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T CELL-REPLACING FACTOR- (TRF) INDUCED IgG SECRETION IN A HUMAN B BLASTOID CELL LINE AND DEMONSTRATION OF ACCEPTORS FOR TRF[1]

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IgG-secretion was induced in a human B blastoid cell line, CESS, by the addition of partially purified T cell-derived helper factor(s) (TRF), which had been obtained from PHA-stimulated human T cells. The number of IgG-producing cells in CESS cells reached its maximal level (10% of total cells) within 48 hr after the addition of TRF. TRF did not affect the proliferation of CESS cells and the block of cell proliferation with hydroxyurea did not inhibit the increase of IgG-producing cells, showing that TRF induced IgG-production in CESS cells without any requirement of cell division. TRF activity was completely removed by CESS cells but TCGF-activity in the same preparation was not absorbed with CESS cells. On the other hand, TCGF-dependent human killer cells absorbed TCGF activity but not TRF activity in the same preparation. The binding of ^{125}I-labeled factor(s) on CESS cells was also demonstrated. These results showed the presence of acceptors for TRF on the surface of CESS cells and this cell line will provide useful means for the chemical characterization of acceptors and for the study of the mechanisms of the signal transmission through acceptors.

The immune system is characterized by an extensive diversity of its cell types and a complexity of cellular interactions between them. One approach toward defining the nature of the cells and their functions is to investigate the regulatory mechanisms of the immune response within a cellular system of limited heterogeneity. Monoclonal tumor cells or established cell lines derived from cells of the immune system, therefore, have provided useful tools for such studies, especially when these cells are affected by certain external signals and subjected to a certain differentiation.

In the previous study (1), we demonstrated the induction of IgG in Epstein Barr (EB)[3] virus-transformed human B blastoid cell lines by co-culture with normal allogeneic T cells. In a subsequent experiment (2), we also showed the induction of the secretion of IgM and IgG with the same idiotype in human chronic B leukemic cells (B-CLL) by co-culture with normal T cells and pokeweed mitogen (PWM) or with PWM-induced helper factor(s). Ig induction in human B leukemic cells has also been shown by Fu *et al.* (3) and by Maino *et al.* (4). In their experiments, IgM (3) or free L chain (4) was induced in B-CLL by the addition of PWM or PHA in the presence of T cells. In murine B cell tumors, Strober *et al.* (5) demonstrated the induction of IgM-secretion in BCL_1 cells with LPS. Induction of κ-chain-specific m-RNA and expression of surface IgM was demonstrated in a pre-B tumor cell line, 70Z/3, by stimulation with LPS (6, 7). From these results, it is conceivable that some B cell tumors or established cell lines are capable of responding to certain external signals for Ig productions.

In the present experiment, we have found a EB virus-transformed cell line (CESS) that is capable of responding to T cell-replacing factor (TRF) and of differentiating into IgG-producing cells without any requirement for cell division. Acceptors for TRF have been demonstrated on the cells by utilizing radiolabeled TRF or by the absorption experiment of TRF activity. These cells will provide useful tools for the studies on the chemical nature of B cell acceptors for T effector molecules and on the mechanisms of the signal transmission through the acceptors.

MATERIALS AND METHODS

Reagents. PHA-P (lot 3110-56) was obtained from Difco. (Detroit, MI.). Agarose was obtained from Nakarai Chemicals LTD. (Kyoto, Japan). Protein A of *Staphylococcus aureus* (Lot EA 11126) was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). Hydroxyurea (HU, Lot 27C-0221) was obtained from Sigma Chemical Co. (St. Louis, MO). Fetal calf serum (FCS, Lot No. 604) was obtained from Centaurus Biological Co. (Santa Ana, CA).

Cells. EB virus-transformed human B blastoid cell line, CESS, was a gift from Dr. P. Ralph (Sloan-Kettering Institute for Cancer Research, Rye, NY). Approximately 10% of cells expressed surface IgG and 0.5 to 1% of cells secreted IgG when measured by reverse plaque assay. They did not bear any IgM or IgD on their surface and none of them secreted any IgM. Myeloma cell line, ARH 77, was a gift from Dr. J. Minowada (Roswell Park Memorial Institute, Buffalo, NY). Cell lines were maintained in RPMI 1640 containing 10% FCS, 100 Uns/ml penicillin, and 100 μg/ml streptomycin. Human peripheral blood lymphocytes (PBL) were separated from heparinized peripheral blood obtained from healthy adult volunteers by centrifugation on Ficoll-Isopaque as described previously (8). B and T cells were separated by the rosette-forming method with neuraminidase-treated sheep erythrocytes as described previously (8). Rosette-forming cells were used as T cells. A T cell growth factor- (TCGF)dependent human allo-killer cell line, PM, was prepared by the modified method of Strausser and Rosenberg (9). Briefly, 5×10^5 responder T cells from normal donor (A.M.) were co-cultured with 5×10^5 allogeneic stimulator cells (CESS) that had been previously exposed to 2000 rads of irradiation. Initial cultures were performed in Corning 25100 flasks in a total volume of 10 ml medium containing 10% FCS. Culture medium was replaced by fresh medium containing 0.5 U/ml of TCGF every 3 days and cells were adjusted to a concentration of 1×10^5 cells/ml. Cells were maintained for more than a month with cytotoxic activity against CESS. The growth of the cells was absolutely dependent upon TCGF and used as a TCGF-dependent human killer cell line.

