Schaniel C, Lemischka IR, Moore K. Induction of a hemogenic program in mouse fibroblasts. Cell Stem Cell. 2013 Aug 1;13(2):205-18. PubMed PMID: 23770078. PMCID: PMC3735774.
96. Feng R, Desbordes SC, Xie H, Tillo ES, Pixley F, Stanley ER, Graf T. PU.1 and C/EBPalpha/beta convert fibroblasts into macrophage-like cells. Proc Natl Acad Sci U S A. 2008 Apr 22;105(16):6057-62. PubMed PMID: 18424555. PMCID: PMC2327209.
97. Szabo E, Rampalli S, Risueno RM, Schnerch A, Mitchell R, Fiebig-Comyn A, Levadoux-Martin M, Bhatia M. Direct conversion of human fibroblasts to multilineage blood progenitors. Nature. 2010 Nov 25;468(7323):521-6. PubMed PMID: 21057492.
98. Elcheva I, Brok-Volchanskaya V, Kumar A, Liu P, Lee JH, Tong L, Vodyanik M, Swanson S, Stewart R, Kyba M, Yakubov E, Cooke J, Thomson JA, Slukvin I. Direct induction of haematoendothelial programs in human pluripotent stem cells by transcriptional regulators. Nat Commun. 2014 Jul 14;5:4372. PubMed PMID: 25019369. PMCID: PMC4107340.
99. Doulatov S, Vo LT, Chou SS, Kim PG, Arora N, Li H, Hadland BK, Bernstein ID, Collins JJ, Zon LI, Daley GQ. Induction of multipotential hematopoietic progenitors from human pluripotent stem cells via respecification of lineage-restricted precursors. Cell Stem Cell. 2013 Oct 3;13(4):459-70. PubMed PMID: 24094326. PMCID: PMC3888026.
100. Kyba M, Perlingeiro RC, Daley GQ. HoxB4 confers definitive lymphoid-myeloid engraftment potential on embryonic stem cell and yolk sac hematopoietic progenitors. Cell. 2002 Apr 5;109(1):29-37. PubMed PMID: 11955444.
101. Sugimura R, Jha DK, Han A, Soria-Valles C, da Rocha EL, Lu YF, Goettel JA, Serrao E, Rowe RG, Malleshaiah M, Wong I, Sousa P, Zhu TN, Ditadi A, Keller G, Engelman AN, Snapper SB, Doulatov S, Daley GQ. Haematopoietic stem and progenitor cells from human pluripotent stem cells. Nature. 2017 May 25;545(7655):432-8. PubMed PMID: 28514439.
102. Wang Y, Yates F, Naveiras O, Ernst P, Daley GQ. Embryonic stem cell-derived hematopoietic stem cells. Proc Natl Acad Sci U S A. 2005 Dec 27;102(52):19081-6. PubMed PMID: 16357205. PMCID: PMC1323159.

