Response Patterns of Hairy Cell Leukemia to B-Cell Mitogens and Growth Factors

By Bruce A. Barut, Maria K. Cochran, Carl O’Hara, and Kenneth C. Anderson

The effect of mitogens and/or recombinant B-cell growth factors (M/GFs) on the in vitro growth of hairy cells was examined. Tumor cells were isolated from the spleen of four patients with hairy cell leukemia (HCL) by Ficoll-Hypaque sedimentation and E-rosetting. Enrichment for tumor cells was confirmed with intracytoplasmic immunoglobulin (Ig) staining, tartrate resistant acid phosphatase (TRAP) staining, and staining using monoclonal antibodies (MoAbs) directed at B, T, myeloid, and monocytoid antigens (Ag) in indirect immunofluorescence assays. Tumor cells were B1(CD20)+ B2(CD21)− B4(CD19)+ IL-2R(CD25)+ PCA-1 TRAP+. HCLs neither synthesized DNA nor secreted Ig in response to culture with granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-1α (IL-1α), IL-1β, IL-2, IL-3, IL-4, IL-5, or IL-6. However, a proliferative response (stimulation index ≥3.0) without Ig secretion was triggered in HCLs by mitogens or combinations of GFs. Specifically, DNA synthesis was induced in 3 days in three of four HCL samples cultured with *Staphylococcus aureus* Cowan A (SAC) or the combination of phorbol ester (TPA) and the calcium ionophore A23187 (Ca21+). DNA synthesis was triggered later (day 7) by tumor necrosis factor (TNF) or by IL-4 and IL-5. In contrast, the fourth patient, a nonresponder to SAC or TPA/Ca2+1, demonstrated increased DNA synthesis at day 3 when cocultured with IL-4 and IL-5. Both autoradiography and staining with antibradycyclorotic antibody (Brdu) MoAb conjugated to fluorescein confirmed DNA synthesis by only a minority (5% to 23%) of tumor cells within each patient. Dual staining confirmed that responsive cells were both Brdu+ and TRAP+. DNA synthesis induced by TPA/Ca2+ was blocked specifically by anti–IL-6 Ab; in contrast, the HCL proliferative response to SAC, TNF, or IL-4 and IL-5 was not inhibited by anti–IL-6 Ab. α-Interferon inhibited the response to TPA/Ca2+, TNF, or IL-4 and IL-5 without any effect on response to SAC. Finally, peroxidase–anti-peroxidase staining demonstrated that HCLs are induced by TPA/Ca2+, but not by SAC, to produce intracytoplasmic IL-6. These data demonstrate IL-4, IL-5, and IL-6 mediated DNA synthesis by HCLs in vitro and suggest a possible in vivo role for these growth factors in the pathophysiology of HCL.

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TNF induced DNA synthesis that does not appear to be related to IL-6. IFN inhibits these responses to IL-4 and IL-5, and to TNF, but does not block responsiveness to SAC.

These studies characterize the in vitro response of HCLs to GFs and support the view that GFs may play a role in the pathophysiology of HCL.

MATERIALS AND METHODS

Preparation of enriched HCL and normal B-cell populations. Splenic samples were obtained from four patients with HCL. The diagnosis of HCL was made on the basis of clinical presentation, as well as histopathologic (Wright-Giemsa) and histochemical (tartrate-resistant acid phosphatase, TRAP) staining of peripheral blood, bone marrow, and spleen. Patient samples were immediately placed in medium containing 5% fetal bovine serum (FBS), minced, extruded through stainless steel mesh, and cryopreserved until use. Ficoll Hypaque mononuclear cell preparations were enriched for tumor cells by E-rosette depletion of T cells and removal of monocytes by adherence to plastic Petri dishes (1 hour, 37°C). Normal spleens were obtained from two patients without any systemic or malignant disease. B cells were prepared as described above.

