Monovalent antibodies directed to transformation-sensitive membrane components inhibit the process of viral transformation

(gangliosides/galactoprotein a/tumor virus/phenotype)

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ABSTRACT Monovalent antibodies (Fab) directed to two classes of transformation-sensitive cell surface components, ganglioside and galactoprotein a (Gap a), inhibit the process of oncogenic viral transformation of cells. Mouse 3T3 cells infected with murine sarcoma virus were not transformed in terms of morphology change and enhancement of sugar uptake when the infected cells were cultured in the presence of monovalent antibodies directed to GM₁ ganglioside or to Gap a. Transformation inhibitory activity of these cell surface ligands was not related to cell growth inhibition because the monovalent antibodies to globoside and divalent Con A were growth inhibitory but did not inhibit oncogenic transformation. Neither anti-GM₁ Fab nor anti-Gap a Fab inhibited virus production. The transformation inhibitory activity of antiganglioside and anti-Gap a Fab was additionally assessed by inhibiting the transformed phenotype in NRK cell lines with mutants of avian sarcoma virus that are temperature sensitive for expression of the transformation phenotype (NRK/LA25). In this cell line, the GM₃ ganglioside (not GM₁) and Gap a were transformation-sensitive cell surface components. The expression at permissive temperature of transformed phenotypes, such as morphology change and capability of growth in 0.3% agar, was inhibited by preincubation of the cells with anti-GM₃ Fab or anti-Gap a Fab.

GM₃ labeling of NRK/LA25 cells decreased at permissive temperature, whereas preincubation of cells with anti-Gap a, which induces the inhibition of transformation after a temperature shift, prevented the decline of GM₃ label on the cell surface. The data suggest a possible correlation between GM₃ and Gap a expression. Application of monovalent antibodies to these transformation-sensitive components may prevent changes of these components on cell surfaces, and thus may result in abortion of phenotypic expression of transformation, although the transforming gene (*src*) has been set active. These results indicate that pericellular structures influence gene expression.

The process of oncogenic transformation in various cells is accompanied by changes in two classes of "transformation-sensitive" molecular species at the cell surface membranes: these are the blocked synthesis of glycolipids (2-4) and the deletion of a high molecular weight glycoprotein, Gap a or LETS (5-8). When transformed cells were cultured in medium supplemented with these transformation-sensitive molecules, a partial restoration of the normal cell phenotype resulted, possibly due to the exogenous addition of the lost components (9-12). A previous study (13) demonstrated that culturing cells in the presence of monovalent anti-GM₃ ganglioside could modify the metabolism, and possibly the function, of target GM₃ and, consequently, inhibit cell growth of mouse 3T3 or NIL cells. Recently, Kemler *et al.* (14) demonstrated that monovalent antibodies to the mouse embryo cell surface antigen (F9) in-

hibited the subsequent developmental changes of the embryo. These studies suggested the possibility that expression of the phenotypic program for oncogenesis or ontogenesis may be blocked by cell surface modification through specific antibodies directed to a specific cell surface component. This paper describes the inhibition of expression of the oncogenic phenotype by monovalent antibodies directed to Gap a or to GM₃ or GM₁ ganglioside.

MATERIALS AND METHODS

Antibodies. Gap a was prepared from the conditioned culture medium of hamster NIL or mouse 3T3 cells by the methods of Engvall and Ruoslahti (15), and the antibodies were raised in rabbits (16, 17). The antibodies to NIL cell Gap a crossreact with that of NRK (rat) and 3T3 (mice) cells on immunodiffusion or counter immunoelectrophoresis. Fab fragment was prepared (18) and concentrated in a "collodion bag" (No. 100, Schleicher & Schuell) with suction to 300 µg/ml. Anti-Gap a Fab did not react with anti-rabbit Fc goat antibody but did react strongly with anti-rabbit Fab goat antibody on Ouchterlony double gel diffusion. The goat antibodies were obtained from Miles. Anti-Gap a Fab inhibited the precipitation of Gap a with whole antibodies when the Gap a antigen was preincubated with the anti-Gap a Fab. This was demonstrated on a double diffusion plate. Antiglycolipid antibodies were raised in rabbits as described (19, 20), and their specificities were well characterized (19-21). The antigloboside titer determined by hemagglutination of trypsin-treated 2% (vol/vol) human erythrocytes was 1:256. The anti-GM₃ titer, determined by hemagglutination of trypsin-treated dog erythrocytes, which contain a large quantity of GM₃, was 1:128. The anti-GM₁ ganglioside titer, determined by hemagglutination of 2% (vol/vol) GM₁-coated human erythrocytes, was 1:256. Monovalent (Fab) antibodies were prepared, and the volume was reduced to the original serum volume in a collodion bag under suction. The Fab fraction of antiglycolipid was further purified by affinity chromatography on a glycolipid column prepared according to the method of Young *et al.* (22). These Fab antibodies were characterized by anti-Fab and anti-Fc goat antibodies as described above, and these antibodies inhibited hemagglutination of dog erythrocytes or of GM₁-coated human erythrocytes caused by whole antibodies.

