Opsonic activity of intravenous immunoglobulin preparations against *Staphylococcus epidermidis*

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SUMMARY Peritoneal dialysis effluent collected from patients undergoing continuous ambulatory peritoneal dialysis had no opsonic activity against *Staphylococcus epidermidis* and contained low concentrations of IgG and C3 (roughly equal to those found in 0.5% normal human serum). An intravenous immunoglobulin preparation that showed no opsonic activity against the same organism, on its own or when added to balanced salt solution or peritoneal dialysis fluid, showed good activity when combined with peritoneal dialysis effluent. This was probably due to the presence of low concentrations of C3 in the effluent as prior heat inactivation at 56°C for 30 minutes eliminated any opsonic activity in the immunoglobulin-dialysis effluent mixture. Examination of a range of immunoglobulin preparations showed that their opsonic activity for *S epidermidis* in the absence of complement varied considerably. Luminol dependent chemiluminescence was unsatisfactory as a method for detecting complement independent immunoglobulin mediated opsonisation. Intravenous immunoglobulin preparations may be useful in boosting the peritoneal defences of those patients undergoing continuous ambulatory peritoneal dialysis (CAPD) who suffer from repeated intraperitoneal infections.

Peritonitis caused by *Staphylococcus epidermidis* is a major complication of continuous ambulatory peritoneal dialysis (CAPD).¹ Persistent or recurrent infection may oblige CAPD to be stopped.

Treatment of peritonitis entails the rapid introduction of large volumes of peritoneal dialysis fluid into the peritoneal cavity. This has been shown to impair phagocytic defences by diluting the amounts of immunoglobulin and complement opsonins.^{2,3} Keane and Peterson⁴ suggested that peritonitis could be managed by enhancing local phagocytic defences. They showed that the addition of purified immunoglobulin to dialysis fluid or effluent increased the opsonic activity of both against *S epidermidis*.

Standard immunoglobulin preparations prepared from human plasma by the classical Cohn alcohol fractionation method have good biological activity. They contain mainly IgG with little IgM (or IgA). They also contain polymeric aggregates, however, which make them unsuitable for intravenous administration and probably for intraperitoneal use. Immunoglobulin preparations suitable for intravenous use

have been prepared using enzyme treatment, chemical modification treatment with polyethylene glycol, or alcohol fractionation at pH 4.^{5,6}

Several of these preparations are now commercially available. In this study we examined the opsonic activity of a range of these for *S epidermidis* when added to balanced salt solution, peritoneal dialysis fluid, and peritoneal dialysis effluent. Our observation that most intravenous immunoglobulin preparations produced maximal opsonic effect when combined with the low concentrations of complement present in dialysis effluent was confirmed using 0.5% normal human serum and antibody free baby rabbit serum as alternative complement sources.

Material and methods

BACTERIA

S epidermidis (strain 1101) isolated from a patient with peritonitis undergoing CAPD was kindly supplied by Dr RR Marples, Division of Hospital Infection, Central Public Health Laboratory, Colindale, London. The organism was stored in 20% glycerol broth in liquid nitrogen and grown overnight at 37°C in brain heart infusion broth (Lab M Ltd). Before use

Table 1 Immunoglobulin preparations used in study

Preparation and batch number	Code number	Company	Method of preparation
Gammaguard 840920 AG11	1	Travenol	DEAE Sephadex
Gammaguard 850124 AG11	2	Travenol	DEAE Sephadex
Intraglobin 401034	3	Biotest Pharma	Beta propiolactone
Intraglobin F 411114	4		
Intraglobin 401054	5		
Intraglobin 402094	6		
Intraglobin 401123	7	Cutter Division of Miles	Reduction and alkylation
Gamimune CS2401A	8		
Sandoglobulin 40003	9	Sandoz	Fractionation at pH 4
Sandoglobulin 23750670	10		
Sandoglobulin 43601010	11		
Sandoglobulin 33751391	12		
Sandoglobulin 43751070	13		
Sandoglobulin 33781490	14		

the bacteria were washed twice in saline (0.9% w/v) and resuspended to a concentration of 3×10^8 colony forming units/ml in Hanks's balanced salt solution without phenol red (HBSS: Gibco Ltd).

OPSONINS

Pooled normal human serum was collected from six apparently healthy donors and frozen at -70°C in 0.5 ml aliquots. Centrifuged, filtered, peritoneal dialysis effluent from uninfected patients undergoing CAPD was also stored at -70°C in 2 ml aliquots. Table 1 lists the intravenous immunoglobulin preparations used in the study.

