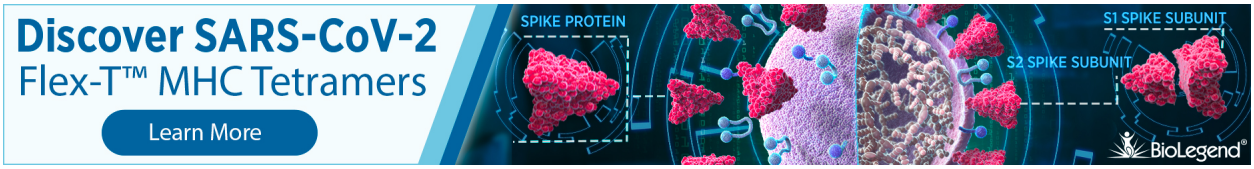

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ESTABLISHMENT AND CHARACTERIZATION OF BALB/c LYMPHOMA LINES WITH B CELL PROPERTIES

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Five spontaneously derived BALB/c tumors and one 1-ethyl-1-nitrosourea induced BALB/c tumor (BALENLM 17), have been established in culture and characterized according to their surface markers. K46, X16C, L10A, and BALENLM 17 are IgM$^+$, IgG$^-$, IgA$^-$, Ig$^+$ (staining with polyvalent anti-Ig), Ia$^+$, Fc receptor$^+$ and complement receptor$^-$. A20 is IgM$^-$, IgG$^-$, IgA$^-$, Ig$^+$, Ia$^+$, Fc receptor$^+$, and complement receptor$^-$. M12 is IgM$^-$, IgG$^-$, IgA$^-$, Ig±, Ia$^-$, Fc receptor$^+$ and complement receptor$^-$. All these lines are Thy 1.2 antigen negative, do not phagocytize latex particles, and grow as stationary suspension cultures. Therefore these six BALB/c lymphoid lines are presumably of B cell origin at various stages of differentiation. All these lines except X16C are tumorigenic in mice within a short period of time and have relatively short generation times in the range of 18 to 26 hr. Karyotype studies show that L10A, K46, X16C, and BALENLM 17 are hyperdiploid to hypotetraploid. A20 and M12 have near diploid chromosome numbers.

Humoral immune responses are mediated by bone marrow-derived lymphoid cells (B cells). These cells have been studied primarily from the aspect of immunoglobulin (Ig) synthesis and secretion after stimulation with antigen or mitogen. B cells have been characterized based upon their expression of various surface markers such as Ia antigens (1, 2), Fc receptors (3, 4), surface immunoglobulins (5–7), and complement (C) receptors (8). However, the developmental relationships among B cell subsets and the mechanism of expression of various surface markers are not fully understood. Homogeneous B lymphoma cell lines with distinct B cell markers would be ideal for such studies. Nonsecreting B-lymphomas have previously been reported in human (9–13), in cattle (14, 15), and in mouse systems (16–21), but these lymphomas have seldom been completely characterized.

In the present study we have characterized BALB/c B cell lines for surface markers such as expression of Ig, Ia antigens, Fc receptors, and C receptors. In order to confirm that the expression of Ig on these tumor cells was not due to proteins absorbed passively, we have adapted these tumors to *in vitro* culture. Tissue culture lines also had the advantage of providing a greater degree of experimental manipulability than *in vivo* tumors. Because these cell lines appear to demonstrate differential expression of B cell markers, they may prove particularly relevant to study of B cell maturation and they may also provide relatively pure sources of B cell antigens.

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MATERIALS AND METHODS

Mice. BALB/c AnN mice were obtained from the Animal Production Unit, National Institutes of Health, Bethesda, Md. and B10.A mice from The Jackson Laboratory, Bar Harbor, Maine.

Tumors. All the tumors except BALENLM[1] 17 arose as spontaneous reticulum cell neoplasms, type B, according to Dunn's classification (22) in old (>15 months) BALB/c AnN mice (unpublished observation). BALENTL 17, which was induced in a BALB/c mouse by i.p. injections of *1*-ethyl-*1*-nitrosourea (23), was the generous gift of Dr. Michael Potter, NIH. All tumors were transplanted in BALB/c mice.

