Subtyping of Factor B by Agarose Gel Electrophoresis

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INTRODUCTION

Factor B (BF) polymorphism has been extended by the occurrence of subtypes in each of two common alleles, BF*F (Teng and Tan 1982; Geserick 1983; Abbal 1985; Nagai 1986; Nakamura 1987; Nishimukai 1987) and BF*S (David 1983; Weidinger 1984). Isoelectric focusing (IEF) has contributed to these studies, but there is a lack of correspondence among the subtypes postulated by each group or their frequencies. We present a BF F subtype using agarose gel electrophoresis (AGE) in Tris/glycine/Veronal buffer, further characterize them by zymosan treatment and by IEF in polyacrylamide gels (PAGIF) and also show a BF S subtype in the Hungarian population.

MATERIALS AND METHODS

EDTA-plasma samples were collected from 325 Japanese, 74 Filipino, 48 Chinese, 72 Thai, 126 Indians, and 219 Hungarian. Some serum samples were also collected for further characterization. Agarose gels (0.8%) were prepared both in the standard Veronal buffer (VB, Teisberg 1970) and in Tris/glycine/Veronal buffer (TGVB, O'Neill 1978). Constant voltage (20V/cm) was applied to gels till HbA marker migrated about 6 cm from the origin. Immunoprecipitated BF protein in the gels was stained with Coomassie Blue R250. Serum samples were treated with zymosan and then subjected to electrophoresis. PAGIF was done in a mixture of Ampholine (pH3.5-9.5, pH4-6, pH5-7) and BF bands were transferred to nitrocellulose filter by capillary blotting.

RESULTS AND DISCUSSION

AGE in TGVB could discriminate a subtype with a slower mobility from the previous type F defined by AGE in the standard VB, thus

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subdividing the BF*F allele into two subtypes which were termed BF*F and BF*Fb1, respectively. BF Fb1 exhibited a mobility slightly cathodic to BF F (Fig. 1), corresponding well to the pattern in PAGIF (Fig. 2). This subtypic pattern was observed only in Chinese and Japanese among the population groups of various ethnic origins. The allele frequency of BF*Fb1 in Chinese (n=88, additionally including 28 Chinese with psoriasis and 12 Chinese in Shanghai) and Japanese (n=325) was calculated at 0.017 and 0.0154, respectively. The serum samples carrying BF*Fb1 were treated with zymosan and then further characterized by AGE. The Ba fragment of BF Fb1 exhibited a different mobility, migrating between that of F and of S (Fig. 3). In addition BF*Fb1 was found to be firmly associated with C4 A3B2 haplotype. Although we have not extensively investigated on Negloid and Caucasoid populations except Indian and Hungarian yet using the method reported here, BF*Fb1 may be characteristic of some of Mongoloid populations.

In the Hungarian population a subtype of BF*S was detected at a low frequency by using PAGIF. This subtype seemed to be similar in the IEF pattern and allele frequency to BF*Sb1 reported by Weidinger (1984) and was designated BF*Sb3. The data obtained in this study were summarized in Table.

Several studies on subtypes of the common BF alleles have been reported thus far, but some discrepancies among subtypic patterns or their frequencies remain to be resolved. We propose thus that (1) all the subtypes postulated by each group are required to be compared with one another, (2) their serum samples must be further characterized by zymosan treatment, and (3) typing method for determining BF subtypes must be standardized.

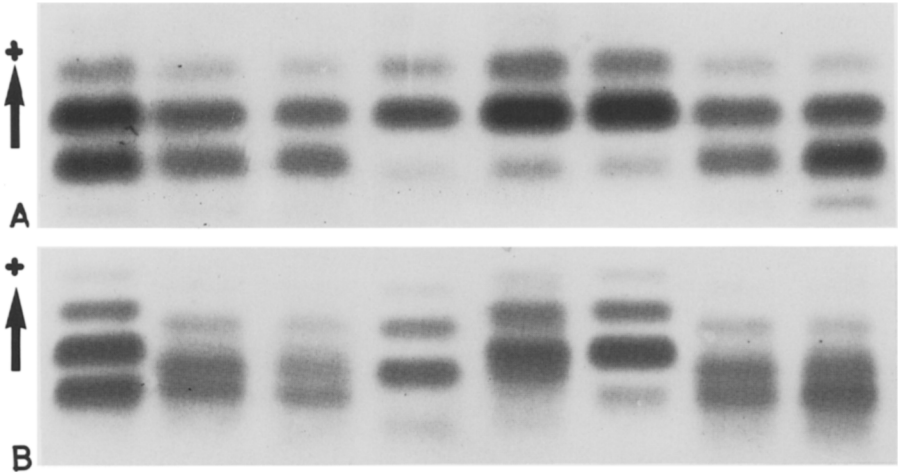

Fig. 1. Electrophoretic pattern of BF Fb1 defined by AGE/VB(A) and by AGE/TGVB(B). No difference in electrophoretic mobility is observed among the samples carrying BF*F by the standard VB but two types of BF F can be discriminated, one (Fb1) migrating a little slower to the other (F). From left to right; FS, Fb1S, Fb1S, Fb1, FFb1, F, Fb1S, Fb1S.

