Brief Definitive Report

T CELL GROWTH FACTOR ABROGATES CONCANAVALIN A-INDUCED SUPPRESSOR CELL FUNCTION*

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Continuous proliferation of T cells after stimulation with antigens or mitogens is maintained by growth factors, released by lectin- or antigen-activated T cells (1–3). T cell growth factor (TCGF) supports only the growth of activated, but not of resting, T cells (2–3). However, resting T cells become sensitive to TCGF when they have been activated by HLA-DR antigens.[1,2] This initial event in T cell triggering does not by itself result in growth or proliferation, because proliferation necessarily requires the presence of TCGF (2–3).[1,2] Once TCGF has been produced and the resting T cells have been rendered sensitive to it, there is no further requirement for antigen, lectin, or HLA-DR-positive accessory cells, and the concentration of TCGF is the only variable determining the extent of proliferation of the TCGF-reactive T cells (2–3).[1,2]

Concanavalin A (Con-A)-induced suppressor cell function has been shown to be caused by T cells (4). We have investigated (*a*) whether Con-A-activated suppressor T cells respond to TCGF stimulation and (*b*) whether these cells cause suppression by decreasing the availability of TCGF produced by the responding cells. The results indicate that Con-A-activated suppressor T cells are sensitive to TCGF and that one mechanism by which these suppressor cells exert suppression is by absorbing TCGF, thereby decreasing the concentration of obligatory growth factors.

Materials and Methods

Cell Separation. Peripheral blood mononuclear cells (MNC) from healthy adult volunteers were obtained as previously described (5). T cells were obtained by passing MNC through nylon wool columns as described elsewhere (6). The cells eluted from nylon fiber columns were washed and resuspended in RPMI-1640 medium (Grand Island Biological Co., Europe, Glasgow, Scotland) supplemented with 10% heat-inactivated fetal calf serum, 20 μg/ml of gentamicin, 2 mM of glutamine, and 1% Hepes buffer solution (Grand Island Biological Co., Europe), which will be referred to as complete culture medium (CCM). To obtain adherent cells, MNC suspended in CCM were incubated at 37°C for 120 min in plastic petri dishes. Nonadherent cells were removed by three washings and the adherent cells were obtained by removing them from the plastic surface with a rubber policeman.

TCGF Production. We have used two different methods for obtaining TCGF. In the first, TCGF was obtained from phytohemagglutinin (PHA)-stimulated MNC as described by

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[1] Palacios, R., G. Möller, L. Claesson, and P. A. Peterson. HLA-DR antigens induce proliferation and cytotoxicity of T cells against haptenated (TNP and FITC) self structures. Manuscript submitted for publication.

[2] Palacios, R., L. Claesson, G. Möller, P. A. Peterson, and E. Möller. Role of HLA-DR antigens in the autologous mixed lymphocyte reaction. I. The heavy nonpolymorphic chain renders resting T cells sensitive to interleukin-2 and both the heavy and the light polymorphic chain participate actively in the production of the growth factor. Manuscript submitted for publication.

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Bonnard et al. (7). In the second method, TCGF was obtained from autologous mixed lymphocyte cultures, by coculturing 3×10^6 T cells with 3×10^6 irradiated autologous non-T cells in 1.5 ml of CCM in 17- × 100-mm round-bottomed plastic tubes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.). After 48 h of incubation at 37°C, the tubes were centrifuged at 2,000 rpm for 15 min, the supernates collected, filtered through 0.45-μm filters (Millipore, S.A., France) and stored at −20°C until used. Those supernatant media that only induced proliferation of activated T cells, but not of resting T cells, were considered to possess TCGF activity.

Generation of Suppressor Cells with Con-A. 3×10^6 T cells supplemented with 3% of irradiated adherent cells were either treated with 20 μg/ml of Con-A (Con-A-activated T cells) or left untreated (nonactivated T cells) at 37°C for 48 h. The cells were harvested and washed three times in CCM. A portion of the cells were treated with mitomycin C (45 μg/ml, Sigma Chemical Co., St. Louis, Mo.) at 37°C for 30 min and tested for suppressor activity on proliferative responses (see below).

