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Immune complexes and the pathogenesis of meningococcal arthritis

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SUMMARY

Immune complex levels were measured in serum and synovial fluid obtained from 10 patients who developed arthritis 3–8 days after the onset of meningococcal meningitis. Mean serum immune complex levels were lower in these patients than in eight age matched control patients with meningococcal disease who did not develop late complications. This observation suggests that meningococcal arthritis follows local formation of immune complexes in the synovium rather than deposition of circulating immune complexes. Purified meningococcal polysaccharide antigen-induced synovitis when injected into the knee of rabbits previously sensitized by i.v. injection with heat killed meningococci.

Keywords immune complexes meningococcal arthritis

INTRODUCTION

Arthritis occurs in 5–10% of patients with invasive meningococcal disease (Schaad, 1980). Arthritis may follow direct bacterial invasion of the synovium during the bacteraemic phase of the illness. In such cases meningococci can usually be isolated from synovial fluid. However, in other patients arthritis develops 3–10 days after the onset of illness at a time when other clinical features are improving. In such patients arthritis may be accompanied by a secondary rise in temperature and by cutaneous vasculitis, episcleritis or pericarditis (Whittle *et al.*, 1973). Synovial fluid from these patients is sterile and negative for meningococcal polysaccharide antigen on countercurrent immunoelectrophoresis. Both direct and indirect evidence implicates the host immune response in the aetiology of late onset 'allergic' meningococcal complications. Arthritis frequently develops at a time when antibody can first be demonstrated in the serum (Greenwood, Whittle & Bryceson, 1973) and the development of arthritis is often accompanied by a transient fall in the serum C3 and by the appearance of split complement components in the serum (Greenwood, Onyewotu & Whittle, 1976). Raised levels of immune complexes have been demonstrated directly in the serum and synovial fluid of three patients with meningococcal arthritis (Davis *et al.*, 1976; Larson *et al.*, 1977; Garrett *et al.*, 1980) and deposits of meningococcal antigen and antibody can be demonstrated in synovial fluid leucocytes obtained from affected patients (Greenwood *et al.*, 1973; Greenwood & Whittle, 1976; Larson *et al.*, 1977).

There is, therefore, strong evidence to implicate immune complexes in the pathogenesis of meningococcal 'allergic' arthritis. However, nephritis, the most frequent clinical manifestation of most forms of post-infectious immune complex disease, is seen only very rarely as a complication of meningococcal disease (Rainford *et al.*, 1978). This clinical observation suggests that the late manifestations of meningococcal disease are due to the formation of immune complexes at sites

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where meningococcal antigens have been deposited during the bacteraemic phase of the illness rather than to the deposition in synovium and skin of circulating immune complexes. In this paper we present some evidence that this is the case.

MATERIALS AND METHODS

Patients. Sera were obtained from 13 patients with meningococcal disease who developed polyarthritis, with or without cutaneous vasculitis, 3–6 days after the onset of their illness. Group A or group C meningococcal polysaccharide antigen was demonstrated in the initial serum sample of each of these patients by countercurrent immunoelectrophoresis. Synovial fluid was obtained from 10 patients; each specimen was sterile and negative for meningococcal polysaccharide antigen. For comparison sera were collected from 13 age matched patients with meningococcal disease who did not develop late complications and whose initial serum was negative for meningococcal antigen. The results of studies of sequential changes in serum levels of meningococcal antigen, antibody and complement in some of these patients have been published previously (Greenwood *et al.*, 1973, 1976).

Aliquots of serum which had not been thawed and refrozen were available for determination of immune complex levels for 10 patients who developed allergic complications and for eight who did not.

Rabbits. Outbred white rabbits were immunized by i.v. injection of increasing volumes (0·1–0·5 ml) of a suspension of heat killed group A meningococci. Rabbits were challenged by injection into the left knee joint of 25 μg of purified group A meningococcal polysaccharide vaccine (Institut Mérieux) diluted in the vaccine solvent. Twenty-four hours after challenge animals were killed and synovium taken from each knee joint for microscopy and for immunofluorescence.

