CELL INTERACTIONS IN THE SUPPRESSION OF IN VITRO ANTIBODY RESPONSES*

By CATHERINE E. CALKINS, SIMONE ORBACH-ARBOUYS, O. STUTMAN, AND R. K. GERSHON

(From Memorial Sloan-Kettering Cancer Center, New York 10021, and the Department of Pathology, Yale Medical School, New Haven, Connecticut 06510)

Downloaded from http://rup.silverchair.com/jem/article-pdf/143/6/1421/1087731/1421.pdf by guest on 30 September 2022

Initiation of humoral immune responses requires a complex series of interactions in which the antibody-forming cell precursors (bone marrow-derived lymphocytes, or B cells) differentiate to synthesize specific antibody after cooperation with "helper" thymus-derived lymphocytes (T cells) (1). Regulation of these responses also involves cellular and humoral interactions in which T cells have been identified as suppressors as well as helpers in several different systems (2, 3). Specific suppressor cells have frequently been observed in hyperimmune or tolerant cell populations (3–8). However, the precise mechanism responsible for the generation and activation of these suppressor cells remains to be elucidated. In the present study we report that unprimed "T" lymphocytes from the spleens of normal syngeneic mice act as specific suppressor cells in vitro when immune B cells are present. These data suggest that antibody (possibly the suppressive signal from the immune B cells) could have a central suppressive effect (9, 10) on immune responses through the activation of suppressor T cells.

Materials and Methods

Animals. C57BL/6, CBA, BALB/C, and BDF_1 (C57BL/6 × DBA/2) mice either from our breeding colony or from The Jackson Laboratory were used. Animals of the same age and sex were used within individual experiments. Normal mice were irradiated by a cesium source, their spleens removed and used within 6 h after the treatment.

Antigens. Sheep (SRBC,[1] from a single donor), horse (HRBC), burro (BRBC), and chicken (CRBC) erythrocytes were obtained from Colorado Serum Co., Denver, Colo. The trinitrophenyl (TNP) hapten was conjugated to CRBC for in vitro immunizations and to HRBC for assay using 2,4,6-trinitrobenzene sulfonic acid as described by Kettman and Dutton (11).

Immunizations in vivo with the erythrocyte antigens used doses of 2×10^8 red cells plus 0.03 ml pertussis vaccine containing 3×10^9 bacteria (Eli Lilly and Company, Indianapolis, Ind.) injected intraperitoneally (i.p.) 8 days before removal of the animals' spleens for culture. In vitro, the standard dose of erythrocytes was 3×10^6 red cells/culture. When TNP was one of the antigens in a particular experiment, both the conjugated and unconjugated erythrocytes were used at a lower dose (3×10^5 red cells/culture) which resulted in responses to SRBC similar to those of the higher dose of the unconjugated antigen.

Cell Suspension. Spleens were gently teased into suspension. Nylon wool purified cells ("T")

* This research was supported by U. S. Public Health Service Grants CA-08748, CA-16889, CA-0959B, and AI-10497.

[1] *Abbreviations used in this paper:* BRBC, burro red blood cells; CRBC, chicken red blood cells; HRBC, horse red blood cells; PFC, plaque-forming cells; SRBC, sheep red blood cells; TNP, trinitrophenyl.

were prepared from the spleen cell suspension according to Julius et al. (12), cell recoveries ranging between 15 and 30%. "B" cells were obtained by incubating 10^8 cells with 1 ml of heat inactivated anti-Thy 1.2 serum in a total volume of 5 ml minimal essential medium (MEM) for 45 min at room temperature. Complement (5 ml of guinea pig serum at 1:5 dilution) was then added for 30 min at 37°C. Cell recoveries ranged between 30 and 45%. The enriched T- and B-cell populations cultured individually were each incapable of mounting an antibody response to SRBC.

