Growth Factor-Mediated Proliferation in B Cell Non-Hodgkin's Lymphomas

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The non-Hodgkin's lymphomas (NHLs) are a heterogenous group of human lymphoid tumors, primarily of B cell lineage, which appear to represent arrested stages in B lymphocyte differentiation. Control of cell proliferation is a fundamentally important but poorly understood area of study in these tumors. We have studied a representative group of B cell NHLs to assess their potential for growth factor-mediated proliferation in vitro. Our results show that purified monoclonal NHL B cells of the small cell (well-differentiated lymphocytic lymphoma, nodular poorly differentiated lymphocytic lymphoma, etc) type, that were positive for the human malignancy-associated nucleolar antigen could be stimulated by human B cell growth factor (BCGF) to proliferate in vitro. Other B cell activators such as insoluble anti-Ig and the mitogen protein A also could stimulate thymidine incorporation in the lymphoma cell populations. In vitro lymphoma cell growth could be maintained in the presence of the growth factor for up to five weeks. The large B cell type NHL, however, appeared to be refractory to in vitro stimulation by BCGF as well as other stimulators of normal B cells. These studies suggest that human B cell lymphoid tumors are not only phenotypically similar to their normal B lymphocyte counterparts, but are also sensitive in some cases, to the same types of immunoregulatory molecules that control normal lymphoid cell growth.

NON-HODGKIN's lymphomas (NHLs) represent a heterogenous group of human lymphoid neoplasms that include both T and B cell types, although B cell type NHLs are more frequently encountered.[1] Understanding of these neoplasms has been hampered by the lack of experimental in vitro systems to study the biology of the neoplastic cells. It has been postulated that neoplastic cells of NHL represent "frozen stages" in lymphocyte differentiation[2] in which the tumor cells continually proliferate without concomitant differentiation. In vitro, however, it has been difficult to establish proliferating lymphoma cell lines, unless Epstein-Barr viral transformation has occurred in the B cell type,[3] or human T cell leukemia virus (HTLV) infection has occurred in the T cell type.[4] Furthermore, it has been shown that many tumor cells retain phenotypic resemblance to their normal lymphoid cell counterparts,[5] and it is therefore conceivable that these tumor cells may also retain some functional capabilities similar to normal lymphoid cells. Recent advances in the characterization of immunoregulatory growth factors, such as interleukin 2[6] (IL 2) or T cell growth factor (TCGF) and more recently B cell growth factor (BCGF),[7] have provided a basis for understanding normal T and B cell proliferation, respectively. The ability to separate and identify neoplastic human lymphoid cells using monoclonal antibodies and recently the human malignancy-associated nucleolar antigen (HMNA),[8] have greatly enhanced the capability of performing experimental studies on defined tumor cell populations. This study was undertaken to investigate the ability of purified human B lymphoma cells to proliferate in vitro in response to soluble lymphoid growth factors. The role of polyclonal activators such as anti-immunoglobulin M (α-μ), and mitogens such as insolubilized protein A-Sepharose (PAS) in activating human lymphoma cells to proliferate was also investigated. The studies reported here indicate that at least some B cell type non-Hodgkin's lymphomas can respond to the homologous growth factor for their putative B cell lineage. The response of the tumor cells to the growth factor results in both proliferation and in vitro cell growth.

MATERIALS AND METHODS

Preparation of Lymphoma Cell Suspensions

Lymph node biopsy specimens were received from surgery under sterile conditions. The specimens were usually diagnostic biopsies from untreated patients. Lymphoma involvement of the lymph nodes was determined by a representative frozen section of a thin slice of the specimen, which was then stained with hematoxylin and eosin (H&E) at the time of tissue procurement. Final pathologic diagnosis was made on H&E-stained paraffin sections, using the modified Rappaport classification[9] after examination by light microscopy. Biopsy tissue was finely minced in Hanks' balanced salt solution (HBSS) and was then filtered through sterile gauze mesh to remove solid particles. The single cell suspension was then washed twice in HBSS, and the T cells and B cells were separated by procedures as previously described in detail for peripheral blood.[10] In brief, the cells were plated on glass Petri dishes for 45 minutes at room temperature to remove adherent cells. The cells were washed and incubated with sheep erythrocytes for two hours at 4 °C, after which rosette-forming T cells were separated from non-rosette-forming B cells on Ficoll-Hypaque (F/H) gradients. The sheep erythrocytes were lysed with Tris-ammonium chloride, and the T and B cell populations were adjusted to the desired cell concentrations in

