Human Immunology 80 (2019) 608–613

Contents lists available at ScienceDirect

Human Immunology

journal homepage: www.elsevier.com/locate/humimm

Injury derived autoimmunity: Anti-perlecan/LG3 antibodies in transplantation

Mélanie Dieudé[a,b,c,*], Héloïse Cardinal[a,b,c,*,1], Marie-Josée Hébert[a,b,c,*,1]

[a] Research Centre, Centre hospitalier de l'Université de Montréal (CRCHUM), Montreal, QC, Canada
[b] Canadian Donation and Transplantation Research Program, Canada
[c] Université de Montréal, Canada

ARTICLE INFO

Keywords:
Autoimmunity
Apoptosis
Tissue injury
Kidney
Transplantation

ABSTRACT

Ischemic, immunologic or pharmacological stressors can induce vascular injury and endothelial apoptosis in organ donors, in transplant candidates due to the impact of end stage organ failure on the vasculature, and in association with peri-transplantation events. Vascular injury may shape innate and adaptive immune responses, leading to dysregulation in the balance between tolerance and immunoreactivity to vascular-derived antigens. Mounting evidence shows that the early stages of apoptosis, characterized by the absence of membrane permeabilization, are prone to trigger various modes of intercellular communication allowing neoantigen production, exposure, or both. In this review, we present the evidence for the release of LG3, an immunogenic fragment of perlecan, as a consequence of caspase-3 dependent vascular apoptosis leading to the genesis of anti-LG3 autoantibodies and the consequences of these autoantibodies in native and transplanted kidneys.

1. Endothelial apoptosis leads to the release of LG3, an immunogenic fragment of perlecan

During the perioperative period, solid organ transplants can be exposed to various types of cellular stresses that compromise tissue viability. Ischemic, immunologic or pharmacological stressors can induce endothelial apoptosis in the allograft, which may shape the innate and adaptive immune response, leading to dysregulation in the balance between tolerance and immune reactivity to vascular derived antigens.

Apoptosis is classically considered an anti-inflammatory and tolerogenic type of cell death [1]. However, autoantibodies reactive to components of apoptotic cells are known to be present in the circulation of transplant candidates and recipients, and their presence is associated with adverse outcomes after transplantation [2,3–6]. This association points to a potential role for apoptosis as a potential trigger for autoantibody production that in turn can increase tissue damage. The early stages of apoptosis, characterized by the absence of membrane permeabilization, are prone to trigger various modes of intercellular communication of importance on local homeostasis and tissue remodeling. The classical dichotomy between "tolerogenic" apoptosis and "inflammatory" necrosis therefore needs to be revisited.

1.1. Paracrine pathways leading to the release of LG3 by apoptotic endothelial cells

An important aspect of the paracrine endothelial apoptotic legacy is the release of proteolytic enzymes that occurs in a highly regulated fashion in the absence of unspecific cell leakage. Cathepsin-L, tissue Plasminogen Activator and ADAMs (metalloendopeptidases) are exported extracellularly during apoptosis through caspase-dependent pathways [7]. Caspase-3 activation is central to the activity of Cathepsin-L on perlecan, generating a truncated C terminal fragment harbouring a laminin G motif (referred to as LG3). The release of LG3 fragment was first characterized using pure endothelial apoptotic culture models in conjunction with multifaceted proteomic strategies [7,8]. Infiltrating leukocytes and aggregating platelets, present at sites of vascular damage and/or inflammation can also contribute to perlecan proteolysis and LG3 release through myeloperoxidase and thrombin production [9–11].

Serum cathepsin-L and LG3 were found to be significantly elevated

Abbreviations: AKI, acute kidney injury; ApoExo, apoptotic exosome-like vesicles; AT_1R, angiotensin II type 1 receptor; ATIR, acute tubulointerstitial rejection; AVR, acute vascular rejection; CNI, calcineurin inhibitors; DGF, delayed graft function; DSA, donor specific antibodies; HLA, human leucocyte antigen; LG3, truncated-c terminal fragment (third Laminin G motif) of perlecan; MHC, major histocompatibility complex; Nabs, natural antibodies; NK cells, natural killer cells; WT, wild type

* Corresponding authors at: Research Centre, Centre hospitalier de l'Université de Montréal (CRCHUM), Montreal, QC, Canada.
E-mail addresses: mdieude@cntrp.ca (M. Dieudé), heloise.cardinal.chum@ssss.gouv.qc.ca (H. Cardinal), marie-josee.hebert@umontreal.ca (M.-J. Hébert).

[1] Héloïse Cardinal and Marie-Josée Hébert have contributed to a similar extent to this manuscript.

https://doi.org/10.1016/j.humimm.2019.04.009
Received 4 February 2019; Received in revised form 8 April 2019; Accepted 13 April 2019
Available online 24 April 2019

in kidney transplant recipients with Banff grade 2 and 3 acute vascular rejection (AVR) in comparison to controls with acute tubulointerstitial rejection (ATIR) or stable graft function [12]. Elevations in urinary levels of LG3 have also been observed in kidney transplant recipients with chronic rejection [13,14] and in patients with severe IgA nephropathy [15]. Furthermore, proteomic approaches have revealed that in patients undergoing cardiac surgery, circulating LG3 increased early in the post-operative period and was a predictor of major adverse renal events [16]. Taken together, these data suggest that LG3 is associated with vascular injury both in transplanted and native kidneys. Whether LG3 is only a biomarker of vascular injury or whether it is actively involved in its pathogenesis is not yet fully resolved. We observed that the LG3 fragment promoted obliterative vascular remodelling and inflammation in a murine model of vascular rejection based on orthotopic transplantation of a fully mismatched aortic graft in absence of immunosuppression [2], which suggests an active role for this fragment in enhancing vascular injury.

