ACCESSORY CELL FUNCTION OF HUMAN B CELLS

I. Production of Both Interleukin 1–like Activity and an Interleukin 1 Inhibitory Factor by an EBV-Transformed Human B Cell Line

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The activation of T lymphocytes for antigen-induced proliferative responses (1), T lymphocyte cytotoxicity (2), delayed-type hypersensitivity (3), and helper function for antibody responses (4) requires their recognition of the nominal antigen on the surface of an antigen-presenting cell (APC)[1] bearing Ia-DR molecules encoded by the I region of the major histocompatibility locus. In addition to the requirement for "joint recognition," the APC provides a second signal for T lymphocyte activation, the cytokine interleukin 1 (IL-1) (5).

Monocytes and other cell types, including dendritic cells, all have been reported to produce IL-1 and to be effective APC (6–8). Recently, normal B cells (9), B cell tumors expressing Ia antigens (10–12), and Epstein-Barr virus (EBV)–transformed B cell lines (13) all have been reported to exert an effective antigen-presenting function for T lymphocyte activation. Furthermore, murine B cell lymphoma cells have been shown to "process" and present antigens (10) in a manner similar to that reported for murine (14) as well as human monocytes (15). These B cell lines, however, have not been reported to secrete IL-1 or any other soluble factors, raising critical questions concerning the importance of such second signals for T cell activation.

In this study we report that ROHA-9, a human EBV-transformed lymphoblastoid cell line that is an effective APC, constitutively produces a soluble factor with biochemical and biological characteristics resembling those of monocyte-derived human IL-1. The IL-1–like activity was undetectable in crude supernatants, but was evident after gel fractionation studies which, in addition, revealed the presence of a coexistent inhibitory factor. The biochemical and biological properties of this inhibitory factor have also been investigated.

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[1] *Abbreviations used in this paper:* APC, antigen-presenting cells; BCGF, B cell growth factor; Con A, concanavalin A; CSF, colony-stimulating factors; EBNA, Epstein-Barr nuclear antigen; EBV, Epstein-Barr virus; HSA, human serum albumin; Ig, immunoglobulin; IL-1, interleukin 1; IL-2, interleukin 2; mAb; monoclonal antibodies; MLR, mixed leukocyte reaction; PBS, phosphate-buffered saline; PEG, polyethylene glycol; PMA, phorbol myristic acetate; SLO, Streptolysin O; SpA, *S. aureus* protein A.

Materials and Methods

Animals. C3H/HeJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME). 6–8-wk old female mice were used. Animals were fed and supplied with water ad libitum.

Reagents. Streptolysin O (SLO) and lipopolysaccharide *Escherichia coli* 055:B55 (LPS) were purchased from Difco Laboratories (Detroit, MI). *Staphylococcus aureus* protein A (SpA) and concanavalin A (Con A) were purchased from Pharmacia (Uppsala, Sweden). ^{3}HTdR (1.9 mCi/mM) was obtained from Amersham (Amersham, UK). RPMI 1640 tissue culture medium was purchased from Gibco laboratories (Grand Island, NY) and supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin (Microbiological Associates, Walkersville, MD), and 2 mM glutamine (Gibco Laboratories). Fetal calf serum was purchased from Hyclone (Logan, VT) and 2-mercaptoethanol from Eastman Kodak (Rochester, NY). Hepes buffer was obtained from Gibco Laboratories.

Production and Biochemical Characterization of Soluble Factors. Human IL-1 was produced as previously described (15) by stimulating adherent monocytes with 25 μg/ml LPS for 48 h in RPMI 1640 medium supplemented with 1 mg/ml human serum albumin (HSA) (generously provided by the American Red Cross, Bethesda, MD), 2 mM glutamine, 100 U penicillin, and 100 μg/ml streptomycin. Supernatants were harvested and the IL-1 was partially purified by sequential $(NH_4)_2SO_4$ purification, DEAE-Sephacel anion exchange chromatography, and Sephacryl S-200 gel filtration as previously described (15).

Supernatants from ROHA-9 cell cultures were collected at the times indicated in the figure legends and processed as follows: for ammonium sulfate precipitation 47.6 g of $(NH_4)_2SO_4$ were added to 100 ml of supernatant under gentle stirring to achieve a final concentration of 75%. The precipitated proteins were collected by centrifugation at 5,000 *g* for 10 min and dialyzed extensively against 20 mM NaCl buffer, pH 7.8 containing 20 mM Tris 0.01% polyethylene glycol (PEG) (6,000 mol wt) and 50 μg/ml of gentamicin (Gibco Laboratories). For DEAE-Sephacel chromatography the samples were centrifuged (15,000 *g* for 10 min) to remove denatured proteins and applied to the anion exchange column at 3 mg of protein per ml of gel. The samples were eluted with a linear NaCl gradient (20 mM to 400 mM), 3-ml fractions were collected, dialyzed extensively in phosphate-buffered saline (PBS) containing 0.01% PEG and antibiotics, and tested as described below.