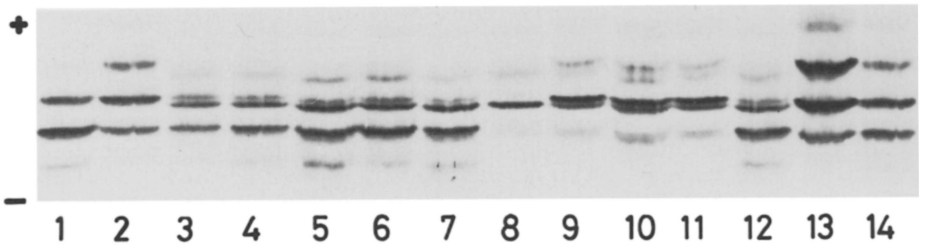

Fig. 2. Characterization of BF Fb1 by PAGIF. (1)S, (2)(14)FS, (3)(4)(12)Fb1S in Japanese, (5)(6)Fb1S in Chinese, (7)F025BS B:Bangkok, (8)Fb1, (9)(10)(11)FFb1, (13)F.

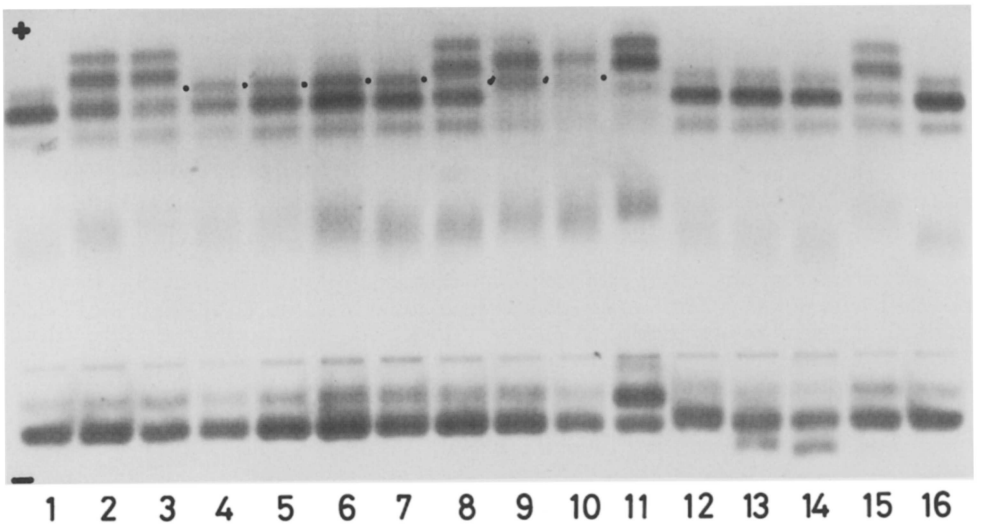

Fig. 3. Characterization of several BF phenotypes by AGE after zymosan treatment. (1)(16)S, (2)(8)FS, (3)(15)F, (4)Fb1S in Japanese, (5)(6)(7)Fb1S in Chinese, (9)(10)FFb1, (11)F075F, (12) F025BS, (13)SS03, (14)SS045. Dots indicate the Ba fragment of BF Fb1.

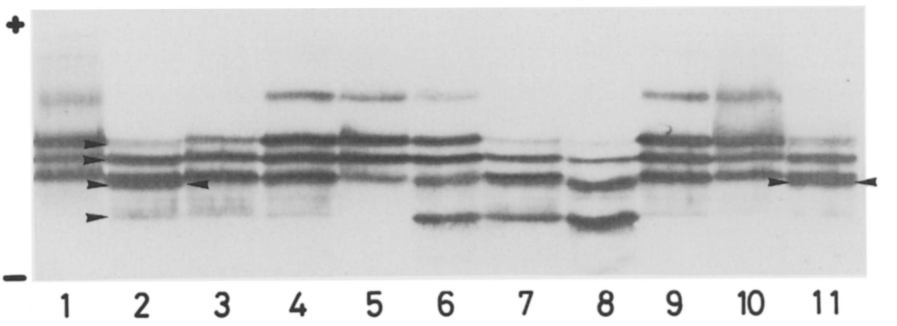

Fig. 4. BF patterns of Hungarian samples characterized by PAGIF. (1)(10)F1S, (2)(11)SSb3, (3)S, (4)(9)FS, (5)F, (6)FS07, (7)SS07, (8)S07. Arrows indicate Sb3 band.

Table 1. Distribution of BF phenotypes and allele frequencies in the six populations

	Japanese	Chinese	Thai	Filipino	Indian	Hungarian
no.	325	48	72	74	126	219
F	6	1	2	6	12	2
FFb1	3	-	-	-	-	-
Fb1S	7	1	-	-	-	-
FS	82	6	18	32	48	54
S	226	37	52	36	63	135
SS07	-	-	-	-	3	19
FS07	-	-	-	-	-	2
F1S	-	-	-	-	-	4
S07	-	-	-	-	-	1
SSb3	-	-	-	-	-	2
Rare[a]	-	3	-	-	-	-
BF*F	0.1492	0.0833	0.1528	0.2973	0.2897	0.1370
BF*S	0.8339	0.8750	0.8472	0.7027	0.6984	0.7968
BF*Fb1	0.0154	0.01	-	-	-	-
BF*F1	-	-	-	-	-	0.0091
BF*S07	-	-	-	-	0.0119	0.0525
BF*Sb3	-	-	-	-	-	0.0046
BF*R[a]	-	0.0313	-	-	-	-

[a] F025BS, SS045, SS03

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