1.2. The importance of apoptotic membrane vesicles in the release of LG3

The classical notion that apoptosis is a non-immunogenic, if not tolerogenic, type of regulated cell death has recently been revisited with the discovery of a novel type of membrane vesicle called the apoptotic exosome-like vesicle (ApoExo). ApoExo, like classical apoptotic bodies, are released through a caspase-3–dependent pathway. However, their size, ultrastructure, enzymatic activity and functions are strikingly different from those of apoptotic bodies [17]. Using unbiased comparative proteomic analysis, we established that different and almost mutually exclusive sets of proteins are present in apoptotic bodies and ApoExo, suggesting that their generation depends on distinct protein- sorting pathways and that their biogenesis is likely different. Detailed proteomic analyses identified perlecan as one of the basement membrane proteins present in apoptotic exosome-like vesicles with specific sequence coverage of the LG3 fragment of perlecan [17]. Western blot analyses confirmed that LG3 fragment of perlecan is enriched in ApoExo [17]. The simultaneous presence of LG3 and an active proteasome 20S core complex was found as a distinctive feature of ApoExo. Proteasome degradation is essential for many cellular processes, including antigen processing. In humans, extracellular proteasomes have been found circulating in the plasma of patients suffering from a variety of inflammatory, autoimmune and neoplastic diseases [18–23]. The concentration of circulating proteasomes correlates with disease activity in certain clinical conditions such as systemic lupus erythematosus, rheumatoid arthritis and hematologic malignancies [24].

1.3. Apoptotic membrane vesicles containing LG3 are immunogenic and fuel anti-LG3 production

In a murine model of mismatched aortic transplantation, we showed that the transfer of ApoExo, but not of apoptotic bodies, triggered the production of anti-LG3 IgG antibodies and heightened the severity of allograft inflammation and vascular rejection [17]. When proteasome activity was blocked in ApoExo prior to their injection in allografted mice, an important reduction in the production anti-LG3 was observed [17]. Inhibiting proteasome activity in parent apoptotic endothelial cells to generate ApoExo without proteasome activity did not modulate the rate or mechanism of endothelial cell death nor the number of released ApoExo, but completely abrogated all proteasome activity in released ApoExo [17]. The presence of LG3 within ApoExo was not reduced by proteasome inhibition and on the contrary tended to be higher, as assessed by mass spectrometry. Despite higher LG3 levels within ApoExo, proteasome activity inhibition abrogated ApoExo's capacity to trigger anti-LG3 production [17]. Taken together, these data suggest that the proteasome activity of ApoExo is a key determinant of their immunogenicity.

These observations provide novel insights into the mechanisms controlling the generation of the LG3 fragment and the production of anti-LG3 autoantibodies. They suggest that vascular and tissue injury prompts the production of ApoExo that can, in turn, foster the production of anti-LG3 autoantibodies. In kidney transplant candidates, vascular injury can occur as a result of uremia, which is known to promote endothelial dysfunction and apoptosis [25]. This could in turn foster the production of anti-LG3 antibodies in susceptible patients awaiting a kidney transplantation. Although definite cut-offs for anti-LG3 positivity are yet to be defined, we found an association between pre-transplant anti-LG3 levels in the upper decile of the distribution and delayed graft function [26]. The renal allograft may also suffer vascular damage in the perioperative period due to ischemia-reperfusion injury and after transplantation as a result of cell- or antibody-mediated alloimmune injury as well as calcineurin inhibitor use, potentially contributing to the release of ApoExo that can in turn enhance anti-LG3 production. We showed that ischemia-reperfusion injury in mice, either induced by renal artery clamping or femoral artery ligation, favour the release of ApoExo in the circulation and trigger the production of anti-LG3 antibodies [17]. Hence, LG3 and anti-LG3 can represent biomarkers of vascular injury in kidney transplant patients and, as will be discussed in the following sections, can also enhance vascular damage.

2. The genesis of anti-LG3 autoimmunity

Classically, the appearance of autoantibodies in the post-transplant context was thought to follow episodes of acute rejection leading to the release or increased immunogenicity/exposure of neoepitopes, including danger-associated molecular patterns (DAMPs), that would in turn favor autoantibody production. Recent data suggest that extracellular matrix components can behave as neoantigens, fuelling humoral responses in association with acute and chronic rejection [27–30]. We first described increases in anti-LG3 levels measured at the time of acute vascular rejection in kidney transplant recipients [2]. We noted correlations between circulating LG3 and anti-LG3 antibody levels in the post-transplant setting. However, anti-LG3 antibodies were of the IgG subclass and the rejection episodes we observed occurred in the early post-transplant period, at a median of 9 days post transplant [2]. This raised the possibility that post-transplant anti-LG3 titers were reflecting a memory response, and not a primary immune response. We then examined pre-transplant anti-LG3 levels in our study cohort. We found an association between increased pre-transplant levels of anti-LG3 and the risk of subsequent rejection involving the graft vasculature (Banff grade $\geq$ 2) [2]. Anti-LG3 antibodies were not associated with the presence of autoimmune disease prior to transplantation.

