$CD4^+/FOXP3^+$ Regulatory T Cells in End-Stage Kidney Disease: Molecular Pathways Trough Cell-Cycle Arrest and Apoptosis

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Abstract: $CD4^+/FOXP3^+$ regulatory T cells (Tregs) are essential for the maintenance of self-tolerance, and Tregs deficiency results in spontaneous autoimmunity in both mice and humans. The forkhead box P3 (FOXP3) expression is required for both survival of Tregs precursors as well as their function. This suggests that Tregs may use multiple mechanisms to limit autoimmunity, and may reflect functional heterogeneity among Tregs subsets that localize to distinct tissue environments. Both cell contact- and cytokine-based immunosuppressive mechanisms would require that Tregs be in close proximity to their targets. The fundamental regulatory activity that can be consistently demonstrated by Tregs *in vivo* and *in vitro* has stimulated great interest in developing novel strategies for treating ongoing inflammatory conditions. Patients with end-stage kidney disease (ESKD) are known to display a cellular immune dysfunction. Uremic solutes that accumulate during ESKD may be involved in these processes. In these patients, oxidative stress induced by oxidized LDL (oxLDL) may increase Tregs sensitivity to Fas-mediated apoptosis in part as a consequence of 26S proteasome activation. The 26S proteasome, an ATP-dependent multisubunit protease complex found in the cytoplasm and in the nucleus of all eukaryotic cells, constitutes the central proteolytic machinery of the ubiquitin/proteasome system. Considering the effect of uremia and oxLDL, Tregs from patients with ESKD exhibit early cell-cycle arrest and become apoptotic. These phenomena are the consequence of the oxLDL inhibited proteasome proteolytic activity of $p27^{Kip1}$ and Bax proteins in Tregs. This may be one mechanistic explanation of the cellular immune dysfunction in patients with ESKD, and may have important implications in clinics, since this response could contribute to the micro-inflammation and atherogenesis encountered in this population.

INTRODUCTION

Alterations of the immune system in patients with end-stage kidney disease (ESKD) represent a complex issue. On one hand, hypercytokinemia is a typical feature of uremia, likely due to accumulation of pro-inflammatory cytokines as a consequence of decreased renal elimination and/or increased generation following induction by uremic toxins, oxidative stress and volume overload [1, 2]. On the other hand, uremia is associated with immunosuppresion due to the impact of the uremic milieu and a variety of associated disorders exerted on immunocompetent cells.

The increased rate of infections, together with an impaired response to vaccination and a common failure of tuberculin skin test to diagnose latent tuberculosis indicate that the adaptive immunity is weakened in the ESKD population [3]. Studies performed *in vitro* show that T-cell proliferation is decreased in the uremic milieu [4, 5]. As mentioned, T helper lymphocytes (Th) play a crucial role in controlling the immune response. Th1 cells, through interferon gamma (IFN-γ) production, regulate antigen presentation and immunity against intracellular pathogens, whereas Th2 cells, which produce interleukin-4 (IL-4), IL-5, and IL-13, mediate certain humoral responses and immunity against parasites [2, 6]. In patients with ESKD, the maturation of both subsets of Th cells is impaired compared with controls. Although the maturation of Th lymphocytes is sustained, ESKD patients present with significantly elevated Th1 levels, leading to an increased Th1/Th2 ratio [6, 7].

The spectrum of $CD4^+/FOXP3^+$ regulatory T cells (Tregs) capable of mediating dominant suppression is expanding to include both naturally arising and inducible subsets. In addition to their expression of CD4 and CD25, these cells are anergic to proliferative responses *in vitro* and do not express key cytokines, including IL-2 or IFN-γ in response to stimulation [8, 9]. Functionally, they are characterized by the capacity to suppress proliferation of effector T cells *in vitro* in a process requiring activation and cell contact but not IL-4, IL-10, or transforming growth factor-β (TGF-β) [10].

Patients with ESKD chronically hemodialyzed present changes not only in T cell immunity but also in lipid profile [11, 12]. Apart from their immune function, circulating T cells may actively participate to atherogenesis, and treatments aimed at reducing T cell activation and apoptosis in patients with ESKD reduce the risk of developing cardiovascular disease [13]. Evidence exists that patients on chronic hemodialysis (HD) are exposed to enhanced oxidative stress that is initiated by the generation of oxygen free radicals, mainly in tissue and probably in the circulation. The most potent O_2-generating proteins are oxidatively modified lipoproteins, mainly oxidized low-density lipoproteins (oxLDL)

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[14]. The pathophysiological relevance of oxLDL-induced Tregs immunodeficiency and pro-atherogenic effect in HD patients remains uncertain, but may be one explanation of the immune dysfunction, illustrated by the high rate of bacterial infections and impaired vaccine response seen in these patients, and may be suggested as a contributor of the micro-inflammation seen in the atherogenesis frequently encountered in patients with ESKD and especially in chronic HD patients [15].

