INHIBITION OF A T-CELL-DEPENDENT IMMUNE RESPONSE IN VITRO BY THYMOMA CELL IMMUNOGLOBULIN*

By JOHN W. STOCKER, JOHN J. MARCHALONIS, AND ALAN W. HARRIS

(From The Walter and Eliza Hall Institute of Medical Research, P. O. Royal Melbourne Hospital, Victoria, 3050, Australia)

(Received for publication 5 December 1973)

Some models of collaboration between T cells and B cells in antibody production propose that T cells release an antigen-specific immunoglobulin (Ig) (1–3) which binds to macrophages resulting in immunogenic presentation of antigen to the receptors on B cells. Evidence presently exists which supports the contention that T-cell surface IgM-like Ig is cytophilic for macrophages whereas that of B cells lacks this property (4–6). If any model of this kind is correct, it may be possible to inhibit antibody formation to a thymus-dependent antigen by introducing large quantities of T-cell Ig of an indifferent specificity. The response to an antigen not requiring T-cell participation should, in contrast, be unaffected by addition of excess T-cell IgM.

Certain cultured lines of murine Thy-1 (Θ) antigen-bearing lymphomas produce, display on their surface, and release Ig (4, 7–9). We report the results of studies designed to determine whether the Ig produced by such cultured cell lines can suppress the in vitro T-cell-dependent production of plaque-forming cells (PFC) secreting antibodies to donkey red blood cells (DRBC). As a control for general cytotoxicity, the T-cell-independent response to dinitrophenylated *Salmonella adelaide* flagellin polymer (DNP-POL) in each culture was measured.

Materials and Methods

Cultured Lymphoma Cell Lines.—Cells derived from murine lymphomas were grown in continuous stationary suspension culture in Dulbecco's modified Eagle's medium plus 10% heat-inactivated fetal calf serum (DME-FCS) as described previously (7, 10). The present work was performed using cells which had been cultured through 300–1,000 successive cell generations.

The in vitro cloned cell lines S49.1 and WEHI-22.1 have been described previously (7, 10). Two additional lines, WEHI-7 (BALB/c) and WEHI-112 (NZB) originated in this Institute as radiation-induced thymic lymphomas (7) and were found to grow well immediately when cultured under the standard conditions from tumors which had undergone 6–10 serial transplantations. Each was cloned in culture by single cell isolations to yield cell lines designated WEHI-7.1 and WEHI-112.1. The C57BL leukemia EL-4 was obtained from Dr. J. C. Cerottini (Swiss Institute for Cancer Research, Lausanne, Switzerland) in 1970 as an ascites tumor, explanted into culture, and a near-tetraploid clone designated EL-4.1 derived by single cell isolation.

Cytotoxic tests with AKR anti-Θ C3H serum have shown all these cell lines to possess the Thy 1.2 cell surface antigen. Culture fluids were prepared free from cells by centrifugation at 1,000 *g* for 10 min.

Treatment of Culture Fluids.—Globulin concentrates were prepared from DME-FCS in which cells had been grown and plain culture medium by precipitation with 45% saturated

* This work was supported by the Anna Fuller Fund, the American Heart Association (72-1050), the Damon Runyon Cancer Society (DRG-1215), the U. S. Public Health Service (AI 10886; AM 11234), and the Australian Research Grants Committee. This is publication no. 1,973 from The Walter and Eliza Hall Institute of Medical Research.

ammonium sulfate at room temperature. The precipitates were resuspended in distilled water to a volume which was 10% that of the original fluid. These globulin concentrates were dialyzed against minimum essential medium and sterilized by filtration through Millipore filters before use in tissue culture.

Immunoadsorbents.—The globulin fraction, prepared by precipitation with 33% saturated $(NH_4)_2SO_4$ of rabbit antiserum to normal mouse IgG was coupled to Sepharose 4B (Pharmacia Fine Chemicals, Inc., Uppsala, Sweden) using the cyanogen bromide method as described by Mannik and Stage (11). Mouse IgG was prepared by zone electrophoresis on starch block (12) followed by gel filtration on Sephadex G-200 (Pharmacia Fine Chemicals, Inc.). It was found to lack contaminants as judged by immunoelectrophoresis and polyacrylamide gel electrophoresis. The rabbit antiserum to this protein contained antibodies directed against light chains and γ-chains. The immunoadsorbent was shown to be specific for mouse Ig through the use of radioiodinated IgG and unlabeled IgG to inhibit binding. FCS was coupled to Sepharose 4B under the same conditions used for rabbit γ-globulin.

