Antigenic relationship between reactivity to hepatitis B e antigen and 19 kDa protein of *Mycobacterium tuberculosis* among the Tibetan settlers in Karnataka

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Abstract.   McGlynn and her co-workers have reported that among the Vietnamese refugees in Philadelphia and among Alaskan natives who are hepatitis B carriers, there is a statistically significant association between a *negative* tuberculin test and the presence of hepatitis B e antigen. A repetition of this work among the population of Bangalore did not yield any significant results because of the very low incidence of hepatitis found among this population. However, on the basis of available data that hepatitis B infection is more prevalent among the Mongolian population than among people of other populations, the work was repeated among Tibetans who had settled down in Karnataka. This set of experiments showed that, contrary to the report of McGlynn *et al,* there is a statistically significant association between a *positive* tuberculin test and the presence of hepatitis B e antigen and that those individuals who showed the presence of hepatitis B e antigen exhibited less severe form of the disease than those who were negative to this antigen. These findings suggested that immunity to tuberculosis and hepatitis B infections may have a common underlying principle. Data bank search revealed a stretch of amino acid sequences which is common to hepatitis B e antigen and 19 kDa antigen of *Mycobacterium tuberculosis.* The significance of these results is discussed.

Keywords.   *Mycobacterium tuberculosis*; hepatitis B; *M. tuberculosis* 19 kDa antigen; hepatitis B e antigen; tuberculin test; nucleotide sequences; amino acid sequences; epidemiology of hepatitis B.

1.   Introduction

McGlynn *et al* (1985, 1987) reported that among hepatitis B carriers in a population of Vietnamese refugees in Philadelphia and among Alaskan natives who are hepatitis B carriers, there is a statistically significant association between a negative tuberculin reaction and hepatitis B e antigen positivity (indicative of viral replication). This suggested to us that there might be a common antigenic region of a protein in hepatitis B virus and another protein in *Mycobacterium tuberculosis,* so that immune response to the latter confers on the individual, immunity to hepatitis B infection. If this is true, it is also likely that since antigen e of hepatitis B virus has been closely related to its DNA polymerase activity, an indicator of viral replication, the common antigenic region in *M. tuberculosis* could reside in its DNA polymerase II (replication enzyme).

   A subsequent report (Tiollais *et al* 1985), that it is the P gene of hepatitis B virus which codes for its DNA polymerase, compelled us to take a new look at the role of antigen e in hepatitis B infection. Our studies on the role of this antigen in a population of Tibetan settlers in Karnataka gave results different from those of

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McGlynn *et al* (1985, 1987) and suggested a role for this antigen in the resistance to hepatitis B.

This paper describes the field experiments leading to the above conclusion. It also describes computer-based studies on data available in the data bank of genes and amino. acid sequences of proteins (either available as such or derived from nucleotide sequences) of hepatitis B and *M. tuberculosis* which show significant base sequence homology between a short stretch each of antigen e of hepatitis B virus and of 19 kDa antigen of M. *tuberculosis.*

2. Materials and methods

Purified protein derivative (PPD) was from BCG Laboratory, Guindy, Madras.
Kits for determination ,of HbsAg and HbeAg by enzyme-linked immunoassay were gifted by Abbott Laboratories, North Chicago, Illinois, USA.

Other reagents, plastic disposable syringes, etc. were commercial grade available in Bangalore.

2.1 *Sample collection*

Non-heparinized blood (5·0 ml) was collected from each individual and allowed to stand at room temperature for 2 h. It was then centrifuged at 1500-2000 rpm, the serum collected, aliquoted and stored at – 20°C until use.

2.2 *Tuberculin testing*

Tuberculin testing was standardized with the assistance of technicians from National Tuberculosis Institute, Bangalore. Standard tuberculin (0·1 ml) was injected intradermally on the forearm of the person and the induration at the spot measured after 72 h. An induration of 10 mm diameter and above was considered tuberculin positive while one smaller than this was considered tuberculin negative.

2.3 *Alanine aminotransferase assay*

Alanine aminotransferase determinations were performed using the spectrophoto-metric method of Wrolewski and La Due (1956) and were measured in international units (IU). Based on the population distribution, 35 IU/litre was defined as the upper limit of normality.

