Effect of Immunoglobulin Light Chains from Hemodialysis and Continuous Ambulatory Peritoneal Dialysis Patients on Polymorphonuclear Leukocyte Functions

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ABSTRACT
Circulating plasma factors accumulating in the serum of uremic patients have the potential to inhibit essential functions of polymorphonuclear leukocytes (PMNL). As a consequence, these factors can contribute to the increased risk for bacterial infections generally found in uremic patients. Free immunoglobulin light chains that are present in the serum of healthy adults at low levels appear in the serum of uremic patients at significantly higher levels. Therefore, κ and λ light chains in their monomeric and dimeric forms were isolated from hemodialysis and continuous ambulatory peritoneal dialysis patients and their potential to inhibit essential PMNL functions in vitro assays was tested. It was found that all isolates tested were able to inhibit deoxyglucose uptake, a measure for the state of activation of PMNL, as well as chemotaxis. In contrast, free immunoglobulin light chains had no influence on the phagocytotic functions of PMNL. It was concluded that free immunoglobulin light chains are able to act as uremic toxins by interfering with essential PMNL functions and that their serum levels and fate during the treatment of uremic patients should be taken into consideration.

Key Words: Uremic toxins, Immunoglobulin light chains, infection, hemodialysis, CAPD

Twenty years ago, bacterial infections were identified as the main cause of death in uremic patients on maintenance dialysis (1). Despite intensive research and better clinical conditions, this situation has so far not improved (2). The increased incidence of infections among patients with chronic renal failure is primarily a consequence of a diminished function of polymorphonuclear leukocytes (PMNL). These cells play a key role in the unspecific immune defense against bacterial infections. Any impairment of one of their essential functions in an inflammatory reaction such as chemotaxis, phagocytosis, and intracellular killing leads to an increased risk for bacterial infections.

Uremic toxins are thought to play a crucial role in inhibiting PMNL functions. They are circulating plasma factors accumulating in the serum of uremic patients primarily as a result of reduced excretion but possibly also the result of increased synthesis in uremia or during the hemodialysis treatment.

Light chains of immunoglobulins (Ig) are produced by B cells slightly excess of Ig heavy chains (3). Therefore, a small amount of light chains exists in free form in the serum, i.e., is not part of an intact Ig (4). Soling (5) found an up to fivefold increase in the level of free Ig light chains in sera from patients with severely reduced kidney function. Wakisugi et al. (6) observed a significantly increased level of free light chains in sera after the start of hemodialysis therapy. Both authors discuss their results with a focus on light chain deposition diseases in the kidney, but do not consider the immune status of uremic patients.

In this study, we isolated Ig light chains from the plasma ultrafiltrate of uremic patients receiving hemodialysis treatment, and from the peritoneal effluent of patients undergoing continuous ambulatory peritoneal dialysis (CAPD) and investigated their ability to inhibit PMNL functions. We found that isolated light chains are capable of inhibiting the uptake of 2-deoxy-D-glucose into PMNL and of reducing PMNL chemotaxis. Therefore, we concluded that one important aspect of Ig light chains in uremia is their contribution to the impairment of the immune defense, which is causing a higher risk of infection in patients with kidney dysfunction.

MATERIAL AND METHODS
Isolation of Ig Light Chains

Patients. Ultrafiltrate was obtained from our patient pool from two randomly chosen hemodialysis patients who had been on hemodialysis for 3 months up to 2 yr. They were dialyzed with polysulfone hemodialyzers three times weekly for 3 to 4 h. They did not have myeloma or any other non-ESRD-related light-chain disease, nor did they have an active infection or inflammation at the time the samples were obtained.

From seven selected CAPD patients treated with 2 L four times daily, the effluent after 4 h dwell-time was collected. As for the hemodialysis patients, care was taken that the CAPD patients chosen for our investigation did not have any infection or light-chain disease interfering with our study.
Informed consent was obtained from all patients. Their dialysates were used separately for the isolation procedure and assays described below.

Affinity columns. The IgG fractions of rabbit-anti-human \( \kappa \) (K-1255; Sigma, St. Louis, MO) or \( \lambda \) (L-7646, Sigma) light chains were coupled onto N-hydroxysuccinimide (NHS)-activated HiTrap affinity columns (Pharmacia Biotech AB, Uppsala, Sweden) according to the instructions from the supplier.

