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NAD-Glycohydrolase Production and *speA* and *speC* Distribution in Group A Streptococcus (GAS) Isolates Do Not Correlate with Severe GAS Diseases in the Australian Population

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Streptococcus pyogenes **isolates from a tropical region and a subtropical region of Australia with high and low incidences of severe streptococcal diseases, respectively, were analyzed for *speA*, *speB*, and *speC* gene distributions and NAD-glycohydrolase expression. No direct correlation of these characteristics with a propensity to cause invasive diseases was observed.**

Group A streptococcus (GAS; *Streptococcus pyogenes*) is generally considered a mucosal or skin pathogen responsible for pharyngitis and impetigo. However, the organism can also cause severe invasive diseases such as necrotizing fasciitis, toxic shock syndrome, and cellulitis; and the incidences of these diseases have increased in developed nations (3, 14). Although the clonal spread of some strains has been noted during some invasive disease outbreaks (8), this is not always observed (5). *S. pyogenes* expresses several excretory proteins which may have a role in virulence and pathogenesis. Whereas genes for many of these virulence factors are present in all GAS strains, other virulence genes have restricted distributions (2, 18, 21). The correlation between the distribution of *speA* (the gene for erythrogenic toxin A) or its expression and the course of streptococcal disease has been investigated and has yielded conflicting results (7, 9, 13, 17, 18, 21). The level of expression of extracellular cysteine protease (SpeB) was reported to be low in GAS isolates from patients with severe disease in Canada (15); however, no such correlation was found in a study performed elsewhere (17).

All GAS strains carry the gene encoding NAD-glycohydrolase (NADase). However, only some strains express this enzyme (1, 20). Ninety-two percent of GAS strains isolated from patients involved in an outbreak of GAS infection in Texas which caused invasive diseases and death produced NADase (1). In addition, serotype M1 isolates obtained prior to 1988, i.e., prior to the resurgence of invasive GAS diseases, are generally negative for NADase production; but M1 isolates obtained after 1988 are generally positive for this phenotype (20). These observations suggest a role for NADase in the pathogenesis of invasive GAS infections.

The Northern Territory (NT) of Australia has the greatest diversity of GAS strains per capita reported in the world (11, 12). In this region the epidemiology of GAS does not appear to follow the epidemiological patterns found elsewhere (4, 5). The rate of streptococcal invasive diseases among the indigenous population is five times that among the general population, and no single *S. pyogenes* clone has been shown to be dominant (5). We now report on the distribution of the genes for three erythrogenic toxins (*speA*, *speB*, and *speC*) and NADase activity in GAS strains from the NT and from southwestern Sydney (SWS), a subtropical region. For this study we selected distinct molecular types of GAS from the two regions to avoid potential representation of multiple clonal isolates in the analysis. The isolates were genotyped (*vir* typed) as described by Gardiner et al. (10). Thirty-seven *vir* types were detected among GAS strains isolated from cultures of blood from patients admitted to Royal Darwin Hospital, a major hospital in the tropical top end of the NT, from 1990 to 1997 for bacteremia ($n = 35$) and necrotizing fasciitis ($n = 2$). Seventy-three percent of the patients with invasive disease were of Aboriginal descent. Thirty-eight *vir* types were detected among GAS strains isolated from patients with uncomplicated infections in the same geographic area during the same period. Thirty-nine *vir* types were found among isolates causing invasive and uncomplicated infections in SWS between 1996 and 1999, with six *vir* types being commonly detected in patients with both types of infections. These six *vir* types were proportionately distributed between patients with the two types of infections. Thus, for this study, 26 distinct *vir* types were obtained from patients with invasive cases of disease and 13 distinct *vir* types were obtained from patients with noninvasive cases of disease. Thus, the 114 genotypes analyzed in this study comprised 63 *vir* types from patients with invasive cases of disease (necrotizing fasciitis, toxic shock syndrome, cellulitis, bacteremia) and 51 *vir* types from patients with uncomplicated infections from the NT and SWS. No dominant GAS clone was identified among patients with invasive cases of disease in either region (5; M. Maley et al., unpublished data). Every isolate within each region had a distinct genotype, as determined by *vir* typing. The genotypes of the isolates recovered from patients in the NT represented nearly three-quarters of the circulating *vir* types identified in the NT, and the genotypes of the isolates recovered from patients in SWS represented 100% of the *vir* types identified in that region from 1996 to 1999. Apart from selecting for a unique genotype as deter-

