Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 3729–3733, June 1983
Cell Biology

Monoclonal antibodies inhibit the adhesion of mouse B 16 melanoma cells *in vitro* and block lung metastasis *in vivo*

(cancer/cell adhesion/syngeneic immunization)

H. PETER VOLLMERS AND WALTER BIRCHMEIER

Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, D-7400 Tübingen, Federal Republic of Germany

Communicated by W. Beermann, March 11, 1983

ABSTRACT **Seven monoclonal antibodies against mouse B 16 melanoma cells (produced in syngeneic C57BL/6 mice) were selected that blocked the adhesion of melanoma cells to tissue culture dishes. These antibodies were found to be directed against antigens on the surface of mouse B 16 melanoma cells but not on normal mouse cells such as 3T3 fibroblasts. Similarly, the antigens could not be detected in normal mouse tissues (e.g., lung, kidney, liver) but were found in lungs colonized by B 16 melanoma cells. Significantly, three of these antibodies virtually abolished lung colonization of highly invasive B 16 sublines injected into the animals' bloodstream. They exerted their effect both when preabsorbed by the melanoma cell *in vitro* and when delivered to the animals prior to the tumor cells. It is suggested that monoclonal antibodies might be a promising tool for preventing metastasis.**

Metastasis represents a crucial and complex event in cancerogenesis. It can be separated into several distinct steps: detachment of some transformed cells from the primary tumor, their migration in the host's bloodstream, adhesion of these migratory cells to vascular endothelia at some distant sites, and their penetration and growth in the stroma at the new areas (1–3). The mouse B 16 melanoma has frequently been used in order to examine the second part—i.e., adhesion, penetration, and growth—of this pathway (2–4). Experimentally, when B 16 cells are injected into the tail veins of syngeneic C57BL/6 mice, dark metastatic lesions in the lungs, brain, and other organs can be detected 10–20 days later (3, 5, 6).

From the original low-metastatic B 16-F 1 cell population, highly metastatic variants have been isolated mainly by two procedures: (*i*) multiple passage through the lungs and other organs *in vivo* [e.g., B 16-F 10; such lines are quite stable (5–7)] and (*ii*) subcloning of B 16-F 1 under limiting-dilution conditions [these lines are somewhat unstable (8, 9)]. It furthermore has been suggested that the differences between such lines might be expressed on their plasma membrane, because the potential for a higher rate of metastasis was transferable to low-metastatic sublines (F 10 to F 1) through fusion with membrane vesicles (10).

The basis of the present work was the idea that, because the attachment of free-floating melanoma cells in the host's bloodstream is a critical event in metastasis, one might be able to suppress this step by use of monoclonal antibodies directed against particular surface antigens. Furthermore, similar surface antigens might also be involved in the adhesion of melanoma cells under certain *in vitro* conditions, and this would permit an easier selection of promising hybridomas. Our laboratory has previously produced a series of monoclonal antibodies that prevented the adhesion of various cell types to tissue culture dishes (11). In immunofluorescence studies, one of these antibodies (anti-FC-1) stained the focal contacts of the cells, organelles known to be involved in the adhesion process (11). With this antibody, we have identifed a new membrane surface molecule (M_r, 60,000) which, *in vivo*, is enriched at cell–substrate interphases of various epithelial monolayers.

In the present investigation we immunized C57BL/6 mice with syngeneic B 16-F 1 melanoma cells and selected seven monoclonal antibodies that prevented the adhesion of melanoma but not other mouse cells *in vitro*. We report here on the effect of these antibodies on metastasis *in vivo*.

MATERIALS AND METHODS

Immunization. C57BL/6 mice (obtained from Zentralinstitut für Versuchstierzucht, Hannover, Federal Republic of Germany) were immunized with 5×10^6 B 16-F 1 melanoma cells (generous gifts of M. Wabl and R. Kemler, Tübingen) that had been scraped from tissue culture dishes and irradiated with 3,500 R (1 R = 2.58×10^{-4} C/kg). The first injections were in complete Freund adjuvant; the following ones were in incomplete adjuvant (for further details and the fusion protocol, see ref. 12).

