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# Immune mechanisms of acute and chronic rejection

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ABSTRACT

With the currently available immunosuppression, severe T-cell mediated rejection has become a rare event. With the introduction of modern antibody-detection techniques, such as the L-SAB technology, acute or hyperacute antibody-mediated rejection of the kidney are also seen infrequently. In contrast, chronic antibody-mediated rejection is considered to be a major contributor to graft loss in the late posttransplant phase. Problems in the management of chronic antibody-mediated rejection are effective prevention of the development of alloantibodies against donor HLA and the early identification of patients at risk for this entity. Finally, today there is still no effective strategy to treat this indolent and slowly progressing form of antibody-mediated rejection. Herein, we review the pathomechanisms of the different forms of rejection and the clinical significance of these entities in human kidney transplantation.



## 1. Introduction

Exposure of an individual to foreign major histocompatibility antigens (MHC or HLA for human leukocyte antigens) can promote activation of the immune system and its memory components, resulting in sensitization. Such an exposure occurs, e.g. after prior transplantations, blood transfusions and pregnancies [1]. If also alloantibodies against HLA antigens that are expressed on foreign donor tissue are generated and if the alloantibody formation is in addition supported by a memory or ongoing T cell response, hyperacute or accelerated rejection of the organ could be the consequence in the early phase after transplantation [2,3]. In later phases after transplantation, especially during episodes of insufficient immunosuppression, *de novo* alloantibodies are generated against mismatched HLA antigen epitopes, leading to episodes of acute or chronic antibody-mediated rejection.

Due to currently available effective T cell-directed immunosuppression, rejection episodes in which only T cells in the absence of antibodies are involved, occur rather rarely and are easily treatable. Even when not promptly detected, for example in case of a subclinical rejection, the long-term significance of these events is still unknown [4,5].

In contrast, alloantibody-mediated rejections are more resistant to therapy and frequently result in graft loss, even when subclinical [4,6]. Therefore, one of the major goals of the histocompatibility testing in HLA laboratories is to precisely identify the organ recipient's HLA antibodies against donor antigens before and after transplantation in order to reduce the immunological risk and recognize and monitor ongoing rejection [7].

## 2. Immunological events during allograft rejection

The rejection process develops in three steps:

a) Recognition of alloantigens on foreign tissue.
b) Generation of alloimmune response.
c) Destruction of transplant tissue.

### 2.1. Recognition of alloantigens on foreign tissue

The key premise for the adequate functionality of the immune system in all vertebrates is the precise differentiation between self, non-self, and the abnormal self. In humans, this process involves the recognition of antigenic epitopes by T cells. Also during the rejection process, recognition of HLA antigens on foreign tissue (allorecognition) is the triggering first step, ultimately resulting in extensive activation of the T-cell response. Inflammation of the kidney after ischemia–reperfusion injury, infections or any condition which leads to an enhanced exposition of surface markers of the donor tissue contribute to this phase.

The allorecognition of foreign HLA occurs via two different pathways, also defined as direct and indirect allorecognition. In the direct

*Abbreviations:* APC, Antigen presenting cell; CD45RO, CD45 180 kD isoform; DSA, Donor specific antibody; HLA, Human leucocyte antigens; L-SAB, Luminex single antigen bead; MFI, Mean fluorescence intensity; MHC, Major histocompatibility complex; MICA, Major-histocompatibility-complex (MHC) class I-related chain A; NK, Natural killer; TNF-α, Tumor necrosis factor alpha.

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allorecognition pathway, the host's T cells interact with foreign HLA presented by "antigen-presenting cells (APCs) of the donor". This process mainly occurs in the early phase of transplantation, when the still viable donor leukocytes migrate to the host's lymph nodes. The relative contribution of this interaction to the alloresponse is, therefore, subject of debate. In the indirect allorecognition pathway, foreign HLA molecules are first processed and then presented in peptide form by "APCs of the patient" to his own helper T cells. After further signaling processes, the formation of a specific T, and in more severe forms, B cell response against foreign HLA is the consequence. These processes can occur not only after ischemia–reperfusion injury but during the allograft's whole lifetime and may also be responsible for chronic rejection events. Moreover, they are not limited to the HLA antigen system and can also be generated against other non-HLA antigen systems [8].

### 2.2. Generation of alloimmune response

Mature APCs also coordinate the further activation and clonal expansion of T cells using other receptor classes. Through secretion of cytokines and chemokines, APCs induce endothelial expression of adhesion molecules, attracting after permeabilization of the vascular endothelium immune cells to the site of the ongoing rejection process. Further cytokine production activates helper T cells. These cells can also generate a sub-population of memory T cells expressing CD45RO, which can rapidly be activated in case of antigen re-exposition [9]. Moreover IL-2, IL-4, IL-5, IL-6 and IL-13 secreted by T cells induce activation and differentiation of antigen-specific B cells. In this process, naïve B cells expressing IgM on its surface undergo immunoglobulin class switching, enabling the production of IgG antibodies with high affinity [10].

