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Fusion Regulation Proteins on the Cell Surface: Isolation and Characterization of Monoclonal Antibodies Which Enhance Giant Polykaryocyte Formation in Newcastle Disease Virus-Infected Cell Lines of Human Origin

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Newcastle disease virus (NDV)-infected HeLa and FL cells showed small polykaryocytes at about 24 h postinfection, while the addition of anti-FL-cell rabbit, rat, or mouse serum to the NDV-infected cells gave rise to giant polykaryocytes at 15 h postinfection. We isolated three monoclonal antibodies (MAbs) (4-5-1, 6-1-13, and 7-2-1) capable of enhancing giant polykaryocyte formation in NDV-infected HeLa cells. These MAbs immunoprecipitated gp80 or gp135, which were detected mainly on the surface of HeLa cells. A functionally intact F protein was essential for antibody-enhanced cell fusion, and hemagglutinating (receptor-binding) activity of HN protein was involved in the fusion at an early stage; that is, the MAbs enhanced NDV-mediated syncytium formation. These molecules were considered to have the ability to regulate NDV-mediated cell fusion and thus were designated fusion regulation protein (FRP)-1 (gp80) and FRP-2 (gp135). Anti-FRP MAbs enhanced the susceptibility of cells to fusion activity of NDV. Anti-FRP-1 MAbs reacted with a molecule on the surface of every cell derived from humans and monkeys but showed no cross-reactivity with mouse or hamster cells. FRP-2 could be detected in limited cell lines of human origin.

All enveloped viruses are potentially capable of inducing syncytia in cultured cells. Induction of syncytium formation depends on the particular combination of virus strain and host cells. Thus, even in the case of infection by the same virus, the degree of syncytium formation varies depending on the host cells. However, virus-induced cell fusion-regulating mechanisms acting in host cells are not well understood. Virus-activating proteases are closely related to induction of cell fusion by viruses, particularly avirulent paramyxovirus (5). In addition, some host cell components play important roles in virus-induced cell fusion; for instance, disturbance of microfilament function suppresses cell fusion, whereas disruption of microtubules enhances cell fusion (20). In addition, virus-induced cell fusion is controlled by host cell lipid composition (13).

Paramyxovirus infections are initiated by the attachment of the virus to sialoconjugated receptors on the cell surface by the hemagglutinin-neuraminidase (HN) glycoprotein, whereas penetration of virus into the cell is mediated by the interaction of the cleaved (activated) fusion (F) glycoprotein with the cell membrane. In the later stages of infection, the newly synthesized glycoproteins accumulate at the cell membrane, resulting in fusion with neighboring cells to produce syncytia. Syncytium formation is a major cytopathic effect induced by infection of paramyxoviruses, and furthermore, by syncytia, infection can pass from cell to cell without the production of infectious virus particles. Thus, investigations as to mechanisms regulating virus-induced syncytium formation in host cells are important for a full understanding of pathogenesis by paramyxoviruses.

We previously reported that infection of a mouse fibroblast cell line (L929 cells) by Newcastle disease virus (NDV) did not induce cell fusion, whereas addition of anti-L929 cell antiserum to the culture medium did result in cell fusion (8). This finding suggested the presence of a factor(s) on the cell surfaces of host cells capable of regulating virus-mediated cell fusion. The aims of this study were as follows: (i) confirming this result in human cell systems and (ii) isolating monoclonal antibodies (MAbs) for identifying a fusion regulation molecule(s) on the cell surface. In several hybridizations, we have isolated three MAbs which enhance cell fusion in NDV-infected human cells. These MAbs immunoprecipitated gp80 or gp135, both of which were detected on the cell surfaces of human and monkey cells. In addition, the mechanism by which cell fusion is induced in the presence of these MAbs has been investigated.

MATERIALS AND METHODS

Cells and virus. FL, HeLa, and baby hamster kidney (BHK) cells were mainly used in this study. The other cell lines used in this study are listed in Table 3. These cells were cultured in Eagle's minimum essential medium (MEM) supplemented with 5% fetal calf serum. The NDV used was the virulent Sato strain. Stock virus was prepared by inoculating 10-day-old embryonated eggs with 0.1 ml of a 10^{-3} dilution of infected allantoic fluid and incubating the eggs for 2 days at 37°C. Harvested allantoic fluid was stored at −80°C. Infectivity of NDV was determined on Vero cells and was expressed as PFU per milliliter.

Antisera. Hyperimmune rabbit, rat, and mouse sera were prepared against intact FL cells. Polyclonal antibodies and MAbs directed against NDV were kindly donated by T. Kohama and S. Umino (National Institute of Health, Tokyo, Japan) and H. Kida (Hokkaido University, Sapporo, Japan). Anti-epithelial membrane antigen (EMA) MAb was purchased from DAKOPATTS a/s, Glostrup, Denmark.

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TABLE 1. Fusion-inducing activities of antibodies

Antibody[a]	Fusion-inducing activity (titer)[b]
MAb 4-5-1	102,400
MAb 6-1-13	6,400
MAb 7-2-1	51,200
Anti-MuV MAb(24-A)	<10
Anti-HPIV-4A MAb(15A)	<10
Anti-MHC class I MAb	<10
Anti-EMA MAb	<2
Anti-fibronectin rabbit serum	<10

[a] The subclass of MAbs 4-5-1, 6-1-13, and 7-2-1 is immunoglobulin G1, and purified MAbs contained 1.72, 1.03, and 1.58 mg of protein per ml, respectively. MHC, major histocompatibility complex.

[b] Expressed as the reciprocal of the highest dilution of the antibody preparation inducing cell fusion in NDV-infected HeLa cells.

Mice. Male BALB/c mice were used for immunization with unfixed whole FL cells and also for ascites preparation.

