INFECTION AND IMMUNITY, Apr. 1983, p. 351-358
0019-9567/83/040351-08$02.00/0

Vol. 40, No. 1

Enhancement of Respiratory Syncytial Virus-Induced Cytopathology by Trypsin, Thrombin, and Plasmin

EDWARD J. DUBOVI,[1]* J. DIETER GERATZ,[2] AND RICHARD R. TIDWELL[2]

Diagnostic Laboratory, New York State College of Veterinary Medicine at Cornell, Ithaca, New York 14850,[1] and Department of Pathology, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514[2]

Received 14 October 1982/Accepted 3 January 1983

A series of proteases of diverse substrate specificity were tested for their effect on respiratory syncytial virus-induced cytopathology. Three of the enzymes, thrombin, plasmin, and trypsin, were able to augment significantly the fusion of virus-infected A549 cells. On a concentration basis, thrombin was the most active promoter, followed by plasmin and then trypsin. Hirudin, a specific thrombin inhibitor, blocked the fusion-enhancing property of thrombin, yet had no influence on the basal rate of fusion in the absence of the enzyme. By contrast, the amidine-type inhibitors of trypsin-like proteases, bis(5-amidino-2-benzimidazolyl)-methane (BABIM), blocked not only the thrombin effect, but also the fusion in the thrombin-free controls. The suppressive activity of BABIM was observed at concentrations so low as to exclude any direct inhibitory effect on thrombin itself. These results make it seem very likely that thrombin advances cell fusion by activating a BABIM-sensitive protease. Plasmin and trypsin can be expected to act in a similar manner.

The involvement of proteolytic enzymes in the replication cycle of many different viruses is now well documented (15, 16). With certain virus groups, the proteolytic enzymes are coded by the viral genome and function to cleave precursor proteins made from polycistronic mRNA (17, 23, 26). With other viruses, the source of the protease(s) appears to be of host cell or culture fluid origin (18, 24, 25, 32), and these enzymes process proteins on the surface of the viral particle which are involved in the initial host cell-virus interactions. Although most cell-derived enzymes have trypsin-like specificity (9, 18, 32), the isolation and characterization of these enzymes have not been achieved.

Recently we identified several low-molecular-weight aromatic amidino compounds as having a striking effect on respiratory syncytial (RS) virus-host cell interactions (6, 7, 36). At noncytotoxic levels, they block RS virus-induced cell fusion and delay the entry of virus into the cells (6, 7). The fact that all of these agents are potent inhibitors of one or more trypsin-like enzymes strongly suggests that the antiviral effect is based on interference with a protease which presumably plays a key role in RS virus-induced cell fusion. The apparent importance of the proteolytic event raised the question whether known proteases, trypsin-like or not, could augment RS virus-induced cell fusion and might, therefore, also participate in the pathogenesis of RS virus-induced disease. The present study supplies evidence that at least three different enzymes, thrombin, plasmin, and trypsin, significantly enhance the rate and extent of RS virus-induced cytopathology. Also, the use of specific inhibitors provides evidence that for thrombin, the enhancing activity is channeled through its activation of a proenzyme.

MATERIALS AND METHODS

Virus and cell cultures. HEp-2, CV-1, MA-104, LLC-MK_2, and A549 cells were propagated in Eagle minimal essential medium (MEM) supplemented with 10% fetal bovine serum. The A549 cell line is derived from a human lung carcinoma and was obtained from J. B. Baseman (University of Texas Health Science Center at San Antonio). These cells were cloned, and one clone (D) was used at passage levels 10 to 25 from the cloning procedure. The A_2 strain of RS virus was propagated and quantitated in HEp-2 cells as previously described (6).

Cell fusion assays. Cells used for fusion assays were grown as monolayers on 15-mm glass cover slips in 24-well trays (Falcon Plastics, Oxnard, Calif.). RS virus ($10^{5.3}$ 50% tissue culture infective doses in 0.25 ml) was adsorbed to cell monolayers for 2 h at 37°C. Monolayers were then rinsed twice with MEM and overlaid with MEM containing various proteolytic enzymes. At 32 to 36 h after infection, the monolayers were fixed with methanol and stained with May-Grunwald-Giemsa stains.

