INFECTION AND IMMUNITY, Sept. 1978, p. 738-746
0019-9567/78/0021-0738$02.00/0

Vol. 21, No. 3
Printed in U.S.A.

Association of Elevated Levels of Extracellular Neuraminidase with Clinical Isolates of Type III Group B Streptococci

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Received for publication 2 May 1978

The level of total extracellular neuraminidase produced by 74 clinical isolates of group B streptococci isolated from diseased or asymptomatically colonized infants was assayed. Extracellular neuraminidase was obtained from concentrated filtrates of exponentially growing cultures of group B streptococci grown in a chemically defined medium (FMC) containing supplemental protein. The total activity of extracellular enzyme produced by these clinical isolates ranged from <10 to 360 nmol of sialic acid released per min per mg of cell dry weight. Strains were arbitrarily classified as either nonproducers (<10 nmol/min per mg of cell dry weight), low producers (>10 to ≤140 nmol/min per mg of cell dry weight), or high producers (>140 to 360 nmol/min per mg of cell dry weight). Type III isolates from diseased infants were significantly more often classified as high producers than strains of group B streptococci of other serotypes from diseased infants ($P \ll 0.001$). Furthermore, the serotype III strains isolated from neonatal infections were more often high producers than those of the same serotype from asymptomatically colonized infants ($P < 0.025$). These results suggest that the ability to produce elevated levels of neuraminidase may be related to the frequent association of type III strains with disease among neonates.

The enzyme neuraminidase (sialidase) has been implicated as a virulence factor in several species of bacteria, particularly those possessing the ability to survive on mucosal surfaces, examples of which include *Corynebacterium diphtheriae, Vibrio cholerae,* and *Streptococcus pyogenes* (19). Gottschalk (13) originally demonstrated that the removal of terminal sialic acid residues from salivary glycoproteins caused a loss in the viscosity and adhesiveness of these secretions, hindering the normal protective function of the epithelial mucous lining (e.g., the entrapment and removal of invading microorganisms). Thus, the enzyme appeared to have an adaptive function in enhancing survival of these microorganisms in the respiratory, intestinal, and urogenital tracts.

In addition to this localized effect, Müller (18) has demonstrated altered electrophoretic patterns of serum glycoproteins in patients suffering from invasive pneumococcal disease. One pathological result from such alteration of serum glycoproteins could be their diminished life span in the circulatory system. In this regard, Morell et al. (17) have shown that orosomucoid, fetuin, ceruloplasmin, haptoglobin, alpha$_2$ macroglobulin, thyroglobulin, lactoferrin, and two gonadotrophic hormones were promptly removed from circulation by hepatic parenchymal cells after being desialylated by *Clostridium perfringens* neuraminidase. Additionally, Aminoff et al. (3) and Woodruff and Gesner (25) have demonstrated similar phenomena in the survival of erythrocytes and lymphocytes in the circulatory system. Neuraminidase-treated erythrocytes were rapidly removed from the circulation by the Kupffer cells of the liver and mononuclear spleen cells. In similar fashion, neuraminidase-treated lymphocytes became trapped in the liver upon transfusion into syngeneic recipients, with a concomitant decreased accumulation in the lymph nodes and spleen. Although this mechanism may be a normal physiological mechanism for removing senescent blood cells from circulation, the process could conceivably be accelerated during systemic disease involving a microbe producing neuraminidase in vivo.

We initially examined extracellular neuraminidase levels in two strains of group B streptococci to define the relationship between the growth of these organisms and the elaboration of the enzyme (16). To obtain evidence for the possible role of this extracellular product in the pathogenesis of serious neonatal disease, we have examined a large number of strains of group B streptococci from both asymptomatically colo-

nized and diseased infants for production of extracellular neuraminidase. The results presented in this study indicate that serotype III strains isolated from neonates with invasive disease are significantly more often capable of elaborating elevated levels of extracellular neuraminidase than type III strains from asymptomatically colonized infants or non-type III strains from infected infants. Since the great majority of neonatal infections in the United States have been shown to be associated with type III group B streptococci (4, 6, 23, 24), it appears that the ability to produce extracellular neuraminidase may be an important microbial factor which contributes to the marked association of type III organisms with neonatal disease.

