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Human complement factor H: expression of an additional truncated gene product of 43 kDa in human liver*

The human complement factor H is an important factor in the control of the alternative pathway and also induces the stimulation of B cells and macrophages *in vitro*. Using a human factor H cDNA clone as probe, two factor H-specific transcripts of 4.4 and 1.8 kb were detected in four human livers. Both mRNAs were found independently of the expression of an acute phase marker SAA, in these livers, indicating that their presence is not linked to an acute phase state. The shorter transcript was cloned in two cDNA plasmids H-19 and H-20, lacking only seven amino acids of the N-terminus of the mature factor H protein. The deduced protein sequence showed that this protein is identical to the N-terminal portion of the large classical factor H of 150 kDa mol. mass. Parallel to the finding that the N-terminal sequence of factor H is expressed by two distinct mRNA species, evidence is presented that the C-terminal sequence is also contained on two different transcripts, the common 4.4-kb mRNA and an additional 1.0-kb mRNA. A novel, short form of human complement factor H of 43 kDa was detected in human sera which represents the translation product of the 1.8-kb factor H-specific mRNA detected in human liver. Five distinct epitopes detected with 6 monoclonal antibodies are present on both the 150-kDa factor H protein and the truncated 43-kDa molecule. We conclude that additional H-specific mRNAs are found in human liver and that at least one of them is translated yielding a truncated form of factor H.

1 Introduction

The human complement factor H accelerates the decay of the alternative pathway convertase C3bBb and serves as a cofactor for the conversion of C3b to hemolytically inactive iC3b by complement factor I [1–3]. H also binds to B cells by means of a receptor [4, 5], and induces proliferation of murine B cells and the release of factor I from human B cells [6, 7] as well as oxygen radicals from human monocytes [8]. The binding site for C3b on H and the cofactor activity of H for factor I are localized on a N-terminal 38-kDa tryptic fragment of H [9]. We have recently isolated cDNA clones for H from a human liver cDNA expression library [10]. One clone, H-19, was sequenced and shown to code for most of the 38-kDa N-terminal fragment of H although the first 34 amino acids were lacking. The clone also encoded an additional 106 amino acids which are derived from the 142 kDa C-terminal tryptic fragment,but as this clone also contained a 212 bp 3' untranslated region with a polyadenylation signal 15 bp upstream of a poly(A+) tail we investigated whether alternative splicing of a factor H gene transcript might explain this factor H cDNA. As two bands of 4.4 and 1.8 kb were consistently observed in human liver, we investigated whether the 1.8-kb mRNA is

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Abbreviations: B: Factor B of the alternative complement pathway **C3bBb:** Alternative pathway convertase **C4-Bp:** C4-binding protein **CR1:** C3b receptor **H:** Regulatory complement factor, formerly β1H **I:** C3b Inactivator **bp:** Base pair **PBS:** Phosphate-buffered saline **PAGE:** Polyacrylamide gel electrophoresis **SDS:** Sodium dodecyl sulfate

only produced during acute phase conditions and we searched for the translation product of the 1.8-kb mRNA in human sera.

2 Materials and methods

2.1 Northern blot analysis

RNA from human liver, murine liver and human tonsils were prepared by the method of Chirgwin et al. [11]. Ten μg of total RNA was separated on a formaldehyde-containing agarose gel and blotted to nylon filters. Agarose gel electrophoresis, RNA transfer and hybridization of blots were done by standard techniques [12]. Due to the limited biopsy material only a small amount of RNA was obtained from normal liver. Poly(A+) RNA was isolated from total RNA preparations of human acute phase liver. No difference in the hybridization pattern with the various probes was observed comparing total RNA with poly(A+) mRNA samples. The isolated inserts of the different plasmids were labeled with ^{32}P using the random priming method [13]. The DNA probes utilized in these studies are H-19, coding for the N-terminus of factor H, the Tth 111 I fragments of H-19, the cDNA H-46 coding for the C-terminus of factor H, and a probe for human serum amyloid A, pA1 [14]. Hybridization was performed at 65 °C in the absence of formamide. The last washing step was done in 0.1% SSC for 1 h at 65 °C. This stringent wash removes any cross-hybridization of the human probe with murine liver RNA.

2.2 Antibodies

Monoclonal antibodies (mAb) to H, MAH 1-3 [15] and MAH-4 were described previously [10]. OX-23 and OX-24, also directed at H, were a kind gift from Dr. R. Sim, Oxford, GB.