Abbreviations: Gangliosides are named according to the designation by Svennerholm (1). Gap a, Galactoprotein a (identical to CSP, SFA, LETS, etc.); NaCl/F₁, 10 mM sodium phosphate, pH 7.2/0.14 M NaCl; Glc-NaCl, 10 mM phosphate, pH 7.2/0.8% NaCl/0.04% KCl/0.1% glucose; Con A, concanavalin A; MSV, murine sarcoma virus (Moloney strain).

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Cells, Cell Culture, Transformation by Virus, and Transformation Inhibition. Subconfluent 3T3 BALB/c cultures (1×10^5 cells per 32-mm petri dish) were infected and transformed by murine sarcoma virus (MSV) according to the modified procedure of Hartley and Rowe (23). The cell sheets were washed twice with 10 mM phosphate, pH 7.2/0.8% NaCl/0.04% KCl/0.1% glucose (Glc-NaCl), incubated with 1 ml of DEAE-dextran (Pharmacia) (12 μ g/ml of Dulbecco's modified Eagle's medium) for 30 min (24), with subsequent addition of 10^3 – 10^4 focus-forming units of virus in 0.1 ml of growth medium, and incubated for 15 min. After infection, cultures were washed with Glc-NaCl and continuously cultured in growth medium with 10% fetal calf serum. The effect of antibodies and lectins on the transformation process was studied by replacing the medium with that containing antibodies or lectins 4 hr after infection. Morphological change, number of foci, and sugar transport were determined 96–120 hr after infection.

Rat kidney cells (NRK), those transformed with wild type avian sarcoma virus Brian strain 77 (NRK/B77), and NRK cell lines transformed with a temperature-sensitive mutant of avian sarcoma virus LA25 and LA31 (NRK/LA25, and NRK/LA31) (25) were donated by P. K. Vogt, University of Southern California, Los Angeles. To observe transformation inhibition, we exposed a subconfluent culture of NRK/LA25 or NRK/LA31 cells to a nonpermissive temperature (39°C) in growth medium containing the monovalent antibodies for at least 48 hr. Then the temperature was shifted to the permissive temperature (32°C) to observe the change in morphology. The concentrated solution of Fab fraction in 10 mM Na phosphate, pH 7.2/0.14 M NaCl (NaCl/P_i) was mixed with twice concentrated growth medium and then used for inhibition studies. To assess the inhibition of transformed phenotype in the NRK cell system, we studied the capability of colony formation in 0.3% soft agar (26).

The virus productivity in MSV-infected 3T3 cell cultures in the presence of antibodies was determined by foci formation assay of the virus pellet separated from the culture medium (23, 24). The presence of viral envelope antigen (gp70) was studied by immunofluorescence with goat antibodies to gp70 glycoprotein (27), which was kindly donated by M. Hatanaka, Frederick Cancer Institute, Frederick, MD.

Cell Surface Labeling. Cell surface carbohydrates were labeled as described (6, 28). The labeled cells were washed, and the cell pellets were analyzed in polyacrylamide gel electrophoresis. A portion of the cell pellets was extracted with chloroform/methanol and subjected to glycolipid analysis (see below).

Glycolipid Analysis and Other Methods. Cells were extracted first with chloroform/methanol (2:1) followed by chloroform/methanol (1:1). Neutral glycolipids and gangliosides were separated by chromatography on DEAE-Sephadex (29) and individual glycolipids were analyzed as described (30). Pure glycolipid samples were prepared as described (30). Hexose uptake was determined with 2- 14 C]deoxyglucose or 3-*O*-methyl 14 C]glucose according to the described procedure (31) except that the condition of sugar uptake was changed to 2 min at room temperature (see Table 1). Divalent (32) and monovalent (33) derivatives of concanavalin A (Con A) were donated by T. Osawa, Tokyo University Faculty of Pharmaceutical Sciences. The cell surface reactivities with anti-GM₃ or anti-Gap a was determined by incubating monolayer cultures with 14 C-labeled monovalent antibodies, which were prepared with 14 C]formaldehyde (34).

