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Immunoglobulin light chains in uremia

GERALD COHEN

Division of Nephrology, Department of Medicine, University of Vienna, Vienna, Austria

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Background. Immunoglobulin light chains (IgLCs) are produced by B cells, slightly in excess of immunoglobulin heavy chains, and therefore are present in the serum of healthy adults in free form at low concentrations. Both the κ and λ form of these polyclonal IgLCs are mainly metabolized by the kidney and appear under normal conditions only in small amounts in the urine. In patients with a reduced or abolished kidney function, IgLC levels are increased. When overproduced in B cell lymphoproliferative disorders and deposited in the kidney, IgLCs can be, by themselves, a causative factor of renal diseases and the development of uremia.

Methods. We compared the effect of treatment with different low- and high-flux membranes on IgLC concentrations. The effect of free IgLCs on neutrophils, cells of the first-line unspecific immune defense, was assessed in in vitro experiments.

Results. We found that IgLC levels in hemodialysis and hemodiafiltration patients were higher than in pre-dialysis patients and that IgLC levels could not be brought into the normal range by currently available hemodialysis or hemodiafiltration treatments. IgLCs interfere with chemotaxis and the activation of glucose uptake, two essential neutrophil functions, and attenuate neutrophil apoptosis, the coordinated cell death that is crucial for the normal resolution of inflammation without tissue destruction.

Conclusion. IgLCs occurring at increased levels in sera of patients with kidney failure modulate essential functions and the apoptotic cell death of neutrophils, and as a consequence contribute to the increased susceptibility to bacterial infections in uremic patients.

Immunoglobulin light chains (IgLCs) of κ- and λ-type are synthesized by B cells, slightly in excess of Ig heavy chains [1]. Therefore, an intracellular pool of free IgLCs exists within the immunoglobulin producing cells. Free IgLCs are also found in human plasma as a result of a secretion parallel to the secretion of intact immunoglobulins (i.e., they originate by de novo synthesis and do not represent degradation products). In plasma of healthy people, free IgLCs exist as monomers (25 kD) and dimers (50 kD) at low concentrations and have a half-life of only 2 to 4 hours [2]. They are mainly metabolized in the kidney and processed in a manner similar to that of other low molecular-weight proteins [3]. After passing the glomerular filtration barrier, free IgLCs are reabsorbed by proximal tubular cells, involving the binding of IgLCs to cubilin [4], a giant glycoprotein receptor, and the megalin-mediated internalization of cubilin and its IgLC ligand [5]. The IgLCs are then catabolized by lysosomal enzymes. As a consequence, under normal conditions only a small amount of these polyclonal light chains appear in the urine.

Key words: hemodialysis, neutrophils, apoptosis, light-chain deposition.

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SERUM CONCENTRATIONS OF FREE IGLCS IN UREMIA

Reduced excretion in patients with renal failure leads to increased serum levels of free IgLCs [2]. Furthermore, a significant raise in serum concentrations of IgLC after the start of hemodialysis (HD) therapy has been reported [6]. Whereas there is a negative correlation between IgLC concentrations and glomerular filtration rate (GFR) [2], there is no significant increase in the concentrations of intact immunoglobulins in uremia [6]. Using a newly developed assay based on Western blotting and enhanced chemiluminescence detection, we measured IgLC levels in chronic renal failure (CRF, pre-dialysis) and dialysis patients [7]. CRF patients had elevated total (κ and λ) IgLC concentrations (106 ± 22 μg/mL) as compared with healthy controls (65 ± 6 μg/mL). In patients on HD and hemodiafiltration (HDF), the treatment values were even higher (157 ± 11 μg/mL). We also compared low- and high-flux polysulfone membranes, low- and high-flux cellulose triacetate membranes, high-flux polymethylmethacrylate (PMMA), and polyacrylonitrile membranes. Only dialysis with PMMA membranes led to a significant reduction in IgLC concentrations, presumably due to adsorption; however, these values did not reach control levels. However, the higher the IgLC concentrations were at the start of the dialysis treatment, the amount of IgLCs removed was also higher. This observation suggests the existence of two different counteracting mechanisms influencing IgLC levels during HD/HDF: the increased rate of IgLC appearance due to the dialysis treatment per se by a yet

unknown mechanism, and the IgLC removal only becoming dominant at very high IgLC concentrations. A similar situation was reported for β_2-microglobulin [8].

