Proteomic profiling of bacterial host adaptation

Racing the Red Queen

Kilsgård, Ola

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LUND
UNIVERSITY

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Abstract

Despite the discovery of antibiotics almost a century ago, infectious diseases continue to be a substantial cause of human mortality and morbidity worldwide, especially in developing countries. The adverse affects of infectious diseases are thought to increase over the coming years as the widespread misuse of antibiotic leads to the emergence of strains for which current therapies are ineffective. The last decades has also seen a large increase of animal pathogens crossing the species barrier to cause disease in humans. To be able to reverse these negative trends we need better knowledge of the events leading to the adaptation of these pathogens to their host. This thesis aspires to increase our understanding of bacterial host adaptation with the hope of finding new targets for diagnostic and therapeutic treatments.

In this thesis the development and application of novel mass spectrometry based methods for investigating bacterial host adaptation is studied. The developed methods are based on state of the art mass spectrometry proteomics, which allows the identification and quantification of in principal any expressed protein from a biological sample. The power of this analysis method was used to simultaneously quantify sets of bacterial and host proteins with a specific role in the infection course. These protein measurements are then used as standardization curves to obtain and account for any variation between biological states. The developed methods are combined to construct a quantitative model, depicting host – pathogen interactions and changes during infection progression. The model was used to determine the degree of host adaptation resulting of sequential passaging of the human pathogen *Streptococcus pyogenes* in a mouse infection model.

In summery, this thesis has increased out understanding of the complex interactions leading to host adaptation of bacterial pathogens by the development of a quantitative model for bacterial infections. In addition, this thesis suggests a new approach for biomarker discovery and validation, by using standardization curves of potential biomarkers. The research conducted in this thesis has the potential to lead to increased clinical diagnostic and treatment opportunities of infectious diseases.

Key words: Proteomics, mass spectrometry, systems biology, biomarker, host adaptation, bacteria, Streptococcus pyogenes

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Proteomic profiling of bacterial host adaptation

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"Nothing is impossible! Not if you can imagine it. That's what being a scientist is all about"

- Professor Hubert J. Farnswoth, Futurama

Content

Original papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Malmström L, Bakochi A, Svensson G, Kilsgård O, Lantz H, Petersson AC, Hauri S, Karlsson C, Malmström J. Quantitative proteogenomics of human pathogens using DIA-MS. J Proteomics, 2015

II. Kilsgård O, Karlsson C, Malmström E, Malmström J. Differential compartmentalization of Streptococcus pyogenes virulence factors and host protein binding properties as a mechanism for host adaptation. Int J Med Microbiol, 2016

III. Malmström E, Kilsgård O, Hauri S, Smeds E, Herwald H, Malmström L, Malmström J. Large-scale inference of protein tissue origin in gram-positive sepsis plasma using quantitative targeted proteomics. Nat Commun, 2015

IV. Kilsgård O, Teleman J, Malmström E, Hauri S, Malmström J. Baccus – determining bacterial load in vivo using targeted mass spectrometry. Manuscript submitted for publication

V. Kilsgård O, Malmström E, Hauri S, Malmström L, Malmström J. Host adaptation of S. pyogenes. Manuscript

My contributions to the papers

My contributions to the papers in this thesis are as follows:

I. I contributed to wet laboratory work by growing the bacterial strains and preparing them for mass spectrometry analysis.

II. I was responsible for the design of the study, performed the experimental procedures and data analysis and wrote the paper.

III. I took active part in the animal experiments conducted in this study.

IV. I was responsible for the design of the study, performed the experimental procedures and data analysis and wrote the paper.

V. I was responsible for the design of the study, performed the experimental procedures and data analysis and wrote the paper.

Excluded publications

I. Carlsson MC, Balog CI, Kilsgård O, Hellmark T, Bakoush O, Segelmark M, Fernö M, Olsson H, Malmström J, Wuhrer M, Leffler H. Different fractions of human serum glycoproteins bind galectin-1 or galectin-8, and their ratio may provide a refined biomarker for pathophysiological conditions in cancer and inflammatory disease. Biochim Biophys Acta, 2012

II. Carlsson MC, Bakoush O, Tengroth L, Kilsgård O, Malmström J, Hellmark T, Segelmark M, Leffler H. Galectin-8 in IgA Nephritis: Decreased Binding of Iga by Galectin-8 Affinity Chromatography and Associated Increased Binding in Non-IgA Serum Glycoproteins. J. Clin. Immunolo, 2011

III. Kilsgård O, Andersson P, Malmsten M, Svensson SL, Linge HM, Eliasson M, Sörenson E, Erjefält JS, Bylund J, Olin AI, Sorensen OE, Egesten A. Peptidylarginine Deiminases Present in the Airways During Tobacco Smoking and Inflammation can Citrullinate the Host Defense Peptide LL-37 Resulting in Altered Activities. Am. J. Respir. Cell Mol. Biol, 2011

Abbreviations

CID	Collision-induced dissociation
Commensal	Normal/indigenous microflora
DDA	Data-dependent acquisition
DIA	Data-independent acquisition
ECD	Electron-capture dissociation
EID	Emerging Infectious Diseases
ESI	Electro-spray ionization
ETD	Electron-transfer dissociation
FTICR	Fourier transform ion cyclotron resonance mass analyzer
GEBA	Genomic Encyclopedia of Bacteria and Archaea
HCD	Higher-energy collisional dissociation
HPLC	High-performance liquid chromatography
iTRAQ	Isobaric tags for relative and absolute quantification
MALDI	Matrix assisted laser desorption ionization
MS	Mass Spectrometry
MS1	mass spectrum of the intact ion
MS2	Mass spectrum of the fragmented ion
Pathogen	Disease causing agent
PRM	Parallel reaction monitoring
PTM	Post translational modification
S. pyogenes	Streptococcus pyogenes
Serotype	A distinct variation within a species of bacteria or virus
SILAC	Stable isotope labeling by amino acids in cell culture
SIRS	Systemic inflammatory response syndrome
SRM	Selected Reaction Monitoring
TMT	Tandem Mass Tags
TOF	Time of flight mass analyzer

Chapter 1

Introduction

The human being plays host to a large microbial community consisting of an estimated 10^{14} bacterial cells, almost ten times as many as the number of human cells in the body. These bacteria colonize a wide range of niches including the skin, intestine, upper respiratory tract and urogenital tract. A large part of these resident bacteria have a beneficial effect on our health and disruptions in the composition of these commensals have been linked to several adverse conditions [1-4]. However, this colonization can also be detrimental to the host when it occurs in sites of the body that should be kept sterile, like the internal organs and lower respiratory tract or when colonized by pathogenic bacteria that do not confer any beneficial effects. Infections caused by these pathogenic bacteria give rise to classical disease symptoms such as localized pain, swelling and fever but can result in the death of the host if the infection is not managed, either by the host itself or by the intervention of medical treatment.

Infectious diseases are a major cause for morbidity and mortality, accounting for more then one fifth of all deaths in adults in South-East Asia and Africa and for half of all child deaths worldwide [5]. In fact, lower respiratory infections were listed as the third leading cause of death in 2004 and this number is expected to rise in coming years as antibiotic resistance leads to the emergence of bacterial strains where the current treatment is ineffective [6]. In order to develop new ways of combating these emerging bacterial strains, we need to better understand how bacterial pathogens adapts to their host to cause infections.