Production and isolation of hybridoma cell lines. The immunization of mice and the fusion of mouse spleen cells with $SP^{2/0}$–AG-14 myeloma cells were performed as described previously (17). The screening procedure for individual wells was carried out by a fusion-enhancing assay using NDV-infected FL or HeLa cells. Cultures of interest were then cloned by soft-agar colony formation and further cloned by limiting dilution. MAbs were purified from ascites by using protein A-Sepharose (Amersham, Tokyo, Japan).

Isotopic labeling of infected cells, RIPA, and SDS-PAGE. Isotopic labeling of infected cells, radioimmunoprecipitation assay (RIPA), and sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were done as described previously (7).

RESULTS

Induction of cell fusion in NDV-infected FL and HeLa cells by anti-FL cell antisera. Either FL or HeLa cells (approximately 10^5 cells per well in a 96-multiwell dish) were infected with NDV at a multiplicity of infection (MOI) of 1. The cells were incubated in MEM supplemented with 5% fetal calf serum, and various concentrations of anti-FL-cell rabbit, rat, or mouse serum were added to the culture fluids 1 h after adsorption. At 15 h postinfection (p.i.), the cells were stained with Giemsa stain solution and observed by microscopy. The addition of any antiserum to NDV-infected cells gave rise to giant polykaryocytes, but neither NDV nor the antiserum alone caused fusion (data not shown). Cell fusion could be detected in anti-FL-cell antiserum-treated cells at 12 h p.i. (data not shown). Addition of 1% anti-FL-cell antiserum resulted in a fusion index of more than 95% at 15 h p.i. (data not shown). On the other hand, small polykaryocytes appeared in the NDV-infected cells incubated with control normal rabbit serum after 24 h p.i. (data not shown). These results confirm our previous study with L929 cells and NDV (8).

Isolation of MAbs that enhance polykaryocyte formation of NDV-infected HeLa cells. Since anti-FL-cell mouse serum enhanced polykaryocyte formation of NDV-infected FL or HeLa cells, we tried to isolate MAbs capable of enhancing giant polykaryocyte formation in NDV-infected HeLa cells. In total, six hybridizations were carried out, and three MAbs (4-5-1, 6-1-13, and 7-2-1) could be isolated on the final hybridization (Table 1 and Fig. 1). These MAbs also enhanced cell fusion in NDV-infected FL cells but not in NDV-infected L929 cells (data not shown). Titers of these purified MAbs were 12,800 (4-5-1), 51,200 (6-1-13), and 9,600 (7-1-2) as measured by enzyme-linked immunosorbent assay (ELISA) with HeLa cells as an antigen. These MAbs and other control antibodies were titrated for fusion-inducing activity (Table 1). Neither anti-mumps virus (MuV) (24A), anti-human parainfluenza virus type 4A (HPIV-4A) (15A), anti-major histocompatibility complex class I, anti-EMA, nor antifibronectin antibody enhanced cell fusion.

In the next experiment, MAb 4-5-1 was digested by immobilized papain, and then Fab fragment was purified by protein A-Sepharose. The ELISA titer of the Fab fragment against HeLa cells was 4,000, but the fusion-enhancing titer was less than 2 (data not shown).

Subsequently, unfixed HeLa, FL, and LLCMK2 cells were immunostained with these MAbs and fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin antiserum. As shown in Fig. 2, these MAbs reacted with the corresponding antigens on the surfaces of these cells, and interestingly, MAb 4-5-1 or 7-2-1 formed large aggregates of HeLa and FL or LLCMK2 cells, respectively. Interestingly, fixed HeLa cells showed a meshlike staining pattern (Fig. 2D). Anti-EMA or anti-fibronectin antibody, neither of which showed an enhancing effect on cell fusion, reacted with unfixed HeLa cells (Fig. 2H and data not shown).

Identification of molecules related to cell fusion. To identify molecules recognized by the MAbs, HeLa cells were labeled with either [^{35}S]methionine or [^{14}C]glucosamine for 24 h. The cells were dissolved in RIPA buffer (15 mM NaCl, 1% deoxycholate, 1% Triton X, 0.1% SDS, 10 mM Tris-HCl [pH 7.4]), and the cell lysates were immunoprecipitated by the MAbs and then analyzed by SDS-PAGE. As shown in Fig. 3, MAbs 4-5-1, 6-1-13, and 7-2-1 precipitated gp80, gp80, and gp135, respectively. The molecule purified by MAb 6-1-13 affinity chromatograpy was also reacted with MAb 4-5-1 but was not reacted with MAb 7-2-1 (data not shown). These molecules were considered to have the ability to regulate NDV-mediated cell fusion and thus were designated fusion regulation protein (FRP)-1 (gp80) and FRP-2 (gp135).

Kinetics of cell fusion in NDV-infected HeLa cells in the presence of MAb 4-5-1. A kinetic study of cell fusion was carried out (Fig. 4). HeLa cells were infected with NDV at an MOI of 1 and incubated in 50 μl of MEM–5% fetal calf serum either without or with 50 μl of MAb 4-5-1, 6-1-13, 7-2-1, 24A, or anti-EMA (fusion-enhancing titers, 64, 128, 64, <2, or <2, respectively). At appropriate times, the cultures were examined for production of infective viruses and morphological alteration of the cells, including cell fusion. The yield of NDV in culture fluids slightly decreased in the presence of anti-FRP MAb (maximum virus yields were 9.5×10^4, 2.7×10^4, 1.2×10^4, 4.5×10^5, and 5.0×10^5 in the presence of MAbs 4-5-1, 6-1-13, 7-2-1, 24A, and anti-EMA, respectively). Cell fusion was distinct in the presence of the anti-FRP MAb at about 12 h p.i., almost all nuclei were found in multinucleated giant cells by about 16 h p.i., nuclei were degenerated by approximately 20 h p.i., and balloon cells appeared at 24 h p.i. The cells became flat in the presence of the MAbs before the appearance of cell fusion (data not shown). When MAb 4-5-1 was added to the culture fluids 11 h after NDV infection, polykaryocytes appeared without a lag period and with a curve similar to that produced when the MAb was added at the start of NDV infection. On the other hand, little cell fusion occurred in the presence of control MAb (MAb 24A or anti-EMA) until 24 h p.i., and thereafter, polykaryocyte formation increased. However, the sizes of syncytia found in the presence of