Ig light chains from hemodialysis patients. The ultrafiltrate obtained from two patients with chronic renal failure during the ultrafiltration period at the beginning of dialysis by using polysulfone membranes (F60; Fresenius, Oberursel, Germany) was concentrated 20-fold with Cuprophan membranes with a cutoff of about 5,000 d (Hemoflow E3 or E4, Fresenius). The concentrate was passed through immunoaffinity columns specific for \( \kappa \) and \( \lambda \) Ig light chains, respectively. After washing the columns with phosphate-buffered saline (PBS, pH 7.4) the corresponding Ig light chains were eluted by lowering the pH (0.2 M Glycine-HCl, pH 2.8). The fractions were neutralized immediately after elution by a 1/5 vol of 1 M Tris-HCl, pH 8.0. Protein containing fractions were identified by the bicinchoninic acid (BCA) protein assay (Pierce, Rockford, IL), pooled and concentrated by centrifugation as described above, and analyzed by nonreducing SDS-PAGE. The purity of the preparations was further checked by Western blotting. After electrophoresis, the proteins from the SDS polyacrylamide gels to a nitrocellulose membrane, the transferred proteins were detected with an antiserum recognizing specific epitopes of human Ig light chains from Nordic (Tilburg, The Netherlands), adjusted to 2 g! 10 l. Monomers and \( \kappa \) chains from Nordic (Tilburg, The Netherlands) were used at a final concentration of 1 X 106 cells/ml. Monomers and \( \lambda \) chains were isolated PMNL were obtained by this procedure was greater than 90% as determined by the trypan blue (Sigma) exclusion test.

PMNL Function Tests

Chemotaxis. The PMNL chemotaxis was determined by the agar-agarose method (9). N-formyl-methionyl-leucyl-phenylalanine methyl-ester (FMLP-M, Sigma) dissolved in Hank’s buffer with Ca\(^{2+}\) and Mg\(^{2+}\) (at a concentration of 10\(^{-6}\) M) was used as a chemoattractant. PMNL were resuspended in Hank’s buffer at a concentration of 2.5 \times 10^6 cells/\mu l. During the PMNL migration step, the agarose plates were incubated for 2 h at 37°C. Then the cells were fixed with methanol and paraformaldehyde and stained with Giemsa (Merck, Darmstadt, Germany). The distance migrated under the agarose was measured under the microscope.

To test the inhibitory activity of Ig light chains, \( \kappa \) or \( \lambda \) preparations were included in the PMNL suspension and preincubated for 20 min at room temperature before starting the assay. The final concentration of light chain dimers was adjusted to 2 \mu g/10 \mu l. Monomers and \( \kappa \) chains from Nordic (Tilburg, The Netherlands) were used at a final concentration of 1 \mu g/10 \mu l. For each type of light chain preparation, between seven and 12 separate assays were performed.

2-Deoxyglucose uptake. Hexose uptake was determined as described by McCall et al. (10). Isolated PMNL were resuspended in PBS with Ca\(^{2+}\) and Mg\(^{2+}\) at a concentration of 1 \times 10^5 cells/ml. 10 \mu l PBS or 10 \mu l FMLP-M in a final concentration of 10\(^{-7}\) M were added to 200 \mu l cell suspension and incubated for 15 min at 37°C in a shaking water bath. After addition of 100 \mu l 2-deoxy-D-[1,3-\text{H}]-glucose (Amersham) stock solution (5 \mu Ci per ml 100 \mu M D-glucose in PBS), the incubation was continued for 60 min. The uptake was stopped by the addition of 1 ml ice-cold PBS and the samples were centrifuged at 20,000 g for 20 s. The pellet was washed once with 1 ml ice-cold PBS. The supernatant was removed and the pellet together with the reaction tube (1.5 ml; Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) was put into a scintillation vial containing 10 ml Optifluor (Packard Instrument B.V.—Chemical Operations, Groningen, The Netherlands). The incorporated radioactivity was determined with a liquid scintillation counter.

In the inhibition experiments, \( \kappa \) and \( \lambda \) light chains were added to the PMNL suspension and preincubated for 15 min at 37°C. Two \mu g of dimers or 1 \mu g of the monomer preparation from the patients, or 1 \mu g of the light chains from Nordic were added to the 200 \mu l PMNL suspension. Between six and
we chemically reduced the A preparation and used the PMNL. Here again the prestimulation of the nonuremic patients inhibits the 2-DOG uptake into eboma patients (Bence Jones proteins) in our in vitro assays. The K preparation consists of a mixture of monomers (Km) and dimers (Kd), λ monomers (Lm), and λ dimers (Ld) were isolated from: (A) hemodialysis patients, (B) CAPD patients, and (C) purchased as Bence Jones proteins isolated from urine. K represents a mixture of K monomers and dimers. Mean value ± SE. * P < 0.05, ** P < 0.01.