Fractionation of CESS. CESS cells were fractionated into surface IgG-bearing ($CESS\gamma^+$) and IgG-negative ($CESS\gamma^-$) population by adherence to anti-γ-coated dishes according to the method of Wysocki and Sato (10). Rabbit anti-human IgG antibody was prepared as described previously (8) and 50 μg/ml of antibody was employed for the coating of dishes. Nonadherent cells were used as $CESS\gamma^-$ cells and adherent cells were recovered from the dishes by flushing with a pasteur pipette after incubation with culture medium, RPMI 1640 containing 10% FCS, at 37°C for 2 hr and used as $CESS\gamma^+$ cells. Unfractionated CESS, $CESS\gamma^+$, and $CESS\gamma^-$ contained about 10, 80, and 4% of IgG-bearing cells, respectively, when measured by direct immunofluorescence technique with FITC-conjugated anti-IgG (11).

Preparation of T-factor(s). T cell-derived soluble factor(s) were prepared by the method of Gillis *et al.* (12). Human palatine tonsils were obtained at tonsillectomy from patients with chronic tonsillitis and were separated into lymphocytes as described previously (13). Cells (1×10^6/ml) were cultured in the medium, RPMI 1640 supplemented with 1% FCS, 100 U/ml penicillin, 100 μg/ml streptomycin, 5×10^{-5} M 2-mercaptoethanol, 2 mM glutamine,

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[1] This work was supported by grants from the Ministry of Education, Science and Culture and from the Ministry of Health and Welfare.
[2] All correspondence should be addressed to T.K.
[3] Abbreviations used in this paper: EB, Epstein Barr; B-CLL, chronic B leukemic cells; PWM, pokeweed mitogen; TRF, T cell-replacing factor; TCGF, T cell growth factor; PHA, phytohemagglutinin; HU, hydroxyurea.

and 0.1% PHA. After 48 hr incubation, cell-free supernatants were recovered, concentrated 10 times with Amicon (Y M-5), and fractionated with 85% saturation of ammonium sulfate. The precipitates were resuspended in 0.01 M sodium phosphate buffer with 0.5 M sodium chloride (pH 7.0) and dialyzed. The sample was applied on Sephadex G-100 column equilibrated in the same buffer and TCGF activity of each fraction was tested by employing a TCGF-dependent T cell line, PM. Fractions eluted between chymotrypsinogen and cytochrome-c (m.w. 15,000 to 20,000), which showed TCGF activity, were collected, concentrated with Amicon (Y M-5), and dialyzed against 0.01 M sodium phosphate buffer with 0.15 M sodium chloride (pH 7.0). This preparation was used as partially purified T-factor(s). The protein content of the preparation was 1.04 mg/ml and TCGF activity was 1200 U/ml. Since the preparation induced IgG-secretion in CESS cells without any requirement for cell division, the preparation including 1 U of TCGF activity was used as 1 U of TRF. The preparation could also induce proliferation as well as differentiation of PWM-stimulated normal B cell, i.e., induction of increase of ^{3}H-thymidine uptake on day 4 and of 250 IgM PFC/10^6 cells on day 6. Thus, TRF preparation employed in this experiment included both TRF and TCGF.

Measurement of TCGF Activity. PM cells maintained in the medium containing TCGF (0.5 U/ml) were used as target cells in TCGF assay. PM cells were washed with TCGF-free medium once and resuspended in RPMI 1640 supplemented with 10% FCS. A standard lot of TCGF obtained from 1×10^6 PHA-stimulated tonsillar lymphocytes was assigned as 1 U of TCGF activity. Ten thousand PM cells in 0.2 ml medium were incubated with serially diluted standard lot or the other test samples in a Micro Test II culture plate (Falcon Plastic Co. Oxnard, CA) in triplicate. After 72 hr incubation, cell counts were performed and the units of TCGF activity in the test samples were determined from the dose-response curve of the standard lot.

Cell culture and detection of Ig-producing cells. CESS cells (10^4 cells/0.2 ml/well) were incubated in RPMI 1640 supplemented with 10% FCS in the absence or the presence of a TRF (Usually 10 U/ml) preparation in triplicate. For the detection of IgG- or IgM-secreting cells, a reverse plaque assay (14) was employed by using protein A-coupled SRBC and purified monospecific anti-γ or anti-μ antiserum. Monospecific rabbit anti-human γ or μ antiserum was prepared as described previously (8).

