International Journal of Medical Microbiology 306 (2016) 504–516

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

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

Differential compartmentalization of *Streptococcus pyogenes* virulence factors and host protein binding properties as a mechanism for host adaptation

Ola Kilsgård[a,b,*], Christofer Karlsson[a], Erik Malmström[a], Johan Malmström[a]

[a] Division of Infection Medicine, Department of Clinical Sciences Lund, Lund University, Lund, Sweden
[b] Department of Immunotechnology, Faculty of Engineering Lund, Lund University, Lund, Sweden

ARTICLE INFO

Article history:
Received 15 March 2016
Received in revised form 28 June 2016
Accepted 28 June 2016

Keywords:
Host adaptation
Proteomics
Streptococcus pyogenes
Pathogenesis

ABSTRACT

Streptococcus pyogenes is an important human pathogen responsible for substantial morbidity and mortality worldwide. Although *S. pyogenes* is a strictly human pathogen with no other known animal reservoir, several murine infection models exist to explore different aspects of the bacterial pathogenesis. Inoculating mice with wild-type *S. pyogenes* strains can result in the generation of new bacterial phenotypes that are hypervirulent compared to the original inoculum. In this study, we used a serial mass spectrometry based proteomics strategy to investigate if these hypervirulent strains have an altered distribution of virulence proteins across the intracellular, surface associated and secreted bacterial compartments and if any change in compartmentalization can alter the protein-protein interaction network between bacteria and host proteins. Quantitative analysis of the *S. pyogenes* surface and secreted proteomes revealed that animal passaged strains are associated with significantly higher amount of virulence factors on the bacterial surface and in the media. This altered virulence factor compartmentalization results in increased binding of several mouse plasma proteins to the bacterial surface, a trend that was consistent for mouse plasma from several different mouse strains. In general, both the wild-type strain and animal passaged strain were capable of binding high amounts of human plasma proteins. However, compared to the non-passaged strains, the animal passaged strains displayed an increased ability to bind mouse plasma proteins, in particular for M protein binders, indicating that the increased affinity for mouse blood plasma proteins is a consequence of host adaptation of this pathogen to a new host. In conclusion, plotting the total amount of virulence factors against the total amount of plasma proteins associated to the bacterial surface could clearly separate out animal passaged strains from wild type strains indicating a virulence model that could predict the virulence of a *S. pyogenes* strain in mice and which could be used to identify key aspects of this bacteria's pathogenesis.

1. Introduction

Streptococcus pyogenes, or Group A Streptococci, is a commonly occurring human bacterial pathogen that can cause a wide range of symptoms ranging from self-limiting infections such as pharyngitis and impetigo to life threatening conditions such as necrotizing fasciitis (NF) and streptococcal toxic shock syndrome (STSS) (Chhatwal and Graham, 2008). Among the over 220 characterized *S. pyogenes* serotypes, a marked increase in incidence and severity of *S. pyogenes* infections since the mid-1980s have been accredited to the now globally disseminated M1T1 clone (Cunningham, 2000). More recent reports have shown that the M1 serotypes can be further distinguished into two groups, the SF370-like and the MGAS5005-like isolates, where horizontal gene transfer in the MGAS5005-like isolates resulted in increased virulence (Nasser et al., 2014).

S. pyogenes is a strictly human pathogen with no other known animal reservoir. Still, murine infection models play an important role when investigating aspects of the pathogenesis and the mechanisms behind *S. pyogenes* host interactions. Inoculating wild-type *S. pyogenes* strains into these murine infection models revealed that the host pressure can alter the composition of the inoculated *S. pyogenes* community, generating phenotypes that differ from the original inoculum (Walker et al., 2007). These new phenotypes exhibit a higher degree of virulence and lethality when

* Corresponding author at: Division of Infection Medicine, Department of Clinical Sciences Lund, Lund University, Lund, Sweden.
E-mail address: ola.kilsgard@immun.lth.se (O. Kilsgård).

http://dx.doi.org/10.1016/j.ijmm.2016.06.007
1438-4221/

re-inoculated into mice (Aziz et al., 2004; Cole et al., 2006; Li et al., 2013; Maamary et al., 2012). A well-characterized pair of M1 isolates used in murine infection models is strain 5448 and the more mouse virulent 5448AP progeny with an inactivated CovRS two component regulatory system (TC-RS) (Aziz et al., 2004; Cole et al., 2006; Li et al., 2013; Maamary et al., 2012). This TC-RS directly or indirectly controls the expression of around 15% of the *S. pyogenes* transcriptome including the bacteriophage-encoded Sda1 DNase (Cole et al., 2010). Strain 5448 is an MGAS5005-like isolate (Maamary et al., 2012) and can cause necrotizing fasciitis (Buchanan et al., 2006), skin and tissue infections in murine models (Cole et al., 2006; Zinkernagel et al., 2012, 2008).

