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DOI: https://doi.org/10.1097/TP.0000000000001215

Chronic Lung Allograft Dysfunction: A Systematic Review of Mechanisms

Pierre-Joseph Royer, PhD,[1] Gustavo Olivera-Botello, PhD,[2] Angela Koutsokera, MD,[3] John-David Aubert, MD,[3] Eric Bernasconi, PhD,[3] Adrien Tissot, MD,[1] Christophe Pison, MD, PhD,[4,5,6] Laurent Nicod, MD,[3] Jean-Pierre Boissel, MD, PhD,[2] and Antoine Magnan, MD[1] on behalf of the SysCLAD consortium

Abstract: Chronic lung allograft dysfunction (CLAD) is the major limitation of long-term survival after lung transplantation. Chronic lung allograft dysfunction manifests as bronchiolitis obliterans syndrome or the recently described restrictive allograft syndrome. Although numerous risk factors have been identified so far, the physiopathological mechanisms of CLAD remain poorly understood. We investigate here the immune mechanisms involved in the development of CLAD after lung transplantation. We explore the innate or adaptive immune reactions induced by the allograft itself or by the environment and how they lead to allograft dysfunction. Because current literature suggests bronchiolitis obliterans syndrome and restrictive allograft syndrome as 2 distinct entities, we focus on the specific factors behind one or the other syndromes. Chronic lung allograft dysfunction is a multifactorial disease that remains irreversible and unpredictable so far. We thus finally discuss the potential of systems-biology approach to predict its occurrence and to better understand its underlying mechanisms.

(*Transplantation* 2016;100: 1803–1814)

THE MULTIPLE FACES OF CHRONIC LUNG ALLOGRAFT DYSFUNCTION

The 2014 report of the International Society for Heart and Lung Transplantation registry accounts for 47 647 adult lung transplantations and for 3772 adult heart-lung transplantations performed up to June 2013.[1] This reflects an average 8% annual increase in the number of adult lung transplantations reported between the years 2001 and 2011. Progresses in surgical techniques and perioperative management have dramatically increased the short-term survival. Yet, long-term survival remains disappointing with a dismal 27% survival rate at 10 years that makes lung transplantation the intervention with the poorest long-term outcome when compared with other solid-organ transplantation such as kidney (58%), liver (70%), heart (56%), pancreas (77%) or intestine (44%).[2] The development of chronic dysfunction or chronic lung allograft dysfunction (CLAD), affecting 50% of patients at 5 years, partly accounts for these clinical pictures.

For a long time, bronchiolitis obliterans, or its surrogate bronchiolitis obliterans syndrome (BOS), was considered to be the only manifestation of chronic lung dysfunction, hence the terms "chronic rejection" and "BOS" were indistinctively used.[3] However, a distinct nosological entity coined under the name of restrictive allograft syndrome (RAS) was characterized in 2011.[4] Since then, the term CLAD has been used to refer to all variants of pulmonary chronic dysfunction, in particular BOS and RAS. In Sato's seminal article, the probability of developing CLAD by 5 years was reported to be around 50%; 35% for the BOS phenotype and 15% for the RAS phenotype (after exclusion of recipients who died within the first 3 months post transplantation).[4] Despite its smaller incidence, the restrictive phenotype seems to imply a poorer prognosis, with a median survival, after disease onset of less than 2 years (compared with around 4 years for BOS phenotype). The survival at 10 years reported in the same monocentric study was then 16% for the RAS group and 31% for the BOS group, which heavily contrasts with the 72% figure reported for the free-from-CLAD group. Histologically, RAS is characterized by a stair-step progression pattern, with tissue damage and fibrotic lesions occurring in the periphery of the lungs (ie, in the visceral pleura, in the alveolar interstitium and in the interlobular septa)[4]; whereas in the case of BOS, the fibrotic lesions are more likely to occur in the bronchioles.[5]

Received 17 July 2014. Revision received 23 February 2016.

Accepted 24 February 2016.

[1] *L'institut du thorax, Nantes, France.*

[2] *Novadiscovery, Lyon, France.*

[3] *Service de Pneumologie, Centre de Transplantation, Lausanne, Switzerland.*

[4] *Clinique Universitaire de Pneumologie, Pôle Thorax et Vaisseaux, CHU Grenoble, France.*

[5] *Inserm1055, France.*

[6] *Université Joseph Fourier Grenoble, France.*

The authors thank the Swiss National Research Fund for supporting the STCS and in particular the lung transplant section (no 3347CO-108795). The SysCLAD study is an EU-funded project, HEALTH-F5-2012, grant agreement 305457, under the Seventh Framework Programme (FP7).

The authors declare no conflicts of interest.

P-J.R. and G.O.-B. conducted systematic literature search, selected and reviewed the article and wrote the article. A.K., J-D.A., E.B., A.T., C.P., L.N., J-P.B., and A. M. critically revised the article.

ClinicalTrials.gov Identifier: NCT00980967.

Correspondence: Pierre-Joseph Royer, L'institut du thorax, INSERM UMR 1087, CNRS UMR 6291, CHU de Nantes, DHU2020, Nantes, France. (pierre-joseph.royer@univ-nantes.fr); Antoine Magnan, L'institut du thorax, INSERM UMR 1087, CNRS UMR 6291, CHU de Nantes, DHU2020, Nantes, France. (antoine.magnan@univ-nantes.fr).

