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Inhibition of neutrophil activation by α_1-acid glycoprotein

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SUMMARY

We report that α_1-acid glycoprotein (AAG), a naturally occurring human plasma protein and acute phase reactant of uncertain biological function, inhibits human neutrophil aggregation and superoxide anion generation induced by a variety of stimuli including zymosan treated serum, formyl–methionyl–leucyl–phenylalanine and phorbol myristate acetate. Inhibition was transient, directly proportional to the glycoprotein concentration and inversely proportional to the concentration of the stimulus added. Desialyzation, resulting in the removal of a substantial portion of the molecule's negative charge, did not alter the effectiveness of AAG. Removal of the penultimate galactose residues from desialyzed AAG resulted in a slight but significant reversal of inhibition, suggesting that the heteropolysaccharide units of AAG may be important for inhibition of cellular function. We therefore suggest that the acute phase glycoprotein AAG may be a significant modulator of neutrophil as well as platelet and lymphocyte function during inflammation.

Keywords α_1-acid glycoprotein neutrophils acute phase reactant desialyzation

INTRODUCTION

Human α_1-acid glycoprotein (AAG) is a constituent of normal plasma which increases up to four-fold during episodes of acute inflammation and cancer, and is therefore recognized as an acute phase reactant. AAG consists of a single completely sequenced polypeptide chain of 39,500 daltons which contains approximately 45% carbohydrate (Schmid, 1975). The liver is the major site of AAG synthesis (Sarcione, 1963) although a precursor form has been reported on the surface of granulocytes, monocytes and allogeneically activated lymphocytes (Gahmberg & Andersson, 1978).

The biological activities experimentally associated with AAG are varied and may or may not depend on the considerable negative charge contributed by sialic acid. Charge dependent interactions include the binding to drugs such as propanolol, perazine and methadone (Piafsky *et al.*, 1978; Schley, Siegert & Muller-Oerlinghausen, 1980; Romach *et al.*, 1981), and interactions with collagen (Franzbeau *et al.*, 1976) and vitamin B_{12} (Heide & Schwick, 1973). Antigenicity and clearance of AAG are definitely charge related (Athinoes, Thornton & Winzler, 1971; Morell *et al.*, 1971). AAG has been reported to inhibit lymphocyte responsiveness (Bennett & Schmid, 1980; Chiu *et al.*, 1977), platelet aggregation (Anderson & Eika, 1980; Costello, Fiedel & Gewurz, 1979), and the phagocytosis of *Staphylococcus aureus* and *Escherichia coli* by neutrophils (PMNs) (Van Oss *et al.*, 1974). Bennett & Schmid (1980) reported that the ability of AAG to inhibit certain murine lymphocyte reactivities was greatly enhanced by removal of both the sialic acid and galactose residues.

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These studies were undertaken to determine the effects of AAG on the superoxide anion (O_2^-) generation and aggregation responses of PMN activated *in vitro* by a variety of soluble stimuli (formyl–methionyl–leucyl–phenylalanine, FMLP; zymosan activated serum, ZTS; phorbol myristate acetate, PMA) and to define the contribution of sialic acid and galactose residues to any regulatory properties of the AAG molecule. Results show that AAG produces a stimulus-independent, transient inhibition of aggregation and O_2^- generation. Inhibitory effects are not a function of the negative charge of AAG but may be related to penultimate galactose residues.

MATERIALS AND METHODS

Preparation of AAG and derivatives. AAG was purified from human pathological ascites and/or pleural fluids by sulphosalicyclic acid precipitation followed by chromatography on sulphopropyl–Sephadex according to a modification of the method of Chiu *et al.* (1977). The protein was dialysed into 0.01 M phosphate buffer (pH 5·0), applied to a DE-52 column (2·0 × 45 cm) equilibrated in phosphate buffer and eluted with a 0·01–0·3 M NaCl gradient. Fractions containing AAG antigen were pooled, concentrated and dialysed into Tris-saline buffer (0·1 M Tris, 0·15 M NaCl, pH 7·4) for storage. Acid precipitation and column chromatography were performed at 4°C. No azide was utilized during storage of fluids or columns or added to buffers in purification.