2.4 *Computer analysis*

This was carried out at the Distributed Information Centre of the Department of Physics, Indian Institute of Science, using a Micro Vax II supermicro computer. The amino acid sequences in the data bank were "back-translated" to nucleotide base sequences employing the codons commonly used by *Escherichia coli,* and in some instances, since mycobacteria are GC rich, employing the GC preferred codons.

For computer analysis three different parameters were employed: (i) to find out the overall percentage of homology of the bases in the genes over the entire stretch, (ii) to find the largest stretch of contiguous bases where "best fit" was available, and the percentage of homology in this region, and (iii) graphical analysis of the homology by "dot plot" (Maizel and Lenk 1981), using smaller stretches of the gene at one time.

3. Results

3.1 *Field studies*

Out of a total of 208 persons from two places in Bangalore—Indian Institute of Science and Escorts Ltd.—181 showed a positive response to PPD. This population consisted of persons in the age group of 20 to 55, the majority (75%) being males. Only 3 persons were found to be positive to HbsAg, and of these, only one was negative to Hbe antigen. The total number of persons having HbsAg and HbeAg was however, too low for statistical analysis.

On the basis of available data that hepatitis B infection is more prevalent among the Mongolian population than among the population of most other races, the above work was repeated among Tibetans who settled down in Bylakuppe in Karnataka State (India) in 1962, and their descendants. In this population of 380 volunteers, comprising of an equal number of men and women of ages 14 and above, the rate of HbsAg (Hbs$^+$) was found to be 17·6%. Among these carriers, the frequency of HbeAg positivity was 32·8%. Among the hepatitis B carriers 89% responded positively to tuberculin. These data are presented in table-1.

Table 1. Results of the surveys at Bylakuppe camps (Tibetan colony).

Total no. of samples analysed	380
No. of samples +ve for PPD	340
No. of samples +ve for HbsAg	67
Hepatitis B carrier rate	17·6%
Frequency of Hbe$^+$ among carrier population	32·8%
Frequency of Hbe$^-$ among carrier population	67·2%

The absence of HbeAg was more prevalent in the age group above 20. The results are presented in table 2.

Table 2. be status and age among HB virus carriers.

Age (years)	Hbe antigen		
	+	−	Total
<20	16	11	27
>20	6	34	40
Total	22	45	67

The correlation between tuberculin' positive (PPD$^+$) and Hbe status in the survey (where equal numbers of age group above and below 20 were included) is given in table 3. It can be seen from this table that there is a positive correlation between tuberculin and Hbe positivities. The correlation is also shown to be statistically significant (P<0·05). In a follow-up study, it was observed that Hbe negative individuals had a more severe form of hepatitis B disease (Chandra Mohan, personal communication). The alanine aminotransferase activity was normal (<IU 35) in all the volunteers tested.

Table 3. PPD response and Hbe status among Hb virus carriers.

PPD	Hbe antigen		
	+	−	Total
+	21	34	55
−	1	11	12
Total	22	45	67

Hbe$^+$ and PPD$^+$ show significant positive association by χ^2 test, *G*-test as well as Fisher's exact test.

3.2 *Computer analysis*

The results in table 3 can be explained if there is a genetic homology between Hbe antigen of hepatitis virus and one of the proteins of *M. tuberculosis,* or if the two proteins have a common antigenic site. These two possibilities were investigated.

The nucleotide sequence of the genome of the hepatitis B virus, including the "gene" for the e antigen, is known (Galibert *et al* 1979). Among the genes coding for the proteins of *M. tuberculosis,* the nucleotide sequence of 65 kDa antigen, 19 kDa antigen (Ashbridge *et al* 1989; Collins *et al* 1990), DNA J protein and tuberculin active protein are known and the amino acid sequence of 10 kDa antigen is also known. The latter was back-translated into nucleotide sequence. Homology searches were made between the nucleotide sequences of Hbs gene and the Hbe "gene" of hepatitis B virus (the nucleotide sequence corresponding to the cleaved off protein of the core antigen in the same reading frame) on the one hand and the nucleotide sequence of each of the above genes of the tubercle bacillus.

