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(54) **MONOCLONAL ANTIBODIES WITH SPECIFICITY FOR FETAL ERYTHROID CELLS**

MONOKLONALE ANTIKÖRPER MIT SPEZIFITÄT FÜR FETALE ERYTHROIDZELLEN

ANTICORPS MONOCLONAUX PRESENTANT UNE SPECIFICITE POUR DES CELLULES ERYTHROCYTES EMBRYONNAIRES

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(56) References cited:
WO-A-02/101387 **US-A1- 2003 180 762**

- **HUIE M A ET AL: "Antibodies to human fetal erythroid cells from a nonimmune phage antibody library" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE, WASHINGTON, DC, US, vol. 98, no. 5, 27 February 2001 (2001-02-27), pages 2682-2687, XP002974719 ISSN: 0027-8424**
- **ALVAREZ F V ET AL: "Development, characterization, and use of monoclonal antibodies made to antigens expressed on the surface of fetal nucleated red blood cells." CLINICAL CHEMISTRY. SEP 1999, vol. 45, no. 9, September 1999 (1999-09), pages 1614-1620, XP002365991 ISSN: 0009-9147**
- **BIANCHI D W ET AL: "ERYTHROID-SPECIFIC ANTIBODIES ENHANCE DETECTION OF FETAL NUCLEATED ERYTHROCYTES IN MATERNAL BLOOD" PRENATAL DIAGNOSIS, CHICHESTER, SUSSEX, GB, vol. 13, no. 4, 1 April 1993 (1993-04-01), pages 293-300, XP000197526 ISSN: 0197-3851**
- **BIANCHI D W: "Fetal cells in the maternal circulation: feasibility for prenatal diagnosis." BRITISH JOURNAL OF HAEMATOLOGY. JUN 1999, vol. 105, no. 3, June 1999 (1999-06), pages 574-583, XP002365992 ISSN: 0007-1048**
- **CHOOLANI ET AL: "Characterization of first trimester fetal erythroblasts for non-invasive prenatal diagnosis" MOLECULAR HUMAN REPRODUCTION APR 2003, vol. 9, no. 4, April 2003 (2003-04), pages 227-235, ENGLAND**

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Remarks:

The file contains technical information submitted after the application was filed and not included in this specification

Description

1 Introduction

[0001] Social developments led to an increase of prenatal investigations. Amniocentesis or less frequently sampling of chorionic villi is performed in every tenth pregnancy for the prenatal analysis of, e.g. trisomy 21. The risk for a chromosomal defect increases with the age of the mother. This is why amniocentesis is performed in more than 50% of pregnant women aged 35 years or older. However, most children with chromosomal or genetic defects are still born by women under the age of 35, if the total number is taken into account. The probability for a trisomy 21 is 0.3% in fetuses of women aged 35 years and older. This has to be seen in the context of a 0.5 % risk to induce an abortion by the amniocentesis procedure. From these numbers it is obvious that there is a great need for an alternative diagnostic procedure which yields the same results without bearing a risk for the unborn. One approach could be the isolation of fetal cells from maternal blood. This would eliminate risks for the fetus.

[0002] It was estimated that one fetal cell can be found in 10^5 to 10^7 maternal nucleated blood cells. Further investigations have shown, that in the presence of chromosomal aberrations more fetal cells can be detected in the maternal circulation. This raises the chance to detect an aneuploid fetus by non-invasive procedures.

Three different types of fetal cells have been identified in maternal blood: lymphocytes, trophoblasts and nucleated red blood cells (NRBCs). Fetal lymphocytes have been detected still one to 5 years after childbirth. This longevity may interfere with the accurate diagnosis in following pregnancies.

Trophoblasts are attractive targets because they can be easily identified by their morphology. However, they can not be easily used for diagnostic purposes, because as placental cells they might differ from cells of the fetus: in about 1% of diagnosed chromosomal aberrations in trophoblasts the fetus turned out to be healthy.

Fetal nucleated red blood cells (NRBCs) appear early in the maternal circulation, however do not persist after birth. Since they have a nucleus they are preferred candidates for chromosomal analysis. However, up to now they can not be distinguished easily and unambiguously from other blood cells.

[0003] They are identified through a marker profile, which is characteristic for erythroid precursor cells and which is different from other blood cell subpopulations. Blood cells are extensively characterized by so-called clusters of differentiation (CD) markers as defined at the 7^{th} Workshop and Conference on Human Leukocyte Differentiation Antigens (Harrogate 2000). Immature erythroid cells express CD71 and they lack CD45 which is expressed on leukocytes. This knowledge can be used to distinguish erythroid precursor cells from other mononuclear cells.

[0004] In order to isolate and identify fetal cells (1 amongst 10^5 to 10^7 maternal nucleated cells) most stringent criteria have to be met. There is no cell surface marker available yet which is exclusively expressed on fetal NRBCs. For the enrichment of fetal cells usually immunomagnetic or flow cytometric cell separation techniques are used either alone or in combination. The results of the chromosomal or genetic analysis of the isolated cells have been compared with the results obtained with amniotic cells. Many investigations have shown the technical feasibility of the non-invasive approach with large cohorts.

However, the existing procedures are still not suitable for routine diagnosis. It has to be assured that the cells under investigation are unambiguously fetal cells. The identification of fetal NRBCs can only be achieved by the recognition of a marker, which is preferentially expressed on fetal erythroid cells or which is expressed or localized in a way that is specific for fetal cells within the blood.