RESULTS

Inhibition of MSV Transformation by Anti-GM₁ Fab and by Anti-Gap a Fab. MSV-transformed 3T3 BALB/c cells were characterized by decreased quantities of Gap a and GM₁ and GD_{1a} ganglioside; hence, these molecules are transformation-sensitive in these cells.[‡] The typical morphological change associated with MSV transformation was completely inhibited in the presence of monovalent antibodies to Gap a or GM₁ and partially inhibited by anti-GM₃ Fab; but it was not inhibited by antigloboside Fab, monovalent Con A, and divalent Con A (see Fig. 1). MSV-transformed cells showed more than 3–4 times higher sugar uptake than nontransformed control 3T3 cells in agreement with Hatanaka *et al.* (35). The sugar uptake of MSV-infected, but transformation-inhibited, cells was in the range of control cells (Table 1). The growth rates of various

[‡] In addition to these changes, increase of asialo GM₂ and lactosylceramide was observed in these transformed cells. In MSV-transformed Swiss 3T3 cells, decrease of GM₁ and GD_{1a} was reported (4).

FIG. 1. Effect of monovalent antibodies on MSV transformation of 3T3 cells. (A) 3T3 BALB/c cells at subconfluency. (B) 3T3 BALB/c cells infected with MSV (5×10^3 focus-forming units) after 72 hr. (C, D, E, F, and G) Cultures infected with MSV and grown under the same condition as B but with the inclusion of various monovalent antibodies. 3T3 BALB/c cells seeded 5×10^4 cells per 35-mm plate were infected with 5×10^3 focus-forming MSV. After 4 hr the growth medium was replaced with that containing the following monovalent antibodies or lectins. (C) Anti-GM₁ Fab; (D) antigloboside Fab; (E) anti-GM₃ Fab; (F) divalent Con A; (G) anti-Gap a Fab; (H) control 3T3 BALB/c cells grown in the presence of anti-Gap a Fab. ($\times 100$).

Table 1. The effect of monovalent antibodies to glycolipid and Gap a on sugar uptake by 3T3 BALB/c and MSV-infected cells

Type of monovalent antibodies	Uptake by infected cells/uptake by non-infected cells	
No addition	4.4*	3.5†
Antigloboside	2.1*	
Anti-GM ₃	1.7*	3.0†
Anti-GM ₁	0.9*	1.0†
Anti-Gap a		1.1†

Cells cultured in growth medium (10^5 cells in 3-cm dishes) were infected with 5×10^3 focus-forming units of MSV as described in the text. After 5 hr the medium was replaced with that containing monovalent antibodies (100 μ g/ml for antiglycolipid Fab and 70 μ g/ml for anti-Gap a Fab). Sugar uptake was determined after 4 days. Noninfected control cells also contained antibodies. After the incubation, cells adhering to dishes were washed five times with ice-cold NaCl/P_i. The cells were dissolved in 2 M NaOH; aliquots were assayed for radioactivity in toluene-based scintillation counting fluid after neutralization with acetic acid, and other aliquots were used for protein determination. These numbers are the average of two to five determinations and represent cpm per mg of protein of MSV-infected cells with or without antibodies divided by cpm per mg of protein of control noninfected cells with or without antibodies. Actual values (cpm/mg of cell protein) for MSV-infected cells were 8,000–12,000, those for control cells were 1500–3000, and those for virus-infected, transformation-inhibited cells were 1200–3500.

* Determined with 0.1 mM 2-deoxy-D-[³H]glucose (10 μ Ci/ml), incubated at 24°C for 2 min.

† Determined with 0.1 mM 3-O-methyl-D-[³H]glucose (10 μ Ci/ml) incubated for 2 min.