***In Vitro* Attachment Assay.** Subconfluent B 16 melanoma cells were treated with trypsin/EDTA for 1 min, the reaction was stopped with excess medium containing 10% serum, and the cells were centrifuged at $200 \times g$. Between 500 and 1,000 cells were then plated in 50–100 μl of hybridoma supernatant or in corresponding control medium (from NS-1 myeloma cells) on tissue culture dishes. The plates were incubated at 37°C in 10% CO_2/90% air for the times indicated, then carefully washed with phosphate-buffered saline. The attached cells were fixed with 3% formaldehyde, and the whole area was examined in a reverse microscope (see also ref. 11).

Assay for Metastasis. Gently trypsinized cells (10^6) of the highly metastatic sublines C 11, C 15, and B 16-F 10 were incubated for 1 hr on ice with 5 ml of the different hybridoma supernatants or with control medium. Cells were then centrifuged and suspended in phosphate-buffered saline, and 10^5 or 2×10^5 living cells were injected into the tail veins of C57BL/6 mice. The animals used for these experiments were sex-matched and always 6–10 weeks old. The lungs of the injected mice were examined for black colonies which were visible on the surface after 10–12 days.

Monoclonal antibodies (as ascites fluids obtained from BALB/c × C57BL/6 mice) were injected intraperitoneally into C57BL/6 mice. Highly metastatic melanoma cells were then delivered intravenously as described above.

Adsorption. Constant amounts of hybridoma supernatant (100 μl) were incubated with different numbers of cells for 1 hr on ice with occasional shaking. The cells were obtained either from tissue culture or from different organs of C57BL/6 mice by

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviation: CEF, chicken embryo fibroblasts.

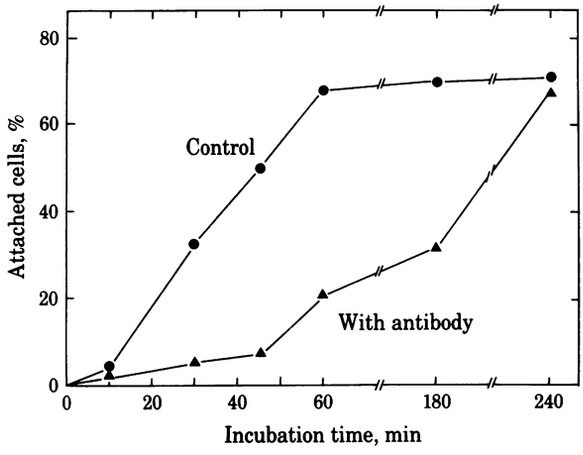

FIG. 1. Attachment of B 16 melanoma cells in the presence of a monoclonal antibody. The assay was performed with subclone C 11 of B 16-F 1 (see Table 2) and hybridoma supernatant 16/43 as antibody (see Table 1). Similar kinetics of inhibition was seen with the other monoclonal antibodies.

gently disrupting them on a tissue sieve (Bellco Glass) to a single-cell suspension. After adsorption the cells were centrifuged and the supernatants were tested for residual activity in the attachment assay with B 16-C 11 melanoma cells as described above.

RESULTS

Preparation and *in Vitro* Testing of Monoclonal Antibodies Against B 16 Melanoma. C57BL/6 mice were immunized with the syngeneic B 16-F 1 melanoma cells and the spleen lymphocytes of these animals were fused with NS-1 myeloma cells (12). The supernatants of the resulting hybridomas were then screened in an *in vitro* attachment assay carried out on tissue culture plates (see also ref. 11). It was found that positive hybridoma supernatants (approximately 1 per 500 hybridomas tested) showed maximal inhibition of cell–substrate interaction at incubation times between 45 and 60 min (Fig. 1). By this procedure we identified seven monoclonal antibodies that reduced the adhesion of B 16-F 1 melanoma cells by 40–90% (Table 1). These antibodies did not prevent the adhesion of mouse 3T3 cells or chicken embryo fibroblasts (CEF), with the exception of antibody 16/56, nor did they block the adherence of differentiated mouse carcinoma cell lines (e.g., lines 944 and PYS-2; similar results on lines 1144 and 1222 are not shown; see also ref. 13). However, some of the antibodies affected the adhesion of embryonal mouse carcinoma cell lines (PCC 4 and F 9). As a control, an alloantiserum against H-2 as well as a monoclonal antibody against β_2-microglobulin had no effect on the adhesion of B 16 melanoma cells *in vitro* (see legend to Table 1). For comparison, Table 1 also illustrates the effect of monoclonal antibodies produced by xenogeneic immunization (BALB/c against CEF), which were selected to inhibit cell–substrate adhesion of CEF. These antibodies generally showed a much wider crossreactivity, inhibiting both differentiated and embryonal mouse cells and B 16 melanoma cells in addition to 3T3 fibroblasts.