[0005] The lack of markers, which specifically identify fetal cells is the crucial obstacle for the development of a reliable non-invasive prenatal diagnostic.

[0006] Huie et al. "Antibodies to human fetal erythroid cells from a nonimmune phage antibody library", Proceedings of the National Academy of Sciences, vol. 98 (5), 2001-02-27, pages 2682-2687, XP002974719 discloses antibodies to human fetal erythroid cells. They were generated by using a new type of nonimmune phage antibody library in which multiple copies of antibody fragments are displayed on each phage.

[0007] The objective of this invention is the generation of antibodies, which allow the discrimination between fetal and adult erythroid cells and the unambiguous identification of fetal cells. Fetal cells recognized by these antibodies preferably should possess at least in part an intact cell nucleus, express the CD71 antigen and should miss the CD45 antigen in line with previously published results.

[0008] It is a further objective to provide a method for the detection or identification of fetal cells and a method for the detection of chromosomal and/or genetic aberrations, defects and/or variants in fetal cells.

[0009] Further, the object of the invention is the generation of monoclonal antibodies, which react specifically with fetal cells as well as a hybridoma cell line, which produces such antibodies, as well as uses thereof. This object is solved by the hybridoma cell according to claim 1, the antibody according to one of claims 2 to 4, the method according to claim 7 and the method according to claim 9. Further improvements of the antibodies, the hybridoma cell and the methods are given in the respective dependent claims.

[0010] For the purpose of the present invention 5 mice have been immunized with isolated erythroid cells from cord

blood (CD71+, CD45-), which carried the "i" antigen as defined by the autoantibody described in DE 100 35 433 A1. The immunization with these cells opens the possibility that besides antibodies against the "i" antigen also antibodies with specificities against new markers could be generated, which could be used to identify erythroid precursor cells. The spleen cells of the immunized mice were fused with a myeloma cell line to produce hybridomas according to standard procedures (Schetters H, Production of Monoclonal Antibodies, in: Methods of Immunological Analysis, Masseyeff RF, Albert WH and Staines NA (Eds.) Vol. 2, Ch. 4.3, 230-245, VCH Weinheim, 1993).

DESIGN AND METHODS IN DETAIL

[0011] In detail, mice were immunized with flow sorted human cord blood cells (CD71+, antigen-i+, CD19- and CD45-). Hybridoma supernatants were screened on pooled mononuclear cord blood cells, whereas the corresponding amount of erythroid precursors was determined by cytochemical staining of blood smears. For the hybridoma screening a six-parameter flow cytometric analysis (four colours, forward and side scatter) was set up for the simultaneous identification of erythroid precursor cells, leukocytes, enucleated erythrocytes and for antibodies reacting specifically with fetal cells. Furthermore, immunohistochemical analyses have been performed with fetal blood smears and fetal liver sections from the 6^{th} up to 38^{th} week of gestation as well as with adult blood, normal adult bone marrow and adult erythrocytes as controls.

RESULTS:

[0012] A clone (accession number DSM ACC 2666 at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ)) with specificity for a surface antigen exclusively expressed on fetal erythroid cells has been identified. The new antibody showed unaltered binding to erythroid cells from fetal blood of early times of gestation (6^{th} week) up to childbirth. Moreover, detailed examinations showed no surface reactivity with adult erythrocytes, erythroblasts or lymphatic and myeloid cells. This antibody did not react with cells of fetal haemolymphatic organs.

CONCLUSIONS:

[0013] The investigation showed that the new monoclonal antibody binds specifically fetal erythroid cells and thus can differentiate between fetal and adult red blood cells. Because of the expression of this fetal antigen in early stages of gestation a non-invasive prenatal diagnostic may be feasible. This antibody can be applied for different enrichment techniques and/or for the identification of fetal erythroid cells.

Detailed analysis of hybridoma cells

Screening for Hybridomas Producing Antibodies Reacting Specifically with Fetal NRBCs

[0014] Since several thousand antibody producing hybridomas have to be screened to find a suitable clone a procedure has been set up permitting a high through-put whilst maintaining the required specificity. A six-parameter analysis (4 fluorescence channels, forward and side scatter) has been established, which enabled the simultaneous identification of erythroid precursor cells, the differentiation of leukocytes from enucleated erythrocytes and the identification of new antibodies in a single step. The analysed cells have been stained with a nucleic acid dye (LDS751, Molecular Probes, cat# 7595) and have been incubated with antibodies of the cloned hybridomas. These antibodies were subjected to a reaction with an antibody directed against them, which was labeled with a fluorescent dye (FITC) (Goat anti mouse IgG (H+L)-FITC, Caltag Laboratories, cat# M35001). In later experiments for antibody characterization the antibodies have been labeled directly with FITC.

[0015] The identification of the erythroid precursor cells is possible due to their light scatter characteristics and by their binding of phycoerythrin labeled CD71 specific antibodies (CD71 PE, Diatec, cat# 3212). Leukocytes could be discerned by their binding to allophycocyanin labeled CD45 specific antibodies (CD45 APC, BD Pharmingen, cat# 555485). Nucleated and enucleated erythroid cells can be distinguished by their binding or absence of binding of the nucleic acid dye. With this procedure it is possible to identify antibodies binding to the intended target cells, i.e. fetal NRBCs, without cross-reaction towards adult erythrocytes or leukocytes (Fig. 1).

