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Proteasome function shapes innate and adaptive immune responses

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RUNNING TITLE:

Proteasomes shape immune responses

ABSTRACT

The proteasome system degrades more than 80% of intracellular proteins into small peptides. Accordingly, the proteasome is involved in many essential cellular functions such as protein quality control, transcription, immune responses, cell signaling, and apoptosis. Moreover, degradation products are loaded onto major histocompatibility (MHC) class I molecules to communicate the intracellular protein composition to the immune system.

The standard 20S proteasome core complex contains three distinct catalytic active sites that are exchanged upon stimulation with inflammatory cytokines to form the so-called immunoproteasome. Immunoproteasomes are constitutively expressed in immune cells and have different proteolytic activities compared to standard proteasomes. They are rapidly induced in parenchymal cells upon intracellular pathogen infection and are crucial for priming effective $CD8^+$ T cell-mediated immune responses against infected cells. Beyond shaping these adaptive immune reactions, immunoproteasomes also regulate the function of immune cells by degradation of inflammatory and immune mediators. Accordingly, they emerge as novel regulators of innate immune responses. The recently unraveled impairment of immunoproteasome function by environmental challenges and by genetic variations of immunoproteasome genes might represent a currently underestimated risk factor for the development and progression of lung diseases. In particular, immunoproteasome dysfunction will dampen resolution of infections thereby promoting exacerbations, may foster autoimmunity in chronic lung diseases, and possibly contributes to immune evasion of tumor cells. Novel pharmacological tools such as site-specific inhibitors of the immunoproteasome as well as activity-based probes, however, hold promises as novel therapeutic drugs for respiratory diseases and biomarker profiling, respectively.

KEY WORDS: immunoproteasome, adaptive immunity, innate immune response, lung disease, immunoproteasome inhibitor

1. Introducing the proteasome system

In the normal course of a protein's lifetime, synthesis and degradation rate determine the half-life of both short- and long-lived proteins for cellular maintenance (119). The proteasome is a huge protease complex and the main protein degradation system within the cell: about 80 % of all cellular proteins are processed by the proteasome into peptides of 3-22 amino acids in length (94). Controlled protein breakdown by the proteasome involves tagging of protein substrates with ubiquitin chains mainly linked at the lysine at position 48 (K48) via a cascade of E1, E2, and E3 ubiquitin-activating, -conjugating, and -ligating enzymes, respectively. However, ubiquitin-independent degradation by the proteasome has also been described (15, 60). Degradation products can be used to recycle amino acids or are loaded onto major histocompatibility (MHC) class I molecules to communicate the intracellular protein composition to the immune system (33, 63): even though less than 0.1 % of the peptides generated by the proteasome are presented at the cell surface as antigens, this system is efficient in eliciting a cytotoxic T cell response towards infected or malignant cells (120). Due to the broad nature of substrates, the proteasome is involved in many essential cellular functions such as protein quality control, transcription, immune responses, cell signaling, and apoptosis (33, 95). Moreover, degradation of damaged and misfolded proteins is also mainly taken over by the ubiquitin-proteasome system (36, 75). This function is of central importance to counteract the cytotoxic potential of damaged proteins that arise upon oxidative modification of amino acids and subsequent exposure of hydrophobic amino acid side chains. In the lung, proteins have been shown to be modified by reactive agents, such as present in pollutants and cigarette smoke, or which are generated at conditions of oxidative stress during immune responses (5). The impact of proteasome dysfunction for protein quality control and proteostasis in chronic lung diseases has recently been covered by several reviews (5, 75, 76,

81). The consequences of proteasome dysfunction for innate and adaptive immune responses in the lung, however, have not been considered so far and will be the focus of this review.

2. The proteasome's catalytic activity

The proteasome consists of a central 20S catalytic core particle, which is activated by proteasome regulators (Figure 1). Several regulators are known that bind to and thus mediate opening of the 20S proteasome for substrate entry (76). The 19S particle is the best studied regulator: it consists of at least 18 different subunits, including ubiquitin receptors and deubiquitinating enzymes, and accounts for ubiquitin- and ATP-dependent degradation of substrates (67). Together with the 20S, it forms the 26S/30S proteasome by binding to one or both sides of the 20S core, respectively. Two 11S-types of regulators are known: the IFNγ-inducible heteroheptameric PA28α/β and the homoheptameric PA28γ, which can only be found in the nucleus. Furthermore, two monomeric regulators, PA200 as well as PI31, have been described. Proteasome regulators have been shown to determine substrate specificity and turnover rate (105).

