# SUPPRESSOR CELL ACTIVITY AFTER CONCANAVALIN A TREATMENT OF LYMPHOCYTES FROM NORMAL DONORS*

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The functional role of suppressor cells in the control of the immune response has now been recognized. This phenomenon may soon be considered an essential aspect of immunity. The role of antigen-specific and nonspecific suppressor lymphocytes in the regulation of antibody production has been demonstrated (1). In vivo experiments have suggested the existence of suppressor cells in some forms of immunologic tolerance (2–5), chronic suppression of alloantibody production (6, 7), antigenic competition (8), and lymphocyte response to stimulation by thymus-independent antigens such as pneumococcal polysaccharide S-III (9–11).

Suppressor cells have also been shown to be involved in the control of cell-mediated immunity in animals (12). Furthermore, inhibition of graft-versus-host reaction by suppressor cells has been demonstrated when thymocytes and spleen cells obtained from young NZB/W mice were transplanted to newborn C3H/He mice (13, 14).

Mouse spleen cells incubated in vitro with the plant lectin, concanavalin A (Con A),[1] will develop suppressor activity in several assay systems. These Con A-activated suppressor cells can inhibit in vitro lymphocyte response to sheep red blood cells (15, 16), allogeneic antigens in mixed lymphocyte cultures (MLC) (17), and cell-mediated cytotoxicity (18).

Recently, increasing evidence has accumulated that suppressor cells in the peripheral blood may be involved in the etiology and pathogenesis of such pathologic states as autoimmune disease in animals (14), and common variable hypogammaglobulinemia (19), Hodgkin's disease (20), and multiple myeloma in man (21).

In this publication, we report the existence of a population of cells present in the peripheral blood of healthy human volunteers which can be induced by Con A to manifest suppressor functions and that this potential may indeed reflect a latent immunologic control.

## Materials and Methods

*Isolation of Lymphocytes.* Lymphocytes were separated from fresh heparinized blood (20 U heparin per ml of blood) from healthy, volunteer adult donors by Ficoll-Hypaque density gradient

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[1] *Abbreviations used in this paper:* Con A, concanavalin A; HBSS, Hanks' balanced salt solution; MC, mitomycin C; MLC, mixed lymphocyte culture; PHA, phytohemagglutinin-P; PPD, tuberculin purified protein derivative; PWM, pokeweed mitogen.

centrifugation using a modification of the technique of Böyum (22). After washing three times with $Ca^{++}$- and $Mg^{++}$-free Hanks' balanced salt solution (HBSS, Grand Island Biological Co., Grand Island, N. Y.) the cells were resuspended in RPMI 1640 medium (Grand Island Biological Co.) containing 15% heat-inactivated pooled human serum and 100 U of penicillin, 50 μg of streptomycin, and 2 μmol of glutamine per ml of medium (RPMI). The final suspension was adjusted to a concentration of either $5 \times 10^5$ cells per ml for the mitogen and antigen stimulation studies or $1.0 \times 10^6$ responder lymphocytes per ml in the (MLC) reactions.

*Con A Pretreatment of Lymphocytes.* A suspension of lymphocytes at a concentration of $3\text{–}5 \times 10^6$ cells per ml of RPMI was cultured in the presence of Con A (60 μg/ml, Difco Laboratories, Detroit, Mich.) in a humidified, 5% $CO_2$ incubator for 48 h. At the end of incubation, the cells were washed three additional times with HBSS and resuspended in RPMI at a concentration equal to that of the responder lymphocytes.

*Cell Culture*

ONE-WAY MIXED LYMPHOCYTE CULTURE. A 0.1-ml aliquot of responder cells was mixed with an equal volume of allogeneic, Con A-pretreated lymphocytes in Microtest plate (Falcon Plastics, Oxnard, Calif.). The cultures were incubated for 5 days in a humidified 5% $CO_2$ atmosphere. For the last 16 h of incubation, 2 μCi of [$^3$H]thymidine (New England Nuclear, Boston, Mass.) were added to each well. The cells were then collected with a multiple automatic sample harvester. Incorporation of the isotope-labeled thymidine into lymphocytes was measured in a liquid scintillation counter using a premixed scintillation cocktail (Aquasol, Packard Instrument Co., Inc., Downers Grove, Ill.).

