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## Original Papers

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### Human Complement Factor B: Functional Properties of a Recombinant Zymogen of the Alternative Activation Pathway Convertase\*

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#### Abstract

The human complement factor B is a centrally important component of the alternative pathway activation of the complement system. Here we report the isolation, characterization and eukaryotic expression of the first full length cDNA transcript for human factor B. In a factor B dependent haemolysis assay, the recombinant human factor B generated by transient COS cell transfection was shown to reconstitute haemolytic activity of factor B depleted human serum. To study the biological activities assigned to factor B, the availability of recombinant polypeptides representing definite portions of the human factor B molecule is desirable.

#### Introduction

The human complement factor B is one of the six components that constitute the alternative pathway activation of the complement system which provides an antibody independent route for the destruction of bacteria, neutralization of viruses and lysis of certain mammalian cells (1, 2). Factor B is a heat-labile, single-chain glycoprotein of 93 kDa which is contained in human plasma in its zymogen form at a concentration of 100–400 mg/l. In the presence of magnesium ions, factor B binds to fluid phase or surface bound C3b or hydrolysed C3 (C3H<sub>2</sub>O) forming a C3bB proenzyme complex. This complex is the substrate for the cleavage of factor B by a serum protease, factor D. Immediately upon removal of a 30 kDa

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+ This work is in partial fulfillment of T. Sokolowski's MD thesis.

° Recipient of a professorship of the Stifterverband für die Deutsche Wissenschaft.

aminoterminal fragment (Ba) of factor B, the remaining C3bBb complex expresses C3 convertase activity. The conversion of C3 to C3a and C3b again forms an important amplification loop as nascent C3b may initiate the generation of other C3bBb complexes. Activation of the alternative pathway ends in the assembly of the C5 convertase which is created by binding of an additional C3b molecule in close proximity to the C3bBb complex. These complexes, C3bBb and C3b<sub>2</sub>Bb, have a short half-life in solution since they are subject to spontaneous as well as factor-H-mediated decay dissociation (3, 4). Therefore, the activation of the alternative pathway is centrally regulated by properdin, a serum component that stabilizes these enzymes (5, 6). Besides the zymogen function, additional biological activities have been observed for both the Ba and the Bb fragments of factor B. Purified human Ba was shown to inhibit proliferation of preactivated human B lymphocytes by binding to a distinct cell surface receptor (7), whereas purified Bb was shown to enhance proliferation of preactivated human B lymphocytes and to act synergistically with IL-2 and B cell growth factor by binding to the high molecular weight B cell growth factor receptor which induces B lymphocyte differentiation into antibody-secreting cells (8). Furthermore, purified Bb was shown to induce the rapid spreading of human peripheral blood monocytes (9), to stimulate lymphocyte blastogenesis (10), to promote the intracellular killing of *Staphylococcus aureus* by human peripheral blood monocytes (11), and to enhance the cell mediated lysis of xenogenic erythrocytes by human peripheral blood monocytes (12).

The structure and organization of the human factor B gene within the class III region of the major histocompatibility complex (MHC) on the short arm of chromosome 6 (13) has been determined by gene cloning (14, 15). The entire amino acid sequence of human factor B has been established combining both peptide sequencing and translation of the partial nucleotide sequences of cDNA and cosmid clones (14–19). In this contribution, we present the first full-length cDNA transcript for human factor B and the functional analysis of the recombinant zymogen obtained by expressing this cDNA in COS cells.

## Materials and Methods

### *Construction and screening of a human cDNA library in the eukaryotic expression vector CDM8*

RNA was prepared from human liver tissue by extraction with guanidinium thiocyanate and centrifugation through a cesium chloride gradient (20). Poly(A)<sup>+</sup> RNA was purified by oligo(dT) cellulose chromatography. Approximately 15 µg poly(A)<sup>+</sup> RNA was employed for cDNA synthesis using a kit supplied by BRL, Karlsruhe, Germany. Size-selected cDNA fragments (> 1 kb) were ligated with Bst XI linker and cloned into purified Bst XI-linearized vector CDM8, following exactly the protocol provided by Dr. B. SEED, Boston, MA, USA (21, 22). About 300,000 colonies were screened with cDNA probe FB1 (kindly provided by Dr. R. D. CAMPBELL, Oxford, UK) representing ca. 500 bp of the human factor B mRNA (14). Positive colonies were subjected to a rescreening procedure which yielded five different cDNA

clones for human factor B, termed pFB1-5. These clones were further characterized by restriction enzyme analysis and cDNA sequencing.