Terminal sialic acid of AAG was removed by incubating AAG and insolubilized *Clostridium perfringens* neuraminidase (2·5 u/10 mg AAG) on a rotator (10 r/min) for 48 h at 37°C in 0·1 M sodium acetate buffer (pH 4·5). The digest was analysed directly for free sialic acid (Warren, 1959) and, after extensive dialysis, for enzymatic contamination. A sample of the dialysed protein (AAG-D) was incubated with 500 μg of fresh AAG (37°C, 1 h), and free sialic acid quantitated. The residual sialic acid content of desialyzed AAG (AAG-D) was assayed as described above after acid hydrolysis in 0·1 N H_2SO_4 at 80°C for 1 h. Desialyzation was monitored by measurement of the relative mobilities on polyacrylamide gels and by immunoelectrophoresis.

To remove galactose, AAG-D and β-galactosidase from *Aspergillus niger* (Sigma Chemical Company, St Louis, Missouri, USA) or bovine testes (a generous gift of Dr J.J. Distler) (50 u/10 mg AAG) were incubated for 120 h at 37°C in 0·05 M sodium acetate buffer (pH 4·0). Free galactose in the AAG-D enzyme digest was determined by the method of Distler & Jourdain (1973). The digest was dialysed into Tris-saline buffer (pH 7·4), layered over Sephadex G-100, and fractions assayed for β-galactosidase activity (Distler & Jourdain, 1973) and AAG antigen. Fractions containing asialo, agalactosyl-AAG (AAG-Agal) were pooled, concentrated, and dialysed against Tris-saline, pH 7·4. Residual galactose was removed by hydrolysis with 0·1 N H_2SO_4 in a boiling water bath for 8 h and assayed as described above.

Characterization of AAG and derivatives. Purity of the AAG preparations was established by SDS-PAGE in the presence and absence of 2-mercaptoethanol (Fairbanks, Steck & Wallach, 1971), as well as by IEF, IEP, and re-chromatography on DE-52 and Sephadex G75. Concentrations of AAG, and derivatives were established by RID, by Lowry protein determination, and by absorbance at 278 nm using an extinction co-efficient for a 1·0% solution of 8·93 for AAG (Schmid, 1975) and 9·85 for AAG-D (Kawahara *et al.*, 1973).

The terminal sialic acid and penultimate galactose concentrations of purified AAG were in agreement with published values (Table 1). Neuraminidase consistently removed > 70% of the sialic acid residues and no enzyme activity was detectable in final AAG-D preparations (≤0·06 u/mg AAG-D). *A. niger* and bovine testicular β-galactosidase removed 80% and 53%, respectively, of the bound galactose from AAG-D (Table 1) and no β-galactosidase activity was detected in either preparation (≤0·1 u/mg AAG-Agal). AAG, AAG-D and AAG-Agal on 7·5% SDS-PAGE under reducing conditions appeared as single bands of 44,000 daltons showing no evidence of molecular degradation (data not shown).

Isolation of human neutrophils (PMN). PMN were prepared from heparinized peripheral blood by Ficoll-Hypaque density gradient centrifugation followed by dextran sedimentation, and at least two cycles of hypotonic lysis. PMN (10^7 cells/ml) in modified Tris-saline buffer (0·15 M NaCl, 0·01 M

Table 1. Sialic acid and galactose concentrations of AAG, AAG-D and AAG-Agal preparations

	Sialic acid* % (wt/wt)	Galactose† % (wt wt)
AAG-21‡	10·3	9·7
AAG-D-21	0·7	9·8
AAG-Agal-21	0·7	1·9
AAG-23	8·9	10·8
AAG-D-23	0·9	9·8
AAG-Agal-23	0·9	4·6

* Sialic acid concentrations were quantitated by the thiobarbituric acid assay of Warren (1959).

† Galactose concentrations were quantitated by the galactose dehydrogenase-dependent reduction of β-NAD^+ according to Distler & Jourdain (1973).

