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Autologous T Cells Control B-Chronic Lymphocytic Leukemia Tumor Progression in Human→Mouse Radiation Chimera[1]

Avichai Shimoni, Hadar Marcus, Benjamin Dekel, Rachel Shkarchi, Fabian Arditti, Lev Shvidel, Mordechai Shtalrid, Wulf Bucher, Allon Canaan, David Ergas, Alain Berrebi, and Yair Reisner[2]

Department of Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel [H. M., B. D., R. S., F. A., W. B., A. C.] and Hematology Institute, Kaplan Hospital, Rehovot, Israel [A. S., L. S., M. S., D. E., A. B.]

ABSTRACT

B-chronic lymphocytic leukemia (B-CLL) is characterized by the clonal accumulation of CD5$^+$ B cells. It has been suggested that CLL cells may be regulated by inhibitory and growth-promoting signals exerted by autologous T cells. We have recently described a model for human B-CLL in which peripheral blood mononuclear cells (PBMCs) are transplanted into the peritoneal cavity of lethally irradiated mice radioprotected with bone marrow from mice with severe combined immunedeficiency. In this model, adoptive transfer of low-stage PBMCs leads to marked engraftment of T cells or combined T and CLL cell engraftment, whereas infusion of high-stage PBMCs leads to dominance of CLL cells with a miniscule level of T-cell engraftment. This mutual exclusive pattern of engraftment indicated that T cells might control the expansion of tumor cells in the peritoneum of recipient BALB/c mice. In the present study, we further investigated this question and we demonstrate that *in vivo* T-cell depletion, using OKT3 antibody, markedly enhances the engraftment of B-CLL cells from patients with early-stage disease. In mice receiving PBMCs from 11 donors with advanced-stage disease, the results were more heterogeneous. In five patients the results were similar to those observed in early stage, whereas in two cases no CLL cell engraftment was found in the absence of T cells. The addition of purified T cells to PBMCs led to a substantial decrease of CLL engraftment in three advanced-stage cases. These results strengthen the working hypothesis that autologous T cells can actively suppress the expansion of the pathological cells in human→mouse radiation chimera. This effect is prominent in early-stage disease, whereas in advanced stage suppresive and/or stimulatory effects may occur in different patients. The interaction of T cells with tumor cells and the potential of autologous T cell/immune-therapy in CLL can be further explored in this model.

INTRODUCTION

B-CLL[3] is a chronic lymphoproliferative disease that is characterized by the accumulation of CD5$^+$ B cells (1–3). T cells may interact with CLL tumor cells in different ways. Early studies suggested that T-cell disregulation may play a role in the increased autoimmunity and hypogamma-globulinemia typical of this disease. *In vitro* studies and studies that compare T cell subpopulations and cytokine levels in different stages of the disease (4–8) suggest that helper T cells may play a role in the support of CLL cell accumulation, probably by certain cytokine production, especially in high-stage disease. CD8 cells may be able to limit disease progression in early-stage disease. In the past few years, SCID mice engrafted with human PBMCs were used as *in vivo* models for studying normal and malignant cells (9), including malignant lymphoid tumors (10). Attempts at the engraftment of CLL cells into SCID mice have generally been unsuccessful. CLL cell lines produced a highly aggressive disease in SCID mice that resembles aggressive or EBV-induced lymphoma and not the stable stage of CLL (11–13). Fresh CLL cells could engraft in the peritoneum of recipient mice (14–16), but EBV transformation of bystander B-cells confounded the results (14, 15).

Lubin *et al.* (14) described a new approach that enables the adoptive transfer of human PBMCs into lethally irradiated normal strains of mice radioprotected with SCID BM. This model has the advantages of rapid dissemination of human cells, rapid human immunoglobulin production, and resistance to EBV-induced transformation of human lymphocytes (15). We have recently described a model for human B-CLL in which PBMCs of CLL patients are transplanted into the peritoneal cavity of these human→mouse radiation chimera (18). In this model, adoptive transfer of low-stage PBMCs leads to marked engraftment of T cells or combined T and CLL cells engraftment, whereas high-stage PBMCs lead to dominance of CLL cells with a miniscule level of T-cell engraftment. This mutually exclusive pattern of engraftment indicated that T cells might control the expansion of tumor cells in the peritoneum of recipient mice.

In the present study, we investigated the effect of manipulation of T cells on the engraftment potential of CLL cells, at different stages of the disease, by manipulating the T cell level in human→mouse chimera following adoptive transfer of PBMCs from CLL patients.

MATERIALS AND METHODS

Mice. Animals used were 6–10 weeks of age. BALB/c mice were obtained from Olac (Shaw's Farm, Blackthorn, Bicester, Oxon., United Kingdom) and from the Weizmann Institute Animal Breeding Center, Rehovot, Israel, outbred immune deficient beige/nude/Xid (BNX) mice were from Harlan Sprague Dawley (Indianapolis, IN), and NOD/SCID mice were from the Weizmann Institute Animal Breeding Center. All mice were kept in small cages (five animals in each cage) and fed sterile food and acid water containing cyprofloxacin (20 μg/ml).

Conditioning Regimen for Mice. BALB/c mice were exposed to split dose (4 Gy, followed 3 days later by 10 Gy) total body irradiation, from a gamma beam 150-A ^{60}Co source (produced by the Atomic Energy of Canada, Kanata, Ontario) with focal skin distance of 75 cm and a dose rate of 0.7 Gy/min. BNX mice were exposed to total body irradiation of 12 Gy (single dose) 1 day before transplantation.

