Mononuclear leucocyte function tests in the assessment of the biocompatibility of peritoneal dialysis fluids

H F H Brulez, P M ter Wee, S V Snijders, A J M Donker, H A Verbrugh

Abstract

***Background*—Previous studies showed that the currently used dextrose based peritoneal dialysis fluids impair several leucocyte functions.**

***Aims*—To determine which in vitro mononuclear leucocyte (monocyte) function tests most clearly reflect the biocompatibility of peritoneal dialysis fluid.**

***Methods*—Monocytes were tested for phagocytic capacity, bactericidal activity, Fc and C3 receptor expression, and chemiluminescence response, and by analysis of the release of interleukin 8 (IL-8) and tumour necrosis factor α (TNFα) in the presence of test fluids. Cytokine release was studied in an alternative dynamic in vitro peritoneal dialysis model in which monocytes were exposed to test fluid that was continuously equilibrated with an interstitial fluid-like medium through a microporous membrane. The chemiluminescence response by stressed monocytes was also tested after an 18 h recovery period. All tests were performed during or after exposure to different degrees of glycerol induced osmotic stress and after exposure to a 1% milk-whey derived, polypeptide enriched test fluid. Cells incubated in 0.1% gel Hanks buffer (GH) served as control.**

***Results*—Osmotic stress induced impairment of leucocyte function was found by the chemiluminescence assay (mean (SEM): 179 (20)% *v* 138 (23)% after 30 minutes in 0.5% and 1.5% glycerol, respectively) and by the analysis of IL-8 released by monocytes (44 (9) ng in 0.7% glycerol *v* 40 (7) ng in 2.0% glycerol). Only the chemiluminescence assay showed a protective effect of polypeptides on leucocyte function (after ≥ 60 minutes). If monocytes were allowed to recover in culture medium after exposure to test fluids, the changes in chemiluminescence response appeared to be reversible after a 30 minute exposure, but became more pronounced after 60 and 120 minutes. The phagocytosis and bacterial killing assays were less sensitive. The observations carried out with the phagocytosis assay did not correspond with the Fc or C3 receptor density data.**

***Conclusions*—The release of IL-8 by peripheral blood monocytes in a two compartment model and their chemiluminescence response are appropriate assays for the assessment of changes in leucocyte function in response to different peritoneal dialysis fluids.**

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Keywords: mononuclear leucocyte function; peritoneal dialysis; biocompatibility

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Bacterial peritonitis remains a major problem in patients with end stage renal failure undergoing continuous peritoneal dialysis. As well as the risk of microbial contamination of the peritoneal cavity by the peritoneal catheter (through either intraluminal or periluminal passage), the high prevalence of peritonitis in these patients may also reflect a reduction in host resistance. Poor host resistance is illustrated by the relatively avirulent nature of the bacteria that commonly cause dialysis related peritonitis, and the finding that some microorganisms may survive and grow within macrophages obtained from peritoneal dialysate.[1 2]

Polymorphonuclear and mononuclear leucocytes are not the only factors concerned with defence against infection of the peritoneal cavity. Diaphragmatic lymphatic uptake, the opsonic activity of the peritoneal fluid, and mesothelial cells and fibroblasts in the peritoneum also play a role in the defence mechanisms.[3] Previous studies have shown that the currently used dextrose based peritoneal dialysis fluids impair most functions of leucocytes, mesothelial cells, and fibroblasts. The poor biocompatibility of these fluids has been attributed to their high osmolarity, their low pH (5) when combined with lactate as a buffering agent, and the cytotoxity of glucose degradation products.[3–8] The diaphragmatic lymphatic uptake and opsonic activity of the peritoneal fluid are affected by the dialysis procedure itself (dilution).[9 10]

To develop more biocompatible peritoneal dialysis fluids, it is important to choose tests that most clearly show the impact of experimental peritoneal dialysis fluids on the host defence systems. In this study we examined assays that estimate several mononuclear leucocyte (monocyte) functions as in vitro measures of peritoneal dialysis fluid biocompatibility. The assays evaluated included a chemiluminescence assay, phagocytosis and killing of microorganisms, IgG and C3 receptor expression, and the release of two chemokines, interleukin 8 (IL-8) and tumour necrosis α (TNFα) by monocytes. The purpose of the study was to investigate the ability of these assays to show differences in biocompatibility when leucocytes were exposed to various experimental test fluids. We used test fluids that were likely to

pose biocompatibility problems as a result of differences in osmolarity and protein content.

The chemiluminescence response, phagocytosis and killing capacity, and receptor expression were tested after 30, 60, and 120 minutes of static exposure to test fluids, while the production and release of IL-8 and TNFα by monocytes was assessed over a six hour exposure period. We assumed that exposure to test fluids in a static model for such a long time would be unlikely to reflect the in vivo situation, as peritoneal dialysis fluid in the peritoneal cavity is subject to continuous change in pH, osmolarity, and protein content.[11] Also, cells in the peritoneal membrane are supported by nutrients in the interstitial fluid. We therefore studied the impact of test fluids on the release of cytokines in a novel in vitro peritoneal dialysis model. In this dynamic double chamber model, a compartment containing test fluid was separated from a compartment containing simulated interstitial fluid by a microporous polycarbonate membrane, with monocytes attached to either side of the membrane. In this setting, monocytes were exposed to test fluid (either directly or through the polycarbonate membrane) and simultaneously supported (fed) by the simulated interstitial fluid. As in the in vivo situation, the test fluids in the double chamber model were continuously equilibrated with the "interstitial fluid" underneath the microporous membrane. In a previous study we showed that equilibration of the osmolarity and pH of the test fluids used in the model were very similar to in vivo data on the equilibration patterns of peritoneal dialysis fluid infused into the peritoneal cavity.[12 13]

