

# Interleukin 3 Is a Growth Factor for Human Follicular B Cell Lymphoma

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## Summary

More than one-half of adults with non-Hodgkin's B cell lymphomas present with low-grade follicular lymphomas. These tumor cells are found in close association with follicular T lymphocytes and dendritic cells, suggesting that the surrounding cells may play a role in the support of follicular tumors. Supernatants from activated human peripheral blood lymphocytes were found to promote the in vitro proliferation of follicular tumor cells. This effect was entirely due to interleukin 3 (IL-3), a factor generally thought to cause the growth and differentiation of immature hematopoietic cells. IL-3 receptors were detected on fresh isolates of all primary follicular cell tumors examined. These findings suggest that follicular cell tumors may be dependent in vivo on IL-3 and that therapies directed against IL-3, its receptor, or the T cells that produce it may be effective treatment for follicular lymphoma.

Follicular lymphoma, one of the most common hematologic malignancies in humans, is usually an indolent although ultimately fatal disease in which the tumor cells grow in close proximity to T cells and follicular dendritic cells. The median survival for all patients with follicular lymphoma is >6 yr (1). For the majority of patients with follicular lymphoma, transformation to a diffuse large cell lymphoma signals the onset of a much more aggressive form of the disease in which the tumor cells appear no longer to require T cells or follicular dendritic cells. In these patients, median survival drops to <3 yr (1). Unfortunately, the biology of follicular lymphoma and the etiology of transformation from follicular lymphoma to the more aggressive large cell lymphoma have been difficult to study because of the low success rate in deriving cell lines in vitro. In this report, we show that the in vitro growth of some follicular lymphomas is dependent upon provision of IL-3.

## Materials and Methods

**Cytokines and Antibodies.** Human rIL-1-6 were purchased from Amgen Biologicals (Thousand Oaks, CA). Supernatant from PHA-P-activated human lymphocytes was prepared as described (2, 3). Neutralizing rabbit anti-IL-3s was obtained from R & D Systems, Inc. (Minneapolis, MN). Expression of IL-3Rs was detected by flow cytometry using reagents from R & D System, Inc. The BL antiidiotype was prepared as described (4). FITC-conjugated goat anti-mouse Ig, PE-conjugated goat anti-mouse Ig, and FITC-conjugated rabbit anti-human Ig were obtained from Tago, Inc. (Burlingame, CA).

**Cells.** The BLT cell line was generated as follows. A female patient presented in 1980 with progressive lymphadenopathy. Lymph node biopsy in 1981 revealed follicular lymphoma, small cleaved type. The patient was treated with combined chemotherapy between 1981 and 1983 with partial response and received antiidiotype immunotherapy and plasmapheresis in 1983. A splenectomy was performed in 1984. A single cell suspension was prepared and aliquots frozen in liquid nitrogen in RPMI 1640 supplemented with 10% DMSO and 20% FCS. Analysis of thawed cells revealed 60% to be tumor B cells, as judged by antiidiotype staining, and the remainder to be T cells. After thawing, spleen cells were cultured in medium supplemented with 10% supernatant from PHA-P-activated peripheral lymphocytes. Growth was observed after ~8 wk. All other tumor cells were obtained as fresh or frozen samples of spleen or lymph node.

**Tumor Cell Growth.** Proliferation ( $^3\text{H}$ ]thymidine incorporation as mean cpm  $\pm$  SE of 12 replicates) of BLT cells or primary tumor cells was determined as follows.  $3 \times 10^3$  BLT cells or  $10^4$  primary tumor cells were cultured in round-bottomed microtiter wells in RPMI 1640 supplemented with 15% FCS (HyClone Laboratories, Ogden, UT) and 2 mM L-glutamine for 7 d. Cultures were pulsed with  $1 \mu\text{Ci}$  [ $^3\text{H}$ ]thymidine for 16 h before harvest and counted in a scintillation counter. rIL-3 was added at a final concentration of 10 U/ml unless otherwise indicated. Supernatant from activated PBL was added at 10% final concentration.

