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Immunologic Suppression of DNA Synthesis in MOPC 104E Plasmacytoma Cells (39490)[1]

C. WILLIAM BARNHILL,[2] VITHAL K. GHANTA, RAYMOND N. HIRAMOTO, AND K. LEMONE YIELDING[3]

Laboratory of Molecular Biology and Department of Microbiology, University of Alabama in Birmingham, Birmingham, Alabama 35294

Lymphocytes respond to various mitogens and antigens by undergoing transformation, a response characterized by increased protein and DNA synthesis followed by cell proliferation (1-3). This type of response can be either augmented or depressed by antibody (4, 5) and the ability of lymphocytes to respond to antigenic stimulation is mediated by an immunoglobulin cell surface receptor (6). For example, Oppenheim found that antigen–antibody complexes (Ab–Ag) either stimulated or suppressed lymphocyte transformation depending on whether antigen or antibody was present in excess (4). Banks (5) demonstrated that the cellular response of ovalbumin (OA) sensitive lymphocytes was consistently depressed when OA was mixed with anti-OA antisera at concentrations equal to equivalence and two times antigen excess with one antiserum and 50 times antibody excess with another antiserum. Other concentrations varying from antigen excess to antibody excess resulted in suppression or augmentation of lymphocyte transformation, respectively. Antibody, therefore, is capable of inhibiting the immune response, either through a peripheral mechanism (action of antibody on potentially immunogenic sites of antigen) or through a central effect (the effect of antibody on antibody-forming cells or their precursors) (7).

Anti-immunoglobulin can also regulate the immune response, as shown by Sell and Gell (8). They demonstrated that anti-immunoglobulin reagents stimulate lymphocyte transformation, and Fanger *et al.* (9) provided evidence that cross-linkage was required in the stimulation of transformation in rabbit peripheral lymphocytes by anti-globulin reagents. Fab and Fc fragments of goat antirabbit Ig were ineffective in the stimulation of transformation but $(Fab')_2$ fragments had stimulatory activity comparable to that of the intact antibody. This work supports that of Woodruff (10), who showed that univalent fragments do not cause transformation of human lymphocytes. Recently, Theis (11) demonstrated suppression of delayed hypersensitivity reactions in chickens by passive administration of anti-IgG and anti-IgM, and Herschowitz (12) showed that anti-IgG serum suppressed the anamnestic response of rabbit lymph node cells *in vitro*. In these studies, anti-IgM treatment did not interfere with antibody formation.

Zatz and Goldstein (13) demonstrated suppression of DNA synthesis in mouse spleen after intravenous administration of sheep erythrocytes, *Salmonella typhi* H, or keyhole limpet hemocyanin (KLH). Depression of DNA synthesis was observed as early as 3 hr after antigen stimulation.

The present studies concern the effect of monospecific goat anti-μ antiserum and a bacterial dextran antigen on the incorporation of tritiated thymidine (^{3}HTdR), as a measure of DNA synthesis, by the MOPC 104E myeloma cells *in vitro*. This bacterial dextran has been demonstrated to have specificity for the IgM synthesized by the MOPC-104E tumor cell (14).

Materials and Methods. Antigen. Dextran, fraction S, from *Leuconostoc mesenteroides* NRRL B-1355 was a generous gift of Dr. Allene Jeanes.

Preparation and purification of IgM. IgM was obtained from ascites fluid pool of MOPC 104E tumor maintained by serial

[1] Supported by NCI Grant No. CA 12538.

[2] Present address: Department of Pediatrics, University of South Alabama Medical School, Mobile, Alabama 36617.

[3] Recipient of RCDA No. GM 22698.

passage of tumor cells into 6-week-old, female Balb/c mice and purified as follows. MOPC 104E ascites fluid was passed through a column of B-1355 dextran conjugated to Sepharose 2B (15). The bound IgM was subsequently eluted from the column with 0.1 *M* glycine-HCl buffer, pH 2.8, followed by simultaneous neutralization with borate-saline buffer. The column was also washed with 0.1 *N* HCl to elute any strongly bound IgM. This technique was possible because the IgM is directed toward certain linkages of bacterial dextran B-1355 and, therefore, reacts specifically with bacterial dextran (14).

