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Role of Neutrophil Degranulation in Streptococcal Leukotoxicity

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Cinemicrography and electron microscopy suggested that leukotoxic *Streptococcus pyogenes* killed polymorphonuclear neutrophils (PMN) by inducing intracytoplasmic rupture of the PMN granules. To further study the relationship between granule rupture and the mode of action of the streptococcal leukotoxin, PMN degranulation was experimentally altered. Exocytosis of PMN granule contents was blocked with 80 mM tetraethylammonium chloride, 2 mM dibutyryl cyclic adenosine monophosphate, or 2 mM magnesium ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetate in calcium-free medium. This treatment did not prevent the granules from firing into the cytoplasm of the PMN, nor did it significantly diminish leukotoxicity. Degranulating the PMN before exposure to the leukotoxic streptococci did partially block leukotoxicity if both the specific and the primary granules were released with either 5 μM calcium ionophore A23187 or 10% zymosan-activated serum plus 5 μg of cytochalasin B per ml. Leukotoxic streptococci stimulated intracytoplasmic rupture of granules, and this granule lysis contributed significantly to the ability of these streptococci to kill PMN.

When polymorphonuclear neutrophils (PMN) phagocytize or come in contact with many strains of *Streptococcus pyogenes* (group A, beta-hemolytic streptococci) the PMN are killed (13, 22). The leukotoxic factor has been identified by previous workers as cell-bound streptolysin S (14). From morphological studies it appears that PMN are killed with 3 min of contact with the microbes. This is associated with disappearance of granules from the PMN. We previously found that when PMN interact with leukotoxic streptococci, there are a six-times-greater β-D-glucuronidase release and a three-times-greater lysozyme release to the extracellular medium than when PMN interact with nonleukotoxic streptococci. Similarly, PMN incubated with leukotoxic streptococci release more granule enzymes to the extracellular medium than when they interact with streptococci in which the toxin has been heat inactivated (19). The purpose of this investigation was to study the mechanisms of streptococcal leukotoxicity and its relationship to PMN granule lysis.

Since one proposed mechanism of leukotoxicity relates to release of granules into the PMN cytoplasm, we attempted to intervene by using several maneuvers. We treated cells with agents that have been shown to affect degranulation. We used substances that blocked exocytosis (discharge of granule contents to the exterior of cells), and we "predegranulated" PMN before interaction with the toxic streptococci.

(This work was presented in part at the National Meeting of the American Federation of Clinical Research in San Francisco, Calif. in May 1978 and published in abstract form [G. W. Sullivan and G. L. Mandell, Clin. Res. **26**:357A, 1978].)

MATERIALS AND METHODS

The bacteria and PMN were prepared as previously reported (19).

Bacteria. *S. pyogenes* strain C203S was obtained from the American Type Culture Collection, Rockville, Md. The parent (C203) of the test strain was originally isolated from a patient with scarlet fever. Strain C203S is an M-protein-poor (T. T. Myoda and E. N. Fox, Bacteriol Proc., p. 94, 1967), streptolysin S-rich strain (14). On standard sheep blood agar plates this strain was beta-hemolytic on the plate surface, indicating the presence of streptolysin S.

The leukotoxicity of this strain was diminished only 6% by 50 μg of cholesterol, a known inhibitor of streptolysin O activity, per ml, but was inhibited 93% by 100 μg of trypan blue, a known streptolysin S inhibitor, per ml (the assay of leukotoxic activity is described below). In addition, there was no release of a leukotoxic factor to the supernatant fluid of bacterial cultures, indicating that the leukotoxicity in these assays was due to cell-bound streptolysin S rather than streptolysin O, which is found in the medium (14). Hemolytic activity was quantitated aerobically by the

method of Ginsburg et al. (7). There was 1 hemolytic unit in 2×10^5 colony-forming units of C203S.

