The Pathogenesis of Arthritis Associated with Acute Hepatitis-B Surface Antigen-Positive Hepatitis

COMPLEMENT ACTIVATION AND CHARACTERIZATION OF CIRCULATING IMMUNE COMPLEXES

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ABSTRACT Circulating immune complexes were identified in cryoproteins isolated from serial samples of serum from six patients with acute viral hepatitis with and without arthritic symptoms. Cryoprecipitates were analyzed for the presence of hepatitis-B surface antigen (HB_sAg) and hepatitis-B surface antibody (anti-HB_s) by hemagglutination inhibition and hemagglutination. Complement components were detected by counter electrophoresis, and immunoglobulins were detected by gel diffusion. HB_sAg, IgG, and IgM were identified in cryoprecipitates from all hepatitis patients, but were higher in concentration in patients with arthritis. Only cryoprecipitates from hepatitis patients with arthritis contained IgA and complement components C3, C4, and C5 as well as IgG and IgM, which disappear with resolution of the arthritis. The subtypes of IgG in these cryoprecipitates were predominantly the complement-fixing IgG1 and IgG3. HB_sAg and anti-HB_s were concentrated several-fold in the cryoprecipitates when compared to the serum concentration. Sequential studies in two patients demonstrated that the initial appearance of anti-HB_s in the cryoprotein complex was associated with the detection in the complex of IgM, suggesting a primary immune response to HB_sAg. The C3 activator fragment (C3A) of the properdin complex was found in fresh serum obtained from three hepatitis patients with arthritis and not in uncomplicated hepatitis. The cryoprecipitable immune complexes from patients with arthritis converted C3PA in fresh normal sera to C3A in vitro whereas cryoprotein isolated from patients with uncomplicated hepatitis had no such effect. Thus, the transient appearance of circulating complement-fixing immune complexes in patients with the arthritis of acute hepatitis is associated with activation of both classical and alternate complement pathways and suggests that they play an important role in the pathogenesis of these serum sickness-like extrahepatic symptoms.

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INTRODUCTION

Acute viral hepatitis is occasionally accompanied by a "serum sickness-like" syndrome characterized by arthralgias, frank arthritis, rash, angioedema, hematuria, and proteinuria (1). These extrahepatic manifestations may present as the dominant early clinical features of hepatitis-B infection (2). Moreover, this symptom complex often abates as the patient becomes jaundiced. An elevated serum glutamic oxaloacetic transaminase enzyme (SGOT)[1] detected during the joint symptoms or rash allows recognition of this syndrome and its relationship to hepatitis (2).

Previous studies have suggested that circulating immune complexes are important in the pathogenesis of the serum sickness syndrome associated with hepatitis B (3–5). We have isolated, purified, and characterized circulating cryoproteins containing immune complexes from patients with the acute arthritis of hepatitis B and compared the composition of these immune complexes to complexes isolated from patients with uncomplicated hepatitis. The role of the classical and alternate complement pathways and their activation by immune com-

[1] *Abbreviations used in this paper:* anti-HB_s, hepatitis-B surface antibody; BSA, bovine serum albumin; HB_sAg, hepatitis-B surface antigen; NHS, normal human serum; PBS, phosphate-buffered saline; SGOT, serum glutamic oxaloacetic transaminase.

