Association between phosphoglucomutase (PGM₁) and group-specific component (Gc) subtypes and tuberculosis

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SUMMARY The polymorphism of PGM₁ and Gc was studied by isoelectric focusing in pulmonary tuberculosis patients and controls. For the Gc system, the phenotypic frequencies did not differ significantly, but the PGM₁ system showed a significant difference in the tuberculosis patients compared to controls. The data presented here show the potential of newly developed techniques to distinguish subjects with susceptibility to disease.

Recent studies have demonstrated an association of tuberculosis with the HLA-B₈ antigen in the Newfoundland population and the HLA-B₁₉ antigen in a Russian population. These findings of increased frequency of tuberculosis in subjects of particular HLA types, together with increased incidence among certain ethnic groups, suggest a genetic component in susceptibility to this disease.

Studies of its association with other single gene characters have not been very conclusive. Associations with ABO blood groups have been examined in 41 studies from various parts of the world, covering some 25,000 patients, analysis of which by Mourant et al indicated an increase of blood group O in patients with pulmonary tuberculosis. In many patients in these studies, the type of tuberculosis was unfortunately not specified, but there is a suggestion that in types other than pulmonary tuberculosis there is an increased frequency of blood group A. Among other genetic systems that have been examined are the Rh and MN blood groups, the secretor and PTC tasting polymorphisms, and the serum protein haptoglobin types, while in the acetylator polymorphism the frequency of the allele controlling slow acetylation (A²) does not differ from that in healthy controls.

There is no study of other polymorphic systems, and the enquiry reported here concerns one serum protein and one red cell enzyme. Instead of using conventional electrophoretic methods to identify the genetic variants of the enzyme phosphoglucomutase (PGM₁) and the serum protein group-specific component (Gc), analysis was performed using the technique of isoelectric focusing, which shows greater heterogeneity at these loci. It is possible that the additional allele so revealed may be more informative with regard to genetic susceptibility to the disease.

Material and method

Blood samples were collected from 68 sputum positive and radiologically confirmed pulmonary tuberculosis patients from the hospitals associated with the Medical College, Lucknow, and 71 controls matched for sex, age, and ethnic group. Samples were transported by air to the Department of Human Genetics, Newcastle upon Tyne, where the blood groups and other genetic markers were studied.

The details of the methods for isoelectric focusing of PGM and Gc in this laboratory have been given by Papiha. In brief, the red cell lysates and plasma samples were examined by thin layer isoelectric focusing in polyacrylamide gels (PAGIF) using an LKB 2117 multiphore system. The polyacrylamide gels with final concentrations of 5% were prepared in glass moulds provided by LKB. For the gel solutions, 10 ml of stock solution (29% acrylamide, Koch-Light and 0.9% bisacrylamide) were added to 40 ml distilled water, 7 ml glycerol, and 3 ml LKB ampholyte (for PGM, pH 5 to 7 and for Gc, pH 4 to 6). The solution polymerised with 1.5 ml of 1% ammonium persulphate. Isoelectric focusing conditions for PGM were 3½ hours at 15 W, and for
### Results and discussion

For Gc and PGM the phenotype numbers and gene frequencies for patients and controls are given in tables 1 and 2. The Gc<sup>3</sup> frequency of 33% in patients is slightly but not significantly higher than that in the control group (26%) and this is at the expense of both the Gc<sup>17</sup> and Gc<sup>15</sup> alleles. The gene frequencies in both these groups are within the north Indian normal range.  

The distribution of the PGM suballeles is more interesting. In indigenous populations of north India, the PGM<sup>1+</sup> gene frequency ranges from 13 to 17%.<sup>11</sup> The frequency of PGM<sup>1+</sup> in the controls fits into this range, whereas the 6% PGM<sup>1+</sup> frequency in tuberculosis patients is very low. This depression is largely made up by increased frequencies of the PGM<sup>2+</sup> and the PGM<sup>1+</sup> alleles.

There is a considerable deficiency observed of the heterozygous phenotype PGM(1+1-) and an excess of phenotype PGM(1+2+). The overall differences between patients and controls are statistically significant ($\chi^2 = 8.80$, p<0.05).

### Association studies

The associations of these systems with the disease may be examined by the method of relative risks following Woolf.<sup>14</sup> Tables 3 and 4 present for the relevant genotypes the relative risks and significance for Gc and PGM respectively. The relative risks for those carrying Gc<sup>17</sup> and Gc<sup>2</sup> are very similar, whereas for PGM<sup>1+</sup> the PGM<sup>1+</sup> gene showed a significant decrease in tuberculosis patients, a relative risk of 0.23 ($\chi^2 = 9.72$, df 1, 0.01>p>0.001).

A similar highly significant result ($\chi^2 = 11.91$) was obtained when the data were examined by Haldane's logarithmic method.<sup>15</sup>

The present study is based on a limited number of subjects and, since it is not yet possible to identify any physiological mechanism for the association of PGM and tuberculosis, the results should be treated with caution. However, the data presented here suggest at least one reason why the search for the association between genetic markers other than the histocompatibility antigens and disease is unrewarding. With conventional electrophoresis, the PGM types do not show any significant association ($\chi^2 = 1.19$, NS), and this is only revealed when the same small samples are subtyped. This suggests that the technique previously used was insufficiently sensitive to detect genes which may be influencing
susceptibility to a particular disorder. The data presented here on PGM and tuberculosis lead one to suspect that discovery of true associations of gene markers and disease must await establishment of more refined techniques of gene identification, such as that of isoelectric focusing used here or of DNA probes currently in process of development.

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References

5 Milunicova A, Dominec M. Blood characteristics and PTC test in young patients suffering from pulmonary tuberculosis and diabetes mellitus. Rozhl Tuberk 1966;26:545-50.

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