Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 4761–4765, August 1982
Immunology

IgG2a monoclonal antibodies inhibit human tumor growth through interaction with effector cells

(macrophages/cytotoxicity/*nude* mice)

DOROTHEE HERLYN AND HILARY KOPROWSKI

The Wistar Institute of Anatomy and Biology, 36th and Spruce Streets, Philadelphia, Pennsylvania 19104

Contributed by Hilary Koprowski, May 14, 1982

ABSTRACT **Monoclonal antibodies of IgG2a isotype specifically inhibited growth of human tumors in *nude* mice. Twenty-three monoclonal antibodies of other isotypes showed no tumoricidal reactivity. Complement depletion of *nude* mice had no effect on tumor suppression by monoclonal antibody. The role of T and killer cells as mediators of the monoclonal antibody effect in *nude* mice was virtually excluded. On the other hand, macrophages were strongly incriminated as effector cells because silica treatment of *nude* mice abolished the tumoricidal effect of monoclonal antibody. IgG2a monoclonal antibody-dependent macrophage-mediated cytotoxicity assays with human tumor cells in culture resulted in specific lysis of tumor cells.**

We have previously reported that hybridoma-derived mouse monoclonal antibodies (MAbs) effectively inhibit growth of human melanomas and colorectal carcinomas in *nude* mice (1, 2). Growth of mouse leukemic cells was also inhibited by MAbs (3–6). The effective MAbs were shown to be of IgG isotype, whereas IgM antibodies of the same binding specificity were less effective (5) or totally ineffective (4, 6, 7). In addition, it was shown (8) that IgG2a but not IgG2b mouse polyclonal antibodies suppressed the growth of murine tumors.

In the present study we tested a large number of monoclonal antibodies produced against human tumors of various origins for their tumor growth-inhibiting properties in *nude* mice. Within the context of this study we have also investigated tumor growth suppression related to a specific isotype of MAb. In parallel studies, MAbs with tumoricidal effects in *nude* mice were tested for cytotoxic effects against tumor targets in tissue culture.

MATERIALS AND METHODS

Human Cell Lines. We included three colorectal carcinomas (CRC), one pancreas carcinoma (PC), two melanomas (MEL), two lung carcinomas (LC), one breast carcinoma (BC), and one epidermoid carcinoma (EC). Most of these cell lines have been described elsewhere (9, 10). The PC line Capan II was kindly provided by T. Fogh (Memorial Sloan–Kettering Cancer Center, New York), and the EC line Hep 2 was obtained from the American Type Culture Collection (Rockville, MD).

Mouse MAbs. The MAbs used in this study are described in Table 1. MAb $H_2$4B5 was kindly provided by W. Gerhard (The Wistar Institute).

Effect of MAb Administration on Tumor Growth in *nude* Mice. Thymus-deficient *nude* mice (*nu/nu*, BALB/c background) 6–8 weeks old (unless otherwise stated) were implanted with tumor cells and injected with anti-tumor MAb or control anti-influenza virus MAb, both in ascitic fluid, as described (2).

Table 1. MAbs used in the present study

Mice immunized with	Binding specificity of MAb*	MAb	Ref.
CRC	GIC	17-1A	11
		19-9	9
		38a	9
		$C_4$14-72	12
		$C_4$20-32	†
MEL	MEL	Nu4-B	1
	MEL‡	37-7	10, 13
LC	LC, CRC, BC	16B-13	14
Influenza virus	Influenza virus	$H_2$4B5	15

GIC, gastrointestinal carcinoma.
* Determined in radioimmunossay (RIA).
† Unpublished results.
‡ Also shows DR antigen specificity.

In some experiments, F(ab')$_2$ fragments of anti-CRC MAb 17-1A, prepared by pepsin digestion of the intact antibody molecule, were injected into tumor-bearing mice. Tumor volumes were monitored as described (2).

Immunosuppressed Mice. C57BL mice were thymectomized, irradiated, and bone marrow-reconstituted as described (16). The effect of MAb treatment on tumor growth in these mice was investigated as described for *nude* mice (see above).

