

COMPETITION BETWEEN CONCAVALIN A-
INDUCED STIMULATORY AND INHIBITORY
EFFECTS IN THE IN VITRO
IMMUNE RESPONSE TO ANTIGEN*

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The requirement for cell cooperation between thymus-derived (T) and bone marrow-derived (B) lymphocytes in the humoral immune response is now well established (1), and it is clear that T cells can play both a "helper" and "suppressor" role (2). T cells have also been shown to perform a variety of other functions such as cell-mediated cytotoxicity and the secretion of various mediators of cellular immunity (e.g., 3). Examples of cell interactions between two populations of T cells have been demonstrated in the development of some of these activities (4, 5). Although it is possible that a single T-cell population could be activated to perform any one of these functions if appropriately triggered it would seem more likely that each function is carried out by a subpopulation of T cells specialized to perform that particular role. The fact that subpopulations of T cells, characterized by differences in half-life, cell surface markers, organ distribution, and responses to mitogens have been demonstrated (6) lends support to this concept. These characteristics, however, would appear to distinguish a short-lived precursor population from a long-lived effector cell population and no clear cut subpopulations of effector cells have as yet been demonstrated.

In the present study we have worked with the in vitro primary response of mouse spleen cell suspensions to sheep erythrocytes (SRBC) and have endeavored to establish whether T-helper effects and T-suppressor effects in the humoral response are mediated by the same or separate populations of T cells. In this work we have utilized the concanavalin A (Con A)-induced stimulatory and inhibited effects (7-9) in the belief that these mitogen-induced T-cell activities parallel the antigen-induced physiological activities of these same cells.

The results of this study show that the inhibitory activity of Con A-induced suppressor T cells can be reversed by the presence of additional Con A-induced stimulator cells. This competitive effect is incompatible with the hypothesis that there is but a single cell type and that suppression is mediated by supraoptimal numbers of stimulator cells. It provides additional evidence that stimulation, which we equate with help, and inhibition, which we equate with suppression, are mediated by separate T-cell populations.

Materials and Methods

Animals. 10- to 14-wk old BDF₁ mice (C57BL/6 female × DBA/2 male) were bred in our own colony. Congenitally athymic (nu/nu) mice were obtained from Dr. James Watson of the Salk Institute, La Jolla, Calif. (See Watson, et al., reference 10, for further details.) Con A, twice crystallized (code 79-001, lot nos. 41, 44, and 111) were obtained from Miles-Yeda Ltd., Miles

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Laboratories, Inc., Kanakakee, Ill. A preparation made by Dr. Stewart Sell (University of California at San Diego, La Jolla, Calif.) was also used. These lots all had optimal activity at $2 \mu\text{g/ml}$ (7). (Later lots nos. 96 and 106, not used in this study, were less active.)

Antigens. SRBC were obtained from the Colorado Serum Co., Denver, Colo.

Immunization. In vitro cultures received 3×10^6 SRBC/culture on day 0.

Cultures. Mouse spleen cells from nu/nu mice were cultured at 3×10^6 cells/culture (11). Varying numbers of inhibitory and stimulatory cells were also added. The total vol was 1 ml. Inhibitory cells were prepared by incubating normal BDF₁ spleen cell suspension at $10^7/\text{ml}$ in the presence of $2 \mu\text{g/ml}$ Con A. The cells were harvested after 48 h, washed, irradiated (1,000 rads), and resuspended in fresh medium. Two types of control for the inhibitory cells were also prepared: (a) cells were preincubated in the absence of Con A, and (b) the cells were treated with rabbit antimouse thymoma antigen and complement (C) (see reference 7) before incubation. Stimulatory cells were fresh, irradiated (1,000 rads) normal BDF₁ spleen cell suspensions. All cultures contained Con A at $2 \mu\text{g/ml}$.