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RPMI 1640 supplemented with 10% fetal calf serum (FCS) (Irvine Scientific, Calif). Control tissue included normal lymph nodes, histologically uninvolved with tumor obtained from various surgical procedures such as radical mastectomy and colectomy, as well as reactive (hyperplastic) lymph nodes removed for diagnosis.

Phenotypic Characterization of Lymphoma Cells

Cytocentrifuge preparations of cell suspensions from biopsy tissues were fixed in methanol-acetone mixture (9:1 ratio, respectively) for ten minutes. The cells were then examined for the presence of HMNA by indirect immunofluorescence (IF) using rabbit anti-HMNA as the primary antibody, and followed by fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG. The procedures for the preparation of anti-HMNA and for staining cells have been previously described in detail.[8] Membrane phenotyping was also performed on cytocentrifuge preparations with a Leitz Orthoplan fluorescence microscope. Monoclonal anti-human IgM, IgG, IgD, anti-κ and anti-λ as well as anti-Leu 1 were purchased from Becton Dickinson (Mountain View, Calif). The pan T cell monoclonal antibody T11 (E-rosette receptor) was purchased from Ortho Diagnostics (Raritan, NJ) The monoclonal antibodies B1, B2[11,12] were kindly provided by Dr Lee Nadler of the Dana-Farber Cancer Center (Boston). Cell suspensions from lymphomatous lymph nodes that had been adherent cell depleted and separated into En^+(T) and En^- (B) cell populations were cell surface phenotyped to determine the putative cell lineage. These procedures consisted of the detection of cell surface Ig (M, G and D) as well as light chain (κ and λ) typing[13] using immunofluorescence staining with monoclonal reagents.

Reagents Used for Activation of Lymphoma Cells

BCGF used in these studies was prepared from conditioned media of 72-hour lectin-stimulated human peripheral blood lymphocytes (LyCM) as previously described.[14] In brief, the LyCM was $(NH_4)_2SO_4$ precipitated, dialyzed, and then chromatographed on diethylaminoethanol (DEAE)-Sephadex column. These steps were followed by gel filtration chromatography first on a Bio-Gel p-30 column (Bio-Rad, Richmond, Calif) and then on a Bio-Gel p100 column. A distinct protein band of 12,000 to 13,000 daltons predominantly supported B cell growth. BCGF preparations contained <5% of the TCGF activity of standard IL 2 preparations on long-term human T cell lines or on PHA-stimulated E^+ cells from lymphomatous lymph nodes. Another protein band of 14,000 to 15,000 daltons predominantly supported T cell growth.

Insolubilized rabbit anti-human IgM (μ chain-specific) beads were obtained from Bio-Rad. The anti-μ beads (α-μ) were used to stimulate neoplastic B lymphoid cell populations at a final concentration of 15 μg/mL.[15] PAS was obtained from Pharmacia (Piscataway, NJ), and was used at 5% vol/vol final concentration.

Proliferative Assays

For the assessment of lymphoma cell proliferation, 2×10^5 purified lymphoma B cells were plated in 0.2 mL of RPMI 1640 supplemented with 10% FCS. The cells were incubated in round-bottom microtiter plates, with or without growth factor and/or anti-μ, or mitogen at 37 °C and 5% CO_2 humidified atmosphere. The incubation period was 96 hours, which included 18 hours of labeling with 0.5 μCi of [^{3}H]-thymidine (6 Ci/mmol, New England Nuclear, Boston). The cells were subsequently harvested onto glass fiber-filters by a Brandel M12 harvester (Gaithersburg, Md) and were counted in a liquid scintillation counter (Beckman LS8100).

