UT Southwestern Medical Center

Internal Medicine Grand Rounds

Immune Mechanisms in Lung Transplantation.

April 21, 2017

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This is to acknowledge that Vaidehi Kaza, M.D. has disclosed that she does not have any financial interests or other relationships with commercial concerns related directly or indirectly to this program. Dr.Vaidehi Kaza will not be discussing off-label uses in her presentation

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Lung Transplantation is a treatment option for end stage lung diseases. However, there are several immune mediated mechanisms that affect long term survival post lung transplantation. My particular interest is in new innovative pathways of immune responses that mediate graft tolerance or rejection.

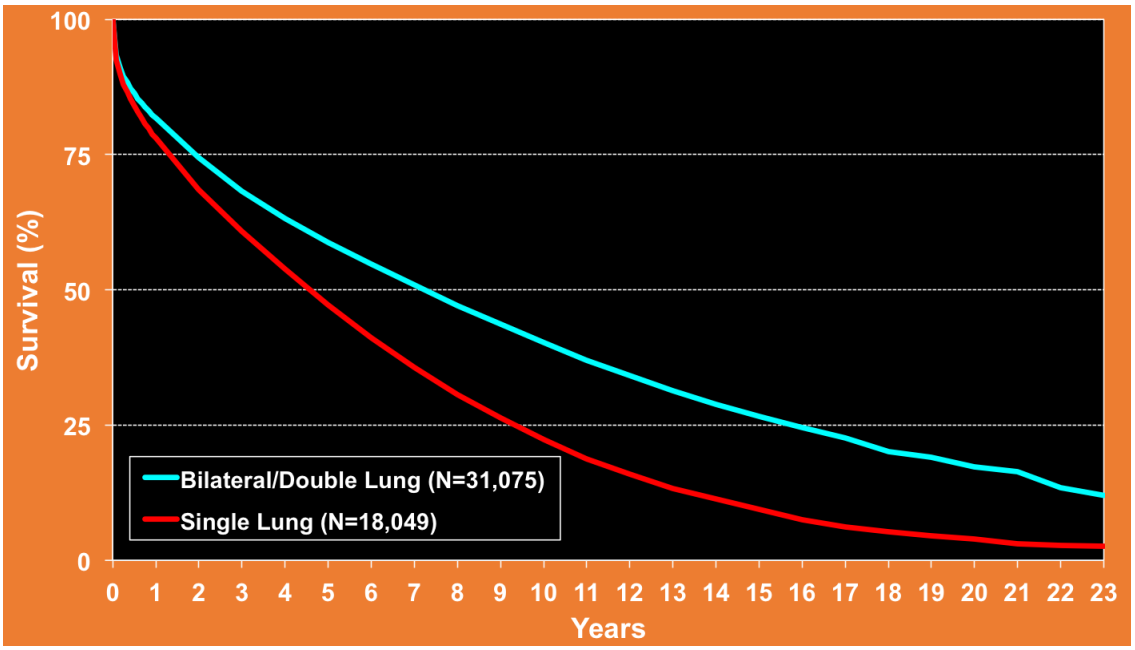
Purpose and Overview:

The purpose of this presentation is to elucidate key aspects of lung transplantation, survival after lung transplantation, mechanisms of allograft injury, various immune mediated antibodies (alloantibodies and autoantibodies) and their influence on the lung allograft.

At the end of this lecture, the listener will be able to

1. Comprehend overview of lung transplantation.
2. Recognize immune mediated allograft injury patterns.
3. Suggest a clinical diagnosis and pathways of treatment.

Lung Transplantation is a treatment option for patients with end stage lung disease [1]. Novel techniques, immunosuppression, extracorporeal membrane oxygenation as bridge to lung transplantation, extended criteria organ donation, ex-vivo lung perfusion have revolutionized lung transplantation. Lung Allocation Scoring allowed sicker patients to be transplanted faster. We have made substantial progress in recipient selection, wait list, donor organ recovery and immediate post-operative management. However, median survival for double lung transplantation is 7.3 years[1]. Chronic Lung Allograft Dysfunction (CLAD) is the most important limiting factor for long term survival after lung transplantation[1]. CLAD is regarded as one of the major reasons that results in lowest three, five and ten year survival is lowest compared to other organs [2]



Median survival (years):
Double Lung = 7.3; Conditional = 9.8 Single Lung = 4.6; Conditional = 6.4

Figure 1: Survival after lung transplantation (reference 1)

Allograft injury presents as primary graft dysfunction, acute cellular injury, lymphocytic bronchiolitis and chronic allograft dysfunction (CLAD) [1]. Acute cellular injury is most common in the first year after lung transplantation [1]. Onset of CLAD is described between 2 and 3 years post lung transplantation. Several factors including acute cellular rejection, gastroesophageal reflux disease, primary graft dysfunction, infections are associated with development of CLAD [3]. Two distinct phenotypes of CLAD exist, obstructive form which is also known as bronchiolitis obliterans syndrome (BOS) and restrictive form known as restrictive allograft syndrome (RAS)[3].