Establishment of BALB/c lymphoid lines. Techniques used to adapt lymphomas into culture have been described elsewhere (24). The medium used was modified Eagle-Hanks medium (C-EHAA, 25) supplemented with 10% heat inactivated fetal calf serum (FCS), 0.135% $NaHCO_3$, 2 mM glutamine, 50 μg streptomycin, and 50 units potassium penicillin-G per ml and 5×10^{-5} M 2-mercaptoethanol. Lymphomas originating in spleen, lymph node, and/or ascites fluid were explanted into culture. Cultures were inspected and fed daily. Once the cells began to proliferate, half of the culture was transferred into dishes containing fresh medium. When tumors were difficult to adapt into culture, *in vitro-in vivo* cycling techniques were used as previously described (26). Cells from cultures that showed poor growth were injected into Pristane (2, 6, 10, 14-tetramethylpentadecane; Aldrich Chemical Co., Milwaukee, Wis.) primed mice (27). Resulting tumors were explanted *in vitro* again. This procedure was repeated if necessary. The established cell lines were frozen in liquid N_2 (28) and also kept in culture as continuous cell lines.

Immunoglobulin reagents. Fluorescein labeled anti-mouse immunoglobulin (Ig) reagents were prepared as described elsewhere (29). Briefly, antisera specific for each class of mouse immunoglobulin were prepared by injecting Fc fragments or intact myeloma proteins into goats or rabbits. Antibodies ob-

[1] Abbreviations used in this paper: ABS, absorption test; BALENLM, BALB/c *1*-ethyl-*1*-nitrosourea induced lymphoma; BSS, balanced salt solution; C-EHAA, complete modified Eagle-Hanks' medium; CT, cytotoxic titer^{-1}; EA, SRBC coded with anti-SRBC serum; EAC, EA sensitized with complement; Ia, I-region associated antigen; IF, immunofluorescence; poly anti-Ig, polyvalent anti-mouse immunoglobulin antibody; S-Ig, surface immunoglobulin.

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tained were further purified by a Sepharose 4B affinity column onto which appropriate purified immunoglobulins had been conjugated. The absorbed antibodies were eluted with weak acid. The polyvalent antiserum to mouse Ig was also prepared and purified on a column containing equal amounts of γ_1, γ_{2a} and γ_{2b} mouse myeloma proteins. The purified polyvalent antibody reacted with all classes of immunoglobulins and with κ- but not λ-light chains. $F(ab')_2$ fragments of the polyvalent reagents were made by digestion with pepsin, followed by passage through a G150 column. Purity of the reagents was determined by SDS polyacrylamide gel electrophoresis. These purified antibodies, goat anti-IgM, goat anti-IgG_1, goat anti-IgG_2, goat anti-IgA, and polyvalent goat or rabbit anti-mouse Ig, were then fluoresceinated as previously described (29) for direct immunofluorescence (IF) labeling. The resulting conjugates had molar F/P (fluorescein to protein) ratios of 2.5 to 4.5.

Antisera against H-2 and Ia antigens. The antisera and immune ascites used for the study of H-2 and Ia antigens on these BALB/c tumors were prepared as previously described (30, 31). The sera used and their activities were as follows: 1) A.SW anti-A.TH (anti-H-2, Cytotoxic $titer^{-1}$ (CT) on BALB/c spleen cells = ≥ 1,000); 2) A.TH anti-A.TL (anti-Ia, CT on BALB/c splenic B cells = 256); 3) (B10.A × A)F_1 anti-B10 (anti-Ia, CT on BALB/c splenic B cells = 32), and 4) (B10 × D2.GD)F_1 anti-B10.D2 absorbed with B10.A (anti-Ia.23, CT on BALB/c splenic B cells = 32). Reagent 1) was used for the detection of H-$2D^d$ antigens, reagent 2) was used as a polyvalent anti-Ia antiserum, reagent 3) was used to detect products of the I-A subregion (Ia.8), and reagent 4) to detect products of *I* subregions to the right of *I-A*, (specifically the I-E subregion antigen, Ia.23). Tumors were tested with these sera by indirect IF, by C-mediated cytotoxicity and by absorption of cytotoxic activity.

