

Establishment and Characterization of Human B-Cell Lymphoma Cell Lines Using B-Cell Growth Factor

By Richard J. Ford, Angela Goodacre, Irma Ramirez, Shashikant R. Mehta, and Fernando Cabanillas

B-cell non-Hodgkin's lymphomas (NHL-B) have been difficult to establish in long-term cell culture using standard techniques. We report the establishment of five representative cell lines from high grade NHL-B using B-cell growth factor (BCGF). The five NHL-B cell lines display the morphologic, immunophenotypic, genotypic, and biologic characteristics of the lymphoma cells present in the original diagnostic specimen. The cell lines showed at least a sevenfold dose-dependent increase in proliferation in vitro over background in the presence of BCGF. Other putative B-cell growth-stimulating cytokines showed no significant proliferative activity or were inhibitory in some cases. NHL-B cell lines secreted growth factor(s) into culture supernatants

that mediated at least a fivefold dose-dependent increase in cell proliferation in autochthonous lymphoma cells and a 10-fold or greater stimulation in growth factor-dependent normal B cell lines in vitro. The cell lines show monoclonal rearrangements of IgH genes and nonrandom chromosomal abnormalities characteristic of NHL-B, while the expression of Epstein-Barr virus associated antigen (EBNA-I) is present in two of the five cell lines. The studies show that lineage-specific growth factors may be used to establish neoplastic B cell lines in vitro, which are important experimental systems for cellular and molecular studies in the NHL-B.

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THE NON-HODGKIN'S lymphomas (NHL) are a heterogeneous group of human lymphoid neoplasms, principally derived (~80%) from the B-lymphoid lineage (NHL-B).¹ The NHL are among the most common human neoplasms, being of particular interest to both hematologists as well as immunologists due to their close phenotypic similarity and association with the lymphoid cells of the immune system.² Although these tumors are often readily accessible to biopsy in peripheral lymphoid tissues, or by bone marrow aspiration, splenectomy, etc, and amenable to suspension cell culture by routine tissue culture techniques, the tumor cells have been difficult if not impossible to reproducibly grow in vitro.³ The single possible exception to this general rule is Burkitt's lymphoma (BL; small noncleaved cell lymphoma), which usually contains the Epstein-Barr virus (EBV) genome that is thought to impart in vitro immortalization as one of the manifestations of the expression of the EBNA gene(s).⁴ The establishment of other NHL cell lines has been sporadically reported since the original studies of Epstein and Kaplan,⁵ but these cell lines have generally arisen "spontaneously" and have, for the most part, provided little information regarding the necessary and sufficient conditions for in vitro establishment. We have reported that fresh NHL-B, obtained from lymph node biopsies, etc, can be stimulated to proliferate in vitro in response to partially purified 12 Kd B-cell growth factor (BCGF).⁶ Furthermore, we have also shown that in the chronic B-cell leukemia, hairy cell leukemia (HCL), BCGF will not only stimulate tritiated thymidine incorporation in vitro, but will also support the growth of the leukemic cells in culture for extended periods.⁷ In the studies reported here, we show that a spectrum of high grade NHL-B, extending from relatively immature to mature B cell phenotypes, can be established in vitro to yield long-term cell lines, using the lineage-specific growth factor, 12 Kd BCGF. These cell lines appear to be representative of the original lymphomatous disease process from which they were derived, and as such provide valid experimental model systems for studying immunologic, molecular, and cell biologic aspects of human B cell lymphoma.

MATERIALS AND METHODS

Patients. Both untreated and relapsing and/or refractory treated patients with NHL-B were studied. Patient samples included bone

marrow aspirations, fine needle aspirations (FNA) from involved lymph nodes, and body cavity fluids containing lymphoma cells. The specimens were usually obtained at the time of initial biopsy or when the patient presented in relapse. A brief description of the patients from which the cell lines were derived is presented in Table 1.

Characterization of lymphoma cells. Patient samples were studied for histopathologic type of lymphoma, using the International Working Formulation (IWF)⁸ and modified Rappaport⁹ classifications by comparing initial cell suspension cytopsin (Shandon Southern, Pittsburgh, PA) preparations with the permanent biopsy paraffin sections, or from the cytologic prepared specimens in the case of fluids or aspirations. Immunophenotypic analysis of single cell suspensions were performed as previously described, using standard T and B lymphocyte-associated panels of murine monoclonal antibodies (MoAbs).¹⁰ Neoplastic B lymphoid cell populations stained for immunofluorescence were analyzed either by fluorescence microscopy (Leitz Orthoplan with Ploem illumination) or using flow cytometry (Becton-Dickinson Fluorescence Analyzer, B-D FACS systems, Mountainview, CA). Immunophenotypic profiles of the established cell lines are also shown in Table 1.

Cytogenetics. Cell cultures were enriched for mitotic cells by a 4°C cold shock inhibition of growth and subsequent release by incubation at 37°C. Aliquots were harvested after 16 hours at 2- to 4-hour intervals, using 0.004 µg/mL colcemid for 15 minutes to disrupt spindles. Cells were exposed to hypotonic KC1 (0.075 mol/L) for 20 minutes followed by several changes of fixative (methanol:acetic acid, 3:1). Air dried slides were made from the cell suspension. Harvests showing the optimal mitotic indices were banded using conventional Q, G, and C banding techniques (Trent et

From the Departments of Molecular Pathology, Hematology, and Pediatrics, M.D. Anderson Cancer Center, The University of Texas, Houston, TX.

