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Suppression of Experimental Lung Colonization of Mouse Colon Adenocarcinoma 26 *in Vivo* by an Anti-Idiotype Monoclonal Antibody Recognizing a Platelet Surface Molecule[1]

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ABSTRACT

The interaction between platelets and tumor cells is important in the formation of pulmonary metastasis. We previously established the 8F11 monoclonal antibody (mAb) by immunizing rats with the NL-17 cell line, a highly metastatic variant of mouse colon adenocarcinoma 26. 8F11 could inhibit the platelet aggregation *in vitro* and suppress the pulmonary metastasis *in vivo* by NL-17 cells. 8F11 recognized the M_r 44,000 sialoglycoprotein (gp44) on NL-17 cells, and the affinity-purified gp44 alone could induce platelet aggregation. Therefore, 8F11 might inhibit gp44-induced platelet aggregation by masking the epitope of gp44 that interacted with unknown molecule(s) on the platelet surface. To identify the platelet antigen that interacted with gp44, we generated anti-idiotype mAbs by immunizing rats with 8F11. Two of the established mAbs, AIP1 and AIP4, recognized not only 8F11 but also the M_r 160,000 platelet surface protein. AIP4 mAb could also inhibit the NL-17 cell-induced platelet aggregation in a dose-dependent manner. Furthermore, pretreatment of mice with AIP4 mAb suppressed the pulmonary metastasis of NL-17 cells *in vivo*. These results suggest that the M_r 160,000 platelet antigen participates in the NL-17 cell-induced platelet aggregation and colonization of NL-17 cells in the lung by interacting with the gp44 of NL-17 cells.

INTRODUCTION

Recent studies on cancer metastasis have shown that some human and animal tumor cells possess platelet-aggregating abilities that correlate with their metastatic potential (1, 2). Actually, several inhibitors of platelet aggregation have been reported to retard tumor metastasis in certain animal models (3–7). Interactions between tumor cells and platelets have been considered to facilitate the arrest of tumor cell cluster in the microcirculation, with the subsequent formation of experimental metastasis.

We previously established several clones possessing different metastatic abilities from a murine colon adenocarcinoma 26 cell line (8). Among these clones, a more highly metastatic clone, NL-17, was found to exhibit a high platelet-aggregating activity, though a less metastatic clone, NL-14, had a marginal platelet-aggregating activity (6, 9). Therefore, the ability to induce platelet aggregation was related to the metastatic potential. Because one of these clones, NL-44, possessing a high platelet-aggregating activity, did not form pulmonary metastasis even after platelet clusters formed, the platelet-aggregating activity alone is not enough for the formation of metastasis (10). NL-44 cells were thereafter found to proliferate *in vivo* more slowly than NL-17 cells. Thus, the ability to proliferate *in vivo* may also be important in the formation of pulmonary metastasis (10). These results indicate that platelet-aggregating ability and *in vivo* growth potential are two major determinants for successful experimental lung metastasis of the colon adenocarcinama 26.

We previously generated mAbs[3] by immunizing rats with NL-17 cells to identify the molecule involved in the resulting platelet aggregation. One of the established mAbs, 8F11, exhibited an inhibitory activity of the platelet aggregation *in vitro* and the lung colonization of NL-17 cells *in vivo* (11, 12). The 8F11 recognized a M_r 44,000 membrane sialoglycoprotein (gp44) on NL-17 cells (11). Using 8F11 affinity column chromatography, we recently purified the gp44 from NL-17 cells (13). The purified gp44 alone could also induce aggregation of washed platelets in a dose-dependent manner, with no requirement for plasma components. These results indicate that 8F11 may recognize the specific epitope of gp44 that interacts with platelets.

In this study, we immunized rats with 8F11 to identify the platelet surface receptor of gp44. Some anti-idiotype mAbs of IgG_{2a} and IgM subclasses were generated, and two of these mAbs, termed AIP1 and AIP4, of IgG_{2a} subclass, recognized not only 8F11 but also a M_r 160,000 platelet surface protein. AIP4 inhibited platelet aggregation by NL-17 cells in a dose-dependent manner. Furthermore, AIP4 inhibited the lung colonization of NL-17 cells *in vivo* as well as 8F11 did. These results suggest that the M_r 160,000 platelet antigen is a counterreceptor of gp44 and plays an important role in colonization of NL-17 cells in the lung.

