

New understanding of the group A *Streptococcus* pathogenesis cycle

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Group A *Streptococcus* (GAS) has long been recognized as a human pathogen causing an exceptionally broad range of infections. Despite intense research, however, the molecular mechanisms of GAS disease remain unclear. Recently, many important discoveries have been made that shed light on GAS pathogenesis and open exciting avenues for future research. Advances in genome sequencing, microarray technology and proteomic analysis, in combination with the development of more suitable animal models, have markedly increased our knowledge of the mechanisms underlying GAS pathogenesis. The information gained from these studies will translate into improved diagnostics and new targets for therapeutic drugs and vaccines.

Epidemiologic versatility of group A *Streptococcus*

The Gram-positive bacterium *Streptococcus pyogenes* (group A *Streptococcus*; GAS) causes a range of human infections from the clinically uncomplicated conditions pharyngitis ('strep throat') and impetigo, to severe invasive diseases such as necrotizing fasciitis ('flesh-eating disease') and streptococcal toxic shock syndrome. In addition, post-infection immune sequelae, including glomerulonephritis and acute rheumatic fever, can occur.

To cause this wide spectrum of disease, GAS must be able to adapt to the diverse physiological conditions encountered in the human host. For example, the pathogen must overcome the first line of physical and immune defense in saliva to colonize the oropharynx successfully. Moreover, GAS must disseminate from the initial site of infection to cause invasive disease – a process that requires the bacterium to break out of the local site of infection and to survive in the blood. To accomplish each of these transitions, GAS has evolved sophisticated strategies and complex regulatory mechanisms that enable it to thwart host defenses and successfully colonize, thrive and/or persist in the host. Many known and putative virulence factors made by GAS contribute to host–pathogen interactions (Figure 1).

Here, we discuss recent advances in our understanding of GAS pathogenesis and focus on the role of various streptococcal virulence factors in the establishment and dissemination of disease. Although the mechanistic basis of the regulation of many of these virulence factors is

extremely important, a complete treatment of this topic is beyond the scope of this review.

Survival of GAS in human saliva

The oropharynx is the primary site of GAS entry into the human host. In this environment, GAS encounters saliva, which contains components of the innate and the acquired immune system including antimicrobial peptides that limit the growth of pathogenic bacteria. It has been known since the 1940s that saliva containing high levels of GAS has a role in the person-to-person spread of this pathogen [1–3]. A recent *in vitro* analysis of the interaction between human saliva and GAS has revealed that the microorganism has evolved a mechanism that enables it to persist in saliva at relatively high viable cell numbers for prolonged periods of time [4]. This phenomenon is specific to saliva, because GAS grown in either limited or defined liquid media does not remain viable for long time periods. In addition, serotype M1 strains of GAS reach higher concentrations in human saliva than do strains of other serotypes [4]. Considering that M1 strains are a leading cause of streptococcal pharyngitis [5], it is possible that the relatively high density achieved in saliva by some GAS serotypes enhances their likelihood of being transmitted to a new host.

Shelburne *et al.* [6] recently discovered that a two-component regulatory system of previously unknown function mediates the persistence of GAS in human saliva. This two-component system, designated *sptR/S* for saliva persistence, directly or indirectly controls the expression of ~8% of the GAS genome during the exponential growth phase and a striking 20% of the genome during the stationary growth phase [6]. Of the genes controlled by *sptR/S*, those involved in carbohydrate metabolism are most abundantly represented.

