Journal of Clinical Pathology, 1978, **31**, 1177-1181

Meningococcal infection and proteolytic control

J. M. STARK, N. MATTHEWS, H. C. RYLEY, B. M. GREENWOOD, L. S. LEWIS, AND H. C. WHITTLE

From the Department of Medical Microbiology, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN, UK, and the Departments of Medicine and Paediatrics, Ahmadu Bello University, Zaria, Nigeria

SUMMARY Cascade enzyme inhibitors (C1-esterase inhibitor, C3b inactivator, antithrombin III) and other major proteolytic enzyme inhibitors ($\alpha_1$trypsin inhibitor, $\alpha_1$chymotrypsin inhibitor, inter-α-trypsin inhibitor, $\alpha_2$macroglobulin) as well as C3 and $\alpha_1$acid glycoprotein, have been examined in the sera of Nigerian patients suffering from meningococcal infection of varied severity.

Patients with meningococcaemia had lower serum concentrations of important inhibitors than did patients with localised meningitic infection. Within the coccaemic group, those who died had the lowest values, notably of antithrombin III and $\alpha_2$macroglobulin (and also of C3).

The clinical end-result of meningococcal infection may be related to the degree of disequilibrium of the linked system of proteolytic control induced by the meningococcal endotoxin.

A previous study (Greenwood *et al.*, 1976) of epidemic meningococcal infection suggested that complement activation was important in the development of the meningococcaemic form of the disease. Once complement activation has been achieved, its subsequent amplification may largely determine the course of the infection with at least three mechanisms playing a part. First, the C3b product itself amplifies complement activation by the alternative pathway; such amplification is controlled by C3b inactivator (C3b INA) (Lachmann and Nicol, 1973) and the β_1H inhibitor (Fearon *et al.*, 1976). Secondly, the C567 byproduct of activation will lyse cells and platelets locally and release from them lysosomal proteases and thromboplastic materials. The released neutral proteases are able to split C3 and activate the alternative pathway (Schorlemmer and Allison, 1976). Thirdly, thrombin formed as a result of endotoxic activation of the coagulation pathway can cleave C3 (Bokisch *et al.*, 1969). The later appearance of plasmin would also result in further activation by splitting of C1 or C3 (Ratnoff and Naff, 1967).

A number of proteolytic inhibitors in plasma could interrupt this amplification. The sera of Nigerian children suffering from epidemic type A meningococcal infection were therefore examined to determine to what extent the concentrations of these inhibitors correlated with the severity of the disease.

Received for publication 8 June 1978

Patients and methods

Randomly selected patients with group A meningococcal disease were studied during an epidemic that affected northern Nigeria in 1977. Sera from 67 patients were studied. Thirty-two patients had meningococcaemia without meningitis, 11 meningitis with group A antigen detectable in the serum by countercurrent immunoelectrophoresis (antigen positive), and 24 meningitis without detectable antigenaemia (antigen negative). Twelve of the patients with meningococcaemia (38%) and two of the patients with meningitis (6%) died. Samples were also examined from 24 relatives of patients with the disease seen in Zaria in 1975. Children attending outpatients with minor ailments provided control samples.

The mean age in years ($\pm$ 1 SD) of the different groups of subjects were: acute meningococcaemia, 6·0 $\pm$ 2·5; acute meningitis (Ag + ve), 8·8 $\pm$ 5·0; acute meningitis (Ag − ve), 10·7 $\pm$ 6·2; convalescents, 11·0 $\pm$ 3·7; relatives, 12·7 $\pm$ 4·5; and controls, 13·2 $\pm$ 5·4. Patients with meningococcaemia were significantly younger than patients with meningitis and controls ($P < 0.001$ for both).