Treatment of *nude* Mice with Anti-Interferon Serum. The anti-interferon serum was kindly provided by I. Gresser (Institut de Recherches Scientifiques Sur le Cancer, Villejuif, France) and was injected 5 days and 1 hr before the injection of tumor cells and MAb as described (17).

Treatment of *nude* Mice with Silica. To study the effect of silica treatment on suppression of tumor growth by MAb, *nude* mice were injected intravenously with 3 mg of silica particles (Whittaker, Clark and Daniels, South Plainfield, NJ; average particle size, 1.5 μm) in 0.3 ml of phosphate-buffered saline (P_i/NaCl) 6 hr before the injection of tumor cells and MAb. The silica preparation was exposed to ultrasonic vibration and subsequent Vortex mixing before injection (18).

Peritoneal Exudate Cells. Ten- to 12-week-old CBA mice were inoculated intraperitoneally with 1 ml of thioglycollate

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Abbreviations: ADLC, antibody-dependent lymphocyte-mediated cytotoxicity; ADMC, antibody-dependent macrophage-mediated cytotoxicity; BC, breast carcinoma; CRC, colorectal carcinoma; EC, epidermoid carcinoma; GIC, gastrointestinal carcinoma; K cells, killer cells; LC, lung carcinoma; MAb, monoclonal antibody; MEL, melanoma; P_i/NaCl, phosphate-buffered saline; PC, pancreas carcinoma; RIA, radioimmunoassay.

broth (Difco). Four days later, peritoneal exudate cells were collected in 10 ml of P_i/NaCl containing 100 units of heparin.

Monoclonal Antibody-Dependent Macrophage-Mediated Cytotoxicity (ADMC) Assays. *Colony formation inhibition assay.* Peritoneal exudate cells were pelleted and resuspended to 5×10^5 cells per ml in Eagle's minimal essential medium containing 10% fetal calf serum. This medium was used throughout the assay. One milliliter of the cell suspension was put in each well of a 24-well tissue culture plate (Linbro FB-16-24-TC). The cells were incubated at 37°C in a 5% CO_2 humidified atmosphere for 2 hr to allow them to attach to the plate. Cells were then washed three times with 1 ml of P_i/NaCl. The adherent cell population consisted of 85% phagocytic cells (average of 10 experiments) as determined by zymosan particle incorporation and nonspecific esterase staining (19, 20). These adherent cells will be referred to as macrophages. One milliliter of MAb in ascitic fluid diluted 1:100 with medium was added to triplicate wells containing macrophages, followed by the addition of human tumor target cells in 1 ml of medium (effector-to-target cell ratio, 50:1). Control wells received macrophages, target cells, and medium or anti-influenza virus MAb. After a 7-day incubation, cells in each well were trypsinized, suspended in 1 ml of a 0.3% agar (agarose, Marine Colloids Division of FMC, Rockland, ME) solution in medium and transferred to a 30-mm Petri dish containing a 0.5% agar bottom layer. The colonies per dish were counted under a light microscope after a 2-week incubation. The colonies growing on agar were solely derived from human tumor cells because macrophages did not form colonies. Colony formation inhibition by MAbs was calculated as percent of controls treated with medium alone.

[methyl-^3H]*Thymidine release assay.* This assay was performed essentially as described by Mantovani *et al.* (21). Briefly, target cells labeled with [*methyl*-^{3}H]thymidine (5 μCi per 10^6 cells; specific activity 82.6 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) were added to wells of microtiter plates containing macrophages (effector-to-target cell ratio, 50:1), followed by the addition of MAbs or medium (controls). After a 3-day incubation of the plates at 37°C in a humidified CO_2 incubator, half of the supernatant was removed for determination of radioactivity. Spontaneous and maximal release of radioactivity by the targets were determined as described (21) and the percentage of specific lysis was calculated (22).

