Host immunity against secondary cytomegalovirus infections. Immunological and genetic studies in kidney transplant candidates and recipients.

Roenhorst, Herman Wilhelm

Chapter 9

SUMMARY AND CONCLUSIONS

Cytomegalovirus (CMV) infections are a major cause of morbidity and even mortality in immunocompromised hosts such as transplant recipients and patients with the Acquired Immunodeficiency Syndrome (AIDS). CMV-seronegative transplant recipients can largely be protected against primary CMV infections by using blood products and grafts from seronegative donors.

Cytomegalovirus has the capacity to establish a latent infection after a primary infection. An intact host cellular immunity is important for keeping CMV in latency. Reactivation of latent CMV can be achieved by affecting the immune system with immunosuppressive drugs or allogeneic stimuli.

Most of the kidney transplant recipients are already CMV-seropositive before allografting, and the majority of active CMV infections after transplantation are secondary CMV infections caused by the suppression of host's cellular immunity after institution of immunosuppressive therapy. Better understanding of host-associated factors for the outcome of secondary CMV infections might contribute to the prevention of serious CMV recurrences after transplantation and give insight in the surveillance mechanisms which keep latent CMV under control in immunocompromised hosts.

The basic concept underlying our study is that pre-existent virus-specific host immunity against CMV-infected cells constitutes an important surveillance mechanism against recurrence of CMV infection in latently CMV-infected hosts. A functionally intact afferent arm of the CMV-specific immune response is needed for the activation of the efferent arm, i.e. the rapid recruitment of CMV-specific cytotoxic T lymphocytes and the production of lymphokines, once reactivation of latent CMV threatens or has already occurred.

Thus we studied in immunocompromised hosts conditions that might exert influences on host immunity against CMV-infected cells or on the outcome of recurrent CMV infections, such as blood transfusions and genetic host factors.

CMV-specific cellular immunity was evaluated with the use of lymphocyte proliferation or reactivity (LR) tests. Cytomegalovirus-specific T memory lymphocytes are activated and proliferate in these in vitro cultures of peripheral blood mononuclear cells after stimulation with cell- associated or cell-free CMV antigens.

In all studies virus-specific cellular immunity against CMV-infected cells was also compared with the immune response against circulating CMV particles in immunocompetent and immunocompromised hosts.

Because of the reciprocal relationship between CMV infection and host's general cellular immunocompetence, also tests for general cellular immunity were performed in our study population.

The study population consisted of immunocompromised hosts who were at risk for a symptomatic CMV recurrence or experienced a secondary CMV infection. They were recruited from CMV-seropositive kidney transplantation candidates and recipients. Immunocompetent hosts who experienced a symptomatic primary CMV infection recently or one to five years before, and healthy latently CMV-infected controls without a history of symptomatic CMV disease, were also investigated.

In *chapter 1* a brief review about CMV infections is presented. Fundamental properties of CMV and clinical significance of CMV infections, in particular after kidney transplantation, are described in the sections 1.1 and 1.2. Non-specific and virus-specific immunological host defence mechanisms against CMV infection, and the virus-induced immunosuppressive effects are briefly discussed in the sections 1.3 and 1.4. In section 1.5 some aspects of CMV latency and recurrence are discussed. The aims of the study are described in section 1.6.

In *chapter 2* the development and maintenance of the cellular immune response against cell-associated CMV antigens (CMVFF) was investigated in non-immunocompromised hosts shortly after outbreak of a symptomatic primary CMV infection, during the year thereafter, and one to five years later.

Lymphocyte responses against CMVFF parallelled the development of responses against cell-free CMV antigens (CMV virions), i.e. severely depressed responses in the first two months of symptomatic CMV infection, followed by a gradual increase to normal levels in the year thereafter.

In the acute phase of the disease qualitative differences existed between lymphocyte responses to CMVFF and CMV virions. This was noticed by a lack of correlation between these responses. Some patients even showed positive lymphocyte responses against CMVFF but no responses to CMV virions. Development of cellular immunity to CMV-induced antigens on infected cells prior to that to CMV virions might be biologically important for getting CMV under control, i.e. in latency.

One to five years after a symptomatic primary CMV infection high lymphocyte reactivities against CMVFF and CMV virions were found, indicating a continuous boostering of CMV-specific cellular immunity by endogenous or exogenous CMV, or a selection of high responders in the group of previously symptomatically CMV-infected immunocompetent hosts.

