

Haptoglobins in chronic lymphatic leukaemia

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Summary. Haptoglobin phenotypes have been determined in 30 chronic lymphatic leukaemia patients and concentrations determined in 21 of these. The number of Hp1 phenotypes is slightly raised but not significantly so compared with a control population and is the same as that in first-degree relatives. Haptoglobin concentrations show higher concentrations in Hp1 than Hp2, as has previously been reported. It is concluded that haptoglobin genes contribute very little to any genetic influence on lymphatic leukaemia.

The possibility that the serum haptoglobin polymorphism, expressed in the three common phenotypes Hp1, Hp2.1, and Hp2 (Smithies and Walker, 1956), is related to disease susceptibility has been the subject of a number of investigations (Giblett, 1969; Sutton, 1970). In particular several reports have suggested an association of haptoglobin phenotype with differential susceptibility to various forms of leukaemia.

In 1960, Latner and Zaki reported an increased number of Hp1 phenotypes among 27 leukaemic patients, particularly marked among cases of chronic lymphatic leukaemia. They concluded that this distribution differed significantly from normal; and moreover there appears to be a significant disequilibrium in their phenotype numbers. A similar study of 35 patients by Galatius-Jensen (1962) showed an increased proportion of Hp1 phenotypes over that in the normal Danish population but not significantly so, and there was no significant phenotype disequilibrium.

Since these early studies, several others have reported conflicting results. Peacock (1966) in Maryland showed an increased incidence of the Hp1 phenotype in each of three different groups of leukaemia (chronic myelogenous, acute myelogenous, and acute lymphocytic), in which the haptoglobin type frequencies did not differ significantly from each other but overall differed significantly from the normal population of Maryland. Larkin (1967) included five leukaemic patients in a group of 36 suffering from reticuloses,

in a study of many different types of cancers, from which she concluded that the overall haptoglobin distribution in cancer patients is substantially similar to that in normal populations but that the reticuloses as a whole tend to have a higher Hp1 gene frequency. Veale and Gunz (1967) showed a decreased incidence of the Hp1 phenotype in 42 cases with frank chronic lymphatic leukaemia significantly different from that in a normal sample and deviating significantly from the number expected under Hardy-Weinberg equilibrium.

Some of these reports are complicated by the heterogeneous nature of the leukaemia groups studied. Moreover, comparison is not straightforward since the patients were drawn from different populations who may vary in their gene frequencies as well as their environments. In view of the inconsistency in the findings, an examination of haptoglobin phenotypes in chronic lymphatic leukaemia was made as part of a larger study of genetic and immunological factors in leukaemia.

Materials and methods

Blood specimens were obtained and the haptoglobin type identified in 30 patients, diagnosed as having chronic lymphatic leukaemia, resident in the north east of England. After separation serum was stored at -20°C until haptoglobin typing and quantitation estimations were carried out.

Haptoglobin phenotypes were determined using horizontal starch gel electrophoresis, following the technique of Smithies using 11% hydrolysed starch (Connaught) with a discontinuous buffer system (Poulik, 1957). Upon completion of electrophoresis, the gel was sliced.

TABLE I
HAPTOGLOBIN PHENOTYPES IN CHRONIC LYMPHATIC LEUKAEMIA PATIENTS

	Numbers	Hp Type				Gene frequency	
		1:1	2:1	2:1 mod.	2:2	Hp1	Hp2
Chronic lymphatic leukaemia	30 obs	7	14	1	8	0.483	0.517
	30 exp (from gene frequency)	6.9	14.2		7.9		
Number expected from normal population	30	5.6	14.4		10.1	0.425	0.575
First-degree relatives of patients	49 obs	10	25	2	12	0.480	0.520
	49 exp (from gene frequency)	11.5	25.2		12.3		

The upper half was stained with amido black to reveal the total protein pattern. The lower half was stained with a freshly prepared mixture of 0.2% benzidine in 0.5% acetic acid containing 0.2 ml 30% w/v hydrogenperoxide. In order to stabilize the phenotype staining, 30–40 mg ammonium chloride was added to the staining mixture. Polyacrilamide gel electrophoresis was used to check the phenotypes.

Quantitative estimates of haptoglobin activity were made on unhaemolysed serum samples using the peroxidase guaiacol method (Owen, Better, and Hoban, 1960).

Data for comparison are available on haptoglobin types in a sample of some 800 normal individuals born in different localities in the north east of England. Since there is local heterogeneity in gene frequency, in haptoglobin type as in ABO blood group (Roberts, 1953; Kopec, 1970), amongst the north-eastern populations, an expected frequency of haptoglobin types was calculated weighted to match the local distribution of the patients. Specimens were also obtained from first-degree relatives of the patients, mainly sibs, in order to provide a second comparable estimate of normal frequencies and to allow intrafamilial comparisons to be made.

Results

The phenotypes found in the 30 chronic lymphatic leukaemia patients are set out in Table I. In a further patient, not included in the present analysis, ahaptoglobinaemia was encountered, but in all the patients of the present study the types were clearly identified. The proportions of the haptoglobin alleles were determined by gene counting, and the equilibrium phenotype numbers expected, also shown in Table I, are very similar to those observed ($\chi^2=1.02$). Making comparison with the normal population, the numbers of phenotypes expected in a sample of 30 individuals, drawn from the same localities as the patients, are again very similar. There is a slight elevation of phenotype Hp1 and diminution of type Hp2 in the patients, but this deviation is not significant ($\chi^2=0.82$). There is a corresponding slight increase in the Hp¹ allele frequency. The phenotype distribution in the first-

