SB Sino Biological
Therapeutic Targets for
Autoimmune Diseases
• Covering Immune Cell Targets, Cytokines, and Kinases
• High Purity and High Activity
Learn More

The Journal of Immunology
RESEARCH ARTICLE | JULY 01 1980

Generation of suppressor cells by concanavalin A: a new perspective. FREE

L A Fernandez; ... et. al

J Immunol (1980) 125 (1): 267–269.
https://doi.org/10.4049/jimmunol.125.1.267

Related Content

Studies in Hypersensitiveness: XXXIII. On the Question of the Specificity of the Protective Effect of the Specific Treatment of Hay Fever

J Immunol (July,1928)

Leu-3+ T cells produce gamma-interferon in patients with recurrent herpes labialis.

J Immunol (January,1984)

Viral, bacterial and host determinants converge on impairment of alveolar macrophage function to promote influenza and *S. pneumoniae* coinfection

J Immunol (May,2017)

Downloaded from http://journals.aai.org/jimmunol/article-pdf/125/1/267/1011110/267.pdf by guest on 24 May 2025

0022-1767/80/1251-0267$02.00/0
THE JOURNAL OF IMMUNOLOGY
Copyright © 1980 by The Williams & Wilkins Co.

Vol. 125, No. 1, July 1980
Printed in U.S.A.

GENERATION OF SUPPRESSOR CELLS BY CONCANAVALIN A: A NEW PERSPECTIVE[1]

LOUIS A. FERNANDEZ AND J. MICHAEL MACSWEEN

From the Department of Medicine, Dalhousie University and Camp Hill Hospital, Halifax, Nova Scotia, Canada, B3H 3G2

Significantly lower mitogenic responses of fresh cells co-cultured with Con A-stimulated cells were found when compared with the responses of fresh cells co-cultured with preincubated control cells. We do not agree with the interpretation that this effect represents the generation of suppressor cells by Con A, since the responses of fresh cells cultured alone were also significantly less than when co-cultured with control cells and the same as when co-cultured with the Con A-stimulated cells. Treatment with mitomycin C was sufficient to prevent the preincubated cells from contributing to the mitogenic response of the fresh cells. The increased responses of fresh cells when co-cultured with preincubated cells seems analogous to the increased mitogenic responses of cells aged *in vitro* by preincubation without mitogen. This effect seems to be transferable to fresh cells in the absence of cell division. Although preincubation in the presence of Con A abrogates this effect, we do not interpret this as the generation of suppressor cells.

The normal immunoregulatory system is a result of a complex balance between helper and suppressor cells and their secretory products (1, 2). In humans, helper and suppressor T cells can be identified by their ability to rosette with ox erythrocytes coated with IgG or IgM antibodies (3). Several functional suppressor cell activities have been demonstrated *in vitro*. One of the most widely used techniques is the generation of suppressor T cells by concanavalin A (Con A), usually for 48 hr (4–9). Control cells were cultured for the same period of time in the absence of Con A. The cells were then treated with mitomycin C to prevent division and washed with α-methyl-D-mannoside to displace cell bound Con A. Suppressor activity in the Con A-stimulated cells was then assessed by culturing fresh (autologous or allogeneic) cells with equal numbers of Con A-stimulated or control cells and measuring their responses to mitogenic or antigenic stimulation. Under these conditions, there were consistently decreased responses of cells cultured in the presence of Con A-stimulated cells compared with control cells. This has been interpreted as an effect of Con A-generated suppressor cells. Such an interpretation presupposes that the control cells have a negligable influence on the responding cells. This assumption is understandable but not necessarily correct. We investigated the phenomenon of Con A-generated suppression by questioning this assumption, and we determined the responses of fresh cells alone as well as their responses when co-cultured with Con A-stimulated or control cells.