Immunofluorescence labeling technique. Approximately 10^6 cells were washed twice in Hanks' medium containing 10% FCS and 0.1% NaN_3 and pelleted by centrifugation at 200 × G for 10 min. For direct IF, 20 λ of fluorescent conjugated reagents (0.5 mg/ml) were added to cell pellets and the mixtures were kept on ice for 30 min. Cells were then washed twice with Hanks' medium containing FCS and NaN_3 and examined with a Leitz fluorescent scope with Ploem illuminator (HBO 100W/2 mercury lamp with BG38 and BG12 excitation filters and an S546 barrier filter). For the indirect IF, cells were first reacted with alloantiserum for 30 min in ice, washed twice, and then reacted with fluorescein-labeled goat anti-mouse IgG_1 or IgG_2 in ice for 30 min, washed twice again, and examined as for direct test.

Cytotoxicity test. Two-stage C-mediated cytotoxicity assays were carried out as described by Sachs *et al.* (32). Briefly, tumor cells or spleen cells were treated with Tris-buffered ammonium chloride to lyse residual red cells, and were washed in Medium 199 and resuspended to 5 × 10^6 cells/ml in Medium 199 containing 0.1% gelatin. Doubling dilutions of serum were made by using 25 λ diluting apparatus (Cook Engineering Co., Alexandria, Va.). Equal volumes of cell suspensions were added into each well, mixed, and incubated at 37°C for 15 min. Cells were then washed again and treated with 25λ of a 1:4 dilution of rabbit C for 30 min at 37°C. The percentage of dead cells was determined microscopically by trypan blue uptake.

Absorption test. Cells were washed twice in Medium 199 and pelleted by centrifugation at 16,000 RPM for 1 min in a Brinkman Model 3200 centrifuge. Cell pellets were mixed with appropriate volumes of antiserum and kept on ice for 30 min. Absorbed antiserum was then obtained by centrifugation at 16,000 RPM for 1 min. The activity remaining in the absorbed antiserum was determined by the cytotoxicity test.

Preparation of antiserum against SRBC. Three procedures of immunization were used in order to obtain anti-SRBC antiserum of different Ig classes. All antisera were raised in (C57BL/6 × BALB/c)F_1 male mice aged 2 to 4 months. IgG_1 class-restricted antiserum was obtained after repeated i.p. injection of 10^8 SRBC per mouse in 0.5 ml of saline every 2 weeks. IgG_1 + IgG_2 anti-SRBC antiserum was raised by one i.p. injection of 10^7 SRBC in complete Freund's adjuvant followed by two intravenous injections of 10^7 SRBC in saline. Antiserum of IgM class was the result of one i.p. immunization with 8 × 10^8 SRBC. Mice were exsanguinated on the 4th day after injection. Hemagglutination titers of IgG_1, IgG_1 + G_2, and IgM antisera were 1000, 256, and 128, respectively. The classes of the antibodies were confirmed by an indirect hemagglutination test as follows: SRBC were presensitized with a subagglutinating dose of each serum and then used in a hemagglutination assay with affinity-purified goat antibodies against different classes of mouse Ig.

Fc rosetting. The technique originally described by Parish and Hayward (33) was used. Equal volumes of an optimal dilution of anti-SRBC antiserum and of a 5% suspension of washed SRBC in PBS were incubated for 30 min at 37°C. The resulting sensitized cells (EA) were then washed three times in PBS. Finally, a 2.5% suspension of EA was made in PBS-10% FCS and kept on ice until used.