Early-stationary-phase (5-h-old) brain heart infusion cultures were used. Bacteria from a 75-ml culture were washed in phosphate-buffered saline and resuspended in 3.6 ml of phosphate-buffered saline containing activation mixture, yielding the following final concentrations: 9 mM glucose, 6 mM $MgSO_4$, and 2 mM cysteine. This mixture enhances the activity of the toxin (14). The leukotoxic factor could be inactivated by heating washed suspensions of *S. pyogenes* to 56°C for 20 min. Heating decreased bacterial viability by 99%. Bacterial counts were performed in a Petroff-Hausser counting chamber and verified with serial dilution and plate counts on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) containing 0.4% defibrinated sheep blood. Microscopic examinations showed that there were 5.5 ± 0.5 (mean ± standard error) streptococci per chain. One colony-forming unit was equivalent to one chain.

PMN. Ninety-five-percent-pure PMN were obtained from normal heparinized (10 U/ml, Connaught Laboratories, Willowdale, Ontario, Canada) human venous blood by Ficoll-Hypaque separation (3) followed by dextran sedimentation and hypotonic lysis of remaining erythrocytes (4).

Assay of leukotoxicity. PMN (2.5×10^6) and streptococci (5×10^8 colony-forming units) were suspended in Hanks balanced salt solution (Microbiological Associates, Bethesda, Md.) and centrifuged at 200 × *g* for 15 min at room temperature to prepare a button. After incubation of the button at 37°C for 30 min, the supernatant fluid was decanted and frozen for later granule enzyme analysis (see below). Trypan blue (0.5% in 0.9% saline) was added to the button, and viable PMN were identified by trypan blue exclusion (5).

Degranulation. Granule enzyme activity released into the supernatant was measured for β-D-glucuronidase by quantitation of the generation of phenolphthalein from phenolphthalein-glucuronic acid (6).

Lysozyme activity was quantitated by measurement of changes in the optical density of a suspension of *Micrococcus lysodeikticus* (18) after addition of the supernatants.

Enzyme activity released to the surrounding medium was compared with amounts of enzyme released from PMN after disruption with 0.1% Triton X-100.

Effect of alterations of degranulation on leukotoxicity. The PMN suspensions were preincubated for 30 min at 37°C in a water bath under the conditions described below before addition of streptococci. PMN samples were simultaneously run without addition of streptococci. After preincubation the samples were assayed for PMN death and granule enzyme release as described above.

(i) Blocked exocytosis. Exocytosis is the process whereby granule contents are secreted to the exterior of the cell. This involves granule lysis. Lysis of granules with discharge of contents into the cytoplasm of the cell is a presumed mechanism of streptolysin S leukotoxicity. We studied agents that decrease exocytosis to clarify the relationship between these two processes that both involve discharge of granule contents. Granule lysis and leukotoxicity were studied with no additions and with the following exocytosis-blocking agents: (i) 80 mM tetraethylammonium chloride (TEA) (it has been previously reported [25] that 80 mM TEA blocks *Staphylococcus* leucocidin activity and its accompanying β-D-glucuronidase release from PMN, presumably by blocking PMN potassium efflux), (ii) 2 mM dibutyryl cyclic adenosine monophosphate–1 mM theophylline (these agents together tend to increase the cellular cyclic adenosine monophosphate level, which in turn blocks particle or soluble agent-stimulated enzyme release [27]) with cytochalasin B (5 μg/ml) added to maximize enzyme release to the outsides of the PMN by blocking phagosome formation (26) (this facilitated measurement of exocytosis), and (iii) Ca^{2+}-free medium plus 2 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetate (external calcium promotes release of granule enzymes [especially β-D-glucuronidase] from stimulated PMN, whereas removal of calcium blocks degranulation [8, 10]).

(ii) Degranulation of PMN before contact with streptococci. Specific granules were released by treatment of PMN with 100 ng of phorbol myristate acetate per ml. Phorbol myristate acetate is a proinflammatory agent which promotes the release of specific granules from PMN (9). Specific and primary granules were released by treatment of PMN with 10% zymosan-activated serum–5 M calcium ionophore A23187. Both of these agents stimulate PMN to release both specific and primary granules. Cytochalasin B (5 μg/ml) was added with the calcium ionophore and zymosan-activated serum because it enhances their activity (8, 10).

Cinemicrography. Cinemicrography was done with a Zeiss photomicroscope fitted with a 16-mm Bolex movie camera as previously reported (4).

