European Journal of Pharmacology 602 (2009) 163–167

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Immunopharmacology and Inflammation

Increased SOCS expression in peripheral blood mononuclear cells of end stage renal disease patients is related to inflammation and dialysis modality

M. Mehdi Rastmanesh [a], Branko Braam [a,b,c], Jaap A. Joles [a], Peter Boer [a], Hans A.R. Bluyssen [d,*]

[a] Department of Nephrology and Hypertension, University Medical Center Utrecht, The Netherlands
[b] Division of Nephrology and Immunology, Department of Medicine, Univ. of Alberta, Edmonton, Canada
[c] Department of Physiology, Univ. of Alberta, Edmonton, Canada
[d] Laboratory of Human Molecular Genetics, Institute of Molecular Biology and Biotechnology, Faculty of Biology, Adam Mickiewicz University, Umultowska 89, 61-614 Poznan, Poland

ARTICLE INFO

Article history:
Received 10 July 2008
Received in revised form 22 October 2008
Accepted 10 November 2008
Available online 17 November 2008

Keywords:
End stage renal disease
Inflammation
SOCS
Peripheral blood mononuclear cells

ABSTRACT

Inflammation is a characteristic of cardiovascular disease and is increased in end-stage renal disease. Suppressors of cytokine signaling (SOCS) inhibit and reflect activation of intracellular inflammatory pathways. We hypothesized that SOCS expression in peripheral blood mononuclear cells of end stage renal disease patients is increased. Whether SOCS expression in peripheral blood mononuclear cells is related to inflammation, dialysis, and dialysis modality was investigated. Monocytes and lymphocytes were isolated from peripheral blood mononuclear cells of patients not on dialysis ($n=8$), on peritoneal dialysis ($n=8$), on hemodialysis ($n=14$) and of healthy control ($n=15$) subjects. SOCS expression was assessed by real-time quantitative PCR and plasma cytokines by ELISA. In end stage renal disease patients monocyte SOCS1, and lymphocyte SOCS1 and cytokine-inducible SH2 containing protein-1 (CIS-1) gene expression were increased along with increased plasma levels of interleukin (IL)-6, tumor necrosis factor (TNF)α ,and C-reactive protein (CRP). Monocyte SOCS1 correlated with IL-6. Lymphocyte CIS-1 was increased in non-dialysis and peritoneal dialysis but not in hemodialysis patients. Lymphocyte CIS-1 in peritoneal dialysis patients correlated with plasma TNFα. Despite the relatively low number of patients studied we observed increased expression of SOCS1 in both monocytes and lymphocytes, and of CIS-1 solely in lymphocytes of the patients. SOCS expression was related to increased systemic inflammation, illustrated by a significant correlation between monocyte SOCS1 and plasma IL-6. SOCS expression in peripheral blood mononuclear cells was also affected by hemodialysis, indicated by increased lymphocyte CIS-1 in non-dialysis and peritoneal dialysis but not in hemodialysis patients. We suggest that increased SOCS expression in peripheral blood mononuclear cells of end stage renal disease patients reflects whether and to which extent systemic inflammation activates the intracellular inflammatory pathways.

1. Introduction

Inflammation is a common feature in end stage renal disease. In fact, C-reactive protein (CRP) and the pro-inflammatory cytokines IL-6, and TNFα are increased at all stages of renal failure (Malaponte et al., 2002; Wong et al., 2007). Inflammation is known to activate endothelial cells, lymphocytes and monocytes and forms an independent risk factor for cardiovascular disease and mortality in renal patients at all stages of renal failure (Carlos et al., 1991; Malaponte et al., 2002). Interestingly, drugs that diminish inflammation, can reduce atherosclerosis and cardiovascular events (Leon and Zuckerman, 2005), pointing to the importance of molecules that inhibit inflammatory signals.

SOCS proteins (SOCS1-7 and CIS-1) are inducible inhibitors of cytokine signaling. Cytokines specifically induce one or more SOCS proteins, which can inhibit the signal initiated by the cytokine itself and potentially by other cytokines (Krebs and Hilton, 2000). Thus, SOCS proteins are not only modulators of cytokine signaling, but also they reflect the activation of intracellular cytokine pathways. Moreover, SOCS have been identified as important regulators of the immune and inflammatory responses, evidenced by the fact that SOCS1 and SOCS3 are frequently increased in inflammatory diseases (Tang et al., 2005; Wong et al., 2006). As such, SOCS expression reflects the initiation of negative feedback responses to an increased inflammatory pressure on cells, resulting in protecting or damaging effects through inhibition of pro- and anti-inflammatory signals, respectively. Differential SOCS expression could therefore be used at the cellular level to test whether patients with end stage renal disease are experiencing increased inflammation.

Renal failure is associated with activation of peripheral blood mononuclear cells. SOCS are involved in differentiation, cytokine

* Corresponding author. Tel.: +48 61 8295832.
E-mail address: johannes.bluijssen@amu.edu.pl (H.A.R. Bluyssen).