Therefore, currently available dialysis treatments are unable to normalize the elevated serum levels of IgLCs in end-stage renal disease (ESRD) patients. In agreement with these data, it has been recently described that neither HD nor HDF treatment with different membranes could bring the high IgLC concentration in a patient with primary amyloidosis even near normal levels, although a higher removal of IgLCs during HDF was observed [9]. Because IgLCs are part of immunoglobulins that exist in an 1000-fold excess in normal human serum, and because free IgLCs represent only one of many factors contributing to a complex mixture of organ dysfunctions in uremic patients [10], attempts to specifically remove free IgLCs seem not to be straightforward. Recently, efforts have been made to use new adsorption technologies to remove uremic retention solutes. Future studies will have to show if these approaches are able to normalize IgLC concentrations.

INFLUENCE OF FREE IGLCS ON FUNCTIONS AND APOPTOSIS OF NEUTROPHILS

Our main interest is the effect of proteins accumulating in uremic sera on the unspecific immune response, with a special focus on neutrophils, cells of the first-line unspecific immune defense. They migrate to the site of infection, attracted by chemotactic stimuli derived from bacteria or from mitochondria of damaged tissues. After the ingestion of invading bacteria by phagocytosis, neutrophils kill the microorganisms by reactive oxygen intermediate and granule constituents in a cooperative manner. Disturbed neutrophil functions play a key role in the increased risk of bacterial infections in kidney failure patients.

We tested the effect of both free polyclonal IgLCs, which have been isolated from dialysis patients and therefore exposed to the uremic milieu, and of commercially available monoclonal IgLCs (Bence Jones proteins [BJP]), originating from the urine of multiple myeloma patients on neutrophils obtained from healthy donors in in vitro assays. We found that both monoclonal and polyclonal IgLCs significantly and irreversibly inhibited the chemotactic movement of neutrophils toward a chemotactic tripeptide, fMLP, in an in vitro assay [11]. IgLCs also reduced the activation of glucose uptake of neutrophils [11], a quantitative measurement of the state of activation of phagocytic cells. In contrast, IgLCs had no influence on the phagocytic functions of neutrophils. The diminished increase in neutrophil glucose uptake upon stimulation was partly a result of the prestimulation caused by IgLCs. We also showed that IgLCs increase the basal levels of neutrophil oxidative metabolism (abstract; *Kidney Blood Press Res* 23:261, 2000). Therefore, free IgLCs contribute to the preactivation of neutrophils characteristic for the chronic state of inflammation described in uremia [12]. Interestingly, in immunoproliferative disorders, such as multiple myeloma, with an overproduction of monoclonal IgLCs, an increased risk of bacterial infections concomitant with decreased neutrophil functions has been reported [13].

Whereas essential neutrophil functions are important for a successful immune response, any discharge of neutrophil cytotoxic products into the extracellular space would lead to prolonged inflammation and, as a consequence, tissue destruction [14]. Therefore, the coordinated removal of senescent neutrophils by macrophages is crucial for the normal resolution of inflammation [15]. Apoptotic neutrophils with an undamaged cell membrane (i.e., not releasing their harmful content into the surrounding tissues) are recognized and taken up by macrophages without the release of pro-inflammatory cytokines. We tested the influence of IgLCs, commercially available and isolated from HD patients, on spontaneous neutrophil apoptosis by detecting morphologic changes under the fluorescent microscope, and DNA strand breaks and the loss of DNA content using flow cytometry (unpublished results) at 30 μg/mL, a concentration typically found for κ and λ IgLCs in the serum of healthy people, and at 100 μg/mL, a concentration measured in uremic sera. In all three apoptosis assays, we observed that IgLCs increased the percentage of viable neutrophils by inhibiting apoptosis in a concentration-dependent manner. There was no difference in the effects of κ and λ IgLCs (Fig. 1). The effect of IgLCs, which has been shown to be concentration-dependent, could be abolished by specific antibodies. Polymyxin B, a cationic peptide that inhibits endotoxin activity [16], had no influence on the IgLC effect. Therefore, the observed effect is not the result of endotoxin contamination. The presence of IgLCs led to a decrease in caspase 3 activity, indicating that IgLCs exert their effect via the modulation of the activity of caspase 3, one of the central executioner enzymes activated during apoptosis in many cell types. Genistein abolished the effect of IgLCs on neutrophil apoptosis, suggesting that tyrosine phosphorylation is essential to increase neutrophil survival. Thus far, no receptor for IgLCs on neutrophils has been characterized. However, it has been reported that IgLCs are able to interact with B-lymphocyte membranes. The reorientation of the IgLCs after binding may facilitate their interaction with integral membrane proteins, leading to the initiation of transmembrane signaling [17].