A recent comparative genomics study revealed how another commonly used M1-type in murine infection models, AP1, contained a substantially increased genetic content (1965 genes) compared to the less virulent 5448 (1858 genes) and SF370 (1830 genes) strains (Fiebig et al., 2015). Strain AP1 is derived from an invasive clinical isolate that binds human IgG via protein H (Åkesson et al., 1990) and evokes a systemic infection in BALB/c (Oehmcke et al., 2013; Oehmcke et al., 2009) and C57BL/6 mice (Shannon et al., 2010). Although AP1 lacks genetic features that drive the selection of hypervirulent clones with inactivated CovRS system, inactivating mutations in *covS* were a common genetic feature of AP1 and 5448AP. However, comparative presence/absence analysis of known virulence factors in SF370, 5448 and AP1 revealed that the isolates differed marginally in the part of the genome that was not phage encoded (Fiebig et al., 2015). It is possible that mutations in *covS* and other genes, such as RofA have a drastic effect on bacterial virulence. How these genetic differences impact post-transcriptional biological processes, in particular control of proteome homeostasis, virulence protein expression levels on a global level, the distribution of virulence factors across bacterial compartments and host-pathogen protein-protein interactions has so far remained elusive. It can be expected that redistribution of proteins between the bacterial compartments will lead to an altered interaction pattern with host proteins that may subsequently influence virulence. There is currently only limited information regarding how the distribution of virulence factors change after animal passage, how this change affects the ratio of virulence factors in specific compartments or between compartments of the bacteria and how these changes will influence the interaction of host proteins.

Mass spectrometry (MS) based proteomics techniques are suitable for bridging the gap between genotype and phenotype. In addition to estimating the relative amount of the present proteins, MS-based proteomics have the ability to estimate protein cellular localization, protein copy number, protein-protein interactions and the organization and distribution of the proteome in time and space (Malmström et al., 2009; Schmidt et al., 2016). For example, a recent study demonstrated how a proteogenomics strategy based on data-independent acquisition mass spectrometry analysis (DIA-MS) of 34 clinical isolates of *S. pyogenes*, identified several significantly differentially expressed proteins between invasive and non-invasive strains (Malmström et al., 2015). The integration of whole genome sequencing with accurately quantified proteomes further advanced the interpretation of the relationship between genomes, proteomes and virulence. Furthermore, highly quantitative selected reaction monitoring mass spectrometry (SRM-MS) analysis (Karlsson et al., 2012) previously revealed the association between an extensive number of human proteins and the *S. pyogenes* bacterial surface, indicating that *S. pyogenes* can form a complex extracellular host-bacteria protein-protein interaction network (Sjöholm et al., 2014). Quantitative analysis of these protein interaction network showed that the plasma protein-binding properties of the wild type, mutant, invasive and non-invasive *S. pyogenes* strains was considerably different, underlining the significance of these protein interactions.

In this study, we used a combination of MS techniques to investigate the changes in compartmentalization of *S. pyogenes* virulence factors between wild type and mouse-passaged strains. Surface proteins, secreted proteins and plasma proteins associated to the bacterial surface were investigated using shotgun MS techniques to define differences in composition of the subproteomes between murine virulent and hypervirulent strains. In addition, we applied quantitative MS analysis using SRM-MS to further evaluate how the changes of the subproteomes altered the bacteriaís ability to bind mouse and human plasma proteins. Collectively these results demonstrate changes that underlie the host adaptation of *S. pyogenes* in mice and add to the understanding of the pathogenesis of these bacteria.

2. Methods

2.1. Bacterial strains and growth conditions

S. pyogenes strain 5448 is a clinical isolate of the M1 serotype, originally isolated from a patient with necrotizing fasciitis and toxic shock syndrome (Chatellier et al., 2000). The 5448AP strain is a *covS* truncated version of the 5448 strain that was isolated after passage in a BALB/c mouse (Aziz et al., 2004). The AP1 strain (strain 40/58 from the WHO Collaborating Centre for Reference and Research on Streptococci, Prague, Czech Republic) is a *covS* truncated clinical isolate of the M1 serotype (Åkesson et al., 1990). Strain SF370 is a M1 serotype, originally isolated from a patient with a wound infection, which have been described earlier (Ferretti et al., 2001).

Single colonies were added to 30 g L^{-1} Todd-Hewitt Broth (BD) and grown until optical density (620 nm) = 0.5 (exponential growth phase) or overnight (stationary growth phase) at 37 °C, 5% CO_2 without shaking. At the correct optical density the bacteria was harvested by centrifugation (2000*g* for 15 min at 4 °C) and washed twice with 20 mM Tris-HCl (Merck), 150 mM NaCl (Sigma-Aldrich, St. Louis, MO, USA) at pH 7.6.

2.2. Animal infection model

Female BALB/c mice were purchased from Janvier labs in France. The animals were housed under standard conditions of light and temperature and all mice were fed laboratory chow and water ad libitum. Experiments were carried out with mice in the age of 10–12 weeks. All procedures used were approved by the local ethics committee (Lund University, Lund, Sweden).

Animals (n = 6) were infected subcutaneously with *S. pyogenes* bacteria (1.1×10^8 in a total volume of 200 μl in the dorsal back), closely monitored for signs and symptoms of infection, and weighed on a daily basis. Moribund animals were euthanized and counted as sacrificed. Animals were euthanized using isofluorane until the animal showed effect, which was followed by cervical dislocation. The animal use protocol (AUP) was approved by the local Malmö/Lund Institutional Animal Care and Use Committee (AUP#M327-12).

2.3. Surface digestion

Bacteria were harvested and resuspended in 4 °C sterile filtered resuspension buffer (1 M d-arabinose (Fluka, Sigma-Aldrich), 20 mM Tris-HCl (Merck), 150 nM NaCl (Sigma-Aldrich, St. Louis, MO, USA), 10 mM CaCl2 (Sigma-Aldrich)) to a concentration of 1.6×10^9 colony forming units (CFU) per ml. 10 μg trypsin (sequence grade modified trypsin Porcin, Promega,) was added per ml solution and incubated for 15 min at 37 °C with 500 rpm shake. The reaction were stopped by putting samples on ice for