Electron micrographs. PMN (1×10^7/ml) and leukotoxic or heated leukotoxic streptococci (1.5×10^8/ml) prepared as described above were tumbled together at 37°C in either Hanks balanced salt solution or calcium-free Hanks balanced salt solution containing 0.2 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetate for 45 min. Samples without streptococci were also prepared. After incubation, samples were withdrawn for trypan blue staining as described above and electron micrograph preparation.

Samples for electron microscopy were fixed in an equal volume of phosphate-buffered saline (pH 7.4) containing 2% glutaraldehyde, centrifuged at 200 × *g* for 5 min, and refrigerated for 24 h at 4°C. After fixation the samples were postfixed in osmium tetroxide for 60 min, dehydrated in ethanol and propylene oxide, and embedded in Epon 812 (Shell Chemical Co., Houston, Tex.). After curing at 55°C for 2 to 3 days, the samples were sliced (650 nm) with an ultramicrotome, stained with uranyl acetate and lead citrate, and observed and photographed with an electron microscope (100-S; JEOL USA, Medford, Mass.).

Chemicals. Ficoll, dibutyryl cyclic adenosine monophosphate, theophylline, cytochalasin B, ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetate, zymosan A and ε-amino-*n*-caproic acid were obtained from Sigma Chemical Co., St. Louis, Mo. Hypaque was purchased from Winthrop Laboratories, New York, N.Y. Dextran was purchased from Pharmacia

Fine Chemicals, Inc., Piscataway, N.J. TEA monohydrate was obtained from Aldrich Chemical Co., Milwaukee, Wis. Phorbol myristate acetate was acquired from Consolidated Midland Corp., Brewster, N.Y. The calcium ionophore A23187 was the generous gift of Robert S. Gordee, Eli Lilly & Co., Indianapolis, Ind.

Stock solutions of cytochalasin B, phorbol myristate acetate, and A23187 were made up at 1,000- to 100,000-fold concentrations in dimethyl sulfoxide. Dimethyl sulfoxide by itself did not significantly diminish leukotoxicity.

Zymosan-activated serum was produced by incubating 1 mg of zymosan A per ml–250 mM ε-amino-*n*-caproic acid for 30 min at 37°C in fresh normal serum. The zymosan was removed by centrifuging the suspension at 3,000 × g for 5 min followed by membrane filtration through a 0.45-μm-pore membrane filter (8) (Gelman Sciences, Inc., Ann Arbor, Mich.).

Statistical analysis. Data were analyzed with Student's *t* test (unpaired data). Results are expressed as means ± standard error.

RESULTS

***Streptococcus*-leukocyte interaction.** Both movie sequences and electron micrographs confirmed that PMN incubated with leukotoxic streptococci show extensive loss of granules (Fig. 1 and 2). PMN that came in contact with leukotoxic streptococci stopped moving, became rounded, and extended cytoplasmic blebs. The granules ceased streaming and then burst. After granule lysis, the PMN swelled and the few remaining granules exhibited Brownian motion. Observations of living cells showed that the plasma membrane remained intact, and there was no evidence of whole, intact granules being released to the external medium. In contrast, phagocytosis of heat-killed leukotoxic streptococci resulted in moderate degranulation and no cytoplasmic disruption (Fig. 2).

Effect of alterations of degranulation on leukotoxicity. (i) Blocked exocytosis. Table 1 shows results of experiments performed without bacteria. There was very little exocytosis and only negligible effects of those agents that block exocytosis. The results (Table 1) indicated that the agents used did not kill large numbers of PMN and that the agents had little effect on exocytosis of resting cells. Cytochalasin B was used to maximize granule discharge to the outside of the cell rather than to a phagosome if a degranulating stimulus was present.

Table 2 shows the results of experiments performed with the potent degranulating stimulus of leukotoxic streptococci. Preincubation with 80 mM TEA reduced the release of β-D-glucuronidase from the PMN incubated with leukotoxic streptococci, indicating that exocytosis from primary granules was blocked. TEA did not block the release of lysozyme. Leukotoxicity was not diminished by TEA.

