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

Armando DelVecchio,1 Michael Maley,2 Bart J. Currie,1,3 and K. S. Sriprakash1,3,4,∗

Menzies School of Health Research1 and Co-operative Research Centre for Aboriginal and Tropical Health,3 Darwin, Queensland Institute of Medical Research, Brisbane,4 and South Western Area Pathology Services, Liverpool, Sydney,2 Australia

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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 extracellular 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 types) 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-
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 × 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% CO2 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 fluorescence reader (FL600; BioTek). The negative control contained 25 μl of growth medium in place of the 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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REFERENCES

10. Gardiner, D., J. Hartas, B. Currie, J. D. Mathews, D. J. Kemp, and K. S.
AUTHORS’ CORRECTIONS

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Menzies School of Health Research and Co-operative Research Centre for Aboriginal and Tropical Health, Darwin, Queensland Institute of Medical Research, Brisbane, and South Western Area Pathology Services, Liverpool, Sydney, Australia

Volume 40, no. 7, p. 2642–2644, 2002. Page 2643, column 1: The first sentence of the second full paragraph should be deleted and replaced with the following text. “The NADase activity in culture supernatants was measured as described by Lutticken et al. (15a), with a few modifications (see below). Overnight cultures of GAS were clarified by centrifugation at 2,000 × g for 10 min.”

Page 2644: The following reference was inadvertently omitted.


Detection of Smallpox Virus DNA by LightCycler PCR

Mark J. Espy, Franklin R. Cockerill III, Richard F. Meyer, Michael D. Bowen, Gregory A. Poland, Ted L. Hadfield, and Thomas F. Smith

Division of Clinical Microbiology, Division of Infectious Diseases, and Division of General Internal Medicine, Mayo Clinic and Foundation, Rochester, Minnesota; Centers for Disease Control and Prevention, Atlanta, Georgia; and Armed Forces Institute of Pathology, Walter Reed Army Medical Center, Washington, D.C.

Volume 40, no. 6, p. 1985–1988, 2002. Page 1985: The nucleic acid sequences of the primer that was used to amplify the smallpox virus hemagglutinin gene target (GenBank accession no. M14783) and of the probe that was used in the assay are as follows: for the primer, 5′-CTA ATA TCA TTA GTA TAC GCT ACA C-3′ (sense) and 5′-GAG TCG TAA GAT ATT TTA TCC-3′ (antisense), and for the probe, 5′-AAT GAT TAT GTC GTT ATG AGT GCT TG-fluorescein-3′ and 5′-RED 640-TAT AAG GAG CCC AAT TCC ATT ATT CT-PHOS-3′.