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# Separation of T cell subpopulations capable of DNA synthesis, lymphotoxin release, and regulation of antigen and phytohemagglutinin responses on the basis of density and adherence properties*

(suppression/potentiation)

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ABSTRACT **T memory cells specifically responsive to ovalbumin and performing the diverse functions of DNA synthesis, lymphotoxin release, and regulation can be isolated in enriched numbers in the most buoyant fractions (A+B) of bovine serum albumin gradients on day 9 after sensitization. At least 20–30% of these cells are capable of mounting a blastogenic response to ovalbumin. A+B cells responding to ovalbumin with DNA synthesis have adherent properties and are further enriched on passage through glass wool. The subpopulations capable of entering into blastogenesis and DNA synthesis and of lymphotoxin release are unresponsive to T mitogens.**

**A+B cells are capable of either potentiating or suppressing DNA synthetic responses to both phytohemagglutinin and antigen when added to $5 \times 10^5$ D cells in different proportions. Potentiation or suppression of phytohemagglutinin responses were observed with $1 \times 10^5$ A+B cells, and total suppression was observed with A+B in the range of $4 \times 10^3$ to $2 \times 10^4$. The response to antigen was sometimes inhibited in the same cell combinations that gave a potentiated response to phytohemagglutinin and vice versa. Regulatory cells in this system were not macrophages since their effect was not mimicked by addition of peritoneal macrophages, and ablation of macrophages by carrageenan affected neither the potentiation nor suppression.**

This study is directed to three lymphocytic functions: the DNA synthetic response after restimulation, lymphotoxin production, and nonspecific suppression of the phytohemagglutinin (PHA) response by antigen. We have previously shown that in rats, after footpad injection with ovalbumin (OA) in complete adjuvant, the DNA synthetic response of draining lymph node cells on restimulation *in vitro* with OA peaks at day 9 (1). A peak in the ability of the same cells to form lymphotoxin in response to OA also is seen at day 9 (2). Finally, the nonspecific suppression of PHA responses by antigen (3) observable as early as 6–12 hr after a primary injection of OA (4) is strongly expressed at 9 days in adjuvant-injected animals and appears to be associated with a population of glass-wool-adherent cells (4, 5).

To further study T cell heterogeneity (reviewed in ref. 6), we have used a combination of techniques based on changes in cell density and adherence properties, associated with clonal expansion and/or activation during the sensitization process, and have isolated a distinct T cell population specifically responsive to OA and capable of performing the different functions described above.

Abbreviations: OA, ovalbumin; PHA, phytohemagglutinin; Con A, concanavalin A; T cell, thymus-derived cell.

* Paper II in the series entitled "Kinetic study of T lymphocytes after sensitization against soluble antigen." Paper I is ref. 1.

## MATERIALS AND METHODS

**Sensitized Cells.** Inbred DA rats (males, 6–8 weeks of age) received OA (ovalbumin, 5 × crystalline, Nutritional Biochemicals Corp., Cleveland, Ohio), 100 μg in Freund's complete adjuvant delivered by multiple injections in both hind footpads (1). For kinetic studies, animals to be compared were immunized with a single batch of antigen in complete Freund's adjuvant. Inguinal lymph node cells were washed and counted and their viability was determined (>98%) by trypan blue dye exclusion (1).

**Separation of OA-Sensitized LNC.** *1. Bovine serum albumin gradients:* LNC were separated on discontinuous gradients (bovine serum albumin, lot 126, Miles Laboratories, Kankakee, Ill.) as described (1). Pools of cells harvested from the density interfaces and related interband regions were designated A, B, C, and D, beginning with the least dense band, and P (pellet) (Fig. 1). Viability of cells after washings was always >95%, excluding P (>85%). Bands A+B were combined because of low yields.

*2. Removal of adherent cells* (5): Three milliliters of warmed cell suspension (1 to $3 \times 10^7$) were incubated on a glass wool column (5) at 37° for 30 min, then eluted with 30 ml of warm medium (*nonadherent* fraction). *Slightly adherent* cells were removed by gentle plunging. The wool was roughly shaken in a siliconized bottle with 50 ml of warm RPMI to remove *moderately adherent* cells. It was then shaken in 50 ml of warm phosphate-buffered saline, pH 7.0, containing 1 mM EDTA (lot 109B-1190, Sigma Chemical Co., St. Louis, Mo.), over 15–30 min at 37° to free *firmly adherent* cells from the wool. The yield of washed nonadherent and slightly adherent cells from whole LNC was 50–70%. Moderately and firmly adherent cells totalled approximately 10%. Comparable yields from A+B cells were 26% and 37%, respectively.

