Pro- and Anti-Inflammatory Cytokines in Chronic Pediatric Dialysis Patients: Effect of Aspirin

Stuart L. Goldstein,* Jocelyn C. Leung,† and Douglas M. Silverstein‡

**Department of Pediatrics, Renal Section, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas; and Department of Pediatrics, Divisions of †Neonatology and ‡Nephrology, Louisiana State University Health Sciences Center and Children's Hospital, New Orleans, Louisiana*

Dialysis provides effective and safe treatment of ESRD in children, but patients who are maintained on chronic dialysis are at risk for cardiovascular disease. One major risk factor for cardiovascular disease in adult patients with ESRD is chronic inflammation. The effect of anti-inflammatory therapy with aspirin on serum cytokine concentration was studied in seven children who were receiving hemodialysis (HD) and seven who were receiving continuous cycling peritoneal dialysis (CCPD or PD). Dialysis vintage was 4.3 ± 4.6 yr; single-pool Kt/V was 1.46 ± 1.4, mean equilibrated Kt/V was 1.27 ± 0.16, and PD weekly Kt/V was 2.45 ± 0.30. Baseline proinflammatory cytokine IL-1β, IL-6, IL-8, and TNF-α serum concentrations were significantly elevated, whereas serum anti-inflammatory cytokine IL-4 and IL-10 concentrations were normal. The patterns of cytokine elevation were similar for patients who were receiving HD *versus* PD. IL-4 and IL-6 concentrations demonstrated strong positive correlation with dialysis vintage (IL-4, $P < 0.03$; IL-6, $P < 0.0001$). Pre-aspirin serum cytokine concentrations did not vary with single-pool Kt/V or equilibrated Kt/V for HD patients or with weekly Kt/V for PD patients. Serum IL-8 and TNF-α concentrations were significantly reduced by aspirin treatment at 4 mo ($P = 0.04$ and $P = 0.007$, respectively). Serum IL-6 concentration decreased with aspirin treatment but not significantly ($P = 0.1$). Serum IL-1β concentration remained unchanged, and IL-4 and IL-10 concentrations remained stable throughout aspirin treatment. The effect of aspirin treatment on serum cytokine concentrations was similar for HD and PD patients. In HD patients, IL-6, IL-8, and TNF-α remained suppressed 1 mo after discontinuation of aspirin. It is concluded that proinflammatory cytokines are elevated in pediatric HD and PD patients without counterbalance from anti-inflammatory cytokines, and aspirin therapy attenuates inflammation.

Clin J Am Soc Nephrol 1: 979–986, 2006. doi: 10.2215/CJN.02291205

Although the advent of dialysis provides effective and safe treatment of ESRD in pediatric patients, children who are maintained on long-term dialysis are at risk for significant cardiovascular disease (CVD). Although many CVD risk factors have not been studied in the pediatric dialysis population, studies in adult patients with ESRD demonstrate that cardiovascular calcifications and inflammation (1), abnormal calcium-phosphorus metabolism (2), and malnutrition (3) play a role in CVD development.

A burgeoning area of research in the past decade has focused on the role of inflammation in dialysis patients. Inflammation, mediated by proinflammatory cytokines, contributes substantially to morbidity and mortality (4–8) in adult patients with ESRD. We previously showed that the proinflammatory cytokines IL-1β and TNF-α increased significantly after hemodialysis (HD), whereas there was only a mild increase in the anti-inflammatory cytokine IL-10, suggesting an imbalance of cytokine production favoring the proinflammatory response. Consistent with the enhanced inflammatory state were the elevated C-reactive protein levels in a majority of the patients whom we studied (9) and that the degree of inflammation correlated with the number of years that patients lived with ESRD (dialysis vintage). A recent study showed that there is increased oxidant stress and early cardiovascular damage in children with various stages of renal insufficiency, although the cohort included only a small number of patients who were receiving dialysis (10). On the basis of our previous studies, we decided to perform a pilot study to assess the anti-inflammatory capacity of daily, low-dosage aspirin on serum cytokine concentrations in children who were receiving maintenance HD or peritoneal dialysis (PD).

Materials and Methods

Patients

All patients who had been receiving either maintenance HD or continuous cycling PD (CCPD) for at least two consecutive months in the Texas Children's Hospital Renal Dialysis Unit were eligible to enroll in the study. Informed consent was obtained from each patient's parent, and assent was obtained from each patient who was older than 14 yr before study enrollment. The Institutional Review Boards from both Baylor College of Medicine and Louisiana State University Health Sciences Center approved the study protocol before its initiation. Patients were selected to ensure inclusion of a wide spectrum of pediatric ages, with similar mean age and range between the two groups that were receiving HD and PD. Patients were excluded from the study for the following reasons: (*1*) Evidence of inflammation and/or infection;

Received December 26, 2005. Accepted June 22, 2006.

Published online ahead of print. Publication date available at www.cjasn.org.