MATERIALS AND METHODS

Animals and Tumor Cells. Female BALB/c mice and SD rats were obtained from Charles River Japan, Inc. (Kanagawa, Japan). Female BALB/c mice, ages 8–12 weeks, were used throughout these experiments. The highly metastatic clone NL-17 was established from mouse colon adenocarcinoma 26, as described previously (8). NL-17 cells were cultured at 37°C in a humidified atmosphere of 5% CO_2-95% air in RPMI 1640 (Nissui Pharmaceutical Co., Tokyo, Japan), supplemented with 10% heat-inactivated fetal bovine serum (Biocell, Carson, CA), 2 mM glutamine, and 100 μg/ml kanamycin (RPMI growth medium).

Hybridoma Production. Six SD rats were immunized by neck s.c. injections of 8F11 with Freund's complete adjuvant (Difco, Detroit, MI). One week later, the secondary i.p. immunizations were performed. The booster injection was given i.v. 3 days before spleen cells were harvested. The spleen cells were fused with mouse myeloma P3U1 cells using polyethylene glycol (M_r 4000), and the hybridomas were grown in RPMI growth medium with hypoxanthine, aminopterin, and thymidine selection medium supplement (Sigma Chemical Co., St. Louis, MO; Ref. 14). The culture supernatants were screened by cellular ELISA for the binding activity to platelets (see below).

Preparation of Mouse PRP and Washed Platelets. PRP was prepared from fresh heparinized blood drawn from BALB/c mice by cardiac puncture, followed by centrifugation at 230 × *g* for 7 min at 23°C. Platelets in PRP were separated from plasma by centrifugation at 400 × *g* for 5 min, washed, and resuspended in PBS (13).

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[3] The abbreviations used are: mAb, monoclonal antibody; PRP, platelet-rich plasma; NR IgG, normal rat IgG.

Cellular ELISA. Platelets were seeded in each well of flat-bottomed 96-well plates and fixed with 0.5% glutaraldehyde. Hybridoma culture supernatants were added to the individual wells and incubated at 23°C for 1 h. After washing with PBS, biotin-labeled goat anti-rat IgG (Fc; American Qualex, La Mirada, CA) was added to the individual wells and incubated for an additional 1 h. Then, alkaline phosphatase-avidin (Zymed, San Francisco, CA) was added to each well. After washing with PBS, the wells were developed with an alkaline phosphatase substrate kit, according to the manufacturer's instructions (Bio-Rad, Richmond, CA), and the absorbance at 405 nm was measured for each well using the model 450 microplate reader (Bio-Rad; Ref. 14).

Purification and Biotinylation of mAbs. Hybridoma cells (AIP1, AIP4, and 8F11) were cultured in RPMI growth medium, harvested by a brief centrifugation, and suspended in PBS. Four-week-old female BALB/c nude mice (Charles River Japan) were given i.p. injections of the hybridoma cells. The mice were sacrificed 7 days later, and ascites fluid was collected. The mAbs were purified using anti-rat IgG column (Sigma) or protein G-Sepharose 4B gel beads (Zymed), dialyzed against PBS, and then used for *in vitro* and *in vivo* assays as follows. The purified 8F11 mAb was biotinylated using an antibody biotinylation kit, according to the manufacturer's instructions (American Qualex).

ELISA. AIP1, AIP4, anti-mouse CD62P (P-selectin) mAb (PharMingen, San Diego, CA), and chromatographically purified NR IgG (Cappel, Durham, NC) were immobilized on 96-well plates by incubation at 23°C for 1 h. After washing with PBS, biotinylated 8F11 (10 μg/ml) was added to the individual wells and incubated for an additional 1 h. Then, alkaline phosphatase-avidin (Zymed) was added to each well, followed by developing with an alkaline phosphatase substrate kit (Bio-Rad), as described above.

Flow Cytometry. Mouse platelets were treated with AIP1, AIP4, 8F11, or NR IgG for 1 h at 4°C and then reacted with FITC-conjugated anti-rat IgG (Cappel). Fluorescence data were collected using a Becton Dickinson (San Jose, CA) FACScan.

Immunoprecipitation and Western Blot Analysis. Platelets were washed with PBS and solubilized at 4°C with lysis buffer [25 mM Tris (pH 7.4), 50 mM NaCl, 0.5% Na deoxycholate, 2% Nonidet P-40, 0.2% SDS, 1 mM phenylmethylsulfonyl fluoride, and 50 μg/ml aprotinin]. The proteins bound to AIP1, AIP4, anti-P-selectin mAb, or NR IgG were immunoprecipitated using protein G-Sepharose 4B gel beads coated with these antibodies. After electrophoresing in reducing conditions using a 4–20% gradient gel polyacrylamide, the immunoprecipitated proteins were transblotted onto a nitrocellulose membrane. The membrane was incubated with 10 μg/ml of these antibodies in blocking buffer [4% skim milk in 25 mM Tris-HCl (pH 8.0), 125 mM NaCl, 0.1% Tween 20, and 0.1% sodium azide] for 1 h. After washing with the blocking buffer, the membrane was incubated with peroxidase-conjugated anti-rat IgG (Amersham, Buckinghamshire, United Kingdom). After washing, the membrane was developed with an enhanced chemiluminescence detection system (Amersham) and Kodak X-Omat AR film.

