This is a postprint of an article published in
Siegert, J., Sastalla, I., Chhatwal, G.S., Medina, E.
Vaccination equally enables both genetically susceptible and resistant mice to control infection with group A streptococci
(2006) Microbes and Infection, 8 (2), pp. 347-353.

Revised mic051587

"Vaccination equally enables both genetically susceptible and resistant mice to control infection with group A streptococci"

Jeannette Siegert, Inka Sastalla, Gursharan Singh Chhatwal, Eva Medina*

Department of Microbial Pathogenesis and Vaccine Research, GBF-German Research Centre for Biotechnology, Mascheroder Weg 1, 38124 Braunschweig, Germany.

***Address correspondence:** Dr. Eva Medina, Infection Immunology Group, GBF-German Research Center for Biotechnology, Mascheroder Weg 1, D-38124 Braunschweig, Germany. Tel: 0531/6181466, Fax: 0531/6181708, e-mail: eme@gbf.de

ABSTRACT

There is substantial evidence that host genetic factors are important in determining susceptibility to infection with group A streptococci (GAS). Similar to humans, several studies have revealed that a genetic component may be important in determining susceptibility to GAS infection in mice. 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 GAS infection. Resistant BALB/c and susceptible C3H/HeN mice were immunized either systemically with heat-killed GAS or trough the mucosal route with an M protein-based subunit vaccine, and challenged with live bacteria. Vaccination elicited in both mouse strains similar levels of bactericidal anti-GAS IgG antibodies and also antigen-specific mucosal IgA. Vaccination provided mice of both strains with an increased and equal capacity to express immunity against GAS as indicated by the reduced level of bacteria in the organs and the ability of vaccinated mice to survive infection. Protection in vaccinated mice was dependent of the presence of T cells-dependent bactericidal antibodies as shown by the ability of serum elicited in immunocompetent mice but not of serum elicited in T cells-deficient nu/nu mice to passively transfer anti-GAS immunity. In conclusion, the results presented here demonstrated that the presence of anti-GAS specific, T cells-dependent bactericidal antibodies elicited after vaccination overcomes the innate genetic susceptibility of C3H/HeN mice and makes both resistant and susceptible mice equally capable to control GAS infection.

Key words: Group A streptococci, vaccination, genetic resistance/susceptibility

1. Introduction

Group A streptococci (GAS; *Streptococcus pyogenes*) are common human pathogens capable of causing a variety of diseases, ranging from very mild infections (*e.g.* pharyngitis, tonsillitis or impetigo) to severe diseases associated with high mortality rates (*e.g.* necrotizing fascitiis, streptococcal toxic shock syndrome) [1].

GAS has re-emerged as a public health threat due to the increased incidence of rapidly progressive severe infections observed since the mid-1980s worldwide [2-4]. Current control measures for treatment or prophylaxis for streptococcal diseases rely on chemotherapy [5, 6]. However, 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-GAS vaccine is thus widely regarded as a major priority, but despite intensive research, a safe and effective anti-GAS 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 GAS, several studies have shown that individuals within a single human population vary genetically in their susceptibility to infection with this pathogen [7-10]. Thus, depending of the genetic makeup, different individuals will develop infections of different severities after encounter with the same streptococcal strain.

As a model for human variation, inbred mouse strains have been critical to our understanding of the role of host genetics in the susceptibility to group A streptococcal infection. Thus, strains of mice with various genetic backgrounds have been shown to differ markedly in their susceptibility to GAS [11, 12]. While some strains of mice (*e.g.* BALB/c) are very resistant to this pathogen, able to clear and survive the infection, other strains (*e.g.* C3H/HeN) are much more susceptible, allowing progressive bacterial multiplication, development of sepsis and death [11, 12].

Similarly to GAS infection, it was previously shown that some strains of mice exhibited a higher level of innate genetic resistance than others to infection with *M. tuberculosis* [13]. In that case, it was found that vaccination completely failed to make genetically susceptible mice as capable as genetically resistant mice to control infection with *M. tuberculosis* [14]. Therefore, genetically susceptible mice remained proportionally more susceptible to tuberculosis after vaccination than genetically resistant mice [14].

The question addressed in this study is whether vaccination equally enables both genetically susceptible and resistant mouse strains to control GAS infection or, as it was the case of tuberculosis infection, susceptible mice remain proportionally much more susceptible to infection after vaccination than those exhibiting higher levels of innate resistance.

