INFECTION AND IMMUNITY, June 1980, p. 842-845
0019-9567/80/06-0842/04$02.00/0

Vol. 28, No. 3

Serum Immunoconglutinin Titers During Acute and Chronic Hepatitis B Virus Infection

G. M. MAKHDOOMI,[1] M. L. TIKU,[1] K. R. BEUTNER,[2] AND P. L. OGRA[3]*

Departments of Pediatrics[1] and Microbiology,[2] State University of New York at Buffalo, and Division of Infectious Diseases, Children's Hospital,[3] Buffalo, New York 14222

Sera from 99 chronic hepatitis B surface antigen carriers, 12 individuals with acute type B hepatitis, 26 hepatitis B surface antibody-seropositive subjects, and 50 hepatitis B surface antigen, hepatitis B surface antibody-seronegative subjects were evaluated for the presence of serum immunoconglutinins (IKs). The mean serum IK titers of hepatitis B surface antibody-seropositive and hepatitis B virus-seronegative subjects were 5.3 and 4.9, respectively. The IK titers of subjects with acute and chronic hepatitis B virus infections were 215.4 and 19.1, respectively. These groups also manifested IK titers greater than or equal to ≥ 16 significantly ($P < 0.005$) more often than controls did. Among chronic hepatitis B surface antigen carriers, high IK titers were associated with low levels of hepatitis B surface antigen. IK titers of individuals chronically infected with hepatitis B virus and having the rheumatoid factor were similar to those of individuals without the rheumatoid factor. Elevated IK titers represent a physiological autoimmune response and may indicate the presence of immune complexes in acute and chronic hepatitis B virus infection.

In the 1930s, Streng (18) and Wartiovaara (23) originally described immunoconglutinin (IK) activity in serum. Subsequently, the IK phenomenon was studied in great detail, as reviewed by Coombs et al. (5) and Lachmann (12). IK antibodies react specifically with fixed third or fourth components or both of the complement system, (5, 10, 12, 13). Because of this specificity for autologous antigens, IK antibodies are considered autoantibodies.

Elevated serum IK titers have been reported after infection (5, 9, 12) and immunization (5, 8, 12), as well as in a number of diseases of presumed autoimmune etiology (3). It is thought that antibodies to the infecting organism, immunizing antigen, or autologous antigen result in the formation of antigen-antibody complexes. These immune complexes fix complement. Subsequent activation of the complement cascade results in an alteration of complement components, which become immunogenic and stimulate the formation of IK antibodies.

Passively and actively acquired IK antibody activity has been shown to enhance immunity to bacterial infection (9) and to accelerate the termination of bacteremia (5). These characteristics have resulted in IK antibodies being considered physiological autoantibodies.

In chronic bacterial infections serum IK titers remain elevated (5). This is thought to reflect the persistence of antigen and hence immune complexes in chronic infections. Little is known about the IK response in chronic viral infections. Hepatitis B virus (HBV) results in acute and chronic infections in humans. The present study was undertaken to study serum IK responses in humans with acute and chronic HBV infections.

MATERIALS AND METHODS

The study population (Table 1) consisted of the following groups of subjects: group 1, 99 chronic hepatitis B surface antigen (HB_sAg) carriers; group 2, 26 subjects seropositive for hepatitis B surface antibody (anti-HB_s); group 3, 12 subjects with acute type B viral hepatitis; and group 4, 50 healthy control subjects who were HBV seronegative (seronegative for HB_sAg and anti-HB_s). A chronic HB_sAg carrier was defined as an individual who was HB_sAg seropositive for greater than 6 months. The diagnosis of acute HBV infection was made based on clinical features, abnormal liver function tests, and the transient presence of HB_sAg in the serum. Subjects in groups 1, 2, and 4 were from the Hepatitis Study Center of an institution for the mentally handicapped. The HBV serological status of these residents has been studied intensively over several cross-sectional and longitudinal epidemiological surveys for a period of 4 to 5 years. Group 3 subjects were recruited from the infectious disease services of local community hospitals. The median ages of the groups were similar. The HBV infection in these patients was related to accidental inoculation by HBV-contaminated needles in a hospital setting or prolonged contact with an HBV-infected spouse or sexual partner. None of these patients had a history of blood transfusion, drug addiction, or homosexuality. The HBV-seronegative group consisted of approximately equal numbers of males and females. However,