MATERIALS AND METHODS

Bacterial strains. Seventy-four clinical isolates of group B streptococci were obtained from asymptomatically colonized or diseased infants hospitalized at various facilities in the Texas Medical Center, Houston. Strains were subsequently serotyped according to the method of Lancefield (14) and frozen in Todd-Hewitt broth at −70°C (8). To obtain cells for determination of levels of extracellular neuraminidase, samples of thawed Todd-Hewitt broth cultures were streaked onto 5% sheep agar plates, and the plates, after overnight incubation at 37°C, were stored at 4°C for up to 3 weeks. Because of the undefined effect of subculture on the ability of group B streptococci to produce neuraminidase, strains were never passaged on blood agar after initial isolation and determination of serotype. All strains were coded and assayed for neuraminidase in single blind fashion. Prototype strains of group B sterptococci representing each of the five serotypes were kindly supplied by Hazel W. Wilkinson, Center for Disease Control, Atlanta, Ga.

Liquid media and growth conditions. The chemically defined medium (FMC) was prepared according to the procedure of Terleckyj et al. (22) with the sodium phosphate concentration adjusted to 0.060 M (15). Glucose and sodium carbonate concentrations were 0.25% (wt/vol) and 0.019 M, respectively, and the final pH of FMC was 7.0. Various supplements were routinely added to FMC, including Todd-Hewitt broth and human serum albumin (HSA). These components were added to FMC, and the entire medium was subsequently filtered through a 0.20-μm sterile Nalgene filter unit to remove any contaminating bacteria. Growth of cultures and quantitation of bacterial mass were performed as previously described (16).

Preparation of culture filtrates. Broth cultures of various group B streptococcal isolates were quickly chilled on ice and harvested by centrifugation at 10,000 × *g* for 30 min at 4°C. After centrifugation, supernatants were routinely filtered through a 0.20-μm sterile Nalgene filter unit, with the exception of those samples labeled "unfiltered" in Table 2. Solid ammonium sulfate to obtain 75% saturation was then added to the filtrates, followed by suspension of the resulting precipitate in a small volume (3 to 4 ml of 10 mM sodium acetate [pH 6.5] per 50 ml of culture fluid) and dialysis against 100 to 200 buffer volumes of the same buffer at 4°C for 24 h.

Enzyme assays. Neuraminidase levels were quantitated as previously described (16) utilizing bovine submaxillary mucin as substrate, except that inclusion of 10 mM $CaCl_2$ in the reaction mixture was omitted after it was determined that calcium ion did not influence the rate of the neuraminidase reaction. Briefly, each assay mixture contained the following components in a volume of 0.5 ml: 1.0 mg of bovine submaxillary mucin (Sigma), 100 mM sodium acetate (pH 6.5), and the appropriate streptococcal preparation in an amount to insure that the bovine submaxillary mucin would be saturating (with 1 mg of mucin the reaction is linear to ca. 0.4 optical density unit when read on a Gilford 250 spectrophotometer at 547 nm). The release of sialic acid was quantitated by the colorimetric thiobarbituric acid assay of Aminoff (2) using *N*-acetyl neuraminic acid (Sigma) as standard. Total neuraminidase activity, expressed as nanomoles per minute per milligram of cell dry weight, was determined as previously described (16).

Statistical methods. Linear regression and chi-square analyses utilized in this study were performed on a Wang model 462 Advanced Statistician Calculator. The Yates correction for continuity (21) was utilized in all chi-square calculations.

RESULTS

Effect of various culture media and filtration on levels of extracellular neuraminidase production by group B streptococci. Previous studies on the recovery of extracellular neuraminidase activity from filtrates of group B streptococci have indicated that supplementation of exogenous protein to the chemically defined growth medium was essential (16). Supplementation of HSA at 250 μg/ml or higher resulted in yields of extracellular neuraminidase comparable to those seen in complete Todd-Hewitt broth and was considered to be necessary for several reasons. The group B streptococci produce extracellular proteases (unpublished data), which could inactivate extracellular neuraminidase during growth at 37°C. The addition of HSA appeared to protect the enzyme at least partially from inactivation by the protease, and, second, HSA functioned as "carrier" protein in the preparation of concentrated filtrates by 0 to 75% ammonium sulfate precipitation. Table 1 illustrates additional information concerning the recovery of group B streptococcal extracellular neuraminidase from filtrates of strain 110 (serotype III) grown in FMC containing various supplements. As expected, growth of strain 110 in FMC resulted in no detectable activity when exogenous protein was absent during the growth cycle and was added only after filtration of the culture supernatant fluid. However, the addition of 1.0 mg of HSA per ml to the supernatant fluid before filtration resulted in recovery of approxi-

