Complement and meningococcal infection

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Summary

Serum C3 levels were measured in 211 patients with meningococcal disease. Low levels were found in 13 patients with acute meningococcaemia, and complement activation may have contributed to the peripheral circulatory collapse that was responsible for nine deaths. The complement profile of these patients suggested activation of both classical and alternative complement pathways. Patients with meningitis had a higher mean serum C3 level than controls. Serial studies in 13 serum antigen-positive patients with meningitis who subsequently developed arthritis or cutaneous vasculitis showed a transient fall in serum C3 in eight. This fall was probably due to the formation of immune complexes that were responsible for their allergic complications.

Introduction

It is now recognised that complement activation, as a result of its interaction with other important biological systems, contributes to the pathogenesis of several infections. Complement activation may play an important part in initiating the peripheral circulatory collapse and intravascular coagulation seen in patients with Gram-negative septicaemia,¹ haemorrhagic dengue,² and *Plasmodium falciparum* malaria.³ Complement is also implicated in the pathogenesis of the immune complex nephritis that may follow streptococcal and many other infections and in the production of the immune complex arthritis seen in patients with infectious hepatitis.⁴

In 1946 Ecker *et al*⁵ noted low serum levels of total haemolytic complement in half of a small group of patients with meningococcal meningitis. This was later confirmed by Hoffman and Edwards,⁶ who found low initial serum C3 levels in patients with meningococcal disease who had meningococcal antigenaemia. Serum C3 levels were considerably depressed in patients with thrombocytopenia. Complement activation via the alternative pathway has recently been reported in a single case of meningococcal septicaemia.⁷ In 1973 we reported the occurrence of a transient fall in serum C3 levels in two out of four patients with meningococcal meningitis who developed arthritis or cutaneous vasculitis and suggested that these complications were due to immune complex formation.⁸

We have since measured serum and synovial fluid complement levels in 211 patients with meningococcal disease, and we report here our results.

Patients and methods

Two-hundred-and-eleven patients with meningococcal infection admitted to Ahmadu Bello University Teaching Hospital, Zaria, during three epidemics in 1972, 1974, and 1975 were studied.

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Meningococcal infection was diagnosed by detecting meningococcal antigen in cerebrospinal fluid (CSF) or serum or by a positive culture from CSF or blood. Altogether 152 patients had group A disease, and the remainder, all seen during 1975, had group C disease. Thirteen patients, all with group C disease, had acute meningococcaemia without meningitis. All of these patients had clinical signs of severe peripheral circulatory collapse and nine died. Circulatory collapse and haemorrhage were rare among the 198 patients with meningitis and were seen in only two patients. Twenty-one of the patients with meningitis developed arthritis, cutaneous vasculitis, or episcleritis, complications that we think have an allergic basis.⁸

Blood donors, healthy adults taking part in a village survey, and children attending outpatients with minor complaints provided control serum samples. Control synovial fluid samples were obtained from 13 patients with various forms of acute inflammatory polyarthritis (acute rheumatoid-like arthritis (4), Reiter's syndrome (2), pneumococcal arthritis (2), acute tropical polyarthritis (2), and miscellaneous forms of polyarthritis (3)).

C3, C4, C1q, factor B, properdin, and albumin levels were measured by radial immunodiffusion using monospecific antisera. Results were expressed as a percentage of a pooled adult Nigerian serum standard. Edetic acid plasma and synovial fluid samples were tested for C3 breakdown products by crossover immunoelectrophoresis. The results of serial C3 determinations in individual patients were assessed by an observer who had no knowledge of the clinical features of the patient.

Meningococcal antigen levels were measured by countercurrent immunoelectrophoresis and antibody levels by an indirect haemagglutination technique using human erythrocytes coated with purified group A or group C meningococcal polysaccharide (Institute Merieux). Cytocentrifuge preparations of synovial fluid leucocytes were examined for meningococcal antigen, immunoglobulin, and complement components by direct immunofluorescence.

