# IgM levels and IgM-mediated immune responses in patients with acute hepatitis A, acute hepatitis B and chronic HB antigenaemia

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## SUMMARY

Immunoglobulin M (IgM) levels and their relationship to isohaemagglutinins, febrile agglutinins, sheep cell agglutinins, and rheumatoid factor were measured in patients with acute hepatitis A, acute hepatitis B, chronic hepatitis B antigenaemia, and normal control populations. Significant IgM elevations were observed in both types of acute hepatitis, but not in chronic hepatitis B antigenaemia.

There was no correlation of the IgM level with either the prevalence or titre of any IgM-mediated immune response studied. These data suggest that the IgM elevations in both types of hepatitis may reflect virus-specific IgM synthesis.

## INTRODUCTION

Smooth muscle and mitochondrial antibodies occur in both acute hepatitis A and B suggesting that their presence may relate to liver cell damage rather than to the type of hepatitis (Vischer, 1970; Farrow *et al.*, 1970; Wright, 1970). Prior to the differentiation of acute hepatitis, high prevalences of rheumatoid factor (RF) were demonstrated in liver disease (Bonomo, LoSpalluto, & Ziff, 1963; Atwater & Jacox, 1963); since then, it has been shown that the prevalence of RF in acute hepatitis B may range from 46 to 94% (Ziegenfuss, Miller & Rossman, 1971; Dudley, O'Shea & Sherlock, 1973). The prevalence of RF in patients with acute hepatitis A has not been reported.

In an earlier report from this laboratory (Peters & Johnson, 1972), a polyclonal increase of IgM was found in patients with acute hepatitis A. Normal control groups and chronically antigenaemic groups were not evaluated; however, others have indicated that IgM elevations probably occurred in both types of hepatitis. Unfortunately the association of IgM elevations with non-specific immune responses such as rheumatoid factor is unknown. If a correlation were present, we could argue that hepatitis causes a non-specific stimulation of the immune system, and that the prevalence of smooth muscle antibodies is a consequence of this stimulation. Conversely, the absence of any correlation would suggest that the elevated IgM was specific anti-viral antibody, a response analogous to that of typhoid immunization (Altemeier, Bellanti & Buescher, 1969). We have therefore expanded our initial study of IgM levels to include chronically antigenaemic patients and controls. In addition, several IgM mediated functions were evaluated in each group of patients.

## MATERIALS AND METHODS

Sera were drawn from patients with acute hepatitis at the Hospital Santo Tomas in Panama, and Gorgas Hospital in the Canal Zone. Chronically hepatitis B (HB) antigenaemic blood donors from Hospital Santo

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Tomas and Gorgas Hospital, and chronically HB antigenaemic Guaymi Indians were also studied; these groups were compared with non-antigenaemic control groups from the same populations. No attempt was made to diagnose or classify the presence or severity of chronic hepatitis. Additional socio-economic and cultural information regarding these hospital and Guaymi populations is published elsewhere (Peters & Johnson, 1972; Reeves *et al.*, 1973).

IgM levels were determined by radial immunodiffusion (Mancini, Carbonara & Heremans, 1965) using antibody-in-agar plates and a standard serum (Meloy Laboratories, Springfield, Va.) as previously described (Peters & Johnson, 1972). The presence of HB$_s$ Ag was determined by a complement fixation test (Purcell *et al.*, 1969) and by radioimmunoprecipitation (Lander, Alter & Purcell, 1971).

All sera were tested for sheep cell agglutinins, isohaemagglutinins, febrile agglutinins, and rheumatoid factor. All of the tests were performed in 'U' shaped microplates (Linbro Chemical Company, New Haven, Conn.), after proper controls assured us that no significant differences were introduced by this adaptation.

*Salmonella* group D, typhoid O (somatic 9, 12, 10t 2483–527), and Typhoid H (flagellar d, lot 2482–535) antigens from Lederle Diagnostics (American Cyanamid Corporation, New York) were used for the determination of anti-typhoid H and O titres.

Isohaemagglutinins were determined using typed human red blood cells at a final concentration of 1·3% as described by Arskine (1963).

Heterophile antibodies were determined using a final concentration of 0·7% washed sheep erythrocytes, but otherwise the method as outlined by Kracke (1941) was followed.

Latex particles (RA-test, lot 0376V009A1) from Hyland Laboratories (Costa Mesa, Calif.) were used to test for rheumatoid factor. Sera were initially screened at a 1:20 dilution in borate-buffered saline, pH 8·2 (Singer & Plotz, 1956) and positives were titred by serial two-fold dilutions of serum in the same buffer.

A Wilcoxon two-sample rank test (Armitage, 1971) was used to compare median titres of each group for significant differences. In addition, a rank correlation coefficient (Armitage, 1971) was computed for IgM and each of the other titres; each correlation was then tested for significance.

