Chronic Lymphocytic Leukemia-reactive T Cells during Disease Progression and after Autologous Tumor Cell Vaccines

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ABSTRACT

Purpose: Tumor-reactive T cells were measured in patients with chronic lymphocytic leukemia (CLL) because vaccines that increase the activity of these cells might lead to better disease control.

Experimental Design: Proliferation and ELISPOT assays (for T cells producing IFN-γ after stimulation by CD40-activated CLL cells) were used to determine the prevalence of tumor-reactive T cells in 25 CLL patients at various stages of disease progression. The effects of vaccines, composed of autologous-oxidized tumor cells, on both the clinical course and tumor-reactive T-cell numbers were then determined in 2 patients.

Results: CLL-reactive T cells were found at frequencies of ≥10−3 in 6 of 11 patients. Significant proliferation was found in 15 of 25 patients and correlated with clinical stage. The inability to measure CLL-reactive T cells in the remaining patients was not uniformly a result of generalized T-cell dysfunction or defective antigen presentation by CD40-activated CLL cells. CLL-reactive T-cell frequencies increased in response to vaccination with oxidized autologous tumor cells in a patient with preexisting CLL-reactive T cells but not in a patient where tumor-reactive T cells were undetectable in the ELISPOT assay.

Conclusions: Tumor-reactive T cells exist in some CLL patients (mainly during earlier stages of disease) and may potentially mediate therapeutic responses if their numbers and activation states can be sufficiently increased by tumor vaccines.

INTRODUCTION

CLL3 is incurable with conventional chemotherapy (1). However, its origin in transformed anergic self-reactive CD5+B cells (2) that are subject to T-cell regulation (3) suggests that immunotherapeutic approaches may lead to better disease control. Other observations that support the use of immunotherapy in CLL include (a) the physical association of CLL and T cells in secondary lymphoid organs, which assures interactions between tumor-reactive T cells and tumor cells (4); (b) spontaneous remissions associated with heightened immune activity because of viral infections (5); (c) clinical responses to immunomodulatory cytokines (6); and (d) long-term disease-free survival after allogeneic bone marrow transplantation (7), possibly from a T-cell-mediated graft-versus-leukemia effect (8).

Immune control of tumors, including CLL, is believed to be mediated mainly by CTLs that recognize tumor antigens (9, 10). Cytotoxic CD8+ T cells are mainly responsible for the destruction of epithelial tumors (11), but both CD4+ and CD8+ CTLs may kill B-cell tumors (3, 12). The purpose of cancer vaccines is to increase the number of these tumor-reactive CTLs and maintain their activity for long enough to clear tumor cells. In theory, cancer vaccines should both promote tumor clearance and prevent relapse by providing long-term antitumor immunity. Unfortunately, tumor vaccines, although occasionally yielding dramatic results, have not generally achieved their objective (10). Our laboratory has been evaluating the requirements for successful vaccination of CLL patients because of the need for new treatments, rationale for vaccines in this disease, and as a general model for immunologically susceptible tumors.

At a minimum, successful cancer vaccines require tumor antigens that can be presented with appropriate costimulatory signals to T cells able to respond to these antigens. With the possible exception of the immunoglobulin idiotype (13), the antigens recognized by CLL-reactive T cells are generally unknown. By analogy with other tumor associated antigens (14), CLL antigens are likely encoded by genes that are mutated or overexpressed during oncogenesis and may be unique to each patient. Problems posed by the unknown character and uniqueness of CLL antigens may be addressed by using vaccines based on the patient’s own tumor cells (15).

Successful cancer vaccines also require T cells that can respond to them. This requirement may not always be satisfied...
for a number of reasons. Tumor antigen-reactive T cells may never have been present [because many tumor antigens are actually self-antigens (16)], they may have been lost during tumor progression (17), or they may have been suppressed by regulatory T cells (18). Tumor vaccines may be improved by inhibiting suppressor cells but are unlikely to be successful in the absence of tumor-reactive T cells.