Preparation and Transplantation of BM Cells. BM cells were obtained from NOD/SCID mice (4–8 weeks of age), according to Levite *et al.* (16). Recipient mice were injected with 2–3x10^6 SCID BM cells (i.v. in 0.2 ml of PBS) 1 day after irradiation.

CLL Patients. The patient group included 22 patients treated in the Kaplan Hospital Hematology Institute (Rehovot, Israel). Diagnosis of CLL was based on sustained lymphocytosis, peripheral blood smear, BM biopsy, and expression of CD5. Some patients had morphological features consistent with activated CLL or prolymphocytoid transformation at diagnosis. Each patient donated 20–75 ml of blood after giving informed consent. Blood was drawn using heparin-washed sterile syringes, and PBMCs were separated as described below within 24 h. The patients' charts were reviewed for age, disease duration, CLL stage according to Rai's classification (17), and current treatment.

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[2] To whom requests for reprints should be addressed, at Department of Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel. Phone: 972-8-9344023; Fax: 972-8-9344145.

[3] The abbreviations used are: B-CLL, B-chronic lymphocytic leukemia; PBMC, peripheral blood mononuclear cell; SCID, severe combined immune deficient; AET, 2-aminoethylisothiouronium bromide hydrobromide; SRBC, sheep RBC; BM, bone marrow; FACS, fluorescence-activated cell-sorter.

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Preparation and Transplantation of Human PBMCs. Whole blood from CLL patients was layered onto Lymphoprep solution (Nycomed, Oslo, Norway) and spun at 2000 *rpm* for 20 min. The interlayer was collected, washed twice, counted, and resuspended in PBS (pH 7.4) to the desired cell concentration. Human PBMCs (120×10^6 cells in 0.5–1.0 ml of PBS) were injected i.p. into recipient mice, conditioned as described above.

Depletion of T Cells from PBMCs. In most of the experiments, T-cell depletion was performed *in vivo* by the i.p. injection of 200 μl of mouse antihuman CD3 (OKT3) ascities (ATCC CRL 8002) on the day of transplantation. In three experiments, using PBMCs of three different patients, PBMCs were depleted of T cells *ex vivo* by SRBC rossetting as described below. Cells from the T cell-enriched cell fraction were than added to unseparated PBMCs, so as to enrich the CLL cells with T cells. In each of these experiments, there were three groups of mice receiving either unseparated, T cell-depleted, or T cell-enriched PBMCs, including the same dose of CLL cells. The latter group received two to three times more T cells compared with the group receiving unseparated cells, whereas in the group receiving T cell-depleted PBMCs only a very low T cell dose was infused.

Preparation of SRBC or Rosetting. AET solution was prepared by dissolving 2 g of 2-aminoethylisothiouronium bromide hydrobromide (Sigma Chemical Co., St. Louis, MO) in 50 ml of sterile preservative-free H_2O. The pH was adjusted to 8.35 with 10 N NaOH ,and the solution was filtered through a 0.2-μ filter. SRBCs were centrifuged for 5 min at 2500 *rpm*. Supernatant and buffy coat were removed, and the cells were washed four times with PBS. Sterile AET solution (20 ml) was added to 5 ml of packed, washed SRBCs in 50-ml polypropylene conical tubes, and incubated at 37°C for 20 min. At the end of the incubation period, 25 ml of cold PBS were added and the tubes were centrifuged at 2500 *rpm* for 5 min. The cell pellets were resuspended and washed five times with PBS until hemolysis stopped. The cells were washed again with M199 and stored at 4°C until use. Before using, the cells were irradiated (2 Gy) and washed three times with cold M199.

Rosetting of T Cells with AET-treated SRBCs. Ficoll separated mononuclear cells (20×10^6 cells/ml, 20 ml) in M199 were mixed with 5 ml of AET-treated SRBCs (4×10^9 cells/ml) in M199. The rosetting mixture (25 ml) was layered on top of 20 ml of Ficoll and centrifuged at 1500 *rpm* for 30 min. After spinning, the B cell-enriched interface was collected and washed twice with PBS. The SRBCs in the pellet were lysed with ammonium chloride lysis buffer (0.155 M ammonium chloride, 0.01 N potassium bicarbonate, and 0.1 mM EDTA), and the cells were washed twice with PBS. Samples from the pellet and the interface were counted and analyzed on a FACS for the presence of T cells and B-CLL cells.

Collection of Cells from Human→Mouse Chimera. Peritoneal cells were obtained by lavage with 10 ml of PBS. Animals were sacrificed by cervical dislocation and the peritoneum was washed again. The cells were then isolated using Lymphoprep and counted.

FACS Analysis of Donors PBMC and Human Cell Engraftment in Chimeric Mice. Single cell suspensions were incubated for 30 min on ice with a mixture of appropriate fluorescently labeled monoclonal antibodies. After washing, two- or three-color flow cytometry analysis of human antigens was performed on a FACScan analyzer (Becton Dickinson, Mountain View, CA). The labeled antibodies used recognize specific surface molecules: CD45-peridinin chlorophyll protein (pan leukocyte antigen); CD3-peridinin chlorophyll protein (pan T cells); CD20-FITC (pan B-cells); CD5-phycoerythrin (pan T-cells and B-1 B-cells); CD4-phycoerythrin (T helper cells); CD8-FITC (T cytotoxic cells); All of the above antibodies were purchased from Becton Dickinson. CD3 (CY-chrome) antibody was purchased from PharMingen (San Diego, CA).

Statistical Analyses. Statistical analyses were performed using the Stat View II program (Abacus Concepts, Inc., Berkeley, CA) on a Macintosh IICi. Student's *t* test, ANOVA correlation, and regression analyses were used to calculate probability (*P*) values. Results are presented as mean ± SE.