The tumor. The MOPC 104E plasmacytoma was obtained through the courtesy of Dr. Michael Potter of the National Institutes of Health. The ascites form of the IgM-producing tumor has since been maintained by serial passage of tumor cells into the peritoneal cavity of normal, 6-week-old, female Balb/c mice (Lab Supply).

Preparation of monospecific goat anti-μ. An immunoabsorbent column of purified MOPC 104E IgM was prepared by coupling to CNBr-activated Sepharose 2B. Goat antimouse IgM serum was passed through the column and the column was washed free of unbound proteins with 0.1 *M* borate-saline buffer, pH 8.6. Absorbed antibody was then eluted from the IgM column with 0.1 *M* glycine-HCl buffer, pH 2.8. The eluates were then passed through a λ-light chain column to resolve the anti-μ and anti-λ antibodies (16).

Tumor cell cultures. Ascites fluid was collected by means of a 2.5-cc plastic syringe from the peritoneal cavity of female Balb/c mice bearing 10-day ascitic MOPC 104E plasmacytoma. The ascites fluid was centrifuged at 300*g* for 5 min in an International Clinical Centrifuge. The supernatant was removed by aspiration and the cells were resuspended in 1 ml of Spinner Modified Medium (BBL) with 20% fetal calf serum (Gibco) and 0.1% penicillin-streptomycin. The cell suspension was diluted with 20% fetal calf serum and viability and cell counts were made in a hemocytometer with trypan blue. The cell suspension was then adjusted to 10^6 tumor cells/ml to serve as stock cell suspension. Samples were then prepared by adding the desired amount of stock cell suspension (0.7 ml) to 16 × 125-mm plastic incubation tubes with screw caps (Falcon Plastics) to obtain a final cell concentration of 10^5 cells/cc. After addition of antigen or antiserum to the appropriate concentration, the samples were mixed well and allowed to stand in ice for 0.5 hr. Subsequent to adding medium to attain the final concentration desired, tritiated thymidine (New England Nuclear, sp act 6.7 mCi/mmole) was added to each culture to a final concentration of 2.68 μCi/ml of medium. Assay for tritium incorporation was made immediately and the samples were placed on a multipurpose rotator (Scientific Industrics, Inc., Model 150V) at 37°. Samples were then taken every 2 hr, up to 10 hr, and assayed for ^{3}HTdR uptake. Viability was also determined at each interval by the trypan blue exclusion technique.

Differential counts. Differential counts were made in order to determine the relative number of tumor cells and other nucleated cells in the stock cell suspension. Cell dilutions were made to a final concentration of 10^4 nucleated cells/ml (0.3 ml) and sedimented onto microscope slides in a cytocentrifuge. The slides were stained with Wright's stain and the tumor cells were counted. Based on the total number of nucleated cells and the percentage of tumor cells, the actual number of tumor cells was calculated.

The plasmacytoma cells are easily distinguished from other cells in the ascites fluid. The nucleus exhibits indentation and lobulation; the cytoplasm is strongly basophilic. Many cells show appearance of plasma cells or plasma blasts, with considerable variation in size and with occasional binucleate forms. Metastatic figures are frequent.

Nucleophore filter technique for uptake of tritiated thymidine. These experiments were based on the nucleopore technique of Evans and Norman (17) with a modification of Gaudin *et al.* (18) which utilizes a counting solution to lyse the cells prior to filtering and washing. Incorporation of ^{3}HTdR was measured by means of a liquid scintillation counter in counts per minute (cpm).

Dextran-conjugated SRBC for the detection of complement (19). Radial hemolysis

in gel plates was performed to compare the complement lysing effect of dextran–IgM complexes in the presence of normal fetal calf serum and guinea pig complement, respectively. Essentially, this method involved oxidation of the dextran (Fraction S from *L. mesenteroides* NRRL B-1355) (20) with periodate and coupling this reaction product to sheep red blood cells. Purified IgM from the MOPC 104E tumor (20 μl) was added to each well of the plate made with the dextran-conjugated SRBC in agarose. The plate was then placed in the refrigerator at 4° for 22 hr to allow the IgM to diffuse out of the well. Twenty microliters of guinea pig complement or other medium components was added to individual wells. The plates were incubated for 2 hr at 37° and photographed to record evidence of cell lysis.

