Immunosuppressive Factors from Adult T-Cell Leukemia Cells

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ABSTRACT

The mechanism of immunodeficiency in adult T-cell leukemia (ATL) patients was studied in vitro. Peripheral blood lymphocytes from ATL patients and ATL cell lines such as Hut 102, MT 1, and MT 2 were not activated by pokeweed mitogen or by the stimulation with concanavalin A and expressed normal lymphocyte proliferative responses induced with concanavalin A when cultured together. The sera from ATL patients and the culture supernatants from ATL cells and ATL cell lines also suppressed normal lymphocyte proliferative responses induced with concanavalin A. By Sephacryl S-200 column chromatography, the suppressive factors were fractionated as a single peak with the molecular weights of 50,000 to 70,000. The suppressive factors were unstable to acid treatment but stable to the treatment with base, heat, freezing-thawing, and trypsin. The factors suppressed the production of interleukin 2 by T-cells and the responsiveness of T-cells to interleukin 2, but not the expression of interleukin 2 receptors on T-cells and the production of interleukin 1 by monocytes. These results suggest that the immunosuppressive factors produced by ATL cells have some roles in the induction of immunodeficient states in ATL patients.

INTRODUCTION

ATL is a unique T-cell malignancy which is caused by HTLV-I and has unique clinical features (1, 2). Patients with ATL often suffer from various kinds of infections, such as Pneumocystis carinii, pathogenic fungi, viruses, and parasites, and these infections often result in death (3). It is reported that in ATL patients cellular immune responses detected in vivo and in vitro were markedly impaired (4). Uchiyama et al. (5) reported that ATL cells had helper T-cell phenotypes (OKT 4 positive), but they functioned as suppressor cells which inhibited pokeweed mitogen-induced immunoglobulin synthesis of normal B-cells by the mixed culture experiment. Morimoto et al. (6) reported that ATL cells worked as suppressor-inducer cells rather than by direct action as suppressor cells. However, the precise cellular and molecular mechanism of the immunosuppression in ATL patients is not fully understood. In this paper, we studied the immunosuppressive activity of the sera from ATL patients and the culture supernatants from ATL cells and ATL cell lines on normal lymphocyte proliferative responses.

MATERIALS AND METHODS

Subjects. Eighteen patients with ATL admitted in our hospital were used for this study. The diagnosis for ATL was performed by the following criteria: neoplastic cells have a highly convoluted nucleus; and are OKT 4 positive; and the patient has anti-ATL-associated antigen antibody in the serum.

Preparation of PBL. PBL were collected from heparinized peripheral blood by a centrifugation over a lymphocyte separation medium (Litton Bionetics, Kensington, MD); washed with 10 mM PBS, pH 7.2; and suspended in a RPMI 1640 medium (Grand Island Biological Co., Grand Island, NY) containing 10% FCS (Grand Island Biological Co.), penicillin (50 units/ml; Flow Laboratories, North Ryde, New South Wales, Australia), and streptomycin (50 µg/ml; Flow Laboratories). We used this medium for in vitro culture in white experiments except as indicated.

ATL Cell Lines. ATL cell lines used in this experiment were Hut 102 established by Dr. A. F. Gazdar (National Cancer Institute-Veterans Administration) (7) and MT 1 and MT 2 established by Dr. I. Miyoshi (Kochi Medical College, Nangoku, Japan) (8). They were maintained in in vitro culture at 37°C in 5% CO2:95% air.

Assay of Lymphocyte Proliferative Response. PBL (1 x 10^6/ml) were incubated in the absence or presence of Con A (10 µg/ml; E-Y Laboratories, San Mateo, CA) in microtiter culture plates (Falcon No. 3072; Falcon Plastics, Oxnard, CA) at 37°C for 72 h in 5% CO2:95% air. The cells were pulsed for the last 24 h with 1 µCi of [3H]dThd (specific activity, 5 Ci/mM; The Radiochemical Center, Amersham, England) and harvested by the aid of an automated cell harvester (Apekagaku Co., Funabashi, Chiba, Japan). The radioactivity incorporated into the cells was counted by a liquid scintillation spectrometer (9). The results were expressed as the mean cpm of [3H]dThd incorporated into cells in triplicate cultures.

