Cryoglobulinaemia in Henoch-Schönlein purpura

MIGUEL GARCIA-FUENTES, CYRIL CHANTLER, D GWYN WILLIAMS

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Summary

Sera from patients with Henoch-Schönlein purpura were examined for cryoglobulinaemia. Thirty patients had acute Henoch-Schönlein purpura, with or without renal disease; 14 had chronic nephritis after a previous episode of purpura; and 17 were well, without urinary abnormalities, after recovering from Henoch-Schönlein purpura. Raised concentrations of cryoglobulins were present in 14 (47%) of those with acute purpura, nine (64%) of those with chronic nephritis, but none of those who had recovered completely from Henoch-Schönlein purpura. This suggests that acute Henoch-Schönlein purpura and the chronic nephritis that sometimes follows it have an immune-complex pathogenesis.

IgA and properdin were found in several cryoglobulins, which suggested that complement had been activated via the alternative pathway, but isolated cryoglobulins capable of splitting C3 *in vitro* did so via the classical pathway.

Introduction

Although the evidence is indirect, Henoch-Schönlein purpura is often considered to be an immune-complex disease. The combination of arthritis, vasculitis, nephritis, and enteritis in this syndrome resembles acute serum sickness, and immunoglobulin and complement components have been shown by immunofluorescence in affected vessels and glomeruli.¹ To examine the immune pathogenesis of Henoch-Schönlein purpura further we studied the incidence and constitution of cryoglobulins in the serum of patients with this disease.

Patients and methods

An episode of Henoch-Schönlein purpura was defined as the typical rash occurring with any two of the three other major manifestations of the syndrome—that is, joint disease; microscopic haematuria or proteinuria, or both; and gastrointestinal disease (abdominal pain or melaena, or both).

Three groups of patients were studied. Thirty patients had active disease and were studied within 30 days of the onset of purpura. Their mean age was 6.7 years (range 2-13 years). Fourteen patients with chronic nephritis were also studied. Each had had an episode of Henoch-Schönlein purpura at least two months earlier and had microscopic haematuria or proteinuria (0.5 g/24 hours), or both, at the time of study. Their mean age at the onset of purpura was 7.8 years (range 5-17 years), and the mean period since they had had Henoch-Schönlein purpura was 3.4 years (range 0.2-12 years). In six patients renal biopsy had shown glomerulonephritis, and all but one had had microscopic haematuria or proteinuria or both at the

time of the acute attack. Seventeen patients who had previously had Henoch-Schönlein purpura but had no urinary abnormalities at the time of study acted as controls for the patients with nephritis. All had had evidence of renal involvement at the time of acute disease. Their mean age at onset of purpura was 6.0 years (range 2-10 years), and the mean period since then was 9.2 years (range 1.5-13 years).

Sera from 15 healthy adults were also collected to establish a control range for cryoglobulin concentration. (It was not considered ethical to take 20-30 ml of blood from normal children for this purpose.) Sera from 31 patients with membranous nephritis, 27 with mesangio-capillary nephritis, and 23 with systemic lupus erythematosus were studied to compare the incidence of IgA-containing cryoglobulins in these diseases with that in Henoch-Schönlein purpura.

Cryoglobulins were isolated as follows: 20-30 ml blood was allowed to clot at 37°C for three to four hours, and serum was separated by centrifuging at 2500 *g* for 20 minutes at 37°C. Sodium azide was added to 0.1% and the serum kept at 4°C for four to seven days. Cryoprecipitate was separated by centrifuging at 300 *g* for 30 minutes at 4°C, then washed four times in phosphate buffered saline at 4°C. The precipitate was dissolved by adding 0.3 ml of sodium chloride 17.5 g/100 ml, mixing for one hour at 37°C, then adding 0.3 ml of distilled water and incubating the solution at 37°C with sodium azide 0.1%. Any insoluble material was removed by centrifuging at 2000 *g* for 10 minutes at 37°C, and the cryoglobulin was then checked for absence of albumin by double immunodiffusion. If albumin was found, the cryoprecipitate was reprecipitated and washed again until free of albumin. IgG, IgA, IgM, C1q, C4, C3, factor B, fibrinogen, and properdin were detected by double immunodiffusion against the appropriate antiserum at 37°C. The protein concentration of the cryoglobulin solution was measured by the Lowry method² (mg/l of original serum).

