Interleukin-1 receptors and receptor antagonist in haemodialysis

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Abstract

Background. Biological activity of interleukin-1 (IL-1) depends on the number and type of IL-1 receptors on target cells and on the amounts of its naturally occurring inhibitor; the IL-1 receptor antagonist (IL-1ra).

Methods. Expression of IL-1 receptor was studied on the peripheral blood mononuclear cells of 20 end-stage renal-disease patients maintained by chronic haemodialysis by means of either polysulphone (10 patients) or cuprophane membranes (10 patients) and compared to that of normal controls. Plasma and cellular levels of IL-1ra and IL-1b were also measured.

Results. The proportion of monocytes expressing the IL-1 receptor was strikingly higher in haemodialysis patients than in the healthy population. This proportion further increased during haemodialysis with cuprophane but not with polysulphone. Expression of the IL-1 receptor on lymphocytes was very low in both controls and dialysed patients; in the latter there was no intradialytic variation. Plasma concentrations of IL-1b and IL-1ra were elevated in haemodialysis patients and undetectable in controls. Whereas plasma IL-1b decreased throughout haemodialysis, IL-1ra further increased, with no significant differences between the two membranes used. Total cellular IL-1b and IL-1ra were also higher in the patient group than in the healthy controls. A further increase of both IL-1b and IL-1ra was detected at the end of the haemodialysis session with any membrane.

Conclusions. Monocytes of haemodialysis patients circulate in a state of activation, which makes them both producer and target of IL-1. Thus there is an autocrine upregulation of IL-1 production. Although IL-1ra levels are high, they are most likely to be expression of monocyte activation rather than represent effective inhibitors of IL-1 activity.

Key words: haemodialysis; interleukin-1b; interleukin-1 receptor; interleukin-1 receptor antagonist

Introduction

Interleukin-1b (IL-1b) is supposed to play a crucial role in the development of both acute and chronic inflammatory changes experienced by uremic patients on maintenance haemodialysis [1–4].

Plasma levels of IL-1b have been studied by a number of investigators and different results have been obtained depending upon the haemodialysis membrane employed [5–9]. Mononuclear cells of uremic patients during haemodialysis have been shown to undergo activation which would account for the elevated IL-1 production [10–16]. In order to explain the mechanisms involved in mononuclear cell activation, several hypotheses have been proposed, such as bacterial contamination of the dialysate [17,18], acetate-containing buffers [19], activated complement fractions [11,20], blood–membrane interaction per se [10,21]. In some studies, however, such elevated plasma IL-1b levels [22–24] and IL-1b production [24–27] were not found.

IL-1 initiates its action in the inflammatory reactions by binding to IL-1 receptors expressed on target cells [28]. IL-1 receptors have been identified on a large variety of human cells including peripheral blood B and T lymphocytes and monocytes [29–31]. The number of IL-1 receptors per human unstimulated lymphocyte as well as the percentage of resting lymphocytes bearing the IL-1 receptor is low [29]. It has been shown that IL-1 receptors are expressed on T cells after activation and that the expression of IL-1 receptor on the T cells requires the presence of activated monocytes [29]. On B cells the IL-1 receptors have been found to be an earliest marker of activation as well [30]. With regards to monocytes, it has been shown that these cells can express the IL-1 receptor too, and that binding of IL-1 to its receptor is followed by activation of monocytes [31]. This observation suggests that monocytes are both a main source and a target of IL-1 resulting in an autocrine regulation of its own production [32]. Two types of IL-1 receptors have been described (type I and type II) [33,34]. The type I receptor (p80) is found on most cells, has a longer
cytoplasmatic segment and appears to be important for transducing the action of IL-1; the type II (p68) receptor is found mainly on neutrophils, monocytes, bone marrow cells, and B lymphocytes, has a shorter cytoplasmatic segment and no intrinsic biological activity [34]. IL-1 bound to IL-1 receptor type I is rapidly internalized, remains inside the cells for as long as 12 h, and only a small amount is degraded, whereas IL-1 bound to type II receptor is poorly internalized, remains long on the surface and is rapidly degraded [33]. Whenever IL-1 is produced, the synthesis of a natural inhibitor [35], which has been named IL-1 receptor antagonist (IL-1ra), occurs as well [36]. The IL-1ra is a polypeptide which is closely related to IL-1. It has the same molecular size and is produced by the same cells. The IL-1ra blocks IL-1 by binding to the cellular receptors without activating them, thus preventing the binding and consequent biological effects of IL-1 [33,34]. Increased production and elevated plasma levels of IL-1ra have been described in haemodialysis patients [9,37] while data on cellular IL-1 receptors are lacking. Biological activities of IL-1 thus results from an interplay and balance between the active molecule, the binding to its cellular receptors, the number and type of receptors expressed by target cells, and the inhibitory action of its antagonists. The aim of the present study was to evaluate the expression of the IL-1 receptor on lymphocytes and monocytes as well as the intradialytic kinetics of plasma and cellular IL-1β and IL-1ra content in uraemic patients undergoing haemodialysis using different membranes.