Characterization of HCL populations. HCL-enriched populations were characterized morphologically, histochemically, and phenotypically before and after culture with M/GFs. Morphology was examined by Wright-Giemsa staining, and HCL-associated histochemistry was defined by TRAP staining. The populations of enriched leukemia cells were also examined using monoclonal antibodies (MoAbs) directed at B, T, and myeloid Ags in indirect immunofluorescence assays as previously described. Monoclonal reagents used to characterize the HCLs included the following: anti-B4(CD19), B1(CD20), B2(CD21), interleukin-2R(IL-2R; CD25), and PCA-1, which are reactive with B cells at various stages of differentiation; anti-T3(CD3), T4(CD4), and T8(CD8), which identify T cells; and anti-Mol(CD11) and Mo2(CD14), which stain monocyteoid cells.

Culture of enriched hairy or normal B-cell populations with M/GFs. The recombinant GFs, granulocyte-macrophage colony stimulating factor (GM-CSF), IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, and IL-6, were either obtained from Genzyme (Boston, MA) or generously provided by Dr Steven Clark at Genetics Institute (Cambridge, MA). TNF was a gift from Dr Donald Kufu (Dana-Farber Cancer Institute, Boston, MA). These factors were used at concentrations of maximal activity in standard assays. Heat-killed formalin-fixed SAC was obtained from Calbiochem-Behring (La Jolla, CA); TPA and calcium ionophore A23187 were obtained from Sigma (St Louis, MO). These reagents were used at titers known to stimulate normal B cells. One hundred-microliter aliquots containing 5 x 10⁶ purified HCLs or normal B lymphocytes in RPMI/10% FBS medium were dispensed into 96-well round-bottomed tissue culture plates (Costar, Cambridge, MA). Cells were cultured in the presence of media or M/GFs at appropriate concentrations to a final culture volume of 200 µL/well.

Assays of DNA synthesis. In vitro DNA synthesis by HCLs cultured in response to M/GFs was measured at days 3, 5, 7, and 10 of culture using 3H-thymidine ([3H]Tdr) uptake. Cells were pulsed with [3H]Tdr during the last 16 hours of incubation (0.2 µCi/well), harvested onto glass filters with the aid of an automatic cell harvester (Cambridge Technology, Cambridge, MA), and counted on a liquid scintillation counter (Packard Tri-Carb 4500, Downers Grove, IL). Significant proliferation was defined as a stimulation index (SI; [3H]Tdr uptake of sample/[3H]Tdr uptake of control) > 3.0. At various intervals, [3H]Tdr-labeled cells were also harvested by cytocentrifuge onto glass slides and prepared for autoradiography. The slides were coated with Kodak (Rochester, NY) NTB-2 nuclear emulsion, exposed at 4°C for 2 to 4 weeks, and then developed and stained with Giemsa. The percentage of [3H]Tdr-labeled cells (greater than five grains per nucleus) was determined. Slides were prepared in duplicate, and 200 cells were counted on each slide.

Cells were also examined at various intervals for bromodeoxyuridine (Brdu) incorporation as another measure of DNA synthesis. Before and after culture with M/GFs, cells were incubated with 10 µmol/L Brdu (Sigma, St Louis, MO) for 30 minutes at 37°C. Cytocentrifuge preparations of cells were then fixed in ethanol, incubated with anti-Brdu Ab (7.5 µg/sample, courtesy of Dr Nick Gonchoroff, Mayo Clinic, Rochester, MN) for 30 minutes, washed, and stained with goat anti-mouse immunoglobulin G (IgG) fluorescein isothiocyanate (FITC) for 30 minutes. The percentage of intranuclear fluorescein-labeled cells was enumerated using a fluorescent microscope.

Radioimmunoassay for IgG. Supernatants were harvested at various intervals after culture with M/GFs and assayed for IgG using a solid-phase radioimmunoassay as previously described. This assay can detect > 5 ng/mL IgG.

Characterization of M/GF-responsive hairy cells. To confirm that those cells responding to M/GFs were HCLs, dual staining for Brdu incorporation and TRAP activity was performed on cells after culture. One hour before sampling, 10 µmol/L Brdu was added to culture media. The cells were harvested by cytocentrifuge onto glass slides and stained for TRAP positivity. The cells were further incubated with an anti-Brdu murine MoAb, which does not require denaturing, and then developed by a standard alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. Cell surface phenotype of responsive HCLs was delineated using the panel of MoAbs described above.

