

University of Groningen

Immunity to human cytomegalovirus after organstransplantation

van Zanten, Jacoba

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version

Publisher's PDF, also known as Version of record

Publication date:

1996

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Zanten, J. V. (1996). Immunity to human cytomegalovirus after organstransplantation s.n.

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

This thesis is written on the immune response to cytomegalovirus in organ transplant recipients. In this summary, the issue of this thesis will be explained, the study design and the results will be described, and the conclusions will be given.

Cytomegalovirus (CMV) is a member of the herpesvirus family. Other, well-known members of this group are herpes virus I (which causes the herpetic lip lesions), Epstein-Barr virus (the cause of Pfeiffer disease), and varicella-zoster virus which causes chicken-pox. Herpes viruses are commonly found in the human population and about 50% of all adults have been infected by CMV during childhood. The majority of these infections are asymptomatic. The main characteristic of the herpes viruses is that after a primary infection they are not eliminated from the body but remain present in a latent form. Consequently, they can cause reactivation.

In general, primary CMV infections in immunocompetent subjects are asymptomatic. However, in immunosuppressed persons CMV infections may cause clinical disease. General symptoms are high fever, malaise, and arthralgias but this can be accompanied by symptoms of organ involvement such as gastritis, hepatitis or pneumonitis. CMV disease is seen in immunosuppressed persons such as AIDS patients and transplant recipients who receive immunosuppressive treatment in order not to reject the graft. About 50% of the renal transplant recipients and 90% of the lung transplant recipients experience active CMV infection after transplantation. Primary CMV infections occur in CMV-seronegative recipients who have received a graft from a CMV-seropositive donor. Secondary CMV infections occur in CMV-seropositive recipients and are caused by reactivation of the hosts strain when the donor of the graft is CMV-seronegative. A superinfection is caused by reactivation of the host strain together with re-infection with a new strain from the CMV-seropositive graft. Generally, primary CMV infections give more clinical problems because the host has no specific, acquired immunity against the virus. However, due to a severe immunosuppressive regimen, secondary CMV infections may also be accompanied by severe CMV disease. CMV disease is treated by reducing the immunosuppressive treatment and/or administration of the antiviral drug ganciclovir. Since CMV itself has also immunosuppressive effects, lowering of immunosuppressive treatment does not lead to rejection of the graft. Although active CMV infection can be treated effectively, long-lasting subclinical CMV infections have been associated with the development of acute and chronic rejection. This implicates that CMV can cause graft failure in the long term.

Thus, CMV can have a direct and an indirect effect on graft function. This suggests that an effective immune system against CMV which keeps the virus under

control, might be important for the success of the transplantation. This thesis is focused on the investigation into the competence of the immune response of immunosuppressed transplant recipients to CMV. Humoral and cellular immune responses are involved in anti-CMV immunity. Cellular immunity is mediated by T helper cells and cytotoxic T cells. Helper T cells play a pivotal role since they provide "help" to the B cells that produce antibodies and also to the cytotoxic T cells. Cytotoxic T cells recognize CMV-infected cells and kill these cells. The humoral as well as the cellular immune responses to CMV were investigated in renal and in lung transplant recipients.

The second aim of this study was to which viral antigens the immune response was directed. CMV is one of the largest viruses known, and in an CMV-infected cell, many virus-induced proteins are present. We questioned the issue which of these proteins are immunogenic and induce a protective immune response. With this knowledge the generation of an effective and protective vaccine to CMV would be one step closer.

In **chapter 1** CMV is introduced. The architecture of the virus and the proteins that can be found in *in vitro* infected cells are described. The effect of the immunosuppressive treatment on the immune system is explained and a short outline is given of what is known about the immune response to CMV. Finally, the scope of this thesis is depicted.

In **chapter 2** the study into the antibody responses of renal transplant recipients against a mixture of CMV antigens is described. These responses were compared with the antibody responses found in healthy CMV-seropositive subjects. It appeared that both groups developed antibodies to the same repertoire of antigens. However, the patients mounted stronger responses to a particular protein and per patient responses to more proteins were seen. In order to compare the antibody response to a clinical parameter, antibody responses were related to the viral load in the patient during active CMV infection. An inverse relationship was seen between viral load and the antibody responses detected in a patient. Patients with a low viral load had strong antibody responses and vice versa. The proteins that induced high antibody responses were proteins with a molecular weight of 150, 104, 94, 66, 50, 38, and 32 kDa. During a primary CMV infection antibodies against the proteins with a molecular weight of 66, 32, 38, and 50 kDa could be detected first.

In **chapter 3** the antibody responses to two individual CMV proteins in renal transplant recipients were investigated. The responses were detected by an "antigen capture" ELISA. The first protein that was used as antigen was the lower matrix protein, or pp65. This is the 66 kDa protein from chapter 2. The second protein was the glycoprotein gB which is present on the virus envelop and on the cell membrane of CMV-infected cells. Antibodies to this protein were considered to be important for the anti-viral defence since gB is able to induce virus neutralizing antibodies *in vitro*. However, results showed that patients developed higher level of antibodies to pp65 than to gB and antibodies directed against gB could be detected only late during infection. Moreover, an inverse relationship could be found between viral load and the level of antibodies to pp65 but not to the level of antibodies to gB. It was concluded that antibodies to pp65 could be of importance in the humoral immune response to CMV.