Exclusion of Antibodies Reacting with Antigens on Adult Erythrocytes Including Common Blood Group Antigens

[0016] Blood group antigens can be found on adult erythrocytes and their precursors in large amounts. Therefore, they might induce a major immune response when used as antigens. Antibodies against these blood group antigens are not suitable for the identification of fetal cells. In order to exclude antibodies binding to antigens on adult erythrocytes including blood group antigens, their binding specificity towards fetal cells is investigated after absorption on erythrocytes.

Erythrocyte with the blood group AB Rh+ have been harvested and stabilized with a reagent supplied by Meridian Diagnostics Europe, Bad Homburg. The antibodies under investigation have been incubated with increasing numbers of erythrocytes and tested before and afterwards for their binding activity for target cells. Reactivity of antibodies towards blood group antigens was thought to be absent, when the intensity of the binding to CD71+, CD45- nucleated erythroid precursor cells was unchanged after the incubation with the erythrocytes (Fig.2). Antibodies selected that way must not react with adult blood cells to enable the correct identification of fetal erythroid precursor cells (Fig. 3).

Specificity Testing of a Selected Monoclonal Antibody

[0017] Hybridoma clone producing a monoclonal antibody of the IgM isotype showing the required binding characteristics in the screening procedure could be identified. It has the designation 4B9 and was deposited by the applicant of the present patent or patent application on July 13, 2004 at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, Braunschweig) under the accession number DSM ACC 2666. A second antibody 4B8 recognizing the same epitope is mentioned in figures 2 and 3.

[0018] Fetal and adult erythroblasts strongly and specifically express glycophorin-A and, therefore, can be identified through this marker protein. The binding of the monoclonal antibody to these cells was visualized by an immunofluorescence double stain.

Protocol for Immunofluorescence Stain

[0019]

1 Fix cytospins or frozen tissue sections in acetone for 10 min
2 Dry for 5 min
3 Apply monoclonal antibody against glycophorin-A ,DAKO M0819 diluted 1:100 in phosphate buffered saline (PBS) containing 1% bovine serum albumin (BSA) for 60 min
4 Rinse with PBS
5 Apply goat anti-mouse antibody F(ab) fragment, Alexa Fluor 488 (Molecular Probes A-21044), green, diluted 1:100 in PBS for 60 min
6 Rinse with PBS
7 Apply monoclonal antibody 4B9 (hybridoma supernatant) for 60 min
8 Rinse with PBS
9 Apply goat anti-mouse IgM, Alexa Fluor 594 (Molecular Probes A-21044), red, for 60 min
10 Rinse with PBS
11 Stain cell nuclei with DAPI (Molecular Probes), blue, diluted 1:300 in PBS for 3 min
12 Rinse with PBS
13 Cover with fluorescence medium (S3023, DA-KO)
14 Visualize with "Universalmikroskop Axioplan", Carl Zeiss, using filter sets 02, 10 and 15 and photograph with a digital camera system, e.g. Visitron Systems GmbH
PBS: 8 g NaCl, -1.3 g Na_2HPO_4, 4 g NaH_2PO_4 in 1 l H_2O, pH 7.4

[0020] An immunoenzymatic method has also been used:

Protocol for Alkaline Phosphatase Anti-alkaline Phosphatase (APAAP) Stain

[0021]

1 Fix cytospins or frozen tissue slices in acetone for 10 min
2 Dry for 5 min
3 Incubate with monoclonal antibody 4B9 (hybridoma supernatant) for 30 min
4 Rinse with Tris buffered saline (TBS)
5 Incubate with APAAP complex (D0651, DAKO), diluted 1:25 in TBS/HS (inactivated human serum) for 30 min
6 Rinse with TBS
7 Repeat steps 5-7 twice for 10 min each
8 Rinse with TBS
9 Develop slides with substrate

i. Prepare solution A: Mix 18 ml 0.2 mol/l 2-amino-2methyl-1,3-propandiol with 50 ml 0.05 mol/l Tris buffer, pH

9.7 and 600 mg NaCl. Add 28 mg levamisol.
ii. Prepare solution B: Dissolve 35 mg naphthol AS-bi-phosphate in 0.42 ml N,N-dimethylformamide.
iii. Prepare solution C: Mix 0.14 ml 5% New Fuchsin with 0.35 freshly prepared 4% sodium nitrite. Stir for 60 sec.
iv. Mix solution A with solution B, then add solution C. Adjust the pH to 8.7. Mix, filter and apply to slides.
v. Incubate for 10-20 min at room temperature.
vi. Rinse with tap water.
vii. Counter stain with Meyer's acid Haemalaun for 5 min.
viii. "Blue" in tap water for 10 min and cover with Kaiser's glycerol gelatine.
TBS (Tris buffered saline): Dissolve 43.33 g NaCl and 39.40 g Tris-HCl in 5 l H_2O dest. Adjust pH to 7.4 with NaOH.
TBS/HS: 9 parts TBS + 1 part inactivated human serum

Negative controls: monoclonal antibody of identical isotype or murine hyper-immune serum.