Platelet Aggregation and Inhibition. Platelet aggregation was measured turbidometrically by an NKK HEMA Tracer I (Niko Bioscientific Co., Tokyo, Japan). A 200-μl aliquot of PRP was incubated with AIP1, AIP4, 8F11, or NR IgG at an appropriate concentration in a cuvette at 37°C under constant stirring in the aggregometer. After incubation for 30 min, 10 μl of NL-17 cell suspension (2×10^7 cells/ml) or 10 μl of 1 mM ADP (Amresco, Solon, OH) were added. The changes in light transmittance were monitored for 10 min (13).

Assay of Experimental Metastasis. NL-17 cells were harvested by brief exposure to HBSS containing 0.05% trypsin and 0.02% EDTA. The cells were washed and resuspended in HBSS supplemented with 1% BALB/c serum. The cells were kept on ice for 10 min and then adjusted to 1.25×10^5 cells/ml. Under these conditions, >95% of the cells were viable. The mice were given i.v. injections of 0.2 ml of the tumor suspension via the lateral tail vein. AIP4, 8F11, or NR IgG was given i.v. 10 min before the tumor inoculation. Lung metastasis was examined on day 14 after the tumor inoculation (12).

Statistics. The two-tailed Student's *t* test was used to compare the control group with the experimental group.

RESULTS

Establishment of Anti-Idiotype mAbs Recognizing a Platelet Antigen. A highly metastatic variant of mouse colon adenocarcinoma 26 cells, the NL-17 cell line, was known to express a platelet aggregation-inducing molecule (gp44) on the cell surface. Because our previously generated rat mAb 8F11 recognized gp44 and suppressed gp44-mediated platelet aggregation (11), it might also recognize the specific epitope of gp44 that would interact with platelets.

To identify the platelet receptor of gp44, we first tried to purify gp44 for making affinity column immobilized of gp44. However, we did not succeed in obtaining a large enough amount of gp44 because gp44 was not stable protein. Then we tried to make anti-idiotype mAbs of 8F11 by immunizing six SD rats with 8F11 and selecting the ability to recognize mouse platelets. We finally obtained six clones of the hybridomas that produced mouse platelet-recognizing mAbs. Among these mAbs, AIP1 and AIP4 were IgG_{2a} subclass, and AIP2, AIP3, AIP5, and AIP6 were IgM subclass (data not shown). Because mAbs of IgM subclass were difficult to purify and characterize, we used the AIP1 and AIP4 of IgG_{2a} subclass throughout this study.

To confirm that the raised AIP1 and AIP4 were anti-idiotype mAbs to 8F11, we examined whether biotin-labeled 8F11 could recognize AIP1 and AIP4, which were immobilized on 96-well plates. 8F11 reacted with the immobilized AIP1 and AIP4 in a dose-dependent manner (Fig. 1, *filled circles* and *filled squares*, respectively). However, 8F11 could not bind to anti-P-selectin mAb or to NR IgG (Fig. 1, *open circles* and *open triangles*, respectively). Therefore, the raised AIP1 and AIP4 were determined to be anti-idiotype mAbs to 8F11.

Then, using flow cytometry, we examined whether AIP1 and AIP4 recognized the membrane surface protein on mouse platelets. As shown in Fig. 2, AIP1 and AIP4 could recognize platelets, but 8F11 could not. To identify the molecular sizes of the recognized protein on mouse platelets, platelet lysates were immunoprecipitated with AIP1, AIP4, anti-P-selectin mAb, or control NR IgG, and then the immunoprecipitated proteins were immunoblotted by these antibodies (Fig. 3). Although AIP1 and AIP4 could immunoprecipitate and immunoblot the M_r 160,000 and M_r 140,000 proteins (Fig. 3, *A* and *B*, *Lanes 1* and *2*, respectively), the M_r 140,000 protein might be nonspecifi-