We went on to investigate how anti-LG3 developed in various models. LG3 immunization in wild type mice showed concomitant increases in anti-LG3 IgM and IgG antibodies, suggesting that the normal immune repertoire is composed of memory B cells specific to LG3. Then, we confirmed the presence of immune memory specific to LG3 by detecting memory B1 cells specific to LG3 in the peritoneal cavity of non-immunized WT mice [31]. Mouse B1 cells are the predominant constituents of peritoneal B cells and are also found in the pleural cavity, the spleen and the bone marrow [32–35]. In contrast to B2 cells, B1 cell development occurs primarily during fetal and peri-natal life. B1 cells are effectors of the innate immune system and the main producers of polyreactive antibodies that bind to both microbial antigens and self-antigens, including neo-self epitopes expressed by apoptotic cells such as annexin IV and phosphorylcholine [36–38]. It has been suggested that natural autoantibodies produced by B1 cells favor the clearance of senescent and apoptotic cells and therefore have protective effector functions [34,39]. As previously mentioned, LG3 is released by apoptotic endothelial cells downstream of caspase-3 activation specifically in ApoExo [7,17,40]. The presence of a predominant B1 cell memory-response to LG3 in naïve mice suggests that memory to LG3 is likely a normal and innate response to components of membrane vesicles

released by apoptotic cells. Interactions between anti-LG3 antibodies and LG3 present on apoptotic membrane vesicles could potentially help the organism clear remnants of apoptotic cells. In consecutive kidney transplant recipients, we also observed that anti-LG3 levels tended to decrease following transplantation, which could be due the initiation of immunosuppression targeting T cells, as our animal studies show that the production of anti-LG3 antibodies requires T cell help [31].

Zorn and colleagues showed that memory B cells producing natural antibodies with reactivity to multiple HLA alleles, DNA and self-antigenic structure were found in the blood of patients with antibody-mediated kidney graft rejection [41]. These autoantibodies were polyreactive to antigens on apoptotic cells, but not on viable cells [5,41]. A subsequent study showed that these polyreactive natural antibodies (Nabs) could be detected prior to transplantation and that their pre-transplant levels were associated with reduced long-term graft survival in kidney transplant patients [5,42,43]. As we found a high frequency of memory B1 cells specific to LG3 in the peritoneal cavity of naïve mice, the possibility that antibodies with reactivity to LG3 also react to a broad range of other antigens cannot be excluded and further studies are under way to evaluate the potential role of polyreactivity in the response to LG3.

In kidney transplant patients, LG3 IgG autoantibodies are almost exclusively with complement fixing and activating properties (IgG1 and IgG3) [2]. Although we had insufficient power to draw a firm conclusion on this topic, anti-LG3 levels were numerically higher in C4d positive patients [2]. In murine models, we showed that LG3 immunization with Freund's adjuvant, which induces a pronounced inflammatory response, leads to the production of high titers of anti-LG3 of complement fixing IgG subtypes whereas the same antigen in a neutral *milieu* (i.e. injected in absence of Freund's adjuvant) triggers the production of non-complement fixing anti-LG3 IgG isotypes [44]. These observations raise the possibility that pro-inflammatory conditions prevalent in patients with end-stage-renal disease leading to a chronic inflammatory response [45–47] could foster immunoglobulin class switching for anti-LG3 antibodies toward complement-fixing isotypes of greater negative impact at the time of transplantation.

Although associations between antibodies to the angiotensin II type 1 receptor (anti-AT_1R) and classical autoimmune diseases have been reported [48], anti-AT_1R as well as polyreactive anti-apoptotic cell Nabs, anti-LG3 antibodies, and anti-vimentin antibodies were also reported in patients awaiting a renal transplantation in the absence of auto-immune diseases [2,5,49,50]. There are no increases in anti-LG3 levels nor in anti-AT_1R in normal pregnancy [51]. This suggests that the conditions associated with the production of anti-LG3 and anti-AT_1R are likely different from allosensitization. Although it is possible that B cell dysregulation gives rise to autoantibody production, the lack of association between anti-LG3, anti-AT_1R and anti-vimentin antibodies [51] suggests that various pathways may be involved in the production of autoantibodies of importance in transplantation.

3. Anti-LG3 antibodies are biomarkers and effectors of immune-mediated kidney vascular damage

In kidney transplant patients, vascular damage caused by rejection is associated with features of complement deposition, lack of response to corticosteroids, and shorter graft survival [52–54]. Although donor-specific anti-HLA antibodies (DSA) are known to attack the transplant macro- and microvasculature, it is increasingly recognized that antibodies directed towards non-HLA epitopes also contribute to acute or chronic allograft rejection in kidney, heart and lung transplant recipients [5,27,55–63]. Antibodies targeting vascular basement membrane components have previously been linked with adverse transplant outcomes. For instance, the presence of anti-agrin antibodies is associated with chronic transplant glomerulopathy and a higher number of prior acute rejection episodes in kidney transplant recipients [27].