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Address reprint requests to Richard J. Ford, MD, PhD, The University of Texas, M.D. Anderson Cancer Center, Department of Molecular Pathology, Box 89, 1515 Holcombe Blvd, Houston, TX 77030.

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Table 1. Description of Lymphoma Patients From Whom NHL-B Cell Lines Were Derived

Cell Line	Pt Age (yrs)/ Race/Sex	Dx	Immunophenotype	Cytogen	Therapy	Clinic Response
CJ	54/W/F	NPDL-DLCL (NSCL) Stage IVB	CD19, 20, 10, Sig, DR+ CD2, 3- Tac (IL-2R)-	t(8, 14) t(14, 18)	CHOP/COP Cis-plat Cytosine Arabinoside	PR; died of refractory lym- phoma
RR	38/W/M	DLCL (IB) Stage IVB	CD19, 20, 22, DR+, Ig, CD2, CD3- Tac(IL-2R)	t(8, 14) del 2, der 17	MIME M-CHOP/VP 16 ARA-C Cis-Plat	CR, relapse/died of refrac- tory lymphoma
JT	70/H/M	DLCL Stage IVB	CD19, 20, Sig, DR+, CD2, 3, 4, 8, IL-R	t(14, 18) del 6, +7, +12, -17	CHOP/Bleo	PR, relapse/died of refractory lymphoma
FM	43/W/M	DMCL(FCC) NLCL Stage IVB	CD19, 20, Ig (k) DR+ CD2, 3	t(14, 18) +3, +9, +12 del 7	CVP, M-BACOD ARA-C Cis-Plat	PR, progress transformation con to high grade refrac- tory lymphoma
RT	16/W/M	ALL(pre-B) with lymph node involvement	CD19, 20, 10, clg+, CD2, 3, Sig-	4(14, 18) t(5, 14), t(1, 17) and multiple clonal abnormalities	ALINC-13 Vincristine Adriamycin Prednisone	PR, CNS/relapse/died of ALL 18 mos post-diagnosis

Abbreviations: PR, partial response; CR, complete remission; NPDL, nodular poorly differentiated lymphocyte; DLCL, diffuse large cell lymphoma; NSCL, nodular small cleaved cell lymphoma; NLCL, nodular large cell lymphoma; ALL, acute lymphoblastic leukemia; CHOP: cytotaxan, Adriamycin, oncovin, prednisone; COP: cytotaxan, oncovin, prednisone; Cis-plat: cis-platinum; MIME: methyl-GAG, ifosfamide, methotrexate, etoposide; ARA-C: cytosine arabinoside; CVP: cytotaxan, vincristine, prednisone; M-BACOD: methotrexate, bleomycin, Adriamycin, oncovin, dexamethasone; ALINC-13: acute leukemia in children #13 (POG), cytotaxan, vincristine, L-asparaginase, prednisone, cytosine arabinoside, methotrexate, 6-mercaptopurine, 6-thioguanine.

al, 1986).¹¹ Karyotypes are described according to ISCN nomenclature (1985).¹²

Cell culture. Single cell suspensions from biopsy or fluid specimens were depleted of T cells by sheep red blood cell (SRBC) E rosetting, and of mononuclear cells (mostly monocytes and/or macrophages) as previously described.⁶ The SRBC negative (E-), nonadherent cell population was cell surface-phenotyped, karyotyped, and prepared for cell culture. Duplicate cell cultures were established in RPMI 1640 media (Irvine Scientific, Irvine, CA) and 20% fetal calf serum (FCS; Hyclone, Logan, UT). BCGF (12 Kd) was used either in the partially purified state as purchased from Cellular Products Inc (Buffalo, NY) or further purified to homogeneity as described by Mehta et al.¹³ Cell cultures were fed twice weekly and monitored for cell growth, cell viability, and growth factor dependence as previously described.¹⁴ Cell lines were monitored for mycoplasma infection on a regular schedule.

Immunoglobulin gene rearrangement studies. Monoclonality of neoplastic B cells was assessed by standard Ig gene rearrangement analysis essentially as described by Cleary et al,¹⁵ using Southern blot analysis for DNA restriction fragments from *Bam*HI and *Hind*III digestions for J_H for IgH, and C_κ and/or C_λ for IgL.

