Kidney International, Vol. 47 (1995), pp. 559–565

Production of interleukin-6, tumor necrosis factor α and interleukin-10 *in vitro* correlates with the clinical immune defect in chronic hemodialysis patients

MATTHIAS GIRNDT, HANS KÖHLER, ERIKA SCHIEDHELM-WEICK, JÖRG F. SCHLAAK, KARL-HERMANN MEYER ZUM BÜSCHENFELDE, and BERNHARD FLEISCHER

Medical Department IV, University of Homburg/Saar, Homburg, and Medical Department I, University of Mainz, Mainz, Germany

Production of interleukin-6, tumor necrosis factor α and interleukin-10 *in vitro* correlates with the clinical immune defect in chronic hemodialysis patients. In patients with chronic renal failure alterations in monokine production are a common feature. Their clinical relevance has not yet been proven. We show here a correlation between an overproduction of interleukin- (IL)-6 and tumor necrosis factor alpha (TNFα) upon stimulation with LPS by mononuclear cells in vitro and the clinical grade of immunodeficiency found in these patients. Higher levels of IL-6 and TNFα were correlated with an immunocompromized state, that is, nonresponsiveness to hepatitis B vaccination, whereas patients with a better immune competence showed the same levels of these cytokines as healthy controls. Only the patients with a good immune function showed a high secretion of IL-10. The feedback mechanism of IL-10 for reducing monokine synthesis seems to be intact in these patients. Thus the secretion of IL-10 might be regarded as a compensatory mechanism which controls monokine induction by chronic renal failure and hemodialysis treatment. Immunocompromized patients who are unresponsive to hepatitis B vaccination seem to be unable to enhance IL-10 synthesis for control of monokine overproduction. This results in higher levels of IL-6 and TNFα that might be involved in the pathogenesis of reduced immune defense.

Various clinical side effects of hemodialysis have been attributed to the systemic release of cytokines like interleukin (IL)-1β, IL-6 and tumor necrosis factor alpha (TNFα) by monocytes [1]. In chronic hemodialysis patients high serum levels of IL-6 [2] and TNF [3] were found before the begin of a hemodialysis treatment with a further increase during the procedure. These results were supported by the finding of a high spontaneous secretion of IL-6 [4] and TNFα [5] by peripheral blood mononuclear cells (PBMC) obtained from these patients during or after dialysis [6]. Upon stimulation by LPS *in vitro* the cells from dialysis patients produce more TNFα than cells from healthy controls [5].

High serum levels of these cytokines are also found in patients with chronic renal failure not yet requiring dialysis [5, 7]. This indicates that monocyte stimulation is a feature of uremia itself and not only of the dialysis procedure. On the other hand Schindler et al [8] showed that hemodialysis with cuprophane dialyzer membranes induces the transcription of mRNA for IL-1 in mononuclear cells. This effect could be attributed mainly to the activation of complement by cuprophane membranes. A similar effect was also shown for the secretion of IL-6 [2].

Chronic renal failure induces a clinical state of immunodeficiency in most patients. This is characterized by impaired efficacy of vaccinations, such as against hepatitis B [9]. The immunodeficiency is based on reduced T cell activating capabilities of the accessory cells, mainly the monocytes [10], and the costimulatory system of the B7/CD28 pathway seems to be involved [11]. Until now a role for the overproduction of soluble mediators by monocytes has not been proven. The *in vivo* immune response of dialysis patients against hepatitis B vaccination correlates well with cellular function *in vitro* [10]. By classifying the patients into nonresponders and responders to a hepatitis B vaccination we show that there is a strong relation between overproduction of monokines upon LPS stimulation and the clinical state of immune responsiveness.

These results focus further interest on regulatory mechanisms of monokine secretion. Interleukin-10 was first identified and cloned as a cytokine synthesis inhibitory factor (CSIF) [12]. One of its major effects is the inhibition of the production of IL-1, IL-6 and TNFα by activated monocytes/macrophages [13]. IL-10 can be induced by stimuli similar to those that induce high cytokine secretion in dialysis patients and an autoregulatory role for monokine induction has been shown [14]. We show here that an enhanced production of IL-10 in cells from responders to hepatitis B vaccination effectively controls monokine overproduction.

Methods

Patients

Twenty-eight patients on chronic hemodialysis using cuprophane dialyzers and bicarbonate dialysate for a mean time of 4.5 hours three times a week were studied. Dialyzers were not reused. None of the patients took medication with immunosuppressive effects including the regular intake of cyclooxygenase inhibitors. Patients were classified as responders or nonresponders according to the efficacy of a standard vaccination against hepatitis B. Antibody titers were determined after four injections of 40 μg hepatitis B vaccine (Engerix B, Smith Kline Dauelsberg, Göttingen, Germany) at 0, 1, 6 and 9 months. Responders had ≥10 IU/ml at two determinations between months 9 and 12, nonresponders had < 10 IU/ml.

Received for publication September 3, 1993
and in revised form September 2, 1994
Accepted for publication September 6, 1994

Table 1. PCR primer sequences and expected product lengths (base pairs)

Oligo	Sense primer	Antisense primer	PCR *bp*	Ref.
β-Actin	5′-TGACGGGGTCACCCACACTGTGCCCATCTA-3′	5′-CTAGAAGCATTTGCGGTGGACGATG-3′	661	
IL-1β	5′-GACACATGGGATAACGAGGC-3′	5′-ACGCAGGACAGGTACAGATT-3′	247	[17]
IL-6	5′-ATGAACTCCTTCTCCACAAGCGC-3′	5′-GAAGAGCCCTCAGGCTGGACTG-3′	628	[18]
TNFα	5′-AGAGGGAAGAGTTCCCCAGGGAC-3′	5′-TGAGTCGGTCACCCTTCTCCAG-3′	450	[14]
IL-10	5′-CTGAGAACCAAGACCCAGACATCAAGG-3′	5′-CAATAAGGTTTCTCAAGGGGCTGGGTC-3′	352	[19]

The control group consisted of 14 healthy individuals from our laboratory personnel.