One of the genes found to aid GAS survival in saliva is *malE*, which encodes a highly conserved maltodextrin-binding protein present in many different GAS strains [6,7]. Two lines of evidence suggests that MalE is required for the successful interaction of GAS with human saliva. First, Shelburne *et al.* [7] have shown that an isogenic *malE* mutant is impaired in its ability to grow and persist in saliva *in vitro*, as compared with the parental GAS strain. These data imply that MalE might play a crucial role in the GAS–saliva interaction by contributing to nutrient acquisition. Because saliva contains only low levels of glucose, GAS depends on additional carbon sources for growth. One

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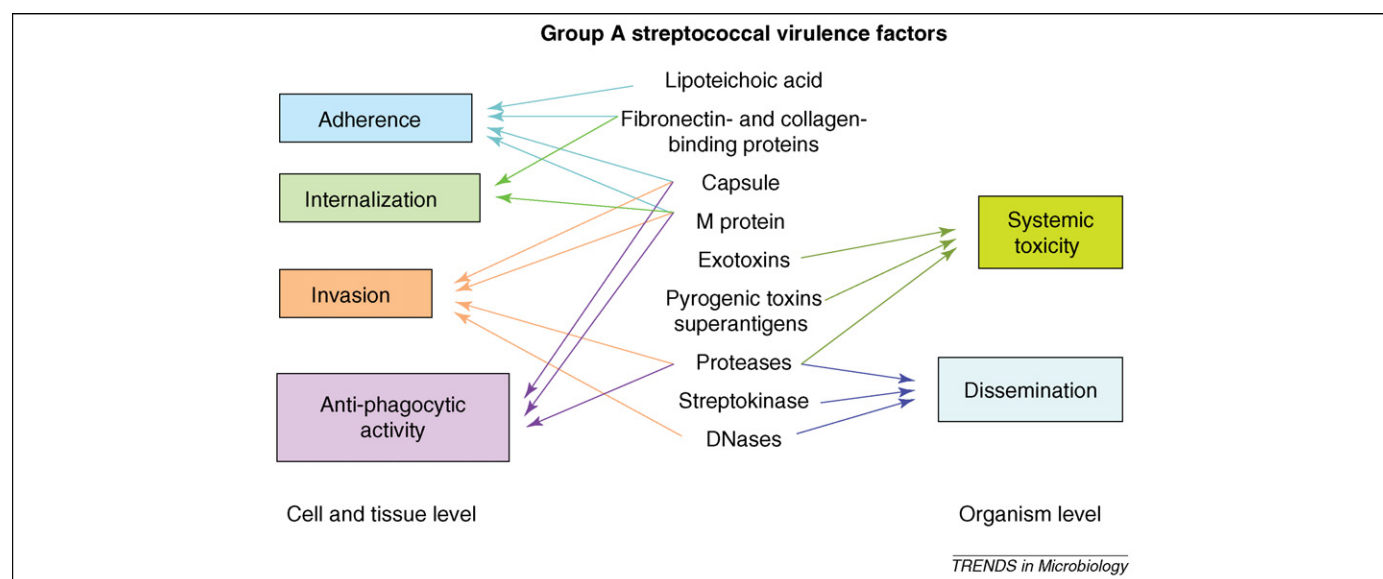


Figure 1. GAS virulence factors interact with the host at many levels. GAS has an arsenal of virulence factors at its disposal that enable it to colonize and thrive successfully in the host. At the cell and tissue level, these factors contribute to the pathogenicity of GAS by mediating adherence to host cells, by promoting internalization and invasion, and by evading phagocytosis. At the organism level, these factors are involved in facilitating dissemination throughout the host and can induce systemic toxicity. Importantly, many of the known streptococcal virulence factors function at several stages of infection.

of the components found in saliva is amylase, which degrades ingested starch to smaller chains of carbohydrates. MalE might enable GAS to use these particular carbohydrates as additional energy sources. Second, MalE might be essential for the earliest stages of GAS colonization, as indicated by the high levels of *malE* transcript expressed early in the GAS–saliva interaction [7]. Thus, human saliva might represent an important reservoir of GAS that is essential for both persistence and transmission of the pathogen.