MITOGEN AND SPECIFIC ANTIGEN STIMULATION OF LYMPHOCYTES. The preparation of lymphocytes and cell culture conditions were as described above. Immediately after mixing the responder cells and the Con A, mitomycin C (MC)-pretreated lymphocytes, 25 μl of various concentrations of either one of the mitogens, phytohemagglutinin-P (PHA, 2.5 μl/ml, Difco Laboratories), Con A (60 μg/ml), or pokeweed mitogen (PWM, 20 μg/ml, Difco Laboratories); or the microbial antigens, tuberculin purified protein derivatives (PPD, Statens Seruminstitut, Copenhagen, Denmark), or *Candida albicans* antigens prepared as described elsewhere (23) were added to each of the wells and the cultures incubated for 5 days. 2 μCi of [$^3$H]thymidine were added per well during the last 16 h of incubation.

Experiments with both MLC and mitogen or antigen-stimulated cells were performed in triplicate or quadruplicate. The data are expressed as mean counts per minute per culture plus and minus the standard error of the mean. Percentage of blastogenic stimulation of responder cells inhibited by Con A-induced suppressor cells was calculated according to the following formula: $(1 - [(C_m^{cA} - C^{cA})/(C_m - C)]) \times 100\%$ equal to percentage of inhibition by Con A-induced suppressor cells, where: $C_m^{cA}$ is counts per minute of normal lymphocytes plus Con A, MC-pretreated cells plus mitogens, antigens, or allogeneic cells; $C^{cA}$ is counts per minute of normal lymphocytes plus Con A, MC-pretreated cells; $C_m$ is counts per minute of normal lymphocytes plus MC-treated cells incubated without Con A plus mitogens, antigens, or allogeneic cells; $C$ is counts per minute of normal lymphocytes plus MC-treated cells incubated without Con A.

## Results

*Effect of Con A-Pretreated Lymphocytes on DNA Synthesis by Normal Human Lymphocytes in Response to Mitogens.* As shown in Table I, the in vitro response of normal human lymphocytes to stimulation with PHA, Con A, and PWM was suppressed by allogeneic, Con A-pretreated lymphocytes, similarly obtained from healthy adult volunteer donors. Although suppression of the mitogenic response was most frequently noted when allogeneic Con A-pretreated normal donor cells were added to responder lymphocytes plus mitogen, other activities ranging from no effect to stimulation were occasionally observed. Suppression, however, was most pronounced when Con A was used as a mitogen and occurred to a lesser extent when PWM was used to stimulate responder cells. It is emphasized that all blastogenic activity was attributable to untreated responder lymphocytes, since the Con A-induced cells had been

TABLE I

*Effect of Con A-Treated Allogeneic Lymphocytes on the Mitogenic Response of Normal Human Lymphocytes*

| Responding cells (subjects)* | Con A pretreatment of allogeneic cells‡ | [$^3$H]TdR incorporation (cpm) in response to mitogens§ | | | | | |
|---|---|---|---|---|---|---|---|
| | | PHA | | Con A | | PWM | |
| | | *cpm* | *% inhibition* | *cpm* | *% inhibition* | *cpm* | *% inhibition* |
| 1 | – | 87,828 ± 6,684 | | 121,521 ± 4,213 | | 37,349 ± 369 | |
| | + | 75,742 ± 5,566 | 13.8 | 88,444 ± 6,356 | 27.2 | 30,401 ± 2,897 | 18.6 |
| 2 | – | 74,450 ± 3,270 | | 65,035 ± 3,227 | | 13,136 ± 354 | |
| | + | 37,576 ± 653 | 49.5 | 34,945 ± 1,753 | 46.3 | 13,979 ± 1,026 | +6.4 |
| 3 | – | 126,324 ± 5,395 | | 85,598 ± 2,878 | | 44,285 ± 1,700 | |
| | + | 90,479 ± 3,772 | 28.4 | 57,860 ± 519 | 32.4 | 27,285 ± 372 | 38.4 |
| 4 | – | 73,138 ± 3,056 | | 94,328 ± 3,366 | | 15,583 ± 1,319 | |
| | + | 59,999 ± 2,375 | 18.0 | 52,759 ± 1,717 | 44.1 | 15,312 ± 467 | 1.7 |
| 5 | – | 38,650 ± 2,850 | | 128,362 ± 6,229 | | 11,445 ± 688 | |
| | + | 28,215 ± 1,417 | 27.0 | 96,491 ± 1,266 | 24.8 | 18,493 ± 1,384 | +61.6 |
| 6 | – | 93,369 ± 930 | | 188,524 ± 4,585 | | 21,278 ± 629 | |
| | + | 58,501 ± 2,903 | 37.3 | 117,377 ± 5,896 | 37.7 | 20,857 ± 1,521 | 2.0 |
| 7 | – | 43,991 ± 3,244 | | 125,332 ± 4,969 | | 19,778 ± 2,068 | |
| | + | 26,533 ± 392 | 39.7 | 69,036 ± 888 | 44.9 | 10,539 ± 713 | 46.7 |
| 8 | – | 60,334 ± 6,856 | | 146,836 ± 6,190 | | 12,390 ± 515 | |
| | + | 38,119 ± 554 | 36.8 | 73,787 ± 3,172 | 49.7 | 16,001 ± 835 | +29.1 |