Results

The experiment illustrated in Fig. 1 is designed to show the competitive effects of stimulatory and inhibitory cells on the response of nude spleen cells to antigen. In preliminary experiments it was shown that Con A-induced inhibitor activity generated by 48 h culture in the presence of $2 \mu\text{g/ml}$ Con A was radioresistant. Such preparations were, therefore, used as a convenient source of inhibitor activity free of any B cells capable of a response to antigen. Previous experiments (7, 8) had shown that irradiated spleen cells incubated in the presence of $2 \mu\text{g/ml}$ Con A manifested predominantly stimulatory activity. These were used as a source of stimulator activity free of functional B cells.

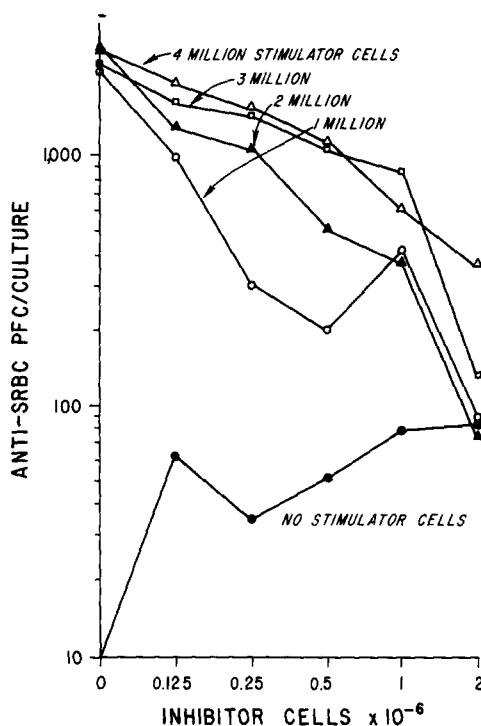


FIG. 1. 3×10^6 Nude spleen cells were incubated with varying numbers of stimulator and suppressor cells as described in the Materials and Methods. The numbers of stimulator cells are indicated on the individual curves and the number of inhibitor cells on the X axis. The number of anti-SRBC PFC per culture were determined on day 4.

It can be seen (along the Y axis) that the addition of even small numbers (10^6) of stimulatory cells in the absence of inhibitory cells raised the response of the nude spleen from zero to several thousand PFC per culture. It would appear that this response was near maximal since increasing numbers of stimulatory cells (2, 3, or 4×10^6) raised the response only slightly. Inhibitory cells in the absence of stimulatory cells exhibited a marginal stimulatory effect but markedly inhibited the responses obtained in the presence of stimulatory cells. This effect was most marked when only small numbers (10^6) of stimulatory cells were present and it can be seen that increasing numbers of stimulatory cells were able to reverse this inhibitory effect. For example, the response of the nude spleen cells in the presence of 10^6 stimulatory cells was progressively inhibited by the addition of 0.125, 0.25, or 0.5 million inhibitor cells. The inhibition at 0.5 million cells could be progressively reversed, however, by raising the number of stimulator cells from 1 to 2 to 3 or 4 million cells.

We have previously demonstrated that both inhibitory and stimulatory effects are mediated by T cells (7, 8). The results of the experiment recorded in Table I confirm that the inhibitory activity of the preincubated irradiated cells in this somewhat modified protocol is also T-cell mediated. Thus, the inhibitory effect is not seen if the cells are treated with rabbit antimouse brain-associated antigen and C before the preincubation with Con A. In another control (not shown) no inhibitory activity was seen if the cells were preincubated and irradiated in the absence of Con A.

TABLE I
The Effect of Goat Antimouse Tymoma Antigen Antiserum on the Activity of Suppressive Populations

Cells	PFC/Culture
3×10^6 nude + 2×10^6 stimulator*	1,285
3×10^6 nude + $\left\{ \begin{array}{l} 2 \times 10^6 \text{ stimulator} \\ 2 \times 10^6 \text{ inhibitor}\ddagger \end{array} \right.$	75
3×10^6 nude + $\left\{ \begin{array}{l} 2 \times 10^6 \text{ stimulator} \\ 2 \times 10^6 \text{ inhibitor treated with antiserum and C}\S \end{array} \right.$	1,210
3×10^6 nude + $\left\{ \begin{array}{l} 2 \times 10^6 \text{ stimulator} \\ 2 \times 10^6 \text{ inhibitor treated with C alone}\ \end{array} \right.$	285

* Stimulator BDF, irradiated with 1,000 rads before culture.

