The Etiology of Chronic Active Hepatitis in Korea

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In a study of apparently normal, healthy Korean Army recruits performed in 1962, we found that 42 of
1,906 screened subjects had elevations of their serum glutamic pyruvate transaminase. Liver biopsies were
obtained from 32 of these subjects and 9 of these had a "novel" antigen present, which reacted specifically
with a convalescent serum from a case of serum hepatitis. We have recently tested frozen serum obtained
from 8/9 of these cases and found that all 8 had HBsAg in their serum which, in some cases, persisted for at
least three months. We reviewed the histological specimens from the original 32 cases using newly defined
criteria: 18 were diagnosed as chronic active hepatitis and the 8 HBsAg positive cases with the "novel"
antigen were in this group. In four of these cases the lesion appeared to progress to cirrhosis during a 3-4
month follow-up period. Since none of the cases had a prior history of hepatitis and no symptoms developed
during the follow-up period, our findings emphasize the significance of chronic hepatitis B virus carrier state
in the etiology of cryptogenic cirrhosis.

INTRODUCTION

In February of 1962 a large-scale serum transaminase survey was carried out
among 1,906 Korean army recruits in a training center in Nonsan, South Korea [1].
Serum glutamic pyruvic transaminase (SGPT) elevations of greater than 50 Sigma
units were found in 42 subjects, 32 of whom were then hospitalized for an average
period of three months. During this time extensive clinical and laboratory investiga-
tions, including three needle liver biopsies, were performed on each patient. These
studies documented a syndrome of subclinical chronic active hepatitis characterized
histologically in the majority of cases by what is now generally termed "chronic active
hepatitis" [2-4]. In four cases subclinical progression to cirrhosis was noted in the
course of serial biopsies.

In 1963 a similar but larger study confirmed the high frequency of this syndrome in
clinically well Oriental populations and further documented the progression of this
disease to typical cryptogenic cirrhosis [5].

To investigate the etiology of this syndrome, Prince, Fuji, and Gershon carried out
immunohistochemical studies on liver biopsies obtained from these patients. These
studies identified hepatitis B associated antigen(s) in the liver cells of nine of the 32
biopsied cases [6]. Antigen was detected by the immunofluorescent complement

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fixation technique, utilizing a pool of convalescent serum from Korean cases of icteric hepatitis as a presumptive source of antibody. It was suggested that the subclinical chronic active hepatitis syndrome was a manifestation of the hepatitis B virus carrier state. However, the possibility that these findings reflected unusual autoantibodies, and that this disease may have been caused by chronic chemical injury, such as that which might be caused by ingestion of mycotoxins, could not be totally excluded at that time.

Due to the lack of a less cumbersome immunologic test for hepatitis B virus (HBV), publication of the above work excited neither great interest nor attempts at independent confirmation. However, the availability of new tests for hepatitis B associated antigens, i.e., the hepatitis B surface antigens (HBsAg) and the hepatitis B core antigen (HBeAg), provided new approaches for reinvestigation of this problem.

Utilizing human and animal antisera-containing antibody against HBsAg (anti-HBs), several workers have investigated the localization of this antigen in livers of patients with type B hepatitis or with the chronic hepatitis B virus carrier state [7–11]. The antigen localization described by most of these workers was strikingly similar to that which had been observed in the Korean study in 1962.

Recently we have had an opportunity to re-examine the 14 remaining original serum specimens of the Korean patients with chronic active hepatitis, including nine in whom cellular localization of the putative hepatitis B specific antigen had been demonstrated.

The present report will review the findings of the Korean study utilizing histologic classification for chronic liver disease, which have been elucidated subsequent to our original publications. We will provide the results of testing the available sera for the hepatitis B related antigens [12–15] and antibodies, i.e., HBsAg and anti-HBs, and antibody to the hepatitis B core antigen (anti-HBe). The report will document that more than half of the cases of chronic anicteric hepatitis in the Korean study had subclinical chronic active hepatitis associated with the chronic hepatitis B virus carrier state.