MATERIALS AND METHODS

Twenty to 30 ml of venous blood were obtained from young healthy individuals. The method of generating Con A suppressor cells has been described in detail previously (4–9). Briefly, mononuclear cells were obtained by centrifuging the blood on Ficoll-Hypaque. The mononuclear cells were washed two times with RPMI 1640. One million cells/ml of RPMI 1640 containing 10% human AB serum, penicillin 100 units/ml, streptomycin 100 μg/ml, and 2 mM glutamine were incubated with different concentrations of Con A (Sigma Chemical Co., Mo.) or without Con A (control cells). After incubation at 37°C for 48, 72, or 96 hr, the cells were treated with mitomycin C (25 mg/ml), washed with α-methyl-D-mannoside (0.3M) and then twice with RPMI 1640.

Fifty thousand control or Con A-stimulated cells were added to aliquots of 50,000 autologous mononuclear cells obtained on the same day. Aliquots of the 50,000 fresh cells were also cultured alone. The cells were cultured for 4 days in microtiter plates containing 0.2 ml of medium 199, 10% fetal calf serum, penicillin 100 units/ml, streptomycin 100 μg/ml, and 2 mM of glutamine without Con A and also in the presence of Con A in concentrations of 25, 50, or 100 μg/ml. All determinations were done in triplicate. The evening before harvesting the cells, 0.5 μCi of tritiated thymidine (specific activity 15 Ci/mM) was added. The next day the cells were harvested onto glass wool filters by using a Titerteck Harvester (Flow Laboratories). The glass wool filters were then placed in vials containing scintillating fluid, and the radioactivity determined in a beta scintillating spectrometer (10).

RESULTS

Con A was used both to generate suppressor activity and also as a mitogenic stimulus for the fresh mononuclear cells. Responses of fresh cells to Con A stimulation was markedly reduced when they were co-cultured with autologous cells preincubated with either 25, 50, 100, or 150 μg of Con A compared with co-culture with control cells preincubated without Con A (Fig. 1). Cells preincubated with 100 μg Con A seemed to produce the greatest degree of suppression, although it was not significantly different from suppression produced by either 50 or 150 μg of Con A. Thus, in all further experiments 100 μg of Con A were used to produce suppressor cells.

The responses of fresh mononuclear cells obtained from four different healthy individuals to stimulation with Con A were measured when cultured alone and also when co-cultured with autologous cells preincubated for 48 hr with or without Con A.

Received for publication February 11, 1980.
Accepted for publication March 24, 1980.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
[1] This work was supported by the National Cancer Institute of Canada.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/125/1/267/1011110/267.pdf by guest on 24 May 2025

The results are shown in Figure 2. The responses of fresh cells co-cultured with Con A-stimulated cells was markedly reduced compared with their responses when co-cultured with control cells ($p < 0.001$ for all concentrations of Con A). This is consistent with previous reports (4–9). However, the responses of fresh cells cultured alone were also significantly reduced compared with their responses when co-cultured with control cells. Furthermore, it was apparent that there was no difference between the responses of fresh cells cultured alone or with the Con A-stimulated cells (Fig. 2). Similar results were obtained when cells from nine healthy individuals were preincubated with Con A for 72 hr (Fig. 3) and in two cases for 96 hr, showing that the effect was not strictly time dependent. Results were similar when human AB serum was substituted for fetal calf serum. The adequacy of mitomycin treatment in preventing cell division was checked by determining the Con A responsiveness of mitomycin-treated preincubated cells in the absence of fresh cells. Negligible proliferation (less than 2,500 counts per minute) resulted when mitomycin-treated cells preincubated with or without Con A were cultured with various concentrations of Con A.