Detection of HLA-DR-positive Monocytes. PBL (1 x 10^6/ml) from a normal donor were cultured in plastic culture dishes (Falcon No. 3002) at 37°C for 2 h. Nonadherent cells were removed by vigorous washes with PBS. Adherent cells were cultured in the presence of the serum from a normal donor or an ATL patient or the culture supernatant of normal PBL, ATL, or MT 2 cells (final concentration, 10%) at 37°C for 72 h. Then, the adherent cells were scraped off with a rubber policeman. The recovered monocytes (3 x 10^5) were treated with a fluoresceinated anti-HLA-DR monoclonal antibody (leu HLADR antibody; Becton Dickinson Co., Mountain View, CA) at 4°C for 30 min. The HLA-DR-positive cells were counted with the Spectrum III flow cytometer (Ortho Diagnostics Systems, Westwood, MA) (10). Results were expressed as the means ± SE of the percentages of triplicate cultures.

Assay of IL 1. The adherent cells (1 x 10^6/ml) prepared as above without incubation were cultured with silica (100 µg/ml; Sigma Chemical Co., St. Louis, MO) in the presence of the serum or the culture supernatant from several sources (final concentration, 10%) in culture tubes (Falcon No. 2057) at 37°C for 48 h. The culture supernatant was recovered by centrifugation. The IL 1 activity was assayed with murine Thymocytes (13). Thymocytes (3 x 10^5) from C3H/He mice (Seiwa Experimental Animal Co., Yoshitomi, Oita, Japan) were cultured with serially diluted IL 1 samples or standard ultrapure human IL 1 (Genzyme Co., Boston, MA) in an EAHAA medium (12) with 10% FCS in microtiter culture plates at 37°C for 72 h. The cells were pulsed with 1 µCi of [3H]dThd for the last 24 h and harvested. The results were expressed as the means ± SE of units of IL 1 produced by monocytes in triplicate cultures per ml.

Assay of Production of IL 2. PBL (5 x 10^6/ml) from a normal donor were cultured with Con A (10 µg/ml) in the presence of the serum or the culture supernatant from several sources in culture tubes at 37°C for 6 h. After washing PBL with PBS 3 times to remove the serum or the culture supernatant, PBL were reincubated in the medium at 37°C for an additional 48 h. The culture supernatant was recovered by centrifugation. The IL 2 activity was assayed with a murine CTLL (13). CTLL (1 x 10^5/well) was cultured with serially diluted IL 2 samples or standard recombinant human IL 2 (Ajinomoto Co., Tokyo, Japan) and are expressed as the means ± SE of the percentages of triplicate cultures.

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: ATL, adult T-cell leukemia; IL 1, interleukin 1; IL 2, interleukin 2; IL 2R, interleukin 2 receptors; PBL, peripheral blood lymphocytes; PBS, phosphate-buffered saline; [3H]dThd, tritiated thymidine; HTLV-I, human T-cell leukemia lymphoma virus 1; FCS, fetal calf serum; Con A, concanavalin A; CTLL, cytotoxic T-cell line.

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Japan) in microtiter culture plates at 37°C for 48 h. The cells were pulsed with 0.25 μCi of [3H]Tdr for the last 24 h and harvested. The results were expressed as the means ± SE of units of IL 2 produced by PBL in triplicate cultures per ml.

Assay of the Expression of IL 2R. PBL (1 × 10^6/ml) from a normal donor were cultured with Con A (10 μg/ml) in the presence of the serum or the culture supernatant from several sources in culture tubes at 37°C for 48 h. The recovered cells (5 × 10^6) were incubated with a fluoresceinated anti-IL 2R monoclonal antibody (Becton Dickinson Co.) at 4°C for 30 min. The IL 2R-positive cells were counted with the Spectrum III flow cytometer (10). The results were expressed as the fluorescence profile and the mean percentage of IL 2R-positive cells in triplicate cultures.

Assay of the Responsiveness of Con A Blasts to IL 2. PBL (1 × 10^6/ml) from a normal donor were cultured with Con A (10 μg/ml) in culture tubes at 37°C for 48 h. The recovered cells were washed with α-methyl-d-mannoside (10 mg/ml; Sigma Chemical Co.) 3 times. The obtained Con A blasts (1 × 10^6/well) were cultured with recombinant IL 2 (150 units/ml) in the presence of the serum or the culture supernatant from several sources in microtiter culture plates at 37°C for 48 h. The cells were pulsed with 1 μCi of [3H]Tdr for the last 24 h and harvested. The results were expressed as the means ± SE of cpm of [3H]Tdr incorporated into Con A blasts in triplicate cultures.

