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ORIGINAL ARTICLE

An Optimized Protocol for the Expression of a Recombinant Protein Fragment of Human Factor B Used For the Production of Polyclonal Antibodies

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ABSTRACT

Background: *Complement is a critical component of the innate immunity bridging both arms of the immune system. Extensive trials are ongoing to develop therapeutics that target complement and hence alleviating complement-mediated disorders.* ***Objectives:*** *Here, we detail our attempts to express a recombinant protein fragment of human factor B (FBfr) in a high yield and solubility in addition to the production of polyclonal antibody against it.* ***Methodology:*** *We tested different expression conditions and host strains to express FBfr. Moreover, we used the expressed Fbfr to immunize rabbits for production of polyclonal antibody and tested its inhibitory properties against the alternative pathway by in vitro assays.* ***Results:*** *We successfully expressed an alternative complement pathway inhibitor FBfr in E. coli using an optimized protocol. The protein was antigenic and led to the production of a high titer of antibodies in sera of the immunized rabbits. The polyclonal antibody was shown to have a moderate inhibition of the alternative complement pathway activity.* ***Conclusion:*** *optimization of expression parameters can greatly improve the expression levels of recombinant proteins. These proteins can have a functional activity and be used for successful immunization process.*

INTRODUCTION

Complement functions mainly in eliminating microbes through target recognition, opsonization, recruitment of immune cells and cell lysis. It also participates in elimination of immune complexes, injured cells and damaged tissues from circulation. The complement cascade comprises nearly 50 soluble and cell surface components ranging from the crucial proteases, cofactors and complement regulators [1-5]. Three activation pathways of complement are well-defined: the classical pathway (CP), the alternative pathway (AP) and the lectin pathway (LP). Regardless of the initiator, the three pathways converge at a fundamental amplifying stage featured by the assembly of the C3 convertase [6].

Either complement insufficiency or dysregulated activation leads to imbalance in complement. This could result in undesirable tissue damage and important pathological consequences. Hence an increasing awareness has placed complement in the heart of approaches of immunomodulation and alleviation of inflammatory conditions [7]. Efforts to develop potent complement inhibitors are anticipated in view of the diversity and impact of complement-mediated diseases [8], yet it is impossible to find a "one-size-fits-all" solution for the management of these disorders. Luckily, the presence of variable intervention points within the complement cascade, the potent inhibitors available in nature and a spike in structure-function insights have all established the rapid advancements in the field of complement-related therapeutics [9-12]. The AP massively amplifies the activation resulting from all pathways. Such character makes the AP a striking target for therapeutic interventions aiming to modulate immunological activation and inflammatory process.

In the current study, we report the attempts made to optimize the expression of a fragment of human Factor B (FBfr) in *E. coli* which we reported its inhibitory activity in our previous study [13]. Additionally, we used this recombinant fragment to produce a novel polyclonal antibody that showed moderate inhibitory activity against the AP.

METHODOLOGY

Ethical approval

This study was approved by the Research Ethics Committee of the Faculty of Pharmacy, Mansoura University, Egypt (Code. 2024-99).

Bacterial strains

Escherichia coli BL21(DE3) (Invitrogen™, Thermofisher Scientific (USA)), BL21(DE3)pLysS (a kind gift from Prof. Mohammed Youssef Ali, School of Biological Sciences, University of Cambridge), *E. coli* BL21-A*I* and Rosetta(DE3)pLysS (kindly provided by Dr. Noha Mansour, Associate Professor of

Biochemistry, Faculty of Pharmacy, Mansoura University) were used throughout this study.

Expression of FBfr protein

For optimization of FBfr expression, time-course expression experiments were initiated using BL21(DE3) and BL21(DE3)pLysS *E. coli* bacterial host strains[14]. In brief, pRSET-B/FBfr construct[13] was transformed into the chemically-competent bacterial host strains. The transformants were selected using the appropriate antibiotic **(Table 1)** and a single colony from each type of host strains was cluttered overnight in 5 ml of LB at 37 ℃ with shaking at 225 rpm. The overnight culture was diluted 1:10 with fresh LB broth in the presence of selection antibiotic. Culture was incubated at 37 ℃ with shaking at 225 rpm until an OD_{600nm} of 0.4-0.6. At this point, IPTG was added to a final concentration of 1 mM. Periodic samples were collected and centrifuged at 6000 rpm for 10 min. The total, insoluble and soluble protein samples were analyzed by 12% SDS-PAGE and Western blot using HRP-conjugated monoclonal mouse anti-polyHistidine antibody to detect the his-tag residues on the recombinant protein. Different bacterial hosts were also tried in expression of FBfr including BL21-AI™ and Rosetta™ (DE3)pLysS.