Address corresponence to: Dr. Douglas Silverstein, Louisiana State University Health Sciences Center, Children's Hospital, New Orleans, LA 70112. Phone: 504-896-9238; Fax: 504-896-9240; E-mail: dsilve@lsuhsc.edu

ISSN: 1555-9041/105-0979

(2) anticipated change in dialysis modality or renal transplantation in the subsequent 6 mo; (3) return to dialysis from renal transplantation or switch in dialysis modality in the preceding 6 mo; or (4) recent or current use of other anti-inflammatory agents (*e.g.*, ibuprofen, corticosteroids) or lipid-lowering agents (*e.g.*, hepatic hydroxymethyl glutaryl CoA reductase inhibitors), the latter of which may affect serum cytokine concentrations (11).

Dialysis Prescription

We performed monthly HD and at least quarterly CCPD adequacy assessment in accordance with the Texas Children's Hospital Renal Dialysis Unit clinical practice during the course of study. We adjusted dialysis prescriptions as needed to deliver a single-pool Kt/V (spKt/V) of ≥1.4 for HD patients and weekly total Kt/V ≥2.1 for CCPD patients.

spKt/V was calculated using formal urea kinetic modeling. Equilibrated Kt/V (eKt/V) was estimated using the logarithmic extrapolation method described by Goldstein and Brewer (12).

Aspirin Intervention

Study patients received 4 mo of daily baby aspirin (81 mg) therapy during the course of study after a positive varicella titer confirmation. All patients tolerated aspirin therapy without any adverse effects (*e.g.*, dyspepsia, nausea, vomiting, gastrointestinal bleeding, rash). During school days, a school nurse verified aspirin administration. On weekends and other nonschool days, families and patients were responsible for aspirin therapy compliance. During this time, the patients were dialyzed exclusively with polymethylmethacrylate membranes.

Cytokine Level Measurement

Blood for analysis of serum cytokines was obtained before a routine, midweek HD treatment. OptEIA assay kits to measure human cytokines were purchased from BD PharMingen (San Diego, CA). The sensitivity/recovery for the ELISA kits were as follows: IL-1β 3.9 pg/ml/80 to 94%; IL-6 2.2 pg/ml/88 to 100%; IL-8 0.8 pg/ml/97 to 102%; TNF-α 2 pg/ml/89 to 93%; IL-2 1 pg/ml/88 to 95%; IL-4 2 pg/ml/89 to 91%; and IL-10 2 pg/ml/89 to 90%. In general, the protocol for the analysis of each cytokine was similar. For each cytokine, all patient samples from each study period were analyzed simultaneously with an identical set of control samples. Briefly, all patient serum samples and reagents were brought to room temperature. First, standards were prepared from a 500-pg/ml stock. The appropriate dilutions were made to establish a standard curve. Fifty microliters of ELISA diluent was pipetted into each well of the 96-well plates. Then, 100 μl of the standards and samples (all done in duplicate) were pipetted into wells and mixed with the diluent. The plate was shaken gently and covered with a plate sealer, and the samples were incubated for 2 h at room temperature. After incubation, the standards and samples were aspirated from the wells and washed with buffer. A total of 100 μl of a solution that contained a detection antibody (avidin–horseradish peroxidase conjugate) preserved in 0.15% Pro-Clin-150 then was added. This was incubated for 1 h at room temperature. The wells then were washed with buffer, and 100 μl of 3,3′,5,5′-tetramethylbenzidine subsequently was added. The plate then was incubated in the dark at room temperature for 30 min. Afterward, 50 μl of a solution that contained 1 M phosphoric acid was added. Absorbance was read at 450 nm. Results initially were calculated by OD and converted to pg/ml by log-log computation with application according to the standard curve (9).

Statistical Analyses

Potential associations between Kt/V, dialysis patient vintage, or serum albumin and pre- and posttreatment serum cytokine concentrations were assessed by linear regression analysis. The effect of aspirin treatment on cytokine production was evaluated by one-way ANOVA or the *t* test, the latter comparing pretreatment cytokine concentration separately with concentration after 2 and 4 mo of aspirin treatment. Significance is defined as $P < 0.05$.

Results

Patient Demographics

Fourteen patients were entered into the study. Seven were receiving HD, and seven were receiving PD. The general patient characteristics are shown in Table 1. Mean dialysis vintage was 4.3 ± 4.6 yr (range 2 mo to 17.2 yr). Mean HD spKt/V was 1.46 ± 1.4, mean eKt/V was 1.27 ± 0.16, and CCPD weekly Kt/V was 2.45 ± 0.30. Kt/V values did not differ significantly for any patient or for all patients in each modality during the course of study.

Baseline Serum Cytokines and Correlation with Dialysis Data

Pre-aspirin treatment serum cytokine concentrations are listed in Table 2. Baseline proinflammatory cytokines IL-1β, IL-6, IL-8, and TNF-α serum concentrations were elevated compared with those of age-matched, published control subjects (13–17). The baseline serum level of the anti-inflammatory cytokine IL-4 was normal; the anti-inflammatory IL-10 was mildly elevated compared with that of published control subjects, although to a lesser extent than the proinflammatory cytokines. The patterns of cytokine elevation were similar for patients who were receiving HD *versus* PD, and no significant difference was observed in mean serum cytokine concentration for any of the cytokines for HD *versus* PD patients.

At baseline, both IL-4 concentration and IL-6 concentrations demonstrated strong positive correlation with dialysis vintage (IL-4 $r^2 = 0.38$, $P < 0.03$; IL-6 $r^2 = 0.90$, $P < 0.0001$), whereas no other serum cytokine level varied with dialysis vintage. Pre-aspirin serum cytokine concentrations did not vary with spKt/V or eKt/V or HD patients or with weekly Kt/V for CCPD patients.

