End-stage organ diseases are responsible for millions of deaths worldwide each year. Organ transplantation has become the treatment of choice, but despite the 120,000 new organ transplantations performed each year, only 1 million persons worldwide have functioning solid-organ transplants because of the lack of improvement in long-term allograft survival over the past few decades and the limited organ supply.

HLA incompatibility between donors and recipients who are not genetically identical has been identified as the main barrier to successful transplantation, mostly because of antibody-mediated rejection, a form of allograft rejection triggered by the production of antibodies directed mainly toward donor (nonself) HLA molecules. This review focuses on current standards for the management of antibody-mediated rejection in transplant recipients and identifies future directions for improving diagnostics and moving toward tailored therapeutics. Such advances require the development of pathogenesis-based approaches that combine precise characterization of the biologic properties of antibodies, noninvasive biomarkers, and allograft gene-expression profiling, which will set the stage for bringing antibody-mediated rejection into the era of precision medicine.

Diagnostic Criteria for Antibody-Mediated Rejection

Antibody-mediated rejection of kidney transplants was introduced as a distinct clinicopathological entity in the 1997 international Banff classification of kidney allograft rejection, with regular updates since then (see the 2017 update in the Supplementary Appendix, available with the full text of this article at NEJM.org).

The recognition of antibody-mediated rejection of kidney allografts led to the development of standardized nomenclature, diagnostic criteria, and a unified reporting scheme that was later adopted and modified for heart, lung, and pancreatic transplantation. In 2016, the reporting scheme was adopted for liver transplantation, reflecting increasing evidence that liver transplants, which had been considered to be resistant to antibody-mediated rejection, could also be a target for antibody-mediated injury. Although the definition of antibody-mediated rejection has undergone many changes over the past several decades, the cardinal features, outlined below, are shared by all classifications in solid-organ transplantation.

Circulating Donor-Specific Antibodies Directed against HLA or Other Antigens

The presence of donor-specific anti-HLA antibodies is a key component of diagnosis of antibody-mediated rejection in kidney, lung, and liver transplants and is currently under consideration as a mandatory criterion for rejection in heart transplants. In addition to preformed donor-specific anti-HLA antibodies — that is, donor-specific anti-HLA antibodies that were present before transplantation — donor-specific anti-HLA antibodies can emerge at any time after transplantation,
often as a result of insufficient immunosuppression or nonadherence to immunosuppressive therapy. Since the first complement-dependent cytotoxicity assays that detect high levels of cytotoxic donor-specific anti-HLA antibodies, newer immunosassays, in which purified HLA antigens are covalently bound to a solid-phase platform, have been developed to improve the sensitivity and specificity of HLA-antibody detection. In addition to HLA antibodies, numerous non-HLA antibodies directed against a heterogeneous subset of both alloantigens and autoantigens mainly expressed by endothelial cells have been identified in relation to allograft rejection in recipients of kidney, heart, and lung transplants, such as major histocompatibility complex I polypeptide-related chain A and agonistic angiotensin II type 1 receptor antibodies in kidney-transplant recipients and collagen autoantibodies in heart-transplant and lung-transplant recipients. Screening for pathogenic non-HLA antibodies may be useful in patients with pathological features of antibody-mediated rejection in the absence of donor-specific anti-HLA antibodies.

**Biopsy Evidence of Current or Recent Antibody–Vascular Endothelium Interaction**

Immunoperoxidase or immunofluorescence techniques are widely used for histologic detection of endothelial membrane–associated complement split product C4d, which provides evidence of antibody interaction with the allograft vasculature. C4d staining is a specific marker of antibody-mediated rejection in heart and kidney allografts when the stain is deposited in the capillaries (Fig. 1). The usefulness of C4d staining in the lung and liver has also been acknowledged in recent classification schema for allograft rejection. However, C4d staining has been shown to have low sensitivity, with negative results in up to 50% of patients with antibody-mediated rejection. In patients with negative C4d staining, the diagnosis of antibody-mediated rejection may be confirmed on the basis of increased expression of gene transcripts or classifiers in the biopsy tissue that are strongly associated with antibody-mediated rejection.

**Histologic Evidence of Acute Tissue Injury in Organ Transplants**

Antibody-mediated rejection is typically observed in the microvasculature of the transplanted organ as capillary dilatation, cytoplasmic swelling or enlargement and vacuolization of the endothelial cells, and the presence of intracapillary activated cells, including monocytes, macrophages, natural killer cells, T cells, neutrophils, and eosinophils (Fig. 1). Thus, microvascular inflammation is a key diagnostic feature of antibody-mediated rejection in all types of organ allografts. The presence of microvascular lesions is increasingly recognized in antibody-mediated rejection, including mild or severe intimal arteritis and mononuclear and lymphocytic inflammation of the intima, with or without transmural necrosis (Fig. 1).