Methods

ISOLATION OF LEUCOCYTES

Peripheral blood monocytes were isolated from the buffy coats of sterile, heparinised venous blood of healthy donors using a Ficoll-Isopaque gradient (Pharmacia), as described earlier.[14] To improve the reproducibility of the test results, the number of monocytes in the suspension (predominantly lymphocytes and monocytes) was measured by labelling with monocyte specific mouse monoclonal CD14, conjugated to phycoerythrin (CLB, Amsterdam), following the manufacturer's instructions, and by subsequent flow cytometric analysis of the number of red fluorescing cells. Finally, the monocytes were resuspended at a concentration of 5×10^6 cells/ml in RPMI-1640 (BioWhittaker) with 10% (vol/vol) fetal calf serum (FCS; Gibco BRL), and stored on ice until use.

TEST FLUIDS

To estimate the sensitivity of the assays used, experimental solutions were chosen that were likely to pose different biocompatibility problems as a result of differences in their osmolarity and protein content. The required osmolarity was obtained by adding glycerol (MW 90) to a lactate buffered electrolyte solution (LcB) (lactate 40, Na 136, Cl 95, Ca 1.75, and Mg 0.25 mmol/l). The glycerol based fluids were tested at 0.5% (332 mmol/l), 1.5% (441 mmol/l), and 2.5% (549 mmol/l). As protein enriched test fluid, we used a 1% (wt/vol) milk whey derived polypeptide (average molecular weight 800[15]; kindly provided by Baxter Healthcare Corporation, Round Lake, Illinois, USA) in the same LcB. The impact on cytokine release by monocytes was tested using three glycerol based solutions (0.7%, 1.15%, and 2.0% glycerol) with an osmolarity similar to that of three commercially available glucose based fluids (1.36%, 2.27%, and 3.86% Dianeal PD1, Baxter Healthcare Corporation). All solutions were prepared under pyrogen-free conditions and filter sterilised immediately after preparation. All fluids were tested at neutral pH (7.4). Cells incubated in Hąnks buffered salt solution with 0.1% (wt/vol) gelatine (GH) served as control. Before exposure all test fluids were prewarmed to 37°C.

BACTERIA

An isolate of coagulase negative staphylococci (V10), obtained from a patient on peritoneal dialysis with peritonitis, and a strain of *Escherichia coli* (ON2) were used. After incubation for 18 hours at 37°C in 5 ml Mueller-Hinton broth (Difco Laboratories), the suspensions were centrifuged at 2000 *g* for 15 minutes, the supernatants discarded, and the pellets washed three times with phosphate buffered saline (PBS). A subset of coagulase negative staphylococci, used for the phagocytosis assay, was fluorescence labelled by incubation in 500 µl PBS containing 0.05 mg fluorescein 5-isothiocyanate (FITC, Sigma) for 45 minutes at 4°C. After labelling, bacteria were washed twice to remove unbound FITC. Finally, all samples were suspended in GH at a concentration of 5×10^8 colony forming units (cfu)/ml and stored on ice until use.

OPSONISATION PROCEDURE

Opsonisation of bacteria was accomplished by incubating 100 µl of the suspensions containing approximately 5×10^7 microorganisms with 900 µl of 5% (vol/vol) human pooled serum in GH for 30 minutes at 37°C. For evaluation of Fc receptor dependent phagocytic capacity, a subset of FITC labelled coagulase negative staphylococci were opsonised using serum that had been heated at 56°C for one hour before use in order to destroy the available heat sensitive complement. Opsonisation was stopped by adding 2.5 ml ice cold PBS, followed by centrifugation at 2000 *g* for 15 minutes, discarding of the supernatants, resuspension of the pellets in 1 ml GH, and storage on ice until use.

PHAGOCYTOSIS ASSAY

Using a fluorometric phagocytosis assay, we tested the impact of test fluids on the capacity of monocytes to phagocytose FITC labelled coagulase negative staphylococci, opsonised with fresh serum (immunoglobulins and complement) or heated serum (immunoglobulins only). The phagocytic capacity of monocytes in the test fluids was tested on cells only exposed briefly to the test fluid (fresh monocytes), and

on those that had already been incubated in the test fluid for 30 minutes before adding the bacteria (stressed monocytes). The procedures used were a modification of a method described earlier by Peterson *et al.*[14] Fresh monocytes (5×10^5 monocytes without medium) were processed by adding 125 µl of test fluid and 5×10^6 bacteria in 25 µl of GH. Stressed monocytes (5×10^5 monocytes that had been exposed for 30 minutes to 200 µl test fluid) were mixed with 5×10^6 bacteria in 150 µl of GH. The 25 µl inoculum had little effect on the osmolarity of the test fluids. However, the addition of a 150 µl bacterial suspension resulted in a decrease in the osmolarity of the test fluids similar to the reduction in osmolarity found in peritoneal dialysis fluids 30 minutes after infusion into the peritoneal cavity. Subsequently, the mixtures were incubated for 30 minutes (fresh and stressed monocytes) or 60 minutes (fresh monocytes only) at 37°C in a shaking water bath. Phagocytosis was stopped by adding 2.5 ml ice cold PBS. After centrifugation at 350 *g* for five minutes, supernatants were discarded and the pellets washed three times with ice cold PBS. Finally, the pellets were resuspended in 200 µl GH and kept on ice until analysis. The samples were analysed in a FACStar flow cytometer (Becton Dickinson) by estimating the mean fluorescence and the percentage of fluorescein positive cells within the scatter gates set for the monocyte fraction. Results are expressed as the relative difference in the proportion of FITC positive monocytes exposed to the test fluid and that of monocytes exposed to GH (control), and as the mean fluorescence relative to that of control cells.

