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Cytomegalovirus infection in patients with solid-organ transplant. Recently reviewed immunologic response and pathogenicity mechanisms

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ABSTRACT. Cytomegalovirus (CMV) infection in immunocompromised host is an important cause of morbidity and mortality. The protective immunity against the virus is both humoral and cellular. Immunologic mechanisms in rejection as in the immune response against CMV infection are similar but there is difficult to separe as histologic and clinically independent events. At least eight different genes of CMV are homologous to human proteins related to the immune response. The potential role of these genes with homology to human genes can be at different levels. The relevance that immunodominant antigens have on the natural control of CMV infection, suggests that the future design of a vaccine directed to protecting from disease those susceptible to primary infection, in an immunocompromised state, should involve a combination of antigens that include pp65, IE1-exon 4 and gB as a recombinant proteins.

Key words: Cytomegalovirus, rejection, immunology, solid organ transplant.

RESUMEN. La infección por citomegalovirus (CMV) en el huésped inmunocomprometido es una causa importante de morbilidad y mortalidad. La inmunidad protectora contra el virus es tanto humoral como celular. Los mecanismos inmunológicos que ocurren en el rechazo como en la respuesta inmune a la infección por CMV son similares y es difícil separarlos como situaciones histológicas y clínicamente independientes. Al menos ocho genes del CMV muestran homología con proteínas humanas relacionadas con la respuesta inmune. El papel potencial de estos genes que muestran homología con genes humanos, puede ser a distintos niveles. La relevancia que tienen los antígenos inmunodominantes en el control natural de la infección por CMV, sugieren que en el futuro, en el diseño de una vacuna dirigida a proteger de enfermedad, a quienes son susceptibles de infección primaria durante una etapa de inmunocompromiso, puede ser el de construir vacunas con una combinación de antígenos que incluya a los antígenos pp65, IE-exon4 y gB como proteínas recombinantes.

Palabras clave: Citomegalovirus, rechazo, inmunología, trasplante de órgano sólido.

INTRODUCTION

Cytomegalovirus (CMV) infection in solid-organ or bone marrow transplant recipients is an important cause of morbidity and mortality.[1,2] Its association with the development of acute or chronic rejection is a matter that has been dealt with for several decades.[3-5] The purpose of this review is to conduct a critical analysis of the most recent findings available in the medical literature with respect to the study of the pathogeny of CMV infection and its association to acute and chronic rejection, as well as the possible implications of the disease caused by this virus and the development of future vaccines.

BIOLOGY OF CMV INFECTION

CMV is a beta herpes virus (65-68 nm) belonging to the herpesviridae family. Its genome is composed by double stranded DNA of approximately 240 base kilopairs (150 million Daltons). An envelope serving as an essential element for the virus´ infectivity surrounds it. It is also known as human herpes 5 (HCMV-5) of slow growth in cell culture. The start of its replication takes about 12 to 24 hours after the cell is infected and the evidence of the cytopathic effect in the cell culture, that is to say the formation of plaques, can be seen 7 to 14 days later.[6]

Just as the rest of the members of the Herpesviridae family (Herpes simplex, Epstein-Barr, Varicella-Zoster, Herpes-6, Herpes-7 and Herpes-8), once the infection has occurred, the viruses remain in the cells for life, even after administering specific antiviral treatment. The permanence of the viral genome in the cell nucleus can be found in two different types of arrangements: circular (episomal) or linear.[7]

The arrangement of the viral genome is closely related to viral replication. During the latent infectious period (permanence of the virus within the infected cell without the replication of its genetic material), the DNA of the CMV is detected in a circular conformation.[7] The linear form exists during the virus´ active replication period and seems to be related to a concatenated genetic arrangement based on concatemeres, necessary for the expression of certain promoters of DNA replication.[7]

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Rev Latinoam Microbiol 2001; 43 (4): 177-182

IMMUNITY AGAINST CMV

Humoral immunity

The protective immunity against the virus is both humoral and cellular. Within the range of antibodies, those outstanding are those against surface glycoproteins (glycoproteins gB and gH) that probably participate in the blockage of cell infection.[8] Immunization with these recombinant proteins shows the development of antibodies capable of decreasing the plaque forming units of the virus *in vitro* in relation to the greater development of antibodies.[9] The administration of specific immunoglobulins against CMV for the prevention of illnesses in SOT recipients is useful in decreasing the incidence of disease.[10] However, humoral immunity by itself is not capable of detaining the development of disease in the absence of cellular immunity as is often seen in bone marrow transplant patients.[11] In these patients, randomized clinical trials show an important effect in the reduction of infection, but not so in its development.[12-14]