POLYMORPHONUCLEAR LEUCOCYTES

Polymorphonuclear leucocytes were separated from heparinised human venous blood by incubation with half the blood volume of dextran (6% w/v) in saline (0.9% w/v) for 45 minutes. The leucocyte rich plasma layer was removed and centrifuged at low speed. Residual red cells were lysed by treatment for 12 minutes with 10 ml Tris buffered ammonium chloride (0.83% w/v). Cells were washed twice and resuspended to a concentration of $1.5 \times 10^6/\text{ml}$ in HBSS supplemented with fetal calf serum (0.1% v/v).

OPSONISATION

S. epidermidis (500 μl) was opsonised with 0.5% or 2.5% normal human serum or 100% peritoneal dialysis effluent, as well as with the immunoglobulin preparations (1–10 mg/ml). The reaction mixture was made

up to 1 ml with HBSS and incubated at 37°C for 15 minutes. The reaction was stopped by the addition of 1 ml HBSS at 4°C . Bacteria were washed twice in phosphate buffered saline and resuspended in HBSS at the original concentration.

PHAGOCYTIC ASSAYS: MICROSCOPY

Opsonised *S. epidermidis* were diluted 1/100 in HBSS, and 200 μl of the suspension were incubated with an equal volume of polymorphonuclear leucocytes at 37°C for 15 minutes on a roller. An aliquot (100 μl) of this mixture was then cytocentrifuged and the smear fixed in methanol and stained by Giemsa's method. Results were expressed as the percentage of polymorphonuclear leucocytes with ingested bacteria.

LUMINOL DEPENDENT CHEMILUMINESCENCE

Suspensions of opsonised bacteria (200 μl) were added to 500 μl of polymorphonuclear leucocytes ($10^6/\text{ml}$) and 900 μl 10^{-5} M luminol (Sigma 6) dissolved in dimethyl sulphoxide, and then diluted in HBSS. The mixture was maintained at 37°C in a Dri-block 08-3 (Technique). After 15 minutes the mixture was placed in the reaction chamber of a 1250 luminometer (LKB) at 37°C and the peak light generated measured in mV at 15 minutes.

COMPLEMENT AND IgG VALUES

These were measured on low concentration radial immunodiffusion plates for C3c and IgG (Behring).

Table 2 Opsonic activity and concentrations of albumin, IgG, and C3c in peritoneal dialysis effluent from 108 patients without evidence of peritonitis (dwell time $\leq$ five hours)

Opsonin source	Chemiluminescence (mV) Mean	Phagocytosis (%) Mean	Albumin (mg/dl) Mean concentration	IgG (mg/dl)	C3c (mg/dl)
2–5% pooled normal human serum	12	12	100	30	1–4
Dialysis effluent	62	44	44	6–4	0–39

Table 3 Effect of addition of pooled normal human serum or intravenous immunoglobulin preparation to Hanks's balanced salt solution or peritoneal dialysis fluid on its capacity to opsonise *S epidermidis*

Medium	Opsonin source (concentration)	Chemiluminescence (mV) Mean (SD)	Phagocytosis (%) Mean (SD)
HBSS:	—	12 (2)	11 (1)
	Normal human serum (2-5%)	48 (6)	49 (7)
	IgG (1 mg/ml)	16 (3)	13 (1)
	IgG (5 mg/ml)	17 (4)	19 (3)
	IgG (10 mg/ml)	17 (4)	25 (5)
Dialysis fluid:	—	11 (1)	11 (1)
	Normal human serum (2-5%)	52 (7)	47 (9)
	IgG (1 mg/ml)	14 (3)	15 (6)
	IgG (5 mg/ml)	19 (5)	18 (9)
	IgG (10 mg/ml)	17 (4)	20 (6)

Table 4 Opsonisation of *S epidermidis* by 2.5% normal human serum and intravenous immunoglobulin preparation (Sandoglobulin) combined with heat inactivated or unheated peritoneal dialysis fluid containing a low concentration of C3*

Opsonin source	Chemiluminescence (mV)	Phagocytosis (%)
Unopsonised	14	8
2.5% normal human serum	57	58
1 g 10 mg/ml and peritoneal dialysis effluent	53	64
1 g 10 mg/ml and heated peritoneal dialysis effluent†	21	26
1 g 5 mg/ml and peritoneal dialysis effluent	47	60
1 g 5 mg/ml and heated peritoneal dialysis effluent	17	18
1 g 1 mg/ml and peritoneal dialysis effluent	25	35
1 g 1 mg/ml and heated peritoneal dialysis effluent	15	14

*Measured by microscopic phagocytic assay and luminol dependent chemiluminescence.