MSV-infected cells cultured for 4 days in the presence or absence of antibodies and lectins were as follows (nontransformed 3T3 cells as 100%): (i) MSV-transformed cells, 70–80%; (ii) MSV-infected cells cultured in monovalent Con A, 100–110%; (iii) MSV-infected cells cultured in divalent Con A, 60%; (iv) MSV-infected cells cultured in anti-GM₃ Fab, 70%; (v) MSV-infected cells cultured in antigloboside Fab, 60%; (vi) MSV-infected cells cultured in anti-GM₁ Fab, 60%; (vii) MSV-infected cells cultured in anti-Gap a Fab, 80%. Only conditions vi and vii resulted in complete inhibition of transformation, whereas divalent Con A and antigloboside significantly inhibited growth but did not inhibit transformation. High concentrations of monovalent antibodies (110 μ g/ml for anti-GM₁ Fab; 70 μ g/ml for anti-Gap a Fab) were required for complete inhibition of transformation. Only partial inhibition was observed at lower concentrations of these reagents. Intact antibodies and tetravalent Con A were cytotoxic in terms of causing morphological change, but they were not transformation inhibitory.

The titer of the MSV virus in focus-forming units of the culture medium from transformed cells was not significantly different from that of cells whose transformed phenotypes were inhibited by either anti-GM₁ Fab or anti-Gap a Fab. The intensity of immunofluorescence by anti-gp70 antibody in MSV-transformed cells was similar to that in MSV-infected transformation-inhibited cells in the presence of anti-GM₁ Fab and anti-Gap a Fab.

Inhibition of Avian Sarcoma Virus-Induced Transformed Phenotype in NRK Cells by Anti-GM₃ Fab and by Anti-Gap a Fab. Avian sarcoma virus-transformed NRK cells have been characterized by a decreased quantity of GM₃, an appearance of a slowly migrating glycolipid, and a deletion of or decrease in Gap a and 170,000-dalton glycoprotein (unpublished data). An enhanced reactivity of NRK/LA25 cells to both anti-GM₃ Fab and anti-Gap a Fab at the nonpermissive temperature was observed, particularly at higher concentrations of antibodies (anti-GM₃ at more than 100 μ g/ml and anti-Gap a at more than

70 μ g/ml) (see Fig. 2). GM₃ and Gap a are, therefore, the transformation-sensitive cell surface molecules in NRK cells in their chemical quantity as well as in antibody reactivities. Consequently, the effect of anti-GM₃ Fab and anti-Gap a Fab on the expression of the transformed phenotype has been studied. NRK/LA25 cells exhibited a fibroblastic and contact-inhibited appearance at 39°C in contrast to the round appearance at 32°C (Fig. 3 A and B). The same cells when preincubated with anti-GM₃ Fab or with anti-Gap a Fab for 48 hr followed by a shift of temperature to 32°C remained at the same morphology as at 39°C (Fig. 3 C and F). The effective minimum doses of anti-GM₃ Fab and anti-Gap a Fab were about 100 and 70 μ g, respectively. However, anti-GM₁ Fab and antigloboside Fab were not transformation inhibitory at any concentration tested (Fig. 3 D and E). In a similar experiment with NRK/LA31 cells, the transformed phenotype was again inhibited by preincubation with anti-GM₃ Fab or anti-Gap a Fab antibodies (data not shown).

Inhibition of transformation by anti-GM₃ Fab and anti-Gap a Fab was further assessed by growth disability in soft agar. NRK/LA25 cells seeded in 0.3% agar in growth medium will grow and form colonies at 32°C but not at 39°C. However, cells grown at 39°C in the presence of anti-GM₃ Fab or anti-Gap a Fab for 48 hr did not grow in agar when the temperature was shifted to 32°C (see Table 2). The most consistent results were obtained when monolayer cultures were preincubated with antibody followed by trypsin release and seeding at 32°C in agar/medium with or without antibody. Effective inhibition of morphological transformation was observed by preincubation of NRK/LA25 cells for 24 hr with anti-Gap a Fab and for 48 hr with anti-GM₃ Fab at the nonpermissive temperature prior to temperature shift to the permissive temperature. Cells treated in a similar manner with the papain fragment of nonimmune γ -globulin, anti-GM₁ Fab, and antigloboside Fab exhibited no inhibition of morphological change or of colony formation at 32°C compared with untreated cultures.

