Volume 314, number 2, 117–121 FEBS 11841 December 1992

Determination of proteinase 3–α_1-antitrypsin complexes in inflammatory fluids

Koert M. Dolman[a], Bart A. van de Wiel[a], Chih-Min Kam[b], Jannie J. Abbink[a], C. Erik Hack[a], Arnoud Sonnenberg[a], James C. Powers[b], Albert E.G.Kr. von dem Borne[a,c] and Roel Goldschmeding[a]

[a]*Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam, The Netherlands,* [b]*School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332, USA* and [c]*Department of Haematology, Academic Medical Centre, University Hospital, Amsterdam, The Netherlands*

Received 9 October 1992

Physiological inhibitors were tested for their in vitro interaction with neutrophil proteinase 3 (PR3). The major plasma proteinase inhibitor of PR3 is α_1AT. We have developed a radioimmunoassay (RIA) for quantitative detection of PR3–α_1AT complexes formed in vivo in inflammatory exudates such as synovial fluid and plasma from patients with sepsis. Levels of PR3–α_1AT complexes correlated significantly with levels of human neutrophil elastase (HNE)–α_1AT complexes. Thus, in vivo α_1AT not only protects against excessive HNE activity, but also against excessive PR3 activity.

Proteinase 3; α_1-Antitrypsin; Inflammatory fluid

1. INTRODUCTION

Proteinase 3 (PR3) is a 29 kDa serine proteinase which is biochemically distinct from human neutrophil elastase (HNE) and cathepsin G [1]. PR3 is at least as prominent in neutrophil azurophilic granules as HNE, and comparison of the active site regions of both enzymes reveals a strong homology [2]. We have recently compared the substrate and inhibitor reactivity of PR3 and HNE and revealed significant differences in the extended substrate binding sites of both enzymes [3]. PR3 degrades a broad spectrum of extracellular matrix proteins [4,5], induces emphysema upon intratracheal instillation in hamsters [1], and is the target antigen of Wegener's granulomatosis (WG) autoantibodies [6–8]. The most important physiological inhibitor of PR3 is α_1-antitrypsin (α_1AT) [5].

In the present study we have determined the in vitro association constants for PR3 with several physiological inhibitors, and compared the values with those previously reported [5]. We have also developed a radioimmunoassay (RIA) for the detection of PR3–α_1AT complexes in plasma and in inflammatory tissue exudates to investigate the in vivo significance of inactivation of PR3 by α_1AT.

Correspondence address: R. Goldschmeding, c/o Publication Secretariat, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, PO Box 9406, 1006 AK Amsterdam, The Netherlands. Fax: (31) (20) 512 3474.

Abbreviations: α_1ACT, alpha$_1$-antichymotrypsin; α_2AP, alpha$_2$-antiplasmin, α_1AT, alpha$_1$-antitrypsin; α_2M, alpha$_2$-macroglobulin; ATIII, antithrombin III; Boc, *t*-butyloxycarbonyl; C-ANCA, cytoplasmic staining-antineutrophil cytoplasmic autoantibodies, C1-inh, C1-inhibitor; MAb, monoclonal antibody; HNE, human neutrophil elastase; *p*NA, *p*-nitroanilide; NPGB, *p*-nitrophenyl 4′-guanidinobenzoate; Nva, norvaline; PBS, phosphate-buffered saline; PDS, 4,4′-dithiodipyridine; PMN, polymorphonuclear leucocyte; PR3, proteinase 3, SBzl, thiobenzyl ester, Suc, succinyl; RA, rheumatoid arthritis; RIA, radioimmunoassay; SF, synovial fluid; WG, Wegener's granulomatosis; Z, benzyloxycarbonyl.

2. MATERIALS AND METHODS

2.1. *Purification of PR3*

PR3 was purified according to the method of Kao et al. [1] from neutrophil granule extract as described [3].

