A HEMOLYTIC PLAQUE ASSAY FOR
ACTIVATED MURINE T CELLS*

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The hemolytic plaque assay (1) for the detection and study of antibody-secreting cells has been a fundamental tool in the armamentarium of cellular immunologists since its introduction almost two decades ago. The basic Jerne technique detects individual cells secreting antibody of a particular specificity. A recently described modification of this assay permits the detection of all immunoglobulin-secreting cells, regardless of their specificity (2). The method employs sheep erythrocytes (SRBC)[1] coated with staphylococcal protein A in a plaquing medium which also contains rabbit anti-mouse Ig, guinea pig complement, and Ig-secreting mouse plasmacytoma cells. Plaques developed around a fraction of the cells, presumably as a consequence of Ig-anti-Ig complexes binding to the protein A-coated RBC and fixing complement. A compelling feature of this procedure is that it permits the detection of any cell secreting a product for which an antiserum is available, provided that the antibody can bind to protein A and can fix complement.

Because splenic T cells activated with Concanavalin A release a variety of products, including soluble helper and suppressor factors (3), it seemed feasible that antisera could be generated against these products and used to detect T cells secreting the products in a similar plaque assay. This communication describes the development of such an assay, provides conclusive evidence that the plaque-forming cells are T lymphocytes and presents quantitative and kinetic data on the T-cell responses which are detected. It will be apparent that this plaque assay detects activated T cells with a facility comparable to that of the classical Jerne technique for B cells.

Materials and Methods

Mice. BDF$_1$ and BALB/c, matched for age and sex, were used through these experiments and were purchased from Simonson Labs, Gilroy, Calif., or were kindly provided by other investigators.

Mitogens. Concanavalin A (Con A) was obtained as a lyophilized powder from Miles Laboratories Inc., Miles Research Products, Elkhart, Ind. Lipopolysaccharide from *Escherichia coli* (LPS) was obtained from Sigma Chemical Co., St. Louis, Mo. Phytohemagglutinin M was purchased from Difco Laboratories, Detroit, Mich.

Culture Conditions. Cells were cultured in Cluster 24 tissue culture plates (Costar Data Packaging, Cambridge, Mass.) at a concentration of 1.0×10^7 spleen cells/ml in 0.5-ml

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[1] *Abbreviations used in this paper*: BSS, balanced saline solution; Con A, concanavalin A; DNP, 2,4-dinitrophenyl; LPS, lipopolysaccharide; PFC, plaque-forming cell; PHA, phytohemagglutinin M; SRBC, sheep erythrocytes.

cultures. Cultures were incubated in plastic boxes in an atmosphere of 10% CO_2, 83% N_2, and 7% O_2.

Medium. The medium used in these experiments was RPMI-1640 supplemented with glutamine, gentamicin, and 5% fetal calf serum.

Preparation of Culture Supernates. Spleen cells were cultured for 48 h in serum-free medium in the presence of 1 μg of Con A. Thereafter, cells were removed by centrifugation and Con A was eliminated by absorbing the supernatant fluids with Sephadex G-50 (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, N. J.) at 4°C at a ratio of 1 ml hydrated Sephadex to 1 ml fluid (4). Supernates were concentrated to 0.2 of the initial volume (in dialysis bags) by perevaporation in a stream of air.

Developing Antisera. Rabbits were given four subcutaneous injections at 10-d intervals of 1 ml of crude Con A-induced supernate in complete Freund's adjuvant (Difco Laboratories). The animals were bled after each boost and sera was stored at −70°C. For some experiments, the IgG fraction of rabbit antiserum was prepared by batch fractionation with DEAE-cellulose.

Absorption of antiserum to remove antibodies directed against mouse immunoglobulins was carried out using an immunoadsorbent consisting of affinity-purified mouse Ig coupled to Sepharose 4B. Antiserum and adsorbent were mixed in equal volumes and incubated overnight at 4°C. The absorbed antiserum was recovered by centrifugation.

Coupling of Protein A to SRBC. Staphylococcal protein A (Sigma Chemical Co.) was coupled to SRBC as described by Gronowicz et al. (2).

Plaque Assay. Cells were harvested and washed twice in cold balanced saline solution (BSS) and adjusted to the desired concentration. One part of prewarmed Eagle's minimal essential medium (Grand Island Biological Co., Grand Island, N. Y.) containing 2.0 mg/ml of DEAE-dextran (Pharmacia, Uppsala, Sweden) was added to an equal volume of 1.4% agar (Difco Laboratories), in H_2O. Two-ml aliquots of the mixture were put in 5-ml plastic tubes at 46°C in a water bath. Thereafter, 0.1 ml of the protein A-coated SRBC diluted 1:4 in BSS, 0.05 ml of the lymphocyte suspension, and 0.1 ml of developing serum (appropriately diluted) were added to each tube. The ingredients were mixed and spread on 9-cm plastic Petri dishes (Falcon Labware, Div. of Becton, Dickinson, & Co., Oxnard, Calif.) which were then incubated at 37°C. 2 ml of guinea pig complement, diluted 1/50, were added to each dish after 1 or 18 h. Plates were incubated for another hour at 37°C. When lymphocytes were stained for Thy-1 antigen, 0.1 ml of a mixture containing agar, lymphocytes, developing serum, SRBC, and complement was put as a drop on a microscope slide and a cover slide immediately put on the drop. This procedure gave a thin layer of agar which facilitated the scoring of stained lymphocytes in the middle of plaques. Plaque-forming cells were counted using indirect light.