Cellular immunity

Among the viral antigens, the 65 KD phosphoprotein (pp65) and the protein that codifies the immediate-early 1-exon 4 are two of the immunodominant dianes in the response of the cytotoxic T lymphocytes.[15,16] These antigens induce the response of the CD8+ memory lymphocytes during the primary infection in about 90% of immunocompetent individuals.[17] Other antigens that induce a cytotoxic cellular response of the T lymphocytes are the gB protein and the pp150 phosphoprotein, although these were only seen in a third of the seropositive patients studied.[17] The cell response to the immunodominant antigens pp65 and the IE1-exon 4 protein is mediated primarily by the class I major histocompatibility complex alleles and therefore, by the induction of the CD8+ lymphocytes.[17,18] However, the formation of CD4+ lymphocytes can also be induced (mediated by class II antigens of the major histocompatibility complex for the pp65 antigen, although its role is still undefined.[17] The induction of the formation of cytotoxic T lymphocytes to these antigens, as already mentioned, is mediated by class I antigens, which means that once the induction for the formation of CD8+ cells has been carried out, these will only recognize those antigens presented by the same alleles that participated in their induction. Therefore, this subsequent response is not transferable (MHC restriction), and therefore can possibly be recovered using autologous cells or lost by cell depletion in early stages of differentiation.[19,20] Therefore, it has been seen that the incidence of CMV disease in seropositive patients that are selectively given CD34 peripheral blood stem cells, have a greater incidence of disease caused by cytomegalovirus than those provided with non-selected stem cells (22.6% *vs* 4.2%).[21] On the other hand, the protection of patients with bone marrow transplants can be carried out through the selection of CD8+ lymphocytes from the patient possessing specificity for recognizing CMV and once administered are capable of recognizing the cells with the viral antigens, protecting the patient from disseminated disease.[22]

Based on the above, patients deprived of artificial cellular immunity, but that have humoral immunity or even those given immunoglobulins immune against CMV, as is the case of bone marrow transplant recipients, require both types of immunities to co-exist in order to impede the development of CMV disease.

CMV AND REJECTION

One of the questions that still needs to be answered is the ability of CMV to induce rejection in solid-organ transplant recipients. This has been a concern of clinical research for a number of years. Both phenomena seems to be closely related and are often seen to occur at an important frequency, but that due to the immunologic mechanisms shared, it is difficult to separate them as independent clinical situations.

IMMUNOLOGIC MECHANISM OF REJECTION

The immune recognition of strange antigens in grafts is mediated by class I and class II MHC antigens. Class I molecules are expressed in all nucleated cells and platelets, while class II are found on B lymphocytes, monocyte-macrophage system cells and dendritic cells.[23] T cells and non-lymphoid cells show class II protein when activated by cytokines.[24,25] Rejection depends on the coordinated activation of T cells and antigen-presenting cells.[23] For example, in cases of kidney rejection, tubulitis is one of the main diagnostic criteria[26] and consists in the invasion of the tubular epithelium by lymphoid cells. The cells that are involved in the development of tubulitis are mainly CD8+ T lymphocytes.[27] These cells are attracted by the secretion of β-chymokines, mainly MCP-1 and MCP-1β (monocyte chemotactic peptides), although also participating are MIP-1α and RANTES (activation regulating peptides, normally expressed in T cells and presumably secreted).[28] In cases of heart transplant rejection, something similar to this occurs.[29] A constant mechanism seen in cells is the expression of their own antigens in order to be recognized and avoid to be damaged.[23] When the expression of class I antigens is lesser than normal, the natural killer cells cause

Rev Latinoam Microbiol 2001; 43 (4): 177-182

the lysis of these cells.[23] When the antigens present are not recognized by the T cell receptors as their own, the mechanism activated is the selection of specific clones that can carry out the cytotoxicity, fundamentally done by CD8+, mainly affecting those cells that show a greater amount such as endothelial or epithelial cells.[25,28,29]

ACUTE AND CHRONIC REJECTION IN SOLID-ORGAN TRANSPLANT RECIPIENTS

Acute rejection seems to be a risk factor for chronic rejection in renal transplants.[30,31] However, many patients having a single episode of acute rejection, do not have subsequent episodes of rejection. On the other hand, those having two or more rejections, are at a greater risk for developing chronic rejection than those having just one episode.[32] This effect is important in terms of graft survival, at least in renal transplants where those without rejection episodes have a 10 year survival rate of 91%, while those having one episode have an 85% survival rate and more than one, 53%.[32] Of these patients, those developing chronic rejection are in a greater proportion than have more than one episode of acute rejection (64.1%) compared to those that have one episode (21.9%) and those that never have acute rejection (3%).

From a tissue compatibility point of view between the donor and the recipient, that is, through the class I and II MHC antigens, there is a very important determining factor, as for example the presence of receptor antibodies, specific against the MHC class I donor antigens. In these cases, the grafts are rapidly and irreversibly rejected.