M/GF-responsive cells containing cytoplasmic immunoglobulin (cIg) were identified by immunofluorescence with either anti-κ or -λ, directly conjugated to fluorescein or rhodamine (DAKO Immunoglobulins, Westminister, PA), as previously described. Directly labeled reagents were tested on myeloma cells of known κ and λ isotype and were found to be specific before use. Cells containing cIg were examined using a Zeiss fluorescent microscope (Carl Zeiss, Inc, New York, NY) to assure that responsive cells were monoclonal tumor cells.

Elucidation of the mechanisms of response by hairy cells to M/GFs. Recombinant α-IFN (Schering Plough Corp, Kenilworth, NJ) and Abs directed against the recombinant GFs GM-CSF, IL-3, and IL-6 (Genetics Institute) were used to determine the mechanisms of response. Purified HCLs were cultured at 5 x 10⁶ cells per 200 µL of media and M/GFs. Either α-IFN at a concentration (1,000 U/mL) known to be cytotoxic to HCLs, or Abs to GM-CSF, IL-3, or IL-6 were added at the initiation of culture. Cells were harvested at intervals of maximal DNA synthesis. The percent inhibition by the IFN or Abs, defined as (SI to M/GF - SI to [M/GF + blocking agent])/SI to M/GF, was determined.

Staining of hairy cells for intracytoplasmic GFs. The purified HCLs were cultured with M/GFs and harvested at various intervals onto glass slides. Hairy cells were probed for intracytoplasmic IL-3 or IL-6 using polyclonal Abs to these growth factors in a horseradish peroxidase antiperoxidase (PAP) technique. Cultured monocytes and the U266 cell line served as positive controls for intracytoplasmic IL-3 and IL-6 staining, respectively.

RESULTS

Morphologic, phenotypic, and histochemical characterization of hairy cell enriched populations. Ficoll Hypaque mononuclear cells were obtained from the spleens of four patients with HCL. T cells and monocytes were depleted by
Table 1. Phenotypic and Histochemical Characterization of Hairy Cell Enriched Populations

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<td>86.5</td>
<td>63.2</td>
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*Mononuclear cells were isolated from the spleens of patients with HCL by Ficoll-Hypaque density sedimentation. E-rosetting and adherence were used to remove T cells and macrophages, respectively. The resulting hairy cell enriched populations were examined using indirect immunofluorescence and flow cytometric analysis.

†Hairy cells were tested for TRAP staining and the percentage of cells expressing positive staining was determined.

E-rosetting followed by adherence to plastic. The E-rosette negative nonadherent cell population demonstrated morphologic features of hairy cells by Wright-Giemsa staining. Stained cells contained an oval nucleus with a reticular nuclear chromatin pattern and had abundant, pale cytoplasm. Cytoplasmic projections were not distinguishable on the cytocentrifuged cells. Cell surface phenotypic analysis in all cases was compatible with the diagnosis of HCL (Table 1, Fig 1). The B-cell restricted (pan B cell) Ags B1 and B4 were strongly expressed on a majority (80%) of cells; in contrast, only a minority of cells (10% to 30%) bore cell surface B2 Ag. The IL-2R, Mol, and the plasma cell associated PCA-1 Ag were present on 60% to 85%, 30% to 90%, and 25% to 50% cells, respectively. T-cell restricted (T3, T4, T8) and monocyte restricted (Mo2) Ags were minimally expressed (4%) on HCL enriched populations. TRAP positive staining was present in all cases (63% to 82%).

Response of hairy cells to M/GFs. The enriched HCL populations were cultured with M/GFs either singularly or in combination and assayed for DNA synthesis after 3, 5, 7, and 10 days of culture. Spontaneous [3H]TdR uptake by the HCLs cultured in media alone was minimal (<500 cpm) (Table 2). At day 3, cells from three of four patients (patients 1 through 3, Table 2) demonstrated increased DNA synthesis when cultured with SAC (SI 3.4 to 10.3) or the combination of TPA and Ca2+ (SI 3.3 to 5.6). Maximal [3H]TdR uptake occurred at day 3, decreased by day 5, and was absent on days 7 and 10 (Fig 2A). Neither TPA nor Ca2+ alone resulted in significant DNA synthesis by HCLs.