2. Materials and Methods

2.1. Bacterial strains and growth conditions. *S. pyogenes* strain A20 (M type 23) is a human isolate obtained from the German Culture Collection (DSM 2071). The *S. pyogenes* strain KTL3 (serotype M1) is a blood isolated described previously [15]. Stocks were maintained at -70°C and were routinely cultured at 37°C in Todd-Hewitt broth (Oxoid, Basingstoke, UK), supplemented with 1% yeast extract for approximately 6 h. Bacteria were collected in mid-log-phase, washed twice with sterile PBS, diluted to the required inoculum and the number of viable bacteria determined by counting CFU after diluting and plating in blood agar plates (GIBCO) containing 5% sheep blood.

2.2. Mouse strains. Inbred BALB/c, C3H/HeN and T cells-deficient BALB/c-*nu* female mice were purchased from Harlan-Winkelmann and used in experiments when they were between 8 and 10 weeks of age. Mice were housed in microisolators cage and given food and water ad libitum. All studies were approved by the appropriate ethical authorities.

2.3. Immunization protocols. Mice were immunized intraperitoneally (i.p.) with 10^7 heat-killed GAS (HK-GAS) or intranasally (i.n.) with 20 μg of M1 protein. Animals were boosted with similar dose at day 7 and 14. Serum samples were collected and monitored for antigen-specific IgG antibodies previous to immunization and 2 weeks after the last boost. For lung lavage, mice were sacrificed 2 weeks after last boost and lung washes were collected after per tracheal cannulation, centrifuged at 3,000 x *g* for 5 min to remove debris and stored at –20°C.

2.4. Challenge protocols. For protection studies, immunized and control mice were challenged with virulent GAS two weeks after the last boost. For intravenous challenge, mice were inoculated with 10^5 CFU of GAS in 0.2 ml of sterile PBS via a lateral tail vein. At selected times after infection, viable bacterial counts were determined in blood of infected

mice by collecting blood samples from the tail vein and plating serial dilutions on blood agar. Infected mice were monitored on daily basis and mortality was recorded. For the intranasal challenge, mice were anesthetized and infected through the respiratory route by administering 10^7 CFU of GAS in a volume of 20 µl of sterile PBS. Mice were sacrificed at 48 h postinoculation, their lungs were removed and homogenized in 5 ml of PBS. Organ homogenates were serially diluted in PBS and plated on blood agar plates.

2.5. Antibodies assay. For the whole-cell ELISA, HK-GAS were suspended in buffer containing 0.1 M $NaHCO_3$ (pH 9.6) to a concentration of 10^8/ml and 50 µl was added in triplicate to wells of a 96-well microtiter plate (Nunc). After overnight incubation at 4°C, the plates were washed with PBS+0.05% Tween 20 and blocked with 200 µl/well of 10%FCS in PBS for 1 h at 37°C. Serum samples were diluted in PBS containing 10% of FCS and 100 µl were added per well. The plates were incubated for 2 h at 37°C. After four washes, appropriate dilutions of either biotinylated γ-chain specific goat anti-mouse IgG antibodies (Sigma) were added and the plates were further incubated for 2 h at 37°C. After four washes, 100µl of peroxidase-conjugated streptavidin (Pharmingen) were added to each well and plates were incubated at room temperature (RT) for 1h. After four washes reactions were developed at RT using ABTS [2,2'-Azino-bis-(3-ethybenzthiazoline-6-sulfonic acid)] in 0.1 M citrate-phosphate buffer (pH 4.35) containing 0.01% H_2O_2. Endpoint titer was expressed as the reciprocal Log_2 of the last dilution, which gave an optical density $\geq$0.1 above the values of the negative controls after a 45 min incubation.

Antibody titers in lung washes were determined by an enzyme-linked immunosorbent assay (ELISA). Nunc-Immuno MaxiSorp™ assay plates (Nunc) were coated with 50 µl/well of M1 protein (5 µg/ml in 0.1 M Na_2HPO_4, pH 9.0) overnight at 4°C. After four washes (0.05% Tween 20 in PBS), they were blocked with 200 µl/well of 10%FCS in PBS for 1 h at 37°C. Lung washes were diluted in PBS containing 10% of FCS and 100 µl was added per

well. The plates were incubated for 2 h at 37°C. After four washes, biotinylated α-chain specific goat anti-mouse IgA antibodies (Sigma) were added and the plates were further incubated for 2 h at 37°C. After four washes, 100µl of peroxidase-conjugated streptavidin (Pharmingen) were added to each well and plates were incubated at room temperature (RT) for 1h. After four washes reactions were developed at RT using ABTS [2,2'-Azino-bis-(3-ethybenzthiazoline-6-sulfonic acid)] in 0.1 M citrate-phosphate buffer (pH 4.35) containing 0.01% H_2O_2. The results are expressed as a percentage of the total IgA present in the lung mucosa.