‡ Numbers refer to various AAG preparations.

Tris, 0·04 μM BSA, 6·0 mM dextrose, $\pm$ 10 mM $MgCl_2$ and 6·0 mM $CaCl_2$, pH 7·4) routinely contained >95% PMN with >95% viability by trypan blue dye exclusion.

PMN aggregation. Aggregation was assayed using a modified method of Craddock, White & Jacob (1978). PMN (1×10^7/ml) in modified Tris-saline buffer, pH 7·4 were incubated with cytochalasin B (5 μg/ml) for 15 min at 37°C with periodic mixing. Cells (0·5 ml) were incubated with 0·1 ml of buffer, AAG, AAG-D, AAG-Agal (0·2–2·0 mg/ml) or HSA (non-specific protein control, HSA control) at 37°C in siliconized glass cuvettes for 3 min with stirring (600 r/min) prior to the addition of 0·1 ml of the activating agent. The Payton Aggregometer (Payton Associates, Inc., Buffalo, New York, USA) was calibrated to record the difference in light transmittance (%T) between $2{\cdot}1 \times 10^6$ PMN/ml (70% T) and $3{\cdot}5 \times 10^6$ PMN/ml (30% T) cell suspensions. Aggregation experiments were repeated at least four times and representative experiments are presented. Zymosan treated serum (ZTS) was prepared by incubation of autologous serum (1 ml) with boiled zymosan A at 5 mg/ml for 30 min at 37°C. FMLP was obtained from Andrulis Biochemical Company, Bethesda, Maryland, USA and PMA from Sigma Chemical Company.

O_2^- generation. O_2^- was detected by its ability to reduce ferricytochrome C according to a modification of the method of Babior, Kipnes & Curnette (1973). Purified PMN (2×10^6/ml) in modified Tris-saline buffer, pH 7·4 were mixed with 0·081 μM ferricytochrome and AAG, AAG-D, AAG-Agal (0·20–2·0 mg/ml) or HSA (0·25–2·0 mg/ml) in a total reaction volume of 0·9 ml on ice. Following the addition of 0·1 ml of PMA (10^{-7}–10^{-9} M) or FMLP (10^{-6}–10^{-8} M), the mixtures were incubated at 37°C with shaking (1–15 min), rapidly cooled, and centrifuged. Reduced ferricytochrome C was measured by absorbance at 550 nm. The addition of superoxide dismutase (175 u) inhibited control cytochrome C reduction by >99%. The effects of time and dose were tested on at least three different donor cell populations and representative experiments presented. All data points were obtained from triplicate determinations and are expressed as means with standard deviations.

RESULTS

Effects on AAG and derivatives on PMN aggregation

AAG, AAG-D and AAG-Agal were tested at normal and acute phase concentrations for effects on ZTS, FMLP and PMA-mediated aggregation responses. Preliminary experiments showed that AAG at 1 mg/ml produced a maximal (>90%) inhibition of control aggregation within 30 s of ZTS addition if added to PMN 60–180 s before stimulation. A progressive decrease in the inhibition resulted when the time of pre-incubation was reduced to 30 s (66%) or 10 s (30%), and AAG added

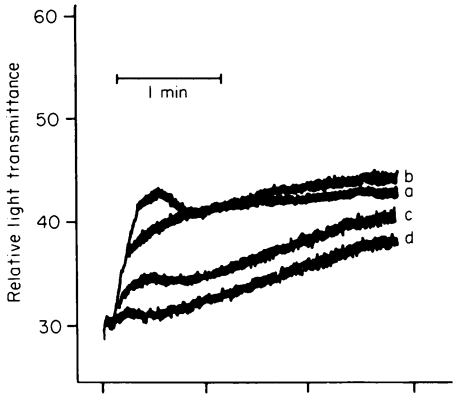

Fig. 1. Effects of pre-incubation in the presence of AAG (1 mg/ml) on inhibition of ZTS-induced PMN aggregation. Curve (a) represents the control aggregation response to 0·1 ml of ZTS while curves (b), (c) and (d) show the inhibition observed after pre-incubation with AAG for 10, 30, and 60–180 s, respectively.