**Macrophage Purification.** "Purified" macrophages were obtained from peritoneal cells of normal donors as described (5), and contained >98% macrophages, as judged by neutral red staining and capacity to phagocytize formalinized sheep erythrocytes. Viability was >90%.

**Cytotoxicity Assay and Selective Removal of T Cells.** A rat T cell-specific antiserum (Black Hooded anti-Lewis thymus), obtained through the kindness of Dr. David Lubaroff (7), was used with complement to lyse T cells (1).

**Lymphocyte Transformation.** Cells were cultured in microtiter plates (8), each well receiving $5 \times 10^5$ unfractionated inguinal LNC, or $5 \times 10^5$ viable cells from subpopulations obtained by either density gradient ultracentrifugation

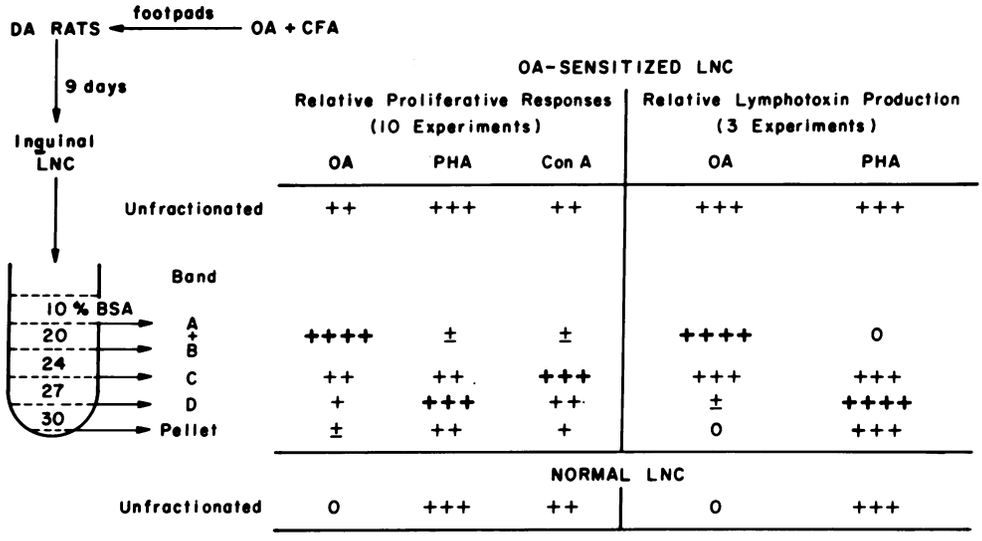


FIG. 1. Separation of OA and PHA responsive subpopulations on bovine serum albumin (BSA) gradients 9 days after sensitization. LNC, lymph node cells; CFA, complete Freund's adjuvant.

or glass wool passage in 0.025 ml of RPMI plus penicillin-streptomycin, 100 units/ml each; 0.025 ml of RPMI plus 24% heat-inactivated normal DA serum; and 0.025 ml of RPMI containing OA (100 $\mu$g/ml), PHA-P (1.1–1.4 $\mu$l/ml) (lot 3110-57), concanavalin A (Con A) (5 $\mu$g/ml) (lot 210073) (mitogens from Difco Laboratories, Inc., Detroit, Mich.), or medium (control). In some experiments, 1–4% normal macrophages were added to the cultures. Dose-response titrations were performed for cells of each band with each stimulant to ensure that the responses reported were maximal. Cultures were incubated for 72 hr and pulsed with [$^3$H]thymidine during the final 24 hr. Results are expressed as mean trichloroacetic acid-precipitable cpm of duplicate experimental cultures minus background ($\Delta$ cpm). In some experiments carrageenan (Sea Kem 9 Carrageenan) purified powder (Marine Colloids, Inc., Passaic, N.J.) was included in cultures at a concentration of 1 mg/ml.