#### *DNA sequence analysis*

To determine whether one or more of the potential factor B clones contained the complete coding sequence of the factor B mRNA, the cDNA insertions from each of the five clones were partially sequenced from both ends, using oligonucleotides, ocdm8-1 (CTGGCTAAC-TAGAGAAC) located 5' and ocdm8-2 (GATCCTCTAGAGTCGC) located 3' of the Bst XI cloning site. DNA sequence analysis was performed with T7 polymerase and the reagent kit supplied by Pharmacia, Freiburg, Germany.

#### *Northern blot analysis*

Total RNA was prepared by the method of CHIRGWIN et al. (20), quantified by measuring the absorbance at 260 nm. 10 µg were separated on a formaldehyde-containing 1.2 % agarose gel and blotted to Hybond N filters. Agarose gel electrophoresis, RNA transfer and hybridization of blots were performed by standard techniques (23). Poly (A<sup>+</sup>) RNA was selected from total RNA preparations using the «mRNA Purification Kit» (Pharmacia). Factor B cDNA was labeled with <sup>32</sup>P-dATP using the random priming method (24). The probe was used at a concentration of 5 × 10<sup>6</sup> cpm of labelled cDNA/ml hybridization solution. Hybridization and washing of the Northern blots was carried out according to the method described by CHURCH and GILBERT (25). The last washing step was performed in 0.3 × SSC/0.1 % SDS for 1 h at 65 °C.

#### *COS cell transfection*

The factor B cDNA in the vector CDM8 was transfected into COS-7 cells via electroporation using a Gene Pulser apparatus (BioRad, Munich, Germany). Prior to transfection, 1 × 10<sup>7</sup> COS cells in suspension were washed in sucrose buffer (272 mM sucrose, 7 mM Na<sub>2</sub>HPO<sub>4</sub> pH 7.4, 1 mM MgCl<sub>2</sub>) and resuspended in 0.8 ml sucrose buffer. The suspension was transferred to a 0.4 cm electroporation kuvette (BioRad) and 30 µg DNA were added. The sample was incubated on ice for 10 min and then electroporated at 25 µF and 400 V. Then the cells were incubated for an additional 10 min on ice and transferred to a cell culture bottle containing 15 ml DMEM and 10 % FCS. After overnight incubation at 37 °C the culture supernatant was collected and centrifuged. The cells were resuspended in 30 ml CG-medium and added to the remaining adherent COS cells. 84 h post transfection, the supernatant was collected and subjected to a Western blot analysis. As a control, supernatant from nontransfected COS cells was loaded on the same gel.

#### *Western blot analysis*

Supernatant of COS cell transfectants (5 µl) were analyzed by SDS-PAGE on a 9-15 % gradient gel under nonreducing conditions (26) and blotted to nitrocellulose according to the method of TOWBIN et al. (27). Blots were stained with a polyclonal goat anti-human factor B antibody (purchased by Quidel, San Diego, CA, USA) and peroxidase-conjugated anti-goat IgG (purchased by Dakopatts, Hamburg, Germany) using standard protocols.