2.6 Preparation of anti-GAS immune serum. Groups of BALB/c and C3H/HeN mice were immunized intraperitoneally with 10^7 heat-killed GAS and boosted with similar dose at day 7 and 14. Serum samples were collected previously and 2 weeks after the last boost and used for the bactericidal assays.

2.7. Bactericidal assays. A 50 µl of a bacterial suspension containing 5×10^3 CFU of GAS was mixed with 100 µl of heat-inactivated either pre-immune or anti-GAS serum and 400 µl of fresh, heparinized blood obtained from non-immune BALB/c or C3H/HeN mice. The blood was rotated for 3 h at 37°C, and viable counts were determined by plating diluted samples onto blood agar. Results are expressed as percentage of killing = [1 – (mean CFU in the presence of anti-GAS serum)/(mean CFU in the presence of pre-immune serum)] × 100.

2.8. Passive transfer of immune serum. To generate polyclonal immune serum, mice were intraperitoneally immunized with either 10^7 HK-GAS microorganisms or PBS as described above. Sera was pooled 10 days after the last boost and used for passive immunization. For passive protection, mice were injected intravenously with 300 µl of anti-GAS polyclonal immune or control serum 1h prior or 24h after bacterial inoculation.

2.9. Construction and purification of recombinant M1 protein. A full-length recombinant M1 protein from GAS strain KTL3 was generated. Restriction and modifying enzymes were purchased from New England Biolabs, PCR reactions were performed using a proof-reading polymerase (GeneCraft) according to the manufacture's instructions. DNA manipulations were performed as described by Sambrook *et al.* [16]. A 1240 bp *BamH*I/*Sal*I fragment encompassing positions 124 to 1363 of the *emm1* sequence was subcloned into pCR2.1 (Invitrogen) as described by the manufacturer, digested with *Sal*I and *Bam*HI, and ligated into vector pQE30 (Qiagen). After transformation of *E. coli*, positive clones were selected and the recombinant His-Tag-protein was overexpressed and purified under native conditions according to Qiagen protocols.

2.10. Statistical Analysis. Comparisons between groups were performed by use of the ANOVA test. Comparison of survival curves was performed by use of the Mann-Whitney (Wilcoxon) W- test. $P < 0.05$ was considered to be statistically significant.

3. Results

3.1. Humoral immune responses elicited in genetically susceptible C3H/HeN and resistant BALB/c mice after systemic vaccination with HK-GAS. It has been suggested that the lack of anti-GAS opsonic antibodies constituted a risk factor towards the development of severe invasive GAS infections [17, 18]. Here, we have here compared the ability of mice previously shown to exhibit a natural resistance to GAS infection with that of mice highly susceptible to this pathogen to generate an anti-GAS systemic and mucosal antibodies after vaccination. To this end, resistant BALB/c and susceptible C3H/HeN mice were immunized i.p. with 10^7 HK-GAS strain A20 microorganisms or PBS, boosted at day 7 and 14, and the elicited anti-GAS IgG antibody response was measured 2 weeks after the last boost. GAS-specific IgG antibodies were determined in serum by a whole cell ELISA. As shown in Fig. 1A, vaccination with HK-GAS elicited comparable high levels of anti-GAS IgG antibodies in both genetically resistant and susceptible mouse strains.

In order to determine whether differences in the genetic background of the mouse strain may influence the bactericidal capacity of the anti-GAS antibodies elicited after vaccination, BALB/c and C3H/HeN mice were vaccinated with HK-GAS strain A20 and the bactericidal capacity of the elicited anti-GAS antibodies was evaluated using the whole blood assay described in the Materials and Methods section. Results in Fig. 1B show that antibodies elicited after vaccination with HK-GAS strain A20 induced more than 90% mean reduction against the homologous strain (A20) in both mouse strains. These results demonstrate that vaccination induced in both, resistant and susceptible mice, anti-GAS bactericidal antibodies.

That bacterial reduction was due to antibody-mediated opsonizing killing and not to bacterial aggregation by specific antibodies was demonstrated by performing a previously described assay [19]. Briefly, 5×10^3 CFU of GAS strain A20 was added to serum isolated from either GAS-vaccinated BALB/c or C3H/HeN mice or non-immune control mice and rotated for 3 h at 37°C. Viable bacteria were determined after incubation by plating serial

dilutions onto blood agar. Similar number of viable bacteria were counted, independently of whether they were incubated with control or anti-GAS serum (data not shown). These results clearly indicate that specific anti-GAS antibodies did not aggregated streptococcal microorganisms in our experimental setting.