Table 1: Bacterial hosts, selection antibiotics and inducers used in the expression of FBfr in LB broth

Bacterial host	Selection antibiotics	Inducer
BL21(DE3)	100 µg/mL ampicillin	1 mM IPTG
BL21(DE3)pLysS	100 µg/mL ampicillin/ 35 µg/mL chloramphenicol	1 mM IPTG
BL21-AI™	100 µg/mL ampicillin	1 mM IPTG /0.2% w/v of L-arabinose
Rosetta™ (DE3)pLysS	100 µg/mL ampicillin/ 35 µg/mL chloramphenicol	1 mM IPTG

An empirical selection of *E. coli* clones that tolerate the expression of FBfr was implemented through plating technique [15]. LB broth supplemented with 1% of sterile-filtered glucose solution was tried in expression of FBfr using BL21(DE3)pLysS host. In addition, FBfr expression was tested in M9 minimal medium supplemented with 1% glucose and 0.01 M $MgSO_4$ [16]. The effect of post induction temperature was assessed in the expression of FBfr at 37 ℃ and 25 ℃ [17].

Enhancing the solubility of the expressed FBfr

To solubilize the inclusion bodies, shaking in presence of solvents such as 8 M urea or 6 M guanidine hydrochloride or the use of mild solubilizer Tris-HCl (pH 8.0) combined with sonication were attempted [18]. Moreover, coexpression with the chaperone plasmids kit (Takara, Japan), listed in **Table 2**, was tried to improve the folding of FBFr. A two-step transformation process was performed, where each chaperone plasmid was separately transformed into the chemically competent BL21(DE3) followed by transformation of the recombinant construct (pRSET-B/ FBfr). Expression was then performed as previously detailed using 1 mM IPTG in addition to the specified chaperone inducers (**Table 2**).

Purification of FBfr

Frozen bacterial pellet from expression experiments was thawed and resuspended in lysis buffer [25 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.5 mg/mL lysozyme (Sigma-Aldrich, USA), 1 mM EDTA]. Following sonication and centrifugation, inclusion bodies and soluble fraction were recovered. Soluble FBfr in the cell lysate was purified using Ni^{+2} Sepharose 6 Fast Flow packed column (GE healthcare, USA) according to the manufacturer's instructions. The inclusion bodies pellet was washed three times by two alternating washing buffers; washing buffer I (25 mM Tris-HCL, pH 8.0 with 0.5% (v/v)Triton X-100) and washing buffer II (25 mM Tris-HCL, pH 8.0 with 1 mg/mL sodium deoxycholate and 1 M urea). Washing was followed by sonication and centrifugation. Finally, a white soft pellet was obtained, and the pellet was resuspended in 25 mM Tris-HCL (pH 8.0) and then purified using Ni^{+2} sepharose 6 Fast Flow pre-packed column.

Table 2: The components of chaperone plasmid set from Takara, Japan.

No	Plasmid	Chaperone	Promoter	Inducer	Resistant marker
1	pG-KJE8	dna K-dna J-grp E gro ES-groEL	araB Pzt-1	L-Arabinose Tetracycline	Cm
2	pGro7	gro ES-gro EL	araB	L-Arabinose	Cm
3	pKJE7	dna K-dna J-grp E	araB	L-Arabinose	Cm
4	pG-Tf2	gro ES-gro EL-tig	Pzt-1	Tetracycline	Cm
5	pTf16	Tig	araB	L-Arabinose	Cm

Cm: chloramphenicol.

Production of anti-FBfr polyclonal antibody

Three female New Zealand rabbits were immunized as previously described [19]. Rabbits were SC injected with 200 µg of the purified FBfr for a total of five injections. The initial dose of the protein was emulsified with an equal volume of complete Freund's adjuvant. Booster doses were given every two weeks in incomplete Freund's adjuvant. Blood samples were withdrawn 7-10 days following each boost for determination of the antibody titer. The rabbits were given the last injection of purified FBfr in saline. A control non-immunized group was also included. Three days after the termination of the immunization schedule, the rabbits were euthanized. Finally, the sera were collected, aliquoted and stored at -80 ℃.

Determination of the antibody titer by indirect ELISA

The titer of the antibody was detected as previously discussed [20]. High-binding flat bottom 96-well plates (CELLSTAR®, Greiner Bio-One, Germany) were coated overnight at 4 ℃ with 1 µg/well of FBfr in 100 µL of coating buffer (15 mM Na_2CO_3 and 35 mM $NaHCO_3$, pH 9.6). On the next day, wells were blocked with 250 µL/well of 1% (w/v) of bovine serum albumin in Tris-buffered saline (TBS) for 2 h at room temperature. The plates were then washed three times with 250 µL/well of TBS containing 0.05% (v/v) Tween 20. The sera were serially diluted starting from 1/100 in TBS containing 2 mM $CaCl_2$ and added to wells in duplicates. Wells receiving buffer only served as a negative control. Plates were incubated for 90 min at 37 ℃, then washed three times. Plates were incubated for 90 min with alkaline phosphatase-conjugated goat anti-rabbit antibody diluted 1:10,000 (v/v) in washing buffer. Plates were washed and finally incubated with Sigma Fast pNPP (Sigma-Aldrich, USA). OD_{405} was recorded using the microtiter plate reader (BioTek instruments El800, 29274, USA).