ESTIMATION OF PROTEIN COMPONENTS

An electroimmunoassay technique (Ryley *et al.*, 1975) was used to estimate C3, C1-esterase inhibitor ($C\bar{1}$ INH), C3b inactivator (C3b INA), antithrombin III (AT III), $\alpha_2$macroglobulin (α_2M), $\alpha_1$chymotrypsin inhibitor (α_1CI), $\alpha_1$trypsin inhibitor (α_1TI), inter-α-trypsin inhibitor (I-α-TI), $\alpha_1$acid glycoprotein (α_1AGP), and C-reactive protein (CRP). Rabbit antisera against C3b INA, α_1CI, α_1TI, and α_2M were prepared in Cardiff from purified antigens. The monospecific sera used in the other tests were obtained from Hoechst Pharmaceuticals Ltd, Hounslow, Middlesex. Absolute values (for all but C3b INA) of the control samples were estimated against the Hoechst standard serum and plasma controls. The final results were expressed as a percentage of the mean value obtained in the control subjects. Concentrations are indicated in the text by square brackets.

Meningococcal antigen titres were measured by countercurrent immunoelectrophoresis on doubling dilutions of serum (Greenwood *et al.*, 1971).

STATISTICS

The comparisons were made by a two-tailed Student's *t* test.

Results

PATIENTS

Complement and complement inhibitors

[C3] and [C3b INA] were significantly reduced ($P < 0.001$ with both) in patients with meningococcaemia but not in patients with meningitis (Fig. 1a, b). Patients with meningococcaemia who died had lower [C3] ($P < 0.01$) and [C3b INA] ($P < 0.05$) than survivors (Table).

Mean [$C\bar{1}$ INH] were significantly raised in both Ag + ve and Ag − ve meningitis patients ($P < 0.02$ and < 0.05, respectively) but not in patients with meningococcaemia (Fig. 1c).

Antithrombin III

[AT III] was significantly depressed ($P < 0.001$) in patients with meningococcaemia but not in patients with meningitis (Fig. 1d). Coccaemic patients who died had a significantly lower ($P < 0.005$) mean [AT III] than patients who survived (Table).

Other protease inhibitors

Each of the four major inhibitors behaved differently.

