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A Genetical Study of the Gm Groups in Human Serum

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Summary. The Gm serum groups in man have been investigated serologically and genetically. Two populations have been studied. In Great Britain the frequency of the Gm (a+) phenotype is 60·98 per cent. In the Ferrara district of Italy the frequency is 40·18 per cent. Family studies in both populations support the hypothesis that the Gm (a+) phenotype is determined by a gene which is expressed in the heterozygote.

INTRODUCTION

The Gm groups of human serum were discovered by Grubb (1956). They can be determined by a serological system which depends upon the agglutination of human red cells, which have been sensitized by certain incomplete Rh (anti-D) antibodies, by sera from selected patients with rheumatoid arthritis. This agglutination reaction is inhibited by human pooled γ globulin, and also by the serum of some normal individuals, and is weakly, or not at all, inhibited by the serum of other normal individuals. This inhibition reaction can be used to divide individuals into two phenotypes, with different γ globulin constitutions, the Gm (a+), inhibitors, and the Gm (a−), non-inhibitors. The differentiation of the two types is not without difficulties, which lie in the selection of the reagents and in the setting up of the reactions.

The purpose of this paper is to give an account of some experiments on the serology of the Gm groups and the results of population and family studies.

EXPERIMENTAL METHODS

(1) SELECTION OF REAGENTS

Only a few per cent of rhesus, anti-D, sera can be used for the cell sensitization and those which are suitable come from donors who are themselves Gm (a+) (Grubb, 1957). The anti-D serum which has been used in these experiments has an albumin titre of 1/128 and a negligible amount of saline agglutinating antibody and for coating cells for Gm grouping it is diluted 1/30. A subsequent sample of serum from the same donor after an interval of 2 years, also provided a suitable antibody for Gm grouping. The antibody arose in this group O Rh–ve Gm (a+) mother during her second pregnancy which resulted in the birth of a child whose red cells gave a positive direct Coombs' test at birth. The red cells which are coated with antibody should be group O Rh positive, but the precise Rh genotype is not an important factor. When possible, in these experiments, O R_2R_2 (cDE/cDE) red cells have been used, but occasionally O R_1r (CDe/cde) cells were used and found to be satisfactory.

Rheumatoid arthritic sera which are suitable for Gm grouping, come usually from patients with long histories of the disease and high positive scores in the serological tests which are used in diagnosis. Patients who are donors of suitable sera are themselves Gm (a−) (Laurell and Grubb, 1958). The Gm group of such patients can be determined

if the sera are first heated to 63° C. for 10 minutes. This treatment prevents the agglutination of the saline controls because the rheumatoid factor is destroyed. The result of this heat treatment slightly improves the inhibitory power of the Gm (a+) donors, and the serum of Gm (a−) donors remains non-inhibitory.

(2) SETTING UP THE REACTIONS

(*a*) Most of the tests were done by the tube technique described by Grubb (1956) using at least two different rheumatoid arthritic sera.

(*b*) *Disagglutination Method.* If suitably sensitized Rh positive red cells are agglutinated on a tile with rheumatoid arthritic serum, the subsequent addition of diluted normal serum from a Gm (a+) individual will, after stirring, cause the dispersal of the agglutinates. The addition of serum from a Gm (a−) individual, after stirring, results in the reappearance of the agglutinates. A saline control must be included to demonstrate that the serum being tested does not agglutinate the sensitized red cells.

This method has been found to give reliable results. Its advantages are that it requires smaller volumes of the reagents and sera under test, and several combinations of cells sensitized with different anti-D sera and several RA sera can be observed simultaneously.

MATERIAL

These techniques have been applied to population and family studies in Great Britain and Ferrara, Italy; samples of serum from mothers and newborn infants, and samples of milk, were investigated.

RESULTS

ITALIAN SAMPLE

The frequency of the Gm (a+) phenotype in a population from Ferrara, Italy, was 40·18 per cent. In this material there were fifty-six two-generation families, the mating being shown in Table 1.

TABLE 1

DISTRIBUTION OF GM MATING TYPES IN FIFTY-SIX ITALIAN FAMILIES LIVING IN THE FERRARA DISTRICT

Mating type	*Number observed*	*Number expected**
Gm (a+) × Gm (a+)	8	9·1
Gm (a+) × Gm (a−)	29	26·9
Gm (a−) × Gm (a−)	19	20·0

Observed phenotype frequencies Gm (a+) 40·18 per cent, Gm (a−) 59·82 per cent.

* Based on random mating with observed phenotype frequencies.

The nineteen families in which both parents were Gm (a−) have between them forty-five Gm (a−) children. The analysis of the thirty-seven families with at least one Gm (a+) child by Fisher's method (as given by Race and Sanger 1958) is shown in Table 2, is consistent with the assumption that the Gm (a+) phenotype is controlled by a gene which

is expressed indistinguishably in the present test in single or double dose. In this method of analysis, using the same gene frequencies as for Table 1, the number of families observed to contain no recessive children is compared with the expected number, and the expectations are calculated separately for each size of family.