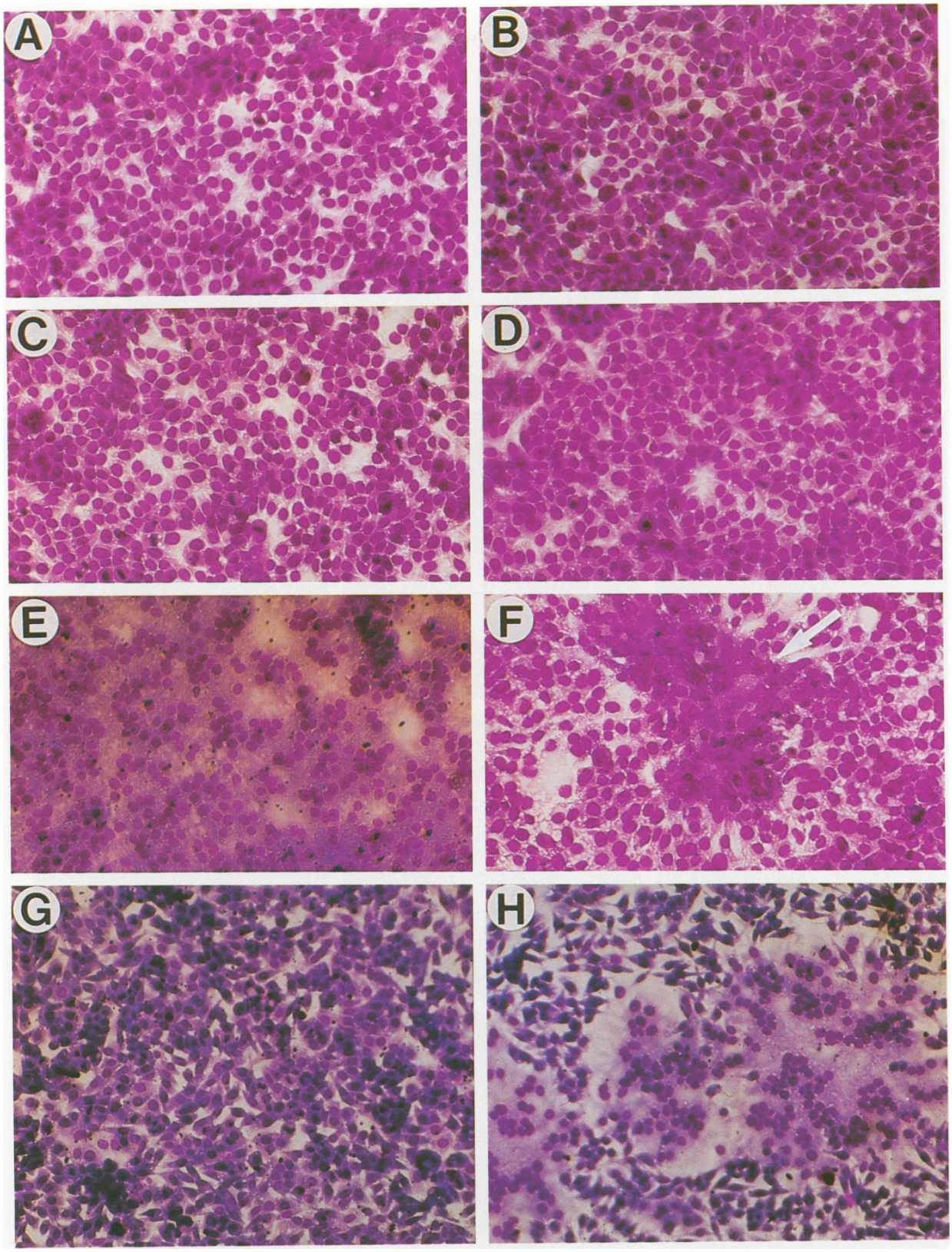

FIG. 1. Induction of cell fusion in NDV-infected HeLa cells by anti-FRP MAbs. (A through F) HeLa cells infected either without (A, C) or with NDV at an MOI of 1 (B, D, E) or 0.01 (F) were cultured in the presence or absence of MAb, and at 15 h p.i., the cells were stained with Giemsa solution. (A) HeLa cells alone; (B) NDV-infected HeLa cells; (C) HeLa cells plus MAb 4-5-1; (D) NDV-infected HeLa cells plus control MAb against EMA; (E and F) NDV-infected HeLa cells plus MAb 4-5-1 (arrow indicates syncytium cell). (G and H) BHK cells infected with NDV at an MOI of 10, incubated at 37°C for 2 h, and then washed three times with MEM. Subsequently, the cells were treated at 37°C with 1% anti-NDV rabbit serum for 1 h, washed three times with MEM, further incubated for 4 h, and then inoculated into monolayers of HeLa cells (the ratio of BHK to HeLa cells was 1:125). After another 10 h of incubation in the presence of MAb against EMA (G) or 4-5-1 (H) at 37°C, the monolayers were fixed and then stained with Giemsa stain solution.

