Genetic regulation of host responses to Group A Streptococcus in mice

Eva Medina and Andreas Lengeling

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Abstract

The group A streptococci (GAS, Streptococcus pyogenes) are important human pathogens which can cause a variety of diseases, ranging from mild infections to very severe invasive diseases. In recent years, evidence has been accumulated that host genetic factors have a major influence on the outcome of streptococcal infections. Variability in the degree of susceptibility of different inbred mouse strains to infection with GAS has demonstrated that the host genetic background largely determines the susceptibility of mice to this pathogen. This information is particularly useful for studying the immune mechanisms underlying disease susceptibility in mice, and provides an entry point for the identification of host defence loci. This paper reviews the recent advances in the characterisation of pathogenic mechanisms associated with the development of GAS-induced septic shock in the mouse model and outlines the current knowledge regarding the genetic control of immune responses to Group A streptococcus in mice.

INTRODUCTION

The group A streptococcus (GAS) — also known as Streptococcus pyogenes — is among the most flexible and prevalent of human pathogens. It is responsible for a wide spectrum of human diseases, ranging from mild, self-limiting infections such as pharyngitis, scarlet fever and impetigo to extremely severe and life-threatening invasive diseases such as necrotising fasciitis and streptococcal toxic shock-like syndrome. Interestingly, GAS can also be carried asymptomatically and, in fact, one-third of all humans are colonised by this pathogen. Besides causing acute diseases, GAS infections can also lead to the development of severe autoimmune sequelae such as acute rheumatic fever, rheumatic heart disease and acute post-streptococcal glomerulonephritis.

Currently, the group A streptococci continue to be a major health problem, due to a worldwide increase in the incidence of severe invasive infections observed since the mid-1980s. Although the reasons for this increased number of severe episodes remain unclear, the emergence of GAS serotypes or clones with increased invasive capacity has been proposed by several groups. In this regard, GAS serotypes M1 and M3 have been isolated from a large number of patients with such severe infections. The fact that these strains also caused increased numbers of non-invasive, less severe infections, however, indicates that association of these serotypes with severe cases simply reflected the prevalence of these strains in the general population. Furthermore, several studies of disease outbreaks have revealed that the same streptococcal strain can be isolated from different patients with infections of varying severity, suggesting a strong influence of host factors in disease pathogenesis. Currently, it is clear that the outcome of GAS infection results from a complex interaction between the microbe, the host and the environment. The dissection of the individual contribution of these factors might provide clues to fundamental questions about the pathogenesis of GAS and the host response. In this regard, animal
models have been used extensively to develop a primary understanding of the mechanisms associated with host defence and for the identification of host susceptibility genes. Several animal models of GAS infection have been developed in recent years to gain a better understanding of host–GAS interactions. Examples include the nematode infection model, which uses the genetically well-characterised model organism Caenorhabditis elegans or the zebrafish model, which can be used to study particular aspects of invasive streptococcal infections in fish. The most commonly used model for studying GAS infection, however, is the murine model. Although GAS is an exclusively human pathogen, the laboratory mouse can be experimentally infected with GAS. The advantage of the murine model of GAS infection is provided by the differential response to GAS exhibited by inbred mouse strains with different genetic backgrounds, mimicking the spectrum of clinical presentations of GAS infections observed in humans. This review will describe different aspects of the murine models of GAS infections.