**Quantitation of OA-Sensitized Cells.** In order to obtain a morphologic estimate of the numbers of cells undergoing early blast transformation in response to antigen, cells were cultured as above with OA, 100 $\mu$g/ml, or with PHA, and harvested at 24 hr. In order to obtain accurate total counts, care was taken to scrape out each well with a rubber policeman, and DNase was added to each cell suspension to reduce clumping. Smears for differential counting were stained with methyl green pyronin. Two hundred cells from each fraction were counted. Only pyroninophilic cells measuring >12 $\mu$m in diameter were counted as blasts.

**Lymphotoxin Assay (2).** Viable inguinal LNC ($1 \times 10^7$) (sensitized or normal) or subpopulations separated on bovine serum albumin gradients, together with either 25 $\mu$g/ml of OA or 1.4 $\mu$l/ml of PHA, were incubated for 24 hr at 37° in petri dishes. The cell-free supernatants were added to flasks containing mouse A9 fibroblast monolayers [A9 is an L-cell derived murine cell line (9)], prepared 1 day earlier by seeding of $4 \times 10^4$ A9 cells in 4 ml of complete medium (minimal Eagle's medium, 10% fetal calf serum, 1% L-glutamine, 100 units/ml each of penicillin-streptomycin). Alternatively, the cells to be tested were added with the appropriate stimulant directly to the flasks. At 72 hr dead cells were removed by gentle washing, and the surviving fibroblasts were then recovered from the flask wall by incubation with 0.25% viokase (Gibco, Grand Island, N.Y.) (2). These were collected and enumerated in a Coulter counter. Cytotoxicity was calculated as % cytotoxicity = A/B × 100, where A = surviving fibroblasts in the presence of sensitized LNC plus OA or PHA, and B = surviving fibroblasts in the presence of normal LNC plus OA or PHA. In some experiments 1–4% normal macrophages were added to the various cultures. Dose-response titrations were performed for cells of each band with each stimulant to ensure that maximal responses were reported.

## RESULTS

**Separation of OA-Sensitized Lymphocytes (1).** Data reported in detail elsewhere (1, 10) establish that the peak DNA synthetic response of unfractionated LNC, on restimulation with specific antigen, is at 9–12 days after sensitization. After separation maximal response to OA on day 9 is associated with cells of fraction A+B, although antigen responsive cells are distributed among all bands. A+B cells give a minimal response to PHA or Con A. The maximal responses to these mitogens on any day after sensitization are always in D and C, respectively (Fig. 1). Addition of 1–4% purified macrophages to the cultures did not affect the levels of response.

**Lymphotoxin Production.** Cells in fraction A+B also showed the capacity to kill virtually all of a target monolayer of syngeneic fibroblasts (Table 1). This response was elicited on stimulation with OA, PHA again proving ineffective. This effect was shown to be mediated by a supernatant factor, presumably lymphotoxin (2). Killing in the presence of PHA was observed with cells of band D which produced little or no lymphotoxin in response to OA. Band C cells were cytotoxic with both stimulants, as were unfractionated LNC. Again, addition of purified macrophages did not affect the levels of responses observed.

**Quantitation of OA-Responsive Cells in Fraction A+B on Day 9.** When LNC are separated on bovine serum albumin gradients on day 9 after sensitization, 5% of the cells obtained are in fraction A+B, cells of C, D, and the pellet representing 32, 29, and 34%, respectively. Whereas only 5% of the unfractionated LNC stimulated with OA became blasts within 24 hr, 20% of the cells of A+B responded to OA with blastogenesis (Fig. 2). If cells measuring >10 $\mu$m are consid-

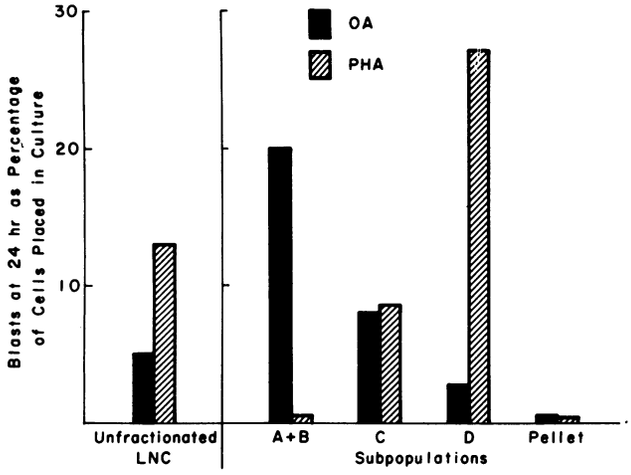


FIG. 2. Enumeration of OA- and PHA-responsive T lymphocytes in subpopulations separated on gradients on day 9. Cells ($5 \times 10^5$) cultured with OA or PHA. Counts at 24 hr after DNase treatment to eliminate clumping. Only pyroninophilic cells >12 $\mu$m in diameter were counted as blasts. LNC, lymph node cells.