#### *Factor B ELISA*

An enzyme-linked immunosorbent assay (ELISA) was carried out for factor B on microtiter plates (78-381-04, Flow Laboratories, UK). The wells were coated overnight at 4 °C with 5 µg/ml of rabbit anti-human factor B IgG (Quidel). The plates were washed five times in PBS/tween-buffer and blocked with PBS/tween-buffer containing BSA (10 mg/ml) for 1 h at room temperature. After washing 5 × with PBS/tween-buffer, the samples (100 µl) containing factor B were applied in PBS/tween-buffer containing BSA (0.5 mg/ml) and dilutions made in the same buffer. To quantify the relative amount of factor B contained in supernatant of the COS cells transfected with cDNA clone pFB2, a standard curve was drawn with dilutions of normal human serum at 1:2000, 1:4000, 1:8000, 1:16,000, 1:32,000, 1:64,000, 1:128,000, and 1:256,000

which correspond to 100, 50, 25, 12.5, 6.25, 3.12, 1.56, and 0.78 ng of serum factor B per ml. The samples were incubated at 4 °C in a moist box overnight. After washing 5× in PBS/tween-buffer, the bound factor B was detected by the addition of biotinylated rabbit anti-human factor B IgG (150 µl of 1:2000 dilution of 10 mg/ml biotinylated antibody IgG fraction) followed by streptavidin-alkaline phosphatase (150 µl of 1:10,000 dilution of 1 mg/ml solution). Finally phosphatase substrate (Sigma 104; 50 µl of 1 mg/ml in pH 9.5 development buffer) was added and the absorbancy was measured at 405 nm. The assay was sensitive to a level of 0.1 ng/100 µl of sample.

#### Haemolytic assay

Factor B activity was determined in a haemolysis assay measuring the formation of C3bBbP on EAC43b cells (28) by the ability of culture supernatant to restore lysis of rabbit erythrocytes by factor B depleted serum. Fifty microliters of supernatant of pFB2 transfected COS cells were mixed with 50 µl of factor B-depleted human serum (purchased from Quidel) and rabbit red blood cells ( $2 \times 10^8$  in 25 µl of PBS-Mg<sup>++</sup>-EGTA), and incubated at 37 °C for 15 min. After addition of ice-cold PBS-Mg<sup>++</sup>-EGTA (1.0 ml), the samples were spun (10 min at 1500 rpm) and the degree of lysis estimated from the E 1 cm/412 nm of the supernatants.

## Results

### Isolation and sequence analysis of the full-length cDNA clone pFB2

Five potential factor B cDNA clones obtained the human liver cDNA library were subjected to a Northern blot analysis. All of them strongly hybridized to a mRNA species of ca. 2.6 kb in human liver RNA (not shown). The orientation of the cDNA clones was determined by digestion with HindIII which cuts in the 5' region of the human factor B cDNA and in the 5' linker sequence of the vector CDM8 (see Fig. 1). Three of the five potential factor B clones were found to contain a cDNA insert in the correct 5' to 3' orientation. Sequencing of the 5' and 3' ends of the inserts confirmed the orientation and revealed that one of these clones, pFB2 potentially comprises the entire coding sequence of the factor B mRNA. Therefore, the complete cDNA sequence of 2447 bp contained in pFB2 was determined. As shown in Figure 2 pFB2 comprises 111 nucleotides of a 5' untranslated region, followed by an open reading frame of 2292 bp,

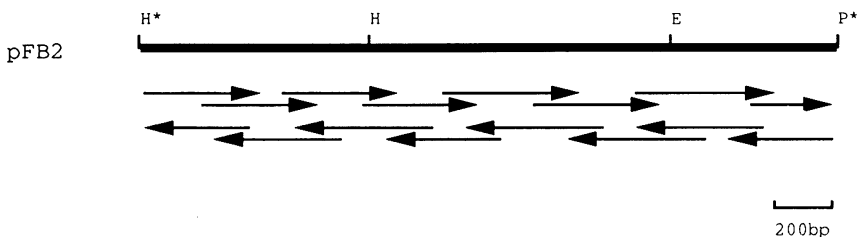


Figure 1. Full-length factor B cDNA pFB2 in the vector CDM8. The restriction map of pFB2 is shown for the following restriction enzymes: E, Eco RI; H, Hind III; P, Pst I. Short horizontal arrows show the direction of DNA sequence determination with oligonucleotides localized in the CDM8 linker region or the internal cDNA sequence. Sites marked with an asterisk are located in the CDM8 polylinker sequence.



encoding a typical leader peptide of 25 amino acids and the entire 739 amino acids of mature factor B. The coding sequence is followed by a stop codon (position 2404) and a 3' untranslated region of 41 bp containing a polyadenylation signal (ATTAAA) 33 bp downstream of the stop codon. A comparison between the nucleotide sequence of pFB2 and previously published partial cDNA and genomic sequences is given in Figure 1.