‡ Inhibitor precultured for 48 h in $2 \mu\text{g/ml}$ Con A, harvested, and irradiated before culture.

§ Inhibitor pretreated with goat antimouse thymus antigen before preculture.

|| C control for footnote §.

Discussion

It has been frequently observed that although small numbers of helper or stimulator cells will enhance the humoral response, this effect will pass through a maximum and then diminish as increasing numbers of helper cells are added (12). This observation has led to the suggestion that inhibition is due to a supraoptimal amount of stimulation. Although this hypothesis is compatible with the observation, other explanations are also possible, especially if it is shown that either the helper or suppressor activity is not linearly related to the number of cells present (13, 14)

The results of this study on the properties of Con A-induced T-cell activity indicate that the increasing activity of an increasing number of inhibitory cells can be reversed if increasing numbers of stimulatory cells are added. This is clearly incompatible with the hypothesis that inhibition is the consequence of too much stimulation since the addition of more activity in a situation where inhibition is already demonstrable could only result in increased inhibition, not stimulation.

It is, thus, more likely that the stimulation and inhibition represent the activities of two subpopulations of T cells specialized to carry out these separate roles. Our previous observations (7-9) are also consistent with this view. Thus, the stimulatory activity is radioresistant, relatively insensitive to C lysis in the presence of anti-T-cell antisera, and disappears slowly from adult thymectomized mice but is absent in the spleens of nu/nu mice. In contrast, the inhibitory activity is radiosensitive (if irradiation occurs before induction), is sensitive to anti-T-cell antisera, and disappears rapidly from adult thymectomized mice.

A number of additional points may be made. First, the present study examines only the mitogen-induced T-cell inhibitory and stimulatory activities. It is our working hypothesis that the mitogen merely elicits the same T-cell activity normally expressed after antigen stimulation. If this is true we would expect that it should be possible to show the same competitive effects between suppressor and helper T cells. In support of this it should be noted that competitive effects have been noted between helper cells and the T cells mediating allotype suppression (15).

Second, it can be seen that in the absence of stimulator cells there is a slight stimulatory effect when inhibitor cells are added (Fig. 1). It is our hypothesis that both populations (stimulator and inhibitor) are impure and contain small numbers of cells exhibiting the opposite activity. In any particular case what is observed is the net balance of two opposing effects.

Third, it seems likely that both stimulation (16) and inhibition (17) are effected by T-cell mediators. The cell target for these mediators, the nature of the mediators, and the cell surface receptors have not yet been identified although some interesting preliminary observations have been made (18, 19). Moreover, there is no clear indication of the relationship between the concentration of either mediator and the size of the effect it produces. Our studies (unpublished observations) suggest that the stimulatory effect is linearly related to the number of stimulator cells present while the inhibitory activity shows a threshold effect. Thus, with small numbers of cells from a cell suspension with both types of cells present, stimulatory effects will predominate while with large numbers, inhibitory effects will be observed.

Summary

The humoral response of nude spleen cells (B cells) to sheep erythrocytes was measured in the presence of varying numbers of concanavalin A (ConA)-activated stimulatory spleen T cells (helper) and Con A-activated inhibitory spleen T cells (suppressor) from BDF₁ mice. It was found that suppressive effects could be reversed by the presence of additional numbers of stimulatory cells. These results seem incompatible with the hypothesis that suppression is mediated by

supraoptimal numbers of stimulatory cells and provides additional evidence that separate populations of T cells mediate stimulation and suppression.

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