Growth factor studies. Tumor cells from the various cell lines were assessed for reactivity to various lymphokine growth factors associated with human lymphocyte growth and differentiation at the initial time of procurement, and after varying lengths of time in vitro. The growth factors used were human BCGF, as described above and, recombinant interleukin-2¹⁶ (rIL-2; a gift from Dr P. Ralph of the Cetus Corp, Emeryville, CA). Other recombinant human cytokines included IL-4 (BSF-1), IL-6 (purchased from Genzyme, Boston, MA), and recombinant lymphotoxin (LT; obtained from Genentech, South San Francisco, CA). Cell lines were also studied for the production of autocrine type B-cell growth factors by collecting culture supernatants from lymphoma cell lines grown in low (fetal calf serum [FCS] 5%) or serum-free conditions (Nutridoma, Boehringer Mannheim, Indianapolis, IN) as previously described.^{17,18} Target cells for assessing autocrine and/or BCGF activity were the lymphoma cell line cells and BCGF-dependent normal human B cell lines (BD-9, RS-1, etc).¹⁴

Southern blot analysis for the presence of EBV genome in lymphoma cell lines. High molecular weight DNA from each of the lymphoma cell lines (10 μg) was digested with the restriction endonuclease *Bam*HI, size-fractionated in 0.8% agarose gel, and transferred to a nitrocellulose filter. The filter was hybridized to an EBNA 1 probe (pEBNA) oligolabeled with P³² to a specific activity of 1 x 10⁹ cpm/μg. The filter was washed under high stringency conditions and exposed on X-ray film for 24 hours at 70°C.

In vitro immunoglobulin secretion by lymphoma cells. Constitutive secretion of immunoglobulins was assayed for by measuring IgM and IgG levels in cell culture supernatants at 48 or 72 hours in vitro. These assays were performed using a standard micro-enzyme linked immunosorbent assay (ELISA) method.¹⁹

RESULTS

Establishment of human NHL-B cells in vitro. Freshly obtained specimens of high grade human NHL-B from various types of biopsy or other diagnostic procedures were received under sterile conditions and processed within 1 hour of removal from the patient. These specimens were representative of the spectrum of high grade type of human B-cell lymphomas. The tumor cells obtained from the patients were usually greater than 90% viable and could be maintained in standard RPMI media and 20% FCS for up to 24 hours without significant loss of tumor cell viability. Cells were aliquoted at this time for immunophenotyping, genotyping by Ig gene rearrangement, and for cytogenetic karyotyping. Tumor cell populations were depleted of T cells by E-rosetting and of monocytes and/or macrophages by plastic adherence. Characteristics of patients' initial lymphoma cell populations are shown in Table 1. Tumor cells were then assayed for functional activities such as proliferation and/or differentiation (Ig secretion) or plated for in vitro cell growth in the presence or absence of the lineage-specific growth factor, BCGF (12 Kd). Cell growth was assessed at 3- to

Table 2. Proliferative Responses of B-Cell Lymphoma Cell Lines to Cytokine Growth Factors

Cell Line	% vol/vol	BCGF*	μ /mL	rIL-2	μ /mL	rIL-4	μ /mL	rIL-6	μ /mL	rLTN
RR	2.5	9,644	50	2,384	50	3,332	50	5,280	30	4,070
	5.0	17,018	100	5,700	100	4,888	100	2,360	60	4,742
	10.0	22,893	150	6,268	150	6,991	150	ND	125	3,142
Control		3,496								
CJ	2.5	82,814	50	3,239	50	746	50	1,648	30	680
	5.0	86,087	100	7,398	100	770	100	2,118	60	1,220
	10.0	96,032	150	2,106	150	840	150	480	125	ND
Control		2,824								
RT	2.5	16,209	50	3,553	100	2,138	50	860	30	163
	5.0	26,632	100	4,774	200	3,522	100	1,110	60	202
	10.0	30,405	150	5,833	300	6,140	150	ND	125	480
Control		4,858								

Proliferation experiments were carried in vitro on the established lymphoma cell lines from the patients described. In these assays 2×10^4 viable lymphoma cells per well in RPMI media plus 5% FCS were cultured in triplicate.^{4,10} Cytokine growth factors, natural product or recombinant, were added at the initiation of cell culture and titered in dose-response-type microwell assays for 72 hours at 37°C. Cells were harvested with an automated harvester and counted in a liquid scintillation spectrometer.

Abbreviation: ND, not determined.

*Mean cpm of triplicate well cultures expressed as experimental minus control (E-C), after 18 hours pulse with 0.5 μ Ci of 3 H-TdR (6 Ci/mmol). SE were less than 10%. Control values are shown for each cell line.

5-day intervals by assaying for tritiated thymidine (3 H-TdR) incorporation, and in representative wells, for increase in viable cell growth by hemacytometer counts using trypan blue dye exclusion. Other lymphoid growth factors such as IL-2, IL-4 (BSF-1), IL-6, or lymphotoxin (LTN) did not stimulate 3 H-TdR incorporation in fresh lymphoma cells, or could not support the growth of freshly explanted or cultured lymphoma cells within the first 10 days in vitro. Once established in vitro, the lymphoma cell cultures showed similar reactivities to the initial responses to growth factors obtained. Established B-cell lymphoma cell lines were then assayed for proliferative responses to BCGF, IL-2, IL-4, IL-6, and LTN in 3 H-TdR incorporation assays. The results of a representative experiment for the B-cell lymphoma cell lines RR, CJ, and RT are shown in Table 2, where it can be observed that BCGF (12 Kd) stimulates a strong proliferative response, while the other cytokines show only weak reactivity.

