CHARACTERIZATION OF A SOLUBLE SUPPRESSOR OF HUMAN B CELL IMMUNOGLOBULIN BIOSYNTHESIS PRODUCED BY A CONTINUOUS HUMAN SUPPRESSOR T CELL LINE

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A variety of soluble mediators produced by lymphocytes (lymphokines) have been reported to play an important role in regulation of the immune response (1–3). Included among the humoral factors that suppress immunologic reactions are: migration inhibitory factor (MIF; 4–6), lymphotoxin (7, 8), interferon (9, 10), histamine-induced suppressor factor (11), and soluble immune response suppressor (SIRS; 12, 13). We have recently studied the immunoregulatory function of supernates generated from concanavalin A (Con A)-activated human peripheral blood mononuclear cells (PBMC) and demonstrated the elaboration of at least two distinct soluble suppressors (14, 15). One factor, soluble immune suppressor of T cell proliferation (SISS-T), inhibits T cell proliferative responses to mitogens and antigens, but does not interfere with a B cell activity. This mediator has a molecular weight of 30,000–45,000, has saccharide binding specificity for N-acetyl-D-glucosamine, and requires monocytes for its generation. A second soluble inhibitor found in these supernates, soluble immune suppressor of immunoglobulin production (SISS-B), inhibits polyclonal B cell immunoglobulin biosynthesis, but not T cell activation. This lymphokine has a molecular weight of 60,000–90,000, has saccharide-binding specificity for L-rhamnose, and is produced by T cells. Because the amounts of these mediators generated in the Con A culture supernates are limited, we investigated the possibility that selected cultured T cell (CTC) lines may generate these or other important mediators in greater amounts. We have identified an interleukin 2-dependent suppressor CTC line that appears to produce large quantities of SISS-B. Pending final confirmation of identity between SISS-B and the soluble suppressor generated by the CTC line, we have designated the CTC line-derived suppressor factor CTC-SISS-B. These data provide evidence that T cell modulation of B cell-immune response may in part be dependent on the

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1 Abbreviations used in this paper: Con A, concanavalin A; CTC, cultured T cell; EBV, Epstein-Barr virus; E:T, effector:target ratio; FCS, fetal calf serum; MIF, migration inhibitory factor; NAG, N-acetyl-D-glucosamine; NWSM, Nocardia water-soluble mitogen; PBMC, peripheral blood mononuclear cells; PHA, purified phytohemagglutinin; PWM, pokeweed mitogen; RIA, radioimmunoassay; SIRS, soluble immune response suppressor (murine); SISS, soluble immune suppressor supernate; SISS-B, SISS of B cell immunoglobulin production (human); SISS-T, SISS of T cell proliferation (human); SRC, sheep erythrocytes.
elaboration of an endogenous lectinlike suppressor factor that interacts with specific glycoprotein or glycolipid receptors.

Materials and Methods

Pokeweed mitogen (PWM; Grand Island Biological Co., Grand Island, N. Y.), purified phytohemagglutinin (PHA; Difco Laboratories, Detroit, Mich.), and Con A (Sigma Chemical Co., St. Louis, Mo.) were prepared as stock solutions in diluent consisting of RPMI-1640 (Grand Island Biological Co.) supplemented with penicillin (50 U/ml), streptomycin (50 µg/ml), and l-glutamine (2.0 mM), and stored at -20°C until used. The B95-8 strain of Epstein-Barr virus (EBV) and Nocardia water-soluble mitogen (NWSM) were prepared and used in selected cultures as previously described (16, 17). Tetanus toxoid (Massachusetts Department of Public Health) and candida antigen (Hollister-Stier, Spokane, Wash.) were extensively dialyzed against diluent before use and stored as stock solutions at 4°C. L-rhamnose, N-acetyl-α-glucosamine (NAG), L-fucose, and D-galactose (all from Sigma Chemical Co.) were prepared as 250-mM stock solutions in diluent and stored at 4°C until used.

