Effect of infection with gonococci on myeloperoxidase activity of leucocytes

WARREN W. SCHULTZ AND DAVID W. THOMAS

Department of Microbiology, Naval Medical Research Institute, Bethesda, Maryland 20014, U.S.A.

The ability of the virulent type of *Neisseria gonorrhoeae* to resist or survive in the presence of the polymorphonuclear leucocyte may play a role in this organism's virulence and in the production of a carrier state. Recent studies (Thongthai and Sawyer, 1973) indicate that the virulent type (Type I) (Kellogg, Peacock, Deacon, Brown, and Pirkle, 1963) of gonococcus is more resistant to phagocytosis by human neutrophils than the avirulent type (Type IV), but both are killed when phagocytosed. In our previous study (Thomas, Hill, and Tyeryar, 1973), it was shown that a greater number of cell-associated virulent Type I (T_1) than avirulent Type IV (T_4) gonococci remain viable after incubation with neutrophils for 2.5 hrs in the presence of heated serum. The viability of virulent gonococci, however, was greatly reduced when unheated serum was used. This suggests that these organisms remained so located as to be susceptible to the bactericidal action of serum.

Myeloperoxidase, a lysosomal enzyme in human neutrophils, acts synergistically with H_2O_2 and a halide to form an active bactericidal system when in direct contact with bacteria (Klebanoff and Hamon, 1972; Sbarra, Paul, Strauss, and Mitchell, 1972). When radioactive iodide is employed as the halide, an iodine compound is formed which fixes to the bacteria, and under certain conditions the degree of iodination parallels bactericidal activity due to lysosomes. This reaction, then, can be used to monitor lysosomal activity. It has also been shown (Dingle, Fell, and Coombs, 1967; Fell, 1969; Schultz, 1972) that lysosomal activation and subsequent enzyme release does not necessarily require or imply phagocytosis. Punsalang and Sawyer (1973) and Swanson (1973) demonstrated that T_1 adheres to certain mammalian cells and is not necessarily phagocytosed. It is possible that T_1 gonococci activate the lysosomal system from their extracellular location. We investigated, therefore, the difference in capabilities of T_1

and T_4 to stimulate the lysosomal system of the leucocytes.

Material and methods

BACTERIAL CULTURES

For our studies the following strains of *Neisseria gonorrhoeae* were used: F62- T_1 and RD5- T_4 (obtained from Dr. D. S. Kellogg, Jr., Center for Disease Control, Atlanta, Ga.), a T_4 isolated in our laboratory from the F62- T_1 culture, and a T_1 and T_4 derived in our laboratory from ATCC-19424. These organisms were grown and stored as previously described (Thomas and others, 1973). The two last-named isolates are resistant to the bactericidal activity of normal human serum. For each experiment, 16 to 18-hr cultures were harvested from plates with modified Hanks's solution (MHS) (Martin and Green, 1953) that contained 0.1 per cent. crystallized bovine serum albumin glucose (HBG). A uniform suspension of bacteria was obtained by agitation for 15 sec. on a Vortex mixer in the presence of Superbrite glass beads (type 070-5005; 3M Co., St. Paul, Minn.). The bacteria were sedimented by centrifugation at 1950 G at 25°C. for 10 min. and re-suspended in HBG to the desired concentration. Serially diluted samples of the working suspension were plated to ensure that bacterial viability was at least 90 per cent. in a given experiment.

LEUCOCYTES AND SERA

Human leucocytes and sera were obtained from adult male donors with no previous history of gonococcal infection and with a negative indirect fluorescent antibody test (H. A. Gaafar and D. D'Arcangelis, Abstr. Annu. Meet. Amer. Soc. Microbiol., p. 114, 1972; performed by Dr. W. D. Lawton, New York State Department of Health). Serum fractions were prepared by standard DEAE-cellulose ion exchange chromatography and ammonium sulphate precipitation. The fractions were tested for relative immunoglobulin composition by the Mancini radial immunodiffusion method (Mancini, Carbonara, and Heremans, 1965). Leucocytes were obtained by dextran sedimentation of peripheral blood. About 35 ml. blood were collected by venepuncture into a plastic syringe containing 20 ml. heparinized (25 U per ml.) MHS containing 3 per cent. dextran 150 (Pharmacia, Uppsala, Sweden). The syringe was placed in an upright position for 30 min. at room temperature. The leucocyte-rich fraction was removed and the cells sedimented by centrifugation at 150 G for 10 min. at 32°C. The pelleted

