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REVIEWS

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LEUKEMIC BLAST CELLS AND CONTROVERSIES IN MODELS OF HEMATOPOIESIS

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Classical and up-to-date models of hematopoietic lineage determination are briefly reviewed with the focus on myeloid-based models challenging the existence of the common progenitor for T cells, B cells and NK cells. The analysis of immunophenotype of leukemic blast cells seems to be a promising approach for interpreting some controversies in the schemes of normal hematopoiesis. The literature data as well as our own findings in the patients with various types of acute leukemias are in favor of the concept postulating that common myeloid-lymphoid progenitors giving rise to T and B cell branches retain the myeloid potential. The similarity of some immunophenotypic features of blast cells in pro-B acute lymphoblastic leukemia and acute monoblastic leukemia is consistent with monocyte origin postulated in the studies of normal hematopoiesis. Study of acute leukemias may be the challenging area of research allowing for new insight into the origin of hematopoietic cell lineages.

Key Words: **models of hematopoiesis, leukemic stem cell, immunophenotype, acute leukemias.**

HISTORICAL BACKGROUND

Unitary theory of hematopoiesis put forward by A. Maximow more than a century ago has an exceptional place in the history of oncohematology [1]. Based on his seminal experimental histological findings, A. Maximow was the first to postulate the existence of a single parental cell common for all three lineages of hematopoiesis, namely lymphoid, myeloid and erythroblastoid. While the question whether or not all blood cells derive from a single precursor cell was a subject of considerable debate over the years, now the monophyletic view of hematopoiesis became generally accepted.

In 1961, the unequivocal evidence proving existence of hematopoietic stem cell (HSC) was obtained in studies with bone marrow in lethally irradiated mice [2]. HSC has been considered as a pluripotent cell being capable of self-renewal and giving rise to the progenitor cells of all lineages of hematopoiesis. Later on, HSC population has been shown to be heterogeneous subdividing into long-term HSCs and short-term HSCs [3]. While originally HSCs were delineated only based on the clonal assays, later they became phenotypically distinguishable [4].

According to the current models of lineage determination in the hematopoietic hierarchies, HSC differentiation gives rise to multipotent progenitors (MPPs) practically devoid of self-renewal ability but retaining the potential for multilineage differentiation. In the classical dichotomy concept, MPP was thought to give rise to two principal lineages of hematopoiesis, namely lymphoid and myeloid-erythroid. Later, hypothetical lineage-restricted progenitors, such as common myeloid progenitor (CMP) and common lymphoid progenitor (CLP) have been identified in experimental studies of hematopoiesis in mice [5, 6].

Classical dichotomic model of myeloid-lymphoid branching vs. myeloid-based model. In classical dichotomic model reinforced by identification of CMP and CLP, lymphoid and myeloid lineages diverge from MPP as a single progenitor in a symmetrical fashion [7]. Therefore, CMP and CLP were regarded as myeloid or lymphoid lineage committed progenitors. Nevertheless, the classical dichotomic model was later modified since the experimental findings suggest that CLP and CMP are generated asymmetrically from different MPPs. In fact, MPPs turned out to be heterogeneous containing the progenitors generating two types of cells and those generating only one type, in addition to progenitors generating myeloid, T and B cells with varying combinations of lymphoid and myeloid potential [8]. Moreover, prior to lymphoid lineage commitment at CLP stage, MPPs lose myeloid lineage differentiation potential in a stepwise fashion. Kawamoto et al. [9] pointed out that lymphoid lineage specification is in fact a gradual process with many intermediate states rather than a pathway with single bifurcation.

The origin of T, B, and NK cell lineages: common or separate progenitors? According to the classical model, CLP is a single progenitor committed to lymphoid lineages, namely T, B or NK cells. In other words, the existence of a single progenitor suggested a common origin of all lymphocytes rather than a separate developmental pathway for each lymphocyte lineage from HSCs. In classical schemes of hematopoiesis, the direct dichotomy of B cell progenitors and T cell progenitors from CLP was postulated [10]. Instead, the myeloid-based model introduces common myeloid-lymphoid progenitor (CMLP) giving rise to T and B cell branches that retain the myeloid potential [9]. The postulated CMLP generates T and B cell progenitors through bipotential myeloid/T progenitor and myeloid/B progenitor stages, respectively [11]. According to the revised models, CMP gives rise to well-defined populations of myelo-erythroid progenitors: granulocyte/macrophage progenitor (GMP) and megakaryocyte/

