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Lymphocyte Commitment and Ikaros Transcription Factors

Mariastefania Antica
Rudjer Boskovic Institute
Croatia

1. Introduction

T lymphocytes like all blood cells are progenies of a single multipotent hematopoietic stem cell (HSC). The existence of HSCs was proven by Till and McCulloch in 1961 when bone marrow cells injected into irradiated mice formed multilineage colonies in their spleens. These cells were called colony forming units (CFU-S) and they have potential for self-renewal and differentiation into all types of blood cells(Till and McCulloch 1961; Wu et al. 1968). For lymphocyte development HSCs migrate from the bone marrow, differentiate in the thymus into immunocompetent cells and seed the peripheral lymphatic organs. This process occurs during fetal development (for review see (Godin and Cumano 2002), but also during the adult life of an individual since T lymphocytes have to be continuously replenished. Transplantation experiments proved that thirty hematopoietic stem cells are sufficient to save 50 percent of lethally irradiated mice, and to reconstitute all blood cell types in vivo (Spangrude et al. 1988). Because it is extremely important to produce immunocompetent T cells the process of their maturation and development is strictly regulated and is succumb to very strict check points at several stages of differentiation. In this context we'll discuss Ikaros family transcription factors as major regulators of T lymphocyte development.

2. Committed lymphocyte precursor

For a long time it has been postulated analogously to committed myeloid precursors that there is a committed lymphocyte precursor. However the first experiments that showed the existence of a committed lymphocyte precursor was described in the early 90tis when a new population of cells in the thymus was found and its features described (Wu 1991; Wu et al. 1991). These cells expressed most markers of the multipotent hematopoietic stem cells like Thy-1^{low}CD44$^+$H2k$^+$Sca-1$^+$ but they expressed Sca-2 and low levels of CD4. These cells were negative for all mature blood cells markers lymphocytes (CD8$^-$CD3$^-$Ig$^-$), macrophages (Mac-1$^-$), granulocytes (Gr-1$^-$) and erhytrocytes (TER119$^-$), lineage negative, (Lin$^-$ cells). Their Ig and TCR receptor genes were in germline configuration. The described precursors represent 0.05% of the cells in an adult mouse thymus. If the cells were sorted and transferred by intrathymic injections (*i.t.*) into lethally irradiated congenic mice they developed into all thymic subpopulations, first CD4$^-$CD8$^-$ (double negatives, DN) thymocytes, than into

$CD4^+CD8^+$ double positives which give rise to mature $CD4^+$ and $CD8^+$ lymphocytes. Mature cells derived from the $CD4^{low}$ precursors seeded the spleen, lymph nodes and bone marrow. The reconstitution potential of the described precursors was lower and the time for development was shorter when compared to the multipotent hematopoietic stem cells from the bone marrow. These experiments proved that the $CD4^{low}$ precursors in the thymus can develop into T lymphocytes. Because of their resemblance to the multipotent progenitors from the bone marrow it was important to answer the question whether their potential was restricted only to develop into T lymphocytes. Therefore we transplanted these cells into the periphery by injecting them intravenously (*i.v.*) into lethally irradiated congenic animals. Their progenies were found to develop into T lymphocytes similarly to the results obtained by direct *i.t.* transplantation experiments. When injected intravenously they also developed into B lymphocytes but there were no myeloid progenies. During fetal development these cells were detected from day 14 in the fetal thymus but only after birth their function was comparable to the adult ones(Antica et al. 1993). In the bone marrow the $CD4^{low}$ precursor cells were described, but their function was not lymphocyte restricted (Antica et al. 1994a). Further studies showed that in the bone marrow the marker that could differentiate the lymphoid precursor cells from the multipotent precursors was IL7Rα (Kondo et al. 1997). This population, called common lymphoid precursor (CLP) is characterized by Lin^- $Thy\text{-}1^-Sca\text{-}1^{lo}c\text{-}Kit^{lo}$ $IL\text{-}7R^+$ and possess rapid and prominent short-term lymphoid-restricted (T, B, and NK cells) reconstitution activity. However, this progenitor population clonally produces both B and T lymphocytes, but have little myeloid potential *in vivo.* A recently developed bioinformatics method, called Mining Developmentally Regulated Genes, which mines the publically available microarray data to identify genes that are up- or down-regulated within a developmental pathway was applied to identify surface proteins that distinguish functional CLPs from other progenitors (Inlay et al. 2009; Sahoo et al. 2010). A surface marker Ly6d dissects the CLP population in two and shows that it consists of a mixture of all lymphoid progenitor cells (ALP) which retain B and T lymphoid potential, and BLP (B cell biased lymphoid progenitors). This manuscript offers strong support for the validity of our earlier conclusions. However, ALPs still keep low myeloid potential indicating that ALPs are either a mixture of the $CD4^{low}$ precursor cells and myeloid progenitors or is a single population but at an earlier developmental stage and still multipotent(Wu et al. 1991; Antica et al. 1993; Antica et al. 1994b; Inlay et al. 2009). Here we also stress the necessity and importance of *in vivo* assays for the determination of physiologic lineage potentials since it has been recently shown that *in vitro* assays can misrepresent *in vivo* lineage potentials of murine lymphoid progenitors(Richie Ehrlich et al. 2011).

From the clinical point of view defining the earliest lymphoid precursor is important for a rapid engraftment and protection from infections after hematopoietic stem cell transplantation in chemotherapy or irradiation compromised patients (Arber et al. 2003; Holländer et al. 2010).

3. Ikaros transcription factors

Mechanisms and factors that regulate lymphocyte development from stem cells have to be very accurate since any alteration of this process may lead to serious diseases like leukemia.

