Diagnostic and Immunological Aspects of the Antibody Response to Human Cytomegalovirus Infection

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The studies described in this thesis were initiated with three main aims:

First, to develop a practical and sensitive method for serodiagnosis of human cytomegalovirus (CMV)-infections and to determine the relative diagnostic value of IgM and IgG antibody responses to distinct CMV-antigens.

Second, to test the hypothesis that antibody responses directed against the CMV-MA complex located on the outer surface of CMV-infected cells would contribute to the recovery from infection, especially in patients receiving immune suppressive therapy and, in addition, to study the relation of the CMV-MA complex to other CMV-antigens.

Third, to determine the molecular nature of CMV-antigens that are recognized by human serum antibodies during acute and latent CMV-infection.

For these studies sera were collected longitudinally over a period of 5 years from individuals with a different immune status background, having either a latent or active CMV-infection. The patients were immunocompromised renal allograft recipients with a primary or secondary CMV-infection and non-compromised patients with community-acquired or post-transfusion CMV-infections. The controls were renal allograft recipients that remained without CMV-infection and healthy laboratory and hospital personnel.

Previous studies from our laboratory had shown that the bulk of CMV-antibodies formed in response to CMV-infection was directed against CMV-antigens of the early (CMV-EA) and late (CMV-LA) antigen complex, mainly located in the nucleus of the infected cell.

Based upon these observations an enzyme-linked immunosorbent assay (ELISA) was developed for the detection of IgM and IgG antibodies to CMV-EA and CMV-LA separately, as described in chapter 2. Increasing the ratio of CMV-antigenic proteins relative to the total protein content in whole cell extracts, used as antigen for the ELISA-test, greatly improved the sensitivity of the technique. A detailed procedure for optimal and reproducible production of the individual CMV-antigens is included.

In this and all subsequent studies it was found that CMV-antigens prepared from CMV-strain AD169 allowed the specific detection of antibodies to human CMV and did not cross-react with antigens from other human herpesviruses. The influence of IgM-rheumatoid factor which may give a false positive CMV-IgM reaction was minimalized. However, it was essential to test all sera for reaction with control antigen from non-infected cells in order to exclude possible false positive reactions due to the presence of "auto-antibodies".

The ELISA-method is shown to be of equal or slightly higher sensitivity in detecting CMV-antibodies compared to the indirect immunofluorescence test and significantly more sensitive compared to the complement-fixation technique. The presence of even low levels of naturally acquired circulating CMV-antibodies reflects previous exposure to CMV and therefore possible latent virus carriership which may not be detected with other serological techniques. Thus, the ELISA-method is recommended for the selection of CMV-seronegative (blood) donors. This type of donor-selection is specially indicated to prevent CMV-transmission to patients at risk of developing a primary CMV-infection.

The reproducibility of the method, including the antigen preparation, facilitates longitudinal studies and abrogates the need of retesting previous serum samples. The application of the CMV-ELISA test for routine CMV-serology is included in chapter 6.

Healthy donors and long-term survivors after renal transplantation showed a stable CMV-serology (less than 4-fold fluctuations of antibody titers in
subsequent sera) over a 2-4 year period. Consequently, changes in antibody titers exceeding the 4-fold level are considered to be of diagnostic value and may indicate ongoing CMV-infection in the patient with concomitant exposure of viral antigens to the immune system. Despite the lack of detectable lymphocyte-reactivity in renal allograft recipients (these data will be subject of the forthcoming thesis of H.W. Roenhorst) antibodies to CMV-antigens appear in the circulation of most patients at or shortly after onset of clinical symptoms of CMV-infection. Their detection may be of help for the diagnosis and the choice of therapy in these patients.

In this respect, antibodies of the IgM- and IgG-class directed against the CMV-LA complex were of diagnostic value and were the earliest antibodies detectable in response to CMV-infection. IgG anti CMV-LA showed a significant increase in all patients and IgM anti CMV-LA was detectable (frequently before IgG) in all cases of primary CMV-infections and in 71% of the secondary CMV-infections. The titer of CMV-LA IgM was positively related to virus-excretion in urine or saliva in these patients.