2.2. *Active site titrations*

Bovine pancreatic trypsin, chymotrypsin, and *p*-nitrophenyl 4′-guanidinobenzoate (NPGB) were purchased from Sigma Chemical Co., St. Louis, MO. Human plasma α_1AT and α_2-macroglobin (α_2M) were from Athens Research and Technology Inc., Athens, GA. α_2-Antiplasmin (α_2AP), α_1-antichymotrypsin (α_1ACT), and antithrombin III (ATIII) were obtained from Calbiochem, San Diego, CA. C1 inhibitor (C1-inh) was a kind gift from Behring Werke AG, Marburg Germany. Substrates Z-Arg-SBzl, Suc-Val-Pro-Phe-*p*NA, and Boc-Ala-Ala-Nva-SBzl were synthesized as previously described [9–11]. Oxidation of α_1AT (α_1AT-ox) was performed as described [12]. Bovine trypsin was titrated with NPGB [13] and was found to be 70% active with respect to total proteins. The trypsin solution was used as a primary standard to determine the active inhibitor concentration of α_1AT. A constant amount of trypsin (0.12 μM) was incubated with various amounts of α_1AT (0.01–0.2 μM) in 0.5 ml of 0.05 M Tris-HCl,

pH 8.0, at 25°C for 5 min, and then a 10 μl aliquot was added to 2.175 ml of buffer containing 8% dimethylsulfoxide (DMSO), 0.34 mM of 4,4'-dithiodipyridine (PDS) and 0.095 mM of Z-Arg-SBzl. The residual trypsin activity was monitored at 324 nm. The active inhibitor concentration was determined from the plot of residual trypsin activity vs α_1AT concentration. The α_1AT solution was used as a secondary standard to determine the active enzyme concentration of PR3 and chymotrypsin using Boc-Ala-Ala-Nva-SBzl and Suc-Val-Pro-Phe-*p*NA as substrates.

2.3. *Interaction of physiologic inhibitors with PR3*

Association rate constant of PR3 with α_1AT was measured under second-order conditions. Equimolar concentrations of PR3 and α_1AT (13.4 nM) were incubated in 0.5 M NaCl, 0.1 M HEPES, pH 7.5, for various periods of time. Residual activity of PR3 was determined by the addition of PDS (0.34 mM) and Boc-Ala-Ala-Nva-SBzl (0.13 mM), and measured spectrophotometrically at 324 nm. This association was also measured by competition experiments using active site titrated chymotrypsin as the second enzyme besides PR3 [14,15].

Equivalence titration of α_2M to PR3 was performed in the presence of α_1AT. A constant amount of PR3 (152 nM) was incubated with increasing amounts of α_2M in 0.33 ml of 0.5 M NaCl, 0.1 M HEPES, pH 7.5, at 25°C for 10 min. Then 20 μl of α_1AT (1.36 μM) was added to the reaction mixture and incubated for another 5 min to inhibit the free PR3. Finally, an aliquot of 0.25 ml was added to 2.175 ml buffer containing PBS (0.34 mM) and Boc-Ala-Ala-Nva-SBzl (0.1 mM) to measure α_2M bound PR3 activity. This experiment was used to determine the α_2M concentration assuming the molar binding ratio of PR3 to α_2M is 1:1 [5,16]. The association rate constant of PR3 with α_2M was measured competitively with α_1AT [14,16]. PR3 (11.5 nM) was added to a mixture of α_2M (12.8 nM) and various amounts of α_1AT (11.5–57.6 nM) in 0.5 M NaCl, 0.1 M HEPES, pH 7.5, at 25°C. After 10 min, 150 μl of 5 mM PDS and 25 μl of 10 mM Boc-Ala-Ala-Nva-SBzl was added, and the residual activity of PR3 bound to α_2M was measured at 324 nm. The association rate constant of α_2M to PR3 was calculated according to the method described previously [14,16].

Interaction of PR3 with α_1ACT, α_2AP, ATIII, C1-inh, and α_1AT-ox was determined under similar conditions in a 1:10 molar excess of inhibitor.

2.4. *Radiolabeling of MAb AT15*

For use in the RIA MAb AT15 (directed against complexed α_1AT) [17] was labeled with ^{125}I (Radiochemical Centre, Amersham, UK) by the Iodo-gen method [18] at a specific activity of 0.30 MBq per μg of protein.