Laboratory methods. Meningococcal polysaccharide antigen levels were determined by countercurrent immunoelectrophoresis on serial dilutions of serum using a rabbit group specific antiserum (Greenwood, Whittle & Rajkovic, 1971). Meningococcal antibody levels were measured by haemagglutination as previously described (Blakebrough *et al.*, 1982). Serum C3 levels were measured by the Mancini technique and the results recorded as a percentage of an adult Nigerian serum standard. Immune complex levels were determined both by a guinea-pig macrophage radiobioassay (Mohammed, Thompson & Holborow, 1977) and by a modified C1q binding assay (Zubler *et al.*, 1976a). Immunofluorescence was carried out on cytocentrifuge preparations of synovial fluid leucocytes and on cryostat sections of snap frozen synovium using both anti-whole immunoglobulin and anti-group A meningococcal polysaccharide antisera conjugated with fluorescin isothiocyanate.

RESULTS

Serum changes in patients with meningococcal disease

Fig. 1 represents a compilation of previously published data from our laboratory and new data on serum antigen, antibody and complement levels in 13 patients with meningococcal arthritis who developed 'allergic' complications and in 13 age matched patients with meningococcal disease who did not. All the patients who developed late complications were initially serum antigen positive. Their antibody response to infection was less than that of patients without complications.

The results of immune complex measurements in patients who developed late complications and in those who did not are shown in Figs 2 & 3. Similar results were obtained with both the radiobioassay and the C1q technique and the overall correlation between the two methods was good ($r = 0{\cdot}78$, $P = < 0{\cdot}001$). Immune complex levels were higher in patients with an uncomplicated course than in patients who developed arthritis but the differences between the two groups are statistically significant only for the radiobioassay on days 1–2 ($P < 0{\cdot}05$). Study of the relationship between immune complex levels and the time that arthritis developed showed that in most patients immune complex levels were highest about 2 days before arthritis was detected.

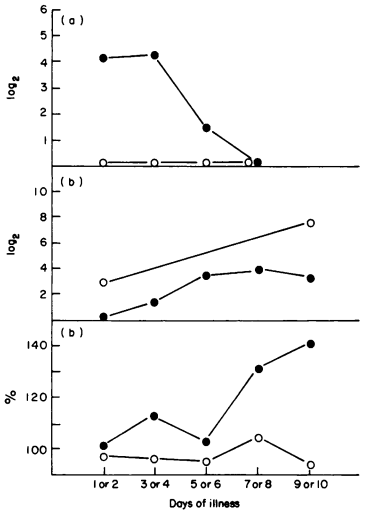

Fig. 1. Mean serum (a) antigen, (b) antibody and (c) C3 levels in 13 patients with meningococcal disease who developed 'allergic' complications (●——●) and in 13 age matched patients who did not (○——○). Antigen and antibody levels are expressed as a reciprocal of the $\log_2$ titre, C3 levels as a % of an adult Nigerian serum pool.

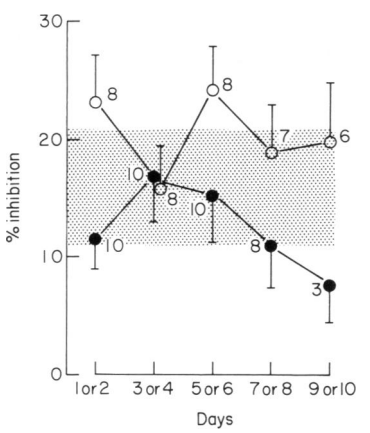

Fig. 2. Mean serum immune complex levels (±1 s.e.) in 10 patients with meningococcal disease who developed 'allergic' complications (●——●) and in eight who did not (○——○) as measured by radiobioassay. Figures indicate the number of observations made. Hatched areas indicate the normal range (mean ± 2 s.d.).

Synovial fluid samples from 10 patients were tested for immune complexes. The mean percentage inhibition obtained in the radioassay was 13·2% (range 2–27%) and the mean percentage binding in the C1q assay was 22·1% (range 8–44%). Synovial fluid from patients with osteoarthritis gave a mean inhibition of 3 ± 5% in the C1q assay (Zubler *et al.*, 1976b).

