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Human Cytomegalovirus (HCMV) – Revised*

Arbeitskreis Blut, Untergruppe «Bewertung Blutassoziierter Krankheitserreger»

1 Current Knowledge about the Pathogen

1.1 Characteristics of HCMV

Together with animal cytomegalovirus, human cytomegalovirus (HCMV), also referred to in recent literature as human herpes virus 5 (HHV-5), belongs to the Herpesviridae family, subfamily Betaherpesviridae, genus Cytomegalovirus. It was isolated in 1956 for the first time. The name is derived from the fact that it causes enlargement of the infected cell (cytomegaly) and induces characteristic inclusion bodies. In the blood, it is predominantly cell-associated, above all with granulocytes and macrophages. The HCMV genome consists of a double-stranded DNA with approximately 230,000 bp. The genome is enclosed by an icosahedral capsid (100–110 nm diameter, 162 capsomers). Between the capsid and the virus envelope is a protein layer known as the tegument. The virus envelope is derived from cell membranes. At least eight different viral glycoproteins are embedded in the lipid bilayer. The mature viral particle has a diameter of 150–200 nm (fig. 1). Like all herpesviruses, HCMV is sensitive to low pH, lipid-dissolving agents, and heat. HCMV has a half-life of approximately 60 min at 37 °C and is relatively unstable at –20 °C. It needs to be stored at at least –70 °C in order to maintain its infectivity.

A distinction is made with herpes viruses between i) the lytic infection cycle and ii) the latency which leads to life-long infection of the organism. Characteristics of the beta-herpes viruses such as also HHV 6 and HHV 7 are their high level of host specificity, their slow replication cycle and the spread of infection from cell to cell in the cell culture even in the presence of neutralising antibodies.

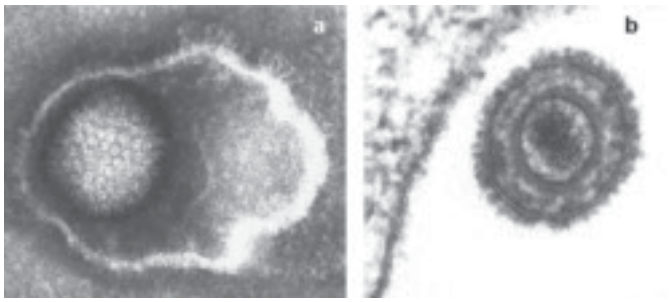

Fig. 1. Electron microscope images of HCMV particles. a Negative contrast: the surface proteins (glycoproteins) are partially visible on the dilated virus envelope. The contrast medium has penetrated the virus particle (deformation of the lipid envelope) and the nucleocapsid with its subunits can be identified in the interior. b Ultrathin section through a virus particle. Inside the virion, the nucleic acid (DNA) is strongly stained with the contrast medium. The protein layer of the tegument can be identified between the capsid and the virus envelope. The dense edge of the glycoproteins can be seen on the lipid bilayer.

The lytic infection of cells can be monitored using protein expression patterns and the replication of nucleic acid. The immediate early (IE) proteins are responsible for the regulation of the early (E) proteins and also for that of the late (L) proteins.

After adsorption of the virus onto the target cell with the aid of viral glycoproteins, the virus envelope fuses with the cell membrane, the capsid is released into the cell and is transported to the nucleus where the genome is released. Transcription of the IE proteins then takes place in the cell nucleus with the aid of the RNA polymerase II of the host cell. Tegument proteins of the infecting virus particle act as transactivators for the IE genes.

The IE proteins regulate the following stages of viral replication and are also involved in cell regulation including the expression and transport of the HLA antigens (class I MHC proteins) to the proteasoma. IE proteins (in particular the

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phosphoprotein pp65) can be used as early markers of the virus infection in cell cultures. The E proteins include the HCMV-coded DNA polymerase interacting with viral nucleotide kinases, the activity of which can be specifically inhibited with antiviral agents.

The synthesis of the structural proteins (L proteins) is regulated via the E proteins. The viral capsids are formed in the cell nucleus; export and enveloping of the viruses take place on the inner nuclear membrane (possibly also on other cell membranes) HCMV shows pronounced cellular association [1].

Studies with monoclonal antibodies point to differences between viral strains and isolates. The most recent studies using primary isolates and the corresponding sera from patients show that strain-specific neutralising antibodies are formed. Whether the variability of antigens is the main cause here as with other virus families or whether there are serological subtypes has not yet been clarified [2].