103. Yamamizu K, Piao Y, Sharov AA, Zsiros V, Yu H, Nakazawa K, Schlessinger D, Ko MS. Identification of transcription factors for lineage-specific ESC differentiation. Stem Cell Reports. 2013;1(6):545-59. PubMed PMID: 24371809. PMCID: PMC3871400.
104. Zaret KS, Carroll JS. Pioneer transcription factors: establishing competence for gene expression. Genes Dev. 2011 Nov 1;25(21):2227-41. PubMed PMID: 22056668. PMCID: PMC3219227.
105. Wapinski OL, Vierbuchen T, Qu K, Lee QY, Chanda S, Fuentes DR, Giresi PG, Ng YH, Marro S, Neff NF, Drechsel D, Martynoga B, Castro DS, Webb AE, Sudhof TC, Brunet A, Guillemot F, Chang HY, Wernig M. Hierarchical mechanisms for direct reprogramming of fibroblasts to neurons. Cell. 2013 Oct 24;155(3):621-35. PubMed PMID: 24243019. PMCID: PMC3871197.
106. Sekiya S, Suzuki A. Direct conversion of mouse fibroblasts to hepatocyte-like cells by defined factors. Nature. 2011 Jun 29;475(7356):390-3. PubMed PMID: 21716291.
107. Lis R, Karrasch CC, Poulos MG, Kunar B, Redmond D, Duran JGB, Badwe CR, Schachterle W, Ginsberg M, Xiang J, Tabrizi AR, Shido K, Rosenwaks Z, Elemento O, Speck NA, Butler JM, Scandura JM, Rafii S. Conversion of adult endothelium to immunocompetent haematopoietic stem cells. Nature. 2017 May 25;545(7655):439-45. PubMed PMID: 28514438.
108. Sandler VM, Lis R, Liu Y, Kedem A, James D, Elemento O, Butler JM, Scandura JM, Rafii S. Reprogramming human endothelial cells to haematopoietic cells requires vascular induction. Nature. 2014 Jul 17;511(7509):312-8. PubMed PMID: 25030167. PMCID: PMC4159670.
109. Vacchelli E, Vitale I, Eggermont A, Fridman WH, Fucikova J, Cremer I, Galon J, Tartour E, Zitvogel L, Kroemer G, Galluzzi L. Trial watch: Dendritic cell-based interventions for cancer therapy. Oncoimmunology. 2013 Oct 1;2(10):e25771. PubMed PMID: 24286020. PMCID: PMC3841205.
110. Zou W, Wolchok JD, Chen L. PD-L1 (B7-H1) and PD-1 pathway blockade for cancer therapy: Mechanisms, response biomarkers, and combinations. Sci Transl Med. 2016 Mar 2;8(328):328rv4. PubMed PMID: 26936508. PMCID: PMC4859220.
111. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, Lebbe C, Baurain JF, Testori A, Grob JJ, Davidson N, Richards J, Maio M, Hauschild A, Miller WH, Jr., Gascon P, Lotem M, Harmankaya K, Ibrahim R, Francis S, Chen TT, Humphrey R, Hoos A, Wolchok JD. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011 Jun 30;364(26):2517-26. PubMed PMID: 21639810.
112. Chodon T, Koya RC, Odunsi K. Active Immunotherapy of Cancer. Immunol Invest. 2015;44(8):817-36. PubMed PMID: 26575466.
113. Lim WA, June CH. The Principles of Engineering Immune Cells to Treat Cancer. Cell. 2017 Feb 9;168(4):724-40. PubMed PMID: 28187291. PMCID: PMC5553442.
114. Sharma P, Hu-Lieskovan S, Wargo JA, Ribas A. Primary, Adaptive, and Acquired Resistance to Cancer Immunotherapy. Cell. 2017 Feb 9;168(4):707-23. PubMed PMID: 28187290. PMCID: PMC5391692.

FIGURE LEGEND

FIGURE 1. The major effects of RUNX3 on the phenotype of various dendritic cell (DC) subsets. The impacts of RUNX3 in TGF-β activated monocyte-derived DCs, in CD4+ splenic DCs and in embryonic stem cell derived dendritic cells (ES-DCs).

FIGURE 2. RUNX3 stimulated CD8+ T cell development. Here, we summarize the effects of RUNX3 on cytotoxic T cells.

FIGURE 3. RUNX3 instructed Th1 polarization. Here, we summarize the effects of RUNX3 upon the development of Th1/Th2 cells.

FIGURE 4. The major effects of RUNX3 on the phenotype of B and NK cells.

