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Production of Three Monoclonal Antibodies – A01, B05, and C11 – Against B-Cell Leukemia

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A. Introduction

The advent of MoAb technology has greatly facilitated the identification of various subsets of lymphoid cells [1, 2]. However, most studies have been concentrated on acute lymphoblastic and myelocytic leukemias [1–4], and relatively few MoAbs that are directed against surface determinants of chronic lymphocytic cells have been reported [5–7]. The reason may be that CLL cell lines are few in number and are not as well characterized as acute lymphocytic or acute myelocytic leukemia cells. In this report we describe the establishment of three hybridomas which secrete MoAbs against an antigen present on malignant B-lymphocytes in the peripheral blood of most CLL and some non-T, non-B ALL patients. These newly established MoAbs have unique patterns of reactivity to normal and leukemic cells distinct from other reported MoAbs and may be useful for the characterization of certain malignant B cells, either for clinical purposes or for the staging of leukemic cells.

B. Methods and Materials

I. Leukemic Cells

All leukemic cell samples were obtained at the onset of disease from patients referred to the Veterans General Hospital (VGH) in Taipei. Initial diagnosis was based upon the morphology and cytochemistry of cells from bone marrow (BM) aspiration. All samples were first separated on Ficoll-Hypaque gradient and further characterized by differentiation-linked markers. The cells were either used within 24 h after collection or cryopreserved in liquid nitrogen until use. The different types of leukemia/lymphoma cell lines listed in Table 1 were kind gifts of Dr. J. Minowada [8] and were cultured in RPMI 1640 medium supplemented with 10% FBS and antibiotics at 37 °C in 5% CO_2.

II. Normal Cells From Various Sources

Blood and bone marrow cells were donated by healthy volunteers. Thymic biopsies and tonsil cells were obtained from children undergoing cardiovascular surgery and tonsillectomy respectively. In some experiments, leukemic cells were separated into B-lymphocytes by a sequential method of filtration through a nylon-wool column, as described by Aota et al. [9]. Red blood cells, platelets, and granulocytes were obtained from normal blood donors using an IBM 2991 separator. Monocytes were separated by a method previously described [10].

III. Hybridomas

Female balb/c mice 8–10 weeks old were immunized i.p. with 1×10^7 leukemic cells from patients with B-cell CLL, and 3 weeks after the first injection the mice were boosted once

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with more of the same cells; fusion was carried out 4 days later using the method of Köhler and Milstein [11]. Mouse spleen cells and NS-1 myeloma cells were induced to fuse in a 50% polyethlene-glycol solution and then cultured in HAT selection medium. After about 4 weeks in culture, supernatants from HAT-resistant cultures were tested for the presence of antibodies reactive to the eliciting CLL cells, leukemic cell lines, and primary leukemic cells by immunofluorescence and immunoperoxidase assays.

IV. Radioimmunoprecipitation

Daudi cells (5×10^7 cells/ml) were surface labeled with 0.5 mCi ^{125}I by the lactoperoxidase technique as described by Lebien et al. [12]. The labeled cells were lysed and added to an equal volume of MoAbs and then incubated overnight at 4 °C. The immune complex formed was adsorbed onto protein A-sepharose CL-4B, and the immunoprecipitated proteins were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), followed by autoradiography.

C. Results

I. Establishment of MoAbs

Three MoAbs, designated A01, B05, and C11, were obtained from three different clones of cells from a successful fusion experiment. The MoAbs were prepared by immunization against cells from a 39-year-old female patient with B-CLL. The patient was admitted to the VGH in February, 1984, with an initial PB cell count of $12 \times 10^4/mm^3$, 90% of which were sIg^+ and Ia^+ lymphoid cells. Clones that secreted the MoAbs had been maintained in vitro for over a year with no apparent change in growth characteristics and properties. All MoAbs were of the IgG_1 subclass with kappa light chain, as revealed by immunodiffusion.