ISSN: 0041-1337/16/10009-1803

DOI: 10.1097/TP.0000000000001215

Nonetheless, both types of lesions may coexist[6] and overall, risk factors are similar between BOS and RAS,[7] suggesting that both syndromes share common physio-pathological mechanisms. However, the specificity of these 2 diseases start to emerge from recent investigations. Diffuse alveolar damage (DAD) for example precedes CLAD,[8,9] but time patterns determine the outcome of the pathology: late-onset DAD has been correlated with RAS, whereas early-onset DAD, diagnosed within the first 3 months after transplantation, has been associated with BOS.[8]

We will present here the mechanisms leading to CLAD after lung transplantation, and we will highlight the specific factors behind BOS or RAS development. The terms "lung transplantation," "chronic rejection," "chronic dysfunction," "CLAD," "BOS," "bronchiolitis obliterans," or "RAS" were used alone and in combination to search in PubMed over the past 25 years 2015. Abstracts from the 2015 American Transplant Congress, International Society for Heart and Lung Transplantation meeting and European Respiratory Society congress were also reviewed. The most relevant and appropriate articles were then hand selected to prepare this review.

INNATE IMMUNE MECHANISMS UNDERLYING CLAD

The Receptors of Innate Immunity: Recognition of Exogenous and Endogenous Molecules

Lungs are continuously exposed to environment. Innate immunity is then repeatedly stimulated by pathogens, allergens, or pollutants. The innate immune system recognizes pathogen associated molecular patterns through the expression of a wide range of pathogen recognition receptors. Among them, the toll like receptors (TLR) comprise a family of 13 members expressed by hematopoietic or parenchymal cells involved in the recognition of pathogens.[10] In humans, an association was suggested between polymorphism of TLR2, TLR4, and TLR9 involved in bacteria or virus recognition, and the probability of developing CLAD.[11,12] In mice, activating TLR4 or TLR3 in the lungs via the administration of repetitive doses of aerosolized LPS[13] or synthetic double-strand RNA[14] results in obliterative bronchiolitis.

The impact of viral, bacterial, or fungal infections in the development on CLAD has been known for a while and undoubtedly increases the risk for chronic rejection.[15-18] Things appear more complex regarding graft colonization. Although colonization by aspergillus is associated with an increase of BOS,[19,20] de novo but not persistent colonization by pseudomonas has been found as a risk factor for BOS,[21] and reestablishment of the pretransplant microbiota can even reduce the risk of chronic rejection.[22] Interestingly, CXCL1 and CXCL5 secretion after pseudomonas colonization determines the transition to chronic rejection.[23] Further works will have to establish if modulation of pseudomonas virulence factors during long-term colonization[24] regulate host immune response and thus susceptibility to CLAD.

Besides pathogens, TLR can be triggered by the endotoxins present in the gastric reflux of lung transplant recipients suffering from gastroesophageal reflux disease.[25] Additionally, pollutants are known now to be strong activators of innate immunity,[26] and attention has been recently drawn to traffic-related air pollution exposure and the risk of developing CLAD.[27,28] Other cytosolic receptors, such as RIG-1 and Mda5, respond to respiratory virus infection by the expression of type I Interferon[29] but their role in the development of CLAD after lung transplantation remains to be investigated.

Graft ischemia time and ischemia-reperfusion injury (after blood recirculation in the devitalized tissue) is a well-known determinant of the long-term survival after lung transplantation.[30] Moreover, during the surgical process of lung transplantation, the bronchial circulation is not routinely reconnected to the main circulation, and although blood vessels can be restored by angiogenesis, reduced blood circulation accounts for ischemia, hypoxia, sensitivity to infection, or defect in immunosuppressive drug delivery, all observed after lung transplantation.[31] Furthermore, transplant arteriosclerosis is common in solid-organ transplantation, and inflammatory cytokines are activators of vascular smooth muscle cells, promoting their proliferation at the intimal level and the abnormal thickening of the microvessel walls.[32]

These defects in blood supply to the small airways[33] or microvascular injuries[34] predispose to chronic dysfunction. Injured cells or tissues released various endogenous factors that can be recognized by pathogen recognition receptor,[35] a great deal of attention has been focused on the recognition of these damage-associated molecular patterns or alarmins by the innate immune system.[36] Innate immunity can be mobilized within the first hours after the transplantation in particular through the release of high-mobility group box 1 (HMGB1). This molecule secreted by necrotic cells after ischemia, signals via TLR or receptor for advanced glycation end products (RAGE). The recognition of HMGB1 by RAGE plays an important role in the development of early pulmonary dysfunction after transplantation, through an IL-17–dependent neutrophil infiltration.[37,38] High-mobility group box 1 and other alarmins, such as S100 proteins,[39] heat shock proteins,[40] the soluble form of RAGE,[41] or hyaluronan,[42] have been found in the bronchoalveolar lavages (BAL) of CLAD patients and are supposed to contribute to CLAD via activation of innate immunity. Interestingly, alarmin profile and especially S100A8, S100A9, S100A12, S100P, and HMGB1 proteins can discriminate between BOS or RAS subtypes suggesting a specific role for these molecules in the development of these 2 pathologies.[39]

Exogenous or endogenous molecules trigger inflammation and the release of cytokines or chemokines resulting in the activation of innate immunity. We will then present the players of innate immunity and detailed the tissue-degrading agents and the chemokines they produced involved in deterioration of lung tissues and activation of adaptive immunity.

