Short Communication

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Dynamics of anti-M antibody response in a mouse model following intranasal infection with group A *Streptococcus* M-18

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Dynamics of anti-M antibody response following intranasal infection with group A *Streptococcus* (GAS) M-18 were investigated in a Swiss albino mouse model. Mice arranged in three groups were inoculated intranasally with 2.0×10^7 c.f.u. ml⁻¹ of GAS M-18 on 1, 2 alternate and 3 alternate days. Plasma collected from the retro-orbital plexus was tested for antibodies by an in-house indirect ELISA. The antibody titres of the plasma samples varied from 1 : 8 to 1 : 1024 in the 1 day dose, from 1 : 4 to 1 : 256 in the 2 day dose and from 1 : 4 to 1 : 128 in the 3 day dose. Peak titres were seen on day 42 or 56 and in all cases the titres had declined by day 84. Swiss albino mouse can thus serve as a useful animal model to study different aspects of type-specific anti-M immune responses against GAS disease when designing candidate streptococcal vaccines.

Introduction

Group A streptococci (GAS) are the most common cause of pharyngitis, scarlet fever and impetigo (Wannamaker, 1970). They are also responsible for important complications, namely acute rheumatic fever/rheumatic heart disease, post-streptococcal glomerulonephritis and streptococcal reactive arthritis (Cunningham, 2000). While the primary infections are innocuous and self-limiting, the sequelae often cause severe morbidity and mortality in developing countries (Koshi *et al.*, 1981; Padmavathi, 1995). Streptococcal toxic shock syndrome, bacteraemia and severe invasive group A streptococcal skin and soft tissue infections have been reported in the USA and Europe (Holm, 1996). The global resurgence of serious streptococcal infections (Kaplan, 1991) and the continued endemicity of fatal post-streptococcal sequelae in developing countries (Padmavathi, 2001) have resulted in renewed interest in GAS, especially in developing vaccines against them (Cunningham, 2000).

Most recent vaccines have targeted either the type-specific N-terminal region of the M protein or the highly conserved C-terminal region of the M protein (Kotloff *et al.*, 2004; Dale *et al.*, 1996; Cunningham, 2000). Studies on mice have shown that vaccination with the N-terminal type-specific region results in protective bactericidal and opsonic antibodies, while vaccination with the C-terminal region protects against colonization with multiple serotypes (Hall *et al.*, 2004; Dale *et al.*, 1999; Medaglini *et al.*, 1995). Animal models have also

been used to identify peptides of streptococcal M proteins that elicit rheumatoid lesions (Huber & Cunningham, 1996). Thus development of a suitable animal model will be of great value when standardizing candidate streptococcal vaccines and before subjecting them to volunteer studies.

Our earlier studies with GAS M-18 and 49 (Jebamani *et al.*, 1998, 1999) showed that Swiss albino mice could serve as a suitable animal model to study the events following experimental infection with throat and skin-associated GAS strains. In the present study, we used this mouse model to study the development and dynamics of type-specific antibodies to GAS M-18. This serotype was chosen because it is a well-recognized rheumatogenic strain that has attracted much attention during the last two decades due to its propensity to cause outbreaks of rheumatic fever (Veasy *et al.*, 2004; Smoot *et al.*, 2002).

Methods

Mice arranged in three groups (groups A, B and C) of 10 were inoculated intranasally with 2.0×10^7 c.f.u. ml⁻¹ of GAS M-18 on 1, 2 or 3 days. Blood samples were collected on days 14, 28, 42, 56, 70 and 84 post-infection for the determination of anti-M antibodies, as described elsewhere (Jebamani *et al.*, 1998, 1999; Babu *et al.*, 1992).

Purified M-18 protein antigen for coating the wells for the ELISA test was prepared according to the CDC procedure (Harrell *et al.*, 1982). The optimum concentration of the M-18 antigen used for coating the microtitre wells in the ELISA test was determined by a checkerboard titration.

Mouse plasma for a positive control was produced by intramuscular

Abbreviation: GAS, group A streptococci.

inoculation with pure M-18 antigen without adjuvant. Samples were pooled and used as a single positive plasma control. Negative controls were prepared by pooling plasma from non-immunized mice.

For coating the wells, 50 µl purified M-18 protein antigen (500 ng ml⁻¹) was placed in microtitre plate wells and incubated overnight at 4 °C. One hundred microlitres of the test plasma sample was added to the wells and incubated at room temperature for 30 min. The wells were then washed, and enzyme conjugate (horse-radish peroxidase conjugated with anti-mouse IgG) was added and incubated at room temperature for 30 min. After washing and incubation with substrate containing the colouring agent (tetramethyl benzidine) for 30 min at room temperature, the reaction was stopped with 1 N H₂SO₄ and the plates were read in a BIOTEK ELISA reader at 450 nm. Each run of the test included positive and negative controls.

The cut-off point to interpret the end point in the ELISA test was calculated using plasma collected from mice prior to infection with GAS M-18. Mean OD₄₅₀ values were calculated for each sample tested on three different occasions in triplicate, and the standard deviation (SD) was calculated from these values. OD₄₅₀ values greater than the mean plus 2 SD were taken as positive for anti-M-18 antibodies.

