PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 121-125 (1976)

Passive Immunity to Murine Plasmacytoma by Rabbit Antiidiotypic Antibody to Myeloma Protein[1] (39157)

Y. CHEN, V. YAKULIS, AND P. HELLER[2]

Veterans Administration West Side Hospital Department of Medicine, and the Abraham Lincoln School of Medicine, University of Illinois, Chicago, Illinois 60612

Plasmacytomas induced in inbred BALB/c mice by mineral oil or plastic material produce monoclonal immunoglobulins that are capable of eliciting in syngeneic normal BALB/c mice the production of antibody specifically reactive to the individually distinctive (idiotypic) determinants of these globulins (1, 2). Thus, plasmacytoma (myeloma) globulins may be considered to be tumor-specific antigens. Lynch *et al.* (3) have shown that BALB/c mice which had been immunized with myeloma globulin develop not only circulating antiidiotypic antibody but also specific resistance to the subsequent challenge by the corresponding plasmacytoma. In a subsequent study from the same laboratory, myeloma globulin was demonstrated on the surface of tumor cells (4). These findings suggested that the antiidiotypic antibody may interact with surface bound myeloma globulins and result in the alteration of the growth of tumors, although the possibility that cell-mediated immunity against the same surface antigen may contribute to the resistance to tumor grafting was also considered (3).

The results of the present study indicate that passively transferred antiidiotypic antibody to myeloma globulin inhibits tumor growth and, thus, strengthen the hypothesis that humoral antibody to a tumor-specific antigen contributes to tumor immunity.

Materials and methods. Female BALB/c mice obtained from the Jackson Memorial Laboratory, Bar Harbor, Maine, weighing approximately 20–30 g were used for tumor implantation. LPC-1, MOPC-300, J606, and MOPC 104E plasmacytomas were obtained from Dr. Michael Potter, National Cancer Institute, National Institutes of Health (Bethesda, Md.), and maintained in our laboratory by successive subcutaneous transplantation into BALB/c mice. The immunoglobulins produced by these tumors are IgG(k), IgF(k), IgG_3(k) and IgM(λ), respectively.

Normal BALB/c and plasmacytoma IgG (LPC-1 and MOPC-300) were isolated by rivanol precipitation (5) followed by Sephadex G-100 gel filtration. Purity of the isolated globulins was determined by immunoelectrophoresis with rabbit anti-mouse serum. Fab and Fc fragments of normal BALB/c IgG and myeloma globulins were prepared by papain digestion (6) followed by agarose gel electrophoresis (7).

Antiidiotypic antisera to plasmacytoma globulins were raised in rabbits by subcutaneous immunization with approximately 2–4 mg of purified globulins in complete Freund's adjuvant, followed by subcutaneous injection of 1 mg of myeloma globulin 4 weeks later and periodically thereafter. The antisera were then absorbed with normal BALB/c immunoglobulins and other plasmacytoma globulins and heated at 56° for 30 min. Antiidiotypic specificity was determined by passive hemagglutination, hemagglutination inhibition, and immunodiffusion in 1% agarose with 4% polyethylene glycol (7, 8). The IgG fractions of the rabbit antiidiotypic antisera were isolated by DEAE-Sephadex G-50 chromatography (9) and used for passive immunization after dilution to a standard hemagglutination titer of 1:1280.

Subcutaneous solid tumors of MOPC-300 and LPC-1 were excised and minced in ice-cold Hank's balanced salt solution (HBSS). The cell suspension was passed through 95% ethanol-washed cotton to remove large tumor fragments, and centrifuged on a Fi-

[1] Supported by Veterans Administration Research Funds and a grant from the Leukemia Research Foundation.