**Chronic Antibody-Mediated Rejection**

As the clinical practice of transplantation evolved and immunosuppressive strategies became increasingly effective, the subtle delayed effects of antibody-mediated rejection were recognized, since acute rejection was no longer a common cause of allograft loss. Transplantation physicians began to focus on late allograft changes, including chronic rejection, which portends serious risks of allograft loss and death among recipients of kidney, heart, and lung transplants. Chronic vascular lesions are a cardinal feature of chronic antibody-mediated rejection in all solid-organ transplants, though manifestations and terminologies vary according to the organ type, as shown in Figure 1. In renal allografts, chronic vascular lesions are seen as glomerulopathy, multilamination of the peritubular capillary basement membrane, and arteriopathy. In cardiac allografts, the vascular injuries are manifested as vasculopathy or arteriosclerosis. In lung transplants, besides the bronchiolitis obliterans syndrome, vascular injuries are characterized by fibrointimal thickening of pulmonary arteries and veins, and liver allografts often develop an obliterative arteriopathy.
Figure 1. Histologic Phenotypes of Antibody-Mediated Rejection in Solid-Organ Transplants.

Histologic images show the histologic features of antibody-mediated rejection in solid-organ transplants. Panel A shows a representative example of kidney transplants, with prominent tubular damage and interstitial inflammation. Panel B shows heart transplants, with prominent cardiac myocyte damage and interstitial inflammation. Panel C shows lung transplants, with prominent interstitial inflammation and bronchiolitis obliterans.

Organ Transplant

- Kidney
- Heart
- Lung
Antibody-Mediated Rejection of Solid-Organ Allografts

Today, we are able to detect circulating donor-specific anti-HLA antibodies that bind complement in organ-transplant recipients, using single-antigen bead assays that detect C1q-binding or C3d-binding donor-specific anti-HLA antibodies or C4d deposition onto the bead surface. There is still debate regarding the role that assessment of complement-activating, donor-specific anti-HLA antibodies should play in clinical practice, although the deleterious effect of these antibodies on allograft outcomes appears to be consistent across populations of patients who have received transplants (kidney, heart, lung, and liver) and across the types of tests used for their detection. Studies have shown that organ recipients with complement-activating, donor-specific anti-HLA antibodies have an increased risk of antibody-mediated rejection, more severe antibody-mediated injury, and overexpression of allograft genes associated with natural killer cells, macrophages, and endothelial activation. The evaluation of complement activation by donor-specific anti-HLA antibodies may allow more rational and pathogenesis-based use of complement-targeting agents.

Besides complement activation, the relevance of complement-independent mechanisms of antibody-mediated allograft injury, which has been primarily shown in animal models, is now increasingly reported in human studies (Fig. S1 in the Supplementary Appendix). This is illustrated by the clinical recognition of C4d-negative antibody-mediated rejection, the observation that the rate of graft survival is lower among patients with non–complement-binding, donor-specific anti-HLA antibodies than among patients without such antibodies, and the failure of complement-inhibition therapies to prevent antibody-mediated rejection in patients with non–complement-binding anti-HLA antibodies.

The complexity of antibody-mediated rejection and the recognized limitations of standard histologic assessment have led to the development of new approaches for improving assessment. Following the example of the field of oncology, in which the measurement of multigene-expression profiles in tissue has been implemented in routine clinical practice, transplantation investigators have scrutinized transcriptome-wide micro-
array data since 2003 and have found associations with discrete phenotypes and lesions. More recently, molecular signatures with evident specificity for antibody-mediated rejection have been identified and are similar in kidney-transplant and heart-transplant recipients, supporting the hypothesis that common mechanisms promote rejection in solid-organ transplants of multiple types. Data from various centers in Europe and North America are consonant, providing common tissue-based measurements of specific pathogenesis-based transcripts (Fig. S2 in the Supplementary Appendix). These data suggest that antibody-mediated rejection involves the presence and activation of natural killer cells, endothelial cells, and macrophages.

The information provided by allograft molecular assessment has led to an improved understanding of the biologic processes that govern antibody-mediated rejection and that have the
Figure 2 (facing page). Prototypical Immune Responses Triggered by Complement-Activating, Donor-Specific Anti-HLA Antibodies in Transplant Recipients with Antibody-Mediated Rejection.