Absorption of TRF activity. TRF preparations including 10 U of activity in 0.5 ml medium were absorbed 3 times with 2×10^7 cells for 2 hr at 4°C or at room temperature. After absorption, TRF activity or TCGF activity was assessed by the ability to induce IgG in the CESSγ^+ population or by the assay of TCGF activity described above.

Binding of radiolabeled TRF. Partially purified TRF preparation was radiolabeled with ^{125}I by the chloramine-T method of Klinman and Taylor (15). One hundred and forty million cpm were found in 100 U of the TRF preparation. Ten million cells in a glass tube were washed with RPMI 1640 containing 10% FCS and 0.05% NaN_3 once and suspended in 100 μl of the same medium. Then, these cells were incubated with 10 μl of a ^{125}I-labeled TRF preparation (total cpm 1.02×10^5) at 4°C for 2 hr and washed with medium 5 times. The radioactivity of the cell pellet was measured with a gamma-counter.

RESULTS

Induction of IgG in CESS cells with TRF. About 10% of CESS cells in the exponential growth expressed surface IgG and the rest of the cells did not have any surface Ig. Thus, CESS cells were fractionated into IgG-bearing (CESSγ^+) and IgG-negative (CESSγ^-) populations and unfractionated CESS, CESSγ^+, and CESSγ^- cells (10^4 cells/0.2 ml/well) were incubated at 37°C in the absence or the presence of 10 or 20 U/ml of TRF. After 48 hr incubation at 37°C, cells were recovered and IgG-producing cells were counted by reverse plaque assay. As shown in Table I, about a 5-fold increase of IgG-producing cells was observed by incubation of CESSγ^+ cells with 10 U/ml of TRF and the number of IgG-producing cells reached 11.4% of total cells cultured. On the other hand, the number of IgG-producing cells in the CESSγ^- cell population, after 48 hr incubation with TRF, was only 0.6% of the total cells. Thus, CESSγ^+ cells were employed for the induction of IgG through all the experiments.

Dose response of TRF in IgG-production. CEESγ^+ cells were cultured in varying concentrations of TRF and the induction of IgG-producing cells was followed. As shown in Figure 1, the maximum response was obtained when CESSγ^+ cells were incubated with 10 U/ml of TRF. When using up to 10 U/ml of TRF, an almost linear relationship between the concentration of TRF and the number of IgG-producing cells was observed. The decrease of IgG-producing cells by the addition of 20 U/ml of TRF might be due to the suppressor factor(s) present in TRF preparation. It is noteworthy that IgM-producing cells were never detected with any concentrations of TRF.

Kinetics of IgG production in CESS cells with TRF. Ten thousand CESSγ^+ cells were cultured in the absence or presence of 10 or 20 U/ml of TRF and the number of IgG-producing cells was followed through day 1 to day 3. The number of IgG-producing cells reached its maximum level on day 2 and the number declined on day 3 (Fig. 2*A*), although the cells were continuously proliferating. The background level of IgG-producing cells was not changed through day 0 to day 3. In contrast with the induction of IgG secretion, TRF preparation did not affect the proliferation of the cells. As shown in Figure 2*B*, the addition of 10 or 20 U/ml of TRF neither inhibited nor augmented the proliferation of CESSγ^+ cells.

Effect of HU on TRF-induced IgG-secretion. The fact that TRF induced an increase of IgG-secreting cells but did not affect the cell proliferation and that the number of IgG-producing cells reached its maximal level within 48 hr suggested that cellular division was not required for the induction of IgG in CESS cells with TRF. In order to study this possibility, cells were cultured with TRF in the presence of HU and the number of IgG-producing cells was followed. The addition of 0.1 mM HU completely blocked the proliferation of the cells (Fig. 3) and the number of the recovered cells after 2 days culture was 0.7 to 0.8×10^4 when 1×10^4 cells were cultured, although more than 90% of cells were viable. However, the addition of HU did not inhibit TRF-induced increase in IgG-producing cells as shown in Table II. The addition of HU without TRF induced about a 2-fold increase of IgG-producing cells, suggesting that inhibition of the proliferation may lead to the IgG production of the cells. The fact that HU inhibited the cell proliferation but did not block the induction of IgG-producing cells indicated that the induction of IgG-producing cells with TRF was not due to the preferential expansion of the cells that had already secreted IgG.

Incubation time of CESS cells with TRF required for IgG induction. In order to study how long TRF should continue to be present with CESS cells for the induction of IgG-producing cells, CESSγ^+ cells were incubated with TRF for various time periods and induction of IgG-producing cells at 48 hr was assessed. As shown in

TABLE I

Induction of IgG-secretion in CESS cells with T cell-derived soluble factor (TRF)

TRF	PFC/Culture (10^4 Cells)[a]		
	Unfractionated CESS	CESSγ^+	CESSγ^-
unit/ml			
0	76 ± 4	256 ± 68	24 ± 3
10	220 ± 1	1140 ± 52	60 ± 8
20	251 ± 13	838 ± 70	33 ± 2

[a] 10^4 cells/0.2 ml were cultured with TRF for 48 hr. IgG-producing cells were measured by reverse plaque assay with protein A-SRBC and anti-γ antibody.

