

KINETIC STUDY OF T LYMPHOCYTES AFTER SENSITIZATION
AGAINST SOLUBLE ANTIGEN

III. Potentiation and Suppression of the PHA Response by
Antigen-Activated Lymphocytes of Low Density*

By JERRY A. BASH,‡ HELEN G. DURKIN,§ AND BYRON H. WAKSMAN

(From the Department of Pathology, Yale University School of Medicine, New Haven,
Connecticut 06510)

Initiation of immune responses may require interactions among several different cell types (1-3), and subsequent regulation of these responses has been shown to involve both helper and suppressor effects exerted by subpopulations distinct from effector cells (4). Whether helper and suppressor cells themselves comprise separate subpopulations is a matter of contention, with conflicting interpretations being derived from results in different experimental systems (5-7). We have previously reported that the proliferative response of sensitized rat lymph node cells stimulated with phytohemagglutinin (PHA) is suppressed by simultaneous stimulation with specific antigen in culture and that fractionation of sensitized lymph node cells on discontinuous bovine serum albumin (BSA) gradients results in the apparent separation of helper and suppressor subpopulations (8, 9). Here we report results obtained by recombining varying numbers of low density cells, which are enriched for antigen-sensitized cells (10) with high density cells, which respond preferentially to PHA. The magnitude of the proliferative response, i.e., the occurrence of potentiation or suppression, appears to depend on the ratio of the interacting cell types.

Materials and Methods

Sensitization and BSA Gradient Separation. Details of all techniques used here are given in (8, 10). Briefly, 5-to-8-wk old DA rats received 100 μ g ovalbumin (OA, 5 times crystalline, Nutritional Biochemicals, Cleveland, Ohio) in 0.1 ml complete Freund's adjuvant in each hind foot pad. Inguinal lymph node cells (LNC) were harvested at 9 days, washed once in cold, sterile RPMI 1640 containing penicillin and streptomycin (Associated Biomedics Systems, Inc., Buffalo, N. Y.) and separated by differential flotation on discontinuous BSA gradients (lot 126, Miles Laboratories, Kankakee, Ill.), 10, 20, 24, 27, and 30% BSA in RPMI. Fractions A, B, C, D, and a pellet were obtained as discrete bands at the BSA interfaces, in order of increasing density, and washed three times, A and B being combined in the experiments reported here. Cells defined as macrophages by their ability to take up neutral red were confined to but made up less than 3% of the A + B

* This work supported by U.S. Public Health Service grants AI-06112 and AI-06455 and contract CB-43926.

‡ Postdoctoral Trainee, NIH grant AI-291-08S1. Present address: Department of Microbiology, University of Cincinnati Medical Center, Cincinnati, Ohio 45221.

§ Fellow, Arthritis Foundation.

fraction. Viability assessed by trypan blue dye exclusion was always < 90% except for the pellet (> 80% viable).

Macrophages. Macrophages were strongly adherent cells, prepared from normal DA rat peritoneal cells after culture for 48 h in plastic petri dishes (8) in the presence of 10% normal DA rat serum. These contained > 95% macrophages, as assessed by their ability to phagocytize formalinized sheep red blood cells.

Lymphocyte Cultures. Cultures in microtiter plates (0.075 ml/well) (8) contained 5×10^5 D cells alone or in combination with varying numbers of A + B cells, C cells or macrophages, PHA-P (Difco Laboratories, Inc., Detroit, Mich.) at optimum stimulatory concentration (1.5 μ l/ml), 8% heat-inactivated normal DA rat serum and RPMI. In some experiments carrageenan (Sea Kem 9 Carrageenan, purified powder, Marine Colloids, Inc., Springfield, N.J.) was included at a final concentration of 1 mg/ml. After 48 h cultures were pulsed with 1 μ Ci [3 H]thymidine (NET-027A, spec act 0.2 Ci/mmol, New England Nuclear, Boston, Mass.), harvested 18–24 h later, and processed for counting (8). All values shown in figures and tables represent peak responses of stimulated cultures minus background (Δ cpm) averaged from counts of duplicate cultures.

Results

In a series of experiments in which low density (A + B) cells were recombined with 5×10^5 high density (D) cells in varying ratios, two findings became apparent (Table I, Fig. 1). With 1, 2, or 5×10^5 A + B cells there was frequently potentiation of the total response by as much as 10-fold. With lower numbers of cells, down to 4×10^3 , there was almost complete suppression. Finally, at 8×10^2 A + B cells there was no effect. Neither striking potentiation nor suppression was seen in cultures to which macrophages were added at a concentration (2%) which approximated that in unfractionated lymph node cells. Furthermore, carrageenan, at a concentration shown to destroy peritoneal macrophage monolayers completely (unpublished data), did not influence the degree of either potentiation or suppression (exp. 5).

