Proc. Natl. Acad. Sci. USA
Vol. 79, pp. 3720–3723, June 1982
Biochemistry

Monoclonal antibody that specifically inhibits a human M_r 52,000 plasminogen-activating enzyme

(hybridomas/screening/serine protease/urokinase)

K. KALTOFT*, L. S. NIELSEN†, J. ZEUTHEN*, AND K. DANØ†‡

*Institute of Human Genetics, The Bartholin Building, University of Aarhus, DK-8000 Aarhus C, Denmark; and †Laboratory of Tumor Biology, University of Copenhagen, Frederik den V's Vej 11, DK-2100 Copenhagen Ø, Denmark

Communicated by George Klein, March 16, 1982

ABSTRACT **Monoclonal antibodies against a human plasminogen activator of M_r ≈52,000 (HPA52) were derived by immunization of mice with an impure preparation of the enzyme (urokinase), subsequent hybridization of spleen cells with NSI-Ag4/1 myeloma cells, and cloning of the hybridomas. Selection of mice for hybridization and screening of hybridomas were based solely on direct inhibition of an enzymatic assay of the plasminogen activator with the impure enzyme preparation. A cloned hybridoma produced IgG1 antibodies that bound to and inhibited the enzymatic activity of HPA52 irrespective of whether the HPA52 was derived from urokinase or from human glioblastoma cells, whereas there was no inhibition of or binding to a plasminogen activator of M_r ≈70,000 from human melanoma cells or a plasminogen activator of M_r ≈36,000 that is a degradation product of HPA52 and present in urokinase. Nor did the anti-HPA52 IgG1 inhibit a murine plasminogen activator of M_r ≈48,000 derived from sarcoma virus-transformed cells. By using affinity chromatography with columns of anti-HPA52 IgG1 bound to Sepharose, HPA52 was purified from urokinase to homogeneity as evaluated by NaDodSO$_4$/polyacrylamide gel electrophoresis. This study demonstrates that inhibitory monoclonal antibodies against enzymes can be derived with the sole use of impure enzyme preparations and shows how such antibodies subsequently can be used for enzyme purification.**

Early work demonstrated a high extracellular proteolytic activity of cultured malignant neoplastic cells (1–4). Later, it was found that transformation of cells by tumor viruses greatly enhanced their proteolytic capacity (5–8). It was shown that proteolysis by neoplastic cells was mainly due to activation of plasminogen by plasminogen activators released from the cells (9–13). Furthermore, it was shown that release of plasminogen activators could be induced by tumor promotors (14), chemical carcinogens (15), and DNA damage (16). Plasminogen activation is also implicated in a wide range of normal physiological processes, such as thrombolysis (17), tissue degradation (18), cell migration (19), and trophoblast invasion (20), and plasminogen activators are released from activated macrophages (21, 22).

In accordance with this diversity of biological function, plasminogen is found in most body fluids. The active enzyme, plasmin, is a serine protease that is formed from plasminogen by proteolytic cleavage (23). At least two types of plasminogen-activating enzymes (both serine proteases) are produced by eukaryotic cells. These differ markedly in M_r and immunological reactivity (13, 24–29). One type has a M_r of ≈70,000 and is strongly implicated as the physiological plasminogen activator active in thrombolysis (29, 30). A different type has a lower M_r (≈48,000 in rodents and ≈52,000 in man) and seems to be primarily involved in other functions—e.g., tissue degradation (18).

The previous findings have stimulated extensive research into the possible role of plasminogen activation in invasive growth of malignant neoplastic cells and the accompanying degradation of normal tissue (28, 31, 32). Studies of plasminogen activators both in relation to neoplasia and to normal physiological processes have been limited mainly to *in vitro* situations because studies on the distribution and function of these enzymes *in vivo* have been hindered by the lack of reagents with sufficient specificity. In this report we describe the derivation by the hybridoma technique (33) of a monoclonal antibody directed against a human plasminogen activator of the M_r 52,000 type.

MATERIALS AND METHODS

Chemicals. Urokinase (34) containing 8,500 Ploug units per mg of protein was a gift from Leo Pharmaceutical (Ballerup, Denmark); diisopropyl fluorophosphate was from Sigma; Freund's incomplete adjuvant was from the State Serum Institute (Copenhagen, Denmark); rabbit IgG anti-mouse IgG was from DAKO Immunoglobulins (Copenhagen, Denmark); polyethylene glycol (M_r 6,000) was from Koch-Light (Colnbrook, England); all other reagents were as described previously or of the best commercially available grade.

