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Human Albumin Genetic Variants: An Attempt at a Classification of European Allotypes

JEAN M. FINE, MICHELINE MARNEUX, AND DANIEL ROCHU

Département d'Immunochimie des Protéines,
Centre National de Transfusion Sanguine-Institut, Paris

SUMMARY

The relative mobility of albumin and proalbumin genetic variants was estimated by means of cellulose acetate electrophoresis performed with three buffer systems at different pH (8.6, 5.0, and 6.9) after addition of a reference protein and dilution of sera. Numerous experiments using samples of reference variants corroborated the accuracy and reproducibility of this technique. The estimation of the variants' relative mobility at three pH allowed us to distinguish three fast-moving variants (Gent, Vanves, and Reading) and five slow-moving variants (Sondrio, Roma, Christchurch, Lille, and B) in the French population. The frequency of alloalbuminemia in this population is .0004 and is characterized by the high occurrence of albumin B and of the two proalbumin variants, Christchurch and Lille. In order to classify the variants of European origin, the methodology that we developed, owing to its more resolutive possibilities, should be employed as a first step in their identification until establishment of a structural nomenclature making mention of the amino acid substitution characterizing each variant.

INTRODUCTION

During the past 30 years a number of genetic variants of human albumin have been described on the basis of their electrophoretic mobility, which can be either faster or slower than that of normal albumin. In most cases, allotypes are expressed in heterozygous condition, providing two albumin components (bisalbuminemia) in electrophoresis. In very rare cases (Petrini et al. 1975;

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Vanzetti et al. 1979), homozygote allotypes are observed, corresponding to a single albumin component with an electrophoretic mobility differing from that of normal albumin.

A first attempt at a classification was performed by Weitkamp et al. (1973*a*, 1973*b*) in 1973. By using comparative starch-gel electrophoresis in three buffer systems (pH 5.0, 6.0, and 6.9), they distinguished 23 albumin variants in a study of serum samples with alloalbuminemia collected in various geographical areas.

In 1982, we developed a new technique allowing for quantitative estimation of the relative mobility of albumin variants (Fine et al. 1982). Owing to the accuracy and reproducibility offered by this technique, a reasoned classification of the variants of European origin can be proposed in the present paper. Indeed, during the past 4 years we have investigated several samples, either of reference variants in which the amino acid substitution was determined or of albumin variants in which the structural change is still unknown.

The frequency of alloalbuminemia in a large population of French blood donors is estimated, and the repartition of each variant type in the French population is reported.

MATERIAL AND METHODS

Reference Albumin and Proalbumin Genetic Variants

Fast-moving variants.—Three variants having an electrophoretic mobility faster than that of normal albumin were used. In order of decreasing mobility at pH 8.6, these were described as albumin Gent, albumin Vanves, and albumin Reading. Albumin Gent, first described by Wieme (1960) and referred to as MI/FG in Italy (Vanzetti et al. 1979), was recently structurally characterized by a 573 Lys→Glu amino acid substitution (Iadarola et al. 1985). Albumin Vanves, which was described in our laboratory (Fine and Lambin 1982), exhibits an amino acid substitution located in the seventh cyanogen bromide fragment (549–585 residues) (Galliano et al. 1986). Albumin Reading was described by Tarnoky and Lestas (1964), and its amino acid substitution is not yet determined.

Slow-moving variants.—Three albumin and two proalbumin variants have been used as slow-moving reference variants. In order of decreasing mobility at pH 8.6, these variants have been described as albumin Sondrio by Porta et al. 1971) and three variants with similar mobility at this pH: albumin Roma, described by Porta et al. (1972); proalbumin Christchurch, described by Brennan and Carrell (1978); and proalbumin Lille, described by Abdo et al. (1981). The slowest variant, albumin B, was firstly described by Earle et al. (1959) and is characterized by a 570 Glu→Lys amino acid substitution (Winter et al. 1972).