Cell preparation and culture conditions

Twenty ml of heparinized venous blood were obtained from patients immediately before the start of hemodialysis. Mononuclear cells were preparated on a Ficoll density gradient (Biochrom, Berlin, Germany). Cells were cultured at 1×10^6 cells/ml in RPMI 1640 (Biochrom) containing 5% fetal calf serum, 2 mM glutamine and 50 μg/ml gentamicin. Cells were stimulated with LPS (S. typhimurium, Sigma, Deisenhofen, Germany) at a concentration of 100 ng/ml. Recombinant human IL-10 (Genzyme, Cambridge, MA, USA) was used at a final concentration of 10 ng/ml unless otherwise indicated. Supernatants were harvested after 24 hours of stimulation and frozen at −20°C. Experiments were always performed on a responder, a nonresponder and a control person on the same day. Anti-IL-10 JES3-9D7 (Pharmingen, San Diego, CA, USA) was used at a final concentration of 10 μg/ml.

Detection of IL-1β, IL-6 and TNFα

IL-1β, IL-6 and TNFα were measured using commercial ELISA test systems. The following ELISA kits were used: Quantikine IL-1β (R&D Systems, Minneapolis, MN, USA; detection limit 4.5 pg/ml), Quantikine IL-6 (R&D Systems; detection limit 3.5 pg/ml) and TNFα ELISA (Hofmann La-Roche, Basel, Switzerland). All supernatants were tested in duplicate and in appropriate dilutions, and all samples for a given assay were tested simultaneously.

Bioassay for IL-10

Human IL-10 was detected using a specific bioassay which has been recently described [15]. Proliferation of the murine mast cell line D36 in the presence of murine IL-4 is strictly dependent on the presence of murine or human IL-10. D36 cells were used at 3×10^3 per well with 2 U/ml of murine IL-4 (provided by C. Hüls, Mainz) and 50 μl/well of the supernatant to be tested in a final volume of 100 μl/well in round bottom microtiter plates (Greiner, Nürnberg, Germany). After 18 to 20 hours, 0.2 μCi of ^{3}H-thymidine were added to each well and incubated for another 16 hours. Finally the cells were harvested and counted in a liquid scintillation counter (BetaPlate 1205, Pharmacia, Sweden). Results were expressed as U/ml with one unit defined as the amount of IL-10 that induces half-maximal proliferation of D36 cells. One U/ml was equal to 1 ng/ml of recombinant human IL-10 (Genzyme). The detection limit was 0.3 U/ml.

Extraction and reverse transcription of mRNA

For the detection of mRNA PBMC were stimulated at 1×10^6/ml for 24 hours with LPS. Reverse transcription and PCR were performed as previously described [8, 16]. Briefly, the cell pellet was lysed with 4 M guanidinium thiocyanate, 0.5 mM sodium citrate (pH 7.0), 0.5% sarcosyl, 20 mM 2-mercaptoethanol and 0.1 M sodium acetate (pH 4.2). RNA was extracted using equal parts of H_2O-saturated phenol and chloroform/isoamylalcohol (24:1). RNA was precipitated from the aqueous phase by 2-propanol and the pellet was washed in 75% and 100% ethanol. mRNA was reverse transcribed into cDNA at 40°C for one hour using 10 μl RNA (1 μg/ml), 4 μl 5 × RT-Buffer (Tris HCl 250 mM, $MgCl_2$ 15 mM, KCl 375 mM), 2 μl dithiothreitol (0.1 M), 1 μl dNTP mix (1 mM of dATP, dCTP, dGTP, dTTP each; Boehringer, Mannheim, Germany), 1 μl RNAse inhibitor (40 U/ml, Boehringer), 1 μl oligo-T_{15}-primer (2 nM), and 1 μl (200 U) MMLV-Superscript reverse transcriptase (Gibco BRL).

Semiquantitative PCR

PCR was performed using the following conditions: 10 μl of cDNA were amplified in a final volume of 100 μl containing Tris HCl 20 mM, KCl 50 mM, $MgCl_2$ 2.5 mM, dNTPs 40 μM each nucleotide, and 400 nM each primer. The mixture was heated to 96°C for three minutes for denaturation and temperature was adjusted to 76°C on the thermal cycler (Omnigene, Hybaid Ldt., Teddington, UK) before 1.5 U of Taq polymerase (Gibco) were added. PCR was performed for three cycles at 96°C for 40 seconds, 58°C for 30 seconds, 72°C for 60 seconds followed by 30 to 36 cycles with an annealing temperature of 54°C. After 20 to 26 cycles an aliquot of 10 μl was taken from the mixture every two cycles. PCR product was visualized on 1.5% agarose gels stained with ethidium bromide. Semiquantitative description of mRNA levels was based on the number of cycles that lead to a visible product in the gel [16]. Additional experiments showed that after a predilution of 1:16 of a known concentration of cDNA four more cycles were required to obtain a visible band of PCR product. Cytoplasmic β-actin was used as a standard and cDNA was prediluted according to the bands appearing for this mRNA before the PCR on cytokines was performed. Primer sequences and expected product lengths are given in Table 1.

Statistics

Calculations were performed using the InStat scientific software (Graphpad, San Diego, CA, USA). Results are given as raw data and mean or as mean ± standard deviation. Significance of the differences in cytokine concentrations was calculated by ANOVA and Bonferroni's multiple comparisons test. Correlations were detected by linear regression analysis.

