INDUCTION OF PLASMINOGEN ACTIVATOR SYNTHESIS BY ANTIBODIES*

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A substantial body of evidence now indicates that plasminogen activator (PA)[1] production is a responsive cellular function that can be modulated within wide limits by a variety of stimuli, including hormones, inflammatory agents, retinoids, and oncogenic factors (1–13). Although enzyme secretion is conspicuously associated with certain processes, such as tissue remodeling (14, 15) or cell migration (6, 16, 17), which can be considered to require localized extracellular proteolysis, the full significance of PA production in vivo and the scope of its hormonal control remain to be defined.

One aspect of interest concerns the modulation of enzyme synthesis in response to cell surface stimuli. Studies of several systems have shown that PA production can be regulated by physiological concentrations of polypeptide hormones (follicle-stimulating hormone, calcitonin, and vasopressin), whose effects are known to be mediated by interaction with specific receptors on the external surface of plasma membranes. In all of the cases so far investigated, enzyme production reflected changes in PA gene expression occurring in response to hormone-stimulated increases in cAMP levels. It is also known that specific antibodies directed against cell surface structures can both mimic polypeptide hormone effects and stimulate processes, such as inflammation (18) and tumor growth (19–21), that are associated with enhanced PA production. A particularly well-studied example is the so-called long-acting thyroid stimulator that is often present in the serum of thyrotoxic patients (22, 23). This molecule has been identified as an immunoglobulin (IgG) that reacts with the receptor for thyroid-stimulating hormone (TSH) and thereby reproduces the effects of TSH. An additional, but less well-analyzed example is the reported ability of immunoglobulin-enriched fractions from sera of pemphigus patients to enhance proteolysis in human keratinocyte cultures (24). The specific receptors that mediate the inflammatory, endocrine, or tumor-enhancing actions of antisera remain to be identified, but it is established beyond any doubt that these effects are also due to IgG.

The present studies were undertaken to determine whether specific antibodies interacting with cell surfaces could modulate PA production, with the following considerations in mind: (*a*) assays for PA are sensitive and rapid and might be a convenient means for detecting antibodies directed against surface constituents, and,

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[1] *Abbreviations used in this paper:* PA, plasminogen activator; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TSH, thyroid-stimulating hormone.

if this proved to be the case, (*b*) the PA response to monoclonal antibodies might ultimately serve to identify a spectrum of surface receptors capable, like hormone receptors, of mediating transmembrane signaling and activating intracellular response systems that regulate gene expression.

Materials and Methods

Materials were purchased from the following sources: fetal bovine serum from Reheis Chemical Co., Chicago, Ill.; Dulbecco's modified Eagle's medium from Gibco Laboratories, Grand Island Biological Co., Grand Island, N. Y.; actinomycin D, cycloheximide, colchicine, soybean trypsin inhibitor, trasylol, leupeptin, and Triton X-100 from Sigma Chemical Co., St. Louis, Mo.; twice-crystallized pepsin from Worthington Biochemical Corp., Freehold, N. J.; Aquacide IIA and bovine fibrinogen from Calbiochem-Behring Corp., American Hoechst Corp., San Diego, Calif.; protein A-Sepharose CL-4B, DEAE-Sepharose from Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J. Human plasminogen was prepared from fresh or outdated plasma by the method of Deutsch and Mertz (25). Plasminogen-depleted serum was prepared as described (16), and fibrinogen was purified according to Strickland and Beers (3). Multiwell culture dishes, model F13 16-24 TC plates were from Linbro Chemical Co., Hamden, Conn., and tissue culture dishes were from Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif. Fibronectin purified from human plasma was a gift from Dr. M. Furie.

Cell Culture. LLC-PK_1 cells (26) were a gift from Dr. J.-D. Vassalli, and KA31 cells (27) were from Dr. D. B. Rifkin, New York University. HEF-MCA (hamster embryo fibroblasts transformed by 3-methylcholanthrene) were from Dr. R. J. Pienta, Frederick Cancer Center, Fort Detrick, Md. TMCK-1 (mouse kidney cells) were obtained from American Type Culture Collection CCL139, human foreskin fibroblasts were prepared by Dr. B. Hosein, and HEF-SV40 and a clone of HEp3 (28) (human epidermoid carcinoma) were as described by Ossowski et al. (12) and Ossowski and Reich (29), respectively.

LLC-PK_1 and KA31 cells were cloned from mass culture according to the method of Puck et al. (30) and were routinely grown in Dulbecco's modified Eagle's medium with 10% fetal bovine serum. For experiments, cells were seeded at 5×10^4 per Linbro well in 0.5 ml medium containing 10% heat-inactivated fetal bovine serum. After a 24-h incubation at 37°C, the cultures were washed three times with 0.5 ml medium. The cells were then incubated in 0.5 ml medium containing 5% heat-inactivated and plasminogen-depleted fetal bovine serum. The IgG in phosphate-buffered saline (PBS) (31) was added to the appropriate wells in volume not exceeding 100 μl.