The infection state is a complex network of interactions

Infections are very complicated interactions between a invading pathogen and the host, the outcome of which depends both on the inherent virulence of the invading pathogen and the susceptibility of the host. Humans and bacteria have coevolved for millions of years resulting in elaborate ways for defending against bacterial infections, in the form of the human immune system [7, 8] and equally elaborate ways of circumventing or overcoming these defenses, in the form of bacterial virulence factors [9, 10]. A lot of research has been conducted in this field, with the hope of unraveling these interactions to understand how bacterial pathogens have adapted to humans to cause disease, a knowledge that would undoubtedly lead to improved therapeutics and diagnostics. However, the complexity of these thousands of interactions between host and pathogen means that in order to gain any understanding, the system need to be deconvoluted and broken down in to more tangible components, often resulting in rudimentary models. These models, though generating a lot of insight into specific parts of the system are unable to convey the full scale of interactions between host and pathogen, leading to incomplete or partial conclusions and subsequently a lack of understanding for pathogen adaptation. In order to further our understanding about host-pathogen adaptations we need to develop more accurate and quantitative models depicting not only parts of, but the full complex network in bacterial infections.

The sum of all parts

Systems biology is a holistic scientific approach that aims to explain a biological system based on all its interacting parts. Instead of reducing a system to individual interactions or components, systems biology studies the interaction between different components of a biological system and relates how these interactions give rise to the function and behavior of a given system. In this way, understanding of the infection state can only come from the study of interactions between host and pathogen, viewing them not as separate entities but rather as different parts of the same system. However, biological systems contain information on many different levels, with a variety of different molecules interacting to keep the system operational. Since the successful sequencing of two complete bacterial genomes in 1995 [11, 12] together with the human genome six years later in 2001 [13,14], our understanding of the genetic blueprint and how mutations of it cause disease

has increased substantially. The biggest impact on genome sequencing has been the reduction in cost and decreased processing time, with third-generation DNA sequencing able to completely sequence a bacterial genome in a few hours [15]. These sequencing procedures have now become routine, resulting in information about tens of thousands of bacterial genomes being available and more being added daily. Despite this vast resource of information, genetic sequencing can only reveal part of the story as the genome is comprised of static information that, with a few exceptions, will remain the same regardless of environmental conditions, which limits information about cell behavior and interactions [16, 17]. The genome contains the information needed for a cell to function but it is the proteins that carry out the actual functions. This information is transcribed from gene to mRNA, which translates the genetic code into a protein consisting of a specific sequence of amino acids. Each gene can give rise to several different proteins [18, 19] and each protein can be modified by potentially hundreds of post-translational modifications (PTMs) [20, 21], giving rise to countless variations that each has a specific place and function in the cell [22, 23]. External and internal perturbations of the cell will result in dynamic changes of the protein abundance and composition thereby defining the current state of the cell and the associated phenotype. By measuring sets of proteins that have distinct cellular functions it is possible to define the phenotype associated with that function. The increased number of genomes in combination with the advancement of proteomic techniques gives a unique possibility to address aspects of systems biology and to apply these system biology strategies to infectious medicine research.

Therefore, the overall aim of this thesis is:

Investigate bacterial adaptation of the human model pathogen S. pyogenes to a murine host using a combination of proteomic and systems biology approach. This allows for a deeper understanding of the infectious state and subsequent host adaptation with the purpose of identifying species-specific events needed for infection establishment and progression. The knowledge of these required events for successful host adaptation and colonization could be used to improve diagnostic and therapeutic interventions.

In order to achieve this aim, a model for the quantitative evaluation of bacterial adaptation and host response musts to be established. This model needs to be able to account for:

Phenotypic changes on the bacterial level related to increased virulence and the effect this have on subsequent host interactions

Host response to infection and the severity of bacterial burden on the host

Dissemination patterns of the bacteria as a result of changed host interaction pattern

To capture these different aspects the constructed model is created using MS based proteomics. This technique has evolved rapidly during the last decades to now be able to principally measure any expressed protein in a sample, making it possible to characterize cellular phenotypes and interactions.

Mass spectrometry based proteomics

There exist several different strategies for studying the proteome of living cells using either biochemical, biophysical or computational methods such as 2-dimensional separation followed by protein staining [24, 25], binding by fluorescently labeled antibodies [26, 27] or mass spectrometry [28, 29]. The work presented in this thesis is based exclusively on mass spectrometry proteomics, which rapid development has profoundly increased our capability of investigating the living proteome by allowing identification, quantification and modifications of in principal any expressed protein [30].

Mass spectrometry can be used to study proteins in their native form in an approached called top-down, which has the advantages of better sequence coverage and more accurate identifications of PTMs [31, 32]. However, challenges with instrumentation and data interpretation have severely limited the general use of this method [33]. The more common way of analyzing proteins with MS is through a bottom-up approach [34], where proteins are enzymatically cleaved into peptides using sequence specific proteases such as trypsin and separated according to hydrophobicity on a reverse-phase liquid-chromatography system. Eluting peptides are ionized and transferred into gaseous form by electrospray ionization (ESI) [35] or alternatively, peptides can be encased in an organic matrix, deposited on solid surface and ionized using matrix assisted laser desorption ionization (MALDI) [36].

Digestion with a suitable enzyme increases the chance of protein identifications by generating peptides that have more favorable ionization abilities than proteins and which occur in higher abundance, which increases the chance of measuring at least one peptide from a protein. Chromatographic separation decreases the amount of peptides eluting at the same time, which means better ionization and fragmentation in the mass spectrometer [37]. In addition, information about retention time can serve as a further parameter for peptide identification.

MS can be combined with various labeling techniques for example isobaric peptide labels such as iTRAQ [38] or TMT [39] and metabolic labeling such as SILAC [40]. The use of a labeling technique drastically reduces technical prejudice, which facilitates data analysis [41]. Label free methods on the other hand are easier to use and is applied to all types of samples by default and can be combined with stable isotope labeled internal standards like AQUA peptides [42], which allow for absolute quantification [43]. The work presented in this thesis was done exclusively using the bottom-up approach with ESI and a label-free workflow.

The defining feature of a mass spectrometer is the ability to separate gas-phase ions according to the mass over charge ratio (m/z). There are different ways of achieving this separation either by; calculating the m/z values based on the flight time of the ion in time-of-flight analyzers (TOF) [44], using oscillating radio frequencies to selectively permit only ions with specific m/z values to pass through in the quadrupole analyzer (Q) [45] or using cylindrical fields to arrange ions into different orbitals in the Fourier transform ion cyclotron resonance (FTICR) [46] and Orbitrap [47] analyzer. These different mass analyzers can be combined in tandem to create hybrid MS/MS instruments like a quadrupole-Orbitrap. Regardless of combination, no single mass spectrometric method can achieve identification and quantification of all theoretical peptides proteolyticaly derived from a sample [48]. This has yielded several different mass spectrometry methods with different strengths and weaknesses.

Mass spectrometry workflows

By combining different mass spectrometry methods the strength of each method is preserved while reducing the associated weakness through overlapping measurements. In this way, observations done with one method can be followed up by another method and thereby increasing the coverage of

each sample. The three different mass spectrometry methods used in this thesis can be seen in Figure 1.1 and are described in the following section.