Antibody-Dependent Lymphocyte-Mediated Cytotoxicity (ADLC). The activity of spleen lymphocytes derived from adult and neonatal *nude* mice and from immunosuppressed C57BL mice in ADLC against ^{51}Cr-labeled target cells was measured as described (22).

RESULTS

Effect of MAb on Established Tumors in *nude* Mice. The tumor-suppressive effect of MAb 17-1A administered simultaneously with CRC cells has been described before (2). The effect of delayed administration of MAb on CRC tumor growth was studied in *nude* mice implanted with CRC cells and injected 4 or 7 days later with MAb 17-1A. Tumor growth was effectively inhibited ($P < 0.05$, t test, when compared to controls), and histological examination showed the absence of viable tumor cells within 2 days after MAb treatment.

Isotypes of Tumor-Inhibiting MAbs. Thirty-three MAbs that bound in RIA to human tumor cells of various origins were tested *in vivo* for tumor growth inhibition activity. Of these, five inhibited growth of tumors in *nude* mice. All five antibodies are of IgG2a isotype, whereas the noninhibiting MAbs belonged to five other immunoglobulin classes (IgG1, IgG2b, IgG3, IgM, and IgA).

Specific destruction of tumor cells by the five IgG2a MAbs is represented in Table 2. The MAb 17-1A, which binds in RIA only to tumor cells of GIC (11), destroyed CRC tumors only and did not destroy MEL or LC. Two other anti-GIC MAbs also destroyed CRC. The MAb 16B-13, which binds in RIA to cells of LC, CRC, and BC (14), suppressed the growth of these tumors but not others. MAb 37-7, which binds to MEL and also shows DR antigen specificity (10, 13), suppressed the growth of DR-antigen-positive MEL but not of Hep 2 cells, which also express this antigen.

Destruction of Tumor Cells in *nude* Mice. Although none of the IgG2a MAbs shows complement-dependent cytotoxicity of tumor cells in culture (results not shown), they bind *nude* mouse complement. To investigate the possible role of complement in tumor suppression by MAbs, adult *nude* mice were implanted with CRC cells, injected with 17-1A MAb, and treated daily with cobra venom factor (Cordis Laboratories, Miami, FL) to deplete the mice of complement as described by Oldstone and Dixon (23). [These experiments were performed by M. Oldstone (Scripps Clinic and Research Foundation, La Jolla, CA) and Z. Steplewski (The Wistar Institute).] Although complement component C3 was undetectable after administration of cobra venom factor, tumor destruction by MAb was as effective as in untreated mice (results not shown). These results suggested involvement of effector cells in the tumor destruction by MAb and that these effector cells must express Fc receptors, because $F(ab')_2$ fragments of the antibody do not show tumor-suppressive activity. It is rather unlikely that T cells would function as effector cells in athymic *nude* mice, and therefore we have investigated the role of killer (K) cells as effector cells. Their role as effector cells was also unlikely, for although K cell activity in adult *nude* mice is high, the MAb inhibited tumor growth in 10- to 12-day-old *nude* mice, spleens of which showed no K cell activity (Fig. 1). Furthermore, treatment of adult *nude* mice with an anti-interferon serum that suppresses K cell activity did not affect inhibition of tumor growth by MAb (results not shown). We were also able to grow CRC cells in immuno-

Table 2. Inhibition of human tumor growth in *nude* mice by MAbs of IgG2a isotype

Mice immunized with	Binding specificity of MAb	MAb	Human tumors implanted into *nude* mice	Mice with suppressed tumor growth
CRC	GIC	17-1A	CRC-1	54/54
			CRC-2	4/4
			MEL-1	0/5
			LC	0/8
		$C_4$14-72	CRC-1	12/12
		$C_4$20-32	CRC-1	11/11
LC	LC, CRC, BC	16B-13	LC-1	5/5
			LC-2	5/5
			CRC	4/4
			BC	5/5
			MEL-1	0/5
MEL	MEL	37-7	MEL-1	6/6
			MEL-2	4/4
			EC	0/5
			CRC	0/5

Immediately after subcutaneous implantation of 5×10^6 tumor cells, mice were treated intraperitoneally with approximately 400 μg of MAb (in ascitic fluid) daily for 5 days and then twice more on the 8th and 12th days. Results are presented as the ratio of mice with suppressed tumor growth to total mice treated.

