Hb J MEXICO IN ALGERIA: ARGUMENTS FOR AN HETEROGENOUS DISTRIBUTION OF α GENES

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1. Introduction

The number of human globin genes has been investigated in the last years by several approaches. The direct isolation and study of the DNA [1,2] is surely the best, but is possible only in a very limited number of cases. A more often used possibility is to investigate the various polypeptide chains which are the final product of the expression of the genes.

From all the data, it is concluded that the β-chain is under the control of one single gene.

Conversely, the existence of at least two γ-genes has been demonstrated by the different primary structures and confirmed by all subsequent genetic studies [3,4].

The problem of the number of α-chain genes is much more controversial and not entirely solved. There are many arguments in favour of the existence of two genes [5], both from direct estimation in cases of α-thalassemia [2] and from the study of the Hungarian probands carrying two α-mutants together with normal Hb A [6].

Nevertheless the data concerning Hb Tongariki [7,8] do not seem to be compatible with this hypothesis, equally in heterozygotes and homozygotes, and favour the existence of a unique α-gene. Furthermore the percentage of α-variants found is often closer to 30 or 40 per cent than the expected 25 per cent. The existence of an associated α-thalassemia has been postulated and allows a possible explanation in almost every case [9]. On the other hand, the varying percentages of the α-mutant Hb G Philadelphia in the same family has suggested a heterogenous distribution of one α-gene and two α-genes [10]. No hypothesis has been convincingly confirmed by biosynthetic studies.

An α-mutant Hb J Mexico α 54 Gln → Glu is relatively frequent in a part of the Algerian population, and this in large families. From these data and biosynthetic studies, we bring forward arguments supporting the model proposed by Rucknagel [10,11], that the chromosomes bear either one or two α -chain genes, at least in this population.

2. Materials and methods

The abnormal haemoglobin was studied in 32 healthy subjects belonging to 7 families. From a partial investigation, it was found to be present in 0.5 per cent of the population in a limited area, 100 km south of Algiers. The haematological data were normal. In some cases the abnormal haemoglobin was associated with a β-thalassemia. The primary structure has been checked by the usual techniques in at least one patient of each family.

The percentage of the abnormal hemoglobin was estimated by several methods. Elution from cellulose acetate strips and spectrophotometric estimation were performed at least in triplicate for all the subjects. It was in several cases confirmed by DEAE-Sephadex chromatography and isoelectric focusing followed by densitometric scanning. A possible contamination by Hb A_3 was excluded.

For biosynthetic studies, the reticulocyte counts were increased by centrifugation. The cells were

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incubated according to Lingrel and Borsook [12] with slight modifications, using [^{3}H]leucine as a marker. Globin was prepared from hemoglobin by acid acetone precipitation and polypeptide chains were separated [13]. The 60 min incubation lysates were passed through a molecular sieve in order to identify possible precursors. The radioactivity was determined by liquid scintillation counting.

3. Results and discussion

3.1. *Expression of the variant Hb J*

On plotting the results of the estimations made by the elution technique, the bimodality of the distribution is apparent with two maxima, one at 31 per cent and the second around 40 per cent (fig.1). These results agree with those given by Rucknagel studying Hb G Philadelphia, and the bimodality is more clearcut.

In each family the distribution was either bimodal, or unimodal around 30 per cent.

Fig.1. Bimodal distribution of the percentages of Hb J in heterozygotes. Each determination was done in triplicate.

3.2. *Biosynthetic studies*

Only three subjects of one family could be fully investigated (table 1). Two heterozygotes (I and II) belonged to groups described above. A third subject (III) was doubly heterozygote for Hb J and β-thalassemia.

In the subjects I and II, the biosynthetic ratio β/α was balanced. In these cases we did not find evidence for α-thalassemia. In the third case, the ratio β/α was 0.5 due to the β-thalassemia. In the three patients, there was a similar decrease between the percentage of abnormal chain synthesized and the percentage expressed in the peripheral blood. This difference was found (table 1) equally with the thalassemic and the non-thalassemic subjects. This result supports the argument that the thalassemic gene does not interfere with the expression of the α^J-gene.

In one patient with the higher percentage of Hb J, the synthetic ratio α^J/α^A was constant (0.85) throughout the various times of incubation, suggesting a slight defect of α^J-chain synthesis.

When plotting the specific activity ratio of both α-chains S.A. α^J/S.A. α^A as a function of the incubation time, a moderate increase suggested a small but discrete destruction of the abnormal chain [14]. This,

Table 1
^{3}H Incorporation into the various chains

Patients	Total activity (cpm) β	α^A	α^J	Total activity ratio $\frac{\beta}{\alpha^A + \alpha^J}$	Total activity ratio $\frac{\alpha}{\alpha^A + \alpha^J}$	Peripheral blood $\frac{\text{Hb J}}{\text{Hb A + J}}$
I	9208	5163	2904	1.14	0.36	0.26
II	16 616	7997	6881	1.12	0.46	0.36
III	55 600	72 546	39 062	0.50	0.35	0.25

After a 60 min incubation, the total activity ratio shows an unbalanced synthesis for the J β-thalassemic patient (III). The synthesis is balanced for both heterozygotes A J.
The α^J synthesis is always higher than the expression in the peripheral blood.

together with the slightly defective synthesis, could explain the difference between the percentages of chains synthesized and the expression in the red cells.

By molecular filtration, a minimal amount of precursors was found in the non-thalassemic subjects. In the thalassemic one, two peaks corresponding to dimers and monomers amounted to more than half of the synthesized subunits. They were pure α-chains, α^A and α^J, the proportion of both components being the same in the precursors as in the whole hemoglobin. Thus even when the β-chains are quantitatively limited, they do not associate preferentially with the normal α-chains.

3.3. *Genetic interpretation*

Our results favour the model proposed by Rucknagel, with a heterogenous distribution of one α-gene and two α-gene chromosomes. This would explain both the bimodal distribution and the biosynthetic data.

A percentage of 40 per cent Hb J would correspond to one α-gene on each chromosome, a percentage of 30 per cent to the abnormal gene on one chromosome and a duplicated normal gene on the other (fig.2).

This would postulate the existence of subjects with four genes. It is possible that in the ethnic group studied, the mutation exists only on the non-duplicated gene, and that the third model with Hb J below 25 per cent cannot be encountered.

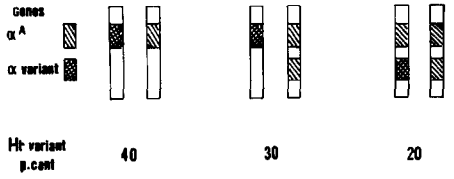

Fig.2. Heterogenous distribution of one and two α-genes. Only the two first patterns have been found for Hb J in Algeria.

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