Rosetting was done in tubes (35 × 12 mm) by adding 0.3 ml of tumor or lymphoid cell suspensions (4 × 10^6 cells/ml in PBS-10% FCS) and 0.3 ml of the EA suspension. Unsensitized SRBC were used as controls. The tubes were placed on a vertical rotator for 15 min at 37°C, after which a 0.1-ml sample was removed, diluted with an equal volume of cold PBS, and immediately stored on ice for counting. Lymphocytes were stained with 2% (v/v) crystal violet, allowing clear distinction between true and false rosettes. A minimum of 200 cells was scored, and only lymphoid cells with more than four SRBC attached were counted as positive.

Complement-rosetting. SRBC were sensitized in the same manner as for the Fc rosetting, with an optimal dilution of heat-inactivated, IgM restricted anti-SRBC antiserum (33). The EA (5% suspension in PBS) was then incubated for 20 min at 37°C with a 1:8 dilution of fresh normal mouse serum, from either (C57BL/6 × BALB/c)F_1 or BALB/c mice as a C source. The cells were then washed twice in cold PBS and kept on ice at a concentration of 2.5% in PBS-10% FCS. The rest of the procedure was similar to the Fc rosetting.

Karyotype study. Chromosome counts were made after cells grew as continuous lines (1 to 6 months). Cells treated overnight with colcemid (0.04 μg/ml) were washed twice in a serum free balanced salt solution (BSS) and treated with an hypotonic serum-distilled water solution (1:5) for 20 min. Cells were then fixed with methanol-acetic acid (3:1), spread on chilled slides, air dried, treated with formamide solution for 30 min at 67°C, fixed in ethanol, and stained with Giemas stain (34, 35).

Generation time. The generation times of established cell lines were determined by counting viable cells during a 1-week period by using trypan blue dye exclusion to assess viability. Generation times were calculated at the exponential growth phase according to the method described by Paul (36).

Latex particle uptake. Cells were harvested, washed twice in HBSS and resuspended in 1 ml of HBSS containing 5% FCS. One-tenth milliliter of latex particle solution (1:100 dilution in saline, size 1.1 μ, Dow Chemical Co., Indianapolis, Ind.) was added to each cell suspension and the mixtures were incubated

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on a vertical rotator for 1 hr at 37°C. Cells were then washed twice in BSS and resuspended in 0.2 ml of FCS, smeared on alcohol clean slides, fixed in methanol, stained with Giemsa stain for 15 min, and examined microscopically.

Esterase staining. Esterase staining was carried out according to the method of Li *et al.* (37). Briefly, cells were smeared on clean slides, air dried, and fixed with formalin-acetone buffer. Fixed smears were then incubated in a reaction mixture containing phosphate buffer, hexazotized pararosanilin, and α-naphthyl for 45 min at room temperature. After washing in distilled water, slides were counterstained with methyl green.

RESULTS

Establishment of six BALB/c lymphoid tumors in cultures. All tumors except BALENLM 17 were derived spontaneously from 15-month-old or older BALB/c mice; BALENLM 17 was induced by *1*-ethyl-*1*-nitrosourea (23). With the exception of K46, tumors were transplanted i.p. into pristane primed BALB/c mice. Since BALB/c mice injected with K46 i.p. died with relatively little tumor growth, this tumor was passaged intramuscularly in mice. Tumors were passaged in mice for 1 to 7 years (23 to 66 generations). All tumors exhibited cell surface staining with a polyvalent anti-mouse immunoglobulin reagent and most except A20 were stained with anti-IgM. In addition, A20 and K46 tumors gave variable fluorescent staining with anti-IgG and anti-IgA, respectively.

X16C and K46 were cycled *in vitro-in vivo* once and twice, respectively, before they were adapted into culture. At the early stage of the adaptation into culture, tumor cells were fed or divided carefully after microscopic examination. Established cell lines were divided in fresh medium every 3 or 4 days. The inoculum was variable depending upon the type of cells and conditions of cultures. All of these cell lines grew as stationary suspension cultures in C-EHAA medium.