PATHOBIOLOGY OF CD4$^+$/FOXP3$^+$ REGULATORY T CELLS

CD4$^+$/FOXP3$^+$ regulatory T cells seem to represent the resurrection of the old suppressor T cells. While most basic knowledge about these cells is derived from animal studies, the recent identification of these cells in humans has further attributed to their characterization by *in vitro* analysis. Results obtained have led to broad speculations about therapeutic potential by interference with these regulatory T cells. These cells are characterized by the expression of CD25 and the forkhead-family transcription factor FOXP3 (forkhead box P3), and they have the capacity to suppress the activation of other T cells in a contact-dependent manner [16-19]. Furthermore, FOXP3 can inhibit activation-induced expression of *IL2* by T cells. However, FOXP3 can target genes other than cytokine genes or genes that are regulated by nuclear factor of activated T cells (NFAT).

CD4$^+$/FOXP3$^+$ regulatory T cells constitute approximately 7-10% of peripheral CD4$^+$ T cells in humans and mice and can suppress T cell function both *in vitro* and *in vivo*. They appear to influence immune responses to self antigens, tumors, and pathogenic organisms. Research mainly from *in vitro* studies has revealed that Tregs can exert suppressive effects against multiple cell types involved in immunity and inflammation [19-21]. These include the induction as well as the effector and memory function of CD4$^+$ and CD8$^+$ T cells, the inhibition of proliferation, immunoglobulin production and class switching of B cells, the inhibition of NK and NK T-cell cytotoxicity, the function and maturation of dendritic cells, as well as effects on the function and survival of neutrophils (Fig. **1**).

CD4$^+$/FOXP3$^+$ regulatory T cells have a specific response to T cell receptor (TCR) engagement. Several studies have shown that Tregs isolated both from humans and from rodents do not proliferate when appropriately activated [22, 23]. They also do not produce cytokines such as IL-2, IL-4 and IFN-γ, as well as other effector molecules such as TNF and TNF-receptor-family members. However, Tregs are not completely unresponsive to TCR-mediated signals, as TCR engagement is required for their ability to suppress the activation of responder T cells.

In addition to making a start at identifying FOXP3-target genes, the precise role of FOXP3 in establishing the Treg-cell differentiation programme is also being worked out. Several recent papers have addressed the role of FOXP3 in Tregs development and function in the thymus and in the periphery. Two groups, Gavin *et al*. and Lin *et al*., generated knock-in mice in which the gene encoding green fluorescent protein (GFP) or enhanced GFP (EGFP) was fused in frame into the *Foxp3* endogenous locus resulting in a fluorescent non-functional Foxp3 protein (referred to here as *Foxp3*^{GFP} knock-in mice) [24, 25]. These studies indicate that Foxp3 is required for the suppressive functions of Tregs, as well as for their anergic state, but there are other factors that have important roles in Tregs development.

FORKHEAD BOX P3 AND T CELL RECEPTOR SIGNALING IN TREGS

CD4$^+$ effector T cells undergo a stereotypical activation programme after engagement of their TCR and appropriate co-stimulation. This programme consists of the activation of specific signaling pathways that result in the induction of effector functions, including the production of IL-2 [26, 27]. It is suggested that generation of Tregs may require higher affinity interaction between the agonist peptides/MHC II and TCR within the thymus in contrast to the process of conventional CD4$^+$ T cells production [28]. Support for this notion has been provided by analyzing Tregs development in mice expressing a transgenic TCR and its cognate ligand in the thymus. Recent studies show that TCR transgenic CD4$^+$ T cells can adopt the regulatory cell phenotype with a higher frequency when they encounter their cognate antigen in the thymus. Based on these observations, engagement of transgenic TCR by a high-affinity self-ligand is expected to initiate signaling cascades that ultimately induce FOXP3 expression and commit thymocytes to Tregs lineage [29].