The studies in this article were carried out to determine whether tumor-reactive T cells could be found in patients at various stages of CLL. ELISPOT (19, 20) and proliferation assays (with autologous-activated CLL cells as stimulators) were used to reveal the presence of tumor-reactive T cells without requiring the precise identification of the antigenic targets. We also determined if CLL-reactive T-cell numbers could be increased in vivo by autologous vaccines composed of oxidized tumor cells.

**PATIENTS AND METHODS**

**Blood Samples.** After obtaining informed consent, heparinized blood (30–40 ml) was taken from 25 CLL patients (Tables 1 and 2). Four blood samples were also obtained from hemochromatosis patients undergoing phlebotomy to prevent iron overload (21). Protocols to obtain these samples were approved by the local Institutional Review Board. Complete blood counts were obtained from the hospital clinical hematology laboratory.

**Cell Purification.** B, CLL, and T cells (and sometimes CD4+ and CD8+ subsets) were immediately isolated from each blood sample using the RosetteSep-negative selection technique (StemCell Technologies, Vancouver, British Columbia, Canada) according to the manufacturer’s instructions (with minor modifications). Briefly, 75% of the plasma volume was removed from heparinized whole blood samples to concentrate the peripheral blood mononuclear cells, increase the yield of isolated cells, and minimize the required antibodies. The collected plasma was HI at 56°C for 40 min for subsequent experimental use and cryopreservation. Total T cells, or CD4+ and CD8+ subsets, were obtained with antibodies against CD16, CD19, CD36, CD56, and glycophorin A or against CD16, CD19, CD36, CD56, CD8 (or CD4), and glycophorin A, with 96, 94, and 92% purity, respectively. Peripheral blood B cells were isolated with antibodies against CD2, CD3, CD14, CD16, CD56, and glycophorin A yielding percentages of CD19+, CD19+/CD5+, and CD3+ cells of >98, >96, and <0.1%, respectively.

**CD40 Activation.** Purified B and CLL cells were made into efficient APCs by activation on NIH-3T3 fibroblasts transfected with human CD40L (t-CD40L-cells; a generous gift of Dr. Joachim Schulzke, Boston, MA), as described previously (22). Wild-type NIH-3T3 cells were used for control cultures. Briefly, CLL cells [in AIM-V media (Life Technologies, Inc., Gaithersburg, MD) supplemented with autologous HI 2% plasma but no FCS or additional cytokines] were cultured for 4–6 days at 37°C in 5% CO2 on irradiated (96 Gy) t-CD40L-cells. CD40L-activated CLL cells (CD40-CLL) were then harvested, activation marker expression was determined (Table 2), and aliquots were cryopreserved in liquid nitrogen in a freezing...
media composed of 10% DMSO, 45% AIM-V, and 45% HI autologous plasma.

**Induction of CLL-specific T-Cell Responses.** Isolated T cells, or CD4⁺ or CD8⁺ subsets (1.5 × 10⁶ cells/ml final concentration in AIM-V media without additional cytokines or serum), were incubated with the following stimulators: autologous unirradiated or irradiated (50 Gy) CD40-CLL, resting CLL serum, or CD4⁺ autologous plasma.

**Materials and Methods.**

**Cellular ELISPOT assays**

ELISPOT Assays. Cellular ELISPOT assays were performed as described elsewhere (23) with minor modifications. Briefly, 96-well nitrocellulose plates (Militite; Millipore, Bedford, MA) were coated with 75 μl of mouse antihuman IFN-γ monoclonal antibody (code 1598-00; Genzyme, Boston, MA) in a concentration of 2 μg/ml in PBS for 2 h at room temperature. Three-fold dilutions of stimulated T-cell cultures (starting from 3 × 10⁵ cells/well) in 0.2 ml were plated in triplicate wells alone or with stimulator CD40-CLL or CLL cells (3 × 10⁵ cells/well) in a final volume of 0.2 ml of AIM-V. T cells in some wells were stimulated with PMA (10 ng/ml; Sigma, St. Louis, MO) and ionomycin (0.5 μg/ml; Calbiochem, San Diego, CA) to indicate the total number of T cells that were capable of making IFN-γ. Wells with CD40-CLL and CLL cells alone were also included in each experiment to rule out alternative sources of IFN-γ. After incubation for 48 h, plates were washed four times with PBS containing 0.05% Tween 20 and 1% BSA at room temperature. Plates were washed six times with PBS-Tween, and extra avidin-alkaline phosphatase (1:5000; Sigma) in blocking buffer solution (PBS buffer plus 1% BSA) for 1 h in blocking buffer solution (PBS buffer plus 1% BSA) at room temperature. Plates were washed under running water and dried by exposure to the air. Blue spots (resulting from localized cytokine production) were counted in an inversion microscope. The number of colored spots/3 × 10⁴ T cells plated.

