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Immunology

Interleukin 5, a T-cell-derived B-cell differentiation factor also induces cytotoxic T lymphocytes

(lymphokine/thymocytes)

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ABSTRACT **We describe an interleukin, termed interleukin 5, that is the recombinant product previously referred to as T-cell-replacing factor (TRF), B-cell growth factor II (BCGF II), or killer-helper factor (KHF). TRF has been defined as a T-cell-derived lymphokine that acts on activated B cells as a B-cell differentiation factor. We have previously demonstrated that TRF is identical to BCGF II and induces expression of receptors for interleukin 2 (IL-2) on activated B cells. We also have reported that KHF can induce not only expression of IL-2 receptors on peanut agglutinin-binding (PNA^+) thymocytes but also generation of cytotoxic T lymphocytes (CTL) in PNA^+ thymocytes in the presence of IL-2. We show here that culture supernatants of T-cell hybridomas that produce TRF as well as TRF purified by high-pressure liquid chromatography (HPLC-TRF) have KHF activity and generate CTL in PNA^+ thymocytes in the presence of stimulator cells and IL-2. Moreover, translation products (recombinant TRF) of *Xenopus* oocytes injected with cDNA encoding for murine TRF (BCGF II) also exert KHF activity. A rat monoclonal anti-TRF antibody TB13 can block generation of CTL by HPLC-TRF or recombinant TRF. These results indicate that TRF acts not only on B cells as BCGF II but also on PNA^+ thymocytes as KHF. In view of the diverse activities and targets of TRF, we propose that TRF refers to a different interleukin, interleukin 5.**

B-cell proliferation and differentiation into immunoglobulin-secreting cells are regulated by several soluble factors derived from macrophages and T cells (1, 2). The T-cell-derived lymphokine, T-cell-replacing factor (TRF), was initially described as a factor that induces terminal differentiation of late-developing B cells to immunoglobulin-secreting cells (3–6). The establishment of a TRF-producing T-cell hybrid B151K12 (B151), which did not secrete any detectable levels of other B-cell stimulatory lymphokines, enabled us to demonstrate that TRF was a lymphokine distinct from interleukins 1, 2, and 3 (IL-1, IL-2, IL-3), B-cell-stimulatory factor type 1 (BSF-1) (interleukin 4, IL-4), and interferon-γ (IFN-γ) (5, 6). Purified TRF from the hybridoma B151 stimulates the differentiation of activated normal B cells and neoplastic B-cell leukemias such as BCL_1 cells into immunoglobulin-secreting cells. It was also found that TRF can stimulate dextran sulfate-activated B cells and BCL_1 cells for proliferation (7–9). This latter activity is referred to as B-cell growth factor type II (BCGF II) (10). Purified B151-TRF as well as cultured supernatants (SN) of B151 exert both TRF and BCGF II activities, suggesting that both activities are associated with a single molecule (7, 8). Purified B151-TRF can also induce an increase in the levels of IL-2 receptor expression on *in vivo* activated B cells as well as BCL_1 cells (unpublished data).

Induction of cytotoxic T lymphocytes (CTL) from thymocytes requires T-cell-derived soluble mediators that have been operationally referred to as killer-helper factor (KHF) (11–14), killer-assisting factor (15), or amplifying factors (16). The KHF is described as a factor that induces generation of CTL from CTL precursors in peanut agglutinin-binding (PNA^+) thymocytes in the presence of stimulator cells and IL-2. Under the experimental conditions, stimulator cells and KHF or IL-2 alone does not induce CTL (11, 12). KHF is purified from SN of the T-cell hybridoma cell line 2Y4 (13) and is shown to be distinct from other T-cell tropic lymphokines such as IL-1, IL-2, IL-3, and IFN-γ. It is also found that purified KHF from 2Y4 can increase expression of receptors for IL-2 on PNA^+ thymocytes (13).

We anticipated that TRF may induce the expression of IL-2 receptors not only on B cells but also on thymocytes and may participate in the generation of CTL. Here we present evidence indicating that TRF purified by high-pressure liquid chromatography (HPLC-TRF) as well as recombinant TRF exhibit KHF activity and participate in the generation of CTL in the presence of stimulator cells and IL-2. The KHF activity in the preparation can be inhibited by a monoclonal anti-TRF antibody. The results indicate that a single lymphokine is involved in B-cell differentiation and generation of CTL.