Dibutyryl cyclic adenosine monophosphate (2 mM) reduced both lysozyme release and β-D-glucuronidase release from PMN that interacted with leukotoxic streptococci. Theophylline (1 mM) blocked only β-D-glucuronidase release. Dibutyryl cyclic adenosine monophosphate plus theophylline plus cytochalasin B significantly blocked the releases of both lysozyme and β-D-glucuronidase, but leukotoxicity was not diminished (Tables 1 and 2).

Calcium-free Hanks balanced salt solution and calcium-free Hanks balanced salt solution plus 2 mM magnesium ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetate significantly blocked both lysozyme and β-D-glucuronidase releases compared with nonblocked PMN, but these conditions did not diminish leukotoxicity. Electron microscopy suggested that leukotoxic

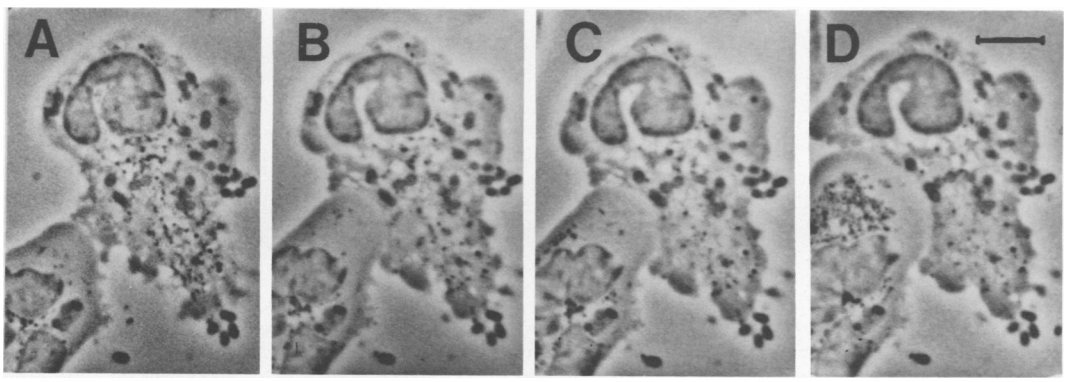

FIG. 1. *Sequence (A through D) of cinemicrographs of PMN interacting with leukotoxic streptococci, showing intracytoplasmic rupture of the granules. The PMN in the center has ingested leukotoxic streptococci. Numbers of granules decreased from (A) to (D). Undamaged PMN enters from lower left corner, and normal numbers can be seen in this cell in (D). Bar represents 5 μm. The elapsed time was about 2 min.*