TABLE 2

ANALYSIS OF THIRTY-SEVEN ITALIAN FAMILIES HAVING AT LEAST ONE GM (a+) PARENT

Class of mating	*Class of family*	*Number of families*		X^2	*d.f.*
		Expected	*Observed*		
Gm (a+) × Gm (a+)	All children Gm (a+)	5·37	6	1·07	1
	Some children Gm (a−)	2·63	2		
Gm (a+) × Gm (a−)	All children Gm (a+)	8·37	6	0·94	1
	Some children Gm (a−)	20·63	23		

ENGLISH SAMPLE

The frequency of the Gm (a+) phenotype in the unrelated English population is 60·98 per cent. Among thirty-nine two-generation families the matings were distributed as shown in Table 3.

TABLE 3

DISTRIBUTION OF GM MATING TYPES IN THIRTY-NINE ENGLISH FAMILIES

Mating type	*Number observed*	*Number expected**
Gm (a+) × Gm (a+)	15	14·50
Gm (a+) × Gm (a−)	20	18·56
Gm (a−) × Gm (a−)	4	5·94

Observed phenotype frequencies Gm (a+) 60·98 per cent, Gm (a−) 39·02 per cent.

* Based on random mating with observed phenotype frequencies.

There were nine Gm (a−) children in the four families involving two Gm (a−) parents. Analysis of thirty-five families, shown in Table 4, with at least one Gm (a+) parent again are compatible with Gm^a gene expression in the heterozygote.

TABLE 4

ANALYSIS OF THIRTY-FIVE ENGLISH FAMILIES HAVING AT LEAST ONE GM (a+) PARENT

Class of mating	*Class of family*	*Number of families*		X^2	*d.f.*
		Expected	*Observed*		
Gm (a+) × Gm (a+)	All children Gm (a+)	11·67	14	2·10	1
	Some children Gm (a−)	3·33	1		
Gm (a+) × Gm (a−)	All children Gm (a+)	9·26	7	1·03	1
	Some children Gm (a−)	10·74	13		

MOTHERS AND NEW-BORN INFANTS

Samples of blood from 100 mothers had the same Gm groups as the cord blood samples of their own infants. The mothers have not been included in the frequency calculation of the English sample because the hospital population was not typical.

MILK SAMPLES

All milk samples, including at least twenty known to have come from Gm (a+) mothers typed as Gm (a−). A few samples of colostrum from Gm (a+) mothers also completely failed to inhibit the reaction between the sensitized red cells and the rheumatoid arthritic sera.

The titre of γ globulin as measured by a γ-globulin consumption test (Mollison, 1956) was between $\frac{1}{2}$–$\frac{1}{8}$ in the milk samples and between $\frac{1}{8}$ and $\frac{1}{64}$ in the samples of colostrum. Sera from these mothers gave inhibition titres in the same test of more than 1/1000.

The titre of the Gm (a) inhibitory substance in the serum of a Gm (a+) individual is between $\frac{1}{16}$ and $\frac{1}{32}$.

DISCUSSION

The family studies reported here indicate that the presence of Gm (a) substance in the normal individual is determined by a gene which is expressed in the heterozygote — i.e. it is a dominant trait. The hypothesis is supported by many other family studies which have been made (Grubb and Laurell, 1956; Moullec, Kherumain, Sutton and Espagnon, 1956; Linnet-Jepson, Galatius-Jenson and Hauge, 1958; Harboe and Lundevall, 1959). There is as yet no evidence of genetical linkage with any of the widely used marker genes. Close linkage between Gm and the ABO, MNS and Rh blood group system is already excluded.

Recently two new developments in the Gm system have been recorded. The Gm (a+) phenotype has been subdivided into two types (Harboe and Lundevall, 1959). A Gm serological system in which an inhibitory reaction is given by sera from all Gm (a−) and some Gm (a+) individuals has been described (Harboe, 1959). These observations could be explained genetically by postulating at least three alleles at the Gm locus. Further confirmation could be provided by family studies. It seems important that people working on Gm groups should interchange reagents so that there may be agreement and uniformity in classification.

Since it is known that the γ globulin of the newborn infant is derived from the mother, the observation that mothers and babies have the same Gm groups (Moullec *et al.*, 1956; Bronnestrom and Nilsson, 1957; and Linnet-Jepson *et al.*, 1958) provides additional evidence that the Gm substance is part of the γ globulin.

The fact that Gm (a) inhibitors are not detectable in human milk or colostrum may be related to the low titres of γ globulin. It is probably for the same reason that patients with agammaglobulinaemia do not show the presence of Gm (a) substance in serum even though they have at least one Gm^a gene.

The determination of the Gm serum groups is more difficult technically than most blood grouping. Nevertheless, the Gm system provides another useful genetic marker.

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