INFECTION AND IMMUNITY, Aug. 1993, p. 3369–3374
0019-9567/93/083369-06$02.00/0

Vol. 61, No. 8

Association of Phenotypic and Genotypic Characteristics of Invasive *Streptococcus pyogenes* Isolates with Clinical Components of Streptococcal Toxic Shock Syndrome

DEBORAH F. TALKINGTON,[1]* BENJAMIN SCHWARTZ,[1] CAROLYN M. BLACK,[1] JAMES K. TODD,[2] JOHN ELLIOTT,[1] ROBERT F. BREIMAN,[1] AND RICHARD R. FACKLAM[1]

Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333,[1] and Department of Pediatrics, University of Colorado School of Medicine, and The Children's Hospital of Denver, Denver, Colorado 80218[2]

Received 31 December 1992/Accepted 6 May 1993

Sixty-two invasive *Streptococcus pyogenes* strains, including 32 strains isolated from patients with streptococcal toxic shock syndrome (STSS), were analyzed for the following phenotypic and genotypic characteristics: M-protein type, serum opacity factor production, protease production, the presence of streptococcal pyrogenic exotoxin (Spe) genes *A*, *B*, and *C*, and in vitro production of SpeA and SpeB. These characteristics were analyzed for possible associations with each other as well as with clinical components of STSS. M-type 1, the most commonly isolated M-type, was significantly associated with protease production. Protease activity was significantly associated with the clinical sign of soft tissue necrosis. M-type 1 and 3 strains from STSS patients were significantly associated with the clinical signs of shock and organ involvement as well as with SpeA production in vitro. Finally, the production of SpeA was significantly associated with the clinical component of shock and organ involvement as well as with rash. These data suggest that STSS does not make up a single syndrome but, rather, that the multiple STSS clinical criteria probably reflect different phenotypic characteristics of individual *S. pyogenes* isolates.

In the 19th century, invasive *Streptococcus pyogenes* infections were a major cause of morbidity and mortality. However, during this century the incidence of severe group A streptococcal infections has declined, especially since the advent of antibiotic therapy. In the mid-1980s, there was a worldwide increase in group A streptococcal sepsis, clusters of rheumatic fever were reported from locations within the United States (3, 4, 7, 11, 34–36), and a streptococcal toxic shock syndrome (STSS) emerged (2, 10, 29). STSS is a rapidly progressing multiorgan illness, characterized by intravascular collapse and shock, with a reported mortality rate of 30% (29). Because of the similarities between clinical features of STSS and those of toxic shock syndrome caused by toxins of *Staphylococcus aureus*, it has been hypothesized that STSS may be caused by one or a combination of streptococcal pyrogenic exotoxins (Spe exotoxins) (2, 10, 15, 23, 29, 41). Investigations into the changing epidemiology of group A streptococcal infections have focused not only on production of pyrogenic exotoxins (15, 16, 23, 29) but also on changes in the M-type distribution (28).

To better characterize the relationship of M-type, streptococcal pyrogenic exotoxins, and proteolytic activity to clinical disease, we tested 62 *S. pyogenes* isolates from patients with invasive streptococcal infection for whom clinical data were available. The strains were M and T typed, assayed for *speA*, *-B*, and *-C* genes, and tested for the in vitro expression of SpeA and SpeB and for proteolytic activity.

MATERIALS AND METHODS

Case definition and sampling framework. From 1989 to 1991, the Centers for Disease Control and Prevention (CDC) coordinated active surveillance for invasive group A streptococcal infections in parts of six states and maintained passive surveillance in the remainder of the country. Clinical data were abstracted onto a standardized form which included demographic data, medical history, and signs and symptoms of the current illness.

A case definition of STSS was formulated by a committee of researchers from academia and public health organizations (42). It includes the isolation of *S. pyogenes* from a normally sterile site and the presence of shock with multiorgan involvement, soft tissue necrosis, or generalized rash. For the purposes of this study, we examined components of this disease by classifying clinical findings into four groups.

(i) Shock and organ involvement were defined by the presence of hypotension (systolic blood pressure, <90 mm Hg) and two or more of the following: renal involvement (creatinine level, >2), thrombocytopenia (platelet count, <100,000, or disseminated intravascular coagulation), hepatic involvement (>2-fold elevation of serum glutamic oxalacetic transaminase or serum glutamic pyruvic transaminase), and the adult respiratory distress syndrome.

(ii) Cutaneous involvement was defined by the presence of a generalized macular erythematous rash or generalized desquamation.

(iii) Soft tissue necrosis was defined by a recorded diagnosis of necrotizing fasciitis or gangrene or by an amputation.