Pharyngeal disease

Although GAS can cause serious invasive disease, pharyngitis is by far the most common infection. For example, ~15 million cases of streptococcal pharyngitis occur annually in the United States alone, resulting in an estimated US\$2 billion of direct healthcare costs [8]. Despite decades of research, however, our knowledge of the precise molecular events mediating GAS pharyngitis remains rudimentary. Recently, three strategies have been used to expand our understanding of the pathogenesis of this infection. In the first, mouse nasal-associated lymphoid tissue (NALT) has yielded new information about GAS–host interactions [9–11]. Use of NALT and a bioluminescent tagged GAS strain suggests that M cells in NALT transport GAS across the epithelial cell layer, perhaps facilitating invasion of deeper tissues [9]. NALT also has been used to study immune responses to GAS [10].

The second strategy is based on gene transcript analysis of GAS in primary throat swabs obtained from patients with pharyngitis. Virtaneva *et al.* [12] used real-time reverse transcription PCR (RT–PCR) to study transcripts of 17 GAS genes in throat swab specimens obtained from 18 children diagnosed with acute pharyngitis. This method enables the analysis of GAS gene expression *in vivo*, thereby opening a new avenue of investigation. Genes belonging to two-component regulatory systems, regulatory genes, and genes encoding known and putative virulence factors were

studied. Although the level of gene transcripts varied among individuals, several notable trends emerged. For example, *fasC*, a gene encoding a member of a gene regulatory system, was highly expressed during acute pharyngitis. The *fasBCA(X)* system regulates the expression of fibronectin-binding proteins, superoxide dismutase, streptolysin-S-associated genes and streptokinase [13]. Another report suggests that the Fas regulon is upregulated under amino acid starvation – a condition that might be encountered during initial contact of the pathogen with the skin and throat or at a site of high cell density in an infection [14].

Virtaneva *et al.* [12] have also shown that the regulatory genes *mga* (multigene activator) and *perR* (ferric uptake regulator) are highly expressed during acute pharyngitis. *mga* controls the expression of genes encoding important GAS virulence factors including *emm* (M protein), *mac* (inhibitor of phagocytosis) and *sic* (streptococcal inhibitor of complement), all of which have been implicated in either adhesion to host cells or evasion from host defenses. Less is known about *perR*, although evidence suggests that it is involved in iron homeostasis and the oxidative stress response. *perR* has been shown to be upregulated *in vitro* under iron-limiting conditions [15], which GAS might encounter in the host during infection where most iron is sequestered by iron-binding proteins and only small amounts of free iron are available [16]. Of the seven extracellular proteins examined, the genes of four (*scpA* encoding C5a peptidase, *bspA* encoding a cell-surface protein, *edin* encoding a homolog of *Staphylococcus aureus* EDIN toxin, and *sda* encoding DNase) were found to be upregulated during acute pharyngitis, as determined by real-time RT–PCR [12].

The third strategy has used a cynomolgus macaque model of pharyngitis [12,17–19]. Owing to the high human host specificity of GAS, rodent models are inadequate in their representation of pharyngitis pathogenesis because they precisely mimic neither human GAS disease nor the host response against GAS infection. Virtaneva *et al.* [17]

used expression microarray analysis to study longitudinal changes of GAS gene expression in 20 cynomolgus macaques experimentally infected with serotype M1 strain MGAS5005. This strain is genetically representative of M1 GAS strains currently responsible for a large percentage of pharyngitis and invasive infections [20]. The monkeys developed acute pharyngitis that was indistinguishable from the human infection. This observation is noteworthy because other non-human primate models, such as baboons [21] and rhesus macaques [22,23], can be successfully colonized with GAS in the upper respiratory tract but do not develop pharyngitis that mimics human disease. The discovery that cynomolgus macaques develop acute streptococcal pharyngitis that phenocopies the human disease has resulted in this animal model being treated as the gold standard for GAS pharyngitis studies.

Emergence of serotype M1T1 GAS strains

Many pathogenic bacteria, including GAS, produce extracellular DNase, and it has been speculated that GAS DNase functions as a virulence factor. The contribution of a phage-encoded DNase to the pathogenesis of infection caused by contemporary M1 strains has an interesting history that warrants a brief review.