Cell Culture. The myeloma line NSI-Ag4/1 (resistant to 0.1 mM 6-thioguanine), which synthesizes but does not secrete κ light chains (35), and the anti-2,4,6-trinitrophenyl IgG1-producing hybridoma Hy2.15 (36) were both the gift of Georges Köhler, Basel Institute for Immunology. Human cell lines (27) derived from a glioblastoma and a melanoma were the gift of E. Dowdle, University of Cape Town, South Africa. These cells and a line of mouse 3T3 cells transformed by mouse sarcoma virus (see ref. 25) were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin, and streptomycin. Serum-free culture medium was prepared from confluent monolayer cultures as described (13).

Production of Hybridomas. On day 0, 7, 14 and 21, BALB/c mice were immunized intradermally with 5,000 Ploug units of urokinase (containing ≈50 μg of M_r 52,000 human plasminogen activator, HPA52) mixed with 200 μl of Freund's incomplete adjuvant. On day 28, a similar dose dissolved in 0.15 M NaCl was administered intravenously, and the spleen was removed 3 days later. Blood was drawn by intracardiac puncture before, during, and after immunization, and the sera were tested for inhibitory effect in the plasminogen-activator assay.

For hybridization, 10^8 spleen cells were mixed with 10^7 NSI-

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviation: HPA52, human M_r 52,000 plasminogen activator.

‡ To whom reprint requests should be addressed.

Ag/1 cells and incubated with 1 ml of 50% (wt/vol) polyethylene glycol in a phosphate-buffered saline solution for 90 sec at 37°C. Dulbecco's modified Eagle's medium (20 ml) was added to the suspension, and the cells were centrifuged at 1,000 × g. The cell pellet was resuspended in 96 ml of hypoxanthine/aminopterin/thymidine medium (37) supplemented with 10% fetal bovine serum and was distributed in the wells of Costar trays (Costar, Cambridge, MA). Medium was changed twice weekly. Hybridomas were cloned by limiting dilution and recloned in soft agar (38). For monitoring the production of inhibitory antibodies, culture fluids were tested for inhibition of plasminogen-activator activity.

Plasminogen-Activator Assay. Plasminogen-activator activity was assayed by using an ^{125}I-labeled fibrin (^{125}I-fibrin) plate method, as described (25). The wells were coated with ^{125}I-fibrin and contained 1 μg of plasminogen in 0.5 ml of 0.1 M Tris·HCl, pH 8.1/0.1% Triton X-100/0.25% gelatin (assay buffer). The incubation time was 1 hr at 37°C. For assay of inhibitory effect, serum or cell culture medium was diluted 1:5 with 0.01 M Tris·HCl, pH 8.1/0.01% Triton X-100; then pH was adjusted to 3.0 by addition of 1.0 M glycine·HCl (pH 3) and 1.0 M HCl, both 1:100 (vol/vol), followed by incubation for 3 hr at 20°C. This treatment inactivates α_2-macroglobulin and prevents a nonspecific inhibition by this serum component. The pH was readjusted to 8.1 by addition of 4 vol of assay buffer and was mixed with an equal volume of assay buffer containing 0.1 Ploug unit/ml of urokinase. This mixture was incubated for 16 hr at 4°C, and 100 μl was assayed in the plasminogen-activator assay.

Characterization and Purification of Antibodies. The class and subclass specificity of the antibody was determined on 10-fold-concentrated culture supernatants by means of immunodiffusion by using pre-prepared Ouchterlony trays (Meloy, Springfield, VA). NaDodSO$_4$/polyacrylamide gel electrophoresis and isoelectric focusing analysis indicated the loss of the NSI-Ag4/1 κ chain in the recloned hybridoma. Antibodies from culture medium were precipitated by addition of $(NH_4)_2SO_4$ [final concentration, 27.5% (wt/vol)], followed by incubation for 16 hr at 4°C. The precipitate was resuspended in 0.1 vol of 0.1 M Tris·HCl (pH 8.1) (application buffer), dialyzed extensively against the same buffer, and applied to a column (1 ml; 9 × 15 mm) of Sepharose-bound rabbit IgG anti-mouse IgG (6.5 mg/ml) prepared by the cyanogen bromide method. The column was washed with 10 ml of the application buffer and 5 ml of the same buffer containing 0.5 M NaCl and then was eluted with 4 ml of 0.1 M glycine·HCl, pH 2.5/0.5 M NaCl. The pH of the eluate was adjusted to 4.5 by addition of 0.1 vol of 1.0 M Tris·HCl (pH 8.1), the purity of the IgG preparation was evaluated by NaDodSO$_4$/polyacrylamide gel electrophoresis, and the IgG concentration was determined by spectrophotometry at 280 nm ($A_{280}^{1\%}$ = 14). Concentrations of IgG in impure solutions were determined by immunodiffusion against rabbit IgG anti-mouse IgG, with purified IgG as a standard.