Purification of polyclonal antibody using HiTrap™ protein G sepharose column

HiTrap™ protein G sepharose column was used to purify rabbit polyclonal anti-FBfr from the hyperimmune sera as described in the manufacturer's manual. Serum samples were mixed with three volumes of the binding buffer (20 mM sodium phosphate, pH 7.0), filtered, loaded onto the column and allowed to flow. Next, the column was washed with 20 mL of binding buffer. Elution was performed using 5 mL of elution buffer (0.1 M Glycine-HCl, pH 3.1) and the eluate was neutralized immediately by 1.0 M Tris-HCl, pH 10. The purity of antibody was assessed by SDS-PAGE analysis. Finally, the specificity of the antibody against FBfr was tested by ELISA and Western blot.

***In vitro* AP activity inhibition assays**

The ability of the purified polyclonal anti-FBfr antibodies to inhibit the AP activity was assessed via C3b deposition ELISA [21] and rabbit erythrocyte hemolytic assay [22, 23]under conditions that only allow the AP activation as previously detailed [13] .

Statistical analysis of data

Two-way ANOVA with Bonferroni post-hoc test was performed for statistical analysis using GraphPad Prism software (version 9.0.0), where $P<0.05$ was considered statistically significant. Each experiment was performed in duplicate and repeated three times, where the mean (±SEM) was used to represent the results.

RESULTS

Expression of recombinant FBfr

Pilot expression experiments showed that FBfr was successfully expressed in BL21(DE3) and BL21(DE3)pLysS strains following induction with IPTG using LB broth medium at 37 ℃, however, the yield was too low (**Fig. 1A and B).** Protein expression was confirmed by Western blot that showed positive protein bands corresponding to the expected molecular weight of FBfr (33 kDa), which was absent in the empty vector control **(Fig. 1C and D).**

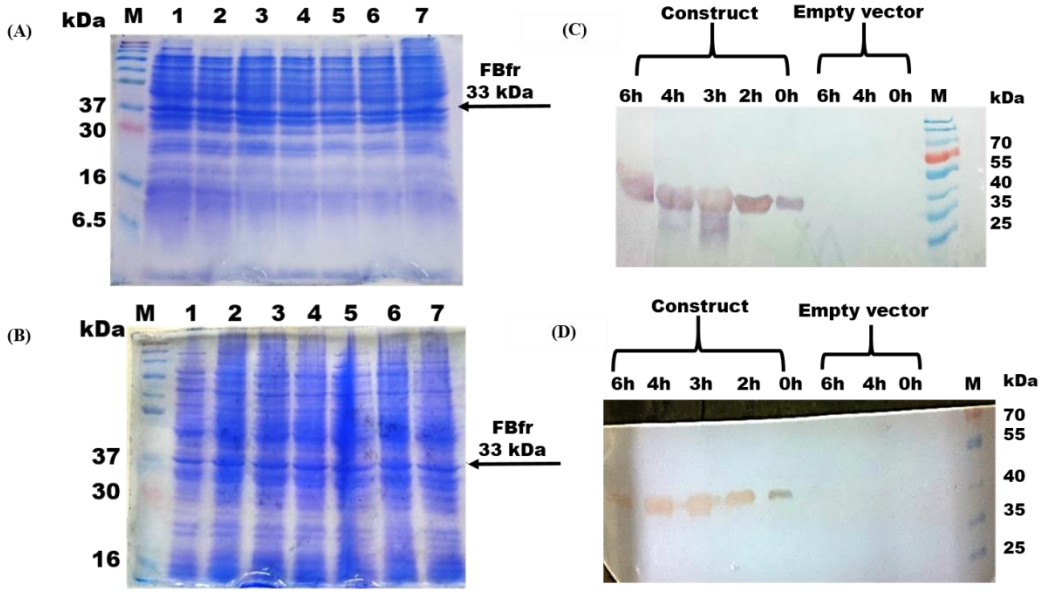

Fig. 1 : Time-course expression of FBfr in LB medium following induction with 1 mM IPTG. (A) and (B) 12% SDS-PAGE analysis in BL21(DE3) and BL21(DE3)pLysS, respectively . Lane M: BLUltra prestained standard protein size marker (BIO-HELIX, Taiwan). Lane 1: the un-induced protein. Lanes (2-7): protein expression at different time points after induction (1, 2, 3, 4, 5 and 6 h). (C) and (D)Western blot analysis for BL21(DE3) and BL21(DE3)pLysS strains, repsectively, using HRP-conjugated monoclonal mouse anti-polyHistidine antibody to detect the his-tag residues in FBfr. Lane M: Page ruler™ prestained protein ladder (10-180 kDa) (Thermo Fisher Scientific, USA).

Attempts to express FBfr in other *E. coli* bacterial hosts including Rosetta™(DE3)pLysS (data not shown) and BL21-AI™ **(Fig. 2A)** did not offer any advantage in terms of expression levels.