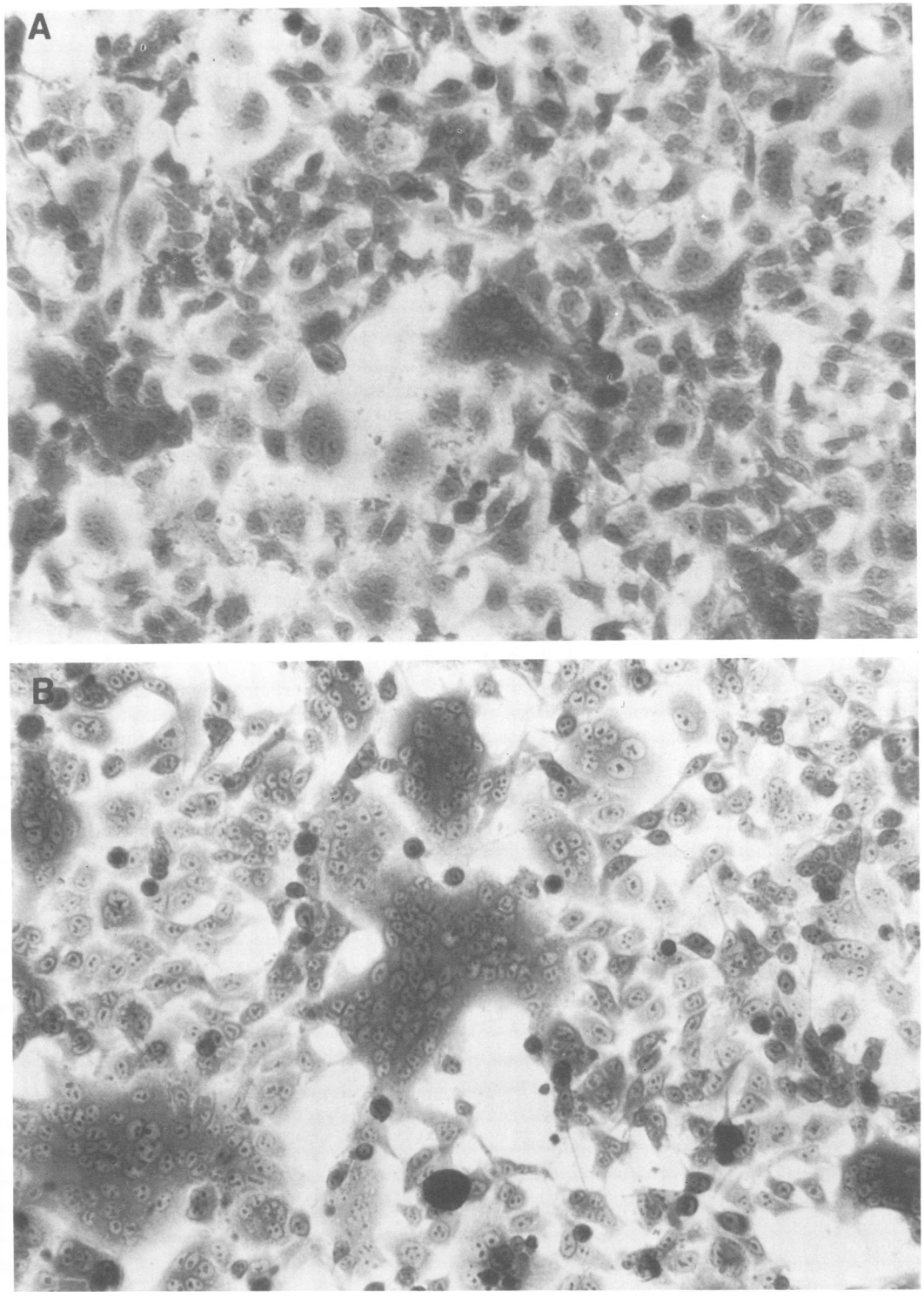

FIG. 1. Proteolytic enhancement of RS virus-induced cell fusion. A549 cells in multiwell trays were infected with RS virus at a multiplicity of infection of 0.1. After virus adsorption, cells were overlaid with MEM containing various proteolytic enzymes. At 36 h postinfection, cells were fixed and stained with May-Grunwald-Giemsa stains. (A) MEM control; (B) trypsin, 0.1 μg; (C) plasmin, 0.2 U; (D) thrombin, 32 U. (174×).