10 s after ZTS had no effect (Fig. 1). Subsequent experiments with AAG and its derivitives utilized a 180 s pre-incubation. All results obtained with ZTS-induced (anti-C5 inhibitable) and FMLP-induced aggregation responses were qualitatively identical and only data for AAG effects on FMLP-induced activation are shown.

The effects of AAG and its derivatives on aggregation induced by FMLP are shown in Fig. 2. Inhibition (30 s post-stimulation) was dose-dependent, with AAG at 0·5, 1·0 and 2·0 mg/ml producing 17%, 33% and 70% inhibition, respectively, of control aggregation (Fig. 2a). The effects of AAG-D at 0·5, 1·0 and 2·0 mg/ml were comparable to those seen with unmodified AAG (Fig. 2b). AAG-Agal was substantially less effective, with 2 mg/ml resulting in only 12% inhibition and 1 mg/ml producing no inhibition (Fig. 2c). The inhibitory effects of AAG and its derivatives were time dependent and significant recovery from an 80% inhibition at 30 s was evident.

Effect of AAG on the prolonged, monophasic, PMA-induced aggregation response (Fig. 3) was compared at 180 s post-stimulation. AAG and AAG-D showed a dose-dependent inhibition reaching 91–100% at 1·0–2·0 mg/ml (Fig. 3a & b) while AAG-Agal was less inhibitory at 1·0 and 2·0 mg/ml, yielding 50% and 81% inhibition, respectively (Fig. 3c). The addition of HSA (2·0 mg/ml) had no effect on aggregation induced by either PMA or FMLP.

Effects of AAG on FMLP- and PMA-induced O_2^- generation

The effects of 1 mg/ml AAG and derivatives, an optimally inhibitory dose in aggregation, on FMLP and PMA-induced O_2^- generation were assayed at intervals up to 15 min. All produced significant

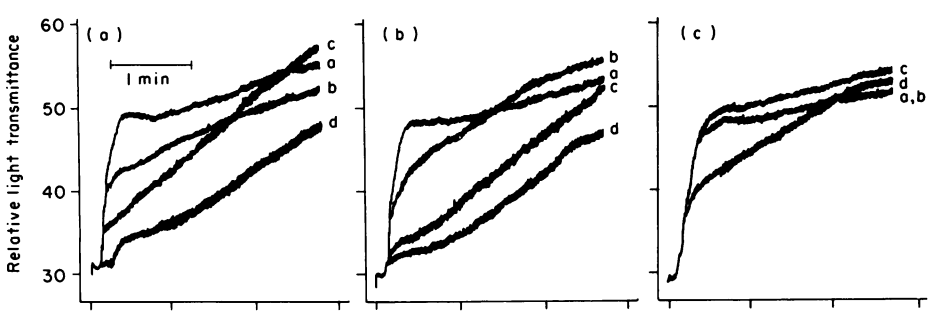

Fig. 2. Effects of (a) AAG, (b) AAG-D and (c) AAG-Agal on FMLP stimulated PMN aggregation. Control aggregation induced by 10^{-5} M FMLP (curve a) is compared to aggregation in the presence of 0·5 (curve b), 1·0 (curve c) and 2·0 mg/ml (curve d) of AAG or derivative.