In turn, acute vascular rejection leads to severe macro and often microvascular injury and enhanced endothelial apoptosis of potential importance in the production or modification of antigenic targets [64,65]. As discussed in section 1, endothelial apoptosis triggers the release of and secretion of ApoExo containing LG3 and proteasome activity, which triggers the production of anti-LG3 antibodies. For this reason, we measured anti-LG3 levels in renal transplant patients with AVR and compared them to levels observed in subjects with Banff grade I rejection or normal allografts. We found elevated post-transplant anti-LG3 levels in kidney transplant recipients with Banff grade ≥ 2 rejection compared to patients with normal allograft function and those who developed Banff grade I rejection. Higher anti-LG3 IgG levels in AVR patients was found not only at the time of rejection, but also prior to transplantation. At both time points, anti-LG3 antibodies were predominantly of the complement-activating IgG1 and IgG3 subclasses. Although this suggested that anti-LG3 could contribute to the pathogenesis of AVR by activating complement, pre-transplant anti-LG3 levels could also reflect the recipient's non-specific propensity to mount an immune response.

To address the possibility that anti-LG3 antibodies could accelerate alloimmune vascular injury, we transferred anti-LG3 IgG in a murine model of vascular rejection based on orthotopic transplantation of an aortic segment between fully-MHC incompatible mice. We exposed the aortic allograft to ischemia before transplantation to reproduce conditions that are similar to clinical transplantation in humans and since, in a non-damaged endothelium, LG3 is embedded within the extracellular matrix and theoretically not readily available for antigenic recognition by circulating antibodies. Passive transfer of anti-LG3 IgGs in ischemic allografts induced striking NK cell infiltration, C4d deposition and obliterative vascular remodelling. These results identified anti-LG3 antibodies as accelerators of inflammation and remodelling in an ischemic vascular allograft [2]. This is especially relevant given the increasing number of organs harvested from extended criteria donors and donors after cardiac arrest having suffered ischemic injury prior to transplantation. Another group has recently reported elevated anti-LG3 levels in hypersensitized patients with no DSA, with 4/11 patients developing early onset antibody-mediated rejection associated with anti-LG3 antibodies [66].

4. Anti-LG3 enhance the risk and severity of ischemia-reperfusion injury in native and transplanted kidneys

Ischemia-reperfusion is an integral component of renal transplantation. As our previous animal studies revealed that anti-LG3 were accelerating immune-mediated damage in ischemic allografts but had no significant effect when the allograft had not suffered from ischemia [2], we evaluated whether anti-LG3 could also enhance ischemia-reperfusion mediated renal damage in absence of rejection. We performed a single center, retrospective cohort study in which we showed that pre-transplant anti-LG3 antibodies titers were associated with an increased risk of delayed graft function (DGF), in the immediate post-transplant period [26]. Also, in patients with DGF but not in those with immediate graft function, anti-LG3 antibodies titers assessed immediately before transplantation predicted lower graft function 1-year after transplantation. This association was also found in patients who did not experience rejection, suggesting that the impact of anti-LG3 antibodies on subsequent renal function in patients with DGF could not be attributed to a concomitant acceleration of rejection [26]. In contrast, anti-AT_1R and anti-vimentin were not associated with DGF or graft function 1-year post-transplant in this cohort [26]. These results suggested that, in addition to their function as accelerators of rejection, anti-LG3 antibodies can also impair renal function through rejection-independent pathways, while anti-AT_1R and anti-vimentin did not.

To investigate how anti-LG3 antibodies aggravate renal dysfunction after ischemia-reperfusion injury, we tested the impact of anti-LG3 infusion on the severity of acute kidney injury (AKI) in a murine model of renal ischemia-reperfusion injury. We chose renal artery clamping of a

native kidney with contralateral nephrectomy as our AKI model to ensure the absence of alloimmune contribution to renal injury. When present in high titers at the time of ischemia-reperfusion, anti-LG3 antibodies enhanced renal dysfunction and prompted activation of the classical complement pathway within peritubular capillaries [26]. We also observed reduced CD31 staining and increased indices of both microvascular and tubular damage in mice transferred with anti-LG3. This was associated, on the long term, with enhanced microvascular dropout and heightened renal fibrosis in mice injected with anti-LG3 antibodies [26]. Taken together, these results suggest that ischemia-reperfusion creates a permissive *milieu* for intra-renal activation of complement by anti-LG3 antibodies, leading to enhanced microvascular injury and involution and fibrosis. This could explain the increased risk of long-term renal dysfunction we observed in kidney transplant recipients who had elevated anti-LG3 levels and DGF.

5. Potential therapeutic avenues to mitigate anti-LG3 autoimmunity in transplantation

Cyclosporin and tacrolimus have been the mainstay of immunosuppression in solid organ transplantation for many decades. Both types of calcineurin inhibitors (CNI) are potent inhibitors of T cell activation [67]. In addition, CNI are known to dampen humoral immunity by inhibiting the differentiation of naïve $CD4^+$ T cells into T follicular helper cells and by suppressing naïve B cells [68]. Mycophenolic acid is the adjuvant immunosuppressant most commonly used in kidney transplant patients [69]. Mycophenolic acid is a recognized treatment for autoimmune diseases such as lupus nephritis and can suppress autoantibody production through early inhibition of B-cell proliferation and differentiation [70]. We assessed how anti-LG3 antibodies behaved in consecutive, unselected patients after kidney transplantation with the onset of immunosuppression. We found that initiation of CNI and mycophenolate-based immunosuppression was associated with decreasing anti-LG3 IgG one month post-transplant [44]. In further support for a role of helper T cells in controlling the production of anti-LG3 antibodies, we also showed that mice depleted in CD4 T cells lose their capacity to produce anti-LG3 antibodies after antigen stimulation [44]. These results suggest that T cell targeting immunosuppression, such as maintenance immunosuppression of common use in clinical transplantation, can dampen humoral immunity specific to LG3. Whether immunosuppressive regimens targeting T cell function could be used prior to transplantation in patients with high anti-LG3 levels as a means of decreasing anti-LG3 levels and potentially preventing the increased risk of DGF, acute rejection and adverse subsequent impact on graft function associated with high pre-transplant anti-LG3 levels remains to be tested [2,26].