3.2. Protective immune response against GAS elicited in genetically resistant and susceptible mice after vaccination. To test whether the immune response elicited after vaccination could make innate susceptible C3H/HeN mice as resistant to GAS as BALB/c mice, animals from both strains were immunized i.p. with 10^7 HK-GAS strain A20 microorganisms and boosted at day 7 and 14. Control animals received PBS. On day 28, mice were intravenously challenged with a lethal dose of live homologous GAS (10^5 CFU) and the survival rates of animals was monitored overtime. As expected, both vaccinated and control resistant BALB/c mice survived GAS infection (Fig. 2A). In contrast, susceptible control C3H/HeN mice underwent a severe infection course, which eventually led to a fatal outcome in all the infected animals by day 3 after bacterial inoculation (Fig. 2A). On the other hand, HK-GAS-immunized susceptible C3H/HeN mice exhibited a level of resistance to GAS similar to that observed in control and vaccinated BALB/c mice (90% survival) (Fig. 2A).

That vaccination makes innate resistant and susceptible mice equally in their capacity to control GAS infection was further demonstrated by the lower bacterial loads in blood of both groups of vaccinated mice at day 2 after bacterial inoculation ($p < 0.05$, ANOVA test) (Fig. 2B).

3.3. Mucosal immune responses elicited in genetically susceptible C3H/HeN and resistant BALB/c mice after intranasal vaccination with a subunit vaccine. Group A streptococcus can very efficiently colonize the mucosal surface of the upper respiratory tract. Therefore, in addition to the systemic immune response, an antigen-specific mucosal immune

responses is also required to achieve full protection against the wide spectrum of streptococcal diseases. This is clearly evidenced by the fact that antigen-specific secretory IgA is required for prevention of pharyngeal GAS infection [20, 21].

It is generally accepted that while systemic vaccination only induced systemic immune responses, vaccination through the mucosal route can induce both mucosal and systemic immune mechanisms [22, 23]. Therefore, we next determine the ability of both resistant BALB/c and susceptible C3H/HeN mice to produce anti-GAS specific IgA mucosal antibodies after intranasal immunization with M1 protein as model of subunit vaccine. The selection of a M1 protein for these experiments is based on the fact that the M1 is one of the most commonly isolated serotype from patients with GAS infections [24, 25]. Mice were immunized i.n. with 20 μg of M1 protein, boosted at day 7 and 14 after the last boost, and the level of anti-M1 IgA antibodies was determined in the lung mucosa at day 28 after the first immunization. Results in Fig. 3A show that mucosal vaccination elicited comparable levels of anti-M1 IgA antibodies in both resistant BALB/c and susceptible C3H/HeN mice.

The ability of anti-M1-specific mucosal antibodies elicited after intranasal vaccination to control bacterial growth after mucosal infection was next evaluated. Vaccinated and control BALB/c and C3H/HeN mice were challenged with GAS strain KTL3 (M-1 serotype) through the respiratory route. Bacterial loads in lungs were determined in the different groups of mice at day 2 after bacterial challenge. Significantly lower amount of bacteria were found in the lungs of vaccinated mice after challenge with virulent GAS of either the resistant or susceptible mouse strains when compared with control mice ($p < 0.05$; ANOVA text) (Fig. 3B).

3.3. Bactericidal anti-GAS antibodies are T cells-dependent. Natural antibodies to toxins, bacteria and viruses are present in the sera of normal, non-immunized humans and mice and they have been shown to play a critical role during infection by enhancing immunogenitiy and

preventing pathogen dissemination to vital organs [26-29]. In order to rule out a role of natural antibodies in the anti-GAS resistance expressed by BALB/c mice, passive-immunization experiments were conducted using BALB/c mice as donors and C3H/HeN mice as recipients. Thus, C3H/HeN mice were injected intravenously with 300 μl of sera from control BALB/c mice followed 1 h later by intravenous challenge with 10^5 CFU of *S. pyogenes* strain A20. C3H/HeN mice receiving the same volume of isogenic serum were used as control. The median survival times for C3H/HeN mice that received the serum from BALB/c mice did not differ significantly than that from the C3H/HeN mice that received isogenic sera ($p = 0.26$; Wilcoxon test) (Fig. 4A). In contrast, C3H/HeNmice receiving serum from immunized BALB/c mice exhibited high levels of protection with 100% of mice surviving infection (Fig. 4A). Thus, resistance of BALB/c mice to GAS seems not to be due to the presence of natural anti-GAS antibodies.

