Kidney International, Vol. 49 (1996), pp. 525–533

Longitudinal evaluation of peritoneal macrophage function and activation during CAPD: Maturity, cytokine synthesis and arachidonic acid metabolism

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Longitudinal evaluation of peritoneal macrophage function and activation during CAPD: Maturity, cytokine synthesis and arachidonic acid metabolism. The release of cytokines and prostaglandins (PG) by peritoneal macrophages (PMØ) may influence the cytokine network controlling peritoneal inflammation and in the long-term the function of the peritoneum as a dialysis membrane. In the present study, an evaluation of the long-term effects of peritoneal dialysis on the release of cytokines and prostaglandins, and the expression of surface markers of cellular maturation on blood and mononuclear cells has been performed in patients during their first year on CAPD. Spontaneous release of tumour necrosis factor α (TNFα) and interleukin 6 (IL-6) by PMØ, after 4 or 24 hours in culture, increased significantly with time on CAPD, while there was a small but significant decrease in release of prostaglandin E_2 (PGE_2). Production of TNFα and IL-6 was enhanced following incubation of the cells with lipopolysaccharide (LPS), but the effect of LPS was proportionally greater on blood monocytes than on PMØ. There was a significant increase in the concentrations of PGE_2 and 6-keto-prostaglandin $F_{1\alpha}$ in overnight dwell peritoneal dialysis effluent with time on CAPD. The levels of TNFα and IL-6 in uninfected PDE were below the detection limit of the immunoassay over the whole time period studied. Expression of CD15, which correlates with immaturity, by PMØ and blood monocytes increased with time on CAPD, while expression of CD11c, a marker of maturation, decreased on blood monocytes, but did not change significantly on PMØ. There was also a slight increase in expression of transferrin receptor in both PMØ and monocytes, but this did not reach statistical significance. These findings suggest that peritoneal macrophages and blood monocytes isolated from CAPD patients over a one year period become increasingly immature with time, and this is accompanied by a significant modulation of their ability to secrete inflammatory cytokines. Dysregulation of macrophage function may have important consequences with respect to inflammatory processes and the long-term function of the peritoneal membrane in CAPD patients.

Continuous ambulatory peritoneal dialysis (CAPD) is now widely used in the treatment of end-stage renal failure. Its increasing use, however, has highlighted the fact that the function of the peritoneal membrane as a dialyzing organ may be adversely affected by long term exposure to the CAPD environment. In particular, loss of ultrafiltration [1] and changes in the morphology of the peritoneum [2] can occur after extended treatment periods, even in patients with no history of peritonitis [3–6]. There is also evidence that immunological changes occur within the peritoneum after the initiation of CAPD [7]. Several studies have demonstrated that PMØ become increasingly immature [8–14], have reduced bactericidal activity as well as increased chemotactic activity and transferrin receptor expression (TfR) compared with controls [8–11]. In addition Betjes et al [14] have recently shown that, compared with cells from normal controls, PMØ from CAPD patients not only had a less mature phenotype, as measured by RFD7 expression, but also showed signs of chronic activation as evidenced by their increased expression of Fc receptors.

Fieren, van den Bemd and Bonta [15] have demonstrated that PMØ isolated from patients with peritonitis are primed for cytokine release following lipopolysaccharide (LPS) stimulation, suggesting that these cells are already in a semi-activated state *in vivo* and only require a second stimulus to become fully activated. It is not known, however, whether PMØ isolated from uninfected CAPD patients show changes in their constitutive and stimulated ability to release cytokines and prostaglandins with increasing time on CAPD.

The present longitudinal study was therefore initiated to examine the potential effects of peritoneal dialysis on: (a) the release by PMØ of the cytokines tumor necrosis factor alpha (TNFα) and interleukin-6 (IL-6) both constitutively and after *in vitro* stimulation; (b) the release of prostaglandins (PGE_2 and 6-keto-$PGF_{1\alpha}$) by PMØ; (c) the levels of cytokines and prostaglandins in peritoneal dialysis effluent (PDE); and (d) the expression of surface markers of cellular maturation on PMØ and peripheral blood mononuclear cells (MNC).

Our data indicate that PMØ isolated from CAPD patients over a one year period become increasingly immature as assessed by surface marker expression. This is accompanied by a significant modulation of their ability to secrete inflammatory cytokines and prostaglandins (PG). Long-term dysregulation of PMØ function may have important consequences and might contribute to the loss of peritoneal membrane function seen in some CAPD patients after long periods on this form of therapy.

Received for publication December 19, 1994
and in revised form September 13, 1995
Accepted for publication September 15, 1995

Table 1. Details of patients in group 1

Patient	Sex	Age	System	Time at which last sample was taken, *months*	No. of peritonitis episodes/cause	Underlying disease	Reason for patient being eliminated before 12 months
1	F	71	Fres.[a]	12	(1) enterococcus (1) coagulase negative *Staphylococcus*	Etiology unknown	—
2	M	17	Bax.[b]	12	(1) *S. aureus*	Hypoplastic renal disease	—
3	F	54	Fres.	12	(0)	Renovascular disease	—
4	M	52	Bax.	3	(1) *S. epidermidis*	Diabetic nephropathy	Changed to hemodialysis
5	M	75	Bax.	9	(1) *S. epidermidis*	Glomerulonephritis	Died
6	M	67	Fres.	9	(0)	Glomerulonephritis	Died
7	M	66	Bax.	12	(0)	Etiology unknown	—
8	F	44	Bax.	9	(0)	Diabetic nephropathy	—
9	F	69	Bax.	12	(0)	Etiology unknown	—
10	F	30	Bax.	12	(0)	Etiology unknown	—
11	M	17	Bax.	12	(1) *S. aureus*	Obstructive nephropathy	—
12	M	58	Bax.	6	(1) *S. epidermidis*	Diabetic nephropathy	Changed to hemodialysis

