Isolated Follicular Lymphoma Cells Are Resistant to Apoptosis and Can Be Grown In Vitro in the CD40/Stromal Cell System


Low-grade follicular non-Hodgkin’s lymphomas are characterized by the presence of a t(14;18) chromosomal translocation that results in deregulation of the B-cell lymphoma (Bcl-2) gene. Studies in cell lines and transgenic animal models have suggested that this results in the suppression of apoptotic cell death in germinal centers. B lymphocytes from normal germinal centers and lymph nodes infiltrated by follicular lymphoma were isolated by immunomagnetic depletion of cells bearing CD4, CD8, or IgD for study in vitro. Follicular lymphoma cells expressing Bcl-2 protein were shown to resist apoptosis after isolation, and could be induced to proliferate in a culture system previously described for the growth of normal B lymphocytes.

The B-cell lymphoma (Bcl-2) proto-oncogene is thought to be involved in the pathogenesis of follicular lymphoma. In the 85% of cases that carry the characteristic t(14;18)(q32;q21) chromosomal translocation, the Bcl-2 gene is juxtaposed to the Ig heavy chain locus,1,2 where it comes under the influence of the Eμ transcriptional enhancer. This results in dysregulation and overproduction of the normal Bcl-2 protein,3-4 the coding sequence being unaffected in the majority of cases.5 Although its precise mechanism of action is unknown, the Bcl-2 protein is thought to suppress apoptosis. Evidence for this includes (1) the inhibition of apoptosis in interleukin-3 (IL-3)–dependent cell lines deprived of growth factors but transfected with a Bcl-2 construct,6 and (2) resistance to apoptosis in serum-deprived Burkitt lymphoma cell lines expressing large amounts of Bcl-2.7

In the process of affinity maturation within secondary lymph node follicles (or germinal centers), large numbers of Bcl-2 negative centrocytes are produced that appear to undergo selection by apoptosis.8 If these cells are artificially rescued by in vitro addition of antibodies such as anti-IgM, CD40, or CD23, they will express Bcl-2.9 Follicular lymphomas are thought to have their origins in the germinal center on the basis of their morphologic and immunophenotypic similarities.10,11 It is generally believed that overproduction of Bcl-2 protein prevents apoptosis in follicular lymphoma cells, although this has not been confirmed experimentally. However, follicular lymphoma specimens show lower numbers of apparent apoptotic figures than do those of benign follicular hyperplasia,12 an observation in line with previous ones describing low numbers of tingible body macrophages in follicular lymphoma.13

Studies in follicular lymphoma would benefit from an in vitro culture system. However only brief proliferative responses have been observed when follicular lymphoma cells are incubated with a variety of cytokines.14 Systems using mixtures of cell types to emulate the microenvironment of the germinal center have been investigated for prolonging the viability of B cells in vitro. Recently, Banchereau et al15 have described a culture system that uses both IL-4 and CD40 antibody presented by a mouse fibroblast (Ltk-) cell layer transfected with the CDw32 Fc receptor. The present study investigates the behavior of isolated follicular lymphoma cells in vitro, in comparison with isolated normal germinal center cells, and analyzes the effects of both IL-4 and CD40 on their growth using the in vitro culture system.

MATERIALS AND METHODS

Isolation of germinal center and follicular lymphoma cells. Benign hyperplastic tonsils were obtained with consent after surgical removal from patients with recurrent tonsilitis. Malignant lymph nodes were obtained from biopsies performed during the routine management of patients with follicular lymphoma. In all cases, the diagnosis was confirmed by morphologic and immunophenotypic analysis of fresh-frozen and paraffin-embedded sections.