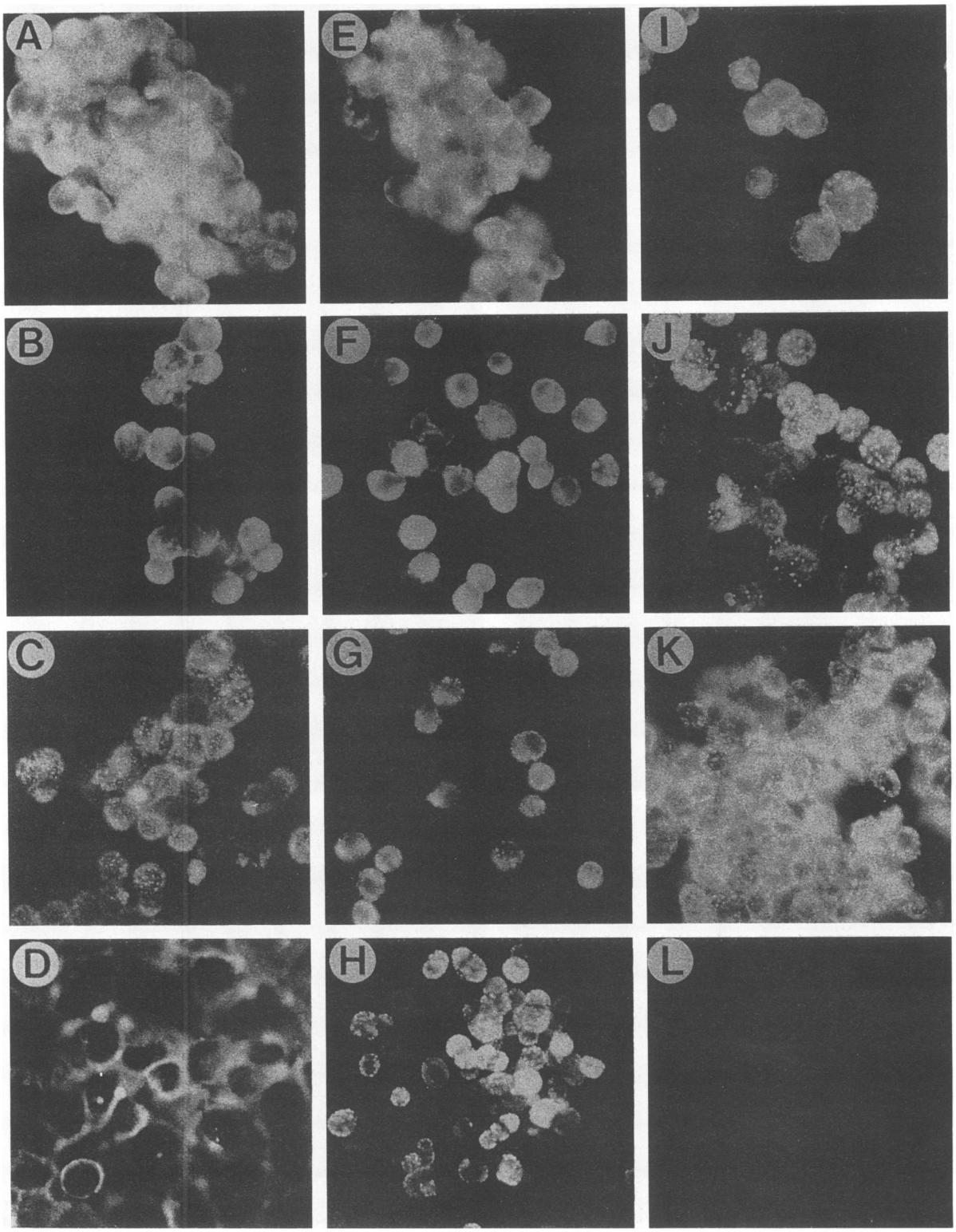

FIG. 2. Indirect immunofluorescent staining of unfixed cells (all panels except D) or acetone-treated cells (D). HeLa (A, B, C, D, H, L), FL (E, F, G), or LLCMK2 (I, J, K) cells were immunostained with MAb 4-5-1 (A, D, E, I), 6-1-13 (B, F, J), 7-2-1 (C, G, K), anti-EMA (H), or 24A (L).

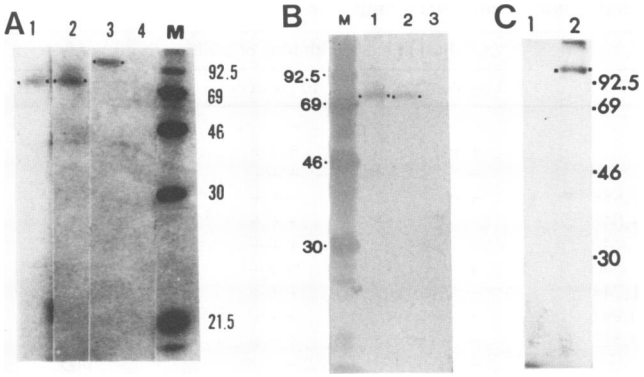

FIG. 3. Radioimmunoprecipitation by anti-FRP MAbs. HeLa cells were labeled with [^{35}S]methionine (A) or [^{14}C]glucosamine (B and C) for 24 h. The cells were dissolved in RIPA buffer, and the cell lysates were immunoprecipitated by MAb 4-5-1 (lanes 1, A and B), 6-1-13 (lanes 2, A and B), 7-2-1 (A, lane 3, and C, lane 2), or control 24A (A, lane 4; B, lane 3; C, lane 1) and then analyzed by SDS-PAGE. Lane M, molecular size markers (in kilodaltons).

control MAb never overtook that in anti-FRP MAb-treated cells (data not shown).