Experimental induction of meningococcal allergic arthritis

Injection of purified meningococcal polysaccharide into the knee of an unsensitised rabbit did not induce inflammation (Table 1). However, when purified meningococcal polysaccharide was injected into the knee of a rabbit which had been immunized with a series of injections of heat killed meningococci and which had developed precipitating antibody, an acute synovitis with effusion was

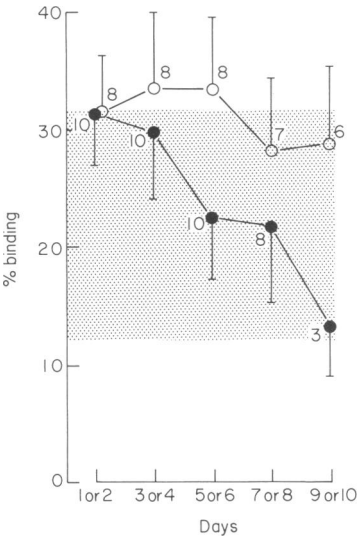

Fig. 3. Mean serum immune complex levels (±1 s.e.) in 10 patients with meningococcal disease who developed 'allergic' complications (●——●) and in eight who did not (○——○) as measured by a C1q binding assay. Figures indicate the number of observations made. Hatched area indicates the normal range (mean ± 2 s.d.).

Table 1. The production of synovitis in rabbits following various schedules of i.v. injections of group A heat killed meningococci and i.a. injection of purified group A meningococcal polysaccharide antigen

Group	Days i.v. immunization given	Day precipitating antibody first detected	Day i.a. injection given	Day joint examined	Synovitis present	
1	—	—	1	2	0/5	
2 (a)	1, 3, 5, 7, 9	7–9	3	10	1/4	3/8
(b)	1, 3, 6, 8, 10, 12	6–10	6	13	2/4	
3 (a)	1, 3, 5, 7, 9, 11	9–11	11	12	3/4	7/8
(b)	1, 3, 6	6	6	7	4/4	

produced. No inflammatory changes were present in the contralateral knee of such animals. In an attempt to mimic more closely the situation that might prevail in man antigen was injected into the knee early in the course of a series of i.v. injections, before precipitating antibody had been produced, and the joint examined 7 days later. Synovitis was seen in some, but not all rabbits, following this procedure but the inflammatory changes produced were less marked than those seen in animals which received an intracellular injection of polysaccharide after precipitating antibodies had appeared in serum.

Inflamed joints showed a variety of inflammatory changes. Oedema and hyperaemia of the synovium were usually present and the synovium was infiltrated with polymorphonuclear neutrophil leucocytes, mononuclear cells and eosinophils (Fig. 4). In two samples obtained from rabbits in group 3 fibrinoid necrosis of small vessels was seen.

Synovial fluid leucocytes and synovium from four rabbits in group 3 were examined by immunofluorescence (Table 2). Meningococcal antigen and immunoglobulin were detected in both polymorphonuclear neutrophil leucocytes and in mononuclear cells. Meningococcal antigen and immunoglobulin were present around small blood vessels in one specimen of synovium. Synovium from two non-sensitized rabbits examined 24 h after injection of meningococcal polysaccharide showed antigen within the synovium but no deposits of immunoglobulin.