Single-cell suspensions were prepared from biopsies and the mononuclear cell fraction isolated by density-gradient centrifugation on Ficoll-Hypaque (Nycomed, Oslo, Norway). The cells were labeled with biotinylated monoclonal antibodies (MoAbs) to CD4 and CD8 and a biotinylated polyclonal anti-IgD. The antibodies used were CD4-IgG1x clone 13B8.2 (Immunotech, Marseille, France) 50 μL/10^6 cells; CD8-IgG3 clone B9.11 (Immunotech) 50 μL/10^6 cells; and IgD (The Binding Site, Birmingham, UK) 15 μL/10^6 cells. Thereafter, streptavidin-coated paramagnetic microbeads (Miltenyi Biotec, Bergisch- Gladbach, Germany) were added at a concentration of 10 μL/10^6 cells and the cells passed down a magnetic cell separator (MACS) column (Miltenyi Biotec) according to the manufacturer’s instructions and collected on ice.

Immunophenotyping of isolated cells. The following antibodies were used: from Immunotech: CD3 clone UCHT1 and CD19 clone J4.119; from Becton Dickinson (Mountain View, CA); CD38 clone HB-7, HLA-DR clone L243, CD14 clone Mo-P9, and mouse IgG1 as a negative control; from Dako (Glostrup, Denmark); CD10 clone SS2/36; from British Biotechnology (Abingdon, UK): vascular cell...
adhesion molecule (VCAM) clone BBA5; from The Binding Site: polyclonal sheep anti-human IgG, x light chains, λ light chains, and anti-rabbit IgG, A, M as a negative control. The CD40 MoAb (clone G28-5) was kindly provided by Dr E. A. Clark and MoAb to Bcl-2 antirabbit IgG, A, M adhesion molecule (VCAM) clone BBA5; from The Binding Site: polyclonal sheep anti-human to 606-nm band-pass filter. HTC-rabbit F(ab), antimouse-Ig (Dako) (clone Bcl-2/100)” by Dr D. Y. Mason. These were grown to 90% confluence in 24-well culture plates (Costar, Boston, MA) before irradiation to 7,500 Gy in a linear accelerator. The isolated B cells described above were suspended in freshly prepared culture medium at a concentration of 5 x 10^5/mL, and 1 mL added to each well. B-cell culture medium was Iscove’s modified Dulbecco’s medium (IMDM; Gibco Biocult, Paisley, UK) with 50 µg/mL human transferrin, 0.5% (wt/vol) bovine serum albumin (BSA), 5 µg/mL bovine insulin, 1 µg/mL of each of oleic, linoleic, and palmitic acids (all from Sigma), 2% (vol/vol) heat-inactivated fetal calf serum (FCS), penicillin, and streptomycin (all from Gibco Biocult). Where appropriate, CD40 antibody was used at a final concentration of 1 µg/mL and recombinant human IL-4 (kindly supplied by Schering-Plough Research, Bloomfield, NJ) at 100 U/mL.

Cells were grown on the same monolayer for 7 to 10 days before splitting. They were removed by gentle pipetting, spun down, and resuspended in fresh medium before replating on a newly irradiated monolayer.

**Cell cycle analysis.** Cells were harvested from culture, fixed in 70% ethanol at -20°C and treated with RNAase (Boehringer-Mannheim, Mannheim, Germany) before staining in propidium iodide (10 µg/mL) with 10^5 cells in 500 µL. Flow cytometric analy-

### Table 1. Clinical Characteristics of Follicular Lymphomas Studied

<table>
<thead>
<tr>
<th>Patient</th>
<th>Clinical Status</th>
<th>Histology</th>
<th>Working Formulation</th>
<th>Light Chains</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.C.</td>
<td>Recurrence</td>
<td>Follicular centroblastic/ centrocytic</td>
<td>B</td>
<td>x</td>
</tr>
<tr>
<td>P.M.</td>
<td>Recurrence</td>
<td>Follicular centroblastic/ centrocytic</td>
<td>B</td>
<td>λ</td>
</tr>
<tr>
<td>D.K.</td>
<td>Presentation</td>
<td>Follicular centroblastic/ centrocytic</td>
<td>B</td>
<td>κ</td>
</tr>
<tr>
<td>J.D.</td>
<td>Recurrence</td>
<td>Transformed follicular</td>
<td>D</td>
<td>κ</td>
</tr>
<tr>
<td>S.W.</td>
<td>Recurrence</td>
<td>Transformed follicular</td>
<td>B and G mixed</td>
<td>λ</td>
</tr>
<tr>
<td>L.S.</td>
<td>Recurrence</td>
<td>Follicular centroblastic/ centrocytic</td>
<td>B</td>
<td>λ</td>
</tr>
<tr>
<td>J.C.</td>
<td>Recurrence</td>
<td>Follicular centroblastic/ centrocytic</td>
<td>B</td>
<td>κ</td>
</tr>
</tbody>
</table>

