Balance between herpes viruses and immunosuppression after lung transplantation

Verschuuren, Erik Alfons Maria

Chapter 13

Summary and future perspective

Introduction

This thesis focuses on the interplay between two Herpes Virus infections and the immunosuppression used after solid organ (and especially lung) transplantation. It starts with the description of diagnostic tools of Cytomegalovirus (CMV) and their therapeutic implications. Then it addresses the major clinical complication of a second herpes virus, the Epstein-Barr virus (EBV), namely Post Transplant Lymphoproliferative Disease (PTLD). These chapters describe the work done to come to a diagnostic tool to predict patients at risk for PTLD and they discuss clinical presentation and treatment of PTLD. We come to the new conclusion that EBV is associated with transplant dysfunction. We subsequently implemented a pre-emptive strategy for PTLD in our lung transplant program, and the results are shown in chapter 11 and 12. Finally, we propose that EBV DNA load can be used as a surrogate marker for over-immunosuppression after lung (and probably also other solid organ) transplantation, and, thus may help to guide and individualize immunosuppression. This summary contains two parts: the first part introduces the different chapters and describes their most important results, the second part summarizes the conclusions and speculates on the clinical implications and future perspective/research.

Summary

After the introduction this thesis starts in chapter 2 with the description of the international standardization of an assay for quantifying Cytomegalovirus in blood. This test, the CMV antigenemia assay, originally developed in Groningen, still forms the basis for the treatment of Cytomegalovirus infection in many hospitals. In this chapter we compare the different methods for assessing CMV antigenemia in different laboratories in Europe. From this comparison it can be concluded that, although there are differences in the way the CMV antigenemia test is carried out in the participating laboratories, the outcomes are similar and results can be compared between different laboratories.

Chapter 3 shows the effect of different immunosuppressive regimens on the clinical manifestations of Cytomegalovirus infection. It illustrates the price paid for the use of immunosuppression that is increasingly effective for preventing rejection. At the start of this study we had the clinical impression that Cytomegalovirus infections became more severe and that the duration became longer. Indeed we could confirm this impression and showed that switch from Sandimmune to Neoral and, later, adding mycophenolate mofetil, was

accompanied by an increased incidence of secondary CMV infections especially in the Neoral treated group. A higher and more prolonged CMV antigenemia was observed especially in the mycophenolate mofetil group. Although the majority of these infections remain asymptomatic, the perseverance of viral activity might result in extended endothelial damage and thus contribute to the development of chronic transplant failure and atherosclerosis. Therapeutically this can be translated into an approach of using more aggressive treatment of CMV infection, e.g. a switch from pre-emptive treatment of CMV infection to CMV prophylaxis.

In chapter 4 and 5 the results are described of new methods to monitor CMV infection and these methods are compared to the CMV antigenemia assay as the standard test. Using nucleic acid based sequence amplification (NASBA), the early stage of CMV activity is detected by quantitative measurements of the RNA of immediate early antigen-1 (IE1 RNA) and qualitative measurements of the RNA of the late expressed antigen (pp67 RNA) is used to demonstrate progression to active systemic CMV infection, allowing fine-tuning of the anti-CMV therapy. These test systems enable us to study the effect of treatment intended to prevent all CMV activity, which may improve long-term allograft outcome. In chapter 5, an active role of viral immune evasion during HCMV infection in vivo is suggested by the observation that immune evasion RNA remained detectable after clinical recovery, often independently of other CMV antigens such as IE1 RNA expression. This indicates that viral activity may persist without clinical manifestations or detectable viral antigens, which may have implications for long-term control of CMV and therapeutic strategies.

From chapter 6 on the studies in this thesis are focussed on Epstein-Barr virus. This part particularly results from our interest in the clinical problem of PTLD. Approximately 10 percent of lung transplant patients develop this serious complication. It forms a major source of morbidity and mortality to the patients. At the time this study started, PTLD was only recognized as it became clinically apparent, and was regarded and treated as a malignancy. With the recognition of the central role of Epstein-Barr virus in the development of PTLD, the diagnostic and therapeutic approach has changed considerably.

Before an effective therapeutic approach for a disease can be developed, effective tools to monitor the disease have to be designed. As in CMV this started with the evaluation of the serologic response to (primary) EBV infection in relation with PTLD (chapter 6). This was the first attempt to come to a clinical tool to understand the central role of EBV infection in the development of PTLD. It showed that the serologic response to EBV is impaired, probably due to the use of immunosuppression, and not useful for monitoring EBV activity for the identification of patients at risk for PTLD after lung transplantation.

In chapter 7 both the cellular and humeral immune responses against EBV were studied in a patient particularly at risk for PTLD. This patient was both EBV- and CMV mismatched at transplantation and developed a persistently active EBV infection with relapsing PTLD. It was shown that even in a patient with relapsing PTLD adequate numbers as well as good effector function of EBV cytotoxic T-lymphocytes (CTLs) can be found in vitro. It was suggested that not lack of EBV-CTL numbers but lack in function induced by immunosuppression is responsible for the development of PTLD after solid organ transplantation. Under the same therapeutic regimen during which control of EBV failed, CMV infection was controlled effectively. Regarding the fragile balance between EBV viral load and levels of immunosuppression we suggest that pre-emptive treatment guided by EBV-DNA levels should be the strategy of choice. An approach that, in our view, could include reduction of immunosuppression.