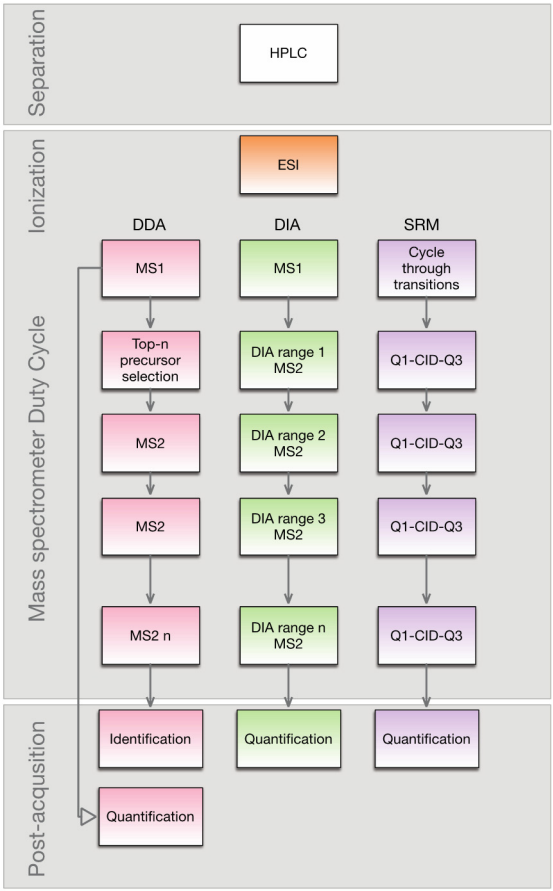

Figure 1.1 Mass spectrometry workflow outline.
After separation (HPLC) and electro spray ionization (ESI), the different workflows diverge. In DDA, the top-N measured MS1 peaks are selected for fragmentation- In DIA, the MS1 range is fragmented in fixed subsets and in SRM, predetermined precursor and fragments m/z values are used to exclusively measure ions of interest. Modified with permission from Johan Teleman, PhD.

Data Dependent Acquisition (DDA), also know as shotgun proteomics [49], is the most wildly used strategy for MS-analysis and focuses on identifying as many peptides as possible at the expense of sensitivity and reproducibility. In this method peptides are separated by HP-LC and analyzed by MS in the full scan and MS/MS modes (Fig 1.1). As the ionized peptides enter the mass

spectrometer they are scanned and chosen for fragmentation using a simple heuristic method. This first detection of the ionized peptides is known as a survey or MS1 scan and in each scan between 3 and 15 peptides are selected for fragmentation. This fragmentation is often performed using CID/HCD [50, 51] but other types of fragmentation such as ETD [52] and ECD [53] also exists. The resulting fragments are detected in the MS2 scan resulting in the product-ion spectra, which together with the information about the precursor ions are used to determine the amino acid composition of the fragmented peptide by either matching the spectra to theoretical mass-spectra created by in silico digestion or to a spectral library containing experiment MS2 spectra of the particular peptide.

Unlike the DDA method, selected reaction monitoring (SRM) [54] and parallel reaction monitoring (PRM) [55] provide very high sensitivity, specificity, reproducibility and accuracy of quantification but limits the amount of analytes that can be measured. These methods are highly hypothesis driven only identifying and quantifying a predetermined set of peptides. In SRM, for each peptide to be measured the m/z of the precursor ion, its retention time and a set of high intensity fragment ions unique to the peptide in question needs to be determined. These parameters constitute a SRM assay for detecting and quantifying the targeted peptide in any sample. Generating high quality SRM assays are paramount for a successful analysis but can be time consuming. Recently, several community-developed tools have been introduced that substantially decreases the generation time of assays creation [56, 57]. SRM experiments are conducted on triple quadrupole instruments where the information contained in these assays will instruct the first and third quadrupole to cycle between preset m/z values corresponding to the chosen peptides instead of scanning across the entire m/z range. Ions of the correct m/z value will be allowed to pass through the first quadrupole into the collision chamber where they are fragmented using CID. Fragments of the correct m/z value will then be transferred through the third quadrupole to the detector. In PRM, the third quadrupole is replaced with a scanning analyzer like an Orbitrap, which enables all fragments of the chosen precursor to be measured and eliminating the need for fragment pre-selection.

Data Independent Acquisition (DIA) aims at acquiring the complete MS/MS subset for every possible mass range, independent on selection or detection of the precursor across a chromatographic resolved sample [58]. DIA moves away from the previous dogma of one MS/MS spectra originating from a

single peptide, which is the foundation of DDA and SRM. Instead DIA-based methods open up the precursor mass window to generates MS2 spectra from all precursor ions falling into a predefined m/z range, meaning the resulting MS2 spectrum is a multiplex spectrum of fragment ions generated from all precursors within the predefined m/z range of the precursor window. This method was proposed several years ago but limitations in instrument performance reduced its usability. If the predefined m/z ranges of the precursor mass window are adjacent each other and cover the entire m/z precursor range of peptides in a sample, the complete fragmentation of every precursor will occur.

Mass spectrometry proteomics is a technique well suited for measuring bacterial proteomes since the relatively small genome size allows the quantitative measurement of the majority of proteins in the bacterial proteome [59] and several studies have already used these techniques to successfully measure how bacterial pathogens adapt to new environments in vitro [60-62].

Choosing a bacterial pathogen

One of the most commonly occurring human bacterial pathogens is Streptococcus pyogenes, or group A Streptococcus (GAS). This gram-positive human pathogen is responsible for a wide range of clinical symptoms, ranging from self-limiting throat infections (pharyngitis) to life threatening invasive disorders such as necrotizing fasciitis and toxic shock syndrome [63]. Repeated infections with this pathogen can lead to severe post infectious sequelae, such as poststreptococcal glomerulonephritis, acute rheumatic fever and rheumatic heart disease [64]. Globally, around 18.1 million people are suffering from severe infections with S. pyogenes, with an estimated 1.78 million new severe infections and over 700 million non-severe infections each year [65]. Despite causing such staggering amounts of infections, “only” around 500 000 results in a fatal outcome, mostly occurring in developing countries. While the superficial infections are easily manageable with antibiotic treatment, invasive infections caused by S. pyogenes are difficult to treat, often requiring considerable surgical intervention and more aggressive antibiotic treatments [66]. There has also been a marked increase in the incident and severity of S. pyogenes infections since the mid-1980s, with possible links to emerging strains of bacteria having a specific virulent phenotype [67] that makes it more well adapted to

cause infections in the human host. This virulent phenotype makes S. pyogenes a very interesting pathogen to study from a host adaptation standpoint, having recently acquired an increased fit in its human host. In addition, despite humans being the exclusive host of this pathogen mice are susceptible to infection, allowing for in vivo studies of host adaptation [68].

Host defenses against bacterial pathogens

When a pathogen first infects its host it will usually be followed by a dramatic activation of the innate and adaptive immune response. These two complementary systems work on different levels, where the innate host response is the first to responds, recognizing a set of specifically conserved structures found in many microorganisms, signaling the host of an impending pathogen invasion [69]. This broad immune response is followed by a species-specific response in the form of the adaptive immune response with the construction of antibodies and cells directed towards the invading pathogen [70]. This response is slower, usually taking a few days to reach full effect but forms an immunological memory that will respond much quicker the next time the same pathogen invades the host.

