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Akademisk avhandling

Streptococcal virulence and the coagulation system

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Abstract <p>This thesis explores streptococcal virulence from both mechanistic and evolutionary perspectives, where the mechanistic studies focus on interactions with the human coagulation system. We describe interactions between streptococcal surface proteins and components of the intrinsic pathway of coagulation and the kallikrein-kinin system in human plasma, as well as to how these surface proteins are produced in different growth phases. The interactions involve activation of the kallikrein-kinin system and inhibition of its antibacterial effects. Inspired by these results, we review the literature, and develop a general model of streptococcal virulence. According to this model, the bacteria have two strategies, which we call asymptomatic colonization and superficial symptomatic infection, respectively, and they are adaptive under different epidemiological conditions. We propose that the bacteria's ability to cause invasive infections, which are the best studied, because they are the most severe, is a side effect of traits that evolved as adaptations for superficial symptomatic infections. This implies that many virulence mechanisms that have been described in invasive infections should have their functions in superficial symptomatic infections. We therefore investigate one such mechanism—the activation of host plasminogen—in conditions simulating pharyngitis, a very common superficial streptococcal infection. Pharyngitis is characterized by the exudation of plasma into an environment with saliva. We find that saliva affects the initiating enzymes of the intrinsic pathway of coagulation, and that this results in the formation of clots that entrap the bacteria. The bacteria escape the clots by activating host plasminogen, a finding that is in concordance with the model. As a whole this thesis underscores the utility of evolutionary analysis for interpreting and guiding mechanistic studies in infection biology, and conversely, the usefulness of mechanistic input for evolutionary theorizing.</p>		
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Streptococcal virulence and the coagulation system

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Lund, 2013

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Anyámnak szeretettel

"Linnaeus and Cuvier have been my two gods, though in very different ways,
but they were mere schoolboys to old Aristotle"
—Charles Darwin

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Paper one

Streptococcal surface proteins activate the contact system and control its antibacterial activity.

Kristofer Wollein Waldetoft, Lisbeth Svensson, Matthias Mörgelin, Anders I. Olin, D. Patric Nitsche-Schmitz, Lars Björck, and Inga-Maria Frick.

The Journal of Biological Chemistry. 2012; 287: 25010-25018.

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Paper two

Surface proteins of the group G *Streptococcus* in different phases of growth: patterns of production and implications for the host-bacteria relationship.

Kristofer Wollein Waldetoft, Christofer Karlsson, Magnus Gram, Johan Malmström, Matthias Mörgelin, Inga-Maria Frick, and Lars Björck.

Submitted

Paper three

To harm or not to harm? On the evolution and expression of virulence in group A streptococci.

Kristofer Wollein Waldetoft and Lars Råberg.

Trends in Microbiology. *Accepted*

Paper four

Saliva activates the intrinsic pathway of coagulation to entrap streptococci in clots and the bacteria escape by activating plasminogen.

Kristofer Wollein Waldetoft, Tirthankar Mohanty, Matthias Mörgelin, Inga-Maria Frick, and Lars Björck.