EFFECTS ON THE Fcγ AND C3 RECEPTOR EXPRESSION

To estimate the impact of experimental peritoneal dialysis fluids on cell surface densities of CD18 (C3bi receptor), CD32 (Fcγ receptor II), and CD64 (Fcγ receptor I), monocytes were labelled with the appropriate FITC conjugated monoclonal antibodies after a 60 minute exposure to the test fluids. Monoclonal CD18 was obtained from CLB (Amsterdam), CD32 and CD64 from Pharmingen (San Diego, California, USA). Labelling was performed according to the manufacturers' guidelines. In brief, 5×10^5 monocytes were incubated for 60 minutes with 200 µl prewarmed test fluid in 96 well culture plates, in a humidified atmosphere at 37°C with 5% CO_2. The test fluids were removed after centrifugation (for five minutes at 250 *g*), and the pellets resuspended in 50 µl PBS with 0.2% (wt/vol) bovine serum albumin (Sigma) and the appropriate monoclonal antibody. Subsequently, the samples were incubated for 30 minutes at 4°C. After incubation with the monoclonal antibodies, the plates were centrifuged, the cells washed with PBS, suspended in GH, and stored at 4°C until analysis. The samples were analysed in a FACStar flow cytometer (Becton Dickinson) by estimating the mean fluorescence of the cells within the scatter gates set for the monocyte fraction.

BACTERICIDAL CAPACITY

The ability of 5×10^5 monocyte to kill *E coli* (2.5×10^6 cfu) in 150 µl of several prewarmed test fluids was estimated after an incubation of 30 and 60 minutes at 37°C in a shaking water bath. Their ability to kill coagulase negative staphylococci (5×10^6 cfu) was measured after a 60 and a 120 minute incubation period. Bactericidal capacity was tested in a modification of the assay described by Peterson *et al.*[14] In brief, after incubation, 100 µl of the suspension of cells and bacteria were added to 5 ml of ice cold distilled water to disrupt the phagocytic process and cells. After serial dilution, samples were plated on Tryptone soy agar (TSA; Oxoid) and the number of viable cfu was counted after overnight incubation at 37°C. Bacterial killing at a given time point is expressed as the percentage decrease in the initial number of viable cfu of *E coli* or coagulase negative staphylococci added to the monocyte suspension.

To obtain more information on the survival and growth of coagulase negative staphylococci and *E coli* in the test fluids alone, the same procedures were followed as described for the killing assay, but in the absence of leucocytes. Results are expressed as the percentage change in the initial number of viable cfu added to the test fluids at time zero.

CHEMILUMINESCENCE ASSAY

The bactericidal activity of leucocytes is characterised by the production of oxygen derived free radicals that are toxic for the ingested microorganisms. As result of this so called respiratory burst excess energy is liberated in the form of light (chemiluminescence), which can be measured using a luminometer.[16] We studied the impact of test fluids on the chemiluminescence response of the monocytes. Before the test, U-shaped 96 well tissue culture plates (Wallac) were filled with 5×10^5 monocytes in GH per well and centrifuged for five minutes at 250 *g*. After removal of the supernatants, the cells were resuspended in 200 µl of test fluid. Cells were exposed to the test fluids for 30, 60, and 120 minutes in a humidified atmosphere at 37°C with 5% CO_2. After exposure, the plates were centrifuged (five minutes at 250 *g*), the test fluids discarded, and the cells resuspended in 100 µl GH. Subsequently, the cells were transferred to a white luminometer plate (EG&G Berthold Instruments). Luminol enhanced chemiluminescence was employed to assess the monocyte respiratory burst, both spontaneously and in response to N-formyl-methionyl-leucyl-phenylalanine (fMLP, Sigma). After addition of 50 µl luminol to the cell suspensions (final concentration 10 ng/ml; Sigma), spontaneous chemiluminescence was measured during 90 seconds per well at 37°C, using a Berthold MicroLumat LB96P automatic luminometer (EG&G Berthold Instruments). To estimate the fMLP induced chemiluminescence, 50 µl (8×10^{-5} M) fMLP were added using the injector available in the luminometer, just before each well was measured. Results are expressed as the number of relative light units (RLU) produced during 90 seconds by 5×10^5 cells divided by the