### Detection of apoptosis

The presence of apoptosis was detected by uptake of low concentrations of the fluorochrome Hoechst 33342 (Sigma, St Louis, MO) as previously described. Cells (10^5) were removed from cultures, spun down, and resuspended in 1 µg/mL Hoechst 33342 and 10 µg/mL propidium iodide (Sigma). After 2 minutes, fluorescence was analyzed using a FACStar plus (Becton Dickinson) with a 100-mW 351-363 nm UV argon ion laser (Spectra Physics, Hemel, Hempstead, UK) to detect excitation of Hoechst 33342 at 420-560 nm and a second 200-mW 488-nm argon ion laser to detect excitation of propidium iodide at over 580 nm. Cell debris was gated out using forward and side light scatter, and dead cells gated on the basis of propidium iodide uptake. Two-dimensional contour plots of forward scatter versus blue fluorescence were used to analyze the proportion of cells taking up Hoechst 33342 using Lysys II software (Becton Dickinson).

### B-cell culture using the CD40 system

Mouse fibroblast Ltk- cells transfected with the CDw32 Fc receptor were obtained from the American Tissue Culture Collection with the kind permission of Dr K. W. Moore. These were grown to 90% confluence in 24-well culture plates (Costar, Boston, MA) before irradiation to 7,500 Gy in a linear accelerator. The isolated B cells described above were suspended in freshly prepared culture medium at a concentration of 5 x 10^5/mL, and 1 mL added to each well. B-cell culture medium was Iscove’s modified Dulbecco’s medium (IMDM; Gibco Biocult, Paisley, UK) with 50 µg/mL human transferrin, 0.5% (wt/vol) bovine serum albumin (BSA), 5 µg/mL bovine insulin, 1 µg/mL of each of oleic, linoleic, and palmitic acids (all from Sigma), 2% (vol/vol) heat-inactivated fetal calf serum (FCS), penicillin, and streptomycin (all from Gibco Biocult). Where appropriate, CD40 antibody was used at a final concentration of 1 µg/mL and recombinant human IL-4 (kindly supplied by Schering-Plough Research, Bloomfield, NJ) at 100 U/mL.

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### Fig 1 Immunophenotype of isolated follicular lymphoma cells (patient S.W.).

- A: Mouse IgG1 negative control; B: CD3; C: CD19; D: IgD; E: VCAM-1; F: x light chains; G: λ light chains; H: CD38. The numbers for each graph indicate the median fluorescence channel, or the relative amount of antibody binding to the cells.
IN VITRO CULTURE OF FOLLICULAR LYMPHOMA

Detection of Epstein-Barr viral (EBV) DNA was performed with primers spanning a 286 bp segment of the BZLF-1 fragment as previously described. Control primers for the presence of amplifiable DNA spanning a 226-bp segment of the Bcl-2 major breakpoint region were used to amplify the untranslocated allele. Visualization of PCR products was performed by electrophoresis on 2% agarose gels and ethidium bromide staining for viewing under UV light.

Sequentialing of t(14;18) PCR products was performed by the di-deoxy chain termination method with a kit using T7 DNA polymerase (Pharmacia, Uppsala, Sweden). PCR products amplified using a biotinylated J consensus primer were held in solid phase for sequencing reactions using streptavidin-coated paramagnetic beads (Dynal, Oslo, Norway). Oligonucleotides (5'TP-labeled) to the Bcl-2 terminal of the PCR product were used for chain initiation. Reaction products were electrophoresed on 5% acrylamide gels.

