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Suppression of lymphocyte activation and functions by a leukemia cell-derived inhibitor

(tumor/inhibition/maturation/lymphokines/interleukin 2)

J. W. CHIAO*, M. HEIL*, Z. ARLIN*, J. D. LUTTON*, Y. S. CHOI†, AND K. LEUNG‡

*Department of Medicine, New York Medical College, Valhalla, NY 10595; †Alton Ochsner Medical Foundation, New Orleans, LA 70121; and ‡Department of Medical Microbiology and Immunology, Ohio State University Medical Center, Columbus, OH 43210

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ABSTRACT **An inhibitor isolated from the serum-free culture medium of human myeloid leukemic HL-60 cells was able to suppress mitogen- and alloantigen-stimulated proliferative responses of normal lymphocytes in a dose-dependent manner. *In vitro* production, concentration, and purification by column chromatography and electrophoresis revealed that the inhibitor was produced constitutively, required RNA synthesis, and had a molecular weight in the range of 40,000–60,000. The inhibitor was also produced *in vitro* by myeloid leukemia cells isolated from patients with acute myelogenous leukemia. In a similar manner, the inhibitory material suppressed proliferative responses of allogeneic and autologous lymphocytes. Suppression was accompanied by drastically reduced production of interleukin 2 and lymphokines, which regulate differentiation of myeloid leukemia cells, and suppression was reversed by addition of exogenous interleukin 2. The inhibitor did not suppress clonogenic proliferation of normal granulocytes and macrophages suggesting that inhibition of production or interference with interleukin 2 activity as a possible mechanism. These interactions between leukemia cells and lymphocytes have shed new light on the immunosuppression and growth advantage of leukemia cells. Inhibitory activity of HL-60 cells was diminished after they were induced to differentiate, indicating that differentiation induced by lymphokines may be an effective means of controlling leukemia.**

Disturbances in the kinetics of cellular proliferation and functions of myeloid and lymphoid cells are typical clinical features associated with acute and chronic myeloid leukemia. Evidence has been accumulating that suggests that leukemia cells or their products may be involved in the suppression of normal hematopoiesis *in vivo* and *in vitro*. The presence of leukemia cell-associated inhibitory activity in cell extracts or conditioned media (CM) has been identified by using normal granulocytes and macrophages as target cells in clonogenic agar cultures or diffusion chambers (1–5). These results suggest a possible explanation for the impaired granulopoiesis often associated with leukemia. Although the role of leukemia cells or leukemia cell-associated inhibitory activity in the regulation of normal lymphocyte functions and growth remains undefined, modulation of leukemic cell differentiation has been achieved by exposure to human and murine lymphokines (6–8). These reports suggest that lymphokines may be potentially important for the immunological surveillance over leukemias. In contrast, the effect of leukemia cells on normal lymphokine production and function has not been demonstrated. In this report we present evidence that human myeloid leukemia cells produce a distinct inhibitor that can suppress normal lymphocyte activation and lymphokine production. Included were lymphokines that are capable of inducing terminal differentiation of myeloid leukemia cells (6–9). Our results clearly show that the inhibitor can block normal lymphocyte regulation of leukemia cell differentiation, thus inhibiting immune regulatory mechanisms and leading to a tumor cell growth advantage.

MATERIALS AND METHODS

Cells and Culture Supernatants. The human leukemia HL-60 line, derived from a patient with acute promyelocytic leukemia (10), was maintained in a serum-free defined medium described by Breitman *et al.* (11). The medium contains a 1:1 mixture of Ham's F-12 and RPMI 1640 (GIBCO) supplemented with 30 nM selenium, transferrin at 5 μg/ml, and insulin at 5 μg/ml (Sigma). Cells at approximately passage 46 were cultured initially at 2×10^5 cells per ml, and the HL-60 CM was collected from cells during the logarithmic growth phase (3–4 days). Cell-free defined medium incubated without cells for 3–4 days was used as a control. HL-60 cells were also exposed to actinomycin D at 0.1 μg/ml (Sigma) in culture medium for 4 hr at 37°C (12). Cells were subsequently washed and resuspended in fresh medium, and actinomyocin D-treated HL-60 CM was collected after 3–4 days.