Large cell lymphomas, B-cell type. Large cell lymphomas are a heterogeneous group of tumors, arising either as spontaneous lymphomas or as transformed variants of primary small cell B-cell lymphomas or B-cell chronic lymphocytic leukemias, as in Richter's syndrome. In the present study, three of the cell lines that were derived represented different histopathologic subtypes (eg, large noncleaved cell, immunoblastic, etc) of large cell lymphoma. While the tumors showed some morphologic (Fig 1A and B) and immunophenotypic heterogeneity, typical of these lymphomas, they also usually displayed quite stable nonrandom chromosomal abnormalities (NRCA) associated with NHL-B, as shown in Figs 3 through 5. In these cell lines, the cell surface immunophenotype and karyotype remained stable in vitro for at least 1 year.

Pre-B-cell leukemia/lymphoma. The cell line reported here (RT) was derived from the bone marrow of a 14-year-old patient with the pre-B cell type of acute lymphocytic

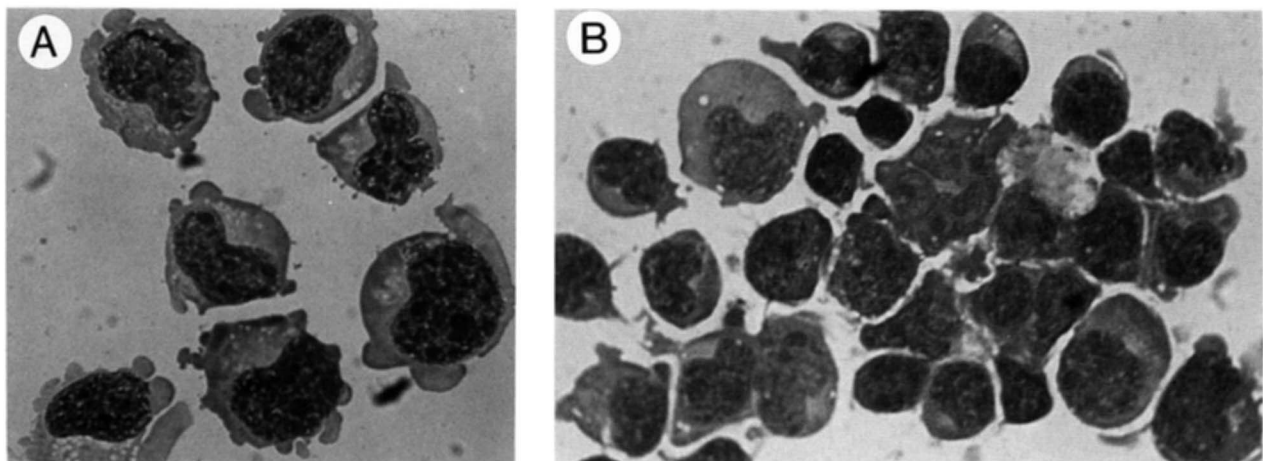


Fig 1. Cytospin preparations of NHL-B cell lines stained with Giemsa, showing characteristic large cell lymphoma morphology with some pleomorphism. A, CJ cell line; B, JT cell line. Magnification, 1000 \times .

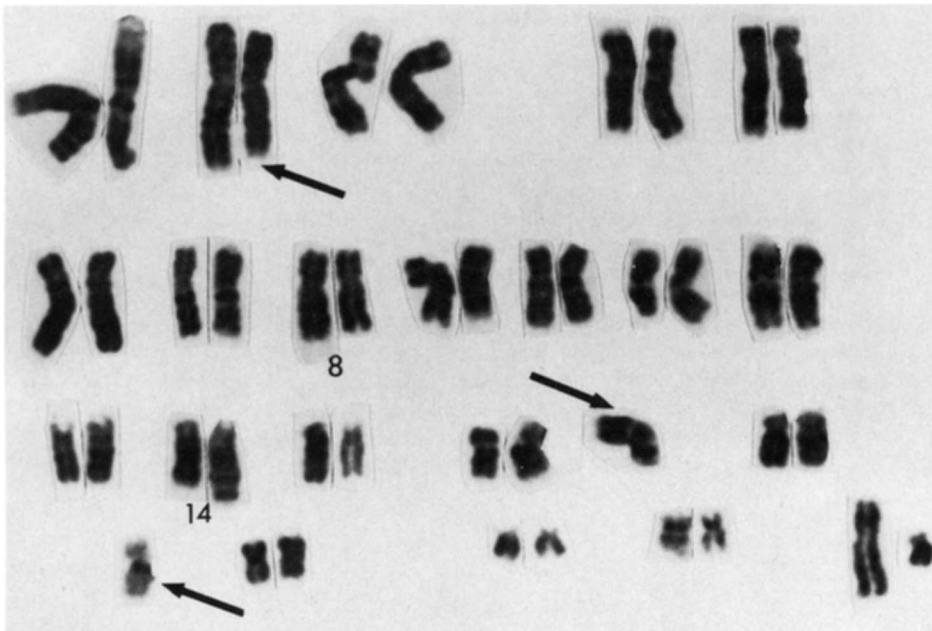


Fig 2. Giemsa-banded karyotype of RR cell line from early in vitro passage, showing 44, XY, -17, -19, del(2)(q₁₃q₂₃), t(8;14)(q₂₄:q₃₂), der(17)t(11;17)(q₁₃:p₁₂) der(19)t(19;7)(q₁₃:?)

leukemia (ALL) with peripheral lymph node involvement. The tumor cells from the initial bone marrow aspirate were typical ALL blasts morphologically and immunophenotypically (Table 1). The tumor cells responded actively to exogenous BCGF on initial exposure, and have continued to respond vigorously to the growth factor for more than a year in vitro (Table 2). The ALL cells had a variety of characteristic cytogenetic abnormalities in the original diagnostic sample that are also present in the cell line (Fig 6).

