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Peritoneal macrophages during peritonitis. Phenotypic studies

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SUMMARY

The expression of a range of surface molecules/receptors that are important in the host response to infection and foreign antigens was examined using peritoneal macrophages isolated from patients on continuous ambulatory peritoneal dialysis (CAPD) with peritonitis. The macrophage phenotypic profile was compared with that of normal peripheral blood monocytes. Consistently there was increased expression by macrophages of CD14, ICAM-1 (CD54), FcγRI (CD64), FcγRII (CDw32), FcγRIII (CD16), transferrin receptors (CD71) and tissue factor. Increased expression of MHC class II was marginally significant. There was no detectable expression of either the p55 (CD25) or p70 chains of the IL-2 receptor. The expression of the complement receptors, CR1 (CD35) and CR3 (CD11b, CD18), was reduced. The activity of well-known inflammatory cytokines, rather than uraemic molecules, can account for the phenotypic profile of these extravasated peritoneal macrophages. The results of this study indicate that peritoneal macrophages from CAPD patients with peritonitis display a phenotype consistent with them being *in vivo*-derived inflammatory macrophages, and that they are appropriate for use in studies of anti-inflammatory agents.

Keywords phenotype peritoneal macrophages inflammation peritonitis

INTRODUCTION

During infection, in response to bacterial antigens and chemotactic products (bacterial- and host-cell-derived), large numbers of macrophages and neutrophils are recuited to the septic site. Both cell types play key roles in the host response to eliminate the invading bacteria by phagocytic and bactericidal processes. The attraction of cells reflects a highly regulated immunological network. Macrophages are the source of a large number of 'pro-inflammatory' molecules that can control the course of infection. Macrophages can produce proteases, as well as bioactive lipids, and a range of monokines, including IL-1, IL-6, IL-8 and tumour necrosis factor-alpha (TNF-α) [1].

There are few studies of the phenotype and function of human macrophages at a site of infection. We have hypothesized that the harvest of macrophages from 'spent' dialysate of patients on continuous ambulatory peritoneal dialysis (CAPD) with peritonitis provides a useful source of cells for the study of the phenotypic and functional profile of macrophages recruited from the circulation to a site of bacterial infection. To enable us to further our studies of molecules that down-regulate macrophage inflammatory activity [2], we have hypothesized that peritoneal macrophages isolated from CAPD patients during peritonitis are appropriate for functional studies of the control of extravasated inflammatory cells by cytokines, drugs and other regulatory mediators.

Here we have examined a large range of surface markers on human peritoneal macrophages during peritonitis. We anticipated that the phenotypic profile would provide a reflection of the functional activity of the cells. Inflammatory peritoneal macrophages were compared directly with blood monocytes, harvested from non-uraemic controls, principally because it had been shown that blood monocytes from controls and CAPD patients do not vary in surface expression of complement and Fc receptors, CD14, MHC class II and the adherence antigen, p150,95 [3]. In order to recognize the factor(s) that may have contributed to changes in macrophage phenotype, phenotypic profiles of peritoneal macrophages were evaluated in the light of our own studies and published reports of the expression of macrophage surface markers following incubation with regulatory molecules, inflammatory or otherwise. Peritoneal macrophages were also compared with blood monocytes cultured overnight with uraemic molecules. For expression of complement receptors, peritoneal macrophages isolated from four CAPD patients without peritonitis were compared with those from patients with peritonitis.

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MATERIALS AND METHODS

Patients

Over a 13-month time period (July 1990–August 1991), peritoneal cells were studied from nine hospitalized CAPD patients

Table 1. Data concerning the nine hospitalized CAPD patients with peritonitis included in this study. The organism(s) detected in the peritoneal effluents from these patients when the peritonitis was first diagnosed is also shown

Patient no.	Renal disease	Date of iniation of CAPD therapy		Organism(s) detected
1	Renal vascular disease	Nov. 1985		*Staphylococcus epidermidis*
2	Balkans nephropathy	Feb. 1981		*St. epidermidis*
3	Reflux nephropathy	July 1990		*St. epidermidis*
4	Polycystic renal disease	Oct. 1990		Chemical peritonitis
5*	Diabetic nephropathy	Jan. 1991	(1)	*St. epidermidis*
			(2)	*St. epidermidis*
6	Uncertain	Apr. 1987		*St. epidermidis*
7	Obstructive uropathy/horseshoe kidney	Sep. 1990		*Klebsiella oxytoca, Acinetobacter calcoaceticus*
8	Mesangial glomerular nephritis	Nov. 1989		*Escherichia coli*
9	Renal vascular disease/hypertension	May 1991		Unknown