Isolated Inhibitor Preparation from Leukemia Cells. The serum-free culture supernatant of HL-60 cells was concentrated using a Millipore Pellicon cassette system with a PTGC membrane (molecular weight cut-off, 10,000) and precipitated with ammonium sulfate at 80% saturation, 4°C. The precipitate was dialyzed against 0.01 M Tris·HCl, pH 8.0, and chromatographed on a DEAE-Sepharose CL-6B column with a linear 0–0.5 M NaCl gradient. The polypeptides from the active fraction were further separated by polyacrylamide gel electrophoresis using the buffer system described by Laemmli (13). The sample was mixed with an equal volume of buffer [4% (wt/vol) $NaDodSO_4$/20% (vol/vol) glycerol/160 mM Tris·HCl, pH 6.8/4 mM phenylmethylsulfonyl fluoride] and applied to a 5–10% polyacrylamide-gradient slab gel. After electrophoresis, gels were stained with Coomassie blue or ground so that the proteins could be eluted in phosphate-buffered saline (10 mM, pH 7.4) containing 1 mg bovine serum albumin at 4°C for 12 hr. Fractions obtained during isolation procedures were prepared in RPMI 1640, millipore filtered, and used as the isolated inhibitor preparation.

Production of Differentiation-Inducing Lymphokines. The preparation of a serum-free medium with differentiation-inducing lymphokines from peripheral blood lymphocytes (PBL) cultures has been reported (10, 14). Pooled normal PBL at 1×10^6 cells per ml were cultured in RPMI 1640 with 0.2% bovine serum albumin, 1% phytohemagglutinin (PHA) (14), and lymphocyte CM collected from 3-day cultures. The

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Abbreviations: CM, conditioned medium; PBL, peripheral blood lymphocytes; PHA, phytohemagglutinin; CTLL, murine cytotoxic T-cell line; IL-2, interleukin 2.

maturation-inducing activity present in lymphocyte CM has been shown to be derived from stimulated T cells (8). Control lymphocyte CM used in each experiment was prepared by adding PHA to PBL from a single donor that had already been cultured for 3 days. Some PBL cultures were cultured for 3 days with additional HL-60 CM or isolated inhibitor preparation. Controls for these PBL cultures were incubated with fresh control medium or RPMI 1640 alone.

Maturation Induction of Leukemic Cells. The differentiation assay of HL-60 promyelocytes into monocytes and macrophages by the maturation-inducing activity from lymphocyte CM (6, 8, 15) was used. HL-60 cells were cultured at 0.25×10^6 cells per ml in serum-free defined medium with 20% (vol/vol) lymphocyte CM or controls. During the culture period, the acquisition of cell membrane complement receptors, phagocytic function, and the development of morphological characteristics, etc., were assessed as described (8, 9). The cell cycle phase distribution was assayed by propidium iodine staining of DNA and analyzed on a 50H cytofluorograph (Ortho Diagnostics) (9). A standard preparation with predetermined maturation-inducer activity was used as a reference. One unit of maturation-inducing activity is defined as the minimal amount required in a given unit volume for 5% development of mature marker-bearing cells (the mean of cells bearing complement receptor, monocyte/macrophage morphology, and phagocytic cells) after 5 days of culture (9). HL-60 cells after being induced for 1–4 days were washed and resuspended in fresh serum-free medium, and HL-60 CM was collected after 3–4 days.

The inhibitor preparation was evaluated for its activity on the proliferation and differentiation of normal bone marrow cells in the soft agar-gel assay (16). The inhibitor preparation was introduced into the lower layer for stimulating 10^5 bone marrow cells that were seeded in the upper layer. The inhibitor or RPMI 1640 was also incorporated into the lower layer containing 10^6 normal leukocytes as a source of colony-stimulating factor. The cultures were incubated for 20 days, and the number of colonies having more than 40 cells was recorded.