To further determine the nature of the anti-GAS protective antibodies elicited after vaccination, naïve susceptible C3H/HeN mice were passively immunized with serum obtained from either unvaccinated or HK-GAS-immunized, immunocompetent or T cells-deficient BALB/c-*nu* mice and challenged with 10^5 CFU of virulent microorganisms 24 h thereafter. BALB/c-*nu* mice lack functional T cells but they retain an intact B cells population, therefore, they can produce T-cell-independent antibodies but not T cell-dependent antibodies in response to vaccination. Results in Fig. 4B show that neither administration of serum obtained from control or immunized BALB/c-*nu* mice were capable to transfer protection to genetically susceptible C3H/HeN mice, indicating the T-cell dependence of protective anti-GAS antibodies.

We next investigated whether administration of polyclonal immune serum at 24 h postinoculation, a time point when bacteria have disseminated and infection is progressing, could rescue genetically susceptible mice from a fatal outcome. Results in Fig. 4C show that

although administration of immune serum could significantly extend the survival time of infected susceptible mice, it failed to rescue the animals from death ($p < 0.05$; Wilcoxon test).

4. Discussion

Several studies have shown that host genetic factors are critical in determining host susceptibility against GAS infection [7-10]. Thus, while some individuals might respond to GAS infection with a mild disease, the same streptococcal strain can cause very severe invasive infection in more susceptible persons.

Severe invasive infections caused by group A streptococci such as necrotizing fascitis (flesh-eating bacterium) or streptococcal toxic shock syndrome have been reported with increased frequency in recent years [2-4]. The reasons for this resurgence of severe GAS infections remain unclear, however, an increased exposure of susceptible individuals after decreased herd immunity due to infrequent exposure to GAS may have contributed to this remarkable rise of severe cases. In fact, several studies have suggested that low levels of isolate-specific as well as superantigen-neutralizing antibodies in infected individuals may increase the risk to develop invasive GAS disease [17, 18, 30, 31]. It is, therefore, plausible that the presence of a serotype–specific immune response elicited after vaccination might rescue genetically susceptible individuals for developing invasive disease after an encounter with this pathogen.

We have recently shown that the ability of different strains of mice to control infection with *S. pyogenes* was genetically determined [11, 12]. Thus, different strains of mice exhibit differential susceptibility to GAS infection, with some strains (*e.g.* BALB/c) being considerable more resistant than others (*e.g.* C3H/HeN). In the study presented here we have assessed whether vaccination equally enables both genetically susceptible and resistant mouse strains to control GAS infection. Our results show that both genetically resistant and susceptible mouse strains generated an antigen-specific immune response against GAS after mucosal or systemic vaccination that makes both mouse strains equally capable to control GAS 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 GAS.

Administration of polyclonal immune serum to infected C3H/HeN mice at 24h 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-GAS 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 vaccination enables both genetically resistance and susceptible mice with an equal capacity to control GAS infection, clearly indicating that resistance to GAS can largely be achieved in the innately susceptible hosts 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.

5. Acknowledgment

We would like to thank Nina Janze for valuable technical assistance.

This work has been supported in part by the Deutsche Forschungsgemeinschaft grant GU 482/2-3, by the Nationales Genonsforschungnetz-2 grant 01GS0404 and by by “Impuls und Vernetzungsfond”, HGF Präsidentenfonds.

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Figure Legends

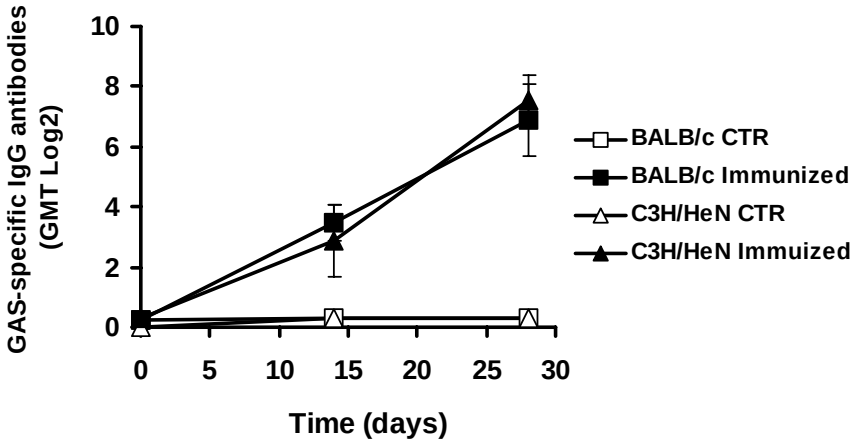
Figure 1. (A) GAS-specific IgG antibodies detected in serum of control (white symbols) and HK-GAS-immunized (black symbols) resistant BALB/c (squares) or susceptible C3H/HeN (triangles) mice. The data shown are the mean ± SD of the reciprocal Log_2 of the geometric mean end-point titer (GMT) (n = 5 mice per group). (B) Bactericidal activity of serum antibodies elicited in resistant BALB/c or susceptible C3H/HeN mice after immunization with HK-GAS strain A20. GAS microorganisms (strain A20) were added to a mix of fresh heparinized blood obtained from non-immune BALB/c or C3H/HeN mice and containing either preimmune or anti-GAS serum elicited after vaccination in the corresponding mouse strain. After rotating for 3 h at 37°C, the CFU were determined after serial plating in blood agar. Results are expressed as percentage of killing = [1 – (mean CFU in the presence of anti-GAS serum)/(mean CFU in the presence of pre-immune serum)] × 100. Each bar represents the mean ± SD of three different measurements.