2.5. *Patients*

Two groups of patients with inflammatory disorders were selected for this study. The first group consisted of 18 patients with rheumatoid arthritis (RA), visiting the outpatient clinic of the Daniël den Hoed Clinic in Rotterdam, The Netherlands. All patients fulfilled the ARA criteria for RA [19]. The patients were part of a larger group that was studied with respect to several inflammatory parameters, including neutrophil activation and determination of HNE–α_1AT complexes in their synovial fluid (SF) [17].

The second group consisted of 14 patients who were admitted to the Intensive Care Unit (ICU) of the Free University Hospital in Amsterdam after clinical diagnosis of severe sepsis. Previously, the levels of HNE–α_1AT complexes had been detected in the same plasma samples of these patients [20]. Patients were selected to cover a range of different SF/plasma levels of HNE–α_1AT complexes.

2.6. *SF samples*

SF samples were taken from the knee joint at therapeutical indication. The SF was aspirated into a plastic syringe and immediately transferred to a siliconized Vacutainer tube, containing 0.05% (w/v) Polybrene and 10 mM EDTA to prevent in vitro activation of the complement and contact system. The samples were stored at −70°C until the tests were performed.

2.7. *Sepsis plasma samples*

Blood from 14 patients with sepsis was obtained, collected and processed as described [20]. Plasma was divided into aliquots and stored in polystyrene tubes at −70°C until the tests were performed.

2.8. *RIA for the detection of PR3–α_1AT and HNE–α_1AT complexes*

Immunoglobulin-enriched fractions of rabbit antiserum against HNE (RIA for HNE–α_1AT) and ascitic fluid of MAb 12.8 directed against PR3 (RIA for PR3–α_1AT) [6], were prepared by 50% ammonium sulphate precipitation and were coupled to CNBr-activated Sepharose (20 mg of protein to 1 g of Sepharose). The Sepharose beads were suspended in PBS containing 10 mM EDTA, 0.1% (v/v) Tween-20, 0.05% (w/v) Polybrene (Janssen Chimica, Beerse, Belgium), 1.5 mM NaN_3, 10 mM benzamidine (Janssen Chimica, Beerse, Belgium) and 0.01% (w/v) soybean trypsin inhibitor (SBTI, BDH Biochemicals Ltd., Poole, UK), pH 7.4, at concentrations of 2 mg/ml (Sepharose/rabbit anti-HNE) and 4 mg/ml (Sepharose/MAb 12.8). Sepharose suspensions of 0.3 ml (RIA for HNE–α_1AT) and of 1.0 ml (RIA for PR3–α_1AT) were incubated in 2 ml polystyrene tubes for 4 h by head-over-head rotation at room temperature with samples diluted in PBS–0.1% (v/v) Tween-20 (50 μl of SF samples (1:25), and sepsis plasma samples (1:5)). Sepharose beads were then washed with PBS (5 times with 1.5 ml) and incubated for 16 h at room temperature with 50 μl of [^{125}I]MAb AT15 (approximately 2–3 ng) together with 0.5 ml of PBS, 0.1% (v/v) Tween-20 and 5% (v/v) bovine serum. Subsequently, the Sepharose was washed with PBS (4 times with 1.5 ml) and the bound radioactivity was measured. Results were expressed as percentage binding of the labeled antibodies added. Levels of PR3–α_1AT and HNE–α_1AT complexes were calculated by using a standard dose–response curve of preformed complexes prepared by incubating purified active site titrated PR3 and HNE with an excess of pooled human plasma. Further details on the assay for the detection of HNE–α_1AT complexes have been described elsewhere [20].