**MODELLING THE SPECTRUM OF HUMAN GAS INFECTIONS IN THE MOUSE**

The pathogenesis of GAS infections is still poorly understood, despite the fact that group A streptococci remain a major cause of human disease. Specifically, very little is known about the molecular mechanisms underlying the different clinical manifestations of GAS infections. This lack of information has been due largely to the limitations of animal models available for experimentation with GAS. By screening different inbred strains of mice for their resistance/susceptibility to GAS, a mouse model of infection has been developed which mimics the spectrum of resistance and susceptibility of human populations to GAS. A remarkable difference in susceptibility to GAS infection was found among different inbred mouse strains after intravenous infection with this pathogen (Figure 1). While CBA/J, C3H/HeN, A/J, PvD/Phe mice were the most susceptible strains, C57BL/6J mice displayed an intermediate susceptibility and DBA/2J and BALB/c mice were the most resistant strains. Thus, while GAS infection in resistant mouse strains may be a good model for mild GAS infection in humans, the course of infection in susceptible mouse strains may resemble that of patients undergoing severe invasive GAS disease. An additional advantage of this mouse model of GAS infection is that the immune mechanisms responsible for resistance/susceptibility can be characterised by simple comparison of the immunological parameters in resistant and susceptible mouse strains during the course of infection. After intravenous infection with GAS, susceptible mouse strains exhibited very high mortality rates and all mice succumbed to infection within two to three days of bacterial inoculation. Mortality was correlated with very high levels of bacterial growth in the blood and internal organs and was independent of the inoculum size (10^3 or 10^5 colony forming units of *S. pyogenes*). These observations suggested that the inability of susceptible mice to control bacterial growth led to enormous bacterial loads in the blood and systemic organs and then to septic shock and death. By contrast, resistant mice were very efficient at controlling GAS infection, most of the mice survived a long observation period. For subsequent studies regarding the identification of immune mechanisms underlying resistance/susceptibility to GAS infection, BALB/c was selected as a prototype resistant mouse strain and C3H/HeN as a prototype susceptible mouse strain. Comparison of the kinetics of bacterial growth in BALB/c and C3H/HeN mice demonstrated that the superior control of bacterial growth
exhibited by BALB/c mice over C3H/HeN was evidenced as early as five hours after bacterial inoculation, with an approximate 2 log10 reduction of bacterial load in the lungs and liver. This difference in susceptibility pattern between C3H/HeN and BALB/c mice was independent of the route of inoculation. Thus, BALB/c mice displayed a superior resistance to GAS infection (>1,000-fold) than C3H/HeN mice after subcutaneous inoculation of GAS in a skin model of infection. The early difference in the control of bacterial growth exhibited by BALB/c and C3H/HeN mice suggested that innate immune effector mechanisms might be especially important for resistance to GAS. Indeed, no differences in bacterial clearance or infection kinetics were observed when T and B cell-deficient SCID mice congenic on a resistant BALB/c genetic background were compared with immunocompetent BALB/c mice. In keeping with these observations, T cell-deficient SCID-C3H/HeN mice were found to be as susceptible to GAS infection as the immunocompetent C3H/HeN mice. This strongly indicated that innate effector rather than adaptive immune mechanisms mediate resistance to GAS.

**IMMUNE MECHANISMS OF HOST DEFENCE: THE ROLE OF INNATE IMMUNE RESPONSES IN GAS INFECTIONS**

Innate immunity to bacterial infections is mainly mediated by resident macrophages, recruited polymorphonuclear leukocytes (PMNs) and natural killer (NK) cells. The combined activity of these cells regulates the host inflammatory response during the early phase of infection. Inflammatory cells recruited at the site of infection are crucial, not only for the killing of the invading pathogens but also for the further recruitment of phagocytic cells through the production of inflammatory mediators. The possibility that the susceptibility exhibited by C3H/HeN mice to GAS was associated with an impaired capacity to mount an initial inflammatory response at a local site of GAS infection was evaluated. To this end, the recruitment of inflammatory PMNs in the peritoneal cavity in response to GAS infection was determined in BALB/c and C3H/HeN mice. Against expectation, C3H/HeN mice responded to GAS infection with a much greater recruitment of inflammatory PMNs than did BALB/c mice. The higher number of recruited PMNs in infected C3H/HeN mice correlated with the production of significantly higher levels of the proinflammatory cytokines interferon gamma (IFN-γ), interleukin (IL)-1α, IL-12 and IL-6 when compared with infected BALB/c mice. The level of nitric oxide (NO) was also significantly greater in the serum of infected C3H/HeN mice than in the serum of infected BALB/c mice. NO is an important inflammatory mediator and has been reported to contribute to the organ failure observed during septic shock in patients.

