



# On the role of the immunoproteasome in transplant rejection

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## Abstract

The immunoproteasome is expressed in cells of hematopoietic origin and is induced during inflammation by IFN- $\gamma$ . Targeting the immunoproteasome with selective inhibitors has been shown to be therapeutically effective in pre-clinical models for autoimmune diseases, colitis-associated cancer formation, and transplantation. Immunoproteasome inhibition prevents activation and proliferation of lymphocytes, lowers MHC class I cell surface expression, reduces the expression of cytokines of activated immune cells, and curtails T helper 1 and 17 cell differentiation. This might explain the *in vivo* efficacy of immunoproteasome inhibition in different pre-clinical disease models for autoimmunity, cancer, and transplantation. In this review, we summarize the effect of immunoproteasome inhibition in different animal models for transplantation.

**Keywords** Proteasome · Immunoproteasome · Antigen processing · Antigen presentation · Transplantation

## Introduction

The proteasome is responsible for the degradation of proteins in the cytoplasm and nuclei of all eukaryotic cells and exerts numerous essential regulatory functions in nearly all cell biological pathways. The 26S proteasome degrades poly-ubiquitylated protein substrates and consists of a 19S regulator and a 20S proteolytic core complex. The 19S regulator bears ubiquitin receptors and an ATPase ring in charge of protein unfolding. The 20S core complex has a barrel-shaped structure consisting of four rings with seven subunits each. The outer two rings consist of  $\alpha$  subunits; the inner two rings of  $\beta$  subunits forming the central proteolytic chamber (Huber

et al. 2012). Depending on the cell type and the presence or absence of the pro-inflammatory cytokine interferon (IFN)- $\gamma$ , the three inducible  $\beta$  subunits of the immunoproteasome low molecular mass polypeptide (LMP)2 ( $\beta$ 1i), multicatalytic endopeptidase complex-like (MECL)-1 ( $\beta$ 2i), and LMP7 ( $\beta$ 5i), can, in addition to the corresponding constitutive subunits  $\beta$ 1c,  $\beta$ 2c, and  $\beta$ 5c, enrich the cellular assortment of catalytically active  $\beta$  subunits (Fig. 1). These exchanges alter the cleavage specificity of the 20S proteasome. In immunoproteasomes, the caspase-like activity, exerted by  $\beta$ 1c in constitutive proteasomes, is strongly reduced, whereas the chymotrypsin-like activity is enhanced. The immunoproteasome-induced changes in the 20S proteasome give rise to more suitable ligands for MHC-I (Basler et al. 2013; Groettrup et al. 2010; Mishto et al. 2014). The altered class I peptidome on immunoproteasome-containing cells influences pathogen-induced cytotoxic T lymphocyte (CTL) responses, pathogen clearance, and shapes the CTL repertoire (Basler et al. 2004, 2006, 2018; Chen et al. 2001; Chou et al. 2008; Ersching et al. 2016; Guimaraes et al. 2018; Hutchinson et al. 2011). Due to the use of immunoproteasome-selective inhibitors in recent years, it became evident that the immunoproteasome has, apart from its role in antigen processing, an important function in T helper cell differentiation (Basler et al. 2018; Guo et al. 2018; Kalim et al. 2012; Muchamuel et al. 2009; Xiao et al. 2017), in macrophage polarization (Chen et al. 2016), in brain inflammation (Kremer et al. 2010; Mundt et al. 2016), in inflammatory cytokine production (Basler et al. 2010, 2014, 2018; Farini