* Patient studied during two episodes of peritonitis, 29 days apart.

with peritonitis. Table 1 gives information concerning the patients' medical history and when they were accepted onto the CAPD programme. The organism(s) detected in peritoneal effluent at the time of first diagnosis of peritonitis is shown. One patient was studied a second time during a separate course of peritonitis, 29 days after the initial study. Cells were isolated from the overnight peritoneal effluent drained on the morning immediately after the detection of peritonitis (or 'cloudy' dialysate) and hospitalization. Peritoneal cells were also studied from the overnight effluent from four non-hospitalized CAPD patients without peritonitis.

Peripheral blood cells for comparison with peritoneal macrophages were obtained from leucocyte-enriched buffy coats kindly provided by the Adelaide Red Cross Blood Bank (South Australia). Mononuclear cell extraction was performed within 4 h of venesection.

Peritoneal macrophage and monocyte isolation

As previously described [4], the cells of the peritoneal effluents (1·3–2·1 *l*) were concentrated by centrifugation (15 min at 400 ***g***) prior to isolation of the mononuclear cells on Lymphoprep density gradients (Nycomed, Oslo, Norway). After a second enrichment for mononuclear cells on Lymphoprep, macrophage populations of >90% purity were obtained [4]; this degree of purification was required for complementary functional studies. In the studies reported here, high purity was not deemed to be so important because it is possible to 'gate' out during flow cytometric analysis those cells that were not staining for the macrophage marker, CD14 (see below). Cells were not transferred to 4°C until after their isolation on Lymphoprep; once reduced in temperature, the cells for phenotypic analysis were not warmed. For studies of peritoneal cells from CAPD patients without peritonitis, the cells obtained after first centrifugation of the total effluent (mean yield $3{\cdot}5 \times 10^6$, range $0{\cdot}6$–$7{\cdot}9 \times 10^6$, $n=4$) were not further purified.

Peripheral blood mononuclear cells were similarly isolated, but on a single Lymphoprep density gradient. Lymphocytes were not physically removed from the monocytes before flow cytometric analysis, or before overnight culture and subsequent examination. As for the peritoneal cells, cells for surface marker analysis were not warmed once they were cooled on ice.

Antibodies

Table 2 details the antibodies used for the phenotypic analysis of human peritoneal macrophages and peripheral blood monocytes. All antibodies were used as azide (0·02%) containing undiluted culture supernatants, with the exception of the following: anti-CD16, ascites diluted 1/100; anti-CD64, purified antibody diluted to 200 μg/ml. Those antibodies not staining macrophages in the studies below, namely those binding to the p55 and p70 chains of the IL-2 receptor, were shown by Professor H. Zola (Flinders Medical Centre, South Australia) in alternative studies with lymphocytes to be active, and to form stable antigen-antibody complexes on the cell surface with formaldehyde fixation. The purified rabbit immunoglobulin to tissue factor [5] was diluted 1/100 for analysis of macrophage expression of tissue factor.

Labelling of cells with marker-specific antibodies

Approximately 5×10^6 cells (derived from peritoneal dialysate or peripheral blood) were centrifuged at 4°C in Nunc Minisorp tubes (cat. no. 466892; Nunc, Roskilde, Denmark) before removal of all fluid. The cell pellet was resuspended in 0·05 ml Sandoglobulin (5 mg/ml in human PBS containing 0·02% sodium azide; Sandoz Australia, Australia). After incubation for 30 min at 4°C, 0·05 ml of the appropriately diluted antibody was added and the tubes were further incubated for 30 min at 4°C. The cells were then washed by centrifugation in human PBS with 0·02% sodium azide, the buffer removed and the cells resuspended in 0·05 ml FITC-labelled anti-mouse or anti-rabbit IgG (Sigma Immunochemicals, St Louis, MO) according to the species of origin of the primary antibody, diluted 1/40 in human PBS containing 0·02% sodium azide. After a further 30-min incubation, the cells were washed twice with the azide-containing buffer described above. The cell pellets were finally resuspended in 0·2 ml PBS supplemented with 1% (v/v) formaldehyde, 2% glucose and 5 mM sodium azide. By direct comparison of the mean fluorescence intensity of antibody-labelled cell populations which were divided into aliquots for formaldehyde