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Abbreviations used: ALL – acute lymphoblastic leukemia; AML – acute myeloid leukemia; BC – blast crisis; CLP – common lymphoid progenitor; CML – chronic myelogenous leukemia; CMLP – common myeloid-lymphoid progenitor; CMP – common myeloid progenitor; DC – dendritic cell; GMP – granulocyte/macrophage progenitor; HSC – hematopoietic stem cell; LSC – leukemic stem cell; MEP – megakaryocyte/erythrocyte progenitor; MLP – multilymphoid progenitor; MPP – multipotent progenitor.

erythrocyte progenitor (MEP) while the existence of CLP as a progenitor with restricted B and T cell output has not been confirmed and MLP (multilymphoid progenitor) giving rise to lymphoid and myelo-monocytic lineages was placed instead of CLP in the scheme of human hematopoiesis [12, 13]. Nevertheless, the precise lineage output of each suggested MLP and the precise points of branching of B, T and NK cell lineages could not be ascertained. The cells possessing the mixed T/B potential were not found out [11]. Moreover, several studies indicate that the divergence of T cell lineage might occur upstream of CLPs in mouse bone marrow [7]. Nevertheless, the exact point of bifurcation giving rise to T cells remains highly questionable.

The exact origin of NK cells also remains questionable. It is still unknown whether the postulated T/NK progenitor exists. While the prevalent idea of common T/NK pathway is supported by a series of the shared T/NK antigens and the similarity of some functions in T and NK cells, a clonogenic lymphoid progenitor with both B cell and NK cell lineage potential has been also identified [14]. This suggests that NK cell pathway may be closer to B cell rather than T cell pathway, and these relations are in fact depicted in some revised schemes of hematopoiesis [12].

Novel myeloid-based model of hematopoiesis and origin of granulocytes, macrophages, and dendritic cells. Since the classical model of hematopoiesis postulating a dichotomous lineage restriction of MPPs into CLPs and CMPs has been challenged by the identification of lymphoid progenitors retaining partial myeloid potential such as MLPs in human hematopoiesis, it was suggested that some cells belonging to myeloid lineage might originate from both myeloid and lymphoid (with myeloid potential) branches [15]. A close relationship between B cells and macrophages has been suggested earlier from the findings that B cells and B-cell lines can be converted into macrophages in transfection experiments [16]. Doulatov et al. [13] presented evidence that MLPs generate not only all lymphoid cell types but also monocytes, macrophages and dendritic cells (DCs). In other words, macrophages and DCs can be derived from both MLPs and GMPs. All these data prompted once again a revision of the model of hematopoiesis.

HSC and leukemic stem cell (LSC). The hierarchic relationships in hematopoietic system have been successfully delineated due to the functional assays based on clonogenic analysis and later on the subtle analysis of immunophenotype of various subpopulations of HSC and progenitor cells. Nevertheless, the interpretation of *in vitro* assays could not be regarded as unequivocal due to various technical factors intrinsic to these assays. Some uncertainties in establishing precise lineage potential and delineating the exact branching points for each lineage as well as presumable pathways and postulated progenitors are demonstrated in various modern modified variants of hematopoiesis schemes [7, 9, 12]. In fact, the detailed features of certain pathways of hematopoiesis and existence of several progenitor cells remain the point of much controversy. Moreover, despite the obvious progress in understanding the functional properties, molecular mechanisms of regulation of proliferation of HSCs and hematopoietic progenitor cells, their cytomorphological and cytochemical features remain underexplored.

In 1997, in a series of meticulous experiments the existence of the LSC was proved for acute myeloblastic leukemia [17]. Later on, LSCs for several other biological forms of leukemias have been identified. Now it is widely recognized that LSC in each type of leukemia corresponds to one or another normal counterpart representing progenitor cells at various stages of lineage commitment and differentiation in the hierarchy of hematopoiesis. In the recent WHO classification of tumors of hematopoietic and lymphoid tissues (2008), the candidate LSCs suggested for each form of leukemias are compared with corresponding stem cells and progenitor cells in normal hematopoiesis [18]. It has been shown that LSCs and HSCs share the gene expression programs [19].

Now it is generally assumed that the study of leukemic analogs of progenitor cells in different biological types of leukemias will be much helpful in elucidating some questionable points in modern scheme of hematopoiesis. The analysis of the blast cells in the patients with hematological disorders, in particularly hematological malignancies, provides important data for elucidating the regulatory mechanisms in hematopoiesis.