0014-2999/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2008.11.014

production and survival of monocytes and lymphocytes. We recently observed increased SOCS in monocytes and lymphocytes of chronic kidney disease patients that correlated with plasma inflammatory cytokines, renal function and cardiovascular disease markers (Rastmanesh et al., 2008). However, no information is available regarding SOCS expression in monocytes and lymphocytes of end stage renal disease patients and its relation to inflammation, dialysis modality, and markers of cardiovascular disease. We hypothesized that SOCS expression in monocytes and lymphocytes of end stage renal disease patients is increased, related to inflammation, dependent on whether dialysis is initiated, and on which dialysis modality is applied. We also tested the correlation between SOCS expression and cardiovascular disease markers or risk factors.

2. Materials and methods

2.1. Subjects and blood samples

Thirty end stage renal disease patients (non-dialysis, $n=8$; hemodialysis, $n=14$; and peritoneal dialysis, $n=8$) and 15 controls were included. Hemodialysis treatment comprised three 4-hour sessions weekly using synthetic polysulfone membrane and bicarbonate dialysate. Peritoneal dialysis patients were treated with regular continuous ambulatory peritoneal dialysis ($n=3$) or continuous cycler peritoneal dialysis ($n=5$). None of the patients had signs of ultrafiltration failure. Exclusion criteria were infection, diabetes mellitus, hepatitis, cancer, recent operation and use of immunosuppressive medication. Blood was sampled (in hemodialysis prior to dialysis and prior to erythropoeitin administration) in EDTA-anticoagulated tubes. Plasma was directly isolated and stored at −80 °C for analysis of albumin, lipids, CRP (CRP values of <5 considered 4.99 and of <7 considered 6.99), glucose, creatinine, and urea using routine laboratory methods. Thiobarbituric acid-reactive substances were measured as described previously (Attia et al., 2002). Hemoglobin was measured with a Multiparameter Haematology Analyzer, Cell Dyn 1700. Glomerular filtration rate (in non-dialysis patients) was calculated using MDRD formula. The study was approved by the Medical Ethics Committee of the Utrecht Medical Center. Informed consent was obtained from all participants.

2.2. Plasma cytokines

Plasma IL-6, IL-10, and TNFα levels were measured using colorimetric ELISA kits and IL-2 levels using a chemiluminescent ELISA (R&D Systems). Detection limits were 0.16–5 pg/ml for IL-6, 0.8–25 pg/ml for IL-10, 15.6–500 pg/ml for IFNγ, 0.5–16 pg/ml for TNFα, and 1.7–1250 pg/ml for IL-2.

2.3. Isolation of peripheral blood mononuclear cells, monocytes and lymphocytes

Peripheral blood mononuclear cells were isolated using Ficoll® (Amersham) according to the manufacturers instructions. Monocytes were isolated using indirect magnetic labeling system (MACS®, Miltenyi Biotec) for the isolation of untouched monocytes from human peripheral blood mononuclear cells. Non-monocytes i.e. T cells, natural killer cells (NK), B cells, dendritic cells and basophils were indirectly magnetically labeled using a cocktail of biotin-conjugated antibodies against CD3, CD7, CD16, CD19, CD56, CD123, glycophorin A, and Anti-Biotin microbeads (Flo et al., 1991). Since lymphocytes form the majority of non-monocytic mononuclear cells, we refer to this fraction as lymphocytes (Schumacher, 1984). Cell separation procedure was performed on ice. For ex-vivo experiments, whole blood was incubated with 20 U/ml IL-6 (Sigma) for 60 min at room temperature before cell isolation was performed.

2.4. Quantitative PCR

RNA was isolated using TRIzol® (Gibco). Quality of isolated total RNA was tested using a Bio-Analyzer™ system (Agilent). RNA from two monocyte and two lymphocyte samples of controls and one lymphocyte sample from the non-dialysis group were excluded because of RNA degradation. One microgram RNA was reverse transcribed (iScript® cDNA synthesis Kit, Bio-Rad). Gene expression was assessed by real-time PCR using pre-designed SOCS1-3, CIS1, GAPDH and 18S primers (Applied Biosystems TaqMan® Assays-on-Demand™). Expression of each target gene in each subject was normalized to endogenous controls GAPDH+18S (ΔCT) and related to a reference (ΔΔCT), being RNA from untreated peripheral blood mononuclear cells of a healthy volunteer. Gene expression is expressed as $(2^{-\Delta\Delta CT} * 100) \pm$S.D.

2.5. Statistics

Results are expressed as mean±S.D. Data were compared using Student's *t*-test, or a one Way ANOVA and the Studentized Newman Keuls post-hoc test, where appropriate. If data were not distributed normally we used the Mann–Whitney rank sum test or Kruskal–Wallis ANOVA on ranks and Dunn's post-hoc test. Correlation coefficients were calculated by Pearson's product moment correlation test (a linear regression test). $P<0.05$ was considered significant.