Immunization of Rabbits and Purification of IgG. Cells used to immunize rabbits were grown to confluency and detached from the culture dishes with 0.25% trypsin in 1 mM EDTA. After several washes in PBS the cells were resuspended in 0.15 N NaCl. Rabbits immunized with KA31 were given two intravenous injections weekly for a total of 4 wk, 10^7 cells per injection, and they were bled 2 d after the last injection. A total of eight rabbits were immunized by this procedure and only three yielded stimulatory antisera. To immunize with LLC-PK_1 cells, 4×10^6 (per rabbit) were mixed with complete Freund's adjuvant and the antigen was introduced by intraperitoneal, intramuscular, and subcutaneous routes. 2 wk later the rabbits were injected with 2×10^6 cells intraperitoneally without adjuvant, and after an additional week they were bled. Antisera from all six rabbits that were immunized stimulated PA production when added to LLC-PK_1 cells, and the magnitude of stimulation varied in the range of 4- to 30-fold. Additional immunization did not increase the degree of stimulation by antisera from rabbits at the lower end of the scale. Preimmune sera were collected from all rabbits before immunization. All sera were filtered, heat inactivated at 60°C for 30 min, and stored frozen at −20°C. To purify IgG, 5–10 ml of rabbit preimmune or immune serum was fractionated by ammonium sulphate (40% saturation at room temperature). The precipitate was dissolved in PBS and dialyzed against PBS overnight at 4°C. The sample was loaded on a protein A-Sepharose-CL-4B column equilibrated with PBS, the column was washed extensively with PBS, and the IgG was eluted with 3 M KCNS in PBS, pH 7.4.

Preparation of $(Fab')_2$ Fragments. Purified IgG (30 mg) was dialyzed overnight at 4°C against

0.07 M acetate buffer, pH 4.0, in 0.05 M NaCl. After dialysis, 200 μg of pepsin was added to 20 mg of IgG, and the solution was incubated for 18 h at 37°C; a portion of IgG (10 mg) was treated identically, except for addition of pepsin, and served as positive control. Digestion was stopped by raising the pH of the solution to 7.6 with 1 N NaOH. The digested antibody was passed over a protein A-Sepharose CL-4B column equilibrated with PBS. The flow-through that contained $F(ab')_2$ fragments was dialyzed overnight against PBS and concentrated by vacuum dialysis, filtered, and stored at −20°C. When analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), a single band of M_r 100,000 was detected.

Adsorption of IgG on LLC-PK1 Cells. 2 mg of immune IgG was mixed with 8×10^7 cells in a total volume of 2.5 ml PBS. The mixture was incubated for 30 min on ice. The cells were pelleted at 1,500 rpm for 15 min, and the supernate was dialyzed overnight against Dulbecco's medium. A sample of immune IgG that was treated identically, except for incubation with cells, served as a positive control. The IgG was filtered and used to stimulate PA in LLC-PK_1 cells.

Assay of PA. To determine extracellular levels of PA, aliquots of conditioned medium were collected at desired times, centrifuged at 1,000 *g* for 15 min, and the supernate was assayed as described by Unkeless et al. (1), using multiwell Linbro dishes coated with ^{125}I-fibrin. The reaction mixture contained 20 μl of conditioned medium and 2 μg of human plasminogen in a total volume of 300 μl of 0.1 M Tris HCl, pH 8.1.

For determination of cell-associated PA, cultures were washed at the indicated times with 2 × (500 μl) volumes of PBS followed by incubation in 500 μl of 0.001 M EDTA in PBS for 10 min at 37°C. The detached cells were pelleted by low speed centrifugation at 1,000 *g* for 10 min, and the pellets were lysed by adding 300 μl per 1×10^5 cells of 0.5% Triton X-100 in 0.1 M Tris HCl, pH 8.1. The cell lysates were assayed for protein content and for PA activity as described above, using 20 μg lysate protein per reaction mixture.

Each experimental point represents an average of two cultures; the extracellular and cell-associated PA of each culture were determined by a duplicate measurement in a fibrin plate assay. The duplicate measurements varied by ≤5%, and the variation between duplicate cultures was <15%. In all experiments, PA activity was compared to the activity of a standard preparation of human urokinase and expressed as Ploug milliunits (mU) per milliliter conditioned medium collected from 10^5 cells for extracellular activity or as milliunits per 10^5 cells for cell-associated activity.