* $5.0 \times 10^4$ normal donor lymphocytes were mixed with an equal number of MC-treated allogeneic control or suppressor cells in RPMI plus designated mitogen and labeled with 2 $\mu$Ci [$^3$H]thymidine ([$^3$H]TdR) as described in Materials and Methods. Each pair of cultures consists of responder lymphocytes from a different subject donor.

‡ Allogeneic lymphocytes were incubated in RPMI plus or minus Con A (60 $\mu$g/ml) for 48 h. Cells were washed, treated with MC (50 $\mu$g/ml) for 30 min, washed again, and resuspended in RPMI. $5.0 \times 10^4$ allogeneic cells were added to each culture well.

§ Counts per minute of [$^3$H]TdR incorporated represents the mean of 3–4 replicate cultures ± the standard error.

previously treated with MC to prevent DNA synthesis.

In these experiments, Con A-pretreated allogeneic lymphocytes functioned as suppressor cells. To determine the amount of stimulation due to recognition of allogeneic lymphocyte-determined antigens, control cultures consisting of normal lymphocytes and Con A-pretreated cells in the absence of exogenous mitogens were set up concomitantly. Considering the complicated reactions which may occur between allogeneic cells in culture, an autologous cell system was developed and tested for suppressor activity. In this syngeneic system, both the untreated responder lymphocytes and the Con A-pretreated cells came from the same individual. It is evident from the data in Table II that comparable suppression of the mitogenic response can also be induced by Con A with autologous cells.

*Effect of Con A Pretreated Lymphocytes on Blastogenic Transformation of Normal Lymphocytes in Response to Specific Antigens.* To determine if the Con A-induced suppressor effect was active on the lymphocyte response to naturally occurring microbial antigens as well as the nonspecific transformation elicited by mitogens, cell extracts of *Candida albicans* and PPD were used to

TABLE II
*Effect of Con A Treatment of Stimulator Lymphocytes on the Allogeneic Response of Normal Human Lymphocytes*

| Responding cells (subjects)* | Con A pretreatment of autologous cells‡ | [$^3$H]TdR incorporation (cpm) in response to mitogens§ | | | | | |
|---|---|---|---|---|---|---|---|
| | | PHA | | Con A | | PWM | |
| | | *cpm* | *% inhibition* | *cpm* | *% inhibition* | *cpm* | *% inhibition* |
| 1 | – | 70,689 ± 4,855 | | 98,718 ± 5,369 | | 20,961 ± 497 | |
| | + | 38,928 ± 1,537 | 44.9 | 48,788 ± 739 | 50.6 | 15,455 ± 618 | 26.3 |
| 2 | – | 39,543 ± 4,542 | | 153,725 ± 1,534 | | 7,152 ± 85 | |
| | + | 23,326 ± 1,236 | 41.0 | 135,043 ± 5,640 | 12.1 | 13,751 ± 363 | +92.3 |
| 3 | – | 108,413 ± 6,667 | | 220,224 ± 20,875 | | 18,780 ± 912 | |
| | + | 85,620 ± 3,614 | 21.0 | 160,887 ± 10,320 | 26.9 | 17,663 ± 490 | 5.9 |
| 4 | – | 114,344 ± 1,885 | | 177,377 ± 13,284 | | 14,636 ± 608 | |
| | + | 118,566 ± 2,200 | +3.7 | 172,470 ± 9,287 | 2.8 | 13,974 ± 860 | 4.5 |
| 5 | – | 65,390 ± 3,006 | | 170,293 ± 7,138 | | 15,883 ± 343 | |
| | + | 53,357 ± 3,206 | 18.4 | 146,380 ± 2,261 | 14.0 | 16,411 ± 684 | +3.3 |
| 6 | – | 68,802 ± 2,356 | | 187,398 ± 4,017 | | 25,188 ± 769 | |
| | + | 47,169 ± 1,150 | 31.4 | 154,919 ± 2,930 | 17.3 | 20,938 ± 346 | 16.9 |