Culture Conditions. The culture conditions used here were described by Mishell and Dutton (13). The medium was additionally supplemented with 5×10^{-5} M mercaptoethanol. Immune spleen cells were cultured at various cell densities in the presence or absence of normal spleen cell populations, whole, or enriched T or B.

Assay. Duplicate cultures were harvested on day 3, 4, and 5 of culture and assayed for direct and indirect antibody-forming cells (PFC) by a slide modification of the Jerne plaque assay (13).

Results

Spleen cells from mice immunized 8 days previously with 2×10^8 SRBC and 0.03 ml pertussis vaccine intraperitoneally, were challenged in vitro with the same antigen. The resultant secondary antibody response (Table I) was depressed by the addition to the cultures of 10^7 normal spleen cells or 5×10^6 nylon wool-passed spleen cells (a procedure shown to enrich for T cells [12]). The response in these cultures was usually less than 30% of the response of 4×10^6 immune spleen cells alone, with approximately equal suppression of IgM and IgG responses (Table I). The addition of lower numbers of unfractionated normal spleen cells (5×10^6 cells) resulted in little if any suppression (Table I). The enrichment of the suppressor cells in the nylon wool-passed cell population suggests that these cells are thymus derived. Comparable results were obtained with either SRBC or BRBC as antigen.

To determine the radiosensitivity of the suppressor population, the "normal" spleens were taken from mice given 300–900 R whole body irradiation. The suppressive capacity of the normal cells was eliminated by 900 R and decreased by 600 R (Table II). Spleen cells from mice receiving 300 R retained their ability to suppress the response of 4×10^6 immune cells.

Because the immune cells and therefore the anti-SRBC antibody were constant in both the suppressed and the nonsuppressed cultures, the suppression could not have been caused by a peripheral removal of antigen by antibody. Alternatively, to determine if the increase in total cell numbers changed the antigen requirements for optimal stimulation, resulting in suboptimal responses, we tried to overcome the suppression by adding five times the standard dose of SRBC to the cultures. Increasing this challenge dose to 10^7 SRBC/culture did not alter the suppressed condition (Table II), indicating that the observed suppression was not due to dilution of the antigen by the higher numbers of cells in culture.

As a control for the effect of cell crowding, higher concentrations (10^7 and 1.4×10^7 cells/culture) of immune cells were cultured separately in each experiment. These cultures, however, reached an earlier peak by day 3 and had already declined to a rather low level by day 5 (Fig. 1). The immune cell concentration used in this study of suppression by normal spleen cells (4×10^6 cells/culture) was chosen because the response of those cultures was still high on day 5, when the normal cells themselves would have been responding in a primary antibody response to the antigen.

Downloaded from http://rup.silverchair.com/jem/article-pdf/143/6/1421/1087731/1421.pdf by guest on 30 September 2022

TABLE I

Suppressive Effect of Normal Spleen Cells on the Primed Antibody Response of Immune Spleen Cells

Immune*	Normal*	Day 5 PFC/culture‡	
		IgM ± SE	IgG ± SE
		% of 4 × 10⁶ imm. resp.	*% of 4 × 10⁶ imm. resp.*
4×10^6		899 ± 167	1,903 ± 469
4×10^6	10^7	145 ± 37 (16)§	387 ± 120 (20)
4×10^6	5×10^6	519 ± 104 (58)	2,244 ± 678 (118)
4×10^6	5×10^6 "T"	265 ± 57 (29)	799 ± 320 (42)

* 4×10^6 immune spleen cells (from BDF_1 mice primed with 2×10^8 SRBC and 0.03 ml pertussis i.p.) were cultured with normal spleen cells or normal spleen cells enriched on a nylon wool column for T cells and challenged with 3×10^6 SRBC. Similar results were obtained using CBA, C57BL/6, and BALB/C mice.

‡ These numbers are the averaged responses from 4–14 experiments.

§ The numbers in parentheses indicate the percent of responses of 4×10^6 challenged immune cells alone.