Results

SERUM

Initial findings—C3 levels were measured in the serum of 211 patients with meningococcal disease collected at the time of their admission to hospital and in the serum of 179 age-matched controls. Thirteen patients with acute meningococcaemia but no meningitis had a much lower mean C3 level ($58\% \pm 19\%$) than the controls, whose mean C3 level was $106\% \pm 29\%$ ($P < 0.001$). In contrast, patients with meningitis had a higher mean C3 level ($115\% \pm 46\%$) than the controls ($P = 0.01$). Among the patients with meningitis 35 whose serum was positive for meningococcal antigen had a lower mean C3 level ($99\% \pm 43\%$) than 163 patients without antigenaemia, whose mean serum C3 level was $119\% \pm 47\%$ ($P = 0.01$).

Serum levels of other complement components were measured in the 13 patients with acute meningococcaemia (fig 1). Levels of all the complement components measured were depressed but especially properdin and factor B. C3 breakdown products were found in five out of eight initial plasma samples obtained from these patients. To ensure that these changes were not due to plasma dilution serum albumin levels were measured in the 13 patients and in 34 age-matched controls. The mean serum albumin levels of the two groups were similar ($103\% \pm 28\%$ for the patients and $110\% \pm 27\%$ for the controls).

Serial findings—C3 levels were followed serially in 10 serum antigen-negative patients with meningitis who made an uneventful recovery and in 13 serum antigen-positive patients who subsequently developed arthritis or cutaneous vasculitis. Many serum antigen-positive patients showed a fall in their serum C3 level on about the sixth day of their illness that was followed by a subsequent rise. The mean serum C3 level on day 6 was significantly lower than the mean C3 level on day 10 ($P = 0.02$). No significant change in mean serum C3 level occurred in the serum antigen-negative controls (table I). Assessment of serial C3 determinations in individual patients showed a transient fall in eight of the 13 patients who developed arthritis or

cutaneous vasculitis but in only one of the 10 controls ($P=0.01$). Transient falls in serum C3 occurred in individual patients between the fourth and the seventh day of their illness, the time at which allergic complications most often appeared (fig 2).

FIG 1—Serum C3, C4, C1q, factor B, and properdin levels in 13 patients with acute meningococcaemia. Hatched areas indicate mean levels ± 1 standard deviation obtained in 24 age-matched controls.

TABLE 1—Mean serial serum C3 levels ($\pm$ SD) in 13 antigen-positive patients who developed allergic complications and in 10 antigen-negative patients with no complications

Day of illness:	1-2	3-4	5-6	7-8	9-10
<i>Antigen-positive patients</i>					
Mean C3 (%)	101 $\pm$ 32	113 $\pm$ 44	104 $\pm$ 39	132 $\pm$ 61	141 $\pm$ 32
No of observations	10	11	12	11	7
<i>Antigen-negative patients</i>					
Mean C3 (%)	97 $\pm$ 46	96 $\pm$ 60	95 $\pm$ 47	105 $\pm$ 47	93 $\pm$ 48
No of observations	7	9	10	8	8

FIG 2—Serial serological changes in 13 patients with meningococcal meningitis who developed arthritis or cutaneous vasculitis. $\times$ = Mean serum C3 level $\pm$ standard error. $\bullet$ = Mean serum antigen titre. $\circ$ = Mean serum antibody titre. Hatched areas indicate number of patients developing arthritis or cutaneous vasculitis.

TABLE 2—Mean levels of C3, C4, and factor B ($\pm$ SD) in synovial fluid of 13 patients with meningococcal arthritis and of 13 patients with other forms of acute inflammatory arthritis

	C3 (%)	C4 (%)	Factor B (%)
Meningococcal arthritis	63 $\pm$ 41	79 $\pm$ 55	67 $\pm$ 25
Other forms of arthritis	72 $\pm$ 58	113 $\pm$ 84	101 $\pm$ 57

Plasma samples collected from eight patients with allergic complications at the time at which these appeared were tested for C3 breakdown products by crossover immunoelectrophoresis. Breakdown products were found in four. In each of these patients serial determinations showed the appearance of increased amounts of C3 breakdown products when the allergic complications developed, followed by their subsequent disappearance.