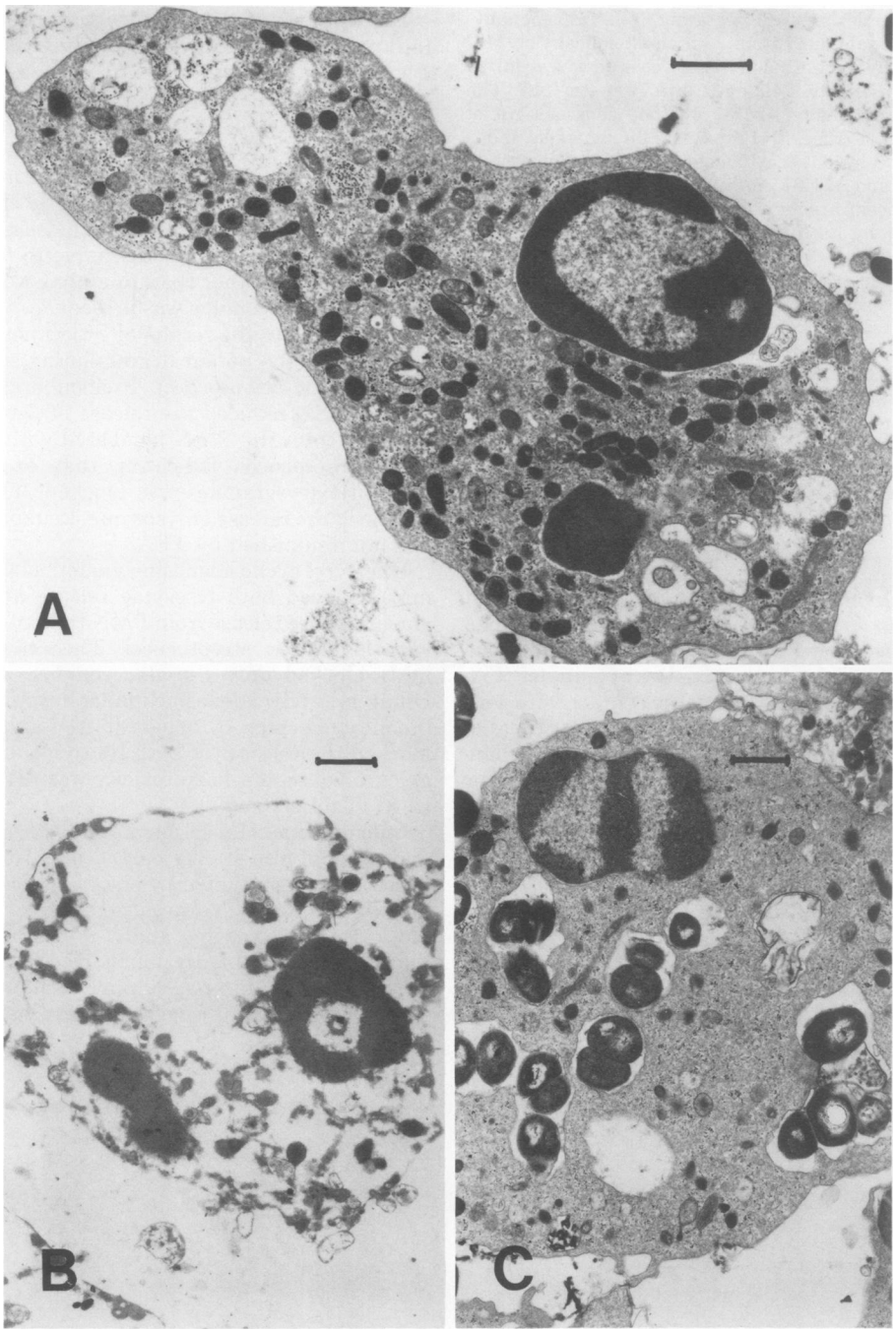

FIG. 2. *Electron microscopy of PMN-Streptococcus interaction. The sample without bacteria (A) showed intact viable PMN (99% trypan blue exclusion) containing many granules. When leukotoxic streptococci were added (B), the PMN became rounded and swollen, and the cell degranulated extensively into the cytoplasm, destroying the cell interior, even though few of the unopsonized leukotoxic bacteria were phagocytized. In contrast, PMN remained intact and viable after phagocytizing unopsonized heat-killed leukotoxic streptococci (C). Bars represent 1 μm.*

TABLE 1. *Blocked exocytosis of PMN with no bacteria*

Addition	Mean % ± standard error[a]		
	Trypan blue uptake	Lysozyme release	β-D-Glucuronidase release
None	3.4 ± 0.9 (9)	12.1 ± 2.1 (8)	6.1 ± 2.8 (8)
80 mM TEA	12.5 ± 3.5 (2)	8.5 ± 1.5 (2)	6.0 ± 2.0 (2)
2 mM dibutyryl cyclic adenosine monophosphate	10.0 ± 0.0 (1)	7.6 ± 0.0 (1)	2.8 ± 0.0 (1)
1 mM theophylline	8.0 ± 0.0 (1)	9.1 ± 0.0 (1)	2.1 ± 0.0 (1)
2 mM dibutyryl cyclic adenosine monophosphate + 1 mM theophylline + 5 μg of cytochalasin B per ml	3.2 ± 1.7 (4)	8.3 ± 0.6 (4)	3.8 ± 0.5 (4)
Ca^{2+}-free Hanks balanced salt solution	5.5 ± 1.0 (6)	9.2 ± 0.9 (5)	1.4 ± 0.8 (5)
Ca^{2+}-free Hanks balanced salt solution + 2 mM magnesium ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetate	7.6 ± 1.9 (5)	10.6 ± 0.8 (5)	5.8 ± 1.1 (5)

[a] Numbers of separate experiments are in parentheses.