Cell Fractionation. B cells were removed from spleen cell suspensions as previously described by allowing the spleen cell suspensions to settle onto plastic Petri plates precoated with affinity-purified rabbit anti-mouse Ig (5, 6). This procedure routinely yielded T-cell preparations that were >95% pure as judged by anti-Thy-1 cytotoxicity and by staining with fluorescent anti-Ig.

Indirect Fluorescence Assay for Thy-1. (Fab′)$_2$ fragments from anti-Thy-1 antibody were prepared by standard procedures and substituted with DNP groups to a level which did not curtail antigen binding. The DNP-(Fab′)$_2$ conjugates were reacted for 30 min at 4°C with spleen cells. The cells were washed and then treated with fluorescein-conjugated (Fab′)$_2$ fragments of rabbit anti-DNP antibody prepared against 2,4-dinitrophenyl (DNP)-ovalbumin conjugates. The immunoglobulin reagents used in this procedure were (Fab′)$_2$ fragments to avoid reactions with Fc receptors on lymphoid cells.

Rosette Formation. Spleen cells from BDF$_1$ mice (2 × 10^7/ml) were reacted at 4°C for 30 min with 1 ml of anti-supernate antiserum diluted 1:20. Thereafter, cells were washed twice and resuspended in 1.0 ml, of BSS. 1 ml of a 0.2% suspension of aseptically coupled protein A-SRBC was added to the lymphocyte suspension and the mixture was incubated for 15 min at 4°C. Cells were then centrifuged at 1,000 rpm for 1 min and gently resuspended in 3 ml of RPMI-1640 medium. Rosetting and nonrosetting cells were separated by Ficoll-Isopaque (Sigma Chemical Co., St. Louis, Mo.) density gradient and the erythrocytes were lysed with ammonium chloride.

T-Cell Long-term Cultures. Spleen cells from BDF$_1$ mice were fractionated on anti-mouse Ig-coated plastic Petri dishes. The nonadherent population (<5% Ig positive) was cultured for 48

h at a density of 10^7 cells/ml in the presence of 2 or 20 µg/ml of Con A. Thereafter, cells were harvested and recultured at 5 × 10^5/ml in RPMI-1640 medium containing 2-mercaptoethanol (5 × 10^{-5} M), 10% fetal calf serum, glutamine (2 mM), gentamicin, and 2 or 20 µg/ml of Con A. At 3-d intervals, each well (containing 0.5 ml of cell suspension) received 0.1 ml Con A-induced culture supernate concentrated fivefold. Cell growth was monitored in a Burker hemocytometer (American Optical Corp., Buffalo, N. Y.) and cells were resuspended at 5 × 10^5/ml every 5 d.

Induction of Cytotoxic Cells.   Allogenically sensitized cytotoxic cells from BALB/c mice (against L929 cells) were kindly provided by Dr. W. Rosenau (Department of Pathology and Cancer Research Institute, University of California, San Francisco). They were prepared according to a modification of the method of Rosenau and Moon (7).

Anti-Thy 1 and Anti-Ly 2.2 Treatment.   Spleen cells from BDF_1 mice were treated for 30 min at 4°C either with anti-mouse Thy 1 antiserum generated in rabbits using mouse brain as antigen and rendered specific as described previously (8) or with a monoclonal anti-Ly 2.2 serum (from Dr. U. Hammerling, Sloan-Kettering Institute, N. Y.). Thereafter, cells were washed in BSS and treated with a selected source of complement for another 30 min at 37°C. Dead cells and erythrocytes were separated by Ficoll-Isopaque density gradient centrifugation.

Results

Production of Antisera against Con-A-induced Culture Supernates.   Each of two rabbits injected four times subcutaneously with 1.0 ml of fivefold concentrated Con A supernate produced antiserum which developed plaques by spleen cells cultured for 48 h in the presence of 10 µg/ml of Con A (Table I, Fig. 1). The two sera differed in potency and plaque formation by Con A-activated lymphocytes was dependent on the concentration of the developing serum. Omission of either the anti-supernate serum or complement from the assay abrogated the appearance of plaques. Negative results were also obtained when serum from normal rabbits was substituted for the anti-supernatant serum, demonstrating the dependence of plaque formation on anti-supernatant antibody. Unless otherwise stated, in the experiments described below, serum from rabbit 2 at a dilution of 1:4 was routinely used.

Response to Selective Mitogens.   The effect of selective T-cell mitogens on the induction of plaque-forming cells (PFC) was investigated. The dose-response curves of spleen cells cultured for 24 h in the presence of various concentrations of Con A and phytohemagglutinin (PHA) revealed that both mitogens induced strong responses at sufficiently high concentrations (Fig. 2 A). In these experiments, complement was added to the plates after 1 h of incubation. It was considered of interest to determine if longer plaquing times would alter the magnitude or quality of the response. Accordingly, the experiments were repeated except that complement was added after 18 h of incubation (Fig. 2 B). It is apparent that the longer plaquing time resulted in a dramatic increase in the number of PFC and in a shift in the response to lower doses of mitogen. The data indicate that high concentrations of mitogen induce a rapid release of the product(s) being detected, whereas lower concentrations induce more gradual secretion by a larger proportion of cells. Consequently, in order to achieve optimal sensitivity all subsequent experiments were performed using an 18-h plaque assay.