RESULTS

CLL Donors. The study included 22 patients (Table 1), 15 men and 7 women with a mean age of 64 ± 2 years (range, 46 to 81). The mean disease duration was 4 ± 0.5 years (range, 2 months to 11 years). The mean WBC count was 80 ± 20 × 10^9/l (14 to 400 × 10^9/l) with 75 ± 3% lymphocytes, 8.5 ± 1.8% of them CD3 positive; 16 had typical CLL and 6 had CLL/PL. Eleven patients were designated early stage [Rai stage 0 or I (Binnet stage A)], and 11 patients were advanced stage (Rai stage II to IV). As expected, these two prognostic groups differed in the patient clinical parameters. The mean age was similar: 66 ± 3 in the early stage and 64 ± 3 in the advanced stage. At the early stage, the disease duration was shorter [3 ± 0.5 years *versus* 7 ± 1 years ($P = 0.002$)], the WBC count was lower [36 ± 7 *versus* 126 ± 3.5 x 10^9/L ($P = 0.01$)], and the CD3 percentage was higher [11.3 ± 2.9 *versus* 3.9 ± 0.8 ($P = 0.004$)]. None of the early stage patients were treated, whereas six of the advanced stage patients received chemotherapy. A typical FACS analysis of donor cells is presented in Fig. 1. CLL cells are $CD20^+CD5^+CD3^-$, whereas T cells are $CD20^-CD5^+CD3^+$.

Engraftment of Human Cells in Human→Mouse Chimera. PBMCs from CLL patients were transplanted into the peritoneal cavity of lethally irradiated BALB/c mice radioprotected with SCID BM. Cells were recovered from the peritoneal cavity 10–14 days after transplantation and analyzed by FACS. These cells were distinguished

Table 1 *Patient characteristics*[a]

Patient no.	Age (yr)	Sex (M/F)	Disease duration	Stage Rai (Binnet)	WBC count ($\times 10^9$/L) (% lymphocytes)	Morphology	%CD3	Treatment
1	59	F	5 years	0	52 (80%)	Typical	12	
2	71	F	4 years	0	78 (83%)	Typical	2	
3	60	M	2 months	I (A)	41 (72%)	Typical	6	
4	78	F	6 years	0	14 (52%)	Typical	2	
5	70	M	3 years	0	15 (46%)	Typical	16	
6	54	M	3 months	0	21 (64%)	CLL/PL[b]	13	
7	70	F	4 years	0	82 (80%)	Typical	2	
8	71	F	3 years	0	32 (81%)	Typical	7	
9	74	M	3 years	0	21 (70%)	Typical	19	
10	71	M	2 years	0	24 (56%)	Typical	NA	
11	46	M	2 years	I (A)	18 (72%)	Typical	24	
12	64	M	10 years	II	44 (87%)	Typical	6	
13	81	M	9 years	II	148 (98%)	CLL/PL	5	CP Sp-ra
14	76	M	4 years	III	140 (88%)	Typical	2	
15	50	M	2 years	IV	400 (96%)	CLL/PL	1	CP Fluda
17	54	M	3 years	II	50 (60%)	CLL/PL	NA	
18	66	M	2 years	IV	78 (77%)	CLL/PL	2	
19	72	F	8 years	IV	35 (87%)	Typical	6	CP Sp-ra
20	55	M	6 years	II	273 (92%)	CLL/PL	2	CP
21	65	M	11 years	IV	48 (82%)	Typical	9	CP CHOP
22	74	F	11 years	II	129 (81%)	Typical	2	CP

[a] The patients were reviewed for age, disease duration, CLL stage according to Rai's classification (17) and current treatment.
[b] PL, prolymphocytic leukemia; CP, chlorambucil and prednisone; Sp-ra, splenic irradiation; Fluda, fludarabine; NA, unavailable; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone.

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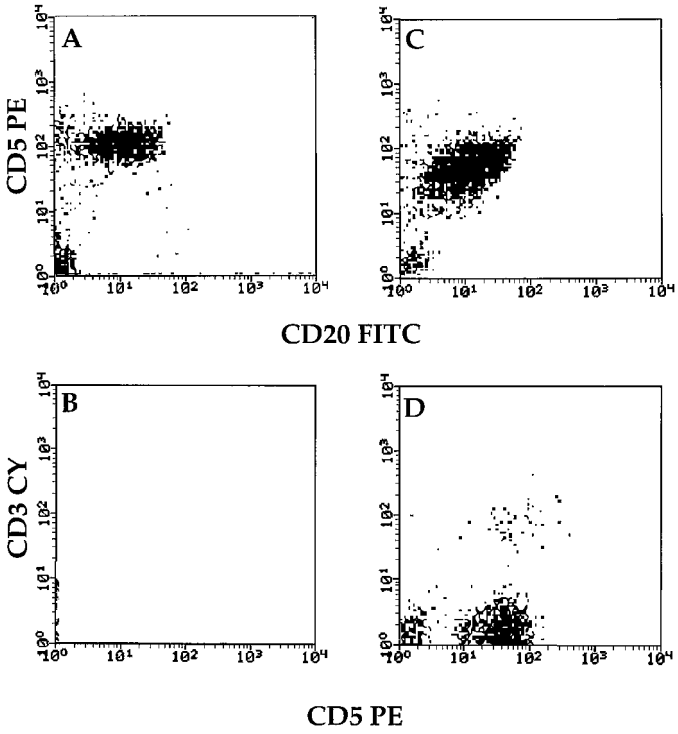

Fig. 1. Typical staining of T cells ($CD3^+CD5^+$) and tumor cells ($CD20^+CD5^+CD3^-$) in PBMCs of B-CLL patients in early stage (*A* and *B*, patient 11) and advanced stage (*C* and *D*, patient 20).