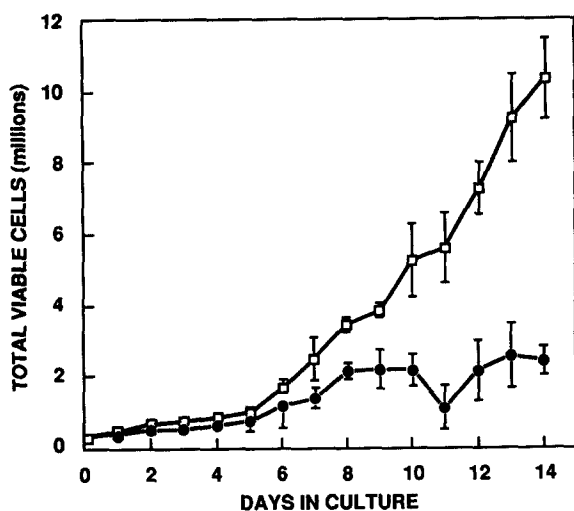
## Results

A cell line designated BLT was established by culturing previously frozen spleen cells isolated from a patient with follicular B cell lymphoma. The growth of this cell line was

completely dependent upon provision of media from mitogen-activated peripheral blood cells (2, 3) (Fig. 1). Exponential growth with a doubling time of  $\sim 4$  d was detected 10 wk after the initiation of culture.

The original splenic tumor expresses an Ig determinant (idiotype) that is unique to that particular tumor. To establish the origin of the cultured cell line, cells were analyzed for expression of the tumor idiotype and Ig isotype on a FACS<sup>®</sup> (Becton Dickinson & Co., Mountain View, CA) (Fig. 2). The majority of the cells in the BLT cell line expressed the tumor idiotype and were surface IgM<sup>+</sup>. However, a portion of the BLT cells were IgM<sup>+</sup>, idiotype<sup>-</sup>. This phenotype was stable over many months of culture. To test whether the BLT cell line was composed of a mixture of tumor and nontumor cells, on two occasions, cells were stained with antiidiotype and sorted on a FACS<sup>®</sup>. The resulting population (>95% idiotype positive) was put back in culture. After 2 wk, the profile was nearly identical to that shown in Fig. 2, with only  $\sim 75\%$  of the cells staining with antiidiotype. Thus, the idiotype-negative population may be due to selective loss of the idiotype determinant during culture or to the outgrowth of a second idiotype-negative population. Further confirmation that the cell line was derived from the original tumor came from Southern analyses of the Ig rearrangements. Identically rearranged bands were detected in the original tumor and the BLT cell line, but not PBL, using probes specific for C<sub>μ</sub> and J<sub>H</sub> (not shown). Finally, the t(14;18) translocation typical of most follicular lymphomas was present in both the original tumor and the BLT cell line (not shown).

As described above, the BLT tumor line was established in media supplemented with supernatants from mitogen-activated PBL. Attempts to grow the cell line in the absence