RESULTS

Immunophenotype of isolated B lymphocytes. Eight lymph node biopsy specimens were used for the isolation of follicular lymphoma cells, with characteristics as shown in Table 1.

After isolation of the cells, all specimens showed a B-cell phenotype (CD19+, CD3−, HLA-DR+) with characteristics of germinal center cells (CD38+, CD10−, IgD−). No CD14+ or VCAM+ cells were detected, confirming the removal of follicular dendritic cells. All the lymphoma samples showed light chain restriction as detailed in Table 1, and all showed strongly positive staining for Bcl-2 protein. Figure 1 shows a representative immunophenotype and Fig 2A the result of immunocytochemical staining for Bcl-2.

By contrast, identically prepared B cells from eight benign hyperplastic tonsils and one reactive lymph node from a patient with follicular lymphoma at other sites showed no light chain restriction and negative results on staining for Bcl-2 (Fig 2B). However, in other respects their phenotype was identical to those from lymphomatous nodes. The uniform expression of CD38 and lack of Bcl-2 expression confirmed the elimination of memory B cells.

Isolated follicular lymphoma cells show prolonged viability without apoptosis. After isolation samples of cells were kept at 37°C. Figure 3 shows the changes in viability seen in these samples. Follicular lymphoma cells showed only a slight decline in viability over 72 hours when kept at 37°C, whereas tonsillar germinal center cells showed a marked decline with almost all cells dead at 72 hours. Neither cell type showed evidence of proliferation after isolation, the numbers falling in accordance with the loss of viability. The mode of death of the germinal center cells was characterized as apoptotic by flow cytometry (Fig 4). After 24 hours at least 50% of the viable cells showed the characteristic increased uptake of Hoechst 33342, whereas the follicular
lymphoma cells showed no appreciable change. That the population with increased Hoechst 33342 uptake was genuinely apoptotic was confirmed by flow-sorting of the two populations and fluorescence microscopy after acridine orange staining. The cells with increased uptake showed the characteristic peripheral condensation of chromatin seen in apoptosis as previously described, whereas the normal population showed diffuse nuclear staining (Fig 2C and D).

Isolated follicular lymphoma cells require the presence of CD40 antibody and IL-4 for growth in vitro. Isolated B cells were plated onto the Ltk- CDw32 fibroblast monolayer in the presence of CD40 antibody and IL-4, with one or the other, or neither. For both germinal center cells and follicular lymphoma cells only the presence of both factors supported a sustained increase in cell numbers as shown in Fig 5. After 3 days of proliferation the numbers of germinal center cells showed a plateau whereas the numbers of follicular lymphoma cells continued to increase. The reasons for this were explored using cell-cycle analysis (Fig 6). While the proportion of follicular lymphoma cells in S and G2 phases continued to increase throughout the first week of culture with CD40 and IL-4, in the cultures of germinal center cells it fell after day 3. This was accompanied by an increase in the proportion of cells with hypodiploid DNA content characteristic of apoptosis. This was seen in the first 2 days of culture where CD40 was absent, but appeared to be delayed by the presence of CD40 so that it only appeared after 72 hours. Less than 10% of follicular lymphoma cells in any sample showed hypodiploid DNA content, even in the absence of CD40.