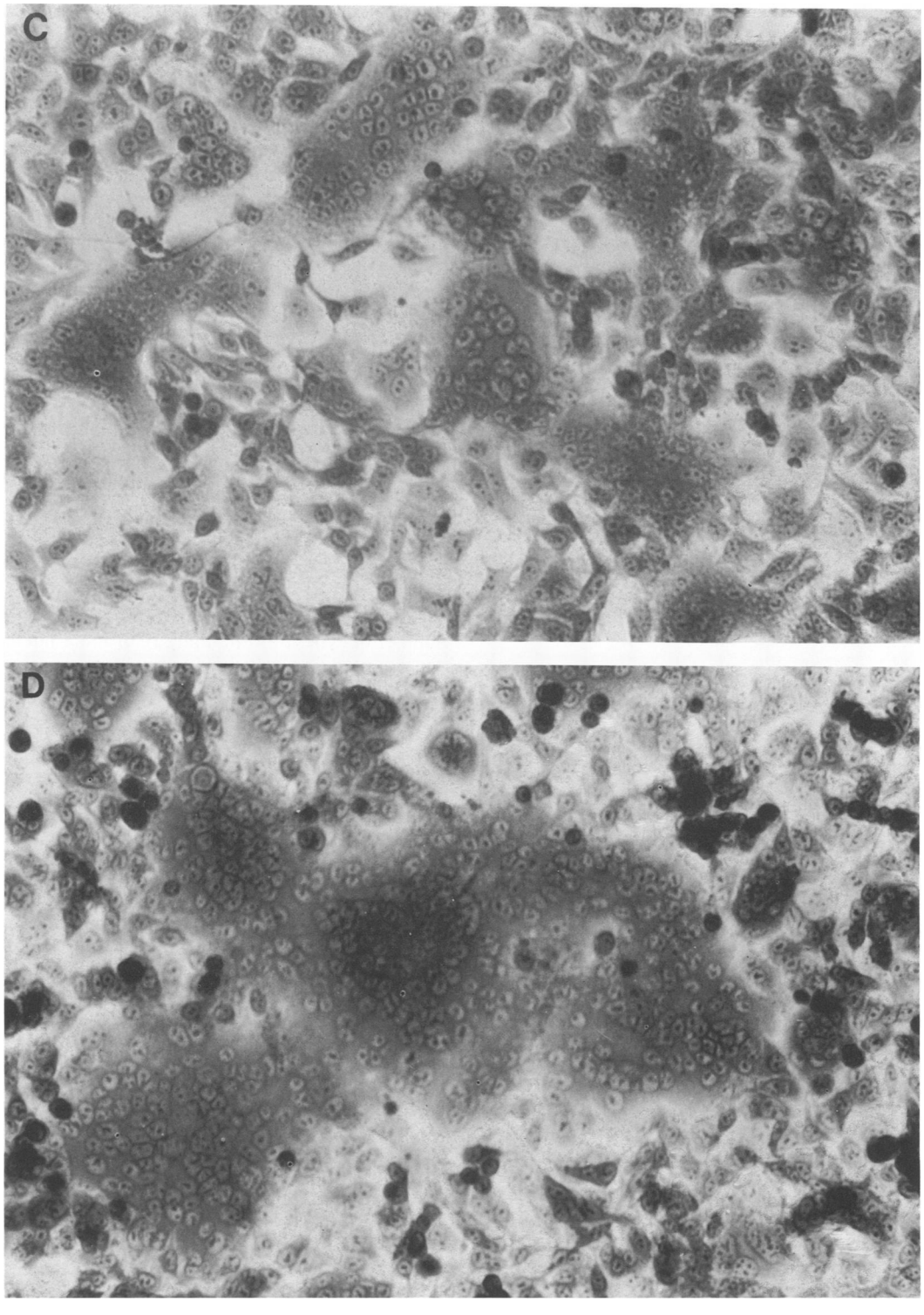

FIG. 1—*Continued*

Although qualitative changes in virus-induced cell fusion were obvious with certain proteases, the following procedure was established to quantify those effects. Syncytia with 10 or more nuclei were counted within 40 microscopic fields (×450) for each coverslip. Syncytia with less than 10 nuclei were not scored because the density and size of the A549 cells did not always permit their unambiguous determination. For the counts, only nuclei actually within a given field were counted even though giant cells at the periphery commonly extended beyond a given field. For all assays, at least triplicate sets of slides were counted. The Student's *t* test was applied for the statistical evaluation of the results.

Enzymes and reagents. Bovine thrombin, bovine trypsin (acetylated), thermolysin, protease V, and hirudin were obtained from Sigma Chemical Co., St. Louis, Mo. Human plasmin and human plasma kallikrein were purchased from Kabi Group, Inc., Greenwich, Conn. Additional enzymes were as follows: porcine pancreatic elastase (Miles Laboratories, Inc., Elkhart, Ind.), bovine chymotrypsin (Worthington Diagnostics, Freehold, N.J.), bromelain and clostridial collagenase (Calbiochem-Behring, La Jolla, Calif.), and human urokinase (Winthrop Laboratories, New York). Human thrombin (4,135 NIH units/mg) was generously supplied by J. W. Fenton II, New York State Department of Health, Albany. The synthesis of bis(5-amidino-2-benzimidazolyl)methane (BABIM) has been described earlier (35).

RESULTS

Selection of cell line suitable for studying trypsin enhancement of RS virus-induced cell fusion. In our earlier studies of the effect of trypsin inhibitors on RS virus cytopathology, we made use nearly exclusively of HEp-2 cells. As these cells undergo rather massive fusion in the presence of the virus, however, they do not lend themselves well for recognition of any superimposed fusion activity of exogenous proteases. For this reason we selected cell lines which exhibit lesser degrees of fusion induced by RS virus and surveyed their response to exposure to the prototype trypsin-like enzyme, i.e., trypsin itself. Three monkey kidney lines (CV-1, MA-104, and LLC-MK_2) and the human lung carcinoma line A549 were included in this screening process. After infection with RS virus, the cells were maintained in either enzyme-free medium or in medium with various concentrations of trypsin. At 36 h after infection, it was clearly evident that the protease had augmented the extent of cell fusion initiated by RS virus in A549 cells (Fig. 1). The number and size of the syncytia were greatly increased in the trypsin-treated cultures. At later times after infection, the trypsin enhancement was evident by the greater amount of cell debris in the culture fluid and by the decrease in the number of cells remaining on the culture dish. A slight increase of RS virus-induced cell fusion was seen in MA-104 cells, while no effect was noted with trypsin-treated LLC-MK_2 or CV-1 cells.