Polykaryocyte formation of HeLa cells cocultured with NDV-infected BHK cells in the presence of anti-FRP MAbs. To determine whether the effect of the MAbs was restricted to virus-infected cells, a new experimental system was devised. When dispersed BHK cells infected with NDV were implanted onto the monolayers of HeLa cells and these cultures were further incubated, small polykaryocytes were found at 24 h p.i.; that is, fusion of uninfected HeLa cells by infected BHK cells occurred. BHK cells did not have corresponding molecules which were recognized by these MAbs (described below), and naturally, these MAbs had no capacity to enhance cell fusion of NDV-infected BHK cells (data not shown). BHK cells were infected with NDV at an MOI of 10, incubated at 37°C for 2 h, and then washed three times with MEM. Subsequently, the cells were treated at 37°C with 1% anti-NDV rabbit serum for 1 h, washed three times with MEM, further incubated for 4 h, and then inoculated into the monolayers of HeLa cells at various ratios of BHK to HeLa cells. After 10 h of further incubation at 37°C in the presence of anti-FRP MAb 4-5-1, 6-1-13, 7-2-1, or anti-EMA, the monolayers were fixed, stained with Giemsa stain solution, and observed by microscopy. All the MAbs against FRPs also enhanced cell fusion in this system, while MAb against EMA did not (Fig. 1 and data not shown). The MAb was considered not to enhance cell fusion of HeLa cells reinfected with released NDV for the following reasons: (i) the infectious titer of NDV was scarcely detected in the culture fluid of virus-infected BHK cells or in the BHK cells per se; (ii) the suspension of NDV-infected BHK cells was diluted at approximately 1:5,000 prior to inoculation of monolayers of HeLa cells; (iii) since HeLa cells were incubated with NDV-infected BHK cells for 10 h, even if HeLa cells were reinfected with released NDV, they showed no cell fusion; and (iv) when HeLa cells were cultured with NDV-infected BHK cells and preincubated for 9 h, polykaryocyte formation could be detected within 4 h (data not shown). Therefore, these findings indicate that anti-FRP MAbs enhance the susceptibility of uninfected cells to the fusion activity of NDV.

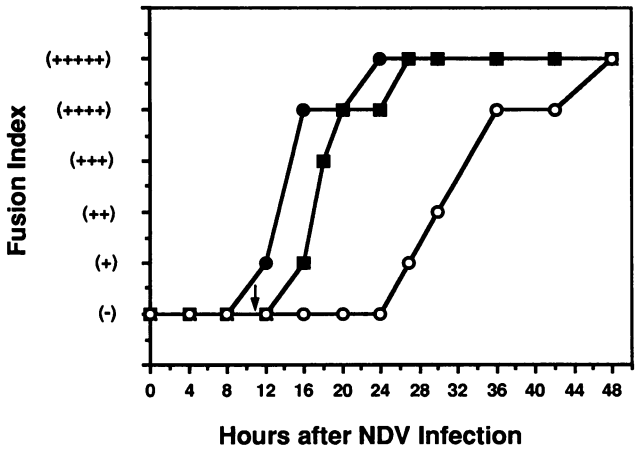

FIG. 4. Kinetic study of cell fusion. NDV-infected HeLa cells were incubated with MAb 4-5-1 (●) or anti-EMA (○). At the times indicated, the cells were stained with Giemsa staining solution and observed under the microscope. MAb 4-5-1 was also added to the culture fluids of NDV-infected cells at 11 h p.i. (↓, ■). Polykaryocytosis: −, <2%; +, 2 to 25%; ++, 25 to 50%; +++, 50 to 75%; ++++, 75 to 100%; +++++, 75 to 100% plus balloon cells.

Effect of anti-NDV antisera on antibody-induced cell fusion. To investigate whether virus-specific polypeptides played an essential role in antibody-enhanced cell fusion, NDV-infected HeLa cells were incubated with antiserum against HN, F, M, or whole virion together with MAb 4-5-1 (system I in Table 2). When added 3 h after infection, anti-HN, -F, and -whole-virion antisera prevented antibody-induced cell fusion. On the other hand, when added 10 h after infection, anti-HN antiserum was no longer effective in preventing cell fusion, whereas anti-F and anti-whole-virion sera inhibited cell fusion. Anti-M of NDV and anti-MuV antisera showed no effect on antibody-enhanced cell fusion. Subsequently, we used MAbs directed against HN or F proteins of NDV. When added 3 h p.i., two anti-HN-protein MAbs having no hemagglutination inhibition activity did not prevent induction of cell fusion, but other anti-HN MAbs with hemagglutination inhibition activity and all the anti-F MAbs inhibited syncytium formation. However, when added 10 h after infection, none of the anti-HN MAbs suppressed syncytium formation, while no cell fusion took place in the presence of any anti-F MAb (Table 2 and Fig. 5). These results indicate that a functionally intact F protein is essential for antibody-induced cell fusion and that the receptor-binding activity of HN protein is involved in fusion at an early stage; that is, anti-FRP MAbs enhance NDV-mediated syncytium formation.

In the next experiment, we tried to add MAb directed against HN or F protein of NDV to the virus-infected BHK cell-HeLa cell system (system II in Table 2; ratio of HeLa cells to NDV-infected BHK cells was 100 to 1). When added 12 h after infection, all the anti-HN MAbs showing hemagglutination inhibition activity considerably suppressed cell fusion (Table 2 and Fig. 5). These findings suggest that HN protein participates at a late stage of cell fusion in this system. The differences in the effects of anti-HN MAbs in systems I and II may be due to some discrepancy in the fusion process; that is, fusions between virus-infected cells are enhanced in system I, and fusions of uninfected cells by virus-infected cells are enhanced in system II.