Kinetic analysis of these mitogen responses disclosed peak PFC values after 1–2 d of culture (Fig. 3). On the other hand, the selective B-cell mitogen lipopolysaccharide (LPS) induced negligible PFC responses over the entire 5-d period studied. The slight enhancement seen on days 2 and 3 is likely a result of a polyclonally induced B-cell

TABLE I

Anti-Con A Supernatant Activity of Rabbit Serum Measured by Protein A Plaque Assay

Rabbit*	No. of injections	PFC/culture						
		Serum dilutions						
		1/1	1/2	1/3	1/4	1/5	1/6	1/7
1	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0
	4	4,160 ± 124	5,640 ± 196	1,224 ± 68	0	0	0	0
2	1	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0
	3	0	3,244 ± 60	2,860 ± 120	0	0	0	0
	4	0	3,600 ± 136	4,800 ± 600	5,600 ± 312	2,080 ± 80		

* Rabbits received the indicated injections of 1.0 ml of concentrated Con A supernate at 10-d intervals. Serum was always collected 7 d after each injection. Activity was measured by PFC response of spleen cells cultured for 48 h in the presence of 10 μg/ml of Con A.

anti-SRBC response, which always occurs after exposure to LPS (8). These findings minimize the likelihood that the PFC in this assay are B cells.

Nature of the PFC. The most immediate priority in this study was to establish the identity of the PFC. For this purpose, the following experiments were carried out.

(a) Cultured cells were assayed for the Thy-1 antigen by the indirect immunofluorescent technique described in Materials and Methods. The cells were plaqued immediately after staining. About 50% of the lymphocytes in the centers of plaques stained strongly for Thy-1. Because the plaque assay is carried out under conditions which lead to capping and internalization of antigen-antibody complexes, this figure represents a minimum value for Thy-1-positive PFC.

(b) Spleen cells were cultured with 10 μg of Con A for 24 h, at which point they were treated with anti-Thy-1 serum and complement, with a monoclonal anti-Ly-2.2 serum and complement, or with complement alone. Dead cells were removed by Ficoll-Isopaque density gradient centrifugation before plaquing to minimize the detection of products released by such cells. Treatment with anti-Thy-1 or with anti-Ly-2.2 virtually eliminated PFC (>95% depletion; Table II). On the other hand, these reagents had no significant effect on the polyclonal response of spleen cells to LPS (data not shown).

(c) Spleen cells were depleted of B cells by adherence to plastic surfaces coated with affinity-purified rabbit anti-mouse Ig antibody. The nonadherent population (<5% Ig-positive by fluorescence) was then cultured for 24 h with 10 μg/ml Con A and assayed for PFC. Removal of B cells doubled the proportion of PFC (Table II), providing additional evidence that at least the vast majority of PFC in the assay are T cells.

(d) Although the inability to detect PFC responses after polyclonal activation of spleen cells with LPS (Fig. 3) provided persuasive evidence that this plaque assay did not detect immunoglobulin-secreting cells, to conclusively eliminate that possibility the anti-supernatant serum was absorbed with a polyvalent mouse Ig immunoadsorbent. This adsorbent, which was very effective for the removal and purification of anti-
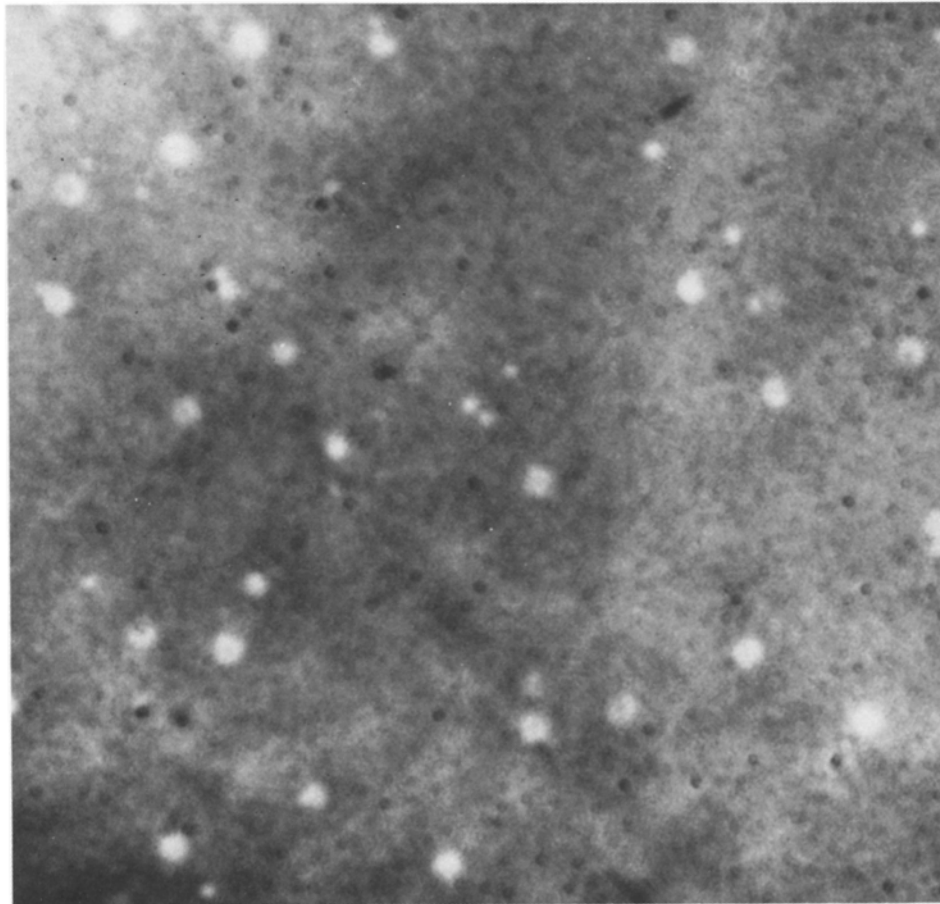
Fig. 1. Plaques formed by spleen cells from BDF₁ mice cultured for 24 h with 10 μg/ml of Con A. Complement was added after 1 h of incubation in the plaquing medium.