Results

Monokine secretion after stimulation by LPS

Cells were obtained from the patients 42 hours after dialysis. The monokines IL-1β, IL-6, TNFα and IL-10 in culture supernatants were determined after 24 hours of culture with or without

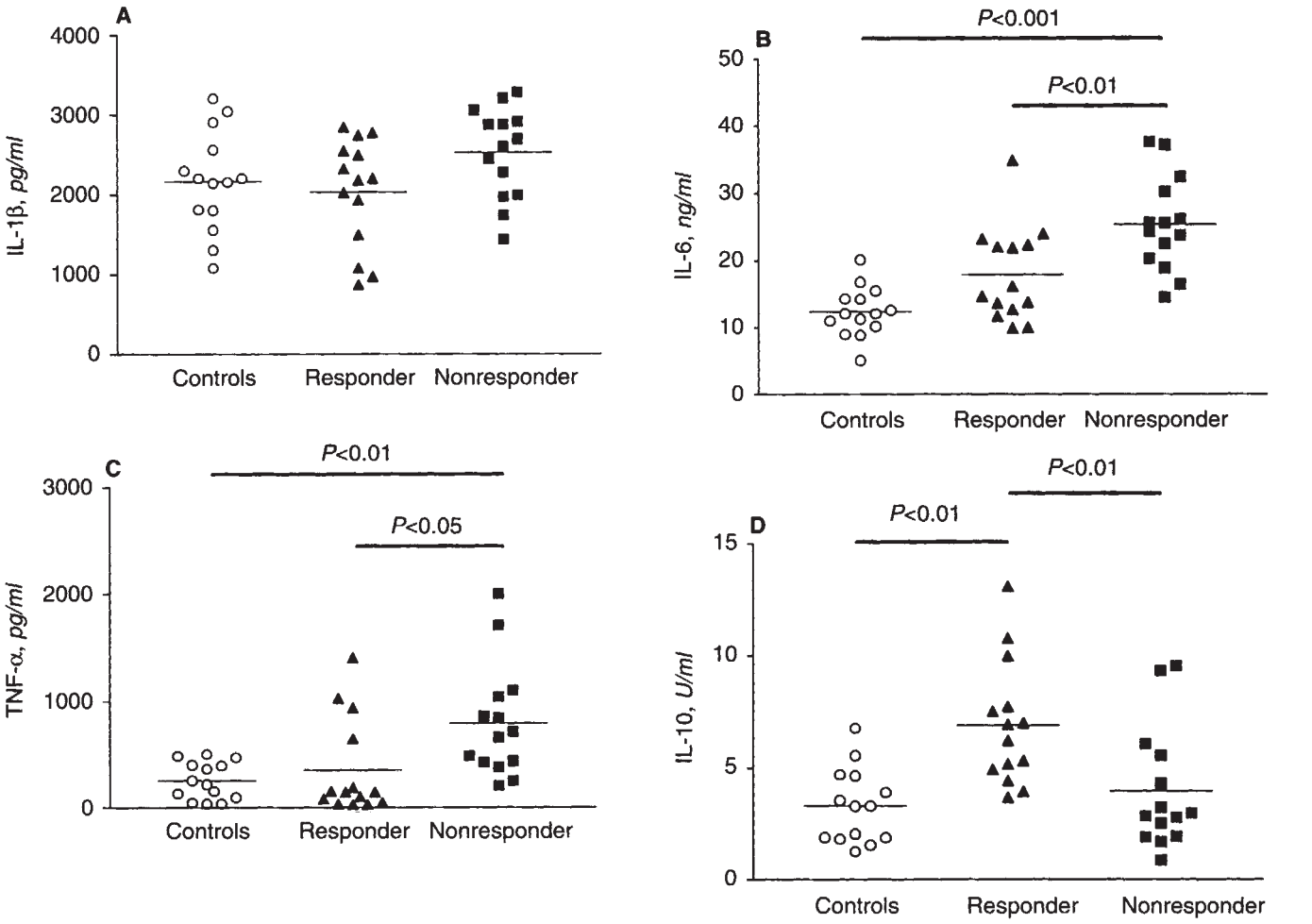

Fig. 1. *Concentrations of IL-1β* (**A**), *IL-6* (**B**), *TNFα* (**C**) *and IL-10* (**D**) *in the culture supernatant of PBMC from hemodialysis patients grouped as nonresponder* (■) *or responder* (▲), *and healthy controls* (○) *after 24 h of stimulation with LPS.* Mean values are given as lines. Significant differences between groups are given as bars on top of each diagram. Cells from nonresponders secrete more IL-6 and TNFα while cells from responders secrete more IL-10 than controls. Production of IL-1β was not different between the groups.

LPS. There were no significant differences in monokine production from unstimulated cells (data not shown). After stimulation by LPS cells from patients clinically classified as nonresponders produced significantly more IL-6 (Fig. 1B) and TNFα (Fig. 1C) than cells from responders or controls. In contrast responders produced more IL-10 (Fig. 1D) than nonresponders who were not different from controls. Secretion of IL-1β did not differ between the groups (Fig. 1A). Although the values for this cytokine were not beyond the normal range, those patients with the higher levels of IL-6 and TNFα also had the highest values for IL-1β (not shown). This relation could not be found in the controls, probably due to the lower cytokine concentrations in this group. The levels of IL-6 and TNFα were correlated in all three groups studied (not shown).