For gel filtration, $(NH_4)SO_4$-precipitated supernatants were dialyzed extensively against PBS containing 0.01% PEG and antibiotics. 2-ml samples were applied to a Sephacryl-S-200 superfine (Pharmacia) column (2.5 × 90 cm) that had been previously calibrated with the following markers: aldolase (158,000 mol wt), bovine serum albumin (67,000 mol wt), ovalbumin (45,000 mol wt), soybean trypsin inhibitor (21,500 mol wt), and cytochrome *c* (12,354 mol wt). Aliquots of 3 ml were collected by elution with PBS supplemented with 0.01% PEG and 50 μg/ml gentamicin at a flow rate of 20 ml/h and then tested as described below. The fractions active in the IL-1 assay or in the assay for inhibitory activity (see below) were pooled, concentrated by Amicon ultrafiltration with PM10 Diaflo membrane (Amicon Corp.; Danvers, MA), and dialyzed extensively against 0.01% PEG. The samples were loaded onto a preparative isoelectric focusing column (LKB) in a 100-ml 5% to 50% sucrose gradient with 0.01% PEG and 2% ampholites, pH 3.5 to 10 (LKB). The pH gradient was formed over a period of 18 h at 1,600 V. 3-ml fractions were collected and the pH was determined. Fractions were dialyzed extensively against PBS (with 0.01% PEG, 50 μg/ml gentamicin), filtered, and tested as described below.

Cell Preparation. The human EBV-transformed B lymphoblastoid cell line ROHA-9 was established and cloned by Hall and Muchmore, as described in detail elsewhere (Hall and Muchmore, submitted for publication). ROHA-9 cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated FCS at 0.2×10^6/ml and grown up to 1.2×10^6 in 6 d. These cells were tested for nonspecific esterase, peroxidase, surface immunoglobulin (Ig), Ig production, and Epstein-Barr Nuclear Antigen (EBNA) positivity by standard techniques. The reactivity of ROHA-9 cells with monoclonal antibodies (mAb) AF10-

(αHLA-DR) (16), αLeu-M1 (17), αLeu M3 (18), αOKM1 (19), and α-Leu 10 (20) was studied by flow cytometry (Ortho Cytofluorograph).

Highly purified T cells were prepared by depletion of adherent cells from PBL by plastic adherence and passage over nylon wool column followed by fractionation on a seven-step discontinuous Percoll (Pharmacia) density gradient ranging from 40% to 57% Percoll with each step varying from the next by 2.5% concentration (21). Lymphocytes recovered from high density fraction 6 were consistently free of contaminating monocytes (<0.1% by nonspecific esterase) and were utilized as responder cells in cocultures with ROHA-9 cells (see below).

Culture Conditions. For Con A, SpA, and mixed leukocyte reaction (MLR) response, purified T lymphocytes were cultured in RPMI 1640 supplemented with 10% heat-inactivated pooled human AB serum, 2 mM glutamine, 10 mM Hepes, and 50 μg/ml gentamicin at 2×10^5 cell/well in 0.2-ml cultures in the presence of the indicated concentrations of ^{137}C-irradiated (5,000 rads) ROHA 9 cells for 3 d (Con A and SpA response) or 7 d (MLR). Cultures were pulsed for the last 16 h with 1 μCi [^{3}H]thymidine and harvested using an automated harvesting system. To obtain an SLO response, 5×10^5 unfractionated PBL were cultured in the same medium in the presence of optimal concentrations of SLO (1.20 final dilution) and linear dilutions of the inhibitory factor as specified in figure legends. IL-1 and IL-2 assays were performed exactly as described previously (15) by using a single cell suspension of C3H/HeJ thymocytes or the IL-2–dependent CT6 cell line (22), respectively.

Results

Surface Marker and Functional Characteristics of ROHA-9 Cells. ROHA-9 cells were 100% EBNA positive, <1% nonspecific esterase and peroxidase positive, 92% surface IgM positive, produced 20 ng/ml of IgM into the medium, reacted strongly (98%) with AF-10, a mAb that recognizes a common determinant on both monocytes and B cell DR molecules, and with αLeu-10 mAb (94%), which is specific for B cells. In contrast, ROHA-9 cells did not react with αLeu-M1 or αOKM1 mAb, both markers of the myelomonocytic lineage.

Functionally, ROHA-9 cells were potent stimulators in the one-way allogeneic MLR. In fact, as shown in Fig. 1, irradiated ROHA-9 cells stimulated a significant proliferative response by allogeneic purified T lymphocytes at a stimulator/responder ratio as low as 1:40. We then tested the capacity of ROHA-9 cells to fulfill an effective accessory function for T lymphocyte responses to Con A or SpA (15, 26). ROHA-9 cells were effective APC for proliferative response of purified T lymphocytes to both Con A or SpA (Fig. 1).

Biochemical Characterization of ROHA-9–derived Interleukin 1. We tested whether ROHA-9 cells, which had the capacity to present alloantigens and polyclonal stimulants to T lymphocytes, might secrete an IL-1–like soluble factor(s). Supernatants from 6-d cultures of unstimulated ROHA-9 cells were concentrated (10×) by Amicon membrane system, dialyzed, and tested for interleukin 1 activity. These crude supernatants showed no detectable IL-1 activity but rather had some inhibitory effects on the response of mouse thymocytes to suboptimal doses of Con A (data not shown). Therefore, we performed gel fractionation studies of the supernatants in the attempt to separate the inhibitory activity from the putative presence of IL-1 activity.

Aliquots of 10-fold concentrated supernatants were applied to Sephacryl S200 columns and the eluted 3-ml fractions were tested for both IL-1–like activity and inhibition of mouse thymocyte response to a standard preparation of IL-1. The