[a] Fresenius Andy
[b] Baxter Solo

Table 2. Details of patients in group 2

Patient	Sex	Age	System	Time at which last sample was taken, *months*	No. of peritonitis episodes/cause	Underlying disease	Reason for patient being eliminated before 12 months
1	F	56	Bax.[a]	12	(1) mixed	Glomerulonephritis	—
2	F	32	Fres.[b]	12	(2) *S. aureus*	Wegener's granulomatosis	—
3	F	33	Fres.	6	(0)	Interstitial nephritis	Transplanted
4	M	58	Fres.	12	(0)	Diabetic nephropathy	—
5	M	55	Bax.	12	(1) *S. epidermidis*	Diabetic nephropathy	—
6	M	43	Bax.	9	(0)	Glomerulonephritis	Changed to hemodialysis
7	F	69	Bax.	9	(2) *S. epidermidis*	Diabetic nephropathy	Died
8	M	18	Fres.	3	(0)	Congenital hypoplastic renal disease	Transplanted
9	M	66	Bax.	12	(0)	Glomerulonephritis	—
10	F	12	Bax.	12	(2) *S. aureus*, *S. epidermidis*	Polycystic kidneys	—

[a] Fresenius Andy
[b] Baxter Solo

Methods

Patients

Two separate trials were conducted, involving a total of 22 patients commencing CAPD, 12 patients in group 1 (Table 1) and 10 in group 2 (Table 2). The first sample was obtained on the day of commencement of dialysis and subsequent samples at one week (group 1 only) and 1, 3, 6, 9 and 12 months. In group 1, 8 patients were monitored for the full period and 6 of those in group 2. Study of the remaining patients was terminated earlier for a variety of reasons (Tables 1 and 2). A total of 7 (group 1) and 8 (group 2) episodes of peritonitis occurred among these patients during the study; all (except group 1, patient 11 at 3 months) occurred at least three weeks before the time when a sample was due to be taken. To ensure that these episodes of peritonitis did not affect the results, the parameters examined were analyzed separately for patients with and without peritonitis. In no case was a significant difference found (data not shown).

Peritoneal equilibrium test (PET). A four-hour hypertonic exchange was performed in each patient at each time interval after one month from the start to assess ultrafiltration. Blood biochemistry was analyzed at the time of each sample to monitor the efficiency of CAPD.

Cell cultures

All the following processes were carried out in a laminar flow cabinet and all buffers and media, etc., had < 12.5 pg/ml contamination with endotoxin.

Peritoneal cells. Overnight PDE was spun in an MSE Mistral centrifuge in 1 liter bottles at 500 × g for 15 minutes. For group 1 the supernatants were removed and stored in aliquots at −70°C until needed for analysis of cytokine and prostaglandin levels. The cells from patients in group 1 were resuspended (10^6 cells/ml) in Ham's F12 medium (ICN Flow Biomedicals Ltd. Rickmansworth, UK) supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), transferrin (5 μg/ml), insulin (5 μg/ml) and hydrocortisone (0.4 μg/ml; all from Sigma, Poole, UK) and 10% fetal calf serum (FCS; ICN Flow). These cells were used for testing constitutive cytokine and PG release. Cells from group 2 patients were resuspended in RPMI 1640, supplemented with penicillin, streptomycin, and FCS as above. Cells from this group were used to measure cytokine release from LPS-stimulated PMØ and also for surface marker studies. Viability, determined by eosin exclusion, was always > 95%. Ham's F12 medium was used for culturing cells from group 1 patients as the supernatants were subsequently added

to mesothelial cell cultures, which require this medium [16]. Otherwise RPMI 1640 was used as in our previous work with PMØ [8].

Blood mononuclear cells. Blood mononuclear cells were obtained from the group 2 patients at the same time as the PMØ and were isolated by centrifugation of heparinized blood over Lymphoprep separating mixture (Nycomed UK Ltd., Birmingham, UK). The cells were removed from the interface layer, washed twice and resuspended at 10^6 cells/ml in RPMI 1640 medium with 10% fetal calf serum.

Preparation of macrophage supernatants from group 1. Peritoneal cells (2×10^6 in 2 ml) were cultured in 35 mm diameter Petri dishes for two hours at 37°C/5% CO_2 to allow adherence of the PMØ. The dishes were then washed twice with warm Ham's F12, the number of non-adherent cells removed in the washings was counted and the number of adherent cells calculated by the difference. Fresh medium (2 ml) was then added to each Petri dish and the incubation continued for a further two hours. The supernatants were then removed and stored in 1 ml aliquots at −70°C until analysis. A further 2 ml of fresh medium was added and cells incubated for a further 20 hours, at which time the supernatants were again collected and stored as above. It should be noted that, in this trial, no external stimulus such as LPS was added to the cells.

Preparation of macrophage and monocyte supernatants from group 2. Aliquots of each above cell suspensions were plated in 24-well plates, non-adherent cells removed after two hours, and the cells then incubated with or without 2 μg/ml of lipopolysaccharide from *E coli* 055.B5 (LPS, Difco, Detroit, MI, USA) in 1 ml of RPMI 1640/10% FCS for 22 hours. Supernatants were then removed, filtered, and stored at −70°C.

Cell surface marker analysis. Peritoneal cells from the second group of patients were incubated in separate 200 μl aliquots for 30 minutes with monoclonal mouse antibodies to transferrin receptor, CD11c and CD15 (all from Serotec). A $F(ab')_2$ rabbit anti mouse FITC conjugate (Serotec) was used as second antibody and the proportion of macrophages and lymphocytes staining positive determined by FACS. Mononuclear cells in peripheral blood obtained from the patients at the same time as PDE were analyzed using Q-Prep (Coulter Electronics Ltd., Luton, UK), a whole blood lysis method, and the same antibodies. The proportion of monocytes and lymphocytes reacting with each antibody was determined.