[α_1CI] was significantly increased in patients with

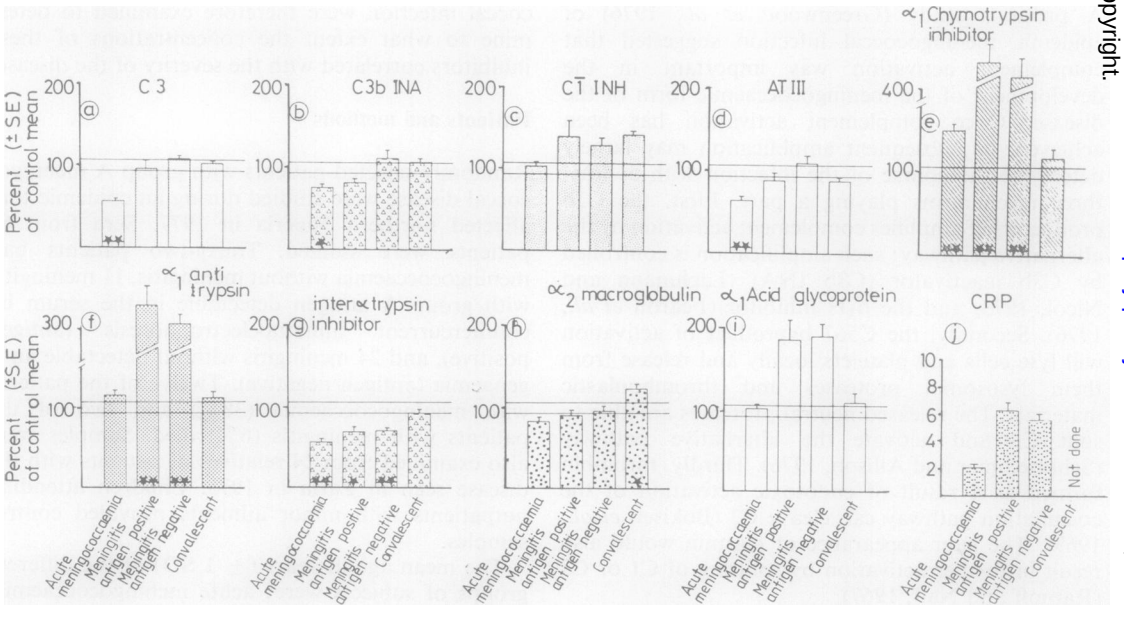

Fig. 1 *Responses of certain plasma protein components in meningococcal infection. In each histogram the groups, left to right are: acute meningococcaemia, antigen positive (Ag + ve) meningitis, antigen negative (Ag − ve) meningitis, and convalescent.* ★ $P < 0.01$ ★★ $P < 0.001$. *The concentrations are expressed as percentages of control mean except for C-reactive protein expressed as mg/dl: (a) C3; (b) C3b inactivator (C3b INA); (c) C1-esterase inhibitor ($C\bar{1}$ INH); (d) Antithrombin III (AT III); (e) $\alpha_1$chymotrypsin inhibitor; (f) $\alpha_1$trypsin inhibitor; (g) Inter-α-trypsin inhibitor; (h) $\alpha_2$macroglobulin; (i) $\alpha_1$acid glycoprotein; (j) C reactive protein (CRP).*

able *Concentration of reactants in fatal and non-fatal acute meningococcaemia (AMC)*

MC	No	Ag titre ****	C3 **	C3b INA ***	C1̄ INH *	AT III ****	α_1CI	α_1TI	I-α-TI	α_2M	α_1AGP	CRP
ıtal	12	4·3 ± 1·3	41·8 ± 24·8	65·5 ± 15·0	84·3 ± 20·8	48·5 ± 15·9	133·1 ± 35·2	102·6 ± 39·2	49·8 ± 15·4	75·6 ± 21·4	101·2 ± 31·2	2·35 ± 1·60
ɔn-fatal	20	1·0 ± 1·7	64·4 ± 20·5	78·2 ± 16·0	107·0 ± 35·5	73·8 ± 21·1	159·1 ± 52·0	117·5 ± 55·1	56·6 ± 15·9	87·5 ± 27·1	106·0 ± 28·8	1·98 ± 1·39

) Results expressed as a percentage of the mean control values ± SD, except for Ag as $\log_2$ of reciprocal titre. Where no antigen detected, $\log_2$ titre taken as 0.
) Antigen detected in all sera from fatal AMC; in 8 of 20 non-fatal AMC sera.
) In comparison between the fatal and non-fatal groups: **** $P < 0.005$
*** $P < 0.025$
** $P < 0.01$
* $P < 0.05$

coccaemia and meningitis (Fig. 1e). This increase, however, was not so great ($P < 0.01$) in patients with coccaemia as in patients with meningitis.

[α_1TI] was significantly raised in patients with meningitis but not in patients with coccaemia (Fig. 1f).

[I-α-TI] was significantly reduced in all groups.

A decrease in the mean serum [α_2M] was found in patients with meningococcaemia (Fig. 1h), this reduction being due to the low concentrations found in patients who subsequently died (Table). In convalescence, an increase ($P < 0.01$) was found only with this inhibitor (Fig. 1h).

Other acute-phase reactants

The behaviour of α_1AGP was similar to that of α_1TI, being significantly increased only in patients with meningitis (Fig. 1i). Significantly raised [CRP] was found in all groups (Fig. 1j) but meningococcaemic patients had a significantly lower concentration than patients with meningitis ($P < 0.001$).

Antigen titre

Circulating antigen was detected in 22 of the patients with meningococcaemia. The mean titre in patients who died was significantly higher than in the survivors (Table).

RELATIVES

Mean serum concentrations of C3, C3b INA, C1̄ INH, AT III, α_1CI, α_1TI, I-α-TI, α_2M, and α_1AGP in relatives of patients with meningococcal infections did not differ significantly from those of controls.

CONTROLS

The absolute values of control sera were (as mg/dl): C3, 85·7 ± 21·4; C1̄ INH, 24·9 ± 8·1; AT III, 40·2 ± 8·5; α_1CI, 57·4 ± 12·2; α_1TI, 191·0 ± 66·6; I-α-TI, 63·4 ± 19·9; α_2M, 341·0 ± 95·6; α_1AGP, 77·7 ± 27·9. CRP, 0·4 ± 0·7.

