

Phagocytosis and killing of *Gardnerella vaginalis* by human neutrophils

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SUMMARY *Gardnerella vaginalis* was ingested and killed by neutrophils in the presence of normal human serum. Heat inactivation of the serum inhibited these processes. The opsonisation of some but not all *G vaginalis* strains was enhanced by immune rabbit serum. Immune serum did not, however, enhance intracellular killing. Blockade of the classical pathway of complement activation had no effect on the opsonic activity of human serum. These results suggest that the opsonisation, phagocytosis, and killing of *G vaginalis* by human serum and neutrophils is primarily mediated by complement activated by the alternative pathway. Serum without neutrophils has little effect on the viability of *G vaginalis*.

Gardnerella vaginalis is closely associated with bacterial vaginosis,¹ which is a superficial infection of the vaginal mucosa. Occasionally, *G vaginalis* causes invasive disease such as endometritis, amnionitis, and bacteraemia.^{2,3} Recently, it has been implicated as a possible cause of preterm labour and other complications of pregnancy.⁴ Although some work has been done on the adherence of *G vaginalis* to cells,⁵⁻⁷ other aspects of its interaction with host defences have not been investigated.

We report here some preliminary studies on the opsonisation and phagocytosis of *G vaginalis* and its killing by human neutrophils, as phagocytosis is likely to be the main defence against invasive gardnerella infections.

Material and methods

BACTERIA

G vaginalis NCTC 10915 and four clinical isolates, strains 33, 35, 36, and 39, were used. Isolates were identified as previously described.⁸ *G vaginalis* was grown in 7% CO₂ either in peptone starch dextrose broth supplemented with 10% (vol/vol) horse serum or on bilayer Columbia agar plates, the upper layer being supplemented with 5% (vol/vol) time expired human blood.

OPSONISATION

For chemiluminescence and phagocytosis experi-

ments a washed 48 h culture of *G vaginalis* was incubated for 30 min at 37°C in Hank's balanced salt solution (HBSS), pH 7.3, buffered with 25 mM HEPES, to which had been added one of the following: 10% pooled human serum; 10% normal human serum heated for 30 min at 56°C; 10% human serum chelated with magnesium sulphate and ethylene glycol tetraacetate; or 5% normal human serum plus 5% immune rabbit serum raised against *G vaginalis*.

Bacteria were then washed and resuspended to the required concentration in HBSS.

PHAGOCYTOSIS

This was measured either by a simple microscopical assay or by luminol dependent chemiluminescence. Human neutrophils were separated from venous blood by dextran sedimentation and residual red cells were lysed by treatment with 0.83% Tris buffered ammonium chloride.⁹ After a wash, neutrophils were counted and resuspended to a concentration of 10⁶ cells/ml in HBSS without phenol red.

For microscopical assays bacteria and cells were incubated at a ratio of 5:1 for 15 min at 37°C. Smears were then prepared using a cytospin centrifuge (Shandon) and stained with Giemsa. The number of neutrophils with ingested bacteria was then counted.

For luminol dependent chemiluminescence a bacterial cell ratio of about 100:1 was used. Luminol was dissolved in dimethyl-sulfoxide and diluted to a final concentration of 2×10^{-5} mol/l. Luminescence

Table 1 *Opsonisation of Gardnerella vaginalis strains measured by microscopical assay and by luminol dependent chemiluminescence (CL)*

Opsonin source	Opsonisation of <i>G vaginalis</i> strain							
	Strain 10915		Strain 33		Strain 35		Strain 36	
	% Phagocytosis	CL (mV)	% Phagocytosis	CL (mV)	% Phagocytosis	CL (mV)	% Phagocytosis	CL (mV)
Unopsonised	31	51	36	36	28	10	42	24
10% normal human serum	72	222	76	74	59	44	90	225
10% normal human serum heated at 56°C for 30 min	33	60	35	32	29	19	40	59
10% human serum chelated with magnesium sulphate and ethylene glycol tetraacetate	69	214	72	74	61	33	88	207
5% normal human serum plus 5% immune rabbit serum	92	306	74	85	82	78	92	208