Cytokine and prostaglandin assays

TNFα and IL-6 were measured by enzyme immunoassay [17–19] and prostaglandin E_2 (PGE_2) and 6-keto-prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) by radioimmunoassay [16, 17, 20] as previously described.

Statistics

Statistical analyses were performed using Minitab (Minitab Inc., State College, PA, USA). If necessary, a logarithmic or a square root transformation was applied to the observations so that they approximated to a normal distribution. The logarithm of the time on dialysis was used when needed to make the sampling interval more uniform. Changes in variables with time on dialysis were estimated by linear regression. Before performing linear regression analyses, the data were adjusted by analysis of variance to have the same grand mean for each patient and for each batch of samples, after which the data were pooled. Degrees of freedom were adjusted accordingly. A paired *t*-test was performed to test for an absolute difference in cytokine concentration due to different experimental treatments of the same cell preparation, irrespective of source. For the *t*-test, observations were pooled by taking the grand mean for all the observations, and the effect of batches, patients and time on cytokine release removed by adding to the grand means the residuals from analysis of variance. Degrees of freedom were adjusted by subtraction of the number of batches, patients and sampling times in the study. A critically significant level of $P = 0.05$ was used throughout the study. Results are given as the mean ± the standard error of the mean.

Results

Patient parameters

No significant changes were seen in creatinine, urea or ultrafiltration values. Initially values were 738 ± 97 μmol/liter, 24.2 ± 2.4 mmol/liter and 670 ± 83 ml, respectively. After one year they were 1042 ± 120 μmol/liter, 21.1 ± 6.8 mmol/liter and 700 ± 82 ml, respectively (P = NS by linear regression, $N = 12$ at day 1 and 8 at 1 year).

Cytokines and prostaglandins in PDE

Prostaglandins. The mean levels of both PGE_2 and 6-keto-$PGF_{1\alpha}$ in PDE at the beginning of the study were 2.35 ± 0.1 ng/ml and 0.87 ± 0.1 ng/ml, respectively (Fig. 1). A significant rise in the levels of both PGs with time on CAPD was observed ($P < 0.001$ by linear regression, $N = 12$ at day 1 and 8 at 1 year).

Cytokines. The levels of TNFα and IL-6 were generally at or beneath the detection limit of the assay (20 and 40 pg/ml for TNFα and IL-6, respectively) throughout the whole time course measured. There was thus no evidence of any change with time on CAPD.

Cytokine and prostaglandin synthesis by PMØ

The constitutive levels of TNFα and IL-6 secreted by PMØ after both 4 or 24 hours in culture increased significantly with increasing time on CAPD (Fig. 2; $P < 0.01$ for IL-6, $P < 0.001$ for TNFα by linear regression, $N = 12$ at day 1 and 8 at 1 year). After four hours constitutive secretion of TNFα increased from 167 ± 106 at day 1 of treatment to 645 ± 338 pg/10^6 PMØ after one year therapy, and for IL-6 from 164 ± 75 to 464 ± 119 pg/10^6 PMØ. After 24 hours in culture PMØ TNFα levels were 74 ± 43 and 388 ± 187 pg/10^6 PMØ at day 1 and one year, respectively, and the corresponding numbers for IL-6 were 331 ± 187 and 929 ± 403 pg/10^6 PMØ. The levels of IL-6 secreted by PMØ after 24 hours were always greater than those at four hours irrespective of the patient or time on CAPD, while for TNFα the reverse was true ($P < 0.001$ by paired *t*-test in both cases, $N = 62$). No significant changes in the constitutive secretion of PGs by PMØ was observed with time on CAPD, other than a significant ($P < 0.05$ by linear regression) decrease in PGE_2 production by PMØ after four hours of culture (data not shown).

Stimulated cytokine and prostaglandin release in PMØ amd MNC

Constitutive TNFα and IL-6 synthesis by PMØ was always significantly greater than by MNC, irrespective of treatment time ($P < 0.01$ by paired *t*-test, $N = 49$), the mean values for TNFα and IL-6 being respectively 106 ± 21.5 and 13704 ± 1593 pg/10^6 cells for PMØ, and 46.7 ± 12.5 and 4119 ± 922 pg/10^6 cells for MNC, respectively. In contrast, the effect of LPS stimulation on cytokine production was significantly greater on peripheral blood MNC