TABLE 1. *Study population, geometric mean serum IK titers, and frequency of IK titers ≥16*

Group tested	No. tested	% Male	% Female	Median age (range)	Geometric mean IK titer	% of subjects with IK titer ≥16
1. Chronic HB_SAg carriers	99	86	14	22 (9–30)	19.1	64[a]
2. Anti-HB_S seropositive	26	77	23	29 (5–58)	5.3	19
3. Acute type B hepatitis	12	67	33	26 (6–31)	215.4	100[a]
4. HBV seronegative	50	44	56	25 (4–62)	4.9	10

[a] $P < 0.005$ by the chi-square test as compared with anti-HB_S seropositive and HBV seronegative groups.

the chronic HB_sAg carriers and the acute type B hepatitis- and anti-HB_s-seropositive groups consisted of more males than females. Specimens of 10 ml of blood were collected, allowed to clot at room temperature for half an hour, and then centrifuged for 10 min. The serum was collected and stored at −70°C before testing. Serum HB_sAg was detected by a solid-phase radioimmunoassay (15) (Ausria II; Abbot Laboratories, North Chicago, Ill.). Samples were also tested for HB_sAg by counterimmune electrophoresis (CEP) (6) employing commercially available horse anti-HB_s (Hyland Laboratories, Inc., Costa Mesa, Calif.). Passive hemagglutination (20) (Electro Nucleonics Inc., Bethesda, Md.) was employed for the detection of anti-HB_s. The rheumatoid factor was detected by latex fixation (Hyland Laboratories). Serum IK titers were determined by the sedimentation method described by Lachmann (11, 14). Alexinated indicator cells were prepared by treating sheep erythrocytes with a subagglutinating dilution of purified rabbit immunoglobulin M anti-sheep erythrocytes (Cordis Laboratories) and then reacting the cells with zymosan-activated fresh normal human serum. Serum samples to be tested for IK activity were heat inactivated for 30 min at 56°C and absorbed with an equal volume of packed sheep erythrocytes overnight at 4°C. Serial twofold dilutions of the heat-inactivated absorbed serum samples were made in U-bottom microtiter (Cook Laboratory Products) plates with Vernol-buffered saline as a diluent. To each serum dilution 25 μl of a 1% suspension of the alexinated cells was added. After overnight incubation at 4°C, the plates were read for agglutination. The IK titer was defined as the reciprocal of the highest serum dilution exhibiting agglutination.

Serum glutamic pyruvic transaminase (SGPT) levels were determined with commercially available kits (Statzyme GPT; Worthington Biochemicals Corp., Freehold, N.J.). SGPT levels were expressed in international units at 30°C. The upper limit of normal in our laboratory was considered as 30 IU.

RESULTS

The individual and mean serum IK titers are presented in Fig. 1. HBV-seronegative and anti-HB_s-seropositive subjects manifested IK titers ranging from 2 to 64. The IK titers of these individuals were most frequently observed between 2 and 8. IK titers of greater than 32 were consistently observed in subjects with acute type B hepatitis. The IK titers of the chronic HB_sAg carriers ranged from 4 to 128.

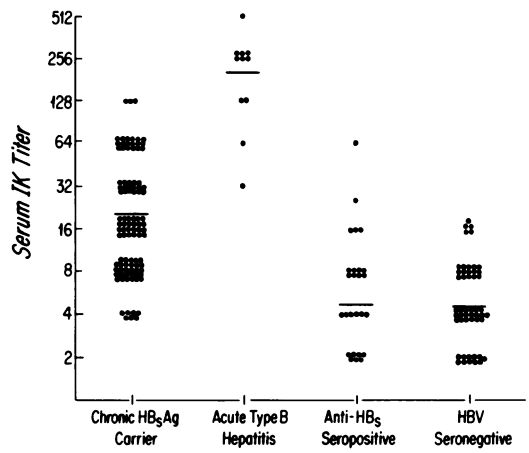

FIG. 1. *Distribution of individual IK titers and geometric mean (——) IK titers of sera obtained from various groups.*

The geometric mean IK titers of the chronic HB_sAg carriers and subjects with acute type B hepatitis were 19.1 and 215.4, respectively. Mean IK titers of 5.3 and 4.9 were observed in the anti-HB_s-seropositive and HBV-seronegative control groups, respectively. The frequency of IK titers ≥16 was significantly ($P < 0.005$) greater in chronic HB_s-Ag carriers (64 out of 99) and acute type B hepatitis (12 out of 12) subjects as compared with anti-HB_s-seropositive (5 out of 26) and HBV-seronegative (5 out of 50) subjects.