Two assay systems were used to detect suppressor activity. In the first, 10^5 mitomycin C-treated, Con-A-activated T cells or their controls were cocultured with 10^5 responding cells in flat-bottomed microtiter plates (Nunclon Delta; A/S Nunc, Copenhagen, Denmark). PHA (1 μg/ml) or 10^5 irradiated (2,500 rad) allogeneic MNC were added in each well in a vol of 0.1 ml of CCM. TCGF or medium was placed in each well at concentrations with final dilutions of 1:2 and 1:4, and were added at different time periods after the initiation of cultures. Proliferation of the responding MNC was measured by the incorporation of [^{3}H]thymidine (0.5 μCi/well, The Radiochemical Centre, Amersham, England) during the last 18 h of 4-d culture for PHA-induced cell proliferation and a 6-d culture for the mixed leukocyte reaction (MLR) as previously described (5). In the second assay system, inhibition of immunoglobulin production was measured. 10^6 responding MNC suspended in CCM containing pokeweed mitogen (PWM) (20 μg/ml) were cocultured with 10^6 Con-A-activated T cells or control cells in the presence or absence of TCGF (final dilutions 1:2 and 1:4). Each culture was carried out in 12- × 75-mm round-bottomed plastic tubes and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air for 7 d. The number of immunoglobulin-secreting cells (PFC) was determined with a reverse hemolytic plaque assay as described by Gronowicz et al. (8). We also did experiments in which Cyclosporin-A (CYA) was added into plastic tubes containing T cells suspended in CCM in the presence of Con-A. After 48 h of incubation at 37°C, the cells were washed four times and resuspended in CCM. CYA-treated or untreated T cells were added to a second culture to test their suppressor activity on both PHA and alloantigen-induced DNA synthesis and PWM-driven immunoglobulin production. We also studied whether CYA-treated T cells were sensitive to TCGF and whether they were able to absorb the growth factor as described before. The degree of inhibition was calculated according to the following formula:

$$\text{percent suppression} = 1 - \left(\frac{A - B}{C - D}\right) \times 100,$$

where A is the counts per minute or PFC in stimulated cultures containing Con-A-activated cells; B is the counts per minute or PFC in nonstimulated cultures containing Con-A-activated cells; C is the counts per minute or PFC in stimulated responding cells; and D is the counts per minute or PFC in nonstimulated responding cells.

Results

TCGF Abrogates Con-A-induced Suppressor Function. Con-A-activated T cells proliferated after addition of TCGF, whereas nonactivated control cells did not, indicating that Con-A-activated cells are susceptible to further stimulation by this factor (Table I), possibly by expressing receptors for TCGF. The suppressor activity of Con-A-activated cells was expressed as a reduced degree of proliferation induced in the responding cells by the addition of PHA or the exposure to allogeneic lymphocytes and a decreased number of immunoglobulin-secreting cells induced by PWM (Table II). Addition of TCGF to the second cultures (indicator systems) resulted in abrogation of the suppressor activity, as determined by both induction of DNA synthesis and of immunoglobulin synthesis (Table II). It seemed possible that Con-A-activated sup-

TABLE I

Response to TCGF of Con-A-activated Peripheral Blood T Cells

Cells	Dilutions of TCGF*		
	1:2	1:4	1:8
Nonactivated T	1.1 ± 0.1§	1.0 ± 0.3	0.8 ± 0.2
Con-A-activated T	10.7 ± 1.5	6.5 ± 0.8	4.9 ± 1.1
Con-A-activated T + CYA‡	1.9 ± 0.4	1.3 ± 0.5	1.1 ± 0.4

* TCGF obtained from PHA-stimulated MNC (see text) was diluted in CCM and incubated with 10^4 cells/well of nonactivated T cells or Con-A-activated T cells for 48 h at 37°C, proliferation was determined by measuring [^{3}H]-thymidine uptake during the last 12 h of culture.

‡ CYA (1 μg/ml) was added together with Con-A to T cells (see text).

§ cpm × 10^{-3}, mean ± SD of four separate experiments, each in triplicate.

TABLE II

Effect of TCGF on the Con-A-induced Suppressor T Cell Function

Cell assay	TCGF dilution*	Percent suppression		
		[^{3}H]thymidine uptake		Ig synthesis
		PHA‡	MLR‡	
Con-A-activated T	—	57.0 ± 2.2	60.8 ± 4.1	52.2 ± 3.8
Con-A-activated T	1:2	−3.7 ± 2.5	−4.9 ± 3.8	12.7 ± 4.1
Con-A-activated T	1:4	−1.1 ± 0.9	−0.8 ± 1.3	20.9 ± 4.4

* TCGF diluted in CCM was added to cell cultures to obtain final dilutions (1:2 and 1:4).

‡ Indicator systems, PHA and alloantigen MLR-induced DNA synthesis and PWM-induced immunoglobulin production. Mean ± SD of four independent experiments, each in triplicate.

pressor cells exerted their function by absorbing TCGF, thereby depriving the responding cells of the necessary growth factors.