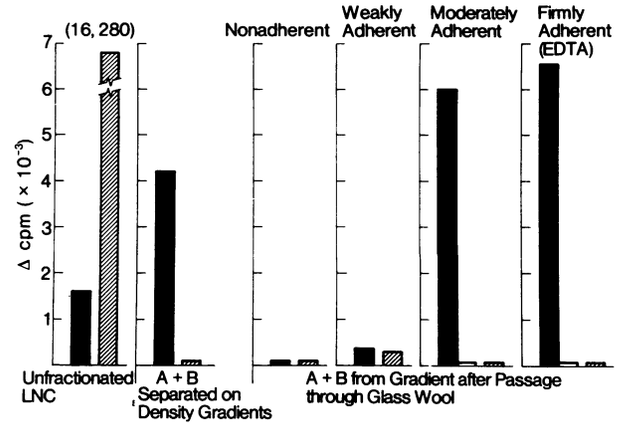


FIG. 3. Enrichment of OA-sensitized T lymphocytes by successive separations on density gradients and glass wool columns. Cells ($5 \times 10^5$) cultured with OA and PHA. ■, OA; □, OA after treatment with T-specific antiserum + C; ▨, PHA.

ered as blasts, approximately 30% of A+B cells entered blastogenesis. This represents a 4- to 6-fold enrichment over the unfractionated LNC population. If any of the OA-sensitized cells, e.g., those producing lymphotoxin in response to restimulation with antigen, do not become morphological blasts, the degree of enrichment may be still greater. Of the unfractionated LNC, 13% responded to PHA with early blastogenesis. There was virtually no blastogenic response to PHA by cells of A+B. PHA-responsive cells again were associated primarily with fraction D (24%). Band C contained intermediate numbers of cells responsive to both stimulants.

**Further Enrichment of OA-Sensitized T Cells on the Basis of Adherence.** When A+B cells were passed through glass wool, virtually all of the cells responding to OA with DNA synthesis were either firmly or moderately adherent (Fig. 3). These cells represent 37% of the total placed on the column; 26% were nonadherent or weakly adherent. Approximately one-third of the cells were lost during fractionation. Assuming that cell losses were nonselective, this procedure must have resulted in a 3-fold enrichment of OA-responsive cells so that most of the final cell population (A+B, adherent) were specifically sensitized cells. The DNA synthetic response to OA was ablated by treatment with T specific antiserum plus C (Fig. 3).

Table 1. Production of lymphotoxin by different subpopulations of sensitized lymph node cells in response to OA and PHA

| Lymph node cells | % Surviving fibroblasts* OA | PHA |
|---|---|---|
| *Sensitized* | | |
| Unfractionated | 6.3 | 0.5 |
| Band: A + B | 2.5 | 100.0 |
| C | 11.2 | 6.3 |
| D | 91.9 | 3.4 |
| Pellet | 100.0 | 1.9 |
| *Normal* | | |
| Unfractionated | 98.0 | 0.9 |

* Counts after 72 hr of culture with $10^7$ sensitized (9 days) or normal lymph node cells + $10^5$ purified normal macrophages (see *Materials and Methods*).

**Regulation of the PHA Response by Cells of Fraction A+B.** When $1 \times 10^5$ A+B cells, themselves unresponsive to PHA, were added to $5 \times 10^5$ sensitized or normal D band cells or to normal nonadherent lymph node or spleen cells, PHA responses by all of these cell types were potentiated 5- to 10-fold (11). In more recent experiments with these cell numbers (Fig. 4), potentiation was sometimes observed (Exps. 1, 2, and 6 in Fig. 4), but occasionally, instead, there was suppression of the PHA response (Exps. 3–5). When $2 \times 10^4$ or $4 \times 10^3$ A+B cells were added to $5 \times 10^5$ sensitized D cells, suppression was always observed and was usually total. When the number of A+B was reduced to $8 \times 10^2$ (Exp. 6), PHA responses returned to the control values or were slightly potentiated. The addition of macrophages (2%—approximately the proportion present in the unfractionated LNC) to these cultures did not result in a significant degree of potentiation or suppression. In Exp. 5, the addition of carrageenan at a concentration capable of completely destroying peritoneal macrophage monolayers (unpublished data) did not affect the levels of potentiation or suppression observed. When varying numbers of cells of intermediate density (C-band), which respond well to PHA, were combined with D-cells, the cultures gave predictably high responses, showing neither potentiation nor suppression.