Synthesis of virus-specific polypeptides in the presence of anti-FRP MAbs. NDV-induced cell fusion is mediated by

TABLE 2. Inhibition of antibody-induced cell fusion by anti-NDV antibodies

Antibody	Titer of antibody[a]				Fusion index with time of addition (h p.i.) of [b]:		
	HI	NI	HLI	NT	3 (system I)	10 (system I)	12 (system II)
Anti-whole virion	200[c]				−	−	−
Anti-M polyclonal	NT				+++	+++	ND
Anti-HN polyclonal	400[c]				−	+++	ND
MAb 388/2	<16	976	512	37,888	++	+++	++
MAb 397/2	2,048	688	1,963	24,046	−	+++	+
MAb 815/4	1,024	154	1,456	<40	−	+++	+
MAb 310/1	<16	<40	<40	<40	+	+++	++
MAb 500/1	512	5,056	580	31,744	−	+++	+
MAb 705/1	512	568	544	20,992	−	+++	+
MAb 301/1	1,024	292	556	13,312	−	+++	+
Anti-F polyclonal	400[c]				−	−	ND
MAb 59/1	<16	<40	656	4,692	−	−	−
MAb 743/1	<16	<40	1,064	3,789	−	−	−
MAb 320/1	<16	<40	3,584	13,312	−	−	−
MAb 70/1	<16	<40	1,136	16,384	−	−	−
Anti-MuV	NT				+++	+++	+++
None						+++	

[a] Biological activities of anti-NDV MAbs are quoted from reference 1. HI, hemagglutination inhibition; NI, neuraminidase inhibition; HLI, hemolysis inhibition; NT, neutral titer.

[b] System I means NDV-infected HeLa cell system, and system II means HeLa cells cocultured with NDV-infected BHK cell system. −, +, ++, and +++ indicate no, weak, intermediate, and strong syncytium formation, respectively. ND, not done. HeLa cells infected with NDV or cocultured with NDV-infected BHK cells were incubated in the presence of MAb 4-5-1 (50% hybridoma culture fluid).

[c] Antibody titer assayed by the fluorescent-antibody staining technique.

interaction between NDV glycoproteins and host cell membranes. One of the possible mechanisms enhancing NDV-mediated cell fusion by anti-FRP MAbs is MAb stimulation of synthesis of F or/and HN protein of NDV. Thus, effects of the MAbs on the syntheses of F and HN proteins were investigated. HeLa cells infected either without or with NDV were incubated with MAb 4-5-1 or anti-EMA, and after 4 h of incubation, these cells were labeled with [^{35}S]methionine in the presence of each MAb for 8 h. Subsequently, they were dissolved in RIPA buffer, and then a portion of cell lysate was analyzed by SDS-PAGE (Fig. 6). MAb 4-5-1 showed no effect on the total macromolecular synthesis of the uninfected or NDV-infected HeLa cells. However, after the appearance of cell fusion (12 h p.i.), macromolecular synthesis was suppressed in the MAb 4-5-1-treated cells, probably because of a secondary effect of their enhancement of polykaryocyte formation (data not shown). MAb 4-5-1 had a slightly suppressive effect on the synthesis of NDV-specific polypeptides until 12 h p.i., indicating that antibody-mediated enhancement of cell fusion is not caused by stimulation of NDV glycoprotein synthesis.

Cell distributions of FRP-1 and FRP-2. For consideration as to the universal significance of FRPs, cell distribution of FRP-1 and FRP-2 was investigated by indirect immunostaining of unfixed cells using MAbs and fluorescein isothiocyanate-conjugated anti-mouse immunoglobulin antiserum (Table 3). MAbs 4-5-1 and 6-1-13 reacted with all the cells derived from humans and monkeys but did not react with mouse or hamster cells, indicating that FRP-1 universally exists in human and monkey cells. These findings do not exclude the presence of FRPs in rodent cells that the MAbs do not cross-react with. On the contrary, FRP-2 could scarcely be detected in some cell lines mainly consisting of B and null cells, suggesting that FRP-2 is expressed in limited cell lines.

DISCUSSION

As for host factors involved in pathogenesis of paramyxovirus, virus receptors on the cell surface (9), virus-activating protease (5, 14), and interferon (6, 12, 19) were intensively studied. Virus receptors for paramyxoviruses were detected in the ciliated cells of the mouse trachea, while very few virus receptors were found in the epithelium of the mouse digestive system (9). These findings agree with the fact that paramyxoviruses are pneumotropic, not enterotropic, in adult mammalian hosts. The F protein of paramyxovirus is synthesized as an inactive precursor, Fo, which is cleaved by host endoproteases, and this proteolytic cleavage is essential for the fusion capacity of the F protein. Gotoh et al. (5) have recently proved that virus-activating protease of chick embryo is identical to blood-clotting factor Xa, a member of the prothrombin family. The involvement of interferon in abortive infection was reported for the infection of L929 cells with NDV (12) or mumps virus (19), in which endogenous interferon interfered with the growth of the viruses. Anti-interferon serum added to the culture medium was able to overcome the abortive replication of these viruses, and consequently, giant polykaryocytes were formed. However, the host factor(s) related to syncytium formation, which is the most important process for cytopathogenesis of several viruses, remains to be clarified. The murine T-cell line BW5147 is defective in the penetration process for mumps virus and is resistant to the fusion activity of mumps virus (19). Wild et al. (18) have recently reported that a double recombinant expressing both measles glycoproteins (H and F) gives extensive syncytia in cells of human and simian origins, but no fusion is observed in mouse, hamster, or chicken cells. They suggest that the membranes of cells other than human or simian cells contain inhibitors of cell fusion. In human immunodeficiency virus infection, the host cell must have not only the CD4 virus receptor (3, 10) but also a second undefined factor(s) in the