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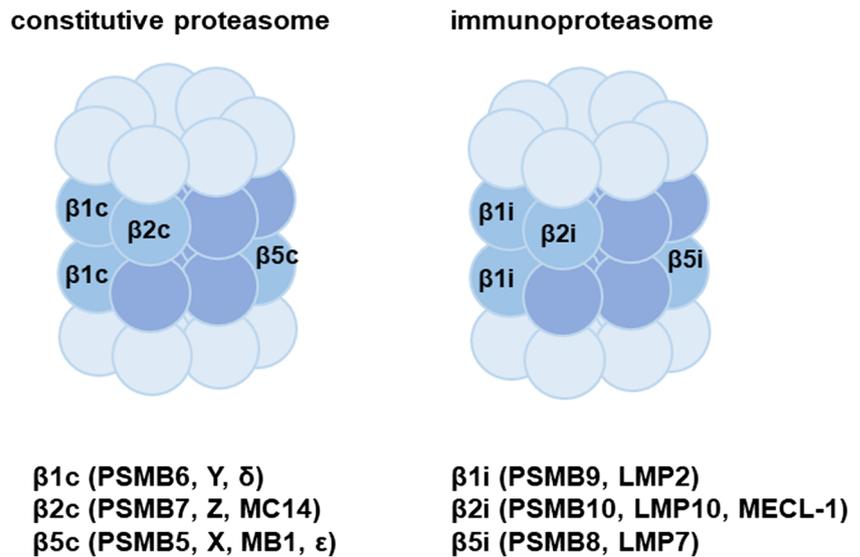
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**Fig. 1** Localization of the active site bearing subunits in the constitutive proteasome and the immunoproteasome. The proteolytic subunits of the constitutive proteasome are  $\beta 1c$  (also known as PSMB6, Y, and  $\delta$ ),  $\beta 2c$  (also known as PSMB7, Z, and MC14), and  $\beta 5c$  (also known as PSMB5, X, MB1, and  $\epsilon$ ). The proteolytic immunoproteasome subunits are  $\beta 1i$  (also known as PSMB9 and LMP2),  $\beta 2i$  (also known as PSMB10, LMP10, and MECL-1), and  $\beta 5i$  (also known as PSMB8 and LMP7)



et al. 2016; Guo et al. 2018; Muchamuel et al. 2009), in autoimmune diseases (Basler et al. 2010, 2014, 2018; Ichikawa et al. 2012; Liu et al. 2017, b; Muchamuel et al. 2009; Nagayama et al. 2012; Xiao et al. 2017), in colitis-associated cancers (Koerner et al. 2017; Vachharajani et al. 2017), in angiogenesis (Chen et al. 2018), in viral myocarditis (Althof et al. 2018), in the activation of lymphocytes (Santos et al. 2017; Sula Karreci et al. 2016), and organ transplantation (Li et al. 2018; Sula Karreci et al. 2016).

Over the course of the last century, organ transplantation has overcome major technical limitations to become the success it is today. However, in the absence of pharmacological immunosuppression, allogeneic organs are acutely rejected. In most cases, adaptive immune responses to proteins of the grafted allogeneic tissues are the major hindrance to successful transplantation. Both cellular (lymphocyte-mediated) and humoral (antibody-mediated) mechanisms are responsible for transplant rejection. Although other cell types are also involved, T cells have been demonstrated to play the central role in the rejection of grafts (Ingulli 2010). To prevent rejection, current immunosuppressive therapies that target T cells non-specifically have to be taken lifelong, leaving patients more susceptible to infections and tumors, in addition to having toxic off-target effects (Hartono et al. 2013). Hence, there is a strong need to develop alternative treatments to classical immunosuppression to induce donor-specific tolerance. Immunoproteasome inhibition might emerge as such a new treatment option. Immunoproteasome expression is restricted to immune cells and to an inflammatory environment, and therefore, selective inhibitors of the immunoproteasome can be applied below their maximally tolerated dose while still retaining their therapeutic efficacy but lacking untoward toxicity. Immunoproteasome inhibition reduces the generation of the T helper cell subsets Th1 and Th17 which are involved in transplant rejection but spares Th2 and regulatory T cells in vitro and in vivo (Basler et al. 2014; Kalim et al. 2012; Muchamuel et al. 2009;

Mundt et al. 2016). This subset preference might contribute to an improved selectivity of immunoproteasome inhibitors as compared to more general immunosuppressants. Furthermore, mice treated with immunoproteasome inhibitors can well control viral infections (Althof et al. 2018; Basler et al. 2011; Muchamuel et al. 2009; Mundt et al. 2016) and immunoproteasome inhibition can even reduce tumor formation (Koerner et al. 2017; Vachharajani et al. 2017). Hence, the immunoproteasome might qualify as a new treatment option to prevent transplant rejection.