[2] To whom all correspondence should be addressed: Senior Medical Investigator, V.A. West Side Hospital, P.O. Box 8195, Chicago, Illinois 60680.

coll-Isopaque gradient (10) at 3000 rpm for 30 min to remove dead cells. Viability as tested by trypan blue exclusion was 90 to 94%. Cell concentrations were adjusted to 5–10 × 10^4/ml with HBSS. Groups of five to seven BALB/c mice were injected subcutaneously in the interscapular area with 1 × 10^4 MOPC-300 cells or 1.2 × 10^4 LPC-1 cells. These tumor dosages were the minimal ones that led to tumor grafting, growth, and death of the animals within 7 weeks. Only very rarely was spontaneous regression observed as indicated in Table 2. Thirty minutes after tumor implantation and 24 and 48 hr later, each animal received an intraperitoneal injection of 0.2 ml of either normal saline or antiidiotypic anti-LPC-1 or anti-MOPC-300 rabbit IgG. The mice were then observed for the growth of tumor.

For the demonstration of the surface globulin on plasmacytoma cells, indirect immunofluorescent staining was used: 10^6 plasmacytoma cells in 0.1 ml of RPMI 1640 with 1% bovine serum albumin and 0.5% sodium azide were incubated with 0.1 ml of antiidiotypic antisera at 4° for 60 min. After three washings they were resuspended in the same solution and 0.1 ml of fluorescein-conjugated goat anti-rabbit IgG antiserum was added. After additional incubation at 4° for 30 min and three washings, surface immunofluorescence was examined with a Zeiss UV microscope.

For the performance of the *in vitro* cytotoxicity test (11), single cell suspensions of plasmacytoma cells were adjusted to 10^7/ml in RPMI 1640 medium with 10% fetal calf serum. Aliquots of 0.1 ml of these suspensions were incubated with 0.1 ml of the antiidiotypic antisera at 37° for 30 min, washed three times and incubated at 37° for 30 min in the presence of guinea pig complement, diluted 1:6 in Earle's medium and absorbed with agarose at 4° for 30 min. Then, 0.2 ml of 0.1% trypan blue was added and the percentage of dead cells determined by counting 400 cells. In the controls, culture medium replaced either complement or antisera or both. Cytotoxicity was also determined in plasma obtained from four normal BALB/c mice that had been given three daily injections of 0.2 ml of antiidiotypic antisera to MOPC-300 or LPC-1 globulins for *in vivo* absorption, the last one 18 hr prior to bleeding. 10^6 plasmacytoma cells were incubated either with 0.1 ml of this plasma at 37° for 30 min, or with heat-inactivated plasma at 37° for 30 min followed by incubation with guinea pig complement.

The results were analzyed by a chi-square test with Yates correction for continuity (12).

Results. By immunodiffusion (Fig. 1) and hemagglutination inhibition (Table I), no cross-reactivity of antiidiotypic antisera with other myeloma globulins, or with sera of normal BALB/c, or of mice carrying other plasmacytomas was observed. These

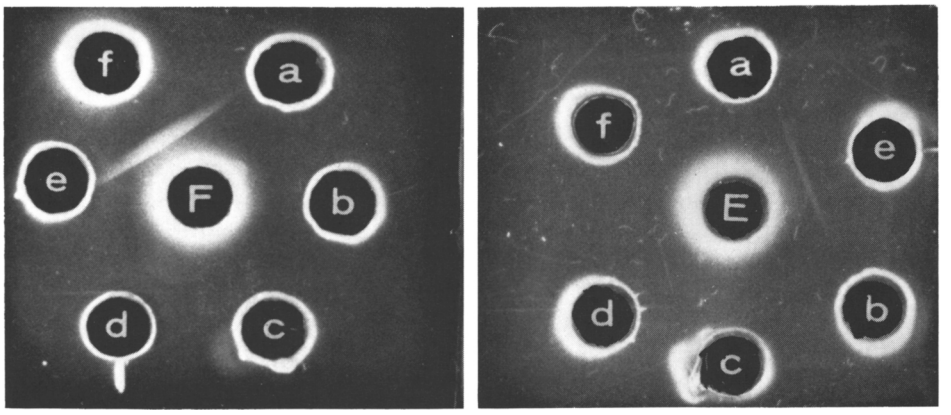

FIG. 1. Specificity of rabbit antiidiotypic antisera as demonstrated by immunodiffusion. E, anti-LPC-1 antiserum; F, anti-MOPC-300 antiserum; a, Normal BALB/c IgG; b, MOPC-195 IgH; c, MOPC-104E IgM; d, MOPC-315 IgA; e, LPC-1 IgG; f, MOPC-300 IgF.

antiidiotypic antisera reacted with Fab, but not with Fc of the respective myeloma globulins.

