ANTIBODY PRODUCING HUMAN-HUMAN HYBRIDOMAS

II. Derivation and Characterization of an Antibody Specific for Human Leukemia Cells

By LENNART OLSSON*, RINETTE B. ANDREASEN*, ÅKE OST‡, BIRGER CHRISTENSEN‡, AND PETER BIBERFELD‡

From the Cancer Biology Laboratory, State University Hospital, DK-2100 Copenhagen, Denmark; and Department of Pathology, Karolinska Hospital, Stockholm, Sweden‡*

Monoclonal antibodies (Mab)[1] against cell surface constituents have the potential to become powerful tools in both diagnosis and therapy of malignant neoplastic diseases (1, 2) and have already proven so in leukemias and lymphomas (3–5). Most of the reported Mab against human malignant cells are of murine or rat origin (6, 7). The rodent hybridoma system has the advantage of high fusion frequencies, high antibody production, and access to optimally antigen primed B lymphocytes. However, it is conceivable that the antigenic spectrum recognized across a xenogeneic barrier (e.g., mouse-recognizing human antigens) is more narrow than across an allogeneic barrier.

The importance of human monoclonal antibody technology in cancer biology is based on the possibility, by this technique of addressing the long prevailing question (8) in tumor immunology: Do human beings recognize and react against specific antigens on autochthonous tumors? Analysis of the autologous tumor reactivity of sera from patients with melanoma (9–11), astrocytoma (12), renal cancer (13), and leukemia (14) revealed three classes of antigens. Some antigens are expressed only on autologous tumor cells, some on autologous *and* allogeneic tumors and on a restricted range of normal cells, and some are distributed on a broad range of normal and malignant cells. It is the first two types of antigens that are of most importance in tumor immunology, because antibodies against these antigens can be expected to have value in several areas of cancer biology and perhaps also in the clinical management of neoplastic diseases.

Human hybridoma technology was recently used to study the humoral immune reactions of melanoma patients against their autologous tumors (15). A number of human Mab were obtained. Most reacted with intracytoplasmatic structures of the malignant cells.

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[1] *Abbreviations used in this paper:* Ab, antibody; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; DMSO, dimethylsulfoxide; EBV, Epstein-Barr virus; ELISA, enzyme-linked immunosorbent assay; FAB, French-American-British; FACS, fluorescence-activated cell sorter; FCS, fetal calf serum; FITC, fluorescein isothiocyanate; HAT, hypoxanthine-aminopterin-thymidine; HT, hypoxanthine-thymidine; Ig, immunoglobulin; Mab, monoclonal antibodies; O.D., optical density; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline; PEG, polyethylene glycol; PMC, peripheral mononuclear cells; PWM, pokeweed mitogen.

We here report the derivation and characterization of a monoclonal human-human hybridoma antibody with high specificity for human leukemia cells.

Materials and Methods

Fusion Partners and Fusion Procedures. Peripheral blood mononuclear cells (PMC) from seven patients with acute myeloid leukemia (AML) in clinical remission were used in nine fusion experiments. PMC from each patient were separated from erythrocytes, granulocytes, and dead cells on a Ficoll-hypaque gradient and subsequently stimulated with pokeweed mitogen (PWM; Gibco Laboratories, Grand Island, NY; final dilution 2.5 μg/10^6 cells/ml) in RPMI-1640 medium with 10% fetal calf serum (FCS) and supplemented with 10^{-4} M hypoxanthine, 10^{-7} M aminopterin, and 10^{-6} M thymidine (HAT medium). Dead cells and debris were removed from the cultures by centrifugation on Ficoll-hypaque 5 d after addition of PWM, viable cells were washed twice in phosphate-buffered saline (PBS), and 1–5 $\times$ 10^7 cells fused with the malignant fusion partner at a cell ratio of 1:1. The malignant fusion partner was in all experiments a human B lymphoma, designated RH-L4, that produces, but does not secrete IgG(k) (15). Fusion procedures have been described in more detail elsewhere (15). Briefly, lymphocytes and lymphoma cells were fused in 0.2 ml 30% (wt/vol) polyethylene glycol (mol wt 1,000; J. T. Baker Chemical Co., Phillipsburg, NJ), washed twice in RPMI-1640 medium and seeded into 96-well plates at a concentration of 2–4 $\times$ 10^5 cells per well in RPMI-1640 medium with 10% FCS. Most of the culture medium was replaced with HAT medium 1–2 d later. The cells were grown for 14–18 d in HAT medium that eventually was replaced by HT medium. Further cultivation and expansion of a number of human hybridomas indicated that highest stability and growth rate was obtained by maintenance of the cultures in HT medium instead of RPMI-1640 medium. The hybridoma cell line designated aml-18 has now been maintained in culture for 24 months and still grows best in HT medium.