Partial exon sequencing data which have been determined from the factor B specific cosmid Cos 10 (15) were aligned over a stretch of 408 bp to the corresponding sequence at the 5' end of pFB2. The comparison revealed that the 5' untranslated region of pFB2 starts 18 bp downstream from the mRNA start site contained in Cos 10. The 5' sequence of pFB2 is in total agreement with the sequence of the two exons analyzed in Cos 10. The pFB2 sequence confirms that no intron interrupts the 5' untranslated and the coding sequence for the signal peptide as previously determined by a S1 nuclease mapping analysis of Cos 10.

Moreover, the 3' portion of pFB2 was colinear with the sequence of eleven exons analyzed in another factor B cosmid, termed Cos1 (14). Within this 1299 bp stretch, one transition (a «G» in Cos1 instead of an «A» in position 1966 of the pFB2 sequence) was observed which results in an amino acid exchange in the derived factor B peptide sequence («GAG» codes for glutamic acid in the Cos1 sequence instead of «AAG coding for lysine in pFB2). Furthermore, the pFB2 sequence was aligned to three other previously published partial cDNA sequences for human factor B, the cDNA FB1 (14) that was used to isolate pFB2, the cDNA pFB3a (15) containing the coding sequence for the Ba fragment of factor B in a reversed orientation, and the cDNA pBfA28 (18) that comprises the coding region for the entire Bb fragment. The alignment of the 515 bp long FB1 sequence shows silent exchanges in two nucleotide positions, one transition at 1326 («T» in the FB1 sequence instead of a «C» in the pFB2 sequence) and a transversion in position 1422 («C» in the FB1 sequence instead of a «G» in pFB2). A comparison of the pFB2 sequence with the 710 bp long coding sequence for the Ba fragment contained in pFB3a revealed total identity. The alignment of the pFB2 sequence with pBfA28 includes a stretch of 2075 bp. Within this length, pBfA28 differs from the pFB2 sequence in sixteen nucleotide positions. The substitutions in the positions 552, 561, 663, 1194, 1401, 1632, 1755, 1770, 1821, and 2316 do not alter the derived amino acid sequence whereas the transversions in the positions 1009 («CTG» coding for leucine in pBfA28 instead of «GTC» coding for valine in pFB2) and

