Kidney International, Vol. 59, Suppl. 78 (2001), pp. S-197–S-205

Apoptosis of leukocytes: Basic concepts and implications in uremia

BERTRAND L. JABER, MIGUEL CENDOROGLO, VAIDYANATHAPURAM S. BALAKRISHNAN, MARY C. PERIANAYAGAM, ANDREW J. KING, and BRIAN J.G. PEREIRA

Division of Nephrology, Department of Medicine, Tupper Research Institute, New England Medical Center, Tufts University School of Medicine, Boston, Massachusetts, USA, and Escola Paulista de Medicina, São Paulo, Brazil

Apoptosis of leukocytes: Basic concepts and implications in uremia. Circulating blood leukocytes have short life expectancies and end their lives by committing programmed cell death or apoptosis. Apoptosis is an active form of cell death that is initiated by a number of stimuli and is intricately regulated. Apoptosis in both excessive and reduced amounts has pathological implications. Evidence suggests that apoptosis may play a role in the pathophysiology of immune dysfunction in uremia. Indeed, accelerated programmed cell death has been observed in lymphocytes, monocytes, and polymorphonuclear leukocytes among patients with chronic renal failure. This may be due in part to the retention of uremic toxins. The aim of this article is to review the evidence for accelerated leukocyte apoptosis, key regulatory apoptotic pathways, and the possible role of this highly organized process in the pathogenesis of immune dysfunction in uremia.

In the past several decades, apoptosis or programmed cell death has been the subject of intense investigation in terms of mechanism, sequence of events, biochemistry, and morphology [1–4]. In contrast to necrosis or accidental cell death, apoptosis is a programmed, active, and highly selective mechanism of cell death, allowing for the removal of cells that are redundant or excessively damaged (Fig. 1) [1]. Apoptosis is initiated by a number of different stimuli, including DNA damage, toxins, or extracellular signals (Table 1). The typical morphological changes of programmed cell death include shrinkage of the cell and the nucleus, condensation and fragmentation of the nuclear chromatin, loss of the nuclear membrane integrity, maintenance of plasma membrane integrity despite membrane blebbing (Fig. 2), and segmentation of the cell into apoptotic bodies that are rapidly ingested by neighboring phagocytic cells. One of the most specific features of apoptosis is the regular fragmentation by an endonuclease of the entire cellular DNA into an oligonucleosome-length unit of 180 base pairs [5]. Assays allowing the quantitation of apoptosis include gel electrophoresis of DNA, immunofluorescence microscopy, and flow cytometry (Fig. 3) [6].

In multicellular organisms, apoptosis is an essential component of development and cellular regulation. Abnormal regulation of apoptosis can lead to disorders such as cancer, lymphocytes depletion in AIDS, and degenerative diseases. Apoptosis in both excessive and reduced amounts has pathological implications. Consequently, control of the apoptotic mechanism may have significant therapeutic implications.

Uremia is associated with alterations in host defense mechanisms, which increase the risk of infection and malignancy. The most striking abnormalities occur in cell-mediated immunity and involve primarily T-lymphocytes. These include lymphocytopenia, impaired delayed skin reactivity, and decreased in vitro lymphocyte proliferation. Alterations in humoral immunity affect B-lymphocytes and result in a decrease in immunoglobulin levels and a depressed antibody response to antigens. Dysregulated cytokine synthesis [7] and impaired macrophage Fc receptor function [8] further impair immune function in uremic patients. Finally, polymorphonuclear leukocytes (PMNLs) exhibit impaired chemotaxis, phagocytosis, and an abnormal respiratory burst [9].

This review summarizes the evidence published to date supporting the hypothesis that the state of uremia is associated with accelerated apoptosis of leukocytes, which in turn, may contribute in part to cellular malfunction.

APOPTOTIC PATHWAYS

Regulation of the apoptotic process is complex and involves several cellular pathways [3]. The Fas (APO-1; CD95)/Fas ligand (FasL) system and members of the *bcl-2* gene family have emerged as key regulators of the apoptotic process (Fig. 4). Fas is a widely expressed

Key words: cell death, monocytes, lymphocytes, neutrophils, cytokines, toxins.

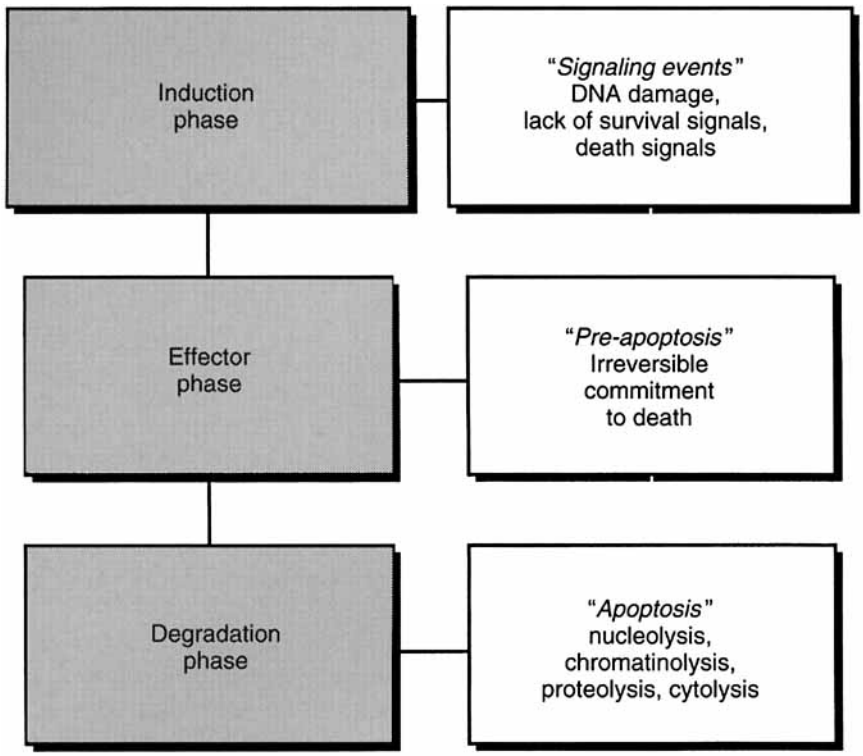

Fig. 1. Phases of programmed cell death.