Gel Electrophoresis. NaDodSO$_4$/polyacrylamide gel electrophoresis was carried out as described (39) under denaturing but nonreducing conditions; slab gels with a linear gradient of 6–16% polyacrylamide were used. Plasminogen activators in polyacrylamide gels were detected by diffusion into agarose gels containing fibrin and plasminogen (25). [^{3}H]Diisopropyl fluorophosphate incorporation into HPA52 and attempts to prevent its incorporation with anti-HPA52 antibodies (50 μg/ml) were assayed by NaDodSO$_4$/polyacrylamide gel electrophoresis and fluorography by a described method (26).

Affinity Chromatography. Monoclonal IgG1 anti-HPA52 or anti-trinitrophenyl was coupled to cyanogen bromide-activated Sepharose by using a coupling buffer of 0.25 M $NaHCO_3$ pH 8.5/0.5 M NaCl. The efficiency of the coupling reaction was always better than 85%. All experiments were performed at 4°C, and the columns were equilibrated with 0.1 M Tris·HCl, pH 8.1/0.1% Triton before use. For the experiments shown in Fig. 2, 0.6-ml columns (9 × 9 mm) containing 100 μg of IgG1 were used. In these experiments, the columns were developed by gravity. Bovine serum albumin (5 mg/ml) was added to the samples to prevent nonspecific absorption, and the columns were washed with 1 ml of the equilibrating buffer containing the same amount of bovine serum albumin. For the experiments shown in Fig. 3, a 1-ml column (9 × 15 mm) containing 498 μg of IgG1 was used. In these experiments, the column was developed with a flow rate of 21 ml/hr. Elution was by reverse flow.

RESULTS

Five mice were immunized with an impure preparation of HPA52 (urokinase; see ref. 34). Sera from all five mice were found to inhibit HPA52 significantly more than preimmunization sera, as tested by the ^{125}I-fibrin plate assay, in which plasminogen activator is measured by conversion of plasminogen to plasmin, which then solubilizes radiolabeled fibrin. Spleen cells from the mouse that showed the strongest inhibitory effect were used for hybridization and distributed into 48 wells. Growth of hybridoma cells was observed in all wells, and the medium in 11 wells was found to inhibit the plasminogen-activator assay. The well showing the strongest inhibition was used for cloning by limiting dilution and subsequent recloning in agar. Based on the inhibitory effect of the culture fluid, a clone producing inhibitory IgG1 as identified by immunodiffusion was selected. This clone, HPA52-2-21-27, had a doubling time of ≈24 hr and produced, with a cell density of between 1×10^5 and 5×10^5 cells per ml, between 10 and 15 μg of IgG1 per ml per 24 hr.

The IgG1 antibodies were purified by salt precipitation and affinity chromatography to homogeneity, as evaluated by NaDodSO$_4$/polyacrylamide gel electrophoresis. The purified antibodies inhibited the fibrinolytic assay (Fig. 1) to approximately the same extent when HPA52 from human urine and from human glioblastoma cells was tested. However, in contrast to this, no inhibition was observed in assays with M_r 70,000 plasminogen activator from human melanoma cells or with M_r 48,000 plasminogen activator from mouse sarcoma virus-transformed mouse cells. This signifies that the antibody, as well as discriminating between the different molecular types of plasminogen activators, does not inhibit plasmin. This was further substantiated by a lack of inhibition when the antibody was tested with preformed plasmin, prepared by activating plasminogen with HPA52 (Fig. 1).