Cytogenetic abnormalities. NRCA associated with the various histopathologic subtype diagnoses have become important parameters in the characterization of NHL-B. The cell lines described here all demonstrated characteristic

NRCA, primarily of the t(14;18) and t(8;14) type in the fresh biopsy specimens, as well as in the subsequent cell line (Figs 2 through 6). The NRCA were concordant in all of the cell lines reported here. In addition to the NRCA, a variety of apparent random chromosomal abnormalities were also identified in the various cell lines (Figs 2 through 6).

Growth factor studies. In addition to providing the initial stimulatory conditions necessary for the establishment of in vitro cell growth in these cell lines, BCGF (12 Kd) sensitivity has been maintained for more than 1 year. Besides retaining growth factor sensitivity, the cell lines also produced a BCGF growth factor activity in the culture supernatants. This growth factor activity displayed autostimulatory

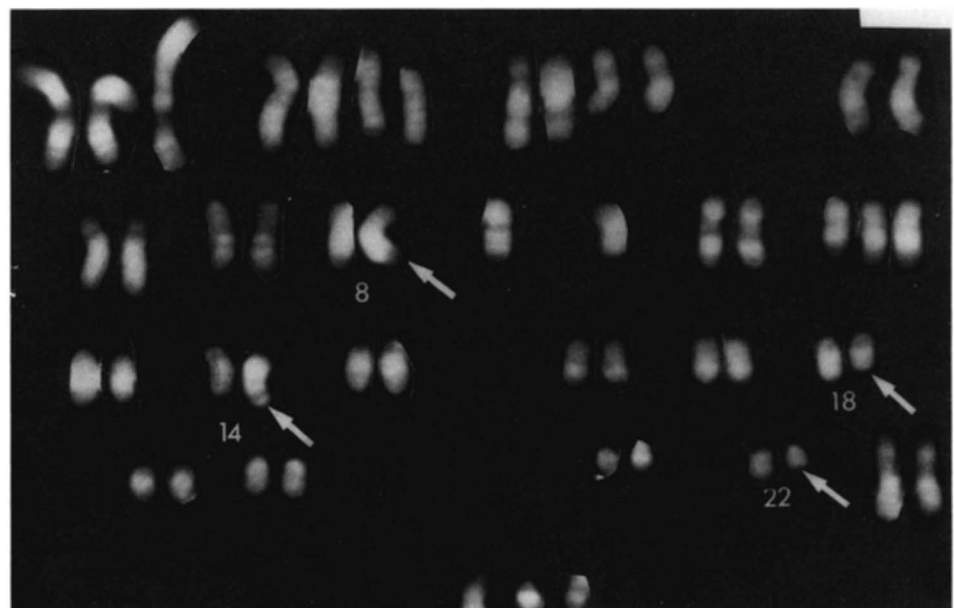


Fig 3. Q-banded karyotype of the large cell lymphoma, NHL-B cell line CJ showing 51, XX, +X, -4+12, +del(2)(p₂₁) +del(2)(q₃₂), +del(3)(q₂₁), +del(3)(q₂₁), der(4)t(1;4)(q₂₃;q₃₅), t(8;22)(q₂₄:q₁₁), del(10)(q₂₂:q₂₄), t(14;18)(q₃₂:q₂₁), and three unidentified markers.