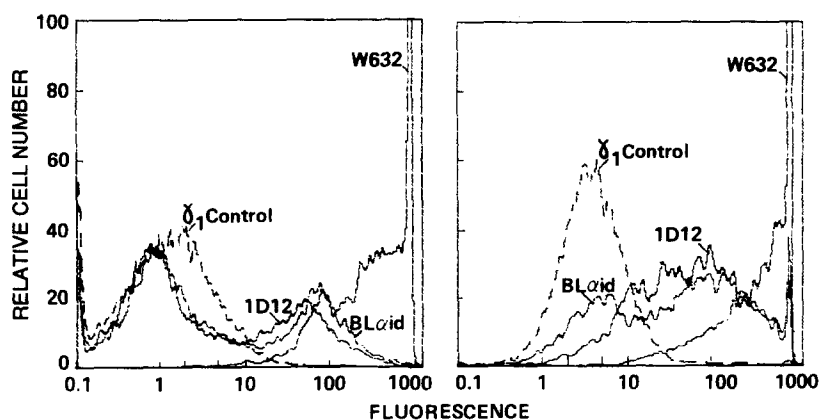


**Figure 1.** Growth of the BLT follicular tumor cell lines requires supernatants from mitogen-activated peripheral blood cells. BLT cells ( $10^6$ /ml) were washed twice in PBS and cultured in three 2-ml wells in the absence (●) or presence (□) of 10% supernatant from PHA-P-activated peripheral blood cells. Aliquots were removed daily and assessed for viability by trypan blue exclusion. Cultures were supplemented with fresh media every 3 d and split as necessary.

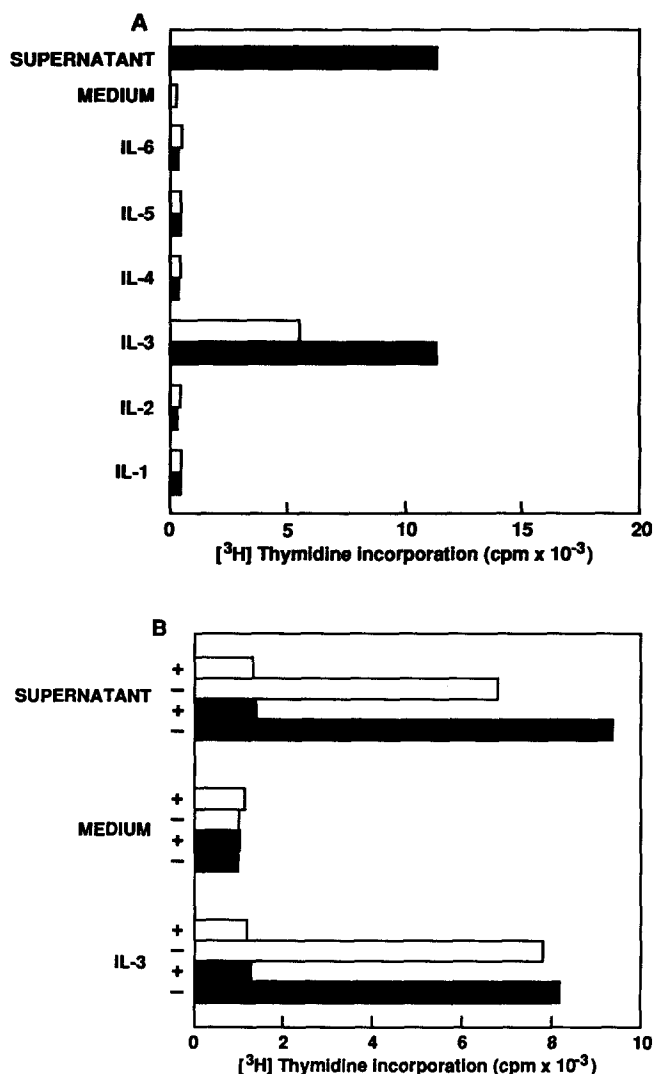
of these supernatants were unsuccessful, indicating that growth of the BLT tumor line required factors produced by activated white blood cells. To determine which, if any, known molecules stimulated the growth of this follicular tumor cell line, BLT tumor cells were cultured in the presence of a panel of recombinant human cytokines, and [<sup>3</sup>H]thymidine incorporation was assessed (Fig. 3 A). IL-3 induced proliferation equivalent to that caused by PBL supernatants. In contrast, IL-1, -2, -4, -5, and -6 did not cause increased [<sup>3</sup>H]thymidine incorporation relative to the medium alone. Moreover, various combinations of IL-1-6 did not result in any alteration in the pattern of proliferation induced by each interleukin alone (not shown).