TABLE II
HAPTOGLOBIN CONCENTRATION

	Hp Type		
	1:1	2:1	2:2
<i>Leukaemia patients</i>			
Present series			
Number studied	5	9	7
Mean Hp concentration* (mg/100 ml)	146	93.1	50.7
Standard deviation (mg/100 ml)	39.12	37.41	31.68
Range (mg/100 ml)	95–200	50–140	25–110
Peacock (1966)			
Number studied	11	23	7
Mean Hp concentration	146	130	128
Standard deviation	78	60	78
Range	75–330	29–238	46–244
<i>Normals</i>			
Smith and Owen (1961)			
Mean	104	102	72
Standard deviation	34	37	38
Nyman (1958)			
Mean	136	108	82
Standard deviation	30	37	34
Bayani-Sioson <i>et al</i> (1962)			
Mean	91	86	69
Standard deviation	21	22	23
Murai (1960)			
Mean	136	133	92

* Expressed as bound merhaemoglobin.

degree relatives, mainly sibs, pooled over all families is likewise similar, with an Hp¹ allele frequency of 0.48, almost identical to that in the patients. It appears therefore that there is no tendency for chronic lymphatic leukaemia to be associated with the Hp1 phenotype or the Hp¹ allele.

The quantitative estimations of haptoglobin concentration in 21 of the chronic lymphatic leukaemia patients are shown in Table II. For comparison the data from normal individuals obtained by Smith and Owen (1961) using the same method, and by other investigators, are included. In the present series there appears a gradient in the mean concentration of haptoglobin, being least in haptoglobin phenotype 2, and greatest in Hp1. There is indeed very little overlap in the concentration in the two homozygous categories, which are significantly different. By comparison with the normal values,

the Hp1 phenotype shows an increased mean haptoglobin concentration, and the Hp2 phenotype shows a marked decrease.

Discussion

The results on the different studies of haptoglobins in leukaemia are summarized in Table III. There is no general tendency for the numbers of phenotype Hp1 to be increased over those expected from the gene frequencies in the sample. There is a general but slight tendency for the Hp1 phenotype frequency to be elevated over that in the corresponding normal population in all studies except that of Veale and Gunz (1967), and in the studies of Peacock (1966) and Latner and Zaki (1960) this elevation is significant giving rise to a slightly increased Hp1 allele frequency. With this slight tendency the present findings are in agreement, but the same tendency is also shown in normal first-degree relatives of the propositi, so it appears that either it is a chance effect or it is characteristic of the families in which cases occur rather than of the patients themselves.

Differentiating types, Latner and Zaki (1960) distinguish chronic lymphatic leukaemia as particularly demonstrating the tendency to increased Hp1 phenotype, Veale and Gunz (1967) find the converse.

If observed and expected phenotype numbers in samples of chronic lymphatic leukaemia patients alone are pooled, there is no significant excess of phenotype Hp1, whether or not the sample of Veale and Gunz (1967) is included.

It is difficult to account for the significance of the findings of Veale and Gunz (1967) and Latner and Zaki (1960). Galatius-Jensen (1962) drew attention to the possibility that ahaptoglobinaemia may be mistaken for the Hp1 phenotype. The lack of exactly comparable gene frequency data for the normal local populations from which the patients were drawn is another possible source of error. But the vagaries due to small sample numbers are notorious, and the small number of cases in each study may well be the explanation.

The gradient in mean haptoglobin concentration is not unexpected. All reports in normal subjects (Allison, 1958; Nyman, 1958; Murai, 1960; Smith and Owen, 1961; Bayani-Sioson *et al*, 1962) show Hp2 to have lower activity than the other phenotypes (the mean level in Hp1 is higher than that in the heterozygote in almost all studies); Nyman (1958) showed a gradient as clear as in the present study. There are no significant differences between our activities and his in phenotypes Hp1 and Hp2.1, but that in our Hp2 phenotype is appreciably lower. The cause of this variation between phenotypes in

TABLE III
HAPTOGLOBIN TYPES IN LEUKAEMIA STUDIES

	Hp Type			Total Tested	Hp Allele Frequency
	1:1	2:1	2:2		
<i>Present study</i>					
No. obs	7	15	8	30	0.483
No. exp from allele frequency	6.9	14.2	7.9		
No. exp from normal population distribution	5.6	14.4	10.1		0.425
<i>Veale and Gunz (1967)</i>					
No. obs	1	26	15	42	0.333
No. exp from allele frequency	4.7	18.7	18.7		
No. exp from normal population distribution*	6.1	19.4	16.4		0.38
<i>Galatius-Jensen (1962)</i>					
No. obs	8	14	9	31	
No. exp from allele frequency	7.3	15.5	8.3		
No. exp from normal population distribution	5	11.4	14.6		0.40
<i>Larkin (1967)</i>					
No. obs	7	17	12	36	0.430
No. exp from allele frequency	6.7	17.7	11.6		
No. exp from normal population distribution	5.4	17.2	13.4		0.389
<i>Latner and Zaki (1960)</i>					
No. obs	12	8	7	26	0.60
No. exp from allele frequency					
No. exp from normal population distribution	2.6	13	10.4		0.35
<i>Peacock (1966)</i>					
No. obs (leukaemia all types)	18	44	17	79	0.506
No. exp from allele frequency	20.2	39.5	19.3		
No. exp from normal population distribution	9.9	34.9	34.1		0.346

* These authors do not state normal frequencies, so figures from other samples of New Zealand whites are inserted here.

normals is not known. Its apparent enhancement in the present study may be a result of the drug therapy received by the patients, as most were receiving prednisone and chlorambucil at the time of blood collection. However, in no patients included in the present study was the activity so low as to inhibit identification of phenotype.

The results therefore indicate that the haptoglobin genes are contributing very little to the genetic influence on the disease previously shown by Miller (1964), Steinberg (1960), and Damsehek and Gunz (1964) in family studies.

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