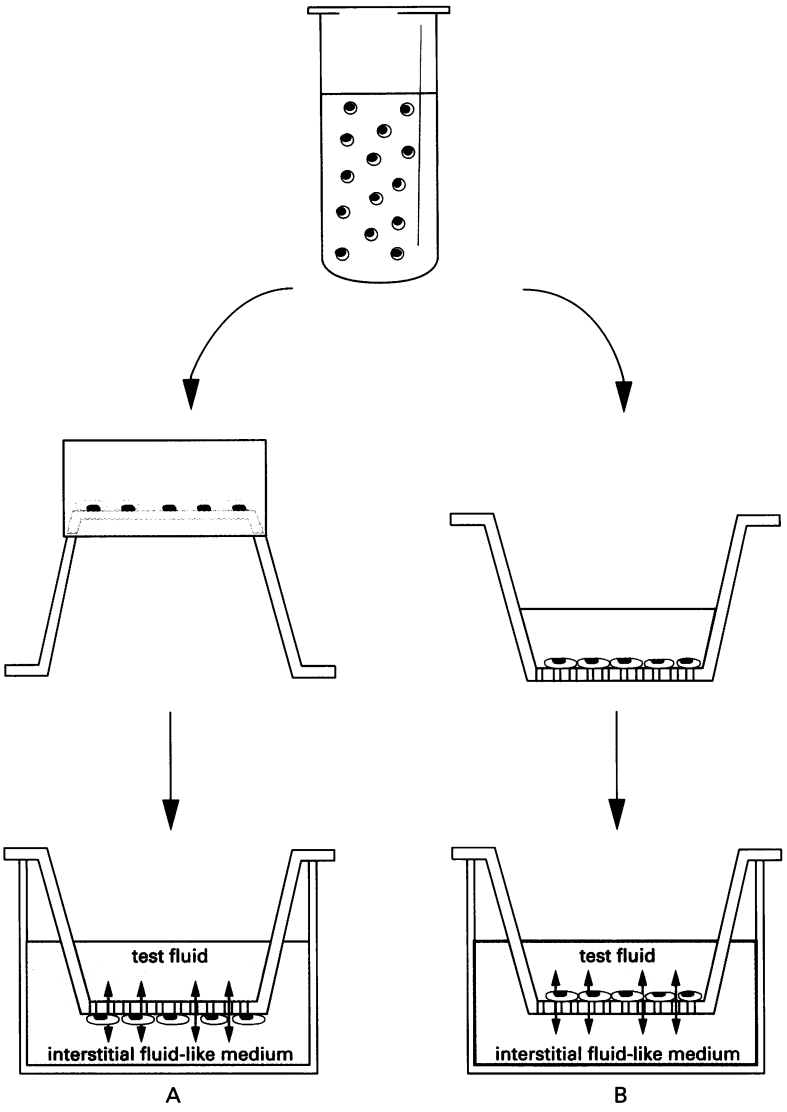

Figure 1 Preparation of a Transwell double chamber model for the assessment of cytokine release by mononuclear leucocytes attached to the lower side (A) and the upper side (B) of a microporous membrane. Procedures are described in Methods.

number RLU by control cells (exposed to GH) × 100%.

CYTOKINE RELEASE

The impact of osmotic stress on monocyte protein synthesis was tested by the assessment of the spontaneous and endotoxin induced production and release of TNFα and IL-8 in a double chamber model, with the test fluid in the upper chamber and simulated interstitial fluid solution in the lower (fig 1).[17] To reflect the situation in the submesothelial interstitium (where most peritoneal macrophages are located[18]) and in the peritoneal cavity, monocytes were tested in two ways—attached to the lower side and then to the upper side of a microporous polycarbonate membrane (0.4 μm pore size) of 6.5 mm Transwell inserts (Corning Costar), respectively. Attachment to the lower side of the membranes was achieved using a modification of the method used by Zeillemaker *et al* to allow mesothelial cells to attach to the lower side of a membrane.[19] In brief, 2.5×10^5 monocytes in 250 μl RPMI-1640 with 20% (vol/vol) FCS were added to an inverted insert to the bottom of which a plastic tube was tightly fixed. After two hours, when about 95% of the monocytes were attached to the membrane (tested by analysis of the total number of CD14 positive cells remaining in the supernatant), the medium with the remaining cells was discarded. After this, the tubes were removed and the inserts placed in the 24 well cluster plates containing 500 μl RPMI-1640 with 20% (vol/vol) FCS per well; 250 μl RPMI-1640 with 20% (vol/vol) FCS were then added to the inserts and the complete test system was stored at 37°C in a humidified atmosphere with 5% CO_2 until the next day. For the assessment of monocyte function at the upper side of the membrane, 2.5×10^5 monocyte in 250 μl RPMI-1640 with 20% (vol/vol) FCS were added to the inserts and also stored at 37°C in a moist chamber with 5% CO_2 until the next day. Before the test, the medium in the inserts (above the membrane) was replaced by 250 μl test fluid and that in the cluster (the compartment below the membrane) by 500 μl RPMI-1640 with 30% (vol/vol) FCS (fig 1). All procedures were performed under sterile circumstances. After one hour of exposure, the "interstitial fluids" were collected and replaced by fresh medium and, where appropriate, 2.5 μg of lipopolysaccharide (LPS O111:B4, Sigma) in 25 μl PBS were added to the test fluids. Three hours after the start of the experiment, the simulated interstitial fluid was also renewed. After six hours of exposure, all fluids were collected and stored in aliquots at −80°C until analysis. The amounts of TNFα and IL-8 released into the fluids were measured by commercially available enzyme linked immunosorbent assays (PeliKine ELISA kit, CLB). The results are expressed as the total amount of TNFα (in pg) or IL-8 (ng) released by 2.5×10^5 monocytes.