TABLE 2. *Blocked exocytosis of PMN with leukotoxic streptococci*

Addition	Trypan blue uptake		Lysozyme release		β-D-Glucuronidase release	
	% (mean ± SEM)[a]	P^b	% (mean ± SEM)[a]	P^b	% (mean ± SEM)[a]	P^b
None	92.8 ± 2.0 (18)		46.2 ± 5.6 (18)		24.7 ± 3.1 (18)	
80 mM TEA	98.5 ± 0.3 (4)	0.104	57.2 ± 6.8 (4)	0.191	2.7 ± 0.8 (4)	0.002
2 mM dibutyryl cyclic adenosine monophosphate	88.5 ± 3.5 (2)	0.253	26.5 ± 2.4 (2)	0.154	4.8 ± 0.0 (2)	0.028
1 mM theophylline	92.0 ± 0.0 (2)	0.450	45.6 ± 9.7 (2)	0.487	4.6 ± 2.1 (2)	0.026
2 mM dibutyryl cyclic adenosine monophosphate + 1 mM theophylline + 5 μg of cytochalasin B per ml	98.5 ± 0.4 (8)	0.039	16.7 ± 1.7 (8)	0.001	7.7 ± 1.2 (8)	0.001
Ca^{2+}-free Hanks balanced salt solution	86.8 ± 9.5 (10)	0.217	19.3 ± 2.9 (11)	<0.001	8.9 ± 0.9 (11)	<0.001
Ca^{2+}-free Hanks balanced salt solution + 2 mM magnesium ethylene glycol-bis(β-aminoethyl ether)-*N,N*-tetraacetate	99.7 ± 0.1 (10)	0.009	9.4 ± 1.1 (10)	<0.001	10.7 ± 1.1 (10)	0.002

[a] SEM, Standard error of the mean. Numbers of separate experiments are in parentheses.
[b] For results compared with those with no addition; calculated by Student's *t* test with unpaired data.

streptococci caused granules to rupture in the cytoplasm of the cell despite blocked exocytosis (Fig. 3).

(ii) Degranulation of PMN before contact with streptococci. Degranulation of PMN specific granules before contact with streptococci was studied. Nonphagocytizing PMN treated with 100 ng of phorbol myristate acetate per ml released significantly more lysozyme than did PMN not exposed to phorbol myristate acetate. There was no increase in β-D-glucuronidase release compared with nonstimulated PMN. Predegranulation of specific granules did not significantly diminish streptococcal toxicity (Table 3).

Predegranulation of PMN specific plus primary granules was also studied. Incubation with 10% zymosan-activated serum plus 5 μg of cytochalasin B per ml released significant amounts of both lysozyme and β-D-glucuronidase from PMN, and leukotoxicity was diminished significantly. Calcium ionophore A23187 plus cytochalasin B (5 μg/ml) stimulated the release of significant amounts of both lysozyme and β-D-glucuronidase from PMN, and leukotoxicity was significantly reduced (Table 3). These agents did not directly affect the toxin, as evidenced by the observations that leukotoxic streptococci pretreated with calcium ionophore and cytochalasin B remained highly leukotoxic.

Predegranulation appeared to reduce leukotoxicity to both aggregated and nonaggregated