Bortezomib is currently being used in the treatment of refractory antibody-mediated rejection as a B cell-depleting agent based on its pro-apoptotic activity on plasma cells and B cells [71,72]. By inhibiting the proteasome activity of ApoExo and therefore lowering their immunogenicity, bortezomib could prevent the formation of anti-LG3 autoantibodies. The use of perfusion machine for administering treatment to the organ prior to transplantation could represent a unique opportunity to treat the allograft through perfusion with pharmacological agents such as bortezomib during the cold ischemic period. Future studies will be needed to assess the impact of such strategies on both early and long-term renal function.

The use of hypothermic machine perfusion has been shown to reduce the risk of DGF, especially when extended criteria donors are used [73,74]. Whether the use of hypothermic machine perfusion could protect the allograft endothelium from apoptosis, thereby diminishing its propensity to release LG3 is presently unknown. If so, the use of hypothermic machine perfusion may be most useful in patients with elevated anti-LG3, preventing anti-LG3–related acceleration of vascular damage in kidney transplant patients.

Last, plasma exchange can remove circulating antibodies and has been used in desentitization protocols and in the treatment of antibody-mediated rejection in kidney transplant patients. Pre-transplant plasma exchange in combination with thymoglobulin and angiotensin-II blockade has resulted in a lower rate of rejection in an Australian cohort with high anti-AT_1R levels when compared to historical controls [75]. Whether plasma exchange could decrease the risk of DGF and/or rejection in transplant candidates with high pre-transplant anti-LG3 remains to be determined.

6. Concluding remarks

Autoantibodies are emerging as important contributors of acute and long-term allograft dysfunction after renal transplantation. Anti-LG3/perlecan antibodies likely represent a type of natural antibodies that target components of dying cells and vascular basement membrane to facilitate clearance of debris at sites of tissue injury. In renal transplant patients however, their presence enhances renal vascular injury, aggravating the risk and negative impact of both DGF and rejection. A better understanding of the mechanisms of production and action of anti-LG3 is instrumental for developing new strategies aimed at blocking their deleterious action on susceptible renal transplants.

Acknowledgements

HC is a research scholar of the Fonds de Recherche du Québec (FRQ) santé, and a KRESCENT new investigator. MJH holds of the Shire Chair in Nephrology, Transplantation and Renal Regeneration of l'Université de Montréal and HC is associate scientist to the Shire Chair. This work was supported by grants from the Canadian Institutes of Health Research (MOP-15447, PJT-148884) and the Kidney Foundation of Canada to HC. We thank the J.-L. Levesque Foundation for renewed support. MJH is the Co-Director of the Canadian Donation and Transplantation Research Program (CDTRP) and MD is the Principal Scientific Officer of the CDTRP. HC, MD and MJH are CDTRP investigators.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2019.04.009.

References

[1] I.K. Poon, C.D. Lucas, A.G. Rossi, K.S. Ravichandran, Apoptotic cell clearance: basic biology and therapeutic potential, Nat. Rev. Immunol. 14 (3) (2014) 166–180.
[2] H. Cardinal, M. Dieude, N. Brassard, et al., Antiperlecan antibodies are novel accelerators of immune-mediated vascular injury, Am. J. Transplant. 13 (4) (2013) 861–874.
[3] L. Li, P. Wadia, R. Chen, et al., Identifying compartment-specific non-HLA targets after renal transplantation by integrating transcriptome and "antibodyome" measures, PNAS 106 (11) (2009) 4148–4153.
[4] T.K. Sigdel, L. Li, T.Q. Tran, et al., Non-HLA antibodies to immunogenic epitopes predict the evolution of chronic renal allograft injury, J. Am. Soc. Nephrol. 23 (4) (2012) 750–763.
[5] B. Gao, C. Moore, F. Porcheray, et al., Pretransplant IgG reactivity to apoptotic cells correlates with late kidney allograft loss, Am. J. Transplant. 14 (7) (2014) 1581–1591.
[6] B. Mahesh, H.S. Leong, A. McCormack, P. Sarathchandra, A. Holder, M.L. Rose, Autoantibodies to vimentin cause accelerated rejection of cardiac allografts, Am. J. Pathol. 170 (4) (2007) 1415–1427.
[7] J.F. Cailhier, I. Sirois, P. Laplante, et al., Caspase-3 activation triggers extracellular cathepsin L release and endorepellin proteolysis, J. Biol. Chem. 283 (40) (2008) 27220–27229.
[8] M.A. Raymond, A. Desormeaux, P. Laplante, et al., Apoptosis of endothelial cells triggers a caspase-dependent anti-apoptotic paracrine loop active on VSMC, FASEB J. 18 (6) (2004) 705–707.
[9] C.A. Owen, E.J. Campbell, The cell biology of leukocyte-mediated proteolysis, J. Leukoc. Biol. 65 (2) (1999) 137–150.
[10] J.M. Whitelock, A.D. Murdoch, R.V. Iozzo, P.A. Underwood, The degradation of human endothelial cell-derived perlecan and release of bound basic fibroblast growth factor by stromelysin, collagenase, plasmin, and heparanases, J. Biol. Chem. 271 (17) (1996) 10079–10086.

