Molecular Immunology 62 (2014) 249–264

Contents lists available at ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

Tuf of *Streptococcus pneumoniae* is a surface displayed human complement regulator binding protein

Sarbani Mohan[a], Christian Hertweck[b], Antje Dudda[a], Sven Hammerschmidt[c], Christine Skerka[a], Teresia Hallström[a], Peter F. Zipfel[a,d,*]

[a] Department of Infection Biology, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Jena, Germany
[b] Department of Biomolecular Chemistry, Leibniz Institute for Natural Product Research and Infection Biology, Hans-Knöll-Institute, Jena, Germany
[c] Department Genetics of Microorganisms, Interfaculty Institute for Genetics and Functional Genomics, Ernst Moritz Arndt University, Greifswald, Germany
[d] Faculty of Biology, Friedrich Schiller University, Jena, Germany

ARTICLE INFO

Article history:
Received 1 May 2014
Received in revised form 21 June 2014
Accepted 23 June 2014

Keywords:
Tuf
Complement
Factor H
Plasminogen
ECM

ABSTRACT

Streptococcus pneumoniae is a Gram-positive bacterium, causing acute sinusitis, otitis media, and severe diseases such as pneumonia, bacteraemia, meningitis and sepsis. Here we identify elongation factor Tu (Tuf) as a new Factor H binding protein of *S. pneumoniae*. The surface protein PspC which also binds a series of other human immune inhibitors, was the first identified pneumococcal Factor H binding protein of *S. pneumoniae*. Pneumococcal Tuf, a 55 kDa pneumococcal moonlighting protein which is displayed on the surface of pneumococci, is also located in the cytoplasm and is detected in the culture supernatant. Tuf binds the human complement inhibitors Factor H, FHL-1, CFHR1 and also the proenzyme plasminogen. Factor H and FHL-1 bound to Tuf, retain their complement regulatory activities. Similarly, plasminogen bound to Tuf was accessible for the activator uPA and activated plasmin cleaved the synthetic chromogenic substrate S-2251 as well as the natural substrates fibrinogen and the complement proteins C3 and C3b. Taken together, Tuf of *S. pneumoniae* is a new multi-functional bacterial virulence factor that helps the pathogen in complement escape and likely also in ECM degradation.

1. Introduction

Streptococcus pneumoniae is a Gram-positive, alpha-hemolytic bacterium, which can cause severe local infections like otitis media, acute sinusitis as well as life threatening invasive diseases such as meningitis, bacteraemia and sepsis (Gillespie, 1989). Young infants, elderly people over 60 years of age and in particular immunocompromised individuals are susceptible to pneumococcal infections (Musher, 1992). According to WHO 2007 about 1.6 million people die every year because of pneumococcal infections and out of which upto a million are young children (Laarman et al., 2011). Pneumococci are considered to be one of the most serious public global health threat mainly because of their growing resistance to available antibiotics and the immediate need for more efficient vaccines (Gamez and Hammerschmidt, 2012; Hair et al., 2010). Studying host-pathogen interactions is thus very important to define strategies that pneumococcus employ to escape from the host immune system and to identify new bacterial targets that may be used for vaccine development.

The innate immune system as the first defence line acts immediately to kill and eliminate the invading microbe (Lambris et al., 2008; Zipfel et al., 2013). The complement cascade when triggered by an infectious agent induces a sequential and highly effective defence response. Host complement is activated by three pathways, the alternative, classical and lectin pathways. All the three pathways form C3 convertases that cleave the central complement component C3, into C3a and C3b. C3b deposits on the surface and when amplified opsonizes this foreign surface. C3a is an anaphylatoxin and has antimicrobial activity.

The complement system is tightly regulated by fluid phase, as well as membrane associated regulators that block complement cascade progression, complement mediated effector function as well as complement mediated damage to host cells. Factor H is a 150 kDa human plasma protein and Factor H-like protein 1

Abbreviations: ε-ACA, ε-amino caproic acid; CFHR1, complement Factor H related protein 1; Tuf, elongation Factor Tu; ECM, extracellular matrix; FHL-1, Factor H like protein-1; MP, membrane protein; NHS, normal human serum; OMP, outer membrane protein; PspC, pneumococcal surface protein C; RT, room temperature; SCR, short concensus repeat; uPA, urokinase-type plasminogen activator.