1.2 Infection and Infectious Diseases

In immunocompetent individuals, most HCMV infections take an asymptomatic course or display minor symptoms not very characteristic for the disease. HCMV finds its way into the body by means of mucous membrane contact or parenterally (via blood components containing cells or via stem cell/organ transplants), and can lead to a general infection with involvement of the organism such as encephalitis, retinitis, hepatitis, nephritis, splenomegaly, and colitis. Transmission of the virus to the foetus/child can be transplacental or via cervical or vaginal secretions and breast milk (peri- and postnatal infection). Also sexual transmission via cervical secretions or semen, or via the saliva [3–6] is possible.

The incubation period is 4–8 weeks. In this phase viraemia occurs; however, the major part of the viruses still remains mainly cell-linked. The cell-associated viral replication can take place in different cell types (e.g. epithelial cells, endothelial cells, various parenchyma cells, mononuclear cells) [7]. Ductal epithelial cells of the salivary glands, but also the renal epithelia and glandular cells of genital organs, are particularly affected by HCMV [8]. During viraemia, HCMV is excreted in the above mentioned secretions. Anyone with an HCMV infection can transmit the virus – even if they are asymptomatic.

Like other herpes viruses, primary HCMV infection, which can be asymptomatic or cause serious illnesses in high-risk patients (see below), enters latency. The following sites are discussed as latency reservoirs in the blood and bone marrow:

- CD34+ haematopoietic progenitor cells
- CD33+ haematopoietic progenitor cells
- Monocytes
- Dendritic cells
- Neutrophil granulocytes
- Macrophages [9–14].

Roback et al. [13] collected leucocytes from mice 6–8 weeks following CMV infection, which they divided into a fraction rich in granulocytes and another fraction rich in monocytes. Both fractions where transfused to CMV-naïve mice. Only the monocytes transmitted CMV in 35–50% of cases; depending on the cell quantity administered. It remains to be analysed to what extent these results can be transferred to humans.

A distinction is necessary in principle between HCMV infection and HCMV disease. Damage to tissues and organs is caused both by direct cytotoxic effects of HCMV by induction of cytotoxic T lymphocytes and interferon-γ production by specific CD4+ lymphocytes, and natural killer cells, and by indirect, HCMV-induced immunopathological phenomena (e.g. immunosuppression, the formation of pro-inflammatory cytokines) [6, 7].

The incidence of congenital HCMV infections is between 0.3 and 1.2% [1]. HCMV transmission thus represents the most frequent congenital infection. Congenital infections are usually caused by a primary infection of the mother during pregnancy with an intrauterine transmission rate of 40–50%. Furthermore, preconceptionally HCMV-seropositive mothers can be infected with an additional HCMV strain (maternal secondary infection) with an infection rate of 1% of all neonates of seropositive mothers [1]. About 7–10% of HCMV-infected infants develop disease involving clinical manifestations such as petechiae, jaundice, hepatosplenomegaly, chorioretinitis and sometimes permanent neurological damage (e.g. mental retardation, impaired hearing or even deafness, motor deficits) the consequences of which are fatal in about 10% of cases [4, 6]. Another route of infection is the breast milk-associated postnatal HCMV transmission, which for instance occurs in 35–40% of the premature newborns of seropositive mothers who have a considerably higher risk regarding HCMV infection than mature newborns, [1, 15, 16]. A marked reduction in the transmission rate can be achieved by freezing the breast milk at approximately –20 °C for at least 24 h since the low temperatures reduce infectivity [17, 18]. In mature newborns, the infection is usually symptom-free.

Important pathogenetic mechanisms for the occurrence of HCMV disease in tissue or organ transplant recipients are lack of immunity and/or immunosuppression on the one hand and reactivation of the latent virus in the event of pre-existing HCMV infection of the recipient on the other [6]. The reactivation of HCMV can be triggered or intensified by, amongst other things, interactions with other viruses (e.g. HHV-6) or increased cytokine production to the point of a 'cytokine storm', during bacterial infections, graft-versus-host disease (GVHD), or treatment with antilymphocytic antibodies. Consequently, in addition to serological status and the type and intensity of immunosuppressive treatment, other risk factors for HCMV disease are intercurrent bacterial and fungal infections, hepatitis following liver transplant, or GVHD following allogenic stem cell transplantation [6, 7]. The type of transplanted organ also affects the development of HCMV disease

(highest risk with lung transplant, lower with heart/liver transplant, lowest with kidney transplant). HCMV pneumonia and, more rarely, gastrointestinal ulcers and retinitis are the major manifestations of HCMV disease in recipients of allogenic stem cell transplants [19]. In case of transplantation of solid organs from HCMV-seropositive donors the disease usually leads to damage of the transplanted organ (e.g. hepatitis following liver transplantation and pneumonia following lung transplantation) [6].