Table 1 Phagocytosis capacity

	Monocyte uptake of staphylococci opsonised with:			
	Fresh serum		*Heated serum*	
Test fluid	*Δ%FITC pos*	*%MF*	*Δ%FITC pos*	*%MF*
GH (control)	0	100	0	100
1% polypeptides	8.4 (3.0)*	89 (4)†	3.3 (1.8)	85 (8)‡
0.5% glycerol	1.6 (1.3)	105 (4)	−1.9 (0.7)	107 (4)
1.5% glycerol	1.2 (1.9)	99 (4)	−1.2 (0.9)	109 (4)
2.5% glycerol	0.7 (0.9)	103 (7)	−2.2 (1.5)	104 (3)

The ability of monocytes to phagocytose fresh and heated, serum opsonised, coagulase negative staphylococci during a 30 min incubation and a 30 min pre-exposure to test fluids. Data expressed mean (SEM) of the relative difference in the number of FITC positive monocytes exposed to test fluid and of monocytes exposed to GH (Δ%FITC pos), and as the mean fluorescence relative to that of control cells (%MF).
*$p < 0.001$ *v* GH and 2.5% glycerol, $p < 0.01$ *v* 0.5% and 1.5% glycerol; †$p < 0.01$ *v* GH and glycerol (all); ‡$p < 0.01$ *v* 0.5% glycerol, $p < 0.05$ *v* GH and 1.5% and 2.5% glycerol.
GH, Hanks buffered salt solution with 0.1% wt/vol gelatine (control); FITC, fluorescein isothiocyanate.

STATISTICAL ANALYSES

Data are presented as mean (SEM). Every test was performed in duplicate and repeated at least three times. All data were compared by analysis of variance for repeated multiple comparison procedures (Student-Newman-Keuls method). A probability of 5% was chosen as the level of significance.

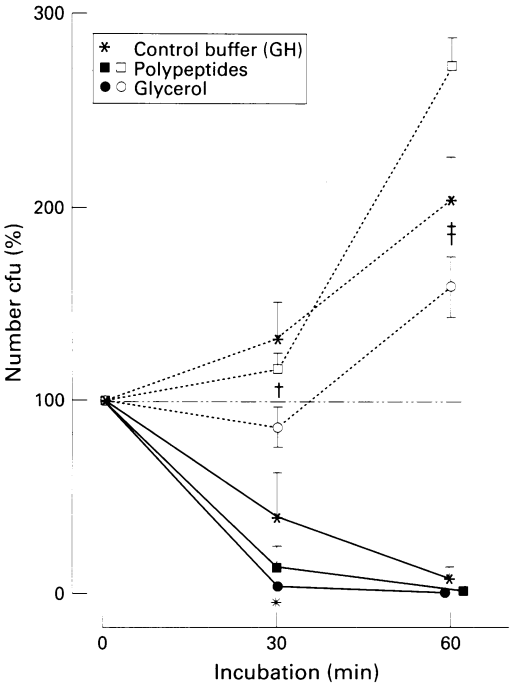

*Figure 2 Survival of E coli in control buffer, 1.0% polypeptides, and 2.5% glycerol in the presence (solid lines) and absence (dashed lines) of mononuclear leucocytes. *Gly v GH: $p < 0.001$; 1.0% PP v GH: $p < 0.01$ (with monocytes, $t = 30$ min); †1% PP and GH v Gly: $p < 0.001$–0.01 (without monocytes, $t = 30$ min); ‡1% PP v GH and Gly: $p < 0.01$–0.05 (without monocytes, $t = 60$ min).*

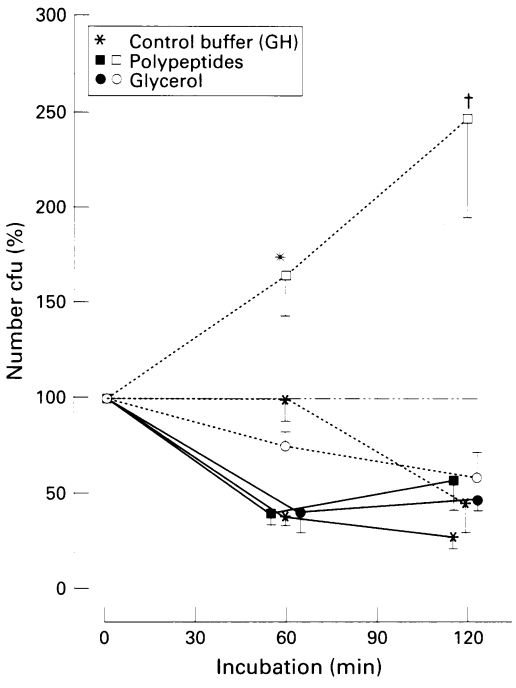

*Figure 3 Survival of coagulase negative staphylococci (strain V10) in control buffer, 1.0% polypeptides, and in 2.5% glycerol in the presence (solid lines) and absence (dashed lines) of mononuclear leucocytes. *1% PP v GH: $p < 0.01$; PP v Gly: $p < 0.001$ (without monocytes, $t = 60$ min); †1% PP v GH and Gly: $p < 0.001$ (without monocytes, $t = 120$ min).*

Results

PHAGOCYTOSIS AND Fcγ AND C3 RECEPTOR EXPRESSION

The impact of test fluids on the uptake of fresh and heated serum opsonised coagulase negative staphylococci by fresh and stressed monocytes was tested. The phagocytosis assay showed that the uptake of opsonised bacteria was not much affected by exposure to the experimental test fluids; in particular, monocyte phagocytosis was not affected significantly by previous exposure to glycerol induced osmotic stress (table 1). Monocytes pre-exposed to 1% polypeptides, however, phagocytosed somewhat smaller numbers of staphylococci though more monocytes seemed to participate in the uptake of the bacteria (table 1). The differences between test fluids were less evident if bacteria were added to the leucocytes at the same time as the test fluids. The preservative effects of polypeptides appeared only after 30 minutes of exposure of the monocytes to the test fluids. After 60 minutes, no differences in phagocytic capacity were found between monocytes exposed to polypeptides or to glycerol (data not shown), neither did we find any difference in phagocytic capacity of monocytes exposed to various degrees of glycerol induced osmotic stress (data not shown).