In 1989, Stevens and colleagues [24] observed that contemporary episodes of severe invasive GAS infections in the Rocky Mountain Region of the United States were associated with scarlet fever toxin A (SpeA), which had long been known to be encoded by a bacteriophage. This crucial observation was confirmed by analyzing clonal diversity and genetic relationships among 108 strains of GAS recovered from affected individuals across the United States [25]. Importantly, Southern blot analysis indicated that there were at least two distinct genotypes of serotype M1 strains: those that were *speA* positive, and those that lacked this gene and presumably the bacteriophage encoding it. Further analysis revealed that contemporary serotype M1 isolates contained a distinct *speA* allele [26]. Cleary *et al.* [27] confirmed that the *speA* gene is associated with severe serotype M1 GAS infections and extended the concept that two distinct subclones of serotype M1 exist. Importantly, their analysis revealed that, relative to *speA*-negative strains, *speA*-positive strains contain ~70 kb of additional DNA. Subsequently, mouse virulence studies showed that *speA*-positive organisms are significantly more virulent [28].

Detailed genetic dissection of 126 GAS strains later demonstrated that considerable genetic diversity exists among M1 strains [20]. This analysis showed that a distinct virulent serotype M1 subclone has emerged recently and has spread across continents. It was then discovered that some contemporary M1 isolates contain a gene encoding a DNase that is absent in the genome of strain SF370 [29]. This DNase gene was linked to a phage gene, providing supportive evidence that contemporary serotype M1 strains are genetically distinct. Sumby *et al.* [18] sequenced the genome of serotype M1 strain MGAS5005 and found that it contains genes encoding three distinct DNases (*spd3*, *sdaD2* and *spd*), two of which are encoded by bacteriophage. Seven isogenic mutant strains were constructed and used to study the role of DNases in host–pathogen interactions *in vivo* [18]. Importantly, it was found that DNase production is

required for normal progression of both pharyngitis and invasive infections. As part of the innate immune response to bacterial infection, neutrophils kill bacteria by entanglement in neutrophil extracellular traps, which consist of DNA and chromatin, and by subsequent destruction of the captured pathogen [30]. Sumby *et al.* [18] demonstrated that GAS DNase activity is crucial in assisting GAS to avoid destruction by neutrophils – a finding that has confirmed by other investigators [31].

Two additional recent publications have closed the loop on some aspects of the genetic differentiation of contemporary and old serotype M1 strains [32,33]. First, the occurrence of prophages encoding DNases in contemporary M1 isolates has been confirmed [32]. Second, Sumby *et al.* [33] have sequenced the genome of a contemporary M1 strain and shown that this *speA*-positive genotype, originally described in 1991, has a 36-kb recombination region involving genes encoding several GAS toxins including streptolysin O and NAD⁺-glycohydrolase. Importantly, this recombination event is associated with a significant increase in the production of these two toxins. Sumby *et al.* [33] have used the genome sequence information, DNA–DNA microarray, high-throughput single nucleotide polymorphism analysis, and expression microarray analysis to reconstruct the molecular evolutionary events that resulted in the genesis of the abundant serotype M1 clone that is now responsible for much human disease worldwide.

Vascular leakage and survival of GAS in plasma and blood

When GAS causes superficial infections, it is exposed to human plasma at sites of inflammation as a consequence of vascular leakage, which is mediated in part by the interaction of M protein and fibrinogen with the β_2 -integrin adhesion molecule on the surface of neutrophils. This complex results in a massive inflammatory response that involves the release of heparin-binding protein and induces vascular leakage [34]. Because augmented vascular permeability is one of the pathophysiological mechanisms underlying shock, crosslinking of the M protein and fibrinogen with β_2 -integrin might be important in the establishment of streptococcal toxic shock syndrome.