3. Results

3.1. General and biochemical data

Systolic and diastolic blood pressure were increased in end stage renal disease patients (Table 1) as compared to healthy controls ($P<0.001$ and $P<0.01$ respectively). Total and LDL cholesterol, albumin and hemoglobin (Hb) were decreased ($P<0.001$). CRP, used as marker of inflammation, was significantly increased in end stage renal disease ($P<0.01$). This was not the case for Thiobarbituric acid-reactive substances a marker of oxidative stress ($P=0.35$). There were no significant differences among the end stage renal disease subgroups except for the systolic blood pressure , which was significantly higher in non-dialysis (148±28 mmHg) and hemodialysis (147±20 mmHg) groups versus both peritoneal dialysis (113±14 mmHg) and controls (121±12 mmHg) ($P<0.01$). Etiologies of renal failure were: unknown ($n=9$), hypertension ($n=5$), Lupus erythematosis ($n=3$), hemolytic uremic syndrome ($n=1$), nephritic syndrome ($n=1$), glomerulonephritis ($n=2$), glomerulosclerosis ($n=2$), Alport's syndrome ($n=1$), Goodpasture's syndrome ($n=1$), autosomal dominant polycystic kidney disease ($n=4$), and microscopic polyangitis ($n=1$). All

Table 1
General and biochemical data of controls and end stage renal disease (ESRD) subjects

Variables	Controls	ESRD	*P*
N	15	30	–
Age (year)	52±14	54±19	0.64
F : M	8 : 7	15 : 15	–
BMI (kg/m^2)	25±4	25±5	0.76
Glomerular filtration rate (1.73 / min / m^2)	80±11	10±4 ($n=8$)	<0.001
Systolic blood pressure (mmHg)	121±12	136±21	<0.05
Diastolic blood pressure (mmHg)	72±6	82±11	<0.01
Cholesterol (mmol/L)	5.6±0.9	3.9±1.0	<0.001
HDL (mmol/L)	1.4±0.5	1.1±0.3	0.08
LDL (mmol/L)	3.6±0.7	2.0±0.8	<0.001
Albumin (g/L)	45.1±2.4	40.5±3.7	<0.001
Glucose (mmol/L)	5.2±0.5	5.3±0.8	0.90
TG (mmol/L)	1.3±0.5	1.8±0.8	0.23
Hb (mmol/L)	9.4±0.8	8.0±1.0	<0.001
Thiobarbituric acid-reactive substances (μM)	3.6±1.0	4.0±1.9	0.35

Table 2
Medication used by end stage renal disease (ESRD) subjects

Drug type	Controls	ESRD
Erythropoietin	None	23 of 30
Statins	None	17 of 30
ARB/ ACEi	None	23 of 30
Other anti-hypertensives	None	22 of 30
Anti-coagulants	None	11 of 30

hemodialysis patients received recombinant erythropoietin (once a week, during hemodialysis), 6 of the 8 peritoneal dialysis patients (once a week), and 3 of the 8 non-dialysis patients (once a month) received recombinant erythropoietin subcutaneously. Medication is summarized in Table 2. n represents the number of patients.

3.2. Increased monocyte SOCS1 and lymphocyte SOCS1 and CIS-1 in end stage renal disease patients

Expression of SOCS1, but not SOCS3 and CIS-1, was increased in monocytes of end stage renal disease patients versus controls ($P<0.05$; Fig. 1A). In contrast, expression of SOCS1 and CIS-1, but not SOCS3, was increased in lymphocytes of end stage renal disease patients ($P<0.05$, and $P<0.01$ respectively; Fig. 1B). SOCS2 expression was highly variable in both cell types (not shown). SOCS expression was also analyzed separately according to dialysis modality and to whether dialysis was initiated or not. We found that monocyte SOCS1 was predominantly increased in hemodialysis patients ($P=0.06$; Fig. 2A), while lymphocyte SOCS1 and CIS-1 in peritoneal dialysis and non-dialysis patients ($P=0.06$, and $P<0.01$ respectively; Fig. 2B).

3.3. Increased plasma levels of IL-6, TNFα and CRP

Plasma levels of IL-6 and TNFα, as well as CRP were significantly increased in end stage renal disease patients ($P<0.001$, $P<0.001$, and $P<0.05$ respectively; Fig. 3A). In contrast, plasma levels of IL-10 were not significantly changed (data not shown). IFNγ and IL-2 levels were

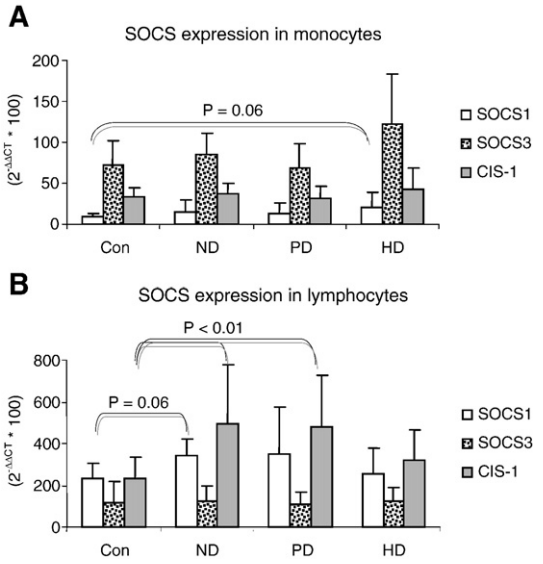

Fig. 2. Increased CIS-1 expression in lymphocytes of non-dialysis and peritoneal dialysis subjects. SOCS expression data of monocytes (A) and lymphocytes (B) of 8 non-dialysis, 8 peritoneal dialysis and 14 hemodialysis and 15 controls were compared to test the effect of dialysis and dialysis modality on SOCS expression in peripheral blood mononuclear cells (RNA from lymphocytes of two control and one non-dialysis subject, and monocytes of two control subjects were excluded because of RNA degradation). $P<0.05$ was considered significant.