Figure 2. (A) Survival curves of genetically resistant BALB/c (squares) and susceptible C3H/HeN (triangles) mice after immunization with HK-GAS (black symbols) or control-PBS (white symbols) and challenged intravenously with 10^5 CFU of GAS strain A20. Animals were immunized i.p. with 10^7 HK-GAS microorganisms or PBS, boosted at day 7 and 14, and challenged intravenously with 10^5 CFU of live homologous bacteria 2 weeks after the last boost. Animals were monitored daily for survival during a period of 14 days. (B) Bacterial loads in the blood of genetically resistant BALB/c and susceptible C3H/HeN mice after immunization with HK-GAS (white bars) or control-PBS (black symbols) at day 2 after intravenous challenge with 10^5 CFU of GAS strain A20. Data are mean ± SD of a total of 15 mice/group obtained from 3 independent experiments. *, Significantly different from mice immunized with HK-GAS ($p < 0.05$, ANOVA test).

Figure 3. (A) Anti-M1-specific IgA antibodies present in the lung washes of genetically resistant BALB/c or susceptible C3H/HeN mice after intranasal immunization with either M1 protein (black bars) or control PBS (white bars). Results are expressed as the mean ± SD of the percentage of M1-specific antibodies with respect to the total IgA detected in the lung washes. *, Significantly different from controls ($p < 0.05$, ANOVA test). (B) Bacterial loads in the lungs of genetically resistant BALB/c and susceptible C3H/HeN mice immunized with either M1 protein (black bars) or PBS (white bars) at day 2 after challenge with virulent GAS though the respiratory route. Mice were intranasally immunized with recombinant M1 protein of PBS and challenged with GAS strain KTL3 (serotype M-1). Data are mean ± SD of a total of 15 mice/group. *, Significantly different from mice immunized with M1 protein ($p < 0.05$, ANOVA test).

Figure 4. (A) Survival curves of genetically susceptible C3H/HeN mice after being passively immunized with serum obtained from either control BALB/c mice (circles), control C3H/HeN mice (squares) or HK-GAS-immunized BALB/c mice (diamonds). Naïve C3H/HeN mice were passively given 300 µl of the corresponding serum and infected after 1 h 10^5 CFU of GAS strain A20 i.v. Mortality was recorded overtime. (B) Survival curves of genetically susceptible C3H/HeN mice after being passively immunized with serum obtained from either controls (white symbols) or HK-GAS-immunized (black symbols) immunocompetent BALB/c (diamonds) or T cells-deficient BALB/c-*nu* mice (squares) and intravenously challenged with GAS. (C) Survival plots of genetically susceptible C3H/HeN mice passively immunized with anti-GAS immune serum (squares), non-immune serum (triangles) or control PBS (diamonds) at 24 h after been inoculated i.v. with GAS strain A20. Mice were passively given 300 µl of the corresponding serum and mortality was recorded in daily basis.

A


GAS-specific IgG antibodies (GMT Log2)
10
8
6
4
2
0
0
5
10
15
20
25
30
Time (days)
BALB/c CTR
BALB/c Immunized
C3H/HeN CTR
C3H/HeN Immuized