**Regulation of the OA Response by Cells of Fraction D.** Cells of fraction D conversely exerted a "regulatory" influence on the response of sensitized lymphocytes in A+B to OA (Table 2), expressed either as potentiation (Exps. 2, 4, and 5) or suppression (Exps. 3 and 6).

## DISCUSSION

This study shows that cells specifically responsive to OA and performing the diverse functions of DNA synthesis, lymphotoxin release, and regulation of specific and nonspecific responses are enriched in the most buoyant fraction from bovine serum antigen gradients on day 9 after sensitization (see also refs. 1 and 10–12). The cells entering DNA synthesis in response to antigen have adherent properties and are further enriched on passage through glass wool. At both levels of purification, these cells are unresponsive to T mitogens. Five percent of unfractionated LNC form blasts in response to OA at 9 days. After successive passage through a bovine

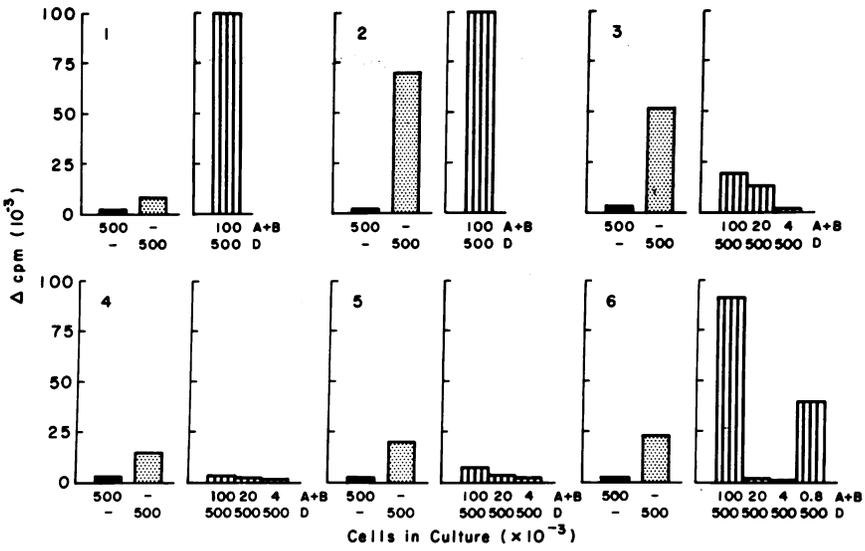


FIG. 4. Helper and suppressor effects of differing numbers of A+B cells added to $5 \times 10^5$ D cells on the PHA response in six successive experiments. In Exp. 5 carrageenan, at a final concentration of 1 mg/ml, was included in cultures.

serum albumin gradient and glass wool, 20–30% of A+B and well over 50% (probably) of A+B, adherent cells are similarly responsive. Such highly enriched populations are ideal for precise studies of surface markers, structural and biochemical peculiarities, and functional or physiologic attributes (e.g., homing properties), as well as for comparison with cells specifically sensitized against histocompatibility antigens (13–16).

The cells of A+B on day 9 are antigen-specific memory cells. They incorporate thymidine and produce lymphotoxin only in response to specific antigen and not in response to PHA. Several lines of evidence suggest that they are T cells (1). Cells that incorporate thymidine are killed by treatment with T-specific antiserum plus C (responses of band D to T mitogens also are ablated). A+B cells are fluorescence negative when stained for membrane-bound immunoglobulin. There are no complement receptor lymphocytes in A+B (unpublished observation). Finally, there are no germinal centers in the lymph nodes from which these cells are obtained.

