B cells and antibodies in transplantation

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Abbreviations:

ABOi, ABO incompatible; AMR, antibody-mediated rejection; ADCC, antibody-dependent cell-mediated cytotoxicity; BCR, B cell receptor; DSA, donor-specific antibodies; IVIg, intravenous immunoglobulins; Treg, regulatory T cells; Tfh, follicular helper T cells;

ABSTRACT

Overlooked for decades, the humoral immune response is increasingly recognized as a leading cause of allograft loss. Improvement in the diagnosis of antibody-mediated rejection has however not yet translated into better outcomes for transplanted patients.

After an update on B cell physiology and antibody generation, the 2015 Beaune Seminar in Transplant Research challenged the conventional view of antibody-mediated rejection pathophysiology, and discussed the latest promising therapeutic approaches.

Introduction

Neglected for decades, humoral alloimmune responses that develop against donor-specific antigens have recently taken centre stage in solid organ transplantation and antibody-mediated rejection (AMR) is now considered as the main cause of allograft loss (for recent review see (1)).

The 15th meeting in the series of "Beaune Seminars in Transplant Research" (www.beaune-seminar.fr), held in the Hospices de Beaune on 21-22 May 2015, focused on the role of B cells and antibodies in transplantation. Diagnosis of AMR has considerably improved with identification of antibody-mediated lesions in graft biopsies, advances made in the detection of circulating donor-specific antibodies (DSA) and development of biomarkers to stratify the risk of graft loss (2). Yet, despite this progress, clinical outcomes of patients diagnosed with AMR remain poor. Thus, there is a need for a better understanding of the molecular mechanisms involved to identify novel targets and strategies that could be used to prevent and cure AMR.

B cells and antibodies: where do they come from?

As reminded by Olivier Thaunat (Lyon, France) in the opening session, to respond to a protein antigen (such as donor HLA molecules), B cells need to receive 2 distinct activation signals in secondary lymphoid organs. The first signal comes from the binding of cognate antigen to surface immunoglobulin of B cells (B cell receptor, BCR). BCR signalling drives the internalisation of bound antigens (donor's HLA molecules) to endosomal compartments, where they are processed. Selected peptides are then loaded onto recipient MHC class II molecules and presented on B cell surface. The provision of signal 1 also drives B cells to migrate toward the B/T-zone boundary where they interact with a particular subset of CD4+ T cells: follicular helper T cells (Tfh). Cognate Tfh cells provide the second co-stimulation signal for

the completion of B cell activation. Activated B cells proliferate and differentiate into either i) short-lived plasmablasts that produce an early burst of germline-encoded low affinity antibodies, or ii) they enter the germinal center. Within the germinal center, selected B cells differentiate into memory B cells or long-lived plasma cells, which are responsible for the delayed (but prolonged) production of switched, mutated high affinity antibodies **(Figure 1)**.

Long-lived plasma cells generated in secondary lymphoid organs (spleen or lymph nodes) express CXCR4, which explains why they respond to CXCL12 gradient secreted by stromal cells and migrate to bone marrow. Claudia Berek (Berlin, Germany) showed interesting data demonstrating that eosinophils are the main source of plasma cells survival factors and are also responsible for keeping plasma cells in bone marrow niches (3) **(Figure 1)**.

The immunosuppressive drugs currently used in clinical practice are mostly targeting T cells. According to the immunologic dogma presented above, they should efficiently prevent DSA generation by inhibiting signal 2 of activation. Yet, up to 10% of renal transplant patients develop *de novo* DSA in the first year post-transplantation, a period during which immunosuppression is maximal and compliance usually good (1). Recent data generated in Dr Thaunat's group in Lyon provide a plausible explanation to this paradox: current maintenance immunosuppression indeed only marginally impacts Tfh cell functionality.

Structure and function of antibodies

IgG molecules are made of 2 functional domains: the $F(ab')_2$ fragment, which is responsible for the antigen recognition; and the Fc fragment, which activates the complement pathway and/or recruits innate immune effector cells. The pathogenicity

of DSA depends not only on the titre (quantity) of DSA, but is also influenced by qualitative factors linked to antibody structure **(Figure 1)**.