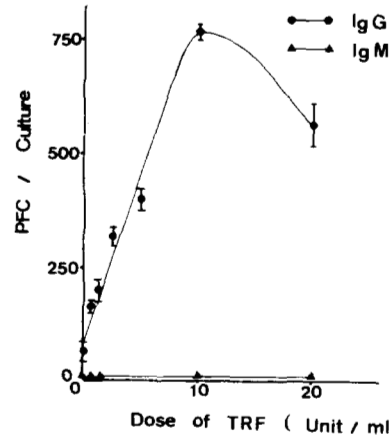

Figure 1. Dose response of TRF in the induction of IgG-secretion in CESS cells. Cells (1×10^4/0.2 ml) were cultured in the presence of varying concentrations of TRF for 48 hr and IgG- (●——●) and IgM- (▲——▲) producing cells were assessed by reverse plaque assay.

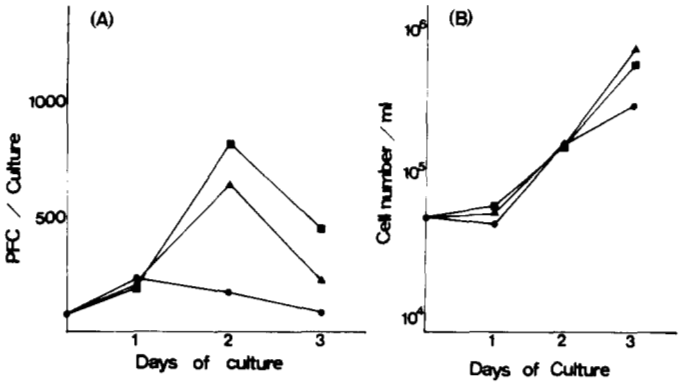

Figure 2. Kinetics of IgG induction (*A*) in CESS cells and proliferation (*B*) of CESS cells in the presence of 10 units/ml (■——■) or 20 units/ml (▲——▲) of TRF or in the absence (●——●) of TRF.

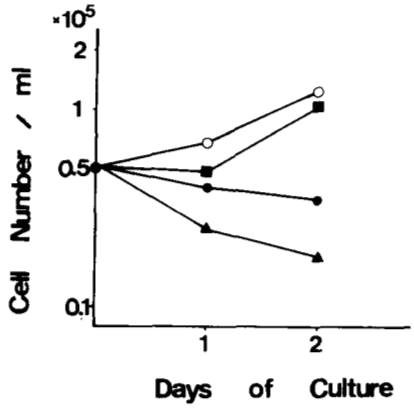

Figure 3. Effect of varying concentrations of HU on the proliferation of CESS cells. 1 mM (▲——▲), 0.1 mM (●——●), 0.01 mM (■——■), and 0 (○——○).

Table III, when the cells were incubated for 8 hr and cultured for another 40 hr without TRF, the induction of IgG-producing cells was comparable to that obtained by the presence of TRF through 48 hr.

Absorption of TRF activity with CESS cells. In order to demonstrate the acceptors for TRF on CESS cells, attempts were made to absorb the activity of TRF with the cells. As a control experiment, a human myeloma cell line, ARH 77, and a TCGF-dependent human killer T cell line, PM, were employed for the absorption of TRF activity. Ten units per milliliter of TRF were absorbed 3 times with 2×10^7 cells at 4°C or at room temperature and the TRF-activity after absorption was assessed by the induction of IgG-production in $CESS\gamma^+$ cells. As shown in Table IV, $CESS\gamma^+$ cells completely removed the activity of 10 U of TRF, when absorbed either at 4°C or at room temperature. The same number of unfractionated CESS cells could absorb about 80% of the activity of 10 U of TRF. On the other hand, the same number of ARH 77 cells or TCGF-dependent killer cells did not absorb the TRF-activity at all. The TRF-preparation also lost the activity to induce differentiation of PWM-stimulated normal B cells after absorption with CESS cells (data not shown).

In contrast, the absorption of factor(s) with CESS cells did not remove the TCGF activity. As shown in Figure 4, the TCGF activity of the preparation that had been tested for the TRF-activity were examined by utilizing a TCGF-dependent cell line, PM. The absorption of factor(s) with a TCGF-dependent cell line completely removed the TCGF-activity. On the other hand, the preparation that had been absorbed with $CESS\gamma^+$ cells and did not show the TRF activity was capable of supporting the growth of a TCGF-dependent cell line. Since the dose-dependent curve of the TCGF activity of the preparation absorbed with $CESS\gamma^+$ cells was almost comparable to that of nonabsorbed factor(s), the result showed that $CESS\gamma^+$ cells did not absorb the TCGF-activity at all. All these results collectively indicated that CESS cells expressed the acceptors for TRF and the acceptors for TRF were not cross-reactive with those for TCGF.