Manuscript

POPULÄRVETENSKAPLIG SAMMANFATTNING

Vi har undersökt hur och varför streptokocker orsakar sjukdom. Vi började med att undersöka hur proteiner på bakterieytan interagerar med det så kallade kontaktsystemet, som är en del av immunförsvaret och blodlevringen. Vi fann att det bildades en inflammatorisk signalmolekyl och ett bakteriedödande proteinfragment men bakteriens ytproteiner skyddade mot det. Att bakterier både utlöser ett immunsvaret (inflammation) och skyddar mot detsamma är ett återkommande tema i studier av hur bakterier orsakar sjukdom, inte minst allvarliga invasiva infektioner. Och ofta verkar det vara så att bakterierna aktivt sätter igång immunsystemet, snarare än bara blir attackerade av det. Det kan verka motsägelsefullt att bakterierna både sätter igång immunsvaret och motverkar det. Dessutom är invasiva infektioner en återvändsgränd för bakterierna, eftersom de inte sprider sig vidare från sådana infektioner, så det är märkligt att bakterierna verkar så väl anpassade för dem. Vi fortsatte därför med att utveckla en teoretisk modell för att förklara evolutionärt varför bakterierna har dessa egenskaper, varför de både sätter igång inflammationen och försvarar sig mot den. I korthet går modellen ut på att det naturliga urvalet gynnar dessa egenskaper, eftersom inflammationen ökar mängden tillgänglig näring för bakterierna när de växer på till exempel halsslemhinnan. Detta kan de utnyttja för att växa och sprida sig, men bara om de samtidigt kan skydda sig mot de antibakteriella komponenterna av inflammationen. Ett exempel på en infektion där streptokocker orsakar inflammation och sprids snabbt från infektionen är halsfluss. Detta kan förklara varför streptokockerna interagerar med immunsystemet på det sätt de gör och varför de har de egenskaper som krävs för det. Oturligt nog fungerar immunförsvaret ungefär likadant i invasiva infektioner som det gör i halsfluss. Därför kan bakterier som är anpassade till halsfluss manipulera immunförsvaret i invasiva infektioner också. Det är en ren bieffekt av bakteriens egenskaper och anpassningar, en väldigt viktig och olycklig bieffekt. Enligt modellen ska alltså bakteriella egenskaper som är viktiga i invasiva infektioner ha sina egentliga funktioner i mer lindriga infektioner som till exempel halsfluss. Vi tillämpade det tänkesättet genom att undersöka streptokockernas förmåga att aktivera plasminogen (ett enzym som löser upp levrat blod och blodplasma). Det är välkänt att den förmågan är viktig vid invasiv sjukdom. Vi beskriver en funktion för den förmågan vid halsfluss. Sammantaget understryker avhandlingen värdet av att kombinera evolutionära och (bio)medicinska perspektiv i studiet av infektioner.

ABSTRACT

This thesis explores streptococcal virulence from both mechanistic and evolutionary perspectives, where the mechanistic studies focus on interactions with the human coagulation system. We describe interactions between streptococcal surface proteins and components of the intrinsic pathway of coagulation and the kallikrein-kinin system in human plasma, as well as to how these surface proteins are produced in different growth phases. The interactions involve activation of the kallikrein-kinin system and inhibition of its antibacterial effects. Inspired by these results, we review the literature, and develop a general model of streptococcal virulence. According to this model, the bacteria have two strategies, which we call asymptomatic colonization and superficial symptomatic infection, respectively, and they are adaptive under different epidemiological conditions. We propose that the bacteria's ability to cause invasive infections, which are the best studied, because they are the most severe, is a side effect of traits that evolved as adaptations for superficial symptomatic infections. This implies that many virulence mechanisms that have been described in invasive infections should have their functions in superficial symptomatic infections. We therefore investigate one such mechanism—the activation of host plasminogen—in conditions simulating pharyngitis, a very common superficial streptococcal infection. Pharyngitis is characterized by the exudation of plasma into an environment with saliva. We find that saliva affects the initiating enzymes of the intrinsic pathway of coagulation, and that this results in the formation of clots that entrap the bacteria. The bacteria escape the clots by activating host plasminogen, a finding that is in concordance with the model. As a whole this thesis underscores the utility of evolutionary analysis for interpreting and guiding mechanistic studies in infection biology, and conversely, the usefulness of mechanistic input for evolutionary theorizing.

INTRODUCTION

Our research tradition, like any other, rests upon assumptions, assumptions that remain largely implicit while we move on with everyday research. And this is probably how it usually should be. But different traditions diverge in what they take for granted, and this may hamper cooperation between them. The present thesis is an enquiry into infection biology. It is grounded in the experimental biomedical tradition, but aims to also join another, the evolutionary tradition, and it does so due to my conviction that integration, and not only decomposition, is an integral part of understanding. The thesis includes studies on molecular virulence mechanisms (**papers one and two**), an evolutionary analysis (**paper three**), and a combination of the two (**paper four**). The discussion will therefore revolve around these two perspectives (biomedical and evolutionary): what they assume, how they differ, and how they may be combined. First, however, I will briefly introduce the subjects: streptococci and coagulation.

