BIOCHEMICAL LETTERS JOURNAL

Present situation of the analysis of Rh genes

The Rh antigens are one of the most important blood-group systems and contain multiple antigenic variants [1]. The major Rh antigens D, C/c and E/e are encoded by at least three distinct but homologous integral proteins of approx. 32 kDa [2,3]. Although the Rhc, D and E polypeptides were found to be highly related, the c and E polypeptides migrated at 33.1 kDa, whereas the D polypeptide migrated at 31.9 kDa on analysis by one-dimensional SDS/PAGE [4]. Also, the RhD and C antigenic reactivities were dependent on a thiol-group located at or near the outer surface of the erythrocyte membrane, because they disappeared after treatment with thiol reagents [5]. This suggests that a labile exofacial cysteine residue is required for the Rh antigenic reactivity.

The N-terminal sequences of the Rh polypeptides were determined [6–8], and then two cDNAs for Rh polypeptides were isolated [9–12]. A group working in Paris reported that the genome of RhD-positive individuals was composed of two strongly related genes, presumably RhD and RhCcEe, whereas one of these two genes was missing in the RhD-negative individuals on Southern-blot analysis [13]. They also argued that multiple mRNA isoforms were produced by alternative splicing from the RhCcEe gene, and that mRNA spliceoforms lacking exon-5 gene gave rise to the RhC or c polypeptide [14,15], whereas full-length transcripts of the Rh CcEe gene exhibited the RhE or e polypeptide. Additionally, they concluded that the RhD gene did not produce alternative splicing isoforms, because they could not detect the mRNA spliceoforms of the RhD gene [12]. These speculations indicate that the RhD and E/e antigens are encoded by the full-length transcripts from the RhD and RhCcEe genes, whereas the RhC/c antigens are encoded by alternative splicing isoforms from the RhCcEe gene. This idea is not in agreement with biochemical data that shows the molecular mass of the three Rh polypeptides to be almost identical. Northern-blot analysis also showed the 1.7 kb Rh full-length transcript, but the spliceoforms were not detectable ([10]; F. Umenishi, E. Kajii and S. Ikemoto, unpublished work), suggesting that all Rh antigens are encoded by full-length transcripts. Using the PCR method, we confirmed the existence of multiple spliceoforms produced by alternative splicing from the RhPII gene in leucocytes [16]. Further, we made sure of the mRNA spliceoforms from both Rh genes by using reticulocyte mRNA; the RhPI and RhPII mRNA spliceoforms in reticulocyte RNA are produced by the presence or absence of exon 4–9 and exon 6–9 respectively (F. Umenishi, E. Kajii, and S. Ikemoto, unpublished work). We previously reported that the spliceoforms from the RhPI gene were produced by immature erythroblasts, but not reticulocytes [17]. These data suggest that the expression of multiple Rh spliceoforms is associated with erythroid differentiation and development rather than Rh antigenicity.

We also revealed genomic polymorphisms concerning the RhC/c phenotypes unrelated to the RhD/d phenotypes [18]. Furthermore, Southern-blot analysis using the RhPI and RhPII cDNA probes showed the same RFLP patterns because of high

nucleotide similarity between the two genes [18]. Recently, it has been reported that the RhE/e antigenic variation is associated with a genomic polymorphism which demonstrates that the cE genes of the cE (r⁺) and cDE (R2) haplotypes are not identical [19]. These data suggest that the Rh polypeptide genes encoding RhD, C/c and E/e antigens are very homologous, but different. Although the RhD/d polymorphism is demonstrated by some of the hybridization bands that are lacking in the RhD-negative DNA, it is obvious that the mRNA transcripts they insist on being RhD gene include its alternative spliceforms. This important observation causes a major conflict in the two-gene model. The two-gene model is based on the existence of multiple Rh spliceoforms produced by the RhCcEe gene, but not the RhD gene [13]. If these two genes are alleles of the same locus, allelic differences can not be explained by alternative splicing.

The Bristol group reported that the RhD polypeptide diverged after amino acid residue 41 compared with the RhC/c and E/e polypeptides, and two PCR products were obtained by using oligonucleotide primers flanking amino acids 29–46 [9]. One of two clones that they isolated (Rh30PCR-B) corresponded exactly to the RhD sequence. For the present, there is no report that the same clone was isolated. As the Rh locus is composed of strongly related genes, it is very difficult to determine a third Rh gene. One possibility is that the 5' moiety of RhD polypeptide makes a great difference compared with two Rh polypeptides. Further information for the RhD polypeptide identical with its peptide fragments will be required to isolate the additional Rh gene.

Bennett et al. [20] reported the application to determine the RhD genotype of fetuses, using two sets of primers. The first set of primers (A1 and A2) yields a 136 bp product common to the RhCcEe and RhD genes, whereas the second set of primers (A3 and A4) yield a 186 bp product only in RhD-positive subjects. However, the Amsterdam group [21] claimed that the determination of RhD genotype by the PCR was not in perfect agreement with a serological RhD phenotype. They attributed the appearance of these discrepancies to the non-specificity for the RhD gene of the primer A4 used. We previously showed in this Journal [22] that a RhPII cDNA clone, which has a strong resemblance to RhXIII, could be amplified by PCR from reticulocyte RNA and genomic DNA of RhD-positive and RhD-negative individuals using the RhPII-specific primers. These results strongly suggest that the RhD typing method reported by them is insufficient and the alternative method may be required.

The greatest problem is that attempts to express Rh protein in eukaryotic cells have so far been unsuccessful [23,24]. The transfected cells produced a 32 kDa Rh protein, but the Rh antigen could not find the cell surface on immunoprecipitation. This result strongly suggests that the insertion of Rh proteins with several proteins (Rh50, LW, GPB, CD47 and Fy5) that constitute the Rh protein complex may require the expression of the Rh antigen. Therefore, it is difficult to determine directly which Rh antigens the two Rh polypeptides encode. Further study is required before successful expression of the Rh genes can be achieved.

Although the analysis of the genes of the Rh system is not yet

complete, we will describe its outline and greatly appreciate being given the opportunity to make objections to the Rh theory of the Paris group [25].

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Received 24 January 1995