



Extracellular, circulating proteasomes and ubiquitin – incidence and relevance

Stephan U. Sixt, Burkhardt Dahlmann

► To cite this version:

Stephan U. Sixt, Burkhardt Dahlmann. Extracellular, circulating proteasomes and ubiquitin – incidence and relevance. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 2008, 1782 (12), pp.817. <10.1016/j.bbadis.2008.06.005>. <hal-00501582>

HAL Id: hal-00501582

<https://hal.science/hal-00501582v1>

Submitted on 12 Jul 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



HAL Authorization

Accepted Manuscript

Extracellular, circulating proteasomes and ubiquitin – incidence and relevance

Stephan U. Sixt, Burkhardt Dahlmann

PII: S0925-4439(08)00128-2
DOI: doi: [10.1016/j.bbadis.2008.06.005](https://doi.org/10.1016/j.bbadis.2008.06.005)
Reference: BBADIS 62823

To appear in: *BBA - Molecular Basis of Disease*

Received date: 17 March 2008
Revised date: 9 June 2008
Accepted date: 10 June 2008



Please cite this article as: Stephan U. Sixt, Burkhardt Dahlmann, Extracellular, circulating proteasomes and ubiquitin – incidence and relevance, *BBA - Molecular Basis of Disease* (2008), doi: [10.1016/j.bbadis.2008.06.005](https://doi.org/10.1016/j.bbadis.2008.06.005)

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Extracellular, circulating proteasomes and ubiquitin – incidence and relevance

Stephan U. Sixt¹ and Burkhardt Dahlmann^{2*}

¹Klinik für Anästhesiologie und Intensivmedizin,

Universität Duisburg-Essen, Universitätsklinikum Essen, Essen, Germany

²Institut für Biochemie/CCM, Charité-Universitätsmedizin-Berlin, Berlin, Germany

* Corresponding author:

Burkhardt Dahlmann

Institut für Biochemie/CCM

Charité-Universitätsmedizin-Berlin

Monbijoustr. 2

10117 Berlin

Tel:+49-(0)30-450528309

Fax:+49-(0)30-450528916

E-mail address: burkhardt.dahlmann@charite.de

Keywords : proteasome, ubiquitin, extracellular, circulating, disease, diagnosis

Summary

The ubiquitin-proteasome-system is the major pathway for intracellular protein degradation and besides is deeply involved in the regulation of most basic cellular processes. Its proteolytic core, the 20S proteasome, has found to be attached also to the cell plasma membrane and certain observations are interpreted as to suggest that they may be released into the extracellular medium, e.g. in the alveolar lining fluid, epididymal fluid and possibly during the acrosome reaction. Proteasomes have also been detected in normal human blood plasma and designated circulating proteasomes; these have a comparatively low specific activity, a distinct pattern of subtypes and their exact origin is still enigmatic. In patients suffering from autoimmune diseases, malignant myeloproliferative syndromes, multiple myeloma, acute and chronic lymphatic leukaemia, solid tumour, sepsis or trauma, respectively, the concentration of circulating proteasomes has found to be elevated, to correlate with the disease state and has even prognostic significance. Similarly, ubiquitin has been discovered as a normal component of human blood and seminal plasma and in ovarian follicular fluid. Increased concentrations were measured in divers pathological situations, not only in blood plasma but also in cerebrospinal fluid, where it may have neuroprotective effects. As defective spermatozoa are covered with ubiquitin in the epididymal fluid, extracellular ubiquitination is proposed to be a mechanism for quality control in spermatogenesis. Growing evidence exists also for a participation of extracellular proteasomes and ubiquitin in the fertilization process.

1. Introduction

The increasing interest in mechanisms of protein degradation during the last four to five decades lead to the realization that there may be as many proteases as there are proteins in an organism. Although this is certainly not the case, the considerable heterogeneity with regard to the protease pool in different cells and tissues was probably suggestive of this idea [1]. However, since the discovery of the ubiquitin-proteasome system (UPS), it is known that each eukaryotic cell contains basic degradation machinery, the proteolytic core facility of which is the cylinder-like 20S proteasome. This is built up by four stacked seven-membered rings; the two outer rings are composed of seven different α -subunits, the two inner of seven different β -subunits. Three β -subunits ($\beta 1$, $\beta 2$, and $\beta 5$) possess an N-terminal threonine residue each forming a peptide-bond hydrolysing catalytic site, all oriented into the inner central chamber of the proteasome barrel and with different substrate specificity, namely chymotrypsin-, trypsin-, and caspase-like activity, respectively [2]. Under the influence of the cytokine interferon- γ standard-proteasomes can be replaced by immuno-proteasomes that have incorporated instead of the three standard β -subunits the immuno-subunits $\beta 1i$ (LMP2), $\beta 2i$ (MECL1) and $\beta 5i$ (LMP7) and thus complete special tasks for antigen processing [3].

All proteins are broken down after their distinct life-time and therefore have to be marked for commitment to degradation. For most intracellular proteins this is managed by their conjugation with a poly-ubiquitin chain, a process implying three steps: the ATP-dependent activation of ubiquitin by a ubiquitin-activating enzyme (E1), transfer of activated ubiquitin to a ubiquitin-conjugating enzyme (E2) and then forming an isopeptide bond between ubiquitin and the substrate protein catalysed by a ubiquitin-ligase (E3). The process is repeated several times with the aim to build up a poly-ubiquitin chain by inter-ubiquitin linkages [4]. The poly-ubiquitinated protein is

then recognized and bound by a 19S regulator complex that is associated to the α -subunit ring of the 20S proteasome thus having formed the 26S proteasome. After binding to the 19S regulator the ubiquitin chain is cleaved off the ubiquitinated substrate protein, the protein is unfolded and then translocated into the central chamber of the 20S proteasome, where it is degraded. The ubiquitin-proteasome system is not only responsible for the breakdown of outlived proteins, but also for regulation of basic cellular processes including cell cycling and proliferation, gene transcription and differentiation, apoptosis, signal transduction, antigen processing and many others [5]. So, it is not unexpected that impairment of the UPS has pathological consequences or rather the UPS is a therapeutic target [6-10].

The present review summarizes discoveries about extraordinary properties of two essential components of the UPS, namely the proteasome and ubiquitin, existing as constituents of extracellular fluids as well as of blood plasma, and it delineates their possible significance for diagnosis and outcome of various malignant and non-malignant diseases.

2. Evidence for association of proteasomes to the cell plasma membrane

The 20S proteasome was originally discovered as a hollow cylinder protein associated to human erythrocyte membranes and designated cylindrin [11-14]. Later on it was also isolated from the cytoplasm of erythrocytes [15]. Today it is known that the major part of proteasomes is located within the cytoplasmic and nuclear compartments while only a minor fraction is associated to membranes [16]. When the major lipopolysaccharide (LPS) component of gram-negative bacteria was added to a membrane preparation from murine macrophages, it was observed to be specifically bound by 20 S proteasomes that are attached to the membranes [17]. The

endoplasmic reticulum (ER) membrane was shown to be the platform for proteasome biogenesis [18] and ER membrane-associated matured proteasomes have been purified and characterized from rat liver cells and HeLa cells [19-21]. A member of the immunophilin family of proteins, FK506-binding protein 38, that resides in the ER and mitochondrial outer membrane was suggested to anchor 26S proteasomes at membranes [22]. In vitro proteasomes have been shown to bind to phospholipid monolayers with a preferred orientation [23] and in vivo they may even be partially buried within the membrane because a certain fraction of ER-associated proteasomes was found to be resistant to degradation by trypsin [19]. The presence of proteasomes at the surface of cell membrane was reported by Bureau and co-workers, who detected proteasomes at the cell surface of a non-permeabilized human leukaemic cell line (U937) and of human T and B lymphocytes by use of proteasome subunit-specific antibodies and flow cytometry [24, 25]. According to these investigations the pattern of proteasomal epitopes detectable at the surface of the cells changed depending on their stage of differentiation after treatment with phorbol-myristate ester, retinoic acid or vitamin D3 and their cluster of differentiation, respectively. The authors could not detect proteasomes in the culture medium of the leukaemic cells [25], but nevertheless speculated that the enzymes are released somehow into the extracellular medium and may function as paracrine factors [24].