(iv) "None" was defined by the lack of any of the three STSS clinical findings listed above. Invasive isolates from this group were designated "background" strains.

Case reports from 248 patients reported through surveillance were entered into a computer file and classified by clinical group. A stratified random sample of 62 patients, which increased the proportion of patients meeting one or more of these clinical criteria, was selected from these reports for further analysis. Isolates were selected for anal-

* Corresponding author.

ysis solely on the basis of the patient's clinical syndrome with no knowledge of serotype, toxin profile, or protease activity.

Bacterial strains. *S. pyogenes* strains from the 62 patients were sent from hospitals where they were initially isolated through state health department laboratories to the Bacterial Reference Laboratory at CDC. All isolates, including background strains, were sterile-site isolates from patients with invasive streptococcal infections. Control strains 594 (SpeA), 86-858 (SpeB), and TP18 (SpeC) used for assay controls and antiserum production were kindly provided by Patrick Schlievert, University of Minnesota.

Grouping and typing. All isolates were grouped for Lancefield carbohydrate antigen and serotyped for T- and M-proteins by the protocols of Moody et al. (22). Carbohydrate grouping and M-types were determined by capillary precipitation testing of Lancefield hot-acid extracts with specific rabbit antisera produced at CDC. Serum opacity factor (OF) reactions were tested by overnight incubation of 7 μl of culture supernatant on appropriate medium (13, 40) at 37°C.

Antiserum production. Hyperimmune rabbit antisera to SpeA and SpeB were prepared from control streptococcal strains in New Zealand White rabbits as described previously (19). Intramuscular immunizations were substituted for subcutaneous injections. Antisera to SpeA and SpeB were absorbed with heterologous whole bacterial cells and with Todd-Hewitt broth powder (Difco Laboratories, Detroit, Mich.).

Culture and exotoxin enrichment. Strains were tested for the ability to produce exotoxin A or B in vitro. Each strain was cultured and toxin enriched by a modification of previously reported methods (19, 26). Isolates were grown to the stationary phase of growth in Todd-Hewitt broth at 37°C, centrifuged at 500 × *g*, and filtered through a 0.22-μm-pore-size, low-protein-binding filter (Millex-GV; Millipore Corp., Bedford, Mass.). The filtered supernatant fluid was precipitated with 4 volumes of cold ethanol and left at 4°C for a minimum of 72 h. The precipitate was collected and resuspended in 1/10 of the original volume of acetate-buffered saline, pH 4.2. The resulting suspension was centrifuged at 2,000 × *g* to remove insoluble material. The protein concentration was measured by the Bio-Rad micro-protein assay (Bio-Rad Laboratories, Richmond, Calif.), with bovine serum albumin as the standard.

Exotoxin testing. Toxin-enriched preparations were mixed with 2× sodium dodecyl sulfate (SDS) sample buffer to yield 30 μg of protein per sample. All samples were run in 12% SDS-polyacrylamide gel electrophoresis, using the Laemmli buffer system (21). Controls for SpeA and SpeB were prepared from strains 594 and 86-858, respectively, as described above and run in each gel. Proteins were transferred to nitrocellulose membranes by established methods (33). Exotoxins were detected by probing with rabbit polyclonal antisera to SpeA or SpeB with the sequential addition of biotinylated goat anti-rabbit immunoglobulin, streptavidin-labeled peroxidase, and 4-chloro-1-naphthol with hydrogen peroxide. Each strain was cultured and tested a minimum of two times. Those strains that were negative initially were treated with 750 U of hyaluronidase at 37°C for 2 h (Sigma Chemical Co., St. Louis, Mo.) and retested.

Polymerase chain reaction. Polymerase chain reaction was performed to detect genes for SpeA, -B, and -C. DNA was extracted for polymerase chain reaction by treatment with 0.45% Nonidet P-40–0.45% Tween 20–20 μg of proteinase K (all from Sigma) at 55°C for 1 h and then at 95°C for 10 min (5). Nested sets of primers were designed by comparing nucleotide sequences, using software by DNAstar, Inc., Madison, Wis., and were synthesized on a DNA synthesizer by using β-cyanoethyl phosphoramidite chemistry. Primer sequences were compared with the staphylococcal toxic shock syndrome toxin 1 gene and staphylococcal enterotoxin genes for SEA, SEB, and SEC.

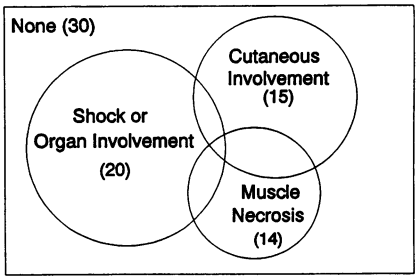

FIG. 1. Venn diagram of clinical signs in patients infected with the 62 invasive isolates of *S. pyogenes* used for these studies. Number in parentheses represents number of patients with clinical sign.