### Effect of the immunoproteasome-associated MHC-I peptidome on transplant rejection

The use of proteasome inhibitors in vitro demonstrated that the proteasome is responsible for the generation of most ligands presented on major histocompatibility complex class I molecules (MHC-I) (Rock et al. 1994). Although proteasomes are required for MHC class I antigen presentation, they actually destroy many more peptides than they generate (Saric et al. 2001). Only 10–15% of the produced peptides are of the appropriate size for MHC class I antigen presentation while most of them are too short to fit into the binding cleft of MHC class I needing further trimming. Proteasome inhibition in vivo leads to reduced MHC-I antigen presentation resulting in an impaired anti-viral CTL response (Basler et al. 2009). Since LMP2 and LMP7 are encoded in the MHC gene locus and LMP2, LMP7, and MECL-1 are induced by IFN- $\gamma$ , a role of immunoproteasomes in optimization of MHC class I antigen processing seems obvious. Indeed, early experiments with LMP2- and LMP7-deficient mice demonstrated an involvement of the immunoproteasome in antigen processing (Van Kaer et al. 1994; Fehling et al. 1994). Later, many more studies showed that the

immunoproteasome is responsible for the generation or destruction of MHC-I ligands (summarized in Basler et al. 2013; Groettrup et al. 2010).

Transplantation presents a life-saving therapy and last resort for end-stage organ failure and can be considered among the major accomplishments of the twentieth century in human health. However, a complex series of interactions involving coordination between both the innate and adaptive immune system can result in the rejection of solid organ allografts. Recipient T cells, central to this process, recognize donor-derived antigen (alloantigens) initiating allograft rejection. In two different pathways, major and minor histocompatibility antigens can activate the immune system against the allograft. (1) T cells can either directly recognize intact non-self donor MHC molecules present on the surface of donor cells or (2) donor MHC molecules can be processed and presented as peptides on self-MHC molecules. Using a model substrate, Toes et al. showed that cells expressing immunoproteasomes display a different peptide repertoire compared to cells expressing constitutive proteasomes (Toes et al. 2001). These changes alter the overall cytotoxic T cell specificity as indicated by the observation that LMP7-deficient mice react against cells derived from LMP7-containing wild-type mice. Skin grafting experiments showed that wild-type mice were not able to reject skin from LMP7<sup>-/-</sup> mice whereas LMP7<sup>-/-</sup> mice rejected skin transplants from wild-type mice with even greater efficiency than observed for the rejection of male skin by female recipients. Similar results were obtained in mice deficient for all immunoproteasome subunits (TKO) (Kincaid et al. 2012). Mass spectrometry analysis of peptides eluted from H-2K<sup>b</sup> or H-2D<sup>b</sup> derived from splenocytes of wild-type and TKO animals revealed changes in the MHC-I peptide repertoire. Approximately one third of peptides was exclusively presented on wild-type splenocytes, one third of peptides was exclusively presented on TKO splenocytes, and one third was presented on both types. The differences in MHC class I restricted Ag presentation between constitutive and immunoproteasomes is probably due to quantitative and not qualitative differences in the proteasome-generated antigenic peptides (Mishto et al. 2014). The altered peptide presentation in TKO resulted in rejection of wild-type splenocytes in TKO mice, but not vice versa (Kincaid et al. 2012). This indicates that wild-type splenocytes present a markedly different repertoire of peptides than those found in either the thymus or periphery of TKO animals, resulting in recognition of this immunoproteasome-derived repertoire as foreign epitopes. The authors conclude that the TKO host has never seen any of the unique peptides generated by immunoproteasomes and therefore would not be tolerant to them. In contrast, wild-type mice express constitutive proteasomes and immunoproteasomes, so the animals would be expected to be tolerant to the peptides generated by both types of proteolytic complexes. Indeed, apart from its influence on class I antigen presentation in antigen-presenting