As a result of immunomodulatory effects of the glycoproteins, HCMV also has pathogenetic significance in transplant patients for acute or chronic rejection reactions. In addition and because of the immunosuppression induced by HCMV, it has pathogenetic significance for bacterial and fungal superinfections or opportunistic infections and Epstein-Barr-associated lymphoproliferative disorders (post-transplantation lymphoproliferative disorder) [7].

HIV-infected individuals are very frequently seropositive for HCMV and usually develop symptoms as a consequence of the reactivation of the latent virus due to progressing immunosuppression and only rarely as a result of primary infection. The occurrence of clinical HCMV manifestations correlates with the severity of the immunosuppression, and patients with CD4+ T lymphocyte levels < 50–100/µl are particularly affected. The most common direct cytotoxic effect caused by HCMV in HIV-infected individuals is HCMV retinitis, which usually starts on one side but later progresses to both sides and can even lead to blindness. The frequency of HCMV retinitis has decreased following the introduction of Highly Active Antiretroviral Therapy (HAART) with protease inhibitors and HIV reverse transcriptase inhibitors. At the same time, however, the occurrence of intraocular inflammation was observed with HAART which has been interpreted as the immune system's response to persistent viral proteins in HCMV lesions of the retina [20]. Other manifestations of illness (e.g. gastroenteritis, colitis pneumonia, meningoencephalitis, polyradiculoneuropathy) are rare.

Patients who receive (poly)chemotherapy and/or irradiation treatment for an underlying malignant disease often experience a transient disturbance of cellular immunity which, however, does not usually progress to HCMV disease as a result of a primary or secondary infection [21].

1.3 Epidemiology

The prevalence of specific antibodies to the ubiquitous HCMV varies hugely depending on the socio-economic standards of a country [22]. In general, the prevalence in industrialised regions such as America, Western Europe and Australia is lower than in Third World countries. The prevalence of HCMV-specific antibodies in adults is said to be up to 100% in many countries in Africa and Asia whilst in industrialised countries it is on average between 40 and 70% [23].

The prevalence increases with age and reaches rates of between 5 and 30% by the 6th year of life. Infection of the population in industrialised countries occurs in two phases: A first peak is reached in the first 2–3 years of life as a result of smear infection, and a second lower peak is reached in adolescence and early adult life, between the ages of 16 and 30 years, as a result of sexual contacts [24]. After that period, the portion of seropositive individuals increases with age up to 50–70% [25].

Following infection, HCMV can be excreted in the urine or saliva for a prolonged period. The reactivation of latent infections may lead to sporadic virus excretion. Neonatally acquired HCMV infection leads to greater and more prolonged chronic virus excretion than later infection.

1.4 Detection Methods and Their Significance

For diagnosing CMV infection, a variety of detection methods are available including direct virus identification by cell culturing, antigen detection of the virus, detection of CMV DNA, detection of IgM and IgG antibodies, or detection of T cell responses against CMV, which can be applied depending on the problem under study [reviewed in 26, 27]. Primary diagnosis of a HCMV infection can be established by antigen detection (pp65), for instance in leucocytes in the blood, saliva and urine, as well as by means of isolating the virus or nucleic acid amplification techniques (NAT) before seroconversion [24, 25]. Seroconversion is confirmed by the detection of HCMV-specific IgM and/or IgG antibodies in the serum. For this purpose, both ELISA and immunofluorescence tests are available.

Diagnostics of the infection is performed serologically based on increases in IgM and IgG titres, which are measured in two serum samples drawn at an interval of about 2 weeks. The domain of the serological tests lies in the definition of the serostatus [28]. A reactivation (or secondary infection) can be detected serologically by means of identifying a significant increase in the titre of HCMV-specific antibodies. In addition, through avidity determination of the IgG antibodies, a distinction can be made between primary infection (low avidity and binding against multivalent antigen) and secondary infection (high avidity). The immunoblot is considered the gold standard for confirmation of IgM antibodies in the serum [29, 30].