TABLE I

Characterization of Cryoprotein Complex in Hepatitis B with Arthritis

	Clinical			Cryoprecipitate								Whole serum	
					Complement			Immunoglobulins					
	Day of study	SGOT	Bili-rubin	Protein*	C3	C4	C5	IgG	IgM	IgA	HB_sAg‡ titer	Anti-HB_s titer	HB_sAg titer	Anti-HI titer
		IU	*mg/100 ml*											
Patient 1														
Angioedema →	1	1,300	12.9	4+	2+	2+	2+	2+	2+	2+	1:32	1:16	1:1,024	0
Rash →														
Arthritis →	3	—	—	3+	2+	+	2+	2+	2+	2+	1:64	1:4	1:1,024	0
	9	800	4.3	2+	2+	0	2+	1+	2+	1+	1:512	0	1:256	0
	14	210	2.7	+	1+	0	0	0	0	0	0	0	0	0
	23	100	1.9	+	1+	0	0	0	0	0	0	0	0	0
	31	42	1.3	+	1+	0	0	0	0	0	0	0	0	0
	44	—	—	ND	0	0	0	0	0	0	0	0	0	0
	109	35	1.0	ND	0	0	0	0	0	0	0	0	0	0
Patient 2														
Arthritis →	1	1,600	2.4	2+	2+	2+	+	+	+	0	0	1:8	0	1:8
	9	800	6.5	2+	2+	2+	+	+	+	+	0	1:4	0	1:8
	16	100	3.1	ND	0	0	0	0	+	0	0	1:8	—	—
	28	56	1.2	ND	0	0	0	0	0	0	0	0	0	1:16
Patient 3														
Rash →	1	800	1.6	+	+	+	+	2+	2+	+	1:4,096	0	1:1,024	0
Arthritis →														
	4	400	1.8	3+	+	+	+	2+	2+	+	1:4,096	0	1:256	0
	6	—	—	2+	0	0	0	2+	2+	0	1:4,096	0	1:2,048	0
	8	370	1.1	3+	+	+	+	2+	2+	+	1:1,024	0	1:128	0
	10	—	—	3+	+	+	+	2+	2+	+	1:512	0	1:32	0
	13	370	3.3	+	+	+	+	2+	2+	+	1:512	0	1:128	0
	15	—	—	2+	+	+	+	2+	2+	0	1:128	0	1:64	0
	17	—	—	3+	+	+	2+	2+	2+	+	1:64	0	1:128	0
Arthritis →														
	22	320	7.3	2+	+	+	2+	+	2+	0	1:2,048	0	1:256	0
	31	1,200	5.8	2+	0	0	0	0	+	0	1:128	0	1:64	0
	39	1,150	7.5	+	0	0	0	0	+	0	1:128	0	1:256	0
	45	380	5.9	ND	0	0	0	0	0	0	1:64	0	0	0
	54	47	2.5	ND	0	0	0	0	0	0	0	1:8	0	0
	60	30	1.3	ND	0	0	0	0	+	0	0	1:16	0	0
	120	25	1.0	ND	0	0	0	0	0	0	0	0	0	1:8

* Cryoprecipitable protein concentration was calculated as the amount of protein measured in the cryoprecipitate resolubilized in 0.5 ml of BSA-containing buffe minus the amount of protein measured in 0.5 ml of the same BSA-containing buffer when no cryoprecipitate was detectable. 4+, 15–20 mg cryoprecipitable protein/0. ml; 3+, 10–15 mg/0.5 ml; 2+, 5–10 mg/0.5 ml; +, 1–5 mg/0.5 ml; ND = < 1.0 mg/0.5 ml.

‡ Measured by hemagglutination and hemagglutination inhibition.

plexes in the pathogenesis of arthritis associated with hepatitis-B infection was studied. Serial studies of the immune complexes were performed and were correlated with the clinical features of the syndrome.

METHODS

Patients. Six patients were studied serially for periods ranging from 2 wk to 4 mo. Three patients had acute hepatitis-B surface antigen (HB_sAg)-positive hepatitis complicated by extrahepatic manifestations of rash, severe polyarthritis, and angioedema, which dominated their clinical presentation. The other three patients had uncomplicated typical acute HB_sAg-positive hepatitis. The clinical features and course of these patients are shown in Tables I and II.