S. pyogenes have developed several mechanisms for evading or subverting the human immune defenses [71], allowing it to establish infections despite mounting host defenses. By establishing the levels of immune activation in response to bacterial infections, it is possible to establish a quantitative baseline for host health, which can be used to determine to progress of infection in a model host system.

Aim of this thesis

Even though we have gained a much greater understanding of the microbial world and its interactions since Antoney van Leeuwenhoek first made the discovery of bacterial organisms more then three centuries ago, our understanding of the complex events behind host adaptation are still not understood. To truly understand this we need to understand the complex interplay between host and pathogen, something that can only be done by identifying and quantifying the molecular actors of these reactions, the proteins. This involves the generation of quantitative protein profiles for a

diverse set of samples across several different species, including bacterial, murine and human.

However, the complexity of the infectious state and the thousand of interactions occurring between host and microbe makes it challenging to investigate, resulting in deconvuloted, simplistic models that only convey part of the story.

Developments in mass spectrometry proteomics have resulted in a high-throughput analytical method capable of identify and precisely quantify thousands of proteins from a complex range of samples, making it a invaluable tool for modeling complex biological systems.

In this thesis, I will describe the research I have conducted during the past five years, which has had the overall goal to improve our current knowledge of bacterial host adaptation by developing a quantitative mass spectrometry based model of bacterial infection using S. pyogenes as a model pathogen. This endeavor has resulted in five scientific publications, describing the various steps taken to develop a quantitative model of bacterial infections, including; mapping how genetic differences affect the composition and expression of bacterial virulence proteins (Paper I), how these specific virulence proteins change after passage of the bacteria in a mouse (Paper II), how the mouse hosts blood plasma proteins change in response to bacterial infection (Paper III) and the creation a tool for estimating bacterial load in complex samples (Paper IV). The four previous steps were then implemented into the construction of a quantitative model of bacterial infections, which used to investigate the adaptation pattern of S. pyogenes to a murine model (Paper V).

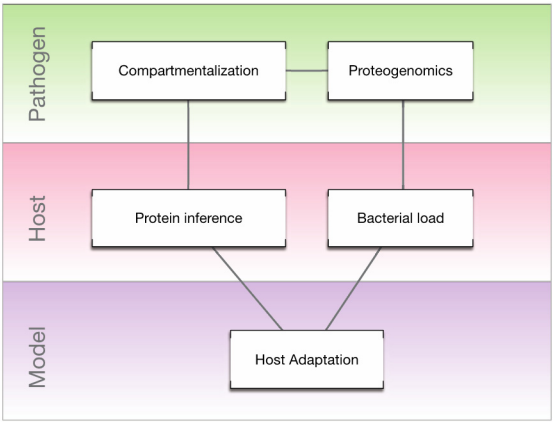

Figure 1.2 Overview of included articles.
Paper I and II investigates virulence changes on the bacteria and modulates a conceptual relationship for bacterial virulence. Paper III and IV describes host effects related to bacterial infections by quantifying changes in blood plasma proteome of infected mice and describes a novel way for estimating bacterial load using MS. Paper V builds upon all previous studies and incorporates them to create a quantitative model for bacterial virulence. The model is used to study the host adaptation of S. pyogenes to mice.

Each chapter of the thesis describes the different aspects of the aims put forth in the introduction, going through the creation of a quantitative infection model to the application to a bacterial adaptation study. The relation between genes and proteins and the role of virulence proteins is discussed in Chapter 2, host interactions and disease burden in Chapter 3 and the use of animal models and biomarkers for disease in Chapter 4. The final chapter of this thesis puts the obtained results in context and describes the impact of the conducted research on a broader scale. Lastly, possible future outcomes of the conducted research are provided, including potential therapeutic and diagnostic impact.

Chapter 2

Bacterial Virulence

To understand a pathogens ability to infect its host we need to understand the molecular factors that governs bacterial virulence. Genomic analysis of bacterial pathogens has revealed that some pathogens can undergo rapid genomic rearrangements, resulting in increased host fitness and virulence [72]. These genomic changes often lead to increased expression of subsets of proteins termed virulence factors, which have a direct role in the pathogens ability to sustain infection. In this chapter, I will try and convey how integration of genomic and proteomic data can be used to yield an increased understanding of the molecular events leading to increased bacterial virulence. Further I will describe the general concept and importance of bacterial virulence factors, together with the proposal of a virulent phenotype of S. pyogenes and the implications this may have for the increased prevalence. The last part of this chapter will detail the studies I conducted into interpretation of proteome changes based on genetic information (Paper I) and the specific changes in expression and distributions of virulence proteins in animal passaged strains (Paper II).

The conducted studies aimed to:

Determine phenotypic changes on the bacterial level related to invasiveness and the effect this have on subsequent host interactions

Red Queen hypothesis

Few events in human history have shaped the evolution of humans as much as infections [73], impacting not only our physiology but also our behavior [74] and social interaction patterns [75, 76]. The complex interactions between host and pathogen can be described as a species arms race, where the host is evolving to resist the invading pathogen, which in turn is under

pressure to evolve mechanisms to overcome host resistance, resulting in a cycle of continuous adaptation and counter-adaptation. This genetic struggle between host and pathogen is generally referred to as a “Red Queen” scenario. A hypothesis first defined by Leigh Van Valen [77] after the character in Lewis Carroll´s novel Through the Looking-Glass, who utters the phrase “It takes all the running you can do, to keep in the same place”, illustrating that each adaptation by a species is matched by a counteracting adaptation in another interacting specie, resulting in a status quo equilibrium [78].

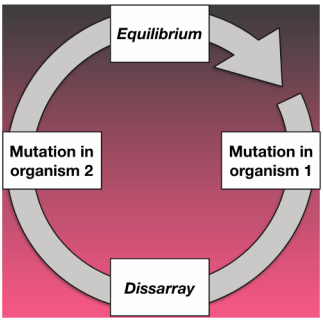

Figure 2.1 The Red Queen hypothesis
A mutation in one interaction organism will be followed by the subsequent mutation of a interaction organism, leading to a evolutionary equilibrium.

Proteogenomics

Fluctuations in this genetic status quo equilibrium can lead to increased fitness of the pathogen, resulting in a breach of the host defenses and subsequent disease emergence. Identification of these genetic events and their resulting protein function is essential for developing predictive strategies for virulence emergence and epidemics. Improvements speed and decreased costs of genetic sequencing have resulted in more then 30 000 sequenced bacterial genomes being publically available (NCBI 2014) with projects like the Genomic Encyclopedia of Bacteria and Archaea (GEBA) leading to increases in both number and diversity of bacterial strains [79]. However, as the number of genomes increases so to does the need for accurately annotated metadata for accurate prediction of loss or gain of function. This vast genomic information has lead to more reliance on in silico annotation methods for predicting protein function, which have several inherent limitations [80]. In proteogenomics, mass spectrometry is used to identify

proteins predicted from genetic sequences and this information is used to improve the genome annotations [81]. Identifications of a protein by one or more peptides add substantial value to the genomic annotation by verifying, correcting or adding missing information making MS-based proteomics a powerful supplement to genomic data. In addition, the use of proteogenomics can help identifying PTMs [82] and verify hypothetical proteins [83]. This latter trait makes proteogenomics a vital tool for identifying new virulence factors.