IL-1α, IL-1β, IL-2, IL-3, IL-5, IL-6, and GM-CSF alone failed to stimulate significant [3H]TdR incorporation by HCLs at intervals up to 10 days of culture. When combinations of two GFs were examined, a significant response to IL-4 and IL-5 was noted in three patients studied: one patient (patient 4, Table 2) demonstrated markedly increased DNA synthesis (SI 4.7) to IL-4 and IL-5 on day 3, which decreased thereafter (Fig 2B); in contrast, two patients (patients 2 and 3, Table 2) did not respond at day 3 to IL-4 and IL-5, but did so on day 7 (Fig 2A). A single patient (patient 1, Table 2) did not respond to IL-4 and IL-5 at day 3 of culture, and was not studied at longer intervals. The response pattern observed to TNF was similar to that triggered by IL-4 and IL-5: [3H]TdR uptake was significantly increased at day 7 for patients 2 and 3 (Fig 2A) and at day 3 for patient 4 (Fig 2B).

No single GF or combinations of GFs other than IL-4 and IL-5 enhanced [3H]TdR uptake by HCLs. Similarly, no enhancement of the SAC or TPA/Ca2+-induced proliferation of HCLs was triggered by the addition of growth factors. Secretion of Ig by HCLs was not induced by M/GFs, even when cultures were extended to 10 days.

Characterization of hairy or normal B cells after culture with B cell M/GFs. DNA synthesis by HCLs in response to SAC, TPA/Ca2+, or IL-4 and IL-5 was confirmed using...


Table 2. Responses of Hairy Cells to B-Cell Ms and Recombinant GFs

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Media</th>
<th>SAC</th>
<th>TPA/Cal⁺</th>
<th>IL-4 + IL-5</th>
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<tr>
<td>Patient 1</td>
<td>256 ± 172</td>
<td>3.4 ± 0.4</td>
<td>3.3 ± 0.2</td>
<td>1.4 ± 0.3</td>
</tr>
<tr>
<td>Patient 2</td>
<td>184 ± 100</td>
<td>10.3 ± 7.3</td>
<td>4.6 ± 1.1</td>
<td>1.0 ± 0.2</td>
</tr>
<tr>
<td>Patient 3</td>
<td>187 ± 94</td>
<td>5.4 ± 1.3</td>
<td>5.6 ± 1.2</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>Patient 4</td>
<td>119 ± 35</td>
<td>1.4 ± 0.7</td>
<td>0.9 ± 0.1</td>
<td>4.7 ± 0.6</td>
</tr>
</tbody>
</table>

Purified hairy cells were suspended at 5 × 10⁵/mL in RPMI/10% FBS and 100 µL aliquots were dispensed in 96-well round bottom tissue culture plates with 100 µL of either media or GF. Cells were pulsed with 0.2 µCi/well [³H]TdR after 48 hours in culture and were harvested onto glass filters and counted on a scintillation counter (mean ± SEM) 18 hours later. SI = [³H]TdR uptake of sample/[³H]TdR uptake of control. Values represent a minimum of three experiments.

[¹H]TdR incorporation by scintillation counting, autoradiography, and bromodeoxyuridine incorporation. As can be seen in Table 3, HCLs that responded to M/GFs comprise a minor subpopulation (2.3% to 23.3%) of cells, with the greatest response occurring in patient 2 to SAC (7.7% of cells BrdU positive at 30 minutes; 23.3% of cells with [¹H]TdR uptake at 18 hours). Up to 14.3% cells (patient 2) responded to TPA/Cal⁺. The combination IL-4 and IL-5 also triggered only a minor subpopulation of cells: 4.1% BrdU at day 3 (patient 4, Table 3), and a mean of 6.5% in patients 2 and 3, who were responsive to IL-4 and IL-5 at day 7.