A mentioned, a key factor known to influence the development of Tregs is IL-2. While IL-2 was discovered as a key stimulatory cytokine of T cells, mice deficient in *Il2* or its receptor *Ilr2a* (*CD25*) or *Il2rb* (*CD122*) show signs of severe immunopathology. However, early studies that relied on CD25 as a Tregs marker had difficulty in determining whether IL-2 has a specific role in Tregs biology because CD25 expression is regulated by IL-2 [30]. Since the identification of FOXP3 as a valid marker of Tregs, several studies have been able to re-examine whether IL-2 has a role in Tregs development. By using mice that carry a *Foxp3*^{gfp} reporter knock-in allele, it has been demonstrated that Foxp3$^+$ Tregs still develop in the thymus — albeit in significantly reduced numbers — in *Il2*$^{-/-}$ and *Ilr2a*$^{-/-}$ mice [31].

It is established that naïve CD4$^+$/CD25$^-$/FOXP3$^-$ T cells can convert into FOXP3$^+$ regulatory T cells (i.e. Tregs). *In vitro* conversion occurs in the presence of TGF-β, typically under conditions of low costimulation [32]. This process requires cytotoxic T lymphocyte antigen (CTLA)-4-mediated negative costimulation. The Tregs resemble naturally occurring Tregs both phenotypically and functionally [33]. *In vivo*, the extrathymic induction of Tregs from naïve CD4$^+$ T cells occurs upon subimmunogenic antigen stimulation [34]. Consistent with *in vitro* studies, TGF-β signaling and B7 costimulation are required for peripheral conversion. One of the key questions is whether and how dendritic cells (DCs) regulate the *de novo* induction of Tregs. To this end, Wang *et al.* examined the capacity of *ex vivo* splenic DC subsets to induce Foxp3 expression in the presence of TGF-β [35]. Their results show that among the splenic DC subsets, the CD8α$^+$ DCs exhibit a superior capacity to drive conversion. Multiple costimulatory and coinhibitory molecules have been identified to nonredundantly regulate this process. In particular, programmed death 1 ligand expression on DCs is required for conversion not only *in vitro* but also in a tumor-induced *in vivo* conversion model. Collectively, this