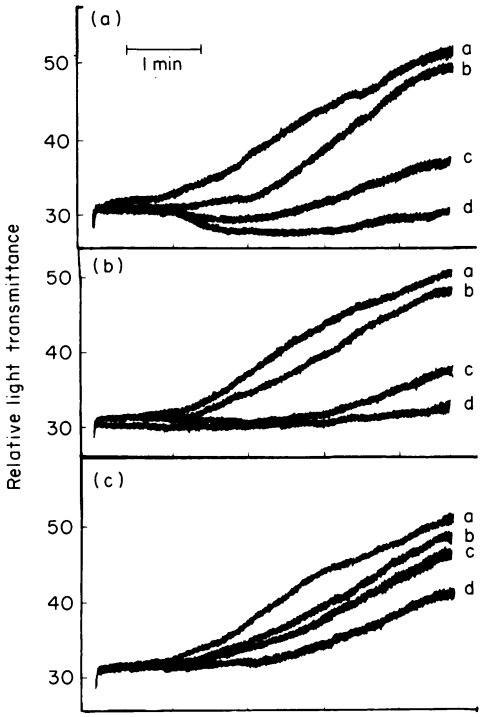

Fig. 3. Effects of (a) AAG, (b) AAG-D and (c) AAG-Agal on PMA-induced PMN aggregation. The control response to 10^{-7} M PMA (curve a) is compared to aggregation in the presence of 0·5 (curve b), 1·0 (curve c) and 2·0 mg/ml (curve d) of AAG or derivative.

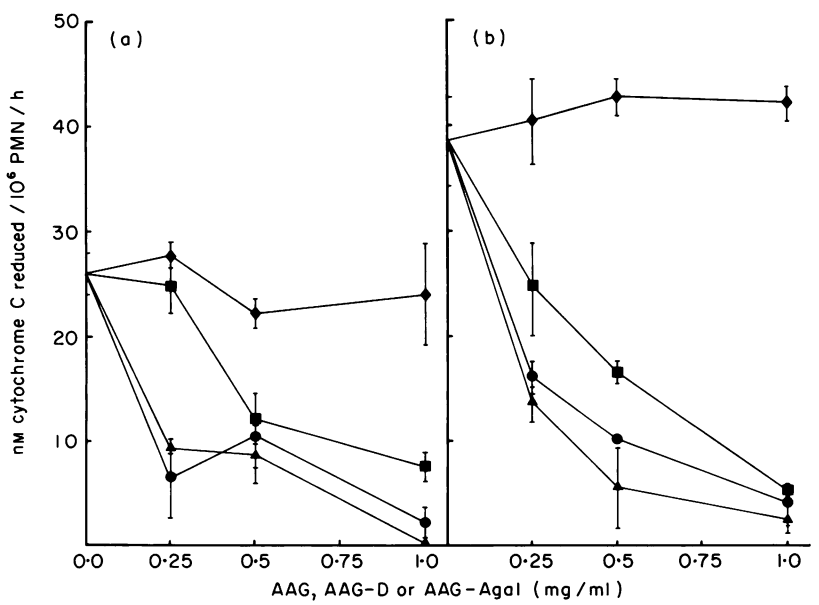

Fig. 4. Dose–response of AAG inhibition of O_2^- generation. The effects of 0·25, 0·50 and 1·0 mg/ml of AAG (▲), AAG-D (●) and AAG-Agal (■) on O_2^- generation 5 min after stimulation by (a) 10^{-7}M FMLP and (b) 10^{-8}M PMA were compared to HSA controls (♦). Concentrations were tested in triplicate and mean nM cytochrome C reduced/10^6PMN/h ± 1 s.d. indicated.

(80–90%) inhibition through 7 min of incubation with evidence of variable recovery between 7 and 15 min post-stimulation (data not shown). The effects of various doses of AAG, AAG-D and AAG-Agal on FMLP- and PMA-induced O_2^- generation were therefore compared 5 min after stimulation (Fig. 4). Inhibition of O_2^- generation by AAG, AAG-D or AAG-Agal was concentration-dependent between 0·25 and 1·0 mg/ml. AAG-Agal was significantly less effective than AAG or AAG-D at lower doses tested in both the FMLP- (Fig. 4a) and PMA- (Fig. 4b) stimulated systems. The significant inhibition (> 70%) of O_2^- generation by 1 mg/ml of all AAG preparations could be at least partially overcome by stimulating with $\geqslant 5 \times 10^{-6}$ M FMLP and $\geqslant 10^{-7}$ M PMA. Again HSA (0·25–2·0 mg/ml) had no effects on FMLP- or PMA-stimulated O_2^- generation at any time interval.