Activation of Airway Epithelial Cells

Airway epithelial cells (AEC) are the first line of defense against airborne pathogens, particulate matter, pollutants or allergens. AEC express a wide range of TLR,[43] nucleotide oligomerization domain-like receptor or retinoic acid-inducible gene-I-like receptor,[44] and their localization make them early responders in case of aggression. Their major impact in pulmonary immunity is now well established.[45] In the context of solid-organ transplantation, several types of allodependent or alloindependent stimuli may induce the secretion of proinflammatory cytokines, chemokines, and growth factors by AEC.[46-48] Among the molecules produced, it is worth mentioning IL-8, associated with the occurrence of alveolar neutrophilia in lung transplant recipients[49]; IL-1α, produced

after pseudomonas infection and responsible for fibroblast activation[50]; CCL2, a monocyte-specific chemoattractant protein upregulated in CLAD patients[51] or the mononuclear cell attractants CXCL9 and CXCL10, produced during acute lung injury and propagating the inflammation within the allograft.[9] Combined with the ability to produce matrix metalloproteinases (MMP)[52] and to upregulate costimulatory or major histocompatibility molecules (MHC) class II molecule expression,[53-55] AEC are endowed with the capacity to attract and activate innate or adaptive immune cells within the graft.

The Role of Neutrophils

Several independent studies have reported abnormally elevated counts of neutrophils, in BAL,[49,56-58] induced sputum[59] and biopsies[60] from lung transplant recipients suffering from CLAD. Regarding the triggers, IL-8 remains the main mediator for neutrophil recruitment and activation after lung transplantation.[46,49] Some of the factors that may induce an upregulation of IL-8 in lung transplant recipients include the presence of bile acids due to concomitant gastroesophageal reflux disease[61] as well as the presence of particulate matter due to exposure to a polluted environment[62] or infections.[48] Neutrophils act through mediators, such as reactive oxygen species[63] or MMP,[64] and other proteases, such as neutrophil elastase. The local persistence of these substances is thought to induce the epithelial damages that precede the excessive scar formation that characterizes CLAD, making alveolar neutrophilia a predictive biomarker for CLAD.[56,65,66]

Although neutrophils have been historically associated with the development of CLAD, the therapeutic use of azithromycin has changed the paradigm. Azithromycin is a macrolide antibiotic able to reverse the decline of lung function in a subset of lung recipients.[67,68] It remains unclear so far whether azithromycin improves lung function due to its antimicrobial or anti-inflammatory properties. Its use defines a new dysfunction phenotype called azithromycin-responsive allograft dysfunction or neutrophilic reversible allograft dysfunction since this phenotype is often (but not always) characterized by BAL neutrophilia (≥15%).[67-72] However, this phenotype is by definition reversible and hence does not fulfill the strict criteria of CLAD.[73]

The Role of NK Cells

Unlike many other components of the immune system, NK cells remain barely affected by the immunosuppressive therapies used in regular clinical practice.[74] That is one of the reasons why they have recently been under the spotlight of the solid-organ transplantation community. NK cells have been associated to both acute[75] and chronic[75,76] rejections after lung transplantation. NK cells use their membrane receptors (CD16, CD32, and CD56) to identify IgG-coated cells via the Fc region of the antibodies.[77] Activation of NK cell may be also antibody-independent. For example, activated endothelial cells express on their surface the chemokine CX3CL1 or fractalkine,[78] which interact with the chemokine receptor CX3CR1 present on NK cells.[79]

In addition, the human MHC class I chain-related proteins MICA and MICB, expressed by epithelial cells under conditions of stress, are known to be ligands for the activating receptor NKG2D on NK cells.[80-82] Once activated, NK cells release a series of cytolytic proteins, such as granzymes A and B, perforin, FasL, TNF-related apoptosis-inducing ligand, and chemotactic cytokines, such as TNF-α and IFN-γ.[83,84] Because of their cytotoxic arsenal and their propensity to migrate in the lungs of patients with chronic rejection,[85] NK cells are ideal culprit for graft destruction. However, recent works have shown their ability to promote graft tolerance through dendritic cells editing.[86,87] In a mouse lung transplantation model, killing of allogeneic dendritic cells by NK induces graft tolerance.[88] Interestingly, in human, lack of activating killer immunoglobulin-like receptor expression by NK cells is associated with the development of BOS,[89] suggesting that NK activity preserves long-term graft function.

Macrophages—Eosinophils

The role of macrophages in the development of CLAD is suggested by their accumulation in human or animal models and by the reduction of allograft dysfunction after blockade of their infiltration.[90-92] In human, temporal variations in macrophage activation profile, either classical (M1) or alternative (M2), in association with alterations of the lung microbiota, have been reported posttransplantation.[93] Whether these variations correlates with the development of CLAD, as suggested in mouse[94] remains to be assessed.

Recent works have shown an association between eosinophilia and allograft dysfunction.[7,95] Eosinophils may promote CLAD through the release of reactive oxygen species, promoting graft destruction, or transforming growth factor-β supporting aberrant remodeling. Interestingly, BAL eosinophilia seems to be correlated with the restrictive phenotype. Further confirmation of this link would provide a potential mechanism leading to this particular CLAD subtype.

Activation of innate immunity and degradation of allograft support the development of adaptive immunity. As we will see, induction of autoimmune reactions along with dysfunction of regulatory mechanisms will then feed a positive feedback loop, responsible for the perpetuation and amplification of the immune response, driving the transition from acute events to a chronic process.