**IFN-γ ELISPOT Assays.** Cellular ELISPOT assays were performed as described elsewhere (23) with minor modifications. Briefly, 96-well nitrocellulose plates (Militite; Millipore, Bedford, MA) were coated with 75 μl of mouse antihuman IFN-γ monoclonal antibody (code 1598-00; Genzyme, Boston, MA) in a concentration of 2 μg/ml in PBS for 2 h at room temperature. Three-fold dilutions of stimulated T-cell cultures (starting from 3 × 10⁵ cells/well) in 0.2 ml were plated in triplicate wells alone or with stimulator CD40-CLL or CLL cells (3 × 10⁵ cells/well) in a final volume of 0.2 ml of AIM-V. T cells in some wells were stimulated with PMA (10 ng/ml; Sigma, St. Louis, MO) and ionomycin (0.5 μg/ml; Calbiochem, San Diego, CA) to indicate the total number of T cells that were capable of making IFN-γ. Wells with CD40-CLL and CLL cells alone were also included in each experiment to rule out alternative sources of IFN-γ. After incubation for 48 h, plates were washed four times with PBS containing 0.05% Tween 20. Wells were then incubated with 100 μl of polyclonal rabbit antihuman IFN-γ antibody (1 μg/ml; code IP-500, 1:250 dilution; Genzyme) for 1 h in blocking buffer solution (PBS buffer plus 1% BSA) at room temperature. Plates were washed six times with PBS-Tween, and extra avidin-alkaline phosphatase (1:5000; Sigma) in blocking buffer solution (100 μl) was added for 30 min. The wells were then washed eight times with PBS/Tween, and 50 μl of substrate [100 mg/ml 3-amino-9-ethylcarbazol (Sigma) dissolved in 10 ml of ddH₂O] was added to each well. The reaction was stopped after 30–40 min, and the plates were then washed under running water and dried by exposure to the air. Blue spots (resulting from localized cytokine production) were counted in an inversion microscope. The number of colored spots divided by the input T cell number was recorded as the CLL-reactive T-cell frequency in each well.
**Immunofluorescence.** FITC- or PE-conjugated CD19, CD5, CD25, CD28, CD69, CD3, CD4, CD8, HLA-DR, CD86 (B7-2), CD54, and CD58 antibodies and the isotype controls, IgG1κ-FITC, IgG1κ-PE, and IgG2a-FITC, were from Coulter (Fullerton, CA) and BD Biosciences (PharMingen Canada, Mississauga, ON), respectively. Anti-human CD3, CD4, or CD8 antibodies, conjugated with TRI-COLOR reagent, were purchased from Caltag Laboratories (Cedarlane, Hornby, ON, Canada). CD80-PE (B7-1) was purchased from Becton Dickinson (San Jose, CA). Fluorescence from specifically reacting antibodies was measured on a FACScan flow cytometer (Becton Dickinson). At least 10,000 events were analyzed in each experiment.

**T-Cell Proliferation Assay.** T-cell responders (1.5 × 10^6 cells/ml, final concentration) were incubated with various stimuli (3.8 × 10^5 cells/ml, final concentration) as described above. Control CD40-CLL, CLL, and T cells were cultured alone at the corresponding final concentrations. On day 6, the cells were resuspended, and 200 μl were transferred into 96-well round-bottomed tissue culture plates and incubated with 20 μl/well Alamar blue (Biosource International, Camarillo, CA) for 48 h (24). Alternatively, Alamar blue was added to cultures set up directly in 96-well plates. Absorbances were determined at wavelengths of 540 nm (reduced state) and 595 nm (oxidized state) using an absorbance colorimeter microplate reader (Molecular Devices, Menlo Park, CA).