Isolation of Highly Metastatic Sublines of B 16 Melanoma and *in Vitro* Testing with the Antibodies. B 16-F 1 melanoma cells were distributed on microtiter plates at 0.3 cells per well, and clones starting from single cells were grown into large cultures (7). The resulting subclones were found to be heterogeneous with respect to their morphology on tissue culture dishes: approximately half of the clones appeared as dense colonies (as from epithelial cells), and the other half were more spindle-shaped (as from fibroblasts).

We selected B 16-F 1 subclones of different shapes and ex-

Table 1. Monoclonal antibodies produced against B 16 melanoma cells and chicken embryo fibroblasts: Effect upon cell–substrate adhesion of different cell lines

Antibody characteristics*		*In vitro* attachment, % of control†						
					Differentiated		Embryonal	
Nomenclature	Ig class‡	B 16-F 1§	3T3	CEF	944	PYS-2	PCC4	F 9
Produced against B 16-F 1:								
19/1	IgM	51	100	100	100	100	92	100
16/56	IgM	40	100	31	100	100	50	0
16/82	IgM	59	100	100	100	100	32	0
16/43	IgG2a	49	95	100	100	92	100	100
16/77	IgG2a	14	93	100	100	100	100	100
16/51	IgG2a	30	100	100	100	100	88	22
16/81	IgG2a	31	100	81	100	100	4	0
Produced against CEF:								
1/78	IgM	36	81	22	25	31	25	0
2/92	IgG2a	1	50	65	100	100	0	0
2/95	IgM	24	69	63	24	100	3	0
2/96	IgM	8	48	49	100	100	0	0
FC-1¶	IgG1	100	43	18	100	100	100	100

An alloantiserum against histocompatibility antigens [specificity *H-2D*^{b}, generous gift of R. Kemler (Tübingen)] and a monoclonal antibody against β_2-microglobulin [the hybridoma supernatant was a generous gift of J. Johnson (Munich)] had no effect on the *in vitro* adhesion of B 16-F 1 melanoma cells.

*The supernatants were taken from hybridoma reclones at densities of 10^6 cells per ml after 3 days of culture. CEF, chicken embryo fibroblasts.

†The attachment assay was performed as in Fig. 1. The incubation times were 60 min for lines PCC4 and F 9 and 30 min for all the others. The data represent means from at least two independent determinations. The mouse teratocarcinoma cell lines were generous gifts of R. Kemler (Tübingen). PCC4 (aza 1) and F 9 (41) are embryonal mouse carcinoma cell lines; 944 (trophoblastoma), PYS-2 (parietal yolk sac carcinoma), 1144 (embryonal fibroblastic cell line), and 1222 (mesenchymal-like cell line) are differentiated cell lines. For details on these cell lines, see ref. 13.

‡Determined by Ouchterlony double-immunodiffusion (14).

¶See ref. 11.

Table 2. Characteristics of sublines of B 16-F 1 melanoma

Subline	Shape*	Growth in agar†	Lung lesions‡
A 6	E	0	0
B 7	E	0	0
H 3	F	11	0
F 3	E	14	0
H 2	E	80	0
G 4	F	332	0
C 10	E	0	1
F 12	E	0	1
F 11	E	0	5
A 4	E	138	8
A 5	E	23	10
B 3	F	77	42
C 5	E	12	44
A 12	F	403	85
C 1	F	215	118
C 18	F	102	146
C 15	F	8	*350*
C 11	F	4	*598*

*E, epithelium-like; F, fibroblast-like.
†Trypsinized cells (1,500) were plated in duplicate on 0.6% agar as described (15) and colonies >0.15 mm were counted microscopically 2 weeks later.
‡Trypsinized cells (2×10^5) from B 16 subclones were injected in duplicate into the tail veins of C57BL/6 mice. Black metastatic lesions were counted microscopically on the surface of the lungs 12 days later.

amined their potential to grow in agar (14) and their ability to form metastatic lesions in lungs of C57BL/6 mice (Table 2). Four of them (C 1, C 11, C 15, and C 18) showed a significantly higher metastatic potential in comparison to the original B 16-F 1 population (≈10 metastatic lesions per lung). Two of the subclones (C 11 and C 15) formed more than 300 metastatic lesions after injection of 2×10^5 cells per animal. The more highly metastatic clones were all found to be of the fibroblastic type. There was no strong correlation between the growth of the different subclones in agar and their capability to form metastatic lesions in lung *in vivo* (cf. ref. 14).