A massive increase in FBfr expression was successfully obtained when the medium was changed from LB to M9 minimal medium supplemented with 1% glucose, 0.01 M $MgSO_4$ and 100 µg/mL ampicillin and post-induction temperature was reduced to 25℃. Among BL21(DE3)pLysS, BL21(DE3) and Rosetta™ (DE3) pLysS host strains, the highest yield of FBfr was achieved using BL21(DE3) under these conditions (**Fig. 2B).**

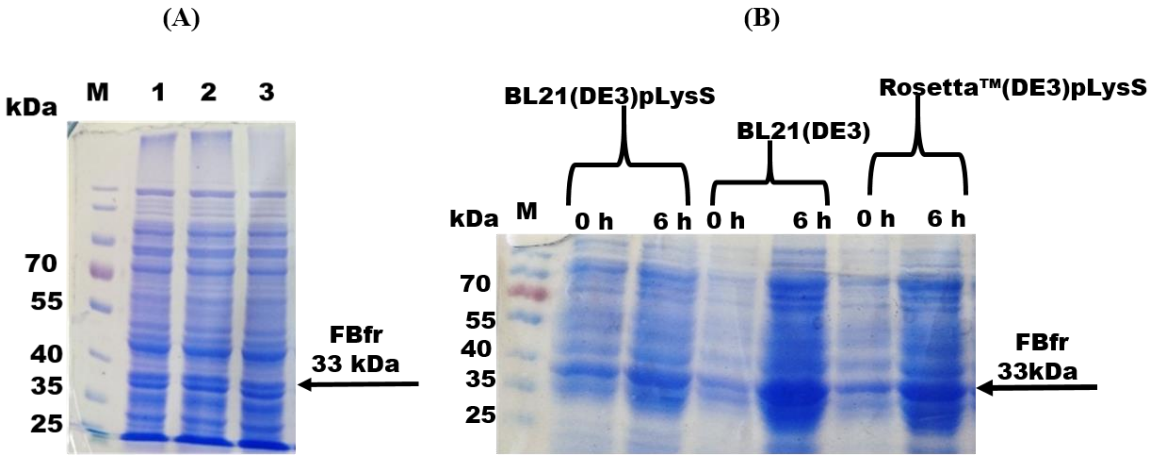

Fig. 2 : SDS-PAGE (12%) stained with Coomassie blue showing the expression of the recombinant FBfr (A) Expression in BL21-AI™. Lane 1: protein expression before induction. Lanes 2-3: protein expression at 3 and 6 h after induction using 1 mM IPTG and 0.2% (w/v) L-arabinose. (B) The use of M9 medium and post induction temperature of 25 ℃ in *E. coli* strains BL21(DE3)pLysS, BL21(DE3) and Rosetta™(DE3)pLysS, where BL21(DE3) showed the highest yield. Lane M: Page ruler™ prestained protein ladder (10-180 kDa) (Thermo Fisher Scientific, USA).

FBfr was produced on a large scale depending on the optimized conditions in BL21(DE3) using M9 medium supplemented with 1% glucose, 0.01 M $MgSO_4$. However, protein overexpression led to the aggregation of most of FBfr into inclusion bodies rather than the soluble form (**Fig. 3).**

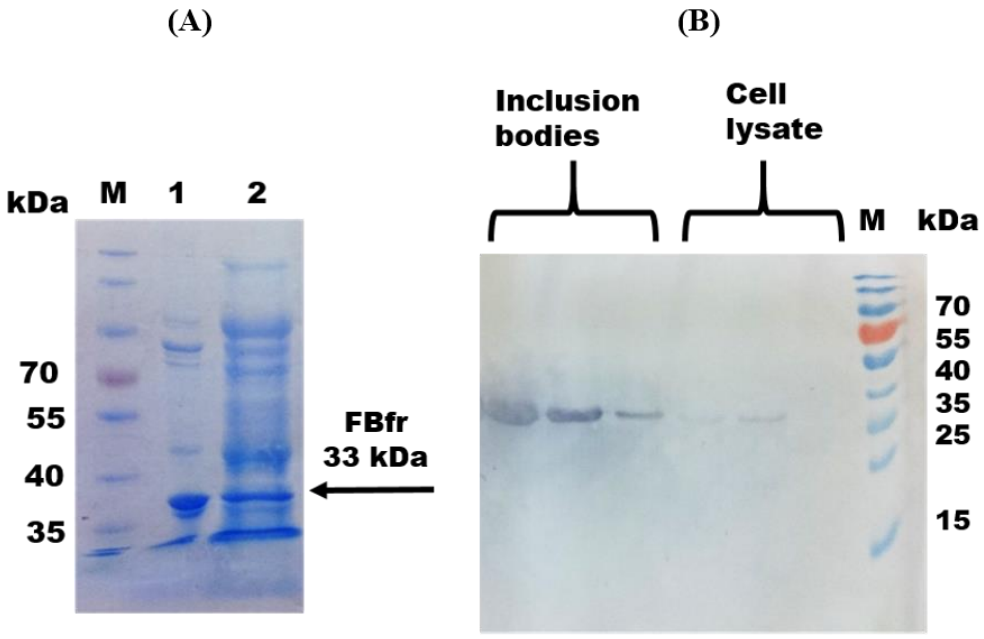

Fig. 3 : Comparison of the expression FBfr in BL21(DE3) using M9 medium in soluble and of insoluble inclusion bodies. (A)SDS-PAGE (12%) stained with Coomassie blue. Lane 1: inclusion bodies. Lane 2: cell lysate containing the soluble protein fraction. (B) Western blot showing the increased levels of FBfr expressed in inclusion bodies compared to the soluble fraction on three separate experiments. Lane M: Page ruler™ prestained protein ladder (10-180 kDa) (Thermo Fisher Scientific, USA).