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cells were re-suspended in heparinized distilled water for 20 sec. to lyse any residual red blood cells and an equal volume of double strength heparinized HBG was then added. The suspension was centrifuged at 250 G for 10 min. at 22°C. and the sedimented leucocytes were then re-suspended to the desired concentration in HBG. Neutrophils represented about 80 to 90 per cent. of the cells in the final suspension and viability ranged from 95 to 99 per cent. as determined by dye exclusion (0.15 per cent eosin Y).

MYELOPEROXIDASE TECHNIQUE

The myeloperoxidase technique employed was a modification of Klebanoff's procedure (Klebanoff, 1967). The reaction mixtures were placed in twelve 75 mm. plastic tubes and contained the following: 3 to 5×10^6 neutrophils, 10 per cent. serum or HBG, gonococci at ratios of 5 to 15 per neutrophil and about 2 μ Ci of carrier-free 125 Iodine in a total volume of 0.5 ml. Each reaction was performed in triplicate. After rotary incubation (110 rpm for 30 min. at 37°C.) the samples were washed four times by centrifugation and re-suspension, once in HBG (250 G for 10 min. at 22°C.) and thrice with ice-cold 5 per cent. trichloroacetic acid (390 G for 10 min. at 5°C.). The counts per minute (cpm) of 125 Iodine in the precipitates after the fourth washing were determined in a gamma counter.

Results

Table I illustrates typical results of leucocyte lysosomal myeloperoxidase activity as determined by the amount of iodination obtained when using serum-

resistant strains of virulent and avirulent gonococci. With the avirulent type much greater myeloperoxidase activity was observed than with the virulent type in the absence of serum. There was a large increase in the activity induced by the virulent type when heated serum was added while there was no increased activity with T_4 . In the case of T_1 , the activity began to approach that of T_4 when complement or other heat labile serum substances were present. When incubation time was extended to 60 min. there was little change in activity induced by T_4 with or without unheated serum, whereas a 2 to 3-fold increase occurred with T_1 .

Table II shows the effect of bacterial multiplicity on myeloperoxidase activity. A pair of serum-sensitive strains were used in this experiment. It can be seen that with T_4 there was increased myeloperoxidase activity, whereas with T_1 there was little further stimulation of activity with higher multiplicity. As was observed with serum-resistant gonococci, T_1 serum-sensitive gonococci stimulated to a much lesser extent than T_4 and serum enhanced the activity induced by T_1 . Unheated serum was not used here because it is bactericidal for these strains in the absence of leucocytes.

An attempt was made to determine which serum fraction was responsible for the increased activity observed when gonococci were incubated in the presence of serum (Table III). Fraction I contained

TABLE I *Myeloperoxidase activity of human leucocytes incubated with gonococci*

Experiment	Serum added (per cent.)		Colonial type ^b			
	Heated ^a	Unheated	T_1		T_4	
			Time (min.)		Time (min.)	
			30	60	30	60
			(cpm $\times 10^{-3}$ of incorporated ^{125}I)			
1	—	—	1.6 ^c		81	
	10	—	32 ^d		69	
	5	5	65		95	
	—	10	86		103	
2	—	—	2.8	7.1	167	159
	—	10	37	71	128	157

^aAt 56° C. for 30 min.

^bDerived from ATCC 19424 and resistant to the bactericidal activity of normal serum. Multiplicity of inoculation in both experiments:5

^cMeans of three samples after subtraction of leucocyte background and gonococcus background

^dThese reactions were inhibited by 0.20 mM sodium cyanide

TABLE II *Myeloperoxidase activity of human leucocytes incubated with increasing numbers of gonococci*

Serum added ^a	Strain ^b	Gonococcal multiplicity per leucocyte		
		5	10	15
		(cpm $\times 10^{-3}$ of incorporated ^{125}I)		
None	F62- T_1	1.3	0.7	2.7
	RD5- T_4	38	72	91
10 per cent.	F62- T_1	25	29	39
	RD5- T_4	46	74	129

^aHeated at 56° C. for 30 min.