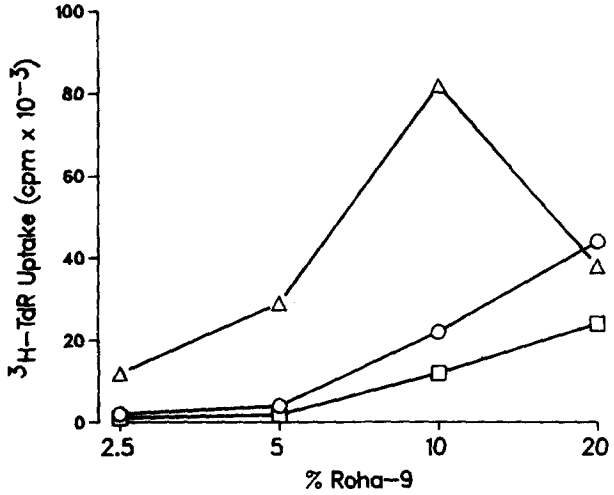

FIGURE 1. Accessory cell function of ROHA-9 cells for T lymphocyte activation. 2.5 × 10^5 monocyte-depleted T lymphocytes were cultured with the indicated percentages of irradiated (5,000 rad) ROHA-9 cells for 3 d in the case of Con A or SpA responses, or for 7 d for one-way MLR. Data are expressed as mean cpm of triplicate cultures. The standard deviations of the experiments shown in this as well as in the next figures were <5–10% (not shown). Results are representative of several experiments. Δ, MLR; O, Con A (3 μg/ml); □, SpA (10 μg/ml).

results (Fig. 2*B*) showed that a major peak of IL-1–like activity eluted in the range of 15–18,000 mol wt, with a second peak of activity in the range of 32–35,000. The active fractions of these two peaks of IL-1–like activity were pooled, concentrated to 2 ml, dialyzed, and reapplied to the same S200 gel column. The eluted samples showed a similar pattern of activity, suggesting that the 32K ROHA-9–derived IL-1 activity was due either to molecular weight heterogeneity or to molecular complexes of 15K IL-1 with each other or with unrelated peptides (data not shown).

The inhibitory activity eluted in a single peak, in the range of 95–100 K mol wt (Fig. 2*C*).

To study whether ROHA-9–derived IL-1–like activity, like monocyte-derived IL-1 (15, 25), also binds to anion exchange resins, 10× concentrated ROHA-9 supernatants were applied to a DEAE-Sephacel column. The IL-1–like activity eluted from DEAE-Sephacel with 50–80 mM NaCl salt concentration and with a second peak eluting with 110–130 mM NaCl (Fig. 3*A*). The second peak may consist of a complex of IL-1 and BSA as observed for monocyte-derived human IL-1 (15) (Fig. 3*A*).

The inhibitory activity in 10× concentrated supernatants was eluted from DEAE-Sephacel in a single peak of activity with 150 mM NaCl (Fig. 3*B*). Next, the major (15–18K mol wt) IL-1 active peak from S200 gel filtration was applied to a preparative isoelectrofocusing column in a 100-ml, 5% to 50% sucrose gradient with 2% ampholites, pH 3.5 to 10. The results (Fig. 4*A*) showed that ROHA-9 IL-1 exhibited IEF heterogeneity similar to that reported for monocyte-derived human IL-1 (26), with a major peak of activity at pI 7.3 and two others at pI 6.1 and 4.1. The 32–35K second peak of activity showed a similar profile on IEF (data not shown).

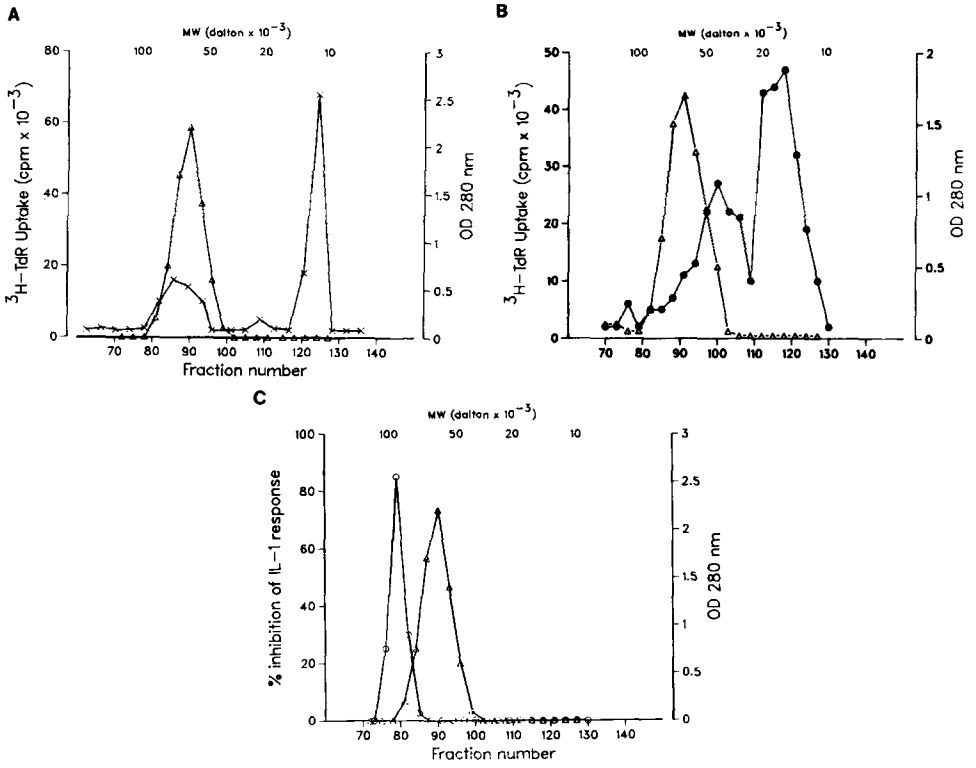

FIGURE 2. Gel fractionation profiles of ROHA-9 cell culture supernatants. $(NH_4)_2SO_4$ precipitated supernatants were dialyzed and applied to Sephacryl S200 superfine packed column. Each fraction was dialyzed extensively and tested at 1:8 final concentration. Panel *A* shows a typical separation pattern of IL-1 generated by LPS stimulated human monocytes (×). Panel *B* shows the ROHA-9 derived IL-1 activity (●). Panel *C* shows the inhibitory activity for IL-1 (○). OD 280 nm (△). Data are expressed as cpm or as percentage of inhibition of triplicate cultures.