from murine cells by staining with human-specific, noncross-reactive monoclonal antibodies. The percentage of positive cells out of the total peritoneal cells is shown in Table 2. Murine cells, mostly macrophages, add to the total 100%. In addition, we have also determined the absolute number of cells in these fractions, recovered by peritoneal lavage (Table 2). As we have previously reported, PBMCs from early-stage patients led to marked engraftment of T cells: 25.6 ± 5.0% of total cells recovered from the peritoneum. CLL cells engrafted with a similar percentage: 20.3 ± 5.4% (Fig. 2, *B* and *E*). This is a pattern similar to the stage I-II pattern we have previously reported. PBMCs from advanced-stage patients led to predominance of tumor cells: 41.5 ± 7.3% ($P = 0.001$ in comparison with early stage) and a low level of T-cells [5.6 ± 2.0% ($P = 0.0001$)], similar to the stage III/IV pattern in our earlier work (Fig. 3, *E* and *F*). CLL cell recoveries from the peritoneum of chimeric mice were 26.8 ± 10.2 × 10^4 CLL cells from mice transplanted with PBMCs from early-stage patients *versus* 103.8 ± 43.8 in those transplanted from advanced-stage patients ($P = 0.05$). T-cell recoveries were 40.1 ± 16.8 × 10^4 *versus* 6.3 ± 2.6 x 10^4 cells ($P = 0.007$), respectively.

Effect of T-Cell Depletion on the Engraftment of Early-Stage CLL. To test the effect of donor T cells on the engraftment and expansion of CLL tumor cells, T-cell depletion was performed *in vivo* by i.p. injection of OKT3 on the day of transplantation. Following this treatment, the recovery of T cells from the peritoneum, 10–14 days after transplantation, was only 0.3 ± 0.1% and resulted in a total of 0.2 ± 0.1 × 10^4 T cells compared with 25.6 ± 5.0% ($P = 0.0006$) or 40.1 ± 16.8 × 10^4 ($P = 0.03$) T cells recovered from the untreated group (Fig. 2). The CLL cell engraftment was markedly affected by the treatment with OKT3. The percentage of B-CLL tumor cells was 44.6 ± 6.7% in the treated group *versus* 20.3 ± 5.4% ($P = 0.02$) in the untreated group, as might be expected based on the removal of T cells. More importantly, the absolute number of recovered tumor cells was elevated to 133.3 ± 55.1 x 10^4 *versus* 26.8 ± 10.2 × 10^4 CLL cells in the control group ($P = 0.05$). In a typical experiment using donor PBMCs from patient number 1, the recovery of CLL cells from control mice transplanted with unmanipulated PBMCs was only 0.7 ± 0.3 × 10^4, and T-cell recovery was 28.8 ± 23.5 × 10^4. Treatment with OKT3 led to marked enhancement of CLL tumor cell recovery that was elevated to 85.0 ± 67.0 × 10^4. As expected, no T cells were recovered (Table 2, Fig. 2). The CLL enhancement ratio, which was defined as the ratio between the total CLL cell count in OKT3-treated mice and the total CLL cell count in the untreated group, was calculated in 11 separate experiments, and the average was 16.5 ± 10.5. T-cell depletion had no effect on CLL cell engraftment (CLL enhancement ratio <1.5) in only 2 of 11 cases.

Effect of T-Cell Depletion on Tumor Engraftment in Advanced-Stage Disease. The effect of T-cell depletion on the engraftment of CLL cells from patients in advanced stage was evaluated in nine cases. As in the early-stage disease, T-cell depletion was efficient and only 0.4 ± 0.3% or a total of 1.1 ± 0.8 × 10^4 T cells were recovered from the OKT3-treated mice *versus* 5.6 ± 2.0% ($P = 0.01$) and 6.3 ± 2.6 × 10^4 ($P = 0.04$) T cells in the untreated group, respectively. The average total CLL cell engraftment was 164.5 ± 72.6 × 10^4 in the treated group *versus* 103.8 ± 43.8 in the untreated group (Table 2). This higher value did not reach statistical significance. The lack of effect was due to the heterogeneous effect of T-cell depletion. In five of nine cases, the CLL enhancement ratio, as defined above, was 4.2:8.6, suggesting that T-cell depletion had the same effect as in early stage. In one experiment, using donor PBMCs from patient number 18, the recovery of CLL cells from control mice transplanted with unmanipulated PBMCs was 27.7 ± 6.2 × 10^4, and T-cell recovery was only 1.0 ± 0.1 × 10^4. Treatment with OKT3 led to marked enhancement of CLL tumor cell recovery that was elevated to 117.2 ± 14.9 × 10^4. As expected, no T cells were recovered (Table 2, Fig. 3). In two patients there was no effect (ratio, 0.9), whereas, interestingly in two groups of mice transplanted with PBMCs of two different patients, T-cell depletion completely abolished CLL engraftment. In mice transplanted with PBMCs from patient number 21, CLL recovery was 32.7 ± 14.3 × 10^4 cells and T cell recovery was only 2.2 ± 1.8 × 10^4. No CLL cells were recovered from the group of mice that was transplanted with PBMCs from the same donor but was treated with OKT3. Altogether, the enhancement of CLL engraftment of early-stage donors on T-cell depletion led to a level of CLL cell recovery that was not statistically different from that found after transplantation of advanced-stage CLL in the absence of T-cell depletion.