Protease assays. Thirty strains were tested for protease activity, using the casein plate assay based on the method of Hynes and Tagg (17). Isolates were grown on a Columbia agar base (GIBCO Laboratories, Grand Island, N.Y.) plus 1.5% sodium caseinate incorporated into the medium. Agar depth was controlled by the addition of a measured volume of agar to each plate. The zone of casein hydrolysis was measured, and protease activity was calculated by dividing the square of the diameter of the zone of hydrolysis by the square of the colony diameter.

Statistical analyses. For dichotomous variables, associations between clinical and laboratory findings were determined by the chi-square test on 2 × 2 tables or, when the sample size of one or more cells was <5, by the two-tailed Fisher exact test.

RESULTS

Of the 62 patients, 44 (65%) were white and 9 (15%) each were black and Hispanic, 35 (57%) were male, and the mean age was 50 years (range, 0 to 99 years). Forty-four patients (71%) had an underlying disease that may have predisposed them to an invasive group A streptococcal infection (including diabetes mellitus [11], surgical or nonsurgical wounds [10], alcohol abuse [7], and chronic lung disease [7]). *S. pyogenes* infection ended fatally in 22 (36%) patients. Strains were isolated from normally sterile sites, including blood (90%), cerebrospinal fluid (8%), and peritoneal fluid (6%).

The criteria for shock with organ involvement were met by 20 (32%) patients, those for cutaneous involvement were met by 15 (24%), and those for soft tissue necrosis were met by 14 (23%); there was some overlap between groups (Fig. 1). Thirty (48%) patients met none of these criteria.

Twenty-seven (43%) of the isolates were M-type 1, 8 (5%) were M-type 3, and 1 each (2%) were M-14 and M-39. Twenty-eight (45%) strains were M nontypeable (MNT). The MNT strains were classified into the OF positive (18 strains) and OF negative (10 strains) groups for comparative purposes. Nine different T types were identified among the 28 MNT strains.

The distribution of streptococcal exotoxins is given in Table 1. The *speA* gene was found in 56% of the isolates, and SpeA was expressed by 32% of the isolates. SpeB was

TABLE 1. Pyrogenic exotoxin profiles of 62 invasive *S. pyogenes* isolates

Toxin	*spe* gene[a]		Spe expression[b]	
	n	%	*n*	%
SpeA	35	56	20	32
SpeB	62	100	38	61
SpeC	17	27	—[c]	—

[a] Three (5%) of the 62 isolates carried both *speA* and *speC* genes; 13 (21%) carried neither gene.
[b] Thirteen (21%) of the 62 strains expressed both SpeA and SpeB; 18 (29%) expressed neither toxin.
[c] —, SpeC antisera not available.

expressed by 61% of the 62 isolates, while the chromosomally associated *speB* gene was carried by every strain. We were unable to assay for production of SpeC the unavailability of SpeC antisera; however, the *speC* gene was found in 27% of the isolates. Of 62 strains, 3 (5%) carried both *speA* and *speC* genes and 13 (21%) carried neither gene.

Table 2 illustrates the relationship between exotoxin expression or gene carriage and M-type or OF production. The two most commonly occurring M-types, 1 and 3, were analyzed together. Ninety-one percent of strains of M-types 1 and 3 carried the *speA* gene, and 50% of these M-types expressed SpeA in vitro. Only 17% of MNT OF^+ strains and 10% of MNT OF^- isolates carried *speA*, and 11 and 10%, respectively, expressed SpeA. Therefore, the presence of the *speA* gene and production of SpeA were significantly associated with M-types 1 and 3 ($P < 0.0001$ and $P = 0.002$, respectively). SpeB was expressed by at least 50% of the isolates in every category. Only 1 (3%) of 32 M-1 and M-3 isolates carried the *speC* gene, yet it was present in 61% of MNT OF^+ strains and 50% of MNT OF^- strains.

Of the 30 strains tested for protease activity, 19 (63%) had some zone of casein hydrolysis, and the mean value of protease activity was 12.2. Only 2 (25%) of 8 isolates from patients with no signs of STSS produced protease, whereas 18 (82%) of 22 isolates from patients with at least one sign of STSS produced protease ($P = 0.0035$). M-type 1 isolates were significantly associated with protease activity compared with isolates of all other M-types (15 of 17 [88%] versus 5 of 13 [38%] isolates; $P < 0.05$). There was no correlation between exotoxin expression and presence of protease activity.