MATERIALS AND METHODS

Mice. Inbred, specific pathogen-free female C3H/HeN and BALB/c mice (obtained from the Shizuoka Animal Center, Hamamatsu, Japan), 6–8 weeks of age, were used for all experiments.

Culture Medium. RPMI-1640 medium (Sigma) supplemented with 10% fetal calf serum (Flow Laboratories), penicillin (100 units/ml), streptomycin (100 μg/ml), and 2-mercaptoethanol (50 μM) was used throughout the experiments.

Cell Lines. The establishment of murine T-cell hybridomas has been described (5). TRF-producing T-cell hybridoma B151 (5) and KHF-producing hybridoma 2Y4 (13) were mainly used in this study. The IL-2-dependent murine cytotoxic T-cell line MTH was kindly provided by Junji Hamuro (Central Laboratory of Ajinomoto Chemical, Yokohama, Japan). BCL_1 tumor, originally provided by Ellen S. Vitetta (University of Texas Health Science Center at Dallas, TX), was maintained by *in vivo* passage in BALB/c

Abbreviations: TRF, T-cell-replacing factor; rTRF, recombinant TRF; BCGF II, B-cell growth factor type II; KHF, killer-helper factor; IL-1, interleukin 1; IL-2, interleukin-2; rIL-2, recombinant IL-2; IL-3, interleukin 3; IL-4 (BSF-1), interleukin 4 (B-cell-stimulatory factor type 1); CTL, cytotoxic T lymphocyte(s); PNA^+, peanut agglutinin binding; TNP, 2,4,6-trinitrophenyl; SN, supernatant(s); pfc, plaque-forming cell(s); IFN-γ, interferon-γ.

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mice. BCL_1 cells were purified by discontinuous density gradient centrifugation with Percoll (Pharmacia) (6) from spleen cells of mice that had carried the BCL_1 tumor >4 weeks.

Preparation of CTL Precursors. Treatment of C3H/HeN thymocytes with PNA and dissociation of the agglutinated cells were performed by described procedures (12). PNA^- thymocytes in preparation of PNA^+ thymocytes was <2%, as determined by fluorescein isothiocyanate-conjugated PNA and flow cytometry.

Preparation of TRF. Cells of various T-cell hybridomas, including the cloned subline of B151, that produce TRF (5) were cultured at 1×10^5 per ml for 48 hr. Culture SN was filtered and aliquots were stored at −70°C. TRF was purified from a large batch of B151-T4 SN by described procedures (6, 13). B151-T4 SN were fractionated by ammonium sulfate precipitation at 50–85% saturation. The precipitate was dialyzed against 10 mM Tris·HCl buffer (pH 8.5) and was applied to a DE-52 (Whatman) column using a linear gradient of NaCl (0–0.25 M) in 10 mM Tris·HCl buffer (pH 8.5). Eluted fractions containing TRF activity were dialyzed against 0.025 M 2,2-bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol hydrochloride buffer (pH 6.3) and applied to a Mono P column (Pharmacia). The samples were eluted with 10% Polybuffer 74 at a pH range between 6.3 and 4.0. TRF active materials from the Mono P column were applied on a Superose 12 column (Pharmacia) for gel permeation and were eluted with 10 mM Hepes-buffered saline (pH 7.0). Finally, TRF active fractions after the gel permeation were applied on a protein C_4 column (Vydac, Hesperia, CA) for reverse-phase HPLC and were eluted with a linear gradient of acetonitrile (0–80%) containing 0.1% trifluoroacetic acid. Murine recombinant TRF (rTRF) was obtained according to described procedures (17). The cDNA for murine TRF (pSP6K-mTRF23) was cleaved with *Sal* I to linearlize plasmid DNA, and mRNAs were synthesized using SP6 RNA polymerase. The synthesized RNAs were injected into *Xenopus* oocytes. Incubation media were collected after 36 hr.