Table 2 *Opsonisation of four strains of Gardnerella vaginalis by normal human serum and three hyperimmune rabbit antisera*

Opsonin source	Opsonisation of <i>G vaginalis</i> strains							
	Strain 10915		Strain 35		Strain 36		Strain 39	
	% Phagocytosis	CL (mV)	% Phagocytosis	CL (mV)	% Phagocytosis	CL (mV)	% Phagocytosis	CL (mV)
Normal human serum	71	141	85	41	100	128	86	103
Normal human serum plus immune rabbit serum-167	91	176	92	65	100	131	97	111
Normal human serum plus immune rabbit serum-166	73	130	89	58	100	175	90	97
Normal human serum plus immune rabbit serum-167	65	124	82	28	100	133	82	76

Table 3 *Killing of Gardnerella vaginalis strains 10915 and 39 by normal human serum, immune rabbit serum, and human neutrophils*

Serum	Neutrophils	% Bacterial survival			
		Strain 10915		Strain 39	
		T ₄₅	T ₉₀	T ₄₅	T ₉₀
—	+	81	42	133	107
Normal human serum	+	18	6	38	12
Normal human serum heated to 56°C for 30 min	+	60	34	110	113
Normal human serum	—	93	59	147	123
Immune rabbit serum	+	22	11	48	26
Immune rabbit serum	—	86	71	100	133

T₄₅ = 45 min incubation; T₉₀ = 90 min incubation.

was measured at a fixed time of 15 min after initial mixing of cells and bacteria. A luminometer 1250 (LKB) was used and the reaction was measured at 37°C.⁹

PHAGOCYTOTIC KILLING

Neutrophils were mixed with *G vaginalis* at a bacterial cell ratio of roughly 5:1. The medium was HBSS supplemented with either 10% normal human serum, 10% normal human serum heated at 56°C for 30 min, or 5% normal human serum plus 5% immune rabbit serum raised against *G vaginalis*. Serum and cell free controls were included. Bacterial counts were made at the start of the experiment

and after 45 and 90 min by taking 0.1 ml aliquots of reaction mixture into 0.9 ml of distilled water and sonicating to disrupt the cells. Surface viable counts were then made on 10-fold dilutions of this sonicate.

IMMUNE RABBIT SERUM

Three immune rabbit sera (IRS) were prepared by intravenous injection of *G vaginalis* into New Zealand white rabbits using the method of Vice and Smaron.¹⁰ IRS-163 was raised against *G vaginalis* NCTC 10915. The other sera IRS-166 and IRS-167 were raised against clinical isolates of *G vaginalis* unrelated to those used in the phagocytosis experiments.

Results

Table 1 shows the phagocytosis of the NCTC strain of *G vaginalis* and the three clinical isolates measured by microscopical assay and chemiluminescence. Both techniques gave similar patterns of results. All four strains were opsonised by normal human serum and the opsonic activity was removed by heat inactivation. Blockade of the classical pathway of complement activation by chelating normal human serum with magnesium sulphate and ethylene glycol tetraacetate had no effect on the opsonisation of *G vaginalis*. The use of IRS-163 raised against *G vaginalis* 10915 produced a moderate enhancement of opsonisation of both this strain and strain 35 but not of strains 33 and 36.

We investigated this apparent heterogeneity of opsonic requirements among *G vaginalis* strains by comparing the opsonic effect of three immune rabbit sera against *G vaginalis* 10915, 35, 36, and 39. IRS-163 again produced a moderate enhancement of opsonisation of strain 10915 in comparison with normal human serum, but neither IRS-166 nor IRS-167 had any such effect. IRS-166 enhanced opsonisation of strains 35 and 36 but not of strain 39. IRS did not enhance the opsonisation of any of the four test strains (Table 2).