Enhancing the solubility and purification of the expressed FBfr

Attempts to express a soluble FBfr were conducted using chaperone plasmids set. Extra protein bands corresponding to the successfully expressed chaperones were observed at 56 kDa for (pG-Tf2 and pTf16) and at 40, 70 kDa for pKJE7 **(Fig. 4A)**. However, co-expression of FBfr with chaperone plasmids pG-Tf2 and pTf16 resulted in a further decrease in the soluble FBfr part and elevation of inclusion bodies part. On the other hand, co-expression with pKJE7 showed no advantage in the level of soluble FBfr compared to expression of FBfr alone as shown in **Fig. 4B.** Different solvents were tried to solubilize FBfr inclusion bodies. However, the best result was obtained using Tris-HCl combined with sonication, where almost all the inclusion bodies were solubilized with avoiding the detrimental effects of denaturing solvents **(Fig. 4C).** This soluble protein was then purified using Ni^{+2} sepharose 6 Fast Flow pre-packed column. SDS-PAGE analysis showed that FBfr was successfully purified **(Fig. 4D).** In addition, western blot analysis confirmed the identity of the purified FBfr by using monoclonal antibody against histidine tag **(Fig. 4E)** as well as using reference rabbit anti-human FB polyclonal antibody **(Fig. 4F)**, where the target protein was observed as a single consistent band.