Results

Three cell types were used, of which two were examined in some detail: (*a*) KA31, a 3T3 cell transformed by Kirsten sarcoma virus (32), which spontaneously produces moderate amounts of PA under ordinary conditions of culture; (*b*) LLC-PK_1, a strain derived from normal pig kidney, which produces very little PA under basal conditions but can be stimulated to high rates of enzyme synthesis by calcitonin or vasopressin;[2] and HEp-3 clone 9, a derivative of the human epithelial HEp-3. Suspensions of each cell type were used to immunize rabbits. Antisera capable of stimulating PA synthesis in KA31 cultures were obtained from a minority of rabbits and then only after prolonged immunization—eight booster injections administered over a period of 4 wk. Stimulatory antisera for LLC-PK_1 cells wee obtained from all rabbits within relatively short periods and after few injections of antigen. PA-inducing rabbit antisera to HEp-3 clone 9 were obtained after two injections during a period of 2 wk.

Stimulation of PA Production: Time-Course and Dose Response. Monolayer cultures of KA31 and LLC-PK_1 cells were exposed to IgG purified from the respective antisera, and PA production in both cases was compared with that in control cultures exposed to IgG isolated from preimmune serum. The results are presented in Fig. 1: under

[2] Dayer, J. M., J.-D. Vassalli, J. L. Bobbitt, R. N. Hull, E. Reich, and S. M. Krane. 1981. Calcitonin stimulates plasminogen activator in porcine renal tubular cells: LLC-PK_1. *J. Cell Biol.* In press.

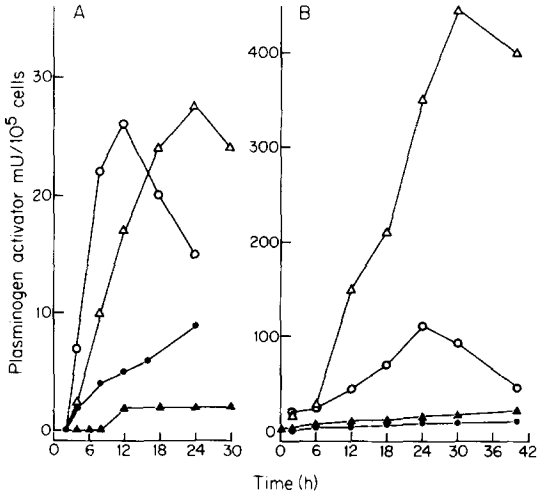


FIG. 1. Stimulation of PA production; time-course. KA31 cells and LLC-PK$_1$ cells were incubated with 1 mg/ml of specific immune IgG or with the same concentration of preimmune IgG; at indicated times, medium from two wells was removed for each experimental point, the cells were detached and lysed, and all samples were frozen. PA content of medium (extracellular activity) and of cell lysates (cell associated activity) were determined as described in Materials and Methods. △, immune IgG, extracellular activity; ○, immune IgG, cell-associated activity; ▲, preimmune IgG, extracellular activity; ●, preimmune IgG, cell-associated activity.

basal conditions, in the presence of preimmune serum, the KA31 cells (Fig. 1 A) contained readily detectable levels of cell-associated enzyme, but the amounts of PA secreted into the medium were very low. After the addition of immune IgG, cell-associated PA rose rapidly; a significant increase was observed within 4 h, reaching a peak after 8–12 h before dropping off slightly. Secreted enzyme lagged slightly behind, with the peak levels in conditioned medium being achieved after 24 h. The increase in PA production over a 24-h period was 13-fold compared with the preimmune IgG-treated control.

In the LLC-PK$_1$ cultures (Fig. 1 B), the basal levels of both cell-associated and extracellular PA were low to undetectable in the presence of preimmune IgG. Here, exposure to immune IgG was followed by a small but detectable increase in both intra- and extracellular enzyme at 2 h. Secreted PA rose rapidly beginning at 6 h and continued to do so until 30 h, reaching levels nearly 20-fold higher than the maximum obtained with KA31 cells. Cell-associated PA increased concurrently but at a lower rate, indicating that the turnover of the intracellular pool was more rapid than in the stimulated mouse cultures. In this experiment, PA production by immune IgG was stimulated ~30-fold; in numerous independent experiments, stimulation varied in the range of 4- to 30-fold.

Under conditions comparable to those used for the experiments in Fig. 1, PA production in cultures of HEp-3 clone 9 exposed to immune rabbit IgG was stimulated ~20-fold above the level in control, preimmune IgG-treated cultures (data not shown).

The dose-response curves for IgG-stimulated PA production are presented in Fig. 2. Appreciable stimulation by immune IgG was observed with both KA31 and LLC-PK$_1$ at the lowest concentration (50 μg/ml) tested. The degree of stimulation increased progressively as a function of IgG concentration, reaching a maximum for both cell