Effect of T-Cell Enrichment in Advanced-Stage B-CLL. Considering that in advanced-stage B-CLL the number of T cells in the initial inoculum is relatively small, it could be reasonable to speculate that this low T-cell level might be responsible for the marked expansion of CLL cells in the chimeric mice after transplantation of advanced-stage B-CLL. To test this possibility, T cells were purified by E-rossetting with SRBCs and were then added to the tumor cell fraction at different ratios (so as to enrich T-cell concentration to two to three times the original T-cell percentage), and the effect of T cell enrichment on the engraftment of the tumor cells was evaluated. As can be seen in Table 3, the average percentage of T cells following T-cell enrichment was 13.8 ± 4.3%, and a total of 6.3 × 10^4 T cells were recovered from the treated group *versus* 2.6 ± 1.2% ($P = 0.02$) and 2.0 ± 0.8 × 10^4 ($P = 0.04$), respectively, in the untreated group. CLL cell recovery was 12.4 ± 4.1 × 10^4 in the T-enriched group *versus* 28.6 ± 5.6 × 10^4 in the untreated group ($P = 0.02$). A

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Table 2 *Effect of T-cell depletion: Recovery of T cells and B-CLL from human→mouse chimera*

	Untreated					Treated with OKT3					
		CLL cells		T cells			CLL cells		T cells		
Patient no.	Number of mice	%[a]	Total ($\times 10^4$)	%[a]	Total ($\times 10^4$)	Number of mice	%[a]	Total ($\times 10^4$)	%[a]	Total ($\times 10^4$)	CLL ratio[b]
Early stage											
1	4	1.0 ± 0.3[c]	0.7 ± 0.3[c]	20.5 ± 9.3	28.8 ± 23.5[c]	2	48.0 ± 13.0	85.0 ± 67.0			121.0
2	3	3.0 ± 0.6[c]	1.4 ± 0.2[c]	37.0 ± 7.5	17.0 ± 1.7[c]	2	20.0 ± 1.0	7.8 ± 3.6	0.7 ± 0.3	0.3 ± 0.3	5.6
3	6	37.6 ± 9.7[c]	94.7 ± 44.3	20.8 ± 3.1	40.3 ± 15.8[c]	6	75.6 ± 3.1	641.5 ± 179.0	0.3 ± 0.2	1.1 ± 0.8	6.7
4	1	1.0	2.0	41.0	88.0	1	12.0	7.0	1.0		3.5
5	1	17.0	7.0	15.0	6.0	2	73.5 ± 6.5	118.0 ± 58.0			16.8
6	1	17.0	10.0	35.0	21.0	1	38.0	30.0			3.0
7	10	41.3 ± 9.0[c]	84.8 ± 30.4[c]	8.5 ± 1.6[c]	7.8 ± 1.9[c]	8	67.6 ± 3.7	162.1 ± 49.4	0.5 ± 0.2	0.6 ± 0.3	1.6
8	4	17.3 ± 9.4	10.6 ± 7.5[c]	23.0 ± 7.1[c]	18.1 ± 12.1	2	50.5 ± 19.5	177.5 ± 25.5			16.7
9	2	41.0 ± 4.0	39.0 ± 12.5	16.5 ± 4.5[c]	18.0 ± 11.0	2	45.0 ± 13.0	41.5 ± 36.5			1.1
10	2	45.0 ± 3.0[c]	38.3 ± 19.0	4.0 ± 0.0[c]	3.3 ± 1.5	1	44.0	189.2			4.9
11	3	2.0 ± 0.6[c]	6.7 ± 2.7	60.7 ± 3.5[c]	193.3 ± 58.1	3	16.3 ± 3.5	6.0 ± 1.2			0.9
Mean ± SE (*P* value)	37	20.3 ± 5.4 (0.005)	26.8 ± 10.2 (0.03)	25.6 ± 5.0 (0.0001)	40.1 ± 16.8 (0.01)	30	44.6 ± 6.7	133.3 ± 55.1	0.3 ± 0.1	0.2 ± 0.1	16.5 ± 10.5
Advanced stage											
12	2	20.0 ± 5.0	1.5 ± 0.5	9.5 ± 1.5[c]	0.6 ± 0.2[c]	2	25.5 ± 9.5	7.5 ± 5.5			5.0
13	6	34.7 ± 5.3[c]	48.8 ± 20.6[c]	21.8 ± 2.6[c]	29.2 ± 14.2[c]	4	56.5 ± 2.3	217.3 ± 67.4	0.5 ± 0.3	1.0 ± 0.7	4.4
14[d]	3	18.3 ± 8.6[c]	7.1 ± 3.0[c]	2.0 ± 0.6[c]	0.9 ± 0.5	3	55.3 ± 4.4	61.3 ± 2430			8.6
15	3	79.7 ± 3.5	292.7 ± 108.4	4.2 ± 0.2[c]	15.0 ± 5.0[c]	5	84.8 ± 3.9	265.8 ± 84.6	0.2 ± 0.2	0.6 ± 0.6	0.9
16	5	84.8 ± 2.8	439.6 ± 257.8	0.4 ± 0.1[c]	1.8 ± 1.0	4	86.3 ± 6.7	687.8 ± 138.5	0.2 ± 0.1	0.9 ± 0.3	0.9
17	2	29.5 ± 18.5	20.7 ± 18.0	7.0 ± 4.0	7.5 ± 1.5	2	38.5 ± 5.0	123.5 ± 57.5	2.5 ± 0.5	7.0 ± 1.0	6.2
18[d]	6	27.6 ± 4.3[c]	27.7 ± 6.2[c]	1.3 ± 0.3[c]	1.0 ± 0.1[c]	6	59.5 ± 5.8	117.2 ± 14.9	0.1 ± 0.1	0.1 ± 0.1	4.2
19	1	22.0	15.0	11.0	7.0						
20	1	34.0	48.0	2.0	3.0						
21	2	40.0 ± 0.0[c]	32.7 ± 14.3	2.5 ± 1.5	2.2 ± 1.8	3	0.3 ± 0.3	0.2 ± 0.2			0.0
22	2	64.5 ± 2.5[c]	209.0 ± 157.8	0.3 ± 0.2	0.9 ± 0.8	2					0.0
Mean ± SE (*P* value)	33	41.5 ± 7.3 (NS[e])	103.8 ± 43.8 (NS)	5.6 ± 2.0 (0.01)	6.3 ± 2.6 (0.04)	31	45.2 ± 10.6	164.5 ± 72.6	0.4 ± 0.3	1.1 ± 0.8	3.4 ± 1.0