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Figure 2 (see next pages). Nucleotide sequence of the full length cDNA clone pFB2 in comparison with all other as yet published cDNA and exon sequence data for human factor B. ► The protein coding region is shown in capital letters. Identity between pFB2 and the compared sequence is indicated by dots. Wherever an aligned sequence differs from the sequence of pFB2, the divergent base is given below. The polyadenylation signal, ATTAAA, in the 3' untranslated region is shown in bold type underlined.

```

10      20      30      40      50      60      70      80      90
caggtctaggtctggagtttcagcttggacactgagccaagcagacaagcaagcaagccaggacacaccatcctgccccaggccagct pFB2
..... Cos10

100     110     120     130     140     150     160     170     180
tctctcctcctccaagcgcATGGGGAGCAATCTCAAGCCCCAACTCTGCCCTGATGCCCTTTATCTTTGGCCCTTGTCTGGAGGTGTG pFB2
..... Cos10
..... pFB3a

190     200     210     220     230     240     250     260     270
ACCACCACTCCATGGTCTTTGGCCCCGGCCCCAGGATCTCTCTCTCGAGGGGTAGAGATCAAAAGCGCTCTTCCGACTTCTCCAA pFB2
..... Cos10
..... pFB3a

280     290     300     310     320     330     340     350     360
CAGGGCCAGGCACTGGAGTACGTGTGTCTTCTGCTTCTAACCCTGACCTGTGCAGACAGTAOCTGCAGATCTACGGGGTCTGGAGC pFB2
..... Cos10
..... pFB3a

370     380     390     400     410     420     430     440     450
ACCCTCAAGACTCAAGACCAAAAACACTGTCAAGGAGCCAGAGTGCAGCAATCCACTGTCCAAAGCAACCAAGCACTTCGACAAAGCGGAA pFB2
..... Cos10
..... pFB3a
..... pBfA28

460     470     480     490     500     510     520     530     540
TACTGGCCCCGGTCTCCCTACTACTAATGTGTGATGATGAGATCTCTTTCCACTGCTATGACGGTTACACTCTCCGGGGCTCTGCCAATGCC pFB2
..... pFB3a
..... pBfA28

550     560     570     580     590     600     610     620     630
AOCCTGCCAAGTCAATGGCCGGTGGAGTGGCCAGACAGCACTCTGTGCAACGGAGCGGGTACTCTCCAAACCCGGGCATCCCAATTGGC pFB2
..... pFB3a
..... pBfA28
..... C..... A.....

640     650     660     670     680     690     700     710     720
ACAAGGAGGTGGCCAGCCAGTACCCGCTTGAAGACAGCGGTCACTACCCTGCGGCCGGCCCTTACCCCTGGGTGGCTCCAGCGGCCA pFB2
..... pFB3a
..... pBfA28
..... G.....

730     740     750     760     770     780     790     800     810
ACGTGTCAAGCAAGTGGCTCTTGGAGCGGGCAAGCGGCTTCTGCCAAGACTCTTCATGTATGACACACCCCTCAAGAGGTGGCCGAACT pFB2
..... pFB3a
..... pBfA28

820     830     840     850     860     870     880     890     900
TTCTCTGTCTTCCCTGACAGACCATAGAGGAGTGCATGCTGAGCATGGGCACGGCCAGGGGAACAACAGAGGGGAAGATCGTCTCTG pFB2
..... pFB3a
..... pBfA28

910     920     930     940     950     960     970     980     990
GACCCCTTCAGGCTCCATGACATCTACCTGCTGCTAGATGATCAACAGCAATTGGCCCGCAACTTCCACAGGACCAAAAAGTGTCTA pFB2
..... pBfA28

1000    1010    1020    1030    1040    1050    1060    1070    1080
GTCAACTTAAATGAGAGGTGGCAAGTATGCTGTGAAGCCAAAGATATGGTCTAGTGCACATATGCCACATACCCCAAAATTTGGGTCAA pFB2
..... pBfA28
..... C.....

1090    1100    1110    1120    1130    1140    1150    1160    1170
GTGTCTGAGGACAGACAGGATATCCAGACTGGGTCAAGGAGGCTCAATGAAATCAATTTATGAAGACCAAGTITGAAGTCAGGCACT pFB2
..... FB1
..... Cos1
..... pBfA28
..... T.....

1180    1190    1200    1210    1220    1230    1240    1250    1260
AACACCAAGAGGCCCTCCAGGCAGTGTACAGCATGATGAGCTGGCCAGATGAAGTCCCTCTGAAAGCTGGAAACCGCACCCGCCATGTCT pFB2
..... FB1
..... Cos1
..... pBfA28
..... G.AG.A..... A.....

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1270	1280	1290	1300	1310	1320	1330	1340	1350	