Surface myeloma globulins of plasmacytoma cells were visualized as numerous fine fluorescent dots by indirect immunofluorescent staining with antiidiotyic antisera. The percentage of LPC-1, MOPC-300, MOPC 104E, and J606 cells stained with anti-LPC-1 antisera were 85, 0, 0, and 1, respectively, and those stained with anti-MOPC-300 were 0, 60, 5, and 0, respectively. Prior immunoabsorption of antisera with the respective myeloma globulins reduced the percentage of specifically stained cells to 1–6%.

The fate of the tumor implants fell into one of three patterns: (1) no growth, (2) tumor became palpable 3 weeks after implantation and regressed in the following 2 weeks completely without recurrence during the observation period of 7–8 weeks, and (3) the tumor grew progressively and killed the host within 7 weeks.

TABLE I. ANTIIDIOTYPIC SPECIFICITY OF RABBIT ANTISERA TO PLASMACYTOMA GLOBULINS AS TESTED BY HEMAGGLUTINATION INHIBITION.[a]

Inhibiting antigen	A	B
Normal BALB/c (serum)	>6300	>6300
Normal BALB/c (IgG)	>280	>310
BALB/c with LPC-1 (serum)	—	>6100
LPC-1 (IgG)	3	>2600
MOPC-195 (IgH)	>800	>2600
BALB/c with MOPC-300 (serum)	>6400	—
MOPC-300 (IgF)	>800	2
Normal BALB/c (IgG Fab)	—	>310
MOPC-300 (Fab)	—	4
LPC-1 (Fab)	4.8	—
LPC-1 (Fc)	>500	—

[a] A, amount (μg) of antigen inhibiting hemagglutination of LPC-1 IgG-coated erythrocytes by antiidiotypic antiserum; B, same for MOPC-300 IgF-coated erythrocytes.

Table II summarizes the effect of the antiidiotypic antisera and of saline on the growth patterns of implanted tumors and the survival of the tumor implanted mice. Ten out of twelve mice implanted with LPC-1 that received specific antiidiotypic antisera survived; the respective survival rate for MOPC-300 was 11/14. Differences between the survival rates of mice treated with specific antisera and of those treated with normal saline or nonspecific antisera were highly significant (Table II). The tumor implant failed to take altogether in a significantly larger number of animals who received specific antiidiotypic antisera (13/26) compared with those receiving normal saline (0/26) or nonspecific antisera (1/23). Also, more implants regressed after initial growth in specific antisera-treated mice (8/13) compared with the saline controls (2/26) and the nonspecific antisera controls (4/22).

Cytotoxicity tests *in vitro* had negative results. The percentage of dead cells in all experiments ranged from 15 to 34 without significant difference between the groups (see Methods). Plasma from mice injected with rabbit antiidiotypic sera containing high titers (1:320 to 1:640) of antiidiotypic antibodies also was not cytotoxic to homologous or heterologous plasmacytoma cells

TABLE II. INHIBITION OF TUMOR GROWTH BY SPECIFIC ANTIIDIOTYPIC ANTISERA TO PLASMACYTOMA GLOBULINS.[a]