DISCUSSION

AAG induced, at low stimulus concentrations, a dose-dependent, transient hyporesponsive state in human PMN that was characterized by the inhibition of early membrane associated responses such as aggregation and O_2^- generation initiated by each of the soluble stimuli utilized. Inhibition could be overcome by increasing the stimulus concentration or by allowing the reaction to proceed for longer time periods. Since the PMN activation systems studied are receptor-mediated (Asuanikumar *et al.*, 1977; Lehrer & Cohen, 1981), cell responses are dependent in part on ligand binding kinetics. Increased stimulus concentrations will result in increased receptor occupancy and enhanced cell responsiveness (Sklar *et al.*, 1981). Apparent recovery from inhibition in the presence of a constant stimulus concentration may reflect cellular adaptation involving functional and receptor recovery. The almost complete reversal of inhibition evident within 2 min in the *in vitro* aggregation assay is probably due to functional metabolic recovery. Modulation involving receptor clearance and/or the expression of new sites for stimulus binding is not complete for 10 min or more (Neidel, Kahane & Cuatracasas, 1979; Sullivan & Zigmond, 1980).

Identification of the carbohydrate composition and/or tertiary structure crucial for the inhibitory effects of orosomucoid needs further investigation. It is clear that the removal of terminal sialic acid residues and thus a substantial portion of the negative charge had no significant effect on AAG-PMN interactions. Removal of the penultimate galactose residues resulted in a slight but reproducible reduction in the inhibitory effectiveness of AAG. Inhibition by AAG-Agal however, did not correlate with the quantity of galactose removed or with the possible effects of contaminating glucosaminidase in the β-galactosidase used. AAG-Agal produced by the highly purified bovine testicular enzyme showed a 53% reduction in bound galactose and was as effective as the preparation made with commercial *A. niger* galactosidase showing 80% galactose removal. These studies suggest that remaining mannose/glucosamine residues may subserve the modulatory effects of AAG on PMN activation. Indeed, Bennett & Schmid (1980) have shown in their studies of lymphocyte function that maximum immunosuppression was obtained with the asialo, agalacto derivative of AAG. Further enzymatic removal of mannose (to $\leqslant 35\%$) and *N*-acetyl glucosamine (to 30%) significantly reduced the inhibitory effects. The apparent discrepancy between the lower potency of AAG compared to its deglycosylated derivatives reported by Bennett & Schmid (1980) and the maximally inhibitory AAG in the PMN studies may be related to *in vivo* molecular heterogeneity. AAG used in the lymphocyte studies was isolated from pooled human serum while material used in these studies was isolated from inflammatory fluids. Forms of AAG with increased affinity for Con A, i.e. increased mannose availability, are elevated during inflammation (Nicollet *et al.*, 1981).

AAG could suppress PMN as well as lymphocyte and platelet responsiveness by interfering in a common activation pathway, perhaps via an interaction with a mannose/glucosamine receptor (Warr, 1980). AAG has been shown to alter membrane fluidity and receptor capping (Cheresh *et al.*, 1982). Such an interaction might interrupt an activation signal(s) at the level of a membrane bound phospholipase (Blackwell *et al.*, 1982) or a membrane glycosyltransferase (Lee & Kim, 1979).

Normal serum levels of AAG ($0{\cdot}650 \pm 0{\cdot}215$ mg/ml) *in vivo* could protect against PMN activation by low levels of spontaneously generated activators. This regulation would be overcome

during inflammation by the generation and accumulation of chemotaxins or other activators. Increased acute phase AAG levels and the production of partially deglycosylated forms could modulate responsiveness when both PMN numbers and stimulus levels are increased. The ability of orosomucoid to affect *in vitro* cell responsiveness, its apparent association with activated cell membranes (Andersen, 1982), and its deposition at sites of inflammation (Jamieson, Turchen & Huebner, 1980) support a role for AAG as a significant modulator of homeostasis during inflammation.