ATCATCCTCATCTGATGGATTGCAACAACATGGGGGGGACCAATTACTGTCAITGATGACATCCGGACTTGTATACAITGGCAAG									pFB2
.....T.....									FBI
.....									Cos1
.....									pBFA28
1360	1370	1380	1390	1400	1410	1420	1430	1440	
GATCGCAAAAACCAAGGGAGCATTATCTCGATGTCIATGTGTTTGGGGTGGGCTTTGGTCAACCAAGTGAACATCAATCCTTTGCT									pFB2
.....C.....									FBI
.....									Cos1
.....G.....									pBFA28
1450	1460	1470	1480	1490	1500	1510	1520	1530	
TCCAAGAAACAATGAGCAACATGTGTTCAAGTCAAGGATATGAAAACCTGCAAGATGTTTTCACCAATGATCAGTGAAGCCAG									pFB2
.....									FBI
.....									Cos1
.....									pBFA28
1540	1550	1560	1570	1580	1590	1600	1610	1620	
TCTCTGAGTCTCTGTGGCATGGTTTGGAAACACAGCAAGGGTACCGATTACCAAGCAACCATGGCAGGCCAAGATCTCAGTCAITGCG									pFB2
.....									FBI
.....									Cos1
.....									pBFA28
1630	1640	1650	1660	1670	1680	1690	1700	1710	
CCTTCAAGGGACACGAGCTGTATGGGGCTGTGGTCTGAGTACTTTGTGCTGACAGCAGCAATTTGTTTCACTGTGGATGACAAG									pFB2
.....									FBI
.....C.....									Cos1
.....									pBFA28
1720	1730	1740	1750	1760	1770	1780	1790	1800	
CAACTCAATCAAGGTACAGGTAGGAGGGGAGAACCGGACCTGGAGATACAAAGTAGTCTTATTTCAACCCCACTCAACATTAATGGG									pFB2
.....C.....A.....									Cos1
.....									pBFA28
1810	1820	1830	1840	1850	1860	1870	1880	1890	
AAAAAGAGGAGCAATTCCTCAATTTTATGACTATGACGTTGCCCTGATCAAGCTCAAGATAAGCTGAAATATGCCAGACTATCAGG									pFB2
.....C.....									Cos1
.....									pBFA28
1900	1910	1920	1930	1940	1950	1960	1970	1980	
CCATTTGTCTCCCTGCACCGAGGGAACTACTGAGCTTTTGGGCTTCTCCAACTACCACTTGCAGCAACAAAAGGAGAGACTGTCTC									pFB2
.....G.....									Cos1
.....									pBFA28
1990	2000	2010	2020	2030	2040	2050	2060	2070	
CCTGCACAGGATATCAAGCTCTGTTTGTGTCTGAAGGAGAAAAAGCTGACTGGGAGGAGTCTACATCAAGATAAGGGGATAAGAAA									pFB2
.....									Cos1
.....									pBFA28
2080	2090	2100	2110	2120	2130	2140	2150	2160	
GCGAGTGTGAGAGATGCTCAATATGCCCGAGGCTATGACAAAGTCAAGCAATCTCAGAGTGGTCAACCCCTGGTTCCTTTGTACT									pFB2
.....									Cos1
.....									pBFA28
2170	2180	2190	2200	2210	2220	2230	2240	2250	
GGAGGAGTGTGCTCCTATGCTGACCCCACTTGCAGAGGTGATCTGGGGGGCTTTCATAGTTCACAAGGAGGTGTTTTCATTCAA									pFB2
.....									Cos1
.....									pBFA28
2260	2270	2280	2290	2300	2310	2320	2330	2340	
GTTGGTGAATCAGCTGGGAGTAGTGTGCTGCAAAAACCAAGAGGGGCAAAAGCAGGTACCTCCTCACCCGACACTTTTCATTC									pFB2
.....C.....									Cos1
.....									pBFA28
2350	2360	2370	2380	2390	2400	2410	2420	2430	
AACCTTTTCAAGTCTGCCCTGGCTGAAAGGAACTCCAGATGAGCATTTGGGTTTCTATAGggggtttctgctggacagggggg									pFB2
.....									Cos1
.....									pBFA28
2440	2450								
tgggattgaattaaac									pFB2
.....									Cos1
.....									pBFA28

1094 («GTC» coding for valine in pBfA28 instead of «GAC» coding for aspartic acid in pFB2) lead to changes in the derived amino acid sequence. In addition, two inversions also lead to changes in the derived amino acid sequence, one in position 1177–1179 («AAG» coding for lysine in pFB2 instead of «GAA» coding for glutamic acid in pBfA28) and another in position 1180–1183 («GAA» coding for glutamic acid in pBfA28 instead of «AAG» coding for lysine in pFB2). The cDNA sequence of pFB2 completes the pBfA28 sequence in sections where the nucleotide sequence was not determined or ambiguity was observed between the cDNA sequence data and the determined protein sequence (see gaps indicated by the omission of dots). Two of these gaps in the pBfA28 sequence were also not filled by any of the other as yet published cDNA or exon sequence data. For these sections, the pFB2 cDNA adds a novel coding sequence which taken together comprises 16 nucleotide residues. The peptides encoded by this added sequence are consistent with the protein sequencing data (15–18).