Supernatants from activated PBL contain many molecules other than IL-1-6 (4). To test whether the proliferation of the BLT line in response to activated PBL medium was solely in response to IL-3, neutralizing antibodies were added to the proliferation assays (Fig. 3 B). [<sup>3</sup>H]Thymidine incorporation was completely abrogated when anti-IL-3 antibodies were added to cultures supplemented with either activated PBL supernatant or rIL-3. These data demonstrate that the *in vitro* proliferation of the BLT follicular lymphoma cell line is dependent upon IL-3.

Although the BLT cell line is clearly derived from the primary follicular tumor, it remained possible that the stimulatory effect of IL-3 on cell proliferation was unique to that cultured cell line. Therefore, the effects of rIL-3 and activated PBL supernatants on the *in vitro* proliferation of a panel of primary follicular lymphomas was examined. In all four cases tested, addition of activated PBL supernatants or rIL-3 resulted in marked increases in [<sup>3</sup>H]thymidine incorporation relative to that induced by medium alone (Table 1). To ensure that the proliferating cells were in fact tumorous, a case was selected for which a mAb specific for the tumor idiotype was available. Cells were obtained from excised spleen and cultured for 4-6 wk in medium supplemented with rIL-3 or with PHA-activated T cell supernatant (Fig. 4). Cells cultured with IL-3 were 100% positive for staining with the antitumor idiotype antibody, whereas only T cells were detectable in parallel cultures grown for 4-6 wk in the presence of T cell supernatants containing IL-2. In our experience, the ability of supernatants from activated T cells to promote the growth of T cells or tumor cells can vary for each patient sample. rIL-3 did not have any effect on the proliferation of peripheral blood cells and normal splenocytes (Table 1), or a variety of other primary B cell tumors, including diffuse large cell, Burkitt's, and chronic lymphocytic leukemias (not shown). Moreover, all samples of primary follicular lymphomas tested expressed high levels of IL-3Rs (Fig. 5). The single case of large cell lymphoma that was examined was also positive for expression of IL-3Rs. In contrast, PBL and normal splenocytes were negative. Two-color FACS<sup>®</sup> analysis was used to show that in a sample of fresh follicular lymphoma, all cells that expressed IL-3Rs coexpressed Ig (Fig. 6). In contrast, a sample of primary Burkitt's lymphoma was surface Ig positive, but IL-3R negative (Fig. 6). Expression of IL-3Rs on an extended panel of other types of primary tumors has not been examined.



**Figure 2.** Expression of tumor-specific idiotype by the primary tumor and BLT tumor cell line. Previously frozen spleen cells containing the primary tumor (*left*) or the BLT tumor line (*right*) were stained with murine mAbs specific for HLA-class I (W632), IgM (1D12), the tumor idiotype (BL anti-id), and an isotype-matched negative control ( $\gamma_1$  control). After washing, cells were stained with FITC-conjugated goat anti-mouse Ig and analyzed on a FACS IV<sup>®</sup> (Becton Dickinson & Co.). The spleen cells (*left*) are a mixture of tumor and nontumor cells, whereas the BLT cell line (*right*) is largely tumor cells.



**Figure 3.** IL-3 stimulates the *in vitro* proliferation of both the BLT follicular cell line and fresh follicular tumor cells. (A) [<sup>3</sup>H]Thymidine incorporation by the BLT follicular cell line. (B) Anti-IL-3 antibody abrogates BLT proliferation induced by either rIL-3 or supernatant from activated peripheral blood cells. 10 U anti-IL-3 (+); no antibody (-). Supernatants were added at a final concentration of 10% (filled bars) or 5% (open bars); lymphokines were added at a final concentration of 10 U/ml (filled bars) or 1 U/ml (open bars).