Assays of phagocytic killing were carried out with strains 10915 and 39. Both strains were resistant to killing by both normal human serum and immune rabbit serum in the absence of neutrophils. Unopsonised organisms were killed poorly by neutrophils. Killing was at its maximum in the presence of normal human serum and neutrophils. The use of IRS-163 in this system did not result in greater killing of *G vaginalis* than that seen with normal human serum. Finally, heat inactivation of serum prevented effective neutrophil killing (Table 3). The clinical strain 39 was more resistant to killing than the NCTC strain.

Discussion

G vaginalis is opsonised and killed by fresh human serum and neutrophils. These processes are complement dependent. None of the strains tested was susceptible to serum killing in the absence of neutrophils. Although complement dependent, opsonisation and phagocytosis were unaffected by blockade of the classical pathway. *G vaginalis* contains lipopolysaccharide, which is a potent activator of the alternative complement pathway.¹¹

The role of antibody is less clear. The hyper-immune rabbit antisera originally raised for immunofluorescence tests produced only moderate enhancement of opsonisation limited to certain strains and had no effect on intracellular killing. The

strain differences in response to antibody mediated opsonisation mirror differences in the immunofluorescence and enzyme linked immunosorbent assay reactions of *G vaginalis* strains that we have observed. This work needs to be extended as it may form the basis of a possible serotyping system.

The effectiveness of cells and serum in killing *G vaginalis* is what one would expect of an organism of low virulence. Yet in some circumstances *G vaginalis* can cause invasive disease, even bacteraemia. We need to see whether strains from such infections are more capable of resisting host defences.

Women and babies with such infections need to be studied to see if they have defects in their humoral or cellular immune responses to *G vaginalis*. We also need to see whether clinical infection results in the production of opsonic or bactericidal antibody and to investigate the surface structures of the organism and their capacity to activate the complement system.

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References

- Gardner HL, Dukes CD. A newly defined specific infection previously classified "non-specific vaginitis" *Am J Obstet Gynecol* 1955; **69**:962-76.
- Monif GRG, Baer H. Haemophilus (Corynebacterium) vaginalis septicemia. *Am J Obstet Gynecol* 1974; **120**:1041-5.
- Gardner HL. Haemophilus vaginalis vaginitis after twenty-five years. *Am J Obstet Gynecol* 1980; **137**:385-90.
- Eschenbach DS, Gravett MG, Chen KCS, Hoyme UB, Holmes KK. Bacterial vaginosis during pregnancy. An association with prematurity and post-partum complications. In: Mårdh P-A, Taylor-Robinson D, eds. *Bacterial Vaginosis*. Stockholm: Almqvist and Wiksell International, 1984:213-22.
- Mårdh P-A, Westrom L. Adherence of bacteria to vaginal epithelium. *Infect Immun* 1976; **13**:661-6.
- Sobel JD, Schneider J, Kaye D, Levison ME. Adherence of bacteria to vaginal epithelial cells at various times of menstrual cycle. *Infect Immun* 1981; **32**:194-7.
- Ison CA, Easmon CSF. Studies on the mechanism of adhesion of *Gardnerella vaginalis* to human erythrocytes. In: Mårdh P-A, Taylor-Robinson D, eds. *Bacterial Vaginosis*. Stockholm: Almqvist and Wiksell International, 1984:191-3.
- Ison CA, Easmon CSF. Carriage of *Gardnerella vaginalis* and anaerobes in semen. *Genito Urinary Medicine* (in press).
- Easmon CSF, Cole PJ, Williams AJ, Hastings MJG. The measurement of opsonic and phagocytic function by luminol-dependent chemiluminescence. *Immunology* 1980; **41**:67-74.
- Vice JL, Smaron MF. Indirect fluorescent-antibody method for the identification of *Corynebacterium vaginale*. *Appl Microbiol* 1973; **25**:908-16.
- Greenwood JR, Pickett MJ. Transfer of *Haemophilus vaginalis* Gardner and Dukes to new genus *Gardnerella G vaginalis* (Gardner and Dukes) comb nov. *Int J System Bacteriol* 1980; **30**:170-8.

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