45 kD type I membrane protein member of the tumor necrosis factor (TNF)/nerve growth factor family of cell surface molecules. The Fas molecule mediates apoptosis in susceptible tissues following interaction with its natural ligand, FasL, a 37 kD type II protein. In contrast to the tissue distribution of Fas, constitutive expression of FasL is relatively limited. The biological importance of the Fas/FasL system has been extensively studied in T lymphocytes, where this pathway plays a critical role in the clonal deletion of autoreactive T cells and activation-induced suicide of T cells. Cytotoxic T cells can deploy FasL as a death effector molecule in their strategies to induce killing of Fas-bearing target cells. It has also been demonstrated that PMNLs coexpress both Fas and FasL and are more susceptible to Fas-mediated apoptosis [10, 11].

The bax and bcl-2 proteins are apoptosis-related molecules that are of major importance as well. These intracellular membrane-bound proteins have opposing effects, with bcl-2 extending cellular survival and bax promoting cell death following apoptotic stimuli. The bcl-2 gene is an oncogene, originally found to be overexpressed in human follicular B-cell lymphoma. The bcl-2 protein is a 25 kD integral membrane protein that lies within the cell rather than on the cell surface. The protein is associated with mitochondria, smooth endoplasmic reticulum, and perinuclear membrane and plays a central role in the inhibition of apoptosis. The *bax* gene encodes a 21 kD protein that has extensive amino acid homology with bcl-2. The bax protein has been found in the mitochondria and perinuclear membranes, suggesting a topographic resemblance with bcl-2. The balance between pro-apoptotic and anti-apoptotic molecules, such as bax and bcl-2, respectively, has been suggested to set the cellular threshold for death following an apoptotic stimulus [12].

Central elements to various apoptotic stimuli comprise the caspase cascade. Caspases belong to a family of cystein proteases (that cleave proteins after an aspartic acid residue) that includes the interleukin-1β–converting enzyme (ICE). The caspases are probably the most important effector molecules that induce apoptosis. They are synthesized as inactive proenzymes and are activated by autocatalytic cleavage or by other proteases. Caspases are essential components of a proteolytic cascade that is triggered in response to a death stimulus and have roles in both the regulation and execution stages of apoptosis (Fig. 4). For example, in Fas-mediated apoptosis, caspase-8 has been implicated in upstream signaling events, while caspase-3 and closely related homologues appear to be involved in the effector phase of apoptosis. These molecules are believed to be responsible for cleavage of crucial homeostatic (involved in DNA repair) as well as structural (involved in both cytoskeletal and nuclear structure) proteins.

Table 1. Inducers of apoptosis

Triggering factors	Examples
DNA and protein damage	Radiation
	Reactive oxygen species
	Enzymatic damage
Growth factor/cytokine deprivation	*Hematopoietic stem cells*
	Interleukin-3
	Granulocyte macrophage colony stimulating factor (GM-CSF)
	Erythropoietin
	T lymphocytes
	Interleukin-2
	Monocytes
	Tumor necrosis factor-α
	Interleukin-1
	Polymorphonuclear leukocytes
	Granulocyte colony stimulating factor (G-CSF)
	Eosinophils
	Interleukin-5
	Endothelial cells
	Basic fibroblast growth factor
	Integrins
	Endothelin-1
Specific ligand binding	Fas-membrane bound Fas ligand (FasL) interaction
	Fas-soluble Fas ligand (sFasL) interaction

APOPTOSIS OF MONONUCLEAR CELLS IN UREMIA

Studies have shown that peripheral blood lymphocytes and monocytes obtained from uremic patients undergo accelerated apoptosis when cultured in vitro [13, 14]. Indeed, Matsumoto et al have observed increased apoptosis of T lymphocytes from both dialyzed and undialyzed patients with advanced chronic renal failure (CRF) [13]. Furthermore, in vivo, these T lymphocytes expressed Fas with higher intensity than control T cells [13], suggesting that apoptosis may be mediated by the Fas system. The authors concluded that the lymphopenia commonly observed in patients with CRF might partly be due to accelerated apoptosis of T lymphocytes. More recently, the same authors have shown that γδ receptor-bearing T lymphocytes are deleted from the peripheral circulation of patients on maintenance hemodialysis (HD) [15]. These cells are usually increased during the course of infection with various intracellular pathogens, such as *Mycobacterium tuberculosis* and *Listeria monocytogenes*, and may play a pathogenic role in cellular responses to such infections. These γδ T cells also had significantly higher levels of Fas expression compared with cells from healthy individuals. The authors concluded that peripheral deletion of this subset of T lymphocytes might be due in part to their increased susceptibility to Fas-mediated apoptosis.

Other studies by Heidenreich et al have demonstrated enhanced apoptosis of uremic monocytes cultured in vitro, and this was accompanied by decreased production of TNF-α and reduced ability to phagocytose *candida albicans* [14]. Furthermore, supplementation of monocytic cultures with exogenous TNF-α decreased apoptosis rates, suggesting that inflammatory mediators may modulate the survival of senescent monocytes [14]. More recently, we have demonstrated that freshly harvested peripheral blood mononuclear cells from patients on maintenance HD exhibit higher apoptotic rates when compared with healthy individuals (abstract; Balakrishnan et al, *J Am Soc Nephrol* 9:242A, 1998). This observation suggests that in vivo, a proportion of circulating mononuclear cells is apoptotic.

Dialysis membranes can modulate the in vitro fate of mononuclear cells. Carracedo et al have demonstrated that upon in vitro exposure to cuprophan (CU) membranes, mononuclear cells undergo accelerated apoptosis, which is greatly reduced when cells are pre-exposed to the *Pertussis* toxin, a guanyl nucleotide-binding protein (or G protein) inhibitor [16, 17]. The authors concluded that apoptotic signal transduction might be coupled to G proteins. Unfortunately, the authors failed to investigate the role of complement activation. In fact, complement activation and generation of component C5a may have had a different impact on cell survival; that is, this activation may counteract the pro-apoptogenic effect of CU.