Serum Cytokines in All Dialysis Patients: Effect of Aspirin Treatment

Proinflammatory Cytokines. The effect of aspirin treatment on serum cytokine concentrations is depicted in Table 2. Serum IL-8 and TNF-α concentrations were significantly reduced by aspirin treatment at 4 mo ($P = 0.04$ and $P = 0.007$,

Table 1. General patient data[a]

Parameter	Value (Mean ± SD)
Age (yr)	14.6 ± 4.3
Weight (kg)	41.7 ± 19.8
Dialysis vintage (yr)	4.3 ± 4.6
spKt/V	1.46 ± 0.14
eKt/V	1.27 ± 0.16
CCPD Kt/V	2.45 ± 0.30

[a]eKt/V, equilibrated Kt/V; spKt/V, single-pool Kt/V; CCPD, continuous cycling peritoneal dialysis.

Table 2. Effect of aspirin on serum cytokines in all dialysis patients[a]

Cytokine	Normal Value for Age (pg/ml)[b]	Pretreatment (pg/ml)	4 Mo of Aspirin Treatment (pg/ml)	*P*
Proinflammatory				
IL-1β	<10.0	20.9 ± 8.7	26.9 ± 12.3	0.7
IL-6	<0.2	10.1 ± 3.7	3.3 ± 0.3	0.1
IL-8	<5.8	69.4 ± 21.1	20.8 ± 2.3	0.04
TNF-α	<0.5	13.6 ± 2.3	4.6 ± 0.9	0.01
IL-2	<0.9	0.9 ± 0.2	0.8 ± 0.2	0.7
Anti-inflammatory				
IL-4	<1.2	1.7 ± 0.1	1.6 ± 0.2	0.8
IL-10	<1.5	6.2 ± 1.4	3.4 ± 1.2	0.07

[a]Compared with published age-matched values, all pretreatment proinflammatory cytokine serum concentrations were elevated; after 4 mo of aspirin therapy, the concentration of several proinflammatory cytokines declined, with significant decreases observed for IL-8 and TNF-α. Serum anti-inflammatory cytokine concentrations were similar to those of age-matched control subjects and were unaffected by aspirin therapy.
[b]References for normal values for age: IL-1β, TNF-α, and IL-6 (13); IL-8 (15); IL-2 (16); IL-4 (17); and IL-10-Katayama (14).

respectively). Serum IL-1β concentration remained unchanged and serum IL-6 concentration was decreased after 2 and 4 mo of aspirin treatment, although the differences were NS ($P = 0.1$). Serum IL-2 concentration was normal pre-aspirin treatment and remained stable throughout the treatment period.

Anti-inflammatory Cytokines. Serum IL-4 and IL-10 concentrations remained stable throughout the aspirin treatment period. None of the changes was significant.

Serum Cytokines in HD versus *PD Patients: Effect of Aspirin Treatment*

Proinflammatory Cytokines. The effect of aspirin treatment on serum cytokine concentrations in patients who were receiving HD and PD are depicted in Figures 1 and 2, respectively, and were no different for any cytokine for patients who were receiving HD *versus* PD. When analyzed by one-way ANOVA, only serum TNF-α concentration decreased significantly with aspirin treatment in HD ($P = 0.001$) and PD ($P = 0.04$) patients. However, when the 2- and 4-mo aspirin treatment cytokine concentrations were compared separately with the pretreatment levels (by *t* test), we observed other interesting results. Serum IL-6 and IL-8 concentrations decreased after 2 and 4 mo of aspirin treatment in HD (but not PD) patients, although the differences were NS. Serum TNF-α concentration decreased significantly after 4 mo of aspirin treatment in HD and PD patients compared with both pretreatment and 2-mo values. In a separate analysis, we measured serum cytokines 1 mo after discontinuation of aspirin treatment in HD patients and found that the proinflammatory cytokines IL-6, IL-8, and TNF-α remained suppressed.

Anti-inflammatory Cytokines. When analyzed either by one-way ANOVA or *t* test analysis (pretreatment *versus* 2- or 4-mo treatment separately), we observed no changes in serum cytokine concentrations in either HD or PD patients. In HD and PD patients, serum IL-4 concentration remained stable throughout the aspirin treatment period. None of the changes was significant. In HD patients, serum IL-10 concentrations were low at all time periods except for 1 mo after completion of aspirin treatment, at which time the absolute value still was relatively low. In PD patients, serum IL-10 concentrations were normal and remained stable throughout the treatment period.

Assessment of the Relationship between Nutritional State and Inflammation

Previous studies showed that there is a potential relationship between nutrition and inflammation in adult patients with ESRD; this topic was reviewed recently (18). This relationship has not been studied in pediatric patients with ESRD. We assessed the correlation between pre- and post-aspirin treatment serum cytokine and albumin (measured before a routine, midweek dialysis treatment) values in our patients with ESRD; these results are shown in Table 3. The only correlation that we observed was a strong negative relationship between post-aspirin treatment serum albumin and IL-4 concentration ($P = 0.01$).