Binding of radiolabeled TRF preparation to CESS cells. In order to demonstrate directly the binding of TRF on CESS cells, an attempt was made to absorb ^{125}I-labeled TRF preparation with CESS cells. Ten million cells were incubated with 10 μl of a ^{125}I-labeled preparation including TRF (total cpm 1.02×10^5) in the presence of 0.05% NaN_3. After 2 hr incubation at 4°C, cells were thoroughly washed and radioactivity bound with the cells was measured. As shown in Table V, approximately 2% of total cpm was bound to $CESS\gamma^+$ cells, whereas the same number of ARH 77 cells, peripheral T cells, or red blood cells did not bind any significant radioactivity. Preincubation of CESS cells with the non-labeled TRF (10 U/ml) preparation reduced the binding of radioactivity to about 20%, indicating the specificity of the binding. The binding of radioactivity in the same preparation to TCGF-dependent cells showed the coexistence of both TRF and TCGF in this preparation.

DISCUSSION

The present study showed the induction of IgG-secretion in a EB virus-transformed human B blastoid cell line (CESS) with soluble factor(s) obtained from mitogen-stimulated T cells. Since 1 to 2% of CESS cells originally secreted IgG without any stimulation, 2 alternative possibilities should be considered for the explanation of IgG induction in CESS cells with T-derived soluble factor(s): i) IgG production was induced in nonproducing cells by stimulation

TABLE II

Effect of HU on the induction of IgG in CESS cells with TRF

HU[a]	TRF[b]	Cells Recovered	PFC/Culture[c]
−	−	2.4×10^4	81 ± 13
+	−	0.7×10^4	157 ± 20
−	+	2.4×10^4	360 ± 9
+	+	0.8×10^4	424 ± 4

[a] The concentration of HU was 0.1 mM.
[b] The concentration of TRF was 10 unit/ml.
[c] One $\times 10^4$ $CESS\gamma^+$ cells in 0.2 ml were cultured for 48 hr and IgG-producing cells were measured by reverse plaque assay.

TABLE III

Effect of incubation time of CESS cells with TRF on the induction of IgG in CESS cells

Incubation Time (hr)[a]	PFC/Culture[b]
0	115 ± 10
1	178 ± 13
4	175 ± 41
8	381 ± 9
24	316 ± 17
48	313 ± 20

[a] One $\times 10^4$ cells were incubated with TRF for various periods of time at 37°C. After incubation, cells were washed to remove TRF and cultured in the absence of TRF.
[b] The number of IgG-producing cells at 48 hr after initiation of culture was measured by reverse plaque assay.

TABLE IV

Absorption of TRF activity with CESS cells

Cells Cultured[a]	TRF[b]	Absorption of TRF[c]	PFC/Culture[d]
$CESS\gamma^+$	−		65 ± 5
$CESS\gamma^+$	+	nonabsorbed	772 ± 4
$CESS\gamma^+$	+	abs c̄ CESS at 4°C[e]	145 ± 3
$CESS\gamma^+$	+	abs c̄ CESS at r.t.[e]	140 ± 9
$CESS\gamma^+$	+	abs c̄ $CESS\gamma^+$ at 4°C[f]	68 ± 9
$CESS\gamma^+$	+	abs c̄ $CESS\gamma^+$ at r.t.	36 ± 7
$CESS\gamma^+$	+	abs c̄ ARH 77 at 4°C	1040 ± 89
$CESS\gamma^+$	+	abs c̄ PM at 4°C[g]	765 ± 52

[a] One $\times 10^4$ cells in 0.2 ml were cultured.
[b] Ten unit/ml of TRF was employed.
[c] Ten unit of TRF was absorbed 3 times with 2×10^7 cells.
[d] IgG-producing cells were measured at 48 hr by reverse plaque assay.
[e] Unfractionated CESS cells containing about 10% of IgG-bearing cells were used for absorption.
[f] IgG-bearing CESS cells were used for absorption.
[g] TCGF-dependent human killer cell line was used for absorption.