mately 22% of maximal activity obtained in the experiment shown in Table 1. Possibly HSA functioned by competing for binding sites on the membrane filter and allowed the remaining active neuraminidase to pass through the filter pores. During the course of these studies, we discovered that the addition of a small amount (final concentration, 2% [vol/vol]) of whole Todd-Hewitt broth to FMC containing 230 μg of HSA per ml brought about the highest and most reproducible recovery of extracellular neuraminidase. Addition of either HSA or Todd-Hewitt broth separately resulted in the recovery of detectable amounts of extracellular neuraminidase, but the addition of HSA resulted in a higher recovery (44% of maximal yield) than did the addition of Todd-Hewitt broth (10% of maximal yield). Since it had been established that filtration in the absence of exogenous protein resulted in loss of neuraminidase activity from culture supernatants (Table 1), we examined six isolates of group B streptococci grown in FMC supplemented with 230 μg of HSA per ml and 2% (vol/vol) Todd-Hewitt broth for total levels of extracellular neuraminidase. Each strain was grown in duplicate; after the cells were harvested by centrifugation, one supernatant was filtered and the other was left unfiltered, and then both were precipitated by 0 to 75% ammonium sulfate saturation. The results (Table 2) indicate that, with every strain tested, subsequent filtration resulted in a decrease in total neuraminidase activity recovered from the harvested supernatants. Although not illustrated in Table 2, experiments were performed in which cell-free filtrates from strain 110 grown in FMC containing 230 μg of HSA per ml and 2% (vol/vol) Todd-Hewitt broth were refiltered through a Nalgene filter unit and found to lose approximately 30 to 40% of recoverable neuraminidase activity (unpublished data). These results indicate that the factors governing recovery of extracellular neuraminidase activity in cell-free filtrates from group B streptococci are more complex than previously proposed (16). We have chosen FMC supplemented with 230 μg of HSA per ml and 2% (vol/vol) Todd-Hewitt broth for the analysis of extracellular neuraminidase in clinical isolates of group B streptococci because these conditions result in the most reproducible recovery of extracellular neuraminidase. Since we are interested in the quantitation of extracellular neuraminidase in group B streptococci, we have chosen to filter the supernatant fluids to insure the removal of any remaining bacterial cells that could contain cell-bound enzyme. It should be noted, however, that preliminary experiments have indicated that cell extracts of group B streptococci contain very little (ca. 10%) active neuraminidase when compared to the enzyme activity present in the supernatant fluid (unpublished data). As will be demonstrated subsequently, this filtration step does not affect our ability to quantitatively examine the association between high neuraminidase activity and serotype III isolates from invasive disease.

TABLE 1. *Effect of various medium components on levels of extracellular neuraminidase produced by group B streptococcus strain 110 (serotype III)*[a]

Addition to FMC medium	When added			Total activity[b]
	During growth	After centrifugation	After filtration	
HSA (1.0 mg/ml)	−	−	+	<4.8 (<1)
HSA (1.0 mg/ml)	−	+	−	79.8 (22)
HSA (230 μg/ml)	+	−	−	160.1 (44)
2% (vol/vol) Todd-Hewitt broth	+	−	−	37.5 (10)
HSA (230 μg/ml) plus 2% (vol/vol) Todd-Hewitt broth	+	−	−	363.6 (100)

[a] Cells were harvested during the late exponential phase of growth (AOD = 400 to 500) and filtered through 0.20-μm Nalgene filters as described in the text.

[b] Expressed as nanomoles of sialic acid released per minute per milligram of cell dry weight as previously described (16); average of duplicate determinations. Numbers in parentheses indicate percentage of total activity.