Exclusion of Antibodies Reacting with CD71

[0022] Antibodies generated with the immunization strategy used may be directed against CD71. To exclude these antibodies, analyses were performed that show whether CD71-antibodies compete for the same binding site. Biotinylated antibody 4B8 was preincubated with mononuclear cells from cord blood. Then unlabeled CD71-specific antibody (Anti-CD71, Clone DF1513, DPC Biermann, Bad Nauheim, Germany) was added. After streptavidin-DTAF-labeling it was analyzed by flow cytometry whether CD71-antibodies had replaced the antibody 4B8. As a positive control sample for this competition experiment, unlabeled antibody 4B8 was added instead of CD71. These analyses showed that antibodies 4B8 and CD71 do not compete for the same epitope whereas the addition of unlabeled antibody 4B8 had diminished the signal.

Results

[0023]

- The 4B9-reactive antigen was expressed on the surface of fetal erythroblasts. This could be demonstrated with fetal cells from the 6. up to the 38th week of gestation. In Fig. 4 the antibody 4B9 recognised all glycophorin-A positive fetal erythroblasts.
- Erythroblasts in normal adult bone marrow were negative for 4B9. In contrast, all erythropoietic cells were positive for glycophorin-A. Only in 1 of 32 cases a intracellular granular expression in the cytoplasm of early basophile erythroblasts was seen.
- The 4B9 reactive antigen was not found on adult and fetal liver hepatocytes. Kupffer cells, macrophages, endothelial and sinusoidal cells were also negative.
- A detailed analysis of haemolymphatic cells in adults showed the absence of reactivity in lymphatic and myeloic cells.
- All haemolymphatic organs of the fetus were negative. This applies for lymphatic as well as myeloic cells.

Table 1. Detailed results of the reactivity of the monoclonal antibody 4B9

Cell or tissue		Adult (n positive / n samples)	Fetal (n positive / n samples)
Granulopoiesis			
Neutrophils			
	Segmented	0/8	0/14
	Rodforms	0/8	0/14
	Metamyelocytes	0/8	0/14
	Myelocytes	0/8	0/14
	Promyelocytes	0/8	0/14
Eosinophils			
	Rodforms	0/8	0/14
	Metamyelocytes	0/8	0/14
	Myelocytes	0/8	0/14
	Promyelocytes	0/8	0/14

(continued)

Cell or tissue		Adult (n positive / n samples)	Fetal (n positive / n samples)
	Basophils	0/8	0/14
	Monocytes		
	Mature monocytes	0/8	0/14
	Promonocytes	0/8	0/14
	Myelocyte	0/8	0/14
	Macrophages	0/8	0/14
Thrombocytopoieses			
	Platelets	0/8	0/14
	Megakaryocytes	0/8	0/14
	Megakaryoblasts	0/8	0/14
Erythrocytopoiesis			
	Erythrocytes	0/8	14/14
	Reticulocytes	0/8	10/10
	Normoblasts	0/8	4/4
	Euchrom. Erythroblasts	1/32	5/5
	Polychrom. Erythroblasts	0/8	10/10
	Basophilic erythroblasts	0/8	4/4
	Proerythroblasts	0/8	4/4
Lymphocytopoiesis			
	B lymphocytes	0/8	0/14
	Plasma cells	0/8	0/14
	T lymphocytes	0/8	0/14
Hepatocytes		0/8	0/4
Kupffer cells		0/8	0/4
Other hepatic cells		0/8	0/8

[0024] In the following, figures 1 to 4 are described in detail

Figures

Figure 1

[0025] Mononuclear cord blood cells were stained with labeled antibodies (anti CD45, anti CD71 and the antibody under investigation, 4B9) and a DNA dye. Antibody binding was measured with a flow cytometer.

a) This figure 1a shows a diagram with the light scatter properties of erythroid precursor cells. For further characterization, the cells characterized by means of their light scatter properties in region R1 were used.
b) Fig. 1b shows a diagram of fluorescence properties of the cells in region R1 and labeled with CD71-antibody and dye LDS751 labeling all nuclei. Region R2 encloses nucleated cells which express or do not express CD71 antigen.
c) Fig. 1c shows a diagram of fluorescence properties of the cells in region R2 incubated with CD71 antibodies and CD45 antibodies. The cells in region R3 express CD71 antigen but not CD45 antigen. This diagram demonstrates the differentiation between CD71 positive nucleated erythroid cells (Region R3) and CD45 positive leukocytes.
d) Fig. 1d shows a diagram of fluorescence properties of the cells in region R2. The cells in region R4 express CD71 antigen and bind to the 4B9 antibody. Thus, antibody 4B9 binds preferentially to CD71 positive cells, which are CD45 negative.

Figure 2

[0026] Fig. 2 discloses absorption of monoclonal antibodies 4B8 and 4B9 with adult erythrocytes, followed by the determination of their binding capability on cord blood cells. It is shown that neither 4B8 antibody nor 4B9 antibody is absorbed by adult red blood cells. For positive and negative controls antibodies against CD71 and glycophorin A were

used.

Figure 3

[0027] Flow cytometric investigation of the binding of the monoclonal antibodies 4B8 and 4B9 on cord blood cells and adult blood cells (x-axis: fluorescence intensity).

a) This histogram shows unstained, negative cord blood cells marked as "unlabeled" and cord blood cells incubated with labeled antibodies 4B8 (marked as 4B8) and 4B9 (marked as 4B9). This demonstrates that cord blood cells are stained by antibodies 4B8 and 4B9.
b) In this figure, adult blood cells show the same fluorescence intensity (x-axes), whether they are incubated with antibodies 4B8 ("4B8") or 4B9 ("4B9") or with no antibody ("unlabeled"). Thus, antibodies 4B8 and 4B9 do not bind to adult blood cells.