Lymphoma Cell Growth In Vitro

For these experiments, purified freshly prepared lymphoma cells were plated in microwell plates at 0.5×10^6 per well in RPMI and 10% FCS, and previously titered growth factor (BCGF) was added at a final concentration of 10% vol/vol at the initiation of the cultures. Control wells contained media and serum alone. Wells were counted in a hemacytometer in duplicate and viability was determined by trypan blue dye exclusion at three- to four-day intervals. Wells showing cell growth were either fed with fresh media and growth factor or had the BCGF deleted to assess factor dependence.

RESULTS

Lymphoma Cell Phenotyping

Freshly obtained cell suspensions of NHL cells from lymph node biopsies were phenotyped for the expression of cell surface antigens to establish the apparent B or T cell lineage of the lymphoma. After adherence to plastic Petri plates to remove adherent cells, the cell suspensions were separated into En^+ and En^- populations by SRBC-rosetting and subsequent centrifugation on F/H gradients. The two populations (En^+, En^-) were then assayed with the HMNA antisera and MCA to various cell surface antigens characteristic of the T cell or B cell lineage. As shown in Table 1 and Fig 1, the B cell lymphoma cells ([En^-] B cell type) showed the expression of HMNA in ≥90% of the cells present. The nontumor cell population (En^+, T cell-enriched) contained ≥90% HMNA-negative cells by immunofluorescence criteria.

Proliferation of Human Small Cell Type Lymphoma Cells In Vitro

Because normal resting (G_0) human T and B lymphocytes have been shown to require activation before growth factor stimulation can be achieved, it was of interest to ascertain if neoplastic lymphoid cells had similar requirements. The effect of insoluble anti-μ (α-μ), an efficient activator for normal human B cells, on the induction of DNA synthesis in malignant B cells

Table 1. HMNA Content of Separated Human Non-Hodgkin's Lymphoma Cell Populations

Patient No.	Dx*	En^- Cells (%)†	En^+ Cells (%)
1	D-PDL-B cell type (T_{11}^-; sIg^+)	95	2
2	D-WDL-B cell type (T_{11}^-; sIg^+, BI^+)	97	2
3	D-large cell lymphoma B cell type (T_{11}^-; sIg^+)	96	3
4	N-PDL-B cell type (T_{11}^-; sIg^+)	84	2
5	D-LCL-B cell type (T_{11}^-, sIg^+)	91	4

*Modified Rappaport classification of H & E-stained paraffin sections.

†Cytocentrifuge preparations of En^+; and En^- cells populations, after F/H separation, were fixed in absolute methanol and stained for the presence of human malignancy-associated nucleolar antigen (HMNA) by indirect immunofluorescence. Five hundred cells were routinely counted in multiple microscopic fields for determination of HMNA reactivity.