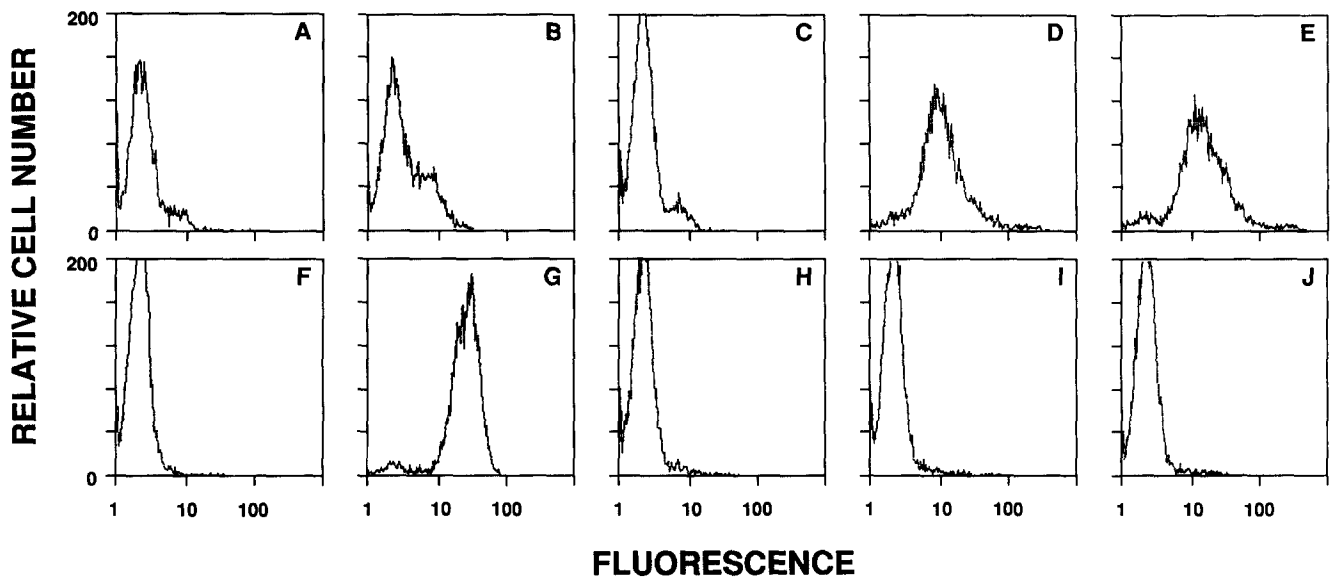
## Discussion

The finding that IL-3 induced proliferation of follicular lymphoma cells *in vitro* is surprising. IL-3, also known as multi-colony stimulating factor (M-CSF), has been demonstrated to promote the survival, proliferation, and development of multi-potential hematopoietic stem cells and committed progenitor cells of the granulocyte/macrophage, erythroid, eosinophil, megakaryocyte, mast cell, and basophile lineages (5-8). IL-3 also enhances myeloid end-cell functions, such as phagocytosis, antibody-dependent cellular cytotoxicity, and monocyte cytotoxicity (9-12). Recently, Le Bien and co-workers showed that both normal and leukemic human B cell precursors proliferate to IL-3. Follicular lymphomas are tumors of mature B cells, as judged by their functionally rearranged Ig genes and cell surface expression of Ig (14-16). However, it appears that the t(14;18) translocation of follicular lymphoma cells occurs at the pre-B cell stage (17-19). The

**Table 1.** Recombinant IL-3 Stimulates Proliferation of Primary Follicular Lymphoma Tumors *In Vitro*

	[ <sup>3</sup> H]TdR incorporation						
	Medium	IL-1	IL-2	IL-3	IL-4	IL-5	IL-6
	<i>cpm</i>						
Lymph node 1*	506	613	411	<u>10,121</u>	581	543	627
Lymph node 2	318	416	309	<u>8,546</u>	475	408	684
Lymph node 3	744	755	672	<u>12,321</u>	519	572	689
Lymph node 4	692	582	543	843	622	927	874
Spleen 1	543	619	892	<u>7,246</u>	629	613	811
Spleen 2	813	724	547	1,013	564	508	714
PBL 1	567	601	612	697	503	782	897
PBL 2	394	511	483	524	597	624	759

\* Lymph nodes 1-3 and spleen 1 were shown by pathologic examination to contain 60-80% follicular lymphoma cells. Lymph node 4 contained diffuse large cell lymphoma cells, while spleen 2 and PBL 1 and 2 were obtained from normal individuals.