Preparation of KHF. Cells of a subline (2Y4-4) of 2Y4 were cultured at 2×10^5 per ml for 24 hr (13). Culture SN was filtered and aliquots were stored at −70°C.

Human Recombinant IL-2 (rIL-2). HPLC-purified human rIL-2 (18) was provided by J. Hamuro. The specific activity of rIL-2 was 5×10^7 units/mg of protein determined by using the IL-2-dependent cytotoxic T-cell line (MTH).

Anti-TRF Antibody. A monoclonal rat IgG1 anti-TRF antibody was obtained from a B-cell hybridoma, TB13, which was constructed by fusion between murine myeloma cells (P3-X63-Ag8.653) and rat spleen cells that had been immunized with HPLC-TRF from B151 SN. Ascitic fluid of mice injected with TB13 was subjected to a protein A-coupled Sepharose CL-4B beads column, and the eluate from the column with 3 M potassium isothiocyanate (pH 8.0) was used as a source of anti-TRF antibody. The purified TB13 antibody (3 mg) was coupled to Sepharose 4B beads (2-ml packed volume) that had been activated with cyanogen bromide (19). TB13-coupled Sepharose 4B was used as an affinity column. As a control, normal rat immunoglobulin was used in place of TB13.

Assessment of TRF Activity. TRF activity was determined by a polyclonal IgM plaque-forming cell (pfc) assay using TRF-responding BCL_1 cells, as described (6). In brief, BCL_1 cells were cultured (1.5×10^5 per 0.2 ml of culture) with appropriate concentrations of a test sample in microtiter plates (no. 3596; Costar, Cambridge, MA) for 2 days. The numbers of IgM-producing cells were enumerated by a reverse pfc assay using protein A-coupled sheep erythrocytes and rabbit anti-IgM antibody. A unit of TRF was defined as the reciprocal dilution yielding a pfc response that was 50% of the maximal response to a standard TRF preparation.

Assessment of KHF Activity. KHF activity was assessed by the method described (11, 12). In brief, PNA^+ thymocytes from C3H/HeN mice ($H\text{-}2^k$) (6×10^6) were mixed with mitomycin C-treated, 2,4,6-trinitrophenyl (TNP)-modified syngeneic spleen cells (2×10^6). Aliquots of the cell suspension were cultured for 5 days with human rIL-2 (10 units/ml) in the presence of a sample to be tested. CTL activity of *in vitro* cultured effector cells was assessed by 4-hr ^{51}Cr release from ^{51}Cr-labeled TNP-modified X5563 ($H\text{-}2^k$) (myeloma cells) as target cells. The percentages of ^{51}Cr release from target cells incubated with various numbers of stimulated and nonstimulated cultured cells were determined and were calculated according to the following formula: % release = (Experimental release − spontaneous release/Maximum release − spontaneous release) × 100. The specific release of ^{51}Cr by PNA^+ thymocytes cultured with stimulator cells and IL-2, KHF, or IL-2 plus KHF (% specific lysis) was calculated by subtracting the percentage released from target cells incubated with PNA^+ thymocytes cultured in the absence of stimulator cells. In some experiments, a dose–response curve was established, and the number of lytic units was calculated according to described methods (11). One lytic unit was arbitrarily defined as the number of lymphocytes needed to achieve 50% lysis of 1×10^4 ^{51}Cr-labeled target cells.

Assay for IL-1, IL-2, IL-3, and BSF-1 (IL-4). To determine the IL-1 activity, 2×10^5 thymocytes of BALB/c mice were cultured in 0.2 ml of culture medium containing phytohemagglutinin at 1 μg/ml and then exposed to [^{3}H]thymidine (specific activity, 15.1 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear) during the last 12 hr of a 72-hr culture. IL-2 activity was assayed as described (6). IL-3 activity was tested in the stimulation of an IL-3-dependent T-cell line, FDC-P1. BSF-1 activity was determined by using purified B cells and purified rabbit anti-mouse IgM antibody as described (6).