Hairy cells that responded to the M/GFs were characterized phenotypically and histochemically. In all four patients studied, incubation with TPA/Cal⁺, SAC, or IL-4 and IL-5 resulted in no consistent changes in B1, B2, B4, Mol, IL-2R, and PCA-I cell surface Ag expression. A representative cell surface phenotype of patient 3 before and after culture with Ms is shown in Table 4. To confirm that those cells responsive to M/GFs were HCLs, dual staining was performed for TRAP reactivity and BrdU incorporation. In all HCL samples responding to SAC, TPA/Cal⁺ or IL-4 plus IL-5, there were minor populations (≤7.7%) of cells which were positive for BrdU staining; a majority (61%) of BrdU positive cells were also TRAP positive (Fig 3). Within responsive HCL populations, ≥93% cells expressed cIg of identical light chain isotype, thus assuring that responsive cells were of B-cell origin and were monoclonal; the remainder of cells did not express any cIg. Culture of normal B cells with TPA/Cal⁺ or SAC for 3 days or with TNF or IL-4 plus IL-5 for 7 days did not induce TRAP positivity.

Mechanisms of response of B-cell M/GFs. Hairy cells were cultured with M/GFs and either α-IFN or antibodies to IL-6, IL-3, or GM-CSF to define potential mechanisms of response. α-IFN was able to inhibit DNA synthesis triggered by TPA/Cal⁺ (42.3% ± 24.8% inhibition) (Fig 4) as well as that induced by IL-4 and IL-5 (69.1% ± 13.4% inhibition). In contrast, α-IFN did not block SAC-induced DNA synthesis by HCLs (11.8% ± 6.8% inhibition). Anti-IL-6 (1:1,000) blocked the response of HCLs to TPA/Cal⁺: DNA synthesis by patients 1, 2, and 3 was inhibited by 22.0% ± 4.5%, 45.0% ± 38.2%, and 47.0% ± 8.5%, respectively, with no abrogation of response by either anti-IL-3 or anti-GM-CSF. In contrast, DNA synthesis noted after culture of HCLs with SAC was not reduced by coculture with Abs directed against IL-6 (Fig 4), IL-3, or GM-CSF.

Because the response of HCLs to TPA/Cal⁺ could be blocked by Ab to IL-6, this population of stimulated HCLs was probed for intracytoplasmic IL-6 using anti-IL-6 Ab and a PAP staining technique. Cytospin slides confirmed the presence of intracellular IL-6 protein in more than 90% HCLs responsive to TPA/Cal⁺. In contrast, HCLs triggered by SAC or by IL-4 and IL-5 did not contain intracytoplasmic IL-6.

Fig 2. Time course of responses of purified hairy cells to B-cell Ms and recombinant GFs. Purified hairy cells were suspended at 5 × 10⁵/mL in RPMI/10% FBS and 100 µL aliquots were dispensed in 96-well round bottom tissue culture plates with 100 µL of either media or GFs. Cells were pulsed with 0.2 µCi/well [³H]TdR after 48 hours in culture and were harvested onto glass filters and counted on a scintillation counter (mean ± SEM) 18 hours later. Stimulation index = [³H]TdR uptake of sample/[³H]TdR uptake of control. Values represent a minimum of three experiments.

Mitogens and growth factors include Staphylococcus aureus Cowan A (M), 12-O-tetradecanoylphorbol-13-acetate/calcium ionophore A23187 (O), IL-4 + IL-5 (E), and TNF (□). Two patterns of response were noted in the hairy cells studied. Patients 1 and 2 displayed patterns of response similar to patient 3 (A). Patient 3 responded to SAC and TPA/Cal⁺ only at day 3. A response to IL-4 and IL-5 was seen at day 7. A response to TNF, a documented stimulus for DNA synthesis by hairy cells, was also seen at day 7. In contrast, patient 4 (B) responded early (day 3) to IL-4/IL-5 and TNF with no response to B-cell mitogens.
RESPONSE OF HAIRY CELLS TO GROWTH FACTORS

TPA/Ca²⁺, 11-4 + 11-5, or media alone. [³H]-thymidine was added to the cultures after 48 hours later onto glass filters fluorescein-labeled Ab against BrdU. The percentage of labeled cells was determined by a scintillation count of the glass filters. SI = [³H]TdR uptake of sample/[³H]TdR uptake of control. The slides were processed for autoradiography and the percentage of labeled cells was determined. At least 200 cells per slide were counted. Cells were incubated with BrdU for 30 minutes harvested onto slides by cytospin, and stained with directly immunoenzymatic technique (APAAP) confirmed that BrdU staining and autoradiography confirmed that hairy cells from patient 3 after 7 days of culture with IL-4 and IL-5 are presented. The cells positive for BrdU incorporation display blue nuclear staining with TRAP activity indicated by red granules in the cytoplasm. In all hairy cell samples studied that demonstrated a positive response to SAC, TPA/Ca²⁺ or IL-4 plus IL-5, there were populations of cells that were positive for both BrdU incorporation and TRAP activity.