Sephacryl S-200 Column Chromatography of the Suppressive Factors. ATL serum (10 ml) and the culture supernatant of MT 2 cells (50 ml) were concentrated with an ultrafiltration device using YM 5 dialysis membrane (Amicon Co., Danvers, MA), applied on a Sephacryl S-200 column (2 × 50 cm; Pharmacia Fine Chemicals, Uppsala, Sweden) equilibrated with PBS, and eluted with PBS at the rate of 10 ml/h. Aliquots of 2 ml were collected. The molecular weight was determined by chromatography of marker proteins: blue dextran (void volume); human immunoglobulin G (M, 150,000); bovine serum albumin (M, 68,000); ovalbumin (M, 44,000); soybean trypsin inhibitor (M, 22,000); and cytochrome c (M, 13,000). The activity of each fraction (final concentration, 10%) was assayed by the lymphocyte proliferative response mentioned above.

Treatment of Suppressive Factors. The active fraction of the Sephacryl S-200 column chromatography of the culture supernatant of MT 2 cells was treated with freezing-thawing 3 times or heated at 60°C for 30 min. The factors were dialyzed against 0.1 M glycine buffer, pH 2.3, or 0.1 M sodium borate buffer, pH 9.0, at room temperature for 12 h and then dialyzed against PBS. The factors were treated with trypsin (50 μg/ml; Sigma Chemical Co.) at 37°C for 30 min, and then soybean trypsin inhibitor (50 μg/ml; Sigma Chemical Co.) was added to stop the action of trypsin. The activity of the factors (final concentration, 10%) was assayed by the lymphocyte proliferative response mentioned above.

RESULTS

PBL from ATL Patients and ATL Cell Lines and Suppression of Normal Lymphocyte Proliferative Response Induced with Con A. To study the immune function of ATL patients, PBL from normal healthy donors and ATL patients were cultured with Con A in vitro. As shown in Table 1, PBL from normal donors responded to Con A by proliferating well. However, PBL from ATL patients did not respond to Con A at all. When PBL from a normal donor were mixed with PBL from an allogeneic normal donor, the responsiveness of PBL was additive. However, when PBL from a normal donor were mixed with PBL from ATL patients, the responsiveness of PBL from a normal donor was markedly suppressed. A similar result was obtained in all 6 ATL patients studied. The surface marker of PBL from 6 ATL patients was OKT 3 M+ 8~11+. These results suggest that the responsiveness of PBL from ATL patients to Con A is markedly impaired and that PBL from ATL patients have the suppressive activity on the proliferative response of normal lymphocytes. The similar suppressive activity on normal lymphocytes was also observed in ATL cell lines, such as Hut 102, MT 1, and MT 2.

Sera from ATL Patients and Culture Supernatants of ATL Cell Lines and Suppression of Normal Lymphocyte Proliferative Response Induced with Con A. Next, we studied the effect of the sera from ATL patients on lymphocyte proliferative response. As shown in Fig. 1, the sera from ATL patients markedly suppressed normal lymphocyte proliferative response induced with Con A, while the sera from normal donors had no suppressive activity. The suppressive activity was observed in all 18 ATL patients studied. Fig. 2A shows a dose-response curve of a representative ATL serum on Con A response. As can be seen, the suppressive activity of the ATL serum was observed even at the concentration of more than 10^-4. A similar result was observed in the culture supernatants of ATL PBL and ATL cell lines as shown in Table 2. The suppressive activity of the culture supernatants was observed at the concentration of more than 10^-4 (Fig. 2B). These results suggest that the sera from ATL patients and the culture supernatants of ATL PBL and ATL cell lines contain immunosuppressive factors and that these factors are produced by ATL cells themselves. In the following experiments, we studied the mechanism of immunosuppression of the sera and the culture supernatants of ATL cells.

<p>| Table 1 Effect of PBL from ATL patients and ATL cell lines on lymphocyte proliferative response |
|---------------------------------|---------------------|---------------------|</p>
<table>
<thead>
<tr>
<th>From Added with</th>
<th>Con A (−)</th>
<th>Con A (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>1</td>
<td>233 ± 69</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>187 ± 42</td>
</tr>
<tr>
<td>ATL</td>
<td>1</td>
<td>2,495 ± 357</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>1,856 ± 103</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>1,578 ± 278</td>
</tr>
<tr>
<td>Normal</td>
<td>Normal PBL</td>
<td>634 ± 82</td>
</tr>
<tr>
<td></td>
<td>ATL PBL</td>
<td>2,894 ± 463</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2,267 ± 125</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>2,203 ± 98</td>
</tr>
<tr>
<td>Hut 102 cells</td>
<td>1</td>
<td>1,034 ± 84</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>932 ± 97</td>
</tr>
<tr>
<td>MT 1 cells</td>
<td>1</td>
<td>726 ± 84</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td></td>
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</tbody>
</table>