Streptococci

The studies reported in this thesis pertain to streptococci of Lancefield groups G and A. The mechanistic studies focus on group G streptococci, because they have been less thoroughly studied in the past, so the need for research is more pressing. The theoretical study is instead focused on group A streptococci, because a wealth of information is available about them. In human infections, group G streptococci are typically *Streptococcus dysgalactiae* subsp. *equisimilis* (Brandt & Spellerberg, 2009), and group A isolates are *Streptococcus pyogenes* (Cole *et al.*, 2011). Both taxa cause a similar spectrum of infections (Brandt & Spellerberg, 2009). They cause symptomatic infections of the skin and upper respiratory tract with activation of immune defences, such as pyoderma and pharyngitis, or asymptomatically colonize the upper airways (Bisno & Stevens, 2005; Brandt & Spellerberg, 2009; Carapetis *et al.*, 2005). They may also cause invasive infections (Brandt & Spellerberg, 2009; Carapetis *et al.*, 2005). These infections are diverse, but the common denominator is that they involve the deeper tissues and/or the bloodstream. The invasive infections are far more severe than the superficial infections, and they are the focus of contemporary research, but they are comparatively rare. Pharyngitis is much more common (Carapetis *et al.*, 2005), but research on it is scarce. Important studies on pharyngitis were performed more than half a century ago (Denny, 1994; Krause,

2002; Rammelkamp, 1956), motivated by a sequela that streptococcal pharyngitis may cause—acute rheumatic fever. In the Western world, the incidence of this sequela has decreased since then, though there was a resurgence in the eighties, but it remains an important problem in many geographic locations (Carapetis *et al.*, 2005; Denny, 1994; Krause, 2002).

Coagulation

The coagulation system prevents blood loss after injury, and plausibly also contributes to immunity (Amara *et al.*, 2008; Esmon *et al.*, 2011; Frick *et al.*, 2006). The system is quite complex, and the picture of it is changing (Hoffman & Monroe, 2001; Smith, 2009), but for the present purposes it may be visualized like this: the hub of the system is thrombin. Thrombin cleaves fibrinogen into fibrin—the material of the clot—and is involved in positive feedback that generates more thrombin (Smith, 2009). The coagulation system can be initiated through two pathways. The extrinsic pathway begins with the formation of a complex between factor VII in the blood and tissue factor on cells surrounding the blood vessel, when these cells are exposed upon injury (Smith, 2009). Tissue factor has also been found in saliva (Berckmans *et al.*, 2011). The intrinsic pathway is instead initiated by activation of factor XII, which activates factor XI (Oehmcke & Herwald, 2010), and factor XI, in turn, is part of the thrombin feedback system mentioned earlier (Smith, 2009). In the end, clots have to be dissolved and cleared, and this is effected by plasmin (McMichael, 2012). A schematic representation of the coagulation system is given in figure 1. In pharyngitis, plasma mixes with saliva, and in **paper four** we find that saliva initiates the clotting of plasma via the extrinsic pathway, and then amplifies it via the intrinsic pathway. We also find that streptococci induce the dissolution of these saliva-plasma

clots by activating plasminogen.

A note on terminology is also in place. ‘The plasma contact phase system’, or ‘the contact system’ for short, is a collective term that includes the intrinsic pathway of coagulation and the proinflammatory kallikrein-kinin system. We use the term ‘the contact system’ in **paper one**, and ‘the intrinsic pathway of coagulation’ in **paper four**.