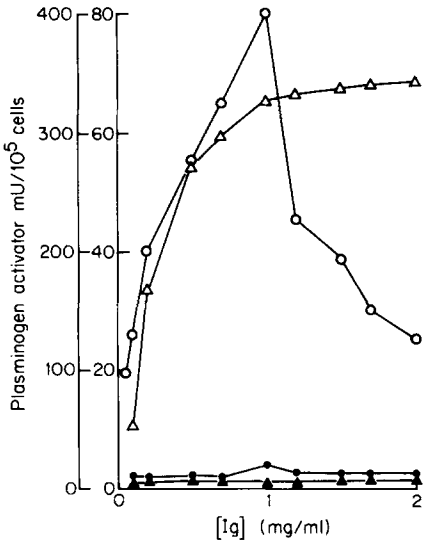
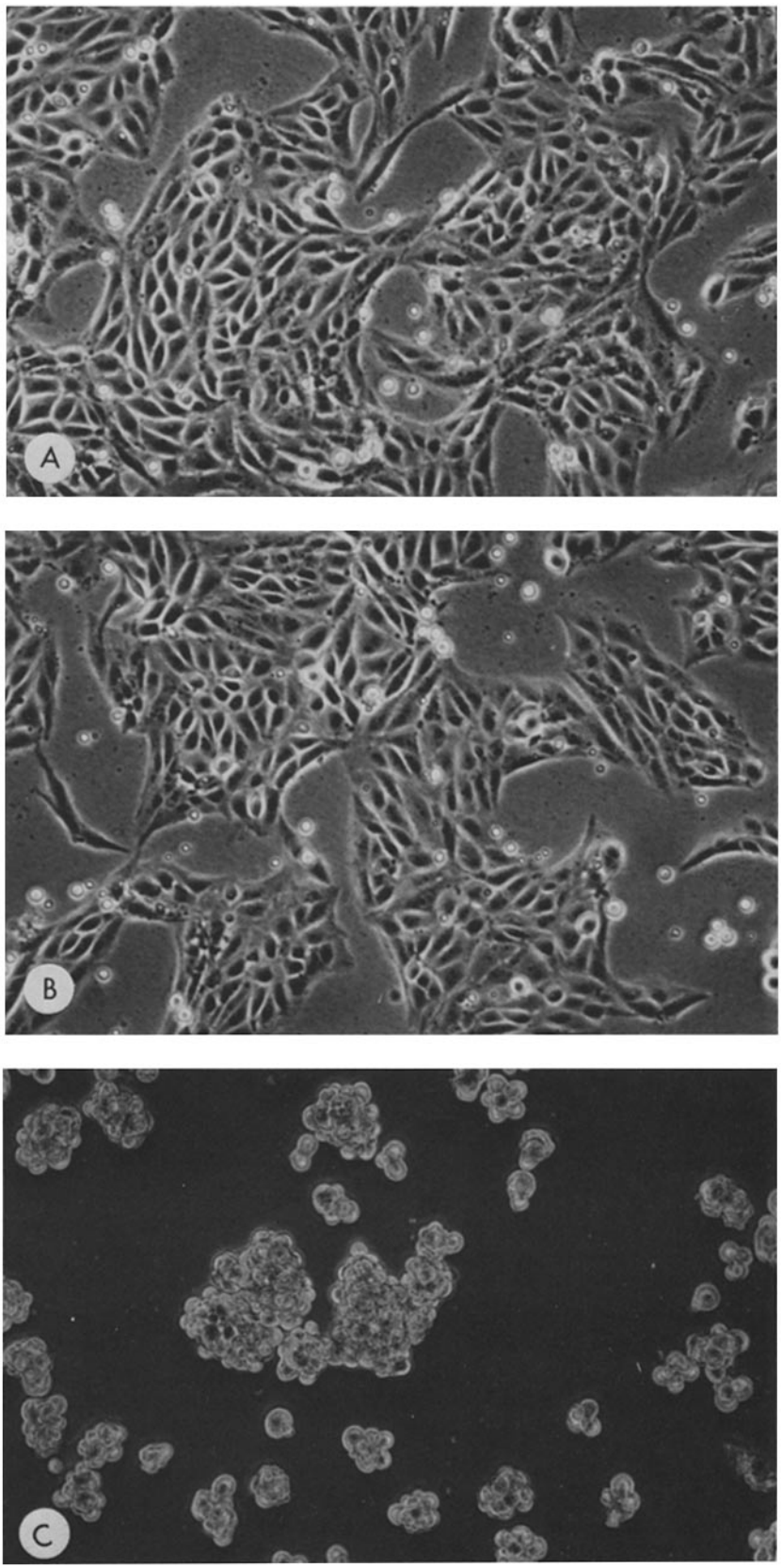


FIG. 2. Stimulation of PA by IgG: dose response. LLC PK_1 cells and KA31 cells were exposed to specific immune IgG or preimmune IgG at concentrations of 50 μg to 2 mg/ml. Medium was collected from all the wells after 24 h of incubation and was assayed for PA content. △, LLC-PK_1 cells with immune IgG (0–400); ○, KA31 cells with immune IgG (0–80); ▲, LLC-PK_1 cells with preimmune IgG; ●, KA31 cells with preimmune IgG.

types at about 1 mg/ml. Further increases in IgG levels produced little or no effect on pig kidney cells, but PA production by the mouse cells showed progressive relative inhibition as the immune IgG was raised above 1 mg/ml. This effect might be due to the separate IgG interactions with individual surface elements that modulate the PA production in opposite directions, respectively; or, alternatively, the pattern might result from interaction at a single class of surface receptors whose effects on PA synthesis, as a function of the degree of complex formation, are inherently biphasic. Preimmune IgG did not stimulate PA production by either culture throughout the range of concentrations tested.