Purification of Human PMBC

PBMC were isolated from heparinized venous blood of healthy adult volunteer donors using a modification of the method of Böyum (18) as previously described (14). PBMC to be used in the assay of in vitro Ig biosynthesis were washed four times over heat-inactivated (56°C for 30 min) fetal calf serum (FCS; Reheis, Phoenix, Ariz.) to remove detectable human serum proteins. The PBMC were suspended in diluent supplemented with 10% (vol:vol) heat-inactivated FCS from selected lots previously screened for support of in vitro Ig production. In selected experiments, PBMC were further fractionated to prepare highly purified B cells and T cells as previously described (19). Briefly, B cells were isolated by elution of cells retained on immunoabsorbent columns composed of rabbit anti-human F(ab')2 Ig insolubilized with cyanogen bromide to Sephadex G-200 beads (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) and further purified using an erythrocyte (E)-rosette depletion step. T cells were isolated by collection of the cells not retained by the immunoabsorbent column, followed by a single E-rosette procedure. Using these techniques, the B cells routinely fail to produce measurable Ig in vitro after PWM stimulation (unless T cells are added back).

Culture of T Cells with Conditioned Medium

T cells derived from the peripheral blood of a patient with a nonleukemic form of mycosis fungoides were cultured in medium containing 20% FCS (vol:vol). To promote continuous growth of T cells, the culture medium was supplemented with 20% (vol:vol) concentrated conditioned medium (produced by coculturing mononuclear cells from 10 donors with PHA; Associated Biomedic Systems, Inc., Buffalo, N. Y.) to provide a source of interleukin 2 as previously described (20, 21). When the cell concentration reached ~1 × 10^6 cells/ml, the cells were diluted to 2.0 × 10^6–3.0 × 10^6 cells/ml using fresh conditioned medium as above. More than 90% of the cultured cells formed spontaneous E-rosettes on periodic testing and cell viability was routinely in excess of 90% as measured by supravital dye exclusion (22).

Generation of CTC-SISS-B

Suppressive supernates were generated by washing the CTC cells (obtained at the time of feeding) five times to remove interleukin 2, PHA, and other agents, and then culturing these washed cells (1 × 10^6) in the absence of conditioned medium for 8–24 h in a humidified atmosphere containing 5% CO₂. At the conclusion of the culture period, the supernates were recovered and contaminating cells were removed by filtration through 0.45-µm membranes (Millipore Corp., Bedford, Mass.) before storage at -20 and -80°C until used. The viability of the T cells elaborating the supernates was the same as that found at the time of feeding (generally >90%).

Reverse Hemolytic Plaque Assay

The technique of coupling protein A (Staphylococcus aureus; Pharmacia Fine Chemicals) to sheep erythrocytes (SRC), the preparation and the characterization of the rabbit antisera to human Ig (IgG, IgA, and IgM), and the method for plaquing are as previously described (23, 24). Briefly, 2 million PBMC suspended in 1 ml of medium were cultured in 5-ml tubes (2058; Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) in the presence and absence of PWM for 6–7 d under the identical culture conditions as above. At the end of the culture period, the cells were washed four times and suspended in 1 ml of balanced salt solution. Duplicate 0.1-ml aliquots of the cell suspension were added to 0.25 ml
of 1.12% Seaplaque agarose (Marine Colloids Inc., Springfield, N. J.), followed by the addition of 0.025 ml of 30% protein A-SRC. The contents of the tubes were then swirled over Petri dishes previously coated with 4 ml of 2.5% agarose. The agarose-cell mixture was allowed to solidify, followed by the addition of 1 ml of appropriately diluted rabbit anti-human Ig, and incubation for 1 h at 37°C in a humidified CO₂ incubator. Plaque formation was initiated by the addition of 1 ml of a 1:8 dilution of SRC-absorbed guinea pig complement (Flow Laboratories Inc., Rockville, Md.) and an additional 2-h incubation at 37°C. After this, the plates were aspirated and held overnight at 4°C. The number of plaques present in the duplicate dishes were enumerated using an automated video plaque counter (Optimax Inc., Hollis, N. H.). The data obtained are expressed as the number of Ig-secreting cells per culture.