The bar graph in Panel A depicts characteristics of a representative case of complement-activating, donor-specific antibody (DSA) targeting HLA-DRB1*03:01 (DR17), including the mean fluorescence intensity (MFI) on the standard pan-IgG, Luminex-based assay and the C1q assay, as well as individual IgG subclass reactivity. Panel B shows the binding of complement-activating DSA to donor endothelium, which involves multiple immune effector mechanisms, largely led by natural killer (NK) cells and monocytes. The primary Fc gamma receptor (FCGR) on NK cells, FCGRIIIa (CD16A), is highly selective for complement-activating IgG, particularly IgG3. NK cells activated through FCGR produce and release effector cytokines interferon-γ (IFNG), tumor necrosis factor (TNF), and granulocyte-macrophage colony-stimulating factor (CSF2), which act on nearby monocytes to enhance their cytotoxicity. Enhancement of monocyte cytotoxic potential translates into increased monocyte-released soluble mediators, such as TNF, interleukin-1, and interleukin-6, which damage and activate endothelial-cell targets and up-regulate adhesion-molecule expression, thus facilitating additional leukocyte adherence. Chemokines CCL4 and CCL3, selectively produced and released by FCGR-activated NK cells, recruit monocytes, whereas IFNG, TNF, and CSF2 induce monocyte CXCL9 and CXCL10 expression, facilitating the recruitment of additional NK cells. NK-cell effector cytokines also act on endothelial targets, directly increasing endothelial HLA expression to provide additional binding targets for DSA. Cytotoxic molecules in NK-cell granules (granulysin [GNLY], perforin [PRF1], and granzymes A and B [GZMA and GZMB]) are also released after FCGR engagement, further increasing endothelial damage through antibody-dependent, cell-mediated cytotoxicity (ADCC). Activation of the classical complement cascade leads to the formation of the membrane attack complex (MAC) C5b–C9, which enhances the extent of endothelial activation. Panel C shows active antibody-mediated rejection, characterized by linear C4d deposition in capillaries (left image) and glomerular and capillary infiltration by monocytes or macrophages (middle image; CD68-positive immunoperoxidase staining) and NK cells (right image; NKP46-positive immunoperoxidase staining).

Because anti-HLA antibodies are well-characterized prognostic biomarkers that predict relevant clinical outcomes for different types of organ transplants, their systematic monitoring is currently part of the consensus guidelines established by the Antibody Consensus Group of the Transplantation Society. However, monitoring patients for the presence of donor-specific anti-HLA antibodies is a limited approach to risk stratification for antibody-mediated rejection and allograft loss because all anti-HLA antibodies are not equally pathogenic. Thus, allograft biopsy remains important for the detection of antibody-mediated rejection when donor-specific anti-HLA antibodies are detected after transplantation, even for patients in stable condition whose allograft function is not compromised. There is growing evidence that characterizing donor-specific anti-HLA antibodies in terms of titer level and the capacity to activate complement or IgG subclass composition might substantially improve our ability to predict antibody-mediated rejection and allograft loss. Moreover, assessment of the kinetics of donor-specific anti-HLA antibody level and complement-activating capacity may predict transplant outcomes after standard-of-care treatment for antibody-mediated rejection, which has important management implications.

Several clinical studies of non-HLA antibodies targeting endothelial cells in kidney-transplant recipients have revealed an association with decreased allograft survival, as well as possibly deleterious synergy with anti-HLA antibodies. However, whether screening or monitoring patients for non-HLA antibodies adds value to the current standard of anti-HLA-antibody assessment for the diagnosis of antibody-mediated rejection and risk stratification is uncertain.
OTHER POTENTIAL BIOMARKERS

Other noninvasive biomarkers in blood or urine have been investigated for their possible usefulness in the diagnosis of rejection in patients with various types of solid-organ transplants, although the majority of the studies were not specifically designed to address antibody-mediated rejection or to cover the broad spectrum of its clinical and histologic manifestations. Changes in leukocyte or whole-blood gene expression, including multigene assays,59,60 have been evaluated in kidney recipients. Several molecules have been assessed as potential biomarkers for kidney-allograft rejection, such as urinary CXCL9 and CXCL10 proteins, perforin, granulysin messenger RNA (mRNA) and blood granzyme B and perforin mRNA.51 In addition, there is evidence that assessment of microRNA (miRNA) molecules may have diagnostic potential. A study showed that specific miRNA molecules in peripheral blood from heart-transplant recipients were associated with allograft rejection.52