Therefore, the extent of organ damage in infected BALB/c and C3H/HeN mice...
was then determined. Histopathological examination revealed extensive areas of tissue destruction in the organs of C3H/HeN but only slight alterations in those of BALB/c mice. Taken together, these observations clearly indicated that the susceptibility to GAS exhibited by C3H/HeN mice was associated with the development of a massive inflammatory reaction in response to infection, which subsequently led to organ damage and death. Production of proinflammatory cytokines during a normal inflammatory response, as was the case with the BALB/c mice, contributes extensively to the recruitment of phagocytic cells capable of killing the invading microorganisms. By contrast, a massive inflammatory reaction, such as that described in the C3H/HeN mice, is potentially autodestructive and may cause tissue damage, septic shock and, eventually, death. Elevated levels of cytokines also have been found in the sera of patients with severe invasive GAS infection such as streptococcal toxic shock syndrome (STSS). Therefore, the course of GAS infection in susceptible C3H/HeN mice strongly resembles many features of patients undergoing STSS.

The primary line of innate defence against most bacterial pathogens is composed of resident macrophages. The potential role played by resident macrophages in host resistance to GAS infection was investigated in resistant BALB/c mice. For this purpose, BALB/c mice were either depleted of macrophages by treatment with carrageenan or rendered deficient in macrophage functional activities by treatment with gadolinium III chloride and subsequently infected with GAS. Depletion or inactivation of macrophages rendered BALB/c mice totally susceptible to GAS. Macrophage-depleted mice exhibited higher bacterial loads and higher mortality rates compared with non-depleted animals. Interestingly, enhanced susceptibility was only observed when macrophage depletion was performed prior to or during the first 24 hours of GAS infection, emphasising the important contribution of this cell population to the early control of infection.

Several studies have shown that patients with a tendency to produce higher levels
of proinflammatory cytokines in response to streptococcal super-antigens (SAgs) developed significantly more severe infections than patients producing lower levels of inflammatory cytokines in the same situation. This genetic predisposition was also reflected in the authors’ mouse model of GAS infection. Spleen cells isolated from uninfected C3H/HeN mice responded to in vitro stimulation with GAS products with stronger proliferative activity and with higher levels of IFN-γ production than those isolated from BALB/c mice. Taken together, susceptibility exhibited by C3H/HeN mice to GAS can be attributed to two factors: 1) the inability to restrict bacterial growth in the early phase of infection, and 2) a genetic predisposition to produce an uncontrolled inflammatory response to bacterial products (Figure 2).

HUMANISING THE MOUSE TO STUDY THE PATHOGENESIS OF GAS DISEASES

Human/mouse chimeras

Scaramuzzino et al. developed a mouse model of streptococcal impetigo, whereby human neonatal foreskin was grafted onto SCID mice. This so-called hu-skin-SCID mouse model strongly mimics the histopathological features of GAS impetigo in humans. This model is very useful for gaining a better understanding of the pathogenic mechanisms associated with GAS skin infection and can also be used to facilitate the development of a vaccine for streptococcal impetigo.

HLA transgensics

It is believed that SAgs made by GAS contribute to the clinical feature of STSS. Several studies have emphasised the importance of host genetic factors in the development of STSS through the regulation of the inflammatory cytokine responses to streptococcal SAgs (GAS-SAgs). SAgs, by virtue of their direct binding to major histocompatibility complex (MHC) and T cell antigen receptors, can activate a large subset of T cells in the absence of conventional antigen presentation. This multiclonal stimulation of T cells results in massive release of proinflammatory cytokines and other inflammatory mediators thought to be important for inducing STSS. The response to SAgs cannot be examined easily in mice because, unlike human lymphocytes, murine cells respond only to extremely high concentrations of these toxins in vitro. Consequently, mice are significantly less susceptible to the toxic effects of GAS-SAgs than are humans. Sriskandan et al. have developed a transgenic humanised mouse that expresses human HLA-DQ molecules, which have been linked with excessive T cell responses in patients. These HLA-congenic mice have been used to dissect the mechanisms by which GAS-SAgs interact with the immune system during bacterial sepsis. These mouse models can be useful for determining the influence of different HLA haplotypes on disease pathogenesis, as well as for the testing of novel therapies.