ADAPTIVE IMMUNE MECHANISMS UNDERLYING CLAD

Th1 Immunity

The role of adaptive cellular immunity in the development of CLAD is highlighted by the association between acute cellular rejection or lymphocytic bronchiolitis and the occurrence of CLAD[7,96,97] or by the incidence of BOS after bone marrow transplantation.[98] The development of cellular immune response against alloantigens (and autoantigens) generally relies on the migration of antigen presenting cells in the secondary lymphoid organs, where they encounter and activate T cells. Additionally, T cell activation within the lung may take place through the formation of de novo lymphoid tissue, such as bronchus-associated lymphoid tissues (BALT).[99,100] Whereas lymphoid neogenesis has been observed in BOS,[101] evidences for organized BALT contribution in CLAD remain scarce.[102] The role of Th1 immunity in the process of CLAD is suggested by an increase in Th1 cells or cytokines and granzyme B levels in blood or lung lavages of BOS recipients.[76,103-106] Furthermore, inhibition of cytotoxic T cells by HLA-G molecules

has been suggested in stable patients.[107] The molecular bases of this allorecognition involve mainly an indirect presentation of donor MHC class I and II molecules.[108-111] However, mouse models have also shown the contribution of minor histocompatibility antigens presentation in the development of obliterative lesions.[112,113]

Th17 Immunity

There is a growing body of evidence that autoimmunity plays an important part in the development of CLAD.[114] Tissue injuries caused by ischemia, primary graft dysfunction (PGD), infections, or alloimmune reactions alter the accessibility of protein antigenic domains. Epitopes normally masked within the protein organization can then be exposed to the immune system, leading to autoimmune responses. In a murine model of lung transplantation, Col(V)-specific T cells found in the lung allograft mediates allograft rejection.[115] This has been confirmed in human, where the Col(V)-specific T cell response intensity correlates with the incidence and the severity of BOS. This Col(V) autoimmune response was found to be dependent of IL-17. Interestingly, adoptive transfer of Col(V)-reactive T cells was sufficient to induce an OB in the absence of alloreactivity.[116] Association between Th17 immunity and the development of CLAD has then been reported by several groups in human[117] or mouse models[118,119] and genetic variation in IL-17 receptor is associated with CLAD.[120] Furthermore, Th17 immunity is linked to both chronic inflammation and neutrophilia in the lungs.[121] In the case of lung transplantation, Th17 immunity may thus favor chronic dysfunction through airway fibrosis, induction of BALT, neutrophil chemotaxis, or expansion of autoantibodies.[114]

Regulatory Cells

Regulatory T (Treg) cells encompass a wide diversity of immunosuppressive populations characterized by specific ontogeny or mechanisms of action.[122] In human, presence of Treg cell in lung allograft or in the blood is correlated with an absence of chronic dysfunction.[65,123-125] More specifically, the Th17/Treg cell balance could determine the fate of lung allograft. Indeed, mouse models have shown a downregulation of Th17 immunity after adoptive transfer of Treg cell.[126,127] On the other hand, plasticity is a feature of Treg cell and inflammatory environment can favor their differentiation into Th17 cells[128] and IL-6, a pivotal factor in the equilibrium of the Th17/Treg cell balance, is a well-known marker of chronic dysfunction.[51] Various experimental approaches have thus been proposed to stimulate the activity of Treg cell and modulate the Th17/Treg cell balance. Independent studies for instance have reported a decrease in the rate of pulmonary function loss in CLAD patients as a result of extracorporeal photopheresis therapy.[130-133] Mechanistically, the mode of action of extracorporeal photopheresis is not fully understood but probably rely on the induction of a regulatory CD4+CD25+ T cell population.[134-136] By contrast, immunosuppressive drugs are thought to impair Treg cell populations and to affect Th17/Treg cell balance, favoring the development of chronic dysfunction.[137,138]

In addition to Treg cell, regulatory B cell, producing IL-10 or TGF-β have been characterized. Regulatory B cells are involved in the control of airway diseases and can inhibit the development of bronchiolitis obliterans in a mouse model of heterotopic tracheal transplantation.[139] A great challenge in the future will be to decipher the impact of these regulatory cell populations on the development of CLAD and to develop immunosuppressive therapies able to maintain or expand these populations, either in vivo or ex vivo.[140]

Humoral Immunity

The association between humoral immunity and the development of CLAD is well documented. Accumulation of B cells is observed in lung tissues of patients with CLAD.[141] The presence of donor-specific HLA antibodies (DSA) is correlated with the development of BOS,[142-148] and DSA targeting therapies lower the incidence of chronic dysfunction.[149,150] The role of antibodies against MICA molecules has been reported as well.[151] Although HLA or MICA/B polymorphism is an evident molecular basis for humoral immunity, recent works have highlighted the role of self-antigen recognition in the humoral immunity associated with BOS. Antibodies directed against col(V) or K-α1 tubulin proteins have been associated with the process of BOS,[115,152] and clearance of these antibodies reduced the risk of BOS, independently of the clearance of DSA.[153]