TABLE 2. *Effect of filtration on levels of extracellular neuraminidase produced by various strains of group B streptococci*[a]

Strain	Serotype	Total activity[b]	
		Filtered	Unfiltered
H36B	Ib	32.3	145.8
110	III	252.4	402.5
134	Ib	325.8	412.6
140	III	<10	<10
168	Ia	57.4	83.3
174	III	214.3	474.7

[a] Cells were harvested during the late exponential phase of growth in FMC supplemented with 230 μg of HSA per ml and 2% (vol/vol) Todd-Hewitt broth.

[b] Expressed as nanomoles of sialic acid released per minute per milligram of cell dry weight as previously described (16); average of duplicate determinations.

Production of extracellular neuraminidase by clinical isolates of group B streptococci. Figure 1 illustrates the relationship between neuraminidase production and the growth cycle in a type III fresh clinical isolate (strain 110) grown in FMC supplemented with 230 μg

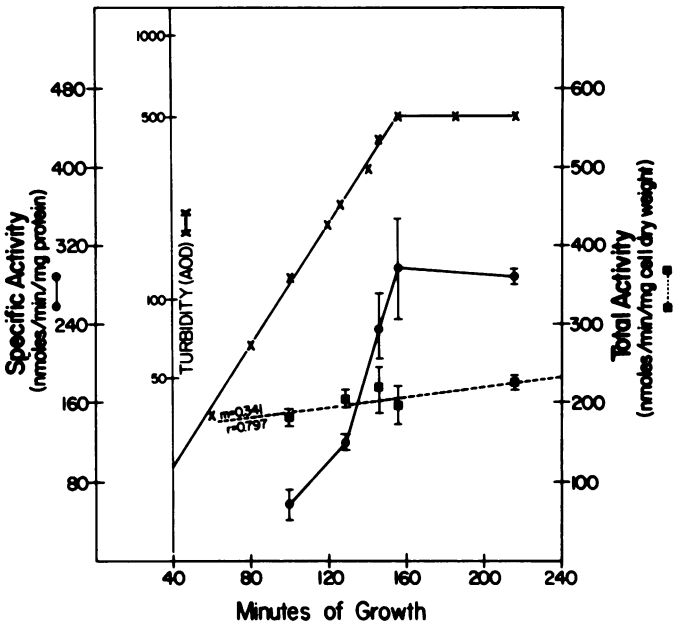

FIG. 1. *Effect of growth phase* (×) *on specific activity* (●) *and total activity per milligram of cell dry weight* (■) *of extracellular neuraminidase from group B streptococcal strain 110. Cells were grown in FMC supplemented with 230 μg of HSA per ml and 2% (vol/vol) Todd-Hewitt broth, and concentrated filtrates obtained at various times during the growth cycle were assayed for neuraminidase as described in the text. Enzyme determinations represent mean values of duplicate assays ± 2 standard deviations.*

of HSA per ml and 2% (vol/vol) Todd-Hewitt broth. At various times in the growth cycle, concentrated supernatants were prepared by 0 to 75% ammonium sulfate precipitation of culture filtrates and assayed for extracellular neuraminidase. It appeared that enzyme synthesis (as measured by increase in specific activity) increased during the exponential phase and that after cessation of growth (stationary phase) no detectable increase in enzyme activity was found. The observation that increase in extracellular neuraminidase was closely correlated to the growth of the organism was also supported by the fact that there was very little change in total extracellular activity per milligram of cell dry weight during the growth cycle. The least-square derivation of this line gave a slope of 0.341, close to what would be expected if this line was completely horizontal. These results were in contrast to the previous findings reported by us (16) which indicated that dramatic decreases in neuraminidase activity occurred during the stationary phase of growth when strain 110 was grown in the dialyzable fraction of Todd-Hewitt broth. However, this discrepancy might be accounted for by our use in this study of a medium (FMC plus HSA and Todd-Hewitt broth) which provides group B streptococcal neuraminidase greater protection against inactivation by group B streptococcal proteases. It should be noted, however, that significant amounts of decay (32 to 52% loss in activity in 30 min) occurred if the dialyzed concentrated samples shown in Fig. 1 were reincubated at 37°C prior to assay for neuraminidase (data not shown). These results stress the importance of performing all preparative procedures (ammonium sulfate precipitation, dialysis, etc.) of group B streptococcal neuraminidase at 0 to 4°C. Unless otherwise stated, in all subsequent data to be presented, enzyme samples were harvested at late exponential phase (AOD = 400 to 500).