3. RESULTS

3.1. *Interaction of physiological inhibitors with PR3*

The interaction of PR3 with α_1AT and α_2M was monitored using Boc-Ala-Ala-Nva-SBzl as the substrate (Table I). The association rate constants for PR3 with α_1AT was measured under second-order conditions and by competition experiments, and were found to be 4.4×10^6 and 5.7×10^6 $M^{-1} \cdot s^{-1}$, respectively. These values are one order of magnitude less than the reported value of HNE (6.5×10^7 $M^{-1} \cdot s^{-1}$) and one order of magnitude

Table I

Association rate constants for the interaction of physiological inhibitors with PR3*

Inhibitor	k_a (M^{-1} s^{-1})	Method
α_1-Antitrypsin	4.4×10^6	second-order conditions
	5.7×10^6	competition experiment
α_2-Macroglobulin	$(1.1 \pm 0.1) \times 10^7$	competition assay
α_1-Antichymotrypsin	no inhibition**	competition assay
α_2-Antiplasmin	no inhibition**	competition assay
Antithrombin III	no inhibition**	competition assay
C1-inhibitor	no inhibition**	competition assay
Oxidized α_1-antitrypsin	no inhibition**	competition assay

*Association rate constants were measured in 0.5 M NaCl, 0.1 M HEPES, pH 7.5, and at 25°C.

**PR3 was incubated with inhibitor in a 1:10 molar excess of inhibitor and no change in the enzyme activity was observed.

higher than that of cathepsin G (4.1×10^5 $M^{-1} \cdot s^{-1}$) [15]. Using the k_a values for PR3 with α_1AT, we determined the association rate constants of α_2M to PR3 by a previously described competition assay [14,16]. The k_a value for PR3 with α_2M was 1.1×10^7 which is 4-fold less than that of HNE (4.1×10^7 $M^{-1} \cdot s^{-1}$) and 3-fold higher than that of cathepsin G (3.7×10^6 $M^{-1} \cdot s^{-1}$) [16]. These association rate constants for PR3 with α_1AT or α_2M were consistent with the previously reported values by Rao et al. [5].

PR3 was not inhibited by either α_1ACT, α_2AP, ATIII, C1-inh, or α_1AT-ox under the conditions employed.

3.2. *Measurement of PR3–α_1AT complexes in a RIA*

In Fig. 1 a dose–response curve is shown that was obtained by testing different dilutions of preformed PR3–α_1AT complexes. These complexes were prepared by the addition of active site titrated PR3 either to an excess of purified α_1AT or to an excess of pooled human plasma. As a control, PR3 was inactivated by DFP prior to incubation with either α_1AT or pooled human plasma. The RIA appeared to be highly specific, sensitive and reproducible: as few as 0.5 nM of PR3–α_1AT complexes could be detected by this RIA, and intra-assay as well as inter-assay coefficients of variation were less than 10%. The difference in the dose–response curves of PR3 mixed with either purified α_1AT or plasma was only slight, indicating that virtually all PR3 added to plasma had formed a complex with its major inhibitor, α_1AT. In addition, these experiments indicated that an excess of irrelevant plasma proteins did not interfere with the detection of the PR3–α_1AT complexes. In plasma to which DFP-treated PR3 was added, a response corresponding to approximately 1 nM of PR3–α_1AT complexes was measured. This response was also observed in plasma without addition of PR3 (not shown), and apparently was due to the presence of low levels of PR3–α_1AT complexes in pooled normal plasma (see below). Preincubation of plasma with methylamine to inactivate α_2M, did not significantly alter this dose–response curve, which indicated that the formation of PR3–α_2M did not detectably influence the formation of PR3–α_1AT complexes in plasma.