Isolation and analysis of cryoprecipitates. 50 ml of venous blood was drawn into warm Vacutainers, allowed to stand for 45 min at 37°C, and then centrifuged for 15 min at 200 *g*. This serum was incubated at 4°C for 3–7 days to allow maximum precipitation. The cryoprecipitates were centrifuged for 1-h at 4°C at 1,000 *g* and resuspended at 37°C in 10 ml of 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS), pH 7.6, with 0.01% sodium azide. These cryoprecipitates were placed at 37°C overnight to resolubilize the true cryoproteins. After centrifugation at 2,000 *g* for 15 min at 37°C, the supernates were placed at 4°C for an additional 3-day period to allow reprecipitation. The cryoproteins were washed and finally redissolved in 0.5 ml of 1% BSA in PBS buffer with 0.01% sodium azide. These purified cryoproteins showed no reaction in double agar gel diffusion (Ouchterlony) at 37°C against anti-human serum albumin, demonstrating the absence of any detectable nonspecific serum contamination. Finally, serial blood samples were drawn during the arthritic and hepatitis phases of the patients illness and also in convalescence to determine the presence, duration, and time of disappearance of circulating cryoprotein immune complexes.

The samples were then analyzed for immunoglobulins IgG, IgM, and IgA by double diffusion in agar gel at 37°C. Complement components C3, C4, and C5 were assayed by counter-immunoelectrophoresis by the method of Gocke and Howe (6). IgG subtypes were measured by radial

TABLE II

Characterization of Cryprotein Complex in Hepatitis without Arthritis

	Clinical			Cryoprecipitate									Whole serum	
					Complement			Immunoglobulins						
	Day of study	SGOT	Bilirubin	Protein*	C3	C4	C5	IgG	IgM	IgA	HB_sAg‡ titer	Anti-HB_s titer	HB_sAg titer	Anti-HB_s titer
		IU	*mg/100 ml*											
Patient 1	1	450	3.8	—	—	—	—	—	—	—	—	—	1:8	0
	5	700	8.6	—	—	—	—	—	—	—	—	—	1:32	0
	8	970	13.0	+	0	0	0	+	2+	0	1:32	0	1:4	0
	11	2,200	17.4	+	0	0	0	+	2+	0	1:32	0	1:64	0
	19	300	6.2	ND	0	0	0	0	0	0	0	0	0	1:16
	25	150	2.1	—	—	—	—	—	—	—	—	—	0	0
Patient 2	1	2,200	4.6	+	0	0	0	+	+	0	1:256	0	1:64	0
	7	350	2.4	ND	0	0	0	0	0	0	0	0	0	0
	14	200	1.7	ND	0	0	0	0	0	0	0	0	0	0
Patient 3	1	600	1.8	—	—	—	—	—	—	—	—	—	1:256	0
	6	1,660	6.0	+	0	0	0	+	+	0	1:512	0	—	—
	13	—	—	—	—	—	—	—	—	—	—	—	1:28	0
	15	—	—	—	—	—	—	—	—	—	—	—	1:64	0
	20	1,100	3.0	+	0	0	0	+	2+	0	1:16	0	0	0
	36	280	1.1	—	—	—	—	—	—	—	—	—	0	0

* Cryoprecipitable protein concentration was calculated as the amount of protein measured in the cryoprecipitate resolubilized in 0.5 ml of BSA-containing buffer minus the amount of protein measured in 0.5 ml of the same BSA-containing buffer when no cryoprecipitate was detectable. 4+, 15–20 mg cryoprecipitable protein/0.5 ml; 3+, 10–15 mg/0.5 ml; 2+, 5–10 mg/0.5 ml; +, 1–5 mg/0.5 ml; ND, <1.0 mg/0.5 ml.

