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Specific Binding of Anti-N-acetyllactosamine Monoclonal Antibody 1B2 to Acute Myeloid Leukaemia Cells

Lydia Campos, Jacques Portoukalian, Sylvie Bonnier, Zhi Hua Shi, Pascale Calmard-Oriol, Danielle Treille and Denis Guyotat

1B2 is an IgM monoclonal antibody binding to glycoconjugates bearing the terminal N-acetyllactosamine structure. It agglutinates human erythrocytes. Various cell lines, peripheral blood leucocytes, normal marrow and blast cells from 179 acute myeloid leukaemia (AML) and 11 acute lymphoblastic leukaemia (ALL) patients were tested for reactivity with 1B2. Myelomonocytic (CFU-GM), erythroid (BFU-E), mixed (CFU-GEMM) and leukaemic (CFU-L) progenitor cells were tested in clonogenic assays. Granulocytes, monocytes, myeloid cell lines and 152 out of 179 AML were positive. All FAB subtypes were equally recognised. Lymphocytes, T-cell and Burkitt's cell lines, and 10 of 11 ALL samples were negative. 1B2 inhibited partially day 7 CFU-GM, whereas it was not toxic for BFU-E, CFU-GEMM and day 14 CFU-GM. Leukaemic clonogenic cells were killed in 33 out of 36 AML (more than 40% growth inhibition). 1B2 identifies the more mature steps of myeloid differentiation. It may be useful in the diagnosis of AML, and is a candidate for remission marrow purging before autologous transplantation.
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INTRODUCTION

MONOCLONAL ANTIBODIES (Mabs) reactive with myeloid antigens are interesting to identify acute myeloid leukaemias (AML) [1] and a few antibodies directed to antigens expressed on AML cells but not on normal precursor cells have been tested for their ability to purge the graft prior to autologous bone marrow transplantation [2–6]. Many tissue-specific and tumour-specific mouse Mabs recognise carbohydrate determinants such as those of cluster of differentiation (CD) 15 [7] and MY28 [8]. The present report describes the reactivity of a mouse monoclonal antibody, 1B2, directed to glycolipids having the nonreducing terminal N-acetyllactosamine structure [9]. 1B2 identified early myeloid cells, monocytes and granulocytes but not the cells of lymphoid lineage. The reactivity with acute myeloblastic (AML) and acute lymphoblastic (ALL) leukaemias was similar to that of CD15 antibodies. Most normal precursor cells were not recognised.

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MATERIALS AND METHODS

Cells

Normal cells were recovered from peripheral blood and marrow from healthy donors. Leucocytes were separated by centrifugation on double Ficoll density gradient (1.077 and 1.092).

Human cell lines. The following cell lines were used: K562, a myeloid stem cell line; HL60, a promyelocytic leukaemia cell line; KG1, a myeloblastic leukaemia cell line; Daudi, a Burkitt's lymphoma line; and MOLT4 and HSB2, two T-cell lines. All cell lines were cultured in RPMI 1640 medium containing 10% heat-inactivated fetal calf serum (FCS) and 20 mmol/l L-glutamine. Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2.

Leukaemic cells were obtained by marrow aspiration from 190 adult patients with newly diagnosed acute leukaemia (179 AML and 11 ALL). Diagnosis was carried out according to usual cytological and cytochemical procedures and classification established according to the FAB criteria [10]. Cells were separated by Ficoll sedimentation (1.077), washed and resuspended in phosphate-buffered saline (PBS). Cytospin samples showed that the percentage of blasts was always higher than 80%.

Surface marker analysis

1B2, a mouse IgM, was prepared as described [9] and purified by ion exchange chromatography on sepharose.

Surface markers were analysed by indirect immunofluorescence, using the following monoclonal antibodies (Mabs): MY7 and My9 (Coulter Immunology, Hialeah, Florida) directed respectively to CD13 and CD33 antigens (expressed on committed progenitors and myelomonocytic lineage cells); CRIS-6 (a gift from Dr Vilella, Barcelona, Spain) directed to CD14 (monocytic) antigen; SMy15a (Biosys, Compiègne, France) to CD15 antigen (expressed on the more mature stages of the granulocytic lineage); My10 (Becton Dickinson) to CD34 (expressed on stem cells and on less than 5% of normal marrow cells). Human AB serum was added to the cell suspension to avoid non-specific fixation of the Mab on Fc receptors. Cells were incubated with Mab at 4°C for 30 min and washed with PBS. Fluorescein-labelled goat anti-mouse Fab'_2 fragments (Bioart, Meudon, France) were added as second layer (4°C, 30 min). Cells were then fixed with 0.1% paraformaldehyde. Cell fluorescence was analysed by flow cytometry using a FACSCAN II (Becton-Dickinson). Controls were performed with a non-reactive antibody (MsIgM and MSIgG kits, Coulter Immunology). The staining was considered positive when 20% cells more than the control were stained.