* PBL (1 × 10^6) from normal donors or ATL patients were cultured with or without Con A (10 μg/ml) at 37°C for 72 h.
* Mean ± SE of cpm of [3H]Tdr incorporated into PBL in triplicate cultures.
* PBL (1 × 10^6) from each donor were added to PBL (1 × 10^6) from Normal Donor 1.
* ATL cell lines (1 × 10^6) which had been treated with mitomycin C (50 μg/ml; Kyowa Hakko Kogyo Co., Tokyo, Japan) at 37°C for 60 min were added.

Fig. 1. Effect of ATL serum on Con A response of normal PBL. PBL (1 × 10^6) from a normal donor were cultured with Con A (10 μg/ml) in the presence of sera from 12 normal donors (O) or 18 ATL patients (□) at the concentration of 10% at 37°C for 72 h. In □, 10% sera from ATL patients marked by suppression of the sera and the culture supernatants of ATL patients. These factors are produced by ATL cells themselves. In the following experiments, we studied the mechanism of immunosuppression of the sera and the culture supernatants of ATL cells.

Fig. 2A. Dose-response curve of a representative ATL serum on Con A response. As can be seen, the suppressive activity of the ATL serum was observed even at the concentration of more than 10^-4. A similar result was observed in the culture supernatants of ATL PBL and ATL cell lines as shown in Table 2. The suppressive activity of the culture supernatants was observed at the concentration of more than 10^-4 (Fig. 2B). These results suggest that the sera from ATL patients and the culture supernatants of ATL PBL and ATL cell lines contain immunosuppressive factors and that these factors are produced by ATL cells themselves. In the following experiments, we studied the mechanism of immunosuppression of the sera and the culture supernatants of ATL cells.
IMMUNOSUPPRESSIVE FACTORS FROM ATL CELLS

Effect of ATL Sera and Culture Supernatants on the Expression of HLA-DR and the Production of IL 1 by Normal Monocytes. The induction of the proliferative response of T-cells with Con A requires the accessory function of monocytes. The accessory function is given by HLA-DR antigens on monocytes and IL 1 produced by monocytes (14). Therefore, we studied the effect of ATL sera and culture supernatants on monocyte functions. When normal monocytes were cultured with ATL serum or the culture supernatant of MT 2 cells in vitro for 3 days, the percentage of HLA-DR-positive cells in the monocyte population was not changed as compared with normal serum or the culture supernatant of normal PBL as shown in Table 3. The production of IL 1 by monocytes was also not affected by the presence of ATL serum or the culture supernatant of MT 2 cells. These results suggest that the factors from ATL cells give no effect on monocyte functions.

Effect of ATL Sera and Culture Supernatants on the Production of IL 2, the Expression of IL 2R, and the Responsiveness to IL 2 of Normal PBL. The next step of T-cell activation with Con A is the production of IL 2 and the expression of IL 2R. Then, we studied the effect of ATL sera and culture supernatants on IL 2 production by normal PBL. As shown in Fig. 3, when normal PBL were cultured with Con A in the presence of ATL serum or the culture supernatants of MT 2 cells or of PBL from an ATL patient, the production of IL 2 by normal PBL was suppressed in a dose-dependent manner. The dose-response curve is almost the same as that of the proliferative response (Fig. 2).

Next, we studied the effect of ATL serum and the culture supernatant of MT 2 cells on the expression of IL 2R on normal PBL stimulated with Con A. As shown in Fig. 4, the expression of IL 2R on normal PBL was not changed by the culture with ATL serum or MT 2 culture supernatant.

We further studied the effect of ATL sera and culture supernatants on the responsiveness of Con A blasts to IL 2. The Con A blasts were prepared by the culture of normal PBL with Con A for 48 h and recultured with recombinant human IL 2 in the presence of ATL sera or culture supernatants. As shown in Fig. 5, the responsiveness of Con A blasts to IL 2 was suppressed by ATL serum and culture supernatants in a dose-dependent manner. These results suggest that the suppression of the Con A response of normal PBL by ATL sera and culture supernatants is induced by the suppression of IL 2 production and the responsiveness to IL 2.