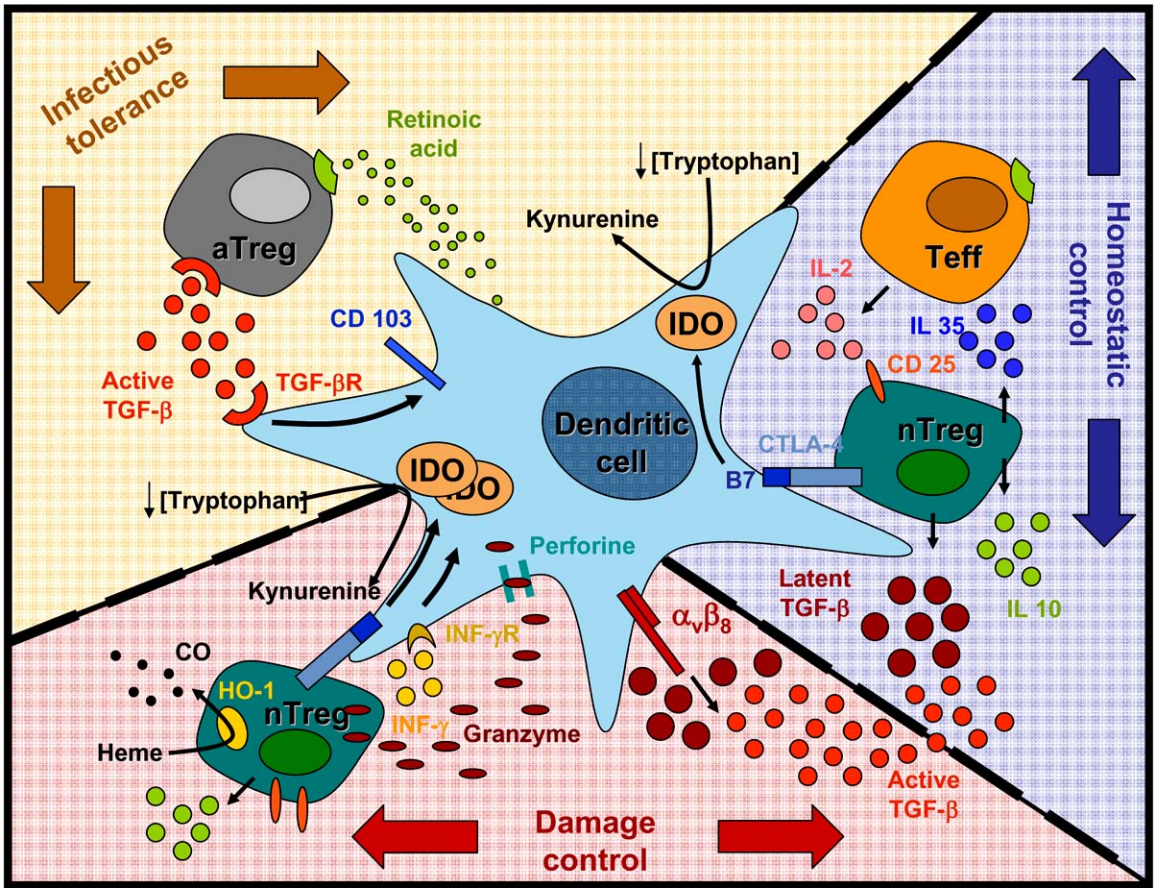

Fig. (1). A three-tiered model of the functions of regulatory T cells in maintaining normal immune homeostasis. This model shows mechanisms potentially used by Treg cells for homeostatic control in the steady state, during 'damage control' at the site of inflammation and for infectious tolerance after the resolution of an immune response.

aTreg: adaptive Tregs; TGF-αR: TGF-α receptor; nTreg: natural Tregs; CO: carbon monoxide; IFN-αR: IFN-α receptor.

study has illuminated the cellular and molecular parameters that regulate the *de novo* generation of Tregs, which might be exploited to prevent tumor-induced immune tolerance [35].

CO-STIMULATORY SIGNALING PATHWAYS AND FOXP3 EXPRESSION

It is widely accepted that T-cell activation requires at least two signals. The first one is specific and initiated by the interaction between TCR and the MHC-peptide complex; the engagement of CD28 by B7 molecules on the antigen presenting cells provides the nonspecific co-stimulatory signals essential for T-cell full activation and function [36]. Similarly, FOXP3 expression and the production of Tregs both in the thymus and in the periphery critically depend on the co-stimulatory signaling. $CD28^{-/-}$ and $B7\text{-}1^{-/-}/B7\text{-}2^{-/-}$ ($CD80^{-/-}/CD86^{-/-}$) mice show significantly reduced numbers of Tregs in the thymus and the periphery, indicating that CD28:B7 interaction is required for the development and maintenance of Tregs. $CD4^{+}/Foxp3^{+}$ regulatory T cells constitutively express the intracellular and surface CTLA-4 [37]. CTLA-4 deficient mice display the phenotypes critically resembling the Foxp3 mutant ones, indicating a close link may exist between CTLA-4 and Tregs [38]. In addition, other co-stimulatory molecules may also contribute to FOXP3 expression and Tregs development and function. Blockade of the programmed death 1 (PD1)-PD-L pathway with the anti-PD1 mAb significantly interrupted the vascular endothelium-induced FOXP3 expression and the conversion into the Tregs from $CD4^{+}/CD25^{-}$ T cells, indicating that PD1-PD-L interaction seems to be critical for Foxp3 expression and the conversion into Tregs in mice [39]. OX40-OX40 ligand (OX40-L) interaction may be important for the development and homeostasis of Tregs, as the significantly reduced number of Tregs in OX40-deficient mice and the increased Tregs in constitutively active OX40-L expressing mice were observed [40].

EFFECTS OF UREMIA AND oxLDL ON $CD4^{+}/CD25^{-}$ T CELLS

Human CD4 regulatory function is observed only when the $CD4^{+}$ T cells expressed high levels of CD25 and the cells are isolated apart from the $CD25^{low}$ T cells. However, patients with ESKD show an early activation of $CD4^{+}$ T cells and impaired proliferation ability in conjunction with an impaired IL-2 pathway [11]. Indeed, part of activated $CD4^{+}$ T

cells from these patients, which did not express the CD25 activation marker, are dysfunctional, do not proliferate and are apoptotic [11]. Thus, in uremic patients, oxLDL seem to play a specific role in the frequencies and phenotypes of $CD4^+/CD25^+/CD127^-$ Tregs, which distinctly discriminates between nTreg and effector cell subsets [15]. Indeed, in these patients, oxLDL inhibit CD25 expression at the cell surface, whereas the expression of CD3 and CD4 (constitutively expressed on T cells), CD69 (early activation antigen) and HLA-DR (late activation antigen) is unchanged [9, 15, 41]. Thus oxLDL do not alter the steps of the signalling pathways triggering CD69 and HLADR expression, but mild oxidation of LDL is sufficient to dramatically disturb CD25 expression as demonstrated in a recent study [15]. Considering these effects, the relative percentage of $CD4^+/CD25^+$ Tregs of the total CD4 population is significantly reduced by incubation with oxLDL compared with a nonsignificant depleting effect on $CD4^+/CD25^-$ T cells.