Prolonged culture of t(14;18)-bearing cells can be performed in the CD40 system. Karyotypic analysis of cell cultures was performed after the third subculture (between 21 and 26 days of culture). The first case of lymphoma analyzed (R.B.) showed the t(14;18) translocation in 10 of 10 metaphases, but in the second (G.C.), occasional normal
cells were seen and only the 14q+ derivative was identified. The third malignant sample grown in culture (D.K.), prepared from a tonsil infiltrated by lymphoma, had 1 normal metaphase with the remaining 9 showing the t(14;18) translocation. After these observations lymphoma cells were kept at 37°C overnight after isolation and before plating out on the fibroblast monolayer, to deplete residual normal germinal center cells by apoptotic selection. No karyotypically normal cells were seen in three lymphoma specimens with the t(14;18) in all cells prepared in this way subsequently (P.M., S.W., J.D.). All the normal germinal center cells (including those from the reactive node from a patient with follicular lymphoma at other sites) and one case of follicular lymphoma showed a normal karyotype (Table 2). PCR studies performed after 1 month of culture confirmed the presence of a t(14;18) in four cases in which it was identified karyotypically and the one in which only 14q+ was seen. One t(14;18) detected by karyotyping could not be amplified (P.M.), presumably because the breakpoint fell outside either of the cluster regions. Comparison of the results of PCR of cultured cells and of biopsy specimens from the same patient showed that the size of amplified segment was the same in both for each case. That the same clone was present in both specimens was further confirmed by direct sequence analysis of the PCR products: In all five cases the Bcl-2 breakpoints, N regions, and Ig heavy chain breakpoints were the same (Table 3). PCR studies of the presence of EBV showed it in 1 of the 8 lymphoma specimens only.

Fig 5. Growth of B lymphocytes in vitro. Viable cell numbers per well in cultures of germinal center cells (A and B) and follicular lymphoma cells (C and D). Cells were grown on the mouse fibroblast Ltk-cell layer in the presence of CD40 antibody, IL-4, both, or neither. Each time-point represents the mean of three observations and two separate experiments are shown. The lymphoma specimens studied were from patients D.K. (C) and S.W. (D). (O) NIL; (m) IL-4; (A) CD40; (O) both.
Fig 6. Cell cycle analysis of cells growing on the Ltk-monolayer in the presence of CD40 antibody, IL-4, both, or neither. (A) and (B) show the S and G2 phase fractions for tonsillar germinal center cells; (C) and (D) show the S and G2 phase fractions for follicular lymphoma cells; (E) and (F) show the hypodiploid fraction for tonsillar germinal center cells. Ten thousand cells were analyzed at each time point and two separate experiments are shown. The lymphoma specimens studied were from patients D.K. (C) and S.W. (D). Symbols are as in Fig 5.
This was the specimen that showed a t(14;18) on karyotyping but that was not amplifiable by PCR.

**Characteristics of follicular lymphoma cells during culture.** The cultured cells grew in discrete colonies on the fibroblast layer. Cytospin preparations stained with May-Grünwald-Giemsa stain showed them to have a blastic morphology with some forms having protein-rich cytoplasm reminiscent of plasma cells. No small cleaved centrocytes could be distinguished after 1 week in culture. The cells remained CD19+ and CD10+ but lost expression of CD38 expression of apoptosis is well supported by the evidence from the studies of viability so that no judgement can be made as to the relationship between the translocation itself and resistance to apoptosis, but their growth characteristics in culture were identical to those of cells with a t(14;18) translocation.

The increased expression of Bcl-2 described in germinal center cells rescued from apoptosis with CD40 antibodies might suggest that cells from follicular lymphomas with constitutive overexpression of Bcl-2 could grow in the absence of the antibodies. This has been shown not to be the case. It must be concluded that the CD40 defined molecule has other roles apart from promoting Bcl-2 expression. Prominent among these may be homo- and hetero-typic adhesion and cell-cycle progression. The recent identification of the CD40 ligand as a type-II transmembrane glycoprotein (GP) on the surface of stimulated T helper cells suggests that it may be involved in T-cell/B-cell interaction within the germinal center and in lymphomas. The cognate interaction of CD4+ T cells with follicular lymphoma cells has been shown to induce short-term proliferation in vitro, an effect augmented by IL-4. In contrast, the use of IL-4 alone was shown not to induce proliferation, and in one study to inhibit the proliferation of lymphoma cells induced by IL-2 or antibodies to IgM. These results suggest that the effects of T-cell surface molecules and cytokines on normal and malignant germinal center B cells are highly complex, with the presence of CD4+ cells (or surrogates in the form of CD40 antibody-presenting fibroblasts) from follicular lymphomas are examined to see whether they support the hypothesis of increased Bcl-2 expression leading to suppression of apoptosis in the malignant tissue. This work has identified a method for the purification of follicular lymphoma cells by immunomagnetic depletion that should facilitate such studies in the future. The finding of improved viability with resistance to apoptosis in the isolated Bcl-2 positive follicular lymphoma cells supports the antiapoptotic hypothesis for the role of gene, although clearly other alterations might affect the malignant phenotype in similar ways. The next step will be to identify agents that can specifically inhibit the expression of Bcl-2 to discover whether they restore the tendency to apoptosis in these cells. The finding of increased Bcl-2 expression in two cases despite the lack of a t(14;18) translocation is in keeping with previous observations made using the same antibody. Unfortunately, these two cases were not included in the studies of viability so that no judgement can be made as to the relationship between the translocation itself and resistance to apoptosis, but their growth characteristics in culture were identical to those of cells with a t(14;18) translocation.