Corresponding to the high secretion of monokines a high level of transcription of mRNA for IL-6 was found in nonresponders whereas responders did not differ from controls. The level of mRNA expression for TNFα was also enhanced in nonresponders while being normal in responders. A typical example of the findings is shown in Figure 2. Expression of mRNA was determined in 6 controls and 12 patients. Overexpression of IL-6 and TNFα mRNA was found in all nonresponders studied. In 5 of 6 responders the levels of mRNA specific for IL-10 tended to be higher than in nonresponders and controls but this was not as impressive as the differences for IL-6 (Fig. 2). As the level of transcription for IL-10 was much lower than that for IL-6 the difference between responders and nonresponders is difficult to be shown by semi-quantitative PCR. The overexpression in most of the responders studied may indicate a difference in transcriptional regulation for this cytokine.

Patients with high secretion of IL-6 in vitro also have high serum levels of IL-6 in vivo

To demonstrate that the *in vitro* monokine secretion is relevant to the *in vivo* situation serum levels of IL-1β, IL-6 and TNFα were determined. IL-6 was detectable in the serum of only 2 of 14 controls. Pre-dialysis serum levels of IL-6 could be detected in 12 of 14 nonresponder patients (mean 7.96 ± 4.7 pg/ml) and in 8 of 14 responders (mean 3.89 ± 3.8 pg/ml, nonresponder vs. responder not significant). The patients with the highest *in vitro* levels of IL-6 upon LPS stimulation also had the highest serum

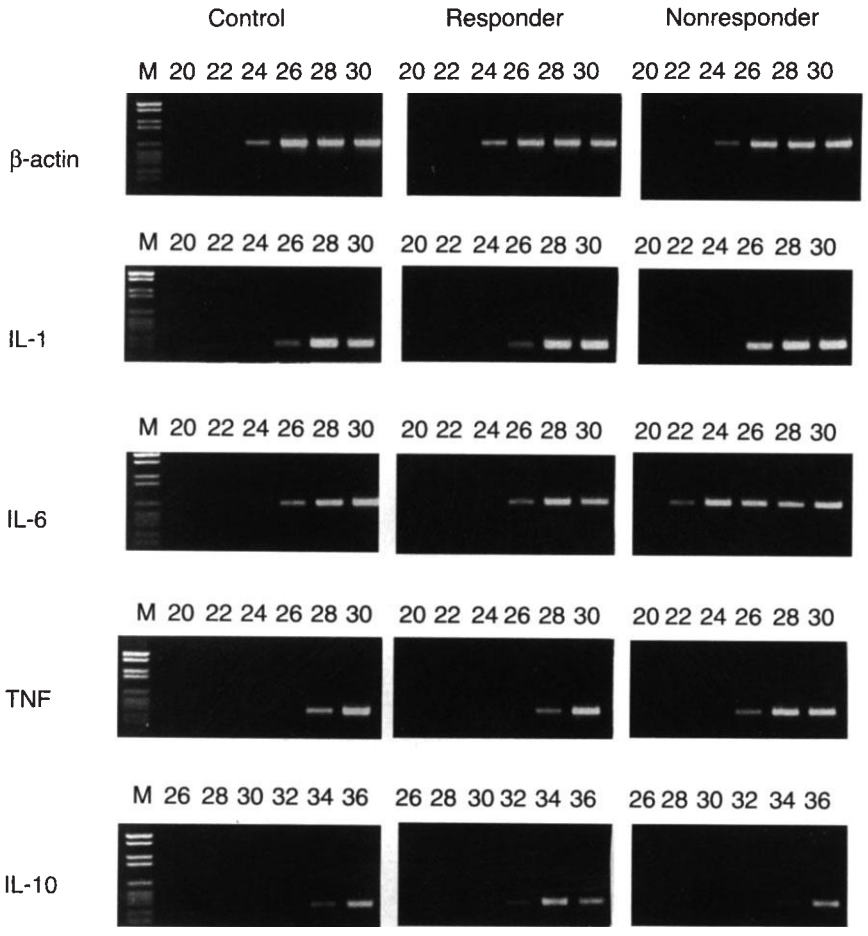

Fig. 2. *Semiquantitative analysis of mRNA levels in PBMC from a control, responder and a nonresponder after 24 hours of stimulation by LPS.* RNA was reverse transcribed and amplified by PCR. Results of one typical experiment out of six are shown. Numbers indicate the PCR cycle of each aliquot analyzed in 1.5% agarose gel stained with ethidium bromide. Samples of cDNA were appropriately diluted before PCR to give equal amounts of product for β-actin. Thus the number of PCR cycle after which a product becomes visible indicates the amount of transcripts in the cells. Detection two cycles earlier reflects the fourfold amount of mRNA. Significantly more transcripts for IL-6 and TNFα in the nonresponder are detected while a slightly higher expression for IL-10 is noted in the responder compared to the control.

levels of IL-6 ($r^2 = 0.62$, $P < 0.001$, Fig. 3). IL-1β and TNFα were found inconsistently in the sera of patients and controls at levels near to the detection limits of the ELISAs. Significant differences between the groups could not be demonstrated (data not shown).

Production of IL-10 is negatively correlated with secretion of IL-6 in responder patients

As IL-10 is a down-regulatory factor for monokine secretion we examined the individual relation of IL-10 and IL-6 in the culture supernatants. In the responder group patients with higher levels of IL-10 upon LPS stimulation have lower levels of IL-6 (Fig. 4, $r^2 = 0.54$, $P < 0.01$). In nonresponders and controls no such correlation between these cytokines could be found. This is mainly due to the fact that the concentrations of IL-10 in the supernatants of these cells were lower than in many of the responder patients. These results may indicate an inhibitory effect of this protein on the production of IL-6. This assumes intact effector mechanisms for IL-10 in dialysis patients.