Lymphocyte Proliferative Responses. Mitogen stimulation of lymphocytes was performed in microtiter plates (Falcon) according to described procedures (17). PBL from normal donors were isolated by Ficoll/Hypaque centrifugation (18) and cultured at 5×10^4 cell per well in RPMI 1640 with 15% (vol/vol) fetal calf serum. Each culture was stimulated with 0.7% (vol/vol) PHA-M (Difco) or Con A at 8 μg/ml (Pharmacia). Some cultures contained various concentrations of the inhibitor preparation or the unseparated culture supernatant. Both defined medium and RPMI 1640 were used in separate cultures as controls. Bidirectional mixed lymphocyte cultures were performed with PBL from two individuals at 0.15×10^6 cells per well. The PHA and Con A cultures were incubated for 2 days, and the mixed lymphocyte culture was incubated for 5 days. Each culture was labeled with 0.4 μCi [^{3}H]thymidine (specific activity, 78.1 Ci/mmol, 1 Ci = 37 GBq, New England Nuclear) 4 hr before termination of culture. The cells were harvested, and incorporation of [^{3}H]thymidine into DNA was measured. The mean counts per minute in triplicate cultures were analyzed.

Cell lines Daudi, SK-DHL, MOLT-4F, U937, KG_1, and K562 were maintained in RPMI 1640 and 15% (vol/vol) fetal calf serum. Cells at 2.5×10^5/ml were placed in wells of microtiter plates with or without supplementation of various concentrations of the inhibitor preparation or RPMI 1640. The cells were cultured for 1–3 days, and proliferation was analyzed by the [^{3}H]thymidine incorporation procedure.

Interleukin 2 (IL-2) Assay. PBL CM was analyzed for IL-2 activity by its ability to support the proliferation of the IL-2-dependent murine cytotoxic T-cell line (CTLL) (19). Each culture of the microtiter well (#76-003-05, Linbro) contained 5×10^3 CTLL cells in 200 μl RPMI 1640 with 15% (vol/vol) fetal calf serum. Medium to be tested was diluted serially from 1:2 to 1:1024, and an IL-2 preparation (rat T-cell polyclone, #40115, Collaborative Research, Waltham, MA), which had an activity of 3200 half-maximal units/ml, was used as a standard. After 20 hr of culture, cells were labeled with [^{3}H]thymidine at 0.5 μCi per culture (20 Ci/mmol, New England Nuclear) for 4 hr. Cultures were treated with 2% (wt/vol) EDTA for 10 min and harvested, and radioactivity was determined. To examine the effect of the inhibitor preparation on IL-2-mediated proliferation, various amounts of the inhibitor preparation or control RPMI 1640 were added to CTLL cultures in the presence of the standard IL-2 at 50 units/ml. Proliferation of CTLL cells was determined by the [^{3}H]thymidine incorporation procedure.

Patient Identification. RI was a male patient who had acute myelogenous leukemia with a leukocyte count of 10,900/mm^3, a hemoglobin concentration of 11.9 g/deciliter, and a platelet count of 47,000/mm^3. Bone marrow examination revealed 60% monoblasts, 6% myelocytes, 5% metamyelocytes, 26% lymphocytes, and 3% monocytes and was consistent with a diagnosis of M5B of the French, American, British (FAB) classification.

Isolation of Immature Leukemia Cells. Immature cells were enriched from the bone marrow of the patient according to the described method (20). Leukocytes were initially isolated by Ficoll/Hypaque centrifugation, and phagocytic cells were removed by carbonyl iron. Cells bearing receptors for complement or IgG were removed by centrifugation after the formation of rosettes. The T cells were isolated by sheep erythrocyte rosettes (20) that yielded 96% purity. The immature and T-cell preparations were cultured at 1×10^6 cells per ml in RPMI 1640 with 15% (vol/vol) fetal calf serum, and T-cell CM was collected after 1 day of incubation. Proliferative response of the T-cell preparation to PHA was similar to that described for PBL.

RESULTS

Demonstration of Leukemia Cell-Derived Lymphocyte Inhibitor. Normal PBL cultures with PHA, Con A, or alloantigen stimulation were supplemented with HL-60 CM or with the fresh defined medium used for culturing HL-60 cells as control. The amounts of mitogens and the duration of culture were determined to produce proliferations of PBL at the ascending portion of the response curve, since the other portion of the response curve gave erratic results. Using these assay conditions, PBL proliferative responses were regularly reduced in the presence of HL-60 CM (Fig. 1). Greater than 50% reduction in proliferative response was observed with the degree of inhibition proportional to the amount of HL-60 CM added (Fig. 1). PHA responses were reduced to 30–65% of control values (5049 ± 102 cpm) when cultured with 20–50% HL-60 culture supernatant. In a similar manner, inhibitor preparations were also found to inhibit the proliferative responses to Con A or alloantigens in mixed lymphocyte cultures. Since cell counts and viability of PBL cultures were found to be unaltered by inhibitor preparations, the reduction in proliferative responses could not be attributed to cell death. To determine whether the production of inhibitory activity was linked to RNA synthesis, HL-60 cells were first treated with actinomycin D, and then the treated HL-60 CM was analyzed. As shown in Fig. 1, the inhibitory activity for lymphocyte proliferation was not detected in these supernatants, indicating the endogenous production of the inhibitor required RNA synthesis.