Expression of cell surface immunoglobulin (Ig). Tumor cell lines gave three patterns of cell surface Ig staining. L10A, K46, X16C, and BALENLM 17 were stained with antiserum specific for IgM and with poly-anti-Ig but not with antibodies against IgG_1, IgG_2, or IgA. A20 and M12 were stained only with poly-anti-Ig. M12 stained less intensely than the other lines. To confirm that fluorescence staining of A20 and M12 with poly-anti-Ig was not due to the Fc receptors on cell membranes, $F(ab')_2$ fragments of polyvalent goat anti-mouse Ig, antibodies were also used for staining. Results obtained with the $F(ab')_2$ portion of anti-mouse Ig antibody were the same as those with intact molecules for all of the cell lines.

Expression of H-2 and Ia. Tumor lines were tested for the presence of major histocompatibility antigens by using three different assay techniques, indirect immunofluorescent (IF) staining, a two-step cytotoxicity test and absorption testing. Table I summarizes the results obtained in such studies. All of the lines were highly reactive with the anti H-2 serum by both IF and cytotoxicity test. L10A, K46, A20, X16C, and BALENLM 17 were >90% IF positive and >71% CT with the polyvalent anti-Ia serum (A.TH anti-A.TL). These lines also expressed the Ia antigens detected by the more restricted anti-Ia reagents, indicating expression of Ia antigens determined by more than one subregion. On the other hand, 30% of M12 cells gave very faint IF staining with the polyvalent anti-Ia antisera and maximum lysis of M12 by the same sera was 24% (19% above C control). Thus, the amounts of Ia antigens present on M12 appeared to be minimal, compared to the other cell lines.

Cell lines grown *in vitro* have been reported to react with anti-viral antisera (24). Therefore in order to determine whether or not positive results obtained from IF staining or cytotoxic $titer^{-1}$ (CT) were due to contaminating antibodies against viral antigens in these sera, absorption studies with anti-Ia sera were performed on the L10A, K46, A20, and M12 lines. The maximum percent lysis by the A.TH anti-A.TL antiserum was 80% on K46 target, 56% on BALB/c spleen cells, and 82% on B10.A spleen cells (Fig. 1). The maximum cytotoxicity produced by this serum after absorption with K46 (5×10^8 cells/ml of antiserum) was less than 10% on K46, and less than 10% on BALB/c spleen cell targets indicating that all anti-Ia antibodies reactive with Ia antigens of the $H\text{-}2^d$ haplotype had been removed. This absorbed serum still lysed 68% of B10.A spleen cells, indicating that anti-Ia^k antibodies not reacting with Ia^d had not been removed, confirming that the absorption of A.TH anti-A.TL antiserum by K46 was specific for Ia^d antigens. Thus, these results confirmed the presence of Ia antigens on K46 tumor and suggested that anti-viral activity, if present in these sera, could not account for the reactivity observed. A similar conclusion was made with A20 and L10A tumors (data not shown). In contrast, absorption with M12 at 10^9 cells/ml, did not reduce the titer of anti-Ia antisera significantly. Therefore it was concluded that Ia antigens on M12, if present, were expressed at very minimal levels on the cell surface.

Fc receptors and complement receptors. Table II shows the percentage of Fc (EA) and C (EAC) rosettes of the various lines. SRBC sensitized with a mixture of IgG_1 and IgG_2 antibodies gave similar percentages of rosettes compared to the IgG_1-coated SRBC. No tumor cells formed rosettes with IgM coated SRBC nor with such cells incubated in fresh mouse

TABLE I

Expression of Ia antigens of BALB/c lymphoma lines

Cell Lines	Anti-H-2D (A.SW anti-A.TH)		Polyvalent Anti-Ia (A.TH anti-A.TL)			Anti-Ia.8($I\text{-}A^d$) (B10.A × A)F_1 anti-B10		Anti-Ia.23($I\text{-}E^d$) (B10 × D2GD)F_1 anti-B10.D2	
	IF	CT	IF	CT	Abs	IF	CT	IF	CT
L10A	>90[a]	>80[b]	>90	82	+	>90	58	>90	>80
K46	>90	>80	>90	>80	+	>90	80	>90	>80
X16C	>90	>80	>90	71	ND[c]	90	71	85F	59
BALENLM 17	90	>80	>90	>80	ND	>90	88	>90	>80
A20	>90	>80	>90	>80	+	>90	80	90	>80
M12	>90	62	30F[d]	24	−	<10	14	<10	<10

[a] Per cent positive fluorescent cells.