The 20S proteasome consists of a barrel-shaped core particle composed of four rings comprising seven subunits each (Figure 1). Seven related, but distinct α-subunits form the two outer α-rings (33). Because the N-termini of the α-subunits close the entry pore and inhibit substrate entry, the 20S core particle only allows entry of unfolded proteins. Three of the seven β-subunits that constitute each of the two inner β-rings are catalytically active and confer the proteolytic capacity of the 20S proteasome. These three β-subunits determine the species of the 20S core particle: depending on the cell-type, cytokine milieu, or activation state of the cell, different β-subunits are expressed and incorporated into mature 20S. The standard 20S proteasome is expressed in every cell-type and integrates the β1, β2, and β5 subunits which cleave after acidic, basic, or hydrophobic amino acids, respectively (45). In

immune cells, however, three different β-subunits are constitutively expressed (103): low molecular mass protein (LMP) 2, multicatalytic endopeptidase complex-like 1 (MECL-1), and LMP7 (also called $\beta1_i$, $\beta2_i$, and $\beta5_i$). In non-immune cells, these three so-called immunosubunits can be induced by interferon (IFN) γ or tumor necrosis factor (TNF) α signaling (2, 40). In addition, several other stimuli have been identified that upregulate immunosubunits including retinoic acid (118), nitric oxide (64), cytokines such as IL-4 (23), Toll-like receptor agonists and type I interferons (101) and mTOR signaling (121). Given the multitude and variety of stimuli that triggers immunoproteasome subunit expression, it is tempting to rename "immunoproteasome" to "inducible proteasome" as these specialized types of proteasomes appear to be not restricted to immune responses anymore. When immunosubunits are expressed, they are preferentially incorporated into newly assembled 20S immunoproteasomes (50, 58). Furthermore, they exhibit altered cleavage preferences compared to standard proteasomes, with a strongly reduced post-acidic cleavage activity based on the β1/LMP2 exchange, leading to generation of peptides that are preferentially loaded onto MHC I molecules compared to peptides derived from standard proteasomes (38). In addition, mixed proteasomes consisting of both standard and immunoproteasome subunits have been described which contribute to an even more diverse peptide pool (24).

3. Immunoproteasomes facilitate $CD8^+$ T-cell mediated resolution of intracellular infections

Immunoproteasomes are of crucial importance for $CD8^+$ T cell-mediated immune responses against intracellular infections (73). Specifically, they play an essential role at three crucial checkpoints: Firstly, immunoproteasomes are important for negative selection of autoreactive $CD8^+$ T cells in the thymus upon development of the immune system: immunoproteasomes are expressed in medullary thymic epithelial cells (mTECs) where they contribute to the

generation of the cellular "self"-peptide repertoire that is presented to developing $CD8^+$ T cells (87). Thereby, selection of only those T cells that do not bind to "self" peptide/MHC I complexes is achieved (3, 39). The remaining naïve $CD8^+$ T cells migrate to lymph nodes and persist until they are activated by antigen-presenting cells (APCs) in order to execute their effector function and combat infections.

Secondly, APCs, especially dendritic cells (DC), mainly express immunoproteasomes (103). DCs are able to engulf necrotic particles of infected cells, and migrate to draining lymph nodes upon maturation. There they present immunoproteasome-derived pathogen-peptides on MHC I together with co-stimulatory molecules to evoke a specific $CD8^+$ T cell responses (so-called cross-presentation). With the help of APCs, intracellular viral or bacterial infections are thus communicated to naïve $CD8^+$ T cells in the lymph nodes to induce a pathogen-specific adaptive immune response. After activation, the $CD8^+$ T cells move to the site of infection and patrol the infected organ in search for their specific antigen bound to MHC I to kill the infected cell (96).

Thirdly, to limit pathogen replication by selective killing of infected cells, cells need to signal their infection status to patrolling activated $CD8^+$ T cells. In order to be recognized by $CD8^+$ T cells, infected cells upregulate immunoproteasome expression to present exactly the same immunoproteasome-generated pathogen antigen as during $CD8^+$ T cell activation by the APC (55, 101) (Figure 2). Importantly, immunoproteasomes are downregulated after the infection is resolved in order to limit possible autoreactivity of $CD8^+$ T cells to non-infected cells (38, 39).

Immunoproteasomes thus enhance antigen presentation by increasing the quantity (32, 78) and/or quality of peptides for MHC I antigen presentation (38, 106). Indeed, immunoproteasomes have been reported to shape the MHC I peptide repertoire which was illustrated by the use of proteasome inhibitors and immunoproteasome knock-out mice, either

of single or of all three immunosubunits (8, 57, 113). Accordingly, immunoproteasomes dictate expansion of $CD8^+$ T cells clones after infection as shown in several mouse models of viral or bacterial infections. In these models, and strongly depending on the immunodominant epitopes of the pathogen, effects of immunoproteasome (subunit) deficiency ranged from no detectable differences in virulence (18, 84), altered antigenic peptide presentation and $CD8^+$ T cell response (6, 37, 46, 48, 89, 97, 98, 102, 110, 123) to even increased morbidity and mortality (85, 109). These studies emphasize the importance of immunoproteasomes during infection to enhance MHC I antigen presentation and to increase generation of pathogen-derived peptides. One well-studied example represents the influenza A virus which is an important trigger of exacerbations in chronic respiratory diseases such as chronic obstructive pulmonary disease (COPD) (100): two immunodominant MHC I epitopes have been shown to be differentially processed by standard and immunoproteasomes in C57BL/6 mice (123). Whether these results can be translated to influenza A human MHC I epitopes, however, has not been investigated so far.