The anti-HB_s-seropositive, 50 randomly selected subjects from the chronic HB_sAg carrier group, and 34 randomly selected HBV-seronegative subjects were also evaluated for the presence of the rheumatoid factor. Anti-HB_s-seropositive subjects and HBV-seronegative subjects failed to manifest the rheumatoid factor activity. Approximately 38% (19 out of 50) of the chronic HB_sAg carriers manifested the rheumatoid factor. The geometric mean IK titers and the frequency of IK titers of ≥16 were 21 and 74% in chronic HB_sAg carriers with positive rheumatoid factor and 13 and 58% in chronic HB_sAg carriers without the rheumatoid factor, respectively.

All chronic HB_sAg carriers were positive for HB_sAg by the radioimmunoassay, but only 61%

were positive for HB_sAg by CEP. In this respect, it is important to note that 50% (31 out of 63) of the chronic HB_sAg carriers with IK titers ≥ 16 were CEP negative as compared with 22% (8 out of 36) of those subjects with IK titers <16 ($P < 0.01$).

The 12 individuals with acute infection were tested serially over the course of their illness. Of these 12 subjects 11 experienced acute infection followed by complete resolution, and 1 subject subsequently became a chronic HB_sAg carrier. In this individual HB_sAg antigenemia and abnormal liver function (SGPT > 30 IU) persisted over the 38 weeks of testing. The results of serial testing of a representative subject with acute HBV infection and the subject who became a chronic HB_sAg carrier are presented in Fig. 2. Transient IK responses were observed with acute HBV infection followed by resolution. IK titers declined after SGPT levels returned to normal but before the termination of antigenemia. In the subject who became a chronic HB_sAg carrier high IK titers, elevated SGPT levels, and HB_sAg antigen persisted for the entire 38 weeks of follow-up.

DISCUSSION

It is important to note that the present study employed a sedimentation method rather than a resuspension method. This would account for the universal appearance of IK activity in normal subjects. With a resuspension method lower IK titers and a lower incidence of IK activity have been noted in normal individuals (5). The low IK titers observed in control (anti-HB_s-seropositive and HBV-seronegative) subjects may represent residual IK antibody from periodic infections which all individuals experience. Coombs et al. (5) serially followed normal subjects over a period of 1 year and demonstrated seasonal variations in IK titers. These variations were attributed to the seasonal occurrence of infections.

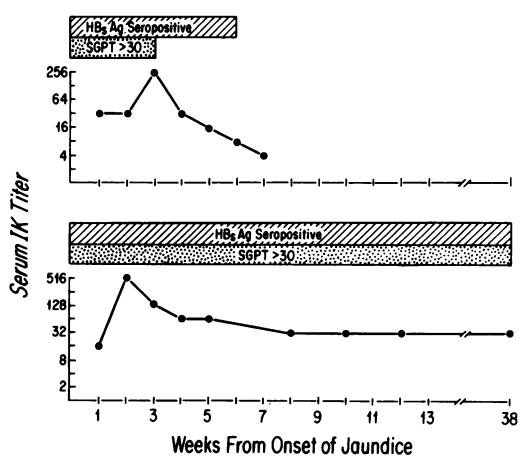

FIG. 2. *Duration of HB_sAg antigenemia, elevated alanine aminotransferase levels, and serum IK titers of two subjects. The upper panel is a representative case of acute HBV infection followed by resolution. The lower panel depicts a subject who presented with acute hepatitis and subsequently became a chronic HB_sAg carrier.*

HBV infection in humans results in a broad spectrum of illness ranging from acute viral hepatitis to an asymptomatic chronic infection. In addition to hepatic disease, immune complex-mediated extrahepatic manifestations of HBV infection have also been noted, including polyarteritis (7), glomerulonephritis (4), polymyalgia rheumatica (2), cryoglobulinemia (16), and a serum sickness-like prodrome (1, 21). Immune complexes consisting of HB_sAg and anti-HB_s have been implicated in hepatic and extrahepatic manifestations of HBV infection (17). Circulating and tissue-bound HB_sAg-anti-HB_s immune complexes have been demonstrated during acute and chronic HBV infections (16, 19, 22).