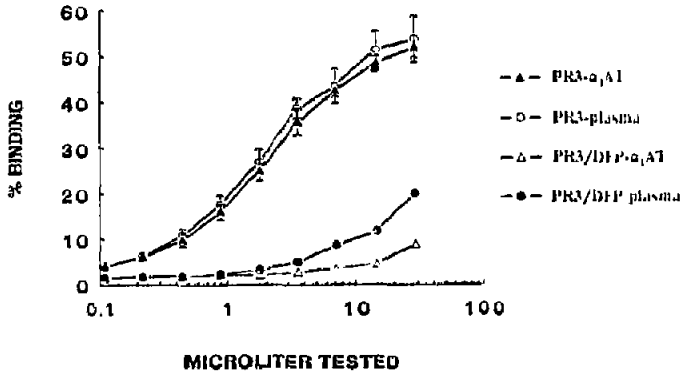

Fig. 1. Detection of PR3–α_1AT complexes by RIA. PR3 (2 μM) was incubated with either 250 μl of pooled human plasma or 250 μl 20 μM α_1AT for 30 min at room temperature. These mixtures were diluted (1:20) in PBS–0.1% (v/v) Tween-20, and subsequently tested in 2-fold serial dilutions by RIA. The amounts of the original mixtures (1:20) expressed as μl tested, are indicated on the abcissa. 1 μl tested contains 1 nM of PR3–α_1AT complexes (▲,○). Control experiments were performed by inhibiting PR3 with DFP (20 μM) prior to incubation with either plasma or α_1AT. In these control experiments 1 μl tested contains 1 nM of DFP treated PR3 (△,●). Results are expressed as percentage of binding of the labeled antibodies added. The experiment shown is representative of 5 experiments performed. The intra-assay coefficient of variation was less than 10%.

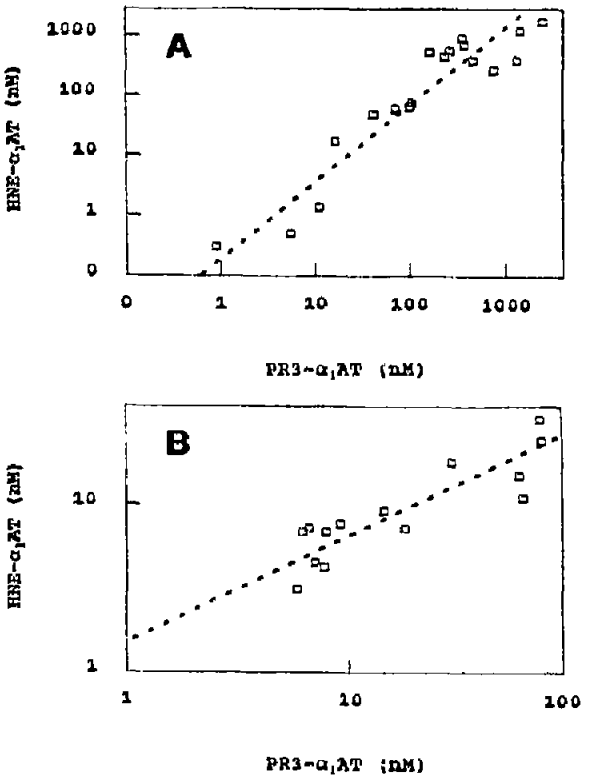

Fig. 2. Relationship between levels of PR3–α_1AT and HNE–α_1AT complexes in inflammatory fluids. In SF obtained from 18 patients with RA (A), and in plasma from 14 patients with sepsis (B), PR3–α_1AT and HNE–α_1AT complexes were detected by RIA. The levels of PR3–α_1AT complexes were plotted against the corresponding levels of HNE–α_1AT complexes, and are shown on a logarithmic scale. The dotted line represents the weighted linear regression line. SF samples $r = 0.94$, $P < 0.01$; sepsis plasma samples: $r = 0.88$, $P < 0.01$.

3.3. *Detection of PR3–α_1AT complexes formed in vivo in SF from arthritic joints and in plasma from patients with sepsis*

To investigate whether formation of PR3–α_1AT complexes also occurs in vivo, we tested SF obtained from 18 patients with RA, and plasma samples from 14 patients with sepsis. In Fig. 2A the levels of PR3–α_1AT complexes in these SF samples are plotted against the corresponding HNE–α_1AT levels. A strong correlation between these levels ($r = 0.93$, $P < 0.01$) was observed. In addition, in plasma samples of patients with sepsis,

PR3–α_1AT complexes could be detected and correlated significantly with HNE–α_1AT levels ($r = 0.88$, $P < 0.01$) (Fig. 2B). Levels of PR3–α_1AT complexes in plasma from healthy donors varied from 0.5–2.5 nM, comparable to the levels of HNE–α_1AT complexes [20]. Thus, also under (patho-)physiological conditions in vivo α_1AT appears to be an inhibitor of PR3.