Several investigations by Sawada and co-workers presented evidence that proteasomes participate in the fertilization process in various marine invertebrates [26-28]. Morales and co-workers reported that human sperm proteasome is also implicated in acrosomal exocytosis [29]. By using Triton-X-114 for partitioning of human sperm extracts into membrane-bound and soluble proteins, the authors found 80% of proteasome activity in a membrane-bound form. After in vivo biotinylation of sperms and subsequent isolation of sperm membranes, more than 80% of the

proteasome activity could be removed by avidin bound to agarose beads, indicating that the major part of proteasome activity is associated with the plasma membrane and only a minor part with intracellular membranes of spermatocytes. Since these proteasomes could be labelled with proteasome-specific antibodies without permeabilization of the sperms, the epitopes seem to be accessible on the surface of sperm heads [30]. These data and similar results obtained with mouse spermatocytes [31] corroborated earlier findings on detection, isolation and identification of proteasomes at membranes of human spermatozoa [32, 33]. Proteasomes thus extracellularly exposed are supposed to participate in the acrosome reaction [34, 35], penetration of the zona pellucida and fertilization [36]. The detection of a ubiquitin-C-terminal hydrolase at the surface of a sperm acrosome [37] and the selective proteasomal degradation of a ubiquitinated zona pelucida glycoprotein during fertilization [38] corroborate this suggestion. Reaction of these surface-exposed proteasomes with proteasome auto-antibodies has been suggested to be causative for autoimmune infertility in man [32]. By means of immunofluorescence microscopy Sutovsky and colleagues showed that proteasomes in boar sperm acrosome contain inducible immunosubunits $\beta 1i$ and $\beta 2i$ [36], a finding recently supported by proteomic analysis of bovine spermatozoa [39] Tengowsky et al. reported about a differential expression of genes encoding constitutive and inducible 20S proteasomes subunits in rat testis and epididymis [40]. Although proteasomes may be released from sperm caps during the acrosome reaction no clear evidence for such a process is presented so far in the literature. Nevertheless, all these data indicate that proteasomes are bound to the plasma membrane and may be even partly exposed to the cell surface.

3. Extracellular proteasomes

The outer-surface of the lung is coated by alveolar fluid called epithelial lining fluid. By using the technique of bronchoalveolar lavage (BAL) [41] Sixt et al [42] collected this extracellular fluid and measured in these fluid samples considerable proteolytic activity that hydrolysed proteasome-specific fluorogenic peptide-substrates and was inhibited by the proteasome-specific inhibitor epoxomicin. Extraction of this proteolytic activity by gel filtration revealed a 660 kDa proteinase that reacted with antibodies to several proteasomal α - and β -subunits. Additionally, mass spectrometric analysis showed the presence of several proteasome α -subunits within the enzyme preparation, indicating that proteasomes are a component of the extracellular alveolar fluid. A critical question was whether these proteasomes are present within the alveolar fluid or originate from cells within the fluid that were damaged during BAL. Depending on the substrate used 94 – 62% of the proteolytic activity was found in the supernatant after precipitation of cellular material by centrifugation. Interestingly, there was neither a positive correlation between cell lysis as measured by LDH activity released during centrifugation of the BAL fluid and proteolytic activity measured in the supernatant, nor between the number of cells stainable with trypan blue in the centrifugation pellet and the proteolytic activity in the supernatant [42]. These data exclude that cell damage is a major source for the proteasomes found in the BAL fluid. The extracellular proteasome exhibited a high cleavage rate of albumin that was ATP- and ubiquitin-independent and inhibited by epoxomicin [42].

Thus, by catalysing alveolar protein degradation the proteasome may possibly assist in maintenance of a low intra-alveolar oncotic pressure. Since the alveolar albumin concentration often increases in lung diseases [43], it was of interest to assess the alveolar proteasomal albumin degradation in lung diseases. The acute respiratory distress syndrome (ARDS) is a clinical syndrome characterized by inflammatory

pulmonary edema, hyaline membranes, diffuse endothelial and epithelial injury and fibrosis [44]. Despite a significantly higher proteasome concentration in the alveolar space of ARDS patients compared to subjects without lung diseases (1069 ± 1194 ng/ml vs. 60 ± 49 ng/ml), ARDS patients had a significantly lower extracellular alveolar proteasome activity as compared to control (Sext, U.S., unpublished data). Whether this reduced activity is a result from or an explanation for the high concentration of albumin found in the extracellular alveolar space of patients suffering from severe ARDS [45] awaits further investigations.

4. Circulating proteasomes

Due to the mutual exchange between blood and alveolar fluid the acellular components of BAL fluid are believed to originate not only from the lung but also from the blood. Therefore it is not a surprise that the concentration of proteasomes in blood plasma was also found to be higher in ARDS patients than in subjects without lung disease, although no direct correlation exists between the extent of increase in blood plasma and BAL fluid (Sext, U.S., unpublished data). By using the enzyme-linked immunosorbent assays technique (ELISA) Wada et al. were the first to detect 20S proteasomes in human serum [46] that nowadays are generally designated 'circulating' proteasomes (c-proteasomes). As shown by electron microscopy purified c-proteasomes are intact 20S proteasome particles that exhibit fluorogenic-peptide hydrolysing activity and are inhibited by lactacystin [47]. Additionally, proteins reacting with proteasome-subunit antibodies were detected in fractions containing low-molecular weight material, when serum was fractionated by sucrose-gradient fractionation, indicating that blood plasma may also contain a certain amount of disintegrated proteasomes [48].

As it was known since the beginning of the last decade that the generation of proteasomes is enhanced in various malignant haematopoietic cell lines [49], Wada et al. measured the proteasome concentrations in serum samples of patients with hematologic malignancies and found a positive correlation between proteasome concentration and the tumour burden of the patients [46]. In patients suffering from acute leukaemia, Non-Hodgkin' lymphoma and multiple myeloma (MM) the elevated serum concentration of proteasomes were significantly lowered after complete remission by chemotherapy but remained unaltered or even increased in patients with no remission [46]. Lavabre-Bertrand and colleagues [50] also measured proteasome levels in blood plasma from patients with malignant hemopoietic disorders and found considerably elevated concentrations of c-proteasomes in chronic myeloproliferative syndrome and myelodysplastic syndrome, whereas in patients with acute myelocytic leukaemia the concentrations was not different from non-diseased control persons. The concentrations of c-proteasomes were statistically even lower in patients with lymphoid hemopoietic malignancies like chronic lymphatic leukaemia (CLL), MM and Non-Hodgkin-lymphoma, except if the disease was in an aggressive phase that was reflected in significantly higher levels of c-proteasomes.

Interdependency between the stage of malignancy and the level of c-proteasomes was also detected in patients with metastatic malignant melanoma [51]. While stage I and II were not significantly different from healthy volunteers, patients with stage III and IV malignant melanomas partially suffering from distant metastases, had up to 4.5 times higher concentrations of c-proteasomes as compared to sera of control persons.

In their investigation Lavabre-Bertrand et al. [50] detected significantly elevated concentrations of circulating proteasomes also in patients with different solid tumors (carcinoma of breast, stomach, kidney, colon, lung, testes and liver, respectively).