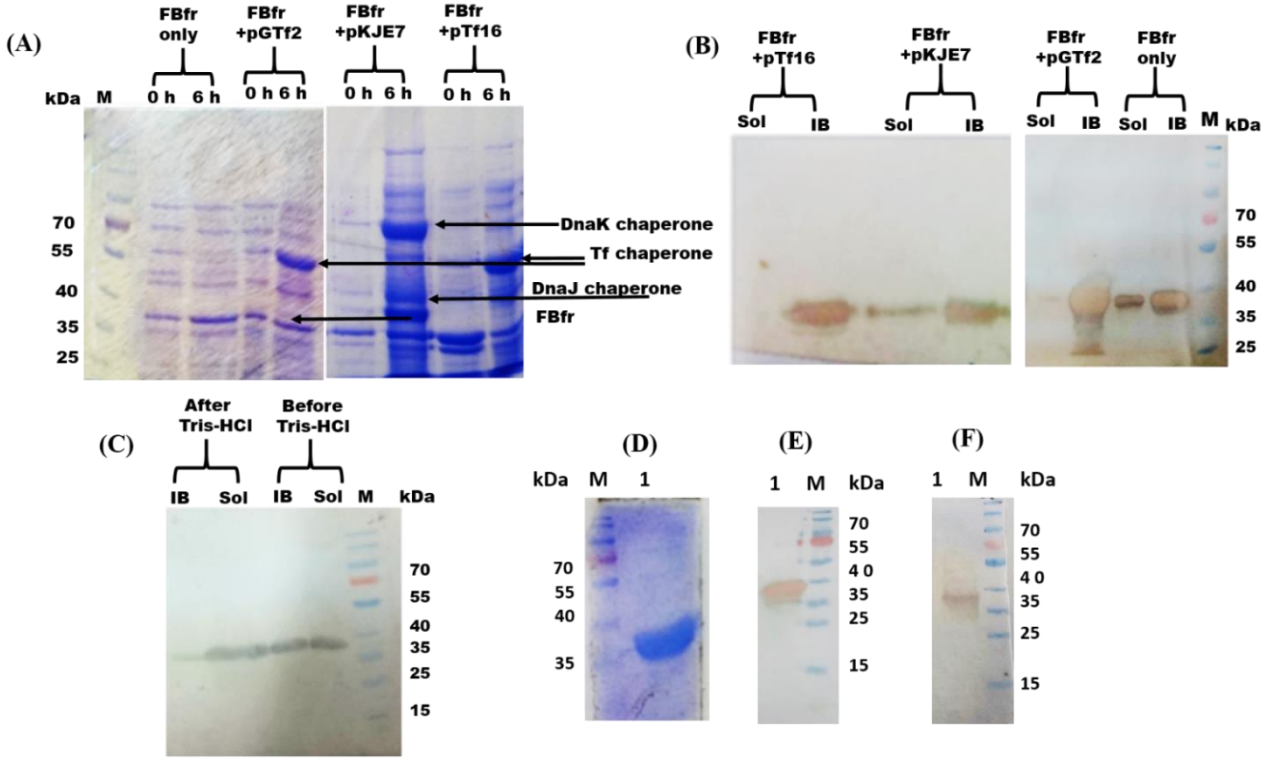

Fig. 4: Expression of FBfr in inclusion bodies (IB) and soluble protein (Sol) following solubilization trials. (A) SDS-PAGE (12%) gel showing the expressed chaperone proteins at approximately 56 kDa for pG-Tf2 and pTf16 and at 40, 70 kDa for pKJE7 and (B) Western blot of the co-expression of FBfr with 3 different chaperones plasmids showing no increase in FBfr soluble part compared to expression of FBfr alone. (C) Western blot showing the solubilizing effect of Tris-HCl on FBfr IB. (D) SDS-PAGE (12%) stained with Coomassie blue showing the purified soluble FBfr protein. (E) and (F) Western blot of FBfr protein using anti-histidine monoclonal antibody and reference rabbit anti-human FB polyclonal antibody, respectively, where lane M: Page ruler™ prestained protein ladder (10-180 kDa), lane 1: FBfr protein.

Determination of the antibody titer by indirect ELISA

All immunized animals showed significantly higher level of antibody titers than the control non-immunized group. The antibody titer against FBfr in the immunized rabbit serum was closely relevant to that of the control anti-human FB rabbit serum and significantly higher than that of the control non-immunized rabbit serum (**Fig. 5A).**

Purification of polyclonal antibodies

The total IgG was purified using HiTrap™ protein G sepharose column from the hyperimmune rabbit sera. Bands corresponding to the heavy and light chains of IgG were shown by SDS-PAGE **(Fig. 5B)**. The purified antibodies reacted towards FBfr as shown by Western blot **(Fig. 5C).**