II. Reactivity to Hematopoietic Cell Lines

Sixteen different established cell lines of various types of leukemia/lymphoma, as listed in Table 1, were examined for their reactivity to MoAbs A01, B05, and C11, and, as shown in the same Table, all three

Table 1. Reactivity of A01, B05, and C11 MoAbs with leukemia/lymphoma cell lines

Cell line	Origin	Compartment at differentiation stage	A01	B05	C11
T cell lines					
Molt-3	ALL	T-blast	–	–	–
CCRF-CEM	ALL	T-blast	–	–	–
B cell lines					
RPMI 6410	ALL	B-blast	–	–	–
BALL	ALL	B-blast	–	–	–
Daudi	BL	B-blast	+	+	+
Nalm-6	ALL	B-blast	–	–	–
Nalmava	ALL	B-blast	–	–	–
SA	ALL	B-blast	–	–	–
Non-T, non cell lines					
NALL	ALL	N-blast	–	–	–
HL-60	APL	Promyelocyte	–	–	–
K562	CML in ABC	Pre-Ery	–	–	–
U-937	Lymphoma	Histiocyte	–	–	–
Kg-1	AML	Myeloblast	–	–	–
CTV-2	AMOL	Monoblast	–	–	–

MoAbs reacted with the B lymphoma cell line Daudi but not with any other cell lines.

III. Reactivity to Normal Hematopoietic Cells

MoAbs A01, B05, and C11 did not react with peripheral blood lymphocytes (PBL), transformed lymphocytes, granulocytes, monocytes, platelets, RBCs, or thymocytes, and only the C11 MoAb reacted with 3%–9% of tonsil cells. The reactivity of the C11 MoAb to tonsil cells was confirmed by immunoperoxidase staining of frozen sections of the tonsil, which showed that a few cells in the follicular center and the intrafollicular areas were reactive to the C11 MoAb but not to A01 or B05 (data not shown).

IV. Reactivity to Leukemic Cells

Specificity to the A01, B05, and C11 MoAbs was tested against a total of 125 cases of various types of leukemia and lymphoma.

Table 2. Reactivity of A01, B05, and C11 MoAbs to cells from patients with different leukemias and lymphomas

	A01 (%)	B05 (%)	C11 (%)
ALL			
cALL	12/36 (33.3)	9/26 (34.6)	5/27 (18.5)
TALL	0/4	0/5	0/3
NALL	1/10 (10.0)	1/8 (12.5)	1/5 (20.0)
CLL			
B	14/16 (87.5)	14/15 (93.3)	15/18 (83.3)
T	0/1	0/1	0/1
Myeloma	0/1	0/1	0/1
AML	0/24	0/23	1/24 (4.17)
AMOL	0/14	0/13	3/19 (15.8)
CML	0/1	0/1	0/1
CML in ABC	0/3	0/2	0/1
B-Lymphoma	1/8 (12.5)	0/5	0/4

Table 3. Reactivity of A01, B05, and C11 MoAbs to cells from selected cALL patients

Patient no.	MoAbs				
	A01	B05	C11	cALL	Ia
1	90	90	90	90	90
2	99	90	0	95	90
3	99	99	0	90	90
4	80	80	5	72	74
5	99	0	90	99	90
6	90	5	80	90	90
7	90	0	95	95	90
8	70	0	65	75	65
9	90	0	0	85	75
10	98	0	0	98	95
11	0	80	0	85	80
12	0	80	2	90	85
13	0	80	0	85	80
14	0	60	0	75	68

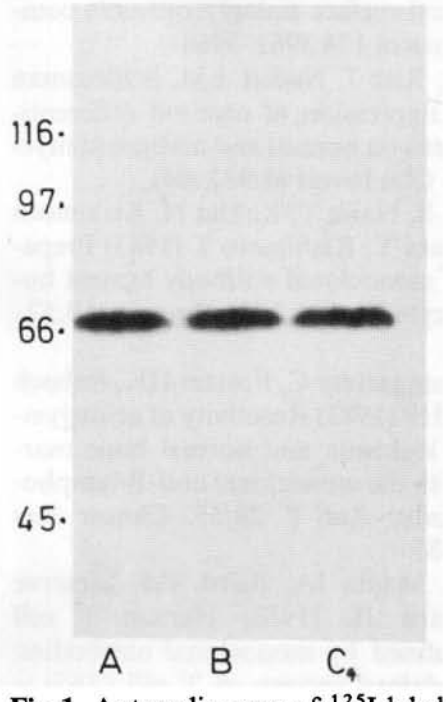

Fig. 1. Autoradiogram of ^{125}I-labeled Daudi cell membrane proteins immunoprecipitated by A01 (*A*), B05 (*B*) and C11 (*C*) monoclonal antibodies. The markers, in descending order, were B-galactosidase (116 K), phosphorylase B (97 K), bovine serum albumin (66 K) and ovalbumin (45 K)