All mAb bind to the 38-kDa N-terminal fragment of H and MAH-1, MAH-2, MAH-4 inhibit the binding of H to C3b as well as the cofactor function of H for I [9, 15, 16]. For some experiments polyclonal goat anti-H antisera were used.

2.3 Factor H

Factor H was purified from fresh human sera by affinity chromatography. One ml of normal human serum was diluted in phosphate-buffered saline (PBS) and run over a 1-ml MAH-4 Sepharose column (2 mg antibody/ml of Sepharose). After five washes in PBS containing either 0.5 M NaCl or 0.01% sodium dodecyl sulfate (SDS), bound proteins were eluted in 5 ml 0.015 M triethanolamine, pH 11.5. In parallel, factor H was also purified by ion-exchange chromatography as described previously [17]. The factor H prepared by ion-exchange chromatography had been exposed to some proteolytic cleavage and the major tryptic fragment of 38 kDa is therefore present in this preparation.

2.4 SDS-polyacrylamide gel electrophoresis (PAGE) and Western blots

One to three µl of human serum or 100 µl of eluate was electrophoresed on a 7.5–15% SDS-PAGE and blotted to nitrocellulose according to the method of Towbin et al. [18]. Blots or individual lanes were stained with mAb to H (MAH-1 to MAH-4, OX-23 and OX-24) and peroxidase-conjugated anti-mouse IgG using standard procedures.

2.5 Construction and screening of a human liver primer extension cDNA library

Recombinant cDNA clones were prepared from 10 µg total liver RNA isolated from human liver (see Fig. 2, lane 4). Synthesis and insertion of double-stranded cDNA by dG : dC homopolymer tailing in the Pst I site of pUC9 used for transformation of E. coli strain DK1 were essentially according to standard protocols [12]. The oligodT primer was replaced by a 36-bp oligonucleotide I derived from H-19 (see Fig. 6) to initiate the synthesis of the first strand. One million colonies were obtained per µg pUC9 vector; 1 × 10^5 were screened with a second 25-bp oligonucleotide II derived from H-19 5' to the first oligonucleotide I (see Fig. 6). The oligonucleotides were labeled with ^{32}P y-ATP and T4-kinase [12]. Thirty cDNA

clones which hybridized to both oligonucleotide probes and contained an internal Bgl II restriction site, present in the very 5' sequence of H-19, were analyzed further. Three clones with the largest insert of approximately 200 bp were sequenced after subcloning in mp 13 vectors by the Sanger dideoxy chain termination method [19].

2.6 Immunoelectrophoresis

Immunoelectrophoresis of acute phase and normal human sera was performed according to Scheidegger [20] in a 1% agarose gel and 0.03 M barbital buffer, pH 8.6. The agarose block was washed and stained with amino black.