The C3b INA control values were 115·2 ± 29·5% of a mean adult Caucasian value.

Discussion

In the meningococcaemic patients the most striking observations were low [C3], [C3b INA], [AT III], and [I-α-TI] with the lowest concentrations in those patients who died, whereas in the meningitic patients the concentrations of these substances (with the exception of I-α-TI) were not reduced, and indeed [α_1TI], [α_1CI], and [α_1AGP] were greatly increased.

The rises in [α_1CI] found in all groups of patients may represent a direct or indirect response to release of cathepsin G, inhibitable by α_1CI (Baugh *et al.*, 1976) from leucocyte lysosomal granules. α_1CI and CRP are known to be early reactants in the acute phase (Laurell and Jeppsson, 1975a). They showed greater rises in meningitic patients than in coccaemic patients, suggesting that the coccaemic patients become seriously ill before these proteins reach their highest values.

The mean serum [α_2M] was significantly reduced in fatal meningococcaemia but increased in convalescent patients, suggesting that there may have been an increased production and consumption of this inhibitor in all groups; once endotoxin ceases to induce enzyme release, the normal [α_2M] is surpassed because supply temporarily exceeds demand.

The pattern with [I-α-TI] was quite different, it being reduced in all types of meningococcal infection. The precise function of this inhibitor *in vivo* has not been established but these observations would suggest an intravascular in addition to the already proposed mucosal role (Laurell and Jeppsson, 1975b).

The normal [C3b INA] in relatives in the 1975 epidemic is some evidence against infection being related to a genetically determined low initial [C3b INA].

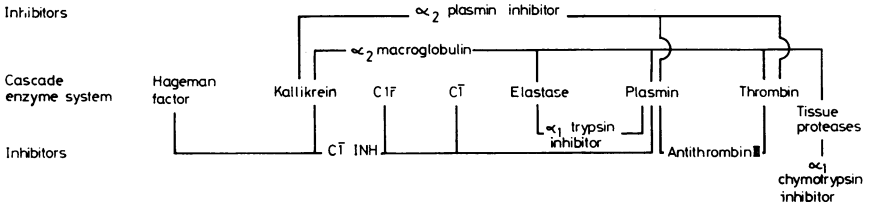

Fig. 2 *Shared specificities of some plasma proteolytic inhibitors ($\alpha_2$macroglobulin, antithrombin III, C1-esterase inhibitor (C1 INH), $\alpha_1$trypsin inhibitor, $\alpha_1$chymotrypsin inhibitor, $\alpha_2$plasmin inhibitor).*

The findings are consistent with the notion that the rate of protease release by endotoxin is important in determining the outcome of meningococcal infection; if the rate is so great as to overwhelm the responding rise in proteolytic inhibitors, the control of proteolysis may be fatally upset.

As well as being affected more directly by endotoxin, important cascades will be activated also by the released proteases and cascade products. Loss of control of the cascades, especially at local sites (Heimburger *et al.*, 1971), will be further accentuated because of the broad specificities of many protease inhibitors (Laurell, 1974; Moroi and Aoki, 1977). These inhibit enzymes of different cascades as well as otherwise unrelated proteases (Fig. 2). Thus, for example, an inhibitor imbalance initiated by proteases released from leucocyte granules may indirectly result in reduced inhibition of kallikrein and thrombin and so end in permeability and coagulation changes. In fatal meningococcaemia, AT III was indeed terminally diminished and associated with shock and collapse to which reduced inhibition of the permeability-altering kallikrein system may also have contributed. Because of these relationships, the stability of proteolytic equilibrium can be seen to affect susceptibility to endotoxin. Any influence either lessening the inhibitor concentrations or increasing the amount of proteases released would tilt the balance towards initial susceptibility and subsequent development of endotoxin shock.