Table 2. Antibodies for analysis of cell marker expression on macrophages isolated from the peritoneal effluents of CAPD patients with peritonitis

Marker	Antibody*	Isotype	Source/reference
Galactocerebroside	—	IgG1	[6]
Unknown	X63	IgG1	[7]
Salmonella antigens	sal 4	IgG2b	[8]
	sal 5	IgG2a	[8]
MHC class I monomorphic determinants	FMC16	IgG2a	[9]
MHC class II monomorphic determinants	FMC4	IgG1	[10]
CD14b	FMC17	IgG2b	[11]
CD25, p55 chain of the IL-2 receptor	Anti-IL-2 receptor (anti Tac)	IgG1	Becton Dickinson, Mountain View, CA (anti-IL-2R)
p70 chain of the IL-2 receptor	Mik-beta 1	IgG2a	[12]
CD54, ICAM-1	1.H.4	IgG2	[13]
CD64, FcγRI	22	IgG1	[14]
CDw32, FcγRII	IV-3	IgG2b	[15]
CD16, FcγRIII	—	IgG2b	[16]
CD35, CR1	MoAb 543	IgG1	ATCC HB 8592
CD11b, CD18, CR3	OKM-1	IgG2b	ATCC CRL8026
CD71, transferrin receptor	5E9CII	IgG1	ATCC HB 21
Tissue factor	RD010	Affinity-purified rabbit immunoglobulin	[5]

* All but antibody RD010 (raised in rabbits) were murine MoAbs.
ATCC, American Type Culture Collection, Rockville, MD.

treatment or kept at 4°C for 1 h in an azide-containing buffer, cell surface antigen-antibody complexes for the antibodies used were shown to be stable under these fixation conditions. Suspensions of human peritoneal macrophages and blood mononuclear cells were equally stored at 4°C until flow cytometric analysis of both populations within 1 week (FACScan; Becton Dickinson, Mountain View, CA).

Culture of blood monocytes

For 24-h culture, mononuclear cells were resuspended in RPMI 1640 medium (Flow Laboratories, McLean, VA) supplemented with 2 mM MOPS (Sigma), 13·3 mM $NaHCO_3$, 2 mM glutamine, 50 μM 2-mercaptoethanol, 100 U/ml penicillin and 100 μg/ml streptomycin (subsequently referred to as complete RPMI), with an osmolality of 290 mmol/kg H_2O. Approximately 2×10^6 mononuclear cells were cultured in 1 ml complete RPMI supplemented with 1% (v/v) fetal calf serum in 70 × 11 mm polyethylene tubes (Nunc Minisorp). The following reagents were added at the initiation of culture to give the indicated final concentrations: PGE_2 (Sigma) 10 ng/ml; urea (Sigma) 10 mM; creatinine (Sigma) 0·5 mM (stock 1 M in 0·1 N HCl); uric acid (Sigma) 0·16 mM (stock 1 mg/ml in $LiCO_3$). The effect of a 'spent' peritoneal dialysate from one of the CAPD patients with peritonitis was also tested at a concentration of 20% (v/v). Duplicate cultures for each test variable were incubated at 37°C in 5% CO_2. To terminate cultures, the contents of the duplicate tubes were pooled, centrifuged and the cell pellets stained with marker-specific antibodies as described above. To confirm the activity of the modulating agents in question, the culture supernatants were frozen at −20°C and assessed for changes in inflammatory mediator production, namely decreased TNF-α production in response to PGE_2 or inflammatory dialysate [2,4; and our unpublished observations].

Statistical analysis

Mean fluorescence intensities of cell populations were compared with the unpaired Student's t-test. In some experiments in which cell responses were heterogeneous, the responses were compared with the non-parametric rank sign test. Results were considered significant if $P < 0{\cdot}05$.

RESULTS

Cell populations under study

We have previously described the isolation of inflammatory macrophages from the 'spent' dialysates of CAPD patients with peritonitis. By staining with Sudan black, these cells were approximately 95% peroxidase positive [4], suggesting a relatively immature population. For flow cytometric analysis of the peritoneal macrophage population, exclusion gates were set such that only the $CD14^+$ population was analysed (CD14 positivity 95 ± 1%, mean ± s.e.m., $n = 10$). The same exclusion gates were used for the analysis of fluorescent-antibody-labelled blood monocytes (mean ± s.e.m. CD14 positivity 87 ± 3%, $n = 10$). The peritoneal macrophage population was sometimes more heterogeneous in size, as determined by increased forward and side scatter. However, less than 25% of the peritoneal cells were larger than the dominant cell in both the peritoneal macrophage and monocyte populations.