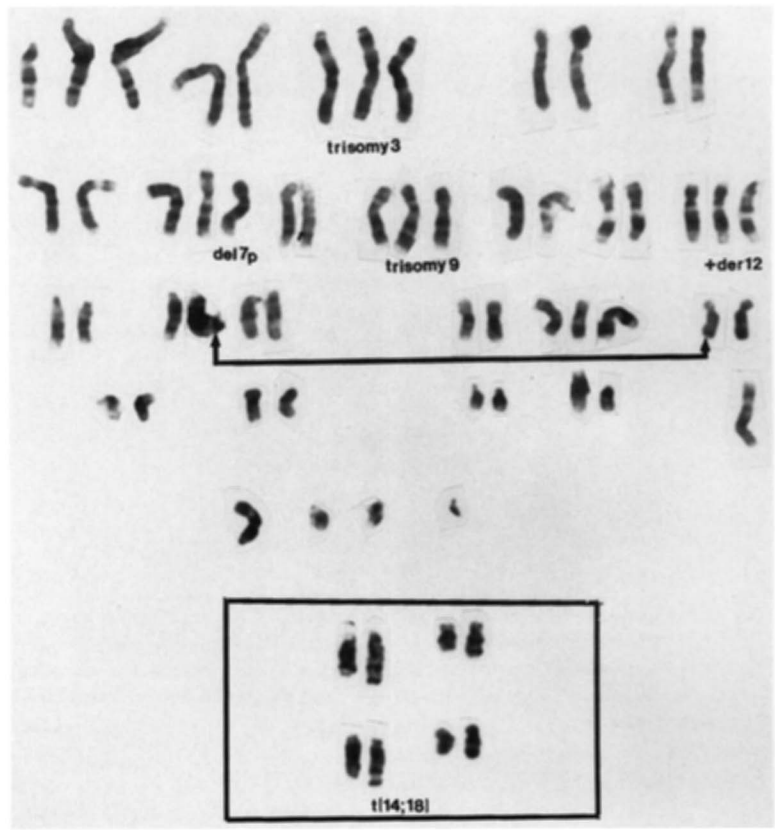


Fig 4. G-banded karyotype of the transformed follicular LCL NHL-B cell line FM, showing 55, XY, +3, +9, +der(1)t(1;3)p₁₁;p₂₁, +del(6)(q₂₁), +del(7)(p₁₃), +der(12)ins(12;7)(p₁₁;7)(t(14;18)(q₃₂;q₂₁), +der(17)t(3;17)p₁₄;p₁₁, and three unidentified markers.

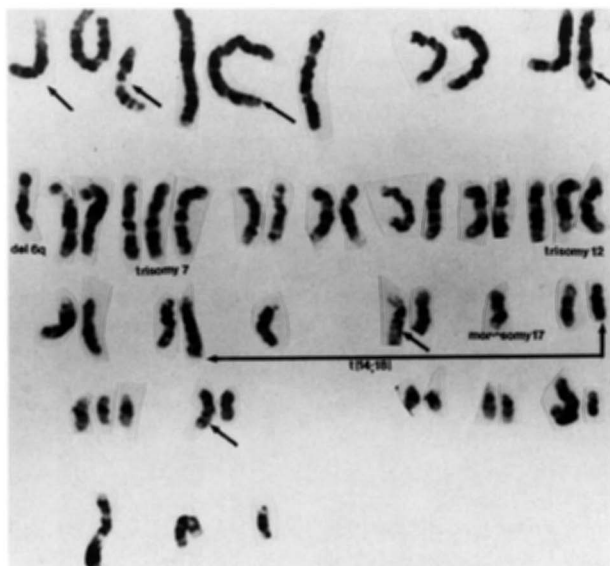


Fig 5. G-banded karyotype of the large cell lymphoma, NHL-B cell line JT showing 51, XY, -3, +7, +12, -15, -17, +19, der(1)t(1;17)(p₁₁;q₂₁), +del(1)(q₂₂)der(2)t(2;12)(q₂₂;q₁₃)der(5)t(3;5)(p₁₄;q₂₁), +del(6)(q₂₁), del(10)(q₂₂;q₂₄), t(14;18)(q₃₂;q₂₁), del(15)(q₁₂;q₂₄), der(16)inv dup(16)(q₁₃;q₂₄), der(20)t(20;7)(q₁₃;7) and three unidentified markers.

activity, as well as growth promoting activity on BCGF-dependent normal B cell lines in vitro (Table 3). The characteristics of the purified autocrine growth factor produced by the RR cell line have been recently described in detail.¹⁸ No proliferative activity was observed on normal IL-2-depend-

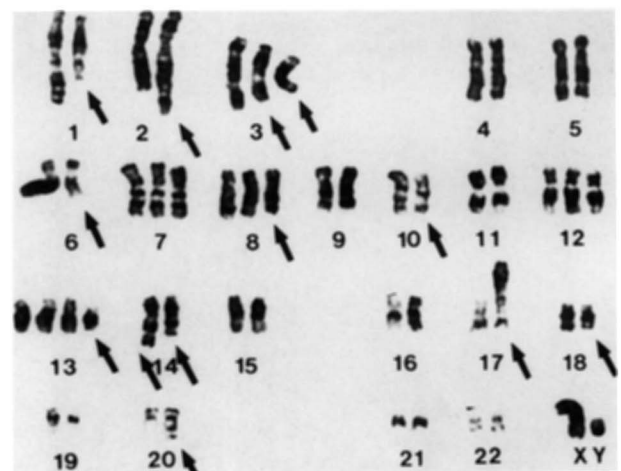


Fig 6. G-banded karyotype of pre-B lymphoma/leukemia RT, showing 52, XY, +7, +12, +13, t(1;17)(q₂₂;p₁₂), t(2;7;12)(q₃₂;q₁₃), +del(3)(p₂₂>cen::q₁₃>cen>p₂₁::q₂₁>qter), iso(3)(p₂₂>cen>p₂₂), der(14)t(5;14)(q₂₂;q₂₃), del(6)(q₂₂), del(8)(q₂₄), +del(8)(q₂₄), del(10)(q₂₂;q₂₄), del(13)(q₁₂;q₁₄), t(14;18)(q₃₂;q₂₁), der(20)t(20;7)(q₁₃;7).