cells in the periphery, immunoproteasome expression in the thymus has been shown to alter CD8 T cell selection. Different immunoproteasome subunit-deficient mice have an altered T cell repertoire influencing CTL responses (Basler et al. 2006, 2018; Chen et al. 2001; Kincaid et al. 2016; Osterloh et al. 2006). Furthermore, a single amino acid exchange in  $\beta 2i$  (G170W) causes severe combined immunodeficiency (SCID) and systemic autoinflammation in G170W mutant mice (Treise et al. 2018). Yeast mutagenesis and crystallographic data suggest that the severe phenotype in G170W mutant mice is due to structural changes in the C-terminal appendage of  $\beta 2i$  that prevent the biogenesis of immuno- and thymoproteasomes (a special class of proteasomes in the thymus). Since proteasomes are essential for cell survival, and defective proteasome assembly causes selective death of cells expressing the mutant  $\beta 2i$ , this probably leads to death of cells responsible for positive and negative selection in the thymus, and thus most likely causes the severe immunological phenotype observed in G170W mutant mice. Taken together, immunoproteasome expression in the thymus and the periphery influences MHC-I antigen presentation and thereby modulates the cytotoxic T cell repertoire and CTL responses. Hence, due to modulating both the cytotoxic T cells and MHC-I ligands, immunoproteasomes play an important role in transplantation.

## The immunoproteasome in graft versus host disease

Hematopoietic stem cell transplantation is used to treat hematologic malignancies. Despite significant advances in tissue typing and donor selection, graft-versus-host disease (GVHD) remains a serious and deadly complication to patients, who undergo hematopoietic stem cell transplantation. GVHD results from donor T lymphocytes activated by host antigen-presenting cells, inducing an inflammatory response against host cells. Bortezomib (also named PS-341, or Velcade; Millennium Pharmaceuticals) is the first clinically approved proteasome inhibitor for relapsed and/or refractory myeloma and mantle cell lymphoma. The boronic acid dipeptide is a reversible broad-spectrum proteasome inhibitor (Adams et al. 1998; Adams et al. 1999). Cells exposed to bortezomib exhibit occupancy of constitutive and immune proteasomal proteolytic subunits, resulting in the rapid accumulation of polyubiquitinated proteins, including several regulatory molecules involved in cell signaling and survival pathways. Both T cells and dendritic cells (DCs) are directly affected by bortezomib treatment. Bortezomib has been shown to suppress T cell activation and production of cytokines (Blanco et al. 2006), T cell mobilization (Liu et al. 2012), and T cell proliferation (Berges et al. 2008; Blanco et al. 2006). In DCs, bortezomib suppresses

maturation, induces apoptosis, suppresses function, and inhibits cytokine production (Arpinati et al. 2009; Nencioni et al. 2006; Straube et al. 2007). Since T cells and DCs are crucial players in GVHD, bortezomib treatment seems a promising strategy in preventing GVHD. Indeed, bortezomib has been shown to be effective in acute graft-versus-host disease in mice (Sun et al. 2004). A reduction in GVHD-associated disease parameters was observed but with no adverse effects on long-term donor reconstitution. Some studies in humans suggest that bortezomib-based GVHD prophylaxis and treatment are feasible and may improve efficacy when added to standard regimens (Al-Homsi et al. 2015, 2017; Koreth et al. 2012, 2015). However, recent randomized phase II results indicated that the bortezomib-based regimens evaluated did not improve outcomes compared with tacrolimus/methotrexate therapy (Koreth et al. 2018). The effect of bortezomib and the high expression of immunoproteasomes in immune cells suggest investigating immunoproteasome inhibition in GVHD. Indeed, the LMP7-selective inhibitor ONX 0914 ameliorated GVHD in an MHC-matched minor histocompatibility antigen-disparate murine model (Zilberberg et al. 2015). In mice experiencing GVHD, ONX 0914 treatment in the B10.BR → CBA MHC-matched/minor histocompatibility antigen (miHA)-disparate murine blood and marrow transplant (BMT) model caused a modest but significant improvement in the survival. Stimulator splenocytes, but not responder T cells, treated with ONX 0914 in lymphocyte cultures resulted in decreased IFN- $\gamma$  production by allogeneic T cells in both MHC-disparate (B10.BR anti-B6) and miHA-mismatched (B10.BR anti-CBA) settings. This indicates that ONX 0914 alters antigen presentation of miHA in this setup.