3 Results

3.1 Expression of additional factor H transcripts in normal and acute phase human liver

Using H-19 to probe Northern blots of human liver RNA two bands were always detected (Fig. 1A, lane a). The band at 4.4 kb has the expected length for a mRNA coding for the 150-kDa factor H whereas the 1.8-kb band could have given rise to cDNA clone H-19 (size: 1.6 kb). To further investigate the two factor H mRNA species from human liver restriction subfragments of H-19 were employed as probes (Fig. 1B). Two Tht 111 I fragments, containing either the 5' or 3' end of the H-19 cDNA, hybridized to both the 4.4-kb and the 1.8-kb message indicating that H-19 is in fact a faithful reverse transcript of the 1.8-kb mRNA (Fig. 1A, lanes b and c). As the 4.4-kb transcript is also detected by both subfragments of H-19, the small mRNA could be an alternatively spliced transcript of the factor H gene. In order to identify regions of H not contained in this alternatively spliced mRNA we probed Northern blots of human RNA with H-46, a cDNA clone isolated from an expression cDNA library by means of a polyclonal antibody and which was found to be derived from the 142-kd C-terminal region of H [10]. This clone was found to hybridize only to the 4.4-kb and not to the 1.8-kb mRNA (Fig. 2). This confirms that the 1.8-kb message is derived from the 5'end of the factor H gene and lacks the sequence coding for the C-terminus of H as does the cDNA clone H-19. Surprisingly, an additional mRNA species of approximately 1-kb was detected with H-46 which does not hybridize to H-19 (Fig. 2). Human liver therefore expresses three distinct factor H derived mRNAs.
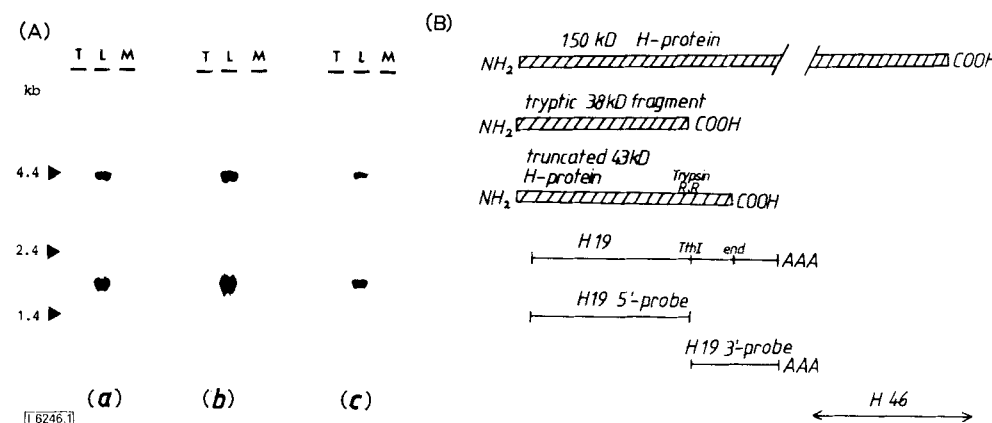
Figure 1. (A) Northern blots of 10 µg total RNA from human liver (L), murine liver (M) and human tonsils (T) hybridized with H-19 (a), the 5'Tht 111 I fragment of H-19 (b) (see (B) and the 3'Tth 111 I fragment of H-19 (c). (B) Schematic representation of the relationship between the complete 150-kDa factor H protein, the 38-kDa tryptic fragment of H carrying the binding site for C3b and the newly described truncated form of H. Also shown are the regions of H coded for by cDNA clones H-19 and H-46 [10] as well as the two Tht 111 I restriction fragments of the H-19 insert used for hybridization of Northern blots in (A).
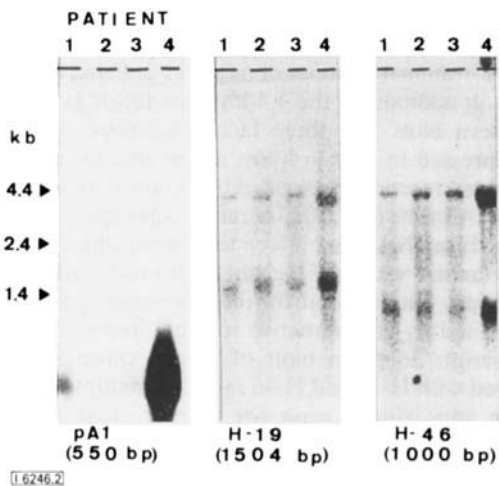
Figure 2. Northern blots of human liver RNA extracted from liver biopsies of three individuals with alcoholic liver disease (lanes 1 to 3) and of one acute phase liver (lane 4). Ten µg of total RNA each was separated on an agarose gel. The central Northern blot was probed with H-19, the right blot with H-46 and the one on the left with a probe for human serum amyloid A (SAA probe pA1).

Figure 3. Western blots of normal (lanes 1–3) and acute phase (lanes 4–6) human sera probed with MAH-4. One µl of human serum was electrophoresed on a 7.5–15% SDS-polyacrylamide gel. The 43-kDa truncated factor H is present in all samples. The molecular mass of the bands is given in kDa.

The first RNA used in these experiments was isolated from acute phase liver. To investigate the possibility that an eventual alternative splicing of factor H mRNA might be linked to acute phase state, as has been postulated by others [21], acute phase liver RNA was compared with liver RNA from biopsies of three patients with alcohol-induced liver disease. The blots were hybridized with H-19 and H-46. As a control for the presence or absence of acute phase reactions in the various liver samples, a probe for human serum amyloid A (SAA probe pA1), a very sensitive acute phase marker in man [22], was included in these experiments. Fig. 2 shows that the level of expression of the two factor H mRNA species is roughly the same in all livers and does not correlate with the expression of the acute phase protein, SAA. This result implies that the 1.8-kb factor H mRNA and the 1.0-kb mRNA are expressed independently of an acute phase reaction and are constitutively synthesized in the liver.