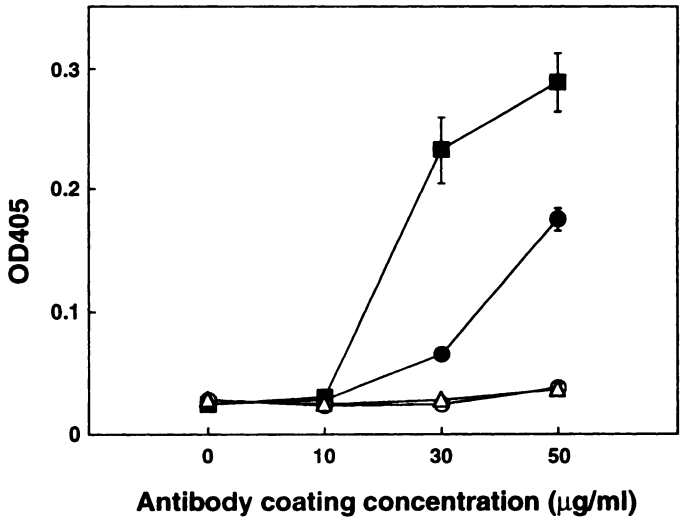

Fig. 1. Identification of AIP1 and AIP4 as anti-idiotype antibodies to 8F11. After immobilization of the indicated concentrations of AIP1 (●), AIP4 (■), anti-P-selectin mAb (○), or NR IgG (△) on 96-well plates, biotinylated 8F11 (10 μg/ml) was added to the individual wells and incubated for 1 h. Alkaline phosphatase-avidin was added to the individual wells, followed by developing with an alkaline phosphatase substrate kit. The absorbance at 405 nm of each well was measured by the model 450 microplate reader (Bio-Rad). *Data points*, mean of triplicate determinations; *bars*, SD.

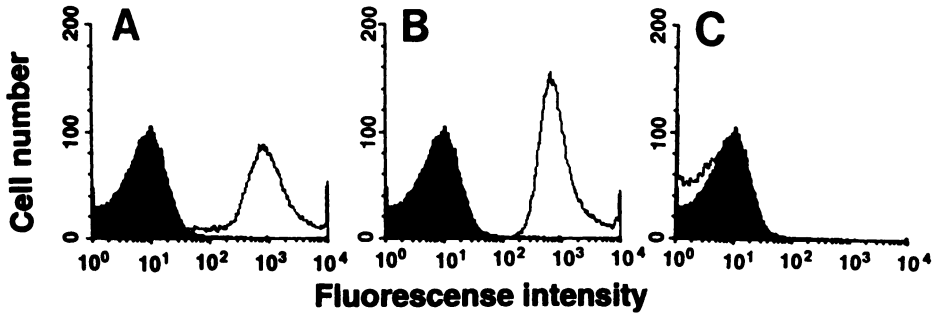

Fig. 2. Recognition of platelet surface proteins by AIP1 and AIP4 but not by 8F11. Mouse platelets were treated with AIP1, AIP4, 8F11, or NR IgG for 1 h at 4°C and then reacted with FITC-conjugated anti-rat IgG. The fluorescence intensity was analyzed using a FACScan. *Open curves*, cells treated with AIP1 (*A*), AIP4 (*B*), or 8F11 (*C*). *Filled curves*, cells treated with NR IgG.

cally immunoprecipitated protein because NR IgG immunoprecipitated the same M_r 140,000 protein (Fig. 3, *A* and *B*, *Lanes 4*). Therefore, AIP1 and AIP4 could recognize the M_r 160,000 protein. M_r 160,000 protein could not be recognized by anti-P-selectin mAb (Fig. 3*C*), indicating that it is not P-selectin.

Suppression of NL-17 Cell-induced Platelet Aggregation by AIP1 and AIP4. NL-17 cells were known to possess platelet aggregation-inducing ability. As 8F11 could suppress the platelet aggregation induced by NL-17 cells, we examined the effect of AIP1 and AIP4 on the aggregation. When platelets were preincubated with AIP1 and AIP4 at 100 μg/ml (Fig. 4*A*, *curves b* and *d*, respectively), NL-17 cell-induced platelet aggregation was inhibited as compared with NR IgG (Fig. 4*A*, *curve a*). The platelet aggregation was also repressed by 8F11 at 100 μg/ml (Fig. 4*A*, *curve c*), as reported previously (11). Moreover, the platelet aggregation was blocked by AIP4 in a dose-dependent manner (Fig. 4*B*). At a concentration of 150 μg/ml, the platelet aggregation was almost completely suppressed (Fig. 4*B*, *curve c*).

