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IN VITRO INDUCTION OF IgM SECRETION AND SWITCHING TO IgG PRODUCTION IN HUMAN B LEUKEMIC CELLS WITH THE HELP OF T CELLS[1]

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***In vitro* stimulation of the B leukemic cells (B-CLL cells) with normal allogeneic T cells plus PWM induced IgM secretion and a switching from IgM to IgG production. Induction of IgM and IgG production in B-CLL cells with T cells was demonstrated by the presence of the same idiotype in induced Ig as that present in the monoclonal IgM protein in the patient's serum. Both T cells and PWM were required for Ig induction in B-CLL cells, and x-irradiated T cells showed the comparable helper effect. T cells and PWM induced not only Ig secretion but proliferation of B-CLL cells. Cell division was essential for the differentiation of the leukemic cells to Ig-producing cells. PWM-induced, antigen-nonspecific helper factor(s) were also effective in the induction of differentiation of the leukemic cells. Variations existed among T cell donors in the capabilities to induce differentiation of the same leukemic cells, suggesting the requirement of matching of acceptors on B-CLL cells and T effector molecules for the induction of Ig production in B-CLL cells.**

Since pokeweed mitogen- (PWM)[3] induced Ig production in human B lymphocytes is highly T dependent (1, 2), this experimental system has been applied for analysis of the mechanisms of the activation of B lymphocytes to Ig-producing cells under the influence of T cells (3–5). Heterogeneity of lymphocytes, however, hindered the molecular analysis of the B cell activation, such as the chemical nature of B cell acceptors for T effector molecules or biochemical events essential for the activation of B lymphocytes. In this situation, monoclonal B tumor cells may provide useful means for such studies if these cells can be affected by signals given by T cells to differentiate Ig-producing cells.

In the previous experiments, we demonstrated IgG induction in Epstein-Barr (EB) virus-transformed human B blastoid cell lines with normal allogeneic T cells (6). Ig induction in human B leukemic cells has also been shown by Fu *et al.* (7) and by Maino *et al.* (8). In their experiments, IgM or free L chain was induced in chronic B leukemic cells by the addition of PWM or PHA in the presence of T cells.

In the present experiments, attempts were made to induce IgG in the chronic B leukemic cells with T cells or soluble T helper factor(s). The results show that stimulation of the B leukemic cells with PWM and normal T cells or T-helper factor(s) induce IgG bearing the same idiotype as that of monoclonal IgM present in the patient's serum. The results also suggest the requirement of matching of a certain cell surface antigen between the B leukemic cells and T cells for the induction of the differentiation of the B leukemic cells.

MATERIALS AND METHODS

Reagents. PWM was obtained from Grand Island Biological Co. (Grand Island, N. Y.). Cytosine arabinoside (Ara-C), hydroxyurea (HU), and neuraminidase were obtained from Sigma Chemical Co. (St. Louis, Mo.).

Isolation of lymphocytes. Peripheral blood lymphocytes (PBL) were isolated by Ficoll-Hypaque gradients from heparinized peripheral blood of normal individuals and a patient with chronic lymphocytic leukemia (CLL) T cells were isolated by rosette methods with neuraminidase-treated SRBC (9). Details of the methods have been described previously (3).

Patient. Patient N.K. was a 75-year-old man who had had CLL for 2 years. His leukocyte count was 29,000/mm^3, and 80.5% of the leukocytes were leukemic cells on his initial presentation (August, 1978). The percentages of erythrocyte (E), erythrocyte-antibody complex (EA), and erythrocyte-antibody-complement complex (EAC) rosette-forming cells in his PBL were 12.3%, 28.5%, and 87.7%, respectively. The leukemic B cells had IgM, IgG, and IgD on their surface. They contained intracellular IgM (μ, κ), but intracellular IgG-positive cells were not detected. A monoclonal IgM protein of 1,539 mg/dl was present in the serum. A monoclonal IgG peak was not present, and the same idiotype as that of the monoclonal IgM was not detected in 7S fraction of the patient's serum. No detectable IgM or IgG was secreted into the culture medium when chronic B lymphocytic leukemia (B-CLL) cells were cultured alone.