* $5.0 \times 10^4$ normal donor lymphocytes were mixed with an equal number of MC-treated autologous control or suppressor cells. Other conditions as in legend to Table I.
‡ $5.0 \times 10^4$ lymphocytes were treated as in the legend for Table I, and were added to freshly prepared autologous normal donor lymphocytes.
§ Same as in legend for Table I.

stimulate lymphocytes in the microtest technique as described in Materials and Methods. The data in Table III demonstrate the suppressor activity of Con A-pretreated lymphocytes on the blastogenic response of normal allogeneic cells to these microbial antigens. Attention is drawn to the fact that all responder cells were stimulated by either the antigens alone or by the allogeneic mitomycin C-treated lymphocytes used for Con A induction of suppressor activity. Inhibition of the transformation response was noted only when the mitomycin C-treated allogeneic cells had also been pretreated with Con A. When autologous lymphocytes were pretreated with Con A and added to cultures of normal responder cells plus mitogen, a suppressor effect similar to that manifested in the allogeneic systems was also observed (Table IV). Untreated responding lymphocytes underwent blastogenesis in the presence of the antigens as previously indicated; however, no stimulatory effect was noted when mitomycin C-treated lymphocytes were mixed with normal, untreated autologous cells. These latter data are not presented.

*Suppression of Allogeneic Stimulation of Responder Cells in MLC by Con A-pretreated Lymphocytes.* The most impressive and consistent suppression of lymphocyte response was observed when Con A-pretreated allogeneic cells were incubated in the one-way MLC. As evidenced in Table V, when Con A-pretreated allogeneic cells are used as stimulators, blast transformation by responder lymphocytes is markedly inhibited. To determine whether this phenomenon was attributable to an active suppressor mechanism or to a decrease in

TABLE III

*Effect of Con-A-Treated Allogeneic Lymphocytes on Blast Transformation by Normal Human Lymphocytes in Response to Antigens*

| Responding cells (subjects)* | Con A pretreatment of allogeneic cells‡ | [$^3$H]TdR incorporation (cpm) in response to antigens§ | | | |
|---|---|---|---|---|---|
| | | PPD | | Candida | |
| | | *cpm* | *% inhibition* | *cpm* | *% inhibition* |
| 1 | – | 100,547 ± 6,876 | | 48,994 ± 6,807 | |
| | + | 24,168 ± 4,115 | 76.0 | 20,450 ± 1,607 | 58.3 |
| 2 | – | 17,644 ± 913 | | 13,660 ± 897 | |
| | + | 2,947 ± 970 | 83.3 | 2,956 ± 644 | 78.4 |
| 3 | – | 48,519 ± 8,116 | | 19,866 ± 4,944 | |
| | + | 23,333 ± 558 | 51.9 | 14,018 ± 2,607 | 29.4 |

* $5.0 \times 10^4$ normal donor lymphocytes were mixed with an equal number of MC-treated allogeneic control or suppressor cells in RPMI plus the designated antigen. Other conditions as in legend for Table I.

‡ Same as in legend for Table I.

§ Same as in legend for Table I.

effective alloantigenicity through induced loss or masking of alloantigens as with steric hindrance, a syngeneic suppressor cell system was devised. Under these conditions, both the responder and suppressor lymphocytes are obtained from the same individual. Table VI shows that in all individuals tested the allogeneic response is suppressed when homologous Con A-pretreated cells are added to the MLC. Furthermore, control cultures consisting of lymphocytes homologous to responder cells preincubated in the absence of Con A but treated with mitomycin C and likewise added to MLC's showed no suppression and occasionally had an enhancing effect. With regard to the latter, all standard control MLC's demonstrated appropriate allogeneic responses. In every case when lymphocytes obtained from normal donors were left untreated, no suppression of mixed leukocyte culture responses was noted.