Transcription factors from the Ikaros family play an essential role in the commitment of hematopoietic progenitors into the lymphoid lineage as well as in the choice of effector functions at later stages of development(Georgopoulos 1994; Sun et al. 1996; Wang et al. 1998; Cortes et al. 1999). Their role has been addressed by gene targeting and such gene inactivation studies have identified Ikaros, Aiolos and Helios as transcription factors required for the maturation of lymphocytes(Morgan 1997; Hahm 1998; Kelley 1998). It has been shown that mice homozygous for a deletion in these genes undergo remarkable changes in their lymphocyte populations and also those ageing animals with the same mutation develop lymphoproliferative disorders. A number of studies show that Ikaros genes in both mice and human malignancies might be deregulated (Winandy et al. 1995; Nichogiannopoulou et al. 1999; Nakase et al. 2000; Nakayama et al. 2000; Nakase et al. 2002; Rebollo and Schmitt 2003; Dovat et al. 2005; Mullighan et al. 2008; Matulic et al. 2009; Billot et al. 2010). Therefore we addressed the question whether a combination of transcription factor failures may contribute to the development of human lymphoma. We amplified human mRNA from formalin fixed paraffin embedded tissues from lymphoma patients in order to have consistent and well defined groups of patients. Hence, we were able to analyze Ikaros, Aiolos and Helios mRNA from archive tissue specimens from patients with Hodgkin's and non- Hodgkin's lymphoma and follicular hyperplasia (Antica et al. 2008; Antica et al. 2010). Further we and others show a deregulation in human leukemia. Acute lymphoblastic leukemia (ALL) is characterized by the Philadelphia chromosome (Ph) which encodes the BCR-ABL1 tyrosine kinase, the most frequent cytogenetic abnormality (~25–30% of cases)(Mancini et al. 2005). Deletion of the IKAROS gene (*IKZF1*) was found in 83.7% cases of BCR-ABL1 ALL, but not in chronic-phase CML(Mullighan et al. 2008). Posttranscriptional regulation of alternative splicing of Ikaros was associated with resistance to tyrosine kinase inhibitors (TKIs) in Ph/positive acute lymphoblastic leukemia (ALL) patients (Iacobucci et al. 2008). Further, *IKZF1* deletions are likely to be a genomic alteration that significantly affects the prognosis of Ph-positive ALL in adults (Martinelli et al. 2009). Further, when Ikaros expression was analyzed by real time RT-PCR the quantitative distribution of mRNA level in hematopoietic cells of patients with lymphocytic leukemia was similar but a clear difference among groups was due to Aiolos lower expression in all types of acute leukemia(Antica et al. 2007). The mechanisms involved have been tested in the mouse model. It has been found that pre-BCR induces Ikaros to inhibit the proliferation of Philadelphia chromosome-positive B-ALL cells (Trageser et al. 2009). Pre–B cell receptor/*IKAROS*-induced cell cycle arrest can be reversed by dominant-negative Ikaros splicing variant IK6(Trageser et al. 2009). A possible mechanism of Ikaros suppression has been described by Ma et al. Their experiments on mice show that Ikaros inhibits c-Myc as a direct target, resulting in inhibition of pre B-lymphocyte proliferation (Ma et al 2010).

In the last ten years a new system, besides fetal thymic organ cultures (FTOC) or reaggregation cultures, for T cell growth *in vitro* has been developed. It has been shown that OP9 stromal cells transfected with the Notch ligand delta like 1 DL1 (OP9-DL1) can support T lymphocyte differentiation *in vitro* (Schmitt and Zúñiga-Pflücker 2002). This new technology provided a powerful tool for analyzing developmental phases from multipotent stem cells to mature T lymphocytes at single cell level *in vitro* and allowed a better understanding of the processes underlying development. However, there are still a lot of unanswered and exciting questions to be solved. Who is regulating Ikaros and

Notch? How transcription factors regulate development with their partners and DNA where and how do they interfere with their partners, and DNA, how can we identify targets for new drugs and finally how can we produce T cells *in vitro* for practical applications and regenerative medicine?

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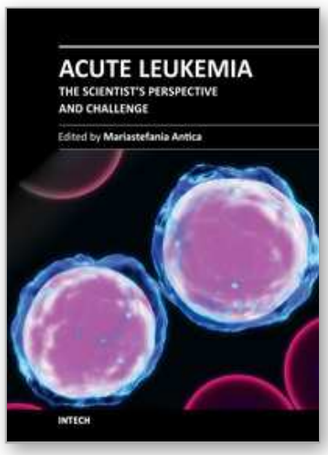

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This book provides a comprehensive overview of he basic mechanisms underlying areas of acute leukemia, current advances, and future directions in management of this disease. The first section discusses the classification of acute leukemia, taking into account diagnoses dependent on techniques that are essential, and thankfully readily available, in the laboratory. The second section concerns recent advances in molecular biology, markers, receptors, and signaling molecules responsible for disease progression, diagnostics based on biochips and other molecular genetic analysis. These advances provide clinicians with important understanding and improved decision making towards the most suitable therapy for acute leukemia. Biochemical, structural, and genetic studies may bring a new era of epigenetic based drugs along with additional molecular targets that will form the basis for novel treatment strategies. Later in the book, pediatric acute leukemia is covered, emphasizing that children are not small adults when it comes to drug development. The last section is a collection of chapters about treatment, as chemotherapy-induced toxicity is still a significant clinical concern. The present challenge lies in reducing the frequency and seriousness of adverse effects while maintaining efficacy and avoiding over-treatment of patients.

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InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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