Morphological Effects of Antisera on Target Cells. Stimulation of PA synthesis by anti-LLC-PK_1 and anti-KA31 IgG was always preceded by morphological alterations of each cell type. In untreated cell cultures and in cultures treated with preimmune rabbit IgG, the cells grew as attached monolayers (Fig. 3A and B). As early as 2 h after addition of the respective antisera, both KA31 and LLC-PK_1 cells became rounded, formed clusters, and ultimately detached from the bottom of the dish (Fig. 3C). The rapidity and the extent of the morphological change were dose dependent: the rounding could be seen within 0.5 h at high concentrations of IgG (1 mg/ml), but up to 16 h were required at lower levels (50 μg/ml). Both cell types remained fully viable throughout the experiments, as judged by trypan blue exclusion and retention of plating efficiency.

We attempted to separate the morphological response to antibody from PA induction by exposing the cultures to a variety of substances. With KA31, neither colchicine (10^{-6} M) nor fibronectin (≤1 mg/ml) prevented the rounding and detachment of cells, and the addition of the protease inhibitors leupeptin (5×10^{-4} M), soybean trypsin inhibitor (50 μg/ml), and Trasylol (100 U/ml) likewise did not block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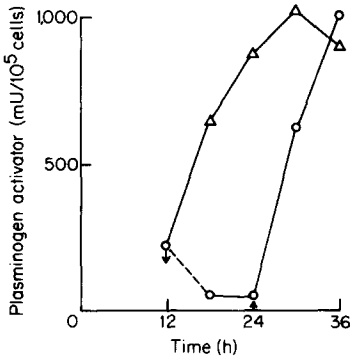

FIG. 4. Reversibility of PA induction. LLC-PK$_1$ were incubated with 1 mg/ml of immune IgG for 12 h. After 12 h the cells were pelleted and replated in fresh medium without IgG (○- - -○, ○——○). The IgG-containing medium was added to fresh, untreated cells (△). The cells removed from the original incubation mixture were restimulated with fresh IgG (shown by upward arrow). Extracellular PA of the various experimental mixtures was determined every 6 h.

the morphological changes, showing that the latter were not due to proteolysis of either the cell surface or substratum.

PA induction and morphological change were uncoupled by actinomycin D (1 μg/ml) or cylcoheximide (10 μg/ml). When cultures of either KA31 or LLC-PK$_1$ cells were exposed to either of these inhibitors concurrently with antibody, the stimulation of PA synthesis was reduced by >85%, but the morphological change was unaffected.

Reversibility of Morphological Effects and PA Induction. That both PA induction and morphological changes are dependent on the continued presence of antibody was shown by the following experiment. A culture of LLC-PK$_1$ cells was exposed for 12 h to a concentration of IgG (1 mg/ml) that induced PA synthesis, cell rounding, and detachment of the monolayer. The suspension of cells and cell clumps was centrifuged, and the IgG-containing supernatant medium and cell pellet were collected separately. The cell pellet was replated in fresh, IgG-free medium: all of the cells reattached and formed a monolayer within 6 h. As seen in Fig. 4 (open circles), PA production declined at first after the cells were separated from the stimulatory IgG. Immediately after a second exposure to IgG (at 12 h after replating), the culture responded rapidly with a burst of PA synthesis. The first IgG-containing medium obtained by centrifugation of the first culture had retained essentially all of the PA stimulatory activity after exposure to the original cells because it induced a prompt synthesis of PA when applied to a fresh cell monolayer (open triangles). Thus, both the PA response and the morphological changes were fully reversible and required the continued presence of the stimulatory IgG. Entirely analogous observations were made with KA31 cells (data not shown).

Identification of IgG as the Stimulatory Agent. It appeared possible that the induction of PA synthesis and morphological changes might have been due to factors contaminating the IgG preparations. Tests for proteolytic or fibrinolytic activity using our standard ^{125}I-fibrin plate (3) and zymographic assays (34) showed no detectable activity in the purified antibody solution (data not shown). The IgG fractions obtained by ammonium sulfate fractionation and adsorption and elution from protein A-

FIG. 3. Effect of IgG on morphology of LLC-PK$_1$ cells. (A) untreated, (B) exposed to 1 mg/ml of preimmune IgG for 24 h, and (C) exposed to 1 mg/ml of immune IgG for 24 h.