**DISCUSSION**

The suggestion that the Bcl-2 proto-oncogene acts by suppression of apoptosis is well supported by the evidence from cell lines and studies of affinity maturation in the normal germinal center. However, its role in the development of follicular lymphoma is more problematic. Bcl-2 transgenic mice do not develop follicular lymphomas, although they do have an expanded polyclonal pool of long-lived memory B cells. Instead, they go on to develop high-grade monoclonal lymphomas of immunoblastic or pre-B cell type, often after further oncogene rearrangements. An additional difficulty is the finding in PCR studies of t(14;18) rearrangements in apparently healthy individuals who do not go on to develop lymphoma, suggesting that a low background rate of recombination may be nonrecognitive.

Therefore, it is important that the characteristics of cells

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**Table 2. Karyotype and t(14;18) PCR Results for Cultured Cells**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Karyotype</th>
<th>t(14;18) PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>G.C.</td>
<td>14q+</td>
<td>MBR/J6</td>
</tr>
<tr>
<td>P.M.</td>
<td>t(14;18)</td>
<td>Negative</td>
</tr>
<tr>
<td>D.K.</td>
<td>t(14;18)</td>
<td>MBR/J6</td>
</tr>
<tr>
<td>R.B.</td>
<td>t(14;18)</td>
<td>MBR/J4</td>
</tr>
<tr>
<td>J.D.</td>
<td>t(14;18)</td>
<td>MCR/J5</td>
</tr>
<tr>
<td>S.W.</td>
<td>t(14;18)</td>
<td>MBR/J6</td>
</tr>
<tr>
<td>L.S.</td>
<td>46, XY</td>
<td>Negative</td>
</tr>
<tr>
<td>J.C.</td>
<td>ND</td>
<td>Negative</td>
</tr>
</tbody>
</table>

Abbreviation: ND, not done.

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**Table 3. Sequence Analysis of t(14;18) Translocations in Cultured Cells**

<table>
<thead>
<tr>
<th>R.B.</th>
<th>Major breakpoint region:</th>
</tr>
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<tr>
<td>TATGAACTCTATGTTTTTACCCACACATGGGCTATGTGTTATGGGTACTGACTACTGGGG</td>
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<tr>
<td>S.W.</td>
<td>Major breakpoint region:</td>
</tr>
<tr>
<td>TGTTGATGAAAGCCAGACTCTCCCCTTATCTACCACGATGGACGT</td>
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</tr>
<tr>
<td>D.K.</td>
<td>Major breakpoint region:</td>
</tr>
<tr>
<td>GGGGGGFIGTTCTGGGCTGGGATCGGACATCTGTGTACTACATACGCC</td>
<td></td>
</tr>
<tr>
<td>G.C.</td>
<td>Major breakpoint region:</td>
</tr>
<tr>
<td>TCCCCGGGGGCTTTCTCATGCTGTACCTACTACGCTATGAGACTCT</td>
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</tr>
<tr>
<td>J.D.</td>
<td>Minor cluster region:</td>
</tr>
<tr>
<td>TGCAAAAACACGGATCTAATGGGCTTTCGCCACTGCCCAGCAGAAC</td>
<td></td>
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</table>