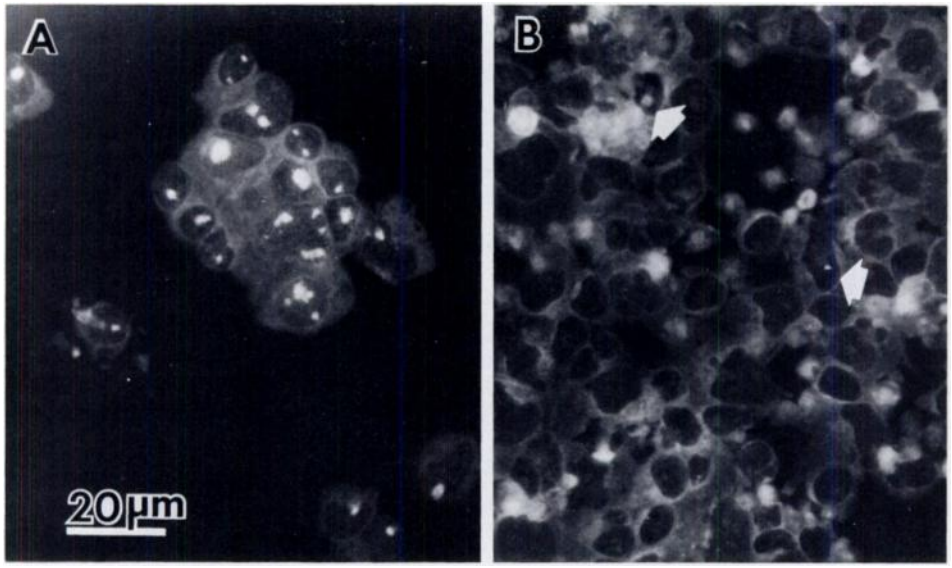

Fig 1. Separation of NHL cell populations into En-positive (reactive T) and En-negative (B cell lymphoma) cells. Cytocentrifuge preparations of a small cell (B cell type) NHL which has been T cell depleted by SRBC-rosetting and macrophage depleted by plastic adherence (A) and then stained with rabbit anti-HMNA by indirect immunofluorescence. Virtually all of the putative lymphoma cells are positive for HMNA, while the rosette-positive (T cell) population (B) is essentially negative, except for occasional HMNA-positive cells (arrows) that probably represent tumor cells that were trapped with the reactive T cells when the Ficoll-Hypaque gradient separation was performed.

was examined in the presence or absence of BCGF. In addition, the effect of insolubilized PAS, a human B cell mitogen,[16] was also examined in this context. Table 2 shows the proliferative capability of HMNA$^+$, monoclonal small B cell type lymphoma populations obtained from various types of NHL. It appears that α-μ alone can induce some proliferation in the B lymphoma cell populations, but the neoplastic cells appear to respond in greater magnitude to BCGF. The magnitude of response to both α-μ and BCGF is

Table 2. Soluble Factor-Mediated Proliferation of Human Small B Cell Lymphomas

Patient No.	Cell Surface Phenotype*	Bkg (cpm)	BCGF†	$\alpha\mu$‡	$\alpha\mu$ + BCGF	PAS§
Well-differentiated lymphocytic, diffuse (WDL) type‖						
1	$\mu\delta\gamma\kappa$ B1, B2, HMNA$^+$T$_{11}$	1,350	11,935¶	1,692	13,620	3,840
2	$\mu\delta\lambda$ B1, B2, B4, HMNA$^+$T$_{11}$	1,168	9,196	846	15,140	8,254
3	$\mu\kappa$ B1, B2, Leu1, HMNA$^+$T$_{11}$	202	4,168	1,886	4,076	2,296
4	$\mu\delta\lambda$ B1, B2, HMNA$^+$T$_{11}$	442	8,159	5,166	20,574	9,226
5	$\mu\delta\kappa$ B1, Leu1, HMNA$^+$T$_{11}$	860	4,471	4,376	11,078	6,753
Nodular poorly differentiated lymphocytic (NPDL) type						
1	$\mu\delta\gamma\kappa$ B1, B2, HMNA$^+$T$_{11}$	256	3,126	2,435	6,794	3,450
2	$\mu\delta\lambda$ B1, HMNA$^+$T$_{11}$	208	5,017	1,625	5,364	28,340
3	$\mu\kappa$ B1, B2, HMNA$^+$T$_{11}$	768	5,768	1,296	6,842	21,860
4	$\mu\gamma\kappa$, Leu1, B1, HMNA$^+$T$_{11}$	788	13,798	2,246	14,687	22,140
5	$\mu\gamma\kappa$ B1, B2, HMNA$^+$T$_{11}$	320	2,708	2,196	6,753	5,880
Nodular mixed cell type						
1	$\mu\kappa$ B1, B2, HMNA$^+$T$_{11}$	492	3,617	2,301	6,791	6,195
2	$\mu\delta\kappa$ B1, HMNA$^+$T$_{11}$	572	4,888	2,233	6,848	15,275

*Tumor cell populations were En rosetted and adherent cell depleted before phenotyping with monoclonal antibodies and HMNA by immunofluorescence. An antigen was considered positive if >80% of the tumor cell population was positive.