This observation was supported by other investigators [52]. But significantly increased levels of c-proteasomes were also found in non-cancerous diseases, for instance in patients suffering from liver diseases like liver cirrhosis, acute or chronic hepatitis and fatty liver [46]. This was also clearly shown in septic and in trauma patients, who had 15-fold or even higher concentrations of c-proteasomes as compared to controls, whereas the level in patients who underwent uncomplicated abdominal surgery the concentration was only twice that of healthy subjects [53]. In a perioperative followup of patients undergoing thoracoscopic thymectomy a biphasic alteration of c-proteasomes was observed with a decrease during the surgical treatment and a postoperative increase [48]. A similar observation was made with coronary artery bypass graft patients [54]. Concentrations of c-proteasomes were also measured in patients with various autoimmune diseases and their levels found to be elevated up to 20-fold especially in patients with systemic and organ-specific autoimmune diseases, among others autoimmune myositis, Jo-1 syndrome, systemic lupus erythematosus, primary Sjögren syndrome, rheumatoid arthritis, vasculitis, systemic scleroderma, autoimmune hepatitis, whereas patients suffering from myasthenia gravis exhibited control levels [48]. Similar to the data obtained with cancer patients [46, 50, 51] the levels of c-proteasomes were related to the disease activity, since according to follow-up studies with patients suffering from rheumatoid arthritis and systemic lupus erythematosus clinical improvement went always along with a reduction if not normalisation of the concentration of c-proteasomes [48].

5. Clinical significance of circulating proteasomes

The relationship between clinical state and concentration of c-proteasomes that was found in patients with systemic lupus erythematosus, rheumatoid arthritis [48] and hematologic malignancies [46] induced Jakob and colleagues to scrutinize

c-proteasome levels in patients with multiple myeloma as well as their alterations in dependence on different forms of chemotherapeutic treatment [55]. In comparison to healthy controls c-proteasome levels were significantly increased in newly diagnosed and untreated patients suffering from monoclonal gammopathies with undetermined significance, from smoldering, asymptomatic and from active multiple myeloma. In agreement with observations in other studies [46] [48] patients with active MM, who positively responded on standard- or high-dose chemotherapy, had significantly lower post-treatment c-proteasome levels, an effect not observed in non-responders. However, even more interesting was that c-proteasome concentrations lower than the median (744 ng/ml) measured in all active MM patients predicted an overall longer survival than those with values above 744 ng/ml serum [55].

Although these data show that the concentration of circulating proteasomes can be used as an independent prognostic factor for survival in MM patients and probably also for other diseases [56], a practical problem arises from the fact that the absolute concentrations of c-proteasomes published are not always in the same order, since different laboratories use different ELISA techniques for quantification of c-proteasomes. While levels in normal subjects were < 400 ng/ml serum in one group of investigators [46, 48, 55], concentrations between 2100 and 2400 ng/ml serum of healthy control persons were measured in other laboratories [50-53]. To overcome such problems Ma et al. [56] developed a method to determine the three proteolytic activities of proteasomes in serum as an alternative to quantifying the c-proteasome concentration by ELISA techniques. More details of this methodology would be helpful in the interest of a wider application, e.g. what were the final substrate concentrations used and why the plasma had to be activated by means of 1% SDS or 1% Tween-20, especially since already 1% SDS was shown to inhibit 20S proteasomes completely [57]. According to this investigations the hydrolytic activities

measured by use of fluorogenic tripeptide-substrates were in the same order as the activities in white blood cell lysates and the chymotrypsin-like activity was inhibited by YU-101, a synthetic tetrapeptide epoxyketone known to inhibit the proteasome [58]. Although not in keeping with unaltered concentrations of c-proteasomes in CLL patients [50], Ma et al [56] found that the three proteasome activities were significantly elevated in plasma from patients with chronic lymphatic leukaemia (CLL) as compared to controls, but the activities were not affected by treatment of the patients. Nevertheless, the caspase-like activity and also to a certain degree the chymotrypsin-like activity measured in the activated plasma from CLL patients are predictors of survival: elevated activities (>1.32 pmol Z-LLE-MCA hydrolysis/sec/ml plasma and >1.28 pmol Suc-LLVY-MCA hydrolysis/sec/ml plasma) correlate with shorter survival; surprisingly, the trypsin-like activity had no predictive value [56].

6. The origin of c-proteasomes

When the concentration and/or the activity of c-proteasomes have a prognostic value for the course of diseases and even survival of a patient, knowledge about the origin of these blood plasma proteasomes is of high interest to develop intervening strategies. As detailed above pathophysiological conditions with rapidly proliferating cells and neoplastic tissue go very often along with significantly elevated levels of c-proteasomes. The proteasome as the central part of the UPS is a major regulator of cell proliferation [59] and enhanced expression of proteasomes has been observed in various cancer cell lines [49, 60, 61]. Therefore, Wada et al. speculated that increased levels of c-proteasomes may be due to their secretion from tumour cells or the enzyme is released from disintegrating tumour cells [46]. Since these are two completely different mechanisms that may contribute to the phenomenon of the existence of c-proteasomes, they should be discernible from each other. If tumour

cells die by other than apoptotic mechanisms, e.g. by mechanical destruction due to their large size, by cell necrosis or by T-cell- or macrophage-induced cytolysis, all sorts of cell components may be released into the extracellular space, amongst others 20S proteasomes. A commonly used marker for this type of cytolysis is the cytoplasmic enzyme lactate dehydrogenase (LDH) the concentration of which was measured in parallel to c-proteasomes in some of the studies mentioned above. Lavabre-Bertand et al [50] calculated the blood plasma LDH/plasma c-proteasome correlation for all (>300) tumours patients inspected in their study and found a positive correlation between both parameters but with numerous exceptions. A poor but significant correlation was also found in MM patients [51]. Similarly, hepatocellular carcinoma goes along with a positive correlation between the activity of serum alanine aminotransferase (ALT) and c-proteasomes [46]. But proliferation and cytolysis of malignant cells cannot be the only source for c-proteasomes, since patients with benign liver diseases like cirrhosis, chronic hepatitis and fatty liver, respectively, showed also a close positive correlation between serum ALT activity and c-proteasome concentration [46]. The post-operative increase of c-proteasome concentration measured in thymectomy patients [48] may support the hypothesis that proteasomes originate from injured cells. On the other hand, the fact that no significantly elevated c-proteasome concentrations were found in patients after abdominal surgery, but in patients suffering from trauma and sepsis, respectively, induced Roth et al. [53] to conclude 'that the immunological activity rather than cellular damage is causative of the early increased 20S proteasome levels in sepsis and severe injury'. This opinion seems to be in line with the finding that remarkably high c-proteasome concentrations were found in patients with systemic and non-systemic autoimmune diseases, like systemic lupus erythematosus (SLE), primary Sjögren's syndrome (pSS), rheumatoid arthritis, autoimmune myositis, and

autoimmune hepatitis [48]. Circulating-proteasomes purified from blood plasma of SLE and pSS contain immuno β -subunits suggesting that they are primarily released from immuno-competent cells. However, this idea was scrutinized by comparing the subtype-pattern of proteasomes purified from monocytes, T-lymphocytes and thrombocytes with that of purified c-proteasomes. Fractionation of 20S proteasomes based on their minimal differences in surface-charge has shown, that standard-proteasomes can be separated from immuno-proteasomes and intermediate-type proteasomes, the latter containing both standard- and immuno-subunits at the same time [21, 62]. No similarity of the subtype-pattern of c-proteasomes, neither from normal subjects nor from patients with SLE and rheumatoid arthritis, respectively, could be shown with the subtype-patterns of 20S proteasomes from monocytes, T-lymphocytes and thrombocytes [47]. On the other hand, all of these proteasome-subtype patterns are different from that obtained with 20S proteasomes from erythrocytes, excluding that c-proteasomes have their origin in red blood cells disintegrating during blood plasma sampling [47]. This conclusion is supported by comparing the specific hydrolytic activities measured with proteasomes from the different origins, showing that c-proteasomes have significantly lower activities than proteasomes from erythrocytes and from other white blood cells (Table 1).