TABLE II

*Suppressive Capacity of Normal Cells at High Antigen Dose and Irradiated Normal Cells**

Antigen dose (SRBC/culture)	PFC/culture, 4×10^6 immune cells‡	Day 5 response			
		Normal‡	300 R§	600 R§	900 R§
		% of uncombined 4 × 10⁶ immune cell response			
3×10^5 or 3×10^6	737 ± 187	24 ± 8	32	58	110
10^7	865 ± 351	31 ± 12	ND	ND	ND

* 10^7 normal or irradiated spleen cells added to the cultures of 4×10^6 immune spleen cells (from BDF_1 mice primed 8 days earlier with SRBC plus pertussis). Spleens were removed from the irradiated animals within 6 h after irradiation.

‡ Averaged responses from three separate experiments, percentages calculated within each experiment and then averaged ± the standard error of the mean.

§ Data from two of the above experiments. Each dosage was only tested once, but duplicate cultures were assayed on each of 2 days with similar relative results on both.

Fig. 1 shows that the suppressive normal cells did not simply change the kinetics of the antibody production to yield an earlier peak response and decline, but suppressed at all stages of the response. The immune cells challenged in vitro with the priming antigen gave an enhanced PFC response with accelerated kinetics and a lower lymphocyte dose requirement (4×10^6 immune cells) for the optimal antibody response relative to that of normal spleen cells cultured at 10^7 cells/dish (Fig. 1). When 10^7 normal or 5×10^6 nylon wool-fractionated spleen cells were added to the cultures of 4×10^6 immune spleen cells, marked suppression was observed by day 3 of culture, subsequently increasing in degree through day 5. The peaks of the suppressed and the unsuppressed cultures of 4×10^6 immune cells differed only in magnitude and not in timing (Fig. 1).

Only the response to the specific antigen to which the immune cells were primed was suppressed by the addition of normal cells (Fig. 2). When 5×10^6 normal spleen "T" cells were added to 4×10^6 spleen cells from animals primed

Downloaded from http://rup.silverchair.com/jem/article-pdf/143/6/1421/1087731/1421.pdf by guest on 30 September 2022

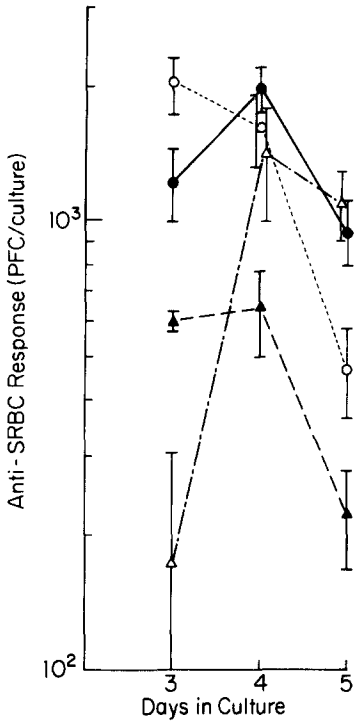

FIG. 1. Suppression of in vitro PFC response to SRBC in cultures containing both 4×10^6 immune spleen cells and 5×10^6 normal "T" cells to a level lower than that of immune cells cultured separately. Normal "T" cells cultured alone with SRBC gave no response. Kinetics of the humoral response to SRBC: (○), 10^7 immune spleen cells; (●), 4×10^6 immune spleen cells; (▲), 4×10^6 immune spleen cells and 5×10^6 normal "T" cells; (△), 10^7 normal spleen cells. Brackets represent the standard error of the mean of 5–8 experiments.

to SRBC and then cultured in the presence of both SRBC and TNP-CRBC, the antibody response to SRBC was depressed as in the previous experiments. The response to TNP, however, in the same cultures was not affected and was equivalent to that of 4×10^6 and 10^7 immune cells receiving the same challenge antigens, indicating specificity in effecting as well as in triggering the suppression (Fig. 2).