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REFERENCES

ANDERSEN, M. (1982) Increased amounts of orosomucoid, alpha-1 antitrypsin and haptoglobin associated with human blood granulocytes during the acute phase reaction. In *Proceedings of the Symposium on Inflammatory Markers* (Abstr.) (ed. by R.M. Suskind & R. Allen) p.111. Walter de Gruyer, Berlin

ANDERSEN, P. & EIKA, C. (1980) Thrombin, epinephrine and collagen induced platelet aggregation inhibited by α_1-acid glycoprotein. Influence of heparin and anti-thrombin III. *Scand. J. Hematol.* **24,** 365.

ASUANIKUMAR, S., CORCORAN, B., SCHIFFMAN, E., DAY, A.R., FREER, R.J., SHOWELL, H.J., BECKER, E.L. & PERT, C.B. (1977) Demonstration of a receptor on rabbit neutrophils for chemotactic peptides. *Biochem. Biophys. Res. Commun.* **74,** 810.

ATHINOES, E., THORNTON, M. & WINZLER, R.J. (1971) Comparative antigenicity of native and desialyzed orosomucoid in rabbits. *Proc. Soc. exp. biol. Med.* **111,** 353.

BABIOR, B.M., KIPNES, R.S. & CURNETTE, J.T. (1973) Biological defense mechanisms. The production by leukocytes of superoxide, a potential bactericidal agent. *J. clin. Invest.* **52,** 741.

BENNETT, M. & SCHMID, K. (1980) Immunosuppression by human α_1-acid glycoprotein: importance of the carbohydrate moiety. *Proc. Natl. Acad. Sci. USA.* **77,** 6109.

BLACKWELL, G.J., CARNUCCIO, R., DIROSA, M., FLOWER, R.J., LANGHAM, C.S.J., PARENTE, L., PERSICO, P., RUSSELL-SMITH, N.C. & STONE, D. (1982) Glucocorticoids induce the formation and release of anti-inflammatory and anti-phospholipase proteins into the peritoneal cavity of the rat. *Br. J. Pharmac.* **76,** 185.

CHERESH, D.A., HAYNES, D.B., PAUL, R.D., LOPEZ, D.M., SILVERMAN, M.A. & DISTASIO, J.A. (1982) Interaction of α_1-acid glycoprotein with the lymphoid membrane: a potential mechanism of immune suppression. *Proc. Am. Soc. Cancer Res.* **23,** 1009.

CHIU, K.M., MORTENSEN, R.F., OSMAND, A.P. & GEWURZ, H. (1977) Interactions of α_1-acid glycoprotein with the immune system. I. Purification and effects upon lymphocyte responsiveness. *Immunology*, **32,** 992.

COSTELLO, M., FIEDEL, B.A. & GEWURZ, H. (1979) Inhibition of platelet aggregation by native and desialized α_1-acid glycoprotein. *Nature*, **281,** 667.

CRADDOCK, P.R., WHITE, J.S. & JACOB, H.S. (1978) Potentiation of complement (C5a)-induced granulocyte aggregation by cytochalasin B. *J. lab. clin. Med.* **91,** 490.

DISTLER, J.J. & JOURDAIN, G.U. (1973) The purification and properties of β-galactosidase from bovine testes. *J. biol. Chem.* **248,** 6772.

FAIRBANKS, S., STECK, T.L. & WALLACH, D.F.H. (1971) Electrophoretic analysis of the major peptides of the human erythrocyte membrane. *Biochemistry*, **10,** 2606.

FRANZBLAU, C., SCHMID, K., FARIS, B., BELDEKAS, J., GARVIN, J., KAGAN, P. & BAUM, B.J. (1976) The interaction of collagen with α_1-acid glycoprotein. *Biochim. Biophys. Acta*, **427,** 302.

GAHMBERG, C.G. & ANDERSSON, L.C. (1978) Leukocyte surface origin of human α_1-acid glycoprotein (orosomucoid). *J. exp. Med.* **148,** 507.

HEIDE, K. & SCHWICK, G. (1973) Chemistry and significance of the carbohydrate moieties of human serum glycoprotein. *Agnew Chem. Int. Ed. Engl.* **12,** 721.