Comparisons of toxin profile by M-type category and the presence of at least one clinical feature of STSS are shown in Table 3. Of the M-type 1 and 3 isolates from patients with at least one STSS disease component, 12 (71%) of 17 isolates expressed SpeA, compared with 4 (27%) of 15 strains isolated from patients with no clinical signs of STSS ($P = 0.02$). Although 100% of the M-1 and M-3 strains from patients with one or more signs of STSS carried the *speA* gene, 80% of M-1 and M-3 strains from patients with no signs of STSS also did. Therefore, the difference between these two patient groups with M-1 and M-3 isolates was significant only in terms of exotoxin A expression, not *speA* gene carriage.

TABLE 2. Associations among study strain type and exotoxin profile

Toxin genotype or phenotype	No. (%) with given characteristic		
	M-types 1 and 3 ($n = 32$)	MNT OF^+ ($n = 18$)	MNT OF^- ($n = 10$)
speA	29 (91)[a]	3 (17)	1 (10)
speB	32 (100)	18 (100)	10 (100)
speC	1 (3)[a]	11 (61)	5 (50)
SpeA	16 (50)[a]	2 (11)	1 (10)
SpeB	19 (59)	11 (61)	5 (50)

[a] Percentage of type 1 and 3 strains with indicated genotype or phenotype significantly different ($P < 0.05$) from percentage of strains in MNT categories.

The associations among M-type, exotoxin, protease activity, and the three clinical components of STSS are presented in Table 4. M-types 1 and 3 were isolated from 70% of patients with shock and organ involvement compared with 43% of patients not meeting these criteria ($P < 0.05$). SpeA was expressed by 10 (50%) of 20 isolates from patients with shock and organ involvement compared with 24% of those from patients without such involvement ($P < 0.05$). Protease activity also was significantly associated with shock and organ involvement ($P < 0.01$). There was no association of any other M-type, OF production, SpeB expression, or the presence of any toxin gene with shock and organ involvement. Cutaneous involvement was significantly associated only with production of SpeA ($P < 0.05$), not with M-type, OF, toxin gene, or protease activity. Soft tissue necrosis was associated only with casein protease activity ($P < 0.05$). The production of SpeB and the carriage of the *speA*, *-B*, or *-C* gene were not associated with any clinical finding.

DISCUSSION

Since 1989, the CDC has collected isolates and clinical data from patients with invasive *S. pyogenes* infections. Isolates included in this report made up a stratified random sample from all isolates collected. The major focus of this work was to determine relationships among streptococcal virulence factors and their association with individual clinical findings for STSS. Although previous descriptions of STSS have included patients with one or more categories of shock with organ involvement, rash, and soft tissue necrosis (11, 16, 24, 27), it is unclear whether all patients included in these studies truly had a single syndrome. The inclusion of patients in published studies of STSS, as well as in this report, who fulfill one or two of these clinical criteria without having all three suggests the possibility of different pathogenic mechanisms for each.

Of the various streptococcal virulence factors, M-1 was the most common (43%) serotype among the strains we analyzed. M-type 1 has been the predominant serotype isolated in several series of studies in patients designated as having STSS (15, 23, 29). In one study, it was shown that M-1 and M-3 serotypes associated with STSS belong to two multilocus genotypes, ET1 and ET2; in another, it was proposed that a unique clone of M-1 carrying the *speA* gene is responsible for the emergence of STSS (9, 23). Although we did not address clonality of our study strains, we did find that M-types 1 and 3 were the predominant serotypes, and they were significantly associated with the STSS clinical component of shock and organ involvement.

A large proportion (45%) of the strains we tested were not M typeable with our 42 available antisera. While these strains are classified as MNT, they are a heterogeneous group. Because the majority of our patients with clinical features of STSS were infected with M-type 1 or 3 and because MNT strains were significantly less likely to be associated with STSS, this large proportion of MNT isolates

TABLE 3. Comparison of exotoxin profiles among *S. pyogenes* isolates from patients with and without clinical signs of STSS

Toxin[a]	No. (%) with given characteristic					
	M-1 and M-3 strains		MNT OF$^+$ strains		MNT OF$^-$ strains	
	At least 1 sign of STSS (n = 17)	No signs of STSS (n = 15)	At least 1 sign of STSS (n = 7)	No signs of STSS (n = 11)	At least 1 sign of STSS (n = 7)	No signs of STSS (n = 3)
speA	17 (100)	12 (80)	1 (14)	2 (18)	0	1 (33)
speC	1 (6)	0	6 (86)[b]	5 (45)	3 (43)	2 (67)
SpeA	12 (71)[b]	4 (27)	1 (14)	1 (9)	0	1 (33)
SpeB	11 (65)	8 (53)	1 (14)[b]	10 (91)	4 (57)	1 (33)