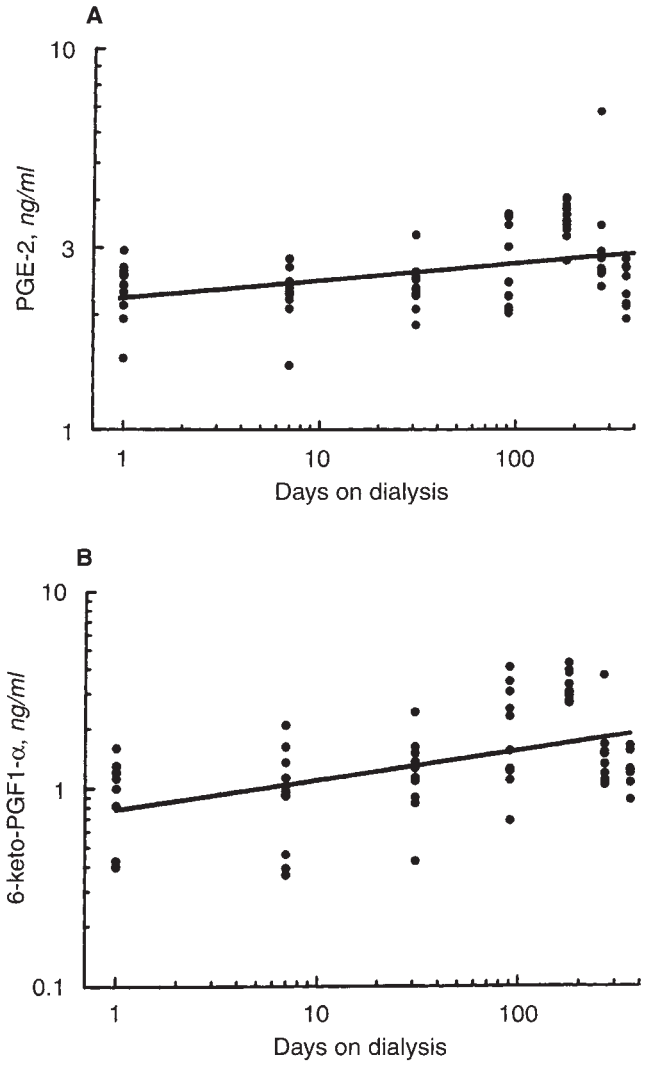

Fig. 1. *Levels of PGE_2 (A) and 6-keto-$PGF_{1\alpha}$ (B) in peritoneal dialysis effluent from patients on CAPD from day of commencement up to one year.* Each point represents the mean value of duplicate determinations. $P < 0.001$.

than on PMØ isolated from the same patient ($P < 0.001$ for TNFα and IL-6, respectively, by paired *t*-test, $N = 47$).

At the start of the study, LPS-stimulated TNFα and IL-6 levels produced by MNC were 35.5 ± 3.58 and and 165.3 ± 7.3 pg/10^6 cells, respectively. There was an increase in both cytokines with time on CAPD, but only in the case of TNFα was this statistically significant ($P < 0.05$ by linear regression). The difference in cytokine production between unstimulated and LPS-stimulated MNC also increased with time on CAPD (Fig. 3 A, B; $P < 0.05$ and $P < 0.002$ for TNFα and IL-6, respectively, by linear regression, $N = 48$).

The initial levels of TNFα and IL-6 produced by LPS-stimulated PMØ were 29.9 ± 3.1 and 157 ± 11.1 pg/10^6 cells, respectively. There was no significant change in the levels with time on CAPD, and there was also no significant change in the relative increase over production by unstimulated cells (Fig. 3 C, D).

Cell surface expression of markers of maturity

The proportion of PMØ expressing CD15 increased significantly with time on CAPD (Fig. 4B, $P < 0.05$ by linear regression, $N = 10$ at day 1 and 6 at 1 year). At time zero 16.1 ± 2.4% of PMØ expressed this marker, and this increased to 36.9 ± 6.2% after one year on CAPD. The mean expression by PMØ of CD11c decreased slightly (Fig. 4A) and that of the TfR (Fig. 4C) increased slightly, but neither of these changes were statistically significant.

In peripheral blood MNC, the expression of CD11c decreased significantly with time on CAPD from a mean of 23.0 ± 10.6% at day 1 to 10.2 ± 2.3% after one year (Fig. 5A; $P < 0.05$ by linear regression, $N = 10$ at day 1 and 4 at 1 year). In contrast, the mean percent expression of CD15 increased significantly ($P < 0.05$; Fig. 5B) from 25.7 ± 5.3% at day 1 to 49.0 ± 3.1% after one year. The level of MNC TfR expression (Fig. 5C) did not change significantly.

Discussion

The process of CAPD is dependent on the long term function of the peritoneal membrane as a dialysing organ. Loss of function of this membrane related to repeated infection or to long term changes in its structure/function can in some cases lead to a change of renal replacement therapy. The mechanisms by which loss of membrane function, as manifested by loss of ultrafiltration, are initiated remain poorly understood, although it has been suggested that chronic inflammatory activation within the peritoneal cavity might be important in its etiology [21]. The present study was therefore initiated to examine the function and maturity of PMØ and peripheral blood MNC isolated from patients over their first year on CAPD.

Previous studies by ourselves and others have indicated that PMØ maturity and function might be altered in patients exposed to long term CAPD [8–11, 14]. In the present study the expression of CD15 (a marker of cell immaturity [22–24]) by PMØ and MNC was significantly increased and the expression of CD11c (a marker of cell maturity [25]) by peripheral blood MNC was significantly reduced.

There was also a slight increase in transferrin receptor expression (CD71; a marker of cell immaturity and/or activation [12, 13]) by both PMØ and MNC with time on CAPD, which did not reach statistical significance in either case. It is possible that had the study been larger this relationship would have become significant, as we have previously reported in a larger number of randomly selected patients [9]. The decrease with time of CD11c expression by MNC supports the concept that the pool of monuclear phagocytes, the precursors of PMØ, is becoming increasingly immature. The CD11c group of integrins is important in MNC phagocytosis [26] and a decrease in its expression might render cells less able to mount an adequate response to bacterial invasion. In this respect a recent study by Betjes et al [27] has demonstrated a malfunction in immunophagocytosis by PMØ immediately prior to the onset of peritonitis.

This increasing immaturity of macrophages and monocytes may explain the results of an earlier study in which the ability of peritoneal cells to kill *Staphylococcus epidermidis* decreased with time on CAPD [28]. Further support for the immaturity of CAPD macrophages comes from the work of Goldstein et al [11], who suggested that PMØ are relatively immature monocytes freshly derived from bone marrow, based on increased chemotaxis and a lower eicosanoid precursor uptake compared with controls. Furthermore, Davies et al [29] found expression of RFD7, a marker of mature tissue macrophages with phagocytic potential, was relatively low on CAPD macrophages, suggesting curtailment of the maturation process, perhaps due to rapid cell turnover [11].