Mouse models of rheumatic fever

Rheumatic fever is a delayed autoimmune sequel to group A streptococcal pharyngitis. It can induce serious heart damage, leading to death or requiring valve replacement. Only a small percentage of individuals develop acute rheumatic fever following acute GAS pharyngitis, clearly suggesting a host genetic susceptibility to rheumatic fever. The molecular basis underlying the development of rheumatic fever remains poorly understood, mainly due to the lack of suitable animal models for studying this disease. The development of antibodies cross-reacting with heart tissue during GAS infection in the susceptible host has been suggested as the potential trigger mechanism of this sequela.

Some studies have shown that genetic differences in mouse strains affected the deposition of mouse anti-myosin
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Figure 2: Summary of the events influencing the outcome of group A streptococcus (GAS) infection in susceptible C3H/HeN and resistant BALB/c mice. Electron microscopy photographs show spleens of BALB/c and C3H/HeN mice 48 hours after GAS infection. Abbreviations: IFN = interferon; IL = interleukin; NK cells = natural killer cells; PMNs = polymorphonuclear leukocytes.

Host genetics in the development of GAS-associated rheumatic heart disease.

Immunisation of mice with epitopes of the streptococcal M protein, which exhibited immunological cross-reactivity with cardiac myosin, has been used extensively to study the development of rheumatic heart disease. Thus, immunisation with these epitopes induced inflammatory heart disease in MRL mice expressing the H2k MHC haplotype. A strong association of the I-Ak molecules with this disease was also shown in these studies. Myocarditis was also induced in BALB/c mice after immunisation with cross-reactive peptides of the M protein.

The development of a mouse model of infection mimicking the development of rheumatic fever following GAS infection is critical for the design of preventive strategies against this devastating sequela.

THE GENETIC COMPONENT OF THE HOST RESPONSE TO GAS

Although immune deficiencies and underlying chronic diseases increase the risk of patients developing severe GAS infection, invasive cases are also common among healthy persons with normal immunity. These observations clearly indicate that host genetic factors have a strong influence on the severity of GAS infection. Genetic linkage studies of susceptibility traits have provided an avenue for identifying host genes that are involved in the control of immune responses to pathogens. In this regard, the animal model of GAS infection offers an excellent system for performing these types of studies. The authors have undertaken a genetic linkage approach to identify GAS susceptibility loci in C3H/HeN mice. To this end, back- and intercross panels of mice were generated using the susceptible C3H/HeN and resistant BALB/c mouse strains. A first genetic linkage analysis in a moderate size backcross identified three quantitative trait loci (QTLs) affecting the survival of backcross progenies and bacterial growth upon infection (unpublished observation). The
QTLs were mapped to proximal mouse chromosome 7 (peak linkage at D7Mit350), proximal mouse chromosome 17 (peak linkage at D17Mit34) and to central mouse chromosome 2 (peak linkage at D2Mit100). Although the candidate gene intervals harbouring the susceptibility loci are still quite large — spanning chromosomal regions from 8–25 centimorgans — the authors were able to confirm the QTL locus mapping to the H2 region on mouse chromosome 17, which contains the mouse MHC gene region. Using congenic BALB/k mice, they were able to show that the H2 haplotype of the susceptible C3H/HeN strain in an otherwise resistant BALB/c background has a major influence on the outcome of GAS infection. BALB/k congenic mice are as susceptible to GAS infection as the H2-donor C3H/HeN mice, demonstrating the presence of a susceptibility locus within the MHC gene region. Interestingly, the QTL on proximal mouse chromosome 7 seems to overlap with a QTL that has been linked with susceptibility to infection with Streptococcus pneumoniae in mice, and has also been mapped approximately to the same region of the chromosome. Because the susceptibility pattern to GAS and S. pneumoniae are very similar in different inbred strains of mice (eg CBA/N, C3H/HeN, A/J mice are susceptible and BALB/c mice are resistant), this suggests that a common locus on mouse chromosome 7 might control host responses to both pathogens.