Intact down-regulation of monokines by IL-10

To see if IL-10 is able to control cytokine production in the cells of dialysis patients recombinant human IL-10 was added to the cultures. The cytokine down-regulated the secretion of IL-6 after stimulation with LPS in cells from patients and controls (Fig. 5). IL-10 was titrated to determine the concentration sufficient to reduce the secretion of IL-6 to half maximal values. This suppression was achieved in both patient's groups at a concentration of 2.5 ng/ml while a concentration of 1 ng/ml IL-10 was sufficient to reduce monokine production in the control group (Fig. 5). The

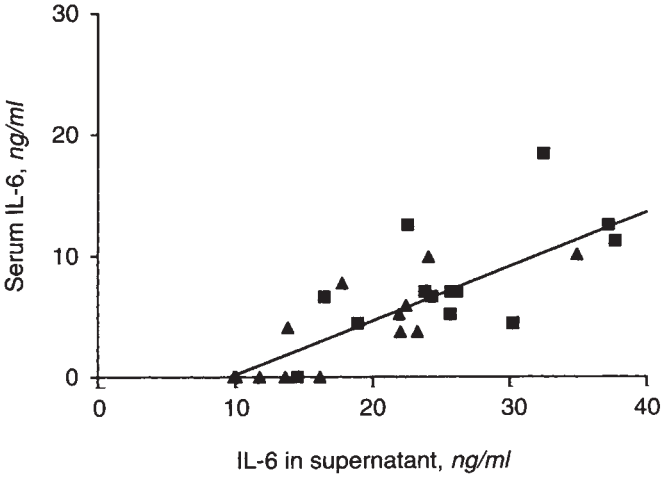

Fig. 3. *Comparison of serum IL-6 concentrations and the* in vitro *secretion of IL-6 upon stimulation of PBMC by LPS in nonresponders (■) and responders (▲).* A linear correlation could be shown between the two parameters that is indicated by the line ($r^2 = 0.62$, $P < 0.001$). Patients whose cells secrete the highest amounts of IL-6 *in vitro* also have high levels of serum IL-6.

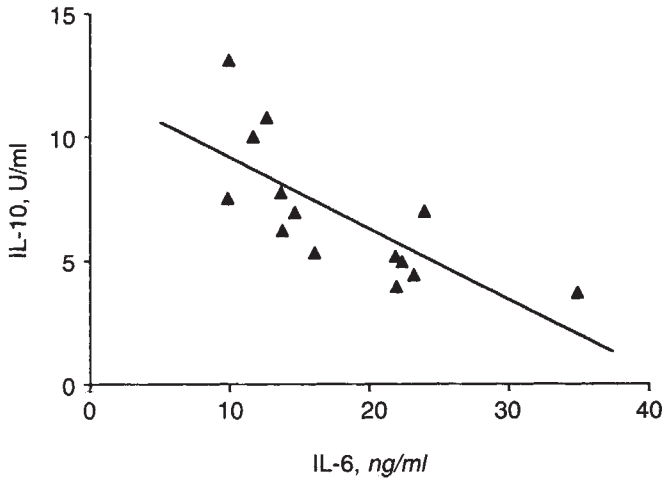

Fig. 4. *Correlation between the concentrations of IL-10 and IL-6 detected in the supernatant of PBMC from responder patients.* Cells were stimulated by LPS for 24 hours. In responder patients high levels of IL-10 occur together with low levels of IL-6 and vice versa ($r^2 = 0.54$, $P < 0.01$).

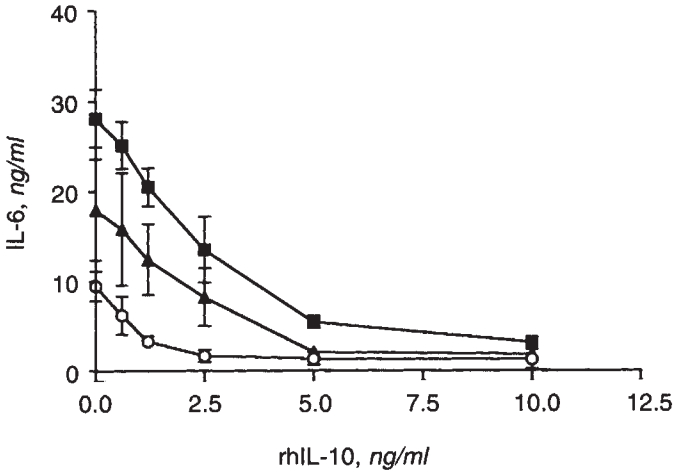

Fig. 5. *Addition of recombinant human IL-10 at different concentrations to cultures of PBMC from healthy controls (○), responders (▲) and nonresponders (■) stimulated by LPS leads to a dose dependent suppression of the secretion of IL-6 in each group.* Half maximal suppression of IL-6 is achieved at about 1 ng/ml IL-10 in controls, whereas about 2.5 ng/ml are needed in responders and nonresponders. Mean values and standard deviation of 5 independent experiments are given.

results indicate an intact down-regulation of monokines by IL-10 and the requirement of higher concentrations in hemodialysis patients. mRNA for IL-6 also was downregulated by recombinant IL-10 while the transcription for β-actin was unaffected (Fig. 6). This shows a specific effect of IL-10 on the cytokines rather than an action as an unspecifically toxic agent.

Production of IL-6 in responders reaches levels of nonresponders after blockade of IL-10 by monoclonal antibody

The production of IL-6 upon stimulation by LPS was compared in the absence and presence of a blocking monoclonal antibody against human IL-10. Without this antibody cells from nonresponders produced more than twice the amount of IL-6 compared to cells from controls. Cells from responders showed an intermediate production. Blocking of IL-10 in this assay lead to an enhanced production of IL-6 in all groups including the controls. But while controls remained at about 25 ng/ml the secretion of IL-6 in cells from responders reached the same level as that of nonresponders (Fig. 7).