TABLE I

Stimulation of PA by Anti-LLC-PK$_1$ and Anti-KA31 IgG of Heterologous Target Cells

Target cells	Cells used for immunization	PA (mU UK/ml)*	
		Preimmune IgG	Immune IgG
HEF-MCA (hamster)	LLC-PK$_1$	35.1	31.5
HEF-SV40 (hamster)	LLC-PK$_1$	113.1	101.8
TMCK (mouse kidney cells)	LLC-PK$_1$	193.1	212.4
KA31 (BALB/KiS)	LLC-PK$_1$	8.8	16.7
HEp-3 (human epidermoid carcinoma)	LLC-PK$_1$	34.0	61.2
Human skin fibroblasts	LLC-PK$_1$	127.8	255.6
LLC-PK$_1$	LLC-PK$_1$	36.2	260.6
Human skin fibroblasts	KA31	160.0	381.0
KA31	KA31	4.0	162.2

* The results show the maximum stimulation of extracellular PA production obtained for each IgG preparation within the range of 0.25–2.00 mg/ml. The different types of target cells were plated at 5×10^4 per Linbro well and incubated at 37°C. 24 h later, either anti-LLC-PK$_1$ or anti-KA31 IgG was added to the cultures, and conditioned medium was assayed for PA activity after 24 h, as described in Materials and Methods.

Sepharose consisted of a single Coomassie-stained band, of apparent mol wt of 150,000, in heavily loaded SDS-PAGE. As a further test for the potential contaminants, the IgG were isolated by the sequence of ammonium sulfate fractionation and passage through DEAE-cellulose columns. The IgG prepared in this way appeared equally pure when examined by SDS-PAGE and were equally active in stimulating PA production and inducing morphological changes in target cells.

The factors that induced PA production by LLC-PK$_1$ cells were reduced by 70% when a solution containing 2 mg of purified IgG was adsorbed by exposure to a suspension of 8×10^7 pig kidney cells, a result consistent with the expected behavior of antibodies directed against cell surface constituents.

The fact that indifferent or preimmune rabbit IgG failed to stimulate PA synthesis indicated that the F_c segment was probably not responsible for this activity. A rigorous confirmation of this expectation was achieved with F(ab')$_2$ fragments prepared from purified IgG that induced PA production by LLC-PK$_1$ cells: the F(ab')$_2$ was almost equipotent on a molar basis with the native IgG, and both preparations stimulated enzyme synthesis by 11- and 13-fold, respectively, at a concentration of 6×10^{-6} M. This is further evidence that enzyme induction is determined by the specificity of the antibody combining site presumably interacting with a surface antigen.

The specificity of PA induction was tested on a series of cell types ostensibly unrelated to those used as immunizing antigen. The results (Table I) show clearly that significant stimulation of PA synthesis was limited to the immunizing cell type against which the antiserum was directed.

Discussion

Our results support the following conclusions:

(*a*) IgG can modulate PA production in at least three cell types. The identity of the

stimulatory molecules as IgG appears to be firmly established by ordinary purification procedures, by their removal on the specific adsorbent protein A, and by the activity of the $F(ab')_2$ fragments.

(*b*) The effect of the antibodies appears to be specific in the sense that stimulation of PA production was observed only in cell types used as the immunizing antigen and did not occur in cultures exposed to preimmune serum. These results, and the retention of full activity by $F(ab')_2$ fragments, exclude the Fc receptor as the mediator for PA induction.

(*c*) The PA-inducing IgG interact with antigenic determinants that are exposed at the cell surface because they are absorbed by cell suspensions under conditions that exclude endocytosis and metabolic degradation.

(*d*) Rounding, clustering, and detachment of cells from the substratum similar to that observed upon addition of anti-LLC-PK_1 or anti-KA31 IgG to their target cells has been described with transformed cell lines grown in the presence of high concentrations of plasminogen (35) with Rous sarcoma virus-transformed chick embryo fibroblasts treated with the tumor promoter phorbol myristate acetate (36) and with cells after exposure to specific antisera (37). The following considerations, however, suggest that the morphological changes caused by anti-LLC-PK_1 and anti-KA31 IgG are not mediated via plasmin, PA, or other proteases: (*i*) the morphological changes are observed in media containing heat-inactivated and plasminogen-depleted serum where they occur with undiminished intensity; (*ii*) protease inhibitors such as Leupeptin, soybean trypsin inhibitor, and Trasylol do not prevent the morphological changes or subsequent cellular detachment; (*iii*) inhibitors of macromolecule synthesis prevent PA production but do not affect the morphological changes; and (*iv*) preparations of anti-LLC-PK_1 and anti-KA31 IgG, apparently free of any detectable protease activity, stimulate PA production and induce detachment of the respective target cells both before and after diisopropylphosphorofluoridate treatment. Whereas the morphological changes produced by the IgG molecules therefore appear to be independent of protease action, it is not evident from the data presented here whether stimulation of PA production and morphological changes are linked in some other way, and further work will be required to define the relationship between them.