### The immunoproteasome in skin allograft transplantation

Different types of CD4<sup>+</sup> T cells play a central role in mediating transplant rejection. Cooperation between CD4<sup>+</sup> and CD8<sup>+</sup> T cells is thought to be required for maximal graft rejection. Th1 cells have been shown to facilitate CD8<sup>+</sup> T cell differentiation by direct cell-to-cell contact or by producing effector cytokines, such as IL-2 and IFN- $\gamma$  that directly support CD8<sup>+</sup> T cell differentiation and killing (Jiang et al. 2004; Shrikant et al. 1999). In recent years, it became evident that, apart from Th1 cells, Th17 cells also mediate acute allograft rejection by recruiting neutrophils and monocytes into the graft promoting transplant inflammation (Gorbacheva et al. 2010; Itoh et al. 2011). LMP7 inhibition was shown to suppress the differentiation of the pro-inflammatory T helper subtypes Th1 and Th17 in vitro (Basler et al. 2018; Kalim et al. 2012; Muchamuel et al. 2009; Mundt et al. 2016) and in vivo (Basler et al. 2014; Mundt et al. 2016). Therefore, the effect of immunoproteasome inhibition was

investigated in skin allograft transplantation using the LMP7-selective inhibitor ONX 0914 (Mundt et al. 2017). In vitro, in a mixed-lymphocyte-reaction (MLR), ONX 0914 reduced allo-specific IL-17 production in T cells. However, in vivo allograft survival in an MHC-disparate (C57BL/6 to BALB/c) and a multiple minor histocompatibility antigen (miHA)-disparate (B10.BR to C3H) model of skin transplantation was not altered in recipient mice treated with ONX 0914. It has been shown that mice treated with ONX 0914 can mount a rather normal virus-induced CTL response (Muchamuel et al. 2009). Alterations can only be observed for CTLs specific for LMP7-dependent class I peptides. Thus, the influence of immunoproteasome inhibition on Th cell differentiation appears to be not strong enough to influence skin allograft rejection which is dominated by CTLs in the used skin allograft models (Mundt et al. 2017). Hence, immunoproteasome inhibition does not appear to be a suitable approach to treat acute T cell-mediated skin allograft rejection.

### The immunoproteasome in kidney transplantation

Kidney transplantation is the most effective therapeutic approach for end-stage renal disease. Immunosuppressive regimes mainly target T cell-mediated acute kidney rejection. However, in contrast to earlier assumptions, long-term kidney rejection is mainly mediated by alloantibodies. Nevertheless, no FDA-approved medications are available for antibody-mediated kidney rejection. Proteasome inhibitors have been shown to be effective in animal models for humoral diseases. Treatment of two mouse strains with lupus-like disease, NZB/W F1 and MRL/lpr mice, with bortezomib-depleted plasma cells producing antibodies to double-stranded DNA, eliminated autoantibody production, ameliorated glomerulonephritis, and prolonged survival (Neubert et al. 2008). Clinical efficacy was also observed in a small clinical study in patients with active, refractory systemic lupus erythematosus (Alexander et al. 2015). Using bortezomib in experimental renal transplantation in the rat, Vogelbacher et al. could show that bortezomib inhibits the chronic active antibody-mediated rejection (Vogelbacher et al. 2010). The production of alloantibodies against components of glomerular basement membrane was reduced and the humoral response was strongly ameliorated, as shown by decreased numbers of IgG-secreting cells, plasma cells, and partially B cells in bortezomib-treated rats. Furthermore, infiltration of the graft with inflammatory cells like cytotoxic T cells, T helper cells, B cells, and macrophages was efficiently blocked by bortezomib treatment. Several incompletely controlled studies have suggested efficacy of the proteasome inhibitor bortezomib in preventing antibody-mediated kidney transplant rejection in humans (Everly et al. 2008; Pearl et al. 2016; Trivedi et al.