[a] Percentage of total cells in peritoneal wash.
[b] Total CLL count in OKT3-treated mice/total CLL count in untreated.
[c] $P < 0.05$ in comparison with the equivalent value in the treated group.
[d] T depletion was performed *ex vivo* by SRBC rosettes.
[e] NS, not significant.

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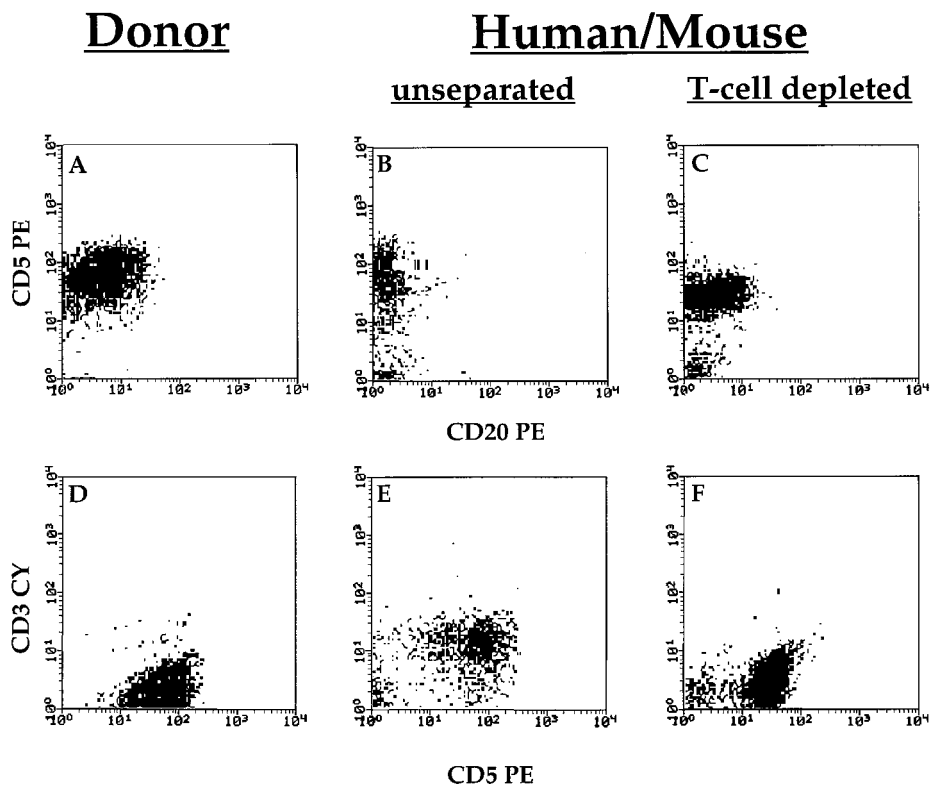

Fig. 2. Phenotypic characterization of donor PBMCs (*A* and *D*) and lymphocytes recovered from human→mouse chimera transplanted with PBMCs (patient number 1) with (*C* and *F*) or without (*B* and *E*) *in vivo* treatment with OKT3 antibody to deplete human T cells. Chimera were prepared by conditioning BALB/c mice with split irradiation, followed by radioprotection with SCID BM; thereafter, the mice were transplanted with 120×10^4 PBMC from B-CLL patients. Some of the mice were treated with OKT3 on the day of PBMC transplantation. Cells were recovered from the peritoneal wash 10–14 days after transplantation. Cells were typed by triple staining with anti CD20, CD5, and CD3 antibodies. T cells were typed as $CD20^-CD5^+CD3^+$, and CLL cells were typed as $CD20^+CD5^+CD3^-$.

typical experiment using PBMCs from patient number 18 is presented in Fig. 3. CLL recovery from the untreated, T-depleted, and T-enriched groups was 27.7 ± 6.2, 117.2 ± 14.9, and 13.2 ± 6.1, respectively.

DISCUSSION

CLL cells from advanced-stage patients have a higher engraftment potential than early-stage CLL cells, whereas T cells have better engraftment potential when derived from patients in early stage (18). This mutually exclusive pattern of engraftment indicated that T cells might control the expansion of tumor cells in the peritoneum of recipient mice, and we speculated that the model reflects a T-cell dysfunction that could be associated with tumor cell expansion in advanced disease.