Other data shown in Fig. 1 involved inducing HL-60 cells to differentiate with lymphocyte CM as a source of lymphokine (6, 8) and then assaying for the presence of inhibitor. In contrast to CM from undifferentiated HL-60

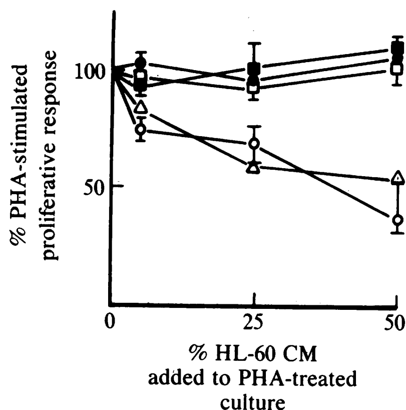

FIG. 1. Reduced proliferative responses of normal PBL by leukemic HL-60 CM. Percent responses of PHA-stimulated PBL when supplemented with HL-60 CM (○), fresh defined HL-60 cell culture medium (●), actinomycin D-treated HL-60 CM (□), or HL-60 CM from HL-60 cells after 1 day of treatment with 20% (vol/vol) lymphocyte CM (8) as a differentiation inducer (■), or with lymphocyte CM control that lacked differentiation-inducer activity (△).

cells, CM from HL-60 cells after differentiating for 1 day or more showed no inhibitory activity. Since differentiation has been shown to be a continuous process following cell-mediated interaction and since lymphocyte CM induces the majority of HL-60 cells to differentiate (8, 14), these results suggest that cells may lose the capacity to produce inhibitory activity following initiation of differentiation.

Purification of Inhibitor and Specificity of Action. The PBL-proliferative-response inhibitor was isolated from serum-free HL-60 CM, concentrated, ammonium sulfate precipitated, and chromatographed on DEAE-Sepharose. The inhibitor activity was found only in the flow through fraction and was not retained by the column. Electrophoresis showed that this fraction contained three bands under nonreducing conditions (Fig. 2). Eluted proteins were then tested for their effects on PBL proliferative responses to PHA. All the activity was found in fraction AIII (Fig. 2), which had a molecular weight range of 40,000–60,000 and a single band at 58,000.

Final concentration of the inhibitor at 1–5 μg/ml resulted in minimal detectable suppression, while 20 μg/ml or more yielded at least 50% suppression. Fig. 3*A* shows that only 17–25% of the control responses of PHA-stimulated PBL were obtained when the inhibitor was present. Since the inhibitor preparation was isolated from a conditioned serum-free defined medium, the medium components including insulin at 5–200/μg/ml, transferrin at 5–500/μg/ml, or concentrated fresh defined medium were also added to PBL cultures and showed that the inhibitory effect was not due to these components of culture medium. The effect of inhibitor on the lymphocyte proliferative response can be overcome by addition of exogenous IL-2. These results of PHA responses are shown in Fig. 3*B* and show that approximately 8 units/ml of IL-2 completely negated the effect of inhibitor at 40 μg/ml.

The IL-2 activity in the supernatant of PHA cultures was analyzed with IL-2-dependent CTLL cells to determine if the inhibitor affected IL-2 production. Media from cultures prepared with or without addition of inhibitor (40 μg/ml) were analyzed with various dilutions in CTLL cell cultures. Results indicate a 57.3–65.6% reduction of IL-2 activity, with the medium used at final dilutions from 1:8 to 1:128 (Fig. 4*A*). Fig. 4*B* shows that the responsiveness of CTLL cells to exogenous IL-2 was not altered in the presence of the inhibitor, indicating that the inhibitor by itself did not affect the IL-2 response. These data suggest that the lower IL-2 activity is attributable to a reduced IL-2 production.