**DISCUSSION**

In this report, we have examined the response of hairy cell enriched populations to a variety of cytokines. Enrichment for tumor cells was confirmed by characteristic phenotypic and histochemical staining. No significant proliferation was noted to GM-CSF, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, or IL-6. However, HCLs did synthesize DNA in response to SAC, TPA/Ca²⁺, TNF, as well as the combination of IL-4 and IL-5. In the majority of cases, maximal response was noted early (day 3) to SAC and TPA/Ca²⁺; in contrast, peak proliferation was triggered by TNF or IL-4 and IL-5 later (day 7). BrdU staining and autoradiography confirmed that a minority (<23%) of HCLs within each patient responded. Responsive cells demonstrated TRAP positivity and bore the cell surface phenotype of HCL. Moreover, dual staining in an immunoenzymatic technique (APAAP) confirmed that BrdU positive cells were also TRAP positive. To elucidate the mechanism of response, either α- or Abs directed to GFs were added to HCL cultures. Both anti-IL-6 Ab and α-IFN inhibited the response to TPA/Ca²⁺ without any effect on the response to SAC; Abs to GM-CSF or IL-3 did not affect the response to either TPA/Ca²⁺ or SAC. Finally, PAP staining demonstrated that HCL populations could be induced by TPA/Ca²⁺, but not by SAC, to produce intracytoplasmic IL-6. These observations give insight to both the biology of hairy cell growth in vitro and the pathophysiology of HCL in vivo.

It has been suggested that the effects of M/GFs on HCLs reported in previous studies are due to “contaminating” normal cells present within the tumor cell populations examined. Most commonly, the increased [³H]TdR incorporation by hairy cells in vitro has been attributed to contaminating T cells. In our studies, the enrichment technique used was E-rosetting to deplete T cells and adhere to plastic to deplete monocytes. Such purification techniques are critical because T cells, macrophages, and B cells may respond to exogenous GFs and confound the analysis. Resultant populations were enriched for tumor cells, confirmed by both the lack of cell surface staining for either T or monocyte Ags and by the expression of cell surface phenotype and

**Table 3. Characterization of M or GF Responsive Hairy Cells**

<table>
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<tr>
<th>Patient</th>
<th>Stimulus*</th>
<th>Stimulation Indices</th>
<th>Percentage of BrdU Positive Cells</th>
<th>Percentage of [³H]-Thymidine Positive Cells</th>
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<td>Media</td>
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<td>TPA/Ca²⁺</td>
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<td>SAC</td>
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</tr>
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<tr>
<td></td>
<td>IL-4 + IL-5</td>
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*Cells were cultured at 2.5 x 10⁹/mL in the presence of SAC, TPA/Ca²⁺, IL-4 + IL-5, or media alone. [³H]-thymidine was added to the cultures after 48 hours of culture and the cells were harvested 18 hours later onto glass filters or slides. Thymidine incorporation was determined by a scintillation count of the glass filters. SI = [³H]TdR uptake of sample/[³H]TdR uptake of control. The slides were processed for autoradiography and the percentage of labeled cells was determined. At least 200 cells per slide were counted. Cells were incubated with BrdU for 30 minutes harvested onto slides by cytospin, and stained with directly immunoenzymatic technique (APAAP) confirmed that BrdU incorporation and TRAP positivity of hairy cells in vitro has been attributed to contaminating T cells. In our studies, the enrichment technique used was E-rosetting to deplete T cells and adhere to plastic to deplete monocytes. Such purification techniques are critical because T cells, macrophages, and B cells may respond to exogenous GFs and confound the analysis. Resultant populations were enriched for tumor cells, confirmed by both the lack of cell surface staining for either T or monocyte Ags and by the expression of cell surface phenotype and