Effect of a variety of proteases on RS virus-induced cytopathology in A549 cells. Having demonstrated the fusion-enhancing activity of trypsin on RS virus-infected A549 cells, we then examined the effect of a series of additional proteases on RS virus-induced cell fusion. The spectrum of enzymes included five with trypsin-like activities (bovine and human thrombin and human plasmin, plasma kallikrein, and urokinase) and six with quite different specificities. At the upper end of the range of concentrations tested, most of the enzymes had a destructive effect on the cell monolayers. Of all of the proteases screened, only the two thrombin preparations and plasmin acted in the same way as trypsin and caused an enhancement of RS virus cytopathology at concentrations which did not remove the cell monolayers (Table 1 and Fig. 1). No cell fusion was seen in uninfected cells treated with any of the enzymes.

Inspection of RS virus-infected monolayers indicated that in the presence of fusion-enhancing proteases, the rate and extent of cell fusion were increased over the enzyme-free controls. To quantitate this effect in a reliable manner, the number of syncytia containing 10 or more nuclei was counted for each slide within 40 microscopic fields (×450). The total number of nuclei in each of those syncytia was also registered. The results of the analysis show that human thrombin, human plasmin, and bovine trypsin significantly increased the number of syncytia as well as the individual size as expressed by the number of nuclei per syncytium (Table 2). From these data it is evident that under the test conditions and within the range of enzyme concentrations used, increasing amounts of proteases produced increasing degrees of fusion. At even higher concentrations or at later times after infection, however, the syncytial count appears to plateau and then to decline because of approaching confluence of all infected cells.

Inhibition of thrombin-enhanced cell fusion by hirudin. Though the human thrombin used in our experiments was a highly purified preparation, we felt it advisable to exclude the possibility that a contaminant, rather than thrombin itself, might have produced the fusion enhancement. For this purpose, the specific antithrombin agent hirudin (20) was used. It was added over a range of concentrations to monolayers supplied with a fixed amount of thrombin. The results indicated that the inhibitor blocked the fusion-enhancing activity of thrombin in a dose-dependent manner, confirming the role of thrombin in the enhancement process (Table 3). From the data it is also evident that hirudin could not reduce the level of endogenous fusion activity occurring in

TABLE 1. Effect of various proteases on RS virus-induced cytopathology

Enzyme	Range of concns per ml	Fusion enhancement
Bromelain	0.1–1.0 μg	–
Chymotrypsin	0.1–1.0 μg	–
Collagenase	1.0–100 μg	–
Elastase	0.1–1.0 μg	–
Kallikrein	0.15–1.5 U[a]	–
Plasmin	0.005–0.08 U[b]	+
Protease V[c]	0.5–50 μg	–
Thermolysin	0.05–0.5 μg	–
Thrombin (bovine)	4–16 U[d]	+
Thrombin (human)	0.25–32 U[d]	+
Trypsin	0.1–2.0 μg	+
Urokinase	2.0–200 U[e]	–

[a] Plasma equivalent units (5).
[b] Sgouris units.
[c] Equivalent to current listing Protease XIV.
[d] NIH units.
[e] International units.

RS virus-infected A549 cells in the absence of added protease. Similarly, hirudin did not affect the endogenous fusion in virus-infected HEp-2 cells (data not shown).

Effect of BABIM on thrombin-enhanced cell fusion. Previously, we reported on the ability of BABIM to block very effectively the RS virus-induced fusion of HEp-2 and CV-1 cells (6, 7). We have now extended the inhibitor study to the A549 cell line to determine how the presence of a protease would influence the performance of the compound. Serial dilutions of BABIM were added to the cells after infection with RS virus. One set of cell monolayers received thrombin in a fixed concentration together with the inhibitor, while another set received inhibitor only. At 36 h after infection, the cells were fixed and examined for fusion events. The quantitative data demonstrated clearly that BABIM proved a powerful antifusion agent also for A549 cells and that the potency of the inhibitor was expressed equally well in the presence or absence of thrombin (Table 4). Even a concentration of 0.1 μM BABIM significantly reduced cell fusion in the thrombin-free cultures (assay 2), while at the same concentration, BABIM reduced cell fusion in the thrombin-treated cultures (assay 7) as compared with the thrombin control (assay 6).