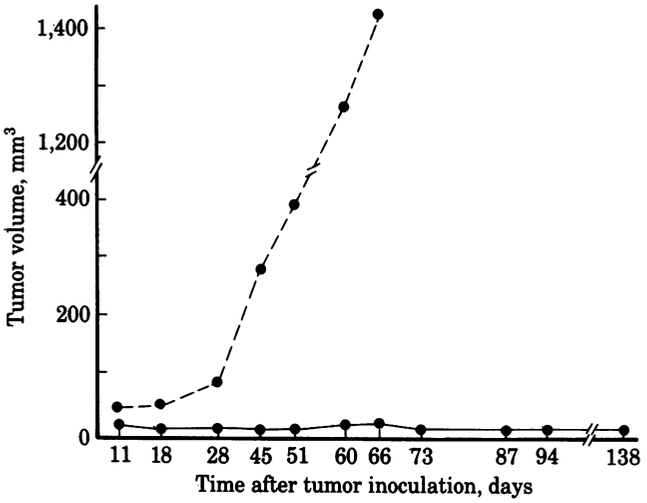

FIG. 1. Inhibition of CRC tumor growth in 10- to 12-day-old *nude* mice by MAb 17-1A. Ten- to 12-day-old *nude* mice were inoculated with CRC and treated with MAb 17-1A (●——●) or control anti-influenza virus antibody $H_2$4B5 (●---●) as described in the legend to Table 2. Six mice were included per treatment group. MAb 17-1A significantly ($P < 0.05$, t test) inhibited tumor growth. Spleen cells of neonatal *nude* mice showed no K cell activity (percent specific lysis with MAb 17-1A = 0.3) in ^{51}Cr release assays (22), in contrast to the high activity in spleens of adult *nude* mice (percent specific lysis with MAb 17-1A = 25).

suppressed C57BL mice. Spleen cells of these mice showed no K cell activity. The MAb effectively inhibited tumor growth in these mice.

Effect of Silica on Inhibition of Tumor Growth in *nude* Mice. As shown in Fig. 2, treatment of *nude* mice with silica abolished the suppressive effect of MAb on the growth of CRC, because tumors in mice treated with silica and MAb 17-1A grew progressively at a rate comparable to that observed in mice treated with anti-influenza virus MAb. Because silica particles of 1.5 μm size seem to primarily inactivate macrophages (18), we have investigated the role of those cells as effector cells in ADMC.

Monoclonal Antibodies in ADMC. Peritoneal macrophages were obtained from CBA mice and mixed with CRC cells and with each of the seven MAbs listed in Table 3, and the mixtures were incubated for 7 days. Destruction of CRC cells was determined by colony inhibition assays and by lysis. Two of the antibodies, 17-1A and 16B-13, which bind in RIA to CRC cells (11, 14), destroyed CRC cells in ADMC. These two MAbs also suppressed the growth of the same CRC tumors in *nude* mice. The three other IgG2a MAbs, which bind either to MEL (37-7 and Nu4-B) or to influenza virus ($H_2$4B5), had no effect in ADMC. The IgG1 MAb 19-9, which binds CRC cells and destroys them in ADMC, had no suppressive effect on the growth of these tumors in *nude* mice. Finally, the MAb 38a of IgM isotype binds CRC cells, but did not show ADMC against those cells and did not suppress the growth of CRC in *nude* mice.

As shown in Table 4, MAb 17-1A, which binds in RIA to tumors of GIC only, mediated an ADMC effect against cells of GIC (three CRC and one PC). MEL or LC cells, which do not bind MAb 17-1A, were not lysed in ADMC.

Similar results were obtained with macrophages obtained from BALB/c and *nude* mice (results not shown).

