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ACUTE MYELOID LEUKAEMIA

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## Early Expression of MCS2 (CD13) in the Cytoplasm of Blast Cells from Acute Myeloid Leukaemia

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**Key Words.** Acute myeloid leukaemia • Cell markers • Monoclonal antibodies

**Abstract.** The expression of two myeloid antigens identified by the monoclonal antibodies (McAb) MCSZ (CD13) and MY9 (CD33) was investigated in 136 cases of leukaemia. MCSZ was positive in blast cells of 78 of 88 (88.5%) and MY9 in 51 of 81 (64%) cases of acute myeloid leukaemia (AML) and chronic granulocytic leukaemia in myeloid blast crisis. One or other McAb, or both, were positive in all but 2 (2.3%) of these cases. MCSZ was more sensitive than MY9 to detect blasts of the myeloid lineage due to its most frequent reactivity in the cytoplasm of fixed cells by the immunoperoxidase (IP) technique compared with its membrane expression on cell suspensions by immunofluorescence (IF). MY9 was not suitable for tests on fixed cells. MCSZ was positive by IP but not by IF in 24% of AML, but the reverse was not observed. This suggests that the antigen detected by MCSZ is expressed in myeloblasts first in the cytoplasm and later on the cell membrane, pattern which is similar to that of the early antigens CD3 and CD22 in T and B lineage lymphoblasts, respectively. MCSZ was always positive in FAB types of AML-involving myeloblasts (M1-M4), including cases of undifferentiated morphology (M0), whilst MY9 was more frequently positive in monocytic leukaemia (M5). On the other hand, MCSZ was positive in 4 of 33 cases of acute lymphoblastic leukaemia and MY9 in 1. We conclude that both McAb, particularly MCS2, contribute to the better characterisation of myeloid leukaemias but that other tests are required to clarify the nature of the blasts when unexpected reactivities are observed.

### Introduction

The classification of acute myeloid leukaemia (AML) has been based largely on morphological and cytochemical findings [1]. The availability of monoclonal antibodies (McAb) with myeloid specificity has provided new insights into the characterisation of AML. Although a relatively large number of McAb identifying myeloid-associated antigens has been described, only a small number react with early myeloid cells and appear to be of value for the phenotyping of AML. One of them is MY9 (CD33), an McAb that reacts with a p67 protein expressed in committed myeloid progenitor cells [2]. Another is MCS2 (CD13), a reagent specific for cells at an early maturation stage along the myeloid pathway [3]. MCSZ has the same specificity as MY7 (CD13) but appears to react with antigens of different molecular weight [4].

We have compared the pattern and reactivity of MY9 and MCSZ using immunofluorescence (IF) and immunoperoxidase (IP) methods and have shown that the IP technique on fixed cells significantly increases the sensitivity of MCSZ for the recognition of AML blast cells and suggests that the p150 antigen detected by MCSZ is expressed in the cytoplasm of myeloblasts before its membrane expression.

### Material and Methods

Cells were obtained from peripheral blood (PB) and/or bone marrow (BM) from 136 patients studied at diagnosis. These included 65 cases of AML, 33 of acute lymphoblastic leukaemia (ALL), 24 of chronic granulocytic leukaemia in blast crisis (CGL-BC), 14 of chronic lymphocytic leukaemia and non-Hodgkin lymphomas. Diagnosis was established by morphological criteria on May-Grünwald-Giemsa-stained BP and BM films and the cyto-

chemical reactions of Sudan black B, a-naphthyl-acetate-esterase and acid phosphatase, as previously described [5]. The diagnosis of ALL was based on cytology and cytochemistry supported by immunological evidence of the presence of E- or T-cell antigens. The diagnosis of AML was based on the FAB criteria [1] as well as on the absence of lymphoid markers. All samples of ALL and AML analysed had more than 50% blast cells.

#### Cell Markers

Mononuclear cells from BP and/or BM were isolated by Lymphoprep (Nyegaard). An indirect IF technique was performed on cell suspensions using McAb, followed by a second layer of a fluorescein-isothiocyanate-conjugated goat antimouse F(ab)2 serum (Cappel). The cells were washed in phosphate-buffered saline (PBS) resuspended in PBS/glycerol (50/50) mounted on slides and examined under a Zeiss 14 fluorescence microscope. A control in which the first layer of McAb was replaced by non-immune mouse ascites was always included. AB Serum at a 2% dilution was used throughout to block non-specific binding to Fc receptors. The following mouse McAb were used: GRB1 (IgG2a), gift from Prof. Garrido-Torres, against class II major histocompatibility complex antigen. MY9 (Coulter-clone, UK IgG2, CD33) [2] and MCSZ (Nichere Corporation, Japan: IgG1, CD13) reactive with immature myeloid and monocytic cells [3]; B4 (Coulter-clone, UK: CD19) against B-lineage blasts, and B-lymphocytes; OKB-CALLA (Orthoclone, UK) and/or Nu-1 (Nichere Corporation, Japan) both McAb against the common ALL antigen (CD10); antiglyco-

phorin A (LICR LON/R10) used to recognise erythroid precursors, (gift from Dr. Edwards; Cl7; IgG1, CD41), against platelet glycoprotein (GP) IIb (gift of Dr. Tetteroo), and ANS1 (IgG, CD42, Dakopatts, UK), which recognises an antigen on GP1b, were used to demonstrate megakaryoblasts. Terminal deoxynucleotidyl transferase (TdT) was assessed by an indirect IF method described by Bollum [6] using a rabbit anti-TdT serum (Sero-lab, UK).