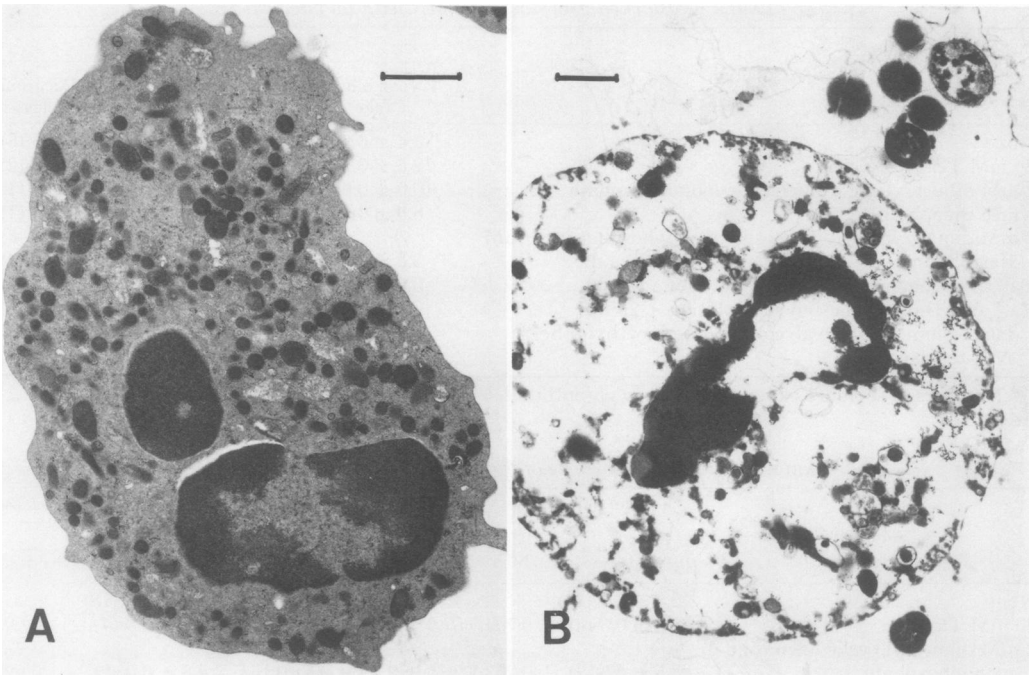

FIG. 3. *Electron micrographs showing the effect of blocked exocytosis on leukotoxicity. Resting PMN in the absence of calcium (A) contained many granules and excluded trypan blue (100%). When the PMN were interacted with leukotoxic streptococci in the absence of calcium (B), the PMN were destroyed and degranulated, although there was little appearance of granule enzymes in the surrounding media (0% trypan blue exclusion). Bars represent 1 μm.*

TABLE 3. *Predegranulation of PMN*

Addition	PMN						Trypan blue uptake of PMN + leukotoxic streptococci	
	Trypan blue uptake (mean % ± SEM)[a]	Lysozyme release		β-D-Glucuronidase release			% (mean ± SEM)[a]	P^b
		% (mean ± SEM)[a]	P^b	% (mean ± SEM)[a]	P^b			
None	3.4 ± 0.9 (9)	12.1 ± 2.1 (8)		6.1 ± 2.8 (8)			92.8 ± 2.0 (18)	
100 ng of phorbol myristate acetate per ml	3.7 ± 2.2 (3)	33.7 ± 2.6 (3)	<0.001	7.3 ± 0.7 (3)	0.402	96.3 ± 1.8 (6)		0.174
5 μg of cytochalasin B per ml	6.3 ± 1.6 (7)	14.4 ± 3.6 (5)	0.246	29.5 ± 2.3 (4)	<0.001	83.9 ± 2.6 (17)		0.005
5 μM A23187	22.6 ± 1.3 (5)	63.2 ± 17.3 (5)	0.002	20.6 ± 3.7 (5)	0.005	70.1 ± 7.9 (10)		<0.001
5 μM A23187 + 5 μg of cytochalasin B per ml	15.7 ± 4.6 (3)	114.0 ± 16.8 (3)	<0.001	35.0 ± 0.0 (3)	<0.001	60.5 ± 2.6 (6)		<0.001
10% zymosan-activated serum + 5 μg of cytochalasin B per ml	4.0 ± 0.6 (3)	43.3 ± 2.8 (3)	<0.001	20.0 ± 2.5 (3)	0.010	71.2 ± 3.0 (6)		<0.001

[a] SEM, Standard error of the mean. Numbers of separate experiments are in parentheses.
[b] For results compared with those with no addition; calculated by Student's *t* test with unpaired data.

PMN. This indicated that these substances prevented toxicity by more than just reducing contact between PMN and bacteria.