The direct link between alloimmunity and autoimmunity has been suggested in a mouse model where injection of anti-MHC class I antibody induced the production of anti-Col(V) and anti-K-α1 tubulin antibodies. Noteworthy, this production was IL-17–dependent and resulted in an obliterative disease.[154,155] In lung transplant recipients, a retrospective analysis showed a correlation between DSA and self-antigen antibody appearance, with DSA preceding the development of self-antigen antibodies.[156] Yet, self-reactive antibodies can be found in the absence of DSA, suggesting a DSA-independent mechanism for their development.[153] In addition, such self-reactive antibodies may already be present by the time of the transplantation.[157] In fact, several types of stimuli (eg, ischemia reperfusion injury, PGD) may increase the expression of these self-proteins and activate the interstitial remodeling machinery, promoting the exposure of the cryptic antigens.[158]

Graft-reactive antibodies induce the activation of the complement system and the degradation of lung tissues. Polymorphism in the complement regulatory protein CD59 has then been associated with the development of BOS,[159] suggesting that harnessing complement activation may control CLAD development. Complement activation has been largely investigated as a potential marker for humoral rejection, via the immunostaining of the complement component 4 (C4d). Although positive results have been reported for kidney,[160] liver,[161] and heart[162] transplantation, it is much debated for lung transplantation.[163]

Continuous immune reaction will cause tissue destruction and dysregulation of epithelium repair. This mechanism is hardly controllable by immunosuppression, and aberrant remodeling process will take place, leading ultimately to loss of graft function (Figure 1).

CONSEQUENCES ON THE LUNG ALLOGRAFT

It is now recognized that the recurrent injuries of the lung allograft, either immune or nonimmune related, result in an excessive scarring and an aberrant healing process responsible for CLAD. Specific features of the respiratory system, and in particular, its continuous exposure to environment, probably favor the perpetuation of acute events

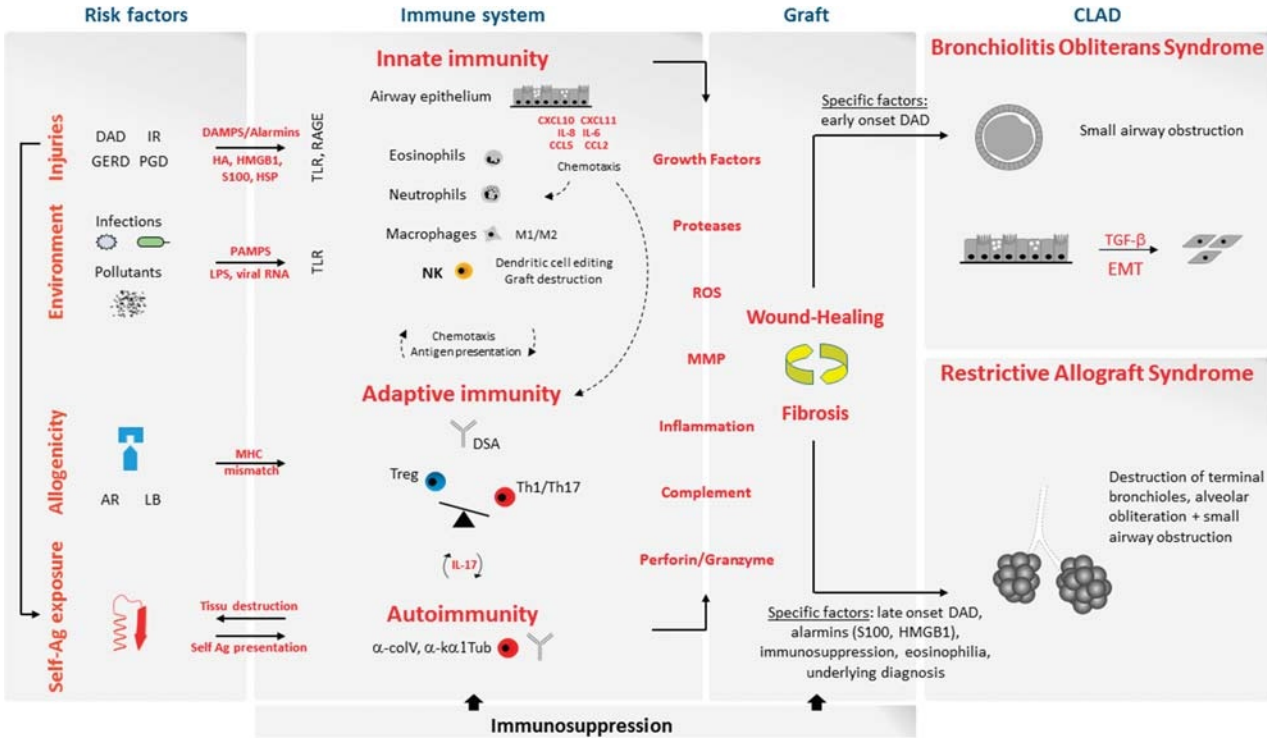

FIGURE 1. Physiopathological mechanisms of CLAD. Endogenous (MHC mismatch, graft injuries, self-antigen exposure) or exogenous (infections, pollutants, allergens) risk factors leading to activation of innate and adaptive immunity after lung transplantation. Continuous exposure to environment and development of autoimmunity promote the persistance of inflammation and tissue injuries. Graft destruction and wound/healing processes promote the remodeling of the lung allograft and the development of CLAD. The specific mechanisms skewing the chronic dysfunction toward BOS or RAS phenotypes are poorly characterized. AR, acute rejection; GERD, gastroesophageal reflux disease; HA, hyaluronan; HSP, heat shock protein; IR, ischemia reperfusion; LB, lymphocytic bronchiolitis.

into chronic injury. Toll like receptors activation for instance may disrupt Treg cell activity and favor a Th1-oriented phenotype.[164] The presence of CXCR3 ligands[9] or inflammatory cytokines and de novo anti-MHC class II DSA[165] may be responsible for the persistence of allograft injury after DAD or PGD, respectively. Overall, this generates and propagates an inflammatory environment and the recruitment of immune cells within the allograft leading to further fibrotic damages. This may explain how very early events, such as PGD or ischemia reperfusion injuries, can be translated several months or years later into chronic dysfunction.