The observed lack of effects of stress on monocytes exposed to the test fluids was largely paralleled by a lack of effect on the density of the Fcγ and C3 receptors on these cells. Compared with control monocytes, an increase of approximately 70% in the density of CD64 (Fcγ-RI) was found, but only after 60 minutes of exposure to the hyperosmolar glycerol based fluids ($p < 0.05$). However, these changes did not result in higher levels of uptake of coagulase negative staphylococci. Expression of CD64 on monocytes exposed to 1% polypeptides, and of CD32 and CD18 on cells from any fluid, was not changed (data not shown).

BACTERIAL SURVIVAL IN TEST FLUIDS WITH AND WITHOUT LEUCOCYTES

In contrast to their phagocytic capacity, the ability of monocytes to kill *E coli* was significantly altered (improved) by incubation in the protein enriched polypeptide solution, as well as in the glycerol based fluids; in particular, the initial rate of killing was higher in these fluids (fig 2). However, after 60 minutes the differences in the total numbers of cfu killed did not differ between monocytes incubated in control fluids and those incubated in test fluids. The bactericidal activity of monocytes for *E coli* was thus not affected by high osmolarity. The ability of monocytes to kill coagulase negative staphylococci was not affected by any of the test fluids at any time.

We also tested survival and growth of *E coli* and coagulase negative staphylococci in the test fluids without monocytes. The numbers of *E coli* cfu increased 2.8-fold after one hour of incubation in 1% polypeptides, compared with a 2.1-fold increase in GH ($p < 0.05$), and a 1.6- to 1.9-fold increase in glycerol based fluids ($p < 0.01$; fig 2). The effects of test fluids on the growth of coagulase negative staphylococci were different. After one hour, the numbers of viable coagulase negative staphylococci gradually decreased from 100% at time zero to 70–77% in glycerol, remained stable in GH, but increased to 168% in 1% polypeptides ($p < 0.01$). Following a two hour exposure, the numbers of viable staphylococci showed no

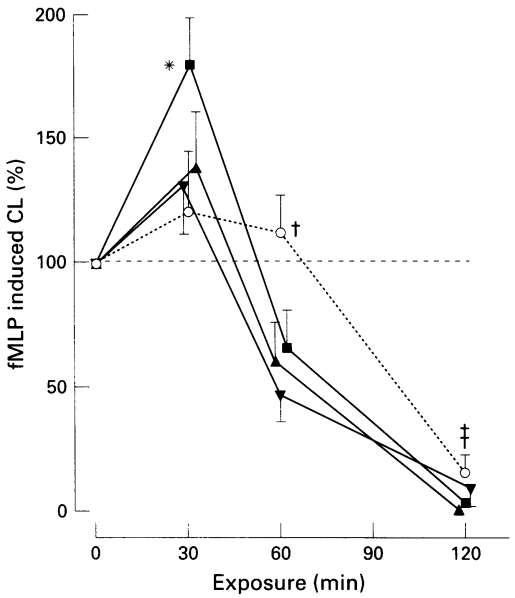

*Figure 4 The relative fMLP induced chemiluminescence response (CL) by monocytes after a 30, 60, and 120 minute exposure to control buffer (=100%), 1.0% polypeptides (PP, ○), and 0.5% (■), 1.5% (▲) and 2.5% (▼) glycerol (Gly). *0.5% Gly v other (t = 30 min): p < 0.05; †2.5% Gly v GH (t = 60): p < 0.05; PP v Gly (all) (t = 60): p < 0.01–0.05; ‡control v other (t = 120): p < 0.001; PP v Gly (all) (t = 120): p < 0.05.*

further decrease in glycerol, but decreased in GH decreased to 46 (14)% of the initial value, and increased in 1.0% polypeptides to 248 (52)% ($p < 0.01$, fig 3).

CHEMILUMINESCENCE ASSAY

The impact of test fluids on the ability of monocytes to mount a respiratory burst, either spontaneously or on stimulation by the tetrapeptide fMLP, was tested in a chemiluminescence assay. As illustrated in fig 4, the fMLP induced chemiluminescence by monocytes after a 30 minute exposure to the test fluids exceeded that of control cells. The greatest response was observed with monocytes exposed to 0.5% glycerol, with a relative chemiluminescence of 179 (20)% (*v* 100% in control cells, $p < 0.01$). With increasing osmotic stress, the relative chemiluminescence decreased ($p < 0.05$ *v* 0.5% glycerol), but still exceeded that of control cells. After 60 minutes, however, the ability of the monocytes to give a respiratory burst became depressed in the glycerol based fluids (2.5% glycerol *v* GH, $p < 0.05$), whereas chemiluminescence in 1% polypeptides remained unaffected ($p < 0.01$–0.05, *v* 0.5–2.5% glycerol). After 120 minutes, the chemiluminescence response of monocytes became significantly depressed in every test fluid ($p < 0.001$ *v* control), although it was still higher after 120 minutes of exposure to 1% polypeptides than to glycerol based solutions ($p < 0.05$). In test fluids the spontaneous chemiluminescence by monocytes only became significantly depressed after 120 minutes of exposure (data not shown).

We also tested whether the changes observed in the spontaneous and fMLP induced chemiluminescence by monocytes were reversible. After centrifugation and discarding of the supernatants, monocytes exposed to the test fluids were resuspended in RPMI-1640 plus 10% (vol/vol) FCS medium and transferred to "low attachment" tissue culture plates (Mat-Tek) and allowed to recover for 18 hours at 37°C in a humidified atmosphere with 5% CO_2. After the recovery period the spontaneous and fMLP induced chemiluminescence were measured and expressed as percentage chemiluminescence relative to that of monocytes exposed to GH.