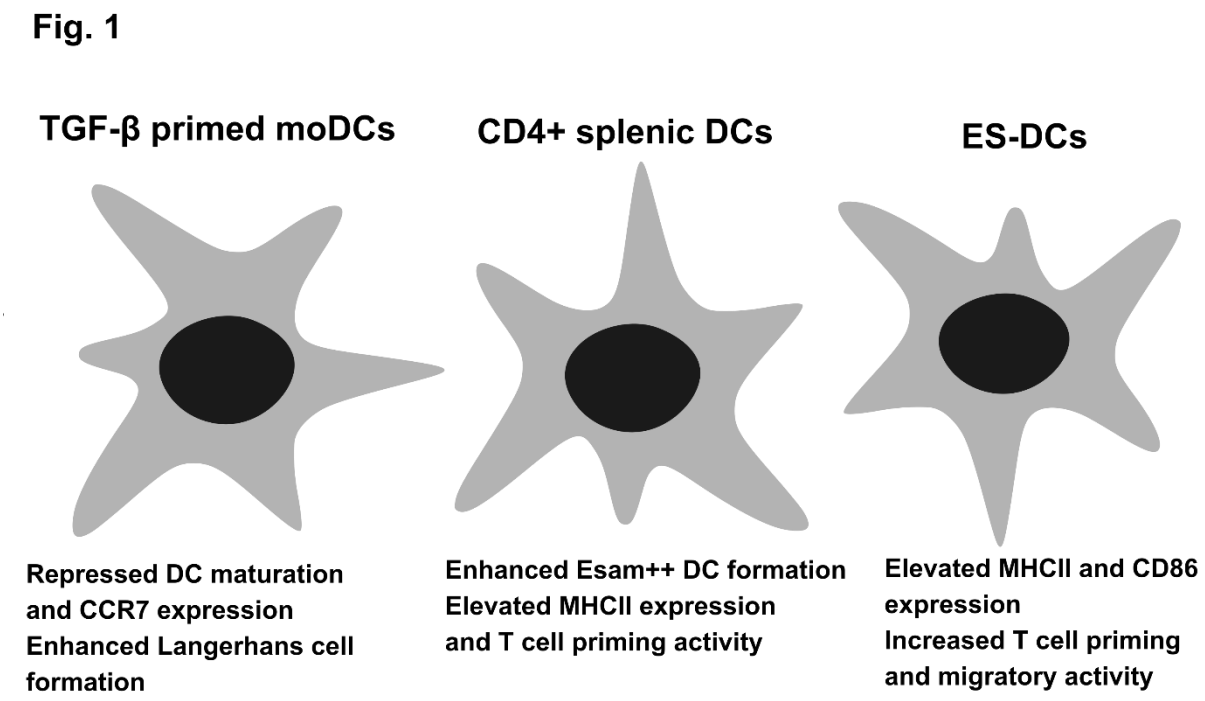


Fig. 2

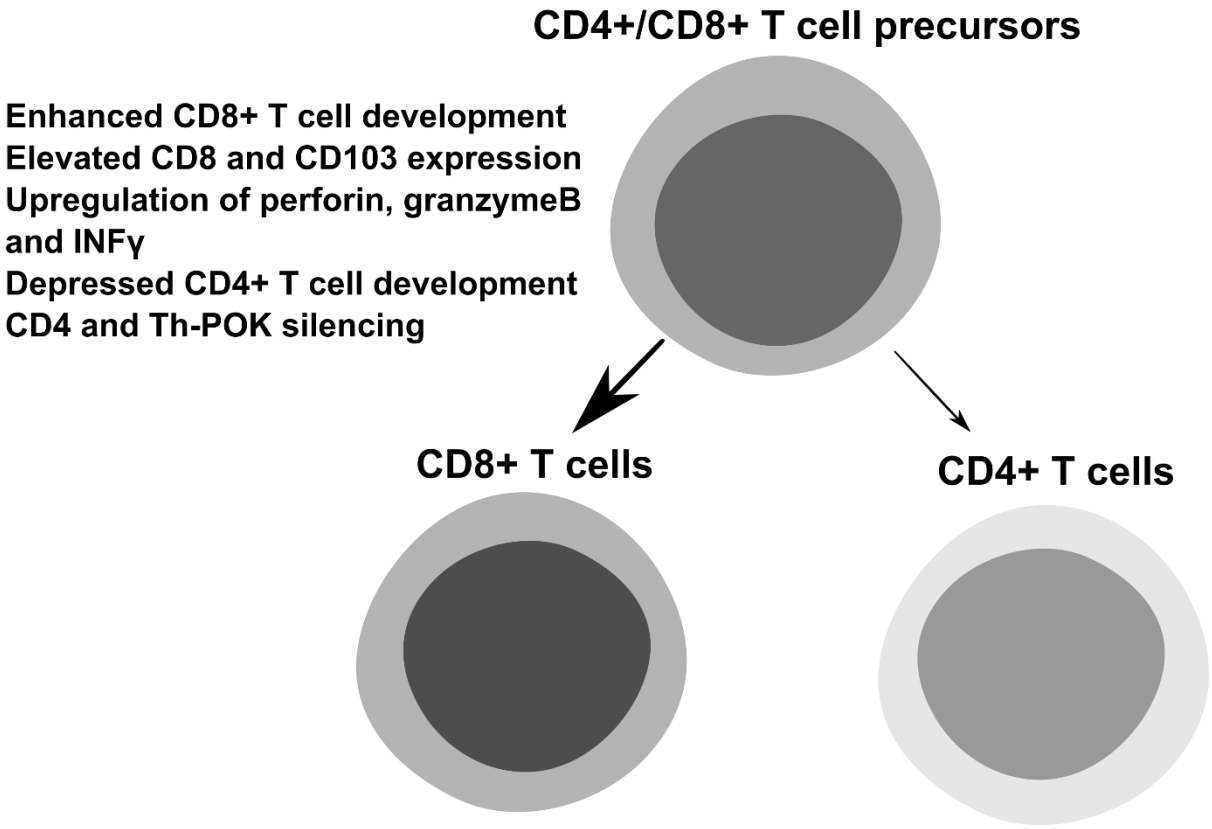

CD4+/CD8+ T cell precursors
Enhanced CD8+ T cell development
Elevated CD8 and CD103 expression
Upregulation of perforin, granzymeB and INFγ
Depressed CD4+ T cell development
CD4 and Th-POK silencing
CD8+ T cells
CD4+ T cells