Cell culture. Cell cultures in total volume of 0.2 ml were set up in a Micro Test II culture plate (Falcon Plastics Co., Oxnard, Calif.) at 37°C with 5% CO_2 in RPMI 1640 supplemented with 10% FCS (Flow Laboratories, Rockville, Md.), 5×10^{-5} M 2-mercaptoethanol (2-ME), and 0.2 mM glutamine. For experiments to induce Ig in the leukemic cells, 1×10^5 B-CLL cells were co-cultured with 1 to 2×10^5 normal T cells and PWM at the concentration of 2.5 μl/ml for 7 days. Cultured cells were

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[1] This work was supported by Grants from the Ministry of Education, Science, and Culture and the Ministry of Health and Welfare.
[2] All correspondence should be addressed to T. K.
[3] Abbreviations used in this paper: PWM, pokeweed mitogen; EB, Epstein-Barr; Ara-C, cytosine arabinoside; HU, hydroxyurea; CLL, chronic lymphocytic leukemia; B-CLL, chronic B lymphocytic leukemia; TdR, thymidine; PBL, peripheral blood lymphocyte; 2-ME, 2-mercaptoethanol.

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then centrifuged, and culture supernatants were recovered for radioimmunoassays. In some experiments, cultured cells were counted, washed, and processed for immunofluorescence microscopy for the determination of Ig-producing cells. For the measurement of DNA synthesis in cultured cells, each culture was pulsed with 0.4 μCi of ^{3}H-TdR (5 to 15 Ci/mmole specific activity, New England Nuclear, Boston, Mass.) for 4 hr. Cells were harvested by Dynatech Automash cell harvester (Dynatech, England), and the radioactivity was measured by a liquid scintillation counter.

Immunofluorescence and radioimmunoassay. Anti-idiotype antibody against the monoclonal IgM_k component in the serum was prepared in New Zealand white rabbits. The monoclonal IgM was purified by euglobulin precipitation and gel filtration on Sephadex G-200. Five hundredths microgram of the purified IgM protein was injected into a rabbit with complete Freund's adjuvant twice at 4-week intervals. Blood was taken 2 weeks after last injection, and serum was absorbed with pooled human serum and the other monoclonal IgM_k protein. In double diffusion in agar, the anti-idiotype antibody reacted with the monoclonal IgM_k purified from the patient's serum but reacted neither with two other monoclonal IgM_k proteins nor with IgG myeloma proteins. The specificity of anti-idiotype antibody was further confirmed by the inhibition radioimmunoassay with ^{125}I-labeled IgM_k from the patient and anti-idiotype antibody. The inhibition curves were shown in Figure 1. Only the IgM_k protein from the patient inhibited the binding of ^{125}I-IgM_k with anti-idiotype antibody.

Antibodies specific for human μ-, γ-, and α-chains were the same preparations as those used in our previous experiments (3, 10). Antibodies specific for human κ- and λ-chains were purchased from Miles Laboratories Inc. (Elkhart, Ind.) and rendered monospecific by absorption with IgG_λ and IgG_κ myeloma proteins, respectively. Details of the method for measuring the amounts of Ig in culture supernatants by radioimmunoassay were described previously (10). FITC-labeled anti-μ and anti-γ antibodies and rhodamine-labeled anti-idiotype antibody were prepared by the method described by Wood *et al.* (11) and Cebra and Goldstein (12), using IgG fractions of anti-μ, anti-γ, and anti-idiotype antisera. Monospecificity of these reagents was confirmed by staining human lymphoblastoid cell lines, i.e., RPMI 1788 cells that had intracellular IgM and CESS cells that had intracellular IgG.