Some antigen binding sites can mimic the active sites of enzymes thus conferring catalytic activity to the immunoglobulin. The role of catalytic antibodies has been documented in various clinical conditions, including sepsis, autoimmunity and resistance to exogenous factor VIII therapy by the group of Sébastien Lacroix-Desmazes (Paris, France). The same group recently reported that IgG endowed with serine protease-like activity, able to hydrolyse coagulation factors VIII and/or IX, were detectable in the circulation of renal transplanted patients. Interestingly, patients with higher levels of circulating catalytic IgG at 3 months had less chronic lesions on 2 years systematic allograft biopsy (4). Based on this observation they hypothesized that hydrolytic IgG may protect the graft by compensating the pro-coagulation state conferred by activated endothelium. They also proposed to use this non-invasive biomarker to identify the patients the more susceptible to develop chronic lesions on their graft (4). This hypothesis is currently under evaluation in the prospective CATAPULT study. More recently, the same group showed that the levels of catalytic antibodies were heterogenous between renal transplanted patients (5). These levels drastically drop following transplantation and then slowly return to pre-transplant values, suggesting that this parameter is an intrinsic property of each individual's immune system (5).

Another possible source of DSA heterogeneity comes from the carbohydrate chains that are attached to the Fc fragment. Studying the mechanisms of action of intravenous immunoglobulins (IVIg), Falk Nimmerjahn (Erlangen, Germany) highlighted the importance of IgG glycosylation (and especially sialylation) for their Fc dependent functions. Sialic acid rich IgG glycoforms have indeed the capacity to

upregulate the expression of inhibitory FcγRIIB and decrease the expression of activating FcγRs on innate immune effectors (6). Moreover, sialylated serum IgG seems to also play a role in immune homeostasis. Analysis of samples from patients and mice suffering from autoimmune and inflammatory diseases indeed revealed increased levels of IgG glycoforms lacking terminal sialic acid and galactose residues (7). In the context of transplantation, the impact of DSA sialylation has not yet been studied but it is reasonable to speculate that this parameter could be a source of clinical heterogeneity in AMR.

Anne S. De Groot (Providence, USA) shared data suggesting that IgG have highly conserved epitope called T regitopes. After IgGs have been internalized and processed by antigen presenting cells, T regitopes are presented in HLA class II DR molecules, which recruit natural regulatory T cells (Tregs) that in turn switch off antigen presenting cell inflammatory functions and promotes the generation of adaptive Tregs. In agreement with this concept, administration of Tregitopes to mice grafted with allogeneic skin graft, promoted the expansion of donor-specific Tregs and reduced the generation of effector T cells, leading to the induction of tolerance (8).

Antibodies and graft rejection or acceptance

Highly polymorphic mismatched HLA molecules represent the most documented targets for DSA. But it is likely that DSA can also be directed against other kinds of molecular targets, including polymorphic minor histocompatibility antigens and non-polymorphic autoantigens (9). Using high-density protein arrays, Minnie Sarwal (San Francisco, USA) and her team identified non-HLA antigens expressed by endothelial cells and provided data suggesting that antibodies directed against these targets, could participate in the development of rejection (10).

Binding of circulating DSA to directly accessible graft endothelial cells can trigger the activation of the classical complement pathway leading to acute humoral rejection (11,12) **(Figure 1)**. Non-complement binding DSA can still promote rejection through the recruitment of innate immune cells, which bind to DSA through their Fc receptors and release lytic enzymes (a mechanism called “antibody-dependent cell-mediated cytotoxicity”, ADCC) **(Figure 1)**. ADCC can be modulated by FcγR polymorphism. This was first demonstrated by the team of Hervé Watier (Tours, France) in the context of therapeutic antibodies, the efficiency of which to induce target cell death was dependent on patients’ genetic variant of FcγRIIIA (13). Elaine Reed (Los Angeles, USA) recently confirmed the importance of FCγR polymorphism in the context of AMR, linking FcγRIIIA polymorphism with leukocyte recruitment to endothelium. Her presentation also highlighted the role of anti-HLA antibodies subclasses in this phenomenon (14).