RNA Transfer Blot Analysis. Total RNA was prepared according to described methods using guanidium thiocyanate (20). The methods used for RNA transfer blotting are essentially the same as those described by Thomas (21). In brief, 20 μg of total RNA isolated from cells was glyoxylated, electrophoresed in a 1.1% agarose gel, and transferred to a nitrocellulose filter (Schleicher & Schuell). The filter was hybridized with a ^{32}P-labeled nick-translated *Pst* I–*Pst* I [414 base pairs (bp)] fragment of pmIL-2R-1 as probe (22, 23).

RESULTS

KHF Activity in TRF-Containing SN. SN from six TRF-producing T-cell hybrid clones and two other TRF-nonproducing T-cell hybrids were tested for their ability to stimulate PNA^+ thymocytes for CTL generation in the presence of TNP-modified syngeneic spleen cells and IL-2. As a positive control, the SN from a subline of KHF-producing T-cell hybrid, 2Y4-4, was also tested. In this assay system, IL-2 itself does not induce a significant number of CTL (Table 1, group 10). The same culture SN were assessed for TRF activity. As shown in Table 1, significant KHF activities were detected in SN from various TRF-producing T-cell hybrids (groups 1–6 vs. group 11). Those SN are unable to induce a significant CTL generation in the absence of IL-2 (data not shown). Because we could not detect IL-2, IL-3, or BSF-1 activities in SN from the cell lines tested (6, 13), there seems to be a significant correlation between TRF and KHF production. The T-cell hybrid B151K12, which was known to produce TRF but none of the IL-1, IL-2, IL-3, BSF-1, or IFN-γ (6), was found to produce KHF. These results suggest that TRF may also exert KHF activity. The only exception was the SN from 2Y4-4, which exerted KHF activity without showing detectable TRF activity (group 9).

Induction of Increase in the Levels of IL-2 Receptor Expression by B151 SN on Thymocytes. In our previous studies, it

Table 1. Activity of SN from different T-cell hybridomas in TRF and KHF assays

Group	Cell line*	TRF,† units/ml	KHF activity,‡ % specific lysis 40:1	20:1
1	B151K12	4.2	41 (1.01)	30 (1.04)
2	B151-T4	3.5	38 (1.03)	24 (1.11)
3	B7	3.1	25 (1.10)	18 (1.09)
4	A32	3.8	46 (1.04)	32 (1.15)
5	A24	2.8	31 (1.03)	16 (1.08)
6	5H6	1.5	23 (1.01)	13 (1.02)
7	5H4	<0.1	12 (1.21)	8 (1.08)
8	A55	<0.1	<1	<1
9	2Y4-4	0.2	52 (1.08)	41 (1.06)
10	IL-2 alone	<0.1	2	<1
11	Medium control	<0.1	<1	<1

*SN from the T-cell hybrids were obtained after the 2-day culture without any antigenic or mitogenic stimulation.
†TRF activity was determined using BCL_1 cells.
‡Aliquots of a cell suspension containing C3H/HeN PNA^+ thymocytes (6×10^6 per ml) and TNP-modified syngeneic spleen cells (2×10^6 per ml) were mixed with an equal volume of a SN. IL-2 (10 units/ml) was then added to the suspension. After 5 days of culture, CTL responses were assayed. KHF activities are expressed as the geometric means and standard errors of the percent specific lysis of triplicate cultures at an effector-to-target ratio of 40:1 and 20:1. Numbers in parentheses show standard errors.

was shown by flow cytometry using fluorescein isothiocyanate-conjugated anti-IL-2 receptor antibody that KHF derived from 2Y4 induces the expression of IL-2 receptors on PNA^+ thymocytes (13). We also obtained similar results using B151 SN (data not shown). To ascertain further the above observation, we determined whether B151 SN induces the synthesis of IL-2 receptor in PNA^+ thymocytes. Steady-state levels of IL-2 receptor mRNA were determined using total RNA extracted from PNA^+ thymocytes that had been stimulated with TNP-modified spleen cells and IL-2 in the presence or absence of B151 SN. RNA from unstimulated parallel cultures served as a control. Aliquots of RNA were subjected to electrophoresis, transferred to a nitrocellulose membrane, and then hybridized with ^{32}P-labeled nick-translated pmIL-2R-1 (22, 23). In samples taken from MTH cells (IL-2-dependent CTL line) autoradiography revealed radioactive bands at the positions expected for mouse IL-2 receptor mRNA [4.5, 3.5, and 2.2 kilobases (kb) and trace amounts of 1.5 kb; Fig. 1, lane 4]. Only a trace amount of 3.5-kb IL-2 receptor mRNA could be detected in IL-2-stimulated cultures (lane 2). In cultures stimulated with B151 SN and IL-2, the IL-2 receptor mRNA(s) (4.5 and 3.5 kb) markedly increased (lane 3). In unstimulated cultures, no significant IL-2 receptor mRNA could be detected (lane 1). Cultures of the cells with B151 SN alone did not give a sufficient number of cells for testing mRNA expression for IL-2 receptor.