Role of Granulocytes in ADMC. We were able to demonstrate that granulocytes were not involved in the ADMC reactions shown in Tables 3 and 4: removal of these cells from peritoneal exudate cells by Ficoll purification prior to the assay had no effect on ADMC.

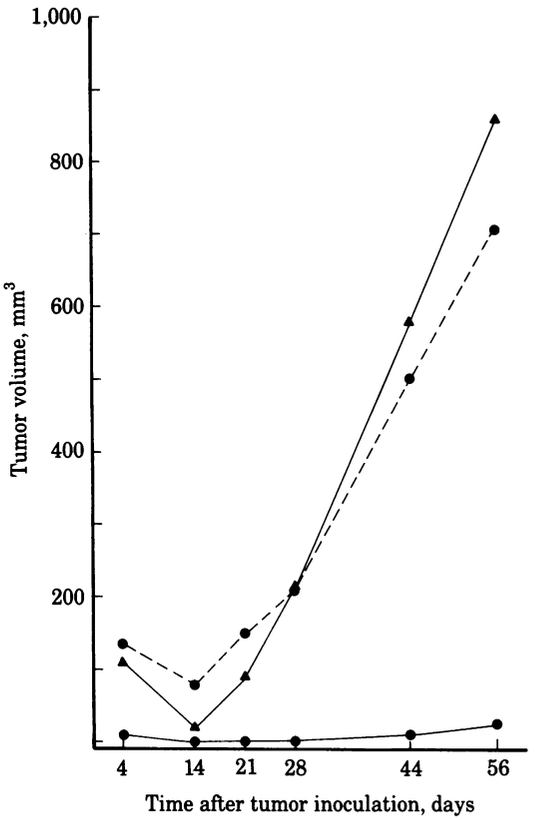

FIG. 2. Effect of silica on MAb-mediated tumor growth inhibition in *nude* mice. Six *nude* mice were injected with CRC cells and MAb 17-1A (●——●) or anti-influenza control antibody (six mice) $H_2$4B5 (●---●), as indicated in the legend to Table 2. Six mice that had been injected with CRC cells and MAb 17-1A were treated with silica 6 hr later (▲——▲). Silica treatment abolished tumor suppression by MAb 17-1A; tumor volumes in these animals did not differ significantly ($P > 0.1$) from control tumors.

Interaction of MAb with Target and Macrophages. CRC cells (1×10^4) were exposed to 4 mg of MAb 17-1A, incubated for 1 hr at room temperature, washed once, and then exposed

Table 3. Effect of MAbs of various binding specificities on growth of CRC in culture and in *nude* mice

Isotype	Binding specificity*	MAb	Colony formation inhibition,† %	Specific lysis,† %	Tumor growth inhibition in *nude* mice
IgG2a	CRC	17-1A	63‡	96‡	+
	CRC§	16B-13	34‡	93‡	+
	MEL¶	37-7	2	−5	−
	MEL	Nu4-B	4	4	−
	Influenza virus	$H_2$4B5	2	8	−
IgG1	CRC	19-9	25‡	91‡	−
IgM	CRC	38a	−2	−7	−

* Determined in RIA.
† Mouse peritoneal macrophages were used as effector cells (effector-to-target cell ratio, 50:1). Results are means of five experiments in line one; all other values in the columns are means of two experiments.
‡ $P < 0.05$ (t test) when compared to controls.
§ Also binds to LC and BC.
¶ Also shows DR specificity.