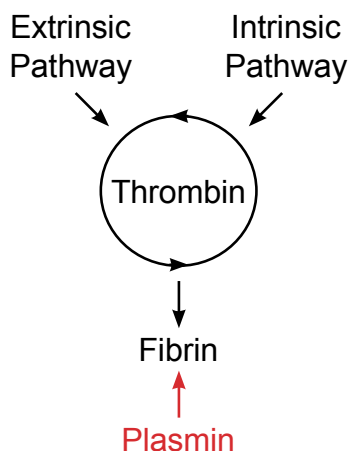


Figure 1
Schematic representation of the coagulation system. Black for components and processes involved in formation of the fibrin clot, and red for dissolution of such clots.

The question

Why are streptococci virulent?

In our biomedical tradition we read this question as ‘what are the molecular mechanisms that cause pathology?’ In the evolutionary tradition it may instead be read as ‘what are the evolutionary forces that cause the existence of such mechanisms?’ But since the force par excellence is natural selection, and mechanisms are typically black-boxed, this is abbreviated to something like ‘how does virulence relate to the fitness of the pathogen?’ where fitness is taken to be, roughly, transmission. The difference between these two modes of thinking was treated by Ernst Mayr in his classic paper ‘Cause and Effect in Biology’ (Mayr, 1961), and his explication has since formed the basis for the understanding of the relationship between mechanistic and evolutionary disciplines, at least among evolutionary biologists. According to Mayr, mechanistic disciplines study *how* organisms work, whereas evolutionary research investigates *why* they are the way they are and do the things they do. Explanations given by the former disciplines he calls ‘proximate’, and those by the latter ‘ultimate’. Needless to say, Mayr himself deals with those ‘ultimate’ questions. Critiques have been raised against Mayr’s account (Ariew, 2003; Francis, 1990), but it remains influential (Laland *et al.*, 2011).

It’s fairly obvious that Mayr’s distinction traces its roots to the Aristotelian account of the four kinds of causes or scientific explanations (Amundsen, 1996, p. 14; Falcon, 2012; Gould, 2002, p. 1194), the ‘proximate’ or mechanistic corresponding to Aristotle’s material and efficient causes, and the ‘ultimate’ to the final cause—what Aristotle calls ‘the for the sake of which’ or ‘the due to which’ or ‘the telos’. The affinity of Aristotle’s formal cause may be less clear. The concept in modern biology closest to it would be that of the genetic code, or perhaps that of developmental program (Johnson, 2005, p. 169), and I would say that *qua* cause it belongs to the ‘proximate’, but as a record of evolutionary history it enters the ‘ultimate’. There is, however, one crucial difference between Aristotelian and modern science. In the latter, the proximate-ultimate distinction defines separate disciplines within biology. But for Aristotle, the different kinds of explanation are parts of one and the same holistic understanding. As he says, ‘...since all knowledge of nature concerns the four causes, it is naturally necessary to demonstrate the reason in all these ways: the matter, the form, the mover, the for the sake of which’ (Aristotle, *Physics* II, cited in (Johnson, 2005, p. 42)). As the present thesis should integrate mechanistic and evolutionary thinking, it will have to pursue the Aristotelian route.

Mechanism, function, and side effect

Since conceptual confusion would lead nowhere, I will make some clarifications. The key lies in appropriate distinctions. Two in particular will be used here. The first is based on Elliot Sober's distinction between selection *of* objects and selection *for* properties (Sober, 1984, pp. 97ff.). Here, I instead distinguish between selection *of* mechanisms and selection *for* functions, where a mechanism is a concrete molecular thing (an object), and a function is something that this thing does. Functions are thus effects of their mechanisms. Not all of a mechanism's effects are proper functions, however, and this is the second distinction, which is inspired by Aristotle. It is the division of the effects into functions and side effects. Selection *for* a function manifests as selection *of* the mechanism causing that function, and therefore the function is a key part of the evolutionary explanation of why the mechanism exists. In contrast, a side effect is any other effect of a mechanism. It is not selected for, and may even be selected against. As opposed to a function, therefore, a side effect is not a positive causal factor for the existence of its mechanism, and thus cannot explain it. If neutral, it isn't part of the explanation at all, and if selected against, it is a negative causal factor.