TABLE 2. Enhancing effect of human thrombin, human plasmin, and bovine trypsin on RS virus-induced cell fusion

Expt	Protease added	No. of syncytia per 40 fields[a]	No. of nuclei per syncytium[a]
1	None	42 ± 7.2	14.9 ± 0.65
	Thrombin (U/ml)		
	0.25	76 ± 15.2[b]	18.5 ± 1.73[b]
	0.5	90 ± 4.0[b]	20.2 ± 1.34[b]
	1	86 ± 11.4[b]	20.2 ± 0.78[b]
	2	118 ± 14.9[b]	22.3 ± 1.9[b]
	4	111 ± 6.3[b]	23.2 ± 1.6[b]
	8	120 ± 6.5[b]	23.0 ± 1.0[b]
	16	146 ± 7.1[b]	22.2 ± 1.35[b]
2	None	29 ± 9.6	13.0 ± 0.69
	Plasmin (U/ml)		
	0.005	25 ± 6.2[c]	13.5 ± 0.59[c]
	0.0075	31 ± 11.9[c]	13.5 ± 0.31[c]
	0.01	44 ± 5.1[d]	14.3 ± 0.2[e]
	0.015	59 ± 11.5[e]	14.2 ± 0.79[d]
	0.02	85 ± 6.5[b]	16.6 ± 1.16[b]
	0.03	97 ± 6.9[b]	15.4 ± 0.54[b]
	Trypsin (μg/ml)		
	0.5	29 ± 5.9[c]	13.1 ± 0.6[c]
	0.75	50 ± 12.3[d]	14.6 ± 0.76[d]
	1.0	92 ± 16.1[b]	18.7 ± 2.6[b]

[a] All values are the means ± the standard deviation where $n = 3$, except for the control in experiment 2 where $n = 4$.
[b] Significantly different from the control at $P < 0.005$.
[c] Not significantly different from the control at $P < 0.05$.
[d] Significantly different from the control at $P < 0.05$.
[e] Significantly different from the control at $P < 0.025$.

DISCUSSION

The results reported here provide the first evidence that extracellular proteases can play an important role in RS virus-induced cell fusion. The investigation was made possible by the initial fortuitous discovery that cytopathology of RS virus-infected A549 cells is greatly enhanced by trypsin. This was in contrast to the absence of any recognizable stimulatory influence on several other cell lines, among them HEp-2 cells, which are commonly used for RS virus cultures. It may well be that the responsiveness of A549 cells, a line of human lung origin, reflects a property of epithelial cells in the respiratory tract, the normal habitat of RS virus. Studies on normal human embryonic lung cells indicate that these cells are capable of producing serine protease(s) comparable to amounts found in transformed cells (28, 30). Should these proteases be expressed at the time of an RS virus infection, then the viral lesions in the respiratory epithelium might be dramatically increased through fusion enhancement. This type of protease-virus interaction might explain the greater morbidity and mortality in children infected with RS virus before 1 year of age.

Cell fusion may convey a survival advantage to a virus which can spread through the syncytial cytoplasmic masses without leaving the cells and thus be protected from neutralizing antibodies. There are several reports of in vitro studies demonstrating virus spread through syncytium formation in the presence of neutralizing antibody (8, 11, 22, 34). The pathophysiological significance of protease-assisted cell fusion is made especially likely by our observation that not only is trypsin able to produce the response, but also thrombin and plasmin. While trypsin is a pancreatic protease normally relegated to the intestinal tract, thrombin and plasmin are present in zymogen form in blood and lymph and therefore ubiquitously in the tissues. Under conditions of congestion and inflammation, the zymogens may be activated through the clotting and fibrinolytic cascades, respectively, and enough active enzyme may become available in the lung parenchyma or the mucosa of the respiratory tract to accelerate fusion of RS virus-bearing cells. The low levels of thrombin and plasmin necessary for the promoting effect in vitro are well within the range that can be expected under in vivo conditions. For human thrombin, 0.25 NIH units/ml represents a concentration of approximately 1.5 nM, while for plasmin, 0.01 units/ml represents a concentration of 5.6 nM. Such a level for thrombin amounts to only 0.1% of the activatable prothrombin per ml of plasma, and for plasmin, the corresponding value amounts to approximately 0.2% of the available plasminogen (29). Perhaps the increased mortality seen in children with RS virus infections and congenital heart defects (19) or exposure to the inactivated RS virus vaccine (13, 14) is due to the interaction of thrombin or plasmin with the virus-infected cells in congested and inflamed lung tissue.

TABLE 3. Effect of the specific thrombin inhibitor hirudin on spontaneous and thrombin-enhanced fusion of RS virus-infected cells

Culture conditions		No. of syncytia per 40 fields[a]	No. of nuclei per syncytium[a]
Thrombin (U/ml)	Hirudin (U/ml)		
0	0	42 ± 7.2	14.9 ± 0.65
16	0	146 ± 7.1[b]	22.2 ± 1.35[b]
16	4	148 ± 16.7[b]	24.4 ± 3.31[b]
16	8	59 ± 22.4[c]	15.5 ± 1.44[c]
16	16	53 ± 7.4[c]	15.9 ± 0.21[c]
16	32	44 ± 4.5[c]	16.7 ± 1.21[c]
16	65	42 ± 5.7[c]	15.8 ± 1.42[c]
0	32	39 ± 13.5[c]	14.5 ± 2.24[c]
0	65	43 ± 8.6[c]	15.0 ± 0.56[c]

[a] All values are the means ± the standard deviation where $n = 3$, except for the thrombin- and inhibitor-free control where $n = 6$.