‡ Measured by hemagglutination and hemagglutination inhibition.

diffusion in agar gel (Mancini). Monospecific antisera to complement components, immunoglobulins, and IgG subtypes were provided by Dr. Peter Schur (Robert B. Brigham Hospital). Protein concentration was measured in the resolubilized cryoprecipitates by the Folin phenol reagent method of Lowry Rosebough, Farr, and Randall (7). The amount of protein in the cryoprecipitate, resolubilized in 0.5 ml of buffer, was measured and the amount of protein in 0.5 ml of the same BSA-containing buffer with no visible cryoprecipitate buffer was deducted to yield the total cryoprecipitable protein concentration.

Activation of the alternate (properdin) complement pathway. Activation of C3 proactivator of the alternate pathway (GBG), in whole sera was determined by the immunoelectrophoretic technique of Scheidegger (8). Fresh normal human serum (NHS) and zymosan-activated NHS served, respectively, as negative and positive controls. Zymosan activation of NHS was achieved by heating 50 mg/ml of zymosan (Sigma Chemical Co., St. Louis, Mo.) in PBS to 100°C for 30 min. Upon cooling, the zymosan was combined with an equal volume of fresh NHS, incubated for 2 h at 37°C and centrifuged at 15,000 *g* for 1 min (pellets were discarded). All serum samples tested were frozen immediately after collection and stored at −70°C until tested. After subjecting control and sample sera to electrophoresis, troughs were filled with anti-C3 activator (Behringwerke AG., Marburg-Lahn, West Germany). Appearance of the C3 activator with gamma mobility, similar to that seen in zymosan-activated serum, indicated activation of C3 proactivator.

Determination of HB_sAg and hepatitis-B antibody (anti-HB_s). Hepatitis-B surface antigen and antibody were determined by hemagglutination inhibition and hemagglutination as described by Vyas and Shulman (9). Hb_sAg-coated human type O Rh-negative red cells were supplied by Electro-Nucleonics (Bethesda, Md.) and titers determined by serial two-fold dilutions using microtiter plates. Titers are expressed as the number of the last well in the plate that showed either an agglutination or inhibition of agglutination pattern. Positive and negative controls for HB_sAg, human type O Rh-negative control cells, and titration of standard anti-HB_s (at 4 agglutinating units) were employed to assure reproducibility. A titer of HB_sAg and anti-HB_s of 1:4 or greater was considered positive.

RESULTS

Characterization of the cryoprotein complex in hepatitis-B patients with arthritis. The protein concentration of immune complexes isolated from patients with hepatitis was highest in those with arthritis and decreased in concentration with resolution of symptoms. Anti-HB_s was detected in all patients with arthritis but was concentrated several-fold in the cryoprotein complex as compared to the whole serum. Anti-HB_s appeared in the cryoprotein complex *before* detectable serum concentrations were reached in patients 1 and 2. Sequential studies in two patients demonstrated that the initial appearance of anti-HB_s in the cryoprotein complex was associated with the appearance of IgM. HB_sAg was also concentrated several-fold in the cryoprotein complex obtained from patients with the arthritis of hepatitis.

Immune complexes consisting of immunoglobulins, anti-HB_s, and HB_sAg, with complement components C3, C4, and C5 were detected during the arthritic phase of patient 1 (Table I). These circulating immune complexes disappeared with resolution of arthritis. All three classes of immunoglobulin, HB_sAg, or anti-HB_s, and