It is common and sometimes useful to take a teleological stance in biological research, but it may also be deceptive. For example, pathogens are studied by considering the end result of the pathogen-host interaction, archetypically an invasive infection, and then laying out the sequence of events, and the corresponding bacterial abilities, required to produce this result. These steps include invasion of deeper tissues, and multiplication at those sites (Smith, 2006). This is perfectly fine if the aim is to find the bacterial traits responsible, but it is objectionable if the aim is to explain why those traits exist. In the latter case one needs to determine whether the end result is a function or a side effect. A study of Aristotle is instructive. As in the biomedical approach, Aristotle starts by considering the end result of a process, and then goes on to ask what is required to produce it (Aristotle, *Parts of animals I*). However, to him, not just any result qualifies as a telos, but a telos must be for the good of the organism. It must help the individual organism to survive and reproduce (Johnson, 2005, pp. 159, 171ff.). The Aristotelian concept of telos is thus related to the Darwinian concept of adaptation, and to the concept of function used in this thesis. A result that is not for the good of the organism (or other relevant entity), in the sense of increasing its fitness, is not a telos/adaptation/function, and thus not explanatory. According to Aristotelian and Darwinian views, therefore, the fact that bacteria need certain mechanisms in order to cause invasive disease does not explain why those mechanisms exist, unless invasion increases the fitness (future genetic representation), which in the case of streptococci is unlikely.

But the teleological approach may deceive in another way too. It may seem to explain causes in terms of their effects. But organisms don't have their traits because

they increase their fitness. Organisms have their traits because they have inherited them from their ancestors, and organisms with certain traits are common, because the traits increased the fitness of those ancestors. The explanatory increase in fitness must be located in the past, lest there be backwards causation. The concepts of adaptation and function are not teleological in this strong sense, and in fact, it has been argued that not even Aristotle's telos is (Johnson, 2005, pp. 56, 166f.).

This is the broader context of **paper three**. It seeks the function of the traits that biomedicine has identified as causative of invasive infection, and it does so in order to explain why those traits exist. In that paper, we argue that it is highly implausible that invasiveness has been selected for. We therefore turn to the superficial symptomatic infections, and propose that rapid growth and transmission in this setting is the function of the mechanisms that cause invasive infection. **Paper four** then follows this line of thought, and investigates the function that streptococcal activation of plasminogen, a known virulence determinant for invasive infection, may have in pharyngitis.

On the origin of virulence

There are different types of question pertaining to this matter. There is the biomedical: 'what is the sequence of events, or set of molecular mechanisms leading to pathology?' Then there is the evolutionary: 'how or why did evolution result in these types of mechanisms?' At least that is the sort of question I, as a student of medicine, would want evolutionary biologists to address. The literature on virulence evolution is, however, rather uninterested in mechanisms, and instead deals with virulence as such, although this may be changing (Brown *et al.*, 2012; Ebert & Bull, 2008; Frank & Schmid-Hempel, 2008). But let's stay for a while with the question of why there is virulence at all.

In medical papers, this question is seldom explicitly addressed, but there are some lines of thought apparent in medical discourse. A recent textbook on bacterial pathogenesis (Wilson *et al.*, 2011, pp. 106f.) summarizes the picture, and states that: 'Perhaps the most widely held view is that disease-causing bacteria evolved specifically to cause human disease. A second view, which has gained more adherents lately, is that disease-causing bacteria are actually trying to achieve an equilibrium with humans that does not result in disease and that disease symptoms result when this equilibrium does not develop.' A third view that humans are an accidental host is also mentioned. An example of the first view is the study by Sun *et al.* (2004) that establishes the activation of plasminogen as a virulence determinant for invasive infection by group A streptococci, and argues that this represents the bacterium hijacking a host system in order to achieve invasion. In **paper four** we describe a function for this virulence determinant in pharyngitis, and suggest that

the effect reported by Sun *et al.* is a side effect. The second view is similar to the ‘conventional wisdom’ (Levin, 1996) that parasites (*sensu lato*) should evolve to not harm their hosts, so as to not bite the hand that feeds them. This view seems common in informal discussions. It is what people in our field have answered when I have asked them.