4. DISCUSSION

Prevention of excessive tissue damage by extracellular elastinolytic enzymes requires neutralization of these enzymes by proteinase inhibitors. We report here on the interaction of PR3 with physiological proteinase inhibitors. We obtained association rate constants that suggest that both α_1AT and α_2M are effective inhibitors of PR3 in vitro. Our kinetic data are in agreement with those previously reported by Rao et al. [5]. In addition, detection of PR3–α_1AT complexes in the SF of RA patients and in plasma of patients with sepsis indicates that also in vivo α_1AT plays an important role as an inhibitor of PR3. The detection of equal amounts of PR3–α_1AT complexes in plasma and in methylamine-treated plasma indicated that, compared to α_1AT, α_2M is of minor importance as an inhibitor of PR3 in plasma.

We have investigated the interaction of PR3 with other plasma proteinase inhibitors, such as α_1ACT, α_2AP, ATIII, and C1-inh. No inhibition of PR3 was detected by these inhibitors. Also, α_1AT-ox did not inhibit PR3, which can be of importance in the microenvironment of activated neutrophils where α_1AT can be inactivated by reactive oxygen derivatives [21].

Patients with α_1AT deficiency, an autosomal recessive disorder characterized by reduced serum levels of functional α_1AT, are particularly susceptible to the development of emphysema [22]. It is generally assumed that these patients produce insufficient α_1AT to protect the alveolar walls from exposure to HNE, which results in progressive destruction of lung tissue, culminating in pulmonary emphysema [23]. Our finding that α_1AT is also the major plasma inhibitor of PR3 suggests that, in α_1AT-deficient patients, in addition to HNE, PR3 may be an important factor in the development of emphysema. This is further emphasized by the previous observations that PR3 is even more abundant in neutrophils than HNE [2], and because it displays strong elastinolytic and emphysema-causing properties [1,4].

PR3 is the antigen recognized by autoantibodies (C-ANCA) that circulate in the blood of patients with WG [6-8]. The possible role of these C-ANCA in the pathogenesis of WG is still unclear. In vitro, these autoantibodies induce primed PMN to degranulate [24]. Recently, we have obtained evidence that binding of C-ANCA to PR3 prevents the enzyme from being inactivated by α_1AT [25]. The resulting unbalanced activity of PR3 may contribute to inflammatory tissue damage, including necrosis and vasculitis. In vitro, neutrophils release HNE upon stimulation by various agonists [26]. High amounts of HNE in arthritic joints indicate that neutrophils are activated and/or damaged in several arthropathies [27]. We found corresponding levels of PR3–α_1AT and HNE–α_1AT in SF of RA patients, as well as in plasma of patients with sepsis, suggesting that these complexes may serve as markers of neutrophil activation. Studies on the levels of PR3–α_1AT complexes in the plasma of WG patients and in other inflammatory diseases are now in progress. In conclusion, we have demonstrated that both α_1AT and α_2M are plasma inhibitors of PR3, and that α_1AT also in vivo is important for the regulation of PR3 activity. These inhibitors should be taken into account in the exploration of the possible role of PR3 in different pathophysiological conditions.

Acknowledgements: Grateful acknowledgement is made to Ms A.M. Kamp for her excellent technical assistance, and to Dr. A.G.J. Swaak and Dr. L.G. Thijs for providing patients material. This research was supported by a grant from The Dutch Kidney Foundation (C88.733), and partially supported by a grant to the Georgia Institute of Technology (GM 42212) from the National Institutes of Health.