[b] Significantly different from the thrombin- and inhibitor-free control ($P < 0.005$).

[c] Not significantly different from the thrombin- and inhibitor-free control ($P > 0.05$).

TABLE 4. Effect of BABIM on spontaneous and thrombin-enhanced fusion of RS virus-infected cells

Assay no.	Culture conditions		No. of syncytia per 40 fields[a]	No. of nuclei per syncytium[a]
	Thrombin (U/ml)	BABIM (μM)		
1	0	0	42 ± 7.2	14.9 ± 0.65
2	0	0.1	12 ± 2.9[b]	13.6 ± 1.0[c]
3	0	1.0	6 ± 3.9[b]	10.9 ± 1.18[b]
4	0	10.0	3 ± 2.2[b]	13.6 ± 2.1[c]
5	0	100.0	5 ± 1.9[b]	13.5 ± 2.1[c]
6	16	0	146 ± 7.1[b]	22.2 ± 1.35[b]
7	16	0.1	83 ± 5.0[d]	16.4 ± 0.87[d]
8	16	1.0	35 ± 8.4[d]	14.3 ± 0.47[d]
9	16	10.0	0[d]	0[d]
10	16	100.0	4 ± 1.0[d]	11.8 ± 0.37[d]

[a] All values are the means ± the standard deviation where $n = 3$, except for the thrombin- and BABIM-free control on line 1 where $n = 6$.

[b] Significantly different from the control on line 1 ($P < 0.005$).

[c] Not significantly different from the control on line 1 ($P > 0.05$).

[d] Significantly different from the thrombin-containing, BABIM-free control on line 6 ($P < 0.005$).

The biological effect of the addition of exogenously added proteases to RS virus does not appear to be identical to the response of paramyxoviruses. If paramyxoviruses, such as Sendai and Newcastle disease virus, are grown in cells deficient in trypsin-like activity, the virions formed will be of low infectivity. However, the number of infectious units can be markedly increased by exposure of the virions to trypsin. The enzyme brings about cleavage of a viral surface glycoprotein which is changed from an inactive precursor into an active fusion (F) glycoprotein. Glycoprotein F, in turn, plays a central role in the penetration of the virus, in the characteristic cell fusion, and in the virus-dependent lysis of erythrocytes (25, 31). This sequence of events is significantly different from the biological effects of exogenous proteases on RS virus where yields from thrombin-containing cultures were similar to those from enzyme-free cultures despite the marked difference in cytopathology (data not shown). An analogous situation was observed for mouse hepatitis virus. Here, trypsin treatment of infected cells also resulted in a considerable increase in syncytium formation without any effect on virus yield (37). It was speculated that the protease was probably directed against a cellular target and not against any component of the virions themselves.

In clarifying the mechanism of thrombin-de-

pendent fusion enhancement, we were aided greatly by the use of two inhibitors, i.e., hirudin and BABIM. The former, a polypeptide which blocks thrombin activity in a highly effective and selective manner (20), unequivocally established thrombin as the fusion-enhancing factor in the thrombin preparation. The latter, a synthetic aromatic diamidine which suppresses fusion of RS virus-infected cells in medium without added protease (6, 7), is also a powerful inhibitor of several trypsin-like enzymes, such as trypsin itself, human plasmin, and urokinase (10, 35). Combination of those two important properties in one and the same compound suggests that they probably are interrelated. At this point we feel that the antifusion effect is another expression of the antiproteolytic potency of BABIM and is caused by a suppression of a cell-bound protease which is involved in the fusion process. Preliminary characterization of this protease with a large series of inhibitors has shown that it is not identical with thrombin, plasmin, urokinase, or trypsin (36). With respect to the current investigation, it is also germane to note that BABIM, despite its powerful inhibition of some proteases, is only a weak inhibitor of human thrombin, with a K_i value of $1.12 \times 10^{-4} \pm 0.44 \times 10^{-4}$ at 37°C (unpublished observation). Since BABIM at concentrations of 10 μM and less is unable to block thrombin to any significant degree but at these same low concentrations can still inhibit fusion in the presence or absence of thrombin, it appears likely that thrombin exerts its fusion-promoting activity through a BABIM-sensitive event, i.e., the previously postulated cell-bound protease. As to the mode of action of thrombin (and of plasmin and trypsin) in the fusion event, we expect it to effect a proteolytic cleavage in an inactive protease and thus bring about its activation. At present, we do not have any information on the exact mechanism of RS virus-induced cell fusion. Conceivably, proteolysis by thrombin may be only the first step in a cascade whose latter steps require the presence of a viral protein for fusion induction.