In a further series of experiments we examined the *in vitro* effect of our anti-B 16 monoclonal antibodies upon the newly isolated B 16 subclones with high metastatic potential. As with B 16-F 1, all antibodies reduced cell–substrate adhesion of clones C 11 and C 15 (Table 3). Similarly, attachment to culture dishes was inhibited in the case of B 16-F 10, a cell line of high metastatic potential derived from F 1 by 10-fold passage through the lungs of C57BL/6 mice. *In vitro*, antibodies 16/82, 16/43, 16/77, and 16/81 were the most effective. The antibodies were not cytotoxic as determined in a cytotoxicity assay with rabbit

Table 3. Effect of anti-B 16 monoclonal antibodies on *in vitro* attachment of highly metastatic melanoma cells

Antibody	Attachment, % of control*		
	C 11	C 15	B 16-F 10†
19/1	56	87	46
16/56	75	83	48
16/82	38	63	39
16/43	35	39	30
16/77	24	48	16
16/51	81	78	90
16/81	16	45	3

*Mean values.
†Kindly provided by J. Mayo (Frederick Cancer Center) through courtesy of G. L. Nicolson.

and guinea pig complement (14), nor did they show any effect upon growth of the melanoma cells in tissue culture (data not shown).

Cell and Tissue Specificities of the Involved Antigens. The monoclonal antibodies described here were produced against a tumor cell line in syngeneic mice and therefore should not crossreact with normal mouse cells or tissue. In fact, the three antibodies examined (16/43, 16/82, and 19/1) could be adsorbed by intact B 16 mouse melanoma cells but not by 3T3 fibroblasts and the differentiated mouse teratocarcinoma cells (Fig. 2*A*). Furthermore, the antibodies were not significantly adsorbed by cells isolated from kidney, liver, and lung tissue of C57BL/6 mice but did react with cells from a lung colonized with B 16 melanoma (Fig. 2*B*). The antibodies also crossreacted with 6 of 10 human melanoma cell lines tested. The molecular weights of the antigens were determined on immunoblots and found to be 40,000–50,000 for antibodies 19/1, 16/43, and 16/82 (unpublished data).

Effect of the Monoclonal Antibodies on B 16 Melanoma *in Vivo*. The attachment of circulating B 16 melanoma cells to vas-