Con-A-activated T Cells Exert Suppression by Absorbing Out TCGF. The ability of TCGF to abrogate T cell suppression could then be a result of binding of TCGF to specific receptors expressed in Con-A-activated T cells. To test this hypothesis, Con-A-activated suppressor T cells were preincubated with TCGF (final dilution 1:2) for 4 h at 37°C. The cells were washed and their suppressor activity tested as described above. The supernates resulting from the incubation of Con-A-activated cells with TCGF were tested for TCGF activity as described before. We also studied whether these supernates would still have the capacity to inhibit the Con-A-induced suppressor cell function. One out of three independent experiments is shown in Tables III and IV. It was found that incubation of Con-A activated suppressor cells with TCGF resulted in a loss of their capacity to suppress both PHA- and alloantigen-induced DNA synthesis, whereas activated suppressor cells preincubated in medium instead of TCGF possessed suppressor activity. On the other hand, the TCGF-supernates that had been added to Con-A-activated cells and subsequently recovered showed a significant decrease in their capacity to abrogate suppressor activity exerted by Con-A-activated T cells. They also lost their capacity to maintain proliferation of activated T cells (Tables III and IV). Thus, it seems likely that Con-A-induced suppressor T cells function by removing TCGF that bind to specific receptors expressed only in activated T cells. The ability of TCGF to abrogate suppressor cells would be achieved by saturating the TCGF-binding receptors on the activated suppressor cells.

Experiments were carried out in the presence of a constant concentration of TCGF (final dilution 1:2) but with increasing numbers of Con-A-activated T cells added to PHA-induced DNA synthesis (indicator system). It was found that there was a close

TABLE III

Effect of TCGF on Con-A-induced Suppressor Cell Function

Cell assays	PHA-induced [^{3}H]-thymidine uptake
	cpm × 10^{-3}
MNC	2.2
MNC + PHA	130.4
MNC + PHA + nonactivated T	139.4
MNC + PHA + Con-A-activated T	67.3
MNC + PHA + Con-A- and CYA-treated T*	124.1
MNC + PHA + Con-A-activated T + TCGF‡	191.5
MNC + PHA + Con-A- and CYA-treated T + TCGF*	206.9
MNC + PHA + Con-A-activated T (preincubated with TCGF)§	157.0
MNC + PHA + Con-A- and CYA-treated T (preincubated with TCGF)*	148.4
MNC + PHA + nonactivated T (preincubated with TCGF)§	141.6
MNC + PHA + Con-A-activated T + TCGF (preincubated with Con-A-activated T)§	73.5
MNC + PHA + Con-A-activated T + TCGF (preincubated with Con-A and CYA-treated T)*	188.6
MNC + PHA + Con-A-activated T (preincubated with TCGF) + TCGF§	210.7
MNC + PHA + Con-A- and CYA- treated T (preincubated with TCGF)* + TCGF	231.4
MNC + PHA + Con-A-activated T (preincubated with TCGF) + TCGF (preincubated Con-A-activated T)	165.1
MNC + PHA + TCGF‡	215.0
MNC + PHA + Con-A-activated T + TCGF (added 48 h before end of culture)	111.9

* CYA (1 μg/ml) together with Con-A was added to T cells to activate suppressor cells.

‡ TCGF added at final dilution 1:2.

§ Con-A-activated T (preincubated with TCGF), Con-A-activated T cells incubated with TCGF for 4 h at 37°C before their addition to the second culture (see text). TCGF (preincubated with Con-A-activated T), supernates resulting from the preincubation of Con-A-activated T cells with TCGF.

TABLE IV

Effect of TCGF on Con-A-induced Suppressor Cell Function

Cell assays	MLR-induced [^{3}H]thymidine uptake
	cpm × 10^{-3}
A*	1.1
A + B*	78.4
A + B + nonactivated T	81.7
A + B + Con-A-activated T	31.6
A + B + Con-A- and CYA-treated T	76.9
A + B + Con-A-activated T + TCGF	188.4
A + B + Con-A- and CYA-treated T + TCGF	236.0
A + B + Con-A-activated T (preincubated with TCGF)	84.1
A + B + Con-A- and CYA-treated T (preincubated with TCGF)	89.9
A + B + Con-A-activated T (preincubated with TCGF) + TCGF	235.6
A + B + Con-A- and CYA-treated T (preincubated with TCGF) + TCGF	245.3
A + B + Con-A-activated T + TCGF (preincubated with Con-A-activated T)	36.3
A + B + Con-A-activated T + TCGF (preincubated with Con-A- and CYA-treated T)	228.7
A + B + TCGF‡	241.0
A + B + TCGF (preincubated with Con-A-activated T)	56.8
A + B + TCGF (preincubated with Con-A- and CYA-treated T)	233.1
A + B + Con-A-activated T (preincubated with TCGF) + TCGF (preincubated with Con-A-activated T)	82.5
A + B + Con-A- and CYA-treated T (preincubated with TCGF) + TCGF (preincubated with Con-A- and CYA-treated T)	227.9
A + B + Con-A-activated T + TCGF (added 906 h before end of culture)	121.3
A + B + Con-A-activated T + TCGF (added 48 h before end of culture)	80.6

* A, responding cells; B, stimulator cells (irradiated).