Enhancement of RS virus-induced cell fusion is another biological activity which can be attributed to thrombin. Besides being intimately involved in various steps of the coagulation process, thrombin can cause release of prostaglandin from endothelial cells (5), initiate platelet aggregation (33) and the release reaction (12), and either alone or in combination with other factors, stimulate cell division in a variety of cells (1, 3, 4, 38). For many of these cell types, high-affinity binding sites for the enzyme have been identified on the cell membranes and have been related to the initiation of the cellular response (2, 21, 27). It would be of interest to determine the presence or absence of thrombin receptors on A549 cells, to ascertain if binding is reversible or irreversible, and also to map the binding sites in relationship to the probably membrane-associated protease involved in the virus-induced fusion process. In light of thrombin's recognized mitogenic activity, one might question whether that property could have been responsible for increased syncytium formation of RS virus-infected cells by stimulating nuclear division in the absence of subsequent cytoplasmic partition. This seems highly unlikely because the morphological arrangement of the syncytia suggested fusion of neighboring cells rather than an expansile growth with compression of adjacent cells and because the occurrence of mitoses within syncytia was a rare event.

ACKNOWLEDGMENT

This work was supported by Public Health Service grant HL 19171-06 from the National Institute of Heart, Lung and Blood.

LITERATURE CITED

1. **Bruhn, H. D.** 1981. Growth regulation of vessel wall cells and of tumor cells by thrombin, factor XIII, and fibronectin. Thromb. Haemostasis **46**:762.
2. **Carney, D. H., and D. D. Cunningham.** 1978. Role of specific cell surface receptors in thrombin-stimulated cell division. Cell **15**:1341–1349.
3. **Chen, L. B., and J. M. Buchanan.** 1975. Mitogenic activity of blood components. I. Thrombin and prothrombin. Proc. Natl. Acad. Sci. U.S.A. **72**:131–135.
4. **Chen, L. B., N. N. H. Teng, and J. M. Buchanan.** 1976. Mitogenicity of thrombin and surface alterations on mouse splenocytes. Exp. Cell Res. **101**:41–46.
5. **Czervionke, R. L., J. B. Smith, J. C. Hoak, G. L. Fry, and D. L. Haycraft.** 1979. Use of a radioimmunoassay to study thrombin-induced release of PGI_2 from cultured endothelium. Thromb. Res. **14**:781–786.
6. **Dubovi, E. J., J. D. Geratz, S. R. Shaver, and R. R. Tidwell.** 1981. Inhibition of respiratory syncytial virus-host cell interactions by mono- and diamidines. Antimicrob. Agents Chemother. **19**:649–656.
7. **Dubovi, E. J., J. D. Geratz, and R. R. Tidwell.** 1980. Inhibition of respiratory syncytial virus by bis(5-amidino-2-benzimidazolyl)methane. Virology **103**:502–505.
8. **Fernie, B. F., and J. L. Gerin.** 1982. Immunochemical identification of viral and nonviral proteins of the respiratory syncytial virus virion. Infect. Immun. **37**:243–249.
9. **Garten, W., F. X. Bosch, D. Linder, R. Rott, and H.-D. Klenk.** 1981. Proteolytic activation of the influenza virus hemagglutinin: the structure of the cleavage site and the enzymes involved in cleavage. Virology **115**:361–374.
10. **Geratz, J. D., S. R. Shaver, and R. R. Tidwell.** 1981. Inhibitory effect of amidino-substituted heterocyclic compounds on the amidase activity of plasmin and of high and low molecular weight urokinase and on urokinase-induced plasminogen activation. Thromb. Res. **24**:73–83.
11. **Hoggan, M. D., and B. Roizman.** 1959. The isolation and properties of a variant of herpes simplex producing multinucleated giant cells in monolayer cultures in the presence of antibody. Am. J. Hyg. **70**:208–219.
12. **Holmsen, H., H. J. Day, and H. Stormorken.** 1969. The blood platelet release reaction. Scand. J. Haematol. Suppl. **8**:1–16.
13. **Kapikian, A. Z., R. H. Mitchell, R. M. Chanock, R. A. Shvedoff, and C. E. Stewart.** 1969. An epidemiologic study of altered clinical reactivity to respiratory syncytial