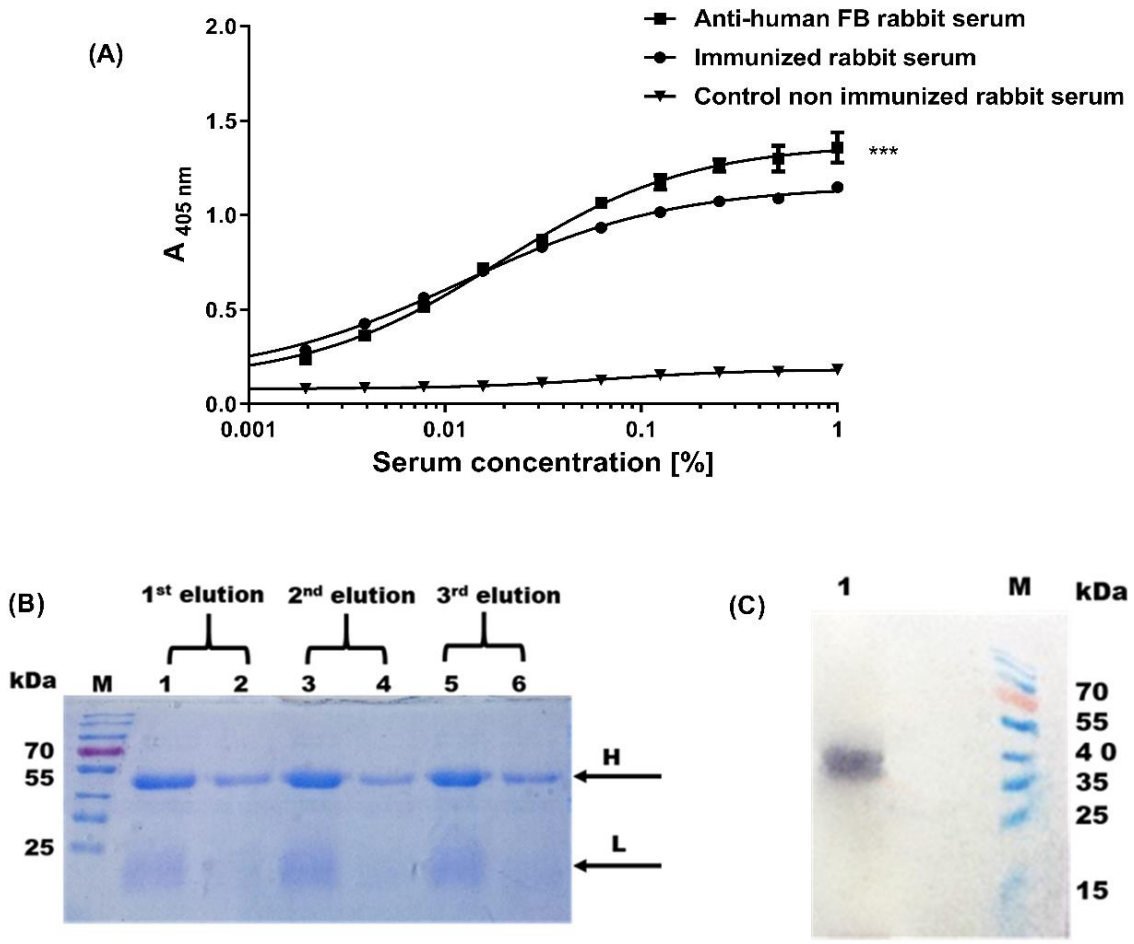

Fig. 5: (A) Determination of the titer of polyclonal anti-FBfr in the sera of immunized and control rabbits compared to reference anti-human FB rabbit serum using ELISA. The results represent duplicate samples of a set of identical experiments and are expressed as means ± SEM. ****P* <0.0001 (B) The purified polyclonal anti-FBfr on 12% SDS-PAGE, stained with Coomassie blue. Arrows denoted H and L, refer to the heavy and light chains of IgG at approximately 50 and 25 kDa, respectively. Lane M: Page ruler™ prestained protein ladder (10-180 kDa) (Thermo Fisher Scientific, USA). (C) Western blot of the purified polyclonal antibodies against FBfr protein. Lane M: Page ruler™ prestained protein ladder (10-180 kDa) (Thermo Fisher Scientific, USA). Lane 1: FBfr protein.

In vitro AP activity inhibition assays

An inhibitory activity on C3b deposition and hence on the AP activity was reported. The antibodies were tested starting from 100 µg/mL using 10% normal human serum (NHS). The rabbit polyclonal anti-FBfr antibody achieved 30.3% of inhibition at 100 µg/mL and only 2.8% at 12.5 µg/mL. The lower concentrations showed no inhibition **(Fig. 6A)**. In the hemolytic assay, the degree of inhibition of hemolysis reached 35.2% for the highest used concentration of rabbit polyclonal antibody as shown in **Fig. 6B**.

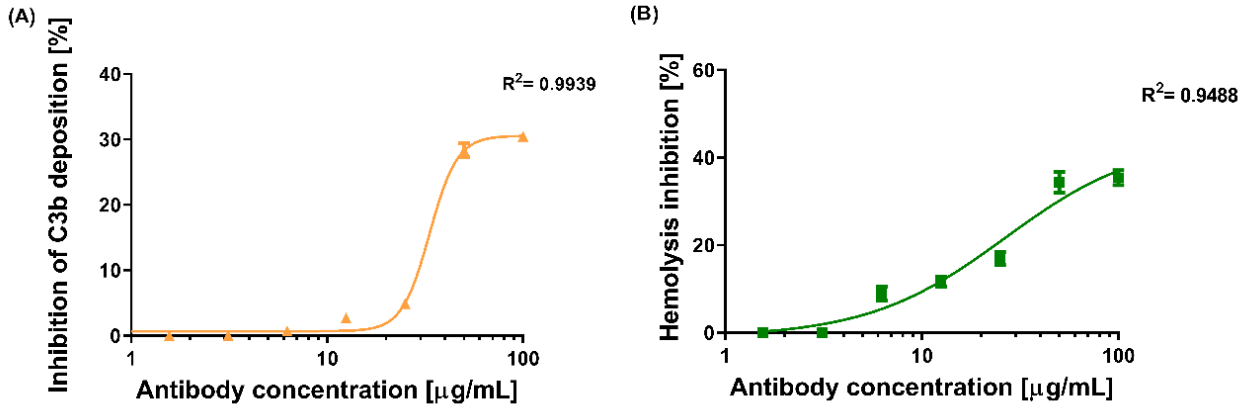

Fig. 6: (A) The inhibitory effect of rabbit anti-FBfr polyclonal antibody on the AP of complement C3b deposition from 10% NHS on mannan-coated ELISA plates. (B) The inhibitory effects of rabbit anti-FBfr polyclonal antibody on hemolysis of rabbit erythrocytes produced by 12.5% normal human serum. Experiments were conducted under conditions permitting only the AP activation. The results represent duplicate samples of a set of identical experiments and are expressed as means ± SEM.

DISCUSSION

The uncontrolled or insufficient regulation of complement clearly contributes to a wide array of inflammatory conditions and accompanying tissue damage [24, 25]. Accordingly, an urgent need is continuously pushing to develop complement-targeted therapeutics. Despite the extensive efforts to develop new complement therapeutics, only few succeeded to get FDA approval. In this study, the light was shed on inhibitors of the AP activity in particular due to its critical role in the complement cascade. By modulating the activity of the AP, this could have benefits in reducing inflammation and tissue damage accompanying diseases in which complement cascade is involved [26].