Subjects and methods

Patients

Two groups of 10 uraemic patients each undergoing maintenance haemodialysis by means of either high-flux polysulphone (1.25 m² F60, Fresenius, Concord, Ca) or cuprophane (1.30 m², Asahi Medical Co., Tokyo, Japan) hollow-fibre haemodialysis membranes were studied. The patients were on renal replacement treatment at the Division of Nephrology of Varese and gave their informed consent to the study. The whole uraemic patient population included eight males and 12 females, their mean age was 65.4 years (range 42–81) and the average duration of haemodialysis was 44 months (range 19–71). There were no differences in gender, age, and duration of haemodialysis between the two groups of patients. Each haemodialysis session had a duration of 4 h 30 min; new dialysers were employed in every case; no critical acute adverse reactions to the dialysis procedure were observed, and the total ultrafiltration was always kept below 3% of the predialysis body weight. All patients were on treatment with recombinant erythropoietin at doses adjusted to keep a constant Hct between 30 and 35%. All patients had an internal a-v fistula as vascular access. Patients dialysed on cuprophane had always been treated by means of the same type of membrane. Patients dialysed on polysulphone had either always been treated with that membrane, or been so treated for a minimum of 6 months preceding the study. They were all free from infections and none of them was taking steroids or other drugs that affect cytokine metabolism. Control samples were obtained partly from healthy volunteers belonging to the personnel of the Division of Nephrology of Varese, and partly from individuals with normal renal function seen periodically because of primary hypertension who were on low-salt diet and were not taking any hypotensive medications. They were 16 females and 11 males. Their average age was 59.2 years (range 25–75).

Plasma preparation

Predialysis samples were taken immediately before the session after the long interval; postdialysis samples were taken from the arterial line just before the end of the session. Any dilution of blood specimens was thus prevented. Plasma for IL-1β and IL-1ra determination was obtained by centrifugation of haeparinized blood. After centrifugation, plasma was aspirated and spun at 4 °C, 5000 g, for 10 min. The supernatant was stored, frozen at −85 °C, until tested for IL-1β and IL-1ra concentration.

Cell preparation

Haeparinized blood was centrifuged on a Ficoll–Hypaque gradient (Pharmacia Diagnostics, Piscataway, NJ). Mononuclear cells were washed three times with medium RPMI 1640 (Gibco, Grand Island, NY) with the addition of polymyxin B (5 mg/ml) and penicillin/streptomycin. Cells were then resuspended in 1 ml of culture medium prepared as above supplemented with 10% fetal calf serum, brought to a concentration of 5 ×10⁶ cells/ml and incubated for 6 h at 37 °C in 5% CO₂ atmosphere. Cultures were harvested and cells resuspended and submitted to three cycles of freezing (−85 °C) and thawing. After the third cycle cells were spun at 5000 g and the supernatant was stored frozen at −85 °C until tested.