The highest IK titers were noted during acute HBV infections. IK activity observed during acute HBV infections may be an epiphenomenon related to the occurrence of immune complexes during the acute phase. Alternatively, IK antibody activity may play a role in the termination of the viremia. In experimental animals, actively or passively acquired IK activity has been shown to accelerate the rate at which salmonella organisms are cleared from the blood (5). There is no evidence to indicate that IK antibodies play a primary role in the termination of HBV infection. However, accelerated clearance of immune complexes may alter the infection by preventing or reducing their tissue deposition and associated immunopathology.

In acute HBV infections, the replication of HBV in hepatocytes results in viremia and antigenemia. Following the formation of HBV-specific antibody, immune complexes are formed which fix complement (1). The altered complement components stimulate the formation of IK antibodies (3). Thus, it is proposed that after or coincident with the termination of the HBV infection, the IK antibody may accelerate the termination of the HB_sAg antigenemia or prevent deposition of immune complexes or both.

Chronic HBV infection results in a persistent antigenemia and the continued presence of immune complexes which stimulate IK antibody. In experimental chronic infections with bacteria, IK titers have been shown to remain elevated (5). In chronic HBV infections, the presence of IK activity may represent a serological indicator of the persistence of immune complexes. Chronic HB_sAg carriers fail to manifest detectable levels of anti-HB_s. The antigenemia observed in

chronic HBV infection is massive, being as great as 10^{13} HB_sAg particles per ml. In light of such an antigenemia it is possible that the anti-HB_s response is undetectable by conventional serological procedures. Serum anti-HB_s in infected subjects may only exist as HB_sAg-anti-HB_s complexes. By cryoprecipitation HB_sAg-anti-HB_s immune complexes have been demonstrated in the serum of patients with chronic HBV infection (19). Alternatively, an antigen-antibody system other than the HB_sAg-anti-HB_s may provide the stimulus for IK antibody formation. Possible candidates for this role include hepatitis B core antigen-hepatitis B core antibody, hepatitis B e antigen-hepatitis B e antigen antibody, and liver-specific antigen-liver-specific antibody systems.

The rheumatoid factor is another autoantibody associated with persistent antigenic exposure. In the present study this factor was observed in the serum of 38% of subjects with chronic HBV infection but was absent in control subjects. The frequency of IK titers $\geq$16 and mean IK titers were similar in rheumatoid factor-positive and -negative chronically infected subjects. This would indicate that IK activity is distinct from the rheumatoid factor.

The sensitivity of CEP for the detection of HB_sAg is relatively low, being 6- to 20-fold less sensitive than the radioimmunoassay (15). Thus, a sample positive for HB_sAg by the radioimmunoassay but negative by CEP has a relatively lower quantity of HB_sAg than a CEP-positive sample. In this manner CEP can serve as a crude method for quantitating HB_sAg. Chronic HB_sAg carriers with IK titers $\geq$16 had a significantly higher prevalence of CEP-negative sera than chronically infected subjects with IK titers <16. This would indicate that with chronic HBV infection high IK titers were associated with lower serum HB_sAg levels (higher incidence of CEP negative). This is consistent with the known ability of IK antibodies to clear antigen (5).

The present study has demonstrated elevated IK titers in humans with acute and chronic HBV infections. The IK response represents a physiological autoimmune response which may be a serological marker for the presence of immune complexes.

ACKNOWLEDGMENTS

This research was supported in part by Public Health Service research contracts and grants from the National Institute of Allergy and Infectious Diseases (AI-32511, AI-42522) and the National Institute of Child Health and Human Development (HD-10088).