Chapter 8 has become a key article as it shows not only the relation between EBV DNA load and PTLD but also that patients who develop PTLD have an elevated EBV DNA load long before the clinical manifestations of PTLD. It also showed that EBV DNA levels fluctuate with levels of immunosuppression, suggesting that pre-emptive treatment of PTLD is feasible. It furthermore shows that the doubling time of EBV DNA can be as short as 56 hours illustrating the speed of EBV driven B-cell proliferation. An argument was made for the use of whole blood as a source of EBV DNA as in parallel obtained serum samples no EBV DNA could be found.

In chapter 9 we describe our first experience with the anti CD20 monoclonal antibody, Rituximab. Rituximab proved to be effective for the treatment of PTLD without progression of transplant dysfunction. Complications were a relapse of PTLD with, partly, CD20-negative B-cells, localized at the site of the diagnostic open lung biopsy, via which the original diagnosis was made. This might suggest that bad penetration of Rituximab due to the recent wound/haematoma was responsible for the relapse. In addition the complication of hypogammaglobulinemia was reported for the first time. So, we advise that attention should be paid to IgG levels especially in patients treated with anti-proliferating agents, such as mycophenolate mofetil, simultaneously with Rituximab.

Chapter 10 is an article studying the relation between transplant (dys-) function and EBV reactivation. The differentiation between infection and rejection as the cause of (lung) transplant dysfunction is often very difficult. Our results described in this chapter suggest that EBV reactivation is associated with transplant dysfunction. Because of the previous lack of diagnostic tools to monitor EBV activity, this association was not recognized previously. The

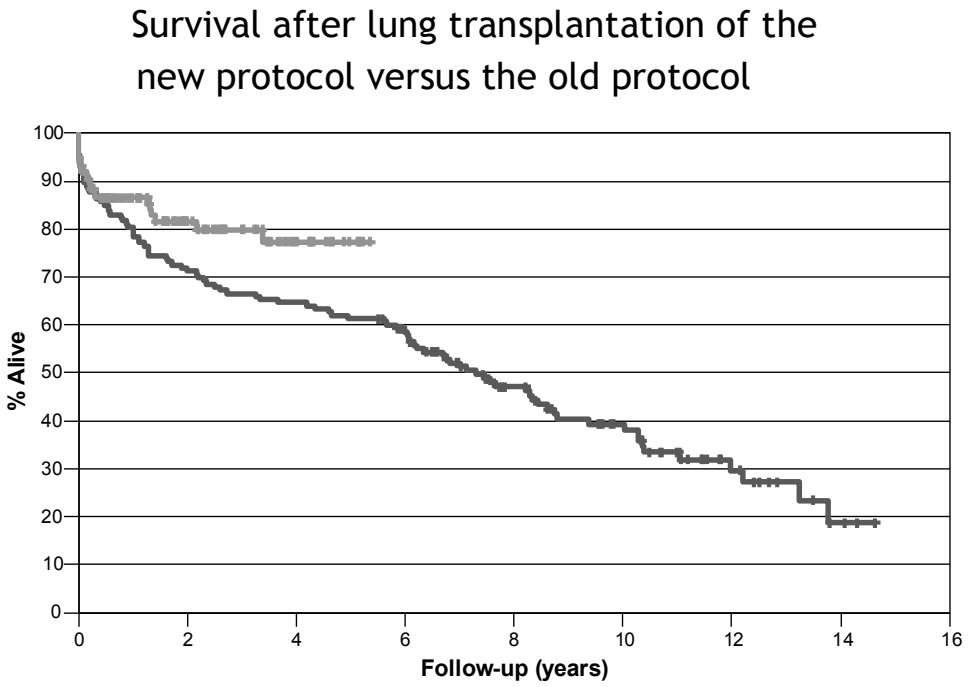
transplant dysfunction, that retrospectively was associated with EBV infection, had often been approached by a rejection treatment, with possible development of PTLD in three of our patients.

Chapter 11 and 12 show the results of the pre-emptive strategy for PTLD that we incorporated in a new treatment protocol starting June 2001. we chose this strategy because 1: the number of transplantations and PTLD was too low to perform a randomised study and 2: the predictive value of the EBV DNA load with regard to the development of PTLD was too strong to not respond to rising EBV DNA titres. The pre-emptive strategy was started to prevent PTLD as the primary objective, but soon we found a strong reduction in the prevalence of chronic transplant dysfunction. This was a bonus that led to the hypothesis of EBV guided immunosuppression, explained in chapter 11. This hypothesis is as follows: EBV DNA will become detectable if the T-cells are suppressed to a level that they cannot control EBV driven B-cell proliferation. The immune response against EBV is carried out by EBV-specific memory T-cells, and can be considered more efficient/stronger than the development of a new (allo-) immune response. Therefore, the failure of the EBV specific T-cell response (detectable EBV DNA) can be considered a biomarker for over-immunosuppression and implicate that it is safe to reduce immunosuppression with respect to rejection. On the other hand transplant dysfunction without detectable EBV DNA could indicate rejection, after exclusion of other causes of transplant dysfunction.