Plasma is a rich medium that supports bacterial growth; however, it also contains many immune system components that GAS must elude to survive, including opsonizing antibodies and complement. Many interactions between plasma proteins and GAS proteins interfere with the proper function of host defenses [5,35]. Moreover, GAS expresses surface proteins that have a high affinity for several human plasma proteins such as albumin, fibrinogen, α_2 -macroglobulin, IgG and plasminogen [5,35–38], which suggests that the pathogen has evolved mechanisms to capture and to use host proteins for enhanced survival *in vivo*.

To examine the effect of plasma on GAS protein expression, Johansson *et al.* [39] compared the proteome of serotype M1 strain AP1 cultured in laboratory medium with that of the same strain grown in human plasma. The expression of 39 protein spots, representing 24 unique GAS proteins, was significantly increased in cells cultured in human plasma. Interestingly, multiple spots corresponding

to the secreted extracellular proteins C5a peptidase and M1 were present in the proteome of plasma-grown bacteria, but were absent in the proteome of strain AP1 grown in laboratory medium. Concurrent with these data, a truncated variant of M1 protein lacking the amino-terminal 13 amino acids of the mature, full-length protein was identified – a finding indicative of the post-translational modification of this key virulence factor in response to plasma exposure [39].

Our knowledge of the differences in protein expression between GAS cultured in blood and GAS cultured in laboratory media is limited; however, Graham *et al.* [40] have demonstrated that GAS undergoes a rapid and extensive remodeling of its transcriptome when grown in human blood *ex vivo*. Within 30 min of the initial contact of GAS with whole blood, the transcripts of 716 genes (representing 37% of the GAS genome) were upregulated, whereas 425 transcripts (22% of the genome) were downregulated. The transcripts of genes belonging to functional categories expected to be important for growth adaptation in blood, including *de novo* synthesis of macromolecular precursors, carbohydrate metabolism, membrane transport, and transcriptional regulation, were increased. The expression of several virulence genes was also upregulated, including the multigene activator *mga* and its regulated transcripts *emm1*, *sic*, streptolysin S and *has* (capsular polysaccharide) [40]. Evidence of the upregulation of GAS capsule expression in blood *in vivo* has been provided by Gryllos *et al.* [41], who showed that there is a temporal relationship between capsule gene expression and proliferation of GAS in the blood of infected mice.

Transition from local to systemic infection

Each year, GAS causes an estimated 700 million cases worldwide of mild non-invasive infections such as pharyngitis and impetigo. Approximately 700 000 of these cases develop into severe invasive disease [42]. The exact mechanism that mediates the switch from a localized to a systemic infection remains to be elucidated. One factor that is thought to contribute to this process is the binding of GAS to human plasminogen and its subsequent activation to the broad-spectrum protease plasmin [43–47]. It has been proposed that plasminogen is supplied by vascular leakage at the site of infection. Evidence indicates that GAS assembles a trimolecular complex, consisting of fibrinogen, plasminogen and streptokinase, that is associated with a propensity for invasive disease [48,49]. Recent studies using a humanized transgenic mouse model have confirmed the important role of human plasminogen in the dissemination of GAS *in vivo* [37,45] and have led to the proposal that GAS might subvert human plasminogen for use as a virulence factor [36,45].

Interestingly, GAS also produces the cysteine protease SpeB, which has been shown to degrade the trimolecular complex directly [48]. Differential regulation of *speB* expression seems to be involved in the transition from localized to invasive disease. The *speB* gene is found in >99% of GAS isolates and is highly conserved, and production of SpeB has been shown to be important in establishing localized GAS infections [37,50,51]. Kansal *et al.* [52] observed that SpeB levels vary greatly in clonally related strains and that

expression of SpeB and human invasive disease severity are inversely related in M1T1 isolates of GAS. In addition, passage of *speB*-expressing GAS strains in mice results in downregulation of *speB*, concomitant with upregulation of the expression of M protein [52]. Moreover, Cole *et al.* [37] have shown that there is a subpopulation of GAS in localized infections that can lose SpeB activity, resulting in an accumulation of plasmin activity on the GAS cell surface and equipping this particular subpopulation with enhanced invasive potential. Ultimately, this might lead to the transition of GAS from the local site of infection to the blood, promoting dissemination to other parts of the host.