In this study the antibody responses to CMV-EA were not found to provide additional information for the serodiagnosis. In comparison to complement-fixation serology the CMV-ELISA test may allow more rapid identification of CMV-infection in renal allograft recipients. This should be further tested in prospective studies.

Interestingly, despite the immune suppressive therapy the group of renal allograft recipients had significantly higher antibody titers compared to the previously healthy, non-immune compromised patients with active symptomatic CMV-infections. This may reflect a higher exposure to CMV-antigens in the former patients and adds to the possible diagnostic value of antibody responses in these patients. On the other hand the increased antibody response may be of value for patient's recovery especially since cell-mediated immune mechanisms may be relatively inefficient in immune suppressed patients. This was the subject of the second part of the investigations.

Considering the cell-associated nature of human CMV it was hypothesized that antibody-mediated recognition and destruction of CMV-infected cells would limit viral spread and thus contribute to the host's recovery. In order to corroborate this hypothesis we monitored the development of IgM and IgG and cytolytic antibodies to CMV-MA in relation to the clinical course of active and latent CMV-infections in patients with a different immune status background.

For the specific detection of IgM and IgG antibodies to CMV-MA it proved to be essential to modify previously published methods as described in chapter 3. A microplate modification of the improved membrane immunofluorescence method that facilitated the simultaneous analysis of multiple samples is also described. The use of viable CMV-infected cells, kept at 0-4°C was required in order to minimize possible reactions with intracellular CMV-antigens and the CMV-induced Fc-receptor. The specificity of the CMV-MA reaction was confirmed by using monoclonal antibodies directed against intracellular CMV-antigens. No relation was found between antibody titers against CMV-MA and those against intracellular CMV-antigens in human sera suggesting that different antigens were bound. This is further extended in chapter 4 and 5.

During these studies we found that healthy, latently infected persons only occasionally had detectable antibodies to CMV-MA whereas antibodies to CMV-EA and CMV-LA were readily detectable in all donors even with very high titers. Interestingly, patients who were recovering from a recent CMV-infection had increased levels of circulating CMV-MA antibodies. These patients sera were molecules on the cell surface including viral DNA-polymerase. Another set of CMV-MMA (CMV-LMA) antigens that were discriminated from CMV-LMA by different reaction with viral DNA-polymerase. The appearance of CMV-MMA was considerably earlier in recovery patients. Their initial antibody response was directed to CMV-EMA whereas the secondary antibody response to CMV-LMA was of IgG-class. The capacity of CMV-MA antibodies, after their initial rise, was determined as well as their presence in patients sera.