* Corresponding author at: Leibniz Institute for Natural Product Research and Infection Biology, Department of Infection Biology, Beutenbergstr 11a, D-07745 Jena, Germany. Tel.: +49 036415321300; fax: +49 036415320807..
E-mail address: peter.zipfel@hki-jena.de (P.F. Zipfel).

http://dx.doi.org/10.1016/j.molimm.2014.06.029
0161-5890/

(FHL-1), which is derived from the *Factor H* gene by alternative splicing is a 42 kDa plasma protein and these two proteins are the major fluid phase inhibitors of the alternative complement pathway (Rodriguez de Cordoba et al., 2004; Zipfel et al., 2008). Both Factor H and FHL-1 function as cofactors in the Factor I mediated degradation and subsequent inactivation of the central complement component C3b. In addition, Factor H and FHL-1 inhibit the formation and accelerates the decay of the C3 convertase. The first four N-terminal SCR domains 1–4 of both Factor H and FHL-1 display complement regulatory activity and the C-terminus of Factor H (SCRs 18–20) mediates binding to cell surfaces and surface recognition (Rodriguez de Cordoba et al., 2004; Zipfel et al., 1999, 2008; Zipfel and Skerka, 1999).

Factor H is the best characterized member of the Factor H family, which in addition to Factor H consists of a Factor H like protein (FHL-1) and five Factor H related proteins (CFHR1–CFHR5) (Jozsi and Zipfel, 2008). CFHR1 is a human complement regulator that inhibits complement at the level of the C5 convertases and blocks assembly of the TCC components (Heinen et al., 2009).

Pathogenic microbes have evolved a number of mechanisms to control and to escape the host complement challenge and they efficiently evade attack by complement effector molecules (Foster et al., 2014; Laarman et al., 2011). Many pathogens mimic self-cell surfaces as they bind and utilize soluble human complement inhibitors to inhibit host complement attack (Zipfel and Skerka, 2014). *S. pneumoniae* binds Factor H with the help of e.g. the surface expressed protein PspC (Dave et al., 2001; Zipfel et al., 2008, 2013). Factor H bound to the pneumococcal surface inhibits complement activation and thus blocks complement mediated elimination (Jarva et al., 2003; Zipfel et al., 2013). Factor H acquisition is a widely used evasion strategy used by *S. pneumoniae* and many other pathogens (Zipfel and Skerka, 2014). Pathogenic microbes often use several Factor H binding and complement acquiring surface proteins. Such microbial immune evasion proteins include Shiga toxin of *Escherichia coli* (Poolpol et al., 2014), NspA of *Neisseria meningitidis* (Lewis et al., 2010), Scl-1 of *Streptococcus pyogens* (Reuter et al., 2010), Sbi, SdrE and Ecb of *Staphylococcus aureus* (Amdahl et al., 2013; Haupt et al., 2008; Sharp et al., 2012), Lpd of *Pseudomonas aeruginosa* (Hallstrom et al., 2012) and Pra-1, Gpd2 and Hgt1p of *Candida albicans* (Lesiak-Markowicz et al., 2011; Luo et al., 2009, 2013; Poltermann et al., 2007).

Pneumococcal surface protein C (PspC), also known as CbpA and SpsA binds the human complement regulator Factor H and assists in complement evasion (Dave et al., 2004; Hammerschmidt et al., 2007). PspC is also an adhesive protein and mediates bacterial attachment to host cells (Hammerschmidt, 2006; Hammerschmidt et al., 1997, 2007; Rennemeier et al., 2007). Pneumococcal histidine triad proteins PhtA, PhtB, PhtD and PhtE are surface proteins that may bind Factor H (Melin et al., 2010; Ogunniyi et al., 2009).

Many microbial Factor H binding proteins bind several human proteins including plasminogen. Plasminogen, is a 92 kDa human glycoprotein synthesized in the liver (Castellino and Ploplis, 2005). Pathogens bind plasminogen and use human as well as bacterial encoded plasminogen activators to generate plasmin that degrades complement components and ECM proteins, takes part in fibrinolysis, wound healing and cell migration (Myohanen and Vaheri, 2004). Plasmin cleaves ECM proteins such as fibrinogen, laminin, fibronectin and vitronectin. (Lahteenmaki et al., 2005). This leads to dissemination of invasive bacteria within the mammalian host (Bergmann and Hammerschmidt, 2007). Acquired plasminogen may also enhance the adhesion of bacteria to human epithelial cells (Pancholi et al., 2003). α-Enolase (Eno), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), choline-binding protein E (CbpE) and endopeptidase O (PepO) are some of the known pneumococcal proteins that bind human plasminogen (Agarwal et al., 2013; Attali et al., 2008; Bergmann et al., 2001, 2004).