To exclude the possibility that AIP1 and AIP4 could nonspecifically inhibit the activation of platelets by binding to the M_r 160,000 platelet antigen, we investigated the effects of AIP1 and AIP4 on the ADP-mediated aggregation of platelets. As shown in Fig. 5, ADP-induced platelet aggregation was not suppressed by the preincubation of platelets with AIP1 or AIP4 (Fig. 5, *curve a* or *c*, respectively). These results suggest that AIP1 and AIP4 specifically inhibit NL-17 cell-induced platelet aggregation by directly interfering with the association between gp44 on the NL-17 cells and the M_r 160,000 protein on the platelets. As 8F11 recognized AIP1 and AIP4 (Fig. 1), AIP1 and AIP4 might recognize the idiotope in the antigen combining site of 8F11 that mimicked the gp44-binding epitope on the M_r 160,000 platelet receptor.

Inhibition of Experimental Lung Colonization of NL-17 Cells by AIP4. As platelet aggregation induced by NL-17 cells was one of the important determinants for the formation of experimental lung metastasis of NL-17 cells (10), we examined the effects of platelet aggregation-inhibiting mAb AIP4 on the formation of pulmonary metastasis *in vivo*. NL-17 cells were given i.v. after 10 min of i.v. inoculation of AIP4 at a dose of 1 mg/mouse. After 14 days, mice were sacrificed, and the nodules in the lung were counted.

Mice given AIP4 and 8F11 showed remarkably suppressed lung colonization of NL-17 cells, as compared to the untreated mice (Fig. 6*A*). The number of pulmonary nodules in the AIP4-treated mice was about 40% of that in the untreated mice (Fig. 6*B*). The lung weights of the AIP4-treated mice were smaller than those of the untreated mice (Fig. 6*C*). These results suggest that AIP4 inhibits the lung metastasis of NL-17 cells *in vivo* by masking the binding epitope of M_r 160,000 protein that interacts with the NL-17 cell surface protein, gp44.

DISCUSSION

The proposed mechanisms of platelet activation by tumor cells are: interactions of tumor cells with platelets via a sialolipoprotein and/or sialoglycoprotein (11, 15); generation of thrombin through a coagulation pathway; activation by ADP (16); activation of the arachidonate metabolism (17); release of cathepsin-B-like protease (18); and a combination of these. Among these mechanisms, several factors in tumor cells and microvesicles shed by tumor cells have been found to induce platelet aggregation through thrombin generation in the presence of plasma components (18–23). Several cancer procoagulants were identified. The procoagulant enzyme, purified from rabbit V2 carcinoma extracts, could directly activate Factor X (18). M_r 35,000 and M_r 28,000, isolated from human ovarian carcinoma, possessed procoagulant activity (20). However, plasma membrane factors that induce platelet aggregation through their direct interactions with platelets had not been identified.

Although molecules responsible for the initial tumor cell-platelet interaction are totally unknown, integrins expressed on both tumor cells and platelets (24–27), platelet membrane glycoprotein Ib/IX (28), and P-selectin (29–31) on platelets are candidates for irreversible formation of platelet-tumor cell aggregates. Several reports have suggested that ligands containing recognition sequences, such as Arg-Gly-Asp (RGD), modulate the affinity of integrins; therefore, resting integrins may be the molecules responsible for the first contact between the tumor cells and platelets (32). In contrast to these mol-

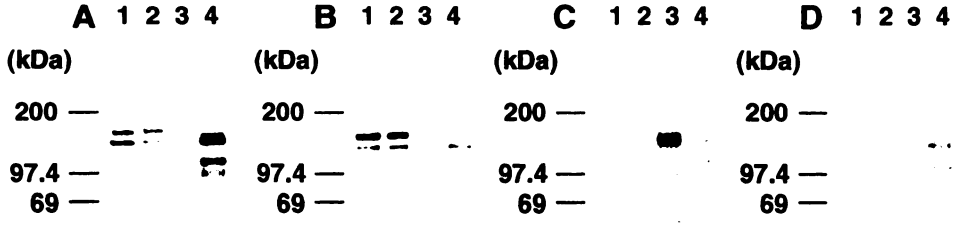

Fig. 3. Recognition of the same M_r 160,000 platelet surface protein by AIP1 and AIP4. The proteins bound to AIP1 (*Lane 1*), AIP4 (*Lane 2*), anti-P-selectin mAb (*Lane 3*), or NR IgG (*Lane 4*) were immunoprecipitated and electrophoresed in reducing conditions using a 4–20% gradient polyacrylamide gel, as described in "Materials and Methods." After blotting onto a nitrocellulose membrane, the membrane was incubated with 10 μg/ml AIP1 (*A*), AIP4 (*B*), anti-P-selectin mAb (*C*), or NR IgG (*D*) for 1 h. After washing, the membrane was incubated with peroxidase-conjugated anti-rat IgG and was developed with an enhanced chemiluminescence detection system (Amersham).