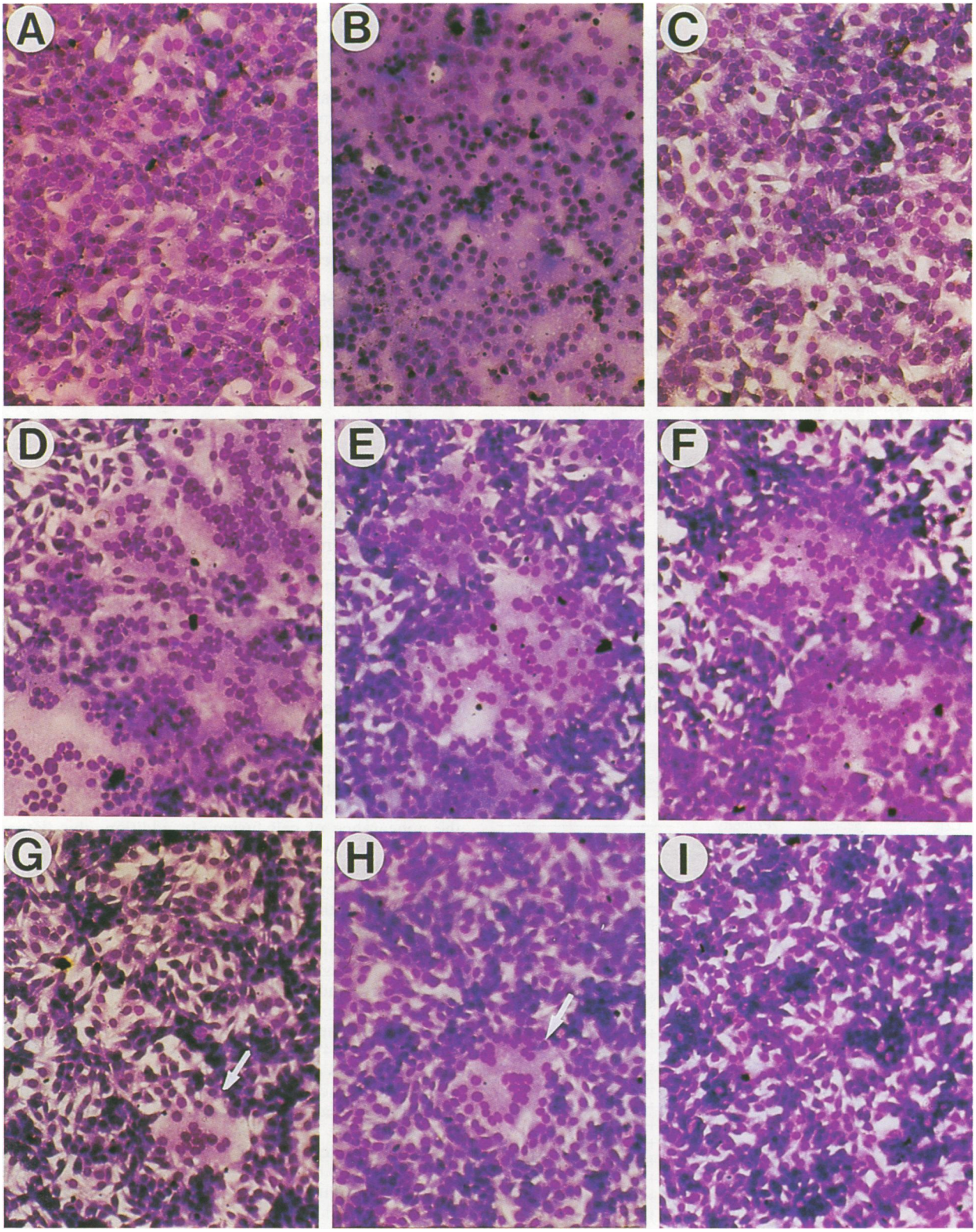

FIG. 5. Effects of anti-NDV MAbs on anti-FRP-induced cell fusion. (A through C) Anti-whole NDV virion (A), anti-HN (MAb 388/2) (B), or anti-F (MAb 320/1) (C) MAb was added to culture fluids of NDV-infected HeLa cells with MAb 4-5-1 at 10 h p.i., and after 5 h more of incubation, the cells were stained with Giemsa solution (system I). (D through I) MAb against MuV (24A) (D), HN (310/1 [E], 388/2 [F], 705/1 [G], 500/1 [H]), or F protein (70/1) (I) of NDV was added to culture fluids of HeLa cells cocultured with NDV-infected BHK cells (ratio of BHK to HeLa cells was 1:100) with MAb 4-5-1 at 12 h after infection, and after 4 h more of incubation, the cells were stained with Giemsa solution (system II). Arrows indicate syncytium cells.

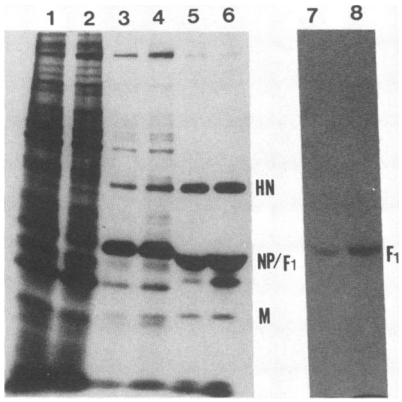

FIG. 6. Synthesis of virus-specific polypeptides in the presence of anti-FRP or anti-EMA MAb. HeLa cells infected either without (lanes 1 and 2) or with (lanes 3 to 8) NDV were incubated with MAb 4-5-1 (lanes 1, 3, 5, and 7) or anti-EMA (lanes 2, 4, 6, and 8), and after 4 h of incubation, these cells were labeled with [^{35}S]methionine in the presence of each MAb for 8 h. Subsequently, these cells were dissolved in 1 ml of RIPA buffer, and then 10 μl of cell lysate was analyzed by SDS-PAGE (lanes 1 through 4). Furthermore, 100 μl of cell lysate of NDV-infected cells was precipitated with anti-NDV virion (lanes 5 and 6) or MAbs against F protein (lanes 7 and 8). The precipitates were analyzed by SDS-PAGE.

cell membrane which determines penetration and syncytium formation (2).