APOPTOSIS OF POLYMORPHONUCLEAR LEUKOCYTES IN UREMIA

Constitutive and inducible apoptosis of PMNLs

In vivo, PMNLs have the shortest half-life among leukocytes [18], and when cultured in vitro, they rapidly die, featuring morphological characteristics of programmed cell death (Fig. 2). This results in the demise of greater than 50% of a population within 48 hours [3, 10]. In vivo, mature PMNLs spend approximately 12 hours in the blood stream, after which time they migrate into normal tissues or are drawn by chemotactic stimuli to inflamed tissues. There is compelling evidence to suggest that once in tissues, PMNLs undergo apoptosis and are recognized and engulfed by tissue-derived macrophages [19]. This highly organized process stands in contrast with accidental cell death or necrosis, where there is a loss of the cell membrane integrity and efflux of intracellular toxic content into host tissues.

We have previously demonstrated that PMNLs harvested from uremic patients and incubated in autologous plasma or fetal calf serum undergo accelerated apoptosis when compared with cells harvested from age- and gender-matched healthy volunteers [20]. Similar trends were also observed among freshly harvested PMNLs from uremic patients versus healthy volunteers (abstract; Balakrishnan et al, ibid), as well as among cells harvested

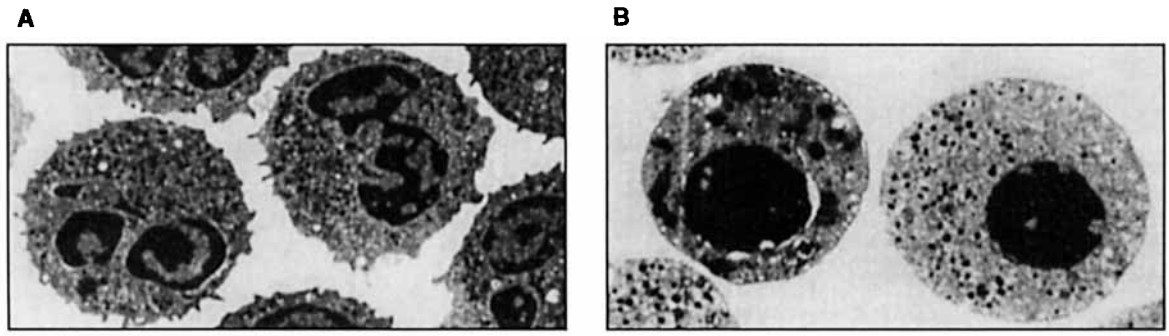

Fig. 2. Transmission electron microscopy of viable (*A*) and apoptotic (*B*) polymorphonuclear leukocytes (PMNLs). The typical features of apoptosis are seen on the right panel, consisting of chromatin condensation, loss of cytoplasmic processes, and round cell shape.

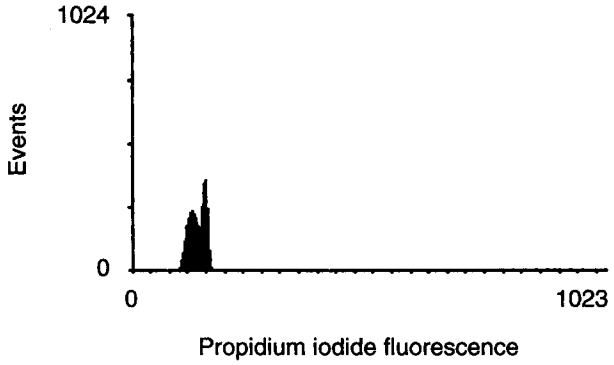

Fig. 3. Flow cytometric analysis of programmed cell death using the nuclear dye propidium iodide (PI). This DNA fluorescence histogram depicts polymorphonuclear leukocytes undergoing apoptosis. The left peak consists of hypodiploid apoptotic cells, which segregate from the right peak that represents diploid surviving cells. *x* axis = DNA content (or PI fluorescence); *y* axis = events (or number of nuclei).

from healthy volunteers and exposed to uremic versus normal plasma [20]. These data suggest that both constitutive as well as soluble factors are responsible for this accelerated cell death. However, the triggering factor(s), mechanisms, and consequences of PMNL apoptosis in uremia need to be further delineated.

PMNL apoptosis and cellular malfunction

Polymorphonuclear leukocytes undergoing apoptosis are dysfunctional [21]. This dysfunctional pattern is similar to that of uremic PMNLs, which demonstrate altered oxidative responses and impaired chemotaxis, aggregation, and phagocytosis [21]. Therefore, it is possible to speculate that "uremia-induced" apoptosis may be partly responsible for the PMNL dysfunction commonly observed in patients with CRF. We have observed that PMNLs exposed to uremic plasma not only undergo accelerated apoptosis, but also exhibit a lower ability to phagocytose bacteria and to produce superoxide in response to formyl methionyl-leucyl-phenylalanine (fMLP), a bacterial wall oligopeptide (Fig. 5) [20]. These data suggest that soluble factors present in uremic plasma induce both apoptosis and dysfunction of PMNLs. It remains to be determined, however, whether PMNL apoptosis is indeed biologically relevant in uremia and whether apoptosis accounts for the uremic dysfunction of PMNLs and to what extent. Of note, Shah et al have suggested that there is a lack of neutrophilia in response to bacterial infections among patients on maintenance HD, which may be due to accelerated apoptosis (abstract; Shah et al, *J Am Soc Nephrol* 10:594A, 1999).

In recent years, a number of uremic toxins that affect PMNL functions have been identified. These include parathyroid hormone, *p*-cresol, polyamines, aminoguanidine products, and a series of granulocyte inhibitory proteins, angiogenin, and complement factor D [22, 23]. Some of these molecules have been examined with respect to their apoptosis-inducing potential.