**Table 4. Cell Surface Phenotype of M Responsive Hairy Cells**

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<td>82.0</td>
<td>21.2</td>
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*Mononuclear cells were isolated from the spleens of HCL patients and enriched for hairy cells by E-rosetting and adherence. Populations of cells were used immediately or cultured at 2.5 x 10⁹/mL in the presence of TPA/Ca²⁺ or SAC for 3 days. Viable cells were isolated by Ficoll-Hypaque density sedimentation. The populations were examined using indirect immunofluorescence and flow cytometric analysis. None of the patients studied displayed significant changes in cell surface phenotype after culture with Ms or GFs.
The Ab to IL-6 of sample /H]TdR uptake of control. The data of one representative
0.2 pCi/well /H]TdR after

GF with and without INF and Abs to GF. Cells were pulsed with
purified hairy cells were suspended at 5 x 10^6/mL in RPMI/10% FBS and 100 µL aliquots were dispensed in
96-well round bottom tissue culture plates with 100 µL of either M
or GF with and without INF and Abs to GF. Cells were pulsed with
0.2 µCi/well /H]TdR after 48 hours in culture and were harvested
onto glass filters and counted on a scintillation counter.

Inhibition of DNA synthesis by purified hairy cells using
INF and Abs to GFs. Purified hairy cells were suspended at 5 x 10^6/mL in RPMI/10% FBS and 100 µL aliquots were dispensed in
96-well round bottom tissue culture plates with 100 µL of either M
or GF with and without INF and Abs to GF. Cells were pulsed with
0.2 µCi/well /H]TdR after 48 hours in culture and were harvested
onto glass filters and counted on a scintillation counter.

The above results demonstrate that approximately two-thirds of the
culture demonstrated that approximately two-thirds of the
minor subset of tumor cells are responsive is analogous to
the phenotype characteristic of HCL. In our previous studies,
we have previously reported that homogeneous populations
of hairy cells proliferate in response to phytomagglutinin-stimulated leukocyte-conditioned media.3 Korsmeyer
et al also have documented a 1.5- to 3.0-fold increase in DNA
synthesis in hairy cells triggered by T-cell GFs.34 Two other
factors, B-cell growth factor (BCGF), and TNF, have
been shown to induce hairy cell proliferation. Finally, Mon
gini et al have demonstrated responses of an HCL cell line to
four known activators of normal human B cells. The availability
of recombinant GFs, coupled with techniques for the
isolation of homogeneous populations of hairy cells, has
permitted us to examine more specifically the role of lympho
dines in regulation of hairy cell growth. Our data suggest
that IL-6, IL-4 and IL-5, and TNF may be GFs for hairy
cells. In particular, hairy cell populations can be induced by
tPA/Ca++ to synthesize IL-6, and either anti-IL-6 or IFN
inhibit DNA synthesis induced in this setting. The
response to IL-4 and IL-5 or TNF, which can also be
blocked by IFN, is not affected by anti-IL-6 Ab and is
therefore unrelated to IL-6. Thus, it would appear that there
are at least two HCL growth regulatory pathways involving
GFs, one of which is IL-6 mediated and a second of which is
IL-4 and IL-5 or TNF mediated. HCL proliferation induced
by SAC, which appears to be unaffected by IFN, may
represent a third mechanism of response. Thus, data to date
suggest that some mitogens (ie, tPA/Ca++) may affect hairy
cell growth via GFs, but that other mitogens (ie, SAC)
trigger hairy cells by as yet undefined mechanisms.35

More detailed studies of the effects of GFs on hairy cells are
needed to define mechanisms of response on both a
cellular and molecular level. Availability of cDNA probes for
GFs and GF receptors will permit assays for GF or GF
receptor mRNA. Abs directed against GFs and their recep
tors will allow measurement of intracytoplasmic or cell
surface GF and receptors. Finally, the availability of GF
dependent cell lines, ie, the B9 IL-6 dependent cell,36 will
permit assessment of GF secretion by HCLs. Such studies
may not only augment our understanding of the biology of
HCL but may also suggest new therapeutic options.

ACKNOWLEDGMENT

We thank Bernadette Miner for secretarial assistance.
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Response patterns of hairy cell leukemia to B-cell mitogens and growth factors

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