To obtain additional information about the GAS genes expressed during skin and soft tissue infection, Graham *et al.* [53] analyzed the transcriptome of GAS in mouse soft tissue infection. The transcriptome of a serotype M1 strain was studied, and array data sets were verified by quantitative real-time RT-PCR and *in situ* immunohistochemistry. Their results demonstrate that the coordinated expression of GAS virulence factors is directed towards overcoming innate host defenses, resulting in severe cellular damage. Furthermore several classes of genes were found to be highly expressed *in vivo*, including those associated with oxidative stress, virulence and complex carbohydrate utilization, in addition to several two-component transcriptional regulators. This study represents the first global analysis of the GAS transcriptome in invasive infection and has produced many new avenues for basic and translational research.

Severe invasive disease

Severe invasive GAS infections have re-emerged on a global level since the mid-1980s. These infections can progress rapidly and are associated with exceedingly high rates of morbidity and mortality [54]. Many strains causing necrotizing fasciitis, an especially severe and destructive infection, are serotype M1 and M3 [54–56], which suggests that particular GAS strains have an increased propensity to cause invasive disease.

Sumby *et al.* [57] have described two distinct transcriptome profiles of GAS that are linked to the types of disease – namely, pharyngitis or invasive infection. The pharyngeal transcriptome profile (PTP) and the invasive transcriptome profile (ITP) differs significantly in expression of ~10% of the genome, including genes of several known and putative virulence factors. Sumby *et al.* [57] found that strains of the PTP were able to transition to the ITP phenotype during invasive infections, whereas strains of the ITP phenotype did not convert to the PTP phenotype under the conditions tested. Strains of the ITP phenotype were more resistant than PTP organisms to phagocytosis and killing by human neutrophils.

To discover the genetic changes underlying the two distinct transcriptome profiles, complete genome re-sequencing of a mouse-derived ITP strain was conducted [57]. The genome of the ITP derivative organism differs from that of the PTP precursor strain by only a 7-bp frameshift mutation located in the gene *covS*, which encodes the sensor kinase of the CovR/S two-component regulatory system. CovR/S directly or indirectly controls ~15% of the GAS transcriptome [58]. The frameshift

mutation truncates CovS, resulting in the de-repression of many genes encoding known virulence factors, including *speA* (streptococcal pyrogenic exotoxin A), *sagA* (streptolysin S), *mac*, *ska* (streptokinase) and *spd3*. Consistent with these findings, Engleberg *et al.* [59] have reported that spontaneous mutations in CovR/S result in increased virulence in murine skin and soft tissue infections. Thus, the transcriptome profile and the virulence character of GAS are intimately linked to the allelic state of the genes encoding the CovR/S two-component system.

Much evidence has recently implicated a GAS extracellular protease (termed ScpC or SpyCEP) that cleaves interleukin-8 (IL-8) in invasive disease [60–62]. IL-8 is a chemoattractant that recruits neutrophils to the site of infection by promoting their migration out of the bloodstream. Thus, the ability of GAS to impair the activity of IL-8 during infection might enhance its survival and aid in its dissemination in the host. SpyCEP specifically cleaves the carboxyl terminus of IL-8, resulting in inactivation of the chemoattractant property of this molecule. This enzyme also cleaves and inactivates the mouse chemokines KC and MIP-2 [62]. Hidalgo-Grass *et al.* [62] have reported that inactivation of the gene encoding SpyCEP impairs clearance of GAS from infected mouse tissue and is associated with a decrease in soft tissue infection.