[11] M.D. Rees, J.M. Whitelock, E. Malle, et al., Myeloperoxidase-derived oxidants selectively disrupt the protein core of the heparan sulfate proteoglycan perlecan, Matrix Biol. 29 (1) (2010) 63–73.
[12] M. Soulez, E.A. Pilon, M. Dieude, et al., The perlecan fragment LG3 is a novel regulator of obliterative remodeling associated with allograft vascular rejection, Circ. Res. 110 (1) (2012) 94–104.
[13] M. Soulez, E.A. Pilon, M. Dieude, et al., The perlecan fragment LG3 is a novel regulator of obliterative remodeling associated with allograft vascular rejection, Circ Res., 110(1), 94–104.
[14] E.O.T. O'Riordan, N. Mendelev, D. Patschan, R. Kemp, P. Chander, R. Hu, G. Hao, S. Gross, R. Iozzo, V. Delaney, M. Goligorsky, Urinary proteomic analysis of chronic allograft nephropathy, Proteomics Clin. Appl. 2 (2008) 1025–1035.
[15] B. Surin, E. Sachon, J.P. Rougier, et al., LG3 fragment of endorepellin is a possible biomarker of severity in IgA nephropathy, Proteomics 13 (1) (2013) 142–152.
[16] M. Haase, R. Bellomo, C. Albert, et al., The identification of three novel biomarkers of major adverse kidney events, Biomarkers Med. 8 (10) (2014) 1207–1217.
[17] M. Dieude, C. Bell, J. Turgeon, et al., The 20S proteasome core, active within apoptotic exosome-like vesicles, induces autoantibody production and accelerates rejection, Sci. Transl. Med. 7 (318) (2015) 318ra200.
[18] M. Majetschak, M. Perez, L.T. Sorell, J. Lam, M.E. Maldonado, R.W. Hoffman, Circulating 20S proteasome levels in patients with mixed connective tissue disease and systemic lupus erythematosus, Clin. Vaccine Immunol. 15 (9) (2008) 1489–1493.
[19] L. Henry, T. Lavabre-Bertrand, T. Douche, et al., Diagnostic value and prognostic significance of plasmatic proteasome level in patients with melanoma, Exp. Dermatol. 19 (12) (2010) 1054–1059.
[20] L. Henry, T. Lavabre-Bertrand, L. Vercambre, et al., Plasma proteasome level is a reliable early marker of malignant transformation of liver cirrhosis, Gut 58 (6) (2009) 833–838.
[21] C. Jakob, K. Egerer, P. Liebisch, et al., Circulating proteasome levels are an independent prognostic factor for survival in multiple myeloma, Blood 109 (5) (2007) 2100–2105.
[22] M. Wada, M. Kosaka, S. Saito, T. Sano, K. Tanaka, 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 (2) (1993) 215–223.
[23] M. Heubner, P. Wimberger, B. Dahlmann, et al., The prognostic impact of circulating proteasome concentrations in patients with epithelial ovarian cancer, Gynecol. Oncol. 120 (2) (2011) 233–238.
[24] S.U. Sixt, B. Dahlmann, Extracellular, circulating proteasomes and ubiquitin – incidence and relevance, Biochim. Biophys. Acta, Proteins Proteomics 1782 (12) (2008) 817–823.
[25] H. Cardinal, M.A. Raymond, M.J. Hebert, F. Madore, Uraemic plasma decreases the expression of ABCA1, ABCG1 and cell-cycle genes in human coronary arterial endothelial cells, Nephrol. Dialysis Transplant. 22 (2) (2007) 409–416.
[26] B. Yang, M. Dieude, K. Hamelin, et al., Anti-LG3 antibodies aggravate renal ischemia-reperfusion injury and long-term renal allograft dysfunction, Am. J. Transplant. 16 (12) (2016) 3416–3429.
[27] S.A. Joosten, Y.W. Sijpkens, V. van Ham, et al., Antibody response against the glomerular basement membrane protein agrin in patients with transplant glomerulopathy, Am. J. Transplant. 5 (2) (2005) 383–393.
[28] Q. Zhang, E.F. Reed, Non-MHC antigenic targets of the humoral immune response in transplantation, Curr. Opin. Immunol., 22(5), 682–688.
[29] D. Dragun, Humoral responses directed against non-human leukocyte antigens in solid-organ transplantation, Transplantation 86 (8) (2008) 1019–1025.
[30] S.A. Joosten, M.G. van Dixhoorn, M.C. Borrias, et al., Antibody response against perlecan and collagen types IV and VI in chronic renal allograft rejection in the rat, Am. J. Pathol. 160 (4) (2002) 1301–1310.
[31] L. Padet, M. Dieude, A. Karakeussian-Rimbaud, et al., New insights into immune mechanisms of antiperlecan/LG3 antibody production: importance of T cells and innate B1 cells, Am. J. Transplant. 19 (3) (2019) 699–712.
[32] R.R. Hardy, B-1 B cell development, J. Immunol. 177 (5) (2006) 2749–2754.
[33] J.M.B. Prieto, M.J.B. Felippe, Development, phenotype, and function of non-conventional B cells, Comp. Immunol. Microbiol. Infect. Dis. 54 (2017) 38–44.