Figure 4

[0028] Immunofluorescent and immunoenzymatic analyses of fetal blood cells.

A) and B) Glycophorin A-positive (marked with "G") fetal erythropoietic cells express the 4B9 antigen (fluorescent, filled black regions in the cells schematically drawn in Fig. 4B). Cell nuclei are stained with DAPI and marked with "B". Obviously, nucleated and enucleated red blood cells are positive for the 4B9 antigen. A1 and B1 show the original fluorescence picture and A2, B2 schematic drawings of A1 and B1 respectively.

Claims

1. Hybridoma cell as deposited under accession number DSM ACC 2666 on July 13, 2004 at the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH in Braunschweig, Germany.

2. Monoclonal antibody expressed by the Hybridoma cell according to claim 1.

3. Monoclonal antibody according to.claim 2 reacting with a surface antigen present on fetal red blood cells and their nucleated precursor cells, and not reacting with surface antigens on adult erythroid cells.

4. Monoclonal antibody according to claim 2 or 3, **characterized in that** it reacts with fetal erythroid cells but not with the CD71 antigen.

5. Use of a monoclonal antibody according to one of claims 2 to 4 for the detection and identification of fetal cells in a sample.

6. Use according to the preceding claim for the detection and identification of fetal cells in a sample of maternal blood.

7. Method for detection or identification of fetal cells in a sample, **characterized in** labeling said fetal cells by an antibody according to one of claims 2 to 4.

8. Method according to the preceding claim, **characterized in that** the sample is maternal blood.

9. Method according to one of claims 7 or 8, **characterized in that** cells binding the monoclonal antibody are separated by one of flow cytometry, solid phase separation, immunomagnetic bead separation and panning on plastic surfaces.

10. Method for the detection of chromosomal and/or genetic aberrations, defects and/or variants in fetal cells comprising the steps of detecting or identifying fetal cells by a method according to one of claims 7 to 9, and subsequent to said detection or identification of fetal cells, analyzing said labeled fetal cells for chromosomal and/or genetic aberrations, defects and/or variants.

Patentansprüche

1. Hybridomzelle, wie hinterlegt unter Zugangsnummer DSM ACC 2666 am 13. Juli 2004 bei der Deutschen Sammlung von Mikroorganismen und Zellkulturen GmbH in Braunschweig, Deutschland.

2. Monoklonaler Antikörper, exprimiert durch die Hybridomzelle nach Anspruch 1.

3. Monoklonaler Antikörper gemäß Anspruch 2, der mit einem Oberflächenantigen reagiert, das auf fötalen roten Blutkörperchen und deren kernhaltigen Vorgänger-Zellen vorhanden ist und der nicht mit Oberflächen-Antigenen auf adulten erythroiden Zellen reagiert.

4. Monoklonaler Antikörper gemäß Anspruch 2 oder 3, **dadurch gekennzeichnet, dass** er mit fötalen erythroiden Zellen, jedoch nicht mit dem CD71-Antigen reagiert.

5. Verwendung eines monoklonalen Antikörpers gemäß einem der Ansprüche 2 bis 4 für die Erfassung und Identifikation von fötalen Zellen in einer Probe.

6. Verwendung gemäß dem vorhergehenden Anspruch zur Erfassung und Identifikation von fötalen Zellen in einer Probe mütterlichen Blutes.

7. Verfahren zur Erfassung oder Identifikation von fötalen Zellen in einer Probe, **gekennzeichnet durch** Markierung der genannten fötalen Zellen mit einem Antikörper gemäß einem der Ansprüche 2 bis 4.

8. Verfahren gemäß dem vorhergehenden Anspruch, **dadurch gekennzeichnet, dass** die Probe mütterliches Blut ist.

9. Verfahren nach einem der Ansprüche 7 oder 8, **dadurch gekennzeichnet, dass** Zellen, die den monoklonalen Antikörper binden, abgetrennt werden durch eines der folgenden Verfahren: Flusszytometrie, Festphasenseparation, Separation mit immunomagnetischen Beads und Panning auf Kunststoffoberflächen.

10. Verfahren zur Erfassung von chromosomalen und/oder genetischen Abweichungen, Defekten und/oder Varianten in fötalen Zellen umfassend die Schritte des
Erfassens oder Identifizierens fötaler Zellen durch ein Verfahren nach einem der Ansprüche 7 bis 9 und
anschließend an die genannte Erfassung oder Identifikation von fötalen Zellen, Analyse der genannten markierten fötalen Zellen für chromosomale und/oder genetische Abweichungen, Defekte und/oder Varianten.

Revendications

1. Cellule d'hybridome déposée sous le numéro d'accès DSM ACC 2666 le 13 juillet 2004 auprès de la Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH à Braunschweig, Allemagne.

2. Anticorps monoclonal exprimé par la cellule d'hybridome selon la revendication 1.

3. Anticorps monoclonal selon la revendication 2 réagissant avec un antigène de surface présent sur les globules rouges foetaux et leurs cellules précurseur nucléées et ne réagissant pas avec les antigènes de surface sur les érythrocytes adultes.

4. Anticorps monoclonal selon la revendication 2 ou 3, **caractérisé en ce qu'**il réagit avec les érythrocytes foetaux mais pas avec l'antigène CD71.