JAMIESON, J.C., TURCHEN, B. & HUEBNER, E. (1980) Evidence for the presence of rat α_1-acid glycoprotein in granuloma tissues: a fluorescence microscopy study. *Can. J. Zool.* **58,** 1513.

KAWAHARA, K., IKENAKA, T., NIMBERG, R.B. & SCHMID, K. (1973) The effect of the sialyl residues on the thermodynamic and hydrodynamic properties of α_1-acid glycoprotein. *Biochim. Biophys. Acta*, **295,** 505.

LEE, E.S. & KIM, S.W. (1979) Adsorbed glycoproteins in platelet adhesion onto polymer surfaces: significance of the terminal galactose units. *Trans. Am. Soc. Artif. Intern. Organs*, **25,** 124.

LEHRER, R.I. & COHEN, L. (1981) Receptor-mediated regulation of superoxide production in human neutrophils stimulated by phorbol myristate acetate. *J. clin. Invest.* **68,** 1314.

MORELL, A., GREGORIADIS, S., SCHIENBERG, H., HICKMAN, J. & ASHWELL, G. (1971) The role of sialic acid in determining the survival of glycoproteins in the circulation. *J. biol. Chem.* **246,** 1461.

NEIDEL, J.E., KAHANE, I. & CUATRECASAS, P. (1979)

Receptor mediated internalization of fluorescent chemotactic peptide by human neutrophils. *Science*, **205,** 1412.

NICOLLET, J., LEHRETON, J.P., FONTAINE, M. & HIRON, M. (1981) Evidence for α_1-acid glycoprotein of different pI values after Con A affinity chromatography. Study of their evolution during inflammation in man. *Biochim. Biophys. Acta*, **668,** 235.

PIAFSKY, K.M., BORGA, O., ODAR-CEDERLOF, I., JOHANSSON, C. & SJOQVIST, F. (1978) Increased plasma protein binding of propranolol and chlorpromazine mediated by disease-induced elevations of plasma α_1-acid glycoprotein. *N. Engl. J. Med.* **299,** 1435.

ROMACH, M.K., PIAFSKY, K.M., ABEL, J.G. & SELLELERS, E.M. (1981) Methadone binding to orosomucoid (alpha-1-acid) glycoprotein): determinant of free fraction in plasma. *Clin. Pharmacol. Ther.* **29,** 211.

SARCIONE, E.J. (1963) Synthesis of α_1-acid glycoprotein by the isolated perfused rat liver. *Arch. Biochim. Biophys.* **100,** 516.

SCHLEY, J., SIEGERT, M. & MULLER-OERLINGHAUSEN, B. (1980) Binding of perazine to α_1-acid glycoprotein. *Eur. J. clin. Pharmacol.* **18,** 501.

SCHMID, K. (1975) α_1-Acid glycoprotein. In *The Plasma Proteins: Structure, Function and Genetic Control.* (ed. by F.W. Putman) p. 163. Vol. I, Chap 4. Academic Press, New York.

SKLAR, L.A., OADES, Z.G. JESAITIS, A.J., PAINTER, R.G. & COCHRANE, C.G. (1981) Fluoresceinated chemotactic peptide and high affinity antifluorescein antibody as a probe of the temporal characteristics of neutrophil stimulation. *Proc. Natl. Acad. Sci. USA.* **78,** 7540.

SULLIVAN, S.J. & ZIGMOND, S.H. (1980) Chemotactic peptide receptor modulation in polymorphonuclear leukocytes. *J. cell Biol.* **85,** 703.

VAN OSS, C.J., GILMAN, C.F., BRONSON, P.M. & BRODER, J.R. (1974) Phagocytosis-inhibiting properties of human α_1-acid glycoprotein. *Immunol. Commun.* **3,** 321.

WARR, G.A. (1980) A macrophage receptor for (mannose/glucosamine) glycoproteins of potential importance in phagocytic activity. *Biochem. Biophys. Res. Commun.* **93,** 737.

WARREN, L. (1959) The thiobarbituric acid assay of sialic acids. *J. biol. Chem.* **234,** 1971.