Challenges in interpreting controversial pathways in modern scheme of hematopoiesis based on studying immunophenotype of leukemia blast cells. In the group of acute leukemias of ambiguous lineage, the recent WHO classification [18] delineates the acute undifferentiated leukemia without expression of lineage-specific antigens and the leukemias co-expressing antigens of several lineages at the levels not sufficient for identifying precisely the lineage of blast cells (acute leukemia with mixed phenotype, including B/myeloid leukemia, T/myeloid leukemia, lymphoblastic leukemia/lymphoma from NK-cells). Study of these leukemias may be the challenging area of research allowing for new insight into the origin of lymphoid cell lineages.

The biphenotypic acute leukemias are believed to arise from MPP cells with the capability of differentiating along both myeloid and lymphoid lineages. Previously, Matutes et al. [20] have been shown that the most common immunophenotype of biphenotypic acute leukemias is a co-expression of B-lymphoid and myeloid markers and much less frequently, T-lymphoid and myeloid markers, while the cases with a B and T lymphoid phenotype or with trilineage differentiation are exceptional. In fact, the same has been demonstrated in our diagnostic studies provided in Ukraine throughout several decades [21]. In some patients with ALL from early B-cells progenitors (pro-B-ALL, ALL of "common" type), the co-expression of a series of myeloid antigens (CD33, CD13, CD15) was detected that is in line with the modern concept of the persistence of myeloid potential in lymphoid lineage branches. Nevertheless, in various immunological variants of ALL of B-cell origin, we have never observed the co-expression of T-cell antigens on the blasts. Similarly, in T-ALL/T-cell lymphoma, the blast cells have never expressed the markers characteristic of B-cell immunophenotype.

Study of blast crisis (BC) in the terminal phase of chronic myelogenous leukemia (CML) is also relevant to the issue of the origin of T cell lineage. This final phase in the evolution of CML behaves like an acute leukemia of myeloid (70% of cases) or lymphoid (30% of cases) types. In lymphoid type of BC CML, blast cells are exclusively of B cell lineage with occasional co-expression of myeloid antigens but very rare if ever expression of T cell antigens. The blast cells of T cell phenotype in BC CML have been never detected in our studies [21]. This is again in line with the data that cells with double expression of B and T cell antigens have been never found in CLP populations in normal hematopoiesis [11]. Therefore, the fact that blast cells simultaneously exhibiting T-cell and B-cell surface antigens have not been observed both in acute leukemias and in BC CML contradicts to the concept of CLP as the common progenitor cell for B cell and T cell lineages.

Some features of acute leukemias may be also considered as the helpful hints of the origin of NK cells. In our studies some similarity between blast cells in pro-B-ALL [MPO^-, HLA-DR^+, $CD34^+$ (less than 50% of cells), $CD19^+$ and $CD33^-$ (or their co-expression), t (4;11), 11q23] and acute monoblastic leukemia (AML M5a) [MPO^-, HLA-$DR^{+/-}$, $CD34^{+/-}$, $CD33^+$ and $CD19^-$ (or their co-expression), t (9;11), 11q23] was found out [21]. Such similarities of immunophenotypic features and cytogenetic abnormalities in blast cells in pro-B-ALL and AML M5a seem to be the hint explaining for us three cases of AML M5a that were presented as a relapse of leukemia in children originally diagnosed as pro-B-ALL [23]. In some acute leukemia patients, the blasts with expression of marker signs of NK-cells and monocytes were detected. In our experience, the aberrant expression of CD7 and CD56 observed in 32% cases of acute monoblastic leukemia was indicative of unfavorable course of the disease.

In our opinion, bipotent cells-progenitors of B-lymphocytes/monocytes and NK-cells/monocytes originate from oligolineage progenitors of lymphocytes/monocytes. At the next stage of development, the unipotent progenitors — pro-B-lymphocytes, pro-NK-cells and promonocytes arise, correspondingly. This is consistent with dual monocyte origin postulated in the studies of normal hematopoiesis [13]. The data on the clonal analysis of human acute leukemias/lymphomas with B cell/myelomonocytic phenotypes [22] also support the possible existence of B cell/myelomonocytic bipotent progenitors.

To sum up, the data based on cytochemical and immunophenotypical signs of leukemic blast cells may be considered as reminiscent of the corresponding data pertinent to normal hematopoiesis. In our opinion, the analysis of the assembly of such data may be useful in solving some controversies in the modern schemes of hematopoiesis and even in amending modern hierarchic model of normal hematopoiesis. On the other hand, the refined schemes of normal hematopoiesis might represent a good basis for improvements and even revisions in up-to-date classifications of the tumors of hematopoietic and lymphoid tissues.

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