This component of host susceptibility does not discount the influence of antibody since patients with low amounts of antibody will more readily admit endotoxin and thereby will have greater lysosomal release and activation of the various cascades. The patient with more antibody will allow the entry of only small amounts of endotoxin, which would test the other postulated protective mechanisms less severely. The significantly lower age of the coccaemic patients reported here would be consistent with the lower natural antibody in this age group.

Host susceptibility will thus be affected not only by antibody but also by the amounts of enzyme present in cells, the readiness with which lysosomes leak such enzymes, and the alacrity with which the host responds with effective proteolytic inhibitory substances. The problem in endotoxaemia, therefore, lies not only in controlling the complement or coagulation systems but in stabilisation of proteolysis in a wide sense.

References

Baugh, R. J., Gaurer, D. L., Johnson, D. A., and Travis, J. (1976). In *Proteolysis and Physiological Regulation (Proceedings of the Miami Winter Symposia)*, edited by D. W. Ribbons and K. Brew, Vol. II, p. 393. Academic Press, New York.

Bokisch, V. A., Müller-Eberhard, H. J., and Cochrane, C. G. (1969). Isolation of a fragment (C3a) of the third component of human complement containing anaphylatoxin and chemotactic activity and description of an anaphylatoxin inactivator of human serum. *Journal of Experimental Medicine*, **129,** 1109-1130.

Fearon, D. T., Daha, M. R., Weiler, J. M., and Austen, K. F. (1976). The natural modulation of the amplification phase of complement activation. *Transplantation Reviews*, **32,** 12-25.

Greenwood, B. M., Onyewotu, I. I., and Whittle, H. C. (1976). Complement and meningococcal infection. *British Medical Journal*, **1,** 797-799.

Greenwood, B. M., Whittle, H. C., and Dominic-Rajkovic, O. (1971). Counter-current immunoelectrophoresis in the diagnosis of meningococcal infections. *Lancet*, **2,** 519-521.

Heimburger, N., Haupt, H., and Schwick, H. G. (1971). In *Proceedings of the International Research Conference on Proteinase Inhibitors*, edited by H. Fritz and H. Tschesche, p. 1. W. de Gruyter, Berlin.

Lachmann, P. J., and Nicol, P. A. E. (1973). Reaction mechanism of the alternative pathway of complement fixation. *Lancet*, **1,** 465-467.

Laurell, C. B. (1974). *Protides of the Biological Fluids*, **22,** 3.

Laurell, C. B., and Jeppsson, J. O. (1975a). In *The Plasma Proteins*, 2nd edition, edited by F. W. Putnam, Vol. 1,

p. 258. Academic Press, New York.
Laurell, C. B., and Jeppsson, J. O. (1975b). In *The Plasma Proteins*, 2nd edition, edited by F. W. Putnam, Vol. 1, p. 257. Academic Press, New York.
Moroi, M., and Aoki, N. (1977). Inhibition of proteases in coagulation, kinin-forming, and complement systems by α_2-plasmin inhibitor. *Journal of Biochemistry*, **82**, 969-972.
Ratnoff, O. D., and Naff, G. B. (1967). The conversion of C'1s to C'1 esterase by plasmin and trypsin. *Journal of Experimental Medicine*, **125**, 337-358.
Ryley, H. C., Neale, M. L., Brogan, T. D., and Bray, P. T. (1975). Screening for cystic fibrosis by analysis of meconium for albumin and protease inhibitors. *Clinica Chimica Acta*, **64**, 117-125.
Schorlemmer, H. U., and Allison, A. C. (1976). Effects of activated complement components on enzyme secretion by macrophages. *Immunology*, **31**, 781-788.

Requests for reprints to: Dr J. M. Stark, Department of Medical Microbiology, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN, UK.

The November 1978 Issue

THE NOVEMBER 1978 ISSUE CONTAINS THE FOLLOWING PAPERS

Copies are still available and may be obtained from the PUBLISHING MANAGER, BRITISH MEDICAL ASSOCIATION, TAVISTOCK SQUARE, LONDON, WC1H 9JR, *price* £3·00, including postage