Neuraminidase treatment

Cells (2×10^7) were suspended in 1 ml PBS containing 0.1 U of neuraminidase (Calbiochem-Behring, La Jolla, California), incubated at 37°C for 30 min and washed. The neuraminidase-treated cells were then analysed by immunofluorescence.

Clonogenic assays

For day 7 granulocyte–monocyte colony-forming units (CFU-GM) assay, 10^5 cells were plated per well (24 Multidish Nunc, Ruskilde, Denmark) containing 0.5 ml Iscove modified Dubelcco's medium (IMDM) supplemented with 30% FCS (Gibco, Paisley, Scotland), 0.8% methylcellulose (Methocel MC, Fluka, Switzerland), and 12% conditioned medium of 5637 human bladder carcinoma cell line (HTB9) [11]. Colonies (more than 50 cells) were counted under an inverse microscope after 7 day incubation at 37°C in humidified atmosphere with 5% CO_2.

For mixed (CFU-GEMM), erythroid (BFU-E) and day 14 granulocyte–macrophage colonies studies, 10^5 cells were cultured in 1 ml IMDM containing 0.8% methyl-cellulose supplemented with 30% FCS, 5×10^{-5} mol/l mercaptoethanol, 1 U/ml erythropoietin (Terry Fox Laboratories, Vancouver, Canada) and 12% 5637 bladder carcinoma cell line supernatant as source of growth factor (12). Erythroid (>50 haemoglobinised cells), granulocyte–macrophage and mixed colonies were scored after 14 days of culture at 37°C in 5% CO_2 humidified atmosphere.

The culture system used to assay leukaemic progenitor cells (CFU-L) was the same as that used for normal CFU-GM.

Depletion of normal bone marrow and leukaemia samples from antibody-reactive clonogenic cells

Antibody-mediated complement cytotoxicity of colony forming units was determined by incubating the marrow mononuclear cells before plating with 1B2 (100 μg/ml) and low toxicity neonate rabbit complement (Centre de Transfusion Sanguine, Besançon) at a final dilution of 1:3 for 30 min at 37°C, followed by two washes with IMDM. Clonogenic cells were considered positive for 1B2 antigen when there was a decrease of 40% or more of the colony recovery by comparison with control with complement alone.

A clonogenic cell lysis was also performed in the same conditions with 8.27, a CD15 IgM antibody (a gift of P. Poncelet, Sanofi Recherche, Montpellier, France). 8.27 was used at a concentration of 100 μg/ml.

RESULTS

Distribution of 1B2 on normal leucocytes

The study was performed on 10 normal peripheral blood and 10 marrow samples. 1B2 reacted with normal granulocytes [mean (S.D.) of reactive cells: 93.4 (3.3), monocytes 66.1 (4.0)] but not with lymphocytes. Flow cytometry analysis of normal cells binding is presented in Fig. 1. In untreated normal marrow, 65.7% (3.1) of cells were recognised, but only 17.2% (2.0) after Ficoll separation.

Neuraminidase treatment increased the intensity of staining of granulocytes and monocytes. Neuraminidase-treated lymphocytes became positive for 1B2 (Fig. 1).

Distribution of 1B2 on leukaemic cell lines and leukaemic cells

The cell lines KG1, HL60 and K562 were positive for 1B2. In contrast, the Burkitt's lymphoma cell line Daudi and two T-cell lines, MOLT4 and HSB2 were negative.

Blast cells of 179 AML patients were studied by indirect immunofluorescence. Of 179 samples, 152 (85%) were positive. All FAB cytological sub-classes were recognised, but the percentage of samples recognised was slightly higher in M2 (93%) and M3 (94%) classes than in M5 (87%), M4 (76%) and M1 (74%) sub-classes (Table 1). A majority of undifferentiated leukaemias (63%) were also stained. The reactivity of 1B2 was compared with that of other myeloid antibodies. CD11, CD13, CD14, CD15, CD33 and CD34 Mabs identified, respectively 31%, 76%, 42%, 70%, 38% and 49% of samples. The concordance (ratio: samples equally positive or negative/samples studied) of 1B2 staining was 38% with CD11, 72% with CD13 ($P = 0.04$), 46% with CD14, 74% with CD15 ($P < 10^{-3}$), 44% with CD33 and 44% with CD34. In combination with CD13 or CD15, 1B2 identified, respectively 169/177 and 170/178 samples studied (96%). This rate was slightly higher than that observed with a combination of CD13 and CD15 (91% of cases recognised), as the pattern of staining of CD13 and CD15 was very similar (concordance rate: 80%, $P < 10^{-5}$).