mouse Ig antibodies (6), had no effect whatsoever on the developing capacity of the antiserum in the PFC assay (Fig. 4), providing conclusive evidence that Ig-secreting cells were not being detected.

The above findings, combined with the selective activation of PFC by T-cell mitogens, convincingly attest to the T-cell nature of PFC detected by this assay.

PFC in Long-term Cultures of T Cells. Recent advances in cell culture techniques have made it possible to selectively grow T or B cells for extended periods of time (9). Taking advantage of the new methodologies, fractionated T cells were maintained in culture for 10 d in the continuous presence of 2 or 20 μg of Con A and T cell growth factor (provided by Con A-induced culture supernate). The method permits only those cells which respond to Con A to survive and divide. About 2% of T cells which had grown for 10 d in the continuous presence of 2 or 20 μg of Con A formed plaques (Fig. 5). Again, it was found that addition of complement after 1 h of plaquing detected only those cells activated by the high dose of mitogen, whereas low doses of mitogen induced much slower secretion detectable after the longer plaquing period.
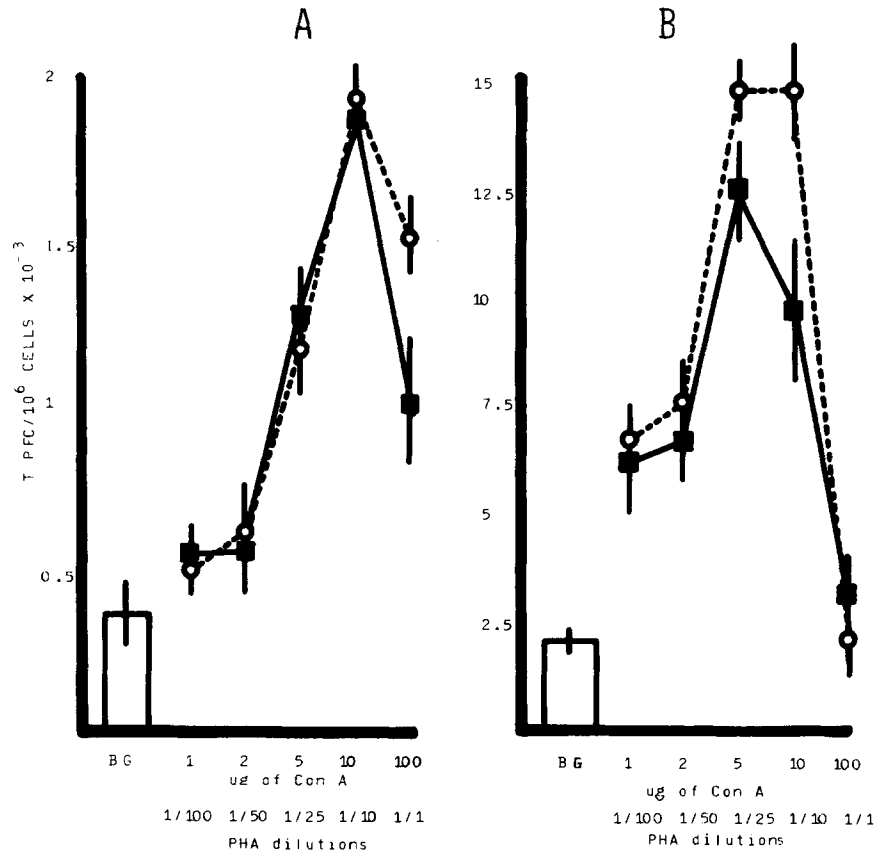
Fig. 2. Spleen cells from BDF₁ mice were cultured for 24 h with the indicated doses of Con A (O) or PHA (■), after which the PFC responses were assayed. Complement was added either 1 h (A) or 18 h (B) after plaquing.

PFC Detection In a Population of Cytotoxic Cells.   It was of interest to investigate whether the anti-supernatant antiserum could detect cells with specific functional activity. For these studies, murine spleen cells were sensitized to alloantigens according to the method of Rosenau and Moon (7). BALB/c mice received one intrasplenic injection of 5×10^6 L929 cells. 1 w later, spleens were removed and cells were incubated for 18 h on a monolayer of L929 cells. Thereafter, nonadherent and adherent (cytotoxic) cells were removed and tested for PFC. As seen in Fig. 6, the adherent cells plaqued with an efficiency of $\cong 10\%$, whereas significant responses were not made by the nonadherent or control populations. It is also noteworthy that the adherent population was >95% Thy-1-positive by fluorescent microscopy. These results indicate that the anti-supernatant antiserum detects products released by T cells activated by allogeneic targets, which are most likely cytotoxic T cells.