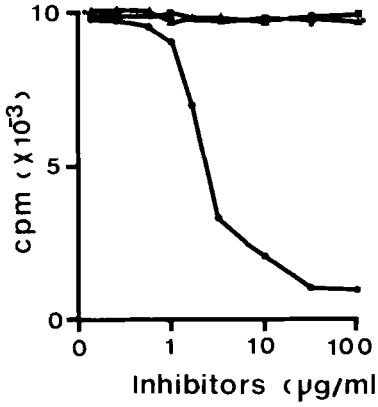

Figure 1. Inhibition radioimmunoassay for studying the specificity of the anti-idiotype antibody. Microtiterplate (Cooke Laboratory Products, Division of Dynatech Laboratories, Inc., Alexandria, Va.) was coated with IgG fraction of anti-idiotype antibody and inhibitions of the binding of ^{125}I-labeled IgM_κ from the patient by several myeloma proteins were measured. Inhibition curve with IgM_κ from the patient (●——●), monoclonal IgM_κ (▲——▲ and ■——■), and monoclonal IgG_κ (★——★) were shown.

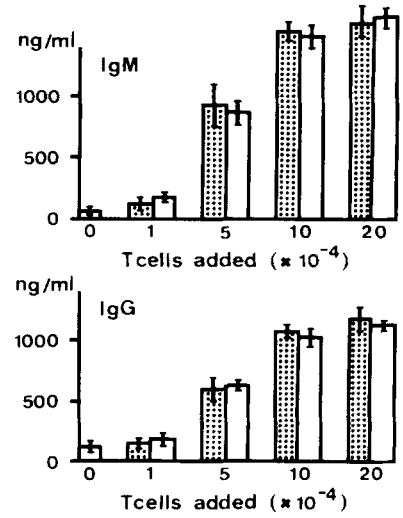

Figure 2. Dose responses of normal allogeneic T cells in the induction of IgM and IgG in B-CLL cells. B-CLL cells (1×10^5) were cocultured with various number of normal (▦) or x-irradiated (□) T cells in the presence of PWM and amounts of IgM and IgG in culture supernatants were measured on day 7.

Preparation of T-helper factor(s). T cells isolated from PBL of healthy donors were incubated with 2.5 μl/ml PWM in culture medium supplemented with 10% FCS and 5×10^{-5} M 2-ME, and after 48 hr incubation cells were centrifuged off and supernatants were recovered and used as helper factor(s).

RESULTS

Induction of IgG and IgM in B-CLL cells with T cells and PWM. B-CLL cells (1×10^5 in 0.2 ml culture medium) were cocultured with various numbers (0.1 to 2.0×10^5) of normal allogeneic T cells in the presence of PWM, and amounts of IgM and IgG in culture supernatants were assessed on day 7. As shown in Figure 2, co-culture of B-CLL cells with T cells and PWM induced both IgM and IgG production, and 1 to 2×10^5 T cells were optimum for the maximum induction of IgM or IgG. Neither T cells alone nor PWM alone induced any detectable IgM or IgG in culture supernatants. Helper function of x-irradiated T cells that had been exposed to 2,000 rads x-irradiation was comparable to that of normal T cells.

Co-culture of B-CLL cells with T cells plus PWM induced comparable amounts of IgM and IgG, but the amount of IgA produced was less than 10% of IgM or IgG (Table I). On the other hand, normal B cells produced not only IgM and IgG but also a comparable amount of IgA when they were cultured with allogeneic T cells plus PWM. The results suggested that Ig induction observed in the B-CLL cell population was not due to the contaminated normal B cells. In normal B cells, the percentage of λ-type Ig produced by T cells and PWM was about 31% of total Ig, whereas the percentage of λ-type Ig induced in the B-CLL cell population was less than 9%, suggesting that only κ-type IgM and IgG were induced in B-CLL cells.

Induction of IgG bearing the same idiotype as that of monoclonal IgM present in the serum. In order to confirm that IgG and IgM were induced in B-CLL cells with T cells and PWM, we examined whether IgG produced by co-culture with T cells had the same idiotype as that of monoclonal IgM isolated from the patient's serum. After co-culturing of B-CLL cells with T

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TABLE I

Induction of κ-type IgM and IgG in B-CLL cells with normal T cells

Cells Cultured[a]	Igs Produced in Culture Supernatants				
	IgM	IgG	IgA	κ-Ig	λ-Ig
			ng/ml		
Bp	110 ± 30	95 ± 41	47 ± 15		
Bp + Tn	2,600 ± 310	3,467 ± 503	257 ± 64	6,000 ± 1,732	700 ± 265
Bn + Tn	3,400 ± 550	4,100 ± 559	4,400 ± 720	13,333 ± 1,528	7,167 ± 2,566