Complement activation by isolated cryoglobulins was examined by incubating cryoglobulins with normal human serum at 37°C and then performing crossed immunoelectrophoresis of the mixture to detect C3 breakdown.³ Cryoglobulins capable of complement activation were tested for their dependence on Ca⁺⁺ and Mg⁺⁺ ions by repeating the reaction in 0.02-M ethylenediamine tetra-acetic acid and 0.02-M ethylene glycol bis-aminoethylether tetra-acetic acid respectively.

Antisera—All antisera were obtained commercially (Behringwerke)

Serum protein concentrations of cryoglobulins in controls and patients with acute or previous Henoch-Schönlein purpura.

Departments of Medicine and Paediatrics, Guy's Hospital, London SE1

MIGUEL GARCIA-FUENTES, MD, research fellow

CYRIL CHANTLER, MD, MRCP, consultant paediatrician

D GWYN WILLIAMS, MD, MRCP, consultant nephrologist and senior lecturer in medicine

except the anti-properdin antiserum, which was a gift from Professor D K Peters, Royal Postgraduate Medical School, London. Each antiserum was shown to be monospecific.

Results

Cryoglobulinaemia—Cryoglobulins were detected in the blood of eight of the 15 controls. The mean protein concentration ($\pm$ SD) was 6 ± 8.5 mg/l. Cryoglobulin protein concentrations greater than 23 mg/l were therefore considered abnormal. Fourteen of the 30 patients with acute disease had raised cryoglobulin concentrations and a further nine had concentrations in the normal range (see figure). Cryoglobulinaemia was not related to the presence or absence of renal disease (table I), but was detected more often in the earlier stages of the illness (table II). Nine of the patients with renal disease and previous Henoch-Schönlein purpura had significant cryoglobulinaemia, while those with previous purpura but no urinary abnormalities had no cryoglobulins or normal levels.

Cryoglobulin analysis—Only IgG, IgM, and IgG-IgM were found in the normal controls. In the patients with acute purpura or chronic nephritis cryoglobulins at normal concentrations were found to contain constituents—IgA, C4, C3, properdin—that were not detected in the normal controls. We therefore considered the results of the analysis of cryoglobulins in all 34 patients with cryoglobulinaemia irrespective of the concentration. Cryoglobulins were single and mixed, and no type was particularly associated with either acute purpura or chronic nephritis or with the presence of renal involvement in the

patients with acute disease. IgA was found in 12 of the 34 patients (35%). C1q, C4, and C3 were found in various combinations in seven patients, all of whom also had IgA. Properdin was found in eight patients, but was not specifically associated with cryoglobulins containing IgA. Factor B was found in one patient with acute purpura, and fibrinogen was found in one patient with acute disease and in three with chronic nephritis.

Cryoglobulin analysis in other forms of nephritis—One out of 15 patients (7%) with membranous nephritis had IgA-containing cryoglobulins, as did two out of 20 (10%) with mesangiocapillary nephritis, and two out of 23 (9%) with systemic lupus erythematosus.

Complement activation by cryoglobulins—Cryoglobulins from 11 patients with acute Henoch-Schönlein purpura and 11 with chronic nephritis were examined for C3-splitting activity. This was found in the cryoglobulins of three patients in each group (cases 3, 7, 21, 25, 26, and 32). In each case the cryoglobulins were dependent on both Ca^{++} and Mg^{++} ions, indicating that each activated complement via the classical pathway.

Discussion

These findings indicate that cryoglobulinaemia is common in both acute Henoch-Schönlein purpura and the chronic nephritis that may follow the acute episode, although other studies⁴⁻⁶ have found only one other patient with Henoch-Schönlein purpura and cryoglobulinaemia.⁶

Cryoglobulins have been shown experimentally to be composed of antigen-antibody complexes, with inflammatory properties of immune complexes.⁷ They occur in diseases with an immune-complex pathogenesis such as systemic lupus erythematosus, glomerulonephritis, vasculitis, and certain chronic infections—for example, leprosy.⁸ Our demonstration of cryoglobulins in acute Henoch-Schönlein purpura and the nephritis succeeding the acute attack therefore provides strong evidence that both conditions are immune-complex diseases. Cryoglobulinaemia was found more often in the early stages of acute Henoch-Schönlein purpura but occurred equally in patients with and without clinical evidence of nephritis. By