In the same cultures, the primary response of the 10^7 normal spleen cells was depressed by the presence of immune spleen cells (Table III). As few as 10^6 immune spleen cells suppressed this response to less than 40% of the normal primary response. When 10^6 spleen cells enriched in T were added, however, the responses were greatly enhanced suggesting that the immune B cell was responsible for the suppression. Treatment of the immune spleen cells with anti-Thy 1.2 antiserum and complement did, in fact, result in a B-cell population more active in suppressing than the unseparated immune cells (Table III).

Discussion

We have observed here the suppression of two types of immune responses which may have related mechanisms (14). Normal T cells specifically sup-

Downloaded from http://rup.silverchair.com/jem/article-pdf/143/6/1421/1087731/1421.pdf by guest on 30 September 2022

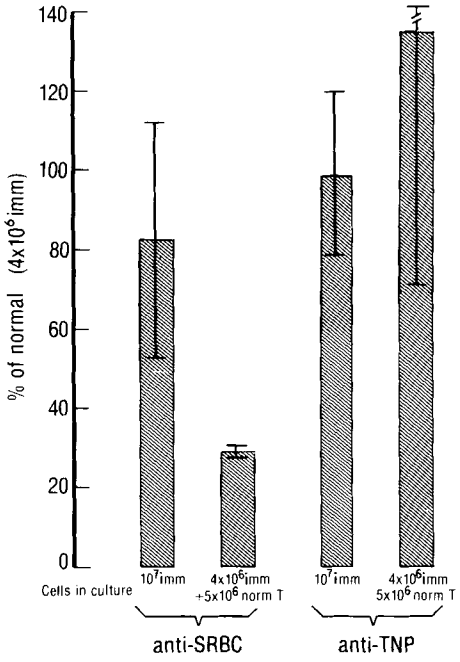

FIG. 2. Antigen specificity of the suppressor activity of 5×10^6 normal "T" cells on the PFC response to SRBC and TNP-CRBC challenge of 4×10^6 SRBC immune spleen cells. Anti-SRBC results are reported as percents of the anti-SRBC response (1,086 PFC/culture) of 4×10^6 SRBC immune spleen cells challenged 5 days earlier with the two antigens. Anti-TNP results are reported as percents of the anti-TNP response (359 PFC/culture) of the same 4×10^6 SRBC immune spleen cells. Percentages were calculated within individual experiments, and these values from three separate experiments were averaged. Brackets represent the standard error of the mean.

pressed secondary responses to SRBC in the presence of cells primed to SRBC. The data presented here do not preclude the possibility that other lymphocytes may have a similar effect. The radiosensitivity of the effect, however, suggests that macrophages are not responsible. These suppressor cells were also nonadherent to glass beads as well as to nylon wool (C. Calkins, unpublished).

Several factors indicate that this suppression was not due to cell overcrowding in the cultures. T-enriched normal cells suppressed to a greater degree than equal numbers (5×10^6 cells) of whole normal spleen cells (Table I). Responses in combination cultures of normal with immune cells were lower even than in cultures of high concentrations (10^7 immune cells/culture) of immune cells at all stages of the response (Fig. 1). Cultures suppressed to SRBC were still responsive at normal levels in a primary response to a noncross-reacting antigen, ruling out a possible toxic effect of too many cells in culture (Fig. 2). The specificity of the suppression suggests the involvement of specific antibody from or on the immune cells in the induction of the suppressor cells from the normal T-cell population.