Evolutionary biology has a ‘conventional wisdom’ of its own, which differs from that of medicine. I will not dwell on the subject here; it is briefly summarized in **paper three**, and has been thoroughly reviewed relatively recently (Alizon *et al.*, 2009). It is also not the sole opinion in that field. Suffice it to say that in this line of thought, virulence not only incurs a cost on the parasite in the decreased duration of the infection, but is also an unavoidable consequence of something (such as high parasite load) that increases the rate of transmission. Virulence is thus coupled to both negative and positive effects for the parasite, and under some reasonable assumptions there is an intermediate optimal level of virulence, towards which pathogens should evolve.

The two pieces of conventional wisdom may not be as irreconcilable as might first appear, because, as argued by Lenski and May (1994), when the pathogen spreads, and the density of susceptible hosts decreases, the optimal level of virulence decreases as well, and less virulent genotypes can take over. Lenski and May state that virulence should thus evolve to be progressively lower, but not reduce to zero, which may seem to preclude commensalism. But the fact that their mathematical model never reaches zero, doesn’t necessarily mean that parasites must retain virulence in any meaningful sense. There is a threshold, below which we don’t call things virulent.

But what about the view that pathogens evolved specifically to cause disease? *Prima facie* this ‘most widely held view’ (Wilson *et al.*, 2011) may seem little short of absurd, but on a liberal interpretation, I think it does have some value. It serves to focus our attention to the fact that pathogens have special factors for pathogenicity, the so called virulence factors, and it prompts the question why those factors are there. This will be discussed in the following section.

Pictures of a pathogen

Perhaps unsurprisingly by now, the picture of a pathogen differs between biomedicine and evolution. Then there are of course variations and nuances within those traditions, but here I will isolate a tendency that is discernable. Evolutionary biologists are holists. They don’t dissect the pathogen in search of virulence factors, but tend to think of virulence as a property of the pathogenic organism as a whole. They also focus on function rather than mere effect. They conceive of pathogens as black boxes that replicate and transmit, and assume that transmission is linked to

virulence (Alizon *et al.*, 2009; Read, 1994). Transmission is the function; virulence is the side effect; and often, replication is what ties them together. The biomedical view is different. Here a pathogen is thought of more as a basic machinery, not pathogenic in itself, with an add-on of virulence factors, that is molecules that cause disease, or at least have some special affinity for the disease process. Function (as the term is used in this thesis) is not in focus. One simply observes a phenomenon, host damage in this case, and identifies the molecular mechanisms that bring it about. Why those mechanisms are there is a different issue.

The strategy guiding experimental research on virulence factors is inspired by Koch's postulates (Falkow, 1988). Roughly, a microbial component is said to be a virulence factor if its removal attenuates virulence, and reconstitution restores it. This is a good strategy, and it has proved very fruitful. It is nonetheless worth noting that on the holist conception of pathogen, it may seem awkward. I will illustrate this with a metaphor. Imagine the pathogen as an aircraft. A holist may characterize the strategy thus: we remove some part of the aircraft, for example the fuel tank, and find that the plane no longer gets off the ground. We then put the tank back, and find that it can fly again. We hence conclude that the fuel tank is a factor for flying. The problem with this approach, it would be argued, is that aircraft are integrated devices, and removing any part is expected to compromise function. The result of such an experiment, therefore, does not warrant the conclusion. The fuel tank is not a special factor for flying; it's just a necessary component of an integrated whole. This is a sort of objection I have encountered more than once.