The question remains open whether the T cells of A+B capable of DNA synthesis, lymphotoxin release, and regulation are distinct differentiation states of cells that initially could respond to T mitogens or subsets with different lineages, as does the question of their identity with each other. Cells responding to OA with DNA synthesis and cells capable of acting as suppressors of PHA responses both are adherent to glass wool (4, 5). The adherence properties of A+B cells producing lymphotoxin are under investigation. In an earlier study from this laboratory, nonadherent sensitized LNC taken at 9 days produced lymphotoxin when stimulated with antigen (17). However, these may have been cells like those of our denser bands which also contained OA-responsive cells. Certainly it is clear that the current widespread practice of using nylon columns to obtain purified T cells (nonadherent) (18) carries the risk that important functional subpopulations like those revealed in the present study and in others' studies (ref. 19; Cone, Askenase, and Gershon, personal communication) may be eliminated.

A particularly interesting finding in the present study was the occurrence of potentiation and suppression of DNA synthetic responses to both PHA and antigen when cells of the subpopulation in A+B interacted in different proportions with those in D (see also ref. 12). In some of our earlier experiments, potentiation was seen with A+B cells and suppression with cells of C (11). It appears probable from the present data that these results simply reflect the use of different numbers or possibly proportions of active cells. Suppression of PHA responses was effected when A+B cells were added to D cells in small aliquots and changed into potentiation when the proportion was increased. This finding resembles that of Haskill and Axelrad, who showed that

Table 2. Evidence of regulation in DNA synthetic responses of cell mixtures to antigen (OA)

| | [$^3$H]Thymidine incorporation (Δcpm) | | | | |
|---|---|---|---|---|---|
| | Before mixing | | Expected additive values | Actual values $1 \times 10^5$ A+B plus $5 \times 10^5$ D | % Expected additive values |
| Exp. no.* | $1 \times 10^5$ A+B | $5 \times 10^5$ D | | | |
| 1 | 1,700 | 1,700 | 3,400 | 3,200 | 94 |
| 2 | 1,900 | 2,800 | 4,700 | 8,700 | 185 |
| 3 | 3,800 | 13,200 | 17,000 | 2,600 | 15 |
| 4 | 3,800 | 200 | 4,000 | 10,200 | 255 |
| 5† | 3,500 | 200 | 3,700 | 6,500 | 177 |
| 6 | 50,000 | 400 | 50,400 | No response | 0 |

* Same experiments as in Fig. 4.
† In Exp. 5, carrageenan, at a final concentration of 1 mg/ml, was included in cultures.

large cells from spleens of mice primed with sheep erythrocytes suppress the plaque-forming cell responses of small lymphocytes when added in small aliquots but fail to do so in larger numbers (20). Kontiainen and Feldman (21) have similarly found carrier-specific suppression with numbers of keyhole limpet hemocyanin-primed cells as low as $1 \times 10^3$ in a system which tests helper activity as the target of suppression. Larger numbers of primed cells gave less suppression. The mixture of A+B cells in the range of $4 \times 10^3$ to $2 \times 10^4$ added to $5 \times 10^5$ D approximates the concentration of these cells in whole sensitized lymph node, and the resulting suppressed condition probably is physiologically significant. The potentiation observed with $1 \times 10^5$ A+B cells is more likely to represent an unphysiologic situation.

The mechanisms involved in the observed potentiation and suppression are probably complex. Cells like those used in the present study, if triggered either with antigen or PHA, release several mediators capable of enhancing and inhibiting DNA synthesis (22, 23). Cells in A+B possess surface receptors for PHA (Cone, Durkin, and Waksman, preliminary observations), and there are antigen-reactive cells in D. Thus the simplest possibility is direct triggering of mediator release by the stimulant, the observed result depending on the number of cells responding and the relative amounts of different mediators. It is equally possible, however, that there are feedback effects (24, 25). Striking examples of such effects involving interaction of immune and normal T cells and resulting in suppression are provided by recent studies of Gershon *et al.* (26), Calkins *et al.* (27), and Eardley and Gershon (28). The complexity of the interactions taking place in our system is underscored by the demonstration that the response to antigen was sometimes inhibited in the same cell combinations that gave a potentiated response to PHA and vice versa. It is clear that both normal and immune T cells are involved in the regulatory function.