### 2.3. Destruction of transplant tissue

After T cell activation, the immune response is directed by T helper cells via cytokine secretion, regulating several effector cell pathways towards a cellular and/or humoral response. Helper T cells are also involved through the secretion of interferon gamma (IFN-γ) in the non-specific activation of both natural killer cells and macrophages, which are able to cause tissue damage by releasing cytotoxic molecules stored in intracellular vesicles. Activated macrophages play an important role in the effector phase of rejection by inducing apoptosis of allogeneic cells through TNF-α secretion, and phagocyting damaged allogeneic cells. Furthermore, macrophages with their Fc receptors are also capable of recognizing the Fc region of alloantibodies bound to foreign tissue, leading to antibody-mediated cellular destruction.

Differently from T cells, which recognize a processed antigen on self-HLA, B cells express immunoglobulin molecules on the cell surface, capable of recognizing and binding a native antigen, which in the consequence leads to the selection of antigen-specific B cell clones.

B cells can also act as APCs. They can internalize the "foreign" antigen, process it to peptide fragments and present these in the antigen binding cleft of self HLA class II molecules to T cells. Furthermore, B cells can cooperate via their CD40 molecules with the CD40 ligand molecules on T helper cells, receiving help for their development towards alloantibody producing plasma cells [11].

HLA antibodies are also able to directly activate the endothelial cells, increasing allograft inflammation. They can bind to foreign HLA, triggering the classical complement pathway and leading to opsonization, chemotaxis and lysis of allogeneic cells. Moreover, the Fc region of antibodies can attract, after antigen binding, besides phagocytes also Fc receptor carrying natural killer (NK) cells of the innate immunity, leading to further destruction of the transplanted tissue [12].

Besides the cytotoxic components of the innate immune system, more effectively, cytotoxic T cells of the adaptive immune system can also do harm to the allogeneic tissue. Helper T cells are primordial for the activation of such cytotoxic T cells and activated cytotoxic T cells can recognize foreign class I MHC molecules on the tissue, releasing agents, such as perforin and granzyme B, which have a direct cytotoxic action leading to rupture of the target cell membrane and induction of apoptosis.

A key strategy to reduce immunogenicity and alloantigen load is to minimize HLA mismatching between donors and recipients, which is effective at not only reducing the number and severity of acute rejection episodes and improving long-term alllograft survival, but also in preventing sensitization in the case of a re-transplantation [13–16].

## 3. Mechanisms of antibody-mediated rejection

### 3.1. Hyperacute, accelerated and acute rejection

Today, hyperacute rejection has become a rare event and is primarily the consequence of high levels of complement-activating donor-specific antibodies at the time of transplantation. It may occur after accidental ABO incompatible kidney transplantation or in the presence of preformed circulating antibodies against donor HLA (DSA), when these are overlooked during the pretransplant crossmatch and antibody screening procedures. Hyperacute rejection may also occur in desensitized patients with a strong early antibody rebound after transplantation. Hereby, alloantibodies bind to vascular endothelium of the graft and activate the complement system, leading to a massive inflammatory response, granulocyte infiltration, micro- and macrovascular thrombosis and mesangiolysis. Thrombosis caused loss of graft perfusion may lead to ischemia, infarction and tissue destruction within minutes to hours in the early posttransplant phase. Histopathological analysis reveals in these cases the deposition in graft capillaries of IgG that are directed against HLA or ABO antigens or in rare cases against other or so far not well characterized antigen systems [17].

Accelerated rejection is a severe form of rejection that shows similar features to hyperacute rejection. The graft function is normal within the first 24–48 h. Thereafter, a rapid decline of graft function due to rapidly progressing antibody-mediated rejection occurs in recipients whose immune system had already been primed to mismatched donor antigens.

(Early) acute antibody-mediated rejection is also an early, during the first 10–14 days occurring aggressive process, which is mostly but not exclusively limited to patients whose immune system had been primed to donor antigen. Peritubular capillaritis and glomerular inflammation are the main signs of acute antibody-mediated graft damage, often accompanied by deposition of the complement split product C4d within peritubular capillaries as a sign of complement activation. In more severe cases, microthrombi and tubular injury are present. Acute antibody-mediated rejection, though often found during the early phase after kidney transplantation, may also occur late, especially during the *de novo* development of DSA, e.g. in patients with non-adherence to immunosuppressive therapy and withdrawal of immunosuppressive medication [18].

### 3.2. Chronic antibody-mediated rejection

During late phases after kidney transplantation, insufficient immunosuppression and stimulation of the memory responses of B- and T-cells by inflammatory events can lead to the *de novo* development of DSA. These antibodies may bind to antigenic structures and result in failure of the transplanted organ due to a more subtle type of injury called chronic antibody-mediated rejection. Further allo- and auto-antibodies that are currently discussed in the evolution of chronic antibody-mediated allograft injury are MHC class I-related chain A (MICA) antibodies, angiotensin II type 1 receptor-activating antibodies and so far not well characterized anti-endothelial cell antibodies [19–21]. During the latest update of the BANFF classification [6], C4d-positivity in peritubular capillaries was not anymore considered as an essential prerequisite for the diagnosis of antibody-mediated rejection. Today, microvascular inflammation or the detection of