Discussion

In the present study we show that PBMC obtained from hemodialysis patients 42 hours after the last dialysis treatment produce high amounts of monokines upon stimulation by LPS. Unlike recent studies investigating the secretion of IL-6 [4] or TNFα [5] by otherwise unstimulated PBMC immediately after dialysis we focused on the long-term alterations arising from uremia and its treatment, as these might be more important for the defect of immune defense commonly seen in the patients. The immune defect of hemodialysis patients is characterized by a reduced T cell activation resulting in a low production of IL-2 [10] while the expression of IL-2 receptors is high [20]. The basis therefore is a defect of the accessory cells [10] that have a reduced capability to provide costimulatory signals for T cell activation. Costimulatory signals arise from surface molecules of the accessory cell or from its soluble mediators. Recently we could show a role for the surface molecule B7 on monocytes [11]. Whether or not soluble mediators are important for the reduced T cell activation has not been shown yet. From theoretical studies this is possible as a suppressive effect of TNFα on the IL-2 dependent clonal expansion of T cells is known [21]. Additionally IL-1 and IL-6 directly enhance the expression of IL-2 receptors on T cells [22] and a correlation between high levels of IL-6 and soluble IL-2 receptor has been shown in HIV patients [23]. Given these effects high levels of TNFα and IL-6 might very well contribute to the characteristics of the immune defect in dialysis patients.

A good indicator for the grade of immunodeficiency of a patient is the *in vivo* response after vaccination against hepatitis B [9]. We thus compared responders and nonresponders to hepatitis B vaccination concerning their monokine profile. Upon stimulation by LPS a clear difference between the two patient groups concerning the secretion of IL-6 and TNFα appeared. Cells from nonresponders to hepatitis B vaccination produced more of these cytokines than responders who mainly behaved like healthy controls. This was confirmed by an overexpression of mRNA for IL-6 and TNFα in the nonresponder patients. The strong relation between the *in vivo* immune status of the patients and the *in vitro* monokine secretion indicates a possible pathogenetical role for the immune defect of these patients. High secretion of TNFα by cells from dialysis patients after stimulation by LPS has been shown before [5]. In contrast Friedlander and coworkers [24] found an impaired production of IL-1β and TNFα. Secretion of IL-6 tended to be higher in their patients although this was not significant. It is difficult to compare these results with ours since bioassays for TNFα and IL-6 were used that may be influenced by soluble inhibitors of the cytokines. Moreover, we can show a heterogeneity of the dialysis patients and we do not know about the clinical immune status of the patients in that study.

The *in vivo* relevance of the *in vitro* findings is further supported by a correlation of high serum levels for IL-6 in those patients whose cells showed the most intense inducibility *in vitro*. Elevated concentrations in serum before dialysis have been shown for IL-1

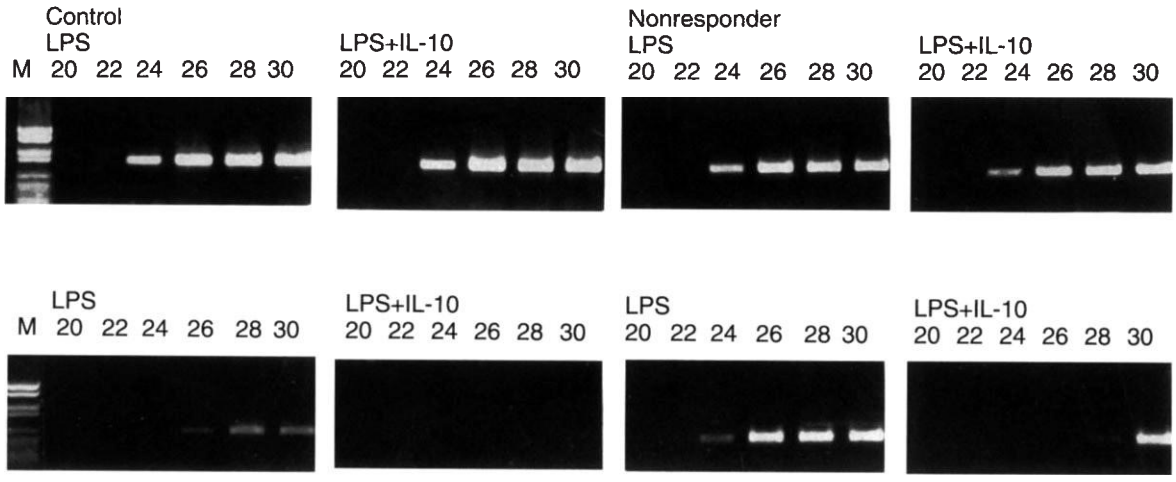

Fig. 6. *Semiquantitative PCR analysis of mRNA levels for IL-6 in PBMC of a healthy control and a nonresponder patient after stimulation by LPS without or with addition of 10 ng/ml recombinant human IL-10.* Results of one typical experiment out of 3 are given. IL-10 specifically reduces the expression of mRNA for IL-6 without affecting the transcription for β-actin.