Anti-supernatant Antibodies Selectively Bind to T Cells.   The capacity of anti-supernatant antibody to bind to lymphocytes was investigated. Unfractionated or B-cell-depleted spleen cells were sequentially reacted with the IgG fraction of the anti-Con A supernatant serum and fluorescent protein A. After this treatment, $\cong 50\%$ of spleen cells and 80% of enriched T cells were positive by visual fluorescent microscopy. The
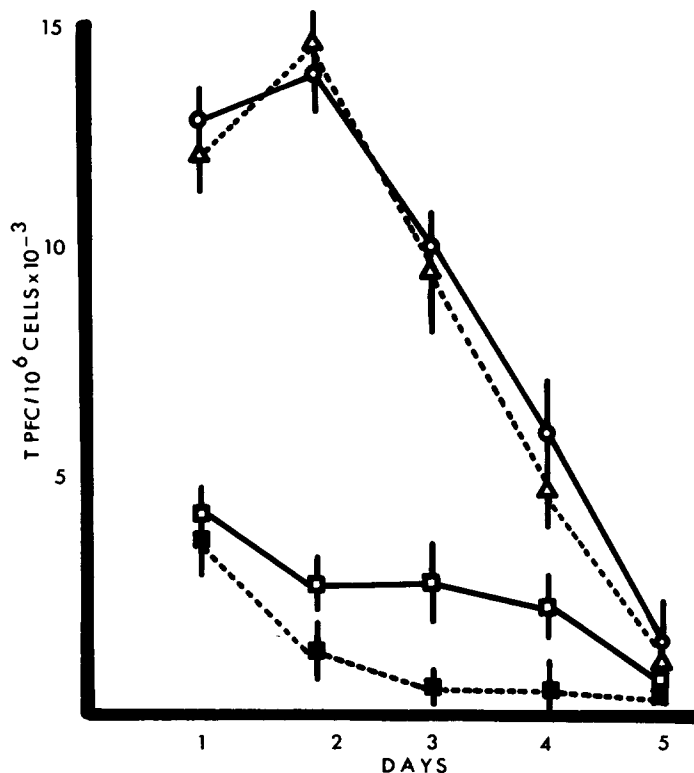
FIG. 3.   Spleen cells of BDF$_1$ mice were cultured for 5 d with nothing (■), 100 μg/ml LPS (□), 10 μg/ml Con A (△), or PHA (○). PFC responses were assayed each day.

TABLE II

Effect of Various Treatments on Plaque-forming Cells

Mitogen	Treatment	PFC/10^6 Cells
—	—	1,800 ± 124
Con A	—	14,400 ± 284
Con A	B-depleted	29,700 ± 697
Con A	Complement	11,720 ± 252
Con A	α-Thy-1 + C	340 ± 55
Con A	α-Ly-2.2 + C	480 ± 62

Spleen cells from BDF$_1$ mice were cultured for 48 h in the presence of 10 μg of Con A. Thereafter, cells were harvested, treated as indicated, and dead cells were removed by Ficoll-Hypaque density gradient centrifugation.

findings, therefore, indicate that the antiserum detects products which are expressed on the surface of a majority of T cells. In a subsequent series of experiments, lymphocytes which bound the anti-Con A supernatant antibody were isolated by rosetting with SRBC coated with protein A. Thereafter, the rosetting and nonrosetting populations were cultured for 24 h in the presence of 10 μg of Con A and then assayed for PFC, which were almost exclusively in the rosetting fraction (Fig. 7). Because this fraction was almost completely comprised of T cells, the findings again support the T-cell nature of the PFC.
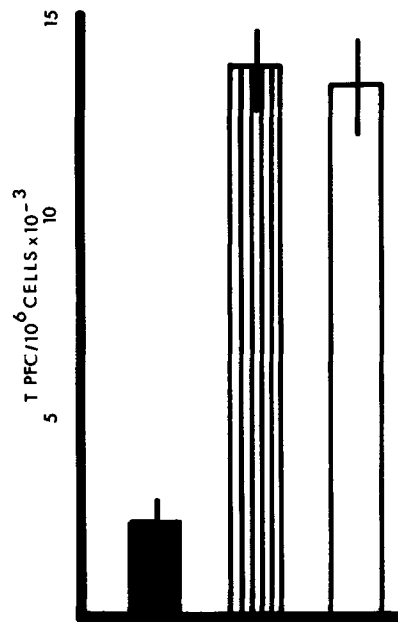
FIG. 4. PFC responses of BDF_1 spleen cells cultured for 24 h with 10 μg/ml of Con A and assayed with unabsorbed ($\boxplus$) or Ig column-absorbed ($\square$) developing serum. The PFC response of cells cultured without Con A and developed with unabsorbed serum is also shown ($\blacksquare$).