In vitro antibody response.—A modified Marbrook method of tissue culture was used (13). 1.5×10^7 spleen cells from female CBA/H Wehi mice, 6–8-wk old, were cultured for 3 days at 37°C in the presence of 100 ng DNP-POL (prepared by the method of Feldmann [14]; 2.3 moles DNP/mole of flagellin monomer), and 3×10^6 DRBC. The culture medium was Eagle's minimum essential medium with nonessential amino acids, obtained from the Grand Island Biological Co., Grand Island, N. Y., supplemented with 5% FCS (C.S.L., Parkville, Victoria, Australia), and equilibrated with a gas phase of 10% CO_2 in air.

Cultures were harvested individually, the cells washed once in Eisen's balanced salt solution at 4°C, and resuspended in 1 ml HEPES-buffered tissue culture medium containing 10% FCS. Direct (i.e., IgM) anti-DRBC and anti-sheep red blood cells (SRBC)PFC were detected using the Cunningham and Szenberg (15) modification of the Jerne hemolytic plaque technique. Anti-DNP PFC were enumerated by subtracting the anti-SRBC background PFC count for each culture from the number of plaques detected in another aliquot of that culture using SRBC coated with DNP-conjugated rabbit anti-SRBC Fab fragments (16).

RESULTS

Culture fluids in which various Thy 1.2-bearing lymphoma cell lines had been grown were tested for suppressive effect on in vitro immune responses to DNP-POL or to DRBC. The lymphoma cell cultures were grown to a concentration of 2×10^6 cells/ml and centrifuged to remove the cells. Undiluted culture fluids were added to tissue cultures containing spleen cells and the antigens at the commencement of the culture period. As shown in Table I culture fluid from the WEHI-22 line caused a marked depression of the response to DRBC and the WEHI-7 fluid brought about a small, but significant suppression. The responses to DNP were not suppressed by addition of any of the test fluids. The results obtained with culture fluid from WEHI-22 were confirmed in a separate experiment in which the culture fluid was diluted serially with tissue culture medium. 94% inhibition was obtained using undiluted culture fluid but suppression was not observed at dilutions of 1:5 or higher.

In an attempt to prepare material of higher suppressive activity, the globulin fraction from WEHI-7, plain medium, WEHI-22, and S49 was concentrated 10-fold by precipitation with 45% saturated ammonium sulfate. Only the WEHI-22 concentrate efficiently suppressed the response to DRBC; no suppressive effect was obtained on PFC to DNP. Fig. 1 depicts a titration curve

TABLE I

Effect of Lymphoma Cell Line Culture Fluids on Thymus-Dependent (DRBC) and Thymus-Independent (DNP) Responses In Vitro

Culture fluid	Anti-DNP response* (PFC/culture)	Anti-DRBC response (PFC/culture)
WEHI-22.1	233 ± 43	190 ± 36
Plain medium	233 ± 35	630 ± 152
EL-4.1	245 ± 32	570 ± 75
WEHI-7.1	218 ± 43	380 ± 76
WEHI-112.1	377 ± 64	796 ± 87
S49.1	437 ± 79	1,020 ± 142

* All immune response results are the mean values from four replicate cultures ±SE of the mean.

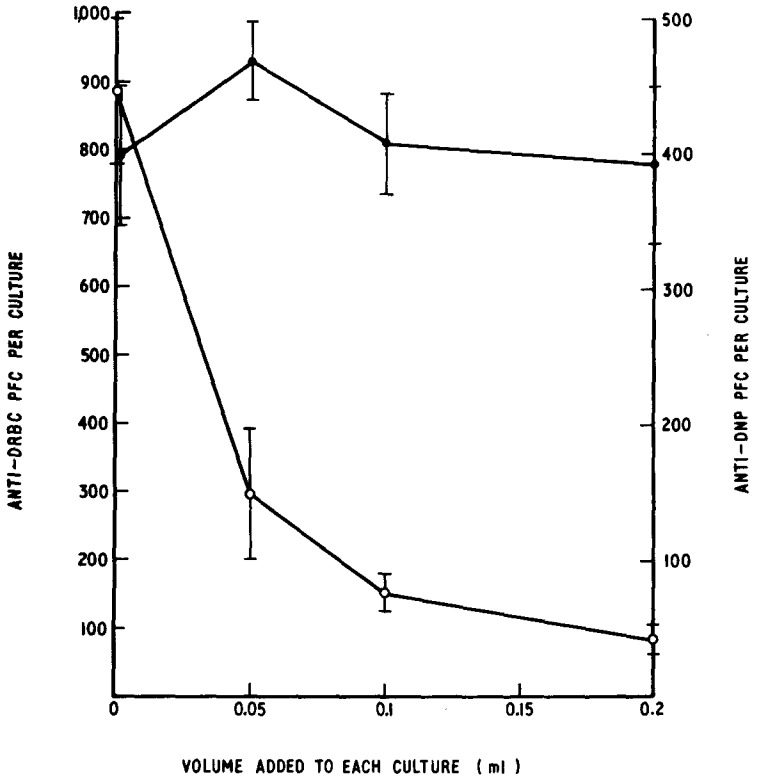

FIG. 1. Effect of different amounts of WEHI-22 culture fluid concentrate on immune responses in vitro. Closed circles anti-DNP PFC, open circles anti-DRBC PFC.