Flow cytometric analysis of peritoneal macrophages from CAPD patients with peritonitis

For the peritoneal macrophage and the monocyte populations, the cells were relatively homogeneous for the level of expression of the surface molecules under study, with a single peak of fluorescence intensity detected. The exception was for expression of CD16 by monocytes (two peaks), and MHC class II antigens by both cell populations (a single broad peak). There was some inter-patient variability in the phenotype of the macrophages isolated from the peritoneal cavities of nine patients during 10 peritonitis episodes. However, there was considerable uniformity in the levels of expression of these inflammatory and differentiative markers by peritoneal macrophages when directly assessed in the same experiment with the expression of the same markers by peripheral blood monocytes from healthy donors (Fig. 1).

The binding of irrelevant mouse antibodies of isotypes IgG1, IgG2b and IgG2a, was examined (Fig. 1a). The latter were particularly important as monocytes/macrophages are FcR-bearing cells, and up-regulation of Fcγ type I (to which mouse IgG2a bind) has been associated with inflammation [14]. In fact, non-specific binding of mouse IgG2a antibodies to human Fc receptors [17] was initially used as a measure of increased FcγRI expression (Fig. 1d). However, cells were incubated with pooled human immunoglobulin (Sandoglobulin) before incubation with antibodies and for this reason non-specific binding of irrelevant antibodies by Fc receptors was limited.

As shown diagrammatically in Fig. 1a–e, FITC-tagged antibody binding was as follows:

CD14 (Fig. 1b): Expression of the macrophage marker, CD14, was increased for nine of the 10 macrophage populations examined (mean fluorescence intensity 835 ± 187 s.e.m. for peritoneal macrophages; 550 ± 138 for blood monocytes; $n = 10$; $P = 0{\cdot}011$ by sign test).

MHC determinants (Fig. 1b): The expression of MHC class I monomorphic determinants was reduced in seven out of 10 peritoneal macrophage populations (mean fluorescence intensity for macrophages, 570 ± 116 s.e.m., for monocytes, 744 ± 168; $n = 10$; $P = 0{\cdot}172$ by the sign test). In contrast, when the broader fluorescence peaks for MHC class II staining of macrophages were compared with the staining of monocytes, antigen expression was increased for eight out of the 10 peritoneal macrophage populations examined (mean fluorescence intensity for macrophages 196 ± 45 s.e.m., for monocytes, 135 ± 29; $n = 10$; $P = 0{\cdot}055$ by the sign test).

ICAM-1/CD54 (Fig. 1b): For the eight comparative studies performed, increased expression by peritoneal macrophages was detected in all studies with the mean fluorescence intensity ($\pm$s.e.m.) increasing from 114 ± 26 on monocytes to 235 ± 42 on peritoneal macrophages ($n = 8$; $P = 0{\cdot}03$ by unpaired Student's t-test).

IL-2 receptors (Fig. 1c): There was no evidence for increased expression of either the α (p55) or β (p70) chains of the IL-2 receptor on peritoneal macrophages. The increased binding to macrophages of the fluorescent antibody to the p55 chain of this receptor (macrophages, 55 ± 5; monocytes, 39 ± 6; mean $\pm$ s.e.m., $n = 5$), and to the p70 chain (macrophages, 70 ± 8; monocytes, 42 ± 9; mean $\pm$ s.e.m., $n = 4$) was not different to that of the non-specific antibodies.

Fc receptors (Fig. 1d): A specific antibody to the type I Fcγ receptor was used in two experiments to show increased receptor expression on peritoneal macrophages (means of 385 and 228 for peritoneal macrophages and monocytes, respectively). However, for six experiments, non-specific binding of an IgG2a antibody, in the absence of cell pre-incubation with human immunoglobulin, was examined and further demonstrated increased FcγRI on peritoneal macrophages (34 ± 13 for monocytes; 139 ± 40 for macrophages; mean $\pm$ s.e.m.; $n = 6$; $P = 0{\cdot}046$ by Student's unpaired t- test).

Small increases in the expression of the FcγRII receptor were detected (monocytes, 410 ± 29; macrophages 511 ± 15; mean $\pm$ s.e.m.; $n = 4$; $P = 0{\cdot}03$ by Student's unpaired t-test).