Virulence factors

For any microorganism, surviving in a specific host niche requires adaptation to the specific conditions encountered therein. For pathogenic microorganisms, these adaptation factors are known as virulence factors as they allow the pathogen to establish colonization in the face of a mounting immune response and transmit the pathogen to another host. These virulence factors are often shared within or between bacteria through horizontal transfer of mobile genetic elements like plasmids, genomic islands and bacteriophages [84-86]. Depending on the host niche of the pathogen, the virulence factors can convey the ability to penetrate the surface epithelial layer, attach the pathogen to cell surfaces and/or the extracellular matrix, invasion of intracellular compartments, accusation of iron, evasion of host-defense mechanisms and transmission to other hosts [87, 88]. The expression of different virulence factors is tightly controlled and often determined by environmental signals from the niche the pathogen occupies, which can result in a varying degree of damage to the host [89]. A large number of S. pyogenes virulence factors have been characterized, many of which show large functional redundancy in the processes of adhesions, colonization, resistance to innate immunity and the capacity to spread within the human host [90]. For examples, the multifunctional virulence factor M protein has a role in both adherence, colonization of the specific niche, resistance to phagocytocis and intracellular entry into host cells. Several of these functions are also provided by other virulence factors such as fibronectin-binding proteins that have roles in adhesion and penetration of host cells. In fact, more than 17 different adhesion candidates have been identified so far and this large redundancy could contribute to S. pyogenes ability to colonize such a wide variety of niches [91]. This pathogens ability to cause severe invasive infections results from its ability to invade to previous sterile sites, like the bloodstream and deep tissue [92]. Systemic spread of the pathogen requires

the very precise and coordinated expression of multiple virulence factors, resulting in tissue destruction, bacterial dissemination and hyper-inflammation.

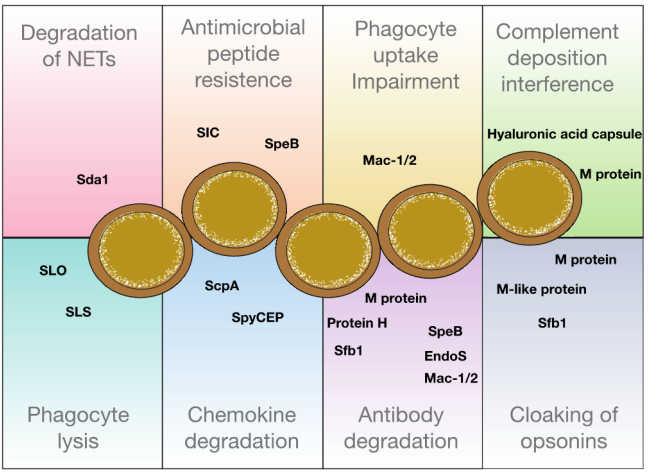

Figure 2.2 Virulence factors of S. pyogenes
Being a human adapted pathogen, S. pyogenes have evolved numerous virulence factors to subvert the human immune defenses. This picture illustrates the many different virulence factors expressed by this pathogen and their role in establishing infection.

Transition to infectious phenotype

Serotype M1 is amongst the most frequently isolated serotypes from both invasive and non-invasive infections of S. pyogenes. The increased incidence of severe infections since the mid-1980s has been linked to the emergence of the M1T1 clonal phenotype, believed to have evolved as a result of diversification of the bacteria and acquisition of new genes that improved the adaptation to the human host [93]. Several studies have shown how this phenotype can be reconstructed by inoculation of the original strain into a mouse, yielding a new hypervirulent phenotype [94]. This passaged strain mimics the genetic changes seen in invasive strains and show increased mortality when reintroduced into a mouse host, leading to speculations that these changes results in a higher fitness of the bacteria in the human host as well, which would explain the global dissemination of this strain and the observed incidence increase [95, 96]. If this hypothesis is correct, the phenotypic changes that occur after passage of S. pyogenes in a mouse is a direct effect of increased virulence independent of host factors and would

indicate that the innate virulence of S. pyogenes is responsible for the incident increase. However, other studies have found no correlation between the specific phenotype and invasive infections, instead citing host and environmental factors as the biggest contributors for the outcome of infection [97, 98].

Contributions

The substantial increase in DNA sequencing speed together with reduction in cost has substantially increased the number of sequenced bacterial genomes. This vast information can be use to investigate mechanisms underlying invasive infectious disease pathogenesis. However, any interpretations require correct annotations of the functional part of genetic changes in order to build accurate disease models. In Paper I, we developed a strategy for integration of genome data with DIA-MS for rapid interrogation of the combined data sets. This method was used to combine data sets from whole genome sequencing, shotgun MS and DIA-MS from 34 clinical isolates of S. pyogenes. The study revealed several proteins that are both affected by mutations and that differ in expression quantity between invasive and non-invasive strains.

Pathogenic bacteria have evolved elaborate mechanism for invading and colonizing the human host using specific virulence factors that enables evasion of host immune defenses. The expression of these virulence factors is tightly controlled and individual proteins are expressed at specific steps of the infection cycle. In Paper II, we investigated the effect animal passage had on the expression profile of virulence factors and the subsequent interaction with mouse plasma proteins. In this study, secreted and surface bound virulence factors were isolated from four strains of S. pyogenes with varying virulence profiles in mice and analyzed with DDA-MS. This experiment identified relatively few but highly abundant virulence factors on the bacterial surface, with a higher proportion on more mouse virulent strains. These changes was followed up by SRM measurements of 41 unique secreted and surface bound virulence factors on two of the strains, including a clinical isolate and the animal passaged version of the same isolate. The SRM analysis showed that the animal passaged strain had a higher proportion of surface bound and secreted virulence factors compared to the original strain. These differences manifested as a changed interaction patterns with mouse and human blood

plasma proteins, with the animal passaged strain showing higher affinity for binding mouse homologues.

Tool creation

The outcome of infection is dependent on both the innate virulence of the invading pathogen and specific host factors. By determining the phenotypic changes between strains with varying virulence in mice, the effect of virulence factor expression off the bacteria could be determined. Changes in virulence profile also resulted in changes in host bloods plasma interaction profiles between strains. These two parameters could be merged into a virulence model depicting quantitative changes to virulence based on the expressed virulence proteins and bound host factors. This virulence model can then be used as a calibration curve to estimate the level of adaptation in other strains of S. pyogenes.

Chapter 3

Host; interactions and defenses

The pathogens success in invading its host depends on its ability to grow and outperform the host defenses. To establish an infection the pathogen must make sure to acquire enough nutrients, overcome any physical forces working against it and prevent any attempts by the innate or adaptive immune system to clear it from the system. In this chapter, I will describe the host response to bacterial infection and how measurements of this can yield quantitative information about host health and status. Further, the concept of bacterial load and its relevance to infections will be discussed. The final part of the chapter is dedicated to the two studies I performed, investigating the changes in the blood proteome composition in response to bacterial infection (Paper III) and the development of a mass spectrometry based method for estimating bacterial load (Paper IV).