Figure 2 illustrates the results obtained on assay of total extracellular neuraminidase activity per milligram of cell dry weight from 74 clinical isolates of group B streptococci. The total amount of detectable extracellular neuraminidase activity produced by these organisms varied from <10 to 360 nmol of sialic acid released per min per mg of cell dry weight, with the strains falling into three apparent categories. Organisms were arbitrarily classified as either nonproducers of neuraminidase (<10 nmol/min per mg of cell dry weight), low producers of neuraminidase (ranging from >10 to ≤140 nmol/min per mg of cell dry weight), or high neuraminidase producers (ranging from >140 to 360 nmol/min per mg of cell dry weight). The

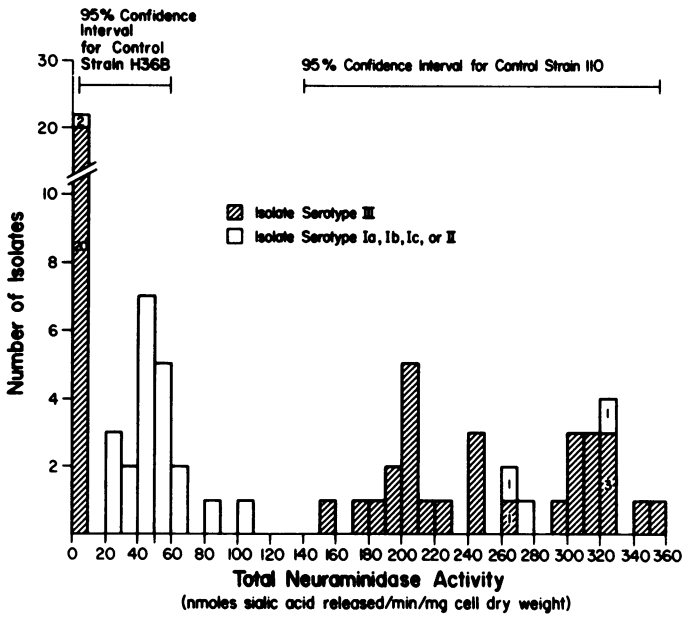

FIG. 2. *Total levels of extracellular neuraminidase of 74 strains of group B streptococci isolated from infants asymptomatically colonized (22 strains) or diseased (54 strains). Concentrated filtrates were prepared from late-exponential-phase cultures grown in FMC supplemented with 230 μg of HSA per ml and 2% (vol/vol) Todd-Hewitt broth and assayed for neuraminidase as described in the text. The numbers of strains of serotype III and of non-serotype III are indicated in those bars which contain both categories of strains.*

value of 140 nmol/min per mg of cell dry weight was chosen as the demarcation point between low and high producers because of its close proximity to the mean value of neuraminidase activity for all isolates (126.0) and also because it appeared to be a natural subdivision between the low and high neuraminidase producers. Additionally, the 95% confidence interval for the two control strains (H36B and 110), included each time a series of clinical isolates was tested, always fell within the low and high producer category, respectively, based on 140 nmol as the demarcation value. After completion of the enzyme assays, relevant clinical information on each isolate was then compiled, including both serotype and diagnosis of the type of disease produced in the human host (early or late onset; meningitis, sepsis, or other manifestation). Without exception, serotype III strains were found to be either nonproducers or higher producers of extracellular neuraminidase, whereas, with few exceptions, non-serotype III strains (Ia, Ib, Ic, II) were observed to produce low levels of the enzyme. Table 3 illustrates levels of neuraminidase and relevant clinical information for serotype III strains from neonatal invasive disease, while Table 4 shows comparable data for serotype III isolates from asymptomatically colonized infants. Table 5 depicts neuraminidase activity of isolates of group B streptococci other than type III from invasive neonatal disease, as well as the type of disease caused by each isolate. Although the data are not illustrated in these tables, all five of the prototype strains were analyzed for extracellular neuraminidase and all were found to be either nonproducers (090 [Ia], A909 [Ic], and D136C [III]; <10 nmol of sialic acid per min per mg of cell dry weight) or low producers of extracellular neuraminidase (H36B [Ib], 18RS21 [II]; 13.6 and 70.1 nmol of sialic acid per min per mg of cell dry weight, respectively). The previous report of high neuraminidase production by control strain H36B (16) has not been shown to be reproducible, and in subsequent experiments only low levels of enzyme have been observed. However, control strain 110 (serotype III from late-onset meningitis) consistently produced high levels of neuraminidase. No other inconsistencies in neuraminidase levels have been observed in any other prototype strain or fresh clinical isolate.