†BCGF preparations were obtained from PHA stimulated LyCM after DEAE, P-30, and P/100 gel filtration,[14] and used at a 10% vol/vol concentration in culture wells containing 0.2 × 10^6 cells per well.

‡$\alpha\mu$-Conjugated agarose beads (Biorad) were added at a concentration of 15 μg/mL final concentration.

§Protein A-Sepharose beads (Pharmacia) were added at a concentration of 5% vol/vol.

‖Modified Rappaport classification.

¶Cells were cultured for 96 hours at 37 °C and labeled with 0.5 μCi of [^{3}H]-Tdr (6 Ci/mmol) for the final 24 hours. [^{3}H]-Tdr incorporation is the mean of triplicate culture wells. Results are expressed as cpm of experimental minus control (E − C). The SEM for triplicates within an individual experiment were approximately 5%.

Table 3. Growth Factor Stimulation of Reactive and Normal Human Lymph Nodes

Pt. No.	Cell Surface Phenotype*	Bkg (cpm)	BCGF†	$\alpha\mu$‡	$\alpha\mu$ + BCGF	PAS§
A. Reactive lymph node hyperplasia						
1	$\mu\delta\gamma\kappa\lambda$, B1, B2$^+$, HMNA$^-$ T_{11}^-	1,786	3,292‖	1,042	18,109	13,296
2	$\mu\delta\gamma\kappa\lambda$, B1$^+$, HMNA$^-$ T_{11}^-	1,038	9,392	19,322	23,543	16,660
3	$\mu\delta\kappa\lambda$, B1$^+$, HMNA$^-$ T_{11}^-	1,372	2,931	4,072	12,024	10,013
4	$\mu\delta\gamma\kappa\lambda$, B1$^+$, HMNA$^-$ T_{11}^-	1,568	4,796	2,750	15,912	7,648
B. Normal lymph nodes						
5	$\mu\delta\gamma\kappa\lambda$, B1$^+$, HMNA$^-$ T_{11}^-	674	5,356	3,819	15,290	10,329
6	$\mu\delta\gamma\kappa\lambda$, B1$^+$, HMNA$^-$ T_{11}^-	1,376	7,709	18,412	37,443	5,041
7	$\mu\delta\gamma\kappa\lambda$, B1$^+$, HMNA$^-$ T_{11}^-	289	2,483	1,297	11,940	11,584

*Lymph node B cells were purified by double En rosetting and adherence. The resulting cell population was assayed for T and B cell associated markers and HMNA by immunofluorescence.

†BCGF was prepared from PHA-stimulated LyCm as before and was lectin free. The BCGF preparation was used at a final concentration of 10% vol/vol.

‡Anti-μ beads (Biorad) were used at a final concentration of 15 μg/mL.

§Protein A-Sepharose beads (Pharmacia) were used at a final concentration of 5% vol/vol.

‖Cells were incubated at 37 °C for 96 hours in a 5% CO_2 atmosphere; 0.5 μCi of [^{3}H]-Tdr (6 Ci/mmol) was added 24 hours prior to harvest; cpm are expressed as mean of triplicate cultures, experimental minus control (E − C). SEM was less than 10% for triplicates within individual experiments.

relatively low when compared with normal lymph node controls (Table 3), but all cases showed the ability to respond to the growth factor to some degree. The small B cell lymphomas generally showed less synergy with α-μ plus BCGF stimulation than is characteristic of normal B cells, but several cases did show at least some synergy. Most tumor cell populations also demonstrated the capability of being stimulated by insoluble PAS, although the magnitude of the response was somewhat variable (Table 2). Additional experiments with IL 1 preparations, greatly purified but not homogeneous IL 2, and cloned γ-interferon (IFN) showed no significant proliferative responses in vitro (data not shown).

