Role of Accessory Cells in Cytokine Production by T Cells in Chronic B-Cell Lymphocytic Leukemia

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We investigated the production of cytokines by highly purified T helper cells from B-cell chronic lymphocytic leukemia (B-CLL) patients stimulated by different activation pathways, and we studied the influence of various accessory cell populations on the pattern of cytokine secretion, including interleukin (IL)-2, IL-4, interferon-γ (IFN-γ), and IL-10. Neither a qualitative nor a quantitative difference in cytokine production and proliferative capacity was observed in CLL-derived purified T cells compared with normal individuals, when T cells were stimulated by different pathways, including CD3, CD2, and costimulation with CD28. Addition of autologous accessory cells (aAC), however, dramatically influenced the cytokine pattern of normal versus B-CLL-derived T cells. CLL cells as aAC caused a marked increase of IL-2, whereas IFN-γ was only slightly induced and IL-4 was not influenced. In contrast, in normal individuals addition of aAC, which predominantly consisted of monocytes, resulted in a significant increase of IFN-γ and a reduction of IL-4 secretion. IL-2 production was inhibited by higher concentrations of aAC. The increased stimulation of IL-2 production by CLL cells was not specific to the leukemic cell population, as purified B cells from normal individuals had the same effect. On the other hand, purified monocytes from CLL patients and controls both induced IFN-γ production and inhibited IL-4 secretion. After antigen-specific stimulation with tetanus toxoid, cytokine secretion was influenced by the type of aAC in a similar pattern. We conclude that T helper cells derived from patients with B-CLL are intrinsically normal and that the predominance of B cells as accessory cells in CLL significantly alters the cytokine profile secreted by the nonmalignant T helper-cell population.

MATERIALS AND METHODS

Cell samples. After informed consent, peripheral blood was obtained from 12 patients with a diagnosis of B-CLL according to clinical and immunophenotypic criteria. Patients were either untreated (n = 10) or had not received cytoreductive chemotherapy for at least 3 months before investigation (n = 2). According to the criteria of Rai et al,27 three patients were in stage 0, two patients were in stage I, five patients were in stage II, and two patients were in stage IV. At the time of the analysis, all patients were clinically stable, free from clinically relevant infectious complications, and undergoing routine clinical out-patient review. For control experiments, blood samples were collected from 10 age-matched healthy volunteers.

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Monoclonal antibodies (MoAbs). VIT 3, a CD3 MoAb of IgM isotype, and VIT13, a CD2 MoAb (IgM), were described previously.\textsuperscript{28} As a second stimulatory CD2 antibody, OKT11 was obtained from Coulter. The other MoAbs used in this study were commercially available: CD28 was obtained from Jansen (Alde, Germany); CD8, CD14, CD19, and CD56 MoAbs, from Coulter (Krefeld, Germany); CD16 MoAb, from Becton Dickinson (Heidelberg, Germany); and CD58 and CD80, from Camiflo (purchased from Becton Dickinson). The CD40 MoAb was provided by S. Saeland (Schering-Plough Research Institute, Darldly, France).

Separation procedures. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood samples by centrifugation over a Ficoll-Hypaque layer (Biochrom, Berlin, Germany) of density 1.077 g/mL. E rosette-negative (E\textsuperscript{−}) and -positive (E\textsuperscript{+}) cells were prepared by rosetting with Neuraminidase (Sigma Chemical Co, St Louis, MO)-treated sheep red blood cells (SRBCs) using standard procedures. E\textsuperscript{−} cells from patients with CLL were greater than 95% CD19-positive and contained less than 1% T cells and less than 3% monocytes, as assessed by direct immunofluorescence using a FACScan (Becton Dickinson). E\textsuperscript{+} cells from normal donors contained greater than 60% monocytes, greater than 30% B cells, and less than 5% T cells. E\textsuperscript{−} cells from both sources will be referred to as autologous accessory cells (aAC).