The two conducted studies aim to resolve:

The host response to infection and the severity of bacterial burden on the host

Dissemination patterns of the bacteria as a result on changed host interaction pattern

Blood plasma

The blood plasma is a very dynamic and complex fluid composed of classical plasma proteins but also a mixture of infused proteins from surrounding tissues and organs. The plasma proteins are mainly produced by the liver and are involved in the blood plasmas main functions such as serving as transport medium, provide colloid osmotic pressure and maintaining homeostasis through the complement and coagulation systems. The dynamic range of

proteins in plasma is huge, commonly cited as 10 orders of magnitude [99] where the top 14 proteins constitutes 98 % of all molecules, making it challenging to analyze the remaining 2%. However, this heterogeneity of the blood plasma can yield significant insights into the current health status of a patient as it contains proteins collected from all sites of the body, in addition to the mediators of the host reaction to infection and inflammation. Together with the relative ease of obtaining blood plasma, makes it an attractive fluid for measuring the host response to infection.

Host response to infection

The host acute-phase reaction can be induced by a number of conditions such as infection, cancer, surgery, trauma and immunological disorders. The acute phase proteins are a heterogeneous group, primarily produced by the liver and mostly found in plasma where their concentration can vary considerably during inflammatory disorders [100, 101]. Main stimulators are pro-inflammatory cytokines, particularly TNF-alpha and IL-6 [102] but the whole reaction involves around 200 proteins, many of which have a direct role in the host defense such as complement factors and coagulation proteins. C reactive protein (CRP) and serum amyloid (SAA) are two very dynamic proteins that both are up regulated 1, 000 fold and have active roles in the inflammatory response [103, 104]. The acute phase proteins reflect the presence and intensity of inflammation and have long been used in the clinic as prognostic biomarkers for invasive infections such as sepsis.

Sepsis

Sepsis is the result of an exaggerated immune response to an invasive infection. The current definition of sepsis is a systemic inflammatory response syndrome (SIRS) to a presumed or known infection. This can lead to the development of sepsis (SIRS caused by an infection), severe sepsis and septic shock were the outcome depends on both host and pathogen characteristics [105, 106]. Sepsis is a very fast progressing disease with a mortality rate of 30-60 % for severe sepsis and septic shock [107], which increases by up to 7,6% for every hour antimicrobial treatment for septic shock is delayed [108]. Early diagnosis of sepsis is therefore crucial for proper treatment and decreased mortality.

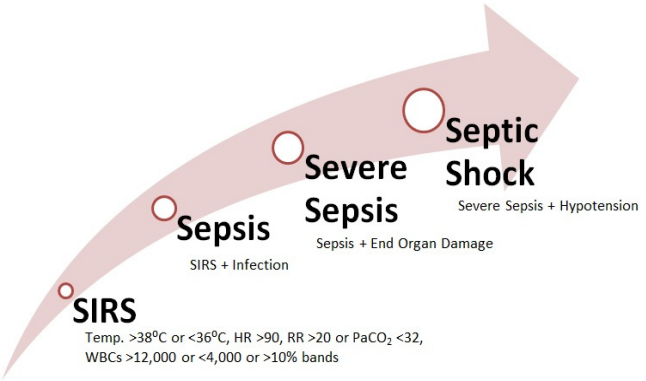

Figure 3.1 Definition of sepsis
The definition of sepsis indicators, according to the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine´s (SCCM).

Unfortunately, sepsis presents with very defuse and heterogeneous symptoms and current biomarkers are not specific and/or sensitive enough to assign an accurate clinical diagnosis [109-111]. This is further complicated with a lack of information concerning the infecting microbe, often overlooked at the initial admittance to health care and the limitation of bacterial cultures, which can be negative in as many as 30-50% of cases [112]. These limitations of current biomarkers have lead to increased focus on finding new and improved prognostic biomarkers for sepsis.

Biomarkers

The term biomarker refers to "a characteristic that is objectively measured and evaluated as an indicator of normal biological courses, pathogenic progressions, or pharmacologic responses to a therapeutic intervention" [113]. The goal of any biomarker is to aid in the treatment of patients through improved diagnostics, prognosis and/or evaluation of treatment efficiency. However, more than 150 000 papers have been put forth presenting thousands of possible biomarkers, with less then 100 of them have been validated for use in routine clinical practice. This large discrepancy between discovery and validation could be a result of a lack of standardization for sample collection and handling, insufficient sampling amounts or a lack of cross-disciplinary knowledge [114]. Biomarkers have the potential to reveal

the health status of patients and could be used as both a diagnostic and therapeutic method.

Bacterial load

Another type of biomarker is the bacterial load, which is defined as the measurable quantity of bacteria in an object, organism or organism compartment. High bacterial loads have been previously shown to correlate with a worse disease progression and increased disease severity in disorders such as sepsis and pneumococcal infections [115]. Unlike conventional biomarkers for infectious diseases, bacterial load puts the focus on the invading pathogen instead on the host response to infection, shifting the application towards a more prognostic approach. However, like host biomarkers such as CRP, bacterial load estimations could be used to gauge response to treatment and guide therapeutic interventions [116-118]. In addition, bacterial load have the potential to identify specific high-risk groups of individuals where the current treatment options are failing [119]. An accurate measurement of bacterial load can also reveal dissemination patterns in its host, revealing subsets of tissue and niches where specific pathogens prefer to reside [120]. The deposition of bacteria in specific tissue could be indicative of a species-specific adaptation to the selected tissue and could explain disease symptoms seen in certain diseases.

Contributions

The easy in which blood samples can be collected and the potential information about patient health status that can be determined from it makes it an attractive fluid to collect in the clinic.

However, the mechanism behind the control of the blood plasma proteome is currently unknown. There is also evidence that the blood plasma proteome of humans are highly variable, further increasing the difficulty of finding prognostic biomarkers [121]. In Paper III, we developed a novel MS-based strategy based for monitoring the dynamics of tissue and cell-specific proteins in the blood plasma by creation of a proteome-wide tissue atlas used for inferring tissue origin of blood plasma proteins. The strategy, based on DIA-MS, allows the quantitative measurement of almost the complete proteome from unfractionated blood plasma samples in a consistent and

precise manner, something that previously would have been technically impossible. This strategy was used to evaluate the rearrangement of blood plasma proteome composition during different stages of sepsis in animal models, revealing different regulation patterns as a consequence of disease severity, were known biomarkers for sepsis such as acute phase proteins, coagulation proteins and cytokines/chemokines showed a dose-dependent increase.

Bacterial load, the amount of bacteria in an organism or organism compartment, has been shown to correlate to disease severity in conditions like sepsis and could be used as a prognostic biomarker for disease progression. However, current methods for estimating bacterial load are limited by slow analysis time, semiquantitative results and an inability to handle bacterial species that do not grow in laboratory settings. In Paper IV, we develop a method using targeted mass spectrometry to rapidly quantify bacterial load in extremely complex samples such as organ tissue. The method, named Baccus is based on measuring stably expressed bacterial proteins as a proxy for bacterial concentrations and relating the measurements of these proteins to established relations of bacterial concentration. Using this method we could accurately estimate the bacterial load in artificially infected mouse organs. When applied to in vivo models of bacterial infections the method revealed information about bacterial adaptation and dissemination unobtainable with previous methods.