[a] 1×10^5 B cells from the patients (Bp) were cultured alone or with 1×10^5 normal allogeneic T cells for 7 days. As a control experiment, 1×10^5 B cells from a healthy donor (Bn) were cultured with 1×10^5 normal allogeneic T cells.

cells and PWM for 7 days, cells were subjected to double staining with fluorescein-labeled anti-γ-chain antibody and rhodamine-labeled anti-idiotype antibody. The percentage of fluorescein-stained cells and rhodamine-stained cells were 7.5% and 18.5%, respectively, and intracellular IgG-positive cells were also rhodamine positive, showing that induced IgG had the same idiotype as that of monoclonal IgM present in the serum. The percentage of intracellular IgM-positive cells was 10.4% when the same cell population was stained with fluorescein-labeled anti-μ-chain antibody. The percentage of E rosette-forming cells in the recovered cells after 7 days' culture was 77%. When cultured cells were stained with anti-idiotype, anti-μ, and anti-γ antibodies after depletion of E rosette-forming cells, the percentages of idiotype-positive, μ-positive, and γ-positive cells were 88%, 70%, and 25%, respectively. These results showed that about 80% of idiotype-negative cells in the co-culture were T cells, and a certain percentage of B-CLL cells switched from IgM to IgG-producing cells with the same idiotype. Radioimmunoassay with anti-idiotype antibody also showed that Ig in culture supernatants of B-CLL cells and normal T cells had the same idiotype (Table II). On the other hand, culture supernatants of normal B and T cells did not have any detectable idiotype-positive Ig, although they had comparable amounts of IgM and IgG. The result that IgG induced with T cells and PWM had the same idiotype was further confirmed by showing the presence of the idiotype on IgG produced in culture supernatants. As shown in Figure 3, culture supernatants were mixed with 1 ml of normal human serum, and they were applied on Sephadex G-200 column. Idiotype-positive Ig in each fraction eluted from the column was examined by radioimmunoassay. Both 19S and 7S fractions contained the idiotype-positive Ig, indicating that IgG induced in B-CLL cells had the same idiotype as that of IgM.

Kinetics of the production of IgM and IgG. B-CLL cells were co-cultured with T cells and PWM, and Ig in culture supernatants were measured on days 3, 5, 7, and 10. As shown in Figure 4, productions of both IgM and IgG were detected on day 5, and they reached the maximal level on Day 7. The similar patterns of kinetics for IgM and IgG productions suggested that IgM-secreting cells and IgG-producing cells derived from separate B-CLL cells.

Kinetics of Ig-production suggested the requirement of proliferation of B-CLL cells for their differentiation to Ig-producing cells, since it took 7 days for the maximum production of Ig. Co-culture of B-CLL cells with x-irradiated T cells plus PWM induced an increase of ^{3}H-TdR uptake on day 3 to 5, as shown in Figure 5. The stimulation of B-CLL cells with PWM in the absence of T cells did not induce any proliferation of these cells. As shown in Table III, the addition of 10^{-3} M HU or 1 μg/ml of Ara-C completely inhibited IgG production. The same concentration of HU or Ara-C inhibited the proliferation of cells but did not affect the viability of B-CLL cells when harvested on

TABLE II

Induction of the idiotype-positive Igs in B-CLL cells with normal T cells

Cells Cultured[a]	Igs Produced in Culture Supernatants		
	IgM	IgG	Id-positive Igs
		ng/ml	
Bp	132 ± 46	192 ± 30	<250
Bp + Tn	7,450 ± 602	4,700 ± 1,150	11,750 ± 450
Bn + Tn	5,650 ± 1,626	3,467 ± 503	<250

[a] As described in Table I, 1×10^5 Bp cells or normal B cells (Bn) were cultured with 1×10^5 normal allogeneic T cells for 7 days.