Induction of T-PFC by Supernates from Con A-activated Spleen Cells. T cells activated by mitogenic doses of Con A (1 μg) release products, some of which have helper or suppressor activities for B cells (3). It was, therefore, of interest to determine if products were also released which influenced the appearance of T-PFC. The supernates from cultures of BDF_1 spleen cells cultured with 1.0 μg of Con A for 48 h were absorbed with Sephadex G-50 to remove residual Con A, concentrated to 0.2 initial volume, and added to fresh spleen cell cultures for 48 h, after which the number of T-PFC were determined (Fig. 8). The Con A supernate did, indeed, induce a T-PFC response as compared to supernates from control cultures without Con A and to positive controls directly induced with Con A. As already indicated, this activity cannot be a result of residual Con A in the absorbed supernates because the amount of Con A required to induce a substantial T-PFC response (at least 2 μg) is higher than that used to induce the supernate (1 μg) and, further, the supernate was absorbed with Sephadex G-50 to remove residual Con A. The absorbed supernate was unable to induce a mitogenic response in high density spleen cell cultures, which are responsive to 1-μg doses of Con A. We tentatively conclude, therefore, that spleen cells (probably T cells) activated by Con A release product(s) which induce the formation of T-PFC.

Discussion

The results presented here establish that a hemolytic plaque assay can be adapted to the detection of activated T lymphocytes secreting soluble products. The products detected by the assay, as used in these studies, are undefined because the developing
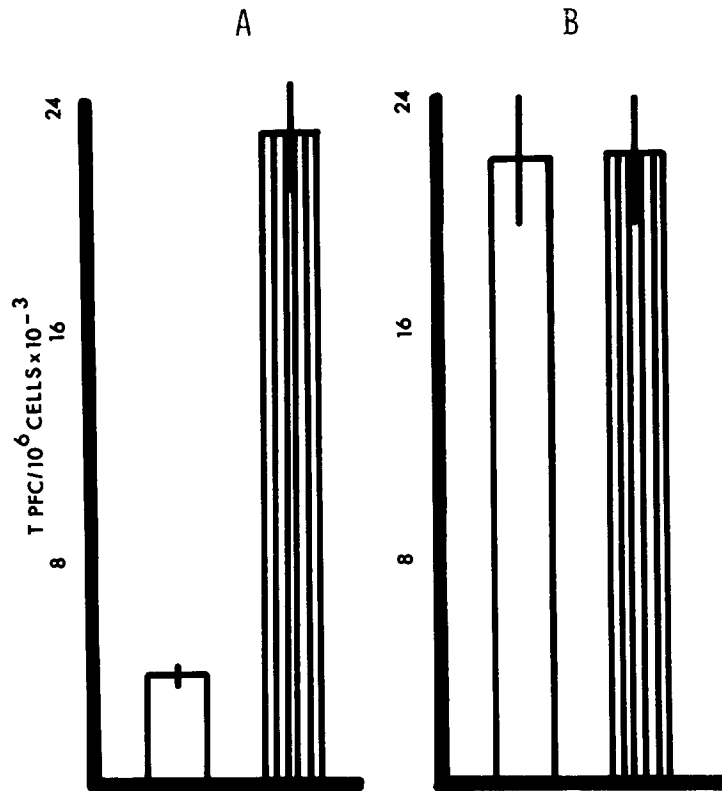
A   B

Fig. 5.   PFC response of enriched T cells from BDF₁ mice cultured for 10 d with 2 (□) or 20 (▥) μg/ml of Con A. Complement was added 1 (A) or 18 (B) h after plaquing.

antiserum was raised against a crude supernate of Con A-activated spleen cells. However, the ground work has been laid whereby antisera specific for T-cell products with defined activity can be used to detect and enumerate the cells secreting the particular mediator in question. Thus, the activities of individual T cells can be explored in a manner analogous to the study of B cells during the past decade or so since the development of the Jerne plaque assay. This technique has enormous potential, for example, for the clonal analysis of lymphokine production. What is needed for this purpose are monospecific (preferably monoclonal) antisera against defined lymphokines which can be used as developing reagents in the plaque assay.

This hemolytic plaque assay for T cells is technically simpler and potentially much more versatile than the viral plaque assay for detecting antigen-activated T cells described some years ago (10). That assay only detected cells releasing viral particles, a property of activated, viral-infected cells, rather than cells releasing specific products of immunological interest.

The evidence that the PFC in this assay are T cells rests on immunofluorescence staining for the Thy-1 marker, depletion of PFC by treatment with anti-Thy-1 and anti-Ly 2.2 sera and complement, their enrichment by removing B cells on anti-Ig-coated plates, their induction by selective T cell, but not by B cell, mitogens, their detection in long-term cultures of T cells, and, finally, by their profound enrichment
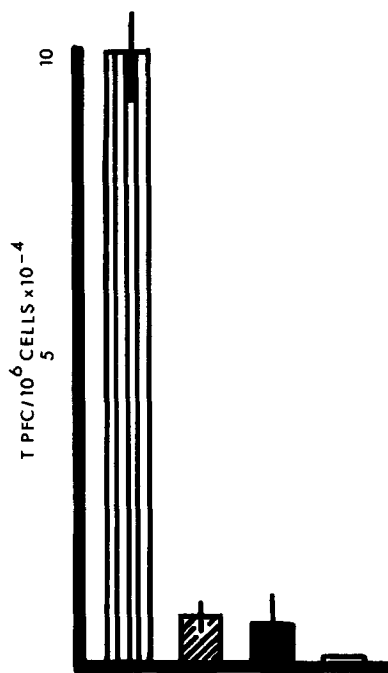
Fig. 6. PFC responses of spleen cells from BALB/c mice sensitized to L929 cells. Target cell adherent population (▭); target cell nonadherent population (▨); spleen cells from immunized mice (■); L929 cells alone (□).

in a selected population of killer cells. In addition, the possibility that the assay detected immunoglobulin-secreting cells was excluded by the strict dependency of plaque formation on the presence of developing serum and by the observation that absorption of the anti-supernatant serum with a polyvalent mouse Ig immunoadsorbent had no effect on the numbers of PFC. These results, therefore, synergize to form a compelling case for the assignment of PFC to the T-cell lineage.