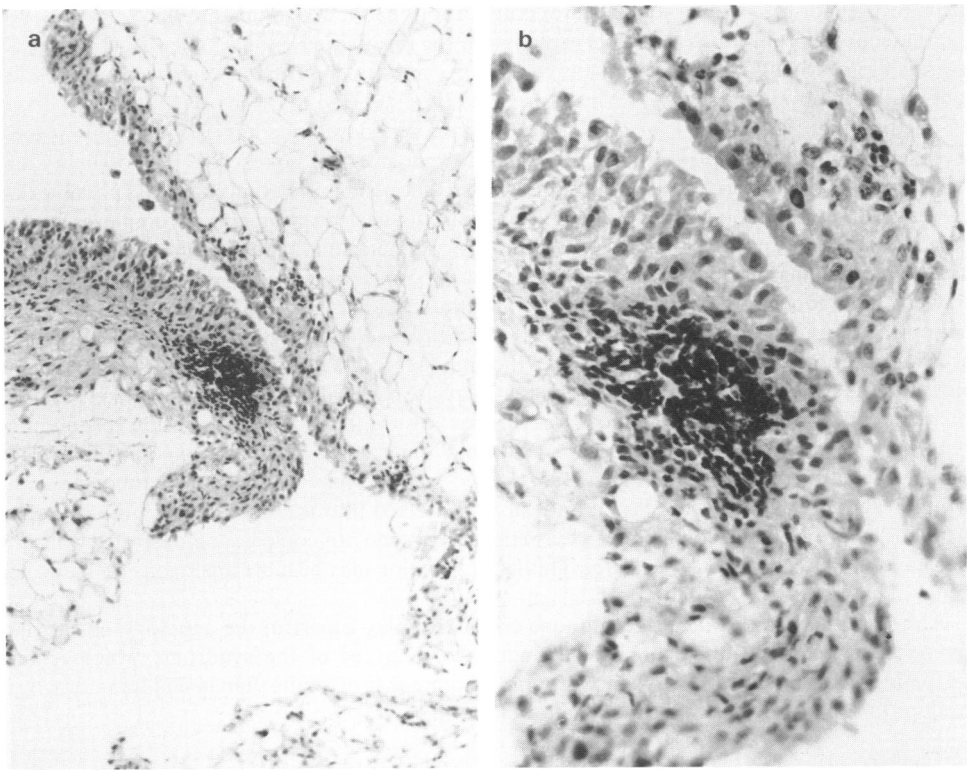

Fig. 4. Photomicrographs of the synovium of a rabbit obtained 24 h after i.a. injection of 25 μg group A meningococcal polysaccharide. The rabbit had been sensitized previously by i.v. injection with killed meningococci. (a) × 250 showing generalized synovial inflammation and (b) × 400 showing a focal collection of inflammatory cells (haematoxylin & eosin).

Table 2. Immunofluorescent findings in synovial fluid leucocytes and synovium from four sensitized rabbits examined 24 h after i.a. injection of 25 μg of purified group A meningococcal polysaccharide antigen

	Positive fluorescence	
	Meningococcal antigen	Immunoglobulin
Synovial fluid leucocytes	3/4	3/4
Synovium	1/4	1/4

DISCUSSION

A variety of methods are now available to measure immune complex levels in biological samples. In this study two standard methods were used which detect the ability of immune complexes to bind to C1q or to bind to receptors for altered immunoglobulin on the surface of guinea pig macrophages. Both of these assays have previously been used successfully to demonstrate abnormalities in patients with a variety of immune complex-mediated conditions. In this study a good correlation between the two assay methods was found and both assays showed that patients with

meningococcal disease who developed late complications had lower serum immune complex levels than patients who did not. A possible explanation for this finding is that patients with 'allergic' arthritis are nearly all initially serum antigen positive and for this, or for other reasons, they are poor antibody producers (Fig. 1) (Whittle *et al.*, 1975).

The fact that patients with meningococcal disease who develop 'allergic' complications have lower serum immune complex levels than patients who do not does not exclude the possibility that their complications are produced by deposition of circulating immune complexes. It is possible that low serum levels of immune complexes reflect extensive tissue deposition or that complexes formed in the serum antigen positive patients are of a size or configuration that makes them more prone to induce an inflammatory response than the complexes formed by patients with an uncomplicated recovery and a good antibody response. This possibility could be investigated. However, we believe that our findings, taken in conjunction with the clinical observations that glomerulonephritis is a rare complication of meningococcal disease and that 'allergic' arthritis sometimes reappears in a joint that was swollen and tender in the bacteraemic phase of the illness, make it more likely that the 'allergic' complications of meningococcal disease result from local formation of immune complexes. This view receives some support from the results of animal experiments. When meningococcal polysaccharide antigen was injected into the knee of sensitised rabbits an acute Arthus reaction was produced. When antigen was injected into the knee early in the course of injections before antibody could be detected in the serum, synovitis could be demonstrated when the injected joint was examined a week later. This latter situation may be more analogous to that which occurs in man.

Although there is evidence that immune complexes play a part in the aetiology of 'allergic' meningococcal arthritis there are still a number of features of the syndrome which remain unexplained. It is not known why the syndrome is commoner in adults than in children nor why it develops in some antigenaemic patients but not in others.

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