The effect of the antibodies on the various plasminogen-activator preparations was further characterized (Fig. 2). Monoclonal IgG1 (anti-HPA52 or, as a control, anti-2,4,6-trinitrophenyl) was coupled to Sepharose. The plasminogen-activator preparations were passed through the column and subsequently analyzed by NaDodSO$_4$/polyacrylamide gel electrophoresis, followed by identification of plasminogen activators by diffusion into an agarose gel containing plasminogen and fibrin. When the plasminogen is activated, it degrades the fibrin, and lysis zones are observed. IgG1 from HPA52-2-21-27 cells bound HPA52 from human urine and from glioblastoma cells. In contrast, it did not bind a plasminogen activator of M_r ≈36,000 found in human urine (the M_r 36,000 molecule is a degradation product of HPA52; see ref. 40), a M_r 70,000 plasminogen activator from human melanoma cells, or a M_r 48,000 plasminogen activator from mouse cells, in good agreement with the results in Fig. 1.

Sepharose coupled with IgG1 from HPA52-2-21-27 cells could be used for simple purification with a good yield (≈44%)

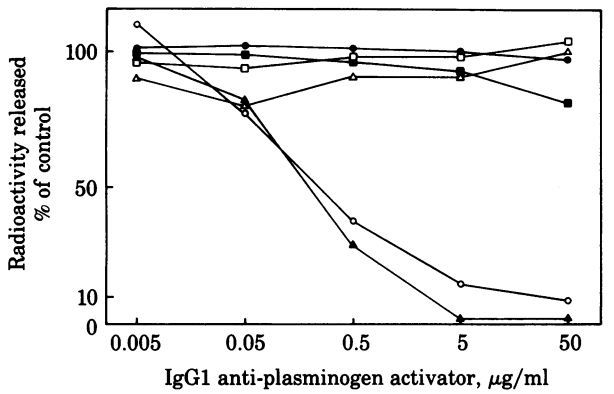

FIG. 1. Inhibition of HPA52 by monoclonal antibody to the enzyme. Release of radioactivity from ^{125}I-fibrin was measured after incubation for 1 hr at 37°C. The assay wells contained (in 0.5 ml of buffer, pH 8.1) 1 μg of human plasminogen, monoclonal mouse IgG1 anti-HPA52 as indicated (final concentration), and one of the following plasminogen activator preparations: 0.005 Ploug unit of urokinase (○); 1 μl of culture fluid from human glioblastoma cells containing 0.007 Ploug unit of HPA52 (▲); 2.5 μl of culture fluid from human melanoma cells containing 0.01 Ploug unit of M_r 70,000 plasminogen activator (□); or 0.5 ng of mouse M_r 48,000 plasminogen activator (△). Monoclonal IgG1 anti-trinitrophenyl was substituted for IgG1 anti-HPA52 (■) in assays with 0.005 Ploug unit of urokinase (compare ■ with ○). Finally, plasminogen was substituted by 10 ng of human plasmin, and no plasminogen activator was added (●). Control assays were performed identically, except that IgG1 was omitted. In the control assays, 4,000–8,000 cpm per well were released, which corresponds to 5–10% of the total radioactivity in each well.