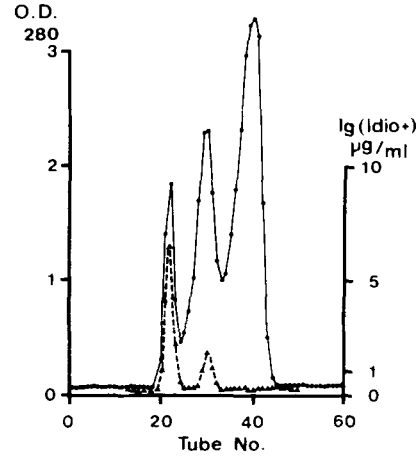

Figure 3. Gel filtration of culture supernatants on Sephadex G-200 and detection of idiotype-positive Ig in 19S and 7S fractions. One milliliter of normal human serum and 12 ml of culture supernatants were applied on Sephadex G-200 column (88 × 1.6) and OD_{280} (●——●) and idiotype-positive Ig (▲——▲) in each tube (2.5 ml/tube) were measured.

day 7. The results suggested that proliferation of B-CLL cells was necessary for Ig induction.

Induction of Ig production and proliferation in B-CLL cells with T-helper factor(s). In order to study whether soluble helper factor(s) can replace the function of T cells in inducing Ig production in B-CLL cells, effect of PWM-induced antigen-nonspecific helper factor(s) was examined. Normal T cells were cultured with PWM for 48 hr, and culture supernatants were recovered as a source of helper factor(s). B-CLL cells were cultured for 7 days in the presence of helper factor(s) at the final concentration of 50% and PWM. As shown in Figure 6, the addition of helper factor(s) (Tn_1-Sup.) and PWM to B-CLL cells induced IgG production comparable to that observed in normal B cells with the same factor(s) plus PWM. The addition of helper factor(s) also induced proliferation of B-CLL cells, as shown in Figure 6. Without helper factor(s), PWM induced neither IgG production nor proliferation in B-CLL cells. On the

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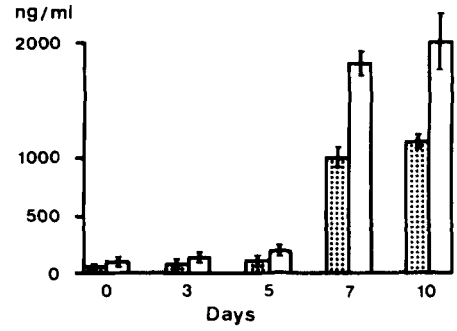

Figure 4. Kinetics of the induction of IgM (□) and IgG (▦) in B-CLL cells with T cells. B-CLL cells (1×10^5) were co-cultured with normal allogeneic T cells (1×10^5) in the presence of PWM and amounts of Ig in culture supernatants were measured on day 0, 3, 5, 7, and 10.

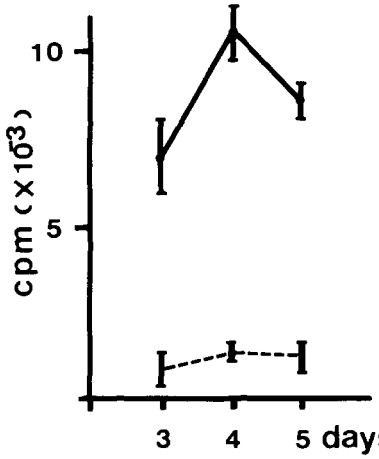

Figure 5. Kinetics of the induction of proliferation of B-CLL cells with T cells plus PWM. B-CLL cells (1×10^5) were cultured with x-irradiated T cells (1×10^5) plus PWM (——) or cultured only with PWM (-----) and uptakes of ^{3}H-TdR on days 3, 4, and 5 were assessed.

other hand, the factor(s) obtained from the other healthy donors (Tn_2-Sup.) induced neither IgG production nor proliferation in B-CLL cells, although IgG production and proliferation induced in normal allogeneic B cells with the factor(s) (Tn_2-Sup.) were comparable to those observed with the other factor(s) (Tn_1-Sup.).