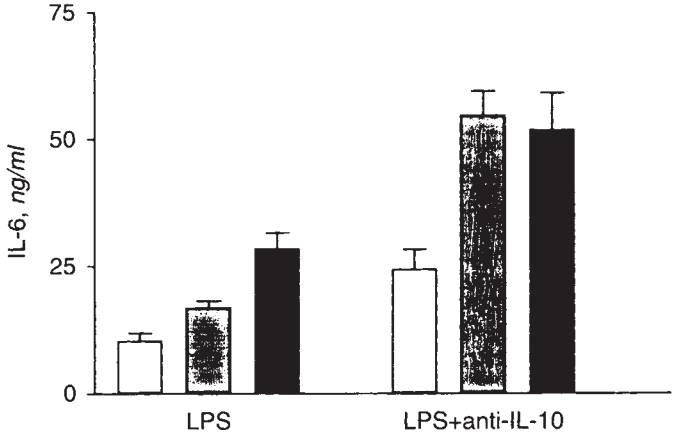

Fig. 7. *Production of IL-6 by cells from healthy controls (□), responders (▨) and nonresponders (■) after stimulation by LPS in the presence or absence of a monoclonal antibody blocking IL-10 activity in culture supernatant (mean ± SD, N = 6 in each group).*

[3], IL-6 [2, 25] and TNFα [3, 5]. While IL-1 and IL-6 remain stable [2, 3, 25] a further increase was noted for serum TNFα during the dialysis procedure [5, 7]. We only detected significantly high serum levels for IL-6. This may be related to the presence of varying amounts of inhibitors for TNFα [26] or IL-1 [27] in the serum of dialysis patients.

The overproduction of monokines by cells from nonresponders was contrasted by higher levels of interleukin-10 from cells of responder patients. IL-10 was measured because this substance, originally referred to as cytokine synthesis inhibitory factor (CSIF) [12], is an interesting candidate for the regulation of monokine secretion. The cytokine can be produced by a number of different cells, among them mainly monocytes, B cells, and T cells [13]. The most important effects of IL-10 are those directed at the antigen presenting cell. At lower concentrations the production of monokines is down-regulated. At higher concentrations (100 U/ml) the antigen presenting function and class II molecule expression are inhibited as well [14]. IL-10 can be induced in monocytes by LPS at the same concentrations that lead to the secretion of IL-1, IL-6 and TNF. Recently an autoregulatory role of IL-10 on monocyte function has been demonstrated [14].

The individual coincidence of high levels of TNFα and IL-6 can be explained by the induction of IL-6 as an effect of TNFα [28]. The reciprocal relation between IL-6 and IL-10, although not proving a causal relationship, indicates that the elevation of IL-10 might be a counterregulatory mechanism to control the monokine overproduction induced by uremia.

This hypothesis assumes that the control of monokine secretion by IL-10 is effective in dialysis patients. We could achieve a nearly complete suppression of IL-6 by the addition of recombinant IL-10 to the cultures in all study groups. Kinetic analysis showed that the concentrations of IL-10 necessary to control the overproduction of IL-6 in the patient's cells are within the range of concentrations usually detected in culture supernatants of stimulated cells. The doses needed for half maximal suppression of IL-6 in the patient groups was about 2.5-fold the dosis of the control group. On the other hand a blockade of IL-10 in the supernatant of stimulated cells by monoclonal antibodies lead to an increased secretion of IL-6 in the responder as well as the nonresponder group while the production in the control group remained much lower. Thus IL-10 can be addressed as the causal factor in controlling monokine secretion in responder patients.

As discussed above it is highly probable that this effect is causally relevant for the clinical immune defect. Additionally IL-10 may directly inhibit the secretion of prostaglandins that act suppressively on T cells. Furthermore, IL-10 exerts directly stimulating effects on B cells and might thus increase the production of specific antibodies *in vivo*. This could be relevant for our criterion of *in vivo* immune function, the response to hepatitis B vaccination.

In conclusion, the enhanced monokine production by PBMC of hemodialysis patients is correlated with the *in vivo* immune status of the patients. Therefore it may contribute to the reduced T cell activating capabilities of accessory cells from dialysis patients. An

enhanced secretion of IL-10 in responders indicates that this factor may play a role in the control of monokine overproduction. It is effective in patients as well as in controls and a moderate increase in its secretion is sufficient to normalize monokine levels. Blocking the IL-10 effect leads to levels of IL-6 in the responder group which compare to those of nonresponders. These results support the notion that the responder dialysis patients can use an elevated secretion of IL-10 for down-regulation of cytokine overproduction while nonresponders seem to lack this mechanism.

Acknowledgments

This work was supported by Bundesminister für Forschung und Technologie grant #01 KI 8914. The authors thank Dr. G. Renschin whose patients were included in the study, S. Peppler for his help with the PCR experiments, and K. Schlich for technical assistance.

Reprint requests to Prof. Dr. H. Köhler, Medical Department IV, University of Homburg, D-66421 Homburg/Saar, Germany.

