Polymorphism and deficiency of human factor H-related proteins p39 and p37

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Abstract. We described previously cDNA clones representing a novel factor H-related 1.4 kilobase mRNA. This mRNA species codes for a doublet of serum proteins of Mₐ 39 000 and 37 000 (p39/p37). The respective recombinant proteins of the three clones H-69, pFH1.4a, and pFH1.4b differ in the expression of the epitope recognized by the monoclonal antibody (mAb) 3D11. This probably reflects the difference of three amino acid residues of the deduced protein sequence. Here we report evidence for corresponding alterations in the native proteins p39/p37 in human sera. Employing mAb 3D11 and a polyclonal factor H-specific antiserum we detected three different patterns in western blot analyses of human sera which we provisionally termed FH1.4p+m+, FH1.4p+m−, and FH1.4p−m−. In the first pattern, p39/p37 were recognized by both antibodies, while in the second pattern the two proteins reacted only with the polyclonal antiserum. Both antibodies failed to detect p39/p37 in the third pattern. These phenotypes are found in the healthy population with frequencies of 0.556, 0.40, and 0.044, respectively. The frequencies of the alleles FH1.4*p+m+, FH1.4*p+m−, and FH1.4*p−m− were estimated to be 0.33, 0.46, and 0.21, respectively, assuming the gene distribution to be in Hardy-Weinberg equilibrium. Studies of 98 members from 27 families revealed an autosomal Mendelian inheritance. Southern blot data support our assumption of a polymorphism of the factor H-related proteins p39 and p37.

Introduction

Factor H, a regulatory protein of the complement system, controls the alternative pathway of complement activation. The control is achieved by competing for the interaction of C3b with factor B and dislodging the Bb fragment of factor B from the active alternative pathway convertase (C3bBb), thus accelerating the decay of this enzyme (Whaley and Ruddy 1976; Conrad et al. 1978). Factor H also serves as a cofactor for the cleavage of C3b to inactivated C3b (iC3b) by factor I (Pangburn et al. 1977). Factor H, a Mₐ 150 000 protein, is composed of twenty short consensus repeats (SCR). Each SCR unit comprises approximately 60 amino acids and shows a characteristic framework of highly conserved residues (Holers et al. 1985; Reid et al. 1989). Factor H shares the SCR feature with several other regulatory proteins of the complement system, for example, CD55 [decay accelerating factor (DAF)], CD35 (CR1), CD21 (CR2), CD46 [membrane cofactor protein (MCP)], the C4-binding protein (C4bp), and noncomplement proteins as clotting factor XIII B, CD25 [interleukin 2 (IL-2) receptor] and β2-globulin (Reid and Day 1989). The genes encoding DAF, CR1, CR2, MCP, C4bp, and factor H have been localized to chromosome 1 where they form a gene cluster termed RCA (the regulator of complement activation; Klickstein et al. 1987; Rodriguez de Cordoba et al. 1985).

Three different mRNA species for human factor H, 4.3 kilobases (kb), 1.8 kb, and 1.4 kb in size, are constitutively expressed in the human liver (Schwaebel et al. 1987). The 4.3 kb mRNA codes for the major factor H serum protein of Mₐ 150 000 (Ripoche et al. 1988; Estaller et al. 1991a). The 1.8 kb mRNA encodes a truncated form of factor H with a Mₐ of 38 500. This protein is present in human serum and also exhibits cofactor activity for factor I (Misasi et al. 1989). It comprises seven SCR completely identical to the first seven N-terminal SCR of factor H plus four unique amino acids at the C-terminus (McAleer et al. 1989).

Recently, we were able to demonstrate that the 1.4 kb mRNA codes for a doublet of serum proteins with Mₐ of about 39 000 and 37 000 (Schwaebel et al. 1991a; Estaller...
et al. 1991b). Hereafter referred to as p39/p37. Both proteins consist of five SCR, but differ in the extent of glycosylation. Their three carboxyterminal SCR are virtually identical to the C-terminus (SCR 18–20) of the M, 150 000 factor H protein. In contrast, the two N-terminal SCR exhibit only a low degree of homology with SCR 6 and 7 of factor H (Schwaeble et al. 1991a; Estaller et al. 1991b). Two factor H-related proteins with very similar characteristics have recently been characterized from human serum where they are found in a concentration of 40 mg/l (Timmann et al. 1991). However, the biological activities of these proteins are still unknown. Factor H-like cofactor activity, in particular, was not associated with these proteins (Timmann et al. 1991).

The cDNA clones pFH1.4a, pFH1.4b, and H-69 are derived from the 1.4 kb mRNA (Estaller et al. 1991b; Schwaeble et al. 1991a). pFH1.4a and b very likely represent allelic variants as they have been found in the same cDNA library. H-69, which was isolated from a distinct cDNA library, shows three amino acid substitutions in the C-terminal three SCR as compared with the pFH1.4 clones (Fig. 1). This difference may give rise to the different immunological properties observed with the respective recombinant proteins: the polypeptides encoded by pFH1.4a and pFH1.4b reacted with a polyclonal factor H-specific antiserum as well as with the monoclonal antibody (mAb) 3D11. In contrast, mAb 3D11 did not react with the translational product of H-69 which was nevertheless recognized by the polyclonal antiserum.