The cells incriminated as suppressors in different systems do not conform to a single description. Suppression is seen with unstimulated rat spleen (5) and thoracic duct (29) cells. After a large systemic dose of antigen, suppressor activity is increased in the spleen and is demonstrable in the lymph node as well (4). Both the unstimulated splenic suppressor cell and the antigen-stimulated suppressor cell share with the sensitized T cell the property of adherence to glass wool (5, 6, 11). These are not macrophages, since their effect is not mimicked by addition of normal peritoneal macrophages or macrophages from rats given a large dose of antigen to the system (4, 5, and present study), nor does ablation of macrophages by carrageenan affect either the potentiation or suppression. Presumably both effects represent direct lymphocytic functions. The possibility of feedback effects between different types of cells implies that different cells will be described as helpers or suppressors depending upon how one looks at them.

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1. Durkin, H. G. & Waksman, B. H. (1975) *J. Immunol.* **115,** 170–176.
2. Ruddle, N. H. & Waksman, B. H. (1968) *J. Exp. Med.* **128,** 1237–1254.
3. Gery, I., Gershon, R. K. & Waksman, B. H. (1972) *J. Exp. Med.* **136,** 128–142.
4. Bash, J. A. & Waksman, B. H. (1975) *J. Immunol.* **114,** 782–787.
5. Folch, H., Yoshinaga, M. & Waksman, B. H. (1973) *J. Immunol.* **110,** 835–839.
6. Raff, M., Owen, J. T. & Greaves, M. (1975) *T and B Lymphocytes* (Academic Press, New York).
7. Lubaroff, D. L. (1973) *Transplantat. Proc.* **5,** 115–119.
8. Williams, R. M. (1973) *Cell. Immunol.* **9,** 435–444.
9. Willecke, K. & Ruddle, F. H. (1975) *Proc. Nat. Acad. Sci. USA* **72,** 1792–1796.
10. Durkin, H. G. & Waksman, B. H. (1973) in *Lymphocyte Recognition and Effector Mechanisms,* eds. Lindahl-Kiessling, K. & Osoba, D. (Academic Press, New York), pp. 599–604.
11. Bash, J. A., Durkin, H. G. & Waksman, B. H. (1975) in *Immune Recognition,* ed. Rosenthal, A. S. (Academic Press, New York), pp. 829–837.
12. Bash, J. A., Durkin, H. G. & Waksman, B. H. (1975) *J. Exp. Med.* **142,** 1017–1022.
13. Ginsburg, H. (1972) *Adv. Cancer Res.* **3,** 213–223.
14. Sprent, J. & Miller, J. F. A. P. (1972) *Cell. Immunol.* **3,** 213–230.
15. Rosenstreich, D. L., Shevach, E., Green, I. & Rosenthal, A. S. (1972) *J. Exp. Med.* **135,** 1037–1048.
16. Häyry, P. & Andersson, L. C. (1974) *Scand. J. Immunol.* **3,** 823–832.
17. Yoshinaga, M. & Waksman, B. H. (1973) *Ann. Immunol. (Inst. Pasteur)* **124C,** 97–120.
18. Julius, M. H., Simpson, E. & Herzenberg, L. A. (1973) *Eur. J. Immunol.* **3,** 645–649.
19. Stutman, O. (1975) *Ann. N.Y. Acad. Sci.* **229,** 89–105.
20. Haskill, S. J. & Axelrad, M. A. (1972) *Nature New Biol.* **237,** 251–253.
21. Kontiainen, S. & Feldman, M. (1975) *J. Immunol.,* in press.
22. Namba, Y. & Waksman, B. H. (1975) *Inflammation* **1,** 5–12.
23. Namba, Y. & Waksman, B. H. (1975) *J. Immunol.,* in press.
24. Harrison, M. R. & Paul, W. E. (1973) *J. Exp. Med.* **138,** 1602–1607.
25. Gershon, R. K., Liebhaber, S. & Ryu, S. (1974) *Immunology* **26,** 909–923.
26. Gershon, R. K., Orbach-Arbouys, S. & Calkins, C. (1974) in *Progress in Immunology II,* eds. Brent, L. & Holborown, J. (American Elsevier Publishing Co., Inc., New York), Vol. 2, pp. 123–133.
27. Calkins, C., Orbach-Arbouys, S., Stutman, O. & Gershon, R. K. (1975) *Fed. Proc.* **34,** abstr. 4642, p. 1037.
28. Eardley, D. & Gershon, R. K. (1975) *J. Exp. Med.,* **142,** 524–529.
29. Tardieu, M., Fradet, Y. & Daguillard, F. (1975) *Cell. Immunol.* **17,** 123–130.