†Heated at 56°C for 30 minutes.

Results

Peritoneal dialysis effluent from uninfected patients (dwell time ≤ 5 hours) was unable to opsonise *S epidermidis*. Concentrations of IgG and C3 in the effluent were low, more or less equivalent to those in 0.5% normal human serum (Table 2). An intravenous immunoglobulin preparation (Sandoglobulin) was used as a source of opsonin. When added to either HBSS or peritoneal dialysis fluid, in concentrations ranging from 1 mg/ml to 10 mg/ml, it failed to enhance the uptake of *S epidermidis* in comparison with 2.5% normal human serum, which gives optimal opsonisation of *S epidermidis* (Table 3). When added to peritoneal dialysis effluent with a low IgG and C3 content (5.0 mg/dl and 0.45 mg/dl, respectively), however, which itself was a poor opsonin, the uptake of *S epidermidis* was enhanced (Table 4). Heating the effluent at 56°C for 30 minutes removed this effect, suggesting that the key factor in the dialysis was the low concentration of C3 in the unheated effluent, which, together with the added immunoglobulin, provided an effective opsonic mixture.

Table 5 shows the opsonic activity of 14 immunoglobulin preparations, representing four different products, against *S epidermidis*, compared with that of 2.5% normal human serum. Two conclusions can be drawn from these results. First, the various preparations differed in their capacity to opsonise *S epider-*

midis in the absence of complement: there was also variation between different batches of the same product. Second, with purified immunoglobulin, luminol dependent chemiluminescence did not seem to be an effective method of monitoring opsonic activity. Even when the microscopic assay showed good bacterial uptake, chemiluminescence remained poor.

The importance of complement for opsonisation was shown by examining the opsonic activity of six immunoglobulin preparations in the presence or absence of complement, provided either by 0.5% or

Table 5 Opsonic activity of 14 IgG preparations against *S epidermidis*

Immunoglobulin source (Ig preparations 5 mg/ml)	Chemiluminescence (mV)	Phagocytosis (%)
—	8	21
2.5% (normal human serum)	45	53
Ig (1)	14	53
Ig (2)	15	41
Ig (3)	12	51
Ig (4)	13	26
Ig (5)	12	22
Ig (6)	14	39
Ig (7)	15	35
Ig (8)	13	39
Ig (9)	12	36
Ig (10)	12	33
Ig (11)	16	32
Ig (12)	16	35
Ig (13)	15	26
Ig (14)	15	28

Table 6 Opsonisation of *S epidermidis* by 1 g preparations 1, 2, 4, 6, 8, and 14 (Table 1) with or without 0.5% normal human serum as measured by microscopic phagocytic assay and luminol dependent chemiluminescence

Opsonin source	Chemiluminescence (mV)	Phagocytosis (%)
Unopsonised	8	22
Normal human serum 0.5%	12	22
Normal human serum 2.5%	45	53
Ig1	14	53
Ig1 and 0.5% normal human serum	33	53
Ig2	17	42
Ig2 and 0.5% normal human serum	38	68
Ig4	15	24
Ig4 and 0.5% normal human serum	15	35
Ig6	15	35
Ig6 and 0.5% normal human serum	15	37
Ig8	15	33
Ig8 and 0.5% normal human serum	37	49
Ig14	17	28
Ig14 and 0.5% normal human serum	39	47

Table 7 Opsonisation of *S epidermidis* by 1 g preparations 1, 2, 4, 6, 8, and 14 (Table 1) with or without peritoneal dialysis effluent or by 0.5% or 2.5% normal human serum*

Opsonin source	Chemiluminescence (mV)	Phagocytosis (%)
Unopsonised	16	16
Normal human serum 0.5%	19	17
Normal human serum 2.5%	69	53
Peritoneal dialysis effluent	18	17
Ig1	27	39
Ig1 and 0.5% peritoneal dialysis effluent	66	50
Ig2	27	39
Ig2 and 0.5% peritoneal dialysis effluent	68	53
Ig4	20	19
Ig4 and 0.5% peritoneal dialysis effluent	20	25
Ig6	20	23
Ig6 and 0.5% peritoneal dialysis effluent	20	30
Ig8	19	19
Ig8 and 0.5% peritoneal dialysis effluent	52	56
Ig14	22	26
Ig14 and 0.5% peritoneal dialysis effluent	64	59

*Measured by microscopic phagocytic assay and luminol dependent chemiluminescence.