5. Utilisation d'un anticorps monoclonal selon l'une des revendications 2 à 4 pour la détection et l'identification de cellules foetales dans un échantillon.

6. Utilisation selon la revendication précédente pour la détection et l'identification de cellules foetales dans un échantillon de sang maternel.

7. Procédé pour la détection ou l'identification de cellules foetales dans un échantillon, **caractérisé par** le marquage desdites cellules foetales par un anticorps selon l'une des revendications 2 à 4.

8. Procédé selon la revendication précédente, **caractérisé en ce que** l'échantillon est du sang maternel.

9. Procédé selon l'une des revendications 7 ou 8, **caractérisé en ce que** les cellules liant l'anticorps monoclonal sont séparées par cytométrie de flux, séparation en phase solide, séparation par billes immunomagnétiques ou adhérence sur surfaces plastiques.

10. Procédé pour la détection d'aberrations chromosomiques et/ou génétiques, de défauts et/ou de variants dans des cellules foetales comprenant les étapes consistant à détecter ou à identifier des cellules foetales par un procédé selon l'une des revendications 7 à 9 et, après ladite détection ou identification de cellules foetales, à analyser lesdites cellules foetales marquées pour détecter des aberrations chromosomiques et/ou génétiques, des défauts et/ou des variants.

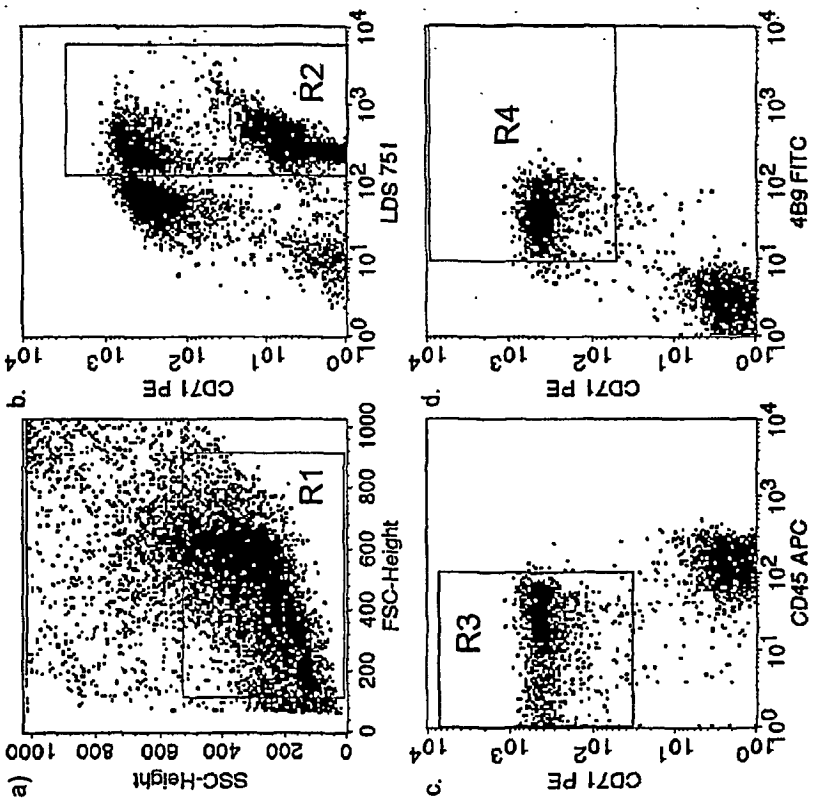

a)
SSC-Height
FSC-Height
R1
b.
CD71 PE
LDS 751
R2
c.
CD71 PE
CD45 APC
R3
d.
CD71 PE
4B9 FITC
R4