Beyond these Fc-dependent mechanisms, other Fc-independent mechanisms are also implicated in graft destruction. Dr Reed demonstrated that crosslinking of MHC class I molecules by DSA stimulates endothelial cell proliferation and migration **(Figure 1)**. Binding of anti-class I antibodies also promote inflammatory activation of endothelial cells, leading to increase expression of P-selectin and secretion of IL-8, MCP-1 that in turn promote leukocyte recruitment. Of note, MHC class I molecules lack an intracellular domain to transduce a signal and must develop a partnership with integrin β4 to do so.

Human endothelial cells, in particular those located in the renal peritubular capillaries and glomeruli, also express (albeit at lesser extent) class II HLA molecules, which can be further enhanced by appropriate stimuli of inflammatory and immunologic origin. Thus, anti-HLA class II DSA also have the capacity to trigger endothelial cell

activation (15), as Nuala Mooney (Paris, France) reminded us . Expression of donor HLA II molecules by graft endothelium turns graft endothelial cells into “antigen presenting cells” for recipient’s CD4 T cells. Secretion of IL-6 by activated endothelial cells polarizes recipient’s alloreactive T CD4+ cells toward the Th17 pathway while repressing Tregs generation (16), which could accelerate graft destruction (17) **(Figure 1)**.

The concept of accommodation (i.e lack of graft injury despite re-appearance of antibodies directed against the graft in recipient’s circulation) emerged in the 90s in ABO incompatible (ABOi) transplantation. Currently, the presence of donor-specific anti-ABO antibodies is determined using hemagglutination assays. Lori West (Edmonton, Canada) questioned the accuracy of this assay by demonstrating that erythrocytes may expressed type II/III/IV based ABH antigens whereas vascular endothelial cells of heart only expressed type II based ABH antigens. Moreover, the level of expression of ABH antigens and their subtype is variable on erythrocytes of different blood types (18). Dr West and her team developed an ABO-subtype antigen microarray where the different subtypes of ABH antigens are incubated with the patients’ plasma. The binding of anti-ABH antibodies is then detected with a fluorescent secondary antibody by flow cytometry. Using this assay, the authors demonstrated that donor-specific anti-ABO antibodies seems to be absent of the plasma of stable ABOi heart graft recipients, meaning that whole concept of accommodation may need to be re-examined.

Challenges in AMR diagnosis

In line with the growing interest in AMR, tools available to detect DSA have considerably improved over the last decades. The introduction of solid-phase membrane-independent assays, and particularly flow bead assays offers the

possibility to detect circulating DSA with exquisite sensitivity and to identify their specificities. As Jean-Luc Taupin (Bordeaux, France) reminded us, these assays may still have some drawbacks: i) the weak correlation with cell-based assays, ii) the conformation of HLA antigens (intact or denatured) on the beads iii) the interference of IgM and complement to detect IgG (the so called “prozone” effect) iv) assay reproducibility and standardization. Recently, new improvements in these assays allow to test the quality of antibodies: i) their capacity to bind C1q or C3d (and so to activate complement *in vivo*) (11,12) ii) the different IgG subclasses of anti-HLA antibodies *(Lefaucheur et al., in press*).

In the same line, it has been shown that DSA bound to the graft (identified in the eluate from needle core graft biopsy) correlated better with the severity of antibody-mediated pathogenic process than circulating DSA (19,20). These new tools will help clinicians to stratify the risk of graft loss in case of AMR **(Figure 1)**.

Usually, different parameters are taken into account separately to stratify the risk of allograft loss after AMR. A. Loupy and his team (Paris, France) have developed an integrative approach (iBOX®; http://www.paristransplantgroup.org) to connect together clinical, histologic, immunological and molecular data to generate multidimentional risk scores for predicting allograft failure (21).