Chemotaxis

We used the under-agarose method (9) to test the influence of Ig light chains on the chemotactic response of PMNL. We found that light chains isolated from hemodialysis patients (Figure 2A) and from patients undergoing CAPD (Figure 2B) inhibit the migration of PMNL towards FMLP-M when added to the PMNL ("cell-directed inhibition"). On the other hand, they do not influence the cell movement when added to the chemotactic factor (data not shown). Bence Jones proteins of the κ and λ type interfere with the PMNL chemotaxis as well (Figure 2C). For the λ dimer preparations, we had to use higher concentrations (fourfold, i.e., 8 μg/μL) to see the inhibitory effect (data not shown).
Figure 2. Effect of Ig light chains on the chemotactic movement of PMNL from healthy subjects toward FMLP-M. Ig light chains were obtained from: (A) hemodialysis patients, (B) CAPD patients, and (C) purchased as Bence Jones proteins isolated from urine. Mean value ± SE. * P < 0.05, ** P < 0.01. See Figure 1 for abbreviations.

Phagocytosis and Intracellular Killing

In our tests of K (N = 8) and λ (N = 14) chains from hemodialysis patients and from pooled urine samples, we do not see any effect of the light chains on phagocytosis and intracellular killing of opsonized E. coli by PMNL. Neither the percentage of phagocytosing PMNL (Figure 3A) nor of the E. coli cells taken up (Figure 3B) or being killed (Figure 3C) by PMNL is influenced by the presence of Ig light chains.

DISCUSSION

The results presented in this paper show that Ig light chains isolated from uremic patients receiving hemodialysis or undergoing CAPD have the potential to inhibit essential functions of PMNL.

One of the main problems in the health care of hemodialysis patients is their susceptibility to bacterial infections. PMNL are cells of first-line unspecific immune defense. A great amount of evidence suggests that the biochemical and functional disturbances of PMNL in uremia contribute to a large extent to the higher risk of infections in uremic patients and, as a consequence, to their increased incidence of morbidity and mortality. PMNL isolated from uremic patients have a disturbed carbohydrate metabolism (13), a reduced chemotactic activity (14), a lower intracellular ATP-level (15), and a reduced oxidative metabolism, leading to a disturbed intracellular killing (16). Furthermore, a significantly reduced cellular response of granulocytes from uremic patients to phagocytic stimuli has been described (17).

Factors that are directly or indirectly responsible for the impaired PMNL functions of uremic patients have been extensively discussed in the literature (18–25): iron overload and zinc deficiency, uremic anemia, increased levels of intracellular calcium, and the hemodialysis treatment per se have been claimed to be
which low molecular weight substances are removed by dialysis membranes or by applying CAPD suggests that molecules above 2000 d are also involved in higher molecular weight toxins by using high-flux ultrafiltration of hemodialysis patients (37,38). Fundamentally, a number of factors effect is thought to be caused by the presence of uremic toxic solutes. Recently, a number of factors have been isolated from the penitoneal effluent of CAPD patients, as well (39). Tschesche et al. (40) isolated angiojenin, a 14.4 kD protein, from the plasma ultrafiltrates of patients with uremia and showed that it has the potential to inhibit degranulation of PMNL. Vanholder et al. (41) identified p-cresol as an uremic solute capable to impair the respiratory burst activity of PMNL.

The 20 N-terminal amino acids of one of the granulocyte inhibitory proteins isolated in our laboratory, GIP I (37), show strong homology to the N-terminus of Ig light chains (80% identity to the k and 40% identity to the l light chain sequence). Furthermore, GIP I is recognized by antibodies raised against k as well as l light chains (42).

The light chains of Ig occur in two main classes, k and l, which are determined by the sequence of the carboxyterminal constant half of the 23 kDa protein. Together with the variable part of the Ig heavy chains, the aminoterminal variable half of the molecule participates in the antigen binding. Ig light chains are synthesized by B cells slightly in excess of Ig heavy chains. The extent of overproduction correlates with the B-cell differentiation (3). Immature B cells produce most of the free light chains that appear as intracellular pool and in small amounts in the serum. Before secretion, two light chains may combine and form a dimer covalently linked by a disulfide bridge (43,44). Whereas the two l chains exist as covalently linked dimers (45), about half of the k chains form dimers and only about half of them are covalently linked.