Tool creation

The outcome of any infection depends on the interactions between host and pathogen. Changes seen in the phenotype of the bacteria is reflected in the reactions of the host and conveys a vital part of the molecular mechanisms of host pathogen interactions. The created DIA-MS strategy allowed us to investigate changes in the blood plasma proteome during different levels of infection and this information can be used to create a dose-response curve for the observed host response. By comparing subsequent host reactions to infections of various bacterial strains to this calibrated curve allows us to estimate the degree of disease in the animal using a quantitative measurement. Infectious diseases tend to disperse from the site of infection and travel via the bloodstream to adjacent tissue. The dissemination pattern of a bacterial pathogen can therefore reveal species-specific adaptations resulting in a preferred niche accumulation. The developed Baccus workflow

allows us to follow the bacterial dissemination pattern of potentially any bacterial pathogen with a high degree of sensitivity and selectivity.

Chapter 4

Virulence model

The last three decades have seen an increased number of diseases characterized by a expansion in geographic range, a change in host specificity, increased morbidity, a change in pathogenesis and/or recently evolved pathogens [122]. These diseases have been termed emerging infectious diseases (EID) and represent one of the major threats to human health [123, 124]. A substantial part of these EIDs are zoonotic i.e. able to infect other host species [125]. Although most zoonotic pathogens are not highly transmissible between humans, the effect of even transient EIDs can be substantial as illustrated by recent outbreaks of the avian flue (H5N1 strain) in 1997 and the Ebola outbreak in West Africa between 2013 and 2016. In this chapter I will describe the steps involved for a pathogen to adapt to a new host, with the focus on animal pathogens adapting to a human host. In addition, the application of mice as a model for human disease and the concept of biomarkers for disease states will be addressed.

The final part of this chapter details the integration of previously developed tools described in the previous chapters to create a quantitative model for evolutionary host adaptation of S. pyogenes (Paper V).

The included study aimed to answer this thesis main goal:

To investigate bacterial adaptation of the human pathogen S. pyogenes to a murine host with the purpose of identifying species-specific events needed for infection establishment and progression.

Bacterial host adaptation

To cross the species barrier and infect a new host type is no easy task and requires a series of adaptation steps in which the pathogen goes from an exclusive animal pathogen to an intermediate form and then finally an exclusive human pathogen [126].

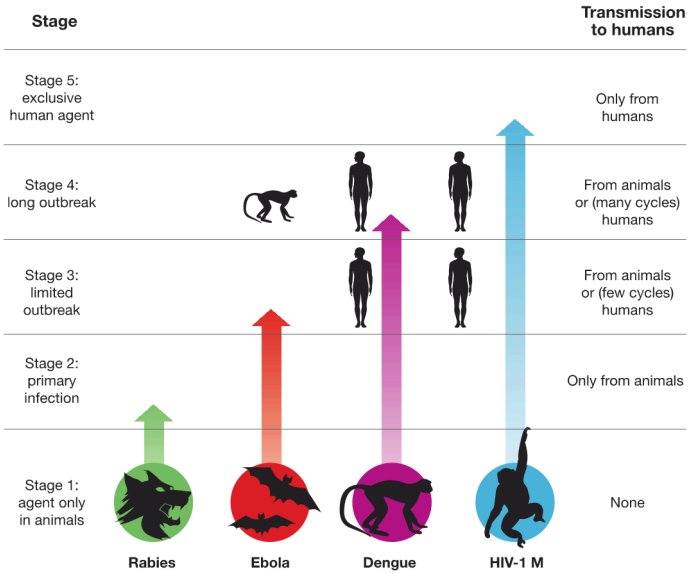

Figure 4.1 Stages of pathogen adaptation
Five stages of pathogen adaptation from animal specific to human specific. Wolfe et al, Origins of major human infectious diseases. Nature Reviews. 2007, doi:10.1038/nature05775

- Stage 1. At this stage the pathogen is present in animals but have not yet been seen in humans under natural conditions (spread could have been seen by artificial means such as blood transfusions or hypodermic needle usage). *Example: most of Malaria Plasmodia*
- Stage 2. At this stage the pathogen remains animal exclusive but can spread from animal to humans ("primary infection") through natural means. However, it cannot be transmitted between humans ("secondary infection"). *Example: Anthrax and Tularemia Bacilli, Rabies and West Nile virus*
- Stage 3. The pathogen can now case secondary transmissions in humans but has a limited life span in the human host, which results in

sporadic outbreaks that soon die out. *Example: Ebola, Marburg and monkeypox virus*

- Stage 4. At this stage the pathogens remains an animal pathogen but has a naturally (sylvatic) cycle of infecting humans through primary transmission from the animal host. These primary infections results in extended periods of secondary transmission between humans without the participation of the animal host. This stage can be further subdivided into three sub-stages, depending on the reliance on primary and secondary transmission.

- Stage 4a. The sylvatic cycle is more important then the direct spread between humans.
- Example: Chagas´disease and yellow fever
- Stage 4b. Both the sylvatic and direct transmission is important.
- Example: Dengue fever
- Stage 4c. Greatest transmission is between humans.
- Example: Influenza A, Cholera, Typhus and West African sleeping sickness

- Stage 5. The pathogen is now infectious exclusively to humans.
- Example: The agents causing Falcirarum malaria, measles, mups, rubella, smallpox and syphilis.

To progress through the different stages depends on a combination of several factors such as; donor (animal) abundance, amount of infected host, the frequencies of encounters between donor and recipient host, the likelihood of transmission of each encounter and the phylogenetic distance between donor and host [127-132]. For example, even though there are few interactions between humans and chimpanzees, the close phylogenetic distance between our two species has resulted in several diseases being passed from chimpanzee to man, most notably AIDS [133]. In contrast, rodents are very distant from humans on a phylogenetic scale but their abundance and multiple interactions with humans have resulted in at least two established diseases, plague and typhus. Even though phylogenetically distant, the genome of mice and humans are both derived from a common mammalian ancestor and only around 100 chromosomal rearrangements separates the two organisms from this ancestor [134]. These genetic similarities have resulted in mice being an important model for human biology and diseases.

Murine models of infectious disease

The use of mice models is a vital asset for studying bacterial pathogenesis using clinically isolated strains from human infections. In these settings, disease parameters such as time of infection, infection route, bacterial strain and host genetics can be controlled [135-138], which reveals disease progression in vivo. Murine models are comparatively cheap, have a fast generation time and offer a homogenous genetic background, which makes mouse models an attractive system to work with. Despite being a human adapted pathogen reports have shown S. pyogenes being able to infect other species and even using them as intermediate reservoirs to spread disease to humans [139]. Studies using murine models have shown that the increased host pressure can alter the composition of the inoculated S. pyogenes strain to reveal a phenotype that differs from the original inoculum and that these emerged phenotypes have a higher degree of lethality when re-inoculated into mice [140]. This phenotypic change allows S. pyogenes to survive in a new host and could represent bacterial changes needed for adaptation to the mouse as a new host.

Contributions

The last decades have seen drastic changes in environmental and human population structure, resulting in closer and more frequent contact with wild animals. These interactions have led to increased chances of zoonotic outbreaks, exemplified by the SARS coronavirus in 2003 [141]. This process, in which animal adapted microorganisms leave their host and adapt to new species are largely unknown and represents a fundamental event for understanding and fighting EIDs. In paper V, we used the tools developed in papers II, III and IV to investigate the degree of host adaptation resulting of sequential passaging of the human pathogen Streptococcus pyogenes in a mouse infection model. At each passage the bacterial virulence, resulting host response and bacterial dissemination pattern was assessed and compared to the developed standardization curves. The results obtained from this study showed a distinct increase in adaptation across each passage, pointing at the observed phenotype changes resulting from passaging as a result of adaptation to the host as a permanent host.