It appeared that the increased fMLP induced chemiluminescence observed after 30 minutes normalised after recovery. Monocytes allowed to recover from a 60 minute exposure yielded depressed chemiluminescence responses that were test fluid independent ($p < 0.01$–0.05), whereas monocytes exposed for 120 minutes to all test fluids except 1% polypeptides were unable to achieve a chemiluminescence response (fig 5). Cells allowed to recover from the 30, 60 and 120 min exposure revealed an unaffected spontaneous (or background) chemiluminescence (data not shown).

CYTOKINE RELEASE

It appeared that osmotic stress from the glycerol based fluids only depressed the

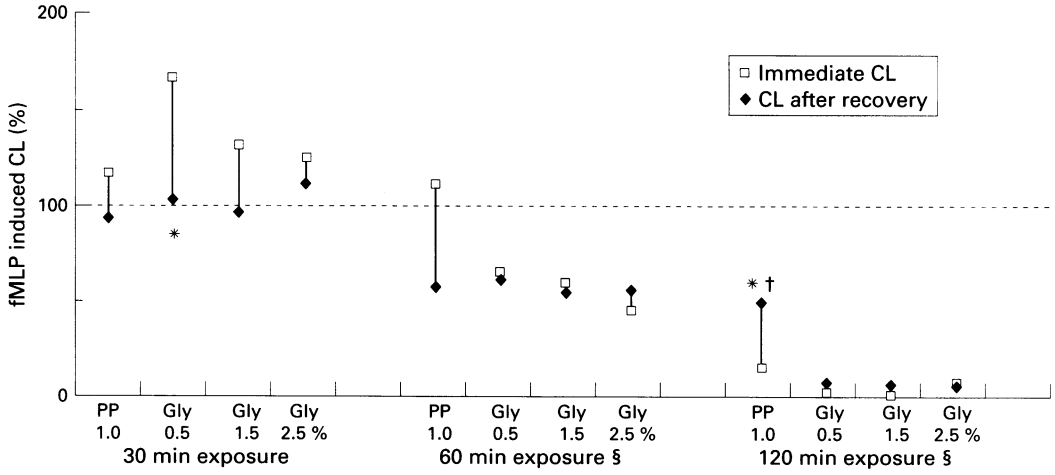

*Figure 5 The relative fMLP induced chemiluminescence response (CL) by monocytes after a 30, 60, and 120 minute exposure to 1% polypeptides (PP), and 0.5–2.5% glycerol (Gly), and after an 18 hour recovery period (control buffer = 100%). *Immediate v CL response after recovery: p < 0.05; †CL after recovery from 1% PP v CL after recovery from Gly: p < 0.05; § control v other: p < 0.05.*

Table 2 Spontaneous and lipopolysaccharide (LPS) induced interleukin 8 release

	Osmolarity (mmol/l)	*Spontaneous release (ng)*	*LPS induced release (ng)*
GH (control)	280	16 (4)	45 (9)
0.7% glycerol	350	15 (4)	44 (9)
1.2% glycerol	404	15 (4)	42 (7)
2.0% glycerol	495	14 (3)	40 (7)*

The spontaneous and LPS induced release of IL-8 by monocytes exposed to control fluid (GH) and 0.7%, 1.2%, and 2.0% glycerol based test fluid (pH 7.4). The cells were attached to the upper side of a microporous membrane located in the upper compartment of a two compartment model. Data are expressed as mean (SEM).
*$p < 0.05$ *v* control and 0.7% glycerol.
GH, Hanks buffered salt solution with 0.1% wt/vol gelatine.

lipopolysaccharide induced release of IL-8 by monocytes attached to the upper side of the microporous membrane. In this setting, the lipopolysaccharide induced release of IL-8 was significantly less on exposure to 2.0% glycerol than on exposure to 0.7% glycerol and GH ($p < 0.05$; table 2). The spontaneous release of IL-8 by monocytes attached to the upper side of the membrane and the spontaneous and lipopolysaccharide induced release of IL-8 by monocytes attached to the basolateral side were not affected by the test fluids. The release of TNFα was not affected at all (data not shown).

The two compartment model studied might be of great value not only in evaluating the impact of several osmotic agents, but also in obtaining information on the exact role of pH in biocompatibility. With that in mind, we also studied the same fluids at pH 5.0. It appeared that low pH had no additional effect on the release of IL-8 or TNFα (data not shown).

Discussion

Dextrose based peritoneal dialysis fluids have several disadvantages. They seem not only to depress host defence function but they may also may provoke a loss of ultrafiltrating capacity by the peritoneum.[3 20] Moreover, they increase adiposity and disturb the serum lipid profile.[21] Host defence functions that are reported to be perturbed in vitro include phagocytic and bactericidal functions of leucocytes and the release of several proinflammatory mediators by leucocytes, mesothelial cells, and fibroblasts.[3] Thus it is of great importance to develop peritoneal dialysis fluids that are more compatible with the normal biological status of body tissues and cells. Any cell function that is altered by a potential peritoneal dialysis fluid is an expression of bioincompatibility. Because there are so many tests available for the in vitro assessment of host defence, it is important to choose tests that most clearly reveal the impact on host defences and thus reflect their biocompatibility. In this study, we focused on assays that estimate mononuclear leucocyte function as a measure of biocompatibility in vitro. We used test fluids that were likely to pose different biocompatibility problems as a result of differences in their osmolarity and protein content. In a previous study we had already shown that protein enriched solutions containing milk whey derived polypeptides were able to preserve polymorphonuclear and mononuclear leucocyte viability during exposure.[22] It was also shown that osmotic stress induced a loss in cellular ATP content.[23 24] Because sufficient intracellular ATP is essential for the maintenance of normal leucocyte function,[24] it was expected that not only the viability but also the function of leukocytes would be preserved by polypeptides but depressed by osmotic stress.