6. Mechanisms of cellular proteasome release

It was suggested that c-proteasomes are released into the blood plasma from apoptotic cells due to an impaired mechanism of phagocytosis in macrophages [63]. Although this could explain the elevated concentrations of c-proteasomes measured in patients suffering from diseases that are going along with increased cell death, the unusual low specific activity of c-proteasomes, their particular subtype pattern and their relatively constant level cast some doubt whether this mechanism holds true

also in non-diseased control subjects. No correlation of c-proteasome levels was detected with gender or age [55]. Some authors mentioned that they have detected high concentrations of proteasomes in culture supernatants of leukaemia cells [46, 64] and speculated that a special secretion mechanism may exist in cancer cells [46]. Proteomic based analysis of the so-called secretome of murine myeloid cells cultured under serum-free conditions as well as of the human visceral adipose tissue also revealed the presence of many 20S proteasome subunits. They were classified under non-classically secreted proteins [65, 66]. Secretion of proteins by 'classical' mechanisms is based on the presence of signal-peptide dependent translocation of newly synthesized proteins into the endoplasmic reticulum and subsequent transportation by the cis/trans-Golgi apparatus to the plasma membrane. Proteasomes have never been detected inside of the ER/Golgi-compartment [67] and thus it is very unlikely that they use this pathway to leave the cell. But 'non-classical', unconventional secretory routes have been described for export of proteins across the plasma membrane of mammalian cells, e.g. by means of membrane-resident transporters or by shedding of plasma membrane-derived exovesicles [68] as detailed in the next chapter [69, 70]. As stated above proteasomes are associated to the plasma membrane and the latter mechanism might be responsible for extruding them into the environment of the cell. Since proteins even larger than the 20S proteasome (700 kD), like α 2-macroglobuline (820 kD) and immunoglobuline M (900kD), were detected in the epithelial lining fluid [71], transport mechanisms for expulsion of these macromolecules must be present in the alveolar epithelial cells that may explain the presence of extracellular alveolar proteasomes [72].

7. Extracellular ubiquitin

Already in 1993 increased blood plasma concentrations of ubiquitin

were measured in patients subjected to hemodialysis [73] and as compared to healthy control persons ubiquitin was also found to be elevated in blood plasma of subjects with parasitic and allergic diseases, respectively [74]. Similarly, increased concentrations of ubiquitin were found in cerebrospinal fluid of patients suffering from Creutzfeldt-Jakob disease [75] and from Alzheimer's disease, respectively [76, 77]. However, a discrimination between mono-ubiquitin and poly-ubiquitin was only possible after establishing a radioimmunoassay detecting exclusively mono-ubiquitin [78]. This assay allowed to detect and determine the concentrations of mono- and poly-ubiquitin in serum of healthy human subjects and to discover that the concentrations of both types of ubiquitin are slightly elevated in patients suffering from rheumatoid arthritis or subjected to hemodialysis [79]. Increased systemic levels of extracellular ubiquitin have also been observed in several very different diseases like alcohol liver cirrhosis and brain atrophy [80], type 2 diabetes [81], chronic hemodialysis [82], hairy cell leukaemia [83], sepsis [84] and severe trauma [85]. Furthermore, in anoxic-ischemic encephalopathy after cardiopulmonary arrest extracellular ubiquitin was observed in the cerebrospinal fluid three days after resuscitation and correlated with outcome [86]. Interestingly, intravenous infusion of ubiquitin into rats after focal cortical contusion brain injury reduces the contusion volume and had neuroprotective effects. Also, during resuscitation from severe trauma intravenous application of ubiquitin reduced the intravenous fluid required to maintain mean arterial pressure [85, 87]. Similarly, infusion of ubiquitin has been shown to have beneficial effects on wound healing [88].

These data indicate that cell damage and hemolysis are the major sources of the increased extracellular ubiquitin concentrations measured under various pathological conditions, especially poly-ubiquitin the concentration of which shows

positive correlation with serum ALT, AST and LDH in patients with acute viral hepatitis [79], [84, 85, 89]. However, these data also suggest that ubiquitin fulfils certain functions in the extracellular space. A similar observation was published already in 1975, when Goldstein et al. discovered that extracellular ubiquitin influences the differentiation of pro-B- and pro-T-lymphocytes [90]. Other investigators observed that extracellular ubiquitin seems to have pleiotropic effects in hematopoietic cells [91] like antimicrobial activity [92], induction of apoptosis [83] as well as inhibition of platelet activities [93], suppression of IgG production in cultured splenocytes [94] and influence on the LPS-induced production of $\text{TNF}\alpha$ in macrophages [95]. Similar to proteasomes low concentrations of mono-ubiquitin (≈ 50 ng/ml) and poly-ubiquitin ($\approx 3\text{-}4$ ng/ml) were measured also in serum samples of healthy control subjects [79]. Therefore, one should also consider active cellular secretion as a source of extracellular ubiquitin [96], an approach that is corroborated by the fact that ubiquitin was detected in the secretome of mouse preimplantation embryos [97].

Epididymal epithelial cells form small membrane-enclosed secretory vesicles designated epididymosomes (equivalent to exosomes) [69] as well as large blebs that are released from the apical surface membrane, therefore designated apical blebs or aposomes [70]. Both were shown to extrude cytoplasmic proteins, including ubiquitin [98] explaining why free mono-ubiquitin was detected in medium of cultured epididymal epithelial cells [99]. Free ubiquitin was also detected in human seminal plasma [100] as well as in bovine preovulatory follicular fluids [101]. Since defective spermatozoa have found to be ubiquitinated on their surface [99], surface-ubiquitination has been interpreted as a quality control mechanism during sperm development [99, 102, 103]. Recently, Sutovsky and coworkers presented evidence by using immuno/western blotting techniques

that beside of ubiquitin the ubiquitin-activating enzyme (E1), a ubiquitin carrier enzyme (E2) and a ubiquitin C-terminal hydrolase is present in bovine epididymal fluid [104]. By means of the same technique as well as by measuring and specifically inhibiting fluorogenic peptide hydrolytic activity a certain evidence was obtained that 26S proteasomes are present in the epididymal fluid, a finding corroborating data published by Jones [103]. The authors hypothesize that in case there is also a ubiquitin-conjugating enzyme (E3) secreted from the epididymal cells, extracellular proteins might be ubiquitinated and degraded by an extracellular UPS [104] that performs sperm quality control [102] and control for proteins secreted by the epididymal epithelial cells [105].

8. Conclusion and next frontiers

The overwhelming amount of data collected on the manifold functions of proteasomes and ubiquitin in the fine-tuned intracellular protein degradation and its involvement in almost all biochemical pathways, eclipses the results elaborated on the existence and significance of extracellular proteasomes (that we suggest to designate e-proteasome in the future) and ubiquitin. Although the sheer fact about their extracellular presence is beyond controversy, their origin and especially their functions in extracellular fluids and blood plasma are still enigmatic. Whether the reproductive system due to its special secretion mechanisms is the exception of the rule that proteasomes and ubiquitin are proteins working almost exclusively inside of the cell or whether other cells, e.g. also those forming the alveolar lining, do also extrude these proteins by peculiar mechanisms has to be thoroughly investigated. If so, the role of these proteins may not necessarily be identical with their intracellular function, since many examples are known for proteins performing multiple, unrelated functions and are thus designated 'moonlighting' proteins [106, 107]. This idea can

easily be imagined for ubiquitin that performs posttranslational modifications of proteins and thus is known to mediate functions other than committing proteins for their degradation, e.g. regulating the cytoplasmic-nuclear trafficking and the endocytic pathway [108-110]. Non-proteolytic functions have been attributed also to proteasomes [111] but have been scarcely substantiated experimentally. Although different models have been proposed for the participation of either a complete extracellular UPS or just acrosomal-released proteasomes in the interaction of sperm and ovarian zona pellucida [36], this aspect may be of importance for treatment of infertility and/or contraceptive research [102]. Another major task should be to generally establish the diagnostic impact of the concentration of c-proteasomes especially with regard to their prognostic significance in critically ill patients or patients suffering from various types of malignant diseases as was also proposed for proteasome antibodies [112].