Since A + B cells give a relatively modest response to PHA, the responses of recombined fractions A + B and D would theoretically approximate the response of D cells alone. Departure of the actual responses from this theoretical summation is shown for exp. 6 in Fig. 2. When varying numbers of cells of intermediate density (C), which respond well to PHA, were recombined with D cells in the same experiment, the combined cultures gave predictably high responses, with slight synergy and no suppression.

Discussion

The findings presented here indicate that low density cells, in large numbers, potentiate the response of high density cells and, in low numbers, completely suppress it. This apparently paradoxical result resembles that of Haskill and Axelrad, which showed that large cells obtained by velocity sedimentation of sheep red blood cell-primed mouse spleen cells suppressed the small lymphocyte plaque-forming cell response when added in small numbers but failed to suppress in larger numbers (11). Similarly, Kontiainen and Feldman have recently demonstrated that specific carrier-primed suppressor cells generated in vitro suppressed plaques to DNP in small numbers but did so less well when added in excess (12). These results are clearly incompatible with the concept of suppression as a result of "too much help" and support Dutton's evidence against such a possibility (5).

TABLE I
 Summary of Experiments with Fractions of Sensitized Lymph Node Cells Cultured with
 Phytohemagglutinin*

| Cell fraction | Peak [³ H]thymidine incorporation (Δ cpm $\times 10^3$) | | | | | | |
|----------------------------------|--|------|-------|------|------|------|------|
| | Exp. | 1 | 2 | 3 | 4 | 5† | 6 |
| D (5×10^5) | | 8.7 | 77.5 | 54.2 | 13.5 | 19.5 | 25.9 |
| A + B (5×10^5) | | 2.4 | 3.0 | 8.8 | 0 | 1.9 | 4.8 |
| D (5×10^5) + A + B: | | | | | | | |
| 5×10^5 | | — | — | — | — | 83.6 | 93.2 |
| 2×10^5 | | — | — | 21.6 | — | — | — |
| 1×10^5 | | 99.1 | 105.3 | 15.5 | 6.2 | 13.2 | 88.0 |
| 5×10^4 | | — | — | 0.4 | — | — | — |
| 2×10^4 | | — | — | — | 2.2 | 2.1 | 0.5 |
| 4×10^3 | | — | — | — | 1.0 | 1.5 | 0 |
| 8×10^2 | | — | — | — | — | — | 38.9 |
| Macrophages‡ (1×10^4) | | 7.0 | 60.3 | 20.9 | 23.8 | 45.7 | 12.5 |

* Optimal concentration = 1.5 μ l/ml.

† Same cell pool as in exp. 4 with carrageenan at 1 mg/ml.

‡ Purified from normal peritoneal cells.