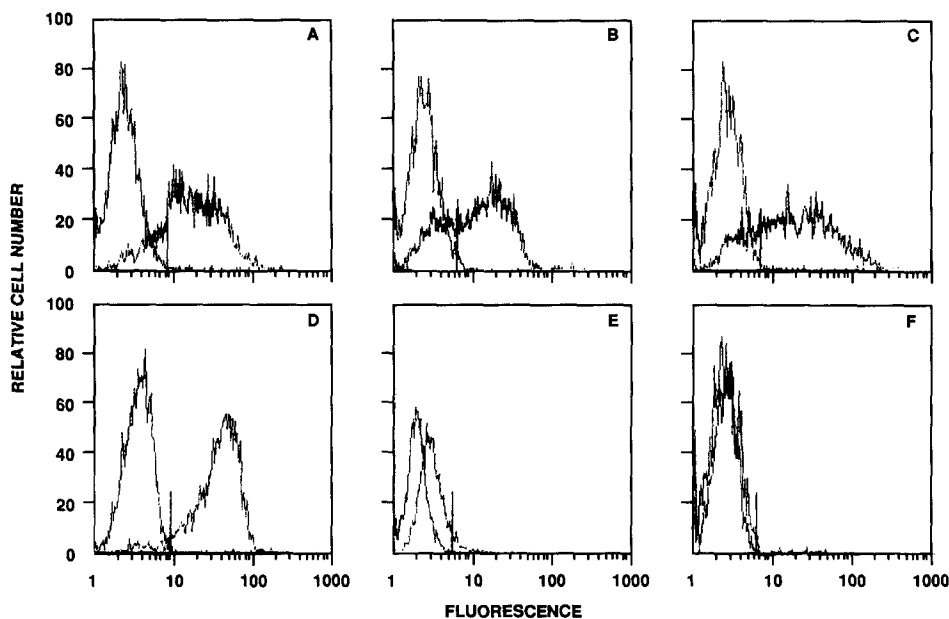


**Figure 4.** Follicular lymphoma cells grown in culture for 6 wk in medium supplemented with IL-3 express tumor idiotype. Cells isolated from the spleen of a patient with follicular lymphoma were grown in medium supplemented with 100 U/ml IL-3 (A-E) or 10% PHA-activated T cell supernatant (F-J). Cells were stained with an irrelevant antibody (A and F), OKT3 (B and G), anti- $\kappa$  (C and H), anti- $\mu$  (D and I), or antiidiotype (E and J), followed by FITC-conjugated goat anti-mouse Ig.