Here, we identified elongation factor Tu (Tuf_{Sp}) as the second Factor H binding protein of *S. pneumoniae*.

2. Materials and methods

2.1. Bacteria and culture conditions

S. pneumoniae strains D39 (NCTC 7466, serotype 2), NCTC 10319 (serotype 35A), G54 (serotype 19F), R800, ATCC 11733 and the mutant strains D39Δ*pspC* and NCTC 10319Δ*pspC* were used. The clinical isolate I was obtained from a patient with *S. pneumoniae* associated atypical haemolytic uremic syndrome (University Children's Hospital, Zürich, Switzerland). *S. pneumoniae* was cultured on blood agar plates (Merck, Darmstadt, Germany) at 37 °C with 5% CO_2, or in Todd-Hewitt broth (Roth, Karlsruhe, Gemany) supplemented with 0.5% yeast extract. Mutants lacking PspC were grown in the same media supplemented with 5 μg/ml Erythromycin (Sigma-Aldrich, Steinheim, Germany). *C. albicans* strain SC5314 was grown in Yeast-extract Peptone Dextrose (YPD; Roth) medium and *P. aeruginosa* strain PAO1 was grown in Nutrient Broth (NB; Roth). *E. coli* expressing Tuf_{Sp} was grown in Luria-Bertani (LB) liquid broth containing 100 μg/ml ampicillin (Sigma-Aldrich).

2.2. Antibodies

Polyclonal rabbit antiserum specific for *S. pneumoniae* Tuf_{Sp} and *P. aeruginosa* Tuf_{Pa} (Kunert et al., 2007) were generated in house. Polyclonal goat Factor H antiserum and polyclonal goat C3 antiserum were purchased from Complement Technology Inc. (Texas, USA). Other antisera used were polyclonal rabbit anti-FH (raised against SCRs 1–4), monoclonal mouse anti-CFHR1 JHD10 (Heinen et al., 2009), polyclonal rabbit anti-CFHR1, monoclonal mouse anti-penta histidine (Qiagen, Hilden, Germany), polyclonal goat anti-plasminogen (Acris antibodies, Herford, Germany) and polyclonal rabbit anti-fibrinogen (Calbiochem, La Jolla, U.S.A). Horseradish peroxidase (HRP)-conjugated rabbit anti-goat, HRP-conjugated goat anti-rabbit and HRP-conjugated goat anti-mouse were obtained from Dako (Glostrup, Denmark). Alexa fluor®-647 conjugated polyclonal goat anti-rabbit and rabbit anti-goat were purchased from Molecular Probes (Eugene, Oregon, USA).

2.3. Serum binding assay

To analyze whether *S. pneumoniae* wild type D39 and NCTC 10319 and PspC deficient mutants D39Δ*pspC* and NCTC 10319Δ*pspC* bind Factor H from normal human serum (NHS), bacteria were grown in broth for 3 h at 37 °C and thereafter incubated with 25% heat inactivated NHS (hiNHS). To remove unbound proteins, bacteria were washed 5 times with DPBS (Lonza, Verviers, Belgium). Thereafter, the cells were lysed by resuspending the bacterial pellets in a lysis buffer containing 0.1% Triton X-100 (Applichem, Darmstadt, Germany) and protease inhibitors (Complete from Roche, Mannheim, Germany). After 30 min incubation at 4 °C, the bacteria were centrifuged and the supernatants were subjected to SDS-PAGE, transferred to a nitrocellulose membrane (Roth) and analysed by Western blotting. Factor H binding was analysed by goat anti-human Factor H (1:1000) followed by HRP-conjugated rabbit anti-goat (1:2500). After additional washing, development was performed with ECL Western blotting detection reagents (Applichem).

2.4. Flow cytometry

S. pneumoniae were grown in broth for about 3 h at 37 °C and then bacterial culture equivalent to 1 ml with $O.D_{600nm}$ 1.00 was