Copurification of TRF and KHF Activities. To determine that the same molecule has TRF and KHF activities, B151 SN were fractionated by methods previously described (6, 13). When precipitates of B151 SN by ammonium sulfate were subjected to DE-52 column chromatography followed by chromatofocusing using a Mono P column, TRF and KHF activities in B151 SN had identical elution profiles (data not shown). Both activities recovered in the fractions of pI 4.5–4.7. Gel permeation of the partially purified TRF using a Superose 12 column showed that the TRF and KHF activities had similar elution patterns (Fig. 2) with a peak equivalent to M_r 45,000. TRF active fractions by gel-permeation chromatography were subjected to reverse-phase HPLC using a protein C_4 column. The two activities were again copurified

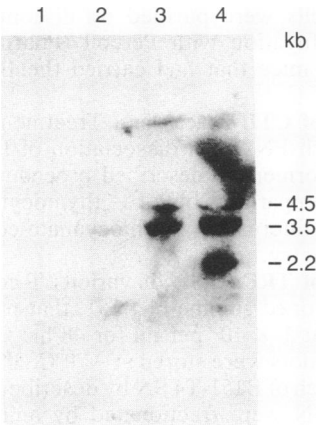

FIG. 1. IL-2 receptor gene expression in PNA^+ thymocytes after stimulation with TNP-modified syngeneic cells in the presence of IL-2 and B151 SN. PNA^+ thymocytes were stimulated with TNP-modified syngeneic spleen cells for 4 days in the presence of 10 units of IL-2 per ml (lane 2) or IL-2 (10 units/ml) plus B151 SN [50% (vol/vol)] (lane 3). As a control, freshly prepared PNA^+ thymocytes (lane 1) or the IL-2-dependent T-cell line MTH (lane 4) was used. Total RNA isolated from 15×10^7 cells was glyoxylated, electrophoresed, and analyzed by RNA blotting (21). Equal amounts of RNA were loaded and hybridizations were performed with the probe for IL-2 receptor as described (22, 23).

(Fig. 3) in the fractions eluted by ≈52–54% acetonitrile in 0.1% trifluoroacetic acid, suggesting that both activities are associated with a single, strongly hydrophobic molecule.

Inhibitory Effect of Monoclonal Anti-TRF Antibodies on KHF Activities. We recently succeeded in producing a rat monoclonal antibody, TB13 (IgG1 class), that can inhibit murine TRF and BCGF II activities of HPLC-TRF. The monoclonal antibody failed to inhibit IL-1, IL-2, IL-3, or BSF-1 activities (unpublished data). Thus, the inhibitory effect of TB13 antibody on KHF activities of HPLC-TRF and 2Y4-4 SN was tested. The results revealed that TB13 antibody significantly inhibited KHF activity in B151 SN and HPLC-TRF in a dose-dependent manner (Table 2). Interest-