Comparisons of each of the available nucleotide sequences of the genes of the two antigens of hepatitis B virus with each of the six genes of *M. tuberculosis* gave overall homologies of only 58% or less. Since only homologies of 60% and above are considered significant, these experiments were repeated using 50 nucleotides of each at a time, since it is more likely that homology existed only between a short stretch of nucleotides corresponding to the antigenic sites of the proteins of the two organisms. When these comparative studies were carried out using dot plot analysis, it was found that there was a significant homology only between bases 1201 to 1400 of the "e gene" of hepatitis B virus and bases 750 to 1300 of the 19 kDa antigen gene of *M. tuberculosis.* These results are given in the dot plot analysis (figure 1A), where it can be seen that diagonals which indicate homology
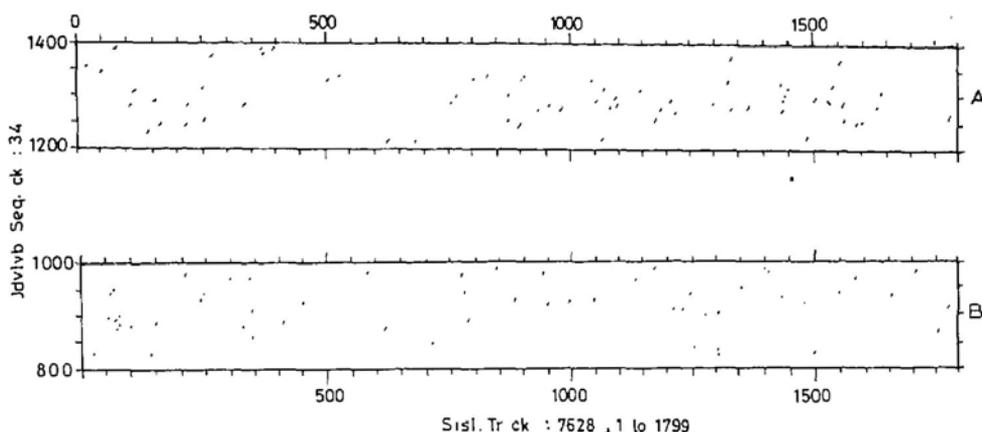
Figure 1. Homology of nucleotide sequences of 19 kDa protein of tubercle bacillus (I-1799) with those of antigen e (DNA polymerase) of hepatitis B virus (80I-1000 and 1201-1400).
  Graphic matrix of region from the nucleotide sequence of hepatitis **B** virus e antigen (Jdv Ivb) on the vertical axis and from the nucleotide sequence of M. *tuberculosis* I9 kDa protein (SiS1. Tr) on the horizontal axis. Each dot represents one base and each hyphen a CUG triplet codon. (**A**) Experimental. (**B**) Control.

between two nucleotides, can be drawn connecting the slanted hyphens (corresponding to CUG, the codon of leucine). A comparison of this plot with the control (figure 1B) where such a diagonal cannot be drawn makes this conclusion clear. With respect to the amino acid sequences, which truly represents the antigenic sites, this homology is reflected in two regions of 20 amino acid stretches which show 60% or more sequence similarity (amino acids 501–520 and 541–560 of hepatitis e antigen with amino acids 434–453 and 586–605 respectively of the mycobacterial 19 kDa protein).

4. Discussion

A number of interesting conclusions can be drawn from the results presented above. Firstly the number of hepatitis B carriers in the Indian population studied at Bangalore is very low (about 1–2%). On the other hand, the frequency of HbsAg positive (the hepatitis B carrier rate) in the Vietnamese refugee population was reported by McGlynn *et al* (1985) to be 12%. According to an Indian Council of Medical Research, New. Delhi report on hepatitis, the prevalence of hepatitis B in India varies from 0·6 to 5% depending on the region, 5% being for Arunachal Pradesh. The majority of viral hepatitis in India is probably caused by C ("non A, non B") type, since even among those in Bangalore who were hospitalized for viral hepatitis, HbsAg and HbeAg could be detected only in very few persons. On the other hand, hepatitis B is widely prevalent in China and southeast Asia, and it is probable that Mongolians are particularly susceptible to infection by this type of virus. This thesis has been borne out by the results of our studies on the Tibetans who have settled down in Karnataka. The Tibetans are Mongolian in origin and the hepatitis B carrier rate among them was found to be around 18%, comparable to the rate found among the Vietnamese refugees in Philadelphia. This difference in