For further isolation of highly purified CD4\textsuperscript{+} cells, after lysing with ammonium chloride, E\textsuperscript{+} cells were incubated with CD8, CD14, CD16, CD19, and CD56 MoAbs. After washing twice, the cells were incubated with sheep antimosue IgG-coated magnetic beads (Dynabeads M450, Dynal, Hamburg, Germany) at a bead-to-cell ratio of 10:1, and the cells attached to the beads were removed after 1 hour using the magnetic particle concentrator according to the manufacturer’s instructions. This separation procedure was repeated once. Such separated cells were greater than 97% CD4+, with some remaining cells of the surface phenotype CD3\textsuperscript{+}, CD2\textsuperscript{+}, CD4\textsuperscript{−}, CD8\textsuperscript{−}. Contamination with monocytes and B cells was less than 1%. B cells were separated from E\textsuperscript{+} cells by incubation with CD19-coated magnetic beads (Dynal) and positive selection using Detachabead (Dynal) according to the manufacturer’s instructions. Such prepared B cells from CLL patients and normals were greater than 99% pure, with no detectable CD4\textsuperscript{+} or CD2\textsuperscript{+} cells.

Monocytes were isolated from E\textsuperscript{−} cells by centrifugation over a Percoll gradient as described.\textsuperscript{29} Monocytes were further enriched by negative selection using Dynabeads M450 Pan T (CD2) and M450 Pan B (CD19) to a purity of greater than 95%.

Stimulation of T cells. Purified CD4\textsuperscript{+} cells were cultured in 96-well flat-bottom plate (Greiner) in a total volume of 200 mL RPMI 1640 medium (Biochrom) supplemented with 10% fetal calf serum (Biochrom), 50 IU/mL penicillin/streptomycin, 1 mmol/L Na-pyruvate, 2 mmol/L L-glutamine, 20 mmol/L L-asparagine, 0.05 mmol/L 2-mercaptoethanol, 10 mmol/L HEPES, and 0.7x modified Eagle’s medium (MEM) nonessential amino acids (Biochrom) at 37°C and 5% CO\textsubscript{2} in a fully humidified atmosphere. T cells were stimulated with combinations of antibodies, including immobilized CD3 (VIT3, 100 ng per well), a combination of soluble CD2 (OKT11, 1 µg/mL; VIT13, 1 µg/mL) and soluble CD28 (0.5 µg/mL) in costimulation with PMA 5 ng/mL (Sigma), or aAC, as indicated in Results. CD3 was immobilized by coating the microtiter wells with 100 µL VIT3 (1 µg/mL) at 4°C overnight. Antigen-dependent response of CD4\textsuperscript{+} T cells was evaluated using tetanus toxoid (Connaught Laboratories, Ontario, Canada). T cells with various types of aAC were stimulated with tetanus toxoid at a final concentration of 10 LF/mL.

For proliferation assays, 3 x 10\textsuperscript{4} CD4\textsuperscript{+} T cells were cultured in triplicate. After 72 hours, the cells were pulsed with H-thymidine, 1 µCi per well (Amersham), and harvested in a PHD-Cell Harvester (Dunn Laborteknik, Asbach, Germany). After 14 hours, thymidine incorporation was quantified in a β-counter (Beckmann, Munich, Germany). For the measurement of cytokine secretion, 1 x 10\textsuperscript{5} T cells per well were incubated for 48 hours, and supernatants of four identical cultures were pooled, filtered, and stored in aliquots at −20°C until use. In experiments using tetanus toxoid for T-cell stimulation, supernatants were obtained after 5 days of incubation, and H-thymidine uptake was determined after 7 days.

In selected experiments, the requirement of direct contact between T cells and B-CLL cells as aAC was tested using culture inserts with semipermeable membranes at the bottom (Millipore, Eschborn, Germany). T cells were plated into culture wells coated with CD3 antibody. B-CLL cells as aAC were added to the culture wells directly, or they were placed in Millipore culture inserts, thus avoiding direct cellular contact between CD4\textsuperscript{+} cells and B-CLL cells. IL-2 was measured in culture supernatants from these cultures.

