

Network: Infection and Inflammation: from Pathogen-induced Signatures to Therapeutic Target Genes**Project: Molecular, Cellular and Genetic Basis of *Streptococcus pyogenes*-induced Sepsis in Mice**

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Introduction

Group A Streptococci (*S. pyogenes*) are important human pathogens responsible for a wide spectrum of human diseases ranging from mild clinical illnesses such as pharyngitis or impetigo, to severe life-threatening diseases such as necrotising fasciitis and streptococcal toxic shock syndrome (STSS) (1). Since the late 1980s, a marked increase in the incidence of severe streptococcal infections, often associated with STSS has been reported worldwide (2). Despite attempts at early diagnosis, the use of antimicrobial treatment, and intensive care support, mortality associated with STSS remains high. The resurgence of severe streptococcal diseases and the associated high mortality rate have renewed the interest in understanding the development of streptococcal toxic shock and in the elucidation of the host immune mechanisms involved in these processes.

STSS is mainly caused by an exaggerated systemic cytokine response during infection with *S. pyogenes*. Challenge of mice with *S. pyogenes* results in either resolution of infection or in a syndrome resembling the septic shock in humans depending of the genetic background of mouse strain (3). While a moderate production of proinflammatory cytokines such as IFN- γ and IL-12 was associated with disease resolution in resistant mice, the uncontrolled production of this cytokines causes several pathophysiological reactions in susceptible mice, which ultimately lead to septic shock and death.

To date, efforts to modulate the inflammatory response during sepsis by inhibition of cytokines have been unsuccessful for gram-positive infection. This leads to the conclusion that the mechanisms leading to shock during streptococcal infection may be multifactorial and perhaps difficult to treat. The available evidences support the conclusion that septic shock during streptococcal infection might not be caused by a single cell population, and that different cellular subsets seem to participate in septic shock in a hierarchical manner. The identification of host cell populations critically involved in the development of septic shock as well as the elucidation of the complex interplay between these cell populations could be highly valuable for future clinical applications.

NK cells are important components of the innate immune response that can be activated during septic shock events and can contribute to the pathogenesis of this reaction through the secretion of high amounts of IFN- γ (4). In this study we have evaluated the contribution of NK cells to the pathogenesis of *S. pyogenes*-induced septic shock in a mouse model of group A streptococcal infection.

Although current measures for treatment or prophylaxis for streptococcal diseases rely on chemotherapy, there is a consensus that in the longer term, vaccination might constitute the most effective strategy to diminish the global disease burden caused by this pathogen. The development of an efficient anti-*S. pyogenes* vaccine is thus widely regarded as a major priority, but despite intensive research, a safe and effective anti-*S. pyogenes* vaccine is not yet available.

An important consideration for the design and implementation of vaccines is the understanding of the contribution of the host genetics to disease susceptibility because the population most in need of protection may be the one which is normally most susceptible to infection. In the particular case of *S. pyogenes*, several studies have

shown that individuals within a single human population vary genetically in their susceptibility to infection with this pathogen (5). Thus, depending of the genetic makeup, different individuals will develop infections of different severities after encounter with the same streptococcal strain. In addition, several studies have suggested that low levels of anti-*S. pyogenes*-specific antibodies in infected individuals may increase the risk to develop severe disease (6), suggesting a potential benefit of pre-existing specific antibodies. In the study presented here we have assessed whether the presence of a serotype-specific immune response elicited after vaccination might rescue the genetically susceptible host for developing sepsis and septic shock after an encounter with *S. pyogenes* using a mouse model of infection.