Polyamines are uremic retention solutes that are generated by intestinal bacteria and include spermine, spermidine, putrescine, and cadevrine [24, 25]. These compounds have been shown to inhibit in vitro hematopoiesis, possibly by apoptosis, and they may contribute to the anemia of CRF [26, 27]. Interestingly, these toxins have also been shown to attenuate PMNL apoptosis [28], but have no impact on cellular functions [29, 30]. Urea is generated during amino acid breakdown, but is a weak uremic toxin. However, it participates in the generation of cyanide and protein carbamylation. These toxic compounds lead to PMNL dysfunction [31], which may be due in part to loss of cell viability [32]. In addition, along with arginine and creatinine, urea participates in the generation of aminoguanidine compounds, such as hydroxyurea, which are toxic to different cell types and may be apoptogenic. Impaired homocysteine metabolism in CRF results in hyperhomocysteinemia. This compound is a pro-oxidant [33] and has been shown to induce apoptosis of leukocyte cell lines [34]. Finally, Cohen et al have demonstrated that glucose-modified proteins that were either generated in vitro or isolated from peritoneal

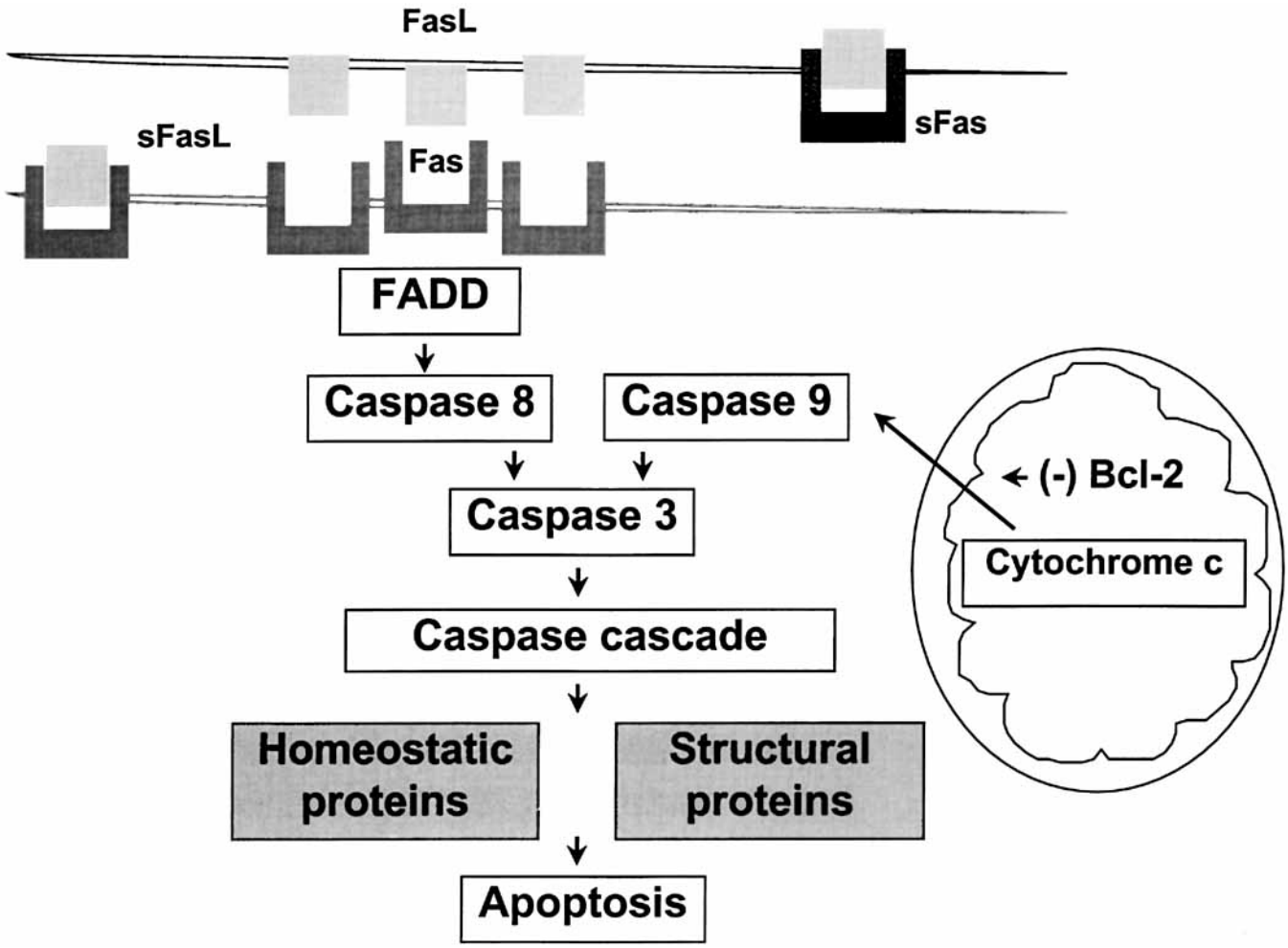

Fig. 4. Simplified scheme of the Fas/Fas ligand system. Abbreviations are: FADD, Fas-associated death domain; FasL, Fas ligand; sFasL, soluble Fas ligand.

dialysate effluents also enhance in vitro apoptosis of PMNLs (abstract; Cohen et al, *J Am Soc Nephrol* 10:509A, 1999). In summary, these data provide evidence for the retention of apoptogenic molecules in the serum of patients with CRF. Further studies are needed to explore the triggering mechanisms and apoptotic signaling pathways.