Antibody Assays. All supernatants were analyzed for human μ-, γ-, κ-, and λ-chains by enzyme-linked immunosorbent assay (ELISA) and for reactivity to AML cells by cell binding ELISA (16). The latter assay was done with a mixture of AML cells from eight different patients. The cells were seeded into 96-well plates, precoated with poly-L-lysin, with 2 $\times$ 10^5 cells per well. Alkaline phosphatase-conjugated rabbit anti-human Ig final dilution 1:2,000; (DAKO, Denmark) was used as second step reagent. O.D. values were <0.150 for negative controls in all experiments. O.D. values of 0.300–0.500 were considered weakly (+) positive, values between 0.500–1.000 were rather strongly (++) positive, and O.D. values >1.000 indicated very strong (+++) reactivity. Controls included cells incubated with (*a*) only RPMI-1640 medium, and (*b*) with only second step antibody (peroxidase-conjugated rabbit anti-human Ig). The highest O.D. values from these groups were used as control values. Moreover, binding to cell surface components on viable AML cells was analyzed by fluorescence-activated cell sorter (FACS). The 2 $\times$ 10^6 AML cells were incubated with 0.2 μl Ig-containing supernatant (diluted 1:1 in PBS) for 45 min at 4°C, washed twice in PBS, and incubated in 0.1 μl FITC-conjugated rabbit anti-human Ig (final dilution 1:20–1:50, Tago, Burlingame, CA) for 30 min at 4°C. The cells were finally washed three times in PBS and analyzed. A 25–50 channel shift in fluorescence on a linear scale was considered weak (+) reactivity, 50–100 channel shift indicated rather strong (++) reactivity, and a shift >100 channels was considered very strong (+++) reactivity. Controls included unstained cells and cells only incubated with FITC-conjugated rabbit anti-human Ig. The latter (the highest fluorescence signal) was used as a control.

Hybridoma cultures secreting Ig with high reactivity to AML cells were cloned by limiting dilution in 96-well plastic plates with human monocytes as feeder cells in a concentration of 10^4–5 $\times$ 10^4 per well. Procedures for isolation of monocyte feeders and details of cloning were described recently (17). Supernatants from cloned hybridoma cultures were screened for Ig content and reactivity to AML cells by the same procedures as the initial culture fluids. Hybridomas that secreted Ig with reactivity to AML cell surface constituents were subsequently tested for reactivity against lymphocytes, mono-

cytes, and erythrocytes of the patient, who provided the lymphocytes for fusion. Lack of binding to these cells resulted in further expansion of the culture that subsequently was analyzed for reactivity against a large panel of normal and malignant cells of both human and animal origin.