There is also evidence that *mtsR*, a gene encoding a metal transport regulator, might have a role in deep-muscle infections [63–65]. Using comparative genome re-sequencing, Beres *et al.* [65] discovered that strains containing a naturally occurring mutation in *mtsR* that yields a severely truncated MtsR protein are significantly underrepresented among GAS isolates recovered from individuals affected with necrotizing fasciitis. These results suggest that MtsR is necessary for the full virulence potential of GAS in invasive disease. Expression microarray analysis found that this truncation mutation is linked to significantly altered transcript levels of multiple genes and operons involved in metal ion homeostasis and response to oxidative stress [65], which perhaps renders the pathogen more vulnerable to the host immune response.

The molecular processes contributing to tissue destruction in necrotizing fasciitis and myonecrosis remain poorly understood. Recent studies have provided important evidence that streptolysin-O-induced platelet or neutrophil complexes have a role in the development of ischemic necrosis of tissue [66]. In related work, Bryant *et al.* [67] have shown that an early step in the pathogenesis of GAS myonecrosis might involve binding of the pathogen to vimentin – a molecule that is upregulated after skeletal muscle injury.

Asymptomatic carriage of GAS

Because GAS is exclusively a human pathogen, it must survive in a human reservoir. Many individuals carry GAS asymptomatically in their upper respiratory tract and other anatomic sites. Over the years, several hypotheses have been proposed to explain the asymptomatic carriage of GAS. In principle, it could arise as a consequence of (i) mutations in the pathogen that downregulate virulence, (ii) a productive immune response by the host that constrains pathogen proliferation, (iii) internalization into

host cells, or (iv) some combination thereof. Relatively little work has been done to study the genetic relationships between strains that cause disease episodes and those of the same M protein serotype that are recovered from asymptomatic carriers. However, this issue can now be studied at the genome-wide level.

For example, Beres *et al.* [68] have used comparative genome re-sequencing to show that several serotype M3 strains from asymptomatic throat carriers contain deletion mutations in the pleiotropic virulence regulatory gene *mga* and the *emm* gene. The occurrence of these mutations in the carrier isolates but not in disease-causing organisms implicates them in downregulating virulence. Consistent with this idea, carrier strains have been shown to be significantly less virulent, as assessed by intraperitoneal injection into mice [68]. Thus, these data support the hypothesis that asymptomatic carriage might arise as a consequence of mutations that downregulate virulence.

Although GAS has long been regarded as an extracellular pathogen, evidence indicates that it can invade non-phagocytic cells [69–72]. This observation has led to conjecture that internalization of GAS by host cells participates in the carrier state [5,70,73]. Internalization of GAS might enable the pathogen to evade some host defenses and might protect it from killing by antibiotics such as penicillin, because this antibiotic does not enter epithelial cells [70]. Consistent with this idea, Sela *et al.* [70] have observed that strains that escape antibiotic killing have significantly higher internalization efficiencies than strains that are successfully eliminated by antibiotics. Other investigators have reported that genes encoding proteins that are linked to internalization, such as the fibronectin-binding proteins F2, SfbI and PfbpI, are significantly more prevalent among persisting GAS strains recovered from asymptomatic carriers [5,73]. By contrast, Brandt *et al.* [74] found no evidence for an increase in the prevalence of the *prtF1* gene among GAS isolates recovered from individuals with pharyngitis for whom antibiotic treatment failed. Thus, the data on this issue remain unclear and additional work is required to elucidate the role of host-cell internalization in asymptomatic carriage and other aspects GAS biology and pathogenesis.

Asymptomatic carriage of GAS also might have a role in some invasive infections. Medina *et al.* [75] observed that the intracellular survival of GAS in neutrophils might result in an increase in bacterial virulence. Using a mouse model, they showed that ingested GAS is transported by neutrophils to distant parts of the body [75]. This finding is notable because it could explain the cases of necrotizing fasciitis that occur in the absence of an entry wound or trauma adjacent to the site of GAS infection.

