Intranasal Vaccination with Streptococcal Fibronectin Binding Protein Sfb1 Fails To Prevent Growth and Dissemination of *Streptococcus pyogenes* in a Murine Skin Infection Model

J. McArthur,1,2 E. Medina,3 A. Mueller,3 J. Chin,4 B. J. Currie,5,6 K. S. Sriprakash,1,5,6 S. R. Talay,3 G. S. Chhatwal,3 and M. J. Walker2*

Bacterial Pathogenesis Laboratory, Queensland Institute of Medical Research, Brisbane,1 NSW Agriculture, Elizabeth Macarthur Agricultural Institute, Camden,4 and Menzies School of Health Research, Charles Darwin University,5 and Co-operative Research Centre for Aboriginal and Tropical Health,6 Darwin, and Department of Biological Sciences, University of Wollongong, Wollongong,2 Australia, and GBF National Research Center for Biotechnology, Braunschweig, Germany6

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Fibronectin binding protein F1 (Sfb1) of *Streptococcus pyogenes* (group A streptococcus [GAS]) is a well-characterized adhesin that has been shown to induce protection in mice against a lethal intranasal GAS challenge after intranasal immunization with cholera toxin B subunit (CTB) as adjuvant. With a murine skin infection model, we have shown that Sfb1/CTB vaccination neither elicits opsonizing antibodies nor prevents systemic bacterial growth and dissemination to internal organs after a subcutaneous GAS challenge. These results indicate that an Sfb1-based vaccine should be complemented with additional protective antigens in order to be used in areas such as the tropical north of Australia, where the skin is the primary route of entry for invasive streptococcal diseases.

Among the aboriginal population living in the Northern Territory of Australia (NT), the incidence and prevalence of streptococcal infection and streptococcal diseases are high. Unlike in Europe and the United States, where the throat is often the primary tissue reservoir, the skin is the major site of infection for the aboriginal population (12). Rates of group A streptococcal (GAS) skin infection are extremely high, owing in part to infection of scabies lesions, with pyoderma prevalence rates in children of up to 70% (13). The rate of streptococcal invasive diseases among the aboriginal population is five times that of the general population, with skin infections underlying most cases (5). The incidence of poststreptococcal sequelae is also high, with rates of acute rheumatic fever in aboriginal populations among the highest reported and acute glomerulonephritis being endemic in many regions (4). Genetic typing of strains causing GAS infection in aboriginal communities has demonstrated that the diversity and turnover rate of these strains are much higher than those reported in other regions and has revealed no evidence of a dominant clone that has been a common cause of GAS invasive infections elsewhere in the world (3, 6). Vaccination might constitute the most suitable strategy to control GAS infections in these communities.

Bacterial adhesins have been proposed as potential vaccine targets for the prevention of infectious diseases (36). In this regard, *Streptococcus pyogenes* produces a number of MSCRAMMs (microbial surface components recognizing adhesive matrix molecules) that are believed to mediate adhesion of the pathogen to host tissue, a critical step in the initial stages of infection (27). A number of GAS MSCRAMMs capable of binding fibronectin have been identified, and they include protein F1/Sfb1 (16, 31), 28-kDa antigen (9), FBP54 (10), serum opacity factor (21, 28), protein F2 (18), PFBP (29), FbaA (33), FbaB (34), and SfbX (19). This diversity of fibronectin binding proteins suggests both the importance of fibronectin binding in the pathogenesis of GAS infection and the possibility that these proteins are differentially expressed at different stages of the infection process (17).

GAS fibronectin binding proteins have been suggested as potential vaccine targets for preventing GAS infections (7). Antibodies directed against such adhesins may prevent bacterial attachment and inhibit colonization (36). Sfb1 is a well-characterized fibronectin binding protein of GAS and is believed to mediate bacterial attachment to host cells and internalization of GAS into nonphagocytic cells (26, 31). Sfb1 has also been shown to interfere with host macrophage-mediated clearance mechanisms by binding to the Fc fragment of human immunoglobulins (24). When adjuvanted with cholera toxin B subunit (CTB), intranasal immunization with Sfb1 induces protection against an intranasal challenge with a lethal dose of GAS (15). Vaccinated mice produce a strong immunoglobulin G (IgG) serum antibody response in a Th2-like pattern (30). However, critical to this level of protection is the elicitation of mucosal immunity in the lungs of vaccinated mice. It is believed that this mucosal immune response may prevent S. *pyogenes* from binding to the upper respiratory epithelium, thereby preventing colonization and establishment of infection (36). Other characteristics that make Sfb1 an attractive vaccine candidate are the presence of highly conserved epitopes and the fact that Sfb1 is expressed on the surface of 70% of clinical GAS isolates belonging to different serotypes and strains, independent of geographic origin (14, 32, 35). Anti-Sfb1 antibodies do not cross-react with heart proteins and therefore may not trigger autoimmune reactions that might be
responsible for poststreptococcal sequelae (35). Although vacci-
nation with Sfb1 conferred protection against mucosal infec-
tion with \textit{S. pyogenes}, the question remains whether it is also
protective against systemic spread subsequent to skin infec-
tions. The objective of this study was therefore to determine
whether the immune response generated against Sfb1 was able
to confer protection against a subcutaneous challenge with \textit{S.
pyogenes}.