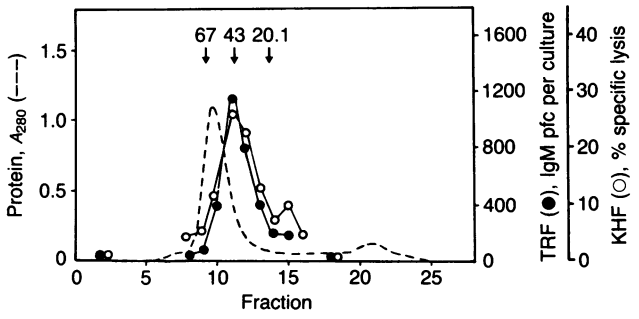

FIG. 2. Copurification of TRF and KHF by gel-permeation chromatography. The TRF active fractions after the isoelectrofocusing (Mono P column) step were concentrated up to 1 ml and then additionally fractionated on a Superose 12 column for gel-permeation chromatography connected with FPLC system (Pharmacia). Each fraction contained 1 ml. TRF and KHF activities in each sample were titrated at 10% concentration (final) by the same methods as described in the legend to Table 1. Shown are TRF activity (●) expressed as mean IgM pfc per culture and KHF activity (○) expressed as % specific lysis of target cells at an effector-to-target ratio of 40:1. The protein profile in the eluting buffer is also shown (----). Positions of apparent molecular weights of reference proteins bovine serum albumin (M_r 67,000), ovalbumin (M_r 43,000), and soybean inhibitor (M_r 20,100) are indicated (as $M_r \times 10^{-3}$) at the arrows.

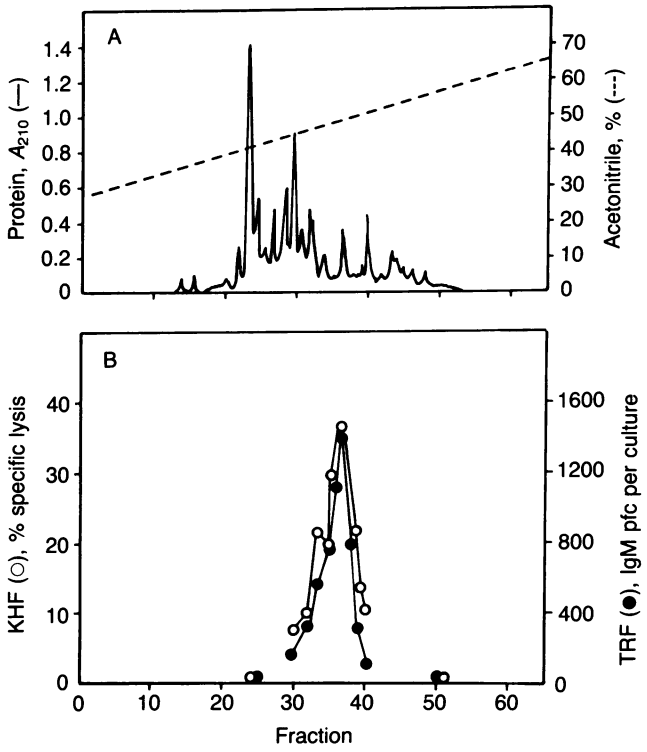

FIG. 3. Copurification of TRF and KHF by reverse-phase HPLC. The TRF active fractions after gel-permeation chromatography were concentrated and then fractionated by reverse-phase HPLC using a protein C_4 column (Vydac, Hesperia, CA). The column was eluted with acetonitrile containing 0.1% trifluoroacetic acid, from 0% to 80% (vol/vol) acetonitrile. (*A*) Protein profile (—) and acetonitrile concentration in the eluting buffer (----). (*B*) Results of assay for TRF (●) and KHF (○) at a dilution of 1:10. KHF activity was expressed as % specific lysis of target cells at an effector-to-target ratio of 40:1.

ingly, the same antibody showed little suppressive effect of KHF activity in 2Y4-4 SN (Table 2, experiment 1).

We then determined whether KHF activity in B151 SN and HPLC-TRF could be absorbed to a TB13-coupled Sepharose 4B column and recovered from the immunoadsorbent by elution with 0.8 M acetic acid. The TRF and KHF activities in the effluent and eluate fractions are shown in Table 3. It is apparent that the eluate from the column exerted TRF and KHF activities, whereas neither activity was detected in the effluent fraction.