References

1. DINARELLO CA: Cytokines: Agents provocateurs in hemodialysis? *Kidney Int* 41:683–694, 1992
2. CAVAILLON JM, POIGNET JL, FITTING C, DELONS S: Serum interleukin-6 in long-term hemodialyzed patients. *Nephron* 60:307–313, 1992
3. 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
4. MEMOLI B, LIBETTA C, RAMPINO T, DE SIMONE W, MECCARIELLO S, STANGHERLIN P, DAL CANTON A, ANDREUCCI VE: Interleukin-6 production of uraemic haemodialysed patients: Effects of different membranes. *Nephrol Dial Transplant* 6 (Suppl 2):96–98, 1991
5. CHOLLET-MARTIN S, STAMATAKIS G, BAILLY S, MERY JP, GOUGEROT-POCIDALO MA: Induction of tumour necrosis factor-alpha during haemodialysis. Influence of the membrane type. *Clin Exp Immunol* 83:329–332, 1991
6. PERTOSA G, MARFELLA C, TARANTINO EA, DI CILLO M, MANNO C, RUSSO R, SCHENA FP: Involvement of peripheral blood monocytes in haemodialysis: In vivo induction of tumour necrosis factor alpha, interleukin 6 and beta 2-microglobulin. *Nephrol Dial Transplant* 6 (Suppl 2):18–23, 1991
7. GHYSEN J, DE PLAEN JF, VAN YPERSELE DE STRIHOU C: The effect of membrane characteristics on tumour necrosis factor kinetics during haemodialysis. *Nephrol Dial Transplant* 5:270–274, 1990
8. SCHINDLER R, LINNENWEBER S, SCHULZE M, OPPERMANN M, DINARELLO CA, SHALDON S, KOCH KM: Gene expression of interleukin-1β during hemodialysis. *Kidney Int* 43:712–721, 1993
9. KÖHLER H, ARNOLD W, RENSCHIN G, DORMEYER HH, MEYER ZUM BÜSCHENFELDE KH: Active hepatitis B vaccination of dialysis patients and medical staff. *Kidney Int* 25:124–128, 1984
10. MEUER SC, HAUER M, KURZ P, MEYER ZUM BÜSCHENFELDE KH, KÖHLER H: Selective blockade of the antigen-receptor-mediated pathway of T cell activation in patients with impaired immune responses. *J Clin Invest* 80:743–749, 1987
11. GIRNDT M, KÖHLER H, SCHIEDHELM-WEICK E, MEYER ZUM BÜSCHENFELDE KH, FLEISCHER B: T-cell activation defect in hemodialysis patients: Evidence for a role of the B7/CD28 pathway. *Kidney Int* 44:359–365, 1993
12. MOORE KW, VIEIRA P, FIORENTINO DF, TROUNSTINE ML, KHAN TA, MOSMANN TR: Homology of cytokine synthesis inhibitory factor (IL-10) to the Epstein Barr virus gene BCRFI. *Science* 248:1230–1234, 1990
13. FIORENTINO DF, ZLOTNIK A, MOSMANN TR, HOWARD M, O'GARRA A: IL-10 inhibits cytokine production by activated macrophages. *J Immunol* 147:3815–3822, 1991
14. DE WAAL MALEFYT R, ABRAMS J, BENNETT B, FIGDOR CG, DE VRIES JE: Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: An autoregulatory role of IL-10 produced by monocytes. *J Exp Med* 174:1209–1220, 1991
15. SCHLAAK JF, SCHMITT E, HÜLS C, MEYER ZUM BÜSCHENFELDE KH, FLEISCHER B: A sensitive and specific bioassay for the detection of human interleukin-10. *J Immunol Meth* 168:49–54, 1994
16. HOOF T, RIORDAN JR, TÜMMLER B: Quantitation of mRNA by the kinetic polymerase chain reaction assay: A tool for monitoring p-glycoprotein expression. *Anal Biochem* 196:161–169, 1991
17. YAMAMURA M, WANG XH, OHMEN JD: Cytokine patterns of immunologically mediated tissue damage. *J Immunol* 149:1470–1475, 1992
18. EHLERS S, SMITH KA: Differentiation of T cell lymphokine gene expression: The in vitro acquisition of T cell memory. *J Exp Med* 173:25–36, 1991
19. VIEIRA P, DE WAAL MALEFYT R, DANG MN, JOHNSON KE, KASTELEIN R, FIORENTINO DF, DE VRIES JE, RONCAROLO MG, MOSMANN TR, MOORE KW: Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: Homology to Epstein-Barr virus open reading frame BCRFI. *Proc Natl Acad Sci USA* 88:1172–1176, 1991
20. DUMANN H, MEUER SC, MEYER ZUM BÜSCHENFELDE KH, KÖHLER H: Hepatitis B vaccination und interleukin 2 receptor expression in chronic renal failure. *Kidney Int* 38:1164–1168, 1990
21. PAWELEC GP, REHBEIN A, SCHAUDT K, BUSCH FW: IL-4-responsive human helper T cell clones are resistant to growth inhibition by tumor necrosis factorα. *J Immunol* 143:902–906, 1989
22. LORRÉ K, VAN DAMME J, VERWILGHEN J, BAROJA ML, CEUPPENS JL: IL-6 is an accessory signal in the alternative CD2-mediated pathway of T cell activation. *J Immunol* 144:4681–4687, 1990
23. HONDA M, KITAMURA K, MIZUTANI Y, OISHI M, ARAI M, OKURA T, IGARAHI K, YASUKAWA K, HIRANO T, KISHIMOTO T, MITSUYASU R, CHERMANN JC, TOKUNAGA T: Quantitative analysis of serum IL-6 and its correlation with increased levels of serum IL-2R in HIV-induced diseases. *J Immunol* 145:4059–4064, 1990
24. FRIEDLANDER MA, HILBERT CM, WU YC, RICH EA: Role of dialysis modality in responses of blood monocytes and peritoneal macrophages to endotoxin stimulation. *Am J Kidney Dis* 22:11–23, 1993
25. HERBELIN A, URENA P, NGUYEN AT, ZINGRAFF J, DESCAMPS-LATSCHA B: Elevated circulating levels of interleukin-6 in patients with chronic renal failure. *Kidney Int* 39:954–960, 1991
26. BROCKHAUS M, BAR-KHAYIM Y, GURWICZ S, FRENSDORFF A, HARAN N: Plasma tumor necrosis factor soluble receptors in chronic renal failure. *Kidney Int* 42:663–667, 1992
27. PEREIRA BJG, POUTSIAKA DD, KUNG AJ, STROM JA, NARAYAN G, LEVEY AS, DINARELLO CA: In vitro production of IL-1 receptor antagonist in chronic renal failure, CAPD and hemodialysis. *Kidney Int* 42:1419–1424, 1992
28. SHERON N, LAU JN, HOFMANN J, WILLIAM R, ALEXANDER GJM: Dose dependent increase in plasma IL-6 after TNF infusion in humans. (abstract) *Clin Exp Immunol* 82:4227–4231, 1990