The persistence of local inflammation results in the emergence of a fibroproliferative phenotype with the secretion of growth factors and dysregulation in the extracellular matrix regeneration process (Figure 1). The binding of anti-HLA class I antibodies on AEC may lead to their death through apoptosis and induces the release profibrotic growth factors, such as platelet-derived growth factor, Insulin-like growth factor-1, and TGF-β.[47] Their upregulation results in the accumulation of fibroblasts and myofibroblasts, the aberrant deposition of collagen fibers (mainly of type I), and the loss of homeostasis in the regeneration of the extracellular matrix. AEC from BOS patients demonstrated an upregulation of mesenchymal markers (S100A4, fibronectin, MMP) along with a drop in epithelial cell marker expression. The epithelial to mesenchymal transition (EMT) has thus been proposed as a general mechanism leading to airway obstruction after lung transplantation.[54,166] TGF-β is the foremost inducer of EMT[167] and has long been associated with the development of BOS after transplantation.[46,104,129,168] Its impact in the EMT triggering has also been shown in vivo in a rat model of airway obliteration where blocking the binding of TGF-β to its receptor reduced intraluminal airway matrix deposition.[169] In human, TGF-β could be the biological link between PGD and BOS,[170] and recent evidence has shown a dysregulation of TGF-β signaling by microRNA-144 in BOS patients.[171] The antifibrotic drug pirfenidone,[172] which acts mainly by suppressing the expression of TGF-β, has thus been proposed as a treatment for BOS[173] or RAS.[174]

Although TGF-β is the main orchestrator of the airway remodeling process after lung transplantation, its effect can be largely modified by an inflammatory environment and cytokines like TNFα or IL-1β.[175,176] Besides, pollutants[177] or immunossupressive drugs[178] have also been described as EMT inducers. Moreover, release of reactive oxygen species by macrophages or neutrophils after lung transplantation[179] associated with a decrease in the counterbalancing factors, such as ascorbic acid, urate, glutathione[57] or Clara Cell Secretory Protein 16,[180] promotes the upregulation of the vascular endothelial growth factor, which may further stimulate fibrosis.[181,182] In an allograft model in rats, the simultaneous blockade of both platelet-derived growth factor and vascular endothelial growth factor could then reduce the severity of CLAD.[183] The respective roles played by these actors during the remodelling process of chronic dysfunction, however, remain to be defined. Moreover, although the role of EMT during the process of BOS development is well established, its relevance to RAS is not described yet, although airway obstruction is observed in this pathology as well. Furthermore, the exact contribution of EMT

TABLE 1.

Innate and adaptive immunity and the development chronic lung allograft dysfunction

	Agents	Triggers	Mediators			Role in CLAD	Association with CLAD	References
			Cytokines	Tissue-degrading factors	Growth factors			
Innate Immunity	Airway Epithelial Cells	PAMP, DAMP	IL-6, IL-8, CCL2, CCL5, CXCL10, CXCL11	MMP	TGF-PDGF, IGF-1	Innate and adaptive immune cell chemoattraction, EMT, matrix remodeling	EMT in BOS. +++	43-55,166
	Neutrophils	PAMP, DAMP, IL-8	IL-8, IL-6, TNF-α	ROS, MMP, proteases		Cytotoxicity, induction of apoptosis, oxidative damage, inflammation	++++	49,56-60,65,66
	NK	MICA, MICB, CX3CL1	TNF-α, IFN-γ	granzyme, perforin, TRAIL, Fas-L		Cytotoxicity; Graft tolerance through dendritic cell editing	DC editing to be confirmed	75,76,88,89
	M1 macrophages	PAMP, DAMP	TNF-α, IL-6, IL-1β	ROS		Induction of apoptosis, oxidative damage, inflammation	M1/M2 polarization to be investigated in human	90-94
	M2 macrophages	PAMP, DAMP	IL-10	MMP	TGF-β	Matrix remodeling		
	Eosinophils	Type II cytokines (IL-5)	IL-8, IL-6, TNF-α	granzyme, ROS	TGF-PDGF	Induction of apoptosis, oxidative damage, inflammation. Matrix remodeling	Associated with RAS +	7-95
Adaptive immunity	Th1 lymphocytes/cytotoxic T cells	CD40L, IL-12p70, IFN-γ	IFN-γ, TNF-α	Granzyme, perforin		Cytotoxic T cell activation; Induction of apoptosis	++	76,98,103-106
	Th17 lymphocytes	IL-6, IL-1β, IL-23	IL-17			Autoimmunity, neutrophil chemotaxis, support fibrosis	+++	115-121
	Treg/Breg cells	IL-2, TGF-β	IL-10		TGF-β	Immunotolerance, graft protection	+	65,123-125,139
	DSA	Allogenicity		Complement		Induction of apoptosis; Induction of profibrotic factors by airway epithelial cells.	++++	141-150
	Autoantibodies	ColV, K-α1 tubulin exposure		Complement		Induction of apoptosis; Induction of profibrotic factors by airway epithelial cells.	+++	115,152,153

Parameters considered for the association with CLAD were the number of studies, the repeatability across independent studies, human study versus animal model and the sample size (for human studies).
PDGF, platelet-derived growth factor; Breg, regulatory B cell; DAMP, damage-associated molecular pattern; PAMP, pathogen-associated molecular pattern.

with regard to other mechanisms, such as the recruitment of circulating fibroblast within the fibrotic lesions,[184] remains poorly documented. Answering these questions will help to develop new strategies for the prevention of CLAD.