To test this hypothesis, we have now compared the effect of T-cell depletion or enrichment on the ability of CLL cells to engraft and

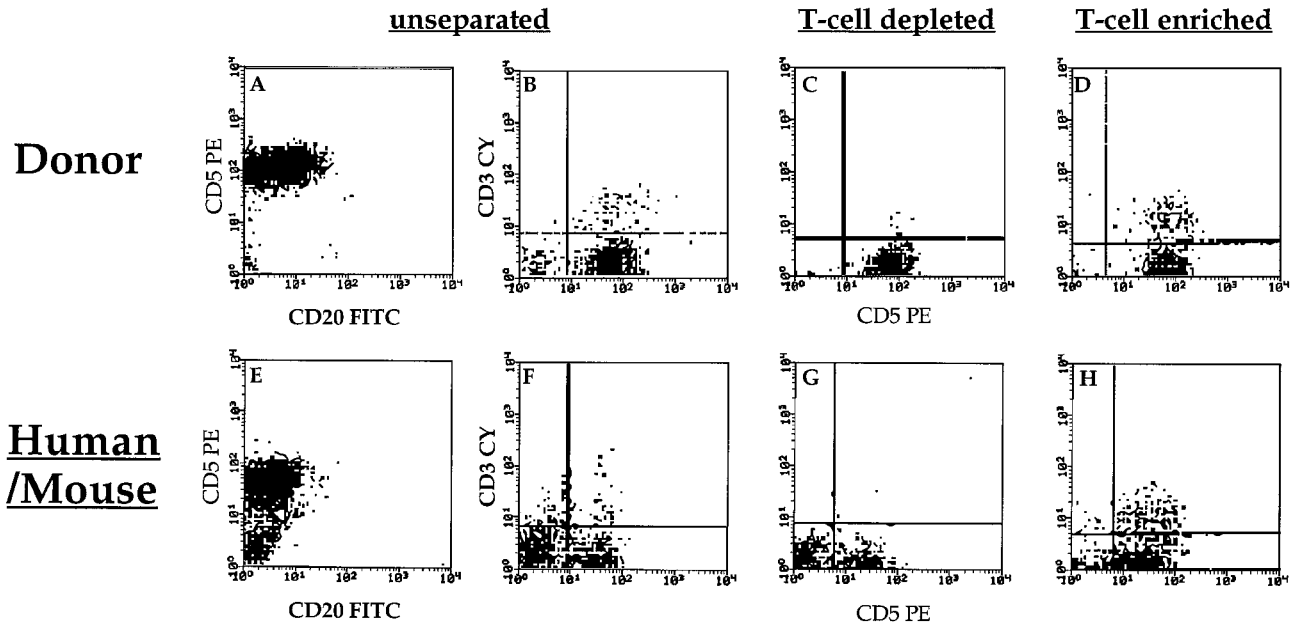

Fig. 3. Phenotypic characterization of donor PBMCs (*A* and *B*), T-depleted (*C*) and T-enriched (*D*) donor PBMCs, as well as the phenotype of lymphocytes recovered from human→mouse chimera transplanted with PBMCs (patient number 18; *E* and *F*), T-depleted (*G*) or T-enriched (*H*) PBMCs. Human→mouse chimera were prepared as described in Fig. 2. T depletion was performed *ex vivo* by E-rosetting with SRBCs. T-cell enrichment was attained by adding to donor PBMC T cells purified by E-rosetting. FACS analysis was performed as described in Fig. 2.

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Table 3 *Effect of T-cell enrichment: Recovery of T cells and B-CLL cells from human→mouse chimera*

	Untreated					Treated with T cell enrichment					
		CLL Cells		T Cells			CLL Cells		T Cells		
Patient no.	Number of mice	%[a]	Total ($\times 10^4$)	%[a]	Total ($\times 10^4$)	Number of mice	%[a]	Total ($\times 10^4$)	%[a]	Total ($\times 10^4$)	CLL ratio[b]
18	6	27.6 ± 4.3	27.7 ± 6.2	1.3 ± 0.3[c]	1.0 ± 0.1	5	32.8 ± 5.8	13.2 ± 6.1	3.6 ± 0.7	1.6 ± 0.7	0.5
19	1	22.0	15.0[c]	11.0[c]	7.0	3	12.6 ± 1.3	5.3 ± 0.3	30.5 ± 0.3	13.3 ± 2.0	0.4
20	1	34.0	48.0	2.0	3.0	1	49.0	30.0	15.0	9.0	0.6
Mean ± SE[d] (P value)	8	27.8 ± 3.3 (NS[e])	28.6 ± 5.6 (0.02)	2.6 ± 1.2 (0.02)	2.0 ± 0.8 (0.04)	9	27.9 ± 5.2	12.4 ± 4.1	13.8 ± 4.3	6.3 ± 2.0	0.5 ± 0.1

[a] Percentage of total cells in peritoneal wash.
[b] Total CLL count in T-enriched mice/total CLL count in untreated.
[c] $P < 0.05$ in comparison with the equivalent value in the treated group.
[d] Sum of all three experiments.
[e] NS, not significant.

expand. By measuring both the percentage and absolute count of CLL cells and T cells in the peritoneum lavage, we have made the following observations. First, T-cell depletion *in vivo* by OKT3 treatment markedly enhanced the expansion of tumor cells after adoptive transfer of B-CLL cells from early-stage patients, such that CLL cell recovery was similar to that found after infusion of cells from advanced-stage patients. Interestingly, in some of the patients with almost no CLL cell engraftment, T-cell depletion restored the ability of the tumor to engraft (as in patient 1, Fig. 2). Second, the results of T-cell depletion in mice transplanted with advanced-stage disease were more heterogeneous. Although in five of nine patients T depletion had the same stimulatory effect on CLL cell engraftment as in early-stage disease, in four cases this effect was not demonstrated. In two patients CLL cells could not engraft in the absence of T cells or were dependent on T cells for their survival in the recipient mice. Therefore, it seems that whereas in some advanced patients T cells are capable of mediating tumor supression, in others the CLL cells exhibit an opposite effect being dependent on T cells for their growth or expansion. Finally, T-cell enrichment before adoptive transfer of CLL cells from advanced-stage patients can lead to a significant reduction of CLL cell engraftment (Table 3).