4. Immunoproteasomes protect from autoimmunity

Intriguingly, the cell type- and tissue-specific distribution of immunoproteasomes is important for protecting the organism from autoimmunity after infection. Immune cells such as APCs constitutively express immunoproteasomes, whereas parenchymal cells only express them in response to inflammatory cytokines such as IFNγ or TNFα (Figure 2). During $CD8^+$ T cell priming in the lymphatic tissues, both immunoproteasome-derived pathogen-, but also "self"-antigens are presented on MHC I by the APC. If a "self"-reactive $CD8^+$ T cell, despite thymic selection, would be activated during infection by an APC, the same immunoproteasome-dependent "self"-antigen might be presented by an infected parenchymal cell. The epitope would cease to be presented by parenchymal cells after the infection is resolved, because

177 immunoproteasomes are gradually replaced by standard proteasomes (42). Certain
178 immunoproteasome-derived “self”-antigens are thus presented to the immune system only
179 during infection, thereby protecting from autoreactive immune responses after resolution of
180 infection (30, 38, 101).
181 Indeed, it has been shown that immunoproteasomes are inappropriately expressed in human
182 autoimmune disorders (29, 35, 65, 77) and experimental models of autoimmunity (7, 14).
183 Accordingly, the use of novel immunoproteasome-specific inhibitors has been proposed for
184 treatment of autoimmune disorders (13, 16, 61, 63, 111). These inhibitors have been proven to
185 successfully counteract autoimmune responses in several experimental models of autoimmune
186 diseases (7, 11, 47, 79, 82, 122). Inhibition of immunoproteasomes in autoimmunity could
187 have two beneficial and synergistic effects: a) presentation of immunoproteasome-dependent
188 “self”-antigens by parenchymal cells might be hindered and b) inflammatory cytokine
189 secretion by immune cells might be dampened (79).
190 Furthermore, single nucleotide polymorphisms (SNP) of proteasome subunits have been
191 associated with autoimmune diseases, however, with partially conflicting results (an overview
192 can be found in Supplementary Table S1 in (76)).
193

194 **5. Immunoproteasomes shape immune cell function and innate immune responses**

195 Beyond shaping adaptive immune reactions, proteasomes and in particular
196 immunoproteasomes regulate the function of immune cells by degradation of inflammatory
197 and immune mediators. Special interest and conflicting data exist on the role of
198 immunoproteasomes in NFκB signaling, which might reflect cell type-specific effects or the
199 outcome of different experimental settings (44, 49, 72). Our own and partially unpublished
200 data indicate that NFκB signaling is not affected by deletion of the immunoproteasome
201 subunits LMP2 or LMP7: NFκB promotor-driven reporter gene as well as NFκB target gene

expression were unchanged in alveolar macrophages of LMP2-deficient mice after LPS and IFNγ-induced macrophage polarization (23). Several mutations in the human *PSMB8* and *PSMB9* genes encoding the LMP7 and LMP2 immunoproteasome subunits, respectively, have been discovered in autoinflammatory disorders (1, 4, 17, 62, 70, 74). In addition, mutations in other 20S proteasome subunits have been identified leading to e.g. reduced expression, misfolding or impaired 20S incorporation of mutated subunits and therefore changes in cellular proteostasis (17). These diseases have been combined in the so-called proteasome-associated autoinflammatory disorders (PRAAS).

Cells of the adaptive immune system have been shown to be regulated by the immunoproteasome subunit LMP7: The differentiation potential of naïve $CD4^+$ T helper cells to Th1/Th17 was impaired while regulatory T cell differentiation was enhanced in the absence or catalytic inhibition of LMP7 due to altered cellular signaling (51). The immunological phenotype of immunoproteasome knock-out mice also points towards altered proteostasis leading to changes in immune cell function: LMP2-deficient mice display less B cells as well as reduced numbers of peripheral $CD4^+$ and $CD8^+$ T cells (44). MECL-1 knock-out (k.o.) mice show an altered T cell repertoire (10) and combined deletion of both MECL-1 and LMP7 leads to hyperproliferation of T cells (22). Inhibitors of LMP7 have also been shown to influence inflammatory cytokine production which might add to their beneficial effects in preclinical models of autoimmunity (79).

Less attention has been paid to the role of immunoproteasomes in innate immunity. Van Helden and colleagues did not observe changes in natural killer (NK) cell education in MECL-1/LMP7 double k.o. mice, but immunoproteasome-deficiency in splenocytes led to their rejection only in virus-infected, but not naïve recipient wildtype mice in an NK-dependent manner (43). Our own study demonstrated altered polarization capacities of alveolar macrophages upon immunoproteasome subunit deficiency. These cells express high

levels of immunoproteasomes (54). While the pro-inflammatory IFNγ/LPS-induced M1 phenotype was not changed in LMP7-deficient primary macrophages, IL-4 treatment of wildtype or LMP7-deficient cells resulted in augmented M2 polarization marker gene expression, increased M2-signalling (via STAT6 and AKT), and an increase in IL4Rα expression already at baseline (23). Catalytic inhibition of LMP7 with the immunoproteasome-specific inhibitor ONX-0914 led to similar results. These data were partially confirmed in a recent study by Kimura and colleagues: compared to wildtype animals, LMP7-deficient mice exhibited increased levels of M2 marker gene expression in white adipose tissue after high-fat-diet while there was no change in M1 marker gene expression (56).