[34] R.R. Hardy, B-1 B cells: development, selection, natural autoantibody and leukemia, Curr. Opin. Immunol. 18 (5) (2006) 547–555.
[35] E. Montecino-Rodriguez, K. Dorshkind, New perspectives in B-1 B cell development and function, Trends Immunol. 27 (9) (2006) 428–433.
[36] L. Kulik, S.D. Fleming, C. Moratz, et al., Pathogenic natural antibodies recognizing annexin IV are required to develop intestinal ischemia-reperfusion injury, J. Immunol. 182 (9) (2009) 5363–5373.
[37] Y. Qian, K.L. Conway, X. Lu, H.M. Seitz, G.K. Matsushima, S.H. Clarke, Autoreactive MZ and B-1 B-cell activation by Faslpr is coincident with an increased frequency of apoptotic lymphocytes and a defect in macrophage clearance, Blood 108 (3) (2006) 974–982.
[38] P.X. Shaw, C.S. Goodyear, M.K. Chang, J.L. Witztum, G.J. Silverman, The autoreactivity of anti-phosphorylcholine antibodies for atherosclerosis-associated neoantigens and apoptotic cells, J. Immunol. 170 (12) (2003) 6151–6157.
[39] N. Baumgarth, The double life of a B-1 cell: self-reactivity selects for protective effector functions, Nat. Rev. Immunol. 11 (1) (2011) 34–46.
[40] P. Laplante, M.A. Raymond, A. Labelle, J. Abe, R.V. Iozzo, M.J. Hebert, Perlecan proteolysis induces an alpha2beta1 integrin- and Src family kinase-dependent anti-apoptotic pathway in fibroblasts in the absence of focal adhesion kinase activation, J. Biol. Chem. 281 (41) (2006) 30383–30392.
[41] F. Porcheray, J. DeVito, Y. Helou, et al., Expansion of polyreactive B cells cross-reactive to HLA and self in the blood of a patient with kidney graft rejection, Am. J. Transplant. 12 (8) (2012) 2088–2097.
[42] F. Porcheray, J.W. Fraser, B. Gao, et al., Polyreactive antibodies developing amidst humoral rejection of human kidney grafts bind apoptotic cells and activate complement, Am. J. Transplant. 13 (10) (2013) 2590–2600.
[43] S.B. See, O. Aubert, A. Loupy, et al., Post-transplant natural antibodies associate with kidney allograft injury and reduced long-term survival, J. Am. Soc. Nephrol. 29 (6) (2018) 1761–1770.
[44] L. Padet, M. Dieude, A. Karakeussian-Rimbaud, et al., New insights into immune mechanisms of antiperlecan/LG3 antibody production: importance of T cells and innate B1 cells, Am. J. Transplant. (2018).
[45] P. Stenvinkel, A. Alvestrand, Inflammation in end-stage renal disease: sources, consequences, and therapy, Semin. Dial. 15 (5) (2002) 329–337.
[46] R. Jofre, P. Rodriguez-Benitez, J.M. Lopez-Gomez, R. Perez-Garcia, Inflammatory syndrome in patients on hemodialysis, J. Am. Soc. Nephrol. 17 (12 Suppl. 3) (2006) S274–280.
[47] O.M. Akchurin, F. Kaskel, Update on inflammation in chronic kidney disease, Blood Purif. 39 (1–3) (2015) 84–92.
[48] O. Cabral-Marques, G. Riemekasten, Vascular hypothesis revisited: role of stimulating antibodies against angiotensin and endothelin receptors in the pathogenesis of systemic sclerosis, Autoimmun. Rev. 15 (7) (2016) 690–694.
[49] M. Giral, Y. Foucher, A. Dufay, et al., Pretransplant sensitization against angiotensin II type 1 receptor is a risk factor for acute rejection and graft loss, Am. J. Transplant. 13 (10) (2013) 2567–2576.
[50] D. Besarani, L. Cerundolo, J.D. Smith, et al., Role of anti-vimentin antibodies in renal transplantation, Transplantation 98 (1) (2014) 72–78.
[51] P. Hirt-Minkowski, M. Roth, G. Honger, P. Amico, H. Hopfer, S. Schaub, Soluble CD30 correlates with clinical but not subclinical renal allograft rejection, Transpl. Int. 26 (1) (2013) 75–83, https://doi.org/10.1111/j.1432-2277.2012.01578.x Epub 02012 Nov 01578.
[52] A. Mueller, P. Schnuelle, R. Waldherr, F.J. van der Woude, Impact of the Banff '97 classification for histological diagnosis of rejection on clinical outcome and renal function parameters after kidney transplantation, Transplantation 69 (6) (2000) 1123–1127.
[53] B. Sis, G. Einecke, J. Chang, et al., Cluster analysis of lesions in nonselected kidney transplant biopsies: microcirculation changes, tubulointerstitial inflammation and scarring, Am. J. Transplant. 10 (2) (2010) 421–430.
[54] M. Haas, Pathologic features of antibody-mediated rejection in renal allografts: an expanding spectrum, Curr. Opin. Nephrol. Hypertens. 21 (3) (2012) 264–271.