B

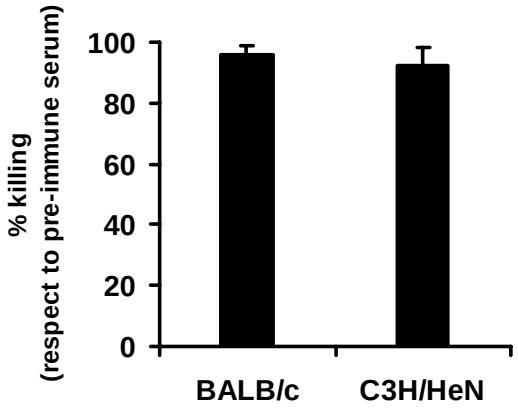
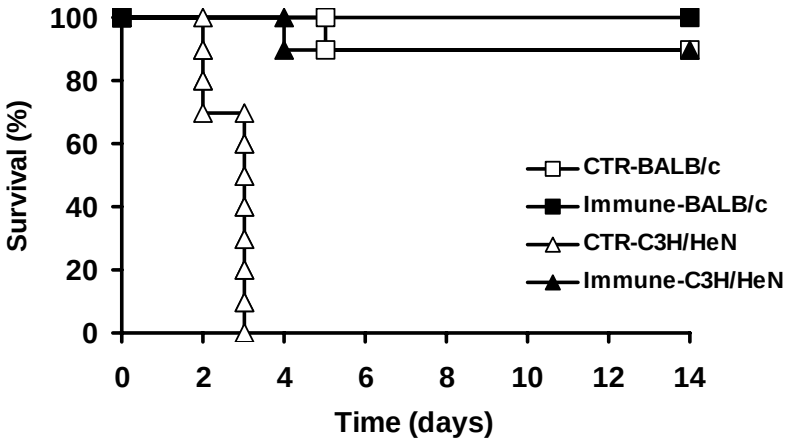

% killing (respect to pre-immune serum)
100
80
60
40
20
0
BALB/c
C3H/HeN

Figure 1

A


100
80
60
40
20
0
Survival (%)
0
2
4
6
8
10
12
14
Time (days)
CTR-BALB/c
Immune-BALB/c
CTR-C3H/HeN
Immune-C3H/HeN

B

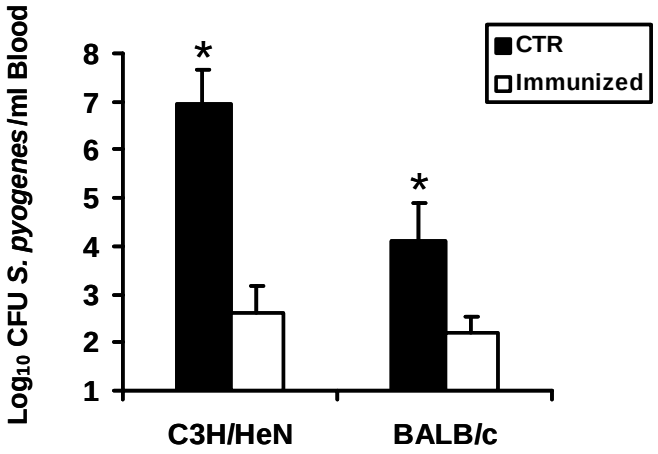
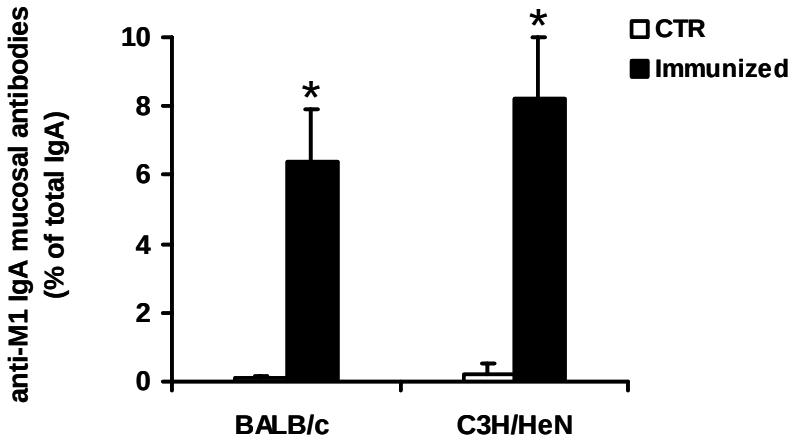

Log10 CFU S. pyogenes/ml Blood
8
7
6
5
4
3
2
1
*
*
CTR
Immunized
C3H/HeN
BALB/c

Figure 2

A


anti-M1 IgA mucosal antibodies
(% of total IgA)
10
8
6
4
2
0
*
*
CTR
Immunized
BALB/c
C3H/HeN