**Assay of Ig Biosynthesis.** The techniques of the culture system for in vitro Ig biosynthesis and the methods for the radioimmunoassay (RIA) determinations of IgG, IgA, and IgM have been previously described (19, 25). Briefly, 2 million PBMC or 5 × 10⁶ highly purified B cells (in the absence and presence of autologous T cells) were cultured in loosely capped 1-dram vials with a polyclonal activator for 12 d in 1 ml of culture medium under identical culture conditions as above. The polyclonal activators used included PWM, EBV, and NWSM. At the termination of the culture period, the supernates were collected and the amounts of IgG, IgA, and IgM synthesized and secreted into the medium were determined by heavy chain-specific, double-antibody RIA (25, 26).

**Lymphocyte Proliferation.** 200,000 lymphocytes suspended in 0.2 ml of medium were cultured in microtiter plates (Falcon Labware) in the presence and absence of the mitogenic lectins PHA (1:2,000 final dilution), Con A (20 μg/ml), or PWM (10 μg/ml) for 3 d, or the recall antigens tetanus toxoid (2.5 Lf/ml) or candida (0.25%) for 6 d at 37°C in a humidified CO₂ incubator. 5 h before the conclusion of culture, 1.0 μCi of [³H]methyl-thymidine (New England Nuclear, Boston, Mass.; 25 Ci/ml sp ac) was added to each microtiter well. Cells were subsequently harvested onto fiberglass filters using a multiple-channel automated cell harvester and washed repeatedly with distilled water. Cell-associated radioactivity was determined by transfer of the filters to the glass vials containing 7.5 ml of scintillant (Ultrafluor, National Diagnostics, Somerville, N. J.) and counting in an automated counter (Beckman Instruments Inc.).

**Allosensitization of PBMC.** Allosensitization of PBMC was performed as described previously (27). Briefly, fresh PBMC and irradiated (2,000 rad) PBMC were suspended at 1 × 10⁶ cells/ml in medium supplemented with 5% human plasma, 4 ml of each population were added to flasks (3013; Falcon Labware), and cultured under identical conditions as described above. Additional cultures of nonirradiated stimulator cells to be used as targets were established at this time, and PHA (1:200) was added to these cultures 72 h before the cytotoxicity assay. On the seventh day, the cells were harvested for the cytotoxicity assay. In addition, triplicate 0.2-ml aliquots of cells were pulsed with 1.0 μCi [³H]methyl-thymidine in microtiter plates (Falcon Labware) and evaluated as above to quantitate allogeneic cell-induced proliferation (mixed lymphocyte reaction [MLR]).

**Cytotoxicity Assay.** On the day of assay, the target cells were suspended in 0.6 ml in culture medium containing 200 μCi of ⁵¹Cr sodium chromate (300 mCi/mg; Amersham Corp., Arlington Heights, Ill.) for 90 min at 37°C with frequent shaking. The labeled cells were then washed and suspended at 1 × 10⁶ cells/ml. Triplicate cultures of putative effector cells and target cells were established by adding 0.1 ml of each cell suspension to the wells of round-bottomed microtiter plates (Linbro Chemical Co., Hamden, Conn.). Control measurements consisted of target cells incubated with medium alone or with 5% Triton X (Research Products International Corp., Elk Grove Village, Ill.). The plates were centrifuged at 50 g for 2 min, incubated at 37°C for 6 h, and recentrifuged at 400 g for 5 min, and the supernates were harvested and counted in a gamma counter (Beckman Instruments Inc.). Percent specific lysis was calculated by the following formula:

\[
\text{Percent specific lysis} = 100 \times \frac{(\text{cpm experimental} - \text{cpm media control})}{(\text{cpm detergent} - \text{cpm media control})}.
\]

Chromium release from the media control was always <20% of the detergent control. Effector cell activity was tested at four different effector:target (E:T) ratios ranging from 40:1 to 0.6:1.
Results

Characteristics of the Human CTC Line. The CTC line that produces the mediator CTC-SISS-B requires the presence of conditioned medium containing interleukin 2 for maintenance. Furthermore, in previous studies we have shown that this CTC line functions as a potent suppressor cell for PWM-induced in vitro immunoglobulin production (21). Evaluation of the phenotype of this T cell line with the fluorescence-activated cell sorter II system (Becton, Dickinson & Co.) demonstrated that the cells were reactive with monoclonal antibodies to OKT3 (Ortho Pharmaceutical Corp., Raritan, N. J.), Tac (an antigen on activated T cells; 28), and Ia-like (DA-2; 29) antigens. The phenotypic characteristics and the cell-mediated suppressor activity of this CTC have remained constant throughout the investigations described below.