susceptibility to certain strains of microorganisms by different populations has been recorded for other diseases in, literature *e.g*., lepromatous leprosy (Cochrane 1947; Job 1965; Berkeley and Berkeley 1970). They have found that the lepromatous leprosy rate in India is 13–15%, while in China it is 40–50% and for Bhutan (another Mongolian country) it is 38%. In the case of hepatitis B also it has been postulated (Sherker and Marion 1991) that genetic and environmental host factors may participate in the development of Hb virus infection.

The results of our studies with Tibetans in Karnataka show that there is a *positive* correlation between tuberculin reaction and the presence of hepatitis B e antigen in this population and this is at variance with those reported by McGlynn *et al* (1985). Since hepatitis B carriers who are Hbe[+] have been found to have less severe hepatitis than those who are Hbe[−], this would imply that those who are resistant to tuberculosis (*i.e*. PPD[+]) exhibit less severe hepatitis B disease.

Computer analyses showed that the basis of the dual immunity could be ascribed to a common stretch of nucleotides, coding for two regions of 20 amino acid stretches, of the "gene" for the e antigen of hepatitis B virus and the gene for the 19 kDa antigen of *M. tuberculosis*. These regions may represent the common antigenic sites of the two organisms. This interpretation could explain our findings in the field study of the Tibetan population of Bylakuppe of a direct correlation between PPD positivity and Hbe antigen positivity.

The divergence between our results and those of McGlynn *et al* (1985) on the relationship between PPD positivity and Hbe antigen status may be explained in the following way. The phenotype Hbe negativity can be caused by mutations in different loci. In its simplest form the mutation could be in the antigenic site preventing it from binding to the antibody. It may also be due to a stop codon mutation in the precore region of hepatitis B virus DNA (Omata *et al* 1991) resulting in its non-cleavage to antigen e and leading to a severe form of hepatitis disease (Liang *et al* 1991). Since antigen e has been shown to be a proteolytic cleavage product of antigen c, the proteolysis could be host-mediated, and a mutation in the proteolytic machinery can also prevent the formation of e antigen. The Hbe negativity in the Vietnamese population may be a reflection of the mutation of the first type whereas Hbe negativity in the Tibetan population may be a reflection of the mutation of the second or third type. The exact role of antigen, however, is still unknown.

It has been noticed that the Tibetan population which was studied by us had normal alanine transaminase levels, even though hepatitis B carrier rate among them was high, showing that they do not have any detectable liver damage. That some individuals have minimal or no histological liver damage, despite the presence of many virus-infected cells, has been reported earlier (Gudat *et al* 1975) and has been postulated to the fact that hepatitis B virus is not directly cytopathic but rather causes liver damage by inducing a host cellular immune response.

In passing, it may be pertinent to comment on certain interesting facts about the derived amino acid sequences of the two proteins—19 kDa antigen of *M. tuberculosis* and antigen e of hepatitis B virus. 19 kDa protein is rich in proline residues (1 out of 7. total residues). Computer data show that the distribution of proline is not random, although we have not yet found a single pattern to account for this. Further, there are 9 to 16 stop codons distributed among the amino acids when the nucleotide sequences are translated into any of the three possible frames; these may represent either the presence of introns—which have not been reported

so far in prokaryotes other than T_4 bacteriophage — or the presence of frame-shift mutations (Blinkowa and Walker 1990). It has also been noticed that antigen e of hepatitis B virus is rich in leucine residues (1 out of 8 total residues), and though these do not show a "leucine zipper" pattern (Landschulz *et al* 1988) or other reported patterns (Suzuki *et al* 1990), the distribution has been found to be non-random. All these aspects are presently under study.

Acknowledgements

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