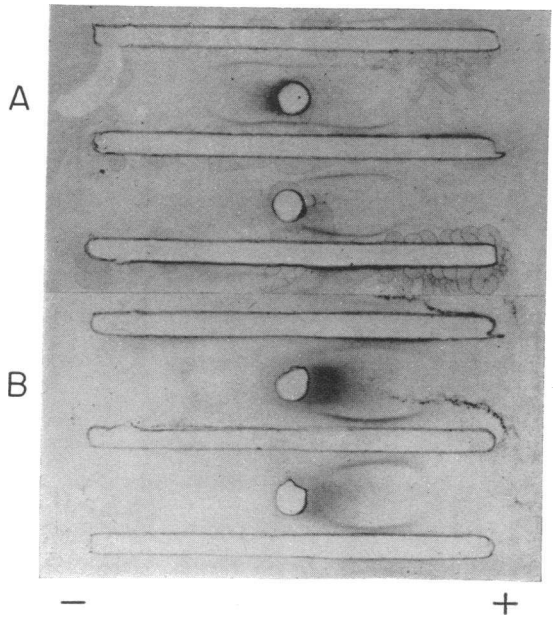

FIGURE 1 (A) C3PA activation in vivo by demonstrating the C3 activator fragment with gamma electrophoretic mobility in serum obtained from patients with hepatitis and arthritis (top). C3 activator fragment was undetectable during convalescence (bottom). (B) C3 activator fragment was also undetectable during the acute phase (top) and during convalescence in patients with uncomplicated hepatitis (bottom).

complement components C3, C4, and C5 were identified in all three patients with arthritis (Table I).

Characterization of the cryoprotein complex in hepatitis-B patients without arthritis. Circulating cryoproteins were also isolated from patients with uncomplicated hepatitis (Table II). These complexes were composed of IgM, IgG, and HB$_s$Ag and disappeared with resolution of hepatitis. In contrast to patients with hepatitis and arthritis, however, IgA and complement components, C3, C4, and C5 were undetectable. Furthermore, the total protein concentration of immune complexes in these patients was lower when compared to arthritis patients.

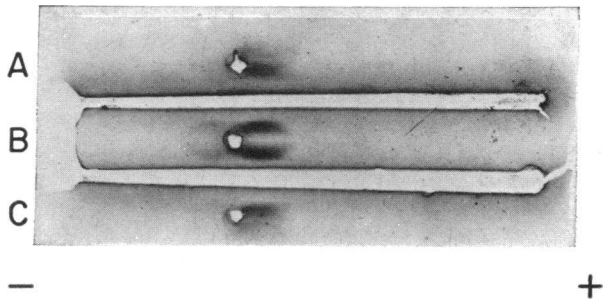

FIGURE 2 Activation of the alternate (properdin) complement pathway in vitro by incubating immune complexes with fresh NHS. (A) Immune complex obtained from a patient with uncomplicated hepatitis (no activation). (B) Immune complexes obtained from a patient with hepatitis complicated by arthritis and rash. Note the appearance of the C3 activator fragment from C3PA. (C) Fresh NHS served as a control.

Determination of IgG subtypes in the cryoprotein complex. The subtypes of IgG in the circulating immune complexes were quantitatively measured by radial diffusion using specific antisera against IgG1, IgG2, IgG3, and IgG4. Table III shows that the subtypes of IgG in the immune complexes obtained from patients with hepatitis and arthritis were predominantly the complement-fixing IgG1 and IgG3. In contrast, there was no consistent pattern of IgG subtypes in immune complexes obtained from patients with uncomplicated hepatitis. Furthermore, the IgG concentration in these complexes was less than in those obtained from patients with arthritis and hepatitis.

In vivo activation of the alternate (properdin) complement pathway. Alternate pathway complement activation was demonstrated in all three patients with hepatitis and arthritis, by the identification of the C3 activator fragment (GGG) of C3 proactivator (properdin factor B or GBG) in acute phase serum. Fig. 1 depicts the C3 activator with gamma electrophoretic mobility in sera from patients only with hepatitis and arthritis and not in sera from patients with uncomplicated hepatitis.