[a] Because 100% of strains carried the *speB* gene, it is not included for comparative purposes.
[b] Percentage of isolates from patients with one or more signs of STSS significantly different ($P < 0.05$) from isolates from patients with no signs of STSS.

does not adversely affect the findings of this study. Another reason for the high proportion of these MNT isolates was our inclusion of background strains or those from patients with severe invasive disease but with no clinical signs of STSS. Other studies have primarily examined those strains associated with STSS, which are more likely to be M-type 1 or to belong to the OF$^-$ group (15, 23, 29). Whether M-protein is in some way directly responsible for STSS or serves as a marker for some other virulence factor(s) has not been determined. However, the former hypothesis is unlikely because the presence of shock and organ involvement is not unique to patients infected with M-types 1 and 3 (Table 4).

Many reports on the pathogenesis of STSS have focused on the role of the Spe exotoxins, primarily SpeA. Not only are they responsible for fever induction and rash and are mitogenic, but they also enhance host susceptibility to endotoxin (1, 6, 12, 14, 27, 37, 38). They are also potent superantigens (32). Therefore, these toxins seem to be likely candidates for a role in STSS. We found that M-1 and M-3 strains were more likely to produce SpeA in vitro than were other serotypes in our study and that SpeA production was significantly associated with rash and with shock and organ involvement. Both of these clinical signs can be explained by known SpeA biological activities. However, as did other investigators (10, 15, 29), we found cases of STSS in which *S. pyogenes* did not express SpeA. Therefore, it is unlikely that all cases of STSS are caused by known streptococcal exotoxins; instead, these toxins may be important in producing one or more of the clinical signs of STSS.

In this study, we found that 91% of all M-1 strains carried the phage-associated *speA* gene; however, only 48% of those carrying the gene expressed the SpeA protein in vitro. In some cases, M-type 1 strains may produce less SpeA than other M-types (15, 20). Also, hyaluronic acid is often copurified with exotoxin and is known to interfere with diffusion of toxins in Ouchterlony assays (15) and may interfere with electrotransfer of toxins to nitrocellulose membranes. Therefore, all strains negative for toxin production were treated with hyaluronidase and retested. We also observed marked differences among strains in the relative amount of exotoxin produced in vitro, and these differences were reproducible despite repeated in vitro passages (30).

It is important to note that toxin production in vitro may not accurately reflect the regulation of protein expression in

TABLE 4. Associations among microbiologic characteristics of study strains and clinical components of STSS

Microbiologic characteristic	No. (%) with given characteristics					
	Shock and organ involvement[a]		Cutaneous involvement[b]		Necrosis[c]	
	Yes (n = 20)	No (n = 42)	Yes (n = 15)	No (n = 47)	Yes (n = 14)	No (n = 48)
M-protein type						
1 and 3	14 (70)[d]	18 (43)	8 (53)	24 (51)	7 (50)	25 (52)
MNT OF$^+$	4 (20)	14 (33)	2 (13)	16 (34)	3 (21)	15 (31)
MNT OF$^-$	2 (10)	8 (19)	4 (27)	6 (13)	3 (21)	7 (15)
Other[e]	0	2 (5)	1 (7)	1 (2)	1 (7)	1 (2)
Toxin						
speA	14 (70)	21 (50)	10 (67)	25 (53)	8 (57)	27 (56)
speC	5 (25)	12 (29)	5 (33)	12 (25)	5 (36)	12 (25)
SpeA	10 (50)[d]	10 (24)	8 (53)[d]	12 (25)	5 (36)	15 (31)
SpeB	12 (60)	25 (59)	8 (53)	29 (62)	7 (50)	30 (62)
Protease production	15/18[f] (83)[d]	5/12 (42)	7/8 (88)	19/22 (86)	7/7 (100)[d]	9/23 (39)

[a] Includes all strains isolated from patients with shock and organ involvement, including those with shock and cutaneous involvement and/or necrosis as a clinical sign.
[b] Includes all strains isolated from patients with cutaneous involvement, including those with cutaneous involvement and shock and/or necrosis as a clinical sign.
[c] Includes all strains isolated from patients with necrosis, including those with necrosis and shock and/or cutaneous involvement as a clinical sign.
[d] $P < 0.05$ versus strains from persons without a clinical component.
[e] Includes one type 14 and one type 39 strain.
[f] Number that produced protease/number tested.

vivo. We found that SpeA production was significantly associated with STSS clinical signs of shock and organ involvement, but no relationship was found between any sign of STSS and the *speA* gene. Our data show that 100% of M-1 or M-3 strains from patients with at least one sign of STSS carry the *speA* gene, but 80% of M-1 and M-3 strains not associated with STSS also carry *speA* (Table 3). These data suggest that studies of exotoxin and STSS should not be limited to carriage of *spe* genes but should also emphasize toxin gene expression.