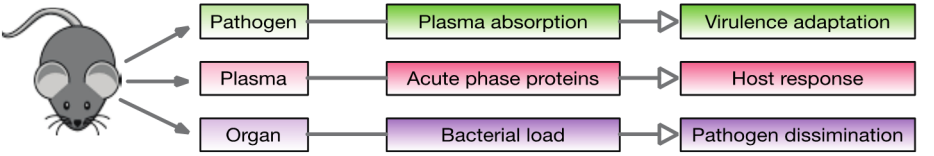

Figure 4.1- Quantitative model of bacterial infection.
The model incorporates several previously described aspects such as the relationship between virulence factors and bound host plasma proteins to estimate virulence adaptation, the calibrated measurement of acute phase proteins to estimate host response and health, and bacterial load estimations in organs and tissue to establish pathogen dissemination in the host.

Chapter 5

Conclusions and relevance

Despite the discovery of antibiotics almost a century ago, infectious diseases continue to be a substantial cause of human mortality and morbidity worldwide, especially in developing countries. The burden of infectious diseases are thought to increase over the coming years as the widespread misuse of antibiotic leads to the emergence of strains for which current therapies are ineffective. The last decades has also seen a large increase of pathogens crossing the species barrier to cause disease in humans, resulting in sporadic epidemic outbreaks flaring up. To be able to reverse these negative trends we need better knowledge of the actual events between host and pathogen leading to host adaptation.

Normally, a research animal is just a surrogate for the human host and substantial effort are made to make sure the animal model is as close a representation as possible of the human condition. However, very little research in the medical field is focused on investigating the effect human pathogens have on the mouse as a host. As the human population increases the contact with wild animals will increase as a result of deforestation and geographical changes. These conditions will force more contact between humans and wild animals, with more chances of a pathogen crossing the species barrier to adapt to the human host. Even though it sounds like a bad movie plot, this is continuously happening but so far very few pathogens are able to spread from a human to human after infection. This thesis represents a first step into what I believe is an unexplored and important field of research. By using large-scale systems biology to investigate the adaptation of pathogens to animal host we not only increase the fundamental knowledge about bacterial evolution, but we also increases our chances of finding species-specific events and mechanisms crucial for infection different species. These would make ideal therapeutic targets and could potentially be used to limit the bacterial spread, and at the same time would reduce our reliance on antibiotics.

This thesis also builds heavy around the concept and use of biomarkers. Not to long ago this was a word rarely spoken in the proteomic world, a consequence of enormous amounts of money being invested in a technique promising much but delivering little. However, it is my belief that the failure of MS to deliver quantitative biomarkers for disease have not been due to a flaw of the technique, but rather a failure of the approach taken to find them. I believe that the approach taken in this thesis, using pre-determined standardization curves to validate the obtained results adds a lot of strength to biomarker identifications. This relatively simples step reduces the error rate seen with so many potential biomarkers by taking into account biological variance and different stages of disease.

A positive yet unexpected side effect of this research is the potential value of the developed model as a clinical diagnostic method. The Baccus method for estimating bacterial load could be expanded to include a multitude of pathogens, building subsets of Baccus methods consisting of pathogens often seen in conditions such as urinary tract infections. The simplicity of the Baccus methods allows other biomarkers to be added, creating a clinical diagnostic method for determining bacterial specie, bacterial load, antibiotic susceptibility and infection progression all in the same MS injection. This approach would drastically reduce the time needed for analysis, which would lead to increased patient care.

The aim of any scientist is to make sense of the world around them. In medical science, we hope that this understanding will lead to an improved care and less human suffering. This thesis have provided a first step into using MS based proteomics to investigate host adaptation of pathogens which could reveal novel adaptation mechanism and have suggested improvements to the development and use of biomarkers for disease. It is my hope that the efforts put into these studies will someday lead to increased diagnostic and therapeutic opportunities in infectious medicine.

Populärvetenskaplig sammanfattning

Bakterier finns överallt omkring oss och sett till antal celler så består en normal människa till större del av bakterier än av mänskliga celler. Dessa bakterier har för det mesta positiva egenskaper för vår hälsa, t.ex. kan de hjälpa oss ta vara på energin från maten vi äter. Men när dessa bakterier hamnar på en plats de inte borde vara på som t.ex. i blodet eller när vi blir invaderade av en bakterie som inte bidrar med någon positiv effekt kan resultatet bli att vi blir sjuka. När detta händer reagerar våra kroppar på bakterierna och försöker bekämpa dem vilket leder till många vanliga sjukdomssymptom som feber och svullnad. Detta kallas för en infektion och huruvida man blir sjuk eller inte beror på hur bakterien och immunförsvaret interagerar med varandra.

Vissa bakterier har anpassat sig till att bara infektera människor medan andra kan infektera både människor och djur. På senare tid att det uppkommit flera olika bakterier och virus som tidigare bara kunde infektera djur men som nu har anpassat sig till att infektera människor. Ett av de mest kända fallen är viruset H5N1 (även kallad fågelinfluensan) som bröt ut i Kina 1997. Bakterier som ”hoppar” mellan arter så här är väldigt farliga eftersom kroppen inte har stött på dem tidigare och inte har något skydd mot dem. För att förstå hur bakterier kan anpassa sig till människor och orsaka sjukdom är det viktigt att vi förstår hur värden och bakterien påverkar varandra. Celler och bakterier interagerar med varandra genom olika proteiner. Dessa proteiner kan vi mäta med hjälp av ett avancerat instrument som kallas för en mass spektrometer, vilket gör det möjligt att studera tusentals olika proteiner i ett biologiskt prov.

Den här avhandlingen är resultatet av nästan fem års forskning där mitt mål har varit att öka vår förståelse för hur bakterier anpassar sig till olika värdar och orsakar sjukdom. Genom att studera vilka proteiner bakterien producerar, hur detta påverkar värden och hur bakterierna sprider sig till olika organ kan vi bygga en modell över hur en infektion går till. Modellen kan vi sedan använda för att undersöka hur olika bakterier förändras när de injiceras i en mus och förändring som sker visar hur väl bakterien har anpassat sig till musen som värd. Samma förändringar som vi mäter i musen kan även användas på människor vilket skulle kunna vara ett sätt att upptäcka bakteriesjukdomar i patienter. Den utvecklade metoden hade kunnat byggas på till att inkludera proteiner som visar om bakterierna är resistent mot antibiotika eller om det finns olika bakteriestammar i samma infektion.

Det övergripande målet med denna avhandling är, att bättre förstå hur bakterier anpassar sig till sin värd och genom denna kunskap öka förståelsen för hur nya sjukdomar uppkommer. Den konstruerade modellen har även potential att användas för klinisk diagnostik av infektionssjukdomar, vilket hade kunnat leda till förbättrat vård och minskat mänskligt lidande.

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“I feel a very unusual sensation – if it is not indigestion, it must be gratitude”

- Benjamin Disraeli

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