MATERIALS AND METHODS

Patients

Patients were selected from 1,906 Korean Army recruits on the basis of results obtained by testing of freshly drawn serum for SGPT as described in detail previously [1]. Thirty-two recruits with SGPT levels greater than 50 Sigma units on two successive bleedings who gave their informed consent were hospitalized for study for an average period of three months. The subjects were males ranging in age from 19 to 32 with an average of 24 years. None had a history of transfusion or illicit or prescribed parenteral drug use.

Immunohistochemical Methods

The detailed immunohistochemical methods employed have been fully described [6]. The immunofluorescent complement fixation technique was employed utilizing a variety of sera as a source for antibody. The initial demonstration of antigen was carried out with a presumptive antibody-containing pool of serum derived from 19 cases of convalescent icteric hepatitis occurring in Korean Army personnel. The specificity of the observed staining was determined in the following manner:

1. Staining required active complement.
PLATE 1. (Case 231) Immunofluorescent staining observed in different areas of the needle biopsy section. A. Nuclear fluorescence (*x 300). B. Nuclear fluorescence (*x 300). The arrow indicates a nucleus containing a crescent-shaped zone of fluorescence which appeared as an amphophilic region when restained with haematoxylin and eosin stain. C. Cytoplasmic staining only (*x 675). D. A focus of cells with both nuclear and cytoplasmic staining (arrow), surrounded by cells with nuclear staining only (circles) (*x 300).
FIG. 1. Serum transaminase in three cases of HB associated chronic aggressive hepatitis. Note transient return of SGPT to normal levels in Case 236.
FIG. 2. Serum transaminase in three cases of HB associated chronic aggressive hepatitis. Note return of SGPT values to normal levels in Case 251 concurrent with development of cirrhosis.
2. Staining was not demonstrated in control liver tissues from patients with normal livers or from patients with acute or chronic liver disease suspected, on the basis of clinical and epidemiologic data, not to be due to hepatitis B infection.

3. Antibody to the demonstrated antigens was not found in sera from cases suspected to be due to infection with hepatitis type A or in conventional gamma globulin. Antibody was, however, found in convalescent serum from a well-documented case of long incubation period post-transfusion hepatitis.

**Serologic Methods**

Sera from the original series of cases which have been stored for 13 years at -70°C were tested for the presence of HBsAg by agar gel diffusion (AGD) [14] and by the Ausria I direct solid phase radioimmunoassay (RIA) [16] according to the instructions of the manufacturers (Abbott Laboratories, Chicago, IL). All positive RIA results were tested for specificity by anti-HBs neutralization [17]. Sera employed as sources of antibody in the original fluorescent antibody experiments were tested for anti-HBs by passive hemagglutination [18] and for anti-HBc by indirect immunofluorescent test [19] utilizing as substrate sections of liver from a chronic HB carrier who died while on renal dialysis from causes unrelated to liver disease.

**RESULTS**

Figures 1 and 2 summarize results of serial transaminase determinations and serial liver biopsies on six of the 12 cases subsequently found to be HBsAg carriers by detection of antigen in their sera.

The predominant pattern was one of continuing transaminase abnormality with occasional fluctuations to normal levels. The histologic findings confirmed the chronic nature of the disease process, even in patients such as Case No. 251 in whom transaminase declined continuously during hospitalization and remained within normal limits for the last six weeks of hospitalization. Serial biopsies in this case revealed chronic active hepatitis with progressive increase in the prominence of fibrous septa. The third biopsy in this case revealed clear cut cirrhosis [3].