Helper effect of T cells from healthy donors on Ig-induction in B-CLL cells. In Ig induction in B-CLL cells with T cells plus PWM, T cells from certain donors consistently showed good helper function, and co-culture of B-CLL cells with those T cells induced large amounts of IgG, as shown in Table IV, groups I and II. On the other hand, T cells from the other donors, group III, did not induce significant IgG production. T cells from any donors, depicted in Table IV, showed comparable helper function in PWM-induced Ig production in normal allogeneic B cells. Donors of T cells that showed good helper function in Ig induction in B-CLL cells could be divided into two groups, groups I and II, according to their proliferative responses against x-irradiated B-CLL cells. As shown in Table IV, T cells from group I donors showed much higher MLR responses against x-irradiated B-CLL cells than T cells from group II donors. All T cells, which did not help Ig induction in B-CLL cells, did not show significant MLR responses against B-CLL cells. On the other hand, T cells from the patient showed high MLR responses against all B cells from donors of groups I, II, and III. The patient's own T cells showed helper function in Ig induction in B-CLL cells but did not show any MLR response against his own B-CLL cells. As shown in Table V, soluble helper factor(s) obtained from group I T cells, which could help Ig-induction in B-CLL cells, induced IgG production as well as proliferation of B-CLL cells, whereas IgG-induction and proliferation in B-CLL cells observed by the addition of the factor(s) from group III T cells, which did not induce IgG in B-CLL cells, was much less than those observed with the effective factor(s). All of these factor(s), i.e., from T cells of group I and T cells of group III, showed comparable helper effect on PWM-induced IgG production as well as proliferation in normal allogeneic B cells. All of these results suggested the requirement of a certain matching of helper factor(s) and acceptors for factor(s) on B-CLL cells for the induction of Ig production as well as proliferation.

DISCUSSION

In the present studies, differentiation of B-CLL cells into IgM- or IgG-producing cells under the influence of T cells and PWM was demonstrated. IgM and IgG detected in the culture supernatants were proved to be produced by the monoclonal B-CLL cells by demonstrating that IgM and IgG produced had the same idiotype as that of the monoclonal IgM protein present

TABLE III

Inhibition of IgG induction in B-CLL cells with hydroxyurea or cytosine arabinoside

Inhibitors[a]		IgG Produced in Culture Supernatants
		ng/ml
H.U.		1060 ± 135
	10^{-3} M	49 ± 10
	5×10^{-3}	<30
Ara-C	0.5 μg/ml	320 ± 82
	1.0	38 ± 3
	2.0	37 ± 0

[a] 1×10^5 B-CLL cells were cultured with 1×10^5 T cells for 7 days in the presence or absence of inhibitors.

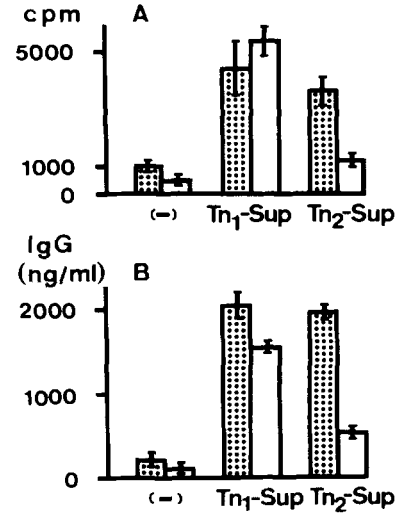

Figure 6. Induction of proliferation (*A*) or IgG-production (*B*) in normal B cells (1×10^5) or B-CLL cells (1×10^5) with helper factor(s) (T-Sup.) and PWM. 1×10^5 of normal B cells (▦) or B-CLL cells (□) were cultured with PWM-induced helper factor(s) from two healthy donors (Tn_1-Sup. and Tn_2-Sup.) and PWM for 7 days for IgG-induction and 4 days for ^{3}H-TdR uptake. As a control experiment, normal B cells or B-CLL cells were cultured only with PWM (−). IgG was not detectable in Tn_1-Sup. and Tn_2-Sup.