**Preparation of Oxidized CLL-cell Vaccines.** Ten ml of the patients’ blood (>90% of CLL cells) were subjected to a combination of oxidative physicochemical stressors in the Model VC7001 blood treatment unit (Vasogen, Toronto, Ontario, Canada), as described previously (25). It was hypothesized that this treatment would release antigen-binding heat shock proteins (26) and free radicals that would activate APCs (27) and increase the immunogenicity of the CLL cells in vivo. Blood was collected into 2 ml of 3.13% sodium citrate and immediately transferred to a sterile, disposable, low-density polyethylene vessel (Model VC7002) for ex vivo treatment involving heat (42.5 ± 1.0°C), a mixture of medical grade oxygen and 14.5 ± 1.0 μg/ml of ozone, and UV-C light at a wavelength of 253.7 nm. At the end of the treatment with all three stressors, the blood was allowed to settle for 7 min. The entire process took ~20 min. The treated blood sample was then immediately transferred to a sterile syringe and injected i.m. into the gluteal region of the patient, together with a local anesthetic (1 ml of 2% Novocain or equivalent).

**Vaccine Protocol.** Permission to use Vasogen’s IMT blood-processing device to treat the blood of these patients and for the schedule of injections was obtained from the Canadian Health Protection Branch. After obtaining informed consent, patients were injected twice weekly for 6 weeks in a schedule that has been used for the treatment of autoimmune diseases (28). Peripheral blood was also collected from each patient on a weekly basis for investigational purposes before each treatment session. Cells were immediately isolated and frozen so that T-cell cultures could be performed at the same time and under the same conditions.

**Statistical Analysis.** Statistical analysis was performed with the SigmaStat program for Windows to test for mean differences by the Student t test. The F test for equality of variances was used to select the corresponding P.

**RESULTS**

**Measurement of CLL-reactive T Cells.** CLL cells, activated through CD40 signaling pathways (22), were used to stimulate autologous T cells because of the inherently poor APC capabilities of circulating CLL cells (29, 30). IFN-γ production was used to indicate the presence of TH1/TC1 cells (31) that are thought to be most associated with effective antitumor responses (9). Stimulator B and responder T cells were cultured for 6–7 days before transfer to the anti-IFN-γ-coated plates because direct incubation on the ELISPOT plates did not produce high numbers of spots (data not shown).

This assay was used to measure CLL-reactive T cells in 11 patients at various stages of their disease (Table 1; patients 1–11). A frequency of 10^-3 IFN-γ-producing T cells (32–98 spots/3 × 10^6 cells, median 50.7) was observed in 6 of 11 patients (Fig. 1), and these patients were judged to have T cells able to respond to their own CLL cells. In the remaining 5 patients, the frequency of IFN-γ-producing T cells (0.5–8 spots/3 × 10^6 cells, median 5.8) was not two SDs above the average results for unstimulated T cells (Fig. 1), and these patients were assumed to be nonresponsive to their CLL cells. The difference between responder and nonresponder CLL-reactive T-cell frequencies was highly significant (P = 0.00002). No spots or very low frequencies were found when resting CLL cells were used to prime autologous T-cell responses in all 8 studied samples (0–9.5 spots/3 × 10^6 cells, median 2).

To provide additional validation of these results, T-cell proliferation (Fig. 2) and activation marker expression (Fig. 3) were assessed with aliquots of the T cells used in the ELISPOT assays. A strong correlation was evident between the presence of CLL-reactive T cells in the ELISPOT assay and the stimulation of T cell proliferation by autologous CD40-CLL cells (Fig. 2). Although T cells from patients that readily produced IFN-γ in the ELISPOT assays also proliferated in culture, T cells that failed to produce IFN-γ in the ELISPOT assays exhibited significantly impaired proliferation after stimulation with autologous CD40-CLL cells (P = 0.00012). No patients whose T-cell proliferation was represented by an A_{595–540nm} reading in the colorimetric assay of <0.150, had evidence of tumor-reactive T cells in the ELISPOT assay. Similarly, enlarged T cells with up-regulated class II MHC expression (estimated by flow cytometry, Fig. 3a) were found only in cultures where IFN-γ production and proliferation were also observed in ELISPOT or colorimetric assays, respectively (Fig. 3b). No significant up-regulation of other T-cell activation markers such as CD25 or CD69 (32) was found in the cultures (data not shown).