There was heterogeneous expression of FcγRIII (CD16) on monocytes. Upon eight analyses, a mean of 13% of the population stained with a fluorescence intensity (mean $\pm$ s.e.m.) of 528 ± 90. The remaining 87% cells showed a fluorescence intensity of 29 ± 9. In contrast, only a single relatively sharp peak of fluorescence intensity was detected when peritoneal macrophages were incubated with an antibody to the FcγRIII (141 ± 41; mean $\pm$ s.e.m.; $n = 8$). For Fig. 1d, antibody binding to the major monocyte population is shown.

Complement receptors (Fig. 1e): The expression of the complement receptors, types 1 and 3, were reduced on peritoneal macrophages from CAPD patients with peritonitis. For four donors, the mean fluorescence intensity for binding of antibodies to the type 1 receptor was reduced from 242 ± 21 (mean $\pm$ s.e.m.) on monocytes, to 167 ± 20 peritoneal macrophages ($P = 0{\cdot}04$, Student's unpaired t-test). Binding of antibody to the anti-CR3 receptor was reduced from a mean fluorescence reading of 907 ± 115 on monocytes to 581 ± 38 on peritoneal macrophages ($n = 4$; $P = 0{\cdot}04$, Student's unpaired t-test).

CD71 (transferrin receptor) (Fig. 1e): The binding of antibody to CD71 was investigated in five experiments with increased fluorescence intensity detected for all five peritoneal macrophage populations (145 ± 26 for macrophages; 58 ± 13 for monocytes; mean $\pm$ s.e.m.; $P = 0{\cdot}02$ by Student's unpaired t-test).

Tissue factor (Fig. 1e): A rabbit polyclonal antibody to tissue factor was used, with a non-specific rabbit antiserum as the control. The latter is not shown in Fig. 1 as negligible binding was detected by either cell population. Monocytes expressed very little of this antigen (fluorescence intensity 34 ± 12; mean $\pm$ s.e.m.; $n = 10$) whereas increased levels were detected on all peritoneal macrophage populations (110 ± 20; $P = 0{\cdot}004$, Student's unpaired t-test).

Thus, in comparison with blood monocytes, there was a significant decrease by peritoneal macrophages from CAPD patients with peritonitis, in the expression of the complement receptors types 1 and 3. However, peritoneal macrophages generally displayed increased expression ($P < 0{\cdot}05$) of CD14, Fc receptors, ICAM-1, transferrin receptors and tissue factor. Increased staining of MHC class II was marginally significant ($P = 0{\cdot}055$). Changes in MHC class I determinants were equivocal.

Comparison with peritonitis-free CAPD macrophages and the phenotypic changes measured after exposure of peripheral blood monocytes to uraemic molecules

There are many reports implicating inflammatory mediators, e.g. cytokines, as the agents responsible for many of the changes in surface antigen expression described above, including de-