TABLE I—Cryoglobulinaemia and renal disease in patients with acute Henoch-Schönlein purpura

	No of patients	Mean No of days since onset (range)	Cryoglobulinaemia	
			>23 mg/l	≤23 mg/l
Patients with renal disease	14	9.6 (1-26)	7	2
Patients without renal disease	16	9.0 (4-18)	7	7
Total	30		14	9

TABLE II—Analysis of cryoglobulins in patients with acute Henoch-Schönlein purpura and nephritis after previous Henoch-Schönlein purpura

Case No	Time since onset	Renal disease	Protein (mg/l)	Cryoprecipitate								
				IgG	IgM	IgA	C1q	C4	C3	B	P	F
Patients with acute Henoch-Schönlein purpura												
1	(days)		19	+	+	+	+	+	+	—	—	—
2	4	—	31	+	+	+	+	—	+	—	+	—
3	5	+	46	+	+	+	+	—	+	—	—	—
4	26	+	21	+	+	+	+	—	+	—	+	—
5	10	+	42	+	+	+	—	—	+	—	+	—
6	1	—	42	+	+	+	—	—	—	+	+	—
7	8	—	9	+	+	+	—	—	—	—	—	+
8	5	+	45	+	+	+	—	—	—	—	+	—
9	4	—	20	+	+	—	—	—	—	—	—	—
10	3	+	25	+	+	—	—	—	—	—	+	—
11	14	—	32	+	+	—	—	—	—	—	—	—
12	10	—	25	+	+	—	—	—	—	—	—	—
13	7	—	21	+	+	—	—	—	—	—	—	—
14	14	—	16	+	+	—	—	—	—	—	—	—
15	28	+	24	—	+	—	—	—	—	—	+	—
16	11	—	59	—	+	—	—	—	—	—	—	—
17	4	—	39	—	+	—	—	—	—	—	—	—
18	5	+	10	—	+	—	—	—	—	—	—	—
19	5	—	30	+	—	—	—	—	—	—	—	—
20	12	+	43	+	—	—	—	—	—	—	—	—
21	18	—	34	+	—	—	—	—	—	—	—	—
22	7	+	16	+	—	—	—	—	—	—	+	—
23	29	—	20	+	—	—	—	—	—	—	—	—
23	30	—	20	+	—	—	—	—	—	—	—	—
Patients with chronic nephritis												
24	(years)		35	+	+	+	—	—	—	—	—	—
25	0.2	Nephrotic syndrome	110	+	+	+	+	—	+	—	—	—
26	2	Proteinuria	132	+	+	+	+	+	+	—	+	—
27	4	CRF	26	+	+	+	—	—	—	—	—	+
28	1	CRF	34	+	+	—	—	—	—	—	—	—
29	1	Haematuria, proteinuria	76	+	+	—	—	—	—	—	—	+
30	1	CRF	8	+	+	—	—	—	—	—	—	—
31	7	Haematuria, proteinuria	10	+	+	—	—	—	—	—	—	—
32	2	Haematuria, proteinuria	44	—	+	—	—	—	—	—	—	—
33	0.5	Haematuria, proteinuria	38	+	—	—	—	—	—	—	—	—
34	2	Haematuria, proteinuria	130	+	—	—	—	—	—	—	—	+
34	12	CRF										

CRF = Chronic renal failure. B = Factor B. P = Properdin. F = Fibrinogen.

contrast, patients who had had Henoch-Schönlein purpura with renal disease and had recovered fully had normal cryoglobulin concentrations, whereas those who had nephritis after an episode of purpura had significant cryoglobulinaemia. These observations suggest that the chronic glomerulonephritis that may follow acute Henoch-Schönlein purpura is a result of persisting immune complexes and that those patients who will develop nephritis may be identified by their continuing cryoglobulinaemia.