Cell Panel to Define Antibody Specificity. Human tumor cell lines: HL-60 (promyelocytic leukemia; 18), K_{562} (erythroleukemia; 19), U-937 (histiocytic lymphoma; 20), RH-L4, (B lymphoma; 15), RH-SLC-L11 (squamous cell lung carcinoma; 21), RH-SCC-L10 (small cell carcinoma; 21), U-266 (human myeloma; 22), Molt-4 (T cell leukemia; 23), DAUDI (Burkitt lymphoma; 24), and human foreskin fibroblasts. All lines were maintained in RPMI-1640 medium with 10–15% FCS and 0.3% L-glutamine. Other human cell types included granulocytes, monocytes, lymphocytes, normal bone marrow aspirates, erythrocytes, and thymocytes (prepared from thymus biopsies of patients undergoing cardiac surgery). Adherent cell lines were prepared for analysis as follows: the cells were detached from the plastic by trypsinization for 10–15 min at 37°C, washed twice in PBS, and cultivated for 24–48 h in RPMI-1640 with 10% FCS in a plastic vessel placed on a rocking platform, thereby preventing reattachment of the cells, but allowing the cells to regain the cell surface attributes they had before trypsinization.

Leukemia Samples. Peripheral blood from patients with cytologically verified acute leukemia was obtained before onset of cytoreductive treatment and classified according to the French-American-British (FAB) classification (25). PMC from samples with leukemia cells were purified by centrifugation on a Ficoll-hypaque gradient, frozen (minus 1°C per minute) in RPMI-1640 medium with 30% FCS and 10% DMSO at a concentration of 2 × 10^7 cells/ml and stored in liquid nitrogen. Cell viability was 80–90% as judged by trypan blue dye exclusion. Differentials of thawed cells were comparable to those before freezing. A total of 63 different leukemia samples were analyzed. AML cell samples often contained cells that varied largely in size, which is reflected in the scatter signal on the FACS. Analysis was done for each sample on medium (~10–15 μm) and large (15–30 μm) sized cells by gating on first the medium-sized cells and subsequently the large cells (Fig. 1). The content of leukemia cells in each of these two fractions was estimated from May-Grünwald-Giemsa stained cytocentrifuge preparations of cells that were isolated from each fraction by FACS. This procedure was only applied in samples with <70% leukemia cells.

Complement-dependent Antibody-mediated Cytotoxicity Assay. One human monoclonal antibody (aml-18) was assayed in a conventional microcytotoxicity assay (26) using 1 μl of antibody, 10^3 target cells suspended in 1 μl RPMI-1640, and 1 μl complement. The complement had to be selected carefully, as most conventional rabbit complement sources

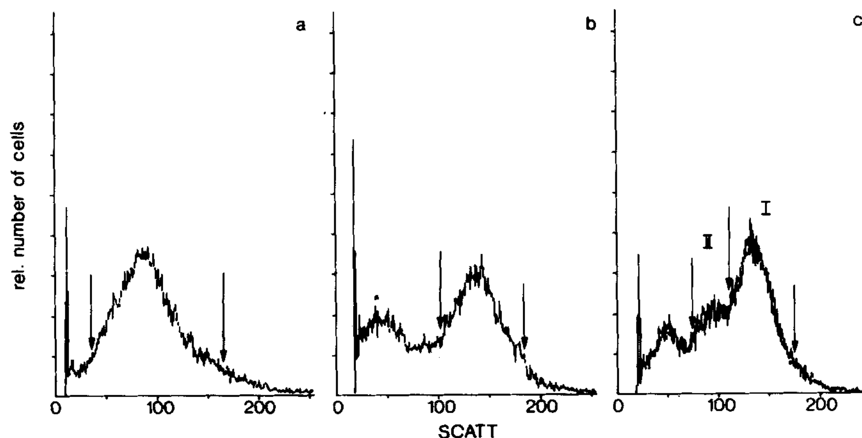

FIGURE 1. Typical scatter signal profiles of AML cell populations. Bars indicate gates for medium (*II*) and large (*I*) cells in the populations with more than one type of cells.

result in nonspecific cytolysis. However, after examination of a number of different types of complement, a mixture of ⅓ rabbit serum and ⅔ human AB-serum was found to be applicable. Antibody absorption with cells was performed as described by others (9).