To monitor reactivation in transplantation patients, quantitative detection of pp65 antigen in neutrophil granulocytes, and, recently to an increasing extent, quantitative HCMV DNA detection from whole blood or plasma are performed. In the case of HCMV encephalitis, HCMV DNA can be detected in the liquor. For long-term treatment with antiviral substances (e.g. in AIDS patients with retinitis), in vitro sensitivity determination of the viruses against medicinal products used or genotype resistance determination by means of sequencing can be sensible diagnostic methods in justified individual cases.

In future, microarrays may complete HCMV diagnostics [31].

2 Blood and Plasma Donors

2.1 Prevalence and Incidence in Donor Populations

The prevalence of antibodies amongst blood donors in Germany and other European countries is between 37 and 65%, depending on the age of the donor populations. In Düsseldorf, the prevalence of HCMV amongst blood donors increased from about 33% in the 18–23 years age group to about 73% amongst 61- to 65-year-olds [32]. In Third World countries, the prevalence can reach 100% [33, 34]. The annual incidence in Germany and other central European countries is 0.8–1.2% [35–38]. In a group of thrombocytapheresis donors in the USA, low incidence rates were recorded. The authors supposed that the main reason for this is the particularly strict selection of donors in the production of cytaphereses, resulting in a lower rate of HCMV infections in these donors [39].

2.2 Definition of Exclusion Criteria

There are no specific exclusion criteria at the present time. In the case of clinical symptoms, the donor is deferred from the donation.

2.3 Donor Testing and Significance

For the following reasons, serological testing for antibodies against HCMV can no longer be recommended:
– high incidence in the donor and recipient population
– low risk for immunocompetent recipients
– obligatory leucocyte depletion with removal of the cell-associated HCMV.

Before the era of leucocyte depletion of red blood cell and platelet concentrates, HCMV-seronegative donors were selected for high-risk patients. Products from HCMV antibody-positive donors were not used for this particular patient group. With the introduction of general leucocyte removal, the HCMV-associated transfusion risk has been reduced to a level as low as that when exclusively HCMV-seronegative products without leucocyte depletion are used.

The use of leucocyte-depleted *and* HCMV-seronegative red blood cell and platelet concentrates can increase the relative risk of transfusion-associated HCMV infections. There is a portion of viraemic donors among those donors tested HCMV-seronegative. The probability of using an undetected viraemic donation for transfusion is higher if the donation was selected from the HCMV-seronegative donor subpopulation only, in comparison with selection from the total population of donors (HCMV-seronegative plus HCMV-seropositive plus HCMV serostatus unknown) [40]. In this context, it is assumed that leucocyte-depleted HCMV-seropositive products practically bear no risk of infection [38].

In the case of blood components which cannot be leucocyte-depleted (e.g. granulocyte and lymphocyte concentrates), donations from HCMV-seronegative donors should be used in HCMV-seronegative patients. For high-risk patients, it is desirable to manufacture these products from donors which have also been tested for the absence of HCMV genome using a sensitive NAT.

2.4 Donor Interviews

Because this virus infection does not show specific symptoms and its course is frequently asymptomatic, interviewing donors in a targeted manner does not provide useful information.

2.5 Donor Information and Counselling

If in the rare cases when HCMV donor testing is carried out, serological evidence of an HCMV infection is obtained, it is not necessary to inform the donor. In view of the high prevalence and the absence of consequences for immunocompetent individuals, no specific advice is planned.

3 Recipients

3.1 Prevalence and Incidence of Blood-Associated Infections and Infectious Diseases in Recipient Populations

The age-dependent prevalence of HCMV infection can be as high as about 70% in Europe. According to Weber and Doerr [34], seroprevalence is not increased in multiply transfused patents and intravenous drug abusers compared with the general population. No European literature is available providing information on the period after the introduction of general leucocyte depletion of red blood cell and platelet concentrates, with the exception of transplantation recipients and some special patient populations.

3.2 Immune Status (Resistance, Existing Immunity, Immune Response, Age, Exogenous Factors)

According to current knowledge, the cellular immune response plays the main role in virus elimination and improvement of clinical symptoms. The significance of the humoral response for the course of the infection is unclear. Re-infections can occur in high-risk individuals such as promiscuous individuals, in-patients, nursing staff caring for sick children etc. Several strains of HCMV have been identified in such individuals in some cases, using the methods of molecular biology. According to van der Meer et al. [5], autologous virus-neutralising antibodies can contribute to accelerated virus elimination or prevention of dissemination.