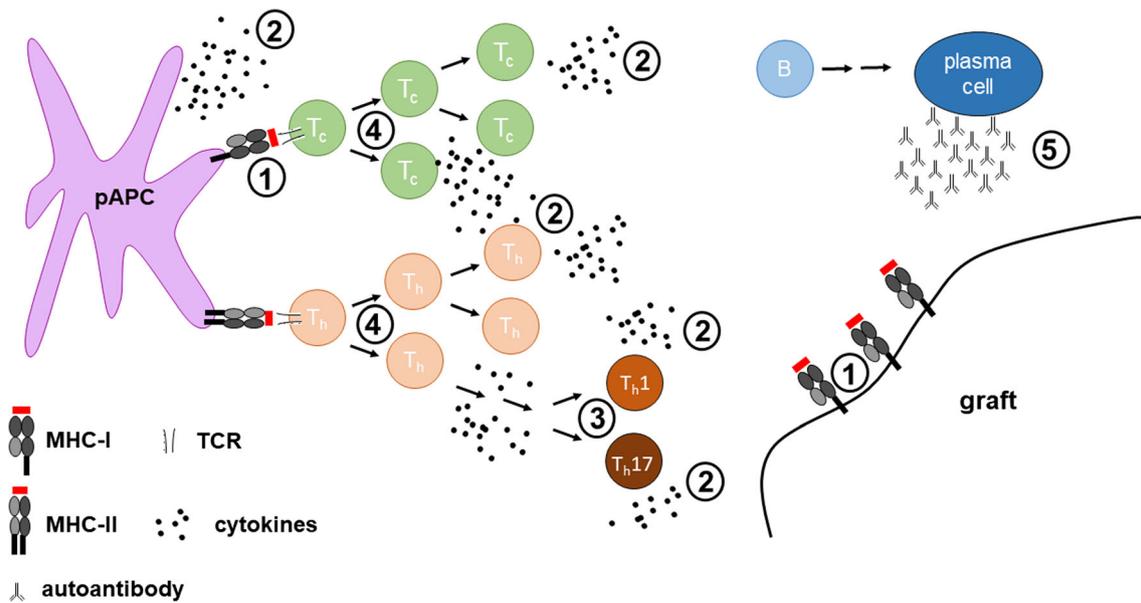
2009; Walsh et al. 2010). However, a recent randomized trial of bortezomib in late antibody-mediated kidney transplant rejection failed to show efficacy (Eskandary et al. 2018).