An interesting finding was the occurrence of antibody-mediated recognition and destruction of CMV-infected cells in patients with a different immune status background. The capacity of CMV-MA antibodies to kill CMV-infected cells was determined as well as their presence in patients sera.
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differences is not known at present. The capacity of CMV-MA antibodies to mediate the destruction of CMV-infected cells was determined in a complement-dependent cytolytic assay based upon Chromium-release as described in chapter 5.
Due to the low density-distribution of CMV-EMA molecules no effective lysis of CMV-infected cells was obtained with human sera early in the infectious cycle. Concurrent with the appearance of CMV-LMA molecules CMV-infected cells were more efficiently lysed and the amount of lysis was related to the antibody titer against CMV-MA in the serum used.
CMV-cytolytic antibodies were never observed in healthy CMV-seropositive controls and were only detectable in patients with a recent CMV-infection. The highest levels of CMV-cytolytic antibodies were found in renal allograft recipients with a secondary CMV-infection. By isolating the IgG fraction from the sera of these patients, we could demonstrate that the lysis of CMV-infected cells is also mediated by human IgG-antibodies, thereby extending previous observations by others.
An interesting finding in this study was the observation that purified CMV-virions were rather inefficient in absorbing CMV-cytolytic antibody activity, whereas non-purified virion-preparations, still containing cell-membrane fragments, were nearly as efficient as intact CMV-infected cells. These data suggest that the antigenic make-up of the virion envelope and the plasma membrane of the infected cell are (in part) different from each other. These findings are supported by additional data as mentioned in chapter 5. However, as to date the precise molecular nature of the CMV-MA complex remains to be determined (see also chapter 7).
Using the above mentioned techniques - ELISA, membrane immunofluorescence and cytolytic antibody assay - we monitored the development of antibody responses to the distinct CMV-antigens, CMV-EA, CMV-LA, and CMV-MA, in relation to clinical and virological parameters as shown in chapter 6. The diagnostic aspects of antibody responses to the intracellular CMV-anti-
gens, CMV-EA, and CMV-LA have been summarized above.
The measurement of antibody responses to CMV-MA did not have diagnostical value since they did not develop in all patients. Therefore the CMV-ELISA technique is preferred for diagnostical purposes. However, besides being of diagnostical value CMV-antibodies contribute to the host’s recovery. In particular, the development of antibody responses to CMV-MA may be of prognostical value since their occurrence appeared to be related to subsequent recovery from CMV-disease.
In this study no cytolytic antibodies were found in the few cases of fatal
CMV-infection. On the other hand the presence of significant levels of anti-
CMV-MA antibodies, particularly the cytolytic ones, related with the sub-
sequent recovery from infection. Such a relation was not found for antibody
responses to intracellular CMV-antigens as measured by ELISA.
The development and level of CMV-MA and cytolytic antibody responses was
positively related to both short-term and long-term recovery from CMV-
infection. For kidney transplant patients with a secondary CMV-infection -
who developed the highest antibody responses to CMV-MA - a significant
relation was found between the level of the cytolytic antibody response and
the graft-function at one year follow-up, being better in patients that had
the highest cytolytic antibody responses.
This relationship was not as clearly found for allograft recipients with a
primary CMV-infection which may be related to a less efficient response due
to the lack of pre-existing CMV-MA memory lymphocytes. In addition, the
relative low level of CMV-MA antibody responses in non-immunocompromised
patients with a CMV-infection suggests that recovery from infection in these
patients may be successfully mediated by cellular processes. However, also
in these patients the development of a high cytolytic antibody response was
paralleled by a more rapid recovery and by the absence of relapse of symp-
toms.
A possible explanation for the observed high frequency of CMV-infections in
renal allograft recipients, especially concerning the secondary infections
due to reactivation of endogenous CMV, is described in the discussion sec-
tion of chapter 6.
Our results together with recent data from other groups indicate an impor-
tant role for immunological recognition of CMV-MA in the recovery from
CMV-infection. It is therefore suggested that improvement of the host's
immunity to these CMV-antigens may significantly contribute to prevent and
limit symptomatic CMV-infections in the human host. This may be achieved by
active immunization through vaccination with CMV-MA containing (subunit)
vaccines, or by passive immunization using hyperimmune human sera containing
CMV-MA cytolytic antibodies. These sera have to be obtained from individuals
recovering from a recent CMV-infection since the sera from healthy latently
infected donors lack the effective antibodies. The methods required for this
serological selection are outlined in the chapters 3 and 5.
In chapter 7 a start was made to characterize the molecular nature of CMV-
antigens recognized by human serum antibodies. It was found that the anti-
body responses of individuals with either a latent or an active and
symptomatic CMV-infection predominantly were directed against antigens pre-

dominantly present in the nucleocapsid fraction of CMV-infected cells. These CMV-antigens
had molecular weights similar to the structural component of the virion,
suggesting possible identity. These observations are in agreement with the
serological data which show that human serum antibodies are predominantly
directed against antigens of the CMV-LA complex, which are mainly located in
the nuclear inclusions of CMV-infected cells being the sites of virion
assembly.
An important observation in this study was the apparently oligoclonal anti-
body response in patients at risk of developing a symptomatic CMV-infection
after renal transplantation and immunosuppression. This was even more
pronounced in those patients that had the most severe clinical course and may
be one of the factors that determine the outcome of CMV-infection, either as
subclinical infection or symptomatic disease. These oligoclonal responses
are not reflected by the serological data which measure "overall" antibody
responses to a complex of CMV-antigens of which some components are clearly
immunodominant by virtue of their abundance.
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Further studies are clearly needed in this field, in particular for the elucidation of the molecular composition of the CMV-MA complex. The development of monoclonal antibodies with defined specificity for CMV-MA molecules may be of great help in this and may elucidate in more detail the role of individual CMV-MA components in host's immunological defence which is a major determinant for recovery from infection and maintenance of life-long CMV-latency.