CTC-SISS-B Production by the CTC Line. The addition of supernates containing CTC-SISS-B to cultures of human PBMC stimulated with PWM resulted in a dose-related suppression of polyclonal Ig production measured by the reverse hemolytic plaque assay as shown in Fig. 1. There is no evidence of class specificity in the inhibitory activity of CTC-SISS-B as demonstrated by the equivalent suppression of IgG, IgA, and IgM production observed when the supernate was added to PWM-stimulated cultures at final concentrations of 1:10 (Fig. 2), 1:50, and 1:250 (data not shown). Furthermore, the addition of CTC-SISS-B at a final concentration of 1:10 to cultures of human PBMC stimulated with the T helper cell-independent polyclonal activator EBV resulted in >80% inhibition of IgG, IgA, and IgM biosynthesis (data not shown). Thus, the suppression by this mediator is neither isotype specific nor dependent on the presence of PWM in the culture system.

Specificity of CTC-SISS-B Suppression. The inhibition mediated by CTC-SISS-B appeared to be specific for Ig production. The addition of this mediator to T cell proliferative assays stimulated either with mitogens, antigens, or allogeneic cells had no suppressive effect on the incorporation of tritiated thymidine by these T cells.

![Graph showing CTC-SISS-B dose-response relationship for suppression of PWM-induced Ig production. Inhibition by varying dilutions of CTC-SISS-B is expressed as the mean percent suppression of the expected number of Ig-secreting cells per culture. Error bars represent the SEM.](image)
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Fig. 2. CTC-SISS-B suppresses equivalently the production of IgG, IgA, and IgM after PWM stimulation. Inhibition of the total amount of IgG, IgA, and IgM secreted into the culture medium by CTC-SISS-B (1:10) is expressed as the mean percent suppression for each specific class of Ig produced in a 12-d culture stimulated with PWM. Error bars represent the SEM.

Table I

| Activator          | [3H]methyl-thymidine incorporation (cpm ± SEM) | Medium | CTC-SI SSI S-B (1:10) |
|--------------------|-----------------------------------------------|--------|----------------------|
| RPMI-1640          | 513 ± 44                                       | 754 ± 66 |
| PHA                | 27,639 ± 288                                   | 43,933 ± 5,542 |
| Con A              | 79,304 ± 6,432                                 | 82,706 ± 2,784 |
| PWM                | 62,486 ± 2,981                                 | 62,694 ± 3,763 |
| Candida            | 33,289 ± 1,480                                 | 34,278 ± 4,571 |
| Tetanus            | 34,995 ± 2,994                                 | 37,213 ± 1,546 |
| Allogeneic cells (MLR) | 34,995 ± 1,383                          | 47,009 ± 3,314 |

(Table I). Furthermore, no inhibition of 51Cr release was observed by the addition of CTC-SISS-B during either the sensitization or effector phase in assays of T cell-mediated allogeneic lympholysis (Table II). These experiments were performed with the supernate at final dilutions of 1:4 and 1:10 in these assays, concentrations that consistently produced >80% suppression of Ig biosynthesis. Direct addition of the supernates to either PBMC or purified T cells at final dilutions of 1:4 and 1:10 did not result in tritiated thymidine incorporation above background; thus there appears to be little possibility that PHA carryover from the conditioned medium has any role in the observed suppression of Ig production by this mediator.

Noncytotoxic Mechanism of CTC-SISS-B Suppression. The effect of delayed addition of CTC-SISS-B on PWM-induced Ig production is shown in Fig. 3. Addition of the supernates after the initial 24–48 h of the 7-d culture period had little or no suppressive effect on the subsequent Ig biosynthesis (five of six experiments). Furthermore, as shown in Fig. 4, CTC-SISS-B addition did not affect the number of Ig-secreting cells that appear early during the PWM-stimulated culture (3–4 d), but produced marked suppression of Ig production during the period of maximal response (5–7 d). These data provide evidence that CTC-SISS-B acts through interference with differentiation
### Table II