N-regions are shown underlined in bold type. A point mutation from the Jh germline in patient S.W. is underlined.
apparently able to turn IL-4 from an antagonistic to an agonistic growth factor.\textsuperscript{13,14,33} This may explain the apparently conflicting results from previous studies, particularly if the efficiency of depletion of tumor-infiltrating CD4\textsuperscript{+} cells was variable. The implications for the therapeutic use of IL-4 as a treatment for follicular lymphoma are uncertain; further in vitro studies using mixed cell populations will be needed before it can be recommended with any confidence.

The resistance of follicular lymphoma cells to apoptosis is further shown during culture in the CD40 system. Whereas normal germinal center cells showed an increasing proportion with hypodiploid DNA content as the culture medium became exhausted by the increase in numbers, the follicular lymphoma cells continued to proliferate despite a comparable expansion, suggesting a growth advantage in this system. However, the two cell types do show a requirement for the same growth factors. The absence of any significant numbers of follicular lymphoma cells with hypodiploid DNA even when IL-4 and/or CD40 were omitted suggests that when death of the cells does occur it may be by necrosis rather than apoptosis. An alternative explanation may be that the low death rate results in the numbers of apoptotic cells at any one time being too low to detect reliably. In either case the system shows a distinction in patterns of growth between normal and malignant B cells that appears to reflect their behavior in vivo to some extent.

That the cells grown in culture originate from follicular lymphoma rather than contaminating normal lymphocytes is suggested by their germinal center B-cell phenotype combined with a high level of Bcl-2 expression at the time of isolation and surface light chain restriction. This was confirmed by karyotypic analysis showing that the dividing cells carried the t(14;18) translocation. That these represent the lymphoma clone rather than de novo recombinations occurring in the lymph node or during culture is shown by the clonal identity between biopsy material and the cultured lymphoma cells continued to proliferate despite a comparable expansion, suggesting a growth advantage in this system. However, the two cell types do show a requirement for the same growth factors. The absence of any significant numbers of follicular lymphoma cells with hypodiploid DNA even when IL-4 and/or CD40 were omitted suggests that when death of the cells does occur it may be by necrosis rather than apoptosis. An alternative explanation may be that the low death rate results in the numbers of apoptotic cells at any one time being too low to detect reliably. In either case the system shows a distinction in patterns of growth between normal and malignant B cells that appears to reflect their behavior in vivo to some extent.

The ability to generate colonies of clonal cells should facilitate the study of mutations. It will also provide a means of culturing normal B cells from benign tissues to examine the incidence of the translocation.

The apparent differentiation of the cultured cells with alteration of their morphology and phenotype makes it unlikely that this system will provide a useful in vitro model for, eg, drug-sensitivity testing. It may be that the use of cytokines other than IL-4 will result in different characteristics being produced.\textsuperscript{37} and further studies to examine this are needed. Another facet of the culture system that has not been tested is the expression of idiotypic Ig on the cells' surface. This clearly has relevance to their possible use for the generation of anti-idiotypic antibodies for therapeutic purposes.

CONCLUSION

Isolated follicular lymphoma cells can be prepared by a rapid process of immunomagnetic depletion from involved lymph nodes. The cells show high levels of Bcl-2 expression, in contrast to similarly isolated cells from benign hyperplastic tonsils, and show enhanced survival when kept at 37°C whereas the Bcl-2 negative cells rapidly die by apoptosis. Cells bearing the t(14;18) rearrangement can be grown on a mouse fibroblast layer presenting CD40 antibodies in the presence of IL-4, and show clonal identity with the lymphoma from which they are derived. These observations provide support for the hypothesis that increased Bcl-2 expression leads to suppression of apoptosis during the development of follicular lymphoma, and the development of an in vitro culture system may allow this relationship to be explored further.

ACKNOWLEDGMENT

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