In heart-transplant recipients, the AlloMap test, which evaluates the expression of 11 genes in peripheral blood and aims to distinguish between rejection and the absence of rejection, may identify patients at risk for acute allograft rejection. Recent efforts to improve the specificity of noninvasive biomarkers for differentiating antibody-mediated rejection from other rejection phenotypes have focused on donor-derived cell-free DNA (dd-cfDNA) in plasma,53-54 HLA-specific memory B cells in peripheral blood,55,56 and donor-specific tissue exosomes released into the recipient’s circulation.57

TREATMENT OF ANTIBODY-MEDIATED REJECTION

The primary aims of therapeutic approaches to antibody-mediated rejection are to remove circulating donor-specific anti-HLA antibodies and to block their effects, reduce their production, or both (Fig. 3). Although no therapeutics have received FDA approval for the treatment of antibody-mediated rejection, the mainstays of contemporary therapy are plasma exchange, intravenous immune globulin, or both and glucocorticoids.63 This therapeutic strategy could be considered the standard of care for patients with active humoral lesions, according to expert consensus at the FDA Antibody-Mediated Rejection Workshop,64 although evidence of the efficacy of these treatments is not strong. Studies have shown that the use of plasma exchange and intravenous immune globulin for the treatment of antibody-mediated rejection improves short-term outcomes.65-68 Unfortunately, long-term outcomes remain poor, underscoring the need for the de-
Antibody-Mediated Rejection of Solid-Organ Allografts

The potential role of the anti-CD20 monoclonal antibody rituximab and the proteasome inhibitor bortezomib in decreasing the production of donor-specific anti-HLA antibodies (by targeting B cells and plasma cells, respectively) and improving allograft survival in patients with antibody-mediated rejection was recently evaluated in two randomized, controlled trials (RITUX ERAH58 and BORTEJECT61), but neither trial showed clinical benefits.

There is growing interest in the potential for...
targeting the complement system to prevent and treat antibody-mediated rejection. The anti-C5 monoclonal antibody eculizumab, which inhibits terminal complement activation, was reported to decrease the incidence of early antibody-mediated rejection in HLA-sensitized renal-transplant recipients, although it failed to prevent chronic antibody-mediated rejection in recipients with persistently high levels of donor-specific anti-HLA antibodies. Two phase 2, unpublished trials — a randomized, open-label, multicenter trial and a single-group, multicenter trial — investigated the efficacy of eculizumab in preventing antibody-mediated rejection in HLA-incompatible kidney-transplant recipients, with no definitive conclusions (ClinicalTrials.gov numbers, NCT01399593 and NCT01567085, respectively). Proximal complement inhibition has also been studied as a therapeutic target. Two pilot studies showed that the plasma C1 esterase inhibitors Berinert (CSL Behring) and Cinryze (Shire ViroPharma) may improve allograft function in kidney recipients with antibody-mediated rejection. Two additional clinical trials evaluating a C1 esterase inhibitor added to plasma exchange and intravenous immune globulin for the treatment of antibody-mediated rejection (NCT02547220) and for the treatment of antibody-mediated rejection that is resistant to plasma exchange and intravenous immune globulin (NCT03221842) in renal-transplant recipients are currently recruiting patients.

Data are even more limited for patients with chronic antibody-mediated rejection, in whom a poor response to the above-mentioned therapies is generally expected. Thus, there is an unmet need for the development of new therapeutic strategies for this patient population. The potential of proinflammatory cytokine blockade in kidney-transplant recipients with chronic antibody-mediated rejection has recently been highlighted. Inhibition of interleukin-6 with the use of tocilizumab, an anti–interleukin-6 receptor monoclonal antibody, may be associated with good outcomes, suggesting that future clinical trials of interleukin-6 and interleukin-6 receptor inhibitors may be indicated in patients with chronic antibody-mediated rejection.

Finally, in small studies, use of the IgG-degrading enzyme of *Streptococcus pyogenes* (IdeS) reduced or eliminated donor-specific anti-HLA antibodies before kidney transplantation in patients who were HLA-incompatible with their donors. However, IdeS has not yet been evaluated in patients with antibody-mediated rejection.

**Summary**

Antibody-mediated rejection has been recognized as a major cause of organ-transplant failure during the past two decades. Through better insights into the pathogenesis of antibody-mediated rejection and an emphasis on anti-HLA antibody characterization and gene-expression profiling in the allograft, precision diagnostics are now possible. The merging of organ-specific approaches to antibody-mediated rejection offers the opportunity to both elucidate the scope of this problem and identify specific pathogenic mechanisms in the various types of organ transplants. However, much work remains to be done in the area of therapeutics to translate our improved understanding of pathophysiological processes into effective personalized treatment for antibody-mediated rejection.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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