Recently humoral immunity and its role in allograft dysfunction are described [4]. Antibody mediated rejection (AMR) is unique pattern of tissue injury and the scope of this presentation describes the immunology, mechanisms of AMR, pathology, clinical diagnosis and monitoring, clinical outcomes and therapy. It is also believed that there are synergistic effects of cellular and antibody mediated immune responses directed against allo-graft[5]

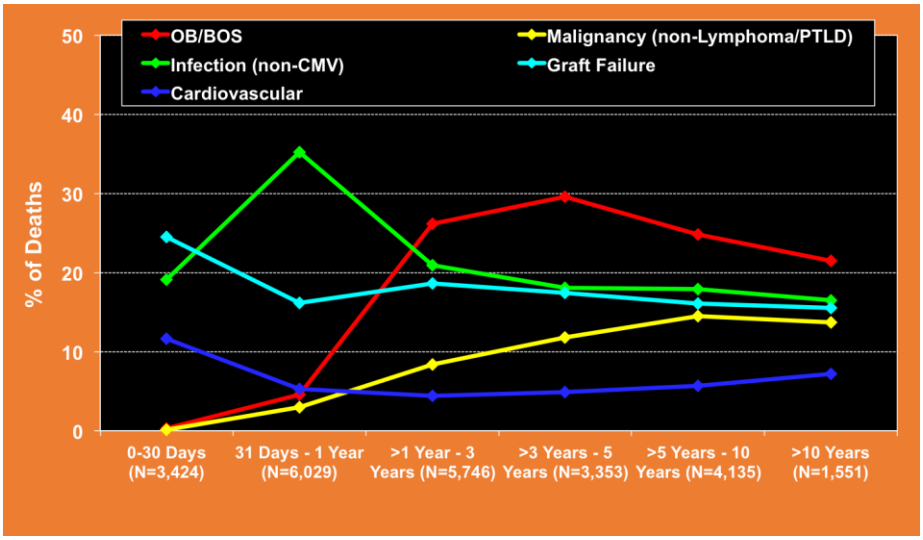


Figure 2: Cause of Death post lung transplantation (reference 1)

Immunology of AMR

Donor specific antibodies (DSA) are anti-human leukocyte antigen antibodies (HLA)[6] . The human leukocyte antigen system comprises of group of cell-surface antigen presenting proteins encoded by a region of short arm of chromosome 6 and is divided into class I and class II molecules. Humans have three class I HLA (A, B, C) that are present on all nucleated cells and six class II HLA (DPA1, DPB1, DQA1, DQB1, DRA, DRB1) that are present only on antigen presenting cells and lymphocytes. Current testing for DSA include solid phase immunoassay. The luminex platform is a solid phase assay that utilizes beads that are coated with HLA. The test serum containing DSA binds to the appropriate molecule. The binding is detected by use of second phycoerythrin (PE) labeled anti-human IgG. Each bead gives a specific signal when excited by lasers built into the Luminex instrument and is measured to be expressed as mean florescence intensity (MFI) [7].

Mechanisms of AMR

The primary event that initiates graft rejection is recognition of HLA on donor cells[6]. Mismatched donor histocompatibility antigens results in both cellular and humoral immune mechanisms, which leads to allograft rejection[4]. Anti- HLA donor specific antibodies (DSA) thus formed result in complement mediated and complement independent graft injury. These DSA induce activation of various transcription factors (NF-κB) involved in activation of various pro-inflammatory cytokines in human endothelial cells[8]. Three pathways are described for allograft recognition. Direct pathway is recognition of donor HLA on donor antigen presenting cells (APC) priming CD4 and CD8 recipient T cells. Indirect pathway is when recipient APC traffic through

transplanted organs, phagocytose allogenic HLA shed from foreign cells through cell necrosis and apoptosis and present processed peptides to their own CD4 cells. Semi-direct pathway is when cell-to-cell contact between donor and recipient APC may transfer intact membrane components including intact allo-HLA, which results in recipient CD4 T cell activation [6,9]