After primary CMV infection general cellular immunocompetence was suppressed. This was revealed by depressed lymphocyte responses to mitogens, allogeneic lymphocytes and microbial recall antigens in the acute phase of the infection, with restoration to normal levels thereafter. We found, in contrast with previous reports, a more prolonged suppression of lymphocyte responses to Concanavalin A (Con A) persisting upto one to five years after a primary CMV infection. This suggests longlasting immunosuppressive effects of a symptoma-tic primary CMV infection on the cellular immunocompetence of non-immunocompromised hosts.

In the *chapters 3 and 4* the maintenance of CMV latency and the long-term immunological consequences of secondary CMV infections were investigated.

The patients studied were CMV-seropositive kidney allograft recipients with a graft survival of at least two years who were on a stable maintenance immunosuppressive regime for more than one year.

The results showed that recipients with an intrinsic capacity to maintain their CMV clinically in latency after transplantation had normal virus-specific cellular immunity to infected target cells i.e. responses comparable with those of healthy CMV-seropositive controls. Long-term renal allograft survivors who experienced a symptomatic secondary CMV infection within the first 6 months after allografting had depressed immunity against CMV-infected cells (chapter 3).

Further results with respect to general host cellular immunocompetence showed that patients with previous symptomatic secondary CMV infections had depressed lymphocyte proliferations to mitogens (phytohemagglutinin, PHA, and pokeweed mitogen, PWM), microbial recall antigens and pooled allogeneic lymphocytes (mixed lymphocyte cultures, MLC).

The alloimmune responses and the lymphocyte responses to cell-associated CMV antigens were positively correlated with each other in these secondarily CMV-infected recipients. Furthermore these responses both increased with prolonged graft survival i.e. time after infection (chapter 4).

These associations are of particular interest, especially since recently an immunologic cross-reactivity between epitopes on immediate-early cell-associated CMV antigens and the β-chain of the HLA-DR molecule has been suggested. This immunologic cross-reactivity may also be reflected in a suppression of the lymphocyte response to CMV-infected cells as well as in a suppression of cellular immune reactivity against class II antigens.

We put forward the hypothesis that this suppression of CMV-specific and general cellular immunity induced by secondary CMV infection appears to exist for years and might contribute to allograft tolerance in kidney transplant recipients.

In a prospective study we analysed the influence of genetic host factors on incidence and severity of CMV infections after kidney transplantation (*chapter 5.*).

The results showed that HLA-DRw6 positive ($DRw6^+$) recipients had a higher susceptibility for posttransplant CMV infections than otherwise comparable DRw6 negative ($DRw6^-$) recipients. Furthermore, $DRw6^+$ secondarily CMV-infected recipients showed an increased incidence of symptomatic CMV disease and CMV excretion during the follow-up period of one year after allografting.

Notably, the other HLA class II antigens were not associated with severity or incidence of posttransplant CMV infections.

We conclude that the DRw6-associated susceptibility is likely a CMV-specific phenomenon because there were no associations with the incidence of (recurrent) herpes simplex virus infections after transplantation.

The influence of blood transfusions on CMV-specific and general cellular immunity was investigated (*chapter 6.*).

The study group consisted of CMV-seropositive candidates for kidney transplantation. The results showed a remarkably high incidence of "non-responders" to non-structural or early (CMVFF-EA) as well as to late or structural cell-associated CMV antigens (CMVFF-LA) among transfused patients.

We found that in the group of previously transfused patients general cellular immunocompetence was also impaired. This was reflected by a high incidence of non-responders in delayed type skin reactivity tests after de novo sensitization with dinitrochlorobenzene (DNCB) and low lymphocyte proliferation to microbial recall antigens and allogeneic lymphocytes.

Blood transfusions (3 volumes of leukocytes containing, packed red cells) were given to never transfused, CMV-seropositive, transplantation candidates with the aim to improve kidney allograft survival. This, incidentally, resulted in a somewhat depressed in vitro lymphocyte response to CMVFF-LA and Con A. The allogeneic interactions induced by blood transfusions, certainly, did not result in a significant boostering of pre-existing CMV-specific cellular immunity in dialysis patients.

Lymphocyte responses to cell-free CMV antigens were not influenced by blood transfusions.

The association between HLA-DR antigens and CMV-specific and general cellular immunity was studied in previously transfused CMV-seropositive kidney transplantation candidates (*chapter 7.*).