Antibody Characterization. The monoclonality of the hybridoma cultures was assured by 2D-analysis of [^{35}S]methionine-labeled Ig products from the hybridomas. 2×10^6 cells were cultivated for 18 h in RPMI-1640 medium (without methionine), but supplemented with 50 μCi [^{35}S]methionine. Supernatants were harvested and Ig products precipitated with rabbit anti-human Ig, and analyzed by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis, or by isoelectric focusing followed by SDS (2D-electrophoresis) (27). The isotype of the Mab was determined by ELISA (16) with antibodies with specificity for the heavy (μ, γ) and light (κ, λ) chains of human Ig. Fab-fragments were prepared by brief papain digestion (28), isolation on a Sephadex G-100 and freed from contamination of Fc-fragments and intact IgG by passage through a protein A-Sepharose column. The Fab-fragments were FITC conjugated by standard procedures.

Results

Fusion Frequency, Hybridoma Yield, and Initial Screening. Nine fusions with PMC from patients with AML in partial or complete remission resulted in 521 wells with hybridoma growth out of 1436 wells seeded (Table I). Ig production was detected in 305 of the 521 wells, but only 26 wells contained supernatants that reacted with AML cells. Specificity analysis showed (Table II) that only five

TABLE I

Hybridoma Yield, and Ig Production in Nine Fusions with RH-L4 Lymphoma Cells with Peripheral Monoclonal Cells from AML Patients in Partial or Complete Remission

No fusions with hybrid growth*/Total no. fusions	No. wells with hybrids/total no. wells	No. wells with IgG production/Total no. wells with hybrids	No. wells with IgM production/Total no. wells with hybrids	No. supernatants with Ig-binding to AML cells/Total no. wells with Ig
7/9	43/164; 97/140; 55/210; 82/278; 110/280; 105/244; 29/120;	18/43; 37/97; 40/55; 42/82; 38/110; 35/105; 11/29;	10/43; 17/97; 6/55; 12/82; 15/110; 17/105; 7/20;	2/28; 5/54; 7/46; 0/42; 1/38; 11/35; 09/28;

* No. wells with hybrid growth and Ig production was scored ~30 d and ~50 d after fusion. The maximal values of these two enumerations are indicated.

TABLE II

Reactivity Pattern of 26 Ig-Containing Supernatants that Bind to AML Cells as Assayed by ELISA and/or FACS

No. supernatants reactive with PMC from the patient from whom lymphocytes for fusion were obtained*	No. supernatants reactive with PMC from normal individuals‡	No. supernatants reactive with foreskin fibroblasts	No. supernatants reactive with malignant cell lines§	No. supernatants reactive with only AML samples
6	11	3	16	5

* The reactivity of the supernatants was analyzed 60–90 d after fusion. Both ELISA and FACS analysis was used to assess the reactivity pattern. The numbers indicate positive reaction in one or both of these two types of analysis.

‡ Tested on PBL from 10 individuals.

§ The cell lines were: RH-L4, SCC-L10, SCL-L11.

hybridoma cultures bound with high specificity to AML cells. However, upon continuous expansion of these five cultures, only one proved stable both in growth properties and Ig production. The four unstable cultures all lost Ig production activity and subsequently growth activity. The fifth hybridoma culture, designated aml-18, has now been stable for >2 years for growth, but rather frequent recloning (every 3–4 months) has been necessary to maintain Ig production.

Characteristics of the Aml-18 Antibody. The aml-18 hybridoma cells secreted the Ig products (γ, κ) of the parental RH-L4 lymphoma and a new γ-chain and κ-light chain. 5–15 μg Ig of which <1 μg is of parental RH-L4 Ig type are produced by 10^6 cells per 24 h. Some of the Ig molecules are permutated, but ~80% of the Ig in the supernatant was the Mab of interest. The aml-18 antibody binds complement and can be used in a cytotoxicity assay. Aml-18 hybridoma cells were injected subcutaneously into nude mice (5–10 × 10^6 cells) mixed with 2 × 10^6 human foreskin fibroblasts. 6 of 10 mice got subcutaneous tumors of which 4 were successfully converted into ascites tumors upon 2–5 passages into other nude mice. However, ascites from three of these tumors did not have significantly increased antibody titer compared with culture supernatants. The fourth tumor gave in some aspirates higher titers, but not in a predictable and reproducible way. Further attempts to grow aml-18 cells as ascites tumors were therefore abandoned.