(RS) virus infection in children previously vaccinated with an inactivated RS virus vaccine. Am. J. Epidemiol. **89**:405–421.
14. **Kim, H. W., J. G. Canchola, C. D. Brandt, G. Pyles, R. M. Chanock, K. Jensen, and R. H. Parrott.** 1969. Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. Am. J. Epidemiol. **89**:422–434.
15. **Korant, B.** 1975. Regulation of animal virus replication by protein cleavage, p. 621–644. *In* E. Reich, D. Rifkin, and E. Shaw (ed.), Proteases and biological control. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
16. **Korant, B.** 1978. Proteolytic events in viral replication, p. 171–224. *In* R. Berlin, H. Herrmann, I. Lepow, and J. Tanzer (ed.), Molecular basis of biological degradative processes. Academic Press, Inc., New York.
17. **Korant, B., N. Chow, M. Lively, and J. Powers.** 1979. Virus-specified protease in poliovirus-infected HeLa cells. Proc. Natl. Acad. Sci. U.S.A. **76**:2992–2995.
18. **Lazarowitz, S. G., A. R. Goldberg, and P. W. Choppin.** 1973. Proteolytic cleavage by plasmin of the HA polypeptides of influenza virus: host cell activation of serum plasminogen. Virology **56**:172–180.
19. **MacDonald, N. E., C. B. Hall, S. C. Suffin, C. Alexson, P. J. Harris, and J. A. Manning.** 1982. Respiratory syncytial viral infection in infants with congenital heart disease. N. Engl. J. Med. **307**:397–400.
20. **Markwardt, F.** 1970. Hirudin as an inhibitor of thrombin. Methods Enzymol. **19**:924–932.
21. **Martin, B. M., W. W. Wasiewski, J. W. Fenton II, and T. C. Detwiler.** 1976. Equilibrium binding of thrombin to platelets. Biochemistry **15**:4886–4893.
22. **Merz, D. C., A. Scheid, and P. W. Choppin.** 1980. Importance of antibodies to the fusion glycoprotein of paramyxoviruses in the prevention of spread of infection. J. Exp. Med. **151**:275–288.
23. **Moelling, K., A. Scott, K. J. Dittmar, and M. Owada.** 1980. Effect of p15-associated protease from an avian RNA tumor virus on avian virus-specific polyprotein precursors. J. Virol. **33**:680–688.
24. **Muramatsu, M., and M. Homma.** 1980. Trypsin action on growth of Sendai virus in tissue cultured cells. V. An activating enzyme for Sendai virus in the chorioallantoic fluid of the embryonated chicken egg. Microbiol. Immunol. **24**:113–122.
25. **Nagai, Y., H.-D. Klenk, and R. Rott.** 1976. Proteolytic cleavage of the viral glycoproteins and its significance for the virulence of Newcastle disease virus. Virology **72**:494–508.
26. **Palmenberg, A. C., M. A. Pallansch, and R. R. Rueckert.** 1979. Protease required for processing picornaviral coat protein residues in the viral replicase gene. J. Virol. **32**:770–778.
27. **Perdue, J. F., W. Lubenskyi, E. Kivity, S. A. Sonder, and J. W. Fenton II.** 1981. Protease mitogenic response of chick embryo fibroblasts and receptor binding/processing of human alpha-thrombin. J. Biol. Chem. **256**:2767–2776.
28. **Rifkin, D. B.** 1978. Plasminogen activator synthesis by cultured human embryonic lung cells: characterization of the suppressive effect of corticosteroids. J. Cell. Physiol. **97**:421–428.
29. **Robbins, K. C., and L. Summaria.** 1970. Human plasminogen and plasmin. Methods Enzymol. **19**:184–199.
30. **Roblin, R., and P. L. Young.** 1980. Dexamethasone regulation of plasminogen activator in embryonic and tumor-derived human cells. Cancer Res. **40**:2706–2713.
31. **Scheid, A., and P. W. Choppin.** 1974. Identification of biological activities of paramyxovirus glycoproteins. Activation of cell fusion, hemolysis, and infectivity by proteolytic cleavage of an inactive precursor protein of Sendai virus. Virology **57**:475–490.
32. **Scheid, A., and P. W. Choppin.** 1976. Protease activation of mutants of Sendai virus. Activation of biological properties by specific proteases. Virology **69**:265–277.
33. **Shermer, R. W., R. G. Mason, R. H. Wagner, and K. M. Brinkhous.** 1961. Studies on thrombin-induced platelet agglutination. J. Exp. Med. **114**:905–920.
34. **Shigeta, S., Y. Hinuma, T. Sato, and N. Ishida.** 1968. The cell to cell infection of respiratory syncytial virus in HEp-2 monolayer culture. J. Gen. Virol. **3**:129–131.
35. **Tidwell, R. R., J. D. Geratz, O. Dann, G. Volz, D. Zeh, and H. Loewe.** 1978. Diarylamidine derivatives with one or both of the aryl moieties consisting of an indole or indole-like ring. Inhibitors of arginine-specific esteroproteases. J. Med. Chem. **21**:613–623.
36. **Tidwell, R. R., J. D. Geratz, and E. J. Dubovi.** 1982. Aromatic amidines-comparison of their ability to block respiratory syncytial virus-induced cell fusion and to inhibit plasmin and urokinase. J. Med. Chem. **26**:294–298.
37. **Yoshikura, H., and S. Tejima.** 1981. Role of protease in mouse hepatitis virus-induced cell fusion. Studies with a cold-sensitive mutant isolated from a persistent infection. Virology **113**:503–511.
38. **Zetter, B. R., and D. Gospodarowicz.** 1977. The effect of thrombin on endothelial cell proliferation, p. 551–560. *In* R. L. Lundblad, J. W. Fenton II, and K. G. Mann (ed.), Chemistry and biology of thrombin. Ann Arbor Science, Ann Arbor, Mich.