Paeschke and colleagues observed reduced levels of the soluble cardio-protective pattern recognition receptor Pentraxin 3 upon LMP7 inhibition or genetic deletion, which was associated with exacerbated coxsackievirus B3 inflammatory injury of heart tissue (88). A recent study investigated the role of ONX-0914 in fungal infection and observed increased susceptibility to systemic candidiasis associated with a possible defect in neutrophil function (80).

Until now, it is unresolved how these changes in immune cell function are mediated. Immunoproteasome deficiency has been shown to influence the transcriptome of immune cells, which might be the result of altered substrate turnover in immunoproteasome-deficient cells (57, 112, 113). One explanation might be that immunoproteasomes increase the general pool of proteasomal catalytic capacity (50). Immunoproteasomes may also have altered substrate specificities due to differential association with proteasome regulators as described for PA28α/β (31). The recently discovered defined interplay of immunoproteasomes with fundamental cell signaling pathways such as mTOR may also specifically regulate immune cell responses (121, 124).

6. Impact of proteasome dysfunction on immune responses in respiratory diseases

As the lung is directly exposed to the environment, environmental stressors such as pollutants, pathogens, and oxidants are continuously challenging proteostasis in lungs cells. By now it is well established that proteasome function is impaired by environmental insults: it has been demonstrated that pesticides, diesel exhaust, and cigarette smoke decrease proteasome activity (59, 92, 93, 116), but also drugs such as ethanol have been shown to impair proteasome function (21, 28, 86). Environmental challenges may affect proteasome function on different levels as summarized recently (76). First, transcriptional regulation of proteasomes has been shown to be part of a protective response to oxidative stress (66). In particular, induction of immunoproteasomes has been suggested to contribute to the degradation of oxidatively modified proteins *in vitro* and *in vivo* (27, 68, 90). Seifert *et al.* demonstrated that immunoproteasome-deficient cells needed more time to resolve IFNγ-induced oxidatively modified, *i.e.* carbonylated, proteins (99). However, these results remain controversial (83). Data from our group refute a protective role of immunoproteasomes in response to cigarette smoke as alveolar macrophages from COPD patients as well as from smoke-exposed mice exhibited reduced immunoproteasome levels similar to lung parenchymal cell lines that had been treated with cigarette smoke extract (52, 53).

Second, dynamic changes in the composition of proteasomal complexes in the cell might serve as a quick means of the cell to cope with environmental stimuli (76). The 26S proteasome was shown to fall apart in response to oxidative stress (71, 108, 115), whereas PA28α/β assembled with 20S proteasomes originating from disassembled 26S proteasomes to protect from oxidative stress (34, 69, 91).

Third, proteasome activity might be directly impaired by oxidative insults. We and others have recently shown that cigarette smoke impairs both standard and immunoproteasome

activity *in vitro* and *in vivo* which correlated with elevated levels of oxidative stress (52, 93, 104). In particular, both standard and immunoproteasome activity was clearly impaired in whole lung homogenates of COPD patients in the absence of transcriptional regulation (52).

In light of the aforementioned role of immunoproteasomes in innate and adaptive immune responses, environmental impairment of immunoproteasome function but also genetic variations in immunoproteasome subunits might represent a currently underestimated risk factor for the development of lung diseases (Figure 3).

Reduced immunoproteasome activity in response to, *e.g.*, cigarette smoke might be harmful during **pulmonary infection and in acute exacerbations**: intracellular human pathogens are efficiently cleared via MHC I-dependent $CD8^+$ T cell responses that kill the infected cell and prevent further amplification of the pathogen and thus tissue damage. The important contribution of immunoproteasomes in mounting an effective adaptive anti-viral immune response has been shown, *e.g.*, for influenza A virus (57, 89, 123). It is well feasible that immunoproteasome dysfunction in patients with COPD might result in impaired antiviral immune responses towards influenza A virus infection thereby contributing to disease exacerbations as previously proposed by us (52).

In a murine model of **asthma**, LMP7-deficient mice showed reduced uptake of ovalbumin and attenuated ovalbumin-induced asthma while responses to house dust mite were comparable (114).

A missense SNP in the PSMB8 gene encoding the immunosubunit LMP7 was associated with an increased risk for the development of pigeon breeder's hypersensitivity pneumonitis, a form of **extrinsic allergic alveolitis** (20). However, whether missense SNPs in proteasomal genes result in alterations of protein levels, impaired 20S proteasome assembly, or altered association of 20S to its regulators, has not been analyzed so far.