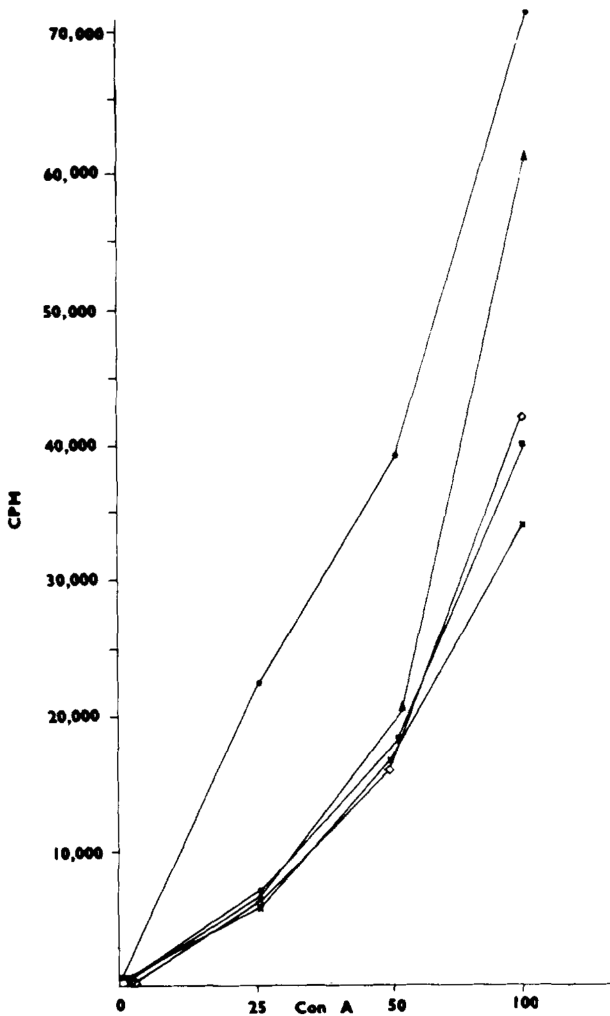

Figure 1. Shows the responses in CPM of fresh cells co-cultured with either control cells or Con A-stimulated cells to various concentrations of Con A. ●——●, Fresh cells + control cells; ▲——▲, fresh cells + 25 μg/ml Con A-stimulated cells; ◇——◇, fresh cells + 50 μg/ml Con A-stimulated cells; ×——×, fresh cells + 100 μg/ml Con A-stimulated cells; ■——■, fresh cells + 150 μg/ml Con A-stimulated cells. Similar results were obtained in three normal individuals.