CONCLUSIONS AND PERSPECTIVES

Although BOS was first considered as the unique manifestation of chronic dysfunction, the identification of RAS phenotype has transformed our perception of this pathology. Why was RAS characterized more than 15 years after the first description of BOS? Was it "under the radar" and has its identification been overlooked for years, or is RAS the result of new immunosuppressive drug regimens? Current works focusing on the specific features of these 2 syndromes will probably answer these questions.

As presented here, BOS or RAS phenotypes can be delineated by infiltrating cells, alarmins or cytokines present within the allograft (Table 1). An article from the Leuven Group shows a specific IL-6, CXCL10 and CXCL11 release in BAL of RAS patients, suggesting a role for B lymphocytes or NK in this pathology.[185] Moreover, the underlying diagnosis and immunosuppression regimens have been recently described as specific risk factors for RAS.[186] Further works will be needed to confirm these data and precisely define the specificities and similarities between the 2 diseases. Noteworthy, some evidences presented in this review have been collected before the description of RAS, that is, on chronic rejection groups where BOS and RAS patients were presumably pooled. Reinvestigating these data in light of our current knowledge will be probably useful to refine the perimeter of both diseases.

Chronic lung allograft dysfunction is irreversible. Therefore, the identification of harbingers of CLAD would allow proactive and targeted strategies to harness the progression of the disease, before degradation of the allograft. Several studies have been carried out to identify predictors. Lung biopsy profiling,[187] BAL composition, neutrophilia,[66,188,189] level of Treg cell,[123,124] cytokines, chemokines,[189] or MMP[190] or blood levels of endothelin-1,[191]CCL17,[192] or KL-6[193] have been proposed as early indicators of CLAD. Interestingly, pretransplant factors may also determine the outcome of the graft.[194,195] Prediction of CLAD is thus presumably achievable. Yet, none of these attempts have demonstrated enough robustness to achieve clinical acceptance. Indeed, CLAD is driven by the additive effect of repeated insults to the graft. The diversity of these insults as well as the donor and recipient genetic burden assign each patient a unique clinical history. Hence, large-scale gene expression profiling[187,196-199] or the powerful systems biology approach,[200-203] which integrates data sets of different nature, represents promising tools to decipher the complex network of factors involved in the development of CLAD.

Lung transplantation appears today as an ideal demonstrator of P4 Systems Medicine (participation, personalization, prediction, and prevention), because all recipients are followed up in well-characterized cohorts for several years, before the occurrence of the disease. Chronic lung allograft dysfunction displays fibrotic processes or alveolar degradation similarly observed in other respiratory diseases such as idiopathic pulmonary fibrosis or chronic obstructive pulmonary disease.[204] Investigating CLAD is thus a mighty lever to better understand other chronic inflammatory lower airway diseases.

ACKNOWLEDGMENTS

The authors are indebted to the "Programme Hospitalier de Recherche Clinique 2008" and to "Vaincre la Mucoviscidose" for supporting this project from its beginning, when 11 French lung transplantation centers gave rise to the Cohort in Lung Transplantation (COLT), "Programme Transplantation 2008", PRTP-13, ClinicalTrials.gov Identifier: NCT00980967.