It is noteworthy that the plaques produced by T cells using this technique differ in several respects from those produced by antibody-secreting cells. One difference is the time of incubation required for the development of plaques. Whereas conventional protein A plaques by B cells require 3–4 h of incubation for detection (2), T-cell plaques, when induced by high doses of mitogen, are fully developed within 45 min. On the other hand, induction of T-PFC by low concentrations of mitogen requires a much longer time of incubation. Another distinctive characteristic of T cell plaques was the apparent relationship of their size and morphology to the concentration of the developing antiserum. Thus, rabbit serum diluted 1:2 produced very small, discrete plaques, whereas at dilutions of 1:5 or 1:6 much larger plaques formed, which in some instances reached a diameter of 3 mm. These larger plaques contained unlysed erythrocytes near their centers, assuming a ring-like or sombrero appearance. This effect may be a result of an excess of antigen (secreted product) in the immediate vicinity of the secreting cell. The plaque size variation as an inverse function of antibody concentration is readily understandable in terms of antigen/antibody ratios.
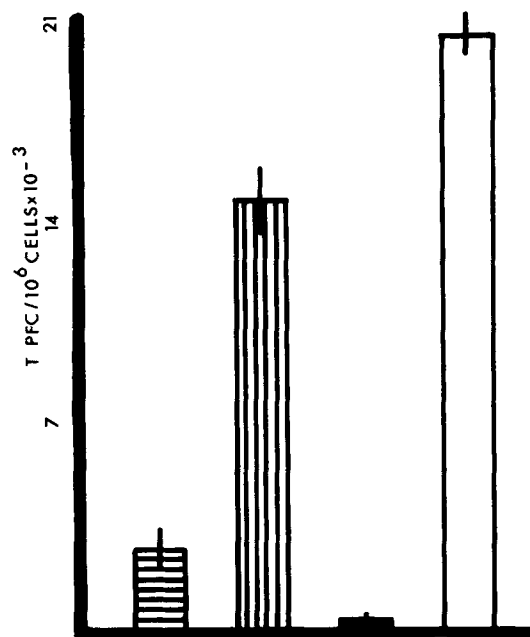
Fig. 7.   Spleen cells from BDF₁ mice were rosetted with SRBC coated with Ig from anti-Con A
supernatant developing serum. The rosetting (□) and nonrosetting (■) populations, as well as
unfractionated spleen cells (▥) were then cultured for 24 h with 10 µg/ml of Con A, after which
the numbers of PFC were assayed. The background response of spleen cells cultured without Con
A (■) is also shown.

At higher concentrations of antibody in the plaquing medium, secreted antigen is
completely bound over a shorter radius of diffusion, giving rise to smaller plaques.

Regarding the mechanism of plaque formation, two possibilities exist. The first is
that activated T cells actively release products which bind to antibody on protein A
SRBC and induce lysis in the presence of complement. The second possibility is that
anti-supernatant antibodies bind to T cells and in the presence of complement cause
their lysis; subsequent passive release of cellular products in a second step could form
complexes with antibody on SRBC, causing plaque formation. We favor the former
hypothesis because the observation that induction of PFC by low doses of mitogen
requires longer times of incubation as compared with high doses is not compatible
with a passive mode of secretion as a result of cell lysis.