Fig. 3

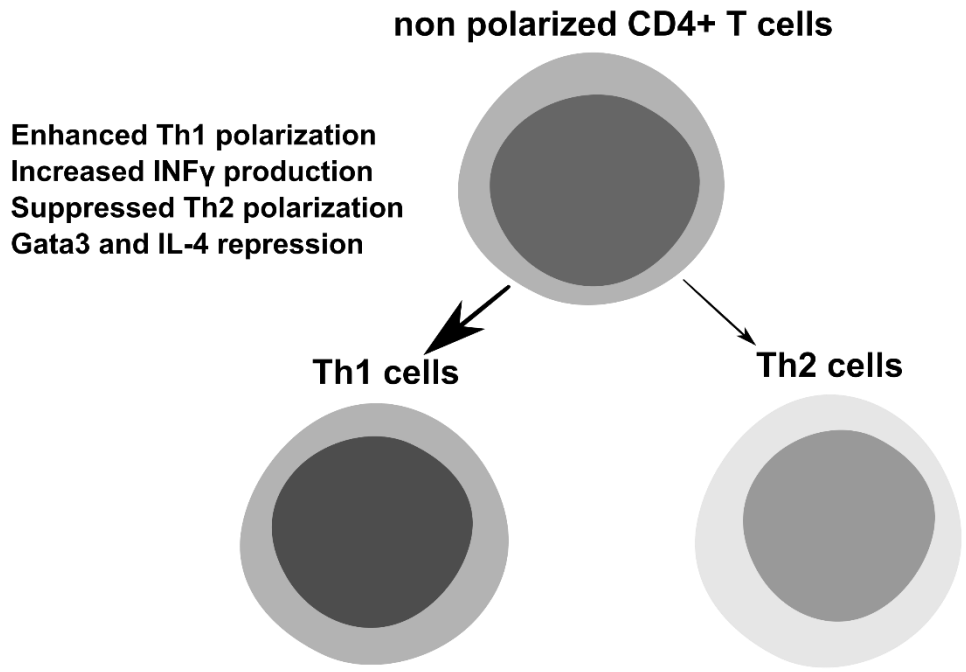
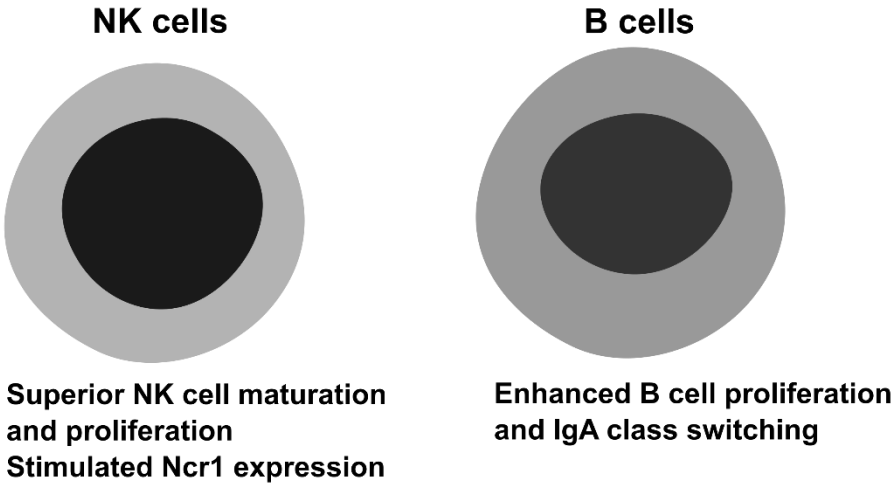

non polarized CD4+ T cells
Enhanced Th1 polarization
Increased INFγ production
Suppressed Th2 polarization
Gata3 and IL-4 repression
Th1 cells
Th2 cells

Fig. 4


NK cells
B cells
Superior NK cell maturation
and proliferation
Stimulated Ncr1 expression
Enhanced B cell proliferation
and IgA class switching