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TABLE IV

Helper effect of T cells from various healthy donors on IgG induction in B-CLL cells

Group	Donor of T Cells	IgG Produced in[a]		MLR Responses against[b]	
		B-CLL cells	Bn cells (N.A)	B-CLL cells	Bn-cells (N.A)
		ng/ml		*cpm*	
I	M.S.	8,333 ± 577	7,233 ± 321	26,777 ± 3,286	39,271 ± 1,326
	O.S.	5,333 ± 721	4,933 ± 1,834	18,771 ± 7,271	19,083 ± 2,046
	T.S.	3,100 ± 503	6,591 ± 864	17,786 ± 2,979	37,388 ± 2,008
II	N.A.	4,467 ± 1,858	4,100 ± 790	519 ± 428	10,647 ± 1,218
	J.H.	4,700 ± 2,150	5,700 ± 2,170	1,432 ± 348	27,285 ± 8,090
	T.F.	6,833 ± 1,559	6,250 ± 1,050	7,740 ± 898	14,227 ± 2,809
III	N.Y.	83 ± 35	7,100 ± 100	1,531 ± 138	39,183 ± 12,304
	T.M.	120 ± 64	7,033 ± 58	2,132 ± 1,169	34,494 ± 2,102
	Patient	8,333 ± 500	7,833 ± 289	727 ± 253	20,511 ± 1,820

[a] 1×10^5 B-CLL cells or normal B cells were cultured with 1×10^5 T cells from various healthy donors for 7 days and IgG in culture supernatants was measured.

[b] 1×10^5 T cells from various healthy donors were cultured with 1×10^5 x-irradiated B-CLL cells for 5 days and uptakes of ^{3}H-TdR were assessed.

TABLE V

Helper effect of T-factor(s) from various healthy donors on induction of IgG and proliferation in B-CLL cells

Group	T factor(s) from[a]	IgG Produced in[b]		Proliferation in[c]	
		B-CLL cells	Bn cells (O.S.)	B-CLL cells	Bn cells (O.S.)
		ng/ml		*cpm*	
I	M.S.	1583 ± 382	1865 ± 197	3265 ± 481	3848 ± 725
	O.S.	1600 ± 265	2140 ± 516	5345 ± 591	4391 ± 1,104
III	N.Y.	543 ± 140	1955 ± 194	1229 ± 163	3666 ± 411
	T.M.	503 ± 285	2050 ± 351	848 ± 115	4159 ± 1150

[a] T cells from several healthy donors were stimulated with PWM for 48 hr and culture supernatants were used as helper factor(s).

[b] 1×10^5 B-CLL cells or normal B cells were cultured with PWM and helper factors at 50% of final concentration for 7 days.

[c] 1×10^5 B-CLL cells or normal B cells were cultured with PWM and helper factors for 3 days and ^{3}H-TdR-uptake was assessed.

in the patient's serum. When B-CLL cells were isolated from the patient, almost 100% of cells were intracellular IgM positive, but they did not secrete any detectable IgM in culture supernatants when they were cultured alone. Thus, the addition of T cells plus PWM induced not only switching of intracellular IgM-positive cells to IgG-producing cells but differentiation of intracellular IgM-positive cells from IgM-secreting cells. Since the patient's own T cells plus PWM could induce IgM and IgG synthesis *in vitro*, a certain percentage of B-CLL cells was differentiated into IgM-producing cells *in vivo*, and they were responsible for the monoclonal IgM protein in the serum. However, a switching from IgM to IgG production *in vivo* was not observed in the present case. *In vivo* differentiation of leukemic cells into Ig-producing cells was demonstrated by Fu *et al.* (13). They showed that certain cases of CLL were associated with monoclonal Ig in the serum, and the surface Ig of the leukemic cells as well as intracellular Ig of plasma cells detected in the patient's PBL were idiotypically identical to the serum monoclonal Ig.

In the present experiment, both T cells and PWM were required for Ig induction in B-CLL cells, and x-irradiated T cells showed comparable helper effect on the leukemic cells in the presence of PWM. Induction of *in vitro* differentiation and Ig synthesis in the leukemic lymphocytes were also demonstrated by Fu *et al.* (7). In their experiments, the B leukemic cells obtained from one patient were induced to differentiate to Ig-producing cells only in the presence of allogeneic T cells, whereas the leukemic cells from the other patient required both T cells and PWM for their differentiation. Our previous experiments (6) showed the induction of IgG in human B lymphoblastoid cells by the addition of allogeneic T cells. In these experiments (6), polyclonal B mitogens, such as PWM, were not required, but x-irradiated T cells did not show the helper function in IgG induction in B blastoid cells. These results obtained by Fu *et al.* (7) and us (6) showed the presence of various subsets of B cells or various stages of differentiation of B cells that required the different signals for their activation to Ig-producing cells. In the studies by Fu *et al.* (7), the B leukemic cells differentiated into Ig-producing cells without any requirement of proliferation. On the other hand, cell division was essential for differentiation of B-CLL cells to Ig-producing cells in the present experiment. The difference between our and their results also suggested the presence of various differentiation stages of the B leukemic cells.