## RESULTS

Patient groups, sample sizes, and IgM levels are shown in Table 1. Although no significant differences were observed between IgM levels of chronic HB antigenaemic groups and their

TABLE 1. IgM levels in sera of acute hepatitis patients, chronic HB antigenaemic patients, and normal control populations

| Population | Patient category | Number tested | Median IgM* | *P* value |
|---|---|---|---|---|
| Hospital Santo Tomas | Normal blood donors | 40 | 184 | |
| | Chronic HB antigenaemia | 22 | 169 | n.s.† |
| | Acute hepatitis B | 22 | 245 | < 0·01 |
| | Acute hepatitis A | 24 | 443 | < 0·01 |
| Gorgas Hospital | Normal blood donors | 42 | 147 | |
| | Chronic HB antigenaemia | 7 | 119 | n.s. |
| | Acute hepatitis B | 30 | 239 | < 0·01 |
| | Acute hepatitis A | 23 | 500 | < 0·01 |
| Guaymi Indians | Normal | 47 | 260‡ | |
| | Chronic HB antigenaemia | 40 | 282 | n.s. |

* IgM expressed in international units/ml.

† n.s. = No significant difference found.

‡ Guaymi Indian median IgM level significantly greater than any other normal population group tested ($P < 0·001$).

appropriate normal controls, IgM levels were significantly greater ($P<0.05$) in donors from Hospital Santo Tomas than in donors from Gorgas Hospital. Normal Guaymi Indians had significantly higher ($P<0.001$) IgM levels than donors from Santo Tomas.

Both hepatitis A and hepatitis B patients from Gorgas and Santo Tomas Hospitals had IgM levels that were significantly greater ($P<0.01$) than blood donors at their respective hospitals. In addition, both groups with hepatitis A had levels of IgM that were significantly higher ($P<0.01$) than groups with hepatitis B from the same hospitals.

Only one significant difference was present with respect to the prevalence of sheep cell agglutinins, febrile agglutinins, isohaemagglutinins, or rheumatoid factor: significantly higher titres of anti-typhoid H were present in Gorgas Hospital HB antigenaemic donors than in their normal controls.

The prevalence of rheumatoid factor (titres $\geq 1:20$) ranged from 10 to 13% among patients with hepatitis, and from 3 to 26% among chronically antigenaemic patients and controls, but no significant differences in prevalence or titre could be demonstrated between any of these groups.

## DISCUSSION

Our data indicate that the IgM elevations that we earlier found associated with acute hepatitis A also occur in acute hepatitis B, although such elevations are significantly lower in hepatitis B than in hepatitis A. We have shown also that IgM levels in three different groups of chronically antigenaemic patients are not elevated, suggesting that these groups may have an altered response to the HB antigen. However, such a hypothesis can only be tested by a prospective study of IgM levels in acute hepatitis B patients developing chronic antigenaemia. Only one such patient was discovered in this study, and his IgM was elevated at the time of onset of his hepatitis.

We were unable to demonstrate any association of elevated IgM and either the prevalence or titre of rheumatoid factor, sheep cell agglutinins, anti-typhoid H, anti-typhoid O or isohaemagglutinins. This observation suggests that the elevated IgM may represent specific immune stimulation, a response analogous to that observed following typhoid immunization (Altemeier *et al.*, 1969). This hypothesis could be tested by absorption of acute sera with the infecting agent; when acute sera from patients vaccinated with typhoid were absorbed with the vaccine, decreases in the elevated IgM could be demonstrated (Altemeier *et al.*, 1969). However, we cannot exclude the possibility that the IgM is non-specific since large increases in IgM may be reflected by only small increases in the parameters of IgM functions that we measured.

The reasons for the observed differences in IgM levels among the normal control populations in our study is not clear. IgM levels show significant variation among black and white races in the United States (Miller *et al.*, 1969; Lichtman, Vaughan & Hames, 1967): in one study (Lichtman *et al.*, 1967), IgG levels were observed to vary independently of socio-economic variables. In a more recent study (Billewicz *et al.*, 1974) genetic determinants appeared to influence IgM, IgG, and IgE levels. Although the significant elevation of IgM levels for the relatively homogeneous population of Guaymi suggests that such genetic factors may also be important determinants of such levels in our populations, socio-economic variations among the groups do not permit any rigorous analysis.

Likewise, we can offer no explanation for the relatively low prevalence of RF in our patients with acute hepatitis B and chronic HB antigenaemia. The prevalence of rheumatoid factor may have racial determinants (Lichtman *et al.*, 1967) but in the absence of other factors, we would expect North Americans in Panama to have a prevalence of RF similar to that observed in the United States.

In the United States, HB antigenic subtype *ay* is found in about half of all cases of acute

sporadic hepatitis, but only infrequently in chronic HB antigenaemia (Holland *et al.*, 1972). Similar rates of subtype *ay* have been observed in persons with chronic HB antigenaemia in Panama City, but the rate in persons with acute sporadic hepatitis is only 19% (Peters *et al.*, 1973). Although these differences in HB subtypes could account for the lower prevalence of RF in our patients with acute hepatitis, the differences do not explain the lower prevalence of RF in our groups with chronic HB antigenaemia.

The only significant difference in the IgM-mediated functions studied, that between anti-typhoid H titres of antigenaemic and normal blood donors at Gorgas Hospital has no suitable explanation, but may be related to sampling errors or differences in the level of typhoid immunization.

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