Chapter 1

General Introduction

1. INTRODUCTION

The number of lung transplantations (LTx) performed every year continues to increase with improved median survival since the start of a successful LTx in 1983 (1, 2). For now, LTx has become an established therapy for progressive end-stage advanced lung diseases. The main reasons for LTx in the Netherlands include emphysema, pulmonary fibrosis, pulmonary hypertension and cystic fibrosis. The most recent International Society for Heart and Lung Transplantation (ISHLT) registry reports a 1 and 5-year survival of 85 and 59%, respectively (3, 4). Due to the improvements in donor selection, organ preservation, perioperative management and better management of postoperative complications, the median survival was 6.7 years in 2010-2017 compared to 4.7 years in 1992-2001 (4-6). Complications following LTx are serious and can be fatal. Graft rejection is a common issue, resulting from immune activation, and is managed by immunosuppressive drugs. However, these drugs can have side effects, including an increased risk of diabetes, kidney damage, and vulnerability to infections. Despite efforts to manage these complications, they can still occur and have significant consequences.

Acute rejection

Acute rejection (AR) is a potential complication that can arise at any time following transplantation, although it predominantly occurs within the first year. Its incidence rate is highest during this initial period, reaching approximately 50% within five years post LTx (7, 8). What's more, it is also a risk factor for subsequent development of Bronchiolitis obliterans syndrome (BOS) and chronic lung allograft dysfunction (CLAD) (9, 10). AR can be manifested as fever, cough, dyspnea and new adventitious lung sounds or even be just clinically silent (11). These nonspecific signs are hardly

distinguishable between rejection and other causes of graft dysfunction. Until now confirmation by histology through lung punction is still the gold standard to assess AR in lung transplantation recipients (LTRs) (12-14). However, this is an invasive method, not very easily performed and it can also cause organ damage. Of note, incidence of AR episodes is increased in LTx compared with other organ transplantations (15, 16). AR is directed by a T cell mediated pathway, which involves direct recognition of donor major histocompatibility complex (MHC) on donor cells by T cells (15, 17). As a result, the identification of biomarkers to predict AR becomes essential. In ***Part 1*** of this thesis, we were searching for approaches to identify non-invasive manners for prediction, early diagnosis, prevention, and treatment of AR by investigating biomarkers in blood.

Infection

Infection is one of the major causes of post LTx morbidity and mortality, especially in the first year after LTx (18). The degree of immunosuppression strongly affects the risk of infection, which is highest during the first six months following LTx to prevent AR (19). After this initial period, the risk of infection generally decreases as the degree of immunosuppression is reduced because the risk of AR decreases. Community acquired infections, such as respiratory and urinary tract infections, are more commonly observed beyond the initial six months (19, 20). Although antimicrobial prophylaxis or microbiologic monitoring are often used to detect common pretransplant pathogens, it is possible for new or resistant pathogens, such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), varicella zoster (VZV), HIV, hepatitis B virus (HBV), hepatitis C virus (HCV), to emerge in LTRs (13, 16, 21). For this reason, LTRs are considered to have vaccinations against microbes such as herpes zoster, influenza and

pneumococci (22-24). Some vaccinations such as VZV are preferably given before transplantation when there is still an intact immune system, while others (e.g. influenza) are given after transplantation because of their limited time-span of protection. Furthermore, LTRs are particularly vulnerable to respiratory infections due to the constantly exposure to inhaled pathogens (25).

Altogether, the primary strategy for reducing morbidity and mortality in LTRs is to minimize the risk of infections. Achieving this goal involves tailoring immunosuppression therapy to balance the risks of both infections and rejections on an individual basis. Furthermore, vaccination is the most effective means of preventing infection.

Immunosuppression

Immunosuppressive treatment includes induction and maintenance therapy. Induction therapy utilizes intensive immunosuppression in the perioperative and the immediate post operative period to reduce the risk of T-cell mediated early rejection (26). The three agents commonly used are Basiliximab, anti-thymocyte globulin (ATG) and Alemtuzumab (27-29). At the UMCG, the preference is to use Basiliximab for induction of immunosuppression. Basiliximab (Simulect®) is a monoclonal antibody which is directed against the interleukin-2 (IL-2) receptor alpha-chain, which inhibits T cell proliferation, resulting in a decrease of circulating T cells without complete depletion (30).