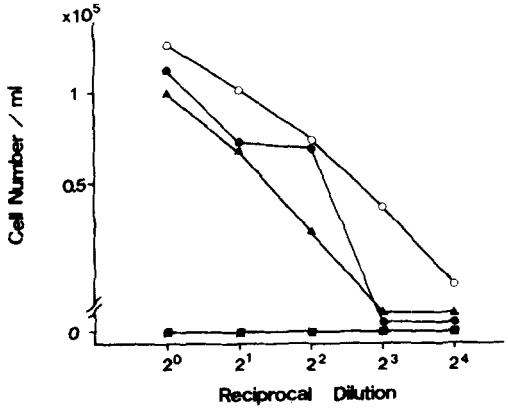

Figure 4. TCGF activity of the factor(s) absorbed with CESS cells at 4°C (●——●), at room temperature (▲——▲), or with a TCGF-dependent cell line (■——■). ○——○ shows the TCGF activity of nonabsorbed factor(s). One × 10^4 of TCGF-dependent human killer T cells (PM) were incubated with serially diluted samples for 72 hr and cell number was measured.

TABLE V

Binding of ^{125}I-labeled TRF preparation with CESS cells

Cells[a]	Binding of Radioactivity (cpm)
$CESS\gamma^+$	1944 ± 80
$CESS\gamma^+$ + TRF (10 Unit/ml)	396 ± 12
PM[b]	815 ± 45
ARH 77[c]	58 ± 16
PBL-T[d]	118 ± 31
Hu-RBC[e]	36 ± 12

[a] Ten million cells were incubated with 10 μl ^{125}I-TRF preparation (1.02 × 10^5 cpm) in the total volume of 0.1 ml for 2 hr at 4°C.
[b] PM is a TCGF-dependent human killer cell line.
[c] ARH 77 is a human myeloma cell line.
[d] PBL-T is a T cell fraction isolated from PBL.
[e] Hu-RBC is human red blood cells.

with soluble factor(s), ii) IgG-producing cells originally present in the nonstimulated cell population were selected by the induction of preferential expansion of those cells with soluble factor(s). As shown in Figure 2, proliferation of CESS cells was not affected by the addition of soluble factor(s), i.e., cell growth was neither inhibited nor enhanced by soluble factors. Furthermore, the complete inhibition of cell growth with HU did not block the factor-induced increase of IgG-producing cells. From these results, it is conceivable that increase of IgG-producing cells with soluble factor(s) is not due to the preferential expansion of IgG-producing cells originally present in the nonstimulated cell population, but might be due to the induction of IgG in nonproducing cells. About 10% of CESS cells bore IgG on their surfaces and the results depicted in Table I showed that IgG production was induced mainly in IgG-bearing cells but not in IgG-negative cells. Thus, it appears that T-derived soluble factor(s) accelerated IgG production in the cells that already had started the production of IgG as membrane receptors.

In the studies of Schimpl and Wecker (16–18) as well as of Askonas *et al.* (19), it was suggested that TRF induced final differentiation of antigen-stimulated B cells into IgG-producing cells without any requirement for cell division. They claimed that antigen binding to Ig receptors induced proliferation of B cells and that TRF was responsible for the final differentiation of antigen-stimulated B cells. In rabbit mesenteric lymph node cells, we showed that anti-Ig stimulation and T-derived soluble factor(s) were capable of inducing Ig production in B cells (20). In those experiments, T-derived soluble factor(s) induced proliferation as well as final differentiation of anti-Ig-stimulated B cells into Ig-producing cells. The result suggested that binding of anti-Ig to Ig-receptors activated B cells into the responsive stage to T-derived soluble factor(s), which induced proliferation as well as differentiation of B cells. Recent application of hybridoma technology succeeded in the establishment of several T-hybrid cell lines secreting factors responsible for proliferation (Interleukin II) (21) or differentiation (TRF) of B cells (22) and revealed that 2 distinct factors, i.e., Interleukin II and TRF, were involved in proliferation and final differentiation of B cells into Ig-producing cells. Thus, it is most conceivable that cross-linkage of Ig receptors activated B cells into the stage responsive to Interleukin II, which induced proliferation of activated B cells and that TRF induced final differentiation of proliferated B cells into Ig-producing cells. If this is the case, CESS cells may represent B cells that are in the final stage of their processes of differentiation into Ig-producing cells, since T-derived soluble factor(s) induced IgG production in those cells without any requirement for cell division. On the other hand, one of the human B-CLL, which have been shown to differentiate into IgG-producing cells by stimulation with normal T cells and PWM (2), may represent B cells at the resting stage, since anti-Ig- or PWM stimulation is required for the induction of proliferation and IgG production in the leukemic B cells with T-derived soluble factor(s) (22A).