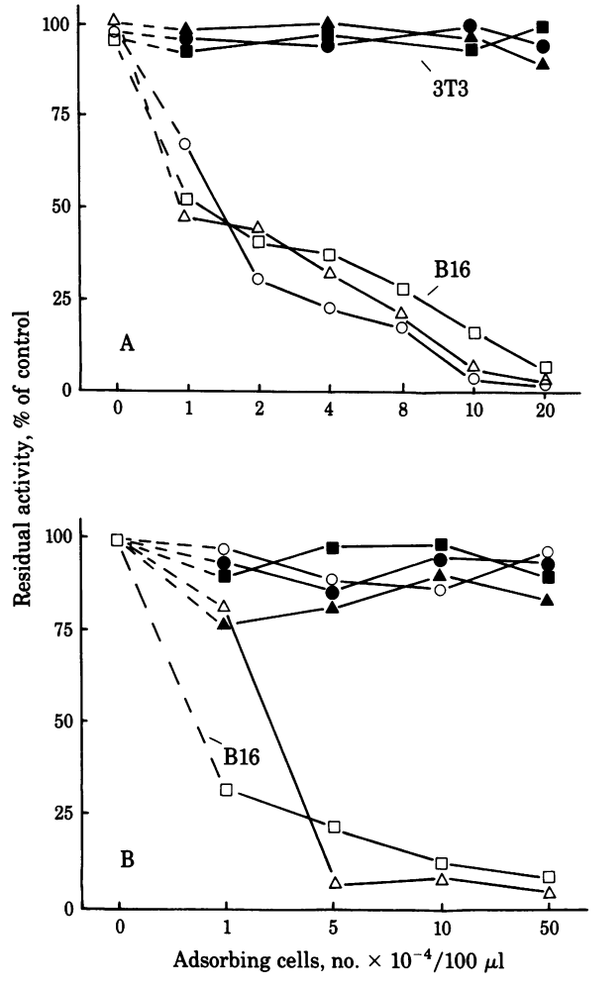

FIG. 2. Adsorption of monoclonal antibodies by different mouse cells and tissues. (*A*) Hybridoma supernatants 16/43 (○, ●), 16/82 (□, ■), and 19/1 (△, ▲) were mixed with different numbers of B 16-C 11 melanoma cells (open symbols) and 3T3 fibroblasts (solid symbols) and then tested for activity. In a similar experiment the differentiated mouse teratocarcinoma cells PYS-2 and 944 (see Table 1) did not adsorb the antibodies. (*B*) Hybridoma supernatant 16/43 was mixed with different numbers of C57BL/6 cells isolated from lung (▲), kidney (●), liver (■), or spleen (not shown) and from a lung that contained 129 metastatic lesions (△, see Table 4). B 16-C 11 melanoma (□) and 3T3 fibroblasts (○) served as positive and negative controls. Similar results were obtained with antibodies 16/82 and 19/1.

Table 4. Effect of monoclonal antibodies on highly metastatic B 16 sublines *in vivo*

Exp.	Cells	Antibody	Lung colonies, no.*
1	C 11	Control	288 (202–245)
		19/1 (αB16)	221 (208–230)
		16/56 (αB16)	221 (202–244)
		16/82 (αB16)	**37** (2–75)
		16/43 (αB16)	**5** (0–10)
		16/77 (αB16)	**10** (6–14)
2	C 11	Control	410
		16/51 (αB16)	202
		16/81 (αB16)	352
		16/82 + 16/43 + 16/77 (αB16)†	80
		1/78 + 2/92 (αCEF)†	380
3	C 15	Control	187
		16/56 (αB16)	120
		16/82 + 16/43 + 16/77 (αB16)†	8
		2/95 + 2/96 (αCEF)†	156
4	B 16-F 10	Control	58
		16/82 (αB16)	5
		16/43 (αB16)	4

Cells of the highly metastatic sublines C 11, C 15, and B 16-F 10 were incubated with the different hybridoma supernatants or with control medium. Living cells (10^5 in Exp. 1 or 2×10^5 in the other experiments) were then injected into the tail veins of C57BL/6 mice, and the lungs of the animals were examined for metastatic lesions after 12 days. The data are means from four and two animals in Exps. 1 and 2–4, respectively.

* In all experiments, other organs such as brain, liver, testis, and spleen were also screened for metastasis but were always found to be negative.

† In these cases, mixtures of 5 ml of each of the indicated hybridoma supernatants were used.

cular endothelia of target tissues is a prerequisite for the generation of metastatic lesions. Therefore, we examined whether the monoclonal antibodies that inhibited cell attachment *in vitro* would also effect adhesion *in vivo*. C 11 and C 15, the two highly metastatic sublines of B 16-F 1, and B 16-F 10 were incubated with a saturating amount of the noncytotoxic antibodies described above and then injected into C57BL/6 mice. The melanoma cells that were exposed to antibodies 16/82, 16/43, and 16/77 showed a significant reduction (down to 5–20%) in the formation of lung colonies (Table 4; Fig. 3). The other four anti-B 16 antibodies (19/1, 16/56, 16/51, and 16/81) as well as the anti-CEF antibodies had no effect.

C57BL/6 mice were preinjected with ascites fluid from different hybridomas, and the potential for lung colonization by B 16 subline C 11 was then examined. Again, the number of lung nodules was significantly reduced in the case of the antibodies 16/43, 16/77, and 16/82 (Table 5). The anti-CEF (e.g., 2/92) and other anti-B 16 antibodies (e.g., 19/1) at the same concentrations had no effect.

DISCUSSION

B 16 melanoma proved to be a suitable experimental system for study of mechanisms involved in the generation of metastatic lesions because (*i*) syngeneic mice (C57BL/6) are available as inbred strains, (*ii*) the formation of metastatic lesions in the lungs and other organs is rapid and highly reproducible, and (*iii*) the metastatic lesions are easily recognized as dark nodules (2, 3). In the present work we produced, by syngeneic immunization, a series of monoclonal antibodies that prevented the adhesion of B 16 melanoma cells to artificial substrates *in vitro*. Three of these antibodies also drastically reduced the formation of lung colonies, most likely by interfering with the initial arrest of the tumor cells at the vascular endothelia of the lungs (Tables 4 and 5; Fig. 3).