1B2 negative samples ($n = 27$) were analysed in detail. Four samples lacked expression of other myeloid surface markers,

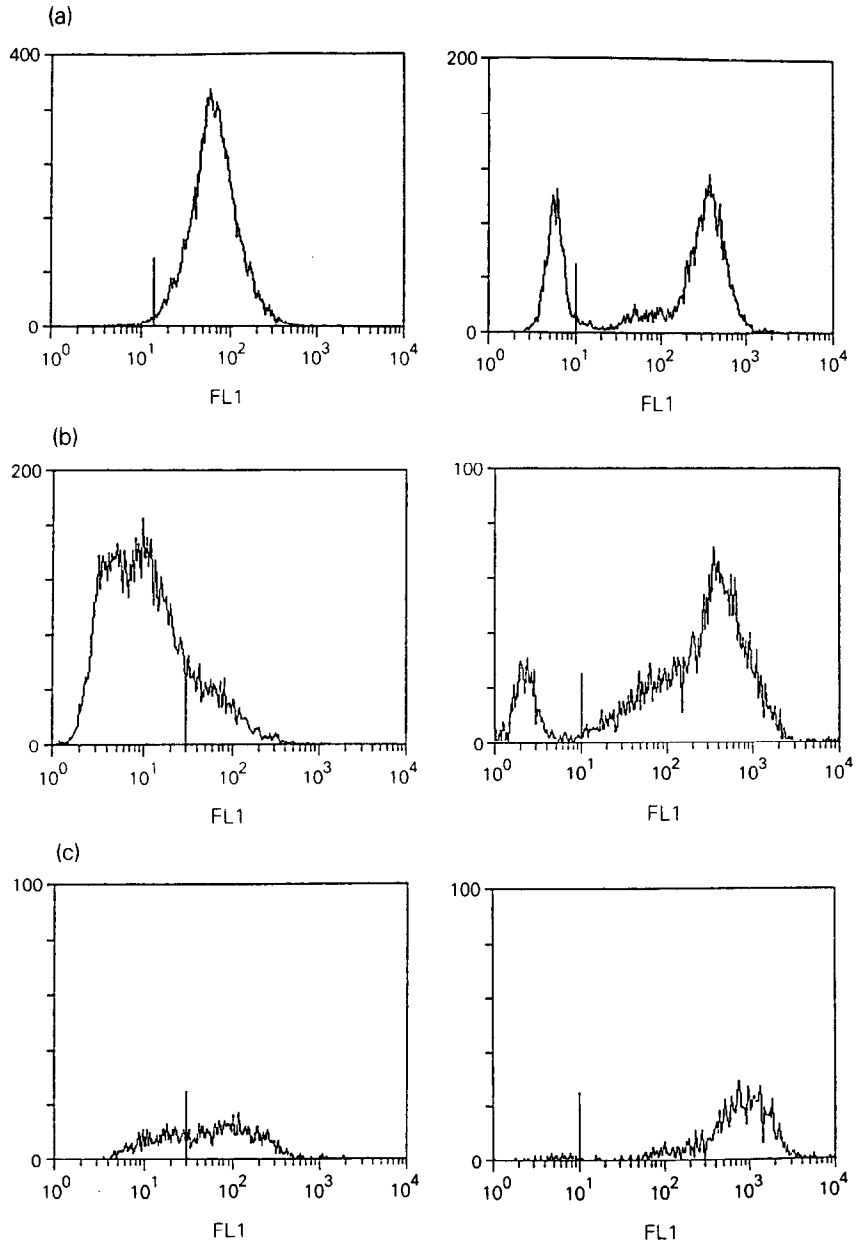

Fig. 1. Flow cytometry analysis of 1B2 binding to normal peripheral granulocytes (a), monocytes (b) and lymphocytes (c). Left = without neuraminidase treatment, right = with neuraminidase treatment.