**Effect of CTC-SISS-B Addition on T Cell Lympholysis**

| Experiment 1 | E:T | Percent $^{51}$Cr release (mean ± SEM) |
|--------------|-----|---------------------------------------|
|              |     | Medium                                       |
| 40:1         |     | 28.2 ± 3.4                                 |
| 10:1         |     | 19.3 ± 3.9                                 |
| 2.5:1        |     | 9.6 ± 2.2                                  |
| 0.6:1        |     | 6.0 ± 1.9                                  |
|              |     | CTC-SISS-B (1:10)                           |
| 40:1         |     | 32.9 ± 2.7                                 |
| 10:1         |     | 25.0 ± 2.3                                 |
| 2.5:1        |     | 15.7 ± 1.9                                 |
| 0.6:1        |     | 9.9 ± 2.1                                  |

| Experiment 2 | E:T | Percent $^{51}$Cr release (mean ± SEM) |
|--------------|-----|---------------------------------------|
|              |     | Medium                                       |
| 10:1         |     | 44.8 ± 4.8                                 |
|              |     | CTC-SISS-B (1:10)                           |
| 10:1         |     | 42.9 ± 5.2                                 |

In experiment 1, CTC-SISS-B was added during the allosensitization (generation) phase. In experiment 2, CTC-SISS-B was added during the cytotoxic effector phase.

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**Fig. 3.** Late addition of CTC-SISS-B. A representative experiment of the inhibition of Ig production observed after addition of CTC-SISS-B (1:10) at various times during the PWM-stimulated culture is shown.

**Fig. 4.** Time-course of PWM-induced Ig production. A representative experiment comparing the time course of PWM-induced Ig production in the presence and absence of CTC-SISS-B (1:10) is shown.
of the B lymphocytes into Ig-secreting cells rather than via a mechanism of direct cytotoxicity. Furthermore, the absence of augmented 51Cr release from labeled lymphocytes in the presence of this mediator is additional evidence against a cytotoxic mechanism of action for CTC-SISS-B.

Target of CTC-SISS-B Suppression. The cellular site of CTC-SISS-B activity was evaluated with experiments in which the supernate was added to T-depleted PBMC (B cells) stimulated by the T helper cell-independent activator, EBV. As seen in Table III, there was equivalent suppression of Ig production whether T cells were absent or present with the B cells and monocytes in these cultures. Similar results were obtained using the activator NWSM as the T-independent polyclonal activator (data not shown). Thus, CTC-SISS-B does not appear to act via a regulatory T cell, but rather suppresses Ig biosynthesis either by direct action on the B cell or, alternatively, through an action on monocytes as has been described for SIRS (13).

Sugar Specificity of CTC-SISS-B. As seen in Fig. 5, the inhibitory effects of CTC-SISS-B were largely abrogated in the presence of the simple sugar L-rhamnose (25 mM). In contrast, the addition of 25 mM NAG had no effect on CTC-SISS-B-mediated suppression, whereas the addition of equimolar concentrations of L-fucose

| ACTIVATOR: PWM | INHIBITOR: CTC-SISS-B | SUGAR: L-RHAMNOSE |
|----------------|-----------------------|-------------------|
| PWM            | CTC-SISS-B            | -                 |
| CTC-SISS-B     |                       | L-RHAMNOSE       |

Fig. 5. Specific saccharide blockade of CTC-SISS-B suppression of PWM-induced Ig production. Mean number of Ig-secreting cells after PWM stimulation in the presence of CTC-SISS-B (1:250) with specific saccharides (25 mM) added at the initiation of the culture. Error bars represent the SEM.

| ACTIVATOR: PWM | INHIBITOR: CTC-SISS-B | SUGAR: L-RHAMNOSE |
|----------------|-----------------------|-------------------|
| PWM            | CTC-SISS-B            | -                 |
| CTC-SISS-B     |                       | L-RHAMNOSE       |