Similar experiments were then carried out on HMNA-positive cells from large cell lymphomas of B cell type. The data are shown in Table 4. In contrast to the small cell NHL, large cell NHL cell populations appear to be generally refractory to activation by either mitogen or growth factor. Of significance is the absence of even a small stimulatory response that can be activated by anti-μ or BCGF alone. Even the response to PAS was minimal as compared with that obtained with small cell NHL.

Table 5 presents results obtained with representative B cell lymphomas, small and large cell type, when dose–response relationships were investigated. Again it can be seen that the small B cell lymphomas respond to the growth factor to varying degrees whereas the large cell lymphomas show little response.

Finally, the response of B cells from normal and hyperplastic lymph nodes to anti-μ was tested in the presence or absence of BCGF. The results of these experiments are shown in Table 5. Synergy between the anti-μ and BCGF responses can be seen as expected. It can also be observed that a subfraction of these cells can be induced into S-phase entry by anti-μ or BCGF alone. In general, B cells from these nodes

Table 4. Proliferation Studies on Human Large Cell Lymphomas (LCL)

Pt. No.	Cell Surface Phenotype*	Dx	Bkg (cpm)	BCGF†	$\alpha\mu$‡	$\alpha\mu$ + BCGF	PAS§
1	$\mu\lambda$, B1, B2, HMNA$^+$ $T_{11}T_3^-$	LCL-B type¶	278‖	660	220	960	1,260
2	$\mu\kappa$, B1, B2, HMNA$^+$ T_{11}^-	LCL-B type	372	1,218	201	1,227	530
3	$\mu\gamma\kappa$, B1, B2, B4, HMNA$^+$ T_{11}^-	LCL-B type	200	256	320	200	3,368
4	$\mu\kappa$, B1, B2, Leu1, HMNA$^+$ T_{11}^-	LCL-B type	110	290	146	310	360
5	$\mu\lambda$, B1, B2, Leu1, HMNA$^+$ T_{11}^-	LCL-B type	240	347	116	690	1,645

*Fresh tumor cells in suspension were enriched for tumor cells by single or double En rosetting and adherent cell depletion. These cell suspensions were then assayed for T and B cell associated antigens with monoclonal antibodies and for HMNA by immunofluorescence.

†BCGF was prepared from PHA-stimulated LyCm after DEAE chromatography and gel filtration as before.

‡Anti-μ beads (Biorad) were used at 15 μg/mL final concentration.

§Protein A-sepharose beads (Pharmacia) were used at 5% final concentration.

‖Cells per microtiter (0.2×10^6) well were incubated at 37 °C for 96 hours; 0.5 μCi [^{3}H]-Tdr (6 Ci/mmol/L was added for the last 24 hours. Results are expressed as cpm Experimental minus control (E − C). SEM for triplicates within an individual experiment were approximately 5%.

¶Diagnosis was made from the combination of H & E-stained paraffin sections plus immunophenotype obtained from either frozen sections or cytospin preparations after immunofluorescent or immunoperoxidase staining with monoclonal antibodies.

Table 5. Dose-Response Characteristics of BCGF-Mediated B Lymphoma Cell Proliferation

BCGF Conc.* (Percentage vol/vol)	Pt. 1† (WDL)	Pt. 2 (WDL)	Pt. 3 (LCL)	Normal‡ $\alpha\mu$-B cells
0	1,036	1,324	108	2,661
1.25	6,626	2,640	216	6,680
2.50	10,638	3,060	446	9,886
5.00	12,960	4,220	729	13,990
10.00	17,424	7,290	1,608	28,718
20.00	19,970	9,770	1,818	33,981

*BCGF was diluted serially into microwells containing 0.2×10^6 cells per well.

†NHL cells were E rosetted and adherent cell depleted as described. Tumor cells were >90% viable at the time of plating, and >95% were monoclonal by Ig light chain typing and expressed the HMNA.

‡Normal B cells were prepared from peripheral blood as previously described,//R10 and treated with anti-μ beads (15 μg/mL).