HLA-DRw6 was associated with depressed immune responses against CMVFF-EA and LA. This phenomenon was not CMV-specific since DRw6 was also associated with disturbances in general cellular immunity such as a high incidence of non-responsiveness to DNCB and depressed lymphocyte responses to microbial recall-antigens.

The presence of HLA-DR4 was associated with high responsiveness to cell-associated CMV antigens. Lymphocyte responses against CMV virions were not associated with DR antigens.

The predictive value of pretransplant memory lymphoycte responses to early and late cell-associated and cell-free CMV antigens for the outcome of secondary CMV infections after kidney transplantation was evaluated in a prospective study (*chapter 8.*).

The absence or presence of pretransplant lymphocyte responses to non-structural or structural cell-associated CMV antigens was not related with the incidence of posttransplant symptomatic CMV recurrences. Absent immune reactivity against non-structural cell-associated CMV antigens contributed to a productive CMV infection once a symptomatic CMV infection had developed.

A pretransplant non-responsiveness to CMV virions was a risk factor for acquiring a symptomatic CMV recurrence after kidney transplantation, independently of the presence of DRw6 antigen. DRw6$^-$ responders to CMV virions had the lowest incidence of symptomatic secondary CMV infections during a follow-up period of one year after transplantation.

The severity of secondary CMV infections was not related to low or high responders state of host's general cellular immunocompetence before allografting.

Concluding remarks

The normal immune responses against cell-associated CMV antigens in hosts who maintain their CMV clinically in latency even under immunosuppressive therapy and allostimulation, and the very high responsiveness against cell-associated CMV antigens in immunocompetent hosts even years after a symptomatic primary CMV infection, support the hypothesis that circulating memory T cells against CMV-infected cells have a surveillance role in the prevention of recurrent CMV disease.

Pretransplant responders to early cell-associated CMV antigens have a much lower incidence of productive CMV infections than non-responders to these antigens. Responders to early and/or late cell-associated CMV antigens are, however, not fully protected from symptomatic CMV recurrences after kidney transplantation.

A genetically determined host factor, i.e. the presence of HLA-DRw6 phenotype, is a more important risk factor for symptomatic secondary CMV infection. DRw6 is not only associated with strongly suppressed immune responses to early and late cell-associated CMV antigens, but also with depressed immune reactivity against other antigens in previously transfused CMV-seropositive candidates for a kidney transplantation. It is possible that previously received blood transfusions or previous primary CMV infections exert longlasting suppressive influences on host's cellular immunocompetence particularly in DRw6 positive patients. Futher studies are needed to explore these possibilities.

The absence of pretransplant responsiveness to circulating CMV particles is another risk factor for symptomatic CMV recurrence, independent of the DRw6 status of the host.

Identification of recipients at risk for a serious CMV recurrence after kidney transplantation might help in the management of these recipients. Prophylactic measures such as specific antiviral drugs, interferon, anti-CMV hyperimmune globulin or interleukin-2 immunotherapy, in order to prevent serious CMV disease after transplantation, could be applied more selectively and more effectively in high risk patients when they are selected on their DRw6 status and the presence or absence of pretransplant memory responses against cell-free and early (non-structural) cell-associated CMV antigens.

SAMENVATTING

Cytomegalovirus (CMV
mortaliteit in immunolc
plantaten of patienten r
deficiency Syndrome, a
bijna volledig tegen pri
bloedprodukten en orga

Cytomegalovirus kar
niteit van de gastheer i
CMV is mogelijk door
catie of allogene stimuli

De meeste ontvange
voor CMV. Het merend
gevolge secundaire CM
immunologische afwee
Een beter begrip van
infecties bepalen, zou
infecties na niertranspla
welke latent CMV onde

Aan onze studie ligt
immuniteit tegen met C
het optreden van sym
patienten. Een functior
response is nodig voor
met een snelle recrutei
van lymfokines, zodra
gevonden.

Derhalve bestudeerd
factoren, welke van ir
teerde cellen of op de
miteerde personen.

De CMV-specifiek
proliferatie- of reactivi
geactiveerd en gaan p
perifere bloed na stimu

De virusspecifieke
tevens vergeleken met
in immunocompetente

Wegens de reciproc
heer werden ook teste
onderzoeksgroepen.

De onderzoekspopu
het risico liepen op e
secundaire CMV infe
seropositieve kandida
personen die recent of
doorgemaakt en gezo
van symptomatische