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B Cell Lineage: Part of the Differentiation Program of Human Pluripotent Stem Cells

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A. Introduction

In the past, developmental methods for examining hematopoiesis began to influence concepts of studying hematologic malignancies. A new approach became possible with the establishment of cell culture procedures for committed and more recently for noncommitted hematopoietic stem cells [1–3]. These primitive progenitors (CFU-GEMMT) derived from human bone marrow can be identified in culture by their ability to form colonies containing different myeloid lineages and T cells of different phenotypes. The observation of a common progenitor of myeloid and lymphoid cells in normal and disturbed hematopoiesis led to the question whether B cells are part of the differentiation program of human stem cells. In the present study, we attempted to induce B cell differentiation of human marrow cells from healthy volunteers.

B. Material and Methods

Bone marrow samples were obtained from four consenting healthy individuals.

I. Preparation of Leukocyte-Conditioned Medium

Conditioned medium was prepared from Leu-5-positive cells of normal individuals [4]. Briefly, 10^6 Leu-5 positive cells were incubated with 1% human serum albumin, IMDM (Iscove's modified Dulbecco's medium) and 1% PHA (Wellcome HA 15). This material, Leu-5-PHA-TCM, was harvested after 4 days of incubation at 37 °C in a humidified atmosphere with 5% CO_2.

II. Preparation of Cell Suspensions

Mononuclear cells of density less than 1.077 g/ml were incubated in antibody-coated dishes using B_1, B_2 (Coulter Immunology, Hialeah), and BA_1 (Hybritech) for 20 min at 4 °C. The supernatant containing nonadherent and B cell-depleted cells was plated as outlined.

III. Colony Assay for Hematopoietic Progenitors

Mixed hematopoietic colonies (CFU-GEMMT) [1–3], erythroid bursts (BFU-E), and granulocytic colonies (CFU-C) [6] were grown as previously described. Nonadherent and B cell-depleted cells were admixed with IMDM, 30% human plasma, 0.9% methylcellulose, and 5% Leu-5-PHA-TCM. Erythropoietin (EPO) 1 U/ml (Step III, Connaught) was added on day 4 of culture. Each dish was examined after an additional 10 days of incubation for the presence of hematopoietic colonies, i.e., BFU-E, CFU-C, and CFU-GEMMT.

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Table 1. Identification of pre-B-cells and B-cell-associated antigen-positive cells in multilineage hematopoietic colonies (CFU-GEMMT)

Patient	Cytoplasmic μ	CFU-GEMMT Positive for B cell-associated antigen B_1/BA_1	Analyzed
N.H.	4	7/3	41
K.G.	9	11/6	74
B.S.	2	3/5	33
S.C.	5	8/9	62

IV. Examination of Hematopoietic Colonies for SIg (M+D), B Cell-Associated Antigen, and Cytoplasmic μ-Positive Cells

Individual colonies (CFU-GEMMT), erythroid bursts (BFU-E), and granulocytic colonies (CFU-C) were aspirated by micropipe from the cultures and washed in NKH buffer (5 min, 300 g). Aliquots were transfered onto poly-L-lysine-coated wells on glass slides. Attached cells were fixed with glutaraldehyde (0.05% in 0.1 *M* phosphate buffer, pH 7.4) to block Fc receptors and to preserve cell morphology. Cells were stained with the monoclonal antibodies IgM and IgD (Bethesda Research Laboratories), with B_1, or with the use of a monoclonal anti-μ-chain (Miles Laboratories). Controls were performed with β_2- microglobulin and the sandwich antisera, i.e., SAR (swine anti-rabbit) and RAM (rabbit anti-mouse) [7].

C. Results

I. Surface Immunoglobulin, B Cell-Associated Antigen, and Cytoplasmic μ-Positive Cells in Multilineage Hematopoietic Colonies

Mixed hematopoietic colonies, erythroid bursts, and granulocytic colonies were grown from nonadherent and B cell-depleted marrow cells of four normal individuals in the presence of a Leu-5-PHA-TCM and EPO. A total of 211 individual mixed hematopoietic colonies were aspirated from the cultures and were examined for the coexistence of lymphopoietic progeny, i.e., pre-B cells, and B cells. Cytoplasmic μ-, B_1-, and SIg-positive cells could be identified in multilineage colonies of each individual (Table 1). The number of pre-B cells observed in the mixed colonies examined ranged from 0 to 45 cells per colony. The number of B_1-positive cells per colony ranged from 0 to 278 cells (173 ± 46, mean ± standard deviation). In contrast, cells derived from individual BFU-E and CFU-C colonies did not stain for cytoplasmic μ, B_1, or surface immunoglobulins.

D. Discussion

A culture assay for human multilineage hematopoietic progenitors has been established. These primitive precursors form mixed colonies containing lymphopoietic and myeloid cells of different lineages. Recloning experiments of multilineage colonies indicated that some fulfill criteria of stem cells [8]. The lymphopoietic component in mixed colonies consisted of T cells of various phenotypes, i.e., OKT 3, OKT 4, OKT 8, or E 2–22 [2, 3, 7]. Until recently, B cells could not be observed in mixed colonies derived from marrow cells of healthy volunteers. The availability of a Leu- 5-PHA-TCM facilitated the formation of mixed colonies containing SIg M+D, B cell-associated antigen, and cytoplasmic μ-positive cells from normal donors. In the murine system, cytogenetic evidence for the coexistence of a pluripotent stem cell capable of differentiating into myeloid and lymphoid progeny, including both B and T lymphocytes has been provided by Abramson et al. [9].

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