Immunoproteasome dysfunction in innate immune cells might also increase susceptibility to diseases associated with type 2 immune responses: increased M2 polarization of alveolar macrophages could facilitate development of **pulmonary fibrosis** (23). Indeed, alveolar macrophages of IPF patients exhibited reduced levels of immunoproteasomes (52).

In **non-small cell lung cancer (NSCLC)**, immunoproteasome expression was recently found to be a prognostic factor in lung cancer patients. Low levels of immunoproteasome-expression were associated with reduced survival and increased recurrence of metastases (107). It is well known that malignant cells actively suppress immunoproteasome function to evade $CD8^+$ T cell surveillance similar to the strategy that is used by several viruses (73). One tactic of cancer cells is expression of a non-functional transcript variant of the LMP7 protein which is not incorporated into mature 20S proteasomes. These cells are thus immunoproteasome-deficient (41). This immunoproteasome repression has been exploited for therapeutic strategies with autologous dendritic cells of tumor patients that were pulsed with tumor antigens and siRNA directed against immunoproteasomes to match the peptidome of the antigen presenting cell with the tumor MHC I peptidome (25, 26).

7. Therapeutic interventions targeting the immunoproteasome in the lung

The cell-type specific expression of immunoproteasomes in immune cells can be utilized to specifically target these cells with immunoproteasome-specific inhibitors. Compared to the FDA-approved proteasome inhibitors such as Bortezomib and Carfilzomib, which do not discriminate between standard and immunoproteasome, these newly developed inhibitors are specific for immunoproteasome subunits (61). As mentioned above, they have successfully been used in several preclinical models of autoimmune diseases to counteract autoimmune responses in first proof-of-concept studies. The anti-inflammatory effect of immunoproteasome-specific inhibition might also prove useful in overshooting

(auto-)inflammatory pulmonary diseases such as acute respiratory distress syndrome (ARDS) or sarcoidosis. Chronic treatment, however, should be avoided due to the suspected side effects of increased susceptibility to virus infections (9) and the potential risk of M2 macrophage-driven fibrotic remodeling (23). For the treatment of lung cancers, immunoproteasome-specific inhibition could represent an attractive target for combinational therapy, however, the levels of active immunoproteasomes should be determined first to maximize the benefit for the patient and to limit side-effects (12, 117),

8. Conclusion

With the recent success of site-specific inhibitors of the immunoproteasome in preclinical models of autoimmune diseases exiting new possibilities have arisen that will allow therapeutic targeting of inappropriate immunoproteasome activity in disease. Moreover, these inhibitors will foster a deeper understanding of the biological role of immunoproteasomes such as the identification of immunoproteasome-specific substrates in immune cells to unravel potential adverse effects on immunoproteasome-specific inhibitors. Furthermore, the possibility to monitor subunit-specific inhibition of the proteasome with activity-based probes (19) raises the prospect of monitoring immunoproteasome activity as a biomarker for susceptibility to infections or cancer prognosis.

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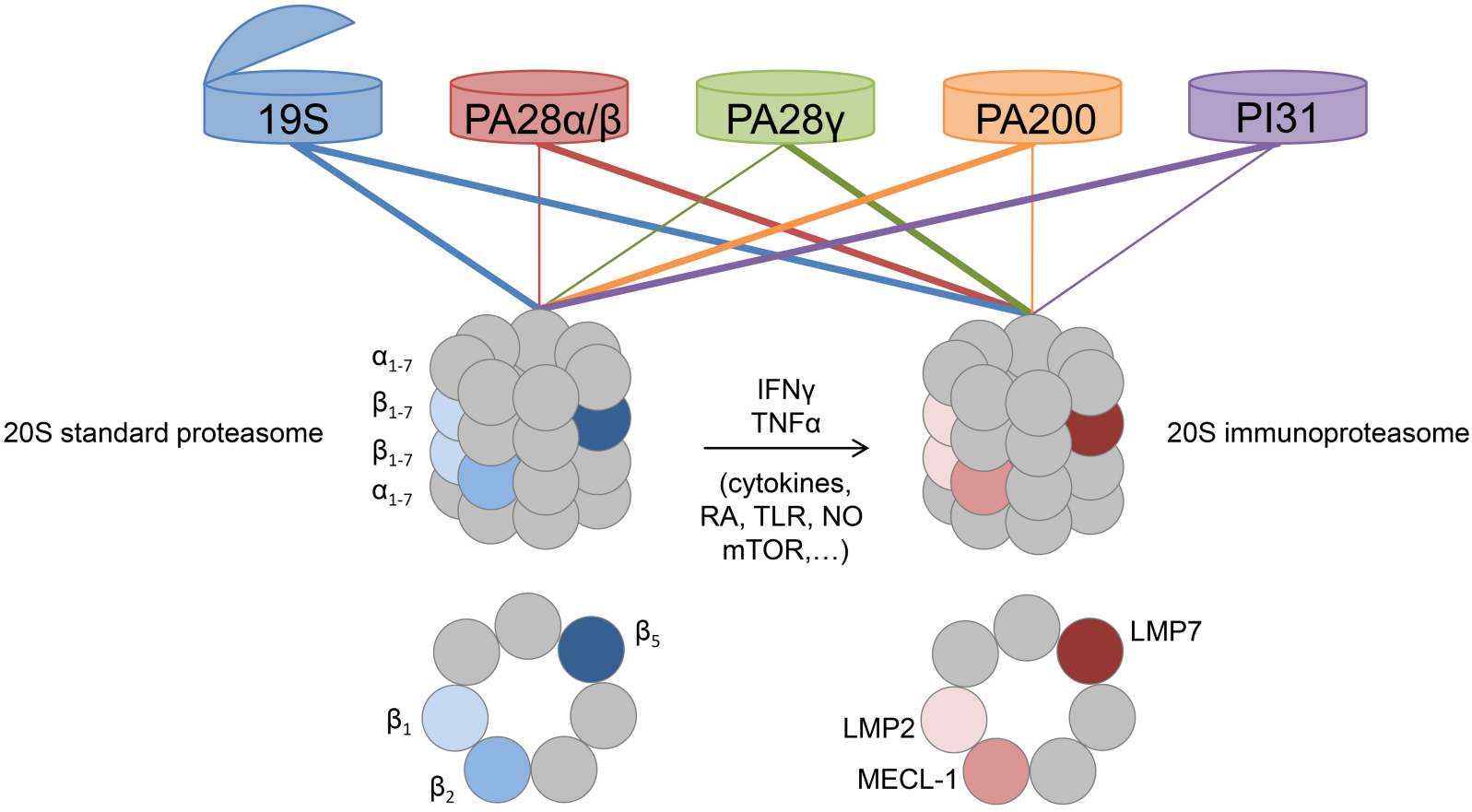
FIGURE LEGENDS