Table 4. Target cell specificity of MAb 17-1A in ADMC

Target cell	Specific lysis,* %
CRC-1	63 ± 4†
CRC-2	22 ± 1†
CRC-3	52 ± 3†
PC	62 ± 2†
MEL	7 ± 1
LC	2 ± 1

* Mean ± SD for triplicate samples.
† $P < 0.05$ (t test) when compared to controls.

to 5×10^5 macrophages. In a parallel experiment, 5×10^5 mouse macrophages were incubated with 2 mg of MAb 17-1A for 1 hr at 37°C and washed once, and 1×10^4 tumor cells were then added. In both experiments a large excess of MAb was used (approximately 1,000 times the saturation concentration). The results of the experiments (Table 5) indicate that the "armed" macrophages are effective in tumor destruction, whereas exposure of tumor cells to MAb before incubation with macrophages does not necessarily, under the conditions of the experiment, result in tumor cell destruction. These results parallel somewhat those obtained in *nude* mice when 5×10^6 cells of CRC were mixed with 15 mg of MAb 17-1A, incubated for 1 hr at room temperature, washed once, and then implanted into mice. Tumor growth was not suppressed under these conditions (Fig. 3).

Scanning Electron Microscopy. Scanning electron micrographs show macrophages in the presence of specific MAb adhering to tumor target cells that were being destroyed (G. Maul, personal communication); in the absence of MAb, macrophages are not adherent to the intact tumor cells (not shown).

DISCUSSION

The results of the experiments presented in this paper and of those previously described (1, 2) clearly indicate that MAbs are specific and powerful inhibitors of growth of human tumors in *nude* mice. Experimental results also show conclusively that only the MAbs of IgG2a isotype are effective in tumor destruction, whereas MAbs of other isotypes are inactive, even though they show binding activity in RIA to the surface of tumor cells that is similar to the binding observed with IgG2a antibodies. Langlois *et al.* (8) have observed that IgG2a antibodies destroy murine adenocarcinomas in mice, in contrast to IgG2b antibodies of the same specificity, which were inactive.

We have virtually excluded the involvement of complement and of T and K cells as effector cells. The possibility that secondary immune responses of the tumor-bearing mouse raised in the course of MAb treatment are involved in the destruction of the tumors seems unlikely because mice cured by MAb supported the growth of a second homologous tumor implant (results not shown).

Table 5. Effect of prior coating with MAb 17-1A of either CRC cells or mouse macrophages on growth of tumor cells in culture

Cells coated with MAb 17-1A	Colony formation inhibition,* %
CRC†	0 ± 2
Macrophages‡	97 ± 3§

* Results are mean ± SD for triplicate samples.
† CRC cells (1×10^4) were exposed to 4 mg of MAb for 1 hr at room temperature before incubation with 5×10^5 macrophages.
‡ Macrophages (5×10^5) were exposed to 2 mg of MAb for 1 hr at 37°C before incubation with 1×10^4 CRC cells.
§ $P < 0.01$ (t test) when compared to controls.

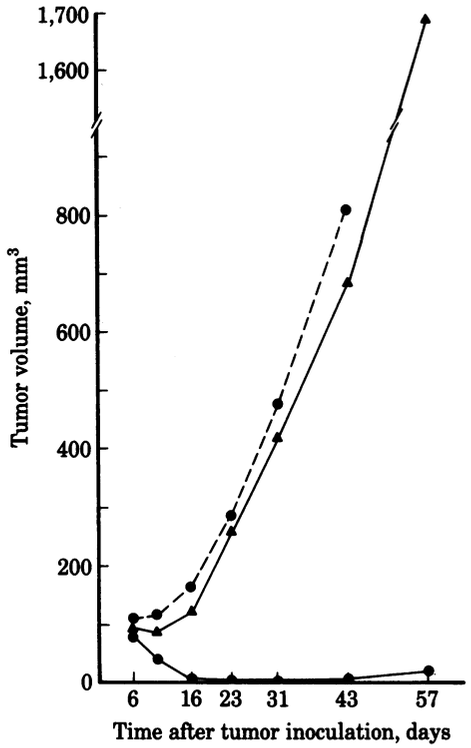

FIG. 3. Effect on tumor growth of coating CRC cells with MAb 17-1A prior to inoculation into *nude* mice. CRC cells (5×10^6) were incubated with 15 mg of MAb 17-1A (▲——▲) or anti-influenza virus MAb $H_2$4B5 (●– – –●), washed once, and injected subcutaneously into *nude* mice. Other animals were injected with 5×10^6 CRC cells subcutaneously and MAb 17-1A intraperitoneally (●——●) as indicated in the legend to Table 2 (six mice were included in each treatment group). The latter treatment resulted in significant ($P < 0.05$, t test) inhibition of tumor growth, whereas the growth of CRC cells precoated with MAb 17-1A did not differ significantly ($P > 0.1$) from the growth of control tumors.