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TABLE 1. Relative distributions of the genes for streptococcal pyrogenic exotoxins A and C and ability to express NADase among strains isolated from patients with invasive and non invasive disease from the NT and SWS regions of Australia

Characteristic	No. (%) of isolates from NT that were positive		*P* value for NT isolates	No. (%) of isolates from SWS that were positive		*P* value for SWS isolates
	Invasive (n = 37)	Noninvasive (n = 38)		Invasive (n = 26)	Noninvasive (n = 13)	
speA	3 (8)	2 (5)	0.67	5 (19)	1 (8)	0.64
speC	7 (19)	11 (29)	0.31	13 (50)	7 (54)	0.82
NADase	15 (41)	19 (50)	0.41	19 (73)	11 (85)	0.69

mined by *vir* typing, no other means of selection was applied to either of the sets of isolates.

The GAS isolates were grown in Todd-Hewitt broth supplemented with 0.02% yeast extract and 20 mM glycine at 37°C, and DNA was extracted as described previously (6, 12). Screening for *speA*, *speB*, and *speC* was carried out by PCR as described previously (7, 19). The isolates of all *vir* types possessed the *speB* gene, confirming the results of studies with strains from other geographic areas (22). The distributions of *speA* or *speC* among the isolates from patients with invasive and noninvasive cases of disease in the NT and in the SWS region were not significantly different (Table 1). Thus, it appears that the presence of *speA* or *speC* does not correlate with severe invasive diseases in the two areas of Australia where isolates for the present study were recovered, one with tropical climatic conditions and the other with subtropical climatic conditions. Large numbers of distinct GAS strains are circulating in both regions, and the populations of these two areas have disparately different life-styles and socioeconomic conditions.

To measure NADase activity in culture supernatants, overnight cultures of GAS were clarified by centrifugation at 2,000 $\times$ *g* for 10 min. The assay mixture contained 25 μl each of 100 mM potassium phosphate buffer (pH 7.3) with 0.1% bovine serum albumin (Sigma), GAS culture supernatant, and NAD (1.8 mg/ml; Sigma). The mixture was poured into the wells of 96-well plates. After incubation at 37°C in 5% CO_2 for 20 min, 50 μl of 5 M NaOH was added to each well and the plates were incubated in the dark for 1 h at room temperature. Fluorescence was measured (excitation, 360 nm; emission, 508 nm) in a microplate fluorescence reader (FL600; BioTek). The negative control contained 25 μl of growth medium in place of culture supernatant in the assay mixture. All samples were assayed in duplicate. A standard curve for the NADase assay was established with commercial NADase (Sigma). NADase-positive strains decreased the amount of NAD in the assay mixture by greater than 30% and were easily distinguished from the negative strains by this assay. Our results, which demonstrated a lack of association between NADase activity and a propensity to cause invasive diseases (Table 1), are in contrast to the results of an earlier study (1). It is possible that host factors and clonal spread (or the lack of it) could explain the contrasting results, but a lack of typing information in the earlier study (1) prevents an appraisal.

Interestingly, overall a significantly greater proportion of the SWS strains than the NT strains were found to harbor the *speC* gene (51 versus 24%, respectively; $P = 0.0034$) or express NADase (77 versus 45%, respectively; $P = 0.0013$), and the results appear to show an inverse correlation between these characteristics and the incidence of severe diseases, as the overall crude incidence of severe GAS-associated diseases in the NT (9.3 per 100,000 population per year [5]) is higher than that in SWS (1.45 per 100,000 population per year [Maley et al., unpublished]). Cytolysin-mediated delivery of NADase into host cells (16), the ability of NADase to induce cellular changes through cyclic ADP-ribose synthesis, and other recent studies (1, 20) implicitly attribute a role for this enzyme in the pathogenesis of invasive diseases. However, the work described here does not lend support to such a simple interpretation. Contributions of host and other bacterial factors may be important in influencing the disease outcomes.

In summary, while we have not observed a correlation between the distribution of *speA* or *speC* or NADase expression and GAS-associated invasive diseases in two locations in Australia, the significantly higher proportions of *speC*- and NADase-positive strains from SWS (where there is a relatively low incidence of GAS-associated invasive diseases) than among strains from the NT (where there is a relatively high incidence of GAS-associated invasive diseases) remain unexplained.

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