**Presence of Tumor-reactive T Cells Is Related to Disease Stage.** Using the technically simpler proliferation assay, the presence of T cells with reactivity against CLL antigens was screened in 14 additional patients (Tables 1 and 2; patients 12–25). Combining the data on all patients, a statistically significant correlation was then observed between the stage of disease and the presence of T cells capable of responding to the tumor cells (P < 0.001; Fig. 4). Only 2 of 13 patients with stage...
0-II disease (patients 1 and 7) did not have significant T-cell proliferation against autologous-activated tumor cells, as measured by an A595–540 nm reading or did not respond in the ELISPOT assay. However, 8 of 12 patients with advanced stage (III-IV) disease did not exhibit significant anti-CLL T-cell reactivity on the basis of proliferation or ELISPOT assays (patients 4, 8, 11, 14, 15, 17, 20, and 23). Although 67% (8 of 12) of these advanced stage patients had been actively treated for their disease at some point, none had been treated for at least 60 days before the in vitro analysis. Moreover, 2 of 4 of the responders with advanced stage disease (patients 5 and 6) had previously been exposed to chemotherapy.

Absence of CLL-reactive T Cells in Vitro Is not because of Costimulatory Defects in CLL Cells. A number of possibilities could account for the apparent absence of CLL-reactive T cells as measured by ELISPOT assays in patients 1, 4, 7, 8, and 11 (Fig. 1) and proliferation assays in patients 14, 15, 17, 20, and 23 (Table 2) including (a) defective APC capabilities of CD40-CLL cells; (b) global T-cell dysfunction; (c) presence of suppressor cells that inhibit tumor-reactive T cells in culture; (d) insensitivity of the assay; and (e) true absence of CLL-reactive T cells. To address the possibility that CD40-CLL cells from these patients were unable to stimulate any T-cell responses, CD40-CLL cells from patients 1 and 4 were used to stimulate allogeneic responses, along with CD40-activated CLL cells from patients 3 and 10, which could elicit autologous CLL-reactive T-cell activity. ELISPOT assays were performed as described above, except that the CD40-activated CLL cells were cultured with T cells from 4 allogeneic hemochromatosis donors. The best response is shown for each donor and patient pair (Fig. 5). CD40-activated CLL cells from all 4 patients were able to stimulate allogeneic responses (90–130 spots/3 × 10⁴ T cells, median 112; Fig. 5), suggesting that defective costimulation on the part of the tumor cells was not responsible for the failure to demonstrate CLL-reactive T cells in patients 1 and 4.

The ability of CD40-activated CLL cells to stimulate tumor-reactive T cells clearly depends on their expression of costimulatory molecules. As shown in Table 2 (columns 6–9), the expression of CD80 (B7-1), CD86 (B7-2), Class II MHC, and/or ICAM-1 increased on activated CLL cells from all patients. However, heterogeneity was observed in both the baseline expression level of these molecules (the denominator of the MFI ratio), as well as the expression level achieved after activation (the numerator of the MFI ratio).
Impaired Allo-responses Do Not Correlate with Absent Antitumor Responses. To address the possibility that the failure to demonstrate reactivity against autologous-activated CLL cells was attributable to global T-cell dysfunction, T cells from a nonresponder (patient 1) were incubated with allogeneic CD40-CLL cells (Fig. 6), and the number of IFN-γ-producing cells was determined in the ELISPOT assay. As before, autologous CLL cells failed to induce evidence of antitumor T-cell responses (Fig. 6a). However, significant T-cell responses were induced by the allogeneic CD40-CLL cells (P = 0.006). Both T-cell cultures produced comparable numbers of spots when additionally stimulated with the mitogens, PMA and ionomycin (Fig. 6, right panel), regardless of previous exposure to autologous or allogeneic CD40-CLL cells (P = 0.12). Similar results were obtained when T cells from patient 4 (also unresponsive to autologous CD40-activated tumor cells) were stimulated with allogeneic CD40-CLL cells (data not shown).