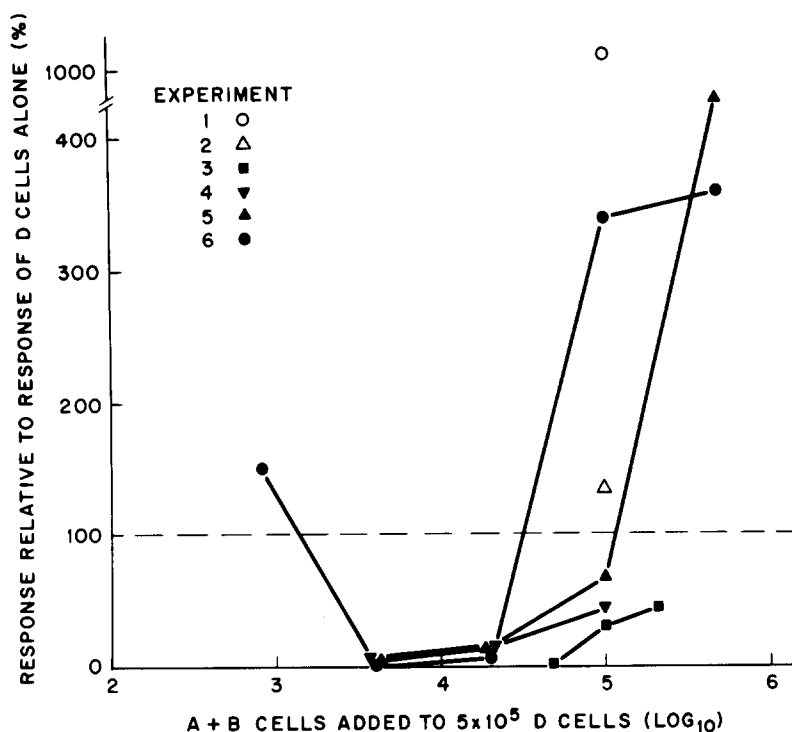


FIG. 1. Summary of data from six experiments showing the effect of recombining varying numbers of A + B cells with a constant number (5×10^5) of D cells on the DNA synthetic response to PHA. Values plotted on the ordinate are $([D + (A + B)]/D) \times 100$, where D + (A + B) and D, respectively, represent mean peak [³H]thymidine incorporation (Δ cpm $\times 10^3$) of the recombined fractions and of D cells cultured alone.

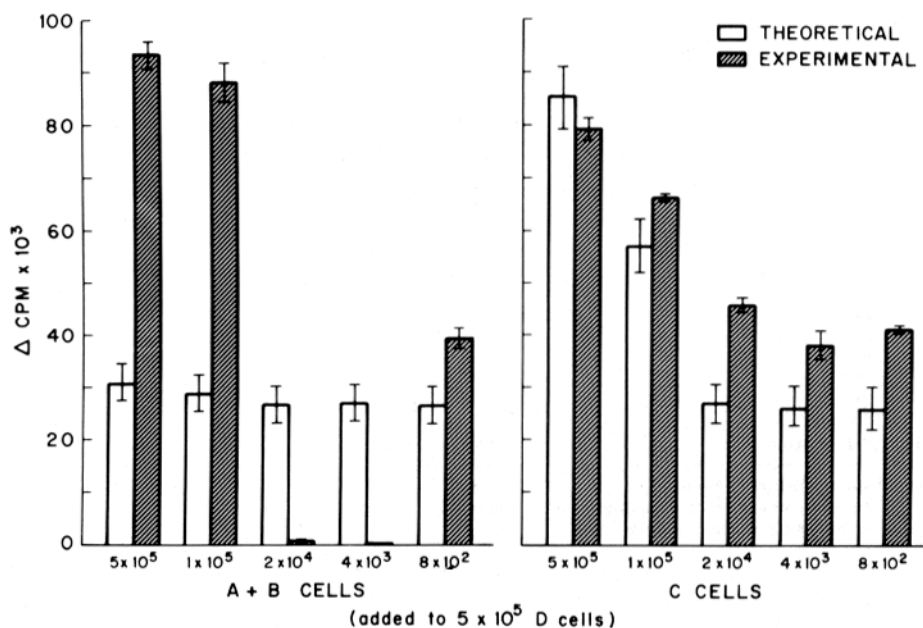


FIG. 2. Results from exp. 6, in which 5×10^5 D cells were cultured with varying numbers of A + B (left) or C cells (right). PHA responses (^3H)thymidine incorporation) of recombined fractions (experimental) are compared with the sum of responses of fractions cultured separately (theoretical).

The regulatory effects observed appear to be produced by sensitized lymphocytes rather than macrophages. Purified macrophages (1×10^4 cells) added to cultures of D cells occasionally gave some enhancement or inhibition of the response, but since macrophages are present in relatively small numbers ($\leq 3\%$) even in fractions enriched in large cells, the finding of profound suppression with as few as 4×10^3 A + B cells argues strongly against macrophage mediation of this effect. In addition, killing of macrophages in the cultures with carrageenan without abrogating either potentiation or suppression is further proof that macrophages do not participate in these phenomena in the system under investigation.

The mechanisms which underlie the regulatory effects seen in these experiments may operate to control immune responses in vivo. Since A + B cells constitute only about 5% of the total LNC population, the potentiation seen with large numbers of cells of this fraction may not be physiologically meaningful. On the other hand, the finding of complete suppression of D-cell responses by A + B cells at a ratio as low as 1:125 may well be meaningful in physiologic terms and is not without precedent. Sensitized lymphocytes have been shown to produce significant inhibition of normal macrophage migration in concentrations down to 0.6% acting by release of migration inhibitory factor (13). The effects seen in the present study may also be mediated by soluble factors. The A + B population is enriched in OA-sensitive cells, which are stimulated by antigen both to proliferate and to release lymphotoxin in culture (10). The

supernate from sensitized lymph node cells triggered with OA in culture also contains both an inhibitor of DNA synthesis and a potentiating or mitogenic factor (14, 15). It is likely that the predominance of potentiation or suppression at a given cell concentration is determined by the different concentration optima of the lymphokines responsible. It remains to be determined whether the different mediators are produced by the same or different cell types within the low density fraction. One may envision more complex mechanisms of control with multiple feedback effects between the subpopulations involved. As an example of this complexity, the response to antigen was sometimes inhibited in the same combinations that gave a potentiated response to PHA (16).