Cytokine measurements. IL-2 production in culture supernatants was assessed using a CTLL bioassy as described.\textsuperscript{18} Briefly, CTLL-2 cells at a cell density of 1 x 10\textsuperscript{5} per well (American Type Culture Collection, Rockville, MD) were cultured with supernatants at different dilutions or various concentrations of recombinant (r) IL-2 to define the standard curve. CTLL cells were incubated for 20 hours, then pulsed with H-thymidine at 1 µCi per well, procured 8 hours later, and counted in a β-counter. The amount of IL-2 in the supernatants was computed by comparison with the standard curve.

IFN-γ concentration was measured using a commercial IFN-γ enzyme-linked immunosorbent assay (ELISA) kit (Medgenix, Hamburg, Germany) according to the manufacturer’s instructions. IL-10 was measured in a sandwich ELISA obtained from Endogen (Biozol, Eching, Germany). IL-4 was determined in a sandwich ELISA as described.\textsuperscript{31} Anti-IL-4 MoAbs 1-41-1 and 1-38-10 were provided by F. Kalthoff, Sandoz Research Institute, Vienna, Austria. The sensitivities of the cytokine tests were as follows: IL-2, 1 IU/mL; IL-4, 30 pg/mL; IFN-γ, 0.5 IU/mL; and IL-10, 30 pg/mL.

Statistical evaluations. All proliferation assays, as well as cytokine measurements in culture supernatants, were set up in triplicate. The production of IL-2, IL-4, IFN-γ, and IL-10 and the proliferative response of T cells were calculated as mean ± SEM of individual experiments. To evaluate the influence of aAC on cytokine production and proliferation, we used the paired t test. All P values are two-tailed, and a P value of less than .05 was considered to indicate statistical significance.

RESULTS

Proliferation of T cells in response to CD3 and CD2 antibodies. Highly purified CD4\textsuperscript{+} T cells from 12 patients with B-CLL and 10 age-matched normal controls were incubated in the presence of various combinations of MoAbs directed at the CD3 molecule by triggering the TCR/CD3 complex\textsuperscript{32} or the alternative pathway via the sheep erythrocyte receptor.\textsuperscript{15} The IgM antibody VIT3 recognizing the CD3 epitope was immobilized by coating to the culture plates. CD2 and CD28 were used in combination with phorbol myristate acetate (PMA). The combination of immobilized CD3 with CD28 or pairs of CD2 antibodies induced the strongest proliferative response (Fig 1). This pattern of stimulation was entirely consistent in all T-cell samples tested. The proliferative capacity of T cells from B-CLL patients was qualitatively and quantitatively identical to the results observed in T cells from normal individuals.

Cytokine production of T cells from B-CLL patients. In cultures stimulated with the above combinations of antibodies, the secretion of IL-2, IL-4, and IFN-γ was measured in purified CD4\textsuperscript{+} T cells from CLL patients and normal controls.
in the absence of aAC (Fig 2). The amount of cytokines measured in the culture supernatants was dependent on the mode of activation. Whereas maximal IL-2 production was detected in cultures stimulated with CD2, CD28, and PMA (Fig 2A), the peak values for IL-4 and IFN-γ were observed in the presence of immobilized CD3 and CD28 (Fig 2B and C). This could be explained by an increased consumption of IL-2 by CD3-stimulated T cells as an autocrine growth factor. However, there was only a minimal difference in thymidine incorporation in such stimulated cultures (Fig 1). Therefore, consumption of IL-2 by CD3-stimulated T cells is unlikely to cause the marked difference in IL-2 production, as observed in Fig 2A. In accordance with the proliferation assays, the capacity for cytokine production by purified CD4+ T cells from B-CLL patients was not different from normal T-cell populations.