References

- [1] D.H. Wolf, Proteinase action in vitro versus proteinase function in vivo: mutants shed light on intracellular proteolysis in yeast, *Trends Biochem. Sci.* 7 (1982) 35-37.
- [2] M. Bochtler, L. Ditzel, M. Groll, C. Hartmann and R. Huber, The proteasome, *Annu. Rev. Biophys. Biomol. Struct.* 28 (1999) 295-317.
- [3] P.M. Kloetzel, The proteasome and MHC class I antigen processing, *Biochim. Biophys. Acta.* 1695 (2004) 225-233.

- [4] A. Hershko and A. Ciechanover, The ubiquitin system, *Annu. Rev. Biochem.* 67 (1998) 425-479.
- [5] M.H. Glickman and A. Ciechanover, The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction, *Physiol. Rev.* 82 (2002) 373-428.
- [6] A. Ciechanover and A.L. Schwartz, The ubiquitin system: pathogenesis of human diseases and drug targeting, *Biochim. Biophys. Acta.* 1695 (2004) 3-17.
- [7] A.F. Kisselev and A.L. Goldberg, Proteasome inhibitors: from research tools to drug candidates, *Chem. Bio.l.* 8 (2001) 739-758.
- [8] S.H. Lecker, A.L. Goldberg and W.E. Mitch, Protein degradation by the ubiquitin-proteasome pathway in normal and disease states, *J. Am. Soc. Nephrol.* 17 (2006) 1807-1819.
- [9] B.R. Wong, F. Parlati, K. Qu, S. Demo, T. Pray, J. Huang, D.G. Payan and M.K. Bennett, Drug discovery in the ubiquitin regulatory pathway, *Drug Discov. Today.* 8 (2003) 746-754.
- [10] J. Golab, T.M. Bauer, V. Daniel and C. Naujokat, Role of the ubiquitin-proteasome pathway in the diagnosis of human diseases, *Clin. Chim. Acta.* 340 (2004) 27-40.
- [11] J.R. Harris, Release of a macromolecular protein component from human erythrocyte ghosts, *Biochim. Biophys. Acta.* 150 (1968) 534-537.
- [12] J.R. Harris, The isolation and purification of a macromolecular protein component from the human erythrocyte ghost, *Biochim. Biophys. Acta.* 188 (1969) 31-42.
- [13] M.D. White and G.B. Ralston, The 'hollow cylinder' protein of erythrocyte membranes, *Biochim. Biophys. Acta.* 554 (1979) 469-478.
- [14] J.R. Harris, Torin and cylindrin, two extrinsic proteins of the erythrocyte membrane: A review, *Nouv. Rev. Fr. Hématol.* 22 (1980) 411-448.

- [15] H.L. Malech and V.T. Marchesi, Hollow cylinder protein in the cytoplasm of human erythrocytes, *Biochim. Biophys. Acta.* 670 (1981) 385-392.
- [16] A.J. Rivett, A. Palmer and E. Knecht, Electron microscopic localization of the multicatalytic proteinase complex in rat liver and in cultured cells, *J. Histochem. Cytochem.* 40 (1992) 1165-1172.
- [17] N. Qureshi, P.Y. Perera, J. Shen, G. Zhang, A. Lenschat, G. Splitter, D.C. Morrison and S.N. Vogel, The proteasome as a lipopolysaccharide-binding protein in macrophages: differential effects of proteasome inhibition on lipopolysaccharide-induced signaling events, *J. Immunol.* 171 (2003) 1515-1525.
- [18] B. Fricke, S. Heink, J. Steffen, P.M. Kloetzel and E. Kruger, The proteasome maturation protein POMP facilitates major steps of 20S proteasome formation at the endoplasmic reticulum, *EMBO Rep.* 8 (2007) 1170-1175.
- [19] H. Hori, T. Nemba, Y. Miyata, T. Hayashi, K. Ueno and T. Koide, Isolation and characterization of two 20S proteasomes from the endoplasmic reticulum of rat liver microsomes, *J. Biochem.* 126 (1999) 722-730.
- [20] M.T. Khan and S.K. Joseph, Characterization of membrane-associated proteasomes in WB rat liver epithelial cells, *Arch. Biochem. Biophys.* 385 (2001) 99-107.
- [21] N. Klare, M. Seeger, K. Janek, P.R. Jungblut and B. Dahlmann, Intermediate-type 20 S proteasomes in HeLa cells: "asymmetric" subunit composition, diversity and adaptation, *J. Mol. Biol.* 373 (2007) 1-10.
- [22] T. Nakagawa, M. Shirane, S. Iemura, T. Natsume and K.I. Nakayama, Anchoring of the 26S proteasome to the organellar membrane by FKBP38, *Genes Cells.* 12 (2007) 709-719.

- [23] R.H. Newman, P. Whitehead, J. Lally, A. Coffey and P. Freemont, 20S human proteasomes bind with a specific orientation to lipid monolayers in vitro, *Biochim. Biophys. Acta.* 1281 (1996) 111-116.
- [24] J.P. Bureau, M. Olink-Coux, N. Brouard, S. Bayle-Julien, M. Huesca, M. Herzberg and K. Scherrer, Characterization of prosomes in human lymphocyte subpopulations and their presence as surface antigens, *Exp. Cell Res.* 231 (1997) 50-60.
- [25] L. Henry, A. Baz, M.T. Chateau, K. Scherrer and J.P. Bureau, Changes in the amount and distribution of prosomal subunits during the differentiation of U937 myeloid cells: high expression of p23K, *Cell Prolif.* 29 (1996) 589-607.
- [26] D.L. Mykles, Intracellular proteinases of invertebrates: calcium-dependent and proteasome/ubiquitin-dependent systems, *Int. Rev. Cytol.* 184 (1998) 157-289.
- [27] N. Sakai, M.T. Sawada and H. Sawada, Non-traditional roles of ubiquitin-proteasome system in fertilization and gametogenesis, *Int. J. Biochem. Cell Biol.* 36 (2004) 776-784.
- [28] N. Yokota and H. Sawada, Sperm proteasomes are responsible for the acrosome reaction and sperm penetration of the vitelline envelope during fertilization of the sea urchin *Pseudocentrotus depressus*, *Dev Biol.* 308 (2007) 222-231.
- [29] P. Morales, M. Kong, E. Pizarro and C. Pasten, Participation of the sperm proteasome in human fertilization, *Hum. Reprod.* 18 (2003) 1010-1017.
- [30] P. Morales, E. Pizarro, M. Kong and M. Jara, Extracellular localization of proteasomes in human sperm, *Mol. Reprod. Dev.* 68 (2004) 115-124.
- [31] C. Pasten, P. Morales and M. Kong, Role of the sperm proteasome during fertilization and gamete interaction in the mouse, *Mol. Reprod. Dev.* 71 (2005) 209-219.