Although the experiments described in this paper were primarily designed to
establish the T-cell nature of the PFC, they also offer important information about
the cells in question. The most remarkable finding was that antibody to the supernate
of Con A-induced spleen cells reacted with the surfaces of T cells which formed
plaques. The implication of this finding is that T cells express on their surface the
product(s) which they are programmed to secrete. This has a very close parallel in the
expression of surface Ig by B cells, and has far-reaching implications because antibody
to specific T-cell products may be used to isolate the cells making the products. The
fact that different doses of mitogen induced secretion at different rates seems to
suggest a functional duality of mitogens with respect to cell activation and secretion.
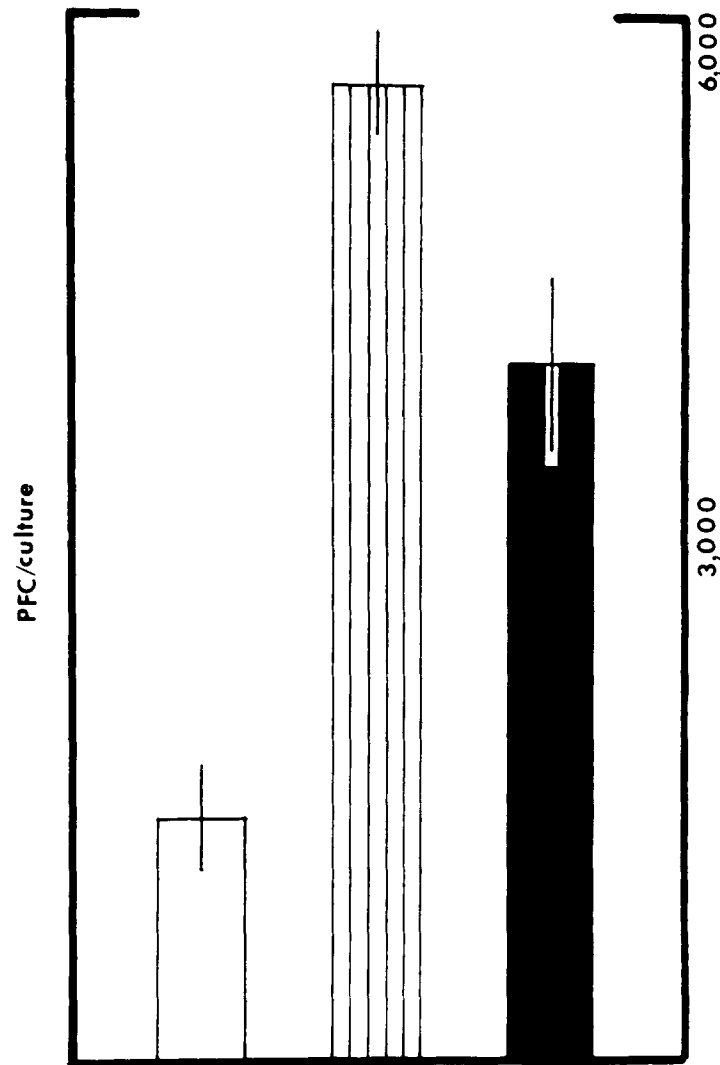
FIG. 8.   Spleen cells from BDF₁ mice were cultured for 48 h with 0.05 ml of BSS (□), 10 μg/ml of Con A (▥), or 0.05 ml of Con A-induced culture supernate (■), after which PFC were assayed.

However, whether the products detected by the assay after activation with different doses of mitogen differ only in rates of secretion remains, at the moment, an important but undecided question. The only safe conclusion that can be made at the present time is that to obtain maximum sensitivity using this assay, it is necessary to run the plaque assay for at least 15 h before adding complement.

Another point of interest was the observation that supernates from spleen cells cultured in the presence of Con A supernate (from which residual Con A was subsequently removed) induced T-PFC responses, which may have important implications for our understanding of immune regulation, because it is consistent with the hypothesis that the immune system may be self-perpetuating.

Finally, the high efficiency of plaque formation (10%) by allogeneically induced cytotoxic cells which can be isolated by adherence to targets seems to suggest that our developing serum contains significant activity against products released by this functional population of T cells.

It is apparent that the work described here represents only a beginning. However, it clearly offers new possibilities for the analysis of T lymphocytes and their products. Defined T-cell products and monospecific antisera can now be almost routinely raised by using hybridoma technology. Experiments aimed at the detection of cells which secrete a defined product are in progress in our laboratory and encouraging results have been obtained in the study of mitogen-activated human peripheral blood T lymphocytes which form plaques with rabbit antiserum to highly purified human lymphotoxin.

Summary

In an earlier report, it was shown that murine spleen cells cultured with concanavalin A (Con A) released into the culture supernatants helper and suppressor substances for antibody production. The present communication describes the production of rabbit antisera against culture supernates from Con A-activated spleen cells and their use in a plaque assay for mitogen-activated T cells. The plaque assay, utilizing SRBC to which Staphylococcal protein A had been coupled, the developing anti-supernatant antiserum and guinea pig complement, readily detected secreting T cells. The T-cell nature of the plaque-forming cells (PFC) was established principally by the following: (a) the majority of lymphocytes in the centers of plaques were Thy-1-positive by fluroescence; (b) spleen cells depleted of B cells by incubation in plastic dishes coated with rabbit anti-mouse Ig antibody gave greatly enriched PFC responses; (c) anti-Thy-1 and anti-Lyt-2.2 treatment of spleen cells almost completely depleted PFC; (d) T-cell mitogens (Con A and phytohemagglutinin) but not B-cell mitogens (lipopolysaccharides) induced PFC responses; (e) T cells maintained in culture for 10 d with Con A and T-cell growth factor yielded PFC.

Kinetic and dose response studies showed that high doses of mitogen induced rapidly appearing T-PFC and the responses peaked at day 1–2 of culture. Lower doses of mitogen-induced PFC required longer periods of incubation for detection, indicating that cell activation and secretion may be different dose-dependent activities of mitogens. Another noteworthy finding was that the antiserum reacted with surface antigens of T-PFC, indicating that secreted products are expressed on the membranes of T cells, offering the possibility of isolating populations of cells with specific secretory potential. Although the precise nature of the T-cell products detected by the antiserum used in this assay are unresolved, 10% of the target-cell-adherent population from spleen cells of BALB/c mice sensitized to L929 cells formed plaques. This suggests that the antiserum has significant activity against the products of cytotoxic T cells, a finding which accords with the activity of anti-Lyt 2.2 serum against mitogen-induced T-PFC. The method clearly offers new possibilities for the analysis of T cells and their products and should provide an important approach to the clonal analysis of lymphokine production.

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