Antibody Specificity. Further details of the results of the fusion from which aml-18 developed are outlined in Table III. 7 of the 11 hybrid supernatants that initially reacted with AML cells were not positive in the first ELISA test for Ig production. This reinforces the insufficiency of an assay for Ig production as the only basis for initial selection of hybrids. Screening on a panel of normal cells (Table IV) revealed that 6 of the 11 hybrids could be of potential interest and were subsequently cloned. Further screening of the cloned cultures clearly indicated that only the aml-18 hybridoma had high specificity for AML cells (Table V).

Analysis of aml-18 reactivity against a panel of cells (Table V) showed that the antibody is highly specific for human leukemia cells. Weak binding was found to normal human bone marrow cells, when undiluted supernatants were used. This reactivity was eliminated in a titer 1:8 (Fig. 2) that gave binding to human leukemia cells. Moreover, Fab fragments of the aml-18 antibody did not bind to

TABLE III

Further Details of Screening Results from the Fusion from Which the aml-18 was Obtained

	No. supernatants with Ig	No. supernatants reacting with AML cells	No. supernatants with reactivity to AML cells/positive for Ig content	Ig-type of 11 supernatants reacting with AML cells
3 wk after fusion	19	2	2/1	IgG: 8
6 wk after fusion	52	11	11/4	IgM: 3

Ig production and Ig subtypes were determined by ELISA. Reactivity with AML cells was determined by ELISA and FACS analysis.

TABLE IV

Reactivity to a Panel of Nonmalignant Cells of 11 Hybrid Supernatants that Reacted with AML Cells

Cell type*	Hybrids										
	AML-3	AML-5	AML-11	AML-17	AML-18	AML-23	AML-24	AML-32	AML-36	AML-41	AML-60
PMC	+[‡]	Neg	Neg	+	Neg	+	+	+	+	Neg	+
Erythrocytes	Neg	+	Neg	+	Neg	Neg	+	Neg	Neg	+	+
Fibroblasts	Neg	Neg	+	+	Neg	+	+	Neg	+	Neg	+
Bone marrow	+	+	Neg	+	Neg	+	+	+	+	+	+
Thymocytes	Neg	Neg	Neg	Neg	Neg	+	+	+	Neg	+	+
Platelets	+	Neg	+	Neg	Neg	Neg	+	Neg	+	+	Neg
Mouse spleen mononuclear cells	Neg	Neg	+	Neg	Neg	Neg	Neg	+	+	Neg	+

* All cells were analyzed with ELISA and FACS for reactivity with supernatants, except for platelets that were analyzed only by FACS.

‡ + indicates significant binding of the antibody as detected by ELISA and/or FACS. O.D. >0.300 was considered significantly increased in the ELISA (background <0.150), and a fluorescence channel shift >25 as compared with cells only incubated with FITC-conjugated second step antibody in FACS analysis.

TABLE V

Reactivity Against a Panel of Normal and Malignant Cells of Ig in Supernatant from the Cloned aml-18 Human Hybridoma

Cell type*	aml-18 antibody dilution		Fab − FITC	
	1:2	1:8	1:2	1:8
Human				
AML	++[‡]	++	++	++
ALL	+[‡]	Neg	+	Neg
RH-SCC-L10	Neg	Neg	Neg	Neg
RH-SCL-L11	Neg	Neg	Neg	Neg
HL-60	++	++	++	++
RH-L4	Neg	Neg	Neg	Neg
K_{562}	+	Neg	+	Neg
DAUDI	Neg	Neg	Neg	Neg
U-266	Neg	Neg	Neg	Neg
U-937	Neg	Neg	Neg	Neg
Molt-3	Neg	Neg	Neg	Neg
Fibroblasts	Neg	Neg	Neg	Neg
Erythrocytes	Neg	Neg	Neg	Neg
PMC	Neg	Neg	Neg	Neg
Granulocytes	(+)[§]	Neg	Neg	Neg
Normal bone marrow	(+)	Neg	Neg	Neg
Platelets	Neg	Neg	Neg	Neg
Mouse				
Spleen cells	Neg	Neg	Neg	Neg
AKR leukemia cells	Neg	Neg	Neg	Neg
Fibroblasts	Neg	Neg	Neg	Neg
Bone marrow	Neg	Neg	Neg	Neg