BALB/c mice were immunized by intranasal inoculation (10
\mu l/nare) with a mixture containing 30 \mu g of Sfb1 and 10 \mu g of
CTB on days 1, 3, 5, and 15 as previously described (15). For
characterization of the immune response, serum samples, lung
washes, and spleen samples were collected 14 days after the
last booster immunization. Lung washes were obtained by tra-
cheal cannulation and gentle washing with 0.7 ml of cold phos-
phate-buffered saline (PBS) containing 2 mM phenylmethyl-
sulfonyl fluoride. To assess the generation of antigen-specific
effector cells by vaccination, lymphocytes were isolated from
the spleens of immunized mice and restimulated in vitro for 3
days in the presence of Sfb1 as previously described (25).
Results in Fig. 1A show that intranasal immunization with Sfb1
elicited a significant serum IgG antibody response to the vac-
cine antigen compared to that of nonvaccinated control mice
\((P = 0.029)\). As shown in Fig. 1B, immunization with Sfb1 also
elicted elevated antigen-specific IgA and IgG antibody re-
sponses in the lung on day 14 postvaccination. Spleen cells
isolated from mice vaccinated with Sfb1, compared with con-
trols, displayed elevated proliferative responses on day 14 post-
vaccination (Fig. 1C).

A previously described mouse skin infection model (23) was
then used to examine the capacity of the immune response
generated after vaccination with Sfb1 to restrain bacterial dis-
semination from the local infection foci at the skin. For this
purpose, groups of Sfb1-vaccinated and nonvaccinated control
mice were challenged with a subcutaneous injection containing
of \textit{S. pyogenes} strain A20 (M type 23) (22) obtained from the
German Culture Collection (DSM 2071) at 10^6 CFU/100 \mu l
administered into the back on day 14 postvaccination. At var-
ious times after infection, mice were sacrificed by CO2 asphyx-
iation, their livers and spleens were removed, and bacterial
loads were determined in homogenates of these organs after
serial dilutions were plated in blood agar.

The results presented in Fig. 2 show that both vaccinated
and nonvaccinated mice had comparable rates of systemic bac-
terial growth and dissemination. Vaccination with Sfb1 had no
effect on the growth of \textit{S. pyogenes} in the blood of challenged
mice (Fig. 2A) and also failed to limit bacterial dissemination
and growth in both the spleen (Fig. 2B) and the liver (Fig. 2C)
in infected animals. By contrast, GAS extracts containing other
antigens were found to protect against systemic infection in
this animal model (E. Medina, personal communication), thus
validating this model system for such vaccination studies.

These results suggest that serum antibodies generated
against Sfb1 might be devoid of bactericidal activity against \textit{S.
pyogenes}. To further demonstrate this assumption, a bacteri-
cidal assay was performed with serum from mice vaccinated
with either PBS, CTB, or CTB/Sfb1. Serum samples obtained
from mice immunized with pepsin-extracted M protein
(PepM) were used as a positive control for the opsonizing
assay. Serum samples were mixed with an inoculum of \textit{S.
pyo-
mined by plating dilutions on blood agar plates. As shown in
Fig. 3, serum samples from mice vaccinated with PBS, CTB, or
CTB/Sfb1 failed to inhibit bacterial growth while serum sam-
ples from mice immunized with pepsin-extracted M protein
from DSM2071 (PepM) was used as a positive control for
the assay. The opsonic effect of rabbit polyclonal anti-Sfb1
serum was also assessed.

As GAS produces a number of MSCRAMMs capable of bind-
ing to a variety of extracellular matrix components, includ-
ing fibronectin, it is likely that the expression of these proteins
is regulated in a coordinated manner during the infection process. It
is not known if Sfb1 is expressed during colonization or inva-
sion through the skin. Protective immunity against GAS infection
is believed to be via two major mechanisms (11). (i) Bacteria can
be prevented from entering the host by blocking attachment and
colonization at mucosal surfaces, and (ii) once GAS has entered
host tissues, infection can be eliminated by opsonization with
specific antibody and complement, followed by phagocytosis. It
has been reported that immune responses against peptides based
on the conserved region of streptococcal M protein confer pro-
tective mucosal immunity against colonization but do not reduce
the rate of mortality due to systemic streptococcal infection and
are also nonopsonic (2). Similarly, while an IgA response against
Sfb1 will protect mice from an intranasal GAS challenge, this
study for the first time has shown that serum IgG against Sfb1 is
not opsonic and does not reduce the growth or dissemination of
GAS in a murine skin infection model. Other GAS fibronectin
binding MSCRAMMs, SOF and FBP54, have been shown to
elicit opsonizing antibodies and protect against a systemic intra-
peritoneal GAS challenge (8, 20). This suggests that GAS fi-
bronectin binding MSCRAMMs, and other potential GAS vac-
cine candidates, should be assessed with both intranasal and
systemic GAS infection models. Furthermore, it remains to be
determined whether the apparent lack of efficacy against a skin
challenge found in this study is also seen for other vaccine can-
didates that have looked promising in oral and nasal challenge
studies (1).

Despite the presence of high anti-Sfb1 titers in the serum of
vaccinated mice, GAS strain A20 was still able to grow and
disseminate at a rate comparable to that in naive mice. In the
NT, the most common focus of invasive infection is the skin
(5). In this population, high serum anti-Sfb1 titers are also seen
in both aboriginal controls and aboriginal patients with defined
streptococcal infections (14), and yet this immune response does not offer protection against systemic GAS infection. Together these observations suggest that high anti-Sfb1 titers in serum do not prevent dissemination of GAS into deeper tissues from the skin. The results of this study appear to reflect epidemiological observations in the NT, where skin infection predisposes to severe GAS infection despite high IgG antibody titers against Sfb1 in the population (14).

In summary, while an anti-Sfb1 immune response protects against pharyngeal colonization (15, 30), this response is inadequate for protection against systemic infections as a consequence of skin colonization. Thus, while Sfb1 may be a useful vaccine candidate in regions where the throat is the primary site of infection, this antigen may not fulfill such a role in regions where the skin is the primary infection site.

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