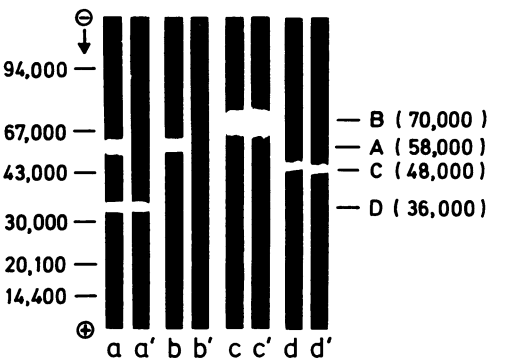

FIG. 2. Binding of various types of plasminogen activators to Sepharose columns coupled with IgG1 anti-HPA52. Before (lanes a, b, c, and d) and after (lanes a', b', c', and d') passage through the columns, samples were electrophoresed in a slab NaDodSO$_4$/polyacrylamide gel under nonreducing conditions, and the gel was layered over an agarose gel containing fibrin and plasminogen. The plasminogen activators diffused into the agarose gel and activated plasminogen to produce visible lysis zones. The following plasminogen-activator preparations were passed through the columns: a', 4 Ploug units of urokinase in 2 ml of 0.1 M Tris·HCl (pH 8.1); b', 2 ml of culture medium from glioblastoma cells containing 5 Ploug units of plasminogen activator; c', 2 ml of culture medium from melanoma cells containing 9 Ploug units of plasminogen activator; and d', 2 ml of culture medium from mouse sarcoma virus-transformed mouse 3T3 cells containing 1.2 μg of M_r 48,000 plasminogen activator. In lanes a, b, and c, 0.05 Ploug unit of the human plasminogen activators was used for electrophoresis; in lane d, 7 ng of the mouse plasminogen activator was used. In lanes a', b', c', and d', the same volume of eluate was electrophoresed as for the corresponding preparations that had not passed the columns. An additional experiment identical to that shown in a and a', except that 300 Ploug units of urokinase was passed through the column, gave identical results. Control experiments in which plasminogen was omitted from the agarose gel showed no lysis zones. In other control experiments, the four plasminogen-activator preparations were passed through columns of Sepharose coupled with monoclonal IgG1 of irrelevant specificity (anti-trinitrophenyl). In all of these experiments, the combined plasminogen-activator activity in the eluate was more than 60% of that added to the column, and electrophoresis of the eluates gave results identical to those shown in lanes a, b, c, and d, respectively. The M_r of marker proteins and the approximate M_r of the plasminogen activators as evaluated by their electrophoretic mobility is indicated. Electrophoresis of HPA52 (band A) under reducing conditions indicates that it has an M_r of ≈52,000 (unpublished observation), instead of the M_r 58,000 estimated in this experiment under nonreducing conditions.

of HPA52 from the impure urokinase preparation. The purified preparation produced only one stained band by NaDodSO$_4$/polyacrylamide gel electrophoresis (Fig. 3). The position of this protein exactly corresponded to enzymatic activity, as determined by the fibrin/plasminogen agarose overlay technique (Fig. 2). This indicates that the monoclonal antibody, among the various proteins in the preparation used for immunization, only binds HPA52 in significant amounts.

When HPA52 purified by this method was coupled to Sepharose, it was found to bind all IgG1 anti-HPA52, as expected for a monoclonal antibody preparation (results not shown).

DISCUSSION

Some characteristics of the monoclonal antibodies against HPA52 should be noted, as distinguished from conventional antibodies. Although lack of crossreaction between the M_r ≈50,000- and ≈70,000-type of plasminogen activators from the same species also is found with conventional antibodies (24, 26–28), conventional antibodies have shown crossreaction between the M_r ≈50,000 plasminogen activators and their M_r 36,000–29,000 degradation products (24, 26, 27). This is in contrast to the lack of crossreaction found with the present monoclonal antibody and indicates a narrower range of specificity present in this reagent.

On the basis of the observation that antibodies can inhibit this type of enzyme (24, 26, 27), the present study illustrates (*i*) how the identification of hybridomas that produce antibodies to an enzyme can be based solely on an enzyme assay and (*ii*) that this screening procedure, in contrast to most other screening procedures (e.g., radioimmunoassays), is independent of the impurity of the enzyme preparation. Many enzymes have never been purified, although their enzymatic activity is well known. For such enzymes (or for activators or inhibitors of enzymes), this might be a valuable approach to obtaining antibodies suitable for immunochemical studies and for the purification of the enzyme. In the case of enzymes that act extracellularly [such as enzymes of the plasminogen activator/plasminogen system (as described here), the complement system, and the blood coagulation system], inhibitory monoclonal antibodies might well have important therapeutic applications.

Recently, a similar approach to production of monoclonal antibodies against the enzyme 5-aminolevulinate dehydrogenase from a plant source has been reported by Liedgens *et al.* (41). In their study, no direct inhibition of the enzyme was reported, but the screening for antibody-producing hybridomas was based on precipitation of the enzyme by a double-antibody technique monitored by enzymatic assay of the supernatant.

In at least one additional enzyme system, β-galactosidase, monoclonal antibodies have been observed to be inhibitory (42). In the present study, two findings suggest that inhibition of the enzyme by the antibodies is not due to a binding of the antibodies to the catalytically active site but rather to an interference with binding of plasminogen to the plasminogen activator, preventing access of plasminogen to the active site: (*i*) Antibodies in concentrations that completely inhibited the enzymatic activity of HPA52 did not inhibit the incorporation of the active-site reagent diisopropyl fluorophosphate in its tritium-labeled form into the enzyme (results not shown). This finding