Modulation of PMNL apoptosis by dialysis membranes and peritoneal dialysis fluids

The role of soluble factors in regulating PMNL apoptosis is indicated by the observation that the life span and functional activity of mature PMNLs can be extended in vitro by incubation with either cytokines [granulocyte colony-stimulating factor, interleukin-2 (IL-2), interleukin-1β or TNF-α], glucocorticoids or complement component C5a [35–37]. These factors appear to inhibit apoptosis. In contrast, IL-10, an anti-inflammatory cytokine, promotes PMNL apoptosis [38]. The generation of complement components and proinflammatory cytokines during dialysis such as C5a, TNF-α, and IL-1β varies between different dialysis membranes and may, therefore, have a different impact on the fate of circulating PMNLs. We have demonstrated that during dialysis, the apoptosis-inducing activity of uremic plasma is modulated by the use of dialyzers with different degrees of biocompatibility [39]. Indeed, compared with PMNLs harvested from healthy volunteers and exposed to predialysis uremic plasma samples, a significantly lower proportion of apoptosis was observed in PMNLs exposed to 15-minute plasma samples obtained from patients dialyzed with CU, but not with cellulose triacetate (CTA) or polysulfone (PS) dialyzers (Fig. 6). Furthermore, there was a significant correlation between PMNL apoptosis and plasma levels of TNF-α and IL-10 [39]. Rosenkranz et al have recently reported on the direct in vitro impact of CU and PS membranes on PMNL survival, in the presence of serum (abstract; Rosenkranz

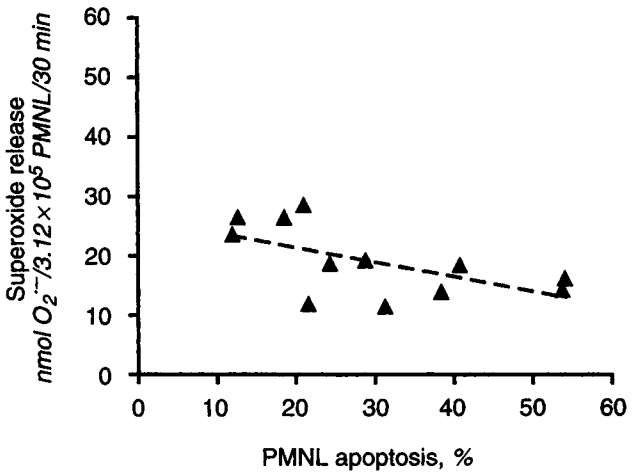

Fig. 5. Inverse correlation between polymorphonuclear leukocyte (PMNL) apoptosis and formyl-methionyl-leucyl-phenylalanine (fMLP)-stimulated superoxide production ($r = -0.60$, $P = 0.04$). Data are from Cendoroglo et al [20].

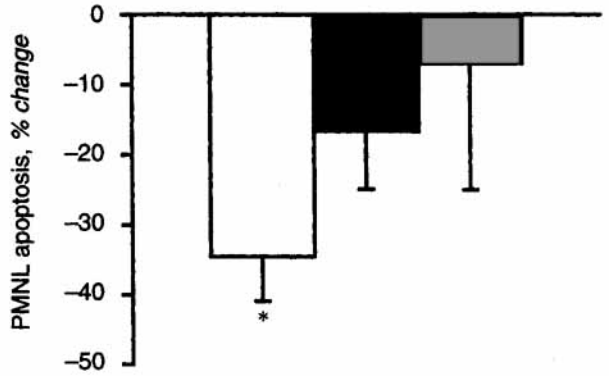

Fig. 6. Effect of uremic plasma collected during HD on PMNL apoptosis. PMNLs harvested from healthy volunteers were incubated with uremic plasma collected from patients at 15 minutes of dialysis with cuprophan (CU; $N = 8$; □), cellulose triacetate (CTA; $N = 8$; ■), or polysulfone (PS; $N = 8$; ■) dialyzers. *$P < 0.001$ compared with plasma obtained prior to dialysis in the CU group. Data are from Jaber et al [39].

et al, *J Am Soc Nephrol* 10:301A, 1999). Apoptosis was most prominent in cells incubated with CU. These findings are in agreement with those by Carracedo et al [17]. Although the authors used serum in their experiments, they failed to investigate the role of complement. It remains to be determined whether in vivo, direct contact with the dialysis membrane is a stronger determinant of the fate of PMNLs than activated soluble factors, which can result in the generation of pro-apoptogenic and anti-apoptogenic molecules.

It can be proposed that the apoptosis-inducing activity of uremic plasma depends on a balance between "death" and "survival" factors. "Death" factors consist of anti-inflammatory molecules such as IL-10 and other yet to be identified uremic toxin(s). On the other hand, "survival" factors consist of proinflammatory molecules generated during dialysis, including C5a, TNF-α, IL-1β, and lipopolysaccharide, a potential dialysate contamination. The fact that uremic plasma retains its apoptotic-inducing potential at the end of dialysis suggests that the apoptosis-inducing molecules are not significantly cleared by dialysis or that their removal may be counterbalanced by the release of apoptosis-inducing factors as a consequence of blood–membrane interactions [39]. Furthermore, heat inactivation of uremic plasma does not abrogate its apoptosis-inducing activity, suggesting that the apoptosis-inducing factors are heat resistant [20]. Tumor necrosis factor-α has been variably reported to either induce, promote, or have no effect on PMNL apoptosis. In fact, recent studies suggest that TNF-α may have a pro-apoptotic effect that is concentration dependent, and which is abolished by TNF-α neutralizing antibodies [40]. In addition, soluble TNF receptors appear to facilitate TNF-α–induced cell death [40]. It is likely that the large number of mediators released during dialysis offset the effects of each other, particularly since soluble TNF receptors are increased as well in dialysis patients [41]. Consequently, taken together, all of these findings provide more complexity for the interactive effect of these soluble factors on the fate of PMNLs.

Studies have shown that conventional peritoneal dialysis fluids adversely affect PMNL function. This dysfunction has been attributed in part to the nonphysiological components of PD fluids, including lactate [42], osmolality [43], and glucose degradation products (GDPs) generated using heat sterilization of the dialysate solutions [44]. In an effort to examine the impact of these solutions on PMNL survival, we have demonstrated that heat sterilization of high glucose-containing, pH-equilibrated PD fluids were predominantly associated with PMNL necrosis, whereas solutions enriched with effective osmolytes such as mannitol or amino acids resulted in cell apoptosis [45]. Similar trends have been observed with monocytes [46]. In summary, the mechanisms involved in PMNL apoptosis induced by various osmolytes merit further investigation.

Oxidative stress and susceptibility of PMNL to apoptosis

Superoxide is a free radical that is derived from molecular oxygen by the addition of a single electron [47]. Reactions that produce superoxide biologically occur under a broad spectrum of physiological and pathological circumstances, including all infectious and inflammatory diseases, as well as in disease processes that involve ischemia and reperfusion. As a component of the bacterial armamentarium, PMNLs possess a reduced nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase that produces superoxide radical when the cell is activated.