B

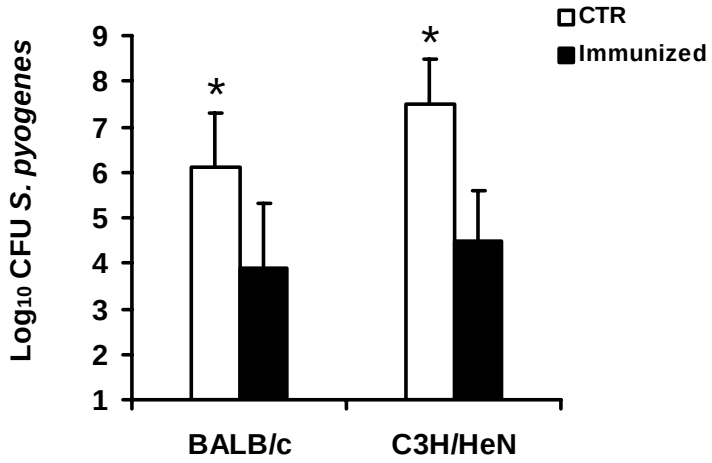
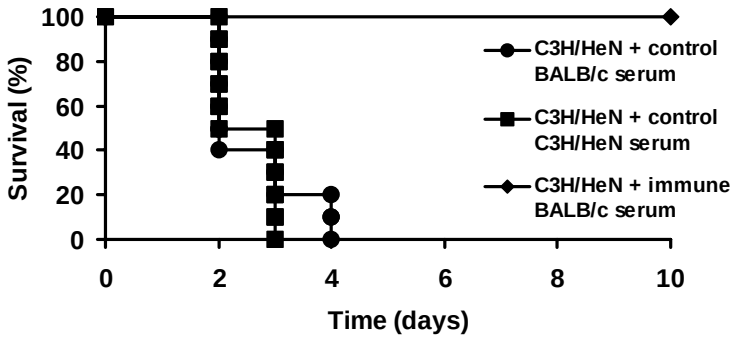
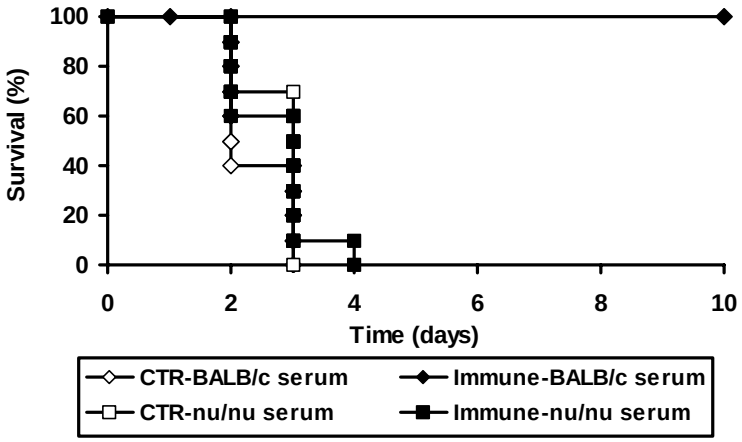

Log10 CFU S. pyogenes
9
8
7
6
5
4
3
2
1
*
*
CTR
Immunized
BALB/c
C3H/HeN

Figure 3

A


100
80
60
40
20
0
Survival (%)
0
2
4
6
8
10
Time (days)
C3H/HeN + control BALB/c serum
C3H/HeN + control C3H/HeN serum
C3H/HeN + immune BALB/c serum

B


100
80
60
40
20
0
Survival (%)
0
2
4
6
8
10
Time (days)
CTR-BALB/c serum
Immune-BALB/c serum
CTR-nu/nu serum
Immune-nu/nu serum

C

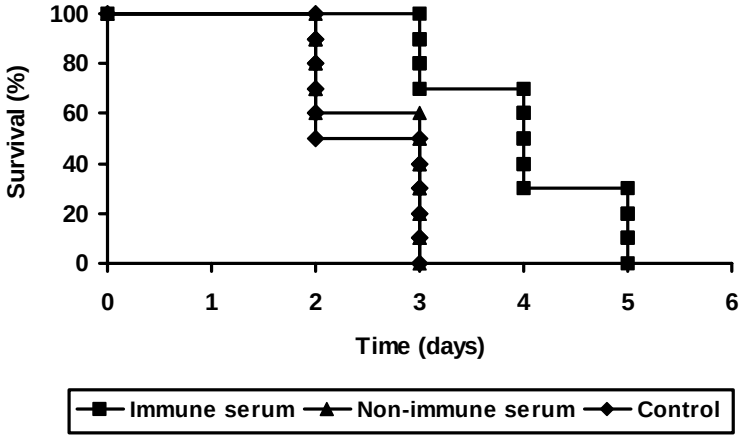

100
80
60
40
20
0
Survival (%)
0
1
2
3
4
5
6
Time (days)
Immune serum
Non-immune serum
Control

Figure 4