- [32] C. Bohring, E. Krause, B. Habermann and W. Krause, Isolation and identification of sperm membrane antigens recognized by antisperm antibodies, and their possible role in immunological infertility disease, *Mol. Hum. Reprod.* 7 (2001) 113-118.
- [33] C. Wojcik, M. Benchaib, J. Lornage, J.C. Czyba and J.F. Guerin, Proteasomes in human spermatozoa, *Int. J. Androl.* 23 (2000) 169-177.
- [34] E.S. Diaz, M. Kong and P. Morales, Effect of fibronectin on proteasome activity, acrosome reaction, tyrosine phosphorylation and intracellular calcium concentrations of human sperm, *Hum. Reprod.* 22 (2007) 1420-1430.
- [35] P. Morales, E.S. Diaz and M. Kong, Proteasome activity and its relationship with protein phosphorylation during capacitation and acrosome reaction in human spermatozoa, *Soc. Reprod. Fertil. Suppl.* 65 (2007) 269-273.
- [36] P. Sutovsky, G. Manandhar, T.C. McCauley, J.N. Caamano, M. Sutovsky, W.E. Thompson and B.N. Day, Proteasomal interference prevents zona pellucida penetration and fertilization in mammals, *Biol. Reprod.* 71 (2004) 1625-1637.
- [37] Y.J. Yi, G. Manandhar, M. Sutovsky, R. Li, V. Jonakova, R. Oko, C.S. Park, R.S. Prather and P. Sutovsky, Ubiquitin C-terminal hydrolase-activity is involved in sperm acrosomal function and anti-polyspermy defense during porcine fertilization, *Biol Reprod.* 77 (2007) 780-793.
- [38] Y.J. Yi, G. Manandhar, R.J. Oko, W.G. Breed and P. Sutovsky, Mechanism of sperm-zona pellucida penetration during mammalian fertilization: 26S proteasome as a candidate egg coat lysin, *Soc Reprod Fertil Suppl.* 63 (2007) 385-408.
- [39] D. Peddinti, B. Nanduri, A. Kaya, J.M. Feugang, S.C. Burgess and E. Memili, Comprehensive proteomic analysis of bovine spermatozoa of varying fertility

- rates and identification of biomarkers associated with fertility, *BMC Syst Biol.* 2 (2008) 19.
- [40] M.W. Tengowski, D. Feng, M. Sutovsky and P. Sutovsky, Differential expression of genes encoding constitutive and inducible 20S proteasomal core subunits in the testis and epididymis of theophylline- or 1,3-dinitrobenzene-exposed rats, *Biol. Reprod.* 76 (2007) 149-163.
- [41] R.P. Baughman, Technical aspects of bronchoalveolar lavage: recommendations for a standard procedure, *Semin. Respir. Crit. Care Med.* 28 (2007) 475-485.
- [42] S.U. Sixt, M. Beiderlinden, H.P. Jennissen and J. Peters, Extracellular proteasome in the human alveolar space: a new housekeeping enzyme?, *Am. J. Physiol. Lung. Cell. Mol. Physiol.* 292 (2007) L1280-1288.
- [43] G. Nakos, J. Pneumatikos, I. Tsangaris, C. Tellis and M. Lekka, Proteins and phospholipids in BAL from patients with hydrostatic pulmonary edema, *Am. J. Respir. Crit. Care Med.* 155 (1997) 945-951.
- [44] G.R. Bernard, A. Artigas, K.L. Brigham, J. Carlet, K. Falke, L. Hudson, M. Lamy, J.R. Legall, A. Morris and R. Spragg, The American-European Consensus Conference on ARDS. Definitions, mechanisms, relevant outcomes, and clinical trial coordination, *Am. J. Respir. Crit. Care Med.* 149 (1994) 818-824.
- [45] M.H. Bishop, J. Jorgens, W.C. Shoemaker, P.L. Appel, A. Fleming, D. Williams, G. Jackson, C.J. Wo, L. Babb, T. Manning and et al., The relationship between ARDS, pulmonary infiltration, fluid balance, and hemodynamics in critically ill surgical patients, *Am. Surg.* 57 (1991) 785-792.
- [46] M. Wada, M. Kosaka, S. Saito, T. Sano, K. Tanaka and A. Ichihara, Serum concentration and localization in tumor cells of proteasomes in patients with hematologic malignancy and their pathophysiologic significance, *J. Lab. Clin. Med.* 121 (1993) 215-223.

- [47] A. Zoeger, M. Blau, K. Egerer, E. Feist and B. Dahlmann, Circulating proteasomes are functional and have a subtype pattern distinct from 20S proteasomes in major blood cells, *Clin. Chem.* 52 (2006) 2079-2086.
- [48] K. Egerer, U. Kuckelkorn, P.E. Rudolph, J.C. Ruckert, T. Dorner, G.R. Burmester, P.M. Kloetzel and E. Feist, Circulating proteasomes are markers of cell damage and immunologic activity in autoimmune diseases, *J. Rheumatol.* 29 (2002) 2045-2052.
- [49] A. Kumatori, K. Tanaka, N. Inamura, S. Sone, T. Ogura, T. Matsumoto, T. Tachikawa, S. Shin and A. Ichihara, Abnormally high expression of proteasomes in human leukemic cells, *Proc. Natl. Acad. Sci. U. S. A.* 87 (1990) 7071-7075.
- [50] T. Lavabre-Bertrand, L. Henry, S. Carillo, I. Guiraud, A. Ouali, D. Dutaud, L. Aubry, J.F. Rossi and J.P. Bureau, Plasma proteasome level is a potential marker in patients with solid tumors and hemopoietic malignancies, *Cancer.* 92 (2001) 2493-2500.
- [51] P.E. Stoeber, T. Lavabre-Bertrand, L. Henry, I. Guiraud, S. Carillo, M. Dandurand, J.M. Joujoux, J.P. Bureau and L. Meunier, High plasma proteasome levels are detected in patients with metastatic malignant melanoma, *Br. J. Dermatol.* 152 (2005) 948-953.
- [52] D. Dutaud, L. Aubry, L. Henry, D. Levieux, K.B. Hendil, L. Kuehn, J.P. Bureau and A. Ouali, Development and evaluation of a sandwich ELISA for quantification of the 20S proteasome in human plasma, *J. Immunol. Methods.* 260 (2002) 183-193.
- [53] G.A. Roth, B. Moser, C. Krenn, F. Roth-Walter, H. Hetz, S. Richter, M. Brunner, E. Jensen-Jarolim, E. Wolner, K. Hoetzenecker, G. Boltz-Nitulescu and H.J. Ankersmit, Heightened levels of circulating 20S proteasome in critically ill patients, *Eur. J. Clin. Invest.* 35 (2005) 399-403.