FIGURE 1

FIGURE 2

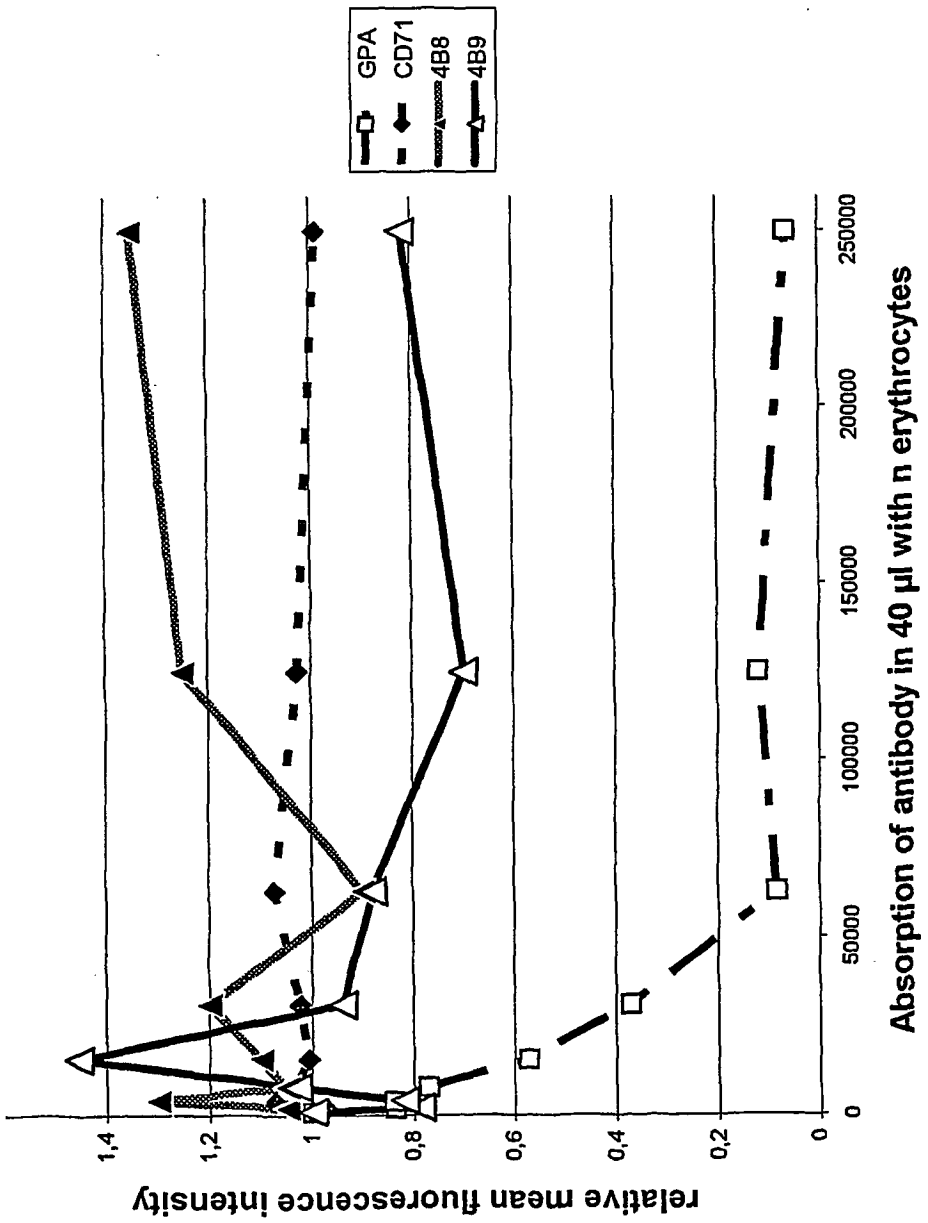
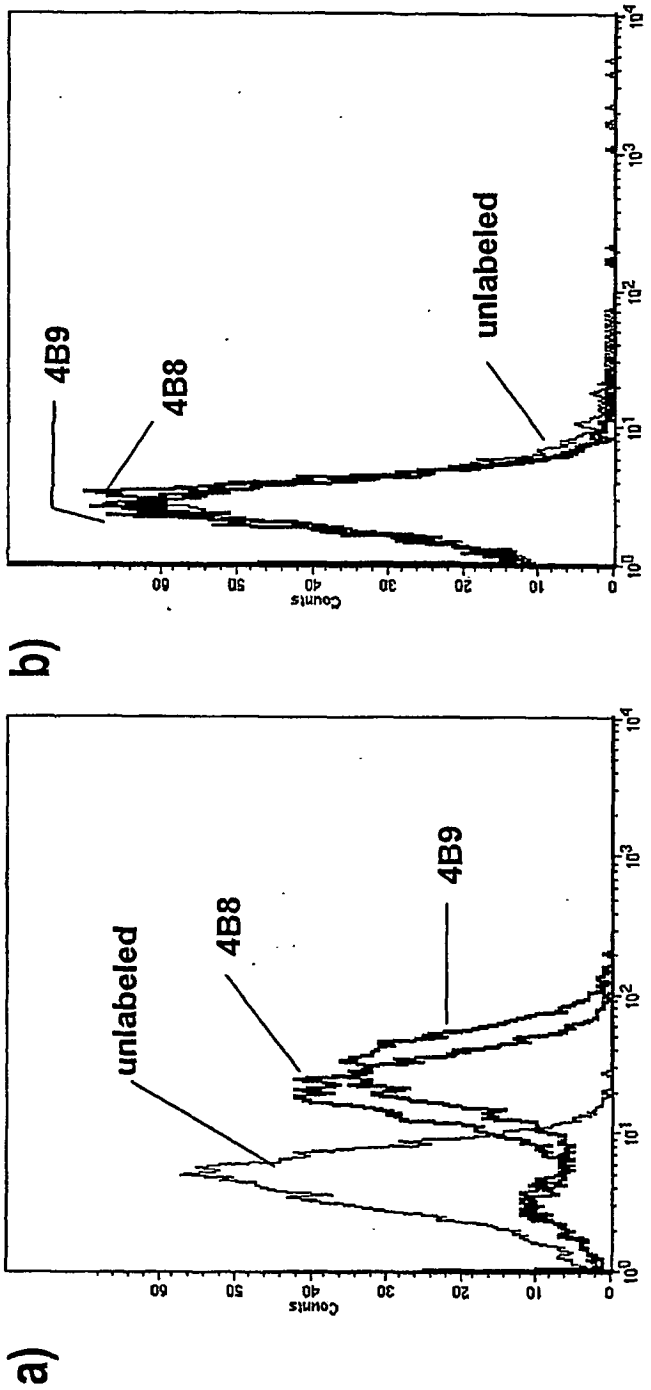

GPA
CD71
4B8
4B9
relative mean fluorescence intensity
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0
50000
100000
150000
200000
250000
Absorption of antibody in 40 µl with n erythrocytes