Post transplant donor specific antibodies produced by B cells are very T- cell dependent and leads to long lasting, anti-donor antibody producing plasma cells. Once bound to the allograft antigen target, a given antibody is then capable of binding complement factor C1q. C1q binding subsequently activated the rest of the complement cascade leading to generation of membrane attract complex and cell lysis[10]. Recently complement independent antibody mediated injury is also described. [11]

Immunoglobulins are tetrameric proteins. Mature B cells express membrane IgM and IgD. Synapse between T and B cell facilitates interactions and cytokines signal the B cell to switch from IgM to IgG. The exact environment under which class switching occurs during transplantation is not explored[10]

During injury to tissue, self –tolerance is maintained at least in part to the action of regulatory T cells, which sense the inflamed environment and dampen the potential autoimmune responses allowing repair and regeneration to proceed. However after transplant self-tolerance is disrupted or inhibited as rejection progresses[12].

Pathology of AMR:

Capillary injury although a non-specific finding is a suggestive of antibody mediated rejection. Findings of small vessel injury with initmitis or endothelialitis along with immunohistochemical demonstration of complement deposition should raise the suspicion for antibody mediated rejection[13]. Histopathological features including neutrophil margination, neutrophil capillaritis and arteritis can be seen in other forms of lung injury. Immunohistochemistry for C4d either by immunofluoresence or immunoperoxidase assays may provide supportive evidence of AMR. However, recent consensus statement [14] indicated C4d staining is no longer obligate criterion of AMR. Pathological findings need to be interpreted in the context of laboratory (DSA) and clinical presentation.

Clinical Diagnosis and Monitoring

Diagnosis of pulmonary AMR is made with multi-disciplinary approach that integrates clinical presentation, immunologic and pathologic diagnostic tools while excluding other causes that can mimic the findings. AMR is defined as clinical or sub-clinical [14].

1. Clinical AMR: The presence of allograft dysfunction defined as alterations in pulmonary physiology, gas exchange properties, radiologic features or deteriorating functional performance associated with AMR.
2. Sub-clinical AMR: Absence of allograft dysfunction with histologic criteria of AMR on surveillance transbronchial biopsies. Isolated DSA without evidence of histology is also under this category.

Clinical and subclinical AMR is staged as definite, probable or possible AMR based on presence of DSA; positive histology suggestive of AMR; positive C4D staining. Definite has all criteria, probable has one criteria missing and possible has two criteria missing.

Clinical Outcomes and Therapy

Treatment is generally directed at interventions that aim to deplete circulating antibodies, suppress B cells and stop further allograft injury. Treatments are individualized and highly dependent on response to each modality of treatment. Current modalities include, B cell depletion (rituximab), plasma cell inhibition (Bortezomib), antibody depletion (plasma exchange, immunoglobulin infusion) or inhibition of antibody function (C5 inhibitor called Ecluzimab inhibits complement mediated intravascular hemolysis, used in paroxysmal nocturnal hemoglobinuria). In addition, T cell therapies such as thymoglobulin (rATG; rabbit antithymocyte globulin is a purified polyclonal gamma immunoglobulin raised in rabbits against human thymocytes) results in T cell depletion, in addition to having an indirect suppressive effect on B cells.

Response to treatment is variable [1] . Definitions for response to treatment of pulmonary AMR are as below.

1. Complete response: return to baseline graft function, abolition of DSA and reversal of pathological changes
2. Partial response: Improvement in graft function if application, but not all parameters return to baseline.
3. Stabilization: No further clinical deterioration

4. No response: Ongoing clinical deterioration and continued abnormal pathology.

It appears now that de novo DSA develop in 25% to 55% of lung transplant recipients and associated with decreased survival and increased incidence of bronchiolitis obliterans syndrome (BOS)[14]. However, it is still unclear when to treat DSA and which class is more detrimental to the graft and important to treat. We also do not know at which strength (MFI) do we start treating DSA. A few recent studies are reviewed below.

DeNicola et al [15] reviewed biopsies from 41 lung transplant recipients. The purpose of the study was to look for an association between DSA and histopathologic findings and development of future morbidity and mortality. Combination of DSA positivity and histopathology suspicious for AMR had stronger association with BOS and mortality.