Table 1. *1B2 reactivity with acute undifferentiated and myeloid leukaemia cells by FAB classification*

	FAB sub-type							
	M0	M1	M2	M3	M4	M5	M6	M7
1B2-positive*	5	17	37	15	19	54	3	2
1B2-negative	3	6	3	1	6	8	0	0
% positive	63	74	93	94	76	87	100	100

*Number of cases positive (20% or more cells stained)

but were CD34 positive. Two of these cases were classified as M1 and two as M5a on cytochemical grounds. All other 1B2 negative samples were positive for at least one myeloid marker (6 for CD11, 17 for CD13, 5 for CD14, 8 for CD15 and 7 for CD33).

Of 11 ALL cases studied (common ALL: 8 cases, T-cell ALL: 3 cases), only one was positive for 1B2. This case disclosed a phenotype of common ALL a t(9;22) chromosomal abnormality and was also positive for CD13, CD15 and CD34.

Expression of 1B2 on normal and leukaemic progenitor cells

The expression of 1B2 antigen on haematopoietic progenitors was studied in 10 normal marrows by analysing the recovery of colonies after treatment of mononuclear cells with 1B2 and

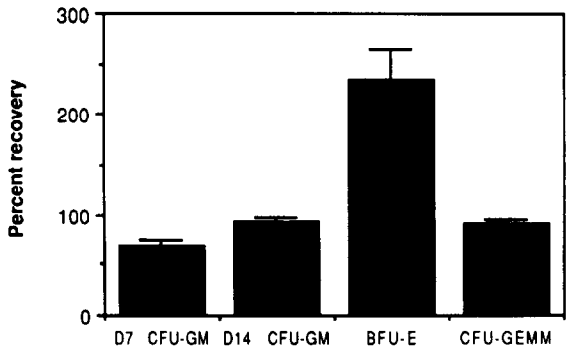

Fig. 2. Toxicity of treatment of normal progenitors by 1B2 and complement.

rabbit complement. The mean recovery (S.D.) was 69% (6) of control for day 7 CFU-GM, 93% (4) for day 14 CFU-GM, 92% (4) for CFU-GEMM and 234% (31%) for BFU-E (Fig. 2).

The expression of 1B2 on leukaemic clonogenic cells was evaluated in 36 AML cases. CFU-L lysis ranged from 10% to 100%. Lysis greater than 40% was observed in 33 out of 36 samples. Lysis equal to or greater than 90% was noted in 13 cases. There was a statistical correlation between the percentage of fresh cell lysis and the percentage of clonogenic cell lysis ($r = 0.80, P < 10^{-5}$) (Fig. 3).

In 28 cases, the CFU-L lysis by 1B2 was compared with that achieved by 8.27, a CD15 Mab, plus complement, and by a cocktail of 8.27 plus 1B2 plus complement. With 8.27 alone, a colony growth inhibition equal to or greater than 90% was obtained in 11 cases (39%). With the cocktail, an inhibition equal to or greater than 90% was observed in 19 cases (68%), and a complete (100%) inhibition in 10 cases (36%). Complete data are presented in Table 2.

DISCUSSION

A number of Mabs have been obtained that recognise surface glycoproteins or glycolipids bearing carbohydrate structures. This paper describes the distribution of Mab 1B2 binding on fresh leukaemia cells, early haematopoietic progenitors and leukaemia progenitors. In a previous paper, it was shown that 1B2 is directed to the terminal N-acetylactosamine carbohydrate structure [9]. Anti-MY-28, which has a pattern of reactivity close to that of 1B2, binds to the lacto-N-neotetraose determinant

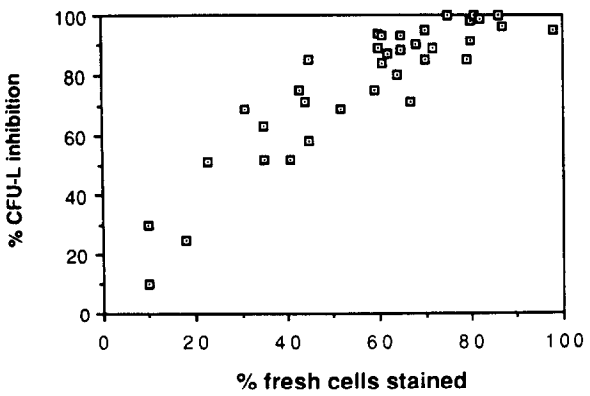

Fig. 3. Plot of percentage of clonogenic leukaemic cell (CFU-L) lysis versus fresh cell lysis.