2.5% normal human serum or peritoneal dialysis effluent, neither of which on their own could opsonise *S epidermidis* (Tables 6 and 7). Similar results were obtained using baby rabbit serum with no detectable antibody to *S epidermidis* as a complement source (results not shown). Where complement was present the results obtained with chemiluminescence matched those of the microscopic assay.

Discussion

Peritoneal dialysis effluents from uninfected patients with a dwell time of five and a half hours or less failed to opsonise *S epidermidis*. These effluents contained low mean concentrations of both IgG and C3, equivalent to those found in 0.5% normal human serum (a concentration also unable to opsonise *S epidermidis*). These findings agree with those of Verbrugh *et al*² and Keane and Peterson.⁴ Verbrugh *et al*² did find some dialysis effluents from uninfected patients that could opsonise staphylococci but these were always

associated with longer peritoneal dwell times of between six and 10 hours.

S epidermidis is an organism of low virulence. The course of peritonitis caused by this organism is relatively benign when compared with that caused by other pathogens. The key factors in CAPD *S epidermidis* peritonitis are firstly the presence of a foreign body, the catheter: this can increase the severity of infection and provides a site on which *S epidermidis* has been shown to adhere and multiply⁷; second, poor technique or management, or both, resulting in contamination of catheter lumen, connectors, and exit site. Both factors help to provide the high inoculum that is probably necessary for infection with *S epidermidis*.

We do not yet know the real importance of peritoneal defence mechanisms in determining whether or not infection occurs in particular patients. Keane *et al*⁸ found that the incidence of *S epidermidis* peritonitis was appreciably lower in those patients with a high degree of opsonic activity in their peritoneal dia-

lysis effluent after overnight exchange. Keane and Peterson⁴ also showed that an immunoglobulin preparation enhanced the uptake of *S epidermidis* when added either to peritoneal dialysis fluid or effluent, and raised the possibility that passive immunisation of the peritoneal cavity might reduce the incidence of peritonitis in patients undergoing CAPD.

We felt that this observation was worth pursuing more systematically. Given the limitations of standard immunoglobulin preparations for anything other than intramuscular administration, we decided to use intravenous immunoglobulin preparations.

Our initial experiments entailed an intravenous immunoglobulin preparation made by alcohol separation at pH 4 (Sandoglobulin). In contrast to Keane and Peterson,⁴ we found that this showed no opsonic activity against *S epidermidis* when added to HBSS or dialysis fluid. It had good opsonic activity, however, when combined with dialysis effluent, which itself was not opsonic for the organism. As heat inactivation of the effluent destroyed the opsonic activity of the immunoglobulin-effluent mixture we concluded that opsonisation resulted from a combination of the high IgG concentration and low complement value.

Testing 14 batches of intravenous immunoglobulin, representing four different preparations, showed that two had good opsonic activity against *S epidermidis*, as measured by a microscopical phagocytic assay, in the absence of complement. When complement was added either as 0.5% normal human serum or peritoneal dialysis effluent, however, four of six preparations tested were good opsonins: interestingly, luminol dependent chemiluminescence was unsatisfactory as an assay for opsonisation by immunoglobulin alone, the amounts seen being uniformly low. When complement was added chemiluminescence correlated well with the microscopical assay of phagocytosis.

Our results show that intravenous immunoglobulin preparations can enhance phagocytosis in the peritoneal cavity. Batches and preparations will need to be screened for optimal opsonic activity, but their safety remains to be proved and they are expensive. As a result, if any clinical work is to be done we feel that careful patient selection will be vital. The host defence approach to controlling CAPD peritonitis will not reduce the prime importance of good technique and improvements in catheter and connector

design. Patients who suffer repeated infections for no apparent reason, however, or in whom we can show opsonic deficiencies in the early phase of peritonitis might be suitable candidates for this approach. We are currently carrying out a prospective study of the opsonic capacity, numbers of phagocytic cells, and values IgG and C3 in the dialysis effluents of both uninfected patients on CAPD and those with early peritonitis. This will allow us to see if we can identify groups in whom the use of intraperitoneal immunoglobulin might be helpful.

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