Effect of Anti-Gap a Fab on the Ganglioside Label on the Cell Surface. Because a possible interaction of Gap a to a particular glycolipid has been suggested (3, 36), the effect of anti-Gap a Fab on the glycolipid surface label in NRK/LA25 cells was studied. A significant decline in GM₃ label but not in GM₂ was observed associated with the expression of the transformation phenotype by a temperature shift from 39 to 32°C

FIG. 2. Cell surface reactivities of NRK-LA25 cells to anti-GM₃ Fab (Left) and to anti-Gap a Fab (Right). Cells were grown for 48 hr at either 32°C (●) or 39°C (▲). Plates were washed and incubated with various doses of ¹⁴C-labeled monovalent antihematoside (anti-GM₃) or anti-Gap a Fab for 1 hr at 37°C. The cells were then washed three times with NaCl/P_i, and the residues were dissolved in sodium dodecyl sulfate and assayed for radioactivity. ¹⁴C-labeled monovalent antibodies were prepared by [¹⁴C]formaldehyde coupling (36). Each point represents the mean value of several determinations.

FIG. 3. Effect of monovalent antibodies on NRK-LA25 cell morphology. Exponential cell cultures were grown in the presence or absence of anti-GM₃ Fab or anti-Gap a Fab at the nonpermissive temperature (39°C) prior to transfer to 32°C. Control cells were treated with 70 μ g of Fab fragment per ml obtained from normal rabbit γ -globulin. (A) Control cells remaining at 39°C; (B) control cells transferred to 32°C; (C) cells grown for 48 hr in the presence of anti-GM₃ Fab prior to the temperature shift; (D) cells grown for 48 hr in the presence of anti-Gap a Fab prior to the temperature shift; (E) cells grown for 48 hr in the presence of anti-GM₁ Fab prior to the temperature shift; (F) cells grown for 48 hr in the presence of anti-Gap a Fab prior to the temperature shift. A similar result was observed when cells were grown for 24 hr in the presence of anti-Gap a Fab prior to the temperature shift. All photographs were taken 48 hr after the temperature shift. ($\times 100$).

(Table 3). The decline in GM₃ label was prevented by anti-Gap a Fab, whereby the expression of transformation phenotype was aborted. GM₃ decline associated with the temperature shift was not prevented by Con A. The chemical quantity of GM₃ in NRK/LA25 cells at 39°C was 19–21 μ g of neuraminic acid per 10 mg of protein and that at 32°C was 8–10 μ g of neuraminic acid per 10 mg of protein (unpublished data). Therefore, the decline of GM₃ surface label must be due to the decline in chemical level.

DISCUSSION

Results of this study clearly indicate that only monovalent antibodies to a specific cell surface component inhibit the process of transformation. Antibodies directed to transformation-insensitive glycolipids and divalent Con A were growth inhibitory but were not transformation-inhibitory; therefore, inhibition of transformation process *per se* is not related to growth inhibition.

The mechanism of the transformation inhibition caused by these Fab antibodies is an interesting subject for future studies. Because both Gap a and GM₃ or GM₁ have been known to be deleted or decreased when the cells were transformed (3–9), the Fab antibodies may alter the metabolism of, and may prevent the decrease of, Gap a and GM₃ or GM₁. A metabolic change in GM₃ induced by Fab anti-GM₃ at low concentration (10 μ g/ml) has been shown (13).

Table 2. Effect of monovalent antibodies on cell growth in soft agar

Cell	Fab antibody	Colonies, %*			
		39°C†	32°C†	39 → 32°C‡	
				Exp. 1	Exp. 2
NRK/LA25	—	3.4	35.6	28.2	30.5
NRK/LA25	Nonimmune	5.7	50.0	35.0	48.0
NRK/LA25	Anti-GM ₁	6.0	52.0	27.2	—
NRK/LA25	Anti-GM ₃	4.0	51.9	0(8)§	3(10)§
NRK/LA25	Anti-Gap a	0	24.0	2.2(0)§	0(0)§
NRK	—	0	0	0	0
NRK/B77	—	40.0	40.0	41.0	65.0
NRK/B77	Anti-Gap a	88.1	53.0	64.1	80.0
NRK/B77	Anti-GM ₃	66.7	52.8	75.5	75.0

* Arithmetic average of percentage of the initial cell inoculum that formed colonies. Each colony was derived from a single cell.

† Control growth in soft agar at nonpermissive and permissive temperatures. Log phase cells grown at 39°C or 32°C were released by trypsin and seeded in 0.3% agar (26) in culture medium with or without monovalent antibodies (75 μ g/ml). The cultures were placed at either 39°C or 32°C and colonies were counted under a microscope after a period of 7 days. Two fields were selected and at least 100 cells were counted.