IMPAIRED $CD4^+/FOXP3^+$ REGULATORY T CELL FUNCTION IN PATIENTS WITH ESKD

In chronic kidney disease, as in other chronic inflammatory diseases, monocytes/macrophages and their mediators make an important contribution to the inflammatory process. Previous findings suggested that in patients with ESKD, a significantly high percentage of activated T cells ultimately did not proliferate but became apoptotic [41, 42]. Circulating T cells from HD patients show clear signs of biological activation, likely caused by the combined action of activated complement components, blood-dialyzer interaction, and such dialysate contaminants as endotoxins. The increase in early activation markers such as CD69 is expected to result in the production of mid, late and then postactivated/memory T cells. The analysis of the other markers such as HLA-DR (late-activated T cells), CD45RO (postactivated memory helper T cells), CD11a (postactivated cytotoxic T cells) and CD28 (postactivated lymphokine-producing cytotoxic T cells) does not provide any argument for further overall T-cell activation. This might indicate that the type of activation observed generally in T cells from uremic patients was somehow aborted or inefficient [11, 42].

In uremic patients as in healthy subjects, monocytes/macrophages (M/M) are not able to prime T cells *de novo*, but rather stimulate effector/memory T cells by the secretion of cytokines, which support T cell proliferation. Their ability to interact with T cells *via* MHC class II–TCR interactions as well as engagement of T cell co-stimulatory receptors on their surface, makes close contact between M/M and Tregs likely to occur *in vivo*. However, only few clear data on this interaction and its relevance in uremia have been exposed [43, 44]. It is possible that the micro-inflammation seen in patients with ESKD may be related to specific M/M, which perform distinct immunological functions. It is conceivable that alternatively activated M/M (i.e. type-II M/M), which have rather immunomodulatory (i.e. suppressive) functions may not be able to correctly suppress the proliferation of activated T cells in uremic patients. Indeed, recent findings indicate, that these M/M are also able to induce Tregs, which additionally account for the drastically reduced T cell proliferation induced by the M/M [45]. *Vice versa*, Tregs respond to M/M since they are able to block M/M maturation [46]. Thus, these data indicate that alternatively activated M/M can induce immunosuppressive Tregs and that in return these Tregs are potent suppressors of M/M maturation. However, further studies in patients with ESKD are needed to confirm the role of these type-II M/M in this population.

PLACE OF oxLDL IN TREGS APOPTOSIS IN PATIENTS WITH ESKD

Apoptosis is a deliberate form of induced cell death distinct from senescence, a process by which unwanted or damaged cells are removed in most multicellular organisms. It requires activation of specific genes that lead to a series of distinctive morphological and biochemical features. These changes include activation of cellular proteases (caspases), mitochondrial depolarization, chromatin condensation, DNA degradation, and cell volume loss or cell fragmentation. The induction of activated Tregs apoptosis from HD patients is dependent on Fas/FasL expression, which leads to a cell contact form of circulating $CD4^+$ T cell self-injury [47]. Furthermore, activated Tregs from HD patients fail to respond adequately to exogenous IL-2. This is due to the down-modulation of surface IL-2 receptor (IL-2R) α-subunits (IL-2Rα) expression, impaired IL-2 signal transduction in $CD4^+$ T cells and/or increased serum levels of soluble IL-2R (sIL-2R) [11]. Decreased proliferative capacity of Tregs from subjects with normal renal function incubated with serum from chronic HD patients and its restoration by normal serum strongly suggests that mediators induced by HD affect transduction mechanisms in the IL-2/IL-2R pathway. Finally, IL-2 seems to inhibit the apoptotic process at many stages by interacting with various proteins [48].

The clinical consequences of the Tregs dysfunction in patients with ESKD are numerous including immune dysregulation, micro-inflammation and atherogenesis [11, 15, 49].