SYNOVIAL FLUID

Synovial fluid was obtained from 13 patients who developed arthritis after meningococcal meningitis. All fluids were sterile and none contained free meningococcal antigen on counter-current immunoelectrophoresis. C3, C4, and factor B levels were measured in these samples and in synovial fluid obtained from 13 patients with other forms of acute inflammatory joint disease. Mean levels of C3, C4, and factor B were lower in the patients with meningococcal arthritis than in the controls (table II), but none of the differences were statistically significant. C3 breakdown products were found in four out of six edetic acid synovial fluid samples obtained from patients with meningococcal arthritis.

Synovial fluid leucocytes obtained from five patients with meningococcal arthritis were examined by direct immunofluorescence. In each case globular cytoplasmic deposits staining for C3, immunoglobulin, and meningococcal antigen were observed. Three samples were studied for the presence of other complement components. Bright staining was given by a C4 conjugate but little staining by conjugates to factor B and properdin.

Discussion

This study has shown that most patients with acute meningococcaemia have depressed serum complement levels. C3, C4, C1q, factor B, and properdin levels were all depressed, especially properdin levels. A similar complement profile has been observed in shocked patients with Gram-negative septicaemia, depression of serum properdin again being especially pronounced.⁹ This complement profile suggests complement activation by both the classical and alternative pathways. Circulating endotoxin is the likely cause of the complement activation found in our patients with acute meningococcaemia, for bacterial endotoxins can activate both the classical and the alternative complement pathways.^{10 11} Endotoxins have a direct action on the properdin pathway; activation of the classical pathway may result from combination of endotoxin with natural antibodies present in many sera.¹⁰ Possibly, our patients with acute meningococcaemia were making sufficient specific antibody to form immune complexes with the circulating capsular antigen detected in each patient and thus to activate the classical complement pathway. Complement activation can lead to the release of vasoactive amines¹² and activation of the coagulation system.¹³ It may therefore play an important part in initiating the circulatory collapse and intravascular coagulation that are prominent features of acute meningococcaemia and which were important causes of the death of nine of our 13 patients.

Patients with meningitis had a slightly higher initial mean serum C3 level than controls but, as noted already,⁶ patients with antigenaemia had a significantly lower mean C3 level than those without antigenaemia. This depression was much less pronounced than that observed in patients with acute meningococcaemia. Endotoxin was probably again concerned in producing the complement activation seen in this group of patients, though surprisingly few had any signs of peripheral circulatory collapse or disseminated intravascular coagulation. Perhaps an irreversible cycle of events is set in progress only after a critical degree of complement activation has occurred.

Eight of the 13 serum antigen-positive patients who developed arthritis or cutaneous vasculitis showed a transient fall in serum C3, and in four this was accompanied by the appearance in the plasma of increased amounts of C3 breakdown products. This fall was followed by a rebound increase. The fall in serum C3 was only short-lived and may have been missed in the remaining

five patients as serum was usually collected only every other day. C3 levels fell around the sixth day of the illness, when antigen was disappearing from the circulation and when free antibody could first be detected in the serum. Hence this transitory fall in serum C3 was probably due to the formation of immune complexes and these complexes were probably responsible for the patients' arthritis and cutaneous vasculitis. This view is supported by our demonstration in synovial fluid leucocytes from patients with meningococcal arthritis of deposits of meningococcal antigen and immunoglobulin that stained strongly for C3 and C4. Sera from patients with arthritis and vasculitis are currently being tested for the presence of immune complexes by more direct methods. Preliminary results suggest that circulating immune complexes are present when allergic complications appear. Possibly, however, immune complexes are also formed locally as a result of trapping of antigen by phagocytic cells during the phase of antigenaemia and the subsequent development of an Arthus reaction.

