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Analysis of Leukemic Cells with Monoclonal Antibodies in Acute Myelomonocytic Leukemia Suggests Abnormality at an Early Differentiation Stage in Certain Cases *

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A. Introduction

Monoclonal antibodies (MoAb) are valuable tools in better defining hematologic malignancies, both for diagnostic purposes and for understanding normal and malignant differentiation [1–5]. In the present study, we characterized the bone marrow cells of a young child with acute myelomonocytic leukemia (AMML). The unusual immunologic phenotype in this case not only allows more insight into myelomonocytic differentiation, but also sheds some light on the origin of the malignant process in certain cases of AMML.

B. Clinical Information

A girl aged 2 years 2 months was admitted to the hospital with a history of low grade fever for several weeks, arthralgias, purpura, tonsillitis, mild generalized lymphadenopathy, and hepatosplenomegaly. Laboratory data showed elevated lactate dehydrogenase, HbF of 4%, and increased urine and serum lysozyme. Her Hb was 5.8 g/dl, thrombocytes $128 \times 10^9/l$, and the white blood cell count $57 \times 10^9/l$ with a differential count of 7% lymphocytes, 6% neutrophils and band forms, 4% metamyelocytes, 1% myelocytes, and 82% myelomonocytic blasts. Bone marrow analysis revealed a high percentage of polymorphic leukemic blasts, predominantly monocytoid with only a small number of cells with promyelocyte appearance. Almost all leukemic cells showed positive myeloperoxidase and nonspecific esterase reaction. Diagnosis of AMML, M4 according to FAB classification [6] was made.

C. Material and Methods

Heparinized bone marrow was layered over a Ficoll–Diatrozoate density gradient and interface cells were obtained after centrifugation as previously described [7]. These bone marrow mononuclear cells were evaluated for staining with MoAb in indirect immunofluorescence staining, using a goat anti-mouse immunoglobulin (Ig) as developing reagent, as described in detail [2, 8]. The following MoAb were used: 3A1 and 4F2 (kindly provided by Dr. A. Fauci) [3]; 9.6 and 20.2 (gift from Dr. J. Hansen) [5]; FMC7 (provided by Dr. H. Zola) [9]; Mo-P9, Mo-P15, Mo-S1, and Mo-S39 [8]; U-28 (gift from Dr. R. Winchester) [10]; the OKT/OKM/OKI series [1, 4]; B1 [11]; BA-1, BA-2, and TA-1 [12–15]; and My-1 [16]. Cytoplasmic Ig was detected on cytocentrifuge preparations stained with heteroantisera. Transmission electron microscopy was performed as previously described [2]. Analysis for lectin binding was done according to a recent paper by Koch et al. [17].

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Table 1. Reactivity of the patient's bone marrow cells with different antibodies

Antibody	Characteristic specificity	Reactive cells (%)
9.6	Pan T cell	22
3A1	Major T cell subset	15
OKT4	Helper T cells	7
OKT8	Suppressor/cytotoxic T cells	10
B1	B cells	9
BA-1	B cells, granulocytes	40
BA-2	Lymphoid precursors	33
FMC7	B cell subset	2
Anti-IgA,G,M,D	Plasma cells	0[a]
Anti-κ/λ	Plasma cells	0[a]
Mo-S39	Monocytes	60
OKI1	HLA-DR-positive cells	60
OKT10	Activation antigen on different lineages, plasma cells	65

[a] Intracytoplasmic staining

Table 2. Reactivity with additional monoclonal antibodies detecting cells of myeloid/monocytoid lineage

Antibody	Characteristic specificity	Reactive cells (%)
Mo-P9	Monocytes	40
Mo-P15	Monocytes	40
Mo-S1	Monocytes	40
20.2	Monocytes, granulocytes	55
OKM1	Monocytes, granulocytes	54
TA-1	T cells, monocytes	45
4F2	Monocytes, activated/proliferating cells (not lineage specific)	80
My-1	Certain stages of myeloid differentiation, including promyelocytes and granulocytes	16
U-28	FAB M1 and some M5 cells	0

D. Results and Discussion

Immunologic characterization of the bone marrow cells of this patient revealed more than 70% leukemic cells, while the remaining population consisted of mature B and T lymphocytes as determined by positive staining with reagents 9.6, OKT4, OKT8, 3A1, and B1 (Table 1). The population of malignant cells reacted with six MoAb which have particular specificity for the monocyte lineage, namely Mo-P9, Mo-P15, Mo-S1, Mo-S39, 20.2, and OKM1, although some have additional reactivity with granulocytes and myeloid precursors (Tables 1 and 2). These cells were also stained by reagent 4F2, detecting monocytes and activated or proliferating cells of different origin. Furthermore, reactivity with MoAb OKT10 was found, an antibody which, besides reacting with plasma cells and certain thymocytes, serves as an activation marker in several lineages. Confirming the AMML differentiation stage, the leukemic cells reacted with TA-1, a MoAb detecting an antigen expressed on mature T cells, monocytes, and AMML cells, but not on cells of acute myelocytic leukemias (AML) [13]. A certain percentage of more immature myeloid cells was detected by antibody My-1,

reactive mainly with FAB M2/M3 cells [16], a finding consistent with the morphological picture of a minor population of promyelocytes. Surprisingly, significant additional reactivity was found with two MoAb, namely BA-1 and BA-2. Antibody BA-1 primarily stains normal and malignant cells of B cell lineage, and, although also binding to granulocytes, is usually distinct unreactive with monocytes and AMML cells [10, 11]. Antibody BA-2 has been found primarily on lymphoid precursor cells and lymphoid malignancies of different differentiation stages, although additional reactivity with some nonlymphoid cells was found, particulary neuroblastoma and certain carcinomas [14, 15]. Finally, lectin binding studies showed 44% of the cells reactive with *Lotus tetragonolobus* agglutinin (LTA), consistent with the presence of myelomonocytic cells [17].

To evaluate whether MoAb BA-1, BA-2, and anti-monocyte MoAb did stain the same cell population, double-staining experiments were performed. Various combinations of two or more of these antibodies added to the same cell preparation did not result in additional positive cells, demonstrating that the corresponding antigens were expressed in the same cell population.

In this case, a rather homogeneous reactivity pattern with MoAb was seen, despite some degree of morphological variation. This has implications for models of myeloid differentiation and would be in favor of an uncommitted promyelocyte, still able to differentiate into the monocyte lineage as has recently been suggested [18]. Furthermore, the pattern of MoAb reactivity would classify this case of AMML as already differentiated primarily into the monocyte lineage.

Finally, the unusual occurrence of antigens normally detected on B lymphocytes and B lymphocyte precursors in these AMML cells suggests that in this case an abnormality may have developed at a very early differentiation stage with subsequent differentiation along the myeloid pathway. This idea would be consistent with genetic analysis of granulocyte/macrophage colonies in a case of AMML, where by karyotyping the malignant cells could be defined as arising shortly after stem cell differentiation. Thus, analysis with monoclonal reagents provides an additional tool in better defining the origin of malignant cells in addition to enzyme markers and chromosomal analysis.

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