3.2 Identification of the translation product of the 1.8-kb factor H mRNA

As the 1.8-kb message is present at a high level in liver, a small protein with factor H reactivity was looked for in human blood which could be encoded by a 1.8-kb mRNA. When normal and acute phase sera were separated directly by SDS-PAGE and analyzed on a Western blot with MAH-4, a mAb to factor H, two distinct bands, one of 150-kDa corresponding to factor H and another of approximately 43-kDa were always detected (Fig. 3). As with the expression of the 1.8-kb mRNA this additional 43-kDa band was found in normal human sera as well as in sera from patients manifesting an acute phase reaction. This 43-kDa protein reacted with six mAb detecting five distinct epitopes on the 38-kDa N-terminal tryptic fragment of factor H [9, 16] (Fig. 4). Of these, MAH-4 and OX-24 had reacted with the recombinant protein produced by clone H-19 [10]. The truncated 43-kDa factor H product contains the same epitopes as they are present on the N-terminal portion of the large factor H molecule.
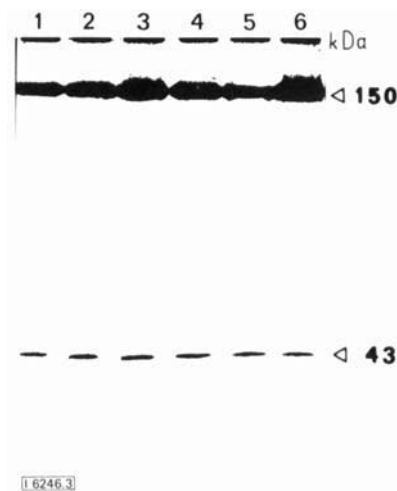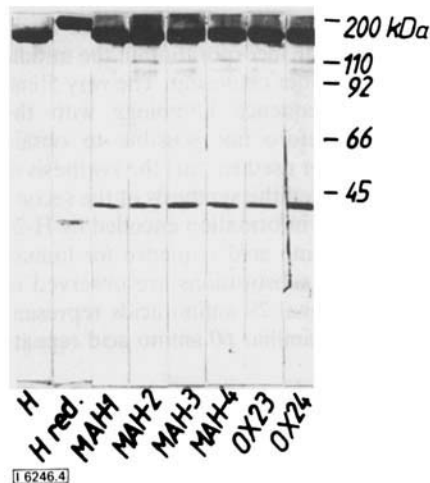
Figure 4. Reactivity of the 43-kDa H-related protein with six mAb to H recognizing five different epitopes. One hundred µl of the affinity-purified eluate was electrophoresed under nonreducing conditions on six lanes of a 7.5–15% SDS-polyacrylamide gel. In parallel, 8 µg of factor H purified by ion-exchange chromatography was run on the same gel under reducing (H red) and nonreducing (H) conditions. Individual lanes were stained with different mAb to H (MAH-1 to MAH-4, OX-23 and OX-24). Lanes H and H red were stained with MAH-4. The factor H prepared by ion-exchange chromatography had been exposed to some proteolytic cleavage and therefore the major tryptic fragment of 38-kDa is visible on these blots under reducing but not under nonreducing conditions. The 43-kDa form of factor H visible in the H preparation obtained by affinity chromatography reacts with all the mAb to factor H and is clearly different in molecular mass from the major tryptic fragment of H at 38-kDa.

In immunoelectrophoresis of human sera two precipitation arcs with β-electrophoretic mobility were found with goat anti-H antisera (Fig. 5). The more acidic precipitation line corresponded to the 150-kDa factor H molecule on SDS-PAGE and Western blot whereas the more basic one represented the novel 43-kDa form factor H (not shown).

Figure 5. Immunoelectrophoresis of acute phase (a) and normal (b) human serum developed with a polyclonal antibody to whole human serum (top through) and a polyclonal antibody to human factor H (central through). Conditions: 1% agarose gel in 0.03 M barbital buffer, pH 8.6, washing and staining with amido black. Anode is on the right.

