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Developmental and Comparative Immunology 30 (2006) 1084–1098
Developmental & Comparative Immunology
www.elsevier.com/locate/devcompimm

Production of human B cells from $CD34^+CD38^-$ T^- B^- progenitors in organ culture by sequential cytokine stimulation

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Received 19 October 2005; received in revised form 13 January 2006; accepted 9 February 2006
Available online 10 March 2006

Abstract

We investigated sequential cytokine addition on human hematopoietic stem cell (HSC) differentiation in murine fetal liver (FL), fetal spleen (FS) and bone marrow (BM) organ cultures (OC). Tissues were colonized with unpurified or FACS sorted $CD34^+CD38^-CD10^-CD19^-CD3^-CD8^-CD4^-$($T^-$ B^-) cells from human cord blood (HUCB). $CD19^+$ cell production and kinetics differed in each tissue. Fetal liver organ cultures (FLOC) inoculated with $CD34^+CD38^-T^-B^-$ cells produced fewer $CD19^+$ cells than fetal liver organ culture (FLOC) cultured with unpurified HUCB. $CD19^+$ cell production was restored in the $CD34^+CD38^-T^-B^-$ organ cultures by treating with SCF, LIF and IL-6 followed by IL-7 and removing all cytokines for the last 3 days of culture (a six-fold increase). FLOC also produced $CD34^+CD38^-T^-B^-$ cells and monocyte-lineage $CD33^+CD14^-$ cells, both of which increased after cytokine treatment. Re-colonization of secondary FLOC with $CD34^+CD38^-T^-B^-$ cells generated in primary FLOC produced additional B-cells, monocytes and $CD34^+CD38^-$ cells suggesting that the primary cells retained HSC activity. Expansion and differentiation of HSCs depended on the microenvironment of the recipient tissue as well as addition of cytokines in the appropriate order.

Keywords: B cell; Organ culture; Hematopoiesis; Cytokine; Hematopoietic; Stem cell; Cord blood; IL-7

1. Introduction

T cells and B cells develop from hematopoietic stem cells (HSCs) in the thymus and bone marrow (BM) microenvironments, respectively [1,2]. Developing B cell precursors must go through several stages in which they are screened for functional Ig chains [3,4]. Further growth and differentiation of B cells in vivo depends on the presence of the appropriate microenvironment, in which stromal and other accessory cells (as found in the avian Bursa of Fabricius or the mammalian BM), as well as T cells (in the secondary lymphoid tissue), can provide the necessary cytokines that ultimately lead to the production of Ig-secreting plasma B-cells. In order to mimic this environment and to improve B-cell production, organ cultures of murine fetal liver (FL), fetal spleen (FS) and adult BM were tested using various sequential cytokine treatments. Unfractionated human cord blood (HUCB), as well as FACS sorted $CD34^+CD38^-$ T^- B^- cells were compared as sources of HSCs.

Although some HSCs may be $CD34^-$ [5–7] most HSCs from humans are contained in the $CD34^+$

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0145-305X/$ - see front matter © 2006 Published by Elsevier Ltd.
doi:10.1016/j.dci.2006.02.003

fraction of hematopoietic precursors, and several methods have been based on the expression of this marker to purify human HSCs [8–10]. It is known, however, that about 50% of $CD34^+$ cells express one or more lineage and/or B cell positive markers, in particular CD19, [11] making it imperative that these B lineage positive (B^+) cells be depleted from the starting population. Several methods of following human B-cell development exist. For example, a culture system utilizing BM stromal cells has been used to produce human $CD19^+$ cells from $CD34^+$ precursors without the involvement of exogenous IL-7 [12–14]. Using a mouse BM stromal cell line, some workers have been able to show early B-cell differentiation in liquid cultures, which is driven by the addition of exogenous cytokines [15–18]. These "defined" in vitro culture systems do not reflect the complex three-dimensional structure of the inductive microenvironment and the cells produced from these cultures appear to lose engraftment potential when they are placed in competition with unmanipulated HSCs [19,20], perhaps due to excessive proliferation induced by cytokine addition [21]. In addition, cultures of HSCs with known cytokines may not include "non-classical" cytokines [22] or other molecules needed to fully support HSC differentiation [23].

In vivo functional assays for human B-cell production, as well as other hematopoietic cell types, have been developed, based on the repopulation of NOD *scid*/*scid* mice with $CD34^+$ $CD38^-$ Lin^- cells [18,24–26]. Recently, intra-hepatic injection of HUCB $CD34^+$ cells into $Rag^{-/-}$ $\gamma_c^{-/-}$ mice resulted in the production of functional human T cells, B cells and dendritic cells [27]. These assays have the advantage of studying the ability of HSCs to reconstitute the entire hematopoietic system. However, they are not easy to manipulate, and they are subject to difficulties in ascertaining the ability of human HSCs to home to the appropriate xenogeneic microenvironment [22]. Therefore, these in vivo methods do not lend themselves to the study of the interactions of cytokines and various microenvironments to influence HSC differentiation.

Murine fetal liver organ cultures (FLOC) were used some time ago [28,29] for studies showing that B-cell differentiation could occur in the FL. Importantly, this work demonstrated that introduced progenitor cells could colonize FLOC, resulting in B-cell development. This system has been used more recently to study the ability of the pre-BCR and the BCR to regulate the development of the various intermediates of B-cell differentiation[13,30–32]. The development of a similar system for the study of human B-cell development would be most useful, not only to determine the number and function of human B-cell precursors, but also to study human B-cell differentiation and how this could be manipulated by cytokines. We treated HUCB $CD34^+$ $CD38^-$ T^- B^- cells in a xenogeneic organ culture system; first with HSC supportive cytokines, then with B-cell supportive cytokines, with intervening washes. This method better enhanced the production of B cells when compared to treatment with the same cytokines added together for the entire culture period. $CD34^+$ $CD38^-$ T^- B^- cells treated with cytokines in this manner were capable of generating B cells, monocyte-lineage $CD33^+$ $CD14^-$ cells, and more $CD34^+$ $CD38^-$ T^- B^- cells in secondary FLOC, suggesting that they retained HSC activity. Our data suggest that the organ culture system can produce B cells as well as HSCs and that the sequential addition of cytokines can strongly increase the production of both of these cell types.

2. Materials and methods

2.1. Human tissues

HUCB samples were obtained from normal full-term deliveries with informed consent. Mononuclear cells were purified from HUCB by Ficoll-Hypaque density gradient (Sigma Chemicals, St. Louis, MO) separation.

2.2. Mice

Timed pregnant C57BL/6 (B6) mice were purchased from the National Cancer Institute (Frederick, MD). C.B-17 *scid*/*scid*, B6 *scid*/*scid* and NOD *scid*/*scid* mouse breeding pairs were the kind gift of Dr. L. Shultz, the Jackson Laboratory (Bar Harbor, ME). These mice were housed in the facilities of the Department of Animal Care, the University of Arizona, and bred to produce timed pregnant animals. The fetuses were removed from pregnant females at the indicated time points (plug day = 0).

2.3. Purification of progenitor cells

Cells were first obtained from Ficoll-separated HUCB. These mononuclear cells were then labeled using a mixture of monoclonal antibodies (mAbs) specific for CD3, CD4, CD8, CD10, and CD19