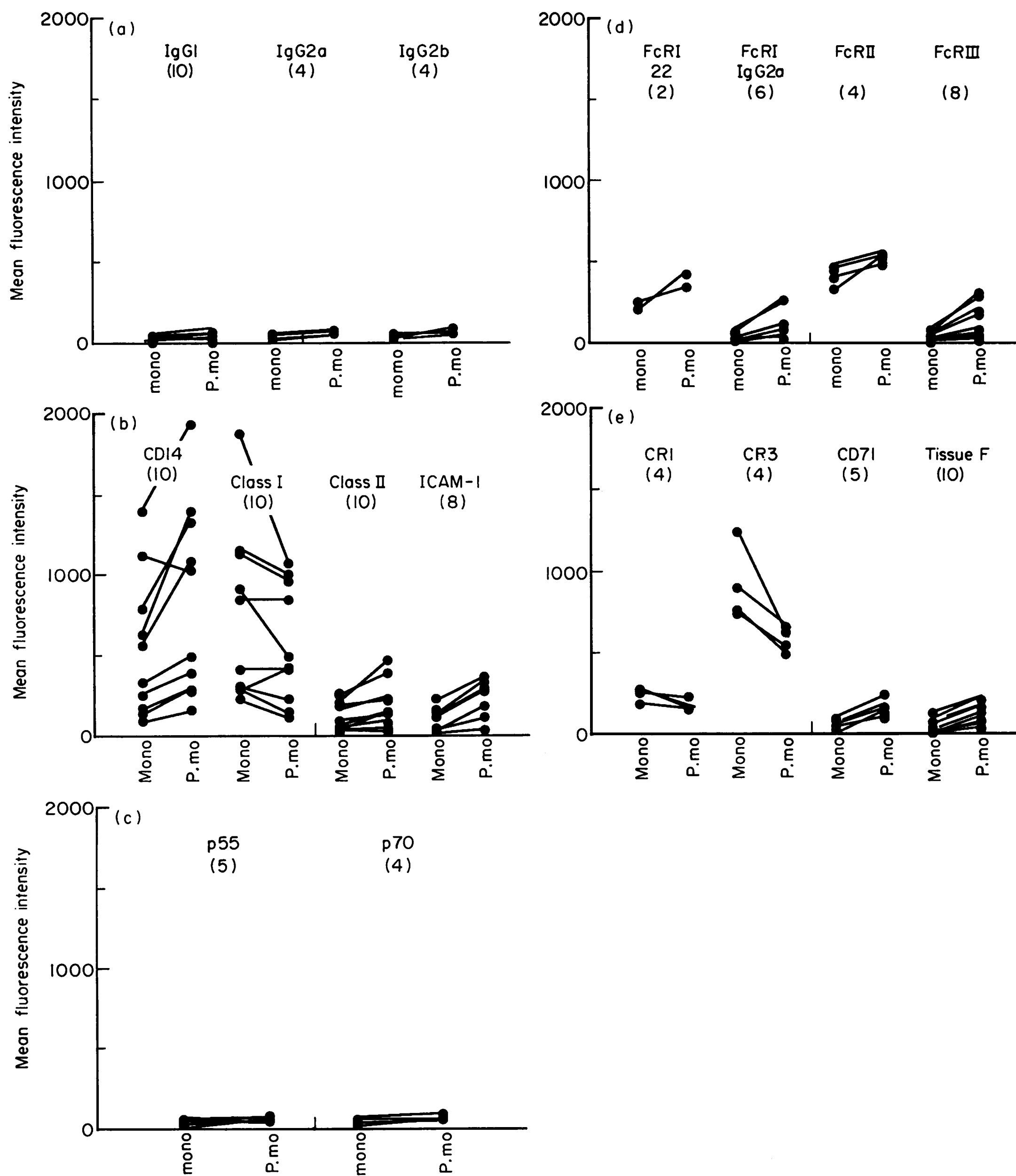

Fig. 1. Flow cytometric analysis of peritoneal macrophages (P.mo) from 10 CAPD patients with peritonitis, and monocytes (Mono) from 10 controls. (a) Binding of non-specific control antibodies; (b) Binding of antibodies to CD14, MHC class I and II, and ICAM-1; (c) binding of antibodies to the p55 and p70 chains of the IL-2 receptor; (d) binding of antibodies to the Fcγ receptors (FcRI, FcRII, FcRIII). FcγRI was measured both with a specific anti-receptor antibody (22) and by the binding of a non-specific IgG2a in the absence of initial incubation with human immunoglobulin; (e) binding of antibodies to the complement receptors (CR1 and CR3), CD71, and tissue factor. Results are expressed as mean fluorescence intensity as determined by the binding of a second fluorescently tagged anti-mouse or anti-rabbit antibody. For each CAPD patient studied, a monocyte population was studied in an identical fashion in the same experiment; the results for each experiment (dots) are joined by a solid line. The number of binding studies for each antibody is indicated in parentheses.

Table 3. Expression of CR3 (CD11b, CD18) by macrophages isolated from the peritoneal effluents of CAPD patients without peritonitis. For comparison, the expression of CR3 by peripheral blood monocytes from two healthy donors was examined

$CD14^+$ cells*	Mean fluorescence intensity†
Monocyte donor 1	617
Monocyte donor 2	697
CAPD patient 1	578
CAPD patient 2	704
CAPD patient 3	640
CAPD patient 4	579

* Monocytes/macrophages in each population were positively selected by expression of CD14. Using these predetermined 'gates', 92% and 89% (donors 1 and 2, respectively) of the selected $CD14^+$ monocytes expressed CR3 as a single sharp peak of fluorescence intensity. Between 80% and 96% ($n=4$) of $CD14^+$ peritoneal macrophages expressed CR3 as a single peak of fluorescence.