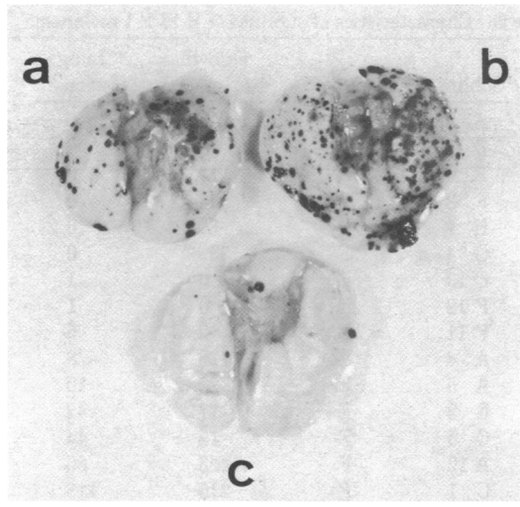

FIG. 3. Lungs from animals that had received highly metastatic B 16 melanoma cells, showing effect of pretreatment with anti-B 16 antibodies. (*a*) Control animal. (*b*) Treated with antibody 16/81. (*c*) Treated with mixture of antibodies 16/82, 16/43, and 16/77.

The procedure of syngeneic immunization has been used in analogous systems by other investigators: e.g., antibodies exclusively directed against the 60,000-dalton *src* gene product were obtained through immunization of BALB/c mice with Rous sarcoma-transformed BALB/c 3T3 cells (16), and monospecific antisera could be raised in rats and mice against tumor cells (15, 17). Similarly, if we consider B 16 melanoma cells as tumorigenic mutants of C57BL/6 melanocytes, syngeneic immunization likely leads to antibodies against possible tumor-associated antigens. Indeed, the seven anti-B 16 monoclonal antibodies reacted with B 16 melanoma when tested in cell adhesion assays and showed crossreaction with embryonal but not with differentiated mouse cells (Table 2). Furthermore, the three antibodies examined more closely in adsorption experiments did not crossreact with normal mouse cells or with cells from four

Table 5. Effect of preinjection of C57BL/6 mice with anti-B 16 antibodies upon lung colonization by highly invasive melanoma subline C 11

Antibody*	Mice, no.	Lesions, % of control†
None	12	100
19/1 (control)	6	95
16/43	12	21
16/77	4	26
16/82	3	31

* Delivered to the mice by intraperitoneal injection of 100 μl of ascites fluid 4–5 hr before the melanoma cells.

† The mean number of metastatic lesions in the control animals was 219.

different mouse tissues (Fig. 2). Because the antibodies are active in adhesion assays and are adsorbable by intact cells, we assume that the corresponding antigens represent specific surface components.

The approach of producing monoclonal antibodies with anti-adhesion activity has recently been developed in our laboratory with fibroblasts (see ref. 11) and by other investigators using myoblasts (18). In these cases, the corresponding antigens were determined to be surface membrane proteins that play a role in cell adhesion. Conceivably, B 16 melanoma expresses new surface antigens not present on normal mouse cells and tissues, and these might be involved in the adhesion of circulating tumor cells to specific foreign tissues. In order to obtain monoclonal antibodies against such unique surface structures it was necessary to use assays that simulate as closely as possible the *in vivo* conditions. Apparently, the attachment assay used in this study fulfills the requirements because it is simple and suitable for screening hundreds of hybridoma supernatants (5,000 in this particular study) in a convenient time period and because three of seven antibodies thus selected were also effective *in vivo*.

It is likely, although not proved (see also below), that these three antibodies selected on the basis of their anti-adhesion activity under tissue culture conditions interfere with a related process *in vivo*—i.e., the adhesion of the melanoma cells to the lung endothelia. The four other anti-B 16 antibodies block adhesion through molecules on melanoma cells which seem not to be important in attachment to lung endothelia; for instance, the corresponding antigens might be involved in adhesion of the melanoma cells to other target organs. The phenomenon that a monoclonal antibody can interfere selectively with the adhesion of cells to particular tissues has also been observed in analogous *in vitro* experiments using different substrates [e.g., concanavalin A, gelatin, and polylysine (unpublished data)]. The anti-CEF antibodies produced xenogeneically and used for comparative purposes (Table 1) react with a further set of adhesion molecules on B 16 cells that are not melanoma-specific components. This corresponds with the broader crossreactivity of these antibodies.