Altogether, our results strongly suggest that T cells of B-CLL, in particular of patients in early stage, are capable of supressing growth and expansion of tumor cells. This suggestion is in accordance with several previous studies. A few reports found oligoclonal expansions within the T-cell repertoire of CLL patients, both CD4 and CD8 (19–22) and some of such clones were found to recognize autologous CLL cells in a cytokine release assay (21). In one case, Sherman *et al.* (23) demonstrated that T cells reacted to a tumor-specific antigen present on CLL cells in a MHC class II-restricted manner. This suggests that a tumor-specific peptide on the cell might be recognized by autologous T cells and could be a target for immunotherapy. However, additional studies with eluted peptides are required before the presence of a tumor-specific antigen can be established.

Whereas the tumor suppressive effect of T cells is regularly found in mice transplanted with CLL cells of early-stage patients and with most donors at advanced stage, it seems that in some patients, with advanced disease, T-cell depletion may lead to ablation of tumor engraftment, indicating T-cell dependency of the CLL cells. This finding is also in accordance with some *in vitro* studies showing that CLL cells in progressive disease, but not in early disease, are dependent on T-cell factors for their growth (6–8). They can respond to signaling through T-cell factors (7) and lose their clonogenic potential in the absence of activated T cells (6).

It, therefore, seems that B-CLL cells may be regulated by the normal T-cell compartment, both negatively by suppressor activity (more prominent on early stage) and positively by helper function (more prominent in advanced stage). Considering this dichotomy, it should be noted that Fludarabine, which is increasingly being used in the treatment of CLL (3), is known to cause severe depletion of T cells, with heterogenous results as might be expected from the dual role of T cells in this disease. Thus, our model could be used to predict the response of patients to Fludarabine and thereby avoid its use in patients in whom it may lead to adverse effect. A second issue raised by our present study is related to the T-cell dysfunction that might be associated with disease progression. B-CLL is characterized by impaired humural and cellular immunity, hypogammaglobulinemia, and autoimmunity (1, 2). Early studies have stressed the dysfunction of T cells in this disease. The total number of T cells is usually increased, there is a reversal of the normal CD4:CD8 ratio, decreased T-cell help, and increased T suppression (24–27) T cells have been found to have a decreased proliferative response to mitogens and in allogeneic and autologous mixed lymphocyte responses (30–33). Decreased cytotoxicity and natural killer activity (28, 29) have also been reported. Different patterns may be observed in typical and atypical CLL cases (30).

Recent studies emphasize that CLL-derived T cells are intrinsically intact, but their interaction with B-CLL cells causes their dysfunction. B-CLL cells, as normal B cells, express the HLA class II molecule and can serve as unprofessional antigen-presenting cells (31). Decker *et al.* (32) found no difference in cytokine production and proliferative response of purified CLL-derived T cells; however, when autologous accessory cells were added, there was a dramatic change. Also, further indication that there is probably no intrinsic T-cell defect was suggested by the demonstration in our model that hypogammaglobulinemia is reversed on adoptive transfer of cells from donors with low-stage disease (18). Our interpretation of these earlier results was that helper T cells, which are anargized in the tumor-bearing patient, can be released of their anargic status by the marked xenoactivation and the subsequent exposure to high levels of interleukin-2 and cytokines experienced in the mouse environment immediately after transfer of human PBMCs.

A major question in this context is how tumor cells escape recognition or anargize specific T cells. In general, the interaction between T cells and B cells is mediated by several costimulatory molecules (33). The most important in this regard is the CD40/CD40 ligand system. Cantwell *et al.* (34) described an acquired CD40 ligand deficiency in CLL-derived T cells. They suggested that CLL cells, by the expression of CD40, down-regulate CD40L on activated T cells and by that impair the immune response. Interestingly, no CD40L could be detected when T cells were <3% of the cells in the culture. By changing the T cell:CLL cell ratio, some of the immune potential can be recovered. This is somewhat similar to the containment of advanced CLL cells in our model by increasing the percentage of T cells in the transplant inoculum. Immune surveilance of tumor cells can also be avoided by manipulation of the B7/CD28 system (41, 42).

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CLL cells can escape immunity as a result of the low expression of B7. In addition, T cells in CLL patients were found to have defective CD28 expression (35). However, exposure of CLL cells to activated T cells might lead to B7 expression. This would lead to improved recognition of these cells by T cells. In our model, a high proportion of T cells are activated due to the murine xeno-environment, and this might be one of the ways T cells improve their ability to recognize and control CLL tumor cells. However, we were unable to demonstrate CD80/86 expression on CLL cells recovered early after transplantation (data not shown). Finally, our most recent results suggest that the FasR/FasL system might explain, in part, how CLL cells escape the effect of T cells (44). Experiments that will compare T cell and CLL cell immunophenotypes, and especially activation markers, in donor cells and in cells recovered from human→mouse chimera will help to further clarify this issue.

Our demonstration that autologous T cells can control CLL cell engraftment and the demonstration that early-stage disease following T-cell depletion was very similar to that of untreated cells from patients with advanced disease, may be relevant to the pathogenesis of disease progression. Thus, it is tempting to speculate that changes in T-cell function, in addition to changes in tumor characteristics, are associated with progression of the disease from an early to an advanced stage. Consequently, it is possible that T cell harvest (perhaps of certain subsets) during early-stage disease with later reinfusion, may afford a beneficial mode of cell therapy in advanced B-CLL patients, in addition to other treatment modalities. In summary, the unique *in vivo* mouse model described in the present study provides a useful tool for the investigation of the pathogenesis and progression of B-CLL, as well as for the development of T-cell therapy in this disease.

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