The purpose of maintenance immunosuppression is to prevent AR and chronic rejection. Immunosuppression is traditionally maintained with a triple therapy consisting of a calcineurin inhibitor (CNI, usually tacrolimus), anti-proliferative immunosuppression (mycophenolate mofetil (MMF) or azathioprine (Aza)) and corticosteroids until one year follow-up post LTx,

according to the 2018 ISHLT registry database report (1). Recently, there is a trend to use more often MMF instead of Aza in combination with tacrolimus, and corticosteroids (29, 31), which is also the preference at the UMCG. Of date, regimen and doses are adjusted over time according to the situation of the LTRs.

Balancing immunosuppression is a key element

LTRs have a higher infection burden due to being intrinsically immunosuppressed (32). Infection is more common in LTx than other solid organ transplantations (SOTs) and remains a significant cause of death (33). As shown in Figure 1**,** reduction of immunosuppressive therapy increases the immune response, and indirectly increases the risk of AR or chronic rejection. On the other side reduction of immunosuppressive therapy might cause risk for infection. It is a key element to carefully balance the immunosuppressive regimen to increase the survival rate of LTRs.

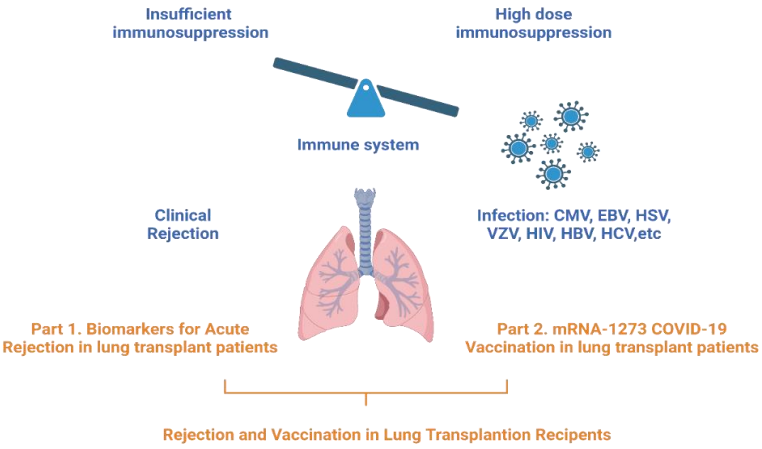

Figure 1. Balancing the immunosuppression is a key element for successful lung transplantation. Cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus (HSV), varicella zoster (VZV), HIV, hepatitis B virus (HBV), hepatitis C virus (HCV).

SARS-CoV-2 infection and vaccination

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) first emerged in Wuhan in 2019 and caused the coronavirus disease 2019 (COVID-19), whose pandemic has lasted for more than two years. There is no specific drug available yet for the medical treatment of those patients (34). Vaccinations are important options for controlling the virus spread. It has been reported that the antibodies response was 95% for the BNT162b2 BioNTech/Pfizer vaccine and 94% for the mRNA-1273 Moderna COVID-19 vaccine in the general population (35). LTRs are immunocompromised because they receive the most intensive immunosuppressive treatment to prevent rejection and, as a consequence are more vulnerable to SARS-CoV-2 infections than other organ transplantation patients (36, 37).

Immunocompromised subjects including LTRs were excluded from Phase III vaccine trials (38, 39). In an observational study, mRNA-1273 vaccination induced a higher seropositivity trend than BNT162b2 in organ transplantation patients (40, 41). However, we still need to be concerned about the duration and mechanism of the mRNA-1273 vaccination and whether an extra booster dose is needed for LTRs.

Therefore, in ***Part 2***, our goal was to explore the effectiveness of the mRNA-1273 vaccine in providing immunity against SARS-CoV-2 infection in LTRs. This included analyzing the impact of infection in the pre-vaccination era as well as conducting a 6-month T cell in depth study after vaccination that also involved kidney transplantation patients.

2. AIM AND OUTLINE OF THE THESIS

This thesis is mainly focused on two contents: Part 1. Biomarkers related to AR in LTRs: To identify specific biomarkers that can greatly aid in early detection, monitoring, and treatment of AR episodes. Part 2. is to investigate the immunogenicity of the mRNA-1273 vaccine in providing immunity against SARS-CoV-2 in LTRs, considering the pre-vaccination infected population and conducting a 6-month T cell in-depth study involving kidney transplantations. By addressing these two areas, this thesis endeavors to understand AR and the implication of mRNA-1273 vaccination in LTRs. The findings from this study may have significant implications for clinical practice, patient management, and the development of targeted interventions to improve outcomes for LTRs.