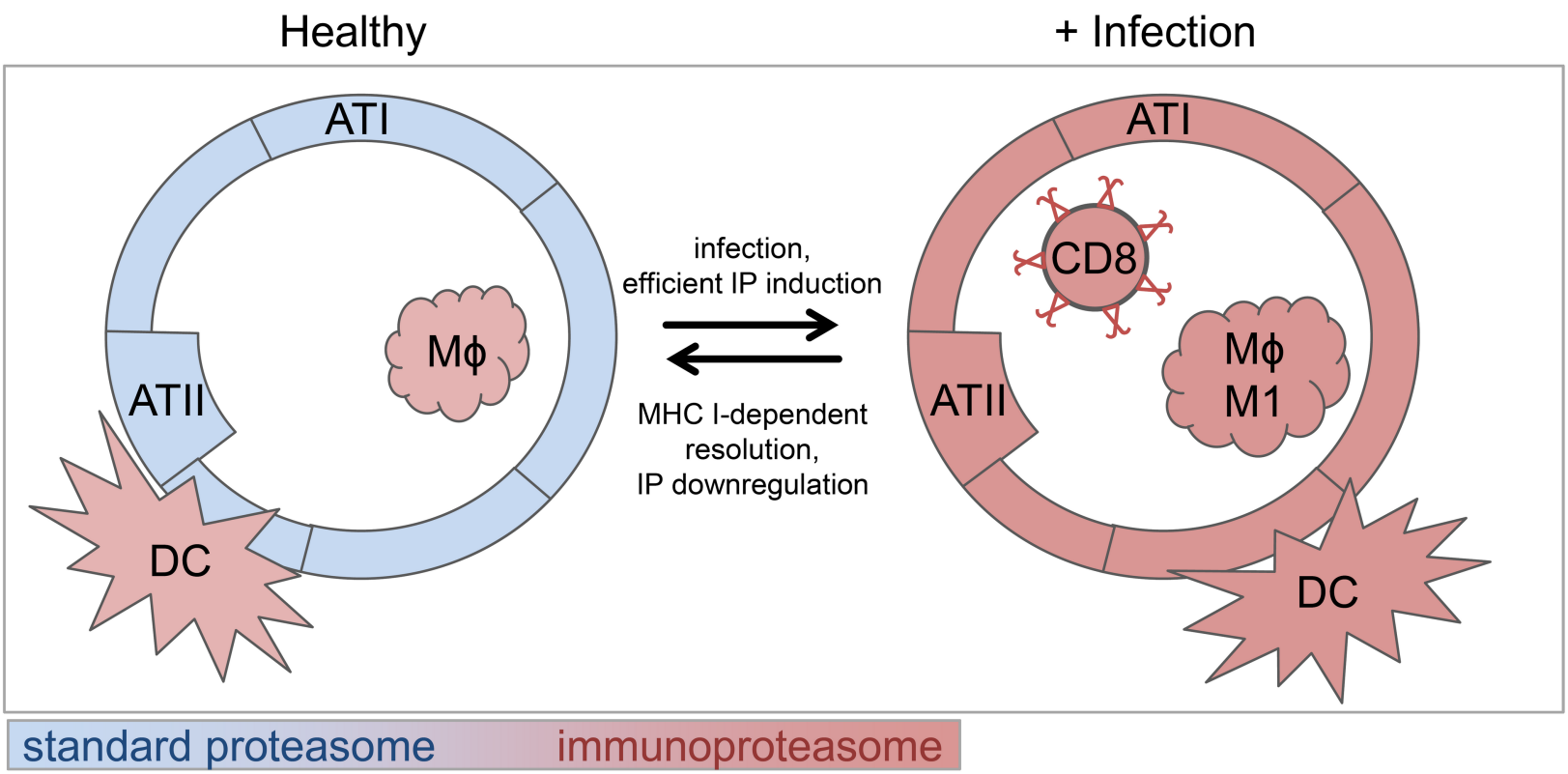
Figure 1: Variety of proteasome complexes. The 20S proteasome catalytic core is composed of four heptameric rings with an $\alpha_{1-7}\beta_{1-7}\beta_{1-7}\alpha_{1-7}$ symmetry and can exist in several forms: the 20S standard proteasome incorporates the catalytic subunits β_1, β_2, and β_5 and is constitutively expressed in every cell. Upon interferon (IFN) γ, tumor necrosis factor (TNF) α signaling or by numerous other triggers, cells upregulate expression of the three immunosubunits low molecular mass protein (LMP) 2, multicatalytic endopeptidase complex-like (MECL) 1, and LMP7 which are incorporated into newly assembled 20S immunoproteasomes. Mixed-type proteasomes are also possible containing both standard and immunosubunits. 20S proteasomes are activated by proteasome regulators. Five different regulators are known that bind to 20S proteasomes and facilitate substrate entry: the multi-subunit 19S regulator mediates ubiquitin-dependent degradation of substrates and is dependent on ATP; two heptameric regulators are PA28α/β (IFNγ-inducible, proposed role in antigen presentation) and PA28γ (found only in the nucleus, implicated in cell cycle regulation); the function of the two monomeric regulators PA200 and PI31 is not well understood. Regulators can bind to one or two sides of 20S proteasomes and may also form hybrid proteasomes consisting of the 20S core and two different activators attached to each side. Depending on the type of 20S proteasome (standard or immuno) preferential association with regulators has been proposed which is indicated by a different line thickness. Abbreviations: mTOR = mammalian target of rapamycin; NO = nitric oxide; PA = proteasome activator; PI = proteasome inhibitor; RA = retinoic acid; TLR = toll-like-receptor agonist.