Two variants, previously described as albumin Gainesville (Lau et al. 1969) and albumin Pollibauer (Weitkamp et al. 1973*b*), were structurally identified, respectively, to proalbumin Christchurch (Fine et al. 1983) and proalbumin Lille (Galliano et al. 1984), in our laboratory. These proalbumin variants are characterized, respectively, by amino acid substitutions −1 Arg→Gln (Christchurch) and −2 Arg→His (Lille), located in the N-terminal additional hexapep-

tide (Brennan and Carrell 1978; Abdo et al. 1981). The identification of these reference variants to proalbumins was ascertained by three distinctive features: the first one was a limited tryptic digestion that converted them into albumin; the second was their inability to bind ^{63}Ni; and the third was the determination of their N-terminal amino acid sequence.

Detection of the genetic variants of human albumin.—Screening of sera containing genetic variants of albumin was realized by routine cellulose acetate electrophoresis at pH 8.6 in a population of 30,000 blood donors. Moreover, we received for identification some samples from other European laboratories.

Estimation of the relative mobilities of albumin variants.—Electrophoresis was carried out on cellulose acetate strips (Cellogel Chemetron, Milano; 57 mm × 140 mm) by means of three buffer systems with a similar conductivity (0.0035 ohm^{-1} cm^{-1} at 20 C) and respective pH's of 8.6, 5.0, and 6.9 (Fine et al. 1982). Each sample submitted to electrophoresis contained 1 vol patient serum, 2 vol purified human transferrin at 10 mg/ml, and 2 vol suitable buffer, giving a fivefold dilution of serum proteins. After electrophoresis, protein staining, and clearing, the densitometry of the electrophoretical pattern was performed. The distances between the summit of the transferrin peak and the summits of the two albumin peaks (normal and variant) were measured and expressed in millimeters. The relative mobility (RM) of each albumin variant was expressed as the ratio of albumin-variant mobility (in millimeters) versus normal albumin mobility (in millimeters), with zero representing the transferrin mobility and one representing the normal albumin mobility.

RESULTS

Accuracy and reproducibility in the determination of variants' relative mobilities.—Eight different types of alloalbuminemia occurring in European populations were submitted to investigation. As shown in figure 1, cellulose acetate electrophoresis, performed according to the conditions described above, allowed, at the pH used, a very good separation of the variant from the normal albumin. By dilution of the serum and addition of purified transferrin, three narrow bands with similar concentrations could be distinguished on the electrophoretic pattern. Consequently, an accurate determination of the relative RM of each variant can be easily performed on the densitometric curves. For example, as shown in figure 2, the RM of the fast-moving variant, albumin Gent, is 1.19 at pH 8.6 and the RM of the slow-moving variant, proalbumin Christchurch, is 0.70 at pH 5.0. The RM of eight reference albumin and proalbumin variants has been measured repeatedly, in numerous experiments of electrophoresis performed in the three buffer systems. For each reference variant, mean value and SD were established. Two major points can be ascertained from the results of these experiments (summarized in table 1). First, the reproducibility of RM calculation is evidenced, since low SDs close to 0.01 are observed in almost all variants analyzed. Second, each albumin or proalbumin variant can be clearly defined by its three RM values. Indeed, the use of a single pH fails to distinguish certain variants. For example, albumin Roma, proalbumin Christchurch, and proalbumin Lille have similar RM values at pH 8.6. By

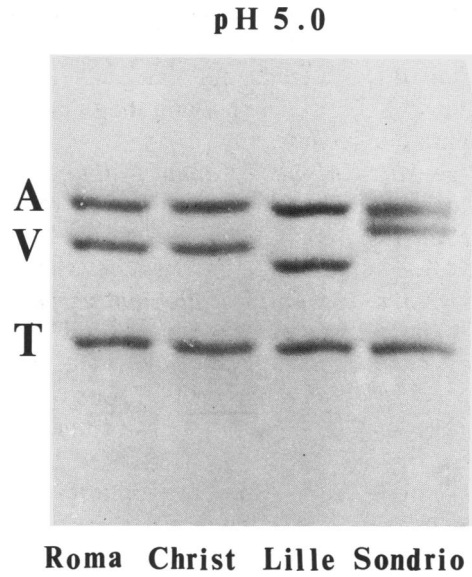

FIG. 1.—Example of cellulose acetate electrophoresis at pH 5.0 of two albumin variants (Roma and Sondrio) and of two proalbumin variants (Christchurch and Lille). A = Normal albumin; V = variant; T = transferrin.

contrast, proalbumin Lille and albumin Roma can be distinguished from the other variants at, respectively, pH 5.0 and pH 6.9.