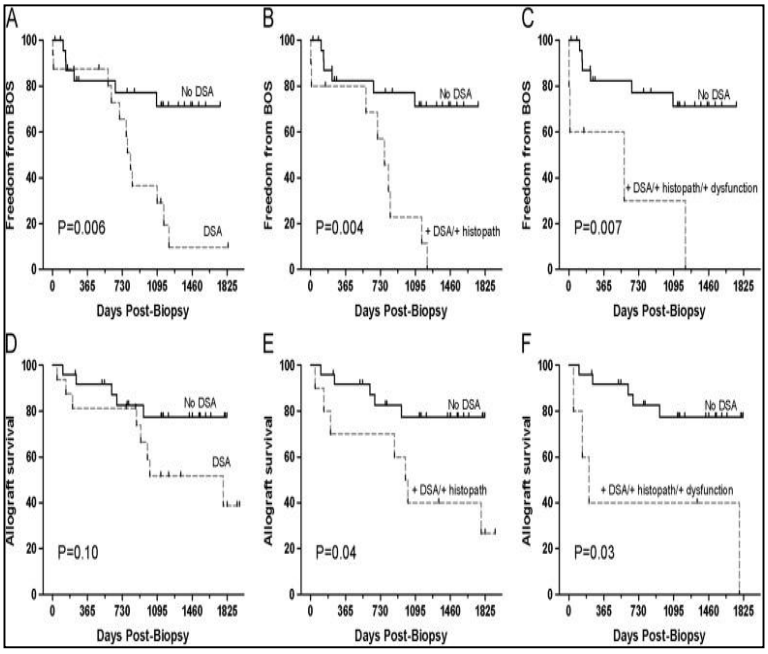


Figure 3: DSA and histopathology findings and association with BOS , Mortality (reference 15).

DSA treatment was evaluated in another study[16] by Hachem et al. No difference in freedom from BOS was found between DSA treatment group with IVIG alone and those with IVIG and rituximab. Likewise, there was no association between DSA class and freedom form BOS. However, recipients with persistent DSA were more likely to develop BOS than those who cleared DSA. There were no differences in infections and other adverse events in the treated group.

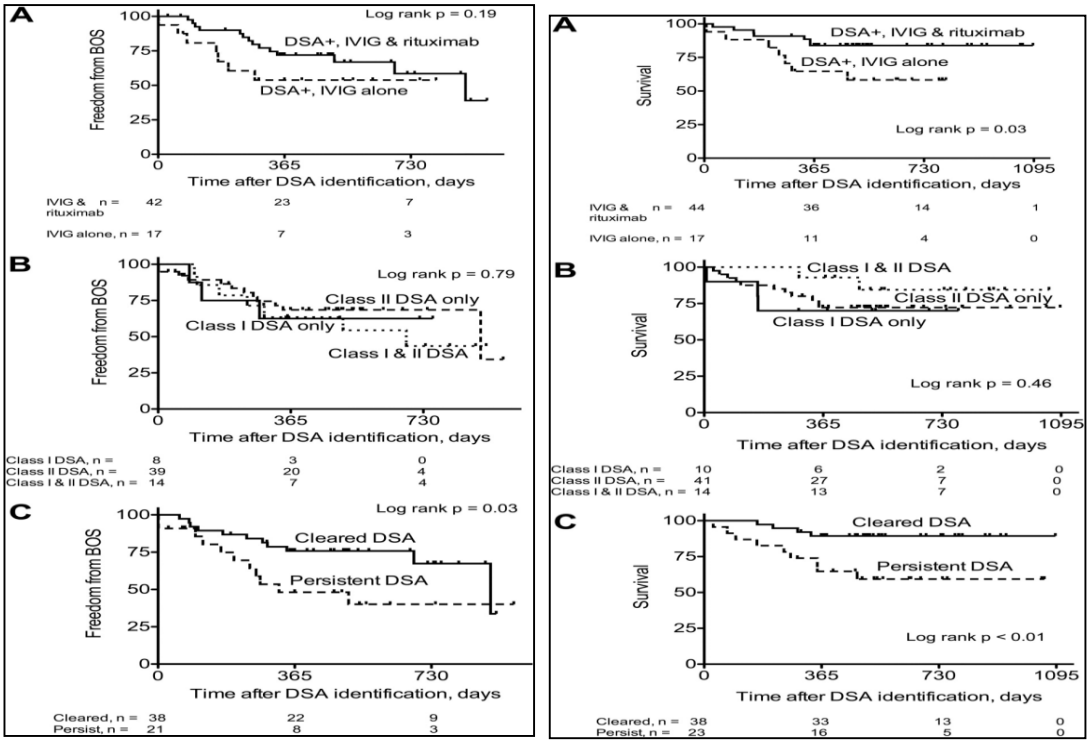


Figure 4: Treatment of DSA and BOS (reference 16)

Another retrospective series[17] with 21 recipients determined to have antibody-mediated rejection by predetermined criteria showed that 93% of them developed chronic allograft dysfunction after antibody mediated rejection. 10 of the 21 recipients died within a year of diagnosis of antibody mediated rejection. Clearance of DSA in this retrospective study showed improved survival.