below the lower detection limit of our method. Further analysis indicated that IL-6 was significantly increased in non-dialysis, peritoneal dialysis, and hemodialysis patients ($P<0.001$), TNFα in peritoneal dialysis and hemodialysis ($P<0.001$), and CRP in hemodialysis ($P<0.001$) (Fig. 3B). Correlation was found between monocyte SOCS1 and IL-6 in all end stage renal disease patients ($r=0.43$, $P<0.05$; Fig. 4A), and between lymphocyte CIS-1 and TNFα in peritoneal dialysis patients

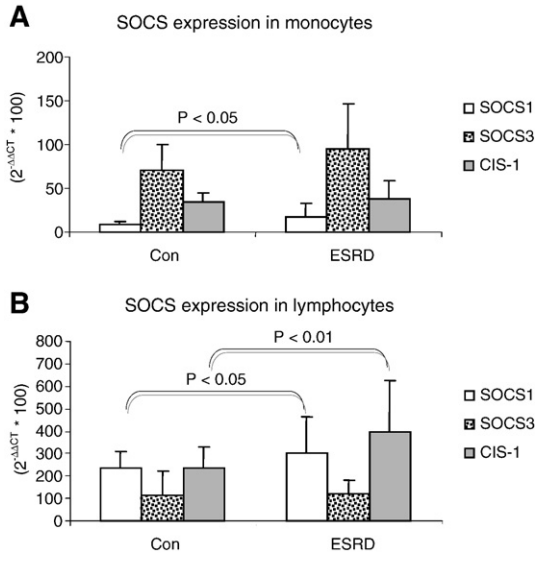

Fig. 1. Increased SOCS expression in monocytes and lymphocytes of end stage renal disease patients. SOCS1, SOC3 and CIS-1 gene expression in monocytes (A) and in lymphocytes (B) of healthy controls and end stage renal disease patients were measured using quantitative real time PCR as described under material and methods. The values are mean $(2^{-\Delta\Delta CT}*100)\pm$S.D. of gene expression in samples from 15 controls and 30 end stage renal disease subjects. RNA from lymphocytes of two controls and one end stage renal disease subjects and from monocytes of two controls were excluded because of RNA degradation. $P<0.05$ was considered significant.

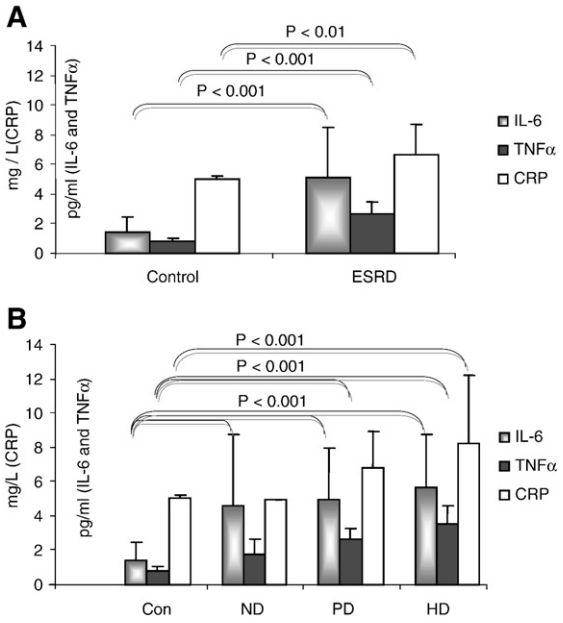

Fig. 3. Increased plasma levels of CRP and inflammatory cytokines in end stage renal disease patients. Plasma levels of IL-6, TNFα and CRP in healthy controls ($n=15$) and end stage renal disease ($n=30$, IL-6 value of one hemodialysis patient was lost) (A) and in non-dialysis ($n=8$), peritoneal dialysis ($n=8$), hemodialysis ($n=14$) and control ($n=15$) subjects (B) were measured as described in the material and methods section. The values are the mean±S.D. n represents the number of subjects in each group.