illustrating the effect of various amounts of concentrated WEHI-22 supernate on the PFC responses to DRBC and DNP. Almost complete inhibition of the response to DRBC was observed when 200 μl of WEHI-22 concentrate were added to the culture, but no effect on anti-DNP PFC was found.

That the inhibitory effect was due to the presence of Ig in the WEHI-22 globulin concentrate, was demonstrated in the following experiment. Equal aliquots of WEHI-22 globulin concentrate were treated, under identical conditions, with rabbit antiserum to mouse Ig bound to Sepharose (RAMIG-S) or FCS bound to Sepharose (FCS-S). As shown in Table II, treatment with antiserum to mouse Ig specifically removed the inhibitory capacity of the WEHI-22 concentrate.

DISCUSSION

It was found that Ig present in the culture fluid in which the thymus-derived lymphoma cell line WEHI-22 had been grown, suppressed the in vitro T-cell-dependent response of CBA spleen lymphocytes to DRBC. On the other hand, no suppressive effect was exerted on the response to DNP-POL which has been shown (17) to be independent of macrophages and T cells. Culture fluids from the other tumor lines tested here did not give uniform suppression of T-cell-dependent responses in vitro. The interpretation of the suppressive effect of WEHI-22 Ig which we favor, is that it binds to macrophages and competes for sites on these cells against antigen-specific T-cell Ig required for induction of B cells. The possibility of some other effect on macrophages or an effect on T cells themselves cannot, however, be excluded on the basis of the present data.

In these experiments, WEHI-22 Ig was present throughout the course of the induction of immunity in vitro. If, however, CBA T cells were first activated to erythrocyte antigens by transfer into lethally irradiated animals in the presence of antigen and poly(A:U) adjuvant, even the most concentrated WEHI-22 culture fluid failed to inhibit a cell cooperation-dependent response in vitro (Stocker and Marchalonis, unpublished observation). This result reflects the probability that the activated T-cell population is enriched in specific helper cells, each of which might release large quantities of specific T-cell collaborative Ig.

Another point which merits comment is the fact that WEHI-22 is of BALB/c

TABLE II

Effect of Treatment of WEHI-22 Culture Fluid Concentrate with Immunoadsorbent

Volume of treated supernate per culture	Adsorbent	Anti-DNP response (PFC/culture)	Anti-DRBC response (PFC/culture)
ml			
0	—	270 ± 51	855 ± 124
0.05	FCS-S	293 ± 52	793 ± 74
0.1	FCS-S	238 ± 63	685 ± 118
0.3	FCS-S	270 ± 49	83 ± 27
0.05	RAMIG-S	258 ± 48	758 ± 147
0.1	RAMIG-S	218 ± 42	803 ± 103
0.3	RAMIG-S	305 ± 33	738 ± 108

origin whereas the spleen cells used in the test were from CBA mice. Thus, the effect of WEHI-22 Ig acts across an *H-2* difference. If our interpretation of the inhibitory mechanism is correct, it indicates that the reported requirement for *H-2* compatibility between collaborating cells (18) operates at some stage other than the binding of T-cell Ig to the macrophage.

Preliminary evidence exists that some of the other cell lines tested here, WEHI-7, WEHI-112, and S49 (D. Haustein, J. J. Marchalonis, and A. W. Harris, unpublished observations), synthesize Ig. The failure of these to inhibit the response to DRBC might be accounted for by the actual quantity of Ig present in the medium, which is a function of rate of release from the cells and rate of degradation. It has recently been found independently by others (19) that Ig from the thymus-derived tumor line EL-4 inhibits T-B cooperation in vitro, although our particular variety of EL-4 was inactive by our tests. These studies offer indirect evidence for T-cell Ig export models of T-B collaboration and indicate that clonal cultured lines of lymphoma cells are useful experimental tools in the study of T-cell Ig.