Autoantibodies: do they have a role?

The variable response to treatment of antibody-mediated rejection raises the question whether there is another process that contributes or negates the process of antibody-mediated rejection. Among the various pathways proposed, formation of preformed autoantibodies before or after allograft implantation influence subsequent graft injury. Autoimmunity is defined as immune response to a self –antigen. It can be cellular or humoral, innate or acquired. Diseases such as rheumatoid arthritis or lupus are thought to occur as a result of the loss of immunogenic tolerance, defined as the ability to react to antigens and ignore self-antigens. After transplantation, exposure of collagen V, K-α1 tubulin triggers autoimmune response and contributes to chronic rejection and obliterative bronchiolitis[18] .

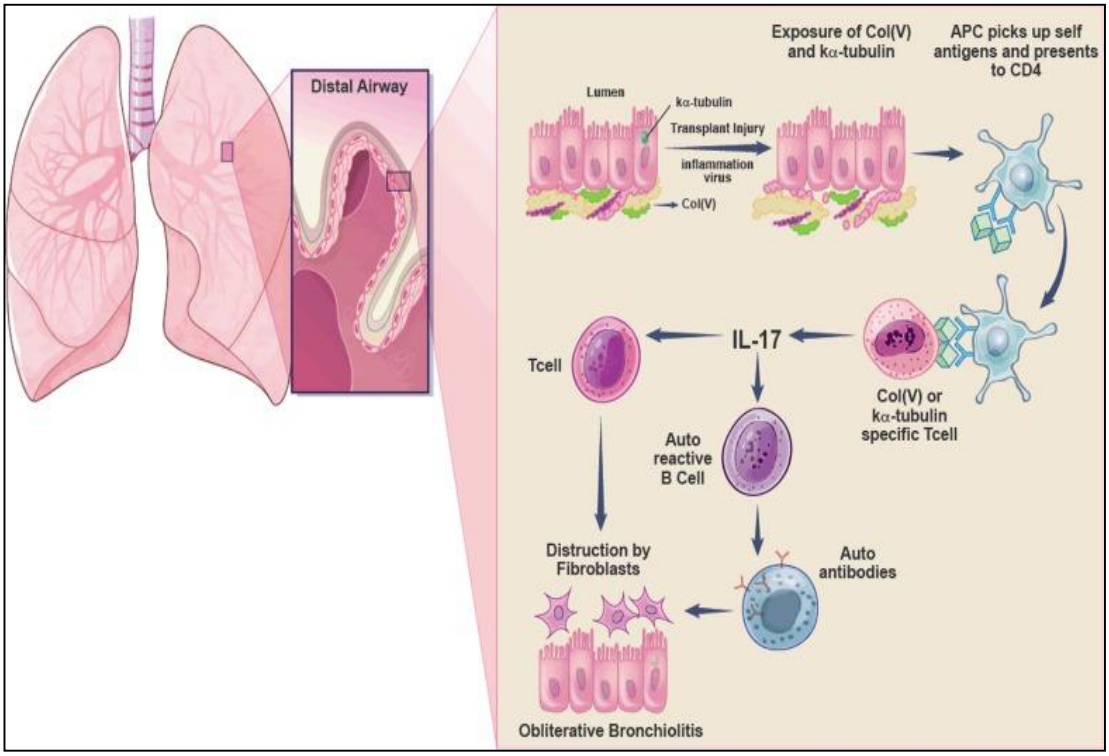


Figure 5: Autoimmunity and mechanisms (reference 18)

It is unclear if auto immunity precedes or follows formation of DSA (alloimmunity). In a retrospective analysis of 42 recipients, strong correlation was demonstrated between development of DSA and autoantibodies. DSA preceded development of autoantibodies[19].

Conclusion.

Graft function and recipient survival after lung transplantation continues to be challenging. Among the different types of immune mechanisms leading to allograft injury, antibody mediated rejection, presence of DSA and their role is in graft injury is still enigmatic. Definitive diagnosis of AMR should be made with clinical, histopathological and laboratory data. Current treatment strategies for AMR and or DSA are limited. Variable responses to treatment prompt the question, whether there are other processes such as formation of autoantibodies that influence graft injury.

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