The fractions, from the S200 gel column, that inhibited the C3H/HeJ mouse thymocyte response to IL-1 were pooled and also applied to a IEF column. The inhibitory activity was recovered from such a preparative IEF column in a single peak at pI 4.75 (see Fig. 4*B*).

Capacity of ROHA-9–derived IL-1 to Enhance the Proliferative Response of T Lymphocytes to Con A. ROHA-9–derived IL-1 partially purified by sequential $(NH_4)_2SO_4$ precipitation, DEAE-Sephacel chromatography, and S200 gel filtration, like monocyte-derived IL-1 (24), enhanced the proliferative response of monocyte-depleted human T lymphocytes to suboptimal doses (1 μg/ml) of Con A (Fig. 5).

Kinetics of Production of IL-1 and of Inhibitory Factor by ROHA-9 Cells. The production of IL-1–like activity and of the inhibitory factor was followed daily for the 6-d period of exponential growth of the ROHA-9 cell line. As shown in Fig. 6, the secretion of IL-1–like activity peaked at day 3 and was stable through day 6.

Murine as well as human monocytic cell lines can be induced by phorbol

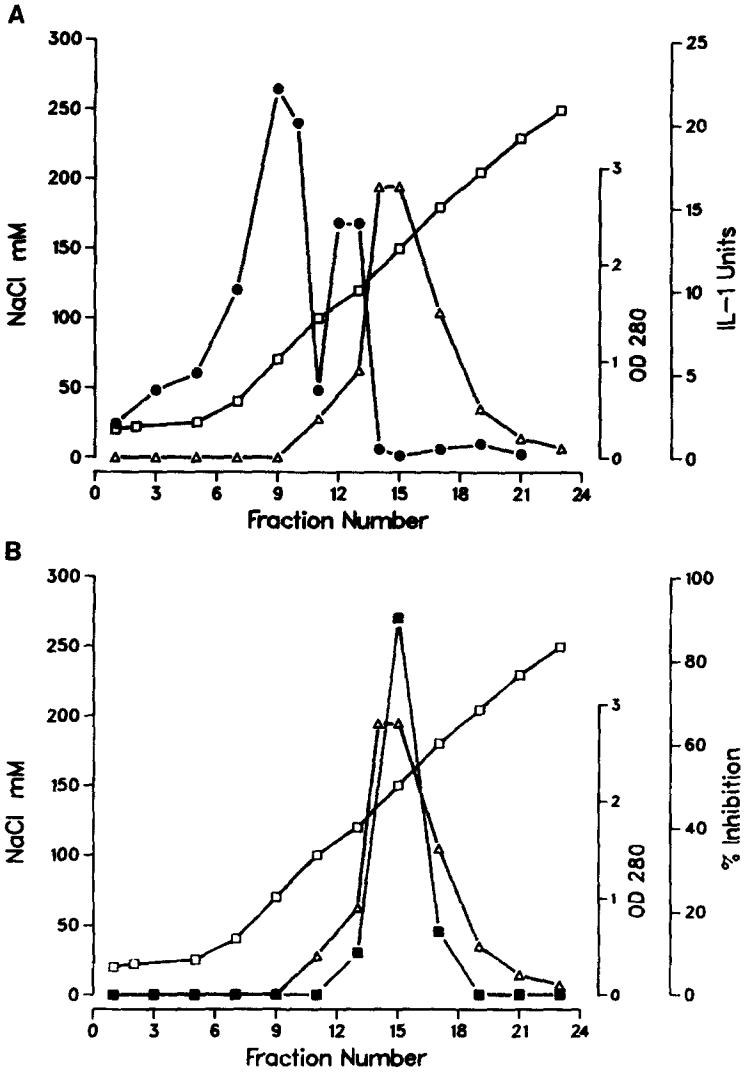

FIGURE 3. DEAE-Sephacel chromatography of ROHA-9 cell culture supernatant. $(NH_4)_2SO_4$ precipitated supernatants were dialyzed, applied to DEAE-Sephacel column, and eluted with a 20–400 mM NaCl gradient (□). The ROHA-9 cell-derived IL-1 activity eluted at 50–80 mM NaCl salt concentration (●) and with a second minor peak of activity at 110–120 NaCl (panel *A*). The inhibitory factor (■) eluted as a single peak of activity at 150 with NaCl (panel *B*) (△). OD (280 mm) of the eluted fractions.

myristic acetate (PMA) to secrete greater levels of IL-1 activity (24, 25). Therefore, we tested whether ROHA-9 cells also could also be induced to produce greater levels of IL-1 activity by PMA. As shown in Fig. 6, unstimulated and PMA-stimulated (20 ng/ml) ROHA-9 cells produced comparable levels of IL-1 activity. No increase in IL-1 production was obtained by stimulating ROHA-9 cells with 50–1,000 ng/ml of PMA (data not shown).