An interesting possibility raised by these findings is that the anergy commonly associated with certain human lymphoid neoplasms is caused by the same phenomenon operating in vivo. Measurements of T-cell-dependent and independent responses in tumor-bearing patients or experimental animals would provide a test of such a proposal.

SUMMARY

Concentrated medium obtained from cultures of a continuous thymus-derived mouse lymphoma cell line (WEHI-22.1) was found to inhibit a T-cell-dependent (antidonkey red blood cell), but not a T-cell-independent (anti-DNP) immune response in vitro. Passage of such a concentrate through an anti-mouse Ig immunoadsorbent column removed its inhibitory activity. It is suggested that the tumor cell Ig can compete with specific normal T-cell Ig in its collaborative function in immune responses. A similar mechanism may account for anergy associated with some human lymphoid neoplasms.

We thank Ms. Elizabeth Anderson for her expert technical assistance.

REFERENCES

1. Bretscher, P. A., and M. Cohn. 1968. Minimal model for the mechanism of antibody induction and paralysis by antigen. *Nature* (*Lond.*). **220**:444.
2. Feldmann, M. 1972. Cell interactions in the immune response in vitro. V. Specific collaboration via complexes of antigen and T cell immunoglobulin. *J. Exp. Med.* **136**:737.
3. Feldmann, M., and G. J. V. Nossal. 1972. Tolerance, enhancement and the regulation of interactions between T cells, B cells and macrophages. *Transplant. Rev.* **13**:3.
4. Marchalonis, J. J., and R. E. Cone. 1973. Biochemical and biological properties of lymphocyte surface immunoglobulins. *Transplant. Rev.* **14**:3.

5. Feldmann, M., R. E. Cone, and J. J. Marchalonis. 1973. Cell interactions in the immune response *in vitro*: VI. Mediation by T cell surface monomeric IgM. *Cell. Immunol.* **9**:1.
6. Cone, R. E., M. Feldmann, J. J. Marchalonis, and G. J. V. Nossal. 1974. Cytophilic properties of surface immunoglobulin of thymus-derived lymphocytes. *Immunology.* **26**:49.
7. Harris, A. W., A. D. Bankhurst, S. Mason, and N. L. Warner. 1973. Differentiated functions expressed by culture mouse lymphoma cells. II. θ antigen, surface immunoglobulin and a receptor for antibody on cells of a thymoma cell line. *J. Immunol.* **110**:431.
8. Boylston, A. W. 1973. Theta antigen and immunoglobulin on a tissue-cultured mouse lymphoma. *Immunology.* **24**:851.
9. Marchalonis, J. J., R. E. Cone, and J. L. Atwell. 1972. Isolation and partial characterization of lymphocyte surface immunoglobulin. *J. Exp. Med.* **135**:956.
10. Horibata, K., and A. W. Harris. 1970. Mouse myelomas and lymphomas in culture. *Exp. Cell Res.* **60**:61.
11. Mannik, M., and D. E. Stage. 1972. Antibody-agarose immunoadsorbents: complete removal of classes of immunoglobulins from serum. *J. Immunol.* **106**:1670.
12. Kunkel, H. G. 1954. Zone electrophoresis. *Methods Biochem. Anal.* **1**:141.
13. Diener, E., and W. D. Armstrong. 1969. Immunological tolerance in vitro: kinetic studies at the cellular level. *J. Exp. Med.* **126**:591.
14. Feldmann, M. 1971. Induction of immunity and tolerance to the dinitrophenyl determinant *in vitro*. *Nat. New Biol.* **231**:21.
15. Cunningham, A. and A. Szenberg. 1968. Further improvements in the plaque technique for detecting single antibody-forming cells. *Immunology.* **14**:599.
16. Strausbach, P., A. Sulica, and D. Givol. 1970. General method for the detection of cells producing antibodies against haptens and proteins. *Nature (Lond.).* **227**:69.
17. Feldmann, M. 1972. Induction of immunity and tolerance *in vitro* by hapten carrier conjugates. II. Carrier independence of the response to dinitrophenylated polymerized flagellin. *Eur. J. Immunol.* **2**:130.
18. Katz, D. H., T. Hamaoka, M. E. Dorf, and B. Benacerraf. 1973. Cell interactions between histoincompatible T and B lymphocytes. The H-2 gene complex determines successful physiologic lymphocyte interactions. *Proc. Natl. Acad. Sci. (U. S. A.).* **70**:2624.
19. Feldmann, M. 1974. Lymphocyte co-operation *in vitro*. *In* The Lymphocyte: Structure and Function. (Unpublished observations of M. Feldmann and A. W. Boylston.) J. J. Marchalonis, editor. Marcel Dekker, Inc., New York. In press.