† Fuorescence measured using a primary antibody to CR3, and a second fluorescently tagged anti-mouse antibody.

creased expression of class I and complement receptors (see below). Because decreased CR1 and CR3 expression may compromise the host's phagocytic and bactericidal processes, we investigated complement receptor expression on peritonitis-free CAPD macrophages. Table 3 shows the mean fluorescence intensity when CR3 expression by macrophages isolated from the overnight effluents from four CAPD patients without peritonitis were compared in the same experiment with CR3 expression by monocytes from two donors. Data in Table 3 suggest that few macrophages from peritonitis-free CAPD patients have reached the threshold level for signalling of reduced CD3 expression.

Monocyte phenotypic changes resulting from exposure to uraemic molecules, namely urea, creatinine and urates, were also investigated by incubation of monocytes overnight with concentrations of urea, creatinine and urates as measured in peritoneal effluents (20 mM, 1 mM and 0·3 mM, respectively). Surface expression of MHC class I and II, complement receptors type 1 and 3, CD14, FcγRII and CD71, the transferrin receptor were not changed (data not shown). PGE_2 (10 ng/ml) was also without effect on expression of the same monocyte activation markers. We were also unable to reproduce the phenotypic changes of macrophages by monocyte incubation for 16 h with a cell-free inflammatory peritoneal effluent (20% v/v) from which peritoneal cells had been isolated.

DISCUSSION

This study began with the premise that peritoneal macrophages isolated from CAPD patients with peritonitis were representative of extravasated inflammatory cells, and because of their abundant supply could be used for extensive study of the actions of anti-inflammatory agents. To this end, we examined the phenotype of inflammatory peritoneal macrophages isolated from the 'spent' effluents of CAPD patients with peritonitis. In each experiment, peripheral blood monocytes from a healthy donor were used as the direct control for macrophage expression of activation markers. Previous studies had suggested little phenotypic difference in monocytes between controls and CAPD patients [3]. In addition, the number of studies performed, and the relative consistency of the results when pairs of cell populations were studied, compensate for interindividual differences in antigen expression and variability in the fluorescence intensity of flow cytometric analyses.

Because of the peritoneal fluid replacement therapy for CAPD patients, the peritoneal cells harvested for analysis in this study must have recently extravasated into the peritoneal cavity. Phenotypic differences between the peritoneal macrophages and blood monocytes were most likely due to the inflammatory process, and possibly recent exposure to bacteria even though cells from at least one patient with chemical peritonitis were included. However, the peritoneal cavity of CAPD patients is considered to reflect a stage of low-grade chronic inflammation [3]. We found no evidence to suggest that uraemic molecules may affect macrophage function as assessed by modulation of surface antigen expression (we have also found that they do not interfere with proinflammatory mediator production [unpublished observation]). This finding would support reports by others who have found that peritoneal macrophages from CAPD patients have normal bactericidal functions as long as they are not exposed *in vitro* to unused, acidic (approximate pH 5·5), hypertonic dialysis medium [18]. We believe that this study confirms that peritoneal macrophages from CAPD patients with peritonitis are not intrinsically different to inflammatory macrophages from other tissues and provide a valid target population for studies of anti-inflammatory agents, e.g. cytokines, drugs.

The peritonitis macrophages displayed many surface markers indicative of cell activation, including increased expression of CD14, MHC class II and Fc receptors. These antigens have previously been shown to be increased on peritoneal cells from CAPD patients without peritonitis [3]. We have also shown increased expression of the inflammatory markers, ICAM-1 and tissue factor, on peritonitis macrophages.

Analysis of their activity both *in vivo* and *in vitro* indicates the role that these surface molecules have in the host response to infection. Both MHC class II molecules and ICAM-1 are important for antigen presentation and the development by the host of an inflammatory response [13, 19]. ICAM-1 binds the LFA-1 molecule of other cells and is important for stabilizing cell–cell interactions [13] and antigen presentation [20]. Not only is CD14 a well-accepted marker for myeloid cells [11], but binding to the receptor can also signal cell activation for production of inflammatory mediators, e.g. TNF-α [21]. This receptor was recently identified to be the receptor for lipopolysaccharide (LPS)-binding protein [21] and thus may also act as an opsonic receptor for promotion of phagocytosis of bacteria or LPS-coated particles. Tissue factor, otherwise known as tissue thromboplastin or tissue procoagulant, is the membrane glycoprotein that serves as the non-enzymatic co-factor for factor VII in the initiation of extravascular fibrin formation and its deposition on inflammatory cells [22].