The most striking correlation evident from the data in Tables 3 and 5 was the predominance of high neuraminidase producers among serotype III isolates from invasive disease. This association is illustrated in Table 6 by a contingency analysis of total neuraminidase activity with serotype of the group B streptococcal isolates. Among isolates from invasive neonatal disease, we tested the hypothesis that there was

TABLE 3. *Neuraminidase activity in 26 strains of type III group B streptococci from neonatal invasive disease*

Strain no.	Total neuraminidase activity[a]	Source of isolate	Type of disease[b]
110	248.8	CSF[c]	Late-onset meningitis
111	231.7	Blood	Early-onset sepsis
112	217.1	CSF	Late-onset meningitis
118	<10	Blood	Early-onset sepsis
119	<10	Blood	Early-onset sepsis
120	307.1	Blood	Late-onset sepsis
121	347.8	Blood	Late-onset sepsis
122	355.4	Blood	Late-onset sepsis
123	194.1	Blood	Late-onset sepsis
124	328.3	Blood	Late-onset sepsis
125	291.6	CSF	Late-onset meningitis
126	328.2	CSF	Late-onset meningitis
127	<10	CSF	Late-onset meningitis
129	<10	CSF	Early-onset meningitis
130	327.0	CSF	Early-onset meningitis
138	159.0	CSF	Late-onset meningitis
139	310.5	Blood	Early-onset sepsis
140	<10	Blood	Early-onset sepsis
142	<10	Blood	Late-onset sepsis
149	311.8	Blood	Early-onset sepsis
150	309.9	Blood	Early-onset sepsis
151	307.5	Joint-fluid	Late-onset septic arthritis
173	211.1	Blood	Late-onset meningitis
174	214.3	CSF	Late-onset meningitis
175	249.5	Blood	Early-onset sepsis
176	268.0	Tracheal aspirate	Early-onset pneumonia

[a] Cells were grown to late exponential phase in FMC supplemented with 230 μg of HSA per ml and 2% (vol/vol) Todd-Hewitt broth. Total activity is expressed as nanomoles of sialic acid released per minute per milligram of cell dry weight; average of duplicate determinatons.

[b] Type of disease classified as early or late onset as previously described (7, 12).

[c] CSF, cerebrospinal fluid.

no relationship between serotype of group B streptococci and the ability to produce high levels of neuraminidase (Table 6). Based on a chi-square value of 19.96, we have rejected the above hypothesis with greater than 99.9% confidence and conclude that the ability of group B streptococci to produce high neuraminidase appears to be associated with strains containing the type III capsular carbohydrate. Because of the established observation (6, 23, 24) that serotype III strains are frequently associated with group B streptococcal meningitis at any age or late-onset disease, we also tested the possible association between high neuraminidase and isolates from either of the above two syndromes. As expected, it was found that a greater proportion of high neuraminidase producers was present in isolates from late-onset disease or meningitis at any age (14/22) than from isolates from early-onset septicemia (9/30). Although the chi-square value of 4.54 was significant, we feel that these results were probably attributable to the predominance of serotype III organisms among isolates from meningitis at any age or late-onset disease rather than any association between high levels of neuraminidase and ability to cause these particular disease states. Table 7 examines the possible association between the ability to produce high neuraminidase and the source of the isolate, i.e., whether the isolate was obtained from asymptomatically colonized or diseased infants. Type III isolates from asymptomatically colonized infants did contain a lower proportion of high neuraminidase producers (8/22) than did type III isolates from invasive disease (20/26). These data in Table 7, which were significant by chi-square analysis ($P < 0.025$), indicate that extracellular neuraminidase may play a role in the pathogenesis of group B streptococcal disease in neonates.