It is known that in T cells, apoptosis plays an important role in maintaining T cell repertoire and deleting autoreactive T and B cells, thus limiting the immune response. Apoptosis is strictly regulated by a number of gene products that promote cell death or extend cell survival. The Fas (CD95) surface receptor mediates apoptosis in a wide variety of cell types. Its ligand (FasL) is predominantly expressed in activated T cells and mediates cell death by cross-linking the Fas receptor in apoptosis sensitive Fas^+ cells. The susceptibility of T cells to apoptosis is controlled by the family of bcl-2 homologues. Overexpression of bcl-2 enhances the survival of T cells. In contrast, Bax heterodimerizes with bcl-2 to counter its antiapoptotic effect and promotes apoptosis.

In HD patients, oxLDL may play a dual role in Tregs. On the one hand, oxLDL activates Tregs and induces Fas expression, thereby initiating a cascade of substrate-specific pro-apoptotic caspases leading to cell cycle arrest [15]. On the other hand, oxLDL alters IL-2/IL-2R pathway and sensitizes activated Tregs from HD patients to exogenous IL-2 explaining the reported Tregs apoptosis in these patients [11]. In activated Tregs from uremic patients and more particularly in those from HD patients, oxLDL induce Fas expression on the cell surface, which corresponds to the early phase of cell apoptosis. The evaluation of intracellular Fas synthesis and DNA fragmentation confirms Fas-mediated

apoptosis in Tregs in response to oxLDL. The percentage of apoptotic cells is related to the copper mildly oxidized LDL concentration. Fas activation induces the recruitment of procaspase-8 to the Fas receptor, and this association triggers the caspase cascade that leads to apoptosis [50]. Overexpression of Fas sensitizes cells to Fas-induced apoptosis, suggesting that increased clustering of Fas on the plasma membrane results in a stronger ability to recruit procaspase-8, which would overcome the sequestering of procaspase-8 by Bcl-2, and could influence the inhibitory function of Bcl-2 or Bcl-xL on Fas-induced apoptosis [51]. Moreover, experiments with blocking antibodies to Fas suggest that mildly oxidized LDL acts mainly by up-regulating expression of Fas [9]. Activation of the Fas pathway results in oligomerization of Fas and recruitment of Fas-associated death domain (FADD) and FADD homologous, IL-1 beta-converting enzyme (ICE)-like protease (FLICE), which then activate caspases. The observation that the FLICE inhibitory protein is down-regulated by oxLDL further supports the involvement of the Fas pathway in oxLDL-induced apoptosis [52-54]. Finally, mildly oxidized LDL causes an overexpression of Fas at the Tregs surface. The stimulation of Fas expression seems to be a key element of this process. Indeed, activation of Tregs induces transient expression of FasL triggering Fas-dependent apoptosis. Therefore, the knowledge of molecular pathways that control and drive Tregs activity is necessary, and the understanding of mechanisms Tregs use for interactions with other subsets of lymphocytes is a key requirement for the development of corrective therapies in the future. The most striking observation recently made is that both Tregs and responder cells can reciprocally regulate T cell survival in cocultures, and that Tregs can be induced to die in cocultures with activated responder cells. Furthermore, Tregs sensitivity or resistance to responder cells-derived death signals appears to be dependent on the IL-2 concentration in cocultures [55].

PLACE OF 26 S PROTEASOME IN T CELL-CYCLE ARREST AND APOTOSIS

The ubiquitin proteasome system is one of the most important proteolytic machineries in eukaryotic cells. It is involved in the regulation of essential cellular processes, such as cell cycle, signal transduction and antigen processing [56].

During the tightly regulated G1/S-phase, the sister chromatids are separated and complemental DNA synthesis and replication takes place. The proper custodial regulation of DNA replication generally refers to the timely ordered progression from G1- to S-phase that constitutes the strict initiation and completion of only one round of DNA replication in each cell cycle. This duplication relies on the coordinated activities of positive regulators, such as cyclins, cyclin-dependent kinases (CDK), CDK-cyclin complexes, E2F and Cdc6, and negative regulators, such as CDK inhibitors (CKI) of the Cip/Kip and INK4 families. The coordinated timely presence and action of these positive and negative regulators is governed by inactivation as a result of proteasomal degradation [57].

During the G2/M-phase, the doubled chromosome set is separated along kinetochore microtubules and divided into two new cells. The ordered progression of the S- and M-phase also highly depends on the spatial and temporal control of cell cycle regulatory proteins by proteasomal degradation that finally ensures proper cell cycle transitions and adequate frequencies of cell division [57].