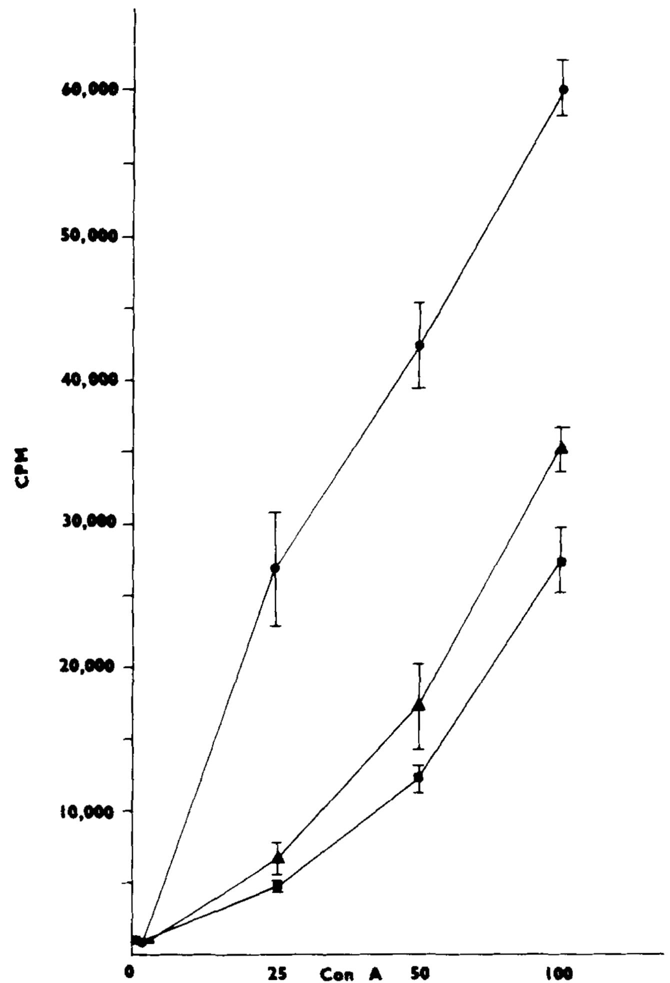

Figure 2. Shows the responses in cpm of fresh cells alone, and fresh cells co-cultured with control cells or with Con A-stimulated cells to 25, 50, and 100 μg/ml of Con A. ■——■, Fresh cells alone; ●——●, fresh cells co-cultured with control cells (preincubated without Con A for 48 hr); ▲——▲, fresh cells co-cultured with cells stimulated with 100 μg/ml Con A for 48 hr. I = S.E.M.

DISCUSSION

In normal human individuals, suppressor T cells have been identified both quantitively (3) and functionally. One of the functional assays of suppressor T cells has been the generation of suppressor T cells by Con A (4–9). On the basis of this *in vitro* test, lack of regulatory suppressor T cells have been observed in disease states (11, 12). In all these studies, the responses of fresh cells mixed with Con A-stimulated cells were compared with fresh cells mixed with cells preincubated without Con A. Our results agree with these reports in that the former responses were consistently lower. It is also clear from our data that the Con A-stimulated cells were not suppressive, since the responses of fresh cells alone were the same as their responses when co-cultured with the Con A-stimulated cells. However, control cells preincubated without Con A clearly enhanced the responses of the fresh cells. Division of the control cells did not contribute to this response, since they were treated with mitomycin C, and this was sufficient to keep them from responding to mitogenic stimulation in the absence of fresh cells.

It has been shown that mitogenic responses of cells that were preincubated without mitogen for 24 to 72 hr were enhanced

Downloaded from http://journals.aai.org/jimmunol/article-pdf/125/1/267/1011110/267.pdf by guest on 24 May 2025

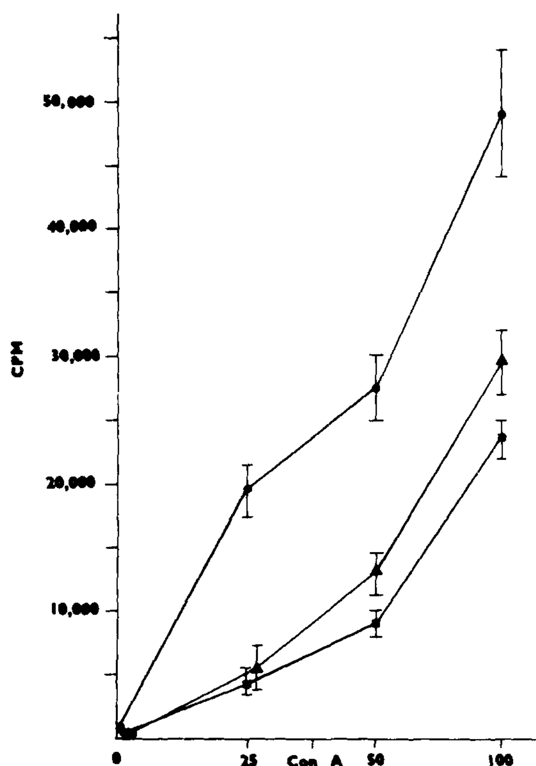

Figure 3. Shows the responses in cpm of fresh cells alone and fresh cells co-cultured with control cells or with Con A-stimulated cells to 25, 50, and 100 μg/ml of Con A. ■——■, Fresh cells alone; ●——●, fresh cells co-cultured with control cells (preincubated without Con A for 72 hr); ▲——▲, fresh cells co-cultured with cells stimulated with 100 μg/ml Con A for 72 hr. I = S.E.M.

compared with responses of fresh cells (13, 14). The enhanced responses were not reduced by the addition of fresh cells (15, 16), leading to the suggestion that preincubation did not inactivate suppressor cells but rather changed the self regulatory activity in the responding cells. This seems to be analogous to the enhanced responses in our system, assuming that the stimulatory effect is transferable to fresh cells. Clearly, it does not require proliferation of the preincubated cells, and may either involve cellular interaction or release of soluble products from the preincubated cells. Preincubation in the presence of Con A seems to abrogate this effect, but it seems inappropriate to refer to this as the generation of suppressor cells.