IGLC DEPOSITION IN THE KIDNEY

In patients with B-cell lymphoproliferative disorders and increased production of free IgLCs (e.g., multiple

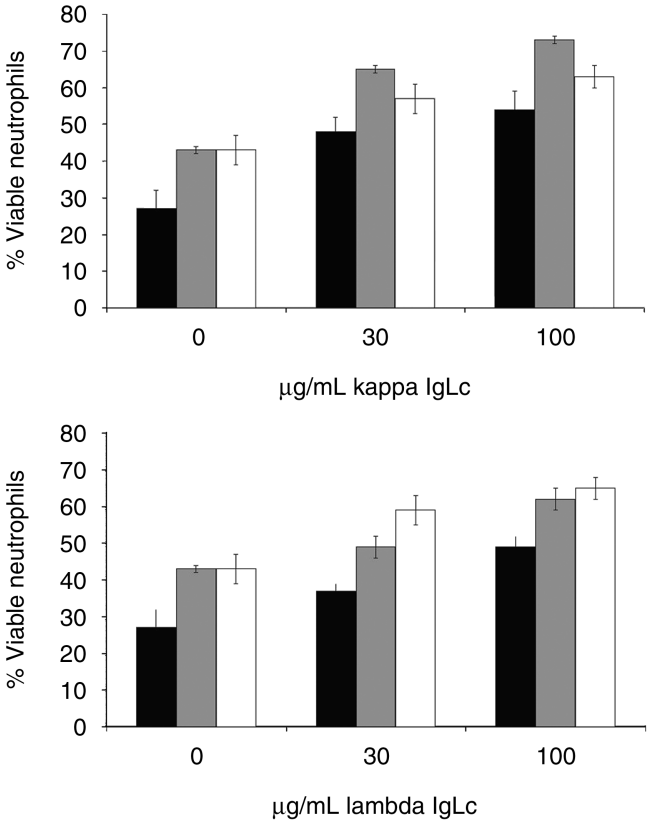

Fig. 1. IgLCs of both κ and λ type increase the percentage of viable neutrophils by attenuating apoptosis in a concentration dependent manner. Neutrophils were incubated with κ IgLCs at final concentrations of 0, 30, and 100 μg/mL (*A*), and with λ IgLCs at the same concentrations (*B*). The percentage of viable neutrophils was determined by assessing morphologic criteria under the fluorescent microscope (black bars), and by flow cytometry, to measure DNA-strand breaks (gray bars) and DNA content (white bars). Mean values ± SEM.

myeloma, malignant lymphoma, and leukemia) the reabsorption capacity of the proximal tubuli is exceeded and monoclonal light chains appear as Bence Jones proteins (BJP) in the urine. The deposition of IgLCs in the kidney can lead to glomerulopathies (AL-type amyloidosis, light chain deposition disease [LCDD]), or tubulointestinal lesions (Fanconi's syndrome, cast nephropathy), and, as a consequence, to renal insufficiency. Special structural features, such as hydrophobic residues exposed to the solvent [18] and unusual glycosylation [19], have been correlated to the nephrotoxicity, leading to LCDD and light chain amyloidosis, respectively. A reduced lysosomal acidification [20] and the inhibition of Na-K-ATPase activity [21] have been described as mechanisms involved in the nephrotoxicity of IgLCs. Furthermore, it has been shown [22] that IgLCs are able to stimulate mesangial cells to produce TGF-β, stimulating matrix synthesis, inhibiting matrix degradation, and stimulating the synthesis of receptors for matrix proteins. Renal solutes such as urea, betain, or sorbitol control the stability of IgLCs, and therefore modulate amyloid fibril formation in the kidney [23].

In conclusion, IgLCs accumulate in patients with chronic renal failure and cannot be sufficiently removed by currently available dialysis treatments. They not only disturb essential neutrophil functions, and thereby an important factor in the unspecific immune response, but modulate neutrophil apoptotic cell death. Furthermore, IgLCs can be by themselves a causative factor of renal diseases and the development of uremia when they are overproduced in B-cell lymphoproliferative disorders.

Reprint requests to Gerald Cohen, Ph.D., Medizinische Universitätsklinik III, Währinger Gürtel 18-20, Vienna, Austria.
E-mail: cohen@nephro.imed3.akh-wien.ac.at

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