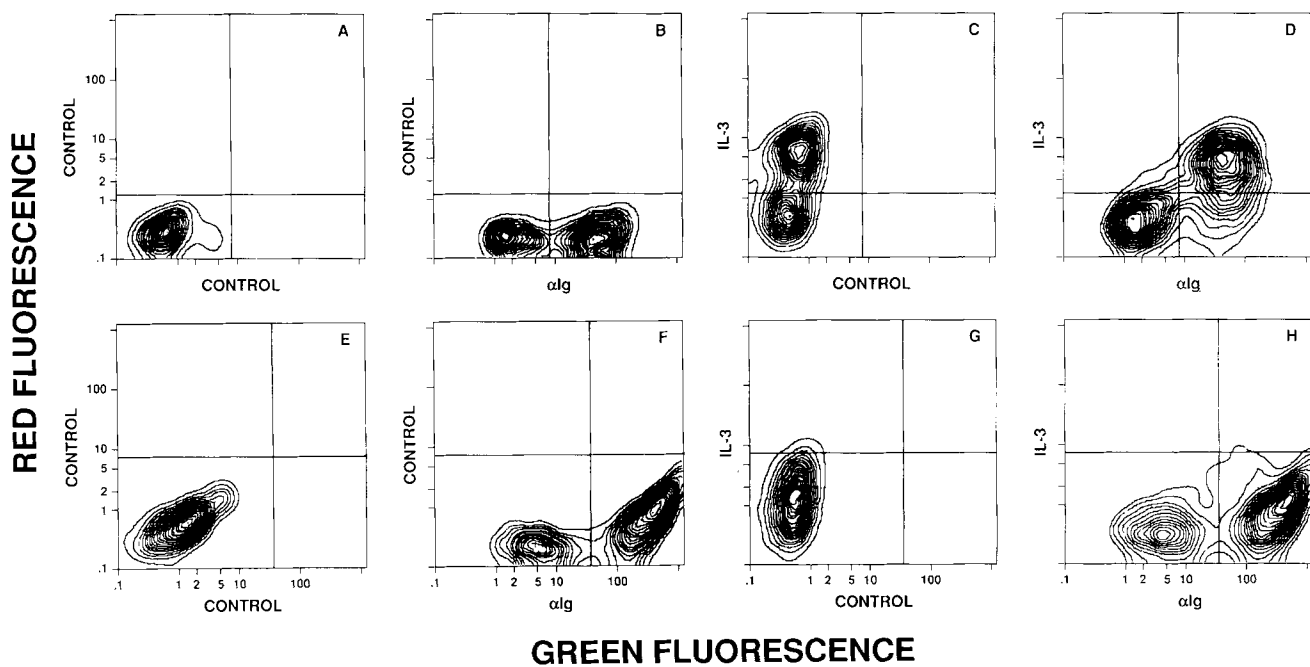
chromosomal breaks are focused at the 5' ends of  $J_H$  regions of the Ig genes, suggesting that the translocation occurred during attempted heavy chain joining at the pre-B cell stage. Thus, it is tempting to speculate that follicular lymphomas have retained some specific characteristics of pre-B cells, such as the ability to respond to IL-3.

Independent evidence supports the notion that T cells and T cell-derived factors may be involved in the maintenance or proliferation of follicular lymphoma tumors. Both  $CD4^+$  and  $CD8^+$  T cells are found in intimate association with fol-

licular lymphoma cells in vivo (1). It has been suggested that these T cells might facilitate the neoplastic process or mount an immune response against it (20). Umetsu et al. (21) showed that an HLA-DR-specific  $CD4^+$  T cell clone induced proliferation of some follicular lymphoma cells in vitro. Hoelzer and colleagues (22) recently conducted a clinical trial using IL-3 in patients with normal hematopoiesis or bone marrow failure. 19 patients with advanced tumors were treated with IL-3 to assess its toxicity and biological effects. Of these, four patients had either follicular or large cell lymphomas.



**Figure 5.** Follicular lymphoma cells express IL-3Rs. Cells isolated from lymph nodes from patients with follicular lymphoma (A-C), large cell lymphoma (D), an EBV-transformed B cell line (E), or PBL from a normal donor (F) were labeled with biotin-conjugated IL-3 followed by FITC-conjugated avidin and analyzed for fluorescence on a FACS IV<sup>®</sup> (Becton Dickinson & Co.). Background staining was assessed using FITC-conjugated avidin alone.



**Figure 6.** Coexpression of IL-3Rs and Ig by a primary follicular tumor. Cells isolated from lymph nodes of a patient diagnosed with follicular lymphoma (A-D) or Burkitt's lymphoma (E-H) were analyzed for expression of surface Ig and IL-3Rs.

Two of these patients experienced significant progression of their disease while receiving IL-3. The progression reversed spontaneously when IL-3 was discontinued.

Collectively, these results indicate a prominent role for IL-3

in the biology of follicular tumors and imply that therapies directed against IL-3, its receptor, or the T cells that produce it may prove effective for these clinically important human tumors.

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