Normal T cells (in the unseparated normal spleen) were able to cooperate with normal B cells in primary in vitro responses in the absence of immune B (Fig. 1,

Downloaded from http://rup.silverchair.com/jem/article-pdf/143/6/1421/1087731/1421.pdf by guest on 30 September 2022

TABLE III

*Suppressive Effect of Immune Spleen Cells on the Primary Antibody Response of Normal Spleen Cells**

Normal	Immune	Day 5 PFC/culture			
		Exp. 1	Exp. 2	Exp. 3	Exp. 4
10^7	–	5,412	570	450	1,414
10^7	4×10^6	175	345	ND	ND
10^7	10^6	860	ND	ND	541
10^7	10^6 "T"‡	ND	8,500	3,000	3,300
10^7	2×10^6 "B"§	ND	100	30	ND

* 10^7 normal spleen cells and SRBC were cultured with "immune" spleen cells from mice primed 8 days earlier with 2×10^8 SRBC and 0.03 ml pertussis.

‡ Enrichment for T cells produced by passage through nylon wool columns.

§ Enrichment for B cells produced by treatment with anti-Thy 1.2 antiserum and C.

Table III). In the system of suppression described above, it is likely that they become suppressors of the primed response through interactions with the immune B cells in the primed spleen population. In the suppression of the normal primary response, as described in Table III, suppression occurred only in the presence of immune B cells. It is possible that this suppression also occurred through the activation of a suppressor T cell from the normal spleen population by the immune B cells or their products.

A central regulatory role of specific antibody has already been noted in other systems (9). Antibody has been implicated acting in a feedback inhibition either alone (9) or as antigen-antibody complexes (10, 15). Experiments in which such complexes were added at defined concentrations to cultures of spleen cells and antigen resulting in specific tolerance are consistent with those presented here and support the notion of a regulatory feedback effect of specific antibody. The ineffectiveness of increased doses of antigen (Table III) in overcoming the suppression in the present system suggests that complexes rather than antibody alone act as the signal for suppression. Feedback suppression has also been suggested in systems where T cells regulate T-cell responses (16, 17). Recent evidence of suppression via anti-idiotype activity (18) suggests the alternative possibility that the normal cells may recognize a foreign idiotype present on the B cells immune to SRBC and react against these cells, preventing their response to the challenge antigen according to the network theory of Jerne (19).

It is also of interest that the primed T-cell population is less likely to suppress in this system, suggesting quantitative or qualitative differences between normal and immune T cells. In the primed animals, the SRBC-sensitive T cells may have been induced to become helper cells so that there is an increased ratio of helper to suppressor and therefore too few potential suppressors to be effective. It is alternatively possible that the potential suppressor T cells in the immune spleen have become insensitive (either temporarily or permanently) to the signal from the immune B cell which activates the suppressor cell. Although this is not yet fully understood, similar observations have been made in different systems (16, 20). In vivo, the nonimmune normal T cells capable of becoming suppressor cells may come from a site relatively less exposed to antigen or come

Downloaded from http://rup.silverchair.com/jem/article-pdf/143/6/1421/1087731/1421.pdf by guest on 30 September 2022

as newly differentiated cells from the thymus and act to shut off ongoing responses.

These data support the concept of an immune system with regulatory cells induced by interaction with an immune cell and/or its product. The mechanisms of the induction and the suppression in this system are being investigated further.

Summary

Normal T and immune B lymphocytes interact in a fashion that leads to suppression of the immune response. Normal spleen cells added to cultures of primed spleen cells specifically suppressed both the IgM and IgG secondary antibody response of the primed cells to less than 30% of the response of the immune cells cultured alone. Cell crowding as a possible in vitro artifact was ruled out. The suppression was specific for the priming antigen, even when the specific and nonspecific antigens were included in the same cultures. Suppression required both normal T and immune B cells to be present in culture. We suggest that the immune population produces a signal that can induce normal T cells to become specific suppressor cells. This form of interaction may represent an important regulatory (homeostatic) mechanism in the immune system.

We wish to thank Dr. Martin Raff for his gift of anti-θ-antiserum to R. K. Gershon. We are also grateful to Lillian Tang for her conscientious, able, and willing assistance.

Received for publication February 1976.