We found no relationship between SpeB production or the carriage of the *speB* or -*C* gene with any clinical manifestations of severe group A streptococcal infection or with any particular M-protein type. The expression of SpeB is highly correlated with in vitro growth conditions and pH regulation; thus, all *S. pyogenes* strains may have the potential to produce SpeB under certain in vitro growth conditions (20). In one study of STSS, strains isolated in Scandinavia produced large amounts of SpeB and -C, but only small amounts of SpeA (25). Later examination of sequences of *speB* alleles from nine electrophoretic type 1 and 2 strains from diverse geographic locations showed no variation within their respective electrophoretic type (24). We have no evidence linking SpeB to any clinical disease component or M-type we tested. SpeB production was, however, inversely associated with MNT OF^+ strains from patients with one or more signs of STSS. The *speC* gene, though found significantly more often in MNT OF^+ strains from patients with one or more signs of STSS, was not associated with any individual clinical component. One interesting finding is that very few (5%) of these strains possess the genes for both SpeA and SpeC, even though these toxin genes are widely dispersed in group A streptococci, and the presence of *speA* and *speC* in these strains is inversely related. Because both *speA* and *speC* are phage mediated, superinfection may be less likely in a strain already possessing one of the toxin genes. However, separately, these genes could be easily dispersed among a wide variety of strains (or particular M-types) that are sensitive to lysogenic conversion.

Necrotizing soft tissue involvement (myositis or fasciitis) was not associated with production of pyrogenic exotoxin, nor is there a reason to expect such an association on the basis of known activities of these toxins. Instead, this STSS clinical finding was associated with protease activity, as determined by the ability to hydrolyze casein. Protease production was also associated with shock and organ involvement. Our finding that protease production was significantly associated with both shock or organ involvement and necrosis is consistent with our finding that a significantly higher percentage of strains from patients with at least one sign of STSS produced protease in comparison with strains from non-STSS patients (82 versus 25%). The mechanism(s) by which proteases could influence these clinical signs is unclear, but others have noted such an association. In a study of a series of patients with group A streptococcal bacteremia in Denver, Colo., Wheeler et al. reported that 8 (53%) of 15 invasive bacteremic isolates had protease activity (39). In our study, two (25%) of eight invasive isolates from patients with no signs of STSS produced protease, a result not significantly different from that reported by Wheeler et al. ($P = 0.193$). Todd et al. (31) have shown that in staphylococci protease activity correlates with the ability of an organism to cause invasive infections; this may be one mechanism promoting invasive disease following a cutaneous infection.

We also confirm earlier observations that there is a correlation between protease production and the OF^- phenotype (39). In our study, the percentage of strains producing protease was significantly higher in OF^- (19 of 23, 83%) versus OF^+ (1 of 7, 14%) strains ($P = 0.0008$).

Host factors are also very likely to affect the expression and severity of clinical disease. However, our ability to demonstrate statistically significant associations between strain characteristics and clinical components of STSS despite the possible contribution of host factors suggests that the strength of these associations is valid.

Our data suggest that clinical features constituting streptococcal STSS may result from different pathogenic mechanisms and may represent multiple clinical entities. A syndrome of shock and organ involvement, in some patients occurring with a generalized erythematous rash or desquamation, may occur with infection by a SpeA-producing strain, most likely an M-1 or an M-3 protein type. A syndrome of shock and organ involvement with necrotizing soft tissue involvement may be mediated by a protease-producing strain.

Further studies with larger numbers of strains are needed to confirm these associations and to elucidate the regulatory control of toxin production. These results also indicate the importance of clearly defining patient groups for analysis on the basis of specific clinical findings in both clinical and epidemiologic studies.

ACKNOWLEDGMENTS

We thank Gail Bosley, Ruth Franklin, Nan Pigott, Valerie Ceasar, and Trudy Messmer for assistance.