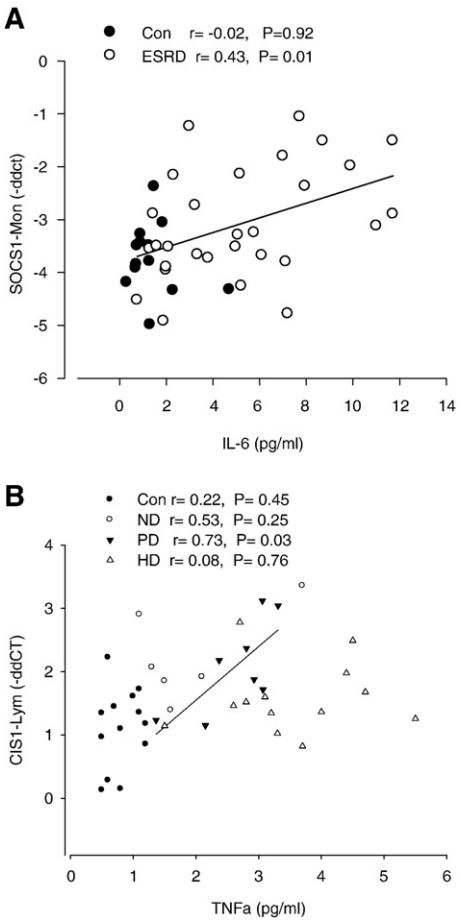

Fig. 4. Correlations between SOCS expression in peripheral blood mononuclear cells and inflammation. Correlations between monocyte SOCS1 and IL-6 in end stage renal disease (ESRD) (n=29) (A), and lymphocyte CIS-1 and plasma TNFα in non-dialysis (n=7), peritoneal dialysis (n=8), hemodialysis (n=14), and controls (n=13). RNA samples of one non-dialysis and two control subjects were excluded because of RNA degradation (B). Correlation coefficients were calculated by Pearson's product moment correlation test (a linear regression test). P<0.05 was considered significant.

only (r=0.73, P<0.05; Fig. 4B). No other significant correlations were found between SOCS expression and the level of inflammatory markers.

4. Discussion

Our study revealed increased monocyte SOCS1 and lymphocyte SOCS1 and CIS-1 in end stage renal disease patients, which was accompanied by increased plasma levels of IL-6, TNFα, and CRP, known biomarkers of inflammation and cardiovascular disease. Interestingly, monocyte SOCS1 correlated with plasma IL-6 levels, linking monocyte SOCS-1 to enhanced activity of this known marker of inflammation and cardiovascular disease. CIS-1 was significantly increased in lymphocytes of non-dialysis and peritoneal dialysis patients but not in hemodialysis, potentially pointing to different inflammatory conditions or cell responsiveness in hemodialysis patients (Dhondt et al., 2000), which could be related to the hemodialysis procedure (Martin-Malo et al., 2000). For instance, hemodialysis membranes can potentially increase inflammation by activating the mononuclear cells (Menegatti et al., 2002), though synthetic polysulfone membrane used in this study has higher biocompatibility and is more efficient in removing the oxidized low density lipoproteins (Walker et al., 2004; Wanner et al., 2004). Indeed CRP was only significantly increased in hemodialysis patients, but not in the other subgroups. We found a significant correlation between lymphocyte CIS-1 and TNFα, confirming a link between systemic inflammation and SOCS expression in mononuclear cells.

Increased SOCS expression is recognized in different inflammatory diseases (Wong et al., 2006). Likewise we report increased SOCS1 and CIS-1 expression in peripheral blood mononuclear cells of end stage renal disease patients, which could possibly reflect initiation or development of cardiovascular disease. SOCS1 and CIS-1 are important mediators of lymphocyte development and differentiation, and activation. SOCS1 is highly expressed in T helper-1 (Th-1) (Egwuagu et al., 2002), while increased CIS-1 correlates with Th2 cell differentiation (Matsumoto et al., 1999). Th-1 cells produce pro-inflammatory cytokines like IFNγ and TNFα; Th-2 cells produce anti-inflammatory cytokines like IL-10 (Tedgui and Mallat, 2006) that antagonize Th-1 effects (Tedgui and Mallat, 2006). Increased lymphocyte CIS-1 in non-dialysis and peritoneal dialysis might point to a predominance of Th2 cells (Egwuagu et al., 2002; Klinger et al., 2002; Matsumoto et al., 1999). It has been suggested that in hemodialysis there is a shift to Th1 cells (Litjens et al., 2006). However, we did not find increased lymphocyte SOCS1 in hemodialysis. That could be related to lymphocyte dysfunction in hemodialysis patients (Meier et al., 2002). Furthermore, insufficient SOCS1 expression in lymphocytes causes hyper-responsiveness of lymphocytes to different inflammatory cytokines (Fujimoto et al., 2004). The expression of CIS-1 is selectively induced in T-cells after T-cell receptor stimulation (Li et al., 2000), and prolongs survival of activated $CD4^+$ T-cells. Interestingly, the number of circulating $CD4^+$ is significantly higher in non-dialysis and peritoneal dialysis *versus* hemodialysis patients (Moser et al., 2003). The increased CIS-1 expression in lymphocytes of peritoneal dialysis and non-dialysis patients but not in hemodialysis, as shown here, could possibly reflect the lower number of $CD4^+$ cells in hemodialysis. Moreover, increase in circulating single positive T cells ($CD4^+$ and $CD8^+$) accompanied by a poor response to erythropoietin (Cooper et al., 2003), could be explained by CIS-1 being an intrinsic inhibitor of erythropoietin signaling (Krebs and Hilton, 2000).