Discussion

Cytokines are substances that play an important role in coordinating the inflammatory response of the body to various external and internal stimuli (19). There are two classes of cytokines: Proinflammatory and anti-inflammatory (20–24). The proinflammatory cytokines are essential to initiate defense against various pathogens. In certain conditions, there is an overproduction of the proinflammatory cytokines, and the result may be counterproductive (25,26). The anti-inflammatory cytokines downregulate the inflammatory process, in part by suppressing production of the proinflammatory cytokines, and therefore help to balance the inflammatory response (27,28). Similar to the proinflammatory cytokines, excess secretion of anti-inflammatory cytokines may have deleterious effects on organ function (29,30). The proinflammatory cytokines include IL-1β, IL-6, IL-8, TNF-α, and IL-2, and the anti-inflammatory cytokines include IL-1 receptor antagonist, IL-4, IL-10, and IL-13 (19–30).

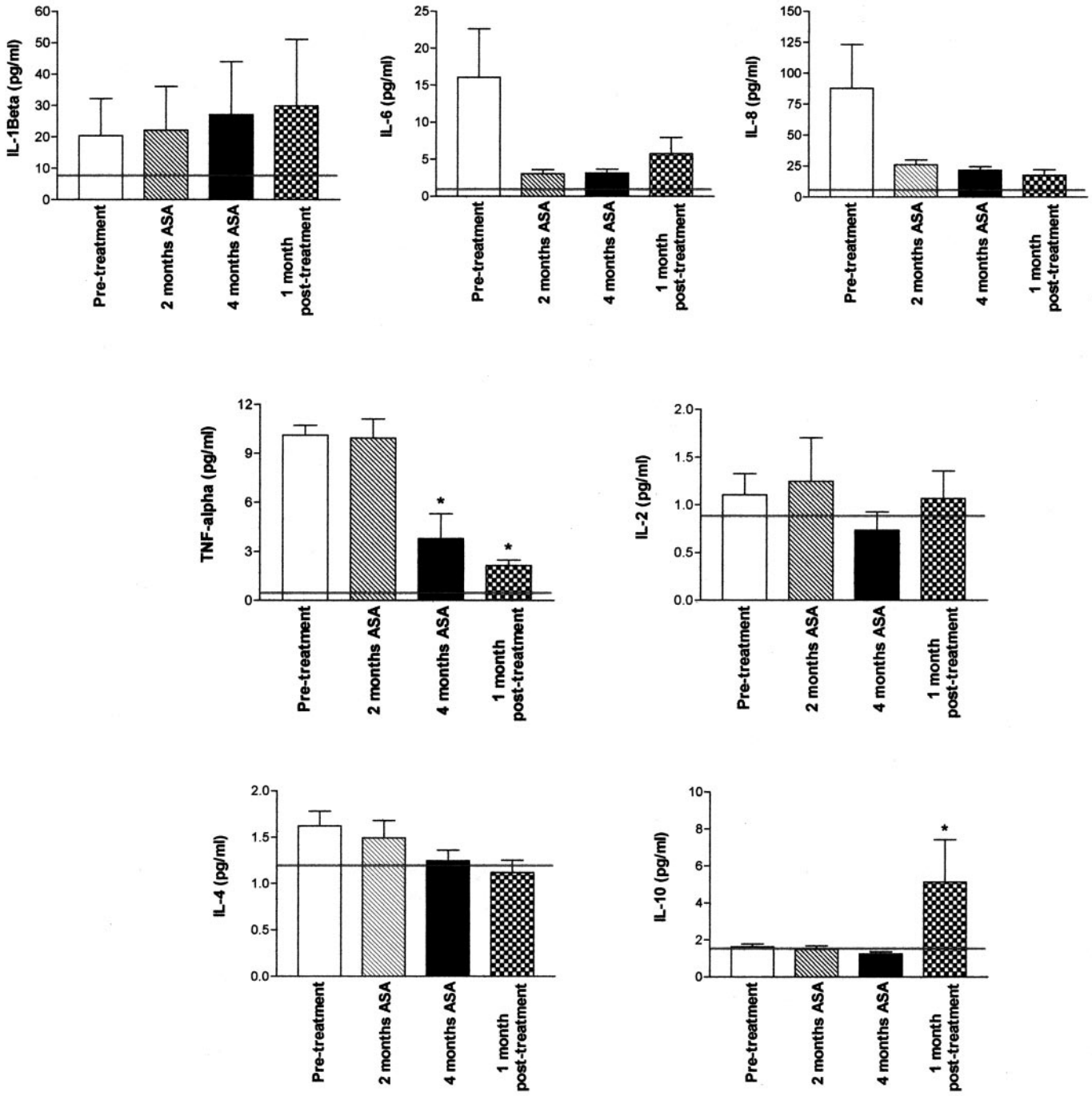

Figure 1. Effect of aspirin (ASA) on serum cytokine concentration in hemodialysis (HD) patients. Serum cytokine concentration were measured in HD patients using polymethylmethacrylate membranes before initiation of aspirin treatment (□) or after 2 (▨) or 4 mo (■) of aspirin treatment or one month after treatment was discontinued (checkered bars). Pretreatment IL-1β, IL-6, IL-8, and TNF-α were significantly higher than those in age-matched control subjects (shown with red horizontal line). Aspirin treatment reduced serum proinflammatory cytokines IL-6, IL-8, and TNF-α concentration, but only the change for TNF-α at 4 mo of aspirin treatment and 1 mo after treatment reached statistical significance (*$P < 0.05$) *versus* the control condition. The 1-mo posttreatment IL-10 level was significantly higher than all other IL-10 concentrations. Data are mean ± SE.