Results/Project Status**Contribution of NK cells to the pathology of septic shock induced by *S. pyogenes* in mice**

Natural Killer (NK) cells are critical components of the innate immune system that have been implicated in the pathogenesis of septic shock (4). In this study, the relative contribution of NK cells to the development of *S. pyogenes*-induced septic shock was investigated in a mouse model. We have previously shown that challenge of C3H/HeN mice with *S. pyogenes* resulted in an exaggerated systemic inflammatory syndrome resembling the streptococcal septic shock in humans (3). Here, we show that depletion of NK cells by treatment with anti-asialo GM1 antibodies significantly improved the survival of susceptible C3H/HeN mice after bacterial inoculation (Fig. 1A), suggesting a potential contribution of these cells to the pathology of *S. pyogenes*-induced septic shock. This assumption was further confirmed by the superior resistance to *S. pyogenes* exhibited by mutant mice devoid of NK cells activity (beige) when compared with the corresponding wild type strain. The augmented resistance to *S. pyogenes* observed in NK cells-depleted mice was associated with much lower levels of proinflammatory cytokines such as IFN- γ , IL-12 and IL-6 in serum during the early phase of infection than those detected in non-depleted control mice (Fig. 1B). We have previously reported that organ damage occurred in C3H/HeN mice during group A streptococcal infection and that liver injury and dysfunction may have been the cause of death in these animals. Histopathological examination performed in liver sections reveals that the liver of infected NK cells-depleted mice appeared relatively undamaged at 48 h of infection with only small areas of focal destruction (Fig. 1C, arrow). In contrast, liver sections of control mice obtain at the same time of infection exhibited large areas of hepatic ischemia with extensive tissue destruction (Fig. 1D, ★).

We conclude that NK cells significantly contribute, by amplifying the inflammatory response, to the progression of *S. pyogenes*-induced septic shock. Thus, our results may contribute to open new avenues for the design of more efficient therapeutic strategies for treatment of gram-positive septic shock.

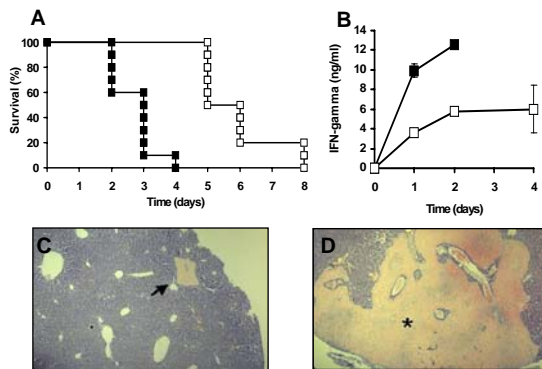


Fig 1: Survival curves (A) and kinetics of IFN- γ production (B) of NK cells-depleted (white symbols) and non-depleted (black symbols) C3H/HeN mice after intravenous infection with 10^5 CFU of *S. pyogenes*. Histopathology of liver tissue from NK cells-depleted (C) and non-depleted (D) C3H/HeN mice taken at 48 h postinoculation with 10^5 CFU of *S. pyogenes*. Livers were harvested, processed for routine histology, and stained with azure blue. Small areas of ischemic tissue can be observed in NK cells-depleted mice (arrow) compared with the large areas of ischemia and necrosis found in the liver of the control mice (*).

Vaccination equally enables both genetically susceptible and resistant mice to control infection with *S. pyogenes*

There is substantial evidence that host genetic factors are important in determining susceptibility to infection with *S. pyogenes*. Similar to humans, we have shown that a genetic component may be important in determining susceptibility to *S. pyogenes* infection in mice (3). Thus, C3H/HeN mice are much more susceptible to streptococcal infection than BALB/c mice. We have determined here whether vaccination makes genetically susceptible mice as capable as genetically resistant mice to control infection with *S. pyogenes*. Resistant BALB/c and susceptible C3H/HeN mice were immunized either systemically with heat-killed *S. pyogenes* or through the mucosal route with an M protein-based subunit vaccine, and challenged with live bacteria. Our results show that both genetically resistant and susceptible mouse strains generated an antigen-specific immune response against *S. pyogenes* after mucosal or systemic vaccination that makes both mouse strains equally capable to control streptococcal infection. Protective antibodies were T cell-dependent as clearly evidenced by the ability of immune serum from immunocompetent but not from T cell-deficient nu/nu mice to transfer immunity to naïve susceptible mice. Thus, it seems that the presence of bactericidal antibodies surpasses the compromised expression of innate genetic resistance exhibited by C3H/HeN mice to *S. pyogenes*. Passive transfer of polyclonal immune serum to infected C3H/HeN mice at 24 h postinoculation, a time point when bacteria have disseminated and infection is progressing, resulted in only a slight attenuation of the severity of infection, extending the survival time of infected animals but failing to rescue them from death. The inability to achieve sterilizing protection after passive transfer at 24 h postchallenge may suggest that the amount of anti-*S. pyogenes* antibodies administered was insufficient against the amount of bacteria present in infected mice at this time of infection. These results suggest that