T-derived soluble factor(s) employed in the present experiment were obtained from PHA-stimulated T cells and were partially purified as described in *Material and Methods*. Thus, the soluble factor(s) included not only TRF but also Interleukin II. Actually, the factor(s) were capable of maintaining the growth of a TCGF-dependent human killer cell line and of inducing proliferation of PWM-stimulated normal B cells (data not shown). The absorption experiment clearly showed that activities of TRF and TCGF were exerted by distinct molecules and acceptors for TRF on a B cell line and those for TCGF on a killer cell line did not have cross-reactivity. Watson and Mochizuki (23) as well as Smith (24) also reported the absorption of the TCGF-activity with mitogen-activated T cells but not with B cells. The absorption of soluble factor(s) with CESS cells removed the TRF-activity but did not affect the TCGF-activity, showing the specificity of the acceptors for TRF on CESS cells and confirming that absorption of the TRF-activity was not due to the nonspecific absorption nor to the degradation by proteolytic enzymes. Utilization of radiolabeled soluble factor(s) suggested the binding of TRF on the surface of CESS cells. Since the addition of excess of soluble factor(s) blocked the binding of radioactivity and the binding of radioactivity was observed at 4°C in the presence of NaN_3, the binding was not due to the nonspecific attachment or endocytosis of the factor(s). Since the absorption of TRF activity and the binding of radioactivity are parallel, i.e., no binding of radioactivity with a myeloma cell, ARH 77, and no absorption of TRF activity with ARH 77, it is conceivable that the binding of radioactivity with CESS cells is due to the specific binding of TRF to CESS cells. Since about 2% of total radioactivity added was detected on CESS cells, at least 2% of total protein in soluble factor(s) may be related with the TRF activity. Several experiments have shown the absorption of the TRF activity with antigen or anti-Ig activated B cells (20, 25). In the absorption of the TCGF activity, mitogen- or antigen-activated T cells but not nonactivated thymocytes or splenocytes could absorb the activity (24, 26). Since CESS cells appear to be at the final activation process of B cells and continuously express acceptors for TRF, the cells may be the most suitable source for the isolation of acceptors for TRF.

Incubation of CESS cells with TRF preparation for 1 or 2 hr either at 4°C or at room temperature could absorb the TRF activity. On the other hand, presence of TRF preparation with CESS cells at least for 8 hr was required for the maximum induction of IgG-producing cells. The result may coincide with those observed in anti-Ig or mitogen-stimulation of B cells. In anti-Ig or mitogen stimulation, the stimulators should be present for more than 18 hr with the lymphocytes, although the bindings of those ligands with lymphocytes may occur within a minute. In L chain induction in a pre-B cell line, 70Z/3, with LPS, we showed that the LPS signal should be given before the G_2-phase and that L chain-specific m-RNA were translated after the M-phase (7). If TRF-signals should be given at a certain phase of the cell cycle, as observed in 70Z/3 cells, presence of TRF for certain periods of time may be required for effective induction, since CESS cells were not synchronized in the present experiment.

Epidermis growth factor was shown to be internalized into cells after the binding with its acceptors on the surface of fibroblasts and the factor was shown to make a complex with chromatin (27). One of the interesting questions is whether TRF can give its signals only by binding with its acceptors of if TRF should go into the inside of the cells. In our preliminary experiments, a monoclonal

antibody specific for B cells could induce IgG secretion in CESS cells, indicating that not only TRF but the binding of certain ligands with certain cell surface molecules could induce final differentiation of B cells and suggesting that the binding of TRF with its acceptors could give signals for the final differentiation of B cells.

In the present experiments, binding of TRF with its acceptor could induce IgG production in more than 10% of CESS cells without any requirement for cell division. Thus, mechanisms of signal transmission through TRF-acceptors can be analyzed by utilizing this experimental system.