DISCUSSION

Wilson (22) analyzed cinemicrographic studies of PMN that had phagocytized leukotoxic streptococci. He noted that leukotoxic streptococci caused PMN death and that this was characterized by several events, including cessation of PMN granule motion, granule lysis with marked increases in random motion of remaining granules, and cell swelling. Others made similar observations when PMN were exposed to the pur-

ified leukotoxic factor, streptolysin S (11). It has been shown also that streptolysin S, unlike the leucocidins from *Pseudomonas aeruginosa* (17) and *Staphylococcus aureus* (23), will lyse isolated granules from rabbit liver cells (21). Our cinemicrographic, electron microscopic, and granule enzyme assays confirm that when PMN interact with leukotoxic streptococci, the PMN degranulate more extensively than when they interact with heated leukotoxic streptococci. To study the relationship between toxin-induced degranulation and PMN death, in certain experiments exocytosis was blocked and in others PMN were degranulated before exposure to the toxic streptococci.

Calcium is essential for phagocytosis-induced degranulation of the PMN primary granules (8, 10) and degranulation stimulated by other leucocidins (16, 20, 23, 24). In the present study, removal of calcium from the external medium effectively prevented the appearance of the granule enzymes (especially β-D-glucuronidase) in the external medium, but still did not prevent PMN death or extensive internal degranulation, as revealed by both movie sequences and electron microscopic examination of PMN that were exposed to leukotoxic streptococci. Thus, exocytosis was prevented, but toxin-induced rupture of the granules into the PMN cytoplasm and subsequent PMN death were not blocked.

Kane et al. (12) found that removal of calcium blocked the lethal effect of silica ingested by macrophages. As we observed with streptolysin S, they observed that degranulation did occur in the absence of calcium, and thus they separated intracellular lysosomal rupture from cell death. But our results differed from those of Kane et al. in that removal of calcium did not protect the PMN from the lethal effects of leukotoxic streptococci, and therefore the streptococcal leukotoxic factor differs from 10 other cytotoxic membrane-active agents whose toxicity is blocked by removal of calcium from the medium (15).

Other blockers of degranulation also failed to prevent PMN death. The TEA ion blocks the potassium channel in nerves (2) and inhibits the leukotoxic activity of staphylococcal leucocidin (25). TEA also inhibits efflux of potassium, release of β-D-glucuronidase, and swelling of PMN. We found that TEA inhibited release of β-D-glucuronidase from PMN interacting with leukotoxic streptococci, but did not diminish leukotoxicity. This discrepancy suggests different modes of action for streptolysin S and staphylococcal leucocidin.

Cyclic adenosine monophosphate inhibits the release of PMN granules when PMN are stimulated by both particles and soluble agents (27). In this study, 2 mM dibutyryl cyclic adenosine monophosphate plus 1 mM theophylline blocked release of both lysozyme and β-D-glucuronidase from leukotoxic-*Streptococcus*-stimulated PMN, but did not diminish PMN death.

Blockage of exocytosis by removal of Ca^{2+} from the external medium or by other means did not prevent PMN death or intracellular rupture of granules, but did prevent the appearance of granule enzymes in the external medium. We think that the breaks in the PMN membrane seen in Fig. 2B and 3B are artifacts, since if they are true "holes," then granule enzymes should escape to the outside of the cells in all cases. The lack of immediate passive leakage of granule enzymes to the outsides of the cells is perhaps a reflection of the relatively small, functional holes produced in membranes by streptolysin S compared with those produced by other membrane-active agents (1). Therefore, toxin-induced granule firing was different from the granule firing that could be blocked by blockers of exocytosis.

We next attempted to fire the granules before incubation with the leukotoxic streptococci. Preincubating the PMN with either zymosan-activated serum or the calcium ionophore A23187 plus cytochalasin B resulted in diminished leukotoxicity. Both of these agents stimulated the release of both specific and primary granules, as expected from previous studies (8, 10). Phorbol myristate acetate, which stimulates release of only the more labile specific granules (9), did not affect leukotoxicity. This implies that it is the primary granules which are important in streptococcal leukotoxicity.

It appears that leukotoxic streptococci induce intracytoplasmic granule lysis (independent of extracellular calcium), which is distinct from phagocytosis-stimulated degranulation. Release of primary granule contents into the cell sap disorganizes cytostructural elements and results in cell death.

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