FcγRI (CD64) is the high-affinity Fc receptor for monomeric mouse IgG2a and IgG3 and human IgG1 and IgG3. FcγRII (CDw32) and FcγRIII (CD16) are low-affinity Fc receptors for complexed IgG [17]. We have found, in agreement with previous

reports [23], that monocytes constitutively express CD64 and CDw32, with a minor subset (< 10%) expressing CD16. As monocytes mature, CD16 expression increases. Fcγ receptors of all subclasses on monocytes/macrophages mediate phagocytosis of immunoglobulin-coated particles [24]. A correlation between a decrease in the number of surface FcγRI and loss in antibody-dependent cellular cytotoxicty (ADCC) ability of cultured monocytes has been reported [25]. Stimulation of monocytes via their FcγRI receptors can induce inflammatory mediator production, including TNF-α and PGE_2 [26].

There is evidence that with inflammation and development of the host immune response, a network of cytokines is responsible at least in part for many of the biochemical, pathological and immunological changes seen. Many of the phenotypic changes seen on peritoneal macrophages are suggestive of a response to interferon-gamma (IFN-γ) (increased MHC class II expression [27], increased ICAM-1 [28], increased FcγRI [14]). Others are suggestive of a response to granulocyte/macrophage colony-stimulating factor (GM-CSF) (increased CD 16 expression [27], FcγRII [27]). Other increases in marker expression may result from the action of less well-characterized cytokines, e.g. expression of tissue factor, which is induced by macrophage procoagulant inducing factor [29]. The response of monocyte CD14 expression to inflammatory cytokines is not settled. We (unpublished) and others [30] have found decreased CD14 expression in response to IFN-γ; others suggest little change in CD14 expression in response to either IFN-γ or GM-CSF [27]. In contrast, inflammation *per se* is stimulatory for CD14 [3].

Expression of CD71, i.e. the transferrin receptor, which is generally considered a marker for proliferating cells, was also increased on peritoneal macrophages. We investigated whether this signified replication of the cells within the peritoneal cavity; however, an analysis of ploidy of peritoneal cells by flow cytometry revealed greater than 95% of the cells in G_0G_1 (data not shown). Transferrin receptor expression on monocytes has been shown to increase upon incubation with both IFN-γ and GM-CSF [27].

Other markers on the peritoneal macrophages from CAPD patients with peritonitis were unchanged, or reduced in comparison to those expressed on blood monocytes. Expression of the α and β chains of the IL-2 receptor was not detected on either blood monocytes or peritoneal macrophages, and appears therefore to preclude the therapeutic use of IL-2 as an immunostimulant during peritonitis. However, IFN-γ has been shown by us (unpublished) and others [31] to increase monocyte expression of the p55 chain of the IL-2 receptor. Therefore, the lack of p55 receptor protein on peritoneal macrophages may reflect insufficient cumulative stimulatory signals for stimulaton of p55 expression.

The expression of the complement receptors, CR1 and CR3, was decreased on peritoneal macrophages. CR1 mediates the adherence of C3b-coated particles to phagocytic cells, whereas iC3b-dependent functions, such as phagocytosis of serum-opsonized particles, are mediated by CR3 [32]. CR3 is also involved in leucocyte adherence, including cell spreading (particularly to surfaces bearing Arg-Gly-Asp-containing ligands) and transendothelial migration, and it has the capacity to bind to polysaccharides present on the surface of unopsonized bacteria [32,33]. Decreased expression of complement receptors may reflect the influence of IFN-γ [27,34,35; and our unpublished observations]. Alternatively, during bacterial peritonitis, decreased receptor number may reflect recent endocytosis of ligand-bound receptors, i.e. C3- and iC3b-opsonized bacteria. Endocytosis is usually accompanied by recycling of internalized receptors; however, for both CR1 and CR3, a selective and long-term loss of expression has been detected following binding of CR1-antibody [36] and serum-opsonized zymosan [32], respectively. The disadvantage, if any, to the host of decreased complement receptor expression on peritoneal macrophages during peritonitis is the subject of further experimentation. Significant decreased peritoneal macrophage CR3 receptor expression was not found for peritonitis-free CAPD patients.

The study of the phenotype of peritoneal macrophages from CAPD patients with peritonitis did not find any evidence for abnormal inflammatory macrophages, but instead a population of macrophages phenotypically very similar to monocytes exposed *in vitro* to a selection of inflammatory cytokines, and other mediators involved in an immune and bactericidal response. From this study, we advocate their use as a target population for the study of the regulation of inflammatory cells.

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