Similarly, T cells from patients 15, 17, and 20 exhibited increased responses to allogeneic activated CLL cells compared with their own tumor cells (Table 2, columns 4 and 5). However, T cells from patient 14 failed to proliferate in response to both autologous and allogeneic-activated CLL cells. These results suggested that the impaired responsiveness of T cells from some CLL patients was not always specific for antigens expressed on their own tumor cells.

Failure to Demonstrate CLL-reactive T Cells in Vitro Is Not Caused by Suppressor Cells. The lack of responsiveness to autologous CD40-activated CLL cells in some patients could potentially be attributable to immunoregulatory CD4+CD25+ or CD8+CD28− T cells that have recently been shown to play a role in CLL-associated immunosuppression (33–35). Accordingly, CD4+ or CD8+ T cells were removed from the purified T cells of patient 1, who did not respond to his own CD40-activated CLL cells, and patient 9, whose T cells did respond (Fig. 1). As shown in Fig. 7a, removal of either subpopulation did not unmask autologous T-cell reactivity to CD40-activated CLL cells in patient 1. Moreover, repetitive stimulation with autologous CD40-activated CLL cells failed to reverse the observed unresponsiveness (data not shown). All T-cell groups produced IFN-γ in response to mitogenic stimulation with PMA and ionomycin (Fig. 7b), suggesting they were potentially capable of responding to antigens.

CD4+ T cells appeared to be the major responders to autologous CD40-CLL cells in patient 9 (Fig. 7a). Interestingly, anti-CLL reactivity appeared to increase when CD8+ T cells were removed, suggesting that suppressor cells may exist within this population.

Changes in CLL-reactive T-Cell Frequencies after Vaccination with Autologous-oxidized Tumor Cells. Because tumor cells continued to circulate in all patients, the clinical relevance of the demonstrated CLL-reactive T cells is unclear. The frequencies of CLL-reactive T cells were low and possibly below the level required to clear tumor cells (36). To determine whether CLL-reactive T-cell frequencies could be increased in vivo, patients 1 and 10 were given 12 injections of autologous...
tumor vaccines over 6 weeks. These vaccines were made from 10 cc of the patients’ blood and consisted mainly of tumor cells made more immunogenic by treatment with a combination of physicochemical stressors, as described in the “Materials and Methods.” Both patients had developed increases in their lymphocyte doubling times or constitutional symptoms that justified an attempt to treat their disease (37).

Although patient 1 felt subjectively better during the treatment, no CLL-reactive T cells were demonstrable before or during the course of the injections. There was also little change in circulating CLL cell numbers as indicated by the total WBC count, which mainly reflects the number of CLL cells (Fig. 8b, right panel).

In contrast, CLL-reactive T cells were demonstrated in the cellular ELISPOT assay in patient 10 (Fig. 1). After four injections of autologous oxidized tumor cells, the frequency of CLL-reactive T cells increased nearly 5-fold (Fig. 8a, left panel, ◆). Although the magnitude of this increase was not sustained, 2-fold increases in CLL-reactive T-cell frequencies were still observed after eight injections. Circulating CLL cells decreased by ~50% between the 4th and 8th week of treatment after the increased frequencies of CLL-reactive T cells (Fig. 8a, left panel, ■). However, this decrease was also not sustained (data not shown).

**DISCUSSION**

The major finding in this article is that T cells able to react against activated tumor cells exist in some CLL patients (Fig. 1, Table 2) and may respond to autologous tumor vaccines (Fig. 8).
In addition, the presence of these cells appears to correlate inversely with disease progression (Fig. 4).

