

INVESTIGATIONS ON THE C8 SYSTEM BY IMMUNOFIXATION ISO-ELECTROFOCUSING

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A genetic polymorphism of the eighth component of human complement (C8) was detected in 1977 by RAUM et al. who used a functional, hemolytic detection system. In the following years the three-chain structure of C8 (C8 α, β, γ) posed numerous questions concerning the formal genetic model. The interpretation of C8 phenotypes was complicated by the fact that activity zones were present at different pI values. In 1983 ALPER et al. reported on a C8 β -subunit polymorphism, subsequently called C82 in contrast to the C81 (α - γ) polymorphism detected 6 years ago. Population data on the C8 systems are scarce.

The aim of our study was the evaluation of various isofocusing parameters for C8 phenotyping of German blood donors by a combined immunofixation agarose gel isofocusing (IAGIF) and immunofixation polyacrylamide gel isofocusing (IPAGIF). In view of a possible replacement of the time-consuming hemolytic assay by a combined immunological/IEF detection system (immunoprint) two pH ranges were employed: pH 3-10 for IPAGIF and pH 5-8 (IAGIF). For the separation of C8 allotypes by IPAGIF fresh serum samples from random blood donors and families were examined on polyacrylamide gels (265x125x1mm; T=5%; C=3%; ampholyte concentration 2.5% w/v). At maximum settings of 1,200 V, 40 mA and 20 W and a cooling temperature of 4°C, 1M H₃PO₄ was used as anolyte, 1N NaOH as catholyte. After 20 min prefocusing, 30 μ l serum were applied on 5x15 mm filter papers (Whatman #1) 1 cm from the anodal edge of the gel, they were removed after 70 min, and focusing was completed after 2.5 h. A CAF was soaked with a 1:2 diluted anti-C8 (C81) serum (ATAB) and placed on the gel surface for 20 min. After washing in saline overnight, the foil was stained in 0.1% CBB R 250 and evaluated.

A model of the C8 (C81, C82) allotypes after IPAGIF at pH 3-10 in different gel regions is shown in fig. 1. The main band patterns of the alleles C81*A and *B (gene products) are present in both A and B regions. In difference, the "total" C8 (α, β, γ = C81 + C82) molecule discloses further heterogeneity in the B region, according to a pH value of 7.0-7.5.

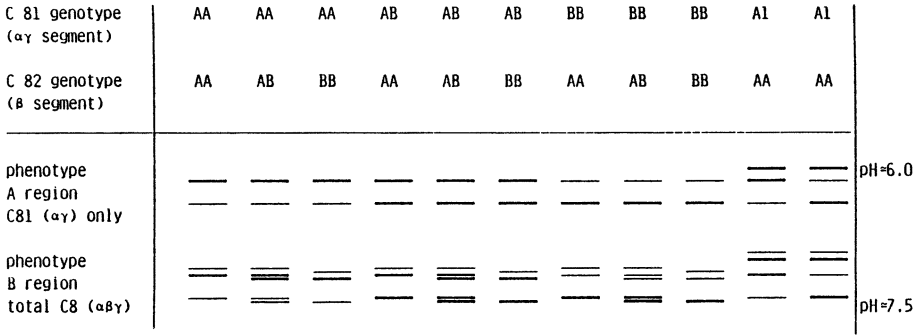


Fig.1: Model of C8(C81,C82) allotypes after IPAGIF at pH3-10

The separation of C8 phenotypes by IAGIF and IPAGIF is shown in fig. 2. The C8 IAGIF in fig. 2a shows the two rare phenotypes A1A and A1B besides the common types AB and B. In the broad pH range of 3-10 the C8 IPAGIF replica (CAF strip soaked with C8-antibody) reveals the two regions A (C81) and B (C81+C82) in a more cathodal position. The fractions which become visible by this technique.