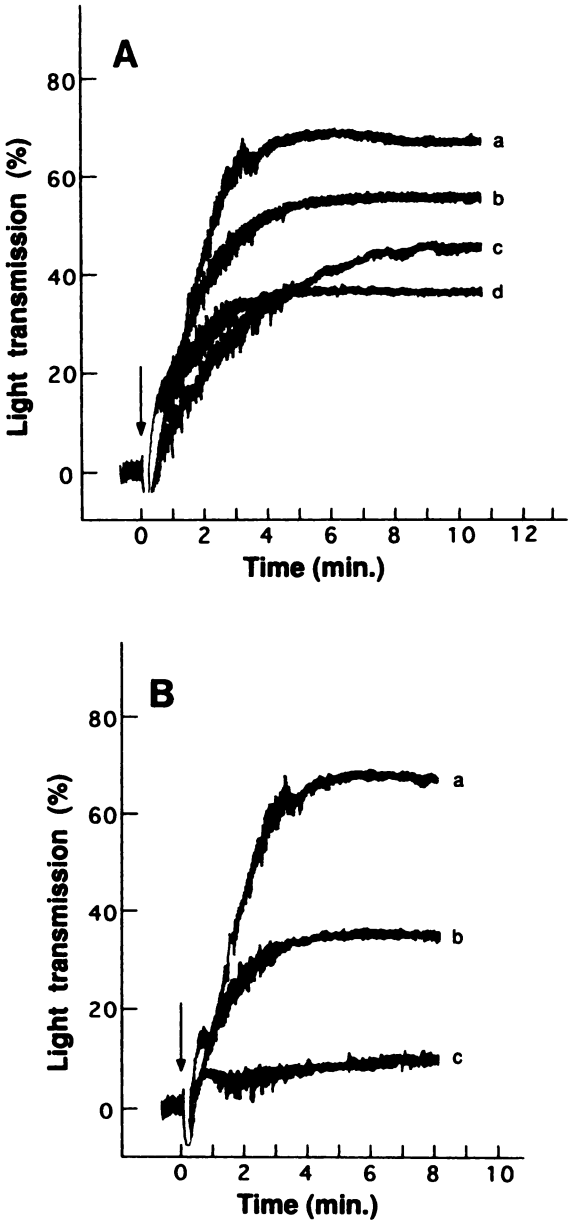

Fig. 4. Suppression of NL-17 cell-induced platelet aggregation by both AIP1 and AIP4. *A*, mouse platelets were preincubated with 100 μg/ml AIP1 (*curve b*), AIP4 (*curve d*), 8F11 (*curve c*), or NR IgG (*curve a*) in a cuvette at 37°C under constant stirring in an NKK HEMA Tracer I, as described in "Materials and Methods." After incubation for 30 min, 2×10^5 cells of the NL-17 cell suspension were added (*arrow*). *B*, mouse platelets were preincubated with 100 μg/ml (*curve b*) or 150 μg/ml (*curve c*) AIP4. They were also preincubated with 100 μg/ml NR IgG (*curve a*). After incubation for 30 min, 2×10^5 cells of the NL-17 cell suspension were added (*arrow*). The changes in light transmittance were monitored for 10 min.

ecules, P-selectin needs activation for its expression, so P-selectin might not be involved in the initial contact (30, 33).

We previously found that the highly metastatic clone NL-17, derived from mouse colon adenocarcinoma 26, had an ability to induce platelet aggregation (8). The NL-17 cell-induced platelet aggregation was mediated by a trypsin-sensitive cell surface protein and not by thrombin generation in a procoagulant mechanism or by ADP release (11). By generating rat mAb 8F11 that could suppress the aggregation, we found that the platelet aggregation-inducing protein on NL-17 cells was a M_r 44,000 membrane glycoprotein (gp44; Ref. 11). We recently purified gp44 from NL-17 cells by two affinity chromatographic methods, wheat germ agglutinin affinity chromatography and 8F11 affinity chromatography (13). Because the purified gp44 alone could induce aggregation of washed platelets containing no plasma components, it could directly interact with platelets and induce aggregation. These results from a sequential enzymatic hydrolysis of carbohydrates linked to gp44 indicate that gp44 contains a large quantity of *O*-glycans (13). Because treatment of gp44 with *O*-glycanase resulted in the loss of the reactivity of 8F11 to gp44, the carbohydrate chains linked to Ser/Thr residues were assumed to be responsible for the gp44-mediated platelet aggregation.