‡ Experimental growth in soft agar after shift from nonpermissive to permissive temperature. Cultures were preincubated with monovalent antibodies at 39°C (48 hr for the antiglycolipid Fab antibodies and the antibody fraction from nonimmune sera; 24 hr for anti-Gap a Fab antibodies) prior to transfer to 32°C. Cells were then released by trypsin and cultured in 0.3% agar with or without antibody. The average values of two separate experiments are shown (Exps. 1 and 2).

§ In parentheses are shown percentages of colony formation of cells preincubated with anti-GM₃ Fab or anti-Gap a Fab followed by inoculation of cells in soft agar without Fab antibodies. Decreased inhibition was observed in the later absence of anti-GM₃ Fab, whereas the same degree of inhibition was observed in the later absence of anti-Gap a Fab. This indicated that inhibition of colony formation was not always dependent on continuous antibody treatment.

On the other hand, antibodies may alter certain organizational structures of Gap a or gangliosides. Gap a or LETS forms a fibrillar meshwork structure on and around cells when cells were confluent. Such an architecture disappeared and only sparse patchy spots remained at the cell contact region when cells were transformed (17, 37). The loss of Gap a or LETS meshwork has been well correlated with tumorigenicity in 45 tumor cell lines (37, 38). In contrast to Gap a, the mode of existence of gangliosides at the cell surface is not known; but a certain organization and orientation must be important in order to maintain normal cell morphology and function. Upon transformation, a derangement of ganglioside organization may

Table 3. Effects of anti-Gap a and Con A on the transformation-associated decline in GM₃

	39°C	39° → 32°C	39° → 32°C	
			With anti-Gap a	With Con A
GM ₃ *	190 ± 9.5	101 ± 5.0	165 ± 8.2	98 ± 4.9
GM ₂ †	38 ± 1.9	36 ± 1.8	37 ± 1.8	36 ± 1.8

Gangliosides were labeled 48 hr after transfer to 32°C in the presence or absence of anti-Gap a Fab or divalent Con A. Cells were grown to confluency in 10-cm plates and surface-labeled with sodium periodate and NaB³H₄. The radiolabeled gangliosides (from 10⁷ cells) were isolated on DEAE-Sephadex.

* The labeled GM₃ which has 3-deoxy-5-acetamidoheptulonic acid instead of sialic acid is indistinguishable from intact GM₃ under usual conditions of thin-layer chromatography.

† The radioactive substance migrated to the same position as GM₂; chemical structure was not determined.

occur as was indicated by the change of "crypticity" (2, 36). It is most likely that the cell surface organization may be fixed by incubating cells with these Fab antibodies; consequently, either meshwork structure of Gap a or a specific organization of ganglioside may become insusceptible to the transformation program coded by an oncogenic gene such as *src* (39, 40). Interestingly, the transformed phenotypes of NRK/LA25 and NRK/LA31 cells were most effectively inhibited when the cells were preincubated with antibodies for at least 24 hr at the nonpermissive temperature. If cells were preincubated with antibodies, the continuous presence of antibodies in the medium was not required for subsequent inhibition of the transformed phenotype.

The inhibition of blastocyte development at an early stage by the action of anti-F9 antibody Fab (14) could be an analogous situation to the transformation inhibition described in this paper; namely, the morphogenesis directed by the genetic program is modified or blocked by surface modification through a specific antibody to a specific surface molecule. In both cases only monovalent antibodies were effective, and multivalent antibodies and Con A derivatives were ineffective. The basis for this phenomenon requires further extensive study.

The present study indicates that changes in Gap a and ganglioside are a crucial step during the process of viral transformation; however, it is not clear whether these surface events relate to tumorigenicity *in vivo*. Genetical analysis of tumorigenicity has been successfully carried out in the Klein-Harris hybrid cell system (41, 42), in which the glycolipid pattern reflects tumorigenicity (43). We anticipate therefore that the phenotype inhibition caused by Fab antibodies may apply to tumorigenicity inhibition as well.

Note Added in Proof. Since this paper was submitted, we found that 3T3 cell transformation by MSV was more effectively inhibited when the antibodies were first preincubated with 3T3 cells as compared to the same concentration of antibodies without preincubation. This finding is consistent with the requirement of antibody preincubation for NRK/LA25 cells to observe transformation inhibition by temperature shift, and indicates that inhibition of the transformation process may be mediated by a cellular factor in addition to the antibody itself.

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