Challenges in AMR treatment

Currently, the number of highly sensitized patients on the renal transplant waiting list is increasing. These patients have difficulty to access to transplantation. To optimize transplantation rate and graft survival after transplantation, Robert Montgomery (Baltimore, USA) demonstrated the interest of combining kidney paired donation and desensitization, especially for recipients who are very difficult to match (AB donors, broadly sensitized) and difficult to desensitize (high titer of DSA) (22).

What should we do when transplanted patients are diagnosed with AMR? Denis Glotz (Paris, France) reviewed the current strategies, which rely on the combination of agents aiming at removing DSA (plasma-exchange), regulating innate immune effectors (IVIg, corticosteroids), depleting B cells (Rituximab) and /or plasma cells (Bortezomib) **(Figure 1)**. These strategies, which have little impact on DSA titer, can unfortunately (at best) only slow down antibody-mediated destruction of the graft and no large controlled trial is available to determine what would be the best combination. Recently, Eculizumab, a humanized monoclonal antibody that targets complement protein C5, was developed to treat paroxysmal nocturnal hemoglobinuria. In transplantation, Christophe Legendre (Paris, France) presented data demonstrating that Eculizumab is effective to prevent post-transplant atypical haemolytic and uremic syndrome and antiphospholipid syndrome recurrences (23). Preliminary data suggest that Eculizumab could also prevent acute AMR in sensitized patients **(Figure 1)**. Its capacity to prevent chronic AMR is currently tested (but might be disappointing given the fact that the drug will not interfere with ADCC, see above).

Gunnar Tufevson (Uppsala, Sweden) presented a new promising drug: the endopeptidase IdeS, which has the ability to degrade all human IgG into a $F(ab')_2$ and Fc fragment (24) **(Figure 1)**.His group tested IdeS in immunized patients on waiting list for a kidney transplant. They showed that IdeS transiently decreased the titer of anti-HLA antibodies, rendering both cytotoxicty and flow cross matches negative. The case of a first patient desensitized with ideS and then transplanted on ATGAM induction plus mycophenolate mofetil, tacrolimus and corticosteroids as maintenance therapy was reported. Graft function remained normal 6 months after transplantation.

Sally Ward (Dallas, USA) presented data about Abdeg, an antibody, which accelerates IgG degradation by binding to neonatal Fc receptor (FcRn) and thus saturating the recycling of IgGs in endothelial, epithelial, and antigen presenting cells **(Figure 1)**. In a mouse model of antibody-dependent arthritis, her group showed both a preventive and a curative effect of Abdeg (25), suggesting that this agent could be of interest to treat AMR.

Of note, the long-term impact of depletion of all IgG (IdeS and Abdeg) and disruption B cell receptor (IdeS) on the risk of infections is not known. Nevertheless, these approaches could open a window to enable highly sensitized patients to receive a transplant. However, it is also important to note that antibody-producing cells are not targeted by these therapies, thus it is likely that they won't be curative of AMR. Therefore, in the absence of efficient curative treatment for AMR, emphasis should be placed on "primary" prevention of *de novo* DSA as Mena Clatworthy (Cambridge, UK) reminded us (26). A future of B cell targeted therapy could be of inhibiting effector B cells while enhancing regulatory B cells **(Figure 1)**.

B cells: not always the bad for the graft

For decades, B cells have been considered exclusively as motor of the rejection process: either as precursor of DSA-producing plasma cells or as antigen presenting cells to allogeneic T lymphocytes. Recently, experimental studies in autoimmune and inflammatory diseases models have highlighted the immune-regulatory potential of B cells (27). Indirect evidence for B cell regulation in transplantation recently emerged from clinical studies (28).

B cells are thought to exert their regulatory function through provision of immune-regulatory cytokines (IL-10, IL-35 and/or TGF-β), inhibition of dendritic cells or CD8 functions, suppression of Th1 and Th17 responses, and promotion of regulatory T

cell differentiation. However, regulatory B cells (if such subset really exist) are heterogeneous across the studies and their phenotypes differ between mouse and human. Identification of B cells with regulatory functions is made difficult by the lack of universal marker (such as Foxp3 for T regulatory cells) and the fact that the signals required to generate these cells are the same as those necessary to generate effector B cells (i.e BCR, CD40 and TLR) (29) **(Figure 1)**.