Results. Dextran B-1355 was shown to inhibit uptake of tritiated thymidine (^{3}HTdR) in MOPC 104E tumor cells. Tumor cell suspensions (10^5 cells/cc) were preincubated at 0° with dextran antigen for 0.5 hr. The tubes were rotated in an incubator at 37° for 8–10 hr. Antigen concentrations varied from 10^{-5} to 10^{-1} mg/ml (excess antigen to excess antibody). Inhibition effects were observed within the range of 10^{-3} to 10^{-1} mg/ml of antigen. Figure 1 shows dextran inhibition of ^{3}HTdR uptake by the MOPC 104E cell suspension. The inhibitory effect became obvious after 2 hr of incubation and there was little further uptake of ^{3}HTdR in the antigen-containing samples (10^{-1} to 10^{-3} mg/ml) subsequent to that time. Conversely, at 10^{-4} mg/ml there was enhancement of ^{3}HTdR uptake.

The effect of anti-μ on ^{3}HTdR incorporation in MOPC 104E tumor cell suspensions is shown in Fig. 2. The experimental conditions are the same as in the dextran antigen experiment above. Note that there was no further incorporation of ^{3}HTdR after a 2 hr (Fig. 2) for 10^{-1} and 10^{-3} mg/ml of samples. At a concentration of 10^{-5} mg/ml of anti-μ there was no inhibition of ^{3}HTdR uptake, but rather a continued uptake of ^{3}HTdR comparable to that of the control sample. Anti-μ caused suppression of ^{3}HTdR incorporation in the concentration range of 10^{-3} to 10^{-1} mg/ml, similar to the results obtained with the dextran antigen.

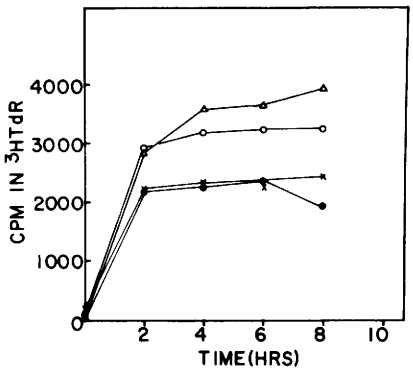

FIG. 1. Inhibition of tritiated thymidine incorporation by dextran B1355 antigen in 80% MOPC 104E tumor cell suspensions (cpm vs time in hr). Each point in time represents one determination of cpm/10^5 cells. ○, 10^{-1} mg/ml of dextran B512, control; ×, 10^{-1} mg/ml of dextran B-1355; ●, 10^{-3} mg/ml of dextran B-1355; △, 10^{-4} mg/ml of dextran B-1355.

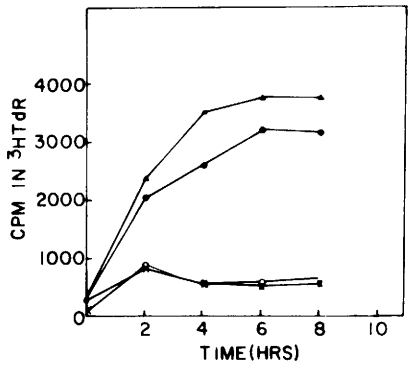

FIG. 2. Inhibition of tritiated thymidine incorporation by monospecific antiserum in 80% MOPC 104E tumor cell suspensions (cpm vs time in hr). Each point in time represents one determination of cpm/10^5 cells. ●, control; ×, 10^{-1} mg/ml of anti-μ; ○, 10^{-3} mg/ml of anti-μ; ▲, 10^{-5} mg/ml of anti-μ.

Viability by the trypan blue exclusion technique was 80% after 8–10 hr of incubation. The inhibitory effects are likely not due to viability differences, since the viability of the control as well as of the antigen-containing samples were approximately the same.