## Discussion

It is becoming increasingly evident that the immune system requires checks and balances to prevent an excessive reaction by any individual element to the multitude of stimulations experienced throughout the lifetime of the organism. Suppressor cells have been proposed as one mechanism that can modulate an immune response. Evidence to date suggests a role for suppressor lymphocytes in the pathogenesis of several disease states in man (19–21); however, others have postulated that suppressor cells are but one of a diverse array of control mechanisms responsible for modulating the normal immune system (1). Thus one would expect such suppressor cells also to be present in most individuals.

As has been shown by others using cells from immunologically active tissues in animals, mitogenic doses of Con A can activate lymphocytes, which subse-

TABLE IV

*Effect of Con A-Treated Autologous Lymphocytes on Blast Transformation by Normal Human Lymphocytes in Response to Antigens*

| Responding cells (subjects)* | Con A pretreatment of autologous cells‡ | [$^3$H]TdR incorporation (cpm) in response to antigens§ | | | |
|---|---|---|---|---|---|
| | | PPD | | Candida | |
| | | *cpm* | *% inhibition* | *cpm* | *% inhibition* |
| 1 | – | 36,617 ± 3,807 | | 63,384 ± 6,279 | |
| | + | 26,473 ± 2,239 | 27.7 | 27,275 ± 1,782 | 57.0 |
| 2 | – | 69,313 ± 4,895 | | 31,188 ± 1,685 | |
| | + | 29,741 ± 1,238 | 57.1 | 13,364 ± 2,062 | 57.1 |
| 3 | – | 34,234 ± 1,929 | | 32,491 ± 920 | |
| | + | 18,651 ± 664 | 45.5 | 15,740 ± 820 | 51.6 |

* $5.0 \times 10^4$ normal donor lymphocytes were mixed with an equal number of MC-treated autologous control or suppressor cells in RPMI plus the designated antigen. Other conditions as in legend for Table I.

‡ $5.0 \times 10^4$ lymphocytes were treated as in legend for Table I and were added to freshly prepared autologous normal donor lymphocytes.

§ Same as in legend for Table I.

quently inhibit antibody secretion in vitro (15, 16); this effect can be demonstrated to be exclusive of cytotoxicity (24). Thomas et al. (25) suggest that such inhibitory or suppressor activity may be due to an antimitotic effect and further propose that such mechanisms may be responsible for inhibiting tumor cell proliferation. By contrast to the animal studies where cells were derived from central immune tissues, the present investigations demonstrate that similar suppressor cells can be induced by Con A treatment of the peripheral blood lymphocytes of healthy adult humans. These suppressor cells can also be shown to exert antimitotic effects. Furthermore, unlike the animal models presented to date, where inhibition of blastogenesis required preliminary sensitization of responder cells in vitro, the suppressor cells described in these studies are active on lymphocytes responding to proliferate to *de novo* stimuli in experiments employing mitogens, antigens, or allogeneic cells. As a final contrast, the animal studies utilized inhibition of a B-cell function, antibody secretion as an indicator of suppressor activity. Since blastogenesis with nonspecific mitogens such as PHA, Con A, and PWM can involve either B or T lymphocytes, in several instances, the degree of suppression noted was greater than could be attributed solely to inhibition of B-cell responses. We conclude, therefore, that the suppressor effect described may be a phenomenon of more general influence involving both major lymphocyte subclasses. From the data presented, it is possible that the effects observed could be attributed to inhibition of T-cell proliferation alone. Our results do not distinguish between these two possibilities. The latter alternative receives support from the observation that suppression of the response to PWM, a potent B-cell stimulator, was generally less than that for the other mitogens.