Quantification of lymphocytes and monocytes expressing the IL-1 receptor

The IL-1 receptor expression was studied by the use of a commercial IL-1β flow cytometry kit (IL-1β Fluorokine kit, R & D Systems Inc. Minneapolis, MN) which measures the binding of IL-1β to cell-surface receptor. PBMC were centrifuged and washed twice with culture medium with the addition of polymyxin B at a concentration of 5 mg/ml. Cells were then resuspended in the same medium and brought to a final concentration of 4 ×10⁶ cells/ml. Cells were incubated with recombinant IL-1β conjugated to biotin for 60 min in siliconized glass tubes and then were washed to remove unbound cytokine. Subsequently cells were incubated with fluorescein-isothiocyanate conjugated avidin. Then they were washed to remove unbound avidin–FITC and resuspended in PBS for flow cytometric analysis by an EPICS Profile flow cytometer (Coulter Corporation, Hieleah, FL). The gates were adjusted to include the lymphocyte and the monocytes clusters. The gates were set up by using monoclonal antibodies against KC562 (pan-leukocyte marker), CD2 (T cells), CD19 (B cells), MO2 (monocyte). The specificity of the binding was tested by preincubating cells with a 20-, 50- and 100-fold molar excess of unconjugated rIL-1β prior to incubation with biotinylated IL-1β. The IL-1 binding by these cells was compared with that of another set of cells that were incubated only with biotinylated IL-1β. As shown in Figure 1, preincubation with different concentrations of unconjugated rIL-1β reduced the percentage of IL-1β-binding
IL-1, IL-1 receptor and IL-1ra interplay in haemodialysis

Expression of IL-1 receptors on peripheral blood mononuclear cells in controls and HD patients

The absolute number as well as the relative proportion of peripheral leukocytes and their subsets after 4 h 30 min of haemodialysis with both cuprophane and polysulphone were indistinguishable from the predialysis values.

The proportion of cells in the lymphocyte clusters that bound recombinant IL-1b on their cytoplasmic membrane in end-stage renal-disease patients \( (n=20) \) was \( 2.9 \pm 0.9\% \). In controls \( (n=27) \) \( 3.1 \pm 1.0\% \) of lymphocytes bound recombinant IL-1. The difference was not significant.

In the monocyte clusters of uraemic patients, however, \( 31.5 \pm 3.5\% \) of the cells bound IL-1 on their cellular membrane compared with \( 8.7 \pm 1.9\% \) IL-1 binding cells in the control population. The difference is extremely significant \( (P<0.0001) \).

IL-1 receptors during haemodialysis

No differences in the expression of IL-1 receptor on both lymphocytes and monocytes were seen between groups of uraemic patients chronically treated by means of cuprophane or polysulphone haemodialysis membranes. During a haemodialysis session there was no significant change in the expression of IL-1 receptor on lymphocytes (Figure 2).

At the end of a haemodialysis session with the use of polysulphone the percentage of cells expressing the IL-1 receptor in the monocyte cluster did not change either \( (28.4 \pm 2.4 \text{ to } 30.1 \pm 2.6) \), whereas it increased when cuprophane was employed \( (32.7 \pm 1.9 \text{ to } 39.5 \pm 2.5, P<0.05) \) (Figure 3). The density of receptors per cell as expressed by fluorescence intensity did not change.
Plasma levels of IL-1ra in controls and HD patients

Non-uraemic controls had plasma levels of IL-1ra below or at the detection limit of the method (23 pg/ml). Plasma levels of IL-1ra in haemodialysis patients were instead extremely high (720 ± 134 pg/ml) ranging from a minimum value of 150 pg/ml to a maximum of 2700 pg/ml.

The difference between the two groups of 10 patients each treated with either cuprophane (797 ± 242 pg/ml) or polysulphone (643 ± 140 pg/ml) was not significant.