- [54] T. Szerafin, K. Hoetzenecker, S. Hacker, A. Horvath, A. Pollreisz, P. Arpad, A. Mangold, T. Wlisczak, M. Dworschak, R. Seitelberger, E. Wolner and H.J. Ankersmit, Heat shock proteins 27, 60, 70, 90alpha, and 20S proteasome in on-pump versus off-pump coronary artery bypass graft patients, *Ann. Thorac. Surg.* 85 (2008) 80-87.
- [55] C. Jakob, K. Egerer, P. Liebisch, S. Turkmen, I. Zavrski, U. Kuckelkorn, U. Heider, M. Kaiser, C. Fleissner, J. Sterz, L. Kleeberg, E. Feist, G.R. Burmester, P.M. Kloetzel and O. Sezer, Circulating proteasome levels are an independent prognostic factor for survival in multiple myeloma, *Blood*. 109 (2007) 2100-2105.
- [56] W. Ma, H. Kantarjian, S. O'Brien, I. Jilani, X. Zhang, Z. Estrov, A. Ferrajoli, M. Keating, F. Giles and M. Albitar, Enzymatic activity of circulating proteasomes correlates with clinical behavior in patients with chronic lymphocytic leukemia, *Cancer*. (2008).
- [57] B. Dahlmann, B. Becher, A. Sobek, C. Ehlers, F. Kopp and L. Kuehn, In vitro activation of the 20S proteasome, *Enzyme Protein*. 47 (1993) 274-284.
- [58] K. Bo Kim, F.N. Fonseca and C.M. Crews, Development and characterization of proteasome inhibitors, *Methods Enzymol*. 399 (2005) 585-609.
- [59] S.I. Reed, Ratchets and clocks: the cell cycle, ubiquitylation and protein turnover, *Nat. Rev. Mol. Cell Biol*. 4 (2003) 855-864.
- [60] A. Bhui-Kaur, A. Therwath, L. Henry, J. Chiesa, A. Kurkure, K. Scherrer and J.P. Bureau, Increased prosomal proteins in breast cancer cells and in neighboring normal cells in Parsi and non-Parsi populations, *J. Cancer Res. Clin. Oncol*. 124 (1998) 117-126.
- [61] H. Kanayama, K. Tanaka, M. Aki, S. Kagawa, H. Miyaji, M. Satoh, F. Okada, S. Sato, N. Shimbara and A. Ichihara, Changes in expressions of proteasome and ubiquitin genes in human renal cancer cells, *Cancer Res*. 51 (1991) 6677-6685.

- [62] B. Dahlmann, T. Ruppert, L. Kuehn, S. Merforth and P.M. Kloetzel, Different proteasome subtypes in a single tissue exhibit different enzymatic properties, *J. Mol. Biol.* 303 (2000) 643-653.
- [63] J.D. Mountz, Significance of increased circulating proteasome in autoimmune disease, *J. Rheumatol.* 29 (2002) 2027-2030.
- [64] T. Lavabre-Bertrand, L. Henry, I. Guiraud, S. Carillo and J.P. Bureau, [The proteasome and malignant hemopathies], *Morphologie.* 84 (2000) 39-43.
- [65] M. Chevallet, H. Diemer, A. Van Dorssealer, C. Villiers and T. Rabilloud, Toward a better analysis of secreted proteins: the example of the myeloid cells secretome, *Proteomics.* 7 (2007) 1757-1770.
- [66] G. Alvarez-Llamas, E. Szalowska, M.P. de Vries, D. Weening, K. Landman, A. Hoek, B.H. Wolffenbuttel, H. Roelofsen and R.J. Vonk, Characterization of the human visceral adipose tissue secretome, *Mol. Cell. Proteomics.* 6 (2007) 589-600.
- [67] C. Wojcik and G.N. DeMartino, Intracellular localization of proteasomes, *Int. J. Biochem. Cell Biol.* 35 (2003) 579-589.
- [68] W. Nickel, Unconventional secretory routes: direct protein export across the plasma membrane of mammalian cells, *Traffic.* 6 (2005) 607-614.
- [69] R. Sullivan, F. Saez, J. Girouard and G. Frenette, Role of exosomes in sperm maturation during the transit along the male reproductive tract, *Blood Cells Mol. Dis.* 35 (2005) 1-10.
- [70] S. Andonian, K. Jarvi, A. Zini and L. Hermo, Ultrastructural features of the vas deferens from patients undergoing vasectomy and vasectomy reversal, *J. Androl.* 23 (2002) 691-701.
- [71] D.L. Delacroix, F.X. Marchandise, C. Francis and Y. Sibille, Alpha-2-macroglobulin, monomeric and polymeric immunoglobulin A, and

- immunoglobulin M in bronchoalveolar lavage, *Am. Rev. Respir. Dis.* 132 (1985) 829-835.
- [72] K.J. Kim and A.B. Malik, Protein transport across the lung epithelial barrier, *Am. J. Physiol. Lung. Cell. Mol. Physiol.* 284 (2003) L247-259.
- [73] M. Okada, S. Miyazaki and Y. Hirasawa, Increase in plasma concentration of ubiquitin in dialysis patients: possible involvement in beta 2-microglobulin amyloidosis, *Clin. Chim. Acta.* 220 (1993) 135-144.
- [74] C. Asseman, V. Pancre, A. Delanoye, A. Capron and C. Auriault, A radioimmunoassay for the quantification of human ubiquitin in biological fluids: application to parasitic and allergic diseases, *J. Immunol. Methods.* 173 (1994) 93-101.
- [75] H. Manaka, T. Kato, K. Kurita, T. Katagiri, Y. Shikama, K. Kujirai, T. Kawanami, Y. Suzuki, K. Nihei, H. Sasaki and et al., Marked increase in cerebrospinal fluid ubiquitin in Creutzfeldt-Jakob disease, *Neurosci. Lett.* 139 (1992) 47-49.
- [76] T. Kudo, K. Iqbal, R. Ravid, D.F. Swaab and I. Grundke-Iqbal, Alzheimer disease: correlation of cerebro-spinal fluid and brain ubiquitin levels, *Brain Res.* 639 (1994) 1-7.
- [77] G.P. Wang, K. Iqbal, G. Bucht, B. Winblad, H.M. Wisniewski and I. Grundke-Iqbal, Alzheimer's disease: paired helical filament immunoreactivity in cerebrospinal fluid, *Acta Neuropathol.* 82 (1991) 6-12.
- [78] K. Takada, N. Hibi, Y. Tsukada, T. Shibasaki and K. Ohkawa, Ability of ubiquitin radioimmunoassay to discriminate between monoubiquitin and multi-ubiquitin chains, *Biochim. Biophys. Acta.* 1290 (1996) 282-288.
- [79] K. Takada, H. Nasu, N. Hibi, Y. Tsukada, T. Shibasaki, K. Fujise, M. Fujimuro, H. Sawada, H. Yokosawa and K. Ohkawa, Serum concentrations of free ubiquitin and multiubiquitin chains, *Clin. Chem.* 43 (1997) 1188-1195.

- [80] M. Takagi, M. Yamauchi, G. Toda, K. Takada, T. Hirakawa and K. Ohkawa, Serum ubiquitin levels in patients with alcoholic liver disease, *Alcohol Clin. Exp. Res.* 23 (1999) 76S-80S.
- [81] E. Akarsu, I. Pirim, I. Capoglu, O. Deniz, G. Akcay and N. Unuvar, Relationship between electroneurographic changes and serum ubiquitin levels in patients with type 2 diabetes, *Diabetes Care.* 24 (2001) 100-103.
- [82] E. Akarsu, I. Pirim, N.Y. Selcuk, H.Z. Tombul and R. Cetinkaya, Relation between serum ubiquitin levels and KT/V in chronic hemodialysis patients, *Nephron.* 88 (2001) 280-282.
- [83] H. Daino, I. Matsumura, K. Takada, J. Odajima, H. Tanaka, S. Ueda, H. Shibayama, H. Ikeda, M. Hibi, T. Machii, T. Hirano and Y. Kanakura, Induction of apoptosis by extracellular ubiquitin in human hematopoietic cells: possible involvement of STAT3 degradation by proteasome pathway in interleukin 6-dependent hematopoietic cells, *Blood.* 95 (2000) 2577-2585.
- [84] M. Majetschak, S.M. Cohn, J.A. Nelson, E.H. Burton, U. Obertacke and K.G. Proctor, Effects of exogenous ubiquitin in lethal endotoxemia, *Surgery.* 135 (2004) 536-543.
- [85] M. Majetschak, S.M. Cohn, U. Obertacke and K.G. Proctor, Therapeutic potential of exogenous ubiquitin during resuscitation from severe trauma, *J. Trauma.* 56 (2004) 991-999; discussion 999-1000.
- [86] M. Kurimura, H. Shirai, K. Takada, H. Manaka and T. Kato, An increase in cerebrospinal fluid ubiquitin in human global brain ischemia--a prognostic marker for anoxic-ischemic encephalopathy, *Rinsho Shinkeigaku.* 37 (1997) 963-968.
- [87] M. Griebnow, P. Casalis, C. Woiciechowsky, M. Majetschak and U.W. Thomale, Ubiquitin reduces contusion volume after controlled cortical impact injury in rats, *J. Neurotrauma.* 24 (2007) 1529-1535.