DISCUSSION

The reproducible recovery of extracellular neuraminidase from group B streptococci was our initial objective in this study before attempting to compare total levels of enzyme produced by various clinical isolates of group B streptococci. These conditions were achieved by the addition of 230 μg of HSA per ml and 2% (vol/vol) whole Todd-Hewitt broth to the chemically defined medium FMC (15). These com-

TABLE 4. *Neuraminidase activity in 22 strains of type III group B streptococci from asymptomatically colonized infants*

Strain no.	Total neuraminidase activity[a]	Source of isolate
177	246.8	Umbilicus
178	223.9	Umbilicus
179	313.3	Throat
180	<10	Rectum
181	<10	Umbilicus
182	<10	Umbilicus
183	<10	Umbilicus
184	<10	Rectum
185	182.6	Umbilicus
186	199.8	Umbilicus
187	<10	Umbilicus
188	<10	Umbilicus
189	216.1	Umbilicus
190	214.5	Umbilicus
191	<10	Umbilicus
192	<10	Umbilicus
193	<10	Umbilicus
194	171.3	Umbilicus
195	<10	Umbilicus
196	<10	Umbilicus
197	<10	Umbilicus
198	<10	Umbilicus

[a] Cells were grown and neuraminidase was assayed as described in Table 3.

TABLE 5. *Neuraminidase activity in 26 isolates of group B streptococci other than type III from invasive neonatal disease*

Strain no.	Serotype	Total neuraminidase activity[a]	Source of isolate	Type of disease
114	Ia	34.8	Blood	Early-onset sepsis
158	Ia	53.0	Blood	Early-onset sepsis
159	Ia	42.2	CSF[b]	Early-onset meningitis
160	Ia	44.3	CSF	Early-onset sepsis with meningitis
161	Ia	68.3	Blood	Early-onset sepsis
167	Ia	58.7	Blood	Late-onset sepsis
168	Ia	57.4	Blood	Late-onset sepsis
131	Ib	23.5	Blood	Early-onset sepsis
132	Ib	40.2	Blood	Early-onset sepsis
133	Ib	<10	Blood	Early-onset sepsis
134	Ib	325.8	Blood	Early-onset sepsis
135	Ib	35.7	Right proximal humerus	Late-onset osteomyelitis
136	Ib	28.7	Blood	Early-onset sepsis
137	Ib	25.5	Blood	Early-onset sepsis
162	Ic	45.0	Blood	Early-onset sepsis
164	Ic	275.3	Blood	Early-onset sepsis
165	Ic	262.0	Blood	Early-onset sepsis
166	Ic	46.3	Blood	Early-onset sepsis
172	Ic	44.4	Blood	Early-onset sepsis
113	II	49.3	Blood	Early-onset sepsis
128	II	52.7	Blood	Early-onset sepsis
155	II	102.3	Blood	Early-onset sepsis
156	II	81.7	Blood	Early-onset sepsis
169	II	58.1	Blood	Early-onset sepsis
170	II	<10	Blood	Early-onset sepsis
171	II	60.1	Blood	Early-onset sepsis

[a] Cells were grown and neuraminidase was assayed as described in Table 3.
[b] CSF, cerebrospinal fluid.

TABLE 6. *Clinical isolates of group B streptococci from invasive neonatal disease classified by serotype and ability to produce extracellular neuraminidase*

Determination	Serotype of isolate (no. of strains)	
	III	Ia, Ib, Ic, or II
Total neuraminidase activity[a]		
>140	20	3
≤140–>10	0	21
<10	6	2
Proportion with activity >140[b]	20/26	3/26

[a] Expressed as nanomoles per minute per milligram of cell dry weight.
[b] χ^2, Yates corrected = 19.96 ($P << 0.001$).

ponents aid in the reproducible recovery of extracellular neuraminidase by (i) functioning as carrier protein during ammonium sulfate precipitation, (ii) acting as competitive substrates for the group B streptococcal protease(s), thus "protecting" the extracellular neuraminidase, and (iii) possibly inhibiting some of the binding of extracellular neuraminidase to the membrane filters used in preparing cell-free supernatants. However, even in the presence of these two