The fact that severe disease courses following HCMV infection occur almost exclusively in patients with impaired cellular immunity emphasises the significance of the prevention of primary infection/reactivation of the latent virus in this patient group. Immediately after primary infection, non-specific mechanisms (e.g. natural killer cells, interferon) initially prevent the spread of HCMV [41]. The specific immune response, mediated by MHC-I-restricted CD8+ cytotoxic T lymphocytes, and also by MHC-II-restricted T helper lymphocytes, is responsible for the early control of the HCMV infection and the development of long-lasting protective immunity [5]. An important target antigen for cytotoxic T lymphocytes is the tegument phosphoprotein pp65, and the epitopes of its split products. CD8+ T lymphocytes directed against IE proteins are responsible for preventing an HCMV disease. Other antigens important for the immune response include pp150 and the glycoprotein B [42, 43]. Up to 25% of all peripheral CD8+ T cells can be involved in the permanent suppression of viral replication. CD4+ T lymphocytes are essential for the induction, replication, and preservation of memory cells [44]. The immunomodulating properties of HCMV such as the down-regulation of MHC-I on the surface of infected cells prevent the presentation of cytotoxic T lymphocytes and favour life-long persistence of the viruses.

3.3 Severity and Course of the Disease

The primary infection of healthy, immunocompetent individuals with HCMV is usually asymptomatic. If symptoms occur, they manifest themselves in the form of a clinical syndrome resembling that of influenza or mononucleosis with fever, relative or absolute lymphocytosis and/or phayryngitis, lymphadenopathy, hepatosplenomegaly, or slight increase in transaminase levels [4]. In contrast, primary infection or reactivation of the latent virus in congenitally infected neonates or immunosuppressed patients, in particular recipients of allogeneic stem cell transplantations and HIV-infected patients, can lead to serious or even fatal clinical manifestations [45, 46]. Other risk groups include pregnant women and nursing mothers with regard to the transmission of the infection to the child [8, 21, 41, 48].

The risk of HCMV transmission is minimised by the compulsory leucocyte depletion of cellular blood components. On the other hand, there is no increased risk of illness from HCMV infection for HCMV-seropositive recipients of autologous or allogeneic stem cell transplantations or organ transplantations, and for full-term infants [reviewed in 8, 49].

3.4 Therapy and Prophylaxis

In general, a distinction is made between the prevention of HCMV infection/disease and the treatment of manifest HCMV disease. Prevention includes prophylactic measures and measures to suppress viral replication. Whereas prophylactic measures are started in patients in whom virus and disease cannot be identified, suppressive measures (also known as 'pre-emptive treatment') by definition relate to patients in whom HCMV infection with viral replication, but not manifest disease, can be detected. The administration of intravenous immunoglobulins (IVIG) or HCMV-specific immunoglobulin come into consideration as a preventive measure only in isolated cases [45, 46]. Studies which confirm the efficacy of IVIG are comparable only to a limited extent due to different HCMV prophylaxis regimens [47]. Nowadays, the administration of IVIG for the prevention of HCMV infection is no longer recommended in stem cell transplantation [49]. Published measures to prevent transmission of HCMV through cellular blood components are transfusion of leucocyte-depleted or HCMV-seronegative blood components, the administration of antiviral agents, and an adapted cellular immunotherapy [6, 43, 44, 50–52]. Although the clinical significance of effective prevention of HCMV disease is undisputed in the above-mentioned at-risk groups (see 3.3) because of the high rates of morbidity and mortality of HCMV disease, there is as yet no consensus on the optimum preventive measures. As far as the transplantation of solid organs is concerned, a prophylaxis with antiviral substances is becoming more and more common [53–55]. However, preventive measures should always be based on the severity of the immunosuppression, the risk of reactivation of a latent HCMV infection, and the tolerability of the antiviral agent [6, 7, 51].