Table 1 summarizes the histologic diagnosis from the first biopsies of the 32 hospitalized cases in accordance with currently accepted histologic criteria [4]. Chronic active hepatitis was the predominant histologic picture and was seen in 18 of the 32 cases. Five cases showed chronic persistent hepatitis, four showed non-specific reactive hepatitis, one case showed a histological picture resembling that of acute hepatitis, and in four cases the biopsy was inadequate for accurate classification.
Results of Immunohistochemical Studies

Nine of the 32 cases examined showed focal nuclear (Plate 1A and B) and/or cytoplasmic staining (Plate 1C and D) of hepatic parenchymal cells when stained with the complete staining system, i.e., convalescent serum from icteric hepatitis cases plus fresh guinea pig serum followed by fluorescent conjugated anti guinea pig globulin. Weaker staining was also seen when serum from the patients who provided the biopsies was used as a source of antibody. However, no staining was seen when conventional gamma globulin, or a variety of other control sera, including acute and convalescent sera from three cases of hepatitis type A, were employed as a source of antibody. Strong staining was observed when serum from a classical case of long incubation post-transfusion hepatitis type B was used as a source of antibody [6]. In addition, staining was blocked by inactivation of the human and guinea pig serum employed (56°C, 30 minutes), thus indicating the complement dependence of the staining system [6].

Further evidence supporting the specificity of the observed staining was derived from utilization of the complete staining system to examine autopsy liver tissues from patients with cirrhosis, Weil's disease, and diseases unrelated to the liver. These tissues did not reveal the staining observed in the biopsies from chronic active hepatitis patients.

It was noted that large foci of stained cells contained cells with nuclear and cytoplasmic staining, or cytoplasmic staining alone, and that cells on the periphery of these foci usually revealed only nuclear staining. The two forms of staining suggested that different stages of infection were being observed.

Results of Tests for Hepatitis B Related Antigens in Patient Sera (Table 2)

In 1973, 13 years after the original study, only 14 sera were available from the 32 hospitalized cases. These included sera from the nine cases in whom the hepatitis B associated antigens had been demonstrated by the fluorescent antibody method. HBsAg was detected in serum from four cases by agar gel diffusion and in an addition eight cases by radioimmunoassay. In two cases there was insufficient serum for definitive testing. Thus, HBsAg was present in at least 12 of the 14 cases, confirming the original postulate that these patients were hepatitis B carriers. In three cases the persistence of detectable HBsAg was documented for at least 90 days and in two additional cases for 60 days.

Results of Testing for Anti-HBs and Anti-HBc in the Antiserum Used for Fluorescent Antibody Tests

The convalescent serum pool used for fluorescent staining in 1962 was retested in 1974 for content of anti-HBs by the passive hemagglutination test, and for anti-HBc by the indirect immunofluorescence test. The antibody titers found in these tests (Table 3) were sufficient to account for the fluorescent antibody staining observed in 1962.

DISCUSSION

The similarity between results presented in recent studies of localization of hepatitis B related antigens in liver tissues [7–11], and those which were obtained in the present study in 1962, as well as data which we had obtained suggesting that the antigens demonstrated in Korean chronic active hepatitis were specific for hepatitis B infection [6] suggested that the antigen described in 1962 and the more recently
TABLE 2
Results of HBsAg Determination in Sera Collected in 1962 from 14 Cases of Anicteric Hepatitis in Korea