To establish the biological specificity of the inhibitor, we next studied the effect of inhibitor (1–80 μg/ml) on the proliferative behavior of several established cell lines. Cell lines included the B cell lines Daudi and SK-DHL, the T-cell line MOLT-4F, the monocytic line U937, myeloid lines KG_1 and HL-60, and the erythroid line K562. No inhibition of [^{3}H]thymidine incorporation could be detected in any of these lines when incubated with the inhibitor for 1–3 days. The inhibitor was also used in the soft agar-gel procedure for proliferation and differentiation of normal bone marrow myeloid colonies. The inhibitor, supplemented at 20, 40, or 80 μg/ml to bone marrow cultures, led to no reduction of colony number during the entire culture period.

Effect of Inhibitor on Differentiation Inducing Lymphokines. To determine if the inhibitor has any effect on the generation of maturation-inducing lymphokines, inhibitor preparation or HL-60 CM was added to pooled PBL cultures with PHA for 3 days. The maturation-inducing activity present in these CMs was quantitated for its capacity to induce the terminal differentiation of HL-60 promyelocytes to monocytic cells. Experimental results in Table 1 indicated that there was a 93% reduction of the maturation-inducing activity in lymphocyte CM from treated PBL (9.6 units/ml) as compared to lymphocyte CM not supplemented with the inhibitor (134.6 units/ml). The activity to mediate growth cessation was reduced as measured by cell cycle analysis,

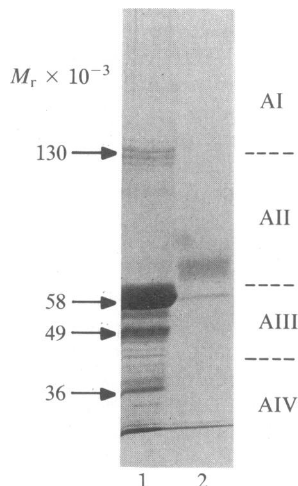

FIG. 2. Nonreducing $NaDodSO_4$/PAGE (5–10% acrylamide gradient). The gel was stained with Coomassie blue. Lane 1, marker proteins (β-galactosidase, catalase, fumarase, and glycerol-3-phosphate dehydrogenase; M_r values are given at *Left*). Lane 2, 15 μg of the early flow-through fraction from DEAE-Sepharose CL-6B; regions tested for inhibitor activity (AI–IV) are delineated.

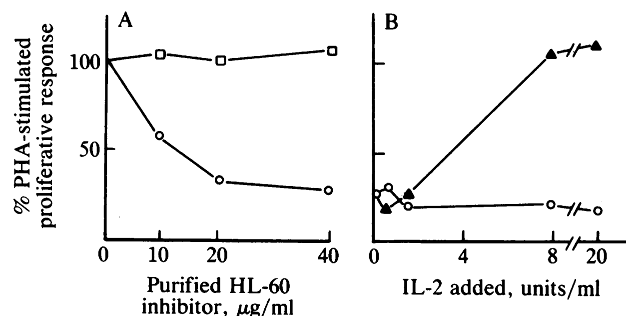

FIG. 3. (*A*) Percentage of PHA responses of PBL in the presence of the inhibitor isolated from HL-60 CM (○) or RPMI 1640 medium (□). (*B*) Percentage of PHA responses of PBL with the addition of purified exogenous IL-2 (▲) or RPMI 1640 (○) in the presence of the inhibitor preparation at 40 μg/ml.

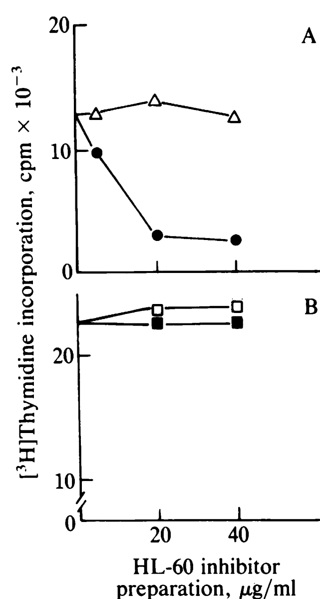

FIG. 4. IL-2 activity of PHA-stimulated lymphocyte CM assayed with IL-2-dependent CTLL cells. (*A*) [^{3}H]Thymidine incorporation of CTLL cells stimulated by a 1:64 dilution of HL-60 CM prepared in the presence of the inhibitor (●) or RPMI 1640 (△). (*B*) [^{3}H]Thymidine incorporation of CTLL cells, as stimulated by IL-2 (50 units/ml) in the presence of the HL-60 inhibitor (■) or RPMI 1640 (□).