Antiviral agents which are currently available for the prevention of HCMV infection or for treatment of overt disease are the nucleoside analogues ganciclovir, valganciclovir and cidofovir, and the pyrophosphate analogue foscarnet sodium. All these substances inhibit HCMV DNA polymerase and therefore HCMV replication. They have to be administered intravenously in order to reach therapeutic plasma concentrations and in some cases can cause severe organ-specific adverse reactions (ganciclovir: myelotoxicity, mainly with neutropenia but more rarely with thrombocytopenia, and, in the case of long-term administration, anaemia; cidofovir: dose-related nephrotoxicity; foscarnet sodium: impaired renal function, electrolyte disturbances). Valganciclovir can be orally administered. The agents listed here slow down the replication of HCMV and are able to suppress clinical symptoms, but they cannot eliminate the viruses. Because of pre-emptive administration of ganciclovir, late manifestations of HCMV disease (>100 days following transplantation compared with manifestation in the first 100 days after transplantation) have become a major problem [56]. Administration of the monoclonal antibody alemtuzumab against CD52+ cells as part of the immunosuppressive regimen can lead to HCMV reactivations which can be prevented by the prophylactic administration of suitable virostatics [57].

A detailed description of the established preventive measures and current treatment strategies for known risk groups

with HCMV disease (i.e. infants with congenital HCMV infection, transplant patients, and HIV-infected patients) can be found in article of de Jong et al. [6], in the published results of the Canadian Consensus Conference on HCMV as well as in the article by Schleiss [58].

The development of vaccines has a high priority, also from the point of view of economic aspects, i.e. costs incurred due to an HCMV disease. A vaccine for routine use is not yet available due to the latent infection cycle and stem-specific variations, lacking induction of vaccination protection, and problems encountered in clinical studies [1, 59–61].

As an alternative to the virostatics which are heavily marred by adverse reactions [6, 51, 52, 62], in the past few years, adoptive immunotherapy with HCMV-specific CD8+ cytotoxic T cells has increasingly developed into an additional option for the treatment of HCMV reactivations showing only few adverse reactions. In principle, the donor's immunity (activated T cells and memory cells) against a particular pathogen is transmitted to the recipient directly by transfusion. The working groups of van den Bosch et al. [43] and Moss and Rickinson [44] have published summaries of the milestones of adoptive immunotherapy. The prerequisite for this is an ex vivo expansion of HCMV-specific cytotoxic T cells and CD4+ T helper cells of the seropositive stem cell donor by restimulation with HCMV epitopes. There is a great number of protocols on ex vivo expansion of the virus-specific T lymphocyte lines [62–69]. Apart from the time required, a disadvantage is the relatively low survival period of the replicated cells by generation of a proapoptotic molecule (CD95) during replication. The selection of antigen-specific T cells from the donor by HLA tetramers makes the collection of a sufficient number of cells possible for direct infusion [69–73]. The avoidance of stimulation with vital viruses by using autologous dendritic cells pulsed with virus lysate (such as pp65 peptide or peptide mixtures) reduces unwanted virus exposure of the recipient [69]. Simultaneous isolation of CD4+ and CD8+ T lymphocytes by means of methods which recognize the cell populations by secreting cytokines is almost ready for clinical application [43, 44].

If the donor is HCMV-seronegative, vaccination of the donor for the purpose of generating HCMV-specific T lymphocytes could be an option for the treatment of primary HCMV infections of the recipient. This method, however, is still in its infancy [43, 63].

According to preliminary research data, autologous T cells after transfer of genes which encode T cell receptors for the required viral peptides could eventually serve as a basis for HCMV-specific T cell lines, if an expansion of virus-specific T lymphocytes in HCMV-naïve transplant donors should not be possible [44].

Adoptive immunotherapy can bring about a rapid reconstitution of the cellular immune response against HCMV, above all after stem cell transplantations, all the more, since some transplantation protocols involve T cell depletion (e.g. in the case of haploidentical transplantation). Some studies were able to provide proof of the efficiency in the treatment and prevention of HCMV diseases [62, 63, 67, 71, 74]. Administration of virus-specific donor T lymphocytes a few weeks after transplantation will further reduce the risk of occurrence of GVHD even though the reasons for this are as yet unknown [44, 75]. It is also still unclear whether antigen-specific T4 or T8 lymphocytes or both cell populations simultaneously should be administered [76].

In the meantime, after new molecular targets have been identified, therapeutic options with a lower spectrum of adverse effects have been developed, such as marabivir, a protein kinase UL97 inhibitor [77–79].