(*e*) PA production by the pig kidney cells used in this study is known to be induced by calcitonin and vasopressin.[2] Because the levels of enzyme synthesis achieved by antibody stimulation were in the same range as those produced by some hormones, one possible explanation of the IgG effect is that the antibodies were interacting with one or both of these hormone receptors in a manner reminiscent of the long-acting thyroid stimulator. It is also possible that interactions with other surface antigens can increase adenyl cyclase activity either specifically or by way of nonspecific membrane effects, thereby inducing enzyme production. These possibilities can be tested and resolved by further work. It will be of interest to explore models of autoimmune and neoplastic conditions to determine whether increases in PA production by target cells can be correlated with immunological enhancement.

Summary

We have purified the immunoglobulins (IgG) from rabbit antisera raised against two cell lines—LLC-PK_1 derived from pig kidney and from Kirsten sarcoma virus-transformed BALB/3T3—and have studied the effects of the IgG on cultures of the

respective target cells. The following observations were made with both cell types: (*a*) The addition of purified IgG produced a rapid change in morphology within 2 h. This consisted of cell rounding, agglutination, and detachment from the surface of the dish. (*b*) Beginning ~2 h after IgG addition there was a progressive rise in plasminogen activator production for 24–36 h. (*c*) Both the morphological change and the induction of plasminogen activator (PA) synthesis were reversible and required the continued presence of IgG for their maintenance. The increase in PA production, but not morphological change, depended on genetic transcription and translation, being inhibited by actinomycin and/or cycloheximide. (*d*) These effects of IgG were specific: they were not observed with preimmune or indifferent IgG and occurred only after interaction between an IgG preparation from antisera and the cells used to generate the particular antiserum. The divalent IgG fragments $F(ab')_2$ retained fully the activities of the native IgG molecules from which they were derived.

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References

1. Beers, W. B., S. Strickland, and E. Reich. 1975. Ovarian plasminogen activator: relationship to ovulation and hormonal regulation. *Cell.* **6**:387.
2. Wigler, M., and I. B. Weinstein. 1976. Tumor promoter induces plasminogen activator. *Nature (Lond.).* **259**:232.
3. Strickland, S., and W. H. Beers. 1976. Studies on the role of plasminogen activator in ovulation. *In vitro* response of granulosa cells to gonadotropins, cyclic nucleotides and prostaglandins. *J. Biol. Chem.* **251**:5694.
4. Lacroix, M., F. E. Smith, and I. B. Fritz. 1977. Secretion of plasminogen activator by Sertoli cells enriched cultures. *Mol. Cell. Endocr.* **9**:227.
5. Lee, L. S., and I. B. Weinstein. 1978. Epidermal growth factor, like phorbol esters, induces plasminogen activator in HeLa cells. *Nature (Lond.).* **274**:696.
6. Vassalli, J.-D., J. Hamilton, and E. Reich. 1976. Macrophage plasminogen activator: modulation of enzyme production by anti-inflammatory steroids, mitotic inhibitors and cyclic nucleotides. *Cell.* **8**:271.
7. Rifkin, D. B. 1978. Plasminogen activator synthesis by cultured human embryonic lung cells: characterization of the suppressive effects of corticosteroids. *J. Cell. Physiol.* **97**:421.
8. Miskin, R., T. Easton, and E. Reich. 1978. Plasminogen activator in chick embryo muscle cells: induction of enzyme by RSV, PMA and retinoic acid. *Cell.* **15**:1301.
9. Wilson, E. L., and E. Reich. 1978. Plasminogen activator in chick embryo fibroblasts: induction of enzyme synthesis by retinoic acid; synergism with viral transformation and phorbol ester. *Cell.* **15**:385.
10. Strickland, S., and V. Mahdavi. 1978. The induction of differentiation in teratocarcinoma stem cells by retinoic acid. *Cell.* **15**:393.
11. Unkeles, J. C., A. Tobia, L. Ossowski, J. P. Quigley, D. B. Rifkin, and E. Reich. 1973. An enzymatic function associated with transformation of fibroblasts by oncogenic viruses. I. Chick embryo fibroblast cultures transformed by avian RNA tumor viruses. *J. Exp. Med.* **137**:85.
12. Ossowski, L., J. C. Unkeless, A. Tobia, J. P. Quigley, D. B. Rifkin, and E. Reich. 1973. An enzymatic function associated with transformation of fibroblasts by oncogenic viruses. II. Mammalian fibroblast cultures transformed by DNA and RNA tumor viruses. *J. Exp. Med.* **137**:112.
13. Sisskin, E. E., I. B. Weinstein, C. H. Evans, and J. A. Di Paolo. 1980. Plasminogen activator

synthesis accompanying chemical carcinogen-induced *in vitro* transformation of syrian hamsters and guinea pig fetal cells. *Int. J. Cancer.* **26:**331.