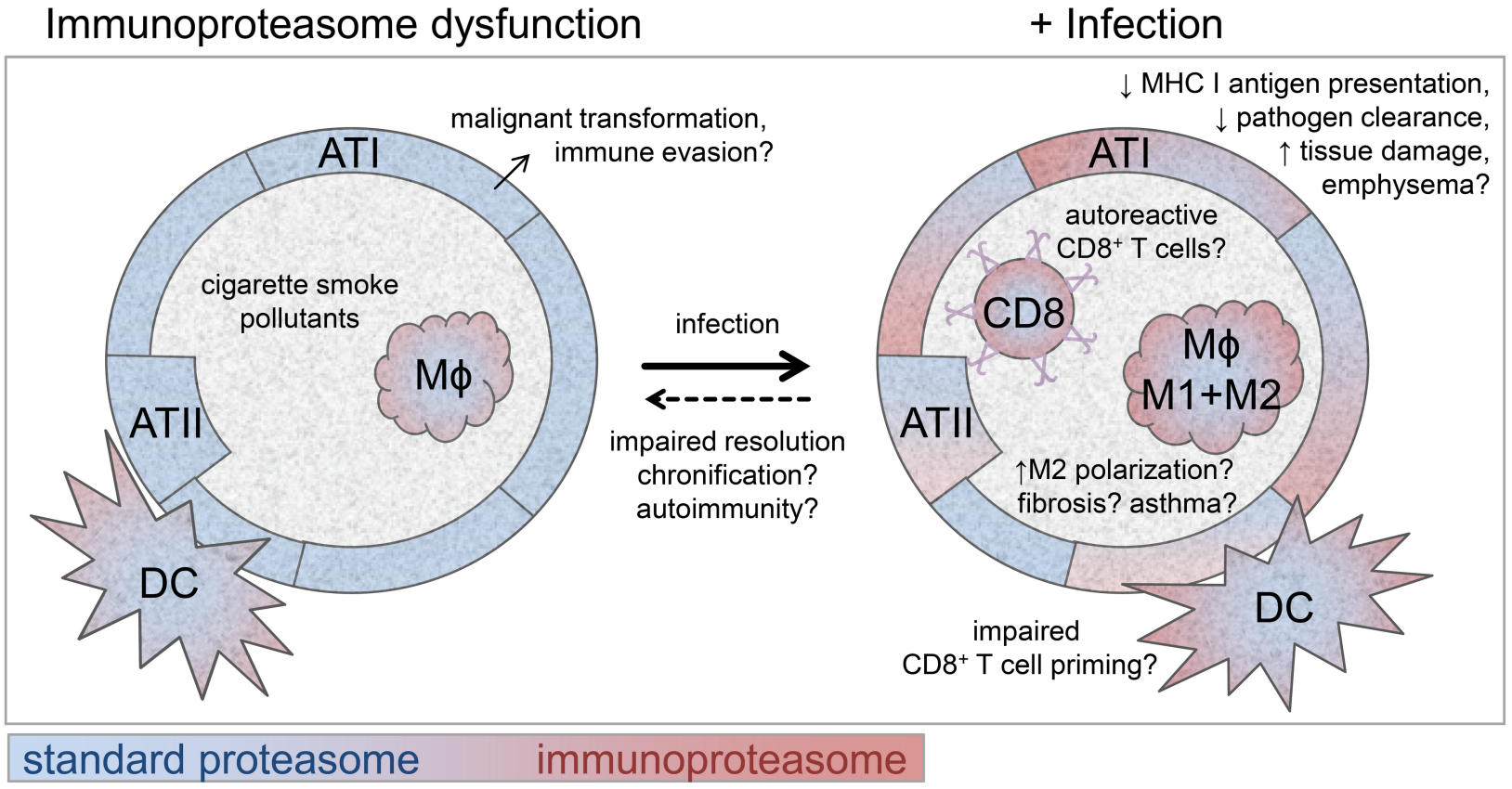
Figure 2: Immunoproteasomes facilitate clearance of respiratory infections. In the healthy lung, immunoproteasome (IP) expression is restricted to immune cells such as