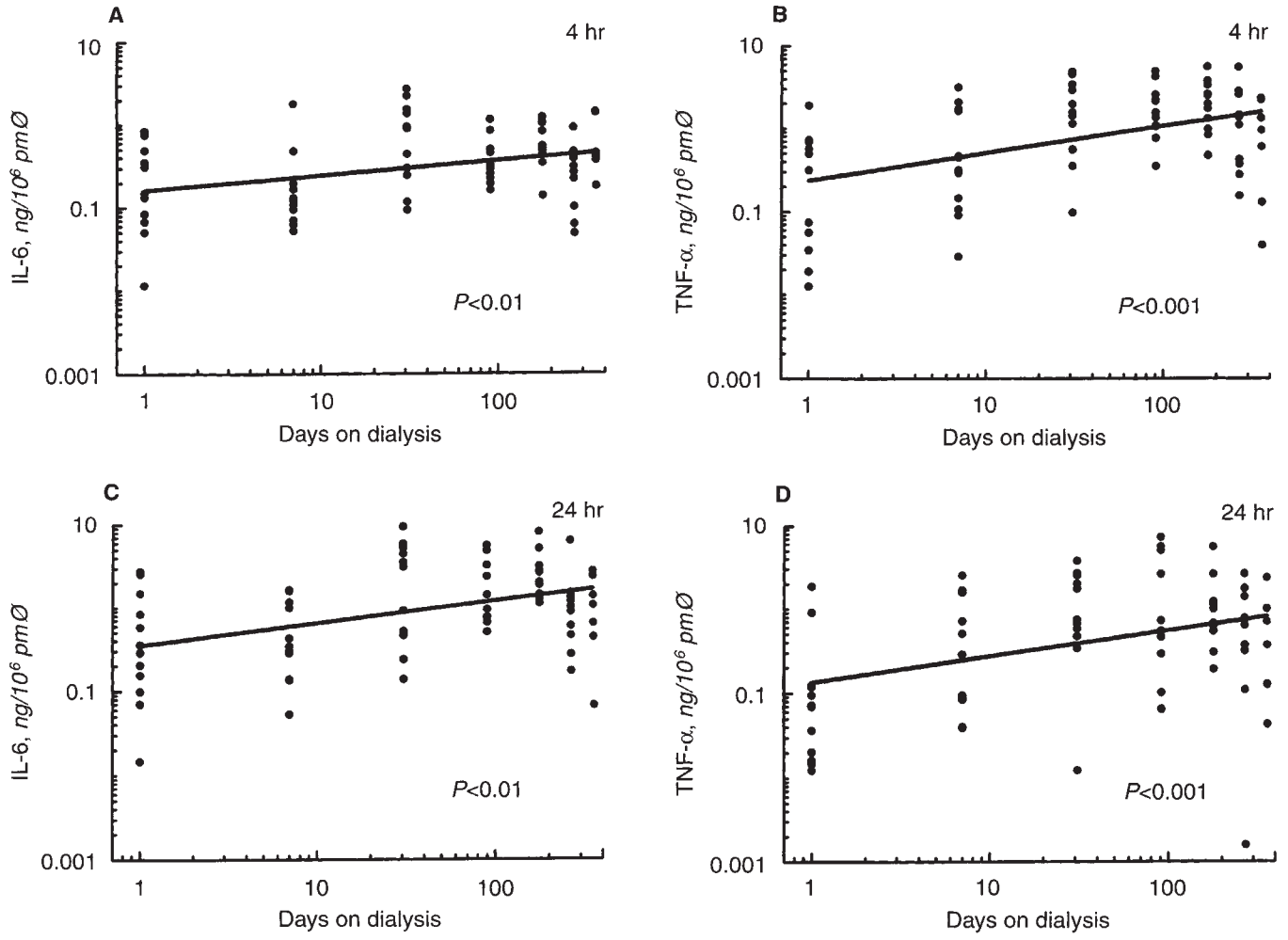

Fig. 2. *Levels of IL-6 (A, C) and TNFα (B, D) in culture supernatants of unstimulated PMØ after 4 (A, B) and 24 (C, D) hours in culture.* The cells were taken from CAPD patients over a one year period from the day of commencement. Each data point represents the mean value of duplicate determinations using PMØ isolated from each patient. (A, $P < 0.01$; B, $P < 0.001$; C, $P < 0.01$; D, $P < 0.001$)

The present study demonstrated increased potential for PMØ to synthesize cytokines (TNFα and IL-6) *ex vivo* with increasing time on CAPD. Although the mechanism underlying this dysregulation of cell function is unclear, it is possible that the increasing immaturity of PMØ with time on CAPD results in a loss of the normal control mechanisms regulating cytokine production. It is known that the release of cytokines from leukocytes is affected by their state of maturation, as well as by activation and/or differentiation [30, 31]. In a recent study, Friedlander et al [32] also found that the constitutive production of PGE_2 and IL-6 in PMØ from CAPD patients was higher than in control cells, which provides evidence that during CAPD chronic inflammatory stimulation of PMØ occurs, and that this may be related to duration of treatment. The effect of LPS on cytokine synthesis by PMØ did not change with time on CAPD, whereas with monocytes an increase did occur. Bos et al [33] have suggested that CAPD changes the normal resident cell population of the peritoneum to an exudate macrophage population, which would indicate that a state of chronic inflammation exists. Nevertheless, inflammatory exudate macrophages may in any case be relatively immature cells [34], so it is possible that both hypotheses are correct. Betjes et al [14] have shown that PMØ from CAPD patients show simultaneous characteristics of immaturity and activation, with expression of a less mature phenotype as measured by RFD7 expression but a higher Fc receptor expression. They also showed that the peritoneal immune system stays at a higher level of activation in high peritonitis-incidence patients which could account for the damage done to the peritoneum after frequent episodes of peritonitis. It should be noted that none of the patients on the present study suffered more than two episodes of peritonitis during the year long study, so none can be considered high peritonitis-incidence patients.