The frequencies of CLL-reactive T cells seen in some patients were found to be of the order of 1/10^3-circulating T cells (Fig. 1). These numbers may actually be lower because they were measured in ELISPOT assays after a brief period of in vitro culture that would allow the proliferation of antigen-activated T cells. The frequency of tumor-reactive T cells may be underestimated in some patients because tumor-reactive TH2/TC2 cells would not be counted in ELISPOT assays that only measure IFN-γ production (38). Regardless, the measured frequencies are one to two orders of magnitude lower than the frequencies associated with protective immunity to viruses (39) or melanoma cells (36).

The role of low frequencies of CLL-reactive T cells is unclear. Normal controls also contain T cells that can respond to CD40-activated B cells (unpublished results), suggesting that these T cells may be involved in normal immunoregulatory processes such as the killing of activated anergic B cells (40). Tumor-reactive T cells in CLL patients may similarly be engaged in protective responses...
against CLL cells, although the magnitude of the response is not enough to completely eliminate the tumor cells. An acquired loss of these cells might then lead to disease progression and account for the inverse correlation that was observed between disease stage and anti-CLL T-cell reactivity (Fig. 4).

On the other hand, Prehn (41) has argued that low numbers of tumor-reactive T cells may actually support tumor growth. The relationship between advanced stage disease and antitumor T-cell reactivity might then reflect genetic events [such as acquired p53 mutations (1)] that allow tumor cells to grow independently of exogenous growth factors. Regardless, the increased CLL-reactive T-cell frequencies after autologous-oxidized CLL cell vaccines (Fig. 8) suggest that it may be possible to manipulate these tumor-reactive T cells so that they have therapeutic effects.

The low or absent numbers of CLL-reactive T cells in some patients may sometimes result from tolerance to tumor antigens that are also self-antigens (16) or the effects of cytotoxic chemotherapeutic or radiation (Table 1, patients 4, 8, 11, 15, 17, and 23). These numbers may also be low because of the development of anergy, senescence, or apoptosis in tumor-reactive T cells (42) or the effects of regulatory T cells (43–45) that either cause, or are associated with, tumor progression. For example, suppressor cells found in the CD8+ T-cell population may have inhibited CLL-reactive CD4+ T cells in patient 9 because the frequency of CLL-reactive T cells increased when the CD8+ T cells were removed (Fig. 3a). Immunosuppressive factors from CLL cells such as soluble MHC molecules (46), CD40 (47), tumor necrosis factor α (48), or transforming growth factor β (49) may also inhibit T-cell activation or interactions with tumor targets. These inhibitors may be involved in causing the general state of immunosuppression in CLL (1) but not the specific defects in anti-CLL T-cell reactivity reported here because our assays were performed in serum-free conditions.

The antigens recognized by responding T cells in the ELISPOT and proliferation assays are currently uncharacterized. We have assumed that circulating CLL cells share common antigens with CD40-activated CLL cells, as well as with oxidized CLL cells in the autologous tumor vaccines. Although the possibility exists that the array of peptide-MHC complexes on CD40-activated CLL cells (which stimulate T cells in ELISPOT assays) may be different from on resting CLL cells, this assumption seems to be justified by the increased CLL-reactive T-cell frequencies observed in the ELISPOT assay (along with decreased circulating CLL cell numbers) after vaccination with oxidized CLL cells (Fig. 8).

Clinical responses have been reported to vaccines of autologous CLL cells made more immunogenic by irradiation (50), alteration of membrane cholesterol levels (51), or infection with CD40L-expressing adenoviruses (52). The results obtained with the autologous oxidized CLL vaccines in patients 1 and 10 have motivated an ongoing Phase VII trial of this approach at our institution. Could measurements of anti-CLL T-cell reactivity be used to guide disease management as well as to monitor responses to vaccines? The failure of patient 1 to respond to vaccination correlated with our inability to demonstrate preexisting CLL-reactive T cells in the cellular ELISPOT assay. If similar results can be documented in other patients, screening for CLL-reactive T cells might be useful to indicate when vaccines are appropriate therapy and when they are not. The ability to choose the patients who might benefit from cancer vaccines would certainly be expected to increase the effective application of this treatment modality.

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REFERENCES

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