Bibliography

1. Möller, G. 1969. Thymus and antigen-reactive cells. *Transplant. Rev.* 1.
2. Gershon, R. K., P. Cohen, R. Hencin, and S. A. Liebhaber. 1970. Suppressor T cells. *J. Immunol.* **108**:586.
3. Gershon, R. K. 1975. A disquistion on suppressor T cells. *Transplant. Rev.* **26**:170.
4. Tada, T., M. Taniguchi, and T. Takemori. 1975. Properties of primed suppressor T cells and their products. *Transplant. Rev.* **26**:106.
5. Ha, T-Y., B. H. Waksman, and H. P. Treffers. 1974. The thymic suppressor cell. I. Separation of subpopulations with suppressor activity. *J. Exp. Med.* **139**:13.
6. Benjamin, D. C. 1975. Evidence for specific suppression in the maintenance of immunologic tolerance. *J. Exp. Med.* **141**:635.
7. Thomas, D. W., W. K. Roberts, and D. W. Talmage. 1975. Regulation of the immune response. Production of a soluble suppressor by immune spleen cells *in vitro*. *J. Immunol.* **114**:1616.
8. Sieckmann, D. G., J. M. Chiller, and W. O. Weigle. 1975. *In vitro* induction of specific suppressor activity in turkey gamma-globulin-primed spleen cell cultures. *Fed. Proc.* **34**:1020 (Abstr. 4543).
9. Bystryn, J-C., I. Schenkein, and J. W. Uhr. 1971. A model for the regulation of antibody synthesis by serum antibody. *Prog. Immunol.* **1**:627.
10. Diener, E., and M. Feldmann. 1972. Relationship between antigen and antibody induced suppression of immunity. *Transplant. Rev.* 8:76.
11. Kettman, J., and R. Dutton. 1970. An *in vitro* primary immune response to 2,4,6-trinitrophenyl substituted erythrocytes: response against carrier and hapten. *J. Immunol.* **104**:1558.

Downloaded from http://rup.silverchair.com/jem/article-pdf/143/6/1421/1087731/1421.pdf by guest on 30 September 2022

12. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3**:645.
13. Mishell, R. I., and R. Dutton. 1967. Immunization of dissociated spleen cell cultures from normal mice. *J. Exp. Med.* **126**:423.
14. Gershon, R. K., S. Orbach-Arbouys, and C. Calkins. 1974. B cell signals which activate suppressor T cells. *Prog. Immunol.* **2**:123.
15. Gorczynski, R., S. Kontiainen, N. A. Mitchison, and R. E. Tigelaar. 1974. Antigen-antibody complexes as blocking factors on the T lymphocyte surface. *In* Cellular Selection and Regulation in the Immune Response. G. M. Edelman, editor. Raven Press, New York. 143.
16. Eardley, D. D., and R. K. Gershon. 1975. Feedback induction of suppressor T-cell activity. *J. Exp. Med.* **142**:524.
17. Durkin, H. G., J. A. Bash, and B. H. Waksman. 1975. 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. *Proc. Natl. Acad. Sci.* **72**:5090.
18. Eichmann, K. 1975. Idiotype suppression. II. Amplification of a suppressor T cell with anti-idiotypic activity. *Eur. J. Immunol.* **5**:511.
19. Jerne, N. K. 1974. Clonal selection in a lymphocyte network. *In* Cellular Selection and Regulation in the Immune Response. G. M. Edelman, editor. Raven Press, New York. 39.
20. Gershon, R. K. 1974. Lack of activity of contra-suppressor T cells as a mechanism of tolerance. *In* Immunological Tolerance: Mechanisms and Potential Therapeutic Application. D. H. Katz and B. Benacerraf, editors. Academic Press, Inc., New York. 441.

Downloaded from http://rup.silverchair.com/jem/article-pdf/143/6/1421/1087731/1421.pdf by guest on 30 September 2022