[55] N. Angaswamy, C. Klein, V. Tiriveedhi, et al., Immune responses to collagen-IV and fibronectin in renal transplant recipients with transplant glomerulopathy, Am. J. Transplant. 14 (3) (2014) 685–693.
[56] D. Dragun, D.N. Muller, J.H. Brasen, et al., Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection, N. Engl. J. Med. 352 (6) (2005) 558–569.
[57] A.T. Linke, B. Marchant, P. Marsh, G. Frampton, J. Murphy, M.L. Rose, Screening of a HUVEC cDNA library with transplant-associated coronary artery disease sera identifies RPL7 as a candidate autoantigen associated with this disease, Clin. Exp. Immunol. 126 (1) (2001) 173–179.
[58] F. Porcheray, J. DeVito, B.Y. Yeap, et al., Chronic humoral rejection of human kidney allografts associates with broad autoantibody responses, Transplantation 89 (10) (2010) 1239–1246.
[59] S. Kalache, R. Dinavahi, S. Pinney, A. Mehrotra, M.W. Cunningham, P.S. Heeger, Anticardiac myosin immunity and chronic allograft vasculopathy in heart transplant recipients, J. Immunol. 187 (2) (2011) 1023–1030.
[60] A. Bharat, D. Saini, N. Steward, et al., Antibodies to self-antigens predispose to primary lung allograft dysfunction and chronic rejection, Ann. Thoracic Surgery 90 (4) (2010) 1094–1101.
[61] S. Jurcevic, M.E. Ainsworth, A. Pomerance, et al., Antivimentin antibodies are an independent predictor of transplant-associated coronary artery disease after cardiac transplantation, Transplantation 71 (7) (2001) 886–892.
[62] A. Morgun, N. Shulzhenko, C.S. Unterkircher, et al., Pre- and post-transplant anti-myosin and anti-heat shock protein antibodies and cardiac transplant outcome, J. Heart Lung Transplant. 23 (2) (2004) 204–209.
[63] Q. Sun, Z. Cheng, D. Cheng, et al., De novo development of circulating anti-endothelial cell antibodies rather than pre-existing antibodies is associated with post-transplant allograft rejection, Kidney Int. 79 (6) (2011) 655–662.
[64] Y. Ishii, T. Sawada, K. Kubota, S. Fuchinoue, S. Teraoka, A. Shimizu, Injury and progressive loss of peritubular capillaries in the development of chronic allograft nephropathy, Kidney Int. 67 (1) (2005) 321–332.
[65] P. Liptak, E. Kemeny, Z. Morvay, et al., Peritubular capillary damage in acute humoral rejection: an ultrastructural study on human renal allografts, Am. J. Transplant. 5 (12) (2005) 2870–2876.
[66] L. Riesco, J. Irure, E. Rodrigo, et al., Anti-perlecan antibodies and acute humoral rejection in hypersensitized patients without forbidden HLA specificities after kidney transplantation, Transpl. Immunol. (2018).
[67] G. Wiederrecht, E. Lam, S. Hung, M. Martin, N. Sigal, The mechanism of action of FK-506 and cyclosporin A, Ann. N. Y. Acad. Sci. 696 (1993) 9–19.
[68] R. De Bruyne, D. Bogaert, N. De Ruyck, et al., Calcineurin inhibitors dampen humoral immunity by acting directly on naive B cells, Clin. Exp. Immunol. 180 (3) (2015) 542–550.
[69] D.B. Kaufman, R. Shapiro, M.R. Lucey, W.S. Cherikh, R. T Bustami, D.B. Dyke, Immunosuppression: practice and trends, Am. J. Transplant. 4 (Suppl. 9) (2004) 38–53.
[70] A.C. Allison, E.M. Eugui, Mechanisms of action of mycophenolate mofetil in preventing acute and chronic allograft rejection, Transplantation 80 (2 Suppl.) (2005) S181–S190.
[71] R.C. Walsh, R.R. Alloway, A.L. Girnita, E.S. Woodle, Proteasome inhibitor-based

therapy for antibody-mediated rejection, Kidney Int. 81 (11) (2012) 1067–1074.
[72] A. Mulder, S. Heidt, M. Vergunst, D.L. Roelen, F.H. Claas, Proteasome inhibition profoundly affects activated human B cells, Transplantation 95 (11) (2013) 1331–1337.
[73] J. Treckmann, C. Moers, J.M. Smits, et al., Machine perfusion versus cold storage for preservation of kidneys from expanded criteria donors after brain death, Transpl. Int. 24 (6) (2011) 548–554.
[74] C. Moers, J.M. Smits, M.H. Maathuis, et al., Machine perfusion or cold storage in deceased-donor kidney transplantation, N. Engl. J. Med. 360 (1) (2009) 7–19.
[75] R.P. Carroll, M. Riceman, C.M. Hope, et al., Angiotensin II type-1 receptor antibody (AT1Rab) associated humoral rejection and the effect of peri operative plasma exchange and candesartan, Hum. Immunol. 77 (12) (2016) 1154–1158.