efficient protection after vaccination could only be acquired by the elicitation of high levels of long-lasting anti-GAS specific antibodies.

The results obtained in this study show that resistance to *S. pyogenes* in the genetically susceptible host may largely be achieved by vaccination. Therefore, the implementation of a functional streptococcal vaccine may significantly contribute to the reduction or elimination of severe cases of invasive streptococcal diseases.

Outlook

Very little is known about the contribution of the host genetic predisposition to the severity of infection caused by *S. pyogenes*. Recent studies have suggested that allelic variations of the major histocompatibility complex (MHC) class II antigens may contribute to susceptibility to or protection from severe streptococcal diseases by its ability to modulate the magnitude of the inflammatory cytokine response elicited to bacterial superantigens. Thus, patients carrying the DRB1*14/DQB1*0503 or DRB1*07/DQB1*0201 haplotypes have an increased risk of developing severe systemic streptococcal disease than those carrying the DRB1*1501/DQB1*0602 haplotype (5). However, significant association of a gene polymorphism with a certain disease susceptibility does not ensure that the gene polymorphism is a primary factor for the disease phenotype. There is a possibility that another polymorphism within closely linked genes may actually determine the disease susceptibility and that the observed association might be a secondary one resulting from linkage disequilibrium. Consequently, it will be important to determine whether genetic susceptibility to group A streptococcal infection is influenced directly by polymorphism of the *H2* locus or by polymorphisms of other genes present in the neighboring regions to this locus.

Besides the highly deleterious exotoxins, *S. pyogenes* contains a number of immunogenic cell wall components, such as lipoteichoic acid and peptidoglycans, capable of stimulating enormous release of pro-inflammatory cytokines and other inflammatory mediators from monocytes/macrophages. The possibility that *S. pyogenes* may induce a differential inflammatory transcriptional profile in macrophages from BALB/c and C3H/HeN mice can establish a new hypothesis regarding the molecular mechanisms underlying the respective resistance and susceptibility exhibited by these two mouse strains to this pathogen.

Lit.: 1. Cunningham MW. Pathogenesis of group A streptococcal infections. *Clin Microbiol Rev.* 2000 Jul;13(3):470-511. 2. Kaplan EL. The resurgence of group A streptococcal infections and their sequelae. *Eur J Clin Microbiol Infect Dis.* 1991 Feb;10(2):55-7. 3. Medina E et al. Genetic control of susceptibility to group A streptococcal infection in mice. *J Infect Dis.* 2001 Oct 1;184(7):846-52. 4. Heremans H et al. Essential role for natural killer cells in the lethal lipopolysaccharide-induced Schwartzman-like reaction in mice. *Eur J Immunol.* 1994 May;24(5):1155-60. 5. Kotb M et al. An immunogenetic and molecular basis for differences in outcomes of invasive group A streptococcal infections. *Nat Med.* 2002 Dec;8(12):1398-404. 6. Basma H et al. Risk factors in the pathogenesis of invasive group A streptococcal infections: role of protective humoral immunity. *Infect Immun.* 1999 Apr;67(4):1871-7.