Frequency of alloalbuminemia.—Using cellulose acetate electrophoresis, we performed a systematic screening of the sera of 30,000 blood donors (all samples collected by the National Center of Blood Transfusion). Twelve samples containing an albumin variant were detected, corresponding to a frequency of .0004. Thus, in the French population one individual in 2,500 exhibits an albumin or proalbumin genetic variant.

Distribution of variants in 44 unrelated subjects.—The examination of 44 sera containing albumin variants (12 sera from the French blood donors noted

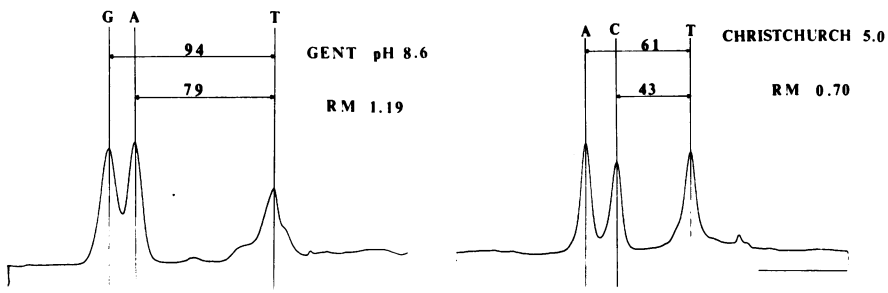

FIG. 2.—Example of quantitative estimation of RMs of albumin Gent at pH 8.6 and of proalbumin Christchurch at pH 5.0. G = albumin Gent; and C = proalbumin Christchurch. Other abbreviations are as in fig. 1.

TABLE 1

MEAN ± SD RELATIVE MOBILITIES OF REFERENCE ALBUMIN VARIANTS OF FRENCH ORIGIN

TYPE	STRUCTURAL CHANGE LOCALIZATION	pH 8.6		pH 5.0		pH 6.9	
		N	RM	*N*	RM	*N*	RM
Gent	573 Lys→Glu (Iadarola et al. 1985)	14	1.19 ± .02	9	1.22 ± .01	5	1.34 ± .05
Vanves	CNBr VII 549–585 (Galliano et al. 1986)	19	1.11 ± .02	10	1.13 ± .02	5	1.11 ± .03
Reading	...	4	1.07 ± .01	6	1.14 ± .01	3	1.16 ± .01
Sondrio	...	24	.90 ± .02	10	0.83 ± .02	3	.85 ± .04
Roma	...	7	.84 ± .01	6	0.73 ± .02	3	.80 ± .02
Proalbumin Christchurch	−1 Arg→Gln (Brennan and Carrell 1978)	14	.82 ± .03	15	0.68 ± .02	4	.86 ± .06
Proalbumin Lille	−2 Arg→His (Abdo et al. 1981)	51	.81 ± .01	22	.59 ± .02	5	.84 ± .02
B	570 Glu→Lys	30	.78 ± .01	9	.69 ± .03	3	.76 ± .02

NOTE.—*N* = number of experiments.

above, 32 sera sent for typing to our department) shows the following distribution: 1 Gent, 2 Vanves, 4 Reading, 4 Sondrio, 1 Roma, 10 Christchurch, 7 Lille, and 15 B type. It is noteworthy that the genetic variants most frequently observed in the French population are of the B type (34%), the Christchurch type (23%), and the Lille type (16%).

DISCUSSION

The determination of the relative mobility of each albumin variant was made with accuracy and reproducibility by using electrophoresis in cellulose acetate. (Owing to low diffusion of proteins in this inert medium, narrow zones are obtained, and we therefore prefer it to agarose for separation according to net charge.)

A previous electrophoretic classification by Weitkamp (1973*a*, 1973*b*), dating from 1973, used starch-gel electrophoresis in three buffer systems. Media such as starch gel or polyacrylamide gel, which introduce a sieving effect, are not required for separation of albumin variants since this separation is based on electric net charge only. On the other hand, highly resolutive systems such as isoelectric focusing cannot be employed, since normal human albumin binds various intrinsic ligands, such as fatty acids, and extrinsic ligands, such as ampholytes. This feature displays a heterogeneous spectrum with more than three bands, even when focusing is performed with an immobilized pH gradient (Gianazza et al. 1984).