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References

- McCabe, W R, *New England Journal of Medicine*, 1973, 288, 21.
- Bokisch, V A, *et al*, *New England Journal of Medicine*, 1973, 289, 996.
- Greenwood, B M, and Brueton, M J, *Clinical and Experimental Immunology*, 1974, 18, 267.
- Alpert, E, Isselbacher, K J, and Schur, P H, *New England Journal of Medicine*, 1971, 285, 185.
- Ecker, E E, *et al*, *Journal of Clinical Investigation*, 1946, 25, 800.
- Hoffman, T A, and Edwards, E A, *Journal of Infectious Diseases*, 1972, 126, 636.
- Johnson, U, and Laurell, A-B, *Acta Pathologica et Microbiologica Scandinavica*, 1975, 83, 285.
- Greenwood, B M, Whittle, H C, and Bryceson, A D M, *British Medical Journal*, 1973, 2, 737.
- Fearon, D T, *et al*, *New England Journal of Medicine*, 1975, 292, 937.
- Kane, M A, May, J E, and Frank, M M, *Journal of Clinical Investigation*, 1973, 52, 370.
- Fine, D P, *Journal of Immunology*, 1974, 112, 763.
- Bokisch, V A, Müller-Eberhard, H J, and Cochrane, C G, *Journal of Experimental Medicine*, 1969, 129, 1109.
- Zimmerman, T S, and Müller-Eberhard, H J, *Journal of Experimental Medicine*, 1971, 134, 1601.

T lymphocytes in kidney allograft recipients

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Summary

The spontaneous sheep erythrocyte (E) rosette test was used on 340 occasions to measure T cell levels in 28 renal allograft recipients. The E rosette counts were reduced by high-dose steroids but usually returned towards normal under maintenance treatment with azathioprine and low-dose prednisone. Unexpectedly, rejection crises were more often associated with a decrease in E rosettes before steroid treatment (12 cases) than with an increase in E rosettes (5 cases). Our results show the need for caution in using the E rosette test and more generally B and T cell markers for monitoring anti-graft immunity in renal allograft recipients.

Introduction

Immunological follow-up of kidney allograft recipients appears to be of great importance for the progress of renal transplantation. Immunosuppressive treatment may not only fail to suppress rejection crises but often induces undesirable side effects. Specific tests for adjusting the dose and duration of treatment according to the level of anti-graft immunity would be of great value. In the absence of such routine procedures, tests of the

non-specific immunity of allograft recipients might show whether azathioprine and steroids are immunosuppressive in a given patient. Furthermore, if it could be shown that rejection occurs when the minimum level of immunosuppression is lost higher doses could be prescribed before the onset of clinical or biological manifestations of rejection.

Serial measurements of T lymphocyte levels in peripheral blood have therefore been performed in 28 renal allograft recipients. T lymphocytes were characterised by their ability to form spontaneous rosettes with sheep erythrocytes.¹

Patients and methods

The 28 patients received their kidney allografts over a period of six months. Fifteen of them were followed up from the day of transplantation for two to six months. Twenty-four had been given cadaver kidneys, and four kidneys from living relatives.

Immunosuppressive treatment—All patients received azathioprine 5 mg/kg daily for the first three days, and then 3 mg/kg daily. Dosage was reduced if leucopenia or a severe infection developed. Steroids were given to the cadaver kidney recipients as follows: methylprednisolone (Solu-Medrol) 1 g intravenously on the day of transplantation, followed by betamethasone (Betnesol) 5 mg/kg (prednisone equivalent) daily by mouth from day 3 for five days, reducing thereafter to 0.25 mg/kg daily over 25 days. Maintenance treatment was with azathioprine 3 mg/kg and prednisone 0.25 mg/kg daily. Steroids were not given routinely to the recipients of kidneys from living donors. Three of these patients, however, had acute rejection crises in the days after transplantation and were therefore given betamethasone in a fashion similar to the cadaver kidney recipients. Thus only one patient received azathioprine without steroids.

Rejection crises—"Acute rejection crisis" was diagnosed when a significant rise in serum creatinine occurred without an obvious urological or infectious cause. In most cases the diagnosis was indicated by either hypcreatininuria or hyponatruuria or unexplained fever or an increase in the size or tenderness of the transplant. The diagnosis was in general only presumptive, which makes difficult the interpretation of individual results. Treatment included an intravenous "pulse" of methylprednisolone 1 g on days 1, 3, and 5. When this appeared to be

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