The results (Table 2) showed that most of our B-CLL cells (83%–93%) were reactive to all three MoAbs, and 12% of our B-lymphoma cells were positive for the A01 reactive antigen. Furthermore, 18%–35% of cells from our cALL patients also reacted with one or all of the MoAbs (Table 2). In addition, MoAb C11 reacted with 4% of AML and 16% of AMOL cells (Table 2). Among the positive cALL cases, 14 were selected for more detailed studies. Table 3 shows that cells from only one cALL patient reacted with all three MoAbs, cells from four patients with only one MoAb, and cells from the remaining nine patients with two types of MoAbs. These results suggest that the MoAbs are of different clonal origins and that all three MoAbs are selective for B-lymphocytes.

V. Characterization of the MoAb Reactive Antigen

In order to determine the molecular weight of the antigen(s) reactive to MoAbs A01, B05, and C11, cell extracts were prepared from surface radiolabeled Daudi cells, mixed with the MoAbs, and sorted out by immunoprecipitation with protein A-Sepharose beads. As shown in Fig. 1, all three MoAbs immunoprecipitated a single protein with an approximate mol.wt. of 66. This suggests that all three MoAbs react with the same surface antigen but presumably with different determinants on Daudi cells.

D. Discussion

This report describes the establishment of three MoAbs, A01, B05, and C11, against cells from a patient with B-CLL. These MoAbs were selected after repeated cloning of hybridomas from different culture wells. All three MoAbs reacted selectively with the B-lymphoma cell line Daudi but not with other tested cell lines (Table 1). This suggests that the MoAbs recognize a unique surface antigen present only on certain B cells. Furthermore, it appears that the antigen reactive to A01, B05, and C11 is not present on normal lymphoid cells, since, with the exception of a few C11-reactive cells in the tonsil, the MoAbs do not react with cells of normal lymphoid tissues (data not shown). Caligaris-Cappio et al. [13] have recently identified an infrequent B-lymphocyte subpopulation in normal tonsil and lymph nodes which carries a 65-K cell-surface determinant. Coincidently, the antigen recognized by C11 as well as by the other two MoAbs has a similar mol.wt. (Fig. 1). Therefore, one might speculate that the few C11-positive cells in the tonsil may be the infrequent B-lymphocytes that are presumably distinct from conventional B-lymphocytes.

When tested against different leukemia and lymphoma cells, the three MoAbs showed distinct patterns of reactivity. The B05 is most selective, in that it recognizes mostly the B-CLL cells and some cALL cells; the A01 reacts with some B-lymphoma cells in addition to B-CLL cells, while the C11 exhibits a more diverse pattern of reactivity, as it can react with both AML (4%) and AMOL (15%) cells other than malignant B cells (Table 2). Furthermore, the three MoAbs react differently to cALL cells from different patients (Table 3). These re-

sults strongly suggest that the MoAbs have different clonal origins and identify antigenic determinants present mostly on certain neoplastic B cells or their putative precursors, which may be very heterogeneous in antigenicity. Although, the three MoAbs are obviously distinct from one another, they have many properties in common. Thus, all three react mostly with B-CLL cells and some cALL cells, and they all immunoprecipitate a single surface antigen from Daudi cells with a mol.wt. of about 66 (Fig. 1). Based on these results, it is very likely that A01, B05, and C11 all recognize the same antigen but at different determinant sites.

The MoAbs reported on in the present study are distinct from those described by other investigators. Thus, the B1 [14], B2 [15], T101 [7, 16], Y 29/55 [6, 17] and BA-1 [18] all recognize malignant B cells, but they also react with normal T or B cells from certain tissues. In conclusion, we have been able to establish three MoAbs which react selectively with B-CLL and some cALL cells but not with lymphocytes from normal tissues. These MoAbs may have unique diagnostic value, in that they can distinguish neoplastic from normal B cells, and may be powerful tools in identifying a small number of abnormal B cells in the peripheral blood, either following chemotherapy or at the subleukemic stage of ongoing diseases. Finally, the A01, B05, and C11 MoAbs may also be useful in distinguishing B-cell leukemia from B-cell lymphoma cells since they all react mostly with leukemic cells.

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