Proinflammatory markers such as C-reactive protein and IL-6 are reliable predictors of CVD in adult dialysis patients (31). There are several reasons to explain the increase in CV risk in ESRD, including alterations in calcium and phosphate metabolism and malnutrition (1–3). In fact, we recently reported two cases of malnourished pediatric dialysis patients who had ESRD and cardiac calcifications and whose underlying disease was ANCA vasculitis. We suggested that the combination of malnutrition, inflammation, and ESRD led to these calcifications, which had not been reported previously in pediatric

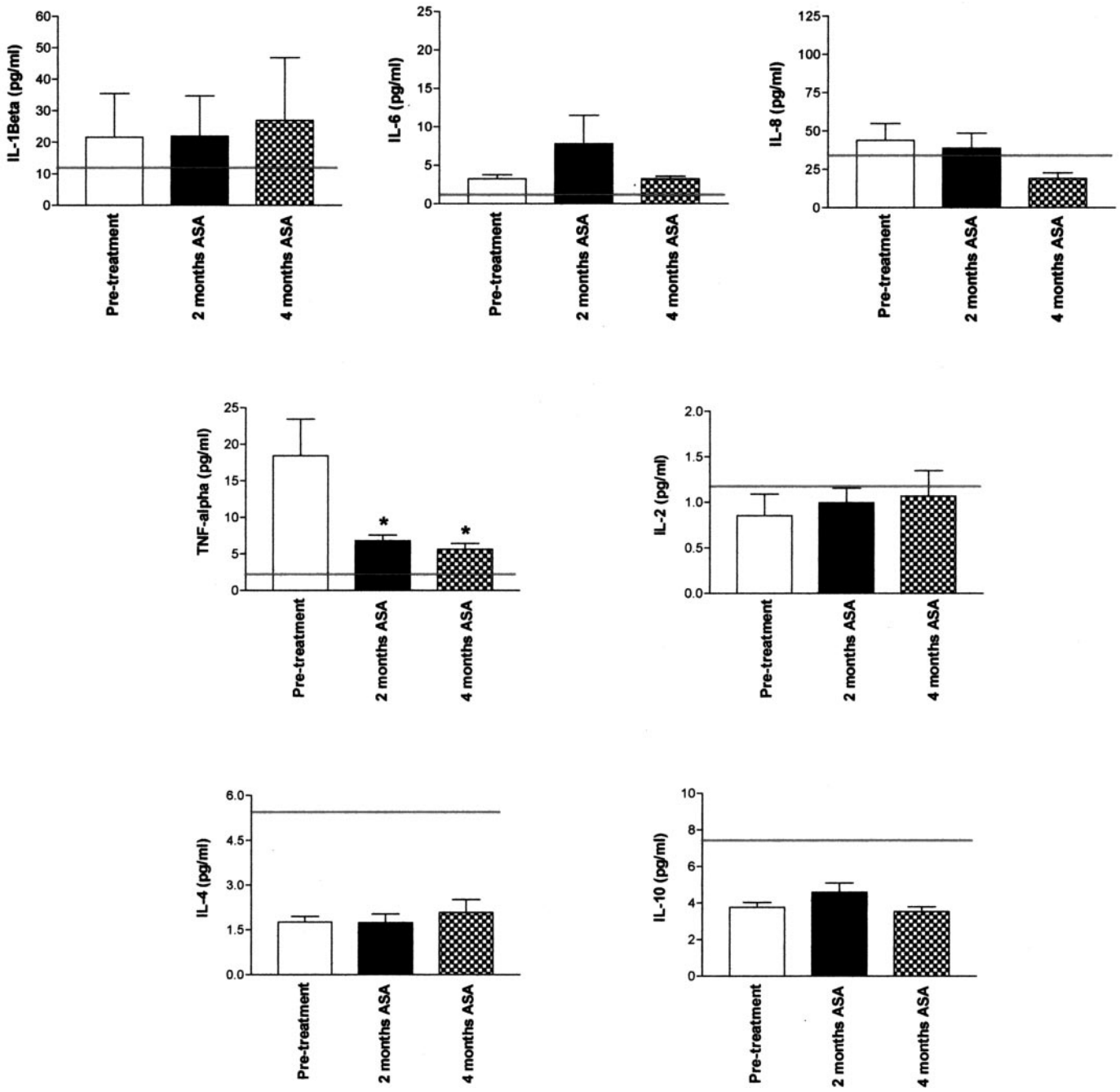

Figure 2. Effect of aspirin (ASA) on serum cytokine concentration in peritoneal dialysis (PD) patients. Serum cytokine concentration were measured in PD patients in the morning after a routine dialysis treatment before initiation of aspirin treatment (□) or after 2 (■) or 4 mo (speckled bars) of aspirin treatment. Pretreatment IL-1β, IL-6, IL-8, and TNF-α were significantly higher than those in age-matched control subjects (shown with red horizontal line). Aspirin treatment reduced serum IL-6, IL-8, and TNF-α concentration, but only the change for TNF-α at 2 and 4 mo of aspirin treatment reached statistical significance. Data are mean ± SE.

patients (32). Recent evidence suggests a cascade of events that result in the chronic proinflammatory condition in ESRD, as a result of persistent stimulation of the immune system. Indeed, our previous study in children showed that immediately and 24 h after dialysis, there is an increase in proinflammatory serum cytokines (9).