14. Strickland, S., E. Reich, and M. I. Sherman. 1976. Plasminogen activator in early embryogenesis: enzyme production by trophoblast and parietal endoderm. *Cell.* **9:** 231.
15. Ossowski, L., D. Biegel, and E. Reich. 1979. Mammary plasminogen activator: correlation with involution, hormonal modulation, and comparison between normal and neoplastic tissue. *Cell.* **16:**929.
16. Ossowski, L., J. P. Quigley, G. M. Kellerman, and E. Reich. 1973. Fibrinolysis associated with oncogenic transformation. Requirement of plasminogen for correlated changes in cellular morphology, colony formation in agar, and cell migration. *J. Exp. Med.* **138:**1056.
17. Ossowski, L., J. P. Quigley, and E. Reich. 1975. Plasminogen, a necessary factor for cell migration *in vitro. In* Proteases and Biological Control. E. Reich et al., editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y. 901–913.
18. Vassalli, J.-D., A. Granelli-Piperno, and E. Reich. 1980. Neutral proteinases of leucocytes and the inflammatory process. *In* Protein Degradation in Health and Diseases. Ciba Foundation 75, Excerpta Medica, Amsterdam, Netherlands. 381–395.
19. Kaliss, N., N. R. StC. Sinclair, and J. L. Cantrell. 1976. Immunological enhancement of murine tumor allograft by passive alloantibody IgG and $F(ab)_2$. *Eur. J. Immunol.* **6:**38.
20. Fink, M. P., C. W. Parker, and W. J. Shearer. 1975. Antibody stimulation of tumor growth in T-cell depleted mice. *Nature (Lond.).* **255:**404.
21. Chard, T. 1968. Immunological enhancement by mouse isoantibodies. The importance of complement fixation. *Immunology.* **14:**583.
22. Kriss, J. P., V. Pleshakov, and J. R. Chien. 1964. Isolation and identification of the long-acting thyroid stimulator and its relation to hyperthyroidism and circumscribed pretibial myxedema. *J. Clin. Endocrinol. Metab.* **24:**1005.
23. Smith, B. R., K. J. Dorrington, and D. S. Munro. 1969. The thyroid stimulating properties of long-acting thyroid stimulator gamma G-globulin subunit. *Biochem. Biophys. Acta.* **192:** 277.
24. Farb, R. M., R. Dykes, and G. S. Lazarus. 1978. Anti-epidermal-cell-surface pemphigus antibody detaches viable epidermal cells from culture plates by activation of proteinase. *Proc. Natl. Acad. Sci. U. S. A.* **75:**459.
25. Deutsch, D. G., and E. T. Mertz. 1970. Plasminogen purification from human plasma by affinity chromatography. *Science (Wash. D.C.).* **170:**1095.
26. Hull, R. N., W. R. Cherry, and G. W. Waver. 1976. The origin and characterization of a pig kidney cell strain, LLC-PK_1. *In Vitro (Rockville).* **12:**670.
27. Rifkin, D. B., and R. Pollack. 1977. Production of plasminogen activator by established cell lines of mouse origin. *J. Cell Biol.* **73:**47.
28. Toolan, H. W. 1954. Transplantable human neoplasms maintained in cortisone-treated laboratory animals: HS#1, H. Ep.#1, H. Ep.#2 H. Ep #3, and H. Emb. Rh.#1. *Cancer Res.* **14:**660.
29. Ossowski, L., and E. Reich. 1980. Loss of malignancy during serial passage of human carcinoma in culture and discordance between malignancy and transformation parameters. *Cancer Res.* **40:**2310.
30. Puck, T. T., P. J. Marcus, and S. J. Cieciuro. 1956. Clonal growth of mammalian cells *in vitro.* Growth characteristic of colonies from single HeLa cells with and without a "feeder layer." *J. Exp. Med.* **103:**273.
31. Dulbecco, R., and M. Vogt. 1954. Plaque formation and isolation of pure lines of poliomyelitis viruses. *J. Exp. Med.* **99:**183.
32. Scolnick, E. M., E. Rands, D. Williams, and W. F. Parks. 1973. Studies on the nucleic acid sequences of Kirsten sarcoma virus: a model for formation of a mammalian RNA-containing sarcoma virus. *J. Virol.* **12:**458.

33. Brandford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. *Anal. Biochem.* **72:**248.
34. Granelli-Peperno, A., and E. Reich. 1978. A study of proteases and protease-inhibitor complexes in biological fluids. *J. Exp. Med.* **148:**223.
35. Ossowski, L., J. P. Quigley, and E. Reich. 1974. Fibrinolysis associated with oncogenic transformation: morphological correlates. *J. Biol. Chem.* **249:**4312.
36. Quigley, J. P. 1979. Phorbol ester induced morphological changes in transformed chick fibroblasts: evidence for direct catalytic involvement of plasminogen activator. *Cell.* **17:**131.
37. Wylie, D. E., C. H. Damsky, and C. A. Buck. 1979. Studies on the function of cell surface glycoprotein. I. Use of antisera to surface membranes in the identification of membrane components relevant to cell-substrate adhesion. *J. Cell Biol.* **80:**385.