The biomedical view is somewhat different. Here, the pathogen would be pictured, not as a normal aircraft (that would be a commensal), but as a bomber one (to conform to the imagery of warfare commonly used for host-bacterium interactions (Martin, 1990)). When the bombs are removed, it still does all the things that aircraft typically do, except that the destruction it inflicts is attenuated. We conclude that bombs are factors for destruction. Here, the result seems to warrant the conclusion. Bacterial exotoxins are cases in point, but the issue may be less clear for other classes of virulence factors.

Can these two sorts of preconception be reconciled? I will now argue that for some parasites they can. The idea is that there are distinct strategies available to parasitic bacteria. These strategies differ in virulence and in the bacterial traits they require, and one strategy can be turned into another by adding or removing traits. Consider a bacterium adapted to the healthy host epithelium. It does not induce an inflammatory response (neither actively nor passively), and when inflammation occurs, for whatever reason, the bacterium does not take advantage of the extra nutrients associated with this response. Perhaps bacterial numbers are instead reduced by the antibacterial components of inflammation. This is a commensal. Now add factors that allow the bacterium to handle the antimicrobial part of inflammation, and exploit the nutrients, so it can take advantage of illness when it occurs. This might be called an opportunist. Compared to the standard concept of

opportunistic, this differs in meaning, but, I would think, less so in denotation. To this add factors that actively induce inflammation, so the bacterium both induces and exploits the host response, and the corresponding illness. This would be a pathogen. In so far as the traits are costly, this could give rise to intransitive fitness relations with ‘rock-paper-scissors-dynamics’, where commensalism, opportunism, and pathogenicity co-exist. The streptococci with which we work seem able to behave as both commensals and pathogens (discussed in **paper three**), and perhaps as opportunists as well.

On this conception, the pathogen is an integrated whole, where the component parts work together, such that removing any one of them will compromise function, where ‘function’ can be construed in terms of bacterial fitness rather than pathogenicity as such. But removal of pathogen-specific parts only compromises pathogen-specific function. The bacterium may still be able to work as an opportunist or commensal. One may therefore conceive of it as a basic machinery, not pathogenic in itself, with add-ons that cause disease (the biomedical view). If the nutrients associated with the inflammatory exudate are used to attain high growth and transmission rates, then this construal allows one to combine the view that pathogens evolved specifically to cause disease (discussed previously), in the form of inflammation, with the principle that natural selection favours transmission. The second view, that virulence is the result of bacterial failure, rather than function, may also be incorporated. Our explanation of invasive disease as a side effect of the traits required to exploit inflammation at the epithelium is of this sort. The induction and evasion of inflammatory responses are key to both pharyngitis and sepsis (**paper three**).

On this view then, there is no selection *for* invasiveness, but there is for attaining high transmission rates in non-invasive infections. This manifests as selection *of* molecular mechanisms that have transmission as their function and invasiveness as their side effect. This is the view developed in **paper three** and applied in **paper four**. **Paper four** is most concrete: in pharyngitis streptococci are entrapped in fibrin clots, and they counteract this by activating plasminogen, which dissolves the clots, and allows the bacteria to avoid clearance and presumably to spread. There is selection *for* escaping entrapment, and it manifests as selection *of* the factor that activates plasminogen (i.e., streptokinase). Plasminogen activation, in turn, contributes to invasive virulence (Sun *et al.*, 2004). This is the side effect.

THE PRESENT INVESTIGATION

The studies reported in this thesis may be thought of as representing one turn around the hermeneutic circle. We begin with a study of molecular mechanisms in **paper one**, complemented by **paper two**. In **paper three**, we develop a general model to make sense of these and other results. Then, in **paper four** we return to the mechanisms, but this time we approach them from the perspective of the general model presented in **paper three**.