**Influence of aAC on cytokine production by T cells.** Autologous accessory cells from normal individuals and CLL patients were added to purified CD4+ cells. In samples of CLL patients, aAC comprised greater than 90% of B-CLL cells, whereas in normal cell samples, monocytic cells were the predominant cell population. Proliferation of CD3-stimulated T cells was slightly enhanced in cultures from normal individuals and from CLL patients; however, the difference was not statistically significant (Fig 3A). In contrast, the pattern of cytokine secretion was markedly influenced by adding aAC. In experiments using cell samples from CLL patients, the production of IL-2 by T cells was significantly enhanced by CLL aAC (P < .003; Fig 3B). CLL-aAC had no influence on IL-4 production (Fig 3C).
whereas IFN-γ secretion (Fig 3D) was also increased \( (P < .001) \). In normal controls, IL-2 was not influenced, IL-4 secretion was inhibited \( (P < .004) \), and IFN-γ production was stimulated \( (P < .001) \) by normal aAC. A comparison of normal and CLL samples showed that, in the presence of aAC, the production of IL-2 and IL-4 was significantly enhanced in CLL samples \( (\text{IL-2}, P = .005; \text{IL-4}, P < .005) \). Although both aAC populations stimulated IFN-γ, the increase was less pronounced in CLL cell samples, resulting in a significantly decreased production of CLL T cells when compared with normal controls \( (P < .01) \). The production of IL-10 in CD3-stimulated T cells from normal individuals and CLL patients was not statistically different. Interestingly, the addition of aAC
Influence of purified B cells and monocytes on cytokine production by T cells. Next we attempted to distinguish whether the differential regulation of cytokines in T cells is specific for B-CLL cells or represents a general feature of B lymphocytes. Monocytes and B cells were separated from blood samples derived from normal individuals and CLL patients and added to CD3-stimulated autologous T cells at various concentrations. Purified CLL B cells stimulated IL-2 production, as found with unseparated aAC, at concentrations of up to $1 \times 10^7$/mL (Fig 4A). In the presence of $4 \times 10^7$ B-CLL cells per milliliter, IL-2 production was almost twice as high as with aAC. In contrast, increasing numbers of monocytes inhibited IL-2 secretion by CD3-stimulated T cells. Therefore, at high cell concentrations, the monocytes present in aAC might counteract B cell-induced enhancement of IL-2 secretion. IL-4 production was not affected whether aAC or purified B cells were added to the cultures (Fig 4B). In the presence of purified monocytes, a bell-shaped curve of IFN-γ production was observed with a more than sixfold enhancement of cytokine secretion at intermediate cell concentrations (Fig 4C). Purified B cells did not significantly influence IFN-γ production. As shown in Fig 5, a similar pattern of cytokine secretion was detected in samples from normal individuals. Purified B cells caused a dose-dependent increase of IL-2 and did not affect the production of IL-4 and IFN-γ. Monocytes inhibited the secretion of IL-2 and IL-4 and stimulated IFN-γ preferentially at low and intermediate cell numbers. A contribution of B-CLL cells in the secretion of these T cell-specific cytokines is highly unlikely. However, to exclude the possibility that T cell-derived signals could induce IL-2 in B cells, we determined IL-2 levels in supernatants of B-CLL cells stimulated with CD40 antibodies presented by Fc receptor, as recently described. In these culture supernatants, we did not detect measurable IL-2 levels (data not shown). These experiments clearly indicate that the differential regulation of T-cell cytokine secretion in CLL patients and normal individuals is caused by the different composition of aAC and does not represent a tumor-specific feature of CLL cells.