* Analyzed by ELISA and FACS, except platelets that only were analyzed by ELISA.

‡ + and ++ indicate significant binding as detected by ELISA and/or FACS.

§ (+) indicates O.D. values of 0.200–0.300 in ELISA and/or a channel shift on 10–25 on the fluorescence signal.

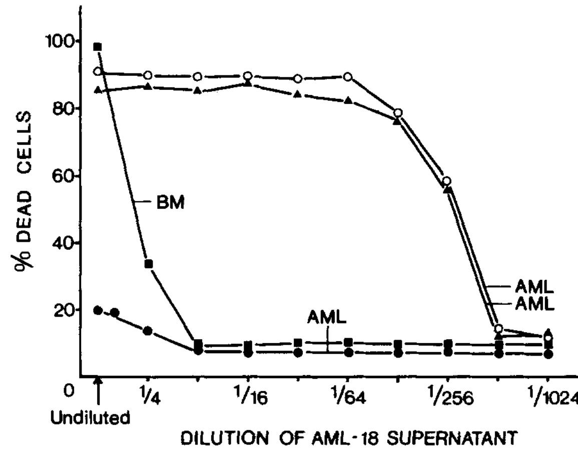

FIGURE 2. Cytotoxic activity of aml-18 hybridoma antibody on an AML cell population with >90% myeloid leukemia cells and on normal myeloid bone marrow cells; (○) aml-18 Ab against AML cells (■) aml-18 Ab against normal bone marrow cells; (▲) aml-18 Ab absorbed with normal bone marrow cells (BM); (●) aml-18 Ab absorbed with myeloid leukemia cells.

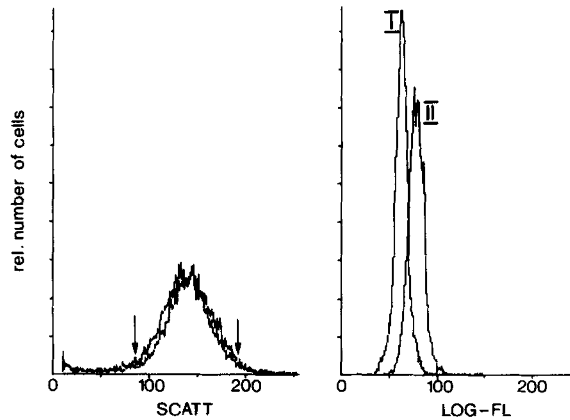

FIGURE 3. FACS analysis of an AML sample (>80% AML cells) stained with aml-18 Ab in a dilution of 1/16. Scatter signal is seen on left histogram and the gating indicated by markers. Fluorescence signal (right histogram) shows control sample (*I*; stained only with FITC-conjugated rabbit anti-human Ig in a dilution 1:20) and the sample stained with aml-18 Ab and subsequently FITC-conjugated rabbit anti-human Ig (*II*).

normal bone marrow cells (Table V). Fig. 3 shows the size of the FACS fluorescence signal that is typically obtained by aml-18 (diluted 1:16), when it binds to AML cells.