Table III

Effect of CTC-SISS-B (1:10 Final Concentration) Addition on EBV-induced Ig Biosynthesis

| Condition                  | IgG (ng/ml) | IgA (ng/ml) | IgM (ng/ml) |
|----------------------------|-------------|-------------|-------------|
| B cells                    | <6          | <6          | <17         |
| B cells + PWM              | <6          | <6          | <17         |
| B cells + EBV              | 69          | 633         | 1,288       |
| B cells + EBV + CTC-SISS-B | <6          | 62          | 115         |
| B + T cells                | <6          | <6          | <17         |
| B + T cells + EBV          | 113         | 734         | 1,393       |
| B + T cells + EBV + CTC-SISS-B | 28      | 124         | 176         |
and d-galactose resulted in a modest decrease in the observed inhibition of Ig production (40% suppression in cultures with these monosaccharides vs. 60% inhibition without). In all these experiments, CTC-SISS-B was used at a final concentration of 1:250 because this was judged to be the most appropriate area on the dilution curve (Fig. 1) to evaluate saccharide blockade of the action of this suppressor. Sugar concentrations used in these experiments did not result in significant suppression of PWM- or EBV-induced Ig production. Preliminary studies suggest that CTC-SISS-B is also retained on columns composed of rhamnose immobilized on agarose bead supports.

Kinetics and Radiosensitivity of CTC-SISS-B Elaboration. CTC-SISS-B is present in the cell-free supernate within 8 h of culture of the washed CTC (in the absence of interleukin 2-containing medium). The elaboration of CTC-SISS-B is partially serum dependent in that generation of the supernate in the absence of FCS results in less suppressor activity (Table IV, part 1). Furthermore, the cells producing this mediator are relatively radioresistant in that 500 or 2,000 rad irradiation after the washing procedure did not interfere with the elaboration of CTC-SISS-B (Table IV, part 2).

Physico-Chemical Characteristics of CTC-SISS-B. A Sephadex G-100 molecular sieve chromatographic profile of CTC-SISS-B is shown in Fig. 6. Inhibitory activity is clearly present as a peak in the molecular weight range of 60,000-90,000. This

| Table IV |
|---------------------------------|-----------------|
| CTC-SISS-B Generation Is Enhanced by the Presence of Serum in the Culture and Is Resistant to At Least 2,000 Rad of Irradiation | Mean percent suppression Ig production ± SEM |
| 1 | CTC-SISS-B (1:4): 10% FCS | 85.3 ± 3.7 |
| 2 | CTC-SISS-B (1:4): no serum | 38.3 ± 19.3 |
| 3 | CTC-SISS-B (1:10): no irradiation | 83.6 ± 3.2 |
| 4 | CTC-SISS-B (1:10): 500 rad | 59.5 ± 31 |
| 5 | CTC-SISS-B (1:10): 2,000 rad | 60.0 ± 28 |

Fig. 6. Molecular sieve chromatography of CTC-SISS-B. CTC-SISS-B was fractionated on a Sephadex G-100 column prepared in phosphate buffer (pH 7.4). Fractions eluted from the chromatographic gel were tested for suppressor activity (○) in the PWM-stimulated culture with the results expressed as the percent suppression of the expected number of Ig-secreting cells per culture. Protein concentration is indicated by the percent absorbance at 280 nm (○) for the applied supernate and the elution profile for the calibration proteins used are indicated by the arrows.
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corresponds to the molecular weight range observed in similar studies of suppressor supernates from Con A-activated human PBMC (15). Furthermore, CTC-SISS-B is acid stable but partially heat labile at 56°C (data not shown), which also correlates with the physico-chemical properties of Con A-generated SISS-B (15).

Discussion

In this paper we present data characterizing a lectin-like soluble suppressor of Ig production that is generated by a cultured human T suppressor line. This mediator appears to be identical to SISS-B produced by Con A-activated peripheral blood T cells. The inhibitory mechanism of this mediator does not involve cell cytotoxicity, as indicated by the lack of inhibition after late addition of the supernate, the failure of CTC-SISS-B to alter early Ig biosynthesis after PWM stimulation (at 3 and 4 d of culture) and the lack of effect on \(^{51}\)Cr release. Furthermore, the suppression observed is not due to PHA carry-over from the conditioned medium as evidenced by: (a) the failure of PBMC or purified T cells to proliferate in response to the supernate, (b) the observed inhibition of Ig production at high dilutions (1:1,250) of the supernate, (c) the demonstration that the suppressor activity has a molecular weight of 60,000–90,000, and (d) the blockade of mediator-induced suppression by L-rhamnose.