Influence of accessory cells on antigen-dependent T-cell stimulation. CD4+ T cells from normal individuals were stimulated with tetanus toxoid using unseparated accessory cells and purified monocytes or B cells as aAC. In three independent experiments (Table 1), the induction of IL-2 production was most pronounced in the presence of high B-cell numbers, whereas monocytes and unseparated aAC stimulated cytokine secretion only moderately. In contrast, IFN-γ induction was most effective with monocytes as aAC and was only minimally stimulated in the presence of B cells. As observed in experiments using immobilized CD3 as T-cell stimulant, the proliferative response of T cells was not influenced by the type of aAC. Thus, the observation that purified B cells promote a different pattern of cytokine synthesis by CD4+ T cells holds true in an antigen-dependent system, supporting its biologic relevance in the presence of a physiologic T-cell stimulant.
Requirement of cellular contact for B-cell-mediated induction of IL-2. To determine the role of direct cellular contact of B cells and T cells in the enhancement of IL-2 production, CD3-stimulated T cells were separated from autologous B cells by culture inserts (Table 2). Direct contact of B-CLL cells with autologous T cells resulted in a more than 10-fold increase of IL-2 secretion. However, when direct contact was prevented by the use of culture inserts, the stimulation of IL-2 secretion above baseline levels was totally prevented. Addition of antibodies directed against the B7 antigen (CD80) to the cultures did not reduce B cell-mediated IL-2 production. Using CD28 antibody, further stimulation of IL-2 secretion was observed, suggesting that T cells can be additionally stimulated by this pathway even in the presence of B cells as aAC. However, CD58 antibodies reduced IL-2 secretion by more than 70%, suggesting that signals mediated by the CD2/CD58 pathway might be involved in the stimulation of IL-2 secretion by B-CLL cells. With CD40 antibody, the interaction between T cells and accessory B cells was inhibited as well; however, a combination of CD40 and CD58 did not result in a synergistic inhibition of IL-2 production. In parallel experiments monocytes were also separated from T cells by culture inserts (data not shown). After physical separation, unstimulated monocytes did not stimulate IFN-γ production. However, adherence of monocytes to glass beads added to the culture inserts induced a stimulatory signal in monocytes, which enhanced IFN-γ production in CD3-stimulated T cells. A similar effect was observed when activated monocytes were replaced by IL-12 at 1 ng/mL (data not shown).

DISCUSSION

We investigated the capacity for cytokine production by freshly isolated T helper cells from B-CLL patients. Neither a quantitative nor a qualitative difference was observed in CLL-derived purified T cells as compared with normal controls, even when T cells were stimulated by different pathways, including soluble and immobilized CD3, CD2, and costimulation with CD28 antibody. In accordance with this normal pattern of cytokine secretion, we found a fully retained proliferative potential of CLL-derived T cells in response to these stimuli with and without accessory cells. The demonstration of normal T helper-cell function in vitro in B-CLL is in contradiction to early reports, which showed an impaired proliferation or response to cytokines. This might be caused by differences in the cell separation and/or stimulatory signals used in the various studies, as suggested by others. However, in normal individuals, addition of aAC caused a significant increase of IFN-γ and a decrease of IL-4 secretion. In contrast, CLL cells as aAC predominantly enhanced IL-2 secretion, whereas IL-4 production was not altered. The increased capacity of B cells to promote IL-2 production as observed in CD3-driven T cells was retained in antigen-dependent T-cell stimulation using tetanus toxoid as more physiologic stimulant. The capacity for IFN production was reduced in the presence of CLL cells as compared with normal aAC. The modulation of cytokine secretion by CLL cells was not specific to the leukemic cell population, as purified B cells from normal controls and B-CLL had the
same effect on the pattern of cytokine production, in contrast with the effect observed with monocytes derived from both groups. Nevertheless, even without a tumor-specific dysfunction of the malignant B-cell clone, the predominance of B cells as accessory cells in CLL appears to result in a profound alteration of the immune function of T helper cells in vitro.

It has been suggested in the context of antigen-driven immune responses in vivo that different accessory cells and their products influence the development of Th1- or Th2-type subpopulations, which then secrete predominantly IFN-γ or IL-4, respectively. Our results with antigen-unspecific stimulation of T cells in combination with accessory cells are in keeping with the regulation observed in antigen-dependent systems. The addition of normal aAC or purified monocytes to CD3-triggered T cells resulted in a typical Th1 pattern of cytokine production, with a marked stimulation of IFN-γ and inhibition of IL-4 secretion. Several mechanisms have been described that might explain the enhancement of IFN-γ production by monocytes, including CD2 signaling and production of IL-1, IL-6, and IL-12. In our experimental system, stimulation of IFN production by monocytes was also observed when the CD3-triggered T cells and activated monocytes were separated by culture inserts with a semipermeable membrane (data not shown), suggesting that soluble products such as IL-12 mediate this effect.