RELATIONSHIP BETWEEN CMV AND ACUTE ORGAN REJECTION

For several years now, a number of clinical studies have shown a probable association between CMV and acute organ rejection.[34-36] However, prospectively it has been difficult to attribute the role directly to the virus, in spite of the fact that one study showed that a group of patients reverted the rejection when treated with gancyclovir.[37] It should be noted that the cells that infiltrate the graft, both in cases of rejection as in CMV disease, are the same, as will be shown below. In this sense, it is difficult to separate both instances. However, differentiating one clinical possibility from another is in practice, possible by measuring antigenemia or viral load. The problem becomes more complicated when both situations co-exist or the disease complicates the management of the rejection. Other studies[38] have not been able to show the exclusive association of CMV with acute rejection, in spite having observed a greater number of rejections in the patient group (n = 57 transplants) with viral reactivation. This study showed an association to rejection only when there was a co-infection with herpes 7 and CMV disease, suggesting that a greater immunologic condition, maybe mediated by rejection and therefore a greater probability of developing the viral disease. To this respect, a group of renal transplant recipients[39] were studied based on the number of rejection episodes and compared with another group of patients that had no rejections. The frequency of CMV disease was greater in the recipient group with the higher number of rejections, which occurred earlier in those given anti-lymphocyte antibodies. Similarly, pancreas-kidney transplant recipients[40] in whom graft rejection was avoided by the induction of anthymocyte globulin, in the absence of steroids, were found to have CMV disease at a similar frequency (28%) to that of the population without induction with these types of preparations.[1] Seen from another prospective, in liver transplant recipients given anti-CMV polyclonal immunoglobulins, the incidence of rejection was significantly less than in those which received them (19% *vs* 48%).[41]

In vitro studies to this respect show that endothelial cells infected by CMV induce the expression of molecules that favor the adhesion of leucocytes to those cells through the induction of the expression of ICAM-1 type molecules, blocking the capacity of expression of VCAM-1 and selectin E, in spite of being stimulated with TNF-alpha.[42] The attraction of these cells, can in such manner, favor vascular rejection, but may also favor dissemination using leucocytes as vehicles.

A meta-analysis study on the search for evidence on a decreased incidence of rejection with the use acyclovir and/or gancyclovir as prophylaxis showed a significant decrease of CMV infection, but was unable to show a significant decrease in the incidence of rejection, while carrying out a separate analysis for each antiviral agent.[43]

CMV AND CHRONIC REJECTION

In a study conducted in liver transplant recipients,[34] CMV infection associated with rejection was only seen in approximately a fourth of all cases (26%), but not in all cases of chronic rejection. Similarly, sharing one or two haplotypes and the presence of CMV infection showed an apparent association and a relative risk of 10, however, the association between the total incompatibility (not sharing any haplotypes) and the absence of CMV infection was not measured. On the other hand, just as in cases of acute rejection, the effects of CMV infection may simulate rejection.[44,45]

Evidence has shown several important data. The group at greater risk for the development of CMV disease, that is the D+/R- combination, is associated to chronic rejection when the viral infection occurs for a longer period of time (detection in over a 30 day period).[45] However, this phenomenon was associated to a greater incompatibility between class I MHC antigens. In this

Rev Latinoam Microbiol 2001; 43 (4): 177-182

case, it is difficult to show that there is a true association between the viral infection and the development of the rejection, in which case the rejection should have occurred more frequently in a group with greater compatibility with prolonged infection. Another study on a large series of patients (n = 1339),[46] did not show an important association between CMV infection and the subsequent development of chronic rejection, with a stronger association, as seen in other studies, between acute rejection and the development of chronic rejection.[47,48]

An effect observed during active infection by CMV, both in heart transplant recipients, as in kidney transplant patients, is the production of antibodies that react with endothelial cells.[49] These antibodies recognize, in addition to the endothelial cell antigens, those expressed on cells such as fibroblasts, keratinocytes, platelets and mononuclear peripheral cells, with a greater amount of antibodies in the sera from patients after the infection than shortly after the start of the infection.[50]

The above would have us believe the probability of a potential role for homologous proteins that codify certain CMV genes, mainly those similar to the class I heavy chain antigens and the beta chymokines due to a cross reaction. It is also possible, that some role can be played by the recognition of specific antigens located mainly in cells where the virus replicates during active infection.

PROBABLE DAMAGING MECHANISMS AND THE EVASION OF THE IMMUNE RESPONSE

The early replication of CMV in polymorphonuclear leukocytes is well known. This has been used as an early diagnostic tool for quantifying antigen expression, such as pp65 (antigenemia).[51] The intensity of the expression of this antigen shows an important correlation to the amount of viral particles in the blood, as well as the severity of the disease.[52,53] The next step after viral replication in the polymorphonuclear leukocytes is the infection of endothelial cells, as has been shown in patients with prolonged antigenemia of over 2 weeks,[54] when the intensity of the antigenemia showed a positive correlation with the intensity of the grade of endothelial cells infection. Similarly, when low levels of antigenemia were found (< 40 cells per 200,000), no endothelial cells were found infected by CMV.[54] It is interesting that the a greater number of infected endothelial cells were found in patients with acute rejection than in those without it (66% *vs* 15%, respectively).