A limit to the therapeutic application of proteasome inhibitors in kidney transplantation is the ubiquitous expression of the proteasome leading to well-known adverse side effects. For instance, although bortezomib has been shown to be effective in SLE, bortezomib treatment leads to a higher mortality rate in lupus model mice (Ikeda et al. 2017). Hence, a potential benefit in terms of graft survival has to be counterweighed with known adverse effects. In contrast to the constitutive proteasome, immunoproteasomes are expressed in immune cells and in an inflammatory environment, and therefore, selective inhibitors of the immunoproteasome can be applied below their maximally tolerated dose while still retaining their therapeutic efficacy but lacking untoward toxicity. Indeed, immunoproteasome inhibitors have been successfully applied in animal models for humoral immunity. ONX 0914, an LMP7-selective inhibitor, has been shown to have beneficial effect in murine lupus (Ichikawa et al. 2012). Inhibition of the immunoproteasome prevented disease progression by targeting two critical pathways in disease pathogenesis, type I IFN activation, and auto-antibody production by plasma cells. Furthermore, in an *in vitro* humoral alloimmunity model, ONX 0914 was shown to decrease alloantibody production (Eleftheriadis et al. 2017). In a recent study, the effect of immunoproteasome inhibition has been investigated in a rat kidney transplantation model (Li et al. 2018). In this transplantation model, kidneys transplanted from Fischer to allogeneic Lewis rats induce a chronic antibody-mediated allograft rejection. Immunoproteasome inhibition by ONX 0914 ameliorated allograft renal function impairment, prolonged the survival of recipients, and alleviated chronic graft nephropathy. Furthermore, ONX 0914 treatment reduced the numbers of B and plasma cells in the bone marrow and spleen, and suppressed donor-specific alloantibody production. Leukocyte infiltration and the inflammatory response in the renal allografts were strongly reduced in recipient rats treated with ONX 0914. This study was performed at a dose (5 mg/kg) fairly below the maximal tolerated dose but still retained their therapeutic efficacy without obvious side effects. This demonstrates that immunoproteasome inhibitors are promising drugs in kidney transplantation with good tolerability, although ONX 0914 has been shown to induce some apoptosis in primary human renal tubular epithelial cells *in vitro* (Eleftheriadis et al. 2016).

### The immunoproteasome in heart allograft survival

Proteasome inhibitors have been applied in animal models of solid organ transplantation. Recently, Sula Karreci et al. described the development of the novel LMP7-selective

inhibitor DPLG3. DPLG3 is a rationally designed, non-covalent reversibly acting N,C-capped dipeptide inhibitor of LMP7 that has a 1000-fold selectivity over the constitutive subunit  $\beta 5c$  (Sula Karreci et al. 2016). DPLG3 inhibited  $\beta 5i$  competitively with an IC<sub>50</sub> of 4.4 nM, with less than 50% inhibition of  $\beta 1i$ ,  $\beta 2c$ ,  $\beta 2i$ , and  $\beta 2c$  at 33.3  $\mu$ M. No cytotoxic activity of DPLG3 was observed on human PBMCs or mouse bone marrow-derived macrophages. DPLG3 significantly inhibited the production of IFN- $\alpha$  and IP-10 by CpG-activated PBMCs without affecting viability. In the clinic, prevention of graft rejection requires suppression of T cell responses. DPLG3 inhibited proliferation of *in vitro* TCR-stimulated CD4<sup>+</sup> and CD8<sup>+</sup> T cells in a concentration-dependent manner. To test whether DPLG3 inhibits T cell responses to alloantigen *in vivo*, CD3<sup>+</sup>CD25<sup>-</sup> T cells (C57BL/6 background) were transferred into RAG<sup>-/-</sup>C57BL/6 mice which were grafted with BALB/c skin 1 day in advance. Mice treated daily with 25 mg/kg DPLG3 showed reduced CD4<sup>+</sup> and CD8<sup>+</sup> effector T cells compared to vehicle-treated mice. Furthermore, exhaustion and co-inhibitory markers PD1, TIM3, and LAG3 on CD4 and CD8 effector cells were enhanced in DPLG3-treated mice. To test the effect of immunoproteasome inhibition on transplantation, Sula Karecci et al. used a stringent, highly reproducible pre-clinical heart transplant mouse model in which BALB/c hearts are transplanted into fully allogeneic C57BL/6 recipients (Sula Karreci et al. 2016). Effector T cells isolated from the spleen on day 7 post-transplantation showed an upregulation of LMP7 in these cells, indicating that LMP7 is a valuable target in allogeneic heart transplantation. Survival of the allograft was significantly prolonged in DPLG3-treated mice compared to vehicle-treated mice (median survival time, day 13 vs. day 7), accompanied by reduced histologic signs of acute allograft rejection. Furthermore, a reduced percentage of effector memory T cells was observed in DPLG3-treated mice. A single-dose of an anti-CTLA4 antibody (sCTLA4-Ig) on day 2 post-transplantation markedly increased the survival of the transplanted heart (median survival time, day 38.5 vs. day 7). Remarkably, mice treated with sCTLA4-Ig (day 2) and DPLG3 (daily for 7 days) significantly prolonged heart survival (median survival time, day 84 vs. day 38.5). Mice receiving sCTLA4-Ig (day 2) and DPLG3 for 14 days even increased the median graft survival time > 100 days. Assessment of alloimmune responses in the periphery also showed a marked reduction in the T cell proliferative response in recipients treated with DPLG3 plus sCTLA4-Ig compared to sCTLA4-Ig alone. Further studies are required to identify the mechanism by which DPLG3 inhibits T cell activation, proliferation, surface marker expression, and secretion of cytokines. Taken together, the results presented in the study by Sula Karecci