Mesothelial cells have been shown to synthesize IL-6, IL-8, IL-1 and prostaglandins [18, 35, 36], and it is possible that if TNFα is produced by the PMØ *in vivo* it could stimulate mesothelial cells, thus altering regulation of the cytokine network [18, 21, 36]. Also of interest is the finding that the levels of IL-6 produced by PMØ were always higher at 24 hours than at four hours after isolation, but for TNFα the reverse was true. This accords with the generally accepted idea that TNFα is produced before IL-6 in an immune response [37]. In this respect the recent study by Zemel et al [38] demonstrates that the levels of TNFα are elevated at the onset of peritonitis while the levels of IL-6 are maximal at a later stage of infection. Recent evidence suggests that IL-6 may be anti- rather

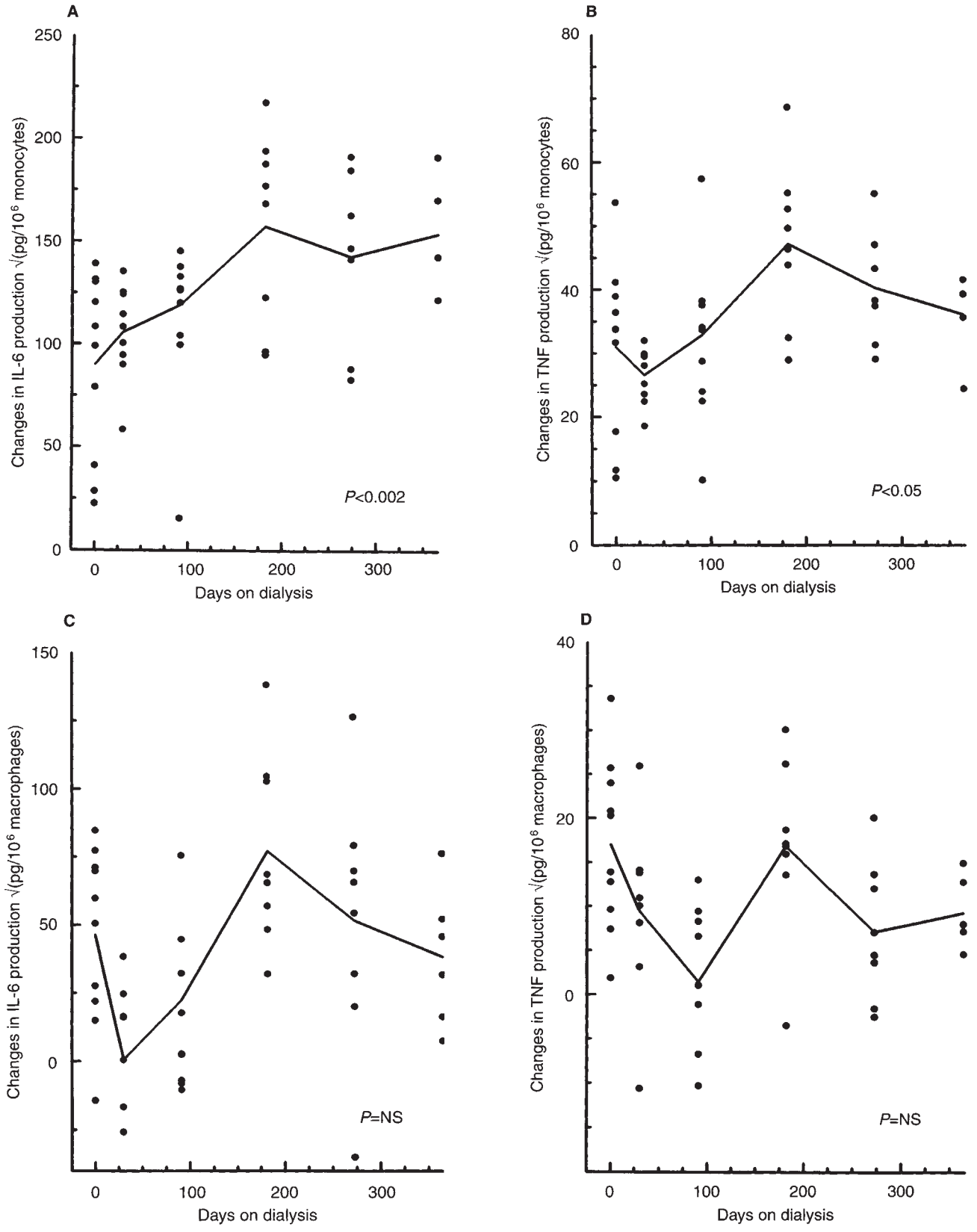

Fig. 3. *The calculated difference between constitutive and LPS-stimulated cytokine levels produced by peripheral blood MNC and PMØ isolated from CAPD patients over one year on CAPD.* MNC and PMØ were exposed to control medium or LPS (2 μg/ml) for 22 hours. Each data point represents the mean change of duplicate determinations in cytokine levels, expressed as pg/10^6 cells. (A, $P < 0.002$; B, $P < 0.05$; C, P = NS; D, P = NS.) A and B depict the change in MNC IL-6 and TNFα levels, respectively. C and D are the changes in PMØ IL-6 and TNFα levels, respectively