At least eight different genes of CMV are homologous to human proteins related to the immune response.[55,56] Some of these homologies occur with proteins as the receptors coupled to the G protein that act as signal transducers mediated by lipids, nucleotides, peptides and proteins. Others are the class I heavy chain of MHC antigens and β-2 microglobulin, but it has also been shown in genes homologous to proteins with chymokine activity, that in the case of the human CMV, these are the alpha-chymokines.[55,56] The potential role of these genes with homology to human genes can occur at different levels: favoring the attraction of immune cells that can be infected, in the case of the chymokines or altering the immune response mechanisms in the presentation of antigens in cases where they are homologous to the class I MHC heavy chain or of β-2 microglobulin, favoring the lysis of the infected cells but also the delivering of viral particles.[56]

One of the mechanisms implicated in the permanence of the virus in the infected cell is the blockage of the stimulating effect of interferon-γ in the presence of antigens mediated by the class I MHC.[57,58] In this case, the viral proteins are transformed into smaller peptides by the proteosome complex in the cytosol. These are then transported by the transporter associated with the processing antigen complex (TAP). The TAP complex is formed by two subunits TAP1 and TAP2, residing in the endoplasmic reticulum and in charge of the coupling of the antigenic viral peptides on the class I MHC heterodimers.[59] These heterodimers are formed by the heavy chain and the β_2-microglobulins and are structural elements of the class I MHC antigens. Once on the cell surface, these structures present the antigens to the T CD8+ lymphocytes.[59] Interferon-γ then increases the effect of this mechanism through the induction of protein synthesis that participate in the presentation of the antigens dependent on class MHC antigens (proteosome proteins, class I MHC heavy chain antigens, β_2-microglobulin, TAP1, TAP2).[60]

Another of the effects observed of the CMV in the mechanism described, is the transduction mechanism of the interferon-γ signal mediated by the Jak1 protein, a tyrosine kinase belonging to the Janus family, whose levels decrease in fibroblasts and infected proteins.[58] The same occurs with the signal transducer and activator of transcription 1 for interferon-γ (named Stat1 protein).

In addition to the commented mechanisms, the CMV US3 protein retains the class I MHC heterodimers in the endoplasmic reticulum in the immediate early stage of infection. The US2 and US11 proteins carry out a retrograde transport of class I heavy chains from the endoplasmic reticulum to the cytosol, where they are degraded by the proteosome complex. In addition, the US6 inhibits the functioning of the TAP complex.[61]

Although many of the mechanisms commented on have been studied *in vitro*, these effects have been recently observed indirectly by following CMV replication kinetics (viral load) in liver and renal transplant recipients[62,63] and the immune response of cytotoxic T lymphocytes (CD8+). These studies were carried out by two different researchers and using the technology behind the construction of tetrameres employ-

Rev Latinoam Microbiol 2001; 43 (4): 177-182

ing heavy chains and those of beta-2 microglobulins of class I antigens, a 9 or 10 amino acid oligopeptide and an enzyme that acts as a signal when linking to the identified cell, in this case, the CD8+ lymphocytes specifically against CMV.[62,63] In both studies, an increase in cytotoxic lymphocytes was seen when there was an increase in viral load and its subsequent control, manifesting the importance of cellular immunity for the control of infections (in recipients seropositive for infection). In certain patients, the response to the increase of CD8+ lymphocytes specific against CMV was not seen as an important viral load and there was only an increase when achieving a decrease in viral load with specific antiviral treatment (ganciclovir), making very clear the role played by the virus on the immunologic response (seronegative receptors).

CONCLUSIONS

The recently studied immune mechanisms and the behavior of CMV show a clearer view on what is known about the pathogeny of the disease caused by this virus in SOT recipients. The role played by CMV, whether in acute or chronic organ rejection, is still controversial. The relevance that immunodominant antigens have on the natural control of CMV infection, suggests that the future design of a vaccine directed to protecting from disease those susceptible to primary infection, in an immunocompromised state, should involve a combination of antigens that include pp65 recombinant proteins, IE1-exon 4 and gB. The stimulation of specific cellular immunity against the virus in these patients, may have a very important impact on three healthcare aspects for SOT recipients: 1) an important decrease in the incidence of the disease due to CMV and therefore of the complications associated to it (rejection, bacterial and fungal infection); 2) the prevention of resistance to antiviral due to their lesser use; 3) an important reduction in costs due to a lesser need for hospitalization and lesser use of drugs.

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