#### *Eukaryotic expression of a recombinant zymogen for human factor B*

As the liver cDNA library was constructed in the eukaryotic expression vector CDM8, the cDNA was tested directly for the encoded protein by transient expression in COS cells (21, 22). Three days after transfection, the

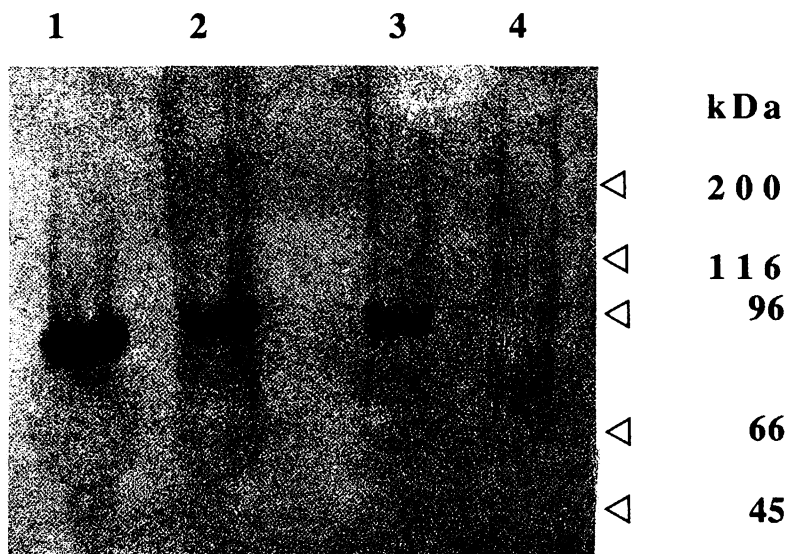


Figure 3. Western blot analysis for factor B expression in Cos cell transfectants. 50  $\mu$ l of supernatant from different transfection assays were subjected to SDS-PAGE under nonreducing conditions and then analyzed by Western blotting with a polyclonal goat anti-human factor B antiserum. Lane 1: 5  $\mu$ l supernatant from  $15 \times 10^6$  COS cells transfected with pFB2, lane 2: 5  $\mu$ l supernatant from  $5 \times 10^6$  COS cells transfected with pFB2, lane 3: 5  $\mu$ l supernatant from  $9 \times 10^6$  COS cells transfected with pFB2, lane 4: 5  $\mu$ l supernatant from  $15 \times 10^6$  nontransfected COS cells. On the right, the position of the sizemarkers is indicated in kDa.

Table 1. Haemolytic activity of serum factor B and the recombinant factor B secreted by pFB2 transfected COS cells

Factor B	( $\mu\text{g/ml}$ ) <sup>a</sup>	(em/ml) <sup>b</sup>	(em/ $\mu\text{g}$ )
Control <sup>c</sup>	0	0	0
Prep 1	7.4	$86.3 \times 10^7$	$11.7 \times 10^7$
Prep 2	1.0	$26.0 \times 10^7$	$26.0 \times 10^7$
Prep 3	2.0	$36.4 \times 10^7$	$18.2 \times 10^7$
Serum	151.0	$5.6 \times 10^{10}$	$37.1 \times 10^7$

<sup>a</sup> Factor B protein concentration ( $\mu\text{g/ml}$ ) measured by ELISA.

<sup>b</sup> Factor B functional activity (expressed as effective molecules/ml) measured by haemolytic assay.

<sup>c</sup> Supernatant from COS cells transfected with the expression vector CDM8 without a cDNA insertion was assayed as a negative control.

supernatants were assayed in duplicate in a factor B ELISA. The relative amount of recombinant factor B secreted by the COS cells was determined in reference to the factor B concentration in a standard dilution of human serum. Supernatants from three pFB2 transfections, done with different numbers of COS cells, were analyzed. In a transfection of  $15 \times 10^6$  COS cells (Prep 1), the relative amount of recombinant factor B was determined to be ca.  $7.4 \mu\text{g/ml}$ . The supernatant from two other transfection assays of  $5 \times 10^6$  and  $9 \times 10^6$  COS cells (Prep 2 and Prep 3) were found to contain a relative amount of  $1.0 \mu\text{g/ml}$  and  $2.0 \mu\text{g/ml}$  factor B respectively. These results correspond to a relative amount of 3–8  $\mu\text{g}$  of recombinant factor B secreted by  $10^6$  COS cells. To confirm the ELISA results, the supernatants were subjected to a Western blot analysis. As shown in Figure 3, in all supernatants from pFB2 transfections, a single band of the expected size of ca. 93 kDa was stained with a polyclonal factor B antiserum (see lanes 1–3). The intensity of staining corresponds to the relative abundance of recombinant factor B measured in the ELISA. As a negative control, supernatant from nontransfected COS cells was loaded in lane 4.