3.5 Transmissibility

In the 1980s, the overall risk of an HCMV infection was in the order of 30–60% for stem cell transplantation patients receiving blood components without taking into account the CMV antibody status and without leucocyte reduction [80, 81]. HCMV transmission to seronegative recipients by seropositive blood was described in 0.4–4% of recipients. It can be concluded that infection is not transmitted from the majority of seropositive donors [8, 48, 82]. As long as non-leucocyte-reduced blood components were administered, the risk of HCMV infection was reduced by HCMV-seronegative cellular blood components [83, 84]. The general use of leucocyte-depleted blood components has also reduced this risk. Spiking experiments have shown a reduction in leucocyte-depleted blood components of about 3 log levels with regard to cellular HCMV DNA [84, 85].

3.6 Frequency of Administration, Type and Amount of Blood Products

Preiksaitis and co-workers [86] have shown for cellular blood products that recipients of transfusions who seroconverted after transfusion had received a higher number of units (50 ± 38.9) than non-seroconverted patients (23.7 ± 15.3). It is not only the quantity of blood products but also the underlying disease of the patients that influences the risk of infection [87].

In a study, Nichols et al. [88] have found an increase in risk of 32% per leucocyte-depleted red blood cell concentrate compared with the basic risk of an HCMV infection, and no increase in risk with leucocyte-depleted platelet concentrates manufactured from apheresis. The results of this study are not undisputed, due to the calculation methods used [89]. Ronghe and co-workers [90] could not find any difference concerning the HCMV seroconversion rate in patients receiving allogenic stem cell transplantations who received either leucocyte-depleted platelet concentrates manufactured by apheresis or

HCMV-seronegative non-leucocyte-depleted platelet concentrates. Red blood cell substitution was performed with non-leucocyte-depleted HCMV-seronegative red blood cell concentrates [90].

Roback and co-workers [13] examined the minimum infectious dose for the transmission of a CMV infection in mice. No CMV transmission was observed in the case of an application of $\leq 10^4$ leucocytes per 25 g mouse weight. Roback concluded that the equivalent human dose should be 4×10^5 leucocytes/kg body weight. Thus, if the limit of 5×10^6 leucocytes/product was observed, this should prevent a transmission of HCMV [13].

No HCMV transmissions have so far been described for therapeutic plasma, regardless of a leucocyte depletion or pathogen inactivation [91, 92].

Based on the production methods, transmission of HCMV by plasma derivatives (such as coagulation factors, immunoglobulins, albumins) is not to be expected.

4 Blood Products

4.1 Infectious Load of the Starting Material and Test Methods

According to studies by Weber et al. [93], about 2–12% of the HCMV antibody-positive blood donors are infectious. According to Sivakumaran et al. [94], free virus particles were found in the blood of HCMV-seropositive blood donors, especially when the samples were left standing for a prolonged period, thereby causing damage to cells.

HCMV is predominantly cell-associated. It is estimated that 0.2% of the leucocytes present in peripheral blood of an HCMV antibody-positive donor are latently infected, i.e. contain HCMV genome. Using sensitive PCR methods, Larsson's working group was able to identify HCMV genome in all seropositive donors [95]. Identification of an antigen as sign of active virus replication, on the other hand, was possible only in about 6% of a group of blood donors, which, however, was small [96].

New examinations with partly improved techniques modify existing findings. In the period between the infection and the time when HCMV antibodies are detectable for the first time (6–8 weeks), viraemia is observed by means of consistent studies [38, 91, 97–99]. During seroconversion, the likelihood of viraemia was indicated to be approximately 1%. The identification of HCMV DNA in leucocytes is rarely successful after seroconversion [100]. A current study by Ziemann and co-workers [38] was able to detect HCMV DNA, partly up to one year after seroconversion, using a very sensitive PCR in 44% of the plasmas examined from seroconverters. Samples before seroconversion were available from 68 donors. In two cases (2.9%), HCMV DNA could be detected in the plasma 68 or 98 days before the first seropositive sample was available ('window phase donation'). In 450 donors who had been seropositive for more than one year, HCMV DNA could not be found in the plasma in any of the cases [38]. This result is comparable with results of other examinations [97, 99, 101]. Considering the calculations by Roback [13] concerning the minimum infectious dose, the low probability of detecting HCMV-infected cells in symptom-free donors one year or later after seroconversion and the efficiency of the leucocyte filters used, the risk of an HCMV transmission by donors who have been HCMV antibody-positive for at least 12 months should be extremely low.

It is not necessary to test plasma for fractionation for HCMV (cf. 3.6). Testing is indicated in individual cases for products containing cells. The above-mentioned procedures/methods (see 1.4) are suitable test methods. NAT methods are currently not yet comparable and their quality should be checked after standards have been established in collaborative studies [102].