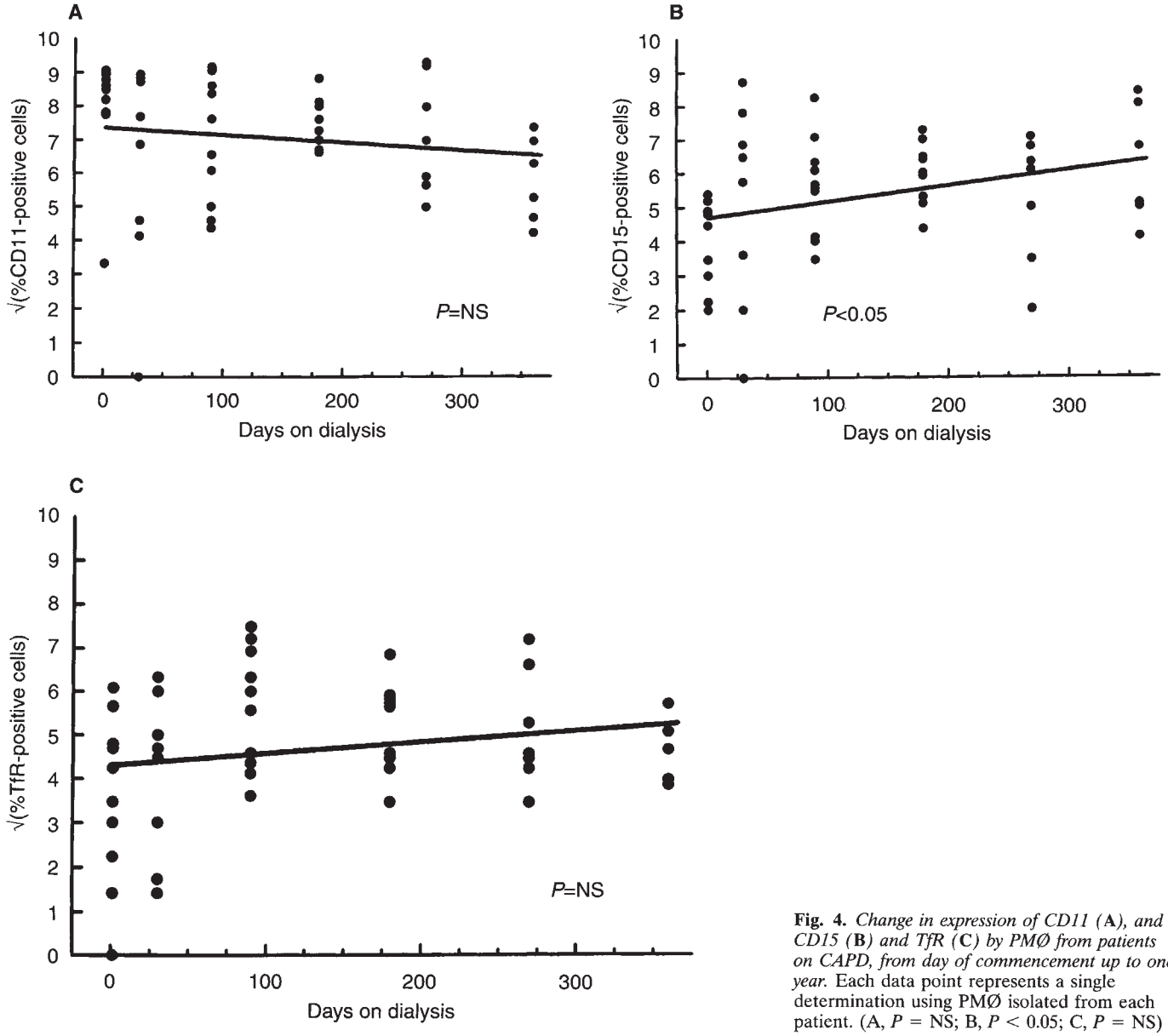

Fig. 4. *Change in expression of CD11* (**A**), *and CD15* (**B**) *and TfR* (**C**) *by PMØ from patients on CAPD, from day of commencement up to one year.* Each data point represents a single determination using PMØ isolated from each patient. (A, P = NS; B, $P < 0.05$; C, P = NS)

than pro-inflammatory as it can suppress the expression of mRNA for other cytokines [39, 40]. In this context IL-6 could act as a negative feedback signal for the production of cytokines by PMØ, thus controlling the inflammatory response.

No significant change in the levels of IL-6 or TNFα in the PDE with time on CAPD was detected but levels were generally at the detection limit of the assay. However, a significant increase did occur in the levels of PGE_2 and 6-keto-$PGF_{1\alpha}$ in PDE. Nevertheless, production of prostaglandin or prostacyclin *in vitro* by cultured PMØ did not change with time on CAPD, suggesting that the changes found in PDE reflect increased secretion by the other resident peritoneal cells. In this respect the mesothelium may be a major source of prostaglandins in the peritoneal cavity and it is likely that the PMØ themselves can directly elevate mesothelial cell arachidonic acid metabolism, at least partly, as a result of their secretion of TNFα [20, 36, 41–43].