TABLE V
*Effect of Con A Treatment of Stimulator Lymphocytes on the Allogeneic Response of Normal Human Lymphocytes*

| Responding cells (subjects)* | Con A pretreatment of allogeneic cells‡ | cpm§ | Percent of inhibition |
|---|---|---|---|
| 1 | – | 6,517 ± 226 | |
| | + | 1,879 ± 134 | 71.2 |
| 2 | – | 5,613 ± 339 | |
| | + | 3,169 ± 176 | 43.5 |
| 3 | – | 3,144 ± 364 | |
| | + | 1,814 ± 6 | 42.3 |
| 4 | – | 8,843 ± 553 | |
| | + | 1,974 ± 89 | 77.7 |
| 5 | – | 8,846 ± 834 | |
| | + | 6,721 ± 283 | 24.0 |
| 6 | – | 13,142 ± 603 | |
| | + | 7,252 ± 23 | 44.8 |
| 7 | – | 26,787 ± 1,059 | |
| | + | 16,207 ± 572 | 39.5 |

* $1.0 \times 10^5$ normal donor lymphocytes were mixed with an equal number of MC-treated allogeneic cells in a one-way MLC as described in Methods. Other conditions as in legend for Table I.

‡ $1.0 \times 10^5$ lymphocytes were treated with or without Con A as in legend for Table I and were added to an equal number of freshly prepared allogeneic normal donor lymphocytes.

§ Same as in legend for Table I.

The postulated existence of suppressor cells capable of modulating immune reactivity in all individuals was further supported by demonstrating that lymphocytes could be induced to inhibit proliferative responses by autologous cells. Such observations are consistent with a model that proposes that individual stimuli are prevented from initiating an unchecked immunologic chain reaction in vivo through the intervention of suppressor cells capable of abrogating the response. Furthermore, the induction of suppressor cells in an entirely autologous system obviates the need to invoke the nonspecific allotype suppression proposed by Jacobson (26).

Since we would define this form of suppression as a reversible inhibition which does not result in cell death, trypan blue dye exclusion analysis of our cells at the termination of incubation revealed greater than 95% viability in either autologous or allogeneic cultures. Several lymphocyte stimulators, including PHA, allogeneic and xenogeneic antigens, and specific cellular antibodies can elicit immunospecific cytotoxicity (27). However, Perlmann et al. (24) have shown that Con A not only fails to generate such cytotoxicity when human

TABLE VI
*Suppression of the Allogeneic Response of Normal Lymphocytes by Con A-Pretreated Autologous Cells*

| Responding cells (subjects)* | Stimulating cells (subjects)‡ | Suppressor cells§ | | cpm | Percent inhibition |
|---|---|---|---|---|---|
| | | Source | Pretreatment | | |
| AG | – | $AG_m$ | – | 555 ± 44 | |
| AG | $AK_m$ | – | – | 18,238 ± 2,070 | |
| AG | $AK_m$ | $AG_m$ | Medium alone | 19,056 ± 1,124 | |
| AG | $AK_m$ | $AG_m$ | Con A | 12,140 ± 1,328 | 36.3 |
| MB | – | $MB_m$ | – | 1,016 ± 110 | |
| MB | $AK_m$ | – | – | 14,801 ± 982 | |
| MB | $AK_m$ | $MB_m$ | Medium alone | 20,972 ± 657 | |
| MB | $AK_m$ | $MB_m$ | Con A | 12,919 ± 1,627 | 38.4 |
| GO | – | $GO_m$ | – | 626 ± 83 | |
| GO | $AK_m$ | – | – | 18,665 ± 1,154 | |
| GO | $AK_m$ | $GO_m$ | Medium alone | 20,850 ± 1,510 | |
| GO | $AK_m$ | $GO_m$ | Con A | 15,603 ± 934 | 25.2 |
| LC | – | $LC_m$ | – | 3,939 ± 436 | |
| LC | $AK_m$ | – | – | 20,537 ± 726 | |
| LC | $AK_m$ | $LC_m$ | Medium alone | 20,211 ± 1,270 | |
| LC | $AK_m$ | $LC_m$ | Con A | 10,577 ± 989 | 47.7 |
| SS | – | $SS_m$ | – | 655 ± 38 | |
| SS | $VM_m$ | – | – | 34,461 ± 698 | |
| SS | $VM_m$ | $SS_m$ | Medium alone | 37,455 ± 1,175 | |
| SS | $VM_m$ | $SS_m$ | Con A | 28,708 ± 1,406 | 23.4 |
| MC | – | $MC_m$ | – | 680 ± 73 | |
| MC | $VM_m$ | – | – | 29,086 ± 1,428 | |
| MC | $VM_m$ | $MC_m$ | Medium alone | 34,213 ± 1,535 | |
| MC | $VM_m$ | $MC_m$ | Con A | 16,878 ± 1,988 | 50.7 |
| EH | – | $EH_m$ | – | 1,346 ± 85 | |
| EH | $VM_m$ | – | – | 48,369 ± 2,371 | |
| EH | $VM_m$ | $EH_m$ | Medium alone | 47,888 ± 988 | |
| EH | $VM_m$ | $EH_m$ | Con A | 37,692 ± 1,107 | 21.3 |

* Same as in legend for Table V.