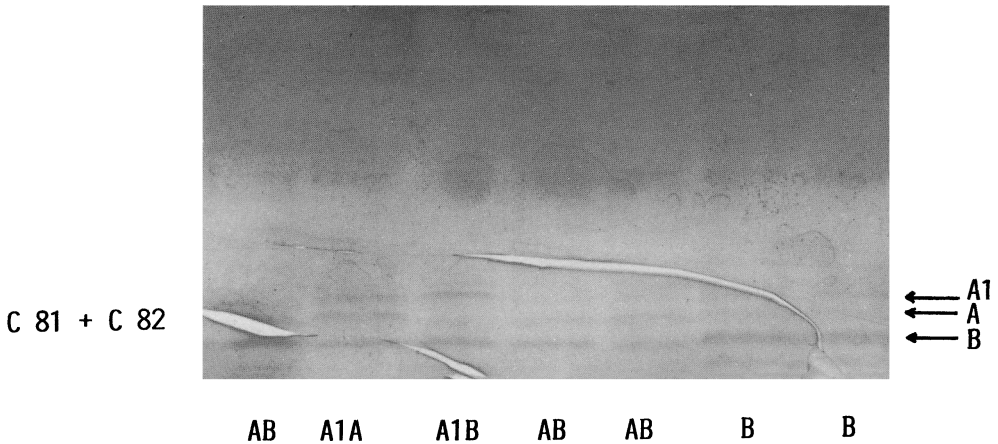


Fig. 2a: C8 IAGIF at pH 5-8

C 8 IPAGIF (CAF) pH 3-10

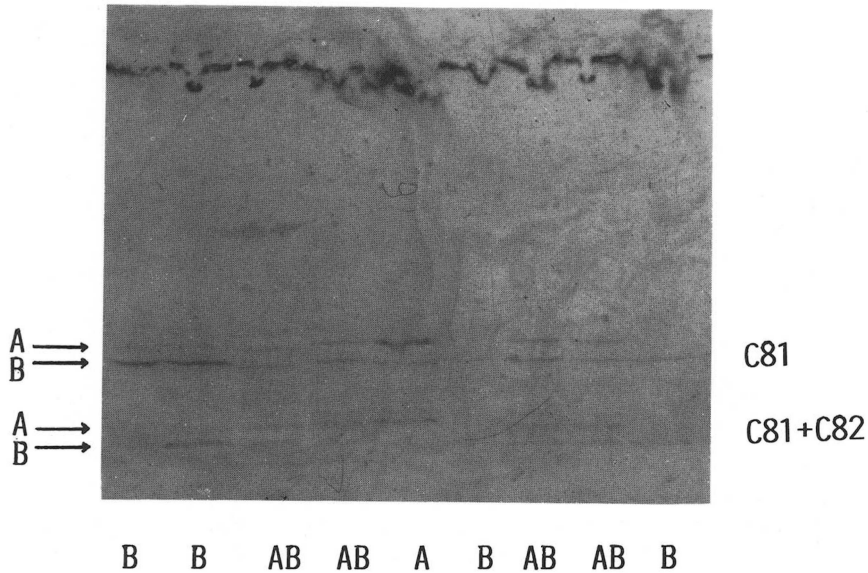


Fig. 2b: C8 IPAGIF (CAF strip) at pH 3-10

Concerning the population data, RAUM et al. published a C81*A frequency of 0.649 (n=165, US Whites); MEVAG et al. (1983) reported a frequency of 0.6 in Norwegians, RITTNER et al. (1984) found a frequency of 0.5536 for C81*A, 0.4286 for *B and 0.0178 for *A1. Our limited population data do not differ significantly from the latter figures (0.57, 0.41 and 0.02, respectively for the alleles C81*A, *B and *A1). Family data support the autosomal codominant inheritance of these C81 alleles.

According to the data of the Human Gene Mapping Conference, Los Angeles 1983, the C8 locus is on the short arm of chromosome # 1 in a distance of 6 centimorgans to PGM1 and 10 cM from the UMPK locus in direction to the centromere. These figures imply no restriction of the informative value of this genetic marker for forensic serological purposes. The exclusion chance for non-fathers hence may be estimated at 19.2%. Further data will be needed to corroborate the hitherto published allele frequencies, family data and in particular the issue of the C82 system, where frequencies of 0.952 (C82*A), 0.044 (C82*B) and 0.004 (C82*A) were reported by ALPER et al. in 1983.

The question, if the contribution of the β chain to the microheterogeneity of the B region can be looked at independently, remains to be investigated in more detail.

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