Table 2. Blocking effect of a rat monoclonal anti-TRF antibody on TRF and KHF activities

Lymphokine	Anti-TRF antibody,* μg/ml	TRF activity, units/ml	KHF activity,† % specific lysis 40:1	20:1
Exp. 1				
Medium	None	<0.1	<1	<1
B151 SN	None	4.0	32 (1.05)	19 (1.03)
	1	<0.1	5 (1.11)	2 (1.08)
	0.2	0.3	11 (1.03)	5 (1.11)
2Y4-4 SN	None	0.2	41 (1.02)	34 (1.11)
	1	<0.1	37 (1.05)	23 (1.06)
Exp. 2				
HPLC-TRF	None	3.5	39 (1.06)	21 (1.13)
	1	<0.1	5 (1.01)	2 (1.09)
	0.2	0.4	10 (1.18)	6 (1.03)

*Affinity-purified rat monoclonal TB13 antibody was added to cultures of BCL_1 cells (TRF assay) or a mixture of PNA^+ thymocytes and stimulator cells (KHF assay) at the onset of the culture.

†KHF activities are expressed as the geometric means with standard errors (see footnote ‡ in Table 1).

Table 3. Absorption of TRF and KHF in B151 SN by anti-TRF antibody-coupled affinity column

Lymphokine	TRF activity, units/ml	KHF activity,* % specific lysis 40:1	20:1
Exp. 1			
None	<0.1	<1	<1
B151-T4 SN	3.8	28 (1.04)	19 (1.11)
Effluent†	<0.1	<1	<1
Eluate‡	2.9	19 (1.03)	15 (1.15)
Exp. 2			
None	<0.1	<1	<1
HPLC-TRF	2.0	42 (1.10)	30 (1.06)
Effluent†	<0.1	<1	<1
Eluate‡	1.6	31 (1.04)	19 (1.06)

*KHF activities were assessed at 50% concentration (final) of lymphokines. Activities are expressed as the geometric means with standard errors (see footnote ‡ in Table 1).

†B151-T4 SN (10 ml) (Exp. 1) or HPLC-TRF (10 ml) (Exp. 2) was passed through a TB13-coupled Sepharose bead (1-ml packed volume) column. The effluent was collected and used for TRF and KHF assay.

‡Samples absorbed to the column were eluted with 0.8 M acetic acid and lyophilized. Then, they were dissolved in the original volume with culture medium and used for TRF and KHF assays.

KHF Activity of rTRF. We tested KHF activity of translation products of *Xenopus* oocytes injected with mRNA from pSP6K-mTRF23 encoding for murine TRF/BCGF II (17). As shown in Table 4, translation products of oocytes exerted TRF and KHF activities, whereas control oocyte SN showed little activity, if any (experiment 1). It was also found that rTRF itself did not generate CTL unless IL-2 was added. Moreover, KHF and TRF activities of rTRF were completely suppressed by the addition of the rat monoclonal anti-TRF antibody to culture of PNA^+ thymocytes or BCL_1 cells (experiment 2).

DISCUSSION

The major finding to emerge from these studies is that TRF-containing B151 SN and HPLC-TRF have KHF activity and generate CTL from CTL precursors in PNA^+ thymo-

Table 4. KHF activity of rTRF

Lymphokine	TRF activity, units/ml	KHF activity, units/ml
Exp. 1		
Medium	<0.1	0
B151-T4 SN	2.6	3.4
5% rTRF*	20	5.5
2.5% rTRF	10	2.1
Oocyte SN†	<0.1	<0.1
5% rTRF	20	<0.1‡
IL-2§	<0.1	<0.1
Exp. 2		
5% rTRF	20	3.9
+ TB13 (1 μg/ml)	<0.1	<0.1
+ TB13 (0.2 μg/ml)	0.3	0.8

*Translation products of *Xenopus* oocytes injected with mRNA derived from pSP6K-mTRF23.

†Translation products of *Xenopus* oocytes injected with buffer.

‡PNA^+ thymocytes and stimulator cells were cultured with rTRF in the absence of IL-2.