				Pattern of tumor growth		
					Initial growth with	
Group (*N*)	Tumor	Treatment	No. of survivors	No growth	Progression	Regression
A (12)	LPC-1	Saline	1	0	11	1
B (12)	"	Anti-LPC-1	10	5	2	5
C (11)	"	Anti-MOPC-300	3	1	8	2
D (14)	MOPC-300	Saline	1	0	13	1
E (14)	"	Anti-MOPC-300	11	8	3	3
F (12)	"	Anti-LPC-1	2	0	10	2

[a] $P(\chi^2)$: comparison of groups. (1) Survival: A/B, <0.005; A/C, >0.1; B/C, <0.025; D/E, <0.005; D/F, >0.25; E/F, <0.01. (2) No growth: A + D/B + E, <0.005; A + D/C + F, >0.1; B + E/C + F, <0.005. (3) Regression: A + D/B + E, <0.005; A + D/C + F, >0.1; B + E/C + F, <0.025.

with or without guinea pig complement. The percentage of dead cells ranged from 0.7 to 10.8.

Discussion. Antiidiotypic antisera used in this study were produced by immunization with purified myeloma globulins. They reacted with the Fab fragments of the respective myeloma globulins, but not with Fc or any component of whole BALB/c serum that did not contain the corresponding myeloma globulin. The demonstration of specific surface staining of plasmacytoma cells with antiidiotypic antisera appears to exclude the possibility of their reacting with other shared plasmacytoma cell surface antigens, such as "differentiation antigens" (13–16), virally related antigen (17) or some components of histocompatibility antigen (13). The results of the blocking experiment on surface staining also makes a reaction with nonimmunoglobulin surface antigens unlikely. These antiidiotypic antisera under the described experimental conditions were shown to suppress the growth of transplanted plasmacytoma in a highly specific manner (Table II). The injection of antiidiotypic antisera into the mice not only prevented the grafting of the implanted tumor in most animals, but in several others led to tumor regression after the grafting and initial growth of the tumor implant had occurred. Myeloma globulin on the tumor cell surface as shown in the present study and by Hannestad *et al.* (4) must have provided the target for immunologic interaction.

The precise mechanism of the inhibition of tumor growth is not clear. Fixation of the antibodies to tumor cells, as demonstrated by *in vitro* immunofluorescent staining, may lead to several possible consequences: (a) with further fixation of complement it may result in the lysis of the target cells. This cytotoxic effect of the antiidiotypic antisera, however, was not observed in our *in vitro* tests; (b) antibody fixation may activate cytotoxic effector cells leading to the killing of the target cells (18); preliminary experiments using splenic lymphocytes and peritoneal macrophages as effector cells, failed to show this antibody-dependent cell-mediated immunity; (c) it is conceivable that the growth of the tumor was only retarded by the antibody and permitted an active host response to be initiated. This possibility remains to be further explored. A similar phenomenon of retardation or inhibition of tumor growth by immunization with a lymphoma 141 specific monoclonal IgM was demonstrated by Sugai *et al.* (19) in NZB/NZW F_1 mice engrafted with lymphoma 141 cells. In their case, splenic cells were found to be cytotoxic for tumor cells.

Manning and Jutila (20) recently reported that antiisotypic anti-Ig (anti-μ and anti-α) sera protected neonatal BALB/c mice from tumor growth when they were challenged approximately 1 month after birth with plasmacytoma cells producing these immunoglobulins. These authors considered it unlikely that antiidiotypic antibody contributed to this suppressive effect. It should be noted, however, that their anti-α antiserum produced by MOPC 406 IgA suppressed the growth of MOPC 406 and did not significantly affect the synthesis of normal IgA, suggesting an antiidiotypic effect. Yutoku *et al.* (21) reported specific suppression of the growth of plasmacytoma with antisera from rabbits immunized with plasmacytoma cells, but they attributed this effect to antibody to non-Ig tumor surface antigen. All these findings suggest that humoral antibodies to plasmacytoma antigens, including monoclonal Ig, may play a role in tumor immunity.