COMPARATIVE GENETICS: A LINK TO MECHANISMS OF GENETIC PREDISPOSITION IN PATIENTS
Studies have shown that the same streptococcal strain can cause infections of varying severity in different individuals, emphasising the influence of host genetic factors in determining the severity and outcome of GAS infections. In this regard, significantly more severe clinical manifestations of streptococcal infections have been observed in patients with a propensity to produce high levels of proinflammatory cytokines in response to GAS products, in addition to the consistent interindividual variation in cytokine response. Patients suffering from STSS or necrotising fasciitis had significantly higher levels of circulating IL-2, IL-6 and tumour necrosis factor-alpha compared with those found in patients with non-severe invasive cases of GAS. This provided evidence for the involvement of cytokines in invasive GAS infections in humans and suggested similarities in the mechanisms underlying disease development in infected C3H/HeN mice. Kotb and colleagues have investigated the influence of allelic variations of the human MHC gene complex in the severity of invasive GAS disease. They found that certain HLA haplotypes contributed to susceptibility to, or protection from, severe streptococcal diseases by their ability to modulate the magnitude of the inflammatory cytokine response elicited to streptococcal super-antigens. The human MHC gene region is located on chromosome 6p21, a region, which is syntenic to the H2 gene region on mouse chromosome 17. The fact that it was possible to map a GAS susceptibility locus to this region suggests the possibility that homologues genes might be involved in regulating GAS infections in humans and mice. Future comparative genetics studies will help to identify candidate genes for infection susceptibility in both species.

CONCLUDING COMMENTS AND OUTLOOK FOR FUTURE STUDIES
The interactions between the host and GAS are multidimensional and very complex. A complete understanding of GAS pathogenesis requires identification and characterisation of host genes that regulate the immune response to this microorganism. Identification of the
different components at the cellular and molecular levels is the first step in understanding how the host deals with this pathogen. Given the potentially large number of factors that contribute to host defence against GAS in humans, gene identification remains a difficult challenge. A combination of different experimental mouse models might help to identify new candidate susceptibility genes for human GAS diseases.

The identification of inbred mouse strains with different levels of susceptibility to GAS (e.g., resistant BALB/c versus susceptible C3H/HeN) provides the appropriate framework for performing these kinds of studies. The BALB/c and C3H/HeN mouse strains have been proven to be instrumental, not only in the detailed characterisation of cellular mechanisms associated with the development of sepsis in the mouse during GAS infection but also in the mapping of loci determining resistance or susceptibility to infection in both mouse strains. To progress the positional identification of susceptibility genes in the future, genomic mapping information can be combined with microarray expression data from immune cells that have been characterised to be crucial for disease development. The generation and characterisation of congenic mice harbouring reduced chromosomal regions of the parental strains will certainly also help in the fine mapping and identification of susceptibility loci.

Another avenue that could be explored in the identification of genes involved in host defence against pathogens in general, is the use of systematic mouse mutagenesis screens.43 If these screens are highly defined in their phenotype outread systems, whole signal transduction cascades for host–pathogen interactions can be dissected genetically.44 A recent example of the successful application of a N-ethyl-N-nitrosourea (ENU) mouse mutagenesis screen is the identification of Cd36 as a sensor for a diacylated bacterial lipopeptide and lipoteichoic acid.45 Mice that are homozygous for an ENU-induced nonsense allele of Cd36 are hypersusceptible to Staphylococcus aureus infections because their macrophages cannot respond to these important pathogen-associated molecular patterns. It might be expected that these large-scale mutagenesis screens are also important for the identification of GAS susceptibility and resistance genes. Newly identified host loci that mediate essential immune defence responses to other Gram-positive bacteria like Staphylococcus aureus and Streptococcus pneumoniae might also be very relevant for a general host defence against Group A Streptococci.

The identification of the cellular and molecular determinants of susceptibility to infection in mice will provide a basis for finding similar pathogenic mechanisms associated with invasive GAS infections in humans. Ultimately, comparative genomics will play a critical role in facilitating the extrapolation of the newly acquired knowledge from the experimental model to a more complete understanding of human host resistance/susceptibility to GAS infection.

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References