Treatment of *nude* mice with silica of 1.5 μm particle size primarily inactivates macrophages (18), and in such mice the MAbs did not suppress tumor growth. Although there is a slight possibility that other phagocytic cells are also inactivated by silica, macrophages have been singled out as playing a major role in anti-tumor activity in ADMC in the presence of MAb specific for a given tumor target cell. Macrophages have been observed to adhere closely to target cells prior to destruction of these cells; it is impossible, however, to determine whether phagocytosis of tumor cells or their destruction by a cytolytic factor (24) secreted by the activated macrophages occurs.

Destruction of tumor cells in ADMC does not always coincide with suppression of tumor growth in *nude* mice. The IgG1 anti-CRC MAb 19-9 (Table 3), for example, is effective in ADMC but ineffective *in vivo*. Similarly, of six MAbs of IgG2a isotype that react in ADMC with melanoma cells, only one suppressed growth of tumors in *nude* mice.

Apparently, attachment of MAb to Fc receptors of the macrophage is essential to effect tumor destruction; however, the precise requirements for this process are still little understood. From our previous studies it seems that many but not all tumor surface molecules may act as effective target structures. For instance, the four anti-CRC IgG2a MAbs described here are clearly of different fine specificities because three MAbs each precipitated a component of different molecular weight (M. Blaszczyk, personal communication) and one failed to immu-

noprecipitate a tumor cell surface component from CRC cell lysates labeled either with ^{125}I by lactoperoxidase or with [^{75}Se]-methionine. On the other hand, of six anti-melanoma MAbs of IgG2a isotype, only the one reactive with DR antigen suppressed melanoma tumor growth *in vivo* (unpublished observations). Also, correct targeting of a macrophage onto a tumor cell may not be the only requirement for subsequent tumor cell lysis. This is suggested by the observation that the IgG2a MAb with anti-DR specificity suppressed growth of melanomas but not growth of the DR-antigen-expressing Hep 2 cells.

Human buffy coat cells are capable of destroying human tumor cells coated with MAb *in vitro* and prior mixing of tumor cells with antibody-coated human peripheral blood lymphocytes will prevent tumor growth in *nude* mice (25). The identity of the human effector cells is, however, a matter of conjecture. Human immunoglobulins of certain isotypes have been shown to bind to rat macrophages (26). It is possible that mouse immunoglobulins bind to Fc receptors on human macrophages and that monoclonal antibodies destroy tumor cells with human macrophages as effector cells. This possibility has far-reaching clinical applications.

We thank Drs. M. Oldstone and Z. Steplewski for investigating the role of complement in tumor destruction by monoclonal antibody and Dr. G. Maul and J. Weibel for the electron microscopy evaluations. We also thank Dr. F. Wheelock for valuable discussion, Drs. W. Gerhard and G. Rovera for reviewing this manuscript, and M. Hoffman for editorial assistance. We are grateful to Drs. I. Gresser and W. Gerhard for supplying materials. The excellent technical assistance of M. Prewett and J. McDonald-Smith is gratefully acknowledged. This study was supported, in part, by Grants CA-25874, CA-21124, and CA-10815 from the National Cancer Institute and RR-05541 from the Division of Research Resources.