Acknowledgments. The authors wish to thank Mrs. D. Robson for technical assistance and Miss Pat Godin for secretarial help.

REFERENCES

1. Namba, Y., B. V. Jegasothy, and B. H. Waksman. 1977. Regulatory substances produced by lymphocytes. J. Immunol. 118:1379.
2. Dutton, R. W. 1975. Suppressor T cells. Transplantation Rev. 26: 39.
3. Moretta, L., S. R. Webb, C. E. Grossi, P. M. Lydard, and M. D. Cooper. 1977. Functional analysis of two human T cell subpopulations; helper and suppressor of B cell responses by T cells bearing receptors for IgM or IgG. J. Exp. Med. 146:184.
4. Shou, L., S. A. Schwartz, and R. A. Good. 1976. Suppressor cell activity after Concanavalin A treatment of lymphocytes from normal donors. J. Exp. Med. 143:1100.
5. Hubert, C., G. Delespesse, and A. Govaerts. 1976. Concanavalin A activated suppressor cells in normal human peripheral blood lymphocytes. Clin. Exp. Immunol. 26:95.
6. Sakane, T., and I. Green. 1977. Human suppressor T cells induced by Concanavalin A: Suppressor T cells belong to distinctive T cell subclasses. J. Immunol. 119:1169.
7. Fineman, S. M., F. B. Mudawwar, and R. S. Geha. 1979. Characteristics and mechanism of action of the Concanavalin A-activated suppressor cell in man. Cell. Immunol. 45:120.
8. Mayumi, M., T. Yoshida, K. Shinomiya, S. Mishikawa, T., Hirata, T. Izumi, and H. Mikawa. Effects of concanavalin A-induced cells on the proliferative response of T cells. J. Immunol. 123:772.
9. Hallgren, H. M., and E. J. Yunis. 1977. Suppressor lymphocytes in young and aged humans. J. Immunol. 118:2004.
10. Hartzman, R. J., M. L. Bach, F. H. Bach, G. B. Thurman, and K. W. Sell. 1972. Precipitation of radioactivity labelled samples: a semi-automatic multiple sample processor. Cell Immunol. 4:182.
11. Hodgson, H. J. F., J. R. Wands, and K. J. Isselbacher. 1978. Decreased suppressor cell activity in inflammatory bowel disease. Clin. Exp. Immunol. 32:451.
12. Hodgson, H. J., J. R. Wands, and K. J. Isselbacher. 1978. Alteration in suppressor cell activity in chronic active hepatitis. Proc. Natl. Acad. Sci. 75:1549.
13. Bresnihan, E., and H. Jasin. 1977. Suppressor function of peripheral blood mononuclear cells in normal individuals and in patients with systemic lupus erythematosis. J. Clin. Invest. 59:106.
14. Feighery, C., C. A. Whelan, D. G., Weir, and J. F. Greally. 1978. In vitro studies of suppressor cell function in human peripheral blood mononuclear cells. Clin. Exp. Immunol. 32:459.
15. McCoombs, C. C., J. P. Michalski, and N. Talal. 1978. Acceleration of human lymphocyte activation after preliminary culture. J. Immunol. 120:532.
16. Sohnle, P. G., and C. Collins-Lech. 1979. Differentiation of the effects of preincubation and indomethacin on lymphocyte transformation. Clin. Immunol. Immunopathol. 13:47.

Downloaded from http://journals.aai.org/jimmunol/article-pdf/125/1/267/1011110/267.pdf by guest on 24 May 2025