An inhibitory factor was detectable in supernatant of unstimulated cultures of ROHA-9 cells starting at day 2 and was present during the entire 6 d of

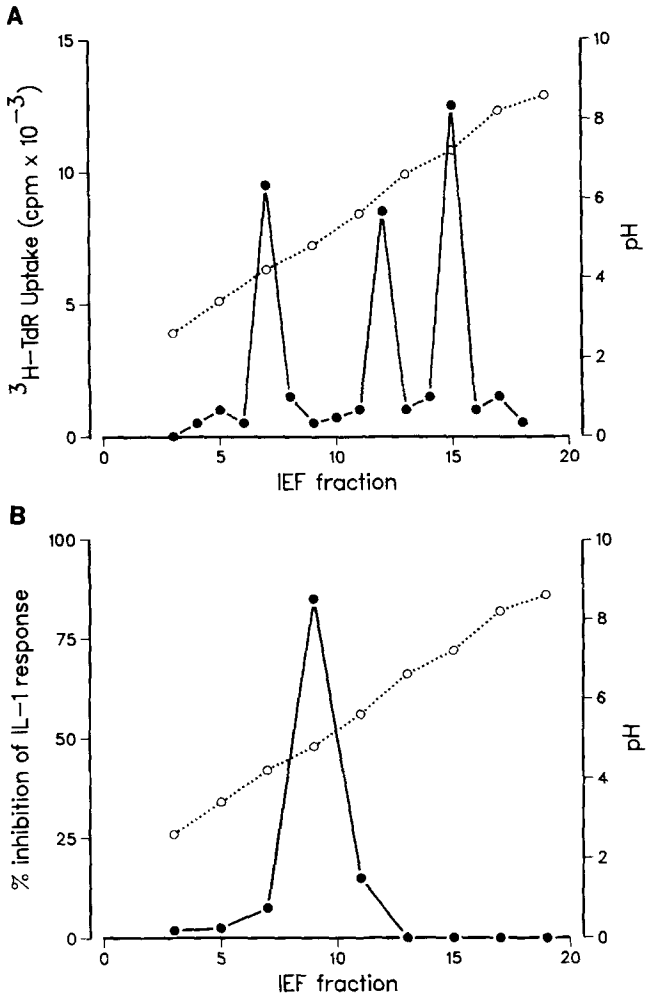

FIGURE 4. Isoelectrofoucsing of ROHA-9 cell supernatants. The 15–18K mol wt fractions of IL-1 activity from S200 gel filtration, which were active in IL-1 assay or the 95–100,000 mol wt inhibitory fractions were applied to a preparative isoelectrofocusing column with a pH gradient (○) from 3.5 to 10. Panel *A* shows ROHA-9 cell-derived IL-1 activity (●). Panel *B* shows the inhibitory activity (●). All fractions were tested at 1:8 final concentration.

logarithmic growth. However, when cultures were stimulated with 20 ng/ml PMA, the inhibitory activity peaked at day 2, but decreased by day 3 and was nearly undetectable by day 4 to 6. It is of interest that 10–100 μg/ml LPS similarly did not increase the production of IL-1 activity, but decreased the production of the inhibitory factor (data not shown). Taken collectively, the above data suggest that "activation" of ROHA-9 cells by PMA or by LPS may lead to a selective block of the secretion of the inhibitory factor, while the production of IL-1 activity remains unaffected.

Biological Characteristics of the Inhibitory Factor Produced by the ROHA-9 Cell Line. Since IL-1 has been reported to be a necessary second signal for T lymphocyte proliferative responses (15), we tested whether the 95K factor

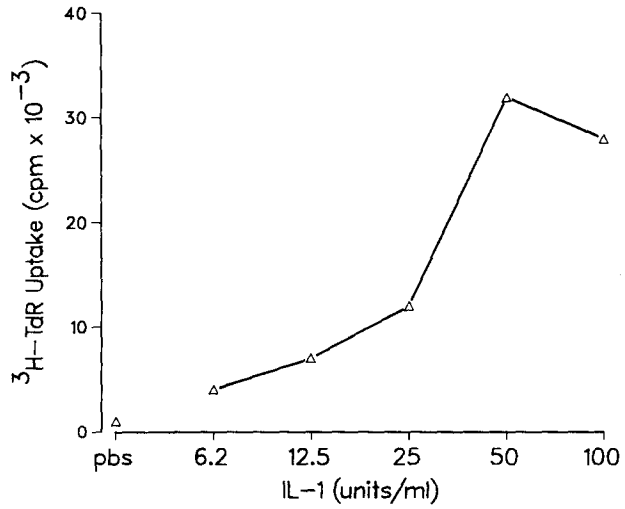

FIGURE 5. Ability of ROHA-9 cell–derived IL-1 to support T lymphocyte response to Con A in the absence of monocytes. 2.5×10^5 monocyte-depleted T lymphocytes from fraction 6 of discontinuous Percoll density gradient were cultured in presence of 1 μg/ml Con A and the indicated concentrations of partially purified IL-1. Data are mean cpm from triplicate cultures. Results are representative of five experiments.

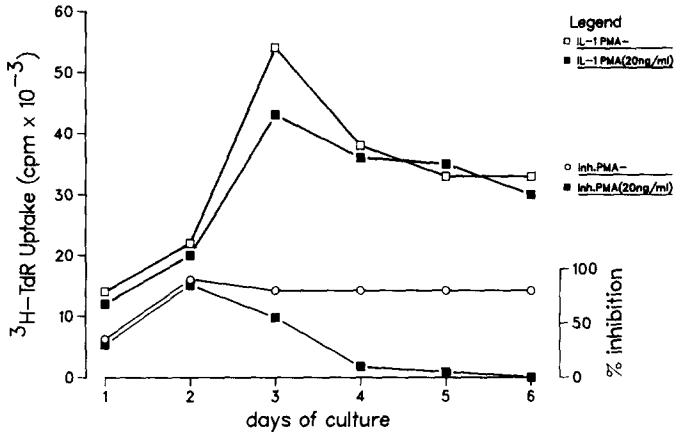

FIGURE 6. Kinetics of production of the IL-1–like activity and of the inhibitory factor by ROHA-9 cells. Supernatants from unstimulated or PMA-stimulated (20 ng/ml) ROHA-9 cell cultures were harvested at the indicated days of the logarithmic growth. $(NH_4)_2SO_4$-precipitated proteins were applied to single DEAE-Sephacel anion exchange columns and eluted by 20–400 mM linear NaCl salt gradient. 2-ml fractions were collected, dialyzed extensively against PBS (with 0.01% PEG and antibiotics), and tested for IL-1–like activity in the C3H/HeJ thymocyte costimulator assay and for the capacity to inhibit the response of C3H/HeJ mouse thymocytes to a standard preparation of IL-1. Fractions were tested at 1:8 final concentration. Results are mean of triplicate cultures. Results are representative of three experiments.