The aim of the present study was to investigate whether a p39/p37 polymorphism, corresponding to these cDNA data, could also be detected in human sera. Furthermore, we attempted to estimate the distribution of these phenotypes in the healthy population and to establish a possible mode of inheritance.

Materials and methods

Antibodies. The mouse mAb 3D11 directed against the C-terminus of factor H has been described elsewhere in detail (Fontaine et al. 1989). A polyclonal goat antiserum raised against the M, 150 000 factor H protein (Schulz et al. 1985) yielded virtually identical results as a commercially available goat IgG specific for factor H (ATAB-81929; Atlantic Antibodies, Stillwater, MN). The mAb MAH-4 recognizes an epitope at the N-terminus of factor H as previously described (Schulz et al. 1986). Peroxidase-conjugated anti-mouse immunoglobulin and peroxidase-conjugated anti-goat immunoglobulin (Dakopatts, Copenhagen, Denmark) were used as second antibodies.

Blood samples. Serum or ethylenediaminetetraacetate (EDTA)-plasma was obtained by standard methods from 252 unrelated healthy volunteers and from 98 members of 27 two- or three-generation families and aliquots were stored at −20 °C. All individuals involved were Caucasians from western Austria.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and western blotting. A 3 μl aliquot of serum or plasma was diluted 1:20 with sample buffer (0.05 M Tris pH 6.8, 10% glycerol, 2% SDS) and proteins were separated under nonreducing conditions by SDS-PAGE (Laemmli 1970) in gels containing either 7.5% or 12% polyacrylamide (BioRad, Richmond, CA). Proteins were transferred to nitrocellulose (Schleicher and Schuell, Dassel, FRG) by standard techniques (Towbin et al. 1979). Following the transfer the filters were treated with blocking-buffer (phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA), 1% nonfat milk powder, 1% gelatin and 0.1% Triton X 100) for 1 h at room temperature (RT). In the next step the filters were incubated with the polyclonal antibodies (pAbs) or mAbs, diluted in blocking buffer, for 1 h at RT. After washing three times with PBS containing 0.05% Tween 20 the filters were incubated with the respective peroxidase-conjugated second antibody for 1 h at RT. The blots were washed again three times and developed with diaminobenzidine (Sigma, St. Louis, MO) under standard conditions (Maniatis 1982).

Probe. The cDNA clone H-69 has been described in detail elsewhere (Schwaeble et al. 1991a). A 5' end 376 base pair (bp) fragment was obtained by digestion of the H-69 insert with Ava II (Pharmacia, Uppsala, Sweden). This fragment is specific for the 1.4 kb mRNA representing SCR 2' and a part of SCR 1'.

Southern blot analysis. Fifty ml of blood were collected in the presence of 5 mM EDTA and plasma was separated by centrifugation. The leukocytes and remaining erythrocytes were mixed with lysis buffer (155 mM NH4Cl, 10 mM KHCO3, 1 mM EDTA) and left with gentle agitation for 30 min. After centrifugation 10 ml of 0.45 M guanidinium thiocyanate (GTCS; Serva, Heidelberg, FRG) solution containing 25 mM sodium citrate, 0.5% sodium laurylsarcosine pH 7, 0.1 M β-mercaptoethanol were added to the pellet and the mixture left for another 2 h at continuous gentle agitation. Protein were removed by extraction with phenol/CHCl3/amyl-alcohol (25:24:1) and DNA was precipitated with ice cold ethanol. 10 μg of DNA were digested using Eco RI, Eco RV, Eco RI, Hind III, or Smal I (Pharmacia). The DNA fragments were electrophoresed on a 0.7% agarose gel for 20 h and transferred to nylon membranes (Hybond N; Amersham, Amersham, UK) by Southern blotting. The blots were hybridized at 65 °C with the 32P-po-ATP-labeled (Feinberg and Vogelstein 1983) probe. After washing under stringent conditions (30 min at RT with 2 x standard sodium citrate (SSC)/0.1% sodium dodecysulfate, 30 min at 65 °C with 2 x SSC/0.1% SDS, 30 min at 65 °C with 1 x SSC/0.1% SDS, 30 min at 65 °C with 0.1 x SSC/0.1 SDS) blots were exposed on X-ray film (Cronex; Du Pont de Nemours, Bad Homburg, FRG) at −70 °C using intensifying screens.

Results

Three different phenotypes of factor H-related proteins p39/p37 were observed in western blot analyses of human sera employing the mAb 3D11 and a polyclonal factor H-specific antiserum (Fig. 2b and c). They were provisionally termed FH1.4p+m+, FH1.4p+m−, and FH1.4p−m−. With FH1.4p+m+ the two bands cor-