An electron transfer from NADPH to molecular oxygen generates superoxide anion, which is rapidly dismutated through superoxide dismutase (SOD) to form hydrogen peroxide. Hydrogen peroxide, in turn, reacts with chloride to generate hypochloric acid. This reaction is promoted by myeloperoxidase (MPO), an enzyme of the PMNL azurophilic granules, but is of most importance in the extracellular space, following cellular degranulation. Hydrogen peroxide is usually detoxified by catalase or glutathione peroxidase. The direct interaction between superoxide and hydrogen peroxide can lead to the generation of highly reactive hydroxyl radicals. This reaction, however, is very slow as compared with competing reactions such as the spontaneous dismutation of superoxide. Rather, a metal-ion–dependent pathway, known as the Haber-Weiss reaction, is responsible for the generation of hydroxyl radicals. This notorious free radical, in turn, indiscriminately tears electrons from bystander molecules to make up for this deficiency. Usually, an initial event generates free radicals, and propagation steps repeated many times perpetuate them. This free radical reaction tends to "snowball" unless held back by antioxidant defenses. The targets of free radicals and ROS include membrane polyunsaturated fatty acids, lipoproteins, proteins, and DNA. Although the consequences are often subtle, damage to membrane receptor proteins may later affect cellular regulatory mechanisms such as signal transduction and cell survival.

Several observations have provided evidence for the presence of oxidative stress in patients with CRF, particularly those on HD [48]. It is multifactorial in origin and is due in part to an increased production of pro-oxidants, mainly ROS generated by activated PMNLs, and improper antioxidant defense mechanisms. Oxidative stress plays an important role in the pathogenesis of a variety of biological processes, including apoptosis. Galli et al have recently described how abnormal apoptosis of peripheral blood leukocytes harvested from uremic patients is associated with oxidative stress, as measured by intracellular thiol depletion [49]. Studies by Kettritz et al suggest that superoxide release is required for both spontaneous and fMLP-mediated PMNL apoptosis [50]. This process is markedly inhibited by reduction of intracellular levels of hydrogen peroxide or hydroxyl radicals, using catalase or desferroxamine, respectively [51, 52]. It has been speculated that these endogenous oxidative products may regulate caspase activity, the mechanism of which remains speculative [53].

Since the mitochondria is the main cellular source of superoxide and other ROS, it may play a crucial role in regulating programmed cell death. Indeed, cytochrome *c*, a mitochondrial heme protein that is primarily involved in electron chain transport, can leak out into the cytosol, where it combines with a putative ICE-like protease [54]. This complex activates caspase-9, which in turn activates caspase-3, the effector death molecule (Fig. 4) [54]. It remains to be elucidated what factor(s) results in the breakdown of the outer membrane of mitochondria, allowing the release of cytochrome *c* into the cytosol. We speculate that superoxide metabolites, in particular, hydroxyl radicals, result in lipid peroxidation of the outer membrane of mitochondria, with consequent increased membrane permeability and leakage of cytochrome *c* into the cytosol. There is compelling evidence to suggest that mitochondrial-associated bcl-2 may regulate apoptosis, at least in part, by attenuating oxidative stress or by modulating the caspase cascade. This is supported by Hockenbery et al, who suggest that bcl-2 blocks lipid membrane peroxidation, and that cells overexpressing bcl-2 still generate peroxides, but do not damage their cellular constituents including membrane lipids [55]. This implies that bcl-2 may exert an antioxidant effect primarily as a free radical scavenger.

Unfortunately, bcl-2 is poorly expressed in mature PMNLs. Since PMNLs are a major source of free radicals, the biochemical risk of excessive superoxide production and its metabolites at the mitochondrial level could result in accelerated apoptosis, when left unopposed by the antioxidant property of bcl-2. This may be operating in PMNLs harvested from uremic patients, where cells have been shown to be primed by the uremic environment, which results in a basal increased production of ROS [56, 57]. Of note, Buemi et al have recently observed that the bcl-2 protein blood concentration is reduced in patients undergoing HD [58]. All of these data implicate oxidative stress as a significant mediator of apoptosis, particularly in professional phagocytes such as PMNLs where ROS play a crucial role in the antibacterial armamentarium.

CONCLUSION

In summary, dysregulation of apoptosis has clearly been demonstrated in leukocytes harvested from patients with CRF. More importantly, this highly selective form of cell death may play a significant role in the pathogenesis of immune dysfunction in uremia. Consequently, a better understanding of the mediation of these events and a better knowledge of the regulatory pathways will further our understanding of the pathophysiology of the acquired immune deficiency of uremia and help tailor therapeutic strategies to some of the important regulatory mechanisms of apoptosis.

ACKNOWLEDGMENTS

This article was supported by a grant (DK 45609) from the National Institutes of Health (to Dr. Pereira), the Baxter Extramural Grant Program (to Dr. Jaber), the Paul Teschan Research Fund, Dialysis Clinic, Inc. (to Dr. Jaber), and the Brazilian National Council for Research (to Dr. Cendoroglo).