MCS2 was also studied in all cases by the IP reaction on fixed cells. Cyto-centrifuged slides were fixed for 10 min with acetone; endogenous peroxidase was inhibited by immersing the slides in chloroform for 10 min at room temperature. After PBS washing for 5 min, a first incubation was made with 2% AB serum, followed by the McAb MCS2 for 30 min used at a 1:20 dilution of the 1:100 diluted ascites used for the IF method. Two layers of horseradish peroxidase-conjugated antibody were employed, rabbit antimouse and swine antirabbit (Dakopatts, UK). After washing, the peroxidase reaction was carried out with 3,3'-diamino-benzidine-tetrahydrochloride (Sigma, UK) in PBS for 10 min in the dark. The slides were counterstained with Harris' hematoxylin for 2 min, dried and mounted in DPX and analysed by comparing with a negative control.

#### Results

A comparison between MCS2 investigated by IF on cells suspensions and by IP on fixed cells was made in samples from 50 cases of AML and CGL-BC (table I). In 76% of cases both methods gave similar results: in 64% both were positive and in 12% both tests were negative. In 12 cases (24%) MCS2 was positive in > 20% of the blast cells when tested by IP whilst it was completely negative by IF. In addition, when both tests were positive, the percentage of reactive cells was 10-80% higher by IP than by IF. In 12

Table I. Expression of MCSZ in AML and CGL-BC tested by two methods

| Number of cases tested | IF+ | IP+ | IF+/IP+ | IF-/IP+ | IF-/IP- |
|------------------------|-----|-----|---------|---------|---------|
| 50                     | 32  | 44  | 32      | 12      | 6       |

Table II. Expression of myeloid antigens detected by MCSZ and MY9 in leukaemic cells

| Leukaemia type | Number of cases tested | MCS2 + <sup>1</sup> | MY9 + | MCSZ +/- MY9 + | MCS2 +/- MY9 - | MCS2 -/ MY9 + | MCS2 -/ MY9 - |
|----------------|------------------------|---------------------|-------|----------------|----------------|---------------|---------------|
| AML            | 65 <sup>2</sup>        | 59                  | 38    | 35             | 20             | 2             | 2             |
| CGL-BC         | 24 <sup>2</sup>        | 19                  | 13    | 13             | 5              | 0             | 0             |
| ALL            | 33 <sup>2</sup>        | 4                   | 1     | 1              | 2              | 0             | 27            |
| NHVCLL         | 14                     | 0                   | 0     | 0              | 0              | 0             | 14            |
| Total          | 136                    | 82                  | 52    | 49             | 27             | 2             | 47            |

NHL = Non-Hodgkin lymphomas; CLL = chronic lymphocytic leukaemia.

<sup>1</sup> MCS2 assessed by IF and/or IP.

<sup>2</sup> MY9 was not investigated in 6 cases and MCS2 was not tested in 1.

<sup>3</sup> MY9 was not studied in 2 CGL-BC and 6 ALL

cases who were positive by IF, MY9 was tested by IP with three different fixatives using various dilutions, but no positive results were obtained by IP. The results that follow will refer to MCSZ tested by IF or IP and to MY9 by IF only.

The results obtained with both McAb on the blast cells from the 136 cases studied are summarised in table II. Overall 88.5% of cases of AML and CGL-BC were positive with MCS2, whereas only 64% of cases were positive with MY9; 60% of cases were positive with both McAb; 31% of cases were positive with MCSZ and negative with MY9 and 2.5% were negative with MCS2 and positive with MY9. Overall one or other McAb were positive in 93% of all cases of myeloid leukaemia. It is of interest that the only cases which were MCS2- and/or MY9-negative involved megakaryoblasts: 2 had AML (M7) and 4 had CGL in megakaryoblastic crisis.

The results in 65 cases of AML analysed according to the FAB classification are shown in table III. The group of very immature myeloblastic leukaemia designated M0 was always MCS2-positive, whereas MY9 was positive in only 40% of cases. Overall MCSZ was more often positive than MY9 in most FAB types, except for M5 (table 111). Although 2 cases of AML (M7) were negative (see above), 3 others were positive in 20–68% of blasts. Evidence for megakaryocytic differentiation in these 5 cases was provided by McAb against platelet GP and the platelet peroxidase reaction at the ultrastructural level.

Of 33 cases of ALL (table IV), 27 were of B-lineage and 6 had T-ALL; in 4 of the 25 cases of adult-ALL, the blasts were positive with one or other McAb in 19–60% of cells. Three (cases 1–3) had c-ALL and one (case 4) T-ALL (table IV).