Our pilot study was designed to expand on our previous observations regarding the proinflammatory condition in pediatric patients with ESRD. We measured the serum concentration of seven cytokines. Five of these cytokines are considered to be proinflammatory (IL-1β, IL-6, IL-8, TNF-α, and IL-2), and two are anti-inflammatory (IL-4 and IL-10) (19–30).

At baseline, both IL-6 and IL-4 were strongly correlated with dialysis patient vintage, which validates data from our previous study. When we combined the data from HD and PD patients, we found that the serum concentration of all of the

Table 3. Correlation (*r*) between serum cytokine concentration and albumin levels before and after aspirin treatment in all dialysis patients[a]

Cytokine	Albumin	
	Before Aspirin	After Aspirin
IL-1β	−0.43	0.55
IL-6	−0.01	−0.18
IL-8	−0.27	0.05
TNF-α	0.43	−0.13
IL-2	−0.42	0.42
IL-4	−0.46	−0.76[b]
IL-10	−0.13	−0.56

[a]There was no correlation between either pre- or posttreatment cytokine and albumin levels except for a strong negative correlation between posttreatment IL-4 and albumin levels.
[b]$P < 0.05$.

proinflammatory cytokines studied except IL-2 were markedly higher than that in established, age-matched control subjects (12,14,15). In contrast, the serum concentration of the two major anti-inflammatory cytokines (IL-4 and IL-10) were similar to that in the age-matched control subjects (14,17). This indicates that in pediatric dialysis patients, there is an imbalance that favors a proinflammatory state. We also observed that aspirin treatment diminishes the concentration of various proinflammatory cytokines, most significantly IL-8 and TNF-α.

When we assessed the inflammatory state in HD patients alone, we found that the baseline concentration of four proinflammatory cytokines was markedly increased. In contrast, the serum concentration of the anti-inflammatory cytokines were either normal (IL-4) or slightly higher than that in age-matched, published control subjects (IL-10), although we could not do a statistical comparison with published values. Therefore, similar to the entire dialysis group, our studies suggest that in pediatric HD patients, there is an imbalance toward a proinflammatory state. Treatment with aspirin resulted in decreased concentration of three proinflammatory cytokines: IL-6, IL-8, and TNF-α. Because of the relatively small number of patients in our pilot study, only serum TNF-α significantly decreased in response to aspirin therapy. However, aspirin therapy induced a large decrease in the serum concentration of other proinflammatory cytokines. For example, among all dialysis patients, IL-6 declined by 67.3%. Similarly, aspirin induced a decrease in IL-6 by 77.4% and of IL-8 by 75.4% in HD patients. Finally, IL-8 concentration decreased by 56.6% in PD patients. We suggest that there is evidence for a proinflammatory state that may be attenuated by aspirin treatment in pediatric patients with ESRD.

When analyzed as a separate group, the inflammatory state in PD patients shows similar results to that observed in HD patients. The baseline concentration of four proinflammatory cytokines was markedly increased whereas the concentration of the anti-inflammatory cytokines was similar to that in age-matched control subjects. There was a clear imbalance toward a proinflammatory state. Treatment with aspirin resulted in decreased concentration of three proinflammatory cytokines: IL-6, IL-8, and TNF-α. Similar to the findings in HD patients, only the changes in TNF-α were significant, but the data clearly show a trend toward a decrease in several proinflammatory cytokines. It is difficult to assess why aspirin had a greater effect on certain proinflammatory cytokine concentrations (*e.g.*, IL-6, IL-8, and TNF-α) whereas treatment had no effect on the proinflammatory cytokine IL-1β. One potential explanation is that although there are stimuli that simultaneously may induce generation of many cytokines, each cytokine is derived from a distinct pathway that may be affected differentially by specific anti-inflammatory agents.

Finally, we considered the possibility that malnutrition may contribute to the proinflammatory state, on the basis of this relationship in adult patients with ESRD (17). However, we generally found no correlation between pro- or anti-inflammatory cytokine concentrations and serum albumin, except for a negative correlation between post-aspirin treatment IL-4 and albumin. We were surprised about the lack of correlation between markers of inflammation and nutrition. One plausible explanation is that albumin may not be the optimal indicator of nutrition in pediatric dialysis patients. Indeed, although albumin often has been used to assess nutrition in adult dialysis patients (18,33), studies in children reveal that serum albumin may be affected by hydration and therefore may not be a consistently reliable indicator of nutritional status (34). Moreover, data from our own unit show serum albumin to be a very poor nutrition status marker in children who have severe protein energy malnutrition and respond to aggressive therapy with intradialytic parenteral nutrition (35,36).