^bBoth strains susceptible to the bactericidal activity of normal serum

only IgG, Fraction II contained a mixture of IgG, IgA, and IgM, and Fraction III contained no measurable immunoglobulins. Crystallized bovine serum albumin was used as an additional control. Fraction I, and to a less extent Fraction II, was stimulatory and this stimulation can be attributed to IgG. Fraction I was at least as active as whole serum despite its much lower total protein. As in previous experiments, activity stimulated by T_4 strains was greater than that by T_1 . Fraction III stimulated activity to a small extent or not at all and in the case of T_1 was comparable to that in the absence of serum (not shown). These results were obtained with both serum-sensitive and serum-resistant strains.

Discussion

Thongthai and Sawyer (1973) reported that T_4 gonococci, which are non-piliated, are phagocytosed more readily than the pilated T_1 . The results of our previous investigation (Thomas and others, 1973) with the same strains of gonococci and the same host cell donors clearly indicate that T_1 gonococci adhere to the outer surface of the neutrophil whereas T_4 are phagocytosed. These results were confirmed by Punsalang and Sawyer (1973) and Swanson (1973) who demonstrated that T_1 actually adhere to various mammalian cells. Our results here are compatible with these findings, that is, T_4 is readily taken into the cell and this event leads to a high level of myeloperoxidase or lysosomal activity, whereas with T_1 a significant fraction of the population adheres to the surface and the results are variable.

In the absence of serum, T_1 stimulated very little or negligible lysosomal activity. Activity was stimulated to a considerable extent, however, in the presence of serum, particularly IgG. This was true of serum-susceptible as well as serum-resistant strains. This effect appeared to be qualitatively different from activity stimulated by T_4 . In the latter case, enhancement by serum was not always observed and, in

contrast to T_1 , was dependent (within limits) on multiplicity of infection. From the results cited above (Thomas and others, 1973; Punsalang and Sawyer, 1973; Swanson, 1973), together with our current findings, it appears that T_1 can stimulate lysosomal activity from its extracellular position. For this stimulation serum (heated or unheated) is required but, in most cases, the activity is less than that observed with intracellular T_4 . Ward, Watt, and Glynn (1970) have shown that gonococci in urethral exudates are serum resistant. The extracellularly stimulated lysosomal activity may, therefore, be a killing mechanism for serum-resistant, membrane-associated gonococci and may account for some of the increased killing of T_1 that we previously observed when these organisms were incubated in the presence of serum.

Summary

Lysosomal myeloperoxidase activity in human phagocytic leucocytes was stimulated by incubation with virulent (T_1) and avirulent (T_4) forms of *Neisseria gonorrhoeae*. The amount of activity, assayed by bacterial iodination (125 I) after 30 min. exposure to the phagocytes in the absence of serum, was about fifty times greater in cells infected with T_4 strains. In the presence of heated human serum, or its IgG component, myeloperoxidase activity increased, but T_1 -stimulated activity was significantly less than that of T_4 and was not proportional to multiplicity of infection. From these results and from those of a previous study we conclude that T_1 can stimulate leucocyte myeloperoxidase activity from an extracellular location, that for this activity a serum fraction is required, and that this may be a mechanism responsible for some of the killing of the membrane associated T_1 .

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TABLE III *Myeloperoxidase activity of human leucocytes incubated with various serum fractions*

Strain	Whole serum ^a	Fractions			Crystallized bovine serum albumin
		I	II	III	
		IgG	49% IgG 29% IgA 22% IgM 8 mg/ml	No IgG IgA or IgM 16 mg/ml	
	81 mg/ml	7 mg/ml			20 mg/ml
(cpm $\times 10^{-3}$ of incorporated 125 I)					
F62- T_1	18	12	11	3.2	1.9
RD5- T_4	40	49	19	21	32
F62- T_1	13	28	6.6	0.1	0.4
F62- T_4	18	45	15	9	8
ATCC- T_1	25	23	4.1	3.2	4.3
ATCC- T_4	79	161	39	Not done	Not done

^aHeated at 56° C. for 30 min.

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