789 dendritic cells (DCs) and macrophages (Mϕ). Their basal levels in parenchymal cells (alveolar
790 epithelial cell type (AT) I and II) are very low. Upon infection and signaling of inflammatory
791 cytokines, parenchymal cells upregulate immunoproteasomes to efficiently present pathogen
792 antigens via major histocompatibility (MHC) class I molecules to matching pathogen-specific
793 $CD8^+$ T cells resulting in killing of infected parenchymal cells. Thus, pathogen amplification
794 is restricted and the infection can be cleared rapidly. After resolution of infection,
795 parenchymal cells gradually replace immunoproteasomes by standard proteasomes. Potential
796 autoreactive $CD8^+$ T cells, which might also have been primed against "self"-antigens, are
797 thus prevented to become activated as the immunoproteasome-dependent MHC I peptide
798 repertoire is switched back to the standard repertoire. Therefore, immunoproteasomes help to
799 protect from autoimmunity.
800
801 **Figure 3: Model of how immunoproteasome dysfunction may predispose to chronic lung**
802 **diseases.** Impaired immunoproteasome function might occur due to genetic variations or to
803 environmental insults such as cigarette smoke or pollution. Such dysfunction will have
804 minimal effects in parenchymal cells under non-infectious conditions but may affect immune
805 surveillance of malignant cells. If immunoproteasomes cannot be induced to sufficient levels
806 upon infection or are impaired in their activity, different outcomes are conceivable. Dendritic
807 cells (DCs) with immunoproteasome dysfunction might not prime $CD8^+$ T cells with the same
808 efficiency or they might prime an altered set of $CD8^+$ T cells. These might also include
809 autoreactive T cells specific for "self"-antigens produced by standard proteasomes that are
810 also presented by parenchymal cells when infection is eventually resolved thereby promoting
811 autoimmunity. Reduced immunoproteasome activity in parenchymal cells such as alveolar
812 epithelial cell type (AT) I and II might lead to delayed resolution of infection, as the infection
813 status cannot be efficiently communicated to the immune system in the form of $CD8^+$ T cells.

Prolonged infection thus could lead to more severe tissue damage and contribute to emphysema formation. Alveolar macrophages with immunoproteasome dysfunction have increased pro-fibrotic M2 polarization capacity and might predispose to tissue remodeling as observed in asthma and pulmonary fibrosis.


19S
PA28α/β
PA28γ
PA200
PI31
α1-7
β1-7
β1-7
α1-7
20S standard proteasome
IFNγ
TNFα
(cytokines,
RA, TLR, NO
mTOR,…)
20S immunoproteasome
β5
β1
β2
LMP7
LMP2
MECL-1


Healthy
+ Infection
ATI
ATII
Mϕ
DC
infection,
efficient IP induction
MHC I-dependent
resolution,
IP downregulation
ATI
CD8
ATII
Mϕ
M1
DC
standard proteasome
immunoproteasome


Immunoproteasome dysfunction
+ Infection
malignant transformation,
immune evasion?
ATI
cigarette smoke
pollutants
Mϕ
ATII
DC
infection
impaired resolution
chronification?
autoimmunity?
↓ MHC I antigen presentation,
↓ pathogen clearance,
↑ tissue damage,
emphysema?
ATI
autoreactive
CD8⁺ T cells?
CD8
Mϕ
M1+M2
ATII
↑M2 polarization?
fibrosis? asthma?
DC
impaired
CD8⁺ T cell priming?
standard proteasome
immunoproteasome