An interesting way to prevent AMR could therefore be to harness regulatory potential of B cells and use them as cell therapy in transplantation. Following this idea Sushma Shankar from the group of Kathryn Wood (Oxford, UK) showed that *in vitro* generated human regulatory B cells could induce graft tolerance in a humanized mouse model of skin graft.

Several questions need however to be answered before translating this strategies in the clinic: i) which regulatory B cell subset should be used, ii) what should be the origin of regulatory B cells (donor? recipient?) ii) what signal combination is the best to generate them, iii) what are their behaviour after transfer in vivo: will they maintain their regulatory phenotype when exposed to inflammatory environment? (30)

Conclusion

B cells have recently taken more central role with the recognition of AMR as the main cause of allograft loss. The ultimate treatment of AMR would shut down DSA production by destroying plasma cells in an antigen-specific fashion. Such therapeutic option is unfortunately not available yet, and since current therapeutic strategies in AMR only achieve 50% graft survival at 3 years (12), it is of utmost importance to focus on primary prevention: the blocking of *de novo* DSA generation. On conventional triple maintenance immunosuppressive regimen (i.e. calcineurin inhibitor + mycophenolate mofetil + corticosteroids), 5-10% of renal transplanted

patients develop *de novo* DSA during the first year post-transplantation, a time when the doses of drugs are maintained at a high level and compliance generally good. Identification of these patients would allow adapting their management before DSA appearance but requires developing innovative biomarkers probing B cell function. What could then be proposed to these patients for whom the classical immunosuppressive approach is insufficient to block DSA generation? New immunosuppressive drugs are currently under development that target the second "costimulation" signal of T and B cell activation. It is tempting to speculate that anti-CD40 monoclonal antibody, which blocks a crucial pathway for B cell differentiation into plasma cells, might prove more efficient than conventional approaches to prevent DSA generation. In line with this hypothesis are the encouraging results of the two phase 3 studies: BENEFIT and BENEFIT-EXT, showing that kidney recipients on belatacept (a blocker of CD28-CD80/CD86 pathway) have surprisingly low rates (5%–6%) of DSA formation at 3 years.

Finally, emergence of experimental data demonstrating that B cells are endowed with potent regulatory roles and that they are central in allograft tolerance, open new perspectives for tolerance induction to allograft.

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Figure 1: B cells and antibodies in transplantation

Therapies currently used are indicated in bold red; drugs under evaluation are in dashed red; future therapies are in dashed orange.

BCR, B cell Receptor; B reg, regulatory B cells; DSA, donor dpecific antibodies; IVIG, intravenous immunoglobulins; MHC, major histocompatibility complex; TLR, toll like receptor; T reg, regulatory T cells;