Although antiidiotypic antisera can clearly prevent tumor grafting, as shown in the present study it is unlikely that such antisera could have a significant effect on tumor growth in a well-established plasmacytoma because of the large excess of circulating antigen. For the same reason, this type of immunotherapy is not suitable in the treatment of human myeloma, but antiidiotypic antisera may be clinically useful in eliminating the residual tumor after chemotherapy.

Summary. Antiidiotypic antisera to LPC-1 and MOPC-300 plasmacytoma globulins were produced in rabbits by immunization with the corresponding antigen and exhaustive immunoabsorption with normal BALB/c plasma and other myeloma globulins. IgG fractions of these antisera when given intraperitoneally on three consecu-

tive days after tumor implantation, protected the animal specifically from the grafting of the corresponding plasmacytoma or induced regression of the tumors after their initial grafting and growth. *In vitro* cytotoxicity of the antisera to plasmacytoma cells was not demonstrated. Plasma of the antisera-treated normal BALB/c mice, though containing antiidiotypic antibody in high titers (320–640), was not cytotoxic to tumor cells. The inhibitory effect on tumor growth can be considered the result of passive immunization against plasmacytoma with xenogeneic humoral antibody.

1. Sirisinha, S., and Eisen, H. N., Proc. Nat. Acad. Sci. USA **68,** 3130 (1971).
2. Yakulis, V., Bhoopalam, N., and Heller, P., J. Immunol. **108,** 1119 (1972).
3. Lynch, R. G., Graff, R. J., Sirisinha, S., Simms, E. S., and Eisen, H. N., Proc. Nat. Acad. Sci. USA **69,** 1540 (1972).
4. Hannestad, K., Kao, M-S., and Eisen, H. N., Proc. Nat. Acad. Sci. USA **69,** 2295 (1972).
5. Horejsi, J., and Smetana, R., Acta Med. Scand. **155,** 65 (1956).
6. Edelman, G. M., and Marchalonis, J. J., "Methods in Immunology and Immunochemistry" (C. A. Williams and M. W. Chase, Eds.), Vol. 1, page 405, Academic Press, New York (1967).
7. Yakulis, V., Costea, N., and Heller, P., J. Immunol. **102,** 488 (1969).
8. Harrington, J. C., Fenton, J. W., II, and Pert, J. H., Immunochemistry **8,** 413 (1971).
9. Levy, H. B., and Sober, H. A. Proc. Soc. Exp. Biol. Med. **103,** 250 (1960).
10. Perper, R. J., Zee, T. W., and Mickelson, M. M., J. Lab. Clin. Med. **72,** 842 (1968).
11. Miller, J. F. A. P., and Sprent, J., Nature New Biol. **230,** 267 (1971).
12. Snedecor, G. W., and Cochran, W. G., "Statistical Methods," 6th ed., Iowa Univ. Press, Ames (1967).
13. Takahashi, T., Old, L. J., and Boyse, E. A., J. Exp. Med. **131,** 1325 (1970).
14. Takahashi, T., Old, L. J., Hsu, C-J, and Boyse, E. A., Eur. J. Immunol. **1,** 478 (1971).
15. Watanabe, T., Yagi, Y., and Pressman, D., J. Immunol. **106,** 1213 (1971).
16. Yutoku, M., Grossberg, A. L., and Pressman, D., J. Immunol. **112,** 1774 (1974).
17. Kuff, E. L., Lueders, K. K., Ozer, H. L., and Wivel, N. A., Proc. Nat. Acad. Sci. USA **69,** 218 (1972).
18. Forman, J., and Möller, G., Transplant Rev. **17,** 108 (1973).
19. Sugai, S., Palmer, D. W., Talal, N., and Witz, I. P., J. Exp. Med. **140,** 1547 (1974).
20. Manning, D. D., and Jutila, J. W., J. Immunol. **113,** 78 (1974).
21. Yutoku, M., Grossberg, A. L., and Pressman, D., J. Nat. Cancer Inst. **53,** 201 (1974).

Received July 24, 1975. P.S.E.B.M. 1975, Vol. 151.