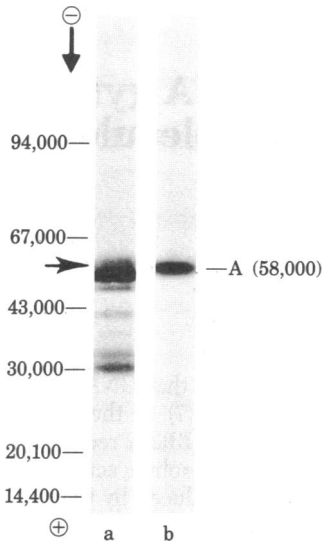

FIG. 3. Purification of HPA52 by affinity chromatography on Sepharose coupled with monoclonal antibody to the enzyme. Urokinase (12,200 Ploug units) was dissolved in 1 ml of Tris·HCl, pH 8.1/0.1% Triton X-100 and applied to a 1-ml Sepharose column coupled with monoclonal IgG1 anti-HPA52 antibody. The column was washed with 10 ml of the application buffer, 5 ml of the same buffer containing 0.5 M NaCl, and eluted with 10 ml of 0.1 M glycine·HCl, pH 2.5/0.5 M NaCl/0.1% Triton X-100. The eluate contained 5,400 Ploug units of plasminogen-activator activity. Samples of the urokinase preparation applied to the column (lane a), and the eluate (lane b), each containing 400 Ploug units of plasminogen activator, were analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis. Gel lanes stained with Coomassie blue are shown. Arrow, HPA52 band in lane a.

is similar to a previous observation with conventional rabbit antibodies to a murine plasminogen activator (26); (*ii*) The antibodies do not react with the $M_r \approx 36{,}000$ degradation product of HPA52, although this degradation product is enzymatically active and, therefore, contains the active site.

The availability of a specific reagent against HPA52 makes it possible to perform studies on the distribution of the enzyme and its function in the human organism under normal and pathological conditions. The present anti-HPA52 monoclonal antibody has already been found to be well suited for immunohistochemical staining of HPA52 in human tissue culture cells (43). The monoclonal antibodies also provide a versatile tool for identification and isolation of HPA52 and its precursors, such as demonstrated in this study, and by a recent isolation of an inactive proenzyme to HPA52 from cultured glioblastoma cells by affinity chromatography with anti-HPA52 IgG1 bound to Sepharose (unpublished data). Recently, an attempt has been reported to clone the gene for HPA52 and express it in bacteria, with conventional antibodies used to identify the gene products (44). Because of their greater specificity, monoclonal antibodies might well be superior to conventional antibodies for such studies.

We thank Drs. E. Dowdle and G. Köhler for gifts of cell lines; Leo Pharmaceutical (Ballerup, Denmark) for the gift of urokinase; and Drs. G. Köhler and B. Foltmann for comments on the manuscript. We are grateful to Kirsten Lund Jakobsen and Jens Peter Stenvang for excellent technical assistance. This work was supported by the Danish Cancer Society, the Danish Medical Research Council, The Novo Foundation, the Carlsberg Foundation, and the NATO Science Programme.