Table 2. AML clonogenic cell (CFU-L) killing by 1B2, 8.27 (CD15) and 1B2 + 8.27 + complement

	% Fresh cell lysis			CFU-L killing		
Case no.	1B2	8.27	1B2 + 8.27	1B2	8.27	1B2 + 8.27
1	35	51	63	63	38	78
2	86	68	ND	100	ND	ND
3	23	59	71	51	10	82
4	67	50	89	71	82	99
5	80	78	85	91	95	93
6	75	50	100	100	90	100
7	65	43	ND	93	76	ND
8	87	ND	ND	96	ND	ND
9	65	59	98	88	92	100
10	81	ND	ND	100	ND	ND
11	31	46	99	69	92	100
12	59	61	83	75	75	85
13	61	70	100	84	93	100
14	80	70	96	98	50	95
15	79	80	82	85	35	79
16	72	65	88	89	16	99
17	68	70	87	90	40	76
18	70	92	ND	85	100	ND
19	60	50	76	94	60	69
20	35	2	39	52	0	66
21	70	35	88	95	59	100
22	43	10	59	75	75	99
23	64	15	72	80	20	95
24	45	34	ND	85	74	ND
25	41	15	ND	52	19	ND
26	45	30	64	58	17	92
27	82	40	100	99	97	100
28	61	40	79	93	91	100
29	60	62	84	89	87	99
30	52	ND	ND	69	ND	ND
31	44	39	57	71	62	87
32	62	85	96	87	97	100
33	10	62	69	10	68	76
34	98	93	100	95	94	100
35	18	80	95	20	74	90
36	10	90	97	30	100	100

ND = not done.

of paragloboside, a glycolipid identified in granulocytes, monocytes, and AML cells and cell lines [8]. The determinant 3-fucosyl-N-acetyllactosamine is recognised by CD15 Mab on the surface of granulocytes, monocytes and up to 85% of AML cells [7].

Our study revealed that the distribution of 1B2 binding on normal haemopoietic cells is close to that of CD15. In immunofluorescence, 1B2 stained all granulocytes and a majority of monocytes. Exposure to neuraminidase allowed the staining of lymphocytes which where almost completely negative before treatment, and increased the staining of myeloid cells. These results are consistent with those obtained with Mab directed to carbohydrate structures [8, 13].

Mabs are now useful tools for the diagnosis and the classification of acute leukaemia [1]. 1B2 recognised most AML cases. Although 1B2 antigen was found on a greater percentage of FAB M2 and M3, 1B2 binding was not predictive of cytological classification. We compared the reactivity of 1B2 to that of widely used myeloid Mab. As previously reported [14, 15], CD13 and CD15 also identified a majority of AML samples.

There was a good but not complete concordance between the results obtained with 1B2 and CD15, and with 1B2 and CD13. In combination with CD13 or CD15 Mab, 1B2 identified 96% of AML samples. This makes 1B2 useful for the routine diagnosis of AML. By contrast, only one ALL case was identified. This sample was also positive for CD15 and CD13. A positivity of some ALL cases for myeloid markers such as CD13 and CD33 has already been reported [16]. We have shown that CD15 antigen (and other myeloid markers) may be expressed on the surface of ALL cells, particularly those with the Philadelphia chromosome [17].

CD15 antigen is expressed on the surface of a minority of normal myeloid progenitors, but not on erythroid or mixed progenitors [18]. Similar results were obtained with 1B2. The expression of 1B2 antigen on progenitor leukemic cells was heterogeneous. We observed a correlation between the percentage of 1B2-positive CFU-L and the percentage of lysis of the whole blast population. This is in contrast to what has been reported for other surface markers, the expression of which is frequently different on fresh leukaemic cells and on CFU-L [19, 20]. Our own studies with CD15 antibodies confirmed this lack of correlation [21]. In the case of 1B2, the study of fresh leukaemic cells was therefore predictive of the CFU-L killing.

Autologous bone marrow transplantation is a promising approach for the treatment of AML. The reinfusion of leukaemic cells with the graft is a possible drawback to this method. Few studies of bone marrow purging by immunological methods have been performed in AML. CD15 [3, 21], CD33 [4] and CD65 [5] Mab have been tested in view to obtain a selective killing of AML cells without toxicity for normal precursors. We show here that a killing of more than 90% of clonogenic cells was achieved by 1B2 and one round of complement in about one third of cases, which is similar to what was reported in the above cited experiments, but is not enough to ensure an efficient purging in the majority of AML. A higher efficiency was obtained by combining 1B2 and 8.27 (CD15) antibodies, which makes this cocktail a good candidate for *ex vivo* bone marrow purging in AML.

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