Proteasomal degradation of the CKI $p27^{Kip1}$ is thought to be required for G1-to-S-phase progression and mainly occurs at the early onset of S-phase), although $p27^{Kip1}$ degradation also can take place at the G0-to-G1-phase transition. Consequently, $p27^{kip1}$ protein is abundant in G0 and G1 cells and is down-regulated in proliferating and S-phase cells. Moreover, ectopic overexpression of mutant $p27^{Kip1}$, but not of wild-type $p27^{Kip1}$, results in cell cycle arrest in the S-phase strongly suggesting that proteasomal degradation of $p27^{Kip1}$ is essential for the entry into S-phase [58]. However, inactivation of $p27^{Kip1}$ function may not only occur by proteasomal degradation, but also *via* alternative pathways such as proteolytic processing.

One candidate mechanism of how proteasomal activity promotes apoptosis at an upstream point of apoptotic signal transduction has been uncovered recently in primary mouse thymocytes: XIAP and c-IAP1, members of the highly conserved family of inhibitors of apoptosis proteins (IAPs) that exert their antiapoptotic activity, at least in part, by inhibiting the activation and enzymatic activity of caspases, and by ubiquitination and targeting of caspase-3 for proteasomal degradation, are autoubiquitinated and subsequently degraded by the 26S proteasome in response to various apoptotic stimuli [59-61].

Another candidate mechanism of providing proapoptotic signals by proteasomal activity has been demonstrated in HUVECs induced to undergo apoptosis by treatment with TNF-α. Early after the initiation of TNF-α treatment of HUVECs, Bcl-2, a mitochondrial membrane-anchored protein capable of blocking apoptosis induced by diverse stimuli, was shown to be specifically degraded by the 26S proteasome [62-64]. This event was demonstrated to be operative in inducing apoptosis, because pretreatment of HUVECs with specific proteasome inhibitors reversed both TNF-α-induced Bcl-2 degradation and induction of apoptosis.

Only few studies have specifically examined proteasome function in primary lymphocytes from human. In fact, there is only one study known to date, that has analyzed the impact of age on proteasome activity in lymphocytes [65]. Other studies analyzed the decreased proteasomal proteolytic activity with age in T cells from the elderly [66]. Recently, Ponnappan *et al*. defined the alterations that occur upon aging in proteasomal subunits and provided an insight into the basis for the loss of catalytic activity [67, 68]. Thus, it appears that while the immunoproteasome is constitutively found in T cells, levels of both immunoproteasome and constitutive catalytic subunits decline with increasing age. In fact, mRNA expression of immunoproteasome subunits is significantly lower in T cells from elderly donors. With regard to functional enzymatic activity, while chymotryptic activity is significantly compromised in T cells from the elderly and post-acidic activity appears to be slightly down regulated, the tryptic activity is least affected. This observation is in keeping with alterations in the ratio of inducible proteasome subunits to constitutive subunits present in the 26S particles of the proteasome. As NFκB is a central mediator of signaling within T cells, and given that the proteasome regulates

the induction of NFκB, it is not too far-fetched to speculate that decreased proteasomal activity in T cells will adversely impact on immune function, thus contributing to immune dysfunction in human, especially in elderly. Additionally, given the role of NFκB as an anti-apoptotic factor/survival factor, altered proteasomal regulation may also be implicated in cell survival.

FOXP3$^+$ REGULATORY T CELL-CYCLE ARREST AND APOPTOSIS IN UREMIA: ROLE OF THE 26 S PROTEASOME

As recently demonstrated, oxLDL inhibit proteasome enzymatic activity of the CKI p27^{Kip1} and the pro-apoptotic molecule Bax [15]. The consequences result in the increased accumulation of these key regulatory proteins in Tregs from HD patients. The mechanisms by which oxLDL modify Tregs proteasome activity in uremic patients remain poorly understood. However, besides enhancing the oxidative damage of proteins such as p27^{kip1} and Bax, oxLDL may lead to accumulation of ubiquitinated proteins *via* inhibition of proteasome enzymatic activity (Fig. **2**). It can be speculated that the oxLDL-related protein damage is responsible for the Tregs cycle arrest at G_1 phase and their apoptosis. Indeed, oxLDL produce a rapid decay of proteasomal proteolysis in inducing derivatization of cell proteins by 4-hydroxynonenal (4-HNE) resulting in an inhibition of the 19S. This is because 4-HNE cross-linked proteins are resistant to proteolysis and are able to inhibit the 26S proteasome and because 26S proteasome is less resistant to H_2O_2-induced oxidative stress than the 20S proteolytic core. The second step (i.e. inhibition of the 20S core) may be a result of the progressing intracellular oxidative stress induced by oxLDL. At this stage, when the proteasome is completely inhibited, Tregs are rapidly dying.