§Data are expressed as mean cpm of triplicate assays; SEM was <10%.

showed a moderate response to PAS, somewhat lower than the usual response of separated human peripheral blood B cells.

Growth of Human Lymphoma Cells In Vitro

The previously presented data indicated that human small cell lymphomas responded to greatly purified interleukin growth factor (BCGF) in tritiated thymidine incorporation proliferative assays. It was also important to ascertain if growth factor-dependent lymphoma cell growth could be obtained in vitro. Figure 2 shows that when E-rosette– and adherent cell-depleted monoclonal populations of well-differentiated lymphocytic lymphoma (WDL), B cell type (sIg^+, $HMNA^+$, $T11^-$), were cultured in vitro in the presence of BCGF, the tumor cell population was observed to increase in cell numbers. The cell surface phenotype of the cells that were found to increase corresponded to the original tumor cells when assayed on a weekly basis, and the cells present after five weeks of in vitro culture were identical in cell surface phenotype (sIgM, D^+, $HMNA^+$, $T11^-$) and morphology to the beginning lymphoma cell population. Growth was observed for up to five weeks in several B cell NHL, and a representative growth curve is shown in Fig 2. In each case, lymphoma cell growth was observed to be growth factor dependent, as lymphoma cells taken off the factor source decreased in number as well as viability, and the cultures usually died out within seven to ten days.

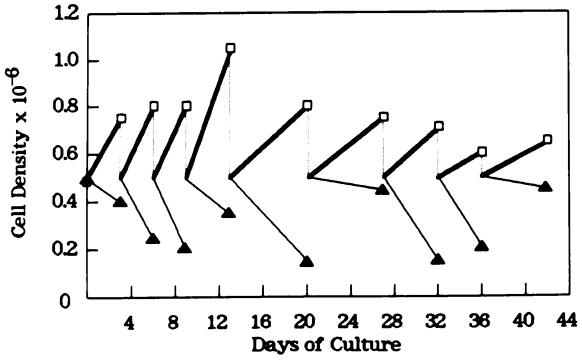

Fig 2. Growth factor-dependent cell growth in a case of well-differentiated lymphocytic lymphoma (WDL) in which the tumor cells were monoclonal ($\mu\kappa$ $B1^+$ $HMNA^+$, T_{11}^-) after removal of En rosette positive and plastic adherent cell populations. The tumor cells were grown in 10% vol/vol BCGF containing media, with cell counts and viability determined at three- to four-day intervals. At termination of the experiment, the cells were both morphologically and phenotypically ($\mu\kappa B1^+$ $HMNA^+$, T_{11}^-) identical to the staining lymphoma cell population. Cells were plated at 0.5×10^6 per well and counted at four-day intervals with growth factor (□) or without growth factor (▲).

DISCUSSION

Control of cell proliferation in neoplastic human lymphoid cells is an area of considerable importance in the biology of these neoplasms. Little information about this subject is currently available, however, except for the empiric observations that the putative tumor cells fail to grow in vitro under usual cell culture conditions.[17] It has been reported that malignant T cells from adult T cell leukemia (ATL) can respond to IL 2 in vitro,[4] and anti-Ig plus T cell factors have been shown to stimulate proliferation in chronic lymphocytic leukemia (CLL) cells.[18] There has been speculation that certain small B cell lymphomas are capable of at least some degree of immunoregulation, because of the large numbers of T cells present in the lesions.[19,20] The present study demonstrates that, under certain defined conditions, some small cell type B lymphoma cells can be induced to proliferate by normal immunoregulatory molecules, such as greatly purified but not homogeneous human B cell growth factor, and to a lesser degree by normal B cell activating signals such as anti-immunoglobulin and mitogenic lectin. From preliminary cytofluorography studies, it was estimated that a relatively small percentage of the tumor cells present in the lymphoma lesions studied responded to stimulation by the growth factor alone. This suggests that a relatively small fraction of the tumor cell population is spontaneously "activated" in vivo to the extent that only one signal (ie, growth factor) appears to be required for induction to S-phase entry. One must consider the possibility, because of the cell separation procedures used, that a subfraction of responding lymphocytes may consist of nonmalignant B cells (or other cells) present within the putative malignant cell population. However, since less than 5% of the tumor cell populations used were nonmalignant cells by our