In vivo and *in vitro* studies indicate a specific role for SOCS1 in inhibition of IFNγ signaling, which has a central role in atherosclerosis (Leon and Zuckerman, 2005). However, studies in SOCS1 transgenic mice indicate that SOCS1 modulates a wider range of cytokine responses (Fujimoto et al., 2000). Monocytes are involved in initiation and progression of atherosclerosis. Therefore, increased SOCS1 in monocytes, the precursors of macrophages, could delay monocyte responses to pro-inflammatory cytokines and delay atherosclerosis. In fact, SOCS1 inhibits IL-6-induced macrophage differentiation (Novak et al., 1999) and IFNγ-induced CD40 production that is involved in initiation of atherosclerosis (Wesemann et al., 2002). However, whether increased SOCS1 in monocytes of end stage renal disease patients is in proportion to increased pro-inflammatory cytokines and whether it could efficiently dampen pro-inflammatory signals remains unclear. Previous studies, however, indicate an increased number of activated monocytes with a pro-inflammatory phenotype in end stage renal disease (Brauner et al., 1998; Heine et al., 2008), which might suggest that increased monocyte SOCS1 is insufficient to dampen increased inflammatory pressure on monocytes. A similar phenomenon has been shown for example in synoviocytes of rheumatoid arthritis patients, which are hyper-responsive to IL-6, because of inadequate levels of SOCS3 (Shouda et al., 2001).

Previously we reported increased monocyte SOCS3 in chronic kidney disease patients (15<GFR>60 ml/min/1.73 m^2) (Rastmanesh et al., 2008). SOCS3 expression in monocytes seems to have pro-atherogenic properties. This notion is based on a recent study that indicated decreased atherosclerosis in $ApoE^{-/-}$ mice, when SOCS3 was deleted from macrophages (Yamamoto et al., 2007). Therefore, increased monocyte SOCS3 in chronic kidney disease patients could be related to higher prevalence of cardiovascular disease. In fact, the chance of cardiovascular death in chronic kidney disease patients is 5–10 times more than the chance of reaching the end stage stadium (Collins et al., 2003). In the present study, we did not find significant changes in SOCS3

expression in end stage renal disease patients (all three subgroups). However, this is a cross-sectioned study and we did not follow the patients over the time. A subpopulation of patients with chronic kidney disease does not reach ESRD, since they die of cardiovascular disease. It can be speculated that this group has different monocyte SOCS3 expression in time than the patients who survive and reach ESRD. Moreover, it could well be that advanced renal failure affects SOCS3 expression differently than milder chronic renal failure.

Theoretically medication could affect SOCS expression in peripheral blood mononuclear cells. For instance both erythropoietin and statins are able to induce SOCS in peripheral blood mononuclear cells (Huang et al., 2003; Krebs and Hilton, 2000). However, resistance to erythropoietin therapy is frequent in end stage renal disease, and this is related to inflammation possibly involving SOCS (Goicoechea et al., 1998; van der Putten et al., 2008). Accordingly, while all hemodialysis patients received erythropoietin, we did not find significant changes in SOCS expression in mononuclear cells of these patients. Furthermore, as we reported previously (Rastmanesh et al., 2008), our preliminary data in 3 chronic kidney disease patients indicated that medical intervention for four weeks did not affect SOCS expression in peripheral blood mononuclear cells significantly. Therefore, it is hard to define the involvement of medications in the changed SOCS expression in mononuclear cells of the patients in this study.

In conclusion, despite the relatively small number of subjects in this study we observed increased SOCS expression in peripheral blood mononuclear cells of end stage renal disease patients, indicating activation of intracellular inflammatory pathways. The SOCS profile in monocytes and lymphocytes was different, which appeared to be related to inflammation and dialysis modality. Unfortunately, we were unable to measure SOCS proteins in peripheral blood mononuclear cells because of technical difficulties. Hence, this should be addressed in follow up studies to confirm our data at the protein level.

Acknowledgments

We gratefully thank W.H. Boer, P.J. Blankestijn, P.F. Vos, N. Willekes and C. Tilburgs for their collaboration. This work was supported by the Dutch Kidney Foundation (NSN C03.2062) and the Genzyme Renal Innovations Program (GRIP).

References

Attia, D.M., Ni, Z.N., Boer, P., Attia, M.A., Goldschmeding, R., Koomans, H.A., Vaziri, N.D., Joles, J.A., 2002. Proteinuria is preceded by decreased nitric oxide synthesis and prevented by a NO donor in cholesterol-fed rats. Kidney Int. 61, 1776–1787.

Brauner, A., Lu, Y., Hallden, G., Hylander, B., Lundahl, J., 1998. Difference in the blood monocyte phenotype between uremic patients and healthy controls: its relation to monocyte differentiation into macrophages in the peritoneal cavity. Inflammation 22, 55–66.