Murine B16 melanoma cell line also induces aggregation of platelets (34). When 8F11 was reacted on highly metastatic variants of B16 melanoma cells, it recognized the M_r 41,000 glycoprotein as an antigen (34). Moreover, 8F11 inhibited platelet aggregation induced by the melanoma cells under conditions that prevent thrombin activity. These data indicate that M_r 41,000 glycoprotein is a platelet aggregation-inducing factor in metastatic variants of B16 melanoma cells.

Because the platelet receptor of gp44 has not yet been identified, we immunized rats with 8F11 to establish anti-idiotype antibody to it. As 8F11 inhibited the gp44-induced platelet aggregation, it might mask the epitope involved in the binding of gp44 to the platelet receptor. Making an anti-idiotype antibody is a useful method of identifying an unknown receptor of the known ligand when the immunizing mAb could suppress the binding between the ligand and the receptor or could mimic the ligand (35, 36). We here report successful generation of anti-idiotype mAbs of IgG_{2a} and IgM subclass that recognize not only 8F11 but also platelet surface molecules (Figs. 1 and 2 and data not shown). Because mAbs of IgM subclass were difficult to purify, we used mAbs of IgG_{2a} subclass (AIP1 and AIP4) for additional experiments. AIP1 and AIP4 recognized the same M_r 160,000 platelet surface protein (Fig. 3). We assumed that AIP1 and AIP4 mAbs might induce platelet aggregation by mimicking gp44. However, they could not induce platelet aggregation by themselves (data not shown). Then, we examined whether AIP1 and AIP4 could inhibit platelet aggregation by NL-17 cells. AIP1 and AIP4 inhibited platelet aggregation induced by NL-17 cells, like 8F11 did (Fig. 4*A*). As ADP-induced platelet aggregation was not inhibited by AIP1 and AIP4 (Fig. 5), their binding to platelets did not lead to platelet inactivation, or blockade of

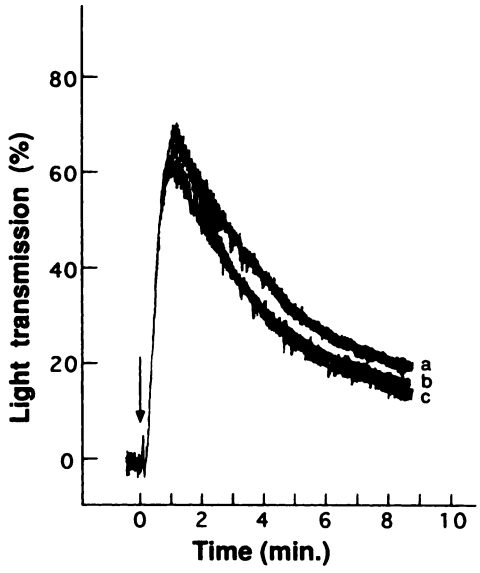

Fig. 5. The effects of AIP1 and AIP4 on ADP-mediated platelet aggregation. Mouse platelets were incubated with 100 μg/ml AIP1 (*curve a*), AIP4 (*curve c*), or NR IgG (*curve b*). After incubation for 30 min, 10 μl of 1 mM ADP was added (*arrow*). The changes in light transmittance were monitored for 10 min.