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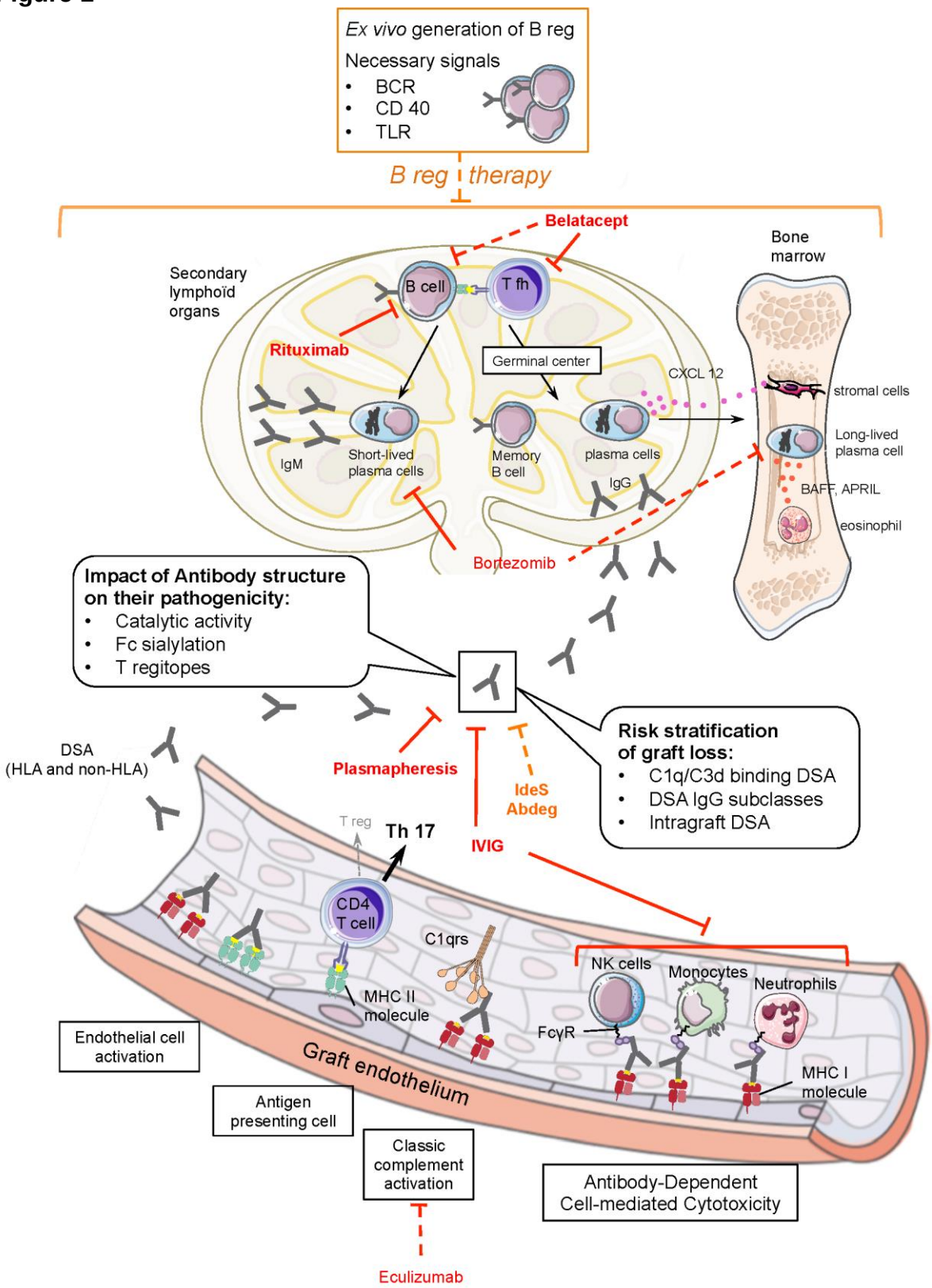
Figure 1


Ex vivo generation of B reg
Necessary signals
• BCR
• CD 40
• TLR
B reg therapy
Belatacept
Secondary lymphoïd organs
B cell
T fh
Rituximab
Germinal center
CXCL 12
Bone marrow
stromal cells
Long-lived plasma cell
BAFF, APRIL
eosinophil
IgM
Short-lived plasma cells
Memory B cell
plasma cells
IgG
Bortezomib
Impact of Antibody structure on their pathogenicity:
• Catalytic activity
• Fc sialylation
• T regitopes
Risk stratification of graft loss:
• C1q/C3d binding DSA
• DSA IgG subclasses
• Intragraft DSA
DSA (HLA and non-HLA)
Plasmapheresis
IdeS Abdeg
IVIG
T reg
Th 17
CD4 T cell
C1qrs
NK cells
Monocytes
Neutrophils
MHC II molecule
FcγR
MHC I molecule
Endothelial cell activation
Graft endothelium
Antigen presenting cell
Classic complement activation
Antibody-Dependent Cell-mediated Cytotoxicity
Eculizumab