In the present study, we have isolated MAbs which have abilities to enhance NDV-mediated cell fusion. These MAbs accelerated and enhanced NDV-mediated cell fusion through enhancement of the susceptibility of cells to fusion activity of NDV. These MAbs reacted with molecules on the surfaces of HeLa cells and immunoprecipitated gp80 or gp135. These findings indicate that there are more than two molecules (gp80 and gp135) on the surfaces of HeLa cells which regulate NDV-mediated cell fusion. These MAbs also enhance cell fusion mediated by another paramyxovirus, measles virus (unpublished data). Thus, these molecules are designated FRP-1 and FRP-2. In addition, MAbs 4-5-1 and 7-2-1 induce syncytium formation in U937-2 cells expressing human immunodeficiency virus gp160 (U2ME-7 cells), which show no spontaneous formation of polykaryocytes (unpublished data). FRP-1 is ubiquitously expressed on the cell surfaces of human cells, suggesting that FRP-1 may play an important role in virus-induced cell fusion or cell fusion in general.

TABLE 3. Reactivities of MAbs to surfaces of various cells

Cell	Species	Property	MAb reactivity[a]		
			4-5	6-1	7-2
FL	Human	Epithelial	+++	+++	+++
HeLa	Human	Epithelial	+++	+++	+++
Jurkat	Human	T cell	+++	+++	+
Molt4	Human	T cell	+++	+++	+
CCRF-CEM	Human	T cell	+++	++	+
Daudi	Human	B cell	+++	+++	−
Raji	Human	B cell	++	+++	−
Ball-1	Human	B cell	+++	+++	−
NALL-1	Human	Null cell	+++	+++	−
NALM-6	Human	Null cell	+++	+++	−
U937	Human	Myelo-mono	+++	+++	+
U937-2	Human	Myelo-mono	+++	+++	+
K562	Human	Myelo-mono	+++	+++	+
HL60	Human	Myelo-mono	+++	+++	+
NOMO-1	Human	Myelo-mono	++	++	−
Becker	Human	Glioma cell	+++	+++	+++
SK-N-SH	Human	Neuroblast	+++	++	+
LLCMK2	Monkey	Epithelial	+++	+++	+++
COS	Monkey	Epithelial	+++	+++	+++
Vero	Monkey	Epithelial	+++	+++	+++
PMK	Monkey	Epithelial	+++	+++	+++
L929	Mouse	Fibroblast	−	−	−
COP5	Mouse	Epithelial	−	−	−
BHK	Hamster	Epithelial	−	−	−

[a] −, +, ++, and +++, no, weak, intermediate, and strong syncytium formation, respectively.

Recently published studies concerning cell fusion by paramyxoviruses indicate that both the hemagglutinin(-neuraminidase) and fusion glycoproteins are required for fusion (4, 11, 15, 18). Morrison et al. (11) expressed the cDNA derived from the F or HN gene of NDV in chick embryo cells by using a retrovirus vector and showed that F-protein-expressing cells would not fuse if mixed with uninfected cells or with cells expressing HN protein and that F- and HN-protein-expressing cells would fuse uninfected cells, indicating that the HN protein must be in the same cell as the F protein. The nature of the contribution of the HN protein in fusion is not clear. Since neither retrovirus *env* protein nor the influenza virus HA protein can substitute for the NDV HN protein in cell fusion (11), a function(s) of HN protein other than hemagglutinating activity is required for cell fusion. Enhancement of NDV-mediated cell fusion by anti-FRP MAb is not accomplished through stimulation of NDV glycoprotein expression. Even when anti-HN-protein MAbs were added at 12 h p.i. to culture fluids of HeLa cells cocultured with NDV-infected BHK cells in the presence of anti-FRP-1 MAb, a large number of anti-HN MAbs considerably suppressed cell fusion, suggesting that the function(s) of HN protein is also involved in this fusion between virus-infected and uninfected cells.

Particularly intriguing is the observation that NDV-infected cells become flat in the presence of MAb 4-5-1 before the appearance of cell fusion. In addition, this flatness of cells is found with uninfected HeLa cells incubated with MAb 4-5-1 (unpublished data), suggesting that the functions of the cytoskeletal system may be secondarily disturbed in cells treated with anti-FRP MAb. Since the Fab fragment of MAb 4-5-1 is not capable of inducing cell fusion and flatness (unpublished data), the disturbance of the cytoskeletal system may be triggered by cross-linking of FRPs with antibody. Furthermore, when various kinds of cells were incubated in the presence of anti-FRP MAbs, very large aggregates formed at 37°C within 30 min of incubation but did not form at 4°C (Fig. 2 and unpublished data), indicating that the MAbs may stimulate expression or activate a function(s) of the adhesion molecule(s). Although cell agglutination may play an important role in antibody-enhanced cell fusion, the promotion of cell-cell aggregation is not considered sufficient to enhance virus-mediated cell fusion, because (i) MAbs 6-1-13 and 7-2-1 showing high fusion-enhancing activity scarcely aggregate HeLa cells; (ii) MAb 7-2-1 did not have a fusion-enhancing effect on NDV-infected Vero cells, while the MAb aggregates Vero cells; and (iii) although MAb 6-1-13 has a strong aggregation ability, the MAb shows no activity inducing cell fusion of U2ME-7 cells (human immunodeficiency virus gp160-expressing U937-2 cells), which form giant polykaryocytes in the presence of MAb 4-5-1 or 7-2-1 (unpublished data).

Giant multinucleated cells are not found physiologically except for osteoclasts, myotubes, and so on. Giant multinucleated cells are associated with granulomatous lesions formed in response to foreign bodies, viruses, and bacteria. For example, the immune response to tubercle bacilli results in a classic granulomatous lesion, which presents as a large number of macrophages and giant multinucleated cells surrounding an infectious focus (16). Cells adhere to surrounding cells in vivo, and some molecules are even exchanged between these cells. However, these cells physiologically show no cell fusion, suggesting the presence of a factor(s) suppressing cell fusion. Since anti-FRP MAbs enhance the susceptibility of cells to fusogens, FRPs found in this study may represent such factors.

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