and the capacity to induce mature cells with complement receptors, phagocytosis, morphological characteristics, etc., was reduced. The degree of the reduction was proportional to the amount of inhibitor added, with a minimal effect at 1 μg/ml and a maximal effect at 20 μg/ml or more. The effect of inhibitor in the presence of untreated lymphocyte CM was examined, and Table 1 shows that the degree of differentiation was not affected by the inhibitor. The reduction of maturation-inducing activity in the CM was, therefore, likely to be the result of suppressed production of the maturation-inducing activity. The inhibitor-supplemented (20 μg/ml) PBL culture containing exogenous IL-2 at 5 units/ml yielded an increase of 40% maturation-inducing activity in this PBL CM compared to that without IL-2.

Inhibitor Activity from Patient-Derived Leukemia Cells. A preparation enriched with leukemia cells was obtained from patient RI with acute myelogenous leukemia. The preparation showed greater than 95% blasts and less than 3% T and B cells. One-day CM from these cells was added to PHA-stimulated lymphocyte cultures. Both allogeneic normal PBL and the isolated autologous T cells from the same patient RI were used for PHA responses (Fig. 5). The PHA responses were reduced significantly with the addition of the leukemia cell CM, and the degree of reduction was proportional to the amount of leukemia cell CM added. When 25% culture supernatant was used, suppression of PHA responses of allogeneic and autologous lymphocyte responses was 92% and 90%, respectively. Control CM, prepared with T cells of patient RI, did not demonstrate any inhibitory activity (Fig. 5). Autologous cell separations were successful with a second patient with acute myelogenous leukemia, and similar results were observed.

DISCUSSION

We have presented evidence for the existence of a leukemia cell-associated inhibitor that suppresses normal lymphocyte activation and immune regulation. Serum-free HL-60 CM and a partially purified preparation of the HL-60 CM were found to inhibit significantly the proliferative response of normal lymphocytes in a dose-dependent manner. In addition, the ability of lymphocytes, exposed to the inhibitor, to generate specific lymphokines was also impaired. These lymphokines, which include γ interferon (21) and the maturation inducer (9, 15), may be components of a potential immune surveillance system over leukemia. Thus, impairment of such a regulator could provide an environment favorable for leukemia cell proliferation. While the role of these inhibitors remains to be determined, the fact that an inhibitory activity produced by leukemic cells of two patients with acute myelogenous leukemia can suppress autologous lymphocyte activation suggests a possible role in human leukemic states. Lymphocytes may function in the regulation of the differentiation of immature myeloid leukemia cells to mature differentiated cells, and the leukemia cell-associated inhibitor is capable of suppressing this differentiation event, shifting the balance of proliferation in favor of the leukemic state.

We (22) and others (23) have reported that some leukemia cells have the capacity to generate their own autostimulatory growth factor. The combination of autostimulatory factor and leukemia-associated inhibitor may in turn amplify the predominance of leukemia cell growth. A better understanding of such cell–mediator interactions may provide new insight for the manipulation of leukemia.

Our experiments indicate that the inhibitor blocks lymphocyte activation by suppressing IL-2 production. Reduced IL-2 production was detected in cultures exposed to inhibitor, and

Table 1. Suppression of maturation-inducing activity from lymphocyte CM by HL-60 CM inhibitor activity

		Day 5 differentiation markers, % suppression					
Inducer	Lymphocyte-CM preparation	S + G_2 + M phases	Complement receptor	Monocytes–macrophages	Promyelocyte morphology	Phagocytic cells	Inducing, unit/ml
None		45 (41–48)*	2 (0–4)	1 (0–2)	98 (95–100)	2 (0–5)	
Lymphocyte CM	PBL CM + inhibitor (40 μg/ml)†	43 (40–46)	12 (10–14)	10 (8–12)	90 (87–93)	7 (6–8)	9.6 (8–14.6)
Lymphocyte CM	PBL CM + 25% (vol/vol) HL-60 CM	40 (37.5–42)	4 (1–6)	8 (6–11)	92 (86–94)	8 (6–11)	6.6 (4.3–9.3)
Lymphocyte CM	PBL CM + control medium	20 (16–25)	50 (43–52)	72 (68–80)	28 (24–31)	80 (75–90)	134.6 (124–148)
Lymphocyte CM + inhibitor (40 μg/ml)	PBL CM + control medium	21 (18–24)	45 (42–48)	68 (65–71)	32 (29–35)	78 (75–81)	127.2 (121.2–133.2)
Inhibitor preparation		42	0	0	92	1	