1. Koprowski, H., Steplewski, Z., Herlyn, D. & Herlyn, M. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3405–3409.
2. Herlyn, D., Steplewski, Z., Herlyn, M. & Koprowski, H. (1980) *Cancer Res.* **40**, 717–721.
3. Bernstein, I. D., Tam, M. R. & Nowinski, R. C. (1980) *Science* **207**, 68–71.
4. Bernstein, I. D., Nowinski, R. C., Tam, M. R., McMaster, B., Houston, L. L. & Clark, E. A. (1980) in *Monoclonal Antibodies. Hybridomas: A New Dimension in Biological Analysis*, eds. Kennett, R. H., McKearn, T. J. & Bechtol, K. B. (Plenum, New York), pp. 275–291.
5. Kirch, M. E. & Hammerling, U. (1981) *J. Immunol.* **127**, 805–810.
6. Young, W. W., Jr., & Hakomori, S.-i. (1981) *Science* **211**, 487–489.
7. Herlyn, D. M. & Koprowski, H. (1981) *Int. J. Cancer* **27**, 769–774.
8. Langlois, A. J., Matthews, T., Rolson, G. J., Thiel, H.-J., Collins, J. J. & Bolognesi, D. P. (1981) *J. Immunol.* **126**, 2337–2341.
9. Koprowski, H., Steplewski, Z., Mitchell, K., Herlyn, M., Herlyn, D. & Fuhrer, P. (1979) *Somat. Cell Genet.* **5**, 957–972.
10. Herlyn, M., Clark, W. H., Jr., Mastrangelo, M. J., Guerry, D., IV, Elder, D. E., LaRossa, D., Hamilton, R., Bondi, E., Tuthill, R., Steplewski, Z. & Koprowski, H. (1980) *Cancer Res.* **40**, 3602–3609.
11. Herlyn, M., Steplewski, Z., Herlyn, D. & Koprowski, H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1438–1442.
12. Herlyn, M., Sears, H. F., Steplewski, Z. & Koprowski, H. (1982) *J. Clin. Immunol.* **2**, 135–140.
13. Mitchell, K. F., Ward, F. E. & Koprowski, H. (1982) *Human Immunol.* **4**, 15–26.
14. Mazauric, T., Mitchell, K. F., Letchworth, G. J., Koprowski, H. & Steplewski, Z. (1982) *Cancer Res.* **42**, 150–154.
15. Gerhard, W., Yewdell, J., Frankel, M., Lopes, D. & Staudt, L. (1980) in *Monoclonal Antibodies. Hybridomas: A New Dimension in Biological Analysis*, eds. Kennett, R. H., McKearn, T. J. & Bechtol, K. B. (Plenum, New York), pp. 317–333.
16. Davies, A. J. S. (1980) in *Immunodeficient Animals for Cancer Research*, ed. Sparrow, S. (Oxford Univ. Press, New York), pp. 1–4.
17. Senik, A., Gresser, I., Maury, C., Gidlund, M., Oru, A. & Wigzell, H. (1979) *Cell. Immunol.* **44**, 186–200.
18. Wirth, J. J., Carney, W. P. & Wheelock, E. F. (1980) *J. Immunol. Methods* **32**, 357–373.
19. Tucker, S. B., Pierre, R. V. & Jordan, R. E. (1977) *J. Immunol. Methods* **14**, 267–269.
20. Boltz-Nitulescu, G. & Foerster, O. (1979) *Immunology* **38**, 621–630.
21. Mantovani, A., Jerrells, T. R., Dean, J. H. & Herberman, B. (1979) *Int. J. Cancer* **23**, 18–27.
22. Herlyn, D., Herlyn, M., Steplewski, Z. & Koprowski, H. (1979) *Eur. J. Immunol.* **9**, 657–659.
23. Oldstone, M. B. A. & Dixon, F. J. (1971) *J. Immunol.* **107**, 1274–1280.
24. Nathan, C., Brukner, L., Kaplan, G., Unkeless, J. & Cohn, Z. (1980) *J. Exp. Med.* **152**, 183–197.
25. Steplewski, Z., Herlyn, D., Maul, G. & Koprowski, H. (1982) *Lancet*, in press.
26. Boltz-Nitulescu, G., Bazin, H. & Spiegelberg, H. L. (1981) *J. Exp. Med.* **154**, 374–384.