Switching from IgM to IgG synthesis (14, 15) or IgG to IgA production (16) in monoclonal leukemic B lymphocytes were suggested in several experiments. In the present experiment, almost 100% of B-CLL cells were stained both with fluorescent-labeled anti-IgM and with rhodamine-labeled anti-idiotype antibody when they were cultured alone. On the other hand, the percentage of intracellular IgM-positive cells was significantly less than that of intracellular idiotype-positive cells when B-CLL cells were cultured with T cells plus PWM. Moreover, about 10% of cells were doubly stained with anti-IgG and anti-idiotype antibody. These results suggest a switching from intracellular IgM-positive cells to IgG-producing cells under the influence of T cells. The results may be essentially the same as that observed in B lymphoblastoid cells, in which the addition of allogeneic T cells induced IgG production in IgM-producing B blastoid cells (6).

The interesting finding in the present study was the presence of donors whose T cells did not induce any Ig production in B-CLL cells. Similar results were also observed in IgG induction in B lymphoblastoid cells, in which T cells from certain donors were consistently superior to T cells from the other donors (6). Chiorazzi *et al.* (17) showed that T cells from certain donors were consistently superior to T cells from the other donors in PWM-induced Ig production in allogeneic B cells. In our pres-

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ent and previous experiments (6), however, T cells, which did not show the helper effect on B-CLL cells or B blastoid cells, showed comparable helper function in PWM-induced Ig production in normal allogeneic B cells. Moreover, T cells that did not induce IgG in the B blastoid cell line (RPMI 1788) induced Ig production in B-CLL cells. These results suggest that the inability of T cells from certain donors to induce IgG in B-CLL cells may not be due to the overfunction of suppressor T cells or dysfunction of helper T cells. The observation that x-irradiated T cells from nonstimulating donors did not show any helper function excluded the possibility that unresponsiveness was due to the presence of x-ray-sensitive suppressor T cells. Thus, the presence of effective and noneffective T cells on Ig induction in B-CLL cells suggests the requirement of matching of certain cell surface antigens between B-CLL cells and T cells for induction in B-CLL cells. As shown in Figure 6, PWM-induced antigen-nonspecific T-helper factor(s) could induce Ig production in B leukemic cells. IgG induced with factor(s) released from T cells, which did not show helper effect on the B leukemic cells, was much less than that induced with the factor(s) from the effective T cells. Both factor(s) induced comparable amounts of IgG in normal allogeneic B cells, as shown in Table V. Moreover, factor(s) from the noneffective T cells induced less proliferation in B-CLL cells than those from effective T cells. The results suggest that the inability of certain T cells or T cell factor(s) to induce IgG or proliferation in B-CLL cells may be due to the dismatching of the acceptors for T-helper factors on B-CLL cells and T-effector molecules.

In PWM-induced Ig production in normal B cells, allogeneic T cells from any healthy donors show helper functions. If we assume that B-CLL cells represent a certain clone of normal B cell populations, the present result suggests that matchings of certain cell surface antigens between T and B cells may be required for the activation of a certain subset of B cells. For example, in the polyclonal activation in B cells, Strelkauskas *et al.* (18) have shown the requirement for identity of some of the MHC-products for the maximum activation of B cells. In our preliminary experiments, however, we observed no correlations between HLA-type and abilities of T cells to induce IgG or proliferation in B-CLL cells. This information suggests that some cell surface antigens other than the major histocompatibility complex products may be involved in the interactions of the acceptors and helper factor(s).

Recently, we have succeeded in establishing a permanent cell line with the same idiotype from B-CLL cells, and the chemical nature of acceptor(s) for T-effector molecules is now under investigation.

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