- [88] M.B. Patel, K.G. Proctor and M. Majetschak, Extracellular ubiquitin increases in packed red blood cell units during storage, *J Surg Res.* 135 (2006) 226-232.
- [89] M. Majetschak, D.R. King, U. Krehmeier, L.T. Busby, C. Thome, S. Vajkoczy and K.G. Proctor, Ubiquitin immunoreactivity in cerebrospinal fluid after traumatic brain injury: clinical and experimental findings, *Crit. Care Med.* 33 (2005) 1589-1594.
- [90] G. Goldstein, M. Scheid, U. Hammerling, D.H. Schlesinger, H.D. Niall and E.A. Boyse, Isolation of a polypeptide that has lymphocyte-differentiating properties and is probably represented universally in living cells, *Proc. Natl. Acad. Sci. U. S. A.* 72 (1975) 11-15.
- [91] M. Majetschak, U. Krehmeier, M. Bardenheuer, C. Denz, M. Quintel, G. Voggenreiter and U. Obertacke, Extracellular ubiquitin inhibits the TNF-alpha response to endotoxin in peripheral blood mononuclear cells and regulates endotoxin hyporesponsiveness in critical illness, *Blood.* 101 (2003) 1882-1890.
- [92] A.E. Kieffer, Y. Goumon, O. Ruh, S. Chasserot-Golaz, G. Nullans, C. Gasnier, D. Aunis and M.H. Metz-Boutigue, The N- and C-terminal fragments of ubiquitin are important for the antimicrobial activities, *Faseb J.* 17 (2003) 776-778.
- [93] V. Pancre, R.J. Pierce, F. Fournier, M. Mehtali, A. Delanoye, A. Capron and C. Auriault, Effect of ubiquitin on platelet functions: possible identity with platelet activity suppressive lymphokine (PASL), *Eur. J. Immunol.* 21 (1991) 2735-2741.
- [94] M. Nakamura, R.M. Xavier and Y. Tanigawa, Ubiquitin-like moiety of the monoclonal nonspecific suppressor factor beta is responsible for its activity, *J. Immunol.* 156 (1996) 532-538.
- [95] T. Nabika, M. Terashima, I. Momose, Y. Hosokawa, N. Nagasue and Y. Tanigawa, Synergistic effect of ubiquitin on lipopolysaccharide-induced TNF-

- alpha production in murine macrophage cell line RAW 264.7 cells, *Biochim. Biophys. Acta.* 1450 (1999) 25-34.
- [96] Y. Ohe, K. Ishikawa, Z. Itoh and K. Tatemoto, Cultured leptomeningeal cells secrete cerebrospinal fluid proteins, *J. Neurochem.* 67 (1996) 964-971.
- [97] M.G. Katz-Jaffe, W.B. Schoolcraft and D.K. Gardner, Analysis of protein expression (secretome) by human and mouse preimplantation embryos, *Fertil Steril.* 86 (2006) 678-685.
- [98] L. Hermo and D. Jacks, Nature's ingenuity: bypassing the classical secretory route via apocrine secretion, *Mol. Reprod. Dev.* 63 (2002) 394-410.
- [99] P. Sutovsky, R. Moreno, J. Ramalho-Santos, T. Dominko, W.E. Thompson and G. Schatten, A putative, ubiquitin-dependent mechanism for the recognition and elimination of defective spermatozoa in the mammalian epididymis, *J. Cell Sci.* 114 (2001) 1665-1675.
- [100] T.H. Lippert, H. Seeger, G. Schieferstein and W. Voelter, Immunoreactive ubiquitin in human seminal plasma, *J Androl.* 14 (1993) 130-131.
- [101] R. Einspanier, H. Schuster and D. Schams, A comparison of hormone levels in follicle-lutein-cysts and in normal bovine ovarian follicles, *Theriogenology.* 40 (1993) 181-188.
- [102] P. Sutovsky, Ubiquitin-dependent proteolysis in mammalian spermatogenesis, fertilization, and sperm quality control: killing three birds with one stone, *Microsc. Res. Tech.* 61 (2003) 88-102.
- [103] R. Jones, Sperm survival versus degradation in the Mammalian epididymis: a hypothesis, *Biol. Reprod.* 71 (2004) 1405-1411.
- [104] K.M. Baska, G. Manandhar, D. Feng, Y. Agca, M.W. Tengowski, M. Sutovsky, Y.J. Yi and P. Sutovsky, Mechanism of extracellular ubiquitination in the mammalian epididymis, *J. Cell Physiol.* (2007).

- [105] G.A. Cornwall, H.H. von Horsten, D. Swartz, S. Johnson, K. Chau and S. Whelly, Extracellular quality control in the epididymis, *Asian J Androl.* 9 (2007) 500-507.
- [106] C.J. Jeffery, Multifunctional proteins: examples of gene sharing, *Ann. Med.* 35 (2003) 28-35.
- [107] P. Tompa, C. Szasz and L. Buday, Structural disorder throws new light on moonlighting, *Trends Biochem. Sci.* 30 (2005) 484-489.
- [108] R.C. Aguilar and B. Wendland, Ubiquitin: not just for proteasomes anymore, *Curr. Opin. Cell Biol.* 15 (2003) 184-190.
- [109] J.D. Schnell and L. Hicke, Non-traditional functions of ubiquitin and ubiquitin-binding proteins, *J. Biol. Chem.* 278 (2003) 35857-35860.
- [110] N. Shcherbik and D.S. Haines, Ub on the move, *J. Cell Biochem.* 93 (2004) 11-19.
- [111] K. Scherrer and F. Bey, The prosomes (multicatalytic proteinases; proteasomes) and their relationship to the untranslated messenger ribonucleoproteins, the cytoskeleton, and cell differentiation, *Prog. Nucleic Acid Res. Mol. Biol.* 49 (1994) 1-64.
- [112] E. Feist, T. Dorner, U. Kuckelkorn, S. Scheffler, G. Burmester and P. Kloetzel, Diagnostic importance of anti-proteasome antibodies, *Int. Arch. Allergy Immunol.* 123 (2000) 92-97.
- [113] A. Zoeger. Vol. PhD, pp. 92, Humboldt-Universität-Berlin, Berlin/Germany 2006.

Table 1.

Activities of 20S proteasomes purified from human plasma and various blood cells.

| | | specific activity (pmol/ng protein x min) | |
|------------------------------|--------------|---|------------|
| purified proteasomes from | Suc-LLVY-MCA | Z-LLE-MCA | Bz-VGR-MCA |
| Erythrocytes | 5.20 | 1.9 | 2.60 |
| Thrombocytes | 1.45 | 0.16 | 1.14 |
| Monocytes | 0.95 | 0.26 | 0.64 |
| T-Lymphocytes | 2.00 | 0.26 | 0.23 |
| Plasma ^{a)} | 0.26 | 0.29 | 0.20 |
| Plasma ^{b)} | 0.026 | 0.028 | 0.020 |

Specific activities are given as pmol substrate hydrolysis per ng purified proteasome per min. Data for proteasomes from erythrocytes, thrombocytes, monocytes and t-lymphocytes are given in [113] and from blood plasma were calculated on the basis of the activities given in [56] either pertained to a mean normal concentration of ^{a)}220 ng/ml [46, 48, 55], or of ^{b)}2200 ng/ml blood plasma [50-53].