[b] Per cent maximum dead cells after treatment with various dilutions of antiserum and rabbit complement. All the complement lyses were ≤23%.

[c] Not determined.

[d] F, faint.

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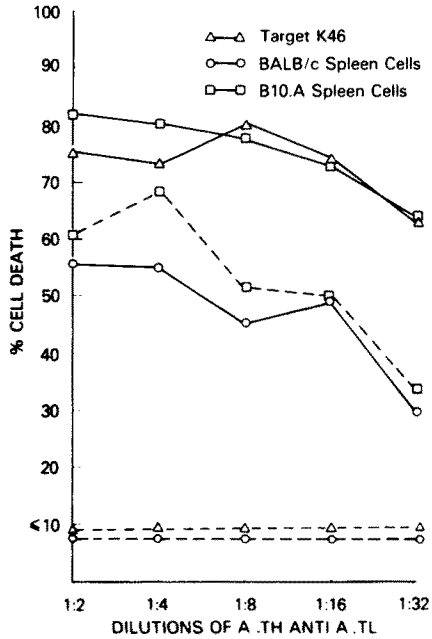

Figure 1. The activity of A.TH anti-A.TL antiserum before (——) and after (- - -) absorption with K46(5×10^8 cells/ml). The absorbed serum did not have residual activity on K46 (△---△) cells nor on BALB/c spleen cells (○——○) but still lysed B10.A spleen cells (□——□). Complement controls were less than 10% and were not subtracted from data shown.

TABLE II

Fc and complement receptors of BALB/c lymphoid lines

Cell Lines	SRBC Sensitized With			
	IgG_1 anti-SRBC[a]	IgG_1-IgG_2 anti-SRBC	IgM anti-SRBC	IgM anti-SRBC[b] + fresh mouse serum (1/8)
BALB/c spleen cells	50	44	3	45
L10A	64.5	76	0	0
K46	96.0	95.5	2	0
X16C	87.7	86.0	1	0
BALENLM 17	94.0	93.0	0	0
A20	91.3	96.5	3	0
M12	81.5	88.5	0	5

[a] Normal SRBC controls were all negative. Figure indicates per cent positive rosette-forming cells; at least 200 cells were scored.

[b] Assay for the detection of complement receptors.

serum (EAC rosettes). Normal spleen contained 45% cells that were EAC+.

Studies of macrophage properties. These lymphoma lines, especially A20 and M12, which did not stain with any antisera against class specific mouse Ig, such as IgM, IgG_1, IgG_2, or IgA, were examined for the possibility that they were macrophage lines rather than B cell lines. Two tests for macrophage properties were employed, the phagocytosis of latex particles and esterase staining. Six lines examined, K46, X16C, L10A, BALENLM 17, A20, and M12, did not take up latex particles and were negative in esterase staining while $P388D_1$, a previously described macrophage line (38), gave positive results in both assays.

Karyotype, generation time, and tumorigenicity. Chromosome numbers of these cell lines could be grouped into diploid and hypotetraploid. The chromosome numbers of L10A, K46, and X16C were hypotetraploid whereas those of BALENLM 17, A20, and M12 were near diploid (Table III). The doubling times of these BALB/c lymphoma lines during exponential growth phase were in the range of 18 to 26 hr (Table III).

All of these established cell lines, except X16C, grown in culture more than 6 months, were still capable of growing in BALB/c mice within 1 month when tumor cells (3×10^6 cells/mouse) were injected i.p. into pristane-primed mice. Tumor cells were detected mainly in spleen, mesenteric lymph nodes, ascites fluid, and occasionally in liver.