#### *Haemolytic assay for functional activity of recombinant factor B*

Recombinant factor B was functionally active as ascertained by haemolytic assay. The haemolytic activity of serum factor B and the culture supernatants from the three different COS cell transfections was assayed in triplicate. The mean values of the assays are shown in Table 1.

When calculating the number of effective molecules per microgramm of factor B, the recombinant human factor B expressed nearly the same functional activity as factor B in normal human serum.

## Discussion

The availability of the first correct full-length cDNA transcript of human factor B mRNA made it possible to generate a recombinant zymogen for

the activation of the alternative pathway of the complement system. The nucleotide sequence of the cDNA contained in clone pFB2 completes the fragmentary cDNA sequence data determined from previously published partial cDNA clones for human factor B. At the 5' end, the pFB2 sequence adds 156 residues to the known cDNA sequence for factor B and confirms the corresponding genomic sequence data obtained from a factor B cosmid. Moreover, the cDNA sequence of pFB2 adds 16 residues of the nucleotide sequence in the coding region of the factor B mRNA which were neither contained in the previous cDNA nor in the genomic sequence data determined for the factor B gene. An alignment between the pFB2 sequence to all the as yet known nucleotide sequence data for factor B revealed a polymorphic structure for factor B mRNA. The pFB2 sequence can be related to the S allele of the factor B gene as in amino acid position seven of the mature protein. pFB2 codes for an arginine, which is specific for the BF S type, instead of a glutamine in the BF F allotype (29, 30). Of the observed nucleotide substitutions, one seen in comparison with the Cos1 sequence and three seen in comparison with the pBfA28 cDNA resulted in changes in the encoded peptide sequence. The transfection assays with pFB2 have shown, that a reasonable amount of recombinant human factor B can be expressed in COS cells. In a factor B-dependent haemolysis assay, the translational product of pFB2 was shown to express functional activity. The number of effective molecules per microgramm of recombinant factor B varied between the different transfection assays, but was nearly the same as that of its natural serum counterpart. A reconstitution of the alternative pathway activation by the recombinantly expressed zymogen requires that the generated peptide can efficiently bind to C3b or C3(H<sub>2</sub>O), that the peptide can be converted subsequently to Bb in a factor D mediated cleavage, and that the Bb portion of this recombinant factor B exhibits serine esterase activity for the conversion of C3 to C3b. As most of the functional sites of factor B have recently been mapped on proteolytic subfragments of the factor B molecule (14, 31), clone pFB2 could be a useful tool to generate definite truncated forms of factor B that express some of the important biological activities of this complement regulatory component.

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