Plasma IL-1ra during haemodialysis

As shown in Figure 4, plasma levels of IL-1ra increased during the haemodialysis session in both groups of patients. The rate of increase was similar regardless of whether cuprophane (from 797 ± 242 pg/ml to 1163 ± 254 pg/ml; \( P < 0.05 \)) or polysulphone (from 643 ± 125 pg/ml to 1012 ± 130 pg/ml; \( P < 0.05 \)) was employed. Changes of plasma IL-1ra, however, were not homogeneous among individual patients, since in two cases of each membrane group IL-1ra concentration decreased.

Total cellular IL-1ra in controls and HD patients

The amount of IL-1ra, expressed as pg/ml per \( 5 \times 10^6 \) cell in 1 ml of medium, measured in control individuals was 544 ± 134 pg/ml vs 941 ± 94 pg/ml in haemodialysis patients (\( P < 0.02 \)). There was no difference between cuprophane- and polysulphone-treated patients (960 ± 143 and 923 ± 131 pg/ml respectively, predialysis values).

Total cellular IL-1ra during haemodialysis

IL-1ra further increased during haemodialysis from 941 ± 94 up to 1595 ± 193 pg/\( 5 \times 10^6 \) cells/ml (\( P < 0.0001 \)). Figure 5 shows that whereas the intradialytic increase of this parameter during haemodialysis was seen in both groups of haemodialysed patients there are differences depending upon the membrane employed. In fact with cuprophane IL-1ra increased from 960 ± 143 to 1,880 ± 324 pg/ml (\( P < 0.0005 \)) while with polysulphone the rate of increase was lower, from 923 ± 131 to 1,310 ± 183 pg/ml (\( P < 0.04 \)).

Plasma levels of IL-1b in controls and in HD patients

Plasma levels of IL-1b in control subjects were at or below the detection limit of the method (31 pg/ml). In haemodialysis patients predialysis plasma concentrations of IL-1b were 1314 ± 210 pg/ml (range 140–3300) with an extremely significant difference with the controls (\( P < 0.0001 \)). Predialysis plasma levels of IL-1b of patients treated by means of two different haemodialysis membranes were slightly but not significantly different (1397 ± 309 pg/ml with cuprophane vs 1231 ± 298 pg/ml with polysulphone).

Plasma IL-1b kinetics during haemodialysis

Figure 6 shows that plasma concentration of IL-1b decreased throughout haemodialysis in every individual patient. The differences of the means were significant in both cases, since IL-1b concentration fell from 1397 ± 309 pg/ml to 491 ± 136 pg/ml with cuprophane (\( P < 0.002 \)) and from 1231 ± 298 pg/ml to 407 ± 80 pg/ml with polysulphone (\( P < 0.02 \)).

Total cellular IL-1b in controls and HD patients

Results of IL-1b concentration are expressed as pg/ml of IL-1b per \( 5 \times 10^6 \) PBMC in 1 ml of medium. The IL-1b concentration was significantly higher in haemodialysis patients than in the control group (598 ± 58 vs 411 ± 65 pg/\( 5 \times 10^6 \) cells respectively; \( P < 0.05 \)).

Total cellular IL-1b during haemodialysis

During cuprophane dialysis IL-1b increased from a predialysis level of 663 ± 86 pg/\( 5 \times 10^6 \) cell/ml to
on their cytoplasmic membrane increases during a haemodialysis session carried out by means of cuprophone but not of polysulphone membranes. IL-1 receptor expression is an early marker of cell activation; thus the results of the present study provide evidence that monocytes are chronically activated in haemodialysis patients. Since IL-1 is produced by activated cells, the high number of monocytes expressing the IL-1 receptor indicates that in haemodialysis there is an increased availability of activated monocytes which are both producers and targets of IL-1.