REFERENCES

1. **Barsumian, E. L., P. M. Schlievert, and D. W. Watson.** 1978. Nonspecific and specific immunological mitogenicity by group A streptococcal pyrogenic exotoxins. Infect. Immun. **22**:681–688.
2. **Bartter, T., A. Dascal, K. Carroll, and F. J. Curley.** 1988. "Toxic Strep Syndrome." A manifestation of group A streptococcal infection. Arch. Intern. Med. **148**:1421–1424.
3. **Bisno, A. L.** 1990. The resurgence of acute rheumatic fever in the United States. Annu. Rev. Med. **41**:319–329.
4. **Bisno, A. L.** 1991. Group A streptococcal infections and acute rheumatic fever. N. Engl. J. Med. **325**:783–793.
5. **Black, C. M., D. F. Talkington, T. O. Messmer, R. R. Facklam, E. Hornes, and O. Olsvik.** Mol. Cell. Probes, in press.
6. **Bohach, G. A., D. J. Fast, R. D. Nelson, and P. M. Schlievert.** 1990. Staphylococcal and streptococcal pyrogenic toxins involved in toxic shock syndrome and related illnesses. Crit. Rev. Microbiol. **17**:251–272.
7. **Centers for Disease Control.** 1988. Acute rheumatic fever among army trainees, Fort Leonard Wood, Missouri, 1987–1988. Morbid. Mortal. Weekly Rep. **37**:519–526.
8. **Chomarat, M., C. Chapuis, A. Lepape, and F. Bernard.** 1990. Two cases of severe infection associated with toxic shock-like syndrome. Eur. J. Clin. Microbiol. Infect. Dis. **9**:901–903.
9. **Cleary, P. P., E. L. Kaplan, J. P. Handley, A. Wlazlo, M. H. Kim, A. R. Hauser, and P. M. Schlievert.** 1992. Clonal basis for resurgence of serious *Streptococcus pyogenes* disease in the 1980s. Lancet **339**:518–521.
10. **Cone, L. A., D. R. Woodard, P. M. Schlievert, and G. S. Tomory.** 1987. Clinical and bacteriologic observations of a toxic shock-like syndrome due to *Streptococcus pyogenes*. N. Engl. J. Med. **317**:146–149.
11. **Congeni, B., C. Rizzo, J. Congeni, and V. V. Sreenivasan.** 1987. Outbreak of acute rheumatic fever in northeast Ohio. J. Pediatr. **111**:176–179.
12. **Dick, G. F., and G. H. Dick.** 1924. Skin test for susceptibility to scarlet fever. JAMA **82**:265–266.
13. **Gooder, H.** 1961. Association of a serum opacity reaction with