In chapter 11 the safety of this approach is shown in terms of incidence of acute and chronic rejection with otherwise a low rate of PTLD, although, probably due to the number of patients, not significantly lower than in our historical cohort. More striking however, is the very low rate of chronic transplant dysfunction which is lower than 10% over 4.5 years. This results in an overall survival in our adult lung transplant program of 76% at three years. Currently (October 21st 2006), the survival is even better with a 5 year survival of 77% (Fig.1)

Chapter 12 shows the same approach of EBV DNA guided reduction of immunosuppression, but now in the patients transplanted before and alive at June 1st 2001, when we introduced the pre-emptive strategy. Originally we had the impression that the strategy only worked early after transplantation but thorough evaluation of this historical cohort showed that also late after transplantation this strategy is safe with respect to acute rejection, acceleration of Bronchiolitis Obliterans Syndrome (BOS, chronic rejection) and survival.

***Figure 1**: Overall survival of all adult lung transplant patients treated according to the new protocol versus the patients treated according to the historical protocol. Survival in the new protocol (upper grey line) is 87% at 4 months and 77% at 5 years. In the old protocol (lower black line) the survival is 87% at 4 months and 61% at 5 years*



Future perspective

From the studies on Cytomegalovirus infection it can be concluded that, although there are differences in the way the CMV antigenemia test is carried out in different laboratories, the outcomes are similar and results can be compared between different laboratories. This enables multicentre studies without the problem of shipping samples.

Chapter 3 shows the price we have to pay for using immunosuppression that is increasingly effective for preventing rejection, namely, increase in severity and duration of CMV infections. This is shown for kidney transplantation, but because immunosuppression after lung transplantation is even more intense these lessons should be taken into account for the lung transplant populations. Indeed the current therapeutic protocol after lung transplantation includes CMV prophylaxis and with the current tools to monitor CMV and the introduction of CMV prophylaxis the clinical problem of CMV infection after lung transplantation

is more or less solved. With this strategy CMV reactivation is postponed until 3 months after lung transplantation when immunosuppression is already at a lower level and rejection becomes less frequent. This reduces the problem of discriminating between CMV reactivation, now postponed until CMV prophylaxis is stopped, and rejection, that mostly presents within the first months after transplantation. The role of chronic subclinical active CMV disease, however, needs to be further addressed, especially with the ongoing discussion in the literature about the role of CMV in the induction of chronic allograft dysfunction. Chapter 4 and 5 further expand on the idea that subclinical CMV infection is important. With these new techniques by which CMV RNA transcription and thus activity is measured in stead of CMV viral load, we might be able to finish this dispute.

As mentioned before, from chapter 6 on the studies in this thesis are focussed on Epstein-Barr virus. With the new tools and strategy described in this thesis we seem to be able to prevent most cases of PTLD.

As to EBV, three main directions are open for further research.

First of all, the hypothesis to use EBV DNA load as a biomarker of over-immunosuppression needs to be validated in other centres and preferably in randomized controlled trials. This should be done in a prospective study in which not only EBV DNA load is monitored, but also EBV specific and alloreactive T-cell responsiveness.

Secondly, the lessons learned in the lung transplant program need to be evaluated in other (solid organ) transplant programs, especially in high volume transplant programs, such as kidney transplantation, and programs with high immunosuppression, such as small bowel transplantation. Not only the pre-emptive strategy to prevent PTLD should be addressed but also the relation between EBV and transplant (dys-)function. If EBV is also associated with transplant dysfunction in other organs, as is suggested by the results of a pilot study done in renal transplant patients 2003, this will have impact on the significance of monitoring of EBV and on the design of treatment protocols of other solid organ transplant programs as well.

Thirdly, the problem of the high incidence of PTLD after primary EBV infection needs to be addressed. As we describe in chapter 11, the only rejection we encountered after EBV DNA guided reduction of immunosuppression, occurred in a patient with primary EBV infection. We still see PTLD in our paediatric lung transplant population (data not shown) mainly due to primary EBV infection. Possibilities for prevention are a pre-emptive strategy not only based on EBV DNA load but also on EBV CTL monitoring (reduction of immunosuppression or pre-emptive administration of Rituximab), long lasting administration of antiviral

drugs, or even, because no vaccine for EBV is available, infecting EBV seronegative patients before transplantation, as occasionally is accidentally done when donor blood transfusions are given before living related kidney transplantation(1).

Reference

1. Babel N, Gabdrakhmanova L, Hammer M et al. Induction of pre-transplant Epstein-Barr virus (EBV) infection by donor blood transfusion in EBV-seronegative recipients may reduce risk of post-transplant lymphoproliferative disease in adolescent renal transplant patients: report of two cases. Transpl Infect Dis 2005:7: 133-136.