The phagocytosis assay seemed to be the least sensitive of the assays studied. Only minor differences in phagocytosis were observed between monocytes from the protein enriched polypeptide solutions and from the other test fluids, and some were even conflicting. A larger percentage of monocytes participated in the phagocytic process but a smaller mean number of bacteria per monocyte was found if monocytes had been exposed to 1% polypeptides compared with the other test fluids. In contrast to what might have been expected, osmotic stress exerted by the glycerol based fluids did not affect phagocytic activity. This finding may be explained in part by the fact that the attachment of opsonised microorganisms to monocytes is a passive, ATP independent process that occurs readily at 0°C, and which is influenced mainly by the number of C3 and Fc receptors expressed on the cell membrane, and the frequency of leucocyte–bacterium interactions.[25]

The differences in phagocytic activity induced by the test fluids could not be explained by a change in the expression of the C3 or Fc receptors. The only receptor that had altered expression on the monocytes with any of the test fluids was CD64 (Fcγ-RI). CD64 receptor surface expression, which was significantly increased after exposure to the glycerol based fluids, mediates antibody dependent cellular cytotoxicity by macrophages and may trigger phagocytosis and superoxide production.[26] None of these cell functions, however, improved after exposure to glycerol based fluids. Kaupke *et al* reported osmolarity dependent depressed expression of the adhesion receptors CD11b and CD18 on granulocytes (neutrophils) and of CD14 on monocytes after a five minute exposure to dextrose or sodium chloride based fluids.[27] In the current study, monocyte expression of CD18, assessed after a 60 minute exposure to the test fluids, was not affected. This discrepancy might be explained by differences in the response to osmotic stress between monocytes and neutrophils (neutrophils are more vulnerable to this type of stress).[6 23]

In contrast to phagocytosis, engulfment and bacterial killing is an energy consuming process that requires active cellular metabolism.[25] Thus it was in agreement with our hypothesis that the killing capacity of monocytes was better in the protein enriched solution (1% polypeptides) than in control buffer (GH) during the first 30 minutes. However, the lack of difference between polypeptides and the glycerol based fluids, and the loss of any differences between GH and the other fluids after 60 minutes, do not support the view that the reduced killing

capacity in GH is related to cell nutrition. The smaller numbers of bacteria killed in GH might be explained in part by an increased ability of monocytes exposed to the other test fluids to provide a respiratory burst, as demonstrated using the chemiluminescence assay.

The ability of stimulated cells to yield a chemiluminescence response tended to decrease with increasing osmolarity. The chemiluminescence assay most clearly showed the beneficial effect of polypeptides. Prolonged exposure to 1% polypeptides revealed a sustained ability to provide a respiratory burst, whereas exposure to the glycerol based fluids impaired the fMLP induced chemiluminescence after an exposure of 60 minutes or more. Thus the chemiluminescence response is a more sensitive assay for assessing the potential detrimental effects of newly designed peritoneal dialysis fluids than determining the phagocytic and killing capacity of the monocytes. The greater sensitivity of the chemiluminescence assay compared with the phagocytosis assay has been reported before.[4] To our knowledge, the difference between the sensitivity of the chemiluminescence assay and the killing capacity of monocytes in response to experimental peritoneal dialysis fluids has not been shown previously.

Our study shows that preservation of leucocyte function by peritoneal dialysis fluid may be more important than suppression of bacterial growth, as illustrated by the better bactericidal capacity of monocytes for *E coli* in 1% polypeptides than in control buffer, despite increased bacterial survival (fig 2). This finding is in contrast with the suggestion that improved biocompatibility of peritoneal dialysis fluids might carry the risk of increased bacterial survival in the peritoneal cavity, thus providing a selective advantage for organisms causing subsequent infection.[28]

The detrimental effects of the low pH (5.2) and high osmolarity of the currently used peritoneal dialysis fluids on leucocyte viability and function in vitro has been demonstrated before.[4-30] Its consequences in terms of biocompatibility in the in vivo situation, however, are much less evident since peritoneal dialysis fluid pH and osmolarity quickly equilibrate with interstitial fluid in the peritoneum. In a previous study we showed that changes in the pH and osmolarity of the test fluids in the double chamber model are very similar to the figures obtained in the in vivo situation.[12] With the novel double chamber model, therefore, it was possible to obtain data about the impact of peritoneal dialysis fluid induced osmotic and acid stress on leucocyte function, in a way that closely mimics the situation in vivo. It appeared that the release of IL-8 by lipopolysaccharide stimulated monocytes was depressed by 2.0% glycerol compared with 0.7% glycerol and control buffer (GH). We also showed that a low pH in the various fluids studied did not affect the release of IL-8 or TNFα by monocytes when tested in the double chamber model.

We conclude that, of the assays studied, the chemiluminescence assay and the estimate of IL-8 release by monocytes in the two compartment model most clearly demonstrated differences in the biocompatibility problems posed for peripheral blood monocytes. We therefore feel that the chemiluminescence and the IL-8 release test are the most appropriate assays for the assessment of changes in mononuclear leucocyte function in response to different potential peritoneal dialysis fluids.

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