TABLE 7. *Clinical isolates of type III group B streptococci classified by ability to produce neuraminidase and whether isolated from asymptomatically colonized or diseased infants*

Determination	Source of isolate (no. of strains)	
	Infant with invasive disease	Asymptomatically colonized infant
Total neuraminidase activity[a]		
>140	20	8
≤140–10	0	0
<10	6	14
Proportion with activity >140[b]	20/26	8/22

[a] Expressed as nanomoles per minute per milligram of cell dry weight.
[b] χ^2, Yates corrected = 6.48 ($P < 0.025$).

supplements, filtration had a marked effect in reducing the level of recoverable enzyme (Table 2) in six different strains of group B streptococci. The reproducibility of our assay is illustrated in Fig. 2, where 95% confidence intervals are shown for low (H36B, serotype Ib) and high (110, serotype III) control strains, which were included in each experiment in which a series of clinical isolates was examined for their ability to produce extracellular neuraminidase. These confidence intervals were obtained from eight separate experiments, and these strains always fell within our established categories of "low" and "high" producers. The effect of the addition of Todd-Hewitt broth deserves comment because a complex mixture of components was being added to the defined medium. Although it is possible that an inducer for neuraminidase might be present in Todd-Hewitt broth, the fact that only 10% of the maximal activity was detected upon the addition of Todd-Hewitt broth alone (Table 1) argues against this conclusion. A particularly intriguing observation arising from this study was the distribution of neuraminidase production among serotype III strains of group B streptococci. These type III isolates either were high producers or produced nondetectable levels of neuraminidase, in marked contrast to the other four serotypes, which, with few exceptions, all produced low but detectable levels of the enzyme (Fig. 2). It would appear, then, that regulation of the production of extracellular neuraminidase in serotype III strains is fundamentally different from the majority of non-type III strains.

The striking association between high neuraminidase production and serotype III strains from neonatal disease warrants discussion for several reasons. Although no significant difference exists in the distribution of serotypes of group B streptococci among infants, women in the third trimester of pregnancy, and hospital personnel (5, 6), serotype III strains have been shown to be particularly prominent among isolates from late-onset disease or meningitis at any age (6, 23, 24). The striking ability of type III strains of group B streptococci to invade and multiply in the leptomeninges of neonates and infants has been suggested to be related to the presence of sialic acid (8) in the capsular carbohydrate of these microbes, as has been shown in *Escherichia coli* K1 strains (20). However, all five serotypes of group B streptococci have been found to contain sialic acid in their capsular carbohydrate (10), while only type III strains are regularly associated with meningitis (6, 23). In our series of isolates from neonatal disease, we have shown the consistent production of high levels of extracellular neuraminidase only by type III strains. It may be that the potential of elaborating high levels of extracellular neuraminidase imparts on serotype III strains an enhanced ability to colonize and invade human infants. This concept has also been supported by the comparison of type III strains from asymptomatically colonized or diseased infants. Serotype III strains from infants with invasive disease contained a higher proportion of high neuraminidase producers (20/26) than type III strains from asymptomatically colonized infants (8/22, $P < 0.025$). These data are pertinent because three independent studies (1, 5, 12) have indicated that a large disparity exists between the rate of asymptomatic colonization of neonates and the rate of invasive disease by group B streptococci. Although the presence of significant levels of serum antibody to type III capsular polysaccharide in the mother may be of profound importance in determining whether an infant will suffer invasive disease (9, 11), it appears that additional microbial factors (e.g., ability to elaborate high levels of neuraminidase) may play an important role in determining whether an infant colonized with group B type III streptococci will subsequently develop systemic disease.

ACKNOWLEDGMENTS

This work was supported by Public Health Service research grant DE-04444 from the National Institute of Dental Research and by Public Health Service grant AI-13249 from the National Institute of Allergy and Infectious Diseases.

We thank Evelyn Oginsky for her continued encouragement and helpful review and criticism of the manuscript. We also thank J. C. Horner for his skilled technical assistance in the laboratory.

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