produced by ROHA-9 cells was inhibitory for T lymphocyte response to polyclonal stimulants such as Con A, for soluble antigens such as SLO, and for the T lymphocyte response to alloantigens. We observed a dose-dependent inhibition of T lymphocyte responses to suboptimal doses of Con A (1 μg/ml), but no inhibition when optimal doses (10 μg/ml) of Con A were used (Fig. 7*A*).

We also observed a dose-dependent inhibition of the response of T lymphocytes to SLO or alloantigens by the partially purified preparation of 95K inhibitory factor (Fig. 7*B*).

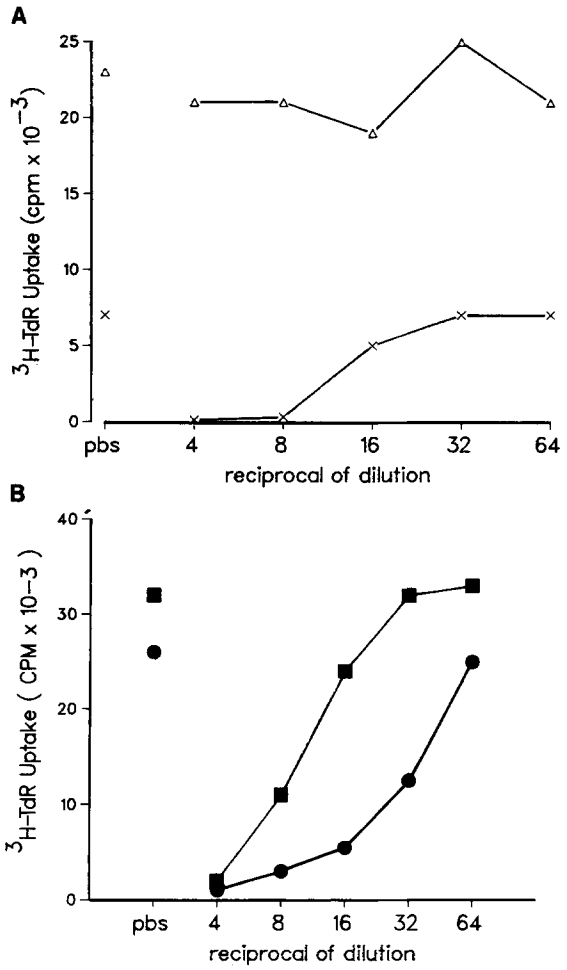

FIGURE 7. Effects of ROHA-9 cell-derived inhibitor factor on T lymphocyte response to stimulants. Panel *A*: 2.5×10^5 unfractionated PBL were cultured for 3 d in the presence of optimal (10 μg/ml, Δ) or suboptimal (1 μg/ml, ×) doses of Con A and various concentration of ROHA-9 cell-derived inhibitory factor partially purified by $(NH_4)_2SO_4$ precipitation and Sephacryl S200 gel filtration. Δ, Con A 10 μg/ml; ×, Con A 1 μg/ml. Panel *B*: 2.5×10^5 unfractionated PBL were cultured for 6 d in the presence of optimal doses of SLO (1.20 final concentration) (16). For MLR studies (panel *B*), 2.5×10^5 purified T lymphocytes were cultured for 7 d with irradiated (3,000 rad) allogeneic monocytes and various concentrations of inhibitory factor. Cultures were pulsed for the last 16 h with 1 μCi ^{3}H-TdR. Results are representative of five experiments. ●, MLR; ■, SLO response.

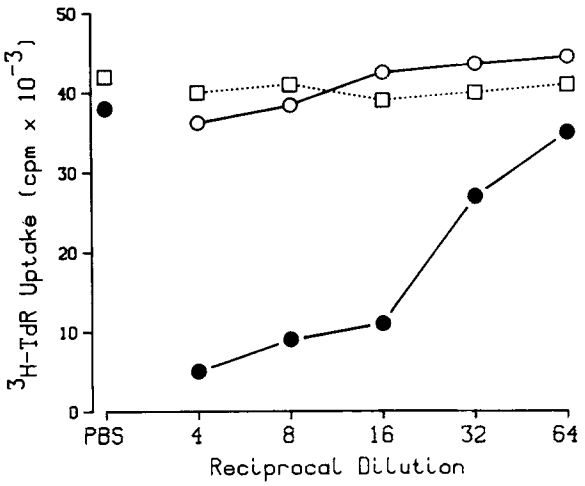

FIGURE 8. Effects of ROHA-9 cell-derived inhibitor on IL-1 and IL-2 responses. 1×10^6 C3H/HeJ mouse thymocytes were cultured for 3 d with suboptimal doses (1 μg/ml) of Con A and a standard preparation of IL-1 (●) or IL-2 (○) in the presence of various concentrations of a preparation of the inhibitor partially purified by $(NH_4)_2SO_4$ precipitation and S200 gel filtration. For IL-2 inhibition, 1×10^4 IL-2-dependent CT6 cells were cultured for 48 h in the presence of a standard preparation of IL-2 and the indicated concentration of the inhibitor factor (□). Cultures were pulsed and harvested as described above. Results are mean ± SEM of triplicate cultures and are representative of several experiments.

