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Balance between herpes viruses and immunosuppression after lung transplantation

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Chapter 1

Aim and outline of this Thesis

Introduction

The ideal of clinical transplantation of solid organs is acceptance of the transplant without impairment of host immunity. The struggle to achieve this goal has started with the first transplantation, and, despite the enormous progress in the field of transplantation, we still are far from that ideal. Transplantation of solid organs depends on immunosuppressive effects of toxic medication, in order to inhibit recognition and rejection of the transplanted organ by the immune system. The underlying concept of immunosuppression encompasses that the immune system is suppressed to a level that rejection is prevented and at the same time the immune system remains capable of controlling infections and cancer.

In clinical medicine we achieve this balance by starting immunosuppression according to a standard protocol, and adapt the level of immunosuppression guided by clinical symptoms of ‘too much’ or ‘too little’ immunosuppression (that is infections or rejection, respectively).

We follow this approach since there is no clinically useful way to measure the capacity of the immune system in such a way that it shows us how to adapt the level of immunosuppression so that rejection is prevented and adequate control of pathogens occurs. Therefore we try empirically to reach a balance in our patients between ‘too much’-immunosuppression, clinically recognized by infectious complications, and ‘too little’ immunosuppression, clinically recognized by rejection of the transplanted organ.

Our ability to “fine-tune” the immunosuppressive medication to the individual needs of the patient determines the outcome of our transplantation protocol. After lung transplantation, not only acute rejection forms an important risk factor for bronchiolitis obliterans syndrome (BOS), the lung transplant presentation of chronic transplant dysfunction, but also infection is widely accepted as a major cause of chronic transplant dysfunction. At the start of the research described in this thesis the percentage of patients developing bronchiolitis obliterans syndrome (BOS), exceeded 40% at 4 years with a median survival of not even 4 years (1). This illustrates our capacity, at that time, to

recognize and adequately respond to the threats to the lung transplant patient. Because rejection after lung transplantation can be patchy and missed by transbronchial biopsies, recognition of infectious complications is of major importance for the treatment of the patient. Often, transplant dysfunction in the absence of (demonstrated) infection is interpreted as rejection and treated as such. This approach only stands if we have sensitive and specific tools to detect infections.

This is especially relevant for infectious agents that are already present in the transplant patient and can reactivate whenever immune control fails. This is the case with the herpes viruses of which two are studied in this thesis. Of all herpes viruses these two, namely the Cytomegalovirus (CMV) and the Epstein-Barr virus (EBV), are currently most recognized as a cause of morbidity and mortality after solid organ transplantation.

In this study we aimed to develop better tools for monitoring of these viruses and to come to better strategies to treat or prevent the associated morbidity and mortality.

Cytomegalovirus

Cytomegalovirus (CMV) infection is the most common viral infection after lung transplantation. It has early and late effects on both the transplanted organ as well as the recipient (2-9). CMV infection can go unnoticed but the initial stage of the infection or reactivation often presents with an acute viral syndrome, with fever and malaise and organ specific symptoms like pneumonitis, hepatitis and entero-colitis (10). Also, CMV has been associated with the development of acute rejection (11,12)

The late effects attributed to CMV infection have been the focus of interest during the nineties (13) and raise still much debate (14). The infection can present itself with transplant dysfunction complicating our clinical strategy to adapt immunosuppression according to clinical signs and symptoms. Without a routine monitoring strategy the infection can be mistaken for transplant rejection, and, if treated so, may seriously worsen the patient's outcome (15-18). Consequently, routine monitoring of CMV viral load is now widely accepted and performed (19-22). The strategy to deal with CMV infection varies and has evolved over time (23,24). Also the current test arsenal to monitor CMV has grown extensively during the last two decades. It evolved from serology, to monitor the humoral immune response (25), to detection of the virus with shell vial culture after clinical suspicion (26), and, nowadays, to direct assessment of pp65 antigenemia (27) to monitor viral derived proteins, and PCR and NASBA to

measure viral DNA or RNA load (20,28). The different tests used to detect CMV infection were mostly in house developed assays and this complicates comparison between studies, interpretation of results and implementation of multi-centre trials (29,30).

A standardization of the most widely used tests was needed and standardization of the CMV antigenemia test forms the second chapter of this thesis.