phenotyping criteria, which could include T cells, macrophages, or normal B cells, we feel that it is unlikely that the observed proliferative responses are caused by a minor contaminating nonmalignant cell population. In addition, from the large cell lymphoma data, in which the tumor cells again are admixed with some normal lymphoid cells, there is no significant proliferation, suggesting that the thymidine incorporation observed in the small cell type lymphoma cell is not accounted for by a residual reactive lymphocyte population.

One interesting observation with B lymphoma cells is the general lack of synergy between anti-μ and BCGF. It has been shown with normal B cells that anti-μ (or other activators) activate a large fraction of the G_0 B cells that subsequently respond to BCGF and are then induced into S-phase entry.[21] Neoplastic human B cells have been reported to be refractory to anti-μ alone but to proliferate when phorbol ester (TPA) is added.[22] The majority of malignant B cells from a lymphomatous lesion in our study appear to be refractory to activation by anti-μ and subsequent response to BCGF, perhaps suggesting that only the proliferating pool of the tumor may be responsive to the growth factor. The majority of cells present in a lymphomatous lesion may no longer be part of the growth fraction and could therefore be refractory to growth factor stimulation. Such a mechanism would imply the presence of specific growth factor receptors on the lymphoma cells and that could be up or down regulated. It is unclear at this time whether the cells responding to anti-μ and/or PAS are also functionally quiescent tumor cells that can be activated in a manner analogous to their normal lymphocyte counterparts. The presence of Tac antigen, which is associated with the IL 2 receptor on normal activated T cells, on the neoplastic B cells of hairy cell leukemia[23] implies that growth factor receptors may play a significant role in the proliferation of neoplastic lymphoid cells. Unfortunately, a monoclonal antibody to the BCGF receptor has not as yet been reported, and the biochemical characteristics of such a receptor are unknown.

Another possibly significant observation from these studies is that the large cell lymphomas (LCLs) in general appear to be relatively unresponsive to either anti-μ or BCGF, as well as to B or T cell mitogens when tested immediately upon removal from the patient. This refractoriness to known lymphoid cell stimulating agents may suggest that some type of autonomous cell growth may occur in vivo in these tumors. Another explanation might involve an increased propensity for cell death in vitro in these lymphomas, although both the large and small cell type tumor cells were usually at least 50% viable at the termination of the assays (96 hours) reported. Other cytokines such as IL 1, IL 2, and γ-IFN also showed no proliferative activity for LCL or for that matter on the small cell type lymphomas as well (data not shown). Epstein and Kaplan were able to establish a number of this type of lymphoma (diffuse "histocytic" or LCL) into permanent tumor cell lines spontaneously by trying various combinations of culture media with a variety of serum supplements.[24] The lack of response to immunoregulatory molecules such as the interleukin growth factors could indicate a lack of regulatory sensitivity in these tumor cells, which may be one reason why the large cell variety of lymphoma generally tends to be more clinically aggressive than most lymphomas of the small cell variety.[25]

The finding that human lymphoma cells can react to normal lymphoid growth factors raises a number of important questions about the control of cell growth in lymphoid neoplasms. At least two possibilities can be considered: (1) that the lymphoma cells can react to growth factors made by normal autochthanous accessory cells (presumably primarily T cells); or (2) the tumor cells can react to a growth factor(s) they make themselves (autocrine stimulation) that is either identical to the normal growth factor or possibly related to it. Because a variety of lymphoblastoid cell lines have been recently described that display autostimulatory characteristics,[26-28] the latter possibility may prove to be valid. These studies, when combined with the large number of phenotypic studies made of human lymphoid neoplasms, imply that many of these tumors not only phenotypically resemble their normal lymphocyte counterparts but also share sensitivity to the same or similar immunoregulatory molecules.

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