The activation of T lymphocytes is the result of a complex cascade of events (27) in which at least two soluble factors, IL-1 and IL-2, sequentially interact so that inhibition of either one or both of them could result in decreases in T lymphocyte proliferation. We therefore tested the capacity of a partially purified preparation of inhibitory factor to inhibit the response of C3H/HeJ mouse thymocytes to a standard preparation of monocyte-derived human IL-1, and to inhibit the effect of a standard preparation of IL-2 on the growth of the IL-2-dependent CT6 cell line. As shown in Fig. 8, we observed a dose-dependent inhibition of the 95K inhibitory factor on mouse thymocyte response to IL-1, while the factor had no effect on the response of CT6 cells. In addition, the inhibitory factor did not inhibit the comitogenic response of thymocytes to a standard preparation of IL-2 (Fig. 8). These data suggest that the inhibitory effect of the 95K factor from ROHA-9 cultures is exerted by interfering with the helper effects of IL-1. On this basis the inhibitory factor will be named hereafter "contra-IL-1."

To better understand the mechanisms of this inhibition, we investigated whether the target cells of contra-IL-1 bear detectable receptors for this inhibitor. C3H/HeJ mouse thymocytes were incubated (37°C, 5% CO_2 with frequent gentle agitation) at 3×10^7/ml with aliquots of the eluted 95K inhibitor factor or with equivalent 90-100K mol wt S200 fractions of FCS-containing medium. After 1 h, cells and supernatants were tested for their capacity to respond to IL-1 or to inhibit the response of untreated thymocytes to the same standard amount of IL-1, respectively. As reported in Fig. 9, the inhibitory effects of the standard preparation of 95K contra-IL-1 were completely absorbed by thymocytes at the

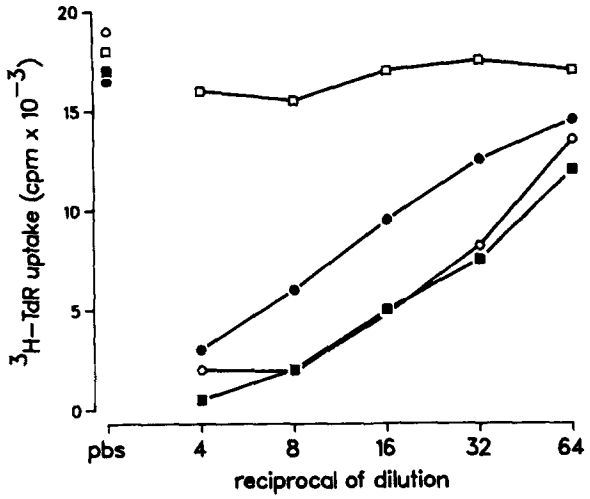

FIGURE 9. Selective absorption of ROHA-9 cell-derived inhibitor. C3H/HeJ mouse thymocytes or CT6 cells were incubated (37°C, 5% CO_2) with various dilutions of a partially purified preparation of inhibitor at 2×10^7/ml. 2 h later, cells and supernatants were collected by centrifugation. Thymocytes were then cultured for 3 d in presence of suboptimal doses of Con A (1 μg/ml), a standard preparation of IL-1 and various dilution of unabsorbed preparations of inhibitor (■). The preparation of inhibitor was tested after thymocyte absorption (□) or after CT6 absorption (○). Thymocytes, which were pulsed with the indicated linear dilution of inhibitor for 2 h, were washed three times with PBS and tested for proliferative response to a standard preparation of IL-1 in presence of suboptimal doses of Con A (1 μg/ml) (●). In experiments done in parallel, thymocytes were incubated with an equivalent 90–100,000 dalton mol wt range preparations from S200 gel columns loaded with FCS-containing medium and showed a normal thymocyte response to the standard preparation of IL-1 (data not shown). Results are representative of four experiments.

dilutions tested. Thymocytes that had absorbed the factor were in turn inhibited from responding to a standard preparation of monocyte-derived IL-1. In contrast, CT6 cells failed to absorb significant levels of the inhibitory activity. The data suggest that the ROHA-9–derived inhibitory factor can be specifically absorbed by thymocytes that also are the target of the biological effects of IL-1.

Discussion

Previous studies by Chestnut and Grey have shown that normal murine B cells (9) as well as Ia-antigen–bearing mouse B cell tumor lines (10–12) could serve as effective APC for antigen-driven T cell activation. Moreover, EBV-transformed human B cells have been reported to present the soluble antigen tetanus toxoid to Ia-DR compatible T lymphocytes (13). In line with these findings we report in the present study that a human EBV-transformed B cell line, ROHA-9, which exhibited many of the phenotypic markers of normal B cells, was an effective APC for T lymphocyte responses to the polyclonal stimulants Con A or SpA and for alloantigens in MLR.

The accessory cell function of APC has been associated with secretion of a soluble factor identified as the 12–18K mol wt IL-1 (5, 15). The data presented in this study also show that a human EBV-transformed B lymphoblast line can constitutively secrete a soluble factor that showed biochemical and biological

characteristics similar to those reported for the monocyte-derived human IL-1 (26). The anion exchange chromatography and IEF profiles of ROHA-9–derived IL-1 were also consistent with that reported for the monocyte-derived human IL-1 (15, 26). Moreover, the major peak of the ROHA-9–derived IL-1 activity eluted in the range of 15K mol wt, compatible with the known characteristics of human IL-1. However, we observed a second peak of activity in the range of 32K. While we cannot rule out that this second peak of activity might be due to molecular aggregation of the 15K mol wt IL-1 activity, it is possible that the 32K peak might be a biochemical variant of human IL-1 secreted by the ROHA-9 lymphoblastoid cell line. A similar molecular weight heterogeneity has been reported for cutaneous T cell lymphoma-derived TCGF (IL-2) (28) and for human interferon that is constitutively produced by virus-infected human lymphoblastoid cell lines (29). The nature and significance of the molecular weight heterogeneity of IL-1 remain unknown since no functional differences were found between these two peaks of activity (data not shown).