Results and Discussion

The optimum antigen concentration for coating the wells was 500 ng ml⁻¹ by the checkerboard titration. The cut-off point was calculated as 0.524 and any OD₄₅₀ value above this was taken as positive for the presence of anti-M-18 antibodies.

Antibody responses of mice to M-18 antigen following intranasal injections with 1 day (Fig. 1a), 2 day (Fig. 1b) and 3 day (Fig. 1c) doses showed that the titres varied from 1 : 8 to 1 : 1024 in the 1 day dose, from 1 : 4 to 1 : 256 in the 2 day dose and from 1 : 4 to 1 : 128 in the 3 day dose. Peak titres varied from 42 to 70 days and in all cases the titres had declined by day 84. This shows that Swiss albino mice elicit a good immune response against M protein antigens following intranasal infection.

The dynamics of the antibody response to M proteins are very complex and varied (Cunningham, 2000). Protection against GAS infections is directly related to the presence of opsonizing antibodies against type-specific M proteins. Such antibodies are necessary for the elimination of GAS and are directed against epitopes in the amino-terminal region of the M protein molecule (Jones & Fischetti, 1988). Antibodies to other parts of the M protein molecule are not opsonic and are therefore not protective (Cunningham, 2000). Studies in mice have shown that mucosal immunity plays a very important role in preventing pharyngeal colonization by GAS (Bessen & Fischetti, 1990). Several studies have shown that IgA-specific mucosal immunity in mice prevents pharyngeal colonization while the opsonic IgG-specific systemic response facilitates clearance of GAS from the host (Cunningham, 2000; Bessen & Fischetti, 1990).

Our study documents the onset of the anti-M-18 antibody response, characterized by development and decline of titres, in Swiss albino mice following intranasal challenge with GAS M-18, over varying periods of time. Variations were observed in antibody titres in different mice, which are probably

Fig. 1. Anti-M-18 antibody response following intranasal inoculation of group A *Streptococcus* M-18.

related to the randomly bred nature of the mice. This also reflects the individual variations in immune responses generally seen in human hosts following infection by GAS. Infection with 2.0×10^7 c.f.u. for 1 day showed a higher magnitude of immune response, while 2 day/3 day doses showed lower immune responses. While it is difficult to explain these differences, they indicate that one episode of streptococcal pharyngitis would be sufficient to elicit specific protective immunity in the host.

The mouse model has been extensively used in the recent past for testing the efficacy of a variety of candidate vaccines against GAS infections (Cunningham, 2000). Intranasal immunization of mice with a recombinant GAS M5 protein fragment protected them against subsequent intraperitoneal challenge with a homologous type (Bessen & Fischetti, 1990). The *emm-5* gene introduced into *Salmonella enterica* serovar *typhimurium* was used for effective oral immunization in BALB/c mice (Pozzi *et al.*, 1992). These animals were protected against M5, but not against heterologous types. Studies have also shown that type-specific IgA delays mortality and decreases mortality rates in mice infected with M6 organisms (Fischetti & Bessen, 1998). Vaccination of mice with conserved regions of M protein peptides conjugated to the cholera toxin B subunit resulted in protection

from oral colonization (Bessen & Fischetti, 1990). The expression of the carboxy-region of the M protein in *Streptococcus gordonii* has been tried as a new vaccine to prevent pharyngeal colonization of GAS M type 6 in mice (Pozzi *et al.*, 1992). This elicited in mice both IgA and IgG responses, which were able to substantially prevent colonization by 78 homologous and heterologous types of GAS. Intranasal immunization of mice with a truncated form of the enzyme C5a peptidase not only resulted in the production of measurable salivary antibody levels but also eliminated streptococci from the pharynx more rapidly (Ji *et al.*, 1997). Recently, Hall *et al.* (2004) have shown the usefulness of the mouse model for the intranasal delivery of adjuvanted multivalent M protein vaccines and subsequent development of protective antibody responses. These studies clearly show the usefulness of mice as convenient laboratory tools to evaluate the protective and type-specific immune response against M proteins. This model will therefore be very valuable to study the potency, safety and protective efficacy of various vaccines based on M proteins before human trials can be initiated.

In conclusion, this study describes an in-house ELISA test to study the dynamics of anti-M antibody responses in mice infected with GAS M-18. Using this system, we have shown that intranasal challenge of Swiss albino mice with M-18 can elicit a good type-specific antibody response to a single challenge with 2.0×10^7 c.f.u. ml⁻¹. Our results also show that Swiss albino mice can serve as a useful animal model to study the different aspects of type-specific immunity to GAS infection. With the reported similarity between mouse and human genomes (Bradley, 2002), this model will definitely serve as one that is scientifically better than other animal models to study the diverse aspects of the pathogenesis of GAS disease, especially rheumatic fever/rheumatic heart disease. Further, knowledge of the dynamics of the anti-M immune response will also help us to modulate immune responses when designing experiments to develop possible experimental cardiac lesions.

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