1. Carrel, A. & Burrows, M. T. (1911) *J. Exp. Med.* **13**, 571–575.
2. Fischer, A. (1925) *J. Cancer Res.* **9**, 62–70.
3. Fischer, A. (1946) *Nature (London)* **157**, 442.
4. Tagnon, H. J., Whitmore, W. F., Schulman, P. & Kravitz, S. C. (1953) *Cancer* **6**, 63–67.
5. Unkeless, J. C., Tobia, A., Ossowski, L., Quigley, J. P., Rifkin, D. B. & Reich, E. (1973) *J. Exp. Med.* **137**, 85–111.
6. Ossowski, L., Unkeless, J. C., Tobia, A., Quigley, J. P., Rifkin, D. B. & Reich, E. (1973) *J. Exp. Med.* **137**, 112–126.
7. Goldberg, A. R. (1974) *Cell* **2**, 95–102.
8. Howett, M. K., High, C. S. & Rapp, F. (1978) *Cancer Res.* **38**, 1075–1078.
9. Cliffton, E. E. & Grossi, C. E. (1955) *Cancer* **8**, 1146–1154.
10. Unkeless, J., Danø, K., Kellerman, G. M. & Reich, E. (1974) *J. Biol. Chem.* **249**, 4295–4305.
11. Quigley, J. P., Ossowski, L. & Reich, E. (1974) *J. Biol. Chem.* **249**, 4306–4311.
12. Rifkin, D. B., Loeb, J. N., Moore, G. & Reich, E. (1974) *J. Exp. Med.* **139**, 1317–1328.
13. Danø, K. & Reich, E. (1978) *J. Exp. Med.* **147**, 745–757.
14. Wigler, M. & Weinstein, I. B. (1976) *Nature (London)* **259**, 232–233.
15. Barrett, J. C., Crawford, B. D., Grady, D. L., Hester, L. D., Jones, P. A., Benedict, W. F. & Ts'o, P. O. P. (1977) *Cancer Res.* **37**, 3815–3823.
16. Miskin, R. & Reich, E. (1980) *Cell* **19**, 217–224.
17. Astrup, T. (1975) in *Proteases and Biological Control*, eds. Reich, E., Rifkin, D. B. & Shaw, E. (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), pp. 343–355.
18. Ossowski, L., Biegel, D. & Reich, E. (1979) *Cell* **16**, 929–940.
19. Ossowski, L., Quigley, J. & Reich, E. (1975) in *Proteases and Biological Control*, eds. Reich, E., Rifkin, D. B. & Shaw, E. (Cold Spring Harbor Laboratory, Cold Spring Harbor, New York), pp. 901–913.
20. Strickland, S., Reich, E. & Sherman, M. I. (1976) *Cell* **9**, 231–240.
21. Unkeless, J. C., Gordon, S. & Reich, E. (1974) *J. Exp. Med.* **139**, 834–850.
22. Gordon, S., Unkeless, J. C. & Cohn, Z. A. (1974) *J. Exp. Med.* **140**, 995–1010.
23. Summaria, L., Hsieh, B. & Robbins, K. C. (1967) *J. Biol. Chem.* **242**, 4279–4283.
24. Åstedt, B. (1979) *Thromb. Res.* **14**, 535–539.
25. Danø, K., Møller, V., Ossowski, L. & Nielsen, L. S. (1980) *Biochim. Biophys. Acta* **613**, 542–555.
26. Danø, K., Nielsen, L. S., Møller, V. & Engelhart, M. (1980) *Biochim. Biophys. Acta* **630**, 146–151.
27. Wilson, E. L., Becker, M. L. B., Hoal, E. G. & Dowdle, E. B. (1980) *Cancer Res.* **40**, 933–938.
28. Roblin, R. & Young, P. L. (1980) *Cancer Res.* **40**, 2706–2713.
29. Matsuo, O., Rijken, D. C. & Collen, D. (1981) *Nature (London)* **291**, 590–591.
30. Mattsson, C., Nyberg-Arrhenius, V. & Wallén, P. (1981) *Thromb. Res.* **21**, 535–545.
31. Reich, E. (1978) in *Molecular Basis of Biological Degradative Processes*, eds. Berlin, R. D., Herman, H., Lepow, J. H. & Tanner, J. M. (Academic, New York), pp. 155–169.
32. Jaken, S. & Black, P. H. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 246–250.
33. Köhler, G. & Milstein, C. (1975) *Nature (London)* **256**, 495–497.
34. Ploug, J. & Kjeldgaard, N. O. (1957) *Biochim. Biophys. Acta* **24**, 278–282.
35. Köhler, G. & Milstein, C. (1976) *Eur. J. Immunol.* **6**, 511–519.
36. Shulman, M., Wilde, C. D. & Köhler, G. (1978) *Nature (London)* **276**, 269–270.
37. Littlefield, J. W. (1964) *Science* **145**, 709–710.
38. Kennett, R. H., McKearn, J. T. & Bechtol, K. B. (1980) *Monoclonal Antibodies. Hybridomas: A New Dimension in Biological Analysis* (Plenum, New York).
39. Skriver, L., Nielsen, L. S., Stephens, R. & Danø, K. *Eur. J. Biochem.*, in press.
40. Lesuk, A., Terminiello, L., Traver, J. H. & Groff, J. L. (1967) *Thromb. Diath. Haemorrh.* **18**, 293–294.
41. Liedgens, W., Grutzmann, R. & Schneider, H. A. W. (1980) *Z. Naturforsch.* **35**, 958–962.
42. Frackelton, A. R. & Rotman, B. (1980) *J. Biol. Chem.* **255**, 5280–5290.
43. Danø, K., Dabelsteen, E., Nielsen, L. S., Kaltoft, K., Wilson, E. L. & Zeuthen, J. *J. Histochem. Cytochem.*, in press.
44. Ratzkin, B., Lee, S. G., Schrenk, W. J., Roychoudhury, R., Chen, M., Hamilton, T. A. & Hung, P. P. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 3313–3317.