Carlos, T., Kovach, N., Schwartz, B., Rosa, M., Newman, B., Wayner, E., Benjamin, C., Osborn, L., Lobb, R., Harlan, J., 1991. Human monocytes bind to two cytokine-induced adhesive ligands on cultured human endothelial cells: endothelial-leukocyte adhesion molecule-1 and vascular cell adhesion molecule-1. Blood 77, 2266–2271.

Collins, A.J., Li, S., Gilbertson, D.T., Liu, J., Chen, S.C., Herzog, C.A., 2003. Chronic kidney disease and cardiovascular disease in the Medicare population. Kidney Int. S24–S31 Suppl.

Cooper, A.C., Breen, C.P., Vyas, B., Ochola, J., Kemeny, D.M., Macdougall, I.C., 2003. Poor response to recombinant erythropoietin is associated with loss of T-lymphocyte CD28 expression and altered interleukin-10 production. Nephrol. Dial. Transplant. 18, 133–140.

Dhondt, A., Vanholder, R., Van Biesen, W., Lameire, N., 2000. The removal of uremic toxins. Kidney Int. Suppl. 76, S47–59.

Egwuagu, C.E., Yu, C.R., Zhang, M., Mahdi, R.M., Kim, S.J., Gery, I., 2002. Suppressors of cytokine signaling proteins are differentially expressed in Th1 and Th2 cells: implications for Th cell lineage commitment and maintenance. J. Immunol. 168, 3181–3187.

Flo, R.W., Naess, A., Lund-Johansen, F., Maehle, B.O., Sjursen, H., Lehmann, V., Solberg, C.O., 1991. Negative selection of human monocytes using magnetic particles covered by anti-lymphocyte antibodies. J. Immunol. Methods 137, 89–94.

Fujimoto, M., Naka, T., Nakagawa, R., Kawazoe, Y., Morita, Y., Tateishi, A., Okumura, K., Narazaki, M., Kishimoto, T., 2000. Defective thymocyte development and perturbed homeostasis of T cells in STAT-induced STAT inhibitor-1/suppressors of cytokine signaling-1 transgenic mice. J. Immunol. 165, 1799–1806.

Fujimoto, M., Tsutsui, H., Xinshou, O., Tokumoto, M., Watanabe, D., Shima, Y., Yoshimoto, T., Hirakata, H., Kawase, I., Nakanishi, K., Kishimoto, T., Naka, T., 2004. Inadequate induction of suppressor of cytokine signaling-1 causes systemic autoimmune diseases. Int. Immunol. 16, 303–314.

Goicoechea, M., Martin, J., de Sequera, P., Quiroga, J.A., Ortiz, A., Carreno, V., Caramelo, C., 1998. Role of cytokines in the response to erythropoietin in hemodialysis patients. Kidney Int. 54, 1337–1343.

Heine, G.H., Ulrich, C., Seibert, E., Seiler, S., Marell, J., Reichart, B., Krause, M., Schlitt, A., Kohler, H., Girndt, M., 2008. CD14(++)CD16+ monocytes but not total monocyte numbers predict cardiovascular events in dialysis patients. Kidney Int. 73, 622–629.

Huang, K.C., Chen, C.W., Chen, J.C., Lin, W.W., 2003. Statins induce suppressor of cytokine signaling-3 in macrophages. FEBS Lett. 555, 385–389.

Klinger, J., Enriquez, J., Arturo, J.A., Delgado, M., Avila, G., Ceballos, O., 2002. Cytokines and peritonitis in continuous ambulatory peritoneal dialysis: immunodeviation and immunodeficiency. Adv. Perit. Dial. 18, 170–176.

Krebs, D.L., Hilton, D.J., 2000. SOCS: physiological suppressors of cytokine signaling. J. Cell Sci. 113 (Pt 16), 2813–2819.

Leon, M.L., Zuckerman, S.H., 2005. Gamma interferon: a central mediator in atherosclerosis. Inflamm. Res. 54, 395–411.

Li, S., Chen, S., Xu, X., Sundstedt, A., Paulsson, K.M., Anderson, P., Karlsson, S., Sjogren, H.O., Wang, P., 2000. Cytokine-induced Src homology 2 protein (CIS) promotes T cell receptor-mediated proliferation and prolongs survival of activated T cells. J. Exp. Med. 191, 985–994.

Litjens, N.H., van Druningen, C.J., Betjes, M.G., 2006. Progressive loss of renal function is associated with activation and depletion of naive T lymphocytes. Clin. Immunol. 118, 83–91.

Malaponte, G., Bevelacqua, V., Fatuzzo, P., Rapisarda, F., Emmanuele, G., Travali, S., Mazzarino, M.C., 2002. IL-1beta, TNF-alpha and IL-6 release from monocytes in haemodialysis patients in relation to dialytic age. Nephrol. Dial. Transplant. 17, 1964–1970.

Martin-Malo, A., Carracedo, J., Ramirez, R., Rodriguez-Benot, A., Soriano, S., Rodriguez, M., Aljama, P., 2000. Effect of uremia and dialysis modality on mononuclear cell apoptosis. J. Am. Soc. Nephrol. 11, 936–942.