In **chapter 4** the antibody responses to different CMV-induced proteins were investigated in lung transplant recipients. The response to pp65 was compared with the responses to three other proteins. To this end prokaryotically expressed recombinant proteins were made. Next to pp65 the immediate early proteins IE1 and IE2 and the DNA binding protein p52 were chosen to serve as antigens. These proteins were chosen because in this way antibody responses were determined to proteins that are abundantly present in an CMV-infected cell (pp65 and p52) and to proteins that are produced in lower quantities but that have important regulatory functions in virus replication (IE1 and IE2). It appeared that pp65 and p52 were the most immunogenic proteins. Lung transplant recipients developed higher amounts of antibody to these proteins than to IE1 and IE2. Here, an inverse relationship was found too between the level of antibodies and viral load. When antibody responses were determined to the same proteins but by an antigen capture ELISA, in which naturally occurring proteins are used as antigen, far higher levels of antibody were found in these patients. No relationship was seen between viral load and the amount of antibody to these naturally occurring (native) antigens. This is possibly due to the high levels all patients had and the minor differences between these responses. So, lung transplant recipients mainly have antibodies to "conformational" epitopes present on naturally occurring proteins and less antibodies to linear epitopes which are present on the recombinant proteins. However, the latter could be of more diagnostic value since they correlate with viral load.

In **chapter 5** it is investigated if B cells obtained from peripheral blood of renal transplant recipients can be used for the generation of human monoclonal antibodies. A new technique is described for the development of these monoclonal antibodies. CMV-specific B cells were selected and cultured. Antibody producing B cells were fused with myeloma cells to yield hybridomas which constantly produce antibodies. The specificity of these antibodies were determined. It appeared that 9 of the 13 hybridomas produced antibodies that reacted with pp65 and 3 were specific for p52. The specificity of one hybridoma remained unknown. From these results it can be concluded that the high antibody levels to pp65 and p52 found during active CMV infection in renal transplant recipients reflects the B cell repertoire in the peripheral blood of these patients.

In **chapter 6** the T helper cell responses to CMV were determined. To this end, the same recombinant proteins as described in chapter four, namely IE1, IE2, pp65, and p52 were used as antigen in proliferation assays. T helper cell responses were detected in renal transplant recipients and in lung transplant recipients. Results were compared with responses found in healthy CMV-seropositive subjects. CMV-infected fibroblasts during late stage of infection which contain all CMV-induced proteins were used as a positive control. Results showed that all healthy subjects responded well to pp65. Also p52 could induce high responses but not every person responded to this protein. Most subjects had proliferative responses to IE1 and IE2 but on the whole, these responses were low. All persons had high responses to the positive control. In contrast, the transplant recipients had low responses to the positive control and no responses at all to the four recombinant proteins. The responses of the lung transplant recipients to the positive control were even more depressed than the renal transplant recipients. This is most likely due to the difference in suppressive regimen. With respect to the immunogenicity of the four proteins the results indicated that the proteins p65 and p52 are not only well recognized by antibodies but also by T helper cells.

In **chapter 7** a third component of the immune response to CMV is investigated, i.e. the cytotoxic T cell responses in lung transplantation recipients. An assay was developed in which the killing of target cells by cytotoxic T cells was determined by measuring the release of ^{51}Cr . CMV-infected autologous fibroblasts were used as target cells. Six lung transplant recipients with a secondary CMV infection, and one healthy CMV-seropositive subjects were tested for the presence of CMV-specific cytotoxic cells. It appeared that only one patient had cytotoxic responses as high as the

healthy subject. The other patients had lower responses. Therefore, it seems that, like the T helper cell responses, also the cytotoxic T cell responses are suppressed in these patients. Nevertheless, the level of these responses correlated with viral load. Patients with a low viral load had higher cytotoxic responses and vice versa. This suggests that cytotoxic T cell responses are important for the control of CMV reactivations.

In **chapter 8** the results that have been described in this thesis are discussed and compared with the findings of others. An outline is given of the immune responses found in the solid organ transplant recipients. As mentioned, this thesis addressed two issues. The first is to investigate the effect of immunosuppressive treatment in these patients on the CMV-specific immune responses. The second issue is to determine the CMV-induced proteins to which the immune responses if focused. With respect to the first issue it can be stated that solid organ transplant recipients are able to develop significant antibody responses but their T helper cell responses as well as the cytotoxic T cell responses are depressed. Due to this hampered T cell reactivity, CMV can reactivate. Presumably, the T helper cell function in patients qualitatively differs from the T helper cell function in healthy CMV-seropositive persons but is just enough to activate B cells so that antibodies can be produced. This results in an immune response predominantly mediated by antibodies. According to the prevalence of CMV infections in immunosuppressed patients this seems to be insufficient to overcome CMV infections. CMV infections are efficiently dealt with when T cell responses can be developed.

With regard to the second issue it can be concluded that of the proteins tested, pp65 and p52 are the most immunogenic proteins. Especially pp65 evokes high antibody responses and T helper cell responses. It was surprising that these intracellularly located proteins induced far higher antibody responses than gB although the latter can be found on the membrane of CMV-infected cells. Moreover, the fact that in spite of high antibody levels CMV infections occur suggests that antibodies play an additional role in the anti-CMV responses and that cytotoxic T cells are more important in this respect. However, antibody responses are valuable for diagnostic reasons.

The ultimate goal of this study is to develop a vaccine against CMV. Transplant recipients who are at risk for a primary CMV infection after transplantation or patients who receive heavy quadruple immunosuppression, like lung transplant recipients, might be helped with such a vaccine. With the findings of this thesis it has become clear that pp65 is a good candidate for this since it induces both cellular and humoral immune responses.