Concluding remarks

We have highlighted some recent advances in our understanding of GAS pathogenesis and its interaction with the human host at various sites of infection. Many of the important findings have been made possible by contemporary genome sequencing, microarray technology and proteome analysis. The combined use of these technologies permits a global interrogation of molecular events that occur during host–pathogen interactions. Indeed,

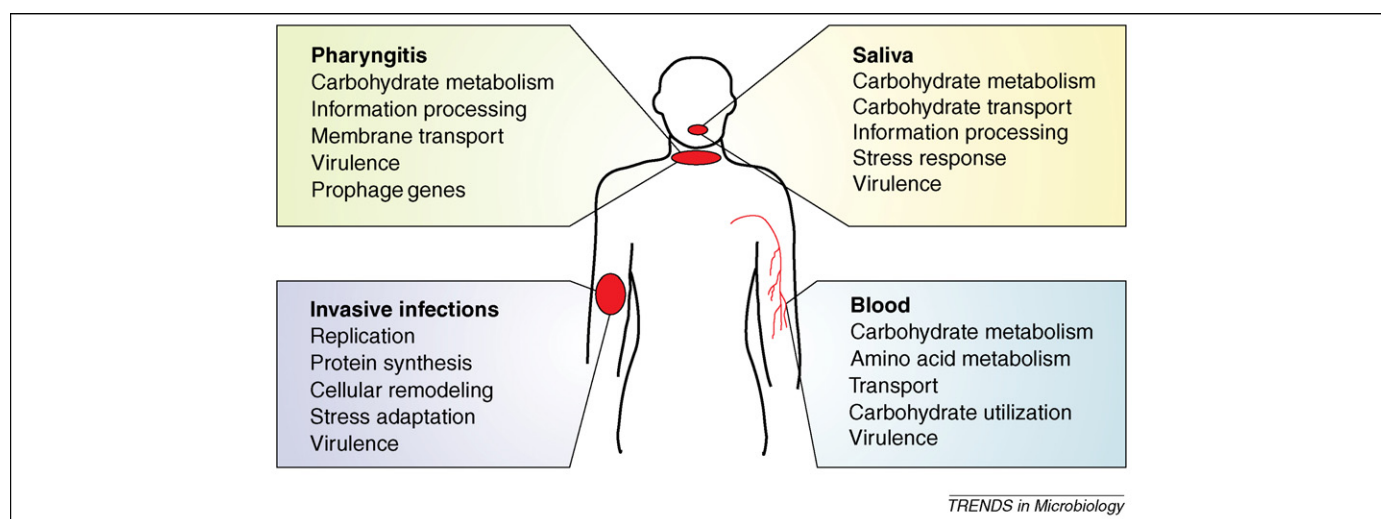


Figure 2. GAS modifies the transcription of genes belonging to diverse functional groups during infection. GAS causes many different human infections, reflecting its ability to adapt to diverse physiological conditions. Several recent studies have investigated GAS transcriptome remodeling in pharyngitis [17] and invasive soft-tissue infection [53], and during growth in saliva [6] and blood [40].

Box 1. Outstanding questions

What are the molecular markers of the initial stages of severe invasive diseases such as streptococcal toxic shock syndrome and necrotizing fasciitis?

What is the molecular mechanism responsible for severe invasive GAS infections?

What host factors are involved in the development of, and the susceptibility to, severe invasive disease?

What are the environmental signals that cause the transcriptional switch from non-invasive to severe invasive streptococcal disease?

these technologies have revealed that GAS responds rapidly and in a regulated manner to the distinct environments encountered in the host during the establishment and dissemination of disease (Figure 2).

In the future, these approaches will be useful in identifying molecular markers specific for each phase of the disease process (Box 1). Such markers are especially important for severe invasive GAS diseases, which progress rapidly and are often identified too late because of the current lack of appropriate diagnostics.

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