Reprint requests to Bertrand L. Jaber, M.D., Division of Nephrology, New England Medical Center, 750 Washington Street, Box 391, Boston, Massachusetts 02111, USA.
E-mail: bjaber@lifespan.org

REFERENCES

1. Kerr JFR, Wyllie AH, Currie AR: Apoptosis: A basic biologic phenomenon with wide-ranging implications in tissue kinetics. *Br J Cancer* 26:239–257, 1972
2. Carson DA, Ribeiro JM: Apoptosis and disease. *Lancet* 341:1251–1254, 1993
3. Squier MKT, Sehnert AJ, Cohen JJ: Apoptosis in leukocytes. *J Leukoc Biol* 57:2–10, 1995
4. Rowan S, Fisher DE: Mechanisms of apoptotic cell death. *Leukemia* 11:457–465, 1997
5. Willie AH: Glucocorticoid-induced thymocyte apoptosis is associated with endogenous endonuclease activation. *Nature* 284:555–556, 1980
6. Cotter TG, Martin SJ: *Techniques in Apoptosis: A User's Guide.* London, Portland Press Ltd., 1996
7. Dinarello C: Cytokines: Agents provocateurs in hemodialysis? *Kidney Int* 41:683–694, 1992
8. Ruiz P, Gomez F, Schreiber A: Impaired function of macrophage Fc gamma receptors in end-stage renal disease. *N Engl J Med* 322:717–722, 1990
9. Lewis SL, van Epps DE: Neutrophil and monocyte alterations in chronic dialysis patients. *Am J Kidney Dis* 9:381–395, 1987
10. Liles WC, Kiener PA, Ledbetter JA, Aruffo A, Klebanoff SJ: Differential expression of Fas (CD95) and Fas ligand on normal human phagocytes: Implications for the regulation of apoptosis in neutrophils. *J Exp Med* 184:429–440, 1996
11. Hsieh S-C, Huang M-H, Tsai C-Y, Tsai Y-Y, Tsai S-T, Sun K-H, Yu H-S, Yu C-L: The expression of genes modulating programmed cell death in normal human polymorphonuclear neutrophils. *Biochem Biophys Res Commun* 233:700–706, 1997
12. Yang E, Korsmeyer SJ: Molecular thanatopsis: A discourse on the BCL2 family and cell death. *Blood* 88:386–401, 1996
13. Matsumoto Y, Shinzato T, Amano I, Takai I, Kimura Y, Morita H, Miwa M, Nakane K, Yoshikai Y, Maeda K: Relationship between susceptibility to apoptosis and Fas expression in peripheral blood T cells from uremic patients: A possible mechanism for lymphopenia in chronic renal failure. *Biochem Biophys Res Commun* 215:98–105, 1995
14. Heidenreich S, Schmidt M, Bachmann J, Harrach B: Apoptosis of monocytes cultured from long-term hemodialysis patients. *Kidney Int* 49:792–799, 1996
15. Matsumoto Y, Shinzato T, Takai I, Nishimoto A, Nakai S, Sakai T, Amano I, Maeda K: Peripheral deletion of gammadelta T cells in haemodialysis patients. *Nephrol Dial Transplant* 13:2861–2866, 1998
16. Carracedo J, Ramirez R, Pintado O, Gomez-Villamandos JC, Martin-Malo A, Rodriguez M, Aljama P: Cell aggregation and apoptosis induced by hemodialysis membranes. *J Am Soc Nephrol* 6:1586–1591, 1995
17. Carracedo J, Ramirez R, Martin-Malo A, Rodriguez M, Aljama P: Nonbiocompatible hemodialysis membranes induce apoptosis in mononuclear cells: The role of G-proteins. *J Am Soc Nephrol* 9:46–53, 1998
18. Sendo F, Tsuchida H, Takeda Y, Gon S, Takei H, Kato T, Hachiya O, Watanabe H: Regulation of neutrophil apoptosis: Its biological significance in inflammation and the immune response. *Hum Cell* 9:215–222, 1996
19. Savill J: Apoptosis in resolution of inflammation. *J Leukoc Biol* 61:375–380, 1997
20. Cendoroglo M, Jaber BL, Balakrishnan VS, Perianayagam MC, King AJ, Pereira BJG: Neutrophil apoptosis and dysfunction in uremia. *J Am Soc Nephrol* 10:93–100, 1999
21. Whyte MK, Meagher LC, Macdermot J, Haslett C: Impairment of function in aging neutrophils is associated with apoptosis. *J Immunol* 150:5124–5134, 1993
22. Hörl WH: Neutrophil function and infections in uremia. *Am J Kidney Dis* 33:45–48, 1999
23. De Smet R, Vanholder R: Pathophysiologic effects of uremic retention solutes. *J Am Soc Nephrol* 10:1815–1823, 1999
24. Takagi T, Chung TG, Saito A: Determination of polyamines in hydrolysates of uremic plasma by high-performance cation-exchange column chromatography. *J Chromatogr* 272:279–285, 1983
25. Saito A, Takagi T, Chung TG, Ohta K: Serum levels of polyamines in patients with chronic renal failure. *Kidney Int* 24(Suppl 16):S234–S237, 1983
26. Segal GM, Stueve T, Adamson JW: Spermine and spermidine are non-specific inhibitors of in vitro hematopoiesis. *Kidney Int* 31:72–76, 1987
27. Kushner D, Beckman B, Nguyen L, Chen S, Della Santina C, Husserl F, Rice J, Fisher JW: Polyamines in the anemia of end-stage renal disease. *Kidney Int* 39:725–732, 1991
28. Ratasirayakorn W, Leone P, Leblebicioglu B, Walters JD: Polyamines found in the inflamed periodontium inhibit priming and apoptosis in human polymorphonuclear leukocytes. *J Periodontol* 70:179–184, 1999
29. Stabellini G, Spisani S, Fiocchi O, Stabellini N, Carletti R, Altobelli A, Farinelli A: Medium molecular weight uremic toxins and endogenous polyamines: Behavior of polymorphonuclear leukocyte chemotaxis with respect to chromatographic peaks of dialysate and standard polyamines. *Boll Soc Ital Biol Sper* 58:1418–1424, 1982
30. Farinelli A, Fiocchi O, Stabellini G, Stabellini N, Marangoni C, Spisani S: Effect on leukocyte locomotion and superoxide production by uremic toxins and polyamines. *Int J Artif Organs* 10:37–40, 1987
31. Kraus LM, Elberger AJ, Handorf CR, Pabstract MJ, Kraus AP Jr: Urea-derived cyanate forms epsilon-amino-carbamoyl-lysine (homocitrulline) in leukocyte proteins in patients with end-stage renal disease on peritoneal dialysis. *J Lab Clin Med* 123:882–891, 1994
32. Hiroi M, Tajima M, Shimojima T, Kashimata M, Miyata T, Sakagami H: Re-evaluation of the culture condition of polymorphonuclear cells for the study of apoptosis induction. *Anticancer Res* 18:1813–1818, 1998
33. Endresen PC, Prytz PS, Lysne S, Aarbakke J: Homocysteine increases the relative number of apoptotic cells and reduces the relative number of apoptotic bodies in HL-60 cells treated with 3-deazaadenosine. *J Pharmacol Exp Ther* 269:1245–1253, 1994
34. Ratter F, Germer M, Fischbach T, Schulze-Osthoff K, Peter Me Droge W, Krammer Ph, Lehmann V: S-adenosyl homocysteine as a physiological modulator of Apo-1-mediated apoptosis. *Int Immunol* 8:1139–1147, 1996
35. Colotta F, Re F, Polentarutti N, Sozzani S, Mantovani A: Modulation of granulocyte survival and programmed cell death by cytokines and bacterial products. *Blood* 80:2012–2020, 1992
36. Lee A, Whyte MKB, Haslett C: Inhibition of apoptosis and prolongation of neutrophil functional longevity by inflammatory mediators. *J Leukoc Biol* 54:283–288, 1993
37. Liles WC, Klebanoff SJ: Regulation of apoptosis in neutrophils-Fas track to death? *J Immunol* 155:3289–3291, 1995
38. Cox G: IL-10 enhances resolution of pulmonary inflammation in vivo by promoting apoptosis of neutrophils. *Am J Physiol* 271: L566–L571, 1996
39. Jaber BB, Balakrishnan VS, Cendoroglo M, Perianayagam M, King AJ, Pereira BJG: Apoptosis-inducing activity of uremic plasma during hemodialysis. *Blood Purif* 16:325–335, 1998
40. Murray J, Barbara JA, Dunkley SA, Lopez AF, Van Ostade X, Condliffe AM Dransfield I, Haslett C, Chilvers ER: Regulation of neutrophil apoptosis by tumor necrosis factor alpha: Requirement for TNFR55 and TNFR75 for induction of apoptosis. *Blood* 90:2772–2783, 1997
41. Ward RA, Gordon L: Soluble tumor necrosis factor receptors are increased in hemodialysis patients. *ASAIO J* 39:M782–M786, 1993
42. Schambye HT, Pedersen FB, Christensen HK, Berthelsen H, Wang P: The cytotoxicity of continuous ambulatory peritoneal dialysis solutions with different bicarbonate/lactate ratios. *Perit Dial Int* 13:S116–S118, 1993
43. Liberek T, Topley N, Jorres A, Coles GA, Gahl GM, Williams