‡ TCGF final dilution 1:2. Con-A-activated cells preincubated with TCGF, Con-A-activated cells that were preincubated with TCGF (4 h, 37°C) and added to the second culture. TCGF preincubated with Con-A-activated cells, supernates resulting from the preincubation of Con-A-activated cells with TCGF; CYA, 1 μg/ml CYA together with Con-A were added to T cells to activate suppressor cells.

TABLE V

Effect of CYA on Con-A-induced Suppressor Cell Function

Cells	TCGF* addition	Percent suppression		
		[^{3}H]thymidine uptake		Ig synthesis
		PHA	MRL	
Con-A-activated T	—	62.0 ± 3.5‡	60.7 ± 4.2	54.2 ± 3.6
Con-A-activated T + 1 μg/ml CYA	—	4.4 ± 1.1	3.8 ± 1.3	7.5 ± 1.9
Con-A-activated T + 3 μg/ml CYA	—	1.2 ± 0.5	1.8 ± 0.4	5.4 ± 2.1
Con-A-activated T + 1 μg/ml CYA	1:2	−9.3 ± 1.6	−10.1 + 2.2	1.8 + 0.7

* TCGF was added together with Con-A- and CYA-treated T cells to the second culture (indicator system).
‡ Mean ± SD of three separate experiments.
§ CYA together with Con-A were added to peripheral blood T cells to induce suppressor cells.

correlation between the number of Con-A activated T cells added and the ability of TCGF to abrogate suppressor cell function. Thus, the capacity of TCGF to abrogate suppressor cell activity decreased with increasing numbers of Con-A-activated cells added to the indicator system (data not shown).

CYA Abrogates Con-A-induced Suppressor Function. CYA seems to inhibit the expression of receptors for TCGF in T cells, as previously suggested by ourselves (9, 10) and by Larsson (11). Accordingly, it was found that CYA effectively inhibited the induction of suppressor T cells by Con-A, whereas the Con-A-activated T cells not exposed to CYA were suppressive (Table V). Moreover, the addition of CYA together with Con-A to T cells made these cells unresponsive to TCGF (Table I) and unable to absorb the growth factor (Tables III and IV).

Discussion

The results presented here show that Con-A-activated suppressor T cells respond to TCGF stimulation, and that TCGF abrogates the suppressor activity exerted by these cells on the proliferation of MNC induced by PHA or alloantigens and on induction of immunoglobulin production driven by PWM.

The most probable mechanism by which TCGF inhibits T cell suppression appears to be by saturating TCGF-specific receptors expressed in the membrane of Con-A-activated T cells, because these cells absorbed TCGF and thereafter lost their capacity to suppress. The supernates obtained after incubation of TCGF with Con-A-activated cells lost TCGF activity as well as the capacity to inhibit Con-A-induced suppression. Moreover, by increasing the number of Con-A-activated T cells added to the indicator system, it was possible to revert the capacity of TCGF to abrogate Con-A suppressor function. Altogether these findings support the notion that Con-A-activated suppressor T cells absorb TCGF and that one mechanism by which these cells exert their function is by decreasing the availability of TCGF elaborated during the activation of the responding cells in the indicator system.

Any procedure that inhibits or blocks the expression of TCGF receptors in T cells or cause a decreased production or availability of this growth factor would impair the proliferation and growth of activated T cells. CYA inhibits the expression of receptors for TCGF, but does not have any effect once these receptors have been expressed (9, 10). Thus, addition of CYA together with Con-A to T cells makes these cells unresponsive to TCGF and unable to absorb the growth factor and abrogates their capacity to exert suppression.

Summary

Concanavalin A (Con-A)-induced suppressor T cells were found to respond to T cell growth factor (TCGF) by proliferation. TCGF abrogated the suppressor activity exerted by these cells on phytohemagglutinin (PHA)- and alloantigen-induced lymphocyte proliferation and on pokeweed mitogen (PWM)-driven immunoglobulin secretion. The Con-A-activated suppressor T cells absorbed the TCGF activity, preincubation of these active suppressor cells with TCGF abolished their suppressor activity and addition of increasing numbers of Con-A-activated T cells reverted the abrogatory effect of TCGF. Altogether, these findings suggest that Con-A-induced suppressor T cells exert their function by decreasing the available levels of TCGF. Cyclosporin-A (CYA), which is known to inhibit the expression of receptors for TCGF on T cells, also inhibited the suppressor activity as determined in both indicator systems, namely PHA- or alloantigen-induced DNA synthesis and PWM-induced immunoglobulin synthesis. CYA made Con-A-treated T cells unresponsive to TCGF and unable to absorb the growth factor, supporting the notion that CYA inhibits the expression of TCGF receptors on T cells, a mechanism by which this drug seems to abrogate Con-A-induced suppressor T cell function.

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