In **paper one** we study the interactions between the two surface proteins that are most abundant on our group G streptococci, FOG and protein G, and the human plasma contact system. We find that FOG, but not protein G, activates the proinflammatory branch of the system, whereas protein G inhibits antimicrobial activity resulting from such activation. I believe that there is more than one possible interpretation of these results. Activation of the contact system may represent host immunity targeting the pathogen by recognizing FOG as a pathogen associated molecular pattern. Alternatively, the bacteria may be taken to ‘actively’ induce inflammation. Both induction and inhibition would thereby be ‘actively’ effected by the bacteria. One common interpretation of this sort of situation is that induction and inhibition oppose each other, and together achieve a ‘fine tuned balance’. This may be so, but I would nevertheless wish to suggest another interpretation in the context of inflammation. The proinflammatory signals of the host, such as TNF, IL-6, and bradykinin, are not very specific, but have a range of effects, some of which should be beneficial for the pathogen (such as a leakage of glucose), while others are detrimental, in line with inflammation participating in host defence. The inhibitory effects exerted by the pathogen may then more specifically target the adverse consequences, leaving the beneficial. The activation of the contact system by FOG releases bradykinin, which is generally proinflammatory (Oehmcke & Herwald, 2010); and protein G, rather than counterbalancing this activation as such, inhibits one of the negative effects. It is, of course, an empirical question as to what the pattern will be when interactions with other systems are added to the picture.

In **paper two**, we follow the expression of FOG and protein G across growth phases, and find that the amount of protein G, but not FOG, increases substantially during the stationary phase. We also measure a number of metabolic proteins, and a picture emerges from the data that the stationary phase of bacterial growth is quite dynamic.

Papers one and two investigate virulence mechanisms, and do not assess the adaptive significance of the pathogen-host interaction. Nevertheless, the results point to the possibility that the pathogen may induce and exploit an inflammatory

response. This was the starting point for **paper three**. In this paper we use theory from evolutionary ecology to paint a broad picture that could make sense of many known virulence mechanisms, including the ones described in **papers one and two**. We suggest that the induction of inflammation, inhibition of its adverse effects, and exploitation of its beneficial effects allow the bacteria to attain high rates of transmission from the host epithelium, but at a cost of infection time span, a strategy that would be adaptive under some conditions. This we propose is the adaptive setting for many (or most) virulence factors. The implication is that, although it is standard to investigate the effects of virulence factors in models of invasive infection, the functions of those factors should rather be found at the inflamed epithelium, for example, in pharyngitis.

In **paper four** we revisit the interactions between streptococci and the contact system in a setting simulating pharyngitis. The results are twofold. Firstly, we describe how the coagulant branch of the contact system contributes to host defence by entrapping bacteria. This is interesting in its own right, since the function of this intrinsic pathway of coagulation has been obscure (Gailani & Renné, 2007). Secondly, in line with the model in **paper three**, it emerges that the bacteria evade this host defence function, by employing a mechanism previously described as crucial for invasive infection.

DISCUSSION

It is only natural that different fields of research should focus on different questions, or approach similar questions from different perspectives. The biomedical approach is decisively reductionist (Fang & Casadevall, 2011). It analyses systems in the literal sense of analysis, which is to break things down into their component parts. Evolutionary reasoning has in contrast an antireductionist tendency (Sober, 1984, p. 155). It seems to me that these two approaches should fit together in a sort of hermeneutic circle, where understanding of both the parts and the whole are pursued in their own right, but are also combined to shed light on each other. Since the two modes of thinking are founded in different intellectual traditions, their occasional combination may decrease the risk that interpretations are misled by preconceptions of a particular field. A problem shared by both traditions, however, is that virulence is described in isolation. The question, ‘why is there virulence?’ is symptomatic. Not all parasitic bacteria are pathogenic, and a comprehensive theory of virulence must explain not only why some are virulent, but also why others are not. Research on commensalism might therefore be enlightening.

FUTURE DIRECTIONS

Our field is heavily focused on data, and it seems likely that progress in the near future will be driven by advances in technologies for data collection. But information in itself is not science. It needs to be structured by theory, and theory is lagging behind. Perhaps the sheer amount of data produced by methods such as mass spectrometry will eventually force the development of theory to handle it. In any case, theory is the direction that I will be heading.

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