Summary

Lymph node cells of ovalbumin-sensitized rats were separated on the basis of buoyant density into fractions reciprocally enriched in cells responsive to ovalbumin or phytohemagglutinin (PHA). Recombination of high density and low density fractions in varying proportions resulted in potentiation or suppression of the DNA synthetic response to PHA in culture. The response of cultures containing equal numbers of high and low density cells was markedly greater than the sum of the two populations stimulated separately. However, when decreasing numbers of low density cells were cultured with a constant number of high density cells, profound suppression was observed.

The technical assistance of Miss Joan M. Carboni is gratefully acknowledged.

Received for publication 21 July 1975.

References

1. Claman, H. N., E. A. Chaperon, and R. F. Triplett. 1966. Thymus-marrow cell combinations. Synergism in antibody production. *Proc. Soc. Exp. Biol. Med.* 122:1167.
2. Mosier, D. E. 1967. A requirement for two cell types for antibody formation *in vitro*. *Science (Wash. D.C.)*. 158:1575.
3. Miller, J. F. A. P., and G. F. Mitchell. 1969. Thymus and antigen-reactive cells. *Transplant. Rev.* 1:3.
4. Gershon, R. K. 1973. T cell control of antibody production. *Contemp. Top. Immunobiol.* 3:1.
5. Dutton, R. W. 1973. Inhibitory and stimulatory effects of Concanavalin A on the response of mouse spleen cell suspensions to antigen. II. Evidence for separate stimulatory and inhibitory cells. *J. Exp. Med.* 138: 1496.
6. Gershon, R. K., S. Orbach-Arbouys, and C. Calkins. 1974. B cell signals which activate suppressor T cells. In *Progress in Immunology II*, Vol. 2. L. Brent and J. Holborow, editors. American Elsevier Publishing Co., Inc., New York. 123.
7. Herzenberg, L. A., and L. A. Herzenberg. 1974. Allotype suppression and production (?) by thymocytes and thymus-derived cells. In *Progress in Immunology II*. L. Brent and J. Holborow, editors. American Elsevier Publishing Co., Inc., New York. 2:111.
8. Bash, J. A., and B. H. Waksman. 1975. The suppressive effect of immunization on the proliferative responses of rat T cells *in vitro*. *J. Immunol.* 114:782.
9. Bash, J. A., H. G. Durkin, and B. H. Waksman. 1975. Suppressor and helper effects of sensitized T-cell subpopulations on proliferative T cell responses. In *Immune*

- Recognition. A. S. Rosenthal, editor. Academic Press, Inc., New York. 829.
10. Durkin, H. G., and B. H. Waksman. 1975. Kinetic study of T lymphocytes following sensitization against soluble antigen. I. Separation on density gradients. *J. Immunol.* 115:171.
 11. Haskill, S. J., and M. A. Axelrad. 1972. Cell-mediated control of an antibody response. *Nat. New Biol.* 237:251.
 12. Kontianinen, S., and M. Feldman. 1975. Specific suppression of in vitro-induced help. *J. Immunol.* In press.
 13. Bloom, B. R., and B. Bennett. 1966. Mechanism of a reaction *in vitro* associated with delayed-type hypersensitivity. *Science (Wash. D.C.)*. 153:80.
 14. Namba, Y., and B. H. Waksman. 1975. Regulatory substances produced by lymphocytes. I. Inhibitor of DNA synthesis in the rat. *Inflammation.* 1:5.
 15. Namba, Y., and B. H. Waksman. 1975. Regulatory substances produced by lymphocytes. II. Lymphotoxin in the rat. *J. Immunol.* In press.
 16. Durkin, H. G., J. A. Bash, and B. H. Waksman. 1975. Kinetic study of T lymphocytes after sensitization against soluble antigen. II. Separation of T cell populations capable of DNA synthesis, lymphotoxin release, and regulation of antigen and PHA responses on the basis of density and adherence. *Proc. Natl. Acad. Sci. U.S.A.* In press.