Matsumoto, A., Seki, Y., Kubo, M., Ohtsuka, S., Suzuki, A., Hayashi, I., Tsuji, K., Nakahata, T., Okabe, M., Yamada, S., Yoshimura, A., 1999. Suppression of STAT5 functions in liver, mammary glands, and T cells in cytokine-inducible SH2-containing protein 1 transgenic mice. Mol. Cell. Biol. 19, 6396–6407.

Meier, P., Dayer, E., Blanc, E., Wauters, J.P., 2002. Early T cell activation correlates with expression of apoptosis markers in patients with end-stage renal disease. J. Am. Soc. Nephrol. 13, 204–212.

Menegatti, E., Rossi, D., Chiara, M., Alpa, M., Sena, L.M., Roccatello, D., 2002. Cytokine release pathway in mononuclear cells stimulated in vitro by dialysis membranes. Am. J. Nephrol. 22, 509–514.

Moser, B., Roth, G., Brunner, M., Lilaj, T., Deicher, R., Wolner, E., Kovarik, J., Boltz-Nitulescu, G., Vychytil, A., Ankersmit, H.J., 2003. Aberrant T cell activation and heightened apoptotic turnover in end-stage renal failure patients: a comparative evaluation between non-dialysis, haemodialysis, and peritoneal dialysis. Biochem. Biophys. Res. Commun. 308, 581–585.

Novak, U., Marks, D., Nicholson, S.E., Hilton, D., Paradiso, L., 1999. Differential ability of SOCS proteins to regulate IL-6 and CSF-1 induced macrophage differentiation. Growth Factors 16, 305–314.

Rastmanesh, M.M., Bluyssen, H.A., Joles, J.A., Boer, P., Willekes, N., Braam, B., 2008. Increased expression of SOCS3 in monocytes and SOCS1 in lymphocytes correlates with progressive loss of renal function and cardiovascular risk factors in chronic kidney disease. Eur. J. Pharmacol. 593, 99–104.

Schumacher, H.R., Garvin, D.F., Triplett, D.A., 1984. Introduction to Laboratory Hematology and Hematopathology, first ed. Alan R. Liss. Inc., New York, NY, pp. 9–107.

Shouda, T., Yoshida, T., Hanada, T., Wakioka, T., Oishi, M., Miyoshi, K., Komiya, S., Kosai, K., Hanakawa, Y., Hashimoto, K., Nagata, K., Yoshimura, A., 2001. Induction of the cytokine signal regulator SOCS3/CIS3 as a therapeutic strategy for treating inflammatory arthritis. J. Clin. Invest. 108, 1781–1788.

Tang, J., Kozaki, K., Farr, A.G., Martin, P.J., Lindahl, P., Betsholtz, C., Raines, E.W., 2005. The absence of platelet-derived growth factor-B in circulating cells promotes immune and inflammatory responses in atherosclerosis-prone ApoE−/− mice. Am J Pathol 167, 901–912.

Tedgui, A., Mallat, Z., 2006. Cytokines in atherosclerosis: pathogenic and regulatory pathways. Physiol. Rev. 86, 515–581.

van der Putten, K., Braam, B., Jie, K.E., Gaillard, C.A., 2008. Mechanisms of disease: erythropoietin resistance in patients with both heart and kidney failure. Nat. Clin. Pract. Nephrol. 4, 47–57.

Walker, R.J., Sutherland, W.H., De Jong, S.A., 2004. Effect of changing from a cellulose acetate to a polysulphone dialysis membrane on protein oxidation and inflammation markers. Clin. Nephrol. 61, 198–206.

Wanner, C., Bahner, U., Mattern, R., Lang, D., Passlick-Deetjen, J., 2004. Effect of dialysis flux and membrane material on dyslipidaemia and inflammation in haemodialysis patients. Nephrol. Dial. Transplant. 19, 2570–2575.

Wesemann, D.R., Dong, Y., O'Keefe, G.M., Nguyen, V.T., Benveniste, E.N., 2002. Suppressor of cytokine signaling 1 inhibits cytokine induction of CD40 expression in macrophages. J. Immunol. 169, 2354–2360.

Wong, C.K., Szeto, C.C., Chan, M.H., Leung, C.B., Li, P.K., Lam, C.W., 2007. Elevation of pro-inflammatory cytokines, C-reactive protein and cardiac troponin T in chronic renal failure patients on dialysis. Immunol. Invest. 36, 47–57.

Wong, P.K., Egan, P.J., Croker, B.A., O'Donnell, K., Sims, N.A., Drake, S., Kiu, H., McManus, E.J., Alexander, W.S., Roberts, A.W., Wicks, I.P., 2006. SOCS-3 negatively regulates innate and adaptive immune mechanisms in acute IL-1-dependent inflammatory arthritis. J. Clin. Invest. 116, 1571–1581.

Yamamoto, T.Y.H., Koga, M., et al., 2007. Lacking the SOCS3 gene in macrophages prevents the development of atherosclerosis in ApoE deficient mice. Circ. J. 71, 170.