Physicochemical Properties of Immunosuppressive Factors. In the previous section, we presented the evidence that ATL PBL and ATL cell lines produced immunosuppressive factors which suppressed proliferative response of normal lymphocytes stimulated with mitogens. Then, we finally studied the physicochemical properties of the immunosuppressive factors. At first, we fractionated the ATL serum and MT 2 culture supernatant using a Sephacryl S-200 column. As shown in Fig. 6A, the immunosuppressive activity of the ATL serum was fractionated as a single peak with the molecular weights of 50,000 to 70,000. The immunosuppressive activity of MT 2 culture supernatant was also fractionated as the same profile as shown in Fig. 6B.
IMMUNOSUPPRESSIVE FACTORS FROM ATL CELLS

Fig. 4. Effect of ATL serum or MT 2 culture supernatant on the expression of IL 2 receptors on normal lymphocytes. PBL (1 x 10^6/ml) from a normal donor were cultured with Con A (10 µg/ml) in the presence of 10% normal serum (A), ATL serum (B), normal PBL culture supernatant (C), or MT 2 culture supernatant (D) at 37°C for 48 h. The recovered cells were treated with a fluoresceinated anti-IL 2R antibody, and IL 2R-positive cells were counted with the Spectrum III flow cytometer. The results are expressed as a fluorescence profile and the mean percentage of IL 2R-positive cells.

Next, we studied the effect of various treatments on the immunosuppressive activities. The active fraction of the Sephacryl S-200 column chromatography of the culture supernatant of MT 2 cells (Fig. 5B) was treated as indicated. PBL (1 x 10^6) from a normal donor were cultured with Con A (10 µg/ml) in the presence or absence of the suppressive factors at 37°C for 72 h.

Table 4 Effect of various treatments on suppressive activity of the culture supernatant of MT 2 cells

<table>
<thead>
<tr>
<th>Treatment of factors</th>
<th>[³H]TdR incorporation (cpm)</th>
<th>% of suppression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ Freezing-thawing (3 times)</td>
<td>1,251 ± 375</td>
<td>95.5</td>
</tr>
<tr>
<td>Heat (60°C, 30 min)</td>
<td>3,475 ± 242</td>
<td>87.4</td>
</tr>
<tr>
<td>Base (pH 9.0, 12 h)</td>
<td>222 ± 20</td>
<td>98.3</td>
</tr>
<tr>
<td>Acid (pH 2.3, 12 h)</td>
<td>20,350 ± 561</td>
<td>25.9</td>
</tr>
<tr>
<td>Trypsin (50 µg/ml, 37°C, 30 min)</td>
<td>1,387 ± 63</td>
<td>95.0</td>
</tr>
</tbody>
</table>

* Mean ± SE of cpm of [³H]TdR incorporation into PBL in triplicate cultures.

DISCUSSION

In ATL patients, opportunistic infections, such as P. carinii, pathogenic fungi, viruses, or parasites, are more commonly observed than in other leukemias (3). Furthermore, almost all ATL patients have negative skin reactions against purified protein derivative of tuberculin and no lymphocyte proliferative responses stimulated with mitogens in vitro (4). This evidence suggests that immune functions are markedly impaired in ATL patients. However, the precise cellular and molecular mechanisms of the immunosuppression are not fully understood. In this paper, we studied immunosuppressive factors from ATL patients and ATL cell lines. One-hundred % of sera from ATL patients (18/18) were suppressive for normal lymphocyte proliferative responses induced with mitogens (Fig. 1). The immunosuppressive activity of the ATL sera is very strong. They showed the immunosuppressive activity even at the concentration of more than 10^-4, and they suppressed up to about 95% of Con A response at the concentration of 10% (Fig. 2). The culture supernatants of ATL cell lines also have suppressive activity on Con A responses (Table 2; Fig. 2). The physicochemical properties of the culture supernatants of ATL cell lines are the same as those of the ATL sera (Table 4; Fig. 6).

Fig. 5. Effect of ATL serum (A) and culture supernatants of ATL PBL and MT 2 cells (B) on the responsiveness of normal lymphocytes. The Con A blasts (1 x 10^4) obtained by the culture of normal PBL with Con A at 37°C for 48 h were cultured with recombinant IL 2 (ISO units/ml) in the presence of serially-diluted serum or culture supernatant at 37°C for 48 h. Points, mean cpm of tritiated thymidine ([³H]dThd) incorporated into Con A blasts in triplicate cultures; bars, SE. O, normal serum; •, ATL serum; A, normal PBL culture supernatant; •, ATL culture supernatant; A, MT 2 culture supernatant.