These same experiments were also run using less homogenous tumor cell populations (12–20% tumor cells based on the total nucleated cells). The results of these experiments were consistent with those reported in this paper, although the relative inhibition using less homogeneous tumors

was slightly less pronounced in the case of the anti-μ experiments. The dextran experiment on less homogeneous cells was comparable to the experiment reported here in terms of the percentage of inhibition of tritiated thymidine uptake.

In view of the demonstrated reproducibility of these experiments, the data reported in this paper were not pooled and thus standard deviations were not reported. Figures 1 and 2 each represent a single experiment. Each point on the curve represents one determination of cpm/10^5 cells.

Future experiments utilizing this system as a model will circumvent this problem of obtaining homogeneous tumor cell populations by first separating the tumor cells on a Ficoll gradient prior to incubation with antigen (21).

The possibility existed that the inhibition could be caused by complement cytotoxicity if complement were present in the fetal calf serum. Dextran–IgM complexes have been previously shown to fix complement in this tumor system (15). Therefore, the radial homolysis technique was utilized to examine this possibility. The results led to the conclusion that the cell suspensions did not contain complement.

Discussion. The present studies demonstrated that both dextran B-1355 antigen and monospecific goat anti-μ inhibit ^{3}HTdR incorporation in suspensions of MOPC 104E tumor cells. This inhibition could not be attributed to viability differences and was shown not to be the result of complement cytotoxicity.

The results of the antigen and antisera studies indicate that both dextran B-1355 and monospecific anti-μ are capable of suppressing DNA synthesis in MOPC 104E tumor cells *in vitro*. The specific dextran antigen might be expected to stimulate G_0 or G_1 tumor cells to enter the S phase more quickly, or monospecific anti-μ might inhibit entry of G_0 cells into the S phase by blocking effects. Whatever the mechanism, either effect would be interesting and would be reflected by the *in vitro* incorporation of ^{3}HTdR. The inhibitory effect was especially interesting since this system would serve as a model for control of neoplastic growth.

A similar inhibitory effect by monospecific antiserum was also noted. This is of considerable interest in view of the potential of blocking antibodies in transplantation therapy (22). The tumors used in these studies contained a wide distribution of cell types (RBC, polymorphonucleocytes, macrophages, lymphocytes, etc., as well as tumor cells). The possibility that the inhibition was due to complement toxicity of fetal calf serum was eliminated for the dextran system as demonstrated by the failure to lyse dextran-conjugated SRBC treated with MOPC 104E IgM in the radial hemolysis technique. Furthermore, Takahashi *et al.* (23) provided firm evidence that anti-μ was not cytotoxic to MOPC 104E tumor cells even in the presence of complement. Viability checks assured that the inhibition was not due to viability differences in the control and the antigen-containing samples.

In the case of the dextran experiments, the fact that this inhibition was immunologically specific was demonstrated by the lack of inhibition by the nonspecific dextran B512 in the control sample. Evidence that the MOPC 104E tumor cells have immunoglobulin receptors on their surface has been demonstrated previously using fluorescein-conjugated dextran and dextran-conjugated SRBC for the detection of these tumor cells (24).

The mechanism by which anti-μ and the dextran antigen (B-1355) exert their effects is not clear at this time. Whether the mechanism of inhibition by monospecific antiserum is due to antigenic modulation, blocking effects, or some other mechanism will be determined by future experiments, as will the mechanism of dextran inhibition.

Antigenic modulation, an antibody-induced process resulting in specific and selective removal of antigen (IgM in this case) from the cell surface, could provide a mechanism for antiserum inhibition of cellular ^{3}HTdR uptake and also provide an explanation for tolerance (23, 25).

The relationship between antigenic modulation and "cap formation" has not been established, but it has been suggested that cap formation might provide the mechanism for the phenomenon of antigenic modulation (26). Whether cap formation occurs with these tumor cells in the presence of

anti-μ antibody has not been investigated, but such studies could provide some evidence linking the phenomenon of antigenic modulation with cap formation; whether cells respond to antigen by undergoing DNA synthesis and further cell division, or simply become nonresponsive to further stimulation also need to be investigated.