The data presented in this study present evidence that over a one year period on CAPD, PMØ become increasingly immature, and that this is associated with an up-regulation of their secretion of pro-inflammatory and immunomodulatory cytokines. These results, together with the recent data of Suassuna et al [44] demonstrating significant numbers of PMØ within the peritoneal membrane during infection-free periods, suggest that the potential for cell activation within the peritoneal membrane, mediated by PMØ products, might increase with time on CAPD. The relationships between changes in macrophage biosynthetic capacity, inflammation and the function of the peritoneal membrane remain to be defined. Recent evidence, however, [44] confirms that PMØ may be in close proximity with the peritoneal membrane during CAPD. It is thus possible that macrophages activated within this environment might have significant effects on the peritoneal membrane. Although in this study we found no changes

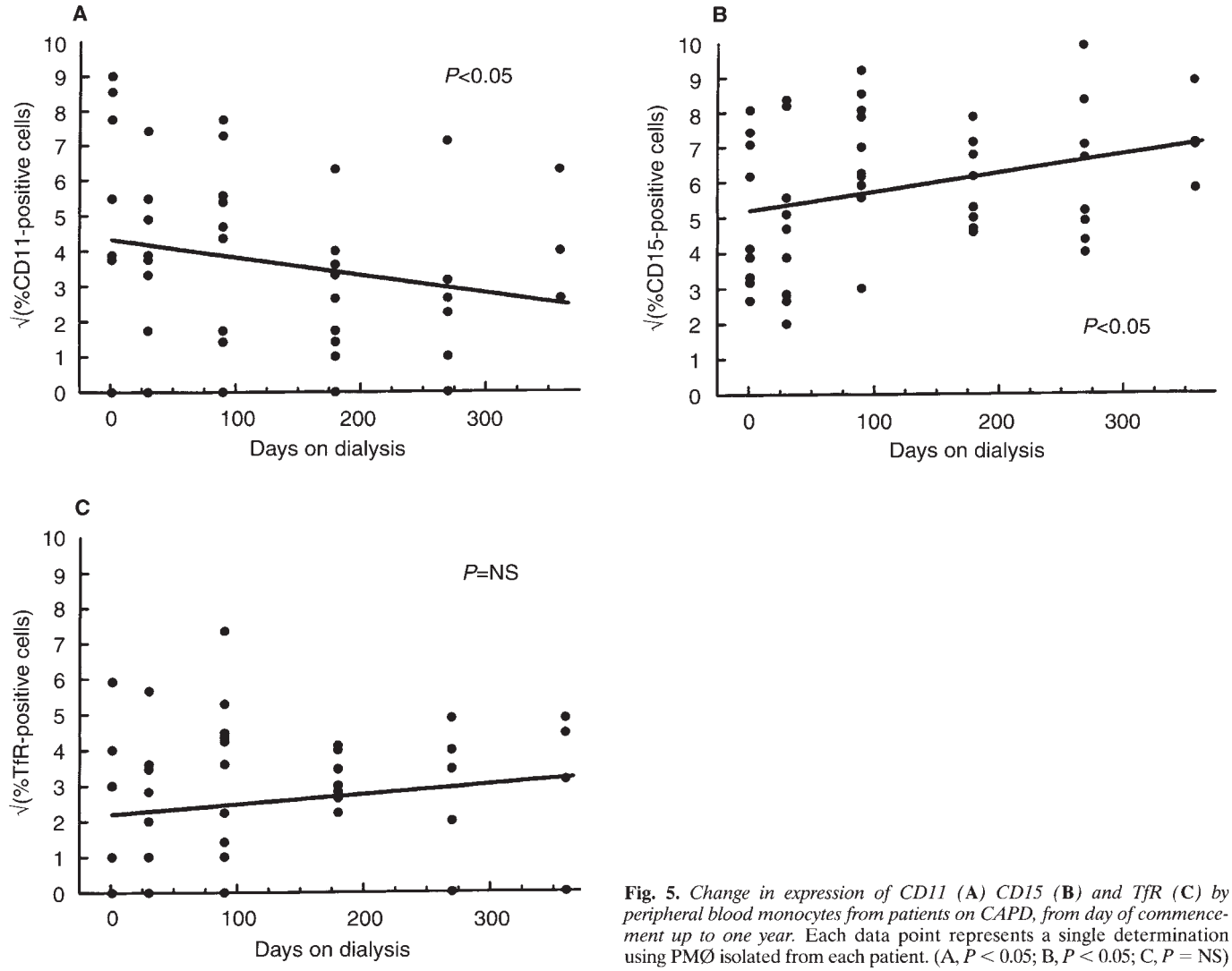

Fig. 5. *Change in expression of CD11* (**A**) *CD15* (**B**) *and TfR* (**C**) *by peripheral blood monocytes from patients on CAPD, from day of commencement up to one year.* Each data point represents a single determination using PMØ isolated from each patient. (A, $P < 0.05$; B, $P < 0.05$; C, P = NS)

in ultrafiltration, it may be that within the first year it is too early to detect such a change which only becomes evident after extended periods using this form of treatment [45].

Although the physiological significance of our results remains to be established, they do suggest that dysregulation of the normally tightly-regulated inflammatory events within the peritoneal membrane may occur, resulting in increased levels of vasodilatory prostaglandins and increased macrophage activation. This may have important consequences for the long term use of the peritoneal membrane as a dialyzing organ.

Acknowledgments

This work was supported by a grant from the National Kidney Research Fund. We especially thank M. McMillan and M. Kennedy, Stobhill Hospital, Glasgow, and C. Donald, M. Timms and staff of the Western Infirmary CAPD Clinic, Glasgow for providing the patient samples. Thanks must also go to S. Fröhlich, B. Mauder and N. Merrifield for technical assistance. N.T. is supported by the Kidney Research Unit Foundation and the Medical Research Council, and N.T. and A.J. are recipients of a British Council/Deutscher Akademischer Austraschdienst Collaborative Grant.

Appendix. Abbreviations

CAPD, continuous ambulatory peritoneal dialysis; MNC, peripheral blood mononuclear cells; PMØ, peritoneal macrophage; PGE_2, prostaglandin E_2; 6-keto $PGF_{1\ \alpha}$, 6-keto prostaglandin $F_{1\alpha}$; PDE, peritoneal dialysis effluent; TNFα, tumor necrosis factor alpha; IL-6, interleukin 6; PG, prostaglandin; LPS, lipopolysaccharide; FACS, fluorescence activated cell sorter; FCS, fetal calf serum;

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