LITERATURE CITED

1. **Alpert, E., K. J. Isselbacher, and P. H. Shur.** 1971. The pathogenesis of arthritis associated with viral hepatitis. N. Engl. J. Med. **285**:185–189.
2. **Bacon, P. A., S. M. Doherty, and A. J. Zuckerman.** 1975. Hepatitis B antibody in polymyalgia rheumatica. Lancet **ii**:476–478.
3. **Bienenstock, J., and K. J. Bloch.** 1967. Immunoconglutinin in various rheumatic diseases and certain diseases suspected of an autoimmune pathogenesis. Arthritis Rheum. **10**:187–198.
4. **Combes, B., P. Stastny, and J. Shorey.** 1971. Glomerulonephritis with deposition of Australia antigen-antibody complexes in glomerular basement membrane. Lancet **ii**:234–237.
5. **Coombs, R. R. A., A. M. Coombs, and D. G. Ingram.** 1961. The serology of conglutination. Blackwell Scientific Publications, Oxford.
6. **Gocke, D. J., and C. Howe.** 1970. Communications: rapid detection of Australia antigen by counterimmunoelectrophoresis. J. Immunol. **104**:1031–1034.
7. **Gocke, D. J., K. Hsu, and S. Bombardieri.** 1970. Association between polyarteritis and Australia antigen. Lancet **ii**:1149–1153.
8. **Henson, P. M.** 1967. Antibody responses to bacteria: the effect of the immunoglobulin stimulation in rabbits and guinea pigs. Immunology **13**:261–274.
9. **Ingram, D. G.** 1959. The conglutination phenomenon. XIV. The resistance enhancing effect of conglutinin and immunoconglutinin in experimental bacterial infections. Immunology **2**:334–345.
10. **Lachmann, P. J.** 1962. A comparison of some properties of bovine conglutinin with those of rabbit immunoconglutinin. Immunology **5**:687–705.
11. **Lachmann, P. J.** 1966. A sedimentation pattern technique for measuring conglutinin: its application to demonstrating immunoconglutinins to C_4. Immunology **11**: 263–271.
12. **Lachmann, P. J.** 1967. Conglutinin and immunoconglutinins. Adv. Immunol. **6**:479–527.
13. **Lachmann, P. J., and R. R. A. Coombs.** 1965. Complement. Ciba Found. Symp., p. 242.
14. **Lachmann, P. J., and R. Liske.** 1966. The preparation and properties of alexinated intermediates that react with conglutinin. II. Equine, rabbit and human complement. Immunology **11**:255–262.
15. **Ling, C. M., and L. R. Overby.** 1972. Prevalence of hepatitis B virus antigen as revealed by direct radioimmune assay with ^{125}I-antibody. J. Immunol. **109**:834–841.
16. **McIntosh, R. M., M. N. Koss, and K. J. Gocke.** 1976. The nature and incidence of cryoproteins in hepatitis B antigen (HB_sAg) positive patients. Q. J. Med. **45**:23–38.
17. **Nowoslaski, A., K. Kravcynyi, T. Nazarewics, and J. Clusarczyk.** 1975. Immunopathological aspects of hepatitis type B. Am. J. Med. Sci. **270**:229–239.
18. **Streng, O.** 1930. Uber Antikomplemente und Immunokonglutinine. Acta Pathol. Microbiol. Scand. **5**(Suppl.): 41.
19. **Tiku, M. L., K. R. Beutner, and P. L. Ogra.** 1979. Immunologic aspects of cryoprecipitates from the sera of chronic HB_sAg carriers. Clin. Exp. Immunol. **36**:54–62.
20. **Vyas, G. N., and N. R. Shulman.** 1970. Hemagglutination assay for antigen and antibody associated with viral hepatitis. Science **170**:332–333.
21. **Wands, J. E., E. Mann, E. Alpert, and K. J. Isselbacher.** 1975. The pathogenesis of arthritis associated with acute hepatitis B surface antigen-positive hepatitis. J. Clin. Invest. **55**:930–936.
22. **Wands, J. R., E. Alpert, and K. J. Isselbacher.** 1973. Arthritis associated with chronic active hepatitis. Complement activation and characterization of circulating immune complexes. Gastroenterology **69**:1286–1291.
23. **Wartiovaara, T. W.** 1932. Uber die Entwicklung der konglutinierenden Egenschaft ber der Immunisierung. Acta Soc. Med. Fenn. Duodecim Ser. B **14**:1–141.