Free Ig light chains are mainly metabolized in the kidney. They are processed in a way similar to that of other low molecular weight proteins (46). While complete Ig and heavy chains are not able to pass the glomerular filtration barrier, free light chains are filtered. Then the light chains are reabsorbed by the cells of the proximal tubulis that display high-capacity, low-affinity binding sites for light chains (47). Finally they are catabolized by lysosomal enzymes (46,48,49). Therefore, only a small amount of these polyclonal light chains appears in the urine. In patients with B-cell lymphoproliferative disorders and increased production of free Ig light chains, such as multiple myeloma and related neoplastic diseases such as malignant lymphoma and leukemia, the reabsorption capacity of the proximal tubulis is exceeded, and monoclonal light chains appear as Bence Jones proteins (BJP) in the urine. BJP represent an important factor in the development of kidney failure (50).

Individual BJP show different degrees of nephrotoxicity depending on features in their primary structure (51–53). An example for a renal defect caused by BJP is the light chain deposition disease that is caused by Ig light chain precipitates on the basal membrane. In general, the Ig light chains represent a huge family of proteins with many common features such as size, sequence homologies, and defined patterns in their primary structure. On the other hand, because of
their biological function in participating in antigen recognition, the polyclonality of Ig light chains leads to proteins with a wide spectrum of characteristics. As a consequence, using isolated Ig light chains in biological assays necessarily leads to a certain variation in their effects.

Elevated levels of free light chains as a result of reduced excretion in patients with impaired kidney function have been reported by Solling (5). Whereas the concentration of free $\kappa$ and $\lambda$ light chains in the sera of healthy adults was found to be $10.5 \pm 2.9$ mg/L and $7.5 \pm 1.1$ mg/L, respectively, the sera of anephric patients had $56.3 \pm 21.7$ mg/L free $\kappa$ and $38.4$ mg/L free $\lambda$ light chains. Furthermore, Solling (5) found a negative correlation between the serum concentration of Ig light chains and the serum creatinine level. Wakasugi et al. (6) found a serum concentration of $K$ free light chains from healthy adults was found to be 10.5 mg/L and $2.9 \text{ mg/L}$ free $K$ in biobog-critical patients with chronic renal failure, and $62 \pm 21$ mg/L in uremic patients in their first year of hemodialysis treatment. The corresponding values for free $\lambda$ light chains were $18 \pm 12$, $36 \pm 10$, and $86 \pm 24$ mg/L. The discrepancy in these values and the data from Solling’s study are explained by the different methods used to measure the protein concentration (dye-binding protein assay of Bradford versus a modified Folin procedure) and to determine the light chain concentrations (nephelometric assay versus gel filtration and RIA). The data from Wakasugi et al. (6) suggest that the elevated light chain concentrations in hemodialysis patients are the result of excess Ig light chain synthesis. However, the mechanism for this increase remains to be clarified. Interestingly, Wakasugi et al. (6) also demonstrate a similar increase in $\beta_2$ microglobulin concentration in sera of hemodialysis patients. In their discussions, Solling (5) and Wakasugi et al. (6) focus on light chain deposition diseases in the kidney but do not consider the immune status of uremic patients. Also, recent publications dealing with the pathologic aspects of Ig light chains in kidney diseases (54,55) describe light chain depositions and the resulting kidney damage as a consequence of the light chain overproduction but do not make any connection between free Ig light chains and the increased susceptibility of uremic patients to infections.

One of the primary interests of our research group is the negative influence of uremic solutes on PMNL functions. In a recently developed assay based on SDS-PAGE, electrotransfer onto nitrocellulose membranes, and chemiluminescence detection (G. Cohen et al., manuscript in preparation), we detected elevated levels of free Ig light chains in the sera of uremic patients. We find free Ig light chains in the sera of healthy individuals in concentrations of $40 \text{ mg/L}$ and $38 \text{ mg/L}$ for free $K$ and free $\lambda$ chains, respectively. Uremic patients before hemodialysis treatment have $71 \text{ mg/L}$ $K$ and $91 \text{ mg/L}$ $\lambda$ light chains in their sera. Therefore, we decided to investigate the influence of Ig light chains from uremic patients on PMNL functions.