Fig. 6. Sephacryl S-200 column chromatography of the immunosuppressive activity of ATL serum (A) and MT 2 culture supernatant (B). ATL serum or MT culture supernatant was concentrated and fractionated by a Sephacryl S-200 column. The molecular weights of each fraction were determined by eluting marker proteins mentioned in "Materials and Methods." The immunosuppressive activity in each fraction was assayed by the lymphocyte proliferative response. Points, mean cpm of tritiated thymidine ([³H]dThd) incorporated into PBL in triplicate cultures.
results suggest that ATL cells themselves produce immunosuppressive factors. There are several reports that sera of cancer patients have immunosuppressive activity. Some of them are immunosuppressive acid protein, prostaglandins, and α-fetoproteins (15–17). However, the immunosuppressive factors derived from ATL cells seem to be different from those agents for the following reasons. Immunosuppressive acid protein is produced by host macrophages or polymorphonuclear cells in the tumor bearer (18), while the ATL factors are produced by ATL cells themselves. α-Fetoprotein is produced by fetal liver cells and hepatoma cells, but not by leukemia cells, and the level of α-fetoprotein in ATL patients is not increased (data not shown). The ATL factors are different from prostaglandins in their molecular weights. Furthermore, the suppressive activity of ATL sera and ATL culture supernatants seems not to be derived from HTLV-I viruses or virus fragments themselves, because the suppressive activity was not removed by an ultracentrifugation (40,000 × g, 120 min) or an absorption of the ATL sera or the culture supernatants with anti-ATL-associated antigen antibodies, anti-HTLV-I envelope or gag protein antibodies, and Staphylococcus aureus Cowan I (data not shown). The most intriguing explanation of the suppressive factors derived from ATL cells is free IL 2R secreted by ATL cells, because ATL cells express many IL 2R on their surface, and IL 2R absorb IL 2 required for the activation of T-cells (19, 20). However, this possibility also seems to be unlikely, because the suppressive activity of the ATL sera and culture supernatants is not absorbed with anti-IL 2R antibody and S. aureus Cowan I (data not shown). The precise character of the suppressive factors from ATL cells is under investigation in relation with oncogene products.

The mechanism of immunosuppression with the ATL sera and the ATL culture supernatants was also studied using lymphocyte proliferative responses induced with Con A as a model system. The suppressive activity of the ATL-derived factors is not the cytotoxic effect on lymphocytes, because the recovered cell number after 3 days of culture is not different between the control group and the suppressed group (data not shown). The induction of proliferative responses of T-cells requires the interaction of T-cells and monocytes and several interleukins, such as IL 1 and IL 2 (14, 21). The ATL sera and the ATL culture supernatants did not suppress the expression of HLA-DR antigens on monocytes and the production of IL 1 by monocytes (Table 3). The ATL sera and the ATL culture supernatants suppressed the production of IL 2 and the responsiveness of T-cells to IL 2, but not the expression of IL 2R on T-cells (Figs. 3 to 5). The failure of the suppression of IL 2R expression on T-cells by ATL sera or culture supernatants from ATL cells or ATL cell lines is not due to the enhanced expression of IL 2R on T-cells by HTLV-I virus in the sera or the culture supernatants, because the sera or the culture supernatants alone without Con A did not induce IL 2R on T-cells in 2-day incubation (data not shown). These results suggest that the mechanism of immunosuppression induced with the ATL sera and ATL culture supernatants is on the production of IL 2 by T-cells and the responsiveness of T-cells to IL 2. The ATL sera and the culture supernatants suppressed not only nonspecific mitogenic responses of T-cells but also specific killer T-cell induction and immunoglobulin synthesis by B-cells. It is reported that both cellular and humoral immune responses are markedly impaired in ATL patients. From our results, it can be speculated that one of the mechanisms of immunodeficiency in ATL patients is caused by immunosuppressive factors produced by ATL cells. However, to reach this conclusion, further physicochemical characterization of the suppressive factors present in ATL serum and produced by ATL cell lines and further analysis of biological, especially in vivo, functions of these factors are required. Although some questions remain unresolved, the evidence reported here will give us a new idea to study the pathogenesis of ATL and to understand the immune states in ATL patients.

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