## Discussion

We have compared the reactivity of two McAb, MCSZ (CD13) and MY9 (CD33) in a series of leukemic samples which involved blast cells. Although a number of reports have appeared, including one from our own group [7], describing the specificity and sensitivity of these reagents for myeloid precursors, almost all studies have been carried out on cell suspensions by IF microscopy or flow cytometry. We have, in addition, used systematically MCSZ by IP on fixed cells and showed that this method is more sensitive than IF to demonstrate this antigen in early myelo-

Table 111. Reactivity of MCS2 and MY9 in FAB types of AML

| FAB type                 | MCSZ        |           |     | MY9         |           |    |
|--------------------------|-------------|-----------|-----|-------------|-----------|----|
|                          | tested<br>n | positive' |     | tested<br>n | positive' |    |
|                          |             | n         | %   |             | n         | %  |
| M0 <sup>2</sup> (n = 10) | 9           | 9         | 100 | 10          | 4         | 40 |
| M1 (n = 16)              | 16          | 16        | 100 | 15          | 10        | 67 |
| M2, M3, M4 (n = 18)      | 18          | 18        | 100 | 17          | 10        | 59 |
| M5 a + b (n = 13)        | 13          | 10        | 77  | 11          | 10        | 91 |
| M6, M7 (n = 8)           | 8           | 6         | 75  | 6           | 4         | 67 |
| Total (n = 65)           | 64          | 59        | 92  | 59          | 38        | 64 |

<sup>1</sup> Positive cases: > 20% positive blast cells.

<sup>2</sup> M0 cases with undifferentiated morphology, negative Sudan Black B, negative lymphoid markers and positive myeloid antigen(s), confirmed by peroxidase at the electron-microscopic level (5 cases).

8 cases (1 M0, 4 M1, 2 M5 and 1 M7) were negative by IF and positive by IP.

Table IV. Cases of ALL with myeloid positive markers (% of positive blasts)

| Cases | Age | FAB             | Ia   | CD19 | CD10 | CD3             | MCS2 | MY9  | TdT |
|-------|-----|-----------------|------|------|------|-----------------|------|------|-----|
| 1     | 36  | L2 <sup>1</sup> | 90   | 92   | 98   | n.d.            | 20   | 19   | 20  |
| 2     | 86  | L2              | 90   | 60   | 60   | neg.            | 40   | neg. | 90  |
| 3     | 20  | L2              | 100  | 47   | 85   | neg.            | 60   | n.d. | 60  |
| 4     | 68  | L2              | neg. | ntg. | neg. | 65 <sup>2</sup> | 60   | neg. | 67  |

<sup>1</sup> Rearrangement of JH Ig gene demonstrated: other cases not tested.

E-rosette test 59% and OKT17 positive in 70% of blasts.

blastic leukaemia. It would appear that as other early antigens, CD22 in the B-lineage [8, 9] and CD3 and in the T-lineage [8, 9], the antigen detected by MCS2 is expressed first in the cytoplasm and later on the cell membrane. Unfortunately, we could not demonstrate the same phenomenon for the protein identified by MY9, as we were unable to show reactivity with this McAb by IP in 12 cases known to be MY9-positive by IF, although we are aware that others showed positivity with alkaline-phosphatase-labelled antibodies [10].

As a result of its greater sensitivity by IP (table I), MCSZ was positive in a higher proportion (88.5%) of

cases of **AML** and **CGL-BC** than **MY9 (64%)**. However, as a result of the preferential reactivity of **MCS2** in **AML** cases involving myeloblasts (**M1-M4**), including poorly differentiated ones (**MO**) and of **MY9** for **monocytic** leukaemia (**M5**), the use of both McAb can recognise 97.7% of these myeloid leukaemias. The diagnostic value of **MCS2** by **IP** was particularly important in **AML** cases designated **MO** where light microscopy, cytochemistry and lymphoid markers are negative. All the 9 cases of **MO** tested were **MCS2**-positive whilst only 4 of them were positive with **MY9**. These cases are of interest because some of them (as well as cases of **MI**) may be **TdT**-positive [11] and have been considered as **ALL** by some authors [12]. In 5 of these cases of **MO** studied by ultrastructural cytochemistry, we have shown myeloperoxidase activity in granules and in membranous structures confirming their early myeloblastic nature. Despite the greater sensitivity and specificity of **MCS2** for the myeloid lineage, 4 (12%) of bonafide cases of **ALL (16% of adult ALL)** showed reactivity with **MCS2** and one with **MY9**. Our limited studies so far with immunoglobulin gene rearrangement (table IV) suggest that these are otherwise typical cases of **ALL**, confirming observations of Sobol et al. [12]. However, further systematic studies by electron microscopy with sensitive methods for myeloperoxidase [13] will be necessary to ascertain whether there is also evidence of myeloid structures on these biphenotypic cases. Nevertheless, the demonstration of myeloid antigens in adult-**ALL** cases appears to be clinically significant and is associated with a worse prognosis [12].

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