There are several limitations to our study. First, our sample size was small, reflecting the general difficulty in performing clinical studies in pediatric dialysis patients while excluding patients with confounding variables (*e.g.*, use of other anti-inflammatory medications) or aiming to study two groups with similar demographic characteristics. Second, instead of measuring cytokine concentrations in our own control subjects, we used age-matched, published control subjects for serum cytokine concentrations. Ideally, we would have preferred to include our own control subjects, but because of frequent minor illnesses in healthy control children, which can affect serum cytokine concentrations, obtaining accurate control subjects in this population can be difficult. However, our patients' mean proinflammatory cytokine levels generally were several-fold greater than those in the published control subjects; therefore, it would be unlikely that our observations were random. Furthermore, these levels are similar to what we saw in our previous study (9). Finally, the main focus of this study was not to assess cytokine values *per se* but to assess the effect of aspirin on their levels; because we could not have justified prescribing aspirin therapy to control children, we could not have compared the values after treatment with those of the control subjects. In other words, the control subjects were not essen-

tial for the treatment arm of the study. Our use of albumin as a marker of nutrition in pediatric patients ESRD, despite its widespread acceptance as a marker of nutrition in adult HD patients (18), has not been established fully in pediatric patients with ESRD. Our future studies are designed to assess the usefulness of various markers of nutrition in pediatric patients with ESRD.

Conclusion

Our results show that the serum concentrations of various proinflammatory cytokines are increased in pediatric dialysis patients who receive either HD or PD. This proinflammatory state is not balanced by enhanced generation of anti-inflammatory cytokines, favoring a balance toward a proinflammatory condition. Our study was the first in either pediatric or adult patients to show that low-dosage aspirin therapy decreases the serum concentration of various proinflammatory cytokines with no adverse effects noted. This provides the basis for larger studies to assess the efficacy and the outcome indicators of modifying the chronic, proinflammatory state observed in pediatric dialysis patients.

Acknowledgments

This study was funded by Toray, Inc. We especially thank David Marsh for support of the project.

References

1. Wang AYM, Woo J, Wang M, Sea MM, Ip R, Li PK, Lui SF, Sanderson JE: Association of inflammation and malnutrition with cardiac valve calcification in continuous ambulatory peritoneal dialysis patients. *J Am Soc Nephrol* 12: 1927–1936, 2001
2. Ganesh SK, Stack AG, Levin NW, Hulbert-Shearon T, Port FK: Association of elevated serum PO(4), Ca × PO(4) product, and parathyroid hormone with cardiac mortality risk in chronic hemodialysis patients. *J Am Soc Nephrol* 12: 2131–2138, 2001
3. Fung F, Sherrard DJ, Gillen DL, Wong C, Kestenbaum B, Seliger S, Ball A, Stehman-Breen C: Increased risk for cardiovascular mortality among malnourished end-stage renal disease patients. *Am J Kidney Dis* 40: 307–314, 2002
4. Horl WH: Hemodialysis membranes: Interleukins, biocompatibility, and middle molecules. *J Am Soc Nephrol* 13[Suppl 1]: S62–S71, 2002
5. Herbelin A, Nguyen AT, Zingraff J, Urena P, Descamps-Latscha B: Influence of uremia and hemodialysis on circulating interleukin-1 and tumor necrosis factor alpha. *Kidney Int* 37: 116–125, 1990
6. Girndt M, Kaul H, Sester U, Ulrich C, Sester M, Georg T, Kohler H: Anti-inflammatory interleukin-10 genotype protects dialysis patients from cardiovascular events. *Kidney Int* 62: 949–955, 2002
7. Nitta K, Akiba T, Kawashima A, Kimata N, Miwa N, Nishida E, Uchida K, Honda K, Yumura W, Nihei H: Characterization of TH1/TH2 profile in uremic patients. *Nephron* 91: 492–495, 2002
8. Herbelin A, Urena P, Nguyen AT, Zingraff J, Descamps-Latscha B: Elevated circulating concentration of interleukin-6 in patients with chronic renal failure. *Kidney Int* 39: 954–960, 1991
9. Goldstein SL, Currier H, Waters L, Hempe JM, Sheth RD, Silverstein DM: Acute and chronic inflammation in pediatric patients receiving hemodialysis. *J Pediatr* 143: 653–657, 2003
10. Ece A, Gurkan F, Kervancioglu, Kocamaz H, Gunes A, Atamer Y, Selek S: Oxidative stress, inflammation and early cardiovascular damage in children with chronic renal failure. *Pediatr Nephrol* 21: 545–552, 2006
11. Schonbeck U, Libby P: Inflammation, immunity, and HMG-CoA reductase inhibitors. Statins as antiinflammatory agents? *Circulation* 109: II-18–II-26, 2004
12. Goldstein SL, Brewer ED: Logarithmic extrapolation of a 15-minute postdialysis BUN to predict equilibrated BUN and calculate double pool KT/V in the pediatric hemodialysis population. *Am J Kidney Dis* 36: 98–104, 2000
13. Sylvester FA, Wyzga N, Hyams JS, Gronowicz GA: Effect of Crohn's disease on bone metabolism in vitro: A role for interleukin-6. *J Bone Miner Res* 17: 695–702, 2002
14. Katayama K, Matsubara T, Fujiwara M, Koga M, Furukawa S: CD14+ CD16+ monocyte subpopulation in Kawasaki disease. *Clin Exp Immunol* 121: 566–570, 2000
15. Yang YH, Lai HJ, Huang CM, Wang LC, Lin YT, Chiang BL: Sera from children with active Henoch-Schonlein purpura can enhance the production of interleukin 8 by human umbilical venous endothelial cells. *Ann Rheum Dis* 63: 1511–1513, 2004
16. Aygun D, Gungor S, Ustundag B, Gurgoze MK, Sen Y: Proinflammatory cytokines and leptin are increased in serum of prepubertal obese children. *Mediators Inflamm* 3: 180–183, 2005
17. Ohshima Y, Katamura K, Miura M, Mikawa H, Mayumi M: Serum levels of interleukin 4 and soluble CD23 in children with allergic disorders. *Eur J Pediatr* 154: 723–728, 1995
18. Kuhlmann MK, Levin NW: Interaction between nutrition and inflammation in hemodialysis patients. *Contrib Nephrol* 149: 200–207, 2005
19. Romagnani S: T-cell subsets (Th1 versus Th2). *Ann Allergy Asthma Immunol* 85: 9–21, 2000
20. Zimmer S, Pollard V, Marshall GD, Garafalo RP, Traber D, Prough D, Herndon DN: The 1996 Moyer Award. Effects of endotoxin on the Th1/Th2 response in humans. *J Burns Care Rehabil* 17: 491–496, 1996
21. van Dissel JT, van Langevelde P, Westendorp RG, Kwappenberg K, Frolich M: Anti-inflammatory cytokine profile and mortality in febrile patients. *Lancet* 351: 950–953, 1998
22. Marchant A, Deviere J, Byl B, De Groote D, Vincent JL, Goldman M: Interleukin-10 production during septicaemia. *Lancet* 343: 707–708, 1994
23. Kasai T, Inada K, Takakuwa T, Yamada Y, Inoue Y, Shimamura T, Taniguchi S, Sato S, Wakabayashi G, Endo S: Anti-inflammatory cytokine levels in patients with septic shock. *Res Commun Mol Pathol Pharmacol* 98: 34–42, 1997
24. Taniguchi T, Koido Y, Aiboshi J, Yamashita T, Suzaki S, Kurokawa A: Change in the ratio of interleukin-6 to interleukin-10 predicts a poor outcome in patients with systemic inflammatory response syndrome. *Crit Care Med* 27: 1262–1264, 1999
25. Pinsky MR, Vincent JL, Deviere J, Alegre M, Kahn RJ, Dupont E: Serum cytokine levels in human septic shock:

Relation to multiple-system organ failure and mortality. *Chest* 103: 565–575, 1993
26. Marty C, Misset B, Tamion F, Fitting C, Carlet J, Cavaillon JM: Circulating interleukin-8 concentrations in patients with multiple organ failure of septic and nonseptic origin. *Crit Care Med* 22: 673–679, 1994
27. Gerard C, Bruyns C, Marchant A, Abramowicz D, Vandenabeele P, Delvaux A, Fiers W, Goldman M, Velu T: Interleukin10 reduces the release of tumor necrosis factor and prevents lethality in experimental endotoxemia. *J Exp Med* 177: 547–550, 1993
28. Howard M, Muchamuel T, Andrade S, Menon S: Interleukin 10 protects mice from lethal endotoxemia. *J Exp Med* 177: 1205–1208, 1993
29. Bone RC: Immunologic dissonance: A continuing evolution in our understanding of the systemic inflammatory response syndrome and the multiple organ dysfunction syndrome. *Ann Intern Med* 125: 690–691, 1996
30. Fisher CJ, Agosti JM, Opal SM, Lowry SF, Balk RA, Sadoff JC, Abraham E, Schein RM, Benjamin E: Treatment of septic shock with the tumour necrosis factor: Fc fusion protein. *N Engl J Med* 334: 1697–1702, 1996
31. Stenvinkel P, Barany P, Heimburger O, Pecoits-Filho R, Lindholm B: Mortality, malnutrition, and atherosclerosis in ESRD: What is the role of interleukin-6? *Kidney Int Suppl* 80: 103–108, 2002
32. Sheth RD, Perez MD, Goldstein SL: Cardiovascular calcifications in pediatric patients receiving maintenance dialysis. *Pediatr Nephrol* 18: 810–813, 2003
33. Kaysen GA, Dubin JA, Muller HG, Mitch WE, Rosales LM, Levin NW: Relationships among inflammation nutrition and physiologic mechanisms establishing albumin levels in hemodialysis patients. *Kidney Int* 61: 2240–2249, 2002
34. Schaefer F, Klaus G, Mehls O; the Mid-European Pediatric Peritoneal Dialysis Study Group: Peritoneal transport properties and dialysis dose affect growth and nutritional status in children on chronic peritoneal dialysis. *J Am Soc Nephrol* 10: 1786–1792, 1999
35. Goldstein SL, Baronette S, Gambrell TV, Currier H, Brewer ED: nPCR assessment and IDPN treatment of malnutrition in pediatric hemodialysis patients. *Pediatr Nephrol* 17: 531–534, 2002
36. Orellana P, Juarez-Cangelosi M, Goldstein SL: Intradialytic parenteral nutrition treatment and biochemical marker assessment for malnutrition in adolescent maintenance hemodialysis patients. *J Ren Nutr* 15: 312–317, 2005