Chapter 2 is a literature review highlights the biological role of HLA-G, and the mechanism of how HLA-G works via receptors present on immune cells. Interestingly, the effect of HLA-G to prevent embryos from being rejected seems similar to the process of transplantation. This review shows the multiple roles of HLA-G during SOT. Monitoring HLA-G expression in tissue and/or serum/plasma indicates a role for HLA-G in tolerance by acting on inhibitory receptors on different immune cells. This review will explain the general characteristics and biological function of HLA-G and summarize the views supporting the tolerogenic and other roles of HLA-G to better understand its role in SOT and its complications. Finally, we will discuss potential future research on the role of HLA-G in prevention, diagnosis, and treatment in SOT.

Chapter 3 is a study to perform a longitudinal analysis measuring the frequencies of T and B cells and their subsets in LTRs with a stable or AR status before and after LTx compared to healthy controls. This chapter

contains data measuring T and B cell subsets using flow cytometry. The longitudinal dynamics were measured at the routine time points, before LTx and 0-3, 3-6, 6-12, and >12 months after LTx. Furthermore, we studied changes within IL-10 producing (regulatory) B cells. This comprehensive approach enabled us to investigate the specific roles of immune cell subsets over time in LTRs, potentially leading to new insights that could improve patient outcomes.

In the study performed in ***Chapter 4***, we monitored gene profiles for AR using the NanoString® nCounter® Analysis System (NanoString Technologies, Seattle, WA, USA) from peripheral blood mononuclear cells (PBMCs) in LTRs. This study found some target expression genes to be differently expressed between stable and AR patients in LTRs. These differential genes were further validated with Quantitative real-time polymerase chain reaction (qRT-PCR, or qPCR). Furthermore, an independent set of validation samples were used to confirm gene related cells and chemokines by flow cytometry and Luminex. For the first time, we tried to monitor AR based on transcriptomic gene expression data to propose a new tool for clinical utilization in management of LTRs in future diagnosis of AR.

Chapter 5, 6, 7 are all belonging to the Covalent (COVID-19 vaccination in lung transplant recipients) study. In ***Chapter 5***, the study mainly focused on the induction and durability of immune responses in patients on the waiting list for LTx and LTRs after SARS-CoV2 vaccination, and showed data on durability of immune responses. We investigated these two groups in this 6-month follow-up study compared to healthy controls. The study describes the generation and the kinetics of the humoral and cellular responses in these groups by determining IgG levels, neutralizing antibodies, and cellular responses by interferon release assays and ELIspot analysis.

In ***Chapter 6***, to investigate the humoral and cellular responses to vaccination after a natural infection, we studied a group of 19 LTRs who had a prior SARS-CoV-2 infection. LTRs, more than 40 days after SARS-CoV-2 infection, were vaccinated twice with the mRNA-1273 vaccine, at a 28 day interval. Blood samples for humoral and cellular analysis were taken before vaccination, at 28 days after the first vaccination, at 28 days and 6 months after the second vaccination. Antibodies to spike (S) and nucleocapsid (N) proteins were measured by ELISA. The capacity of antibodies neutralizing the D614G in Wuhan strain and BA.5 in Omicron was measured using plaque reduction neutralization test (PRNT50). SARS-CoV-2 specific T cell activity was measured using Interferon Gamma Release Assay (IGRA), ELIspot, and flow cytometry.

Chapter 7 is a follow-up study mainly focusing on the spike-specific CD4+ T cell response and phenotypes both in kidney and lung transplant recipients compared to a control group at the timepoints of 28 days and 6 months after vaccination. T cell responses were evaluated by IFN-γ ELISpot assay, and activation induced T cell markers assays. Besides, we investigated the production of the following Th cell cytokines IL-2, IL4, IL5, IL9, IL10, IL13, IL17A, IL17F, IL22, IFN-γ and TNF-α, which are respectively secreted by Th1, Th2, Th9, Th17, and Th22 cells. Furthermore, we investigated the T cell phenotype profile of T cell responders or non-responders in kidney and lung recipients both at 28 days and 6 months after vaccination.

Finally, the results and implications of this thesis were summarized, and the future perspectives were discussed in ***Chapter 8***.

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