In contrast with the findings with monocytes, the cytokine profile secreted by T cells in the presence of B cells as aAC does not fit so well in a Th1/Th2 pattern. In fact, IFN-γ and IL-4 production are not affected by B cells. However, the role of our findings in the pathophysiology of B-CLL remains to be determined. Infectious complications associated with severe reduction of serum Ig levels cause considerable morbidity and mortality in CLL patients. In addition to the humoral immunodeficiency, an alteration of cytokine production by CD4 cells might influence the cellular immune

### Table 1. Influence of Accessory Cells on Antigen-Dependent T-Cell Stimulation

<table>
<thead>
<tr>
<th>Type of Accessory Cells</th>
<th>Proliferation (cpm/2 × 10⁶ T cells)</th>
<th>IFN-γ Secretion (U/mL)</th>
<th>IL-2 Secretion (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp 1</td>
<td>Exp 2</td>
<td>Exp 3</td>
</tr>
<tr>
<td>None</td>
<td>9,670</td>
<td>2,710</td>
<td>5,412</td>
</tr>
<tr>
<td>Unseparated aAC</td>
<td>48,609</td>
<td>36,820</td>
<td>56,371</td>
</tr>
<tr>
<td>Monocytes (4 × 10⁶)</td>
<td>45,342</td>
<td>24,111</td>
<td>25,266</td>
</tr>
<tr>
<td>B cells (4 × 10⁶)</td>
<td>39,620</td>
<td>36,870</td>
<td>46,716</td>
</tr>
<tr>
<td>CD80 (10 µg/mL)</td>
<td>43,217</td>
<td>36,248</td>
<td>49,773</td>
</tr>
<tr>
<td>CD40 (10 µg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B cells (4 × 10⁶)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD40, +CD58</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

CD4-positive T cells at a cell density of 1 × 10⁶ per well stimulated with tetanus toxoid in the presence of various kinds and concentrations of aAC. Results of ³H-thymidine incorporation and secretion of IL-2 and IFN-γ are given for three independent experiments (Exp).

CD4.T cells were incubated with or without autologous B-CLL cells in the presence of the antibodies listed, and IL-2 secretion was measured in culture supernatants. IgG1 was used as isotype-matched control antibody.

Abbreviation: ND, not determined.

*B cells were physically separated from CD4.T cells by a culture insert with a semipermeable membrane, as described in Materials and Methods.

### Table 2. Direct Cellular Contact Is Required for Induction of IL-2 Secretion by B-CLL Cells: Influence of Antibodies Against B-Cell Ligands

<table>
<thead>
<tr>
<th>Antibody</th>
<th>B Cells as a AC</th>
<th>IL-2 (U/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exp 1</td>
<td>Exp 2</td>
</tr>
<tr>
<td>None</td>
<td>5.5</td>
<td>2.5</td>
</tr>
<tr>
<td>CD80, 10 µg/mL</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD80, 10 µg/mL</td>
<td>4.5</td>
<td>3.3</td>
</tr>
<tr>
<td>CD58, 10 µg/mL</td>
<td>6.3</td>
<td>3.1</td>
</tr>
<tr>
<td>CD40, 10 µg/mL</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>None</td>
<td>72.3</td>
<td>39.7</td>
</tr>
<tr>
<td>None</td>
<td>6.0</td>
<td>3.5</td>
</tr>
<tr>
<td>IgG1, 10 µg/mL</td>
<td>77.5</td>
<td>44.9</td>
</tr>
<tr>
<td>CD28, 10 µg/mL</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD80, 2 µg/mL</td>
<td>69.4</td>
<td>38.3</td>
</tr>
<tr>
<td>CD80, 10 µg/mL</td>
<td>70.1</td>
<td>34.6</td>
</tr>
<tr>
<td>CD58, 2 µg/mL</td>
<td>58.7</td>
<td>34.6</td>
</tr>
<tr>
<td>CD40, 10 µg/mL</td>
<td>17.5</td>
<td>16.1</td>
</tr>
<tr>
<td>CD58</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>CD40, +CD58</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