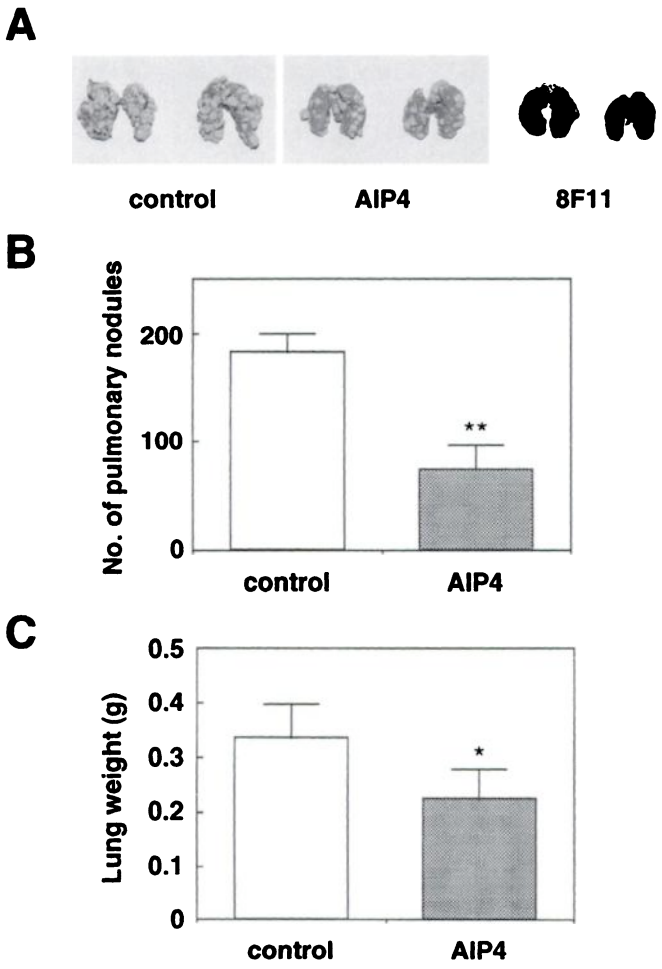

Fig. 6. Suppression of pulmonary metastasis of NL-17 cells *in vivo* by AIP4. NL-17 cells were harvested and resuspended in HBSS supplemented with 1% BALB/c serum. The mice were given i.v. injections of 0.2 ml (2.5×10^4 cells) of the tumor suspension via the lateral tail vein. AIP4 (1 mg/mouse) or 8F11 (500 μg/mouse) was given i.v. 10 min before the tumor inoculation. Lung metastasis was examined on day 14 after the tumor inoculation. Six mice were used for each group. *A*, photographs of lungs. *B*, nodules in the lungs were counted; **, $P < 0.01$. *C*, lung weights were measured; *, $P < 0.05$.

the binding between platelets. We speculate that AIP1 and AIP4 may recognize the epitope close to the gp44 binding domain of M_r 160,000 platelet receptor. By combining such epitope on the platelet receptor, AIP1 and AIP4 could suppress the gp44 binding to the platelet receptor by conformationally interfering the binding, though AIP1 and AIP4 could not induce platelet aggregation by themselves.

Pulmonary metastasis of NL-17 cells was inhibited by 8F11 when the antibody was administered before tumor inoculation (12). The effect was diminished when the antibody was given after tumor inoculation. These results indicated that 8F11 suppressed the lung colonization of NL-17 cells by interfering with the initial arrest of tumor cells in the lung vasculature by inhibiting tumor cell-platelet interaction. When we administered AIP4 and 8F11 into mice 10 min before the i.v. inoculation of NL-17 cells, it inhibited pulmonary metastasis of NL-17 cells as well as 8F11 did (Fig. 6*A*). This inhibition was dependent on the dose of AIP4 (data not shown). This indicates that AIP4 suppresses the lung colonization of NL-17 cells by blocking the initial arrest of tumor cells in the lung vasculature through the inhibition of tumor cell-platelet interaction.

The possibility exists that AIP4 bound to mouse platelets and the immune-mediated destruction of platelets could occur. Rabbit anti-mouse platelet antibody decreased the platelet count from 1.5×10^6/ml to 0.12×10^6/ml at 6 h when injected i.p. and suppressed the pulmonary metastasis of CT26 colon adenocarcinoma (37). Because we injected AIP4 10 min before tumor inoculation in this study, the suppression of pulmonary metastasis by AIP4 seemed to be independent of immune-mediated destruction of platelets. Further studies are needed to determine the involvement of immune-mediated destruction by making $F(ab')_2$ fragments of AIP4. As with 8F11-mediated suppression of lung colonization by NL-17 cells, $F(ab')_2$ fragments of 8F11 also inhibited the lung colonization (12). Therefore, mechanisms unrelated to immune-mediated destruction may be involved in the 8F11 suppression of pulmonary metastasis. Therefore, we expected that the immune-mediated destruction of platelets might not be involved in the AIP4-mediated suppression of lung colonization of NL-17 cells.

In summary, we here identified the M_r 160,000 platelet surface component as the receptor of the platelet aggregation-inducing factor, gp44 of NL-17 cells, by generating anti-idiotype mAbs to 8F11. One of these mAbs, AIP4, possesses the ability to suppress the gp44-mediated platelet aggregation and the pulmonary metastasis of NL-17 cells. Because some human tumor cells possess the platelet-aggregating abilities that correlate with their metastatic potential, further studies are needed to identify the human homologue of the M_r 160,000 platelet antigen.

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