§PNA^+ thymocytes and stimulator cells were cultured only in the presence of IL-2 (10 units/ml).

cytes in the presence of IL-2. TRF and KHF activities in the SN were copurified and barely separated from each other by various purification methods, and rTRF could also exert KHF activity. We have obtained a monoclonal anti-TRF TB13 antibody that inhibited differentiation induced by HPLC-TRF or rTRF of activated B cells as well as BCL_1 cells but failed to inhibit IL-1, IL-2, IL-3, or BSF-1 (IL-4) activities (unpublished data). The addition of the monoclonal anti-TRF TB13 antibody to our CTL assay system caused strong suppression of CTL generation induced by HPLC-TRF or rTRF (Tables 2 and 3). Moreover, TRF purified from B151 SN by using the anti-TRF antibody-coupled affinity column exerted TRF as well as KHF activities (Table 3). These results indicate that TRF itself has KHF activity. However, these results do not exclude a possibility that another lymphokine that has KHF activity but has no TRF activity exists. As shown in Table 1, 2Y4-4 SN exerts KHF activity with little TRF activity. Moreover, the anti-TRF antibody hardly suppresses KHF activity in 2Y4-4 SN (Table 2). These results suggest that there exist at least two different lymphokines that have KHF activity: one is identical to B151-TRF and the other may be unrelated molecules to B151-TRF.

The generation of CTL is thought to involve an antigen signal as well as signals delivered by soluble lymphokines. Early studies by Raulet and Bevan (24) and other investigators strongly suggested that a non-IL-2 lymphokine was required for the generation of CTL (25–29). With our approach, using PNA^+ thymocytes, the generation of CTL against TNP-modified syngeneic spleen cells in the presence of low concentrations of IL-2 (10 units/ml) requires the presence of the other factor, KHF (12–14). Although the involvement of KHF in CTL generation from thymocytes is well established, the precise role of KHF in CTL generation has not yet been elucidated. We demonstrated that KHF is required in the early period of culture (within 48 hr), and IL-2 is required for the late stages of the culture (3–5 days of a 5-day culture) (12), suggesting that KHF may augment the expression of receptors for IL-2 or may change the characteristic of IL-2 receptors already expressed. We also reported that the purified KHF from 2Y4 SN is able to augment not only proliferation of thymocytes but also the expression of IL-2 receptors on PNA^+ thymocytes in synergy with IL-2 (13). As shown in Fig. 1, TRF can induce IL-2 receptor expression in PNA^+ thymocytes in the presence of stimulator cells and IL-2, whereas IL-2 alone does not induce IL-2 receptor expression. This may support the former possibility, although the latter possibility cannot be excluded at this time.

At present, we do not have conclusive evidence concerning the mode of action of KHF other than inducing IL-2 receptor expression. In preliminary experiments, we obtained data showing that IL-1 induces IL-2 receptor expression on thymocytes in synergy with IL-2, whereas addition of IL-1 and IL-2 to the culture of PNA^+ thymocytes and stimulator cells does not induce CTL generation. Thus, the data reported here clearly suggest that KHF may play an additional augmenting role in the CTL generation in PNA^+ thymocytes in addition to induction of IL-2 receptor expression.

Several groups have described KHF-like lymphokines that are needed for CTL generation. Wagner and colleagues (28) reported that the receptor-inducing factor that induces IL-2 receptors is required for induction of CTL from resting $Lyt\text{-}2^+$ splenic T cells. Falk *et al.* (29), using PNA^- thymocytes as responders and glutaraldehyde-fixed TNP-modified spleen cells as stimulators, also found it necessary to add T-cell cytotoxic inducing factor type 1 (TCF1) other than IL-2 to generate TNP-specific CTL. TCF1 substantially induces an increase of IL-2 receptor expression. We do not know whether these lymphokines are the same or distinct molecules from the TRF and KHF described here.

In conclusion, our data strongly suggest that HPLC-TRF and rTRF act not only on B cells as a growth and differentiation factor (TRF/BCGF II) but also on PNA^+ thymocytes as KHF. Recently it was shown by Klaus and colleagues (30, 31) that eosinophil differentiation factor (EDF), which also has BCGF II activity, induces growth and differentiation of preactivated normal mouse B cells (30, 31). In view of the diverse activities and targets of TRF/BCGF II, TRF/BCGF II is classified as a different interleukin. Therefore, we propose to identify TRF/BCGF II as interleukin 5 (17).

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