REFERENCES

1. Kishimoto, T., T. Hirano, T. Kuritani, Y. Yamamura, P. Ralph, and R. A. Good. 1978. Induction of IgG production in human B lymphoblastoid cell lines with normal human T cells. Nature 271:756.
2. Saiki, O., T. Kishimoto, T. Kuritani, A. Muraguchi, and Y. Yamamura. 1980. *In vitro* induction of IgM-secretion and switching to IgG-production in human B leukemic cells with the help of T cells. J. Immunol. 124:2609.
3. Fu, S. M., N. Chiorazzi, H. G. Kunkel, J. P. Halper, and S. R. Harris. 1978. Induction of *in vitro* differentiation and immunoglobulin synthesis of human leukemic B lymphocytes. J. Exp. Med. 148:1570.
4. Maino, V. C., J. T. Kurnick, R. T. Kubo, and H. M. Grey. 1977. Mitogen activation of hyman chronic lymphocytic leukemia cells. I. Synthesis and secretion of immunoglobulin. J. Immunol. 118:742.
5. Strober, S., E. S. Gronowicz, M. R. Knapp, S. Slarin, E. S. Vitetta, R. A. Warnke, B. Kotzin, and J. Schröder. 1979. Immunobiology of a spontaneous murine B cell leukemia (BCL_1). Immunol. Rev. 48:169.
6. Perry, R. P., and D. E. Kelley. 1979. Immunoglobulin messenger RNAs in murine cell lines that have characteristics of immature B lymphocytes. Cell 18:1333.
7. Sakaguchi, N., T. Kishimoto, H. Kikutani, T. Watanabe, N. Yoshida, A. Shimizu, Y. Yamawaki-Kataoka, T. Honjo, and Y. Yamamura. 1980. Induction and regulation of immunoglobulin expression in a murine pre-B cell line, 70Z/3. I. Cell cycle-associated induction of sIgM expression and κ-chain synthesis in 70Z/3 cells by LPS stimulation. J. Immunol. 125:2654.
8. Hirano, T., T. Kuritani, T. Kishimoto, and Y. Yamamura. 1977. *In vitro* immune response of human peripheral lymphocyte. I. The mechanism(s) involved in T cell helper functions in the pokeweed mitogen-induced differentiation and proliferation of B cells. J. Immunol. 119:1235.
9. Strausser, J. L., and S. A. Rosenberg. 1978. *In vitro* growth of cytotoxic human lymphocytes. I. Growth of cells sensitized *in vitro* to alloantigens. J. Immunol. 121:1491.
10. Wysocki, L. J., and V. L. Sato. 1978 "Panning" for lymphocytes: a method for cell selection. Proc. Natl. Acad. Sci. 75:2844.
11. Muraguchi, A., T. Kishimoto, T. Kuritani, T. Watanabe, and Y. Yamamura. 1980. *In vitro* immune response of human peripheral lymphocytes. V. PHA- and protein A-induced human B colony formation and analysis of subpopulations of B cells. J. Immunol. 125:564.
12. Gillis, S., K. A. Smith, and J. Watson. 1980. Biochemical characterization of lymphocyte regulatory molecules. II. Purification of a class of rat and human lymphokines. J. Immunol. 124:1954.
13. Watanabe, T., K. Yoshizaki, T. Yagura, and Y. Yamamura. 1974. *In vitro* antibody formation by human tonsil lymphocytes. J. Immunol. 113:608.
14. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting Ig and a given type or class. Eur. J. Immunol 6:588.
15. Klinman, N. R., and R. B. Taylor. 1969. General methods for the study of cells and serum during the immune response: the response to dinitrophenyl in mice. Clin. Exp. Immunol. 4:473.
16. Schimpl, A., and E. Wecker. 1973. Stimulation of IgG antibody formation *in vitro* by T cell-replacing factor. J. Exp. Med. 137:547.
17. Schimpl, A., and E. Wecker. 1972. Replacement of T cell function by a T cell product. Nature (New Biol.) 237:15.
18. Schimpl, A., and E. Wecker. 1975. A third signal in B cell activation given by TRF. Transplant. Rev. 23:176.
19. Askonas, B. A., A. Schimpl, and E. Wecker. 1974. The differentiation function of T cell-replacing factor in nu/nu spleen cell cultures. Eur. J. Immunol. 4:164.
20. Kishimoto, T., T. Miyake, Y. Nishizawa, T. Watanabe, and Y. Yamamura. 1975. Triggering mechanism of B lymphocytes. I. Effect of antiimmunoglobulin and enhancing soluble factor on differentiation and proliferation of B cells. J. Immunol. 115:1179.
21. Harwell, L., B. Skidmore, P. Marrack, and J. Kappler. 1980. Concanavalin A-inducible, Interleukin-2-producing T cell hybridoma. J. Exp. Med. 152: 893.
22. Takatsu, K., K. Tanaka, A. Tominaga, Y. Kumahara, and T. Hamaoka. 1980. Antigen-induced T cell-replacing factor (TRF). III. Establishment of T cell hybrid clone continuously producing TRF and functional analysis of released TRF. J. Immunol. 125:2646.

22A. Kishimoto, T., K. Yoshizaki, T. Kuritani, A. Muraguchi, Y. Miki, T. Nakagawa, and Y. Yamamura. 1981. Induction of proliferation and Ig-secretion in human monoclonal B cells with anti-immunoglobulin and T cell products. *In* B lymphocytes in the Immune Response. Edited by Klinman, Mosier, Sher, and Vitetta. Elsevier North Holland, Amsterdam, P. 515.

23. Watson, J., and D. Mochizuki. 1980. Interleukin 2: a class of T cell growth factors. Immunol. Rev. 51:257.
24. Smith, K. A. 1980. T cell growth factor. Immunol. Rev. 51:337.
25. Tominaga, A., K. Takatsu, and T. Hamaoka. 1980. Antigen-induced T cell-replacing factor (TRF). II. X-linked gene control for the expression of TRF-acceptor site(s) on B lymphocytes and preparation of specific antiserum to that acceptor. J. Immunol. 124:2423.
26. Smith, K. A., S. Gillis, P. G. Baker, D. Mckenzie, and F. W. Ruscetti. 1979. T cell growth factor-mediated T-cell proliferation. Ann. N.Y. Acad. Sci. 332: 423.
27. Johnson, L. K., I. Vlodvsky, J. D. Baxter, and D. Gospodarozicz. 1980. Nuclear accumulation of epidermal growth factor in cultured rat pituitary cells. Nature 287:320.