‡ $1.0 \times 10^5$ MC-treated (designated by subscript "m") lymphocytes were added to an equal number of freshly prepared allogeneic normal donor cells.

§ An additional $1.0 \times 10^5$ MC-treated lymphocytes autologous to the untreated responder cells previously incubated for 48 h with or without Con A were added to each MLC as indicated.

§ Same as in legend for Table I.

peripheral blood lymphocyte proliferation is stimulated, but can even inhibit the PHA-induced cytotoxic effect. This may be taken as additional evidence that Con A induces a suppressor influence which can operate under several different circumstances.

Throughout the studies we observed occasional individuals whose lymphocytes did not manifest suppressor activity after Con A treatment (Table II, subject no. 4). This failure is not attributable to the absence of receptors for Con A, since the cells transformed well in response to the mitogen. It is possible that Con A can act through more than a single receptor; one site responsible for triggering blast transformation and another for initiating suppressor activity. Thus, cells that cannot be induced to manifest suppressor functions, but can

undergo blast transformation, may lack a second receptor. Such individuals may have a diminished potential suppressor cell population as occurs later in the NZB/W mouse (14) and consequently might be increasingly susceptible to autoimmune disease. The variability in the degree of inducible suppression from person to person and in the same individual at various times (data not presented) probably reflects the state of immunologic reactivity at that moment. That is, few suppressor cells would be required or expected early in an immune response, but later a greater suppressor activity would be necessary to prevent immunologic excesses.

Since we have shown that Con A can induce a suppressor response of autologous cells, the total amount of blast transformation observed after treatment with this mitogen might not reflect the full proliferative potential of the cell population. Indeed, if suppressor cells were selectively removed from a suspension of lymphocytes, one might observe an even greater mitogenic effect with equivalent amounts of Con A.

Of major importance is the establishment of the molecular mechanism of the Con A-induced suppressor effect reported here. Search for a soluble mediator for suppression of human lymphoid cells like that demonstrated in several animal studies (25, 28) is necessary as are experiments to determine whether direct cell-cell contact is needed for this influence. Recent investigations by other workers have raised the possibility that latent viruses induced by immunologic stimuli such as mitogens, in particular Con A, and allogenic responses may contribute to immunosuppression (29). It is therefore possible that Con A could be acting by activation of latent intracellular viruses with concomitant immunosuppressive effects. If so, such a virus or viruses are widely distributed in the blood cells of healthy men.

It is tempting to conclude that the presence of cells capable, upon appropriate stimulation, of manifesting immune suppression in the peripheral blood of healthy adults probably represents a normal control mechanism which can contribute to immunologic homeostasis and stability. An analysis of variations of such suppressor cells during ontogeny, aging, and disease seems urgent.

## Summary

Pretreatment of normal human peripheral blood lymphocytes with the plant lectin, concanavalin A (Con A), results in inhibition of blast transformation and [$^3$H]thymidine incorporation by untreated allogeneic lymphocytes from healthy volunteer donors in one-way mixed leukocyte culture. Similarly, responses to mitogens, certain microbial antigens, and allogeneic lymphocytes are inhibited by Con A-treated. allogeneic cells. Con A pretreated autologous lymphocytes can also be induced to manifest suppressor activities. This antimitotic effect occurs without evidence of cytotoxicity and is active on *de novo* lymphocyte responses and does not require prior sensitization of the cells being tested.

Suppression of the lymphocyte response to pokeweed mitogen, a potent B-cell stimulator, by Con A-pretreated suppressor cells was not as consistent as was inhibition of response to other mitogens, including phytohemagglutinin and Con A. Furthermore, suppression of lymphocyte transformation to the microbial antigens, tuberculin purified protein derivative, and *Candida albicans* extracts

could be similarly induced by Con A pretreatment of either allogeneic or autologous cells.

Induction of autologous suppressor activity in lymphocytes from healthy donors is compatible with a model that includes a role for suppressor cells in the modulation of the normal immune response.

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