A protein fragment of FB (FBfr) was successfully cloned and expressed in *E. coli* in our lab that had an inhibitory activity on the AP [13]. In this work, different expression host strains were employed to find the best producing strain, however BL21(DE3) achieved the highest yield. FBFr was initially thought to be toxic to the host cells, thus trials to minimize its basal expression were employed. This can be reduced by production of T7 lysozyme to inhibit the T7-RNA polymerase (RNAP) as in BL21(DE3) pLysS. Glucose effect in inducing the catabolic repression was also considered. The use of BL21-AI™, which is known to express toxic proteins failed to be produced in BL21(DE3), was adopted. The BL21-AI™ strain allows the expression of T7-RNAP to be regulated by the araBAD promoter. Yet, this strain did not enhance the yield of FBFr protein, which may refer to the lack of toxicity. Other problems were addressed. The Codon Adaptation Index (CAI) of FBfr was found to be 0.67, which indicates a slight codon bias. Therefore, the *E. coli* strain Rosetta™(DE3)pLysS was tried for expression. The tRNAs for rare codons are expressed by Rosetta™ (DE3)pLysS on a compatible chloramphenicol resistance (Cm^r) plasmid [27]. However, the lack of improvement in protein level when this strain was used can be explained by the low percentage of rare codons in FBfr protein.

The likelihood that the protein production can be improved by changing the expression conditions was investigated as a final resort. The use of M9 minimal medium supplemented with 1% glucose and 0.01 M MgSO4 in addition to reducing the post-induction temperature to 25 ℃ was found to enhance the yield. The induction at reduced temperature aimed to minimize the formation of inclusion bodies [17]. This modified expression protocol also included the empirical selection of transformed clones by plating on solid medium for several cycles in order to get the most stable producer of FBfr. Such procedure depends on the incidence of silent mutations over generations of growth that enables the host to handle the heterologous protein.

As a major drawback of protein overexpression, the larger fraction of FBfr protein was expressed as inclusion bodies. Therefore, the TAKARA chaperone plasmid set was used in an attempt to increase the solubility of the expressed FBfr. However, this attempt showed a little success. From previous studies, the beneficial effect of co-expression with molecular chaperones is unpredictable. It is a matter of trial and error to find out which of the chaperones could be appropriate and effective for a given protein [28]. In a previous study [29], the co-expression of cold-active lipase (Lip-948) with different chaperones either led to an increase or a decrease in the formation of soluble LIP-

948 in varying degrees. The co-expression of Lip-948 with chaperone pTf16 and pGro7 decreased the amount of soluble LIP-948, while the soluble expression was enhanced when Lip-948 was co-expressed with ‘‘chaperone team’’ plasmids (pKJE7, pG-Tf2, pG-KJE8) [29].

Using another experimental approach, FBfr was successfully solubilized using non-denaturing solvent (Tris-HCl), which later on proved to be active without the need for refolding. Similarly, previous studies reported the production of active inclusion bodies using non-denaturing solvents [30-32]. Those biologically active inclusion body aggregates are referred to as non-classical and do not require refolding [18, 33].

The purified FBfr protein was used to actively immunize rabbits to produce a polyclonal antibody. The high affinity of the produced antibody makes them good candidates to be used for detection of FB for diagnostic purposes. In this regard, the polyclonal anti-FBfr reacted in ELISA and Western blot indicating that they can recognize their epitopes in native as well as denatured form of FBfr.

The produced anti-FBfr antibody was tested in *in vitro* assays. In C3b deposition assay, the polyclonal antibody achieved a maximal inhibition of 30.3% at 100 µg/mL, while concentrations lower than 12.5 µg/mL did not produce any inhibition. The hemolysis of rabbit erythrocytes was monitored in the hemolytic assay. Polyclonal rabbit anti-FBfr produced a maximum of 35.3% of hemolysis inhibition. It is suggested that this antibody potentially bind to the neoepitope on human FB revealed upon conformational changes that would prevent the binding between factor D and its substrate (FB) through binding of anti-FBfr antibody to the sites surrounding and harboring the scissile bond. Another epitope could be the Von Willebrand factor A (VWA) domain, so the antibodies would initially hinder the C3b binding to FB.

This novel antibody produced in the current study represents a new trial added to the few antibodies that target FB and subsequently inhibits the AP activity. The first antibody against FB was reported in 2005 by Thurman *et al.* [34], the monoclonal antibody (mAb) denoted 1379 was developed against the second and the third SCR domains of mouse FB. This antibody was able to inhibit the formation of the AP convertase, through which it provided protection to mouse model against anti-phospholipid antibody-induced fetal loss. In 2014, Subías *et al.*[35] reported the production of FB28.4.2, the mAb against human FB. The produced antibody was proven to target a conserved epitope in the Ba fragment of human FB. Therefore, both of the previous antibodies 1379 and FB28.4.2 were directed against the Ba portion of either mouse FB or human FB, respectively [34, 35]. Accordingly, anti-FBfr antibodies differed from the previously reported ones in that they were directed against epitopes in the Bb portion of human FB particularly the newly formed epitope, the scissile bond, or VWA domain included in the design of FBfr.

In conclusion, polyclonal antibodies produced in the current study represent a first step in the road that could lead to the development of a new and more specific therapeutic in the form of mAb against FBfr. The therapeutic implications can be further explored to design new drug leads that could participate in the current state of anti-complement drugs.

Declarations:

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Conflict of interest: The authors declare no conflict of interest related to this work. All authors confirm that this article is original and has not been published elsewhere, nor is currently under consideration for publication elsewhere and approve the version submitted.

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