63 leukemia samples were analyzed with aml-18 and the reactivity pattern related to the FAB classification of these leukemias (Table VI). 24 samples (40%) were positive, including two of four acute lymphoblastic leukemia (ALL) samples. No correlation in frequency of positive samples and the FAB classification was seen. No morphological differences were seen between cells that bind the aml-18 antibody compared to those that were negative for the antibody. Estimation of the percentage of positive cells within each of the 24 leukemia samples revealed

TABLE VI

Reactivity Pattern of HuMab-aml-18 to a Panel of Leukemia Cell Samples

Types of leukemias*	No samples reactive with aml-18 mab/Total no. samples
AML-M1	3/7
AML-M2	8/21
AML-M3	—
AML-M4	5/9
AML-M5	6/17
ALL	2/4
AUL	0/2
AL, total	24/60
Others	
CMML	0/1
PCL	0/1
L1 + M4	0/1
Total	24/63

* ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; AUL, acute unclassifiable leukemia; CMML, chronic myelomonocytic leukemia; PCL, plasma cell leukemia; L1 + M4, mixed type of leukemia.

TABLE VII

Percentage of Leukemia Cells Reactive with aml-18 in Positive AML Samples

Leukemia type	Percentage of reactive cells*	No. of reactive samples		
		+	++	+++
	%			
AML-M1 (Total:7)				
Mean	22	2	5	
Range	11–43			
AML-M2 (Total:21)				
Mean	44	9	10	2
Range	21–78			
AML-M4 (Total:9)				
Mean	50	5	4	
Range	26–86			
AML-M5 (Total:17)				
Mean	33	3	13	1
Range	15–53			
ALL (Total:4)				
Mean	37	2		
Range	10–64			

* Taken from the gated population with most leukemia cells (if more than one).

‡ Reactivity grading as described in Materials and Methods.

a pronounced intratumoral heterogeneity with many leukemia cells being negative for the aml-18 epitope within the leukemia cell populations that were scored positive for aml-18 (Table VII).

Discussion

Immortalization of human B lymphocytes by fusion with human malignant lymphoma cells/myeloma cells/lymphoblastoid cells, EBV transformation or fusion with mouse myeloma cells are all feasible approaches to produce human Mab (16, 29–35). Each of these techniques all have drawbacks like chromosomal instability, low Ig secretion, and low hybrid yield, which are some of the main reasons why production of human Mab still is a more laborious and tedious process than the mouse hybridoma antibody production. In addition, in vitro antigen-priming systems are required for a broad applicability of the human hybridoma technology, but these techniques are not yet developed to a stage that allows both primary and secondary immune response in vitro to a broad and predefined antigenic spectrum.

The possibility of dissecting the antigenic repertoire recognized by human B cells on different biological components, including human tumors, is one of the major biological aspects of human monoclonal antibody technology. In the case of human tumors, it allows one of the basic questions in cancer immunology to be addressed, whether human malignant cells express antigens that are recognized as foreign by the autologous host (8). A few studies on human Mab reactive with antigens on tumor cells have been reported (15, 36–38), but none of these antigens seems specific for neoplastic cells. It was further found in one of these studies (15) that melanoma patients mostly react with intracellular structures, rather than cell surface constituents. This probably reflects the high and abnormal cell decay rate of malignant tumors, which exposes the autologous host to a number of intracellular structures that are not normally encountered by the immune system of healthy persons.

Mab are already used in diagnosis of human acute lymphoid leukemias. A similar approach has not yet been successful in AML because attempts to generate Mab against defined subsets of myeloid cells have encountered considerable difficulties. Mouse Mab with high specificity for myeloid cells seem almost invariably also to bind to monocytes (or subsets) (39–41), and this hampers the use of such Mab as diagnostic tools in AML. The possibility that some human B lymphocvtes in AML patients recognize antigens that are expressed on some or all the leukemia cells, but not on the normal cell populations in the patients was the logistic background for our attempts to generate human Mab, because immortalization of such lymphocytes could lead to generation of Mab with high specificity for leukemia cells.

PMC from AML patients were used as B lymphocyte source in all experiments. The patients were in partial or complete remission, because the hybrid yield was found to be extremely low, when PMC from patients with circulating leukemia cells were used. However, the hybrid yield was also very low with PMC from patients in remission compared to a mouse-mouse fusion with optimally antigen-primed lymphocytes. Cocultivation in vitro of PMC with AML cells for 3–10 d did not result in improved hybrid yield, rather the opposite (data not shown).