| Case No. | Histologic Diagnosis                        | AGD | Tests for HB Antigen | Radioimmunoassay Immunofluorescence (1962) |
|---------|---------------------------------------------|-----|----------------------|--------------------------------------------|
|         |                                             |     | # +/ -               | Nuclear (% Cells)                           |
|         |                                             |     | # Tested             | Cytoplasm (% Cells)                         |
| 230     | Acute hepatitis                             | +   | QNS                  |                                            |
| 231     | Chronic active hepatitis (mild)             | -   | QNS                  |                                            |
| 234     | Chronic active hepatitis (severe)           | -   | 1/1                  | 104                                        |
| 235     | Chronic active hepatitis (severe)           | -   | 1/1                  | 12.9                                       |
| 236     | Chronic active hepatitis (severe)           | +   | QNS                  |                                            |
| 237     | Inadequate biopsy                           | +   | QNS                  |                                            |
| 239     | Chronic active hepatitis (mild)             | -   | 1/1                  | 121                                        |
| 240     | Chronic active hepatitis (severe)           | -   | 3/3                  | 152                                        |
|         | with cirrhosis in final biopsy only         |     |                      |                                            |
| 244     | Chronic active hepatitis (severe)           | -   | 1/1                  | 121                                        |
| 249     | Chronic active hepatitis (mild)             | -   | QNS                  |                                            |
| 251     | Chronic active hepatitis (severe)           | +   | 3/3                  | 127                                        |
|         | with cirrhosis in final biopsy only         |     |                      |                                            |
| 252     | Chronic active hepatitis (mild)             | -   | 2/2                  | 87                                         |
| 254     | Chronic active hepatitis (severe)           | -   | 3/3                  | 122                                        |
| 257     | Chronic active hepatitis (mild)             | -   | 2/2                  | 108                                        |
|         |                                             |     |                      |                                            |
| a       | Standard deviations from mean of negative controls.

defined hepatitis B related antigens might be identical. The presently reported serologic data indicate that 12 of the 14 cases examined had detectable hepatitis B antigen in their serum, providing additional evidence that the cases under study in 1962 were due to type B infection.

In the light of our present understanding [19] it seems likely that the cytoplasmic staining observed in 1962 represented localization of HBsAg (now thought to represent excess viral membrane) in the cytoplasm of infected liver cells and that the nuclear staining represented localization of the core antigen [20] (HBcAg) of the 42 nm Dane particle which is thought to represent the complete hepatitis B virion [21]. The fact that the centrally located cells of stainable foci showed both antigens, while the cells on the periphery of these foci, which were probably more recently infected, showed only nuclear staining, would be consistent with the expectation that synthesis of nuclear nucleocapsid antigens precedes that of the cytoplasmic surface antigens.

TABLE 3
Assay for HB Related Antibodies in the Convalescent Serum Pool Used for Immunofluorescent Staining in 1962

| Antibody | Test                | Titer |
|----------|---------------------|-------|
| Anti-HBs | Passive hemagglutination | 1:160 |
| Anti-HBc | Indirect immunofluorescence | 1:40  |
The presence of anti-HBc and anti-HBs in the convalescent serum pool used for fluorescent antibody staining is not surprising in the light of recent reports. It is now known that a strong anti-HBc response develops during the acute illness in HBV infections [20,22]; anti-HBs usually appears somewhat later but does not generally rise to high levels in primary HBV infection; high titers of anti-HBs are often, however, observed in reinfected or chronically infected patients [23]. The convalescent serum pool probably contained sera from cases of all of the above types. Due to the high sensitivity of the indirect fluorescent antibody techniques for detection of antibody it is now not surprising that antibodies to both of these HB associated specificities were readily detectable.

The documentation of a rapid progression of HB associated chronic active hepatitis to cirrhosis among some of the patients studied in Korea in 1962, as well as the already published detailed clinical, laboratory, and histologic characterization of these patients [2,3] contributes to our emerging understanding of the medical significance of the hepatitis B carrier state. These results indicate that the chronic hepatitis B carrier state may be a significant etiologic factor in the development of chronic active hepatitis and liver cirrhosis. The close association between these entities [24] cannot be readily explained by superinfection of chronic liver disease patients with hepatitis B virus since most chronic HBV carriers in high prevalence regions acquire their infection very early in life, i.e., prior to development of chronic liver disease [25]. Furthermore, there are now many well-documented cases in which primary hepatitis B infection of newborn and older patients results in the sequence of acute hepatitis, unresolved hepatitis, chronic active hepatitis, and cirrhosis. In some cases the end result of this sequence is primary liver cancer.

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