TABLE III
IgG Subtypes in the Cryoprotein Complexes

	Hepatitis with arthritis				Hepatitis without arthritis			
Patient	IgG1	IgG2	IgG3	IgG4	IgG1	IgG2	IgG3	IgG4
	mg/ml				*mg/ml*			
1	0.21 (3)*	0	0.10	0	0.10 (8)	0	0.10	0
2	0.23 (9)	0	0.10	0	0.142 (1)	0.10	0.10	0.16
3	0.33 (8)	0.143	0.10	0	0.10 (20)	0	0	0.185

* Day of study (Tables I and II)

In vitro activation of the properdin complement pathway. Alternate pathway activation by isolated immune complexes was tested in vitro, since the alternate pathway was activated in vivo only when detectable immune complexes were present. Fresh serum (0.5 ml) was obtained from a healthy donor and incubated for 1 h at 37°C with 100 μl of solubilized cryoprotein complex obtained from a patient with uncomplicated hepatitis (containing HB$_s$Ag, IgG subtypes 1 and 4, and IgM) and from a patient with arthritis (containing HB$_s$Ag, IgM, IgG subtypes 1 and 3, IgA, C3, C4, and C5). Fresh NHS served as a control. The incubation mixture was then electrophoresed and troughs were filled with anti-C3 activator. Fig. 2 shows the appearance of C3 activator fragment of C3PA in fresh NHS when incubated with immune complexes in cryoprecipitates obtained from patients with arthritis and hepatitis, demonstrating alternate pathway activation in vitro. However, C3 activator was undetectable when fresh NHS was incubated with cryoproteins obtained from patients with uncomplicated hepatitis.

DISCUSSION

Circulating immune complexes are important in the pathogenesis of rash, arthritis, and angioedema associated with antigen-antibody-induced experimental serum sickness (10). Evidence for a similar pathogenesis in the serum sickness associated with hepatitis in man includes: (*a*) low serum (CH_{50}, C3 and C4) and joint fluid (CH_{50}) complement values have been detected during the active arthritic phase with return of depressed levels to normal when joint symptoms are no longer present (3–5). (*b*) Hepatitis-B antigen titers were highest during joint symptoms consistent with the presence of soluble circulating immune complexes in antigen excess (4, 11). Furthermore, HB$_s$Ag was present in joint fluid in association with depressed joint fluid complement (5). (*c*) Finally, the early appearance of anti-HB$_s$ in the serum was observed at the time of arthritis (5). Thus, these studies suggest that circulating immune complexes composed of HB$_s$Ag in antigen excess and anti-HB$_s$ with subsequent consumption of complement components were responsible for the serum sickness-like syndromes occasionally observe in hepatitis-B infection.

Immune complexes composed of HB$_s$Ag immunoglobulin, and complement have also been identified by immunoflourescent techniques in HB$_s$Ag-positive patients with polyarteritis nodosa and glomerulonephritis (12–14). Infusion of high-titer anti-HB$_s$ into patients with HB$_s$Ag-positive chronic active hepatitis produced a fall in HB$_s$Ag serum titers and transient hematuria but no arthritis or rash (15). Chromatography on DEAE-cellulose was used for dissociation of immune complexes obtained from sera of patients with uncomplicated acute hepatitis. Hepatitis-B antigen, anti-HB$_s$, IgG, IgM, and IgA were separated by this technique, but complement components were undetectable (16). More recently, circulating immune complexes were identified by cryoprecipitation in patients with acute hepatitis (17). There is, however, little information concerning the isolation, quantitation, and characterization of these complexes and their relationship to clinical symptoms in man.

Our sequential study in patients with hepatitis-B infection demonstrates that circulating immune complexes are present in hepatitis patients both with and without arthritis. The physical characteristic of cryoprecipitation has allowed us to isolate, purify (e.g., no serum albumin), and characterize the components in circulating cryoprotein-immune complexes. More importantly, the presence, composition, and concentration of these circulating immune complexes correlates with the clinical findings of rash, arthritis, and angioedema, which strongly suggests an etiological relationship. Although circulating immune complexes are present in both acute hepatitis with and without arthritis, their composition differs significantly in several important respects. First, complement components C3, C4, and C5 and IgA were detected *only* in cryoproteins isolated from patients with arthritis complicating hepatitis, and the IgG subtypes in these immune complexes were predominantly the complement-fixing IgG1 and IgG3 (18). Secondly, the IgG concentration of immune complexes was greater in patients with acute hepatitis B complicated by the serum sickness-like syndrome. Finally, anti-HB$_s$ was detected in the cryoprotein complex in all patients with arthritis. Indeed, anti-HB$_s$ appeared before detectable serum levels and was concentrated several-fold when compared to serum titers. Of particular interest were the sequential studies in two patients demonstrating that the initial appearance of anti-HB$_s$ in the cryoprotein complex was associated only with detectable IgM, suggesting the primary immune response to HB$_s$Ag (19, 20).