Consortium, SysCLAD consortium

Cohort Of Lung Transplantation-COLT (associating surgeons; anaesthetists-intensivists; physicians, research staff) ***Bordeaux*:** J. Jougon, J.-F. Velly; H. Rozé; E. Blanchard, C. Dromer; ***Bruxelles*:** M. Antoine, M. Cappello, R. Souilamas, M. Ruiz, Y. Sokolow, F. Vanden Eynden, G. Van Nooten; L. Barvais, J. Berré, S. Brimioulle, D. De Backer, J. Créteur, E. Engelman, I. Huybrechts, B. Ickx, T. J.C. Preiser, T. Tuna, L. Van Obberghe, N. Vancutsem, J.-L. Vincent; P. De Vuyst, I. Etienne, F. Féry, F. Jacobs, C. Knoop, J.L. Vachiéry, P. Van den Borne, I. Wellemans; G. Amand, L. Collignon, M. Giroux; ***Grenoble*** V. Bach, P.-Y. Brichon, P. Chaffanjon, O. Chavanon, S. Guigard, A. Pirvu, P. Porcu, R. Hacini; P. Albaladejo, C. Allègre, A. Bataillard, D. Bedague, E. Briot, M. Casez-Brasseur, D. Colas, G. Dessertaine, M. Durand, G. Francony, A. Hebrard, M.R Marino, B. Oummahan, D. Protar, D. Rehm, S. Robin, M. Rossi-Blancher; P. Bedouch, A. Boignard, H. Bouvaist, A. Briault, B. Camara, S. Chanoine, M. Dubuc, S. Quêtant, J. Maurizi, P. Pavèse, C. Pison, C. Saint-Raymond, N. Wion; C. Chérion; ***Lyon*** R. Grima, O. Jegaden, J.-M. Maury, F. Tronc; C. Flamens, S. Paulus; J.-F. Mornex, F. Philit, A. Senechal, J.-C. Glérant, S. Turquier; D. Gamondes; L. Chalabresse, F. Thivolet-Bejui; C Barnel, C. Dubois, A. Tiberghien; ***Paris, Hôpital Européen Georges Pompidou*** F. Le Pimpec-Barthes, A. Bel, P. Mordant, P. Achouh; V. Boussaud; R. Guillemain, D. Méléard, M.O. Bricourt, B. Cholley; V. Pezella; ***Marseille*:** G. Brioude, X.B. D'Journo, C. Doddoli. P. Thomas, D. Trousse; S. Dizier, M. Leone, L. Papazian; F. Bregeon, B. Coltey, N. Dufeu, H. Dutau, S. Garcia, JY. Gaubert, C. Gomez, S. Laroumagne, A. Nieves, L, C. Picard, F. Rey, M. Reynaud-Gaubert, JM. Rolain, E. Sampol, V. Secq; I. Bouvin; ***Nantes*** O. Baron, P. Lacoste, C. Perigaud, J.C. Roussel; I. Danner, A Haloun A. Magnan, A Tissot; T. Lepoivre, M. Treilhaud; K. Botturi-Cavaillès, S. Brouard, R. Danger, J. Loy, M. Morisset, M. Pain, S. Pares, D. Reboulleau, P.-J. Royer; ***Le Plessis Robinson, Hôpital Marie Lannelongue*** Ph. Dartevelle, D. Fabre, E. Fadel, O. Mercier, S. Mussot; F. Stephan, P. Viard; J. Cerrina, P. Dorfmuller, S. Feuillet M. Ghigna, Ph. Hervén F. Le Roy Ladurie, J. Le Pavec, V. Thomas de Montpreville; L. Lamrani; ***Paris, Hôpital Bichat*** Y. Castier, P. Cerceau, F. Francis, G. Lesèche; N. Allou, P. Augustin, S. Boudinet, M. Desmard, G. Dufour, P. Montravers; O. Brugière, G. Dauriat, G. Jébrak, H. Mal, A. Marceau, A.-C. Métivier, G. Thabut; B. Ait Ilalne; ***Strasbourg*** P. Falcoz, G. Massard, N. Santelmo; G. Ajob, O. Collange O. Helms, J. Hentz, A. Roche; B. Bakouboula, T. Degot, A. Dory, S. Hirschi, S. Ohlmann-Caillard, L. Kessler, R. Kessler, A. Schuller; K. Bennedif, S. Vargas; ***Suresnes*** P. Bonnette, A. Chapelier, P. Puyo, E. Sage; J. Bresson, V. Caille, C. Cerf, J. Devaquet, V. Dumans-Nizard, ML. Felten, M. Fischler, AG. Si Larbi, M. Leguen, L. Ley,

N. Liu, G. Trebbia; S. De Miranda, B. Douvry, F. Gonin, D. Grenet, A.M. Hamid, H. Neveu, F. Parquin, C. Picard, A. Roux, M. Stern; F. Bouillioud, P. Cahen, M. Colombat, C. Dautricourt, M. Delahousse, B. D'Urso, J. Gravisse, A. Guth, S. Hillaire, P. Honderlick, M. Lequintrec, E. Longchampt, F. Mellot, A. Scherrer, L. Temagoult, L. Tricot; M. Vasse, C. Veyrie, L. Zemoura; ***Toulouse*** J. Berjaud, L. Brouchet, M. Dahan; F. Le Balle, O. Mathe; H. Benahoua, A. Didier, A.L. Goin, M. Murris; L. Crognier, O. Fourcade.

Swiss Transplant Cohort Study-STCS *Genève-Lausanne* T. Krueger, H.B. Ris, M. Gonzalez; J.-D. Aubert, L.P. Nicod, B.J. Marsland, T.C. Berutto, T. Rochat, P. Soccal, Ph. Jolliet, A. Koutsokera, C. Marcucci, O. Manuel; E. Bernasconi, M. Chollet, F. Gronchi C Courbon; ***Zurich*** S. Hillinger, I. Inci, P. Kestenholz, W. Weder; R. Schuepbach, M. Zalunardo; C. Benden, U. Buergi, L.C. Huber; B. Isenring, M.M. Schuurmans; A. Gaspert, D. Holzmann, N. Müller, C. Schmid, B. Vrugt, T. Rechsteiner.

SME and Platforms *Biomax, Germany* A. Fritz, D. Maier; ***Finovatis, Lyon, France*** K. Desplanche, D. Koubi; ***GATC, Germany*** F. Ernst, T. Paprotka, M. Schmitt, B. Wahl; **Novasdicovery, Lyon, France** J.-P. Boissel, G. Olivera-Botello; ***Prométhée Proteomics Platform, Grenoble, France*** C. Trocmé, B. Toussaint, S. Bourgoin-Voillard, M. Séve; ***Inserm U823, Université Joseph Fourier, Grenoble*, France** M. Benmerad, V. Siroux, R. Slama; ***European Institute for Systems Biology & Medicine, Lyon, France*** C. Auffray, D. Charron, J. Pellet, C. Pison.

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