REFERENCES

[1] Kao, R.C., Wehner, N.G., Skubitz, K.M., Gray, B.H. and Holdal, J.R. (1988) J. Clin. Invest. 82, 1963–1973.
[2] Campanelli, D., Melchior, M., Fu, Y., Nakata, M., Shuman, H., Nathan, C. and Gabay, J.E. (1990) J. Exp. Med. 172, 1709–1715.
[3] Kam, C.-M., Kerrigan, J.E., Dolman, K.M., Goldschmeding, R., Von dem Borne, A.E.G.Kr. and Powers, J.C. (1992) FEBS Lett. 297, 119–123.
[4] Lüdemann, J., Utecht, B. and Gross, W.L. (1990) J. Exp. Med. 171, 357–362.
[5] Rao, N.V., Wehner, N.G., Marshall, B.C., Gray, W.R., Gray, B.H. and Holdal, J.R. (1991) J. Biol. Chem. 266, 9540–9548.
[6] Goldschmeding, R., van der Schoot, C.E., ten Bokkel Huinink, D., Hack, C.E., Van den Ende, M.E., Kallenberg, C.G.M. and Von dem Borne, A.E.G.Kr. (1989) J. Clin Invest. 84, 1577–1587.
[7] Niles, J.L., McCluskey, R.T., Ahmad, M.F. and Arnaout, M.A. (1989) Blood 74, 1888–1893.
[8] Jennette, J.C., Holdal, J.R. and Falk, R.J. (1990) Blood 75, 2263–2265.
[9] Kam, C.-M., McRae, B.J., Harper, J.W., Niemann, M.A., Volanakis, J.E. and Powers, J.C. (1987) J. Biol. Chem. 262, 3444–3451.
[10] Tanaka, T., Minematsu, Y., Reilly, C.F., Travis, J. and Powers, J.C. (1985) Biochemistry 24, 2040–2047.
[11] Harper, J.W., Cook, R.R., Roberts, J., Mclaughlin, B.J. and Powers, J.C. (1984) Biochemistry 23, 2995–3002.
[12] Matheson, N.R., Wong, P.S. and Travis, J. (1979) Biochem. Biophys. Res. Commun. 88, 402–409.
[13] Kezdy, F.T. and Kaiser, E.T. (1970) Methods Enzymol. 19, 3–20.
[14] Vincent, J.-P. and Lazdunski, M. (1972) Biochemistry 11, 2967–2977.
[15] Beatty, K., Bieth, J. and Travis, J. (1980) J. Biol. Chem. 255, 3931–3934.
[16] Virca, G.D. and Travis, J. (1984) J. Biol. Chem. 259, 8870–8874.
[17] Abbink, J.J., Kamp, A.M., Swaak, A.J.G. and Hack, C.E. (1992) J. Immunol. Methods 143, 197–208.
[18] Fraker, P.J. and Speck, J.C. (1978) Biochem. Biophys. Res. Commun. 80, 849–857.
[19] Arnett, F.C., Edworthy, S.M., Bloch, D.A., McShane, D.J., Fries, J.F., Cooper, N.S., Healey, L.A., Kaplan, S.R., Liang,

M.H., Luthra, H.S., Medsger Jr., T.A., Mitchell, D.M., Neustadt, D.H., Pinals, R.S., Schaller, J.G., Sharp, J.T., Wilder, R.L. and Hunder, G.G. (1988) Arthritis Rheum. 31, 315–324.
[20] Nuijens, J.H., Abbink, J.J., Wachtfogel, Y.T., Colman, R.W., Eerenberg, A.J.M., Dors, D., Kamp, A.M., Strack van Schijndel, R.J.M., Thijs, L.G. and Hack, C.E. (1992) J. Lab Clin. Med. 119, 159–168.
[21] Weiss, S.J. (1989) N. Engl. J. Med. 320, 365–376.
[22] Laurell, C.B. and Ericksson, S. (1963) Scand. J. Clin. Lab. Invest. 15, 132–140.
[23] Wevers, M.D., Casolaro, M.A., Sellers, S., Swayze, S.C., McPhaul, K.M. and Crystal, R.G. (1987) N. Engl. J. Med. 316, 1055–1062.
[24] Falk, R.J., Terrel, R.S., Charles, L.A. and Jennette, J.C. (1990) Proc. Natl. Acad. Sci. USA 87, 4115–4119.
[25] Van de Wiel, B.A., Dolman, K.M., Van der Meer-Gerritsen, C.H., Hack, C.E., Von dem Borne, A.E.G.Kr. and Goldschmeding, R. (1992) J. Clin. Exp. Immunol. (in press).
[26] Weissmann, G., Smolen, J.E. and Korchak, H.M. (1980) N. Engl. J. Med. 303, 27–34.
[27] Virca, G.D., Mallya, R.K., Pepys, M.B. and Schnebli, H.P. (1984) Adv. Exp. Med. Biol. 167, 345–353.