DISCUSSION

Table IV summarized all of the surface markers of the BALB/c B lymphoid lines detected in this study. These cell lines can be separated into three groups based upon these cell surface markers: 1) L10A, X16C, K46, and BALENLM 17 bear IgM, Fc receptors, and Ia antigens. 2) A20 expresses Fc receptors and Ia antigens. 3) M12 has only Fc receptors. All of these cell lines bear surface Ig and none bears C receptors. All these lines stained with intact molecules or with $F(ab')_2$ fractions of anti-mouse Ig antibodies, however staining of M12 with these reagents was faint.

The positive staining of A20 or M12 with poly-anti-Ig but not with anti-IgM, anti-IgG, or anti-IgA could reflect the presence of IgD, free light chains, another as yet undefined class of Ig molecules, or a scant amount of a known class of Ig molecules. These possibilities are under investigation. Often, cultured cells did not express the same pattern of Ig as *in vivo* tumor cells:

TABLE III

General properties of BALB/c lymphoma lines

Cell Lines	Chromosome Counts[a]		Generation Times[b]	Tumorigenicity
	Range	Modal No.		
			hr	
L10A	36–85	70 (13 months)	26	+ (Sp, LN, LV)[c]
K46	48–71	62 (5 months)	19	+ (Sp, LN, AS)
X16c	39–84	78 (9 months)	18	ND
BALENLM 17	37–53	48 (5 months)	ND	+ (Sp, LN)
A20	33–38	37 (5 months)	18	+ (Sp, LN, LV, AS)
M12	27–67	37 (7 months)	26	+ (Sp, LN, LV, AS)

[a] Approximately 20 metaphases were counted.

[b] Generation times of cell lines were determined at approximately the same time as karyotype study.

[c] Sites where tumors appear. Sp, spleen; LN, lymph nodes; LV, liver; AS, ascites fluids.

TABLE IV

Summary of the surface markers of B cell lines

Tumor Lines	Surface Ig	Ia	Fc	C
L10A	Ig^+,[a] μ^+	+	+	–
K46	Ig^+, μ^+	+	+	–
X16 C	Ig^+, μ^+	+	+	–
BALENLM 17	Ig^+, μ^+	+	+	–
A-20	Ig^+	+	+	–
M-12	$Ig^{\pm}$[b]	–	+	–

[a] Stains with a purified polyvalent anti-Ig antibody (See *Materials and Methods*).

[b] Weak staining; (See *Results*).

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K46, A20, and M12 lost the ability to express IgA, IgG, and IgM on the cell surface after adaptation into culture. These changes could be interpreted in several ways: 1) an *in vitro* selection of certain types of tumor cells, 2) spontaneous mutation, and 3) false positive Ig staining of *in vivo* tumor cells due to passive Ig adsorption.

K46, X16C, L10A, and BALENLM 17, which bear a typical B cell monomeric IgM on the cell surface (P. E. McKeever, observation) along with other B cell markers, are presumably of B cell origin. Other lines such as M12 and A20 do not have any class specific S-Ig. About 20 to 30% of macrophages have Fc receptors and Ia antigens (39, 40). Thus, the possibility that M12 and A20 are of macrophage origin was investigated. Neither A20 or M12 phagocytized latex particles, nor were they positive for esterase staining; however, P388D_1, a known macrophage line, was positive in both assays. All cell lines grow as stationary suspension cultures and do not appear to be morphologically macrophage-like. A20, a uniformly round cell with Ia antigens and Fc receptors, is thought to be of B cell origin but less differentiated than K46, X16C, L10A, and BALENLM 17. M12 bears only Fc receptors and possibly originated from a B cell that was less mature than any of the other lymphoma lines.

IgM bearing culture lines, L10A, K46, X16C and BALENLM 17, have chromosome numbers in the range of hyperdiploid to hypotetraploid. On the other hand, B cell-like lines without S-Ig, such as A20 and M12, have hypodiploid numbers. It is not known whether the karyotype of the cell lines has any importance in the expression of Ig on the cell surface. Further studies of Ig synthesis and induction of Ig secretion by these B cell lines are in progress in our laboratory and could provide valuable information concerning B cell maturation.

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