CD4.T cells were incubated with or without autologous B-CLL cells in the presence of the antibodies listed, and IL-2 secretion was measured in culture supernatants. IgG1 was used as isotype-matched control antibody.

Abbreviation: ND, not determined.

*B cells were physically separated from CD4.T cells by a culture insert with a semipermeable membrane, as described in Materials and Methods.
reaction. IFN-γ has been shown to play a crucial role in the elimination of infectious pathogens.\textsuperscript{51,42} IL-4 and IL-10 both display immunosuppressive properties and have been shown to synergize to inhibit cell-mediated immunity.\textsuperscript{43,44} Replacement of monocytes by B cells as aAC results in reduced production of IFN-γ and relatively enhanced IL-4 secretion. Therefore, it is tempting to speculate that such a cytokine pattern of T helper cells in vivo would provide suboptimal stimulation of a cellular immune reaction and might further aggravate the impaired response to infectious pathogens in B-CLL patients.

Interaction with T cells plays a crucial role in the activation of normal T cells\textsuperscript{45} and in the regulation of proliferation and survival of low-grade B-cell malignancies in vitro.\textsuperscript{2,46,47} Furthermore, signals mediated by cellular contact with T cells can reverse the biologic response to certain cytokines observed with B-CLL cells alone: proliferation of B-CLL cells induced by IL-2 and polyclonal B-cell mitogens is inhibited by IL-4. However, IL-4 acts as a stimulating substance when CLL cells are induced with CD40 antibody presented by CDW32-transfected L cells\textsuperscript{2} or when CLL cells are incubated with activated T cells (Trettler et al., unpublished observation, June 1994). Differential biologic effects on CLL cells have also been observed for IL-10 in the presence or absence of T-cell derived signals. Whereas IL-10 appears to induce apoptosis on purified CLL cells,\textsuperscript{48} IL-10 is a potent differentation factor for CLL cells in co-stimulation with triggering the CD40 pathway.\textsuperscript{49} Thus, the importance of T cells for regulation of clonal B cells is well documented, whereas the impact of that interaction on T-cell biology is less obvious. If B-CLL cells have a major impact on the paracrine production of cytokines by T cells in vivo, this interaction in turn might regulate their own expansion. In B-CLL, an increased absolute number of circulating T cells has been observed. CLL cells residing in the lymphoid tissue appear to have a considerable proliferation rate,\textsuperscript{50} indicating the requirement of stimulatory signals, which might be provided by interaction with T cells. Stimulation of IL-2 secretion by B-CLL cells as observed in our experiments might be responsible for the expansion of the T-cell population, as well as for the stimulation of the malignant cell clone. Monocytes as aAC caused inhibition of IL-4 secretion, whereas B cells were at least permissive for maximal IL-4 production. Interestingly, increased serum levels of IL-4 have been reported in CLL patients.\textsuperscript{51} A relative enhancement of IL-4 production by T cells in B-CLL patients might contribute to prevention of apoptosis by bcl-2 induction\textsuperscript{52} or might even further enhance B-cell proliferation in direct contact with T cells.

In conclusion, we show that the in vitro biologic response of CD4-positive T cells to stimulatory signals is highly dependent on the composition of the surrounding cells. Although B-CLL cells were comparable with normal B cells in their function as aAC, the predominance of the malignant B cells profoundly influences immune functions of the non-malignant T-cell population. Further studies are required to investigate the role of this observation for progression of B-CLL in vivo.

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Role of accessory cells in cytokine production by T cells in chronic B-cell lymphocytic leukemia

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