serological type in *Streptococcus pyogenes*. J. Gen. Microbiol. **25**:347–352.
14. **Hanna, E. E., and D. W. Watson.** 1968. Host-parasite relationships among group A streptococci. IV. Suppression of antibody response by streptococcal pyrogenic exotoxin. J. Bacteriol. **95**:14–21.
15. **Hauser, A. R., D. L. Stevens, E. L. Kaplan, and P. M. Schlievert.** 1991. Molecular analysis of pyrogenic exotoxins from *Streptococcus pyogenes* isolates associated with toxic shock-like syndrome. J. Clin. Microbiol. **29**:1562–1567.
16. **Hoge, C. W., B. Schwartz, D. F. Talkington, R. F. Breiman, E. M. MacNeill, and S. J. Englender.** 1993. The changing epidemiology of invasive group A streptococcal infections and the emergence of streptococcal toxic shock-like syndrome: a retrospective population-based study. JAMA **269**:384–389.
17. **Hynes, W. L., and J. R. Tagg.** 1985. A simple plate assay for detection of group A streptococcus proteinase. J. Microbiol. Methods **4**:25–31.
18. **Johnson, D. R., D. L. Stevens, and E. L. Kaplan.** 1992. Epidemiologic analysis of group A streptococcal serotypes associated with severe systemic infections, rheumatic fever, or uncomplicated pharyngitis. J. Infect. Dis. **166**:374–382.
19. **Kim, Y. B., and D. W. Watson.** 1970. A purified group A streptococcal pyrogenic exotoxin. Physiochemical and biological properties including the enhancement of susceptibility to endotoxin lethal shock. J. Exp. Med. **131**:611–628.
20. **Köhler, W., D. Gerlach, and H. Knöll.** 1987. Streptococcal outbreaks and erythrogenic toxin type A. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A **266**:104–115.
21. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
22. **Moody, M. D., J. Padula, D. Lizama, and C. T. Hall.** 1965. Epidemiologic characterization of group A streptococci by T-agglutination and M-precipitation tests in the public health laboratory. Health Lab. Sci. **2**:149–162.
23. **Musser, J. M., A. R. Hauser, M. H. Kim, P. M. Schlievert, K. Nelson, and R. K. Selander.** 1991. *Streptococcus pyogenes* causing toxic shock-like syndrome and other invasive diseases: clonal diversity and pyrogenic exotoxin expression. Proc. Natl. Acad. Sci. USA **88**:2668–2672.
24. **Musser, J. M., V. Kapur, S. Kanjilal, U. Shah, D. M. Musher, N. L. Barg, K. H. Johnston, P. M. Schlievert, J. Henrichsen, D. Gerlach, R. M. Rakita, A. Tanna, B. D. Cookson, and J. C. Huang.** 1993. Geographic and temporal distribution and molecular characterization of two highly pathogenic clones of *Streptococcus pyogenes* expressing allelic variants of pyrogenic exotoxin A (scarlet fever toxin). J. Infect. Dis. **167**:337–346.
25. **Norgren, M., A. Norrby, and S. E. Holm.** 1992. Genetic diversity in T1M1 group A streptococci in relation to clinical outcome of infection. J. Infect. Dis. **166**:1014–1020.
26. **Schlievert, P. M., K. M. Bettin, and D. W. Watson.** 1979. Production of pyrogenic exotoxin by groups of streptococci: association with group A. J. Infect. Dis. **140**:676–681.
27. **Schlievert, P. M., and D. W. Watson.** 1978. Group A streptococcal pyrogenic exotoxin: pyrogenicity, alteration of blood-brain barrier, and separation of sites for pyrogenicity and enhancement of lethal endotoxin shock. Infect. Immun. **21**:753–763.
28. **Schwartz, B., R. R. Facklam, and R. F. Breiman.** 1990. Changing epidemiology of group A streptococcal infection in the USA. Lancet **336**:1167–1171.
29. **Stevens, D. L., M. H. Tanner, J. Winship, R. Swarts, K. M. Ries, P. M. Schlievert, and E. Kaplan.** 1989. Severe group A streptococcal infections associated with a toxic shock-like syndrome and scarlet fever toxin A. N. Engl. J. Med. **321**:1–7.
30. **Talkington, D. F., and R. R. Facklam.** Unpublished data.
31. **Todd, J., S. Roberson, and M. Roe.** 1991. Relationship of protease production to invasiveness of *Staphylococcus aureus* strains, abstr. 275. Program Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, D.C.
32. **Tomai, M., P. M. Schlievert, and M. Kotb.** 1992. Distinct T cell receptor Vβ gene usage by T lymphocytes stimulated with the streptococcal pyrogenic exotoxins and M protein, E-9, p. 145. Abstr. 92nd Gen. Meet. Am. Soc. Microbiol. 1992. American Society for Microbiology, Washington, D.C.
33. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedures and some applications. Proc. Natl. Acad. Sci. USA **76**:4350–4354.
34. **Veasy, G. L., S. E. Wiedmeier, G. S. Orsmond, et al.** 1987. Resurgence of acute rheumatic fever in the intermountain area of the United States. N. Engl. J. Med. **316**:421–428.
35. **Wald, E. R., B. Dashefsky, C. Feidt, D. Chiponis, and C. Byers.** 1987. Acute rheumatic fever in western Pennsylvania and the Tristate area. Pediatrics **80**:371–374.
36. **Wallace, M. R., P. D. Garst, T. J. Papadimos, and E. C. Oldfield III.** 1989. The return of acute rheumatic fever in young adults. JAMA **262**:2557–2561.
37. **Wannamaker, L. W., and P. M. Schlievert.** 1988. Exotoxins of group A streptococci, p. 267–296. *In* M. C. Hardegree and A. T. Tu (ed.), Bacterial toxins. Marcel Dekker, Inc., New York.
38. **Watson, D. W.** 1960. Host-parasite factors in group A streptococcal infections. Pyrogenic and other effects of immunologic distinct exotoxins related to scarlet fever toxins. J. Exp. Med. **111**:255–284.
39. **Wheeler, M. C., M. H. Roe, E. L. Kaplan, P. M. Schlievert, and J. K. Todd.** 1991. Outbreak of group A streptococcus septicemia in children. JAMA **266**:533–537.
40. **Widdowson, J. P., W. R. Maxted, and D. L. Grant.** 1970. The production of opacity in serum by group A streptococci and its relationship with the presence of M antigen. J. Gen. Microbiol. **61**:343–353.
41. **Willoughby, R., and R. N. Greenberg.** 1983. The toxic shock syndrome and streptococcal pyrogenic exotoxins. Ann. Intern. Med. **98**:559.
42. **Working Group on Severe Streptococcal Infections.** 1993. Defining the group A streptococcal toxic shock syndrome. Rationale and consensus definition. JAMA **269**:390–391.