4.2 Methods for Removal and Inactivation of the Infectious Agent

The use of non-leucocyte-depleted blood products from HCMV-seronegative starting material reduces the incidence of infection to 1–4% in HCMV-seronegative bone marrow and organ recipients. Although the results from studies on the equivalence of leucocyte reduction compared to the application of seronegative blood are contradictory, the evaluation of all results shows that the leucocyte elimination and the use of HCMV-seronegative non-leucocyte-depleted blood components are equivalent [40, 41, 87, 88, 103–110]. Viraemia during the pre-seroconversion phase, which is a major cause of transfusion-associated HCMV infections that are still recorded to a small extent, can be controlled neither by leucocyte depletion nor by exclusive use of HCMV antibody-negative blood components [111].

Leucocyte depletion for red blood cell and platelet products is compulsory in Germany. Thus, an identification of the HCMV donor status is unnecessary, apart from special cases of cellular blood components which cannot leucocyte-depleted. Moreover, the selection of exclusively HCMV-seronegative donors with their higher relative portion of viraemic donors compared with the population of total donors can increase the risk of HCMV transmission (see also 2.3).

HCMV transmissions by therapeutic plasma are as yet unknown. This may be due to the instability of the virus in a cold environment around –20 °C, an opinion which is based on the experience gained with frozen breast milk [17, 18].

According to several results of studies which agree with each other, there are various pathogen inactivation methods which reliably inactivate HCMV [112–115]. As HCMV is one of the viruses with a lipid envelope, any measures which attack the lipid envelope (e.g. S/D process) lead to the inactivation of HCMV. HCMV is also inactivated by heat treatment

(e.g. pasteurisation). Moreover there are filters which retain the HCMV measuring 150–200 nm, and which can be used for the manufacture of plasma-derived products. HCMV transmission by plasma-derived products can be ruled out on the basis of current knowledge.

4.3 Feasibility and Validation of Procedures for Removal/Inactivation of Infectious Agents

The significance of leucocyte depletion for the safety of cellular components has already been dealt with under 4.2. HCMV cannot be used directly for the experimental demonstration of the viral safety of plasma-derived products as it cannot be cultured in high titres in cell cultures and is difficult to titrate. In addition, antibodies in the plasma would react with the virus and distort the results. For this reason, animal herpes viruses are used to test the process as model viruses [112, 116, 117]. The most frequently used virus is the Pseudorabies virus (herpes virus of the pig). It is comparable to HCMV in its characteristics, e.g. thermolability and sensitivity to lipid solvents and extreme pH values.

5 Assessment

HCMV infections show low pathogenicity in healthy, immunocompetent individuals and cause only mild, if any, symptoms. HCMV leads to latent (persistent) infections with the possibility of recurrence with viral replication (with and without symptoms). Severe HCMV illness is observed with increased frequency, however, in individuals whose immune systems are impaired or who are immunoincompetent. Although antiviral chemotherapy and, in some cases, adoptive immunotherapy is possible, transmission of HCMV by blood components should be avoided as far as possible for certain recipient groups. These include pregnant women, premature infants, anyone with acquired or genetic immune impairment, and individuals undergoing massive immunosuppression with significant T cell number reduction for therapeutic reasons (such as recipients of stem cell transplantations and (homologous) transplants). The removal or reduction of leucocytes by filtration (leucocyte depletion) has proved to be a successful method of minimising the risk of transfusion-associated HCMV infection. Leucocyte depletion is not effective in viraemic donors in the seroconversion phase since the virus is not fully cell-associated.

If leucocyte-depleted cellular blood products are used, a selection of seronegative donors is no longer useful. On the contrary, the selection of HCMV antibody-seronegative donors could increase the risk of HCMV transmission. The reason for this is that in acutely infected still seronegative individuals, a non-cell-associated viraemia may be present.

If non cellular blood components which cannot be leucocyte-depleted are administered, e.g. granulocyte or lymphocyte concentrates, donations should be used from donors without HCMV antibodies. In such cases, the HCMV antibody examination should be completed by an HCMV NAT if possible. Regarding the use of HCMV NAT, there is a particular need for research to establish threshold values and national as well as international standards.

Transmission of HCMV by therapeutic fresh frozen plasma derivatives (such as coagulation factors, immunoglobulins, or albumin) is not to be expected.

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