Lymphocyte CM was prepared from activated normal PBL in a serum-free medium.
*Values in parentheses represent the range.
†The inhibitor was isolated from HL-60 CM.

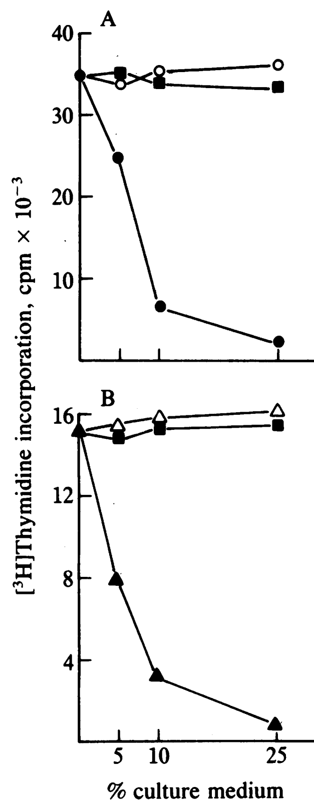

FIG. 5. Inhibitor activity of leukemia cells, isolated from patient RI, reduces [^{3}H]thymidine incorporation of PHA responses. (*A*) Allogeneic normal PBL responses in the presence of the isolated RI leukemia cell CM (●), fresh culture medium (■), or CM from isolated RI T-cell preparation (○). (*B*) PHA response of RI T cells in the presence of the CM from isolated RI leukemia cells (▲), culture medium (■), or CM from RI T-cell preparation (△).

addition of exogenous IL-2 was able to completely abolish the inhibitory action. A similar mechanism has been described for a melanoma cell-derived inhibitor (24). The leukemia cell inhibitor does not appear to lyse or alter the viability of myeloid or lymphoid cells, nor does it appear to directly inhibit DNA replication. Furthermore, the inhibitor does not affect the clonogenic proliferation and differentiation of normal granulocytes and macrophages. Gel electrophoresis of the isolated inhibitor preparation indicated a molecular weight range between 40,000–60,000 with one M_r 58,000 band. These results suggest that the leukemia cell-associated lymphocyte inhibitor may be a different molecular entity than the granulopoiesis inhibitors derived from human leukemia cells (1–5). Those inhibitors were detected by their inhibition of bone marrow granulocyte and macrophage colony formation (1–3, 25). In contrast to the lymphocyte inhibitor, whose production requires RNA synthesis, the granulocyte inhibitor is insensitive to RNA inhibition (25). The granulocyte inhibitors have apparent molecular weights of greater than 300,000 or 500,000 with subunits in the range of 150,000–170,000 (2, 25, 29). Broxmeyer *et al.* (25) have suggested that acidic isoferritins are the important molecules mediating the suppression of granulopoiesis. Suppressor molecules for the production of IL-2 have been described including prostaglandin E (26), adherent cell products from leprosy patients (27), and certain serum proteins from pregnant women (28). Since IL-2 plays a central role in both cellular and humoral immune responses, it remains possible that the leukemia cell inhibitor could cause immune suppression at both T- and B-cell levels.

Our contention that production of the inhibitor is associated with the leukemic state is supported by two lines of evidence. First, the inhibitor was produced only by leukemia cells, not by normal lymphocytes from normal donors and by PBL from patients. Second, HL-60 cells after being induced to differentiate for 1 day or more lost their capacity to produce the inhibitor. The inability of differentiated HL-60 cells to produce the inhibitor may be associated with the early events of cellular differentiation such as the reduction of cellular RNA content (8). Induction of the differentiation process by lymphokines may represent an effective means of controlling the production of leukemia cell-associated inhibitor. Eventually this induction process could possibly eliminate the accumulation of inhibitors and favor normal hematopoietic homeostasis.

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