The classical complement system is composed of a group of serum proteins that interact sequentially to form a series of active enzyme products. The biological consequences of the complement-reaction sequence include: (*a*) inflammation mediated by increased vascular permeability and attraction of polymorphonuclear leukocytes, (*b*) viral neutralization, (*c*) immune adherence promoting phagocytosis, and (*d*) alteration in cell membranes leading to cell lysis. The products of the complement activation sequence have been studied extensively in vitro but conclusions about their in vivo significance remains to be determined (21).

An alternate pathway of complement activation (properdin) has been described that bypasses the early

components (C1, C4, and C2) of the classical pathway (22). This system is composed of several unique serum proteins, and activation in vitro is detectable by the serological identification of the C3 activator fragment of C3 proactivator (C3PA, properdin factor B, or GBG). C3 activator then activates C3, splitting it into several fragments (21, 23, 24). Thus, the terminal components of the classical pathway may be sequentially activated in this manner and produce the known in vitro biological consequences of the reaction (24).

Recent studies have emphasized the importance of alternate complement pathway activation in the pathogenesis of several diseases in man. Alternate complement pathway activation was demonstrated in sera of patients with hypocomplementemic mesangiocapillary glomerulonephritis and in blister fluid of patients with pemphigus vulgaris (25–27). Depressed serum levels of C3 with normal levels of the earlier components were detected in patients with acute poststreptococcal glomerulonephritis and chronic membranoproliferative glomerulonephritis, and C3 and properdin factor B has been identified along the glomerular basement membrane in such patients suggesting complement activation through the alternate pathway (28). Furthermore, a defect in alternate complement pathway activation was reported in children with sickle cell anemia associated with reduced bacterial opsinization (29).

Preformed antigen-antibody immune complexes activate both the alternate and classical complement pathways in vitro (21, 30). We have demonstrated that circulating immune complexes in man may activate both the classical and alternate complement pathways in vivo since C4, an early component of the complement sequence, was found in the cryoprotein immune complex, and the C3 activator fragment of the properidin complex was demonstrable in the serum only from patients with arthritis and not in uncomplicated hepatitis. Furthermore, purified immune complexes obtained from a patient with arthritis activated the alternate complement pathway in normal serum in vitro. Although cryoproteins composed of HB$_s$Ag, IgG, and IgM were detected in patients with uncomplicated hepatitis, we could not demonstrate classical and alternate complement pathway activation either in vivo or in vitro. Immune complexes may not activate the alternate and classical complement pathways under these circumstances because of the absence of specific complement-fixing IgG subtypes, specific antibody (anti-HB$_s$), or inappropriate antigen-antibody ratio in the circulating cryoprotein immune complex. The presence of IgA in the cryoprotein complex, found only in the patients with arthritis, may also be important, since unaggregated natural polymers of IgA are thought to be weak activators of the alternate complement pathway in vitro (22).

These serial studies demonstrate the transient appearance of circulating immune complexes composed of IgG (complement-fixing subtypes IgG1 and IgG3) IgM, IgA, C3, C4, C5, HB$_s$Ag, and anti-HB$_s$ with activation of both classical and alternate complement pathways in patients with arthritis and acute hepatitis. These immune complexes disappear with resolution of joint symptoms and are probably responsible for the serum sickness syndrome occasionally complicating viral hepatitis.

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