JD: Peritoneal dialysis fluid inhibition of phagocyte function: Effects of osmolality and glucose concentration. *J Am Soc Nephrol* 3:1508–1515, 1993
44. Nilsson-Thorell CB, Muscalu N, Andren AHG, Kjellstrand PTT, Wieslander AP: Heat sterilization for peritoneal dialysis gives rise to aldehydes. *Perit Dial Int* 1993:208–213, 1993
45. Cendoroglo M, Sundaram S, Groves C, Ucci AA, Jaber BL, Pereira BL: Necrosis and apoptosis of polymorphonuclear cells exposed to peritoneal dialysis fluids *in vitro*. *Kidney Int* 52:1626–1634, 1997
46. Plum J, Lordnejad MR, Grabensee B: Effect of alternative peritoneal dialysis solutions on cell viability, apoptosis/necrosis and cytokine expression in human monocytes. *Kidney Int* 54:224–235, 1998
47. Klebanoff SJ: Oxygen metabolism and the toxic properties of phagocytes. *Ann Intern Med* 93:480–489, 1980
48. Galli F, Canestrari F, Buoncristiani U: Biological effects of oxidant stress in haemodialysis: The possible roles of vitamin E. *Blood Purif* 17:79–94, 1999
49. Galli F, Rovidati S, Chiarantini L, Kulurianu H, Canestrari F, Buoncristiani U: Bioreactivity and biocompatibility of a vitamin E-modified multi-layer hemodialysis filter. *Kidney Int* 54:580–589, 1998
50. Kettritz R, Falk RJ, Jennette JC, Gaido ML: Neutrophil superoxide release is required for spontaneous and FMLP-mediated but not for TNF alpha-mediated apoptosis. *J Am Soc Nephrol* 8:1091–1100, 1997
51. Kasahara Y, Iwai K, Yashie A, Ohta K, Konno A, Seki H, Miyawaki T, Tanigushi N: Involvement of reactive oxygen intermediates in spontaneous and CD95 (Fas/APO-1)-mediated apoptosis in neutrophils. *Blood* 89:1748–1753, 1997
52. Rollet-Labelle E, Grange M-J, Elbim C, Marquetty C, Gougerot-Pocidalo M-A, Pasquier C: Hydroxyl radical as a potential intracellular mediator of polymorphonuclear neutrophil apoptosis. *Free Radic Biol Med* 24:563–572, 1998
53. Fadeel B, Ahlin A, Henter J-I, Orrenius S, Hampton MB: Involvement of caspases in neutrophil apoptosis: Regulation by reactive oxygen species. *Blood* 92:4808–4818, 1998
54. Skulachev VP: Cytochrome c in the apoptotic and antioxidant cascades. *FEBS Lett* 423:275–280, 1998
55. Hockenbery DM, Oltvai ZN, Yin X-M, Milliman CL, Korsmeyer SJ: Bcl-2 functions in an antioxidant pathway to prevent apoptosis. *Cell* 75:241–251, 1993
56. Ward RA, McLeish KR: Polymorphonuclear leukocyte oxidative burst is enhanced in patients with chronic renal insufficiency. *J Am Soc Nephrol* 5:1697–1702, 1995
57. Klein JB, Mcleish KR, Ward RA: Transplantation, not dialysis, corrects azotemia-dependent priming of the neutrophil oxidative burst. *Am J Kidney Dis* 33:483–491, 1999
58. Buemi M, Allegra A, Corica F, Ruello M, Di Pasquale G, Aloisi C, Giaccobe MS, Romeo A, Frisina N: Reduced blood bcl-2 protein concentration in patients on hemodialysis. *Blood Purif* 16:312–316, 1998