In vitro, the anti-B 16 antibodies were found to be most active within the first hours (Fig. 1); we therefore assume that such a short time period is critical for the initial arrest of circulating melanoma cells *in vivo* as well. In agreement with this is the observation that injection of antibody 16/43 into C57BL/6 mice 1 day after injection of the melanoma cells had no effect on lung colonization (data not shown). Those cells that are initially prevented from adhering to the lungs by our antibodies did not grow in other organs (see legend to Table 4). Furthermore, we attempted to exclude the possibility that the initial effect of our monoclonal antibodies was nonspecific—e.g., leads to killing or opsonization of the melanoma cells. The antibodies were not cytotoxic, and other antibodies of the same Ig class and giving the same *in vitro* effects [e.g., the anti-fibroblast antibodies (Tables 1, 4, and 5)] did not reduce metastasis.

Our data indicate that preselection of monoclonal antibodies by suitable *in vitro* assays might lead to therapeutically interesting reagents. It is obvious from the results presented that similar procedures are applicable for other metastasizing tumors as well.

Note Added in Proof. We have recently found that the monoclonal antibodies described react with a variety of other tumor cell lines (melanoma, carcinoma, neuroblastoma) but not untransformed lines or serum components from humans or mice. The antigens are also present on simian virus 40-transformed mouse fibroblasts (3T3 fibroblasts are negative) and are enriched, at the permissive but not the nonpermissive temperature, on chicken fibroblasts transformed with a temperature-sensitive Rous sarcoma virus.

The authors thank Annette Hiesel and Susanne Braun for excellent technical assistance, Bernhard Luescher, student of the Swiss Federal Institute of Technology (Zurich), who was involved in the beginning phase of the work, and Carmen Birchmeier for critically reading the manuscripts.

1. Zeidman, I. (1957) *Cancer Res.* **17,** 157–162.
2. Poste, G. & Fidler, I. J. (1980) *Nature (London)* **283,** 139–146.
3. Nicolson, G. L. (1979) *Sci. Am.* **240** (3), 50–60.
4. Kramer, R. H. & Nicolson, G. L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5704–5708.
5. Brunson, K. W., Beattie, G. & Nicolson, G. L. (1978) *Nature (London)* **272,** 543–545.
6. Tao, T. W., Matter, A., Vogel, K. & Burger, M. M. (1979) *Int. J. Cancer* **23,** 854–857.
7. Fidler, I. J. (1975) *Cancer Res.* **35,** 218–224.
8. Fidler, I. J. & Kripke, M. L. (1977) *Science* **197,** 893–895.
9. Poste, G., Doll, J. & Fidler, I. J. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6226–6230.
10. Poste, G. & Nicolson, G. L. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 399–403.
11. Oesch, B. & Birchmeier, W. (1982) *Cell* **31,** 671–679.
12. Vollmers, H. P., Eulitz, M. & Götze, D. (1979) *Immunogenetics* **8,** 447–459.
13. Kemler, R., Morello, D. & Jacob, F. (1979) in *Cell Lineage, Stem Cells and Cell Determination*, INSERM Symposium, ed. Le Douarin, N. (North-Holland, Amsterdam), Vol. 10, pp. 101–113.
14. Cifone, M. A. & Fidler, I. J. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 1039–1043.
15. Dean, C. J., Hobbs, S. M., Hopkins, J. U., North, S. M. & Styles, J. M. (1982) *Br. J. Cancer* **46,** 190–197.
16. Parsons, S. J., Riley, S. C., Mullen, E. E., Brock, E. J., Benjamin, D. C., Kuehl, W. M. & Parsons, J. T. (1979) *J. Virol.* **32,** 40–46.
17. Seto, M., Umemoto, N., Masuho, Y., Hara, T. & Takahashi, T. (1982) *Cancer Res.* **42,** 5209–5215.
18. Neff, N. T., Lowrey, C., Decker, C., Tovar, A., Damsky, C., Buck, C. & Horwitz, A. F. (1982) *J. Cell Biol.* **95,** 654–666.