Thus, only one hybridoma (aml-18) out of 305 had acceptable specificity for leukemia cells, which clearly indicates the urgent need for improved in vitro antigen-priming systems.

The aml-18 Mab bound to ~38% of the leukemia samples that were analyzed, but not to a variety of other malignant or normal human cells. The weak reaction with normal bone marrow could easily be eliminated by titration. As FITC-conjugated Fab fragments of the aml-18 antibody did not bind to normal cells, we consider the weak binding of aml-18 antibody to be nonspecific reactivity. This is substantiated by recent experiments on the characteristics and modulation pattern of the antigen recognized by the aml-18 antibody (Olsson et al., in preparation). These experiments indicate that the antigen is a cellular protein with a M_r ~58,000, and Western-blotting experiments have failed to demonstrate its presence on 11 samples of normal bone marrow cells. It is also of interest that the aml-18 Mab does not bind to murine malignant cells, including mouse leukemia. The antibody is not specific for AML, as two samples of ALL also were positive. The diagnosis of ALL was primarily based on cytology. All AML were FAB classified, and no correlation between FAB classification and aml-18 reactivity was seen. Fluorescence microscopy showed that the antigen can be detected only on the cell surface (ring fluorescence).

Each individual leukemia sample was found to contain variable amounts of aml-18 positive cells. Intratumoral antigenic heterogeneity was thus pronounced in respect to the aml-18 epitope in line with previous studies on other antigens expressed on murine (42) or human (43, 44) leukemia cells and on human melanoma cells (45). The variability in antigen expression in respect to both the antigen density as evaluated by FACS analysis, and the existence of antigen-negative leukemia cells in a sample with antigen-positive cells, suggests that the leukemic cells may be phenotypically diversified in respect to a number of other features. This diversification is not reflected in the FAB classification and may be one reason for the lack of success in the many attempts to generate mouse antibodies specific for AML.

The reactivity pattern of aml-18 does not conform to any known antigen system or differentiation pathway. The biological function of the antigen is unknown, but its high specificity for leukemia cells suggests that the gene encoding for the antigen is associated with genes directly responsible for the leukemic behavior. The gene coding for the aml-18 antigen is therefore now being characterized and searched for in both leukemic and normal bone marrow cells.

Summary

Human-human hybridoma technology was used to immortalize human B lymphocytes from patients with acute myeloid leukemia (AML) to study the antigenic repertoire of the humoral immune response against the patients' own leukemia cells and against leukemic cells from other patients. Nine fusions were done with lymphocytes from seven AML patients, and all with the human RH-L4 B lymphoma line as malignant fusion partner. A total of 305 Ig-producing hybrids were obtained. 26 reacted with cell surface components on AML cells, but 21 were found not to be specific for leukemia cells, when screened for

reactivity against a panel of normal and malignant cells of both human and murine origin. Five hybridomas secreted Ig with high specificity for human leukemia cells, but only one hybridoma culture, aml-18, was stable in respect to Ig-production and growth upon repeated clonings and expansion in liquid cultures. A method was developed to grow human hybridomas as ascites tumors in nude mice, but the ascites fluid did not contain increased amount of antibody.

The reactivity of the aml-18 antibody (γ, κ) was analyzed against samples of mononuclear cells from peripheral blood of 63 patients with leukemia and with cytologically verified leukemia cells in the blood. 22 of 54 AML samples reacted with aml-18. The reactivity pattern was not correlated to any categories of the French-American-British (FAB) classification; two of four ALL were positive. Moreover, a pronounced intratumoral antigenic heterogeneity in regard to aml-18 reactivity was seen and indicates a high degree of diversity in the immunological phenotype within individual AML cell populations.

The study demonstrates that some patients with AML generate an immune response against their autologous malignant cells, and that the antigenic determinant in the case of aml-18 is also expressed specifically on leukemic cells from other patients.

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