It should also be interesting to ascertain in what phase of the cell cycle antigen and antiserum inhibition occur. Is the effect directly on the S phase or does it simply involve some mechanism whereby cells are prevented from making the G_1/S transition. The probability that cells were inhibited in late G_2 is unlikely since inhibition of ^{3}HTdR uptake relative to the control was seen early, and the time of G_2 + time of mitosis was less than 2.5 hr (unpublished observation, this laboratory). Cells would not have had time to progress from early G_2 into S phase since G_2 + M + G_1 was found to be about 7 hr.

Another interesting experiment would be to treat myeloma-bearing mice with the dextran or antiserum and to see if this treatment would either ameliorate or accelerate growth of the tumor. Further experiments involving the use of a mitotic inhibitor could be utilized to determine in what phase of the cell cycle the inhibition effect is exerted.

1. Elfenbein, G. J., Shevack, E. M., and Green, I., J. Immunol. **109,** 870 (1972).
2. Elfenbein, G. J., Harrison, M. R., and Green, I., J. Immunol. **110,** 1334 (1973).
3. Elfenbein, G. J., Harrison, M. R., and Mage, R. G., J. Immunol. **110,** 1340 (1973).
4. Oppenheim, J. J., Cell. Immunol. **3,** 341 (1972).
5. Banks, K. L., J. Immunol. **110,** 709 (1973).
6. Greaves, M. F., Transplant (Rev.) **5,** 45 (1970).
7. Schwartz, R. S., Prog. Immunol. **1,** 1081 (1971).
8. Sell, S., and Gell, P. G. H., J. Exp. Med. **122,** 423 (1965).
9. Fanger, M. W., Hart, D. A., Wells, V. J., and Nosonoff, A., J. Immunol. **105,** 1484 (1970).
10. Woodruff, M. F. A., Reid, B., and James, K., Nature (London) **215,** 1591 (1967).
11. Theis, G. A., and Thorbecke, G. J., J. Immunol. **110,** 1312 (1973).
12. Herschowitz, H. B., J. Immunol. **110,** 444 (1973).
13. Zatz, M. M., and Goldstein, A. L., J. Immunol. **110,** 1312 (1973).
14. Leon, M. A., Young, N. M., and McIntire, K. R., Biochem. **9,** 1023 (1970).
15. Hiramoto, R., Ghanta, V. K., McGhee, J. R., Schrohenloher, R., and Hamlin, N. M., Immunochemistry **9,** 1251 (1972).
16. Hiramoto, R. N., Ghanta, V. K., McGhee, J. R., and Hamlin, N. M., J. Immunol. **111,** 1546 (1973).
17. Evans, R. G., and Norman, A., Rad. Res. **36,** 287 (1968).
18. Gaudin, D., Gregg, R. S., and Yielding, K. L., Biochem. Biophys. Res. Comm. **45,** 630 (1971).
19. Ghanta, V. K., Hamlin, N. M., Pretlow, T. G., and Hiramoto, R. N., J. Immunol. **109,** 810 (1972).
20. Jeanes, A., Haynes, W. C., Wilham, C. A., Rankin, J. C., Melvin, E. H., Austin, M. J., Cluskey, J. E., Fischer, B. E., Tsuchuja, H. M., and Rist, C. A., J. Amer. Chem. Soc. **76,** 5041 (1954).
21. Stewart, M. J., Pretlow, T. G., and Hiramoto, R. N., Amer. J. Pathol. **68,** 163 (1972).
22. Feldman, J. D., Adv. Immunol. **15,** 167 (1972).
23. Takahashi, T., Old, L. J., McIntire, K. R., and Boyse, E. A., J. Exp. Med. **134,** 815 (1971).
24. Ghanta, V. K., Hamlin, N. M., and Hiramoto, R. N., Immunochemistry **10,** 51 (1973).
25. Old, L. J., Stockert, E., Boyse, E. A., and Kim, J. H., J. Exp. Med. **127,** 523 (1968).
26. Hutteroth, T. H., Cleve, H., and Litwin, S. D., J. Immunol. **110,** 1325 (1973).

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