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Research Article

Unique monoclonal antibodies specifically bind surface structures on human fetal erythroid blood cells ☆

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ABSTRACT

Background: Continuing efforts in development of non-invasive prenatal genetic tests have focused on the isolation of fetal nucleated red blood cells (NRBCs) from maternal blood for decades. Because no fetal cell-specific antibody has been described so far, the present study focused on the development of monoclonal antibodies (mAbs) to antigens that are expressed exclusively on fetal NRBCs.

Methods: Mice were immunized with fetal erythroid cell membranes and hybridomas screened for Abs using a multi-parameter fluorescence-activated cell sorting (FACS). Selected mAbs were evaluated by comparative FACS analysis involving Abs known to bind erythroid cell surface markers (CD71, CD36, CD34), antigen-i, galactose, or glycophorin-A (GPA). Specificity was further confirmed by extensive immunohistological and immunocytological analyses of NRBCs from umbilical cord blood and fetal and adult cells from liver, bone marrow, peripheral blood, and lymphoid tissues.

Results: Screening of 690 hybridomas yielded three clones of which Abs from 4B8 and 4B9 clones demonstrated the desired specificity for a novel antigenic structure expressed on fetal erythroblast cell membranes. The antigenic structure identified is different from known surface markers (CD36, CD71, GPA, antigen-i, and galactose), and is not present on circulating adult erythroid cells, except for occasional detectability in adult bone marrow cells.

Abbreviations, in order cited: NRBC, Nucleated red blood cell; mAb, Monoclonal antibody; FACS, Fluorescence-activated cell sorter; CD, Cluster of differentiation; GPA, Glycophorin A; BFU—E, Burst-forming unit—erythoid; SBA, Soybean agglutinin; PCR, Polymerase chain reaction; FISH, Fluorescence in situ hybridization; DNA, Deoxyribonucleic acid; STR, Short tandem repeats; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (German Collection of Microorganisms and Cell Cultures); MNCs, Mononuclear cells; FCS, Fetal calf serum; DMSO, Dimethylsulfoxide; FSC, Forward scattered light; SSC, Side scattered light; APC, Allophycocyanine; PE, Phycoerythrin; FITC, Fluoresceinthiocyanate; PBS, Phosphate-buffered saline; FcR, FC receptor; RT, Room temperature; APAAP, Alkaline phosphatase anti-alkaline phosphatase; Tris, Tris(hydroxymethyl)aminomethane; TBS, Tris-buffered saline; HRPO, Horseradish peroxidase; BSA, Bovine serum albumin; DAPI, 4′-6-Diamidino-2-phenylindol; GalNAc, Terminal α- or β-*N*-acetylgalactosamine.

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*Conclusions:*The new mAbs specifically bind the same or highly overlapping epitopes of a surface antigen that is almost exclusively expressed on fetal erythroid cells. The high specificity of the mAbs should facilitate development of simple methods for reliable isolation of fetal NRBCs and their use in non-invasive prenatal diagnosis of fetal genetic status.

Introduction

Development of a reliable non-invasive alternative to prenatal diagnostic procedures such as amniocentesis and chorionic villous sampling used to detect fetal aneuploidies has been the goal of numerous scientific groups. Aside from fetal nucleated red blood cells (NRBC, also known as erythroblasts), trophoblasts [1,2] or fetal cell-free DNA [3,4] have also been considered as objects for prenatal diagnostics out of maternal blood. Methods used to enrich the rare fetal NRBCs (about 1–8 fetal erythroblasts in 2 mL maternal blood) are amongst others combinations of cell sorting with magnetic particles or flow cytometry [5,6], density gradient centrifugation [7], selective cell lysis [8], or depletion of unwanted cell populations (5). Cells from very early developmental stages such as the colony forming definitive erythroid progenitors (BFU-E) can be isolated from umbilical cord blood using CD34 [9]. To enrich and identify erythroid cells from later developmental stages, surface markers such as CD71 [10], glycophorin A [11], CD36 [12] and intracellularly expressed hemoglobins have been used [13,14].

Since Bianchi et al. [15] first used antibodies to CD71 to isolate fetal erythroid cells from maternal peripheral blood, the transferrin receptor has become one of the most frequently used selection markers [16]. Soybean agglutinin (SBA) has also been used to isolate fetal erythroid precursors from blood of pregnant women because the formation of membrane galactose is closely linked to the development and maturation of erythroid precursor cells [17].

The fetal origin of the isolated cells can be proved by PCR-amplification of Y-chromosome-specific sequences [18], by fluorescence in situ hybridization (FISH), by detecting ε- and γ-globin, or by comparing DNA-polymorphisms with STR (short tandem repeats)-markers from mother and child [19]. However, the Y-chromosome detection method excludes the examination of pregnancies with a female fetus, fetal globins are also formed in adults that have hematologic diseases, and STR-markers do not always detect a DNA-polymorphism between mother and child [20].

The identification and isolation of fetal cells is still difficult because no surface antigens are known that are exclusively expressed on these cells. Therefore, the aim of this study was the development of a fetal cell-specific monoclonal antibody which enables the characterization of fetal erythroid cells as well as their differentiation and isolation from adult erythroid blood cells and leucocytes.

Materials and methods

Samples

To test the specificity of the new mAbs for fetal erythroid cells, cytospin preparations with mononuclear cells from term umbilical cord blood, blood smears with fetal blood, fetal and adult bone marrow, and frozen sections of yolk sac tissue, fetal and adult liver, fetal and adult lymphatic tissue were investigated (Table 1). The samples were provided, stained and analyzed by Professor Reza Parwaresch at the Institute for Hematopathology of the University of Kiel, Germany. Umbilical cord blood samples that were used for flow cytometric tests were provided by the Paracelsius Clinic birth unit in Langenhagen, Germany. Samples were collected after informed consents were obtained from blood and tissue donors.

Cell lines K-562 and KMOE-2 were purchased from DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH).

Development of monoclonal antibodies: Antigen preparation and immunizaton

The isolation of fetal erythroid cell membranes used for immunization was performed by Genelab (Gelsenkirchen, Germany). Erythroid cord blood cells from term pregnancies were isolated based on differential expression of known surface antigens using multi-parameter FACS. Isolated cells were CD71+ and antigen i+ and negative for CD3, CD14, CD19, and CD45. Those cells were lysed and the cell membranes separated from the nuclei by centrifugation. Five mice were immunized with cell membranes

Table 1 – Overview of number and provenance of embryonic, fetal, and adult tissue samples.

Tissue	Total number of samples	Gestation week	Analyses performed
Yolk sac	4	6 and 8	Microscope
Fetal liver	8	6, 8, 20 and 30–38	Microscope
Fetal bone marrow	4	10, 20 and 30–38	Microscope
Fetal blood	14	6, 8, 20 and 30–38	Microscope
Umbilical cord blood	35	38–40	FACS
Fetal lymph node	14	6, 8, 20 and 30–38	Microscope
Adult bone marrow	32	N/A	Microscope
Adult liver	8	N/A	Microscope
Adult peripheral blood	5	N/A	FACS
Adult lymph node	8	N/A	Microscope