Another advantage of the method that we propose is the use of pH 8.6 as the third pH, instead of the pH 6.0 previously employed. Indeed, for studying albumin variants on the basis of their electrophoretic mobilities, the use of different pH's, which provide various ionizations of the protein, is essential. A buffer at pH 8.6 can play this role in more alkaline conditions, and this one is routinely employed for cellulose acetate electrophoresis. So, by using pH 5.0, 6.9, and 8.6, a wide range of ionizations of albumin can be investigated, thus allowing differentiation of all the variants detected up to the present time.

Finally, dilution of sera, together with addition of a reference protein (human transferrin), allowed us to offer quantitative measurements of mobility. This procedure is much more reproducible than side-by-side comparisons performed according to other electrophoresis systems that have been published for the same purpose.

In order to compare the relative frequency reported here for each type of French variant with those summarized in Europeans by Weitkamp (Weitkamp 1973), we subtracted seven of our samples that were included in his statistic. Our results were not significantly modified by this fact, and the major differences are the higher frequency of proalbumin variants (.40 vs. .15) and the lower frequency of the B variant (.27 vs. .51) observed in our study. It seems likely that the better separations achieved by our method on cellulose acetate allow a much finer discrimination within four of the slow-moving variants—i.e., Roma, Christchurch, Lille, and B—which were not easy to distinguish by means of the methods previously described.

With the technique that we developed, a clear classification of albumin vari-

TABLE 2

CLASSIFICATION OF ALBUMIN VARIANTS OF FRENCH ORIGIN AND THEIR SYNONYMS

Proposed Nomenclature	Synonyms
Gent (Wieme 1960)	Very fast (Weitkamp et al. 1969), MIMI fast (Porta et al. 1972), MI/FG (Vanzetti et al. 1979), Milano fast (Camera and Muttini 1968)
Vanves (Fine and Lambin 1982)	...
Reading (Tarnoky and Lestas 1964)	Fast (Weitkamp et al. 1967), Syracuse (Schneiderman et al. 1968), New Guinea (Weitkamp et al. 1969), Westcott (Weitkamp et al. 1973*b*), CN/CN (Vacca et al. 1974), Cuneo (Burlina et al. 1985)
Sondrio (Porta et al. 1971)	SO/BS (Porta et al. 1971), D (Fine et al. 1982)
Roma (Porta et al. 1972)	...
Proalbumin Christchurch (Brennan and Carrell 1978)	Gainesville (Lau et al. 1969)
Proalbumin Lille (Abdo et al. 1981)	Pollibauer (Weitkamp et al. 1973*b*)
B (Earle et al. 1959) ...	Ann Arbor (Adams 1966), Jensen (Weitkamp et al. 1966), Oliphant (Weitkamp et al. 1966), Verona (Bonazzi 1968), SO/CZ (Porta et al. 1972)

ants found in Europe seems possible. The first condition required prior to classifying the genetic variants of human albumin and proalbumin is clarification of the confusion introduced into the literature by the use of several synonyms. The Albumin Study Group of the CISMEL (Italian Committee for Standardization in Hematology and Clinical Biology) has recently proposed a new classification of inherited albumin variants in man (Burlina et al. 1985) on the basis of electrophoretic relative mobilities at the three pH that we have used above. Table 2 summarizes the nomenclature proposed for European albumin variants. These are listed in order of decreasing mobility at pH 8.6, with the denomination corresponding to their first citation in the literature and the various synonyms of each variant mentioned with their sources.

We suggest the use of this nomenclature, defined in terms of the electrophoretic relative mobilities of the variants, to all the authors publishing reports concerning variants of European origin. In the future, the denominations proposed here and adopted by the CISMEL Albumin Study Group will be progressively replaced by a structural nomenclature. Automated peptide mapping (Takahashi et al. 1986) or cyanogen bromide–fragment analysis (Franklin et al. 1980; Iadarola et al. 1985) could play a role in the ultimate characterization of the genetic variants of human albumin, by mentioning the localization and nature of the structural change. In anticipation of this final nomenclature, the proposals reported here allow rapid screening and systematic typing of the variants.

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