In further studies, we have been able to demonstrate that six of seven other EBV-transformed human B cell lines that produced factors with biological and biochemical characteristics of IL-1 can be effective APC. Moreover, another EBV-B cell line constitutively secreted an IL-1 activity as well as an inhibitory factor (Kuang et al., submitted for publication). Furthermore, preliminary results (G. Scala, work in progress) also indicate that when appropriately stimulated highly purified human normal B cells may secrete a soluble factor with some of the characteristics of IL-1.

In addition to the above-mentioned role in antigen presentation, IL-1 is involved in the generation of cytotoxic effector cells (30), acts synergistically with IL-2 and γ-IFN in boosting natural killer activity (31), stimulates fibroblast proliferation (26) and collagenase production (32), and induces fever (33) and the production of acute phase proteins (34). Although the B cell line IL-1–like factor needs to be tested for these other effects, it is likely that B cells may actively participate in the same mechanisms of immunity and inflammation in which IL-1 is also involved. Furthermore, IL-1 has been recently reported to enhance B cell activation synergistically with T cell–derived factor such as B cell growth factor (BCGF) (35), thus suggesting that B cell–derived IL-1 may exert a positive self-regulatory effect on B cell activation.

The other new observation was that ROHA-9 cells constitutively produced an inhibitory factor. This inhibitory factor appeared to inhibit the C3H/HeJ mouse thymocyte response to a standard preparation of IL-1, but did not inhibit the response of either mouse thymocytes or of CT6 cells to a standard preparation of IL-2. Furthermore, the inhibitory factor was selectively absorbed by mouse thymocytes but not by CT6 cells, suggesting a selective interference with the mechanism of thymocyte activation by IL-1. As expected, partially purified preparations of contra IL-1 also inhibited in a dose-dependent manner the response of PBL to the soluble antigen SLO or the response of purified T lymphocytes to alloantigens in a one-way allogeneic MLR. Furthermore, contra-IL-1 inhibited the response of PBL to suboptimal (1 μg/ml) but not optimal (10 μg/ml) doses of Con A, raising the question of whether, as suggested by recent observations (36), the activation of T lymphocytes by large amounts of Con A

might circumvent the requirement for IL-1, or whether the factor was not sufficiently potent to overcome the amount of factor(s) (namely IL-1) produced during activation of PBL with optimal doses of Con A.

It was of interest that in ROHA-9 cultures stimulated with PMA or LPS the production of the inhibitor decreased by day 3, becoming then undetectable. The data suggested that stimulation of ROHA-9 cells might lead to a selective suppression of the synthesis of the inhibitory factor, and consequently a relative increase in IL-1–like activity. These results may explain the paradoxical observation that these cells can act as APC even though they constitutively produce an inhibitory factor.

It is of note that, while positive regulation of IL-1 production by monocytes through secretion of CSF (37) or γ-IFN by T cells (38) is established, little data have been reported on negative regulation of IL-1 secretion and/or action by soluble factors. Recently, an inhibitory factor, which specifically inhibited IL-1 activity, has been identified in urine from patients with fever (39). The cellular source of such inhibitory factors, however, was unknown. The data presented in this paper suggest that the IL-1–secreting cells may also negatively regulate the action of IL-1 through secretion of inhibitory factor(s) such as contra IL-1.

Summary

In the present paper we report that the ROHA-9 cell line, an Epstein-Barr virus (EBV)-transformed human B cell line with accessory cell capabilities, constitutively secretes a soluble factor with the biochemical and biological characteristics of human monocyte-derived IL-1. The IL-1 derived from ROHA-9 augmented murine thymocyte proliferation and enhanced the proliferative response of human T lymphocytes to concanavalin A (Con A). The ROHA-9–derived IL-1 activity eluted from Sephacryl S-200 in two peaks, at 15–18K and 32–35K mol wt, eluted from DEAE-Sephacel at 50–80 and 110–130 mM NaCl, and showed charge heterogeneity with peaks at pI 7.3, 6.1, and 4.1 on isoelectrofocusing (IEF). These findings suggest that B cells may elaborate an IL-1–like activity. During the logarithmic growth of ROHA-9 cells, a inhibitory factor that inhibited the response of mouse thymocytes to IL-1 was also produced. This factor had a mol wt of 95K on Sephacryl S-200, eluted at 150 mM NaCl on DEAE-Sephacel and showed a peak of pI 4.7 on preparative IEF. The inhibitory factor appeared to be selective in its effects on IL-1 responses, since it did not inhibit the activity of IL-2 on mouse thymocytes or on the growth of the IL-2–dependent CT6 cell line. This "contra-IL-1" inhibited the response of murine thymocytes to suboptimal (1 μg/ml) but not optimal (10 μg/ml) doses of Con A and the response of human peripheral blood lymphocytes to streptolysin O (SLO) or to alloantigens. Moreover, the factor could be absorbed by mouse thymocytes but not by CT6 cells, and such thymocytes pretreated with contra-IL-1 failed to respond to IL-1. Although this inhibitor is the product of a transformed B cell line, it may be representative of regulatory substances that normally control IL-1 activities either at the extracellular or intracellular level.

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