We chose three different in vitro tests to assess the inhibitory activity of our light chain isolates. The uptake and accumulation of 2-deoxy-D[1-3H]-glucose serves as a quantitative measurement of the state of activation of phagocytic cells (12). PMNL take up 2-deoxy-D-glucose by facilitated diffusion through the same sites as D-glucose (10), and afterwards both substances are phosphorylated. Whereas D-glucose-6-phosphate enters the metabolic pathways, the corresponding deoxy compound, added in trace amounts to the assay, accumulates in the cell. N-Formylmethionyl peptides are released by microorganisms and represent the main chemoattractants in bacterial infections. Therefore, we used FMLP-M as a chemoattractant and stimulant in the chemotaxis and uptake assay, respectively.

Figures 1A and 1B show that Ig light chains isolated from hemodialysis patients and from CAPD patients reduce the ability of PMNL to be activated in response to FMLP-M. We observed a certain variation in the degree of inhibition between the different light chain groups tested. As discussed above, this variation has to be expected when isolated polyclonal Ig light chains are tested in functional assays.

Moving along a chemotactic gradient and accumulating at sites of infection and injury are crucial functions of leukocytes, and involve many coordinated cellular functions (56). To test whether this first step of unspecific immune response is impaired by Ig light chains, we added the light chain preparations to PMNL from healthy donors in our chemotaxis assay. As Figure 2 shows, all $K$ and $\lambda$ Ig light chains isolated from uremic patients exert a cell-directed inhibitory effect on PMNL migration at the concentrations used in our in vitro assay. For $K$ monomers (Km) and $\lambda$ dimers (Ld), we performed range tests with light chain concentrations in the range of serum levels of healthy adults, 10 and 20 $\mu$g/mL, respectively (data not shown). We did not observe any effect on PMNL chemotaxis under those conditions. In contrast, 100 $\mu$g/mL for Km and 200 $\mu$g/mL for Ld show significant effects (Figure 2). As these numbers are only about twice the reported mean of in vivo concentrations and fall into the range of the highest reported light chain concentrations in uremic patients, we believe that the data obtained in these tests are of potential clinical relevance. This inhibition observed in the chemotaxis assays was not reversible; however, we found by the trypan blue exclusion test that the PMNL have been still viable after the assay conditions used (data not shown). When we added the Ig light chains to the chemoattractants, no inhibition was observed (data not shown). Ig light chains also do not act as chemoattractants by themselves in our assay (data not shown). Granulocytes depend on anaerobic glycolysis for the energy required for chemotaxis and phagocytosis (57). Whereas chemotaxin-induced movement provokes accelerated uptake of exogenous glucose, phagocytic
stimuli do not enhance glucose uptake. These findings are consistent with our observations that Ig light chains are able to reduce both chemotactic and glucose uptake activities of PMNL.

After PMNL reach the site of infection, they ingest bacteria by phagocytosis and destroy them by degradation with proteolytic enzymes and by the production of toxic oxygen radicals during the oxidative burst. Figure 3 shows that neither phagocytosis nor intracellular killing of E. coli by PMNL is influenced by Ig light chains even at high concentrations up to 200 mg/L. The different functions of PMNL depend on a complex network of individual signaling, each step being a potential target of modulation and inhibition. This may explain the finding that some inhibitors of one PMNL function do not influence another one.

In order to determine if the ability to inhibit PMNL activity is a general feature of Ig light chains, we used commercially available Ig light chains and Bence Jones proteins, isolated from pooled urine samples, in our in vitro assays. As shown in Figure 1C and Figure 2C, bothκ and λ-type Bence Jones proteins inhibit 2-deoxy-D-glucose uptake and chemotaxis. λ-type Bence Jones proteins that occur as dimers influence the chemotactic response of PMNL only when added at much higher concentrations. However, when we reduced this preparation, the obtained λ monomer inhibited chemotaxis to a similar degree as κ Bence Jones proteins consisting of monomers and dimers.

We found no prior reports regarding this inhibitory effect of unmodified free Ig light chains on PMNL. However, in in vivo experiments, Mimura et al. investigated the anti-inflammatory effect of chemically modified Ig light chains (58), as well as their effect on leukocyte functions (59). They showed in a rat model that Ig light and heavy chains produced from Ig light chains are able to reduce both chemotactic and hexose uptake activities of PMNL.

REFERENCES