Both B and T lymphocytes express the IL-1 receptor following activation [29,30]. Our study shows that the proportion of peripheral lymphocytes of uraemic patients on maintenance haemodialysis expressing the IL-1 receptor is low and similar to that of healthy individuals. No change is induced by the haemodialysis session regardless of whether a cellulose or a synthetic haemodialysis membrane is employed. Since IL-1 cannot exert its activity in the absence of its receptor on cellular membrane, our data indicate that the high levels of IL-1 do not play a direct role in the changes of lymphocyte function associated with chronic haemodialysis. These results are consistent with earlier studies showing no signs of activation on resting lymphocytes of uraemic patients, both in vivo and in vitro [38]. Our data also support the previous observation that the upregulation of the high-affinity IL-2 receptor induced in vitro by the uraemic serum on normal lymphocytes occurs by a mechanism which is independent by circulating IL-1 [39].

Discussion

The biological activity of IL-1 depends on the number and type of its receptors on target cells and the amount of its naturally occurring inhibitors [28,33,34]. The present study demonstrates that the autocrine loop of IL-1 is activated in monocytes but not in lymphocytes of patients undergoing chronic maintenance haemodialysis. In our study we show that in haemodialysed patients the number of monocytes binding recombinant IL-1 is significantly higher than in healthy individuals. As far as the role of the haemodialysis membrane is concerned, the small number of cases per group does not permit definite conclusions; however, there is a trend which suggests that the proportion of monocytes expressing the IL-1 receptor on their cytoplasmic membrane increases during a haemodialysis session carried out by means of cuprophone but not of polysulphone membranes. IL-1 receptor expression is an early marker of cell activation; thus the results of the present study provide evidence that monocytes are chronically activated in haemodialysis patients. Since IL-1 is produced by activated cells, the high number of monocytes expressing the IL-1 receptor indicates that in haemodialysis there is an increased availability of activated monocytes which are both producers and targets of IL-1.

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Upon activation, monocytes produce both IL-1 and its inhibitor, the IL-1 receptor antagonist [34,35]. In our study the average of both IL-1β and IL-1ra plasma levels are significantly higher in haemodialysis patients than in non-uraemic individuals whose plasma levels of the cytokines are undetectable. However, there is an extremely large distribution of values of both cytokines among individuals, which prevents our results from having a general application to the condition of chronic haemodialysis as such. Implications of cytokine measurements during haemodialysis have been recently reviewed [40]. Some investigators found high predialysis plasma levels of IL-1β [5–9,12,41]; other groups of investigators, however, did not find such elevated plasma IL-1β levels [22–24]. These discrepancies may have several explanations.

Firstly, the method employed. Bioassays based on T cell, B cell, or thymocyte proliferations are highly sensitive; however, other cytokines possibly present in plasma (mainly IL-6 but also IL-2, IL-4, and IL-7) can stimulate cell proliferation in a bioassay [3,26] with consequent overestimation of the results. Conversely, low IL-1 activity in a bioassay may result from the presence of inhibitors in plasma of haemodialysis patients. IL-1 levels measured by the use of radioimmune-assays (RIA) may give lower values than those determined with enzyme-linked immunosorbent assays (ELISA), since RIAs appear to be up to 10 times less sensitive [7]. ELISAs have been shown to be sensitive and highly correlated with bioassays whose...
specificity for IL-1\(\beta\) activity was tested by the use of a neutralization test with an antibody against human IL-1 or with experiments comparing activity of human recombinant IL-1\(\beta\) with that of rIL-4 and rIL-6 [7]. In order to check the specificity or other possible disturbances of the immuno assay, we retested for IL-1\(\beta\) and IL-1ra random plasma and medium from cell culture samples after they had been incubated for 2 h in anti-IL-1\(\beta\), IL-1ra, IL-2 precoated or uncoated wells. After incubation with anti-IL-1\(\beta\) and anti-IL-1ra, concentrations of IL-1\(\beta\) and IL-1ra were on average 30% of their original value, whereas there were no changes of IL-1\(\beta\) and IL-1ra in the samples preincubated with anti-IL-2 or in uncoated wells (Figure 8).