Table 3. Production of Growth Factor Activity by B-Cell Lymphoma Cell Lines

Cell Line Supernatant	Target Cells			
	Autochthonous Lymphoma Cells		Normal B Cell Line	
	SN %	cpm*	SN %	cpm*
RR	0	2,418	0	940
	2.5	42,958	5	3,282
	5	60,662	10	10,644
	10	76,200	20	14,254
CJ	0	453	0	642
	5	2,336	5	2,316
	10	4,265	10	3,050
	20	5,476	20	7,722
FM	0	8,020	0	860
	5	32,245	5	15,401
	10	44,487	10	17,230
	20	47,536	20	26,179
JT	0	1,512	0	330
	5	4,105	5	5,456
	10	5,137	10	7,228
	20	6,190	20	9,654

Cell lines were cultured at 10^6 /mL for 24 hours in RPMI 1640, 5% FCS. The supernatants were collected, dialyzed overnight at 4°C against RPMI, and concentrated 10-fold with an Amicon concentrator, using YM 5 membranes. Autochthonous lymphoma cells or normal BCGF-dependent human B cells (10^4 per well) were washed, exposed to serial dilutions of the above supernatants, and cultured for 72 hours at 37°C. The microwells were pulsed with 3 H-Tdr for the final 18 hours in vitro. No activity above background was observed on IL-2-dependent normal human T cells or T-ALL cells exposed to the supernatants for 72 hours.

Abbreviation: SN, supernatant.

*Mean cpm of triplicate microwell cultures. SE were less than 10%.

dent human T cells or T-ALL cells in vitro when exposed to supernatant from the B-cell lymphoma cell lines.

EBV expression in NHL-B cell lines. Due to the ubiquitous nature of EBV expression in most well-known human B-cell lymphoma cell lines (particularly Burkitt's lymphoma cell lines), our cell lines were evaluated for EBNA-1 by Southern blot analysis. In the five lines studied, two were positive and three were negative (Fig 7).

Immunoglobulin secretion by lymphoma cell lines. The cell lines reported represent different apparent stages in B-cell differentiation showing characteristic Ig gene rear-

angement by Southern blot analysis (Fig 8), including fairly late (mature) stages of differentiation, where Ig secretion occurs in normal B cells and many EBNA positive lymphoblastoid cell lines. One of the cell lines (Fig 8, lane 5) shows only a single allele present, probably indicating that one of the alleles has been deleted. It was also of interest to determine if the NHL-B cell lines secreted Ig in vitro. Micro-ELISA analysis for IgM and IgG secretion was performed on cell culture supernatants after various lengths of time in vitro. As shown in Fig 9, most of the cell lines did not spontaneously secrete IgM or IgG when cultured under standard conditions in vitro.

DISCUSSION

The non-Hodgkin's lymphomas are an important, heterogeneous group of common human lymphoid neoplasms that have provided a great deal of interest in a variety of areas of cancer research.²⁰ The NHL are, in fact, among the most studied human tumors, due in part to the relative ease in obtaining representative specimens from lymph node biopsies and other commonly performed diagnostic procedures. Such specimens, if properly handled, will usually provide a source of fairly homogeneous, viable tumor cells that can be not only readily characterized in considerable detail and further purified if necessary, but can also provide excellent experimental systems for a wide range of studies. The recent development of extensive panels of lineage and in some cases stage-specific or associated MoAbs for antigens expressed on normal human B cells, which are also expressed on most if not all neoplastic B cells, has provided for the most compre-

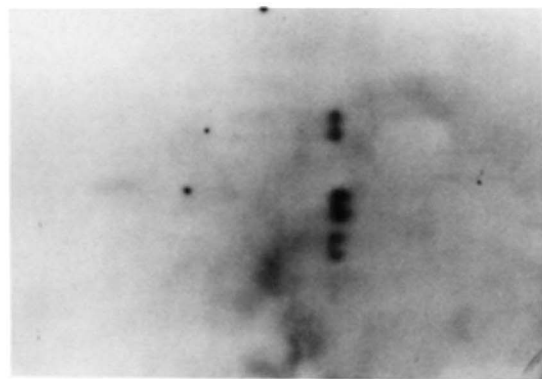


Fig 7. Southern blot analysis of NHL-B cell line DNA cut with the *Bam*HI restriction endonuclease, and then hybridized with the pEBNA probe showing a single 5 kb band in the cell line RS-1 (normal lymphoblastoid B cell line) and four NHL-B cell lines RR, LT, CJ, and SS.

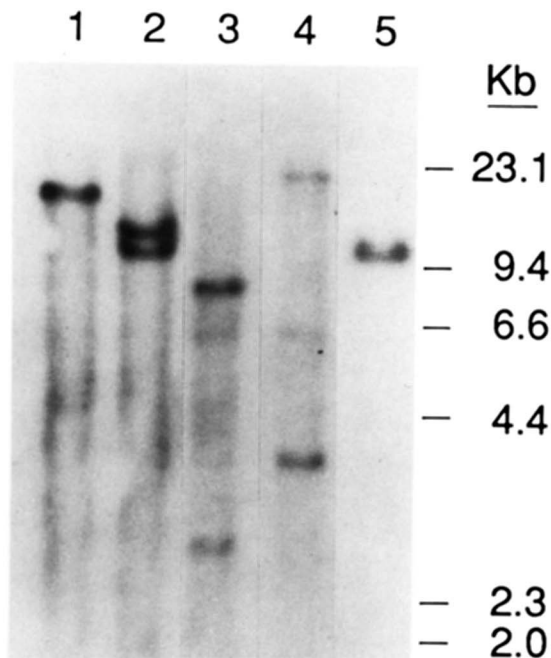


Fig 8. Southern blot analysis showing IgH gene rearrangements in NHL-B cell lines. Isolated cell line DNA was cut with the restriction endonuclease *Bam*HI, and hybridized with a J_H probe that recognizes a germline 17 kb *Bam*HI fragment. Lane 1, placental DNA; lane 2, RR NHL-B cell line; lane 3, CJ NHL-B cell line; lane 4, FM NHL-B cell line; lane 5, JT NHL-B cell line.

hensive immunophenotypic analysis of any group of human tumors.²¹ When such extensive immunophenotyping is combined with genotypic analysis by current cytogenetic and molecular techniques,²² the resulting biologic data base is probably the most comprehensive available for any type of human tumor. Such a comprehensive data base for tumor cell characterization can in turn allow for correlation with a wide variety of experimental parameters.