On the other hand, oxLDL affect the anti-apoptotic protein Bcl-xL degradation by increasing its removal in parallel with the activation of the 20 S and 26 S proteasome in Phytohemagglutinin (PHA)-stimulated Tregs from healthy subjects treated with various concentrations of oxLDL or cultured with uremic serum from HD patients [15, 69, 70].

CONCLUDING REMARKS

Accumulating evidence suggests an important role for Tregs in the control of immunity and some inflammatory diseases development and/or progression. Future studies should aim at the delineation of the critical subtypes of Tregs responsible for these protective effects, the factors and molecular mechanisms involved in their survival, migration and suppressive function especially in patients with ESKD. Indeed, the modified Tregs number and function seen in this patient population are the consequences of the p27^{Kip1} and Bax accumulation in these CD4$^+$ T cell subtypes, which was due to oxLDL proteasome activity alteration. Proteolytic degradation of cell proteins by the 26S proteasome is a highly complex and tightly regulated process that plays pivotal roles in the regulation of basic cellular processes, including differentiation, proliferation, cell cycling, apoptosis, gene expression, and signal transduction. From a mechanistic view, the 26S proteasome is capable of governing strictly opposite biologic features that crucially determine the fate of a cell, proliferation, and apoptosis. Because proteasomal protein degradation is a highly ordered and elaborated process, it is obvious that this process also can underlie deregulation as observed in several human diseases that exhibit an imbalance of proliferation and apoptosis as a fundamental pathogenetic feature as encountered in patients with ESKD. A fact which may have important implications in clinics, since this response could contribute to the CD4$^+$ T

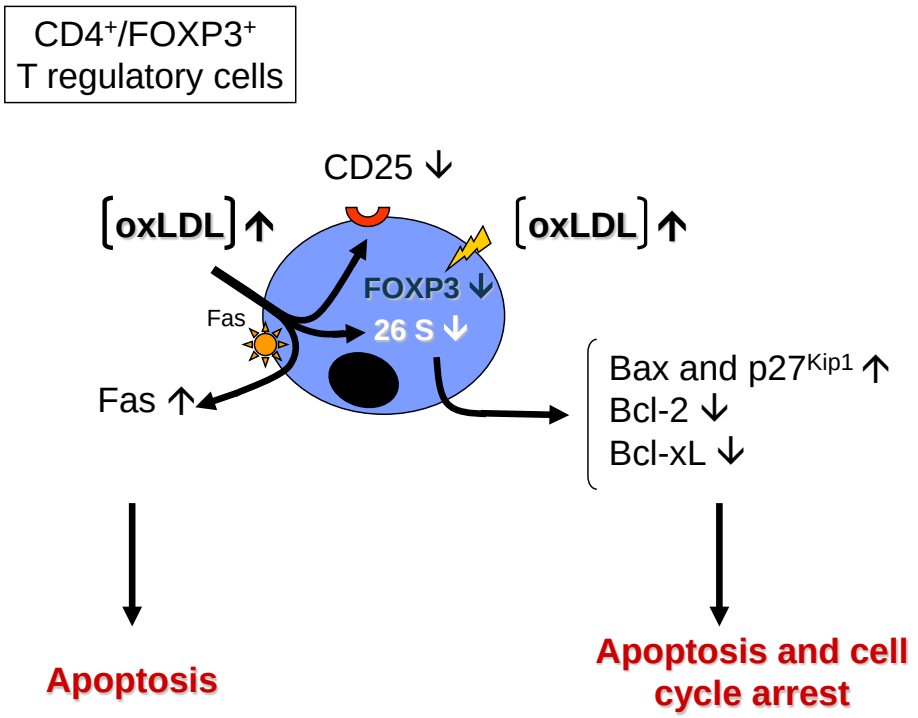

Fig. (2). Model for 26 S proteasome-mediated regulation of FOXP3$^+$ regulatory T cells apoptosis in uremia.

oxLDL: oxidized LDL.

cell immune dysfunction in patients with ESKD including micro-inflammation and atherogenesis.

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