Secondly, the selection of patients and the already mentioned large interpatient variability of results. In the present study plasma IL-1\(\beta\) concentration in haemodialysis patients ranged from 140 to 3300 pg/ml. Other investigators found IL-1\(\beta\) values ranging from 160 to 1700 pg/ml [9] or from undetectable values to nearly 20 000 pg/ml [12].

Thirdly, the large distribution of values may result from differences in the duration of treatment with haemodialysis. In the present work we investigated patients who had undergone haemodialysis for a minimum of 19 months. Earlier studies have shown that uraemic patients not yet dialysed have no detectable IL-1\(\beta\) in their plasma and that levels of the cytokine progressively increase with the duration of regular haemodialysis treatment [7,8].

Fourthly, poorly nourished and anaemic patients have an impaired ability to produce cytokines [3] which is significantly improved by treatment with erythropoietin [42]. The high levels of IL-1\(\beta\) in our study may then also be explained by the fact that all the patients were receiving erythropoietin.

Lastly, other factors such as coexisting infectious or autoimmune diseases, immunosuppressive or hypotensive therapies, or blood transfusions might explain the diverse results among studies.

In our study the spontaneous production of IL-1\(\beta\) in haemodialysis patients is greater than in the control group. Mononuclear cells obtained after 4 h 30 min of haemodialysis are associated with higher amounts of IL-1\(\beta\) and IL-1ra than the predialysis samples. This indicates a de novo production of both cytokines during the course of the haemodialysis encounter. The absolute concentrations as well as rates of intradialytic increase of IL-1ra are higher than those of IL-1\(\beta\). Predialysis IL-1ra plasma levels, however, are lower than IL-1\(\beta\). During the course of a haemodialysis encounter, IL-1\(\beta\) and IL-1ra have different kinetics. Whereas plasma concentration of IL-1\(\beta\) decreases in every individual patient, with an average of 35% of the predialysis value, plasma concentration of IL-1ra further increases. It has been shown that once IL-1\(\beta\) is produced, relevant amounts of it remain in the intracellular compartments and its release may require up to 72 h to equal the intracellular rate [8,33]. Some random samples of cell cultures of the present study were tested in order to measure separately the intracellular and extracellular IL-1\(\beta\) and IL-1ra concentrations. The amount of IL-1ra released in the supernatant of the cell cultures was relatively higher than that of IL-1\(\beta\) (39 vs 18% of the total cytokine measured), which is consistent with results of other investigators who have shown that the synthesis of IL-1\(\beta\) and IL-1ra by mononuclear cells does not follow a unique pathway and that IL-1ra is much more readily secreted than IL-1\(\beta\) [43]. It is then likely that postdialysis plasma samples contain much of the de novo IL-1ra produced, whereas release of IL-1\(\beta\) occurs mostly during the interdialytic stretches.

Haemodialysis membranes are able to remove circulating IL-1\(\beta\) by mechanisms which involve ultrafiltration and adsorption [21]. In our study plasma IL-1\(\beta\) levels decreased during haemodialysis sessions with both low-flux cuprophone and high-flux polysulphone, while others found no change with cuprophone and slight, non-significant decrease with polysulphone [12], or intradialytic increase with both more or less biocompatible membranes [7] or opposite kinetics during dialysis with cuprophone and polyacrylonitrile membranes [6]. The discrepancies with data of other studies are to be explained.

Firstly, as already stated, the large variability of predialysis levels impairs the generalizability of our results, and such limitation is even stronger in the case of analysis of membrane groups which include small number of patients. Secondly, the predialysis level may affect the results of the intradialytic kinetics. Thirdly, an important role can be played by the rate of dehydration applied during the haemodialysis session that in our study was very low (less than 3% of body weight). Moreover, despite the different clearance by convective transport between cuprophone and polysulphone membranes, adsorption of IL-1\(\beta\) may occur with all membranes contributing to remove the cyto-

![Figure 8](image-url)
kine from circulation as some studies have shown [8,21,44].