The lectin-like characteristics of CTC-SISS-B are demonstrated by the reversal of suppression with addition of the simple sugar L-rhamnose. This reversal appears to be relatively specific in that other saccharides, including NAG, L-fucose, and D-galactose, had little or no effect on the suppression of Ig production. Because L-rhamnose is not found in mammalian cells, it is likely that this saccharide closely resembles a saccharide constituent of the glycoprotein or glycolipid surface receptor that CTC-SISS-B interacts with. We are currently screening other oligosaccharides to further define this interaction. CTC-SISS-B suppresses Ig production in cultures of T-depleted PBMC stimulated with the T-independent activator EBV, indicating that the mediator SISS-B acts either directly on the B cell or via action on the monocyte. Irradiation of the CTC with 500 or 2,000 rad does not interfere with the elaboration of CTC-SISS-B. Furthermore, elaboration of the supernate occurs within 8–16 h after the initiation of the culture, and generation of CTC-SISS-B is partially serum dependent in that more suppressor activity is found in supernates produced in the presence of serum. The molecular weight, saccharide specificity, site and mode of action, and generation characteristics of CTC-SISS-B are the same as those we have previously described for SISS-B generated from Con A-activated PBMC (15). Thus, it appears likely that these two supernates contain the same suppressor factor. Based on the dilution curves, it appears that the CTC line is enriched for the cells producing this factor in that there is ~50–100-fold more suppressor activity in the CTC (1 × 10^6 cells/ml) supernate than in the Con A-activated PBMC (2.5 × 10^6 cells/ml) supernate.

CTC-SISS-B has certain characteristics that distinguish it from other humoral suppressors found in the supernates of lymphoid cell lines. For example, although MIF is blocked by L-rhamnose (30), it differs from CTC-SISS-B based on the reported molecular weight for MIF of the 17,000–25,000 mol wt (31). Human lymphotoxins also differ from CTC-SISS-B based on molecular weight as well as in terms of their cytotoxic mode of action (32).

Interferon has been described to inhibit specific antibody production in both the murine model and man (9, 10) as well as having been implicated as a mediator of
Con A-activated suppression of proliferative responses in man (33). Studies in our laboratory have demonstrated that lymphoblastoid-derived interferon (human alpha interferon) inhibits PWM-induced Ig biosynthesis. However, we have not observed reversal of the interferon-mediated suppression by L-rhamnose (T. A. Fleisher and W. C. Greene, unpublished data). Thus, it does not appear that CTC-SISS-B suppression results from interferon activity. Finally, because CTC-SISS-B has no effect on proliferative responses to either mitogens or antigens by either PBMC or T cells, it is unlikely that the supernate contains either the lymphocyte-stimulating or -inhibiting factors (34).

These data suggest that a long-term-cultured human T suppressor cell elaborates the same humoral suppressor factor as is generated by Con A-activated human PBMC. Furthermore, this mediator specifically modulates B cell Ig production in the absence of T cells via recognition and binding to defined carbohydrate-containing surface receptors.

Summary

A human suppressor T cell maintained in long-term culture with conditioned medium containing interleukin 2 elaborates a suppressor factor(s) that specifically inhibits human polyclonal B cell immunoglobulin biosynthesis. This soluble immune suppressor supernate of immunoglobulin production (CTC-SISS-B) shares a number of features with the previously described suppressive mediator elaborated by concanavalin A-activated human peripheral T cells (SISS-B) including: (a) the inhibition by a noncytotoxic mechanism, (b) the suppression of immunoglobulin biosynthesis either through direct action on the B cell or indirect action via the monocyte, (c) the loss of inhibition in the presence of the monosaccharide l-rhamnose, (d) the elaboration by cells irradiated with 500 or 2,000 rad, and (e) molecular weights of 60,000-90,000. Furthermore, the suppression by this mediator appears to be specific for B cell immunoglobulin production in that CTC-SISS-B has no effect on T cell proliferation to mitogens, antigens, and allogeneic cells or on T cell-mediated cytotoxicity. These data indicate that one possible mechanism of suppressor T cell inhibition of human immunoglobulin production is via the generation of a lectinlike suppressor lymphokine that interacts with defined saccharide determinants on the cell surface of either the B cell or monocyte.

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