Given the experimental potential of human NHL systems, the availability of long-term cell lines from representative subtypes of NHL-B should be particularly useful in delineating the biology of these lymphomas. Since studies by many groups, including our own, have shown that NHL cells do not usually grow spontaneously in vitro, under standard in vitro culture conditions for human lymphoid cells,²³⁻²⁵ the use of exogenous lineage-specific growth factor (BCGF) for initial growth stimulation is of considerable importance from a technical perspective. Our studies over the past 2 years have indicated that we could grow the majority (~70%) of fresh NHL-B for at least 4 weeks in vitro in a growth factor-dependent manner. It has been possible to establish a significant number of the intermediate and high grade (aggressive) NHL-B into long-term cell lines that subsequently become independent of exogenously provided BCGF, due at least in part to the secretion of a BCGF-like autocrine growth factor molecule by the lymphoma cells.^{17,26} We have used one of these cell lines to purify and characterize this growth factor molecule, which cross-reacts with the high

molecular weight (70 Kd) intracellular BCGF molecule found in normal human T lymphocytes.¹⁸ These findings imply that the gene for BCGF, which is not usually expressed in normal B cells but which is apparently turned on in EBV-transformed lymphoblastoid cell lines,²⁷ is activated in the high grade NHL-B. Preliminary studies on these NHL-B cell lines, using the cDNA for the 12 Kd BCGF gene,²⁷ indicate that this may in fact be the case.²⁸

A further consideration involves the issue of BCGFs in general, which has sparked considerable controversy recently. A rather wide array of cytokines, including IL-2, IL-4, IL-6, and LTN²⁹ in particular, have been reported to stimulate proliferation in human B cells of one source or another. We have tested all of these (as well as a wide spectrum of other natural products and recombinant cytokines on NHL-B), both from freshly plated biopsy specimens and on established NHL-B cell lines (data not shown), and find significant and reproducible growth stimulation only by low (12 Kd) and/or high (60 Kd) BCGF. Another consideration has been the role of the CD23 antigen and other agonistic MoAbs in human B cell growth stimulation.^{30,31} The NHL-B cells and cell lines that have expressed EBNA-1 in our experience have shown moderate amounts of ³H-TdR incorporation in vitro after stimulation with anti-CD23 MoAb, but have not been able to support tumor cell growth in vitro. Those NHL-B that have been EBNA negative (greater than 50%) have been CD23 negative on immunophenotypic analysis and have shown neither ³H-TdR incorporation nor cell growth in vitro in the presence of anti-CD23 MoAb.

The presence of EBNA negative NHL-B cell lines suggests again, as in the Burkitt lymphoma cell lines previously described that have shown the lack of expression of this antigen, that transformation is not necessary for establish-

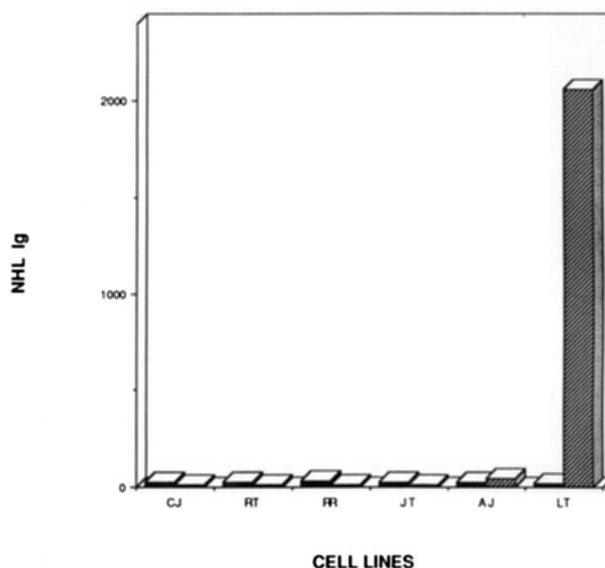


Fig 9. Spontaneous secretion of ng/mL IgM (□) or IgG (■) secretion in vitro from 72-hour supernatants of NHL-B cell lines, measured by micro-ELISA assay.

ment of NHL-B cell lines. Finally, it should be noted that our greatest success in establishing NHL-B has been in those patients with relapsing, relatively chemotherapeutically resistant disease. The establishment of such lines has augured a particularly poor prognosis in these patients, in that all have died within 6 months of our establishing the cell lines. Whether the various treatment modalities used in these patients have contributed to the apparent increased in vitro

sensitivity to growth factor(s) or, alternatively, have led to aberrant expression of autocrine growth factor genes by the lymphoma cells is of particular interest.

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