Using this test differences in frequency and severity of CMV reactivation was recognized not only between the different solid organ transplant programs but also over time with the evaluation of immunosuppressive protocols. The impact of different immunosuppressive regimes on frequency and severity of CMV reactivation was studied in chapter 3. A final problem of CMV infection, assessed in this thesis, was the problem of subclinical CMV infection. It has been suggested that chronic subclinical reactivation of CMV causes chronic transplant dysfunction by way of chronic inflammation not only resulting in damage to the transplant but also upregulating alloreactivity (31,32).

Using a new method, nucleic acid sequence based amplification (NASBA), which measures viral RNA and thus virus activation/transcription, and not just the presence of the virus, we looked for better tools to monitor CMV activity in the lung transplant patient (chapter 4 and 5).

Epstein-Barr virus

With the implementation of new antiviral drugs such as ganciclovir and valganciclovir CMV infection, if clinically managed well, has largely ceased to be a clinical problem (33,34). However, with the use of increasingly more effective immunosuppression, EBV has gained importance as a cause of morbidity and mortality (35-38).

In contrast to CMV, EBV has two ways of replication. First, the lytic replication, causing lysis of the host cell, which takes place in the nasopharyngeal cavity and causes shedding of infectious virus with saliva (39). Second, the latent replication when EBV infects B-cells and transforms these cells into large lymphoblasts that can proliferate indefinitely. This leads to the clinical presentation of post transplant lymphoproliferative disease (PTLD) (40).

While the lytic replication responds well to antiviral drugs (41), B-cell proliferation does not respond and asks for an alternative approach. Generally, EBV driven B-cell proliferation in the human host is considered a consequence of (iatrogenic) impairment of EBV-specific T-cells (42-46), as EBV specific T-cells have been demonstrated to be most important for control of EBV.

Aim and outline

When we started the studies reported in this thesis, PTLD was considered a malignancy (47,48). It was recognized only after its clinical presentation as a mass or nodular lesions(37), and was treated with polychemotherapy (49-51). Depending on which organ is transplanted the incidence of PTLD varies between approximately 1% after kidney transplantation up to 10% in lung transplant patients (48,52-54)

The diagnosis of PTLD means for a patient a high mortality with a range up to 50-80% (48,52-55).

To approach this clinical problem we started various laboratory and clinical studies.

First, monitoring tools for EBV were introduced and evaluated as described in chapters 6 to 8. The laboratory studies evolved from EBV specific serology with synthetic peptides to EBV DNA load measurements and measurement of the EBV specific T-cell immunity.

Secondly, with the recognition of the presence of CD20 on the cell membrane of B-cells that constitute most PTLD, Rituximab, an anti-CD20 chimeric monoclonal antibody, was successfully introduced in the treatment of PTLD (chapter 9).

Clinical observations led to the suspicion that EBV reactivation was associated with transplant dysfunction. This observation is important in the clinical approach of lung transplant patients as transplant dysfunction in absence of an infectious explanation is often regarded as rejection and treated as such. In case of an EBV associated/ induced transplant dysfunction treatment directed on rejection could lead to further over-immunosuppression and possibly PTLD. The relation between EBV and transplant dysfunction was therefore evaluated in chapter 10.

Lessons learned from these studies were then implemented in the clinic. In 2001 the immunosuppressive protocol of the lung transplant program was changed and a pre-emptive strategy for PTLD was incorporated in the treatment protocol. This led to what we now call EBV DNA guided immunosuppression, in which the level of immunosuppression is pre-emptively reduced, in case of increasing EBV-DNA load measured in peripheral blood.

Primary objective of this pre-emptive approach is reduction in the prevalence of PTLD. The improvement in outcome, particularly the reduction of BOS, which we observed since the introduction of this approach, led to the hypothesis presented in chapter 11. This hypothesis states, that the extent of EBV reactivation, as measured by the peripheral blood EBV DNA load, reflects the balance of the immune system between infection and rejection. As such, peripheral blood EBV DNA load can be used to individualize immunosuppression

after lung (and possibly other solid organ) transplantation. In chapter 11 the results of this pre-emptive approach are described in all adult lung transplant patients transplanted since June 1st, 2001, enabling early intervention in EBV reactivation at the time of initial rise of EBV DNA load, and, thus, in the beginning of EBV reactivation. In chapter 12 the results of this approach, are reported in the adult lung transplant patients transplanted before, and alive at, June 1st, 2001. In this more heterogeneous cohort of patients, safety and efficacy of the pre-emptive approach on patients late after transplantation was studied.

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Aim and outline