FIGURE 3



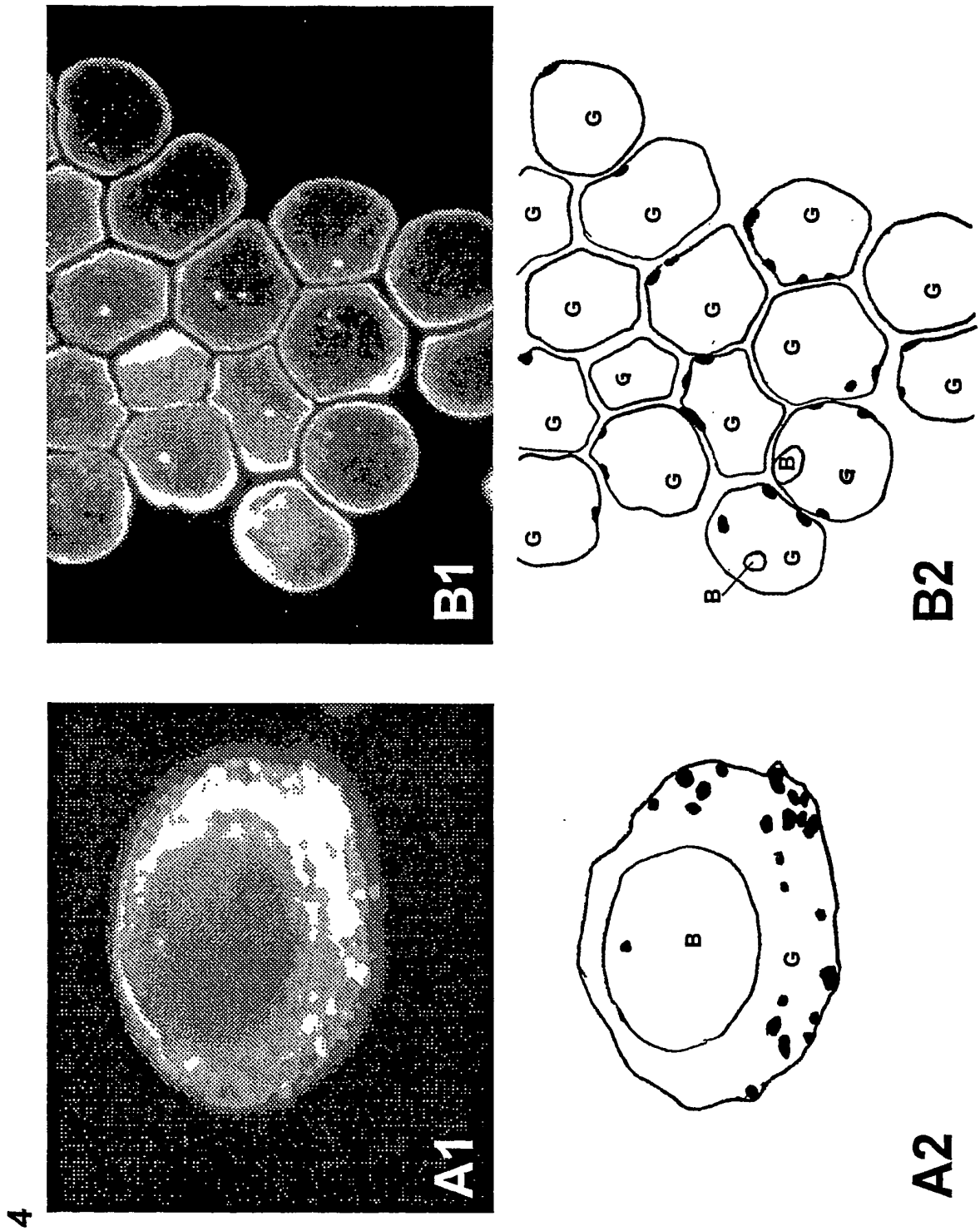


FIGURE 4

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- DE 10035433 A1 **[0010]**

Non-patent literature cited in the description

- **Huie et al.** Antibodies to human fetal erythroid cells from a nonimmune phage antibody library. *Proceedings of the National Academy of Sciences,* 27 February 2001, vol. 98 (5), 2682-2687 **[0006]**
- **Schetters H.** Production of Monoclonal Antibodies, in: Methods of Immunological Analysis. VCH Weinheim, 1993, vol. 2, 230-245 **[0010]**

专利名称(译)	对胎儿红系细胞具有特异性的单克隆抗体		
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[标]申请(专利权)人(译)	ADNAGEN		
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CPC分类号	G01N33/56966 C07K16/28 C07K2317/14 G01N33/5094 G01N33/577 G01N33/80 G01N2333/70582 G01N2469/10 Y10T436/25125 Y10T436/25375		
优先权	2004007811 2004-03-31 EP		
其他公开文献	EP1732951A2		
外部链接	Espacenet		

摘要(译)

本发明涉及单克隆抗体和相应的杂交瘤细胞和适用于从母体血液中分离胎儿细胞的抗原。单克隆抗体与胎儿红细胞（包括其有核前体细胞）上存在的表面抗原反应，但不与成体红细胞上的表面抗原反应。

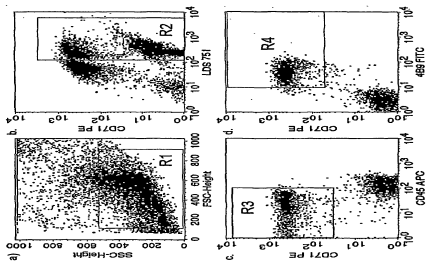

FIGURE 1