**Fig. 2** Immunoproteasome inhibition in transplantation can interfere at multiple levels with transplant rejection affecting different pathways: (1) antigen presentation, (2) cytokine production, (3) T helper cell differentiation, (4) T cell activation and proliferation, and (5) prevention

of alloantibody production. pAPC, professional antigen-presenting cell; T<sub>c</sub>, cytotoxic T cell; T<sub>h</sub>, T helper cell; T<sub>h</sub>1, T helper 1 cell; T<sub>h</sub>17, T helper 17 cell; B, B cell

et al. show that a brief post-transplant treatment with immunoproteasome inhibitors as part of an immunomodulatory regimen prolongs allograft survival without broadly cytotoxic or immunosuppressive effects.

## Conclusion

Immunoproteasome inhibition seems to be a promising approach for preventing transplant rejection. Different studies indicated that immunoproteasome inhibition in transplantation can interfere at different levels affecting different pathways: (1) antigen presentation, (2) cytokine production, (3) T helper cell differentiation, (4) T cell activation and proliferation, and (5) prevention of alloantibody production (Fig. 2).

1. Immunoproteasome inhibition has been shown to affect antigen presentation. Therefore, immunoproteasome inhibition might alter the presentation of donor antigens. Whether these changes influence transplantation efficiency has barely been investigated and remains elusive.
2. Immunoproteasome inhibition has been shown to reduce cytokine secretion of different immune cells. Since cytokines regulate activation and effector functions of all immune cells involved in transplantation, immunoproteasome inhibition will dampen allograft specific immune responses.

3. Depending on the cytokine environment, T helper cells can differentiate into different subsets. While the role of Th17 cell in transplantation is less clear, Th1 cells play a crucial role in the rejection process. Th1 cells are characterized by the production of pro-inflammatory cytokines IFN- $\gamma$ , IL-2, IL-12, TNF, and GM-CSF. This cytokine profile activates macrophages, natural killer cells, and cytotoxic T cells which are drawn to the graft. Hence, a reduced Th1 differentiation mediated by immunoproteasome inhibition can positively influence graft survival.
4. Both activation and proliferation of CD4<sup>+</sup> and CD8<sup>+</sup> have been shown to be reduced in immunoproteasome inhibitor-treated mice (Sula Karreci et al. 2016). Since both cell types are crucially involved in rejection, immunoproteasome inhibition will improve graft survival.
5. Alloantibodies are responsible for long-term rejection of allografts. Bortezomib enhances the accumulation of unfolded proteins via the induction of ER stress and the unfolded protein response, eventually leading to apoptotic cell death. Plasma cells that synthesize large amounts of immunoglobulins have a relatively high sensitivity to bortezomib, which partly explains the clinical success of bortezomib in treating plasma cell-derived multiple myelomas. Li et al. showed that immunoproteasome inhibition in rats prevented alloantibody production and reduced the number of plasma cells in an antibody-mediated kidney allograft

rejection model (Li et al. 2018). Hence, it seems likely that immunoproteasome inhibition in plasma cells induces ER stress leading to apoptotic plasma cell death and prevention of alloantibody production.

Taken together, multiple modes of actions on different pathways involved in transplant rejection render the immunoproteasome a highly valuable target. Indeed, the first pre-clinical studies with immunoproteasome inhibitors in animal models of transplantations are encouraging.

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### Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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