Although some patients with purpura or nephritis had normal cryoglobulin concentrations, their cryoglobulins contained proteins such as IgA, C4, C3, and properdin, which are never, or only rarely, detected in cryoglobulins isolated from normal subjects.⁵⁻⁹ The constitution of a cryoglobulin may therefore be as important in indicating its pathogenetic significance as its serum concentration. Among our patients with nephritis IgA was much more prevalent (35%) than in other types of nephritis,^{11,12} though not more so than in vasculitis.³ The high incidence in Henoch-Schönlein purpura is of obvious interest in view of the frequent demonstration of IgA in the diseased glomeruli^{11,12} and skin lesions of patients with Henoch-Schönlein purpura^{13,14} and their raised serum IgA levels.^{15,16} The role of IgA in Henoch-Schönlein purpura is not clear; it may be present in affected tissues and cryoglobulins as antibody against an infectious organism or other antigen which has entered via the respiratory or gastrointestinal mucosa. Alternatively the IgA may be altered by an infectious organism so that it becomes antigenic and causes immune-complex formation. This mechanism has been implicated in cases of poststreptococcal nephritis in which the IgG component of some cryoglobulins lacked sialic acid: the streptococci were found to be capable of producing this chemical change in vitro.¹⁰ IgG so altered becomes autoantigenic, and experimentally induces nephritis and cryoglobulinaemia.¹⁰ Bacteria can remove sialic acid from IgG by their secretion of neuraminidase, and this property may therefore allow immune-complex disease to follow infections. Although respiratory tract infections often precede Henoch-Schönlein purpura, no organisms have been specifically associated with the disease, and if the condition has an immune-complex aetiology the antigen awaits definition.

The finding that C1q, C4, and C3 were present in cryoglobulins only in association with IgA also suggests that IgA has some particular role in Henoch-Schönlein purpura. IgA can activate complement via the alternative pathway¹⁷ or the classical pathway.¹⁸ Furthermore, an IgA-IgG-IgM cryoglobulin which activated complement via both classical and alternative pathways has been described.¹⁹ In our in-vitro studies the IgA-containing cryoglobulins activated complement exclusively by the classical pathway, but this does not exclude the possibility that they may activate the alternative complement pathway in vivo. That this occurs in Henoch-Schönlein purpura has been

postulated from the high prevalence of glomerular properdin (a constituent of the alternative pathway) and IgA in patients with Henoch-Schönlein nephritis,²⁰ and, indirectly, from the occurrence of Henoch-Schönlein purpura in patients with C2 deficiency.²¹⁻²³

Our finding of properdin in cryoglobulins from eight patients supports the view that the alternative pathway is also activated, although properdin was not specifically associated with IgA-containing cryoglobulins. Recent experiments have indicated that properdin may attach non-specifically to activated C3.²⁴ This mechanism may explain its presence in the glomeruli and cryoglobulins in Henoch-Schönlein purpura, though it does not account for the higher incidence of glomerular properdin in this condition than in other forms of nephritis.

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SHORT REPORTS

Paralysis of palate in a child

Isolated paralysis of the palate in children is now a rarity. Here I report a case in which this was possibly caused by a Coxsackie A9 infection.

Case report

A boy of 12 began to have difficulty with swallowing, and fluids he took regurgitated through his nose. At the same time his voice assumed a nasal intonation. He was referred to the otorhinolaryngology clinic, where it was found that his soft palate was virtually paralysed, there being only a slight

flicker of movement on the left side. His voice was distinctly nasal, and an enlarged, non-tender lymph node was felt at each angle of the jaw. Apart from this he had no complaints, and showed no abnormal signs. He had never been seriously ill, and had received standard inoculation for diphtheria and poliomyelitis. Towards the end of last summer he had camped with a troop of Boy Scouts for two weeks before the onset of his palatal paralysis, and during that time he had bathed in the river Tame. None of his companions at the camp was adversely affected. Within a week of being seen in out-patients his difficulty in swallowing disappeared and only slight right palatal weakness and barely perceptible twang of his voice remained. Soon afterwards the palate appeared to be back to normal, but the enlarged nodes were slow to subside.

Investigations included culture of postnasal swab, which did not produce any pathogens; routine complement fixation tests in the virus laboratory (not including those for the Coxsackie viruses), which showed no abnormalities; and culture of a sample of stool, which yielded Coxsackie virus type A9.