3.3 Primer extension clones derived from the 1.8-kb mRNA

In order to have the complete 1.8-kb mRNA isolated in cDNA clones a primer extension liver cDNA library was constructed using the information provided by H-19. One primer extension clone, H-20, was characterized which extends the sequence by 28 amino acids. H-19 and H-20 differ by 4 nucleotides at the very 5' end of H-19. We think that the H-19 sequence is most likely incorrect in this position as this is the start of the second strand in cDNA synthesis. In our experience we have never observed any incorrect nucleotide incorporation in the middle of cDNA clones obtained by primer extension. The very 5'end of the 1.8-kb mRNA has sequence homology with the oligonucleotide I. It was therefore not possible to obtain larger inserts, as the same primer used to start the synthesis of the reverse transcript also initiated the synthesis of the second strand. The additional sequence information encoded by H-20 overlaps with the published amino acid sequence for human factor H [23]. Two amino acid substitutions are observed in position 12 and 16. The additional 28 amino acids represent roughly one half of one of the familiar 60 amino acid repeats found in factor H [10].

4 Discussion

The cDNA clone H-19 which was previously thought to be derived from the 4.4-kb mRNA coding for the 150-kDa fac-

tor H protein [10] is a faithful transcript of a 1.8-kb mRNA constitutively expressed in human liver. A 1-kb cDNA (H-46) coding for the C-terminal epitopes of factor H detected a third 1.0-kb mRNA in addition to the 4.4-kb large factor H transcript or Northern blots. All three factor H-derived mRNA species are expressed in human livers in the absence of any sign of acute phase reaction as assessed by a control hybridization with the SAA probe (Fig. 2). Therefore, the expression of the short factor H mRNA is not linked to an acute phase state. The question arises whether the three distinct factor H-specific transcripts are encoded by three separate genes or might be explained by an alternative splicing process of one factor H transcript. Southern blots of human chromosomal DNA hybridized with H-19 and H-46 revealed multiple bands pointing to an approximate gene size of more than 50 kb. From these data we cannot exclude that the three mRNA species are derived from separate genes. In addition the N-terminal sequence obtained for the 43-kDa protein through the isolation of the primer extension clone H-20 differs from the published amino acid sequence of factor H in two positions (see Fig. 6). The differences might be explained by protein sequencing errors or allelic variation [24], but may also indicate that the two factor H proteins are encoded by separate genes. As DNA sequence data of H-46 are not yet available, we concentrated on the expression of the H-19-encoded protein product which should share reactivities with the 150-kDa factor H. In all sera tested a 43-kDa factor H protein was detected. We consider it highly unlikely that this newly described 43-kDa form of factor H is a proteolytic cleavage product of the 150-kDa factor H for the following reasons: (a) the major proteolytic fragment of H sometimes encountered in aged sera is only seen on Western blots under reduced conditions and has a molecular mass of 38-kDa which is clearly distinct from the 43-kDa form of H (see Fig. 4). (b) Using polyclonal antibodies to factor H we never detected the 142-kDa C-terminal tryptic fragment of H (which is complementary to the 38-kDa N-terminal fragment) on unreduced Western blots of human sera (not shown). (c) The 43-kDa band was found whether or not protease inhibitors had been added to plasma immediately after separation of blood cells and its expression did not increase in plasma that had been left at room temperature for 48 h (not shown). The size of this new form of H (43 kDa) is in good agreement with the 1.8-kb mRNA found in human liver.

Figure 6. Sequence of the primer extension cDNA clone, H-20, compared with H-19 and the 17-amino acids sequence obtained from the N-terminus of factor H [23]. Two mismatches with the published amino acid sequence (shown on top) were found in position 11 and 15. The sequence of the oligonucleotide I used to construct the primer extension cDNA library is marked with * * * and the oligonucleotide II employed for screening this library is indicated with · · · beneath the H-19 sequence. Amino acids are shown by the single-letter code and are numbered according to the published factor H protein sequence [23].

We therefore conclude that a novel form of human complement factor H with a molecular mass of 43-kDa can be found in human serum and represents the translation product of an additional H-derived mRNA in human liver. This truncated form of factor H is synthesized under normal conditions and represents that portion of whole factor H which carries the cofactor activity for factor I and the binding for C3b. As to the physiological function of the truncated 43-kDa H protein we speculate that some of the many known functions of whole H (binding to C3b, cofactor activity for I, B cell and monocyte stimulation) may be more efficiently mediated by the 43-kDa molecule. These hypotheses are at present under investigation.

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