Whereas synthetic membranes, in particular hydrophobic membranes, adsorb more proteins than cellulose membranes [4], it has been shown that during normal use of a new cellulosic dialyser, as in the present study, coating of the membrane with a protein film occurs as well [45,46], making otherwise different membranes similar in terms of permeability to some substances [47]. According to our study such clearing and binding properties of the haemodialysis membranes do not seem to apply to IL-1ra. Although IL-1b and IL-1ra share many similarities in terms of chemical structure and molecular size, IL-1ra might not necessarily have the same affinity for the dialysis membranes as IL-1b.

Since elevated amounts of IL-1 receptor antagonist are produced by mononuclear cells of haemodialysed patients, circulate in their plasma, and compete for binding to the cellular IL-1 receptors, balance between IL-1b and IL-1ra is crucial to allow the biological activity of IL-1b. Based on the current available studies we should conclude that the IL-1b effects largely prevail on those of IL-1ra. In fact it has been shown that as much as 100-fold excess of IL-1ra is required to inhibit IL-1-induced responses of T lymphocytes and of other target cells [48,49]. Those values are much higher than those we measured in our study even in the postdialysis period when the IL-1b/IL-1ra ratio decreases.

Mechanisms underlying monocyte activation which leads to IL-1 production are still a matter of debate. In our study the differences between cuprophane and polysulphone membranes never reached the levels of statistical significance, but the intradialytic changes of monocytes expressing the IL-1 receptor. These results make it unlikely that activated complement fractions are a relevant stimulus to the IL-1 production. Earlier studies have concluded that no IL-1 production can occur in the absence of bacterial contamination [26]. We performed our experiments under sterile conditions in the presence of polymyxin B in order to prevent any possible environmental endotoxin contamination; this, however, does not allow us definitely to rule out bacterial contamination, which at a subclinical level might have occurred during the course of the dialysis sessions, and which can happen through any dialysers, including cellulose [18]. Our patients were on a bicarbonate-dialysis programme. In bicarbonate-haemodialysis the dialysate contains low concentrations of acetate as well, which is known to induce IL-1 production [19]. However, we have previously shown that IL-1 can be produced even in the complete absence of acetate-containing buffers in vitro [8].

In conclusion, our study shows that elevated levels of IL-1b can be found in haemodialysis patients as a result of synthesis by chronically activated monocytes, which are both producers and targets of IL-1, allowing constant autocrine upregulation of IL-1 synthesis. During haemodialysis, large amounts of circulating IL-1 are cleared. Conversely plasma IL-1ra levels do not decrease. However, production and kinetics of IL-1b and IL-1ra are not uniform and do not follow a unique pathway. Accordingly any conclusion must be drawn with caution and may not be always suitable for general application.

During the course of a haemodialysis session uraemic patients often suffer from otherwise unexplained hypotensive episodes, sudden occurrence of chills, or fever. Chronically treated haemodialysis patients are, in high proportion, affected by osteopenia, loss of body mass, accelerated atherosclerosis, and a unique form of β2-microglobulin amyloidosis. Given its peculiar intradialytic kinetics it is likely that, at least in stable patients, free from acute massive bacterial contamination, IL-1 is more involved in the chronic than in the acute complications of the extracorporeal renal replacement treatments. Changes of immunocompetence of chronic haemodialysis might not be mediated by the abnormal IL-1 activity. The high IL-1ra levels appear to be mostly an expression of the inflammatory state which haemodialysis somewhat resembles, rather than to have a protective role. Besides monocytes and lymphocytes, a large variety of cells express the IL-1 receptor, including endothelial cells, chondrocytes, fibroblasts, and synovial lining cells [33]. Studies of such cells as target of IL-1 action might be useful in the understanding of the multifactorial pathology which accompanies uraemia.

References


43. Poutsiaia DD, Clark DD, Vannier E, Dinarello CA. Production of interleukin-1 receptor antagonist and interleukin-1b by peripheral blood mononuclear cells is differentially regulated. Blood 1991; 78: 1275–1281


